

## Eicosapentaenoic Acid Regulates Scallop (*Placopecten magellanicus*) Membrane Fluidity in Response to Cold

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*The lipid core of a biological membrane requires a certain degree of structural rigidity, but it must also be sufficiently fluid to permit lateral movement of the constituent lipids and embedded proteins. Ectotherms can counteract the ordering effects of reduced temperature by changing the structure of their membranes, a process known as homeoviscous adaptation (1). Although the content of unsaturated fatty acids in the membranes of ectothermic animals is generally known to increase in response to cold (2), no clear and direct relationship between unsaturated fatty acids and membrane fluidity has been established in marine organisms. For example, phospholipid molecular species containing docosahexaenoic acid (22:6 $\omega$ 3) are believed to be important in controlling finfish membrane fluidity (3–6), but a direct correlation between 22:6 $\omega$ 3 and membrane fluidity has not been found (4, 5, 7, 8). In contrast, we show here a simple but very strong relationship between fluidity and a single polyunsaturated fatty acid, eicosapentaenoic acid (20:5 $\omega$ 3), in gill membranes from a marine bivalve mollusc, the sea scallop *Placopecten magellanicus*.*

Phospholipids are the main structural elements of biological membranes, and their physical characteristics are key determinants of membrane structure and function. Many vital cell activities that depend on the optimal functioning of membranes are therefore sensitive to the chemistry of the membrane lipids (9) and to environmental conditions, such as temperature and pressure, that perturb the phase behavior and dynamics of lipids in membranes (10). Under extreme or variable conditions, organisms can exploit the tremen-

dous chemical diversity among membrane lipids to defend the physical properties of the membrane (10). Thus in ectotherms, where changes in temperature cause important membrane perturbations, the usual adaptive response includes a modification of lipid composition (11).

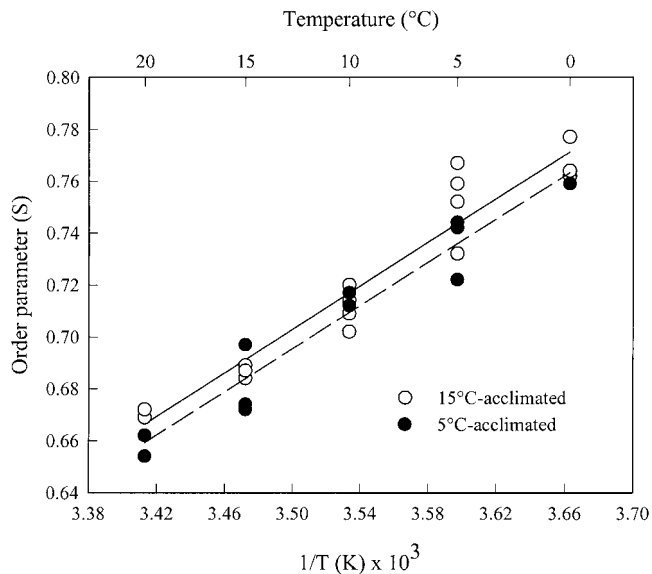
Sessile animals living in Newfoundland waters must maintain membrane structure and function in the face of extreme cold in deep waters (as low as  $-1.4^{\circ}\text{C}$ ) or seasonally highly variable conditions in surface waters (as much as  $22^{\circ}\text{C}$  in 6 months) (12). In the present study, we exposed sea scallops to a  $10^{\circ}\text{C}$  decrease in temperature for up to 3 weeks and then examined the relationship between the fatty acid composition of branchial phospholipids and membrane fluidity.

Vesicles were prepared from the gills of scallops acclimated to temperatures of 15 and  $5^{\circ}\text{C}$ . After three weeks of thermal acclimation, the structural order of the phospholipids was measured by electron spin resonance (ESR) spectroscopy at five temperatures ( $0$ – $20^{\circ}\text{C}$ ) that span the physiological range of *Placopecten magellanicus* (Fig. 1). The vesicles prepared from gills of  $5^{\circ}\text{C}$ -acclimated scallops were significantly (ANCOVA,  $P = 0.03$ ) less ordered than vesicles from  $15^{\circ}\text{C}$ -acclimated scallops. Temperature acclimation had shifted the order parameter curve  $1$ – $2^{\circ}\text{C}$  toward lower assay temperatures, giving a homeoviscous efficacy (13) of 14%. Such a partial adjustment towards an ideal or complete homeoviscous response has also been found in crabs (14) and crayfish (15). In these invertebrates, the costs of perfect compensation may be too high, or the benefits too low. On the other hand, the ESR measurements in this study were made with the spin probe 5-doxyl stearic acid, reflecting the homeoviscous response in the outer region of the purified lipid bilayer. It is possible that the response deeper in the bilayer, in the actual region of alkenyl chain unsaturation, would have been greater (16).

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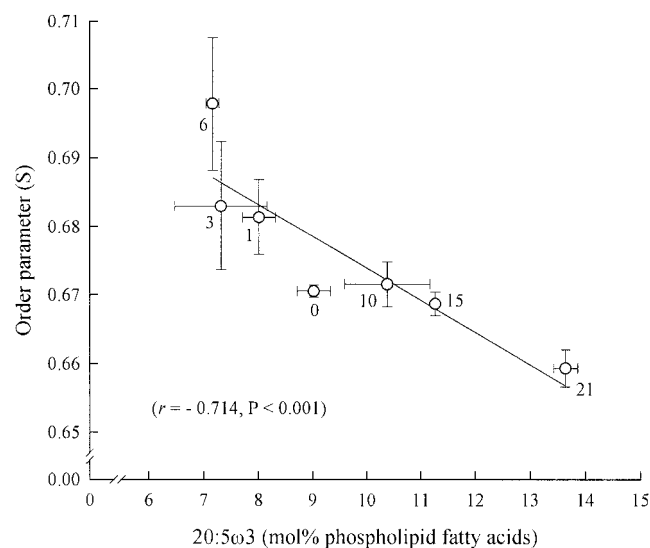


**Figure 1.** Temperature dependence of the structural order of phospholipids in vesicles prepared from scallop gills. The order parameter ( $S$ ) was measured by electron spin resonance (ESR) using 5-doxyl stearic acid incorporated into the hydrated phospholipid vesicles. Gills were obtained from scallops acclimated in the laboratory for three weeks to 5°C ( $n = 3$ ) and 15°C ( $n = 4$ ). Each data point represents a single animal. The solid regression line represents 15°C acclimation, the broken line 5°C acclimation. *Methods:* Twenty-eight sea scallops of 8–12 cm shell height were collected in Placentia Bay, Newfoundland, and held at 14–15°C for 4 weeks. They were fed four times weekly a diet of two microalgae, *Isochrysis galbana* (T-Iso) and *Nanochloropsis* sp. In *I. galbana*, the major long-chain polyunsaturated fatty acid was 22:6 $\omega$ 3, accounting for 8.6%  $\pm$  0.4% of total fatty acids, while in *Nanochloropsis* the major fatty acid was 20:5 $\omega$ 3, accounting for 34%  $\pm$  1%. (Shorthand notation for fatty acids gives the ratio of carbon atoms to double bonds and the position of the first double bond relative to the terminal methyl group.) Four scallops were randomly selected, and their gills were sampled at 15°C; the remaining animals were then transferred directly to temperature-controlled aquaria (80 l) and maintained at 5  $\pm$  0.5°C for up to 21 days. Four randomly selected scallops were sampled periodically from day 1 to day 21 of the experiment. The gills were excised, the phospholipids were extracted and purified according to Bligh and Dyer (28), and the fatty acid composition was determined by gas chromatography (22). Phospholipids were separated from the neutral lipids by silica column chromatography, the neutral fraction being eluted with chloroform: methanol: formic acid (9.9:0.1:0.1 by vol.) and the polar fraction with methanol (22). The spin label 5-doxyl stearic acid (29) was incorporated into the phospholipids at <1 mol%, and vesicles were prepared by sonication in Tris-HCl (6, 30). Labeled vesicles were drawn into a capillary tube, which was sealed, then centrifuged, and finally inserted into a quartz tube for ESR spectroscopy in a Bruker ESP-300 spectrometer. Spectra were obtained from 0 to 20°C at 5°C intervals, and the samples were maintained in the instrument at each temperature for 15 min before measurement. The outer and inner hyperfine splitting values were used to calculate the order parameter (16), which was interpreted to be inversely proportional to membrane fluidity. Values of the order parameter obtained for different acclimation groups were compared statistically by analysis of covariance (ANCOVA).

Membrane order in gills of thermally acclimated scallops was strongly and negatively correlated ( $r = -0.714$ ,  $P < 0.001$ ) with the proportion of 20:5 $\omega$ 3 in gill phospholipids

(Fig. 2). This finding is consistent with a role for 20:5 $\omega$ 3 in regulating gill phospholipid structure, demonstrating one important function of this essential metabolite (17, 18), which is also believed to be an essential nutrient in scallops, because they are unable to synthesize it from precursors (19–21). The relationship between 20:5 $\omega$ 3 and low acclimation temperature explains, at least in part, the high proportions of this polyunsaturated fatty acid found in bivalves living permanently at sub-zero temperatures in Newfoundland waters (22).

In contrast to 20:5 $\omega$ 3, the proportion of 22:6 $\omega$ 3 was not significantly correlated with membrane gill phospholipid order. This suggests that 22:6 $\omega$ 3 in scallop gills may have a function other than regulating membrane fluidity, whereas finfish seem to rely mainly on changes in 22:6 $\omega$ 3 levels to regulate bilayer order (3, 6, 17, 23). Although the melting point of 20:5 $\omega$ 3 is 10°C lower (24) than that of 22:6 $\omega$ 3, the biological importance of 20:5 $\omega$ 3 is usually associated with its role as a precursor of biologically active metabolites, including prostaglandins (25, 26). The possible dual function of this fatty acid in scallop gill membranes would explain the paradoxical increase in membrane order during the first 6 days of exposure to cold (Fig. 2), since 20:5 $\omega$ 3 in scallops may also serve as a substrate for prostaglandin biosynthesis as a stress response to the acute drop in temperature. Gill tissues isolated from marine bivalves are known to synthesize prostaglandins in response to hypos-



**Figure 2.** Relative changes in membrane fluidity as a function of the proportion of eicosapentaenoic acid (20:5 $\omega$ 3) in gill phospholipids of scallops during acclimation from 15 to 5°C. Data are mean  $\pm$  SEM for 3–4 individuals. Membrane fluidity is expressed as an order parameter ( $S$ ) using 5-doxyl stearic acid incorporated into hydrated phospholipid vesicles prepared from excised gills. Order parameter measurements were made in duplicate at an assay temperature of 20°C, and 50% of the variance in the measurements was accounted for by 20:5 $\omega$ 3. The numbers associated with the data points represent the time, in days, after the temperature change from 15 to 5°C.

motric stress (27), but little is known about the modes of action of these compounds in marine invertebrates (26).

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### Literature Cited

1. **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.
2. **Hazel, J. R. 1988.** Homeoviscous adaptation in animal cell membranes. Pp. 149–188 in *Physiological Regulation of Membrane Fluidity*, R. C. Aloia, C. C. Curtain, and L. M. Gordon, eds. A. R. Liss, New York.
3. **Hazel, J. R., E. E. Williams, R. Livermore, and N. Mozingo. 1991.** Thermal adaptation in biological membranes: functional significance of changes in phospholipid molecular species composition. *Lipids* **26**: 277–282.
4. **Dey, I., C. Buda, T. Wiik, J. E. Halver, and T. Farkas. 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.
5. **Buda, C., I. Dey, N. Balogh, L. I. Horvath, K. Maderspach, M. Juhasz, Y. K. Yeo, and T. Farkas. 1994.** Structural order of membranes and composition of phospholipids in fish brain cells during thermal acclimatization. *Proc. Natl. Acad. Sci. USA* **91**: 8234–8238.
6. **Fodor, E., R. Jones, C. Buda, K. Kitajka, I. Dey., and T. Farkas. 1995.** Molecular architecture and biophysical properties of phospholipids during thermal adaptation in fish: an experimental and model study. *Lipids* **30**: 1119–1126.
7. **Farkas, T., I. Dey, C. Buda, and J. E. Halver. 1994.** Role of phospholipid molecular species in maintaining lipid membrane structure in response to temperature. *Biophys. Chem.* **50**: 147–155.
8. **Bowden, L. A., C. J. Restall, and A. F. Rowley. 1996.** The influence of environmental temperature on membrane fluidity, fatty acid composition and lipoxygenase product generation in head kidney leucocytes of the rainbow trout, *Oncorhynchus mykiss*. *Comp. Biochem. Physiol.* **115B**: 375–382.
9. **Spector, A. A., and M. A. Yorek. 1985.** Membrane lipid composition and cellular function. *J. Lipid Res.* **26**: 1015–1035.
10. **Hazel, J. R., and E. E. Williams. 1990.** The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. *Prog. Lipid Res.* **29**: 167–227.
11. **Hazel, J. R. 1995.** Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? *Annu. Rev. Physiol.* **57**: 19–42.
12. **deYoung B., and B. Sanderson. 1995.** The circulation and hydrography of Conception Bay, Newfoundland. *Atmos.-Ocean* **33**: 135–162.
13. **Cossins, A. R. 1994.** Homeoviscous adaptation of biological membranes and its functional significance. Pp. 63–76 in *Temperature Adaptation of Biological Membranes*, A. R. Cossins, ed. Portland Press, London.
14. **Cuculescu, M., D. Hyde, and K. Bowler. 1995.** Temperature acclimation of marine crabs: changes in plasma membrane fluidity and lipid composition. *J. Therm. Biol.* **20**: 207–222.
15. **Lehti-Koivunen, S. M., and L. A. Kivivuori. 1998.** Fluidity of neuronal membranes of crayfish (*Astacus astacus* L.) acclimated to 5°C and 20°C. *Comp. Biochem. Physiol.* **119A**: 773–779.
16. **Kamada, T., and S. Otsuji. 1983.** Lower levels of erythrocyte membrane fluidity in diabetic patients: a spin label study. *Diabetes* **32**: 585–591.
17. **Bell, M. V., R. J. Henderson, and J. R. Sargent. 1986.** The role of polyunsaturated fatty acids in fish. *Comp. Biochem. Physiol.* **83B**: 711–719.
18. **Gurr, M. I., and J. L. Harwood. 1991.** *Lipid Biochemistry: an Introduction*. 4th ed. Chapman-Hall, London.
19. **Whyte, J. N. C., N. Bourne, and C. A. Hodgson. 1989.** Influence of algal diets on biochemical composition and energy reserves in *Patinopecten yessoensis* (Jay) larvae. *Aquaculture* **78**: 333–347.
20. **Marty, Y., F. Delaunay, J. Moal, and J.-F. Samain. 1992.** Changes in the fatty acid composition of *Pecten maximus* (L.) during larval development. *J. Exp. Mar. Biol. Ecol.* **163**: 221–234.
21. **Delaunay, F., Y. Marty, J. Moal, and J.-F. Samain. 1993.** The effect of monospecific algal diets on growth and fatty acid composition of *Pecten maximus* (L.) larvae. *J. Exp. Mar. Biol. Ecol.* **173**: 163–179.
22. **Parrish, C. C., Z. Yang, A. Lau, and R. J. Thompson. 1996.** Lipid composition of *Yoldia hyperborea* (Protobranchia), *Nephtys ciliata* (Nephtyidae) and *Artacama proboscidea* (Terebellidae) living at sub-zero temperatures. *Comp. Biochem. Physiol.* **114B**: 59–67.
23. **Behar, D., U. Cogan, S. Viola, and S. Mokaday. 1989.** Dietary fish oil augments the function and fluidity of the intestinal brush-border membrane of the carp. *Lipids* **24**: 737–742.
24. **Fasman, G. D., ed. 1975.** *CRC Handbook of Biochemistry and Molecular Biology. Lipids, Carbohydrates, Steroids*. 3rd ed. CRC Press, Cleveland.
25. **Stanley-Samuels, D. W. 1994.** The biological significance of prostaglandins and related eicosanoids in invertebrates. *Am. Zool.* **34**: 589–598.
26. **Stanley, D. W., and R. W. Howard. 1998.** The biology of prostaglandins and related eicosanoids in invertebrates: cellular, organismal and ecological actions. *Am. Zool.* **38**: 369–381.
27. **Freas, W., and S. Grollman. 1980.** Ionic and osmotic influences on prostaglandin release from the gill tissue of a marine bivalve, *Modiolus demissus*. *J. Exp. Biol.* **84**: 169–185.
28. **Bligh, E. G., and W. J. Dyer. 1959.** A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**: 911–917.
29. **Schreier, S., C. F. Polnaszek, and I. C. P. Smith. 1978.** Spin labels in membranes: problems in practice. *Biochim. Biophys. Acta* **515**: 375–436.
30. **Williams, E. E., and G. N. Somero. 1996.** Seasonal-, tidal-cycle- and microhabitat-related variation in membrane order of phospholipid vesicles from gills of the intertidal mussel *Mytilus californianus*. *J. Exp. Biol.* **199**: 1587–1596.