Acid Tolerance of Japanese Dace (a Cyprinid Teleost) in Lake Osorezan, a Remarkable Acid Lake

Authors: Kaneko, Toyoji, Hasegawa, Sanae, Uchida, Katsuhisa, Ogasawara, Tsuyoshi, Oyagi, Akira, et al.

Source: Zoological Science, 16(6) : 871-877

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.16.871

ZOOLOGICAL SCIENCE **16**: 871–877 (1999)

Acid Tolerance of Japanese Dace (a Cyprinid Teleost) in Lake Osorezan, a Remarkable Acid Lake

Toyoji Kaneko[1]*, Sanae Hasegawa[1], Katsuhisa Uchida[1], Tsuyoshi Ogasawara[2], Akira Oyagi[3] and Tetsuya Hirano[1]

[1]*Ocean Research Institute, University of Tokyo, Nakano, Tokyo 164-8639, Japan,*
[2]*Faculty of Science, Kanagawa University, Hiratsuka, Kanagawa 259-1293, Japan and*
[3]*Tanabu High School, Mutsu, Aomori 035-0054, Japan*

ABSTRACT—The Japanese dace (*Tribolodon hakonensis*) is the only teleost species that inhabits Lake Osorezan, a remarkable acid lake in Japan with the water pH of 3.4–3.8. In the present study, physiological changes following transfer of the dace acclimated to neutral stream water (pH 6.8–7.2) to acid lake water (pH 3.6–3.7) were examined with special reference to changes in gill chloride cell morphology. The dace survived direct transfer to acid lake water for 3 days. Plasma [Na^+] and [Cl^-] showed transient decreases at 1 hr after transfer; however, the decrease in [Na^+] was greater than that in [Cl^-]. The recovery of [Cl^-] was more evident than that of [Na^+]. The transient decreases of plasma [Na^+] and [Cl^-] were followed by acidosis. Blood pH was decreased by 0.13 unit at 6 hr, but partially restored by 24 hr. In the dace acclimated to neutral stream water, chloride cells were scattered in the gill filament. Following transfer to acid lake water, however, well-developed chloride cells were arranged in a radial fashion, forming follicular or gland-like structures in the gills. Each chloride cell was equipped with an apical pit, which faced a common lumen leading to the external environment. These findings provide morphological evidence for a significant role(s) of gill chloride cells in ion and acid-base regulation in the acid-tolerant dace.

INTRODUCTION

Lake Osorezan is located in Shimokita Peninsula, lying in the northernmost district of Honshu, the mainland of Japan. This is a remarkable acid lake, and strong acidity of pH 3.4–3.8 is derived from hot springs containing sulfuric acid (Masiko, 1940; Satake *et al.* 1995). The lake is fed by several streams, originating from the surrounding mountains. The water pH is neutral in most streams, except for some small ones flowing into the northern region of the lake. The lake empties its water into the Pacific Ocean through the Shozu River, the only outlet from the lake.

Among teleosts that are generally intolerant of acid environments, the Japanese dace (*Tribolodon hakonensis*) is the only species inhabiting this acid lake (Masiko, 1940). Thus, the dace is expected to possess excellent acid tolerant ability. Exposure of teleost fish to acid environments has been reported to alter gill chloride cell number, distribution and morphology (Jagoe and Haines, 1990; Leino and McCormick, 1984; Leino *et al.*, 1987; Wendelaar Bonga *et al.*, 1990), implying the involvement of chloride cells in adaptation to acidic environments. Mashiko *et al.* (1973) have described well-developed complexes of chloride cells in the gills of the dace in Lake Osorezan; chloride cells are arranged in a radial fashion, forming a gland-like structure. Occurrence of these chloride cell complexes may be related to the strong acid tolerance of the dace in Lake Osorezan. However, little information is available on the acid-tolerant mechanism of the dace.

Considering the strong acid-tolerant ability, the dace would be a suitable model to search for mechanisms of adaptation to acid environments in teleosts. In the present study, to gain a better understanding of acid-tolerant mechanisms and to clarify the involvement of branchial chloride cells in acid-base regulation, we examined physiological and morphological changes in response to environmental acidification in Osorezan dace.

MATERIALS AND METHODS

Fish

During the spawning period of the dace in Lake Osorezan extending from mid-May to early August, mature fish migrate from the acid lake up to neutral streams. The fish used in this study were caught by means of netting in one of neutral streams, Maruyamasawa, in early August 1994. The fish caught at the end of the spawning season were considered to be acclimated well to neutral stream water, presumably having spent in the stream for several weeks. They were stocked in fish cages (60 *l*) immersed in the stream water.

* Corresponding author: Tel. +81-3-5351-6531;
FAX. +81-3-5351-6531.
E-mail: kaneko@ori.u-tokyo.ac.jp

Transfer experiment

The dace stocked in the stream for 2 days were directly transferred to acid lake water. The pH and ion concentrations of lake and stream waters are shown in Table 1. About 80 fish weighing 13-34 g were used for the transfer experiment. Two 60-*l* cages containing about 40 fish each were immersed in the lake. The dace were sampled at 0 (before transfer), 1, 3, 6, 12, 24, 48 and 72 hr after transfer.

At sampling, fish were removed from the cage and anesthetized with 2-phenoxyethanol. After measurement of the body weight, blood was drawn into a syringe pretreated with heparin-ammonium from the caudal vessels. Blood samples in the syringes were kept in an ice box and subjected to blood pH determination within 30 min. Blood was then centrifuged at 2,000 g for 5 min to obtain plasma, which were kept frozen at –40°C until measurement of ion concentrations. For histological observations, the gills were sampled at 0, 24 and 72 hr.

Table 1. The pH and ion concentrations of Lake Osorezan and stream waters

Parameter	Lake water	Stream water
pH	3.6–3.7	6.8–7.2
Ion concentration (*mM*)		
Na+	0.92	0.40
Cl^-	0.84	0.25
K^+	0.06	0.02
Ca^{2+}	0.21	0.11
Mg^{2+}	0.07	0.05
SO_4^{2-}	0.49	0.03

Blood analyses

Blood pH was measured using a pH analyzer (AVL 9110, Graz, Austria). Plasma [Na^+] was determined by atomic absorption spectrophotometry (Hitachi 180-50, Tokyo), and plasma [Cl^-] with a chloridometer (Buchler 4-2500).

Immunocytochemistry

The gills were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 24 hr and preserved in 70% ethanol. Later, the tissues were dehydrated in ethanol and embedded in paraplast. Cross sections (4 μm thickness) of gill filaments were mounted on gelatin-coated slides. To detect chloride cells, the gill sections were immunocytochemically stained with an antiserum specific for Na^+, K^+-ATPase according to Uchida *et al.* (1996). The sections were incubated overnight with the antiserum raised against a synthetic peptide corresponding to part of the highly conserved region of the Na^+, K^+-ATPase α-subunit (Ura *et al.*, 1996) at a dilution of 1:500. The specificity of the immunoreaction has been confirmed previously (Uchida *et al.*, 1996; Ura *et al.*, 1996).

For the quantitative analysis, the percentage of the total chloride cell area to the cross sectional area of the gill filament (% chloride cell area) was used as criteria to describe chloride cell activity. Nine representative cross sections of gill filaments were photographed from three individuals for each group. The cross sectional area of the gill filament (filament area) and the total area of chloride cells (chloride cell area) were measured on microphotographs using a digitizer (KD 4600, Graphtec, Tokyo). The cartilage tissue and central venous sinus located in the center of the gill filament were excluded from the filament area. The % chloride cell area was calculated as chloride cell area ×100 / filament area.

Transmission and scanning electron microscopy

For transmission and scanning electron microscopic observations on gill chloride cells, the gills at 0 and 72 hr after transfer were fixed in 2% paraformaldehyde-2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 4 hr. For the transmission electron microscopy, some gill tissues were cut into small pieces, postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 1 h, and preserved in 70% ethanol. The postfixed gill fragments were then dehydrated in ethanol and embedded in Spurr's resin. Ultrathin sections, cut with a diamond knife, were mounted on grids. The sections were stained with uranyl acetate and lead citrate, and examined with a Hitachi H-7100 transmission electron microscope.

For the scanning electron microscopy, the gill tissues fixed in 2% paraformaldehyde-2% glutaraldehyde were dehydrated in ethanol, transferred to *t*-butylalcohol, and dried using a JEOL freeze drying device (JFD-300). The gill tissue was mounted on a specimen stub, coated with gold in a JEOL ion sputter (JFC-1100), and examined with a Hitachi S-2150 scanning electron microscope.

Statistics

Numerical data were expressed as the mean±SEM. For statistical analysis, Student's *t*-test or Cochran-Cox test was applied after F-test for variance analysis.

RESULTS

Transfer to acid lake water

The dace survived direct transfer from neutral stream water to acid lake water; no fish died during 72 hr after transfer. Plasma [Na^+] was significantly decreased at 1 hr after trans-

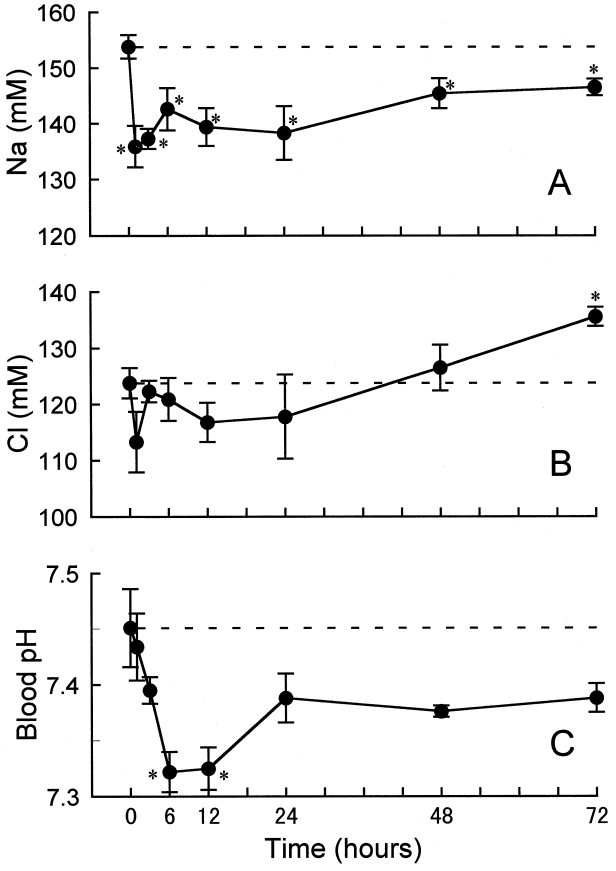

Fig 1. Changes in plasma [Na^+] (**A**), [Cl^-] (**B**) and blood pH (**C**) following transfer of Osorezan dace from neutral stream water (pH 6.8–7.2) to acid lake water (pH 3.6–3.7). Data are expressed as the mean±SEM (n=7–11). Asterisks indicate significant difference ($p<0.05$) from the initial values.

fer, and then increased at 6 hr. The increased levels, still significantly lower than those before transfer, were maintained until 72 hr (Fig. 1A). Plasma [Cl^-] also showed a transient decrease at 1 hr. However, the decrease in [Cl^-] was less evident than that in [Na^+]. The decreased [Cl^-] was restored to the initial levels by 3 hr, followed by a significant increase at 72 hr (Fig. 1B). In contrast with rapid decreases in plasma [Na^+] and [Cl^-], the blood pH was decreased significantly by 0.13 unit at 6 hr after transfer, and partially restored by 24 hr (Fig. 1C).

Morphological changes in gill chloride cells

Figure 2 shows cross sections of gill filaments stained with the anti-Na^+, K^+-ATPase at 0, 24 and 72 hr. Na^+, K^+-ATPase-immunoreactive chloride cells were observed in the outer layer of the gill filaments. In fish acclimated to neutral water, chloride cells appeared individually in the gills (Fig. 2A). At 72 hr after transfer to acid water, well-developed chloride cells were arranged in a radial fashion, forming a follicular or gland-like structure (Fig. 2C). The follicular arrangement of chloride cells was observed more abundantly on the afferent vascular side than on the efferent. These structures are mostly located in the interlamellar regions and in the afferent edge of the gill filament. A large follicular structure consists of more than 10 chloride cells on the section with an expanded lumen in the center. Follicular structures were also observed at 24 hr after transfer, but less developed than those observed at 72 hr (Fig. 2B). In accordance with these morphological changes, the % chloride cell area was significantly increased at 72 hr (Fig. 3).

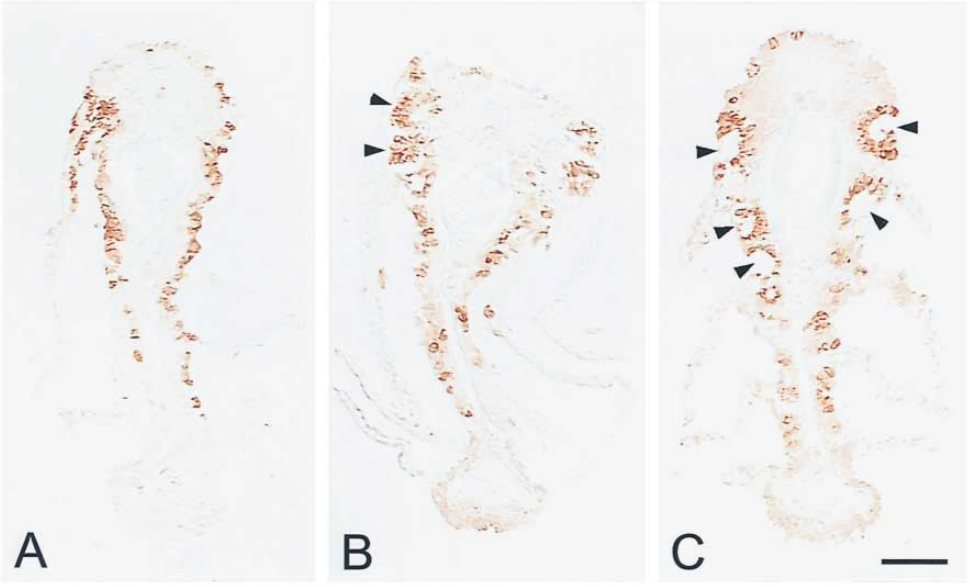

Fig. 2. Cross sections of gill filaments stained with anti-Na^+, K^+-ATPase in Osorezan dace at 0 hr (**A**), 24 hr (**B**) and 72 hr (**C**) after transfer to acid lake water. Immunoreactive chloride cells form follicular structures (arrowheads) following transfer. Follicular structures of chloride cells are more abundant on the afferent vascular side (top) than on the efferent (bottom). Bar, 50 μm.

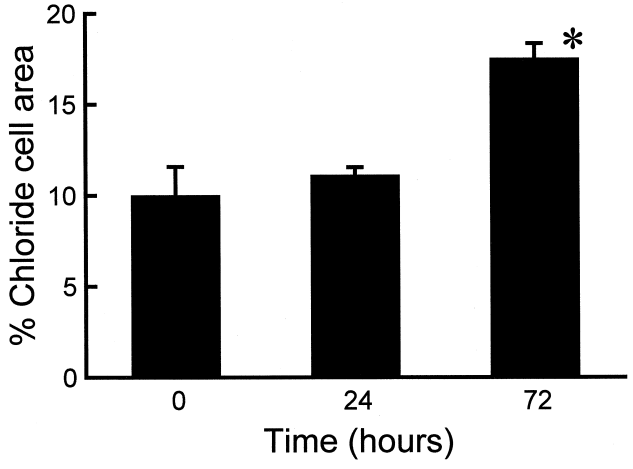

Fig. 3. Change in % chloride cell area following transfer of Osorezan dace from neutral stream water (pH 6.8–7.2) to acid lake water (pH 3.6–3.7). Data are expressed as the mean±SEM (n=9). An asterisk indicates significant difference ($p<0.05$) from the initial value.

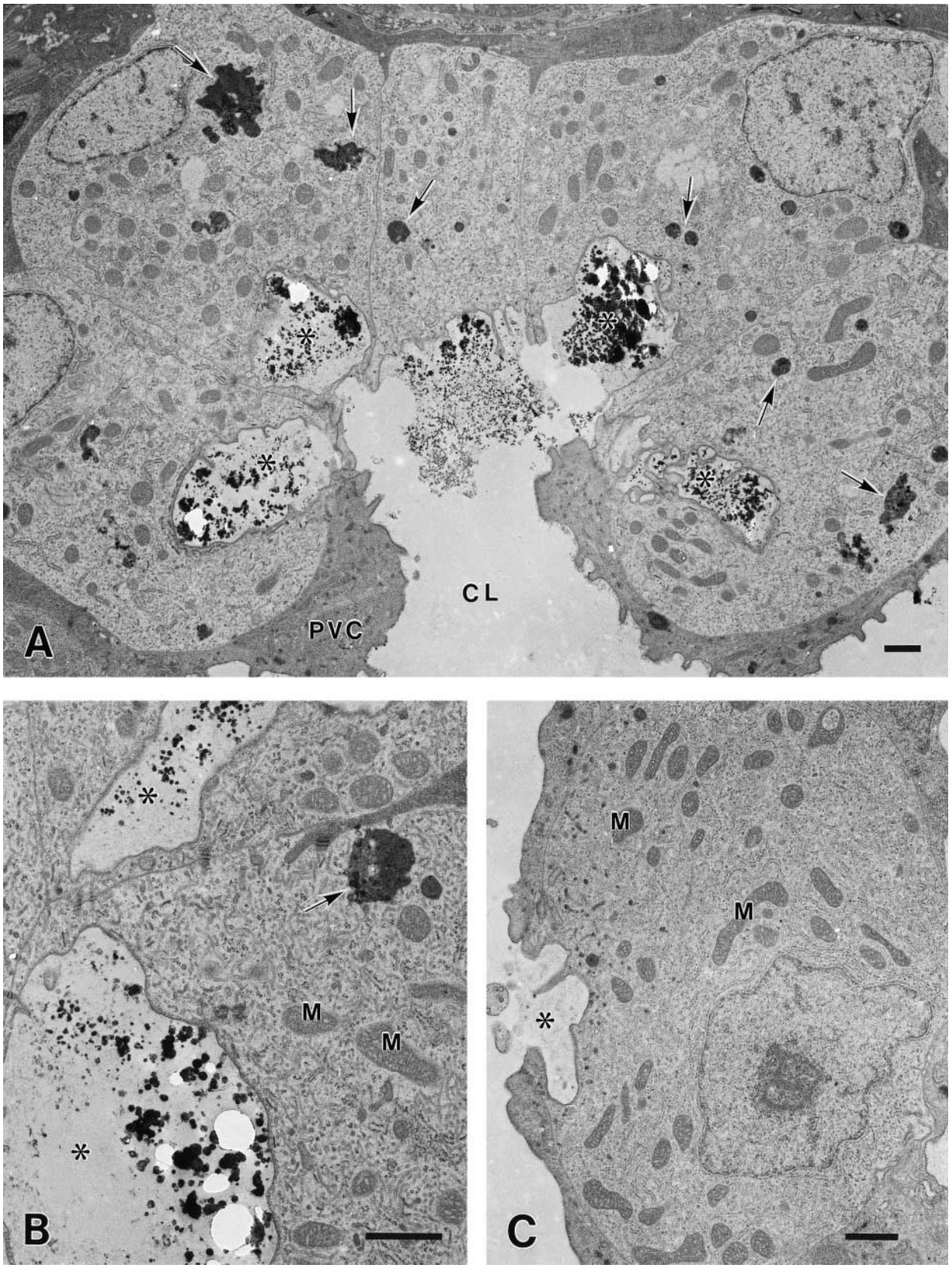

Fig. 4. Transmission electron micrographs of follicular arrangement of chloride cells in Osorezan dace transferred to acid lake water (**A** and **B**), and an individual chloride cell in those acclimated to neutral stream water (**C**). Follicular chloride cells are equipped with an apical pit (asterisk), which faces a common lumen (CL) leading to the external environment. Arrows indicate electron-dense bodies in the cytoplasm of follicular chloride cells. M, mitochondria; PVC, pavement cell. Bars, 1 μm.

Electron microscopic observations on gill chloride cells

The follicular structures of gill chloride cells were observed in the dace at 72 hr after transfer to acid lake water by the transmission electron microscopy (Fig. 4A, B). As shown by the light microscopy, chloride cells are arranged in a radial fashion (Fig. 4A). Chloride cells are linked to each other by tight junctions (Fig. 4B), and no cytoplasmic interdigitation is formed between chloride cells. In each cell, the tubular system and mitochondria are moderately developed in the cytoplasm, and a round nucleus is usually located in the basal side. The cells are equipped with an apical pit, which faces a common lumen leading to the external environment. The apical pits and the common lumen often contain electron-dense bodies and electron-lucent materials. Similar electron-dense bodies of irregular shape are also scattered in the cytoplasm. In the fish adapted to neutral stream water, the follicular arrangement of chloride cells is not developed; chloride cells are sparsely located in the gill filament (Fig. 4C). Although these chloride cells also possess an apical pit, electron-dense bodies and electron-lucent materials observed in the transferred fish are not evident in the apical pits. Furthermore, electron-dense bodies are absent in the cytoplasm.

By the scanning electron microscopy, the common lumens of the follicular chloride cells appear as large pits on the gill filament (Fig. 5). These openings of chloride cell follicles, 10–20 μm in diameter, are located among pavement cells in the interlamellar region of the afferent vascular side and the afferent edge of the filament, but not in the efferent vascular side. Such structures are absent in the fish acclimated to neutral water.

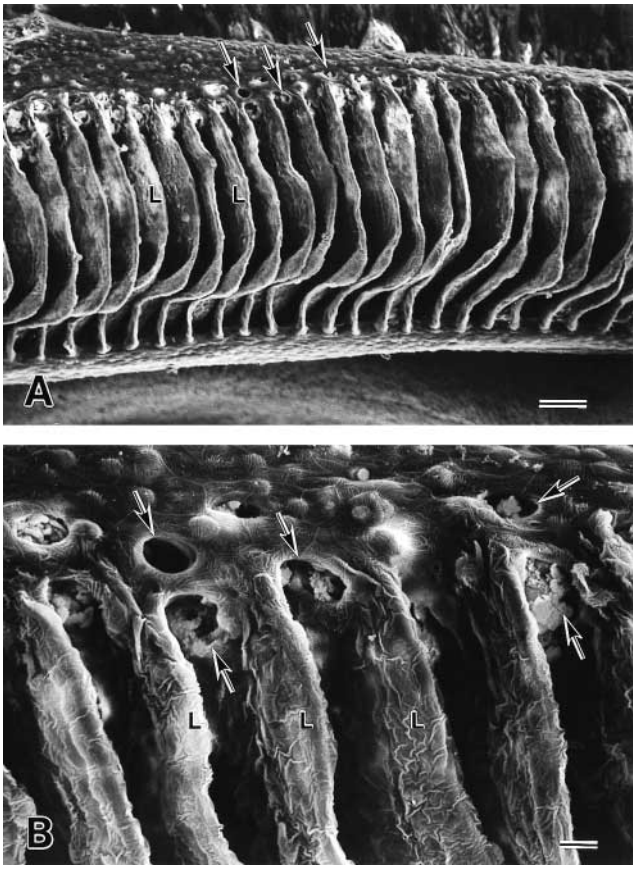

Fig. 5. Scanning electron micrographs of a gill filament in Osorezan dace transferred to acid lake water. Common lumens of follicular chloride cells appear as large openings (arrows) on the afferent vascular side (top) of the gill filament. L, gill lamella. Bars: A, 50 μm; B, 10 μm.

DISCUSSION

The genus *Tribolodon* belonging to the family Cyprinidae is widely distributed from rivers to coastal regions of the sea in Japan and the adjacent countries, whereas most cyprinids are typically freshwater fish. The Japanese dace (*T. hakonensis*), one of *Tribolodon* species in Japan, shows greater geographic differentiation. Based on allozymic variation analysis, Hanzawa *et al.* (1987, 1988) have indicated that populations of different local groups are highly differentiated from each other. In the present study, Osorezan dace acclimated to neutral stream water survived direct transfer to acid lake water. In contrast, the same species of the dace caught in the neighboring area and a geographically distinct area could neither survive transfer to the acid water of Lake Osorezan, nor develop the follicular arrangement of chloride cells (our unpublished data). Thus, Osorezan dace seems more tolerant of acid environments than other strains of the same species, indicating strain-specific difference in acid-tolerant ability.

Ion and acid-base regulation in acid water

In the present study, plasma [Na^+] and [Cl^-] showed decreases shortly after transfer, which were corrected by 6 hr. Exposure to acid environments has been reported to cause ion losses in fish, and plasma ion levels have proved to be a fairly reliable indicator of sublethal acid stress (Goss *et al.*, 1995; Leivestad and Muniz, 1976). The observed decreases in plasma ion levels may be caused by increased membrane permeability in the acid environment (Goss *et al.*, 1995; McDonald *et al.*, 1991; Wood, 1989). Decrease in [Na^+] was more evident than that in [Cl^-] shortly after transfer. Moreover, plasma [Cl^-] was significantly higher at 72 hr than the initial level, whereas stabilized levels of plasma [Na^+] at 72 hr was lower than the initial. Thus, a greater loss of [Na^+] than [Cl^-] is evident in the dace transferred to acid lake.

Transient decreases in plasma [Na^+] and [Cl^-] were followed by acidosis; a significant decrease in blood pH was first observed at 6 hr after transfer to acid water, whereas plasma [Na^+] was significantly decreased as early as 1 hr after transfer. It is well documented that the gills alter Na^+/H^+ and Cl^-/HCO_3^- exchanges in response to acid-base disturbance (Cameron, 1976; Goss *et al.*, 1992b; Maetz, 1973). The observed acidosis could be in part the result of an increase in Cl^-/HCO_3^- exchange; Cl^- uptake occurs in exchange for HCO_3^-, and the resultant decrease in HCO_3^- may cause blood acidosis. The Na^+/H^+ exchange, which uptakes Na^+ concomitant with H^+ secretion, seems less active than the Cl^-/HCO_3^-

exchange, because the observed recovery of [Cl^-] was more evident than that of [Na^+]. As a combined result of those ion transport activities, blood pH is likely to decrease in compensation for the recovery of [Cl^-] and [Na^+] in Osorezan dace.

Involvement of chloride cells in ion and acid-base regulation

In general, chloride cells have diverse functions in ion regulation in both fresh water and seawater (McCormick, 1995). Chloride cells secrete excess Na^+ and Cl^- to maintain ionic balance in seawater. Foskett and Scheffey (1982) provided direct evidence for chloride cells as the site of Cl^- secretion in seawater. On the other hand, proliferation in response to low ion content in fresh water has suggested an active role of chloride cells in ion uptake (Avella *et al.*, 1987; Flik *et al.*, 1995; Greco *et al.*, 1996; Laurent and Dunel, 1980; Laurent *et al.*, 1985; Perry and Laurent, 1989).

As suggested previously by Mashiko *et al.* (1973), it is most probable that the excellent acid-tolerant ability of Osorezan dace is closely related to well-developed chloride cells. Following transfer, the % chloride cell area increased and follicular arrangement of chloride cells became evident in Osorezan dace. Morphological changes in gill chloride cells during acid-base disturbance caused by acid environments have been documented in several species. Exposure to acidified water has been shown to increase the number and distribution of gill chloride cells and to alter their morphology (Jagoe and Haines, 1990; Leino and McCormick, 1984; Leino *et al.*, 1987; Wendelaar Bonga *et al.*, 1990). Besides Osorezan dace, however, the yellow perch (*Perca flavescens*, Percidae) is the only species that exhibits follicular or gland-like arrangement of chloride cells in acid water (Leino *et al.*, 1987). Interestingly, the yellow perch also shows great acid tolerance. Thus, exposure to acid water induces development of chloride cells to varying degrees, and the acid-tolerant ability may depend in part on potential capability of development and functional differentiation of chloride cells in response to water acidification.

Functional significance of follicular arrangement of chloride cells

In Osorezan dace, an increased number of chloride cells form follicular structures, which results in a great increase in total apical surface area of chloride cells without reducing the gill respiratory surface. A great increase in chloride cell population without forming follicles would considerably reduce the respiratory surface of pavement cells (Greco *et al.*, 1996). Thus, the follicular formation could be an adaptive response to increase ion transporting activity of chloride cells without affecting gas exchange efficiency. Well-developed chloride cells with increased apical surface area in Osorezan dace suggest their active roles in H^+ secretion and/or HCO_3^- retention to correct blood acidosis. On the other hand, during respiratory acidosis induced by hypercapnia or hyperoxia, the surface area of chloride cells exposed to the water is reduced by being covered by adjacent pavement cells in catfish and rainbow trout (Goss *et al.*,1992a, b, 1994, 1995). Such morphological changes may serve to reduce Cl^-/HCO_3^- exchange activity in the apical surface of chloride cells, and thus lower the rate of HCO_3^- excretion, which is the predominant response to correct acidosis.

Chloride cells and accessory cells often form a multicellular complex in seawater fish (Hootman and Philpott, 1980; Sardet *et al.*,1979; Shiraishi *et al.*, 1997). The multicellular complex has been considered to provide leaky paracellular pathways, which may be important in Na^+ secretion in seawater fish (Degnan and Zadunaisky, 1980). Hwang (1988) also found multicellular complexes of chloride cells in some freshwater teleosts. The follicular arrangement of chloride cells observed in Osorezan dace is distinct from those multicellular complexes: First, the cells in the follicles are mostly similar to one another in their morphology, and thus the follicles may not be simply the complex of chloride cells and accessory cells. Second, chloride cells in the follicles are linked by tight junctions without forming interdigitation, whereas chloride cells and accessory cells interdigitate each other and junctions between them are shallow and leaky (Hootman and Philpott, 1980; Sardet *et al.*, 1979; Shiraishi *et al.*, 1997).

The chloride cell number reflects a balance between differentiation and degeneration of chloride cells (Uchida and Kaneko, 1996). Following transfer of Osorezan dace to acid water, chloride cell differentiation may predominate over degeneration, resulting in the increase in the cell number. The accessory cells have been interpreted as immature, differentiating chloride cells (Hootman and Philpott, 1980; Sardet *et al.*, 1979; Wendelaar Bonga *et al.*, 1990). Because accessory cells share their apical pits with mature chloride cells, rapid recruitment of chloride cells without degeneration of preexisting chloride cells would lead to formation of the follicular arrangement. Thus, the development of follicular arrangement may imply increasing demand for chloride cells in acid environments.

ACKNOWLEDGEMENTS

We express our gratitude to Prof. K. Jozuka, University of Kanazawa, for his encouragement during the course of this study. We are grateful to Prof. K. Yamauchi, Hokkaido University, for providing us with anti-Na^+, K^+-ATPase. This study was supported in part by Grants-in-Aid from Ministry of Education, and Global Environment Research Promotion Fund, Environment Agency of Japan.

REFERENCES

Avella M, Mason A, Bornancin M, Mayer-Gostan N (1987) Gill morphology and sodium influx in the rainbow trout (*Salmo gairdneri*) acclimated artificial freshwater environments. J Exp Zool 241: 159–169

Cameron JN (1976) Branchial ion uptake in Arctic grayling: Resting values and effects of acid-base disturbance. J Exp Biol 64: 711–725

Degnan KJ, Zadunaisky JA (1980) Passive sodium movements across the opercular epithelium: The paracellular shunt pathway and ionic conductance. J Membr Biol 55: 175–185

Flik G, Verbost PM, Wendelaar Bonga SE (1995) Calcium transport processes in fishes. In "Cellular and Molecular Approaches to Fish Ionic Regulation" Ed by CM Wood, TJ Shuttleworth, Academic Press, San Diego, pp 317–342

Foskett JK, Scheffey C (1982) The chloride cell: Difinitive identification as the salt-secretory cell in teleosts. Science 215: 164–166

Goss GG, Laurent P, Perry SF (1992a) Evidence for a morphological component in acid-base regulation during environmental hypercapnia in the brown bullhead (*Ictalurus nebulosus*). Cell Tissue Res 268: 539–552

Goss GG, Perry SF, Wood CM, Laurent P (1992b) Mechanisms of ion and acid-base regulation at the gills of freshwater fish. J Exp Zool. 263: 143–159

Goss G, Perry S, Laurent P (1995) Ultrastructural and morphometric studies on ion and acid-base transport processes in freshwater fish. In "Cellular and Molecular Approaches to Fish Ionic Regulation" Ed by CM Wood, TJ Shuttleworth, Academic Press, San Diego, pp 257–284

Goss GG, Wood CM, Laurent P, Perry SF (1994) Morphological responses of the rainbow trout (*Oncorhynchus mykiss*) gill to hyperoxia, base ($NaHCO_3$) and acid (HCl) infusions. Fish Physiol Biochem 12: 465–477

Greco AM, Fenwick JC, Perry, SF (1996) The effects of soft-water acclimation on gill structure in the rainbow trout *Oncorhynchus mykiss*. Cell Tissue Res 285: 75–82

Hanzawa N, Yonekawa H, Numachi K (1987) Variability of mitochondrial DNA in Japanese dace, *Tribolodon hakonensis* (Cyprinidae). Jpn J Genet 62: 27–38

Hanzawa N, Taniguchi N, Numachi K (1988) Geographic differentiation in populations of Japanese dace *Tribolodon hakonensis* deduced from allozymic variation. Zool Sci 5: 449–461

Hootman SR, Philpott CW (1980) Accessory cells in teleost branchial epithelium. Am J Physiol 238: R199–R206

Hwang PP (1988) Multicellular complex of chloride cells in the gills of freshwater teleosts. J Morphol 196: 15–22

Lin H, Randall D (1995) Proton pumps in fish gills. In "Cellular and Molecular Approaches to Fish Ionic Regulation" Ed by CM Wood, TJ Shuttleworth, Academic Press, San Diego, pp 229–255

Jagoe CH, Haines TA (1990) Morphometric effects of low pH and limed water on the gills of Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 47: 2451–2460

Laurent P, Dunel S (1980) Morphology of gill epithelia in fish. Am J Physiol 238: R147–R159

Laurent P, Hobe H, Dunel-Erb S (1985) The role of environmental sodium and chloride relative to calcium in gill morphology of freshwater salmonid fish. Cell Tissue Res 240: 675–692

Leino RL, McCormick JH (1984) Morphological and morphometrical changes in chloride cells of the gills of *Pimephales promelas* after chronic exposure to acid water. Cell Tissue Res 236: 121–128

Leino RL, McCormick JH, Jensen KM (1987) Changes in gill histology of fathead minnows and yellow perch transferred to soft water or acidified soft water with particular reference to chloride cells. Cell Tissue Res 250: 389–399

Leivestad H, Muniz IP (1976) Fish kill at low pH in a Norwegian river. Nature 259: 391–392

Maetz J (1973) Na^+/NH_4^+, Na^+/H^+ exchanges and NH_3 movement across the gill of *Carassius auratus*. J Exp Biol 58: 255–275

Masiko K (1940) Limnological study of Lake Osoresan-ko, a remarkable acidotrophic lake in Japan. Sci Rep Tohoku Imp Univ, 4th Ser, Biology 15: 331–356

Mashiko K, Jozuka K, Asakura K (1973) Different type of chloride cells in the gills of *Tribolodon hakonensis* from Lake Osoresanko. Ann Rep Noto Mar Lab 13: 33–37

McCormick SD (1995) Hormonal control of gill Na^+, K^+-ATPase and chloride cell function. In "Cellular and Molecular Approaches to Fish Ionic Regulation" Ed by CM Wood, TJ Shuttleworth, Academic Press, San Diego, pp 285–315

McDonald DG, Freda J, Cavdek V, Gonzalez R, Zia S (1991) Interspecific differences in gill morphology of freshwater fish in relation to tolerance of low-pH environments. Physiol Zool 64: 124–144

Perry SF, Laurent P (1989) Adaptational responses of rainbow trout to lowered external NaCl concentration: Contribution of the branchial chloride cell. J Exp Biol 147: 147–168

Sardet C, Pisam M, Maetz J (1979) The surface epithelium of teleostean fish gills. J Cell Biol 80: 96–117

Satake K, Oyagi A, Iwao Y (1995) Natural acidification of lakes and rivers in Japan: The ecosystem of Lake Usoriko (pH 3.4–3.8). Water Air Soil Pollution 85: 511–516

Shiraishi K, Kaneko T, Hasegawa S, Hirano T (1997) Development of multicellular complexes of chloride cells in the yolk-sac membrane of tilapia (*Oreochromis mossambicus*) embryos and larvae in seawater. Cell Tissue Res 288: 583–590

Uchida K, Kaneko T (1996) Enhanced chloride cell turnover in the gills of chum salmon fry in seawater. Zool Sci 13: 655–660

Uchida K, Kaneko T, Yamauchi K, Hirano T (1996) Morphometrical analysis of chloride cell activity in the gill filaments and lamellae and changes in Na^+, K^+-ATPase activity during seawater adaptation in chum salmon fry. J Exp Zool 276: 193–200

Ura K, Soyano K, Omoto N, Adachi S, Yamauchi K (1996) Localization of Na^+, K^+-ATPase in tissues of rabbit and teleosts using an antiserum directed against a partial sequence of the α-subunit. Zool Sci 13: 219–227

Wendelaar Bonga SE, Flik G, Balm PHM, van der Meij JA (1990) The ultrastructure of chloride cells in the gills of the teleost *Oreochromis mossambicus* during exposure to acidified water. Cell Tissue Res 259: 575–585

Wood CM (1989) The physiological problems of fish in acid waters. In "Acid Toxicity and Aquatic Animals" Ed by R Morris, EW Taylor, DJA Brown, JA Brown, Cambridge University Press, Cambridge, pp 125–152

(Received May 21, 1999 / Accepted July 9, 1999)