

Metamorphosis Alters Contaminants and Chemical Tracers in Insects: Implications for Food Webs

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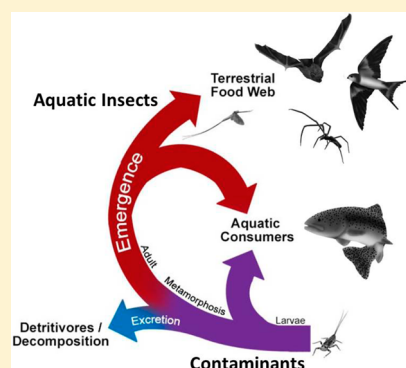
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S Supporting Information

ABSTRACT: Insects are integral to most freshwater and terrestrial food webs, but due to their accumulation of environmental pollutants they are also contaminant vectors that threaten reproduction, development, and survival of consumers. Metamorphosis from larvae to adult can cause large chemical changes in insects, altering contaminant concentrations and fractionation of chemical tracers used to establish contaminant biomagnification in food webs, but no framework exists for predicting and managing these effects. We analyzed data from 39 studies of 68 analytes (stable isotopes and contaminants), and found that metamorphosis effects varied greatly. $\delta^{15}\text{N}$, widely used to estimate relative trophic position in biomagnification studies, was enriched by $\sim 1\%$ during metamorphosis, while $\delta^{13}\text{C}$ used to estimate diet, was similar in larvae and adults. Metals and polycyclic aromatic hydrocarbons (PAHs) were predominantly lost during metamorphosis leading to ~ 2 to 125-fold higher larval concentrations and higher exposure risks for predators of larvae compared to predators of adults. In contrast, manufactured organic contaminants (such as polychlorinated biphenyls) were retained and concentrated in adults, causing up to ~ 3 -fold higher adult concentrations and higher exposure risks to predators of adult insects. Both food web studies and contaminant management and mitigation strategies need to consider how metamorphosis affects the movement of materials between habitats and ecosystems, with special regard for aquatic-terrestrial linkages.



INTRODUCTION

Food webs are linked across ecosystem and habitat boundaries through movements of animals; in particular, insects that emerge from freshwaters can be important vectors of materials from one ecosystem to another.^{1–3} Due to their ubiquity and quality as prey, insects provide a critical resource for consumers in multiple habitats, but they can also spatially propagate contaminants and the effects of contaminants across ecosystem boundaries.^{4–6} The transport of contaminants via animal movement is of increasing environmental concern,^{7–9} but little is known about how metamorphosis, the physiological transition from juvenile to adult, determines the transport and transformation of chemicals and their effects on recipient consumers.

Insects are integral components of most nonmarine food webs, providing large biomass of critical resources to consumers.^{10,11} For example, emerging aquatic insects export ~ 6800 t of carbon from freshwaters annually in one U.S. state (Wisconsin) alone.¹² Because of their ubiquity and importance in food webs, insects can be a predominant exposure pathway to consumers, concentrating contaminants as larvae and during

metamorphosis and exporting them as adults to recipient food webs. For example, aquatic insect adults can export organochlorines from polluted freshwater systems in concentrations high enough to cause physiological and reproductive effects in terrestrial bats, birds, and spiders that prey on them.^{5,13,14} Metamorphosis can also alter the chemical tracers (stable isotopes) used to elucidate food web connections. Interest in these processes has increased dramatically over the last several decades (from 6 papers on food webs, insects and contaminants published in the 1960s to 3770 from 2000 to 2014). Although our understanding of how insect metamorphosis affects chemistry has received some attention (50 studies total have measured isotopes or contaminants in both larvae and adults), no synthesis or general predictive framework exists. The impacts of insect metamorphosis on chemical tracers, toxic elements and compounds vary widely. Some contaminants are

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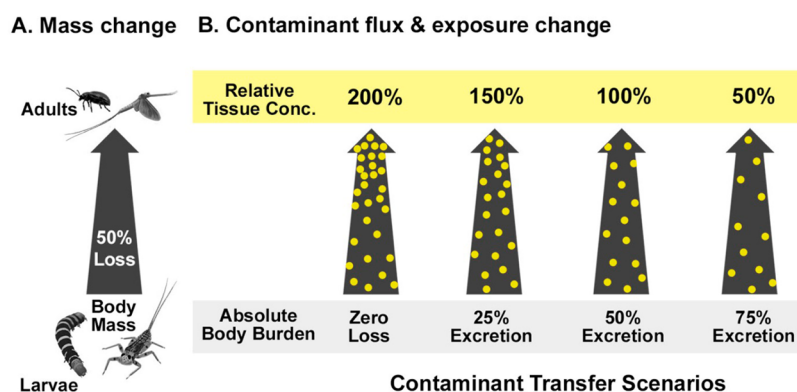


Figure 1. Potential effects of insect metamorphosis on transfer and concentration of contaminants under four excretion scenarios. Insects lose mass during metamorphosis ($\sim 50\%$ on average, Supporting Information (SI) Table S4) (A); thus an increase in concentration can result from either no change or slight loss of body burden, while no change or decrease in concentration can result only from a loss in body burden (i.e., excretion of contaminant mass) (B).

transferred readily from larval to adult stages while others are virtually eliminated, and some insect species concentrate contaminants more than others. Because diet is one of the major pathways of contaminant exposure for wildlife, the effects of insect metamorphosis on contaminants may be key regulators of exposure and contaminant flux to food webs.^{12,15,16}

Metamorphosis changes body chemistry in insects by altering protein and lipid content, metabolizing stored resources (no feeding occurs during this period), and breaking-down cellular structures. These changes alter stable isotope signatures as well as contaminant body burdens (contaminant mass/individual) and concentrations (contaminant mass/insect mass, Figure 1;^{17,18}). For example, nitrogen isotopic signatures ($\delta^{15}\text{N}$) can become enriched as a result of protein catabolism during metamorphosis and nitrogen excretion after adult eclosion (ref 18, i.e., the insect is essentially eating itself). Given that $\delta^{15}\text{N}$ is widely used to estimate trophic level in bioaccumulation studies,¹⁹ metamorphosis could contribute error in trophic position assignment and lead to erroneous conclusions about the pathways by which contaminants accumulate in food webs. For contaminants, metal-containing granules formed in lysosomes to detoxify metals in larvae are excreted by adults into the gut lumen during metamorphosis, along with large proportions of metal body burdens.^{20,21} In contrast, some organochlorines (e.g., PCBs) are mostly retained within the insect body during metamorphosis, leading to increased concentrations in adults, which are smaller in mass than larvae.^{5,22} We conducted a comprehensive literature review to develop a unified model of the underlying mechanisms driving changes in contaminants and stable isotopes via metamorphosis. Understanding these differences is key to predicting the cross life-stage and habitat propagation of contaminant effects.

To understand the effects of insect metamorphosis on a range of contaminants and isotopic signatures, and the impact of these changes on wildlife exposure, we applied meta-analysis to studies that measured the paired chemistry of larval and adult insects ($N = 39$ studies, 382 observations). Our analysis considers the effects of taxonomy, study design, and exposure levels on our findings. We hypothesized that isotopes that are more likely to be fractionated during trophic processes ($\delta^{15}\text{N}$) would also be more likely to fractionate during metamorphosis because of tissue catabolism, thus impacting their use as food web tracers of contaminant biomagnification. Similarly, we

expected contaminants that are regulated by insects and tend not to biomagnify in food webs (e.g., some trace metals,^{20,23}) would more likely be lost in adults, leading to higher exposure risk (i.e., the probability an organism will be exposed to harmful doses of contaminants) for predators of larval insects. In contrast, trophically conserved isotopes, like $\delta^{13}\text{C}$ used to estimate diet, might be less likely to fractionate during metamorphosis. Furthermore, contaminants that are conserved metabolically and retained in food webs²⁴ such as some organochlorines would similarly “persist” across metamorphosis, leading to higher exposure risk for predators of adult insects. This meta-analysis provides the first comprehensive review of the effects of metamorphosis on the chemistry of insects and has broad implications for food web and contaminant research.

MATERIALS AND METHODS

We used Google Scholar to find articles containing information on contaminant concentrations, contaminant body burdens, or stable isotopes for larval and adult insects (search terms provided in the Supporting Information Appendix). Citations of relevant papers and papers citing relevant papers were also searched along with papers or unpublished data known personally to the authors. We identified 50 relevant studies, 39 of which contained sufficient data for meta-analysis (e.g., mean, error, and sample size for both larval and adult insects, SI Appendix;²⁵). Studies contained data on either contaminants or stable isotopes, so we were unable to evaluate interactions between these factors. We defined metamorphosis as the transition from larva to adult (or winged subadult for mayflies), and only included studies that contained data on both life stages. Insects were identified to order for all studies and to species in 36 of 39 studies. Larvae were late instar for 232 observations, but were of unidentified or mixed age for 125 observations. Adults were collected shortly after emerging and before feeding, except for some field studies where adults were captured near larval habitats ($N = 12$ studies and 245 observations, 163 of which were of nonfeeding adults). Our method could obtain an incorrect estimate of effects of metamorphosis on adult chemistry for field-collected individuals if adults immigrated from elsewhere or consumed noncontaminated food; however, no differences in our data could be attributed to these effects (SI Appendix, results from field vs laboratory studies did not differ significantly).

Raw chemistry values were extracted from figures and tables using ImageJ software²⁶ and were collected separately for each study, analyte, exposure level, taxon, and sex. For contaminant studies, only exposure observations were included (i.e., not controls or levels similar to controls). We treated each permutation of these categories within a study independently (see SI Table S1 for number of observations extracted per study). We adopted this approach to identify potential differences in effects of metamorphosis within these categories (e.g., test for differences among analytes, taxa and exposure levels) and to increase power to detect effects.²⁵ Roughly half of the studies that measured contaminant concentrations in adult and larval insect tissues contained data from multiple exposure levels (14 studies of 29), which yielded a total of 90 observations for inclusion in the meta-analysis.

We used meta-analytical mixed models to test our main hypotheses regarding differences in the effects of metamorphosis on (1) change in contaminant concentration and body burden among classes (metals vs organics) and subclasses of contaminants (essential vs nonessential for metals; PCBs, dioxin, pesticides, and PAHs for organics), and (2) fractionation of stable isotopes among elements (MetaWin 2.0,²⁷). The mixed models assume that there is random variation among studies in the effect of interest.²⁵ In meta-analysis, the null hypothesis that all effect sizes are equal are evaluated in comparison to the alternative hypothesis (that at least one of the true effect sizes differ from the rest) using the homogeneity statistic Q .²⁵ Q has an approximately χ^2 -distribution and is partitioned into within-class and between-class homogeneity similar to ANOVA.²⁵ We report the between-class Q (i.e., Q_B), which is a measure of the variation between classes in mean effect size, along with between-class degrees of freedom and total degrees of freedom for the comparison.

We also used linear and curvilinear regression to test for relationships between retention (i.e., persistence) across metamorphosis and physical–chemical properties of individual metals and organic compounds. Specifically, we compared persistence across metamorphosis to two physical–chemical measures that are known to alter the bioactivity of chemicals: (1) ionic softness index for metals ($-\sigma_{\text{CON}}$,²⁸), and (2) hydrophobicity for organics (octanol–water partition coefficient, $\log K_{\text{OW}}$). For metals, the softness index (i.e., [coordinate bond energy of the metal fluoride–coordinate bond energy of the metal iodide]/coordinate bond energy of the metal fluoride) is an ionic characteristic that tends to correlate well with bioactivity of trace elements including protein binding and toxicity in single parameter models.^{29–31} The “softer” ions (the smaller numbers on this scale) are more likely to bioaccumulate in biota,^{31,32} leading to a negative relationship between the softness index and persistence. For organics, K_{OW} generally reflects chemical affinity for lipids in organisms,³³ and for most organic compounds, is positively correlated with bioaccumulation in animals.^{33,34} When $\log K_{\text{OW}} > 5$, K_{OW} becomes an increasingly reliable predictor of the trophic magnification factor for a given compound, which is a potential metric of biomagnification within a food web.³⁴ In general, we predict that persistence of a contaminant across metamorphosis would increase with either increasing softness or increasing hydrophobicity.

For effect sizes, we used standardized mean differences (Hedges' d , [mean of experimental group–mean of control group]/[pooled standard deviation of the control and

experimental groups]) for isotopes because we were interested in absolute differences in isotopic signatures from larva to adult and log response ratios (\ln [adult mean/larval mean]) for contaminants because we were interested in proportional changes.²⁵ We postulated exposure potentials for consumers of adults vs larvae by plotting back-transformed contaminant ratios where adults had higher concentrations of contaminants than larvae (i.e., ratio >1) above the x -axis and plotting the negative inverse of ratios where larvae concentrated contaminants more than adults below the x -axis. We only considered natural abundance-level stable isotope studies on bulk tissue measurements ($N = 9$ studies, 25 observations) because artificial isotopic enrichment ($\delta^{15}\text{N}$ and δD , 1 study each, 8 observations total,^{35,36}) and compound-specific measurements (i.e., individual amino-acids; 1 study, 18 observations,³⁷) were few and varied in isotopic fractionation during metamorphosis. Specifically, the absolute changes in isotopic signature across metamorphosis for both enriched $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in isotopic enrichment studies were much larger and variable than with natural abundance ($\Delta^{15}\text{N} = -52$ to 79‰ and $\Delta^{13}\text{C} = 2$ to 32‰ vs $\sim 1\text{‰}$ and $\sim 0\text{‰}$, respectively,³⁵). Enriched δD showed depletion across metamorphosis ($\Delta\text{D} = -11.5\text{‰}$,³⁶). For compound specific analyses, seven of nine amino acids analyzed were depleted in $\delta^{15}\text{N}$ during metamorphosis in contrast to enrichment of bulk measurements. Most isotope samples were whole body homogenates of individual insects (see SI for details).

We extracted information on other factors that could cause heterogeneity in effect sizes in order to examine the effects of these potential covariates with respect to our primary analyses and as a strategy for handling possible differences in study quality.³⁸ These included taxonomy (order, family, species), contaminant exposure levels (low vs high as reported within studies), experimental design (experiment vs survey, laboratory vs field), metamorphosis type (holo- vs hemimetabolous), larval origin (aquatic vs terrestrial), larval trophic level (herbivore, detritivore, predator), population source (cultured vs natural), sex, and potential for preadaptation to contaminated conditions (previously exposed to contaminant vs naïve populations). The effects of these factors on contaminant accumulation or isotopic enrichment were evaluated one at a time within the meta-analytical framework. Only taxonomy, exposure level, experimental design, and sex had sufficient sample size and resolution for this statistical testing. Limitations and results for other factors are provided in SI Appendix. We further summarized available data from these papers on changes in mass of insects during metamorphosis and mechanisms for loss of contaminant (i.e., where the contaminants went and in what percentages). We ran standard diagnostic tests of publication bias (i.e., bias against publishing nonsignificant results usually based on small samples with low statistical power) funnel plots, Kendall's rank correlation between effect size and variance), which suggested no publication bias in all cases (SI Appendix,³⁸). Very few of the studies explicitly tested the hypothesis of contaminant change across metamorphosis. Instead, most studies simply reported concentrations in adults and larvae as descriptive data, which were ancillary to the main focus of a given paper. This pattern likely contributed to the lack of publication bias.

■ RESULTS

Metamorphosis effects varied greatly among chemical tracers and contaminants (Figure 2, SI Table S2), which may in some

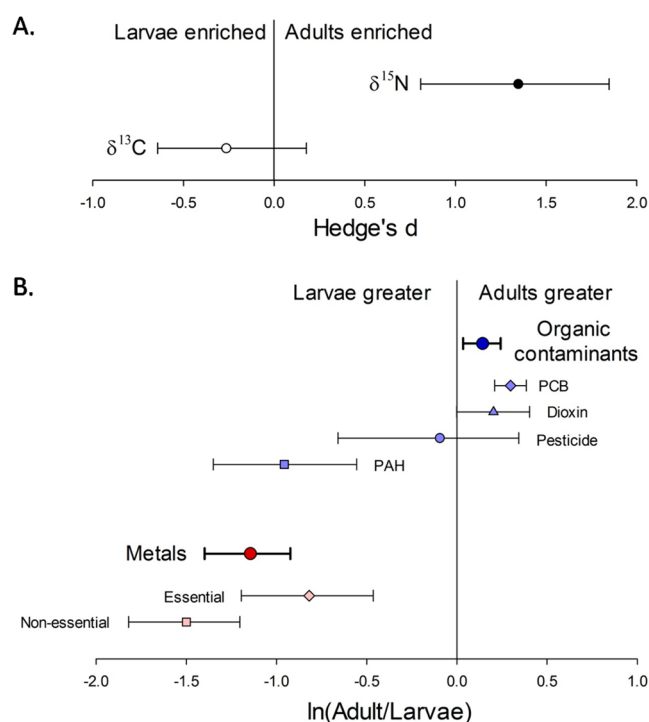


Figure 2. Mean effect size (Hedges' d and \ln response ratio, respectively) of metamorphosis on isotopic signatures (A) and contaminant concentrations (B) of insects. Bars represent 95% confidence intervals. Sample sizes presented in Appendix SI Table S2.

cases profoundly influence our interpretation of food web connections and differences in exposure risk for consumers of larval versus adult insects (Figure 3, and SI Figure S1). $\delta^{15}\text{N}$ fractionated during metamorphosis, becoming enriched in adults ($1.0 \pm 0.3\text{‰}$, mean \pm SE, $N = 15$), while $\delta^{13}\text{C}$ was unchanged ($-0.2 \pm 0.5\text{‰}$, $N = 10$; Figure 2, SI Table S2). Metals were 3.1-fold more concentrated in larvae than adults, while organic contaminants were slightly but significantly more concentrated in adults than larvae (1.2-fold; Figure 2; SI Table S2). For subclasses of contaminants, nonessential metals were more concentrated in larvae compared to adults than essential metals (4.5 vs 2.3-fold higher in larvae than adults; Figure 2, SI Table S2). PAH concentrations were 2.9-fold higher in larvae, dioxins and most pesticides (mostly insecticides) were similarly concentrated in adults and larvae, and PCBs were 1.3-fold higher in adults on average (Figure 2, SI Table S2). Body burden in larvae and adults was only reported for lead, zinc, copper, cadmium, nickel, and mercury, and 39–94% of body burdens for these metals were lost during metamorphosis (SI Figure S2). Information on change in body mass during metamorphosis that would have allowed us to calculate body burden from concentration data was missing from most papers. Isotope fractionation and percent loss of contaminant body burdens during metamorphosis directly calculated by authors of the original papers range from 0.3–1.9‰ for $\delta^{15}\text{N}$, 0–100% for metals (including some reports of increases in contaminant burden) and 0–17% for organics (SI Table S3).

Concentration changes for individual compounds and elements also varied widely (Figures 3, S1). For example, metals and PAHs were generally 2- to 10-fold higher in larvae than adults, with some extreme examples 24- to 127-fold higher (e.g., manganese). In contrast, individual PCBs and pesticides were around 1- to 3-fold higher in adults than larvae. These

differences between larval and adult concentrations in contaminants were predictable to varying degrees according to their physical-chemical properties. Change in concentrations of metals across metamorphosis were related to ion softness: softer metals (i.e., those with a lower softness index) were more likely to be conserved in adults (Figure 4; $R^2 = 0.47$ excluding outlier [Mn, studentized residuals >2.0], $F_{1,12} = 10.85$, $P = 0.006$). For organic compounds, persistence across metamorphosis increased nonlinearly with hydrophobicity ($\log K_{\text{OW}}$) when $\log K_{\text{OW}} > 5.0$ (Figure 4; $R^2 = 0.34$; χ^2 , $F_{1,29} = 8.28$, $P = 0.008$; χ^2 , $F_{1,29} = 7.19$, $P = 0.012$). For organics where $\log K_{\text{OW}} < 5.0$ (mainly PAHs), persistence decreased with this metric of hydrophobicity (which also correlates positively with molecular weight; Figure 4; $R^2 = 0.96$, $F_{1,4} = 100.0$, $P < 0.001$).

Insect larvae exposed to reportedly low levels of metals had similar concentrations in larvae and adults, while larvae exposed to high levels had ~ 4.8 -fold higher metal concentrations than adults (SI Table S2). This suggests that proportionately more metals are lost during metamorphosis as larval exposure levels increase. Changes in metal concentrations during metamorphosis differed significantly by taxonomic order (SI Table S2), apparently driven by variation in mass loss during metamorphosis. For example, three metals (Cu, Cd, and Zn) were the only contaminants studied in more than two taxa. For those metals, moths and dipterans showed increased concentrations in adults (~ 1.5 -fold), wasps showed similar concentrations in adults and larvae and mayflies showed higher concentrations in larvae (~ 2.2 times, mayflies; $Q_{1,50} = 34.8$, $P = 0.001$). These patterns are consistent with moths and dipterans losing much greater mass than mayflies during metamorphosis (SI Table S4).

Despite taxonomic variation and contaminant bias (i.e., which contaminants are studied for which taxa, SI Table S5), the overall patterns of contaminant persistence during metamorphosis reported here are similar within taxonomic groups as well (SI Figure S3). Laboratory and field results did not differ for the 8 of 16 metals studied in both scenarios ($Q_{1,107} = 2.72$, $P = 0.250$). The comparison could be not be made for organic contaminants because of lack of data. Metamorphosis did not have significantly different effects on isotopic fractionation or concentrations of organic contaminants in late instar vs mixed-age larvae, but there were marginal differences for concentrations of metals (SI Appendix). Sex had no effect on metal concentrations through metamorphosis ($Q_{1,46} = 0.00$, $P = 0.99$), but males had significantly higher increases in organic contaminant concentrations than females (1.53 times higher than larvae vs 1.17 times higher, respectively; $Q_{1,108} = 9.34$, $P = 0.004$), likely due to larger losses of body mass by males compared to mixed sex larvae. Insects lost mass during metamorphosis (2–90% of dry or wet mass among taxa, SI Table S3), and exuvia and first adult excrement (meconium) were found to be the major pathways for contaminant excretion (SI Table S3).

DISCUSSION

Metamorphosing insects compose 65% of all animal species,³⁹ and are integral to nearly every nonmarine food web. Metamorphosis is physiologically complex, often resulting in drastic changes in morphology, chemistry, and habitat as insects develop from larvae to adults (e.g., aquatic insects, migrating butterflies). Ecologists have only recently begun to quantitatively incorporate the role of these complex life cycles in linking food webs in different habitats and niches (e.g., aquatic-

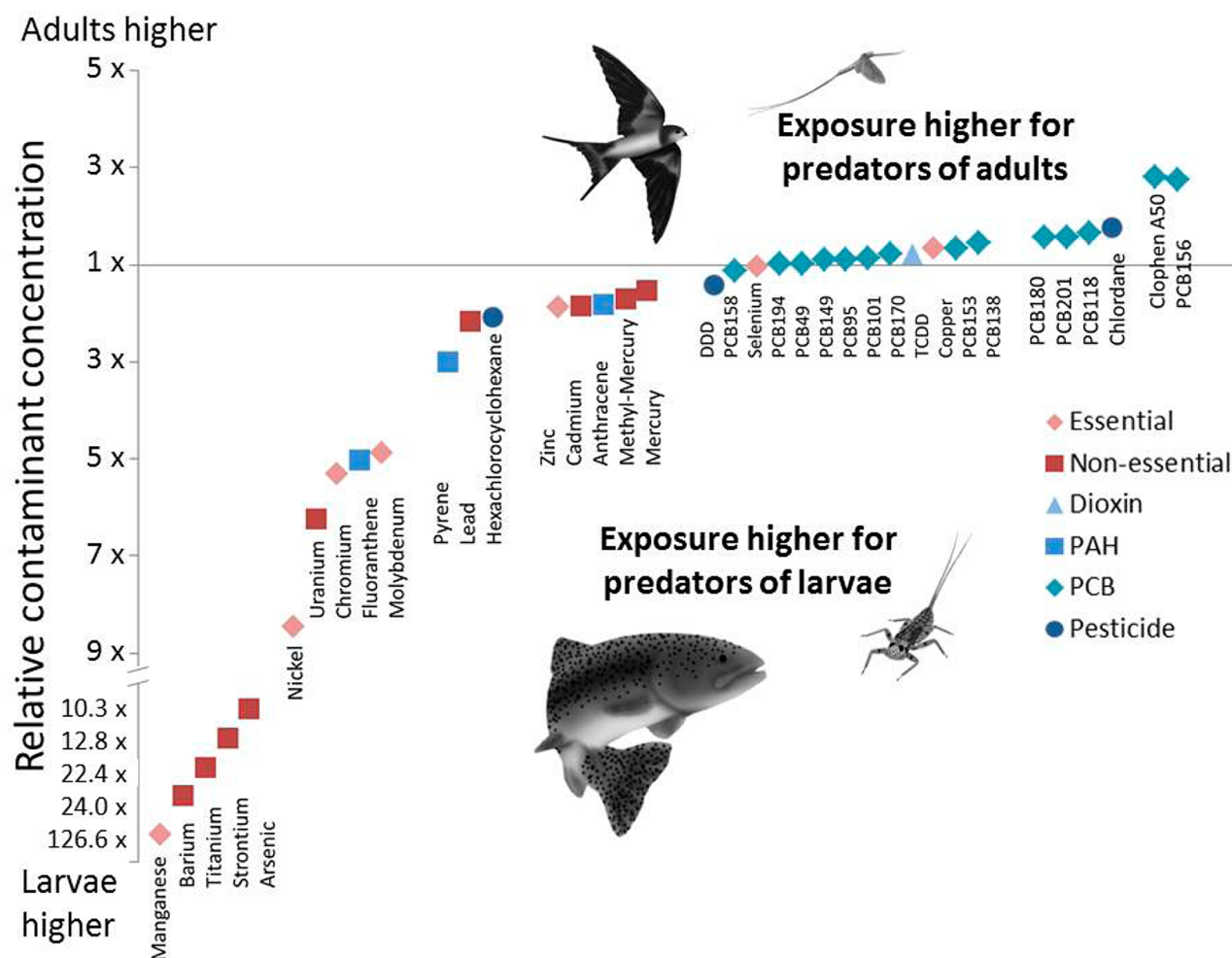


Figure 3. Relative potential contaminant exposure for consumers of larval or adult insects. Above x -axis = concentration in adults/larvae; Below x -axis = concentration in larvae/adults; 1 x = larval and adult concentrations are equal. Weighted means for contaminants with multiple observations are presented. Note: values below axis break are not to scale.

terrestrial, forest-field), revealing substantial energy and contaminant fluxes between ecosystems.^{2,4,7,42} Our results suggest that metamorphosis affects stable isotopes and contaminants in a manner generally consistent with their ability to be regulated by insects (i.e., excreted, detoxified, or metabolized as a result of their chemical properties) and transferred within food webs. These effects influence our estimates of food web connectivity, the magnitude of contaminant flux among food webs and ecosystems, and the concentration of contaminants within insect bodies to which consumers could potentially be exposed.

For example, N^{14} , trace metals and PAHs are internally regulated or metabolized.^{40,41} These were lost during metamorphosis (up to 96% of burden), leading to $\delta^{15}N$ isotopic enrichment, decreased contaminant concentrations, and reduced exposure risk to predators of adult insects compared to predators of insect larvae (e.g., ref 42). The effects of metamorphosis on metal concentrations are exposure-dependent; metal concentration decreases during metamorphosis at high, but not low, larval exposure levels. Thus, proportionally more metals may be excreted during metamorphosis as larval exposure increases, similar to the finding that metal bioaccumulation decreases as environmental concentrations increase.²³ C^{12} and PCBs, on the other hand, are mostly conserved across metamorphosis (this study,^{14,43}).

As a result, $\delta^{13}C$ is similar for larvae and adults, and PCBs can be transferred across ecosystem boundaries at concentrations that are potentially harmful to some consumers (e.g., ref 5). These patterns are similar to trophic transfer of these chemicals: $\delta^{15}N$ is enriched with trophic level when light nitrogen is differentially excreted by consumers, many trace metals do not biomagnify (ref 19 and 23), PAHs are diluted as trophic levels increase (refs 44 and 45 although toxic metabolites may be transferred,⁴⁶), and PCB concentrations increase with trophic level.^{34,45}

Metamorphosis effects on isotopic signatures and contaminant concentrations likely affect predictions of food web exposure because metamorphosing insects often occupy different niches and food webs as larvae and adults (e.g., adult aquatic insects are eaten by terrestrial consumers and migrating butterflies fall prey far from natal feeding grounds;⁴⁷). Similar to Tibbets et al.,¹⁸ we found that $\delta^{15}N$, an indicator of trophic position, became enriched on average 1.0‰ during metamorphosis. Compared to a trophic enrichment (i.e., fractionation) factor of 3.4‰ for $\delta^{15}N$ typically used in contaminant studies (e.g., ref 34), this nontrophic enrichment could lead to a 30% overestimation of trophic position if nitrogen isotopes of larval prey were used to estimate trophic position of consumers of adult prey. Such an error in contaminant studies would cause underestimation of contam-

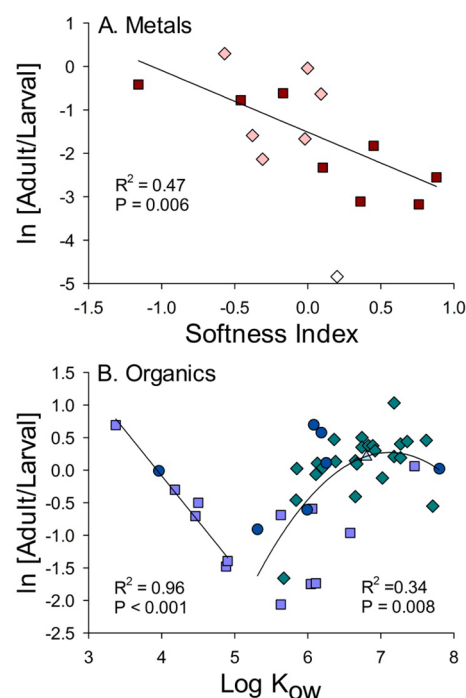


Figure 4. Relationship between persistence and physical-chemical properties for metal (A) and organic (B) contaminants. The softness index is the negative normalized consensus of softness indices calculated by Kinraide (28), except for Se and Ar which were calculated from Kinraide's equations; smaller numbers represent softer elements. Log K_{OW} is the octanol–water partitioning coefficient. Symbols are same as Figure 3. Mn (white diamond, A) was an outlier and excluded from this analysis.

inant biomagnification in food webs and exposure risk to higher order predators by 23% by reducing the slope of the relationship between trophic position and contaminant concentration (i.e., the trophic magnification factor, TMF). If trophic fractionation is actually less than $\delta^{15}N = 3.4\text{‰}$ (e.g., 1.4‰ for consumers of invertebrates,⁴⁷), the potential underestimation would be further exacerbated. This potential for underestimation exists wherever assumptions about fractionation are made, even if biomagnification is estimated at the individual level.

Actual contaminant exposure and flux among food webs are determined both by the concentration in insects and by the effects of contaminants on insect survival to emergence.⁴² Lethal levels of any contaminant reduce total exposure and flux of that contaminant and prey availability for predators (Figure 1 in ref 42). For example, increased trace metal concentrations (Zn, Cu, Cd) montane streams of the Intermountain West (U.S.) can lead to up to 97% loss of adult emergence biomass, which reduces both prey and metal transfer to riparian spiders.⁴² Elements like zinc have strong effects on adult densities because of the physiological and metabolic stress of metamorphosis following development in a contaminated larval environment.^{48,49} Because these elements are also lost during metamorphosis, their primary impact on adult food webs is through reduction of prey quantity rather than direct contaminant transfer.⁴² Alternatively, areas affected by contaminants with high persistence across metamorphosis and low effects on adult survival will represent “hotspots” of contaminant exposure and flux.⁵ For example, PCBs and selenium have limited impacts on larval survival in aquatic

systems, but high propensity to be conserved during metamorphosis. As a result, adult insects expose consumers to high concentrations of these contaminants where larval exposure is high, and predator exposure in adult insect food webs scales with contamination of the larval environment.⁵ Thus, the persistence of contaminants during metamorphosis and the relationship between contaminant persistence and survival to adult emergence will determine whether the contaminant effects propagated by adults to recipient food webs are driven by contaminant exposure or reduction of adult insect prey biomass.

In general, we found that the behavior of metals and organochlorines across metamorphosis was strikingly similar to their behavior during trophic transfer. That is, contaminants were lost or retained through metamorphosis according to their propensity to be metabolized or bioaccumulate in food webs, and this variation was explained by the physical-chemical properties of the contaminants. Softness, for example, is a metric of ion bioactivity related to bioaccumulation,³² protein binding,³¹ probability of forming covalent bonds,²⁸ and toxicity,³⁰ and this metric explained 47% of the variance in persistence of metals across metamorphosis. Snodgrass et al.⁶ report a similar pattern in amphibians, suggesting that the percent change in trace metal concentration through frog metamorphosis may be controlled by the tendency of the ions to bind to biomolecules. This metric is also related to essentiality of the metals included in the meta-analysis and could explain why nonessential metals as a group were lost at greater rates during metamorphosis compared to essential metals. Differences in the need for essential and nonessential metals are invoked to explain differences in metabolic regulation among metals (e.g., ref 50), yet metals often utilize the same cellular machinery for uptake into the body (e.g., facilitated diffusion, active pumps;⁴¹). Thus, the effects of metamorphosis on essential and nonessential metals as a group are likely due to the softness of individual metals, rather than because of some inherent difference in the metabolic regulation of essential vs nonessential metals as a group.

Likewise for organic contaminants, the octanol–water partition coefficient ($\log K_{OW}$), which measures hydrophobicity and predicts biomagnification of many organic contaminants within food webs for lipophilic compounds ($\log K_{OW} > 5$;^{22,34}), increased nonlinearly with contaminant persistence across metamorphosis. The relationship between $\log K_{OW}$ and persistence is strikingly similar to relationships between $\log K_{OW}$ and bioaccumulation across food webs and bioretention in consumers of organochlorines with $\log K_{OW} > 5.0$.^{32,33} For compounds with low hydrophobicity ($\log K_{OW} < 5$), different physical–chemical properties may drive patterns of persistence both during metamorphosis and trophic transfer. Because molecular weight generally increases with $\log K_{OW}$, the behavior of compounds with $\log K_{OW} < 5$ which are mainly PAHs in this study may be driven more by molecular weight or ability to be metabolized (i.e., biotransformation⁵¹) rather than hydrophobicity. Similar to our findings, Wan et al.⁴⁴ found no or negative relationships between bioaccumulation across the food web and hydrophobicity of PAHs with $\log K_{OW} < 5$, but a positive relationship for $\log K_{OW} > 5$. However, this pattern is not consistent among studies. Takeuchi et al.⁴⁵ show no relationship (or trending negative) for all PAHs measured ($\log K_{OW}$: ~ 4.5 – 7.5). Interestingly, the metabolism of PAHs during metamorphosis could contribute to estimates of trophic dilution, particularly as it occurs during the aquatic–terrestrial

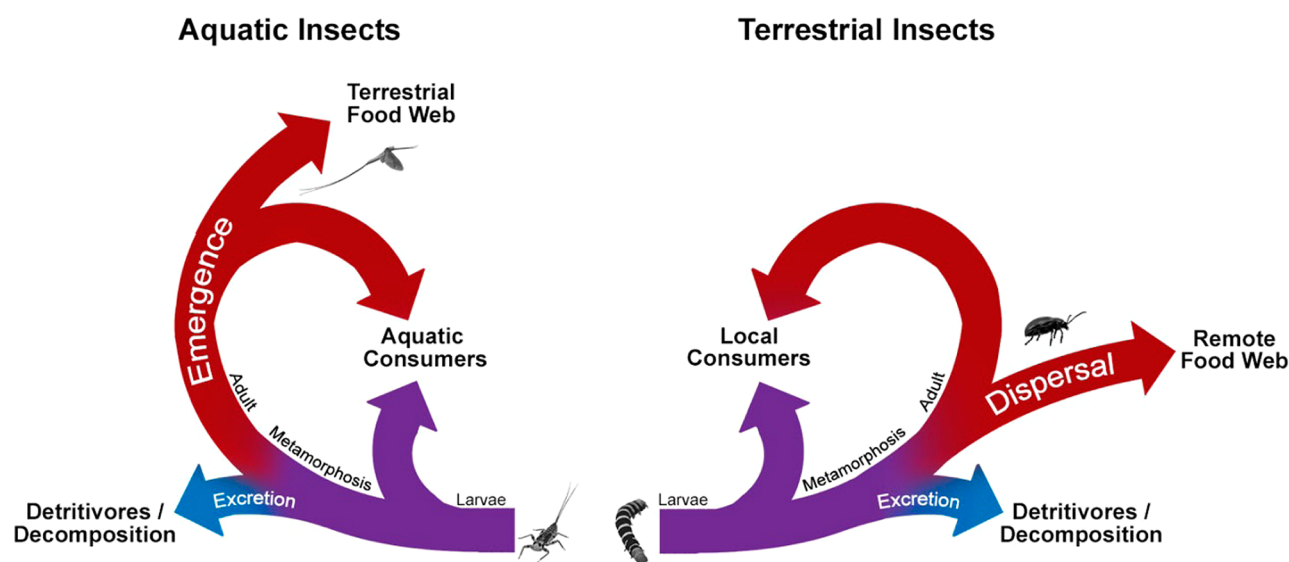


Figure 5. A generalized diagram showing transformation of contaminant flows within and across ecosystem boundaries by insect metamorphosis.

transfer of PAHs. These patterns may be specific to PAHs; more data are needed to evaluate other biotransformable organic compounds.

Metamorphosis is a time of tissue catabolism; insects do not eat during this time but do expend energy. Mainly fats and some carbohydrates fuel the breakdown of larval proteins (histolysis) to generate new adult structures (histogenesis).^{17,52} The analogous metabolic processes that occur during consumption and the similar impacts of metamorphosis and trophic transfer on both stable isotope signatures and contaminant accumulation suggest that metamorphosis may in some ways be viewed as a trophic process. Given the wide array of insects considered (multiple studies and different propensities to lose biomass during metamorphosis), the similarity of these patterns to patterns for trophic processes is compelling. However, although several lines of evidence point to predictability in patterns of metamorphosis effects on chemical tracers and contaminants, some outcomes could not be as well explained. For example, mercury and methyl-mercury were lost during metamorphosis in all but one study (Supporting Information).¹⁶ This result was counterintuitive given the persistence of mercury in the body and magnification in food webs.^{24,53} Further study of these and other contaminants is needed to better understand their behavior during metamorphosis, especially given the low number of studies for mercury and some other analytes. The current work illuminates data gaps in terms of our understanding of specific chemical tracers and contaminants, taxonomic coverage of those analytes (e.g., effects of metamorphosis on concentrations of organic contaminants are only measured aquatic insects), and even the change in mass that occurs during metamorphosis from larval to adult insects.

The loss of contaminant burdens during metamorphosis generally aligns with the ability of insects to metabolize the contaminant and the anthropogenic origins of the contaminant (PCBs are man-made vs metals and some PAHs which are not). Mechanisms of contaminant losses include the exoskeleton (exuvia), which is shed by final larval instars or pupae, and the excrement (meconium) of last instar larvae or newly emerged adults. The relative importance of these pathways appears to depend on the contaminant. Most of the loss of

organic contaminants is accounted for by contaminant burden within exuvia,^{14,54} but for most metals (where up to 98% of burdens can be lost during metamorphosis) the meconium appears a more likely mode of contaminant loss. Less than 10% of the metal burden lost during metamorphosis is attributed to loss through exuvia whereas typically > ~50% of metal is lost through meconium (SI Table S4). This pattern is consistent with metals being excreted into the gut lumen during metamorphosis,²¹ and suggests that meconium may be the predominant excretory pathway in metal-contaminated systems, although the ecological fate of contaminants contained in the meconium is unknown.

Understanding changes in insect chemistry related to metamorphosis will help predict and mitigate the potential cascading effects of contaminants across ecological boundaries and will improve risk management of contaminated sites. For example, the U.S. Environmental Protection Agency manages hundreds of highly contaminated aquatic sites (e.g., Superfund sites and Great Lakes Areas of Concern), many of which contain a mixture of contaminants including metals, PCBs, PAHs, and pesticides. Predicting which compounds and elements are likely to cross the water-land boundary or affect remote food webs (e.g., Figure 5) in concentrations and quantities that exceed safe limits for wildlife is a critical first step in assessing and managing risks to riparian and wetland ecosystems. Likewise, analytical chemistry costs comprise a substantial portion of the management budget for these sites (e.g., high-resolution, broad-spectrum organic analyses are commonly upward of \$1000 U.S. per sample). Thus, targeting specific contaminants, habitats, and life stages to sample for tissue analyses would serve the dual purpose of improving risk analyses while shepherding limited management resources. Finally, correctly assigning tracer values to potential food sources will support correct interpretation of the food web relationships responsible for cross-habitat energy and contaminant transfer.

■ ASSOCIATED CONTENT

■ Supporting Information

Contents include search term information, statistical analyses, and data for body burdens. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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