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The effect of metal metabolism on uptake, disposition and toxicity in fish

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Heavy metals are regulated through water quality criteria which do not recognize important interactions between the fate and effect of heavy metals. For example: (1) mercury methylation by sediment bacteria increases its lipophilicity and accumulation by fish; (2) safe levels of waterborne selenium can be concentrated by benthic invertebrates and become lethal to benthic-feeding fish; (3) metal-binding proteins induced by metal exposure can increase the tolerance of fish to heavy metals and change the normal metabolism of nutrients such as zinc.

Management of metal contamination requires more understanding of metal uptake and metabolism in fish and the establishment of criteria for loadings as well as concentration.

Key words: Metal; Criteria; Metabolism; Accumulation; Management; Loading

INTRODUCTION

Metallic elements are important causes of environmental pollution. They are ubiquitous, readily dissolved in and transported by water, readily taken up by aquatic organisms, and strongly bound by sulfhydryl groups of proteins. Sulfhydryl binding changes the structure and enzymatic activities of proteins and causes toxic effects evident at the whole organism level.

The metals most often associated with pollution problems are those that are toxic and that are mined and used in high volumes. These include aluminum, arsenic, beryllium, cadmium, chromium, copper, iron, nickel, mercury, lead, silver,

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selenium, tin, and zinc. They are usually released in liquid wastes, or leach from solid waste disposed on land. Metals associated with fossil fuels, either as contaminants in coal and oil or as fuel additives (e.g. lead compounds), enter aquatic ecosystems via rain or dustfall. Therefore, metal contamination is usually associated with mines and metal smelters, urban and industrial development, atmospheric deposition near major highways, cities, and industries, and with regions of North America receiving acid deposition. In these areas, metals may be leached by acid from soil or bedrock.

Once in water, metals may remain in solution as free ions or as soluble complexes of organic and inorganic anions. Insoluble complexes with organic particulates or inorganic anions, such as carbonates, precipitate to the sediments. Therefore, exposure of aquatic biota is most often via direct uptake of free ions from water, usually across respiratory surfaces, or directly across cell membranes of plants and bacteria. Some metals move through the food chain as the organic form (e.g. mercury), and others are taken up by benthos with ingested sediments (e.g. selenium).

Many of the metals are not only toxic when in sufficient amounts, but, at low levels in the diet or water, are considered micronutrients. Signs of deficiencies in fish can be induced by inadequate intake of calcium, iron, magnesium, selenium and zinc (Hilton and Slinger, 1981) while chromium, nickel, vanadium, arsenic and silicon are nutrients in other organisms (Coombs, 1980). Other metals have no nutritional role (e.g. cadmium, lead, mercury) or their role is still a matter of controversy. Nutrient metals are usually key elements in metalloenzymes or cofactors in enzymatic reactions.

The toxic effects of metals on aquatic ecosystems range from a complete loss of biota to subtle effects on rates of population reproduction, growth and mortality. Gross effects are typical of pollution from older mines and milling plants that used waste disposal practices unacceptable today. Good examples include the lead-zinc mines of Wales (Jones, 1940) and Missouri's old lead belt (Schmitt et al., 1984). Lead and zinc were discharged to streams with mine water and with eroding tailings. The acidification of streams caused by the exposure and oxidation of sulfur-containing rock and the smothering of benthos by finely ground particulates enhanced metal effects and all life was eliminated from large stretches of streams. These impacts first occurred in the 1800s and persisted into the 1900s, often long after the mines had ceased to operate. Despite some recovery, their effects are still evident in changes in species composition of benthos downstream of old mines or tailings deposits (Gale et al., 1982), and in contamination of fish by lead (Gale et al., 1982; Schmitt et al., 1984).

More subtle effects of metals have been observed with better managed tailings dumps and pollution control measures; a chronic release of low levels of metals can still have significant impacts. A good example was the effect of copper and zinc in rivers of New Brunswick, Canada, downstream from active mines. While levels were below those associated with lethality, they were sufficiently high that migrating

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(Sprague et al., 1965).

MANAGEMENT - THE WAT

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TABLE I

Water quality criteria for filterab

Hardness (mg/L as $CaCO_3$)
10
50
100
300

adult Atlantic salmon (*Salmo salar*) avoided the contaminated water. During periods of high metal levels, spawning runs were reversed and large sections of stream that would normally be highly productive of juvenile salmon were unused (Sprague et al., 1965).

MANAGEMENT - THE WATER QUALITY APPROACH

The traditional approach to managing most pollution problems, including those of toxic metals, has been to establish water quality criteria. Criteria seek to protect beneficial uses of water by limiting the concentrations of metals in water according to observed correlations of cause and effect. A criterion for a metal level in water to protect aquatic biota would be an estimate of the 'safe' concentration, i.e. the concentration permitting long term survival, growth, and reproduction.

There are numerous criticisms of this approach, among them that criteria are based on single species responses in highly controlled experiments isolated from any modifying factors (Hodson, 1986). Nevertheless, some criteria recognize the modifying effects of water quality. For example, Alabaster and Lloyd (1980) recommended higher 'safe' levels of copper as hardness or alkalinity increased (Table I). This accounted for the role of inorganic complexation in reducing solubility, availability and toxicity of copper. Since criteria 'predict' effects at a given level of metal, the recognition of complexation is an attempt to explain variability between predicted and observed toxicity.

Despite modified criteria, we cannot consistently predict effects in the field from laboratory testing. One reason is that aquatic biota, both conceptually and experimentally, are generally viewed as animate 'black boxes'. Exposures in toxicity tests are well characterized and understood to establish 'cause', but 'effects' are estimated from the responses of an organism that is poorly understood, assumed to be 'standard' in its physiology, health and sensitivity to toxic chemicals, and rarely described beyond species, age, and size. Population growth, reproduction and mortality rates form the basis for establishing effects, but the mechanisms by which metals act and interact with metabolism, health, and life stage are rarely studied or understood. The ecological consequences of toxicity are equally misunderstood or

TABLE I

Water quality criteria for filterable copper to protect rainbow trout (Alabaster and Lloyd, 1980).

Hardness (mg/L as $CaCO_3$)	Copper criterion (μg/L)
10	1
50	6
100	10
300	28

Un

unstudied (Levin, 1982). Hence predicted responses rarely equal those observed (e.g. Reash and Berra, 1986) and management of metals and their ecological impacts is impaired.

The water quality approach has been accepted by environmental managers and waste treatment engineers because it supports a quick engineering 'fix' to the problem. Resource managers accept it because they manage the productivity of populations of organisms; unlike human beings, the death of an individual fish is of little consequence. Similarly, ecologists concern themselves with system properties and individual organisms are lost from sight. Nevertheless, metal toxicity must begin with an effect on an individual organism, usually as a molecular interaction between the metal and some biological substrate. The effect on the individual, and hence the subsequent responses of populations and ecosystems, will ultimately depend upon factors that modify this initial molecular interaction (Hodson, 1986). Therefore, to manage metals in aquatic ecosystems, a *toxicological* view is required. The appropriate model should consider not only environmental factors that affect the exposure of organisms (e.g. water quality), but also those factors affecting uptake of metals, their distribution within the organism, and their molecular interactions.

The literature that supports this thesis is rather broad. Hence, this paper will be restricted to a brief review of three examples of metabolic transformations of metals that affect uptake, distribution and effects in fish. More examples, pertaining to marine organisms, are given in an excellent review by Coombs (1980).

MERCURY METHYLATION

The mercury crisis was a vivid demonstration of the importance of metabolic transformations of a metal. In Canada, the discharge of mercury from chlor-alkali plants, the largest single source (NRCC, 1979), was regarded with little concern. Inorganic mercury has a very low water solubility, is readily complexed or adsorbed to particulates, and precipitates to sediments (NRCC, 1979). Therefore, it was assumed to move quickly from sources to sediment sinks. The difficulty of detecting mercury in water and the observed enrichment of mercury in sediments near sources confirmed that view. However, widespread contamination of aquatic biota, in particular fish, and the poisoning of native consumers of fish changed that view. Research revealed that mercury was not contaminating aquatic food chains as inorganic mercury via uptake of ionic or elemental mercury. Rather, mercury occurred primarily as the organic form, methyl mercury, and was biomagnified in food chains. Furthermore, methyl mercury was generated in particulates, surface sediments and even fish intestines by the action of aquatic bacteria (Rudd et al., 1983).

The methylation of mercury by bacteria is highly significant from a number of standpoints. First, methylation drastically alters the properties of mercury. It loses polarity and ceases to behave as a typical metallic ion: it becomes much less water

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soluble and much more fat soluble (Craig, 1986a). This affects environmental fate: sediments become a source instead of a sink. Instead of ionic bonding of inorganic mercury to particulates, methyl mercury becomes mobile, entering any substrate containing fat, particularly aquatic organisms. In fish, methyl mercury may comprise 80% or more of total mercury and it occurs primarily in muscle as a cysteine complex (Craig, 1986b; Coombs, 1980). Since methyl mercury is conserved while energy is lost with each trophic transfer, methyl mercury is biomagnified in aquatic food chains.

Toxicologically, increased fat solubility means not only rapid uptake and retention of mercury, but more rapid penetration of sensitive tissues, particularly the lipid membranes of neurons (Craig, 1986a). The high affinity of methyl mercury for sulfhydryl groups causes significant neurotoxic effects by complexing with cysteine-containing proteins. With biomagnification, toxic effects occur at the first trophic level acquiring a neurotoxic dose – in this case man.

This situation had enormous implications for water quality management. The assumption that sediments were a sink for all metals had to be abandoned, and also the notion of a tolerable or 'safe' concentration. Sediments became a source of mercury, and a highly mobile one at that. In the Great Lakes, mercury discharged to the St. Clair River was traced far downstream in the sediments of Lake St. Clair and Lake Erie (Allan, 1986). As the sediments were moved by currents, they contributed to mercury contamination of fish and an important walleye fishery was ultimately closed. A similar situation on the English-Wabigoon River system in Northwestern Ontario led to mercury poisoning of native consumers of fish (NRCC, 1979).

The effects of mercury can only be managed by limiting the loading of mercury to aquatic ecosystems and not its concentration in water. The important factor linking loading to contamination is the rate of mercury methylation by sediment bacteria balanced against the rate of its degradation (Wood, 1974; Craig, 1986b). The rate of methylation is a function of mercury concentration, microbial activity (i.e. nutrient levels) and dissolved oxygen levels (Rudd et al., 1983). In Canada, the mercury problem has been reduced by virtually eliminating the use of mercury in chloralkali plants and reducing loads of mercury from this source by more than 99% (NRCC, 1979).

This situation is not unique. Metals that can be methylated by bacteria include tin, palladium, platinum, gold and thallium as well as the metalloids selenium, arsenic, tellurium and sulfur (Wood, 1974). The biological methylation of lead has also been demonstrated and methyl-lead compounds measured in environmental samples. However, the major problems to date have been associated with the industrial production of alkyl-lead compounds for gasoline additives. Similarly tin may be methylated but occurs as an environmental contaminant primarily as organotin biocides used in agriculture, wood preservatives and boat antifouling compounds (Blunden and Chapman, 1986). Nevertheless, the result is generally the

same, a very marked increase in bioaccumulation and toxicity of alkyl metals relative to their inorganic ions (Craig, 1986a; Table II).

SELENIUM ACCUMULATION

The toxicity of selenium to fish is also amended by metabolism, but the evidence is much less direct than is the case with mercury. Selenium can be enriched in aquatic ecosystems during its recovery as a byproduct of copper mining or its use in the manufacture of glass and electronic components, and in photocopying, pigments and animal feed additives (Chau and Wong, 1986). It also occurs in fossil fuels and the fly-ash from some coal-fired power plants (Andren et al., 1975) and flooding of seleniferous soils by reservoirs may mobilize selenium.

Where selenium levels in water are elevated, there have been declines in fish populations due to reproductive failure and occasional acute fish kills. This has been a fairly common occurrence in lakes in the southern and western United States. In all cases, however, the levels of waterborne selenium appear far lower than those associated with selenium poisoning of fish in laboratory experiments. Nevertheless, the symptoms of dying fish are those of selenosis, with enrichment of tissue selenium levels, particularly in ovaries. Centrarchids are amongst the most sensitive species, accumulating up to 10 times the levels of fish from control lakes (Baumann and Gillespie, 1986).

Dietary selenium (as sodium selenite) is relatively more toxic than waterborne selenium in the same form (Table III). Relative to background levels of waterborne selenium, toxic concentrations are >600 times higher. In contrast, the lethal concentrations of dietary selenium are <37 times higher than the average level of selenium in natural or artificial fish diets.

Field studies by Cumbie and Van Horn (1978) and Cumbie (1980) support the hypothesis that environmental impacts of selenium are due to dietary intake. In Belews Lake, a reservoir containing sublethal concentrations of waterborne

TABLE II

The effect of methylation on mercury and lead accumulation and toxicity to fish.

Metal	Bioconcentration Factor		Toxicity (μg/L)		Author
	Inorganic	Organic	Inorganic	Organic	
mercury	10	32000	50 (sublethal)	1–10	IJC 1975. MacLeod and Pessah, 1973.
lead	<1000	10000	1000–6500 (LC50)	50–230	Hodson et al. 1984b.

TABLE III

Selenium toxicity to fish.[a]

Source	Toxicity Acute	C
Waterborne (μg/L)	35 000	>
Dietary (μg/g)	?*	≥

*see Goetll and Davies, 1

[a]Hodson et al., 1984a. Background phytoplankton, zooplankton and fis

selenium, there was sediment cumulated selenium concentra for fish. Bluegills (*Lepomis* selenosis after consuming bent longer, dying only after consun and passed through the cages. of toxicity of Belews Lake bent 1985). In bluegills accumulatir ovaries so that developing eggs are unaffected, the young are e be teratogenic since waterbor Baumann, 1986).

Why is dietary selenium mor to the metabolism of seleniur rapidly across the gills of rainb tion is about 29 days, regardle (Gissel-Nielsen and Gissel-Niel diffusion model, in which both by the concentration gradient

In contrast, the accumulatio dietary concentration. Half-live increased concentration in the concentrations suggests an acti at dietary concentrations appro that a greater proportion of int selenium changes dramatically, relative to the kidneys (Hilton e

TABLE III

Selenium toxicity to fish.[a]

Source	Toxicity		Background[a] concentrations	Ratio of toxicity to background concentrations	
	Acute	Chronic		Acute	Chronic
Waterborne (μg/L)	35 000	> 50.0	< 0.1	> 6000	> 500
Dietary (μg/g)	?*	≥ 3.7	0.1–0.9	?	< 37

*see Goetll and Davies, 1978

[a]Hodson et al., 1984a. Background concentrations refer to levels observed in Great Lakes Water, phytoplankton, zooplankton and fish (wet weight basis).

selenium, there was sediment enrichment of selenium; benthic invertebrates accumulated selenium concentrations up to 10 times the lethal dietary concentration for fish. Bluegills (*Lepomis macrochirus*) released into this reservoir died of selenosis after consuming benthic invertebrates, whereas caged fish survived much longer, dying only after consuming benthic insects that emerged from the sediments and passed through the cages. These observations have been supported by reports of toxicity of Belews Lake benthos when fed to bluegills in lab experiments (Finley, 1985). In bluegills accumulating dietary selenium, high proportions occur in the ovaries so that developing eggs are also contaminated. While fertilization and hatch are unaffected, the young are edematous and do not survive. This effect appears to be teratogenic since waterborne selenium is non-toxic to eggs (Gillespie and Baumann, 1986).

Why is dietary selenium more toxic than waterborne? The answer may be related o the metabolism of selenium by fish. Waterborne sodium selenite is taken up apidly across the gills of rainbow trout (*Salmo gairdneri*) and the half-life for excreon is about 29 days, regardless of the level of exposure or of selenium in tissues Gissel-Nielsen and Gissel-Nielsen, 1978). This behaviour corresponds to a passive ffusion model, in which both uptake and excretion rates of selenium are governed the concentration gradient between fish and water.

In contrast, the accumulation of dietary selenite by trout appears independent of tary concentration. Half-lives are a function of dietary loading, decreasing with reased concentration in the diet. An accelerated excretion rate at higher dietary centrations suggests an active or energy-requiring excretory process. However, ietary concentrations approaching the lethal level, half-lives increase again, so a greater proportion of intake is retained. Furthermore, tissue distribution of ium changes dramatically, with a much higher concentration in the liver ive to the kidneys (Hilton et al., 1982).

Hodson and Hilton (1983) proposed different models for metabolism of waterborne and dietary selenium; for the latter, excretion was governed by a rate-limiting mechanism. When this mechanism was overloaded, selenium accumulation rates increased and toxicity occurred. The rate-limiting mechanism appeared to reside in the liver, based on high levels of liver selenium during intoxication, and the pattern of blood flow associated with dietary intake. The liver of fish receives its blood supply from an intestinal portal system in contrast to other tissues which are supplied directly from the gills. According to Hodson and Hilton's model, waterborne selenium taken up across the gills would circulate through the majority of tissues of fish before reaching the liver. Hence only a small portion of waterborne selenium would pass through the liver and the majority would be available for uptake into other tissues in the form taken up from water. Dietary selenium, however, would all pass through the liver after intestinal absorption. Before reaching other tissues, there would be ample opportunity for liver accumulation. Hence, dietary selenium may be metabolized to a form unlike that accumulated from water.

There may also be a relationship between the compounds of selenium formed and toxicity. Seleno methionine appeared more toxic to zebrafish (*Brachydanio rerio*) than did selenite or selenate (Niimi and LaHam 1976), suggesting that metabolism of selenium to an organic form may enhance toxicity. Selenium transformations in sediments by bacteria or benthic invertebrates also cannot be ruled out in environmental exposures, since microbial activity in freshwater sediments caused the methylation of selenite, selenate, selenocystine, selenourea, and seleno-DL-methionine (Chau et al., 1976).

Unfortunately, there are few data on the forms of selenium in fish muscle other than a study by Cappon and Smith (1981). In freshwater and marine fish products, they found that 15–35% of total selenium was selenate (SeVI); the remainder was selenite (SeIV) and selenide (SeII) but the proportions could not be discriminated. When the tissue was divided into three major fractions, solid residue (cell fragments), TCA precipitate (large proteins) and aqueous extracts (small proteins, peptides, amino acids and ionic or neutral selenium), 55 to 80% of total selenium was in the aqueous extract and selenate was more extractable than selenite or selenide. While these data represent a starting point in studies of selenium metabolism, it does not say much about transformations within fish.

Selenium in aquatic environments may also reduce the toxicity of accumulated mercury. Because of a higher affinity for selenium than for sulfur, mercury compounds can be complexed preferentially by selenium to prevent complexation with sulfur-containing proteins (Craig, 1986b). Selenium in fish food organisms also reduces the uptake of mercury by predatory fish (Rudd et al., 1983). Therefore, the addition of selenium to low-selenium, mercury-contaminated areas has been proposed as one method to reduce both the contamination of fish and the risks to fish consumers of mercury poisoning (Rudd et al., 1983). Clearly, however, the experience in selenium-contaminated reservoirs indicates that a very good under-

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METALLOTHIONEIN

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standing of selenium metabolism by aquatic biota and selenium cycling in aquatic ecosystems is needed before such an addition: the elimination of mercury in fish is the desired end, not the elimination of fish from mercury-laden ecosystems!

This example again illustrates weaknesses in the traditional water quality approach to the control of toxic metals. To limit the effects of selenium on fish populations, knowledge is required of selenium cycling in the environment and of selenium metabolism in fish. Basing water quality criteria on standard aqueous toxicity tests is clearly inadequate. While the picture for selenium is becoming better known, other related metals could behave in a similar manner (Chau and Wong, 1986).

METALLOTHIONEIN

Metallothionein (MT) is a low molecular weight protein, rich in cysteine and poor in aromatic amino acids, that occurs in virtually all organisms (Ley et al., 1983). A high cysteine content gives MT a very high binding capacity for metals; all SH groups are available for metal binding and there is no disulfide bonding (Ley et al., 1983). MT is heat stable, soluble in acid and resistant to precipitation by alcohol and trichloroacetic acid (Eaton and Toal, 1982).

In mammals, MT is found in most tissues and its synthesis is induced by exposure to metals. The highest levels of MT and lowest inducibility are in the intestine. In contrast, low levels occur in liver and kidney, but considerable induction occurs after exposure to cadmium, copper, zinc, and mercury. Within 20 hours of exposure to cadmium, the concentration of MT increases and reaches a plateau (Olafson, 1981). The relative potency of induction in mice liver is Cd >Cu >Zn (Olafson, 1981). The appearance of MT is usually associated with increased RNA concentrations which confirms that new protein is, indeed, synthesized.

MT may play a role in the normal metabolism of metals. In rainbow trout, MT levels in livers of females increase with the onset of sexual maturation (Olsson et al., 1986). As vitellogenin synthesis increases in the late fall and early winter to support oogenesis, there are parallel increases in hepatic zinc and MT. Since zinc is implicated in mammals as an essential element in DNA/RNA synthesis, it is likely that the requirements for zinc increase with vitellogenesis and that MT provides a means of scavenging and storing zinc (Olsson et al., 1986).

Elevated levels of MT have also been observed in fish exposed to metals in the field. Roch et al. (1982) sampled rainbow trout from British Columbia lakes contaminated with copper, cadmium and zinc. Relative to fish from control lakes, hepatic metallothionein was enriched by about 5 times and copper and cadmium levels by 15 and 4 times respectively. Zinc was not elevated on a whole liver basis, but was enriched by about 2 times in high molecular weight cell fractions. The authors speculated that this zinc was displaced from MT by copper. Similar results have been observed for perch (*Perca fluviatilis*) sampled from a cadmium-polluted

river in Sweden. In this case, only cadmium was elevated in water, liver and low molecular weight proteins corresponding to MT (Olsson and Haux, 1986).

While these studies suggest a strong role for MT in the metabolism of metals, there is, as yet, no proven function. The most common theory is that MT acts to detoxify heavy metals through competitive binding and transport for excretion. The detoxification of metals in aquatic organisms was expressed by Brown and Parsons (1978) as a 'spillover' hypothesis. They and others have demonstrated that exposure of aquatic organisms to cadmium or mercury leads to increasing concentrations of MT and bound metal in liver. At high metal exposure levels, MT reaches a plateau, and further exposure to metals results in acute toxicity to the exposed organisms. The 'spillover' hypothesis states that MT acts as a 'mop' for free metals in the cytosol. As metal exposure increases, increasing amounts of MT maintain low levels of free metal in the cytosol. However, when the rate of synthesis of MT reaches a maximum, excess free metal is no longer mopped up and is available for binding to other proteins to cause enzyme inhibition and toxicity.

This hypothesis may be incorrect, however. Roch and McCarter (1984a) found enhanced levels of metals in the high molecular weight cell fractions of trout exposed to cadmium, copper and zinc but not showing symptoms of toxicity. Lauren and McDonald (1985) demonstrated that acute copper toxicity to trout is primarily due to effects on gills through disruption of sodium regulation. Induction of metal binding proteins was observed in the liver but not in the gills, suggesting that MT induction may only be coincidental to metal exposure (Lauren, 1986).

In aquatic organisms, the induction of MT after exposure to sublethal levels of copper, cadmium or zinc is associated with an increased tolerance to subsequent exposure (Table IV; also see review by Klaverkamp et al., 1984). In controlled laboratory experiments, pre-exposure of rainbow trout to copper and zinc enhances tolerance by 2–2.5 times relative to non pre-exposed control fish. Bradley et al. (1985) demonstrated that this tolerance was accompanied by increased levels of hepatic proteins corresponding to MT. When trout were transferred back to uncontaminated water, both tolerance and levels of MT-like proteins declined to control levels. Duncan and Klaverkamp (1983) and Thomas et al. (1985) have also shown that tolerance to one metal (e.g. Cd) can be induced by exposure to another (e.g. Zn), usually with a corresponding increase in hepatic metallothionein.

In field studies, white suckers from a lake contaminated with cadmium showed elevated protein binding of metals in liver and a greater tolerance of lethal cadmium exposures than fish from control lakes (Klaverkamp et al., 1984). In a recent review of metal acclimation, Chapman (1985) found that the degree of acclimation decreased in the following order: Zn > Cu > Cd > Cr. This is in reverse order of binding affinities: in vitro binding studies indicated that the affinity of MT for metals was in the order of Ag > Hg > Cd > Cu > Zn (Eaton, 1985). However, Chapman (1985) concluded that changes in acute toxicity due to acclimation were within the range of variability caused by other factors and may be unimportant ecologically.

TABLE IV

Metal tolerance of fish after pre-

Species	Toxic Metal	Inc To Ac Co
Rainbow trout	zinc	2.3
Rainbow trout	copper	1.7
White sucker (lab)	cadmium	2.5
White sucker (field)	cadmium	2.3
Coho salmon	copper	2
Rainbow trout (lab study)	cadmium, copper, zinc	1.5
Rainbow trout (field study)	cadmium, copper, zinc	1.0
Rainbow trout	aluminium	1.8

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TABLE IV

Metal tolerance of fish after pre-exposure to sublethal concentrations.

Species	Toxic Metal	Induced Tolerance Acclimated LC_{50} / Control LC_{50}	Metallothionein induction	Author
Rainbow trout	zinc	2.3–2.7	yes	Bradley et al., 1985.
Rainbow trout	copper	1.7–2	yes	Dixon and Sprague, 1981a,b
White sucker (lab)	cadmium	2.5	?	Duncan and Klaverkamp, 1983.
White sucker (field)	cadmium	2.3	yes	Klaverkamp et al., 1984.
Coho salmon	copper	2	yes	McCarter and Roch, 1983.
Rainbow trout (lab study)	cadmium, copper, zinc	1.5	yes	Roch and McCarter, 1984a.
Rainbow trout (field study)	cadmium, copper, zinc	1.0	yes	Roch and McCarter, 1984b.
Rainbow trout	aluminium	1.8	?	Orr et al., 1986.

Since MT always exists in a metal-saturated form, it is assumed that MT can detoxify metals only through de novo synthesis or if one metal can displace another (Brown and Parsons, 1978). A third alternative is the synthesis of different metal binding proteins. For example, hepatic cadmium associated with low molecular weight cell fractions was assumed to be complexed by MT but recent studies by Kay et al. (1986) have demonstrated synthesis of other low-molecular weight metal binding proteins. Using exhaustive protein separation techniques, they showed that endogenous MT in livers of rainbow trout failed to bind cadmium. Exposure of fish to cadmium induced the synthesis of a smaller protein that bound calcium exclusively and that differed from MT (Table V). Cadmium could only be bound to MT when there was a pre- or co-exposure to zinc; in this case, newly synthesized MT, induced by the zinc exposure, would bind cadmium preferentially.

Further evidence for unique cadmium binding proteins was the observation by Olafson (1981) that metal binding proteins of mice induced by cadmium did not break down quickly. They persisted for at least 10–20 days following a single injection, in contrast to copper or zinc binding proteins that disappeared within 40–60

TABLE V

Characteristics of protein synthesized in rainbow trout liver following exposure to copper, zinc and cadmium (Kay, et al., 1986; Olsson and Haux, 1985).

Peaks	Cadmium		Copper, Zinc	
	1	2	1	2
Molecular weight	< 10000	< 10000	5989	5955
Metal content (g atom/mole)	2	1		
Isoelectric points	4.0†	3.4	2.5 4.9	4.5 4.7
Amino acids (residues/mole)				
Cysteine	5	3	20	17
Phenylalanine	3	5	0	0
Cysteine/metal (mole/mole)			2.55	2.38
Metals bound	Cd (not Cu, Zn, Ca, Ni, Fe)	Cd	Cu,Zn	Cu,Zn

hours of induction. Stone and Overnell (1985) reviewed the literature and reported a variety of cadmium binding proteins in different organisms, but none corresponded to metallothionein. There is also a considerable species effect on tissue distribution, structure, induction and turnover rates of MT. The physiological and toxicological significance of these differences is essentially unknown although patterns of cadmium accumulation and MT synthesis in rainbow trout, loach and stone loach correspond to species differences in metal sensitivity (Kay et al., 1985).

Other indications of the complexity of metal and MT metabolism were the studies of Roch and McCarter (1984a,b). A four week exposure of trout to copper, cadmium and zinc induced both elevated hepatic MT levels and tolerance to lethal metal exposure. However, a similar exposure of fish caged in a naturally-contaminated lake caused MT induction but no changes in tolerance. Aluminum tolerance can be induced in fish (Orr et al., 1986) but aluminum exposure does not induce synthesis of metal binding proteins.

While Chapman (1985) concluded that the degree of acute metal tolerance by fish associated with MT induction was insignificant relative to other modifying factors, there are enough unanswered questions that the role of MT in chronic metal toxicity and regulation cannot yet be dismissed. Small changes in metal metabolism may have significant effects on fish population survival in situations of marginal metal contamination.

One of the most important conclusions to be drawn from these studies is that the role of MT will not be completely understood until the identity and role of other metal binding proteins is also understood. MT should be studied in conjunction with other metal binding proteins during both normal and pathological exposures to metals.

TABLE VI

Environmentally important metals r

Metals having widespread environmental effects
Aluminium
Arsenic
Beryllium
Cadmium
Chromium
Copper
Iron
Nickel
Mercury
Lead
Silver
Selenium
Thallium
Tin
Vanadium
Zinc

[a]Metabolism refers to incorporation o
of proteins that sequester metals by p

CONCLUSIONS

Metal metabolism by aquatic
distribution in tissues, and toxi
effect of mercury and selenium
pact of the metabolized forms
evident for a large proportion
VI). It is obvious, therefore,
models of waterborne, ionic
distribution in aquatic ecosy
thoroughly understood to desig
quire new and innovative r
biochemistry, pharmacokineti
rates to aquatic ecosystems m

REFERENCES

Alabaster, J.S. and R. Lloyd, 1980. \
Allan, R.J., 1986. The limnological un
their role in the source and aquatic fa

iver following exposure to copper, zinc and cad-

m		Copper, Zinc	
	2	1	2
)	<10000	5989	5955
	1	2.5	4.5
	3.4	4.9	4.7
	3	20	17
	5	0	0
		2.55	2.38
	Cd	Cu,Zn	Cu,Zn
t Cu, Zn,			
., Ni, Fe)			

85) reviewed the literature and reported fferent organisms, but none correspond- derable species effect on tissue distribu- rates of MT. The physiological and ces is essentially unknown although pat- nthesis in rainbow trout, loach and stone metal sensitivity (Kay et al., 1985). etal and MT metabolism were the studies week exposure of trout to copper, cad- ic MT levels and tolerance to lethal metal fish caged in a naturally-contaminated in tolerance. Aluminum tolerance can be inum exposure does not induce synthesis

he degree of acute metal tolerance by fish icant relative to other modifying factors, at the role of MT in chronic metal toxicity Small changes in metal metabolism may survival in situations of marginal metal

to be drawn from these studies is that the stood until the identity and role of other MT should be studied in conjunction with h normal and pathological exposures to

TABLE VI

Environmentally important metals metabolized[a] by aquatic biota.

Metals having widespread environmental effects	Metabolism by aquatic or marine organisms	
	co-valent bonding	complexation (e.g. MT)
Aluminium	–	–
Arsenic	X	–
Beryllium	–	–
Cadmium	–	X
Chromium	X	–
Copper	X	X
Iron	X	X
Nickel	–	?
Mercury	X	X
Lead	–	?
Silver	–	?
Selenium	X	–
Thallium	?	?
Tin	X	–
Vanadium	?	–
Zinc	X	X

[a]Metabolism refers to incorporation of metals into organic compounds by covalent bonding or synthesis of proteins that sequester metals by polar or ionic bonding (see Craig, 1986a).

CONCLUSIONS

Metal metabolism by aquatic biota has significant effects on metal accumulation, distribution in tissues, and toxic effects on fish. The environmental behaviour and effect of mercury and selenium are strongly influenced by metabolism, and the impact of the metabolized forms far outweigh that of the simple ions. Metabolism is evident for a large proportion of metals having environmental significance (Table VI). It is obvious, therefore, that metal regulation must expand beyond simple models of waterborne, ionic metals. The effects of biotic metabolism on metal distribution in aquatic ecosystems, uptake by biota and toxic effects must be thoroughly understood to design the most appropriate control schemes. This will require new and innovative research in toxicity testing, metal speciation and biochemistry, pharmacokinetics and mathematical modelling. Limits on loading rates to aquatic ecosystems must replace or supplement limits on concentration.

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