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Lipid profiles of hydrothermal vent shrimps

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Introduction

Since their discovery in 1977, hydrothermal vents have presented us with numerous new and unusual taxa of marine organisms. Although there have been many in depth studies of the physiology of some of these animals, the life history strategies of relatively few vent species have been elucidated.

Rimicaris exoculata Williams & Rona, 1986 is the dominant vent shrimp at the deeper Mid-Atlantic Ridge hydrothermal vent sites. Stable isotope studies indicate that adult shrimps are reliant on a non-photosynthetic source of organic carbon, and nitrogen isotopes are consistent with the role of shrimps as primary consumers (Van Dover et al., 1988). A strong but circumstantial case has been developed for the importance of epibiotic bacteria in the nutrition of the shrimps, but they may also feed on bacteria on sulphide surfaces. Significant gene flow exists between populations at the TAG and Broken Spur vent fields (Creasey et al., 1996). *Nematocarcinus gracilis* Spence Bate, 1888 is a deep-sea caridean shrimp found in the Arabian Sea. It is a benthic scavenger and predator collected in an area of seasonal upwelling and is used as a comparator in this study.

Lipids are distinguished by their solubility in hydrocarbons and insolubility in water. They include a variety of compounds with diverse functions. Lipids can be classed according to their physical and structural properties. Polar lipids are soluble in polar organic solvents and include phospholipids and glycolipids. These constitute the bulk of membrane lipids in an organism. Triglycerides and wax esters are neutral lipids that are used for energy storage. Fatty acids are components of many types of lipid. Naturally occurring fatty acids vary in chain length and degree of unsaturation and certain fatty acids can only be produced by plants and some bacteria. As a result of this, fatty acids may be used as biomarkers of sources of organic carbon. The lipid profiling technique used here combines total lipid, lipid class and fatty acid composition of an organism to inform us about lipid function and sources of organic matter.

The lipid profiles of the organisms studied are discussed in terms of their ecological significance to the life history strategy of hydrothermal vent shrimps.

Methods

Rimicaris exoculata were collected from the TAG hydrothermal mound using the *Mir* submersibles during the BRAVEX/94 expedition to the Mid-Atlantic Ridge. Postlarval bresiliids were collected from the water column above the Broken Spur vent field during the FLUXES1 (CD95) cruise in 1995. Allozyme analysis (Creasey et al., 1996) confirmed that the small, oily, orange bresiliids collected at TAG were juvenile *R. exoculata*. The identity of the postlarval bresiliids collected from above the Broken Spur vent field is uncertain. They probably included *R. exoculata, Chorocaris chacei* (Williams & Rona, 1986) and *Alvinocaris markensis* Williams, 1988 (see Dixon & Dixon, 1996). *Nematocarcinus gracilis* were collected from a depth of 900 m in the Arabian Sea using an epibenthic sled during RRS *Discovery* cruise 211 in October 1994.

Total lipid was extracted from tissue samples after Folch et al. (1957). Lipid class and total lipid concentrations were determined by Thin Layer Chromatography (TLC) with Flame Ionisation Detection. Wax or sterol esters, triglycerides, free fatty acids and sterols were developed in 85:15 hexane:chloroform containing 5% i-propanol and 0.5% formic acid. SIII Chromarods were partially scanned and then phosphatidyl ethanolamine, phosphatidyl choline, lysophosphatidyl choline and sphingomyelin were separated in 70:30:3.5 chloroform:methanol:water and the rods fully scanned. SIII Chromarods were individually calibrated for each lipid class. Total lipid was dissolved in toluene and

transmethylated by heating with 1.5% sulphuric acid in dry methanol at 50°C for 16 hours. The resulting fatty acid methyl esters (FAME) were purified by TLC. Fatty alcohols were separated at this stage and esterified by heating to 37°C with 2:1 pyridine:acetic anhydride for 15 minutes, then purified by TLC. FAME and fatty alcohol acetates were analysed by GC using a PE 8500 gas chromatograph equipped with a BPX70 column (50 m, 0.25 µm i.d., SGE) using hydrogen as a carrier gas. Peaks were identified by reference to known standards and by GC-MS using a VG7070 Micromass fitted with a BPX5 column (25 m, 0.25 mm i.d., SGE) using helium as the carrier gas.

Results

Mean total lipid and lipid class composition for large adult, small adult and juvenile *Rimicaris exoculata,* postlarval bresiliids and *Nematocarcinus gracilis* are presented in Table 1. Juvenile *R. exoculata* and postlarval bresiliids had elevated levels of total lipid tissue concentration compared to adults and non-vent shrimps. This increase is accounted for by very high levels of wax esters per gram dry weight of tissue. Wax esters were not detected in *N. gracilis* and wax ester levels in adult *R. exoculata* decreased with increasing size. Triglyceride was the major neutral lipid in *N. gracilis* and adult *R. exoculata*.

Table 1. Shrimps lipid class composition is quoted in mg g^{-1} dry weight of tissue ± S.E. (S.E. = $\sigma/\sqrt{n}$). Abbreviations: WE, wax ester; TG, triglyceride; FA, free fatty acid; S, sterol; PL total polar lipid; TL, total lipid; CL, carapace length; n.d., not detected.

Shrimps	WE	TG	FA & S	PL	TL
R. exoculata >16 mm CL (n = 54)	0.88±0.20	42.6±2.0	6.7±0.4	17.8±0.8	68.0±2.7
R. exoculata <16 mm CL (n = 54)	4.3±0.6	17.3±1.2	5.5±0.4	20.8±0.6	47.8±1.9
Juvenile *R. exoculata* (n = 12)	239±23	43.5±9.0	n.d.	12.6±0.9	295±13
Postlarval bresiliids (n = 18)	277±9.0	25.5±7.0	n.d.	13.7±0.4	316±10
N. gracilis (n = 84)	n.d.	52.5±1.5	8.9±0.4	20.4±0.5	81.8±1.8

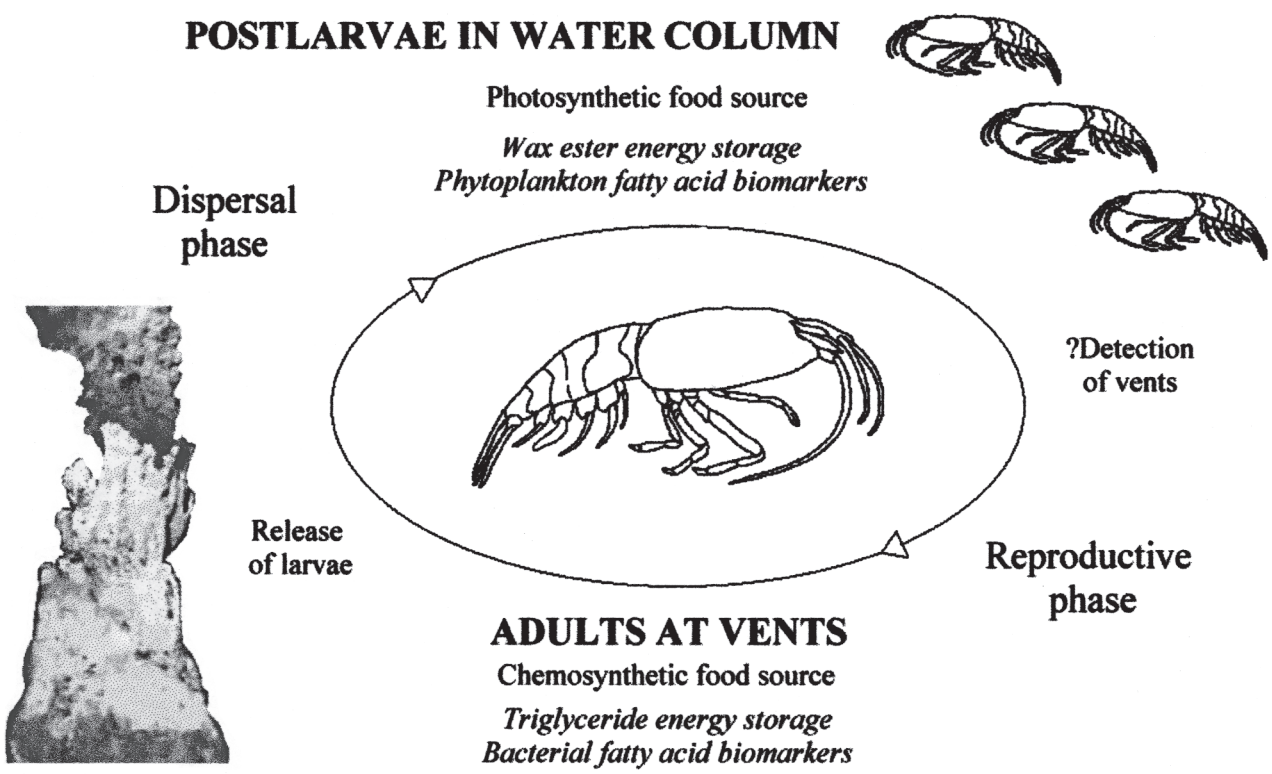

Figure 1. Proposed life cycle for *Rimicaris exoculata*.

The major fatty acids(1) detected in adult *R. exoculata* were 16:0, 16:1 n-7, 16:2 n-4, 18:1 n-7 and 18:2 n-4. Where detected in adult *R. exoculata,* the highly unsaturated fatty acids 20:5 n-3 and 22:6 n-3 were present only in small amounts. Juvenile *R. exoculata* and postlarval bresiliids were distinguished by fatty acid profiles with 16:0, 16:1 n-7, 18:1 n-9, 20:5 n-3 and 22:6 n-3 as their major components. Fatty alcohols identified as 16:0, 18:0 and 18:1 were detected in juvenile and postlarval bresiliids and in wax ester containing adult *R. exoculata*. *N. gracilis* had similar fatty acid profiles to the juvenile and postlarval samples examined, with major fatty acids comprising 16:0, 16:1 n-7, 18:1 n-9, 20:5 n-3 and 22:6 n-3.

Discussion

The lipid profiles described, combined with the results of reproductive and genetic studies, allow the proposal of a life history strategy for a hydrothermal vent shrimp shown in Fig. 1. Wax esters are common storage lipids in marine organisms such as copepods that live in marine environments with intermittent food supplies (Sargent, 1976). Results for *N. gracilis* reinforce that the usual form of storage lipid in deep-sea benthic shrimps is triglyceride. Neutral lipid class results here show that postlarval and juvenile vent shrimps use wax esters as their main storage lipid and that, as adults living at the vents, they change over to triglyceride as storage lipid. From this, it may be inferred that postlarval and juvenile vent shrimps are adapted to live with an intermittent food source, while adults rely on a more constant food source, similar to other deep-sea benthic shrimps.

The fatty acid profile of juvenile and postlarval bresiliids suggests that they rely on a photosynthetic food source. Although the HUFA 20:5 n-3 and 22:6 n-3 can be produced by some deep-sea bacteria, they are usually indicative of an ultimately phytoplanktonic food source. The combination of the occurrence of these fatty acids and wax esters in juvenile and postlarval vent shrimps and their discovery in the water column above the Broken Spur hydrothermal vents suggests that they spend part of their lifetime feeding on phytodetritus in the water column.

Although 16:2 n-4 is known to be produced by some species of diatom, 18:2 n-4 is an unusual fatty acid. Marine organisms rarely possess the necessary desaturases to produce polyunsaturated fatty acids. Carbon stable isotope values reported by Van Dover et al. (1988) are consistent with a non-photosynthetic source of organic carbon for adult *R. exoculata,* so it can be inferred that 16:2 n-4 and 18:2 n-4 fatty acids are produced by chemoautotrophic vent bacteria and are bacterial biomarkers.

In summary, it is proposed that adults *R. exoculata,* living at hydrothermal vents, feed on chemosynthetic bacteria and use triglyceride as their energy storage medium. Their larvae feed on phytodetritus in the water column and use wax esters for energy storage. This larval stage facilitates dispersal between vent sites evidenced by genetic studies (Creasey et al., 1996), although the mechanism for detection of an active vent remains uncertain.

(1) Fatty acid nomenclature is simplified using the following shorthand 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) and C denotes the position of unsaturation (number of carbon atoms from the ω (alkyl) end of the carbon chain).

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