Global Ecology and Conservation 24 (2020) e01292

Contents lists available at ScienceDirect

Global Ecology and Conservation

journal homepage: http://www.elsevier.com/locate/gecco

Original Research Article

Bioaccumulation of the pesticide imidacloprid in stream organisms and sublethal effects on salamanders

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ARTICLE INFO

Article history:
Received 2 December 2019
Received in revised form 22 September 2020
Accepted 22 September 2020

Keywords:
Imidacloprid
Bioaccumulation
Stream salamanders
Hemlock woolly adelgid
Corticosterone
Body condition index

ABSTRACT

Neonicotinoids are one of the most widely used classes of insecticides in the world. The neonicotinoid imidacloprid is commonly applied to hemlock (*Tsuga* spp.) stands in eastern North America to reduce tree mortality from infestations of the invasive hemlock woolly adelgid (HWA; *Adelges tsugae*). While laboratory and mesocosm studies have determined that imidacloprid can bioaccumulate in anurans and cause sublethal effects, no field studies have investigated whether salamanders or insects in streams adjacent to HWA treatments bioaccumulate imidacloprid or if sublethal effects are detectable in wild salamanders. We assessed relationships between imidacloprid exposure and stream salamander health in West Virginia, USA, using concentration of the stress hormone corticosterone and body condition indices (BCI) as response variables. Of 107 *Desmognathus* salamanders from 11 sites tested for bioaccumulation, we detected imidacloprid in 47 salamanders. Of 15 benthic macroinvertebrate samples tested, we detected imidacloprid, imidacloprid-urea, and imidacloprid-olefin in 15, 13, and 1 sample, respectively. Based on 115 *Desmognathus* salamanders sampled at 11 sites for stress hormone responses, corticosterone concentration increased with imidacloprid concentration in stream water. For 802 salamanders sampled at 48 sites, BCI decreased as concentration of imidacloprid in stream water increased, but explanatory power was low. Our study suggests that chronic leaching of imidacloprid from treated hemlock stands into adjacent streams has the potential to negatively affect aquatic organisms and may provide a route of exposure to higher trophic levels.

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https://doi.org/10.1016/j.gecco.2020.e01292

1. Introduction

Neonicotinoids are one of the most widely used classes of insecticides in the world and have applications in agriculture, household use, and protection of native trees from invasive pests (Jeschke et al., 2010; Webb et al., 2003). Although neonicotinoids are highly selective for insect nicotinic acetylcholine receptors, many studies have documented negative effects of neonicotinoids on the health and survival of vertebrates (Gibbons et al., 2015). Neonicotinoids are generally not lethal to adult vertebrates at concentrations typically found in the environment, but studies have found a variety of sublethal effects on mammals (Berheim et al., 2019), birds (Hallmann et al., 2014), and frogs (Ade et al., 2010; Feng et al., 2004; Pérez-Iglesias et al., 2014).

In eastern North America, the neonicotinoid imidacloprid is the most common and effective method for reducing mortality in eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*T. caroliniana*) from infestations of the invasive pest, hemlock woolly adelgid (HWA; *Adelges tsugae;* Havill et al., 2014, Webb et al., 2003). Hemlocks are ecologically important trees that provide unique microhabitat conditions used by diverse invertebrate and vertebrate taxa (Becker et al., 2008; Ellison, 2014; Snyder et al., 2002; Tingley et al., 2002). Hemlock trees exert a strong influence on the abiotic environment by creating deep shade that reduces ground and stream temperatures and stabilizes soil moisture levels (Snyder et al., 2002).

Previous studies found that imidacloprid can leach from HWA treated areas into adjacent streams (Benton et al., 2016; Churchel et al., 2011; Wiggins et al., 2018), thus potentially exposing stream salamanders to the insecticide. Imidacloprid was detected in water from one stream in Chattahoochee National Forest (Georgia, USA) nearly two years after imidacloprid application (<1000 pg/mL); Churchel et al., (2011). Water imidacloprid concentrations of 53–833 pg/mL were detected in three streams adjacent to HWA treatment areas in Big South Fork National River and Recreational Area (Kentucky and Tennessee, USA) six months after imidacloprid application (Wiggins et al., 2018). In the Great Smoky Mountains National Park (North Carolina and Tennessee, USA), imidacloprid was detected in water from 7 of 10 sites downstream of treated hemlock trees with concentrations of 28.5–379.1 pg/mL (Benton et al., 2015). Imidacloprid degrades by a number of processes into numerous metabolites, some of which also have insecticidal properties (Wamhoff and Schneider, 1999). Imidacloprid-urea is a metabolite commonly detected in soil that has been exposed to imidacloprid (Dai et al., 2006) and imidacloprid-olefin is a metabolite that was found to be 10 times more toxic than imidacloprid to an insect pest (*Bemisia tabaci*; Nauen et al., 1998).

North America is a global hotspot for salamander diversity (Yap et al., 2015), particularly the Appalachian Mountains in the eastern USA (Petranka and Murray, 2001). In headwater streams, salamanders are often the dominant vertebrates in abundance and biomass (Davic and Welsh, 2004). However, research investigating the physiological and ecological consequences of imidacloprid exposure on salamanders is lacking. Bioaccumulation is the uptake of substances and contaminants from the environment into the tissues, as compared to biomagnification, which is the process by which substances and contaminants are passed through food to an organism which will have a higher concentration of that substance than the source of the substance (Zenker et al., 2014). Amphibians are likely to bioaccumulate substances because of their thin, highly permeable skin (Van Meter et al., 2014). Bioaccumulation of imidacloprid has been investigated in anurans, and four species had measurable levels of imidacloprid in their tissues after 8 h of exposure to imidacloprid in a laboratory (Glinski et al., 2018; Van Meter et al., 2014, 2015). There is fairly limited published data for imidacloprid LC_{50} in amphibians: 52.6 mg/L (Montevideo tree frog [*Hypsiboas pulchellus*]; Pérez-Iglesias et al., 2014), 165 mg/L (alpine cricket frog [*Fejervarya limnocharis*]; Feng et al., 2004) and 219 mg/L (dark-spotted frog [*Pelophylax nigromaculatus*]; Feng et al., 2004). However, other studies have found that imidacloprid causes mortality at lower concentrations: 0.5 μg/L in spotted marsh frog tadpoles (*Limnodynastes tasmaniensis*; Sievers et al., 2018) and 9 mg/L in northern cricket frogs (*Acris crepitans;* Ade et al., 2010). Additionally, sublethal effects of imidacloprid exposure in amphibians include DNA damage in Montevideo tree frogs at 12.5–37.5 mg/L (Pérez-Iglesias et al., 2014), and reduced swimming speed, distance, and predator avoidance behavior in spotted marsh frog tadpoles at 0.5 μg/L (Sievers et al., 2018).

Sublethal effects of environmental stressors are associated with hormone level changes in amphibians, particularly the hormone corticosterone, which is associated with reproduction, development, growth, and stress (Romero, 2004). Long-term elevation of corticosterone levels induced by chronic stressors can have negative effects, including suppression of the immune system and growth (Romero, 2004). Multiple studies have documented increases in corticosterone levels in salamanders due to environmental stressors such as competition for habitat (Cooperman et al., 2004), food limitation (Hopkins et al., 1997), increased temperature (Novarro et al., 2018), reduced moisture (Charbonnier et al., 2018), increased acidity (Chambers et al., 2013), and vernal pool size (Millikin et al., 2019). Importantly, Hopkins et al. (1997) found that anuran corticosterone levels were elevated when exposed to environmental contaminants.

Benthic macroinvertebrates are the primary food source of stream salamanders (reviewed by Petranka, 1998) and thus are a potential route of pesticide exposure for salamanders and other vertebrates. Aquatic invertebrates are used for biomonitoring because they are known to bioaccumulate contaminants such as heavy metals (Kiffney and Clements, 1993; Goodyear and McNeill, 1999) and because they live in the stream sediments and have lifespans of months–years, thus allowing them time to take up contaminants into their bodies (Goodyear and McNeill, 1999). Among benthic macroinvertebrates, laboratory studies have documented sublethal effects such as inhibited feeding of mayflies (Order Ephemeroptera) at 0.5 μg/L (Alexander et al., 2007) and inhibited feeding of leaf-shredding invertebrates at 18–30 μg/mL (Kreutzweiser et al., 2009). Lethal effects of imidacloprid exposure have been documented on mayflies at 2.1 μg/L (Alexander et al., 2007), and on midges at 2.3 μg/L (Colombo et al., 2013). Population-level effects of imidacloprid on aquatic insects may be detected above 1–2 μg/L (Sánchez-Bayo and Goka, 2006; Pestana et al., 2009).

Although previous studies reported numerous effects of imidacloprid in agricultural systems on invertebrates, birds, mammals, and other taxa (e.g. Cresswell, 2011; Hallmann et al., 2014; Millot et al., 2017), to our knowledge, no studies have investigated whether imidacloprid application for native tree conservation is resulting in imidacloprid bioaccumulation and sublethal effects in wild stream salamanders. The purpose of our study was to determine if salamanders inhabiting streams adjacent to imidacloprid-treated hemlock stands (hereafter HWA treatments) are bioaccumulating imidacloprid and if there are detectable sublethal effects on individuals. We also assessed bioaccumulation of imidacloprid and its metabolites in benthic macroinvertebrates. Corticosterone levels and body condition indices (BCI) were used to assess potential sublethal effects of imidacloprid exposure on salamanders. We hypothesized that (1) salamanders and benthic macroinvertebrates in streams adjacent to treated stands would have imidacloprid in their tissues, and (2) health metrics of individual salamanders would be negatively associated with imidacloprid exposure.

2. Methods

2.1. Study sites

This study was conducted in the Monongahela National Forest (MNF) and two units of the National Park Service (NPS): Gauley River National Recreational Area (GARI) and New River Gorge National River (NERI), in West Virginia, USA (Fig. 1). Hemlock trees in the MNF were treated with a single application of imidacloprid in 2013, 2014, or 2015. At MNF sites, the majority (approximately 70%) of trees were treated with soil injections (imidacloprid mixed with water was injected near the roots) and the remainder were treated with stem injections (imidacloprid was injected directly into the tree's cambium). Hemlock stand treatments in the NPS units began in 2006 and continued annually, including repeated applications at 10 of the sampling locations. At NPS sites, approximately 5% of trees were treated with stem injections, 12% were treated with soil drenches (imidacloprid mixed with water was poured on the ground for the roots to uptake), and 83% were treated with soil injections (Strickler, 2014). In water, imidacloprid photodegrades and has a half-life of 1 h–3 days (Wamhoff and Schneider, 1999), however, continual leaching into stream systems may maintain imidacloprid presence in stream systems near treated hemlocks (Benton et al., 2016). For NPS, treatment sites were not selected if the last treatment occurred prior to 2011 due to the eventual breakdown of imidacloprid in the environment. A geographic information system (ArcMAP® 10.4, ESRI, Redland, CA) was used to identify candidate first or second order headwater streams based on proximity to HWA treatments. Candidate streams were visited to determine whether the stream depth, stream substrate, and water flow speeds were suitable for sampling salamanders and stream invertebrates. Final study sites were selected based on the suitability of the streams for sampling, the proximity of treated trees (and flow direction for non-treated sites), the proximity of treated and non-treated sites, and similarity in habitat characteristics such as stream size, water flow, water chemistry, and surrounding vegetative community (Crayton, 2019).

We sampled 24 sites in MNF, 14 sites in NERI, and 10 sites in GARI. Of these 48 sites, 27 were adjacent to HWA treatments and 21 were not. In the sites adjacent to HWA treatments, on average 151.9 ± 83.6 hemlock trees were treated (range = 5–3993). Hemlock trees that were treated multiple years were counted as multiple trees to reflect treatment intensity (i.e. a tree that was treated for two years was counted as two trees). Sites that were not adjacent to HWA treatments were either a minimum of 100 m upstream of imidacloprid application or were in a watershed without known HWA treatments. We selected 100 m as a minimum distance because Benton et al. (2016) did not detect imidacloprid in stream sites that were 10–100 m upstream of treated trees and because *Desmognathus* spp. have fairly small average home range sizes (e.g., 23.7 m^2; Barbour et al., 1969).

2.2. Water and sediment sampling

Water samples were collected adjacent to the salamander sampling plots from all NPS sites in June 2017 and from all MNF sites in June–July 2018. Two L of water were collected from each site in 1 L plastic bottles (Thermo Scientific Nalgene™ labware, Rochester, New York, USA) without disturbing the stream sediment. Stream sediment was collected from all sites in September 2018 from multiple spots in the site with a trowel. Enough sediment was collected to fill one quart-sized plastic bag per site. If the stream bottom did not have any sediment, sediment was collected from the sides of the stream bank. The trowel was wiped with 95% ethanol between each use to prevent contamination. The bottles of water and bags of sediment were placed in black bags and a backpack to prevent light exposure from metabolizing imidacloprid until the samples could be placed in a dark cooler with ice. Imidacloprid is a thermally stable compound (Liu et al., 2006) and was therefore stored at 4 °C and out of sunlight from the day of collection until extraction procedures began. Water samples were stored in air-tight containers to prevent loss by evaporation. The water collected in summer 2017 was processed in August 2018 and the water collected in summer 2018 was processed in October 2018. The sediment collected in September 2018 was processed in February 2019.

2.3. Salamander and habitat sampling

Within each of the 48 sites, we established three 6.6 m (3.3 m by 2 m) subplots for a total plot area of 20 m (10 m by 2 m). One meter of the subplot width was on the bank and 1 m was within the wetted stream channel. Subplots were placed

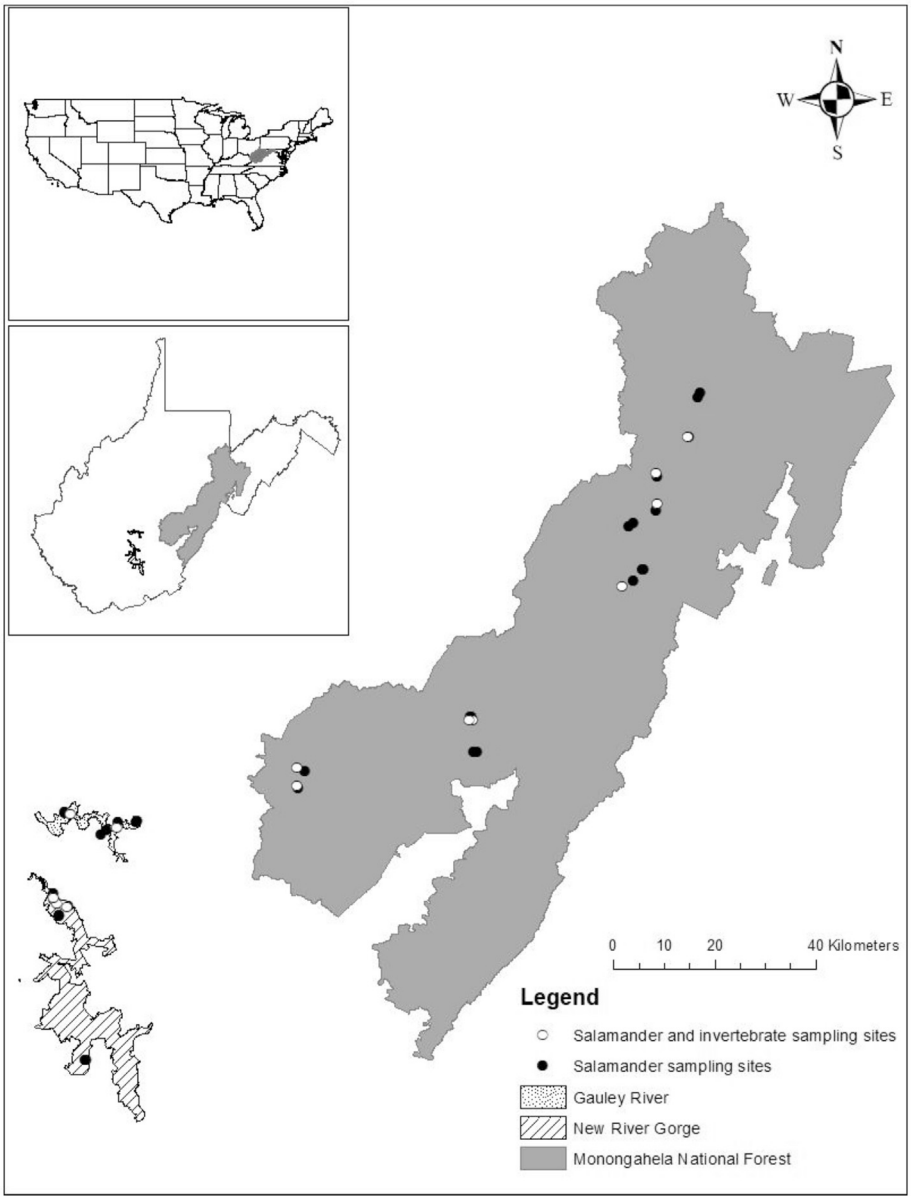


Fig. 1. Locations of study sites used to sample stream water and sediment, salamanders, and benthic macroinvertebrates for presence and concentration of the pesticide imidacloprid and its metabolites, and to investigate potential sublethal impacts of imidacloprid on salamanders. Stream water and sediment was collected from every site. Study sites were located in West Virginia, USA (inset) in the New River Gorge National River (New River Gorge), Gauley River National Recreational Area (Gauley River), and Monongahela National Forest.

primarily in riffles but were occasionally placed in runs or pools if the site did not have riffle habitat. Across sites, subplots were similar in terms of stream depth, substrate, canopy cover, vegetative community, and flow regime. We sampled salamanders April–July 2017 in the NPS sites and April–July 2018 in the MNF sites, sampling each site six to seven times during the year during baseflow conditions. While moving upstream to prevent stream sediment from flowing downstream and obscuring our view, we flipped every cover object greater than 50 mm in diameter and searched through leaf packs.

All captured salamanders were placed into individual plastic bags and identified to species, or genus when identification to species was not possible. Captured salamanders were weighed to the nearest 0.1 g with a spring scale (Pesola Precision Scales, Schindellegi, Switzerland), and snout-vent length (SVL) and total length were measured to the nearest 0.1 mm with dial calipers (Wiha Tools, Monticello, Minnesota, USA). Salamanders were measured using a salamander stick to maximize accuracy (Margenau et al., 2018), and notes were made of any missing limbs or tails and if salamanders were gravid. After processing, the salamanders were released at their point of capture.

After every sampling event, we measured stream depth (cm) in the center of the plot. Canopy cover was measured at each subplot once during each sampling season after full leaf-out using a 25 cm × 25 cm plexiglass grid which was divided into 5 cm × 5 cm cells. We held the plexiglass grid overhead and visually estimated how many cells were covered by the tree and shrub canopy and calculated % canopy cover (Haché et al., 2013).

2.4. Salamander sampling for corticosterone concentration and imidacloprid bioaccumulation

After sampling salamanders for BCI (section 2.3), we collected adult individuals of the salamander genus *Desmognathus* to quantify imidacloprid bioaccumulation and corticosterone levels from 11 of the 48 sampled sites (n = 7 for GARI, n = 4 for NERI). We selected sites that had high densities of large adult seal salamanders (*D. monticola*) and northern dusky salamanders (*D. fuscus*) to minimize potential impacts of removal of individuals on the populations and to obtain a large enough volume of tissue for imidacloprid testing. Seven of the eleven sites were directly adjacent to HWA treatments and four were not. The seven sites adjacent to HWA treatments had an average of 242.2 ± 80.1 treated hemlock trees (range = 17–494).

We hand captured 167 *D. monticola* and *D. fuscus* by flipping rocks and other cover objects in the stream between 8 July and November 24, 2017. Of the 167 salamanders captured, 109 salamanders were from sites treated with imidacloprid and 58 were from untreated sites. We quantified plasma levels of corticosterone for a subset of the salamanders (n = 119; 61 from sites treated with imidacloprid and 58 from untreated sites). For these samples, salamanders were decapitated in the field and a minimum blood sample of 2 μL was collected within 3 min of initial disturbance of the salamander to minimize the influence of capture stress on corticosterone level (Romero and Reed, 2005). The samples were stored in coolers until they were transferred to the laboratory. In the laboratory, we centrifuged blood samples for 5 min and plasma was collected and stored at −20 °C until analysis. Plasma samples were packed in a cooler on dry ice and sent to the Endocrine Technology Laboratory at the Oregon National Primate Research Center and assayed for corticosterone using radioimmunoassay (RIA; Thomas and Woodley, 2017). Recovery was 98.8% and intra-assay coefficient of variation (CV) was 8.1%. Of the 119 salamanders sacrificed for corticosterone quantification, 59 were also used to quantify whole body imidacloprid concentration. An additional 48 salamanders were captured only for imidacloprid quantification, all of which were collected from sites treated with imidacloprid. We measured and weighed the salamanders in individual plastic bags, transferred them to the laboratory, euthanized them through exposure to carbon dioxide followed by decapitation, and then froze them at −20 °C until processing for imidacloprid extraction. Our total sample size of salamanders for bioaccumulation analyses was 107 individuals.

2.5. Benthic macroinvertebrate sampling

We collected benthic macroinvertebrate samples between 17 September and November 20, 2018 from 15 of the 48 sites with five of the sites in NERI, two in GARI, and eight in MNF. Sites were selected from the three localities to be representative of multiple regions within West Virginia, and we selected sites adjacent to HWA treatments to evaluate the potential for bioaccumulation. Due to time and funding constraints, we only selected sites with abundant benthic macroinvertebrate populations to enable us to collect the minimum invertebrate tissue volume needed for chemical analyses. These 15 sites had an average of 371.2 ± 260.2 treated hemlock trees (range = 34–3993).

We collected benthic macroinvertebrate samples by placing a D-net (Bioquip Inc., Rancho Dominguez, CA) flush with the stream bottom and disturbing the substrate upstream or by sweeping the D-net under stream overhangs. Samples included organisms of the orders Ephemeroptera (mayfly), Plecoptera (stonefly), Trichoptera (caddisfly), Megaloptera (dobsonfly), Coleoptera (beetles), Diptera (flies), and Decapoda (crayfish). All invertebrates from each site were stored together in a tube containing 75% ethanol and covered with foil to prevent light exposure until imidacloprid extraction. Only crayfish <2.5 cm were included in the samples to ensure that they were of a size that could be consumed by a salamander.

2.6. Water and sediment imidacloprid extraction and quantification

Water and sediment extraction procedures were adopted from Baskaran et al. (1997). One L of water from each site was filtered through 0.22-μm filters. The water samples were then filtered through pre-conditioned C18 solid-phase extraction (SPE; Restek Corporation, State College, PA, USA) cartridges on a vacuum manifold. The imidacloprid was eluted from the cartridges with 5 mL acetone into 15-mL glass test tubes. Then, the eluent was dried under nitrogen at 40 °C and the residue was reconstituted in 0.5 mL of acetonitrile. The reconstituted samples were then filtered through 0.20-μm filters into liquid chromatography (LC) vials.

We dried sediment samples at 60 °C for 3 days before fracturing and sieving the samples (Kagabu and Medej, 1995, Thuyet et al., 2010). The sediment samples were weighed to 30 g, 100 mL of deionized (DI) water was added, and the solution was stirred for 1 min. The samples were filtered through cheese cloth and a 0.22-μm filter and the extract was transferred to a separatory funnel with 25 mL of chloroform. The solution was mixed vigorously before extracting the chloroform layer through anhydrous sodium sulfate. We repeated this process twice before drying the solution under nitrogen at 40 °C and reconstituting the residue in 0.5 mL of acetonitrile. We filtered the reconstituted samples through pre-conditioned Florisil cartridges, eluted the imidacloprid using 5 mL of acetonitrile, and dried the eluent under nitrogen at 100 °C. The residue was reconstituted in 0.5 mL of acetonitrile and filtered through 0.20 μm filters into LC vials.

We quantified the concentration of imidacloprid in the stream sediment and water samples using ultra high-performance liquid chromatography-mass spectrometry (UHPLC-MS). The chromatographic and mass spectrometry conditions were adapted from Galeano et al. (2013). We used the Exion LC AD UHPLC system in tandem with an AB Sciex Qtrap 5500 triple quadrupole AcQuRate CEM detector. The compounds imidacloprid, imidacloprid-urea, and imidacloprid-olefin and external standards were separated on a Kromasil C_{-18} (M05CLD05) column (2.1 $\times$ 50 mm) and maintained at an oven temperature of 40 °C. The autosampler temperature was maintained at 10 °C and the injection volume was 2 μL. The MS/MS detection of the compounds was performed by electrospray ionization (ESI) source operated in positive ion mode.

We used multiple reaction monitoring (MRM) for the detection and quantification of imidacloprid and metabolites. MRM parameters were as follows: imidacloprid, Q1 mass 256.000 Da, Q3 mass 209.000 Da, 50.0 msec; imidacloprid urea, Q1 mass 213.200 Da, Q3 mass 129.000, 50.0 msec; imidacloprid olefin, Q1 mass 254.100 Da, Q3 mass 171.000 Da, 50 msec. The Ion-Spray voltage and source temperature were maintained at 4.50 kV and 450 °C, respectively. The LC-MS/MS software Analyst (AB Sciex, version 1.6.3) was used for data acquisition and processing.

Due to time and funding constraints, we were unable to quantify recovery success of imidacloprid from sediment, and thus we treated sediment data as presence-absence only for analyses. We note that estimated imidacloprid concentrations in sediment were minor compared to estimated concentrations in stream water (i.e., typically <15% of the concentration in stream water). The estimated recovery rate for imidacloprid, imidacloprid-urea, and imidacloprid-olefin in water was approximately 100%, and the recovery rates for imidacloprid, imidacloprid-urea, and imidacloprid-olefin in salamanders and insects were estimated at 63%, 56%, and 42%, respectively. We did not test for presence/absence of metabolites in water for sites at NERI and GARI because chemical analyses for those sites were completed before the study methods evolved to include metabolite analyses.

2.7. Salamander and invertebrate imidacloprid extraction and quantification

We quantified imidacloprid concentrations in 107 *Desmognathus* salamanders (34 *D. fuscus* and 73 *D. monticola*). Pesticide extraction and chromatographic and mass spectrometry conditions were adapted from procedures developed by Lehotay (2006) and Galeano et al. (2013). Salamanders were placed individually into 50-mL tubes and macroinvertebrates samples were combined by site before being placed into 50-mL tubes. Samples were flash-frozen in liquid nitrogen and placed in a freeze dryer. Because of the high lipid content of the salamander tissues, freeze drying took 3 days (imidacloprid and its metabolites are stable at −40 °C; Kagabu and Medej, 1995; Spomer et al., 2009). After freeze drying, we placed 3 5-mm steel beads into each 50-mL tube and ground the salamanders and invertebrates in a Retsch MixerMill (MM 400, Haan, Germany) for 3 min. The samples were sonicated for 20 min before the addition of Quick Easy Cheap Effective Rugged Safe (QuEChERS) Mylar salt pouches (ECQUEU7-MP, United Chemical Technologies, Bristol, PA) to each sample. Samples were centrifuged at 2200 relative centrifugal force for 5 min. We assembled a high-throughput vacuum apparatus with clean-up cartridges (ECPSAC1856, United Chemical Technologies, Bristol, PA) and conditioned with 5 mL of acetonitrile. A total of 8 mL of the organic layer (acetonitrile) of each sample was collected and cleaned through the cartridges. The test tubes were then dried under nitrogen at 50 °C, reconstituted in 0.5 mL of acetonitrile, and filtered through PTFE Whatman Mini-UniPrep Syringeless Filter vials. We followed the same procedure to quantify imidacloprid concentration as described above for water and sediment.

The number of significant figures for each concentration was determined, first, by calculating the average uncertainty (i.e., averaging the absolute value of each residual divided by each prepared concentration for the calibration standards), and second, by finding the smallest digit in the raw number that was at least three times the uncertainty and then eliminating all digits to the right of it as non-significant digits (Agut et al., 2006). Finally, we added one digit to represent the uncertainty in the data (least significant digit + 1; Sorenson, 2002). For all sample types, we calculated limit of detection (LOD) values from an external standard solution containing imidacloprid, imidacloprid-urea, and imidacloprid-olefin ranging from 5 to 300 ng/mL in LC-MS grade acetonitrile. We performed a linear regression on the data points in the concentration range ($n = 7$) and calculated the LOD using formula $3 \times (SE/R^2)$, where SE is standard error and R^2 is coefficient of determination). LOD values were determined for each sample type and differed for each round of quantification. LOD for imidacloprid in water samples was either 10 ng/mL or 5 ng/mL in the concentrated sample, depending on whether the sample was analyzed in the first or second run. LOD for imidacloprid in salamander tissues was either 2.5 ng/g or 5 ng/g. Insect samples were all run at once, and LOD was 2.5 ng/g for imidacloprid and 20 ng/g for imidacloprid-urea and imidacloprid-olefin. Data below the LOD were recorded as zero.

2.8. Statistical analyses

We used multiple linear regressions and a model selection approach to examine relationships between imidacloprid exposure and the following response variables: (1) water imidacloprid concentration, (2) bioaccumulation in salamanders, (3) bioaccumulation in benthic macroinvertebrates, (4) salamander corticosterone concentration, and (5) salamander BCI. Candidate models were ranked using Akaike's Information Criterion corrected for small sample size (AIC_c; Burnham et al., 2011; Zuur et al., 2009). We created linear regressions using the lm function in the base package stats (version 3.6.2) in program R (R Foundation for Statistical Computing; version 3.6.3). We assessed model support based on ΔAIC_c and Akaike weight (w_i), and considered candidate models to have strong support when $\Delta AIC_c < 2$ and some support when $\Delta AIC_c < 7$

Table 1
Model selection results for (1) influence of treatment intensity in the last treatment year (Trt Intensity - Last Tx Yr) and years since last treatment (YST) on water imidacloprid concentration (Water IMI Concentration) at the time of sampling (n = 25), (2) influence of water imidacloprid concentration on whole body imidacloprid concentration of individual *Desmognathus* (*D. fuscus* and *D. monticola*) salamanders (n = 47), (3) influence of water imidacloprid concentration on site-level samples of benthic macroinvertebrates (n = 15), (4) influence of five measures of imidacloprid exposure on corticosterone concentration in *D. fuscus* and *D. monticola* salamanders (n = 115), and (5) influence of five measures of imidacloprid exposure on body condition index (BCI) score of stream salamanders (n = 802). We used Akaike's Information Criterion corrected for small sample size (AIC_c) to rank candidate models. The null model is shown as (.) and includes only the intercept. Akaike weights are represented as w_i. Only candidate models with some support ($\Delta AICc \leq 7$) are included; see Table S1 for the full model selection results. Treatment intensity refers to number of treated trees in adjacent eastern hemlock (*Tsuga canadensis*) stands across all treatment events (Trt Intensity - Total) or during the last year treated (Trt Intensity - Last Tx Yr). For the corticosterone and BCI model selections, we also assessed the influence of whether the site was adjacent to treated trees (Trees), and whether imidacloprid was detected in the environment (Presence). For corticosterone, we accounted for the influence of species, sex, and snout-vent length (SVL). For BCI, we standardized scores for each life stage (larva or adult/sub-adult) within each species (*D. fuscus*, *D. monticola*, *D. ochrophaeus*, *Eurycea* spp. [*E. bislineata* and *E. cirrigera*], and *Gyrinophilus porphyriticus*), and accounted for the potential influence of species, life stage, and habitat characteristics (i.e., mean stream water depth and percent canopy cover).

Model	Parameters	AICc	ΔAICc	Adj-R2	w_i
Water Imidacloprid Concentration					
Trt Intensity - Last Tx Yr	3	106.12	0.00	0.52	0.73
YST + Trt Intensity - Last Tx Yr	4	108.06	1.94	0.51	0.27
(.)	2	122.82	16.70	NA	0.00
YST	3	125.02	18.90	−0.03	0.00
Salamander bioaccumulation					
(.)	2	34.87	0.00	NA	0.76
Water IMI Concentration	3	37.15	2.28	−0.02	0.24
Benthic macroinvertebrate bioaccumulation					
Water IMI Concentration	3	147.31	0.00	0.32	0.83
(.)	2	150.46	3.14	NA	0.17
Salamander corticosterone concentration					
Species + Sex + SVL × Water IMI Concentration	8	293.24	0.00	0.29	0.84
Species + Sex + SVL + Water IMI Concentration	7	298.98	5.75	0.25	0.05
Species + Sex + SVL + Trt Intensity - Last Tx Yr	7	299.42	6.19	0.24	0.04
Species + Sex + SVL + Trt Intensity - Total	7	300.00	6.77	0.24	0.03
Salamander BCI					
Canopy Cover + Water IMI Concentration	4	2247.56	0.00	0.03	0.52
Canopy Cover	3	2247.73	0.16	0.03	0.48

(Burnham and Anderson, 2004; Burnham et al., 2011). For treated sites only, we assessed if number of years since treatment (YST) and number of trees treated in the last treatment year (treatment intensity - last treatment year) influenced imidacloprid concentration in stream water at the time of sampling. For all sites sampled, we assessed if imidacloprid concentration in stream water was an influential predictor of imidacloprid concentration in salamanders and benthic macroinvertebrates. For salamander bioaccumulation analyses, we did not test species separately because of the small sample size. Due to lack of normality with the full dataset caused by severe zero-inflation, we restricted the analysis to individuals with detectable levels of imidacloprid (n = 47). To represent the full dataset (n = 107), we also tested if salamander tissue concentration differed between sites with and without imidacloprid detected in the water using a non-parametric Mann–Whitney *U* test (Sokal and Rohlf, 1995).

For corticosterone, we conducted a two-step model selection. First, we identified the most parsimonious demography model by ranking models containing all combinations of sex and SVL (species included in all models except the null model) because demographic variables can strongly influence corticosterone concentration (Dickens and Romero, 2013). We retained the most parsimonious model as the null model for the second model selection, which ranked predictors of imidacloprid exposure. For BCI, we accounted for the potential influence of species, life stage (larva or adult/sub-adult), and site habitat characteristics (mean stream water depth and percent canopy cover). For both analyses, we ranked five predictors of imidacloprid exposure: water imidacloprid concentration, presence of treated trees, treatment intensity - total, treatment intensity - last treatment year, and presence of imidacloprid in the environment. A site was considered to be present for imidacloprid if the site was located adjacent to a treated stand or if imidacloprid or one of its metabolites (imidacloprid-urea and imidacloprid-olefin) was detected in either stream water or sediment.

We calculated BCI scores by regressing (log) SVL on (log) weight (Schulte-Hostedde et al., 2005) for 802 individuals of 5 species. Positive residuals indicate a higher-than-average weight for a given SVL. We did not include salamanders missing portions of their tails or legs in BCI analyses. We created separate BCIs for five salamander species, and for gravid females within-species, including *D. fuscus* (n = 207), *D. monticola* (n = 274), *D. ochrophaeus* (Allegheny Mountain dusky salamander; n = 141), *Eurycea* spp. (northern and southern two-lined salamanders; n = 59), and *Gyrinophilus porphyriticus* (spring salamander; n = 121). We did not include sex as a candidate predictor because we could not reliably identify sex for many of the

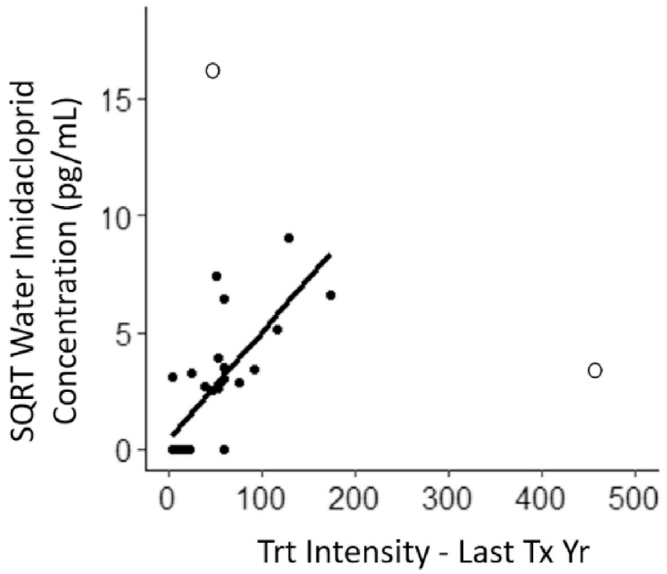


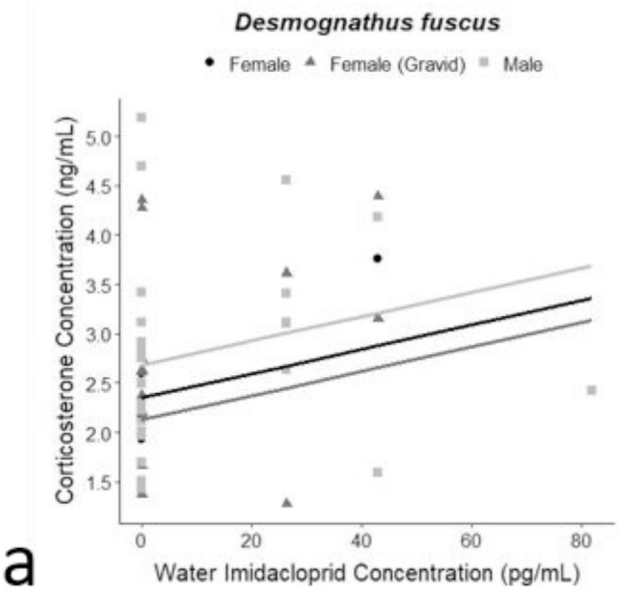
Fig. 2. Model-estimated relationship between number of trees treated with imidacloprid in the last treated year (Trt Intensity - Last Tx Year) and (square root transformed) water imidacloprid concentration at the time in headwater streams located in the New River Gorge National River (New River Gorge), Gauley River National Recreational Area (Gauley River), and Monongahela National Forest, West Virginia, USA. For the analysis, water concentrations were square root transformed and two outlier sites (open circles) were removed from analysis to satisfy model assumptions.

captures. We excluded larval salamanders that weighed ≤0.1 g and all larval *Eurycea* spp. because SVL was not a strong predictor of weight. The data were z-score transformed so that standard deviations were equal across species and life stages (Legendre and Legendre, 2012).

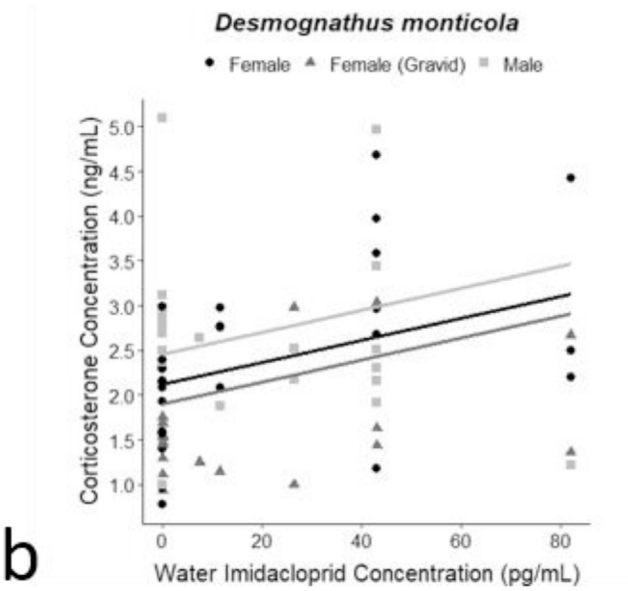
For all analyses, we assessed assumptions of normality using quantile-quantile plots and homoscedasticity using residual plots (Zuur et al., 2009, 2010). For the water imidacloprid concentration models, we excluded the highest water concentration site and square root transformed the response data to satisfy the assumption of normality. We also excluded the highest treatment intensity site because the site was an extreme outlier with high leverage. We note that inclusion of this outlier did not influence the selected top model or the directional relationship. For the salamander bioaccumulation dataset that was reduced to include only salamanders with imidacloprid in their tissues, we log transformed the response data to satisfy the

Table 2
Coefficient (β) estimates and 95% confidence intervals (CI) for supported linear regression models in this study investigating potential sublethal effects of imidacloprid on salamanders in West Virginia, USA. Models include number of trees treated in the most recent year of imidacloprid application (Treatment Intensity - Last Tx Year) as a predictor of water imidacloprid concentration, and water imidacloprid concentration (Water IMI Concentration) as a predictor of salamander corticosterone concentration and salamander body condition index (BCI), with influential demographic and environmental variables included in the models.

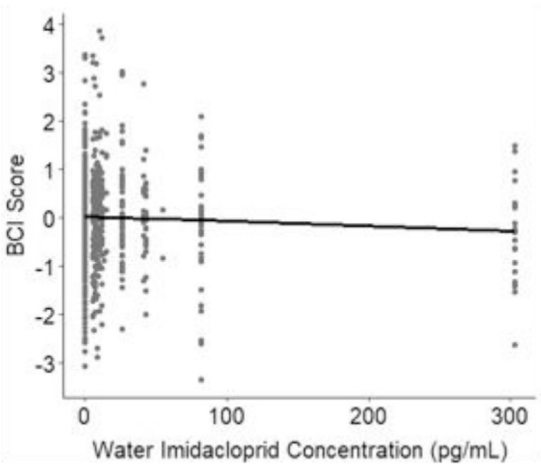
Variable	β	Lower CI	Upper CI	Partial-R^2
Water imidacloprid concentration				
Intercept	0.3379	−0.8810	1.5567	
Treatment Intensity - Last Tx Year	0.0467	0.0280	0.0653	0.5378
Intercept	17.2112	−10.9094	45.3319	
Water IMI Concentration	2.4820	0.6467	4.3172	0.3693
Salamander corticosterone concentration				
Intercept	2.3500	1.8538	2.8461	
Species (DEMO)	−0.2229	−0.6445	0.1987	0.0035
Sex (G)	−0.2228	−0.6733	0.2278	0.0667
Sex (M)	0.3318	−0.1071	0.7706	0.0667
SVL	−0.0197	−0.0441	0.0048	0.1818
Water IMI Concentration	0.0123	0.0052	0.0194	0.0318
SVL × Water IMI Concentration	−0.0009	−0.0015	−0.0002	0.0851
Salamander BCI				
Intercept	0.3458	0.199	0.4927	
% Total Canopy	−0.0052	−0.0072	−0.0031	0.0322
Water IMI Concentration	−0.001	−0.0023	0.0003	0.0062


Desmognathus fuscus
Female
Female (Gravid)
Male
Corticosterone Concentration (ng/mL)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
0
20
40
60
80
Water Imidacloprid Concentration (pg/mL)

a


Desmognathus monticola
Female
Female (Gravid)
Male
Corticosterone Concentration (ng/mL)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0
20
40
60
80
Water Imidacloprid Concentration (pg/mL)

b


BCI Score
4
3
2
1
0
-1
-2
-3
0
100
200
300
Water Imidacloprid Concentration (pg/mL)

c

assumption of normality. For the macroinvertebrate bioaccumulation data set, we identified one macroinvertebrate imidacloprid concentration sample as an extreme outlier and removed it to satisfy the assumption of homoscedasticity. For the corticosterone data set, we removed the four highest corticosterone concentration samples to satisfy the assumption of normality.

3. Results

3.1. Water and sediment imidacloprid concentration

Of the 48 sampled sites, 27 were adjacent to HWA treatments (Table S1). Imidacloprid was detected in the stream water at 23 sites, with a mean concentration of 30.7 ± 13.0 pg/mL (range = 5.29–303). Imidacloprid was detected in sediment at eight sites, five of which were adjacent to HWA treatments, and six of which had imidacloprid detected in the stream water. Imidacloprid-urea and imidacloprid-olefin were not detected in the stream water or sediment at any site. Sites adjacent to HWA treatments where we detected imidacloprid in the water or sediment on average had been treated 2.8 ± 0.3 (range = 1–5) years prior to sampling. The model containing treatment intensity - last treatment year was the most supported model (w_i = 0.73; Table 1) with water imidacloprid concentration increasing as treatment intensity in the last treatment year increased ($\beta = 0.0467$, $R^2 = 0.54$; Fig. 2).

3.2. Salamander imidacloprid bioaccumulation

Of the 107 salamanders tested for bioaccumulation, 47 had detectable levels of imidacloprid, 3 of which were from a site without known imidacloprid treatments or detectable imidacloprid in the stream water or sediment. These three salamanders were captured 350 m away from and in a different drainage from the nearest known treatment site. We detected imidacloprid in the tissues of 31 *D. monticola* and 16 *D. fuscus*, with a mean concentration of 20.6 ± 2.9 ng/g (range = 3.8–86.3). Of the 60 salamanders in which imidacloprid was not detected, 53 salamanders were from sites where imidacloprid was detected in the stream water. We did not detect imidacloprid-urea or imidacloprid-olefin in the tissues of any salamanders.

For the subset of the bioaccumulation dataset that only included salamanders with imidacloprid detected in their tissues, the null model received more support than the model containing concentration of imidacloprid in stream water (w_i = 0.76; Table 1), indicating little support that imidacloprid concentrations in stream water was related to concentration in salamander tissues. The coefficient was negative ($\beta = -0.0002$), indicating concentration in salamanders decreased as concentration in stream water increased, but the 95% confidence interval (CI) broadly overlapped zero (−0.004–0.004). For the full bioaccumulation dataset, concentration of imidacloprid in salamanders differed between samples taken from sites with and without imidacloprid detected in stream water ($W = 1317.5$, $p = 0.003$). For samples from sites with water imidacloprid detected (n = 84), imidacloprid was detected in 37% of salamanders, median concentration was 0 ng/g, and mean concentration was 7.3 ng/g. For samples from sites without water imidacloprid detected (n = 23), 70% of salamanders had detectable imidacloprid, median concentration was 12.6 ng/g, and mean concentration was 15.7 ng/g.

3.3. Imidacloprid bioaccumulation in benthic macroinvertebrates

We detected imidacloprid in all 15 benthic macroinvertebrate samples, with a mean concentration of 46.0 ± 11.4 ng/g (range = 9.27–185.47; Fig. S1). We also detected imidacloprid-urea in 13 of the samples and imidacloprid-olefin in one of the samples. The average detected concentration of imidacloprid-urea was 275 ± 29.9 ng/g (range = 125–445.3); concentration of imidacloprid-olefin in the one sample was 28.9 ng/g. For three sites adjacent to HWA treatments, imidacloprid was not detected in the water or sediment, but was detected in the invertebrate samples (mean invertebrate concentration = 43.5 ± 17.8 ng/g; Table S2). The model containing water imidacloprid concentration was the most supported model (w_i = 0.83; Table 1). Benthic macroinvertebrate imidacloprid concentration was positively associated with water imidacloprid concentration ($\beta = 2.4820$, 95% CI: 0.6467–4.3172; Table 2).

3.4. Sublethal effects of imidacloprid on salamanders

For corticosterone, average plasma concentration was 2.8 ± 2.6 ng/mL (range = 0.8–28.4). For the demography model selection, an additive model with species, sex, and SVL had the highest support. For the imidacloprid model selection, the most-supported model contained an interaction effect between SVL and water imidacloprid concentration (w_i = 0.84; Table 1). Water imidacloprid concentration was positively correlated with corticosterone concentration ($\beta = 0.0123$, 95% CI: 0.005–0.019; Fig. 3a and b; Table 2), with a decreasing effect as salamander SVL increased (interaction $\beta = -0.0009$),

Fig. 3. Potential sublethal effects of imidacloprid exposure on stream salamanders in West Virginia, USA. Model-estimated relationship between water imidacloprid concentration and concentration of the hormone corticosterone for male, non-gravid female, and gravid female (a) *Desmognathus fuscus* (n = 46) and (b) *Desmognathus monticola* (n = 69). (c) Model-estimated relationship between standardized salamander body condition index (BCI) score and concentration of imidacloprid in stream water with canopy cover (%) held at the mean (63.4%; n = 802 individuals representing 5 species).

indicating the positive relationship between corticosterone and water imidacloprid concentrations was stronger for smaller salamanders. The second most supported model contained an additive effect between SVL and water imidacloprid concentration ($w_i = 0.05$).

For the BCI model selection, the model with the strongest support contained an additive effect with canopy cover and concentration of imidacloprid in stream water ($w_i = 0.52$), and the model with the second strongest support contained canopy cover as a predictor ($w_i = 0.48$). After accounting for canopy cover, BCI decreased as concentration of imidacloprid in stream water increased, however the 95% CI overlapped zero ($\beta = -0.001$, 95% CI: -0.0023− 0.0003; Table 2). Additionally, explanatory power was very low (partial $R^2 = 0.003$), and the relationship was largely driven by the site with the highest imidacloprid concentration in the water (Fig. 3c). For all five species, mean BCI score was lower at sites with presence of imidacloprid in the environment, and median BCI score was lower for four of the species (Fig. 4).

4. Discussion

Our results add to a growing body of literature documenting presence of imidacloprid in streams adjacent to treated hemlock stands, indicating that persistent leaching from treated stands is prevalent. The range of imidacloprid concentration that we found in stream water (5.29–303 pg/mL) was within the range of imidacloprid concentrations detected in stream water from three other studies in hemlock systems (Churchel et al., 2011; Benton et al., 2015; Wiggins et al., 2018). We detected imidacloprid in the stream sediment of only 8 sites compared to the 23 at which we detected imidacloprid in the water, likely due to the high water-solubility of imidacloprid (US EPA, 2003). Information about the persistence of imidacloprid at these sites, which were treated up to five years prior to sampling, may assist land managers when assessing potential risks of treating hemlock stands.

The primary goal of this study was to determine whether imidacloprid is bioaccumulating in the tissues of stream organisms and potentially resulting in sublethal effects. Consistent with our hypotheses, we found evidence that stream salamanders and stream macroinvertebrates uptake imidacloprid that leaches into their environment from nearby treated hemlock stands. For amphibians, the two primary pathways for imidacloprid uptake include direct oral/dermal uptake and the consumption of contaminated prey (Van Meter et al., 2014, 2015; Pisa et al., 2015; Glinski et al., 2018). Anurans can uptake imidacloprid dermally (Van Meter et al., 2014, 2015), and we assume that stream salamanders in contact with contaminated water or sediment can do so as well. Benthic macroinvertebrates, including crayfish, are important food sources of stream salamanders and other aquatic and terrestrial vertebrates, and all composite invertebrate samples from streams adjacent to treated hemlocks contained imidacloprid. The general composition of invertebrate taxa we collected are likely to be consumed by stream salamanders (Keitzer and Goforth, 2013; Trice et al., 2015). Thus, prey consumption may represent a route of imidacloprid exposure for salamanders and other vertebrate species. Interestingly, we did not find a strong relationship between imidacloprid concentration in stream water and imidacloprid concentration in salamander tissues, likely because point samples of water imidacloprid can vary over time with differing amounts of precipitation (Wiggins et al., 2018) and because our water samples were not collected at the same time as the salamander samples. Additionally, retention time of imidacloprid in salamander or macroinvertebrate tissues is not known, so additional work is needed to understand relationships between exposure concentrations and tissue concentrations.

Corticosterone in *D. monticola* and *D. fuscus* was positively associated with imidacloprid concentration in stream water, indicating that exposure to imidacloprid has an effect on the health of individual stream salamanders. Additionally, water

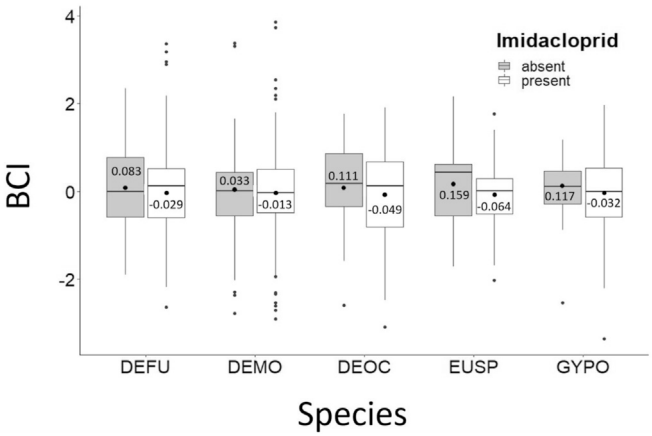


Fig. 4. Boxplot summaries of body condition index (BCI) values assessing potential sublethal effects of imidacloprid exposure on *Desmognathus fuscus* (DEFU; absent, n = 54, present, n = 153), *D. monticola* (DEMO; absent, n = 79, present, n = 195), *D. ochrophaeus* (DEOC; absent, n = 43, present, n = 98), *Eurycea* spp. adults (EUSP; absent, n = 17, present, n = 42), and *Gyrinophilus porphyriticus* (GYPO; absent, n = 26, present, n = 95). Mean BCI is indicated with a circle and median BCI is indicated with a line.

imidacloprid concentration more strongly affected corticosterone in smaller salamanders, meaning that imidacloprid exposure may have greater effects on the health of younger individuals. Negative effects on the health of individuals can potentially result in population-level effects through impacts on survival and reproduction (Hayes et al., 2010; Willson et al., 2012). Conclusions from previous studies investigating pesticide-associated changes in corticosterone levels are conflicted. For example, larval western tiger salamanders (*Ambystoma mavortium*) had higher corticosterone levels in agricultural wetlands in South Dakota with elevated levels of neonicotinoid insecticides, compared to reference wetlands (Davis et al., 2019). In contrast, wood frog (*Lithobates sylvaticus*) tadpoles experimentally exposed to environmentally relevant concentrations of the neonicotinoid thiamethoxam had lower corticosterone concentrations, and there was no difference in corticosterone concentrations in juveniles (Gavel et al., 2019). In addition, we acknowledge that much of the variance in corticosterone concentration was not explained by water imidacloprid concentration. This is not surprising given the reliance on point samples as a proxy for dynamic imidacloprid exposure and the complexity of natural systems with many stressors.

Body condition is an important indicator of amphibian health and correlates with survival and productivity (e.g., Reading, 2007; Roznik et al., 2015). For example, larger body size is advantageous in mate competition (Howard et al., 1997). We found that salamander BCI decreased with higher imidacloprid concentration in stream water, and that mean BCI was lower in streams with environmental imidacloprid for all five species sampled. As with corticosterone concentration, we acknowledge that most of the variance in BCI scores was not captured by our predictors of imidacloprid exposure and suggest that more controlled studies be conducted to further clarify the relationship.

We detected imidacloprid in the tissues of macroinvertebrates from three sites adjacent to treated hemlock stands but where we did not detect imidacloprid in the stream water or sediment. Presence of imidacloprid in stream water varies temporally and increases after rain events (Churchel et al., 2011; Cowles, 2009; Wiggins et al., 2018), but can break down quickly in the presence of sunlight (Ding et al., 2011). Grab sampling of stream water for pesticide runoff research does not account for spatial and temporal variation and can lead to underestimates of pesticide residues (Xing et al., 2013). Our results suggest that sampling stream invertebrates may be more reliable than sampling stream water to confirm presence of imidacloprid in streams. Additionally, downstream drift and upstream movement commonly occur in aquatic macroinvertebrate communities, and the dispersal of macroinvertebrates with imidacloprid bioaccumulation may contribute to the spread of environmental imidacloprid in riparian environments (Bird and Hynes, 1981). In this study, we were unable to assess whether imidacloprid concentrations in benthic macroinvertebrate tissues varied by guild, body size, feeding type, or family. Additional research investigating the relationships between imidacloprid concentration in tissues and macroinvertebrate classifications is warranted. Previously, two studies found no differences in aquatic invertebrate communities between streams exposed to HWA treatments and control streams (Churchel et al., 2011, Benton et al., 2016). The maximum water imidacloprid concentrations detected in these previous studies (<1000 pg/mL and 379.1 pg/mL) are higher than those detected in our study, in which the maximum imidacloprid concentration in water was 303 pg/mL. Additionally, the concentrations detected in this study are below those previously found to cause sublethal and lethal effects in aquatic invertebrates (i.e. Sánchez-Bayo and Goka, 2006; Alexander et al., 2007; Kreutzweiser et al., 2009; Pestana et al., 2009; Colombo et al., 2013). Further work is needed to determine the effect bioaccumulation may have on population-level changes in benthic macroinvertebrates.

Two sites that were not directly adjacent to HWA treatments contained salamanders with detectable levels of imidacloprid. One of these sites was located downstream of farmland, and we did detect imidacloprid in the stream sediment. Imidacloprid is commonly used in agriculture (Elbert et al., 2008), and thus leaching from upstream farmland may explain presence of imidacloprid at this site. Similarly, another site was near private homes, and imidacloprid is used in residential areas to treat pests such as termites (Parman and Vargo, 2010). However, we did not detect imidacloprid in the stream water or sediment at this site. It is possible that sampling aquatic salamanders, like sampling benthic macroinvertebrates, is a more reliable method to assess potential imidacloprid leaching than collecting grab samples of stream water, but further research is needed to clarify the origin of imidacloprid residues when treated trees are not directly adjacent to the stream. Furthermore, we note that although *Desmognathus* spp. typically have small home ranges, they are capable of dispersal via movements within the stream channel and over land (Grant et al., 2010; Miller et al., 2015), and may increase their home range sizes in response to environmental stressors such as drought (Ashton, 1975).

Imidacloprid is one of the world's most widely used pesticides, with application in agricultural, urban, and forest systems (Elbert et al., 2008; Havill et al., 2014). Global concern about the pervasiveness of imidacloprid in the environment have prompted numerous studies showing effects on non-target species. In summary, our study suggests that treating forest hemlock stands with imidacloprid can result in persistent leaching of the pesticide into adjacent streams, uptake by aquatic organisms, and potential sublethal effects on salamanders. However, hemlocks are a foundation species and numerous plant and animal species depend on healthy hemlock stands, making the control of HWA critical. There is a strong need for additional research to improve our understanding of non-target effects and movement of imidacloprid in aquatic and terrestrial food webs.

Data availability

The data used in this manuscript are publicly available on the research data repository Dryad. The shareable link to view and download the data is https://doi.org/10.5061/dryad.1ns1rn8s2.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank C. Arnott and J. Perez for assistance with proposal development. We thank M. Kim, S. Woodley, A. Fulcher, S. Wilson, K. Sudol, Z. Dienes, J. Kim, D. Hoffer, D. Erikson, L. Paul, L. Strickler, C. Johnson, I. Allen, and R.A. Wilkes for assistance with data collection or chemical analyses. We thank D. Davis and two anonymous reviewers for providing valuable suggestions on earlier drafts of this manuscript, and we thank R. Tyl for reviewing data and metadata. We thank D. Erikson and the staff at the Endocrine Technologies Core at the Oregon National Primate Research Center, which is supported by National Institutes of Health Grant P51 OD011092. This research was supported by the U.S. Geological Survey-National Park Service Water Quality Partnership Program, the Foundation for the Conservation of Salamanders, and the West Virginia University Program to Stimulate Competitive Research.

Capture and handling methods were approved by the West Virginia Division of Natural Resources (Permit #: 2017.073, 2017.096), National Park Service (Permit #: NERI-2017-SCI-0002 and GARI-2017-SCI-0001), and West Virginia University Institutional Animal Care Use Committee (Protocol #: 1602000309_R1). Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gecco.2020.e01292.

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