P.S.Z.N.: Marine Ecology, 22 (3): 241–253 (2001)

Accepted: February 20, 2001
ISSN 0173-9565

Lipid Partitioning in the Hydrothermal Vent Shrimp *Rimicaris exoculata*

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With 1 figure and 5 tables

Keywords: Caridean shrimp, chemoautotrophic bacteria, fatty acid, hydrothermal vents, lipid, reproduction, *Rimicaris exoculata*.

Abstract. This study examines the composition and partitioning of lipids in the alvinocarid shrimp *Rimicaris exoculata* from Mid-Atlantic hydrothermal vents. Juveniles and adults at different stages of reproductive development were dissected into abdomen, branchial and ovary/hepatopancreas tissues. Each of these tissues was analysed for total lipid and lipid class composition, and fatty acids and fatty alcohols were identified using GC and GC-MS. Adult and juvenile shrimp differ in the partitioning of lipids between tissues. Juveniles store lipids in the abdomen as wax ester droplets and may use phosphatidyl choline as an additional reserve. Adult shrimp use triglycerides as an energy store, and triglycerides and polar lipids accumulate in ovary and hepatopancreas tissue during reproductive development. The wax ester storage droplets of juvenile shrimp contain high concentrations of n-3 fatty acids, which are photosynthetically-derived and thought to be important for reproductive development in crustaceans. These n-3 fatty acids are concentrated in the ovary and hepatopancreas of adults compared to other tissues. The n-3 fatty acid content of these adult tissues is well within that estimated for whole juvenile shrimp, supporting the hypothesis that the n-3 fatty acids putatively required for adult reproduction are stored from the juvenile stage.

Problem

Rimicaris exoculata Williams & Rona 1986 is a species of alvinocarid shrimp found in varying abundance at hydrothermal vent sites on the Mid-Atlantic Ridge (MAR) (Van Dover, 1995). Adult *R. exoculata* feed on bacteria, either growing epibiotically within the shrimps' carapace on modified appendages known as scaphagnothites (Casanova *et*

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al., 1993; Gebruk *et al.*, 1993; Gebruk *et al.*, 2000), or scraped from the surfaces of sulphides around the vents (Van Dover *et al.*, 1988; Segonzac *et al.*, 1993). Recent stable isotope studies, however, suggest that the latter source may not be significant in the nutrition of the shrimp (Pond *et al.*, 2000). It has also been postulated that adult shrimp gain some of their nutrition from endosymbiotic gut bacteria that are thought to oxidise polymetallic sulphide particles ingested by the shrimp (Polz *et al.*, 1998). Whatever the source, the fatty acid composition of adult *R. exoculata* reflects their dependence on bacterially-derived nutrition (Pond *et al.*, 1998; Allen Copley *et al.*, 1998; Rieley *et al.*, 1999; Pond *et al.*, 2000) and adult shrimp store lipid in the form of triglyceride (Allen Copley *et al.*, 1998).

In contrast, juvenile *R. exoculata* are rich in wax esters and photosynthetically-derived fatty acids (Pond *et al.*, 1997; Allen Copley *et al.*, 1998). *R. exoculata* produces lipid-rich eggs (Ramirez *et al.*, 2000) and planktotrophic larvae (Tyler & Young, 1999). There is no evidence of gametogenic synchrony (Ramirez Llodra *et al.*, 2000), but a polymodal population structure for *R. exoculata* suggests periodic recruitment to the vents (Copley, 1998). Postlarvae have been discovered in the water column at considerable distances from known sites of hydrothermal venting (Herring & Dixon, 1998) and allozyme analysis has shown a high level of gene flow between *R. exoculata* at the TAG and Broken Spur vent sites, which are 380 km apart on the MAR (Creasey *et al.*, 1996; Shank *et al.*, 1998). Reports of high levels of wax esters and phototrophically-derived fatty acids in the tissues of postlarval alvinocarid shrimp (Pond *et al.*, 1997; Allen Copley *et al.*, 1998) also support a pelagic, planktotrophic existence at this life stage.

Although primary production by chemosynthetic bacteria at hydrothermal vents constitutes a rich source of organic carbon in the deep sea, an entirely bacterial diet may not meet all the nutritional requirements of adult *R. exoculata*. Highly unsaturated fatty acids (HUFA) such as 20:5 n-3 and 22:6 n-3 are precursors to prostaglandins and play an important role in crustacean reproduction. They have been shown to induce hatching in barnacle eggs (Clare & Walker, 1986) and 20:5 n-3 has been shown to be necessary for vitellogenesis in *Penaeus japonicus* (Muriana *et al.*, 1993). Shrimp are unable to synthesise HUFA *de novo* (Middleditch *et al.*, 1980) and therefore require them from a dietary source. HUFA are relatively scarce in the deep sea in general (*e.g.,* Wakeham *et al.*, 1997) and although several recent studies have shown that some bacteria are capable of producing 20:5 n-3 and 22:6 n-3 (reviewed by Russell & Nichols, 1999), these fatty acids have not been detected in bacterial mat samples collected at MAR vents (Pond *et al.*, 2000) or in bacteria scraped from the scaphagnothites of *R. exoculata* (Rieley *et al.*, 1999). It has therefore been suggested that phototrophically-derived HUFA may be accumulated by juvenile shrimp and preferentially stored for later use in reproduction by the adults (Gebruk *et al.*, 2000; Pond *et al.*, 2000).

The work presented here examines the lipid composition of juvenile and adult *R. exoculata* in order to address the following questions: (1) Are there differences in the partitioning of lipids between different tissues in juvenile and adult *R. exoculata*? (2) Does lipid composition vary with reproductive state? (3) Is there evidence that juvenile shrimp store photosynthetically-derived fatty acids for later reproductive use?

Material and Methods

1. Specimen collection

Rimicaris exoculata Williams & Rona 1986 were collected during the MAR/97 cruise to all known hydrothermal vent sites on the Mid-Atlantic Ridge (MAR) south of the Azores, aboard the R/V 'Atlantis', in July 1997. Specimens used in this study were collected from the White Button structure in the Broken Spur vent field (29°10.0' N, 43°10.4' W, MAR, ~3050 m depth) using a slurp gun deployed by 'Alvin'. Adult specimens were sexed and their reproductive state scored: reproductive state scoring was on the basis of visible gonad size (after removal of the carapace/dorsal organ) in females (Fig. 1, after Chang & Shih, 1995). Muscle tissue from the abdomen, tissue from the branchial area (scaphagnothites and associated bacteria) and combined ovary and hepatopancreas were immediately dissected (separate dissection of ovary from hepatopancreas proved difficult, especially at earlier reproductive stages). Muscle tissue from the abdomen and tissue from the branchial area were also dissected for juvenile specimens (discriminated by their smaller size and bright orange coloration (Creasey *et al.*, 1996; Shank *et al.*, 1998). All tissue samples were wrapped in aluminium foil and placed in labeled ziplock bags in a cryo-freezer at –70 °C.

2. Lipid extraction

Tissues from three individuals of the same sex and reproductive state were combined to provide enough total lipid for analysis: tissue from twenty-four female, nine male and nine juvenile individuals were combined to provide eight, three and three samples for lipid analysis, respectively. There was insufficient combined ovary and hepatopancreas tissue from shrimp at the 0 and I stages of reproductive development for analysis. Lyophi-

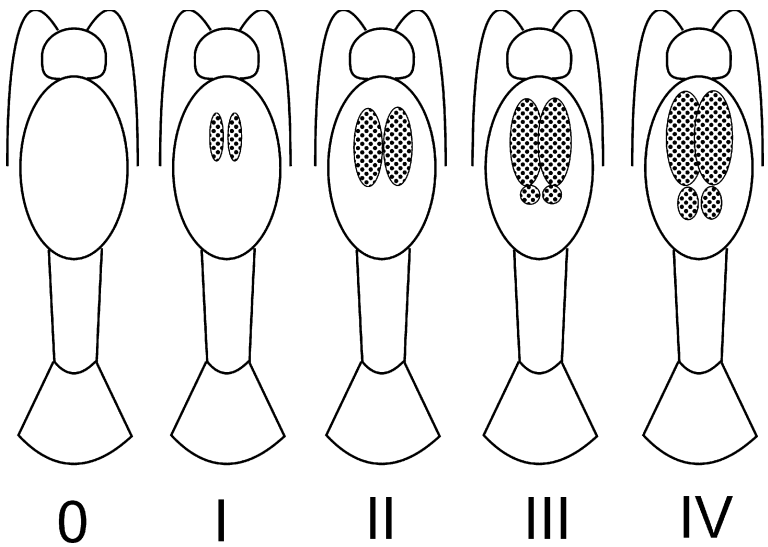

Fig. 1. Reproductive state scoring used for female *R. exoculata* (after Chang & Shih, 1995). Shaded areas represent ovarian tissue, which is cream in colour and lies on top of the yellow/orange hepatopancreas. Stages 0 (no ovarian development) and V (egg carrying) show no ovarian tissue. At stage I 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.

lised tissue was weighed, re-hydrated to ensure efficient triglyceride extraction (Dunstan *et al.*, 1993), extracted with 2:1 chloroform/methanol and washed according to the procedure of Folch *et al.* (1957). Total lipid extracts were evaporated to dryness under nitrogen, weighed, re-dissolved in a small amount of chloroform containing 0.01 % BHT and stored under nitrogen at –20 °C until analysis.

3. Lipid class analysis

Lipid class composition was determined by thin layer chromatography with flame ionisation detection (TLC-FID). Neutral lipids were eluted in 85:15 hexane/chloroform containing 5 % i-propanol and 0.5 % formic acid (Shantha & Ackman, 1990). SIII Chromarods were partially (90 %) scanned using an Mk IV Iatroscan, then polar lipids were eluted in 70 : 30 : 3.5 chloroform/methanol/water (Fraser *et al.*, 1985) before a full Chromarod scan. Standards were run on two of the ten Chromarods to allow sample peak identification by comparison. Quantification was achieved by constructing of a calibration curve over the range of observed concentrations for each lipid class.

4. Fatty acid analysis

Aliquots of total lipid were dissolved in toluene and transmethylated with 1.5 % sulphuric acid in methanol under nitrogen at 50 °C for a minimum of 16 h (Christie, 1982). Fatty acid methyl esters (FAME) were extracted and purified by thin layer chromatography (TLC) before analysis by gas chromatography (GC) on a PE8500 gas chromatograph equipped with a BPX 70 fused silica capillary column (50 m × 0.22 mm ID, SGE), using hydrogen as a carrier gas. FAME were identified by reference to a cod liver oil standard and *R. exoculata* FAME samples of composition previously determined by GC-MS (mass spectrometry) (Allen Copley *et al.*, 1998; Pond *et al.*, 1998). Fatty alcohols released during transmethylation were extracted from TLC plates at the FAME purification step. Fatty alcohol acetates were produced by addition of 1 : 2 acetic anhydride/pyridine to the dry fatty alcohols under nitrogen and heating to 37 °C with occasional shaking for 15 min (Farquhar, 1962). Fatty alcohol acetates were analysed by GC in the same way as FAME and identified by GC-MS.

Results

1. Total lipid and lipid class composition

The highest levels of total lipid were detected in ovary/hepatopancreas tissue in adult *R. exoculata* and in abdomen tissue of juveniles (Table 1). In hepatopancreas/ovary tissue, most of this lipid constituted triglyceride, while wax esters were the major lipid component in juvenile abdomen tissue. Adult abdomen tissue comprised mostly polar lipids. In both adult and juvenile shrimp, branchial area tissue was composed of around 50 % polar lipids. Significant levels of storage lipids in the form of triglyceride (adult shrimp) and wax esters (juvenile shrimp) were also detected in branchial area tissue.

Phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) were the major phospholipid classes detected in all tissues of *R. exoculata* analysed. Lysophosphatidyl choline and sphingomyelin were also present at low concentrations. Non-detection probably reflects the detection limits of the TLC-FID method rather than true absence in the tissues concerned. The detection limits for these compounds were typically in the range of 0.5 to 1 % dry tissue weight. In addition, other polar lipids such as phosphatidyl serine and cardiolipin may have been present in the samples analysed – but at levels below method detection limits. Juvenile specimens contained lower levels of PE than

adults (Table 1). Adult specimens had approximately equal tissue concentrations of PC and PE, but juveniles contained a higher proportion of the former.

There was no clear trend in lipid tissue concentrations for combined ovary and hepatopancreas tissue at different stages of development (Table 2). However, the highest triglyceride and polar lipid tissue concentrations were detected in tissue at reproductive stage IV.

2. Fatty acid and fatty alcohol composition

There were no significant differences in the fatty acid composition of abdomen or branchial area tissues from male and female shrimp. Saturated fatty acids comprised around 15 % by weight of the fatty acids of all tissue types of adult *R. exoculata* (Table 3). A lower proportion of saturates (around 7 %) was found in the abdomen region of juvenile *R. exoculata*, although this value was around 18 % in juvenile branchial tissue. Adult tissues bore higher ratios of 18:1 n-7/n-9 than juvenile tissues. This ratio was also significantly higher in juvenile branchial tissue than in juvenile abdomen tissue. Non-methylene interrupted dienoic (NMID) fatty acids were more abundant in adult tissue and present only at low levels in juveniles.

Proportions of n-4 PUFA (polyunsaturated fatty acids) were much higher in adult *R. exoculata* than in juveniles. Among adults, ovary and hepatopancreas tissue contained the highest proportion of n-4 fatty acids, followed by branchial tissue and then abdomen. In contrast, branchial tissue contained the highest proportion of n-4 fatty acids in juveniles (Table 3).

The highest percentages of n-3 fatty acids were found in juvenile abdomen tissue (Table 3). Their proportion in adult abdomen tissue was approximately the same as that of n-4 fatty acids, while n-3 fatty acids constituted a smaller proportion of branchial area and combined ovary and hepatopancreas tissues. The n-6 fatty acids were also most abundant in juvenile abdomen tissue, with lower levels in juvenile branchial tissue and low levels in all adult tissue types.

It was not possible to determine absolute amounts of fatty acids in the reproductive tissue of individual shrimp because tissue from several shrimp was combined to provide sufficient lipids for the analyses. However, in order to compare the relative amounts of different fatty acids in different tissues, approximate tissue concentrations were calculated by multiplying proportions of fatty acids by mean total lipids [mg·g (dry weight)$^{-1}$] for the appropriate tissue (Table 4).

The highest tissue concentrations of n-3 fatty acids were detected in juvenile shrimp tissues. In adult shrimp the highest concentrations were found in ovary and hepatopancreas tissue. Although the proportion of saturated fatty acids was lower in juvenile shrimp abdomen, tissue concentrations of these fatty acids were actually higher than those in adult shrimp.

There were few apparent differences in the percentage fatty acid composition of ovary and hepatopancreas tissue from shrimp at different reproductive stages (Table 3). The percentage of n-4 fatty acids seemed to be higher in shrimp at reproductive stage II versus stages III and IV. The percentage of n-3 fatty acids appeared to be lower at stage II than stages III and IV and the reverse was true for n-6 fatty acids. Tissue concentrations of n-3 fatty acids appeared to increase with reproductive development, as did

Table 1. Mean lipid class composition [mg·g (dw)$^{-1}$] ± SE ($\sigma/\sqrt{n}$) of different tissues of adult and juvenile *R. exoculata*. (female abdomen and branchial tissues, n = 8; male and juvenile tissues, n = 3; combined ovary and hepatopancreas tissue, n = 6). WE, wax esters; TG, triglycerides; FA, free fatty acids; S, sterols; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline; PL, polar lipids; TL, total lipids.

	WE	TG	FA	S	PE	PC	SM	LPC	PL	TL
female abdomen	nd	0.93 ± 0.10	1.10 ± 0.08	3.22 ± 0.54	9.69 ± 0.64	10.5 ± 0.70	0.28 ± 0.05	0.99 ± 0.24	21.5 ± 1.46	26.7 ± 2.05
male abdomen	nd	0.60 ± 0.60	1.56 ± 0.05	3.62 ± 0.47	10.2 ± 0.93	12.0 ± 1.04	0.24 ± 0.02	0.69 ± 0.18	23.2 ± 1.49	29.0 ± 2.55
juvenile abdomen	89.8 ± 26.1	1.66 ± 0.42	1.94 ± 0.27	1.29 ± 0.27	4.13 ± 0.16	8.53 ± 0.60	nd	nd	12.7 ± 0.95	107 ± 27.4
female branchial	nd	17.4 ± 6.07	2.78 ± 0.17	2.80 ± 0.25	12.8 ± 1.35	11.5 ± 1.32	0.52 ± 0.06	0.74 ± 0.28	25.5 ± 2.71	48.2 ± 8.07
male branchial	nd	8.09 ± 2.27	3.17 ± 0.10	2.90 ± 1.18	12.6 ± 1.12	12.7 ± 1.07	0.54 ± 0.02	1.46 ± 0.16	27.3 ± 2.04	41.4 ± 4.86
juvenile branchial	32.0 ± 9.54	2.73 ± 0.75	3.01 ± 1.59	1.90 ± 1.17	5.83 ± 2.92	15.2 ± 0.92	nd	nd	21.1 ± 2.07	37.1 ± 7.05
ovary/hepatopancreas	nd	116 ± 11.1	4.99 ± 0.75	2.92 ± 0.98	8.82 ± 1.61	21.8 ± 6.14	0.33 ± 0.12	0.86 ± 0.36	31.9 ± 8.12	156 ± 20.4

Table 2. Lipid class composition [mg·g (dw tissue)$^{-1}$], means and ranges in parentheses where n > 1 (F II, n = 1; F III, n = 2; F IV, n = 3) for combined hepatopancreas/ovary tissue of *R. exoculata* at different stages of reproductive development. TG, triglycerides; FA, free fatty acids; S, sterols; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; SM, sphingomyelin; LPC, lysophosphatidyl choline; PL, polar lipids; TL, total lipids.

	TG	FA	S	PE	PC	SM	LPC	PL	TL
female II	107	3.45	n.d.	5.13	11.9	n.d.	n.d.	17.0	127
female III	104	5.35	2.06	7.56	16.4	0.37	0.83	24.5	136
	(97.2,111)	(4.74, 5.97)	(1.21, 2.91)	(5.76, 9.37)	(11.8, 20.9)			(18.8, 30.3)	(122, 150)
female IV	128	5.26	4.47	10.9	28.8	0.54	1.44	41.7	179
	(95, 169)	(2.96, 8.01)	(2.99, 7.10)	(8.19, 16.2)	(15.8, 51.6)	(0.35, 0.8)	(0.84, 2.40)	(25.5, 71.0)	(129, 255)

Table 3. Mean percentage fatty acid composition of different tissues (F, female; M, male; J, juvenile; ab, abdomen; br, branchial; II, III & IV, hepatopancreas/ovary tissue at respective reproductive development stage) of *R. exoculata*. Standard errors are omitted for clarity but were generally less than 5 % of the mean. SFA, saturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids.

fatty acids	F ab	M ab	J ab	F br	M br	J br	F II	F III	F IV
14:0	0.55	0.23	0.27	3.81	3.47	1.64	4.32	4.71	4.14
14:1	0.44	0.32	0.34	2.86	1.68	1.56	3.42	3.68	3.77
15:0	0.14	0.16	0.13	0.27	0.19	1.84	0.68	0.07	0.03
16:0	10.7	12.4	4.99	8.34	8.66	11.3	5.89	8.57	9.30
16:1 n-7	15.6	13.5	15.1	23.4	21.9	19.5	21.8	24.3	25.5
16:2 n-4	1.29	1.11	0.26	7.06	6.29	0.39	6.71	8.71	8.29
16:3 n-3	nd	nd	0.47	nd	nd	0.96	nd	nd	nd
18:0	2.69	3.20	1.40	1.91	2.39	3.61	0.45	0.97	1.08
18:1 n-9	5.25	4.43	20.6	3.73	3.55	20.2	2.82	2.68	2.43
18:1 n-7	21.0	23.6	9.45	13.7	15.1	18.8	11.2	11.7	12.1
18:2 n-6	1.10	0.91	2.01	0.95	1.00	1.96	1.30	0.95	1.05
18:2 n-4	13.9	13.7	0.85	14.7	14.4	2.36	18.6	19.5	19.2
18:3 n-6	nd	nd	0.30	5.00	5.09	0.10	nd	nd	nd
18:3 n-3	7.14	5.84	0.27	1.91	1.95	0.35	5.30	5.50	6.03
18:4 n-4	1.13	1.56	0.34	0.23	0.23	1.60	12.4	0.88	0.13
20:1 n-9	0.49	0.30	0.74	0.40	0.39	0.26	0.00	0.17	0.14
20:1 n-7	0.26	0.27	0.20	nd	nd	0.54	0.82	0.20	0.04
20:2	3.62	1.87	0.06	1.80	1.35	0.32	1.82	1.70	1.93
20:3 n-6	0.33	0.33	0.04	0.49	0.49	0.29	1.77	0.50	0.44
20:3 n-3	1.78	0.90	0.18	0.80	0.59	0.18	0.29	0.75	0.91
20:4 n-6	2.01	2.75	6.59	1.64	2.77	3.84	0.71	0.40	0.44
20:4 n-4	0.50	0.24	0.19	0.37	0.54	0.35	0.00	0.11	0.05
20:4 n-3	nd	nd	0.26	nd	nd	0.15	nd	nd	nd
22:1 n-11	0.90	0.86	0.26	0.89	0.96	0.72	0.41	0.85	0.60
20:5 n-3	3.38	4.03	8.13	1.97	2.73	1.34	0.17	0.49	0.48
22:2	0.65	0.39	0.06	0.81	0.37	0.18	0.38	1.08	0.64
22:5 n-6	nd	nd	5.69	nd	nd	2.38	nd	nd	nd
24:1	0.34	0.66	0.03	0.18	0.35	0.10	0.00	0.00	0.04
22:5 n-3	0.51	0.38	1.10	0.74	0.44	0.29	0.55	0.94	0.75
22:6 n-3	4.28	6.08	19.66	2.01	3.11	2.84	0.13	0.62	0.50
SFA	14.1	16.0	6.79	14.3	14.7	18.4	11.4	14.3	14.6
MFA	44.3	43.9	46.7	45.4	44.1	61.7	40.5	43.5	44.6
NMID	4.27	2.26	0.12	2.60	1.72	0.50	2.20	2.78	2.56
n-4 PUFA	16.8	16.6	1.65	24.0	23.2	4.69	38.0	29.2	27.7
n-6 PUFA	3.44	3.99	14.6	3.08	4.25	8.58	3.77	1.85	1.93
n-3 PUFA	17.1	17.2	30.1	10.5	12.0	6.11	6.44	8.31	8.67
18:1 n-7/n-9	4.0	5.32	0.46	3.68	4.26	0.93	3.97	4.35	4.95

Table 4. Approximate tissue concentrations [mg · g (dw)$^{-1}$] of fatty acid classes in different tissues of *R. exoculata* (F, female; M, male; J, juvenile; ab, abdomen; br, branchial; II, III & IV, hepatopancreas/ovary tissue at respective reproductive development stage). Standard errors are omitted for clarity, but were generally less than 5 % of the mean. SFA, saturated fatty acids; MFA, monounsaturated fatty acids; NMID, non-methylene interrupted dienoic fatty acids; PUFA, polyunsaturated fatty acids.

fatty acids	F ab	M ab	J ab	F br	M br	J br	F II	F III	F IV
SFA	3.8	4.6	7.3	6.9	6.0	6.8	14.5	19.4	26.1
MFA	12.0	12.7	50.0	21.8	18.1	22.8	51.4	59.2	79.8
NMID	1.2	0.7	0.1	1.2	0.7	0.2	2.8	3.8	4.6
n-4 PUFA	4.5	4.8	1.8	11.5	9.5	1.7	48.3	39.7	49.6
n-6 PUFA	0.9	1.2	15.6	1.5	1.7	3.2	4.8	2.5	3.5
n-3 PUFA	4.6	5.0	32.2	5.0	4.9	2.3	8.2	11.3	15.5

Table 5. Mean percentage (by weight) fatty alcohol composition of abdomen and branchial region tissues in juvenile *R. exoculata* ± SE ($\sigma/\sqrt{n}$); n = 3.

	abdomen tissue	branchial tissue
14:0 alcohol	0.75 ± 0.18	3.54 ± 0.54
14:1 alcohol	1.74 ± 0.93	6.17 ± 0.66
16:0 alcohol	48.2 ± 1.36	43.7 ± 1.11
16:1 alcohol	0.90 ± 0.34	nd
18:0 alcohol	8.86 ± 0.34	7.99 ± 0.24
18:1 n-9 alcohol	15.6 ± 0.81	11.9 ± 0.28
18:1 n-7 alcohol	22.0 ± 0.93	20.7 ± 1.18
18:2 alcohol	1.29 ± 0.67	6.05 ± 1.03
20:1 alcohol	0.64 ± 0.19	nd
saturated fatty alcohol	57.8 ± 0.69	55.2 ± 0.47
monounsaturated fatty alcohol	41.0 ± 0.72	38.8 ± 0.71

those of saturated and monounsaturated fatty acids, but in all cases, the small sample size did not allow distinction of significant differences.

The major fatty alcohols detected in tissues of juvenile *R. exoculata* 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 (Table 5). The composition of abdomen and branchial regions was broadly similar, although higher levels of 14 : 0 and 14 : 1 fatty alcohols were detected in the branchial region.

Discussion

1. Lipid partitioning in adult and juvenile *R. exoculata*

Juvenile shrimp apparently concentrate lipid in the form of wax esters in their abdomen region, whereas adult shrimp store lipid in the form of triglyceride in the hepatopancreas/ovary. The high concentrations of wax esters detected in the abdomen and bran-

chial tissues of juvenile shrimp correspond with the presence of an oily substance observed while dissecting these tissues. On dissection, the hepatopancreas of juvenile shrimp was barely visible and it seemed that their lipid is stored mostly in the abdomen as wax esters in droplets. Dissected adult male shrimp possessed only a small hepatopancreas – similar in size to that of female shrimp at early reproductive stages – yielding insufficient tissue to analyse the lipid composition of hepatopancreas in males and females at early reproductive stages. At later female reproductive stages, hepatopancreas size increased, and in adult females, lipids in the form of triglycerides appeared to be stored primarily in combined hepatopancreas and ovary tissues.

The detection of high n-3 fatty acid concentrations in the wax-ester-rich abdomen of juvenile shrimp is consistent with recent reports of very high levels of these fatty acids in the wax esters of juvenile vent shrimp (Pond *et al.*, 2000). As expected, higher proportions of bacterial biomarker fatty acids such as 18 : 1 n-7, 18 : 2 n-4 and non-methylene interrupted dienoic (NMID) fatty acids (Pond *et al.*, 1998; Pond *et al.*, 2000) were detected in the tissue of adult versus juvenile shrimp. Ratios of 18 : 1 n-7/n-9 were also higher in adults than in juveniles, reflecting the increased proportion of bacterially derived organic matter in adults.

Free fatty acids comprised less than 10 % of total lipid in all tissues, suggesting that samples were in a good state of preservation (Jeckel *et al.*, 1989). Free fatty acid levels were highest in combined ovary and hepatopancreas tissue; this reflects the digestive function of hepatopancreas tissue and increased metabolic activity here compared with branchial and abdominal tissues.

Decapod crustaceans have been shown to accumulate phosphatidyl choline, as well as neutral storage lipids, during periods of high food availability (Mayzaud *et al.*, 1998). The higher levels of PC than PE in juvenile *R. exoculata* may therefore indicate their use of PC as an additional lipid store.

Branchial area tissue of both adult and juvenile shrimp contained higher proportions of bacterially-derived n-4 fatty acids than abdomen tissue, probably reflecting the presence of bacteria here. Note also that the proportion of n-4 fatty acids is highest in tissues of adult shrimp that are richest in triglycerides. This is consistent with a significant portion of adult shrimp storage lipids having been derived from a bacterial source and therefore accumulated after the shrimp have recruited to the vent ecosystem.

Triglyceride detected in the branchial area tissue of adults may represent contamination from combined ovary and hepatopancreas tissue, but the observed levels seem a little high for this to be the case. Under certain conditions, marine bacteria have been shown to store lipids in the form of triglycerides (Alvarez *et al.*, 1997), so it is conceivable that triglyceride detected in adult vent shrimp gills represents lipids from bacterial epibionts. Histological staining of carefully prepared sections of branchial tissue might confirm whether epibionts contain triglyceride stores, without the possibility of contamination from other tissues.

2. Variations in lipid composition with reproductive development

Although the small sample size did not allow the identification of clear trends in the lipid class composition of ovary and hepatopancreas tissue with reproductive development, the highest concentrations of polar lipids and triglycerides were found in this tissue at reproductive stage IV. It might be speculated that, as in other marine shrimp (Jeckel *et al.*, 1989; Teshima *et al.*, 1989; Mourente & Rodriguez, 1991), triglycerides and polar lipids do accumulate with reproductive development. Previous studies have reported a reciprocal relationship between lipid composition of hepatopancreas and ovary tissues (Adiyodi & Subrahmanian, 1983; Spaargaren & Haefner, 1994), but the analysis of combined ovary and hepatopancreas tissues here would have integrated any such variations.

3. Phototrophically-derived, highly unsaturated n-3 fatty acids

Highly unsaturated n-3 fatty acids have been shown to play an important role in crustacean reproduction (*e.g.,* Clare & Walker, 1986; Muriana *et al.*, 1993). In the study reported here, a lower proportion of n-3 fatty acids was detected in ovary and hepatopancreas tissue than in abdomen tissue of adult shrimp.

This is initially puzzling as it seems to suggest that these fatty acids were not preferentially stored in reproductive tissue as hypothesised. This apparent pattern may reflect the different lipid class composition of these two tissue types: abdomen lipid comprised mostly polar lipid, while ovary and hepatopancreas is predominantly triglyceride. In a recent study of the fatty acid composition of different lipid classes of *R. exoculata*, Pond *et al.* (2000) reported that polar lipids are generally richer in n-3 fatty acids than neutral lipids. However, if we consider fatty acids in terms of tissue concentrations (Table 4), n-3 fatty acids are clearly concentrated in ovary and hepatopancreas tissue in comparison with other adult tissues.

Could the n-3 fatty acids concentrated in ovary and hepatopancreas tissue have been stored from a juvenile stage? In a previous study of the fatty acid composition of whole *R. exoculata* (Allen, 1998), the n-3 fatty acid concentration in whole juveniles was 27 mg·g (dry weight)$^{-1}$ for individuals that weighed about 0.1 g. An individual juvenile shrimp therefore contains an estimated 2.7 mg of n-3 fatty acids. In the same study (Allen, 1998), the tissue concentration of n-3 fatty acids in whole adult female *R. exoculata* with dry weights of up to 0.58 g was *ca.* 3 mg·g (dry weight)$^{-1}$. This suggests a total amount of around 1.7 mg of n-3 fatty acids per adult. Combined ovary and hepatopancreas tissue weights were not recorded for individual adult shrimp in the present study – but an extrapolation from the combined weights indicated a dry weight of up to 0.03 g for a single individual at reproductive stage IV. Combining this value with the approximate tissue concentrations presented in Table 4, it can be estimated that an individual adult might have stored a total of 0.5 mg of n-3 fatty acids in ovary and hepatopancreas tissue at the most advanced reproductive stage. This is well within the total amount of n-3 fatty acids estimated for juvenile shrimp (2.7 mg) and supports the hypothesis that

n-3 fatty acids required by the adults for reproduction could be stored from a juvenile stage.

It has been suggested that *R. exoculata* are iteroparous (Ramirez Llodra *et al.*, 2000), so if these shrimp do store n-3 fatty acids for reproduction, the proportion of these fatty acids present in ovary and hepatopancreas would be expected to drop with each reproductive output. There is no way of determining what the previous reproductive output of the individual *R. exoculata* studied here might have been. However, the estimated amount of n-3 fatty acids per shrimp (stage IV) ovary/hepatopancreas (0.5 mg) is less than one fifth of the estimated amount that could be stored by juvenile shrimp (2.7 mg). Thus, the argument that juvenile shrimp store sufficient fatty acids for use in reproductive development still seems reasonable.

Summary

Neutral lipid in the form of triglycerides is concentrated in ovary and hepatopancreas tissue of adult *Rimicaris exoculata*. Adult shrimp abdomen tissue comprises mostly polar lipids, whereas in juvenile shrimp this tissue is rich in wax esters. In adult *R. exoculata*, the highest tissue concentration of n-3 fatty acids thought to be required for reproduction was detected in ovary and hepatopancreas tissue.

Although small sample sizes did not allow distinction of statistically significant differences in lipid composition of ovary and hepatopancreas tissue at different reproductive stages, stage IV tissue appeared to contain the highest triglyceride and polar lipid tissue concentrations.

Estimates of the levels of putatively phototrophically-derived n-3 fatty acids in individual adult and juvenile shrimp suggest that juvenile shrimp do contain enough of these fatty acids to account for the levels detected in adult shrimp.

Acknowledgements

Thanks to the Captain & Crew of RV 'Atlantis' and 'Alvin', to Rich Lutz, Bob Vrijenhoek and the Scientific Party of MAR/97 for their co-operation with sample collection. Thanks also to Eva Ramirez for useful discussions on reproductive biology in *R. exoculata*. CEA was supported by a University of Southampton Ph. D. Studentship and NERC BRIDGE grant 97 (GST/02/1492). Ship time and 'Alvin' dives to collect the specimens for this study were also funded by NERC's BRIDGE programme.

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