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Effects of Temperature on Fatty Acids of Crayfish Tissues

by
Esther Wilson Will*

ABSTRACT

Crayfish *(Orconectes nais)* were collected from locations in Lyon County, Kansas, during each season of 1981. Also, summer-collected crayfish were acclimated in the laboratory to temperatures of 7, 14, and 27 C. The fatty acid patterns of total lipids from gill, muscle, and hepatopancreas tissues were determined. The gill and hepatopancreas lipids demonstrated an overall trend of increased unsaturation of fatty acids with a decrease in temperature. The greatest variations were evident in the relative content of $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{20:4}$, and $C_{20:5}$ fatty acids. The muscle tissue did not show significant differences between concentration values of fatty acids. The study of the effects of temperature on crayfish lipid composition indicated that different lipid populations are formed during exposure to varying temperatures. It has been noted that the primary role of an increase in unsaturated fatty acids is to maintain membrane fluidity at lower temperatures which is essential for proper functioning and control of membrane-bound enzyme activity.

INTRODUCTION

Much research has been done on the relationship between changes in environmental temperature and alterations that occur in the lipid composition in many biological systems. A correlation has been established between lower environmental temperatures and an increased degree of unsaturation of fatty acids in the tissues of many poikilotherms. Several studies have been done on the common goldfish, *Carassius auratus*. Hoar and Cottle (1952) reported that the degree of unsaturation of goldfish body lipids varied inversely with temperature. They suggested that these changes influence metabolism and insure the integrity of cell membrane properties. A study by Johnston and Roots (1964) on goldfish brain lipids also indicated a trend to more unsaturation with lower temperatures. This was especially true for the 18-carbon acids and the long-chain polyunsaturated acids such as docosapentaenoic (22:5), docosahexaenoic (22:6), and arachidonic (20:4) acids. Roots (1968) also examined the composition of phospholipids from goldfish brain tissue. After cold acclimation, there were no significant changes in phosphatide composition. However, a major phosphatide, phosphatidylethanolamine, did show an increase in

*This study originated as a master's thesis under the direction of Dr. Gaylen Neufeld in the Division of Biological Sciences at Emporia State University. The author is currently a member of the faculty of the University of Wisconsin - Parkside, Kenosha, WI 53140.

fatty acid unsaturation at cool temperatures due to an increase of palmitoleic acid (16:1) and a decrease in palmitic acid (16:0). Fatty acids from cold-acclimated goldfish muscle exhibited an increase in unsaturation due primarily to changes in the C_{16} and C_{18} fatty acids (Knipprath and Mead 1967). This same tendency of changes in fatty acid composition has been reported for goldfish intestinal lipids (Kemp and Smith 1970; Smith and Kemp 1971).

Several investigators have demonstrated similar changes in fatty acid patterns of total lipids in aquatic crustaceans (Chapelle 1978; Chapelle et al. 1977). Farkas (1970) found differences in fatty acid composition between two species of freshwater crustaceans. The species that was able to live through the winter had a higher amount of lipid unsaturation. Farkas and Herodek (1964) reported that lipids from planktonic copepods from Lake Balaton, Hungary, had a melting point lower than the environmental temperature thoughout the year due to more unsaturation of long-chain fatty acids.

Other evidence from poikilotherms includes a study on mosquito-fish and guppies which showed increased unsaturation at low temperatures (Knipprath and Mead 1966). The major changes were an increase of docosahexaenoic acid and decrease of both palmitic and stearic acids. Lewis (1962) observed this same relationship in separated populations of marine ectotherms, one from temperate waters and the other from Arctic waters. During cold adaptation in frogs, unsaturated fatty acids increased in both liver and adipose tissues (Baranska and Wlodawer 1969). Wodtke (1978) presented evidence for lipid adaptation in carp liver mitochondria. Such alterations induced by changes in temperature help control the fluidity of the membrane.

There seems to be a general agreement that many organisms change the chemistry of their lipids in order to maintain their membranes in a specific liquid-crystalline state (Caldwell and Vernberg 1970; Thomson et al. 1977). Many investigators have examined how temperature acclimation affects membrane-bound enzyme activity. Freed (1965) showed that cytochrome oxidase activity in goldfish muscle increased during cold-acclimation and decreased during warm-acclimation. Similarly, Caldwell (1969) reported that alterations in goldfish gill mitochondria lipids at low temperatures may influence cytochrome oxidase and other electron transport enzymes since they exist in lipoprotein complexes. These lipid alterations may have an effect on the affinity properties of membrane-bound enzymes. Studies by Hazel (1972) indicated that

temperature-induced changes in membrane lipids may influence the normal catalytic function of another membrane-bound enzyme, succinic dehydrogenase, from the epaxial muscle of the common goldfish. It has also been shown that the enzyme adenosine triphophatase is influenced by temperature (Smith et al. 1968) and that the degree of fatty acid unsaturation in association with this enzyme significantly affects its response to temperature (Kimelberg and Papahadjopoulos 1974).

Several microorganisms have been studied which display this trend of increased lipid unsaturation with low temperature. Cultures of the yeast, *Candida utilis,* showed an increased synthesis of unsaturated fatty acids with a decrease in temperature (Brown and Rose 1969). This same tendency was demonstrated in the bacterium, *Escherichia coli* (Marr and Ingrahm 1962; Haest et al. 1969), as well as in the cold-tolerant bacterium, *Pseudomonas fluorescens* (Cullen et al. 1971). However, varying responses have been reported in lipids of *Bacillus stearothermophilus* (Long and Williams 1960). These authors found more unsaturation in lipids of 55 C spores than spore lipids from 37 C.

In addition to poikilotherms, hibernating mammals have received attention as unique homeotherms that undergo biochemical and physiological changes to survive the cold. Goldman (1975) found that hibernating hamsters exhibited an increase in the proportion of unsaturated fatty acids in most brain phospholipids. These lipid changes were suggested as important adjustments needs for cold-resistance. Similar increases in lipid unsaturation resulting from cold acclimation have also been reported for hamster white and brown adipose tissue (Minor et al. 1973). Ground squirrels also have been reported to have lipid changes during hibernation (Rotermund and Veltman 1981; Aloia et al. 1974).

Lipids are vital to an organism because they play an important role in the structure and physiology of membranes. It seems probable that the more unsaturated fatty acids may help assure the liquid state of membrane lipids because these fatty acids remain in a fluid state at low environmental temperatures. Therefore, the organism would benefit from using unsaturated fatty acids as structural lipids during the cooler seasons (O'Conner and Gilbert 1968).

The present study investigated the effects of temperature upon the lipid constituents of local freshwater crayfish, *Orconectes nais* (Faxon). The fatty acid composition of total lipids from several different tissues was compared between crayfish groups collected

during the middle of each of the four seasons. In addition, lipid constituents from crayfish which had been acclimated in the laboratory to various temperatures were compared. This species was chosen because freshwater ecosystems show a great degree of seasonal temperature variation. The crayfish, being common in the freshwater habitats of Lyon County, Kansas, is a typical inhabitant which would be subjected to these temperature variations.

METHODS AND MATERIALS

Crayfish *(Orconectes nais)* were collected from freshwater streams and rivers in Lyon County, Kansas, during winter, spring, summer, and fall of 1981. Water temperatures at the time of collection for each season were 9, 17, 25, and 13 C, respectively. In addition, three groups of 12 crayfish each were collected in the summer and acclimated in environmental chambers at 7, 14, and 27 C for 21 days. They were fed TetraMin fish food during the acclimation period. Photoperiods were controlled with incandescent lighting. They were chosen to coincide with the appropriate seasonal daylength and were LD 11:13 for 7 C, LD 12:12 for 14 C, and LD 13:11 for 27 C. The crayfish were kept in large aquaria with sand and rock substrates, two-inch deep dechlorinated tapwater, and with constant aeration.

At designated times, the crayfish were sacrificed and gill, hepatopancreas, and muscle tissues isolated. Lipids were either extracted immediately or the tissues were frozen for later processing. Each organism's tissues were frozen separately to minimize pooling. Extracts were stored at -10 C under nitrogen.

The procedure for extraction of total lipids was based on Kates (1972). One to two g of tissue were combined with 0.5 ml of water and blended in a Potter-Elvehjem homogenizer for 30 seconds. Five ml of methanol-chloroform (2:1, v/v) were added to this mixture and blended for one minute. The homogenate was centrifuged for 5 minutes, the supernatant decanted, and the residue re-extracted with 6.3 ml of methanol-chloroform-water (2:1:0.8) by homogenization for one minute. The homogenate was centrifuged for 5 minutes, the supernatants combined and diluted with 3.3 ml each of chloroform and water. The phases were separated either by centrifugation or in a separatory funnel. The lower chloroform phase was withdrawn and concentrated under a hood using air outflow to evaporate the solvent. The residue was redissolved in a suitable volume (e.g., 3 ml) of chloroform.

Fatty acids from the lipid extracts were esterified for later analysis by gas-liquid chromatography (GLC). Three ml of 2% H_2SO_4 in methanol were added to the residue from the extraction procedure. The direct methylation of fatty acids was accomplished by gently boiling the mixture in a test tube stoppered loosely with a marble and placed in a heated sand bath. The boiling continued for a minimum of three hours. After boiling the mixture, 8 ml of distilled water were added and the fatty acid methyl esters extracted three times with 3 ml of petroleum ether. Extracts were collected and evaporated to dryness by placing the test tube in the heated sand bath. Residual methyl esters were dissolved in a suitable volume (e.g., 0.15 ml) of hexane. All analyses were made with a Varian Aerograph Gas Chromatograph 1440 equipped with a flame ionization detector and a nickel column (6 feet in length and 1/8 inch in diameter) packed with 12% Stabilized DEGS on 90/100 mesh Anakrom Q (Analabs, Inc.). Nitrogen was used as the carrier gas with a flow rate of 20 ml/min and a pressure of 60 psi. The column temperature was maintained at 185 C; the injector and detector temperatures were set at 350 C. Flow rate for the hydrogen was 30 ml/min; the air flow rate was set at 240 ml/min. Chart speed used was 1.25 cm/min.

Suitable aliquots (e.g., 0.5 - 1.0 µl) of the fatty acid methyl ester preparations were injected into the chromatograph. The fatty acid methyl esters were identified from the comparison of their retention times with those of known standards. Peak area of each component was estimated as the product of peak height time retention time (Kates 1972). Percentage composition of the fatty acids was determined from the measured area under all observed peaks expressed as 100 per cent ("international normalization") (Purnell 1962). The data were statistically analyzed and compared using the Student t-test at the 0.05 level of significance.

RESULTS

Fatty acid composition of total lipids from crayfish acclimated to varying environmental conditions was determined in this study. Individual fatty acids are classified into three groups, saturated, monounsaturated, and polyunsaturated. Results are expressed as concentrations of each individual fatty acid. Eicosapentaenoic acid (20:5) was not available as a reference standard so its peak was identified by its relative retention time. Other unidentified fatty acids were detected but they made up less than 1% of the total and were not examined further.

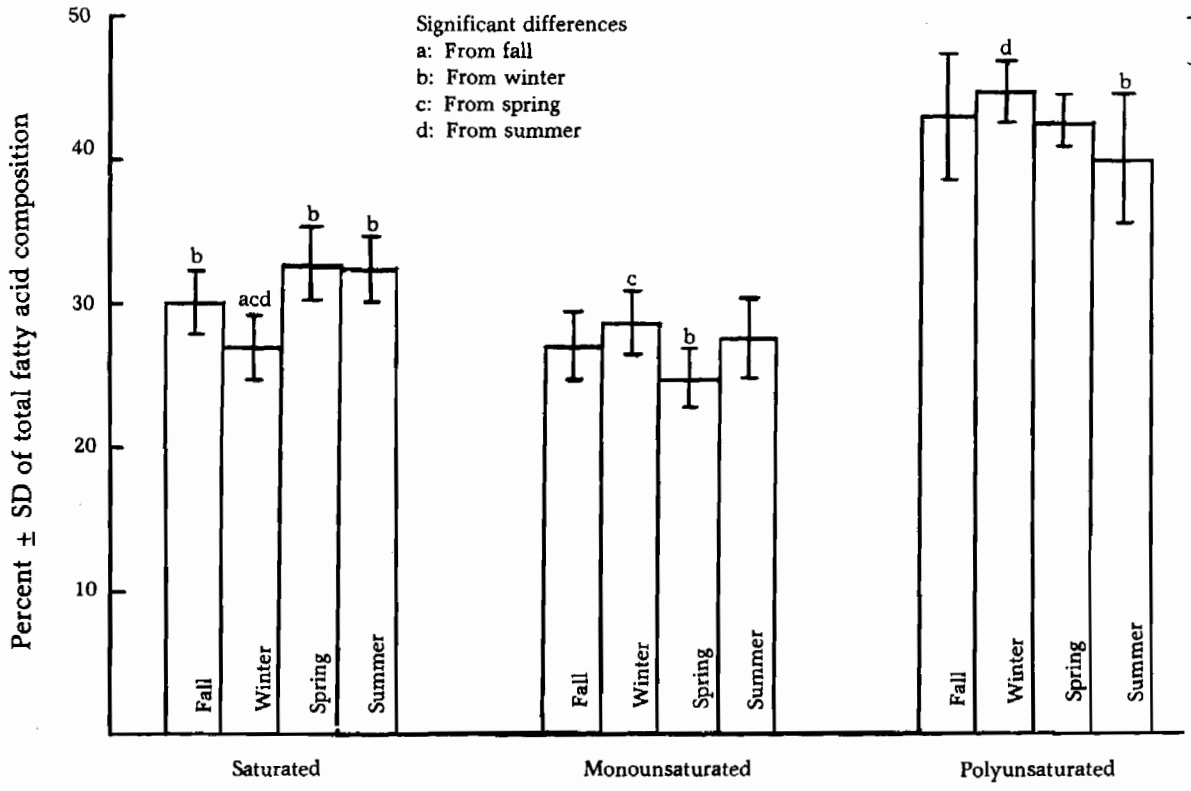

Figure 1. Classes of fatty acids from gills of crayfish collected during the four seasons. Saturated fatty acids include myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0); monounsaturated fatty acids include palmitoleic acid (16:1) and oleic acid (18:1); polyunsaturated fatty acids include linoleic acid (18:2), linolenic acid (18:3), arachidonic acid (20:4), and eicosapentaenoic acid (20:5).

Table 1. Fatty acid composition of total gill lipids extracted from crayfish, *Orconectes nais*, collected during each season (N = 7).

	Percent ± SD of Total			
Fatty Acids	Fall	Winter	Spring	Summer
Myristic acid (14:0)	1.51 ± 0.53	1.75 ± 0.54	1.43 ± 0.20	1.79 ± 0.47
Palmitic acid (16:0)	15.93 ± 1.85	15.72 ± 1.29^{cd}	17.99 ± 2.11^{b}	17.47 ± 1.50^{b}
Palmitoleic acid (16:1)	4.77 ± 1.64^{b}	7.18 ± 1.02^{acd}	5.38 ± 0.56^{b}	4.61 ± 0.62^{b}
Stearic acid (18:0)	12.68 ± 2.21^{b}	9.55 ± 0.94^{acd}	13.38 ± 1.75^{b}	13.37 ± 0.88^{b}
Oleic acid (18:1)	22.14 ± 1.87^{c}	21.36 ± 1.54	19.29 ± 2.06^{ad}	22.92 ± 2.50^{c}
Linoleic acid (18:2)	11.13 ± 5.30^{bcd}	3.81 ± 1.48^{ad}	3.91 ± 0.83^{ad}	6.02 ± 1.29^{abc}
Linolenic acid (18:3)	2.71 ± 1.24^{d}	3.34 ± 0.93	3.79 ± 0.86	4.46 ± 1.04^{a}
Arachidonic acid (20:4)	15.43 ± 2.28^{c}	14.60 ± 1.90	12.54 ± 2.21^{a}	12.97 ± 2.27
Eicosapentaenoic acid (20:5)	13.71 ± 3.96^{bc}	22.70 ± 3.16^{ad}	22.30 ± 2.10^{ad}	16.40 ± 3.17^{bc}

a = significantly different from fall
b = significantly different from winter
c = significantly different from spring
d = significantly different from summer

The effect of temperature on fatty acid composition of total lipids from crayfish gill tissue is shown in Figure 1 and Table 1. In general the total of saturated fatty acids ($C_{14:0}$, $C_{16:0}$, and $C_{18:0}$) was lowest for winter, significantly differing from all other seasonal groups (Fig. 1). The monounsaturated fatty acid total ($C_{16:1}$ and $C_{18:1}$) was highest for the winter group. Also, total polyunsaturated fatty acids ($C_{18:2}$, $C_{18:3}$, $C_{20:4}$, and $C_{20:5}$) were highest in the winter crayfish tissue, differing significantly from the summer polyunsaturated acid total. Fall and spring concentrations appeared to be in transition between winter and summer.

Table 1 shows that the proportions of the major saturated fatty acids, palmitic and stearic acids, were reduced in winter crayfish gills. There was a significantly higher amount of palmitic acid in both spring and summer crayfish groups over the winter group. Also, there was significantly more stearic acid in spring, summer, and fall than in the winter group. One major monounsaturated fatty acid, palmitoleic acid, increased significantly at the winter temperature. In contrast, the other major monounsaturated fatty acid, oleic acid, increased at warmer fall and summer temperatures. Of the polyunsaturated acids, both arachidonic and eicosapentaenoic acids became more abundant at the winter temperature. Linoleic acid showed a significant increase in the fall and summer while the concentration of linolenic acid remained relatively constant. Environmental temperature did have a definite effect on particular fatty acid levels. It can be seen that lipids from the gills of winter crayfish had a decreased level of saturated fatty acids which was compensated by increased levels of monounsaturated and polyunsaturated fatty acids.

The effect of laboratory temperature acclimation on the fatty acid composition of total lipids from crayfish gills is shown in Figure 2 and Table 2. Generally, the results showed the same trend as results from the seasonal environmental groups. The saturated fatty acid total was significantly less at the lower acclimation temperature. Yet, both the monounsaturated and polyunsaturated totals showed increased concentrations at the low temperature. The specific changes (Table 2) did not completely correspond to the natural environmental changes. Of the saturated fatty acids, palmitic acid remained stable while the amount of stearic acid decreased at the low temperature. Palmitoleic acid remained stable while oleic acid tended to be most abundant at 7 C. Both linolenic and arachidonic acids remained constant. However, the linoleic acid concentration increased at the low temperature while

eicosapentaenoic acid concentration increased at the warm temperature.

Figure 2. Classes of fatty acids from gills of crayfish acclimated to different temperatures. The fatty acids in each category are identified in the legend for Figure 1.

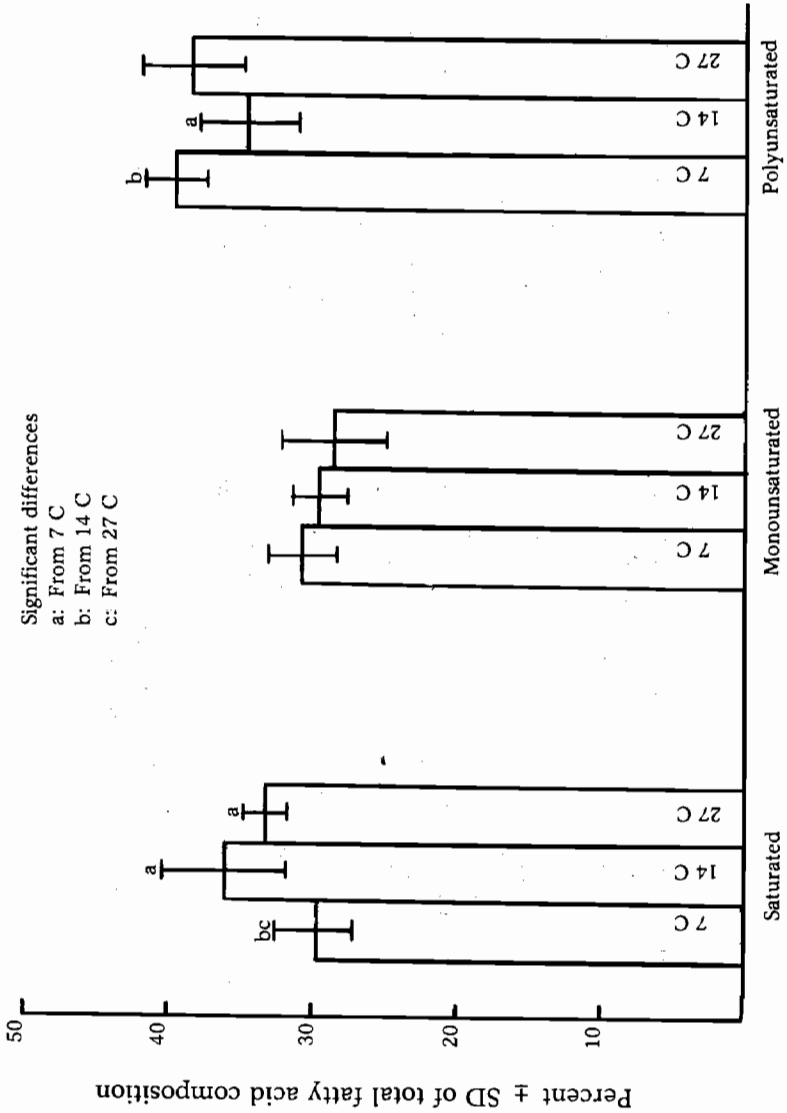

Table 2. Fatty acid composition of total gill lipids extracted from crayfish, *Orconectes nais*, acclimated to different temperatures (N = 7).

Fatty Acids	Percent ± SD of Total		
	7 C	14 C	27 C
Myristic acid (14:0)	1.77±0.80	2.79±1.40	1.88±0.92
Palmitic acid (16:0)	16.60±1.42	18.68±2.45	16.83±1.29
Palmitoleic acid (16:1)	4.81±1.38	4.87±0.97	5.63±1.19
Stearic acid (18:0)	11.45±1.57[bc]	14.56±1.90[a]	14.49±1.41[a]
Oleic acid (18:1)	25.88±3.43	24.64±2.17	22.82±3.51
Linoleic acid (18:2)	7.72±1.22[bc]	5.61±2.11[a]	4.98±2.81[a]
Linolenic acid (18:3)	3.55±1.06[c]	3.26±1.71	2.23±0.63[a]
Arachidonic acid (20:4)	16.43±1.48	15.12±3.14	16.49±4.38
Eicosapentaenoic acid (20:5)	11.81±2.36	10.47±2.21[c]	14.64±2.75[b]

a = significantly different from 7 C
b = significantly different from 14 C
c = significantly different from 27 C

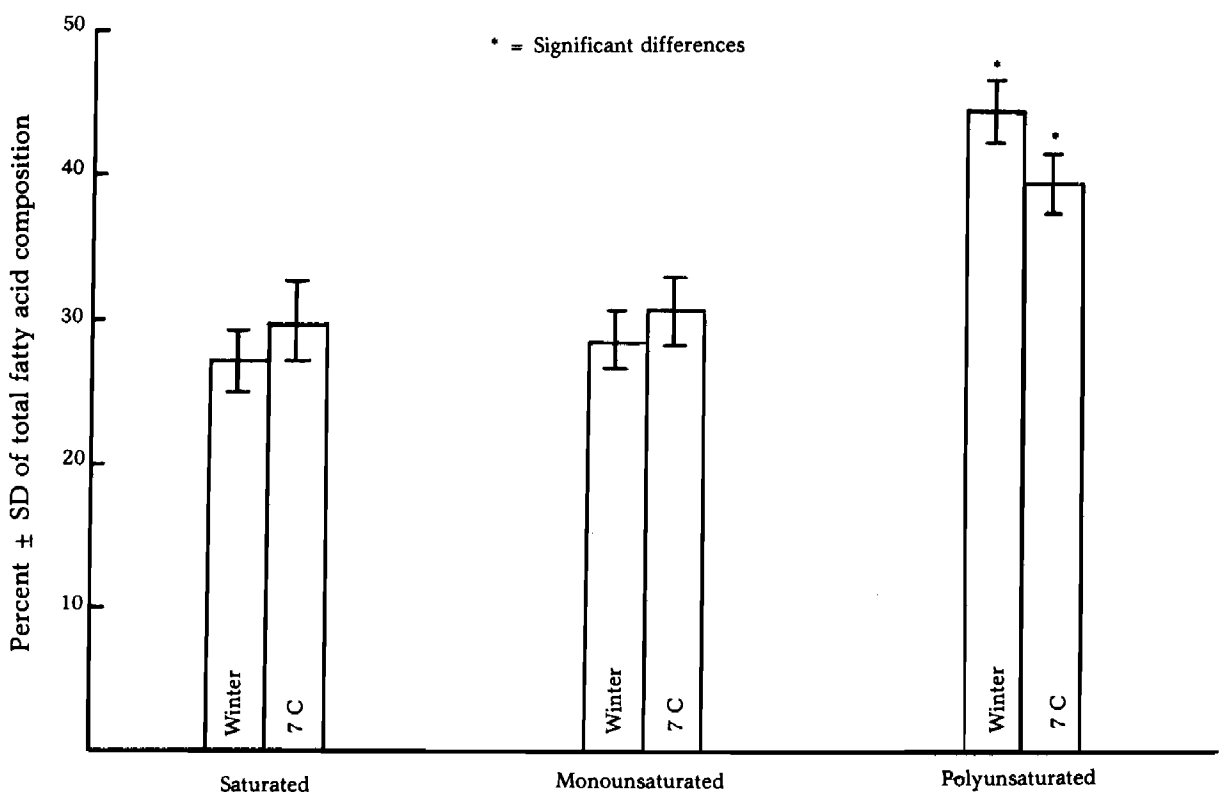

Figure 3. Classes of fatty acids from gills of crayfish collected during winter and acclimated to 7 C. The fatty acids in each category are identified in the legend for figure 1.

Table 3. Fatty acid composition of total gill lipids extracted from crayfish, *Orconectes nais*, collected during winter and acclimated to 7 C (N = 7).

	Percent ± SD of Total	
Fatty Acids	Winter	7 C
Myristic acid (14:0)	1.75±0.54	1.77±0.80
Palmitic acid (16:0)	15.72±1.29	16.60±1.42
Palmitoleic acid (16:1)	7.18±1.02*	4.81±1.38*
Stearic acid (18:0)	9.55±0.94*	11.45±1.57*
Oleic acid (18:1)	21.36±1.54*	25.88±3.43*
Linoleic acid (18:2)	3.81±1.48*	7.72±1.22*
Linolenic acid (18:3)	3.34±0.93	3.55±1.06
Arachidonic acid (20:4)	14.60±1.90	16.43±1.48
Eicosapentaenoic acid (20:5)	22.70±3.16*	11.81±2.36*

* = significantly different from 7 C

The effect of laboratory temperature acclimation on the fatty acid composition of total lipids from crayfish gills is shown in Figure 2 and Table 2. Generally, the results showed the same trend as results from the seasonal environmental groups. The saturated fatty acid total was significantly less at the lower acclimation temperature. Yet, both the monounsaturated and polyunsaturated totals showed increased concentrations at the low temperature. The specific changes (Table 2) did not completely correspond to the natural environmental changes. Of the saturated fatty acids, palmitic acid remained stable while the amount of stearic acid decreased at the low temperature. Palmitoleic acid remained stable while oleic acid tended to be most abundant at 7 C. Both linolenic and arachidonic acids remained constant. However, the linoleic acid concentration increased at the low temperature while eicosapentaenoic acid concentration increased at the warm temperature.

Data in Figures 3, 4, and 5 and in Tables 3, 4, and 5, compare lipids from the environmental crayfish groups to the lipids of the corresponding acclimated groups. Figure 3 and Table 3 show the comparison of winter crayfish to the group acclimated to 7 C. Lipids from the winter group had a significantly higher concentration of polyunsaturated fatty acids than the low temperature acclimated group (Fig. 3). Gill lipids from the winter group showed

increased levels of palmitoleic and eicosapentaenoic acids (Table 3). However, there were lower levels of oleic, linoleic, linolenic, and arachidonic acids.

Comparison of the summer crayfish group to the group acclimated in the laboratory to 27 C is shown in Figure 4 and Table 4. The data showed very few significant differences. The only changes which occurred in the lipids of the acclimated group were an increase in the level of palmitoleic acid and a decrease in linolenic acid concentration.

Figure 4. Classes of fatty acids from gills of crayfish collected during summer and acclimated to 27 C. The fatty acids in each category are identified in the legend for Figure 1.

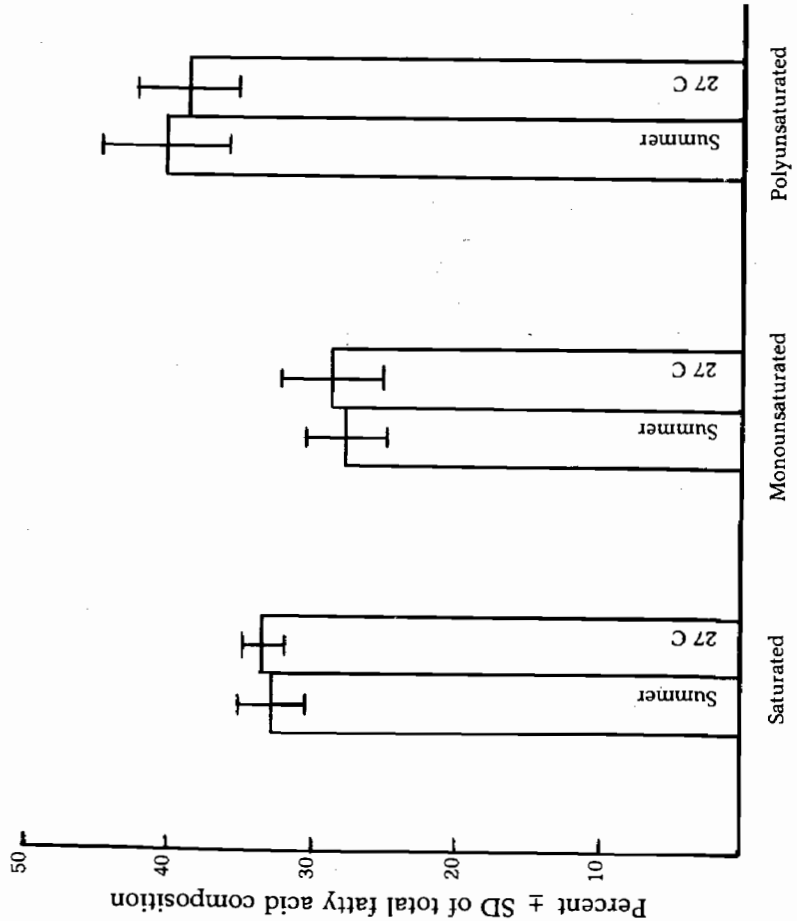

Table 4. Fatty acid composition of total gill lipids extracted from crayfish, *Orconectes nais,* collected during summer and acclimated to 27 C (N = 7).

Fatty Acids	Percent ± SD of Total	
	Summer	27 C
Myristic acid (14:0)	1.79±0.47	1.88±0.92
Palmitic acid (16:0)	17.47±1.50	16.83±1.29
Palmitoleic acid (16:1)	4.61±0.62*	5.63±1.19*
Stearic acid (18:0)	13.37±0.88	14.49±1.41
Oleic acid (18:1)	22.92±2.50	22.82±3.51
Linoleic acid (18:2)	6.02±1.29	4.98±2.81
Linolenic acid (18:3)	4.46±1.04*	2.23±0.63*
Arachidonic acid (20:4)	12.97±2.27	16.49±4.38
Eicosapentaenoic acid (20:5)	16.40±3.17	14.64±2.75

* = significantly different

Figure 5 and Table 5 show the comparison of environmental groups from spring and fall to the group acclimated in the laboratory to 14 C. In general, the acclimated group had higher totals of both saturated and monounsaturated fatty acids and a lower polyunsaturated fatty acid total (Fig. 5). There were increased levels of myristic, palmitic, and oleic acids in the acclimated group and a decreased level of eicosapentaenoic acid (Table 5).

Data presented in Figure 6 and Table 6 report the influence of acclimation temperatures on fatty acid patterns in muscle tissue lipids. There is only one significant difference within the groups. The polyunsaturated total was highest for the 27 C group (Fig. 6). The amount of eicosapentaenoic acid was much greater from the warm temperature group (Table 6).

Fatty acid concentrations from hepatopancreas tissue of crayfish acclimated to various temperatures are illustrated in Figure 7 and Table 7. These results follow the same trend as the lipid changes from gill tissue. The saturated fatty acid total was highest for the warm temperature group while the highest amount of total polyunsaturated fatty acids was from the cooler temperature group (Fig. 7). Specifically, the warm temperature group had the most palmitic acid (Table 7). Of the polyunsaturated acids, arachidonic and eicosapentaenoic acids were most abundant from the coolest temperature.

Figure 5. Classes of fatty acids from gills of crayfish collected during fall, spring, and acclimated to 14 C. The fatty acids in each category are identified in the legend for Figure 1.

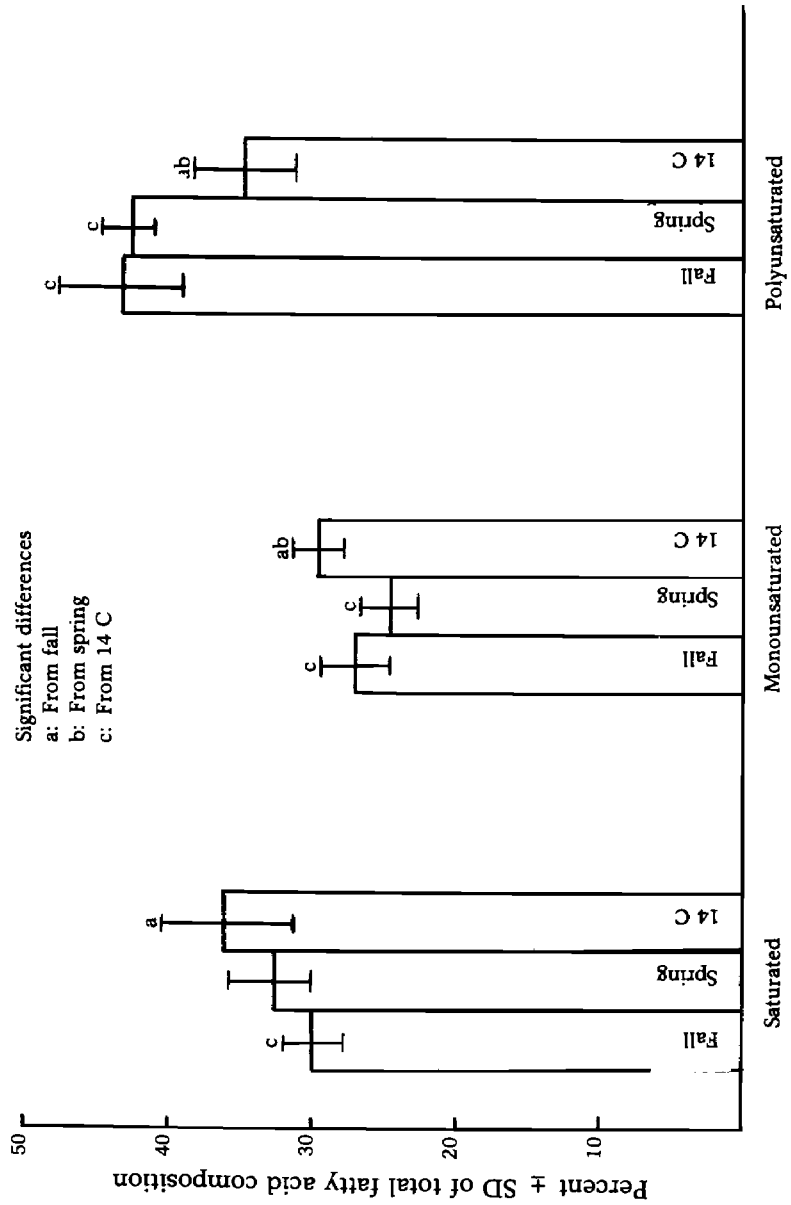

Table 5. Fatty acid composition of total gill lipids extracted from crayfish, *Orconectes nais*, collected during fall, spring, and acclimated to 14 C.

Fatty Acids	Percent ± SD of Total		
	Fall	Spring	14 C
Myristic acid (14:0)	1.51 ± 0.53[c]	1.43 ± 0.20[c]	2.79 ± 1.40[ab]
Palmitic acid (16:0)	15.93 ± 1.85[c]	17.99 ± 2.11	18.68 ± 2.45[a]
Palmitoleic acid (16:1)	4.77 ± 1.64	5.38 ± 0.56	4.87 ± 0.97
Stearic acid (18:0)	12.68 ± 2.21	13.38 ± 1.75	14.56 ± 1.90
Oleic acid (18:1)	22.14 ± 1.87[bc]	19.29 ± 2.06[ac]	24.64 ± 2.17[ab]
Linoleic acid (18:2)	11.13 ± 5.30[bc]	3.91 ± 0.83[a]	5.61 ± 2.11[a]
Linolenic acid (18:3)	2.71 ± 1.24	3.79 ± 0.86	3.26 ± 1.71
Arachidonic acid (20:4)	15.43 ± 2.28	12.54 ± 2.21	15.21 ± 3.14
Eicosapentaenoic acid (20:5)	13.72 ± 3.96[b]	22.30 ± 2.10[ac]	10.47 ± 2.21[b]

a = significantly different from fall
b = significantly different from spring
c = significantly different from 14 C

Figure 6. Classes of fatty acids from muscle of crayfish acclimated to different temperatures. The fatty acids in each category are identified in the legend for Figure 1.

Table 6. Fatty acid composition of total muscle lipids extracted from crayfish, *Orconectes nais*, acclimated to different temperatures (N = 5).

Fatty Acids	Percent ± SD of Total		
	7C	14 C	27 C
Myristic acid (14:0)	1.68±0.64	1.43±0.34	1.66±0.73
Palmitic acid (16:0)	21.32±1.31[c]	20.87±1.33[c]	18.41±1.94[ab]
Palmitoleic acid (16:1)	5.86±0.53[b]	4.32±1.10[a]	5.35±0.94
Stearic acid (18:0)	9.43±0.89	10.68±1.44	8.34±2.95
Oleic acid (18:1)	22.19±1.29	23.29±1.49[c]	19.88±2.56[b]
Linoleic acid (18:2)	6.49±1.99	5.83±0.73	4.57±1.48
Linolenic acid (18:3)	3.59±0.86[b]	2.45±0.57[a]	3.61±1.54
Arachidonic acid (20:4)	6.70±1.23	8.25±1.31	8.33±2.27
Eicosapentaenoic acid (20:5)	22.74±4.05[c]	22.89±2.33[c]	29.84±4.63[ab]

a = significantly different from 7 C
b = significantly different from 14 C
c = significantly different from 27 C

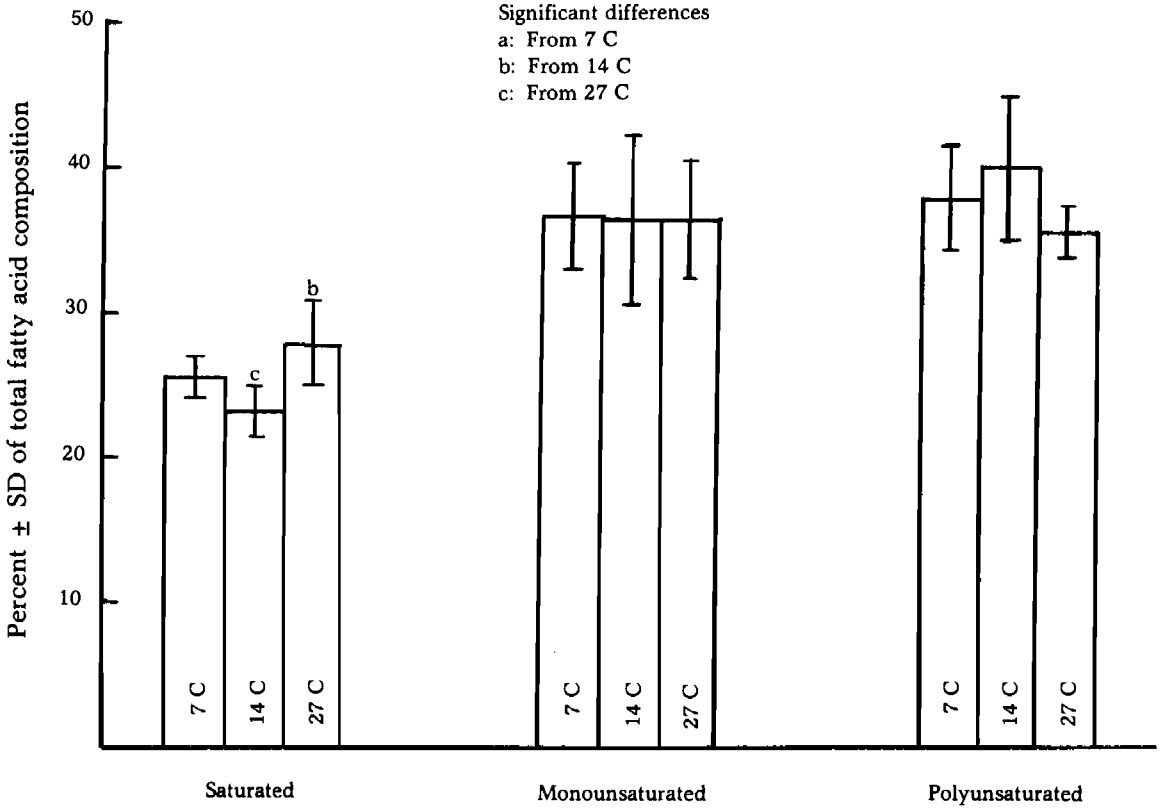

Figure 7. Classes of fatty acids from hepatopancreas of crayfish acclimated to different temperatures. The fatty acids in each category are identified in the legend for Figure 1.

Table 7. Fatty acid composition of total hepatopancreas lipids extracted from crayfish, *Orconectes nais,* acclimated to different temperatures.

Fatty Acids	Percent ± SD of Total		
	7C	14 C	27 C
Myristic acid (14:0)	1.07 ± 0.46	1.55 ± 0.70	2.06 ± 1.17
Palmitic acid (16:0)	17.13 ± 0.85	14.75 ± 1.83[c]	17.29 ± 1.48[b]
Palmitoleic acid (16:1)	8.29 ± 1.37	7.70 ± 2.56	7.58 ± 3.93
Stearic acid (18:0)	7.20 ± 1.39	6.96 ± 1.90	8.38 ± 3.64
Oleic acid (18:1)	28.42 ± 3.84	28.83 ± 4.47	29.00 ± 1.88
Linoleic acid (18:2)	11.30 ± 2.30[c]	16.76 ± 2.44	19.04 ± 4.47[a]
Linolenic acid (18:3)	4.80 ± 1.33	3.91 ± 1.61	3.75 ± 1.66
Arachidonic acid (20:4)	8.06 ± 1.25	6.78 ± 2.03	5.10 ± 3.02
Eicosapentaenoic acid (20:5)	13.74 ± 4.36	12.76 ± 3.88	7.79 ± 4.11

a = significantly different from 7 C
b = significantly different from 14 C
c = significantly different from 27 C

DISCUSSION

Generally, results of the present work on the crayfish, *Orconectes nais,* correlate with previous studies which have demonstrated lipid compositional changes. These changes showed a trend towards a greater degree of unsaturated fatty acids in the organisms at low temperatures. In addition to all the species previously decribed, a recent observation was made by Brichon et al. (1980). These authors observed a slightly higher amount of polyunsaturated fatty acids in lipids extracted from hemolymph of *Carcinus maenas* living at low temperatures.

In the present study, fatty acids of the gill lipids from the seasonal crayfish (Fig. 1 and Table 1) as well as from laboratory acclimated crayfish (Fig. 2 and Table 2) showed this same trend. The decreases in palmitic and stearic acids at low temperatures are in agreement with studies on crabs, goldfish, and mosquitofish (Chapelle 1978; Kemp and Smith 1970; Knipprath and Mead 1966). A study by Wodtke (1978) on carp liver mitochondria reported similar alterations in fatty acids. For example, decreased environmental temperatures caused a decrease in palmitic acid and an increase in arachidonic acid. Finally, the present study showed an increase in eicosapentaenoic acid for the winter and 7 C acclimated crayfish groups. This observation corresponds to results from the crab gill tissue exposed to cool temperatures (Chappelle 1978). These lipid composition changes suggest the importance of temperature on the degree of lipid unsaturation. However, this study did not examine other possible influencing factors such as photoperiod and diet.

When the low temperature acclimated group was compared to the corresponding winter group (Fig. 3), it had a lower polyunsaturated fatty acid total. This result may suggest that the acclimation period was not long enough to allow complete lipid compositional changes to occur. Chapelle (1978) demonstrated higher degrees of unsaturation with longer cold acclimation times. The animals were acclimated for 3 and 9 days. Similarly, the comparison of the 14 C acclimated group to the fall and spring groups also showed less lipid unsaturation in the acclimated group. Again, this result might be accounted for by the length of acclimation time.

There exists a possiblity that various tissues may adapt differently to temperature changes, perhaps, due to functional differences of their component membranes. A previous study by Roots (1968) showed that in goldfish brain tissue there was no obvious increase at low temperatures in unsaturation of C_{20} and C_{22}

fatty acids. However, in goldfish mucosal phospholipids (Kemp and Smith 1970), there were major changes in these long-chain unsaturated fatty acids. The present study demonstrated that lipids from gill and hepathopancreas tissues of *Orconectes nais* have a higher amount of unsaturation from both the winter group (gills) and the 7 C acclimated groups (gills and hepatopancreas). Lipids from muscle tissue of crayfish acclimated at the lowest temperature, however, did not show significant changes. These results suggest that, perhaps, the composition alterations are different for this tissue.

Physiologically, these lipid alterations may help to explain how the organisms adapt to cold temperatures. The fluid nature of the cell membrane greatly affects the activity of several membrane-bound enzymes. It has been shown that enriching the membrane lipids with fatty acids with low melting points can improve membrane flexibility at cool temperatures. These changes are necessary for the proper functioning of lipid-dependent enzymes (Raison et al. 1971; Sanderman 1978). This evidence suggests important reasons for the changes that occur in lipid composition. The changes observed in this study were not large. It may be that small and subtle changes are sufficient to maintain control over membrane fluidity and hence enzyme function. The current study does not attempt to prove the reason for the lipid changes. Instead, an observation of a system is presented where there was an increase in unsaturation of lipids with a decrease in laboratory acclimation as well as environmental temperatures. Further investigation is needed to determine the biochemical mechanisms for the lipid changes and also the resulting consequences from these alterations.

LITERATURE CITED

Aloia, R.C., E.T. Pengelley, J.L. Bolen, and G. Rouser. 1974. Changes in phospholipid composition in hibernating ground squirrel, *Citellis lateralis,* and their relationships to membrane function at reduced temperatures. Lipids. 9:993-999.

Baranska, J. and P. Wlodawer. 1969. Influence of temperature on the composition of fatty acids and on lipogenesis in frog tissues. Comp. Biochem. Physiol. 28:553-570.

Brichon, G., S. Chapelle, and G. Zwingelstein. 1980. Phospholipids composition and metabolism in the hemolymph of *Carcinus maenas* (Crustacea, Decapoda) - Effect of temperature. Comp. Biochem. Physiol. 67B:647-652.

Brown, C.M. and A.H. Rose. 1969. Fatty-acid composition of *Candida utilis* as affected by growth temperature and dissolved-oxygen tension. J. Bacteriol. 99:371-378.

Caldwell, R.S. 1969. Thermal compensation of respiratory enzymes in tissues of the goldfish (*Carassius auratus* L.). Comp. Biochem. Physiol. 31:79-90.

_____ and F.J. Vernberg. 1970. The influence of acclimation temperature on the lipid composition of fish gill mitochondria. Comp. Biochem. Physiol. 34:179-191.

Chapelle, S. 1978. The influence of acclimation temperature on the fatty acid composition of an aquatic crustacean *(Carcinus maenas).* J. Exp. Zool. 204:337-346.

_____, R. Meiser, G. Brichon, and G. Zwingelstein. 1977. Influence of temperature on the phospholipid metabolism of various tissues from the crab *(Carcinus maenas).* Comp. Biochem. Physiol. 58:413-417.

Cullen, J., M.C. Phillips, and G.G. Shipley. 1971. The effects of temperature on the composition and physical properties of the lipids of *Pseudomonas fluorescens.* Biochem. J. 125:733-742.

Farkas, T. 1970. Fats in fresh water crustaceans: Fatty acid composition of lipid obtained from *Eudiaptomus gracilis* G. O. Sars (Copepoda) and *Daphnia cucullata* G. O. Sars (Cladocera). Acta Biol. Acad. Sci. Hugn. 21:225-233.

_____ and S. Herodek. 1964. The effect of environmental temperature on the fatty acid composition of crustacean plankton. J. Lipid Res. 5:369-379.

Freed, J.M. 1965. Changes in activity of cytochrome oxidase during adaptation of goldfish to different temperatures. Comp. Biochem. Physiol. 14:651-659.

Goldman, S.S. 1975. Cold resistance of the brain during hibernation. III. Evidence of a lipid adaptation. Am. J. Physiol. 228:834-838.

Haest, C.W.M., J. DeGier, and L.L.M. van Deenen. 1969. Changes in the chemical and the barrier properties of the membrane lipids of *E. coli* by variation of the temperature of growth. Chem. Phys. Lipids. 3:413-417.

Hazel, J.R. 1972. The effect of temperature acclimation upon succinic dehydrogenase activity from the epaxial muscle of the common goldfish (*Carassius auratus* L). I. Properties of the enzyme and the effect of lipid extraction. Comp. Biochem. Physiol. 43B:837-861.

Hoar, S. and M.K. Cottle. 1952. Some effects of temperature and acclimatization on the chemical constitution of goldfish tissues. Can. J. Zool. 30:49-55.

Johnston, P.V. and B.I. Roots. 1964. Brain lipid fatty acid and temperature acclimation. Comp. Biochem. Physiol. 11:303-309.

Kates, M. 1972. Techniques of lipidology. T.S. Work and E.J. Work, eds. North-Holland Publishing Co. Amsterdam, 610 pp.

Kemp, P. and M.W. Smith. 1970. Effect of temperature acclimatization on the fatty acid composition of goldfish intestinal lipids. Biochem. J. 117:9-15.

Kimelberg, H.K. and D. Papahadjopoulos. 1974. Effects of phospholipid acyl chain fluidity, phase transitions, and cholesterol on ($Na^+ + K^+$)-stimulated adenosine triphosphatase. J. Biol. Chem. 249:1071-1080.

Knipprath, W.G. and J.F. Mead. 1966. Influence of temperature on fatty acid pattern of mosquitofish *(Gambusia affinis)* and guppies *(Lebistes reticulatus)*. Lipids. 1:113-117.

_____. 1967. The effect of environmental temperature on the fatty acid composition and on the in vivo incorporation of I-^{14}C-acetate in goldfish (*Carassius auratus*, L.) Lipids. 3:121-128.

Lewis, R.W. 1962. Temperature and pressure effects on the fatty acids of some marine ectotherms. Comp. Biochem. Physiol. 6:75-89.

Long, S.K. and O.B. Williams. 1960. Lipids of *Bacillus stearothermophilus*. J. Bacteriol. 79:629-637.

Marr, A.G. and J.L. Ingraham. 1962. Effect of temperature on the composition of fatty acids in *Escherichia coli*. J. Bacteriol. 84:1260-1267.

Minor, J.G., G.E. Folk, and R.L. Dryer. 1973. Changes in triglyceride composition of white and brown adipose tissues during developing cold acclimation of the golden hamster *Mesocricetus auratus*. Comp. Biochem. Physiol. 46B:375-385.

O'Conner, J.D. and L.I. Gilbert. 1968. Aspect of lipid metabolism in crustaceans. Am. Zool. 8:529-539.

Purnell, J.H. 1962. Gas chromatography. Wiley, New York, 441 pp.

Raison, J.K., J.M. Lyons, R.J. Mehlhorn, and A.D. Keith. 1971. Temperature-induced phase changes in mitochondrial membranes detected by spin labeling. J. Biol. Chem. 246:4036-4040.

Roots, B.I. 1968. Phospholipids of goldfish (*Carassius auratus* L.) brain: The influence of environmental temperature. Comp. Biochem. Physiol. 25:457-466.

Rotermund, A.J. and J.C. Veltman. 1981. Modification of membrane-bound lipids in erythrocytes of cold-acclimated and hibernating 13-lined ground squirrels. Comp. Biochem. Physiol. 69B:523-528.

Sanderman, H. Jr. 1978. Regulation of membrane enzymes by lipids. Biochim. Biophys. Acta. 515:209-237.

Smith, M.W., V.E. Colombo, and E.A. Munn. 1968. Influence of temperature acclimatization on the ionic activation of goldfish intestinal adenosine triphosphatase. Biochem. J. 107:691-698.

_____ and P. Kemp. 1971. Parallel temperature-induced changes in membrane fatty acids and in transport of amino acids by the intestine of goldfish (*Carassius auratus* L.). Comp. Biochem. Physiol. 39B:357-366.

Thomson, A.J., J.R. Sargent, and J.M. Owen. 1977. Influence of acclimatization temperature and salinity on ($Na^+ + K^+$)-dependent adenosine triphosphatase and fatty acid composition in the gills of the eel, *(Anguilla anguilla)*. Comp. Biochem. Physiol. 56B:223-228.

Wodtke, E. 1978. Lipid adaptation in liver mitochondrial membranes of carp acclimated to different environmental temperatures-Phospholipid composition, fatty acid pattern and cholesterol content. Biochim. Biophys. Acta. 529:280-291.