Mitochondrial Membrane Composition of Two Arctic Marine Bivalve Mollusks, *Serripes groenlandicus* and *Mya truncata*

Todd E. Gillis[a] and James S. Ballantyne[b,*]

[a]Cardiac Membrane Research Laboratory, School of Kinesiology, Simon Fraser University, Burnaby, British Columbia V5A 1S6, and [b]Department of Zoology, University of Guelph, Guelph, Ontario, N1G-2W1 Canada

ABSTRACT: The phospholipid and fatty acid composition of gill mitochondria membranes from two Arctic marine bivalve mollusks, *Mya truncata* and *Serripes groenlandicus*, were examined. These animals were collected from the Arctic Ocean, where waters remain below 0°C throughout the year. In both species, the primary membrane phospholipids were phosphatidylcholine, and phosphatidylethanolamine. Although a low ratio of bilayer-stabilizing phospholipids to bilayer-destabilizing phospholipids is frequently associated with cold acclimation in temperate species, this ratio is very different between the two species. The monounsaturated fatty acid 20:1 was abundant in the membranes of both Arctic species equaling 13.0% of the fatty acid composition in *S. groenlandicus*, and 17.7% in *M. truncata*. Polyunsaturated fatty acids were relatively low in the Arctic species, equaling 35.9% of total membrane fatty acids compared to that of temperate zone mollusks. It is suggested that monoenes are common in the tissues of Arctic species since they play a role in maintaining membrane function at subzero temperatures.

Paper no. L7759 in *Lipids 34*, 53–57 (January 1999).

Polar marine environments are among the most thermally stable regions of the world. Such stability resulted in many organisms becoming highly specialized to function at subzero temperatures (1). The properties of biological membranes in particular must be adapted to function under these conditions. The composition of cellular membranes can be manipulated to maintain membrane properties and function. Membrane phospholipid and fatty acid composition are two of the components that can be altered to maintain membrane function (2–4). Study of temperate, ectothermic species demonstrated that the bilayer-destabilizing phospholipid phosphatidylethanolamine (PE), as well as long-chain polyunsaturated fatty acids (PUFA) accumulate within cellular membranes upon acclimation to low temperatures (2,3,5–9). Such manipulation of membrane composition aids in maintaining membrane properties (2–4,6,9).

Several studies indicate that the lipids of polar organisms differ from those of temperate zone organisms. In particular, whole tissue lipids of Arctic and Antarctic copepods contain unusually high amounts of monounsaturated fatty acids (monoenes) (10). It is not known if these differences apply to membrane lipids. Chronic exposure to very low temperature such as occurs in polar organisms may require membrane adaptational strategies previously unidentified by thermal acclimation studies of temperate species.

The purpose of this study is to characterize the mitochondrial membranes of the two Arctic bivalve mollusk species *Serripes groenlandicus* and *Mya truncata*. As a result of their limited ability for locomotion, these species remain in subzero waters throughout the year in the Canadian Arctic. By comparing the membrane composition of these animals to those of cold-adapted temperate species, it may be possible to establish if mollusk species from the Arctic and Temperate zones utilize similar methods of membrane modification in overcoming the challenge of living at low temperatures.

MATERIALS AND METHODS

Experimental animals. Serripes groenlandicus and *M. truncata* were collected by scuba divers in depths of 7–18 m from the waters of the Arctic Ocean surrounding Igloolik Island (81°45′ W, 69°23′ N), Northwest Territories. The water temperature was −1.5–0.0°C, and the animals were observed to be feeding at time of capture. The animals were held in an aerated marine aquarium, maintained below 0°C for a maximum of 24 h prior to tissue processing.

Mitochondrial isolation. The gills were excised and placed in ice-cold isolation medium consisting of 400 mM sucrose, 1 mM EDTA, 2 mM EGTA, 10 mM HEPES pH 7.4 at 20°C, and 1% bovine serum albumin (essentially fatty acid-free). The gills of two animals were pooled to obtain a sufficient mass of tissue. The tissue was then weighed, minced, and suspended in ice-cold isolation medium in a ratio of 1 g of tissue to 5 mL of isolation medium. This mixture was homogenized

*To whom correspondence should be addressed.
E-mail: jballant@uoguelph.ca.

Abbreviations: CL, cardiolipin; monoene, monounsaturated fatty acid; PC, phosphatidylcholine; PC/(PE + CL), phosphatidylcholine to (phosphatidylethanolamine + cardiolipin) ratio; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SPH, sphingomyelin.

with three passes of a loosely-fitting Teflon pestle in a Potter-Elvehjem homogenizer. The homogenate was then centrifuged for 15 min at 1200 × *g*. The resulting supernatant was centrifuged for 15 min at 13600 × *g*, the pellet was removed and resuspended in 1 mL isolation medium, stored at −20°C for 3–7 d, and transported to the laboratory at the University of Guelph where it was stored at −80.0°C until further processing.

Measurement of marker enzymes. Marker enzymes were measured in both the initial homogenate and in the mitochondrial preparation. The following were used as markers for cellular membranes: cytochrome C oxidase (mitochondrial membrane), glucose 6-phosphatase (endoplasmic reticulum), Na^+,K^+-ATPase (plasma membrane), and peroxidase (peroxisomes). The activities of these marker enzymes were measured as described by Gillis and Ballantyne (11).

Analysis of mitochondrial membrane phospholipid composition. The mitochondrial membrane phospholipids were extracted, separated, and analyzed as described by Gillis and Ballantyne (11)

Protein determination. Protein was determined by the method of Bradford (12) using bovine serum albumin as a standard. Mitochondrial and tissue protein was determined by measuring the difference between the protein concentration in the isolation medium and the mitochondrial suspension.

Chemicals. The lipid standard used (Nu-Chek-Prep., Inc. Elysian, MN) was augmented by the addition of menhaden oil extract. Menhaden oil contains fatty acids not found in the commercial standard, but which are present in the lipids of most marine and freshwater organisms. Solvents were obtained from Fisher Scientific Ltd. (Whitby, Ontario) and were of American Chemical Society-certified grade. All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) and were of the highest purity available.

RESULTS

Characterization of mitochondrial membrane purity. The isolation procedure used results in a substantial enrichment of the mitochondrial membrane marker cytochrome C oxidase in relation to the other marker enzymes for plasma membrane, peroxisomes, and endoplasmic reticulum (Table 1), indicating little contamination of the mitochondria with these cellular membranes. A second indication of membrane purity was the almost complete absence of sphingomyelin (SPH) (<1.0%). This phospholipid is found in trace amounts in the mitochondrial membrane (1%) while it is prevalent in lysosomes, plasma membranes, and Golgi membranes, representing as much as 20, 16, and 8%, respectively, of total phospholipid in these membranes (13). The very low proportion of this phospholipid is further evidence that there was minimal contamination of the membrane preparations by these organelles in the present study.

Phospholipid composition. In *M. truncata,* PE was the dominant phospholipid, representing 47.8% of the total membrane phospholipids, while phosphatidylcholine (PC) and phosphatidylinositol (PI) made up 22.4 and 19.5%, respectively, of the membrane phospholipid composition (Table 2). In *S. groenlandicus*, PE and PC were the two most abundant phospholipids, equaling 38.4 and 40.4% of the total membrane phospholipids, while PI represented 13.3% (Table 2). Cardiolipin (CL) and phosphatidylserine (PS) were minor components in the membrane phospholipids of both bivalve species. The ratio of bilayer-stabilizing (PC) to bilayer-destabilizing phospholipids (PE, CL) equaled 1.01 in *S. groenlandicus* and 0.45 in *M. truncata*. The phospholipid SPH was present in trace amounts in the mitochondrial membranes of both species but in all preparations represented less than 1% of total membrane phospholipid.

TABLE 1
Fold Purification of Marker Enzymes in Mitochondrial Fraction Isolated from Gill Tissue of *Mya truncata* and *Serripes groenlandicus*[a]

	M. truncata	*S. groenlandicus*
Na^+/K^+-ATPase	0.074 ± 0.030	0.080 ± 0.028
Cytochrome C oxidase	13.62 ± 3.62	15.22 ± 4.09
Peroxidase	0.060 ± 0.028	0.059 ± 0.007
Glucose 6-phosphatase	0.30 ± 0.053	0.22 ± 0.057

[a]Values are means ± SE for $n = 4$. Fold purification = (activity in mitochondrial pellet/mg protein)/(activity in homogenate/mg protein).

Gill mitochondrial membrane fatty acid composition. The predominant fatty acids in the cumulative fatty acid composition of membrane phospholipids in both bivalve species were 16:0, 20:1, and 20:5n-3 (Table 3), equaling a combined total of 45.9% in *S. groenlandicus* and 56.1% in *M. truncata*. Saturated fatty acids (SFA) were the most prevalent class of fatty acids equaling 42% in *S. groenlandicus* and 38% in *M. truncata* (Table 3).

In both species of mollusk, SFA, monoenes, and PUFA represented similar proportions of the fatty acids in the phospholipid PE (Table 4). The main individual fatty acids in this phospholipid from both species were 20:0 and 20:1. In *S. groenlandicus,* 20:0 equaled 24.3 ± 3.2% of total fatty acid content while 20:1 equaled 23.1 ± 3.3%. In *M. truncata* these fatty acids equaled 18.5 ± 4.7 and 29.4 ± 3.6%, respectively.

In mitochondrial PC of *S. groenlandicus*, SFA and PUFA were the prevalent classes of fatty acids (Table 4); this was

TABLE 2
Percentage of Phospholipids in Gill Mitochondria from *Serripes groenlandicus* and *Mya truncata*[a]

Phospholipid	*S. groenlandicus* ($n = 8$) (mol %)	*M. truncata* ($n = 8$) (mol %)
Cardiolipin	2.82 ± 0.40	4.90 ± 0.74
Phosphatidylethanolamine	38.48 ± 1.50	47.78 ± 2.43
Phosphatidylinositol	13.34 ± 0.66	19.49 ± 1.84
Phosphatidylserine	4.87 ± 0.83	5.93 ± 1.63
Phosphatidylcholine	40.48 ± 1.63	22.37 ± 0.76
PC/(PE + CL)[b]	1.01 ± 0.098	0.45 ± 0.038

[a]Values are presented as means ± SEM.
[b]PC = phosphatidylcholine, PE = phosphatidylethanolamine, CL = cardiolipin.

TABLE 3
Cumulative Percentages of Individual Fatty Acids in Gill Mitochondria from *Serripes groenlandicus* and *Mya truncata*[a]

Fatty acid	*S. groenlandicus* (*n*=8) (mol %)	*M. truncata* (*n* = 8) (mol %)
14:0	7.70 ± 0.57	0.95 ± 0.12
14:1	0.53 ± 0.15	0.23 ± 0.08
16:0	17.14 ± 0.99	22.14 ± 0.93
16:1	4.48 ± 0.32	4.97 ± 0.30
18:0	7.90 ± 0.32	4.29 ± 0.26
18:1n-9	3.36 ± 0.38	3.05 ± 0.29
18:2n-6	0.21 ± 0.06	n.d.[b]
18:3n-3	0.40 ± 0.09	0.69 ± 0.10
18:4n-3	0.56 ± 0.15	0.36 ± 0.03
20:0	9.20 ± 1.15	9.90 ± 2.14
20:1	13.02 ± 1.18	17.68 ± 1.28
20:2n-6	0.16 ± 0.04	0.08 ± 0.03[c]
20:3n-6	0.93 ± 0.29	1.55 ± 0.13
20:4n-6	1.66 ± 0.28	1.09 ± 0.15
20:3n-3	0.08 ± 0.04[c]	0.02 ± 0.01[c]
20:4n-3	0.31 ± 0.05	0.34 ± 0.07
20:5n-3	15.86 ± 1.71	16.32 ± 0.66
22:0	0.24 ± 0.06	0.20 ± 0.04
22:1	0.10 ± 0.03	0.10 ± 0.02
22:2n-6	4.83 ± 0.13	1.94 ± 0.11
23:0	0.39 ± 0.08	0.48 ± 0.02
22:4n-6	0.88 ± 0.09	1.92 ± 0.09
22:5n-6	0.09 ± 0.06[c]	0.04 ± 0.03[c]
22:5n-3	2.77 ± 0.18	2.83 ± 0.16
22:6n-3	7.10 ± 0.25	8.72 ± 0.43
Saturated fatty acids	42.59 ± 1.25	37.99 ± 1.36
Monounsaturated fatty acids	21.51 ± 1.15	26.05 ± 1.11
Polyunsaturated fatty acids	35.90 ± 1.74	35.96 ± 1.34
n-3 Polyunsaturated fatty acids	27.10 ± 1.69	29.31 ± 1.16
n-6 Polyunsaturated fatty acids	8.79 ± 0.37	6.64 ± 0.32
n-3/n-6 Polyunsaturated fatty acids	3.12 ± 0.24	4.44 ± 0.19
Unsaturation index[d]	185.60 ± 8.04	199.50 ± 6.33
Chain length[e]	18.61 ± 0.10	18.87 ± 0.05

[a]Values are presented as means ± SEM.
[b]Not detectable.
[c]Three or more values expressed in mean were not detectable therefore included as zeroes.
[d]Unsaturation index = $\Sigma m_i \pm n_i$, where m_i is the mole percentage and n_i is the number of C-C double bonds of the fatty acid$_i$.
[e]Mean chain length = $\Sigma f_i \pm c_i$, where f_i is the mole fraction and c_i is the number of carbon atoms of the fatty acid $_i$.

due to the large proportions of 14:0, 16:0, and 20:5n-3, equaling 17.8 ± 1.3, 21.0 ± 1.1, and 18.4 ± 4.4%, respectively, of the fatty acids in this phospholipid. In *M. truncata* PUFA represented the largest class of fatty acids in PC due to the high levels of 20:5n-3 (33.7 ± 0.6%) in this phospholipid (Table 4).

PUFA was the largest class of fatty acids in CL from *S. groenlandicus* (Table 4). This was due to 20:5n-3, equaling 28.7 ± 3.9% of the fatty acids in this phospholipid. The monoene 20:1 was also prominent in this phospholipid, equaling 19.9 ± 6.5% of the fatty acid content. SFA and PUFA were the most common fatty acids in CL from *M. truncata* due to the high levels of 16:0 and 20:5n-3. These two fatty acids equaled 26.6 ± 1.4 and 24.9 ± 1.2%, respectively.

In PS from *S. groenlandicus*, SFA and PUFA were the dominant fatty acids (Table 4) as a result of the levels of 18:0 and 20:5n-3. These two fatty acids equaled 30.5 ± 2.2 and 17.9 ± 2.5%, respectively. In *M. truncata*, SFA was the most common class of fatty acids in this phospholipid due to the levels of 16:0 and 20:0. These two fatty acids equaled 19.6 ± 3.8 and 13.3 ± 1.2%, respectively. The monoene 20:1 was also prominent in PS, equaling 16.0 ± 2.6% of the fatty acid content.

In the phospholipid PI, SFA was the prevalent class of fatty acids in both species (Table 4). This result is due to the high levels of 16:0 in this phospholipid, equaling 53.7 ± 1.5% in *S. groenlandicus* and 71.2 ± 3.8% in *M. truncata*.

DISCUSSION

In the mitochondrial membranes of both *S. groenlandicus* and *M. truncata,* PE and PC were the main phospholipids, typical of most mitochondrial membranes (13). These two phospholipids play an important role in regulating membrane properties in the biological membranes of temperate zone organisms (2,6,14). The ratio of PC to PE decreases in many ectothermic animals acclimated to lower temperatures (2). PC stabilizes the bilayer as it favors the formation of a laminar bilayer, while PE destabilizes the bilayer by keeping the membrane close to the phase transition between laminar and hexagonal (H_{II}) phase conformations (15). For example, acclimation of trout *Salmo gairdneri* from 20 to 5°C lowered the ratio of PC/PE from 1.71 to 0.78 (16). Mitochondria contain a second bilayer-destabilizing phospholipid, CL. This phospholipid adopts the H_{II} phase conformation in the presence of Ca^{2+} (17,18) and the membrane protein cytochrome C oxidase (19), both of which are associated with the inner mitochondrial membrane. The ratio of PC/(PE + CL) in *M. truncata* was about half that of *S. groenlandicus.* This difference is as a result of twofold higher proportion of PC in the membranes of *S. groenlandicus*. Such interspecific differences in this ratio are not uncommon in temperate marine bivalves. In the oyster, *Crassostrea virginica,* this ratio is twice that of the quahog, *Mercenaria mercenaria* (11,20), and similar to that of *S. groenlandicus* in the present study. These results suggest that a low PC/(PE + CL) ratio is not a unique characteristic of Arctic marine mollusks.

The monounsaturated fatty acid (monoene) 20:1 was one of the three primary fatty acids in the cumulative totals of the mitochondrial membrane phospholipids in both bivalve species. Accordingly, this fatty acid was dominant in the phospholipids PE, CL, and PI in both species as well as in PS of *M. truncata*. This is different from the fatty acid composition of temperate marine bivalves where the predominant fatty acids are SFA and PUFA (11,20). Other cold-water organisms have high levels of monoenes. The monoene 18:1 is reported to be prevalent in red muscle mitochondrial membranes of Arctic char *Salvelinus alpinus* sampled from 4°C waters of the Arctic Ocean (21). The monoenes 20:1 and 22:1 were also reported in unusually high proportion in the whole tissue lipids of Arctic and Antarctic copepods (10).

The combination of the monoene 18:1 in the *sn*-1 position and a long-chain PUFA in the *sn*-2 position in the phospho-

TABLE 4
Percentages of Fatty Acid Classes in the Membrane Phospholipids from the Gill Mitochondria of *Serripes groenlandicus* and *Mya truncata*[a]

Species	Class	PE	PC	PS	PI	CL
S.	SFA	37.6 ± 1.3	43.6 ± 2.5	48.9 ± 3.7	64.9 ± 1.7	20.0 ± 4.6
groenlandicus	Monoenes	28.8 ± 2.2	15.9 ± 1.5	8.7 ± 2.5	16.5 ± 2.2	28.3 ± 6.7
(*n* = 8)	PUFA	33.7 ± 2.9	40.5 ± 3.6	42.4 ± 3.8	18.6 ± 0.9	51.6 ± 5.2
	n-3 PUFA	21.8 ± 2.2	34.3 ± 3.8	26.1 ± 2.8	10.4 ± 0.6	41.5 ± 6.7
	n-6 PUFA	11.8 ± 0.9	6.2 ± 0.4	16.3 ± 1.4	8.2 ± 0.4	10.1 ± 2.2
	Unsat ind	171.1 ± 10.8	212.8 ± 17.0	182.8 ± 16.6	94.5 ± 3.2	270.8 ± 27.3
M.	SFA	28.2 ± 4.0	22.1 ± 0.7	42.2 ± 2.8	77.8 ± 2.8	35.9 ± 1.9
truncata	Monoenes	35.2 ± 3.0	15.3 ± 0.6	28.3 ± 3.6	11.6 ± 1.5	19.1 ± 1.5
(*n* = 8)	PUFA	36.5 ± 3.9	62.6 ± 0.8	29.5 ± 4.6	10.7 ± 1.4	45.1± 2.7
	n-3 PUFA	28.2 ± 3.1	55.8 ± 0.7	24.7 ± 5.0	6.8 ± 1.3	39.6 ± 2.6
	n-6 PUFA	8.3 ± 0.8	6.7 ± 0.4	4.8 ± 0.9	3.9 ± 0.3	5.5 ± 1.1
	Unsat. Ind[b]	206.2 ± 17.7	324.9 ± 3.4	179.6 ± 22.2	59.2 ± 8.9	248.5 ± 13.7

[a]Values are presented as means ± SEM. [b]PS, phosphatidylserine; PI, phosphatidylinositol; SFA, saturated fatty acids; monoenes, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. See Table 2 for other abbreviations.
[b](Unsat) index = $\Sigma m_i \cdot n_i$, where m_i is the mole percentage and n_i is the number of C-C double bonds of the fatty acid $_i$.

lipid PE in cold-adapted and acclimated teleosts was suggested to play a role in fluidizing the biological membrane (13,22). These authors suggest that the presence of these specific fatty acids in PE is at least partially responsible for the increased disorder and lower transition temperatures of membranes of fish either evolutionarily or seasonally adapted to low temperatures. In PE of *S. groenlandicus,* the monoene 20:1 was the predominant fatty acid while in *M. truncata* it and 20:0 were of equally high proportion. Compared to the levels in PE of the temperate marine bivalve *C. virginica*, 20:1 was at least five times as abundant while PUFA content was one-half in this phospholipid in the Arctic species (20). This result suggests that this monoene is of particular importance to this phospholipid in the membranes of Arctic organisms.

While monoenes are in abundance in the mitochondrial membranes of the Arctic bivalves, PUFA are not. The PUFA content in the mitochondrial membranes of the Arctic animals is at least one-third less than that reported for the mitochondrial membranes of the temperate marine bivalve species acclimated to either −1, 10, or 12°C (11,20). It is the comparatively low levels of the fatty acid 22:6n-3 in a number of the membrane phospholipids of the Arctic bivalve species that can account for this difference. This PUFA was demonstrated to be accumulated in the membranes of both cold-adapted and cold-acclimated teleost fish (6,14,22) and is the primary fatty acid in the mitochondrial membranes of *S. alpinus* caught from 4°C water of the Arctic Ocean (21). The low level of 22:6n-3 in the Arctic bivalves is not due to a limited supply of its n-3 precursors as the level of the fatty acid 20:5n-3 is similar to that of temperate species (20). This implies that high levels of 22:6n-3 are not required for optimal membrane function at low temperatures.

The SFA content in the mitochondrial membranes of the Arctic bivalves is higher than any reported values for the mitochondrial membranes of marine and freshwater teleost fish (8,21,23), or of tissue phospholipids of marine and freshwater crustaceans (5,7). However, this finding is similar to what has been reported for the gill mitochondrial membranes of the temperate marine bivalves *C. virginica* and *M. mercenaria* acclimated to either −1.5, 10, or 12°C (4,11). This suggests that high concentrations of SFA, in particular 16:0, in mitochondrial membranes may be a common characteristic of marine mollusks. Having high proportions of SFA in the membranes of animals from very cold temperatures does not conform to what is predicted by acclimation studies of teleost fish (2,8, 9,22). As membrane fluidity was not measured in our study, the thermotropic phase transition temperatures of the membranes are unknown. However, as the Arctic species were active and feeding at time of capture, their membranes must have been sufficiently fluid to be functioning.

Diet plays a role in determining the fatty acid composition of biological membranes (24). The prevalence of monoenes in the tissues of Arctic copepod plankton (10) indicates that these fatty acids are common in the Arctic food chain. These fatty acids would be of adaptive value to the copepods, and when transferred to the mollusks feeding on them would require little modification to confer optimal membrane properties of the mollusks.

Overall the membranes of the Arctic species are very different from what would be expected based on previously published studies of animals acclimated to the cold. This may be due to the chronic lower temperatures faced by Arctic organisms. High levels of monoenes appear to be characteristic of polar animals and may play a functional role in the maintenance of membrane fluidity. The high proportion of 20:1 in PE may support the fluidizing effect of this phospholipid on the membrane. The low levels of PUFA, specifically 22:6n-3, compared to temperate species in the Arctic bivalves suggest that these fatty acids are not essential to membrane function at low temperature.

ACKNOWLEDGMENTS

This research was funded by a Natural Sciences and Engineering Research Council of Canada operating grant to JSB and a Northern

Training Grant to TEG. We also thank Johnathon Hall and Jan DenDalk for field assistance and John MacDonald of the Igloolik Research Center for field support.

REFERENCES

1. Somero, G.N. (1991) Biochemical Mechanisms of Cold Adaptation and Stenothermality in Antarctic Fish, in *Biology of Antarctic Fish* (di Prisco, G., Maresca, B., and Tota, B., eds.) pp. 232–247, Springer-Verlag, New York.
2. Hazel, J.R. (1995) Thermal Adaptation in Biological Membranes: Is Homeoviscous Adaptation the Explanation? *Annu. Rev. Physiol. 57*, 19–42.
3. Sinensky, M. (1974) Homeoviscous Adaptation—A Homeostatic Process That Regulates the Viscosity of Membrane Lipids in *Escherichia coli, Proc. Natl. Acad. Sci. USA 71*, 522–525.
4. Coolbear, K.P., Berde, C.B., and Keough, K.M.W. (1983) Gel to Liquid-Crystalline Phase Transitions of Aqueous Dispersions of Polyunsaturated Mixed-Acid Phosphatidylcholines, *Biochemistry, 22*, 1466–1473.
5. Pruitt, N.L. (1988) Membrane Lipid Composition and Overwintering Strategy in Thermally Acclimated Crayfish, *Am. J. Physiol. 254*, R870–R876.
6. Hazel, J.R., and Zerba, E. (1986) Adaptation of Biological Membranes to Temperature: Molecular Species Compositions of Phosphatidylcholine and Phosphatidylethanolamine in Mitochondrial and Microsomal Membranes of Liver from Thermally-Acclimated Rainbow Trout, *J. Comp. Physiol. 156B*, 665–674.
7. 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.
8. Wodtke, E. (1981) The Effects of Acclimation Temperature on the Unsaturation of the Main Neutral and Charged Phospholipids in Mitochondrial Membranes of the Carp, *Cyprinus carpio, Biochim. Biophys. Acta 640*, 698–709.
9. Hazel, J.R. (1983) The Incorporation of Unsaturated Fatty Acids of the n-9, n-6, and n-3 Families into Individual Phospholipids by Isolated Hepatocytes of Thermally-Acclimated Rainbow Trout, *Salmo gairdneri*, *J. Exp. Zool. 227*, 167–176.
10. Kattner, G., and Hagen, W. (1995) Polar Herbivorous Copepods—Different Pathways in Lipid Biosynthesis, *ICES J. Mar. Sci. 52*, 329–335.
11. Gillis, T.E., and Ballentyne, J.S. (1999) Influences of Subzero Thermal Acclimation on Mitochondrial Membrane Composition of Temperate Zone Marine Bivalve Mollusks, *Lipids 34*, 59–66.
12. Bradford, M.M. (1976) A Rapid Sensitive Method for Quantification of Microgram Quantities of Protein Using the Principle of Protein Dye Binding, *Anal. Biochem. 72*, 248–254.
13. Daum, G. (1985) Lipids of Mitochondria, *Biochim. Biophys. Acta 822*, 1–42.
14. Dey, I., Buda, C., Wiik, T., Halver, J.E., and Farkas, T. (1993) Molecular and Structural Composition of Phospholipid Membranes in Livers of Marine and Freshwater Fish in Relation to Temperature, *Proc. Natl. Acad. Sci. USA 90*, 7498–7502.
15. Thurmond, R.L., and Lindblom, G. (1997) NMR Studies of Membrane Lipid Properties. in *Current Topics in Membranes* (Epand, R., ed.) Vol. 44, pp. 103–166, Academic Press, New York.
16. Hazel, J.R., and Landrey, S.R. (1988) Time Course of Thermal Adaptation in Plasma Membranes of Trout Kidney. I Headgroup Composition, *Am. J. Physiol. 255*, R622–R627.
17. Rand, R.P., and Sengupta, S. (1972) Cardiolipin Forms Hexagonal Structures with Divalent Cations, *Biochim. Biophys. Acta 255*, 484–492.
18. Cullis, P.R., Verkleij, A.J., and Ververgaert, P.H.J.Th. (1978) Polymorphic Phase Behaviour of Cardiolipin as Detected by ^{31}P NMR and Freeze-Fracture Techniques, *Biochim. Biophys. Acta 513*, 11–20.
19. De Kruijff, B., and Cullis, P.R. (1980) Cytochrome c Specifically Induces Non-Bilayer Structures in Cardiolipin-Containing Model Membranes, *Biochim. Biophys. Acta 602*, 477–490.
20. Glemet, H.C., and Ballantyne, J.S. (1995) Influences of Environmental Salinity on the Structure and Function of Gill Mitochondria Membranes of an Osmoconforming Invertebrate, *Crassostrea virginica, Mar. Biol. 121*, 673–683.
21. Glemet, H.C., Gerrits, M.F., and Ballantyne, J.S. (1997) Membrane Lipids of Red Muscle Mitochondria from Land-Locked and Sea Run Arctic Char, *Salvelinus alpinus, Mar Biol. 129*, 673–679.
22. Fodor, E., Jones, R.H., Buda, C., Kitajka, K., Dey, I., and Farkas, T. (1995) Molecular Architecture and Biophysical Properties of Phospholipids During Thermal Adaptation in Fish: An Experimental and Model Study, *Lipids 30*, 1119–1126.
23. Glemet, H.C., and Ballantyne, J.S. (1996) Comparisons of Liver Mitochondrial Membranes from an Agnathan, *Myxine glutinosa*, an Elasmobranch, *Raja erinacea*, and a Teleost Fish, *Pleuronectes americanus, Mar. Biol. 124*, 509–518.
24. Sargent, J.R., Bell, M.V., Henderson, R.J., and Tocher, D.R. (1990) Polyunsaturated Fatty Acids in Marine and Terrestrial Food Webs, in *Comparative Physiology* (Kinne, R.K.H., Kinne-Saffran, E., and Beyenbach, K.W., eds.) Vol. 6, pp. 11–23, S. Karger, Basel.

[Received August 11, 1997, and in final revised form October 22, 1998; revision accepted December 17, 1998]