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## Biochemical contents of the ovary and hepatopancreas of *Uca longisignalis* and *Uca* nr. *minax*

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**SUMMARY:** Biochemical composition of ovary and hepatopancreas tissues in wild populations of *Uca longisignalis* and *Uca* nr. *minax* were monitored during the reproductive season. Total lipid (concentration and content), C (carbon), N (nitrogen), and C:N ratios of the ovary and hepatopancreas were quantified over the course of ovarian maturation. Ovary lipid and C concentration varied significantly over the course of ovarian maturation for both species, but there was no relationship between lipid concentration or hepatopancreas content and the stage of ovarian development in females. Hepatopancreatic lipid and C concentration did not differ between sexes of *U. nr. minax*. Lipid demands of ovarian maturation thus appear to be met in large part by increased dietary intake and not purely by translocating lipid stores from the hepatopancreas. In both *Uca longisignalis* and *U. nr. minax*, the color of the hepatopancreas may be used as an indicator of the lipid and C levels of the hepatopancreas. Cadmium-yellow and lemon-yellow hepatopancreas tissues had the highest lipid concentrations. No evidence could be found to demonstrate depletion of lipid or C concentrations in the hepatopancreas concomitant with ovarian maturation.

**Keywords:** crustacean, gonad maturation, Gulf of Mexico, lipid, hepatopancreas.

**RESUMEN:** CONTENIDO BIOQUÍMICO DEL OVARIO Y HEPATOPÁNCREAS DE *UCA LONGISIGNALIS* Y *UCA* NR. *MINAX*. – Se analizó la composición bioquímica de tejidos del ovario y hepatopáncreas de poblaciones naturales de *Uca longisignalis* and *Uca* nr. *minax* durante la época reproductiva. Se cuantificaron lípidos totales (concentración y contenido), C (carbono), N (nitrógeno), y los cocientes C:N del ovario y hepatopáncreas durante el proceso de maduración del ovario. Los lípidos y la concentración de C variaron significativamente durante el curso de maduración del ovario en ambas especies, pero no se encontró ninguna relación entre la concentración o contenido de lípidos del hepatopáncreas con el estadio de desarrollo del ovario en hembras. Tampoco se encontró ninguna diferencia en los lípidos del hepatopáncreas y la concentración de C entre sexos de la especie *Uca* nr. *minax*. La demanda de lípidos durante la maduración del ovario, parece ser aportada en gran parte por un incremento en la dieta diaria y no solamente por la translocación de reservas de lípidos desde el hepatopáncreas. En ambas especies, *Uca longisignalis* y *U. nr. minax*, el color del hepatopáncreas puede ser usado como un indicador de los niveles de lípidos y C en el hepatopáncreas. Los tejidos del hepatopáncreas con color amarillo-cadmio y amarillo-limón tuvieron las más altas concentraciones de lípidos. No se encontró ninguna evidencia para demostrar que la disminución en la concentración de lípidos y C en el hepatopáncreas es concomitante con la maduración del óvulo.

**Palabras clave:** crustáceos, maduración gónadas, Golfo de México, lípidos, hepatopáncreas.

### INTRODUCTION

Crustaceans use lipids for numerous biological structures and processes (Allen, 1976). Lipids provide energy for metabolic processes, maintain the structural and physiological integrity of cellular and

sub-cellular membranes, and transport substrates via the circulatory system (O'Connor and Gilbert, 1968). The type and quantity of lipid moieties oscillate throughout the life cycle (Farkas and Nemezc, 1984; Spaargaren and Haefner, 1994; Zhukova *et al.*, 1998).

Typically, the organs with the highest lipid content are the hepatopancreas and the ovary. In females, the total lipid in the ovary is influenced by the stage of ovarian development. During gonadal maturation and vitellogenesis lipids are deposited in the ovaries (Morris, 1973; Gehring, 1974; Mourente *et al.*, 1994; Lubzens *et al.*, 1995; Spaziani and Hinsch, 1997). It appears that these ovarian lipids may be derived from the diet or, in some decapods, lipids may be accumulated and later transported to the ovaries during gonad maturation (Spaargaren and Haefer, 1994). While the hepatopancreas is the universal organic reserve organ, not all decapods shuttle measurable lipid reserves from it to the ovaries (Heath and Barnes, 1970; Pillay and Nair, 1973; Castille and Lawrence, 1989).

*Uca longisignalis* Salmon and Atsides is the most common fiddler crab in Louisiana estuaries and is restricted to northern Gulf of Mexico warm-temperate habitats, where temperatures are similar to those of the Carolinian biogeographic province on the North Atlantic coast. These fiddler crabs prefer areas with low clay soils, such as those from lower portions of marshes, and have adaptations in feeding setae that may reflect specialization for this zone (Mouton and Felder, 1995). *U. longisignalis* exhibits physiological plasticity and commonly inhabits areas with salinities from 5 to >35 (Thurman, 1982). Its reproductive cycle is restricted seasonally in Louisiana, with ovarian maturation from February to July. Ovigerous females first occur in April and peak occurrence of ovigerous females is in summer (June) (Mouton and Felder, 1995).

*Uca* nr. *minax*, an unnamed Gulf of Mexico endemic population near (nr.) *Uca minax* (LeConte), occurs throughout coastal Louisiana (Felder and Staton, 1994). Both the Gulf populations of *U. nr. minax* and Atlantic populations of *U. minax* are found along tidal stream banks and in marsh habitats usually some distance from high salinity water. They are widely distributed in upper estuaries dominated by *Spartina*, including those of low salinity (Miller, 1961; Williams, 1984). The burrows of *U. nr. minax* are usually on banks above the high tide level and descend to the water table (Williams, 1984; Felder and Staton, 1994). *U. nr. minax* and *U. minax* deposit feed by sorting the substrate and appear to eat bacterial and algal coatings on decaying plant matter along with decaying plant matter itself, in lower parts of the marsh where the mud is wet (Miller, 1961; Felder and Staton, 1994; Pratt *et al.*, 2002).

At our sampling site burrows of the two species were in close proximity to one another and crabs could sometimes be seen wandering the marsh in mixed groups. Burrows of *U. nr. minax* were usually further removed from water than those of *U. longisignalis*, but not usually by more than a few meters.

In this study we quantified total lipid, N (nitrogen), C (carbon), C:N ratios, and water content of the ovary and hepatopancreas throughout different stages of ovarian maturation for females of both species. We compared these measures to those for the hepatopancreas of males. In doing so we sought to clarify roles of these biochemical constituents in the life histories of *U. longisignalis* and *U. nr. minax*. We hypothesized that lipid and C concentration would increase in the ovary and would decrease in the hepatopancreas throughout the course of ovarian maturation. Biochemical constituents commonly increase in the ovary during maturation and it has been suggested that the hepatopancreas is a source of stored lipid that is shuttled to the ovary during maturation (Pillay and Nair, 1973). Dietary and behavioral differences between the two species suggested that the amounts of lipid in the ovary and hepatopancreas of the two species should differ (Felder and Staton, 1994; Mouton and Felder, 1996). We also postulated that there would be differences in the biochemical components of the hepatopancreas between males and females, with females exhibiting lower hepatopancreas lipid concentrations concomitant with the increasing lipid requirements of ovarian maturation.

## MATERIALS AND METHODS

### Sampling

Adult intermolt specimens of *U. longisignalis* and *U. nr. minax* were sampled during the reproductive season on 19, 26 March, 13, 16 April, 15 May, 19, 26 June and 4, 6 July 2001. Crabs were collected from coastal wetlands between Cypremort Bayou and Vermilion Bay near Cypremort Point, Louisiana. Specimens were removed from the upper reaches of burrows or caught as they ran for cover. Crabs <10 mm carapace width were not included in the sample because they were usually immature. Crabs were placed in individual perforated vials for transport to the laboratory.

### Morphometric analysis

Carapace width (CW) was measured with dial calipers ( $\pm 0.1$  mm), and body wet weight (WW) was measured with an analytical balance ( $\pm 0.1$  g). Wet weights of dissected ovary and hepatopancreas were determined with an analytical balance ( $\pm 0.0004$  g). Tissues were blotted on paper prior to weighing to remove surface water. Ovary and hepatopancreas tissues were then lyophilized and stored in glass vials with Teflon tops at  $-80^{\circ}\text{C}$  for further analyses. Gonadosomatic index (GSI) was equal to the ovary WW divided by the total body WW multiplied by 100. Digestive gland index (DGI) for females was equal to the hepatopancreas WW divided by the total body weight minus ovary WW and then multiplied by 100. Digestive gland index (DGI) for males was equal to the hepatopancreas WW divided by the total body WW and then multiplied by 100 (Clarke, 1977).

### Ovary stage determination

Ovaries were classified into three developmental stages on the basis of color and size (Ajmal Khan and Natarajan, 1980; Mourente *et al.*, 1994). For both species, stage I ovaries were slender, stage III ovaries filled the body cavity and stage II ovaries were of intermediate thickness between stage I and III. Ovary colors are approximated by Pantone® Color Matching System (Pantone Inc., Carlstadt, New Jersey, 07072 USA). Stage I ovaries were largely lavender (257CVC) or peach (163 CVC) color and sometimes almost translucent (1205CVC/203 CVC); in stage II, ovaries were mauve (194CVC) or rose color (1805 CVC); in stage III, ovaries were liver (4975 CVC) or maroon color (195 CVC).

### Hepatopancreas color determination

During dissection, the hepatopancreas was classified into one of four color classes. Each class approximated a color in the Pantone® Color Matching System (Pantone Inc., Carlstadt, New Jersey, 07072 USA; colors as numbered below) found in Adobe Photoshop® (Adobe Systems Inc., San Jose, CA 95110 USA). The classes were brown (419CV), in which the general mass of the hepatopancreas ranged from a dark yellowish-brown to brown-black with black or brown areas

along tubules; yellow-ochre (145CV), in which the general mass of the hepatopancreas ranged from uniform brownish-yellow or dark-yellow sometimes with brown spots throughout tubules; lemon-yellow (129CV), 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 to orange-yellow.

### Lipid extraction and determination

Lipids were extracted from tissues of individual animals. The total amount of lipid was extracted from ovary and hepatopancreas tissues using Parrish's modification of the Folch chloroform/methanol extraction (Parrish, 1999). Total lipids were determined by measuring mg ( $\pm 0.1$  mg) of lipid of  $\text{g}^{-1}$  dry weight (DW) gravimetrically, with an analytical balance. Lipids were stored in chloroform at  $-80^{\circ}\text{C}$  for further analysis.

### CHN analysis

All CHN analyses were conducted with tissues from individual animals. Tissues were re-dried for 30 minutes prior to analysis at  $60^{\circ}\text{C}$ . Each sample was analyzed in triplicate (analytical replicates); analytical replicates that varied  $>5\%$  were removed from testing. A CE Instruments NC2500 Elemental Analyzer (Lakewood, New Jersey, 08701) was used for CHN analysis with EDTA as the standard.

### Data analysis

JMP 4.0 statistical package (SAS Institute Inc., Cary, North Carolina) was used to analyze all data. Concentration data for biochemical constituents were expressed as  $\text{mg g}^{-1}$  DW of tissue and then transformed logarithmically to form a normal distribution. Content data for biochemical constituents were expressed as  $\text{mg g}^{-1}$  DW of tissue multiplied by the DW of the whole organ and then transformed for normality. Water concentration was expressed as  $\text{mg g}^{-1}$  wet weight tissue (WW) 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 ovari-

TABLE 1. – *Uca longisignalis* and *Uca nr. minax*. Biometric means  $\pm$  SD for females at three stages of ovarian maturation (I, II and III). 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  $\times$  100. Values in a row with different superscripts are significantly different ( $p < 0.05$ ).

<i>Uca longisignalis</i>	ovary stages		
	I (n = 12)	II (n = 5)	III (n = 16)
CW	24.1 $\pm$ 1.1	22.6 $\pm$ 5.4	23.4 $\pm$ 1.9
WW	4.85 $\pm$ 0.65	4.34 $\pm$ 1.42	4.6 $\pm$ 1.22
ovary WW	0.033 $\pm$ 0.016 <sup>a</sup>	0.063 $\pm$ 0.015 <sup>a</sup>	0.23 $\pm$ 0.13 <sup>b</sup>
GSI	0.68 $\pm$ 0.33 <sup>a</sup>	1.55 $\pm$ 0.36 <sup>b</sup>	5.19 $\pm$ 2.78 <sup>c</sup>
hepatopancreas WW	0.24 $\pm$ 0.065	0.21 $\pm$ 0.056	0.23 $\pm$ .082
<i>Uca nr. minax</i>	I (n = 14)	II (n = 6)	III (n = 5)
Carapace width	26.0 $\pm$ 2.8	28.4 $\pm$ 3.8	27.9 $\pm$ 2.9
total WW	7.33 $\pm$ 2.56	8.41 $\pm$ 2.67	7.87 $\pm$ 2.43
ovary WW	0.041 $\pm$ 0.032 <sup>a</sup>	0.082 $\pm$ 0.029 <sup>a</sup>	0.24 $\pm$ 0.19 <sup>b</sup>
GSI	0.56 $\pm$ 0.29 <sup>a</sup>	0.99 $\pm$ 0.21 <sup>a</sup>	3.13 $\pm$ 2.18 <sup>b</sup>
hepatopancreas WW	0.30 $\pm$ 0.11	0.48 $\pm$ 0.17	0.46 $\pm$ 0.22

an, as well as hepatopancreas, biochemical constituents was assessed with regression analysis. Two-factor ANOVA was used to test for differences in hepatopancreatic biochemical constituents with sex and hepatopancreas color as factors. It was not possible to conduct two-factor ANOVA for differences in biochemical components of the hepatopancreas of *U. longisignalis* with sex and hepatopancreas color as factors, as no females with brown hepatopancreas tissue were collected; in this case separate one-factor ANOVA for sex and hepatopancreas color was conducted. The relationship between DGI and hepatopancreas biochemical constituents was assessed with regression analysis. In all tests, when significant differences were observed, a Tukey HSD 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.

## RESULTS

### Biometric data

Female CW, WW, and hepatopancreas WW did not differ among animals of different ovary stages for either species (Table 1). Ovary WW and GSI increased significantly during ovarian maturation in *U. longisignalis* ( $F = 16.49$ ,  $df = 30$ ,  $2 p < 0.0001$ ;  $F = 35.71$ ,  $df = 28$ ,  $2 p < 0.0001$  respectively) and *U. nr. minax* ( $F = 10.03$ ,  $df = 22$ ,  $2 p = 0.0008$ ;  $F = 13.83$ ,  $df = 20$ ,  $2 p = 0.0002$  respectively) (Table 1). GSI values ranged from 0.28 to 9.9. Ovigerous females of both species were collected in all months (March-July).

### Biochemical composition of the ovary

There were no differences in ovary lipid concentration, C, N, C:N ratio, and water concentration between the two species during any of the three ovary stages (Table 2). Ovary N concentration did not change in either species during ovarian maturation.

TABLE 2. – *Uca longisignalis* and *U. nr. minax*. Results of two-factor ANOVA tests for differences in ovarian biochemical concentrations with variation in ovary stage and gonadosomatic index GSI. GSI = ovary WW/total WW  $\times$  100. NS = not significant at 0.05 level.

Component	Source of variation	df	F ratio	p value
Lipid	species	1	1.0713	NS
	ovary stage	2	11.5485	0.0001
	species and ovary stage	2	1.2451	NS
	species	1	0.0134	NS
	GSI	1	11.9309	0.0013
nitrogen	species and GSI	1	3.8109	NS
	species	1	0.0166	NS
	ovary stage	2	3.6216	NS
	species and ovary stage	2	0.476	NS
	species	1	1.0702	NS
Carbon	GSI	1	5.84	NS
	species and GSI	1	0.6779	NS
	species	1	0.8726	NS
	ovary stage	2	13.895	0.0001
	species and ovary stage	2	0.6632	NS
C:N ratio	species	1	0.5137	NS
	GSI	1	25.508	<0.0001
	species and GSI	1	1.3253	NS
	species	1	0.5234	NS
	ovary stage	2	0.5954	NS
Water	species and ovary stage	2	1.3697	NS
	species	1	1.1861	NS
	GSI	1	17.769	0.0003
	species and GSI	1	1.3325	NS
	species	1	0.0735	NS
	ovary stage	2	29.3554	<0.001
	species and ovary stage	2	0.1374	NS
	species	1	0.1492	NS
	GSI	1	32.0239	<0.0001
	species and GSI	1	0.0072	NS

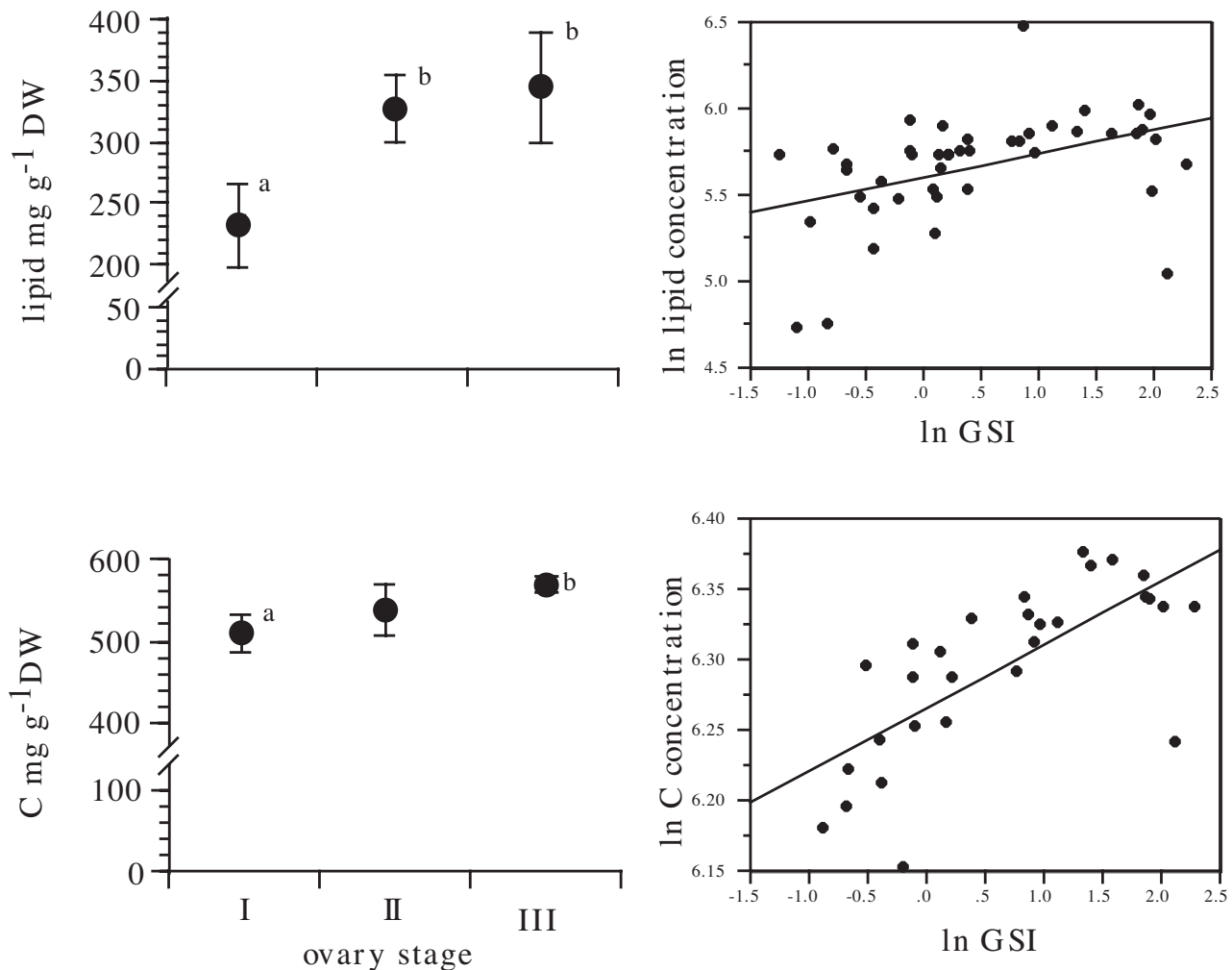


FIG. 1. – *Uca longisignalis* and *U. nr. minax*. Pooled mean  $\pm$  95% CI ovary lipid and carbon (C) concentration during three stages of ovary maturation (I, II and III) and logarithmic regressions of ovary lipid and C concentrations against gonadosomatic index GSI. Means with different letters are significantly different at 0.05 level. DW = dry weight.

tion (Table 2). Ovary lipid ( $r^2 = 0.18$ ,  $p = 0.004$ ) and C concentration ( $r^2 = 0.51$ ,  $p < 0.0001$ ) increased significantly in ovary tissue as ovary stage advanced and as the GSI increased (Fig. 1, Table 2). Ovary water concentration decreased throughout ovary maturation, but ovary C:N ratio did not differ with ovary stage. In contrast, there was a positive relationship between the GSI and C:N ratio ( $r^2 = 0.42$ ,  $p = 0.0004$ , Table 2).

**Color and biometric changes in the hepatopancreas**

Hepatopancreas color in both male and female specimens of *U. longisignalis* did not differ with respect to CW, WW, ovary WW, GSI, or hepatopancreas WW (Table 3). Hepatopancreas color and the

DGI in female specimens of *U. longisignalis* were not related; however, the DGI of male specimens covaried with respect to hepatopancreas color (Table 3). Males with brown or yellow-ochre color hepatopancreatic tissues had lower DGI than those with lemon-yellow or cadmium-yellow coloration.

Hepatopancreas color of female specimens of *U. nr. minax* was not significantly related to CW, WW, ovary WW, GSI, hepatopancreas WW, and DGI (Table 4). For *U. nr. minax* males there was no evidence of a significant relationship between hepatopancreas coloration and hepatopancreas WW or DGI; however, there were differences in CW and WW with respect to hepatopancreas color (Table 4). Males with cadmium-yellow hepatopancreas tissues were smaller (CW) than those with other hepatopancreas colors. Males with lemon-yellow and cadmi-

TABLE 3. – *Uca longisignalis*. Biometric means  $\pm$  SD for crabs with different hepatopancreas colors. 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  $\times$  100. Digestive gland index GDI for females = hepatopancreas (WW/total WW – ovary WW)  $\times$  100. For males DGI = hepatopancreas WW/total WW  $\times$  100. Values in a row with different superscripts are significantly different ( $p < 0.05$ ).

		hepatopancreas color			
		brown	yellow-ochre	lemon-yellow	cadmium-yellow
CW	f	none	22.94 $\pm$ 1.83	23.18 $\pm$ 3.094	23.57 $\pm$ 1.83
	m	26.50 $\pm$ 0.57	25.05 $\pm$ 1.86	22.13 $\pm$ 3.97	23.05 $\pm$ 1.93
total WW	f	none	4.35 $\pm$ 0.60	4.41 $\pm$ 1.27	4.67 $\pm$ 1.060
	m	8.55 $\pm$ 0.64	7.90 $\pm$ 1.82	5.74 $\pm$ 2.19	5.53 $\pm$ 1.94
ovary WW	f	none	0.099 $\pm$ 0.10	0.099 $\pm$ 0.11	0.19 $\pm$ 0.15
GSI	f	none	2.45 $\pm$ 2.85	2.41 $\pm$ 2.77	4.018 $\pm$ 3.051
hepatopancreas WW	f	none	0.21 $\pm$ 0.048	0.22 $\pm$ 0.068	0.24 $\pm$ 0.082
	m	0.17 $\pm$ 0.063	0.24 $\pm$ 0.079	0.25 $\pm$ 0.087	0.32 $\pm$ 0.10
DGI	f	none	4.8 $\pm$ 0.79	5.40 $\pm$ 1.87	5.30 $\pm$ 1.56
	m	2.071 $\pm$ 0.89 <sup>a</sup>	3.079 $\pm$ 0.93 <sup>a</sup>	4.66 $\pm$ 1.35 <sup>b</sup>	6.12 $\pm$ 2.061 <sup>b</sup>
N		f 0, m 2	f 5, m 6	f 19, m 25	f 10, m 6

TABLE 4. – *Uca nr. minax*. Biometric means  $\pm$  SD for crabs with different hepatopancreas colors. Carapace width CW is expressed in mm. Total WW, ovary and hepatopancreas WW are expressed in g. Gonadosomatic index GSI = ovary WW/total WW  $\times$  100. Digestive gland index GDI for females = hepatopancreas (WW/total WW – ovary WW)  $\times$  100. For males DGI = hepatopancreas WW/total WW  $\times$  100. Values in a row with different superscripts are significantly different ( $P < 0.05$ ).

		hepatopancreas color			
		brown	yellow-ochre	lemon-yellow	cadmium-yellow
CW	f	28.63 $\pm$ 1.43	26.48 $\pm$ 4.48	25.86 $\pm$ 3.98	27.02 $\pm$ 3.093
	m	33.90 $\pm$ 0.71 <sup>a</sup>	31.56 $\pm$ 2.53 <sup>a</sup>	30.63 $\pm$ 2.40 <sup>a</sup>	25.16 $\pm$ 1.060 <sup>b</sup>
total WW	f	8.05 $\pm$ 1.3	7.38 $\pm$ 2.90	6.50 $\pm$ 2.80	7.86 $\pm$ 2.53
	m	16.90 $\pm$ 0.71 <sup>a</sup>	16.29 $\pm$ 4.31 <sup>a</sup>	13.55 $\pm$ 3.57 <sup>b</sup>	7.60 $\pm$ 1.74 <sup>b</sup>
Ovary WW		0.030	0.057 $\pm$ .056	0.057 $\pm$ 0.034	0.16 $\pm$ 0.18
GSI	f	0.41	0.59 $\pm$ 0.51	0.76 $\pm$ 0.30	2.31 $\pm$ 2.13
hepatopancreas WW	f	0.29 $\pm$ 0.014	0.35 $\pm$ 0.14	0.36 $\pm$ 0.18	0.39 $\pm$ 0.19
	m	0.53 $\pm$ 0.18	0.53 $\pm$ 0.15	0.61 $\pm$ 0.20	0.39 $\pm$ 0.24
DGI	f	3.88	4.26 $\pm$ 1.49	5.50 $\pm$ 1.30	5.060 $\pm$ 1.65
	m	3.11 $\pm$ 0.92	3.34 $\pm$ 0.94	4.65 $\pm$ 1.37	4.69 $\pm$ 1.82
N		f 3, m 2	f 4, m 8	f 10, m 11	f 9, m 3

um-yellow hepatopancreas weighed less (WW) than males with other hepatopancreas colors.

### Biochemical composition of the hepatopancreas

The DGI of female specimens of *U. longisignalis* and *U. nr. minax* was not significantly correlated to either hepatopancreatic lipid or N concentration. The DGI of female specimens of *U. longisignalis* was not correlated to hepatopancreatic C concentration or C:N ratio; however, for female specimens of *U. nr. minax*, there was a positive correlation between DGI and both hepatopancreatic C concentration and C:N ratio ( $r^2 = 0.28$ ,  $p = 0.0079$ ;  $r^2 = 0.24$ ,  $p = 0.0145$  respectively).

The DGI of male specimens of *U. longisignalis* and *U. nr. minax* respectively, was correlated positively to hepatopancreatic lipid concentration ( $r^2 = 0.43$ ,  $p < 0.0001$ , Fig. 2;  $r^2 = 0.44$ ,  $p = 0.0004$ , Fig. 3), C concentration ( $r^2 = 0.19$ ,  $p = 0.0171$ , Fig.

2;  $r^2 = 0.36$ ,  $p = 0.0025$ , Fig. 3), and C:N ratio ( $r^2 = 0.27$ ,  $p = 0.0043$ ;  $r^2 = 0.44$ ,  $p = 0.0006$ ). For males of the two species respectively, DGI and N concentration of the hepatopancreas were correlated negatively ( $r^2 = 0.27$ ,  $p = 0.004$ , Fig. 2;  $r^2 = 0.42$ ,  $p = 0.0008$ , Fig. 3).

Hepatopancreas color was not related to either the lipid or C concentration of the hepatopancreas in female specimens of *U. longisignalis*. For males there was a significant relationship between both lipid and C concentration and the color of the hepatopancreas. In males, brown colored hepatopancreas tissues had significantly lower lipid concentration than those of other colors (Fig. 2, Table 5). Hepatopancreatic N concentration of male and female specimens of *U. longisignalis* was significantly greater in yellow-ochre colored hepatopancreas tissue than in those of other colors (Fig. 2, Table 5). C:N ratio of yellow-ochre colored hepatopancreatic tissues was less than in those of

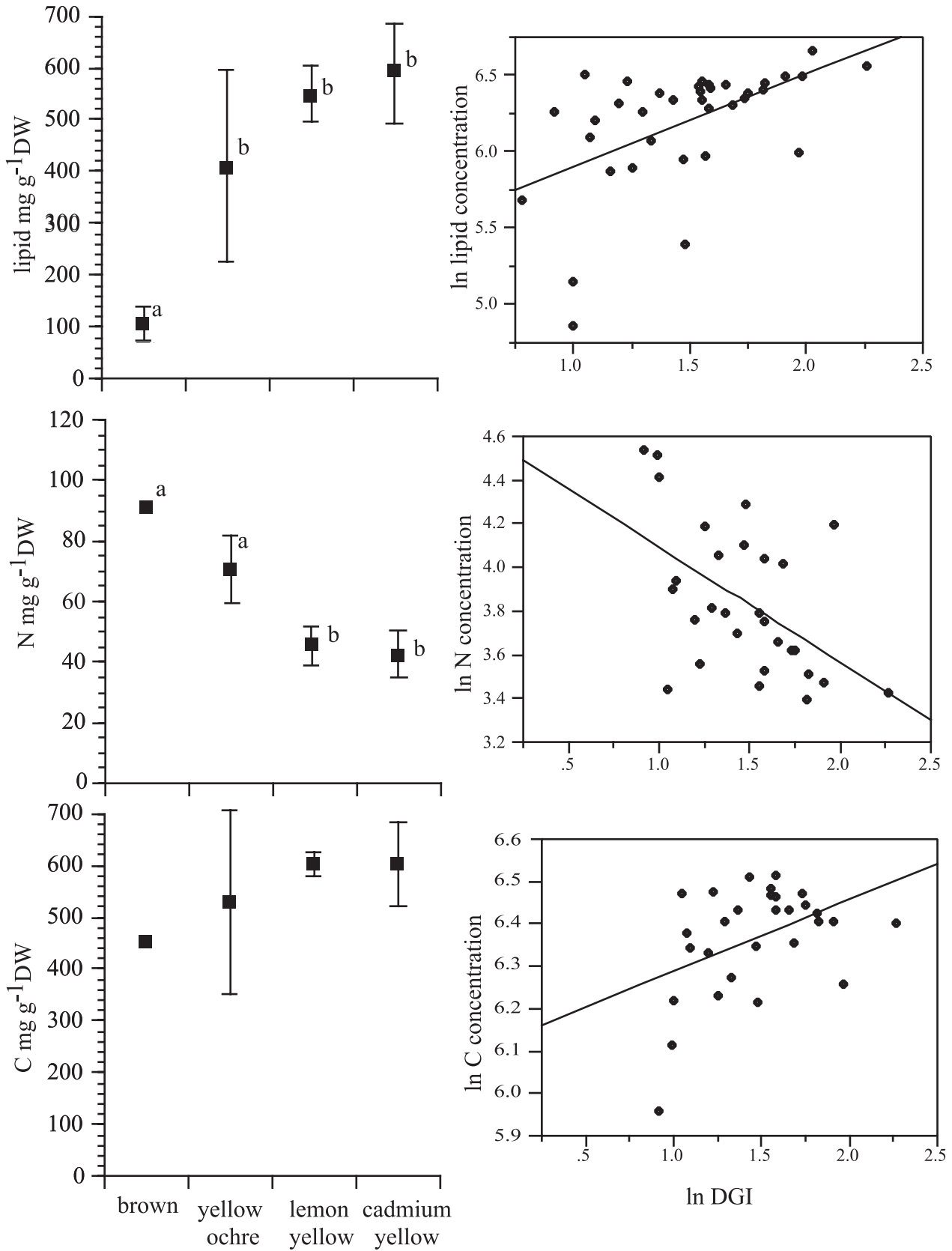


FIG. 2. – *Uca longisignalis*. Mean  $\pm$  95% CI biochemical concentration of the hepatopancreas of male specimens of *U. longisignalis* and log-arithmic regressions of digestive gland index (DGI) and lipid, nitrogen (N), and carbon (C) concentration. Means with different letters are significantly different.

TABLE 5. – *Uca longisignalis*. Results of one-factor ANOVA test for differences in lipid, carbon C, and nitrogen N concentrations, as well as the C:N ratio, as varied over four hepatopancreas tissue colors. Letter m = male; f = female; NS = not significant at the 0.05 level.

	sex	n	df	F ratio	p value
lipid	m	37	3	19.4646	<0.0001
	f	31	2	1.3308	NS
Nitrogen	m	29	3	5.6227	0.0042
	f	28	2	4.7418	0.0176
carbon	m	29	3	3.7954	0.0221
	f	28	2	2.5791	NS
C:N ratio	m	29	3	5.5920	0.0043
	f	28	2	4.4789	NS

other colors in male specimens of *U. longisignalis* (Fig. 2, Table 5).

Hepatopancreatic nutrient concentrations did not differ between male and female specimens of *U. nr. minax* within any one color classification (Table 6). Lipid concentrations of the hepatopancreas in *U. nr. minax* increased significantly along a gradient from brown, yellow-ochre, lemon-yellow, to cadmium-yellow (Fig. 3, Table 6). Hepatopancreas C concentrations showed the same overall trend, but brown and yellow ochre tissues had lower C concentrations than in lemon yellow and cadmium yellow tissues (Fig 3). N concentration had the opposite trend, as N concentration in brown and yellow ochre hepatopancreatic tissues was greater than in specimens of other hepatopancreas colors (Fig. 3, Table 6). The relationship between C:N ratio and hepatopancreas color was similar to the trend seen with both lipid and C concentration; brown and yellow-ochre colored hepatopancreas tissues had smaller C:N ratios than did hepatopancreas tissue of other colors (Fig. 3, Table 6).

### Relationship between ovarian maturation and hepatopancreas

As ovarian maturation progressed, both the total lipid ( $F = 5.057$ ,  $df = 2,32$ ,  $p = 0.0131$ ) and C ( $F = 3.8206$ ,  $df = 2,29$ ,  $p = 0.0081$ ) concentration of the hepatopancreas of *U. longisignalis* increased. Females with a stage III ovary had higher hepatopancreatic lipid concentrations (mean =  $347.9 \pm 106$  mg g<sup>-1</sup> DW) than those with a stage I ovary (mean =  $255.6 \pm 57$  mg g<sup>-1</sup> DW). Hepatopancreatic C:N ratios of *U. longisignalis* also increased with ovarian maturation. N concentration of the hepatopancreas did not differ with respect to ovary

TABLE 6. – *Uca nr. minax*. Results of two-factor ANOVA tests for effects of sex and hepatopancreas color (hep. color) on differences in hepatopancreas lipid, carbon C, and nitrogen N concentrations, as well as the C:N ratio. NS = not significant at 0.05 level.

	Source of variation	df	F value	P value
Lipid	sex	1	1.57	NS
	hep. color	3	42.359	<0.0001
	sex and hep. color	3	0.579	NS
nitrogen	sex	1	4.6432	NS
	hep. color	3	13.9219	<0.0001
	sex and hep. color	3	0.3698	NS
Carbon	sex	1	0.4623	NS
	hep. color	3	17.3251	<0.0001
	sex and hep. color	3	0.514	NS
C:N ratio	sex	1	3.1238	NS
	hep. color	3	8.4639	0.0001
	sex and hep. color	3	0.0436	NS

stage in *U. longisignalis*. In contrast, during ovarian maturation of *U. nr. minax*, the lipid, C, and N concentrations and the C:N ratio of the hepatopancreas did not change.

Hepatopancreatic lipid and C content, as opposed to concentration, increased throughout ovarian maturation in *U. longisignalis* ( $F = 7.585$ ,  $df = 2,30$ ,  $p = 0.0024$ ;  $F = 5.274$ ,  $df = 2,29$ ,  $p = 0.0119$ , respectively; stage I mean =  $37 \pm 18.2$  mg and stage III mean =  $71 \pm 30.5$  mg; stage I mean =  $45.5 \pm 17.2$  mg and stage III mean =  $74.4 \pm 30.9$  mg respectively). The pattern was the same for *U. nr. minax* ( $F = 6.934$ ,  $df = 2,26$ ,  $p = 0.0044$ ;  $F = 7.766$ ,  $df = 2,26$ ,  $p = 0.0026$  respectively; stage I mean =  $40.6 \pm 38$  mg and stage III mean =  $116.2 \pm 40$  mg; stage I mean =  $50.9 \pm 33.9$  mg and stage III mean =  $124.6 \pm 42.9$  mg respectively). The N content of the hepatopancreas did not change during ovarian maturation of either species.

As GSI increased, hepatopancreatic lipid and C concentration, as well as nutrient content of the hepatopancreas, also increased. There was a positive relationship between the GSI of *U. longisignalis* and both the lipid and C concentration of the hepatopancreas ( $r^2 = 0.18$ ,  $p = 0.017$ ;  $r^2 = 0.23$   $p = 0.009$  respectively, Fig. 4). GSI of *U. longisignalis* was not correlated to lipid or C content of the hepatopancreas. The GSI of *U. nr. minax* was not correlated to hepatopancreatic lipid concentration but was positively correlated to C concentration of the hepatopancreas ( $r^2 = 0.27$ ,  $p = 0.01$ , Fig. 4). GSI of *U. nr. minax* was not correlated to the lipid content of the hepatopancreas. However, there was a positive correlation between GSI and the C content of the hepatopancreas ( $r^2 = 0.18$ ,  $p = 0.0416$ , Fig. 4).



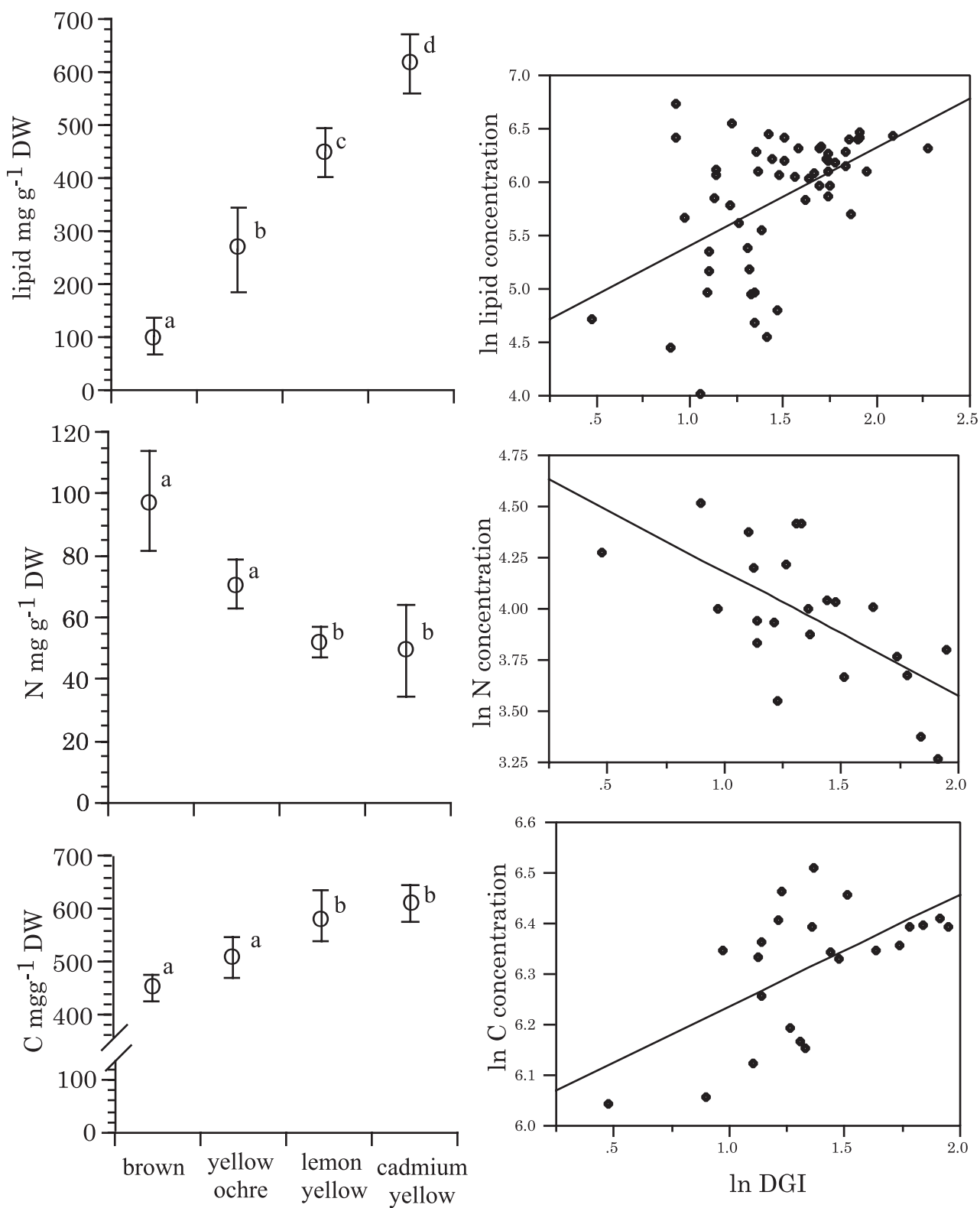


FIG. 3. – *Uca nr. minax*. Mean ± 95% CI total hepatopancreas lipid concentration of pooled male and female specimens with differently colored hepatopancreas tissues (on left) and logarithmic regression of hepatopancreatic lipid, nitrogen (N) and carbon (C) concentration and digestive gland index (DGI) of male specimens (on right). Means with different letters are significantly different at  $p < 0.05$ .

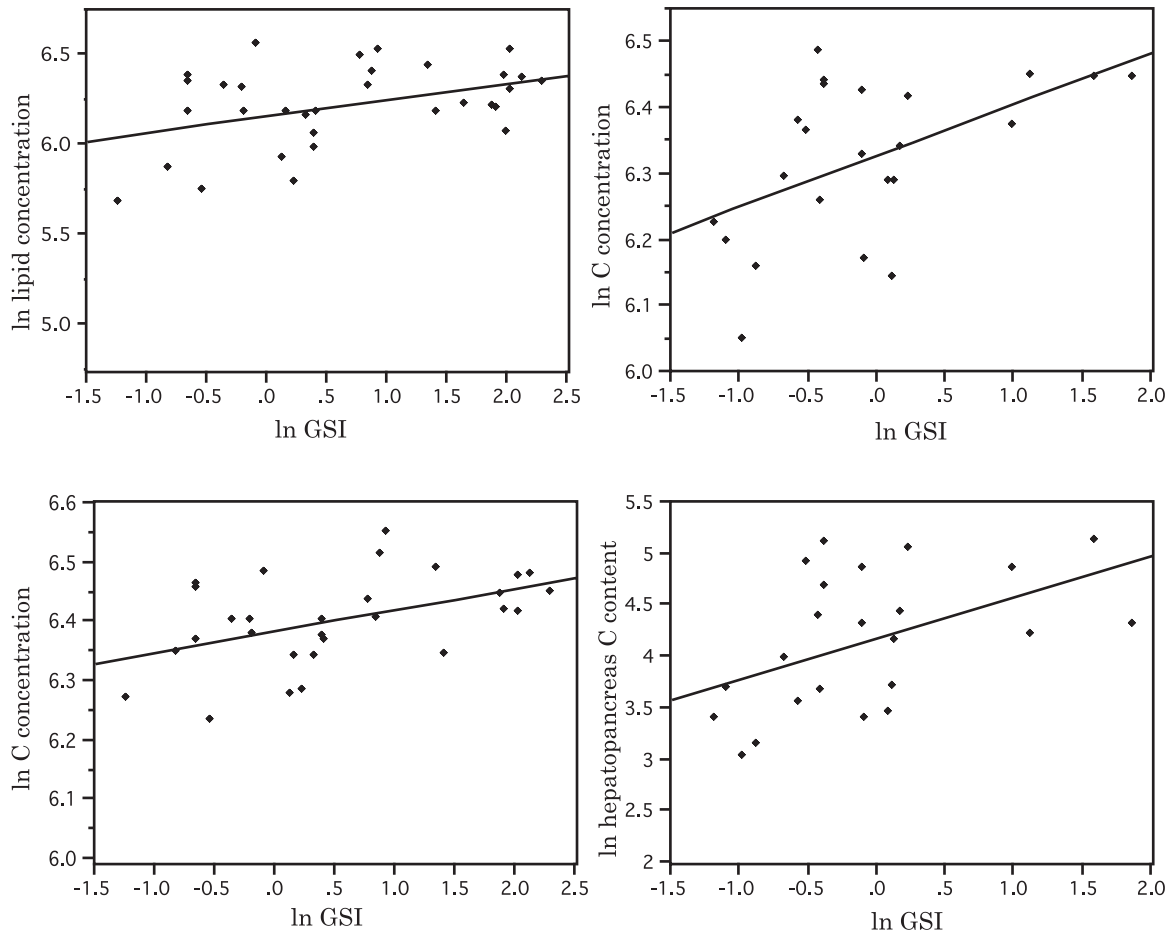


FIG. 4. – *Uca longisignalis* (left column) and *U. nr. minax* (right column). Logarithmic regression of hepatopancreas lipid concentration and carbon (C) concentration with gonadosomatic index (GSI) (on left). Logarithmic regression of hepatopancreas C concentration and content with GSI (on right).

## DISCUSSION

The most studied decapod crustaceans in terms of lipid concentrations and ovarian maturation are penaeid shrimp (Kulkarni and Nagabhushanam, 1979; Middleditch *et al.*, 1980; Read and Caulton, 1980; Castille and Lawrence, 1989; Mourente and Rodriguez, 1991; Lubzens *et al.*, 1995). There are few studies documenting increases in lipid concentrations with ovarian maturation for semi-terrestrial or intertidal crabs (Mourente *et al.*, 1994; Wen *et al.*, 2001). The present study is the first to examine the lipid concentration of fiddler crabs from the warm temperate western Atlantic or Gulf of Mexico. As in other studies, this work documents an increase in total ovary lipid and C concentration with ovarian maturation. This increase was similar in *U. longisignalis* and *U. nr. minax*, with species exhibiting similar lipid and C concentrations in each ovarian stage. These similarities are perhaps not surprising

because the sampled populations of these two closely related species were sampled at close proximity to one another in the marshes of Cypremort Point, Louisiana.

Both *U. longisignalis* and *U. nr. minax* were found to have higher ovarian lipid concentrations than previously reported for *U. tangeri* Eydoux (106 - 197 mg g<sup>-1</sup> DW) from the Bay of Cádiz in southwestern Spain; even so, the GSI reported for *U. tangeri* was very similar to those reported for the two Gulf of Mexico species (Mourente *et al.*, 1994). As a percentage of total ovary weight, total ovarian lipid of *Clibanarius longitarsus* (De Haan), an estuarine hermit crab, ranged from 10 to 40% DW (Ajmal Khan and Natarajan, 1980). In comparison, total ovary lipid of *U. longisignalis* and *U. nr. minax* ranged from 21 to 38% DW, which is similar to the 12 to 37% range reported in marine nephropid lobsters (Tuck *et al.*, 1997; Rosa and Nunes, 2002). The range for *Uca* in the present study is slightly higher

than the range of 23 to 30% DW of two grapsoid crabs, *Armases cinereum* (Bosc) and *Sesarma nr. reticulatum* (Say), from Louisiana (Hasek and Felder, 2005). Thus, these values range widely among the few taxa examined to date and do not suggest a pattern that can be readily correlated to phylogeny or habitat.

*U. longisignalis* and *U. nr. minax* also exhibited similar ovary N concentrations, which did not differ over the course of ovarian maturation, suggesting relatively stable protein levels. In the deep-sea crab, *Chaceon quinquedens* (Smith), protein levels also did not differ between ovaries of mature and advanced stages (Biesiot and Perry, 1995). In contrast, protein contents of other species such as *Clibanarius longitarsus* showed evidence of decrease as ovarian maturation progressed (Ajmal Khan and Natarajan, 1980). Thus, reported patterns in this measure over the course of ovarian maturation also vary among decapods.

Water concentration of the ovaries decreased with ovarian maturation to an equal extent in both *U. longisignalis* and *U. nr. minax* (Table 2). In comparison, water concentration of the ovaries of *C. longitarsus* also decreased with ovarian maturation and the water concentration of the entire animal decreased with ovarian maturation of the shrimp *Fenneropenaeus indicus* (H. Milne Edwards) (Ajmal Khan and Natarajan, 1980; Read and Caulton, 1980). From examples known to date, this appears to be a consistent pattern in decapod embryogenesis.

Previous reports have noted that color of the hepatopancreas varies among and within species (Herring, 1973; Gibson and Barker, 1979; Herreid and Full, 1988), as well as that measurable pigment contents of the hepatopancreas can vary (Castillo and Negre-Sadargues, 1995). Since only intermolt crabs were sampled in the present study, the color differences we observed are relatively independent of imminent pre-ecdysis or post-ecdysis. In general, brachyuran crabs exhibit ovarian growth and ecdysis as mutually exclusive processes and ovarian maturation occurs between reoccurring molts (Adiyodi, 1988). Furthermore, we observed these same colors in both sexes and species over a continuous year of monthly sampling (unpublished data).

Previous studies have not measured total lipid of the hepatopancreas in relation to hepatopancreatic color (Kucharski and Da Silva, 1991; Wen *et al.*, 2001). To determine the relationship between bio-

chemical composition and season, Health and Barnes (1970) only used crabs with "hepatopancreas of normal color and consistency" but failed to mention specific criteria. Our findings indicate that there is potential value to stating color of analyzed hepatopancreatic tissues in future works, especially if determined empirically and expressed by standardized color terminology.

Clearly, the range of total hepatopancreatic lipid varies widely among and within species. Mean hepatopancreatic lipid concentration for three species of hermit crabs from the Marshall Islands ranged from 54 to 57% DW (Lawrence, 1976). Total lipid of the hepatopancreas of *Paratelphusa hydrodromous* (Herbst) ranged from 60 to 70% (Adiyodi and Adiyodi, 1971). In comparison, hepatopancreatic lipid concentration of *U. longisignalis* and *U. nr. minax* ranged from 9 to 65% DW over the entire course of the present study (Figs. 2 and 3).

Changes in total hepatopancreatic lipid levels may occur during late intermolt and early premolt (Giese, 1966; O'Connor and Gilbert, 1968; Bollenbacher *et al.*, 1972; Cockcroft, 1997). For example, Cockcroft (1997) found lipid concentration was lowest during the molt period of the rock lobster and highest during intermolt and premolt. While our molt staging was not fine-scaled enough to differentiate the phase of intermolt for our sampled crabs, it was clear the population we sampled was not in a molt period. Further work would be required to determine whether differently colored hepatopancreas tissues represent different phases of the intermolt period. There can be large variations in the lipid content of the hepatopancreas independent of the molt cycle. Male lobsters in molt-stage C<sub>4</sub> had hepatopancreas lipid contents that ranged from 16 to 61% DW (Dall, 1981), similar to the range we found with *U. longisignalis* and *U. nr. minax*.

Previous workers do not mention hepatopancreatic color differences with respect to phases of the intermolt cycle or the molt cycle in general. *Uca* with brown or yellow-ochre hepatopancreases may have been in the earliest stages of intermolt, when feeding has resumed after the fasting periods during ecdysis and postmolt. Many of the females with darker hepatopancreases were in the early stage of ovarian development, a process that only occurs during intermolt.

Another possible explanation of the different colors and lipid contents is fluctuating food sources. These intertidal crabs occupy habitats in constant

flux, unlike those of many other crustaceans in which lipid levels have been studied. During ovarian maturation the hepatopancreas of *Carcinus* becomes pale compared to the normal yellowish-brown or orange color (Goodwin, 1960). Normal coloration can be restored to the hepatopancreas by feeding the crab with a carotenoid-rich diet (Goodwin, 1960). The hepatopancreas of *Clibanarius erythropus* Latreille, when animals are fed astaxanthin diets in the laboratory, is light pink; for specimens fed  $\beta$ -carotene and canthaxanthin diets, it is visually colorless (Castillo and Negre-Sadargues, 1995).

There were significant morphometric relationships between DGI and hepatopancreas color among males of *U. longisignalis*, but not among female specimens. Biometric relationship between CW or WW and hepatopancreas color was also found among males of *U. nr. minax*, but again not among females. Morphometric relationships with biochemical components were found for male crabs of both species. Females did not show morphometric relationships to lipid concentration and only in *U. nr. minax* was hepatopancreatic C concentration correlated to DGI. This suggests that some processes specific to males may influence lipid concentration of the hepatopancreas. As DGI increased the lipid concentration increased. This may indicate that when the hepatopancreas has a lower lipid content it is smaller in relation to body size compared to when it is lipid-rich. As males increase in CW and invest resources in allometric growth of a massive chela, DGI might be expected to decrease. Insight as to why lipid concentration increases as the DGI increases may come from studies that monitor seasonal lipid content as well as morphometric data.

In some crustaceans hepatopancreatic lipid levels decrease as ovaries mature (Pillay and Nair, 1973). In others hepatopancreatic lipid levels increase with ovarian maturation (Mourente *et al.*, 1994) or initially increase and then decrease (Wen *et al.*, 2001). Hepatopancreatic lipid and C concentrations of *U. longisignalis* increased as ovaries matured. In *U. nr. minax* hepatopancreatic lipid and C concentrations were not related significantly to ovarian maturation, as was the case with *Melicertus kerathurus* Forskål and *Carcinus maenas* (Heath and Barnes, 1970; Mourente and Rodriguez, 1991). The relationship between the GSI and lipid or C concentration of the hepatopancreas was positive for *U. longisignalis*; but only C concentration was significantly related to

GSI of *U. nr. minax*. However, there was no significant relationship between hepatopancreatic lipid or C content and GSI of *U. longisignalis*. Only C content of the hepatopancreas of *U. nr. minax* increased as the GSI increased. These findings suggest there is no mobilization of lipids from the hepatopancreas to the ovary during ovarian maturation. Most probably the biochemical needs of ovarian maturation are met by increased dietary intake and not by translocating lipid stores from the hepatopancreas. As these fiddler crabs live in temperate environs where food sources may fluctuate in source and quality it appears there is no need to deplete hepatopancreas lipid stores for ovarian maturation. Alternatively, if mobilization occurred it might not have been detected because dietary intake may have been increased sufficiently to compensate for it during ovarian maturation; thus, there would be no concomitant depletion of the hepatopancreas lipid store.

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