Prolactin Cell Activity and Sodium Balance in the Acid-Tolerant Mudminnow Umbra pygmaea in Acid and Neutral Water

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In East-American mudminnows, obtained from lakes and bogs of different water pH levels, prolactin cell size and ultrastructure reflect higher secretory activity in neutral water than in water of pH 3.5–6.5. This contrasts with observations on other species in which prolactin cell activity is higher at low water pH. Laboratory experiments involving acute exposure of mudminnows from pH 5.5 for 48 hr to different pH levels showed that prolactin secretion increased in water below pH 3 and above pH 6.5, which could be correlated with losses of blood electrolytes. No osmoregulatory stress was noticeable in the pH range of 3.5 to 5.5. Acclimation of fish for 5 to 6 months to either pH 4.5 or 7.2 confirmed that prolactin cell activity, as estimated with ultrastructural morphometry, was significantly higher in water of neutral pH than of pH 4.5. The growth rate was significantly higher at the lower pH. Determination of whole body sodium fluxes, with 24Na as a tracer, showed that both groups had a positive sodium balance. However, total body Na+ influx as well as efflux values were slightly but significantly reduced at pH 7.0 when compared to pH 4.5. The reduction of Na+ efflux at pH 7.0 is in line with increased secretion of prolactin since this hormone is known to limit the branchial permeability to water and ions, including Na+. The results show that the mudminnow is extremely acid tolerant, having an optimum water pH range of about 3.5 to 6.0, which is consistent with ecological observations. Prolactin secretion is at a minimum in this range. Exposure to neutral water represents osmoregulatory stress for the mudminnow, in contrast to all other teleost species examined so far.

The progressive anthropogenic acidification of rivers and lakes in Northern and Western parts of Europe and in North America results in decline or even disappearance of fish populations. Under laboratory conditions, acute exposure to acid water leads to osmoregulatory stress caused by high diffusional losses across the branchial epithelium. Such losses are considered the main cause of death of fish in acutely and severely acidified water (Packer and Dunson, 1970; McDonald, 1983; Krout and Dunson, 1985; Wendelaar Bonga et al., 1987). On prolonged exposure, several teleost species are able to survive in moderately acid water and to compensate the initial ion losses. Hormones are most likely involved in this acclimation process. We have studied acclimation to acid water in the African cichlid fish Oreochromis mossambicus. This species also shows enhanced sodium losses after acute reduction of water pH, which is reflected by reduced plasma osmolarity and sodium levels in the first days in acid water. However, at pH 4.0 or higher, the initial drop in plasma sodium is restored in the course of the first week in acid water (Wendelaar Bonga et al., 1984), probably as a result of reduction of the acid-induced increase in branchial permeability to ions. We have shown that after long-term acclimation the
branchial permeability to sodium was even reduced to levels significantly below control values (Flik et al., 1989). This decrease was associated with a substantial increase in prolactin cell activity. Since the main function of prolactin is the control of the branchial permeability to water and ions, in particular sodium (Hirano, 1986; Wendelaar Bonga et al., 1989), we have interpreted the increased prolactin cell activity in acid water as an appropriate endocrine response for maintaining water and ion balance in acid water (Wendelaar Bonga et al., 1987; Flik et al., 1989). We have evidence that prolactin is also involved in the acclimation to acid water of eels (Wendelaar Bonga and Balm, 1989).

A survey of recently acidified freshwater lakes and bogs in the Netherlands has revealed that in water below pH 5 only one species is able to survive: the East-American mudminnow (Dederen et al., 1986; Leuven and Oyen, 1987). It was introduced in Europe some 70 years ago and at present it frequently occurs in high densities in acid bog lakes at a pH as low as 3.1. Many acidifying soft water bodies have been invaded by this species. In the present paper we report on the size and ultrastructure of the prolactin cells of mudminnows from lakes and pools with a water pH ranging from 3.2 to 7.0. We further report on prolactin cell activity and plasma electrolytes in fish from a large group of fish collected from an acid lake (pH 5.5) and ranging in body weight from 1.5 to 9.4 g were used for experiments. They were kept in well-aerated aquaria at 15°. The concentration of the major ions was (in mM): Na⁺, 0.5; K⁺, 0.06; Ca²⁺, 0.2; Mg²⁺, 0.2; Cl⁻, 0.76; and SO₄²⁻, 0.2. The water was acidified with H₂SO₄. Water pH was maintained by pH-stat equipment, using H₂SO₄ or NaOH (Radiometer PHM83). The fish were fed oligochaetes (Tubifex). They were kept at pH 5.5 for at least the first 3 weeks in the laboratory and subsequently used for experiments:

(a) Acute exposure. Groups of fish (4–6 g body wt) were exposed for 48 hr to pH 2.8, 3.5, 4.5, 5.5 (control group), or 7.2. Water pH was raised (with NaOH) or lowered (with H₂SO₄) from pH 5.5 to the desired pH in about 30 min. After 48 hr, blood was collected and the pituitary glands were dissected and fixed for electron microscopy.

(b) Chronic exposure. Fish of 3–6 g body wt were exposed to water of pH 4.5 or 7.2. The fish were fed fixed rations of Tubifex. After an exposure period of 180 days, part of the fish was used for determination of plasma osmolality and sodium concentration, body sodium content, body weight, and for ultrastructural examination of the pituitary gland. Another part of the fish was exposed for 170 days to pH 4.5 or 7.0 and used for determination of sodium fluxes.

Electron Microscopy and Morphometry

Fish of about 4 g body wt were anesthetized with MS-222. The pituitary glands were dissected and fixed for 1 hr in a cacodylate-buffered (pH 7.3) mixture of 2% osmium tetroxide, 3% glutaraldehyde, and 3% potassium dichromate (Wendelaar Bonga and Van der Meij, 1980). After dehydration in ethanol the tissues were embedded in Spurr's resin. Ultrathin sections were stained with lead citrate and examined in a Philips EM 200 electron microscope.

Cell and nuclear sizes were estimated lightmicroscopically (400×) in semithin sections by determining the surface area of 100 cells (only cells with a nuclear profile were taken into account) and nuclei per fish. Electron micrographs of prolactin cells of fish per group and at a final magnification of 13000× were analyzed morphometrically with Kontron Digiplan equipment for determining the fractional cytoplasmic volumes of mitochondria, Golgi areas, and granular endoplasmic reticulum. The sampling area per fish amounted to 500 μm². The numerical density of the secretory granules was also determined.

MATERIALS AND METHODS

Fish and Exposure System

East-American mudminnows (Umbra pygmaea) of both sexes were collected in late summer, after the reproductive period, in different lakes and bogs in the Netherlands. The pH of the water at the time of collection differed from 3.2 to 7.2. After capture by netting, the fish were transferred to the laboratory. Pituitary glands of fish of about 5 g were dissected immediately and fixed for electron microscopy.

A large group of fish collected from an acid lake (pH 5.5) and ranging in body weight from 1.5 to 9.4 g were used for experiments. They were kept in well-aerated aquaria at 15°. The concentration of the major ions was (in mM): Na⁺, 0.5; K⁺, 0.06; Ca²⁺, 0.2; Mg²⁺, 0.2; Cl⁻, 0.76; and SO₄²⁻, 0.2. The water was acidified with H₂SO₄. Water pH was maintained by pH-stat equipment, using H₂SO₄ or NaOH (Radiometer PHM83). The fish were fed oligochaetes (Tubifex). They were kept at pH 5.5 for at least the first 3 weeks in the laboratory and subsequently used for experiments:

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Determination of Plasma Osmolarity and Sodium, Total Body Sodium, and Sodium Fluxes

For the determination of plasma osmolarity and Na, the tail of the fish was severed and blood was collected from the caudal peduncle into NH4-heparinized capillaries. Plasma was separated from cells by centrifugation (30 sec at 9000g). Plasma osmolarity was determined in a Vogel microosmometer. Total Na of plasma and water samples was determined by flame spectrophotometry (Thermo Jarrell Ash, U.S.A.). The Na content of whole fish was determined by digesting fish in concentrated nitric acid. After neutralization and appropriate dilution, sodium concentration was determined by atomic absorption spectrophotometry.

Sodium fluxes were determined as recently described in detail (Flik et al., 1989). Briefly, 24Na produced by neutron activation of 23Na2CO3 was used as tracer. 24Na activity was measured in the well of a 3 x 3" NaI (Tl) scintillation crystal equipped with an appropriate γ-spectrometer. Influx was calculated on the basis of 24Na uptake from the water. After addition of 0.185 MBq tracer to 400 ml recirculating water, radioactivity was monitored over 305 periods for 2 hr. After this period, 24Na activity in the fish was measured.

Na efflux was estimated by determining 24Na appearance in the water from fish that had been loaded with 24Na by exposing them for 24 hr to 1.85 MBq 24Na in 700 ml water. This period was sufficiently long for obtaining an equal 24Na-specific activity in blood plasma and in the whole fish. The fish were transferred to 400 ml circulating water, and tracer appearance was monitored for 24 hr. After this period, blood was collected and plasma 24Na activity and, after 10 half-lives, plasma sodium content determined. Fluxes are normalized to body weight by linear extrapolation and are expressed in nmol · hr⁻¹ · g⁻¹. Data were statistically analyzed by the Mann–Whitney U test, and statistical significance was accepted at the 5% level.

RESULTS

1. Prolactin Cells in Mudminnows from Lakes of Different pH

Prolactin cells of mudminnows collected from different lakes and pools were examined with the electron microscope. As in other teleosts, the prolactin cells were concentrated in the rostral pars distalis of the pituitary gland. They are separated from each other by stellate cells, which distinguishes them from the other cell types of the rostral and proximal pars distalis, and show the ultrastructural characteristics described for teleost prolactin cells in general: granular endoplasmic reticulum and Golgi areas, and many electron-dense secretory granules. Their identity was confirmed by demonstration in these cells of immunoreactivity to a goldfish anti-prolactin serum (results not shown). Mean prolactin cell size varied with water pH (Fig. 1). Lowest values were observed in fish from water of pH 3.5–5.5. In fish from water of pH 6.5 and 7.0 the cells were significantly larger (P < 0.05). In the cells of fish from pH 7.0, the granular endoplasmic reticulum and the Golgi areas were much more prominent, and the number of secretory granules per unit of surface area was lower than in the cells of fish from pH 5.5 (Figs. 2 and 3). Additional observations were made in fish exposed in the laboratory for 4 to 6 months to water of pH 3.0, 4.5, 5.5, and 7.2. The values for prolactin cell size of fish from the latter three pH levels were comparable to those of fish from natural water of similar pH. In fish from pH 3.0 (natural water of
Fig. 2. Prolactin cell of mudminnow from natural water of pH 5.5; 12,350×.

Fig. 3. Prolactin cells of mudminnow from natural water of pH 7.2; cells are larger and granular endoplasmic reticulum (er) and Golgi areas (Ga) are more prominent than in water of pH 5.5; 12,350×.
TABLE 1

<table>
<thead>
<tr>
<th>Water pH</th>
<th>Osmolarity (mOsmol • liter⁻¹)</th>
<th>Na⁺ (mM)</th>
<th>PRL granulation (n • µm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.8</td>
<td>271 ± 6**</td>
<td>121 ± 9*</td>
<td>8.0 ± 3.1*</td>
</tr>
<tr>
<td>3.5</td>
<td>295 ± 7</td>
<td>131 ± 11</td>
<td>15.6 ± 4.3</td>
</tr>
<tr>
<td>4.5</td>
<td>306 ± 9</td>
<td>139 ± 8</td>
<td>16.2 ± 3.4</td>
</tr>
<tr>
<td>5.5</td>
<td>308 ± 11</td>
<td>140 ± 9</td>
<td>15.7 ± 3.1</td>
</tr>
<tr>
<td>7.2</td>
<td>278 ± 8**</td>
<td>117 ± 11*</td>
<td>7.2 ± 2.1**</td>
</tr>
</tbody>
</table>

Note: Means ± SD of six fish per group. Significantly different from controls (pH 5.5), *P < 0.05; **P < 0.01.

this pH was not available), prolactin cells were significantly larger (P < 0.05) than those of fish from pH 3.5–5.5 (Fig. 1).

2. Exposure Experiments

Exposure for 48 hr of fish fully adapted to pH 5.5 to water of pH 2.8, 3.5, 4.5, or 7.2 induced differences only in fish of the first and the latter pH group: at pH 2.8 and 7.2, plasma osmolarity and plasma sodium levels were significantly reduced, and the prolactin cells of these fish had degranulated (Table 1). The Golgi areas had enlarged and presecretory granules were more frequently observed (Figs. 4–6). In the fish from pH 3.5 and 4.5, no differences were observed for these parameters when compared to the control group (pH 5.5).

3. Chronic Exposure

Fish exposed for 180 days to water of pH 4.5 or 7.2 were compared. Morphometrical analysis at the ultrastructural level showed that the mean cell area of the prolactin cells was more than twofold higher in the fish from pH 7.2 (Figs. 7, 8, and 9), which corresponds to a more than threefold higher cell volume. The values for nuclear areas, the fractional volumes of mitochondria, Golgi areas, and granular endoplasmic reticulum were all significantly higher in the fish from pH 7.2 than in those from pH 4.5. The numerical density of the secretory granules present in the cytoplasm was 50% lower in the cells of the fish from neutral water (Fig. 9).

As shown in Table 2, plasma osmolarity, plasma sodium, and body sodium content were not significantly different. The body sodium content at the end was similar to that at the start of the experiment (42.9 ± 2.4 mmol • kg⁻¹; n = 6). Both groups increased in body weight, but the growth rate was almost three times higher at pH 4.5 than at pH 7.2. In mudminnows kept under very similar conditions (pH 4.5 or 7.0 for 170 days), Na⁺ exchange with the water was determined. In the fish from pH 7.0, Na⁺ influx and Na⁺ efflux were both slightly but significantly lower than in the fish from pH 4.5. At both pH levels the fish established a positive sodium balance: a net whole body influx was observed (Fig. 10).

DISCUSSION

Prolactin Cell Activity and Water pH

In mudminnows from different locations, prolactin cell size, which is in general a reliable parameter for prolactin cell activity, was lowest in water of pH 3.5 to 5.5. At pH 3.2, the lowest water pH value included in our survey, there was a tendency for prolactin cell size to increase. Also at pH 6.5–7.0, prolactin cell size was significantly higher than in fish from pH 3.5 to 5.5. The water pH is certainly not the only factor
that may influence prolactin cell activity in freshwater fish: water salinity and calcium concentration (Ball and Ingleton, 1973; Olivereau et al., 1983; Ruijter et al., 1984; Wendelaar Bonga et al., 1980, 1985; Fu et al., 1989), and probably the aluminum concentration (personal observation), which may all vary with water pH, are possible factors that influence prolactin cell activity of fish in natural waters. However, our laboratory data on chronically exposed fish showed a similar relationship between prolactin cell size and water pH as found in fish from natural waters. Thus, in the latter fish, water pH was probably the dominating factor determining prolactin cell size and therefore prolactin cell activity. In the laboratory experiment

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**Fig. 4.** Prolactin cell of mudminnow from natural water of pH 5.5 and exposed in the laboratory for 48 hr to water of pH 7.2; only few secretory granules are present; 11,000×.

**Fig. 5.** Prolactin cells of mudminnow from natural water of pH 5.5 and exposed in the laboratory for 48 hr to water of the same pH; compared to Fig. 4 the cells contain more secretory granules; sc, stellate cell; 11,000×.

**Fig. 6.** Part of prolactin cell of mudminnow exposed in the laboratory for 180 days to water of pH 7.2; the Golgi areas (Ga) contain presecretory material (arrows); 17,000×.

**Fig. 7.** Prolactin cells of mudminnow exposed in the laboratory for 180 days to water of pH 4.5; secretory granules are common; granular endoplasmic reticulum and Golgi areas are scarce; 11,000×.
Fig. 9. Morphometrical analysis of electron micrographs of prolactin cells of mudminnows exposed for 180 days to water of pH 7.2 (left bars) or pH 4.5 (right bars); fractional volumes of mitochondria, Golgi areas and granular endoplasmic reticulum (GER) are expressed as percentages of the cytoplasmic area; density of the secretory granules is expressed as number of granules per unit of cytoplasmic area; means ± SD of seven fish per group. *Statistically different from pH 7.2; \( P < 0.05 \); **statistically different from pH 7.2, \( P < 0.01 \).

TABLE 2

<table>
<thead>
<tr>
<th>Effects of Exposure for 180 Days of Mudminnows to pH 4.5 or 7.2 on Plasma Osmolarity, Plasma Sodium, Body Sodium Content, and Body Weight</th>
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<tbody>
<tr>
<td><strong>pH 4.5</strong></td>
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<td>-------------------</td>
</tr>
<tr>
<td>Plasma osmolarity (mOsmol · liter(^{-1}))</td>
</tr>
<tr>
<td>Plasma sodium (mEq)</td>
</tr>
<tr>
<td>Body sodium (mmol · kg(^{-1}))</td>
</tr>
<tr>
<td>Body weight, Day 180 (g)</td>
</tr>
<tr>
<td>Growth rate (%)</td>
</tr>
</tbody>
</table>

*Note. Means ± SD; numbers of fish in parentheses. Significantly different from pH 7.2; \(* P < 0.001\).
increase of Na\(^+\) efflux in fish exposed to water below pH 3.2. For the acid resistant holostean fish *Lepisosteus platyrhynchus*, this was found below pH 3.6 and in brown trout already at pH 5.0 (McWilliams and Potts, 1978). In two other acid resistant-teleosts, *Enneacanthus ebosus* and *Leptomis gibbosus*, increased sodium losses became evident at pH 4.0 and 3.5, respectively (Gonzalez and Dunson, 1987). These data illustrate the high acid tolerance of mudminnows, which can prevent sodium loss at 5 to 10 times higher H\(^+\) concentrations than other acid-tolerant species.

Activation of prolactin cells in acid water, as observed in mudminnows exposed for 48 hr to pH 2.8, has also been reported for other species, albeit at higher pH. We have reported activation of prolactin cells of the cichlid *O. mossambicus* acutely or chronically exposed to water of pH 4.5 or lower (Wendelaar Bonga et al., 1984, 1987, 1988). In this species and in the carp, *Cyprinus carpio*, activation becomes noticeable at pH 5.0, i.e., at a H\(^+\) concentration that is almost two orders of magnitude lower than the concentration that activates the prolactin cells of mudminnows (our unpublished results). Thus, similar to the disturbance of water and ion regulation, it is not the activation of the prolactin cells that makes mudminnows noteworthy, but the very low ambient pH level at which this phenomenon becomes apparent.

Neutral Water

There is another phenomenon that distinguishes the mudminnow from other teleosts, viz the activation of its prolactin cells in neutral water. Activation was indicated by the degranulation of the prolactin cells of fish exposed for 48 hr to pH 7.5 and also by the morphometrical analysis of the prolactin cells of fish chronically exposed to neutral water. This observation is in marked contrast with data on other species such as *O. mossambicus*, carp and eel, kept...
under comparable conditions (Wendelaar Bonga et al., 1984, 1987; Wendelaar Bonga and Balm, 1989). In these species, prolactin cell activity is lower at neutral pH and increases progressively with decreasing water pH. Our short-term experiment established that neutralization of the water induces a drop in plasma electrolytes in mudminnows kept at pH 5.5. No such an effect was observed in O. mossambicus subjected to a similar protocol (unpublished observation). Apparently, for the mudminnow, neutral water represents an osmoregulatory stress and the activation of the prolactin cells may be interpreted as a response to this stress. After more than 5 months in neutral water, prolactin cells had the same large size as those of fish from natural water of the same pH. In these fish the plasma electrolyte levels were similar to those of the fish acclimated to pH 4.5. This implies that the drop in plasma electrolytes observed in fish kept for 48 hr in neutral water is only transient: the fish seem to adapt to neutral water. However, the high prolactin cell activity, displayed by these fish even after 5 months, demonstrates that neutral water remains a continuous osmoregulatory challenge.

That the fish do indeed experience more stress under this condition than at pH 4.5 is supported by their growth rate, which is significantly higher at pH 4.5 than at neutral pH. In fish other than the mudminnow, growth rates are usually inversely related to water pH (Kwain and Rose, 1985; Tam and Payson, 1986). In O. mossambicus kept for 4 months under the same controlled conditions as the mudminnows in the present experiment, the growth rate was higher at pH 7.0 than at pH 4.5 (Flik et al., 1989). We consider the higher growth rate of mudminnows in acid water as further evidence for the exceptional acid tolerance of these fish. The reduced growth rate at pH 7 may reflect increased metabolic demands created by difficulties in ionic regulation, which reduce the energy available for growth (cf. McDonald, 1983).

Sodium Balance in Acid and Neutral Water

After exposure of mudminnows for about 6 months to water of pH 4.5 or pH 7.0, differences were evident between both groups with respect to sodium fluxes. Influx as well as efflux were slightly but significantly lower at the higher pH level. With respect to sodium efflux, this finding is consistent with the higher prolactin secretion rate in neutral water since prolactin controls Na$^+$ efflux in freshwater teleost fish (Hirano, 1986). At first glance the higher Na$^+$ efflux at the lower pH is in line with data on other species: Na$^+$ efflux increases rapidly after a sudden drop of water pH, as has been reported for salmonids (Packer and Dunson, 1970; McDonald, 1983), garpike (Krout and Dunson, 1985), and sunfish (Gonzalez and Dunson, 1987). This has also been observed in the mudminnow (Krout and Dunson, 1985). However, since these observations all concern acute exposure experiments, the data are not comparable to those of our flux measurements on chronically exposed fish. Recently, we have published Na$^+$ flux data on chronically exposed O. mossambicus (Flik et al., 1989) and found that Na$^+$ efflux was lower at pH 4.5 than at pH 7.0, which contrasts with the present data on the mudminnow. Similar to the mudminnow, however, the Na$^+$ efflux was inversely related to prolactin cell activity. It seems, therefore, as if chronic osmoregulatory stress experienced by O. mossambicus at pH 4.5 and by mudminnows at pH 7.0 leads to reduced Na$^+$ efflux, via increased prolactin secretion. A comparison of the responses of mudminnows and O. mossambicus to acid water is presented in Table 3.

Na$^+$ influx. In chronically exposed mudminnows, Na$^+$ influx is higher at pH 4.5
TABLE 3
Comparison of Differences in Some Parameters Measured in Tilapia (from: Flik et al., 1989) and Mudminnows (This Study)

<table>
<thead>
<tr>
<th></th>
<th>O. mossambicus</th>
<th>U. pygmaea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water pH</td>
<td>4.5</td>
<td>7.0</td>
</tr>
<tr>
<td>Na⁺ efflux</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Na⁺ influx</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Na⁺ net influx*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PRL cell activity</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Body growth rate</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

Note. Both species were chronically exposed under the same conditions to acid and neutral water.

a + , slightly positive; + + + , highly positive.

than at pH 7.0. In similarly treated O. mossambicus the reverse was found (Flik et al., 1989). Also, in brown trout, Na⁺ influx was lower in acid water than in neutral water, after short-term as well as after long-term adaptation (McWilliams, 1980). Thus, the relationship between Na⁺ influx and water pH is different from that in other species.

Net Na⁺ flux. In chronically exposed mudminnows the net Na⁺ flux is similar and positive at pH 4.5 and pH 7.0. A net sodium uptake over the total exposure period is indicated by the sustained growth and increase in total body sodium content as determined in a parallel experiment (Table 2): there is a net gain in body weight with similar body Na⁺ concentrations at both pHs. The calculated net Na⁺ accumulation over the 6-month exposure period was substantially higher at pH 4.5 than at neutral pH.

Ecological Implications

The observation that prolactin cell activity is at a minimum in the mudminnow from water of pH 3.5-5.5 indicates that this is the optimum pH range for this species with respect to water and ion regulation. It may represent the optimum pH range in general for this species. In a survey of about 100 lakes, pools, and ditches with a water pH varying from 3.5 to 8.1, mudminnows were most abundant in water of pH 3.5-5.5. They were the only species encountered in water of pH < 5, compared to a relative abundance of only 1.6% in water of pH > 5 (Leuven and Oyen, 1987). In contrast to other teleosts, which are unable to reproduce in water of pH < 5, mudminnows successfully reproduce even at pH 3.5 (Dederen et al., 1986). The present data show that the unusual acid tolerance of mudminnows is also reflected by the pH dependency of prolactin secretion.

REFERENCES


