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Effects of mining industry waste waters on fish in the Kostomuksha area, NW Karelia, Russia

by
Victoria Tkatcheva

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ACADEMIC DISSERTATION

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Key words: environment, perch, roach, rainbow trout, heavy metal concentration, liver, gills, histological structure, enzyme activities, lipid composition.

The problem of detecting the effects of heavy metals on nature is receiving considerable attention in North America, Europe and Russia. This thesis studies the morphological, physiological and ecotoxicological effects of elevated metal concentrations and changed water quality on individual fish and their populations.

The changes are attributable to mining industry wastewaters in the forest lakes of the Kenti river system (North-west Karelia, Russia). Two fish species were studied, the piscivorous perch (*Perca fluviatilis*) and the detritivorous- herbivorous roach (*Rutilus rutilus*). Concentrations of copper, zinc, cadmium, mercury and chromium were analyzed in the water, sediment and fish from the Kenti river system. The combined effects of the alkali metals, lithium and potassium, and of lithium alone, were investigated in laboratory experiments using juvenile rainbow trout (*Oncorhynchus mykiss*). Fish liver and gill structure, lipids and enzyme activities were studied in response to metal concentrations, seasonal factors, and the environment.

The release of wastewater caused changes in the water quality and sediment composition. In addition to the abiotic factors in their habitat, the feeding habits and life style of the fish were important factors in their metal uptake. A lower mercury concentration in fish was registered in spring before snowmelt. In spite of the low metal bioavailability caused by the high water hardness and pH in L. Poppalijärvi, chronic Cu exposure was pronounced due to its high concentration in water, sediments and roach. Physiological changes in the gill chloride cell structure imply an increase in osmoregulatory work in L. Poppalijärvi. Cholesterol was shown to be the most adaptive compound in the gill membrane, both in the field and in the laboratory. Lithium may affect fish through diffusive sodium ions losses at the gills and by reduced enzymatic activity of the gills, possibly related to observed lower concentrations of free fatty acids and cholesterol in gill tissue. However, no destructive effects of Li on Na^+,K^+-ATPase activity were found in the presence of potassium.

Content

List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I Tkatcheva V., Holopainen I.J., Hyvärinen H.. 2002. Effects of mining waste waters on fish in lakes of NW Russia. Verh. int. Ver. Limnol. 28, 484-487.

II Tkatcheva, V.G., Holopainen, I.J., Hyvärinen, H. 2000. Heavy metals in perch (Perca fluviatilis) from the Kostomuksha region (north-west Karelia, Russia). Boreal Environment Research 5, 209-220.

III Tkatcheva V., Holopainen, I J., Hyvärinen, H., Ryzhkov, L.P., Kukkonen J. 2004. Toxic effects of mining effluents on fish gills in a subarctic lake system in NW Russia, Ecotoxicology and Environmental Safety, 57, 278-289.

IV Tkatcheva, V., Holopainen, I.J., Hyvärinen, H., Kukkonen, J.V.K. The response of rainbow trout gills to high potassium and lithium in water. Ecotoxicology and Environmental Safety (in press).

V Tkatcheva, V., Franklin, N.M., McClelland, G.B., Smith, R.W., Holopainen, I.J., Wood, C.M. Physiological and biochemical effects of lithium in rainbow trout. Manuscript accepted for publication (December 2006) in Archives of Environmental Contamination and Toxicology.

Some unpublished results are also presented and discussed.

1. Introduction

Metals, such as mercury, cadmium, copper and zinc, form major types of toxic compounds that are released into many watercourses by the mining industry (Mason, 2002). Both air emissions and wastewaters are sources of metal pollution in the Kenti river system (Morozov, 1998; Lozovik *et al.*, 2001, Kalinkina *et al.*, 2003). In the late 1990s, metal pollution of the Kenti river system caused by the mining industry was not investigated to any great extent, and the heavy metal contamination of its fish had not been studied. Nevertheless, one of the few studies from the area has shown that molluscs (*Lymnaea stagnalis* L. and *Sphaerium* sp.) had elevated levels of metallothionein in the upper part of the Kenti river system (Regerand, 1995), thus indicating exposure to potentially toxic concentrations of metals (Amiard *et al.*, 2006). In addition, preliminary results from the years 1997-1998 showed that fish from the upper part of the water system had higher tissue concentrations of metals. The studies in this thesis were undertaken to investigate the environmental impacts of metal pollution caused by the release of wastewater into the Kenti river system by Kostomuksha Mining Plant (KMP).

The main purpose of this thesis was to find out how metals accumulate and affect feral fish in the Kenti river system. In 2000, the project was initiated as an investigation of metal impact on the biota of the natural Kenti river system. Holopainen *et al.* (2003a, b) explained changes in the biotic community from bacteria to fish. Perch and roach, which have different feeding habits and lifestyles, were chosen to demonstrate the differences in metal uptake and the interrelations with abiotic factors in the Kenti river system. Concentrations of metal were studied in fish muscle and liver tissue (I, II) and in the gills (III). Since high concentrations of metals do not imply that the metals have a toxic effect (Pettersen *et al.*, 2002), toxicity of metals was mostly associated with vital physiological functions, such as enzyme activity, modifications in membrane lipid composition, and changes in tissue structures. The last part of this work focused on alkali metals, which were abnormally high in the Kenti river system. The effects of alkali metals on the gills of juvenile rainbow trout (IV, V) were studied in the laboratory.

The objectives of this thesis were to find out:

1. The effects of metal pollution on individual fish and fish populations in successive lakes downstream from the Kostomuksha Mining Plant (I, II, III)
2. Possible changes in the structure of fish liver and gills in response to metal concentrations (II, III).
3. Possible interaction between gill enzyme activity and lipid composition under wastewater impact, and the role of the lipids in seasonal and environmental adaptation (III).
4. The combined effects of the alkali metals, lithium and potassium, on gill structure, lipid composition and enzyme activity in juvenile rainbow trout in the laboratory (IV).
5. The effects of lithium on the physiology of juvenile rainbow trout in the laboratory (V).

2. Background

2.1. Kenti river system and mining industry pollution

The Kenti river system is an example of the beautiful, but vulnerable natural northern river systems of the Republic of Karelia, Russia. Historically, the study area belongs to Belomorskaja (White Sea) Karelia, where Elias Lönnrot collected the old Karelian poems, charms and conjurations that formed the basis for the

Finnish national epos, the Kalevala (The Kalevala, 2004).

One of the ecological problems in this area is the wastewater discharge from the Kostomuksha Mining Plant (KMP). The production operations have severe effects on the environment in the form of air pollution (SO_2 and dust) and wastewater emissions (Anonymous, 1995; Kuharev *et al*., 1995; Fedorets *et al.*, 1998; Virtanen *et al*., 2000; Lozovik *et al*., 2001). The mining effluents have been deposited in the closed basin since 1982. Since 1994, the overflow of wastewaters into the Kenti river system has been allowed. Formerly the depository was a natural lake, L. Kostamus (or Kostomuksha). Water from Lake Kostamus flows out to the northeast through the Kenti river system into the larger Kuito Lakes and the White Sea (see map in articles I-III). The contamination of this area has been studied since KMP started to use the upper lake of this system as a waste basin. Water quality is monitored by the Karelian Science Center (Northern Water Research Institute, Petrozavodsk, Russia) and by the Kainuu Regional Environment Centre (Finland).

The Kenti watercourse is a typical boreal lake-river system with low mineral content, soft brown water with a slightly acidic pH, and with a low average annual temperature (Lozovik *et al*., 2001; Holopainen *et al*., 2003b). The main ion composition of the water is altered in the upper part of the Kenti system by a considerable rise in total nitrogen and in Mg, Ca, Na, Cl, SO_4, K and Li, which has changed the water pH from acidic to alkaline and increased the hardness of the water. It seems that the effect of water quality on solubility and speciation will decrease the availability of metals for uptake by biota and thus help to make the metal pollution less severe (Lozovik *et al.*, 2001; Platonov and Lozovik, 2003). It has been suggested that the wastewaters cause a threat to biodiversity, impair local fishing and contaminate drinking water (e.g. Kalinkina *et al*., 2003).

The effects of wastewater pollution on biota as described by Holopainen *et al* (2003b) are the following: firstly, the primary producers (including picocyanobacteria) and the fish in the lakes downstream from the mine had double the biomass compared to fish in the reference lake; secondly, zooplankton and zoobenthos biomasses are highest in L. Kento, the lake in the middle of the outflow series; thirdly, the fish fauna is the same in all the lakes and is dominated by perch and roach. Both grow well, and the roach show the best growth in L. Poppalijärvi, next to the waste basin. Since the phosphorus levels are equally low in all the studies of the lakes, the high nitrogen content (source: quarry explosives) in the Kenti river system might be partly responsible for the higher phytoplankton and picocyanobacteria biomasses, an important food for *Ciliata*, zooplankton and zoobenthos, which in turn are important food sources for the fish. The high biomass of benthic animals was assumed to be more important for the predominant roach than that of the pelagic zooplankton (Holopainen *et al*., 2007). The dominant fish, perch and roach, were considered to be suitable organisms for demonstrating metal accumulation.

The natural source of metals for the Kenti river system is the chemical weathering of rocks and soils. This territory is part of the Baltic or Scandinavian Shield area, and the ancient Precambrian silicate rocks are rich in metals, especially mercury (Startchev, 1985). Another source of metals in the lake water are the wastewaters from mining processes (Lozovik *et al.*, 2001). For example, the following concentrations of metals have been measured from the precipitation basin in the liquid and solid phases (Lozovik *et al*., 2001): iron 270 mg l^{-1} in the liquid phase and 1500 mg l^{-1} in the solid phase, chromium 0.09 to 2.9 and zinc 0.08 to 1.2 mg l^{-1}, in the liquid and

solid phases respectively. The alkali metals potassium and lithium are found in concentrations of 143 to 330, and 0.16 to 0.53 mg l^{-1}, the divalent cations calcium in concentrations of 18 to 630 and magnesium in concentrations of 9 to 130 mg l^{-1}, respectively. Two canals bring metals into the Kenti river system. The South canal flows directly into L. Poppalijärvi and collects waters from the drainage area of the depository, including the flow from four rivers contaminated heavily by metals from the tailings of the open pit mine (Lozovik *et al*., 2001).

In addition to our own results from the field study, data from Morozov (1998), Virtanen and Markkanen (2000) and Lozovik *et al.* (2001) have been used, and they are presented in the chapter entitled "Results" (Table 4). The water samples collected at the same time as the fish tissue samples were collected in the years 2000 and 2001.

The high concentrations of metals in the sediment of L. Poppalijärvi (Table 5) reflected the binding of metals by absorption and coprecipitation with hydrous Fe and Mn oxides, and with Ca and Mg carbonates (III). Although, immobilisation of metals seems to be very high in the lake next to the waste basin, mobilisation of metals is registered downstream in L. Kento. This is attributable to changes in the water conditions towards lower pH and low mineral content, including low complexation or chelation by DOC.

Perch (*Perca fluviatilis* L.) and roach (*Rutilus rutilus* L.) are common freshwater species found widely throughout Europe (Lappalainen *et al.*, 2001). Both are food generalists and they have no special requirements for spawning areas. The investigation by Lappalainen *et al.* (2001) demonstrated that in the Tvärminne area in SW Finland, for over twenty years there was an increase in roach stocks (main food molluscs) in comparison to perch (main food macro-crustaceans and fish). Eutrophication was suggested as the main cause of the increase in the roach population. This agrees with the study of Persson (1983), according to which roach are adapted to utilising molluscs, and they can crush their food mechanically in the dark, whereas perch are visual hunters dependent on vision, as reported by Diehl (1988). Perch, being carnivorous predators of relatively large and mobile prey, expend a lot of energy per capture, whereas roach as an omnivorous species have a feeding strategy that minimises energy expenditure (Persson, 1983; Lappalainen *et al*., 2001).

In the Kenti river system, roach (comprising 35–51% of biomass) and perch (32–59%) were the most numerous fish (Holopainen *et al.,* 2003b). The mean size of roach in L. Upper Kuito (the reference lake) appeared to be smaller than that in L. Kento (7 km from the depository) or in L. Poppalijärvi (3 km, from the mining plant). Yet young fish <10 cm were found in equal numbers in all lakes, suggesting normal reproduction. The growth rate of perch appeared to be similar in all three lakes in both 2000 and 2001. On average, perch reached a length of 17 cm at the age of 4+ years. Roach grew well in both the impacted lakes, reaching a length of 18 cm at the age of 4+, but showed a lower growth rate in the reference lake, with a length of only 15 cm at the age of 4+ years. A preliminary stomach analysis of a small number of young perch from all the lakes suggested a diet of insect larvae (e.g., *Ephemera vulgata*) and pupae. A large number of *Chydoriidae (Cladocera)* was found in L. Kento and L. Upper Kuito. The roach diet, however, appeared to differ between the lakes. *Cladocera* and *Chironomidae* predominated in L. Upper Kuito, but the larger *Ephemera* and *Sphaeriidae (Pisidium sp.)* were predominant in the two other lakes. This finding is in agreement with littoral zoobenthos samples, where the proportion of molluscs was highest in L. Poppalijärvi (Holopainen *et al*., 2003a, 2007; Aroviita *et al.,* 2006).

2.2. Accumulation and toxicity of metals in fish

Mercury (Hg) and cadmium (Cd) have no known biological function (Di Giulio *et al.,* 1995), but are able to bioaccumulate in the food chain (Mason *et al*. 1994; 1999; 2000). They can be toxic in small quantities and are present at high levels even in the Arctic region, far from most anthropogenic sources (AMAP, 2002).

Mercury:

Mercury accumulation in fish depends on the following: its chemical form (the most toxic being methylmercury), the water temperature, chlorine ions, dissolved organic matter (DOM), dissolved organic carbon (DOC) and hardness (Grieb *et al*., 1990; Newman and Jagoe, 1994; Hamilton, 1995; Downs *et al.,* 1998; Mason *et al.*, 2000; Wood, 2001; Duffy and Zhang, 2002). Selenium (Se) can reduce the toxicity of Hg in aquatic organisms by competing for protein binding (Kai *et al.,* 1995; Dorea *et al*., 1998; Nuutinen and Kukkonen, 1998). Methylmercury (MeHg) is mostly bound in protein via association with the thiol group in the muscle of the fish, while inorganic Hg is usually concentrated in the detoxifying organs, such as the kidney and the liver (Mason *et al.*, 2000). The gills are not the dominant uptake routes for MeHg, and blood rapidly transfers and distributes MeHg to internal organs (Mason *et al.*, 2000). Inorganic Hg accelerates the uptake of methylmercury by an unknown mechanism in the gills (Rodgers and Beamish, 1983; Wood, 2001). The loss mechanism for metalloids is depuration. Inorganic Hg is more effectively eliminated than methylmercury in clams (Inza *et al*., 1998).

For both forms of mercury, a failure in osmoregulation appears to be the proximate cause of acute toxicity (Heath, 1995). In freshwater fish the concentration-dependent decrease may occur in plasma Na^+, Cl^- concentrations and osmolality (Lock *et al*., 1981). These negative effects have two separate mechanisms: an increase in plasma-membrane osmotic water permeability (at a level well below 96-h LC50), and an inhibition of gills Na^+,K^+-ATPase, which reduces Na^+ uptake at concentrations close to the 96-h LC50 (in the range of 30-700 µg Hg_{inorg} l^{-1}) (Lock *et al.*, 1981; Spry and Wiener, 1991). Mercury has a strong affinity for sulfhydryl groups, which explains its ability to inhibit branchial Na^+,K^+-ATPase (Mason *et al.*, 2000).

The specific morphologic response of freshwater trout exposed to methylmercury was an increase in the frequency of mitotic figures in pavement cells, while seawater windowpane flounder that were chronically exposed to inorganic mercury showed chloride cell proliferation (Wood, 2001). Other non-specific responses could be swelling and hyperplasia of pavement cells, necrosis, excess secretion of mucus and mucous cell proliferation, epithelial rupture and fusion of lamellae. Fish exposed to high mercury levels may suffer damage to their gills, blindness and they may develop a reduced ability to absorb nutrients through the intestine. Mercury levels in fish are related to their age and size (Bodaly *et al*., 1993).

Cadmium:

Cadmium is taken up mainly through the intestine in fish and to a much lesser extentthrough their gills (AMAP, 2002). Fish kidneys and gills are organs for depuration, and they are also areas where metals accumulate, either directly from the water or from the food (Mason *et al*., 2000). Dietary cadmium is initially bound to the albumin and the blood cells, and then to metallothionein in the liver and kidneys.

Cadmium is considered to be a respiratory toxicant (Hollis *et al.*, 1999) and a nephrotoxicant for fish (Sangalang and Freeman, 1979). Lethal concentrations of cadmium in water may vary greatly, since Cd accumulation in fish depends on

water quality (pH, DOC etc., Table 1) and the sensitivity of the fish species (Hollis *et al.*, 1999, 2001). Toxic Cd concentration is below 100 µg l^{-1} in freshwater fish (Wood, 2001). In fish gills, both Cd uptake and toxicity occur via the chloride cells (Wicklund-Glynn *et al.*, 1994, Wood, 2001). Cadmium is an antagonist of Ca^{2+} uptake in fish gills, because the intracellular free Cd^{2+} ions competitively inhibit the basolateral Ca^{2+}-ATPase (Reid and McDonald, 1988; Verbost *et al.*, 1989; Hollis *et al.*, 2000). Uptake and plasma concentrations of Na^+ and Cl^- are less important, but may occur at higher threshold levels of Cd. These contribute to the damage to or death of chloride cells by both apoptosis and necrosis, and an associated loss of Na^+,K^+-ATPase activity. The recent investigation by Niyogi and Wood (2004) has shown that branchial influxes of Ca^{2+} and Cd^{2+} occur through common pathways. In the analyses of inhibitor constants for branchial Cd^{2+} uptake by waterborne Ca^{2+}, they indicated that the inhibition was three times more potent in yellow perch than in rainbow trout.

A respiratory effect during sublethal exposure was reported as a common morphological response, namely increased blood-to-water diffusion distance in the gills resulting from cellular proliferation, and hyperplasia of the chloride cells (Wendelaar Bonga and Lock, 1992, Hollis *et al.*, 1999). In contrast to Hg, there is no metallothionein induction in the gills by Cd (Hollis *et al.*, 2001).

Chromium:

Chromium is present in the water as various anions of Cr(III) and Cr(VI). The gills, liver, kidney and the digestive tract are the organs that accumulate the Cr at alkaline pH and in high environmental concentrations. At neutral pH only the gills accumulate Cr (Eisler, 1986). Adverse effects of chromium have been documented at 10.0 µg l^{-1} of Cr(VI) and 30 µg l^{-1} of Cr(III), in freshwater, for sensitive species (Eisler, 1986). No biomagnification of Cr has been observed in food chains, and its concentration is usually highest at the lowest trophic level. Arillo *et al.* (1982) reported that only male rainbow trout showed changes in liver enzyme activities (0.2 ppm Cr(VI) for 6 months). The effects were more intensive when Ni and Cd ions were present. In trout, Cr(VI) uptake increased when 10 ppb of ionic Cd was also present in the solution (Calamari *et al.*, 1982). In fish gills Cr(III) is directly coupled with the transfer of oxygen, and this reaction is more rapid at pH 6.5 than at alkaline pH (Van der Putte and Part, 1982; Eisler, 1986).

Copper and Zinc:

Essential metals such as copper (Cu) and zinc (Zn) are regulated by specific transport mechanisms in the gills and gut (Wood, 2001; Bury *et al.*, 2003).

Copper is used in numerous enzymatic processes because of its capability to accept or donate ions (redox reactions) (Hochachka and Somero, 2002, Bury *et al.*, 2003). However, Cu^{2+} is an ionoregulatory toxicant in fresh water, and pathophysiological effects are registered at total concentrations below 100 µg l^{-1} (Wood, 2001). Copper induces ionoregulatory dysfunction in the gills by inhibiting Na^+ and Cl^- influx (insensitive to $[H^+]$) and the effects are similar to those of the more toxic silver and aluminum (Wood, 2001). Copper has a strong affinity for the sulfhydryl group, which explains its ability to inhibit branchial Na^+,K^+-ATPase activity both in vitro and in vivo (Pelgrom *et al.*, 1995; Li *et al.*, 1996). Copper may increase gill transcellular permeability, since K^+ losses were disproportionately high relative to Na^+ and Cl^- losses (Lauren and McDonald, 1985). In addition, copper may induce lamellar damage and mucification in the gills (Wilson and Taylor, 1993).

According to Wood (2001), the blood pH level of fish was increased as a result of metabolic alkalosis in copper sublethal exposure, which may reflect an internal build-up of ammonia in the plasma and tissue, a phenomenon which has been implicated in reduced swimming performance (Lauren and McDonald, 1985; Taylor *et al.*, 1996; Wang *et al.*, 1998; Beaumont *et al.*, 1995a,b). Furthermore, the decreased aerobic swimming performance of the fish may reflect changes in gill morphology, such as epithelial lifting, lamellar fusion, necrosis and apoptosis caused by cortisol (Bury *et al.*, 1998). This is in contrast to the necrosis that is caused by copper directly (Li *et al.*, 1998; Bury *et al.*, 1998, Wood, 2001). Repair during chronic exposure involved proliferation of chloride cells and mucous cells with an accompanying increase in the total Na^+,K^+-ATPase activity of the gills, a reduction in diffusive ion losses and a restoration of internal ionic level (Pelgrom *et al.*, 1995; Li *et al.*, 1998). In contrast to Hg and similar to Cd, there is no metallothionein induction in the gills or change in the gill copper uptake or binding (Grosell *et al.*, 1996, 1997; Wood, 2001; Hollis *et al.*, 2001).

More than 200 zinc based enzymes or other proteins have been identified from various organisms. In contrast to Cu (and iron), zinc does not form free radical ions and has antioxidant properties (Powell, 2000, cited by Bury *et al.*, 2003). Zinc inhibits active Ca^{2+} uptake across the branchial epithelium and vice versa. One result may be hypocalcemia, since Ca^{2+} and Zn^{2+} share a common uptake pathway via the chloride cells (Wood, 1992; Hogstrand *et al.*, 1998; Wood, 2001). In contrast to copper, zinc does not inhibit net Na^+ and Cl^- uptake in the gills, so that the plasma level of these ions is not affected. Zn induces metallothionein in branchial tissue (Hogstrand *et al.*, 1995). Chloride cell proliferation on the respiratory lamellae has been reported at high chronic zinc levels, explaining the long-term increases in branchial Na^+,K^+-ATPase activity, and the repair of structural damage in the gills appears to proceed in the same way as described for copper (Watson and Beamish, 1980; Thomas *et al.*, 1985; Spry and Wood, 1989; Hogstrand *et al.*, 1995; Wood, 2001).

Potassium:

Potassium is an essential mineral for animals due to its role in electrolyte and acid-base balance, which helps to maintain plasma viscosity and osmotic pressure (Emsley, 2003). Red blood cells contain the most potassium, followed by the muscles and brain tissue. The most important functions of K in the cells are: regulating intracellular fluids, solubilising proteins, operating nerve impulses and contracting the muscles. Potassium, being a K^+ ion, concentrates inside the cells, and 95% of the body's K is located in the cells, unlike sodium and calcium, which are more abundant outside the cells. Cell membranes have channels for selective transport of Na^+ and K^+ ions against the concentration gradient (Hochachka and Somero, 2002). Information about the physiological effects of potassium on fish seems to be very sparse (Fielder *et al.*, 2001).

Lithium:

The lethal concentration of dissolved Li for human ingestion is 5 g l^{-1} (Kszos and Stewart, 2003). Corbella and Vieta (2003) reviewed more than 300 studies on the long-term effects of Li on humans. The main effects of Li are neuropsychiatric indications, such as acute mania, depression, aggression, schizophrenia, and teratogenic (Ebstein's anomaly) and metabolic effects. Lithium can stimulate glycogen synthesis in mammals through the activation of glycogen synthase and by mimicking insulin action, which causes weight gain. Lithium has teratogenic effects in amphibians (Boğa *et al.*, 2000) and may affect embryonic development (Stachel *et al.*, 1993). Lazou and Beis

(1993) found that Li affected the plasma membrane protein pattern in amphibian embryos. Bury *et al*. (2003) in their review of metal uptake in teleost fish supposed that Li passes via a putative epithelial sodium channel, which is probably also an important pathway in sodium-sensitive copper uptake (Grosell and Wood, 2002).

2.3. Effects of temperature, pH level, water hardness, and iron deposition on metal uptake and toxicity

The toxicity of metal ions depends on water quality, ion composition and the acid-base equivalents, as well as on the tolerance of the fish or other aquatic organisms, and digestive or respiratory metal uptake and excretion (Newman and Jagoe, 1994; Hamilton, 1995; Downs *et al*., 1998; Mason *et al*., 2000; Wood, 2001; Mason, 2002; Duffy and Zhang, 2002). The effects of abiotic factors, such as temperature, are of great importance as reflected in Table 1. In general, the higher the temperature of the water, the more toxic are metals such as Hg, Ni, Cr and K (Eisler, 1986; Fisher *et al*., 1991; Hamilton, 1995; Mason *et al*., 2000; Wood, 2001; Duffy and Zhang, 2002).

Campbell and Stokes (1985) described two contrasting responses of an organism to metal toxicity combined with a declining pH level:

1) a reduction in competition for binding sites with hydrogen ions (if the change in speciation is small and the metal binding is weak at the biological surface),
2) an increase in the metal availability (if there is a marked effect on speciation and strong binding of the metal at the biological surface).

In general, metals such Cd, Cu, Cr are more bioavailable and toxic for aquatic organisms in acidic water (Bradley and Sprague, 1985; Eisler, 1986; Lauren and McDonald, 1986; Playle *et al*., 1993*;* Hamilton, 1995; Pelgrom *et al*., 1995; Mason *et al*., 2000; Hollis *et al., 2000;* Wood, 2001; Naddy *et al*., 2002; Bury *et al*., 2003).

The ion transport and permeability characteristics of fish gills are known to be extremely sensitive to water chemistry, particularly to hardness. Hardness is caused by main ions such as calcium (Ca) and magnesium (Mg), and for most toxicants, Ca^{2+} exerts a greater protective effect than Mg^{2+} (Wood, 2001). Calcium regulates the permeability and stability of membrane proteins in fish gills (Flik and Verbost, 1993). The higher Ca^{2+} (and Mg^{2+}) concentrations in water lead to lower availability of metals. Similarly, a high level of dissolved organic carbon (DOC) reduces Hg, Cd, and Cu bioavailability (Playle *et al*., 1993; Hamilton, 1995; Mason *et al*., 2000; Niyogi and Wood, 2004). In addition, compounds such as chlorine ion and dissolved organic matter (DOM) have been found to limit Hg bioavailability (Newman and Jagoe, 1994). High water alkalinity reduces the toxicity of Cd, Cr and Cu (Carre *et al*., 1983; Linnik and Nabivanetch, 1986; Niyogi and Wood, 2004). High concentrations of sodium ions were found to reduce Cd and Li toxicity in water habitats (Kszos *et al*., 2003; Niyogi and Wood, 2004). Hardness is thus a surrogate for the protective aspects of water chemistry. It also has effects on the diffusive gradients for passive ion loss, substrate concentrations for active ion uptake, speciation on the toxicant or charged groups on the branchial surface (Wood, 2001).

It has been recognized that iron in the form of freshly precipitated ferric hydroxide $Fe(OH)_3$ quickly scavenges many heavy metals, organics, and phosphorus (Jones Lee and Lee, 2005). Martinez and McBride (2001) reported that Cu, Cd, Pb, and Zn were coprecipitated with ferric hydroxide, and that the binding of the metal to the ferric hydroxide depended on the type of metal, and for some metals, on the age of the precipitate.

2.4. Mechanism of metal toxicity in gills of freshwater organisms

The gills are the most vulnerable fish organs for metal toxicity. The extremely thin (0.5-10 μm) diffusion distance between water and blood and the effectiveness of the diffusive exchange, is maximised by the counter current flow of water versus blood at the exchange sites (Wood, 2001). The apical epithelial surfaces of gills are rich in the cells involved in ion exchange (pavement and chloride cells) and in cells that attract specific ions, such as water-borne metals, by their external mucous layer being charged negatively (mucous cells) or neutrally (rodlet cells) (Mallatt, 1985; Matey, 1990; Reid and McDonald, 1991; Wood, 2001; Evans *et al*., 2005). These facts explain why fish gills are the first sites of uptake for many water-borne toxicants such as metals.

The similarities and differences in metal toxicity mechanisms for freshwater fish gills are summarized in Table 1. The table is organized according to binding affinities (Log K) (Niyogi and Wood, 2004). Roughly, all other factors being the same, the Cd^{2+} is more toxic to gills than Cu^{2+}, which in turn is more toxic than Zn^{2+} and so on. Consequently, the higher binding affinities (log K) bring greater toxicity for a particular metal. Wood (2001) concluded that the toxic mechanisms at the gills have diversities for different contaminants at environmentally realistic concentrations, and therefore no general mechanism of toxicant action in the gills can be proposed. The main toxic site of metal actions in the gills is the Ca^{2+} membrane channel for Cd^{2+}, Zn^{2+} and methylmercury. Copper acts through the Na^{+} channel, and Li^{+} is assumed to act similarly. Ionoregulatory toxicants, such as Cd^{2+}, inhibited Ca^{2+} or Na^{+} and Cl^{-} influx, as did Cu^{2+} and both forms of mercury. These toxicants affect the enzymes Ca^{2+}- or Na^{+}, K^{+}-ATPase activity. The latter enzyme appears to be a key toxic site for the Na^{+} antagonist. However, it was also inhibited by mercury, which acts (or is assumed to act for inorganic Hg) through the Ca^{2+} membrane channel. Thus it is possible to observe that the metabolic pathway does not depend on the toxic site of action. In other words, mercury can use both the Na^{+} and Ca^{2+} transport ion channels to penetrate the gill membranes (Playle, 1998).

Since abiotic factors have a high importance for metal availability (as described above), short notes are also given in Table 1 for each metal (for references see chapter 2.2.).

Table 1. *Metal toxicity mechanisms for fish gills in freshwater.*

	Log K	The point of toxic action in gills	Metal uptake localisation	Mechanism of action	References	Effects of abiotic factors on metal bioavailability
Cd	8.6	Ca^{2+} transport	Chloride cells	Inhibits Ca^{2+} influx and Ca^{2+}-ATP activity, while binding to calmodulin protein, no metallothionein induction.	Wicklund-Glynn *et al.*, 1994; Hollis, 1999, 2000; 2001; Wood, 2001; Niyogi and Wood, 2004	pH, alkalinity, DOC, Ca^{2+}, Mg^{2+}, Na^{+}, Zn^{2+}
Cu	7.4-8.0	Na^{+} transport, can be Na^{+} insensitive	Chloride cells	Ionoregulatory toxicant: inhibits Na^{+} and Cl^{-} influx, raises transcellular permeability, Na^{+},K^{+}-ATPase inhibitor by high affinity to SH-group, stimulates mucous cell secretion.	Lauren and McDonald, 1985; Pelgrom *et al.*, 1995; Li *et al.* 1996; Wood, 2001, Grosell *et al.*, 2004a,b	pH, alkalinity, DOC, Ca^{2+}, Zn^{2+}**, Ni^{+}**, Cu^{2+}**
Zn	5.3-5.5	Ca^{2+} transport	Chloride cells	Inhibitor of carbonic anhydrase and Ca^{2+}-ATPase activity	Hogstrand *et al.*, 1995; Hogstrand and Wood, 1996; Wood, 2001	pH, Ca^{2+}, Mg^{2+}, Ni^{+**}, Cu^{2+**}
Cr(III); Cr(VI)	U/n	U/n	U/n	More toxic at higher temperatures	Eisler, 1986; Pawlisz *et al.*, 1997	$t^{o}C$*, pH, alkalinity, DOC, Ca^{2+}, Mg^{2+}, Ni^{+}**, Cd^{2+}**
MeHg	U/n	Penetrates by diffusion; Ca^{2+} transport	Chloride cells	Ionoregulatory toxicant: Lipophilic, inhibits Na^{+} and Cl^{-} influx, raises transcellular permeability; Na^{+},K^{+}-ATPase inhibitor by high affinity to SH-group	Lock *et al.*, 1981; Heath, 1995; Playle, 1998; Wood, 2001	$t^{o}C$*, DOC, DOM, Ca^{2+}, Mg^{2+}, Cl^{-}, Se^{2+}
Hg	U/n	U/n, assumed Ca^{2+} transport	Distributed diffusely in gill epithelium	Ionoregulatory toxicant: Permeability increase, inhibits Na^{+} and Cl^{-} influx, Na^{+},K^{+}-ATPase inhibition, stimulates mucous cells proliferation and secretion		
Li	U/n	Reportedly by Na^{+} transport	Assumed by chloride cells	Assumed as an ionoregulatory toxicant. Water ions K^{+} and Na^{+} play a protective role against Li	Grosell and Wood, 2002; Kszos *et al.*, 2003; V.	Na^{+}, K^{+}

*U/n – unknown; * the lower the factor the lower the metal bioavailability; **-additive toxicity effect*

3. Material and methods

3.1 Field sampling

Three lakes in the Kenti river system – L. Poppalijärvi, L. Kento and L. Upper Kuito were sampled in early August in 1997, 1998, 2000 and 2001. Some samples were from April 1997 and 2000 and some were taken from L. Kamennoe (in the year 1997 and 1998). L. Kamennoe and L. Upper Kuito are reference lakes not affected by mining waters. Samples for water quality were taken and analyzed according to Finnish standard methods (SFS Standards) in the water laboratory of the University of Joensuu. For all sediment samples only the first 4 cm of the surface were used. The sediments were stored frozen for 2 months before the analyses.

Multimesh gillnets were used to monitor the abundance and structure of the fish fauna (e.g. Kurkilahti and Rask, 1996). Stratified random sampling was carried out in each lake with nine nets (3 inshore or 0-3 m, 3 on the surface and 3 on the bottom offshore) for 12 h. The fish were identified, counted, measured and weighed according to species, and sampled for scales and opercula for growth analysis (Holopainen *et al*., 2003b; I). The samples for chemical and histological analyses were taken immediately after catching. Descriptions of the fish sampled from different lakes are given in (III), except for the fish from the year 2001. The results for these fish are unpublished and given in Table 2.

Table 2. *Characteristics of fish sampled from the Kostomuksha area. Mean ± S.E., values with no common letter (superscript) are different when compared by ANOVA followed by the Post Hoc LSD-test ($p<0.05$).*

	Poppalijärvi	Kento	Upper Kuito
Summer 2001	August 2-3	August 8-9	August 1-2
Perch			
n	15	15	15
Fork length (mm)	163±4[a]	175±5[a]	169±4[a]
Weight (g)	54±4[a]	97±25[b]	61±6[a]
Liver somatic index	0.64±0.05[a]	0.77±0.08[a]	0.97±0.06[b]
Roach			
n	15	15	15
Fork length (mm)	173±3[a]	166±3[b]	143±5[c]
Weight (g)	79±5[a]	69±5a	41±4b
Liver somatic index	0.63±0.06[a]	0.94±0.16[a]	0.8±0.06[a]

3.2. Laboratory experiments

The effects of a Li&K mixture (Experiment I) and Li only (Experiment II) were studied in juvenile rainbow trout (*Oncorhynchus mykiss)*. This is a fish species with a high metabolic level suitable for adequate response, even in a short-term laboratory experiment. In both experiments rainbow trout were obtained from a local fish farm, brought to the laboratory and acclimated to the local tap water with comparable pH and oxygenated levels (IV, V). The fish were fed with granulated industrial dry food to an amount of about 1% of fish mass. During the experiments the fish were not fed. The total fasting period in both experiments was three weeks.

The differences in the experiments were water hardness (soft in the Li&K mix and medium hard in the Li exposure), temperature (12 and 13°C in the Li&K mix; between 14 and 15°C in the Li exposure), standing water (Li&K) and flowing water (Li), fish weight, and Li concentration (Table 3). Nevertheless, concentrations of Li were comparable between the exposures and similar to the condition of L. Poppalijärvi regarding the Na^+/Li^+ log ratio, and fish sizes were also maximally comparable (Table 3.).

Table 3. *Water and fish characteristics during the laboratory exposure. Rainbow trout were exposed to the Li&K mixture in standing water (Feb. 2002, Joensuu) and in flowing water to the Li only (a step-up exposure, July-August, 2004, Hamilton). Mean ±S.E.*

	T°C	Ca mg l^{-1}	Mg mg l^{-1}	Li µg l^{-1}	Na mg l^{-1}	K mg l^{-1}	Log Na/Li	Log K/Li	Weight, g	Fork length mm
Exp.I	12-13	16	1.9-2.6	27±4	2.3-2.9	115-126	0.35	-0.2	19±1	11.9±0.2
Exp.II	14-15	40	4.9	66±27; 528±165	13.6	2	0.07; 0.2	1.2; 1.2	13±2	10.5±0.5

3.3. Analyses

3.3.1. Heavy metals

After taking fork length (AC) measurements and weights of all the fish caught from each lake, 15 fish were randomly selected. Samples of skeletal muscle (2-3 g) were dissected from the left side (between the dorsal fin and lateral line) of each fish. The whole gill arches and whole livers were dissected (with hands dressed in powderless surgical gloves). Each tissue sample was collected into a separate uncoloured polyethylene vial. Before sampling, each vial was cleaned in 10% HNO_3 for 5 hours and washed thoroughly with de-ionized water, rinsed 3 times, dried, labeled, preweighed and packed in two clean paper boxes with lids. Samples for metals, histology, enzymes, and lipid analyses were taken from each fish simultaneously and placed in separate vials. The samples were kept on ice (1997, 1998) and in liquid nitrogen (2000, 2001) until arrival at the laboratory. Samples were kept in the freezer for up to two days (-20°C, samples from 1997 and 1998) and for up to two months (-20°C inorganic samples from 2000 and 2001). Then the samples were dried at 105°C for about 12 hours. The dry samples were kept in the same vials at +4°C until they were analyzed less than 6 months later (samples from the years 1997-1998) or analyzed immediately (all other samples). The dried samples were digested in a microwave digestion unit (Milestone 1200 mega) in a mixture of HNO_3 and H_2O_2 in proportions of 4:1 for fish, and 5:1 for sediment. Cadmium, chromium and nickel in water, sediment and fish samples were analyzed by graphite furnace AAS (Hitachi Z-9000). Copper and zinc were measured by flame AAS and mercury concentrations by a gold-film mercury analyzer (Jerome Inst. Corp. Model S-11). Water potassium and Li concentrations were analyzed by atomic absorption spectrometry (Perkin Elmer AAS). Minimum detection limits (ng ml^{-1}) according to the technical notes were: Cd=0.0014; Cr=0.0038; Ni=0.072; Zn=0.8; Cu=1; Hg=0.009. The reagents blank value and standard solutions with known concentration were used for the quality control.

3.3.2. Histology and histochemical analyses

A transmission electron microscope was used to study the structure of the liver (II)

and the gills (III, IV) in Epon section. Light microscopy was used to count iron deposits in the gills after histochemical staining in paraffin section (III).

3.3.3. Enzyme analyses

The field and Experiment I: total ATPase and Na^+,K^+-ATPase activities in the gill were measured using a kinetic method described by Johnson *et al.* (1977). Proteins were measured by standard methods with the Folin phenol reagent (Lowry *et al.*1951). In the gills of Li-exposed fish (V), total ATPase and Na^+,K^+-ATPase activities were measured using a kinetic microassay run in 96-well microplates, as outlined by McCormick (1993). The latter method is preferred because it requires less sample tissue and chemicals. However, the first method measured the total ATPase activity (III, IV), which is not the case in the second method (V).

The citrate synthase (CS) activity was measured using a kinetic microassay run in 96-well microplates as described by Leonard and McCormick (1999). Protein concentrations were determined by the Bradford method (Biorad) (V).

3.3.4. Lipid analysis

All lipids (about 100 mg) were extracted from the gill tissue (separated from the cartilaginous arches, left part of the gills) and homogenized in a chloroform-methanol (2:1, vol/vol) solution (Blind and Dyer, 1959). Then the lipids were extracted at room temperature during 40 minutes in the dark and collected as described in articles III, IV and V. However, after the extraction, the lipids were redissolved and analyzed differently (III-V).

A thin layer chromatography, flame ionization detection system (Iatroscan, Iatron Laboratories Inc., Japan) was used at the University of Joensuu (Finland) for gill lipid determination. All the extracts (samples, standard, blank) were scanned in duplicate and then calculated in relation to the internal standard (cholesterol acetate) (IV and unpublished data in the Summary). Since Iatroscan was not available at McMaster University (Canada), we used enzymatic colorimetric methods for the quantitative determination of total free fatty acids and total cholesterol with commercial diagnostic kits (NFFA C and Cho E, Wako Chem.) (V). The latter method is less time-consuming, but it limited the diversity of lipids to those for which the kit was designed.

3.3.5. Osmolality and ionic concentrations of plasma

Blood samples were obtained only in Experiment II. Plasma measurement of osmolality, total Na^+, K^+ and Cl^- concentrations were made in order to assess the effect of Li exposure on juvenile rainbow trout (V).

3.3.6. Apolipoprotein AI analysis

A semi-quantitative Enzyme Linked Immunosorbent Assay (ELISA) was used to assess ApoAI in blood plasma, since purified trout apolipoprotein AI (apoAI) was not commercially available for a fully quantitative assay. The apoAI was analyzed in plasma samples from the second step of exposure at the higher Li concentration. A detailed description of the method is given in article V.

3.3.7. Statistics

All heavy metal concentrations in tissues ($\mu g\ g^{-1}$) are expressed on the basis of dry weight. The heavy metal data were $\log_{10}$ transformed prior to statistical analysis. A non-parametric Kruskal-Wallis test used to compare three independent groups of sampled data (Zar, 1999). For statistical similarities and differences, and to find out whether these are due to the effect of metals or are changes depending on time or the lake in question, fish morphometric

parameters, concentrations of plasma ions, osmolality, lipids and enzymes values were compared by one-way analysis of variance (ANOVA), followed by the Least Significant Difference test (LSD). The Kolmogorov-Smirnov test was used in both the exposed and the control group for determining statistical significance as against Li added directly to the media for Na^+,K^+-ATPase analyses. The Student's unpaired two-tailed t-test was performed with control fish and gill lipids sampled at the same time from L. Poppalijärvi and L. U. Kuito. The Student's t-test was used for 405 nm absorbance at each dilution in the ELISA assay to compare the mean area and describe the effect of Li along the plasma dilution curve. SPSS 10.0 and Excel 2000 computer programs were used for statistical analyses.

4. Results

4.1. Fish communities

Seven species of fish were caught in L. Poppalijärvi and L. Kento. In the reference lake the number of species was eight; dace (*Leuciscus leuciscus*) was missing from the impacted lakes (I). Roach and perch were most numerous, and roach were predominant in the catch from all lakes. The total biomass per unit effort was twice as high in the impacted lakes (Holopainen *et al*., 2003b; I).

4.2. Metals in the water and sediment

Table 4 summarizes the water quality data from the field studies in the years 1997-2001. Copper shows a higher concentration in L. Poppalijärvi in the spring than in the summer. Chromium was higher in the uppermost L. Ahvenjärvi than in L. Poppalijärvi or in the other lakes. High concentrations of Cr (91 μg l^{-1}) were found in the wastewater data of Virtanen and Markkanen (2000). Cadmium primarily affected L. Poppalijärvi.

Aluminium, Zn and Fe were low in the L. Poppalijärvi water when compared to the reference lake, L. Upper Kuito (Table 4). Iron content fluctuated a lot in summer and early spring, and was, for example, about ten times higher in early April 2000.

The alkali metals Li and K were found at much higher concentrations in L. Poppalijärvi compared to the reference lake (Table 4).

Sediment analysis demonstrated high concentrations of Hg, Cd, Zn, Cu and Fe in L. Poppalijärvi in 2000 (Table 5). The reference lake had a higher value for Cr deposition than the study lakes. The sediments of L. Kento appear to have been contaminated more heavily in the past since the top layer had a low metal concentration (Table 5), except for Fe. Iron showed a higher concentration in the surface layer (0-2cm) than in the deeper layer (2-4 cm). While the average concentrations of Cu and Cr were low, the Hg, Cd, Fe and Zn showed elevated concentrations in the studied lakes.

Table 4. *Lake description and surface water quality in the Kenti river system in 1997-2001. Year 1997 – data from Morozov (1998) and Virtanen and Markkanen (2000). A – results from L. Ahvenjärvi. No asterisk – data from August 2000 and 2001, * data from April 2000, **data from Lozovik et al., 2001 (L. Middle Kuito), ***data from August 2002.*

	Lakes from the Kenti River System						Reference lakes		
	Poppalijärvi			Kento			Kamennoe	Upper Kuito	
Surface area (km^2)	1.7			27.1			95.5	197.6	
Maximum depth, m	11			24			29	44	
Year	1997	2000	2001	1997	2000	2001	1997	2000	2001
pH	7.6-8.2	8.3	8.4	6.6	7.7	7.7	6.4-7.0	6.8	6.7
Oxygen, surf./ bot., % of saturation ***		107/95	94/97	97/74	96/85			115/95	90/94
Conduct. mS m^{-1}	-	39.1	39.4	-	14.9	14.0	2.6	2.5	2.4
Color, Pt mg^{-1}	50	35 (50*)	-	60	40	-	25	40 (70*)	-
TOC mg l^{-1}	6.2	7.7*	-	7.2	-	-	6.1	11.6*	-
IC mg l^{-1}	-	17.8*	-	-	-	-	-	2.3*	-
Tot. Phosph. µg l^{-1}	8	6	9	7	8	8	7	14	11
Tot. Nitrogen, µg l^{-1}	2200	3888	4038	270	797	697	220-350**	263	581
SO_4^{2-} mg l^{-1}	56	82.1	-	23	20.6	-	2	2.2	3.6**
Cl^- mg l^{-1}	3.7	3.7	-	1.5	1.5	-	0.8	0.8	-
Na mg l^{-1}	6.0	6.2 (8*)	6.4	2.0	2.8	2.6	1.2	1.2 (2.1*)	0.8
K mg l^{-1}	60	60 (62*)	60	13	20	20	0.4	0.5 (1.0*)	0.4
Li µg l^{-1}	17-38	20	-	7	7*	-	0.2	0.2	-
Ca mg l^{-1}	35	21 (18*)	23	6.2	7.9	8.4	1.6	1.9 (1.6*)	1.9
Mg mg l^{-1}	8.0**	8.3	7.7	3.0**	3.1	3.2	-	0.8	0.6
Al µg l^{-1}	5.9A	11	-	-	17	-	-	57	35-60**
Zn µg l^{-1}	0.63A	6 (8*)	-	0.45	9	-	5	16 (0*)	-
Cu µg l^{-1}	0.49A	4.31*	-	0.33	-	-	1	0.93*	-
Cr µg l^{-1}	8.3A	0.38*	-	0.20	-	-	<1.0	0.37*	0.55-0.58**
Cd µg l^{-1}	<0.03	0.20 (0.19*)	-	<0.03	0.1	-	<0.03	0.1 (0.07*)	-
Fe µg l^{-1}	≈600	38-45 (434*)	58	≈200	98-104	123	300	202-266 (269*)	240

Table 5. *Heavy metal contents and pH in sediment in the Kenti river system in August 2000 (µg g^{-1} dry wt, for iron mg g^{-1}).*

	pH	Hg	Cd	Cr	Cu	Fe	Zn	Organic matter	C org *
L. Poppalijärvi	6.7	0.125	1.8	221	17	216	140	0.89	12.6
L. Kento, AV	5.5	0.085	1.2	180	7	140	109	0.58	14.4
L. Kento 0-2 cm			0.9	139	4	205	68		
L. Kento 2-4 cm			1.5	222	11	74	149		
L. U. Kuito (ref.)	5.7	0.075	0.5	345	14	49	106	0.55	3.13**

* data from Lozovik *et al.* (2001), % of dry wt **L. Middle Kuito.

*** The results are higher than the standards, even after some dilution.

4.3. Metal concentrations in the fish

Mercury and Cadmium: Table 6 gives an overview of the heavy metal concentrations in the fish organs in articles I-III as well as unpublished data from the year 2000. Only the concentrations in August are presented in Table 6. The highest Hg concentrations were found in perch muscle, liver and gills in L. Poppalijärvi in 1997 (Table 6) and in the sediment in 2000. In 2000, more Hg was accumulated in perch muscle, liver and gills in the reference lake, L. Upper Kuito. Roach accumulated less Hg than perch. However, roach from L. Poppalijärvi basically accumulated more Hg than roach from the reference lake. This correlates with the Hg sediment concentration (Table 5). Cadmium accumulated more in fish from the reference lakes (Table 6) than in the Kenti river system, despite the higher depositions in the water and sediment in the upper part of the Kenti river system (Tables 4 and 5). Cadmium concentration was lower in roach liver, while in the gills the concentration was similar to or higher than in perch.

Copper and Zinc in the liver: Significantly more copper was accumulated in liver of roach than of perch. The highest Cu concentration was in roach livers from L. Poppalijärvi (Table 6). This is thus associated with the higher Cu concentration in the water and also in the sediment (Table 4 and 5). Zinc showed higher concentrations in the water and roach liver from the reference L. U. Kuito (Table 6), regardless of the low Zn deposition in the sediments.

Chromium and Zinc in the gills: Chromium concentrations varied in fish gills from year to year. For example, Cr was higher in the water and in the fish in L. Poppalijärvi (and Koivas – see article III) in the summer of 1997. In the year 2000, Cr was low in the fish and in the sediment in L. Poppalijärvi (Table 6 and 5), and it was about the same in the water compared to the reference L. U. Kuito (Table 4). Zinc was high in roach gills from L. Poppalijärvi in 1997, but it declined in the year 2000. The roach accumulated much more Zn in the gills than did the perch.

Table 6. *Comparison of the heavy metal concentrations in perch and roach organs (µg g^{-1} dry weight, medians) between the three lakes from the Kenti river system (n=15, except for the year 1997 (n from 10 to 19). The control lakes were L. Kamennoe in 1997 and L. Upper Kuito in 2000.*

Metal	organ	Study year	L. Poppalijärvi		L. Kento		Reference lake		Kruskal-Wallis test	
			perch	roach	perch	roach	perch	roach	perch	roach
Hg	Muscle	1997	1.45	0.82	0.49	0.44	0.84	-	p<0.05	p<0.05
		2000	0.46	0.40	0.56	0.24	0.84	0.32	p<0.05	p<0.05
	Liver	1997	1.13	0.18	0.42	0.12	0.43	-	p<0.05	p<0.05
		2000	0.58	0.15	0.51	0.14	0.65	0.15	p<0.05	ns
	Gill	1997	0.55	0.23	0.23	0.17	0.32	-	p<0.05	p<0.05
		2000	0.21	0.11	0.18	0.11	0.29	0.07	p<0.05	p<0.05
Cd	Liver	1997	1.76	0.14	1.73	0.24	2.34	-	p<0.05	p<0.05
		2000	1.06	0.16	2.16	0.17	1.79	0.36	p<0.05	p<0.05
	Gill	1997	0.038	0.033	0.055	0.046	0.159	-	p<0.05	p<0.05
		2000	0.008	0.012	0.031	0.051	0.087	0.18	p<0.05	p<0.05
Cr	Gill	1997	0.55	1.83	0.44	0.26	0.26	-	p<0.05	p<0.05
		2000	0.09	0.12	0.04	0.07	0.39	0.35	p<0.05	p<0.05
Cu	Liver	1997	10	30	9	20	17	-	p<0.05	p<0.05
		2000	9	34	12	25	11	27	p<0.05	p<0.05
Zn	Liver	1997	115	172	119	175	104	-	p<0.05	p<0.05
		2000	106	117	108	137	124	166	p<0.05	p<0.05
	Gill	1997	92	536	94	392	104	-	p<0.05	p<0.05
		2000	93	369	83	438	91	442	p<0.05	p<0.05

- There were no roach in L. Kamennoe

4.4. Fish tissue damages

Liver: Perch liver somatic indexes were increased in L. Poppalijärvi for both males and females (III), except in 2001 (Table 2). The main changes in perch liver cells were an increased distance between hepatocytes and glycogen inclusions, a decrease in hepatocyte size and in the size of their nuclei and mitochondria (II).

Gills: Morphological changes in perch gill structure in L. Poppalijärvi (III) were the following:

a) changes in the pavement cell shapes caused by weakly organized microridges,

b) the activities of the secretory cells (both mucous and rodlet) were very high,

c) the mucous and rodlet cells were numerous in L. Poppalijärvi,

d) the epithelium of the secondary lamellae was increased in thickness because of the thicker basement membranes and the increased size of chloride cells.

The chloride cells (CC) in perch gills in L. Poppalijärvi increased their size and the length of their open areas. The changes in their apical membrane and mitochondria were the following:

a) higher electronic density and increased size of coated vesicles,

b) absence of the apical cavities,

c) the tubular reticulum was organized weakly and had only a few reciprocal contacts

d) the mitochondria were much larger and more numerous

e) the morphology of the mitochondria was abnormal and the cristae were very sparse

Experiment I, with the Li&K mixture, evoked a significant decrease in the mitochondria numbers and in the nuclear size of the trout gill chloride cells. However, the rainbow trout gill tissue did not show any change in the CC size or in the length of its open area. The microridges of the CCs were numerous and high in the control fish (IV).

There were few iron deposits in the epithelium of the perch and roach gills, and the lowest numbers were found in L. Poppalijärvi in 2001 (III). Fish from the reference lake with neutral pH accumulated more iron granules in one visual field, regardless of the lower Fe concentration in the sediments.

4.4. Lipids in the gills

Lipids in the perch and roach gills showed the following results: a) a prevalence of triglycerides in the gills in early spring and b) low cholesterol and a higher concentration of sphingomyelin and phosphatidylcholine in fish gills from L. Poppalijärvi in summer 2000 (III).

Unpublished data on fish caught in August 2001 show that total lipid content in L. Poppalijärvi was higher for roach, but lower for perch (100 and 70 µg/g of tissue respectively) when compared to the reference lake (50 and 120 µg/g of tissue respectively). Perch gill lipids did not show much difference between L. Poppalijärvi and the reference lake, with the exception of cholesterol, where there was a difference both in weight and in proportion. The cholesterol concentration was low in perch from L. Poppalijärvi, as was the relative concentration in summer 2000 (III).

The data from 2001 (Table 7) show that roach gills in L. Poppalijärvi had significantly higher concentrations of the following lipids:

a) cholesterol (Cho),

b) phosphatidyl-ethanolamine and phosphatidyl-serine (PE+PS),

c) free fatty acids (FFA).

The proportion of triglyceride (TG) was significantly low in roach gills in the upper lake.

Table 7. *The concentrations (µg/g of tissue) and proportions (%) of triglyceride (TG), free fatty acids (FFA), cholesterol (Cho), sphingomyelin (SM) and phospholipids phosphatidyl-choline (PC) and phosphatidylethanolamine and -serine (PE+PS) in the gills of perch and roach in August 2001 (mean ± S.E., t-test).*

Fish/Lake	Poppalijärvi	U. Kuito	t-test	Poppalijärvi	U. Kuito	t-test
Perch	*µg/g tissue*	*µg/g tissue*		*%*	*%*	
TG	4.9±1.8	7.1±2.0	ns	5.13±11	7.4±1.2	ns
FFA	2.2±0.03	2.8±0.5	ns	3.3±0.5	3.1±0.7	ns
Cho	14±1.2	21.4±3.6	p<0.1	23.7±2.5	22.4±1.8	ns
SM	2.5±0.3	2.8±1.0	ns	2.8±0.5	8.5±0.4	ns
PE+PS	26.1±4.5	32.2±4.4	ns	37.7±2.5	34.6±2.1	ns
PC	23.1±2.8	31.7±8.6	ns	27.5±2.9	29.5±2.5	ns
Roach						
TG	12.5±3.6	18.8±3.7	ns	15.7±2.6	33.3±4.6	p<0.05
FFA	3.9±0.5	0.6±0.2	p<0.05	5.9±1.2	1.8±0.2	p<0.05
Cho	27.7±4.8	5.8±1.2	p<0.05	21.7±3.5	12.0±1.4	p<0.05
SM	2.9±2.8	1.5±1.7	p<0.05	4.4±1.1	2.8±0.4	p<0.1
PE+PS	49.5±10.0	11±1.5	p<0.05	32.9±4.3	26.0±2.9	ns
PC	21.6±2.8	12.3±1.7	p<0.05	18.9±2.3	24.1±2.0	ns

Thus gill lipid compositions are comparable between the years 2000 and 2001, except for roach cholesterol (which was low in L. Poppalijärvi in August 2000, and nominally and proportionally higher in August 2001). Significant correlations between lipids are presented in Table 8 for fish gills from L. Poppalijärvi and the reference L. Upper Kuito.

Table 8. *Positive correlations were found between lipids, and between cholesterol and liver indices in fish gills from L. Poppalijärvi and L. U. Kuito in August 2001. Triglyceride (TG), free fatty acids (FFA), cholesterol (Cho), sphingomyelin (SM) and phospholipids phosphatidylcholine (PC) and phosphatidylethanolamine and -serine (PE+PS).*

	Correlation, perch, n=22	p<	Correlation, roach, n=28	p<
Cho, PE+PS	0.687	0.000	0.601	0.001
Cho, PC	0.613	0.002	0.592	0.001
PC, PE+PS	0.593	0.004	0.603	0.001
TG, PC	0.509	0.016		
TG, SM	0.630	0.002		
PC, SM	0.836	0.000		
FFA, SM			0.371	0.052
FFA,PE+PS			0.468	0.012
PE+PS, SM			0.470	0.012
Cho, liver index			0.431	0.022

The correlations between cholesterol (Cho) and phospholipids for both perch and roach are coincident. Other lipids correlated differently. In perch gills, triglyceride (TG) correlated with phosphatidylcholine (PC), and the same lipids correlated with sphingomyelin (SM). In roach, free fatty acids (FFA) correlated with the compositions of phosphatidyl-ethanolamine and -serine (PE+PS), and with sphingomyelin (SM), as did PE+PS with SM. Roach liver index also correlated with the cholesterol concentration. The cholesterol to phospholipids ratio shows that in both lakes the gill membrane composition is very different in spring compared to summer (Table 9). The most important difference in fish gills between L. Poppalijärvi and the reference lake U. Kuito is the cholesterol concentration.

The total lipid weight was reduced during Experiment I because both groups of fish were starved (IV). However, the weights of all phospholipids (PC and PS+PS) were significantly lower in the Li&K-exposed group when compared to the control. The gills of Li&K-exposed fish showed increased phosphatidylethanolamine and -serine (PE+PS) due to a drop in the cholesterol level on day 7. Consequently, the correlation of PC to (PE+PS) was the same during the experiment for both groups, except on day 7 in the control gills (Table 3 in article IV). The concentrations of SM had diminished significantly after day 7 of the exposure. The cholesterol to total phospholipids molar ratio (Cho/Pho) decreased on day 7 of the exposure in the Li&K mixture, whereas it did not change in the control.

In spite of the different methods used for the lipid analyses in Experiment II with Li-exposure (see material and methods), the data shows results that are comparable with those of the previous laboratory and field studies. Cholesterol was low towards the end of the exposure, as were the free fatty acids in the gills of the Li- exposed fish. Three days after increasing the Li concentration in the experimental water, the gills showed a sudden increase in cholesterol concentration. In the experiment, a significant positive correlation was registered between free fatty acids and citrate synthase (CS) activity in the gills (V).

Table 9. *Fish gills: The ratios between cholesterol and the amounts of sphingomyelin and phosphatidylcholine (Cho/SM+PC) and total phospholipids (Cho/Pho), and between PC and phosphatidylethanolamine and -serine (PC/PE+PS). Mean±S.E., values with no common letter (superscript) are different when compared by ANOVA followed by the Post Hoc LSD test ($p<0.05$).*

	L. Poppalijärvi			L. U. Kuito		
Year	2000		2001	2000		2001
	Spring	Summer	Summer	Spring	Summer	Summer
Perch	n=5	n=6	n=11	n=5	n=5	n=11
Cho/SM+PC	1.6 ± 0.3^{a}	0.7 ± 0.1^{b}	0.60 ± 0.03^{b}	1.8 ± 0.1^{a}	0.8 ± 0.1^{b}	0.76 ± 0.1^{b}
PC/PE+PS	2.0 ± 0.7^{a}	4.2 ± 0.3^{b}	1.13 ± 0.24^{a}	0.8 ± 0.3^{a}	1.7 ± 0.3^{a}	0.9 ± 0.1^{a}
Cho/Pho	1.5 ± 0.3^{a}	0.6 ± 0.1^{b}	0.27 ± 0.03^{b}	1.6 ± 0.2^{a}	0.6 ± 0.1^{b}	0.34 ± 0.03^{b}
Roach		n=5	n=14		n=5	n=14
Cho/SM+PC	-	0.46 ± 0.01^{a}	1.3 ± 0.4^{b}	-	1.2 ± 0.1^{b}	0.4 ± 0.1^{a}
PC/PE+PS	-	3.2 ± 0.7^{a}	0.6 ± 0.1^{b}	-	2.7 ± 1.1^{a}	1.2 ± 0.2^{a}
Cho/Pho	-	0.38 ± 0.03^{a}	0.4 ± 0.1^{a}	-	0.8 ± 0.1^{b}	0.21 ± 0.03^{a}

Note: the proportion was calculated between cholesterol and the membrane phospholipids; triglyceride and free fatty acids were not included in the calculation.

4.5. Enzyme activity in gills

Perch and roach: The activities of total ATPase and Na^+,K^+-ATPase in perch and roach gills from the year 2000 did not differ significantly (III).

Rainbow trout: Total ATPase activity was higher in the gills exposed to the Li&K compared to the control gills. However, no change was found in Na^+,K^+-ATPase activity between these groups (IV). The Na^+,K^+-ATPase activity in the gills of the Li-exposed group was significantly lower than in the control group at the end of exposure. Nevertheless, when LiCl was added directly to the Na^+,K^+-ATPase assay media (in concentrations from 0.1 pmol to 1 mmol), there was no significant inhibitory effect on the Na^+,K^+-ATPase activity of the gills (V).

The citrate synthase activity in the gills was clearly higher in the second part of Experiment II. This was related to a lower gill protein content towards the end of the experiment in both groups of fish (V).

4.6. Plasma ion concentrations under Li exposure

A significant decrease was observed in Na^+ concentration in rainbow trout plasma after two days of Li exposure (V).

4.7. Apolipoprotein AI under Li exposure

The ELISA assay showed an increase in plasma apoAI between 13 and 15 days from the onset of the experiment (V).

5. Discussion

Clear gradients were seen in water mineral content, pH level and hardness, from high values in the most polluted L. Poppalijärvi to more natural values in L. Kento (Results, Table 4 and 5). Obviously the changes in values have affected the composition of the fish community, their food chain structure, and the health of the fish in the lakes (Holopainen *et al*., 2003b; I-III).

5.1. Metal concentration in the fish as affected by season, fish feeding behavior, water and sediment parameters

Mercury concentration: Mining activity is assumed to be the local source of mercury in the Kenti river system. The total Hg concentration was nearly twice as high in L. Poppalijärvi sediment in comparison to that in the reference lake (Table 4). However, it is not clear whether the mining plant wastewater is the main source of Hg in this territory. It may also be a result of the following:

a) atmospheric deposition,
b) snowmelt,
c) biological pathways of Hg transportation in the migrating fish.

The biological transportation of Hg (AMAP, 2002) is probably not a very significant influence in L. Poppalijärvi, since the main spawning grounds are in L. Kento and in L. Alajärvi downstream. Moreover, White Sea salmon do not necessarily die after spawning, in contrast to the Alaskan or Kamchatka salmon.

Atmospheric deposition and snowmelt may be sources of bioavailable mercury, which is in agreement with the findings on contamination of moss samples reported by Rühling and Steinnes (1998).

However, the fish showed lower concentrations of Hg in spring than in summer, mainly because of the cold water and the low metabolic rate of the fish (I-III). Total Hg concentration in the fish varied as follows:

a) higher in perch in L. Poppalijärvi in 1997, but lower in 2000 compared to the other studied lakes,
b) higher in roach muscles and gills in L. Poppalijärvi in 1997 and 2000, and equal in livers from all the lakes.

The results showed a lower bioavailability of Hg in L. Poppalijärvi, despite the high concentration of Hg in the sediment. This agrees with other studies, where results show that piscivorous fish, such as perch and pike, contain less Hg in neutral than in acidic waters (Cope *et al.,* 1990; Verta, 1990; Haines *et al.,* 1995), and that the raised mineral concentrations and high concentrations of chlorine can reduce Hg uptake in fish (Newman and Jagoe, 1994; Watras *et al.,* 1995).

The third result may be connected with roach diet (presence of molluscs *Pisidium sp*.) and their larger size (Holopainen *et al*., 2003b). It is suggested that *Pisidium* accumulate more methyl mercury (MeHg) due to the fact that they live inside the contaminated sediment in L. Poppalijärvi. Methylation takes place mostly due to sulfate-reducing bacteria (Mason *et al.*, 2000). These bacteria might be very active in the upper lakes of the Kenti river system due to their high sulfate content. This, however, needs to be verified.

The Hg concentration in perch muscle in 1997 (II) was close to the highest values of Hg found in fish muscle in Arctic freshwaters (0.32 μg g^{-1} wet wt in Finnish Lapland, 0.25 in Norway and 0.28 in Sweden), but lower than 0.62 in Alaska (AMAP, 1998, 2000; Duffy and Zhang, 2002). However, it is higher than permitted for human consumption, i.e. 0.2 μg g^{-1} wet weight for subsistence users consuming large quantities of fish (WASP, 1995; US EPA, 1997).

Cadmium concentrations in the studied fish were higher than those given in most of the other studies (e.g. Harrison and Klaverkamp, 1990; Allen-Gill *et al.,* 1997; Tulonen *et al.*, 2006; for a review in Arctic areas see AMAP 1998, 2000 and 2002). However, it was lower than the Cd accumulation in brown trout (*Salmo trutta*) liver and gills from the River Naustebekken (Norway), in spite of a comparable water concentration of Cd (Olsvik *et al*., 2001).

In the year 1997, the highest values were found in L. Kamennoe (>0.36 μg g^{-1} wet wt) and in L. Poppalijärvi perch liver (>0.26 μg g^{-1} wet wt). This is high when compared to cadmium concentrations in northern pike liver from Manitoban lakes in Canada (0.13 μg g^{-1} wet weight), but not as high as the 0.55 μg g^{-1} wet weight in the «Flin Flon lakes» contaminated by smelting operations (Harrison and Klaverkamp, 1990). Perch and brown trout accumulated more cadmium in acidic water and sediment (Hollis *et al*., 2000; Mason *et al.*, 2000; Olsvik *et al*., 2001; Tulonen *et al.*, 2006), as was also the case downstream in the Kenti river system (I-III).

The Cd concentrations in perch and roach gills were considerably lower in L. Poppalijärvi compared to the reference lake (Table 6; III). This is probably due to the interference of water Ca in fish gills in the hard water of L. Poppalijärvi, since calcium competition is a dominant protective factor preventing Cd from accumulating in the gills in hard water (Carrol *et al.*, 1979; Hollis *et al.*, 1997, 1999; Wood, 2001; Niyogi and Wood, 2004). Also binding with the Cl^- ion may reduce the bioavailability of Cd in L. Poppalijärvi water (Newman and Jagoe, 1994). Since Cd uptake also depends on calcium concentration in the food (Franklin *et al.*, 2005), this may have reduced Cd accumulation in fish liver in L. Poppalijärvi.

Thus Cd was deposited in a high concentration downstream from L. Poppalijärvi, which demonstrates the potential risk for biota in the study area. Since Cd can reduce the growth and reproduction of invertebrates (Mason *et al.*, 2000), it is possible that Cd concentration is also related to the lower biomass of zooplankton in L. Poppalijärvi compared to the lakes downstream (Holopainen *et al.*, 2003b, 2007; I).

Chromium concentration: The concentrations in the muscle and liver of the fish were much lower in all studied

lakes compared to the data from small boreal lakes in Finland (Tulonen *et al*., 2006). However, according to Platonov and Lozovik (2003), Cr reached the highest concentration in water from the Kostomuksha area compared to other regions in Russian Karelia, but was still apparently below the toxic level (Buljan, 1996).

The total Cr in fish gills varied between the study years (Table 6, and articles II and III). In 2000, the higher concentration of Cr in roach and perch gills in the reference lake could be explained by the higher concentration of acidic Cr-rich sediment in L. Upper Kuito. This also suggests a natural source of Cr in this geographic region. It is possible that the soft, acidic water in L. Upper Kuito contributes to dissolution of Cr. Trivalent chromium salts can be used as a tanning agent for the transformation of hides and skins into leather. However, no tannery is known in the small Karelian village, which is the closest settlement to L. Upper Kuito. However the contamination may have taken place long ago, as at Tannery Bay in White Lake (USA) (Tuchman, 2004).

Copper and Zn concentration: The reason for the highest concentrations of copper in the water and in the fish of L. Kamennoe is unclear. Local geological features could be involved, since anomalous high copper concentrations were registered in the Kamennoe surroundings (Koljonen, 1992). However, the concentration of Cu in the studied fish was lower compared to that of trout from Norwegian rivers (Olsvik *et al*., 2001).

Copper levels in fish liver reflect the concentration of this metal in the diet and in the water (Kamunde *et al*., 2002, 2003). Thus the highest concentration of Cu in roach liver in L. Poppalijärvi agrees with the higher Cu concentrations found in the water and sediment there. The presence of *Pisidium sp*. in the roach diet may also be considered a reason for this, since higher concentrations of Cu have been found in trout feeding on snails (Allen-Gill *et al*., 1997). It is known that molluscs in sediments may accumulate twice the concentration of Cu from food than from the water (Croteau and Luoma, 2005), and then transfer it to the fish (Bury *et al.*, 2003).

On the other hand, the concentration of Cu in the gills did not indicate any difference between the lakes. This agrees with the observation by Wood (2001) that gills show no marked changes in Cu uptake or binding, and the gill copper burden remains stable over time (established in a study by Lauren and McDonald, 1987; Grosell *et al.*, 1996, 1997). In the branchial uptake of Cu, Grosell and Wood (2002) identified two branchial copper uptake processes: sodium-sensitive and sodium-insensitive pathways, which implies that the presence of high sodium concentration in the upper part of the Kenti river system may inhibit Cu uptake via the gills.

Cu concentration is also affected by the water bicarbonate ion (HCO_3) (Linnik and Nabivanets, 1986). According to Lozovik *et al.* (2001), the values of HCO_3 in the water (mean 165 mg l^{-1}) were extremely high in the KMP Depository and in the south and northwest canals that flow into the Kenti River system (60 and 45 mg l^{-1} respectively). This might explain the binding of Cu ions and their being deposited in sediments. At the same time, the high sulphate ion concentration and the increased amount of Cu in the water may form copper sulphate, an algaecidic compound. Article II shows that Cu concentration did not exceed the natural level in the Kenti river system. However, after the year 2000, it was found that Cu accumulated more in the sediment in the upper lake than it did downstream.

Zinc was found in very high and toxic concentrations in waste and mining waters (Virtanen and Markkanen, 2000; Lozovik *et al.*, 2001). Nevertheless, in the upper lakes of the Kenti river system Zn concentrations were never as high in fish gills and liver as those in the contaminated River Naustebekken (Norway), and Zn

concentrations were about the same as in the River Rugla (Norway), which was not contaminated by Zn (Olsvik *et al*., 2001). The possible reasons for this relatively low concentration of Zn in the studied fish are the following:

a) the high concentration of Ca^{2+} keeps the water alkaline and competitively inhibits Zn uptake (Spry and Wood, 1989),

b) the complexation and precipitation of Zn in the upper part of the Kenti river system (seen as high Zn concentration in the sediment).

Moreover, the presence of water-borne Zn may competitively inhibit Cd uptake (Wicklund-Glynn, 2001). Consequently, it is possible that Cd availability, which was discussed above, is inhibited not only by high Ca^{2+}, but also by high Zn.

The concentration of Zn in roach gills, which was four times greater than in perch gills, is possibly related to their genetic differences and different feeding habits. Roach are bottom feeders, feeding on detritus and benthic organisms, which are exposed to metal in the sediment (Allen-Gil *et al*., 1997; Peuranen, 2000). Nevertheless, Zn concentrations did not exceed the natural levels in the Kenti river system (I-III), i.e. the KMP causes no increase in concentrations of this metal in fish.

Iron precipitation in the gill tissue: The gills may play a vital role in iron homeostasis after yolk-sac absorption and prior to feeding (Bury *et al*., 2003). A potential mechanism of iron toxicity includes its role in DNA and membrane damage, especially in the gills (Peuranen, 2000). According to Vuori (1995), iron has both direct and indirect toxic effects on aquatic species. The direct effects occur from the uptake of iron from the water (Fe^{2+}) and food (Fe^{3+}). The indirect effects of iron are apparent as ferric hydroxide precipitation (ochre), which may clog fish gills and egg pores, and also affect the diversity and abundance of benthic invertebrates. This in turn will also affect fish. Iron uptake in fish gills may presuppose that bacteria (Vibro sp.) on the gill surface secrete "siderphore-like" protein, and that the compounds that make up branchial mucus play a key role in sequestering water-borne iron (Muiňo *et al*., 2001). According to Steffens *et al.* (1992), the safe total iron concentration for rainbow trout is as high as 5-10 mg l^{-1} at a water pH close to neutral, which is much lower than the concentration in L. Poppalijärvi (Table 4).

In L. Poppalijärvi, the water iron content was ten times higher in early spring compared to August (Table 4). Lozovik *et al.* (2001) have shown a decrease in the oxygen level under the ice in winter in L. Poppalijärvi, which may increase the solubility of iron as Fe^{2+}. This suggests that the high concentration of iron is a result of late winter hypoxia.

In August 2002, both L. Poppalijärvi and L. Kento were stratified, with a low oxygen content close to the bottom when compared to L. Upper Kuito (Table 4). However, the water iron concentration in summer was lower in L. Poppalijärvi, and was assumed to be precipitated as a ferric hydroxide owing to the following factors:

a) high pH level,

b) high oxygen concentration,

c) low TOC (Lozovik *et al*., 2001).

Blood comprises about half of the gill's weight, and iron in the bloodstream is a topping element in gas exchange (Wood, 2001; Bury *et al*., 2003). Direct deposition in the gill tissue was studied by the histochemistry of the Turnbull Blue method (III). Iron showed the lowest deposition on fish gills from L. Poppalijärvi compared to the highest deposition in the reference lake. In spite of the higher concentration of iron in the sediment (Table 5), the amount of iron granules in fish gills in L. Poppalijärvi was only ca. ten percent of that in the reference lake for both the studied species (III). Note that the bottom-feeding roach accumulated

ca. ten times more iron granules than the perch, but the same difference prevailed between the lakes, which shows that the changed water chemistry in L. Poppalijärvi (high pH, oxygen and water hardness, and low TOC) protects the fish from iron deposition.

5.2. Liver damage, histology aspect

In L. Poppalijärvi, increased liver somatic indexes and histological changes were discovered in perch (II). The combined effects of all the environmental factors led to changes in the liver cell structure in the years 1997 and 1998 (II). The later study suggests that the large number of glycogen inclusions in hepatocytes probably reflects two more factors, among others, which led to changes in cell structure:

a) higher food ability in L. Poppalijärvi (Holopainen *et al.*, 2003; I)

b) chronic exposure to the Li-rich water, Li being known as a stimulant for glycogen synthesis (Corbella and Vieta, 2003).

In conclusion, the changes in fish liver in L. Poppalijärvi protected cell viability and indicated the presence of toxic influence in the upper part of the Kenti river system. However, the causes of these changes were difficult to identify.

5.3. Gill responses

Morphological changes in the epithelium as a strategy to cope with contaminated water

The changes in water chemistry not only reduced the bioavailability of heavy metals to the gills, but also caused changes in the gill structures, which are considered to be protective reactions. For example, the almost round pavement cells observed in perch from L. Poppalijärvi in 1997 (III) probably occurred when they were pulled back to uncover increased chloride cell-surface area in response to acid-base and ionic disturbances, as described in the literature (Goss *et al*., 1995; Wood, 2001; Evans *et al.*, 2005).

Mucus secretion - a strategy to prevent metal accumulation in the gills:

Mucus secretion can provide protection from some metal toxicants, such as copper and inorganic mercury (Wood, 2001), which were present in L. Poppalijärvi's water and sediment. Considerable chronic metal exposure was evident in the upper Kenti river system, since high activities and numbers of mucous and rodlet cells were recorded in perch gills close to the factory (III). This may be the initial response by the gills to metal exposure (McDonald and Wood, 1993). Moreover, mucus can prevent metal toxicity in the gills through its ability to depurate metals by sloughing (Handy *et al.*, 1989; Handy and Eddy, 1991; Playle and Wood, 1991; Peuranen, 2000; Wood, 2001), and also by slowing down the diffusion rate of metals (Pärt and Lock, 1983).

However, Wood (2001) mentioned that the thickened mucous layer may block water flow between the respiratory lamellae, and it may magnify the diffusion distance between water and blood, exactly as was seen in perch gill lamellae in L. Poppalijärvi (III). This magnification slows down the diffusion of oxygen by about 30% compared to the normal rate (Ultsch and Gros, 1979 cited by Wood, 2001).

The higher iron deposition on gills did not stimulate mucus production in fish gills in the reference lake (III). This is in agreement with Peuranen (2000). Although, initially, lithium and potassium were assumed to be the causes of the high gill mucification in L. Poppalijärvi (III), the latest experiment shows that Li&K-exposed gills did not show mucous cell proliferation, nor an increase in cell numbers (IV). Thus, the reasons for the more active mucus secretion by perch gills in L. Poppalijärvi are not known. The changed water quality and possibly some unmeasured toxicants might be "background factors".

Changes in the chloride cells (CC) as compensation for ion loss:

Proliferation of CC in L. Poppalijärvi was probably connected with the ability of mucus to increase ion concentrations, such as Ca^{2+}, close to the gill surface (Handy, 1989; Laurent and Perry, 1991, 1995). High K^+ and Ca^{2+} concentrations in the water may interfere with the uptake of Na^+, so that hypertrophy of CC becomes a compensatory response to the ion loss (Goss and Wood, 1990a b; Perry, 1997). In L. Poppalijärvi, therefore, proliferations of chloride and pavement cells cause inundation of the lamellae; in addition they are covered with the thickened mucous layer. As a result this response might concurrently impair gas transfer, owing to the longer blood-to-water diffusion barrier as described by Wood (2001).

Detrimental changes in the chloride cell mitochondria and tubular reticulum, together with increased sizes and numbers of the mitochondria and vesicles, suggested an active increase in osmoregulatory work in the gills in fish from L. Poppalijärvi.

5.4. Lipid responses in the gills

As a reaction to metal exposure, gills may increase membrane permeability to inorganic ions and inhibit Na^+,K^+-ATPase activity (Lock *et al*., 1981; Lauren and McDonald, 1985; Spry and Wiener, 1991; Flik and Verbost, 1993; Gabryelak *et al*., 2000). The changes in gill structures and in the thickness of the CC membrane in L. Poppalijärvi fish (III) aroused our interest in the lipids biochemistry. Since they play a role in cell membrane physiological adaptation (Hochachka and Somero, 2002), changes in the lipids composition could be considered to be subtle toxicity endpoints in fish.

Changes in cholesterol concentration

Towards the end of winter, cholesterol level in the gills decreased due to an increase in the proportion of triglycerides (III). Cholesterol was much lower in L. Poppalijärvi fish (both perch and roach). However, the ratios of cholesterol to neutrally charged SM and PC were the same between the lakes, but much higher in spring than in summer (Table 9). The differences in pH and ion concentrations between the studied lakes did not affect this ratio. Cholesterol is of high importance in the cold season because it provides adaptive possibilities by modulating the properties of the membranes in temperature-compensatory manners (Robertson and Hazel, 1995, 1997 Hochachka and Somero, 2002).

In summer 2001, there was a positive correlation between the increased gill cholesterol and the liver somatic index of the roach (Table 8). This may also reflect the higher availability of food in L. Poppalijärvi (Holopainen *et al*., 2003ab). The changes in 2001 were different compared with the low cholesterol found in the roach in summer 2000 (III). Since the amount of cholesterol in cellular membranes may depend on oxygen levels (Hochachka and Somero, 2002), the changes in oxygen concentrations close to the sediment in L. Poppalijärvi (discussed above for Cu accumulation) can be assumed to be a possible reason for the change in cholesterol between the years.

It was assumed that the presence of alkali metals in lakes was related to changes in the lipid membranes of the gill tissue, and this hypothesis was tested with rainbow trout in laboratory experiments: in Experiment I (Li&K mixture, IV) and Experiment II (Li-rich water, V). In both cases the fish were starved, which as such decreased the amount of lipids in the gills. However, in Experiment I the gill cholesterol dropped first in the Li&K-rich water in both weight and proportion, as it did in perch gills from L. Poppalijärvi in a field study (III and Tables 7 and 9). In

Experiment II, after four days at 0.528 mg Li l^{-1} (water with the same Na/Li ratio 0.20 as in L. Poppalijärvi), the gills responded by increasing cholesterol concentrations and decreased it only after a further two days of exposure. The changes in gill cholesterol, accompanied by an increase in blood plasma apoAI, probably suggest a relation between them, as well as gill apical membrane protection in Li-containing water (Smith, 2005; V).

Changes in the phospholipid composition:

The significant correlation between phospholipids for perch and roach gills in 2001 (Table 8) according to Hazel and Williams (1990) a sign of temperature acclimation. Zwingelstein *et al.* (1998) also suggested that changes in environmental conditions could stimulate cell surface receptors, which elicit a degradation cascade of membrane phospholipids in the tissues of fish.

A decrease in the numbers of mitochondria in the gills of exposed rainbow trout, following the decline in phosphatidylcholine, can be assumed to be a coupled reaction attributable to Li&K exposure (IV). Since the phospholipids are indicators of the synthesis of mitochondria (Chapelle and Zwingelstein, 1984) and the principal function of mitochondria is to produce the necessary energy, e.g. for Na^+ uptake, the Li in water may possibly affect Na^+ uptake in gills. Experiment II studied this possibility and showed that Li may have affected rainbow trout through increased diffusive Na^+ losses at the gills at the beginning of Li-exposure, and decreased branchial citrate synthase activity (V).

Lipid composition as a strategy to regulate the inner environment of the gills:

According to Perry (1997), the majority of Na^+,K^+-ATPase activity in the gills of freshwater fish occurs in the basolateral membranes of chloride cells (CC). Lipid restructuring at the basolateral membranes does not contribute to increases in this activity during the seawater-acclimation of yellow-phase eels (*Anguilla rostrata*) (Crockett, 1999). However, changes in lipids play some role in altering the catalytic properties of enzymes (Crocket, 1999; Hochachka and Somero, 2002; Else *et al*., 2003). Crockett (1999) also heightens the importance of apical membranes compared to the basolateral domains of the gill's epithelium. Therefore, we may assume that changes in the gill lipids and the apical membrane of CC in fish from L. Poppalijärvi, can be considered as an osmoregulatory adaptation. The low variability in Na^+,K^+-ATPase activity in the field (III) and in Experiment I demonstrated that CCs were able to regulate intracellular stability with the presence of high K in the water. In Experiment II (V), Li affected rainbow trout by decreasing the lipids of cellular membranes in the gills, which in turn reduced the gill's enzymatic activities and caused detrimental effects on the fish. Thus, K (as well as Na, Kszos *et al*., 2003) is able to play a protective role against Li.

6. Conclusions

The most important effects of wastewaters from KMP and the heavy metal pollution in the upper part of the Kenti river system were the following:

- Wastewaters have changed the natural water and sediment chemistry, and affect the biota extensively, right up to the fish community.
- Metals accumulated less in fish in water with a high pH, accompanied with high chloride, calcium, sulphur and iron concentrations, than in the sub-neutral water downstream and in the reference lake. The risk of metal effects was increased in the lakes downstream.
- The high Cu concentration in water, sediments and fish (roach) in L. Poppalijärvi demonstrates its high impact, but also the high capacity of Cu for complexation and precipitation in the

upper part of the Kenti river system. The role of Cu as an algaecide in lakes should also be taken into consideration.

- Iron accumulates directly in bottom-feeding fish.
- From the public health perspective, fishing in the downstream lakes, Koivas, Kento, and Middle Kuito, is considered safer in winter, since the Hg concentrations in fish tissue were lower in the ice-covered lakes than in summer.
- Changes in the perch liver and gill structures indicated responses by the fish tissues to changed water quality, exposure to metals, and possibly also to the presence of some unmeasured toxicants.
- Food availability, differences in the diet between lakes due to a changed aquatic community and the oxygen levels in water affected roach metal concentration and gill lipid composition.
- The low variability in gill Na^+,K^+-ATPase activity in the presence of high K^+ suggests that gill intracellular stability is achieved through adjustment in membrane lipids and in the chloride cell structure.
- Lithium seems to affect rainbow trout through increased sodium loss at the gills. Lithium also caused a decrease in Na^+,K^+-ATPase activity by causing a drop in lipids in the gill cell membranes.
- The effects of lithium are possibly diminished by the protective role of high concentrations of potassium and sodium in the upper part of the Kenti river system.

Future research needs

As was seen in the roach gills in L. Poppalijärvi, total Hg concentration increased in relation to changes in water quality. This may be connected with the presence of the mollusc *Pisidium sp*. in the roach's diet, which was also a reason for the higher total lipid concentration. It is suggested that *Pisidium* accumulated much more MeHg due to the fact that they live inside the contaminated sediment and in water with a high sulfate content (I). The Hg methylation process under the pressure of KMP wastewater can be studied in L. Poppalijärvi, as well as diet-derived adjustment of the lipid metabolism in metal-exposed fish.

Lithium pollution is very potent in a water environment, as reviewed by Kszos *et al*. (2003). The mechanism of Li effects on fish gills in general and in the Kenti River system, which is not known at present, seems to be important. The main question is how Li slows down enzyme activity. Is it by changes in phospholipid composition, which affect free fatty acids synthesis? Or could it be a mechanism that affects gill ionoregulation in the presence of Li?

The role of waterborne K as a key physiological ion should be studied, since K^+ is involved in many transport mechanisms and may play an important role in the metal toxicity expressed in the Kenti River system.

Furthermore, the relation between gill cholesterol and plasma apolipoprotein AI should be studied in more depth, since it may be part of a membrane protection mechanism.

The interaction of Li with other ions should be studied further, assuming that Li is an ionoregulatory toxicant.

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