Vol. 151: 205–218, 1997 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published May 22

Adaptations of the Antarctic silverfish *Pleuragramma antarcticum* (Pisces: Nototheniidae) to pelagic life in high-Antarctic waters

Andreas P. A. Wöhrmann, Wilhelm Hagen*, Andreas Kunzmann

Institut für Polarökologie, Wischhofstr. 1–3, Gebäude 12, University of Kiel, D-24148 Kiel, Germany

ABSTRACT: Most fishes in Antarctic waters belong to the suborder Notothenioidei, highly developed perciform fishes which have been able to occupy not only a variety of benthic but also pelagic niches in Antarctic waters. One of the rare truly pelagic fish species, *Pleuragramma antarcticum*, plays a pivotal role in high-Antarctic food webs, due to its exceptional abundance. To investigate the life history of *P. antarcticum* more than 16 000 specimens were collected in the Weddell Sea during various cruises with RV 'Polarstern'. Apart from the more general life cycle adaptations with respect to reproduction, migrations, and feeding behaviour, *P. antarcticum* has developed a number of specific biochemical and physiological adaptations to cope with the environmental conditions in these permanently cold and highly seasonal Antarctic waters. During its second summer *P. antarcticum* starts to accumulate large lipid deposits, mainly in the form of triacylglycerols. These low-density compounds provide the species with hydrostatic lift, an important factor for a pelagic fish without a swim bladder. The lipid stores may also serve as energy reserves. Highly polar brain gangliosides suggest wide-ranging neurophysiological adaptations to ensure proper functioning of the nervous system in icy waters. To avoid freezing in the presence of frazil ice *P. antarcticum* contains efficient antifreeze glycoproteins. A newly discovered glycoprotein acts as an additional antifreeze agent. Although pelagic, adult *P. antarcticum* are rather sluggish, which is indicated by the small total gill area as well as blood physiological characteristics. Such behaviour diminishes routine energy costs. Blood viscosity is reduced and at least 2 major haemoglobins are found. Provided that these haemoglobins are functionally different, they indicate a strong relationship between physiological and biochemical adaptations of the oxygen transport system and life style. *P. antarcticum* represents a prime example of the complexity of adaptations necessary to thrive in the pelagic realm of Antarctic shelf waters, a niche largely unoccupied by other fish species.

KEY WORDS: High-Antarctic · Notothenioid fish · *Pleuragramma antarcticum* · Life history · Energetics · Lipids · Gangliosides · Antifreeze glycoproteins · Gill · Haemoglobins

INTRODUCTION

High-Antarctic shelf waters are characterized by particularly low and constant temperatures between −1.6 and −2.1°C, high oxygen levels of more than 95% saturation (Hellmer & Bersch 1985), and a pronounced seasonality in both the ice regime and in pelagic production (Clarke 1988). In addition, hydrographic components such as a complex current system, ice drift, summer stratification due to melting processes and a highly specific distribution of water masses on the shelf, all strongly affect the life cycle of fish in the Weddell Sea (Hubold 1991). While temperature and production decrease towards the south, stability of temperature and predictability of hydrographic features increase in the permanent pack-ice zone surrounding the continent (Hempel 1990). This stability is important for adaptive processes (Clarke 1990).

The originally benthic notothenioids have evolved in Antarctic waters since the Tertiary (Andersen 1984). Their comprehensive set of morphological, physiological and life history adaptations to a permanently ice-covered, highly seasonal ecosystem has enabled them to clearly dominate the high-Antarctic fish fauna (Andriashev 1987, Macdonald et al. 1987, Eastman 1993). Today we find a high degree of stenothermy

*Addressee for correspondence. E-mail: whagen@awi-bremerhaven.de

and endemism among the notothenioids. In high-Antarctic waters such as the Weddell Sea, more than 70% of the species (Hubold 1991, 1992) and 98% of all demersal fish belong to one perciform suborder, the Notothenioidei (Ekau 1988). As confirmed for the major high-Antarctic shelf regions, the Weddell Sea (Hubold & Ekau 1987), the Ross Sea (DeWitt 1970) and the Prydz Bay area (Williams 1985), *Pleuragramma antarcticum* is the only pelagic fish species which is present in all sizes throughout the water column. The major stimulus for this change in its mode of life may have been nutritional competition among demersal species, on the one hand, as well as the rich and largely unexploited food sources in the pelagic habitat, on the other hand. To successfully occupy this pelagic niche *P. antarcticum* had to combine the more general adaptations of all notothenioids with specializations necessary to survive in the water column.

It is well known that lipids play an extraordinary role in high latitude ecosystems (e.g Clarke 1983, Falk-Petersen et al. 1990, Hagen 1996). In Antarctic fishes lipids may be used as energy reserves to buffer seasonal differences in food availability. These low-density compounds also provide considerable hydrostatic lift. The latter is of major importance for a migrating and pelagic species such as *Pleuragramma antarcticum*, which lacks a swim bladder to regulate its buoyancy (Eastman & DeVries 1982, Clarke et al. 1984, Friedrich & Hagen 1994). In addition, larger amounts of highly unsaturated phospholipid classes increase the fluidity and hence ensure the proper functioning of biomembranes at freezing temperatures (Dey et al. 1993, Cossins 1994, Clarke & Johnston 1996). Exposure to such low ambient temperatures also requires neurophysiological adjustments (Macdonald et al. 1987, Maggio et al. 1990, Montgomery et al. 1990). Alterations in the composition of gangliosides may contribute to the functioning of the nervous system and brain in icy waters (Becker et al. 1995).

An important physiological adaptation in a freezing environment is the presence of macromolecular antifreeze compounds, which have been detected in the body fluids of most Antarctic fishes. Antifreezes are glycopeptides or peptides which evolved in a number of unrelated lineages of cold water teleosts including notothenioids, zoarcids, cottids, gadids, pleuronectids, clupeids and osmerids (DeVries 1988, Cheng & DeVries 1991, Eastman 1993, Wöhrmann 1993). However, no antifreeze agents were found in *Pleuragramma antarcticum* during earlier investigations (Haschemeyer & Jannasch 1983).

The respiratory system of fishes, particularly their haemoglobins, constitutes an excellent model for studies on environmental adaptations (Powers 1980). These adaptations may occur at the morphological, physiological or molecular level. Gill morphometrics reflect oxygen requirements, indicate activity levels, and permit conclusions about the mode of life of a particular species. Of special interest in this respect are gill surface area, water-blood diffusion distance and the general gill structure (Hughes 1984). Most fish species adjust certain haematological parameters according to environmental conditions (Val et al. 1990) Respiratory blood properties, especially oxygen carrying capacity and oxygen affinity, respond to evolutionary selective pressure (Wells et al. 1980, 1989)

It is the purpose of this article to compile our results about the adaptations of *Pleuragramma antarcticum* to pelagic life in the extremely cold shelf waters of the Weddell Sea. This large marine ecosystem has been the scene of studies over several years to better understand the biology of this dominant fish species in a unique marine environment. Consequently, various biochemical, physiological and ecological aspects of the biology of *P. antarcticum* were investigated in detail, which reflect adjustments to the pelagic mode of life and may provide information on the evolution of this teleost.

MATERIALS AND METHODS

Specimens of *Pleuragramma antarcticum* were caught with various nets such as the bongo (fish larvae), krill trawl, Agassiz trawl, benthopelagic trawl and bottom trawl (Hubold 1992). More than 14 000 larvae, postlarvae and juveniles and more than 2000 adults were collected in the Weddell Sea region by RV 'Polarstern' during the following expeditions: ANT III/3 (Jan–Mar 1985), ANT V/3 (Oct–Dec 1986), ANT VII/4 (Jan–Mar 1989), ANT IX/3 (Jan–Mar 1991) and ANT X/3 (Mar–May 1992) (Fig. 1). The standard length (SL) of the specimens ranged from 8 mm to about 80 mm for larvae, postlarvae, and juveniles and up to 255 mm for adults. Exact positions and depths of all hauls are given in the respective cruise reports (Ekau & Hubold 1985, Ekau et al. 1987, Hureau et al. 1990, Wöhrmann & Zimmermann 1992, di Prisco et al. 1993).

The fish specimens were usually sorted, counted, measured, weighed and frozen at -30°C or fixed in 4% buffered formalin immediately after the catch came on board. Gills were fixed in glutaraldehyde and osmium tetroxide (Kunzmann 1990). Samples of muscle, brain, blood and liver (antifreeze) and samples for more detailed lipid analyses were deep-frozen at -80°C.

Analysis of total lipid content and composition. In the home laboratory the specimens were lyophilized for 48 h and their dry weight determined. The lipid fraction was extracted with chloroform/methanol [2:1, v:v, plus 0.01% butylhydroxytoluene (BHT) as anti-

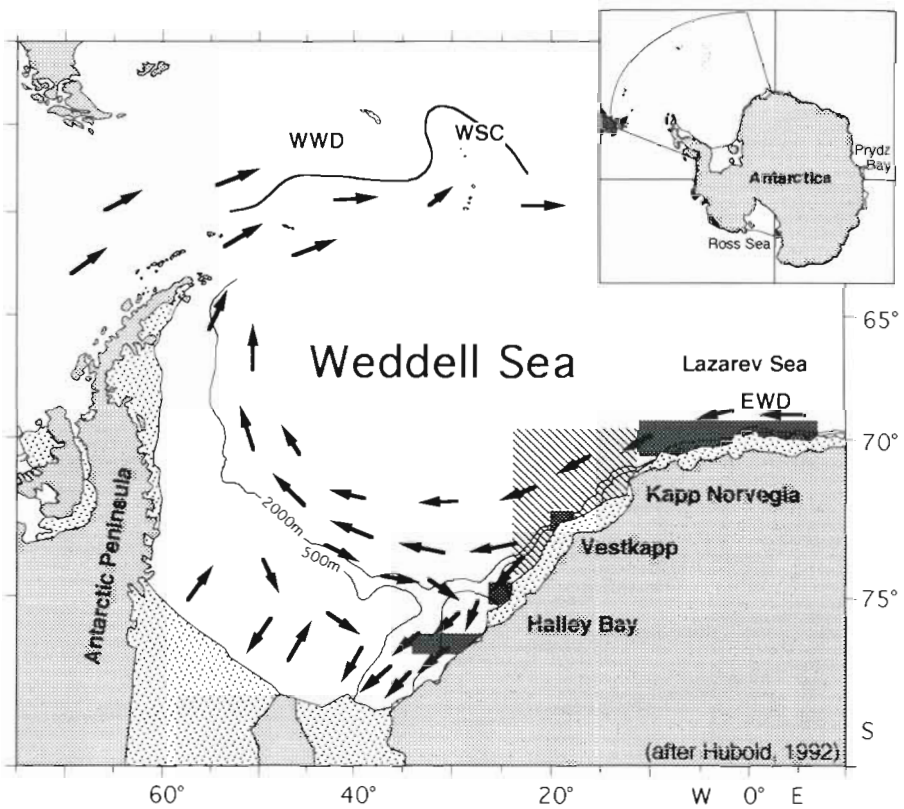

Fig. 1 Investigation areas (shaded boxes) during the various 'Polarstern' expeditions in the Weddell Sea and the Lazarev Sea. Currents (arrows) according to Hubold (1992). WSC = Weddell Scotia Confluence; WWD = West Wind Drift; EWD = East Wind Drift

oxidant] and the total lipid content (expressed in percent of dry weight, %DW) was determined gravimetrically according to Folch et al. (1957). The lipid class composition (phospholipids, triacylglycerols etc.) was analyzed according to Fraser et al. (1985) by thin-layer chromatography/flame ionization detection with an IATROSCAN. Different standard mixtures were prepared for calibration which approximated the lipid class composition of the samples analyzed. For details see Hagen (1988) and Friedrich & Hagen (1994).

Extraction of gangliosides. The whole brain was removed from the anaesthezised fish, weighed and deep-frozen at −80°C. The extraction and purification of gangliosides was performed according to Svennerholm & Fredman (1980). Ganglioside content and composition were examined in the combined aqueous phases as well as in the organic phase. Quantitative estimates of sialic acid were carried out according to Svennerholm (1957) as modified by Miettinen & Takki-Lukkainen (1959) Aliquots of purified ganglioside mixtures were separated by thin-layer chromatography on pre-coated silica gel plates (high-performance thin-layer chromatography, HPTLC, 0.2 mm, Merck). The ganglioside spots were visualized with resorcinol reagent (Svennerholm 1957) and identified in comparison to co-chromatographed known standards.

Two-dimensional HPTLC (2-D-HPTLC) was performed in order to separate alkali-labile from alkali-stable gangliosides. Fractionation of the ganglioside mixtures was attained by modifying the methods of Ledeen et al. (1981), Ghidoni et al. (1984) and Sonnino et al. (1984).

Extraction of antifreeze compounds. Fish or tissues were homogenized in 50% ethanol. Ethanol was removed by evaporation under vacuum. After centrifugation the supernatant was pre-treated with 10% trichloroacetic acid (TCA) at 0°C and then centrifuged at 5000 × *g*. Removal of TCA by extensive dialysis yields a preparation greatly enriched in antifreeze glycoproteins (AFGP). The eluates were concentrated by ultrafiltration and subsequently freeze-dried. Samples were passed over Bio-Gel TSK DEAE 5PW (Bio-Rad) ion exchange resin (column 75 × 7.5 mm i.d.) equilibrated with 20 mM Tris-HCl, pH 9.5 and a NaCl gradient (0 to 0.8 M). The freeze-dried glycoprotein fractions were desalted by extensive dialysis. Finally, the samples were resolved by reversed-phase HPLC using a C4 column (5 µm, Bischoff) with a 0.1% trifluoroacetic acid/acetonitrile gradient (Wöhrmann & Haselbeck 1992).

The amino acid analysis was accomplished on an 'Applied Biosystems' model 420A derivatizer-analyzer system. The carbohydrate residues were analyzed

using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) or on dot blots. Circular dichroism (CD) spectra were obtained with a Jasco J-600 spectropolarimeter with a 0.1 mm cell and a temperature-controlled sample compartment. Molecular weight was determined by polyacrylamide gel electrophoresis, plasma desorption mass spectrometry and laser light scattering. Solutions of antifreeze glycoproteins were analyzed for activity by differential scanning calorimetry (Perkin-Elmer DSC-7). For detailed references of all procedures see Wöhrmann (1993, 1995, 1996).

Analysis of blood samples. Blood samples were drawn from the caudal vein of unanaesthetized specimens by means of heparinized syringes. The general blood parameters were investigated immediately on board the 'Polarstern'. They comprised pH, pO_2 and pCO_2 (partial pressure of oxygen and carbon dioxide), RBC (number of red blood cells), Hct (haematocrit), Hb (haemoglobin concentration), MCH (mean cellular haemoglobin) and MCHC (mean corpuscular haemoglobin concentration). Detailed descriptions of all methods are given in Kunzmann (1991).

Structural and functional studies were carried out on frozen blood cells at the Institute of Protein Biochemistry and Enzymology (IBPE) in Naples, Italy. Haemolysates were prepared according to previously described methods (D'Avino & di Prisco 1988) with 20 mM Tris-HCl at pH 8.0. For the detection of multiple haemoglobin components electrophoretic analyses of haemolysates were carried out on cellulose acetate in Tris-glycine at pH 9.0 with a chamber from 'Gelman Sciences'. Assessment of globin molecular weight was achieved by means of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, which was run on a 'Bio-Rad' system PROTEAN II together with a standard of known molecular weight. Both procedures were described in detail by D'Avino & di Prisco (1988).

RESULTS

Lipids

Postlarvae (age class, AC = 0; 15 to 20 mm) have a low lipid content (12 to 15% DW), which consists mainly of structural lipids, e.g phospholipids (Figs. 2 & 3). This is a typical pattern in early pelagic life stages, where energy is primarily directed towards growth, i.e. protein build-up. During the second year lipids increase drastically and early juveniles (AC 1; 45 to 48 mm) have lipid contents of about 20 to 32% DW. The lipid accumulation curve (Fig. 2) gradually levels off after the second year and older specimens (AC 2; 65 to 70 mm) with clearly visible lipid sacs reach

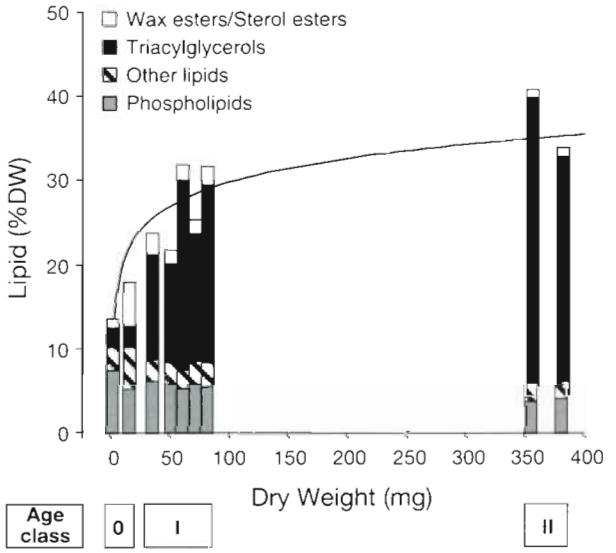

Fig. 2. *Pleuragramma antarcticum*. Total lipid content (%DW = % of dry weight) and lipid class composition as a function of dry weight (age classes 0, I and II)

a lipid content of 33 to 41% DW (Fig 2). The lipid increase in AC 1 and 2 is due to a massive accumulation of triacylglycerols (Fig. 3).

Brain gangliosides

Brain ganglioside contents of notothenioids range from 1622 µg g^{-1} DW in *Trematomus lepidorhinus* to 2099 µg g^{-1} DW in *Dolloidraco longedorsalis*. The content in brains of adult *Pleuragramma antarcticum* is 2085 µg neuraminic acid (NeuAc) per g DW or 4.21 µg NeuAc per g protein (Table 1). The molecular compositions of the individual brain ganglioside fractions

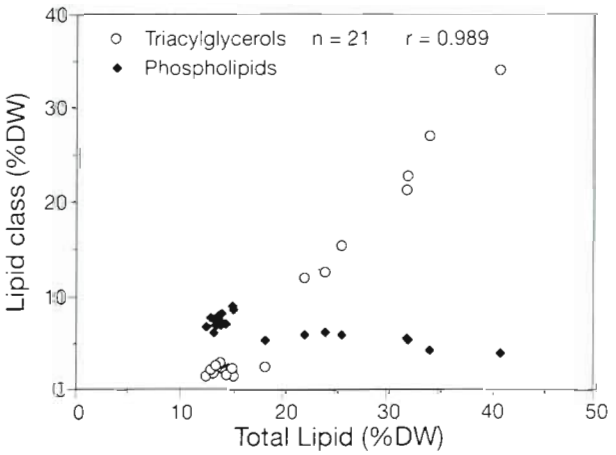

Fig. 3. *Pleuragramma antarcticum*. Lipid classes (%DW) versus total lipid content (%DW). Note the linear accumulation of triacylglycerols (in %DW) with increasing total lipid content

Table 1. Content of brain gangliosides of selected Antarctic fish species from the Weddell Sea (data of 2 eurythermic fish species have been added for comparison). Artedidraconids, bathydraconids and nototheniids are red-blooded notothenioids; channichthyids are white-blooded notothenioids. The content of neuraminic acid (µg Neu-Ac) is given in relation to dry weight (g) and protein (g) of the investigated brain. Alkali-labile gangliosides are given in percent of total gangliosides

Family	Species	Ambient water temp. (°C)	µg Neu-Ac g^{-1} DW	µg Neu-Ac g^{-1} protein	% alkali-labile fractions
Cold stenothermic species (Antarctic notothenioids)					
Nototheniidae	*Pleuragramma antarcticum*	−1.5	2085 ± 132	4.21 ± 0.21	63.60 ± 6.45
	Aethotaxis mitopteryx	−1.0	2034 ± 384	4.32 ± 0.36	
	Pagothenia bernacchii	−1.8	1997 ± 259	3.94 ± 0.48	
	Trematomus nicolai	−1.8	1899 ± 169	3.90 ± 0.42	
	Trematomus lepidorhinus	−1.8	1622 ± 146	3.66 ± 0.43	65.21 ± 7.05
Artedidraconidae	*Dolloidraco longedorsalis*	−1.0	2099 ± 234	4.07 ± 0.34	
	Artedidraco orianae	−1.8	1866 ± 211	3.61 ± 4.01	
Bathydraconidae	*Cygnodraco mawsoni*	−1.8	1874 ± 172	3.79 ± 0.27	
Channichthyidae	*Chionodraco hamatus*	−0.5	1862 ± 176	3.80 ± 0.29	56.45 ± 6.22
	Pagetopsis maculatus	−1.8	1723 ± 274	3.51 ± 0.27	
Eurythermic species					
Cichlidae	*Oreochromis mossambicus*	28	2483 ± 197	4.26 ± 0.37	34.15 ± 2.99
Cyprinidae	*Cyprinus carpio*	22	1607 ± 146	3.16 ± 0.31	38.43 ± 3.28

differ in their number of sialic acid residues and show remarkable differences between the cold-stenothermic notothenioids and warm-adapted fish species. The brains of a number of notothenioids, mostly nototheniids and bathydraconids, even have gangliosides with more than 5 sialic acid residues. The brains of adult *P. antarcticum*, which are exposed to extremely low temperatures in the Eastern Shelf Water, contain the most polar penta- and hexasialogangliosides (1.5%) ever found in a vertebrate brain (Fig. 4). In contrast, the brains of the sub-Antarctic species *Notothenia gibberifrons* and the icefish *Neopagetopsis ionah*, which live in less frigid waters (+0.5°C), do not contain any hexasialogangliosides.

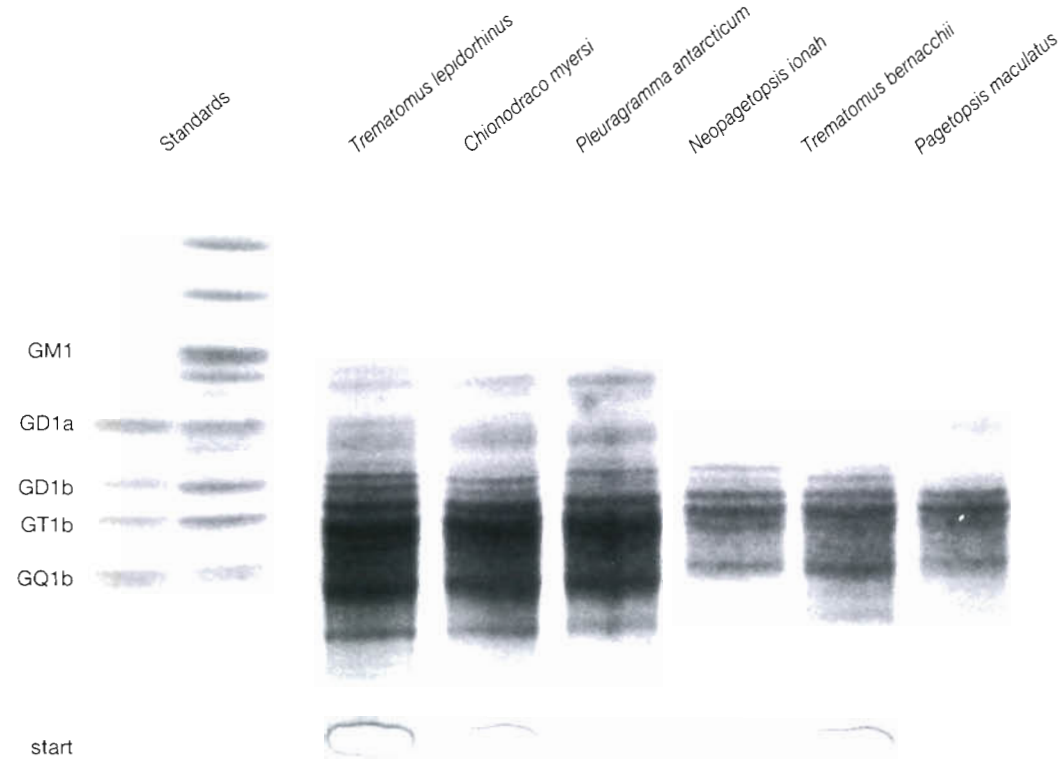

Fig. 4. High-performance thin-layer chromatography (HPTLC) of brain ganglioside extracts from various high-Antarctic notothenioids. A standard mixture and a chicken brain standard were used for the identification of the different fractions. G = ganglioside; M/D/T/Q = content of sialic acids

In a second set of analyses alkali-labile (O-acetylated/lactonized) were separated from alkali-stable gangliosides. The percentage of alkali-labile gangliosides correlates with the ecophysiological state of thermal adaptation (Table 1). Smaller amounts of alkali-labile brain gangliosides (34%) are found in warm-stenothermic species, whereas the brains of notothenioids contain up to 65% of such types of glycosphingolipids. Individual differences among the notothenioids were small. Highest values were found in the brains of high-Antarctic nototheniids, with 64% alkali-labile brain gangliosides in *Pleuragramma antarcticum*.

Antifreeze glycoproteins

The AFGPs isolated from *Pleuragramma antarcticum* have an amino acid (AA) composition of alanine and threonine in a 2:1 ratio, and some proline occurs in the lower molecular weight glycopeptide fractions (AFGP 6–8) (Fig. 5). Carbohydrate analysis revealed the presence of galactosamine (GalNAc) and galactose (Gal) in a 1:1 ratio. The AFGP concentration in adult specimens of *P. antarcticum* (267 mg kg^{-1} fresh weight) is about 80% lower as compared to other notothenioids from the same region of the Weddell Sea, e.g. *Trematomus lepidorhinus* (1351 mg kg^{-1} fresh weight). However, an additional glycopeptide was discovered in *P. antarcticum* which occurs in similar concentrations as the AFGPs (Fig. 5). Amino acid analysis of this novel glycopeptide, called '*Pleuragramma*-antifreeze glycopeptide' (PAGP), revealed a composition of 30.1 mol% glycine, 14.7 mol% aspartic acid, 15.5 mol% glutamic acid, and 11.3 mol% alanine, as well as smaller amounts of threonine, lysine, leucine, and valine. Sugar analysis by HPAEC-PAD identified N-acetylglucosamine as carbohydrate residue (Fig. 5). The molecular weight of PAGP is approximately 150 kDa, the root mean square radius 57.3 nm as determined by laser-light scattering and therefore similar to the root mean square radius of the high molecular weight AFGP 1.

The analysis of CD for the PAGP, obtained at a temperature of 20°C, shows β-sheet (56%), α-helical (19%) and random chain (25%) characteristics. This suggests the existence of a definite secondary structure. The CD spectrum strongly deviates from that of AFGP. The results reveal AFGP as having a left-handed helical structure. Both AFGP and PAGP have an expanded secondary structure. The antifreeze compounds comprise 2.46 mg ml^{-1} blood serum in adult specimens (22 cm SL). The AFGP/PAGP contents vary depending on the age of the fish, but no relationship was observed between AFGP/PAGP concentration and depth of catch (Table 2).

The antifreeze activity was measured by differential scanning calorimetry (Fig. 6). The sample was frozen at −40°C, then taken to various annealing temperatures (between 0.2 and −1.0°C for 5 min) and cooled again. At −0.2°C no ice was present, and the sample supercooled and crystallized around −17°C. While water, non-antifreeze peptides or glycopeptides freeze immediately upon cooling when ice is present, AFGP revealed an initial shoulder immediately upon cooling, followed by a delay before the onset of freezing. For a detailed description of this method see Wöhrmann (1996). The antifreeze activity increased with decreasing ice content (Fig. 7), with an observed maximum hysteretic activity of 1.2°C in the case of AFGP. However, no shoulder was observed when cooling blood serum or PAGP.

In contrast to AFGP, the PAGP antifreeze activity showed a very pronounced increase with decreasing ice content and glycoprotein concentration. A thermal hysteresis maximum of 1.23°C was determined at a

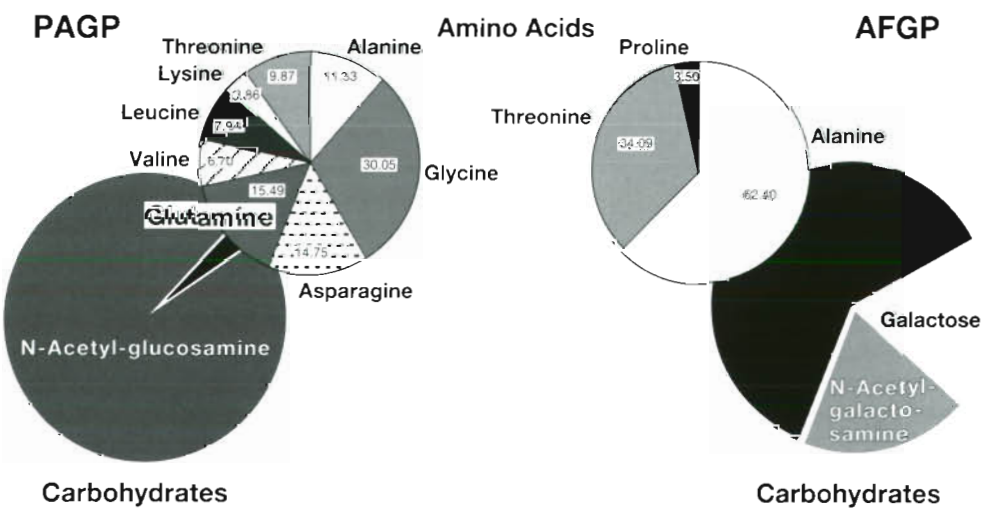

Fig. 5. *Pleuragramma antarcticum*. Amino acid (AA) and carbohydrate composition of antifreeze glycoproteins (AFGP) and the novel *Pleuragramma* antifreeze glycoprotein (PAGP). The values depict the mole percentage of AA

Table 2. Glycoprotein fractions (AFGP and PAGP) of *Pleuragramma antarcticum* in relation to the age of the specimens from various depths. SL = standard length (cm); AFGP = antifreeze glycoprotein; PAGP = *Pleuragramma* antifreeze glycoprotein (mg glycoprotein per kg fresh weight); MW = molecular weight; n = number of specimens. Age was determined indirectly using the standard length according to Hubold & Tomo (1989)

SL (cm)	Age (yr)	Depth (m)	Low MW AFGP (mg kg^{-1})	High MW AFGP (mg kg^{-1})	PAGP (mg kg^{-1})	n
9.5	4	620	150	133	219	32
14.5	8	570	109	170	139	9
17.0	10	450	105	187	179	10
22.0	14	620	101	194	188	3

concentration of 30 mg ml^{-1}. Hence, PAGP functions as an additional antifreeze agent to the known AFGP. When the colligative freezing point depression effects of plasma solutes (0.89°C) are added to the thermal hysteretic effect of blood serum (1.06°C), the resulting protection from freezing (≈ −1.9°C) does not reach the lowest water temperature experienced by adult *Pleuragramma antarcticum* (<−2.1°C). AFGP showed a higher activity at very high ice contents (>50%), an important factor in the ice-laden waters of the high-Antarctic region. This is complemented by PAGP, which exhibited the highest activity at low ice contents (Fig. 7).

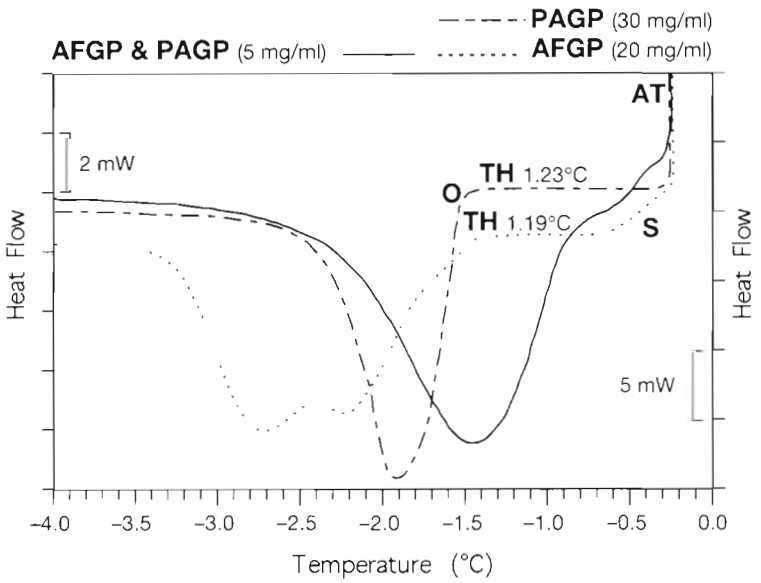

Fig. 6. *Pleuragramma antarcticum*. Thermal hysteresis (°C) of AFGP, PAGP and a combination of both. Glycoprotein solutions of 5, 20 and 30 mg ml^{-1} were tested for antifreeze activity. The scale bar represents heat flow measured in milliwatts (mW) for AFGP and PAGP (left) and both compounds separately (right). AT = annealing temperature; TH = thermal hysteresis; O = onset; S = shoulder

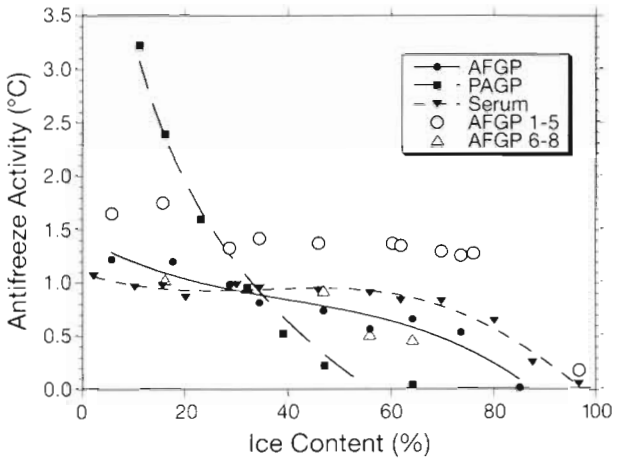

Fig. 7. *Pleuragramma antarcticum*. Antifreeze activity (°C) of AFGP, PAGP, high molecular weight AFGP 1–5, low molecular weight AFGP 6–8 and blood serum of an adult specimen (SL = 22 cm) in relation to the ice content (%) in the sample

Gill morphometrics

Gill dimensions of 27 juvenile and adult *Pleuragramma antarcticum* were determined (Fig. 8). The moderate number of short filaments results in a low average value of 1000 mm total filament length (TFL) for *P. antarcticum*. Lamellar density decreases with increasing body weight. With an average of 21 lamellae per mm filament, the lamellar density in *P. antarcticum* is higher than in other Antarctic notothenioids known so far and higher than in some sluggish species (e.g. *Lophius piscatorius* and *Anguilla anguilla*), but distinctly lower as compared to active species such as *Scomber scombrus* (Kunzmann 1990). The total gill area (TGA = $a \times W^{dg}$; a = gill area index, GAI, W = body weight, and dg = slope of log TGA/log W in Fig. 8) is estimated to be 597 to 6583 mm^2 (mean of 2070 mm^2) for *P. antarcticum*. Its standardized unit gill area (UGA), i.e. the total gill area per gram of body weight, ranges from 75 to 167 mm^2 g^{-1} (mean of 105 mm^2 g^{-1}). Other sluggish Antarctic species have a unit gill area between 100 and 300 mm^2 g^{-1}, whereas active species such as *S. scombrus* have values beyond 1000 mm^2 g^{-1}. The gill area index (GAI) and exponent dg in the relationship of total gill area to weight, as presented in Fig. 8, were found to be 1.38 cm^2 and 0.90, respectively, for *P. antarcticum*. The water-blood distance (WBD) has a mean value of 3.3 µm (SD ± 0.8). This distance is higher in slug-

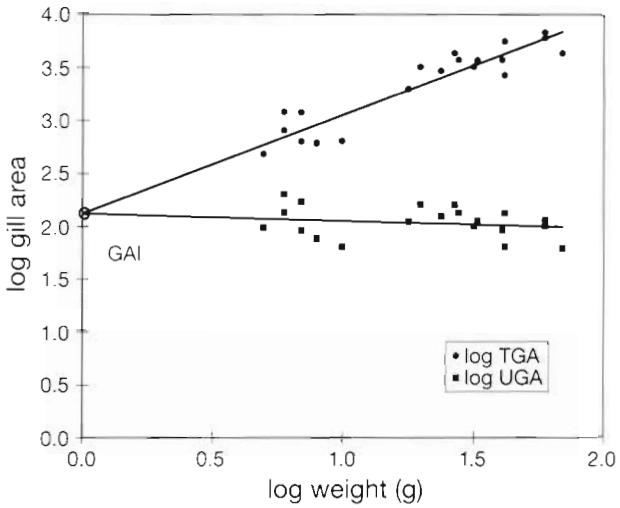

Fig. 8. *Pleuragramma antarcticum*. Weight-relationship of total gill area (TGA, mm^2) and unit gill area (UGA, $mm^2\ g^{-1}$). GAI = gill area index

gish species from temperate regions but lower in active species.

Blood physiology

Table 3 shows the most important blood parameters of *Pleuragramma antarcticum*. Blood viscosity is clearly reduced. The values for the number of red blood cells (RBC = $0.43 \pm 0.17 \times 10^{12}\ l^{-1}$) and the haemoglobin concentration (Hb = 26.5 ± 8.1 g l^{-1}) are at the lower end of the range known of Antarctic fishes (Kunzmann 1991) Similar results were found for *Aethotaxis mitopteryx* (RBC = $0.39 \pm 0.09 \times 10^{12}\ l^{-1}$, Hb = 27.8 ± 4.7 g l^{-1}), a benthopelagic nototheniid which also has a sluggish mode of life. Species with little or moderate activity also have low RBC numbers (e.g *Gerlachea australis*: $0.38 \pm 0.09 \times 10^{12}\ l^{-1}$). The oxygen affinity is moderate in *P. antarcticum* (Table 3) and at least 2 major haemoglobin components are present in functionally relevant concentrations (Fig 9), together with 1 minor haemoglobin component. In general, pelagic species have rather low oxygen affinities. The presence of differing Root effects in Hb 1 and Hb 2 (Fig 10) and an only moderate Bohr effect of the haemolysate of *P. antarcticum* differ considerably from data of *Aethotaxis mitopteryx*, where a Root effect is absent and only 1 haemoglobin is present (Kunzmann 1991).

Table 3. Blood parameters of *Pleuragramma antarcticum*. Samples had to be taken from stressed specimens, since the species cannot be kept alive in aquaria over long periods of time. Hb = Haemoglobin; MCH = mean cellular Hb content; MCHC = mean corpuscular Hb concentration; PO_2, PCO_2 = partial pressure of O_2 and CO_2; P_{50} oxygen pressure for 50% saturation; n^1_2 = cooperativity of Hb subunits

Parameter	Value
Haematocrit	16.6%
Number of erythrocytes	0.43×10^{12}
Haemoglobin concentration	26.5 g l^{-1}
Hb content (MCH)	61.6 pg
Hb concentration (MCHC)	159.6 g l^{-1}
pH	7.66
PO_2	30 mm Hg
PCO_2	3 mm Hg
P_{50} and n^1_2	12.7 mm Hg; 1.5[a]
ϕ (log P_{50}/log pH)	−0.74[a]

[a]At −1.5°C and under influence of effectors

DISCUSSION

In high-Antarctic waters such as the Weddell Sea the pelagic fish community represents a relatively young component of the ichthyofauna, which evolved during the interglacial periods (DeWitt 1970, Hubold & Ekau 1987). As one of very few fish species, *Pleuragramma antarcticum* succeeded in occupying the pelagic habitat (Eastman 1993). This species benefits from the higher productivity in the water column and achieves larger stock sizes than the strictly benthic species in this region (Hubold 1992). During the various phases of its life cycle *P. antarcticum* makes full use of the high-Antarctic shelf regions. Below we will follow the different stages of its life history, describe the extreme

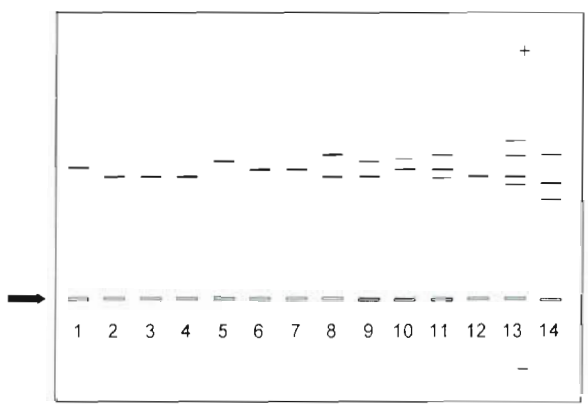

Fig. 9. Cellulose acetate electrophoresis of haemoglobin components of high-Antarctic fishes. Note the 2 large bands in *Pleuragramma antarcticum*. The arrow indicates origin; + and – refer to polarity. 1: *Bathydraco marri*; 2: *B. macrolepis*; 3: *Racovitzia glacialis*; 4: *Gerlachea australis*; 5: *Pogonophryne* sp., 6: *Dissostichus mawsoni*; 7: *Aethotaxis mitopteryx*; 8: *P. antarcticum*; 9: *Trematomus lepidorhinus*; 10: *T. eulepidotus*; 11: *T. scotti*; 12: *Pagothenia hansoni*; 13: *Anotopterus pharao*; 14: *Macrourus holotrachys*

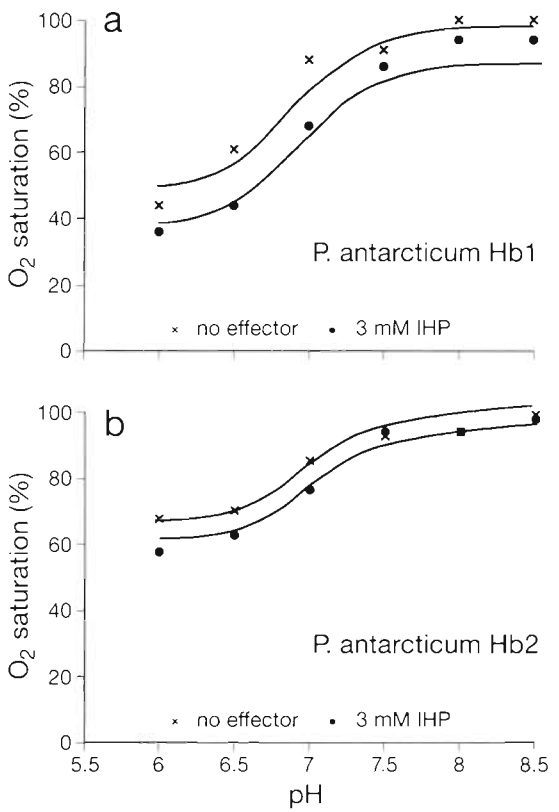

Fig. 10. *Pleuragramma antarcticum*. Oxygen saturation as a function of pH (Root effect). The difference in saturation between (a) Hb 1 and (b) Hb 2 at low pH is about 15 to 20%. IHP = Inositol hexaphosphate

environmental conditions in the pelagic habitat that the species has to cope with and further highlight some of the extraordinary adaptations which enable *P. antarcticum* to thrive in these polar waters.

Life history

Larvae/postlarvae

The reproductive cycle of *Pleuragramma antarcticum* is closely coupled to the seasonal zooplankton production during the short spring and summer period (Hagen & Schnack-Schiel 1996). Transport of larvae with the coastal current suggests upstream spawning migrations of the adults which spawn numerous small eggs (13 000 per female; 2 mm in diameter) in winter (August). Such a relatively high fecundity and the small eggs are unusual for notothenioids (Hubold 1991, 1992). The eggs probably float benthopelagically over the slope of the shelf (in the Warm Deep Water; G. Hubold pers. comm.). By November the larvae hatch at depths below 500 m on the eastern shelf in the coastal current upstream from juvenile and adult populations. The yolk sac larvae ascend to the stabilized surface layer while consuming their internal energy reserves. Along the Antarctic continent the local hydrographical features of the Weddell Sea coastal current provide stable advection and retention structures for the larvae. These assure larval transport from the hatching areas on the eastern shelf to the nursery grounds in the South. During this drift vertical and horizontal segregation of postlarvae, juveniles and adults prevents cannibalism (Hubold 1984, 1985a).

The early postlarvae exhibit low lipid levels. These compounds consist mainly of structural lipids (phospholipids), whereas reserve lipids (triacylglycerols) are scant. Hence, these postlarvae cannot rely on their energy stores; they require external energy sources. At this time of the year — if the timing is right — some of the dominant copepod species start to reproduce (Schnack-Schiel & Hagen 1994, 1995, Hagen & Schnack-Schiel 1996) and their offspring matches the trophic requirements of the *Pleuragramma* postlarvae. Major prey organisms are therefore copepod nauplii between 0.1 and 0.5 mm but also cyclopoid copepods ranging from 0.2 to 0.8 mm (Dorrien 1989, Hubold & Hagen 1997). Larval development is rather rapid (0.15 mm d^{-1}), comparable to herring larvae in the North Sea, since it takes place in the relatively warm and productive summer surface layer of the shelf water (Hubold 1985a, Hubold & Tomo 1989).

During their first year the postlarvae seem to 'invest' dietary energy mainly in somatic growth (protein build-up). However, during their second summer they start to accumulate large lipid deposits, mainly in the form of triacylglycerols. This incorporation of lipids in specific ventral oil sacs coincides with ossification and sclerification (DeVries & Eastman 1978). Since *Pleuragramma antarcticum* lacks a swim bladder to regulate its buoyancy, these low-density compounds will provide hydrostatic lift to prevent the fishes from sinking, a crucial factor for such a strong pelagic migrant. Indeed, *P. antarcticum* is so well-adapted that it almost attains neutral buoyancy due to lipid deposition and reduction of heavy elements (DeVries & Eastman 1978, Eastman & DeVries 1982). The triacylglycerols may to some degree also serve as energy stores during times of food paucity, although major catabolism of lipids would inevitably cause buoyancy problems (Friedrich & Hagen 1994).

Maximum amounts of antifreeze glycoproteins, both AFGP and PAGP, were detected in the postlarvae. Postlarvae were found all along the coast of the Weddell Sea, as far west as 60° W. The early stages of *Pleuragramma antarcticum* are most abundant in the upper 100 m (Hubold 1990). Highest concentrations of postlarvae of *P. antarcticum* and other dominant spe-

cies (e.g. *Chionodraco myersi* and *Aethotaxis mitopteryx*) were associated with the strongest horizontal currents (White & Piatkowski 1993). The overall pattern of distribution appears to be associated with the cold water (-1.4 to <-2.0°C) of the ice shelf (Hubold 1985a). The postlarvae are also abundant over the deep trenches (Filchner depression and the trench off Camp Norway). These trenches or 'inner-shelf depressions' were defined as a 'pseudo-abyssal zone' by Andriashev (1965) and belong to the cold ice-shelf ecosystem. Hydrographic observations made during the 'European Polarstern Study' (ANT VII/4; summer 1989) suggested marked vertical mixing and possible upwelling of cold ice-shelf water at the shelf-break front (Rohardt et al. 1990). The high antifreeze contents in the postlarvae of *P. antarcticum* seem to be an obvious adaptation to life in surface waters, where these specimens may be exposed to freezing temperatures and are most likely to come in close contact with ice-formation processes.

Juveniles

During a prolonged juvenile phase, *Pleuragramma antarcticum* fingerlings spread over a wide area towards lower latitudes, e.g. the Antarctic Peninsula, where they feed on the abundant copepod and larval krill resources (Hubold & Ekau 1990). Juveniles feed on a variety of small zooplankton species like calanoid copepods (0.3 to 15 mm) and euphausiid larvae (1.5 to 5 mm) and have a broader nutritional basis than other notothenioids (Hubold & Hagen 1997). *P. antarcticum* specimens have been reported as by-catch in hauls of the Antarctic krill *Euphausia superba*. In this case they often had juvenile euphausiids in their stomachs (Rembiszewski et al. 1978). Hence, it is likely that young fish leave the cold waters of the shelf to feed on krill at depths of 100 to 400 m in the warmer East Wind Drift. Recruitment of 5 to 7 yr old fishes to the southern adult stocks thus includes input of allochthonous energy into the high latitude shelf system. Growth of juveniles is much slower than in the postlarvae, and the juvenile phase extends over several years (Hubold 1991, 1992).

Juveniles of *Pleuragramma antarcticum* continue to deposit extensive amounts of lipid (Friedrich & Hagen 1994). Other Antarctic notothenioids also show substantial corporeal accumulations of lipid, which are considered to be buoyancy adaptations in this swim bladderless group (Eastman & DeVries 1982). However, lipids are the primary fuel for energy metabolism of Antarctic fish (Sidell 1991). The antifreeze compounds AFGP and PAGP in juvenile specimens show the lowest concentrations of all investigated age classes. In their natural deeper water habitat, juveniles of *P. antarcticum* are in little danger of freezing. The temperatures (e.g. -0.5°C at the continental slope in the Weddell Sea) are higher and the effect of hydrostatic pressure lowers their freezing point to -2.3°C (at 500 m); hence, these waters remain in a supercooled state.

Subadults and adults

In contrast to the postlarvae, growth of subadult and adult *Pleuragramma antarcticum* is amongst the slowest known of marine fish (Hubold & Tomo 1989). The combination of a low growth parameter k (0.07) and a low infinite length (31 cm) results in a low growth performance index (P = 1.08). Slow growth of *P. antarcticum* is considered an adaptation to make optimum use of the productive mesoplankton fraction (copepods) over most of the fishes' life span (Hubold 1990). Instead of investing it in somatic growth, this metabolic energy can fuel reproduction, activity (including spawning migrations), freezing resistance and lipid deposition (Hubold 1991). In addition to the food items of juveniles, adult *P. antarcticum* ingest a higher proportion of larger euphausiids (10 to 40 mm). This dietary flexibility enables *P. antarcticum* to cope with the strong seasonal as well as spatial variability of the high-Antarctic zooplankton. Thus, adult *P. antarcticum* utilize a much wider range of different food items than any other notothenioid species (Hubold 1985b, Hubold & Hagen 1997). The high fecundity and the fast growth of postlarvae, as well as the subsequent slow growth of juveniles and adults consuming a wide range of different food organisms, seem to be key adaptations of *P. antarcticum*, which may explain the predominance of this species throughout the high-Antarctic shelf waters.

However, the dominance of *Pleuragramma antarcticum* in the pelagic realm would not have been possible without pronounced biochemical and physiological adjustments. We have already discussed the importance of lipids in buoyancy regulation and as a possible energy reserve. Lipid compounds represent an important metabolic fuel for aerobic respiration of *P. antarcticum*. The substantial amounts of lipid in the muscles of Antarctic fish have chemical properties that may also increase the intracellular flux of oxygen (Sidell 1991).

Low temperatures also require neurophysiological alterations. In Antarctic fish action potentials may occur down to the disruptive freezing of membranes (-5°C). There are stable and relatively high conduction velocities and a lowered upper thermal limit of spike activity (Macdonald et al. 1987, Montgomery et al. 1990). In specific zones of the cell membrane, ganglio-

sides aggregate in clusters and appear to influence the fluidity and/or the polarity of these areas. The ability of gangliosides to complex with calcium enables these glycosphingolipids to open ion channels for the influx of calcium ions (Rahmann 1992). Due to these specific interactions with calcium, gangliosides are assumed to modulate functional membraneous proteins. However, the extremely high polarity of brain gangliosides in *Pleuragramma antarcticum* causes a high binding affinity with calcium. This could be shown with an energy-filtering transmission electron microscope for Image Electron Energy-Loss Spectroscopy (Wöhrmann unpubl.). Thus, normal conduction velocities of the neuronal membranes are maintained even below the freezing point (Rahmann et al. 1984, Becker et al. 1995). Furthermore, the high proportions of lactonization and O-acetylation of the brain gangliosides in *P. antarcticum* represent an important modulation of the surface electrostatics of the neuronal membranes to ensure a regular functioning of action potentials.

A crucial adaptation to the cold Antarctic environment is the presence of antifreeze proteins in most Antarctic fishes (Macdonald et al. 1987, DeVries 1988, Wöhrmann 1997a). In contrast to earlier reports, our findings have shown that this holds true also for *Pleuragramma antarcticum*. This is not surprising, since this species, especially the early developmental stages, may frequently be exposed to freezing conditions. Maturing adults possess low molecular weight AFGP 6–8 and PAGP in concentrations similar to those found in the postlarvae, but they have high amounts of the high molecular weight AFGP 1–5. This is an important feature for the survivel of *P. antarcticum* in the cold deep waters. AFGP 6–8 and PAGP are much less effective in depressing the freezing temperature. These compounds have a greatly reduced level of activity when ice is developing by nucleation during supercooling conditions at depth (Feeney & Yeh 1993, Wöhrmann 1995, 1996). In adult *P. antarcticum* the freezing point of the blood serum (≈ −1.9°C) is above the lowest water temperature experienced by this species (< −2.0°C). At depths below 500 m these specimens live in a supercooled state, but in surface waters they may freeze when contacting ice crystals. This may explain the occurrence of frozen specimens of *P. antarcticum* after being hauled onboard 'Polarstern' from greater depths.

The year-round synthesis of high amounts of antifreeze glycopeptides (>20 mg ml^{-1} blood serum) causes high metabolic costs in species like the cryopelagic *Pagothenia borchgrevinki* and the benthic *Pagetopsis macropterus*. To minimize these metabolic demands, antifreeze peptides are only produced in minimum essential concentrations by the more active and the pelagic species, e.g. *Neopagetopsis ionah* and *Aethotaxis mitopteryx* (Wöhrmann 1997b). The synthesis of different antifreeze glycopeptides and peptides in rather low concentrations as well as the high efficiency of these compounds is an adaptation characteristic of the fully pelagic *Pleuragramma antarcticum*.

Gill morphometric data indicate that in spite of its pelagic life style *Pleuragramma antarcticum* belongs to the sluggish species with low routine energy costs. This is suggested by the long diffusion distances of *P. antarcticum* and its very low mean unit gill area. The latter is similar to sluggish species of temperate regions (Gray 1954, Hughes 1966, Hughes & Morgan 1973), but much lower as compared to active, pelagic species (Gray 1954). The same holds true for the low haematocrit of *P. antarcticum* (Everson & Ralph 1968, Love 1980, Wells et al. 1980). The idea of rather inactive behaviour is also supported by other characteristics of *P. antarcticum*, such as neutral buoyancy due to lipid storage, and a poorly developed motoric system (Johnston et al. 1988). However, the closely packed gill lamellae indicate that *P. antarcticum* has at least a potential for more active behaviour, which is in contrast to other sluggish Antarctic fish species.

Adaptations in the respiratory system of *Pleuragramma antarcticum* allow this species to maintain a fairly stable oxygen affinity. In combination with the saturation properties of haemoglobin Hb 2 these adapatations ensure a sufficient oxygen supply upon encountering water masses with a decreased oxygen content. This situation may occur when older specimens descend to the Warm Deep Water In comparison to other notothenioids the presence of 2 or more haemoglobins in higher amounts is unusual (Kunzmann 1991, Kunzmann et al. 1992). We do not know as yet whether Hb 1, Hb 2 and Hb 3 are functionally different haemoglobins. This is particularly interesting in *P. antarcticum* because of its seasonal migrations. Different water masses are probably encountered during such migrations and functionally different haemoglobins could be advantageous if ambient oxygen concentrations vary. More detailed functional studies on the haemoglobins Hb 2 and Hb 3 and the sequencing of all 3 haemoglobins may clarify this phenomenon.

In summary, gill morphometrics and the various blood parameters indicate that adult *Pleuragramma antarcticum* have a rather sluggish mode of life (Kunzmann 1990), as compared to other fish species (Hughes 1984). This notion is quite different from our conventional concept of pelagic schooling fishes from warmer regions, which usually have high activity levels. On the other hand, it is in good agreement with data from a close relative, *Aethotaxis mitopteryx*, a benthopelagic species from high-Antarctic waters (Kunzmann & Zimmermann 1992, Kunzmann et al. 1992). Grigg (1967) and Hureau et al. (1977) confirm these

data for other Antarctic species such as *Trematomus pennellii* or *T. bernacchii*.

Conversely, *Pleuragramma antarcticum* and *Aethotaxis mitopteryx* share the unusual feature that the oxygen affinity and the level of oxygenation does not change considerably under the influence of effectors such as organic phosphates or Cl^- ions. The presence of such effectors can dramatically increase the Bohr effect and the Root effect in other fish species (Riggs 1988). *P. antarcticum* and *A. mitopteryx* belong to the tribe Pleuragramminii, together with *Cryothenia peninsulae* and *Gvozdarus svetovidovi*. This tribe is believed to have developed rather recently, less than 10 million years ago (Andersen 1984, Eastman 1985) and it is the most advanced group amongst the Nototheniidae. All 4 species are more or less confined to a pelagic/benthopelagic mode of life.

Some special adaptations (e.g. neutral buoyancy, antifreeze, blood characteristics) may be of relatively recent origin (Andersen 1984) and could be assigned to recent changes in life style. Apparently, this unique mode of life for fish, i.e. pelagic *and* sluggish, seems to be an energy-saving adaptation (Kunzmann 1991, Kunzmann & Zimmermann 1992). Such economic behaviour could provide advantages in the frigid high-Antarctic waters, which have a much higher viscosity and may turn fast swimming into an energy-intensive struggle (Hubold 1992). Sluggishness in the few existing pelagic Antarctic fish species may be a relict of their benthic ancestors, which selective pressures never forced them to abandon.

CONCLUSIONS

Pleuragramma antarcticum has evolved from a bottom-dwelling, solitary species to a pelagic schooling fish with a circum-Antarctic distribution. It occurs in epi- and mesopelagic layers and was confronted with various new problems, which the species managed to overcome. In this paper we have highlighted some of these adaptations which help to explain the overwhelming dominance of *P. antarcticum* in the high-Antarctic pelagic habitat. Subadult and adult *P. antarcticum* have a very high mean lipid content, second only to *Aethotaxis mitopteryx*. These potential energy stores greatly improve the buoyancy characteristics of *P. antarcticum*. The synthesis of various efficient antifreeze glycopeptides and peptides in relatively low concentrations is another extraordinary adaptation to life in polar waters, which only the fully pelagic *P. antarcticum* has developed. Morphological, ultrastructural and blood physiological studies suggest that pelagic notothenioids like *P. antarcticum* are rather sluggish with a low scope of activity and low metabolic demands. *P. antarcticum* is a prime example of the complex adaptations required to successfully occupy such a new habitat. This 'pioneering' fish species has impressively filled the pelagic niche in high-Antarctic shelf waters.

Acknowledgements. The skillful support of the captains and crews of the RV 'Polarstern' is greatly appreciated. It allowed the collection of Antarctic fishes during a period of more than 10 yr under very difficult conditions. We thank Prof. Gerd Hubold for his valuable comments on an earlier draft. The manuscript also benefited from constructive suggestions by Dr Christopher Zimmermann and 3 anonymous referees. Part of this work was financed by the 'Deutsche Forschungsgemeinschaft' (He89/55), the 'EPOS' expedition (ANT VII/4) was funded by the 'European Science Foundation'

LITERATURE CITED

Andersen NC (1984) Genera and subfamilies of the family Nototheniidae from the Antarctic and Sub-Antarctic. Steenstrupia 10:1–34

Andriashev AP (1965) A general review of the Antarctic fish fauna. Monogr Biol 25:491–550

Andriashev AP (1987) A general review of the Antarctic bottom fish fauna. In: Kullander SO, Fernholm B (eds) Proc 5th Cong Eur Ichthyol. Mus Nat Hist, Stockholm, p 357–372

Becker K, Wöhrmann APA, Rahmann H (1995) Brain gangliosides and cold-adaptation in high-Antarctic fish. Biochem Syst Ecol 23:695–707

Cheng CC, DeVries AL (1991) The role of antifreeze glycopeptides and peptides in the freezing avoidance of cold-water fish. In: di Prisco G (ed) Life under extreme conditions. Springer, Berlin, p 1–15

Clarke A (1983) Life in cold water: the physiological ecology of polar marine ectotherms. Oceanogr Mar Biol Annu Rev 21:341–453

Clarke A (1988) Seasonality in the Antarctic environment. Comp Biochem Physiol B 90:461–473

Clarke A (1990) Temperature and evolution: Southern Ocean cooling and the Antarctic marine fauna. In: Kerry KR, Hempel G (eds) Antarctic ecosystems: change and conservation. Springer, Berlin, p 9–22

Clarke A, Doherty N, DeVries AL, Eastman JT (1984) Lipid content and composition of three species of Antarctic fish in relation to buoyancy. Polar Biol 3:77–83

Clarke A, Johnston IA (1996) Evolution and adaptive radiation of Antarctic fishes. Trends Ecol Evol 11:212–218

Cossins AR (1994) Homeoviscous adaptation of biological membranes and its functional significance. In: Cossins AR (ed) Temperature adaptation of biological membranes. Portland Press, Portugal, OR, p 63–75

D'Avino R, di Prisco G (1988) Antarctic fish hemoglobin: an outline of the molecular structure and oxygen binding properties. I. Molecular structure. Comp Biochem Physiol B 90:579–584

DeVries AL (1988) The role of antifreeze glycopeptides in the freezing avoidance of Antarctic fishes. Comp Biochem Physiol B 90:611–621

DeVries AL, Eastman JT (1978) Lipid sacs as a buoyancy adaptation in an Antarctic fish. Nature 271:352–353

DeWitt HH (1970) The character of the midwater fish fauna of the Ross Sea, Antarctica. In: Holdgate MW (ed) Antarctic ecology, Vol 1. Academic Press, London, p 305–314

Dey I, Buda C, Wiik T, Halver JE, 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

di Prisco G, Tamburrini M, Kunzmann A (1993) Fisheries biology and the structure of haemoglobins. Ber Polarforsch 121:92–98

Dornen CF v (1989) Ichthyoplankton in relation to hydrography and zooplankton in the Weddell Sea. MSc thesis, Kiel University (in German)

Eastman JT (1985) The evolution of neutrally buoyant notothenioid fishes: their specialisations and potential interactions in the Antarctic marine food web. In: Siegfried WR, Condy PR, Laws RM (eds) Antarctic nutrient cycles and food webs. Springer, Berlin, p 430–436

Eastman JT (1993) Antarctic fish biology. Academic Press, San Diego

Eastman JT, DeVries AL (1982) Buoyancy studies of notothenioid fishes in McMurdo Sound, Antarctica. Copeia 1982: 385–393

Ekau W (1988) Ecomorphology of nototheniid fishes from the Weddell Sea, Antarctica. Ber Polarforsch 51:1–140 (in German)

Ekau W, Hubold G (1985) Fish and fish larvae. Ber Polarforsch 25:121–129

Ekau W, Hubold G, Wöhrmann APA (1987) Fish and fish larvae. Ber Polarforsch 39:210–218

Everson I, Ralph R (1968) Blood analysis of some Antarctic fish. Br Antarct Surv Bull 15:59–62

Falk-Petersen S, Hopkins CCE, Sargent JR (1990) Trophic relationships in the pelagic Arctic food web. In: Barnes M, Gibson RN (eds) Trophic relationships in the marine environment: Proc 24th Eur Mar Biol Symp. University Press, Aberdeen, p 315–333

Feeney RE, Yeh Y (1993) Antifreeze proteins: properties, mechanism of action, and possible applications. Food Techn 1/93:82–90

Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509

Fraser AJ, Tocher DR, Sargent JR (1985) Thin-layer chromatography—flame ionization detection and the quantitation of marine neutral lipids and phospholipids. J Exp Mar Biol Ecol 88:91–100

Friedrich C, Hagen W (1994) Lipid contents of five species of notothenioid fish from high-Antarctic waters and ecological implications. Polar Biol 14:359–369

Ghidoni R, Sonnino S, Chigorno V, Malesci A, Tettamanti G (1984) Comparative developmental behavior of alkali-labile gangliosides in the brain. Adv Exp Med Biol 174: 307–764

Gray IE (1954) Comparative study of the gill area of marine fishes. Biol Bull 107:219–225

Grigg GC (1967) Some respiratory properties of the blood of four species of Antarctic fishes. Comp Biochem Physiol 23:139–148

Hagen W (1988) On the significance of lipids in Antarctic zooplankton. Ber Polarforsch 49:1–129 (in German). English version (1989): Can Transl Fish Aquat Sci 5458:1–149

Hagen W (1996) The role of lipids in the ecology of polar plankton and nekton—a synopsis. Habilitation thesis, Kiel University

Hagen W, Schnack-Schiel SB (1996) Seasonal lipid dynamics in dominant Antarctic copepods: energy for overwintering or reproduction? Deep Sea Res 43:139–158

Haschemeyer AEV, Jannasch HW (1983) Antifreeze glycopeptides of Antarctic fishes. Comp Biochem Physiol B 76:545–548

Hellmer HH, Bersch M (1985) The Southern Ocean. Ber Polarforsch 26:1–115

Hempel G (1990) The Weddell Sea: a high polar ecosystem. In: Sherman K, Alexander LM, Gold BD (eds) Large marine ecosystems—patterns, processes, and yields. Publ No 90-30S, American Association for the Advancement of Science (AAAS), Washington DC, p 5–18

Hubold G (1984) Spatial distribution of *Pleuragramma antarcticum* (Pisces: Nototheniidae) near the Filchner and Larsen Ice Shelves (Weddell Sea/Antarctica). Polar Biol 3: 231–236

Hubold G (1985a) On the early life history of the high-Antarctic silverfish *Pleuragramma antarcticum*. In: Siegfried WR, Condy PR, Laws RM (eds) Antarctic nutrient cycles and food webs. Springer, Berlin, p 445–451

Hubold G (1985b) Stomach contents of the Antarctic silverfish *Pleuragramma antarcticum* from the southern and eastern Weddell Sea (Antarctica). Polar Biol 5:43–48

Hubold G (1990) Seasonal patterns of larval fish distribution in the Weddell Sea. Proc 5th SCAR Symp on Antarctic Biology; Hobart, Australia; 29 Aug – 3 Sept 1988. Springer, Berlin, p 149–158

Hubold G (1991) Ecology of notothenioid fish in the Weddell Sea. In: di Prisco G, Maresca B, Tota B (eds) Biology of Antarctic fish. Springer, Berlin, p 3–23

Hubold G (1992) The ecology of fishes in the Weddell Sea. Ber Polarforsch 103:1–157 (in German)

Hubold G, Ekau W (1987) Midwater fish fauna of the Weddell Sea, Antarctica. In: Kullander SO, Fernholm B (eds) Proc 5th Congr Eur Ichthyol. Museum of Natural History, Stockholm, p 391–396

Hubold G, Ekau W (1990) Feeding patterns of postlarval and juvenile notothenioids in the southern Weddell Sea (Antarctica). Polar Biol 10:255–260

Hubold G, Hagen W (1997) Seasonality of feeding and lipid content in juvenile *Pleuragramma antarcticum* (Pisces: Nototheniidae) from the southern Weddell Sea. In: Battaglia B, Valencia J, Walton DWH (eds) Antarctic communities: species, structure and survival. University Press, Cambridge (in press)

Hubold G, Tomo AP (1989) Age and growth of Antarctic silverfish *Pleuragramma antarcticum* Boulenger, 1902, from the Southern Weddell Sea and Antarctic Peninsula. Polar Biol 9:205–212

Hughes GM (1966) The dimensions of fish gills in relation to their function. J Exp Biol 45:177–195

Hughes GM (1984) Measurement of gill area in fishes: practices and problems. J Mar Biol Assoc UK 64:637–655

Hughes GM, Morgan M (1973) The structure of fish gills in relation to their respiratory function. Biol Rev 48:419–475

Hureau JC, Balgerias E, Duhamel G, Kock KH, Ozouf-Costaz C, White M (1990) Fish fauna of the Eastern Weddell Sea. Ber Polarforsch 68:130–139

Hureau JC, Petit D, Fine JM, Marneux M (1977) New cytological, biochemical, and physiological data on the colorless blood of the Channichthyidae (Pisces, Teleosteans, Perciformes). In: Llano GA (ed) Adaptations within Antarctic ecosystems. Gulf Publ Co, Houston, p 459–477

Johnston IA, Camm JP, White M (1988) Specialisations of swimming muscles in the pelagic Antarctic fish *Pleuragramma antarcticum*. Mar Biol 100:3–12

Kunzmann A (1990) Gill morphometrics of two Antarctic fish species, *Pleuragramma antarcticum* and *Notothenia gibberifrons*. Polar Biol 11:9–18

Kunzmann A (1991) Blood physiology and ecological consequences in Weddell Sea fishes. Ber Polarforsch 91:1–79

Kunzmann A, Fago A, D'Avino R, di Prisco G (1992) Haema-

tological studies on *Aethotaxis mitopteryx* DeWitt, a high-Antarctic fish with a single haemoglobin. Polar Biol 12: 141–145

Kunzmann A, Zimmermann C (1992) *Aethotaxis mitopteryx*, a high-Antarctic fish with benthopelagic mode of life. Mar Ecol Prog Ser 88:33–40

Ledeen RW, Haley JE, Skrivanek JA (1981) Study of ganglioside patterns with two-dimensional thin-layer-chromatography and radiography of new fucogangliosides and other minor species in rabbit brain. Anal Biochem 112: 135–142

Love RM (1980) The chemical biology of fishes, Vol 2. Advances 1968–77. Academic Press, London

Macdonald JA, Montgomery JC, Wells RMG (1987) Comparative physiology of Antarctic fishes. Adv Mar Biol 24: 321–388

Maggio B, Ariga T, Yu RK (1990) Ganglioside GD3-lactones: polar head group mediated control of the intermolecular organization. Biochem 29:8729–8734

Miettinen T, Takki-Lukkainen JT (1959) Use of butylacetate in determination of sialic acid. Acta Chem Scand 13: 856–858

Montgomery JC, Macdonald JA, Housely GD (1990) Brain function in Antarctic fish: frequency response analysis of central vestibular units. J Comp Physiol A 166:407–410

Powers DA (1980) Molecular ecology of teleost fish hemoglobins: strategies for adapting to changing environments. Am Zool 20:139–162

Rahmann H (1992) Calcium—ganglioside interaction and modulation of neuronal functions. In: Osborne NN (ed) Current aspects of the neurosciences. Macmillan Press, London, p 87–125

Rahmann H, Schneppenheim R, Hilbig R, Lauke G (1984) Variability in brain ganglioside composition: a further molecular mechanism beside serum antifreeze glycoproteins for adaptation to cold in antarctic and arctic-boreal fishes. Polar Biol 3:119–125

Rembizewski JM, Krzeptowski M, Linkowski TB (1978) Fishes as by-catch in fisheries of krill *Euphausia superba* Dana (Euphausiacea - Crustacea). Pol Arch Hydrobiol 25: 677–695

Riggs AF (1988) The Bohr effect. Annu Rev Physiol 50: 181–204

Rohardt G, Ruhland G, Schleif U (1990) The Halley Bay—Kapp Norvegia comparison. Ber Polarforsch 68:39–49

Schnack-Schiel SB, Hagen W (1994) Life cycle strategies and seasonal variations in distribution and population structure of four dominant calanoid copepod species in the eastern Weddell Sea, Antarctica. J Plankton Res 16: 1543–1566

Schnack-Schiel SB, Hagen W (1995) Life cycle strategies of *Calanoides acutus*, *Calanus propinquus*, and *Metridia gerlachei* (Copepoda: Calanoida) in the eastern Weddell Sea, Antarctica. ICES J Mar Sci 52:541–548

Sidell BD (1991) Physiological roles of high lipid content in tissues of Antarctic fish species. In: di Prisco G, Maresca B, Tota B (eds) Biology of Antarctic fish. Springer, Berlin, p 220–232

Sonnino S, Ghidoni R, Malesci A, Tettamanti G, Marx J, Hilbig R, Rahmann H (1984) Nervous system ganglioside composition of normothermic and hibernating dormice (*Glis glis*). Neurochem Int 6:677–684

Svennerholm L (1957) Quantitative estimation of sialic acids. A colorimetric resocinol-hydrochloride acid method. Biochem Biophys Acta 24:604–611

Svennerholm L, Fredman PA (1980) A procedure for the quantitative isolation of brain gangliosides. Biochem Biophys Acta 617:97–109

Val AL, Almeida-Val VMF, Affonso EG (1990) Adaptive features of Amazonian fishes: haemoglobins, haematology, intraerythrocytic phosphates and whole blood Bohr effect of *Pterygolichthys multiradiatus* (Siluriformes). Comp Biochem Physiol B 97:435–444

Wells RM, Ashby MD, Duncan SJ, Macdonald JA (1980) Comparative study of the erythrocytes and haemoglobins in notothenid fishes from Antarctica. J Fish Biol 17:517–527

Wells RMG, Grigg GC, Beard LA, Summers G (1989) Hypoxic responses in a fish from a stable environment: blood oxygen transport in the Antarctic fish *Pagothenia borchgrevinki*. J Exp Biol 141:97–111

White MG, Piatkowski U (1993) Abundance, horizontal and vertical distribution of fish in eastern Weddell Sea micronekton. Polar Biol 13:41–53

Williams R (1985) Trophic relationship between pelagic fish and euphausiids in Antarctic waters. In: Siegfried WR, Condy PR, Laws RM (eds) Antarctic nutrient cycles and food webs. Springer, Berlin, p 452–459

Wöhrmann APA (1993) Freezing resistance in Antarctic and Arctic fishes. Ber Polarforsch 119:1–99 (in German)

Wöhrmann APA (1995) Antifreeze glycopeptides of the high-Antarctic silverfish *Pleuragramma antarcticum* (Notothenioidei). Comp Biochem Physiol C 111:121–129

Wöhrmann APA (1996) Antifreeze glycopeptides and peptides in Antarctic fish species from the Weddell Sea and the Lazarev Sea. Mar Ecol Prog Ser 130:47–59

Wöhrmann APA (1997a) Freezing resistance in Antarctic fish. In: Battaglia B, Valencia J, Walton D (eds) Antarctic communities: species, structure and survival. University Press, Cambridge (in press)

Wöhrmann APA (1997b) Antifreeze glycopeptides and peptides in the freezing avoidance of Weddell Sea fishes: its relation to mode of life, depth distribution and evolution. In: Pörtner HO, Playle R (eds) Cold ocean physiology. University Press, Cambridge (in press)

Wöhrmann APA, Haselbeck A (1992) Characterization of antifreeze glycoproteins of *Pleuragramma antarcticum* (Pisces: Notothenioidei). Biol Chem Hoppe-Seyler 373:854

Wöhrmann APA, Zimmermann C (1992) Comparative investigations on fishes of the Weddell Sea and the Lazarev Sea. Ber Polarforsch 100:208–222

This article was submitted to the editor

Manuscript first received: November 24, 1995
Revised version accepted: March 16, 1997