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SEASONAL DIFFERENCE OF THE PLASMA OSMOLALITIES OF SOME TELEOSTS IN HIGH-LATITUDE COLD WATER IN JAPAN

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Abstract: Antifreeze glycoprotein (AFGP) and/or antifreeze proteins (AFPs) have been found not only in Antarctic teleosts but also in high-latitude cold water fish in the Northern Hemisphere. In our previous study of saffron cod, a significantly higher plasma osmolality was observed in winter than in summer fish. The plasma osmolality and Na concentration were determined in the four teleosts, *Liopsetta pinnifasciata*, *Myoxocephalus brandti*, *Hypomesus pretiosus japonicus* and *Zoarces elongatus*, collected from Notsuke Bay on the Okhotsk Sea side of Hokkaido. Both plasma osmolality and Na concentration were higher during winter than during summer. The high plasma osmolalities of the winter fishes may be mainly due to the increased AFGP and/or AFP contents.

1. Introduction

Most Antarctic teleosts have been reported to have antifreeze glycoprotein (AFGP) in their sera and aglomerular kidneys (DEVRIES *et al.*, 1970; DOBBS *et al.*, 1974). AFGP and/or antifreeze proteins (AFPs) have been observed not only in Antarctic fishes but also in several high-latitude cold-water fish in the Northern Hemisphere (DAVIES and HEW, 1990). These antifreeze agents depress the freezing point of the body fluids or tissues for adaptation to cold-water environments (DEVRIES, 1984). It has also been noted that the antifreeze levels in the blood of cold-water fish show seasonal changes (FLETCHER, 1977).

In Japan, of the teleosts surviving in the ice-laden coastal water during winter, the saffron cod, *Eleginus gracilis*, has been reported to possess a higher concentration of AFGP in the serum than during summer (BURCHAM *et al.*, 1984). The plasma osmolality of this fish also increased during winter. The high plasma osmolality of the winter fish may be due to the increased AFGP contents (OGAWA *et al.*, 1993).

In the present study, therefore, an attempt has been made to observed the seasonal difference of the plasma osmolality of some teleosts in high-latitude cold-water areas of Japan, and to confirm the possibility of fish possessing the antifreezes.

2. Materials and Methods

The seasonal variations of plasma osmolality and sodium (Na) concentration were investigated. In this observation, the following four teleosts were collected in February and June, 1993, from Notsuke Bay, on the Okhotsk Sea side of Hokkaido: Barfin plaice, *Liopsetta pinnifasciata*, snowy sculpin, *Myoxocephalus brandti*, surf smelt, *Hypomesus pretiosus japonicus* and notched-fin eelpout, *Zoarces elongatus*. The numbers and standard lengths of these fishes are shown in Table 1. No attempt was made to separate the sexes.

Blood samples of 1.5–2.0 m*l*, were taken from the caudal vessels by cutting the tail. Fresh blood was immediately centrifuged at 2000 g for 5 minutes. The plasma was decanted into a 1.5 m*l*, clean polyethylene sampling tube and was stored frozen at $-20°C$ until analysis. The blood cells were discarded.

A Micro-Osmometer (Hermann Roebling, TY 13DR) and an Ion-Meter (Horiba, Model N-8F) were employed for determination of plasma osmolality and Na concentration, respectively.

For histological observation, the kidneys were fixed with Bouin's fixative, embedded in paraffin and sectioned at 6 μm using routine procedures. Sections were stained with hematoxylin and eosin.

3. Results and Discussion

The plasma osmolalities were significantly higher during winter than during summer in all the fishes examined, although only a limited number of *L. pinnifasciata* were available (Table 1). The Na concentrations also increased in winter

Table 1. Seasonal differences in the plasma osmolality and Na concentration of teleosts in high-latitude cold water.

Species	No. of fish (SL, mm)	Plasma osmolality mOsm/kg	Na concentration mEq/L
Liopsetta pinnifasciata	W3 (180–195)	740.3+109.8	224.6+18.6
	S2 (265–275)	525.7+ 22.0	183.6+ 3.0
Myoxocephalus brandti	W9 (145–230)	851.8+ 14.8	212.2+ 6.2
	S7 (170–200)	422.2+ 3.9**	161.8+ 2.0**
Hypomesus pretiosus japonicus	W5 (175–200)	793.2+ 6.4	213.2+ 7.4
	S4 (170–185)	482.9+ 14.5**	115.8+ 6.0**
Zoarces elongatus	W7 (290–390)	1061.0+ 28.3	206.3+ 6.5
	S6 (320–360)	704.2+ 30.1**	165.6+ 7.9*

W: Winter, S: Summer.
*: Significantly different from corresponding values for winter fish at $p<0.01$.
**: Significantly different from corresponding values for winter fish at $p<0.001$.

compared with those in summer (Table 1). Such high plasma osmolality during winter seems to be unexplainable by the increased Na content alone. Based on the stability of the ionic composition of plasma in teleosts (HOLMES and DONALDSON, 1969), the contents of Cl and other ions, such as Mg and Ca, were estimated at roughly 95% and 35%, respectively, of Na contents. This paper suggests that the possibility that the high plasma osmolality of these winter fishes may be mainly due to the increased antifreeze contents rather than the increased Na and other ionic concentrations should be considered (Fig. 1).

Antarctic fish with AFGP have aglomerular kidney (DOBBS *et al.*, 1974). It is known that the shorthorn sculpin, *Myoxocephalus scorpius*, has an aglomerular kidney (GRAFFLIN, 1933) and also AFP in the serum (HEW *et al.*, 1980), while the closely related snowy sculpin, *M. brandti*, shows good glomerular development. All the fishes examined in this study, including *M. brandti*, have the glomerular kidney and their glomeruli are well developed.

Four distinct macromolecular antifreezes, one type of AFGP, and three types of AFPs, have been isolated and characterized from different marine fishes. AFGP

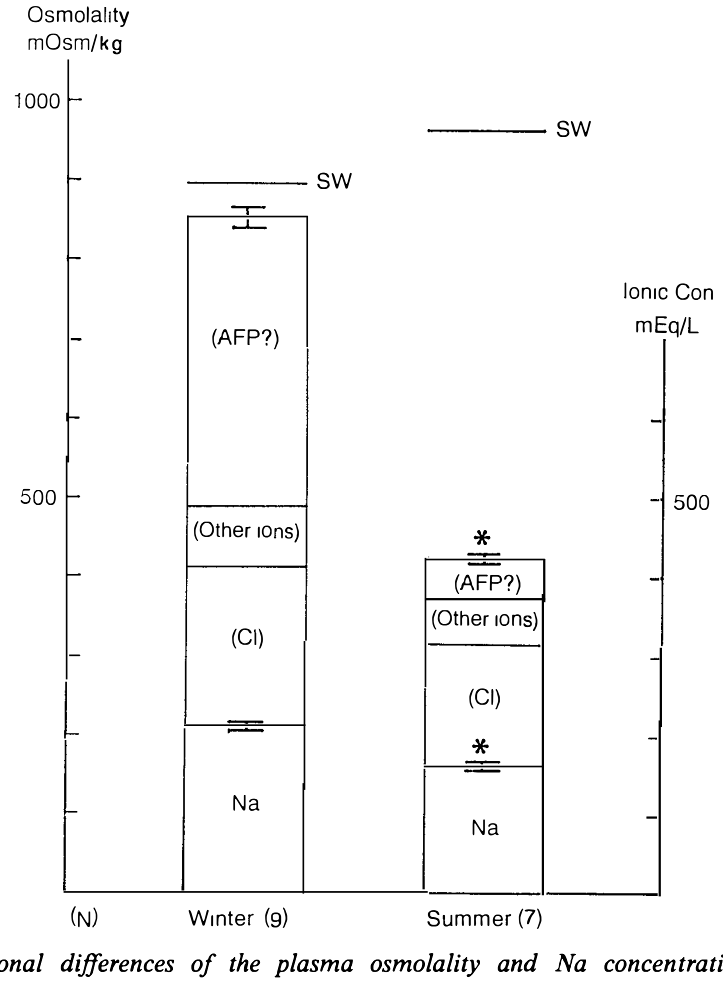

Fig. 1. Seasonal differences of the plasma osmolality and Na concentration in plasma of snowy sculpin, Myoxocephalus brandti. *SW: Osmolality of sea water. ( ): Estimated substance and its value. *: Significantly different from corresponding values for winter fish at p < 0.001.*

is made up of a repeating tripeptide with a disaccharide attached to the threonyl residues. Among AFPs, type I is an alanine-rich, amphiphilic and α-helix; type II is a larger protein with a high content of reverse turns and disulfide bridges; and type III is intermediate in size with no distinguishing features of secondary structure (DAVIES and HEW, 1990). The AFGP has been reported in Antarctic teleosts (DEVRIES *et al.*, 1970) and polar cod (OSUGA and FEENEY, 1978). Type I AFP has been described in winter flounder (DUMAN and DEVRIES, 1974) and short-horn sculpin (HEW *et al.*, 1980). Type II AFP was first isolated from the sea raven (SLAUGHTER *et al.*, 1981). Type III AFP was isolated from the eelpout (ANANTHANARAYANAN *et al.*, 1986). There is a close phylogenic relationship between the fish groups and their antifreezes.

At present in Japan, AFGP has been reported only in saffron cod (BURCHAM *et al.*, 1984). In all teleosts examined in this study, both plasma osmolality and Na concentration were higher in winter than in summer. The plasma osmolalities of the winter fish may, as has been suggested, be mainly due to the increased antifreezes, AFGP and/or AFPs. It remains to be determined what kind of antifreezes they have. From their phylogenic relation, however, it seems that *Liopsetta pinnifasciata* and *Myoxocephalus brandti* have a type I AFP, *Hypomesus pretiosus japonicus* has a type II AFP and *Zoarces elongatus* has a type III AFP. More studies are necessary to establish the presence of antifreezes and to identify their antifreezes.

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