RESPONSE OF ZOOPLANKTON, BENTHOS, AND FISH TO ACIDIFICATION: AN OVERVIEW

MAGDA HAVAS[1] AND BJØRN OLAV ROSSELAND[2]

[1]Environmental & Resource Studies, Trent University, Peterborough, Ontario, Canada, K9J 7B8
[2]Norwegian Institute for Water Research, P.O. Box 173, Kjelsas, 0411 Oslo, Norway

Abstract: This paper presents an overview of the response to acidification of aquatic fauna with special emphasis on zooplankton, benthos, and fishes. Changes in behavior, body chemistry, reproduction, and species diversity are presented based on laboratory experiments and field studies in both Europe and North America. Differences in species sensitivity are discussed as they relate, not only to acidification but also to low calcium concentrations in the water, elevated aluminum concentrations, and presence of naturally occurring organic acids. The mechanisms—behavioral, physiological and ecological—enabling aquatic fauna to survive in acidified waters are discussed.

Key Words: fish, invertebrates, osmoregulation, ionoregulation, aluminum, calcium, organic acids, low pH, acid lakes, refugia, mixing zones

1. Introduction

The environmental problem of aquatic acidification involves effects on organisms at all trophic levels. Early studies focused on the loss of fish populations, especially the salmonids. Later studies reported that zooplankton, insect larvae, benthic invertebrates, especially crayfish, snails and freshwater mussels, are sensitive and are often reduced or absent from acidified lakes and streams (see review by Havas 1986a). Water hardness, represented by the calcium concentration, significantly affects the distribution of species and their ability to survive in acidified environments. Early studies that failed to report water hardness or those that used hard water for bioassays often report considerably higher tolerance to acid than is observed in nature. Aluminum, leached by acid precipitation from the drainage basin, complicated the picture considerably since the various forms of aluminum differ in their toxicity. Aluminum and hydrogen ions interact both synergistically and antagonististically depending on conditions (Muniz and Leivestad 1980, Havas 1985, Herrmann 1993, Rosseland and Staurnes, 1994) and, in the presence of naturally occurring organic acids, aluminum toxicity can be neutralized.

The importance of these water quality variables emerged as scientists across two continents tried to understand why some circumneutral lakes are almost devoid of life while other, more acidic lakes are teaming with life. In an attempt to explain apparent discrepancies the importance of water quality, especially pH, Ca, Al, and organic acids became better understood.

CRITICAL WATER QUALITY

SAFE PLACES (REFUGIA): CHRONIC TOXICITY

Other discrepancies in the presence and distribution of species began to emerge that could not be explained by surface water quality. Fishes, in particular, and possibly other species, can sense the pH of their environment in so far as they can avoid areas of low pH. Since water quality parameters in lakes are seldom uniform from top to bottom, organisms that are mobile and can sense the pH of their environment can move to more favorable areas. These refugia can be found just above or within the top few

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cm of sediments, in sheltered bays among macrophytes, or in areas that receive alkaline inputs from surface or groundwater drainage. Hence we learned that spatial distribution of pH and other water quality parameters was important for explaining the presence/absence of species within spatially heterogeneous environments. Often symptoms of stress in these environments resembled chronic toxicity.

TOXIC PLACES/EVENTS (EPISODES AND MIXING ZONES): ACUTE TOXICITY

Just as spatially heterogeneous environments or refugia enable some species to survive in otherwise unfavorable chemical conditions, temporal heterogeneity often has the opposite effect. During periods of high runoff, following spring snow melt, autumn or summer showers, waters of different chemical composition come together to produce chemically distinct episodes. When acid, Al-rich water mixes with alkaline water Al precipitates as $Al(OH_3)$. This precipitate can then accumulate on the gills and filtering apparatus of aquatic organisms causing acute toxicity. Since aluminum hydroxide is an insoluble precipitate it will eventually settle or otherwise be removed from the water column hence this acute toxicity is short-lived and limited in space. Even short-term exposure, in the matter of a few hours and, in some cases, a few minutes, can be lethal.

The consequences of acidic episodes, particularly spring snow melt, have been well documented. Mixing zones, however, have received less attention despite the fact that they can be acutely toxic to aquatic biota. This appears at first to be a contradiction to the concept of refugia. Whether an area of acidic water that comes in contact with alkaline water is a safe haven or a toxic zones depends on many parameters but one of the most important may very well be the amount and form of Al produced at the boundaries.

BIOCHEMICAL/PHYSIOLOGICAL STUDIES

SENSITIVE SPECIES

We learned from the early studies that some species are particularly sensitive to acid water, these include Atlantic salmon (*Salmo salar*), Brown trout (*Salmo trutta*), Arctic char (*Salvelinus alpinus*), and among the invertebrates gastropods, crustaceans, and some species of insect larvae (Havas 1986a). Much of this sensitivity could be explained by biochemical/physiological failures to regulate osmotically essential ions. Interestingly, the biochemical/physiological response of fishes and invertebrates is similar. The identification and understanding of these failures became of fundamental importance for predicting future changes related to reductions/increases in pollution loads and to the optimalization of mitigation techniques in relation to recolonization, liming, and restocking strategies. For the ecophysiologist, the key focus was to identify these biochemical/physiological failures, and thereby contribute to the explanation of the decline or disappearance of biota in acidic waters.

Attention has also been paid to mechanisms of acid-resistance or acid-tolerance. At the individual level the effects of chemical toxicants can be reduced in a number of ways (Calow 1991) including avoidance or escape reactions; exclusion (e.g. excretion of more mucous onto exposed surfaces of aquatic animals); removal (in-coming toxicants might be actively pumped out); neutralization (i.e. by complexation with protective proteins, e.g. metalothionins); excretion, and/or repair of damage caused by the toxicant.

SENSITIVE LIFE STAGES AND LIFE PROCESSES

A life history study of fish populations and other aquatic biota in a lake undergoing acidification, involves all the physiological mechanisms in the area of toxic effects and resistance. All temporary and chronic changes in water chemistry can have different impacts on organisms and populations depending on species and the life history stages represented. For fish, the previous acid-acclimation history, year class composition, the population size, spawning strategy and spawning facilities as well as competition between other fish species in the lake are also important. Controlled, short-term, laboratory studies, which have given us most of the detailed insight into the complex chemistry involved, can represent only a small fraction of what happens in nature. As we discover the complexities of nature we become aware of the limitations of these short-term laboratory studies. However, it is these very limitations that provide us with clues that enable us to see a larger portion of the whole picture.

The purpose of this paper is to provide an overview of the research on the response of fishes and aquatic invertebrates to airborne acid deposition and the subsequent acidification of freshwater systems. Special emphasis is placed on biochemical and physiological responses.

2. Critical Water Quality

Today, the three elements, hydrogen ions (pH), aluminum (Al), and calcium (Ca), are still considered to be of most importance for the toxicity of acid water to freshwater biota (reviewed by Havas 1986a, Wood and McDonald 1987, Rosseland et al. 1990, Rosseland and Staurnes, 1994).

The effects of H^+ and Al are dependent not only on animal species, but also on the life history stage of the animals and previous acclimation history. In the field, the effects of Al alone are difficult to isolate from a variety of potentially interrelated adverse factors. During episodes of high water flow, and in lakes and streams where different water qualities mix, large variations in pH, Al-species, Ca and other ions and metals, and organic substances occur (Henriksen et al. 1984, Skogheim et al. 1984, Lydersen et al. 1994). When pH of an acidic water body increases, for example, or when acid Al-rich water mixes with limed or neutral water, low molecular inorganic forms of Al are transformed to high molecular weight forms and hence precipitate. In such mixing zones, rapid Al-precipitation onto fish gills and the filtering apparatus of invertebrates, combined with osmoregulation failure, inhibition of enzyme activities, and gill lesions have been observed. Hence, water in the mixing zone is often more toxic than the original acid water (Rosseland et al. 1992, Poléo et al. 1994) and in rivers fish seems to avoid or have disappeared from such areas (Åtland and Barlaup 1995).

The situation is made even more complex by the fact that the organisms themselves can influence their chemical micro environment near their body surface (see Exley et al. 1991). The basic chemistry of the external water may not necessarily represent the true toxic components relevant to the organism. Our knowledge about an exact chemical threshold level for a certain physiological response, might therefore be based on wrong assumptions.

Calcium has a fundamental biological importance for water breathing animals and plays a key role in membrane permeability. In many acidified areas, the Ca concentration is so low that it is close to the concentration limit for snails and mussels and even for the softwater-tolerant salmonid species. In such areas, one must be aware of any substance that can have an adverse effect on Ca metabolism.

ENVIRONMENTAL VARIABILITY

STREAMS

Streams are spatially heterogeneous and temporally dynamic environments and many of the organisms that inhabit streams have learned to tolerate or adapt to the constant chemical fluxes. When the chemical stress becomes extreme, many invertebrates can avoid the stress by drifting down stream to more favorable conditions. Fishes, that require first order streams to spawn in the spring or fall are not nearly as fortunate and often find themselves moving into more and more acidic and hence unfavorable environments. In the event that adverse chemical conditions of low pH and elevated Al concentrations persist downstream and avoidance is not possible, the consequence can be seen as massive fish kills and the loss of the invertebrate population.

LAKES

In lake systems there are more escape possibilities than in rivers for fishes. In accordance with this, massive fish kills have primarily been reported from rivers and brooks. Most brown trout populations spend their early low mobility stages in brooks and streams. Thus, the effects on these stages seem to be the most important for the fish decline. River-dwelling species/stocks have very restricted escape possibilities, and this fact renders them especially vulnerable to acidic waters. For anadromous fish the situation is even worse since they have the smolt stage that is extremely sensitive to acidic waters.

Invertebrates can minimize exposure to stressful water quality in lakes by living close to the water/sediment interface where the sediments offer some buffering. They can also drift to more favorable conditions among macrophytes and filamentous algae that can increase the pH of the surrounding medium as they photosynthesize.

ESTUARIES

For the anadromous fish populations, the situation is more complex, and effects on older life history stages are possibly of greater importance. The smolt stage is considered to be the most sensitive one, especially for Atlantic salmon, but spawning fish also have a low tolerance (see reviews by Rosseland 1986, Rosseland et al. 1990). During the short smoltification period when the bottom-dwelling and territorial freshwater parr change to become smolt prepared for a pelagic marine life, the gill epithelium gradually change toward that of a marine fish; characterized by great increase in membrane permeability, chloride cell number and structure complexity, and activity of the ion pump enzyme Na-K-ATPase (reviewed by e.g. Hoar 1988). These changes render the smolt epithelium extremely sensitive to the deleterious effects from H^+ and Al complexes. Returning fish from sea (e.g. spawners) also have a seawater-like gill epithelium, rendering them very vulnerable for acidic/Al stress. Atlantic salmon smolts demonstrate inhibition of gill Na-K-ATPase activity to parr level, ionoregulatory failure and complete loss of seawater tolerance exposed in acidic water with a low Al concentration. These results indicate

impaired smoltification that might cause poorer seawater survival or cause the fish not to migrate (organism level: life performance). This will certainly cause effects on the salmon population and river community. Since the results are from a laboratory study, they are just indicative of what happens in nature. There is thus a need for future verification under natural conditions for interpretation of possible environmental implications (See Staurnes et al. 1995).

3. Physiological Response

A toxicant exerts its effects when it comes in contact with an organism. In fish and other water-breathing animals in acidic water, the primary target organs for toxicants are sensory organs and the gills. In early life history stages of some species the skin is also used for major regulatory processes between the animal and the environment. When the gills and the circulatory system develop, these functions are gradually taken over by the highly specialized gill organ, and the skin becomes nearly impermeable.

EFFECTS ON SENSE ORGANS

Superficial sense organs, like olfactory and taste organs, are not protected by "external barriers", and are therefore vulnerable to pollutants. The toxicants may disrupt normal chemosensory functions by masking or counteracting biologically relevant chemical signals, or they may cause direct morphological and physiological damage to the receptors. Such effects on olfactory and taste organs are likely to cause behavioral effects, e.g. interfere with avoidance and escape reactions related to chemical perception (which are primary resistance mechanisms), social interactions (e.g. reproduction), schooling behavior, predator avoidance, territoriality, as well as interfering with the ability to search for food items and maybe also contribute to a reduced appetite.

Another possible disturbance with great environmental implication, is the inhibition of the olfactory system in seaward migrating smolts of anadromous species. As olfaction is one of the main senses believed to be important for homing in Atlantic salmon, a reduced imprinting caused by acidic or "mixing zone" water quality on leaving their home river, may be hypothesized to increase straying on their return. Effects on chemoreception and chemical communication are sublethal per se, but may have important implications for long-term survival of fish populations.

EFFECTS ON GILLS AND HOMEOSTASIS

In water-breathing animals, the gills are the primary organ for respiration, ionoregulation, osmoregulation, acid-base balance, and excretion of nitrogenous waste. Any environmental stressor that influences the function of this organ may therefore cause homeostatic disorder. Breakdown of some key physiological functions may result in rapid death or induce physiological compensatory responses to maintain homeostasis at least for some time, thus greatly interfering with the overall life performance of the animal. Toxicity thresholds are specific to species and life history stages, but the resultant physiological disturbances are generally similar across species. Prominent physiological disturbances for fish and many invertebrates exposed to acid waters are iono/osmoregulatory failure, acid-base regulatory failure, respiratory and circulatory failure. Most of these effects can directly be attributed to effects on gill functions or

structure in the case of fish and the gill equivalent (chloride cells, anal papillae, etc.) in the case of invertebrates.

Environmental irritants, including toxicants, can affect gill functions by direct interference with biochemical/physiological processes and by causing structural changes (gill lesions). When in contact with such an irritant, one of the first responses is increased mucous secretion to protect the epithelial cells and prevent entrance of the toxicant. Prominent structural changes including epithelial necrosis, hypertrophy, hyperplasia, lamellar fusion, bulbing of lamella, as well as changes in chloride cells (number and size) have been observed (Mallat 1985). Most of these lesions can be documented in fish exposed to acid Al-rich waters both in laboratory and field (Rosseland et al. 1990), where the main target of Al/H^+ effects is the chloride cells. Since chloride cells seem to be the main targets in invertebrates, similar studies could provide useful insights regarding acid/Al sensitivity. However, except for mucous secretion and resulting respiratory effects, studies on direct effects of such gill lesions on biochemical/physiological processes are few and warrant further research.

TOXIC EFFECTS OF H^+

The acute toxicity of H^+ in acidic waters (reviewed by e.g. McDonald 1983, Havas 1986a, Exley and Phillip 1988) results in the loss of Ca from important binding sites in the gill epithelium, which reduces the ability of the gill to control membrane permeability causing ionregulatory disturbance and loss of ions (primarily Na^+ and Cl^-). An additional effect of the H^+ is blood acidosis. Very high acidity (found in acid mine drainage ponds, and in a few extremely acidic environments, see Havas and Hutchinson 1983a) causes interlamellar mucous clogging and resulting hypoxia and also severe gill lesions. While acidity alone has been held responsible for the decline of aquatic biota in some acidic waters, Al is now recognized as the principal toxicant.

SYMPTOMS AND MODE OF ALUMINUM TOXICITY

The toxic effects of Al on fish and invertebrate physiology are numerous (reviewed by Havas 1986b, Wood and McDonald 1987, Exley and Phillips 1988, Rosseland et al. 1990, Rosseland and Staurnes 1994). The main consequences, all contributing to mortality when exposure is acute, appear to be respiratory disturbances (due to interlamellar mucous clogging and Al precipitation and reduced membrane fluidity) and iono/osmoregulatory disturbances (due to decreased uptake and increased loss of Na^+, Cl^- and Ca^{2+} caused by Al binding to gill surface). Circulatory disturbances, characterized by very high hematocrit due to reduced blood plasma volume and erythrocyte swelling, increases the blood viscosity (Mazeaud and Mazeaud 1981).

At the present state of knowledge then, the main factor in H^+ toxicity is thought to be iono-regulatory failure, whereas in Al/H^+-toxicity, respiratory and circulatory distress are added.

Whereas the consequences of Al to some extent are characterized, the toxic mechanisms are yet poorly understood. Aluminum is found on the epithelial surface and inside the epithelium cells (see e.g. Rosseland et al. 1990). Several potential sites of Al interaction are suggested in the toxic mechanism model recently suggested by Exley et al. (1991).

Important biochemical properties of the gill epithelium for the explanation of Al precipitation and binding, are its net negative charge, the glycoproteins and sialic acid in

mucous, the phosphate groups on membrane phospholipids, carboxylate groups on the membrane proteins, and the binding structure of membrane transport proteins (Exley and Phillips 1988, Exley et al. 1991). Of crucial importance is also the fact that the gill boundary layer closest to the epithelium, is both chemically and physically dissimilar to water outside this layer. The chemistry of boundary layer is very much influenced by the organism itself. The pH of the layer is determined by the hydration equilibrium of expiration products (mainly carbon dioxide and ammonia) and diffusion into and out of the boundary layer of acid or base equivalents from both the water and the organism. An important property is the facility to maintain a circumneutral boundary layer pH even at low environmental pH. This protects biota in acidic waters from deleterious effects of acidity per se. When organisms are exposed to non-acutely toxic pH, there is an initial increase in ion efflux that is followed by a relatively rapid recovery of the efflux (McDonald 1983, Havas 1986b). The mechanisms involved are unknown. Since the boundary layer is the first surface to come in contact with the environment, this boundary layer should receive more attention since it may help us to better understand acclimation effects and acid tolerance, including species, strain and life history stage differences.

The toxicity of Al is caused by inorganic monomeric Al-species, and presence of Al-complexing ligands other than OH^- such as fluoride, sialic acid, organic substances such as humic acids and citrate etc., generally reduces the toxicity of aluminum (see review by Havas 1986b Rosseland et al. 1990 and Rosseland and Staurnes 1994). At pH about 5, the sialic acid in the mucous is primarily negatively charged, and positively charged Al-hydroxides may therefore bind to mucous. The polymerization of Al may cause irritation of the gill epithelium, stimulation of mucous secretion that along with the Al-precipitation, causes interlamellar clogging and several of the gill lesions. Since polymerization kinetics are temperature dependent, it is not surprising that Al toxicity is reduced at low temperatures (Poleo et al. 1991).

On gill membrane Al binds to small electronegative species such as common biological oxygen-based functional groups (phosphates, carboxylates, carboxyls and hydroxyls) (see Exley et al. 1991). The circumneutrality of the boundary layer at moderate pH (4.5 - 5.5) favors binding to such oxygen-based functional groups. Thus, Al may bind to and neutralize the charge of both the phosphate groups on membrane phospholipids and carboxylate groups on the membrane proteins, subsequently reducing the membrane fluidity. Exley et al. (1991) also suggested that Al may substitute for metal co-factors in the functional domains of transport proteins or also for the transport species per se. All these effects on apical cell surface may be summarized in the prediction of interference with both important transcellular diffusional and active processes, of which could be the uptake of ions (Na^+, Cl^-, Ca^{2+}), excretion of waste products (NH^{4+}, HCO^{3-}) and diffusion of respiratory gases (O_2, CO_2). Al has also been suggested to substitute for Ca^{2+} in the intercellular cement. Increased paracellular permeability caused by H^+ and Al-induced removal of Ca from the anionic sites in the cement, especially between chloride cells and adjacent pavement epithelial cells, probably contributes to the increased efflux of ions in acidic water. The ameliorating effect of Ca on Al and pH response is probably by tightening of the junctions, thereby preventing the passive loss of ions (Wood and McDonald 1987). Prolactin production increases after a chronic exposure to sublethal acidic waters, mainly as a response to a

drop in plasma electrolytes (Wendelaar Bonga et al. 1987). Prolactin reduces the gill epithelium permeability, and this time-dependent increase is clearly a mechanism of resistance.

Chemical analysis of invertebrates suggests that there is a poor relationship between total Al content and Al toxicity (Havas and Hutchinson 1983b). Histochemical staining for Al shows that Al can bind to the carapace (physiologically inactive) and be shed with each molt. Aluminum can be associated with filtering appendages as a particulate and hence more Al can be accumulated by active than inactive individuals. Aluminum can also be found on chloride cells, but the surface area of chloride cells is considerably smaller than that of the carapace which may account for the lack of correlation between toxicity and Al content (Havas 1986c).

How Al comes into the cells is not known. Excley et al. (1991) suggested, based on in vitro experiments with phospholipid vesicles, that apical bound Al alters membrane permeability to allow the intracellular accumulation of Al. The possibility for entrance via carrier systems should be investigated. Inside the cell, citrate might be an excellent ligand to Al and act as an intermediate chelator, passing Al to groups with higher affinity. The gill activities of the enzymes carbonic acid anhydrase and Na-K-ATPase are inhibited in acid/Al-exposed salmonids (Staurnes et al. 1984, Rosseland et al. 1990, 1992). The interaction between Al and ATP (mainly the tendency for ATP to form stronger complexes with Al than with Mg) may imply that Al can affect many enzymes reactions where ATP is a substrate, and possibly disturb the energy metabolism of the cells (Ganrot 1986). Aluminum also binds to calmodulin, which is a multifunctional, Ca-dependent protein that regulates a variety of cellular reactions, including regulation of many enzymes (Ganrot 1986). The cytosolic plasma Ca^{2+} concentration is well regulated, and higher concentrations can breakdown cellular functions (Wiercinski 1989). The overall effects of the possible interference of Al with basic processes in the gill epithelial cells may thus be severe effects on the epithelial barrier properties (Exley et al. 1991).

In spite of the importance of the sensory system, however, studies on the effect of acidic waters on sense organs are relatively few and should be encouraged. However, low pH alone has been found to reduce the olfactory response to amino acids and increase the mucous layer in the olfactory organ (Klaprat et al. 1988). Adding Al to the water depresses olfactory response even more and causes histopathological changes such as swelling and dysformation of the olfactory epithelium (Klaprat et al. 1988). Even in cases where no changes in structure of the chemosensory tissue have been observed, complete elimination of feeding response have occurred at low pH (Lemly and Smith 1987). Behavioral effects can also be caused by homeostatic changes. In accordance with this, avoidance reactions to low pH waters have been observed when plasma cation concentrations have been moderately reduced by acidic water exposures (see Rosseland et al. 1990).

HORMONES RELATED TO OSMOREGULATION

Prolactin and cortisol are important hormones related to osmoregulation; prolactin reduces ion permeability and increases mucous production, while cortisol stimulates the onset of cellular proliferation and differentiation in the primary gill epithelium, and increases the specific activity of Na-K-ATPase. Both hormones are affected by acidic waters (Wendelaar Bonga et al. 1987, Witters et al. 1991). Plasma cortisol increases in

fish exposed to low pH only when aluminum is present at high concentration, presumably as a response to compensate the H^+/Al-toxicity (Witters et al. 1991), as well as in fish exposed to low external NaCl-concentration (Perry and Laurent 1989) and chronic stress in general (Pottinger and Pickering 1992). Prolactin production increases after a chronic exposure to sublethal acidic waters, mainly as a response to a drop in plasma electrolytes (Wendelaar Bonga et al. 1987). As this is a time dependent increase, an increased prolactin production is clearly a mechanism of resistance.

Although hormones play an important role in various resistance mechanism towards acid waters (Exley and Phillips 1988), an important and yet undiscussed aspect, is the potential negative effects of an increased level of cortisol as a response to prolonged (chronic) exposure to acid aluminum-rich waters. As a permanent increased level of cortisol has a negative effect on the immune system (Mazeaud and Mazeaud 1981, Pickering and Pottinger 1985), such a response might thus have a negative effect on the health status of fish populations in acid lakes in general. Another important aspect is a possible post-episodic effect. A combination of a primary sublethal physiological stress (osmoregulatory and circulatory problems) and a secondary reduced immunity caused by a cortisol response, might lead to an increased mortality over a long period. The overall effect might thus be substantially greater than the direct observed mortality during and shortly after an episode or an exposure to a "mixing zone chemistry" (Rosseland and Staurnes 1994). If such a relation exists, it might explain the phenomena of post spawning mortality and "juvenilization", where brown trout post spawners disappears in some populations after their first spawning resulting in lack of older year-classes in the population (see Rosseland 1986).

METABOLISM AND GROWTH

Metabolic activity, measured as oxygen uptake, is not affected by moderate H^+ concentrations alone, but increases as a response to Al in the water. The increased respiratory and heart rate observed in acidic waters are not believed to cause the increased energy expenditure per se, as the increased metabolism rather reflects the increased activity of the intrinsic compensatory mechanism trying to restore homeostasis. Hyperventilation in acidic waters seems to be a specific response to the labile Al-concentration, as the addition of chelator such as citrate depresses hyperventilation (see reviews by Wood and McDonald 1987, Rosseland et al. 1990).

In long-term experiments, even low concentrations of Al have reduced growth (Sadler and Turnpenny 1986). Results from a study of stocking brown trout in the limed Lake Hovvatn to study the growth during the reacidification period, have documented the relation between reduced growth and increased mortality, and critical levels of pH, Al concentrations and Ca concentrations (Barlaup et al. 1994). A decreased appetite have been observed in brook trout exposed to acid water (see Rosseland et al. 1990). The growth reduction response seen in Lake Hovvatn might thus have been caused by a combination of both an increased metabolism as the lake water reacidified, combined with a general decrease in appetite and food conversion rate.

SENSITIVE LIFE STAGES AND PROCESSES

REPRODUCTION AND EARLY LIFE STAGE: OOGENESIS

Among fish and invertebrate oogenesis and fertilization period have been pointed to as sensitive to low pH. Reduced serum and plasma Ca in female fish from acidic lakes have been reported, indicating a probability for failure in producing viable eggs. A depletion of Ca from bone and increased numbers of females with unshed eggs have also been reported. However, several studies indicate that females from acidic lakes develop eggs and spawn normally, even though the plasma Ca during oogenesis has been low (see reviews by Rosseland 1986, Muniz 1991).

EGG STAGE

After fertilization, the embryo seems to be susceptible to acidic waters throughout the whole period of development, although the period shortly after fertilization and also prior to hatching seems to be most critical (Rosseland 1986). For a long period of time, H^+ alone was considered to be the major toxicant at the egg stage, although some effects of Al at intermediate pH-ranges was demonstrated. Low pH in the surrounding waters results in pH-depression inside the egg (in the perivitelline fluid), leading to either a prolongation of the hatching or to a reduced hatching success. The low pH of the perivitelline fluid also depresses the activity of the hatching enzyme, chorionic dehydrogenase, which then reduces an effective break down of the eggshell (chorion). Species and strain differences in sensitivity to acid waters at hatching may therefore reflect variation in levels of inactivation of the hatching enzyme. A chorion thickening, probably a protein denaturation due to the surrounding low pH, and a reduced activity of the embryo inside the egg (reduced mechanical breakdown) due to the low pH of the perivitteline fluid, enforces these negative effects.

Daphnids, ventilate their brood pouch so that developing embryos are exposed to the external medium before their are released. Hence, many die within the brood pouch if conditions are unfavorable.

ALEVIN STAGE

An increasing negative influence of Al with age occurs after hatch (Baker and Schofield 1982, Wood and McDonald 1982). The reason for this is still unclear, but it might have something to do with the changes that take place in the respiratory system/organ. Alevins shortly after hatch still respire through their skin but gradually gills become the primary organ of gas and ion exchange. The sensitivity related to the gill as a target organ thus gradually develops in the alevin. This might therefore explain the gradual importance of Al as a toxicant after hatch.

Al and pH are known to interfere with whole body mineral content and skeletal calcification at the embryo and fry stage (Sayer et al. 1991). Recent studies on strain of brown trout having different sensitivity to acidic waters seem to indicate differences in calcification rate at the alevin stage (Dalziel et al. 1995). In spite of a comparable total body Ca, the most resistant strains had the lowest calcification rate of finrays and skeletal. This phenomenon might thus indicate an important resistant mechanism for embryo survival before swimup, giving priority to a high plasma/serum Ca to ensure Ca and electrolyte homeostasis. Development of screening techniques to investigate strain tolerance in fish based on early calcification rate, is under way in the ReFish project (Dalziel et al. 1995).

A reduced metabolism, indicated by an increased number of degree-days from fertilization to hatch and through the yolksack period, have been suggested as a mechanism of resistance. By increasing the period until swimup, the chance to avoid acid episodes in the stream habitats will be reduced, thus enhancing the chance to survive (Rosseland 1986).

4. Conclusions

What really causes the loss of fishes and invertebrates in acidified water is probably a sum of a variety of disturbances of which we know but a few. Laboratory studies and field studies, at the subcellular to the ecosystem level, spanning a broad set of dynamic water chemistry challenges and including sensitive stages of an organisms life-cycle have been conducted. Many of the early studies focused on the physiological effects of acid and aluminum in softwater environments. Information regarding environment heterogeneity and the presence of both refugia as well as toxic episodes and mixing zones has improved our understanding of the subtle factors controlling the presence and absence of species in a particular environment. In the future scientists will deal with even more subtle factors of competition, and predator-prey interactions and their role in influencing species composition, population biomass, and community structure as lakes either continue to acidify or begin to recover as they have in parts of North America and Scandinavia.

To link the toxic substances involved to the ecological effects observed, calls for cooperation between chemists, geologists, atmospheric scientists, ecophysiologists, and ecologist. And to link ecological effects with regulation, legislation, and management strategies requires even broader cooperation between scientists and policy makers.

It seems that the observations and theories of the effects of acid deposition on aquatic systems formulated by Developed Countries will soon be tested in Developing Countries as their use of and dependence on fossil fuels increase.

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