Proc. NIPR Symp. Polar Biol., 4, 30–35, 1991

ON THE KIDNEY AND THE ADRENOCORTICAL TISSUE OF TOOTHED SMELT, *OSMERUS MORDAX DENTEX* AND SURF SMELT, *HYPOMESUS PRETIOSUS JAPONICUS* AND THEIR COLD ADAPTATION

Mizuho OGAWA[1], Mayumi FUKUDA[2], Ayumi HAYASHIDA[2] and Mitsuo FUKUCHI[3]

[1]*Graduate School of Policy Science, Saitama University, 255, Shimo-Ookubo, Urawa 338*
[2]*Faculty of Education, Saitama University, 255, Shimo-Ookubo, Urawa 338*
[3]*National Institute of Polar Research, 9–10, Kaga 1-chome, Itabashi-ku, Tokyo 173*

Abstract: The kindneys and the adrenocortical tissues of toothed smelt, *Osmerus mordax dentex* and surf smelt, *Hypomesus pretiosus japonicus* were investigated by light microscopy.

Their kidneys, which are glomerular, show no changes in the Bowman's capsules and glomeruli between the fishes caught in summer and those in winter. In contrast, the saffron cod, *Eleginus gracilis* previously observed, showed seasonal difference of the glomerulus which is considered related to the conservation of antifreeze serum glycoprotein. The present results suggest that toothed smelt and surf smelt possess a different adaptive mechanism for cold water from that of the saffron cod.

The adrenocortical tissue is located around the posterior cardinal veins in the head kidney. No seasonal difference in the cross-sectional areas of the nuclei of adrenocortical cells was observed in either the toothed smelt or the surf smelt. It is concluded that the adrenocortical cells are probably not related directly to cold adaptation in these fishes.

1. Introduction

Most of the Antarctic fishes have freezing-point-depressing glycoproteins (antifreeze glycoproteins) in their sera (DEVRIES *et al.*, 1970). Many species of Antarctic teleosts have also been reported to have aglomerular kidneys (DOBBS *et al.*, 1974; DOBBS and DEVRIES, 1975a). Such aglomerularism in the Antarctic fishes may be related to the conservation of the serum glycoprotein with "antifreeze" properties (DOBBS *et al.*, 1974; DOBBS and DEVRIES, 1975b).

Antifreeze glycoproteins have been reported not only in Antarctic fishes but also in high latitude cold water fishs (VAN VOORHIES *et al.*, 1978; OSUGA and FEENEY, 1978); further it has been noted that the properties of these substances change seasonally (DUMAN and DEVRIES, 1974a, b; FLETCHER, 1977; HEW *et al.*, 1981). Seasonal variations in the concentration of antifreeze glycoproteins have been reported in the saffron cod, *Eleginus gracilis* (BURCHMAN *et al.*, 1984). This fish also shows seasonal variations in kidney histology (KITAGAWA *et al.*, 1990). In contrast to the well-vascularied glomeruli seen in saffron cod collected during summer, the glomeruli of fish collected during winter appeared to be remarkably atrophic—physiologically similar to the aglomerular kidney. These morphological variations in kidneys of the saffron cod may be related to the conservation of antifreeze glycoproteins, an adaptation for low winter temperatures.

In the winter flounder, *Pseudopleuronectes americanus*, seasonal variations in the concentration of antifreeze glycoprotein are evidently regulated by endogenous annual cycles (FLETCHER, 1981). Pituitary has been shown to be necessary for the seasonal disappearance of the antifreeze glycoprotein (FLETCHER *et al.*, 1978; HEW and FLETCHER, 1979). Further investigation of the endocrinological control on the antifreeze glycoprotein level is required.

It would be interesting to know whether seasonal differences in the kidney structure occur in other species of fishes living in the same area as the saffron cod. In the present study, therefore, an attempt was made to observe the kidney structures in the toothed smelt, *Osmerus mordax dentex* and the surf smelt, *Hypomesus pretiosus japonicus*, and to compare them in winter and summer. The adrenocortical cells in both fishes were also investigated.

2. Materials and Methods

Toothed smelt, *Osmerus mordax dentex* (weighing 150–218 g) and surf smelt, *Hypomesus pretiosus japonicus* (weighing 117–170 g) were collected in March and July 1989 from lagoon Saroma Ko located on the Okhotsk Sea coast of Hokkaido, Japan. The water temperatures of collected point were 0.4°C in March and 13.0°C in July. No attempt was made to separate the sexes. These smelts have almost the same habitat as the saffron cod except that the toothed smelt enters rivers in spring for spawning.

The head kidney including the adrenocortical tissue and the kidney were fixed in Zenker-formol, embedded in paraffin and sectioned at 6 μm using routine procedures. Sections were stained with hematoxylin and eosin.

The measurements of Bowman's capsule and glomerular tuft were made on 20 sections from five different toothed smelts and three different surf smelts in each season. Similarly, the measurements of the cross-sectional area of adrenocortical cell nuclei were made on 20 cells from each of five toothed smelts and three surf smelts. The statistical analyses of data from the two groups (the winter fish and summer fish), were performed by Student T test.

3. Results and Discussion

3.1. Kidney

In *Osmerus mordax dentex* and *Hypomesus pretiosus japonicus*, there is a complete fusion of two sides of the kidney from the bifurcated anterior kidney (head kidney) to the posterior end of the kidney (trunk kidney). The external shape of the kidneys corresponds to Type I in the classification of gross structure of teleostean kidneys (OGAWA, 1961). The head kidney is composed of lympoid tissue and contains no renal tubules. In the anterior part of the kidney, renal tubules were rare.

In these fishes, each nephron is composed of a renal corpuscle (glomerulus and Bowman's capsule) and a tubule subdivided into segments. In the Bowman's capsules, the glomerulus is well vascularized; some blood cells were observed in the glomeruli of both of the winter and summer fish (Figs. 1 and 2). Sizes of the glomeruli and Bowman's capsules of winter and summer fish are similar (Table 1). Thus, no seasonal difference of the renal corpuscles was evident.

The tubular portion of the nephron is specialized into a ciliated neck segment and

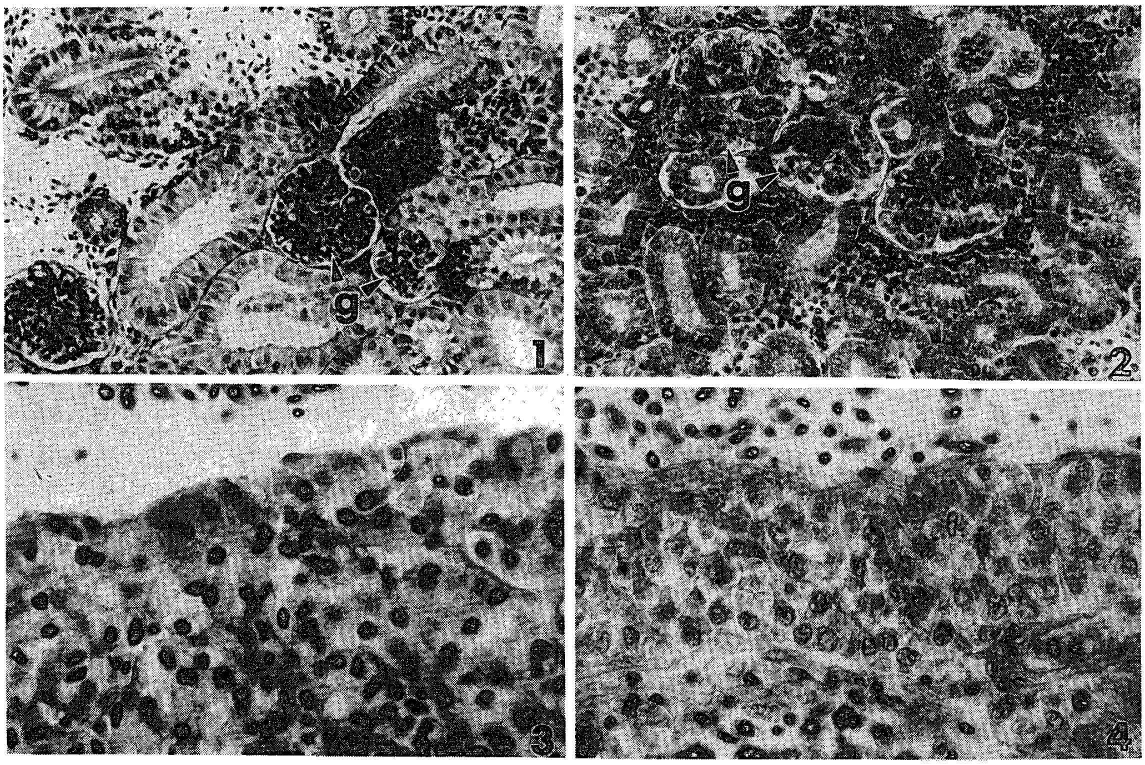

Fig. 1. Kidney of the surf smelt, Hypomesus pretiosus japonicus *collected in March. Note the well-vascularized glomerulus and some blood cells in the glomerulus. g: glomerulus. ×260.*

Fig. 2. Kidney of the surf smelt collected in July. g: glomerulus. ×260.

Fig. 3. Adrenocortical cells of the surf smelt collected in March. ×650.

Fig. 4. Adrenocortical cells of the surf smelt collected in July. ×650.

Table 1. Seasonal differences of the measurements of Bowman's capsules and glomerular tufts in the kidneys of toothed smelt, Osmerus mordax dentex *and surf smelt,* Hypomesus pretiosus japonicus.

No. of fish (SL, mm)		Bowman's capsule (μm)	Glomerular tuft (μm)
Toothed smelt, *Osmerus mordax dentex*			
winter fish	5 (160–200)	68.8±1.0	60.8±1.0
summer fish	5 (150–218)	80.3±0.8*	67.8±0.8
Surf smelt, *Hypomesus pretiosus japonicus*			
winter fish	3 (160–178)	73.9±1.1	67.1±1.0
summer fish	3 (117–132)	77.5±1.2	66.2±1.1

All values are means and standard error ($n=100$ for toothed smelt and $n=60$ for surf smelt).

SL: Standard length.

* Significantly different from corresponding those of winter fish at $P<0.01$.

two proximal segments before entering the collecting tubule. There is no distal segment. Thus, the nephron of these fishes is typical of marine teleosts. The short ciliated neck segment is continuous with Bowman's space at the urinary pole. The proximal segment is lined by cuboidal cells with abundant cytoplasm and characteristic brush borders at the apical surface. The brush border in the first portion is relatively higher than that of the second portion. The nuclei in the first portion of the proximal segment are oval and located in the basal part of the cells. On the contrary, the nuclei in the second portion are round and located in the middle. No morphological difference in nephrons of winter and summer fish was observed.

Seasonal variation in antifreeze glycoproteins was reported in the saffron cod, *Eleginus gracilis* with a five-fold higher concentration of these serum proteins in fish collected during March compared with fish collected in November (BURCHMAN *et al.*, 1984). In the saffron cod, the histological observations showed shrunken glomerular tufts during winter in which the glomeruli would be non-functional similar to aglomerular kidneys for conservation of antifreeze serum glycoproteins (KITAGAWA *et al.*, 1990). Antifreeze glycoprotein has not yet been identified in either the toothed smelt or the surf smelt. In contrast with the saffron cod, the present results suggest that these smelts must possess a different adaptive mechanism for cold water in their glomeruli if they are found to have antifreeze serum glycoproteins.

Many high latitude cold water fishes with antifreeze glycoprotein have functional glomeruli. In these, it has been suggested that the acidic antifreeze glycoprotein is conserved in the circulation because it is repelled from the negative-charged basement membrane of the glomerular capillary wall (DEVRIES, 1982). Therefore, electron-microscopical studies on the basement membrane of glomerular capillaries in each season are also required.

3.2. Adrenocortical tissue

In both species of smelts, adrenocortical tissues are located around the posterior cardinal veins in the head kidney and also extensively in the more anterior portion of the kidney. Adrenocortical cells are completely separated from the mass of chromaffin cells. This finding corresponds to Type III in the classification of distribution of the chromaffin and adrenocortical cells of teleost fishes (OGURI and HIBIYA, 1957).

Adrenocortical cells are irregular in shape and possess a spherical or oval nucleus and vacuolated cytoplasm. In both species, the cross-sectional measurements of adrenocortical cell nuclei in winter fish were similar to those of summer fish with no significant difference (Table 2). Although in the winter fishes many nuclei of adrenocortical cells were stained homogeneous with hematoxylin, the summer fishes possessed the light stained nuclei with prominant nucleoli and some chromatin scattered peripherally (Figs. 3 and 4). Such structural difference of the nuclei may be related to their functional activity. However, in both species, no seasonal difference in the cross-sectional area of adrenocortical cell nuclei was observed in comparisons of winter and summer fish. Therefore, the results suggest that the adrenocortical tissue may not be involved directly in cold adaptation.

It is well known that the adrenocortical hormone of fishes is involved in osmoregulation, especially in sea water adaptation and also that increased plasma adrenocorticoid

Table 2. Seasonal differences in the cross-sectional areas (μm^2) of adrenocortical cell nuclei in the interrenal tissue of toothed smelt, Osmerus mordax dentex *and surf smelt,* Hypomesus pretiosus japonicus.

No. of fish (SL, mm)	Area of nucleus (μm^2)
Toothed smelt, *Osmerus mordax dentex*	
winter fish 5 (160–200)	14.4±0.3
summer fish 5 (150–218)	13.5±0.3
Surf smelt, *Hypomesus pretiosus japonicus*	
winter fish 3 (160–170)	11.6±0.3
summer fish 3 (117–132)	11.5±0.3

All values are means and standard error ($n=100$ for toothed smelt and $n=60$ for surf smelt). SL: Standard length.

levels are found in a variety of stress and shock situations (HENDERSON and GARLAND, 1980). When the freshwater tropical teleost, *Colisa fasciatus* was exposed to the cold water of about 2°C, hypertrophy of the interrenal tissue was observed (AGRAWAL and SRIVASTAVA, 1978).

Further, it has been shown that the pituitary is necessary for the seasonal disappearance of the antifreeze glycoprotein from the plasma of the winter flounder during the summer (FLETCHER *et al.*, 1978; HEW and FLETCHER, 1979). Hence, further studies of the endocrine organs other than adrenocortical tissue are also desirable in two different seasons.

Acknowledgments

We are grateful to Mr. S. MIURA for the collection of fishes used in this study. We wish to express our gratitude to Emeritus Professor W. S. HOAR, University of British Columbia, for his critical reading of the manuscript.

References

AGRAWAL, U. and SRIVASTAVA, A. K. (1978): Cold-shock induced changes in the interrenal tissue of a fresh water tropical teleost, *Colisa fasciatus*. Arch. Anat. Microsc. Morphol. Exp., **67**, 1–10.

BURCHMAN, T. S., OSUGA, D. T., CHINO, H. and FEENEY, R. E. (1984): Analysis of antifreeze glycoproteins in fish serum. Anal. Biochem., **139**, 197–204.

DEVRIES, A. L. (1982): Biological antifreeze agents in cold water fishes. Comp. Biochem. Physiol., **73A**, 627–640.

DEVRIES, A. L., KOMATSU, S. K. and FEENEY, R. E. (1970): Chemical and physical properties of freezing-point-depressing glycoproteins from Antarctic fishes. J. Biol. Chem., **245**, 2901–2908.

DOBBS, G. H., III and DEVRIES, A. L. (1975a): The aglomerular nephron of Antarctic teleosts; A light and electron microscopic study. Tissue Cell, **7**, 159–179.

DOBBS, G. H., III and DEVRIES, A. L. (1975b): Renal function in Antarctic fishes; Serum and urine composition. Mar. Biol., **29**, 59–70.

DOBBS, G. H., III, LIN, Y. and DEVRIES, A. L. (1974): Aglomerularism in Antarctic fish. Science, **185**, 793–794.

DUMAN, J. G. and DEVRIES, A. L. (1974a): Freezing resistance in winter flounder *Pseudopleuronectes americanus*. Nature, **247**, 237–238.

DUMAN, J. G. and DEVRIES, A. L. (1974b): The effects of temperature and photoperiod on antifreeze production in cold water fishes. J. Exp. Zool., **190**, 89–97.

FLETCHER, G. L. (1977): Circannual cycles of blood plasma freezing point and Na and Cl concentrations in Newfoundland winter flounder (*Pseudopleuronectes americanus*); Correlation with water temperature and photoperiod. Can. J. Zool., **55**, 789–795.

FLETCHER, G. L. (1981): Effects of temperature and photoperiod on the plasma freezing point depression, Cl^- concentration, and protein "antifreeze" in winter flounder. Can. J. Zool., **59**, 193–201.

FLETCHER, G. L., CAMPBELL, C. M. and HEW, C. L. (1978): The effects of hypophysectomy on seasonal changes in plasma freezing-point depression, protein "antifreeze", and Na^+ and Cl^- concentrations of winter flounder (*Pseudopleuronectes americanus*). Can. J. Zool., **56**, 109–113.

HENDERSON, I. W. and GARLAND, H. O. (1980): The interrenal gland in Pisces, Part 2, Physiology. General, Comparative and Clinical Endocrinology of the Adrenal Cortex, Vol. 3, ed. by I. CHESTER JONES and I. W. HENDERSON. London, Academic Press, 473–523.

HEW, C. L. and FLETCHER, G. L. (1979): The role of pituitary in regulating antifreeze protien synthesis in the winter flounder. FEBS Lett., **99**, 337–339.

HEW, C. L., SLAUGHTER, D., FLETCHER, G. L. and JOSHI, S. B. (1981): Antifreeze glycoproteins in the plasma of Newfoundland Atlantic cod (*Gaudus morhua*). Can. J. Zool., **59**, 2186–2192.

KITAGAWA, Y., OGAWA, M. and FUKUCHI, M. (1990): On the kidney of the saffron cod, *Eleginus gracilis* and its cold adaptation. Proc. NIPR Symp. Polar Biol., **3**, 71–75.

OGAWA, M. (1961): Comparative study of the external shape of the teleostean kidney with relation to phylogeny. Sci. Rep. Tokyo Kyoiku Univ., **10**, 61–88.

OGURI, M. and HIBIYA, T. (1957): Studies on the adrenal gland of teleosts. II. On the adrenal tissues in 15 species of fishes. Nippon Suisan Gakkaishi (Bull. Jpn. Soc. Sci. Fish.), **23**, 144–149.

OSUGA, D. T. and FEENEY, R. E. (1978): Antifreeze glycoproteins from Arctic fish. J. Biol. Chem., **253**, 5338–5343.

VAN VOORHIES, W. V., RAYMOND, J. A. and DEVRIES, A. L. (1978): Glycoproteins as biological antifreeze agents in the cod, *Gadus ogac* (RICHARDSON). Physiol. Zool., **51**, 347–353.

(*Received April 6, 1990; Revised manuscript received July 19, 1990*)