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Chronic Engineered Nanoparticle Additions Alter Insect Emergence and Result in Metal Flux from Aquatic Ecosystems into Riparian Food Webs

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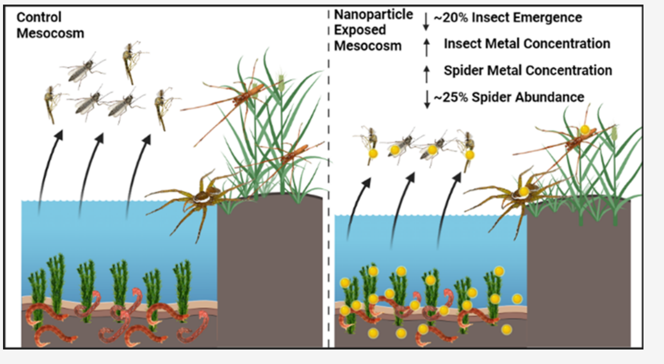
ABSTRACT: Freshwater ecosystems are exposed to engineered nanoparticles (NPs) through discharge from wastewater and agricultural runoff. We conducted a 9-month mesocosm experiment to examine the combined effects of chronic NP additions on insect emergence and insect-mediated contaminant flux to riparian spiders. Two NPs (copper, gold, plus controls) were crossed by two levels of nutrients in 18 outdoor mesocosms open to natural insect and spider colonization. We collected adult insects and two riparian spider genera, *Tetragnatha* and *Dolomedes*, for 1 week on a monthly basis. We estimated a significant decrease in cumulative insect emergence of 19% and 24% after exposure to copper and gold NPs, irrespective of nutrient level. NP treatments led to elevated copper and gold tissue concentrations in adult insects, which resulted in terrestrial fluxes of metals. These metal fluxes were associated with increased gold and copper tissue concentrations for both spider genera. We also observed about 25% fewer spiders in the NP mesocosms, likely due to reduced insect emergence and/or NP toxicity. These results demonstrate the transfer of NPs from aquatic to terrestrial ecosystems via emergence of aquatic insects and predation by riparian spiders, as well as significant reductions in insect and spider abundance in response to NP additions.



KEYWORDS: *aquatic−riparian linkages, food web, nanoparticles, trophic transfer, riparian spiders*

■ INTRODUCTION

Engineered nanoparticles (NPs), particles with novel properties that are less than 100 nm in at least one dimension, are frequently used in consumer products and enter aquatic ecosystems through consumer waste streams. It has been previously estimated that 21,000 t of NPs annually enter aquatic ecosystems, primarily through agricultural runoff and wastewater treatment plants.[1,2] Once NPs are released into the environment, they interact with complex aquatic ecosystems where they have been shown to have a variety of ecosystem effects, from increased primary productivity to accumulation in aquatic organisms, including aquatic insects.[3−6] Aquatic insects are essential components of aquatic ecosystems and are an important subsidy to both terrestrial and aquatic food webs.[7] Previous studies have evaluated the effects of NPs on aquatic insects, but most have focused on short-term laboratory exposures using model organisms.[8−10] Less research has been conducted on naturally assembled aquatic insect communities in outdoor aquatic ecosystems, in particular tracking how NP additions affect insect emergence.

Exposure to NPs has been shown to have adverse effects on all life stages of insects. Many studies have found effects ranging from gene expression and oxidative stress responses in larval stages to shorter life span in adults and a myriad of other lethal and sublethal toxicity responses in between.[11,12] Gold (Au) NPs have been observed to have detrimental effects on insect life history, including inhibiting trypsin production and altering development and reproduction.[11,13−15] Fewer studies have been completed on a copper (Cu) NP mode of action in insects, though Cu salts and minerals have been extensively used as biocontrol of insects in agricultural environments, especially in organic farming and vineyard applications.[16−18] Several field and mesocosm studies have found significant declines in insect emergence after prolonged dissolved Cu

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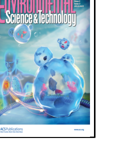

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exposure in several taxa including chironomids, mayflies, and stoneflies.[19−22] While the exact mechanisms of chronic CuNP toxicity have not been resolved in larval aquatic insects, studies comparing CuNP and dissolved Cu toxicity have found CuNPs to be more toxic to benthic invertebrates.[23] However, the effects of AuNPs and CuNPs on natural insect communities through metamorphosis remain to be addressed.

Accumulation of NPs in larval insects could result in a transfer of NPs from the aquatic to the riparian food web if NPs are retained to any extent through metamorphosis. Contaminant flux through insect subsidy has been previously observed in contaminants such as NPs (AgNPs, AuNPs, TiO_2NPs), heavy metals, PCBs, methylmercury, selenium, per- and polyfluoroalkyl substances, and pharmaceuticals.[3,15,24−28] However, the retention of contaminants through insect metamorphosis varies by contaminant.[29] For Cu, concentrations are generally similar between larval insects and adults.[29,30] Data on metamorphic retention of metals from CuNPs and AuNPs are lacking, but one study found that caddisflies retain some Au from AuNPs through emergence as adults.[15]

Insect emergence into the riparian food web provides an essential prey subsidy for riparian consumers, such as spiders and songbirds, and can contribute to the detrital food web in the terrestrial environment.[7,31,32] Riparian spiders, such as *Tetragnatha* and *Dolomedes*, are important links for the transport of contaminants from aquatic insects to terrestrial ecosystems.[33−38] Tetragnathids have been shown to accumulate aquatic contaminants, including metals in other polluted ecosystems.[26,38,39] They have life history characteristics that make them especially compatible with tracing the flux of contaminants from aquatic to terrestrial ecosystems since they are globally distributed, relatively sedentary as adults, and specialize in the consumption of aquatic insects.[33,37]

NPs are likely to enter aquatic ecosystems primarily via wastewater treatment and agricultural runoff due to their inclusion in consumer and industrial products. They are likely to co-occur with excess nutrients, primarily nitrogen and phosphorus. Surface waters have become nutrient-enriched largely due to urban and agricultural practices, and interaction between NPs and nutrients are likely frequent occurrences in the environment. Previous components of this mesocosm study found that Cu from CuNPs associated with the sediments while Au from AuNPs were mostly associated with the aquatic macrophytes (Table S1). Nutrients did not have any effect on metal accumulation into various mesocosm compartments, but we observed interactions between nutrients and nanoparticles resulting in increased primary productivity.[5,40] Here, we studied the interactive effects of additions of copper hydroxide nanoparticles (CuNPs) or gold nanoparticles (AuNPs) and nutrient enrichment on the rate of insect emergence, metal accumulation in adult insects, and flux of metals into riparian spiders in a 9-month wetland mesocosm experiment. We added CuNPs found in a commercially available nanopesticide at the application dose to investigate particle and biotic interactions likely to be occurring in aquatic ecosystems. We used AuNPs as a model nanoparticle because it is easily detectable in complex environmental matrices, in part because of its low natural background, enabling us to study the fate of these particles.

■ MATERIALS AND METHODS

Wetland Mesocosm Setup and Experimental Design. The mesocosms used in this study were located at the Center for Environmental Implications of NanoTechnology (CEINT) facility in the Duke Forest in Durham County, North Carolina, USA. They were constructed above ground with pressure treated lumber to dimensions 3.66 m × 1.22 m × 0.81 m, spaced 1 m apart, and have been used in previous studies.[5,41,42] Prior to the addition of ∼250 L of groundwater, a layer of sand was added to the boxes and then mesocosms were lined with a fish-safe ethylene propylene diene monomer rubber liner (PondGard; Firestone, Nashville, TN) overlaid by a high-density polyethylene material (Permalon; REEF Industries,Inc., Houston, TX), making mesocosms watertight.[42] In each of the mesocosms, there was an upland zone that never flooded, a transitional zone with occasional flooding depending on rainfall, and an aquatic zone that was permanently flooded. The relative size of each zone varied as mesocosm volumes fluctuated seasonally, but typically the permanently flooded zone covered about 70% of the total surface area of each mesocosm.[41] In July 2015 the mesocosms were inoculated with zooplankton and periphyton, then in September 2015 equal numbers of mosquitofish (*Gambusia holbrooki*), and aquatic snails (*Physella acuta* and *Lymnaea* sp.) were added to mesocosms to standardize predation pressure. All organisms acclimated to mesocosm conditions for several months (summer and fall seasons) prior to the start of dosing in January 2016. After September 2015, the mesocosms were open to allow for natural colonization of insects and spiders.

Mesocosms were randomly assigned to a combination of NP and Nutrient status with three replicates for each of the six treatments: control-ambient, control-enriched, CuNP-ambient, CuNP-enriched, AuNP-ambient, and AuNP-enriched. Nutrient loading started in September 2015. The ambient treatments did not receive any additional nutrient supplement, while the enriched received 1 L of mesocosm water supplemented with 88 mg of N (as KNO_3) and 35 mg of P (as KH_2PO_4), weekly. While the mesocosm water volume varied seasonally, the weekly dose remained consistent throughout the study. Dosing was throughout the entire aquatic zone just below the surface of the water using a modified Mariotte's bottle and distributed in a grid like pattern. Weekly NP dosing began in January 2016. In total, 18 boxes were established and dosed with both nutrients and NPs weekly for nine months.[5]

The CuNP used in this study is a commercially available nanoenabled fungicide/bactericide, sold as a powder (KOCIDE 3000, DuPont, Wilmington, DE). Kocide is ∼46% $Cu(OH)_2$, which is ∼27% Cu by dry weight, 1−5% of (undefined) clays, 5−10% 2-propenoic acid, and 38.9−47.9% of "other proprietary ingredients".[6,43] Kocide contains nanoparticles and microparticles of Cu, as well as nanosheets composed of $Cu(OH)_2$.[44,45] Size fractionation studies of Kocide found that all Cu species were smaller than 0.45 μm, with 20% passing through a 0.1 μm filter in water.[6,43] In our study, Kocide was first spiked (347 mg), and then 34.67 mg was added weekly, representing a cumulative Cu dose of 450 mg after 9 months. Inflows of agricultural runoff are episodic, occurring during rainfall or irrigation, so this scenario is not atypical. We assumed an application rate of 20 kg/ha, the manufacturer recommended intermediate application rate. We assumed a 10:1 ratio between cultivated land and the aquatic compartment of our mesocosms and a 6% rate of loss of agrochemicals from surface soils.[46] When scaled to the 9-month window of our experiment, this gave an addition rate of 450 mg of Cu per mesocosm.[5] In contrast, Au is more likely to be released from wastewater treatment plants and in this study was used as a model nanoparticle that could be easily detected in complex environmental media due to the low natural Au background. We chose to maintain dosing concentrations at levels we expected to be greater than instrument detection limits. Citrate-coated AuNPs were synthesized at Duke University following a previously established protocol.[47] The AuNP mesocosms received a weekly dose of 19 mg Au resulting in a total dose of 750 mg Au per AuNP mesocosm.[65,6] Additional NP characterization, weekly water column metal concentrations, and metal masses in all biological compartments are reported in Avellan et al. and repeated in the Supporting Information (Figures S1 and S2 and Tables S1 and S2).[6] Additional details about mesocosm setup, water chemistry, and NP dissolution from this mesocosm study are reported in Simonin et al. and Avellan et al.[5,6]

To briefly summarize mesocosm fate and transport results reported in Avellan et al., large quantities (>50%) of Cu transferred into the nonflooded compartments (transition and upland soils) while Au was mostly retained (65−90%) in the aquatic compartment associated with macrophytes or in the floc and aquatic sediment. Kocide had a fast dissolution rate in mesocosm water (half-life of 8 h).[48] In our study, Cu was concentrated in the water column, more labile, and thus amenable to distribution into the upland and transition zones as water levels fluctuated. In contrast, Au is relatively stable against dissolution, thus remained in the aquatic portion of the mesocosms and associated with the macrophytes and aquatic sediments.[6]

Emergence traps were placed over the entire aquatic surface area of each mesocosm to collect all adult insects that emerged during deployment. Traps were deployed for about 1 week of every month during the nine-month experiment. Sampling dates were Week 12 (April 11−17), Week 17 (May 19−22), Week 22 (June 20−26), Week 27 (July 25−31), Week 32 (August 29−September 4), and Week 38 (October 10−17). Emergence traps were pyramid shaped, constructed from untreated pine lumber, and lined with fiberglass mesh stapled to the wooden frame. The base of the emergence traps was 1.22 m × 1.22 m and about 1 m in height with a mosquito breeder at the top. Mosquito breeders are often used for rearing field collected mosquito larvae in lab and consist of an inverted funnel opening into an enclosed plastic housing (Bioquip, Rancho Domingo, CA). Insects were collected from the trap twice per week using a hand-held insect vacuum (Bioquip 2820GA, SKIL, Naperville, USA). Weekly dosing occurred immediately prior to placing emergence traps.

We did not sample the entirety of the experiment, but we captured all the emerging adult insects while traps were deployed, cumulatively representing about 20% of the days across the 9-month study period. As such, the result was a spatially and temporally integrative sampling strategy, giving us a strong inference of the total flux and larger sample masses that enabled our metal and isotope measurements.

Spiders were collected from the mesocosms as they were observed during the time the emergence traps were deployed. A sterile, 50 mL falcon tube was used to collect the *Tetragnatha* spiders from their webs in the planted transition zones, while *Dolomedes* were more commonly collected from the surface of the water and placed into a 50 mL falcon tube.

Eastern mosquitofish (*Gambusia holbrooki*) were harvested using a net in Week 38. Mosquitofish were immediately euthanized with tricaine methanesulfonate buffered with bicarbonate and frozen (Duke University Institutional Animal Care and Use Committee protocol no. A135-16-06).

Insect and Spider Identification. We sampled over 8,700 insects and 200 spiders over six sampling events during the study. All insects were first separated by order, and Chironomidae were sorted to morphospecies. The eight most abundant morphospecies by number were identified to the lowest possible taxon (Table S3), and spiders were sorted to genus.[49,50] Chironomidae represented 92% of emerging insects; therefore, they were the only insects to be analyzed for metals. *Tetragnatha* and *Dolomedes* were sufficiently abundant among all mesocosms thus were included in metal analyses. One limitation of this study is that morphospecies IDs are not sufficiently reliable to determine underlying patterns in community composition when the majority of morphospecies are Chironomidae. Future studies should consider DNA barcoding to conclusively identify emerged adult taxa.

Metal Digestion. Insects, spiders, and mosquitofish were analyzed for NP uptake by quantifying concentration of Cu and Au using inductively coupled plasma mass spectrometry (ICP-MS). Chironomidae were combined by sampling event, oven-dried at 60 °C, pulverized, rehydrated with ultrapure water (18.2 MΩ-cm), and fully acid digested. Spiders and mosquitofish were processed individually in the same manner. Digestions followed a previously published protocol.[6,40] Cu and Au were quantified using an Agilent 7900 ICP-MS generally following U.S. EPA Method 6020A.[51] All analytical runs included reagent blanks, fortified water and tissue-mixed spike recovery samples, and standard reference material recovery (NIST 1643e). Blank verification, drift checks and intercalibration/cross-calibration verification were performed for every 10 samples. Additional ICP-MS methods are detailed in the SI, section "ICP-MS Methods".

We calculated the daily metal "flux" by dividing the mass of metal accumulated in the emerged aquatic insects by the number of days the emergence traps were deployed. We then estimated the cumulative flux by multiplying daily metal flux by the number of days present in the sampled month. We used a cumulative sum over the course of the experiment to obtain the estimated cumulative flux (see Figure 2B,D). Overall, we measured total Au and Cu metal concentration in the whole-body tissue, and we assumed that the difference in metal concentration between the control and NP mesocosms was related to the NP dosing.

Statistical Analysis. Insect emergence and metal accumulation in insect tissue were modeled using generalized linear mixed models (GLMMs) and linear mixed models (LMM), respectively, to determine the effects of NP exposure, Nutrient and Sampling event (hereafter called Week). NP Treatment (Control, Au, Cu) and Nutrient treatment (Ambient and Enriched) were nested within Week.[52] Mesocosm number (each mesocosm was assigned a unique numerical identifier) was treated as a random effect, which accounted for repeated observations from the same mesocosms over time and subsequently modeled the random effect of each mesocosm on these values. Insect emergence was fit using the "glmer.nb" function while metal accumulation and mass models were fit using the "lmer" function in the lme4 package in RStudio using R (version 4.2.2).[53,54] Spider abundance was modeled using a generalized linear model (glm). Model fits were evaluated

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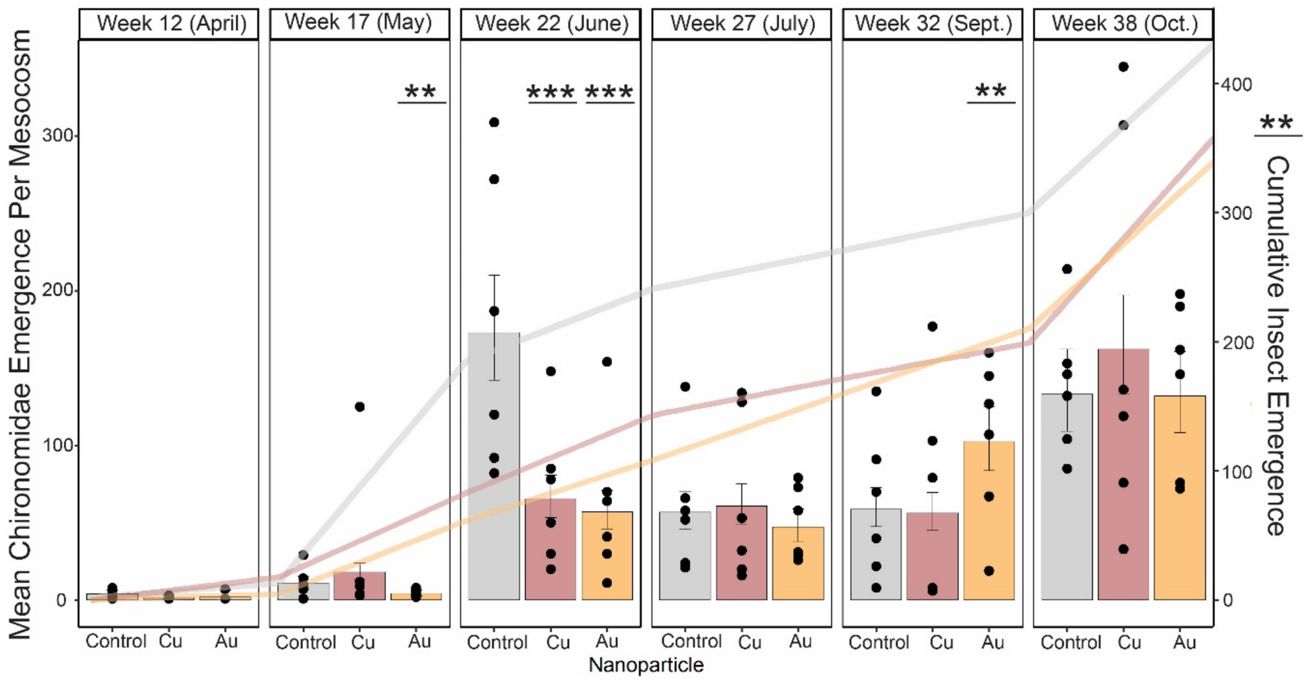

Figure 1. Mean counts ±95% CI of Chironomidae emerging from mesocosms by sampling week and treatment. Points represent individual mesocosm emergence. NP levels are distinguished by bar colors. Cumulative Chironomidae emergence numbers by NP level are illustrated by lines (right *y*-axis) and are distinguished by color.

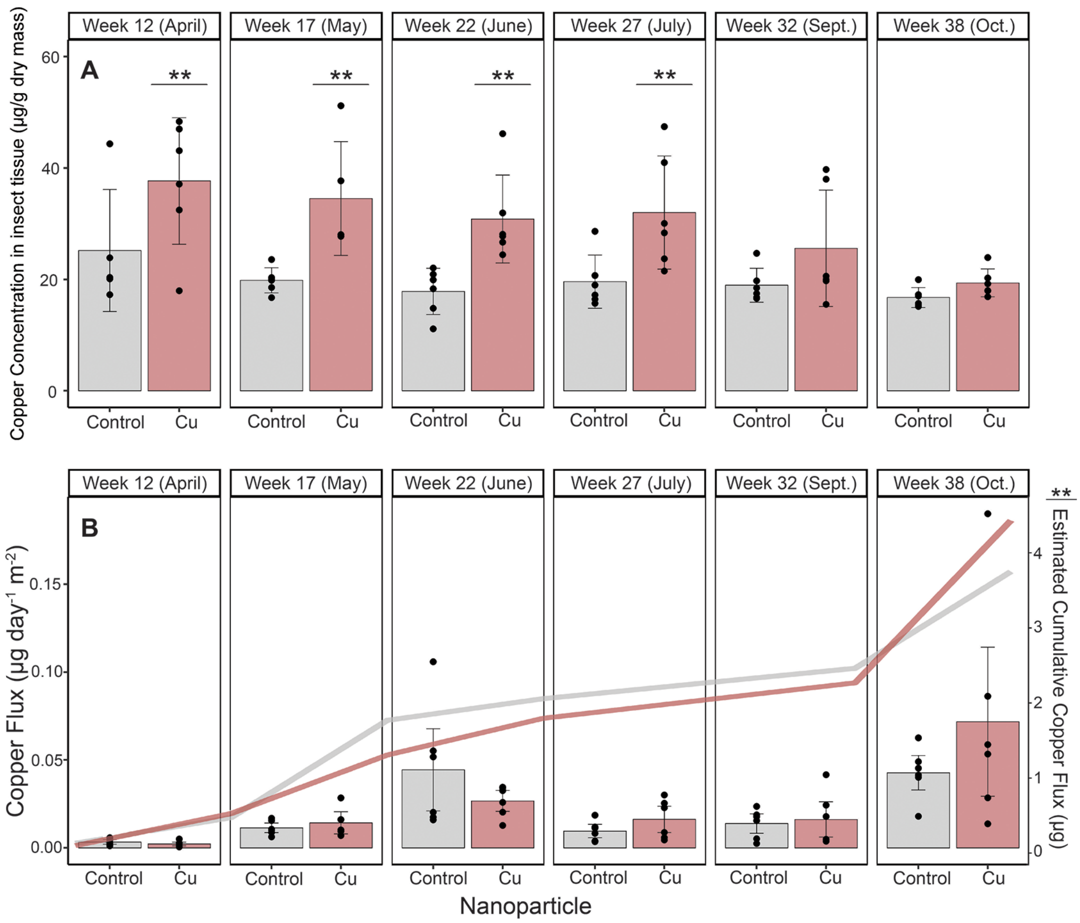

Figure 2. Mean ± SD of Cu concentration in (A) Chironomidae emerging from mesocosms and (B) daily flux from aquatic to terrestrial ecosystem by sampling week and treatment. Points represent individual mesocosm emergence. NP levels are distinguished by bar colors. Cumulative Cu fluxes by NP level are illustrated by lines (right *y*-axis) and are distinguished by color.

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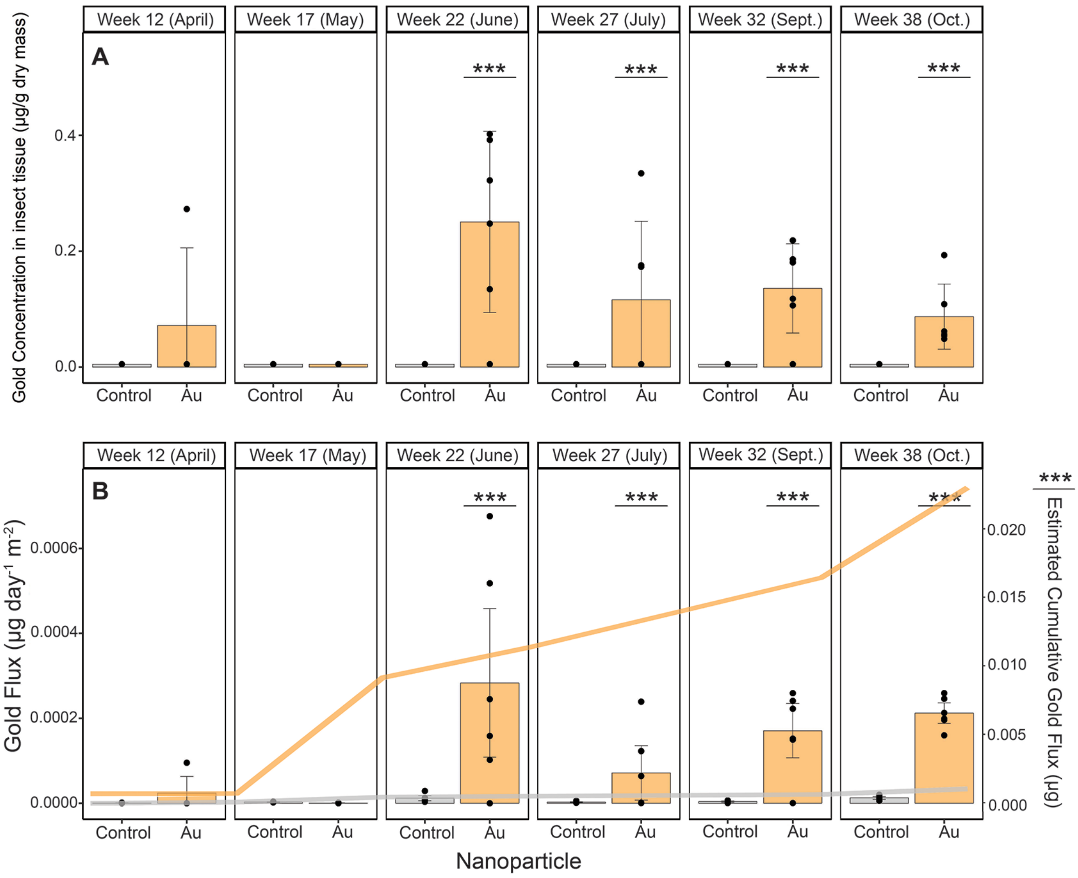

Figure 3. Mean ± SD of Au concentration in (A) Chironomidae emerging from mesocosms and (B) daily flux from aquatic to terrestrial ecosystem by sampling week and treatment. Points represent individual mesocosm emergence. NP levels are distinguished by bar colors. Cumulative Au fluxes by NP level are illustrated by lines (right y-axis) and are distinguished by color.

using residual plots.[52] Following model fitting and evaluation, significance of main effects and interactions were estimated using the "Anova" function in the car package and means, confidence intervals, and *post hoc* tests completed using the "contrasts" function in the R package emmeans.[55]

The effect of NP, nutrient status, and sampling week (NP, Nutrient, and Week) on the emerging insect community composition was analyzed using nonmetric multidimensional scaling (NMDS), in the vegan package with the "metaMDS" function using the Bray−Curtis distance as the dissimilarity measure.[56] Count data was log transformed prior to the composition assessment to give more weight to composition and less to total abundance of the dominant taxa (Chironomidae). Additionally, we assessed the effects of NP, nutrient status and NP × Nutrient interaction on insect community composition using the "manyglm" function in the model-based multivariate abundance data (mvabund) package.[57] Models were evaluated for fit using residual plots.

Riparian diet was analyzed using carbon and nitrogen stable isotope ratios of spiders and sources (Figure S3) and methods are reported in the "Stable Isotope Analysis" section of the SI. To compare the stable isotope data from basal resources (i.e., periphyton, sediment, *E. densa*), chironomidae, riparian spiders, and mosquitofish sampled in Week 38, we used the "adonis" function in the vegan package.[56] For this analysis, we used measured δ13C and δ15N and created a dissimilarity matrix using Bray−Curtis distances and assessed NP and taxa as main effects. If the adonis function yielded significant results, we used the pairwise.adonis package to determine which NP treatments were different from control groups and which taxa were distinct.[58]

■ RESULTS

Effects of Gold and Copper NP Exposures on Insect Emergence. While nutrient status did not have significant effects on insect emergence in either isolation or through interactions with NP or time ($P = 0.4$ for Nutrient and $P = 0.4$ for NP × Nutrient), there was a significant Nanoparticle effect on insect emergence in both the CuNP and AuNP mesocosms in Week 22 (Figure 1, $P = 0.0118, 0.0026$).

There was a significant decrease in cumulative insect emergence in CuNP and AuNP mesocosms over the 9-month period, resulting in a 19% and 24% decrease in total insect emergence compared to control mesocosms, respectively (Figure 1, $p = 0.03$). This overall cumulative decrease was completely driven by the decreased emergence in NP treatments compared to controls in Week 22.

There was no effect of NPs or Nutrient status on the community composition of emerging insects, though Week was a significant factor ($p < 0.001$, $R^2 = 0.50$, Figures S4−S6).

Copper and Gold Nanoparticle Accumulation in Emerging Insects and Flux to Riparian Ecosystem. There was a significant difference in Cu concentration in insect tissue between CuNP and control mesocosms in Week 12, Week 17, Week 22, and Week 27 ($p < 0.01$, Figure 2A). In these weeks, the mean percent increase in insect tissue concentration between the CuNP and control mesocosms was

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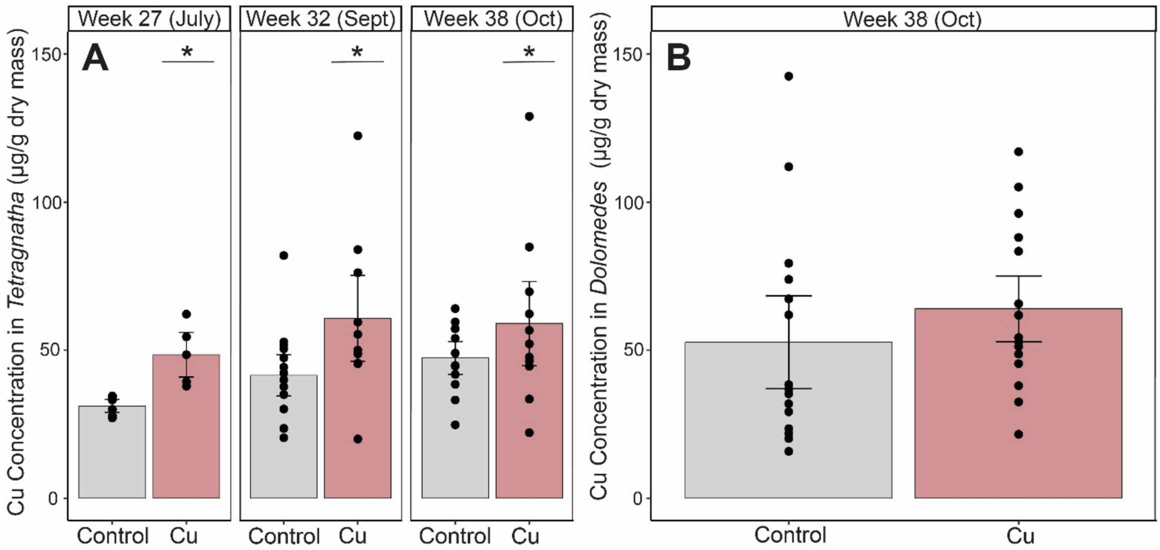

Figure 4. Mean Cu concentration by sampling week and treatment ±95% CI in *Tetragnatha* (A) and *Dolomedes* (B). Points represent observed individual values and NP treatments are distinguished by colors.

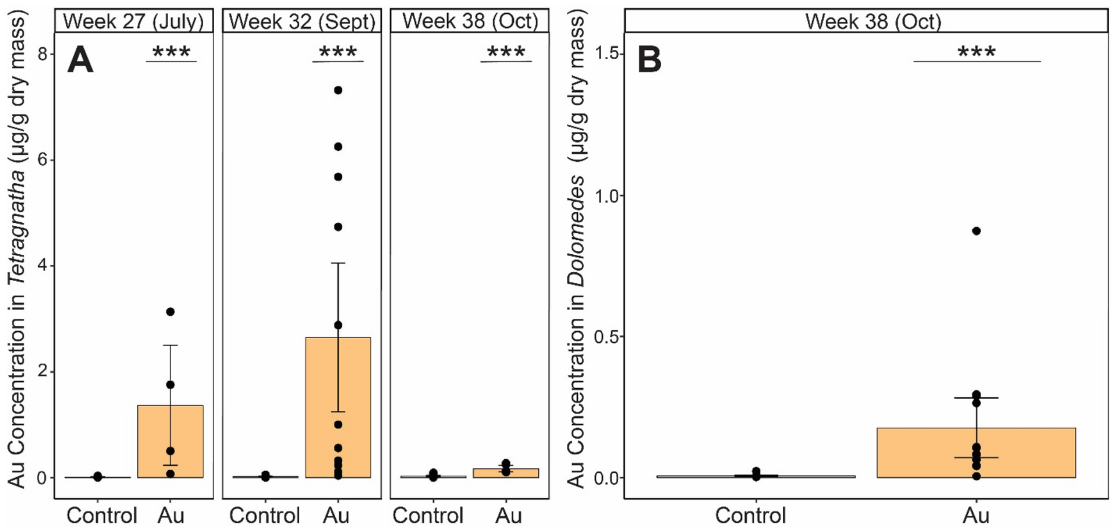

Figure 5. Mean Au concentration by sampling week and treatment ±95% CI in *Tetragnatha* (A) and *Dolomedes* (B). Points represent observed individual values and NP treatments are distinguished by colors.

64%. The Week 12 sampling event had the highest mean Chironomidae tissue Cu concentration at 38 μg Cu g^{-1} (all concentrations in tissues are expressed per unit dry mass) and the Week 38 sampling event had the lowest Cu concentration at 19 μg Cu g^{-1}(Figure 2A). There was no effect of Nutrient status on Cu concentrations in insect tissue (p = 0.78) or Nutrient × Week interaction (p = 0.90).

When comparing the cumulative flux of Cu over the sampled dates, there was a significant, 36% increase in the flux of Cu from the CuNP mesocosms compared to the Control mesocosms (Figure 2B, $p < 0.01$). However, there was not a significant difference between treatments when comparing the daily flux of Cu between the CuNP and Control mesocosms in any individual week (Figure 2B). There was a higher amount of Cu leaving from the Control mesocosms in Week 22 due to the greater number of insects emerging from Control mesocosms compared to CuNP mesocosms. Sampling week was a significant factor in the GLMM for both Cu concentration and the daily flux of Cu to the riparian ecosystem ($p < 0.001$). In contrast to metal concentration in Chironomidae tissue, Week 38 had the highest Cu daily flux from Chironomidae where 0.04 μg of Cu left the aquatic ecosystem, and Week 12 had the lowest flux of Cu where only 0.001 μg of Cu left the aquatic ecosystem.

There was a significant difference in Au concentration between AuNP and Control mesocosms when comparing individual Au concentration and daily Au flux from aquatic to riparian ecosystems weekly (Figure 3A,B, $p < 0.001$). All Control samples were below detection limit on the instrument. Week 22 had the highest Au tissue concentration at 0.25 μg Au g^{-1}, and Week 12 and Week 17 had the lowest Au tissue concentration as Chironomidae samples were below the MDL. There was no effect of Nutrient status on Au concentrations in insect tissue (p = 0.63) or Nutrient × Month interaction (p = 0.17).

Au flux was significantly higher in Au mesocosms compared to Control mesocosms in all weeks, except Week 12 and Week 17. The daily flux of Au ranged from 0.07 ng in Week 27 to

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0.03 ng Au in Week 22 (Figure 3A, $p < 0.001$). When comparing the cumulative flux of Au over the sampled dates, there was a flux of 0.023 μg of Au from the AuNP mesocosms compared to the Control mesocosms (Figure 3B, $p < 0.001$). Week was also a significant factor in the GLMM model for both the concentration of Au in individuals and for the daily flux of Au to the riparian ecosystem ($p < 0.05$).

Comparatively, Cu accumulated similarly in both emerging insects and mosquitofish in Week 38. On average, mosquitofish collected in Week 38 from the CuNP mesocosms contained 29.53 μg Cu g^{-1}, while mosquitofish tissue collected from the Au mesocosms contained 1.05 μg Au g^{-1}. In the Control mesocosms, mosquitofish contained 7.6 μg Cu g^{-1} and Au was below instrument detection.

Copper and Gold Nanoparticle Accumulation in Spider and Abundance. Spiders naturally colonized the mesocosms and their counts and presence during sampling events varied during the study. *Tetragnatha* were collected in all treatments during sampling weeks 27, 32 and 38, while *Dolomedes* were collected in Week 38. In total, 39 *Tetragnatha* were collected from Control mesocosms, 32 from CuNP mesocosms and 23 from AuNP mesocosms. We collected fewer *Dolomedes*, totaling 20 from Control mesocosms, 22 from CuNP mesocosms and 12 from AuNP mesocosms. (Table S4) There were significantly fewer spiders collected from AuNP mesocosms ($p < 0.05$) compared to the Control mesocosms. In total, we collected fewer spiders in CuNP mesocosms compared to Control mesocosms ($P = 0.09$).

There was a significant difference in Cu concentration (μg/g) between *Tetragnatha* tissue in CuNP and Control mesocosms for spiders collected in sampling weeks 27, 32, and 38 (Figure 4A, $p < 0.05$). There was not a significant difference in Cu concentration (μg/g) between *Dolomedes* tissue in CuNP and Control mesocosms for spiders collected in Week 38 (Figure 4B, $P = 0.13$).

There was a significant difference in Au (μg/g dry mass) concentration in tissues from both spider genera between the AuNP and Control mesocosms in all weeks (Figure 5A,B, $p < 0.001$). All samples collected from Control mesocosms were below detection limit. In *Tetragnatha* there was an interactive effect between Week and Treatment (Figure 5A, $p < 0.001$). In *Dolomedes* there was a significant NP effect (Figure 5B, $p < 0.001$).

Stable Isotope Analysis. There was a significant difference between sources and riparian spiders ($p < 0.001$, Figure S3), and further, there was a significant distinction between isotopic signatures of all sources ($p < 0.05$). *Dolomedes* had a distinct isotopic ratio from all sources except *Gambusia*. *Tetragnatha* had a distinct isotopic ratio from all sources except from Chironomidae ($p < 0.9$). *Dolomedes* and *Tetragnatha* had a distinct isotopic ratio from each other ($p < 0.05$). There was no distinction between isotopic signatures within NP treatments ($p < 0.8$). Additional results are detailed in the SI, section "Stable Isotope Analysis".

DISCUSSION

We found that 9-month additions of Cu and Au NPs decreased cumulative insect emergence and resulted in a flux of metals from aquatic ecosystems into riparian predators. Both Cu and Au NPs had similar magnitudes of effects on insect emergence over time, though Cu concentrations in insect tissue were higher than Au concentrations. We found that nutrient enrichment did not alter insect emergence, community composition, or insect tissue concentration of metals. In riparian spiders, we saw increased accumulation of Cu and Au in respective NP mesocosms and fewer spiders likely due to reduced insect emergence and/or NP toxicity. This study suggests that even at environmentally realistic contaminant exposures we see ecosystem-level effects of Cu and Au added as NPs on cumulative insect emergence, riparian predator abundance, and transfer of aquatic contaminants into predators within terrestrial food webs.

Nutrient status did not have a significant effect on total insect emergence or community composition. Previous studies found that systems with higher nutrient inputs have a higher biomass of emergence, largely driven by chironomids; thus we were expecting a higher emergence of midges from our nutrient enriched treatment.[59−61] While our community composition was dominated by midges, we did not observe differences in emergence or community composition patterns between ambient and enriched nutrient statuses. Insects naturally colonized our mesocosms; thus, there may have been underlying habitat preferences or prey-avoidance strategies influencing the initial colonization community and eventual emergence observed in our mesocosms.[62] Mosquitofish likely had a significant effect on habitat colonization and insect emergence.[63,64] Mosquitofish have a voracious appetite, and previous studies have found the inclusion of mosquitofish predators to negate any nutrient effect on insect emergence and significantly reduce biomass.[65,66] Lastly, our mesocosms were densely planted with macrophytes which limited light penetration in the water column and restricted periphytic algae growth, a key basal resource in wetland food webs.[67] While a previously published component of this mesocosm study determined that nutrients and NPs had an interactive effect increasing primary production in our mesocosms, we do not see similar patterns in secondary production.[5]

In our study, exposure to NPs decreased emergence significantly in some weeks, but cumulative emergence over the course of the experiment resulted in a 19% and 24% decrease in insect emergence in CuNP and AuNP mesocosms compared to the Control mesocosms. Environmental factors such as temperature, humidity and light regulate insect emergence, and were the likely drivers of emergence in the control mesocosms.[68] While the NP mesocosms experienced these same environmental factors, the inclusion of NPs altered insect emergence, perhaps explained by decreased larval survival and altered developmental time.[32,69] The reduction of insect emergence after exposure to Cu complements the results of other studies, which showed reductions in dipteran and ephemeropteran emergence after chronic Cu exposure.[39,70] In AuNP mesocosms, there was reduced emergence in Week 27 and increased emergence in Week 32, consistent with the delayed emergence pattern previously described.[15,27]

Throughout the experiment, we observed shifts in the driver of contaminant flux across time. In Week 12 through Week 27, we observed metal concentrations driving the patterns of contaminant flux, where high insect tissue concentrations were coupled with low emergence. Conversely, in Week 32 and Week 38 in the CuNP mesocosms, overall emergence was high, but concentrations in insect tissue were low, suggesting that the increased contaminant flux was driven by increased emergence. Metal exposure throughout growth, development, and metamorphosis likely results in an overall reduction of emergence and may have implications on the timing and dietary quality of the subsidy emerging to the terrestrial

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ecosystem.[15,27] This overall reduction in emerging insects could result in an imbalance of prey items for predators that specialize in consumption of emerging insects.

We observed differential accumulation of the two metals added as NPs in adult insect tissue, where Cu tissue concentrations were 10 times higher than Au tissue concentrations. Cu is an essential element and found in detectable quantities in insect tissue under noncontaminated conditions. Au is not an essential element, and not likely to be encountered by insects in aquatic ecosystems. Further the overall decrease in Cu tissue concentration over time may demonstrate copper tolerance mechanisms.[27,70] Cu is regulated by insects to balance toxicity and essentiality. Chironomids have demonstrated tolerance mechanisms to adapt to metal-contaminated environments enhancing survival, but there is a high energetic cost to detoxifying metals.[71] The elevated Cu tissue concentrations in Weeks 12 and 17 coincide with higher water Cu concentrations measured in the first two months of the experiment.

Reduced emergent insect abundance has been shown to reduce terrestrial food web density, particularly for nonmobile predators, such as spiders.[72,73] We collected fewer spiders from the NP mesocosms compared to the Control mesocosms likely due to reduced insect emergence and/or NP toxicity (Table S4). Further, in collected spiders there is evidence of trophic transfer of metals from aquatic prey into the riparian spider consumers, as there are elevated concentrations of Cu and Au in tissues from both genera of collected spiders from NP mesocosms. Sampling week was an important covariate in Au accumulation in *Tetragnatha*. In Week 32, elevated Au tissue concentrations were measured in small-bodied individuals and perhaps the reduced abundance recorded in Week 38 may be a toxicity response to elevated tissue concentrations in Week 32 (Figure S7). Overall, spiders collected from CuNP mesocosms had higher tissue concentrations and contained more Cu than spiders collected from the Control mesocosms. At spider tissue concentrations found in this study, toxicity effects such as increased mortality and decreased body mass change have been reported after dietary Cu exposure.[74,75]

Both spider genera used in our study are relatively sedentary and frequently used as sentinels of chemical contaminants in aquatic ecosystems, but very little is known about their home ranges so it is possible that spiders could have moved between mesocosms during sampling.[37] However, we did not observe any evidence of movement, as Au was below the instrument detection limit for all Control and CuNP spiders. Our stable isotope analysis indicated that *Tetragnatha* aligned more closely with Chironomidae, suggesting that this was its most common prey item, while *Dolomedes* was more closely associated with mosquitofish (*Gambusia*, Figure S3). Despite differences in diet, the two spider taxa had similar metal accumulation. This could indicate that metal accumulation from NPs could be widespread across diverse spider taxa with varied ecologies and diet preferences.

Overall, the addition of NPs had significant effects on aquatic ecosystems and resulted in decreased insect emergence and increased transport of metals to the riparian ecosystem via common, pollution tolerant Chironomidae. In both Cu and Au mesocosms, increased metal fluxes into riparian ecosystems were driven by increased tissue concentrations, which were sufficient to overcome reduced insect emergence. This has critical implications for both aquatic and riparian food webs. In addition to the increased transport of metals from aquatic ecosystems into terrestrial ecosystems, reductions in biomass mean fewer prey items, fewer riparian spiders, altered food webs, and altered energy and nutrient flows.[32,39,72,76] Additionally, this study adds to the ever-increasing evidence of strong linkages between aquatic and terrestrial ecosystems. These data also support the importance of trophic transfer among complex food webs as an important component of the environmental fate and transport of nanoparticles and other contaminants of emerging concern.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c00620.

Additional information concerning the zeta potential and hydrodynamic diameters, mass balance (from ref 6), water chemistry, ICP-MS methods, common morpho-species identifications, stable isotope analysis, insect community, and spider abundance (PDF)

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Notes

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