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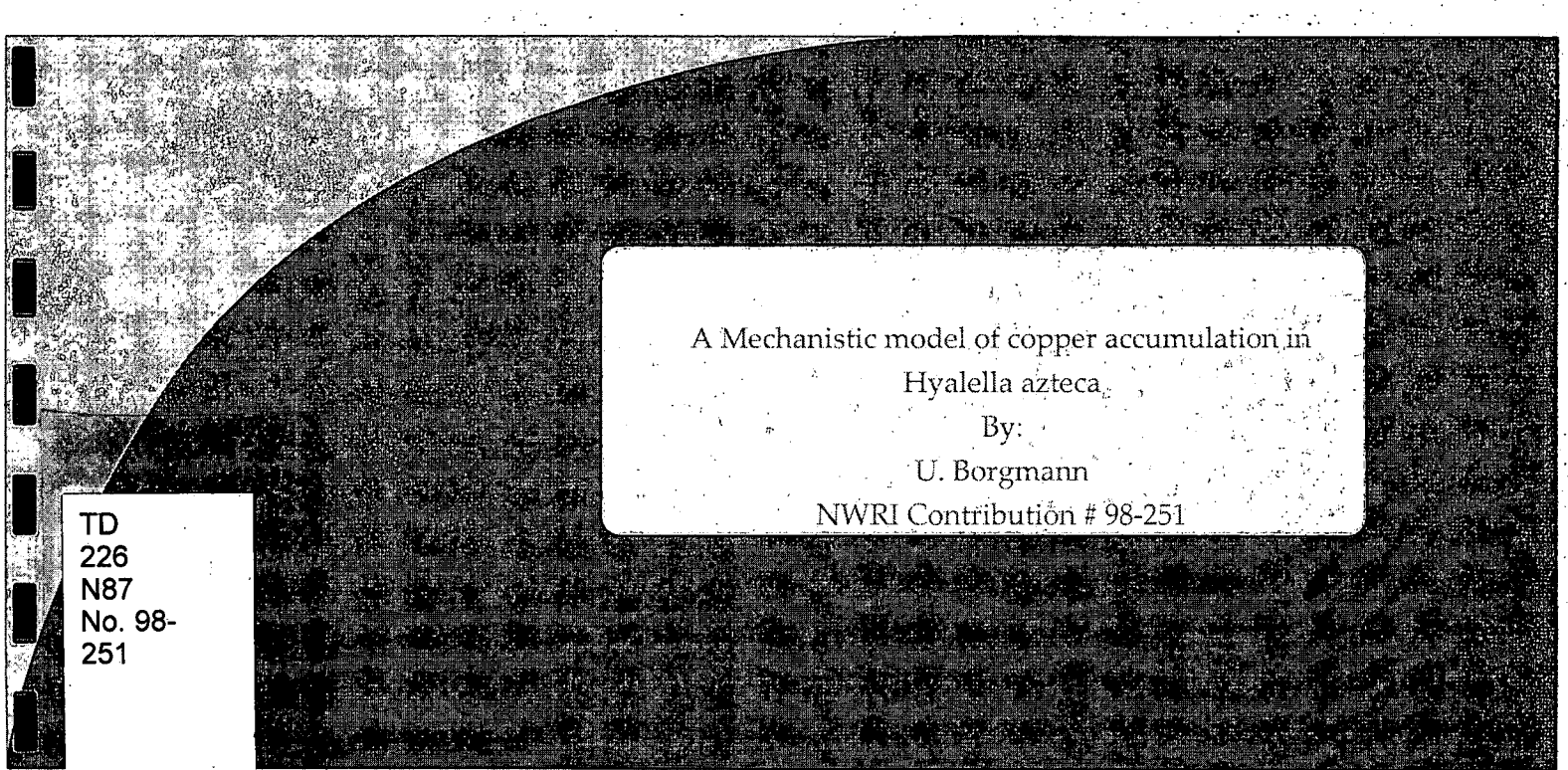

A Mechanistic model of copper accumulation in Hyalella azteca

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MANAGEMENT PERSPECTIVE

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EC Priority/Issue: This is part of Environment Canada's Action Plan (Conserving Canada's Ecosystems). It proposes a model to explain how and why copper is accumulated in a freshwater crustacean at the levels observed in previous experimental studies.

Current Status: This paper proposes that copper accumulation, as observed in *Hyalella*, is due to diffusion of the metal into the body followed by binding to an internal molecule (ligand "X") which probably has a physiological role in transferring copper to essential copper-requiring macromolecules. Such a model explains why body concentrations increase rapidly and level off quickly in the presence of high copper concentrations in the water, but at the same time decrease very slowing when previously exposed animals are placed in clean water. Changes in X also explain how the animal regulates body copper in long term exposures. This model allows comparison of the mechanisms of copper accumulation in an invertebrate over long term exposures with the mechanism of copper uptake by fish gills in short term exposures. The latter models are quickly gaining wide scientific interest for their capacity to explain and predict acute metal toxicity to fish, but they cannot predict chronic toxicity. The proposed model for *Hyalella* helps fill this need.

Next Steps: The above paper is the basis for further research on modelling of metal uptake and prediction of chronic toxicity in aquatic ecosystems. This approach forms the basis for a recent Metals in the Environment submission to NSERC for joint work between NWRI and U. of Waterloo. The ultimate objective is to be able to accurately predict biological effects of metals, even though their bioavailability varies considerably from lake to lake.

A MECHANISTIC MODEL OF COPPER ACCUMULATION IN *HYALELLA AZTECA*

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Abstract

A mechanistic model is proposed for describing the accumulation of copper in *Hyalella azteca*. The metal is assumed to diffuse passively into and out of the animal. Once inside, it binds primarily to a ligand (X), the function of which is probably to supply copper to copper-requiring essential macromolecules. Once X is saturated, no further copper is accumulated. The rate of approach to the steady-state is much faster during uptake than during depuration because the number of binding sites (X_t) is limited. Diffusion across the animal's body surface does not seem to be rate limiting. The binding strength of copper to X (K_{Cu-X}) is stronger than the binding of copper to fish gills, but this is not necessarily a valid comparison because K_{Cu-X} is the product of several constants, including the equilibrium for diffusion across the animal's surface and the strength of the internal binding site. Prolonged exposure to elevated copper in the water gradually reduces the concentration of X_t, primarily through growth dilution. Regulation of body copper appears to be through control of the concentration of X_t, rather than through control of copper influx or efflux rates, and chronic mortality is not affected by changes in X_t.

Introduction

The modeling of metal bioaccumulation by aquatic biota has recently received increased attention as a means of better defining the effects of metals on aquatic ecosystems. It is well known that total metal concentrations in the environment, whether in water or sediment, provide little indication of their potential biological effects because not all of the metal is bioavailable. One of the most promising new approaches in the development of a better means of predicting toxicity discussed at the Pellston Workshop on the Reassessment of Metals Criteria for Aquatic Life Protection was the use of models of metal binding to fish gills in predicting acute toxicity (Wood et al. 1997). Acute toxicity of metals to fish is largely the result of damage to the gill (e.g. inhibition of Na^+ or Ca^{++} uptake). It is logical, therefore, that understanding the uptake mechanisms of metals by the gill, and how this is affected by competing ions (Ca^{++}, Na^+, H^+), can help in the prediction of acute metal toxicity. One of the major recommendations of the workshop was that such studies be extended to invertebrates and to the prediction of chronic toxicity, with the hope that this will eventually lead to a tissue-residue, mechanism-based approach to the regulation of metals in the environment (Wood et al. 1997).

Detailed information on the uptake kinetics of copper and zinc from water to the freshwater amphipod *Hyalella azteca*, along with chronic toxicity data, have recently been obtained (Borgmann et al. 1993, Borgmann and Norwood 1995a, 1995b). Borgmann and Norwood (1995a) presented a simple mathematical model to describe metal uptake kinetics, but they did not present a mechanistic model to explain their results. Such a model is presented below for copper, along with an estimation of the binding affinity of copper to *Hyalella*, for comparison to the fish gill model. The model differs from the fish gill model in that it is a whole animal uptake model, and metal accumulation is internal and not on the animal's surface. This has some impact on the nature of the kinetics and on the interpretation of the meaning of the metal-animal binding constants obtained. The model considers only copper uptake from water. Copper accumulation and toxicity from contaminated sediments differs from water exposure, presumably because of increased uptake through the gut (Borgmann and Norwood 1997). Data on copper uptake kinetics from sediment are, unfortunately, insufficient at this time to be included in a detailed mechanistic model of copper accumulation in *Hyalella*.

Model Description

The proposed model of copper uptake in *Hyalella* (Fig. 1) consists of the external water concentration of

the metal and three internal compartments; the free (or loosely bound) metal (M), metal bound to biologically essential macromolecules (ME) and metal bound to a ligand which allows accumulation of the metal above the essential background concentration (MX). The metal diffuses into the animal, probably through specific ion channels, and leaves via a similar process. Both processes are proportional to the free, or loosely bound, metal concentration (M) with rate constants given by k_1 and k_{-1} respectively. Once inside, the metal can complex with ligand X to form MX. This complex can then supply the metal to metal-requiring essential macromolecules (E) to produce the active form of these molecules (ME). In metal deficient environments the concentration of MX may be low and much of the ligand X may be uncomplexed by copper. Subsequent exposure to higher metal levels will result in increases in MX and increases in total body metal. The essential complexes (ME), on the other hand, are normally probably fully complexed because they would be non-functional otherwise. The concentrations of ME, unlike those of MX, probably vary relatively little with changes in external metal concentrations.

Mathematically, the rate of change in the three internal components can be written as

$$\begin{aligned} d(ME)/dt &= k_3 \cdot MX \cdot E - k_{-3} \cdot ME \cdot X - g \cdot ME \\ &= k_3 \cdot MX \cdot (E_t - ME) - k_{-3} \cdot ME \cdot (X_t - MX) - g \cdot ME \\ &= k_3 \cdot E_t \cdot MX - (k_{-3} \cdot X_t + g) \cdot ME + (k_{-3} - k_3) \cdot MX \cdot ME \end{aligned} \quad (1)$$

$$\begin{aligned} d(MX)/dt &= k_2 \cdot M_b \cdot X - k_{-2} \cdot MX - d(ME)/dt - g \cdot (MX + ME) \\ &= k_2 \cdot M_b \cdot (X_t - MX) - k_{-2} \cdot MX - d(ME)/dt - g \cdot (MX + ME) \\ &= k_2 \cdot M_b \cdot X_t - (k_2 \cdot M_b + k_{-2} + g) \cdot MX - d(ME)/dt - g \cdot ME \end{aligned} \quad (2)$$

$$d(M_b)/dt = k_1 \cdot C_w - k_{-1} \cdot M_b - k_2 \cdot M_b \cdot X + k_{-2} \cdot MX - g \cdot M_b \quad (3)$$

where ME, MX and M_b, represent the concentrations of ME, MX and the internal free M respectively, C_w is the external water concentration, E_t and X_t are the total concentrations (bound to metal plus unbound) of the two types of binding sites($X_t = X + MX$, $E_t = E + ME$) and g is the instantaneous growth rate. Under steady-state conditions, or if the rate of diffusion into and out of the animal are fast relative to the other rates, then equation 3 simplifies to

$$M_b = k_1 \cdot C_w / k_{-1} \quad (4)$$

If ME is relatively constant ($d(ME)/dt = 0$), as generally seems to be the case for copper and zinc in *Hyalella* (Borgmann and Norwood 1995b), and if equation 4 is valid, then equation 2 simplifies to

$$d(MX)/dt = k_1 \cdot k_2 \cdot C_w \cdot X_t / k_{-1} - g \cdot ME - (k_1 \cdot k_2 \cdot C_w / k_{-1} + k_{-2} + g) \cdot MX \quad (5)$$

If g and ME are constant, this integrates to give

$$\begin{aligned} MX = {} & (k_1 \cdot k_2 \cdot C_w \cdot X_t / k_{-1} - g \cdot ME) / (k_1 \cdot k_2 \cdot C_w / k_{-1} + k_{-2} + g) \cdot (1 - \exp(-(k_1 \cdot k_2 \cdot C_w / k_{-1} + k_{-2} + g) \cdot t)) \\ & + MX_o \cdot \exp(-(k_1 \cdot k_2 \cdot C_w / k_{-1} + k_{-2} + g) \cdot t)) \end{aligned} \quad (6)$$

where MX_o is the initial concentration of MX at $t = 0$. Borgmann and Norwood (1995a) used a similar equation to describe copper and zinc uptake kinetics but did not present a specific model (e.g. Fig. 1) to explain their results. In their terminology $k_e = k_{-2}$, $k_u = k_1 \cdot k_2 / k_{-1}$, $C_{Xmax} = X_t$ and $C_{Xs} = MX$. Their model did not, however, contain the growth dilution term $-g \cdot ME$ in equation 5. This term results from the assumption that the concentration of ME remains constant. If this is true, then new ME must be synthesized as the animal grows, and the metal for this requirement comes from MX. If the model (Fig. 1) is correct, if equation 4 holds, and if growth is negligible during the exposure period ($g \approx 0$), then the apparent uptake rate constant for copper (k_u) is the product of the rate of binding of M to X (k_2) and the equilibrium between internal and external unbound, or loosely bound, copper (k_1/k_{-1}). The apparent elimination rate (k_e) under these conditions is really the rate of dissociation of the MX complex (k_{-2}) with no contribution from the actual diffusion of metal out of the animal (k_{-1}). If growth is significant ($g >> 0$), then

appropriate corrections for growth dilution must made to obtain accurate estimates of uptake and elimination rates.

Under steady-state conditions (d(ME)/dt and d(MX)/dt = 0), and if g is substantially smaller than the other rate constants and can be ignored, then equations 2 and 1 simplify to

$$MX = X_t \cdot C_w / (k_{-1} \cdot k_{-2}/(k_1 \cdot k_2) + C_w) \quad (7)$$

$$ME = k_3/(k_3 - k_{-3}) \cdot E_t \cdot MX / (k_{-3}/(k_3 - k_{-3}) \cdot X_t + MX) \quad (8)$$

Combining equations 7 and 8 gives

$$ME = E_t \cdot C_w / (k_{-1} \cdot k_{-2} \cdot k_{-3}/(k_1 \cdot k_2 \cdot k_3) + C_w) \quad (9)$$

Equations 7, 8 and 9 are all of the form

$$\text{Bound metal} = \text{Maximum} \cdot C / (K_{0.5} + C) \quad (10)$$

where C is C_w or MX and $K_{0.5}$ is the concentration of metal in the water or the concentration of MX which results in metal binding to one half of maximum (i.e. if $C = K_{0.5}$ then $MX = 0.5 \cdot X_t$ or $ME = 0.5 \cdot E_t$).

Short Term Model Predictions and Comparison to Observation

The proposed model (Fig. 1 with rapid equilibration between free or loosely bound M inside and outside the animal) is consistent with a number of observations. First, copper concentrations are independent of body size, as expected if binding is internal and not due to adsorption to the exterior of the animal, and not controlled by a surface diffusion rate. Secondly, the steady-state metal concentration reaches a maximum with increasing metal concentration in the water (equation 7). Third, the rate at which the steady-state is approached is much faster during metal uptake from water which is spiked with metal than during depuration in clean water. This results because the exponential rate of approach to the steady-state ($k_1 \cdot k_2 \cdot C_w/k_{-1} + k_{-2} + g$ in equation 6) is a function of the water concentration C_w which is 0 during depuration. These model predictions are all consistent with observation (Borgmann and Norwood 1995a).

The model in Fig. 1 also provides a possible explanation for the existence of ligand X. *Hyalella* appears to require maintenance of stable concentrations of essential copper and zinc (ME). It can maintain constant body concentrations even in the presence of substantial concentrations of complexing agents in the external water resulting in low external free metal concentrations (Borgmann and Norwood 1995b). This is due to a small value for $K_{0.5}$ (equations 9 and 10), representing a very strong effective binding of metal in ME. The value of $K_{0.5}$ for the ME complex is the product of 3 equilibria (i.e. $K_{0.5} = (k_{-1}/k_1) \cdot (k_{-2}/k_2) \cdot (k_{-3}/k_3)$, equation 9). If ME were produced from direct equilibria between internal M and E, then the value of $K_{0.5}$ would be the product of only 2 equilibria ($(k_{-1}/k_1) \cdot (k_{-3}/k_3)$). Inclusion of the additional step in the formation of ME results in an apparent binding strength of metal to E which is stronger than possible by direct formation of ME from M and E. This allows the animal to maintain essential metal concentrations to lower environmental free metal concentrations than otherwise possible, and may extend the range of habitats in which *Hyalella* can survive.

Long Term Model Predictions and Regulation of Body Copper

Long term exposures of *Hyalella* to copper provide additional insights into the function of the ligand X. During chronic exposure to constant elevated copper in water, the body concentration increases at first, but then gradually declines so that after 10 weeks of exposure the body copper concentration is indistinguishable between exposed and control animals (Borgmann et al. 1993, Borgmann and Norwood 1995a). This means that the concentration of MX has declined to negligible levels. This could be achieved through a decrease in uptake rates

(k_1), or an increase in metal elimination (k_{-1}), or a reduction in the total amount of X present (X_t). It is not possible to distinguish among these options based on the kinetic data alone. However, survival rates of *Hyalella* during long term exposure to copper or zinc did not decrease over time (Borgmann et al. 1993). The apparent "regulation" of copper during long term exposure did not result in reduced toxicity. This means that copper toxicity is not related to the concentration of MX, which declines through time. Toxicity is probably associated with the concentration of free or loosely bound internal copper (M_b). Since toxicity did not decline over time, M_b must have remained elevated during chronic exposure. A decrease in uptake rates (k_1) or increase in metal elimination (k_{-1}) would have reduced M_b. The most likely explanation for the apparent "regulation" of body copper during long term exposure is, therefore, a reduction in X_t.

Why would long term exposure result in a reduction in X_t? The concentration of ME is a hyperbolic function of MX (equations 8 & 10). Since ME is relatively constant, MX must be sufficiently high to cause E to be saturated ($ME \approx E_t$). The concentration of MX, therefore, normally exceeds the $K_{0.5}$ for equation 8. When the external copper concentration is increased, MX increases (equation 7). However, since E is already saturated with copper, the additional MX serves no beneficial function. There is, therefore, no need for the organism to continue synthesis of X, and the concentration of X_t can be allowed to decrease. Since the rate of decrease in body copper during continued exposure is slower than the rate of growth, the total body burden of copper (concentration of copper • body mass) does not decrease (Borgmann and Norwood 1995a). The decrease in the concentration of X_t with time can be attributed to growth dilution alone and does not imply active destruction of X_t. The apparent "regulation" of body copper concentrations over time is, therefore, consistent with a physiological function of the ligand X, such as proposed in the model (Fig. 1). Since X is involved in supplying copper to produce ME, and not in the control of copper toxicity, there is no correlation between long term regulation of body copper and copper toxicity.

Comparison to Alternate Models

Several alternative models have been considered and rejected as the best explanation for the process of copper accumulation in *Hyalella*. The most plausible alternate model is one in which ME is not formed from the reaction of E with MX, but is formed directly from E and M. Such a model would give the same short term kinetics, but it fails to explain why the ligand X is present. The model in Fig. 1, on the other hand, suggests that X allows maintenance of ME concentrations to lower external copper concentrations by decreasing the value of $K_{0.5}$. Furthermore, the apparent regulation of copper during long term exposures serves no benefit unless MX is used in the formation of ME. It seems logical, therefore, that X should serve a physiological function, such as shown in Fig. 1.

Another model considered was one in which copper accumulation was controlled by the rate of uptake, rather than by internal binding. If uptake is through specific ion channels which can be saturated (i.e. k_1, Fig. 1, decreases at high external copper) then the steady-state internal copper concentration would reach a maximum as external concentrations increase. However, under these conditions the rate at which the steady-state is approached would be similar, or slower, during the uptake phase than during depuration. This is inconsistent with observations; the steady-state is approached much faster during uptake than during depuration (Borgmann and Norwood 1995a). Control of copper accumulation through modulation of uptake rates, rather than through internal binding, is also inconsistent with the observation that copper accumulation is constant over large changes in body mass (Borgmann and Norwood 1995a). This would only be possible if the density of metal uptake channels on the body surface increases with body mass to a degree that compensates exactly for the decease in surface area per unit body mass in larger organisms.

The proposed model of copper accumulation (Fig. 1) is simple and is consistent with observations, to date, of copper uptake from water in *Hyalella*. The other models tested were not. This does not prove that the model is correct, but it does provide a plausible explanation of copper uptake control mechanisms which can be tested in future studies.

Estimation of Model Parameters

Several of the model parameters can be estimated from the data in Borgmann and Norwood (1995a). The values of X_t and E_t, equivalent to C_{Xmax} and C_{Bk} in the notation of Borgmann and Norwood (1995a), are 3.6 and 1.2 µmole/g dry weight respectively. The values of k_1, k_{-1} and k_2 cannot be estimated independently, but the value of $k_1 \bullet k_2/k_{-1}$ (= k_u in Borgmann and Norwood 1995a) ranges from 0.55-0.67 $L \bullet \mu mol^{-1} \bullet day^{-1}$ for exposure to copper in Lake Ontario water, depending on which data are used in the calculations. (Growth is minimal during the short time required to reach steady-state during copper uptake and can be ignored.) The values of k_3 and k_{-3} also cannot be calculated, but it is clear that k_3 is large and k_{-3} is small, resulting in complete saturation of E at most times.

Estimation of k_{-2} is possible from data on copper depuration. During depuration $C_w = 0$, and equations 5 and 6 simplify to

$$d(MX)/dt = -g \bullet ME - (k_{-2} + g) \bullet MX \quad (11)$$

$$MX = (-g \bullet ME) / (k_{-2} + g) \bullet (1 - \exp(-(k_{-2} + g) \bullet t)) + MX_o \bullet \exp(-(k_{-2} + g) \bullet t)) \quad (12)$$

The right hand term in equations 11 and 12 is similar to most equations of contaminant depuration. The left hand term, containing g•ME and not present in most depuration models, occurs because MX loses copper to E in order to keep ME constant as the animal grows. Borgmann and Norwood (1995a) estimated $k_e = 0.16$ day^{-1} using a formula equivalent to equation 11, but without the g•ME term. If *Hyalella* obtained copper from the food for synthesis of ME during the depuration phase, then k_{-2} would be equal to k_e. However, if copper for the synthesis of ME came largely from MX (Fig. 1), then k_{-2} would be smaller than the estimate of k_e calculated by Borgmann and Norwood (1995a). Computer simulation of copper depuration over a 2 week period, based on equation 12 with iteration steps of 0.1 days, showed that a value of $k_{-2} = 0.11$ result in a depuration curve similar to that obtained when k_{-2} is set to 0.16 and the term with g•ME is omitted. Unfortunately, data on copper accumulation from food and its incorporation into ME during the depuration experiment are not available. If, however, copper for synthesis of ME came from MX, then the value of k_{-2} would be about 0.11 day^{-1}.

Combining the above estimates of $k_1 \bullet k_2/k_{-1}$ and k_{-2} gives

$$K_{Cu\text{-}X} = (k_1 \bullet k_2) / (k_{-1} \bullet k_{-2}) = 1 / K_{0.5} \quad (13)$$

where $K_{0.5}$ is the concentration of copper in water resulting in half saturation of the ligand X and $K_{Cu\text{-}X}$, the inverse of $K_{0.5}$, is the strength of binding of copper to X. Note that $K_{Cu\text{-}X}$ is the product of several equilibria and includes constants for both diffusion into the animal and binding to the internal site. Using $k_{-2} = 0.11$ day^{-1} gives a value of $K_{Cu\text{-}X} = 5.0$ to 6.9 L/µmol ($K_{0.5} = 0.14$-0.20 µmol/L), based on total copper in Lake Ontario water. Extrapolating off the graph of free copper ion, as measured using a cupric ion electrode, against total copper added to Lake Ontario water given in Borgmann and Charlton (1984), a total copper concentration of 0.14-0.20 µmol/L ($K_{0.5}$) would equal about 10^{-10} M free Cu^{++}. Assuming the complexing capacity was similar in our experiments, this would give a $K_{Cu\text{-}X}$ of about 10^{10} L/mol based on free copper ion. This is considerably stronger than the binding capacity of copper to the gills of fathead minnows ($K_{Cu\text{-}gill} = 10^{7.4}$, Playle et al. 1993), possibly because $K_{Cu\text{-}X}$ is the product of several equilibria and not just external binding, or possibly because X has a physiological function in copper regulation in *Hyalella* and has, therefore, a higher binding affinity for copper.

Accumulation of Other Metals by *Hyalella azteca*

The uptake and depuration kinetics of zinc in *Hyalella* are very similar to those of copper, although the actual values of the various constants differ. Zinc uptake reaches a maximum at high waterborne zinc levels, the steady-state is approached more rapidly during uptake than depuration, and uptake is independent of body size (Borgmann and Norwood 1995a). The model used for copper (Fig. 1) might also apply for zinc. The estimate of X_t

for zinc (3.55 µmol/g) is very close to that of copper (3.60). Copper and zinc did not interfere with accumulation of each other, indicating no competition in binding and suggesting that X for copper and X for zinc are different ligands. Estimates of $k_1 \cdot k_2/k_{-1}$ (0.29-0.63 $L \cdot \mu mol^{-1} \cdot day^{-1}$) are similar to those of copper, but k_{-2} ($k_e = 0.68$ day^{-1}) is considerably larger (Borgmann and Norwood 1995a). Ignoring the effect of growth because k_e is much larger than g gives estimates of $K_{Zn\text{-}X}$ of about 0.4-0.9 L/µmol ($K_{0.5}$ = 1.1-2.3 µmol/L), based on total zinc in Lake Ontario water. This is somewhat weaker than the binding of copper to *Hyalella*. Zinc is, however, usually more abundant in the environment, and the binding strength need not be as strong in order to result in the same level of metal accumulation. Long term exposure to zinc does not, however, result in the same level of regulation as observed for copper (Borgmann and Norwood 1995a), indicating that there is less feedback control over MX through reduced X synthesis for zinc than for copper.

Uptake kinetics of lead in *Hyalella* differed from copper and zinc kinetics in some respects (MacLean et al. 1996). Background concentrations of lead were, as expected, much lower for this non-essential metal (i.e. $E_t = 0$). In addition, however, the rate at which the steady-state was approached was almost as fast during depuration as during lead uptake, and a clear saturation of body lead at high waterborne lead was not observed. Excessive mortality occurred before maximum body lead concentrations could be achieved. The depuration rate for lead (k_e = 0.52 day^{-1}) was, however, similar to that of zinc. Lead accumulation was not directly proportional to the water concentration; the bioaccumulation factor decreased slightly at higher lead concentrations in water. When a hyperbolic model (equation 10) was fit to the data, the $K_{0.5}$ values ranged from 0.6 to 7.5 µmol/L for the smallest to largest amphipods respectively ($K_{Pb\text{-}X}$ = 1.7-0.13 L/µmol for total Pb in Lake Ontario water). This is in the same range as the binding affinity for zinc. Maximum binding capacity (X_t) ranged from 2.6 to 25 µmol/g for small to large amphipods (MacLean et al. 1996). This also overlaps X_t values for copper and zinc. Unlike copper and zinc, however, short term (1 week) lethal concentrations are lower than the value of $K_{0.5}$. Therefore, while the model in Fig. 1 is consistent with lead accumulation (with E and ME = 0), most uptake kinetics of interest occur well below water concentrations near the $K_{0.5}$. Under these conditions, uptake kinetics can be approximated using a one-compartment first order kinetics model (i.e. omitting X, MX, E and ME in Fig. 1).

Comparison to Other Species

The model presented here is similar, in some respects, to the model of metal binding to fish gills (Playle et al. 1993). In both cases a metal binding constant can be calculated and saturation of binding sites occurs at high metal concentrations. However, whereas the gill model describes short term (3 hr) binding, the *Hyalella* model describes long term binding (equilibria are reached after about a week of exposure). The gill model deals with surface binding, whereas the *Hyalella* model deals with internal binding. The former can be used when investigating acute toxicity, whereas the latter is useful when studying chronic toxicity. The models contrast in that the gill binding constant is modeled as a single constant, whereas the binding of metal to internal sites in *Hyalella* is clearly a product of several processes (diffusion into the animal and internal binding, equation 13). This could affect the modeling of calcium-metal interactions in *Hyalella*. If calcium uptake is proportional to calcium concentrations in the water, calcium-metal interactions might be similar to those in fish gills. If, however, internal dissolved calcium is actively regulated and controlled within tight limits, then external calcium concentrations would be expected to have a much smaller effect on metal binding to internal sites in *Hyalella*. Detailed studies on calcium-metal interaction are needed and might shed further light on the accuracy of the proposed model (Fig. 1).

The model of metal uptake used for *Hyalella* is not necessarily appropriate for other crustacean species, especially larger animals. The amphipod *Gammarus pulex*, which reaches larger sizes than *Hyalella*, approached steady-state at the same rate during zinc uptake as during depuration (Xu and Pascoe 1993). Zinc accumulation in this amphipod can be modeled using a simple one-compartment first order kinetics model. This may simply be because the zinc concentrations tested were well below $K_{0.5}$ (e.g. see the discussion above on lead uptake in *Hyalella*), or it could imply a different metal control mechanism (e.g. control of uptake and elimination rates rather than internal binding). In larger crustaceans, such as decapods, control of the essential metals copper and zinc clearly differs from the *Hyalella* model. Body concentrations of shrimp are often maintained at constant levels until

external water concentrations exceed some critical threshold, after which body concentrations increase (White and Rainbow 1982). In the lobster, a very large crustacean, zinc regulation appears to involve active control of zinc excretion (Bryan et al. 1986).

The difference in control mechanisms between *Hyalella* and these larger Crustacea could be due to their different sizes, and the much larger surface to volume ratios in *Hyalella*. Because of its small size, it would be much more difficult for *Hyalella* than for decapods to control both the diffusion of metals through the body surface and the internal distribution of metals among different tissues. Diffusion is a relatively slower process in larger species, which therefore have a more elaborate circulatory system with more highly specialized tissues for absorption and excretion of metabolites. It might, therefore, be simpler for a small crustacean like *Hyalella* to control the internal binding sites for essential metals, rather than attempt to control uptake and excretion mechanisms. Active (i.e. energy requiring) uptake for metals, although feasible, is not necessary to achieve essential copper and zinc concentrations in aquatic invertebrates because of the high affinity of these metals for organic ligands (Rainbow and Dallinger 1993). Passive diffusion and binding to specific internal ligands (e.g. Fig. 1) is, therefore, an logical mechanism for regulation of essential metals in *Hyalella*.

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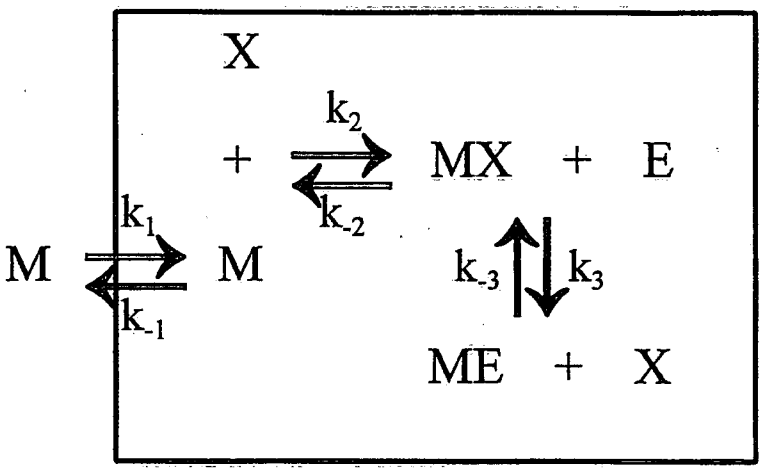
List of Figures

Figure 1. Proposed model for copper uptake by *Hyalella*. The box represents the body surface. M, free for loosely bound metal; X, internal ligand to which most of the non-essential copper is bound during exposure to elevated copper in water; E, essential copper-requiring macromolecules.



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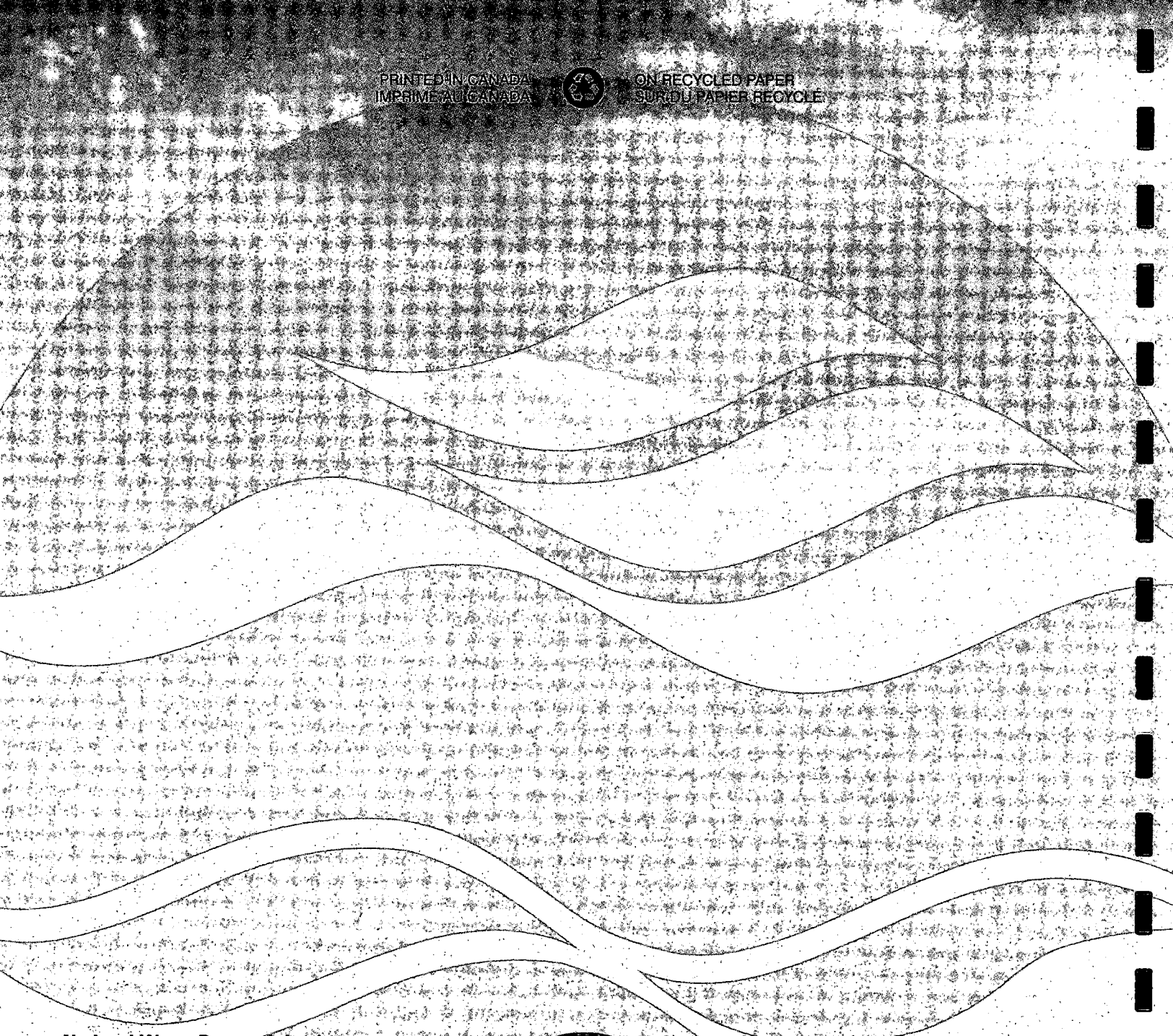

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