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Biochemical composition of ovary, embryo, and hepatopancreas in the grapsoid crabs *Armases cinereum* and *Sesarma* nr. *reticulatum* (Crustacea, Decapoda)

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Abstract

Biochemical composition of ovary, embryo, and hepatopancreas tissues in wild populations of *Armases cinereum* and *Sesarma* nr. *reticulatum* were monitored during the reproductive season. Total lipid, carbon, nitrogen, C:N ratio, and water concentration of the ovary, hepatopancreas and embryos were quantified over the course of ovarian maturation. Ovary nitrogen concentration decreased as ovaries matured. Ovary lipid and carbon concentration differed significantly over the course of ovarian maturation for both species, but there was no relationship between the concentration or total content of hepatopancreas lipid and the stage of ovarian development in females. Neither species showed a relationship between measures of hepatopancreas lipid and the gonadosomatic index. There was also no simultaneously measurable net decrease in mass of the females' hepatopancreas. Lipid demands of ovarian maturation thus appear to be met in large part by increased dietary intake rather than by substantial draw down of pre-existing lipid stores from the hepatopancreas. While these temperate grapsoid crabs live with putatively fluctuating quality and quantity of food resources, no evidence could be found to demonstrate depletion of lipid concentrations in the hepatopancreas concomitant with ovarian maturation.
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1. Introduction

Populations of *Armases cinereum* Bosc and *Sesarma* nr. *reticulatum* Say are found commonly in intertidal areas near bayous and in well-drained salt marshes of Louisiana (Zimmerman and Felder, 1991). *S.* nr. *reticulatum*, a species from the Gulf of Mexico near (nr.) *S. reticulatum*, is morphologically, genetically, and physiologically distinct from *S. reticulatum* of the Atlantic coast (Zimmerman and Felder, 1991; Staton and Felder, 1992; Felder and Staton, 1994; Mangum and McKenney, 1996). Burrows of *S.* nr. *reticulatum* are characterized by an above-ground hood and

often connect with burrows of other species (Zimmerman and Felder, 1991). *S.* nr. *reticulatum* occurs in densities as high as 25 crabs m^{-2} and is thought to feed on live vegetation such as *Spartina alterniflora* as well as fiddler crabs, detritus, and algae (Zimmerman and Felder, 1991). In populations of *S. reticulatum* on the southeastern US Atlantic coast a mature male and several females will defend their interconnected burrows (Allen and Curran, 1974; Seiple, 1979). Burrow guarding behaviors have not been studied in Gulf populations of *S.* nr. *reticulatum*, though we have observed that these crabs also live communally in burrow systems. In Louisiana, the reproductive season of *S.* nr. *reticulatum* occurs from April to September (Zimmerman and Felder, 1991).

A. cinereum prefers to live along intertidal margins of marshes under debris and dense vegetation or constructs shallow burrows (Seiple, 1979). Commonly, *A. cinereum* will occupy the abandoned burrow of other species

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(Seiple, 1979). This species does not defend permanent burrows and spends less time in social interactions than does *S. reticulatum* (Seiple and Salmon, 1982). *A. cinereum* appears to be a scavenger that feeds on decaying plant matter and animals as well as shoots of *Spartina* (Seiple and Salmon, 1982). It is primarily nocturnal and during the day hides under debris and vegetation (Seiple and Salmon, 1982).

The lipid and relative fatty acid concentration in the larvae of *A. cinereum* and *S. reticulatum* have been studied in laboratory-hatched broods (Staton and Sulkin, 1991). Larvae of *A. cinereum* had a lower lipid concentration (9%) than did those of *S. reticulatum* (12%) (Staton and Sulkin, 1991). Staton and Sulkin (1991) suggested that *S. reticulatum* had high nutritional flexibility, which they defined as an ability to develop without high levels of PUFA (polyunsaturated fatty acid) in the diet. *S. reticulatum* showed resistance to starvation, while *A. cinereum* showed a complete lack of nutritional flexibility (Staton and Sulkin, 1991). It is not known if the lipid concentrations in Atlantic populations of *S. reticulatum* and the Gulf coast populations of *S. nr. reticulatum* are similar.

Few studies document the dynamic between lipid concentration and ovarian maturation for semi-terrestrial or intertidal crabs (Pillay and Nair, 1973; Mourente et al., 1994; Wen et al., 2001), and the present study is the first investigation of lipid concentration and content in grapsoid crabs from specifically the northwestern Gulf of Mexico. The purpose of this study was to measure the lipid, carbon (C), nitrogen (N), and water concentration of the ovary, hepatopancreas and embryos of wild caught crabs. Measurement of lipid concentration throughout the stages of ovarian maturation provides insight to the role of lipids in crustacean life histories and can indicate whether or not a given crustacean transfers lipid from the hepatopancreas to the ovary during gonad maturation. We hypothesized that lipid and C in the hepatopancreas would decrease as ovarian maturation progressed. We expected the amounts of lipid and C in the ovary, hepatopancreas and embryos would differ between these two species with putatively differing habitat and dietary preferences. Finally, we hypothesized there would be differences between males and females in the biochemical constituents of the hepatopancreas, most probably a result of ovarian maturation influencing the biochemical contents of the hepatopancreas.

2. Materials and methods

2.1. Sampling

Intermolt adult specimens of *A. cinereum* and *S. nr. reticulatum* (Crustacea, Decapoda, Sesarmidae) were sampled during their reproductive season in March–July 2001. Crabs were collected from coastal wetlands between Cypremort Bayou and Vermilion Bay near Cypremort Point, LA, USA (29°43′ 57.9″N; 91°50′ 37.3″W). Specimens were removed from burrows with shovels or caught as they ran for cover. Crabs were placed in individual vials and transported to the laboratory for analysis.

2.2. Morphometric analysis and ovary stage determination

Carapace width (CW) was measured with dial calipers (± 0.1 mm). Body wet weight WW (WW, ± 0.1 g), as well as dissected ovary and hepatopancreas tissue WW (± 0.0001 g) were determined with an analytical balance. Embryos on pleopods of ovigerous females were removed and weighed. All tissues were lightly blotted with tissue paper prior to weighing to remove surface water. Ovary, embryo, and hepatopancreas tissues were then lyophilized and stored in glass vials with Teflon® tops at -80 °C for further analyses. Gonadosomatic index (GSI) was equal to the ovary WW divided by the total body WW multiplied by 100 (Giese, 1966). Digestive gland index (DGI) for females was equal to the hepatopancreas WW divided by the total body WW minus ovary WW multiplied by 100 (Clarke, 1977). DGI for males was equal to the hepatopancreas WW divided by the total body WW multiplied by 100. Ovaries were classified into three developmental stages (I, II and III) on the basis of color and size (Ajmal Khan and Natarajan, 1980; Mourente et al., 1994).

2.3. Hepatopancreas color determination

During dissection, the hepatopancreas was classified into one of five color classes. Each class approximated a color in the Pantone® Color Matching System (Pantone, Carlstadt, NJ 07072, USA; colors as numbered below) found in Adobe Photoshop® (Adobe Systems, San Jose, CA 95110 USA). The classes were brown (419CV), which ranged to a brown-black with black patches; yellow-ochre (145CV), in which the general mass of the hepatopancreas ranged to uniform dark brown-yellow or dark-yellow with brown spots throughout tubules; white (5875CV), in which hepatopancreas tissues were uniform white or cream; lemon-yellow (129CV), in which the general mass of the hepatopancreas and tubules were a uniform yellow; and cadmium-yellow (102CV), in which the general mass of the hepatopancreas and tubules were a uniform high intensity yellow with little white.

2.4. Lipid extraction and CHN analysis

Lipids were extracted from tissues of individual animals using Parrish's modification of the Folch, Lees, and Sloane Stanley chloroform/methanol extraction method (Parrish, 1999). Lipid concentrations were determined gravimetrically by measuring mg of lipid (± 0.1 mg) g^{-1} dry weight (DW) tissue with an analytical balance. Total lipid content was determined by multiplying lipid concentration by organ

Table 1
Biometric means±SD for females at different stages of ovarian maturation

Armases cinereum	Ovary stages		
	I $(n=6)$	II $(n=4)$	III $(n=16)$
CW	16.5±4	18.2±1.2	17.2±3.3
Total WW	3.3±1	3.7±1.1	3.2±1.1
Ovary WW	0.04±0.01[a]	0.1±0.04	0.16±0.06[b]
GSI	1.2±0.5[a]	2.7±1[a]	5.3±2[b]
Hepatopancreas WW	0.18±0.07	0.3±0.1	0.16±0.04
Sesarma nr. *reticulatum*	I $(n=10)$	II $(n=13)$	III $(n=15)$
CW	18±3.6	22.1±1.6	21.2±3.3
Total WW	3.5±1.4	6.1±2.3	5.7±2
Ovary WW	0.04±0.03[a]	0.12±0.05[a]	0.26±0.1[b]
GSI	1.1±0.6[a]	2.1±0.8[b]	4.9±2[c]
Hepatopancreas WW	0.3±0.1	0.3±0.07	0.2±0.1

Carapace width CW is expressed in mm. Total wet weight WW, ovary and hepatopancreas WW are expressed in grams. Gonadosomatic index GSI=ovary WW/total WW×100. Values in a row with different superscripts are significantly different ($P<0.05$). Values without a superscript are not significantly different.

DW. All CHN analyses were conducted with tissues from individual animals. Tissues were dried prior to analysis at 60 °C. Each sample was analyzed in triplicate; analytical replicates that varied >5% were excluded from analyses. A CE Instruments NC2500 Elemental Analyzer (Lakewood, New Jersey, 08701) was used for CHN analysis with EDTA as the standard.

2.5. Data analyses

JMP® 4.0 statistical package (SAS Institute, Cary, NC) was used to analyze all data. Data for lipid, C and N were expressed as mg g^{-1} tissue DW and then transformed for normality. Water concentration was expressed as mg g^{-1} WW tissue and then transformed for normality. One-factor analysis of variance (ANOVA) was used to test for biometric differences among ovary stages of each species. Two-factor ANOVA was used to test for differences in biochemical components with species and ovary stage as factors. The relationship between GSI and ovarian biochemical constituents was assessed with regression analysis. Differences between embryo biochemical components of the two species were tested with one-factor ANOVA. One-factor ANOVA was used to test for biometric differences among crabs in different hepatopancreas color classes. Regression analysis was used to test for relationships between biometric data and lipid, C, and N concentration. Two-factor ANOVA was used to test for differences in hepatopancreatic biochemical constituents with species and sex as factors. If factors were not significant they were pooled to test for the relationship between hepatopancreas color and nutrient concentration. If factors were significant, they were tested separately with one-factor ANOVA. In all tests, when significant differences were observed, a Tukey test (multiple comparison procedure) was conducted to see

which factors were different. A significance level of 0.05 was applied for all tests in this study. One-factor ANOVA was used to test for differences between ovary stage and hepatopancreatic nutrient concentration as well as total organ content; regression analyses was used to determine the relationship between GSI and hepatopancreatic nutrient concentration, as well as total organ content.

3. Results

3.1. Biometric data

CW, body WW, and hepatopancreas WW did not differ among female specimens of *A. cinereum* of different ovary stages (Table 1). Ovary WW and GSI increased significantly during ovarian maturation in *A. cinereum* ($F=21.486$, $df=2$, $P<0.0001$; $F=38.81$, $df=2$, $P<0.0001$, respectively). CW, body WW, and hepatopancreas WW did not differ among female specimens of *S.* nr. *reticulatum* of different ovary stages. Ovary WW and GSI increased significantly during ovarian maturation in *S.* nr. *reticulatum* ($F=24.13$, $df=2$, $P<0.0001$; $F=40.24$, $df=2$, $P<0.0001$, respectively) (Table 1).

3.2. Biochemical composition of the ovary

There were no differences between the two species for measurements of ovary lipid concentration, carbon (C), nitrogen (N), C:N ratio and water concentration during any of the three ovary stages (Table 2). Ovary lipid and C concentration increased significantly in ovary tissue as ovary stage advanced and as the GSI increased (Fig. 1 and Table 2). C:N ratio increased significantly in ovary tissue as ovary stage advanced and as the GSI increased. N concentration decreased significantly in ovary tissue as

Table 2
Two-factor ANOVA results of tests for differences in biochemical concentration of the ovaries of *A. cinereum* and *S.* nr. *reticulatum*

| Nutrient	Source of variation	df	F ratio	P value
Lipid	species	1	0.1274	NS
	ovary stage	2	7.3122	0.0017
	species*ovary stage	2	1.1748	NS
Carbon (C)	species	1	0.0192	NS
	ovary stage	2	5.0831	0.0105
	species*ovary stage	2	1.5374	NS
Nitrogen (N)	species	1	1.6647	NS
	ovary stage	2	5.8324	0.0059
	species*ovary stage	2	0.7993	NS
C:N ratio	species	1	2.3567	NS
	ovary stage	2	17.9539	<0.0001
	species*ovary stage	2	1.8437	NS
Water	species	1	0.4755	NS
	ovary stage	2	41.8385	<0.001
	species*ovary stage	2	7.1908	NS

Significant at $P<0.05$ level; NS = not significant.
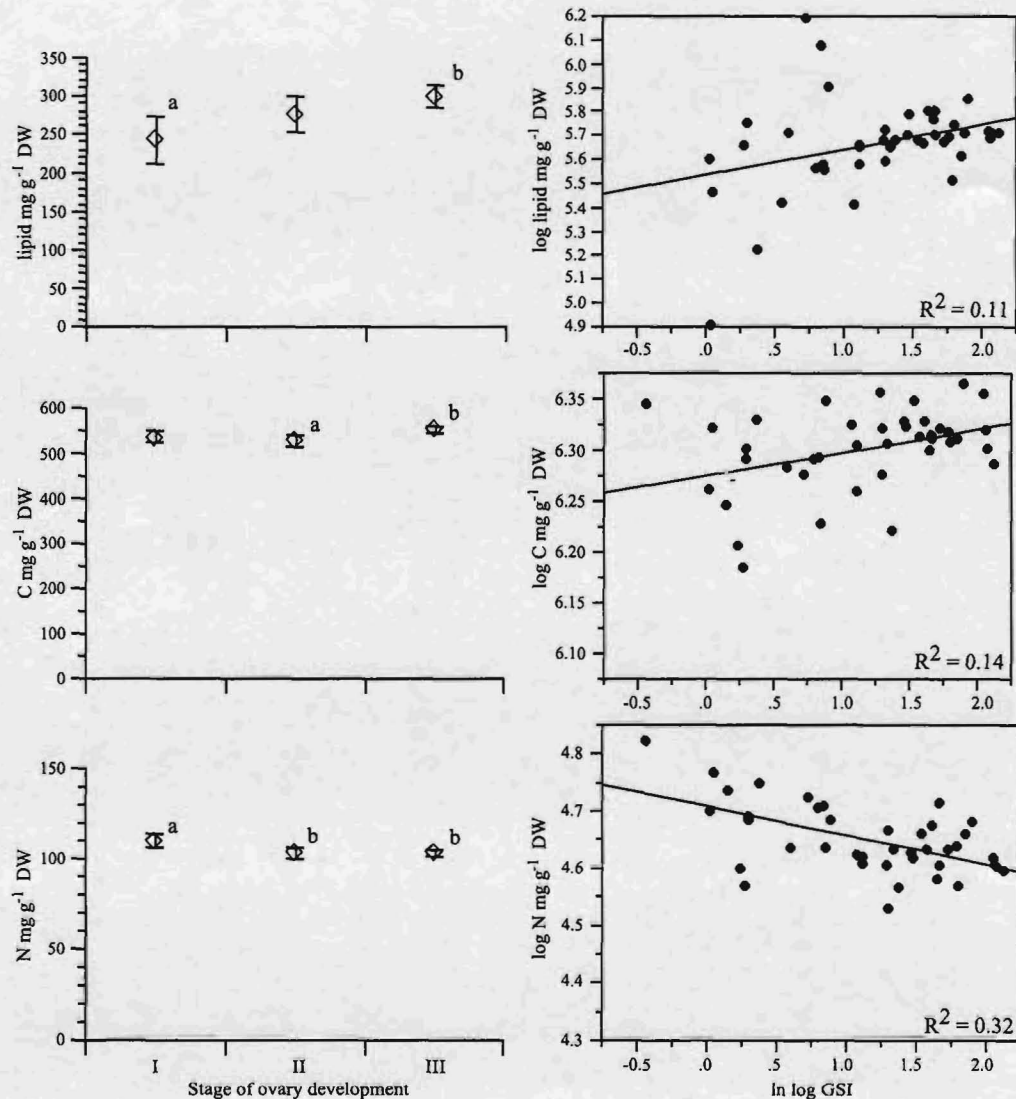
Fig. 1. Pooled mean (±95% confidence interval) ovary lipid, nitrogen N, and carbon C of *A. cinereum* and *S.* nr. *reticulatum* in three progressive stages of ovary development, with stage III as the most mature. Logarithmic ovary lipid, N, and C regressed against logarithmic gonadosomatic index GSI. Means with different letters are significantly different at *P*<0.05 level.

ovary stage advanced and as the GSI increased (Fig. 1 and Table 2). Ovary water concentration decreased significantly throughout ovarian maturation.

3.3. Color and biometric changes in the hepatopancreas

Hepatopancreas color in both male and female specimens of *A. cinereum* did not differ with respect to CW, WW, ovary WW, GSI, hepatopancreas WW or DGI (Table 3). Hepatopancreas color in female specimens of *S.* nr. *reticulatum* did not differ with respect to CW, WW, ovary WW, GSI, hepatopancreas WW or DGI. Hepatopancreas color in male specimens of *S.* nr. *reticulatum* was not related to hepatopancreas WW or DGI; however, the CW and WW of male specimens differed in specimens of differing

hepatopancreas color (F=4.94, df=2, P=0.008; F=8.63, df=2, P=0.0005, respectively). Males with brown or yellow-ochre colored hepatopancreas tissues were of greater size, evident in CW and WW, than were those with lemon-yellow and cadmium-yellow hepatopancreas tissue coloration (Table 3).

3.4. Biochemical composition embryos

The mean lipid content of the embryos of *A. cinereum* (μ=292 mg g^{-1} DW ±110 SD) did not differ from that in embryos of *S.* nr. *reticulatum* (μ=177 mg g^{-1} DW ±56 SD) ($F_{2,17}$=7.3093, P=0.0163, power=0.71). Concentration of C in embryos of *A. cinereum* (μ=538 mg g^{-1} DW ±35 SD) was significantly higher than in embryos of *S.* nr.

Table 3
Biometric means±SD for crabs with different hepatopancreas colors

		Hepatopancreas color				
		Brown	Yellow-ochre	White	Lemon-yellow	Cadmium-yellow
Armases cinereum						
Carapace width	f	19±2.7	17.1±4.1		17.9±2.5	13.4±4.3
	m	15.6±0.07	18.9±1.7	16.6±2.7	16.6±3.9	
Total WW	f	3.6±1.4	3.4±1.4		3±0.6	2±0.14
	m	2.5±0.07	4.2±0.9	2.7±1.1	3.7±1.7	
Ovary WW	f	0.1±0.11	0.13±0.1		0.12±0.1	0.1±0.1
GSI	f	2.5±1.8	4.2±2.8		4.1±2.2	4.6±4.8
Hepatopancreas WW	f	0.18±0.03	0.16±0.07	0.2±0.1	0.21±0.1	0.07±0.02
	m	0.1±0.004	0.18±0.03	0.2±0.1	0.2±0.1	
DGI	f	5.6±1.4	4.9±1.1		7±1.8	3.6±1.2
	m	3.7±0.3	4.5±0.9	5.8±1.6	5.3±1.3	
n		f 3, m 2	f 8, m 3	m 4	f 14, m 13	f 2, m 0
Sesarma nr. *reticulatum*						
Carapace width	f		19.4±4.7	22.3±1.9	20.7±3.1	19.8±3.5
	m	26.7±0.2a	27.2±0.9[a]	25±2.7[a]	23.8±2.2	22.5±2.1
Total WW	f		5±1.4	7.1±2.1	5±2.2	4.8±1.9
	m	14.3±1.0 a	15.7±1.9[a]	9.2±2.9	9.6±3	8±2.4
Ovary WW	f		0.1±0.06	0.2±0.04	0.2±0.2	0.1±0.1
GSI	f		3.5±0.9	3.3±1.9	4±2.4	2.7±1.6
Hepatopancreas WW	f		0.3±0.1	0.3±0.1	0.3±0.1	0.3±0.8
	m	0.5±0.1	0.6±0.2	0.4±0.1	0.4±0.2	0.4±0.1
DGI	f		5.5±0.9	3.7±0.9	5.8±1.9	5.8±2.1
	m	3.4±0.3	3.8±0.8	4.7±0.03	4.6±1.3	4.7±1.7
n		f 0, m 3	f 4, m 7	f 6, m 2	f 22, m 9	f 9, m 4

Carapace width CW is expressed in mm. Total wet weight WW, ovary and hepatopancreas WW are expressed in g. Gonadosomatic index GSI=ovary WW/total WW×100. Digestive gland index GDI for females=hepatopancreas (WW/total WW−ovary WW)×100. For males DGI=hepatopancreas WW/total WW×100. Values in a row with different superscripts are significantly different ($P<0.05$). f = female and m = male.

reticulatum (μ=462 mg g^{-1} DW ±25 SD) ($F_{2,14}$=16.4274, P=0.0016). The mean N content in embryos of *A. cinereum* (μ=109 mg g^{-1} DW ±6 SD) was higher than in embryos of *S.* nr. *reticulatum* (μ=98 mg g^{-1} DW ±4 SD) differed ($F_{2,14}$=11.4419, P=0.0054). There was no difference between the C:N ratios in embryos of the two species ($F_{2,14}$=0.7212, P=0.4124). Water concentration did not differ between embryos of the two species ($F_{2,14}$=2.4556, P=0.1394).

3.5. Lipid, C, and N concentrations of the hepatopancreas

The DGI of female specimens of *A. cinereum* was correlated positively to hepatopancreas lipid and C concentration and C:N ratio (r^2=0.16, P=0.0418; r^2=0.22, P=0.0284, r^2=0.42, P=0.0011, respectively). The DGI of female specimens of *A. cinereum* was correlated negatively to hepatopancreas N (r^2=0.45, P=0.0007). CW, WW, and hepatopancreas WW of female specimens of *A. cinereum* were not related to hepatopancreas lipid, C, N, or C:N ratio.

The DGI of male specimens of *A. cinereum* was correlated positively to hepatopancreas lipid and C concentration and C:N ratio (r^2=0.2, P=0.0403; r^2=0.22, P=0.0454, r^2=0.28, P=0.019, respectively). The DGI of male specimens of *A. cinereum* was correlated negatively to hepatopancreas

N (r^2=0.28, P=0.0202). CW, WW, and hepatopancreas WW of male specimens of *A. cinereum* were not correlated to hepatopancreas lipid, C, N, or C:N ratio.

CW, WW, DGI, and hepatopancreas WW of female specimens of *S.* nr. *reticulatum* were not correlated to hepatopancreas lipid, C, and N concentrations, or C:N ratio. CW, WW, DGI, and hepatopancreas WW of male specimens of *S.* nr. *reticulatum* were not correlated to hepatopancreas lipid and C concentration. CW and WW of male specimens of S. nr. *reticulatum* were positively correlated to hepatopancreas N concentration (r^2=0.31, P=0.0212; r^2=0.39, P=0.0076, respectively). CW and WW of male specimens of *S.* nr. *reticulatum* were negatively correlated to hepatopancreas C:N ratio (r^2=0.28, P=0.0274; r^2=0.3, P=0.0218, respectively).

Hepatopancreas lipid and C concentrations were not correlated to sex but did differ between species (Table 4). Hepatopancreas tissue in *A. cinereum* had higher lipid (mean=466±17 and 373±15 mg g^{-1} DW, respectively) and C concentrations (mean=569±7 and 526±8 mg g^{-1} DW, respectively) than did hepatopancreas tissue in *S.* nr. *reticulatum*. Hepatopancreas N concentration and C:N ratio were not correlated to sex and did not differ between species (Table 4). Hepatopancreas water concentration was not correlated to sex but differed between species (Table 4).

3.6. Hepatopancreas nutrients and color

Hepatopancreas lipid concentration in *A. cinereum* differed among individuals with differing hepatopancreas colors (F=5.9772, df = 4,48, P=0.0007), though C concentration did not (Fig. 2). Hepatopancreas lipid and C concentrations in *S.* nr. *reticulatum* did not differ among individuals with differing hepatopancreas tissue colors. Hepatopancreas N concentration differed among hepatopancreas tissues of different color classes of both species (F=5.1744, df = 4, P=0.0009). Brown and yellow-ochre colored hepatopancreas tissues had a higher N concentration than did lemon-yellow colored tissues. C:N ratios of the hepatopancreas differed among hepatopancreas color classes (F=3.9083, df = 4, P=0.0058). Brown and yellow-ochre hepatopancreas tissues had lower C:N ratios than did lemon-yellow colored tissues. Hepatopancreas water concentration did not differ among hepatopancreas tissues of differing colors in *A. cinereum*. In contrast, white and yellow-ochre colored hepatopancreas tissues in *S.* nr. *reticulatum* had higher water concentrations than did cadmium-yellow tissues.

3.7. Relationship between hepatopancreas and ovarian maturation

There was no relationship between ovary stage and measures of hepatopancreas lipid and C in *A. cinereum*. As ovarian maturation progressed, lipid concentration (F=6.5473, df = 2, P=0.0041) of the hepatopancreas of *S.* nr. *reticulatum* increased. However, there was no relationship between ovary stage and hepatopancreas lipid content or C concentration and content. Female specimens of *S.* nr. *reticulatum* with a stage III ovary had higher hepatopancreas lipid concentrations than did those with a stage I ovary. N concentration and content, as well as C:N ratios of the hepatopancreas, did not differ with respect to ovary
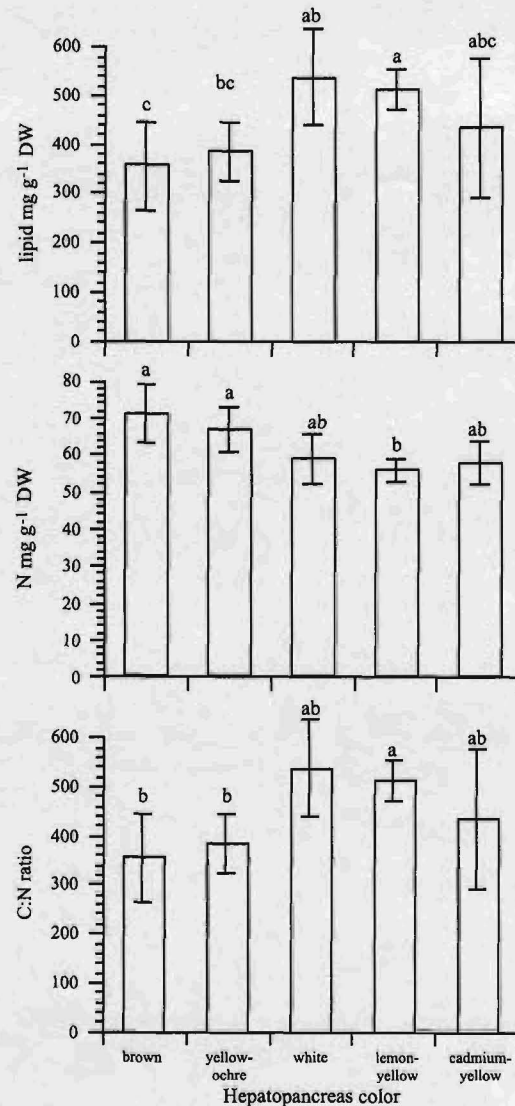

Fig. 2. Mean (±95% confidence interval) hepatopancreas lipid concentration in *A. cinereum* and mean N concentration and C:N ratios of pooled *A. cinereum* and *S.* nr. *reticulatum*. Means with different letters are significantly different at $P{\le}0.05$ levels.

stage in either species. There were no relationships for either species between GSI and measures of hepatopancreas lipid, C, and N or C:N ratio.

4. Discussion

As in other studies, this work documents an increase in measures of ovary lipid and C with ovarian maturation (Castille and Lawrence, 1989; Mourente and Rodriguez, 1991; Mourente et al., 1994; Tuck et al., 1997; Wen et al., 2001; Rosa and Nunes, 2002), and the pattern of increase was similar in *A. cinereum* and *S.* nr. *reticulatum*. These two species also had similar lipid levels in each ovary stage. Concentrations of ovary lipids range widely within decapod

Table 4
Two-factor ANOVA results of tests for differences in nutrient concentration of the hepatopancreas of *A. cinereum* and *S.*nr. *reticulatum*

Nutrient	Source of variation	df	F ratio	P value
Total lipid	species	1	23.1161	<0.001
	sex	1	2.6119	NS
	species*sex	1	8.1951	0.0051
Carbon (C)	species	1	19.1583	<0.0001
	sex	1	1.0334	NS
	species*sex	1	2.441	NS
Nitrogen (N)	species	1	0.0008	NS
	sex	1	0.2004	NS
	species*sex	1	4.6637	0.0337
C:N ratio	species	1	0.7362	NS
	sex	1	0.2288	NS
	species*sex	1	0.1856	NS
Water	species	1	8.3708	0.0046
	sex	1	1.3734	NS
	species*sex	1	0.9898	NS

Significant at the 0.05 level; NS=not significant.

species studied to date, as might be expected under the influence of maturation and reproductive cycling. Variation from 23–30% DW in *A. cinereum* and *S.* nr. *reticulatum* is closely comparable to the 19–39% DW range we found in two species of *Uca* from Louisiana. The two species of *Uca* represent a logical ecological comparison to the present study as crabs were collected from the same location and time. Similarities between the ovary lipid concentration range of the two grapsoid and two ocypodid species indicates that ovary lipid concentration may not reflect intrinsic differences in phylogeny or ecology. Rather, sampling constraints such as the inclusion of both under-fed and well-feed animals, young and old specimens, or the effects of tidal cycles and local weather events (e.g., heat stress) probably best explain the range of lipid and C reported in various studies.

Overall variation in ovary lipid concentrations reported in the present study are similar to the 12–37% range reported in marine nephropid lobsters (Tuck et al., 1997; Rosa and Nunes, 2002). However, this is less than half the range reported in an estuarine hermit crab (Ajmal Khan and Natarajan, 1980) and does not reach the low extremes of about 11% reported for hermit crabs and an intertidal fiddler crab (Ajmal Khan and Natarajan, 1980; Mourente et al., 1994). In the case of a tropical hermit crab, lipid concentration of the hepatopancreas declined during ovarian maturation (Ajmal Khan and Natarajan, 1980). Hepatopancreas lipids also declined during portions of the ovarian maturation cycle in a fiddler crab (Mourente et al., 1994). In the present study we found no evidence of hepatopancreas lipid decline during the course of ovarian maturation.

A. cinereum and *S.* nr. *reticulatum* had decreasing ovary N concentrations and increasing C:N ratios as ovaries matured, which indicates that during ovarian maturation the ovary of these species accumulated lipid based stores for successful ovarian maturation rather than protein reserves. A similar pattern was found in Louisiana fiddler crabs. In contrast, in the deep-sea crab, *Chaceon quinquedens*, lipid:protein ratios did not differ between ovaries of mature and advanced stages (Biesiot and Perry, 1995). Biesiot and Perry (1995) suggested that patterns of organic reserve storage in deep-sea crustaceans may be related to the food-limited conditions of the bathyal region. While the quality of food in coastal marshes varies throughout the year, the grapsoid crabs in the present study did not inhabit food-limited environs. Ovary tissue in decapods that inhabit deeper waters may exhibit different energy allocation patterns than do those of intertidal areas. *A. cinereum* and *S.* nr. *reticulatum* also had lower ovary N concentration than did penaeid shrimp. Specimens of *Farfantepenaeus aztecus*, *Litopenaeus setiferus* and *Metapenaeus affinis* had higher ovary protein than lipid concentrations (Pillay and Nair, 1973; Castille and Lawrence, 1989). Differences in the level of lipid and protein throughout ovarian maturation may be an indication that some species exhibit a mainly lipid based nutrient maturation process, while other species

may have higher protein requirements for successful vitellogenesis.

Lipid concentration of the embryos did not differ (low power) between species in this study. The embryos sampled in this study were not staged, so embryos may have been days or weeks from hatching. In a laboratory study, the larvae of *S. reticulatum* had a higher percentage of lipid than did larvae of *A. cinereum* (Staton and Sulkin, 1991). The authors suggested that *S. reticulatum* demonstrated resistance to starvation while *A. cinereum* showed a complete lack of nutritional flexibility (Staton and Sulkin, 1991). Comparisons between our study and that of Staton and Sulkin (1991) are difficult because *S. reticulatum* and *S.* nr. *reticulatum* appear to be genetically diverged and we did not measure larval nutrient concentrations.

In specimens of *A. cinereum*, hepatopancreatic lipid, N concentration, and C:N ratio were correlated to hepatopancreas color. The cause of the different colors and the relationship between color and biochemical constituents is not clear, but other workers have also noted that the color of the hepatopancreas can vary among and within species (George et al., 1956; Herring, 1973; Gibson and Barker, 1979; Herreid and Full, 1988) and that the pigment contents of the hepatopancreas can vary (Castillo and Negre-Sadargues, 1995).

In this study, lipids were extracted from only intermolt crabs, so the observed colors were not related to imminent ecdysis or post-molt period. Hepatopancreatic lipid levels may change during intermolt and early premolt (Giese, 1966; O'Connor and Gilbert, 1968; Bollenbacher et al., 1972; Chandumpai et al., 1991; Cockcroft, 1997). While our molt staging did not determine a specific phase of intermolt, it was clear the population sampled was not in ecdysis.

There can be large ranges in the lipid content of the hepatopancreas independent of the molt cycle. Male lobsters in molt-stage C_4 had hepatopancreas lipid contents that ranged from 16% to 61% DW (Dall, 1981). This is similar to the range we found with *A. cinereum* and *S.* nr. *reticulatum*. Large variations in lipid content may not simply reflect the different phases of intermolt, but rather other related or unrelated factors, such as diet. As suggested above, the range of hepatopancreas lipid concentration may be a reflection of sampling animals that were for some reason under-fed or of mixed nutritional states along with well-fed animals.

Fluctuating food sources or food sources with varying nutritive quality may influence lipid levels. *A. cinereum* and *S.* nr. *reticulatum* live in a habitat of constant flux, unlike the habitats of many other crustaceans in which lipid quantities have been studied. Food sources and food quality may vary from day to day depending on the tidal cycle. Other diet-dependent changes in the color of the hepatopancreas include the presence of carotenoids such as astaxanthin and β-carotene. The color of the hepatopancreas may fluctuate in wild caught crabs because of the presence of carotenoids in the diet and how these pigments

are metabolized. In some crustaceans carotenoids are mobilized from the hepatopancreas to the gonad (Goodwin, 1950).

A pattern of correlation was observable between hepatopancreatic water concentration and hepatopancreas color. For *S*. nr. *reticulatum* water concentration decreased along a continuum of hepatopancreas color from brown (with the greatest water concentration) to cadmium-yellow tissues (with the lowest water concentration). This suggests that hepatopancreas color is related to nutritional status. During starvation or periods of nutritional stress the water concentration of the hepatopancreas increases (Dall, 1974; Trendall and Prescott, 1989). During nutritional stress hepatopancreas C concentration decreases while N concentration increases (Trendall and Prescott, 1989). This is the same pattern we found for hepatopancreas tissue of varied color along the continuum from brown to cadmium-yellow. Increasing water and N concentration along with decreasing C concentration pattern (seen during nutritional stress) may also explain the biochemical differences in differently colored hepatopancreases.

Because there was no relationship between hepatopancreas lipid concentration and ovarian maturation for either species, stored hepatopancreas reserves do not appear to be a primary source of lipids essential to the gonadal maturation cycle. Rather, it appears that any draw upon lipids from the hepatopancreas for this process is offset by simultaneous feeding. It would be of interest to ascertain whether *A. cinereum* and *S*. nr. *reticulatum* simply increase food intake during this period or whether they instead turn more heavily to lipid-rich substrates. As there was no measurable difference between the hepatopancreas lipid concentration and content of males and females in this study, it is possible that such increased nutritional intake would be sex specific. Other female decapod crustaceans have been reported to double food consumption during ovarian maturation (Teshima et al., 1986), but no such observations have been reported for grapsoid crabs.

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