

# Ecotoxicological Studies Indicate That Sublethal and Lethal Processes Limit Insect-Mediated Contaminant Flux

Connor I. Olson,<sup>a,\*</sup> Gale B. Beaubien,<sup>b</sup> Ryan R. Otter,<sup>c,d</sup> David M. Walters,<sup>e</sup> and Marc A. Mills<sup>b</sup>

<sup>a</sup>Department of Civil and Environmental Engineering, Syracuse University, Syracuse, New York, USA

<sup>b</sup>Center for Environmental Solutions and Emergency Response, US Environmental Protection Agency, Cincinnati, Ohio

<sup>c</sup>Data Science Institute, Middle Tennessee State University, Murfreesboro, Tennessee, USA

<sup>d</sup>Department of Biology, Middle Tennessee State University, Murfreesboro, Tennessee, USA

<sup>e</sup>Columbia Environmental Research Center, US Geological Survey, Columbia, Missouri

**Abstract:** Merolimnic insects can accumulate and transport considerable amounts of aquatic contaminants to terrestrial systems. The rate of contaminant biotransport, termed *insect-mediated contaminant flux* (IMCF), depends on emergent insect biomass and contaminant accumulation, both functions of environmental concentration. We developed a mathematical model of IMCF and apply it to three ecotoxicological studies obtained through the US Environmental Protection Agency's ECOTOX database to determine at which concentration maximum IMCF occurs. Model results demonstrate that the maximum IMCF depends on competing rates of biomass loss and contaminant accumulation and does not necessarily occur at the highest insect or environmental contaminant concentration. In addition, modeling results suggest that sublethal contaminant effects (e.g., decreased growth) on insect biomass can be an important and potentially underappreciated control on IMCF. *Environ Toxicol Chem* 2023;00:1–10. © 2023 SETAC. This article has been contributed to by U.S. Government employees and their work is in the public domain in the USA.

**Keywords:** Aquatic invertebrates; Biotransport; Ecotoxicology; Environmental transport

## INTRODUCTION

The impact of environmental contamination is heavily mediated by biological processes, ranging from the microbial (e.g., biotransformation, biodegradation) to the ecosystem scale (e.g., biomagnification, biotransport; Schiesari et al., 2018). One such example is the movement of contaminants from aquatic to terrestrial environments accompanying the emergence of merolimnic insects, a long-recognized exposure vector for terrestrial consumers (Bundschuh et al., 2019, 2022; Carson, 1962; Koch et al., 2020; Menzie, 1980; Walters et al., 2008, 2020). As larvae, merolimnic insects live in aquatic ecosystems where they accumulate aquatic contaminants. Once the larval aquatic insects undergo metamorphosis, they emerge from the aquatic system as winged adult aquatic insects and may retain contaminants from their larval stage. Aquatic contaminants can thus be transported between compartments, and this transport serves as an exposure route to terrestrial and aerial insectivores (Beaubien

et al., 2020; Cristol et al., 2008; Howie et al., 2018; Walters et al., 2010). The rate of this biotransport for a given area is referred to as *insect-mediated contaminant flux* (IMCF; definitions of key terms used in this article are provided in Textbox 1).

Otter et al. (2020) developed a theoretical model based on the mechanisms governing IMCF and provided a framework to better understand how IMCF changes across increasing environmental contaminant concentrations. From this model, three postulates were produced regarding IMCF:

1. IMCF can only occur if a contaminant accumulates in larval aquatic insect tissues and is at least partially retained through metamorphosis.
2. As contaminant concentrations increase, contaminants that cause larval mortality will limit emergent insect biomass and eventually decrease IMCF to 0.
3. In cases where contaminant stress inhibits metamorphosis, total mortality (both larval and emergent mortality) will decrease IMCF to a greater degree than effects on larval mortality alone would suggest.

Based on these postulates, Otter et al. (2020) hypothesized that the maximum flux possible for a system would occur at the

This article includes online-only Supporting Information.

\* Address correspondence to [connor.i.olson@gmail.com](mailto:connor.i.olson@gmail.com)

Published online 30 January 2023 in Wiley Online Library ([wileyonlinelibrary.com](http://wileyonlinelibrary.com)).

DOI: 10.1002/etc.5574

## TEXTBOX 1: Definitions for important terms

<b>Merolimnic insects</b>	Insects with an aquatic larval life stage that undergo metamorphosis to enter a terrestrial/aerial life stage.
<b>Larval aquatic insects</b>	<b>Merolimnic insects</b> in a premetamorphic aquatic larval life stage.
<b>Adult aquatic insects</b>	<b>Merolimnic insects</b> in a postmetamorphic terrestrial/aerial life stage.
<b>Emergence</b>	The movement of <b>merolimnic insects</b> from the aquatic to the terrestrial environment coincident with metamorphosis.
<b>Larval mortality</b>	The death of a <b>larval aquatic insect</b> prior—and unrelated—to metamorphosis.
<b>Emergent mortality</b>	The death of a <b>merolimnic insect</b> during metamorphosis.
<b>Total mortality</b>	The death of a <b>merolimnic insect</b> before metamorphosing into an <b>adult aquatic insect</b> . This term includes both <b>larval mortality</b> and <b>emergent mortality</b> .
<b>Emergent biomass</b>	The total mass of <b>adult aquatic insects</b> completing <b>emergence</b> .
<b>Biotransport</b>	The transfer of a contaminant between ecosystem compartments via a biological vector (e.g., the <b>emergence</b> of <b>merolimnic insects</b> ).
<b>Insect-mediated contaminant flux</b>	Calculation of total contaminant <b>biotransport</b> via the <b>emergence</b> of <b>merolimnic insects</b> per unit area and time.

highest environmental concentration where all larval aquatic insects survive and complete metamorphosis (i.e., no effects on emergence are observed). It follows that in systems where contaminant concentrations exceed no-effect levels, maximum IMCF would not occur at the highest environmental concentration. These conclusions have important implications for risk assessors and resource managers but are currently only theoretical, somewhat limiting the practical implementation of the IMCF model. To further develop the IMCF model, an empirical framework is needed.

We developed a mathematical framework to examine IMCF over varying contaminant concentrations. Based on Otter et al. (2020), we hypothesize that maximum flux does not occur at the highest environmental contaminant concentration but at the first instance of inhibited emergence. Testing this hypothesis requires data sets in which contaminant accumulation and effects are quantified over a gradient of contaminant concentrations. Conveniently, the typical dose–response design of ecotoxicological studies produces data sets closely aligned with these needs. Utilizing peer-reviewed ecotoxicological data sets, we (1) develop a flexible mathematical model of IMCF, (2) apply our mathematical model to three case studies (selenium,

fluoranthene, cadmium) to determine the environmental concentrations at which maximum flux occurs, and (3) use the mathematical model to examine the drivers of IMCF.

## IMCF MODEL BASIS

For any given concentration, IMCF is defined as

$$\text{IMCF} = \frac{\bar{C}_{\text{adult}} \times B_{\text{total}}}{A \times T} \quad (1)$$

where IMCF is insect-mediated contaminant flux (ng/m<sup>2</sup>/d or ng/m/d),  $\bar{C}_{\text{adult}}$  is the mean adult aquatic insect contaminant concentration (ng/mg),  $B_{\text{total}}$  is the total emergent biomass (mg),  $A$  is surface area (m<sup>2</sup>) or linear area from which the biomass is emerging (meters of shoreline; see Walters et al., 2020), and  $T$  is time (days). To assess the concentration at which maximum flux (IMCF<sub>max</sub>) occurs, it is necessary to examine Equation 1 as a function of an environmental contaminant concentration (e.g., water, sediment, or dietary concentration), denoted hereafter as  $C_{\text{env}}$ . Neither  $A$  nor  $T$  are affected by  $C_{\text{env}}$ , so only the contaminant effect on  $\bar{C}_{\text{adult}}$  and  $B_{\text{total}}$  must be considered. When these variables are measured directly, calculating IMCF is straightforward. When direct measurements are unavailable, surrogate measures (most often larval endpoints) may be used instead. Below, we examine the ways in which  $\bar{C}_{\text{adult}}$  and  $B_{\text{total}}$  may be calculated and then expressed as a function of  $C_{\text{env}}$ . We conclude with a mathematical representation of our hypothesis which will be evaluated in subsequent sections.

### $\bar{C}_{\text{adult}}$

The measurement of contaminant concentrations in adult aquatic insects is the most direct approach for calculating  $\bar{C}_{\text{adult}}$  and allows for direct estimation of IMCF. However, these data are somewhat uncommon (Schmidt et al., 2013) because measuring contaminant concentrations in adult aquatic insects can be difficult, depending on the analytical constraints of the target contaminant (e.g., cost of analysis, biomass requirements). Merolimnic insects can metabolize contaminants and lose mass during metamorphosis, further compounding these analytical challenges. For these reasons, among others, larval aquatic insects are often measured preferentially as a surrogate.

When only larval concentrations are available,  $\bar{C}_{\text{adult}}$  can be calculated using

$$\bar{C}_{\text{adult}} = \bar{C}_{\text{larval}} \times M_c \quad (2)$$

where  $\bar{C}_{\text{larval}}$  is the average larval aquatic insect contaminant concentration (nanogram per milligram) and  $M_c$  is the metamorphic contaminant retention ratio, which represents

$$M_c = \bar{C}_{\text{adult}} / \bar{C}_{\text{larval}} \quad (3)$$

$M_c$  accounts for the change in contaminant concentration that may occur during metamorphosis and must be either assumed or referenced from the literature (if  $\bar{C}_{\text{adult}}$  is known,  $M_c$  is not

needed). However, the degree to which contaminants are retained in tissues or depurated, through either exuviae or the meconium, is contaminant-dependent, and for many contaminants these data are sparse, if available (see Bundschuh et al., 2022; Kraus et al., 2014).

Once  $\bar{C}_{adult}$  is calculated, it must be expressed as a function of  $C_{env}$ , using either adult or larval endpoints. As will be demonstrated in subsequent sections, the nature of this relationship (e.g., linear, exponential) will vary based on the contaminant and organism in question. For simplicity, the generalized equation

$$\bar{C}_{adult} = f_{adult}(C_{env}) \quad (4)$$

or

$$\bar{C}_{larval} = f_{larval}(C_{env}) \quad (5)$$

is used, where  $\bar{C}_{adult}$  and  $\bar{C}_{larval}$  are some functions (denoted  $f_{adult}$  and  $f_{larval}$ , respectively) of  $C_{env}$ .

### $B_{total}$

Emergent biomass is the total mass of adult aquatic insects completing emergence. Measurements of biomass are simpler and less costly than measuring contaminant concentration and are limited by collection time, processing, and scale sensitivity. Like contaminant concentration, direct measurements of emergent biomass allow for direct flux calculations. Unlike contaminant concentration, emergent biomass is not the focus of most ecotoxicological studies and is often unpublished.

Emergent biomass measurements may take the form of a sum total or a calculation based on mean individual mass,

$$B_{total} = \Sigma(B_{adult}) \quad (6)$$

or

$$B_{total} = \bar{B}_{adult} \times n_{adult} \quad (7)$$

respectively, where  $B_{adult}$  is the individual adult aquatic insect mass (milligrams),  $\bar{B}_{adult}$  is the mean individual adult aquatic insect mass (milligrams), and  $n_{adult}$  is the number of adult aquatic insects.

In instances where only larval aquatic insect biomass is measured,  $B_{total}$  can be calculated using the formula

$$B_{total} = \Sigma(B_{larval}) \times M_b \times S \quad (8)$$

where  $\Sigma(B_{larval})$  is the summation of individual larval insect masses (milligrams),  $M_b$  is the metamorphic biomass ratio, and  $S$  is the percentage of emergence. The metamorphic biomass ratio ( $M_b$ ) accounts for the mass retained by adult aquatic insects after the completion of metamorphosis and represents

$$M_b = \bar{B}_{adult} / \bar{B}_{larval} \quad (9)$$

where  $\bar{B}_{larval}$  is the mean mass of an individual larval aquatic insect (milligrams). Like  $M_c$ ,  $M_b$  is only needed when  $B_{adult}$  is

unavailable and therefore must be either assumed or referenced from the literature.  $S$  is the fraction of merolimnic insects that complete metamorphosis to reach adulthood and is calculated as

$$S = n_{adult} / n_{total} \quad (10)$$

where  $n_{total}$  is the total number of organisms in the system.  $S$  accounts for the lethal effects that occur from environmental conditions which may or may not include the contaminant of interest. If only the mean individual larval biomass (i.e.,  $\bar{B}_{larval}$ ) and total number of insects is measured, Equation 7 is easily adjusted to become

$$B_{total} = \bar{B}_{larval} \times M_b \times S \times n_{total} \quad (11)$$

Expressing  $B_{total}$  as a function of  $C_{env}$  is complicated by the effect of contaminants on emergent biomass. It is well appreciated that, for select contaminants of concern, increasing environmental contamination will lead to insect mortality, decreasing the number of individuals capable of emergence (Wesner et al., 2014). Less appreciated is the complex relationship between contaminant exposure and growth. Growth is often described as a modulator of contaminant concentration in individuals (i.e., growth dilution; Schiesari et al., 2018). Conversely, increasing contaminant exposure may reduce growth because of the energetic cost of contaminant sequestration and depuration, ultimately leading to smaller individuals. To account for both growth and total mortality, insect mass ( $B_{total}$ ,  $\bar{B}_{adult}$ ,  $B_{larval}$ ,  $\bar{B}_{larval}$ ) and survival ( $S$ ) are treated as a function of  $C_{env}$ ,

$$B_{total} = g_{adult}(C_{env}) \quad (12)$$

$$\bar{B}_{adult} = j_{adult}(C_{env}) \quad (13)$$

$$\Sigma B_{larval} = g_{larval}(C_{env}) \quad (14)$$

$$\bar{B}_{larval} = j_{larval}(C_{env}) \quad (15)$$

$$S = s(C_{env}) \quad (16)$$

where  $B_{total}$ ,  $\bar{B}_{adult}$ ,  $B_{larval}$ ,  $\bar{B}_{larval}$ , and  $S$  are functions (denoted  $g_{adult}$ ,  $j_{adult}$ ,  $g_{larval}$ ,  $j_{larval}$ , and  $s$ , respectively) of the environmental concentration,  $C_{env}$ . The exact relationship between these variables will differ by contaminant, organism, and other environmental variables, so only generalized equations are provided. Once these relationships are established,  $B_{total}$  may be calculated by the substitution of Equations 13–16 into Equations 7–11.

### Effective dose for $x\%$ of the population

Once calculated, the concentration at which  $IMCF_{max}$  occurs may be used to evaluate our hypothesis. This is achieved by comparing  $IMCF_{max}$  to the highest concentration where no effect on emergence is observed. Mathematically, the latter term may be approximated by the effective dose (ED) at which 1% of the merolimnic insect population died before

completing metamorphosis (i.e.,  $ED_1 = C_{env}$  at which 99% of organisms emerge). Thus, our hypothesis may be stated as follows:

$$IMCF_{max} \approx ED_1 \quad (17)$$

## CONTAMINANT IDENTIFICATION

Modeling data sets were obtained from peer-reviewed articles identified via the US Environmental Protection Agency's (2022) ECOTOX database. We searched aquatic data records using the "All Effects" search tab and the search terms *emerg* and *metamorph* to ensure that substrings of each respective term would be retrieved. Queries were limited to include only the predefined species group "Insects/Spiders" and included "All Chemicals," "All Endpoints," "All Test Conditions," and "All Publication Options." These searches produced 80 references, of which 26 were removed from consideration: One was a duplicate, eight had not undergone peer review, 10 did not measure effects on merolimnic insects (e.g., tadpoles, fish, and brine shrimp), and seven tested the effectiveness of an insect growth regulator that inhibits mosquito emergence (hexaflumuron).

The remaining 54 candidate publications included compounds from eight contaminant classes (Supporting Information, Figure S1): metals (21), neonicotinoids (29), organochlorine pesticides (5), perchlorates (1), per- and polyfluoroalkyl substances (9), pharmaceuticals (7), polycyclic aromatic hydrocarbons (3), and phthalates (2; Supporting Information, Figure S1). Because single manuscripts often considered multiple contaminants (e.g., multiple neonicotinoids assessed in one study), there were more contaminants (77) than publications (54).

We reviewed each of the 54 publications to determine suitability for modeling IMCF. Our criteria for inclusion were that the publications (1) presented contaminant effects data as a dose–response relationship or in such a way that dose response could be meaningfully interpreted and (2) provided tissue concentration data alongside effects data. Of the 54 studies considered, only four met these criteria: Sildanchandra and Crane (2000; cadmium), Stewart and Thompson (1995; fluoranthene), Conley et al. (2011; selenium), and Conley et al. (2013; selenium). The latter two studies shared similar experimental conditions and designs, so only Conley et al. (2011) is presented.

All modeling was completed in R (Ver 3.6.2); dose–response fitting was accomplished using the package "drc" (Ritz et al., 2015); model visualization was accomplished using the package "ggplot2" (Wickham, 2016). Contaminant-specific assumptions and/or mathematical considerations are discussed in each respective section.

## IMCF MODEL CASE STUDIES

In the subsequent sections, each contaminant data set is assessed in turn. First, a brief description of the data set and modeling assumptions is presented. Second, all relevant model

parameters are generated, and IMCF is calculated. Finally, the calculated  $IMCF_{max}$  is compared with the  $ED_1$  to evaluate Equation 17.

### Selenium

Conley et al. (2011) examined the effect of Se (as selenite) on the mayfly *Centroptilum triangulifer* (now *Neocloeon triangulifer*; Jacobus & Wiersema, 2014) in a periphyton feeding study. Several toxicologically relevant endpoints were measured over varying periphyton concentrations (micrograms per gram dry wt), including emergent biomass (micrograms dry wt), effects on emergence (percentage) as total mortality, and Se concentration in adult mayflies (micrograms dry wt). Because concentrations of Se in periphyton and in the dissolved phase exhibited a strong linear relationship (adjusted  $R^2 = 0.90$ ,  $p < 0.05$ ,  $F = 126.1$ ,  $df = 1.13$ ), periphyton Se concentration was used as  $C_{env}$  to calculate IMCF. Because both  $\bar{C}_{adult}$  and  $B_{total}$  were presented, the IMCF calculations were relatively simple following Equation 1, and no assumptions were needed. All replicates adhered to standardized experimental designs such that A and T were constant across all conditions; values were normalized for convenience (i.e.,  $A \times T = 1 \text{ m}^2 \text{ d}$ ).

#### Se– $\bar{C}_{adult}$

$\bar{C}_{adult}$  was calculated using Equation 4, where  $f_{adult}$  was calculated using a simple linear regression of the adult mayfly Se concentration (micrograms per gram dry wt) and periphyton Se concentration ( $C_{env}$ , micrograms per gram dry wt; adjusted  $R^2 = 0.66$ ,  $p < 0.05$ ,  $F = 101.9$ ,  $df = 1.50$ ; Figure 1A, black line; Supporting Information, Figure S2a):

$$\bar{C}_{adult} = f_{adult}(C_{env}) = 1.9853C_{env} + 6.8129 \quad (18)$$

#### Se– $B_{total}$

$B_{total}$  was calculated using Equation 12, where  $g_{adult}$  was modeled using a simple linear regression of the emergent biomass ( $\mu\text{g}$  dry wt) and periphyton Se concentration ( $\mu\text{g/g}$  dry wt; adjusted  $R^2 = 0.53$ ,  $p < 0.05$ ,  $F = 16.72$ ,  $df = 1.13$ ; Figure 1B, purple line; Supporting Information, Figure S2b)

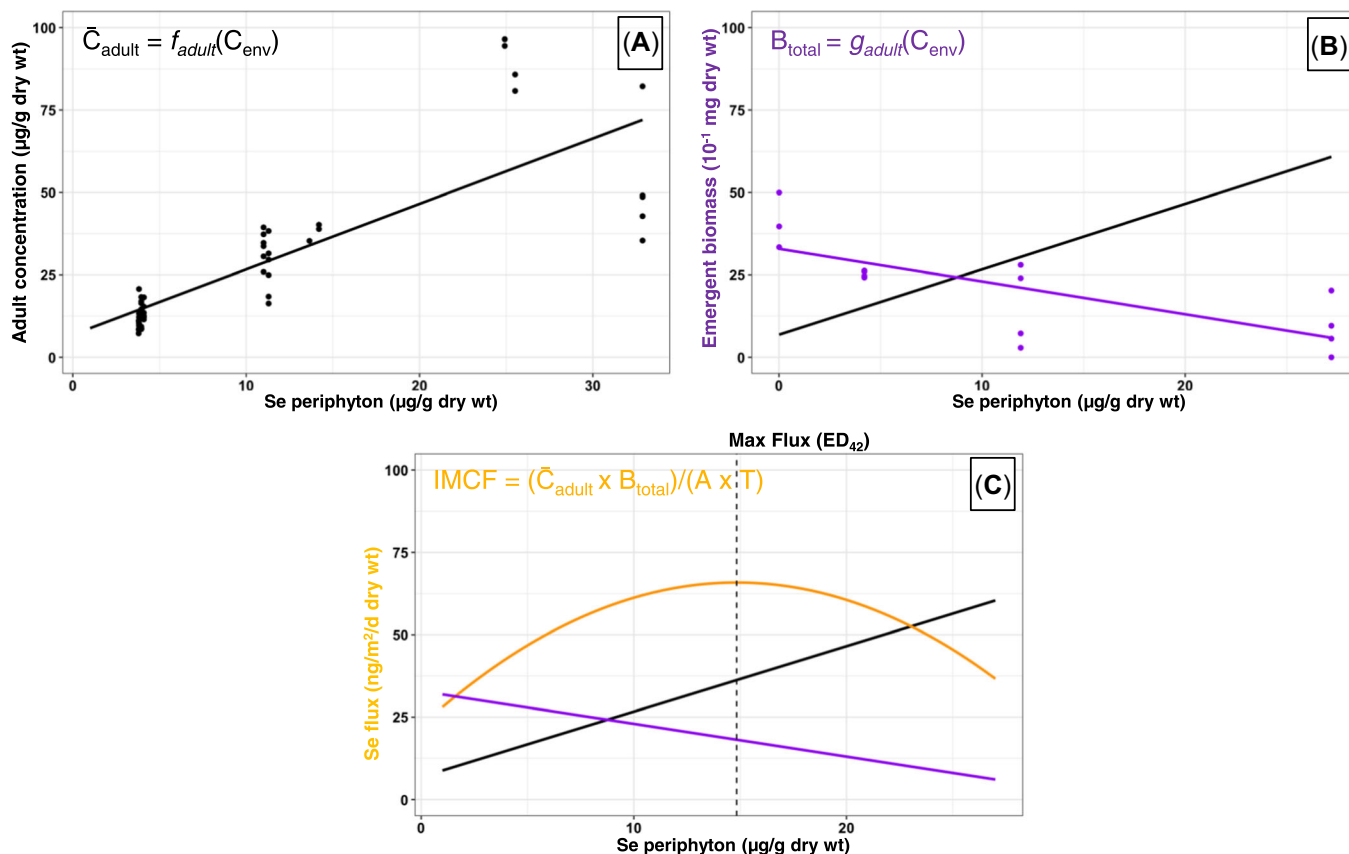
$$B_{total} = g_{adult}(C_{env}) = -99.42C_{env} + 3291.06 \quad (19)$$

#### Se–IMCF

Selenium flux was calculated by substituting Equations 18 and 19 into Equation (1):

$$IMCF = [f_{adult}(C_{env}) \times g_{adult}(C_{env})]/1000 \quad (20)$$

The resultant flux curve (Figure 1C, orange line) demonstrated that the highest Se flux of  $65.86 \text{ ng/m}^2/\text{day}$  occurred at a periphyton Se concentration of  $14.99 \mu\text{g/g}$  ( $ED_{42}$ ), rather than the maximum concentration tested,  $27.2 \mu\text{g/g}$  ( $35.69 \text{ ng/m}^2/\text{day}$ ). This was driven by decreasing emergent biomass, though whether the loss of biomass was due to mortality, growth effects, or both was not discernable.



**FIGURE 1:** Select insect-mediated contaminant flux model parameters and output. (A) Adult mayfly Se concentration (µg/g dry wt); (B) total emergent biomass (10<sup>-1</sup> mg dry wt); (C) Se flux over varying periphyton Se concentrations (ng/m<sup>2</sup>/d dry wt). Labeling on the y-axis corresponds to the newest line added in subsequent panels. Periphyton Se concentration correlates linearly with dissolved Se (as selenite). All data (points) are from Conley et al. (2011).  $\bar{C}_{adult}$  = mean adult concentration;  $f_{adult}$  = function of  $C_{env}$ ;  $C_{env}$  = environmental concentration;  $B_{total}$  = total emergent biomass;  $g_{adult}$  = function of  $C_{env}$ ;  $ED_{42}$  = effective dose where 42% of the population dies prior to emergence; IMCF = insect-mediated contaminant flux; A = surface area; T = time.

## Se—Effects on emergence

Effects on emergence were presented in Conley et al. (2011) and measured as total mortality. The Se dose–response curve for emergence was modeled using simple linear regression (adjusted  $R^2 = 0.40$ ,  $p < 0.05$ ,  $F = 10.2$ ,  $df = 1.13$ ; Supporting Information, Figure S2c)

$$S = s(C_{env}) = -1.6698 C_{env} + 59.6304 \quad (21)$$

Equation 21 results in an  $ED_1$  of 0.36 µg/g dry weight, much less than the observed  $IMCF_{max}$  of 14.99 µg/g dry weight ( $ED_{42}$ ;

**TABLE 1:** Summary of relevant concentrations for the Se, fluoranthene, and Cd case studies

Contaminant	$ED_1$	$IMCF_{max}$	Maximum $C_{env}$
Se (µg/g dry wt)	0.36	14.99 ( $ED_{42}$ )	27.2
Fluoranthene (mg/kg dry wt)	45.50	83.54 ( $ED_{21}$ )	222.0
Cd (mg/L)	2251.52	2307.86 ( $ED_1$ )	25 600

$ED_1$  is the effective dose at which 99% of organisms emerge.  $IMCF_{max}$  is the concentration where insect-mediated contaminant flux is highest;  $[ED_x]$  is the percent of emergence inhibited at  $IMCF_{max}$ . Maximum  $C_{env}$  is the highest concentration tested.  $ED_x$  = effective dose for x%;  $IMCF_{max}$  = maximum insect-mediated contaminant flux;  $C_{env}$  = environmental concentration.

Table 1; Supporting Information, Table S1). In this case  $IMCF_{max}$  did not occur at the first instance of inhibited emergence because emergent biomass loss associated with toxicity was offset by the higher contaminant concentrations in surviving adult insects. Thus, the Conley et al. (2011) model only partially matched our hypothesis;  $IMCF_{max}$  did not occur at the highest environmental concentration but did occur after the first instance of decreased emergence.

## Fluoranthene

Stewart and Thompson (1995) measured the effect of fluoranthene on the nonbiting midge *Chironomus riparius*. The data presented include nominal sediment fluoranthene concentration (milligrams per kilogram dry wt), adult midge concentration (milligrams per kilogram dry wt), and effects on emergence (percentage) as total mortality. No data on the mass of adult insects were presented, and no effects of growth were noted. Therefore, for modeling purposes, we assumed that individual body mass was unaffected by fluoranthene in that study and that all adult test organisms were 1 mg dry weight in size,  $j_{adult}(C_{env}) = 1$  mg. No other assumptions were necessary for the calculation. Because test conditions were



standardized, A and T were held constant (i.e.,  $A \times T = 1 \text{ m}^2$  day) throughout calculations.

### Fluoranthene– $\bar{C}_{adult}$

$\bar{C}_{adult}$  was calculated using Equation 4, where the parameters for  $f_{adult}$  were provided in Table 1 of Stewart and Thompson (1995);  $f_{adult}$  relates the log-normalized adult fluoranthene concentration (milligrams per kilogram dry wt) and the log-normalized nominal sediment concentration ( $C_{env}$ , mg/kg dry wt; adjusted  $R^2 = 0.98$ ,  $p < 0.05$ ,  $F = 197.6$ ,  $df = 1.3$ ; Figure 2A, black line; Supporting Information, Figure S3a)

$$\bar{C}_{adult} = f_{adult}(C_{env}) = 10^{0.1608 + 1.16232 \log_{10}(C_{env})} \quad (22)$$

### Fluoranthene– $B_{total}$

The value of  $B_{total}$  was calculated by substituting Equation 13 into Equation 7 (Figure 2B, purple line; Supporting Information, Figure S3b):

$$B_{total} = j_{adult}(C_{env}) \times n_{adult} \quad (23)$$

Growth effects were not tested, nor were mass values reported, so mass was assumed across all concentrations:

$$j_{adult}(C_{env}) = 1 \text{ mg} \quad (24)$$

The number of adults was calculated as

$$n_{adult} = n_{total} \times S \quad (25)$$

where  $n_{total}$  was reported (60) and survival was modeled using the three-parameter log-logistic function:

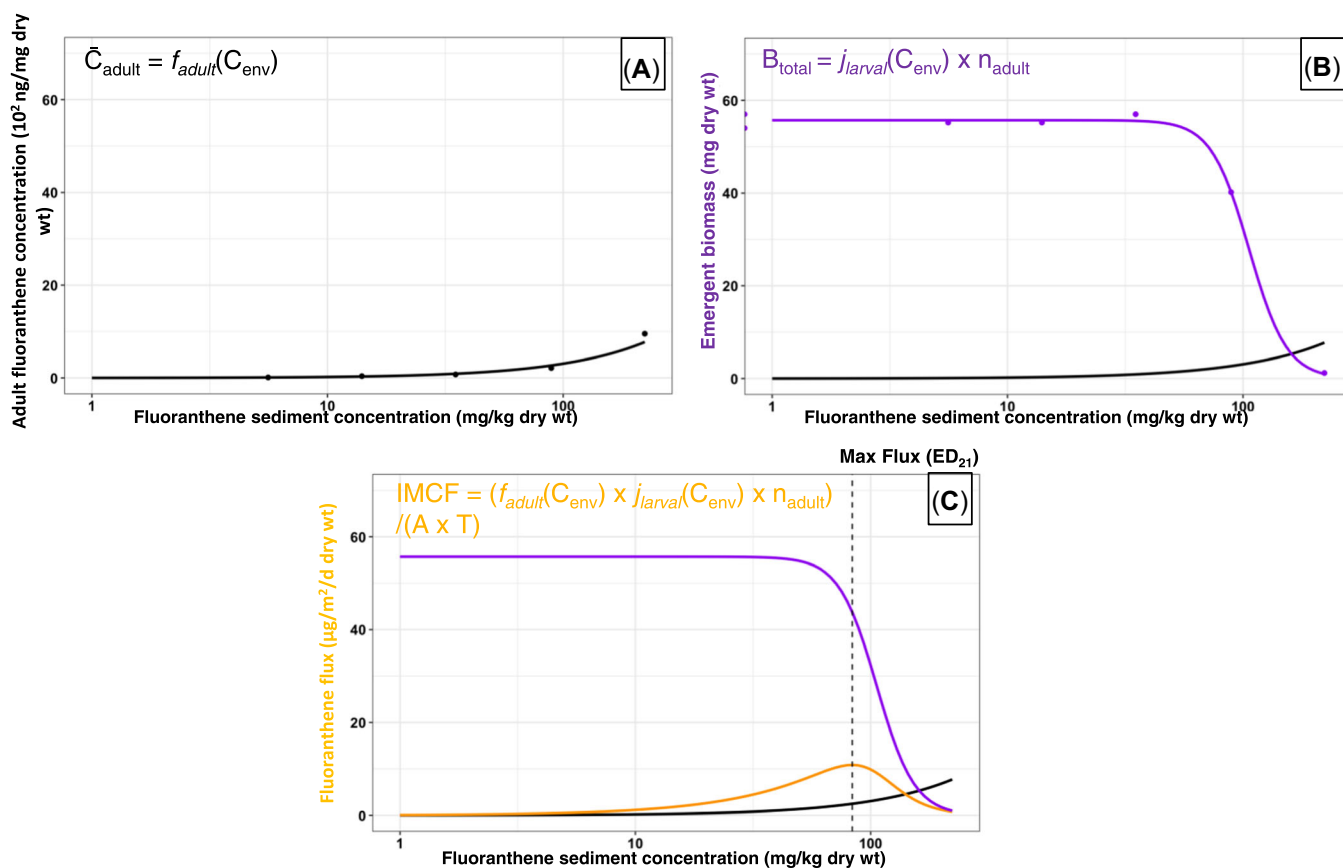
$$S = s(C_{env}) = 0 + (0.9284 - 0) / (1 + e^{5.424[\ln(C_{env}) - \ln(106.1536)]}) \quad (26)$$

### Fluoranthene–IMCF

By substituting Equations 22 and 23 into Equation 1, flux was calculated as follows:

$$\text{IMCF} = [f_{adult}(C_{env}) \times j_{adult}(C_{env}) \times n_{adult}] / 1000 \quad (27)$$

The calculated flux function (Figure 2C, orange line) had a maximum of value of  $10.86 \mu\text{g}/\text{m}^2/\text{day}$  ( $\text{ED}_{21}$ ) occurring at a sediment concentration of  $83.54 \text{ mg}/\text{kg}$  dry weight, which fell between the  $\text{ED}_1$  ( $45.5 \text{ mg}/\text{kg}$ ) and the maximum concentration ( $222 \text{ mg}/\text{kg}$ ; Table 1; Supporting Information, Table S2). Maximum flux did not occur at the highest concentration per our hypothesis, nor at the first instance of depressed



**FIGURE 2:** Select insect-mediated contaminant flux model parameters and output. (A) Adult chironomid fluoranthene concentration ( $10^2 \text{ ng}/\text{mg}$  dry wt); (B) total emergent biomass (mg dry wt); (C) fluoranthene flux over varying sediment concentrations ( $\mu\text{g}/\text{m}^2/\text{d}$  dry wt). Labeling on the y-axis corresponds to the newest line added in subsequent panels. All data (points) are from Stewart and Thompson (1995).  $\bar{C}_{adult}$  = adult concentration;  $f_{adult}$  = function of  $C_{env}$ ;  $C_{env}$  = environmental concentration;  $B_{total}$  = total emergent biomass;  $j_{larval}$  = function of  $C_{env}$ ;  $n_{adult}$  = number of adults;  $\text{ED}_{21}$  = effective dose where 21% of the population dies prior to emergence; IMCF = insect-mediated contaminant flux; A = surface area; T = time.

emergence (i.e., ED<sub>1</sub>). As was the case for Se, higher concentrations of fluoranthene in surviving insects offset lower total emergent biomass resulting from insect mortality caused by fluoranthene toxicity. Our model based on the Stewart and Thompson (1995) data only partially aligned with our hypothesis.

## Cadmium

Sildanchandra and Crane (2000) examined the effect of Cd on *Chironomus riparius*. Sufficient data were presented to develop an IMCF model with minimal assumptions. Data reported included nominal Cd doses (milligrams per liter), adult Cd concentration (micrograms per kilogram dry wt), larval mortality (10-d mortality [number of animals]), and effects on emergence (number of animals) as total mortality. Insect contaminant concentration was converted to wet weight using a dry weight-to-wet weight ratio of 0.2 for modeling purposes. A value of 0.1 was added to all Cd concentrations prior to normalization to allow for log transformation. Relative IMCF flux was not affected because these conversions were applied to all values.

In addition, Sildanchandra and Crane (2000) provided an empirical relationship between nominal Cd doses (milligrams per liter) and the individual larval aquatic insect mass. No

information was provided on the mass of adult aquatic insects, so  $M_b$  was assigned a value of 1 (i.e., no mass was lost during metamorphosis). The  $M_b$  value was arbitrary and did not affect relative IMCF calculations, assuming the amount of mass loss through metamorphosis was not a function of environmental concentration:  $M_b \neq f(C_{env})$ . Finally, because test conditions standardized area and time, both A and T were constant throughout the calculations (i.e.,  $A \times T = 1 \text{ m}^2 \text{ day}$ ).

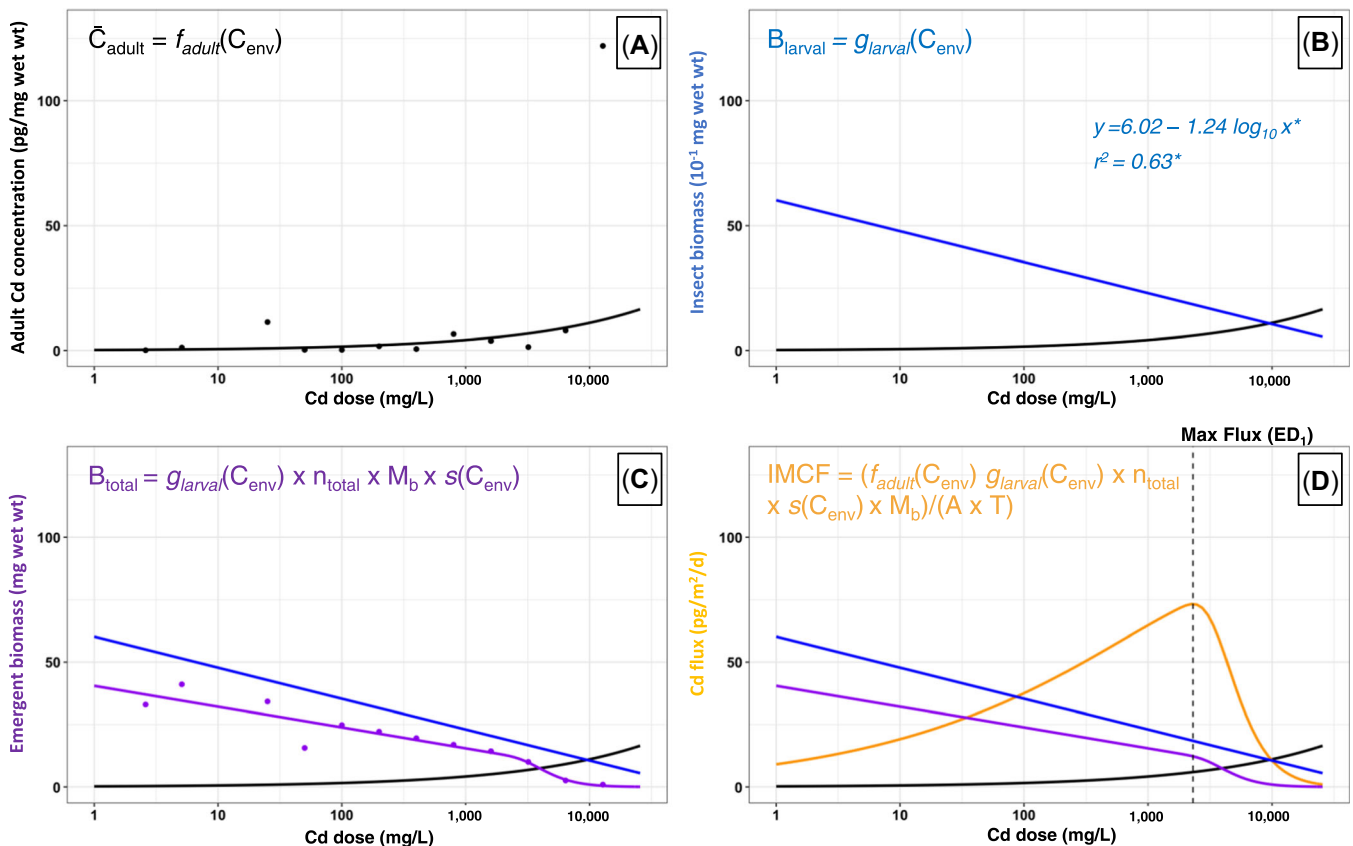
### Cd– $\bar{C}_{adult}$

$\bar{C}_{adult}$  was calculated using Equation 4, where  $f_{adult}$  was calculated using a simple linear regression from the log-normalized reported concentrations of Cd in adult aquatic insects ( $\mu\text{g}/\text{kg}$ ) and log-normalized Cd dose ( $\text{mg}/\text{L}$ ;  $p < 0.05$ ; adjusted  $R^2 = 0.56$ ;  $F = 17.47$ ;  $df = 1, 12$ ; Figure 3A, black line; Supporting Information, Figure S4a):

$$\bar{C}_{adult} = f_{adult}(C_{env}) = 10^{-0.6497 + 0.4237 \log_{10}(C_{env} + 0.1)} \quad (28)$$

### Cd– $B_{total}$

The value of  $B_{total}$  was calculated by substituting Equations 15 and 16 into Equation 11,



**FIGURE 3:** Select insect-mediated contaminant flux model parameters and output. (A) Adult chironomid Cd concentration (pg/mg wet wt); (B) average larval biomass ( $10^{-1}$  mg wet wt); (C) total emergent biomass (mg wet wt); (D) Cd flux (pg/m<sup>2</sup>/d) over varying Cd dosages (mg/L). Labeling on the y-axis corresponds to the newest line added. All data (\*including equation and  $r^2$ ) are from Sildanchandra and Crane (2000).  $\bar{C}_{adult}$  = adult concentration;  $f_{adult}$  = function of  $C_{env}$ ;  $C_{env}$  = environmental concentration;  $B_{larval}$  = larval biomass;  $g_{larval}$  = function of  $C_{env}$ ;  $B_{total}$  = total emergent biomass;  $n_{total}$  = total number;  $M_b$  = metamorphic biomass ratio;  $s$  = percentage of emergence; ED<sub>1</sub> = effective dose where 1% of the population dies prior to emergence; IMCF = insect-mediated contaminant flux; A = surface area; T = time.

$$B_{\text{total}} = g_{\text{larval}}(C_{\text{env}}) \times n_{\text{total}} \times M_b \times s(C_{\text{env}}) \quad (29)$$

where  $g_{\text{larval}}(C_{\text{env}})$  was provided as a logarithmic function, with larval aquatic insect mass (milligrams wet wt) decreasing as the Cd dosage increased (Figure 3B, blue line):

$$g_{\text{larval}}(C_{\text{env}}) = 6.02 - 1.24 \log_{10}(C_{\text{env}} + 0.1) \quad (30)$$

and

$$n_{\text{total}} = 10 \quad (31)$$

The value of  $s(C_{\text{env}})$  was calculated by fitting a dose–response curve to the number of emergent adult aquatic insects at the varying Cd doses. A three-parameter Weibull function was used to generate a dose–response equation for these data (Supporting Information, Figure S4b):

$$s(C_{\text{env}}) = 0.6735 \times (1 - e^{-2.326[\ln(C_{\text{env}}) - \ln(4340.8077)]}) \quad (32)$$

The value of  $B_{\text{total}}$  was calculated by substituting Equations 30–32 into Equation 29 (Figure 3C, purple line).

### Cd–IMCF

Finally, flux was calculated by substituting Equations 28 and 29 into Equation 1 (Figure 3D, orange line):

$$\text{IMCF} = [f_{\text{adult}}(C_{\text{env}}) \times g_{\text{larval}}(C_{\text{env}}) \times n_{\text{total}} \times s(C_{\text{env}}) \times M_b] / 1 \quad (33)$$

The lowest flux calculated occurred at the highest Cd dose (25,600 mg/L). The highest value of Cd flux occurred at an environmental concentration of 2307.86 mg/L (73.29 pg/m<sup>2</sup>/day), which roughly coincided with the ED<sub>1</sub> (2251.52 mg/L; Table 1; Supporting Information, Table S3). Thus, the Sildanchandra and Crane (2000) model aligned with our hypothesis that IMCF<sub>max</sub> would occur at the first instance of inhibited emergence.

### Drivers of IMCF<sub>max</sub>

The principal aim of the present study was to evaluate at which environmental concentration IMCF<sub>max</sub> would occur. We hypothesized that rather than occurring at the highest environmental concentration, IMCF<sub>max</sub> would occur at the highest environmental concentration at which no effects on emergence occurred. The case studies modeled above demonstrate that our hypothesis was only partially accurate because IMCF<sub>max</sub> occurred at concentrations exceeding the ED<sub>1</sub> for two of three contaminants (Table 1). This then raises the question “Where does maximum flux occur?”

To answer this question, it is useful to reexamine the flux curves of the three modeled contaminants. In each case, area and time are standardized and are, therefore, irrelevant to IMCF<sub>max</sub>, leaving emergent biomass and adult aquatic insect contaminant concentration as the variables driving flux

magnitude. In cases where these variables are described by a decreasing and increasing function, respectively, maximum flux occurs at the first concentration, where

$$\left| \frac{f'(B_{\text{total}})}{f(B_{\text{total}})} \right| = \left| \frac{f'(C_{\text{adult}})}{f(C_{\text{adult}})} \right| \quad (34)$$

or, put simply, the concentration at which the relative rate of decreasing emergent biomass equals the relative rate of increasing contaminant accumulation and retention (Supporting Information, Figure S5 demonstrates this relationship graphically for fluoranthene). This relationship demonstrates why the IMCF<sub>max</sub> values for Se and fluoranthene did not occur at the ED<sub>1</sub> because the relative rate of declining biomass was more than offset by the increasing rate of contaminant accumulation.

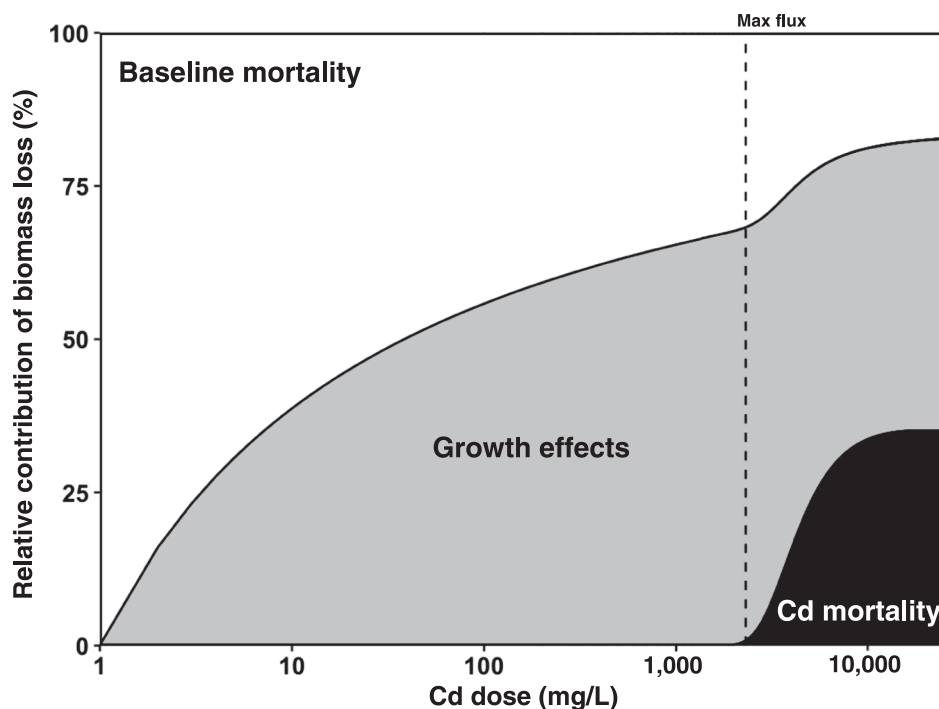
Provided that data are available to estimate the relationships among bioaccumulation, emergent biomass loss, and contaminant concentration for a system of interest, this approach allows for the modeling and prediction of IMCF<sub>max</sub>. It should be noted that the relationship outlined in Equation 34 holds true only if (1) the relative rates of emergent biomass loss and contaminant uptake intersect within the experimental domain and (2) contaminant uptake and emergent biomass are strictly increasing and decreasing functions, respectively. For example, a micronutrient with a U-shaped dose–response curve would require a different formulation for calculating IMCF<sub>max</sub>.

Equation 34 demonstrates that IMCF<sub>max</sub> depends on the competing rates of contaminant uptake and biomass loss. While the processes governing contaminant uptake are beyond the scope of the present study, Sildanchandra and Crane (2000) provide multiple mechanisms contributing to emergent biomass loss including growth effects, baseline mortality (i.e., the y-intercept of the dose–response curve), and mortality associated with increasing Cd concentration. Using the current mathematical approach, each of these drivers may be modeled separately to examine their relative contribution to biomass loss and, thus, decreasing IMCF. Using

$$\text{Percentage of loss} = (1 - \text{ED}_x) / \sum_{i=1}^n (1 - \text{ED}_i) \quad (35)$$

where percentage of loss is the relative contribution of biomass loss (percentage) by a given contaminant effect at a given concentration (ED<sub>x</sub>); the importance of each parameter may be examined for the entire range of Cd concentrations modeled from Sildanchandra and Crane (2000; Figure 4). At lower concentrations of Cd, baseline mortality was more responsible for overall biomass loss than the Cd-related effects on growth or mortality. The effects of Cd on growth surpassed baseline mortality as the main factor leading to biomass loss as environmental concentrations of Cd increased. Notably, growth effects were a stronger driver of biomass loss than Cd-related mortality for all environmental concentrations tested, including the concentration at which maximum IMCF occurs. Alternatively stated, at the concentration of maximum IMCF, inhibited growth was a stronger driver of biomass loss than mortality.





**FIGURE 4:** Relative contribution of individual toxic effects on biomass loss: baseline mortality (white), growth effects (gray), Cd mortality (black). Dashed line indicates maximum insect-mediated contaminant flux. All data are from Sildanchandra and Crane (2000).

## IMPLICATIONS

The mathematical framework presented was developed to answer a relatively narrow question (“At which concentration does maximum contaminant flux occur?”), yet our findings have important implications for ecotoxicological studies and risk management of toxic chemicals in the environment. A growing body of literature now concerns itself with risks faced by terrestrial organisms to aquatic contaminant biotransport, and therefore, it is critical to understand drivers of this exposure (Beaubien et al., 2020; Walters et al., 2010). Although the IMCF model cannot predict exposure, which is predicated on factors like dietary selection and dispersion, the model can help identify areas of potential concern. For example, Otter et al. (2020) highlighted the importance of sampling location in site management because maximum IMCF may not occur at areas of high environmental concentrations (e.g., sediment “hotspots”). The results of the present work echo this finding because all of the modeled fluxes reached their zenith at intermediate concentrations. Future development of the IMCF model to include additional considerations, such as temporal variations in emergence, will enable more informed decision-making when targeting sites of maximum flux. As more data become available, application of the IMCF model will provide greater insight into the variability of IMCF drivers and the importance of contaminant class and community structure on flux. Finally, although the IMCF model was developed chiefly to examine contaminant fluxes, the mathematical framework developed in the present study would be easily amended to investigate the effect of aquatic contaminants on the insect-mediated transfer of nutrients, such as fatty acids (Pietz et al., 2023).

Despite the few studies available for analysis, the IMCF model has already demonstrated its utility by highlighting the potential importance of sublethal contaminant effects on IMCF. Toxicological studies often emphasize lethal effects of contaminants rather than sublethal effects, which include decreased growth, changes in the timing of emergence, and lower fecundity (Lee et al., 2016; Richmond et al., 2016; Schuler et al., 2007; Sildanchandra & Crane, 2000). This is likely because animal death is considered more impactful to contaminant biotransport than other effects of contaminant exposure; however, sublethal contaminant effects could have important implications for IMCF and subsequent risk for riparian insectivores. We found that in the case of Cd exposure sublethal, negative effects on growth had a stronger influence on  $IMCF_{max}$  than did Cd-related mortality. Whether this observation occurs for other contaminants or in natural rather than laboratory settings is unresolved, given the paucity of available data.

The data requirements for the IMCF model are not inherently aligned with those of the ECOTOX database, so it is perhaps not surprising that of >50 000 references, only four suited the needs of this modeling effort. Critically, many of the candidate studies were unusable because they did not report accumulation data alongside effects on adult insect emergence. Similarly, few studies quantified growth effects. Given that these endpoints are relatively easy to measure and can provide added insight into contaminant effects, we encourage their consideration in future toxicological studies. Further, the use of any published data is predicated on it being available and accessible. The present study serves as an example of the importance of data sharing and database curation in tandem with the publication of results in journal articles and reports.

## CONCLUSION

To fully understand the movement of contaminants through ecosystems, it is necessary to expand the focus of toxicological studies beyond traditional metrics like contaminant concentration and mortality to include factors that govern the accrual and loss of organismal biomass. The latter has traditionally fallen more within the realm of ecologists than toxicologists, and the challenge is now to integrate the efforts of these two disciplines. In our study, we have shown that the concentration where maximum IMCF occurs depends on competing rates of biomass loss and contaminant accumulation and does not necessarily occur at the highest instance of emergent biomass or insect contaminant concentration. Further, we have demonstrated that sublethal contaminant effects on insect biomass can be an important and potentially underappreciated control on IMCF.

**Supporting Information**—The Supporting Information is available on the Wiley Online Library at <https://10.1002/etc.5574>.

**Acknowledgments**—We thank J. Conley for providing raw data needed for modeling. We thank J. Gibbons, R. Yeardley, and D. Timberlake for their critical review. The present study was partially funded by the US Environmental Protection Agency's Great Lakes Restoration Initiative grant (to D. M. Walters) and through the US Geological Survey's Environmental Health Program.

**Disclaimer**—The present study was subjected to US Environmental Protection Agency and US Geological Survey review and approved for publication. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US government.

**Author Contributions Statement**—**Connor I. Olson**: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Software; Supervision; Validation; Visualization; Writing—original draft; Writing—review & editing. **Gale B. Beaubien**: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Supervision; Validation; Visualization; Writing—original draft; Writing—review & editing. **Ryan R. Otter**: Conceptualization; Data curation; Investigation; Methodology; Visualization; Writing—review & editing. **David Walters**: Conceptualization; Funding acquisition; Investigation; Methodology; Visualization; Writing—review & editing. **Marc Mills**: Conceptualization; Investigation; Methodology; Supervision; Visualization; Writing—review & editing.

**Data Availability Statement**—All data analyzed in the present study were acquired from previously published work. These data may be accessed via the cited publications and/or through correspondence with the publications' authors. All mathematical approaches used in the present study are described in the *IMCF Model Basis* section and are available from the corresponding author ([connor.i.olson@gmail.com](mailto:connor.i.olson@gmail.com)).

## REFERENCES

- Beaubien, G. B., Olson, C. I., Todd, A. C., & Otter, R. R. (2020). The spider exposure pathway and the potential risk to arachnivoracious birds. *Environmental Toxicology and Chemistry*, 39(11), 2314–2324. <https://doi.org/10.1002/etc.4848>
- Bundschuh, M., Englert, D., Rosenfeldt, R. R., Bundschuh, R., Feckler, A., Lüderwald, S., Seitz, F., Zubrod, J. P., & Schulz, R. (2019). Nanoparticles transported from aquatic to terrestrial ecosystems via emerging aquatic insects compromise subsidy quality. *Scientific Reports*, 9(1), Article 15676. <https://doi.org/10.1038/s41598-019-52096-7>
- Bundschuh, M., Pietz, S., Roodt, A. P., & Kraus, J. M. (2022). Contaminant fluxes across ecosystems mediated by aquatic insects. *Current Opinion in Insect Science*, 50, Article 100885. <https://doi.org/10.1016/j.cois.2022.100885>
- Carson, R. (1962). *Silent spring*. Houghton Mifflin.
- Conley, J. M., Funk, D. H., Cariello, N. J., & Buchwalter, D. B. (2011). Food rationing affects dietary selenium bioaccumulation and life cycle performance in the mayfly *Centroptilum triangulifer*. *Ecotoxicology*, 20(8), 1840–1851. <https://doi.org/10.1007/s10646-011-0722-1>
- Conley, J. M., Funk, D. H., Hesterberg, D. H., Hsu, L. C., Kan, J., Liu, Y. T., & Buchwalter, D. B. (2013). Bioconcentration and biotransformation of selenite versus selenate exposed periphyton and subsequent toxicity to the mayfly *Centroptilum triangulifer*. *Environmental Science & Technology*, 47(14), 7965–7973. <https://doi.org/10.1021/es400643x>
- Cristol, D. A., Brasso, R. L., Condon, A. M., Fovargue, R. E., Friedman, S. L., Hallinger, K. K., Monroe, A. P., & White, A. E. (2008). The movement of aquatic mercury through terrestrial food webs. *Science*, 320(5874), 335. <https://doi.org/10.1126/science.1154082>
- Howie, M. G., Jackson, A. K., & Cristol, D. A. (2018). Spatial extent of mercury contamination in birds and their prey on the floodplain of a contaminated river. *Science of the Total Environment*, 630, 1446–1452. <https://doi.org/10.1016/j.scitotenv.2018.02.272>
- Jacobus, L. M., & Wiersema, N. A. (2014). The genera *Anafroptilum* Kluge, 2011 and *Neocloeon* Traver, 1932, reinstated status, in North America, with remarks about the global composition of *Centroptilum* Eaton, 1869 (Ephemeroptera: Baetidae). *Zootaxa*, 3814(3), 385–391. <https://doi.org/10.11646/zootaxa.3814.3.5>
- Koch, A., Jonsson, M., Yeung, L. W. Y., Kärrman, A., Ahrens, L., Ekblad, A., & Wang, T. (2020). Per- and polyfluoroalkyl-contaminated freshwater impacts adjacent riparian food webs. *Environmental Science & Technology*, 54(19), 11951–11960. <https://doi.org/10.1021/acs.est.0c01640>
- Kraus, J. M., Walters, D. M., Wesner, J. S., Stricker, C. A., Schmidt, T. S., & Zuellig, R. E. (2014). Metamorphosis alters contaminants and chemical tracers in insects: Implications for food webs. *Environmental Science & Technology*, 48(18), 10957–10965. <https://doi.org/10.1021/es502970b>
- Lee, S. S., Paspalof, A. M., Snow, D. D., Richmond, E. K., Rosi-Marshall, E. J., & Kelly, J. J. (2016). Occurrence and potential biological effects of amphetamine on stream communities. *Environmental Science & Technology*, 50(17), 9727–9735. <https://doi.org/10.1021/acs.est.6b03717>
- Menzie, C. A. (1980). Potential significance of insects in the removal of contaminants from aquatic systems. *Water, Air, and Soil Pollution*, 13, 473–479.
- Otter, R. R., Beaubien, G. B., Olson, C. I., Walters, D. M., & Mills, M. A. (2020). Practical considerations for the incorporation of insect-mediated contaminant flux into ecological risk assessments. In *Contaminants and ecological subsidies* (pp. 179–195). Springer. [https://doi.org/10.1007/978-3-030-49480-3\\_9](https://doi.org/10.1007/978-3-030-49480-3_9)
- Pietz, S., Kainz, M. J., Schröder, H., Manfrin, A., Schäfer, R. B., Zubrod, J. P., & Bundschuh, M. (2023). Metal exposure and sex shape the fatty acid profile of midges and reduce the aquatic subsidy to terrestrial food webs. *Environmental Science & Technology*, 57, 951–962. <https://doi.org/10.1021/acs.est.2c05495>
- R: A language and environment for statistical computing (Version 3.6.2) [Computer software]. R Foundation for Statistical Computing.
- Richmond, E. K., Rosi-Marshall, E. J., Lee, S. S., Thompson, R. M., & Grace, M. R. (2016). Antidepressants in stream ecosystems: Influence of selective serotonin reuptake inhibitors (SSRIs) on algal production and insect emergence. *Freshwater Science*, 35(3), 845–855. <https://doi.org/10.1086/687841>

- Ritz, C., Baty, F., Streibig, J. C., & Gerhard, D. (2015). Dose–response analysis using R. *PLOS ONE*, *10*(12), Article e0146021. <https://doi.org/10.1371/journal.pone.0146021>
- Schiesari, L., Leibold, M. A., & Burton, G. A. (2018). Metacommunities, metaecosystems and the environmental fate of chemical contaminants. *Journal of Applied Ecology*, *55*(3), 1553–1563. <https://doi.org/10.1111/1365-2664.13054>
- Schmidt, T. S., Kraus, J. M., Walters, D. M., & Wanty, R. B. (2013). Emergence flux declines disproportionately to larval density along a stream metals gradient. *Environmental Science & Technology*, *47*(15), 8784–8792. <https://doi.org/10.1021/es3051857>
- Schuler, L. J., Landrum, P. F., & Lydy, M. J. (2007). Response spectrum of pentachlorobenzene and fluoranthene for *Chironomus tentans* and *Hyalella azteca*. *Environmental Toxicology and Chemistry*, *26*(6), 1248–1257. <https://doi.org/10.1897/06-535R.1>
- Sildanchandra, W., & Crane, M. (2000). Influence of sexual dimorphism in *Chironomus riparius* Meigen on toxic effects of cadmium. *Environmental Toxicology and Chemistry*, *19*(9), 2309–2313. <https://doi.org/10.1002/etc.5620190921>
- Stewart, K. M., & Thompson, R. S. (1995). Fluoranthene as a model toxicant in sediment studies with *Chironomus riparius*. *Journal of Aquatic Ecosystem Health*, *4*(4), 231–238. <https://doi.org/10.1007/BF00118003>
- US Environmental Protection Agency. (2022). *ECOTOX user guide: ECOTOXicology Knowledgebase System* (Version 5.3). <http://www.epa.gov/ecotox>
- Walters, D. M., Cross, W. F., Kennedy, T. A., Baxter, C. V., Hall, R. O., Jr., & Rosi, E. J. (2020). Food web controls on mercury fluxes and fate in the Colorado River, Grand Canyon. *Science Advances*, *6*(20), Article eaaz4880. <https://doi.org/10.1126/sciadv.aaz4880>
- Walters, D. M., Fritz, K. M., & Otter, R. R. (2008). The dark side of subsidies: Adult stream insects export organic contaminants to riparian predators. *Ecological Applications*, *18*(8), 1835–1841. <https://doi.org/10.1890/08-0354.1>
- Walters, D. M., Mills, M. A., Fritz, K. M., & Raikow, D. F. (2010). Spider-mediated flux of PCBs from contaminated sediments to terrestrial ecosystems and potential risks to arachnivorous birds. *Environmental Science & Technology*, *44*(8), 2849–2856. <https://doi.org/10.1021/es9023139>
- Wesner, J. S., Kraus, J. M., Schmidt, T. S., Walters, D. M., & Clements, W. H. (2014). Metamorphosis enhances the effects of metal exposure on the mayfly, *Centroptilum triangulifer*. *Environmental Science & Technology*, *48*(17), 10415–10422. <https://doi.org/10.1021/es501914y>
- Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag.