J Membrane Biol (2009) 230:125–131
DOI 10.1007/s00232-009-9192-2

Blood Lipids in Antarctic and in Temperate-Water Fish Species

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Received: 26 January 2009 / Accepted: 28 July 2009 / Published online: 21 August 2009

Abstract Antarctic fish live in very cold water and have adapted to this exceptional environment. Hemoglobin is absent or very low; yet these fish still have erythrocytes, and from these we prepared ghost-like membranes. We studied for the first time the lipid composition of ghost membranes and of plasma in Antarctic fish (*C. hamatus* and *T. bernacchii*) and compared our results with those obtained for temperate-water fish (*C. auratus* and *A. anguilla* taken from Lake Trasimeno, Perugia, Italy). The membranes of Antarctic fish were richer in glycerophospholipid (especially phosphatidylethanolamine), whereas the membranes of temperate-water fish were richer in sphingomyelin. Unsaturated fatty acids were particularly abundant in Antarctic fish: *C. hamatus* had long-chain unsaturated fatty acid (especially C22:6 ω-3), whereas *T. bernacchii* had shorter unsaturated fatty acyl chains (c16:1, ω-7). On the other hand, *C. auratus* and *A. anguilla* were particularly rich in C16:0, which constituted more than one-half of the total fatty acid. Plasma lipids (both phospholipid and cholesterol) were much more abundant in temperate-water fish. The differences in phospholipid content were mainly due to choline glycerolipids. Measures of membrane fluidity inferred from the fluorescence anisotropy of DPH indicated that the membranes from Antarctic fish were more fluid at any measured temperature than those obtained from fish living in temperate waters. The ability to live in a very cold environment has therefore been achieved by the two Antarctic species tested in this paper by different strategies, but with the same results on fluidity.

Keywords Antarctic fish · Cholesterol · Glycerophospholipid · Fatty acid · Membrane fluidity

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The Antarctic Sea habitat (southern Ross Sea) is characterized by a very cold and constant temperature (−1.87°C), as the result of the equilibrium between ice and seawater. Nototheniidae are the chief fish in the Antarctic Sea and are characterized by an extreme stenothermy; they die if the water temperature exceeds 4°C (Somero and DeVries 1967). The ecological adaptations of Antarctic fish are important for understanding complex ecosystems such as those present at low latitudes. *Chionodraco hamatus* and *Trematomus bernacchii* (Hureau et al. 1977) belong to the same suborder (Notothenioidei) and represent the highest specialization to cold adaptation (Eastman and Eakiun 2000).

The solubility of oxygen in seawater is inversely proportional to the temperature and therefore the concentration of oxygen in the Antarctic Sea is high. Moreover, the metabolic demand of fish living at cold temperatures is low (Sidell and O'Brien 2006). These facts may decrease the importance of hemoglobin in Antarctic fish, which have less hemoglobin than fish living in warmer waters. In particular, *C. hamatus* (family Channichthyidae) is devoid of hemoglobin. The blood of this fish is white, whereas the blood of *T. bernacchii* (family Nototheniidae) is red, even

though its hemoglobin content is low compared to that found in fish living in warmer waters (Ruud 1954).

Lipids, especially phospholipid, are very important for determining membrane properties, such as fluidity (Shinitzky 1984a). Therefore, it is reasonable that fish living in very cold waters may have a different lipid pattern from those living in temperate waters. The most consistent biochemical response of poikilothermic organisms to a cold environment is an increase in fatty acid unsaturation of both membrane and depot lipids (Cossins 1977a, b; Hazel 1990; Hazel and Williams 1990; Logue et al. 2000). The composition of membrane lipid may be affected both by adaptive mechanisms in the long or the short term and by genetic differences among various species. The lipid composition of red blood cell membranes (ghost-like membranes) of the hemoglobinless teleost, *C. hamatus*, and that of the red-blooded member of the same Antarctic suborder (*T. bernacchii*) were studied for the first time and were compared with *A. anguilla* and *C. auratus*, teleosts of temperate zones. On the other hand, plasma lipid may be important to determine the overall blood viscosity in *C. hamatus*, which is devoid of hemoglobin. For this reason, we also investigated the plasma lipid composition.

Materials and Methods

Collection of Fish Blood

Antarctic fish (*Chionodraco hamatus*, *Trematomus bernacchii*) blood was obtained from the Italian Antarctic base (Terranova Bay, southern Sea of Ross). Blood samples were centrifuged (800*g* × 10 min) to separate cells from plasma, frozen at −80°C, and shipped to our laboratory. Lake Trasimeno (Italy) fish (*Anguilla anguilla*, *Carassius auratus*) blood was taken during the month of July, centrifuged (800*g* × 10 min) to separate red cells from plasma. Red blood cells and plasma were immediately frozen at −80°C.

Preparation of Ghost-Like Membranes

Erythrocyte membranes were prepared as described (Steck and Kant 1974): cells (corresponding to 1 ml of blood) were mixed (1:200, v/v) with 5 mM phosphate buffer (pH 8.0) also containing 2% (w/v) EDTA. Red blood cell ghost-like particles were collected by centrifugation (300*g* × 10 min). They were then washed with 20 ml of 8 mM phosphate buffer (pH 8) also containing 150 mM NaCl. Ghosts were recovered by centrifugation at 3500*g* for 10 min. The washing procedure was repeated twice. The pellet was suspended in 40 ml of 5 mM phosphate buffer (pH 8) and was centrifuged at 35,000*g* for 10 min. Collected membranes were suspended in 1 ml of 0.32 M sucrose + 2 mM Hepes, pH 7.

Fluorescence Anisotropy Determination

DPH (1,6-diphenyl–1,3,5-hexatriene; Sigma Chemical Company) was dissolved in tetrahydrofuran to have a 2 mM solution that was added to ghost-like preparations. The molar ratio between phospholipid and the probe was ≤200:1. Samples were kept in the dark for 2 h before use. They were then poured in a cuvette already containing 0.32 M sucrose + 2 mM Hepes adjusted to pH 7 with HCl. Fluorescence was measured with a Shimadzu F5000 spectrophotofluorimeter equipped with a polarizer (Shimadzu). Measurements were performed at 365 nm (excitation) and at 430 nm (emission). Temperature was varied from 4°C to 40°C with a thermostatic apparatus. The actual temperature was assessed through a probe inserted in the sample with an accuracy of ±0.1°C. Fluorescence anisotropy was calculated by the equation of Shinitzky and Barenholz (1978).

Lipid Extraction

Lipids were extracted following published procedures (Folch et al. 1957): samples (corresponding to 1 ml of blood) were mixed with 20 vol of chloroform:methanol (2:1, v/v) + 4 vol of 0.9% (w/v) NaCl. The chloroformic phase was collected by centrifugation at 2,000 *g* for 5 min and washed two times with 4 vol of methanol:water (1:1, v/vol) each time. The upper phases were discarded, whereas the organic phase was reduced in volume under a gentle N_2 flux, and the extract was dissolved in 150 µl of chloroform and used for further analyses.

Fatty Acid Methylation

The lipid extract (150 µl) was dried under a gentle nitrogen flux, mixed with 2 ml of 3% H_2SO_4 (w/v) in methanol, and kept at 80°C for 1 h. A few milligrams of BHT (2,6-D-tert-butyl-4-methylphenol) was added to prevent unwanted oxidation. The reaction was stopped by placing the samples in a melting ice bath and by adding 100 µl of water to each sample. The methylated fatty acids formed in the reaction were extracted with 5 ml of *n*-hexane. Samples were then centrifuged at 1,000 *g* for 10 min, dried, dissolved in 30 µl of *n*-hexane, and used for gas-chromatographic analysis.

Gas-Chromatographic Analysis

Samples were analyzed with a Carlo Erba (Milan, Italy) gas chromatograph (HRG 5300 Mega Series) equipped with a 30-m-long (0.53-mm-internal diameter) capillary column (Supelco; Sigma-Aldrich, Milan, Italy). The stationary

phase was poly-alkylene glycol (PAG). The carrier gas was He (1 ml/min). Samples (1 µl) were then injected. The injector temperature was 230°C and that of the detector was 250°C. The temperature gradient was started at 150°C and increased up to 190°C in 40 min.

Eluted fatty acids were identified with pure reference standards and results are expressed as the percentage of each fatty acid/total fatty acid.

Phospholipid Separation and Analysis

Lipid extract aliquots were chromatographed on TLC plates (TLC aluminum sheets, Silica Gel 60, 20 × 20 cm; Merck, Darmstadt, Germany). The bidimensional chromatography was developed first with chloroform:methanol (1.6 M):ammonia (35:15:2.5, v/v) and then with chloroform:methanol:acetone:acetic acid:water (35:10:15:7.5:3.75, v/v) (Horrocks 1968).

Dried plates were colored with iodine vapors. Lipid classes were identified by a comparison with chromatographic plates run with pure reference standards: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, cardiolipin, and phosphatidic acid (all produced by Sigma Chemical Co). The silica corresponding to phospholipid classes was scraped off the plate and lipid determined as phosphate after digestion with perchloric acid.

Cholesterol Determination

Folch lipid extracts were dried under a gentle N_2 flux and suspended in 2 ml of 3.58 mM *o*-phthalaldehyde in acetic acid. Concentrated sulfuric acid (1 ml) was then added and samples were stirred for 10 min at room temperature. Absorbance was then determined with a spectrophotometer (Cary 50; Varian) at 550 nm (Rudel and Morris 1973). Cholesterol concentration was determined with cholesterol pure reference standards.

Determination of Lipid Phosphorus

Lipid phosphorus was determined as described by Bartlett (1959): briefly, dried lipid extracts were digested for 1 h with 0.7 ml of 70% (w/v) perchloric acid at 180°C, and phosphorus determined as described (Fiske and Subbarow 1929).

Protein Determination

Protein was determined as described by Lowry et al. (1951).

Results

We determined total lipid phosphorus and cholesterol on a protein basis in isolated ghost-like membranes and in blood plasma (Tables 1 and 2). Lipid phosphorus was much higher in Antarctic fish than in temperate-water fish, irrespective of the examined species. On the other hand, cholesterol seemed to depend more on the species than on the environmental temperature.

The values of the same parameters were much different in plasma, where cholesterol was about 4–8 times and phospholipid 10–20 times higher in temperate-water fish than in Antarctic species (Table 2). Therefore, the differences between plasma lipids were very large and appeared to be related to environmental conditions.

To further investigate the phospholipid composition of Antarctic and temperate-water fish species, we separated phospholipid classes from ghost-like membrane lipid extracts (Table 3). Comparing Tables 1 and 3, we observed

Table 1 Total lipid phosphorus and cholesterol in erythrocyte ghost-like membranes of four species of fish: nmol mg^{-1} protein ± SE

	C. hamatus	*T. bernacchii*	*C. auratus*	*A. anguilla*
Total lipid phosphorus	420 ± 23 (a)	225 ± 12 (b)	78 ± 5 (c)	145 ± 8 (d)
Cholesterol	253 ± 11 (a)	64 ± 2 (b)	24 ± 1 (c)	118 ± 64 (d)

Note: Differences among species for each parameter were calculated by one-way ANOVA followed by Scheffé post hoc test at $P = 0.05$ level. Values belonging to the same subset are followed by the same letter in parentheses

Table 2 Total lipid phosphorus and cholesterol in the plasma of four species of fish: nmol mg^{-1} protein ± SE

	C. hamatus	*T. bernacchii*	*C. auratus*	*A. anguilla*
Total lipid phosphorus	25 ± 1 (a)	20 ± 1 (a)	479 ± 31 (b)	538 ± 30 (b)
Cholesterol	69 ± 3 (a)	69 ± 2 (a)	391 ± 17 (b)	588 ± 18 (c)

Note: Differences among species for each parameter were calculated by one-way ANOVA followed by Scheffé post hoc test at $P = 0.05$ level. Values belonging to the same subset are followed by the same letter in parentheses

that the higher content of lipid phosphorus in Antarctic fish was due to phosphatidylethanolamine and, to a lesser extent, to phosphatidylcholine (Table 3). The only phospholipid lower in Antarctic fish was sphingomyelin. This was in broad agreement with data obtained by others (Stornelli et al. 1998), who compared intestinal mucosa obtained from Antarctic fish and from *A. anguilla*. The plasma phospholipids of temperate-water fish were mainly choline lipids. These were less represented in Antarctic fish. The trend of the differences in blood plasma was therefore roughly opposite to that found in ghost-like membranes (Table 4).

We also measured the fatty acid composition of ghost-like particle total phospholipid (Table 5). Palmitic acid was the most abundant in temperate-water fish lipids, whereas Antarctic species had a larger amount of unsaturated fatty acid. However, the two Antarctic fish species examined by us differed largely from one another. *C. hamatus* showed a very large amount of 22:6 ω-3, practically absent in other species. Moreover, C. *hamatus* possessed large amounts of long-chain unsaturated fatty acids. On the other hand, *T. bernacchii* was rich in 16:1 ω-7 and 16:2 ω-3. The ω-6 species were absent in Antarctic fish and in *A. anguilla*. Only small proportions of 18:2, ω-6 and of 18:3 ω-6 were found in *C. auratus*.

Lipid and protein composition is known to affect membrane fluidity (Shinitzky 1984b). We measured the fluorescence anisotropy of diphenylhexatriene (DPH) in ghost-like membranes as an indication of fluidity (Shinitzky and Barenholz 1978). In our hands, the curve of fluorescence anisotropy versus temperature (5–40°C) fit well into a straight-line model (r^2, 0.99–0.95) in all examined samples (Fig. 1). The slope of the lines was higher for Antarctic fish, the contrary being true for the intercept.

We calculated the parameters of the straight-line best-fitting experimental data and obtained the following correlation equations (fa, fluorescence anisotropy; CI, 0.95 confidence interval; T, temperature): *C. hamatus*, fa = 0.239 (CI, 0.237–0.241) − 0.003T; *T. bernacchii*, fa = 0.235 (CI, 0.231–0.238) − 0.003T; *C. auratus*, fa = 0.249 (CI, 0.246–0.251) − 0.002T; and *A. anguilla*, fa = 0.245 (CI, 0.242–0.247) − 0.002 T.

At all examined temperatures, fluorescence anisotropy was lower in Antarctic than in temperate-water fish; this meant that the temperature had to be lower in Antarctic fish to have the same fluorescence anisotropy and it may represent an adaptation of biological membranes to low temperatures. In solving the above-reported equations, it may be noted that the fluidity of Antarctic fish membranes

Table 3 Phospholipid classes in erythrocyte ghost-like membrane lipids in four species of fish: percentage of total lipid phosphorus in each lipid class (mean of four determinations) ± SE

	C. hamatus	*T. bernacchii*	*C. auratus*	*A. anguilla*
Lysophosphatidylcholine	6 ± 1.4 (a)	4 ± 0.3 (b)	9 ± 0.5 (c)	6 ± 0.6 (a)
Phosphtidylcholine	45 ± 5.0 (a)	48 ± 2.9 (a)	29 ± 1.5 (b)	45 ± 5.6 (a)
Phosphatidylethanolamine	26 ± 4.3 (a)	28 ± 2.2 (a)	10 ± 1.4 (b)	8 ± 1.2 (b)
Sphingomyelin	4 ± 1.1 (a)	3 ± 0.5 (a)	27 ± 3.4 (b)	27 ± 5.0 (b)
Phosphatidylserine	10 ± 1.1 (a)	9 ± 0.4 (a)	13 ± 1.4 (a)	11 ± 2.4 (a)
Phosphatidylinositol	6 ± 0.7 (a)	5 ± 0.3 (a)	7 ± 1.0 (a)	6 ± 0.4 (a)

Note: Differences among species for each parameter were calculated by one-way ANOVA followed by Scheffé post hoc test at $P = 0.05$ level. Values belonging to the same subset are followed by the same letter in parentheses

Table 4 Phospholipid classes in blood plasma in four species of fish: percentage of total lipid phosphorus in each lipid class (mean of four determinations) ± SE

	C. hamatus	*T. bernacchii*	*C. auratus*	*A. anguilla*
Lysophosphatidylcholine	6 ± 1.0 (a,b)	4 ± 1.1 (a)	3 ± 0.6 (a)	7 ± 1.8 (b)
Phosphatidylcholine	53 ± 6.3 (a)	50 ± 3.7 (a)	86 ± 5.6 (b)	64 ± 9.3 (a)
Phosphatidylethanolamine	12 ± 1.5 (a)	8 ± 1.3 (a)	4 ± 0.9 (b)	5 ± 0.2 (b)
Sphingomyelin	23 ± 3.7 (a,b)	28 ± 4.0 (b)	2 ± 0.4 (c)	17 ± 2.3 (a)
Phosphatidylserine	1 ± 0.1 (a,b)	5 ± 0.9 (c)	2 ± 0.1 (b)	0.3 ± 0.1 (a)
Phosphatidylinositol	0.2 ± 0.1 (a)	2 ± 0.3 (b)	0.1 ± 0.1 (a)	0.1 ± 0.1 (a)

Note: Differences among species for each parameter were calculated by one-way ANOVA followed by Scheffé post hoc test at $P = 0.05$ level. Values belonging to the same subset are followed by the same letter in parentheses

Table 5 Fatty acid distribution in the ghost phospholipids of four species of fish: percentage of each fatty acid (mean of four determinations) ± SE

	C. hamatus	*T. bernacchii*	*C. auratus*	*A. anguilla*
14:0	6.5 ± 0.5 (a)	11.9 ± 0.7 (b)	9.0 ± 1.0 (a,b)	13.7 ± 0.7 (b)
14:1, ω-3	16.0 ± 1.1 (a)	–	4.6 ± 0.8 (a,b)	13.8 ± 3.8 (a)
16:0	8.6 ± 0.6 (a)	32.1 ± 0.5 (b)	67.5 ± 3.5 (c)	56.3 ± 0.7 (d)
16:1, ω-7	4.2 ± 0.8 (a)	24.6 ± 1.1 (b)	10.5 ± 3.5 (a)	4.6 ± 0.6 (a)
16:2, ω-3	–	10.3 ± 1.4 (a)	–	1.1 ± 0.1 (b)
18:0	4.1 ± 0.1 (a)	14.2 ± 1.7 (b)	1.7 ± 0.2 (a)	4.5 ± 0.8 (a)
18:1, ω-9	6.6 ± 0.8 (a,b)	8.2 ± 1.1 (b)	1.5 ± 0.5 (b,c)	2.5 ± 0.5 (b)
18:2, ω-6	–	–	6.3 ± 2.1	–
18:3, ω-6	–	–	1.1 ± 0.2	–
20:1, ω-9	13.9 ± 1.1 (a)	–	0.8 ± 0.3 (b)	–
20:5, ω-3	11.3 ± 0.7	–	–	–
22:1, ω-11	9.6 ± 0.3	–	–	–
22:6, ω-3	20.5 ± 1.5	–	–	–
Average double-bonds per fatty acyl chain[a]	2.30	0.53	0.32	0.23

Note: Differences among species for each parameter were calculated by one-way ANOVA followed by Scheffé post hoc test at $P = 0.05$ level. Values belonging to the same subset are followed by the same letter in parentheses

[a] The sum of the percentage of each unsaturated fatty acid × number of double bonds/100

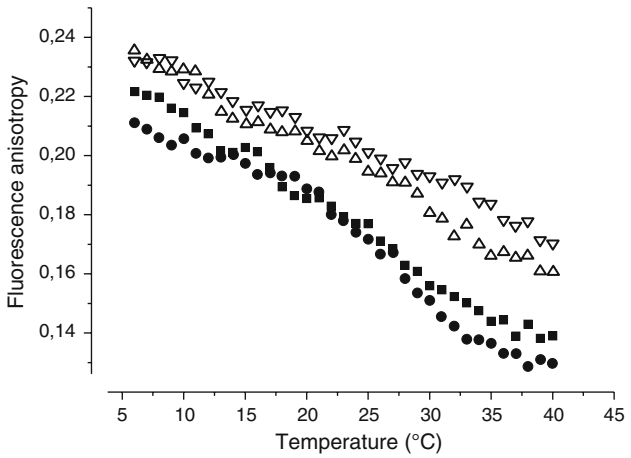

Fig. 1 Fluorescence anisotropy (fa) of diphenylhexatriene scan from 5°C to 40°C. ●-●, *T. bernacchii*; ■-■, *C. hamatus*; △-△, *C. auratus*; △-△, *A. Anguilla*. We calculated the parameters of the straight-line (two samples for each fish) best-fitting experimental data and obtained the following correlation equations (T = temperature): *C. hamatus*, fa = 0.239 − 0.003T; *T. bernachii*, fa = 0.235 − 0.003T; *C. auratus*, fa = 0.249 − 0.002T; and *A. anguilla*, fa = 0.245 − 0.002T

(extrapolated to −1.8°C) was similar to that of temperate-water fish at about 15–20°C.

Discussion

Antarctic fish species live at a constant temperature of −1.8°C (southern Ross Sea) (Somero and DeVries 1967); this requires an adaptation to cold that, theoretically, might be achieved by changing the body composition in a number of ways (variations of lipid classes, cholesterol, fatty acid instauration, and proteins, decrease in hematocrit, etc.) (McDonald et al. 1987). This may explain why fish belonging to different species have developed dissimilar biological responses to solve the common problem of living at low environmental temperatures.

We utilized two Antarctic fish teleost species, namely, *C. hamatus* and *T. bernacchii*. In these species, hemoglobin is absent and reduced, respectively (Andriashev 1987; Ruud 1954); moreover, some other adaptations to a cold environment are present, such as cutaneous respiration, enlarged blood vessels, and greater amounts of fat. The blood of Antarctic fish contains few cells and membranes that are deemed important to decrease blood fluidity (McDonald and Wells 1991). However, this material can be separated from the blood following the procedure utilized for erythrocyte ghost preparation. For this reason, we use the phrase "ghost-like" throughout this work. The literature on Antarctic fish blood lipid is rather limited for obvious reasons; previously published work has mainly been done on organs or tissues other than blood (Logue et al. 2000; Londraville and Sidell 1995; Somero and DeVries 1967; Stornelli et al. 1998).

Differences in membrane lipid composition are present among the species we used, as expected (Table 1). Phospholipid phosphorus is higher in Antarctic fish. Differences in other membrane components, such as cholesterol, depend more on the species than on the adaptation to

a cold environment. The higher phospholipid level in Antarctic fish ghost-like membranes is due primarily to phosphatidylethanolamine. The data appear to be in agreement with those reported by others on intestinal mucosa of *T. bernacchii* and *A. anguilla* (Stornelli et al. 1998). These data fit well with the reported viscosity behavior of different phospholipid classes (Shinitzky 1984a). The shape of molecules may affect the ordering of membrane lipids. A conical molecule (phosphatidylethanolamine) would destabilize the membrane and make it more fluid. Phosphatidylcholine is a more cylindrical molecule and would destabilize membranes less than phosphatidylethanolamine (Gruner 1985; Logue et al. 2000). The fatty acid composition of membrane lipid is important for membrane fluidity, mainly because unsaturated fatty acids decrease the order of the membrane lipids, thereby decreasing viscosity (Shinitzky 1984a).

Our results may be interesting from this point of view because the ghost-like particles of the two Antarctic species present a very different fatty acid composition. This means that the adaptation to a cold environment is obtained in two different ways by *T. bernacchii* and *C. hamatus*. *C. hamatus* has much more unsaturated fatty acid than any other species examined in this study. The mean double-bond number for each fatty acid is 2.30, whereas it is 0.53 for *T. bernacchii* and still lower for *C. auratus* and *A. anguilla* (0.32 and 0.23, respectively) (Table 4). It is interesting to observe that the very high level of instauration present in *C. hamatus* fatty acids is due to long chain fatty acids (C:20–C:22), absent in *T. bernacchii*. On the other hand, *T. bernacchii* does not have fatty acids longer than C:18, and instaurations are chiefly present in C:16 and C:18. The main fatty acid in temperate species is palmitate, which, alone, accounts for more than one-half of the total fatty acid. Therefore, we observe a greater number of instaurations in Antarctic fish, but the distribution of double-bonds is different and the long-chain unsaturated fatty acid might compensate for the less unsaturated, shorter-chain lipids in ghost-like membranes of *T. bernacchii*.

The viscosity of plasma may be important to determine overall blood viscosity in Antarctic fish because of the low hematocrit (McDonald and Wells 1991), the presence of antifreeze proteins (Harding et al. 2003), and the high content of triglyceride-rich lipoproteins (Lund and Sidell 1992). In this study, we found very low amounts of plasma phospholipid in Antarctic fish compared to fish living in temperate waters. This may entail a decreased viscosity (McDonald and Wells 1991), which could be useful to compensate the viscosity increase due to triacylglycerol and to the antifreeze protein necessary for survival at very low temperatures.

Membrane viscosity is determined by many factors and it is difficult to draw a priori conclusions from compositional data. Therefore, we thought it necessary to experimentally evaluate the viscosity of membranes. To do this, we used the DPH anisotropy fluorescence, assumed to be a parameter correlating with membrane fluidity. DPH probably is the most widely used reagent for this type of work and it probes the core of the bilayer. With pure lipids its fluorescence anisotropy shows a transition point; in natural membranes, a definite transition point cannot be observed because these biological structures contain a mixture of different lipids (Arienti et al. 1996; Carlini et al. 1997).

The fluorescence anisotropy of Antarctic fish is lower, at any given temperature, than that of temperate fish species; this is in agreement with the previously reported data on brain synaptic membranes (Logue et al. 2000). Therefore, the ghost-like membranes of various fish species have similar viscosity properties if data are extrapolated at their environmental temperatures (that is, −1.8°C for Antarctic species and 15–20° for temperate-water fish). A direct comparison is not possible because Antarctic fish would not survive at 15−20°C, and on the other hand, *C. auratus* and *A. anguilla* would not survive at −1.8°C. Comparing results obtained by measuring DPH anisotropy and data on membrane composition, we conclude that membrane viscosity is similar in both Antarctic species but, also, that this similarity is achieved by means of different strategies.

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