2種のティラピア、オレオクロミス・ニロチクスとモザンビクスの酸性環境への寛容性の違い

誌名	中央水産研究所研究報告
ISSN	09158014
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発行元	水産庁中央水産研究所
巻/号	9号
掲載ページ	p. 11-18
発行年月	1997年2月

中央水研研報 第9号 11～18頁 平成9年2月
Bull. Natl. Res. Inst. Fish. Sci., No.9, 11～18 , Feb. 1997

Differences in Tolerance to Acidic Environments between Two Species of Tilapia, *Oreochromis niloticus* and *O. mossambicus*.

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Abstract : Studies were undertaken to examine the difference in adaptability to low pH environment between two species of tilapia, *Oreochromis niloticus* and *O. mossambicus*.

Three days after transfer from neutral water to acidic water of pH 4. 5, 4.0 or 3.5, *O. niloticus* below pH 4. 5 showed a significant decrease in plasma sodium levels.

In *O. mossambicvs*, decreased levels of plasma sodium were observed only at pH 3. 5. The time course of changes in plasma sodium of *O. niloticus* showed a continuous decrease after the exposure to acidic water of pH 3. 5. On the other hand, plasma sodium of *O. mossambicus* reached the lowest level 3 days after the exposure and then showed a tendency to increase to the control levels. In both species, gill Na^+, K^+- ATPase activity increased after the exposure to acidic water. These results indicate that *O. mossambicus* has a greater ability to maintain plasma sodium in acidic water than *O. niloticus*.

Received : March 27, 1996
Contribution A No. 54 from the National Research Institute of Fisheries Science.

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Introduction

The toxicity of low pH environment to fishes has been studied with reference to water pollution (Fromm, 1980; Wood, 1989; Goss *et al.*, 1995). Field data show a correlation between a decrease in the number of fish species observed in a lake and a degree of water acidification (Brown and Sadler, 1989; Turnpenny, 1989). The fact indicates that fish exhibit profound species-specific differences in acid tolerance. On the other hand, there are some similarities in the physiological response of fish to water acidification (Beamish, 1976; Turnpenny, 1989). Water acidification results in a large increase in the net sodium loss mainly from the gills (Fromm, 1980; Wood, 1989; Goss *et al.*, 1995). A continuous increase in sodium loss is followed by a large shift of fluid into cells increasing the intracellular fluid volume and a severe hypovolaemia, which is thought to be a main cause of death of fish exposed to acidic environments (Wood, 1989). In laboratory studies, the maintenance of plasma sodium levels in acidic water seems to be related to the activity of gill Na^+, K^+-ATPase (Saunders *et al.*, 1983; McKeown *et al.*, 1985; Powell and McKeown 1986). It is well known that the activity of gill Na^+, K^+-ATPase which is localized in the chloride cells has an important role in the excretion of excess salt in sea water (see reviews by De Renzis and Bornancin, 1984; Zadunaisky, 1984). On the other hand, in freshwater fish, gill Na^+, K^+-ATPase activity is thought to be important for sodium uptake (Randall *et al.*, 1982; Evans, 1993; Goss *et al.*, 1995; McCormick, 1995). An activation of gill Na^+, K^+-ATPase in response to acidic water seems to be involved in the maintenance of plasma sodium levels in rainbow trout (*Oncorhynchus mykiss*) exposed to acidic water (McKeown *et al.*, 1985).

Two species of tilapia of the genus *Oreochromis*, *O. mossambicus* and *O. niloticus*, show a difference in their hypoosmoregulatory ability in sea water. *Oreochromis mossambicus* can survive a direct transfer from fresh water to full-strength sea water, whereas *O. niloticus* can not (Balarin and Hatton, 1979). In studies on hyperosmoregulation in acidic water, *O. mossambicus* has been examined repeatedly (see review by Wendelaar Bonga and Balm, 1989), while there is little attention to *O. niloticus*. To investigate species-specific differences in the physiological response to water acidification, changes in plasma sodium levels and gill Na^+, K^+-ATPase activity of the two species of tilapia after exposure to acidic water were compared.

Materials and methods

Oreochromis niloticus and *O. mossambicus* were hatched and reared in concrete ponds supplied with spring water (27-30℃) at the National Research Institute of Fisheries Science, Ueda Station. They were fed a commercially prepared dry diet, and were maintained under natural photoperiod. Immature fish, weighing 3-5g, were transferred from the ponds to 200-liter rectangular tanks containing fresh water at 27℃, and were reared for several weeks,

pH was adjusted to 7.0 by a pH controller (NPH-6800, Nissin, Tokyo) connected to a peristaltic pump (AC-2110, Atto, Tokyo) with 0.1 N sulfuric acid. The lighting regime was set at 14 hr light and 10 hr dark.

To investigate any dose-dependent effects of environmental pH on the plasma sodium levels, 4-7 fish were transferred to tanks containing fresh water at 27℃, pH being adjusted to 3.5, 4.0 or 4.5 using the pH controller described above. Three days after transfer, blood samples were collected directly from the caudal artery of the fish as described by Hasegawa *et al.* (1987). Plasma samples were immediately separated after centrifugation at 3,000 rpm for 5 min, and frozen at −20℃ until analysis. Plasma sodium concentration was measured by atomic absorption spectrophotometry (AA-855, Nippon Jarrell-Ash, Kyoto).

For time course experiments of the changes in plasma sodium levels and gill Na^+, K^+-ATPase activity, fish were transferred to tanks containing fresh water at pH 3.5 or 7.0, and were sacrificed at various intervals. Gill Na^+, K^+-ATPase was measured essentially following the procedures described by McCormick (1993). In brief, gill filaments were cut from the ceratobranchials immediately after blood sampling, placed in 100 $\mu\ell$ of ice-cold sucrose-EDTA-imidazole solution (150 mM sucrose, 10 mM disodium ethylenediamine tetraacetate, 50 mM imidazole, pH 7.3), and frozen within 30 min. They were stored at −80℃ and analyzed within 3 months of sampling. The ouabain-sensitive hydrolysis of adenosine triphosphate (ATP) is enzymatically coupled to the oxidation of nicotinamide adenine dinucleotide (reduced form, NADH), which was directly measured in a microplate reader (MTP-120, Corona Electronics, Ibaragi). Protein concentration was measured using a Protein Assay Kit (Bio-Rad, CA) with bovine serum albumin as standard.

Differences in plasma sodium levels and gill Na^+, K^+-ATPase activities were analyzed by ANOVA followed by Duncan's multiple range test. Calculations were performed using the computer program, VisualStat (Design Technologies Incorporation, Tokyo).

Results

Three days after transfer to acidic water of pH 3.5, 4.0 or 4.5, *O. niloticus* showed a significant decrease in plasma levels of sodium (**Fig. 1**). On the other hand, *O. mossambicus* showed decreased levels of plasma sodium only at pH 3.5. **Figure 2** shows the time course of changes in plasma sodium after exposure to acidic water of pH 3.5. Plasma sodium of *O. niloticus* continuously decreased for 7 days, and there was no tendency to recovery to the levels of the fish in neutral water. In *O. mossambicus*, plasma sodium also decreased after the initial exposure. In contrast to *O. niloticus*, the levels increased again on day 7 to the same level as day 3. In both *O. niloticus* and *O. mossambicus*, gill Na^+, K^+-ATPase activity increased significantly a day after the initial exposure to acidic water (**Fig. 3**). On days 3 and 7, there was no significant difference between the gill Na^+, K^+-ATPase activities in acidic water and neutral water in both species.

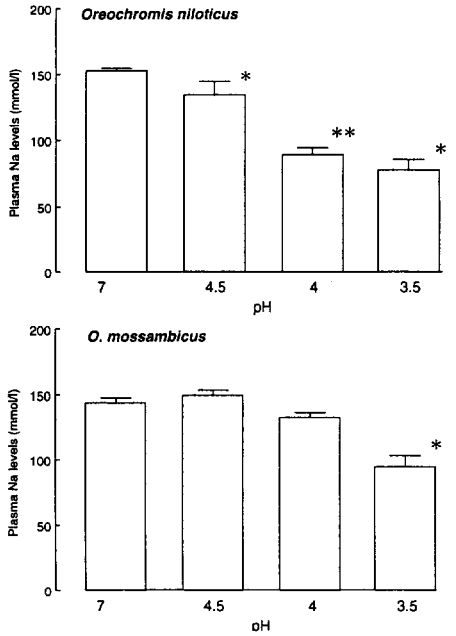

Fig. 1. Changes in plasma sodium levels of *O. niloticus* and *O. mossambicus* 3 days after exposure to neutral water at pH 7.0 or acidic water at pH 4.5, 4.0 or 3.5. Data are expressed as means ± SEM (n=4-7). *,** Significantly different from the levels in neutral water at $P < 0.05$ and $P < 0.01$, respectively.

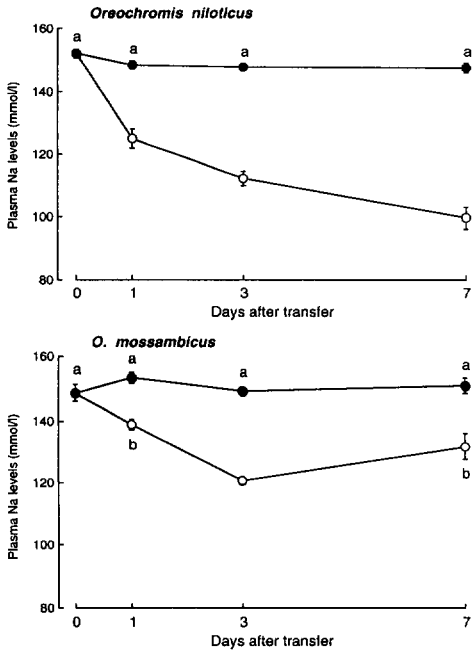

Fig. 2. Changes in plasma sodium levels of *O. niloticus* and *O. mossambicus* after exposure to neutral water at pH 7.0 (●) or acidic water at pH 3.5 (○). Vertical bars represent the mean ± SEM (n=5-7). Values with shared symbols are not significantly different ($P > 0.05$).

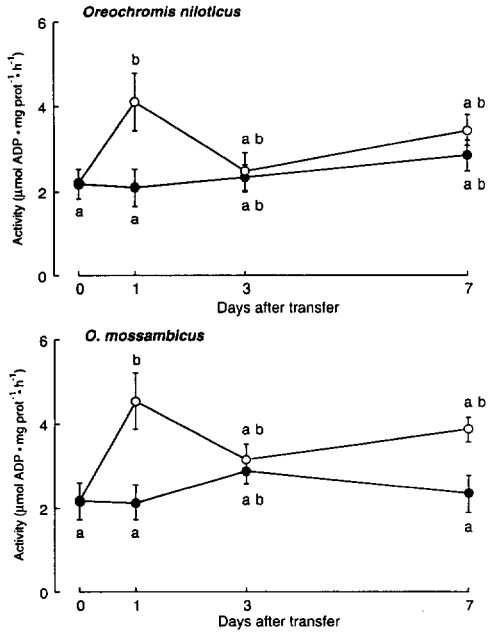

Fig. 3. Changes in gill Na^+, K^+-ATPase activity of *O. niloticus* and *O. mossambicus* after exposure to neutral water at pH 7.0 (●) or acidic water at pH 3.5 (○). Vertical bars represent the mean ± SEM (n=5-7). Values with shared symbols are not significantly different ($P>0.05$).

Discussion

We demonstrated that *Oreochromis mossambicus* has a greater hyperosmoregulatory ability to retain plasma sodium levels in acidic water than *O. niloticus*. Previous studies have shown that *O. mossambicus* is more tolerant to sea water than *O. niloticus* (Baralin and Hatton, 1979; Ayson *et al.*, 1993). Species-specific differences in acid tolerance between *O. niloticus* and *O. mossambicus* seems, therefore, to show similar trends to salinity tolerance.

A positive relationship between a hypoosmoregulatory ability to maintain plasma sodium levels in sea water and the activity of gill Na^+, K^+-ATPase is well documented in euryhaline teleosts, and the elevation of activity of this enzyme is thought to correspond to an activation of salt excretion from the gill (De Renzis and Bornancin, 1984; Zadunaisky, 1984; McCormick *et al.*, 1989). The role of Na^+, K^+-ATPase in sodium uptake in "neutral" fresh water is less certain than its role in ion excretion in sea water (McCormick, 1995). On the other hand, in "acidified" fresh water, gill Na^+, K^+-ATPase activity and sodium-retaining ability seem to be positively related. An increase in gill Na^+, K^+-ATPase activity was observed in rainbow trout after exposure to acidic water, but not in largescale sucker (*Catostomus macrocheilus*) (McKeown *et al.*, 1985). The difference in the response of gill Na^+, K^+-ATPase to the exposure is correlated to the ability in the trout to regulate plasma ion levels better than the sucker in acidic water. In coho salmon (*Oncorhynchus kisutch*) and Atlantic salmon (*Salmo*

salar), decreased plasma sodium levels were observed after exposure to acidic water in relation to the lack of activation of gill Na^+, K^+-ATPase (Saunders *et al.*, 1983; Powell and McKeown, 1986). In the present study, both *O. mossambicus* and *O. niloticus* showed a similar pattern of changes in the gill Na^+, K^+-ATPase activity, while there was a difference in the sodium-retaining ability in acidic water between the two species. In *O. niloticus*, gill Na^+, K^+-ATPase activity increased and attained a peak one day after the exposure, whereas plasma sodium levels decreased continuously for a week. In *O. mossambicus*, plasma sodium levels showed a recovery at 7 days after the exposure, whereas the activity of gill Na^+, K^+-ATPase showed no significant difference to the control levels. These results indicate that, in the two species, the activity of gill Na^+, K^+-ATPase does not coincide with the sodium-retaining ability in acidic water.

In the surviving rainbow trout in acidic water, the decreased influx rates of sodium exhibited only a small recovery, however, the increased efflux was markedly corrected (McDonald *et al.*, 1983). McDonald *et al.* (1991) suggested that species-specific differences in acid tolerance are correlated to the density of gill chloride cells and the length of the tight junctions between the adjacent gill pavement cells. In this study, there is an inconsistency of gill Na^+, K^+-ATPase activity with acid tolerance. Thus, the species-specific difference in acid tolerance between *O. mossambicus* and *O. niloticus* is possibly caused by a difference in the mechanism to block the branchial sodium efflux. A comparison between the two species of tilapia seems to be suitable for further studies on the regulatory mechanisms to suppress sodium loss in acidic environments.

Acknowledgments

This study was supported in part by grants-in-aid from the Ministry of Agriculture, Forestry and Fisheries, and also from Environment Agency, Japan.

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2種のティラピア、オレオクロミス・ニロチクスとモザンビクスの酸性環境への寛容性の違い

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摘　　要

2種のティラピア、オレオクロミス・ニロチクスとモザンビクスについて、低pH環境への適応能力の違いを調べるための実験を行った。中性の環境水からpH 4.5，4.0，3.5の酸性環境水へと移行させてから3日後に、ニロチクスでは血中ナトリウム濃度の有意な低下がpH 4.5以下でみられた。モザンビクスでは、血中ナトリウム濃度の低下はpH 3.5でしかみられなかった。ニロチクスの血中ナトリウムの経時変化をみると、pH 3.5の酸性環境水への曝露後には低下し続けていた。一方モザンビクスの血中ナトリウムは、曝露後3日目に最も低い値を示した後、対照のレベルへの回復傾向がみられた。両方の種において鰓の Na^+, K^+-ATPase活性は、酸性環境水への曝露後には上昇していた。これらの結果はモザンビクスがニロチクスよりも、酸性環境水中での血中ナトリウムを保持する高い能力を持つことを示している。