ABSTRACT

The sodium balance of the East American mudminnow was studied in fish well acclimated to water of pH 7.0 or pH 4.5. The fish were growing and actively feeding and had been exposed to the experimental media for at least two months. It was found that the mudminnow grows faster at pH 4.5 than at pH 7.0, and that growth at pH 4.5 is not influenced when ambient Na+ is varied between 0.25 and 1.0 mH. In neutral water though, growth was significantly slower and more so with decreasing ambient Na+. Whole body sodium exchange studies revealed that Na+ inflow is stimulated at pH 4.5 as compared to pH 7.0 and that outflow of Na+ is not influenced by ambient pH. Very well developed chloride cells in the gills of the fish adapted to pH 4.5 were observed and these cells may be the morphological correlate of the enhanced Na+ uptake rates observed.

It is concluded that the successful regulation of active Na+ uptake mechanisms underlies the success of the mudminnow in acid waters.

INTRODUCTION

It is becoming clear now that the numbers of fish and of fish species have decreased considerably in acidified waters, and more so with decreasing water pH (Packer & Dunson, 1970; Munis & Leivestad, 1980; Rosseland et al., 1980; Krout & Dunson, 1985). In natural waters acidified beyond pH 4.0 often no fish are found at all. Disturbed ecosystems are the concomitants of such acidification and this may explain for a part the disappearance of fish. However, for a variety of fish species evidence is growing that acidification of the ambient water imposes a primary stress on the osmoregulation of the fish (McDonald & Wood, 1981; McWilliams, 1982; Wendelaar Bonga et al., 1984a,b). Some fish appear to cope with this stress. The extra energy to be invested in the process of osmoregulation in acidifying waters is, however, eventually reflected by hampered growth and reproduction (Rosseland et al., 1980). Decreases in ventilation and overall activity are considered apparent.
but secondary effects of acid stress.

Certain fish species appear resistant to acid stress. Among the best studied fish that may survive in acid waters are the tilapia, *Oreochromis mossambicus* (Wendelaar Bonga et al., 1981a,b) and the trout (McDonald & Wood, 1981; McWilliams, 1982). The adaptive response of the tilapia to acid stress is certainly impressive and involves the recruitment of a larger part of the osmoregulatory potential, as indicated by the responses of the cortisol and prolactin producing cells (Wendelaar Bonga et al., this volume). The laboratories working on the topic of acid stress in salmonids have focussed considerably less on the endocrines involved in the adaptive response to low ambient pH. However, from the studies of these laboratories the general view arises that control of branchial ion exchange (Na⁺, Cl⁻ & Ca²⁺) is pivotal in the success of the fish to survive the deleterious effects of high ambient proton levels.

A particularly interesting fish species that recently has been the focus of research in our laboratories is the East American mudminnow, *Umbra pygmaea*. This fish has been introduced in Europe some 70 years ago. It is often found as the single fish species in natural waters with pH values below 4.0 (Dederen et al., 1986). The fish is considered extremely acid resistant (Kroyt & Dunson, 1985). The fact that these fish live and reproduce successfully in strongly acidified waters has prompted us to investigate the sodium balance of this fish under acid and neutral water conditions. The strategy followed was to acclimatize the fish for long periods of time (at least two months) to well defined, artificially prepared water. Then, the sodium balance of the fish was analyzed by determining whole body Na⁺ exchange, total Na content and growth related sodium accumulation.
MATERIALS AND METHODS

Fish and water conditions. East American mudminnow (Umbra pygmaea, De Kay; further called mudminnow) of both sexes, ranging in body weight from 1.5 to 8.5 g were collected in natural freshwater ponds in the southern provinces of the Netherlands. Upon transfer to the laboratory the fish were kept in 100 l all glass aquaria supplied with Nijmegen tapwater (15°C) and fed with the oligochaete Tubifex. The photoperiod was 12 h light alternating with 12 h darkness. For experiments fish were transferred to 20 l tanks containing artificially prepared water consisting of demineralized water to which were added: 0.25, 0.50 or 1.00 mM NaCl, 0.20 mM CaCl2, 0.20 mM MgSO4, and 0.06 mM KCl; the water pH was maintained at pH 4.5 by the addition of \( \text{H}_2\text{SO}_4 \) using pH-stat equipment; to guarantee water with a constant pH 7.0 a 2.5 mM capacity Tris buffer was added.

Analytical methods. Total Na of plasma and water samples was determined by flame spectrophotometry. The Na content of whole fish was determined by neutron activation analysis. To weigh the fish during growth experiments, they were gently blotted on wet tissue paper to prevent skin damage.

Radiotracer techniques. \( ^{24}\text{Na}^+ \) was used to follow Na exchange in whole fish. \( ^{24}\text{Na}^+ \) was produced by neutron activation of \( ^{23}\text{Na}_2\text{CO}_3 \) dissolved in water in the "Hoger Onderwijs Reactor" at the Interuniversity Reactor Institute, Delft. The specific activity averaged 70 GBq/mole. \( ^{24}\text{Na}^+ \) inflow was determined on the basis of the rate of uptake of \( ^{24}\text{Na}^+ \) by the fish from the water. \( ^{24}\text{Na}^+ \) outflow was determined on the basis of \( ^{24}\text{Na}^+ \) flow to the water from fish previously loaded with \( ^{24}\text{Na}^+ \) after exposure to the tracer in the water. The procedures followed and the flow calculations have been described in detail elsewhere (Flik et al., 1985 & 1987).

Electron microscopy. Freshly excised gill filaments were fixed for electron microscope examination as described in detail by Wendelaar Bonga & Van der Meij (1980). Fixed materials were embedded in Spurr's resin and
ultrathin sections contrasted with Reynolds's lead citrate were examined in a Philips 300 electron microscope. The electron microscope results will be presented in this paper as a graphical compilation of the electron micrographs made.

Statistics and calculations. Data were statistically analysed by the Mann-Whitney U-test or Student's t-test, where appropriate. Significance was accepted at the 5% level. Linear regression analysis was based on the least squares method. Data are presented as means ± S.E., unless otherwise stated.

RESULTS

As shown in Fig. 1, the mudminnow grows faster in water of pH 4.5 as compared to water of pH 7.0. At the latter pH growth is dependent on ambient Na⁺ levels, as indicated by retarded growth at 0.25 mM Na⁺ in the water. No such dependency of growth on external Na⁺ was observed at pH 4.5.

Fig. 1. Growth of the mudminnow at an ambient pH 7.0 or 4.5 and 0.25, 0.50 or 1.00 mM NaCl in the water. Growth was followed over a 170 days period. Growth was constant and is expressed as the relative increase in weight (AW/W₀ × 100%). The change in total body Na was calculated as the increase in total body Na, using the relationship for body weight and total body Na: Qₜ(Na) = 27.5W + 15.24 μmol (Pilk et al., 1987) to calculate Qₜ(Na) at t₀ of the experiment.

The increase in total body Na proved proportional to the increase in body
weight. This observation indicates that the fish had established a positive Na balance under all conditions tested and suggests that a constant Na content of the body is a requirement for growth. No differences were found in the total body sodium contents of fish kept at either pH 7.0 or 4.5; the Na content of the fish proved to be linearly related to body weight and typically amounted to 33 ± 1 umol/g (n = 22) at pH 7.0 and 36 ± 2 umol/g (n = 27) at pH 4.5. The increase in weight of the fish proved to be linear in time in all cases (data not shown) and, therefore, it is justified to convert growth rates in sodium accumulation rates on the basis of the increases in total body Na per unit of time. It was calculated that at an ambient pH 4.5, Na⁺ accumulation rates are 35% higher than those at pH 7.0 at an ambient 0.50 mM Na⁺ (the Na⁺ concentration used in the flow experiments).

The plasma Na levels amounted to 154 ± 15 mM (n = 5) and 140 ± 22 nM (n = 9) in fish kept at pH 7.0 and at pH 4.5, respectively, and are not significantly different.

No differences existed in the mean body weights of the groups tested for Na⁺ flow or growth rate determinations. As shown in Table I, inflow of Na⁺ is significantly higher in fish adapted to pH 4.5 as compared to pH 7.0. No differences were observed in Na⁺ outflow values. Calculated net flow of Na⁺, according to the conservation equation \( F_{\text{net}} = F_{\text{in}} - F_{\text{out}} \), was not significantly different from 0 at either pH tested.

<table>
<thead>
<tr>
<th>WEIGHT (g)</th>
<th>( F_{\text{in}} ) (umol/h)</th>
<th>( F_{\text{out}} ) (umol/h)</th>
<th>GROWTH RATE (nmol/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.0</td>
<td>3.43 ± 1.13</td>
<td>664 ± 58 (11)</td>
<td>662 ± 256 (4)</td>
</tr>
<tr>
<td>pH 4.5</td>
<td>3.05 ± 0.47</td>
<td>1227 ± 108 (11)</td>
<td>1120 ± 263 (5)</td>
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</tbody>
</table>

Table I. Na⁺ flow and growth rates in mudminnow well acclimatized to pH 7.0 or 4.5. Na⁺ inflow is stimulated at pH 4.5. Growth rates presented have the dimension nmol/h; they were calculated for fish kept under identical conditions as the fish used for flow determinations as the (linear) increase in total body Na per unit of time.
Calculated growth rates expressed as net Na accumulation rates were 2.1 and 4.1 mmol/h at pH 7.0 and at pH 4.5, respectively, indicating a nearly doubled growth rate in the low pH adapted fish.

Fig. 2 shows a compilation of ultrastructural observations on the chloride cells of the branchial epithelium of the mudminnow kept at pH 7.0 or 4.5.

The most remarkable observation was the occurrence at pH 4.5 of very well developed chloride cells with funnel-shaped apical crypts. The branchial chloride cells in the pH 4.5 adapted fish were generally found more deeply located in the epithelium; also, on the lamellae as well as on the primary filaments a thicker epithelium was found in the pH 4.5 adapted fish. At pH 7.0, the epithelium appeared thinner and the chloride cells were located more superficially in the epithelium and the chloride cells exhibited spherical apical crypts.
DISCUSSION

From the studies presented here, the mudminnow emerges as a teleost with a remarkable potential to live in acid waters. Unexpected and peculiar physiological adaptations appear to underlie the ability of the species to resist acid stress: it was found that growth rates in acid waters surpass those in neutral waters; enhanced branchial Na\(^+\) uptake may form the basis of this adaptation. The funnel-shaped apical crypts of the chloride cells of the branchial epithelium indicate that these cells may structurally adapt to the strenuous conditions of acid environments and create thereby a highly effective Na\(^+\) uptake system. We hypothesize that the elaborate tubular system and strong expansion of the apical membrane area, in which membranes the Na\(^+\) exchangers reside, augment the fish's capacity for active Na\(^+\) uptake. Moreover, one may imagine that the funnel-shaped apical crypts allow the formation of favorable ion (H\(^+\), Na\(^+\), Cl\(^-\), Ca\(^{2+}\)) gradients for ion exchange over the apical membrane.

The observation of stimulated Na\(^+\) uptake at low ambient pH presented in this paper adds new to the understanding of the tolerance of the mudminnow to acid stress. From their studies in which fish were subjected to more acute pH changes, Krout and Dunson (1985) concluded that "the high degree of tolerance of the mudminnow to acidic waters is associated with a capacity to resist the acceleration in sodium efflux", that normally occurs in fish exposed to high ambient proton levels. Clearly, our results on the outflow of Na\(^+\), which appeared independent of ambient pH in well acclimatized fish, indicate that eventually the successful regulation of Na\(^+\) uptake determines whether or not the species may live and grow in acid waters. In this respect the mudminnow differs from the tilapia, which fish establishes a positive Na balance under low ambient pH conditions by a strong reduction of Na\(^+\) outflow (Flik et al., 1987). An attractive hypothesis would be that for its
osmoregulation the mudminnow is more dependent on the mineralocorticoid effects of cortisol, that stimulates active Na⁺ uptake, whereas the tilapia depends more strongly on prolactin for the regulation of integumental permeability to water and ions. In general decreased Na⁺ inflow is observed when fish (also the mudminnow) are acutely exposed to acid waters (McDonald & Wood, 1981; Krout & Dunson, 1985). The enhanced Na⁺ inflow reported here for the mudminnow seems a result of a total acclimatization of the fish to the low pH conditions, structurally characterized by the development of specialized chloride cells.

The values for outflow of Na⁺ (normalized to weight for reasons of comparison with values in the literature) come to 400 nmol/h.g at pH 4.5 and to 283 nmol/h.g at pH 7.0. These values are in line with values of 240 nmol/h.g reported by Krout & Dunson (1985) for the same species kept at pH 8.3.

From the rather low growth rate of the mudminnow, it may be anticipated that the net flow of Na⁺ between fish and water will be negligible. Indeed, no significant net flow of Na⁺ could be established on the basis of the unidirectional Na⁺ flows. Yet, the fact that whole body inflow and outflow of Na⁺ were equal indicates that the fish established Na⁺ balance and that the values for unidirectional flows must be realistic. Finally, the fact that no differences in plasma Na⁺ were observed are a further indication that the fish controlled Na⁺ balance by branchial Na⁺ exchange, for it has been reported for a variety of fish including the mudminnow that acute acidification induces hypernatremia via enhanced Na⁺ outflow (McDonald & Wood, 1981; McWilliams, 1982; Mendel and Bonga et al., 1984a, Krout & Dunson, 1985; McKeown et al., 1985).

We conclude that the successful regulation of active Na⁺ uptake mechanisms may explain the success of the mudminnow in acidified waters. The results presented show that for the evaluation of the impact of acid stress on the physiology of the fish long term experiments are warranted.
REFERENCES


Wendelaar Bonga, SE, Meij, JCA van der, Krabben, WAMA van der & Flik, G (1984b)