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"Lipid profiles of deep-sea organisms"

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ABSTRACT

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LIPID PROFILES OF DEEP-SEA ORGANISMS

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Lipids are a group of biologically important compounds that may be used as indicators of relationships between an organism and its environment. Lipid class composition identifies types of storage lipid which can suggest a pelagic or benthic existence and may be used to measure the condition of an organism. Fatty acid composition may reflect trophic ecology. Lipid profiling has been used to elucidate elements of the ecology of organisms from Mid-Atlantic and Juan de Fuca Ridge hydrothermal vents and shrimp from below a permanent oxygen minimum zone. Lipid profiles of these organisms from extreme deep-sea environments are compared.

Lipid profiles of adult Mid-Atlantic Ridge hydrothermal vent shrimp, Rimicaris exoculata, support a benthic existence and primarily chemoautotrophic bacterial nutrition. Postlarval alvinocarid shrimp lipid profiles suggest a pelagic life cycle stage with a reliance on phototrophically derived organic matter. This result supports previous molecular evidence that Rimicaris exoculata travels between hydrothermal vents on the Mid-Atlantic Ridge. The branchial area of Rimicaris exoculata has been reported to be colonised with bacteria and elevated levels of bacterial fatty acids are present in these tissues. Lipid analyses of the reproductive organs of Rimicaris exoculata reveal increases in triglycerides, sterols, phosphatidyl choline and the proportion of n-3 fatty acids with advancing reproductive maturity. Preliminary studies of the lipid profiles of the Mid-Atlantic vent shrimp Alvinocaris markensis support the hypothesis that it is a scavenger and contains a lower proportion of bacterially derived fatty acids.

The lipid profiles of the deep-sea shrimp, Nematocarcinus gracilis, from the Indian Ocean, were consistent with a benthic lifestyle, opportunistic feeding and a reliance on phototrophically derived organic matter. Despite the presence of a permanent oxygen minimum zone above the habitat of the shrimp, Nematocarcinus gracilis contain low levels of highly unsaturated fatty acids, reflecting a reduction in the availability of labile organic matter with depth.

The hydrothermal vent tube worm Ridgeia piscesae contains only low levels of storage lipid, reflecting an extreme reliance on endosymbiotic bacteria. Wax esters detected are thought to be stored in oocytes. The fatty acid profile of Ridgeia piscesae reveal mainly bacterial biomarker fatty acids, but some phototrophically derived fatty acids are also present. Adult Ridgeia piscesae have no gut, so the mechanism by which these fatty acids are assimilated is uncertain.

Lipid profiles of the hydrothermal vent palm worm, Paralvinella palmiformis vary with the conditions of the different microenvironments in which specimens were found. Fatty acids reflect a mixed diet based mainly on chemoautotrophic bacteria, but with significant inputs of phototrophically derived organic matter, such as diatom debris.

Phototrophically derived fatty acids were also detected in the hydrothermal vent clam Calyptogena pacifica, suggesting that the reduced filter-feeding ability of this species is used to supplement nutrition from endosymbiotic bacteria. Endosymbiont-bearing gill tissues contain the highest proportion of bacterial fatty acids and also high concentrations of triglycerides. The presence of triglycerides in gill tissues suggests that energy may be transferred from symbionts by hydrolysis of bacterial symbiont membrane lipids and their conversion to triglyceride.

The lipid profiles presented in this work increase understanding of the life history strategies and ecology of the species studied. This thesis shows that even the most highly adapted hydrothermal vent organisms do not rely solely on a chemoautotrophic source of nutrition, but also appear to require phototrophically derived elements in their diet.

CONTENTS

Chapter 1 - Introduction, background and aims

Chapter 2 - Materials and methods

Chapter 3 - Lipid profiles of Mid-Atlantic Ridge vent shrimp

Chapter 4 - Lipid profiles of *Nematocarcinus gracilis*

Chapter 5 - Lipid profiles of Juan de Fuca Ridge invertebrates

Chapter 6 - Conclusions and suggestions for future research

REFERENCES

APPENDICES

Raw data for calibrations presented in chapter 2 and results presented in chapters 3 to 5 are presented on the appendix CD-ROM. Details of data files, formats and structure are described in the readme.txt file in the root directory.

Acknowledgements

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Chapter 1: Introduction

1.1 The deep sea and extreme environments

Exploration and study, since pioneering 19th century expeditions, have extended our understanding of physical, chemical and biological processes in the deep sea, although still only a tiny fraction of its volume has been examined. Before this time, knowledge of the oceans was essentially a combination of pieces of information amassed by seafarers since man first built seagoing vessels. Wyville Thomson's *Challenger* Expedition (1872-76) was the first concerted multidisciplinary effort to describe the physical, chemical and biological properties of the world's oceans. Every ocean except the Arctic Ocean was visited during this expedition and it marks the beginning of modern oceanography. It was also the first comprehensive study of deep-sea fauna and finally established the habitual existence of life in the deep oceans.

Major environmental variables affecting life in the deep oceans include salinity, temperature, pressure and dissolved oxygen concentrations (Tyler, 1995). Temperature and salinity are relatively homogeneous in the deep-sea. Typical temperatures range from -1°C to 4°C and salinities are generally close to 34.7 ‰ (except in the deep Mediterranean and Red Seas, where both are somewhat higher). Dissolved oxygen concentrations below the oxygen minimum are approximately 3.6 mg ml^{-1}, although levels are a little lower in the deep North Pacific than in the deep Atlantic owing to biological utilisation, thermohaline circulation and oceanic residence times. Pressure increases with depth and organisms living at depth experience increased pressures of 1 atmosphere for every 10 metres increase in depth. Gradients of pressure decrease as depth increases and adaptations of membranes and enzymes (Somero, 1992) allow organisms to overcome the biochemical constraints of living in these conditions (see section 1.3).

The idea that the deep ocean is an environment homogeneous in space and time has only relatively recently been contradicted with discoveries such as the seasonal input of organic matter (Billett *et al.*, 1983), benthic storms (Aller, 1989) and animals associated with hydrothermal vent and cold seep environments (Corliss *et al.*, 1979, Kennicutt *et*

al., 1985). Exceptions to general levels of deep-sea temperature, salinity and dissolved oxygen may be found in hydrothermal vent environments, at cold seeps and deep-sea oxygen minima: in the Arabian Sea, for example, the oxygen minimum zone (dissolved oxygen concentrations around 0.5 mg ml^{-1}) extends to a depth of around 1 km; at hydrothermal vents anoxic, black smoker fluids can reach temperatures of 360°C; at cold seeps, brine pools may be formed, with salinities in the region of 121‰.

Although the physical conditions in the deep sea might be extreme to organisms adapted to live on land or even in shallow water, the deep sea is not an extreme environment for the organisms that are adapted to live there. An extreme marine environment can be defined as one that exhibits large variations or fluctuations in conditions. There are areas of the deep sea that fall into this category: sharp gradients in seawater temperature and chemical composition are characteristic of deep-sea hydrothermal vents; another deep-sea environment exhibiting a marked variation in chemical conditions is in the region of oxygen minima.

1.1.1 Deep-sea hydrothermal vents

Biological communities associated with hydrothermal vent fauna were first discovered in the Pacific on the Galápagos Rift (Corliss *et al.*, 1979). The sulphide-rich waters emanating from hydrothermal vents support chemoautotrophic bacteria that are the primary producers of vent communities (Karl *et al.*, 1980). Eastern Pacific vents are typically occupied by beds of tubeworms, clams and mussels that demonstrate extraordinary symbioses with sulphur-oxidising bacteria (Cavanaugh *et al.*, 1981; Cavanaugh, 1985). A variety of polychaete worms, gastropods and other invertebrates are found amongst the beds of tubeworms. Mid-Atlantic hydrothermal vent communities are strikingly different. Although mussels have been found at most sites, many sites are dominated by swarms of shrimp and extensive beds of anemones (Van Dover, 1995). The hydrothermal vent environment is characterised by extremes of temperature and chemical gradients between oxygen deficient, trace metal and sulphide-rich hot water and ambient sea water. Animals living in this environment have adapted to withstand these conditions and these adaptations may be reflected in their lipid composition (See section 1.3).

Mid-Atlantic Ridge hydrothermal vent shrimp

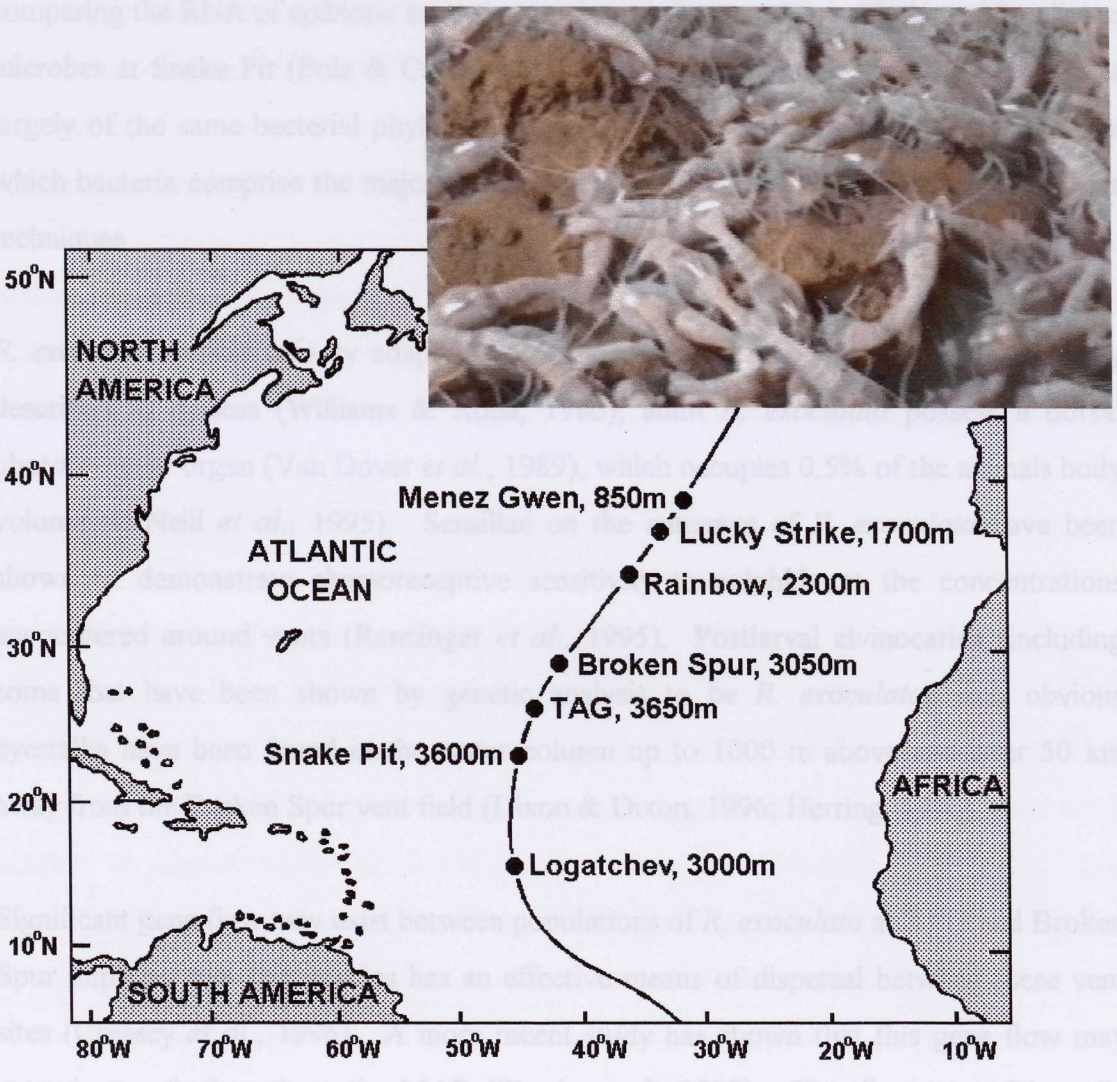


Figure 1.1 Locations and approximate depths of the known sites of hydrothermal venting on the Mid-Atlantic Ridge South of the Azores. Inset: *R. exoculata* at the TAG Central Black Smoker Complex.

Rimicaris exoculata is a species of alvinocarid shrimp found in varying abundance at hydrothermal vent sites on the Mid-Atlantic Ridge (MAR) (Figure 1.1). It occurs in dense aggregations around sites of high temperature venting (Van Dover, 1995). Carbon stable isotope studies indicate that adult *R. exoculata* are dependent on a non-photosynthetic source of organic carbon and nitrogen stable isotopes are consistent with the role of the shrimp as a primary consumer of chemosynthetic bacteria (Van Dover *et al.*, 1988). A strong but circumstantial case has been developed for the importance of epibiotic bacteria in the nutrition of the shrimp (Van Dover *et al.*, 1988, Gebruk *et al.*,

1993, reviewed by Van Dover, 1995), but they may also feed on bacteria living on sulphide surfaces (Van Dover *et al.*, 1988, Segonzac *et al.*, 1993). A recent study comparing the RNA of epibiotic bacteria growing on *R. exoculata* with that of free living microbes at Snake Pit (Polz & Cavanaugh, 1995) has shown that the microbes consist largely of the same bacterial phylogenetic type. It may therefore be difficult to prove which bacteria comprise the major source of nutrition for *R. exoculata* using molecular techniques.

R. exoculata exhibit sensory adaptations to the vent environment. Although originally described as eyeless (Williams & Rona, 1986), adult *R. exoculata* possess a dorsal photoreceptor organ (Van Dover *et al.*, 1989), which occupies 0.5% of the animals body volume (O'Neill *et al.*, 1995). Sensillae on the antennae of *R. exoculata* have been shown to demonstrate chemoreceptive sensitivity to sulphide at the concentrations encountered around vents (Renninger *et al.*, 1995). Postlarval alvinocarids (including some that have been shown by genetic analysis to be *R. exoculata*) with obvious eyestalks have been found in the water column up to 1000 m above and over 50 km away from the Broken Spur vent field (Dixon & Dixon, 1996; Herring, 1996).

Significant gene flow may exist between populations of *R. exoculata* at TAG and Broken Spur implying that this species has an effective means of dispersal between these vent sites (Creasey *et al.*, 1996). A more recent study has shown that this gene flow may extend even further along the MAR (Shank *et al.*, 1998). The discovery of juvenile alvinocarids in the water column above Broken Spur (Dixon & Dixon, 1996; Herring, 1996) has shown that juvenile vent shrimp are planktonic.

Other caridean shrimp species found at Mid-Atlantic Ridge hydrothermal vents include *Alvinocaris markensis, Chorocaris chacei* and two proposed species of *Mirocaris* (Williams, 1988; Segonzac *et al.*, 1993; Williams & Rona, 1986; Martin & Christiansen, 1995; Vereschaka, 1997). At the deeper vent sites on the Mid-Atlantic Ridge (Broken Spur, TAG, Snake Pit and Logatchev) these shrimp occur at lower densities than *R. exoculata* in areas of high temperature venting and are more abundant in peripheral areas of vent fields. *Mirocaris fortunata* appears to be the dominant shrimp at the shallower vent sites (Lucky Strike and Menez Gwen), although *R. exoculata* are also present in

low numbers at Lucky Strike. The dorsal organ is reduced in *C. chacei* and *Mirocaris* species relative to *R. exoculata*, and is much smaller in *A. markensis*. *A. markensis* have been found to have larger stomachs than *Chorocaris chacei*, which have larger stomachs than *R. exoculata* (Segonzac *et al.*, 1993). Together with other morphological differences, this has been suggested to reflect scavenging habits in *A. markensis* and optional scavenging habits in *C. chacei* (Segonzac *et al.*, 1993). Quantification of the relative importance of these species as primary and secondary consumers is unlikely to be within the scope of lipid biomarker studies, but may be possible using compound specific stable isotope methodology.

Juan de Fuca Ridge invertebrates

Ridgeia piscesae

Ridgeia piscesae is a vestimentiferan tubeworm found at hydrothermal vents in the north eastern Pacific (Jones, 1985). It occurs in clumps in areas of diffuse flow around black smokers in tubes typically between 10 and 150 cm long (Tunnicliffe *et al.*, 1991). The wide variation in size and morphology of *Ridgeia piscesae* originally led to the description of two species of vestimentifera at Juan de Fuca vents (*Ridgeia piscesae* and *Ridgeia phaeophile*) (Jones, 1985). However, allozyme analysis has since suggested that the two morphotypes in fact belong to the same species (Southward *et al.*, 1995) and it is presumed that the variation in morphology is a phenotypic response to environmental factors. There has been little specific work on the physiology of *Ridgeia piscesae*, but it is generally considered to be similar to that of the more intensively studied south eastern Pacific vestimentiferan *Riftia pachyptila* (Fisher, 1990). Of all known chemoautotrophic symbioses, vestimentiferan tubeworms appear to have the highest degree of physiological and morphological adaptation for symbiosis. Their morphology is fairly simple (Figure 1.2). The obturaculum supports a branchial plume for gas exchange and is the only part of the worm that protrudes from the chitinous tube (it may be retracted at will). The muscular vestimentum is posterior to the obturaculum. It holds the worm in the tube and secretes more tube as the worm grows. The vestimentum is connected to the trunk region that extends down the tube and contains the trophosome and gonads. The

trophosome is a highly vascularised organ that contains bacteriocytes, i.e. cells containing symbiotic sulphur-oxidising bacteria. The opisthosome is a short region at the base of the trunk that anchors it to the base of the

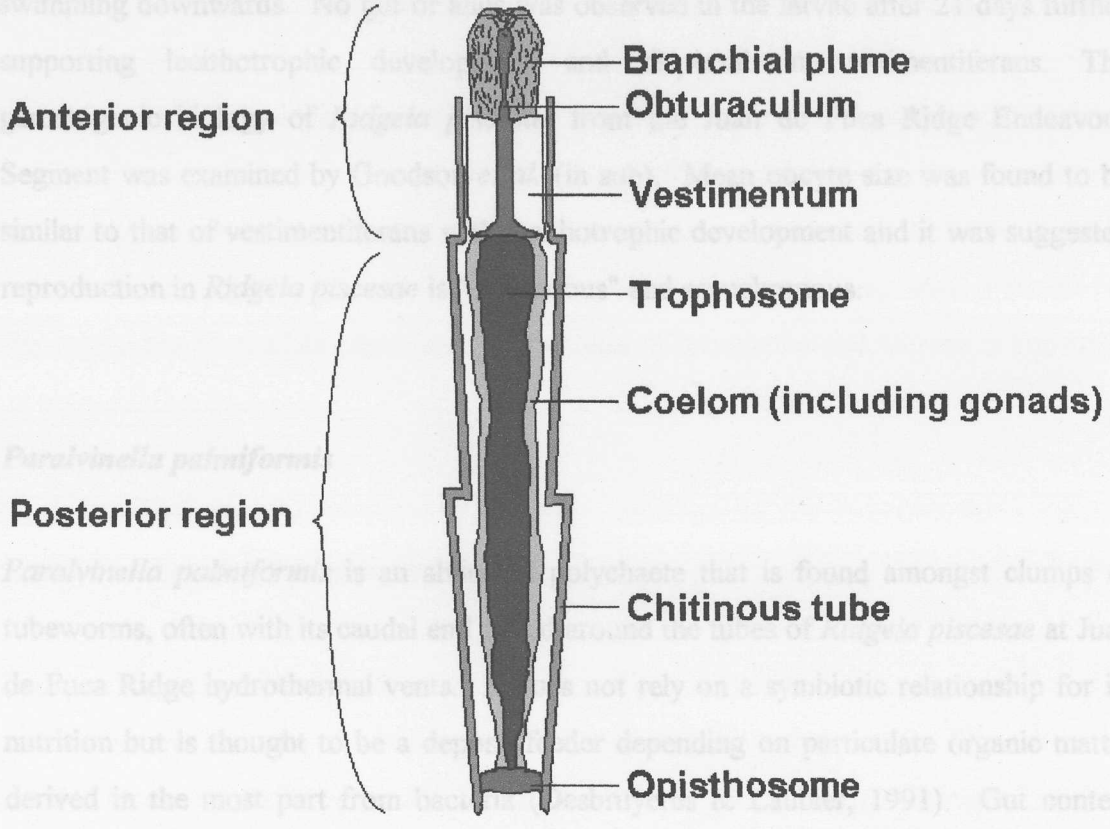


Figure 1.2 A diagrammatic representation of the morphology of a vestimentiferan tubeworm.

tube. Adult vestimentiferans have no mouth, gut or anus. They possess a modified blood haemoglobin system to accommodate the needs of their sulphur-oxidising symbionts. Free-living larval *Ridgeia piscesae* have yet to be captured, but newly settled postlarvae possess a mouth and a gut with a digestive function (Southward, 1988). As they grow larger (from 150μm length to >300μm length) bacteria appear in gut cells and the trophosome develops from the mid gut region (Southward, 1988). The observation of lipid droplets in the oocytes of *Riftia pachyptila* (Cary *et al.*, 1989) and the small size of *Ridgeia piscesae* postlarvae (Southward *et al.*, 1996) suggest that larvae exhibit non-feeding lecithotrophic development. Recent studies of the eggs of *Lamellibrachia*, a cold seep vestimentiferan from the Gulf of Mexico, have revealed that they are rich in wax esters (Craig Young, Harbour Branch Oceanographic Institution, pers. comm.). In

laboratory studies (Young *et al.*, 1996), the eggs and larvae of *Lamellibrachia* and *Seepia* (another cold seep vestimentiferan) were found to be positively buoyant, floating up in the water column. After several days these larvae developed cilia and began swimming downwards. No gut or anus was observed in the larvae after 21 days further supporting lecithotrophic development and dispersal in vestimentiferans. The gametogenic biology of *Ridgeia piscesae* from the Juan de Fuca Ridge Endeavour Segment was examined by Goodson *et al.* (in sub). Mean oocyte size was found to be similar to that of vestimentiferans with lecithotrophic development and it was suggested reproduction in *Ridgeia piscesae* is "continuous" and asynchronous.

Paralvinella palmiformis

Paralvinella palmiformis is an alvinellid polychaete that is found amongst clumps of tubeworms, often with its caudal end coiled around the tubes of *Ridgeia piscesae* at Juan de Fuca Ridge hydrothermal vents. It does not rely on a symbiotic relationship for its nutrition but is thought to be a deposit feeder depending on particulate organic matter derived in the most part from bacteria (Desbruyeres & Laubier, 1991). Gut content analysis revealed filamentous bacteria, mucus and diatomaceous debris (McHugh, 1989). The reproductive biology of *P. palmiformis* from Endeavour Segment Main Field vents was the subject of a study by McHugh (1989) and has more recently been examined by Copley (1998). Synchronous, discontinuous reproduction and recruitment were inferred from body and oocyte size frequency distributions. Reproductive development in *P. palmiformis* was found to be patchy within a vent field (Copley, 1998). It was suggested that this patchiness is probably governed by a combination of two factors: discrete breeding periods on lunar or semi-lunar timescales and differences in environmental conditions leading to different stages of reproductive development.

Calyptogena pacifica

Vesicomyid clams are conspicuous members of the fauna surrounding Eastern Pacific hydrothermal vents. The vesicomyids found at Juan de Fuca Ridge Endeavour Segment

hydrothermal vents have recently been shown to belong to a group of genetically similar species which include *Calyptogena pacifica* (name used here) and *Vesicomya lepta* (Peek *et al.*, 1997). The physiology of *C. pacifica* has not been studied in detail, but is thought to be similar to that of *Calyptogena magnifica*. It lives with the foot buried in sediments which are flushed with hydrothermal fluids and the siphon opens to ambient seawater. Vesicomyid clams possess a reduced and simple digestive system, and modified haemoglobin to transport chemicals to symbiotic sulphide-oxidising bacteria in bacteriocytes in the gills (Fisher, 1990). *C. pacifica* occurs in beds at some distance from the main sites of high temperature venting on the Endeavour segment. Perhaps for this reason there have been few ecological studies of the species and nothing is known of its reproductive biology.

1.1.2 The Arabian Sea oxygen minimum zone

Dissolved oxygen distribution in the oceans reflects a balance between surface inputs (diffusion), biological processes and physical transport (advection). The extent of oxygen inputs by surface gas exchange depends upon the solubility of oxygen that is in turn dependent on seawater temperature. Oxygen is less soluble and therefore dissolved oxygen concentrations are lower in warm water. Dissolved oxygen is released during photosynthesis, but consumed during respiration so these processes are also important. Finally, the physical transport of water with dissolved oxygen concentrations determined by surface gas exchange and biological processes characterises oceanic dissolved oxygen distribution. Oxygen minima are formed where biological oxygen utilisation exceeds the rate of replenishment by advection and diffusion. In regions where upwelling occurs, the constant replenishment of otherwise limiting (to primary production) nutrients results in high surface production. The subsequent downward flux of organic matter is associated with high levels of respiration, which can result in the formation of an oxygen minimum layer.

The Arabian Sea is one of the most productive areas of the world's oceans and also boasts an extensive oxygen minimum zone (OMZ; Currie *et al.*, 1973). During the South West monsoon period from June to August, offshore Ekman transport of surface

waters results in strong upwelling of nutrient-rich deep waters and consequently intense primary production (Currie *et al.*, 1973). The combination of the subsequent high downward flux of organic matter (Nair *et al.*, 1989) and sluggish intermediate water circulation rates (Swallow, 1984; Olsen *et al.*, 1994) is responsible for an intense and permanent oxygen minimum. The large fluxes of organic carbon are preserved in surface sediments where the oxygen minimum zone intersects the slope (between 150 and 900m depth). However, it should be pointed out that the high sediment organic carbon content may be a function of the high levels of input and post-depositional reworking by hydrodynamic influences rather than a result of the OMZ (Pedersen *et al.*, 1993).

A high biomass of benthic megafauna is found associated with the high levels of sedimentary organic carbon below the OMZ. The spider crab, *Encephaloides armstrongi* has been observed at densities of over 100 individuals per square metre at the oxic/anoxic boundary at around 975m in the Arabian Sea (Gage *et al.*, 1995). This crab is one of a number of organisms that appear to be adapted to migrate into and out of the oxygen minimum zone (Creasey *et al.*, 1997), taking advantage of the elevated levels of organic matter: polychaetes with bright red gills and tentacles and morid fish with large, bright red gills were caught in trawls in the Arabian Sea oxygen minimum zone (Herring, 1994). The mysid *Gnathophausia ingens* has been shown to be adapted to survive in an oxygen minimum zone of the South California basin. It possesses haemocyanin with elevated affinity and cooperativity for oxygen compared with other cold-water living crustaceans (Sanders & Childress, 1990). *Nematocarcinus gracilis* does not show these types of adaptation, so is probably less tolerant of hypoxic conditions and avoids the oxygen deficient waters.

Nematocarcinus gracilis

Nematocarcinus gracilis is a bright red species of deep-sea caridean shrimp that occurs in the Indian Ocean. In general, concentrations of shrimp are relatively low in the ocean, but it has been noted that their numbers increase near the sea floor (Domanski, 1986). Nematocarcinidae are characterised by three pairs of extremely long pereiopods that have been suggested to be used like stilts for walking on soft sediments (Calman, 1911). Relatively little is known about *N. gracilis*, but the congeneric species *Nematocarcinus*

exilis, found on the western Mediterranean slope, has been examined in detail. Stomach content analyses of this species (Cartes, 1993) indicated that it is a scavenger and predator, consuming mainly fish remains and slow-moving benthic organisms. It does not seem unreasonable that *N. gracilis* would exhibit similar feeding strategies. In a study of deep-water caridean shrimp in the Pacific, King and Butler (1985) found results suggesting that reproductive lifespan, degree of iteroparity and mean egg size all increase with depth of distribution. A recent investigation (Eva Ramierez, University of Southampton, pers. comm.) has shown that *N. gracilis* produce oval eggs (0.5 to 0.65 mm length), and it is proposed that they may achieve several reproductive outputs during a relatively long (two to three years) reproductive lifespan.

1.2 Lipid structure and biochemistry

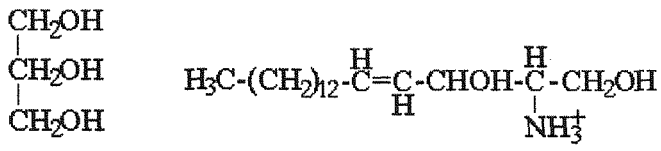
Lipids are a group of biologically important compounds that are distinguished by their solubility in hydrocarbons and insolubility in water. This group includes a variety of compounds with diverse biological functions. Functional adaptations to high pressures and low temperatures in the deep sea have resulted in characteristic lipids in deep-sea organisms (Somero, 1992). Lipids may therefore be used as indicators of the physiological relationship between an organism and its environment.

Lipids have a variety of biological roles in marine organisms: they serve as highly concentrated energy stores, membrane components, signal molecules and hormones. The use of lipids for energy storage obviates the expenditure of energy on osmoregulation that would be required with hydrated proteins or carbohydrates. Neutral lipids are less dense than water and may also be used to attain neutral or positive buoyancy (Sargent, 1976). Membrane lipids must act as a barrier between two environments and be able to exclude water and other molecules. A lipid combining a hydrophilic group and a hydrophobic moiety is ideally suited to this. There are three main groups of membrane lipids:

Membrane lipids

i) Phospholipids are derived either from glycerol or sphingosine (Figure 1.3) esterified or amide-linked to a hydrophilic phosphoric acid group and a hydrophobic fatty

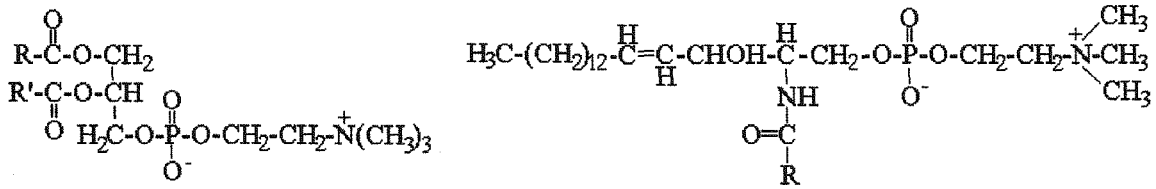
acid chain.



Glycerol Sphingosine

Figure 1.3 The chemical structures of glycerol and sphingosine

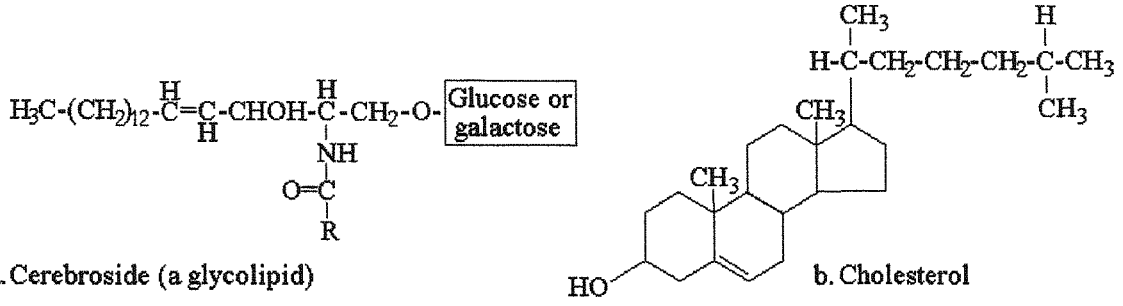
The phosphoric acid group is usually further esterified to an alcohol component such as choline or ethanolamine (Figure 1.4).



Phosphatidyl choline Sphingomyelin

Figure 1.4. The chemical structures of two types of phospholipids (R and R' represent hydrophobic, long-chain hydrocarbon moeties)

ii) *Glycolipids* are sugar containing lipids. They are derived from sphingosine that is amide-linked to a fatty acid and ester-linked to one or more sugar residues (Figure 1.5a).



a. Cerebroside (a glycolipid) b. Cholesterol

Figure 1.5. The chemical structures of a glycolipid and cholesterol

iii) *Sterols*, such as cholesterol (Figure 1.5b) are present in eukaryotes, but not in most prokaryotes (Stryer, 1988).

Membrane fluidity is controlled to a large extent by fatty acid composition. Saturated fatty acyl residues have straight chains that fit together favouring a rigid state at high pressures and low temperatures. Membrane lipids need to be sufficiently fluid to allow

transport of ions and small molecules. Unsaturated fatty acids with *cis* double bonds have a bend in their chains, interfering with the ordered packing of fatty acyl moieties and thereby increasing the membrane fluidity (Figure 1.6). The position of unsaturation is also important: a fatty acid with a double bond in the middle of the alkyl chain has a lower melting point (and therefore conveys greater fluidity) than one with the double bond at the end of the chain. Sterol, protein content and phospholipid class composition complicate the relationship between membrane fluidity and degree of unsaturation (Bell *et al.*, 1986). For example, the cholesterol hydroxyl group can bond to the carbonyl moiety of a phospholipid head group and insert its hydrocarbon "tail" between the alkyl chains of the phospholipid fatty acids increasing membrane fluidity. Alternatively, sterols can be positioned such that they sterically block large motions of unsaturated fatty acid chains, reducing fluidity (Stryer, 1988).

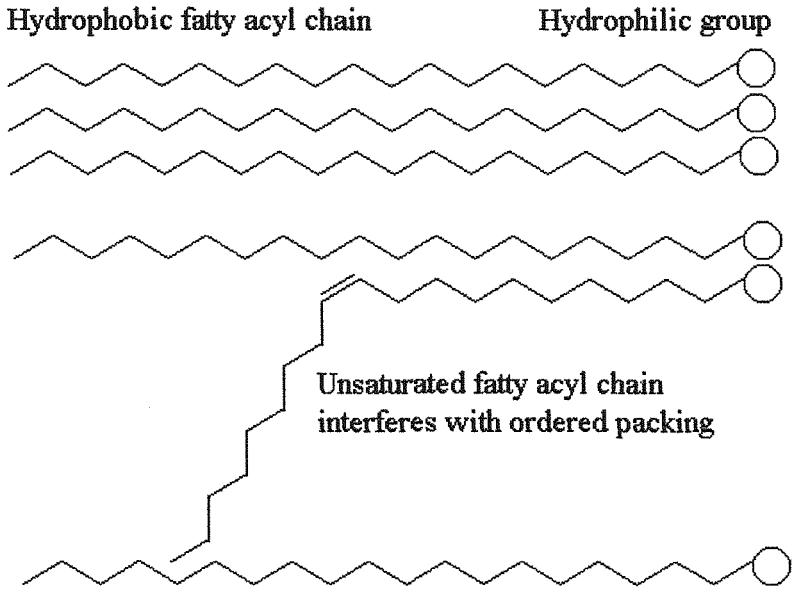


Figure 1.6. Membrane fluidity is regulated by fatty acid composition

Energy storage lipids

Triacylglycerols are the major long-term energy storage lipid in terrestrial organisms. In marine organisms however, *alkyldiacylglycerols* and *cholesteryl* and *wax esters* are also important food stores (Sargent & Whittle, 1981). Triacylglycerols and alkyldiacylglycerols are derived from glycerol esterified or ether-linked to fatty acids or alcohols. Sterol and wax esters comprise a sterol or fatty alcohol molecule esterified to a fatty acid (Figure 1.7). Storage lipids are broken down first by enzyme hydrolysis by lipases to release free fatty acids. The β-oxidation of fatty acids yields large amounts of

energy in the form of ATP. Lipids are much more efficient energy stores than the highly hydrated proteins and carbohydrates, because they are much more highly reduced and being non-polar are stored in an almost anhydrous form.

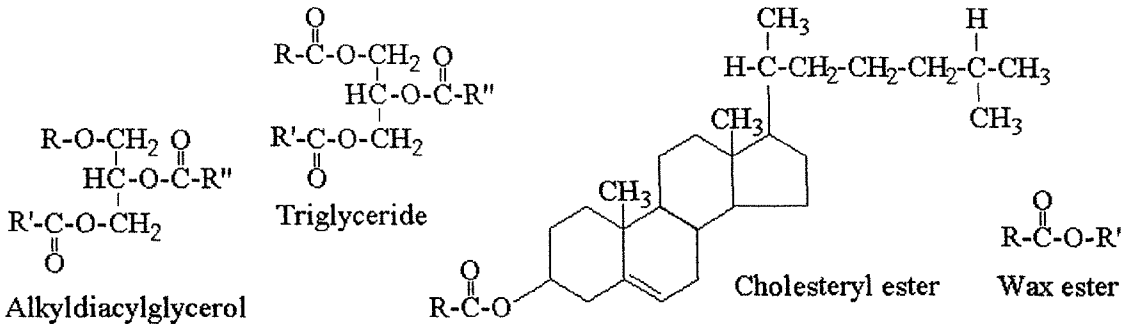


Figure 1.7. The chemical structures of some neutral lipids. R, R' and R" are alkyl chains.

Signal lipids and hormone precursors

Phosphatidyl inositol is an example of a phospholipid signal molecule that may be important in marine organisms. On reception of an extracellular stimulus (such as the binding of a hormone to an extracellular receptor) an enzyme is activated that hydrolyses it into two messenger units - a phosphorylated inositol unit that increases cellular calcium ion concentrations and a diacylglycerol that in mammals usually has arachidonate (20:4 n-6)[1] in the 2-position of the glycerol backbone.

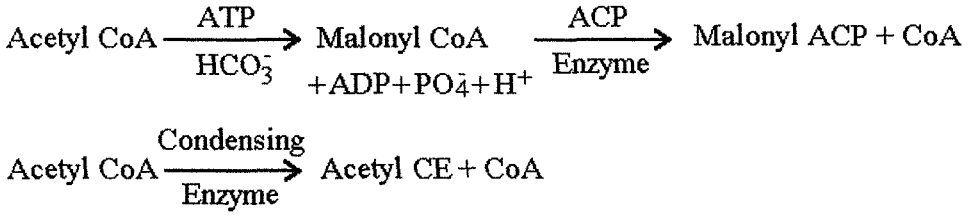
Prostaglandin hormones are derived from eicosanoids, such as arachidonic acid. In mammals, prostaglandins are synthesised primarily from arachidonic acid (20:4 n-6). It is thought that the precursors to prostaglandins in marine fish are eicosapentaenoic (20:5 n-3) and docosahexaenoic (22:6 n-3) acids derived from phosphatidyl inositol (Bell *et al.*, 1986).

1.2.1 Fatty acid synthesis

Fatty acids are a major component of lipids in general. The general pathway of *de novo*

[1] Fatty acid nomenclature is simplified using the following notation: a:b n-c where a is the fatty acid chain length (number of carbon atoms), b is the degree of unsaturation (number of double bonds), n signifies number of carbon atoms, c from the ω (alkyl i.e. hydrocarbon chain) end of the chain to the first double bond (position of unsaturation). Double bonds are cis-conformation unless otherwise indicated and interrupted by methylene groups. Δ# indicates the carbon number from the α (carboxylate) end of the chain. The use of i- or a- prefixes (e.g. i-19:0) indicates iso- (methylene on the α carbon) or anteiso- (methylene on the β carbon) branching of the alkyl chain.

synthesis of fatty acids is similar for all organisms. Although individual fatty acid synthetases vary greatly in structure, their metabolic function is broadly the same. Fatty acids are synthesised using acetyl coenzyme A (an enzyme), adenosine triphosphate



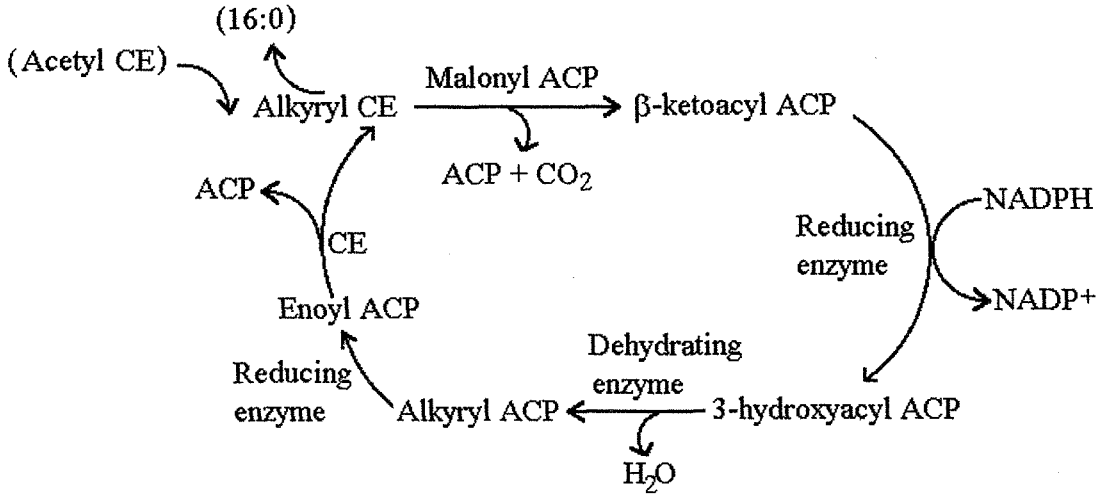


Figure 1.8. Fatty acid synthesis. (Abbreviations: CE, condensing enzyme; ACP, acyl carrier protein)

(ATP), nicotinamide adenine dinucleotide phosphate (NADPH) (energy molecules) and bicarbonate (HCO_3^-). Precursors are activated by substituting an acyl carrier protein (ACP), then there are basically four steps in a cycle (condensation, reduction, dehydration, reduction) adding on two carbon units per cycle to the fatty acid precursor (See Table 1.1) until it is 16 carbons long (Figure 1.8).

Accessory enzyme systems are responsible for further elongation and unsaturation reactions (Stryer, 1988). Eukaryotes elongate and desaturate their fatty acids aerobically. Some of the possible pathways for these reactions are shown in figure 1.9 (page 1.15). Most higher marine organisms lack Δ12 and Δ15 desaturases and are therefore unable to produce n-3 and n-6 polyunsaturated fatty acids *de novo*. They have an essential requirement for 18:2 n-6 and 18:3 n-3 fatty acids in order to produce the highly unsaturated fatty acids (HUFA; Kanazawa *et al.*, 1979). In some cases marine organisms also lack Δ5 desaturases and have an essential requirement for highly

unsaturated fatty acids (Bell *et al.*, 1986).

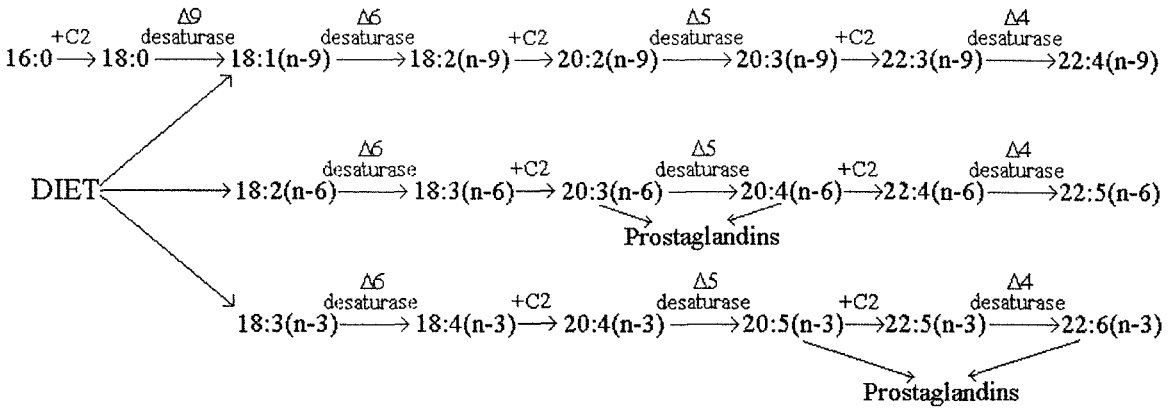


Figure 1.9. Pathways of aerobic elongation and desaturation of (n-9), (n-6) and (n-3) fatty acids.

Although prokaryotes are capable of aerobic desaturation, they can also desaturate their fatty acids anaerobically. The main end product of this desaturation is 16:1 n-7, which is the same as the aerobic product. However, in the absence of the oxygen-dependent conversion of 18:0 to 18:1 n-9, the only source of 18:1 is the chain elongation of 16:1 n-7. This leads to the production of 18:1 n-7 which is considered to be a characteristic fatty acid of prokaryotes.

Another characteristic of prokaryotic fatty acid synthesis is the production of branched-chain fatty acids. These result from the use of different primer molecules in the synthesis of precursors: coenzyme A is esterified to an acyl primer derived from an amino acid instead of pyruvate. Table 1.1 shows the type of branched chain fatty acids that result from different precursor primers (Fullarton, 1993).

Table 1.1 Branched chain fatty acid precursors

Acyl coA precursor	Acyl group	Fatty acid alkyl chain type
Pyruvate	Acetyl	Straight chain
Leucine	3-methyl butyryl	iso- odd numbered chain
Isoleucine	2-methyl butyryl	anteiso- odd numbered chain
Valine	2-methyl propyl	iso-even numbered chain

An unusual group of fatty acids has been observed in symbiotic organisms and some

molluscs (Joseph, 1982, Conway & McDowell Capuzzo, 1991). Non-methylene interrupted dienoic (NMID) fatty acids[2] have two double bonds separated by more than one methylene group. Figure 1.10 shows the proposed pathways of production of two of the more common NMID fatty acids (Zhukova,1991).

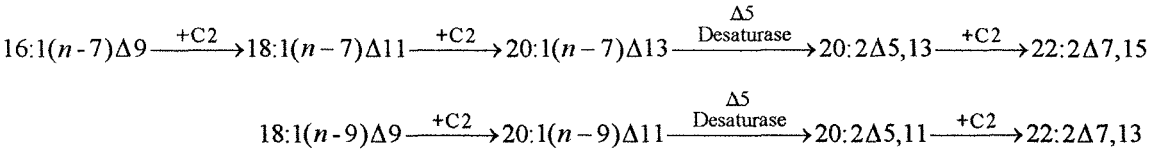


Figure 1.10. Pathways of elongation and desaturation of NMID fatty acids (after Zhukova, 1991).

1.3 Adaptations of lipids in marine organisms

The low temperatures and high pressures experienced by marine organisms in the deep sea require adaptations of their lipids in order that they function normally. The maintenance of membrane fluidity is a good example of this. At high pressures and low temperatures lipid membranes containing saturated fatty acids become less fluid as a result of their higher melting points and more ordered packing. Membranes containing more polyunsaturated fatty acids (PUFA) with lower melting points are fluid under these conditions and allow the proper function of the membrane. Phenotypic acclimation and evolutionary adaptation are both important in adaptations of deep-sea organisms to high pressures and low temperatures (Somero, 1992).

Deep-sea prokaryotes are able to vary the chain length and saturation of their fatty acid components to accommodate high pressures and lower temperatures and maintain membrane fluidity (Delong & Yayanos, 1986). Unlike their shallow water counterparts that are usually rich in short and branched chain fatty acids, deep-sea bacteria of the *Vibrio* genus have been shown to contain polyunsaturated fatty acids especially 20:5 n-3 and 22:6 n-3 (Yano *et al.*, 1994). It has also been reported that some species of *Vibrio* alter their PUFA levels with environmental conditions: increasing the concentration of

[2] NMID nomenclature differs slightly from that for single methylene interrupted fatty acids: positions of unsaturation are denoted by the number of carbon atoms from the carboxylate end of the alkyl chain: e.g. 20:2 Δ5,11 represents a 20 carbon chain fatty acid with 2 double bonds between the 5th and 6th and 11th and 12th carbon atoms from the carboxyl end of the chain.

PUFA with decreasing temperature (Delong & Yayanos, 1986). Fish and other deep-sea organisms are adapted to incorporate dietary PUFA into their membranes (Bell *et al.*, 1986) and to use cholesterol to regulate membrane fluidity as described earlier. Fish acclimated to cold temperatures have been shown to selectively incorporate PUFA into their phospholipids (Bell *et al.*, 1986).

Highly unsaturated fatty acids (HUFA) and their precursors have been found to be an essential dietary requirement for some marine organisms (Bell *et al.*, 1986). Marine symbiotic organisms have been shown to produce non-methylene interrupted fatty acids (NMID) (Conway & McDowell Capuzzo, 1991). The function of NMID fatty acids is uncertain, but they are produced where there is an abundance of 16:1 n-7 and 18:1 n-7 fatty acids and a paucity of HUFA (e.g. in organisms with symbiotic bacteria), and it has been suggested that they might act as a substitute for HUFA (Fullarton, 1993).

Besides being excellent energy storage molecules, neutral lipids such as triglycerides and especially wax esters are less dense than water and may be used by benthic organisms with lecithotrophic development to aid dispersal. Macrourid fish have been shown to incorporate an oil globule into their eggs that makes them buoyant and allows wide dispersal (Merrett, 1986).

It has recently been shown that Arctic phytoplankton alter the types of lipid they synthesise according to the availability of nutrients such as nitrate and silicate (Smith *et al.*, 1997). As the nutrient concentrations declined, it was observed that lipid synthesis shifted towards production of neutral lipids and glycolipids and away from phospholipids.

1.4 The lipid composition of shrimp

Early studies of lipid composition of shrimp concerned oceanic caridean decapods from the North East Atlantic (Culkin & Morris, 1969; Morris, 1973; Morris & Sargent, 1973, Herring, 1973). They found that the major fatty acids in *Acanthephyra purpurea* were 16:0, 16:1, 18:1 20:1, 22:1, 20:5 and 22:6 (Culkin & Morris, 1969), that female *A. purpurea* contained higher levels of monounsaturated fatty acids (MUFA) than male or

juvenile specimens of the same species and that *A. purpurea* eggs (from berried females) were rich in triglycerides and phospholipids (Morris, 1973). Morris and Sargent (1973) demonstrated that *A. purpurea* could synthesise triglycerides and phospholipids and noted that this species contained only small amounts of wax esters compared with mysid and euphausid crustaceans.

Herring (1973) studied the gross lipid and lipid class content of some oceanic decapods. He reported total lipid levels varying between 3 % and 20 % of wet weight. In general, shallow pelagic and deep benthic shrimp species (including *Nematocarcinus cursor*) were found to contain low levels of wax esters and to use triglyceride as their major energy storage lipid. Conversely, most deep pelagic shrimp, comprised wax esters as important if not dominant components of their total lipid. Eggs analysed were all generally found to be rich in triglyceride.

Clarke (1979) analysed the lipid class and fatty acid composition of the shallow water caridean *Pandalus montagui*. He found the major fatty acids were 16:0, 16:1, 18:1, 20:5 and 22:6, typical of a benthic crustacean. Major lipid classes were triglycerides, free sterols and phospholipids, with phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) comprising the major phospholipids. The phospholipids were also found to contain more PUFA than the triglycerides.

Perhaps as a result of their greater economic significance, there have been a large number of studies of penaeid shrimp. The major fatty acids found in penaeid shrimp are 16:0, 16:1 n-7, 18:1 n-9, 20:5 n-3 and 22:6 n-3 (Jeckel *et al.*, 1989; Teshima *et al.*, 1989; Mourente & Rodriguez, 1991; Muriana *et al.*, 1993a; Muriana *et al.*, 1993b; Rodriguez *et al.*, 1994).

Kanazawa *et al.* (1979) studied the dietary requirement for 18:2 n-6, 18:3 n-3, 20:5 n-3 and 22:6 n-3 in *Penaeus japonicus*. They found that 20:5 n-3 and 22:6 n-3 were more effective than 18:3 n-3 that, in turn, was more effective than 18:2 n-6 as essential fatty acids (EFA). Marine organisms have been shown to be inferior to aquatic fish in their capacity for elongation and desaturation of 18:2 n-6 and 18:3 n-3 to highly unsaturated fatty acids (HUFA) such as 20:5 n-3 and 22:6 n-3. It has been implied that this

bioconversion ability is related to the availability of dietary HUFA in the environment (Bell *et al.*, 1986). It was later discovered that penaeid shrimp are unable to synthesise 20:5 n-3 and 22:6 n-3 and therefore have a dietary requirement for them (Teshima & Kanazawa, 1983).

Jeckel *et al.* (1989), performed a seasonal study of the lipid composition of the ovaries of *Pleoticus muelleri.* They noted increases in both polar and neutral lipids during the development of the ovary. Triglycerides were the major neutral fraction and their fatty acid composition varied seasonally, while that of the polar lipids did not. It was suggested that relatively high levels of free fatty acids (up to 9%) found in tissues did not result from post-mortem hydrolysis, but from their being a common metabolic product of this species.

Teshima *et al.* (1989) and Mourente & Rodriguez (1991) characterised lipid profiles of *Penaeus japonicus* and *Penaeus kerathurus* during maturation. Higher concentrations of neutral lipid than polar lipid were found in both ovaries and hepatopancreas by Teshima *et al.* (1989), but Mourente & Rodriguez (1991) found greater concentrations of polar lipid than neutral lipid in the ovaries. Both groups of workers found that concentrations of triglycerides in the hepatopancreas increased during maturation and that concentrations of free sterols decreased (Teshima *et al.*, 1989), while those of sterol esters increased (Mourente & Rodriguez, 1991). Unlike Jeckel *et al.* (1989), Teshima *et al.* (1989) noted marked variations in the fatty acid composition of both neutral and polar lipids during maturation: triglyceride HUFA concentrations remained approximately the same, while levels of 16:1 and 18:1 increased; phosphatidyl choline HUFA concentrations increased while 16:1 and 18:1 decreased.

Sterol esters were found to be particularly abundant in the hepatopancreas and ovaries of *Penaeus monodon* (Young *et al.*, 1992). Crustaceans are incapable of *de novo* sterol synthesis (Goad, 1981) and HUFA synthesis (Teshima and Kanazawa, 1983) and it has been suggested that sterol esters are a storage lipid form of these dietary components. It has been shown that only hepatopancreas tissues can esterify sterols (Young *et al.*, 1992) and it was proposed that sterol esters may be transported in esterified form to the ovaries where they can be hydrolysed to HUFA and free sterols and fulfil important roles in reproductive biochemistry.

Phospholipids are the principal lipid component of crustacean tissue and haemolymph, except in the hepatopancreas where stored lipid is composed mainly of neutral lipids. Storage lipids are used for vitellogenesis and moulting and therefore associated with reproductive effort. It has been suggested (Mourente & Rodriguez, 1991) that neutral storage lipids are converted into polar lipids for transport to the hepatopancreas to the ovaries during maturation. The increase in concentration of HUFA in polar lipids may be a result of their mobilisation from storage lipids and transport to the phospholipid-rich ovaries. HUFA are important as precursors to prostaglandins and play an important role in crustacean reproduction. HUFA have been shown to induce hatching in barnacle eggs (Mourente & Rodriguez, 1991) and 20:5 n-3 has been shown to be necessary for vitellogenesis in *Penaeus japonicus* (Muriana *et al.*, 1993a). Muriana *et al.* (1993b) examined the fatty acid composition of different types of phospholipids and found that phosphatidyl choline contained mainly 16:0, 18:1 n-9, 20:5 n-3 and 22:6 n-3. Phosphatidyl ethanolamine was found to comprise mainly 20:5 n-3 and significant amounts of 22:6 n-3, while sphingomyelin was found to contain mainly saturated 18:0.

There have been relatively few recent studies of caridean shrimp lipid composition. Bell and Dick (1990) examined the phospholipid composition of the eye membranes of *Pandalus borealis*. They found the phospholipids comprised mainly phosphatidyl ethanolamine and phosphatidyl choline with smaller amounts of phosphatidyl serine, sphingomyelin, cardiolipin and phosphatidyl inositol. The most abundant fatty acid was 20:5 n-3 with significant amounts of 22:6 n-3 also present in phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine. The total lipid composition of *P. borealis* was examined by Hopkins *et al.* (1993). Lipid was found to comprise 10 to 40% of the animal's dry weight and triglycerides were the largest lipid class. The major fatty acids were 16:0, 16:1 n-7, 18:1 n-9 an 18:1 n-7, with lesser amounts of 20:5 n-3 and 22:6 n-3.

A study of the eggs and larval stages of crangonid shrimp in the North Sea (Kattner *et al.*, 1994) showed unusual patterns in lipid composition reflecting unusual weather conditions in the area. It was inferred that the absence of PUFA in larvae was a consequence of unfavourable nutritional conditions during the developmental stages of the shrimp. This study also examined the phospholipid composition of eggs and larvae.

The major phospholipid in both was phosphatidyl ethanolamine, but eggs also contained phosphatidyl choline and phosphatidyl serine. It was inferred from the absence of phosphatidyl choline and phosphatidyl serine in early larval stages that these were used during embryonic development.

In summary, the major fatty acids found in marine shrimp are 16:0, 16:1 n-7, 18:1 n-9, 20:5 n-3 and 22:6 n-3. The bulk of their lipid is usually in the form of neutral storage lipids, with significant amounts of phospholipids. Shrimp are unable to synthesise sterols and HUFA *de novo*, so must obtain these from a dietary source. They are then stored in the hepatopancreas and transported to organs such as the gonads where they probably play an important role in reproduction.

1.5 The lipid composition of hydrothermal vent fauna

There has been little work on the lipid composition of hydrothermal vent organisms, probably as a result of the limited availability of specimens from this environment. Taghon (1988) examined the phospholipid fatty acid composition of *Paralvinella palmiformis* from hydrothermal vents in the Endeavour segment of the Juan de Fuca Ridge. The dominant fatty acids were 16:0, 16:1, 18:1, 20:1 and 22:6 (positions of unsaturation not specified). Specimens from a high temperature site were shown to contain significantly lower levels of PUFA than those from a low temperature site and *P. palmiformis* were also shown to contain higher levels of PUFA than two shallow water confamilial species.

Ben Mlih *et al.* (1992) compared the lipids in a littoral mussel with those in *Bathymodiolus thermophilus* from two sites in the Pacific, the Galápagos vents and 13°N on the East Pacific Rise. They also examined the lipids of *Calyptogena magnifica* from the Galápagos vents. A total of eight specimens were used in this study. In the littoral mussel 16:0, 20:5 n-3 and 22:6 n-3 were the predominant fatty acids. *B. thermophilus* samples from 13°N contained predominantly 16:1 n-7, i-19:0 and 20:1 n-7, but with significant amounts of 20:5 n-3. The *C. magnifica* and *B. thermophilus* from the Galápagos vents however contained predominantly 16:0, 16:1 n-7 and 20:2

(positions of unsaturation not specified). The absence of HUFA and predominance of n-7 monounsaturated fatty acids (MUFA) in the Galápagos vent animals confirms the existence of an alternative food source i.e. one based on bacterial chemosynthetic and not phytoplanktonic photosynthetic primary production. The difference in lipid composition between hydrothermal vent sites was attributed to the presence of phytoplankton derived organic matter in the water column around the 13°N vents. The i-19:0 in *B. thermophilus* from 13°N was identified as an unusual bacterial fatty acid and proposed as a biomarker for a type of vent bacteria.

The lipid composition of mud chimney scrapings and bacterial mats from hydrothermal vents in the Endeavour segment of the Juan de Fuca Ridge were determined by Hedrick *et al.* (1992). They used lipid compositions to study the *in situ* microbial ecology. The major bacterial lipids identified were 16:0, 16:1 n-7 and 18:1 n-7. The presence of ether lipids indicated that there were archaebacteria in some of the chimney and flange scrapings and there was an unusually high proportion of cyclopropyl 17:0 fatty acids in some flange scrapings. Significant amounts of 20:5 n-3 were also present, especially in the bacterial mat samples. Delong and Yayanos (1986) showed that barophilic bacteria produce 20:5 n-3, but compound specific stable isotope data would be needed to discover whether or not PUFA are produced by chemoautotrophs at hydrothermal vents.

Rieley *et al.* (1995) analysed the tubeworm, *Riftia pachyptila*, the mussel, *Bathymodiolus thermophilus*, and the amphipod *Halice hesmonectes* from 9°N on the East Pacific Rise. The major fatty acids in *Riftia pachyptila* comprised 16:0, 16:1 n-7 and 18:1 n-7, with small amounts of the NMID fatty acids 20:2 Δ5,13 and 22:2 Δ7,15. It was inferred from the very small concentrations of branched chain fatty acids present that these fatty acids are not significant components of the sulphur oxidising symbiont of *Riftia pachyptila*. No single methylene-interrupted polyunsaturated (SMIP) fatty acids were detected, confirming the fact that *Riftia pachyptila* relies completely on symbiotic bacteria for nutrition. By contrast, *B. thermophilus* was distinguished by its large concentrations of NMID fatty acids, large amounts of 16:0 and 18:0 and small amounts of SMIP fatty acids. This fatty acid profile is consistent with an input of phototrophically-derived organic matter by filter feeding. The unusual i-19:0 branched chain fatty acid was also identified in these *B. thermophilus*. *H. hesmonectes* contained

high concentrations of wax esters, in common with other deep sea amphipods. However, like the vent mussels, it contained few SMIP fatty acids and a high proportion of NMID fatty acids.

Rieley *et al.* (1995) also examined the sterol and carbon stable isotope composition of these vent organisms. *Riftia pachyptila* was found to contain mainly cholesterol, cholest-5,24-dienol and 24-methylcholesta-5,24(28)-dienol. The $\delta^{13}C$ values for these sterols indicated that they were probably produced *de novo* by the tubeworm. Small amounts of phytoplankton derived sterols were also identified. It was suggested that these may indicate a small amount of dissolved organic carbon assimilation by the tubeworm, but it is perhaps more likely that they were ingested during a juvenile stage. *B. thermophilus* contained mainly cholesterol and a variety of minor sterols (including a number that are indicative of algae, such as cholesta-5,22-dienol). Stable isotope and electron microscope studies suggest that this mussel does not synthesise sterols *de novo*, but obtains them from dietary sources. A large variety of sterols were observed in *H. hesmonectes* and their $\delta^{13}C$ values fell into two categories: one similar to that of the tubeworms and one similar to that of the mussels. Since crustacea are unable to synthesise sterols *de novo*, this result must indicate two dietary sources of sterols: a phytodetrital-derived and a chemoautotrophic-derived source.

The fatty acid composition of the hydrothermal vent tubeworm *Ridgeia piscesae* collected from the Axial Seamount on the Juan de Fuca Ridge in the Pacific was examined as part of a study by Fullarton *et al.* (1995a). In the six tissue samples analysed, the major fatty acids present in the trophosome and vestimentum were 16:0, 16:1 n-7 and 18:1 n-7. There were also substantial amounts of 20:2 and 22:2 NMID fatty acids and the HUFA 20:5 n-3. Unlike the tubeworms from 9°N (Rieley *et al.*, 1995) *Ridgeia piscesae* contained significant amounts of SMIP fatty acids. It was suggested that these were assimilated at a juvenile stage, although the size difference between the first stage settled larvae and the adults implies that careful conservation of PUFA would be necessary to allow this. However, as *Ridgeia piscesae* is non-motile and has a poorly developed neural system, it is conceivable that it would have a reduced requirement for these fatty acids. The requirement for these fatty acids may be for

reproduction, especially the production of motile sperm. The possibility of trans-epidermal absorption of PUFA was rejected given that the high external concentrations necessary for this are unlikely in an oxidising medium. In a vent environment however, reducing conditions may make this a possibility.

A comparative study of the polar and neutral lipid fatty acid composition of a littoral mussel and two species of bathymodiolid mussel from Lau and Fiji back arc basin hydrothermal vents was recently published (Pranal *et al.*, 1997). Endosymbiotic bacterial biomass was estimated using 16:1 n-7 and 18:1 n-7 polar lipid fatty acids and a few rather broad assumptions. The major fatty acids included 16:0, 16:1 n-7, 20:1 n-7, 20:1 n-9, 18:3, 20:2 NMID, 20:3, and 22:2 NMID. It was noted that the levels of neutral lipid were elevated in the gills of vent mussels compared to littoral mussels and suggested that this reflects the important metabolic role of the gills in symbiotic mussels. Neutral lipid (and hence stored energy) levels were higher in all in vent mussel tissues and it was proposed that this might allow the animals to withstand periods of hydrothermal quiescence. Consequently, the ratio of triglyceride to phospholipid was used as a stress index to compare the condition of mussels from different vent sites: low values were indicative of poor condition of mussels and correlated with low bacterial biomass estimations.

The fatty acid and compound-specific stable isotope composition of vent mussels from the Menez Gwen site on the Mid-Atlantic Ridge was the subject of a recent study by Pond *et al.* (1998). The combination of fatty acid and stable isotope methods confirmed previous suggestions that these mussels bear two different types of symbiont and allowed identification of biomarkers specific to methanotrophic and thiotrophic bacteria: Methanotrophs were characterised by 16:1 n-9, n-8 and n-7, 18:1 n-13, n-9 and n-8, while fatty acids with stable isotope values typical of thiotrophs were 14:0, 18:0, 18:1 n-7, 20:1 n-7, 18:3 n-7 and 20:3 n-7.

Since the beginning of this work, there have been other studies of hydrothermal vent shrimp from the Mid-Atlantic Ridge. Pond *et al.*, (1997a) reported the presence of high concentrations of wax esters in the postlarvae of alvinocarid shrimp collected in the water column above hydrothermal vents on the Mid-Atlantic Ridge. In a subsequent

paper, Pond *et al.*, (1997b) analysed the fatty acid and compound specific stable isotope composition of four *R. exoculata* from the TAG hydrothermal vent field and three *Alvinocaris markensis* from Snake Pit. *R. exoculata* was found to contain unusual n-4 dienoic fatty acids, with stable isotope signatures reflecting chemoautotrophic bacterial origin. These fatty acids comprised a higher proportion of the total in digestive gland compared with tail muscle. Significant amounts of the bacterial biomarkers, 16:1 n-7 and 18:1 n-7 and perhaps surprisingly phototrophically-derived HUFA 20:5 n-3 and 22:6 n-3 were also reported for *R. exoculata*. *A. markensis* contained a higher proportion of photosynthetically-derived HUFA as well as substantial amounts of 18:1 n-9. The unusual bacterially-derived fatty acids were present, but in much smaller quantities. These results are consistent with *R. exoculata* being essentially bacterivorous, while *A. markensis* is a scavenger.

In another study, Pond *et al.* (1997c) examined the lipid and compound specific stable isotope composition of adult, small adult and eggs of *Mirocaris fortunata* from the Lucky Strike vent field. The highest concentrations of phytoplankton derived PUFA were found in small, highly pigmented adults with lower amounts found in small adults with less pigment and the lowest concentrations found in large adults. The small, highly pigmented shrimp also contained higher concentrations of 18:1 n-9 compared with 18:1 n-7. Larger, paler shrimp contained more of the bacterial NMID and n-4 dienoic fatty acid biomarkers. However, these were present at lower levels than in *R. exoculata* from TAG. The adult shrimp were found to use triglyceride for energy storage, while the smaller shrimp contained significant amounts of wax ester. Eggs contained tryglyceride and over 70% of fatty acids comprised MUFA, with less than 5% comprising PUFA.

1.6 Aims and hypotheses

The aim of this thesis is to examine and compare lipid profiles of marine organisms from different environments and relate them to their ecology. In general it appears that hydrothermal vent fauna are rich in the bacterial biomarker fatty acids 16:1 n-7 and 18:1 n-7 and contain NMID and other unusual fatty acids. SMIP fatty acids may be present in vent organisms, but stable isotope evidence does not suggest that they are produced

by vent bacteria so their presence is taken to be an indication of the input of photosynthetically derived organic matter. In summary, triglycerides are typical storage lipids in deep-sea benthic organisms while wax esters generally reflect a planktonic lifestyle. Polar lipids comprise mainly structural lipid and are the major lipid constituent of bacteria.

Hypotheses underpinning this study

i) The fatty acid composition of *R. exoculata* will reflect its proposed reliance on a bacterial food source. A benthic lifestyle will be confirmed by the use of triglyceride as the principle major storage lipid. The lipid composition of different tissue types will reflect the function of the tissue. The branchial area is expected to contain a higher proportion of bacterial fatty acid biomarkers; gonad tissue will be rich in storage lipid and may contain elevated levels of unsaturated fatty acids required for reproduction in other marine organisms; abdomen tissue will contain mainly structural lipid.

ii) The benthic shrimp, *N. gracilis* will exhibit phytoplankton biomarker fatty acids and a lipid class composition commensurate with a benthic lifestyle.

iii) The lipid profiles of *Ridgeia piscesae* will reflect its proposed life cycle: wax esters may be detected in the trophosome of females containing well-developed oocytes and fatty acid profiles will be consistent with a chemoautotrophic bacterial source of nutrition. Phytoplankton biomarkers will be absent or present at very low levels.

iv) The fatty acid composition of *P. palmiformis* will reflect both chemoautotrophic bacterial and phototrophically derived sources of nutrition. The lipid class composition may reflect reproductive developmental state, with elevated levels of storage lipid present in specimens at a late stage in development.

v) The fatty acid composition of *C. pacifica* will reflect its reliance on bacterial symbionts. Phytoplankton biomarkers will indicate whether additional sources of nutrition are significant. The partitioning of lipid classes between tissues will reflect their function.

Chapter 2: Materials and methods

2.1 Sample collection and preparation

2.1.1 Alvinocarid shrimp from the Mid-Atlantic Ridge

Individuals of *Rimicaris exoculata* were collected during the BRAVEX/94 cruise to the TAG hydrothermal vent field (26°08.3' N 44°49.6' W, Mid-Atlantic Ridge, 3620-3650 m depth) in August and September 1994, aboard the R/V *Akademik Mstislav Keldysh.* Specimens were collected using nets and a slurp gun deployed by the Mir submersibles in the swarms of shrimp around the central black smoker complex. On recovery, blotting paper was used to remove excess seawater from individual shrimps which were frozen on trays in a -70°C freezer, then placed in ziplock bags and stored at -70°C. Sex and carapace length were determined before analysis.

Postlarval alvinocarid specimens were collected from the water column above and around the Broken Spur Vent field (29°10.0' N 43°10.4' W, Mid-Atlantic Ridge) using plankton nets deployed during the RRS *Charles Darwin* # 95 cruise (CD95) in August 1995. Nets were fished at depths from 2000 to 3000 m both above the vent site and up to 50 km away from it. Specimens were tentatively identified on the basis of morphology, frozen separately and stored at -70°C.

Further vent shrimp samples were collected during the MAR/97 cruise to all known vent sites on the Mid-Atlantic Ridge, aboard the R/V *Atlantis,* in July 1997. Specimens of *R. exoculata* were collected from the White Button structure in the Broken Spur vent field (29°10.0' N 43°10.4' W, Mid-Atlantic Ridge, ~3050 m depth) using a slurp gun deployed by ALVIN. Adult specimens (Figure 2.1) were sexed, their carapace length measured and their reproductive state scored: reproductive state scoring was on the basis of visible gonad size in females (after Copley, 1998; Figure 2.3) and presence or absence of spermatophore in males. Specimens were then dissected into abdomen, branchial area, gonad and "rest" (included carapace, pereiopods, and antennae).

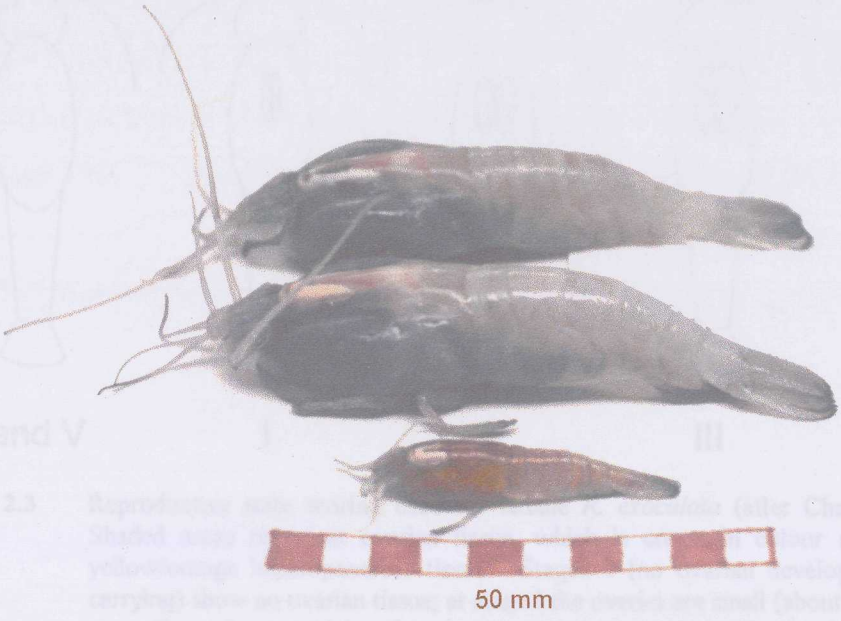


Figure 2.1 Adult and juvenile *R. exoculata* from the Broken Spur hydrothermal vent field.

Figure 2.2 *Alvinocaris markensis* from the Snake Pit hydrothermal vent field.

Juvenile specimens (discriminated by their smaller size and bright orange colouration) were measured and dissected into abdomen, branchial area and "rest" portions. All tissue samples were wrapped in aluminium foil and placed in labelled ziplock bags in a freezer at -70°C. Individuals of *Alvinocaris markensis* (Figure 2.2) were collected from the Snake Pit (23°22.1' N 44°57.0' W, Mid-Atlantic Ridge, ~3600 m depth) and TAG hydrothermal vent fields. They were sexed, their carapace length measured and then were dissected into abdomen, hepatopancreas, and "rest" portions. Eggs were also collected from the pleopods of some specimens. All tissue samples were wrapped in aluminium foil and frozen to -70°C.

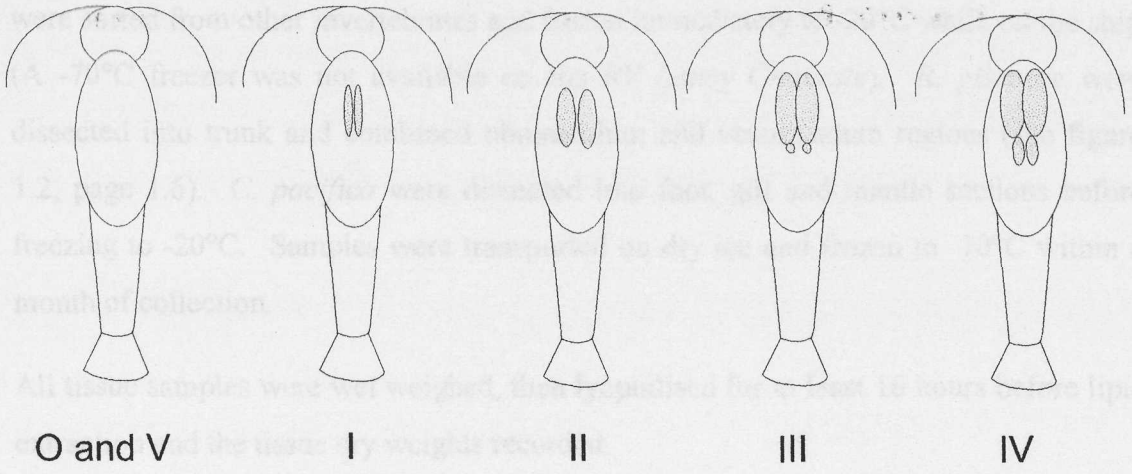


Figure 2.3 Reproductive state scoring used for female *R. exoculata* (after Chang & Shih, 1995). Shaded areas represent ovarian tissue, which is cream in colour and lies on top of yellow/orange hepatopancreas tissue: Stages 0 (no ovarian development) and V (egg carrying) show no ovarian tissue; at stage I the ovaries are small (about 3 oocytes thick); at stage II ovaries are thicker, but do not extend more than half way along the carapace; at stage III, posterior lobes are visible and the ovary extends half to three quarters of the way along the carapace; stage IV represents full ovarian development. Stage V (egg carrying) *R. exoculata* have rarely been caught and none were available for this study.

2.1.2 Epibenthic shrimp from the Arabian Sea

Nematocarcinus gracilis were collected during RRS *Discovery* 211/94 cruise to the north Arabian Sea in October 1994. Specimens were collected using an Agassiz Trawl just below the area where the oxygen minimum intersects the slope (19°15' N, 58°27' W, 933 - 1100 m depth) and on recovery, individuals were immediately frozen to -70°C. Sex (where possible) and carapace length were determined before analysis.

2.1.3 Juan de Fuca Ridge hydrothermal vent invertebrates

Specimens of the vestimentiferan tubeworm, *Ridgeia piscesae*, the polychaete palm worm *Paralvinella palmiformis* and the hydrothermal vent clam *Calyptogena pacifica* were collected during the High Rise '95 cruise to the High Rise hydrothermal vent field, Juan de Fuca Ridge Endeavour Segment (48°57.1' N, 129°05.2' W, ~ 2200 m depth). *R. piscesae* and *P. palmiformis* were collected using the manipulators of the US Navy's deep submergence vehicle *Seacliff* to grab clumps of worms. Large *R. piscesae* were collected from the "Fairy Castle" chimney structure and *P. palmiformis* were collected from "Godzilla" and "Park Place". Individual *C. pacifica* were collected from the Clam Bed site, south of the High Rise vent field (47°57.7' N, 129°05.6' W) using the more delicate manipulators of the US Navy's remote operated vehicle ATV. *P. palmiformis*

were sorted from other invertebrates and frozen immediately to -20°C while on the ship (A -70°C freezer was not available on the *RV Laney Choueste*). *R. piscesae* were dissected into trunk and combined obturaculum and vestimentum regions (see figure 1.2, page 1.6). *C. pacifica* were dissected into foot, gill and mantle sections before freezing to -20°C. Samples were transported on dry ice and frozen to -70°C within a month of collection.

All tissue samples were wet weighed, then lyophilised for at least 16 hours before lipid extraction and the tissue dry weights recorded.

2.2 Laboratory procedures

Glassware was cleaned, before use, by soaking overnight in 2% Micro™ solution, rinsing with reverse osmosis (RO) water (i.e. tap water purified using a semi-permeable RO membrane to remove colloids, bacteria etc.). Initially, glassware was also cleaned by placing in an acid bath (50% nitric acid) overnight then rinsing with RO water, drying in an oven at 100°C. All glassware was stored in clean drawers until use. Vinyl gloves were worn throughout the analysis to reduce contamination and full procedural blanks were carried out.

2.2.1 Organic solvents

HPLC or Distol grade solvents were used throughout. Solvents used were as follows:

Chloroform	Methanol
Diethyl ether	i-Propanol
Hexane	Toluene
Pyridine	

2.2.2 Reagents

Analytical grade reagents were used. Reagents used were as follows:

Concentrated sulphuric acid	Formic acid
Acetic anhydride	Sodium chloride
Potassium chloride	Potassium hydrogen carbonate
Glacial acetic acid	Butylated hydroxytoluene (BHT)
2,7-Dichlorofluoroscein	

2.2.3 Gases

High purity (99.99%) hydrogen and helium were used for the gas chromatography and nitrogen of a lower purity (99.9%) was used for the evaporation of solvents.

2.2.4 Standards

Standards used were as follows:

37 fatty acid methyl ester standard (Sigma)
Bacterial acid methyl ester standard (Radleys)
Dihydrocholesterol
Tripentadecanoin
Phosphatidyl ethanolamine
Tricosanoic acid methyl ester (23:0)
Palmitic acid stearyl ester
Cod liver oil
Cholesteryl myristate
Myristic acid
Phosphatidyl choline
Lysophosphatidyl choline
Sphingomyelin
Heptadecanol (17:0 alcohol)

2.3 Lipid extraction

Freeze-dried tissue samples were rehydrated by adding the mass of water that was lost during lyophilisation (or three times the dry weight of tissue) to ensure efficient triglyceride extraction (Dunstan *et al.*, 1993). Lipids were extracted after the procedure of Folch *et al.*, (1957). The rehydrated tissue sample was homogenised with methanol (5 ml) in a mortar and pestle. Chloroform (10 ml) was added and homogenisation continued. The homogenate was filtered through a Whatman No.1 filter and the filtrate reserved in a centrifuge tube. The residue was re-homogenised with methanol (5 ml), then chloroform (10 ml) was added and homogenisation continued. The homogenate was filtered through the same filter paper and the residue washed with 2:1 (v/v) chloroform/methanol (6 ml). The filtrates were combined in the centrifuge tube and this lipid solution washed by first adding a quarter of the total volume of lipid extract of 0.88% potassium chloride solution (9 ml), centrifuging, aspirating off and discarding the upper aqueous layer, then adding a quarter of the total volume of lipid extract of 1:1 (v/v) methanol/water (9 ml), centrifuging, aspirating off and discarding the upper, aqueous layer. The solvents were evaporated off the resulting washed total lipid extract using a stream of nitrogen and the lipid residues dried in a vacuum dessicator then

redissolved in chloroform containing 0.01% BHT and stored under nitrogen in the freezer at -20°C.

2.4 Lipid class composition

2.4.1 Thin Layer Chromatography with Flame Ionisation Detection (TLC-FID)

Thin layer chromatography with flame ionisation detection combines the resolution capabilities of thin layer chromatography with the possibility of quantification by using a flame ionisation detector. Separation of lipid classes is achieved on silica-coated rods known as Chromarods, which are then passed through a flame ionisation detector (FID). Careful preparation and calibration of Chromarods is required to ensure reliable, consistent results (Ackman *et al.*, 1990).

2.4.2 Iatroscan operating conditions

The Iatroscan Mark IV analyser was operated with an air flow of 2000 ml min^{-1}, hydrogen flow of 160 ml min^{-1} and at a scan speed of 4.2 mm s^{-1} The signal from the FID was transmitted via a Nelson 760 series interface to a PC with Turbochrom (version 3.1; PE Nelson Systems) data acquisition software.

2.4.3 Chromarod preparation and calibration

SIII Chromarods (Iatron Laboratories Inc., Japan) were stored in a dessicator and activated prior to use by passing twice through the Iatroscan flame ionisation detector (FID). Each rod was calibrated individually, for each lipid class, to account for inter-rod variability and differences in the response to different compound classes. Lipid standards were prepared at concentrations in the range expected for the samples to be analysed, based on other shrimp lipid data. A lipid standard was prepared by dissolving neutral lipids: palmitic acid stearyl ester (wax ester, WE); tripentadecanoin (triglyceride, TG); myristic acid (free fatty acid, FA); dihydrocholesterol (sterol, S); and phospholipids: phosphatidyl ethanolamine (PE); phosphatidyl choline (PC); sphingomyelin (SM); and lyso-phosphatidyl choline (LPC); in chloroform. This standard solution comprised concentrations of between 0.5 and 20 mg ml^{-1} with respect

to each lipid class and different amounts were applied to Chromarods allowing construction of calibration curves for each lipid class for each Chromarod.

2.4.4 Sample application and development

A rack of ten SIII Chromarods were blank-scanned in the Iatroscan FID twice before application of lipid solutions using a GC syringe or a 1 µl Socorex glass micropipette (Camlab). The rods were transferred to a humidity chamber containing a saturated solution of calcium chloride to achieve a humidity of approximately 32% (Delmas *et al.*, 1984) for 15 minutes. This ensured the same degree of hydration of the silica on the rods for each analysis. Development took place in a lined chamber containing the developing solvent (100 ml) that had been allowed to saturate the atmosphere within the chamber for at least 15 minutes. The humidified rods were suspended above the solvent in the developing chamber for 15 minutes to allow equilibration of the solvent with the rods, and then lowered into the solvent to develop until the solvent front reached a point approximately 10 cm above the origin. This took 30 minutes for the neutral lipid solvent and 45 minutes for the phospholipid solvent. The rods were then taken out of the chamber and immediately dried in an oven at 100°C for 2 minutes to remove the solvent before scanning in the Iatroscan Analyser. Neutral lipids were developed in a 85:15 mixture of hexane/chloroform containing 5% iso-propanol and 0.5% formic acid (Shantha & Ackman, 1990). Chromarods were partially scanned after neutral lipid separation (PPS 5 program). Rods were then placed in the humidity chamber for 15 minutes before equilibration and development of phospholipids in a 70:30:3.5 mixture of chloroform, methanol and water (Fraser *et al.*, 1985). Rods were fully scanned after phospholipid development and then blank-scanned at least twice before the next analysis. Figure 2.4 shows typical scans of lipid standards developed using these solvent systems and illustrates lipid class elution order.

Two (where consistent) or three replicates of each lipid sample were analysed. Where necessary, different amounts of sample were applied to achieve responses in the range calibrated for each lipid class.

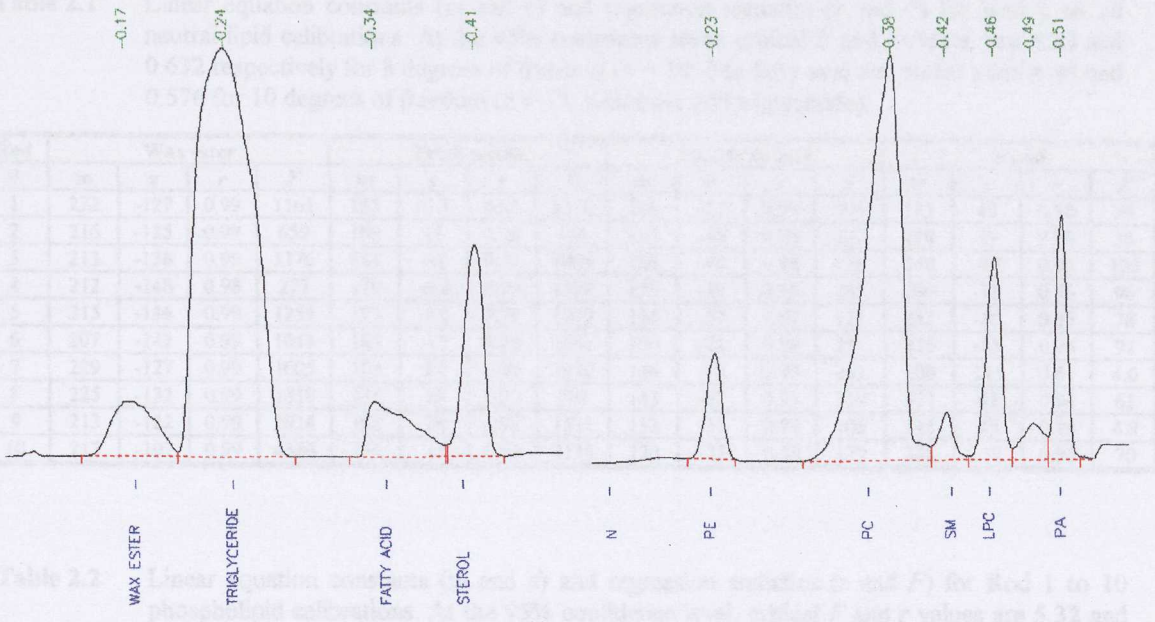


Figure 2.4 TLC-FID scans of neutral lipid (left of diagram) and phospholipid (right of diagram) standards. Names and abbreviations (N, neutrals; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lyso-phosphatidyl choline; PA, point of application) in blue refer to lipid classes. Red dotted lines delineate the baseline above which peak areas are integrated. Green numbers represent the decimal time in minutes from the beginning of the Chromarod scan to the detection of the peak.

2.4.5 TLC-FID data handling

Peak retention times and areas were exported from Turbochrom into a spreadsheet. Each Chromarod was individually calibrated for each lipid class by linear regression of peak areas on concentrations. The correlation coefficient was calculated and ANOVA was performed on each set of calibration data. *F* statistics and *r* values for each rod and each lipid class are presented along with the linear equation constants in Tables 2.1 and 2.2. Figures 2.5 to 2.8 present typical calibration curves for each lipid class on a Chromarod. Raw data (peak areas and corresponding amounts of lipid) are recorded in the CD-ROM appendix. Calibration was repeated when a new batch of samples were run to account for changes in the response of Chromarods: other calibration data are recorded in the CD-ROM appendix.

Sample lipid class concentrations were calculated by substituting peak areas into the relevant linear equation. The lipid solution concentrations and sample dry weights were used to calculate lipid class tissue concentrations (mg lipid g dry weight tissue^{-1}) and percentage lipid class compositions (lipid class % total lipid) for each sample.

Table 2.1 Linear equation constants (m and c) and regression statistics (r and F) for Rod 1 to 10 neutral lipid calibrations. At the 95% confidence level, critical F and r values are 5.32 and 0.632 respectively for 8 degrees of freedom (n = 10; free fatty acid and sterol) and 4.96 and 0.576 for 10 degrees of freedom (n = 12; wax ester and triglyceride).

Rod	Wax ester				Triglyceride				Free fatty acid				Sterol			
#	m	c	*r*	*F*	m	c	*r*	*F*	m	c	*r*	*F*	m	c	*r*	*F*
1	222	-127	0.99	1161	185	-0.3	0.99	2214	126	-4.5	0.99	506	181	49	0.88	28
2	216	-125	0.99	659	189	45	0.99	316	135	-30	0.98	222	218	29	0.88	28
3	213	-136	0.99	1376	184	-38	0.99	1809	126	-40	0.98	178	248	-47	0.96	105
4	212	-148	0.98	277	179	-0.4	0.99	1598	139	-39	0.98	235	280	-75	0.96	96
5	215	-136	0.99	1255	179	-9.3	0.99	1540	124	-57	0.97	121	233	-40	0.95	78
6	207	-142	0.99	1043	185	-11	0.99	1481	160	-71	0.98	221	325	-83	0.96	91
7	229	-127	0.99	1605	184	37	0.99	1910	146	-48	0.99	261	108	141	0.61	4.6
8	225	-132	0.99	1818	185	14	0.99	902	143	-57	0.99	318	171	62	0.94	61
9	213	-122	0.99	1914	182	28	0.99	1511	133	-31	0.96	105	145	78	0.93	4.9
10	217	-102	0.99	4366	181	15	0.99	2136	120	-23	0.98	179	238	-17	0.95	70

Table 2.2 Linear equation constants (m and c) and regression statistics (r and F) for Rod 1 to 10 phospholipid calibrations. At the 95% confidence level, critical F and r values are 5.32 and 0.632 respectively for 8 degrees of freedom (n = 10).

Rod	Phosphatidyl ethanolamine				Phosphatidyl choline				Sphingomyelin				Lysophosphatidyl choline			
#	m	c	r	F	m	c	r	F	m	c	r	F	m	c	r	F
1	90	-28	0.96	97	132	-103	0.99	454	156	-14	0.98	178	135	-9.3	0.97	127
2	94	-30	0.96	110	136	-77	0.98	239	142	-9.5	0.93	48	136	2.2	0.95	72
3	102	-35	0.95	71	145	-117	0.99	614	197	-20	0.97	113	158	-28	0.98	224
4	76	-21	0.98	171	129	-75	0.99	1288	229	-13	0.95	77	188	12	0.98	188
5	100	-36	0.97	114	133	-99	0.99	454	147	-9	0.93	53	125	-10	0.99	314
6	86	-28	0.97	150	144	-94	0.99	574	199	3.5	0.9	35	225	18	0.97	145
7	107	-34	0.97	111	139	-96	0.99	1136	164	-13	0.95	78	199	-33	0.96	88
8	113	-44	0.96	96	150	-148	0.99	901	148	-14	0.92	46	145	-39	0.98	190
9	110	-48	0.93	55	146	-137	0.99	620	144	-13	0.93	50	137	-28	0.98	206
10	113	-40	0.96	84	141	-131	0.99	615	126	-11	0.91	39	140	-35	0.98	216

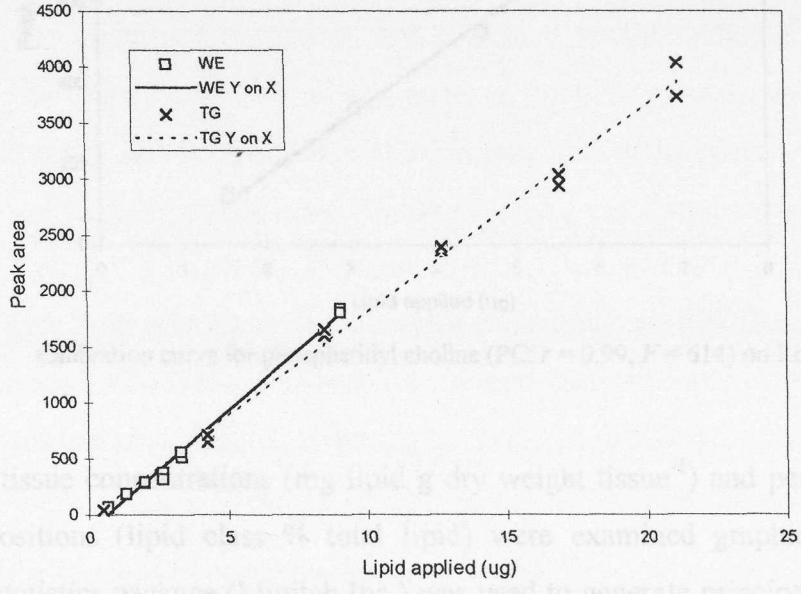


Figure 2.5 Calibration curves for wax ester (WE; r = 0.99; F = 1380) and triglyceride (TG; r = 0.99, F = 1810) on Rod 3

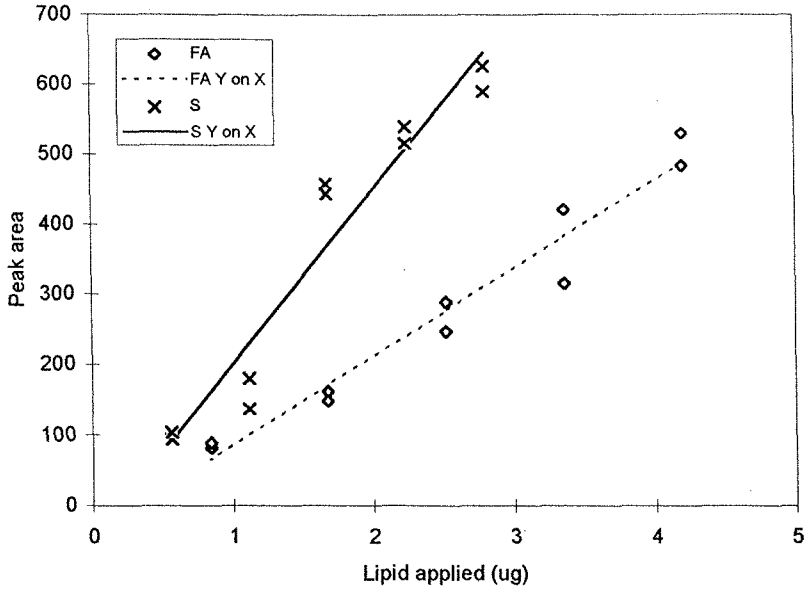


Figure 2.6 Calibration curves for free fatty acids (FA; $r = 0.98$, $F = 178$) and sterols (S; $r = 0.96$; $F = 105$) on Rod 3

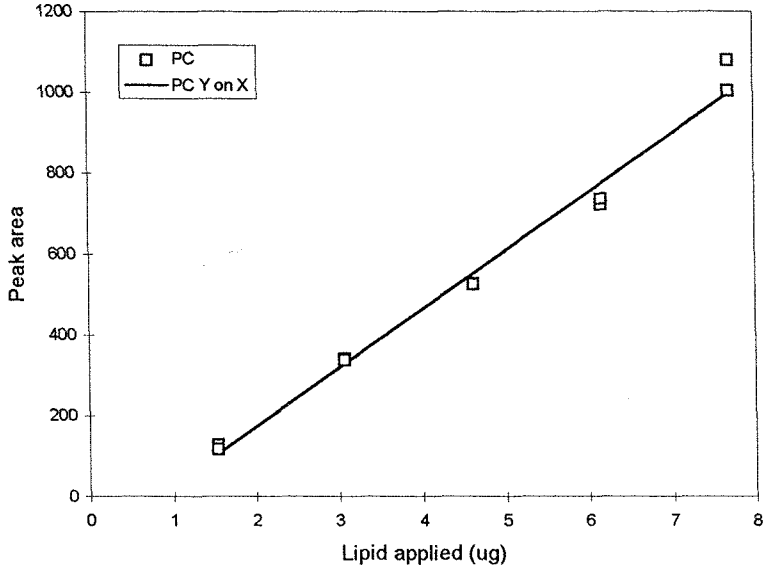


Figure 2.7 Calibration curve for phosphatidyl choline (PC; $r = 0.99$, $F = 614$) on Rod 3

Lipid class tissue concentrations (mg lipid g dry weight tissue^{-1}) and percentage lipid class compositions (lipid class % total lipid) were examined graphically and the MINITAB statistics package (Minitab Inc.) was used to generate principal components from a correlation matrix of lipid class compositions for each species and of mean lipid class compositions of samples from different sites.

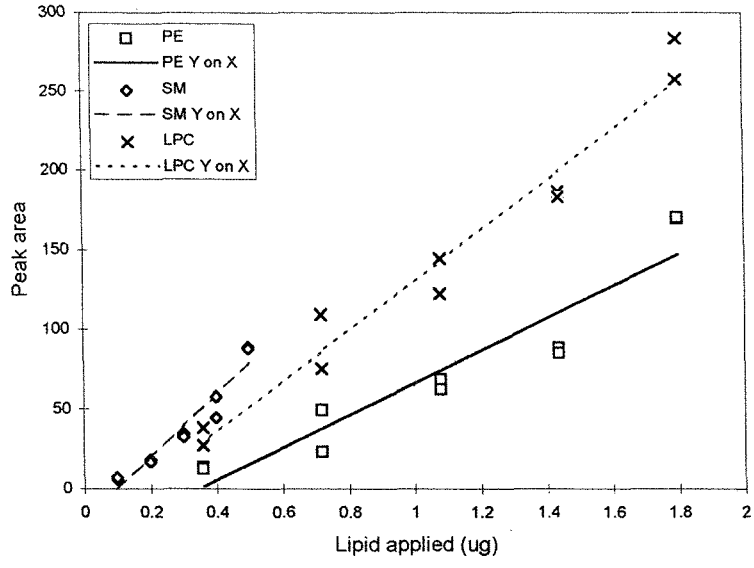


Figure 2.8 Calibration curves for phosphatidyl ethanolamine (PE), sphingomyelin (SM) and lyso-phosphatidyl choline (LPC) on Rod 3

2.5 Fatty acid and alcohol composition

2.5.1 Preparation of fatty acid methyl esters (FAME)

A known quantity of 23:0 methyl ester was dissolved in hexane to a concentration of approximately 1 mg ml^{-1}. This internal standard (100 µl) was added to an aliquot of total lipid solution containing approximately 0.5 mg lipid. The solution was evaporated to dryness and redissolved in toluene (0.5 ml) in a Sovirell tube. Dry methanol containing 1.5% sulphuric acid (2 ml) was added to the lipid solution, the tube flushed with nitrogen, sealed and heated overnight, for a minimum of 16 hours, at 50°C. After allowing to cool to room temperature, RO water (2 ml) was added to the solution and the fatty acid methyl esters were extracted with 1:1 hexane/diethyl ether containing 0.01% BHT (2 x 3 ml aliquots). The extract was washed with 2% aqueous potassium hydrogen carbonate (2 ml) then evaporated to dryness under nitrogen. The lipid residue was redissolved in a small volume of hexane containing 0.01% BHT.

2.5.2 Purification of fatty acid methyl esters (FAME)

The transmethylated lipid solution was purified by thin layer chromatography (TLC). Concentrated lipid solutions in hexane were applied to Merck 60 silica TLC plates (20

x 20 cm) in 2 cm wide bands 1.5 cm apart. Plates were developed in a solution of hexane/diethyl ether/acetic acid (90:10:1) for 45 minutes, dried and sprayed with 2,7-dichlorofluoroscein in 95% methanol. They were dessicated in a vacuum dessicator for at least an hour. The fatty acid methyl ester bands (and fatty alcohol bands, where present - see section 2.5.3) were visualised under UV light and scraped into centrifuge tubes containing 4 ml 1:1 hexane/diethyl ether. The methyl ester containing silica residue and solvent were rotamixed with 2% potassium hydrogen carbonate (2 ml) and centrifuged. The upper organic layer was transferred to a second tube. A further aliquot of 1:1 hexane/diethyl ether (4 ml) was added to the aqueous silica residue, the tube rotamixed and centrifuged, and the upper organic layer added to the first extract. The solvent was evaporated off and the FAME residue redissolved in hexane containing 0.01% BHT (100 µl). Samples were stored in the freezer at -20°C until analysis by gas chromatography and gas chromatography with mass spectrometry.

2.5.3 Preparation and purification of fatty alcohol acetates

Transmethylation of wax esters releases fatty alcohols, as well as producing FAME, which were separated from FAME at the TLC purification stage of FAME preparation. A known quantity of heptadecanol (17:0 alcohol) was dissolved in hexane to a concentration of approximately 1 mg ml^{-1}. This internal standard (50 or 100 µl) was added to the total lipid to be transmethylated, where wax esters were thought to be present in the sample. After transmethylation, fatty alcohols were extracted along with FAME as described in section 2.5.2. Fatty alcohols eluted separately from FAME during the TLC purification stage and were scraped separately from the TLC plates and extracted into 1:1 hexane/diethyl ether as for FAME. The pure fatty alcohol solution in 1:1 hexane/diethyl ether was evaporated to dryness in a Sovirell tube. A mixture of 1:2 acetic anhydride/pyridine (3 ml) was added, the tube flushed with nitrogen, sealed and heated to 37°C for 15 minutes with occasional shaking. After cooling, RO water (1 ml) was added and the solution extracted with 1:1 hexane/diethyl ether (2 x 4 ml aliquots). The organic layer was thoroughly washed with RO water to remove all traces of pyridine, then evaporated to dryness under nitrogen. Resulting fatty alcohol acetates were redissolved in hexane containing 0.01% BHT (50 or 100 µl) and stored as FAME until analysis by GC.

2.5.4 Gas chromatography of fatty acid methyl esters and fatty alcohol acetates

Gas chromatography was carried out using a Perkin Elmer 8500 gas chromatograph equipped with a Nelson 900 series interface connected to a PC running Turbochrom (version 3.1; PE Nelson Systems) data acquisition software. The split/splitless injector and flame ionisation detector were operated at 250°C and 300°C respectively and injection was manual and in split mode. Flame gases, hydrogen and air were run at pressures of 8 psi and 18 psi respectively. The temperature programme used for all analyses started with a 6°C min^{-1} ramp from 120°C to 150°C, followed by a 4°C min^{-1} ramp up to 185°C then a 2°C min^{-1} ramp to 195°C and finally a 4°C min^{-1} ramp to 230°C holding isothermal at 230°C for 2.5 minutes. The carrier gas was hydrogen running at a pressure of 23 psi, yielding a carrier gas velocity of 38 to 43 cm s^{-1} (near the optimum for good separation at the programmed temperatures). The flow rate through the split vent and carrier gas flow rates were found using a bubble flow meter and these values used to calculate the split ratio, which was between 40 and 50 units:

Equation 2.1

$$\text{Split ratio} = \frac{\text{Flow rate through split vent (ml min}^{-1})}{\text{Carrier gas flow rate (ml min}^{-1})}$$

Chromatography was carried out using a BPX70 (SGE) very polar capillary column: a 50 m x 0.22 mm (I.D.) fused silica column coated with a 70% cyanopropyl equivalent modified siloxane phase (film thickness 0.25 μm) stable at high temperatures (maximum operating temperature 290-300°C, maximum continuous operating temperature 260°C). FAME and fatty alcohols were identified by reference to the retention times of known standards under the same conditions (column, temperature, gas pressure): a lipid standard containing 37 FAME (Sigma); a bacterial acid methyl ester (BAME) standard (Radleys); a fatty alcohol standard containing 14:0, 16:0, 17:0 and 18:0 fatty alcohols; marinol standards and GC-MS characterised fatty acid methyl esters derived from hydrothermal vent organisms (University of Stirling). FAME and fatty alcohol identities were also confirmed by GC-MS. Figure 2.9 is a gas chromatogram of the 37 FAME lipid standard, illustrating the elution order and retention times of FAME on a 50 m BPX70 column under the conditions mentioned earlier.

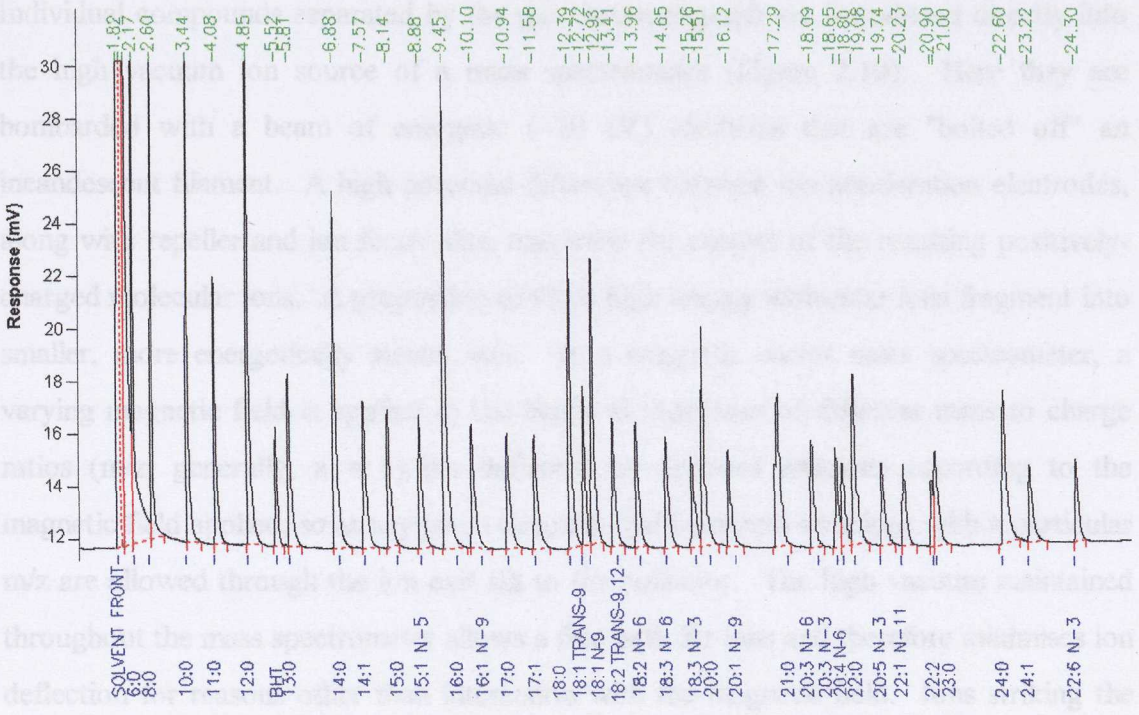


Figure 2.9 Gas chromatogram of the 37 FAME standard. Peak identifications are in blue. Retention times are in green and the red dotted line delineates the baseline above which peaks are integrated.

2.5.5 Gas Chromatography with Mass Spectrometry (GC-MS)

GC-MS was carried out using a HP5890 GC equipped with a J & W on-column injector and a column directly interfaced to the electron ionisation (EI) source of a VG 7070 Micromass magnetic sector double focussing mass spectrometer set to scan 55 to 550 atomic mass units (a.m.u.) at 1.5 seconds decade^{-1}. Data were collected using a PC with Mass Spectrometry Services (MSS) version 12.1 software. The carrier gas used was helium (99.99999% pure) and typically 0.2 to 0.4 µl of sample were injected. Samples were analysed using a BPX5 column (a 25 m x 0.32 mm I.D. fused silica column with a 5% phenyl equivalent modified siloxane phase, film thickness 0.25 µm). The temperature program used was as follows: 60°C isothermal for 5 min, then ramped to 120°C at 20°C min^{-1}, 120°C to 250°C at 2°C min^{-1} and isothermal at 250°C for 10 min.

2.5.6 Fatty acid and fatty alcohol data handling

Mass spectrometry

GC-MS allows the identification of individual molecules separated by gas chromatography. Compounds effect characteristic mass spectra from which their structure may be deduced, or that can be identified with reference to known spectra.

Individual compounds separated by the gas chromatograph are introduced directly into the high vacuum ion source of a mass spectrometer (Figure 2.10). Here they are bombarded with a beam of energetic (~70 eV) electrons that are "boiled off" an incandescent filament. A high potential difference between ion acceleration electrodes, along with repeller and ion focus slits, maximise the current of the resulting positively-charged molecular ions. A proportion of these high energy molecular ions fragment into smaller, more energetically stable, ions. In a magnetic sector mass spectrometer, a varying magnetic field is applied to the beam of ions: ions of different mass to charge ratios (m/z; generally, z = 1) are deflected by different amounts according to the magnetic field applied, so at any given magnetic field strength only ions with a particular m/z are allowed through the ion exit slit to the collector. The high vacuum maintained throughout the mass spectrometer allows a free path for ions and therefore minimises ion deflection for reasons other than interaction with the magnetic field. Ions striking the collector electrode result in a flow of neutralising electrons that is proportional to the ion abundance. This current is measured accurately and amplified to allow sensitive ion abundance measurement. The result is a total ion current trace that is approximately equivalent to a gas chromatogram trace and individual mass spectra for each compound that elutes from the gas chromatograph (e.g. figure 2.13).

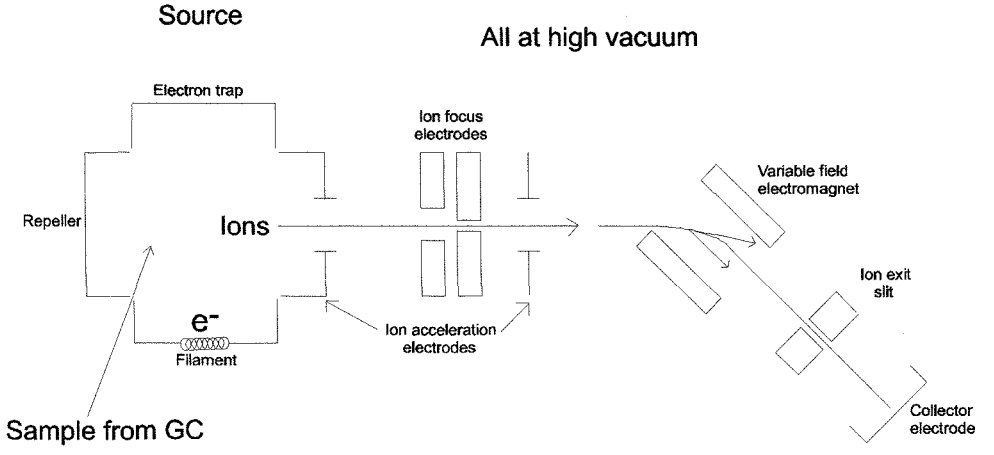


Figure 2.10 Schematic diagram of a mass spectrometer

The most common ion fragment in an individual m/z spectrum is used as the 100% intensity standard against which the intensities of all other ions are measured. This is referred to as the base peak. The ion fragment with the same mass as the original compound (and consequently, usually the highest m/z) is known as the molecular ion.

For example, the molecular ion of a 16:0 methyl ester ($C_{17}H_{34}O_2$) has a mass of 270 a.m.u.

Identification of fatty acid methyl esters was achieved using a combination of a database of known FAME mass spectra (NIST library software, supplier) and personal knowledge of the fragmentation of fatty acid methyl esters:

Characteristic peaks owing to ions formed during the fragmentation of saturated FAME include that with a mass to charge ratio (m/z) of 74, resulting from a McClafferty rearrangement (McClafferty & Turecek, 1993; Figure 2.11). This is usually the base peak in the mass spectrum of saturated FAME.

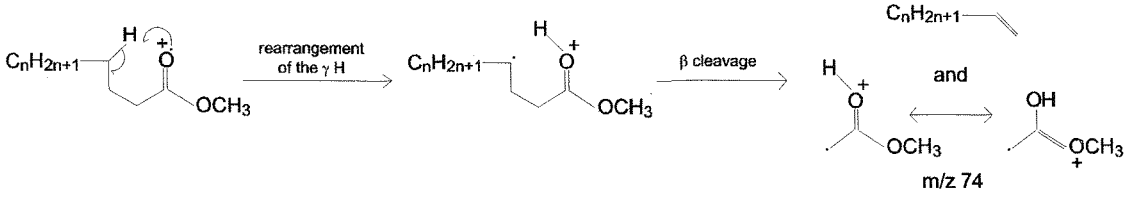


Figure 2.11 McClafferty rearrangement and saturated FAME fragmentation

The molecular ion is usually also present in the spectra of saturated FAME. Fragmentation of the alkyl chain of the molecule results in a series of ions with m/z ratios separated by 14 or 28 (the mass of methylene (CH_2) or ethylene (CH_2CH_2) groups) a.m.u. An ion with m/z ratio of M-31 a.m.u (where M is the mass of the molecular ion) is also characteristic of saturated fatty acid methyl esters. This is a result of the loss of the CH_3O- moiety of the methyl ester. Figure 2.13 (page 2.17) shows the typical mass spectrum of a saturated FAME.

The ion at m/z 87 is the result of a different hydrogen atom migration and subsequent cleavage (Figure 2.12, McClafferty & Turecek, 1993).

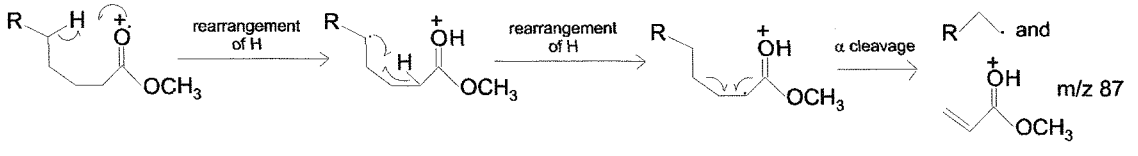


Figure 2.12 FAME fragmentation giving rise to an ion with m/z 87

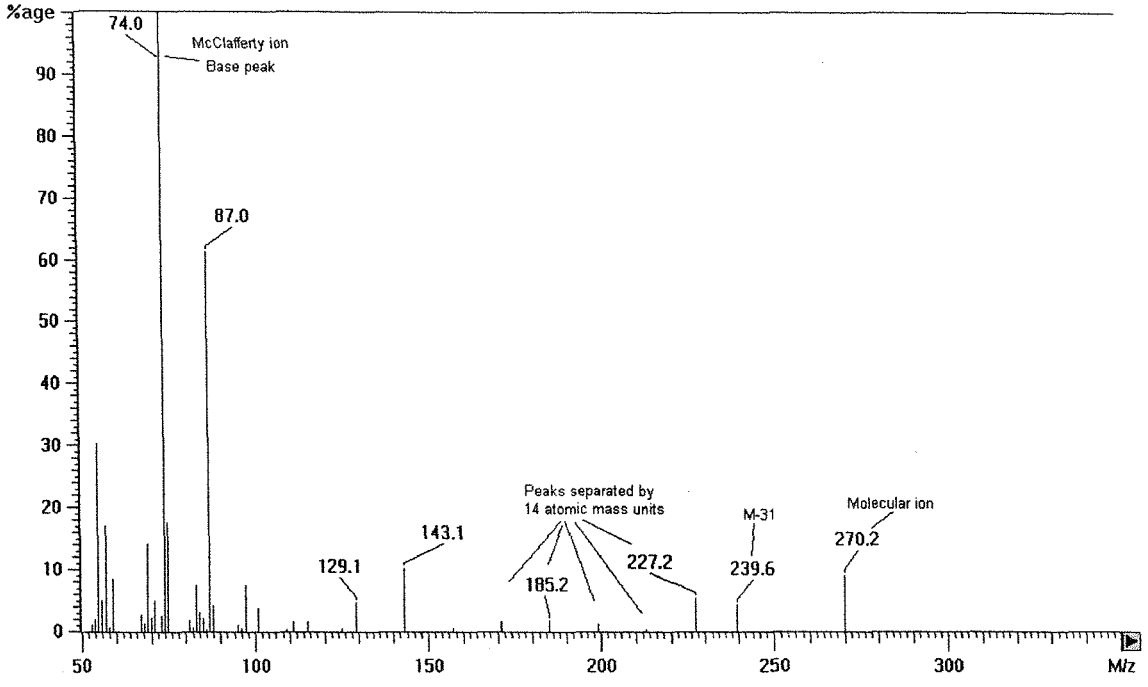


Figure 2.13 Annotated mass spectrum for 16:0 methyl ester

The molecular ion is less prominent, sometimes absent, in the mass spectra of unsaturated FAME: monounsaturated fatty acids are characterised by an ion with a m/z of M-32 a.m.u. e.g. 18:1 FAME spectra have an ion at m/z 264: the mass of the molecular ion is 296. This is a result of the fragmentation and ionisation shown in Figure 2.14. The loss of a methanol molecule is favoured by the double bond stabilisation of the radical on the alkyl chain fragment (McClafferty & Turecek, 1993).

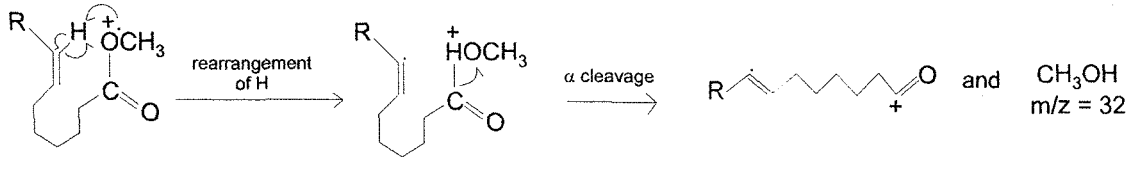


Figure 2.14 Monounsaturated FAME fragmentation

Figure 2.15 (page 2.18) shows the mass spectrum for an 18:1 fatty acid methyl ester. It is not possible to determine the position of unsaturation from this spectrum, owing to the double bond migration during ionisation in the mass spectrometer.

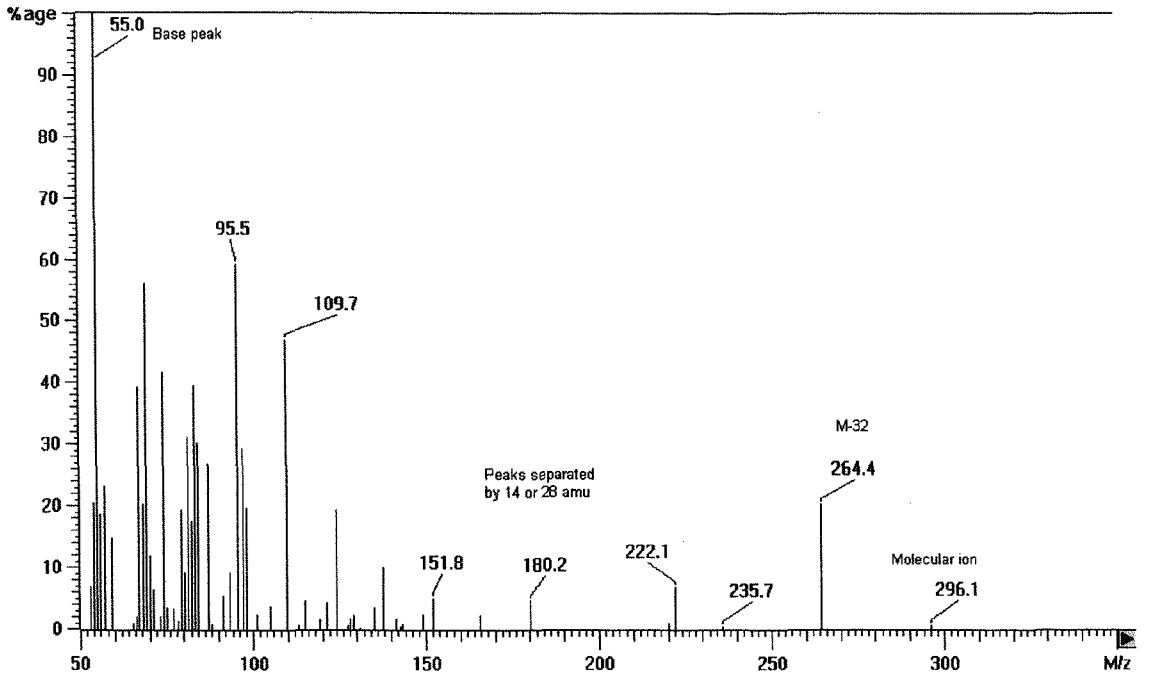


Figure 2.15 Annotated mass spectrum for a monounsaturated fatty acid methyl ester, 18:1

Characterisation of double bond position in unsaturated fatty acids is possible by the mass spectrometry of fatty acid derivatives such as fatty acid picolinyl esters (Harvey, 1982; Harvey, 1984). With these derivatives, interactions with the derivatising group prevent the rearrangement of double bond positions during ionisation and characteristic ions are formed when fragmentation occurs. In this study, fatty acid double bond positions were confirmed by comparison of retention times with those of known fatty acids at the University of Stirling. Characteristic mass spectra were used in conjunction with retention time information to confirm chain length and degree of unsaturation of FAME.

Fatty acid methyl ester and fatty alcohol quantification

Peak identities were assigned to chromatograms and internal standard data entered using Turbochrom software. Reports, including retention time, assigned peak identification, raw area amounts and FAME or fatty alcohol concentrations, calculated with reference to the FAME or fatty alcohol internal standard using equation 2.2 (page 2.19), were

exported into a spreadsheet (CD-ROM appendix). These data were used to calculate percentage FAME or fatty alcohol composition (Equation 2.3) for each sample.

$$\text{FAME concentration (mg ml}^{-1}\text{)} = \frac{\text{Concentration of internal standard (mg ml}^{-1}\text{)}}{\text{Area of internal standard peak}} \times \text{Area of FAME peak}$$

Equation 2.2 Calculation of FAME concentrations by reference to an internal standard

$$\text{Percentage FAME} = \frac{\text{FAME concentration}}{(\sum \text{FAME concentrations}) - \text{internal standard concentration}} \times 100$$

Equation 2.3 Calculation of percentage FAME composition

Each of these equations also applies to the calculation of fatty alcohol concentrations or percentage compositions.

Chapter 3: Lipid profiles of Mid-Atlantic Ridge vent shrimp

Whole animal lipid profiles were determined for thirty six adult and four juvenile specimens of *Rimicaris exoculata* from TAG and for six sets of two or three individuals of postlarval alvinocarid specimens from the water column above Broken Spur. Juvenile *R. exoculata* were distinguished by their smaller size, bright orange colouration and oily texture. The exact identity, however, of the postlarvae remains uncertain. They were originally divided morphologically into an *Alvinocaris* type, a *Chorocaris* type and a third type named "type A". It was suggested that this third type was *Rimicaris exoculata*, but this was disputed because the specimens possessed eyes that are absent in the adults. Subsequent genetic studies of other postlarvae collected with these specimens found that "type A" included all three genera, the *Chorocaris* type included *Rimicaris* and *Chorocaris* species and the *Alvinocaris* type were *Alvinocaris* only (Dixon & Dixon, 1996). However, whatever the exact identities of the postlarval specimens, there were no significant differences in their lipid composition, so these data have been pooled.

Lipid profiles were also determined for dissected *R. exoculata* from Broken Spur and for dissected adult *Alvinocaris markensis* from TAG and Snakepit. In order that sufficient lipid was available for analysis, samples consisted of combined dissected tissues from two or three shrimp of the same reproductive state classification (see Chapter 2.1). Dissection of the gonad in male *R. exoculata* proved difficult, as this organ was usually very small and infiltrated other tissues. Insufficient tissue was available for quantitative lipid analysis in two of the three samples and it is possible that the one male gonad sample may also have contained other tissue from within the cephalothorax. Ovary and hepatopancreas were difficult to separate in female *R. exoculata*, so these tissues were combined for analysis: here they are generally referred to as gonad tissue for brevity.

Tables of mean lipid class composition are presented in Appendix 1. Raw data are stored in files on the appendix CD-ROM; a guide to these files is saved in a readme file: appendix.txt.

3.1 Lipid profiles of whole *R. exoculata* from TAG and postlarval alvinocarids from the water column above Broken Spur

All individual lipid class tissue concentrations were summed to provide total lipid tissue concentrations for adult and juvenile *R. exoculata* and for postlarval alvinocarids. Similarly, polar lipid tissue concentrations were calculated by summing individual phospholipid class concentrations. Mean total lipid and total polar lipid tissue concentrations are presented in Table 3.1.1. When carapace length was accounted for, there was no significant difference in the total lipid concentration of male and female adult *R. exoculata*. A Mann-Whitney Rank Sum Test was performed on [lipid tissue concentration (mg g dwt^{-1}) divided by carapace length (mm)] values for male and female specimens. The differences in the median values among the two groups were not great enough to exclude the possibility that the difference was a result of random sampling variability; there was no statistically significant difference ($P = 0.883$).

Table 3.1.1 Mean total lipid and polar lipid concentrations for whole hydrothermal vent shrimp (mean tissue concentrations mg g dry $weight^{-1}$ ± S.E. ($\sigma/\sqrt{n}$)

Shrimp	Mean total lipid ± S.E ($\sigma/\sqrt{n}$) (mg g dry $weight^{-1}$)	Mean polar lipid ± S.E ($\sigma/\sqrt{n}$) (mg g dry $weight^{-1}$)
Adult *R. exoculata* (n^1 = 108)	58.7 ± 1.9	19.3 ± 0.5
Juvenile *R. exoculata* (n = 12)	295 ± 31	12.6 ± 0.9
Postlarval alvinocarids (n = 18)	316 ± 10	13.7 ± 0.4

It is apparent that juvenile and postlarval shrimp have much higher total lipid tissue concentrations than adult shrimp. The major difference in tissue lipid concentration is not in the levels of polar lipids, so must be a result of variations in neutral lipid composition (see section 3.1.1). There is no statistically significant difference in the total polar lipids of juvenile *R. exoculata* and postlarval alvinocarids (Mann-Whitney Rank Sum Test, $P = 0.568$), but there is a significant difference between the total polar lipids of adult *R. exoculata* and those of juvenile and postlarval alvinocarids (Mann-Whitney Rank Sum Test, $P < 0.0001$).

[1] n refers to the number of lipid class results used to calculate the sample mean. It does not refer to the number of shrimp, which is n/3, because each sample was assayed three times.

3.1.1 Lipid class composition of whole *R. exoculata* from TAG and postlarval alvinocarids from the water column above Broken Spur

Figure 3.1.1 shows the mean contribution of each neutral lipid class to the total lipid of vent shrimp studied. The most obvious feature of these data is the greatly elevated levels of wax esters in juvenile and postlarval shrimp when compared to adults. There is also a decrease in wax ester tissue concentration (Figure 3.1.1) and an increase in triglyceride tissue concentration with increasing carapace length in adult *R. exoculata*

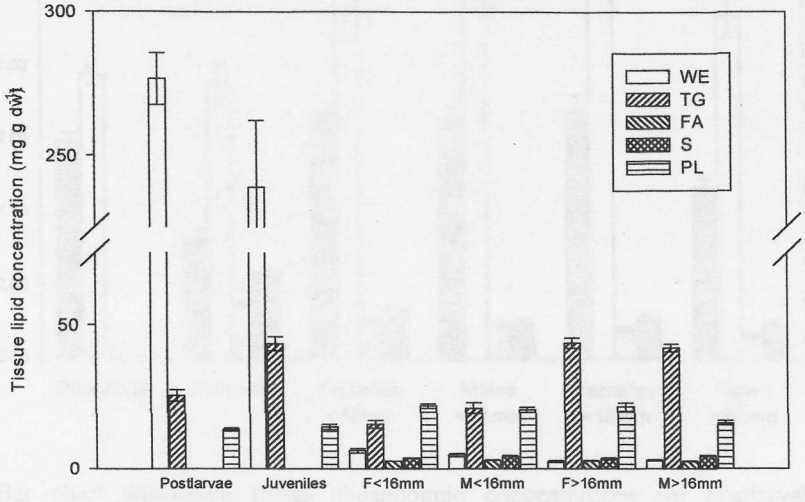


Figure 3.1.1 Bar chart showing the lipid class composition of postlarval alvinocarids, juvenile, small and large adult male and female *R. exoculata*. Error bars indicate standard errors of the mean (S.E. = $\sigma/\sqrt{n}$). WE, wax ester; TG, triglyceride; FA, free fatty acid; S, sterol; PL, polar lipids. Postlarvae, n = 18; juveniles, n = 12; adult female <16 mm, n = 36; adult male < 16 mm, n = 18; adult female >16 mm, n = 21; adult male >16 mm, n = 33.

(Figure 3.1.1, Figure 3.1.2). To highlight this fact, adult *R. exoculata* were split into two size fractions, each containing approximately the same number of specimens, and their lipid class composition data were pooled.

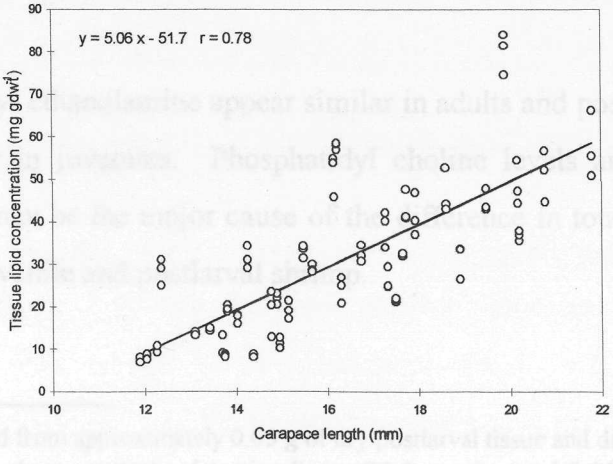


Figure 3.1.2 Triglyceride tissue concentration v/s carapace length for adult *R. exoculata* (n = 108)

Data for separate sexes were pooled. However, there were no apparent differences between data for male and female specimens in the same size fraction. Figure 3.1.3 presents phospholipid class composition for whole vent shrimp.

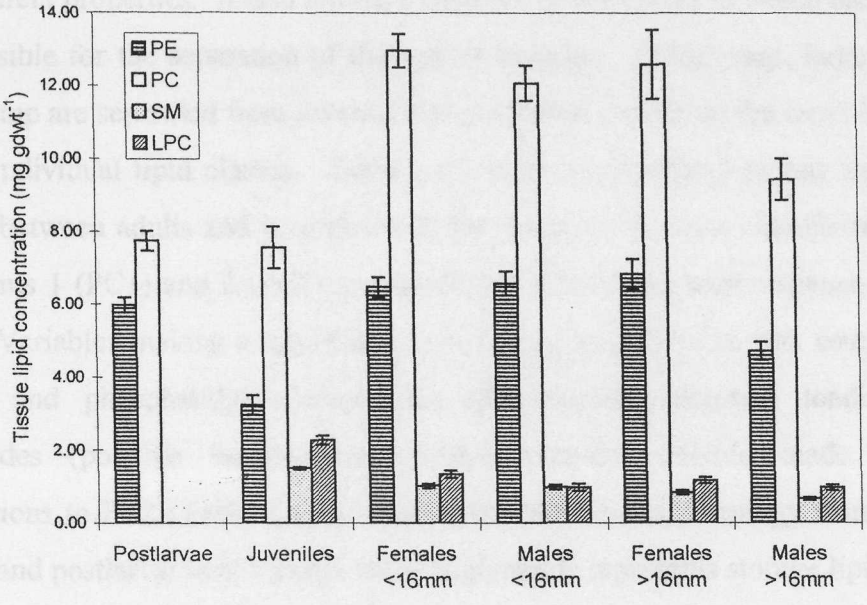


Figure 3.1.3 Bar chart illustrating tissue phospholipid concentrations for postlarval alvinocarids, juvenile, small and large adult male and female *R. exoculata*. Error bars represent standard error of the mean (S.E. = $\sigma/\sqrt{n}$), PE = phosphatidyl ethanolamine; PC = phosphatidyl choline; SM = sphingomyelin; LPC = lysophosphatidyl choline. Postlarvae, n = 18; juveniles, n = 12; adult female <16mm, n = 36; adult male < 16mm, n = 18; adult female >16mm, n = 21; adult male >16mm, n = 33.

Levels of sphingomyelin (SM) and lysophosphatidyl choline (LPC) appear fairly constant: their apparent absence in postlarvae is probably a function of the method detection limits, rather than a reflection of actual tissue concentrations[2].

Levels of phosphatidyl ethanolamine appear similar in adults and postlarvae, while they are somewhat lower in juveniles. Phosphatidyl choline levels are highest in adult specimens and this may be the major cause of the difference in total polar lipid levels between adult and juvenile and postlarval shrimp.

[2] Total lipid was extracted from approximately 0.05 g of dry postlarval tissue and dissolved in 1 ml solvent. In order for e.g. LPC levels to reach the detection limit of 0.3 µg, the total lipid solution would need a concentration of approximately 0.3 mg ml^{-1} LPC. If it did contain this level of LPC, that would correspond to a tissue concentration of [0.3 mg (in 1 ml solvent)/0.05g (dry weight tissue)] = 6 mg $gdwt^{-1}$. This is more than twice the level found in other tissue samples.

Principal component analysis of a correlation matrix of the percentage lipid class composition of hydrothermal vent shrimp was performed using the Minitab (Minitab Inc.) statistical analysis package. Principal component analysis allows simple illustration of the separation of a set of samples on the basis of variation in and between their different properties. It also returns a measure of the extent to which each property is responsible for the separation of the sets of samples. In this case, large and small adult shrimp are separated from juvenile and postlarval shrimp on the basis of variation in eight individual lipid classes. Table 3.1.2 identifies the lipid classes in which the variance between adults and juveniles and postlarvae is the most significant: principal components 1 (PC1) and 2 (PC2) accounted for 72% of the total variance within the matrix. Variables making a significant contribution to PC1 were wax esters (positive loading) and phosphatidyl choline and ethanolamine (negative loading), while triglycerides (positive loading) and lysophosphatidyl choline made significant contributions to PC2 (Table 3.1.2). Wax esters are the major energy storage lipid in juvenile and postlarval vent shrimp, while triglyceride represents storage lipid in adults. Phospholipids generally represent structural lipid.

Table 3.1.2 Summary of the key variables contributing to the first three principal components from a multivariate lipid class analysis of hydrothermal vent shrimp. WE, wax ester; TG, triglyceride; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; LPC, lysophosphatidyl choline; SM, sphingomyelin.

PC	Loading	Variable	Proportion of variance (%)	Cumulative variance (%)
PC1	+ -	WE PE, PC	53.0	53.0
PC2	+ -	TG LPC	19.1	72.0
PC3	+ -	SM LPC, TG	9.6	81.7

Ordination of all the samples on PC1 and PC2 produced clear separation of adult and juvenile shrimp (Figure 3.1.4) with the more positive values of PC1 reflecting the higher proportion of wax ester in juvenile and postlarval shrimp compared to adult shrimp. Variation in PC2 reflected the increase in triglyceride concentrations in larger adult shrimp compared to smaller adults. There was no clear separation of male and female adult shrimp.

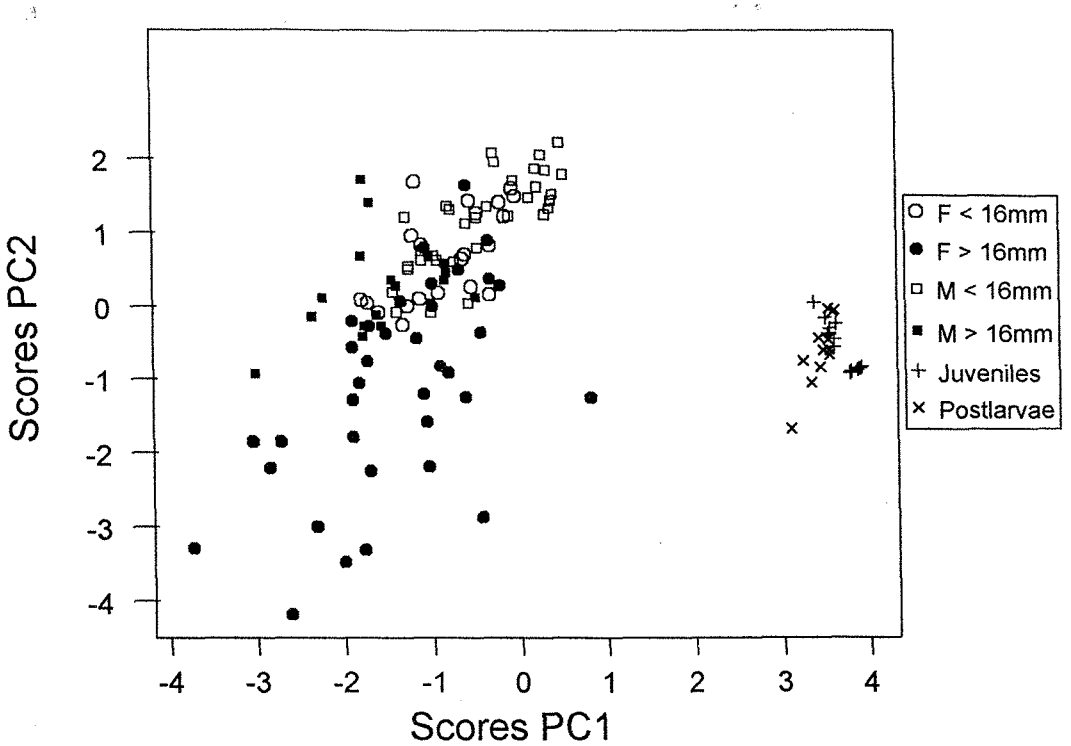


Figure 3.1.4 Ordinations of the scores of variables derived from percentage lipid class composition of hydrothermal vent shrimp (M = adult males; F = adult females). Postlarvae, n = 18; juveniles, n = 12; F <16mm, n = 36; M < 16mm, n = 18; F >16mm, n = 21; M >16mm, n = 33.

3.1.2 Fatty acid composition of whole *R. exoculata* from TAG and postlarval alvinocarids from the water column above Broken Spur

Mean fatty acid composition values have been calculated for large (>16 mm carapace length) and small (<16 mm carapace length) male and female adult *R. exoculata*, for juvenile *R. exoculata* and for postlarval alvinocarids. In addition to mean individual fatty acid percentage (weight) composition, the percentage composition of certain fatty acid types have been summed and their mean composition is also presented.

Fatty acid methyl ester tissue concentrations (mg g dry weight^{-1}) of different fatty acid types are also presented (Table 3.1.5). However, these results must be treated with some caution, because the actual total yields of FAME were low in some cases. Estimated expected FAME yields have been calculated from individual lipid class composition results (Equation 3.1).

Equation 3.1 Calculation of expected FAME yields from lipid class concentrations

Expected yield of FAME from lipid class = lipid class concentration x lipid class factor

The lipid class factors were calculated for the nonadecanoic acid derivatives (19:0 fatty acid; a mid-range molecular weight) of each lipid class: for example, the triglyceride factor was calculated as follows (Equation 3.2):

Equation 3.2 Calculation of triglyceride class factor to estimate yield of FAME

$$\text{Triglyceride factor} = \frac{\text{Molecular mass of 19:0 FAME x No of fatty acids per molecule}}{\text{Molecular mass of 19:0 triglyceride}} = \frac{312 \text{ g mol}^{-1} \text{ x } 3}{932 \text{ g mol}^{-1}} = 0.996$$

The wax ester factor was calculated for a 16:0 alcohol esterified to a 19:0 fatty acid. Factors used were as follows: wax ester, 0.60; triglyceride, 1.00; free fatty acid, 1.02; phosphatidyl ethanolamine, 0.81; phosphatidyl choline, 0.76; sphingomyelin, 0.41; lysophosphatidyl choline, 0.57. Total expected FAME yields were calculated by summing expected yields for lipid classes. Table 3.1.3 presents actual and expected mean FAME yields for adult and juvenile *R. exoculata* and postlarval alvinocarids. It was not possible to deduce standard deviations on calculated expected yields owing to the summation of the mean lipid class expected yields. However, a paired t-test was run on observed and estimated FAME yields for adult *R. exoculata* showing no significant difference in the two sets of data ($P = 0.182$). A paired t-test on observed and expected yields for juvenile *R. exoculata* and postlarval alvinocarids suggested that they are significantly different ($P = 0.013$, 95% confidence).

Table 3.1.3 Total FAME yields by gas chromatography (actual yield) and FAME yields estimated by calculation from lipid class tissue concentrations (estimated yield) for whole adult and juvenile *R. exoculata* and postlarval alvinocarids. Actual yields are quoted ± S.E. ($\sigma/\sqrt{n}$): Postlarvae, n = 6; juveniles, n = 4; adult female <16 mm, n = 12; adult male < 16 mm, n = 6; adult female >16 mm, n = 7; adult male >16 mm, n = 11.

	Postlarvae	Juveniles	Females <16mm	Males <16mm	Females >16mm	Males >16mm
Actual yield	81.5 ± 36.5	76.1 ± 25.6	28.4 ± 5.7	38.7 ± 11.3	58.3 ± 30.0	44.3 ± 10.4
Estimated yield	203	203	32.6	43.2	58.2	61.7

Table 3.1.4 presents the mean percentage fatty acid composition for whole vent shrimp. The principal fatty acids in adult *R. exoculata* comprise 16:0, 16:1 n-7, 18:1 n-9, 18:1 n-

7, 16:2 n-4 and 18:2 n-4, while juveniles and postlarvae are richest in 16:0, 16:1 n-7, 18:1 n-9, 18:1 n-7, 20:5 n-3 and 22:6 n-3.

Table 3.1.4 Mean percentage fatty acid composition of whole vent shrimp. Standard errors omitted for clarity. SFA, saturated fatty acids; UFA, unsaturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids.

	Postlarval alvinocarids	Juvenile *R. exoculata*	Female *R. exoculata* < 16 mm	Male *R. exoculata* < 16 mm	Female *R. exoculata* > 16 mm	Male *R. exoculata* > 16 mm
14:0	0.78	0.49	2.90	3.55	4.68	4.47
14:1	0.18	0.28	2.51	2.88	4.68	4.97
15:0	0.19	0.09	0.29	0.28	0.21	0.17
16:0	4.56	4.77	8.33	8.15	8.56	8.31
16:1 n-7	25.76	15.29	16.25	17.66	18.50	21.01
16:2 n-4	0.30	0.32	7.16	9.18	12.51	11.94
16:3 n-3	0.69	0.33	nd	nd	nd	nd
18:0	0.68	0.72	2.95	2.40	1.86	1.54
18:1 n-9	24.01	25.63	4.36	6.38	5.27	4.95
18:1 n-7	8.90	4.61	15.16	12.10	10.70	12.38
18:2 n-6	2.36	1.78	0.32	0.37	0.35	0.38
18:2 n-4	0.13	0.68	17.94	18.29	20.69	18.60
18:3 n-6	0.40	0.15	nd	nd	nd	nd
18:3 n-3	0.86	0.32	0.56	0.61	0.53	0.49
18:4 n-4	0.51	1.89	0.22	0.16	0.11	0.12
20:1 n-9	1.22	0.49	0.40	0.26	0.24	0.28
20:1 n-7	0.51	0.21	0.28	0.26	0.19	0.22
20:2 NMID	0.23	0.05	0.73	0.89	1.00	0.90
20:3 n-6	0.03	0.06	0.88	0.86	0.84	0.79
20:3 n-3	0.19	0.06	0.52	0.55	0.61	0.58
20:4 n-6	4.77	2.18	1.94	1.62	0.72	0.64
20:4 n-4	0.21	0.18	0.49	0.27	0.34	0.23
20:4 n-3	0.32	0.66	nd	nd	nd	nd
22:1 n-11	0.12	0.05	0.68	0.75	0.47	0.53
20:5 n-3	4.58	11.23	4.74	3.84	1.50	1.10
22:2 NMID	0.07	0.07	0.80	0.83	0.85	0.81
22:5 n-6	5.28	1.98	nd	nd	nd	nd
24:1	0.04	0.05	0.39	0.26	0.14	0.09
22:5 n-3	0.61	1.39	0.57	0.59	0.55	0.50
22:6 n-3	11.55	24.08	6.81	4.78	1.57	1.37
SFA	6.21	6.06	14.74	14.70	15.67	14.87
UFA	93.79	93.94	85.26	85.30	84.33	85.13
MFA	60.71	46.57	40.78	41.48	41.19	45.62
NMID	0.30	0.12	1.56	1.76	1.90	1.75
n-4 PUFA	1.15	3.07	26.30	28.56	34.45	31.75
n-6 PUFA	12.82	6.14	3.20	2.91	1.95	1.86
n-3 PUFA	18.80	38.04	13.43	10.59	4.85	4.15
18:1 n-7/n-9	0.43	0.19	3.49	3.19	3.38	3.26

Juvenile and postlarval shrimp contain only 6% saturated fatty acids, while mean adult percentage saturates are around 15%. However, ANOVA on the fatty acid tissue

Juvenile and postlarval shrimp contain only 6% saturated fatty acids, while mean adult percentage saturates are around 15%. However, ANOVA on the fatty acid tissue concentrations of SFA (Table 3.1.5) shows no significant differences in their amounts (P = 0.691). Levels of monounsaturated fatty acids are relatively high in postlarvae and juveniles compared to adults. The difference in the type of monounsaturates, evident in the ratio of 18:1 n-7 to 18:1 n-9 fatty acids is particularly striking. Juvenile and postlarval shrimp have significantly lower 18:1 n-7/n-9 ratios (reflecting higher levels of n-9 fatty acids) than adults (t-test, $P < 0.001$).

Table 3.1.5 Fatty acid methyl ester tissue concentrations of whole vent shrimp (mean tissue concentrations mg g dry weight^{-1} ± S.E ($\sigma/\sqrt{n}$). SFA, saturated fatty acids; UFA, unsaturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids (four or more double bonds). Postlarvae, n = 6; juveniles, n = 4; adult female <16 mm, n = 12; adult male < 16 mm, n = 6; adult female >16 mm, n = 7; adult male >16 mm, n = 11.

	Postlarval alvinocarids	Juvenile *R. exoculata*	Female *R. exoculata* < 16 mm	Male *R. exoculata* < 16 mm	Female *R. exoculata* > 16 mm	Male *R. exoculata* > 16 mm
Totals	81.5 ± 36.5	76.1 ± 25.6	28.4 ± 5.69	38.7 ± 11.3	58.3 ± 29.9	44.3 ± 10.4
SFA	5.16 ± 2.62	5.01 ± 2.02	4.19 ± 0.83	5.53 ± 1.54	9.05 ± 4.45	6.46 ± 1.52
UFA	76.3 ± 34.0	71.1 ± 23.7	24.2 ± 4.87	33.2 ± 9.81	49.2 ± 25.5	37.9 ± 8.88
MFA	45.5 ± 19.1	38.0 ± 14.5	11.8 ± 2.40	16.0 ± 4.66	24.2 ± 13.1	20.8 ± 5.17
NMID	0.12 ± 0.04	0.08 ± 0.02	0.46 ± 0.11	0.68 ± 0.21	1.01 ± 0.48	0.71 ± 0.15
n-4 PUFA	0.93 ± 0.54	1.95 ± 0.55	7.60 ± 1.62	11.6 ± 3.75	20.5 ± 10.5	13.5 ± 3.16
n-6 PUFA	11.3 ± 5.53	4.47 ± 1.39	0.93 ± 0.21	1.04 ± 0.26	0.97 ± 0.42	0.80 ± 0.22
n-3 PUFA	18.5 ± 9.24	26.6 ± 8.53	3.38 ± 0.65	3.88 ± 1.03	2.57 ± 1.23	2.03 ± 0.71
PUFA	30.8 ± 15.3	33.1 ± 10.3	12.4 ± 2.48	17.2 ± 5.17	25.0 ± 12.5	17.1 ± 3.91
HUFA	26.3 ± 13.3	29.1 ± 9.07	3.76 ± 0.72	4.08 ± 1.03	2.37 ± 1.09	1.98 ± 0.79

Adult *R. exoculata* contain relatively high levels of n-4 polyunsaturated fatty acids that are present in only small amounts in juvenile and postlarval shrimp. In addition to the increase in the percentage of n-4 polyunsaturated fatty acids from postlarvae to adults, there is a general decrease in the percentage of n-6 and n-3 polyunsaturated fatty acids. Figure 3.1.4 illustrates these trends. Levels of n-3 fatty acids are highest in juvenile *R. exoculata* and levels found in postlarval alvinocarids also exceed those found in adult *R. exoculata*. NMID fatty acids are present, if at low concentrations, in all adult *R. exoculata*, but are only present in trace amounts in juveniles and postlarvae.

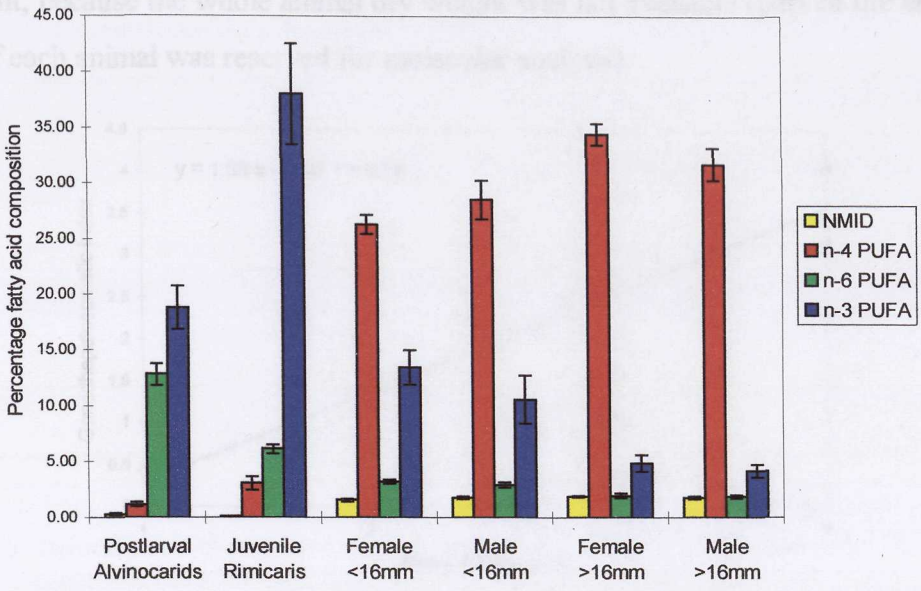


Figure 3.1.5 Bar chart showing mean percentage unsaturated fatty acid type composition of whole vent shrimp. NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids. Error bars indicate standard errors (SE = $\sigma/\sqrt{n}$). Postlarvae, n = 6; juveniles, n = 4; adult female <16mm, n = 12; adult male < 16mm, n = 6; adult female >16mm, n = 7; adult male >16mm, n = 11.

3.2 Lipid profiles of different tissues of *R. exoculata* from Broken Spur

The mean carapace lengths of *R. exoculata* dissected and combined for analysis are presented in Table 3.2.1.

Table 3.2.1 Mean carapace lengths of dissected *R. exoculata*

Sample type (number of specimens)	Mean carapace length ± S.E ($\sigma/\sqrt{n}$)
Juvenile (n = 9)	8.89 ± 0.27
Male (n = 9)	17.6 ± 0.50
Female (n = 24)	19.5 ± 0.32
F I (n = 3)	17.3 ± 0.15
F II (n = 5)	18.1 ± 0.52
F III (n = 6)	19.8 ± 0.25
F IV (n = 10)	20.7 ± 0.15

An index of combined ovary and hepatopancreas growth (gonadohepatosomatic index) was calculated by dividing the dry weight of ovary and hepatopancreas tissue by the mean carapace length of the animals from which the tissue was taken and multiplying by 1000 (to get numbers that were greater than one and therefore easier to interpret). Carapace length was used as a measure of the size of an individual, instead of total tissue

dry weight, because the whole animal dry weight was not available (part of the abdomen region of each animal was reserved for molecular analysis).

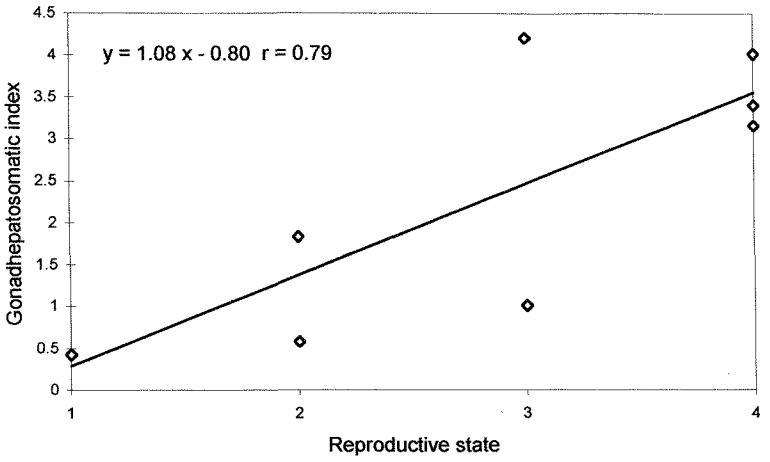


Figure 3.2.1 Gonadohepatosomatic index of female *R. exoculata* versus reproductive state. There is a statistically significant positive correlation (r = 0.79, n = 8, $P < 0.02$)

There was a positive correlation between the gonadohepatosomatic index and reproductive stage, although the points did not lie along a straight line (Figure 3.2.1).

Individual lipid class tissue concentrations were summed to give total lipid tissue concentrations for each tissue sample. These values were compared with total lipid tissue concentrations determined gravimetrically for the same samples. A Wilcoxon Signed Rank Test revealed that there was no statistically significant difference (P = 0.142) between the two sets of values. Table 3.2.2 presents total lipid tissue concentration (calculated by summing individual lipid class tissue concentrations) and polar lipid tissue concentration (calculated by summing individual polar lipid tissue concentrations) for different tissue types in male, female and juvenile *R. exoculata*.

It is apparent that high levels of lipid found in juvenile *R. exoculata* are stored in the abdomen region. Polar lipid levels are significantly lower in juvenile abdomen tissue than in adults (Mann-Whitney Rank Sum Test, $P < 0.0001$), while there is no significant difference between male and female polar lipid abdomen tissue concentrations (t-test, d.f. = 20, $P = 0.330$; 95% confidence limits). In adult *R. exoculata*, abdomen tissue was composed mainly of polar lipid. There were no significant differences in the tissue lipid concentrations of branchial area tissue of *R. exoculata* (Table 3.2.3).

Table 3.2.2 Mean total lipid and polar lipid concentrations for dissected *R. exoculata* (mean tissue concentrations mg g dry weight^{-1} ± S.E. ($\sigma/\sqrt{n}$))

Tissue Type	Sex	Mean total lipid ± S.E ($\sigma/\sqrt{n}$) (mg g dry weight^{-1})	Polar lipid ± S.E ($\sigma/\sqrt{n}$) (mg g dry weight^{-1})
Abdomen	Male (n[3] = 6)	29.0 ± 1.64	23.2 ± 0.98
	Female (n = 16)	26.7 ± 1.25	21.5 ± 0.97
	Juvenile (n = 6)	107 ± 17.4	12.7 ± 0.66
Branchial area	Male (n = 6)	41.4 ± 3.10	27.3 ± 1.31
	Female (n = 16)	45.6 ± 5.50	24.7 ± 1.81
	Juvenile (n = 6)	37.1 ± 12.2	21.1 ± 1.37
Gonad	Male (n = 2)	50.9 ± 0.78	36.8 ± 0.40
	Female (n = 16)	112 ± 9.11	30.5 ± 4.11

Table 3.2.3 Summary of the results of statistical comparisons of branchial area lipid tissue concentrations of *R. exoculata*. TL = total lipid; PL = polar lipid.

Shrimp and lipid compared	Sig diff?	Statistical test results
Adult male and female *R. exoculata* TL	No	Mann-Whitney Rank Sum test: $P = 0.740$
Adult and juvenile *R. exoculata* TL	No	Mann-Whitney Rank Sum test: $P = 0.675$
Adult male and female *R. exoculata* PL	No	t-test, 95% confidence: $P = 0.425$
Adult and juvenile *R. exoculata* PL	No	t-test, 95% confidence: $P = 0.124$

Ovarian (including hepatopancreas) tissue is rich in lipid and the level of polar lipid indicates that it is mainly composed of neutral lipid. However, there is a high standard error on both total lipid and polar lipid means for ovarian tissue. Table 3.2.4 shows total and polar lipid levels for ovarian tissue at different reproductive stages (See figure 2.3, page 2.3, for a description of reproductive stage scoring).

Table 3.2.4 Mean total lipid and polar lipid concentrations ovary (and hepatopancreas) tissue of *R. exoculata* at different stages of reproductive development (mean tissue concentrations mg g dry weight^{-1} ± S.E. ($\sigma/\sqrt{n}$)

Reproductive Stage	Mean total lipid ± S.E ($\sigma/\sqrt{n}$) (mg g dry weight^{-1})	Polar lipid ± S.E ($\sigma/\sqrt{n}$) (mg g dry weight^{-1})
F 0 & I (n = 2)	77.5 ± 6.61	27.5 ± 1.11
F II (n = 4)	87.1 ± 23.1	21.4 ± 2.57
F III (n = 4)	136 ± 8.17	24.5 ± 3.37
F IV (n = 6)	123 ± 12.6	41.7 ± 9.30

[3] n refers to the number of lipid class results used to calculate the sample mean value. It does not refer to the number of tissue samples, which is n/2, because each sample was assayed twice.

There is no clear trend in either total lipid or polar lipid concentrations for ovarian tissue at different stages of development. However, this table helps to explain the high standard errors of mean total and polar lipid concentrations for all ovarian tissue.

3.2.1 Lipid class composition of different tissues of *R. exoculata* from Broken Spur

Lipid class tissue concentrations for different tissue types are presented in Figure 3.2.2.

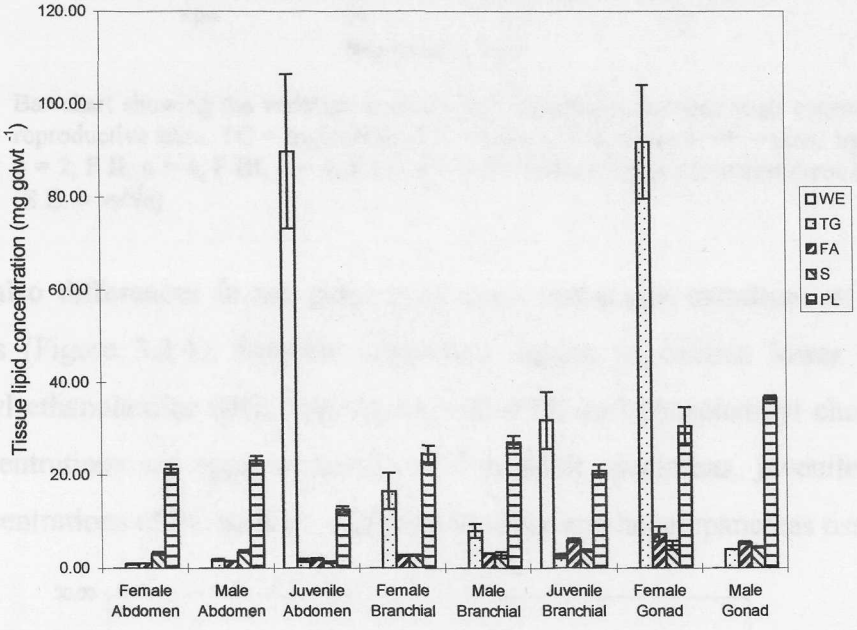


Figure 3.2.2 Bar chart illustrating tissue lipid concentrations in abdomen, branchial and gonad tissue for male, female and juvenile *R. exoculata*. Error bars represent standard error of the mean (S.E. = $\sigma/\sqrt{n}$), WE = wax ester; TG = triglyceride; FA = fatty acid; S = sterol; PL = polar lipid. Female tissue samples, n = 16; juvenile tissue samples and male tissue samples except gonad, n = 6; male gonad sample, n = 2.

Wax ester was detected in the tissue of juvenile specimens only. Triglyceride levels were low in abdomen tissue, somewhat higher in branchial tissue and highest in ovarian and hepatopancreas tissue. The variation in the concentration of triglyceride in ovarian tissue, reflected in the large standard error evident in Figure 3.2.2, is accounted for in Figure 3.2.3. It is evident that lipid class tissue concentration varies greatly with reproductive state.

There is an notable increase in triglyceride tissue concentration with reproductive development. Tissue concentrations of sterols and free fatty acids appear to decrease after stages 0 and I and polar lipid levels are highest in tissue at reproductive stage IV.

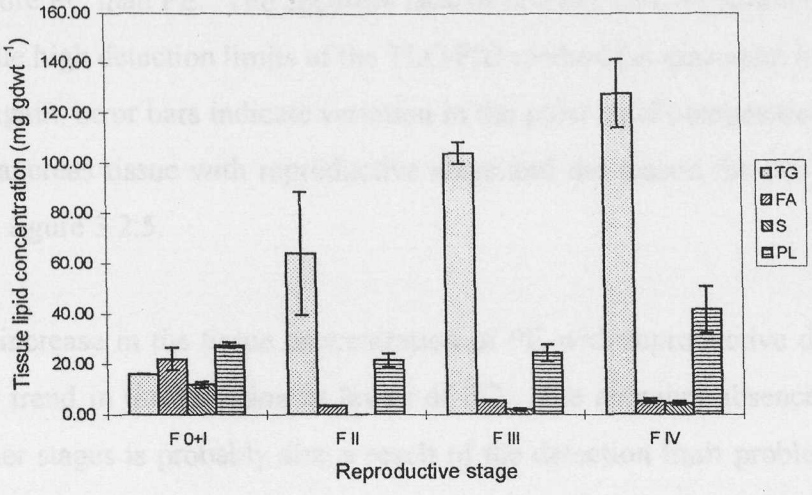


Figure 3.2.3 Bar chart showing the variation in ovary and hepatopancreas lipid class composition with reproductive state. TG = triglyceride; FA = fatty acid; S = sterol; PL = polar lipid. F 0 + I, n = 2; F II, n = 4; F III, n = 4; F IV, n = 6. Error bars indicate standard error of the mean (S.E. = $\sigma/\sqrt{n}$)

There are also differences in the polar lipid class tissue concentrations of different tissue types (Figure 3.2.4). Juvenile specimens appear to contain lower levels of phosphatidyl ethanolamine (PE) than adults: while PE and phosphatidyl choline (PC) tissue concentrations are approximately equal in adult specimens, juveniles contain higher concentrations of PC than PE. Combined ovary and hepatopancreas tissue also

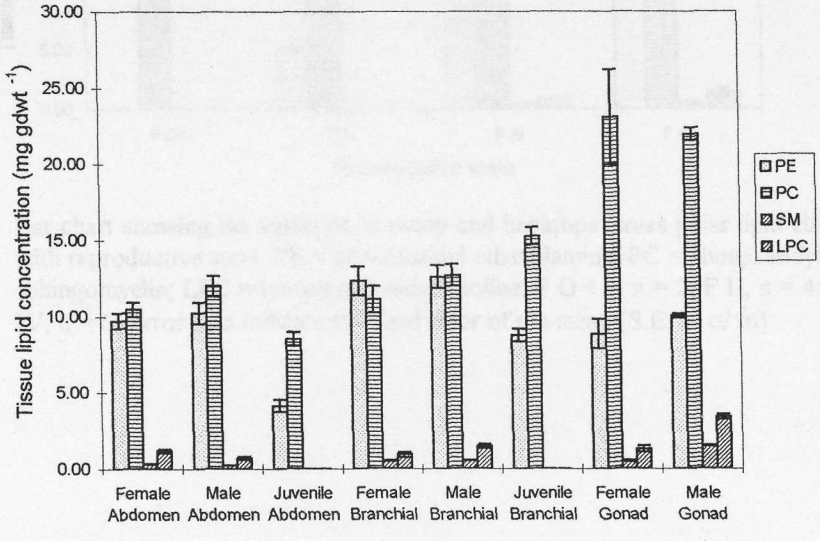


Figure 3.2.4 Bar chart illustrating polar lipid tissue concentrations in abdomen, branchial and gonad tissue of male, female and juvenile *R. exoculata*. Error bars represent standard error of the mean (S.E. = $\sigma/\sqrt{n}$), PE = phosphatidyl ethanolamine; PC = phosphatidyl choline; SM = sphingomyelin; LPC = lysophosphatidyl choline. Female tissue samples, n = 16; juvenile tissue samples and male tissue samples except gonad, n = 6; male gonad sample, n = 2.

comprises more PC than PE. The apparent lack of SM and LPC in juvenile tissue may result from the high detection limits of the TLC-FID method (as discussed in footnote 2, page 3.4). Again, error bars indicate variation in the polar lipid composition of ovarian and hepatopancreas tissue with reproductive stage and the reason for this variation is illustrated in figure 3.2.5.

There is an increase in the tissue concentration of PE with reproductive development, but no clear trend in the variation in levels of PC. The apparent absence of SM and LPC in earlier stages is probably also a result of the detection limit problem discussed earlier (footnote 2, page 3.4).

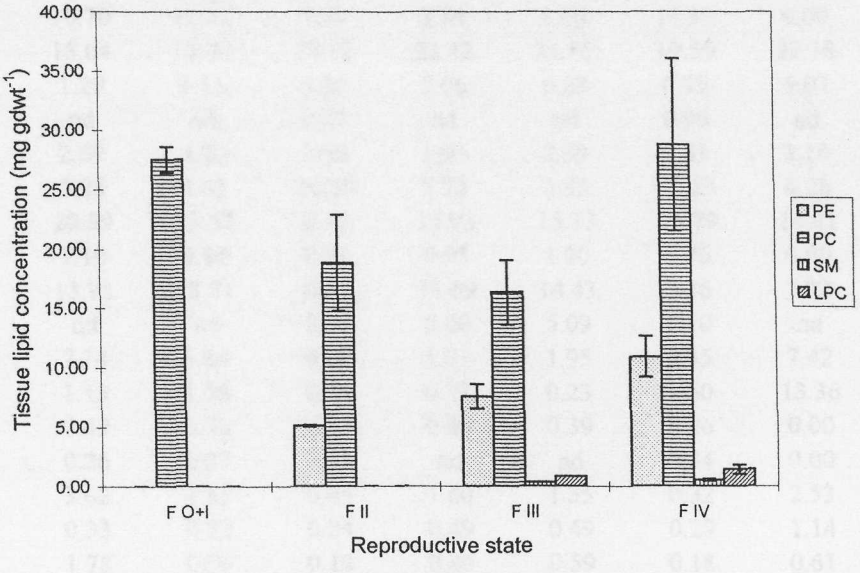


Figure 3.2.5 Bar chart showing the variation in ovary and hepatopancreas polar lipid class composition with reproductive state. PE = phosphatidyl ethanolamine; PC = phosphatidyl choline; SM = sphingomyelin; LPC = lysophosphatidyl choline. F O + I, n = 2; F II, n = 4; F III, n = 4; F IV, n = 6. Error bars indicate standard error of the mean (S.E. = $\sigma/\sqrt{n}$)

3.2.2 Fatty acid composition of different tissues of *R. exoculata* from Broken Spur

Mean individual fatty acid percentage (weight) composition values and the mean percentage composition of certain fatty acid types for whole *R. exoculata* and postlarval alvinocarids are presented in Table 3.2.5.

Table 3.2.5 Mean percentage fatty acid composition of different tissues of *R. exoculata*. Standard errors omitted for clarity. SFA, saturated fatty acids; UFA, unsaturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids.

	Female Abdomen	Male Abdomen	Juvenile Abdomen	Female Branchial	Male Branchial	Juvenile Branchial	Male Gonad	Female Gonad
14:0	0.55	0.23	0.27	3.81	3.47	1.64	2.01	4.35
14:1	0.44	0.32	0.34	2.86	1.68	1.56	2.45	3.64
15:0	0.14	0.16	0.13	0.27	0.19	1.84	1.05	0.23
16:0	10.70	12.42	4.99	8.34	8.66	11.33	9.00	8.12
16:1 n-7	15.64	13.49	15.12	23.42	21.85	19.50	22.18	24.10
16:2 n-4	1.29	1.11	0.26	7.06	6.29	0.39	5.07	7.96
16:3 n-3	nd	nd	0.47	nd	nd	0.96	nd	nd
18:0	2.69	3.20	1.40	1.91	2.39	3.61	2.10	0.87
18:1 n-9	5.25	4.43	20.60	3.73	3.55	20.23	4.28	2.04
18:1 n-7	20.99	23.57	9.45	13.73	15.13	18.79	14.41	11.70
18:2 n-6	1.10	0.91	2.01	0.95	1.00	1.96	0.00	1.09
18:2 n-4	13.91	13.71	0.85	14.69	14.43	2.36	1.92	19.14
18:3 n-6	nd	nd	0.30	5.00	5.09	0.10	nd	nd
18:3 n-3	7.14	5.84	0.27	1.91	1.95	0.35	7.42	5.67
18:4 n-4	1.13	1.56	0.34	0.23	0.23	1.60	13.36	3.86
20:1 n-9	0.49	0.30	0.74	0.40	0.39	0.26	0.00	0.11
20:1 n-7	0.26	0.27	0.20	nd	nd	0.54	0.00	0.31
20:2 NMID	3.62	1.87	0.06	1.80	1.35	0.32	2.53	1.83
20:3 n-6	0.33	0.33	0.04	0.49	0.49	0.29	1.14	0.83
20:3 n-3	1.78	0.90	0.18	0.80	0.59	0.18	0.61	0.69
20:4 n-6	2.01	2.75	6.59	1.64	2.77	3.84	4.19	0.51
20:4 n-4	0.50	0.24	0.19	0.37	0.54	0.35	1.14	0.05
20:4 n-3	nd	nd	0.26	nd	nd	0.15	nd	nd
22:1 n-11	0.90	0.86	0.26	0.89	0.96	0.72	0.00	0.62
20:5 n-3	3.38	4.03	8.13	1.97	2.73	1.34	1.48	0.39
22:2 NMID	0.65	0.39	0.06	0.81	0.37	0.18	2.36	0.69
22:5 n-6	nd	nd	5.69	nd	nd	2.38	nd	nd
24:1	0.34	0.66	0.03	0.18	0.35	0.10	0.00	0.02
22:5 n-3	0.51	0.38	1.10	0.74	0.44	0.29	0.00	0.75
22:6 n-3	4.28	6.08	19.66	2.01	3.11	2.84	1.31	0.43
SFA	14.07	16.01	6.79	14.33	14.71	18.43	14.15	13.57
UFA	85.93	83.99	93.21	85.67	85.29	81.57	85.85	86.43
MFA	44.30	43.90	46.73	45.44	44.14	61.69	43.32	42.53
NMID	4.27	2.26	0.12	2.60	1.72	0.50	4.89	2.52
n-4 PUFA	16.83	16.62	1.65	24.03	23.21	4.69	21.48	31.01
n-6 PUFA	3.44	3.99	14.63	3.08	4.25	8.58	5.33	2.43
n-3 PUFA	17.09	17.23	30.07	10.52	11.96	6.11	10.83	7.93
18:1 n-7/n-9	4.00	5.32	0.46	3.68	4.26	0.93	3.37	5.74

Fatty acid methyl ester tissue concentrations of different fatty acid types are also presented (Table 3.2.7). However, as discussed in section 3.1.2, these results must be treated with some caution. Estimated expected FAME yields have been calculated from individual lipid class composition results (see section 3.2.1) and are presented in Table 3.2.6. Although a Wilcoxon Signed Rank test indicated no significant difference between the two sets of data ($P = 0.742$), actual total yields of FAME are lower than is expected in some cases.

Table 3.2.6 Total FAME yields by gas chromatography (actual yield) and FAME yields estimated by calculation from lipid class tissue concentrations (estimated yield) for dissected *R. exoculata*. Actual yields are quoted ± S.E. ($\sigma/\sqrt{n}$): Female tissue samples except ovary, n = 8; juvenile and male tissue samples (except gonad), n = 6; male gonad, n = 1; ovary, n = 7.

	Female Abdomen	Male Abdomen	Juvenile Abdomen	Female Branchial	Male Branchial	Juvenile Branchial	Male Gonad	Female Gonad
Actual yield	15.8±0.75	9.83±2.84	72.9 ± 27.7	34.7 ± 18.0	36.4 ± 9.9	24.7 ± 2.36	18.2	220 ± 102
Estimated yield	18.69	21.30	67.27	38.62	32.24	46.72	39.03	125.55

Saturated fatty acids comprise around 15% by weight of the fatty acids of all tissue types of adult *R. exoculata*. A lower proportion of saturates (around 7%) is found in the abdomen region of juvenile *R. exoculata*, although this value is around 18% in juvenile branchial tissue. There is no significant difference in percentage monounsaturate composition for all tissue types except juvenile branchial tissue (ANOVA, $P = 0.834$).

Table 3.2.7 Fatty acid methyl ester tissue concentrations of dissected *R. exoculata* (mean tissue concentrations mg g dry weight^{-1} ± S.E ($\sigma/\sqrt{n}$). SFA, saturated fatty acids; UFA, unsaturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids (four or more double bonds). Female tissue samples except ovary, n = 8; juvenile and male tissue samples (except gonad), n = 6; male gonad, n = 1; ovary, n = 7.

	Female Abdomen	Male Abdomen	Juvenile Abdomen	Female Branchial	Male Branchial	Juvenile Branchial	Male Gonad	Female Gonad
Totals	15.8±0.75	9.83±2.84	72.9 ± 27.7	34.7 ± 18.0	36.4 ± 9.9	24.7 ± 2.36	18.2	220 ± 102
SFA	2.53±0.13	1.36±0.38	3.73±0.47	5.19±2.72	5.19±1.40	4.50±0.19	2.57	23.8±6.73
UFA	13.3±0.63	8.47±2.46	69.2±27.3	29.5±15.3	31.2±8.50	20.2±2.18	15.6	196±95.2
MFA	6.94±0.38	4.37±1.30	31.6±10.7	15.3±8.02	16.8±4.68	15.2±1.89	7.87	86.9±35.0
NMID	0.36±0.08	0.42±0.14	0.09±0.03	0.54±0.24	0.92±0.25	0.12±0.04	0.89	5.27±2.35
n-4 PUFA	2.63±0.15	1.68±0.51	0.94±0.25	8.29±4.49	8.80±2.31	1.14±0.07	3.90	80.5±46.4
n-6 PUFA	0.63±0.04	0.34±0.09	10.4±3.50	1.47±0.71	1.07±0.30	2.16±0.50	0.97	8.61±6.33
n-3 PUFA	2.74±0.55	1.66±0.45	26.3±13.2	3.93±1.88	3.68±0.99	1.53±0.65	1.97	15.2±5.42
PUFA	6.36±0.53	4.10±1.17	37.7±16.7	14.2±7.30	14.5±3.83	4.95±1.18	7.73	110±60.4
HUFA	2.39±0.66	1.10±0.28	34.6±15.8	3.87±1.83	2.89±0.86	3.22±1.06	3.90	31.0±27.7

However, monounsaturate tissue concentrations in the branchial tissue of adults and juveniles are not significantly different from each other (ANOVA, $P = 0.992$; around 15 mg g dwt^{-1}). Adult tissues bear higher 18:1 n-7/n-9 ratios than juveniles and this ratio is significantly higher in juvenile branchial tissue than in juvenile abdomen tissue (t-test, 95% confidence, $P = 0.040$). Percentage compositions of polyunsaturated fatty acids are illustrated in figure 3.2.6. NMID fatty acids are more abundant in female than in male tissue (t-test, 95% confidence, $P = 0.009$ (abdomen) and $P = 0.005$ (branchial)) and in female branchial tissue than abdomen tissue (t-test, 95% confidence, $P < 0.001$). NMID fatty acids are present only at low levels in juveniles.

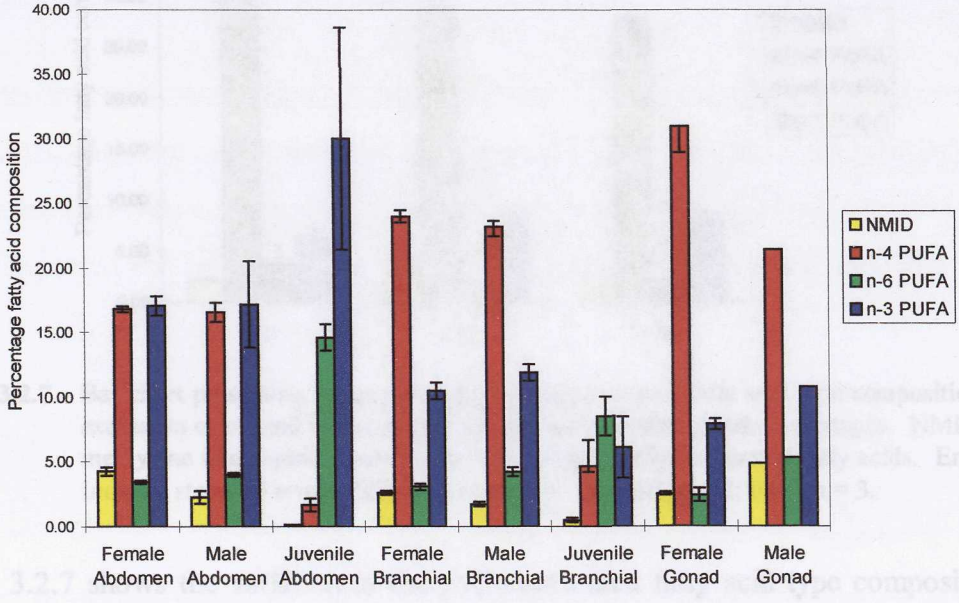


Figure 3.2.6 Bar chart presenting mean percentage polyunsaturated fatty acid type composition of dissected *R. exoculata*. NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids. Error bars indicate standard errors (SE = $\sigma/\sqrt{n}$). Female tissue samples except ovary, n = 8; juvenile and male tissue samples (except gonad), n = 3; male gonad, n = 1; ovary, n = 7.

Proportions of n-4 PUFA are much higher in adult *R. exoculata* (17-30%) than in juveniles, with the highest proportions in juveniles found in branchial tissue (5%) being significantly higher than those in juvenile abdomen tissue (t-test, 95% confidence, $P = 0.011$). Adult branchial tissue comprises a significantly higher percentage of n-4 fatty acids (~24%) than adult abdomen tissue (~17%) (t-test, 95% confidence, $P < 0.001$) and levels are also high in ovary and hepatopancreas tissue (21-31%).

The highest percentages of n-3 fatty acids are found in juvenile abdomen tissue (30%). Levels in adult abdomen tissue are approximately the same as those of n-4 fatty acids (~17%), while lower levels of n-3 fatty acids are present in branchial and gonad tissues (6-12%). The n-6 fatty acids are also most abundant in juvenile abdomen tissue (15%) with lower levels in juvenile branchial tissue (9%) and low levels (around 5%) found in all adult tissue types.

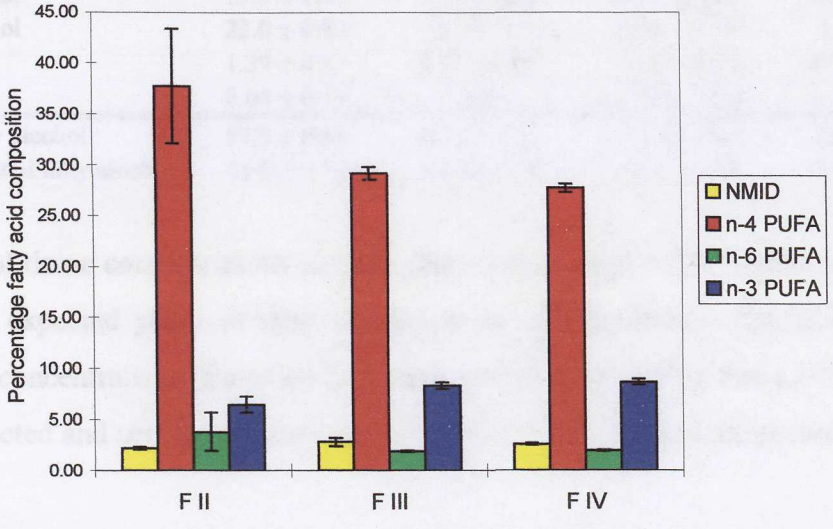


Figure 3.2.7 Bar chart presenting mean percentage polyunsaturated fatty acid type composition of *R. exoculata* ovary and hepatopancreas tissue at different reproductive stages. NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids. Error bars indicate standard errors (SE = $\sigma/\sqrt{n}$); F II, n = 2, F III, n = 2; F IV, n = 3.

Figure 3.2.7 shows the variation in the polyunsaturated fatty acid type composition of ovarian and hepatopancreas tissue at different stages of reproductive development. The percentage of n-4 fatty acids appears to decrease with reproductive development ($r_{0.755}$ = -0.76, n = 7; 95% confidence), while the percentage of n-3 fatty acids increases ($r_{0.755}$ = 0.83, n = 7; 95% confidence).

The percentage compositions of fatty alcohols detected in tissues of juvenile *R. exoculata* are presented in Table 3.2.8. The major fatty alcohols detected were 16:0, 18:1 n-7 and 18:1 n-9, with a higher percentage of 18:1 n-9 than 18:1 n-7. Composition of abdomen and branchial regions were broadly similar, although higher levels of 14:0 and 14:1 fatty alcohols were detected in the branchial region.

Table 3.2.8 Mean percentage fatty alcohol composition and tissue concentration (by weight and mg g dry weight^{-1} ± S.E. ($\sigma/\sqrt{n}$)) of dissected juvenile *R. exoculata*. Abdomen and branchial tissues, n = 3.

	Juvenile abdomen (% weight)	Juvenile branchial (% weight)	Juvenile abdomen (mg g d wt^{-1})	Juvenile branchial (mg g d wt^{-1})
14:0 alcohol	0.75 ± 0.18	3.54 ± 0.54	0.10 ± 0.03	0.20 ± 0.01
14:1 alcohol	1.74 ± 0.93	6.17 ± 0.66	0.19 ± 0.04	0.37 ± 0.06
16:0 alcohol	48.2 ± 1.36	43.7 ± 1.11	6.40 ± 1.93	2.72 ± 0.63
16:1 alcohol	0.90 ± 0.34	nd	0.13 ± 0.06	nd
18:0 alcohol	8.86 ± 0.34	7.99 ± 0.24	1.15 ± 0.31	0.49 ± 0.09
18:1 n-9 alcohol	15.6 ± 0.81	11.9 ± 0.28	2.10 ± 0.65	0.73 ± 0.15
18:1 n-7 alcohol	22.0 ± 0.93	20.7 ± 1.18	2.90 ± 0.79	1.3 ± 0.32
18:2 alcohol	1.29 ± 0.67	6.05 ± 1.03	0.14 ± 0.00	0.35 ± 0.01
20:1 alcohol	0.64 ± 0.19	nd	0.09 ± 0.04	nd
Saturated fatty alcohol	57.8 ± 0.69	55.2 ± 0.47	7.65 ± 2.26	3.41 ± 0.74
Monounsaturated fatty alcohol	41.0 ± 0.72	38.8 ± 0.71	5.41 ± 1.53	2.40 ± 0.53

Fatty alcohol tissue concentrations are also presented in table 3.2.8. In the same way as for FAME, expected yields of fatty alcohols were calculated from the TLC-FID wax ester tissue concentrations (Equation 3.3) using a 16:0 fatty alcohol and a 16:0/19:0 wax ester. Expected and actual total fatty alcohol tissue concentrations are presented in table 3.2.9.

Equation 3.3 Calculation of expected fatty alcohol yields from wax ester (WE) tissue concentrations

$$\text{Expected yield} = \text{WE by TLC-FID (mg gdwt}^{-1}\text{)} \times \frac{\text{RMM 16:0 fatty alcohol}}{\text{RMM 16:0/19:0 wax ester}}$$

Total fatty alcohol yields by GC appear lower than estimated yields from TLC-FID, although there is no statistically significant difference between the means (t-test, 95% confidence, $P = 0.068$ (abdomen); $P = 0.122$ (branchial)).

Table 3.2.9 Total fatty alcohol yields and (mg g dry weight^{-1}) by gas chromatography (actual yield) and fatty alcohol yields estimated by calculation from wax ester tissue concentrations (estimated yield) for dissected juvenile *R. exoculata*. Yields are quoted ± S.E. ($\sigma/\sqrt{n}$).

	Abdomen	Branchial
Actual yield (n = 3)	13.2 ± 3.78	6.16 ± 1.28
Estimated yield (n = 9)	41.5 ± 7.65	14.8 ± 2.82

3.3 Lipid profiles of *Alvinocaris markensis*

Total lipid and polar lipid tissue concentrations of *A. markensis* were calculated as for *R. exoculata* for each tissue type. Whole body lipid tissue concentrations (mg g dwt^{-1}) were calculated by summing total lipid amounts (mg) for each tissue then dividing by the sum of the tissue dry weights (g). These data are presented in Table 3.3.1.

Table 3.3.1 Mean total lipid and polar lipid concentrations for *A. markensis* (mean tissue concentrations mg g dry $weight^{-1}$ ± S.E. ($\sigma/\sqrt{n}$); "whole body" excludes eggs)

Tissue	Mean total lipid ± S.E ($\sigma/\sqrt{n}$) (mg g dry $weight^{-1}$)	Polar lipid ± S.E ($\sigma/\sqrt{n}$) (mg g dry $weight^{-1}$)
Abdomen (n = 2)	28.6 ± 0.85	19.0 ± 0.13
Hepatopancreas (n = 2)	316 ± 0.99	16.2 ± 0.94
"Rest" (n = 2)	76.7 ± 0.68	14.7 ± 0.73
Eggs (n = 2)	374 ± 8.80	49.5 ± 0.94
Whole body (n = 2)	97.5 ± 0.70	17.0 ± 0.52

A. markensis whole body lipid tissue concentration (97.5 ± 0.70 mg g dry $weight^{-1}$) appears high compared to that of adult *R. exoculata* (59 ± 1.9 mg g dwt^{-1} SE, n = 108) and is statistically significantly different, (t-test, P = 0.008, 95% confidence), but is similar to that of the deep-sea caridean shrimp, *Nematocarcinus gracilis* (82 ± 1.8 mg g dwt^{-1} SE, n = 84; see also chapter 4) and not statistically significantly different (t-test, P = 0.205, 95% confidence). Hepatopancreas tissue and eggs are richest in lipid. Eggs contain a higher polar lipid tissue concentration than other tissues that have levels similar to those found in adult *R. exoculata* (Table 2.1.1, 2.2.1). There is no significant difference in the whole body polar lipid tissue concentration of *A. markensis* and that of whole adult *R. exoculata* (t-test, P = 0.535, 95% confidence). The lowest total lipid levels are found in abdomen tissue, with abdomen total lipid and polar lipid levels being similar to those found in adult *R. exoculata* (Table 3.2.1).

3.3.1 Lipid class composition of *A. markensis*

Whole body lipid class profiles of *A. markensis* are similar to those of adult *R. exoculata* with triglyceride, the dominant storage lipid, found at highest concentrations

in the hepatopancreas and lowest concentrations in the abdomen region. Levels of fatty acids and sterols are similar in all body tissue types except for hepatopancreas which has lower sterol concentrations (Figure 3.3.1).

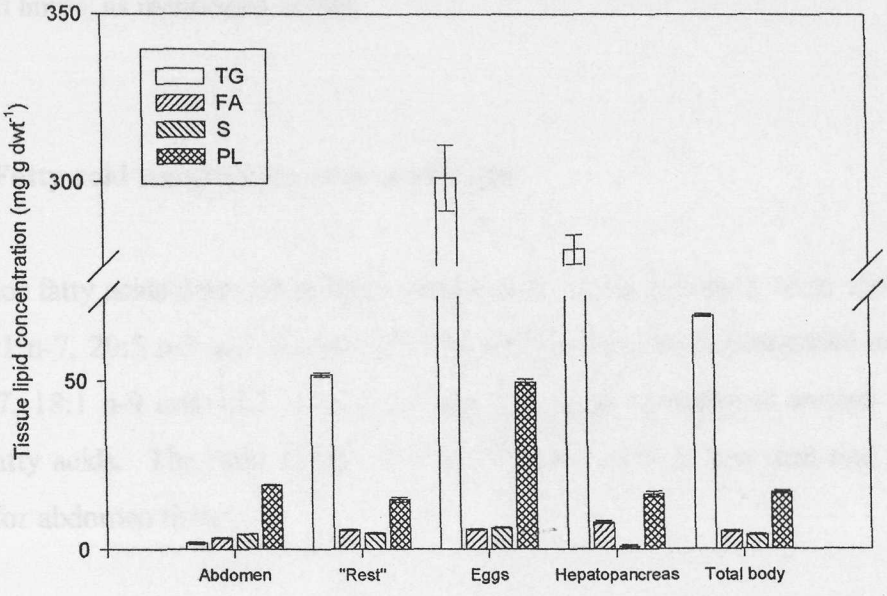


Figure 3.3.1 Bar chart illustrating tissue lipid concentrations in abdomen, "rest", hepatopancreas, eggs and whole body tissues of *Alvinocaris markensis*. Error bars represent standard error of the mean (S.E. = $\sigma/\sqrt{n}$), n = 2 for all samples. WE = wax ester; TG = triglyceride; FA = fatty acid; S = sterol; PL = polar lipid.

Eggs contain very high levels of triglyceride (Figure 3.3.1) and increased polar lipid concentrations (Figure 3.3.2).

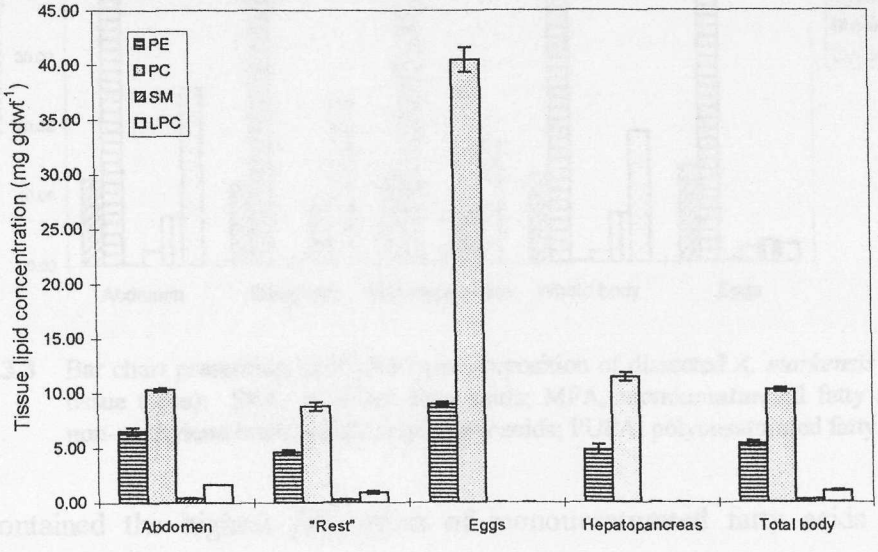


Figure 3.3.2 Bar chart illustrating polar lipid tissue concentrations in abdomen, "rest", hepatopancreas, eggs and whole body tissues of *Alvinocaris markensis*. Error bars represent standard error of the mean (S.E. = $\sigma/\sqrt{n}$): n = 2 for all samples. PE = phosphatidyl ethanolamine; PC = phosphatidyl choline; SM = sphingomyelin; LPC = lysophosphatidyl choline.

Figure 3.3.2 shows that PC is the most abundant phospholipid in eggs. Phospholipid composition is otherwise similar to that of whole adult *R. exoculata* with PC more abundant than PE. The apparent absence of SM and LPC in eggs may be a function of detection limits, as mentioned earlier.

3.3.2 Fatty acid composition of *A. markensis*

The major fatty acids detected in body tissues of *A. markensis* were 16:0, 16:1 n-7, 18:1 n-9, 18:1 n-7, 20:5 n-3 and 22:6 n-3 (Table 3.3.2). Egg tissue comprised mainly 16:0, 16:1 n-7, 18:1 n-9 and 18:1 n-7. Saturated fatty acids comprised around 13% of all tissue fatty acids. The ratio of 18:1 n-7 to n-9 fatty acids is less than one in all cases except for abdomen tissue.

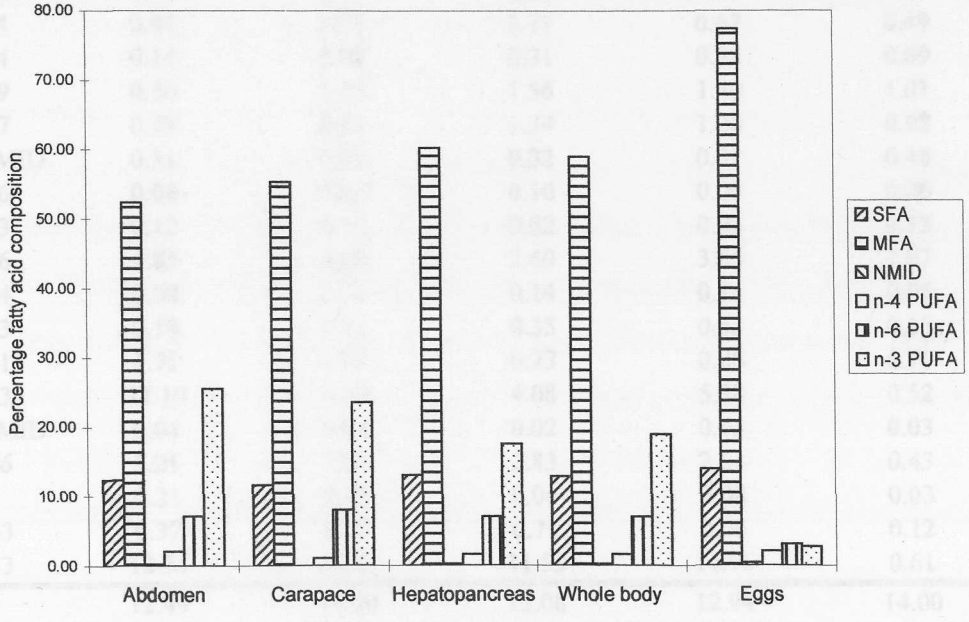


Figure 3.3.3 Bar chart presenting fatty acid type composition of dissected *A. markensis* (n = 1 for all tissue types). SFA, saturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids.

Eggs contained the highest proportion of monounsaturated fatty acids and lowest proportion of n-3 PUFA (Figure 3.3.3). NMID fatty acids are only present in small amounts and the most abundant PUFA are n-3 PUFA in all cases except eggs. Low

levels of n-4 PUFA are also present, but at levels similar to those found in juvenile *R. exoculata* rather than adults.

Table 3.3.2 Percentage (by weight) fatty acid composition of dissected *A. markensis* (n = 1 for each tissue type). SFA, saturated fatty acids; UFA, unsaturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids.

	Abdomen	Carapace	Hepatopancreas	Whole body	Eggs
14:0	0.45	0.46	0.51	0.50	0.55
14:1	0.53	0.15	0.06	0.13	0.28
15:0	0.14	0.08	0.15	0.15	0.09
16:0	8.72	9.22	10.71	10.37	12.14
16:1 n-7	8.28	13.94	15.33	14.29	23.60
16:2 n-4	0.17	0.26	0.57	0.50	0.46
16:3 n-3	0.96	0.49	0.22	0.33	0.79
18:0	3.18	1.94	1.71	1.93	1.22
18:1 n-9	13.00	21.22	27.18	24.96	33.14
18:1 n-7	27.62	16.89	13.99	16.01	17.70
18:2 n-6	0.84	1.11	1.20	1.15	1.35
18:2 n-4	1.70	0.46	0.63	0.78	1.56
18:3 n-6	0.20	0.08	0.24	0.23	0.21
18:3 n-4	0.44	0.75	0.71	0.67	0.49
18:4 n-4	0.14	0.08	0.31	0.28	0.09
20:1 n-9	0.50	1.19	1.56	1.40	1.01
20:1 n-7	0.49	0.83	1.34	1.20	0.92
20:2 NMID	0.31	0.05	0.32	0.31	0.46
20:3 n-6	0.06	0.05	0.10	0.09	0.06
20:3 n-3	0.12	0.13	0.02	0.03	0.15
20:4 n-6	4.85	4.00	2.69	3.05	1.07
20:4 n-4	0.08	0.31	0.14	0.14	0.06
20:4 n-3	0.19	0.31	0.35	0.33	0.15
22:1 n-11	1.75	0.98	0.73	0.88	0.70
20:5 n-3	11.10	6.84	4.08	5.18	0.52
22:2 NMID	0.04	0.03	0.02	0.03	0.03
22:5 n-6	1.21	2.87	2.83	2.61	0.43
24:1	0.21	0.15	0.06	0.08	0.03
22:5 n-3	0.37	0.75	0.71	0.66	0.12
22:6 n-3	12.35	14.38	11.53	11.76	0.61
SFA	12.49	11.70	13.08	12.94	14.00
UFA	87.51	88.30	86.92	87.06	86.00
MFA	52.38	55.36	60.25	58.95	77.38
NMID	0.35	0.08	0.35	0.34	0.49
n-4 PUFA	2.09	1.11	1.65	1.69	2.17
n-6 PUFA	7.17	8.11	7.06	7.12	3.12
n-3 PUFA	25.53	23.65	17.61	18.97	2.84
18:1 n-7/n-9	2.12	0.80	0.51	0.65	0.53

3.4 Discussion of the lipid profiles of hydrothermal vent shrimp

3.4.1 Potential errors in the lipid profiles of hydrothermal vent shrimp

The low detection limits of the TLC-FID procedure (around 0.5 μg lipid depending on lipid class) leading to low levels of certain lipid classes going undetected in some samples, have been mentioned in section 3.1 (page 3.4). This might lead to some margin of error in total lipid tissue concentration values, especially in low tissue weight samples. However, where total lipid tissue concentrations were determined gravimetrically (dissected *R. exoculata*, section 3.2), they were compared with total lipids determined by summation of lipid class tissue concentrations determined by TLC-FID and shown to be not statistically significantly different. It is, therefore, assumed that the undetected lipid classes were indeed of only little significance. Other lipid classes, such as partial acylglycerols and cardiolipin, might also have been present at low levels in the samples studied, but these were not detected using the TLC-FID technique and it is assumed that if present, they were also of minor significance.

There are several possible explanations for the, apparently, low FAME yields in whole juvenile and postlarval specimens and tissues with high level lipid tissue concentrations. It is possible that the TLC-FID lipid class composition figures are higher than they should be. Flame ionisation of lipids containing unsaturated fatty acids may elicit higher responses in the Iatroscan detector than those containing saturated fatty acids. The lipid standards used to calibrate the TLC-FID method contained only saturated fatty acids, so this may have led to overestimates of lipid class composition in samples with high proportions of unsaturated fatty acids: juvenile and postlarval specimens did contain high levels of unsaturated fatty acids, and significantly higher levels of n-3 fatty acids than adult shrimp. This problem could be overcome by hydrogenating all samples (using hydrogen gas and a platinum catalyst) before lipid class analysis. Time constraints prevented this experiment as part of the current study.

Another possible reason for low FAME yields could be incomplete transmethylation of total lipid. The fact that actual FAME yields were lower than expected for juveniles and postlarvae suggests that transmethylation of wax esters might be the problem (this was the major lipid class in these samples). However, the transmethylation method is

an established one, the reagents were in excess and this problem has not been reported before. In addition, lower than expected FAME yields also occurred in samples with high tissue lipid concentrations and without wax esters, so this is probably not the reason for the low yields.

The most likely reason for the inconsistent results is probably a function of the errors occurring with a small sample size that are multiplied up when calculating tissue concentrations. Samples with the biggest differences in expected and actual FAME yield contained high tissue concentrations of lipid and were often derived from low dry weights of tissue. Relatively small errors in total lipid levels divided by small tissue dry weights could lead to large errors in tissue concentrations. The problem is compounded when measuring lipid class concentrations of concentrated lipid solutions: smaller amounts must be applied to Chromarods to get peak areas within the range of the calibration curves. To calculate the amount of lipid in the original sample, the amount of lipid in a small volume must be multiplied up more times than for samples where a larger volume has been applied. For example, if 0.2 µl of total lipid sample is applied to a Chromarod, then the amount of lipid detected must be multiplied by 5000 to calculate the amount in 1 ml (i.e. the whole sample) whereas if 2 µl of total lipid sample is applied to a Chromarod, the amount of lipid detected is multiplied by 500 to get the amount of lipid in 1 ml. It is clear that errors might be up to tens of times greater in some samples than others, simply as a result of constraints of the method. To improve the method, approximate lipid concentration should be determined before analysing lipid class composition and samples should be diluted to similar concentrations before analysing. This was carried out in the case of dissected *R. exoculata* samples. However, it still presented difficulties when the proportions of lipid classes in samples differ and the preliminary studies were very time consuming.

3.4.2 Lipid profiles of whole *R. exoculata* from TAG and postlarval alvinocarids from the water column above Broken Spur

There have been few reports of total lipid tissue concentration per gram dry weight in caridean shrimp. More frequently, total lipid tissue concentrations have been reported

in terms of shrimp wet weight. Assuming that shrimp wet weight generally equates to approximately four times wet weight (as was the case for *P. borealis* (Hopkins *et al.*, 1993); *R. exoculata* and *N. gracilis*: see CD-ROM appendix) it is possible to compare previously published shrimp total lipid tissue concentrations with those found in this study (Table 3.4.1).

Table 3.4.1 Table summarising some reported total lipid tissue concentrations of caridean shrimp and deep water zooplankton: where * percentage dry weight or † percentage wet weight figures were quoted in the literature, these have been adjusted to mg g dry weight^{-1} figures for comparison (assuming dry weight ≈ 1/4 wet weight)

Species	Total lipid (mg g dwt^{-1})	Reference	Comments/depths
Mixed zooplankton included decapod larvae, copepods, ostracods, amphipods, euphausiids, chaetognaths, pteropods, polychaetes	30 - 160	Herring, 1972†	Sampled from 350 - 600 m
	170 - 440		Sampled from 600 - 900 m
Acanthephyra	150 - 380	Culkin & Morris, 1969†	940 m to surface
acanthitelsonis	240	Herring 1973†	All species pelagic
Acanthephyra purpurea	90	Culkin & Morris, 1969†	1000 m to surface
	130	Herring 1973†	(shallow species)
Oplophorus spinosus	180	Culkin & Morris, 1969†	265 m to surface
Sergestes corniculum	70	Culkin & Morris, 1969†	750 m to surface
Systellapsis debilis	220	Culkin & Morris, 1969†	750, 265 and 190 m to surface
	140	Herring 1973†	
Parapasiphaea sulcatifrons	600	Herring 1973†	Pelagic species
Nematocarcinus cursor	70	Herring 1973†	Benthic or epibenthic species
Acanthephyra aximia	150		
Plesionika martia	100		
Chorismus antarcticus	55 - 200	Clarke, 1977†	Varied seasonally and with degree of maturity
Pandalus montagui	40 - 240	Clarke, 1979†	Shallow water shrimp Varied seasonally
Pandalus borealis	100 - 400	Hopkins *et al.*, 1993*	Varied seasonally Trawled from 185 m

The total lipid content of adult *R. exoculata* is lower than that found in other benthic and epibenthic deep-sea shrimp, and closer to the lower levels found in shallow water and antarctic species. The benthic and epibenthic deep-sea shrimp are probably mostly opportunistic feeders that may not have a regular food supply, while the shallow water and antarctic species have regular, if seasonally variable food supplies. Total lipid levels in juveniles and postlarvae are similar to those much higher levels found in deep, pelagic crustaceans. Herring (1972) reports that the total lipid concentration of zooplankton increases with depth and suggests that this is congruent with an increase in the scarcity of food. The type of storage lipid used also varies with environment and

food availability. Deep, pelagic caridean shrimp have been reported to contain wax esters, while shallow-dwelling, benthic and epibenthic shrimp use triglyceride as their major storage lipid (Herring, 1973). It has been suggested that wax esters are used as storage lipid by organisms in areas where food is plentiful for short periods and scarce for long periods, especially the deep sea and polar regions (Sargent & Whittle, 1981). Wax esters comprised 75 to 82 % of total lipid in postlarval alvinocarids assayed by Pond *et al.* (1997a), and 88 ± 1.9 % (SE; n = 18) of total lipid in postlarvae analysed in this study. The high total lipid content of vent shrimp postlarvae and their use of wax esters as the major storage lipid, along with the depths of the trawls used to catch them, suggest a deep pelagic lifestyle for postlarval vent shrimp (Pond *et al.*, 1997a). The shrimp described here as juvenile *R. exoculata* bear similar total lipid and neutral lipid class compositions to postlarval alvinocarids. They differ from postlarvae in their appearance, the dorsal organ being present and its shape being half way between that found in large postlarvae and adult *R. exoculata* (Shank *et al.*, 1998). In addition, they were actually collected at the vent site, from amongst swarms of adult shrimp. They contained a slightly lower percentage wax esters (82 ± 1.1 %; SE; n = 12) than postlarvae analysed in this study.

Principal Component Analysis highlighted the difference in lipid class composition of postlarval and juvenile shrimp and large and small adults. Where there is a constant supply of food, it is intuitive that large energy stores are less important. Copepods that feed all year round have been found to use triglyceride as their major storage lipid (Sargent & Whittle, 1981). It therefore seems likely that the moderate levels of triglyceride in adult *R. exoculata* reflect the constant food source at active hydrothermal vents. Triglyceride levels in whole adult *R. exoculata* (~ 35% in adults < 16mm CL and ~ 62% in adults > 16 mm CL) were comparable with those reported for *P. borealis* (Hopkins *et al.*, 1993) that reached around 40% in mature shrimp. The increase in tissue concentration of triglyceride in adult *R. exoculata* with increasing carapace length seems likely to be a consequence of the change over from the use of wax ester as the major lipid storage medium to triglyceride as juvenile shrimp mature into adults. The low levels of wax esters detected in small adult shrimp, generally decreasing with increasing shrimp size, support this hypothesis.

Sterols comprised around 6 % of lipid in all adult *R. exoculata*, a figure comparable with the 8 - 10% found in *Pandalus montagui* (Clarke, 1979). It is unclear whether the level of sterols found in *R. exoculata* is significantly lower than that reported in *P. montagui*. However shrimp are known to be unable to synthesise sterols *de novo* (Goad, 1981) and usually gain sterols from a phytoplankton source and therefore adult vent shrimp may have less access to dietary sterols.

Free fatty acids comprised less than 5 % of total lipid in all whole vent shrimp samples analysed, suggesting that the specimens were in good condition. A high percentage of free fatty acids (more than 10 % of total lipid) may be a sign of enzyme degradation of lipids (Jeckel *et al.*, 1989).

The higher polar lipid tissue concentrations of adult vent shrimp compared with juvenile and postlarval vent shrimp may be a reflection of the presence of epibiotic bacteria on the carapace and in the branchial area of the adults. However, approximate polar lipid tissue concentrations of *P. montagui* (20 - 30 mg g dwt^{-1}; calculated by assuming a wet weight dry weight ratio of 4:1) are not dissimilar to those found in adult *R. exoculata* (~ 20 mg g dry $weight^{-1}$) so perhaps there is another reason for the discrepancy. The problems with the detection limits of the TLC-FID method and small tissue samples discussed earlier may have resulted in an underestimate of the polar lipid concentrations in postlarval or juvenile shrimp. Further work would be necessary to verify this, perhaps using a different method of lipid class analysis such as scanning densitometry.

Postlarval shrimp polar lipids comprised a larger percentage of phosphatidyl ethanolamine (PE) than was found in adults. The eggs and larvae of caridean shrimp are usually richer in PE than in phosphatidyl choline (PC; Kattner *et al.*, 1994) and it has been suggested that PE is used during larval development (Kattner *et al.*, 1994). PC tissue concentration increases during larval development of *Crangon* species and this is probably also the case here. The percentage PE is higher in postlarvae (44 %) than in juveniles (26%), supporting this hypothesis. The percentage PE and PC in the polar lipid of adult *R. exoculata* (~ 31% PE; ~ 60% PC) is similar to that found in *Pandalus montagui* (PE 33 - 36 % polar lipid; PC 45 - 48 % polar lipid; Clarke, 1979). The

slightly higher percentage PC may be related to the epibiotic bacteria in adult *R. exoculata*, but is perhaps more likely to be a reflection of fewer polar lipid classes detected here than for *P. montagui*.

The lipid class profiles of adult and juvenile *R. exoculata* are similar to those reported for *Mirocaris fortunata*, found at the Lucky Strike vent site (37°17' N, 32°16' W Mid-Atlantic Ridge, 1750 m; Pond *et al.*, 1997c). Small, highly pigmented *M. fortunata* (equivalent to "juvenile" *R. exoculata*) comprised around 60% wax esters, while adults used triglyceride as their major storage lipid. *M. fortunata* polar lipids comprised 17 to 30 % of total lipid in adults, while highly pigmented small adults contained lower polar lipid levels of 10 - 11% of total lipid.

The principal fatty acids found in juvenile and postlarval shrimp (16:0, 16:1 n-7, 18:1 n-9, 20:5 n-3 and 22:6 n-3) were similar to those found in other caridean shrimp that rely on a photosynthetically-derived food source (Culkin & Morris, 1969; Clarke, 1979; Hopkins, 1993). The major fatty acids found in adult *R. exoculata* were quite different (16:0, 16:1 n-7, 16:2 n-4, 18:1 n-7 and 18:2 n-4) and the same as those reported in *R. exoculata* (Pond *et al.*, 1997b) and similar to those in *M. fortunata* (Pond *et al.* 1997c).

Table 3.4.2 Percentage saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) of total fatty acids by weight in caridean shrimp in some previous studies. N.B. *P. montagui* is a protandrous hermaphrodite

Study and samples	SFA	MUFA	PUFA
Culkin & Morris, 1969	18 - 28 %	47 - 65 %	18 - 40 %
Deep-sea caridean shrimp			
Clarke, 1979	transitionals 27 - 38 %	transitionals 16 - 41 %	transitionals 30 - 40 %
Pandalus montagui	males 21 - 24 %	males 30 - 40 %	males 22 - 41 %
Hopkins *et al.*, 1993	27 - 39 %	40 - 59 %	7 - 24 %
Pandalus borealis			
Kattner *et al.*, 1994	~ 30 %	20 - 22%	48 - 50 %
Larval Crangonids			
Pond *et al.*, 1997c	adults 16 - 20 %	adults 57 - 64 %	adults 17 - 24 %
Mirocaris fortunata	"juveniles" ~ 6%	"juveniles" ~ 31%	"juveniles" ~ 63 %
Pond *et al.*, 1997b	13 - 17 %	36 - 40 %	43 - 49 %
Rimicaris exoculata			
Pond *et al.*, 1997a	9 %	54 %	36 %
Postlarval alvinocarids			

Levels of saturated fatty acids (6 - 15%) were lower than those reported for other deep-sea and shallow water caridean shrimp (Table 3.4.2), but in the same region as those reported for *M. fortunata* (Pond *et al.*, 1997c) and *R. exoculata* (Pond *et al.*, 1997b).

Postlarval and juvenile shrimp contained higher levels of monounsaturated fatty acids than adults and these levels (Table 3.4.2) also compared well with those found for *M. fortunata*. Elevated levels of monounsaturates have been reported in the eggs and larval stages of many caridean shrimp (Clarke, 1979; Hopkins *et al.*, 1993; Kattner *et al.*, 1994).

The type of monounsaturated fatty acids present in an organisms can be related to dietary input. Eukaryotes are not generally able to synthesise 18:1 n-7, so this fatty acid is a biomarker for bacteria. The ratio of 18:1 n-7 to 18:1 n-9 fatty acids can therefore be used as an indication of the contribution of bacteria to the nutrition of an organism (Sargent *et al.*, 1987). The higher the ratio of 18:1 n-7 to 18:1 n-9, the greater the input of bacterially derived organic matter. Adult 18:1 n-7/n-9 ratios here were similar to those reported by Pond *et al.*, (1997b; 2 - 5) and somewhat higher than those reported for *M. fortunata* (Pond *et al.*, 1997c ~ 2). *M. fortunata* is a vent shrimp found in highest abundance at the shallower sites south of the Azores along the Mid-Atlantic Ridge. At shallower vent sites there is probably more phytodetrital organic matter to supplement the nutrition of vent shrimp than there is at the deeper sites. The 18:1 n-7/n-9 ratios for juvenile and postlarval shrimp were nearer to those calculated for *P. borealis* (Hopkins *et al.*,1993; 0.3 to 0.6) and typical of a low dietary input of bacteria.

Adult *R. exoculata* contained unusual n-4 fatty acids. Although 16:2 n-4 is a fatty acid detected in some diatom species (Volkman *et al.*, 1989) it is not generally known to be elongated to 18:2 n-4. In addition to this, compound specific stable isotope evidence (Pond *et al.*, 1997b) indicates that 16:2 n-4 and 18:2 n-4 fatty acids in *R. exoculata* are derived from a chemosynthetic precursor, possibly 16:1 n-7. Levels of n-4 fatty acids detected in adult *R. exoculata* here were similar to those found in *R. exoculata* by Pond *et al.* (1997b) and somewhat higher than those found in *M. fortunata* (Pond *et al.*, 1997c).

NMID fatty acids are characteristic of molluscs and other organisms with symbiotic bacteria and low levels of highly unsaturated fatty acids (Joseph, 1982, Fullarton *et al.*, 1995a & b). NMID fatty acids comprised a lower proportion of fatty acids in adult vent shrimp than are found in deep vent bivalves, for example (Pranal *et al.*, 1997). This

may be a result of the use of n-4 fatty acids. The percentage total PUFA (including n-4 and NMID fatty acids) in adult *R. exoculata* are similar to those found for other caridean shrimp (Table 3.4.3). This concurs with the hypothesis that n-4 and NMID fatty acids act as substitutes for n-3 and n-6 PUFA in their absence. Long chain unsaturated fatty acids are probably necessary for effective membrane operation in deep-sea organisms and n-4 PUFA and NMID fatty acids may fulfil this role in the absence of n-3 and n-6 PUFA (Pond *et al.*, 1997b). The decrease in levels of phytoplankton derived n-3 and n-6 fatty acids and concomitant increase in n-4 PUFA and NMID fatty acids in vent shrimp from postlarvae to large adults also agrees with this theory.

The presence of significant amounts of the highly unsaturated fatty acids 20:5 n-3 (EPA) and 22:6 n-3 (DHA) in postlarval alvinocarids and juvenile *R. exoculata* suggests that they derive a large amount of their organic carbon from a photosynthetic source. EPA and DHA have been known to be produced by deep-sea bacteria (Delong & Yayanos, 1986), but the high total lipid content and wax ester storage lipids in these shrimp coupled with the presence of EPA and DHA point to a deep-pelagic existence and a dependence on phototrophically derived organic matter.

Fatty acid profiles of postlarval alvinocarids suggest that they subsist on an ultimately phototrophically derived source of organic carbon, while adults primarily rely on a bacterial source of nutrition. Juvenile *R. exoculata* fatty acid profiles lie between the two, indicating the change over to an organic carbon source derived ultimately from a photosynthetic source to a chemosynthetic source.

The lipid profiles of whole *R. exoculata* and postlarval alvinocarid shrimp support a proposed life cycle for vent shrimp (Figure 3.4.1) that also provides a possible explanation for the extensive gene flow between populations of shrimp at different hydrothermal vent sites (Creasey *et al.*, 1996; Shank *et al.*, 1998). Alvinocarid larvae reach the deep pelagic region in some manner. Larvae may be transported to the deep pelagic region in hydrothermal plumes, possibly assisted by a buoyant wax ester ene store. It has been suggested that the eggs of *R. exoculata* may be released dire the water column (Vereschaka, 1997). Although this would be extrem

because caridean decapods bear eggs on the pleopods until they hatch (Chace, 1992), it might be an adaptation to prevent the exposure of eggs to the extreme conditions of

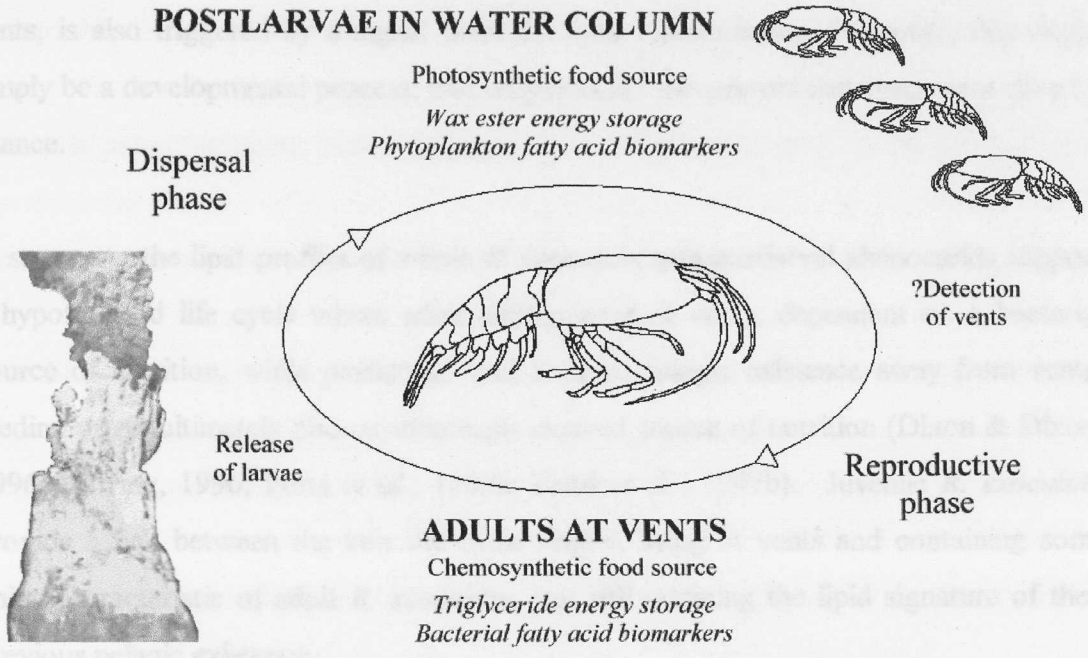


Figure 3.4.1 Proposed life cycle for *R. exoculata*

temperature and the high concentrations of heavy metals at vents. Very few female *R. exoculata* have been found with eggs on their pleopods and this might appear to support the hypothesis that eggs are released directly into the water column. It is perhaps more likely that female *R. exoculata* with eggs distance themselves from active vents, to protect the eggs from the vent environment and consequently have not been captured. In the absence of further information, it is impossible to do more than speculate on the mechanisms of transport of larvae to the deep pelagic zone. Once there, it is possible to imagine a stock of alvinocarid postlarvae, at highest concentrations above active vents, but subject to water movement and deep ocean currents and therefore possibly transported and spread along the Mid-Atlantic Ridge. The postlarvae may have some mechanism for detecting an active vent. Perhaps like us, they sniff out vents by sensing increased levels of particulates in hydrothermal plumes, or maybe they have a more sensitive detection system, and can trace the presence of metals such as reduced manganese. Alternatively, postlarvae may not detect the vents at all, but simply arrive at vents by chance or not at all. It is not known how long postlarvae exist in the pelagic zone, but they metamorphose either before, or on arrival at hydrothermal vents.

Juvenile shrimp appear to be at an intermediate stage, with a dorsal organ shape and general morphology and lipid composition between that of postlarvae and adults. It may be speculated that this metamorphosis, to a form suited to an existence at hydrothermal vents, is also triggered by a signal from the vent environment. However, this might simply be a developmental process, that only benefits the animals that reach vent sites by chance.

In summary, the lipid profiles of whole *R. exoculata* and postlarval alvinocarids support a hypothesised life cycle where adult shrimp exist at vents, dependent on a bacterial source of nutrition, while postlarvae lead a deep, pelagic existence away from vents, feeding on an ultimately photosynthetically derived source of nutrition (Dixon & Dixon, 1996; Herring, 1996; Pond *et al.*, 1997a; Pond *et al.*, 1997b). Juvenile *R. exoculata* provide a link between the two life cycle stages, living at vents and containing some lipids characteristic of adult *R. exoculata*, but still retaining the lipid signature of their previous pelagic existence.

3.4.3 Lipid profiles of dissected *R. exoculata* from Broken Spur

The total lipid and lipid class composition of different tissue types of adult and juvenile *R. exoculata* can provide information about energy partitioning in different life cycle stages. The high concentrations of lipid, in the form of wax esters, found in juvenile shrimp are stored mainly in the abdomen region, while adult shrimp triglyceride energy stores are concentrated in ovary and hepatopancreas tissue. Polar lipid levels are similar in adult abdomen and branchial area tissues, where they probably represent mainly structural lipid. They are slightly higher in ovary and hepatopancreas tissue, where they may play a role in metabolism and reproductive development.

The branchial area tissue of *R. exoculata* includes large gills that are covered in filamentous bacteria (Casanova *et al.*, 1993) and often small black particles of sulphides. Wax esters detected in the branchial area of juvenile shrimp almost certainly represent contamination of the branchial area tissue. Juvenile shrimp were very oily and it was difficult to keep this oil from getting into branchial area tissue during dissection. Similarly, triglyceride detected in branchial area tissue of adult shrimp may represent

contamination from ovary and hepatopancreas tissue, although levels seem a little high for this to be the case. Hydrothermal vent mussels and clams with symbiotic bacteria are known to store triglyceride in their gills (Pranal *et al.*, 1997; Chapter 5.3) so it is conceivable that triglyceride in adult vent shrimp gills represents a store of bacterial lipids. The largest lipid component in adult abdomen tissue of both male and female shrimp is polar, structural lipid. As mentioned earlier, polar lipid levels are similar in branchial and abdomen tissue and the overall higher concentration of total lipid in adult branchial tissue is a consequence of the presence of triglyceride.

Free fatty acids comprise less than 10 % of total lipid in all tissue types suggesting that the samples were in a good state of preservation (Jeckel *et al.*, 1989). However, free fatty acid and sterol tissue concentrations are higher in ovary and hepatopancreas tissue than in branchial area tissue and free fatty acid concentrations are higher in branchial area tissue than in abdomen tissue. This probably reflects elevated levels of metabolic activity in ovary and hepatopancreas tissue and possibly also in branchial area tissue.

There was no difference in the principal fatty acid composition of dissected shrimp tissues and whole adult or juvenile *R. exoculata*. Adult *R. exoculata* branchial and abdomen tissues contained similar levels of saturated fatty acids and as for whole shrimp, adult tissues contained a higher proportion of SFA than juvenile tissues. This is probably a reflection of elevated levels of dietary polyunsaturated fatty acids in juvenile shrimp. Juvenile *R. exoculata* branchial region tissue contained a higher percentage of saturated fatty acids than abdomen tissue. This may be a related to the lower levels of wax ester in this region and the presence of bacteria.

The proportion of monounsaturates was highest in juvenile branchial regions, although tissue concentrations of MFA in juvenile and adult branchial regions were all approximately the same. This probably reflects the presence of bacteria in branchial region tissue. In juveniles, this is supported by a higher 18:1 n-7/n-9 ratio in branchial regions than in the abdomen region reflecting a larger bacterial contribution to the fatty acid profile. It is initially surprising that the ratios of 18:1 n-7/n-9 are lower for adult branchial regions than for abdomen regions, but this can be explained when considered in conjunction with polyunsaturated fatty acid type. It has already been suggested (section

3.4.1, Pond *et al.*, 1997b) that n-4 fatty acids might be synthesised from n-7 monounsaturates. The reduced level of 18:1 n-7 fatty acids compared with 18:1 n-9 fatty acids in branchial tissue may be a result of their conversion to n-4 fatty acids. There is a higher proportion of n-4 fatty acids in branchial tissue than in abdomen tissue.

In contrast to the n-4 (bacterial biomarker) fatty acids, n-3 PUFA (phytoplankton biomarkers) comprise a larger proportion of abdomen tissue than branchial tissue in both adult and juvenile *R. exoculata*. The lower proportion of n-3 PUFA in adult and juvenile branchial tissue than abdomen tissue may simply be ascribed to the association of bacteria and their product fatty acids with this tissue type. There is a lower proportion of n-3 PUFA in juvenile branchial tissue than in adult branchial tissue, but tissue concentrations are similar and so this is probably just a reflection of the high levels of monounsaturates in juvenile branchial tissue. The lower proportion of n-6 PUFA in juvenile branchial tissue than abdomen tissue is probably also a reflection of the bacterial contribution to branchial tissue.

Wax esters detected in juvenile shrimp abdomen tissue contained mainly sixteen and eighteen carbon saturated and monounsaturated fatty alcohols. Graeve *et al.* (1994) described such short chain fatty alcohols as biomarkers for omnivorous and opportunistic feeding in Antarctic copepods. Herbivorous antarctic copepods bear longer chain twenty and twenty-two carbon saturated and monounsaturated fatty alcohols (Albers *et al.*, 1996). It might therefore be inferred that during their planktonic existence, juvenile *R. exoculata* are omnivorous and opportunistic feeders.

The lipid profiles of the branchial regions of *R. exoculata* reflect the presence of bacteria in these regions and triglyceride levels suggest that this tissue may also contain an extra lipid store. Elevated levels of free fatty acids suggest metabolic activity and high concentrations of n-4 and NMID fatty acids and lower 18:1 n-7/n-9 ratios may suggest that these fatty acids are synthesised here from n-7 fatty acid precursors. The fatty acid profiles of juvenile branchial regions are more redolent of those of adult *R. exoculata* than those of juvenile abdomen tissue. This might be a result of the bacterial colonisation of the growing gills in juvenile *R. exoculata* during their development and metamorphosis into adults.

There have been few studies of the variation in lipid profiles of hepatopancreas and ovary tissue with reproductive state in caridean shrimp. Consequently, results of some investigations into the reproductive cycles and lipids of penaeid shrimp are also referred to here. It must be borne in mind, however, that penaeids are taxonomically quite different than caridean shrimp and have a different reproductive strategy (e.g. they do not bear eggs on pleopods) and might therefore also display different patterns of reproductive development. Hepatopancreas and ovary tissue were analysed together in this study, so transfers of energy stores from one organ to the other would not be distinguishable. In two species of caridean shrimp studied (*Chorismus antarcticus*, Clarke, 1982; *Crangon crangon*, Spaargaren & Haefner, 1994) it has been shown that total lipid concentration increases in both ovary and hepatopancreas throughout oogonial development, previtellogenesis and primary vitellogenesis. Total lipid concentration also increases in ovaries during primary and secondary vitellogenesis, but decreases in hepatopancreas tissue during secondary vitellogenesis, suggesting transfer of stored lipid from hepatopancreas to ovary at this stage. Reproductive stages described here correspond approximately to oogenesis and previtellogenesis at stage I, and vitellogenesis and maturation from stages II to IV (Copley, 1998).

The gonadohepatosomatic index calculated showed a general increase in the proportion of hepatopancreas and ovary tissue with reproductive development in *R. exoculata.* Each index value was derived from the combined weights of tissue from two to three individuals, divided by the mean carapace length of those individuals. Considering these approximations and the low number of samples available, the index should be viewed with some reservations. However, assuming that, in caridean shrimp, the proportion of ovary and hepatopancreas tissue increases with reproductive state (Clarke, 1977; Clarke, 1982; Spaargaren & Haefner, 1994), then the general increase in the gonadohepatosomatic index supported the method of identification of reproductive stages.

A general increase in total lipid tissue concentration of ovary and hepatopancreas tissue is seen from reproductive stages I to III in *R. exoculata.* There is little difference in the mean total tissue lipid concentration of ovary and hepatopancreas tissue at stages III and IV but polar lipid levels are higher in stage IV tissue than in tissue at reproductive stages

I to III. Triglyceride, phosphatidyl choline and phosphatidyl ethanolamine concentrations were suggested to be responsible for the increases in ovarian tissue lipid concentrations in *P. japonicus* (Teshima *et al.*, 1989), while tissue lipid concentration increases in hepatopancreas tissue were attributed to triglycerides. In addition to this, Mourente & Rodriguez (1991) reported that ovarian tissue in *Penaeus kerathurus* was dominated by polar lipid and that polar lipid tissue concentrations in the hepatopancreas were constant throughout reproductive development. It is therefore conceivable, that the elevated levels of polar lipid in ovary and hepatopancreas tissue at stage IV in *R. exoculata* reflect an increased proportion of ovarian lipid. It has been suggested that neutral lipid in the hepatopancreas is converted to polar lipids and transported to the ovary in mature female shrimp (Mourente & Rodriguez, 1991). It appears that this may occur in *R. exoculata*, although further work (examination of the lipids of separate hepatopancreas and ovarian tissue) would be required to verify this.

Combined ovary and hepatopancreas tissue contains a higher proportion of n-4 fatty acids and lower proportion of n-3 fatty acids than abdomen or branchial tissue lipid. This may be related to the types of lipid in the different tissues. Abdomen and branchial region tissues contain higher proportions of polar, structural lipid than hepatopancreas and ovary tissue. Storage lipid is comprised of triglyceride containing dietary fatty acids and their derivatives. As the lipid store in *R. exoculata* is relatively small (section 3.4.2), it seems likely that there is a relatively rapid turnover of this lipid in adult shrimp. The turnover of structural lipids is probably slower and therefore a higher proportion of the lipid laid down at postlarval and juvenile stages, when dietary n-3 fatty acids are available, might be expected. Other workers have reported higher proportions of n-3 fatty acids in phospholipids, such as phosphatidyl choline, than in triglycerides in the ovaries and hepatopancreas of penaeid shrimp (Teshima *et al.*, 1989; Mourente & Rodriguez, 1991). This might be related to the structural properties of these fatty acids and their use in membranes, or their function in reproduction and development.

The decrease in the proportion of n-4 fatty acids and concomitant increase in the proportion of n-3 fatty acids in combined ovary and hepatopancreas tissue with reproductive development might also be a reflection of the type of lipid stored. It has already been mentioned that polar lipid tends to contain a higher proportion of highly unsaturated fatty acids than neutral lipid and the increase in n-3 fatty acids coincides with

the increase in polar lipid levels. In addition, n-3 fatty acids are known to be required for vitellogenesis in penaeid shrimp (Middleditch *et al.*, 1980). This may also be the case here. It is uncertain whether the n-4 and NMID fatty acids suggested to be substitutes for n-3 PUFA (section 3.4.2) are able to fulfil the roles of n-3 PUFA in reproduction. It is possible that n-3 fatty acids, possibly gained during postlarval, pelagic stages of the life cycle of the shrimp, are required for use in reproduction.

3.4.4 Lipid profiles of *A. markensis*

The limited data set of lipid profiles for *A. markensis* means that only preliminary conclusions may be drawn from the results presented. The total lipid tissue concentration of whole *A. markensis* is higher than that of *R. exoculata* and similar to that of other deep-sea benthic shrimp (see table 3.4.1, page 3.27 and chapter 4). This perhaps reflects the lesser degree of adaptation of *A. markensis* to the constant food source at hydrothermal vents than is seen in *R. exoculata*. Lipid class composition is similar to that of *R. exoculata*, with triglyceride comprising the majority of all body lipid, stored mainly in hepatopancreas tissue. Polar, structural lipids comprised the highest percentage of abdomen tissue and their composition was also similar to that found in *R. exoculata*. However, the fatty acid composition of *A. markensis* was markedly different than that found in *R. exoculata*.

The ratios of 18:1 n-7/n-9 generally reflected a low input of bacterially derived organic matter in *A. markensis*, and agreed with the results of Pond *et al.*, 1997b. However, ratios were higher than those found for the non-vent shrimp *N. gracilis* (section 4.3, page 4.5) probably reflecting the importance of bacterivorous organisms or their remains in the diet of *A. markensis* or perhaps an opportunistic diet that includes bacteria. The particularly high 18:1 n-7/n-9 ratio (~2) reported for the abdomen portion of *A. markensis* might reflect the presence of bacteria on the outside of the abdomen. However, if that were the case, such high ratios would be expected in the "rest" portion of the shrimp that included the carapace as well. As only one sample was available for analysis, it is difficult to know whether this result was real or an artefact. Pond *et al.*,

(1997b) did not report such high ratios for *A. markensis*, so it is perhaps likely that this is not a representative result.

The unusual n-4 and NMID fatty acids detected in *R. exoculata* were present in *A. markensis*, but at much lower levels. In addition, a higher proportion of n-3 fatty acids were present in *A. markensis* than were found in *R. exoculata.* This concurs with the hypothesis that *A. markensis* is an opportunistic feeder, with a diet that includes the remains of *R. exoculata* and other vent shrimp, but also some amount of phytoplankton derived organic matter (Segonzac *et al.*, 1993; Pond *et al.*, 1997b).

Deep-sea caridean shrimp eggs are generally rich in triglycerides and phospholipids and bear only low levels, if any, of wax esters (Herring 1974). The eggs of *A. markensis* are no exception to this rule, comprising mainly triglyceride and a fairly significant proportion of polar lipid, that is mainly phosphatidyl choline. The high proportion of monounsaturated fatty acids is a characteristic of shrimp egg fatty acids (Clarke, 1979; Hopkins *et al.* 1993, Kattner *et al.*, 1994).

4: Lipid profiles of benthic shrimp from the Arabian Sea

Lipid profiles were determined for 28 specimens of the deep-sea benthic shrimp, *Nematocarcinus gracilis*, collected from the Arabian Sea. These samples included 6 females, 7 males, 7 berried (i.e. egg carrying) females and 8 specimens whose sex could not be determined owing to the absence of pleopods one and two. All specimens were analysed whole, as they had been previously deep frozen.

Tables of mean lipid class composition are presented in Appendix 2. Raw data are stored in files on the appendix CD-ROM; a guide to these files is saved in a readme file: appendix.txt.

4.1 Total lipid and polar lipid profiles of *N. gracilis*

Total lipid and polar lipid tissue concentrations were calculated as for hydrothermal vent shrimp. Mean total lipid and polar lipid tissue concentrations are presented for each group of specimens and for all *N. gracilis* in Table 4.1.1. There was no significant difference in the total lipid concentrations of males, females, berried females and specimens of undetermined sex (ANOVA, 95% confidence, $P = 0.388$). The total lipid tissue concentration of *N. gracilis* was significantly higher than the total lipid tissue concentration of whole adult *R. exoculata* (57.9 ± 1.9 mg g dry weight^{-1} SE; Mann-Whitney Rank Sum test, $P < 0.0001$).

Table 4.1.1 Mean total lipid and polar lipid concentrations for *N. gracilis* (mean tissue concentrations mg g dry weight^{-1} ± S.E. ($\sigma/\sqrt{n}$)

Shrimp	Mean total lipid ± SE ($\sigma/\sqrt{n}$) (mg g dry weight $^{-1}$)	Mean polar lipid ± SE ($\sigma/\sqrt{n}$) (mg g dry weight $^{-1}$)
Males (n[1] = 21)	82.3 ± 2.24	18.1 ± 0.46
Females (n = 18)	79.8 ± 4.28	19.3 ± 0.78
Berried females (n = 21)	78.1 ± 2.36	20.8 ± 0.56
Indeterminate sex (n = 24)	86.3 ± 4.69	22.8 ± 1.35
All specimens (n = 84)	81.8 ± 1.82	20.4 ± 0.49

Mean polar lipid tissue concentration of all *N. gracilis* specimens was not significantly different from that of adult *R. exoculata* (19.3 ± 0.5 mg g dry weight^{-1}; t-test, 95% confidence, $P = 0.125$). Differences in mean polar lipid values for different groups of

[1] n refers to the number of lipid class results used to calculate the sample mean. It does not refer to the number of shrimp, which is n/3, because each sample was assayed three times.

N. gracilis were statistically significantly different from each other (ANOVA, $P = 0.002$) and a multiple pairwise comparison (Bonferonni's test) indicated that mean polar lipid was significantly lower in male *N. gracilis* than in *N. gracilis* of indeterminate sex.

4.2 Lipid class composition of *N. gracilis*

Figure 4.2.1 illustrates the mean contribution of each neutral lipid class to the total lipid of groups of male, female, berried female and specimens of undetermined sex as well as the mean lipid class composition of all specimens analysed.

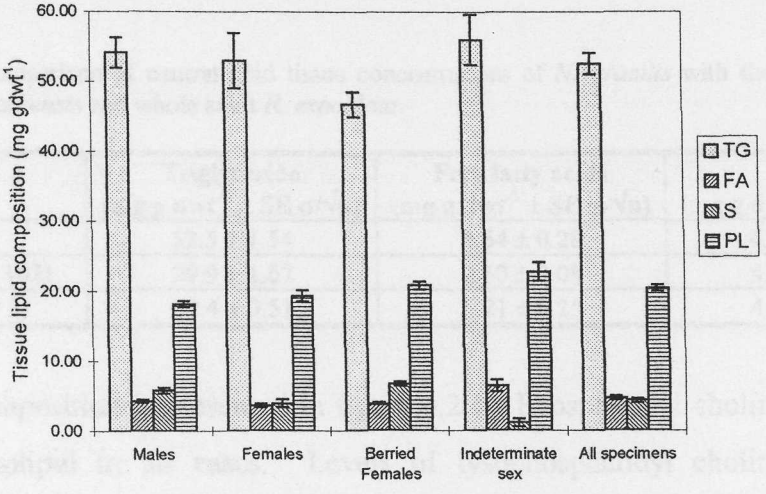


Figure 4.2.1 Bar chart showing the lipid class composition of *N. gracilis*. Error bars indicate standard errors of the mean (S.E. = $\sigma/\sqrt{n}$). TG, triglyceride; FA, free fatty acid; S, sterol; PL, polar lipids. Males, n = 21; females, n = 18; berried females, n = 21; indeterminate sex, n = 24; all specimens *N. gracilis*, n = 84.

As in adult vent shrimp, triglyceride is the major storage lipid, but triglyceride tissue concentrations do not increase with increasing carapace length (Figure 4.2.2).

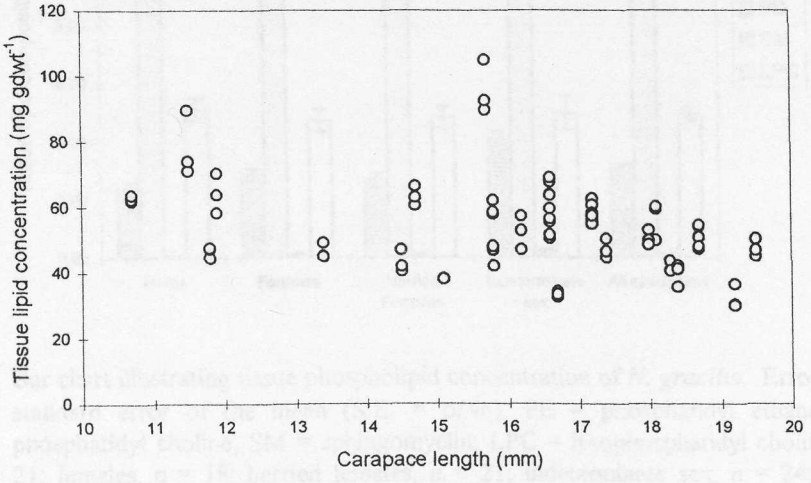


Figure 4.2.2 Triglyceride tissue concentration v/s carapace length for *N. gracilis*

Mean triglyceride tissue concentration for all *N. gracilis* (Table 4.2.1) is significantly higher than that found in adult *R. exoculata* (Mann-Whitney Rank Sum test, $P < 0.0001$). However, triglyceride tissue concentrations are not significantly different than those found in *A. markensis* (t-test, 95% confidence, $P = 0.095$). Sterol tissue concentration is not significantly different than that found in *R. exoculata* and *A. markensis* (Kruskal Wallis ANOVA on ranks, $P = 0.175$). Levels of free fatty acids are similar to those found in *A. markensis* (Mann-Whitney Rank Sum test, $P = 0.330$), but statistically different than those found in adult *R. exoculata* (Mann-Whitney Rank Sum test, $P = 0.017$).

Table 4.2.1 Comparison of neutral lipid tissue concentrations of *N. gracilis* with those of whole *A. markensis* and whole adult *R. exoculata*.

Shrimp species	Triglyceride (mg g dwt^{-1} ± SE σ/√n)	Free fatty acids (mg g dwt^{-1} ± SE σ/√n)	Sterols (mg g dwt^{-1} ± SE σ/√n)
N. gracilis	52.5 ± 1.54	4.64 ± 0.28	4.29 ± 0.34
R. exoculata (n = 102)	29.9 ± 1.67	2.60 ± 0.08	4.33 ± 0.16
A. markensis (n = 2)	69.4 ± 0.51	5.21 ± 0.25	4.20 ± 0.23

Polar lipid composition is presented in figure 4.2.3. Phosphatidyl choline (PC) is the major phospholipid in all cases. Levels of lysophosphatidyl choline (LPC) are significantly higher than levels in vent shrimp (Table 4.2.2; Kruskal-Wallis ANOVA on

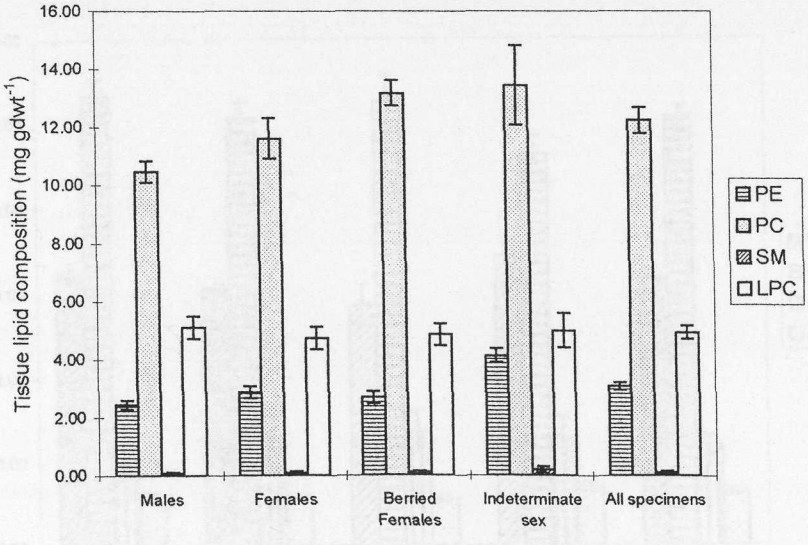


Figure 4.2.3 Bar chart illustrating tissue phospholipid concentration of *N. gracilis*. Error bars represent standard error of the mean (S.E. = σ/√n), PE = phosphatidyl ethanolamine; PC = phosphatidyl choline; SM = sphingomyelin; LPC = lysophosphatidyl choline. Males, n = 21; females, n = 18; berried females, n = 21; indeterminate sex, n = 24; all *N. gracilis* specimens, n = 84.

ranks, $P < 0.001$) and LPC is more abundant than phosphatidyl ethanolamine (PE) in all cases. Sphingomyelin is present, but at low levels similar to those found in vent shrimp. PC tissue concentration is not significantly different than that found in vent shrimp (Kruskal-Wallis ANOVA on ranks, $P = 0.580$) but PE levels are significantly lower (Kruskal-Wallis ANOVA on ranks, $P < 0.001$).

Table 4.2.2 Comparison of polar lipid tissue concentrations of *N. gracilis*, whole *A. markensis* and whole adult *R. exoculata* (SE = $\sigma/\sqrt{n}$); PE = phosphatidyl ethanolamine; PC = phosphatidyl choline; SM = sphingomyelin; LPC = lysophosphatidyl choline.

Shrimp species	PE (mg g dwt^{-1} ± SE)	PC (mg g dwt^{-1} ± SE)	SM (mg g dwt^{-1} ± SE)	LPC (mg g dwt^{-1} ± SE)
N. gracilis (n = 84)	3.07 ± 0.13	12.2 ± 0.46	0.12 ± 0.04	4.93 ± 0.23
R. exoculata (n=102)	6.01 ± 0.17	11.7 ± 0.34	0.86 ± 0.03	1.12 ± 0.05
A. markensis (n = 2)	5.36 ± 0.28	10.28 ± 0.18	0.31 ± 0.01	1.02 ± 0.07

4.3 Fatty acid composition of *N. gracilis*

The major fatty acids detected in *N. gracilis* were 16:0, 16:1 n-7, 18:0, 18:1 n-9 and 18:1 n-7 (Table 4.3.1, page 4.5). The percentage fatty acid composition of male, female, berried female and indeterminate sex specimens was similar and the highly unsaturated fatty acids 20:5 n-3 and 22:6 n-3 comprised less than 5% each of all fatty acids. Figure 4.3.1 illustrates the contributions of different types of fatty acids to the fatty acid profile of *N. gracilis*.

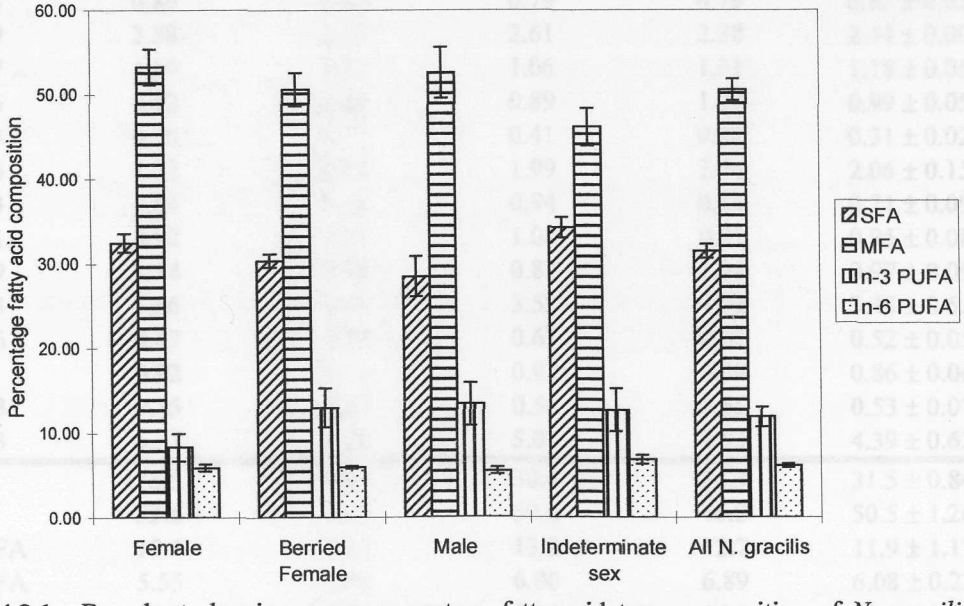


Figure 4.3.1 Bar chart showing mean percentage fatty acid type composition of *N. gracilis*. SFA, saturated fatty acids; MFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Error bars indicate standard errors (SE = $\sigma/\sqrt{n}$). Males, n = 7; females, n = 6; berried females, n = 7; indeterminate sex, n = 8; all *N. gracilis*, n = 28.

Saturated fatty acids comprised over 30% of all fatty acids, a higher proportion than found in adult or juvenile vent shrimp (table 4.3.2), while monounsaturates were the most abundant type of fatty acid. The ratio of 18:1 n-7/n-9 fatty acids (Table 4.3.1) is low for all specimens, with levels not significantly different than those for juvenile and

Table 4.3.1 Mean percentage fatty acid composition of *N. gracilis*. Standard errors omitted for clarity, except for all *N. gracilis* where mean ± SE is quoted (SE = $\sigma/\sqrt{n}$; n = 28). SFA, saturated fatty acids; MFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HUFA highly unsaturated fatty acids (> 4 double bonds).

	Male	Female	Berried female	Indeterminate sex	All *N. gracilis*
14:0	3.67	3.61	3.58	3.48	3.56 ± 0.12
14:1	0.43	0.64	0.61	0.53	0.54 ± 0.07
15:0	1.07	1.05	1.08	1.16	1.09 ± 0.04
15:1	0.52	0.60	0.56	0.87	0.64 ± 0.04
16:0	17.7	21.9	19.7	22.1	20.4 ± 0.84
16:1 n-9	1.14	1.42	1.17	1.56	1.33 ± 0.05
16:1 n-7	9.59	9.70	8.76	7.90	8.93 ± 0.31
16:2 n-6	0.62	0.69	0.74	0.79	0.71 ± 0.04
17:0	0.83	0.87	0.81	1.15	0.93 ± 0.05
16:3 n-3	1.11	1.12	1.06	0.96	1.05 ± 0.04
18:0	4.69	4.69	4.58	5.85	4.99 ± 0.20
18:1 n-9	27.5	28.4	27.1	21.5	26.0 ± 0.96
18:1 n-7	6.63	6.74	5.98	7.27	6.67 ± 0.21
18:2 n-6	1.25	1.22	1.23	1.26	1.23 ± 0.04
18:2 n-3	0.43	0.49	0.48	0.40	0.45 ± 0.03
18:3 n-6	0.57	0.61	0.51	0.56	0.56 ± 0.03
18:3 n-3	0.18	0.23	0.21	0.18	0.19 ± 0.01
20:0	0.54	0.35	0.58	0.52	0.49 ± 0.04
18:4 n-3	0.89	0.80	0.79	0.79	0.82 ± 0.03
20:1 n-9	2.58	2.16	2.61	2.38	2.44 ± 0.09
20:1 n-7	1.19	1.13	1.06	1.31	1.18 ± 0.06
20:3 n-6	0.92	0.89	0.89	1.22	0.99 ± 0.05
20:3 n-3	0.36	0.27	0.41	0.22	0.31 ± 0.02
20:4 n-6	1.73	2.11	1.99	2.44	2.06 ± 0.15
20:4 n-3	0.84	0.53	0.94	0.63	0.71 ± 0.09
22:1 n-11	0.92	0.95	1.08	0.82	0.95 ± 0.08
22:1 n-9	1.14	0.77	0.82	1.08	0.97 ± 0.09
20:5 n-3	3.66	1.98	3.52	4.33	3.45 ± 0.53
22:5 n-6	0.47	0.38	0.61	0.62	0.52 ± 0.05
24:1	0.92	0.72	0.82	0.89	0.86 ± 0.06
22:5 n-3	0.55	0.32	0.56	0.65	0.53 ± 0.07
22:6 n-3	5.37	2.58	5.07	4.53	4.39 ± 0.62
SFA	28.5	32.5	30.4	34.3	31.5 ± 0.84
MFA	52.6	53.3	50.6	46.2	50.5 ± 1.26
n-3 PUFA	13.4	8.33	13.0	12.7	11.9 ± 1.17
n-6 PUFA	5.55	5.90	6.00	6.89	6.08 ± 0.22
HUFA	13.5	8.71	13.5	14.0	12.5 ± 1.32
18:1 n-7/n-9	0.24	0.24	0.22	0.37	0.27 ± 0.02

postlarval *R. exoculata* (table 4.3.2). The polyunsaturated fatty acids found in *N. gracilis* were mainly n-3 PUFA. NMID and n-4 fatty acids were not detected. Table 4.3.2 summarises the results of statistical comparisons of fatty acid group percentage compositions of *N. gracilis* with adult and juvenile *R. exoculata*.

Table 4.3.2 Comparison of percentage FAME group composition of *N. gracilis* with adult and juvenile *R. exoculata*, all quoted ± SE (standard error, $\sigma/\sqrt{n}$). *N. gracilis*, n = 28; Adult *R. exoculata*, n = 36; juvenile and postlarval *R. exoculata*, n = 10. SFA saturated fatty acids; MFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; statistical tests: Mann-Whitney Rank Sum test; t-test (95% confidence).

	N. gracilis	Adult *Rimicaris* Juv *Rimicaris*	Statistical test	Significant difference? & probability
% SFA	31.5 ± 0.84	15.0 ± 0.21 6.15 ± 0.70	Mann-Whitney t-test	Yes, $P < 0.0001$ Yes, $P < 0.0001$
% MFA	50.5 ± 1.26	42.5 ± 0.71 55.1 ± 3.22	Mann-Whitney t-test	Yes, $P < 0.0001$ No, $P = 0.119$
% n-3 PUFA	11.9 ± 1.17	8.45 ± 0.95 26.5 ± 3.73	Mann-Whitney t-test	Yes, $P = 0.001$ Yes, $P < 0.0001$
% n-6 PUFA	6.08 ± 0.22	2.50 ± 0.14 10.1 ± 1.24	t-test Mann-Whitney	Yes, $P < 0.0001$ Yes, $P = 0.004$
% HUFA	12.5 ± 1.32	9.12 ± 1.10 33.1 ± 3.26	Mann-Whitney Mann-Whitney	Yes, $P = 0.023$ Yes, $P < 0.0001$
18:1 n-7/n-9 ratio	0.27 ± 0.02	3.35 ± 0.19 0.33 ± 0.06	Mann-Whitney Mann-Whitney	Yes, $P < 0.0001$ No, $P = 0.703$

Levels of monounsaturates are similar in *N. gracilis* and juvenile and postlarval alvinocarids, but otherwise, percentage fatty acid composition is quite different. The presence or absence of significant amounts of two different groups of polyunsaturated fatty acids (NMID and n-4 fatty acids) in adult shrimp is probably largely responsible for this difference, but there are also relatively low levels of HUFA in *N. gracilis* compared to juvenile alvinocarids.

Table 4.3.3 Mean expected and actual FAME tissue concentrations for *N. gracilis* and results of statistical comparisons. SE = $\sigma/\sqrt{n}$; conc'n = concentration.

Specimens	Mean ± SE expected FAME tissue conc'n (mg g dry weight^{-1})	Mean ± SE actual FAME tissue conc'n (mg g dry weight^{-1})	Statistical test, significant difference? & probability
All *N. gracilis*	72.2 ± 1.79	50.2 ± 6.10	Wilcoxon Signed Rank Significant difference $P < 0.0001$
N. gracilis specimens 2, 4, 7 & 23	72.3 ± 2.02	70.4 ± 1.59	t-test, 95% confidence No significant difference $P = 0.299$
N. gracilis except 2, 4, 7 & 23	72.1 ± 2.07	46.8 ± 7.04	Wilcoxon Signed Rank Significant difference $P < 0.0001$

Fatty acid tissue concentrations are not presented here because there was a significant difference in mean actual and expected FAME yields (Table 4.3.3) for all specimens, calculated as described in chapter 3 (section 3.1.2, page 3.7). However, there was no significant difference (Table 4.3.3) in the actual and expected FAME yields of four of the shrimp specimens. The mean percentage fatty acid composition of these four individuals was compared with the mean percentage fatty acid composition of all other individuals and no significant differences were found in the proportions of fatty acid types (results of statistical tests summarised in table 4.3.4).

Table 4.3.4 Summary of the results of statistical comparisons of mean fatty acid type composition of *N. gracilis* with high and low FAME yields. SFA, saturated fatty acids; MFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Fatty acid type	Significant difference?	Statistical test results
SFA	No	t-test, 95% confidence: $P = 0.528$
MFA	No	t-test, 95% confidence: $P = 0.964$
n-3 PUFA	No	t-test, 95% confidence: $P = 0.368$
n-6 PUFA	No	t-test, 95% confidence: $P = 0.692$

Therefore, although fatty acid tissue concentrations cannot be used here and despite low total FAME yields in most specimens, the mean percentage fatty acid compositions of all shrimp may be considered to be unaffected.

4.4 Discussion of the lipid profiles of *N. gracilis*

4.4.1 Potential errors in the lipid profiles of *N. gracilis*

Relatively high levels of lysophosphatidyl choline (LPC; around five times higher than those found in *R. exoculata* or *A. markensis*) were detected in all specimens of *N. gracilis*. LPC has the same basic structure as phosphatidyl choline (PC), but where PC has two fatty acids and a phosphate function on a glycerol backbone, LPC has only one fatty acid, a hydroxyl group and a phosphate function on a glycerol backbone (see also Chapter 1.2). LPC is an intermediate in the metabolism of PC, so levels of LPC can reflect metabolic activity. However, high levels of LPC can also indicate enzymatic degradation of PC, that sometimes occurs in specimens that are not frozen quickly enough after recovery, or that are frozen to temperatures warmer than -70°C. Such high levels may also be associated with increased levels of free fatty acids that have been released from PC during enzymatic degradation to LPC. In the case of *N. gracilis*

studied here, however, levels of free fatty acids are not particularly high (< 6 % of total lipid), which suggests that if enzymatic degradation has occurred, then the fatty acids released have been lost, perhaps by the auto-oxidation of unsaturated fatty acids. Levels of unsaturated fatty acids may therefore have been underestimated and this must be taken into consideration before interpreting the fatty acid profiles of *N. gracilis*.

Assuming that approximately 4 mg g dry weight^{-1} of the LPC detected is a product of the post-mortem enzymatic degradation of PC (this assumes that there should be similar levels of LPC in *N. gracilis* as in *R. exoculata* and *A. markensis*), approximately 2.3 mg g dry weight^{-1} of FAME must have been released. (This is calculated from the proportion of the molecular mass of LPC that comprises fatty acids: there is one fatty acid in LPC, where there are two in PC). The total mean FAME tissue concentration (expected) in *N. gracilis* is ~ 72 mg g dry weight^{-1}. Of this ~ 18 % are polyunsaturated fatty acids (PUFA). This corresponds to 13 mg g dry weight^{-1}. Adding the extra 2.3 mg g dry weight^{-1} PUFA to both figures allows calculation of a new percentage PUFA, including those that might have been lost by auto-oxidation. 15.3 mg g dry weight^{-1} divided by 74.3 mg g dry weight^{-1} multiplied by 100 equals 20.6% PUFA. This suggests that the difference in percentage PUFA would be less than 3% of total fatty acids. It may therefore be concluded that the loss of PUFA by auto-oxidation of free fatty acids released by enzyme degradation of LPC is not large enough to affect percentage fatty acid composition considerably.

The causes of apparently low yields of fatty acid methyl esters in some cases have already been discussed (Chapter 3.4.1). In *N. gracilis* the problem of low FAME yields affected most of the samples analysed. However, although fatty acid tissue concentrations may not be meaningfully considered here, results indicated no significant difference in the percentage fatty acid composition of specimens with low yields of FAME and specimens with FAME yields that were not significantly different than expected yields. Consequently, percentage results have been considered unaffected by FAME yield levels.

4.4.2 Discussion of the lipid profiles of *N. gracilis*

N. gracilis total lipid tissue concentrations were at the low end of the range of those reported for other deep-sea benthic and epibenthic shrimp species (Culkin & Morris, 1969; Herring, 1973; Hopkins *et al.*, 1993; see table 3.4.1, p. 3.27). The reasons for variations in total lipid contents of deep-sea shrimp reported in early papers (Culkin & Morris, 1969; Herring, 1973) were not accounted for. However, variations in the total lipid content of *P. borealis* (Hopkins *et al.*, 1993) were seasonal and related to reproductive state. The samples studied here were collected about two months after the South West Monsoon period, during which extensive upwelling and consequently primary production occurs (Nair *et al.*, 1989). It might therefore be expected that the lipid profiles of *N. gracilis* would reflect the resulting input of organic matter in some manner. An increase in the level of total stored lipid might be expected, but seasonal studies of this species are necessary to reveal whether or not this is the case.

The major storage lipid in *N. gracilis* was triglyceride, which is typical of a crustacean with a benthic lifestyle. Triglyceride levels were within the seasonally varying range reported for *P. borealis* (Hopkins *et al.*, 1993; 40 to 160 mg g dry weight^{-1}) that is a carnivorous-omnivorous feeder. Triglyceride levels were also similar to those reported for *A. markensis* that is thought to be a scavenger and opportunistic feeder (Segonzac *et al.*, 1993), so perhaps storage lipid levels reflect this kind of lifestyle. Levels of triglyceride were higher in *N. gracilis* than in *R. exoculata* and this may reflect either the difference between primary and secondary consumers or the seasonal variability in food supply for *N. gracilis* compared to the relatively constant supply of food at vents.

Unlike *R. exoculata*, in *N. gracilis* there was no variation in triglyceride level with carapace length. This is in line with the argument, that the increase in triglyceride concentration in *R. exoculata* is a consequence of the changeover in lipid storage type, from wax ester to triglyceride. Wax esters were not detected in any of the specimens of *N. gracilis* studied.

Free fatty acid levels were less than 6 % of total lipid, suggesting that the samples were in a relatively good state of preservation (Jeckel *et al.*, 1989), although levels of

lysophosphatidyl choline appear to belie this (See also section 4.4.1 and discussion of fatty acid composition).

Tissue concentrations of sterols were similar to those found in *R. exoculata* and *A. markensis* and at around 5% of total lipid a little lower than those reported for *P. montagui* and *P. borealis* (Clarke, 1979, 8 - 10%; Hopkins *et al.*, 1993, ~10 %). This is probably a reflection of the depth at which *N. gracilis* exists (*P. montagui* is a shallow water species and *P. borealis* was collected at depths of ~ 100 m) and the proportion of its diet that includes unaltered phytoplankton derived material. Fatty acid composition also reflects a lower dietary input of phytoplankton derived material and this feature is discussed in more depth later.

Total polar lipid tissue concentrations of *N. gracilis* were similar to those of *R. exoculata* and *A. markensis.* This concurs with polar lipids comprising mainly structural lipid. The types of polar lipid differed, however. Levels of phosphatidyl choline (PC) in *N. gracilis* were similar to those found in *R. exoculata* and *A. markensis*, while tissue concentrations of lysophosphatidyl choline (LPC) were higher and phosphatidyl ethanolamine (PE) were lower. Assuming that the higher levels of LPC are a result of enzymatic degradation of PC, as mentioned in section 4.4.1, the original proportion of PC was probably actually higher in *N. gracilis* than in vent shrimp and may have comprised as much as 70 % of polar lipid. This is also a higher proportion than reported for *P. montagui* (Clarke, 1979). In the absence of reports of phospholipid composition of deep-sea caridean shrimp, it might be speculated that this is an adaptation to life in the deep sea. However, further studies would be necessary to prove that actual tissue concentrations of PC in *N. gracilis* are this high, as well as to test the hypothesis that PC is more abundant in deep-sea shrimp species.

The five most abundant fatty acids in *N. gracilis* do not include the highly unsaturated fatty acids 20:5 n-3 and 22:6 n-3 considered characteristic of marine organisms, although these fatty acids are present at around 5 % of total fatty acids in *N. gracilis.* Levels of different fatty acid types in *N. gracilis* were generally similar to those reported by Culkin & Morris (1969) for deep-sea caridean shrimp (see table 3.4.2, page 3.30). Saturated fatty acids were present in *N. gracilis* at higher levels than found in

vent shrimp, but at similar levels to those reported in other caridean shrimp (table 3.4.2). Monounsaturated fatty acids comprised around 50 % of the fatty acids of *N. gracilis* studied. The ratio of 18:1 n-7/n-9 fatty acids is low for all *N. gracilis*, indicating a low level of bacterial dietary input. This is perhaps surprising considering the proximity of the shrimp to the oxygen minimum zone and the presumable availability of anaerobic bacteria. This result suggests that the anaerobic bacteria, or organisms that feed on anaerobes, do not form a significant part of the diet of *N. gracilis*.

Levels of polyunsaturated fatty acids were lower than those detected in the shallow water species, *Pandalus montagui* (Clarke, 1979) and larval crangonids (Kattner *et al.*, 1994), but similar to those reported for *Pandalus borealis* (Hopkins *et al.*, 1993) and deep-sea caridean shrimp (Culkin & Morris, 1969; table 3.4.2).

The combination of lower levels of sterols and polyunsaturated fatty acids, neither of which can be synthesised *de novo* by shrimp, (Teshima & Kanazawa, 1983; Goad, 1981) in this species of deep-sea shrimp is probably related to the availability of labile organic matter in the deep sea. Studies of fluxes of particulate organic matter in the deep sea have shown that labile organic material, including polyunsaturated fatty acids, is recycled into shallower water, in the form of zooplankton, eggs and other buoyant particles (Grimalt *et al.*, 1990), while organic matter reaching the deep sea tends to be more refractory and depleted in highly unsaturated fatty acids (Reemstma *et al.*, 1990). Even in anoxic regions of the ocean, particulate organic matter (POM) from deeper waters has been shown to be depleted in highly unsaturated fatty acids (Wakeham, 1995) compared with surface waters, although to a lesser extent than POM from deep oxic water. In addition to this, the reported lack of enhanced preservation of organic matter in sediments under the oxygen minimum zone in the Arabian Sea (Pedersen *et al.*, 1993), supports the suggestion that labile organic matter is scarce in the region where *N. gracilis* is found.

It is perhaps surprising that the lipid profiles of berried female shrimp are similar to those of other *N. gracilis* studied. As caridean shrimp eggs are known to be particularly rich in triglycerides, phosphatidyl ethanolamine and monounsaturated fatty acids

(Herring, 1974; Clarke, 1979; Hopkins *et al.* 1993, Kattner *et al.*, 1994; Section 3.3), it might be expected that higher concentrations of these lipid components would be detected in the tissues of berried female shrimp. It seems likely, however, that the proportion of tissue and of total lipid that comprises eggs was too low to have an effect on the overall lipid profile. It was not possible to separate eggs from the pleopods of the frozen shrimp analysed here. In order to determine egg lipid composition, it would be necessary to remove the eggs after sampling and freeze them for analysis separately.

Chapter 5: Lipid profiles of Juan de Fuca Ridge hydrothermal vent invertebrates

Tables of mean lipid class composition for all Juan de Fuca Ridge invertebrates are presented in Appendix 3. Raw data are stored in files on the appendix CD-ROM; a guide to these files is saved in a readme file: appendix.txt.

5.1 Lipid profiles of the hydrothermal vent vestimentiferan, *Ridgeia piscesae*

Lipid profiles were determined for the anterior and posterior portions of five large specimens of *Ridgeia piscesae* (tubes approximately 10 mm diameter, up to 1.5 m long; total soft tissue dry weights 0.2 to 0.5 g) from the Fairy Castle structure in the High Rise vent field, Juan de Fuca Ridge. The anterior portion included the obturaculum, branchial plume and muscular vestimentum region of the worm, while the posterior portion comprised mainly trophosome, but also contained gonad and opisthosome.

5.1.1 Total lipid and polar lipid profiles of *Ridgeia piscesae*

Total lipid and polar lipid tissue concentrations were calculated as for hydrothermal vent shrimp. Whole organism lipid tissue concentrations (mg g dry weight^{-1}) were calculated by summing the actual amounts of lipid extracted from the anterior and posterior portions of the same specimen (mg) and dividing by the sum of the tissue dry weights (g). Mean total and polar lipid tissue concentrations for the whole organism and anterior and posterior portions are presented in Table 5.1.1

Table 5.1.1 Mean total lipid and polar lipid concentrations for *Ridgeia piscesae* (mean tissue concentrations mg g dry weight^{-1} ± S.E. ($\sigma/\sqrt{n}$))

Tissue	Mean total lipid ± S.E ($\sigma/\sqrt{n}$) (mg g dry weight^{-1})	Mean polar lipid ± S.E ($\sigma/\sqrt{n}$) (mg g dry weight^{-1})
Anterior portion (n [1]= 15)	33.4 ± 1.42	23.3 ± 1.24
Posterior portion (n = 15)	40.0 ± 1.26	24.5 ± 0.95
Whole organism (n = 15)	37.4 ± 0.59	24.2 ± 0.50

[1] n refers to the number of lipid class results used to calculate the sample mean. It does not refer to the number of samples, which is n/3, because each sample was assayed three times.

Mean total lipid tissue concentration is higher in the posterior portion than the anterior portion of the tubeworm (Mann-Whitney Rank Sum test, $P < 0.001$), but there is no significant difference in the polar lipid tissue concentrations of the different tissue types (t-test, 95% confidence, $P = 0.439$).

5.1.2 Lipid class composition of *Ridgeia piscesae*

Mean neutral lipid class tissue concentrations for whole animals, anterior and posterior regions are presented in Figure 5.1.1. Sterols comprised the major neutral lipid in *Ridgeia piscesae* tissue. Storage lipid in the form of triglyceride or wax or sterol ester[2] was not detected in anterior region tissue, but was present at low concentrations in tissue from the posterior region.

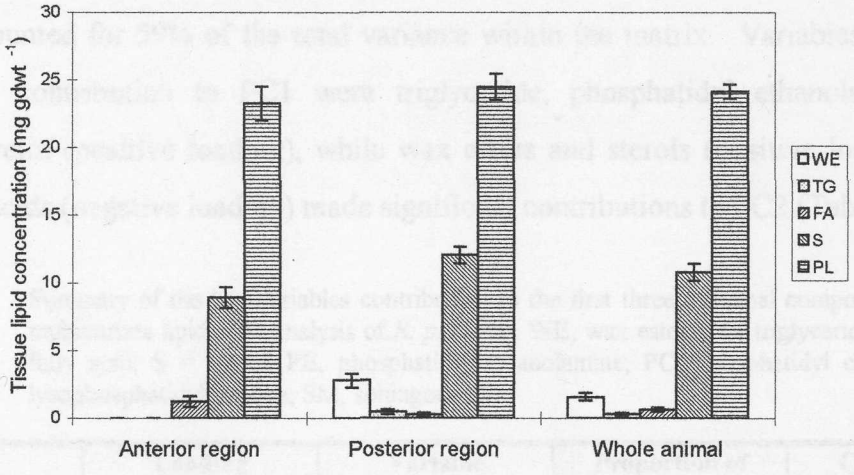


Figure 5.1.1 Bar chart showing the lipid class composition of *R. piscesae*. Error bars indicate standard errors of the mean (S.E. = $\sigma/\sqrt{n}$). WE, wax ester; TG, triglyceride; FA, free fatty acid; S, sterol; PL, polar lipids. Anterior region, n = 15; posterior region, n = 15; whole body, n = 15.

Polar lipids constitute over 60% of tissue lipids in *Ridgeia piscesae*. There was no significant difference in the phospholipid class composition of tissue from anterior and posterior regions (Figure 5.1.2; Wilcoxon Signed Rank Test, $P = 0.791$). Lysophosphatidyl choline was the predominant phospholipid, followed by phosphatidyl choline. Phosphatidyl ethanolamine and sphingomyelin were present at approximately the same levels.

[2] Wax and sterol esters elute in the same position on the Chromarod with the solvent system used here. Fatty alcohols were released during transmethylation of lipid from the posterior region of *Ridgeia piscesae* identifying the lipid class eluting in the wax/sterol ester position here as wax esters.

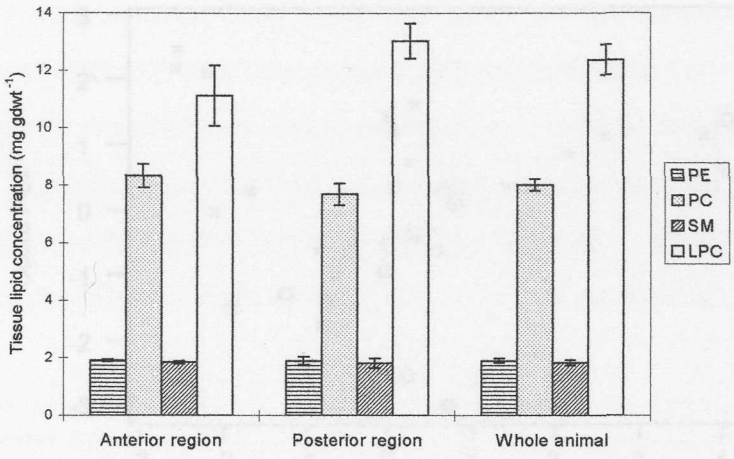


Figure 5.1.2 Bar chart illustrating tissue phospholipid concentrations for *R. piscesae*. Error bars represent standard error of the mean (S.E. = $\sigma/\sqrt{n}$), PE = phosphatidyl ethanolamine; PC = phosphatidyl choline; SM = sphingomyelin; LPC = lysophosphatidyl choline. Anterior region, n = 15; posterior region, n = 15; whole body, n = 15.

Principal component analysis was performed on a correlation matrix of *Ridgeia piscesae* anterior and posterior lipid class tissue concentration. Principal components 1 and 2 accounted for 59% of the total variance within the matrix. Variables making a significant contribution to PC1 were triglyceride, phosphatidyl ethanolamine and sphingomyelin (positive loading), while wax esters and sterols (positive loading) and free fatty acids (negative loading) made significant contributions to PC2 (Table 5.1.2).

Table 5.1.2 Summary of the key variables contributing to the first three principal components from a multivariate lipid class analysis of *R. piscesae*. WE, wax ester; TG, triglyceride; FA = free fatty acid; S = sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; LPC, lysophosphatidyl choline; SM, sphingomyelin.

PC	Loading	Variable	Proportion of variance (%)	Cumulative variance (%)
PC1	+ -	TG, PE, SM	35.1	35.1
PC2	+ -	WE, S FA	23.4	58.5
PC3	+ -	PC WE, FA, LPC	15.7	74.2

Ordination of all the samples on PC1 and PC2 separates anterior and posterior tissues fairly well (Figure 5.1.3), with the separation on the PC2 axis reflecting the elevated concentrations of sterols and wax esters and reduced levels of free fatty acids in the posterior region compared to the anterior region.

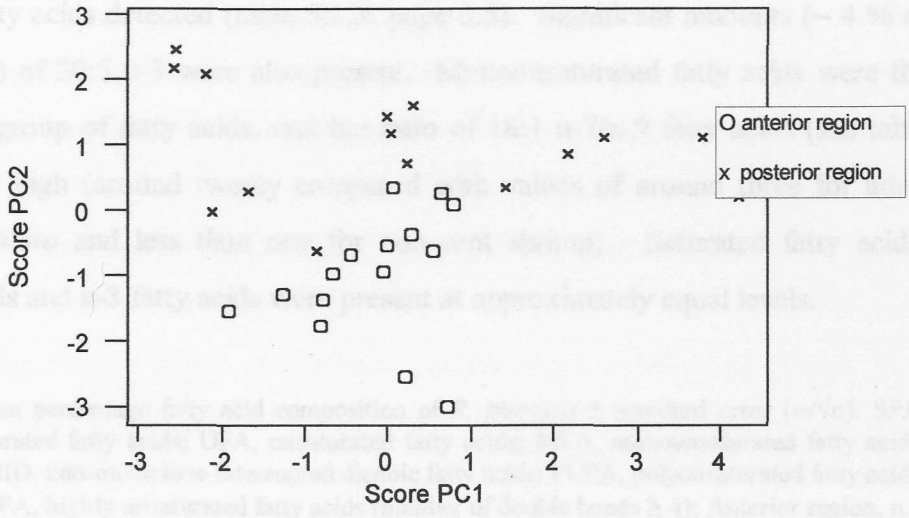


Figure 5.1.3 Ordinations of the scores of variables derived from anterior and posterior tissue lipid class tissue concentration (mg g dry weight^{-1}) of *R. piscesae*

5.1.3 Fatty acid composition of *Ridgeia piscesae*

Mean percentage (by weight) fatty acid composition has been calculated for whole organisms, anterior region and posterior region tissue of *Ridgeia piscesae*. Whole organism fatty acid composition was calculated by summing amounts of fatty acids (mg) for anterior and posterior regions and dividing by the sum of tissue dry weights (g).

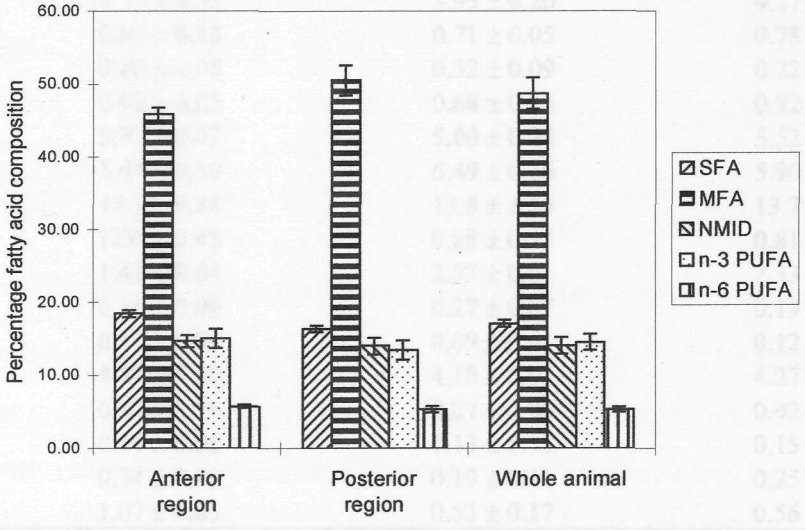


Figure 5.1.4 Bar chart presenting mean percentage fatty acid type composition of *R. piscesae*. SFA, saturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids. Error bars indicate standard errors (SE = $\sigma/\sqrt{n}$). Anterior region, n = 5; posterior region, n = 5; whole body, n = 5.

The fatty acid composition of the tissue from anterior and posterior regions of *Ridgeia piscesae* was similar, with 16:0, 16:1 n-7, 18:1 n-7, 18:4 n-3, 20:1 n-7 and 20:2 Δ5,11[3]

[3] Reminder: Δn,n notation indicates the positions of double bonds in NMID fatty acids where n is the number of carbon atoms from the ω (carboxylate) end of the fatty acid alkyl chain.

as the major fatty acids detected (table 5.1.3, page 5.5). Significant amounts (~ 4 % of total fatty acids) of 20:5 n-3 were also present. Monounsaturated fatty acids were the most abundant group of fatty acids, and the ratio of 18:1 n-7/n-9 fatty acids (see table 5.1.3) was very high (around twenty compared with values of around three for adult *Rimicaris exoculata* and less than one for non-vent shrimp). Saturated fatty acids, NMID fatty acids and n-3 fatty acids were present at approximately equal levels.

Table 5.1.3 Mean percentage fatty acid composition of *R. piscesae* ± standard error ($\sigma/\sqrt{n}$). SFA, saturated fatty acids; UFA, unsaturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids. HUFA, highly unsaturated fatty acids (number of double bonds ≥ 4); Anterior region, n = 5; posterior region, n = 5; whole body, n = 5.

	Anterior region	Posterior region	Whole animal
14:0	0.23 ± 0.04	0.26 ± 0.03	0.24 ± 0.02
14:1	0.37 ± 0.12	0.16 ± 0.04	0.27 ± 0.06
15:0	0.62 ± 0.21	0.39 ± 0.17	0.50 ± 0.19
16:0	13.7 ± 0.40	12.3 ± 0.44	12.8 ± 0.40
16:1 n-9	1.51 ± 0.32	0.86 ± 0.11	1.25 ± 0.28
16:1 n-7	10.8 ± 0.71	15.2 ± 2.40	14.0 ± 2.61
17:0	0.23 ± 0.06	0.21 ± 0.03	0.22 ± 0.03
18:0	3.70 ± 0.82	3.27 ± 0.65	3.34 ± 0.40
18:1 n-9	1.40 ± 0.07	1.34 ± 0.14	1.34 ± 0.04
18:1 n-7	26.1 ± 0.83	26.3 ± 1.46	25.8 ± 0.71
18:2 n-6	4.35 ± 0.33	3.95 ± 0.26	4.17 ± 0.32
18:2 n-3	0.86 ± 0.13	0.71 ± 0.05	0.78 ± 0.10
18:3 n-6	0.20 ± 0.08	0.32 ± 0.09	0.22 ± 0.03
18:3 n-3	0.98 ± 0.25	0.68 ± 0.16	0.92 ± 0.23
18:4 n-3	5.97 ± 0.42	5.00 ± 0.28	5.52 ± 0.49
20:1 n-7	5.44 ± 0.39	6.49 ± 0.56	5.90 ± 0.36
20:2 Δ5,11	14.1 ± 0.84	13.8 ± 1.16	13.7 ± 1.13
20:3 n-6	1.06 ± 0.48	0.88 ± 0.33	0.81 ± 0.39
20:3 n-3	1.41 ± 0.64	2.33 ± 0.61	2.33 ± 0.61
20:4 n-6	0.16 ± 0.04	0.27 ± 0.07	0.19 ± 0.02
22:1 n-11	0.11 ± 0.04	0.09 ± 0.01	0.12 ± 0.03
20:5 n-3	4.47 ± 0.20	4.10 ± 0.93	4.27 ± 0.43
22:2 Δ7,15	0.62 ± 0.14	0.27 ± 0.06	0.42 ± 0.01
24:1	0.14 ± 0.06	0.13 ± 0.03	0.15 ± 0.03
22:5 n-3	0.34 ± 0.29	0.19 ± 0.11	0.25 ± 0.14
22:6 n-3	1.07 ± 0.65	0.53 ± 0.17	0.56 ± 0.18
SFA	18.5 ± 0.51	16.4 ± 0.48	17.1 ± 0.47
MFA	45.9 ± 0.91	50.6 ± 2.08	48.8 ± 2.15
NMID	14.7 ± 0.83	14.1 ± 1.14	14.1 ± 1.14
n-3 PUFA	15.1 ± 1.27	13.5 ± 1.36	14.6 ± 1.10
n-6 PUFA	5.77 ± 0.26	5.41 ± 0.42	5.39 ± 0.37
UFA	81.5 ± 0.51	83.6 ± 0.48	82.9 ± 0.47
HUFA	7.01 ± 0.90	5.76 ± 0.84	6.18 ± 0.35
18:1 n-7/n-9	18.9 ± 1.03	20.8 ± 3.04	19.3 ± 1.10

Fatty alcohols (14:0, 16:0, 16:1 n-9 and 18:1 n-9) were detected in four of the five posterior tissue portions. The most abundant fatty alcohols were 14:0 (46.7 ± 11.7% SE) and 16:0 (38.5 ± 4.41% SE), but total levels were low (table 5.1.4, page 5.6).

Expected FAME and fatty alcohol yields were calculated from lipid class tissue concentrations, as described in Chapter 3 (Section 3.1.2; page 3.7). Actual mean FAME yields of anterior region tissue and whole animals were significantly lower than expected yields (table 5.1.4). Posterior region fatty alcohol yields were also lower than expected, although there was no statistically significant difference in expected and actual FAME yields in posterior region tissue. As for *N. gracilis*, there was no significant difference in the percentage composition of fatty acid groups of individuals with significantly lower FAME yields than those with high FAME yields, so fatty acid

Table 5.1.4 Expected and actual total fatty acid and fatty alcohol yields for *R. piscesae* ± SE (standard error, $\sigma/\sqrt{n}$).

Specimens	Mean ± SE expected FAME tissue conc (mg g dry weight^{-1})	Mean ± SE actual FAME tissue conc (mg g dry weight^{-1})	Statistical test, significant difference? & probability
Anterior region	16.2 ± 0.89	9.91 ± 1.13	t-test, 95% confidence Significant difference $P = 0.0002$
Posterior region	17.5 ± 0.65	11.9 ± 3.48	Wilcoxon Signed Rank No significant difference $P = 0.330$
Whole *R. piscesae*	17.1 ± 0.22	11.4 ± 1.61	Wilcoxon Signed Rank Significant difference $P = 0.0084$
Posterior region fatty alcohols	1.28 ± 0.24	0.25 ± 0.06	t-test, 95% confidence Significant difference $P = 0.0005$

percentage composition of all individuals can confidently be considered to be representative. Table 5.1.5 summarises the results of statistical comparisons of percentage fatty acid type composition in anterior and posterior region tissues.

Table 5.1.5 Summary of the results of statistical comparisons of mean fatty acid type composition of *R. piscesae* anterior and posterior regions (see also table 5.1.3). SFA, saturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted fatty acids; PUFA, polyunsaturated fatty acids.

Fatty acid type	Significant difference?	Statistical test results
SFA	No	t-test, 95% confidence: $P = 0.942$
MFA	No	t-test, 95% confidence: $P = 0.620$
NMID	No	t-test, 95% confidence: $P = 0.864$
n-3 PUFA	No	t-test, 95% confidence: $P = 0.912$
n-6 PUFA	No	t-test, 95% confidence: $P = 1.000$

5.2 Lipid profiles of the hydrothermal vent polychaete, *Paralvinella palmiformis*

Lipid class composition was determined for five whole specimens of *Paralvinella palmiformis* from the Park Place structure (sample # 174 from Marker # 67) and six larger, whole specimens from the Godzilla structure (sample # 296) in the High Rise vent field, Juan de Fuca Ridge. Copley (1998) examined the reproductive development of specimens of *P. palmiformis* from the same submersible grabs: oocytes were present in the coelom of female specimens in the samples from Godzilla and Park Place marker 67; reproductive synchrony was inferred between 63% of the female specimens examined. The size frequency distribution of samples demonstrated that individuals from Godzilla were significantly larger than those from Park Place (Copley, 1998).

5.2.1 Total lipid and polar lipid profiles of *P. palmiformis*

Total lipid and polar lipid tissue concentrations were determined as for hydrothermal vent shrimp and mean data for specimens from the two sites and for pooled specimens are presented in table 5.2.1.

Table 5.2.1 Mean total lipid and polar lipid concentrations for *Paralvinella palmiformis* (mean tissue concentrations mg g dry weight^{-1} ± S.E. ($\sigma/\sqrt{n}$))

Tissue	Mean total lipid ± S.E ($\sigma/\sqrt{n}$) (mg g dry weight^{-1})	Polar lipid ± S.E. ($\sigma/\sqrt{n}$) (mg g dry weight^{-1})
P. palmiformis # 296 from Godzilla (n = 18)	46.8 ± 1.73	20.9 ± 0.88
P. palmiformis # 174 from Park Place (n = 15)	44.4 ± 1.51	23.4 ± 0.86
All *P. palmiformis* (n = 33)	45.7 ± 1.19	22.1 ± 0.66

There was no significant difference in the mean total lipid (t-test, $P = 0.323$) and mean polar lipid (t-test, $P = 0.058$) tissue concentrations of *P. palmiformis* from Park Place and Godzilla. Although statistically significantly different, mean polar lipid tissue concentration was similar to that found in whole *Ridgeia piscesae* (24.2 ± 0.50 mg g dry weight^{-1} SE ($\sigma/\sqrt{n}$); Mann-Whitney Rank Sum test, $P = 0.033$). Total lipid tissue concentration was significantly higher (37.4 ± 0.59 mg g dry weight^{-1} SE ($\sigma/\sqrt{n}$); Mann-Whitney Rank Sum test, $P < 0.001$) than that found in *Ridgeia piscesae*. This suggests that total neutral lipid levels in *P. palmiformis* are higher than those of *Ridgeia piscesae*.

5.2.2 Lipid class composition of *P. palmiformis*

Mean lipid class composition was determined for *P. palmiformis* from Godzilla and Park Place and for all *P. palmiformis* and these data are presented in figure 5.2.1 (page 5.8). Sterols are the major neutral lipid, as is the case in *Ridgeia piscesae*. However, triglycerides are also present at around 5 mg g dry weight^{-1} and low levels of sterol esters[4] were also detected. There were no significant differences (statistical test results summarised in Table 5.2.2) in the sterol ester, free fatty acid and sterol composition of *P. palmiformis* specimens from Park Place and Godzilla vents, but the triglyceride

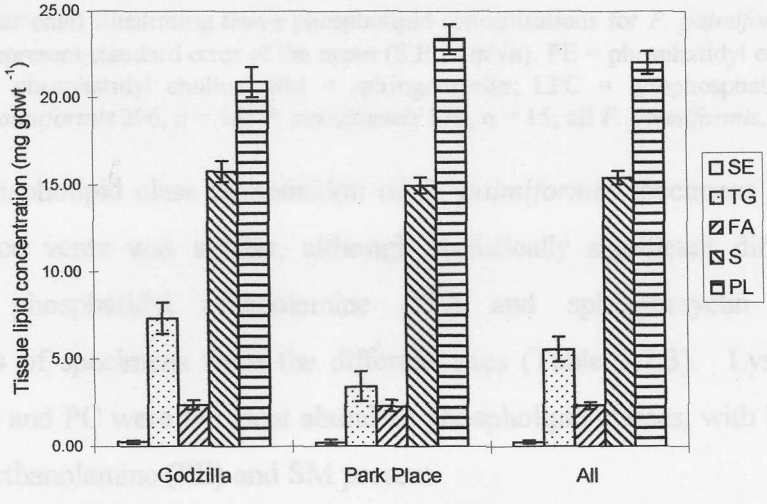


Figure 5.2.1 Bar chart showing the lipid class composition of *P. palmiformis*. Error bars indicate standard errors of the mean (S.E. = $\sigma/\sqrt{n}$). SE, sterol ester; TG, triglyceride; FA, free fatty acid; S, sterol; PL, polar lipids. Godzilla, *P. palmiformis* # 296, n = 18; Park Place, *P. palmiformis* # 176, n = 15; All *P. palmiformis*, n = 33.

Table 5.2.2 Results of statistical comparisons of the neutral lipid class composition (see also appendix 3) of *P. palmiformis* from Godzilla (# 296) and Park Place (# 174) vent structures.

Lipid class	Significant difference?	Statistical test results
Sterol esters	No	Mann-Whitney Rank Sum test: $P = 0.456$
Triglycerides	Yes	Mann-Whitney Rank Sum test: $P = 0.004$
Free fatty acids	No	Mann-Whitney Rank Sum test: $P = 0.957$
Sterols	No	t-test, 95% confidence: $P = 0.309$

tissue concentrations of *P. palmiformis* from Godzilla were significantly higher than those of samples from Park Place.

[4] Fatty alcohols were not detected in the transmethylated lipid of *P. palmiformis*, so it is inferred that sterol esters and not wax esters are the lipid eluting in this position on the Chromarod (See also footnote 2).

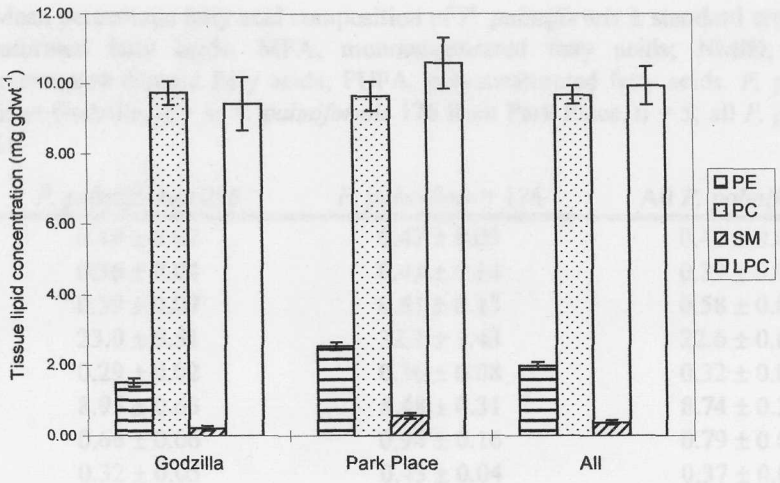


Figure 5.2.2 Bar chart illustrating tissue phospholipid concentrations for *P. palmiformis*. Error bars represent standard error of the mean (S.E. = $\sigma/\sqrt{n}$). PE = phosphatidyl ethanolamine; PC = phosphatidyl choline; SM = sphingomyelin; LPC = lysophosphatidyl choline. *P. palmiformis* 296, n = 18; *P. palmiformis* 176, n = 15; all *P. palmiformis*, n = 33.

The mean phospholipid class composition of *P. palmiformis* specimens from Godzilla and Park Place vents was similar, although statistically significant differences were detected in phosphatidyl ethanolamine (PE) and sphingomyelin (SM) tissue concentrations of specimens from the different sites (Table 5.2.3). Lysophosphatidyl choline (LPC) and PC were the most abundant phospholipid classes, with lower levels of phosphatidyl ethanolamine (PE) and SM present.

Table 5.2.3 Results of statistical comparisons of the phospholipid class composition of *P. palmiformis* from Godzilla (# 296) and Park Place (# 174) vent structures (see also appendix #). PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

Lipid class	Significant difference?	Statistical test results
PE	Yes	Mann-Whitney Rank Sum test: $P < 0.001$
PC	No	t-test, 95% confidence: $P = 0.890$
SM	Yes	Mann-Whitney Rank Sum test: $P = 0.003$
LPC	No	t-test, 95% confidence: $P = 0.288$

5.2.3 Fatty acid composition of *P. palmiformis*

The major fatty acids found in *P. palmiformis* were 16:0, 16:1 n-7, 18:1 n-9, 18:1 n-7 and 18:4 n-3 (Table 5.2.4, page 5.10). Significant amounts of n-3 and NMID fatty acids were also detected. Monounsaturated fatty acids were the most abundant group of fatty acids and the ratio of 18:1 n-7 to 18:1 n-9 was lower than that for *Ridgeia piscesae* and nearer to that for adult *Rimicaris exoculata*. Figure 5.2.3 demonstrates that there was

Table 5.2.4 Mean percentage fatty acid composition of *P. palmiformis* ± standard error ($\sigma/\sqrt{n}$). SFA, saturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids. *P. palmiformis* 296 from Godzilla, n = 6; *P. palmiformis* 176 from Park Place, n = 5; all *P. palmiformis*, n = 11.

	P. palmiformis 296	*P. palmiformis* 176	All *P. palmiformis*
14:0	0.49 ± 0.07	0.47 ± 0.03	0.48 ± 0.04
14:1	0.36 ± 0.04	0.43 ± 0.14	0.39 ± 0.06
15:0	0.39 ± 0.07	0.81 ± 0.13	0.58 ± 0.09
16:0	23.0 ± 0.51	22.1 ± 1.43	22.6 ± 0.68
16:1 n-9	0.29 ± 0.02	0.36 ± 0.08	0.32 ± 0.04
16:1 n-7	8.95 ± 0.63	8.48 ± 0.31	8.74 ± 0.36
16:2 n-6	0.66 ± 0.06	0.94 ± 0.16	0.79 ± 0.09
16:2 n-4	0.32 ± 0.05	0.43 ± 0.04	0.37 ± 0.04
17:0	0.46 ± 0.10	0.14 ± 0.04	0.31 ± 0.08
17:1	0.18 ± 0.03	0.28 ± 0.06	0.23 ± 0.03
18:0	3.39 ± 0.17	3.53 ± 0.18	3.45 ± 0.12
18:1 trans-9	2.69 ± 0.36	3.02 ± 0.17	2.84 ± 0.21
18:1 n-9	4.96 ± 0.14	4.71 ± 0.39	4.85 ± 0.19
18:1 n-7	21.8 ± 1.20	18.9 ± 1.65	20.5 ± 1.05
18:2 n-6	1.36 ± 0.09	1.71 ± 0.13	1.52 ± 0.09
18:2 n-4	2.04 ± 0.13	2.03 ± 0.28	2.03 ± 0.14
18:2 n-3	4.24 ± 0.43	2.27 ± 0.61	3.34 ± 0.46
18:3 n-6	0.46 ± 0.09	0.53 ± 0.30	0.49 ± 0.14
18:3 n-3	0.17 ± 0.03	0.23 ± 0.11	0.20 ± 0.05
18:4 n-3	5.32 ± 0.44	6.31 ± 0.40	5.77 ± 0.33
20:1 n-9	0.96 ± 0.13	1.31 ± 0.22	1.12 ± 0.13
20:1 n-7	0.50 ± 0.28	0.43 ± 0.21	0.47 ± 0.17
20:2 Δ5,11	0.67 ± 0.10	1.28 ± 0.13	0.95 ± 0.12
20:2 Δ5,13	0.68 ± 0.10	0.94 ± 0.08	0.80 ± 0.07
20:3 n-9	0.77 ± 0.06	0.82 ± 0.14	0.80 ± 0.07
20:3 n-7	0.27 ± 0.04	0.30 ± 0.04	0.29 ± 0.03
20:3 n-6	0.43 ± 0.04	0.42 ± 0.06	0.43 ± 0.03
20:3 n-3	0.58 ± 0.05	0.92 ± 0.27	0.73 ± 0.13
20:4 n-6	1.83 ± 0.22	2.40 ± 0.14	2.09 ± 0.16
20:4 n-3	0.56 ± 0.09	0.82 ± 0.10	0.68 ± 0.08
22:1 n-11	0.24 ± 0.07	0.59 ± 0.23	0.40 ± 0.12
20:5 n-3	3.27 ± 0.74	3.66 ± 0.59	3.44 ± 0.46
22:2 Δ7,13	3.01 ± 0.18	3.42 ± 0.08	3.20 ± 0.12
22:2 Δ7,15	1.83 ± 0.24	2.23 ± 0.21	2.01 ± 0.17
22:3 n-3	0.73 ± 0.10	0.96 ± 0.10	0.83 ± 0.08
22:5 n-3	1.16 ± 0.43	1.13 ± 0.26	1.15 ± 0.25
22:6 n-3	0.96 ± 0.25	0.70 ± 0.21	0.84 ± 0.17
SFA	27.8 ± 0.50	27.1 ± 1.47	27.4 ± 0.69
MFA	40.9 ± 1.53	38.5 ± 2.12	39.8 ± 1.26
NMID	6.19 ± 0.45	7.87 ± 0.25	6.95 ± 0.37
n-4 PUFA	2.35 ± 0.17	2.45 ± 0.25	2.40 ± 0.14
n-3 PUFA	17.0 ± 1.16	17.0 ± 0.83	17.0 ± 0.70
n-6 PUFA	4.74 ± 0.33	6.00 ± 0.48	5.31 ± 0.33
HUFA	13.1 ± 1.25	15.0 ± 0.65	14.0 ± 0.77
18:1n-7/n-9	4.41 ± 0.27	4.08 ± 0.38	4.26 ± 0.22

little difference in the fatty acid type composition of *P. palmiformis* from Godzilla and Park Place.

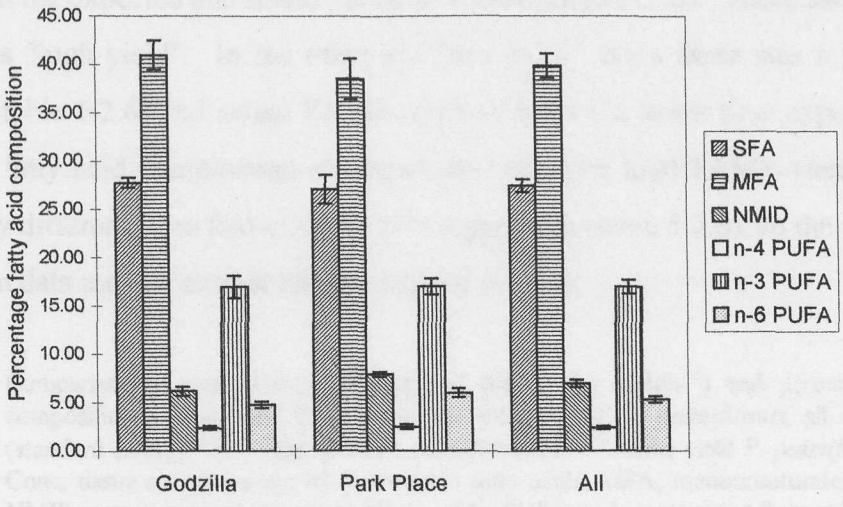


Figure 5.2.3 Bar chart presenting mean percentage fatty acid type composition of *P. palmiformis*. SFA, saturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids. Error bars indicate standard errors ($SE = \sigma/\sqrt{n}$). *P. palmiformis* 296 from Godzilla, n = 6; *P. palmiformis* 176 from Park Place, n = 5; all *P. palmiformis*, n = 11.

The most abundant polyunsaturates were n-3 PUFA, including the highly unsaturated fatty acid 20:5 n-3. Two types each of 20:2 and 22:2 NMID fatty acids were detected. There was a significantly higher percentage of saturated fatty acids in all *P. palmiformis* than in whole *Ridgeia piscesae* (statistics summarised in table 5.2.5) and lower proportions of both monounsaturates and NMID fatty acids. There was no significant difference in the levels of n-3 and n-6 PUFA in specimens of the two species, but n-4 fatty acids were detected in *P. palmiformis* and not in *Ridgeia piscesae*. *P. palmiformis* also contained significantly higher levels of HUFA than *Ridgeia piscesae*.

Table 5.2.5 Summary of the results of statistical comparisons of mean fatty acid type composition of *P. palmiformis* (table 5.2.4, p5.5) and whole *R. piscesae* (table 5.1.3, p5.10). SFA, saturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids.

Fatty acid type	Significant difference?	Statistical test results
SFA	Yes	t-test, 95% confidence: $P < 0.0001$
MFA	Yes	t-test, 95% confidence: $P = 0.002$
NMID	Yes	t-test, 95% confidence: $P < 0.0001$
n-3 PUFA	No	t-test, 95% confidence: $P = 0.083$
n-6 PUFA	No	t-test, 95% confidence: $P = 0.891$
HUFA	Yes	t-test, 95% confidence: $P < 0.0001$

FAME tissue concentration data are not presented here because there were discrepancies in some of the observed and expected FAME yields calculated, as described in chapter 3 (Section 3.1.2; page 3.7). In five out of the eleven lipid samples (two from Godzilla and three from Park Place), a paired t-test showed no significant

difference in the expected and actual yields of FAME (table 5.2.6). These samples were described as "high yield". In the other six "low yield" cases there was a significant difference (table 5.2.6) and actual FAME yields were much lower than expected. The percentage fatty acid composition of specimens with low total FAME yields was not significantly different from that of those with high yields (table 5.2.6), so the percentage composition data may be confidently considered reliable.

Table 5.2.6 Comparison of total tissue concentration (mg g dry weight^{-1}) and percentage FAME composition of high yield FAME and low yield FAME *P. palmiformis*, all quoted ± SE (standard error, $\sigma/\sqrt{n}$). High yield *P. palmiformis*, n = 5; Low yield *P. palmiformis*, n = 6. Conc, tissue concentration; SFA saturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted fatty acids; PUFA, polyunsaturated fatty acids; statistical tests: Wilcoxon signed rank test; Mann-Whitney Rank Sum test, t-test (95% confidence).

	High yield *P. palmiformis*	Low yield *P. palmiformis*	Statistical test	Significant difference? & probability
Expected FAME conc	23.4 ± 1.54	22.7 ± 1.14	paired t-test	No, $P = 0.327$
Actual FAME conc	22.6 ± 1.72	5.01 ± 0.50	Wilcoxon	Yes, $P < 0.0001$
% SFA	23.8 ± 3.62	27.4 ± 1.27	Mann-Whitney	No, $P = 0.329$
% MFA	33.2 ± 4.46	40.3 ± 1.74	t-test	No, $P = 0.143$
% NMID	5.77 ± 1.14	7.40 ± 0.38	t-test	No, $P = 0.175$
% n-3 PUFA	2.02 ± 0.38	2.47 ± 0.20	t-test	No, $P = 0.307$
% n-4 PUFA	15.7 ± 2.89	16.6 ± 0.81	t-test	No, $P = 0.762$
% n-6 PUFA	5.15 ± 1.01	4.93 ± 0.37	t-test	No, $P = 0.831$

5.3. Lipid profiles of the hydrothermal vent clam, *Calyptogena pacifica*

Lipid profiles were determined for mantle, gill and foot portions of five specimens of *Calyptogena pacifica* from the Clam Bed site, south of the High Rise vent field, Juan de Fuca Ridge. Gill tissue and its lipid extracts were a dark reddish brown colour. The lipid extracts from one each of the foot and mantle samples were also tinged brown, suggesting that gill tissue may have contaminated these samples.

5.3.1 Total lipid and polar lipid profiles of *Calyptogena pacifica*

Total lipid and polar lipid concentrations were calculated as for hydrothermal vent shrimp. Whole organism tissue concentrations (mg g dry weight^{-1}) were calculated by summing actual amounts of lipid (mg) extracted from foot, mantle and gill sections of the same specimen and dividing by the sum of the tissue dry weights (g). Mean total

lipid and polar lipid concentrations for each tissue type and for whole specimens are presented in table 5.3.1.

Table 5.3.1 Mean total lipid and polar lipid concentrations for *Calyptogena pacifica* (mean tissue concentrations mg g dry weight^{-1} ± S.E. ($\sigma/\sqrt{n}$))

Tissue	Mean total lipid ± S.E ($\sigma/\sqrt{n}$) (mg g dry weight^{-1})	Polar lipid ± S.E ($\sigma/\sqrt{n}$) (mg g dry weight^{-1})
Foot (n = 15)	27.2 ±1.18	22.2 ± 1.15
Gill (n = 15)	48.4 ± 3.01	29.0 ± 2.42
Mantle (n = 15)	56.7 ± 4.38	47.2 ± 3.51
Whole organism (n = 15)	47.8 ± 1.62	32.7 ± 0.96

The highest mean total lipid tissue concentrations were found in gill and mantle tissues, that contained almost twice as much lipid as foot tissue. Mantle tissue contained the highest polar lipid tissue concentration. Mantle and foot tissues comprised over 80 % polar lipid, while gill tissue comprised around 60% polar lipid.

5.3.2 Lipid class composition of *Calyptogena pacifica*

Mean lipid class composition was determined for the separate tissue types and for the whole organism and these data are presented in figures 5.3.1 and 5.3.3 (page 5.12).

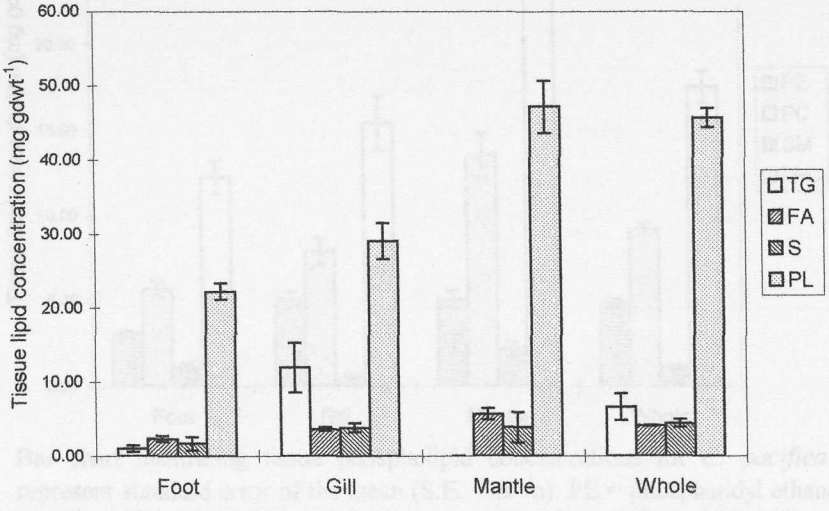


Figure 5.3.1 Bar chart showing the neutral lipid class composition of *C. pacifica*. Error bars indicate standard errors of the mean (S.E. = $\sigma/\sqrt{n}$). TG, triglyceride; FA, free fatty acid; S, sterol; PL, polar lipids. Foot, n = 15; gill, n = 15; mantle, n = 15; whole organism, n = 15.

Wax or sterol esters were not detected in *C. pacifica*. Gill tissue contained significantly more triglyceride than foot tissue (Mann-Whitney Rank Sum test, $P < 0.0001$) and triglyceride was not detected in mantle tissue. There was a large variance in

triglyceride levels in gill tissue and Figure 5.3.2 illustrates the reason for this: triglyceride levels were very high in gill tissue of specimens two and five, but low in the

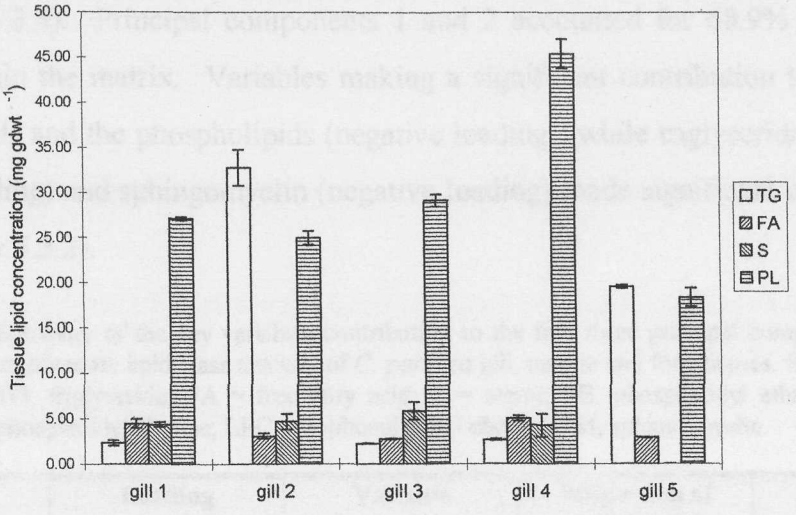


Figure 5.3.2 Bar chart illustrating tissue lipid concentrations for *C. pacifica* gills. Error bars represent standard error of the mean (S.E. = $\sigma/\sqrt{n}$). TG, triglyceride; FA, free fatty acid; S, sterol; PL, polar lipid. Gill 1-5, n = 3.

other three specimens. The lack of sterols in the gill tissue of specimen five was probably a function of method detection limits, as discussed earlier.

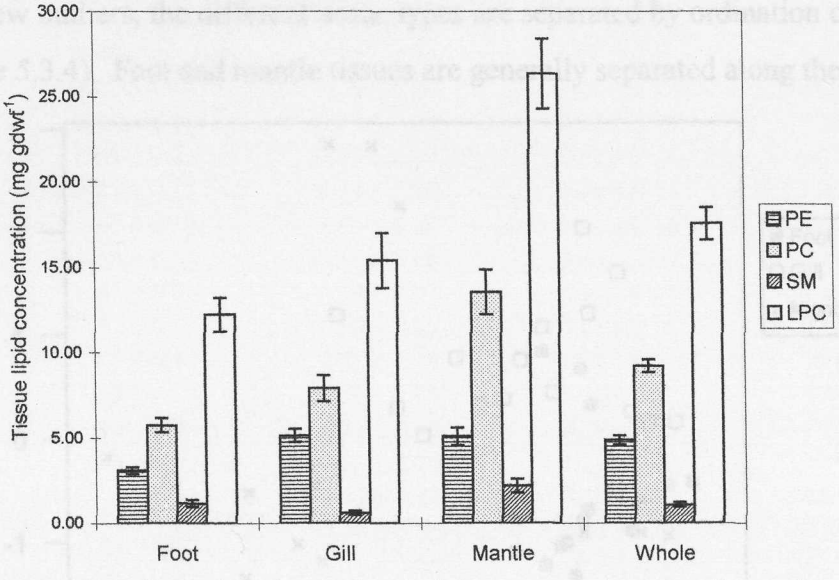


Figure 5.3.3 Bar chart illustrating tissue phospholipid concentrations for *C. pacifica*. Error bars represent standard error of the mean (S.E. = $\sigma/\sqrt{n}$). PE = phosphatidyl ethanolamine; PC = phosphatidyl choline; SM = sphingomyelin; LPC = lysophosphatidyl choline. Foot, n = 15; gill, n = 15; mantle, n = 15; whole organism, n = 15.

The most abundant phospholipid detected in *C. pacifica* tissues was lysophosphatidyl choline (LPC), followed by phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE). Sphingomyelin (SM) was also present at low levels.

Principal component analysis was performed on a correlation matrix of the lipid class tissue concentration of foot, gill and mantle tissues of *C. pacifica* as described for vent shrimp (page 3.5). Principal components 1 and 2 accounted for 68.9% of the total variance within the matrix. Variables making a significant contribution to PC1 were free fatty acids and the phospholipids (negative loading), while triglyceride and sterols (positive loading) and sphingomyelin (negative loading) made significant contributions to PC2 (Table 5.3.2).

Table 5.3.2 Summary of the key variables contributing to the first three principal components from a multivariate lipid class analysis of *C. pacifica* gill, mantle and foot tissues. SE, sterol ester; TG, triglyceride; FA = free fatty acid; S = sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; LPC, lysophosphatidyl choline; SM, sphingomyelin.

PC	Loading	Variable	Proportion of variance (%)	Cumulative variance (%)
PC1	+	-	50.9	50.9
	-	FA, PE, PC, SM, LPC		
PC2	+	TG, S	18.0	68.9
	-	SM		
PC3	+	S	13.5	82.4
	-	TG		

Despite a few outliers, the different tissue types are separated by ordination on PC1 and PC2 (Figure 5.3.4). Foot and mantle tissues are generally separated along the PC1 axis,

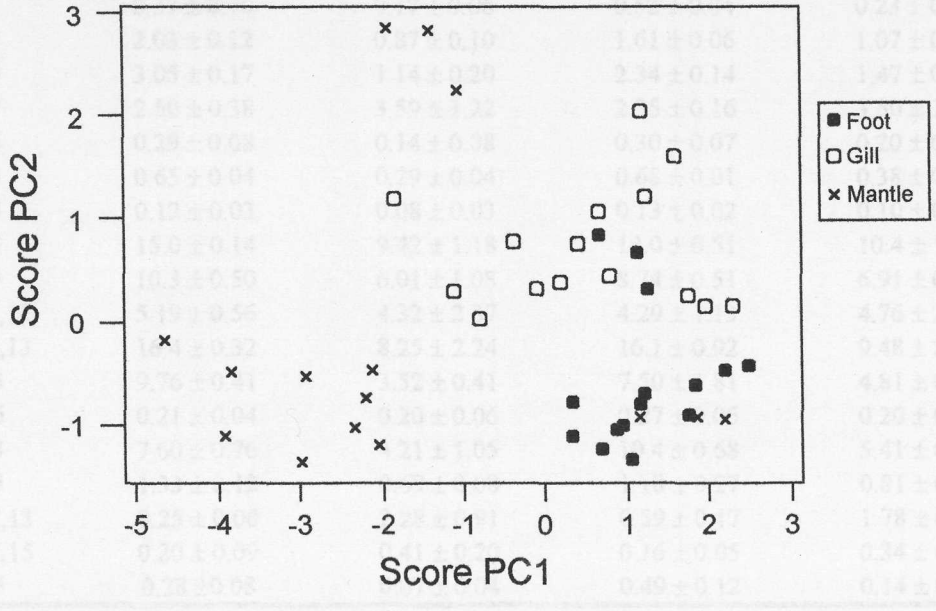


Figure 5.3.4 Ordinations of the scores of variables derived from gill, mantle and foot lipid class tissue concentration (mg g dry weight^{-1}) of *C. pacifica*

according to differences in their phospholipid (structural lipid) composition. Gill tissue is generally separated from the foot and mantle tissues on PC2, reflecting higher levels

of neutral lipid compared to the other tissue types. Exceptions to these trends are the two mantle samples (three replicates each) where the points ordinate nearer gill and foot tissue samples and one foot sample, that appears nearer gill samples.

5.3.3 Fatty acid composition of *Calyptogena pacifica*

Mean percentage fatty acid composition (by weight) has been calculated for foot, gill, and mantle tissues and for whole *C. pacifica* (Table 5.3.3, page 5.16). Whole organism composition was calculated by summing amounts of fatty acids (mg) for foot, gill and mantle regions and dividing by the sum of tissue dry weights (g). The major fatty acids

Table 5.3.3 Mean percentage fatty acid composition of *C. pacifica* ± standard error ($\sigma/\sqrt{n}$). SFA, saturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids (≥ 4 double bonds). Foot, n = 15; gill, n = 15; mantle, n = 15; whole organism, n = 15.

	Foot	Gill	Mantle	Whole
14:0	1.35 ± 0.08	4.13 ± 0.72	1.49 ± 0.13	3.34 ±0.44
14:1	0.62 ± 0.09	0.95 ± 0.28	0.43 ± 0.04	0.78 ± 0.17
15:0	0.77 ± 0.12	0.38 ± 0.09	0.77 ± 0.18	0.47 ± 0.08
16:0	7.00 ± 0.56	7.54 ± 1.39	6.97 ± 0.22	7.55 ± 1.12
16:1 n-7	14.7 ± 0.32	41.4 ± 2.52	18.7 ± 0.92	35.8 ± 2.96
17:0	0.37 ± 0.10	0.17 ± 0.06	0.52 ± 0.04	0.23 ± 0.05
18:0	2.03 ± 0.12	0.87 ± 0.10	1.61 ± 0.06	1.07 ± 0.09
18:1 n-9	3.05 ± 0.17	1.14 ± 0.20	2.34 ± 0.14	1.47 ± 0.13
18:1 n-7	2.50 ± 0.38	3.59 ± 1.22	2.35 ± 0.16	3.50 ± 1.00
18:2 n-6	0.29 ± 0.08	0.14 ± 0.08	0.30 ± 0.07	0.20 ± 0.07
18:2 n-3	0.65 ± 0.04	0.29 ± 0.04	0.68 ± 0.01	0.38 ± 0.04
18:3 n-3	0.12 ± 0.03	0.08 ± 0.03	0.13 ± 0.02	0.10 ± 0.02
18:4 n-3	15.0 ± 0.14	9.42 ± 1.18	14.0 ± 0.51	10.4 ± 1.12
20:1 n-9	10.3 ± 0.50	6.01 ± 1.08	8.74 ± 0.51	6.91 ± 0.75
20:2 Δ5,11	5.19 ± 0.56	4.32 ± 2.37	4.29 ± 1.13	4.76 ± 2.12
20:2 Δ5,13	16.4 ± 0.32	8.25 ± 2.24	16.1 ± 0.92	9.48 ± 2.19
20:3 n-3	9.76 ± 0.41	3.52 ± 0.41	7.50 ± 1.81	4.81 ± 0.61
20:4 n-6	0.21 ± 0.04	0.20 ± 0.06	0.27 ± 0.06	0.20 ± 0.05
20:4 n-3	7.60 ± 0.76	4.21 ± 1.05	10.4 ± 0.68	5.41 ± 0.96
20:5 n-3	1.33 ± 0.12	0.69 ± 0.08	1.18 ± 0.27	0.81 ± 0.09
22:2 Δ7,13	0.25 ± 0.06	2.28 ± 0.91	0.59 ± 0.17	1.78 ± 0.65
22:2 Δ7,15	0.20 ± 0.09	0.41 ± 0.20	0.16 ± 0.05	0.34 ± 0.16
22:6 n-3	0.28 ±0.08	0.07 ± 0.04	0.49 ± 0.12	0.14 ± 0.06
SFA	11.5 ± 0.64	13.1 ± 0.78	11.4 ± 0.11	12.7 ± 0.70
MFA	31.1 ± 1.19	53.0 ± 3.88	32.5 ± 1.57	48.5 ± 4.04
NMID	22.1 ± 0.41	15.3 ± 2.59	21.2 ± 1.57	16.4 ± 2.27
n-3 PUFA	34.8 ± 1.02	18.3 ± 2.50	34.4 ± 1.91	22.1 ± 2.59
n-6 PUFA	0.50 ± 0.08	0.34 ± 0.11	0.57 ± 0.09	0.40 ± 0.10
HUFA	24.5 ± 0.65	14.6 ± 2.15	26.3 ± 0.39	17.0 ± 2.06
18:1 n-7/n-9	0.81 ± 0.09	3.33 ± 0.94	1.01 ± 0.06	2.46 ± 0.71

in *C. pacifica* were 16:0, 16:1 n-7, 18:4 n-3, 20:1 n-9, 20:2 Δ5,13, 20:3 n-3 and 20:4 n-3. The fatty acid profiles of mantle and foot tissue were similar but differed from that of gill tissue (Figure 5.3.5, p5.17). Saturated fatty acids comprised around 12% of tissue lipids in all cases. Monounsaturated fatty acids constituted the major fatty acid group in gill tissue and the 18:1 n-7/n-9 ratio was highest in this tissue at 3.3 ± 0.9. The ratio of 18:1 n-7/n-9 fatty acids was lowest for foot tissue at 0.8 ± 0.1. NMID fatty acids and n-3 PUFA comprised over 50% of the fatty acids in foot and mantle tissue. A lower proportion of these fatty acids was also present in gill tissue. Trace amounts of n-6 fatty acids were detected in all tissue types.

Mean expected and actual FAME yields were calculated as described in Chapter 3 (section 3.1.2, page 3.7) and are presented in table 5.3.4. There was a significant difference in the mean observed and expected yield of FAME in mantle tissue, but not in foot tissue, gill tissue or whole organism. Where mean FAME yields were significantly lower than expected (mantle tissue), there were several tissue samples with FAME yields nearer the expected yield and some with much lower yields. Comparison

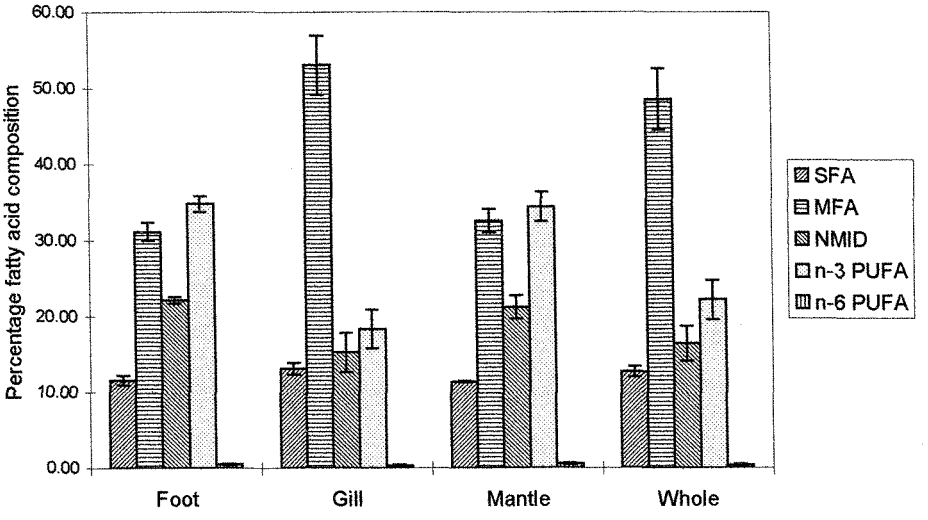


Figure 5.3.5 Bar chart presenting mean percentage fatty acid type composition of *C. pacifica*. SFA, saturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids. Error bars indicate standard errors (SE = $\sigma/\sqrt{n}$). Foot, n = 15; gill, n = 15; mantle, n = 15; whole organism, n = 15.

of the proportions of FAME in high and low yield samples revealed no significant differences (Table 5.3.5; except for MFA: this difference is probably a function of gill contamination, that is reflected in the lipid class composition of one of the "high yield" mantle samples) so as with other species, percentage fatty acid composition has been considered with confidence for all samples.

Table 5.3.4 Expected and actual total FAME yields for *C. pacifica* ± SE (standard error, $\sigma/\sqrt{n}$) mg g dry weight^{-1}; statistical tests: Wilcoxon Signed Rank test; paired t-test, 95% confidence.

	Mean ± SE expected FAME tissue conc	Mean ± SE actual FAME tissue conc	Statistical test	Significant difference? & probability
Foot	17.7 ± 0.56	16.9 ± 2.92	paired t-test	No, P = 0.816
Gill	34.8 ± 2.82	44.9 ± 11.4	paired t-test	No, P = 0.293
Mantle	36.0 ± 2.92	14.3 ± 3.29	Wilcoxon	Yes, P < 0.0001
Whole	32.2 ± 1.75	30.5 ± 6.96	Wilcoxon	No, P = 0.198

Table 5.3.5 Comparison of percentage FAME composition of high yield FAME and low yield FAME *C. pacifica* mantle tissue all quoted ± SE (standard error, $\sigma/\sqrt{n}$). High yield mantle, n = 2; Low yield mantle, n = 3. Conc, tissue concentration; SFA saturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted fatty acids; PUFA, polyunsaturated fatty acids; statistical tests: Wilcoxon Signed Rank test; Mann-Whitney Rank Sum test, t-test and paired t-test (95% confidence).

	High yield mantle	Low yield mantle	Statistical test	Significant difference? & probability
Expected FAME conc	43.7 ± 2.32	30.9 ± 3.79	paired t-test	Yes, P = 0.0002
Actual FAME conc	29.2 ± 1.21	4.40 ± 0.34	paired t-test	Yes, P < 0.0001
% SFA	11.4 ± 0.16	11.3 ± 0.18	t-test	No, P = 0.877
% MFA	28.9 ± 0.40	35.0 ± 0.89	t-test	Yes, P = 0.014
% NMID	22.2 ± 0.37	20.5 ± 2.76	Mann-Whitney	No, P = 0.800
% n-3 PUFA	37.0 ± 0.44	32.7 ± 2.91	t-test	No, P = 0.339
% n-6 PUFA	0.62 ± 0.17	0.53 ± 0.13	t-test	No, P = 0.692

5.4 Discussion of lipid profiles of Juan de Fuca Ridge invertebrates

5.4.1 Potential errors in the lipid profiles of Juan de Fuca Ridge invertebrates

There were relatively high levels of lysophosphatidyl choline (LPC) in all samples of invertebrates from the Juan de Fuca Ridge. As has already been mentioned, this phospholipid can be indicative of post mortem enzymatic degradation of phosphatidyl choline (PC), which may occur if samples are not stored at a low enough temperature after collection. Juan de Fuca Ridge samples were stored in a -20°C freezer directly after collection, that might have allowed sample degradation. They were also transported, by a courier, in dry ice from the USA to the UK. On arrival the samples appeared still to be frozen, but little dry ice remained in the packaging, so it is possible that sample degradation occurred at this point.

Levels of free fatty acids were low (< 6 %) in all Juan de Fuca Ridge invertebrate samples, so if PC had degraded to LPC, then the fatty acids released were lost, probably through auto-oxidation of polyunsaturated fatty acids (PUFA). The possible loss of PUFA through auto-oxidation of free fatty acids released by degradation of PC may be estimated, assuming that LPC should be present in tissues at around 10 % of polar lipid (a conservative estimate, but similar to that found for well preserved *Rimicaris exoculata*). This calculation has been explained for *N. gracilis* (Section 4.4.1) and is summarised in equations 5.1 and 5.2.

$$n = (\text{Observed LPC} - \text{Expected LPC}) \times \text{LPC factor}$$

Equation 5.1 Calculation of tissue concentration of possible lost free fatty acids (n), where LPC factor is the amount of fatty acid methyl esters released per amount of LPC, calculated as 0.57; see also equation 3.1, page 3.7.

$$\text{Percentage PUFA} = \frac{n + \text{total PUFA tissue concentration}}{n + \text{total fatty acid tissue concentration}} \times 100$$

Equation 5.2 Calculation of possible percentage PUFA, accounting for lost free fatty acids

Levels of LPC thought to have been produced by enzymatic degradation of PC, possible percentage PUFA, and actual percentage PUFA for *Ridgeia piscesae*, *P. palmiformis* and *C. pacifica* are summarised in table 5.4.1.

Table 5.4.1 Estimated possible percentage PUFA, accounting for that which might have been lost by auto-oxidation of free fatty acids released by enzymatic degradation of LPC.

Samples	Excess LPC (mg g dry weight-1)	Possible PUFA (percentage total FAME)	Reported PUFA (percentage total FAME)
Whole R. piscesae	9.7	50 %	34 %
All P. palmiformis	7.8	34 %	22 %
Whole C. pacifica	14.3	38 %	51 %

In summary, up to 16 % of polyunsaturated fatty acids may have been lost as a result of auto-oxidation of free fatty acids. This means that relative levels of fatty acids should be treated with some caution.

Low yields of fatty acid methyl esters, restricting discussion of fatty acid methyl ester tissue concentrations have been discussed previously (section 3.4.1, page 3.25). Again, although fatty acid tissue concentrations may not be meaningfully considered, results indicated no significant difference in the percentage fatty acid composition of specimens with low yields of FAME and specimens with FAME yields that were not significantly different than expected yields. Consequently, percentage results have been considered unaffected by FAME yield levels.

5.4.2 Discussion of the lipid profiles of *Ridgeia piscesae*

The total lipid tissue concentrations of *Ridgeia piscesae* tissues from the High Rise vent field, reported here, were in the same region as those reported by Fullarton *et al.* (1995a) for *Ridgeia piscesae* from the Main Endeavour Field, Juan de Fuca Ridge. However, Fullarton *et al.* (1995a) suggested that polar lipids comprised over 90 % of the total lipid in *Ridgeia piscesae*, whereas polar lipids comprised a maximum of around 70 % of total lipid in the tissues examined here. Posterior region tissue comprised as little as 60 % polar lipid and contained considerable amounts of wax esters and sterols.

Principal component analysis of the lipid class composition of *Ridgeia piscesae* tissues highlighted the difference in the composition of anterior and posterior region tissues, reflecting the higher concentrations of wax esters and triglycerides in posterior region tissue and higher levels of free fatty acids in anterior region tissue.

Energy storage lipids present in posterior region tissue and not present in anterior region tissue were probably stored in gonad tissue in the posterior region. Wax ester-rich eggs have been reported in *Lamellibrachia*, a cold seep vestimentiferan (Craig Young, Harbour Branch Oceanographic Institution, pers. comm.) and Cary *et al.* (1989) reported lipid rich droplets in the oocytes of the giant tubeworm, *Riftia pachyptila*. It seems likely, therefore, that the wax esters detected here indicate the presence of oocytes or mature eggs in posterior region tissues. Dissection of gonad tissue from trophosome tissue in the posterior region would be extremely difficult in *Ridgeia piscesae*. Histology of sections of posterior region tissue of *Ridgeia piscesae*, staining to reveal stored lipid, might be an alternative method of determining whether gonad tissue comprises the only lipid store.

Compound-specific stable isotope analysis of the sterols in the giant tubeworm, *Riftia pachyptila*, suggested that most of the sterols were synthesised *de novo* by the worm, although some sterols with a phytoplanktonic stable isotope signature were also detected (Rieley *et al.*, 1995). The high levels of sterols detected in *Ridgeia piscesae* seem therefore most likely to have been synthesised by the worm *de novo*. However,

compound-specific stable isotope analysis of sterols in *Ridgeia piscesae* would be necessary to confirm this hypothesis.

Polar lipids comprised mainly phosphatidyl choline (PC) and lysophosphatidyl choline (LPC). The presence of high levels of LPC has been discussed in section 5.4.1. It is possible that the high LPC levels indicate, in part, a high level of metabolic activity that might be expected in tissue containing symbiotic sulphur-oxidising bacteria. However, it seems likely that most of the LPC is a product of *post mortem* enzymatic degradation of PC and that PC would have been the major polar lipid in the tissues of *Ridgeia piscesae*.

There were no significant differences in the fatty acid compositions of anterior and posterior region tissues of *Ridgeia piscesae*. This is perhaps surprising, given the large proportion of bacteria in posterior region tissue, but bacteria were almost certainly also abundant on the branchial plume surfaces of anterior region tissue. Moreover, the fatty acid composition of all tissues of *Ridgeia piscesae* is governed by its principal fatty acid source, that is endosymbiotic bacteria. The three principal fatty acids detected in *Ridgeia piscesae* from the High Rise Vent Field (16:0, 16:1 n-7 and 18:1 n-7) were the same as those identified by Fullarton *et al.* (1995a) in *Ridgeia piscesae* from the Main Endeavour Field and those in the giant tubeworm, *Riftia pachyptila*, from the East Pacific Rise (Rieley *et al.*, 1995). Levels of total saturated, monounsaturated and n-6 polyunsaturated fatty acids were similar to those reported for *Ridgeia piscesae* by Fullarton *et al.* (1995a). However, non-methylene interrupted dienoic (NMID) fatty acids comprised a larger proportion of fatty acids than was reported by Fullarton *et al.* (1995a) and levels of n-3 PUFA were lower than previously reported. This variation might be a result of different conditions at the two different vent sites. However, given the limited number of comparable samples analysed by Fullarton *et al.* (1995a; one trunk and one vestimentum region of adult *Ridgeia piscesae*) and the likelihood of loss of PUFA through auto-oxidation in this study, discussion of this variation in fatty acid composition is speculative at best.

The general fatty acid profile of *Ridgeia piscesae* was markedly different than that reported for the giant tubeworm, *Riftia pachyptila*, from the East Pacific Rise (Rieley *et*

al., 1995). *Riftia pachyptila* comprised over 70 % MUFA and only 3 to 8 % PUFA that were mainly NMID fatty acids. It was suggested that *Riftia pachyptila* derived none of its adult energy requirements from a non-chemoautotrophic energy source. The presence of significant quantities of n-3 PUFA in *Ridgeia piscesae* suggests that this is not the case here.

The ratio of 18:1 n-7/n-9 fatty acids in the tissue of *Ridgeia piscesae* is very high at around 20. This is much higher than was found in *Ridgeia piscesae* from the main Endeavour vent field (Fullarton *et al.*, 1995a; ~ 3 - 6 in adults) and somewhat lower than found in *Riftia pachyptila* from 9°N East Pacific Rise (Rieley *et al.*, 1995; ~ 30 to 40). This concurs with the hypothesis that *Ridgeia piscesae* from the High Rise vent field rely less on a bacterial source of nutrition than *Riftia pachyptila*, but more than *Ridgeia piscesae* from the main Endeavour Field, although again, conclusions are limited by sample sizes in the previous studies. The ratio of 18:1 n-7/n-9 fatty acids for *Ridgeia piscesae* is higher than this ratio for *P. palmiformis* or for *C. pacifica* from the same vent field, reflecting the greater degree of reliance on bacterial symbiosis in *Ridgeia piscesae* compared with other vent organisms.

NMID fatty acids have already been mentioned as possible substitutes for n-3 and n-6 PUFA in the absence of photosynthetically derived precursors required for the production of n-3 and n-6 PUFA (Section 3.4.2) and are common in organisms harbouring symbiotic bacteria (Conway & McDowell Capuzzo, 1991; Ben-Mlih *et al.*, 1992; Fullarton *et al.*, 1995a). The NMID fatty acids detected have been suggested to be derived from chain elongation and (Δ5) desaturation of 16:1 n-7 and 18:1 n-9 fatty acid precursors (Zhukova, 1991; Fullarton *et al.*, 1995a; Figure 1.10, page 1.16).

Compound-specific stable isotope analyses of *Ridgeia piscesae* fatty acids (Pond *et al.*, in preparation) suggest that n-3 PUFA in *Ridgeia piscesae* might be derived from photosynthetically produced fatty acids. Adult *Ridgeia piscesae* possess no gut, so any photosynthetically derived fatty acids in their tissues must either have been assimilated at a juvenile stage, when a gut was present (Southward, 1988), and preserved in adult tissues, or have been actively taken up in the form of dissolved organic matter (DOM).

There were slightly higher levels of free fatty acids in the anterior region tissue than in posterior region tissue that might indicate uptake of fatty acids in the form of DOM in the plume region of the worm (this region has a large surface area in direct contact with seawater). Marine oligochaete and echiuran worms have been shown to be able to accumulate and assimilate free fatty acids dissolved in seawater across their body walls (Testermann, 1972) and the uptake of palmitic acid and other dissolved organic compounds has been demonstrated in pogonophoran worms (Southward & Southward, 1970; Southward & Southward, 1980). It is, therefore, likely that vestimentiferans are able to absorb free fatty acids dissolved in seawater. A problem with this method of fatty acid accumulation is the availability of n-3 PUFA in seawater. Polyunsaturated fatty acids are very susceptible to auto-oxidation in oxic conditions and are present only at small concentrations in particulate organic matter in deep water, even in areas of anoxia (Wakeham, 1995). However, Saliot & Marty (1988) have reported elevated concentrations of dissolved fatty acids, including PUFA, in water collected at hydrothermal vents on the East Pacific Rise from above colonies of alvinellid and vestimentiferan worms. It is therefore conceivable that accumulation of n-3 PUFA occurs by direct absorption of DOM from the water around vents, although it is not certain that this could account for all of the n-3 and n-6 PUFA in adult *Ridgeia piscesae*.

In the previous study of the fatty acid composition of *Ridgeia piscesae*, Fullarton *et al.* (1995a) also examined the fatty acid composition of three portions of tissue from juvenile *Ridgeia piscesae*. Juvenile tissue contained less NMID fatty acids than adult tissue, but there were wide and inconsistent variations in the levels of n-3 and n-6 fatty acids in the different tissue types. It has been suggested that *Ridgeia piscesae* supports lecithotrophic development (Southward *et al.*, 1996; Goodson *et al.*, in sub.), but it also seems likely that a trochophore larval stage of *Ridgeia piscesae* feeds on particulate organic matter (Southward *et al.*, 1988). Phytoplankton derived fatty acids might be accumulated at this life stage. Assuming that NMID fatty acids are produced as substitutes for n-3 and n-6 PUFA, the low levels of NMID found in juvenile compared with adult *Ridgeia piscesae*, suggest that juveniles possess sufficient n-3 and n-6 PUFA not to require NMID fatty acids.

The method by which *Ridgeia piscesae* maintain highly unsaturated fatty acids (HUFA) in their tissues remains uncertain. The evidence so far points to the conservation of HUFA from a juvenile stage, but cannot rule out the possibility of direct absorption and assimilation of free fatty acids from water around the tubeworms.

5.4.3 Discussion of the lipid profiles of *P. palmiformis*

Total lipid tissue concentration in *P. palmiformis* was similar to that found for the shallow water polychaete *Nereis virens* (Lemieux *et al.*, 1997; ~ 40 - 50 mg g dry weight^{-1} assuming a wet weight/dry weight ratio of 4:1). Significant levels of triglyceride were found in all *P. palmiformis* examined, but higher tissue concentrations were found in the specimens from the Godzilla vent than in those from the Park Place vent. The specimens from Godzilla were also larger than those from Park Place. It has been suggested that specimens collected from the Park Place vent were in a more marginal habitat for *P. palmiformis* than those collected at the Godzilla vent (Copley, 1998) and consequently Park Place *P. palmiformis* bear higher energy maintenance costs for their size than those at Godzilla. This concurs with a model of successional colonisation of vent structures at Juan de Fuca Ridge hydrothermal vents where *P. palmiformis* can exist over a range of environmental conditions, but certain conditions favour optimal growth and reproduction (Sarrazin *et al.*, 1997). *P. palmiformis* from Godzilla have been able to store more energy in the form of triglyceride than those at Park Place, suggesting that Godzilla *P. palmiformis* exist in more favourable conditions than those at Park Place. Another difference in the lipid class composition of *P. palmiformis* from Godzilla and Park Place is that levels of phosphatidyl ethanolamine (PE) and sphingomyelin (SM) are higher in specimens from Park Place than in those from Godzilla. This may also be a reflection of the condition of the worms in different micro-environments at the two vents within the vent field.

Sterols constituted the major lipid class in *P. palmiformis*. Compound-specific stable isotope analysis would be necessary to determine whether these sterols were derived from phytoplankton or synthesised by the worms *de novo*.

Polar lipids comprised mainly phosphatidyl choline (PC) and lysophosphatidyl choline (LPC). The presence of large amounts of lysophosphatidyl choline has already been discussed, and it seems likely that PC would have been the major lipid in *P. palmiformis*.

The major fatty acids detected in *P. palmiformis* include both bacterial biomarkers, 18:1 n-7 and phytoplankton biomarkers, 18:1 n-9 and 18:4 n-3. It was difficult to compare the fatty acid profiles of *P. palmiformis* examined here with those reported by Taghon (1988), because the previous study dealt only with phospholipid fatty acids. Similar fatty acids were found in *P. palmiformis* studied here, but in different proportions than reported by Taghon (1988). There were no apparent differences in the fatty acid compositions of *P. palmiformis* from Godzilla and Park Place vents.

The most abundant fatty acids detected in *P. palmiformis* were monounsaturated fatty acids. The mean ratio of 18:1 n-7/n-9 fatty acids in all *P. palmiformis* reflects a reliance on a chemoautotrophic bacterial source of nutrition and supports the assertion that *P. palmiformis* is a primary consumer of vent bacteria (McHugh, 1989; Desbruyères & Laubier, 1991). *P. palmiformis* were also found to contain two other types of fatty acids that can be considered to be indicators of a bacterial source of nutrition. Significant amounts of non-methylene interrupted fatty acids and small amounts of n-4 dienoic fatty acids, also found in *Rimicaris exoculata* from the Mid-Atlantic Ridge, were detected in *P. palmiformis*. NMID fatty acids have already been suggested to be possible substitutes for n-3 and n-6 PUFA, and are thought to be synthesised from 16:1 n-7 and 18:1 n-7 fatty acid precursors (Fullarton *et al.*, 1995a). 16:2 n-4 has been detected in diatoms (Volkman *et al.*, 1989), that are known to form part of the diet of *P. palmiformis* (McHugh, 1989). However, it has been suggested that n-4 fatty acids in *Rimicaris exoculata* were produced from bacterially derived n-7 monounsaturates. Compound-specific stable isotope analyses would confirm whether n-4 fatty acids in *P. palmiformis* have been synthesised from chemoautotrophically or phototrophically derived precursors.

Significant amounts of n-3 PUFA were detected in *P. palmiformis*, in accordance with evidence that a proportion of the nutrition of these worms is ultimately phytoplankton derived. The stomach contents of *P. palmiformis* have been found to include

diatomaceous debris as well as bacteria (McHugh, 1989). In addition, 20:5 n-3 has been found in bacterial samples (especially bacterial mats) scraped off chimneys at Juan de Fuca Ridge Endeavour segment hydrothermal vents (Hedrick *et al.*, 1992). Although deep-sea species of the *Vibrio* genus of bacteria have been reported to produce 20:5 n-3 (Delong & Yayanos, 1986), it seems likely that the 20:5 n-3 detected in the bacterial samples analysed by Hedrick *et al.* (1992) was a result of phototrophically derived POM contamination. So the fatty acid profile of *P. palmiformis* supports the suggestion that it is a deposit feeder that consumes POM that comprises mainly chemoautotrophic bacteria (Desbruyères & Laubier, 1991), but also some phototrophically derived organic matter. Chemoautotrophic bacteria comprise a more abundant source of organic matter at hydrothermal vents than phototrophically derived organic matter. Consequently, the lipid profiles of *P. palmiformis* reflect the bacterial source of nutrition more strongly than their phototrophically derived dietary source of organic matter.

5.4.4 Discussion of the lipid profiles of *C. pacifica*

Glycogen is generally considered to be the major energy store in bivalves (Holland, 1978), but lipids are also an important energy reserve, especially during periods of nutrient insufficiency (Beninger & Lucas, 1984). Total lipid and lipid class composition have not previously been reported for vesicomyid clams, so the total lipid and lipid class profiles of *C. pacifica* can only be compared with lipids reported for shallow water clams. The total lipid of two clam species, *Tapes decussatus* and *Tapes philippinarum* ranged from 58 to 80 mg g dry weight^{-1}, varying seasonally with lowest levels in Winter and highest levels prior to spawning (Beninger & Lucas, 1984). In a more recent study, Ferreira & Vale (1998) reported total lipid levels of around 65 mg g dry weight^{-1} for the clam *Ruditapes decussatus*, but lower levels in animals that had been exposed to stress in the form of polychlorinated biphenols (PCBs). Total lipid levels found in *C. pacifica* (48 ± 1.6 mg g dry weight^{-1}) were lower than the normal range of total lipids reported for these shallow water species.

The highest total lipid tissue concentrations were found in the gill and mantle tissue of *C. pacifica*. Lipid constituted mainly polar lipids in all *C. pacifica* tissues, perhaps reflecting the reduced requirement of lipid for energy storage in bivalves (Holland, 1978). The principal component analysis (PCA) of different tissues of *C. pacifica* separated most of the tissue samples well, on the basis of their different lipid class compositions. Removal of all traces of gill tissue from other tissues during dissection was difficult, so contamination was probably responsible for the ordination of one mantle and one foot sample near to gill samples.

Gill tissue contained more triglyceride than foot or mantle tissue, suggesting that this tissue is used for energy storage. The concentration of triglyceride in gill tissue varied widely in different *C. pacifica* individuals. This is probably an indication of the condition of the individual clams. Elevated lipid may be an indication of reproductive maturity, or simply of good condition of an individual in a favourable position within the clam bed, with access to all required nutrients. The gill tissue lipids of symbiont-bearing bathymodiolid mussels from south-west Pacific hydrothermal vents have been reported to be composed of around 20 % triglyceride (Pranal *et al.*, 1997). It therefore seems likely that energy gained from symbiotic intracellular bacteria is stored in the form of triglyceride in the gills of hydrothermal vent bivalves. The exact mechanisms by which energy from bacterial symbionts is converted to energy for the host in vesicomyid clams have yet to be elucidated (Fisher, 1990). One possible mechanism involving the digestion of bacterial symbionts by the host has been reported in *Bathymodiolus thermophilus* (Fiala-Médioni *et al.*, 1986). It is possible that neutral lipids might be formed from the hydrolysis of phospholipids during degradation of bacterial membranes (Pranal *et al.*, 1997).

Fatty acid profiles of foot and mantle tissue were similar, but differed from that of gill tissue. Gill tissue contained a higher proportion of monounsaturated fatty acids and consequently lower proportion of n-3 PUFA and NMID fatty acids than foot and mantle tissue. The ratio of 18:1 n-7/n-9 fatty acids, that can be used as a measure of the extent to which bacterial fatty acids contribute to a tissue, was also highest in gill tissue. These differences in fatty acid profiles are a result of the high concentration of bacteria in specially modified cells in the gills of *C. pacifica*, that have been reported in the gills

of vesicomyid clams in general (Nelson & Fisher, 1995). Bacteria comprise a significant proportion of the dry weight of gill tissue, so their fatty acids naturally comprise a large proportion of gill tissue fatty acids. A bacterial fatty acid signature is also present in foot and mantle tissue, but it is diluted by other clam tissue lipids.

All vesicomyid clams contain intracellular sulphur-oxidising bacteria in the gills, but also have limited filter-feeding ability and a reduced digestive tract and stomach (Nelson & Fisher, 1995). Diatom remains have been reported in the digestive tract of vesicomyids from the Japan subduction zone (LePennec & Fiala-Médioni, 1988) and it has been suggested that filter-feeding ability allows the supply of essential nutrients not provided by symbiotic bacteria such as the HUFA, 20:5 n-3 and 22:6 n-3 (Nelson & Fisher, 1995). It has also been suggested that vesicomyid clams transmit symbiotic bacteria to their offspring at the primary oocyte stage of development (Endow & Ohta, 1990).

Vesicomyid clams are thought to produce lecithotrophic larvae (Lutz *et al.*, 1984) and if these larvae have no planktotrophic stage in their development then they have no other means of obtaining required phototrophically produced nutrients than filter feeding as adults. All *C. pacifica* tissues examined contained significant amounts of n-3 PUFA that are indicative of an ultimately phototrophic source of organic matter. Compound-specific stable isotope studies are required to establish that these n-3 fatty acids are phototrophically derived, but as chemoautotrophically derived n-3 fatty acids have yet to be discovered, it seems likely that this is the case. This suggests that the *C. pacifica* does use its limited filter feeding system to supplement its symbiotic nutrition system.

The other unsaturated fatty acids present in *C. pacifica* include non-methylene interrupted fatty acids (NMID), that are widespread in bivalves with symbiotic bacteria (Conway & McDowell Capuzzo, 1991; Ben-Mlih *et al.*, 1992; Zhukova *et al.*, 1992; Fullarton *et al.*, 1995a). As has been noted above (section 5.4.2; 5.4.3), these fatty acids are thought to be synthesised by animals deficient in phototrophically derived 20:5 n-3 and 22:6 n-3 or their precursors (Zhukova, 1991; Fullarton *et al.*, 1995a). Ben Mlih *et al.* (1992) reported the fatty acid composition of *C. magnifica* from Galapagos hydrothermal vents. The major polyunsaturated fatty acids detected were 20:2 and 20:3

fatty acids (positions of unsaturation unidentified, but 20:2 were probably NMID fatty acids) and 20:4 (not n-6) fatty acids. The highly unsaturated phytoplankton biomarker fatty acids 20:5 n-3 and 22:6 n-3 were not detected. A similar polyunsaturated fatty acid profile was found in *C. pacifica*. The highly unsaturated fatty acids 20:5 n-3 and 22:6 n-3 were detected at low concentrations in *C. pacifica*, but major polyunsaturated fatty acids were 20:2 NMIDs and 18:4 n-3, 20:3 n-3, and 20:4 n-3. Low levels of the HUFA, 20:5 n-3 and 22:6 n-3, were also found in bathymodiolid mussels from hydrothermal vents on the Mid-Atlantic Ridge (Pranal *et al.*, 1997; Pond *et al.*, 1998). High levels of 18:4 n-3 and 20:3 n-3 were not reported in studies of bathymodiolid fatty acids however (Pranal *et al.*, 1997; Pond *et al.*, 1998).

C. pacifica contains only low levels of n-6 PUFA. This has also been reported in *C. magnifica*, the giant clam from the Galapagos vents, (Ben Mlih *et al.*, 1992) and in bathymodiolid mussels from the East Pacific Rise, the Mid-Atlantic Ridge and south-western Pacific hydrothermal vents (Ben Mlih *et al.*, 1992; Pranal *et al.*, 1997; Pond *et al.*, 1998). This deficiency might be a result of the reduced availability of these fatty acids in the deep sea. However, n-6 PUFA do comprise a significant proportion of the fatty acids of other vent organisms (see sections 5.1.3 and 5.2.3), so there is perhaps another reason for this lack of n-6 PUFA in vent bivalves.

Fatty acid biomarkers of both thiotrophic and methanotrophic bacteria were found in the gills of bathymodiolid mussels from the Mid-Atlantic Ridge (Pond *et al.*, 1998). Although methanotrophic bacteria were reported in vesicomyids from the Juan de Fuca Ridge (Gal'chenko *et al.*, 1988), the presence of methanotrophic symbionts in vesicomyid clams has not yet been demonstrated (Nelson & Fisher, 1995) and stable isotope studies of vesicomyids from the western Pacific (Saino & Ohta, 1989; Fiala-Médioni *et al.*, 1993) do not suggest that organic carbon derived from methanotrophic bacteria is a major source of nutrition for vent and seep clams. The absence of n-8 fatty acids, that have been used as biomarkers for methanotrophs in previous studies (Jahnke *et al.*, 1995; Pond *et al.*, 1998), in *C. pacifica* supports the hypothesis that methanotrophs do not form a significant part of their nutrition.

5.4.5 Comparison of the lipid class profiles of hydrothermal vent invertebrates in this study

Certain aspects of lipid profiles, such as levels of total lipid and lipid class composition, may be phylogenetically constrained, so care must be taken when comparing the lipid profiles of organisms from different phyla. However, fatty acid profiles depend largely on sources of organic matter, so the fatty acid profiles of different organisms may be compared with a view to relating their trophic ecology.

The percentage fatty acid composition of different types of fatty acids and the ratio of 18:1 n-7/n-9 fatty acids for some of the hydrothermal vent organisms studied in this thesis are compared in table 5.4.2.

Table 5.4.2 Mean percentage fatty acid type composition and 18:1 n-7/n-9 ratios for all whole *R. exoculata* from TAG, whole *Ridgeia piscesae*, *P. palmiformis* and *C. pacifica* from the Juan de Fuca Ridge. MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids; standard errors excluded for clarity.

Species	% MFA	% NMID	% n-3 PUFA	% n-4 PUFA	% n-6 PUFA	18:1 n-7/n-9
Rimicaris exoculata	42.5	1.72	8.45	29.9	2.50	3.34
Ridgeia piscesae	48.8	14.1	14.6	-	5.39	19.3
Paralvinella palmiformis	39.8	6.95	17.0	2.40	5.31	4.26
Calyptogena pacifica	48.5	16.4	22.1	-	0.40	2.46

It is perhaps unsurprising that the highest proportions of monounsaturated fatty acids were found in organisms with endosymbiotic bacteria: the vestimentiferan tubeworm, *Ridgeia piscesae* and the vesicomyid clam, *C. pacifica*. Levels of NMID fatty acids were also highest in these two organisms, reflecting their reliance on a bacterial source of nutrition. The very high 18:1 n-7/n-9 ratio found in *Ridgeia piscesae* reflects the extreme degree of symbiosis known from physiological studies of this and other vestimentiferan tubeworms (reviewed in Fisher, 1990). The much lower ratio of 18:1 n-7/n-9 for *C. pacifica* was, therefore, surprising considering the extent of endo-symbiosis known in vesicomyid clams (reviewed in Fisher, 1990). Along with relatively high levels of n-3 PUFA in *C. pacifica*, the 18:1 n-7/n-9 ratio seems to suggest that *C. pacifica* is more reliant on external inputs of phototrophically derived organic matter than previously thought. However, both n-3 and n-6 PUFA may be biomarkers

of phototrophically derived organic matter and there is little difference in the sum of n-3 and n-6 PUFA in *R. piscesae* and *C. pacifica*, suggesting that *C. pacifica* does not rely significantly more on phototrophically derived organic matter than *R. piscesae*. *C. pacifica* may either derive fatty acids from different quality organic matter (poor in n-6 PUFA) or selectively assimilate n-3 PUFA.

The mean ratio of 18:1 n-7/n-9 fatty acids in the palm worm *Paralvinella palmiformis* was similar to that of the Mid-Atlantic vent shrimp *Rimicaris exoculata*, and a lot lower than that of the tubeworm *Ridgeia piscesae*, reflecting a lesser degree of reliance on a bacterial source of nutrition in *Rimicaris exoculata* and *Paralvinella palmiformis* than in *Ridgeia piscesae*. The $\delta^{15}N$ values of *Rimicaris exoculata* and *Paralvinella grasslei*, a species of paralvinellid polychaete that occupies a similar niche to *P. palmiformis* at the geographically distinct hydrothermal vent sites on the East Pacific Rise, indicate that these species occupy a similar trophic level at hydrothermal vents (Van Dover & Fry, 1989). Similar ratios of 18:1 n-7/n-9 fatty acids appear to support the hypothesis that *P. palmiformis* and *Rimicaris exoculata* occupy a similar trophic niche at hydrothermal vents in that they exhibit a similar degree of nutritional reliance on chemoautotrophic bacteria. It was also interesting that n-4 PUFA were detected in both *P. palmiformis* and *R. exoculata*, although at very different proportions of total fatty acids. It would be interesting to discover whether the n-4 fatty acids detected in *P. palmiformis* were also derived ultimately from diatoms or, as in the case of *R. exoculata*, from an ultimately chemosynthetic source of organic carbon (Pond *et al.*, 1997b). Although similar groups of fatty acids were found in *R. exoculata* and *P. palmiformis*, the relative proportions of polyunsaturated fatty acids were very different. The major PUFA class in *R. exoculata* was n-4 PUFA, while the PUFA in *P. palmiformis* comprised much higher proportions of n-3 PUFA and NMID fatty acids. The difference in levels of n-3 PUFA might be related to the availability of labile organic carbon at the different hydrothermal vent sites from which *R. exoculata* and *P. palmiformis* were collected. *R. exoculata* were collected from the TAG hydrothermal vent site at a depth of around 3600 m, while *P. palmiformis* were collected from Juan de Fuca Ridge Endeavour Segment vents at a depth of approximately 2200 m. The TAG hydrothermal vent field is situated in the North Atlantic subtropical gyral province, with seasonally varying primary production rates of between 5 and 15 g

carbon m^{-2} $month^{-1}$, while the Juan de Fuca Ridge vents are on the edge of the California current province, with primary production rates of 15 to 45 g carbon m^{-2} $month^{-1}$ (Longhurst, 1998). Consequently, labile organic carbon is probably more scarce at TAG than at Juan de Fuca Ridge vents. Further speculation as to the reasons for differences in the type of PUFA in *P. palmiformis* and *R. exoculata* is restricted by the possibility that in addition to likely differences in the quality of organic matter input, there may be phylogenetically constrained differences in biosynthetic abilities of each species to produce NMID and n-4 PUFA.

6: Conclusions and suggestions for future research

6.1 Experimental methods - conclusions

The lipid extraction techniques used here are well tried and tested, but required attention to detail in order that they worked efficiently. Thin Layer Chromatography with Flame Ionisation Detection (TLC-FID) analysis of lipid class composition was time-consuming to calibrate, but once calibrated, returned a lot of information about a sample in a relatively short time. The problems with TLC-FID detection limits in this study might be overcome by using larger amounts of tissue, or more concentrated lipid solutions. TLC-FID accuracy might be improved by hydrogenation of samples before analysis, although this would be time consuming. It would perhaps be quicker, and no less accurate, to use thin layer chromatography with scanning densitometry for the determination of lipid class composition, given suitable equipment.

If the problem with FAME yields described in chapters 3, 4 and 5 was owing to incomplete derivatisation of lipids, then it might be easily avoided by using a free fatty acid internal standard, instead of a fatty acid methyl ester. This would account for inefficiency in the derivatisation procedure and allow confident presentation and discussion of fatty acid tissue concentrations. However, it seems more likely that the apparent FAME yield problem was caused by small inaccuracies in lipid levels used in calculations that were multiplied up and became large inaccuracies.

6.2 Lipid profiles of hydrothermal vent shrimp - conclusions

Lipid profiles of whole hydrothermal vent shrimp supported a life history pattern where postlarval shrimp lead a pelagic existence, as evidenced by wax ester storage lipid, and are reliant on a photosynthetic source of nutrition, that is substantiated by the presence of phytoplankton biomarker fatty acids. Adult shrimp are distinguished by their use of triglycerides as storage lipids, reflecting a benthic lifestyle and a fatty acid profile that verifies a primarily chemoautotrophic bacterial source of nutrition.

The lipid analysis of different tissues of hydrothermal vent shrimp revealed that in juveniles wax ester storage lipid was stored mainly in the abdomen region, while adults stored the majority of their triglyceride storage lipid in hepatopancreas and ovary tissue. Branchial area tissues were characterised by elevated levels of bacterial fatty acids compared with abdomen tissue, reflecting the colonies of bacteria found on and around the gills of *R. exoculata*. Ovary and hepatopancreas tissue lipid composition varied with reproductive state. Total lipid tissue concentration in ovary and hepatopancreas tissue generally increased with maturity. Triglycerides, phosphatidyl choline and phosphatidyl ethanolamine were the principal lipid classes responsible for this increase. The fatty acid profile of ovary and hepatopancreas tissue also changed with maturity. The proportion of n-3 fatty acids increased and the proportion of n-4 fatty acids decreased with advancing reproductive state. It is uncertain whether this was simply a result of the increased proportion of phospholipids in mature ovary and hepatopancreas tissue, or a reflection of the requirement of n-3 fatty acids in reproductive development.

The lipid profile of *A. markensis* supported a different proposed feeding regime to that of *R. exoculata*. Elevated levels of triglyceride compared with *R. exoculata* might suggest a less reliable food source for *A. markensis*. Although bacterial fatty acid biomarkers were present, *A. markensis* fatty acid profiles reflected a higher dietary input of photosynthetically-derived organic matter than was found in adult *R. exoculata*, and concurred with the hypothesis that *A. markensis* is an opportunistic feeder, scavenging vent shrimp, or deep-sea organisms with a phototrophic lipid signature as well as perhaps bacteria and other particulate organic matter.

6.3 Lipid profiles of *N. gracilis* - conclusions

The major storage lipid in *N. gracilis* was triglyceride, supporting the hypothesis that this species exists in benthic regions. Neutral lipid levels were commensurate with *N. gracilis* being an opportunistic feeder. Fatty acid composition was typical of an organism with a diet based on an ultimately photosynthetic source of organic carbon, but also reflected the reduction in the availability of labile organic carbon (in the case of lipid, highly unsaturated fatty acids) in the deep sea.

6.4 Lipid profiles of Juan de Fuca Ridge invertebrates - conclusions

The lipid profile of the tubeworm, *Ridgeia piscesae*, reflected the extreme degree of reliance on symbiotic bacteria of this species, both in the low levels of storage lipid present and in the high ratio of 18:1 n-7/n-9 bacterial to non-bacterial fatty acids. Wax esters detected in the trunk region of some specimens of *Ridgeia piscesae* might reflect the presence of oocytes in the gonad.

In addition to bacterial biomarker fatty acids, certain fatty acid biomarkers that are consistent with photosynthetic primary production were present in significant amounts in the tissues of *Ridgeia piscesae.* These fatty acids may be a nutritional requirement for *Ridgeia piscesae.* The mechanism of assimilation of photosynthetically-derived fatty acids by *Ridgeia piscesae* is uncertain, but it seems likely that they are either absorbed directly from sea water or perhaps preserved in the tissues from a juvenile life cycle stage.

The lipid profiles of the palm worm, *Paralvinella palmiformis* collected from different areas within a hydrothermal vent field reflected small scale variations in environmental conditions. Higher tissue lipid concentrations of triglyceride were associated with more favourable environmental conditions. The fatty acid profiles of *P. palmiformis* were consistent with a mixed diet of chemoautotrophic bacteria and phototrophically derived organic matter. The types of fatty acids found in *P. palmiformis* were similar to those found in the vent shrimp *Rimicaris exoculata.* Along with similar 18:1 n-7/n-9 ratios, this supports the idea that these two species occupy a similar trophic niche at North Eastern Pacific and Mid-Atlantic vents.

Wide variations in the triglyceride levels of individual hydrothermal vent clams, *Calyptogena pacifica*, probably also reflected small scale variations in environmental conditions. High concentrations of triglyceride found in gill tissue, concurred with the theory that symbiotic bacterial phospholipids are hydrolysed and converted to storage lipids by the host clam. The 18:1 n-7/n-9 ratio of *C. pacifica* gill tissue reflected the high concentrations of bacteria in this tissue type. Fatty acid profiles of foot and mantle tissue reflected a lesser contribution of bacterial fatty acids to the total fatty acids in

these tissues. In addition to bacterial fatty acids, significant levels of n-3 fatty acids were found that implied a potential photosynthetic input of organic matter. These fatty acids may be a nutritional requirement for *C. pacifica* and it seems likely that the reduced filter-feeding ability of these clams allows the assimilation of external organic matter to fulfil this requirement. Biomarker fatty acids characteristic of methanotrophs were not found in *C. pacifica*, suggesting that it relies purely on thiotrophic symbionts.

6.5 Suggestions for further work

Further questions have arisen during this work that were outside the scope of this thesis. The following suggestions are just a few of the possibilities for further lipid analyses to help elucidate life history strategies, trophic ecology and the fate of organic matter in the systems described here.

The analysis of the fatty acid composition of different lipid classes would be interesting. It might be expected that the polar, membrane lipid composition of vent organisms would reflect the temperature of the water in which they exist, as was shown for *P. palmiformis* (Taghon, 1988). For example, *R. exoculata* inhabits areas of the Mid-Atlantic hydrothermal vents near to the black smokers, while *Alvinocaris markensis* is a more peripheral species. The membrane lipids of *Rimicaris exoculata* might therefore be expected to contain lower levels of unsaturated fatty acids than those of *Alvinocaris markensis*.

A seasonal study of the lipid composition of deep-sea shrimp such as *N. gracilis* might reveal a correlation between influxes of organic matter and lipid levels. Gonadosomatic or hepatosomatic indices might also be measured and related to seasonal differences in the availability of organic matter. However, the costs involved with this sort of study are probably prohibitive.

When coupled with lipid analysis, compound specific stable isotope analysis is a very powerful tool in the determination of the trophic ecology of marine organisms, especially those that may rely in some part on a chemoautotrophic source of organic

matter. Examination of individual fatty acids and sterols and their stable isotope composition may help shed more light on the trophic ecology of deep-sea organisms. It would be particularly interesting to further examine the fatty acid stable isotope composition and the sterol composition of Juan de Fuca Ridge invertebrates. Given the high concentrations of sterols present in tubeworms and palm worms from the Juan de Fuca Ridge, it would be very interesting to discover whether the worms are synthesising these compounds *de novo*, or gaining them from another source (e.g. phytoplankton). It would also be interesting to ascertain whether the n-4 fatty acids detected in *P. palmiformis* have a chemosynthetic origin, as they do in *Rimicaris exoculata*. Likewise, compound-specific stable isotope analysis of *C. pacifica* could determine the source of n-3 polyunsaturated fatty acids.

The presence of photosynthetically derived organic matter in *Ridgeia piscesae* is an fascinating puzzle. This puzzle might be best tackled from three directions. Analysis of newly settled juvenile *R. piscesae* would confirm that they contain higher levels of phototrophically derived fatty acids than adult specimens. It might then be estimated whether these levels are high enough to explain levels found in adult *Ridgeia piscesae*. *In vivo* experiments using radioactively labelled fatty acids might be used to determine whether adult *Ridgeia piscesae* is capable of active assimilation of dissolved organic matter. Finally, analysis of dissolved and particulate organic matter in the water around beds of adult and newly settled tubeworms would reveal whether phototrophically derived fatty acids and precursors to n-3 PUFA are available for assimilation.

References

Ackman, R.G., McCleod, C.A. & Banerjee, A.K. (1990). An overview of analyses by Chromarod Iatroscan Thin Layer Chromatography-Flame Ionisation Detection. *Journal of Planar Chromatography*, **3**, 450-90.

Albers, C.S., Kattner, G. & Hagen, W. (1996). The composition of wax esters, triacylglycerols and phospholipids in Arctic and Antarctic copepods: evidence of energetic adaptations. *Marine Chemistry*, **55**, 347-58.

Aller, J.Y. (1989). Quantifying sediment disturbances by bottom currents and its effect on benthic communities in a deep-sea western boundary zone. *Deep-Sea Research*, **36A**, 901-34.

Bell, M.V. & Dick, J.R. (1990). The fatty acid composition of phospholipids from the eyes of the northern deepwater prawn, *Pandalus borealis*. *Biochemical Society Transactions*, **18**, 907-8.

Bell, M.V., Henderson, R.J. & Sargent, J.R. (1986). The role of polyunsaturated fatty acids in fish. *Comparative Biochemistry and Physiology B*, **83(4)**, 711-9.

Beninger, P.G & Lucas, A. (1984). Seasonal variations in condition, reproductive activity and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus L.* (Jeffreys) and *Tapes philippinarum* (Adams & Reeve). *Journal of Experimental Marine Biology and Ecology*, **79**, 19-37.

Ben-Mlih, F., Marty, J.-C. & Fiala-Médioni, A. (1992). Fatty acid composition in deep hydrothermal vent symbiotic bivalves. *Journal of Lipid Research*, **33**, 1797-1806.

Billet, D.S.M., Lampitt, R.S., Rice, A.L. & Mantoura, R.F.C. (1983). Seasonal sedimentation of phytoplankton to the deep-sea benthos. *Nature*, **302**, 520-2.

Calman, W.T. (1911). The Life of Crustacea. Methuen & Co., London, 289pp.

Cartes, J.E. (1993). The diets of two deep-sea decapods - *Nematocarcinus exilis* (Caridea: Nematocarcinidae) and *Munida tenuimana* (Anomura, Galatheidae) on the western Mediterranean Slope. *Ophelia*, **37**, 213-29.

Cary, S. C., Felbeck, H. & Holland, N. D. (1989). Observations on the reproductive biology of the hydrothermal vent tubeworm *Riftia pachyptila*. *Marine Ecology Progress Series*, **52**, 89-94.

Casanova, B., Brunet, M. & Segonzac, M. (1993). L'impact d'une epibiose bacterienne sur la morphologie fonctionelle des crevettes associeés a l'hydrothermalisme médio-atlantique. *Cahiers de Biologie Marine*, **34**, 573-588.

Cavanaugh, C.M., Gardiner, S.L., & Jones, M.L., Jannasch, H.W. & Waterbury, J.B. (1981). Prokaryotic cells in the hydrothermal vent tube worm *R. pachyptila* Jones: possible chemoautotrophic symbionts. *Science*, **213**, 340-42.

Cavanaugh, C.M. (1985). Symbiosis of chemoautotrophic bacteria and marine invertebrates from hydrothermal vents and reducing sediments. *Bulletin of the Biological Society of Washington*, **6**, 373-88.

Chace, F.L. (1992). On the classification of the Caridea (Decapoda). *Crustaceana*, **63**, 70-80.

Chang, C.-F. & Shih, T.-W. (1995). Reproductive cycle of ovarian development and vitellogenin profiles in freshwater prawns, *Macrobrachium rosenbergii*. *Invertebrate Reproduction and Development*, **27**, 11-20.

Clarke, A. (1977). Seasonal variations in the total lipid content of *Chorismus antarcticus* (Pfeffer) (Crustacea: Decapoda) at South Georgia. *Journal of Experimental Marine Biology and Ecology*, **27**, 93-106.

Clarke, A. (1979). Lipid content and composition of the pink shrimp, *Pandalus montagui* (Leach) (Crustacea: Decapoda). *Journal of Experimental Marine Biology and Ecology*, **38**, 1-17.

Clarke, A. (1982). Lipid synthesis and reproduction in the polar shrimp *Chorismus antarcticus*. *Marine Ecology-Progress Series*, **9**, 81-90.

Conway, N. & McDowell Capuzzo, J. (1991). Incorporation and utilisation of bacterial lipids in the *Solemya velum* symbiosis. *Marine Biology*, **108**, 277-91

Copley, J.T.P. (1998). Ecology of deep-sea hydrothermal vents. PhD Thesis. University of Southampton, Department of Oceanography.

Corliss J.B., Dymond, J., Gordo, L.I., Edmond, J.M., von Herzen, R.P., Ballard, R.D., Green, K., Williams, D., Bainbridge, A., Crane, K., & van Andel, T.H. (1979). Submarine thermal springs on the Galapagos rift. *Science,* **203**, 1073-83.

Creasey, S., Rogers, A.D. & Tyler, P.A. (1996). A genetic comparison of two populations of the deep-sea vent shrimp *Rimicaris exoculata* (Decapoda:Caridea:Bresiliidae) from the Mid-Atlantic Ridge. *Marine Biology*, **125**, 473-82.

Creasey, S., Rogers, A.D., Tyler, P.A., Young, C.M & Gage, J. (1997). The population biology and genetics of the deep-sea spider crab, *Encephaloides armstrongi* Wood-Mason 1891 (Decapoda:majidae). *Philosophical Transactions of the Royal Society of London, B - Biological Sciences*, **352**, 365-79.

Culkin, F., & Morris, R.J. (1969). The fatty acids of some marine crustaceans. *Deep-Sea Research*, **16**, 109-116.

Currie, R.I., Fisher, A.E. Hargreaves, P.M. (1973). "Arabian Sea upwelling" *In* Zeitschel, B. (Ed.), *The biology of the Indian Ocean*, Chapman & Hall Ltd., London, 549pp.

Delmas, R.P., Parrish, C.C. & Ackman, R.G. (1984). Determination of lipid class concentrations in seawater by Thin Layer Chromatography-Flame Ionisation Detection. *Analytical Chemistry*, **56**, 1272-6.

Delong, E.F. & Yayanos, A.A. (1986). Biochemical function and ecological significance of novel bacterial lipids in deep-sea prokaryotes. *Applied and Environmental Microbiology*, **51**, 730-7.

Desbruyères, D. & Laubier, L. (1991). Systematics, phylogeny, ecology and distribution of the Alvinellidae (Polychaeta) from deep-sea hydrothermal vents. *Ophelia Supplement*, **5**, 31-45.

Dixon, D.R. & Dixon, L.R.J. (1996). Results of DNA analyses conducted on vent shrimp postlarvae collected above the Broken Spur vent field during the CD95 cruise, August 1995. *BRIDGE Newsletter*, **11**, 9-15.

Domanski, P. (1986). The near bottom shrimp faunas (Decapoda:Natantia) at two abyssal sites in the north-east Atlantic Ocean. *Marine Biology*, **93**, 171-80.

Dunstan, G.A., Volkman, J.K. & Barrett, S.M. (1993). The effect of lyophilisation on the solvent extraction of lipid classes, fatty acids and sterols from the oyster *Crassostrea gigas*. *Lipids*, **28**, 937-944.

Endow, K. & Ohta, S. (1990). Occurrence of bacteria in the primary oocytes of the vesicomyid clam, *Calyptogena soyoae*. *Marine Ecology-Progress Series*, **64**, 309-11.

Ferreira, A.M. & Vale, C. (1998). PCB accumulation and alteration of lipids of two length classes of the oyster *Crassostrea angulata* and of the clam *Ruditapes decussatus*. *Marine Environmental Research*, **45**, 259-68.

Fiala-Médioni, A., Métivier, C., Hervy, A. & LePennec, M. (1986). Ultrastructure of the gill of the hydrothermal vent mytilid *Bathymodiolus sp. Marine Biology*, **92**, 65-72.

Fiala-Médioni, A., Boulègue, J., Ohta, S., Felbeck, H. & Mariotti, A. (1993). Source of energy sustaining *Calyptogena* populations from deep trenches in subduction zones off Japan. *Deep-Sea Research I*, **40**, 1241-58.

Fisher, C.R. (1990). Chemoautotrophic and methanotrophic symbioses in marine invertebrates. *Reviews in Aquatic Sciences*, **2**, 399-436.

Folch, J., Lees, M. & Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipid from animal tissue. *Journal of Biological Chemistry*, **226**, 497-509.

Fraser, A.J., Tocher, D.R. & Sargent, J.R. (1985). Thin-layer chromatography-flame ionisation detection and the quantification of marine neutral lipids and phospholipids. *Journal of Experimental Marine Biology and Ecology*, **88**, 91-99.

Fullarton, J.G. (1993). Fatty acid biomarkers in symbiotic species. PhD thesis, University of Stirling, Institute of Aquaculture, DX180328.

Fullarton, J.G., Dando, P.R., Sargent, J.R., Southward, A.J. & Southward, E.C. (1995a). Fatty acids of hydrothermal vent *Ridgeia piscesae* and inshore bivalves containing symbiotic bacteria. *Journal of the Marine Biological Association of the United Kingdom*, **75**, 455-68.

Fullarton, J.G., Wood, A.P., Sargent, J.R. (1995b). Fatty acid composition of lipids from sulphur-oxidising and methylotrophic bacteria from thyasirid and lucinid bivalves. *Journal of the Marine Biological Association of the United Kingdom*, **75**, 445-454.

Gage, J.D. & Tyler, P.A. (1991). *Deep-sea biology*, Cambridge University Press, 504pp.

Gage, J.D., Bett, B.J., Campbell, A.C., Crooks, J., Debenham, N.J., Gooday, A., Hoover, D., Levin, L., Meadows, A., Meadows, P.S., Murray, J., Oliver, G., Patience, A.J., Rogers, A., Scheltema, A., Squire, G., Smallman, D., Tyler, P.A., Wolff, G.A., Young, C.M. (1995). Benthic community and fluxes in relation to the oxygen minimum zone in the Arabian Sea. *Discovery 211/94 Cruise Report, 9th October-11th November 1994.*

Gal'chenko, V.F., Galkin, S.V., Lein, A.I., Moskalev, L.I. & Ivanov, M.V. (1988). Role of bacterial symbionts in nutrition of invertebrates from areas of active underwater hydrotherms. *Oceanology*, **28**, 786-94.

Gebruk, A.V., Pimenov, N.V. & Savvichev, A.S. (1993). Feeding specialisation of bresiliid shrimps in the TAG site hydrothermal community. *Marine Ecology Progress Series*, **98**, 247-53.

Goad, L.J. (1981). The steroids of marine algae and invertebrate animals. *In* D.C. Malins & J.R. Sargent (eds), *Biochemical and Biophysical Perspectives in Marine Biology*. Volume 3, 213-318.

Goodson, M., Tyler, P.A., Copley, J.T.P. & Van Dover, C.L. (in submission). Reproductive biology and ecology of *Ridgeia piscesae* from the Juan de Fuca Ridge Endeavour Segment. Submitted to *Marine Ecology Progress Series.*

Graeve, M., Hagen, W. & Kattner, G. (1994). Herbivorous or omnivorous? On the significance of lipid compositions as trophic markers in Antarctic copepods. *Deep-Sea Research I*, **41**, 915-24.

Grimalt, J.O., Simoneit, B.R.T., Gómez-Belinchón, Fischer, K. & Dymond, J. (1990). Ascending and descending fluxes of lipid compounds in North Atlantic and North Pacific abyssal waters. *Nature*, **345**, 147-150.

Harvey, D.J. (1982). Picolinyl esters as derivatives for the structural determination of long chain branched and unsaturated fatty acids. *Biomedical Mass Spectrometry*, **9**, 33-38.

Harvey, D.J. (1984). Picolinyl derivatives for the structural determination of fatty acids by mass spectrometry: Applications to polyenoic acids, hydroxy acids, di-acids and related compounds. *Biomedical Mass Spectrometry*, **11**, 340-47.

Hedrick, D.B., Pledger, R.D., White, D.C. & Baross, J.A. (1992). *In situ* microbial ecology of hydrothermal vent sediments. *FEMS Microbiology and Ecology*, **101**, 1-10.

Herring, P.J. (1972). Depth distribution of the carotenoid pigments and lipids of some oceanic animals. 1. Mixed zooplankton, copepods and euphausiids. *Journal of the Marine Biological Association of the United Kingdom*, **52**, 179-89.

Herring, P.J. (1973). Depth distribution of the carotenoid pigments and lipids of some oceanic animals. 2. Decapod crustaceans. *Journal of the Marine Biological Association of the United Kingdom*, **53**, 539-62.

Herring, P.J. (1974). Size, density and lipid content of some decapod eggs. *Deep-Sea Research*, **21**, 91-94.

Herring, P.J. (1994). Cruise Report of the Institute of Oceanographic Science Deacon Laboratory 1994, No 244, 55pp.

Herring, P.J. (1996). Travelling shrimp. *BRIDGE Newsletter*, **11**, 6-9.

Holland, D.L. (1978). Larvae of benthic marine invertebrates. *In* D.C. Malins & J.R. Sargent (eds), *Biochemical and Biophysical Perspectives in Marine Biology.* Volume 4, 85-123.

Hopkins, C.C.E., Sargent, J.R. & Nilssen, E.M. (1993). Total lipid content and lipid and fatty acid composition of the deep-water prawn, *Pandalus borealis* from Balsfjord, northern Norway: growth and feeding relationships. *Marine Ecology Progress Series*, **96**, 217-28.

Jahnke, L.L., Summons, R.E., Dowling, L.M. & Zahiralis, K.D. (1995). Identification of methanotrophic lipid biomarkers in cold-seep mussel gills: chemical and isotopic analysis. *Applied and Environmental Microbiology*, **61**, 576-582.

Jeckel, W.H., De Moreno, J.E.A. & Moreno, V.J. (1989). Biochemical composition, lipid classes and fatty acids in the ovary of the shrimp, *Pleoticus muelleri* Bate. *Comparative Biochemistry and Physiology B*, **92**, 271-6.

Jones, M. L. (1985). On the Vestimentifera, new phylum: six species, and other taxa, from hydrothermal vents and elsewhere. *Bulletin of the Biological Society of Washington*, **6**, 117-158.

Joseph, J.D. (1982). Lipid composition of marine and estuarine invertebrates. *Progress in Lipid Research*, **21**, 109-153.

Kanazawa, Teshima, S.-I. & Ono, K. (1979). Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. *Comparative Biochemistry and Physiology B*, **63**, 295-8.

Karl, D., Wirsen, C. & Jannasch, H. (1980). Deep-sea primary production at the Galapagos hydrothermal vents. *Science,* **207**, 1345-7.

Kattner, G., Wehrtmann, I.S. & Merck, T. (1994). Interannual variations of lipids and fatty acids during larval development of *Crangon* spp. in the German Bight, North Sea. *Comparative Biochemistry and Physiology B*, **107**, 103-110.

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*, London, **317**, 351-3.

King, M.G. & Butler, A.J. (1985). Relationship of life history patterns to depth in deep-water caridean shrimps (Crustacea:Natantia). *Marine Biology*, **86**, 129-38.

Lemieux, H., Blier, P.U., Dufresne, F., & Désrosiers, G. (1997). Metabolism and habitat competition in *Nereis virens*. *Marine Ecology-Progress Series*, **156**, 151-6.

LePennec, M. & Fiala-Médioni, A. (1988). The role of the digestive tract of *Calyptogena laubieri* and *C. phaseoliformis*, vesicomyid bivalves of the subduction zone of Japan. *Oceanologica Acta*, **11**, 193-9

Longhurst, A. (1998). *Ecological geography of the sea*. Academic Press, 398pp.

Lutz, R.A., Jablonsk, D. & Turner, R.D. (1984). Larval development and dispersal at deep-sea hydrothermal vents. *Science*, **226**, 1451-3.

Martin, J.W. & Christiansen, J.C. (1995). A new species of the shrimp genus *Chorocaris* Martin & Hessler, 1990, from hydrothermal vents along the Mid-Atlantic Ridge. *Proceedings of the Biological Society of Washington*, **108**, 220-227.

McClafferty, F.W. & Turecek, F. (1993). *Interpretation of Mass Spectra*, 4th Edition. University Science Books, California, 371pp.

McHugh, D. (1989). Population structure and reproductive biology of two sympatric hydrothermal vent polychaetes, *Paralvinella pandorae* and *P. palmiformis*. *Marine Biology*, **103**, 95-106.

Merrett, N.R. (1986). Macrouridae of the eastern North Atlantic. *Fiche d'Identification du Plancton*, Fiche No.173/174/175, 14pp.

Middleditch, B.S., Missler, S.R., Hines, H.B., McVey, J.P., Brown, A., Ward, D.E. & Lawrence, A.L. (1980). Metabolic profiles of penaeid shrimp: Dietary lipids and ovarian maturation. *Journal of Chromatography*, **195**, 359-68.

Morris, R.J. & Sargent, J.R. (1973). Studies on the lipid metabolism of some oceanic crustaceans. *Marine Biology*, **22**, 77-83.

Morris, R.J. (1973). Relationships between the sex and degree of maturity of marine crustaceans and their lipid compositions. *Journal of the Marine Biological Association of the United Kingdom*, **53**, 27-37.

Mourente, G. & Rodriguez, A. (1991). Variation in lipid content of wild caught females of the marine shrimp, *Penaeus kerathurus* during sexual maturation. *Marine Biology*, **110**, 21-28.

Muriana, F.J.G., Ruiz Gutierrez, V. & Bolufer, J. (1993a). Phospholipid fatty acid composition of hepatopancreas and muscle from the prawn, *Penaeus japonicus. Journal of Biochemistry*, **114**, 404-7.

Muriana, F.J.G., Ruiz Gutierrez, V., Gallardo-Guerrero, M.L. & Minguez-Mosquera, M.I. (1993b). A study of the lipids and carotenoprotein in the prawn, *Penaeus japonicus. Journal of Biochemistry*, **114**, 223-9.

Murray, J. The Caridean Shrimp. *Reports of John Murray Expedition 1933-34,* Volume VI, No 4, 196.

Nair, R.R., Ittekkot, V., Manganini, S.J., Ramaswamy, V., Haake, B., Degens, E.T., Desai, B.N. & Honjo, S. (1989). Increased particle flux to the deep ocean related to monsoons. *Nature*, **338**, 749-51.

Nelson, D.C. & Fisher, C.R. (1995). Chemoautotrophic and methanoautotrophic endosymbiotic bacteria at deep-sea vents and seeps. *In* Karl, D.M. (Ed.), *The microbiology of deep-sea hydrothermal vents*, CRC, 125-67.

O'Neill, P.J., Jinks, R.N., Herzog, E.D., Battelle, B.-A., Kass, L., Renninger, G.H. & Chamberlain, S.C. (1995). The morphology of the dorsal eye of the hydrothermal vent shrimp *Rimicaris exoculata. Visual Neuroscience*, **12**, 861-75.

Olsen, D.B., Hitchcock, G.L., Fine R.S. & Warren, B.A. (1994). Maintenance of the low oxygen layer in the central Arabian Sea. *Deep-Sea Research II*, **40**, 673-85.

Pedersen, T.F., Shimmield, G.B. & Price, N.S. (1993). Lack of enhanced preservation of organic matter in sediments under the oxygen minimum on the Oman margin. *Geochimica et Cosmochimica Acta*, **56**, 545-51.

Peek, A.S., Gustafson, R.G., Lutz, R.A.& Vrijenhoek, R.C. (1997). Evolutionary relationships of deep-sea hydrothermal vent and cold-water seep clams (Bivalvia: Vesicomyidae): results from mitochondrial cytochrome oxidase subunit I. *Marine Biology*, **130**, 151-61.

Polz, M.F. & Cavanaugh, C.M. (1995). Dominance of one bacterial phylotype at a Mid-Atlantic Ridge hydrothermal vent site. *Proceedings of the National Academy of Sciences of the USA*, **92**, 7232-6.

Pond, D.P., Bell, M.V., Dixon, D.R., Fallick, A.E., Segonzac, M. & Sargent, J. (1998). Stable-carbon-isotope composition of fatty acids in hydrothermal vent mussels containing methanotrophic and thiotrophic bacterial endosymbionts. *Applied and Environmental Microbiology*, **64**, 370-5.

Pond, D.P., Dixon, D.R. & Sargent, J.R. (1997a). Wax-ester reserves facilitate dispersal of hydrothermal vent shrimps. *Marine Ecology Progress Series*, **146**, 289-90.

Pond, D.P., Dixon, D.R., Bell, M., Fallick, A. & Sargent, J.R. (1997b). Occurrence of 16:2 (n-4) and 18:2 (n-4) fatty acids in the lipids of the hydrothermal vent shrimps *Rimicaris exoculata* and *Alvinocaris markensis*: nutritional and trophic implications. *Marine Ecology Progress Series*, **156**, 167-74.

Pond., D.P., Allen Copley, C.E., Fallick., A.E., Sargent, J.R. & Van Dover, C.L. (In preparation). Fatty acid stable isotope composition of *R. piscesae* from the High Rise Vent Field, Juan de Fuca Ridge Endeavour Segment. Evidence of a non-chemoautotrophic energy source for a hydrothermal vent vestimentiferan.

Pond., D.P., Segonzac, M., Bell, M.V., Dixon, D.R., Fallick, A.E. & Sargent, J.R. (1997c). Lipid and lipid carbon stable isotope composition of the hydrothermal vent shrimp *Mirocaris fortunata*: evidence for nutritional dependence on photosynthetically fixed carbon. *Marine Ecology Progress Series*, **157**, 221-231.

Pranal, V., Fiala-Medioni, A. & Guezennec, J. (1997). Fatty acid characteristics in two symbiont bearing mussels from deep-sea hydrothermal vents of the south-western Pacific. *Journal of the Marine Biological Association of the United Kingdom*, 77, 473-92.

Reemstma, T., Haake, B., Ittekkot, I., Nair, R.R. & Brockmann, U. (1990). Downward flux of particulate fatty acids in the central Arabian sea. *Marine Chemistry*, **29**, 183-202.

Renninger, G. H., Kass, L., Gleeson, R. A., Van Dover, C. L., Battelle, B.-A., Jinks, R. N., Herzog, E. D. & Chamberlain, S. C. (1995). Sulfide as a chemical stimulus for deep-sea hydrothermal vent shrimp. *Biological Bulletin*, **189**, 69-76.

Rieley, G., Van Dover, C.L., Hedrick, D.B., White, D.C. & Eglinton, G. (1995). Lipid characteristics of hydrothermal organisms from 9°N East Pacific Rise. *In* Parson, L.M., Walker, C.L. & Dixon, D.R. (eds), *Hydrothermal vents and processes*, Geological Society Special Publication No 87, 329-342.

Rodriguez, A., Le Vay, L., Mourente, G. & Jones, D.A. (1994). Biochemical composition and digestive enzyme activity in larvae and postlarvae of *Penaeus japonicus* during herbivorous and carnivorous feeding. *Marine Biology*, **118**, 45-51.

Saino, T. & Ohta, S. (1989). $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of vesicomyid clams and a vestimentiferan tubeworm in the subduction zone east of Japan. *Palaeogeography, Palaeoclimatology, Palaeoecology*. **71**, 169-78.

Saliot, A. & Marty, J.C. (1988). Lipides dissous dans les eax de mer. Etude des relations entre acides gras et organismes marins. *Océanis*, **14**, 321-330.

Sanders, N.K.& Childress, J.J. (1990). Adaptations to the oxygen minimum layer: oxygen binding by the haemocyanin of the bathypelagic mysid, *Gnathophausia ingens* Dohrn. *Biological Bulletin*, **178**, 286-94.

Sarrazin, J., Robigou, V., Juniper, S.K. & Delaney, J.R. (1997). Biological and geological dynamics over four years on a high-temperature sulphide structure at the Juan de Fuca Ridge hydrothermal observatory. *Marine Ecology - Progress Series*, **153**, 5-24.

Sargent, J.R. (1976). The structure, metabolism and function of lipids in marine organisms. *In* D.C. Malins & J.R. Sargent (eds), *Biochemical and Biophysical Perspectives in Marine Biology*. Volume 3, 149-212.

Sargent, J.R. & Whittle, K.J. (1981). Lipids and hydrocarbons in the marine food web. *In* A.R.Longhurst (ed) *Analysis of marine ecosystems*, Academic, 491-533.

Sargent J.R., Parkes, R.J., Mueller-Harvey, I. & Henderson, R.J. (1987). Lipid biomarkers in marine ecology. In *Microbes in the Sea*, ed. M.A. Sleigh, Ellis Horwood, 119-138.

Segonzac, M., de Saint Laurent, M. & Casanova, B. (1993). L'enigme du comportement trophique des crevettes Alvinocarididae des site hydothermaux de la dorsale medio-atlantique. *Cahiers de Biologie Marine*, **34**, 535-71.

Shank, T.M., Lutz, R.A. & Vrijenhoek, R.C. (1998). Molecular systematics of shrimp (Decapoda:bresiliidae) from deep-sea hydrothermal vents, I. Enigmatic "small orange" shrimp from the Mid-Atlantic Ridge are juvenile *Rimicaris exoculata*. *Molecular Marine Biology and Biotechnology*, **7**, 88-94.

Shantha, N.C. & Ackman R.G. (1990). Advantages of total lipid hydrogenation prior to lipid class determination on Chromarods SIII. *Lipids*, **25**, 570-4.

Smith, R.E.H., Gosselin, M., Kattner, G., Legendre, L. & Pesant, S. (1997). Biosynthesis of macromolecular and lipid classes by phytoplankton in the Northeast Water Polynya. *Marine Ecology Progress Series*, **147**, 231-42.

Somero, G.N. (1992). Biochemical ecology of deep-sea animals, *Experientia*, **48**, 537-43.

Southward, A.J. & Southward, E.C. (1970). Observations on the role of dissolved organic compounds in the nutrition of benthic invertebrates. Experiments on three species of Pogonophora. *Sarsia*, **45**, 69-90.

Southward A.J. & Southward, E.C. (1980). The significance of dissolved organic compounds in the nutrition of *Siboglinum ekmani* and other small species of Pogonophora. *Journal of the Marine Biological Association of the United Kingdom*, **60**, 1005-34.

Southward, E. C. (1988). Development of the gut and segmentation of newly settled stages of *Ridgeia* (Vestimentifera): implications for relationship between Vestimentifera and Pogonophora. *Journal of the Marine Biological Association of the UK*, **68**, 465-487.

Southward, E. C., Tunnicliffe, V. & Black, M. B. (1995). Revision of the species of *Ridgeia* from northeast Pacific hydrothermal vents, with a redescription of *Ridgeia piscesae* Jones (Pogonophora: Obturata = Vestimentifera). *Canadian Journal of Zoology*, **73**, 282-295.

Southward. E. C., Tunnicliffe, V., Black, M. B., Dixon, D. R. & Dixon, L. R. J. (1996). Ocean-ridge segmentation and vent tubeworms (Vestimentifera) in the NE Pacific. In *Tectonic, Magmatic, Hydrothermal and Biological Segmentation of Mid-Ocean Ridges*, eds. MacLeod, C. J., Tyler, P. A. & Walker, C. L., Geological Society of London Special Publication No. 118, 211-224.

Spaargaren, D.H. & Haefner, P.A. Jr. (1994). Interactions of ovary and hepatopancreas during the reproductive cycle of *Crangon crangon* (L.). II. Biochemical relationships. *Journal of Crustacean Biology*, **14**, 6-19.

Stryer, L. (1988). Biochemistry, W.H. Freeman & Co., NewYork, 3rd Edition.

Swallow, J.C. (1984). Some aspects of the physical oceanography of the Indian Ocean. *Deep-Sea Research*, **31**, 639-50.

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 confamilial species. *Comparative Biochemistry and Physiology B*, **91**, 593-6.

Teshima, S. -I. & Kanazawa, A. (1983). Variation in lipid compositions during the ovarian maturation of the prawn. *Nippon Suisan Gakkaishi*, **49**, 957-962.

Teshima, S. -I., Kanazawa, A., Koshio, S. & Horinouchi, K. (1989). Lipid metabolism of the prawn, *Penaeus japonicus* during maturation: variation in lipid profiles of the ovary and hepatopancreas. *Comparative Biochemistry and Physiology B*, **92**, 45-9.

Testermann, J.K. (1972). Accumulation of free fatty acids from seawater by marine invertebrates. *Biological Bulletin*, **142**, 160-177.

Tunnicliffe, V. (1991). The biology of hydrothermal vents: ecology and evolution. *Oceanography and Marine Biology Annual Review*, **29**, 319-407.

Tyler, P.A. (1995). Conditions for the existence of life at the deep-sea floor: an update. *Oceanography and Marine Biology: an Annual Review*, **33**, 221-44.

Van Dover, C.L. (1995). Ecology of Mid-Atlantic Ridge hydrothermal vents. *In* Parson, L.M., Walker, C.L. & Dixon, D.R. (eds), *Hydrothermal vents and processes*, Geological Society Special Publication No 87, 257-94.

Van Dover, C.L & Fry, B. (1989). Stable isotopic compositions of hydrothermal vent organisms. *Marine Biology*, **102**, 257-63.

Van Dover, C.L., Szuts, E.Z, Chamberlain, S.C. & Cann, J.R. (1989). A novel eye in "eyeless" shrimp from hydrothermal vents of the Mid-Atlantic Ridge. *Nature*, **337**, 458-60.

Van Dover, C.L., Fry, B., Grassle, J.F., Humphris, S. & Rona, P.A. (1988). Feeding biology of the shrimp *Rimicaris exoculata* at hydrothermal vents on the Mid-Atlantic Ridge. *Marine Biology*, **98**, 209-16.

Vereschaka, A.L. (1997). Comparative studies on four populations of the shrimp *Rimicaris exoculata* from the Mid-Atlantic Ridge. *Deep-Sea Research*, **44**, 1905-21.

Volkmann, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I. & Garland, C.D. (1989). Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *Journal of Experimental Marine Biology and Ecology*, **128**, 219-240.

Wakeham, S.G. (1995). Lipid biomarkers for heterotrophic alteration of suspended particulate organic matter in oxygenated and anoxic water columns of the ocean. *Deep-Sea Research I*, **42**, 1749-1771.

Williams, A.B. (1988). New marine decapod crustaceans from waters affected by hydrothermal discharge, brine and hydrocarbon seepage. *Fisheries Bulletin*, **86**, 263-87.

Williams, A.B. & Rona, P.A. (1986). Two new caridean shrimps (Bresiliidae) from a hydrothermal field on the Mid-Atlantic Ridge. *Journal of Crustacean Biology*, **6**, 446-62.

Yano, Y., Nakayama, A., Saito, H. & Ishikara, K. (1994). Production of docosahexaenoic acid by marine bacteria isolated from deep-sea fish. *Lipids*, **29**, 527-8.

Young, C.M., Vásquez, E., Metaxas, A. & Tyler, P.A. (1996). Embryology of vestimentiferan tube worms from deep-sea methane/sulphide seeps. *Nature*, **381**, 514-516.

Young, N.J., Quinlan, P.T. & Goad, L.J. (1992). Cholesteryl esters in the decapod crustacean, *Penaeus monodon*. *Comparative Biochemistry and Physiology B*, **102**, 761-768.

Zhukova, N.V. (1991). The pathway of the biosynthesis of non-methylene interrupted dienoic fatty acids in molluscs. *Comparative Biochemistry and Physiology B*, **83**, 643-646.

Zhukova, N.V. Kharlamenko, V.I., Svetashev, V.I. & Rodionov, I.A. (1992). Fatty acids as markers of bacterial symbionts of marine bivalve molluscs. *Journal of Experimental Marine Biology and Ecology*, **162**, 253-263.

APPENDICES

A1: Mean lipid class composition of *R. exoculata*

Tables A1.1 and A1.2 show the mean lipid class compositions (tissue concentration and percentage composition respectively) of whole adult and juvenile *R. exoculata* from TAG and postlarval alvinocarids from the water column above Broken Spur.

Table A1.1 Mean lipid class tissue concentrations for whole adult and juvenile *R. exoculata* from TAG and for postlarval alvinocarids from the water column above Broken Spur (mean tissue concentrations mg g dry weight^{-1} ± S.E. ($\sigma/\sqrt{n}$), n.d. = not detected). WE, wax ester; TG, triglyceride; FA, free fatty acid; S, sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

Specimens	WE	TG	FA	S	PE	PC	SM	LPC
Females CL < 16mm (n = 36)	6.3 ± 0.8	15.4 ± 1.4	2.6 ± 0.1	3.6 ± 0.2	6.5 ± 0.3	13.0 ± 0.5	1.0 ± 0.1	1.3 ± 0.1
Females CL > 16mm (n = 21)	2.4 ± 0.4	43.7 ± 4.3	2.6 ± 0.2	5.3 ± 0.6	6.8 ± 0.4	12.6 ± 1.0	0.8 ± 0.1	1.2 ± 0.1
Males CL < 16mm (n = 18)	4.7 ± 0.6	21.1 ± 1.9	2.9 ± 0.2	4.4 ± 0.3	6.6 ± 0.3	12.1 ± 0.5	1.0 ± 0.1	1.0 ± 0.1
Males CL > 16mm (n = 33)	2.8 ± 0.2	41.9 ± 1.8	2.5 ± 0.2	4.3 ± 0.2	4.7 ± 0.3	9.4 ± 0.6	0.7 ± 0.1	1.0 ± 0.1
Juveniles (n = 12)	239 ± 23	43.5 ± 9.0	n.d.	n.d.	3.2 ± 0.2	7.6 ± 0.6	n.d.	1.2 ± 0.4
Postlarvae (n = 18)	277 ± 9.0	25.4 ± 7.0	n.d.	n.d.	6.0 ± 0.2	7.7 ± 0.3	n.d.	n.d.

Table A1.2 Mean percentage lipid class compositions for whole adult and juvenile *R. exoculata* from TAG and for postlarval alvinocarids from the water column above Broken Spur (lipid classes as a percentage of total lipid ± S.E. ($\sigma/\sqrt{n}$), n.d. = not detected). WE, wax ester; TG, triglyceride; FA, free fatty acid; S, sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

Specimens	WE	TG	FA	S	PE	PC	SM	LPC
Females CL < 16mm (n = 36)	12.6 ± 1.5	31.1 ± 2.7	5.2 ± 0.2	7.2 ± 0.3	13.0 ± 0.6	26.1 ± 0.9	2.0 ± 0.1	2.7 ± 0.2
Females CL > 16mm (n = 21)	3.2 ± 0.5	57.9 ± 5.7	3.5 ± 0.3	7.1 ± 0.8	9.0 ± 0.6	16.7 ± 1.3	1.1 ± 0.1	1.5 ± 0.1
Males CL < 16mm (n = 18)	8.7 ± 1.0	39.3 ± 3.6	5.4 ± 0.4	8.3 ± 0.5	12.2 ± 0.6	22.5 ± 0.9	1.8 ± 0.1	1.8 ± 0.2
Males CL > 16mm (n = 33)	4.1 ± 0.3	62.3 ± 2.6	3.6 ± 0.2	6.4 ± 0.3	7.0 ± 0.4	14.0 ± 0.8	1.0 ± 0.1	1.4 ± 0.1
Juveniles (n = 12)	80.4 ± 7.9	14.7 ± 3.0	n.d.	n.d.	1.1 ± 0.1	2.5 ± 0.2	n.d.	0.8 ± 0.1
Postlarvae (n = 18)	87.6 ± 2.8	8.1 ± 2.2	n.d.	n.d.	1.9 ± 0.1	2.5 ± 0.1	n.d.	n.d.

Tables A1.3 and A1.4 show the mean lipid class compositions (tissue concentration and percentage composition respectively) of dissected *R. exoculata* from Broken Spur.

Table A1.3 Mean lipid class tissue concentrations for different tissues of *R. exoculata* from Broken Spur (mean tissue concentrations mg g dry weight^{-1} ± S.E. ($\sigma/\sqrt{n}$), n.d. = not detected). WE, wax ester; TG, triglyceride; FA, free fatty acid; S, sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

	WE	TG	FA	S	PE	PC	SM	LPC
Female Abdomen (n = 16)	nd	0.97 ± 0.07	1.08 ± 0.06	3.16 ± 0.35	9.71 ± 0.51	10.5 ± 0.47	0.33 ± 0.02	1.16 ± 0.13
Male Abdomen (n = 6)	nd	1.79 ± 0.19	1.56 ± 0.07	3.62 ± 0.30	10.2 ± 0.71	12.0 ± 0.69	0.24 ± 0.02	0.69 ± 0.12
Juvenile Abdomen (n = 6)	89.8 ± 16.6	1.66 ± 0.35	1.94 ± 0.19	1.29 ± 0.23	4.13 ± 0.40	8.53 ± 0.43	nd	nd
Female Branchial (n = 16)	nd	16.6 ± 3.89	2.73 ± 0.12	2.73 ± 0.17	12.3 ± 0.94	11.1 ± 0.88	0.51 ± 0.04	0.91 ± 0.18
Male Branchial (n = 6)	nd	8.09 ± 1.44	3.17 ± 0.09	2.90 ± 0.75	12.6 ± 0.75	12.7 ± 0.71	0.54 ± 0.02	1.46 ± 0.12
Juvenile Branchial (n = 6)	32.0 ± 6.10	2.73 ± 0.49	6.02 ± 0.46	3.81 ± 0.19	8.75 ± 0.43	15.2 ± 0.59	nd	nd
Ovary stage 0+I (n = 2)	nd	16.1 ± 0.15	22.0 ± 4.47	11.9 ± 1.18	nd	27.5 ± 1.11	nd	nd
Ovary stage II (n = 4)	nd	64.0 ± 24.6	3.45 ± 0.12	nd	5.13 ± 0.08	18.9 ± 4.04	nd	nd
Ovary stage III (n = 4)	nd	104 ± 4.23	5.35 ± 0.37	2.06 ± 0.49	7.56 ± 1.04	16.4 ± 2.70	0.37 ± 0.00	0.83 ± 0.00
Ovary stage IV (n = 6)	nd	128 ± 13.6	5.26 ± 0.94	4.47 ± 0.85	10.9 ± 1.73	28.8 ± 7.25	0.54 ± 0.09	1.44 ± 0.38
Testes (n = 2)	nd	4.10 ± 0.04	5.50 ± 0.28	4.51 ± 0.14	10.0 ± 0.13	21.9 ± 0.40	1.49 ± 0.05	3.39 ± 0.18

Table A1.4 Mean percentage lipid class compositions for different tissues of *R. exoculata* from Broken Spur (lipid classes as a percentage of total lipid ± S.E. ($\sigma/\sqrt{n}$), n.d. = not detected). WE, wax ester; TG, triglyceride; FA, free fatty acid; S, sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

	WE	TG	FA	S	PE	PC	SM	LPC
Female Abdomen (n = 16)	nd	3.62 ± 0.27	4.02 ± 0.21	11.8 ± 1.30	36.1 ± 1.89	39.0 ± 1.75	1.22 ± 0.07	4.31 ± 0.47
Male Abdomen (n = 6)	nd	5.93 ± 0.65	5.17 ± 0.23	12.0 ± 1.00	33.9 ± 2.35	39.9 ± 2.27	0.80 ± 0.07	2.29 ± 0.39
Juvenile Abdomen (n = 6)	83.7 ± 15.4	1.54 ± 0.33	1.81 ± 0.18	1.20 ± 0.21	3.84 ± 0.37	7.94 ± 0.40	nd	nd
Female Branchial (n = 16)	nd	35.4 ± 8.28	5.81 ± 0.27	5.82 ± 0.35	26.3 ± 2.01	23.7 ± 1.87	1.09 ± 0.09	1.94 ± 0.38
Male Branchial (n = 6)	nd	19.5 ± 3.47	7.66 ± 0.21	6.99 ± 1.82	30.3 ± 1.81	30.6 ± 1.72	1.31 ± 0.04	3.52 ± 0.28
Juvenile Branchial (n = 6)	46.7 ± 8.90	3.98 ± 0.72	8.78 ± 0.68	5.56 ± 0.28	12.8 ± 0.63	22.2 ± 0.86	nd	nd
Ovary stage 0+I (n = 2)	nd	20.7 ± 0.20	28.4 ± 5.77	15.3 ± 1.52	nd	35.5 ± 1.44	nd	nd
Ovary stage II (n = 4)	nd	70.0 ± 26.9	3.77 ± 0.13	nd	5.61 ± 0.09	20.6 ± 4.42	nd	nd
Ovary stage III (n = 4)	nd	76.2 ± 3.09	3.92 ± 0.27	1.51 ± 0.36	5.54 ± 0.76	12.0 ± 1.98	0.27 ± 0.00	0.61 ± 0.00
Ovary stage IV (n = 6)	nd	71.3 ± 7.61	2.94 ± 0.52	2.49 ± 0.48	6.08 ± 0.96	16.1 ± 4.04	0.30 ± 0.05	0.80 ± 0.21
Testes (n = 2)	nd	8.04 ± 0.07	10.8 ± 0.55	8.85 ± 0.27	19.7 ± 0.25	43.1 ± 0.79	2.92 ± 0.10	6.65 ± 0.36

Table A1.5 Mean lipid class tissue concentrations for different tissues of *A. markensis* (mean tissue concentrations mg g dry weight^{-1} ± S.E. ($\sigma/\sqrt{n}$), n.d. = not detected). WE, wax ester; TG, triglyceride; FA, free fatty acid; S, sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

	WE	TG	FA	S	PE	PC	SM	LPC
Abdomen (n = 2)	nd	1.82 ± 0.39	3.31 ± 0.20	4.51 ± 0.13	6.54 ± 0.35	10.4 ± 0.16	0.44 ± 0.05	1.66 ± 0.01
"Rest" (n = 2)	nd	51.6 ± 0.55	5.68 ± 0.24	4.74 ± 0.26	4.64 ± 0.19	8.80 ± 0.40	0.32 ± 0.01	0.92 ± 0.14
Eggs (n = 2)	nd	301 ± 9.81	5.79 ± 0.22	6.34 ± 0.07	8.98 ± 0.19	40.5 ± 1.14	nd	nd
Hepatopancreas (n = 2)	nd	280 ± 4.34	7.95 ± 0.45	0.78 ± 0.41	4.81 ± 0.49	11.4 ± 0.38	nd	nd
Whole body (n = 2)	nd	69.4 ± 0.51	5.21 ± 0.25	4.20 ± 0.23	5.36 ± 0.28	10.3 ± 0.18	0.31 ± 0.01	1.02 ± 0.07

Table A1.6 Mean percentage lipid class compositions for different tissues of *A. markensis* (lipid classes as a percentage of total lipid ± S.E. ($\sigma/\sqrt{n}$), n.d. = not detected). WE, wax ester; TG, triglyceride; FA, free fatty acid; S, sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

	WE	TG	FA	S	PE	PC	SM	LPC
Abdomen (n = 2)	nd	6.34 ±1.35	11.6 ± 0.70	15.7 ± 0.46	22.9 ± 1.23	36.2 ± 0.58	1.54 ± 0.16	5.78 ± 0.04
"Rest" (n = 2)	nd	67.3 ± 0.72	7.40 ± 0.32	6.18 ± 0.34	6.05 ± 0.25	11.5 ± 0.52	0.42 ± 0.01	1.20 ± 0.18
Eggs (n = 2)	nd	83.0 ± 2.71	1.60 ± 0.06	1.75 ± 0.02	2.48 ± 0.05	11.2 ± 0.31	nd	nd
Hepatopancreas (n = 2)	nd	91.8 ± 1.42	2.61 ± 0.15	0.26 ± 0.13	1.58 ± 0.16	3.75 ± 0.12	nd	nd
Whole body (n = 2)	nd	72.5 ± 0.53	5.44 ± 0.26	4.39 ± 0.25	5.59 ± 0.29	10.7 ± 0.19	0.32 ± 0.01	1.07 ± 0.07

A2: Mean lipid class composition of *N. gracilis*

Tables A2.1 and A2.2 show the mean lipid class compositions (tissue concentration and percentage composition respectively) of *N. gracilis*.

Table A2.1 Mean lipid class tissue concentrations for *N. gracilis* (mean tissue concentrations mg g dry weight^{-1} ± S.E. ($\sigma/\sqrt{n}$). WE, wax ester; TG, triglyceride; FA, free fatty acid; S, sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

Specimens	WE	TG	FA	S	PE	PC	SM	LPC
Females (n = 18)	0	53.0 ± 4.0	3.6 ± 0.3	3.9 ± 0.6	2.9 ± 0.2	11.6 ± 0.7	0.1 ± 0.1	4.7 ± 0.4
Berried females (n = 21)	0	46.7 ± 1.8	3.9 ± 0.1	6.7 ± 0.3	2.7 ± 0.2	13.2 ± 0.4	0.1 ± 0.1	4.9 ± 0.4
Males (n = 21)	0	54.1 ± 2.2	4.3 ± 0.2	5.8 ± 0.4	2.4 ± 0.2	10.5 ± 0.4	0.1 ± 0.1	5.1 ± 0.4
Sex not determined (n = 24)	0	56.0 ± 3.6	6.4 ± 0.8	1.1 ± 0.5	4.1 ± 0.2	13.4 ± 1.4	0.2 ± 0.1	5.0 ± 0.6
All samples (n = 84)	0	52.5 ± 1.5	4.6 ± 0.3	4.3 ± 0.3	3.0 ± 0.1	12.2 ± 0.5	0.1 ± 0.1	4.9 ± 0.2

Table A2.2 Mean percentage lipid class compositions for *N. gracilis* (lipid classes as a percentage of total lipid ± S.E. ($\sigma/\sqrt{n}$). WE, wax ester; TG, triglyceride; FA, free fatty acid; S, sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

Specimens	WE	TG	FA	S	PE	PC	SM	LPC
Females (n = 18)	0	66.4 ± 5.0	4.6 ± 0.4	4.9 ± 0.7	3.6 ± 0.3	14.5 ± 0.9	0.1 ± 0.1	5.9 ± 0.5
Berried females (n = 21)	0	59.8 ± 2.3	5.0 ± 0.2	8.6 ± 0.4	3.4 ± 0.3	16.9 ± 0.6	0.1 ± 0.1	6.2 ± 0.5
Males (n = 21)	0	65.8 ± 2.6	5.2 ± 0.3	7.1 ± 0.5	3.0 ± 0.2	12.7 ± 0.4	0.1 ± 0.1	6.2 ± 0.5
Sex not determined (n = 24)	0	64.9 ± 4.2	7.4 ± 1.0	1.3 ± 0.6	4.8 ± 0.3	15.6 ± 1.6	0.2 ± 0.1	5.8 ± 0.7
All samples (n = 84)	0	64.2 ± 1.9	5.7 ± 0.4	5.3 ± 0.4	3.8 ± 0.2	15.0 ± 0.6	0.2 ± 0.1	6.0 ± 0.3

A3: Mean lipid class composition of Juan de Fuca Ridge invertebrates

Tables A3.1 and A3.2 show the mean lipid class compositions (tissue concentration and percentage composition respectively) of *R. piscesae*.

Table A3.1 Mean lipid class tissue concentrations for *R. piscesae* (mean tissue concentrations mg g dry weight^{-1} ± S.E. ($\sigma/\sqrt{n}$), n.d. = not detected). SE, sterol ester; TG, triglyceride; FA, free fatty acid; S, sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

Specimen	SE	TG	FA	S	PE	PC	SM	LPC
Anterior (n = 15)	n.d.	n.d.	1.2 ± 0.4	8.9 ± 0.8	1.9 ± 0.1	8.3 ± 0.4	1.9 ± 0.1	11.1 ± 1.4
Posterior (n = 15)	2.8 ± 0.5	0.4 ± 0.2	0.3 ± 0.1	12.0 ± 0.6	1.9 ± 0.1	7.7 ± 0.4	1.8 ± 0.2	13.0 ± 0.6
Whole organism (n = 15)	1.4 ± 0.4	0.2 ± 0.1	0.7 ± 0.2	10.5 ± 0.6	1.9 ± 0.1	8.0 ± 0.3	1.8 ± 0.1	12.1 ± 0.6

Table A3.2 Mean percentage lipid class compositions for *R. piscesae* (lipid classes as a percentage of total lipid ± S.E. ($\sigma/\sqrt{n}$), n.d. = not detected). SE, sterol ester; TG, triglyceride; FA, free fatty acid; S, sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

Specimen	SE	TG	FA	S	PE	PC	SM	LPC
Anterior (n = 15)	n.d.	n.d.	3.6 ± 1.2	26.7 ± 2.3	5.7 ± 0.1	25.0 ± 1.2	5.6 ± 0.1	33.4 ± 3.24
Posterior (n = 15)	6.9 ± 1.3	1.1 ± 0.5	0.6 ± 0.3	30.1 ± 1.5	4.8 ± 0.4	19.3 ± 1.0	4.6 ± 0.4	32.6 ± 1.5
Whole organism (n = 15)	3.8 ± 1.0	0.6 ± 0.3	2.0 ± 0.6	28.6 ± 1.5	5.2 ± 0.2	21.9 ± 0.8	5.0 ± 0.2	33.0 ± 1.7

Tables A3.3 and A3.4 show the mean lipid class compositions (tissue concentration and percentage composition respectively) of *P. palmiformis*.

Table A3.3 Mean lipid class tissue concentrations for *P. palmiformis* (mean tissue concentrations mg g dry weight^{-1} ± S.E. ($\sigma/\sqrt{n}$)). SE, sterol ester; TG, triglyceride; FA, free fatty acid; S, sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

Specimens	SE	TG	FA	S	PE	PC	SM	LPC
P. palmiformis from Godzilla 296 (n = 18)	0.3 ± 0.1	7.4 ± 0.9	2.4 ± 0.3	15.8 ± 0.6	1.5 ± 0.1	9.7 ± 0.3	0.2 ± 0.1	9.4 ± 0.8
P. palmiformis from Park Place 174 (n = 15)	0.2 ± 0.2	3.4 ± 0.9	2.3 ± 0.3	15.0 ± 0.5	2.6 ± 0.1	9.7 ± 0.4	0.6 ± 0.1	10.6 ± 0.7
All *P. palmiformis* (n = 33)	0.3 ± 0.1	5.6 ± 0.7	2.4 ± 0.2	15.4 ± 0.4	2.0 ± 0.1	9.7 ± 0.3	0.4 ± 0.1	10.0 ± 0.6

Table A3.4 Mean percentage lipid class compositions for *P. palmiformis* (lipid classes as a percentage of total lipid ± S.E. ($\sigma/\sqrt{n}$)). SE, sterol ester; TG, triglyceride; FA, free fatty acid; S, sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

Specimens	SE	TG	FA	S	PE	PC	SM	LPC
P. palmiformis from Godzilla 296 (n = 18)	0.6 ± 0.2	15.7 ± 1.9	5.1 ± 0.6	33.8 ± 1.3	3.3 ± 0.2	20.8 ± 0.7	0.5 ± 0.1	20.2 ± 1.6
P. palmiformis from Park Place 174 (n = 15)	0.5 ± 0.4	7.8 ± 2.0	5.3 ± 0.7	33.8 ± 1.1	5.7 ± 0.2	21.8 ± 1.0	1.3 ± 0.1	23.9 ± 1.7
All *P. palmiformis* (n = 33)	0.6 ± 0.2	12.2 ± 1.6	5.2 ± 0.5	33.8 ± 0.9	4.4 ± 0.3	21.3 ± 0.6	0.8 ± 0.1	21.8 ± 1.2

Tables A3.5 and A3.6 show the mean lipid class compositions (tissue concentration and percentage composition respectively) of *C. pacifica.*

Table A3.5 Mean lipid class tissue concentrations for *C. pacifica* (mean tissue concentrations mg g dry weight^{-1} ± S.E. ($\sigma/\sqrt{n}$), n.d. = not detected). SE, sterol ester; TG, triglyceride; FA, free fatty acid; S, sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

Specimens	SE	TG	FA	S	PE	PC	SM	LPC
Foot (n = 15)	n.d.	1.0 ± 0.4	2.3 ± 0.4	1.7 ± 0.9	3.1 ± 0.2	5.7 ± 0.4	1.1 ± 0.2	12.2 ± 1.0
Gill (n = 15)	n.d.	11.9 ± 3.3	3.6 ± 0.3	3.8 ± 0.6	5.1 ± 0.4	7.9 ± 0.8	0.6 ± 0.1	15.4 ± 1.6
Mantle (n = 15)	n.d.	n.d.	5.6 ± 0.8	3.9 ± 2.1	5.1 ± 0.5	13.6 ± 1.3	2.2 ± 0.4	26.3 ± 2.1
Whole organism (n = 15)	n.d.	6.6 ± 1.8	4.1 ± 0.1	4.4 ± 0.6	4.9 ± 0.3	9.2 ± 0.4	1.1 ± 0.1	17.6 ± 1.0

Table A3.6 Mean percentage lipid class compositions for *C. pacifica* (lipid classes as a percentage of total lipid ± S.E. ($\sigma/\sqrt{n}$), n.d. = not detected). SE, sterol ester; TG, triglyceride; FA, free fatty acid; S, sterol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline.

Specimens	SE	TG	FA	S	PE	PC	SM	LPC
Foot (n = 15)	n.d.	3.7 ± 1.6	8.5 ± 1.4	6.2 ± 3.4	11.3 ± 0.8	21.0 ± 1.6	4.3 ± 0.7	44.9 ± 3.7
Gill (n = 15)	n.d.	24.6 ± 6.9	7.5 ± 0.6	7.9 ± 1.2	10.6 ± 0.8	16.3 ± 1.6	1.2 ± 0.3	31.8 ± 3.3
Mantle (n = 15)	n.d.	n.d.	9.9 ± 1.4	6.8 ± 3.7	9.0 ± 0.9	23.9 ± 2.3	3.9 ± 0.7	46.5 ± 3.6
Whole animal (n = 15)	n.d.	13.8 ± 3.8	8.6 ± 0.2	9.3 ± 1.2	10.2 ± 0.6	19.2 ± 0.8	2.2 ± 0.3	36.7 ± 2.0

A4: Taxonomy of species analysed

Crustacea Natantia Decapoda Caridea Alvinocarididae *Rimicaris exoculata*, Williams & Rona, 1986.

Crustacea Natantia Decapoda Caridea Alvinocarididae *Alvinocaris markensis*, Williams, 1988.

Crustacea Natantia Decapoda Caridea Nematocarcinidae *Nematocarcinus gracilis*, Spence Bate, 1888.

Pogonophora Vestimentifera Ridgeiidae *Ridgeia piscesae*, Jones, 1985.

Annelida Polychaeta Ampharetidae *Paralvinella palmiformis*, Desbruyeres & Laubier, 1986.

Mollusca Bivalvia Vesicomyidae *Calyptogena pacifica*, Specific status in doubt: see Peek *et al.* 1997