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Review

The sensitivity of aquatic insects to divalent metals: A comparative analysis of laboratory and field data

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ABSTRACT

Laboratory studies have traditionally indicated that aquatic insects are relatively insensitive to metals while field studies have suggested them to be among the most sensitive aquatic invertebrate taxa. We reviewed and synthesized available studies in the literature to critically assess why this discrepancy exists. Despite the intense effort to study the effects of metals on aquatic biota over the past several decades, we found studies specific to insects to still be relatively limited. In general, the discrepancy between laboratory and field studies continues with few efforts having been made to elucidate the ecological and physiological mechanisms that underlie the relative sensitivity (or insensitivity) of aquatic insects to metals. However, given the limited data available, it appears that aquatic insects are indeed relatively insensitive to acute metal exposures. In contrast, we suggest that some aquatic insect taxa may be quite sensitive to chronic metal exposure and in some cases may not be protected by existing water quality criteria for metals. The discrepancy between laboratory and field studies with respect to chronic sensitivity appears to largely be driven by the relatively short exposure periods in laboratory studies as compared to field studies. It also appears that, in some cases, the sensitivity of aquatic insects in field studies may be the result of direct effects on primary producers, which lead to indirect effects via the food chain on aquatic insects. Finally, available evidence suggests that diet is an important source of metal accumulation in insects, but to date there have been no conclusive studies evaluating whether dietary metal accumulation causes toxicity. There is a clear need for developing a more mechanistic understanding of aquatic insect sensitivity to metals in long-term laboratory and field studies.

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1. Introduction

Over the past 40 years, the acute, and to a lesser extent chronic, sensitivity of aquatic organisms to metals has been relatively well characterized in laboratory studies. A number of different meta-analysis studies have been performed summarizing the comparative sensitivity of different groups of aquatic organisms (Brix et al., 2001; Forbes and Calow, 2002; Brix et al., 2005). One of the conclusions from these analyses is that, largely based on acute toxicity data, aquatic insects are relatively insensitive to metals compared to other taxonomic groups (Brix et al., 2005). Previously, no reliable conclusions could be made regarding the relative sensitivity of aquatic insects chronically exposed to metals due to the paucity of data, although in general the inter-specific range of chronic metal sensitivity for all aquatic taxa is much less than observed for acute toxicity (Brix et al., 2001, 2005).

In contrast to laboratory data, some field studies suggest that specific aquatic insect taxa (e.g., some species of Ephemeroptera) are the most sensitive taxa to metal exposure in many aquatic systems (Clements et al., 2000). These are typically bioassessment studies in which abundance and diversity of aquatic insect taxa are characterized as a function of one or more metals along either a concentration gradient within a stream or between streams, where one stream is used as a reference stream and has metal concentrations characteristic of background conditions. Determining specific effect levels for aquatic insects in these studies is often difficult due to a number of factors, including multi-metal exposures (Clements et al., 2000; Schmidt et al., 2010), natural variability in insect abundance and diversity (Clements and Kiffney, 1995; Johnson, 1998; Linke et al., 1999), and the complex ecological interactions (e.g., predation, competition, facilitation) that can occur when a natural ecosystem is subjected to a stressor such as elevated metal concentrations (Clements et al., 1989; Hare, 1992; Clements, 1999).

Consequently, there is significant discordance in our understanding of the relative sensitivity of aquatic insects to metals based on short-term single species, single metal experiments in the laboratory and long-term multi-species, often multi-metal studies in the field. Reconciling these disparities is of significant import. Current water quality regulations are almost exclusively based on laboratory toxicity studies, where there is a preponderance of testing with just a few taxa. In general, cladocerans and salmonid fish species are significantly over-represented in toxicity data sets for metals, while aquatic insects are significantly under-represented when compared to the diversity of taxa typically found in a wide range of aquatic habitats (Brix et al., 2005). This bias in test organisms has lead to a corresponding bias in our water quality regulations, although it appears to be largely conservative with respect to ensuring protection of aquatic ecosystems given that cladocera and salmonids are relatively sensitive to metals. Similarly, on an acute basis the under-representation of insensitive aquatic insects is a conservative bias (Brix et al., 2005).

It is unclear, however, how the under-representation of insects in chronic toxicity data sets influences water quality criteria. Indeed, given the limited testing, the relative sensitivity of aquatic insects chronically exposed to metals is highly uncertain. Aquatic insects can comprise up to 90% of the taxa present in lotic systems and play a critical role in stream ecology providing a mechanism for the mobilization of energy from primary production and detrital pools into higher levels of the foodweb (Anderson, 1979; Cummins and Klug, 1979; Wallace and Webster, 1996; Covich et al., 1999). Therefore improving our understanding of what is causing the discrepancy between laboratory and field studies on aquatic insects is of obvious import.

The objective of this paper is to synthesize acute and chronic metal toxicity data for aquatic insects with the intent of trying to assess their relative sensitivity to metals with respect to laboratory, mesocosm, and field exposures.

2. Methods and materials

Three types of data were compiled for this meta-analysis; single-species toxicity tests conducted in the laboratory; multi-species outdoor mesocosm studies; and field bioassessment studies. In general, we took an inclusive rather than exclusive approach to screening data for use in this meta-analysis, the details of which are provided below. This was necessary to allow for any sort of robust analysis given the general paucity of data available for these taxa. This approach obviously introduces uncertainty into our analysis, particularly with respect to controlling variables that may influence organism sensitivity. However, we concluded that the uncertainty introduced using this approach was less than what would be incurred had we excluded more data by establishing a stricter screening protocol.

2.1. Laboratory toxicity data sets

Acute and chronic toxicity data sets based on laboratory studies were compiled for aquatic insects by conducting a comprehensive search of published literature. Five metals (Cd, Cu, Pb, Ni, and Zn) were selected for this review. These metals were selected because they represent the most common metal pollutants in aquatic ecosystems, and the metals for which the largest amount of toxicity data are available. For all toxicity tests, we required measured test concentrations and, at a minimum, measurement of water hardness. All acute studies were 96 h in duration and used survival as the biological endpoint. Chronic studies ranged from 4 to 240 days in duration and used any combination of survival, growth, molting, emergence and reproduction as endpoints. It is recognized that 4 days is normally not considered of sufficient duration to be considered a chronic exposure, but again, we opted to be as inclusive as possible in our data analysis. The LC50 was used as the statistical endpoint for acute studies while the EC20 was the preferred endpoint for chronic studies. In cases where insufficient information was available, the geometric mean of the NOEC and LOEC was used as an alternative to the EC20 for chronic studies.

Efforts were made to normalize data for differences in test water chemistry to the extent that information was available. Acute toxicity values were either hardness-normalized or, where sufficient data were available, acute values were normalized using the Biotic Ligand Model (BLM) (HydroQual, 2003). Chronic toxicity values were compared to the US hardness-based water quality criteria (WQC) and when sufficient water quality data were available they were compared to US (Cu) and EU (Cu, Ni, and Zn) BLM-based WQC (USEPA, 1996, 2001, 2007, 2009; Van Sprang et al., 2009). As there are currently no formal EU-wide WQC, we used the EU BLM-estimated HC5 (5th percentile) as a surrogate. All data were normalized to a default water chemistry (1.14 mM Na^+, 0.35 mM Ca^{2+}, 0.50 mM Mg^{2+}, 0.05 mM K^+, 0.05 mM Cl^-, 0.04 mM, 0.85 mM SO_4^{2-}, 0.5 mg l^{-1} DOC, pH 7.5, 65 mg l^{-1} alkalinity, 85 mg l^{-1} hardness) using either hardness-based regressions or the BLM, as appropriate.

Table 1
Summary of acute metal toxicity data for aquatic insects. All LC50s normalized to a hardness of 85 mg l^{-1} or using the BLM default water quality parameters (USEPA, 2007).

Species	Taxa	LC50 (μg l^{-1})	Reference
Cadmium			
Agapetus fuscipes	Trichoptera	149,326	(McCahon et al., 1989)
Baetis tricaudatus	Ephemeroptera	531	(Irving et al., 2003)
Chironomus plumulosus	Diptera	13,599	(Fargasova, 2001)
Chironomus riparius	Diptera	10,243	(Williams et al., 1986)
Chironomus riparius	Diptera	1264	(Watts and Pascoe, 2000)
Chironomus tentans	Diptera	1206	(Watts and Pascoe, 2000)
Chironomus tentans	Diptera	43,631	(Suedel et al., 1997)
Ephemerella subvaria	Ephemeroptera	2545	(Warnick and Bell, 1969)
Ranatra elongate	Hemiptera	211	(Shukla et al., 1983)
Rithrogena hageni	Ephemeroptera	20,005	(Brinkman and Johnston, 2008)
Trichoptera sp.	Trichoptera	6186	(Rehwoldt et al., 1973)
Zygoptera sp.	Odonata	7584	(Rehwoldt et al., 1973)
Copper			
Acroneuria lycorias	Plecoptera	20,636	(Warnick and Bell, 1969)
Adenophlebia auriculata	Ephemeroptera	152	(Gerhardt and Palmer, 1998)
Chironomus decorus	Diptera	1987	(Kosalwat and Knight, 1987)
Chironomus plumulosus	Diptera	159	(Fargasova, 2001)
Chironomus riparius	Diptera	407	(Taylor et al., 1991)
Cyrnus trimaculatus	Trichoptera	355	(Van der Geest et al., 2000a)
Ephoron virgo	Ephemeroptera	37	(Van der Geest et al., 2000a)
Ephoron virgo	Ephemeroptera	36	(Van der Geest et al., 2000b)
Hydropsyche augustipennis	Trichoptera	163	(Van der Geest et al., 2000b)
Rithrogena hageni	Ephemeroptera	235	(Brinkman and Johnston, 2008)
Trichoptera sp.	Trichoptera	10,222	(Rehwoldt et al., 1973)
Zygoptera sp.	Odonata	7584	(Rehwoldt et al., 1973)
Lead			
Baetis tricaudatus	Ephemeroptera	3937	(Mebane et al., 2008)
Benacus sp.	Diptera	50,107	(Oladimeji and Offem, 1989)
Chironomus tentans	Diptera	27,414	(Oladimeji and Offem, 1989)
Chironomus tentans	Diptera	11,525	(Mebane et al., 2008)
Nickel			
Acroneuria lycorias	Plecoptera	37,326	(Warnick and Bell, 1969)
Chironomus sp.	Diptera	13,473	(Rehwoldt et al., 1973)
Ephemeralla subvaria	Ephemeroptera	4636	(Warnick and Bell, 1969)
Trichoptera sp.	Trichoptera	47,312	(Rehwoldt et al., 1973)
Zygoptera sp.	Odonata	33,212	(Rehwoldt et al., 1973)
Zinc			
Chironomus plumulosus	Diptera	34,318	(Fargasova, 2001)
Ranatra elongata	Hemiptera	1312	(Shukla et al., 1983)
Rithrogena hageni	Ephemeroptera	88,225	(Brinkman and Johnston, 2008)
Trichoptera sp.	Trichoptera	91,083	(Rehwoldt et al., 1973)
Zygoptera sp.	Odonata	41,073	(Rehwoldt et al., 1973)

2.2. Mesocosm studies

Toxicity data from mesocosm studies were used if the study evaluated a single metal (i.e., mixture studies were excluded), had measured test concentrations, and at a minimum, a measurement of water hardness. By definition, mesocosm studies involved assessment of simulated aquatic communities of which insects were only a component. In these studies, the specific sensitivity of insects was identified, as well as the sensitivity of other community indices that might influence the response of insects. The EC20 was the preferred statistical endpoint for mesocosm studies for our assessment. In cases where insufficient information was available, the geometric mean of the NOEC and LOEC was used as an alternative to the EC20. Results were normalized for water quality to the extent data was available, as described for the laboratory toxicity data.

2.3. Field bioassessment studies

Field bioassessment studies were used only if the study evaluated a single metal or if there was reasonable confidence that only a single metal was the source of effects to a system. The specific sensitivity of insects was identified as well as the sensitivity of other community indices that might influence the response of insects. In many field studies, a large number of insect taxa were assessed. In these cases, data from sensitive representatives of major phylogenetic groupings (e.g., Ephemeroptera, Plecoptera, Trichoptera, Diptera) were used to summarize the relative sensitivity of different insect taxa. The statistical endpoints used for field studies involved a variety of different bioassessment metrics of abundance and diversity. Because raw data from these studies were not available, we were constrained to use the metrics reported for individual studies and as a result have a variety of different endpoints to represent the chronic sensitivity of insect taxa in the field. Results were normalized for water quality to the extent data was available, as described for the laboratory toxicity data.

3. Results

3.1. Laboratory toxicity data

Generally consistent with previous analyses of data for all aquatic species, the aquatic insect acute data sets for Cd and Cu (10–11 species) were significantly larger than for Ni, Pb, and Zn (3–5 species) (Brix et al., 2005) (Table 1). Across all metals, 84% of all tests were performed using species from 4 orders – Diptera, Ephemeroptera, Plecoptera, and

Table 2
Summary of chronic metal toxicity data for aquatic insects. All values normalized to a hardness of 85 mg l^{-1}. LC = life cycle; ELS = early life stage; S = survival; G = growth; E = emergence; R = reproduction; and M = molting.

Species	Taxa	Test type	Endpoints	Duration	Chronic value[a] (μg l^{-1})	Reference
Cadmium						
Chironomus riparius	Diptera	LC	S, G	26 days	42	(Pascoe et al., 1989)
Chironomus riparius	Diptera	LC	S, G	56 days	5.9	(Postma et al., 1994)
Chironomus tentans	Diptera	ELS	S	10 days	588	(Watts and Pascoe, 2000)
Chironomus tentans	Diptera	ELS	S, G	14 days	<100	(Suedel et al., 1997)
Ephemerella sp.	Ephemeroptera	ELS	S	28 days	<4.7	(Spehar et al., 1978)
Hydropsyche betteni	Trichoptera	ELS	S	28 days	>375	(Spehar et al., 1978)
Polypedilum nubifer	Diptera	LC	S, G, E, R	32 days	25	(Hatakeyama, 1987)
Pteronarcys dorsata	Plecoptera	ELS	S	28 days	>375	(Spehar et al., 1978)
Tanytarsus dissimilis	Diptera	ELS	S, G	10 days	6.1	(Anderson et al., 1980)
Copper						
Baetis sp.	Ephemeroptera	ELS	S	8 days	4.3	(Leland et al., 1989)
Chironomus tentans	Diptera	ELS	S	10 days	90	(Phipps et al., 1995)
Chironomus tentans	Diptera	ELS	S, G	10 days	621	(Karouna-Renier and Zehr, 2003)
Clistoronia magnifica	Trichoptera	LC	S, E, R	240 days	21	(Nebeker et al., 1984)
Epeorus latifolium	Ephemeroptera	ELS	G, E	57 days	18	(Hatakeyama, 1989)
Epeorus longimanus	Ephemeroptera	ELS	S	8 days	3.9	(Leland et al., 1989)
Ephemerella infrequens	Ephemeroptera	ELS	S	8 days	3.2	(Leland et al., 1989)
Ironodes lepidus	Ephemeroptera	ELS	S	8 days	6.1	(Leland et al., 1989)
Paraleptophlebia pallipus	Ephemeroptera	ELS	S	8 days	4.3	(Leland et al., 1989)
Paratanytarsus parthenogenticus	Diptera	LC	S, G, E, R	14 days	341	(Hatakeyama and Yasuno, 1981)
Polypedilum nubifer	Diptera	LC	E, R	32 days	11	(Hatakeyama, 1988)
Tanytarsus dissimilis	Diptera	ELS	S, G	10 days	27	(Anderson et al., 1980)
Lead						
Baetis tricaudatus	Ephemeroptera	ELS	M	10 days	66	(Mebane et al., 2008)
Chironomus tentans	Diptera	ELS	S, E	27 days	261	(Grosell et al., 2006)
Chironomus tentans	Diptera	LC	S, G, E, R	55 days	28	(Mebane et al., 2008)
Hydropsyche betteni	Trichoptera	ELS	S	28 days	>565	(Spehar et al., 1978)
Pteronarcys dorsalis	Plecoptera	ELS	S	28 days	>565	(Spehar et al., 1978)
Tanytarsus dissimilis	Diptera	ELS	S, G	10 days	258	(Anderson et al., 1980)
Nickel						
Clistoronia magnifica	Caddisfly	LC	S, E, R	240 days	92	(Nebeker et al., 1984)
Zinc						
Chironomus riparius	Diptera	ELS	G	4 days	88	(Miller and Hendricks, 1996)
Chironomus tentans	Diptera	ELS	S	10 days	1125	(Phipps et al., 1995)
Clistoronia magnifica	Trichoptera	LC	S, E, R	240 days	>5243	(Nebeker et al., 1984)
Tanytarsus dissimilis	Diptera	ELS	S, G	10 days	37	(Anderson et al., 1980)

[a] The preferred chronic value is the EC20, but if insufficient information were available, the geometric mean of the NOEC and LOEC were used instead.

Trichoptera – of the 13 insect orders with aquatic life stages (Merritt and Cummins, 1996). The chronic toxicity data sets for Cd and Cu were comparable in size to the acute data sets (7 and 11 species, respectively), with smaller data sets for the other metals (1–4 species) (Table 2). When acute and chronic laboratory toxicity data for insects were plotted as species sensitivity distributions, there was no indication that insects were not protected by existing USEPA WQC or EU HC5s with the exception of Zn, where two species of chironomids (*Chironomus riparius* and *Tanytarsus dissimilis*) appear to be chronically sensitive at concentrations below the USEPA WQC, but well above (>4-fold) the EU HC5 (Fig. 1). The limited samples sizes made it difficult to assess trends in relative sensitivity between taxa for a given metal (Fig. 2). However, across metal acute toxicity data sets, Ephemeroptera and Hemiptera were normally the most sensitive taxa tested. Similarly, for chronic toxicity data sets, Ephemeroptera were clearly the most sensitive taxa when tested (Cd, Cu, Pb), while Diptera (represented exclusively by chironomids) were the second most sensitive taxa despite being acutely insensitive to all of the metals (Fig. 2).

3.2. Mesocosm studies

While there has been a relatively large number of mesocosm studies performed with metals, the majority of these studies utilized a phytoplankton–daphnid–fish food chain and did not consider insects. Additionally, several mesocosm studies with invertebrates used metal mixtures (n = 5) preventing metal-specific analysis of insect sensitivity. Ultimately, only eight mesocosm studies that included insects and used single metal exposures were identified. Of these, six studies were on Cu and two on Zn. For Cu, one study (Hedtke, 1984) was a pond mesocosm in which chironomids were the primary insect taxa, one study was a stream mesocosm in which chironomids were again the insect taxa (Roussel, 2005), while the other mesocosm studies (one pond and three stream) all focused on Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa (Clements et al., 1988, 1989; Moore and Winner, 1989; Clements et al., 1990). The two study on Zn also focused on EPT taxa (Clements, 2004; Hickey and Golding, 2002).

Of the six Cu studies, two of the studies suggest that the USEPA WQC for Cu is not protective of the insect taxa present in the mesocosms (Hedtke, 1984; Clements et al., 1989), while insects are protected in the two studies with sufficient information to calculate the EU HC5 (Fig. 3). In the Hedtke (1984) study, chironomid emergence was significantly reduced at 5.6 μg l^{-1} Cu, while the hardness- and BLM-based USEPA WQC for this study were 21 and 9.3 μg l^{-1} Cu, respectively. In Clements et al. (1989), increased predation of caddisflies by stoneflies was observed at 5.5 μg l^{-1} Cu, while the corresponding hardness-based USEPA WQC was 8.4 μg l^{-1} Cu. The other four studies all indicate the

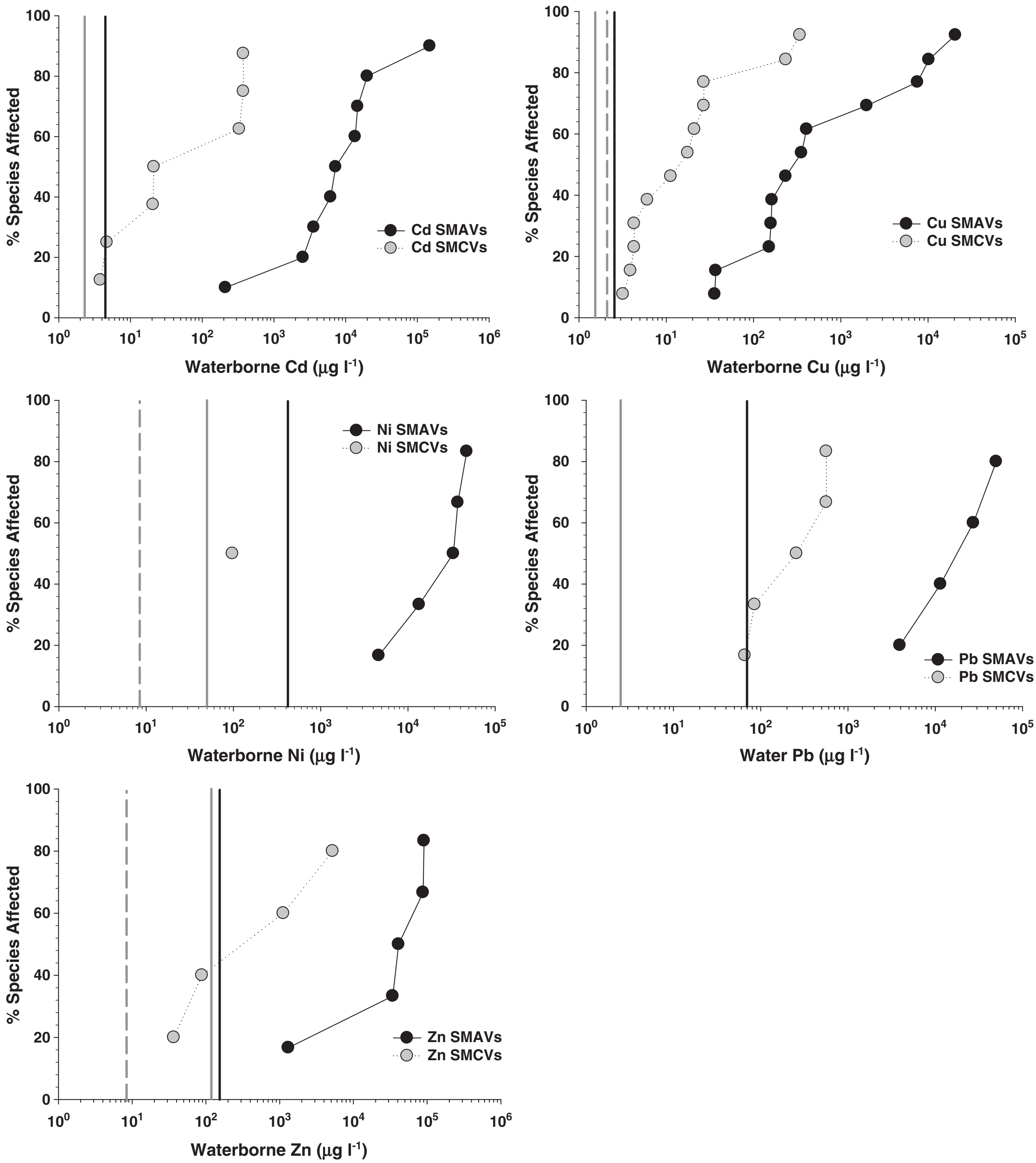

Fig. 1. Acute and chronic species sensitivity distributions for aquatic insects for Cd, Cu, Ni, Pb and Zn. All values based on the geometric mean of available toxicity data for a given species and normalized to a hardness of 85 mg l^{-1} or using the default water quality parameters for the BLM. SMAV = Species Mean Acute Value, and SMCV = Species Mean Chronic Value. Bars represent acute and chronic WQC under the same water quality conditions. Black bars represent the acute USEPA WQC. Solid gray bar represent the chronic USEPA WQC and dashed gray bar represents the chronic EU BLM-based HC5 (see Methods and materials for details).

USEPA WQC for Cu is protective of insects although for two of the studies (Clements et al., 1988, 1990), effects were observed at the lowest Cu concentration tested (Fig. 3).

The two mesocosm studies on Zn, also indicated Zn WQC are protective of insects. Clements (2004) identified an effect concentration of >792 μg l^{-1} Zn at a corresponding USEPA WQC of 34 μg l^{-1}. However, this was a relatively short-term study (10 days) that may not have fully characterized the chronic toxicity of Zn to insects in the mesocosm. The second mesocosm study on Zn (Hickey and Golding, 2002) was a 34 day exposure on stream macroinvertebrates and was actually a mixture study with Zn and Cu. This mixture makes it difficult to assess the relative importance of Zn and Cu in treatments where effects were observed as both metals were above USEPA WQC. However, in the lowest treatment, no effects were observed on a variety of indices at a Zn concentration of 72 μg l^{-1} compared to the corresponding USEPA WQC of 50 μg l^{-1}, suggesting this criterion is protective of

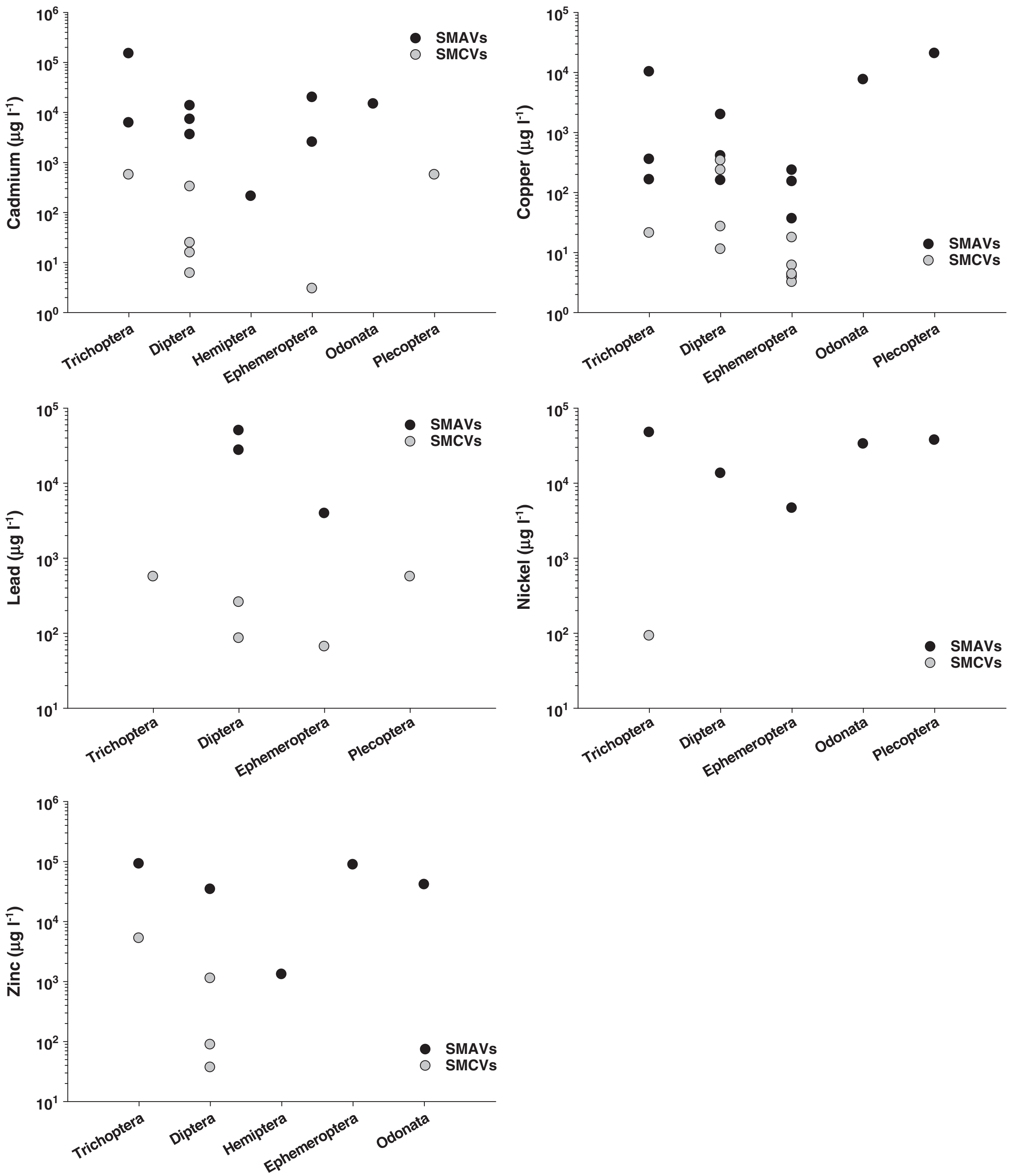

Fig. 2. Distribution of SMAVs and SMCVs for Cd and Cu organized by insect order. All values based on the geometric mean of available toxicity data for a given species and normalized to a hardness of 85 mg l^{-1} or using the default water quality parameters for the BLM.

insects. Copper concentrations in this treatment were below the USEPA WQC and so inferences about protection cannot be made.

3.3. Field bioassessment studies

We identified a relatively large number ($n = 23$) of field bioassessment studies investigating the affects of metals on aquatic insects (Table S1). In all but 4 of these studies insects were exposed to elevated concentrations of multiple metals or other classes of toxicants. Evaluation of whether chronic WQC are protective of metal mixtures is complex. We chose not to assume additivity because it is not clear if the chronic toxicity of divalent metals is indeed additive (most research on this issue has focused on acute toxicity). However, we evaluated whether any of the field studies we identified included examples where metals concentrations were all below their respective WQC, but effects on insect populations or communities were observed. Conversely, we also evaluated whether there were examples where metals concentrations exceeded their respective WQC, but no effects on insect

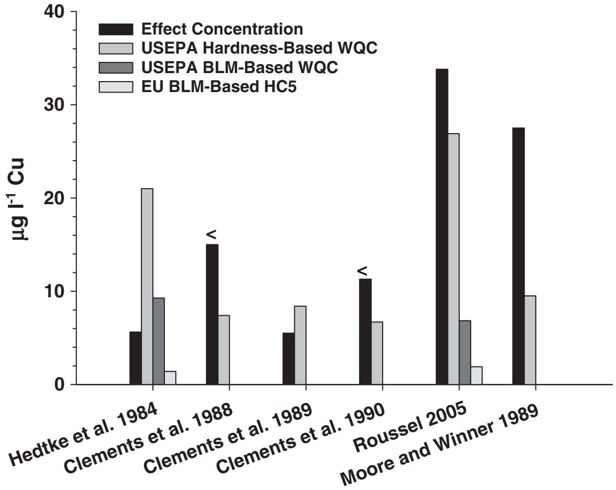

Fig. 3. Comparison of estimated Cu effect concentrations and corresponding chronic WQC for mesocosm studies. Effect concentrations are compared to hardness-based and BLM-based WQC normalized to the water quality conditions of the study. Missing bars indicate insufficient water quality data was available to derive BLM-based WQC. Estimated effects concentrations for Clements et al. (1988, 1990) were below the lowest concentration tested.

populations were observed. Overall, we found that most examples of metals-related effects on insects in the field were associated with metals concentrations that exceeded WQC concentrations, and often substantially. In many of these cases, impacts on insect populations and communities were also often severe. Accordingly, limited field data were identified where possible impacts on insect communities or metrics were observed at metals concentrations below WQC levels. The often severe impacts also observed at high metals concentrations relative to criteria also did not provide much information on whether insects in the field may be sensitive to metals near WQC concentrations.

As noted, 4 field studies were identified in which aquatic insects were primarily exposed to elevated concentrations of a single metal. In the first of these, Clements and Kiffney (1995) studied the effects of elevated Zn concentrations across a range of streams in Colorado (USA) receiving wastewater from various mining activities. Concentrations of other metals (e.g., Cu and Cd) were nearly always below detection. Streams were grouped into categories of low, medium and high Zn and a variety of insect bioassessment parameters were evaluated. Overall, the most sensitive endpoint was Ephemeroptera richness with the lowest effects observed at 50 $\mu g\ l^{-1}$, compared against a hardness normalized chronic WQC for 72 $\mu g\ l^{-1}$ Zn. A further analysis of these data by stepwise multiple regression showed that significant amounts of the partitioned variance in several bioassessment endpoints (total Ephemeroptera richness and abundance, Heptageniidae and *Baetis* sp. abundance, as well as Chloroperlidae abundance) were all negatively correlated with Zn concentrations.

In the second field study, Leland and Carter (1984, 1985) and Leland et al. (1989) dosed three separate channels of Convict Creek in the high Sierra Nevada Mountains with 2.5, 5–7 or 10–15 $\mu g\ l^{-1}$ Cu for approximately one year. A fourth un-dosed channel served as a control. During and at the end of the exposure period, a variety of bioassessment metrics for both periphyton and insects were measured to characterize the potential effects of the Cu exposure. The researchers observed significant declines in periphyton production (Fig. 5A) at all Cu concentrations tested while declines in aquatic insect standing stock were observed in the 5–7 and 10–15 $\mu g\ l^{-1}$ Cu treatments (Fig. 5B), resulting in a chronic value of 3.5–4.2 $\mu g\ l^{-1}$ Cu for insects. In contrast to these observed effects, the hardness-normalized USEPA WQC for Cu in this stream is 10.7 $\mu g\ l^{-1}$ while the BLM-based WQC are 2.3 and 1.9 $\mu g\ l^{-1}$ Cu using US and EU derived BLMs, respectively.

In a third study, Cain et al. (2004) evaluated the distribution of aquatic insect taxa along a metals contamination gradient in the Clark Fork (Montana, USA). The insect species composition change over the contamination gradient, with, for example, the mayflies *Epeorus albertae* and *Serratella tibialis* occurring at less contaminated sites but being rare or absent from the most contaminated sites. Conversely, the metals-tolerant caddisfly *Hydropsyche* spp. and mayfly *Baetis* spp. were widely distributed and dominant in the most contaminated sections of the river. Based on metals bioaccumulation data, the authors suggested that the compositional changes in aquatic insect over the contamination gradient was more closely related to Cu than other metals. In addition, Cu concentrations exceeded chronic hardness-normalized USEPA WQC by a factor of approximately 3 to 8, while Cd and Zn concentrations were below their respective WQC.

Finally, Carlisle and Clements (2005) evaluated secondary production of leaf-shredding insects in several Colorado (USA) streams predominantly stressed by Zn. They found that total secondary production of shredders was negatively associated with Zn concentrations. The dissolved Zn concentrations in the non-reference streams were 117, 256, and 366 $\mu g\ l^{-1}$, which exceeded hardness-normalized USEPA WQC by factors of approximately 2 to 8.

4. Discussion

The overall objective of this data synthesis was to evaluate whether the relative sensitivity of aquatic insects to metals could be assessed by comparing data from single species toxicity tests, multi-species mesocosm studies, and field bioassessments. Such a comparison might allow for the determination of whether aquatic insects are protected by WQC for metals, which are derived from laboratory toxicity data sets where insects are grossly under-represented. Results from this synthesis clearly demonstrate the continued lack of concerted effort to study the effects of metals on aquatic insects. For instance, we were only able to identify 9 laboratory studies published on the aqueous toxicity of the five metals considered in this review for aquatic insects from the year 2000 to present as compared to the hundreds of papers published over the same time frame on other aquatic taxa. While there has been a more intensive effort to study the effects of metals on aquatic insects in the field, the vast majority of these studies are in systems where multiple metals are elevated, a scenario that is not conducive to deriving effects data for single metal WQC. It is also worth noting that a relatively large body of data on metal bioaccumulation in aquatic insects has been developed (Hare, 1992; Jarvinen and Ankley, 1999), including a number of studies evaluating the mechanisms and phylogenetic relationships observed with respect to metal bioaccumulation (Buchwalter and Luoma, 2005; Buchwalter et al., 2008). Unfortunately, to date, no clear links between metal bioaccumulation and toxicity have been made. Despite these limitations, there are several important inferences that can be made about the relative sensitivity of aquatic insects to metals, the mechanisms which likely contribute to their sensitivity, and whether they are protected by existing WQC.

4.1. Relative sensitivity of aquatic insects

In general, laboratory studies have concluded that aquatic insects are relatively insensitive to metals when compared with other aquatic organisms (Brix et al., 2001, 2005) while field studies have frequently inferred them to be sensitive indicators of stream impairment in many mining-impacted areas (Hare, 1992). The literature review conducted for this assessment generally supports these earlier observations. With the exception of two studies for Zn, laboratory toxicity studies continue to indicate aquatic insects are relatively insensitive, with the most sensitive insect species tested typically at least a factor of 3 (chronic) to more than an order of magnitude (acute) less sensitive than existing acute and chronic USEPA WQC, while all insect laboratory studies were less sensitive than EU HC5s. In contrast, two of six mesocosm studies indicate effects below the USEPA hardness-based WQC for Cu and one of two studies indicate effects below the USEPA BLM-based criteria (BLM-based criteria could not be calculated for all six mesocosm studies; Fig. 3) as does the field study for Cu, and one Zn field study indicated effects at a mean Zn concentration approximately 68% above the WQC.

There are several possible reasons for these differences. First, with respect to acute toxicity, aquatic insects may indeed by relatively insensitive to metals due to certain physiological mechanisms which will be discussed later. For chronic toxicity, however, the discrepancy between laboratory and mesocosm/field studies may be related to exposure duration and the endpoints evaluated. For this review, we considered any study >4 days in duration as a chronic exposure. The mean study duration was 44 days, but the mode was only 10 days (i.e., 3 studies each 240 days in duration strongly bias the mean). Use of a more rigorous definition of chronic toxicity, such as requiring a full life cycle study (Stephan et al., 1985) would have resulted in the exclusion of 17 of the 28 studies used in this analysis. In contrast, the mesocosm and field studies ranged in duration from 4 to 548 days, with the studies indicating insects are sensitive below the WQC being 14, 224, 365 and 365 days in duration respectively. While by no means conclusive, these data are at least suggestive that study duration is a potentially important factor and that current laboratory studies are of insufficient duration (or fail to include sensitive life stages) to fully assess aquatic insect sensitivity to metals. It should also be noted, however, that there are examples of insect taxa being very sensitive to metals in short-term exposures. For example, Leland et al. (1989) in 8-day toxicity tests estimated EC20s between 3 and 6 $\mu g\ l^{-1}$ Cu for several species of Ephemeroptera. Hence the selection of sensitive taxa is clearly as important as study duration on laboratory toxicity studies.

4.2. Mechanisms underlying acute insect sensitivity to metals

The physiological mechanisms underlying the sensitivity of aquatic organisms to acute metal exposure is relatively well understood. In general, metals inhibit the uptake of major ions (Na^+, Ca^{2+}, Mg^{2+}, Cl^-) by freshwater organisms through either competitive or direct inhibition. Freshwater organisms actively take up these ions in response to diffusive loss to the hypo-osmotic environment. Consequently, the uptake rates of these ions in different organisms are in response to the rates of diffusive loss, such that $J_{in} = J_{out}$ when the organism is at ionoregulatory equilibrium. The loss rate (J_{out}) and consequently the uptake rate (J_{in}) is largely a function of the permeable surface area to volume ratio and so in general follows allometric scaling with smaller organisms being more sensitive. Several studies with Ag and Cu have shown that the sensitivity of aquatic organisms can be directly related to Na^+ turnover rates (Bianchini et al., 2002; Grosell et al., 2002).

Given this construct, the relative insensitivity of aquatic insects to acute metal exposures is not surprising given their generally large size (and therefore presumably low ion turnover rates) relative to many other aquatic invertebrates (e.g., daphnids) that are routinely tested. However, based on size alone it is somewhat surprising that aquatic insects are generally less sensitive than fish to acute metal exposure, suggesting that they may have unusually low size-independent ion turnover rates. Unfortunately, with the exception of several studies on mosquito larvae (Patrick et al., 2001, 2002a,b), for which there is no corresponding toxicity data, we were unable to identify any data on ion uptake or loss rates in aquatic insects. There is a clear need to develop such data to test whether the generally understood mechanisms of acute metal toxicity apply to aquatic insects as well.

4.3. Mechanisms underlying chronic insect sensitivity to metals

With respect to chronic metal toxicity, our general understanding of the mechanisms of action is very limited. In a few cases, ionoregulatory disturbance, as in acute exposures, may be the primary mechanism for observed chronic effects (Grosell and Brix, 2009). However, in general, this does not appear to be the case with a wide range of potential mechanisms including energetic costs of detoxification, target organ dysfunction, behavioral dysfunction, and endocrine effects being hypothesized (Bergman and Doward-King, 1997). Given the limited data available, it is not surprising that there are no mechanistic studies on the effects of chronic metal exposure to aquatic insects. However, the mesocosm and field studies discussed in this review may provide some interesting insights into some potential mechanisms.

Unlike standard laboratory studies where environmental variables other than the concentration of the toxicant are normally closely controlled, mesocosm and field studies frequently have variable conditions with respect to water quality, temperature, and food supply. While they may be more environmentally realistic, data interpretation in such studies is often complicated by these variables unless they are closely measured. The mesocosm study by Hedtke (1984) and the field study by Leland and Carter (1984, 1985), Leland et al. (1989) both closely characterized the effects of Cu on not only aquatic insect communities, but also their food sources. In the Hedtke (1984) study, the estimated EC20 for chironomid emergence was 5.6 $\mu g\ l^{-1}$ Cu compared with USEPA and EU BLM estimated chronic WQC/HC5s of 9.3 and 1.4 $\mu g\ l^{-1}$ Cu, respectively. In contrast, laboratory studies on Cu toxicity to chironomids have estimated water quality normalized EC20s ranging from 11.3 to 89 $\mu g\ l^{-1}$ Cu, suggesting they are less sensitive in laboratory exposures.

Interestingly, Hedtke (1984) also measured several parameters to characterize productivity in the mesocosm systems and found that gross primary productivity was also significantly inhibited by Cu exposure. We plotted chironomid emergence as a function of gross primary productivity and found a strong correlation ($r^2 = 0.95$) between the two factors (Fig. 4). These data suggest that observed effects on chironomids in the mesocosm may be related to limited food resources rather than direct Cu toxicity. This hypothesis is supported by the relatively lower sensitivity of chironomids to Cu when exposed in the laboratory where food resources are not limited.

In the Leland et al. study, an effect concentration of 3.5 $\mu g\ l^{-1}$ Cu (geometric mean of 2.5 $\mu g\ l^{-1}$ NOEC and 5 $\mu g\ l^{-1}$ LOEC) was estimated based on reduced insect species richness and diversity. This effect concentration is higher than the USEPA and EU BLM estimated WQC/HC5 of 2.3 and 1.1 $\mu g\ l^{-1}$ Cu, but much lower than the USEPA hardness-based WQC of 18.4 $\mu g\ l^{-1}$ Cu. Somewhat similar to the Hedtke (1984) study, a closer examination of the biological data from this study suggests the possibility that effects on primary production may be the cause of the observed effects on some insect taxa. A significant impact on periphyton biomass was observed in all Cu treated streams in this study (Fig. 5A). A significant decrease in insect standing stock was also

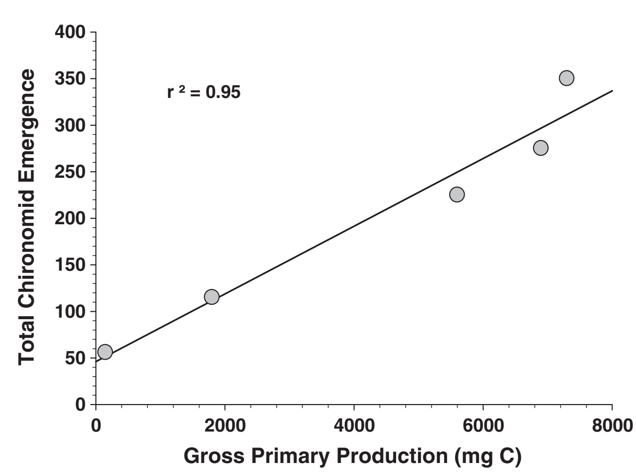

Fig. 4. Relationship between gross primary productivity (mg C) and total chironomid emergence in the mesocosm study by Hedtke (1984).

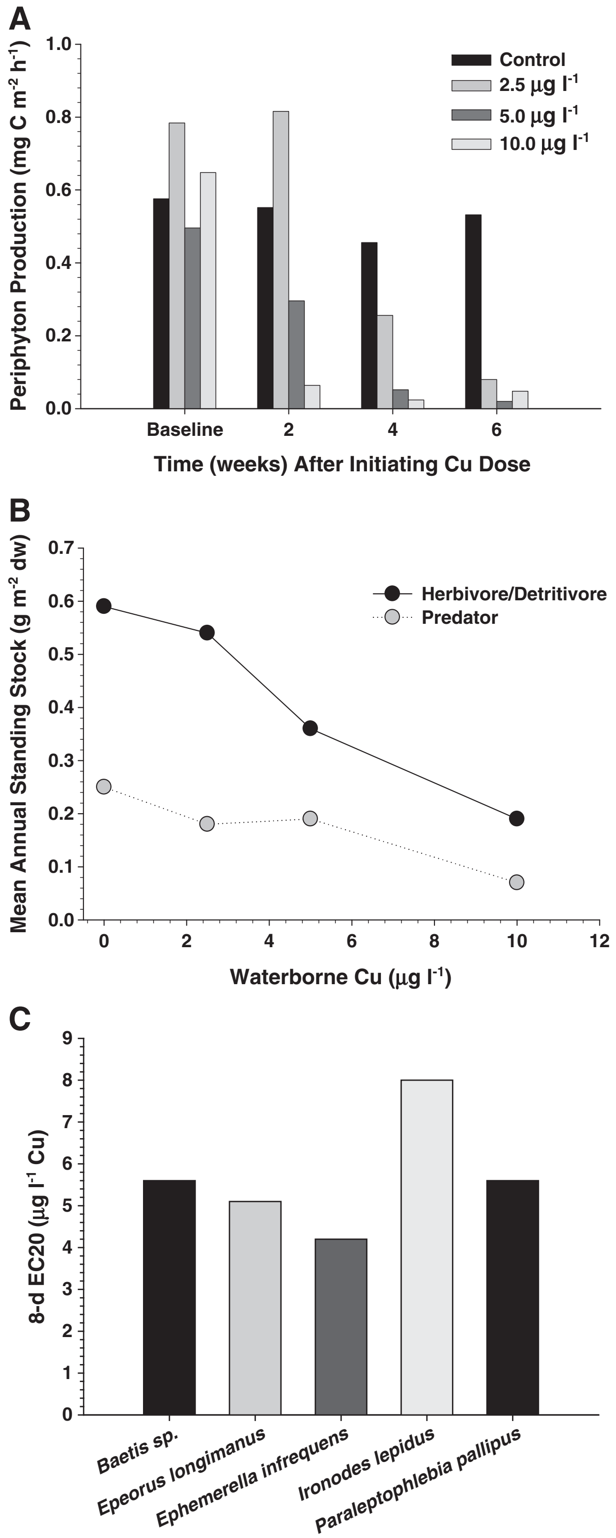

Fig. 5. Effects of Cu in the stream field study by Leland et al. (1984, 1985, 1989). A. Effects of Cu on algal production (mg C m^{-2} h^{-1}). B. Effects of Cu on aquatic insect standing stock (g m^{-2} dw) after 1 year of Cu exposure. C. Estimated 8-day EC20s for key aquatic insect species exposed in the laboratory.

observed in the 5 µg l^{-1} Cu treatment for those taxa in the herbivore/detritivore guild, while insects in the predator guild were only significantly impacted at 10 µg l^{-1} Cu (Fig. 5B). These data suggest the possibility that the observed effects on insects may be the result of a trophic cascade that began with impacts periphyton biomass, limiting available resources for higher trophic levels. However, contrary to this hypothesis, Leland et al. (1989) conducted 8-day laboratory toxicity tests on key insect taxa from their field study and found all species to be sensitive to Cu with EC20s for survival ranging from 3 to 6 µg l^{-1} Cu, suggesting direct Cu toxicity could also explain effects observed in the field study.

4.4. The importance of dietary metal exposure

A final possibility to consider in explaining the discrepancies between chronic laboratory and field study sensitivity of aquatic insects to metals is the role of dietary metal exposure. A number of studies have examined the relative importance of waterborne versus dietary pathways for Cd exposure in insects. These studies have demonstrated that for most aquatic insects, the diet is the primary Cd exposure pathway (Timmermans et al., 1992; Munger and Hare, 1997; Roy and Hare, 1999; Xie et al., 2010). For example, Martin et al. (2007) demonstrated that the diet contributed 25–93% of Cd accumulation in stoneflies and was the dominant pathway for 5 of 7 species examined. We only identified one study on a metal other than Cd exploring the importance of dietary metal exposure. Timmermans et al. (1992) examined dietary exposure to Zn in caddisfly larvae and found water to be the dominant exposure pathway.

While the available data suggests, at least for Cd, that the diet is the dominant exposure pathway, data demonstrating that dietary metal exposure actually causes toxicity is quite limited. Only two studies have attempted to evaluate this issue with aquatic insects. Irving et al. (2003) exposed the mayfly *Baetis tricaudatus* to dietary Cd (periphyton) for 13 days and observed a significant reduction in mayfly body mass at 10 µg g^{-1} dw Cd in the periphyton. Unfortunately, the periphyton diets were developed by short-term (15 min) exposures to very high aqueous Cd (100 µg l^{-1} for the 10 µg g^{-1} dw diet) and so it is difficult to relate observed effects to a environmentally relevant waterborne Cd concentration. Xie et al. (2010) also investigated dietary Cd toxicity to a mayfly (*Centroptilum triangulifer*). They exposed periphyton to environmentally realistic aqueous Cd concentrations for 6–7 days prior to feeding to mayflies for ~30 days. The authors ran three experiments, but results were inconsistent between trials making it difficult to conclude whether dietary Cd toxicity was observed.

The efforts to date provide no definitive answer on the importance of dietary metals with respect to causing toxicity to aquatic insects. However, given that the dietary exposure pathway has been shown to be important and that a number of other studies have shown dietary metals to cause toxicity to invertebrates at low exposure levels (Hook and Fisher, 2001; Bielmyer et al., 2006), additional study of this issue is clearly needed.

4.5. Are insects protected by current water quality standards?

This review was largely prompted by contradicting data from the laboratory and field on the relative sensitivity of aquatic insects. A number of field studies have suggested that aquatic insects are sensitive to metals at concentrations below existing WQC. Our review of the available literature suggests this is clearly not the case based on the EU BLM-based HC5s, but there is some indication that USEPA WQC, particularly hardness-based criteria, may be under-protective in some cases. In general, laboratory studies continue to support previous characterizations that aquatic insects are relatively insensitive and therefore protected by existing WQC. However, these studies may be underestimating aquatic insect sensitivity in chronic metal exposures due to the generally short study durations that may be missing key sensitive life stages.

We found interpretation of the majority of field studies to be difficult as most involved simultaneous exposure to elevated concentrations of multiple metals. While we do not discount the importance of considering multi-metal exposures in assessing environmental impacts, current WQC are based on individual metals and we have limited our evaluation accordingly. Using these criteria, we were only able to identify a few single metal mesocosm and field bioassessment studies. Of these, 3 out of 12 studies identified effects on aquatic insects at Cu concentrations below the USEPA hardness-based WQC, while one study indicated the USEPA BLM-based WQC was also under-protective. All three studies that indicated under-protection were characterized by high (>200 mg l^{-1}) water hardness, suggesting the possibility that the slope of the USEPA hardness-based WQC may be overestimating the degree of protection provided by increasing hardness for insects in chronic Cu exposures. A similar observation was recently made when comparatively evaluating the relative protection provided by BLM-based and hardness-based Cu criteria for effects on salmonid olfaction (Meyer and Adams, 2010). Zinc was the only other metal evaluated in the mesocosm and field studies. Although limited in number, no effects were observed on insects at Zn concentrations below WQC while several studies showed effects at Zn concentrations ~5-fold higher than current WQC.

While the total number of studies is small, these results certainly suggest that aquatic insects are indeed very sensitive to some metals and in some cases may not be protected by existing WQC, particularly the hardness-based Cu WQC that are largely derived in the absence of any long-term studies on aquatic insects. At a minimum, these results should be interpreted as highlighting an immediate need for significant research efforts into evaluating the sensitivity, including the underlying ecological and physiological mechanisms, of aquatic insects in long-term metal exposures.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2011.06.061.

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