Effects of acidified water on fish
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The acidification of water leads to disturbances in the water and ion metabolism of fish, including the acid-base balance. Many fish probably possess physiological mechanisms to eliminate these disturbances completely or in part. However successful they may be in the short term, such adaptation mechanisms nevertheless require additional energy, thus causing growth and reproduction to be retarded. Taking into account the high sensitivity of fish eggs and larvae to acidification, these phenomena are probably a major contributory factor to the decline of fish stocks in acidified water.

As water becomes acidified, fish stocks fall substantially. The variety of species is reduced, as are the populations of those who are left. At acidities below pH 4 virtually no species are found in nature [1, 2]. The causes are complex, one factor being the disturbance of the ecosystem resulting in a partial or total disappearance of plants and animals serving as food. However, water acidification also has a direct impact on the physiology of fish, in particular water and ion regulation and closely connected with this - respiration, acid-base balance, growth, and reproduction. Research over the past ten years has revealed that the disturbance of the water and ion metabolism of fish is the major cause of the mortality that occurs when water becomes acidified suddenly, as can happen, for example with discharges of acid waste [3, 4, 5, 6]. It may be asked whether this is also the reason for the decline in fish stocks where acidification proceeds slowly, as normally happens in nature. So far, little research has been done into the physiological consequences of chronic acidification. However, it is known that trout populations can survive in rivers with acid levels that are harmful, if not lethal, to trout that have developed in neutral water. This phenomenon indicates that trout can adapt to acidified water [7, 8, 9]. Research conducted at our laboratory shows that this phenomenon can be observed with other species of fish [5, 6]. This raises four fundamental questions:

1. What causes the water and ion metabolism to be disturbed?
2. What are the consequences for other physiological processes such as respiration, growth, and reproduction?
3. To what extent are fish able to adapt physiologically to acidified water?
4. Can it be assumed from such adaptation processes that fish stocks in acidified waters could recover in the long term?

Effects on water and ion metabolism

Most fish are totally reliant on their gills for respiratory gas exchange. Gills consist of arches, each with primary and secondary lamellae (figure 1). The secondary lamellae provide the actual gas-exchange surface. They are plate-shaped and generously supplied with blood, which is separated from the ambient water only by an extremely thin layer of skin. Although this thin epithelium permits efficient gas exchange, water and most ions have difficulty penetrating it under normal circumstances. This is also essential for fish to be able to live in fresh water. The large difference in osmotic value and ion composition between blood (= 300 m0sm/l; = 140 mM Na+ /l) and water (usually less than 5 m0sm/l and 3 mM Na+ /l) means that fish are constantly susceptible to taking up water by osmosis and losing ions through diffusion via the surface of the skin, notably the gills. In normal circumstances, these processes occur on only a minor scale due to the limited permeability of the skin epithelium.

Any water that does nevertheless penetrate the gills is discharged via the kidneys. The loss of ions (in particular Na+ and Cl-) is compensated for by an active ion uptake - to a small extent in the gut, but mainly by the gills from the ambient water, via the chloride cells (figures 2 and 3). These are specialized cells in the skin epithelium at the boundary between the primary and secondary lamellae and distributed over the secondary lamellae. Chloride cells contain mechanisms for transporting ions from water to blood and vice versa. The active uptake of Na+ ions is driven by the enzyme Na+/K+-ATPase [10] and probably also the enzyme Na+/H+-ATPase [11]. These are ion pumps able to pump Na+ ions from the cytoplasm of the chloride cells in exchange for K+ ions. They are located in the tubular system of the chloride cells and thus in fact form part of the baso-lateral membrane of these cells (figure 4). In the apical cell membrane, at the water/chloride cell boundary, similar ion exchanges occur due to the presence of carrier molecules.

In contrast with transport to the basal side of the cell, transport to the apical side does not require ATP. Cl- ions can, for example, be taken up in exchange for HCO3 ions (figure 3).

Acidified water attacks the surface of the skin, resulting in a substantial increase in permeability to water and ions (figure 5a, b). One of the factors affecting permeability is the extent to which bivalent ions, in particular calcium, are bound to the surface of the skin [12, 13]; see also figure 2. Water acidification reduces the binding of calcium ions, as a result of which the...
skin is no longer water-tight. This increases osmotic water uptake, an increase in the excretion of urine is required, and this in turn leads to additional ion loss. With acute exposure to acidified water, the result is a fall in the osmotic value of the blood: the lower the pH of water, the steeper the fall (figure 6). This drop is due mainly to the loss of Na⁺ and Cl⁻ ions, which together make up some 90 per cent of the total ion content of the blood. In addition, the blood is acidified by the penetration of H⁺ ions. The exposure of the skin to acid makes it more susceptible to mycotic infections and other diseases [4, 5, 6, 14]. If the osmotic value of the blood drops too far, the fish will die. With sublethal water acidification, the osmotic value often remains at very low levels for weeks, as has been observed with many species of trout and, as we have found, with goldfish (figure 7). With an African fish, Oreochromis mossambicus, however, a fairly rapid recovery normally takes place (figure 7), which indicates that this species can fairly easily adapt to acidified water. Such adaptation will probably occur with many other species, although over a much longer term than with Oreochromis mossambicus (figure 8).

The loss of body salts is a major cause of mortality following acute acidification, as is shown by experiments in which Oreochromis mossambicus is adapted to water with increased Na⁺ and Cl⁻ levels some weeks prior to acidification. When concentrations of these ions in water are virtually equal to those in the blood (such a solution has an osmotic value practically identical to that of blood), acute acidification no longer leads to any appreciable mortality or any drop in the osmotic value of the blood. In water with increased levels of Ca²⁺ ions, mortality is less than under control conditions. This provides support for the hypothesis that the main cause of ion loss from the blood (this loss is also less in water containing elevated Ca²⁺ concentrations) is the reduced binding of Ca²⁺ ions to the skin epithelium (figure 9).

**The effect on other physiological processes**

**Oxygen transport.** Water acidification has a particular effect on the transport of oxygen to tissue via the blood and on growth and reproduction. In an acid environment the pH of the blood falls. Although this drop is limited to no more than a few tenths of pH units, one effect is to cause more calcium carbonate to be released from the blood. Moreover, in acidified water, the CO₂ tension is generally increased, thus raising the CO₂ tension in the blood [15, 16, 17]. The transport of oxygen to tissue via the blood is inhibited by both the drop in pH and the increase in CO₂ levels in the blood:

1. The lower blood pH results in a fall in the affinity of haemoglobin for oxygen.
2. As a result of the high CO₂ tension in the blood, the haemoglobin remains relatively unsaturated with oxygen.

The overall capacity of the blood for transporting oxygen is hence reduced. Acute acidification leads to a rise in mucus production by the skin, which may result in an accumulation of mucus in the gills and elsewhere. This phenomenon is found particularly in trout. At present, it is assumed that all these processes can obstruct the supply of oxygen to tissue, though not to an extent that is in itself lethal [4]. Except in very extreme cases of acidification, the buffer capacity of fish appears to be...
sufficient to cope with the consequences of the increased H⁺ influx in acidified water.

**Growth.** The loss of body salts that occurs in acidified water has to be compensated for by an active uptake of ions, in particular Na⁺ and Cl⁻, by the gills. This process requires additional energy. Whereas a fish will devote less than 20 per cent of its energy to regulating its ion and water metabolism in normal freshwater conditions, this percentage can more than double in acidified water. The result is a fall in the growth rate. One factor behind this fall may be the calcium loss that occurs in acidified water. Figure 9 shows that bone calcium in *Oreochromis mossambicus* decreases in the initial weeks of exposure to acidified water. Recovery then takes place, however. With trout, hardly any loss of bone calcium has been observed [18], but their rate of growth is also inhibited by water acidification [8, 18, 19], although no effects have been observed on the growth of brown trout [20]. Data on the effect of acidification on fish growth are still scarce, however.

**Reproduction.** Water acidification inhibits reproduction in various ways – by reducing egg production and increasing egg mortality, and by retarding the development and increasing the mortality of larvae.

In 1979 it was noted [21] that the egg production of trout decreased in acidified water and this was ascribed to a disturbance of the calcium metabolism, because calcium levels in the blood plasma of females with maturing eggs were much lower than normal. Usually, calcium levels double during maturation of the ovary, because many vitellogenins (calcium-binding proteins) are transported by the blood from the liver, where they are synthesized, to the maturing eggs in the ovaries. In water with pH 5, the quantity of circulating vitellogenins proved to be much lower than normal, thus retarding, or even entirely inhibiting, the maturation of the eggs.

*Oreochromis mossambicus* also suffers retarded egg development. Normally, the female of this species lays eggs at intervals of about twenty days. In acidified water, these intervals increase as the pH level falls. At pH 4, no mature eggs have been observed (Table 1). The inability of many fish to lay eggs in highly acidified water is also frequently observed in nature [21].

Table 2 shows that in acidified water not only are there fewer eggs laid, but egg mortality increases as well. The eggs are at their most vulnerable in the first few hours after laying, when the chorion has not yet hardened [22]. The percentage of eggs that hatch falls substantially as acidification increases. Table 2 illustrates the considerable variation in the sensitivity of eggs from one species to another. Below pH 4, however, virtually no eggs hatch. The only species of fish in the Netherlands that can successfully reproduce below pH 4 is the small mud-minnow (*Umbra pymeia*), which was introduced at the beginning of the century from North America and is the only fish with excellent resistance to high water acidification [2].

With regard to the brook trout, it should be noted that the egg mortality observed by R. Menendez [19] (Table 2) is much greater than that found by Trojnar (1977) with the same species. J. R. Trojnar [23] exposed eggs from normal animals to acidified water immediately after laying. Menendez,
TABLE 1 THE INFLUENCE OF WATER pH ON THE PERCENTAGE OF EGG-LAYING FEMALES AND THE EGG-LAYING INTERVAL WITH *OREOCHROMIS MOSSAMBICUS* (= 20 FEMALES PER GROUP).

<table>
<thead>
<tr>
<th>Water pH</th>
<th>Egg-laying females</th>
<th>Egg-laying interval (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.4</td>
<td>72%</td>
<td>19.3 ± 3.1</td>
</tr>
<tr>
<td>6</td>
<td>55%</td>
<td>21.3 ± 5.2</td>
</tr>
<tr>
<td>5</td>
<td>15%</td>
<td>32.4 ± 7.1</td>
</tr>
<tr>
<td>4</td>
<td>0%</td>
<td>—</td>
</tr>
</tbody>
</table>

The water was acidified immediately after laying, after which the interval until the next laying was determined.

TABLE 2 RELATIVE SURVIVAL RATE FOR THE EGG OF VARIOUS FISH IN ACIDIFIED WATER

<table>
<thead>
<tr>
<th>pH</th>
<th>Overall percentage</th>
<th>Atlantic salmon (Salmo salar [27])</th>
<th>Brook trout (Salvelinus fontinalis [19])</th>
<th>Cyprinodon nevadensis [25]</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0</td>
<td>100</td>
<td>100</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>6.5</td>
<td>n.a.</td>
<td>82</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td>97</td>
<td>74</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>5.5</td>
<td>70</td>
<td>59</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>60</td>
<td>54</td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>63</td>
<td>26</td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>3</td>
<td>n.a.</td>
<td>n.a.</td>
<td></td>
</tr>
</tbody>
</table>

Note: 100% = control conditions (pH 7 for salmon and trout; pH 8 for Cyprinodon nevadensis). n.a. = not available.

Discussion

Although there is no doubt that the water and ion metabolism of fish is seriously disturbed by acute exposure to acidified water, the long-term effects are unclear. Species such as *Oreochromis mossambicus* recover rapidly from the effects of acidification: ten days after the onset of acidification the blood's osmotic value (figure 7) and the Na⁺ and Cl⁻ concentrations in the blood have already returned to control levels [5]. Furthermore, the loss of calcium from the body, in the shape of a decrease in bone calcium, is restored within a few weeks (figure 9). This recovery is probably regulated by hormones. Following acidification, there is a substantial increase in the secretion of cortisol by the interrenal cells [11] and prolactin by the hypophysis [5]. Both hormones are involved in water and ion regulation and in the calcium metabolism. Cortisol stimulates the uptake of Na⁺ by the chloride cells in the gills and causes these cells to increase in number. Prolactin causes the skin to thicken and makes the gill epithelium less permeable to water and ions. All these phenomena have been observed in the weeks immediately following acidification [5, 11] (figure 8). Such physiological adaptation processes will undoubtedly occur with other species of fish, although probably at a much slower rate. Goldfish (figure 7) and trout show only a very slight recovery during the first weeks in however, studied eggs from fish kept at low pH levels for a number of months. This difference in mortality is a further indication that the development and maturation of eggs in the ovaries is hindered by water acidification. In addition, the experiments by Menendez and Trojnar show that the survival of trout larvae and fry in the initial months after hatching is proportional to the pH of the water. Below pH 5, mortality in the first three months proved to be virtually 100 per cent.
acidified water. With chronic acidification, however, trout show little sign of a disturbed water and ion metabolism as long as the pH level does not fall below 5, although it has been established that the energy 'cost' of regulation is much higher than in neutral water [24, 25]. It is, therefore, questionable whether such adaptation phenomena will be of particular importance in the long term for the survival of the species, especially as fish exposed to long-term acidification also appear to be more susceptible to all kinds of infection and less able to survive the stress that occurs, for example, during the reproduction period [26].

In addition, growth and production of eggs appear to be retarded in the longer term. However, the greatest threat to fish stocks is undoubtedly the fact that the eggs of many species are extremely sensitive to water acidification in the initial hours after laying. Only when fish are able to develop acid-resistant eggs will they be able to survive in acidified water over the long term. That this is not impossible is shown by the fact that stable trout populations have been found living for at least several decades, and probably much longer, in acidified water in rivers in Britain, North America, and elsewhere [7, 9]. In laboratory experiments, such trout have turned out to be much better able to maintain the ion balance in their blood at low pH levels. There appears to have been genetic adaptation in this case. However, in Norway, in particular, the size and number of trout populations in highly acidified water have fallen substantially over the past eighty years [3]. Furthermore, the small number of fish species found in acidified water in the Netherlands does not in the least indicate an ability to adapt successfully on the part of native species [2]. It thus appears that genetic adaptation to acidification is by no means general. The ability of the small mud-minnow to survive successfully in water at pH 3.5 is an outstanding exception, and has probably arisen in the course of a long period of evolution. Acidification is currently increasing too fast to allow such adaptation to be expected on the part of other species. Moreover, the decline in fish stocks is caused by acidification in combination with other factors. The decrease in fish populations in Norway, for example, is due mainly to acidification combined with an increase in aluminium content [3]. Aluminium is particularly toxic at pH 5. An increase in aluminium levels in acidified water has also been observed in the Netherlands [27]. Continuing acidification will thus almost certainly lead to further decline in fish stocks.

References