Species- and Element-Specific Patterns of Metal Flux from Contaminated Wetlands versus Metals Shed with Exuviae in Emerging Dragonflies

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ABSTRACT

Dragonfly adults and their aquatic immature stages are important parts of food webs and provide a link between aquatic and terrestrial components. During emergence, contaminants can be exported into terrestrial food webs as immature adults fly away or be shed with their exuviae and remain in the wetland. Our previous work established metals accumulating in dragonfly nymphs throughout a contaminated constructed wetland designed to regulate pH and sequester trace metals from an industrial effluent line. Here, we evaluated the concentration and mass of metals leaving the wetland in flying emergents versus remaining in the wetland with the shed exuviae in 10 species of dragonflies belonging to 8 genera. Nine elements (Cu, Zn, Cd, Mn, V, Mg, Fe, Al, Pb) were evaluated that include essential and nonessential elements as well as trace and major metals. Metal concentrations in the emergent body and exuviae can differ by orders of magnitude. Aluminum, Fe, Mn, and Pb were largely shed in the exuviae. Vanadium and Cd were more variable among species but also tended to be shed with the exuviae. In contrast, Cu, Zn, and Mg showed a higher tendency to leave the wetland with an emerging dragonfly. Metals shed in dragonfly exuviae can moderate the transport of metals from contaminated wetlands. Taxonomic- and metal-specific variability in daily metal flux from the wetland depended upon concentration accumulated, individual body mass, and number of individuals emerging, with each factor's relative importance often differing among species. This illustrates the importance of evaluating the mass of metals in an individual and not only concentrations. Furthermore, differences in numbers of each species emerging will magnify differences in individual metal flux when calculating community metal flux. A better understanding of the variability of metal accumulation in nymphs/larvae and metal shedding during metamorphosis among both metals and species is needed.

Keywords: aquatic insect; dragonfly; metal; contamination; insect emergence; aquatic-terrestrial linkages

Highlights:

- 9 metals evaluated in emerging dragonflies and their exuviae from contaminated wetland
- Larger amounts of some metals (Cu, Zn, and Mg) were retained in the emergent dragonflies
- Some elements (Al, Fe, Mn, Pb) were largely shed with exuviae
- Metals shed in exuviae can moderate metal export from wetlands
- Importance of factors influencing metal flux differed among species

1. Introduction

Aquatic insects commonly exhibit complex life cycles with both terrestrial and aquatic phases. Terrestrial adults lay eggs in water that hatch into aquatic larvae. Larvae feed and grow for a few months to several years before undergoing metamorphosis and emerging from the wetland as flying juvenile or adult individuals. The aquatic larvae of many species have been shown to accumulate both essential (e.g. Cu, Zn, Mn, Ni) and nonessential (e.g. Cd, Hg, Pb) trace elements when exposed to diverse pollutant sources (Rainbow, 2002; Karimi and Folt, 2006; Rainbow, 2007). Although the terrestrial flying adult insects can travel many kilometers, the aquatic larvae are restricted to the inhabited waterbody and consequently accumulate contaminants from their local environment (Bonada et al., 2006; May and Matthews, 2008; Kharitonov and Popova, 2011). In addition to the spatial integration of contaminant exposure within their home ranges, an advantage of using aquatic invertebrates as biomonitors is the temporal integration of exposure that is less susceptible to being influenced by short-term fluctuations in contaminant concentrations, as will single or infrequent collections of only water and/or sediment (Fletcher et al., 2019). Consequently, aquatic invertebrates are extensively used as biomonitors of diverse pollutants.

Aquatic insects provide both a food source and a potential source of trophically transferred contaminants to terrestrial predators that feed on insects in or on the water (Schulz and Bundschuh, 2020). However, the emergence of flying insects from a wetland provides an additional food source to terrestrial predators that live along stream, lake, or wetland margins and cause increased productivity near the wetland (Baxter et al., 2005; Burdon and Harding, 2008; Gratton and Vander Zanden, 2009; Schulz and Bundschuh, 2020). This linkage of aquatic to terrestrial food webs also presents the potential of exporting contaminants accumulated in the aquatic larvae from the contaminated wetland or stream to terrestrial food webs (Walters et al., 2008, 2010, 2020; Kraus et al., 2014). Consequently, riparian species such as spiders, birds, and bats can accumulate contaminants from emerging aquatic insects (Walters et al., 2008; Walters et al., 2010; Hernout et al., 2018; Ramos-H et al., 2020; Schulz and Bundschuh, 2020). Dietary exposure represents a primary route of uptake of contaminants to diverse bird species, putting adults and nestlings at risk of adverse physiological effects; contaminant exposure can include trace metals, and prey often includes emerged aquatic insects (Hernout et al., 2018; Lidman et al., 2020). For example, gut content analyses revealed 184 bird species to have eaten dragonfly nymphs or adults in the continental United States

(Kennedy, 1950). Similarly, insectivorous bats have been shown to accumulate contaminants from diverse sources such as vehicular traffic, industrial activities, and sewage disposal (Ramos-H et al., 2020). In addition to direct predation of emerging insects, aquatic contamination can pass from emerging aquatic insects, through riparian spiders to birds preying upon spiders (Cristol et al., 2008; Walters et al., 2010; Schulz and Bundschuh, 2020).

Adding to the complexity, changes of the contaminant body burden in insects can occur during metamorphosis and emergence into flying adults/subadults (Krantzberg and Stokes, 1988; Timmermans and Walker, 1989; Kraus et al., 2014; Wanty et al., 2015; Kraus and Pomeranz, 2020). Thus, aquatic larvae contaminant body burdens do not necessarily reflect what terrestrial predators may be exposed to by emerging insects (Kraus et al., 2014; Wesner et al., 2017; Kraus and Pomeranz, 2020; Kraus et al., 2021). Furthermore, whether contaminants accumulating in the aquatic nymph are transferred through metamorphosis into the flying subadult/adult can vary among different species, contaminants, sites, and levels of contamination (Timmermans and Walker, 1989; Groenendijk et al., 1999; Cid et al., 2010). For example, some contaminants such as polychlorinated biphenyls (Walters et al., 2008; Daley et al., 2011; Kraus, 2019) or MeHg (Chételat et al., 2008) can be largely transferred from the aquatic larvae to the emergent. Moreover, contaminants can become more concentrated in emergents when an extensive transfer of contaminants to the emergents is combined with a substantial mass loss during metamorphosis/emergence (Chételat et al., 2008; Kraus et al., 2014). In contrast, metamorphosis and emergence can dramatically reduce contaminants in the flying emergent (Kraus et al., 2014; Wesner et al., 2017; Kraus, 2019; Kraus and Pomeranz, 2020). It has long been established that the level of metals accumulated in syntopic aquatic invertebrate taxa can vary in both taxa- and metal-specific patterns (Cain et al., 1992; Rainbow, 2002; Luoma and Rainbow, 2005), and it is becoming increasingly apparent that the proportion of a metal that is incorporated into the emergent insect from the aquatic larvae does as well (e.g. Timmermans and Walker, 1989; Kraus et al., 2014; Wesner et al., 2020). Overall, the metal- and taxa-specific patterns in the export of metals from contaminated wetlands via emerging insects are not well known.

Metals eliminated during the process of emergence may be shed with the discarded larvae exoskeleton (exuviae), nymph defecation, and/or meconium (Timmermans and Walker, 1989; Sarica et al., 2005, Kraus et al., 2014). Contaminants shed by these mechanisms remain in the contaminated wetland. The role of the exuviae in the shedding of metals during emergence differs among metals and taxa (Timmermans and Walker, 1989). We used dragonflies to evaluate the potential export of metals from a constructed wetland where aquatic

nymphs have been previously shown to accumulate elevated levels of metals (Fletcher et al., 2020). Capturing dragonflies as they emerged from the wetland, combined with collecting their shed exuviae, allowed evaluation of metals leaving the wetland in the emerging dragonfly (teneral) versus shed in their exuviae and consequently returned to the wetland. Concurrent evaluation of individual body mass and numbers of individuals of each species emerging allowed us to compare how metal flux from the wetland differed among species. Specifically, our objectives were first to evaluate, within a species, how different metals vary in their propensity to be incorporated into the teneral body and leave the wetland versus being shed within the exuviae and remain in the wetland. Nine elements (Cu, Zn, Mg, Cd, Mn, V, Fe, Al, Pb) were evaluated that include essential and nonessential elements as well as major and trace elements. Second, we evaluated how the patterns of accumulation in tenerals versus exuviae differed among different syntopic species of dragonflies. The evaluations included 10 species of dragonflies belonging to 8 genera. Third, we evaluated how element concentration, individual body mass, and numbers of individuals influenced inter-specific patterns of metal flux from the wetland.

2. Methods

2.1. Study site

The Savannah River Site is an 801 km^2 National Environmental Research Park established in 1951 and operated by the United States Department of Energy to produce and process materials for nuclear weapons. The H-02 Constructed Wetland, described in detail and illustrated in Fletcher et al. (2020), was constructed to treat effluents composed of cooling water, laboratory drain waste, steam condensate, and storm water from roof and ground drains from a nuclear materials (tritium) processing facility (Halverson, 2004; Huddleston and Nelson, 2008). The H-02 constructed wetland system consists of a 0.48 ha retention basin, two 0.213 ha wetland treatment cells, a 0.058 ha effluent pool, and a discharge stream. The retention basin was selected as a study site due to its accessibility, abundance of dragonflies, and because several genera of dragonfly nymphs were shown to accumulate higher levels of metals in it than from unpolluted waters (Fletcher et al., 2020).

Untreated effluents primarily enter the retention basin via two pipes located in the southwest and southeast basin corners. Three other pipes discharge lesser volumes of water into the basin and primarily after rain events. Water leaves the retention basin through a

166 standpipe and flows through a splitter box to the treatment wetlands. These effluents are
167 regulated through the National Pollutant Discharge Elimination System (NPDES) managed by
168 the US Environmental Protection Agency with permits issued by the South Carolina Department
169 of Health and Environmental Control (permit: SC0000175). Radionuclides have not been
170 released through this outfall, but elevated pH and levels of metals were identified in H-02
171 effluents before construction of the wetland (Halverson, 2004; Huddleston and Nelson, 2008).
172 Consequent to these previous evaluations, the permit specifies monitoring pH as well as Cu, Zn,
173 and Pb concentrations. With an effluent sampling frequency of once per month, permit limits for
174 pH are 6 - 8.5; additionally, the monthly average and daily maximum concentrations (from 24 hr
175 composite) (mg/L) for Cu is 0.032 and 0.042, and Zn 0.11 and 0.11. Limits are not specified for
176 Pb, but levels must be monitored and reported. Effluent lead concentrations have been
177 consistently low, so Cu and Zn are the focus of routine monthly water quality monitoring (Xu and
178 Mills, 2018; Xu et al., 2019). In 2014–2016 based on monthly water grab samples,
179 total/dissolved Cu water concentrations in the retention basin averaged 23.8/22.1 ug/L and
180 total/dissolved averaged Zn concentrations 30.5/22.5 ug/L, respectively (Xu and Mills, 2018).
181 Additionally, over this period, pH in effluents from the retention basin ranged from 7.6–9.2 (Xu
182 and Mills, 2018). However, the constructed wetland sequesters additional metals from the
183 diverse water sources of the influents. Substantial interspecific variability occurred among 5
184 dragonfly genera, but some genera were found to have elevated metal concentrations (e.g. Cu,
185 Zn, Mn, Al, Cd) throughout the H-02 wetland system compared to reference sites (Fletcher et
186 al., 2020). Thus, the level of contamination and the abundance of dragonfly nymphs presented
187 a risk of metal export in emerging dragonflies.
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189 *2.2. Study organisms*
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191 Dragonfly adults lay their eggs in aquatic habitats. Aquatic nymphs may live underwater for
192 relatively short periods of time in multivoltine species that produce multiple generations per year
193 or up to several years in semivoltine species before emerging as flying juveniles (Corbet et al.,
194 2006). The predaceous aquatic nymphs represent prominent components of aquatic food webs
195 and can be influential in shaping aquatic communities (Benke, 1978; Morin, 1984; Thorp and
196 Cothran, 1984; Knight et al., 2005; Crumrine et al., 2008). The relatively long-lived dragonfly
197 nymphs that inhabit relatively small home ranges accumulate metals/metalloids from
198 contamination in their local aquatic habitats (e.g. Karouna-Renier and Sparling, 2001; Nummelin
199 et al., 2007; Corbi et al., 2008, 2010, 2011a,b; Wiener et al., 2008; Tollett et al., 2009; Lavilla et

al., 2010; Fletcher et al., 2017, 2020). Moreover, this tendency of dragonfly nymphs to accumulate contaminants, while being tolerant enough to survive in wetlands designed to sequester contaminants, make them suitable biomonitors for constructed wetlands (Fletcher et al., 2020). Odonates have been found to significantly contribute to the export of biomagnifying MeHg from wetlands (Tweedy et al., 2013; Williams et al., 2017; Chumchal and Drenner, 2020). Here we further explore the utility of these versatile organisms in evaluating the export of 9 metals from aquatic to terrestrial habitats. A total of 10 species of Libellulid dragonfly tenerals distributed across 5 subfamilies and 8 genera were collected in sufficient numbers to statistically compare metal accumulation in the teneral body and shed exuviae (Table 1). Libellulids in the local area are typically univoltine and only overwinter once as an aquatic nymph (Benke, 1972; Benke and Benke, 1975). Our data set also provided the unique opportunity to compare metal accumulation patterns in different dragonfly species within the same genus, which has been rarely done in the literature (Fletcher et al., 2017). Here we compare 2 species of *Libellula* and 2 species of *Pantala*.

2.3. Sample collection and processing

Even though the retention basin is only 30 m wide, both littoral and limnetic habitats were sampled to acquire as many dragonfly species as possible. Traps consisted of vertical or near-vertical 3.05 m long screens that provided an emergence substrate. Six cages, each 45.7 cm wide, were installed over each screen to prevent tenerals from flying away (Fig. SI 1). Installing six individual cages on each screen reduced the number of individuals emerging in the same trap, which reduced cannibalism and facilitated matching a teneral to its exuviae. Of available individuals, preference was given to individuals in cages without conspecifics. On a few occasions, conspecifics in a cage were matched based on body/exuviae size. Screen height was adjusted according to water depth to ensure air space to avoid drowning the emerging tenerals. A total of 12 screens with 72 traps were monitored; two littoral and two limnetic screens were placed in locations in the deep (west), middle (center), and shallow (east) portions of the retention basin.

Traps were monitored daily for one week every 2-3 weeks between 14 June to 5 September 2015 and the weeks of 18 April and 23 May 2016. Most samples used for metal analyses were selected from the weeks of 9 August 2015 and 5 September 2015 for this study to reduce potential influences of temporal variability. For calculating metal flux from the wetland, quantitative data of numbers of dragonflies emerging was available for a total of 32

trap-days distributed over seven weeks. Only tenerals or exuviae left by an escaped or preyed upon teneral were counted from within the traps. Occasionally frog and turtle predation occurred, so only the exuviae remained. Since some species, such as *Perythemis tenera*, tended to emerge on the outside of our cages, exuviae collected from the outside of the cages were also counted. Counts of emerging dragonflies were taken from 12 screens, each 3.05 m long for a total of 36.6 m in length of the artificial substrate. We used the number of individuals of each species emerging from these structures over the sampling period as a representative subsample of individuals emerging from the wetland. Because we cannot know how far individuals traveled to reach the screens and because artificial substrates such as screens or platforms do not provide comparable habitat as a bed of emergent macrophytes, wood debris, or shoreline vegetation, we did not convert the numbers to a density. Such analyses of true dragonfly emergence density would require counting exuviae on natural substrate.

Tenerals and their exuviae were individually put into plastic bags and held on ice until returned to the lab and frozen. Dragonfly tenerals were later thawed long enough to identify species using dichotomous keys (Merritt et al., 2008; Needham et al., 2014) and their sex noted. To maintain good condition, samples were held on ice in enclosed insolated containers, except when viewing on a dissecting scope. After identification, individuals were refrozen. Later, individuals were thawed, rinsed with Milli-Q (18 MΩ) water, transferred to glass vials, refrozen, lyophilized to a constant weight, and weighed to the nearest 0.1 mg. Tenerals of *Bachymesia gravida*, *Libellula auripennis*, *L. luctuosa*, *Orthemis ferruginea*, *Plathemis lydia*, *Pantala flavescens*, *P. hymanaea*, and *Tramea lacerata* were paired with their own shed exuviae. Exuviae of the two smaller species, *Pachydiplax longipennis* and *Perythemis tenera*, were of insufficient mass to conduct trace metal analyses on individual exuviae. Consequently, exuviae from the inside or outside of the cages were composited to acquire enough mass for analyses. Composites were formed from individuals from within the same retention basin section, habitat, and week.

2.4. Trace element analyses

Trace metal analyses (Cu, Zn, Mg, Cd, Mn, V, Fe, Al, and Pb) were conducted on 191 teneral and 191 exuviae samples (Table 1) by inductively coupled plasma mass spectroscopy (NexIon 300X ICP-MS; Perkin Elmer, Norwalk, CT) according to standard QC protocols described by Fletcher et al. (2019, 2020) and detailed in Text S1. Blanks, duplicates, and certified reference materials were used as quality control measures (Fletcher et al., 2020; Text S1). Efforts were

made to select multiple elements from each element category: essential trace elements (Cu, Zn, Mn, and V), nonessential trace elements (Cd, Pb), essential major elements (Mg and Fe), and nonessential major elements (Al). Comparison of multiple elements within metal categories allowed the evaluation of the consistency of patterns within a category. Metal accumulation is highly variable among dragonfly taxa, but additional criteria included accumulation of the metal in dragonflies living in the constructed wetland and elevation of the metal concentration in dragonflies inhabiting local disturbed streams, including the receiving stream, compared to reference streams (Cu, Zn, Cd, Mn, V, Fe, Al, and Pb) (Fletcher et al., 2017, 2019, 2020; Fletcher, unpublished data). Magnesium concentrations can be reduced rather than elevated in dragonflies from disturbed streams (Fletcher, unpublished data) but were included to allow the comparison of multiple major elements. All element concentrations in text, tables, and figures are presented on a dry mass basis as mg/kg. No teneral or exuviae individual concentrations were below method detection limits for Cu, Zn, Mg, Fe, V, or Mn. No exuviae and only 1% of the tenerals had Pb concentrations below the method detection limits. No exuviae Al concentrations were below method detection limits, but 48% of the tenerals were. Cadmium concentrations were below method detection limits for 86% of the tenerals, but only 3.7% of the exuviae.

Mean percent recovery of elements in the TORT-3 certified reference material in the microwave-assisted digestions of tenerals ranged from 94-107% in the 6 elements that overlap in TORT-3 and our presented analyses (Table S1). Mean percent recovery for elements in the TORT-3 certified reference material in hot block digestions or tenerals ranged from 86-114 % (Table S1). Mean percent recovery of elements in TORT-3 certified reference materials in the hot block digestions of exuviae ranged from 89-97% (Table S1).

2.5. Statistical analyses

For statistical analyses, we replaced concentrations below the detection limit with 50% of the mean detection limit. Statistical distributions of element concentrations were improved by Log_e transformation prior to statistical analyses. Statistical comparisons were conducted with SYSTAT® statistical package (version 13.00.05/2009, SYSTAT Software Inc., San Jose). A $p < 0.05$ significance level was exclusively used in all statistical analyses. To determine which samples could validly be grouped, we first explored the possible influence of sex, habitat type, and section of the retention basin on metal accumulation in the teneral body versus exuviae. Due to the lack of consistent or extensive influence of sex, habitat, and section on the focal

questions of this study, these variables were not included in the comparisons of concentrations or mass of each element in the teneral versus the exuviae (summarized in Text S2; Figure S2-S10). Wilcoxon Signed-Rank tests were employed to compare metal concentrations and masses of metals in tenerals and exuviae for the 8 species in which the teneral was paired with its own exuviae. Mann-Whitney U tests were used to make these comparisons in *P. longipennis* and *P. tenera* because exuviae were composited and thus not natural pairs. The percentage of each metal that was shed with the exuviae upon emergence was calculated by dividing the element mass in the exuviae by the combined mass of element found in the emerged teneral and shed exuviae. For each species, average number of individuals emerging per day was calculated by dividing the total number of individuals collected by the 32 trapping days. Average biomass flux per day of each species was calculated by multiplying the average individual dry body mass by the average number of individuals emerging per day. Similarly, average metal flux per day was calculated by multiplying the average individual metal body burden by the average number of individuals emerging per day.

3. Results

3.1. Metal concentration and metal mass in individuals

The percentage of total mass (teneral + exuviae) of the emerging individual that is shed with the exuviae was generally consistent among the 10 study species, with only 13-17% of the total mass being shed with the exuviae (Table 2). Copper concentrations were significantly higher in exuviae than tenerals in 8 of 10 species (Fig. 1; $p<0.05$) but did not differ in *P. hymenaea* and *T. lacerata* (Fig. 1; $p>0.05$). Despite often higher concentrations in exuviae, Cu mass was greater in the heavier teneral bodies than in the relatively light exuviae of all 10 species (Fig. 1; $p<0.05$). Copper concentrations in exuviae differed more among species than Cu concentrations in tenerals (Fig. 1). Concentrations exceeding 100 mg/kg in exuviae of the 4 Libellulinae species, *P. longipennis,* and *P. tenera* resulted in higher percentages of Cu being shed in their exuviae (Table 2). The percentage of combined Cu mass in the teneral and exuviae (teneral + exuviae metal mass, hereafter referred to as total) shed in exuviae averaged from 33-41% in these 6 species (Table 2), but only 16-19% in the 3 Trameinae species and 23% in *B. gravida*. Overall, higher Cu concentrations in exuviae resulted in a greater percentage of Cu shed during emergence.

Zn concentrations in tenerals versus exuviae significantly differed in 8 species (Fig. 1; $p<0.05$). Again, concentrations were higher in exuviae than teneral in the 4 Libellulinae species, *P. longipennis,* and *P. tenera* but interestingly were higher in tenerals than exuviae of the 2 *Pantala* species (Fig. 1; $p<0.05$). Zinc concentrations in the teneral body and exuviae of *B. gravida* and *T. lacerata* did not differ (Fig. 1; $p>0.05$). Similar to Cu, Zn concentrations varied more among species in the exuviae than in tenerals. The variation among species included Zn concentrations in *L. luctuosa* exuviae being 1.7 times higher than those of *L. auripennis*. Greater Zn mass accumulated in teneral bodies than was shed in the exuviae of all 10 species. Elevated Zn concentrations in exuviae of the 4 Libellulinae species, *P. longipennis,* and *P. tenera* resulted in 23 to 35% of the total Zn being shed with the exuviae, whereas less than 16% was shed with exuviae of the 3 Trameinae species and *B. gravida*. (Fig. 1; Table 2).

All ten species accumulated less than 3.5 mg/kg Mn in teneral bodies, but 33.0-135 mg/kg in their exuviae. Corresponding with the substantially higher concentrations in exuviae, greater Mn mass was also shed in exuviae than retained by the tenerals (Fig. 1, $p<0.05$), with species averages of 64-90% of the total Mn being shed with the exuviae (Table 2). Again, variation among species included exuviae of *L. luctuosa* having 1.6 times higher concentrations and 3.8 times higher mass of Mn than those of *L. auripennis*. Higher concentrations of V also accumulated in the exuviae than the teneral body of all 10 species, with a greater mass of V in the exuviae than the teneral body of 8 species (Fig. 2; $p<0.05$). Consequently, 45-90% of the V was shed with the exuviae (Table 2). Of the 10 species, *L. auripennis* shed the lowest percentage of V with their exuviae (45%), whereas *L. luctuosa* shed 80%, which was more than some members of other subfamilies.

Despite generally low concentrations in both exuviae and teneral bodies, higher Cd concentrations occurred in exuviae than teneral bodies in 9 of the 10 species (Fig. 2; $p<0.05$). The one exception was that *L. auripenni*s teneral and exuviae Cd concentrations did not differ (Fig. 2; $p>0.05$). The magnitude of difference of Cd concentrations in teneral vs exuviae is highlighted by Cd being below detection limits in 86% of the tenerals, but only 3.7% of the exuviae. However, the elevated accumulation of Cd in *L. auripennis* and *P. longipennis* tenerals is further illustrated by these species having no individuals with concentrations below detection limits. Greater Cd mass was shed in the exuviae than retained in the teneral body of *O. ferruginea*, *P. lydia*, *P. tenera*, and *P. hymenaea* (Fig. 2; $p<0.05$). However, higher Cd concentrations in *L. auripennis* and *P. longipennis* tenerals resulted in greater Cd masses being retained in the teneral than being shed in their exuviae (Fig. 2; $p<0.05$); Cd mass did not differ between the teneral and exuviae in the other 4 species ($p>0.05$). *L. auripennis* shed only 20%

of the total Cd at emergence due to both higher concentrations in tenerals and lower concentrations in the exuviae (Fig. 2; Table 2). This also illustrates how substantially different metal dynamics can be in two congeneric species of *Libellula*.

Species average Pb concentrations ranged 2.94-10.7 mg/kg in shed exuviae but were less than 0.4 mg/kg in tenerals. Correspondingly, greater Pb mass was shed in the exuviae than accumulated in the teneral bodies of all 10 species (Fig. 2; $p<0.05$), with averages of 80-95% of the Pb being shed at emergence (Table 2).

Magnesium concentrations averaged > 900 mg/kg in teneral bodies of all 10 species as opposed to 310-738 mg/kg in shed exuviae. Correspondingly, greater Mg masses were retained in the teneral than were shed in exuviae (Fig. 3; $p<0.05$). In contrast, species average Fe concentrations in exuviae ranged from 1,674-13,014 mg/kg but were less than 250 mg/kg in tenerals. This resulted in all 10 species accumulating a greater mass of Fe in their exuviae than teneral bodies (Fig. 3; $p<0.05$) with averages of 63-94% of the total Fe shed with the exuviae (Table 2). Similarly, Al concentrations averaged 941-3217 mg/kg in the shed exuviae, but only 6.04-190 mg/kg in teneral bodies (Fig. 3). Consequently, 85-98% of the Al was shed in the exuviae (Table 2). Despite both shedding more Fe and Al than retained in the teneral, the two *Libellula* differed substantially in the percentage shed.

3.2. Metal flux from the wetland

A gradient in average teneral dry body mass among species ranged from *P. tenera* and *P. longipennis* (12.5 and 27.2 mg, respectively) being the smallest species, up to the 2 large *Pantala* species (77.0 and 90.9 mg, respectively) (Fig. 4). Average teneral dry body mass of the other 4 species ranged from 54.0 to 72.0 mg. Over the course of 32 sampling days distributed over the flying season, a total of 1265 individuals represented by 655 tenerals and 602 exuviae (exuviae not accompanied with a teneral) were collected from in or on the emergence cages. *Perythemis tenera* and *P. lydia* were collected in the greatest numbers (495 and 454, respectively), followed by 126 *L. auripennis*, 83 *P. longipennis*, and 54 *P. hymenaea* (Fig. 4). Less than 20 individuals of the other 4 species were collected over the 32 days. All species combined, emergence would have averaged a daily export of 1526 mg of dry dragonfly biomass from the emergence structures. Despite large numbers emerging on the structures, *P. tenera* represented only the third-highest flux of biomass per day (193 mg/day; Fig. 4) due to their small individual body mass. Biomass export was dominated by the moderate-sized *P. lydia,* which exported over one-half of the daily observed total dragonfly biomass. A total of 454 *P.*

lydia individuals (14 individuals/day) exported 766 mg of biomass/day. The second greatest export of biomass from the structures was 229 mg/day by a total of 126 *L. auripennis* (3.9 individuals/day; Fig. 4). The large body size of *P. hymenaea* resulted in a total of 54 individuals (1.7 individuals/day) exporting 153 mg/day of biomass from the wetland.

Comparison of how the daily flux of each element was distributed among species illustrates how individual body mass and numbers of individuals emerging per day influences dragonfly biomass daily flux that interacts with metal concentration to determine daily metal flux (Fig 4). Patterns in the daily flux of Cu, Zn, Mn, and Mg follow the pattern in the daily flux of biomass (Fig. 4). The greatest flux of these elements occurred in *P. lydia*, followed by *L. auripennis* and *P. tenera* as in the daily flux of body biomass (Fig. 4). Additionally, Fe follows this general pattern, but with greater flux in *P. lydia*, which reduces the percentage of other species such as *P. tenera* and *P. hymenaea*. In contrast, elevated concentrations of Cd, V, Al, and to a lesser extent Pb in *L. auripennis* tenerals (Figs. 2 and 3) increased the daily metal flux of *L. auripennis* compared to other species despite only 3.9 individuals collected from the traps each day (Fig. 4).

4. Discussion

4.1. Metal accumulation in nymphs versus tenerals and exuviae

We previously evaluated metal concentrations in six genera of aquatic dragonfly nymphs throughout the H-02 constructed wetland (Fletcher et al., 2020; Table S4). Nymphs analyzed in this previous study were held individually for 24 hours to clear their guts prior to sacrificing to provide an evaluation of what is incorporated into the nymph rather than just passing through their gut. During the Fletcher et al. (2020) nymph collections, the H-02 retention basin had cattails lining the basin margins and dense submergent vegetation in limnetic habitats. Two years later, when this emergent trapping was conducted, the regular application of aquatic herbicide had removed all vascular vegetation. Dragonfly community species composition appeared to have changed, but sufficient numbers of *Plathemis*, *Libellula, Tramea, and Pachydiplax* were present in both studies for comparison.

However, concentrations of Cu, Zn, Pb, Cd, Al, Fe, and V, in the shed exuviae tended to not only be higher than their associated teneral, but also substantially higher than nymphs collected prior to vegetation removal. The change in environmental conditions limited the utility of these comparisons. Visual inspection of nymphs and exuviae indicated that our rinsing

procedure removed surface sediments from both types of samples equally well. Additionally, no deposits were visible under a dissecting scope on the inside of the exuviae. However, some tissue remained in the apex of the abdomen. Proportionally more metals are shed during metamorphosis/emergence in habitats where metal exposure is increased (Groenendijk et al., 1999; Kim et al., 2012; Kraus et al., 2014; Kraus and Pomeranz, 2020). Accordingly, higher concentrations of both essential and nonessential elements may have resulted in shedding the observed large amounts in the exuviae. Alternatively, metals not visible by a dissecting scope or lodged in the apex of the abdomen could be inflating concentrations. The effects of vegetation removal on metal dynamics in invertebrates represent a needed area of study. Nevertheless, it appears evident that substantial portions of the nymph body burden could be shed with the exuviae during emergence.

4.2. Variability in Metal Export/Shedding

The shedding of metals by aquatic insects during metamorphosis/emergence has been shown to be highly variable, with up to 125 times higher concentrations in aquatic nymphs than flying adults (Kraus et al., 2014; Kraus et al., 2020; Kraus et al., 2021). This broad variability can result from both differences among taxa and among metals. Differences in biology, ecology, and physiology can influence rates of trace element uptake from diet and/or water along with efflux rates resulting in differential accumulation of contaminants among aquatic taxa inhabiting the same waterbody (Cain et al., 1992; Hare, 1992; Rainbow, 2002; Luoma and Rainbow, 2005; Buchwalter et al., 2008; Poteat et al., 2013). Correspondingly, it is becoming increasingly apparent that the degree to which contaminants are shed during metamorphosis/emergence versus exported from the wetland in emerging insects also often varies by taxa (Timmermans and Walker, 1989; Kraus et al., 2014; Wesner et al., 2020). Additionally, substantial proportions of both essential and nonessential metals may be shed by aquatic insects during metamorphosis, but this can differ between and within these categories (Kraus et al., 2014). However, differences between the retention of essential and nonessential metals may be due to other factors such as ionic softness rather than related to metabolic regulation of the elements (Kraus et al., 2014) as softer Class B soft ions tend to enter biota and be more readily transferred to the adults than Class A hard ions (Kraus et al., 2014; Cetinić et al., 2021). An ionic softness index explained 47% of the variation after removing Mn as an outlier in a meta-analysis (Kraus et al., 2014). Thus, ionic softness can be a significant influence, but unexplained variability remains to be understood. Below we discuss variability in the shedding

of metals as it relates to our study. Previous studies have shown that body mass of emerging odonates and the numbers emerging can influence metal flux from wetlands (Williams et al., 2017; Chumchal and Drenner, 2020). Overall, the amount of contaminant being exported from a wetland by emerging insects can be influenced by the contaminant concentration in individuals, individual body mass, and the number of individuals emerging throughout a flying season. This study showed how each of these factors can play a role in creating species-specific patterns of metal export from a contaminated wetland by emerging dragonflies.

4.2.1. Essential Trace Elements (Cu, Zn, Mn, V)

In the H-02 constructed wetlands, variability in both concentrations and body burdens of essential trace metals and taxa were prevalent. Metal concentrations in exuviae tended to vary more among taxa than in tenerals, and body mass influenced body burden. Higher Cu concentrations occurred in dragonfly exuviae than in tenerals. However, across species, the teneral contained 60 to 84% of the total Cu mass present in the teneral and lighter exuviae combined. This resulted in 1.4 to 5.4 times more Cu mass leaving the wetland in the teneral than being shed with the exuviae. Moreover, heavier dragonfly species tended to export more Cu in an individual (Fig. 5). These patterns of Cu and Zn illustrate why it is important to evaluate the mass of metals in an individual and not just concentrations, when evaluating contaminant flux from a wetland. Moreover, the Cu and Zn flux from the wetland was influenced by dragonfly biomass leaving the wetland, as shown by the mass flux carried by large numbers of moderate-sized *P. lydia* versus large numbers of small *P. tenera* with higher concentrations. Mass balances influenced by concentration combined with overall mass of each emerging species will establish the species- and metal-specific flux from a wetland. Consequences of this species specificity are evident by comparing total daily Zn flux calculated from an overall average of Zn flux per individual (all nine species combined) x the average number of individuals emerging per day, to summing the daily Zn flux of the individual species. Compositing species as a single taxon results in a daily Zn flux calculation that is 1.6 times higher than the actual. Whether these types of differences will be over- or under-estimated would depend upon species composition of the emerging dragonflies.

Manganese and V illustrate how extremely high differences in metal concentrations between shed exuviae and emerging tenerals can influence overall metal flux—despite the exuviae being much lighter than the teneral. Manganese is an essential element that was largely but variably shed during emergence of the H-02 dragonflies. Manganese concentrations were 11 to 54 times higher in the exuviae than teneral, which resulted in 1.8 to 11.0 times more

505 Mn being shed in the exuviae than leaving the wetland with the emergent. The 5 species that
506 averaged discarding over 80% of their total Mn mass with the exuviae also all had higher
507 concentrations in their exuviae than the other four species. Similarly, in the 8 species that shed
508 more V in their exuviae than retained in the teneral, V concentrations in exuviae averaged 13 to
509 47 times higher than in the teneral. In contrast, however, the two species with a similar mass of
510 V in tenerals and exuviae had elevated concentration levels in the tenerals. Similar to Cu and
511 Zn, Mn flux from the wetland was highest in *P. lydia*. However, the elevated V concentrations in
512 *L. auripennis* tenerals resulted in a higher flux in this species.
513 Among nymphs, exuviae, and free-ranging-feeding adults of the dragonfly *Gomphus*
514 *flavipes*, whether the greater metal mass was present in the nymphs or feeding adults varied by
515 site (Simon et al., 2019). However, similar to H-02 dragonflies, both nymphs and adults tended
516 to have greater masses of Cu and Zn but lesser masses of Mn than in the exuviae within which
517 much Mn was shed. Another dragonfly study showed that whether nymphs or tenerals had
518 higher Cu and Zn concentrations differed between sites, but nymphs consistently had higher
519 concentrations of V and Mn than tenerals, with nymphs having substantially higher
520 concentrations of Mn (Cetinić et al., 2021). These studies again illustrate the more prevalent
521 shedding of V and Mn than Cu and Zn.
522 Similar to dragonflies, mayflies (Ephemeroptera) have a hemimetabolous life cycle that
523 lacks a pupal phase during metamorphosis, although they undergo an additional molt after
524 emergence. Mayfly *Ephoron virgo* adult males and their exuviae had similar Cu concentrations,
525 but both were higher than those found in nymphs (Cid et al., 2010). Metals that are transferred
526 through metamorphosis/emergence to the emergent can become concentrated in emergent
527 species that lose weight during these developmental phases. In contrast, adult males had shed
528 Zn and Mn during metamorphosis/emergence with elevated Mn concentrations found in their
529 exuviae. In laboratory treatments, the mayfly *Neocloeon* (*Centroptilum) triangulifer* shed Zn
530 during metamorphosis/emergence with over 80% more Zn in nymphs than adults in their higher
531 exposure concentration treatments; however, the percentage shed varied according to exposure
532 level (Kim et al., 2012). Similarly, when the mayfly *Neocloeon triangulifer* was fed Zn enriched
533 diatoms, over 70% of the Zn nymph body burden was shed at emergence (Wanty et al., 2015).
534 Based on isotopically heavy Zn being retained by the emergent and the lighter Zn being shed, it
535 was suggested that as a detoxification strategy, light Zn is detoxified Zn that is shed, and the
536 heavier Zn represents metabolically needed Zn retained during metamorphosis/emergence
537 (Wanty et al., 2017). When the mayfly *Baetis tricaudatus* was exposed to Zn in the laboratory,
538 emergents had 7x lower Zn concentrations than larvae, but sex-dependent relationships were

observed (Wesner et al., 2017). In these hemimetabolous insects, Mn and V appear to be shed during metamorphosis to a greater extent than Cu and Zn, but Zn, in particular, can be highly variable among taxa and metal exposure levels. Manganese is often associated with the shed exuviae.

Insects with a holometabolous life cycle may have higher metal concentrations in adults than larvae due to mass loss during the extended, nonfeeding, pupal, and in some taxa, a nonfeeding adult phase, as was observed for Cu and Zn in trichopterans (Cetinić et al., 2021). Field and laboratory experiments illustrated taxonomic variability in the shedding of Cu during metamorphosis and emergence in chironomids that also have a holometabolous life cycle (Timmermans and Walker, 1989). Field-collected chironomid *Stictochironomus histrio* larvae had higher Cu and Zn body burdens than emergents with little shed in the exuviae, but *Chironomus anthracinus* body burdens did not differ between life stages (Timmermans and Walker, 1989). Additionally, in lab exposure experiments, *S. histrio* shed Cu during metamorphosis resulting in greater Cu body burdens in larvae than in pupae and adults with similar Cu burdens, so the Cu shedding occurred before emergence. Metal-specific patterns were illustrated in these individuals by Zn body burden not only being reduced during metamorphosis, but again at emergence as evident by higher Zn mass in pupae than adults. Further taxonomic variability within chironomids was evident by *C. riparius* larvae and adults having similar Cu and Zn body burdens (Timmermans and Walker, 1989). Larvae of the chironomid genus *Chironomus* accumulated a greater mass of Cu, Zn, and Mn than retained in adults caught by emergence and light traps in all of four sampled lakes (Krantzberg and Stokes, 1988). The proportion of these essential trace metals also varies in taxa- and metal-specific patterns in holometabolous insects.

Ionic softness may influence the proportion of metal retained by an emergent; “harder” Class A ions may tend to be shed (Kraus et al., 2014; Cetinić et al., 2021). Borderline elements between the Class A (hard) and Class B (soft) categories can exhibit characteristics of either type depending upon the situation. The ionic softness of Cu^{2+} and Zn^{2+} have been classified as borderline (Kinraide, 2009) or borderline A (Newman, 2010). These elements can be highly variable in the proportion shed during metamorphosis/emergence. $Manganese^{2+}$ has been classified as hard ions (Kinraide, 2009) and borderline A (Newman 2010), which may be consistent with the tendency for Mn to be shed. Additionally, Mn concentrations and masses are generally substantially higher in exuviae than emergents in ours and several of the above-cited studies. However, Lavilla et al. (2010) reported 69 to 73% of nymph Mn body burden to be bound to the surface of the nymph in three dragonfly species as evidenced by Mn being washed

573 off by sequential washes with ultrapure water, EDTA, ultrapure water, HCl, and ultrapure water
574 as opposed to only washing with ultrapure water. The Mn-binding was concluded to be
575 associated with the deposition of oxyhydroxides on the nymph's surface. Moreover, scanning
576 electron microscopy revealed the removal of inorganic particles of 15 µm maximum size from
577 the surface of these nymphs by acid washing. It stands to reason that surface-bound Mn would
578 have been shed with the exuviae at emergence. In contrast, Cu and Zn were not removed by
579 washing and were considered inside the organism. Multiple processes may be involved, but the
580 role of surface binding in the shedding of Mn should be further evaluated in more taxa. If
581 differences in concentrations in exuviae and tenerals are small, such as is often the case in Cu
582 and Zn, then the heavy mass of the teneral may determine whether more metals leave the
583 wetland or are shed with the exuviae. In contrast, substantially higher Mn concentrations in the
584 exuviae compared to the teneral resulted in most of the Mn being shed. Similarly, elevated
585 metal concentrations in the teneral, as with V in *L. auripennis,* can produce higher masses
586 leaving the wetland.

587

588 *4.2.2. Nonessential trace Elements (Cd, Pb)*

589 Cadmium is a toxic, nonessential element that generally accumulates to low concentrations in
590 aquatic insects (e.g. Cain et al., 1992; Lavilla et al., 2010). Greater concentrations accumulated
591 in the exuviae than tenerals of most dragonfly species, but whether a greater portion left the
592 wetland in the teneral or was shed in the exuviae differed among species. With averages
593 ranging from 20 to 76% across species, the percentage of Cd shed in the dragonfly exuviae
594 varied more among species than most other elements. However, it should be noted that with
595 these small masses of Cd, relatively small absolute differences in masses can result in a large
596 difference in the percentage shed as opposed to elements such as Fe or Mg that accumulate to
597 substantially greater masses. Across all of our dragonfly species, Pb accumulated in the
598 exuviae to concentrations that were 17 to 96 times higher than those retained in the teneral.
599 Correspondingly, 3 to 20 times more Pb remained in the wetland with the exuviae than left with
600 the emergent.

601 Exuviae of the dragonfly *G. flavipes* contained a greater mass of Pb than feeding adults
602 at three of four sites and more than nymphs at two sites (Simon et al., 2019); the shedding of Pb
603 at emergence was variable, but significant levels of Pb were generally shed in the exuviae.
604 Masses were not evaluated, but average Cd concentrations in dragonfly larvae (six composited
605 genera) were higher than those in adults (two composited genera) from a contaminated lake,
606 indicating Cd was shed during emergence or accumulation was reduced in feeding adults

(Currie et al., 1997). Dragonfly nymphs had higher concentrations of Pb than tenerals at 2 study sites and Cd at one of 2 sites (Cetinić et al., 2021). Mayfly *Ephoron virgo* Cd and Pb concentrations were higher in nymphs than adult males and their exuviae. However, between exuviae and adults, shed exuviae had lower Cd concentrations but higher Pb concentrations than adult males (Cid et al., 2010). Similarly, less than 10% of the larval Pb body burden in the caddisfly *Leptocerus americanus* was transferred to the emerging adult (Ryan et al., 2019). In contrast to their results for Cu and Zn, Timmermans and Walker (1989) found that field-collected chironomids (*S. histrio* and *C. anthracinu*s) shed substantial amounts of Cd in their exuviae when the larvae emerged to an adult (Timmermans and Walker, 1989). C*hironomus* larvae also accumulated a greater mass of Cd and Pb than found in adults caught by emergence and light traps in four of four examined lakes (Krantzberg and Stokes, 1988). *Chironomus riparius* larvae from polluted sites accumulated more Cd than from reference sites, but sufficient Cd was shed during metamorphosis/emergence that adults from polluted and reference sites had similar burdens of Cd (Groenendijk et al., 1999).

Lead and cadmium can be largely shed during emergence/metamorphosis, but particularly Cd can be variable. Such variability was found with a greater percentage of the Cd body burden being shed in the polluted sites; nearly 100% was shed during metamorphosis/emergence in contaminated sites, but 50 and 90% were shed in reference sites (Groenendijk et al., 1999). Moreover, when Pb and Cd are shed during emergence, there is a greater tendency for it to be shed with the exuviae than elements such as Cu and Zn. In three species of dragonfly nymphs, 38-80% of the Pb and 50-52% of the Cd were found to be associated with the nymph's exoskeleton (Lavilla et al., 2010). The role of surface binding warrants further investigation. However, regardless of whether Pb and Cd are binding to the surface or being expelled to the inside of the exuviae, both metals are often associated with the shed exuviae during emergence.

4.2.3. Essential major elements (Mg, Fe)

Though Mg is essential and can naturally accumulate to high concentrations, aquatic exposure to excessive Mg can potentially be toxic to aquatic organisms (Hogan et al., 2013). Less than 15% of the total Mg being shed in the exuviae of all dragonfly species, resulted in 6 to 18 times more Mg leaving the wetland in the teneral than being shed in the exuviae. In contrast, while Fe also accumulated to high concentrations (exceeded 10,000 mg/kg in some species), 1.6 to 16.6 times more Fe remained in the wetland with the exuviae than left with the emerging teneral. This illustrates distinctly different patterns in accumulation between these metals. Similarly, the

exuviae of *G. flavipes* contained a greater mass of Fe than in nymphs; nymphs, in turn, contained more Fe than feeding adults in all four study sites (Simon et al., 2019). Lower Fe concentrations in dragonfly tenerals than nymphs also suggested Fe shedding during metamorphosis/emergence at 2 study sites (Cetinić et al., 2021). *Chironomus* larvae also accumulated a greater mass of Fe than found in adults caught by emergence and light traps in four of four study lakes (Krantzberg and Stokes, 1988).

Magnesium has been classified as a Class A hard ion (Kinraide, 2009; Newman, 2010), which would suggest shedding of the element (Kraus et al., 2014). The incorporation of Mg into the teneral suggests that different factors must be involved in the transfer of this essential major element to the emergent. Fe^{2+} has been classified as a borderline or borderline Class A ion (Kinraide, 2009; Newman, 2010). This is consistent with observed patterns of Fe being shed. Like Mn, Fe has been shown to extensively surface bind to the nymph exoskeleton, with 68 to 71% of the nymph Fe body burden being surface bound in three species of dragonfly nymphs (Lavilla et al., 2010). The role of surface binding in the shedding of Fe during emergence should be further evaluated as well as the variability in Fe accumulation at a given size. Overall, these two essential major elements showed nearly opposite patterns of shedding during emergence.

4.2.4. Nonessential major element (Al)

Average concentrations of the nonessential element Al in the shed exuviae across species ranged from 9 to 281 times higher than in the associated tenerals. This resulted in 2 to 45 times more Al being shed in the exuviae and remaining in the wetland than leaving with the teneral. The exuviae of *G. flavipes* also contained a greater mass of Al than nymphs which contained more Al than feeding adults in four of four studied sites (Simon et al., 2019). Similarly, aluminum concentrations in dragonflies were substantially reduced during metamorphosis/emergence at 2 study sites (Cetinić et al., 2021). *Chironomus* larvae accumulated a greater mass of Al than found in adults caught by emergence and light traps in three of four study lakes and a similar mass in one lake (Krantzberg and Stokes, 1988). Aluminum has been classified as a Class A/hard ion (Kinraide, 2009; Newman, 2010), which is consistent with the observed shedding of Al. Overall, a large portion of the Al can be lost during metamorphosis/emergence, and a large portion of this loss can be associated with the exuviae. The possibility of Al surface binding remains to be evaluated.

5. Conclusions

675
676 The amount of metal leaving the wetland in an emerging dragonfly and the amount left behind in
677 the exuviae were shown to vary among both metals and species. Moreover, taxonomic- and
678 metal-specific variability in daily metal flux from the wetland depended upon concentration
679 accumulated, individual body mass, and number of individuals emerging, with each factor's
680 relative importance often differing among species. These patterns illustrate why it is important
681 to evaluate the mass of metals in an individual and not only concentrations when evaluating
682 body metal distributions or metal flux from a wetland. Similar to others' findings (e.g. Kraus et
683 al., 2014), whether elements were transferred to the teneral or shed with the exuviae was not
684 consistent within the categories of essential and nonessential trace and major elements. Often-
685 shed elements include both essential (Fe, Mn, and V) and nonessential (Al, Cd, and Pb)
686 elements, as well as elements that commonly accumulate to low levels (Cd and Pb) in/on
687 aquatic nymphs and elements that often accumulate to higher levels (Fe, Mn, and Al).
688 However, of our analyzed elements, all elements that showed a higher tendency to leave the
689 wetland with the teneral were all essential (Cu, Zn, Mg). Some patterns were consistent with
690 previous studies of ionic softness (Kinraide, 2009; Kraus et al., 2014; Cetinić et al., 2021) and
691 surface binding (Lavilla et al., 2010). Whether metals were bound to the surface of the exuviae,
692 incorporated into the exuviae matrix, or expelled into the inside of it during/after metamorphosis
693 was outside of the scope of our present study, but this remains an area of needed further
694 research as is whether additional metal mass is expelled from the nymph during
695 metamorphosis/emergence that is not retained in the exuviae. Species-specific patterns of
696 individual metal flux included substantial differences between the two similar-sized members of
697 the genus *Libellula*—even though the species accumulating or shedding highest metal amounts
698 varied among metals. Furthermore, differences in numbers of each species emerging will
699 magnify the species-specific differences in individual metal flux when evaluating overall metal
700 flux. Although patterns within groups of species will occur and site-specific contamination levels
701 will influence patterns, species-specific differences have the potential to hamper evaluations of
702 metal flux when multiple species are composited into a single taxon. Overall, metals shed in
703 dragonfly exuviae have the potential to substantially moderate the transport of metals from
704 contaminated wetlands, but the species- and metal-specific patterns need to be better
705 understood.
706
707 **Appendix A. Supplementary data**
708

Supplementary data to this article can be found online at https://_____.

Acknowledgements

We thank Danielle Pitt and Brooke Lindell for laboratory and field assistance. This work was supported by the National Nuclear Security Administration and the Department of Energy under award number DE-EM0004391.

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931 **Table 1.** Numbers of tenerals and exuviae analyzed for each
932 dragonfly species.

Species	Teneral	Exuviae
Libellulidae		
Leucorrhiniinae		
Brachymesia gravida	14	14
Libellulinae		
Libellula auripennis	10	10
Libellula luctuosa	5	5
Orthemis ferruginea	12	12
Plathemis lydia	43	43
Sympetrinae		
*Pachydiplax longipennis**	8	19
Palpopleurinae		
*Perithemis tenera**	33	22
Trameinae		
Pantala flavescens	15	15
Pantala hymenaea	46	46
Tramea lacerata	5	5
Total	191	191

933 *Multiple exuviae of this species were composited to attain sufficient mass.
934

Table 2. Percent of total teneral + exuviae mass composed of the exuviae and the percent of each element shed with the exuviae upon emergence. Percent shed is coded by color: yellow 0-25%, peach 26-50%, blue 51-75%, violet 76-100%.

Subfamily	Species	Mass[1]	Cu	Zn	Mg	Cd	Mn	V	Fe	Al	Pb
Essential/Nonessential[2]			E	E	E	N	E	E	E	N	N
Leucorrhiniinae	*B. gravida*	15	23	15	7	49	72	51	76	95	83
Libellulinae	*L. auripennis*	16	38	23	8	20	70	45	79	85	80
	L. luctuosa	17	41	34	12	69	90	80	94	97	93
	O. ferruginea	17	34	35	14	76	89	90	93	97	95
	P. lydia	16	40	32	12	75	87	84	90	95	94
Sympetrinae	*P. longipennis*	13	38	29	8	36	87	85	88	98	87
Palpopleurinae	*P. tenera*	17	33	33	11	66	84	86	87	98	89
Trameinae	*P. flavescens*	13	16	10	5	44	66	77	70	91	81
	P. hymenaea	14	16	12	6	54	64	72	63	92	85
	T. lacerata	15	19	13	5	38	74	71	73	94	88

[1][exuviae dry mass/(teneral dry mass + exuviae dry mass)] x 100

[2]E = Essential; N = Nonessential (Mertz 1981)

xes

Figure Legends

Figure 1. Comparison of the concentrations and mass of Cu, Zn, and Mn in the teneral body and exuviae of *B. gravida*, *L. auripennis*, *L. luctuosa*, *O ferruginea*, *P. lydia*, *P. longipennis*, *P. tenera*, *P. flavescens*, *P. hymenaea*, and *T. lacerata*. * denotes comparisons within a species significant at $p<0.05$.

Figure 2. Comparison of the concentrations and mass of V, Cd, and Pb in the teneral body and exuviae of *B. gravida*, *L. auripennis*, *L. luctuosa*, *O ferruginea*, *P. lydia*, *P. longipennis*, *P. tenera*, *P. flavescens*, *P. hymenaea*, and *T. lacerata*. * denotes comparisons within a species significant at $p<0.05$.

Figure 3. Comparison of the concentrations and mass of Mg, Fe, and Al in the teneral body and exuviae of *B. gravida*, *L. auripennis*, *L. luctuosa*, *O ferruginea*, *P. lydia*, *P. longipennis*, *P. tenera*, *P. flavescens*, *P. hymenaea*, and *T. lacerata*. * denotes comparisons within a species significant at $p<0.05$.

Figure 4. Distribution of individual dry body mass, numbers of individuals emerging, daily body mass flux, and the daily flux of Cu, Zn, Mg, Mn, Fe, Pb, Cd, V, and Al among species.

Figure 5. Relationships of the mass of Cu, Zn, and Fe in the teneral body with dry teneral body mass in *B. gravida*, *L. auripennis*, *L. luctuosa*, *O ferruginea*, *P. lydia*, *P. longipennis*, *P. tenera*, *P. flavescens*, *P. hymenaea*, and *T. lacerata*.

Figure 1

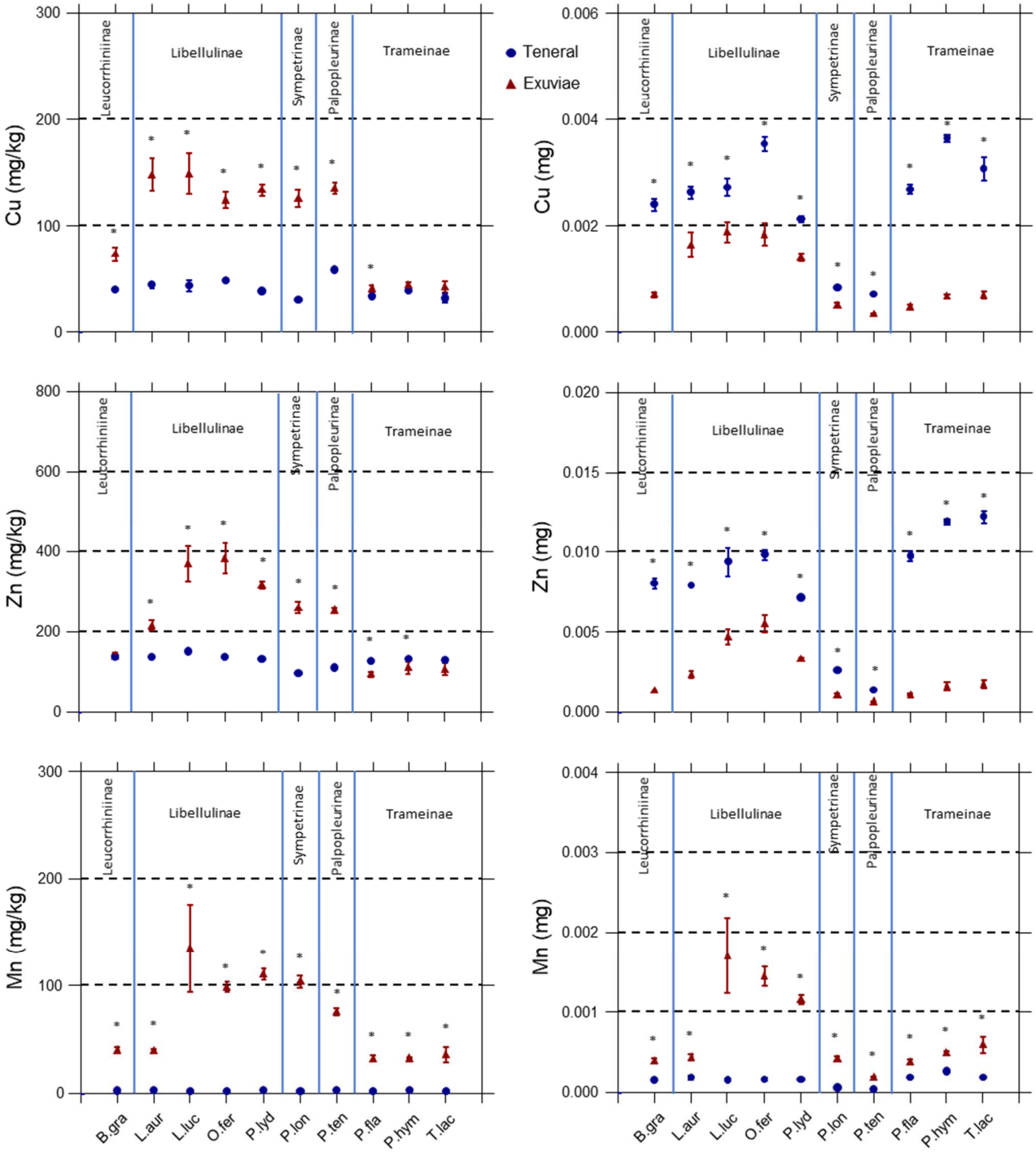

Teneral
Exuviae
Cu (mg/kg)
Cu (mg)
Zn (mg/kg)
Zn (mg)
Mn (mg/kg)
Mn (mg)
Leucorrhiniinae
Libellulinae
Sympetrinae
Palpopleurinae
Trameinae
B.gra
L.aur
L.luc
O.fer
P.lyd
P.lon
P.ten
P.fla
P.hym
T.lac

Figure 2

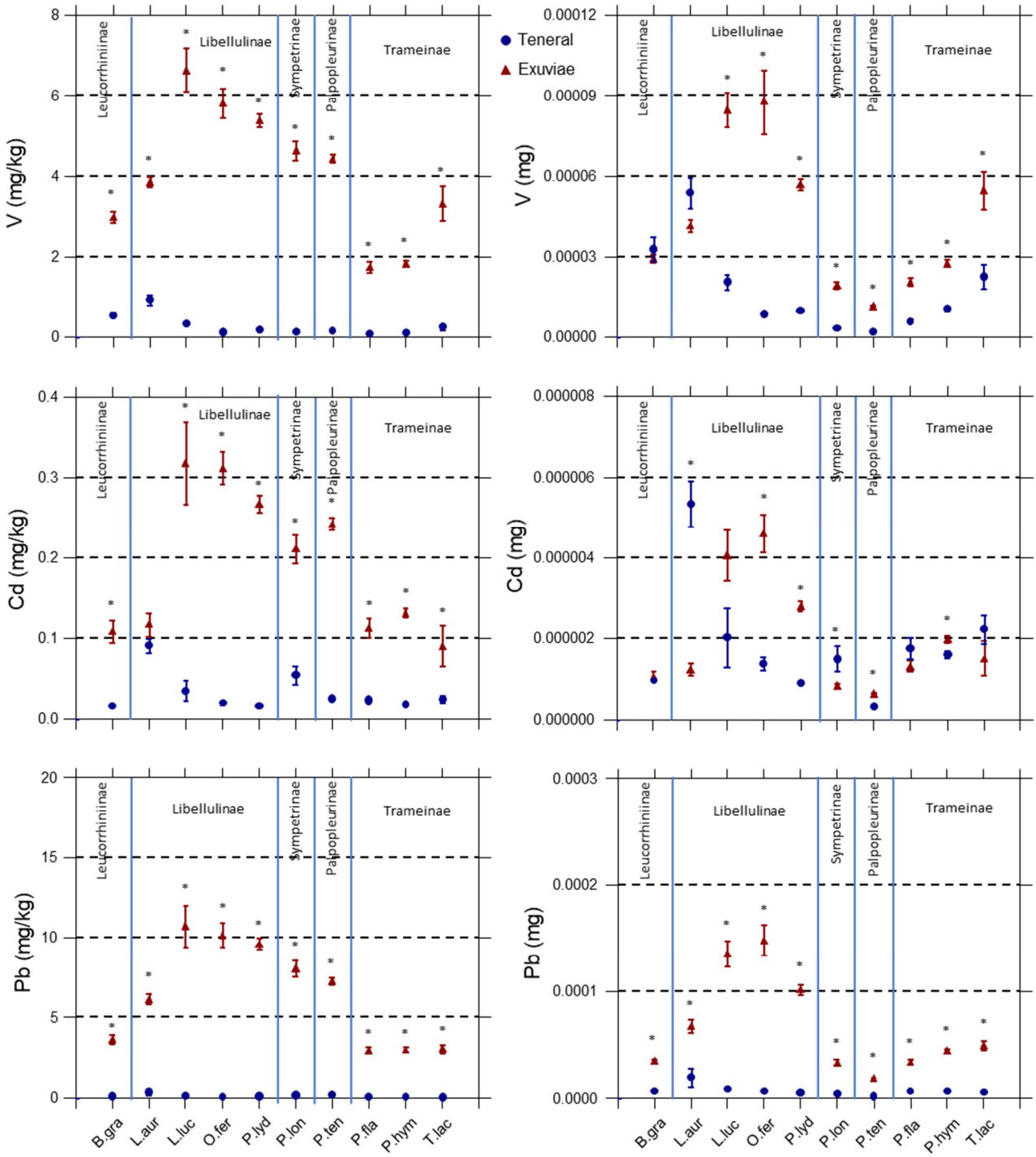

Teneral
Exuviae
V (mg/kg)
V (mg)
Cd (mg/kg)
Cd (mg)
Pb (mg/kg)
Pb (mg)
Leucorrhiniinae
Libellulinae
Sympetrinae
Palpopleurinae
Trameinae
B.gra
L.aur
L.luc
O.fer
P.lyd
P.lon
P.ten
P.fla
P.hym
T.lac

Figure 3

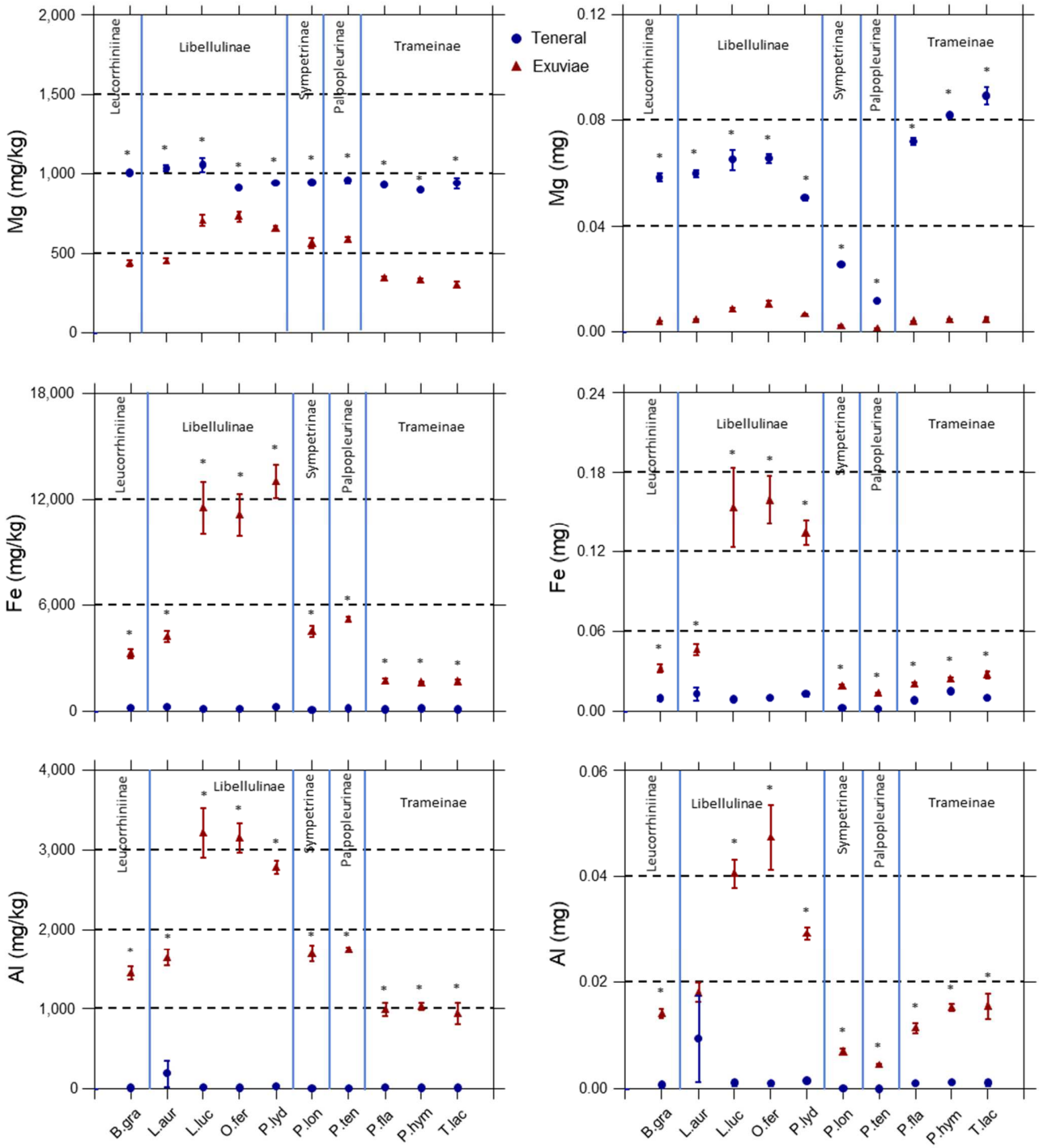
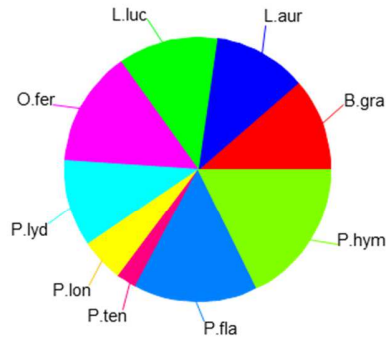
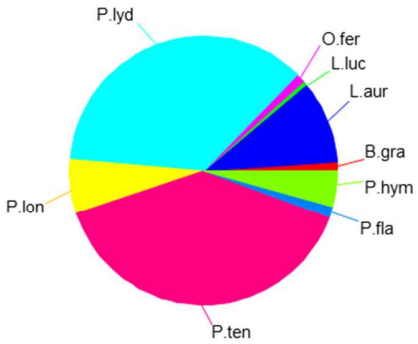

Teneral
Exuviae
Mg (mg/kg)
Mg (mg)
Fe (mg/kg)
Fe (mg)
Al (mg/kg)
Al (mg)
Leucorrhiniinae
Libellulinae
Sympetrinae
Palpopleurinae
Trameinae
B.gra
L.aur
L.luc
O.fer
P.lyd
P.lon
P.ten
P.fla
P.hym
T.lac

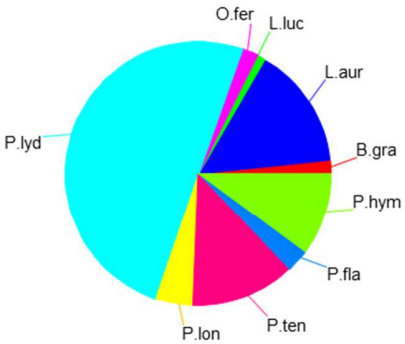
Figure 4



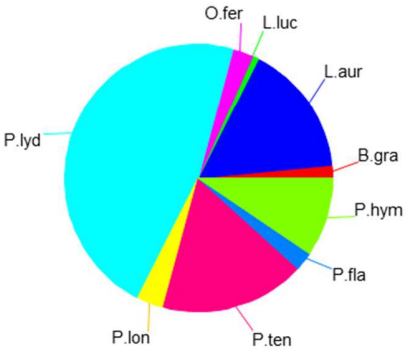
Individual Dry Body Mass (mg)



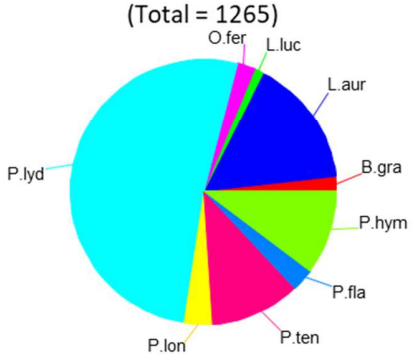
Numbers
(Total = 1265)



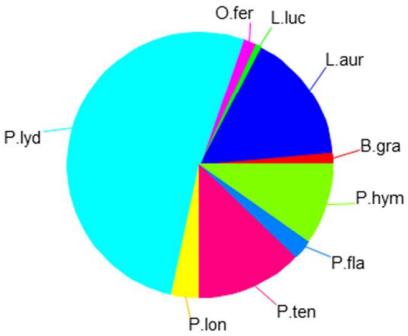
Dry Body Mass Flux
(Total = 1526 mg/day)



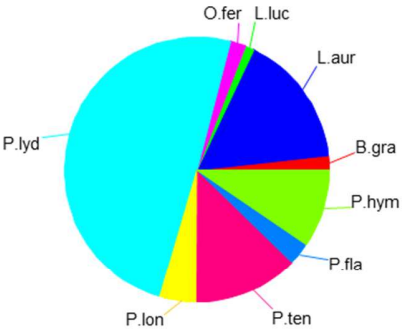
Cu flux
(Total = 0.0653 mg/day)



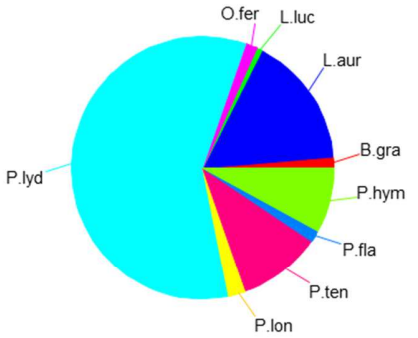
Zn flux
(Total = 0.196 mg/day)



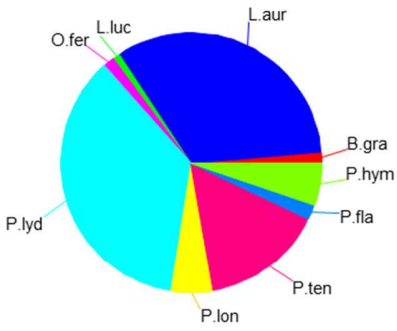
Mn flux
(Total = 0.00467 mg/day)



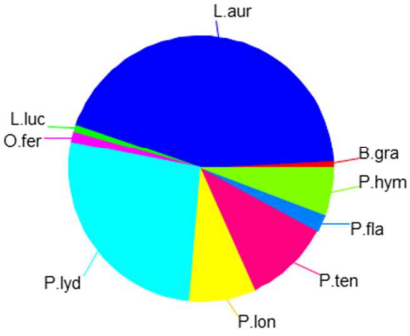
Mg flux mg/day
(Total = 1.460 mg/day)



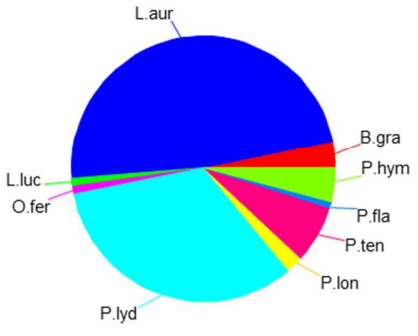
Fe flux
(Total = 0.322 mg/day)



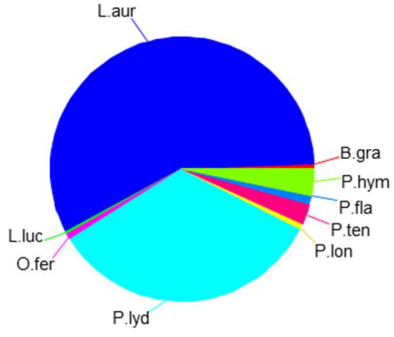
Pb flux
(Total = 0.000239 mg/day)



Cd flux
(Total = 0.0000481 mg/day)

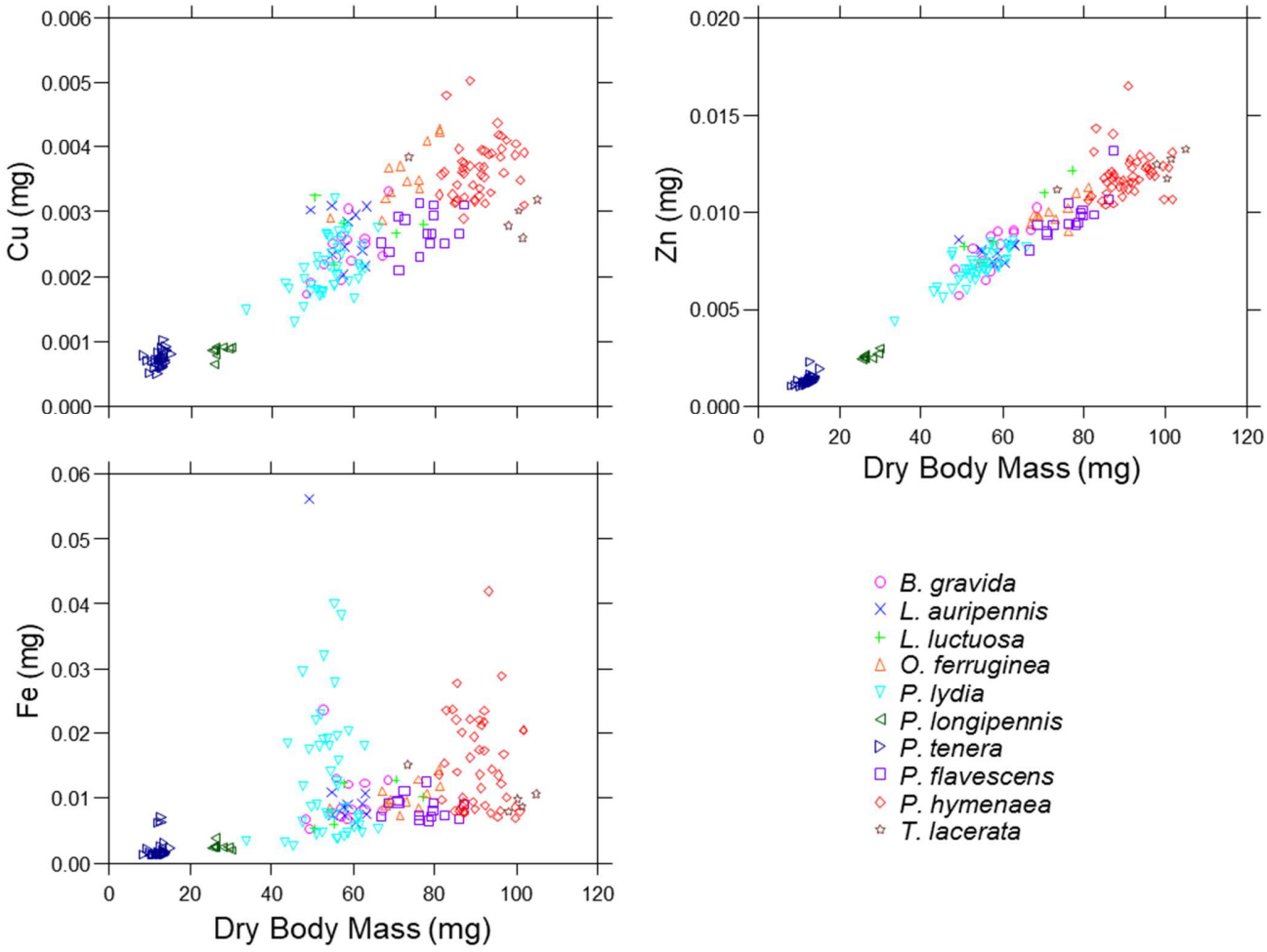


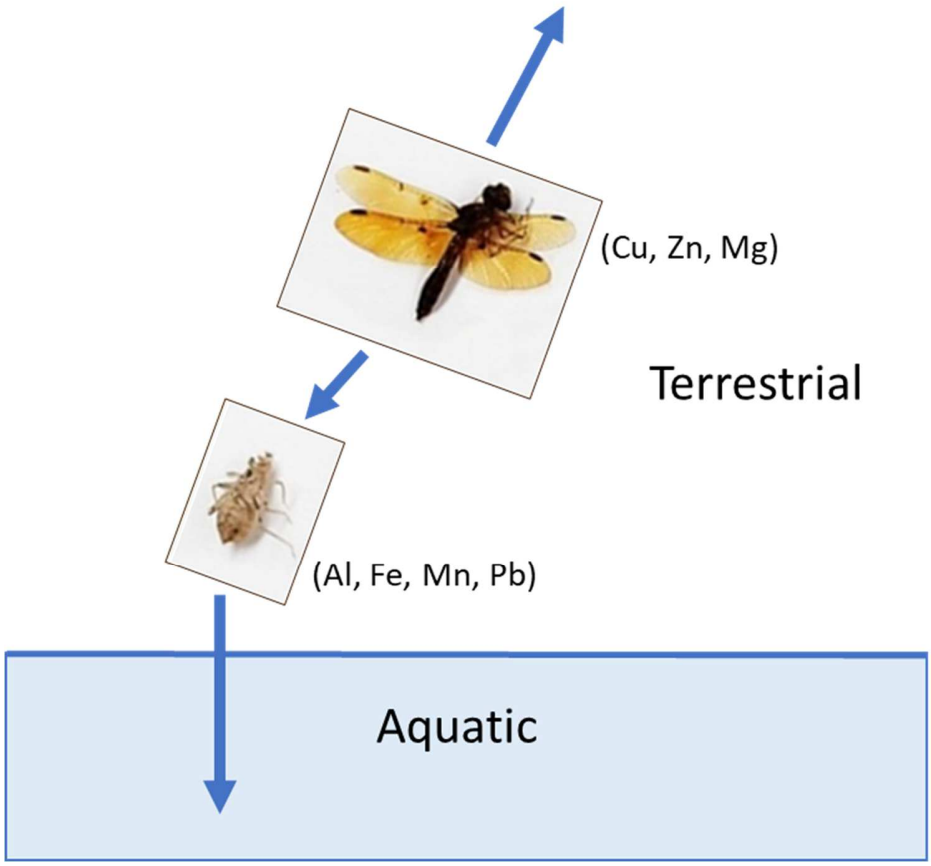
V flux
Total =(0.000442 mg/day)



Al flux
(Total = 0.0655 mg/day)

Figure 5


Cu (mg)
0.006
0.005
0.004
0.003
0.002
0.001
0.000
Zn (mg)
0.020
0.015
0.010
0.005
0.000
Fe (mg)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
20
40
60
80
100
120
Dry Body Mass (mg)
Dry Body Mass (mg)
B. gravida
L. auripennis
L. luctuosa
O. ferruginea
P. lydia
P. longipennis
P. tenera
P. flavescens
P. hymenaea
T. lacerata


(Cu, Zn, Mg)
Terrestrial
(Al, Fe, Mn, Pb)
Aquatic