SEASONAL CHANGES IN THE LIPID COMPOSITION OF GILL TISSUE FROM THE FRESHWATER MUSSEL CARUNCULINA TEXASENSIS[1]

ARTHUR F. HAGAR AND THOMAS H. DIETZ

Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803

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The lipid composition of gill tissue from the freshwater mussel *Carunculina texasensis* was determined. Particular attention was paid to the distribution of arachidonic acid, because the arachidonic acid metabolite prostaglandin E_2 appears to be involved in the regulation of Na uptake by gills of freshwater mussels. Arachidonic acid is the most abundant fatty acid in the phospholipid fraction of gill tissue, but it is not prevalent in the neutral lipid fractions. In contrast with marine molluscs, in which fatty acids of the n-3 family predominate, fatty acids of the n-6 family predominate in the phospholipid fraction from *C. texasensis* gills. The effects of season on lipid composition also were investigated. Gill tissue from mussels collected in winter contained higher proportions of polyunsaturates and lower amounts of saturates than did gill tissue from animals collected in summer. The percentage of arachidonic acid, however, was uniformly high throughout the year, suggesting that this fatty acid serves an important function in which precursor stability is required. Seasonal alterations in the proportions of free sterols and triglycerides and in the phosphatide composition were observed. The functional significance of the various changes is not known, but they may be related to some of the aspects of gill function other than ion regulation.

INTRODUCTION

One of the principal problems faced by freshwater animals is the maintenance of ionic balance. In freshwater mussels the primary site of Na uptake is the gills (Dietz and Graves 1981). Gills of unionid mussels, however, are complex organ systems. In addition to being the primary site of ion transport, the gills also are involved in food procurement and gas exchange and serve as a brooding chamber for the larval glochidia in females. Thus the gills serve many different functions the relative importance of which may vary during the year.

Phospholipids are the main structural elements of cellular membranes and, as such, may be expected to undergo changes in composition in order to accommodate changes in gill function. Additionally, poikilothermic organisms often undergo alterations in membrane composition seasonally in order to compensate for changes in environmental temperature (Hazel and Sellner 1980). While lipid composition and metabolism have been extensively studied in marine molluscs, little work has been done on freshwater forms and even less on gill tissue. The primary goals of this study were to characterize the lipid composition of gill tissue from the unionid mussel *Carunculina texasensis* (Lea) and to determine whether seasonal changes occur in the phosphatide and fatty acid compositions of membrane phospholipids.

Particular attention was paid to arachidonic acid [20:4(n-6)], because this acid is the substrate for the production of the diene prostaglandins through the cyclooxygenase pathway (Kuehl and Egan 1980). Investigation of the mechanisms regulating Na uptake in the freshwater mussel *Ligumia subrostrata* (Say) has implicated prostaglandin E_2 (PGE_2) as a negative modulator of uptake (Saintsing, Hwang, and Dietz 1983). While marine molluscs possess little 20:4(n-6), the few freshwater bivalves so far investigated contain relatively high levels of this fatty acid. Arachidonic acid was found to be the most abundant fatty acid in a total lipid extract of *L. subrostrata* gills (Saintsing et al. 1983) and was reported to be a major component of a whole-animal extract of the South American freshwater mussel *Diplodom patagonicus* (Pollero, Brenner, and Gros 1981). Since the concentration of free arachidonate is thought to control the production of prostaglandins

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in mammalian systems (Irvine 1982), the distribution of endogenous 20:4(n-6) in gill tissues of freshwater mussels is of considerable interest in determining how PGE_2 synthesis may be regulated in this tissue.

MATERIAL AND METHODS

ANIMALS

Male *Carunculina texasensis* used in this study were collected from freshwater ponds near Baton Rouge, Louisiana. Field temperatures ranged from 4 in the winter to 30 C in the summer. Mussels were maintained in the laboratory in aquaria containing artificial pond water, as previously described (Saintsing et al. 1983).

LIPID EXTRACTION AND SEPARATION

Gill tissue was excised and blotted dry, and the wet weight was determined. The tissue was then cut into small pieces on a beaker inverted in ice and the lipids extracted by the procedure of Bligh and Dyer (1959). The chloroform/methanol solution used to extract the lipids contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant, and all organic solvents used in the study were gassed with N_2 before use. An aliquot of the total lipid extract was transferred to a preweighed vial, the solvent evaporated under N_2, and the total lipid weight determined gravimetrically.

The lipid extract was spotted on a Whatman K5 thin-layer plate and resolved into the various lipid classes by development in hexane/diethyl ether/acetic acid (80:20:2). The plate was removed from the tank and the solvent evaporated briefly under a stream of N_2. The origin, which contained the phospholipids, was immediately scraped from the plate and the silica gel extracted three times with 2 ml of chloroform/methanol/water (5:5:1). The neutral lipids were visualized by spraying the plate with 2′,7′-dichlorofluorescein and identified by comparison to R_f-values of a standard mixture containing phospholipid ($R_f = 0.0$), cholesterol ($R_f = 0.18$), free fatty acid ($R_f = 0.35$), triglyceride ($R_f = 0.51$), fatty acid methyl ester ($R_f = 0.67$), and cholesterol ester ($R_f = 0.80$). The identity of cholesterol and cholesterol ester were confirmed by the development of a dark red color following application of an acid ferric chloride solution to the plate (Christie 1982). Neutral lipid fractions were scraped from the thin-layer plates and extracted from the silica gel three times with 2 ml of chloroform/methanol (9:1). The extracts were washed twice with 1.5 ml of 0.05 M Tris, pH 9, to remove the dichlorofluorescein. The dry weights of each lipid class were then determined gravimetrically.

FATTY ACID ANALYSIS

The lipid classes were dissolved in 1.0 ml of benzene and fatty acid methyl esters prepared by transesterification in 2.0 ml of 5% methanolic HCl by refluxing for 2 h at 66 C (Christie 1982). The methyl esters were separated by gas-liquid chromatography on a 3.6-m stainless steel column packed with silar-10C using a Hewlett-Packard model 830A gas chromatograph. Fatty acids were identified by comparing relative retention times to those of standard mixtures, by semilog plot and separation factor analysis (Ackman 1969), and by separation of the methyl esters into fractions of varying degrees of unsaturation by thin-layer chromatography on silver nitrate–impregnated plates. A sample chromatogram is shown in figure 1. The fatty acid shorthand notation employed in this paper conforms to that suggested by the IUPAC-IUB Commission on Biochemical Nomenclature (1977). For example, the shorthand designation for arachidonic acid is 20:4(n-6), which describes a 20-carbon fatty acid with four double bonds in which the terminal olefinic bond is six carbons from the methyl end of the fatty acid chain.

PHOSPHOLIPID COMPOSITION

The phospholipid fraction was resolved into various phosphatide classes by thin-layer chromatography in one of two solvent systems: (1) chloroform/petroleum ether/methanol/acetic acid/boric acid (40:30:20:10:1.8, v:v:v:v:w) (Gilfillan et al. 1983), or (2) chloroform/acetone/methanol/acetic acid/water (10:4:2:2:1) (Rouser, Siakotos, and Fleischer 1966). The phospholipids were visualized by exposure to I_2 vapors and identified by comparison to R_f-values of standards. Phosphatide separation and identification were independently confirmed by two-dimensional thin-layer chromatography employing chloroform/methanol/28% aqueous ammonia (65:35:

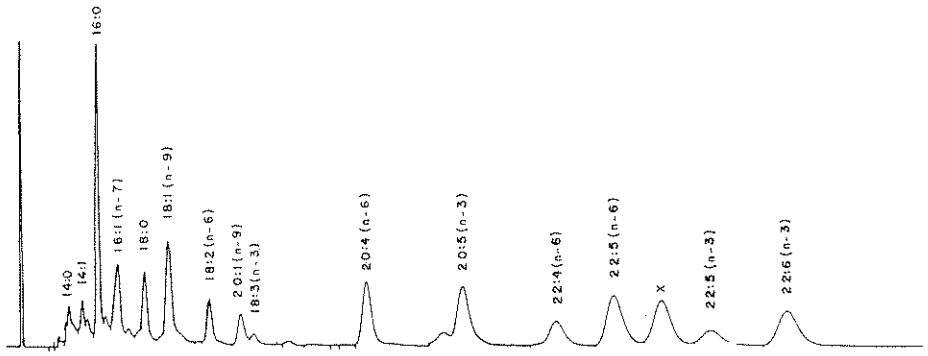

FIG. 1.—Sample chromatogram of fatty acids extracted from gill tissue of a male *Carunculina texasensis*. Analysis was performed on a 3.6-m stainless steel column packed with silar-10C. The peak labeled *X* is the unknown with an R_f-value of 5.87. Data are from August.

5) in the first direction and chloroform/acetone/methanol/acetic acid/water (10:4:2:2:1) in the second direction (Rouser et al. 1966). The amount of each phospholipid class was determined by the method of Rouser, Fleischer, and Yamamoto (1970).

STATISTICS

Tests for statistical differences between means for the lipid composition (table 1), and phospholipid fatty acid (table 3) data were made using a *t*-test. Seasonal changes in phosphatide percentages (fig. 2) were analyzed by an analysis of variance, and differences between means were tested for significance using the Tukey-Kramer method for unplanned comparisons (Sokal and Rohlf 1981).

MATERIALS

All chemicals and solvents were of analytical reagent grade, and all aqueous so-

TABLE 1

LIPID COMPOSITION OF GILL TISSUE FROM MALE *Carunculina texasensis*

	Spring (April)	Summer (July)	Autumn (October)	Winter (December)
Total lipid (TL; mg)[f]	11.1 ± 3.5	11.9 ± 1.0	11.4 ± .6	8.4 ± 1.1
mg/g gill[b,c,d,e,f]	11.3 ± 1.7	11.2 ± .8	15.7 ± .6	7.7 ± .8
Phospholipids[b,d,f]	5.9 ± 1.0	6.1 ± .7	9.0 ± .4	4.2 ± .3
% TL	52.3 ± 2.7	54.5 ± 2.3	60.2 ± 3.4	54.8 ± 2.2
Sterols[a,b,e,f]	.9 ± .4	3.2 ± .4	2.7 ± .3	.8 ± .1
% TL[a,b,d,e,f]	7.1 ± 2.0	29.0 ± 1.3	18.0 ± 2.0	10.6 ± 1.1
Fatty acids	.2 ± .1	.3 ± .0	.8 ± .2	.2 ± .0
% TL	2.2 ± 1.1	2.8 ± .4	5.1 ± .9	2.0 ± .2
Triglycerides[a,b,c,e]	3.6 ± .3	.2 ± .0	.8 ± .2	1.7 ± .4
% TL[a,b,e,f]	32.4 ± 2.4	2.3 ± .3	5.1 ± 1.4	21.4 ± 2.4
Sterol esters	.7 ± .2	1.2 ± .1	1.7 ± .5	.8 ± .1
% TL	6.0 ± 1.9	10.6 ± 1.5	11.2 ± 3.2	10.5 ± .4

NOTE.—Values are means ± SEM ($N = 3$), in which each replicate was composed of tissue from three animals. Data on lipid classes are presented as mg of lipid/g of gill tissue and as percent of TL.

Significant differences ($P < .05$):

[a] Spring vs. summer.
[b] Spring vs. autumn.
[c] Spring vs. winter.
[d] Summer vs. autumn.
[e] Summer vs. winter.
[f] Autumn vs. winter.

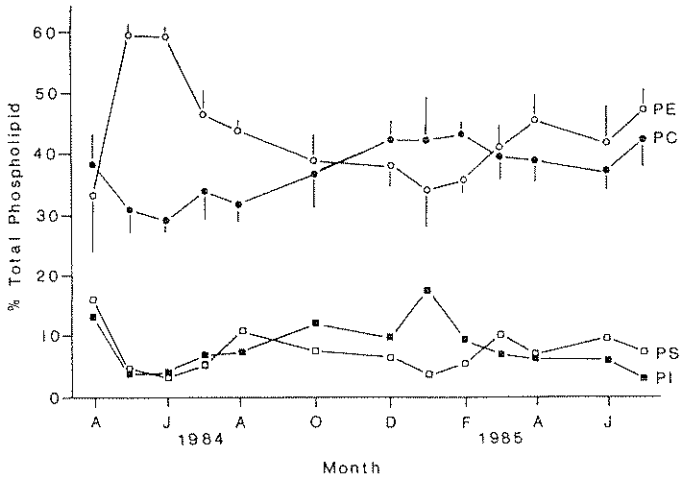

FIG. 2.—Seasonal variation in the phosphatide composition of gill tissue from male *Carunculina texasensis*. Data are the means of three to four animals and are presented as the percent of the total phospholipid. Vertical bars represent SEM for PC and PE. No error measurements are given for PI and PS because they are typically smaller than the size of the symbols.

lutions were prepared in deionized water. Thin-layer chromatography plates were purchased from Whatman, Inc. Thin-layer and gas-liquid chromatographic standard mixtures were obtained from Nu-Check Prep, and phospholipid standards were obtained from Sigma Chemical Co. Silar-10C was purchased from Applied Science.

RESULTS

LIPID COMPOSITION

Male *Carunculina texasensis* collected in spring 1984 contained 11.3 ± 1.7 mg of lipid/g wet weight of gill tissue (table 1). Approximately half of the total lipid extract was phospholipid, and about one-third was in the form of triglyceride. Free sterols and sterol esters accounted for 7.1% and 6.0%, respectively. The free fatty acid content of gill tissue was low (2.2%), and only trace amounts of diglyceride were found. The major phosphatide classes in gill tissue of *C. texasensis* were phosphatidylcholine (37%) and phosphatidylethanolamine (32%); phosphatidylinositol (13%), phosphatidylserine (16%), and cardiolipin (2%) were present in lesser amounts.

The fatty acid compositions of the lipid classes are presented in table 2. The gas chromatography program employed did not separate 20:1(n-9) and 18:3(n-6) on silar-10C. Separation of the fatty acid methyl esters by argentation thin-layer chromatography prior to injection, however, indicated that over 90% of this peak was composed of 20:1(n-9). Arachidonic acid was the most abundant fatty acid in the phospholipid fraction; other major components were 18:2(n-6), 18:0, 16:0, 18:1(n-9), 20:1(n-9), and 16:1(n-7). Polyunsaturated fatty acids (PUFA) accounted for almost one-half of the total, while saturates and monoenes each made up about 25%; the ratio of unsaturates to saturates (U/S) was 3:1. Arachidonic acid is produced from linoleic acid [18:2(n-6)] through desaturation and elongation, and these two fatty acids summed to 25% of the total fatty acids in the phospholipid fraction. Thus gill phospholipids of *C. texasensis* contain an abundant supply of substrate for the production of PGE_2.

The neutral lipid fractions contained higher proportions of saturated fatty acids and lower amounts of monoenes than did the phospholipid fraction (table 2). This was particularly true for the triglyceride fraction, in which saturates accounted for almost 50% of the total. The triglyceride and sterol ester classes also had lower percentages of PUFA, as compared to the phospholipids, but the large amount of 22:5(n-3) in the free fatty acid fraction resulted in this class having a proportion of PUFA that was equivalent to that found in the phospholipid fraction. The sterol ester fraction was

marked by a significant amount of an unidentified component with a relative retention time of 0.64 min. None of the neutral lipid fractions contained large amounts of 20:4(n-6). Another major difference between the phospholipids and the neutral lipids was that PUFA derived from 18:2(n-6), that is, n-6 fatty acids, predominated in the phospholipid fraction, while PUFA derived from 18:3(n-3), that is, n-3 fatty acids, predominated in the neutral lipid fractions. Consequently, while the (n-6)/(n-3) ratio was 2.35 for the phospholipids, neutral lipids had ratios that ranged from 0.2 to 0.9.

SEASONAL CHANGES IN LIPID COMPOSITION

The total lipid content of gill tissue from *C. texasensis* varied seasonally, being highest in autumn and lowest in winter (table 1). No significant change in the relative proportions of phospholipids (52%–60%), free fatty acids (2%–5%), or sterol esters (6%–11%) were noted. Large fluctuations, however, were observed in the free sterol and triglyceride fractions. The percentage of free sterols peaked in the summer, declined through the autumn and winter, and was at a minimum in the spring. The triglyceride fraction exhibited the opposite trend. While there were no significant seasonal changes in the total amount of phospholipid, fluctuations in the relative proportions of the major phospholipid classes were observed (fig. 2). The percentage of phosphatidylethanolamine (PE) increased during the spring and declined in the late summer, while the percentage of phosphatidylinositol (PI) varied in the opposite di-

TABLE 2

FATTY ACID COMPOSITIONAL ANALYSIS OF THE LIPID CLASSES FROM GILL TISSUE OF MALE *Carunculina texasensis*

Fatty Acid	Phospholipids	Triglycerides	Fatty Acids	Sterol Esters
14:0	4.22	17.39	13.08	13.65
14:1	.67	1.88	2.16	1.68
X_1 (RRT = .64)	0	0	0	10.05
16:0	9.08	15.57	9.95	10.52
16:1(n-9)	1.25	3.46	.45	1.26
16:1(n-7)	6.33	1.30	1.06	0
18:0	9.31	13.04	8.54	10.12
18:1(n-9)	8.51	8.38	4.06	9.70
18:2(n-6)	10.36	7.08	3.48	5.12
20:1(n-9)[a]	7.94	2.19	2.19	1.06
18:3(n-3)	1.70	0	.20	.29
20:2(n-6)	.14	0	2.82	0
22:0	.14	0	2.82	0
20:4(n-6)	15.13	4.11	.74	.92
20:4(n-3)	.40	0	1.95	.21
24:0	.40	0	0	6.76
20:5(n-3)	5.50	2.06	10.85	1.60
24:1	.40	0	0	0
22:4(n-6)	2.88	.20	.68	0
22:5(n-6)	2.41	.66	.44	1.78
22:5(n-3)	3.84	9.45	27.77	6.08
22:6(n-3)	1.69	1.76	.32	4.79
Saturates	23.01	46.00	34.39	41.05
Monoenes	25.10	17.21	9.92	13.70
PUFA[b]	44.05	25.32	49.25	20.79
U/S	3.01	.92	1.72	.84
(n-6)/(n-3)	2.35	.91	.20	.60

NOTE.—Collected in spring 1984. Data are presented as weight percents. Phospholipid values are means of quadruplicate determinations; neutral lipid values are means of duplicate determinations. PUFA = polyunsaturated fatty acids; U/S = (monoenes + PUFA)/saturates.

[a] 20:1(n-9) and 18:3(n-6) were not separated.

[b] Two to six double bonds.

rection. Phosphatidylcholine (PC) exhibited a pattern similar to that of PI, but the ANOVA indicated that the differences were significant at only the 0.08 level. Significant fluctuations occurred in the level of phosphatidylserine (PS), but no seasonal pattern was observed. The percentage of cardiolipin was relatively constant at 2%–5% (data not shown) throughout the year.

Fatty acid compositional data for the phospholipid fractions from animals collected in different seasons are presented in table 3. Winter-acclimatized mussels had (1) a lower amount of saturates and monoenes, (2) a higher proportion of PUFA, and (3) higher values of U/S and unsaturation index (UI) than did summer-acclimatized animals. The reduced amount of saturates was due to decreases in 14:0 and 16:0, while the increased proportion of monoenes in the summer was due to increases in 18:1(n-9) and 20:1(n-9). The increase in PUFA in winter animals was caused by small, but significant, increases in the amounts of almost all of the eicosa- and docosanoic acids, particularly the n-3 fatty acids.

Mussels collected in winter also had high levels of two unidentified components, which had relative retention time (RRT) of 0.59 and 5.87 min on silar-10C. The former compound accounted for 7%, 11%, and 13% of the total fatty acids in summer, au-

TABLE 3

FATTY ACID COMPOSITIONAL ANALYSIS OF THE PHOSPHOLIPID FRACTION FROM GILL TISSUE OF MALE *Carunculina texasensis*

Fatty Acid	Spring (April)	Summer (July)	Autumn (October)	Winter (December)
14:0[a]	4.22 ± .32	6.52 ± .02	1.93 ± .73	2.84 ± .11
14:1[a]	.67 ± .07	2.81 ± .07	1.50 ± .28	.74 ± .45
X_1 (RRT = .59)[a]	0	6.93 ± .13	10.66 ± 1.36	12.72 ± .61
16:0[a]	9.08 ± 1.00	9.21 ± .14	8.35 ± .69	6.78 ± .21
16:1(n-9)	1.25 ± .46	2.14 ± .10	2.52 ± .48	2.68 ± .04
16:1(n-7)	6.33 ± .69	4.62 ± .29	5.03 ± .11	4.40 ± .08
18:0	9.31 ± 1.88	5.50 ± .27	6.20 ± .23	5.13 ± .12
18:1(n-9)[a]	8.51 ± 1.66	6.96 ± .10	4.41 ± .29	3.68 ± .08
18:2(n-6)[a]	10.36 ± 2.72	5.21 ± .10	3.74 ± .38	4.15 ± .12
20:1(n-9)[a,b]	7.94 ± 1.41	18.42 ± .53	12.68 ± .44	13.33 ± .27
18:3(n-3)[a]	1.70 ± .10	1.18 ± .18	2.23 ± .14	2.81 ± .07
20:2(n-6)[a]	.14 ± .10	.11 ± .03	1.29 ± .03	1.32 ± .04
22:0	4.71 ± 2.55	0	0	0
20:4(n-6)	15.13 ± 3.44	17.72 ± .48	15.19 ± 1.08	17.78 ± .42
20:4(n-3)[a]	.40 ± .14	.09 ± .01	2.01 ± .75	1.62 ± .17
24:0	.40 ± .14	0	0	0
20:5(n-3)[a]	5.50 ± 1.95	1.96 ± .07	2.33 ± .31	3.46 ± .11
24:1[a]	.40 ± .14	.74 ± .06	3.66 ± .68	1.38 ± .02
22:4(n-6)	2.88 ± .47	3.54 ± .06	3.38 ± .21	3.71 ± .08
22:5(n-6)[a]	2.41 ± .28	3.05 ± .08	2.70 ± .12	3.82 ± .07
X_2 (RRT = 5.87)[a]	0	.48 ± .06	4.09 ± .38	1.63 ± .26
22:5(n-3)[a]	3.84 ± 1.02	1.18 ± .01	1.34 ± .01	2.16 ± .06
22:6 (n-3)[a]	1.69 ± .35	1.23 ± .05	1.86 ± .30	2.32 ± .08
Saturates[a]	23.01 ± 2.77	21.23 ± .41	16.48 ± 1.10	14.75 ± .46
Monoenes[a]	25.10 ± .99	35.69 ± .34	29.80 ± .13	26.21 ± .22
PUFA[a]	44.05 ± 4.63	35.27 ± .78	36.33 ± 2.72	43.33 ± .34
U/S[a]	3.01 ± .59	3.34 ± .10	4.01 ± .25	4.71 ± .14
UI[a]	1.90 ± .24	1.73 ± .02	1.68 ± .10	1.98 ± .01
(n-6)/(n-3)[a]	2.35 ± .68	5.25 ± .16	2.69 ± .08	2.49 ± .09

NOTE.—Collected during different times of the year. Data are presented as weight percents. Values are means ± SEM of three to four animals. Fatty acids are ordered by their retention times on silar-10C. For the unidentified components, the relative retention times are given in parentheses. UI = Unsaturation index (average number of double bonds per fatty acid).

[a] Significant ($P < .05$) difference between summer- and winter-acclimatized animals.

[b] 20:1(n-9) and 18:3(n-6) were not separated.

tumn, and winter animals, respectively, but was absent from gill tissue from animals collected in spring. The latter compound was much less abundant and was also not found in gill tissue from spring animals.

Mussels collected in the spring and autumn generally had higher variances with respect to fatty acid composition than did animals collected in the summer and winter. This is not unexpected since these are periods of greater change in water temperature, photoperiod, and reproductive condition, and all of the animals may not necessarily be physiologically synchronized. Spring animals were characterized by a large amount of 18:2(n-6), which resulted in the amount of PUFA and the value of UI being similar to winter animals. The other major 18-carbon fatty acids [18:0 and 18:1(n-9)] were also elevated in these animals, while the amount of 20:1(n-9) was reduced. Mussels collected in autumn had values of U/S and UI that were similar to those of summer animals. The autumn animals were also characterized by high amounts of the unidentified components with RRT of 0.59 and 5.87 min.

DISCUSSION

Limited data have been reported on the lipid composition of freshwater bivalves, and no information is available on the lipid composition of gill tissue. Voogt (1975) found that the soft tissues of *Anodonta cygnea* contained about 10 mg lipid/g wet weight, and Pollero, Irazu, and Brenner (1983) reported values of 9.4 and 12.0 for gonadal tissue from nonreproductive male and female *Diplodon delodontus*. Values ranging from 3.7 to 24 mg/g have been reported for marine bivalves (Sampugna et al. 1972; Watanabe and Ackman 1974; Moreno, Moreno, and Brenner 1976), and 13.5 mg/g for the terrestrial snail *Cepaea nemoralis* (Van der Horst 1970). The values of 7.7–15.7 mg of lipid/g of gill tissue reported herein for *Carunculina texasensis* are thus typical of molluscan tissues.

The total lipid content of gill tissue was highest in autumn and lowest in winter (table 1). This pattern could represent a storage of energy prior to an anticipated food shortage. This is unlikely, however, because metabolism in freshwater bivalves is primarily carbohydrate based. Also, the amount of triglyceride, which is the primary storage form of lipid, is actually lower in autumn than in winter. Seasonal changes in total lipid content and lipid class composition have been observed previously in bivalves and have usually been correlated with the reproductive cycle (Ansell 1974*a*, 1974*b*; Nagabhushanam and Dhamne 1977; Pollero, Re, and Brenner 1979; Moreno et al. 1980; Swift, White, and Ghassemieh 1980). In general, total lipid content is highest immediately prior to spawning and subsequently declines. In unionids fertilized eggs leave the gonads and are then retained in the gill marsupium, where they develop into larval glochidia. In *C. texasensis* the glochidia are released in the spring, but fertilization occurs a month or more prior to release.

Phosphatidylcholine and phosphatidylethanolamine were the most abundant phosphatides in gill tissue from male *C. texasensis*. These two phospholipid classes are generally the most abundant in animal tissues, including molluscan tissues (Thompson and Hanahan 1963; Shieh 1968; Van der Horst, Kingma, and Oudejans 1973; Moreno et al. 1976; Pollero et al. 1979). However, the specific phosphatide composition of *C. texasensis* gill tissue differs slightly from a whole-animal analysis of another unionid, *Diplodom patogonicus*, in which sphingomyelin constituted 10% of the total phospholipid and no PI or PS was apparently found (Pollero et al. 1981).

Significant seasonal fluctuations in the percentages of PE and PI occurred. In the spring of both 1984 and 1985 there was an increase in the relative proportion of PE in the gills (fig. 2). Neither the functional significance of the seasonal changes in phospholipid class composition nor the difference in the relative amounts of PE in the springs of 1984 and 1985 is known. In *C. texasensis*, however, the larval glochidia are released in the spring, which coincides with the time at which the PE content of the gill is elevated. The PE content of another freshwater unionid, *D. patagonicus*, was approximately 33% higher in the spring and summer than in the autumn (Pollero et al. 1981), which compares favorably with our data from 1985 (34% increase between January and April). It thus appears that

changes in gill phosphatide composition may be related to reproduction in *C. texasensis* and other unionids.

The fatty acid profile of the gill phospholipid fraction from *C. texasensis* was similar to lipid preparations from other freshwater molluscs. In total lipid extracts from gill tissue of *Ligumia subrostrata* 20:4(n-6) was the most abundant fatty acid, and 20:4(n-6) + 18:2(n-6) accounted for 23.5% of the total (Saintsing et al. 1983). In whole-animal extracts of *D. patagonicus*, 20:4(n-6) constituted 13%–17% of the total fatty acids, and 20:4(n-6) + 18:2(n-6) summed to 23%–26% (Pollero et al. 1981). The data on freshwater mussels differ considerably from those of marine molluscs. Arachidonic acid accounts for only 0%–5% of the total fatty acids in marine bivalves (Gardner and Riley 1972; Watanabe and Ackman 1974; Paradis and Ackman 1977; Moreno et al. 1980; Joseph 1982) and 5%–10% in marine gastropods (Paradis and Ackman 1977; Johns, Nichols, and Perry 1980; Joseph 1982). Marine molluscs are generally rich in fatty acids of the n-3 (i.e., α-linolenic acid) family, primarily 20:5(n-3) and 22:6(n-3). Freshwater mussels, however, contain a greater proportion of fatty acids of the n-6 (i.e., linoleic acid) family. While freshwater mussels have (n-6)/(n-3) ratios of 2–4 (tables 2, 3; Saintsing et al. [1983]), marine molluscs have ratios of 0.1–1.0. Fatty acids of the phospholipid fraction from the terrestrial snail *C. nemoralis* had a 20:4(n-6) content of 16%, a 20:4(n-6) + 18:2(n-6) total of 28%, and a (n-6)/(n-3) ratio of 6–9 (Van der Horst 1970; Van der Horst and Zandee 1973). Thus freshwater mussels have a fatty acid profile that corresponds more closely to terrestrial molluscs than to marine molluscs. The differences in the fatty acid profiles of marine and freshwater molluscs may be due to dietary differences since marine plankton are rich in n-3 acids, while n-6 acids predominate in terrestrial plants (Sargent 1976).

The data in table 2 indicate that there is some control over the distribution of fatty acids between the different lipid classes in gill tissue of *C. texasensis*. For example, 20:4(n-6) is preferentially channeled into phospholipids, while the n-3 acids are directed into the neutral lipid fractions. The relatively low levels of 20:4(n-6) in the neutral lipid fractions combined with the high levels of the n-3 acids resulted in (n-6)/(n-3) ratios that were similar to those found in total lipid and phospholipid extracts from marine molluscs. Since 20:4(n-6) is found almost exclusively in the phospholipid fraction, the enzymes most likely to be responsible for liberating 20:4(n-6) for PGE_2 synthesis are the phospholipase A's or phospholipase C coupled with a diglyceride lipase (Irvine 1982).

Two unidentified fatty acids were present in the phospholipid fraction. Both of these unknowns exhibited seasonal variations in abundance, suggesting that they may be of dietary origin. The first had an RRT of 0.59, which elutes between 14:1 and 15:0. This compound could be a branched fatty acid, which are known to occur in some molluscs (Voogt 1983). The second unknown (RRT = 5.87) eluted between 22:5(n-6) and 22:5(n-3). Nonmethylene-interrupted (NMI) fatty acids have been reported in both marine (Paradis and Ackman 1975) and freshwater (Pollero et al. 1981) bivalves. While this possibility cannot be excluded, the relative retention time of unknown no. 2 is much greater than that reported by these authors for 20:2 and 22:2 NMI acids.

The changes in the fatty acid composition of gill phospholipids from winter- and summer-acclimatized *C. texasensis* are in agreement with data on other poikilothermic organisms in that mussels collected in winter contained more PUFA and had higher indices of membrane unsaturation than did animals collected in summer (Hazel and Sellner 1980). The increase in the degree of unsaturation which results from these changes has been interpreted as one of the mechanisms by which poikilotherms maintain the degree of fluidity of biomembranes relatively independent of temperature. Preservation of membrane fluidity is crucial for the maintenance of intra- and extracellular ion concentrations and for the proper functioning of membrane-bound enzymes, transport proteins, and receptors. The data on *C. texasensis* contrast with those on *D. patagonicus* in this regard. While seasonal changes in the fatty acid composition of the latter species occurred, there were no significant changes in the proportions of saturates, monoenes, or PUFA, or in measures of membrane un-

saturation. The authors concluded that dietary intake overrides the limited temperature range (6–13.5 C) experienced by this species in determining the fatty acid composition in *D. patagonicus* (Pollero et al. 1981). A similar conclusion was also reached for the marine bivalves *Mesodesma mactroides* (Moreno et al. 1976), *Chlamys tehuelcha* (Pollero et al. 1979), and *Mytilus platensis* (Moreno et al. 1980). The lipid composition of the diet of *C. texasensis* has not been investigated. Thus, it is not known whether the changes in table 3 represent adaptation on the part of the mussel or whether they represent changes in the fatty acid composition of the diet. As a filter feeder, *C. texasensis* would be expected to feed heavily on algae. The most abundant algae in ponds in the Baton Rouge area are blue-greens, with green algae being secondarily abundant (Gigante 1979). Freshwater members of these taxa contain virtually no 20- or 22-carbon fatty acids (Wood 1974). Thus the composition of the eicosa- and docosanoic acids are likely to reflect endogenous synthesis. Most of the local algal species exhibit spring blooms (Gigante 1979), which may explain the relatively high proportions of 18-carbon fatty acids found in gill phospholipids at this time of the year.

Although 20:4(n-6) is the most abundant fatty acid in the phospholipid fraction, the amount of this acid is independent of season (table 3). If seasonal alterations in the fatty acid composition of gill membranes are related primarily to the maintenance of membrane fluidity, it would be reasonable to anticipate changes in the relative abundance of this fatty acid. An analogous situation occurs in freshwater trout in which 22:6(n-3) is the most abundant fatty acid and undergoes the largest seasonal change (Hazel 1979). It is unlikely that the dietary intake of arachidonate is constant throughout the year, and, if algae constitute the primary food source, the dietary intake of 20:4(n-6) would be expected to be quite low. It therefore appears that the amount of 20:4(n-6) is actively regulated at high levels, which suggests a nonstructural function for this fatty acid. Since the production of PGE_2 from exogenous 20:4(n-6) has been demonstrated using gill homogenates of another freshwater unionid (*L. subrostrata*), and since PGE_2 has been implicated in the regulation of Na uptake by *L. subrostrata* gills, it seems likely that the amount of 20:4(n-6) is maintained at relatively high and constant levels in order to ensure an adequate supply of substrate for the production of PGE_2.

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