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RISK ASSESSMENT OF IMIDACLOPRID USE IN FOREST SETTINGS ON THE AQUATIC MACROINVERTEBRATE COMMUNITY

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Abstract: The isolated effects of a single insecticide can be difficult to assess in natural settings because of the presence of numerous pollutants in many watersheds. Imidacloprid use for suppressing hemlock woolly adelgid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae), in forests offers a rare opportunity to assess potential impacts on aquatic macroinvertebrates in relatively pristine landscapes. Aquatic macroinvertebrate communities were assessed in 9 streams in Great Smoky Mountains National Park (southern Appalachian Mountains, USA). The streams flow through hemlock conservation areas where imidacloprid soil drench treatments were applied for hemlock woolly adelgid suppression. Sites were located upstream and downstream of the imidacloprid treatments. Baseline species presence data (pre-imidacloprid treatment) were available from previous sample collections at downstream sites. Downstream and upstream sites did not vary in numerous community measures. Although comparisons of paired upstream and downstream sites showed differences in diversity in 7 streams, higher diversity was found more often in downstream sites. Macroinvertebrate functional feeding groups and life habits were similar between downstream and upstream sites. Downstream and baseline stream samples were similar. While some functional feeding group and life habit species richness categories varied, variations did not indicate poorer quality downstream communities. Imidacloprid treatments applied according to US Environmental Protection Agency federal restrictions did not result in negative effects to aquatic macroinvertebrate communities, which indicates that risks of imidacloprid use in forest settings are low. *Environ Toxicol Chem* 2017;9999:1–12. © 2017 SETAC

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INTRODUCTION

Neonicotinoid insecticides are currently under much scrutiny because of environmental risks to nontarget species. Before the 1990s, carbamates, pyrethroids, and organophosphates constituted the majority of insecticides used in agricultural systems. Neonicotinoids were developed in response to concern about the chronic and acute mammalian toxicity of the other insecticide classes [1]. Low toxicity to vertebrates occurs because neonicotinoids are less selective toward nerve receptors in vertebrates compared with insects [2]. Thus, vertebrate safety profiles for neonicotinoids are much better compared with other classes of insecticides. However, concern for nontarget effects of neonicotinoids, especially imidacloprid, has increased. Factors of particular interest include environmental persistence, potential to leach into surface waters, toxicity to aquatic macroinvertebrates, and possible role in pollinator decline [3–6].

The movement of imidacloprid through the soil is a route of potential impact to surface water [3]. Imidacloprid photodegrades in water, where it has a half-life ranging from 1 h to 3 d [7–9]. Negative effects of aquatic macroinvertebrate exposure to imidacloprid pollution in surface water are likely chronic rather than acute [10]. Chronic effects are sublethal effects after exposure to a lower concentration of a pollutant over a longer time frame. Acute effects are lethal effects after a short-term, high-concentration exposure to a pollutant. Chronic exposure to imidacloprid in sufficiently high concentrations is expected to result in cumulative and usually long-term effects [11].

The US Environmental Protection Agency (USEPA) lists imidacloprid as highly toxic to aquatic macroinvertebrates [3]. Caddisflies (Trichoptera), mayflies (Ephemeroptera), and true flies (Diptera) have been documented as the most sensitive taxa [12,13]. Aquatic macroinvertebrate toxicity data are most often generated from dose–response single-species laboratory assays. These studies are commonly based on responses to short-term exposure times of 24 to 96 h. Median lethal concentrations (LC50s) for true flies (*Simulium latigonium* Rubtsov [Diptera: Simuliidae]) and mayflies (*Epeorus longimanus* Eaton [Family Heptageniidae]) as low as 3.73 and 0.65 μg/L, respectively, have been documented [14,15].

A 28-d assessment was conducted to determine chronic effects of imidacloprid over longer time frames to gauge likely impacts in natural systems [16]. The LC50 values were 0.195 and 0.316 μg/L for the mayflies *Cloeon dipterum* (L.) (Ephemeroptera: Baetidae) and *Caenis horaria* (L.) (Ephemeroptera: Caenidae), respectively. Immobilization (median effect concentration [EC50]) was observed at 0.123 and 0.126 ppb, respectively [16]. Unfortunately, laboratory experiments consisting of single-species analyses are not adequate to fully gauge potential ecological threats [17].

Microcosm and mesocosm studies have been conducted to assess the effects of imidacloprid on macroinvertebrate

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communities in settings replicative of natural conditions [12,18]. Aquatic macroinvertebrate communities were exposed to 12 μg/L imidacloprid pulses to simulate stormflow peaks in imidacloprid pollution. Negative long-term effects of imidacloprid pulses included reduction in abundance of chironomids (Diptera: Chironomidae), increased abundance of tolerant gastropods (Hygrophila: Limnaeidae), decreased emergence of adult mayflies (Ephemeroptera: Caenidae), less overall taxa emergence in the summer, and a decline in caddisfly nets (Trichoptera: Polycentropodidae) [12,18]. However, no difference was detected in the overall abundance of larval mayflies, caddisflies, and true flies [12,18]. Water quality protection is essential given the chronic and acute effects of imidacloprid on aquatic macroinvertebrate taxa.

Many governments have set limits on imidacloprid concentrations in surface waters to protect water quality from potential detrimental effects of imidacloprid pollution. Chronic and acute aquatic life benchmarks set by the USEPA are 1.05 and 34.5 μg/L, respectively [19]. Methods of USEPA limit determination are not specified. The limit in Canada is 0.23 μg/L for chronic exposure, which is based on the 28-d effect concentration for 15% of a *Chironomus riparius* Meigen (Diptera: Chironomidae) population of 2.25 μg/L, divided by a safety factor of 10 [20]. Dutch standards for chronic and acute imidacloprid concentrations are 0.067 and 0.2 μg/L, respectively [21], based on the no-observable-adverse-effect concentration (NOAEC) of 0.67 μg/L for *Chironomus tetans* F. (Diptera: Chironomidae). The NOAEC was divided by a safety factor of 10 and 3 for the chronic and acute limits, respectively [21].

Neonicotinoids, including imidacloprid, have been detected in surface waters in numerous studies. Concentrations of neonicotinoids documented in surface water samples in a review of 29 studies conducted in numerous countries showed average ambient and peak concentrations of 0.13 and 0.63 μg/L, respectively [22]. Sampled streams in the United States with positive imidacloprid detections have concentrations ranging from 0.05 to 0.67 μg/L [6,23–25].

Imidacloprid has been documented in surface waters in concentrations that may negatively affect aquatic macroinvertebrates. The presence of numerous pollutants in most watersheds has prohibited assessing the isolated effects of imidacloprid [26]. However, the use of imidacloprid for suppression of hemlock woolly adelgid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae), offers a unique opportunity to assess imidacloprid in relatively pristine landscapes.

Imidacloprid has been widely used for suppression of hemlock woolly adelgid, an invasive species threatening eastern hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelmann (Pinales: Pinaceae), resources in forests in the eastern United States. Eastern hemlock is a foundation species, providing many ecological services in forest settings [27,28]. Hemlocks provide habitat for more than 400 species of canopy arthropods and are a source of food and shelter for wildlife [29–32]. Hemlock-dominated riparian areas have distinctive water temperature regimes and aquatic macroinvertebrate species composition [33,34]. The loss of this shade-tolerant conifer will have cascading ecological effects, as the role of hemlock cannot be filled by any other native evergreen tree species [27,28]. The use of imidacloprid is critical for the preservation of this iconic foundation species, and 1 imidacloprid application can provide up to 7 yr of hemlock woolly adelgid suppression [35].

Great Smoky Mountains National Park, which is located in the southern Appalachian Mountains in eastern North America, is an extensive 211 418-ha national park. Elevation in Great Smoky Mountains National Park ranges from 227 to 2025 m. More than 1175 km of fish-bearing streams, as well as another 2092 km of tributaries, flow through the Park. Great Smoky Mountains National Park is typified by extensive tracts of wilderness, and there are only 10 developed campgrounds in the more than 200 000 ha of natural resources [36–38]. Environmental conditions for the extensive tracts of wilderness areas are nearly pristine.

Eastern hemlock ranges throughout Great Smoky Mountains National Park and occupied greater than 55 500 ha before hemlock woolly adelgid–induced mortality. Within this area, more than 5665 ha contained hemlock-dominant forests [39]. Personnel in Great Smoky Mountains National Park have implemented an extensive hemlock woolly adelgid integrated pest management program. Imidacloprid soil drench treatments have been applied to more than 250 000 individual hemlocks, with 4249 kg of imidacloprid applied to more than 4470 ha of hemlock forests.

Since the establishment of the Park in 1934, few chemical insecticide applications have occurred. In the 1960s and 1970s, DDT and lindane were applied over 405 and 24 ha, respectively. The only recent insecticide applications in Great Smoky Mountains National Park have been associated with hemlock woolly adelgid suppression. Given the limited use of insecticides in Great Smoky Mountains National Park, the hemlock woolly adelgid integrated pest management program offers a unique opportunity to assess imidacloprid effects in watersheds isolated from other insecticidal pollutants. Imidacloprid concentrations have been assessed in the streams used in the present study. Positive detections occurred in 7 of 10 sampled streams within hemlock conservation areas where imidacloprid soil drench treatments were applied (Table 1) [25]. The highest concentration was 0.379 μg/L, and all other detections were below 0.100 μg/L. The highest concentration detection did not exceed USEPA benchmarks. However, imidacloprid was present in many streams during ambient conditions. The possible consistent presence of imidacloprid in streams in Great Smoky Mountains National Park associated with hemlock woolly adelgid conservation areas increases concern for potential chronic effects, because toxicity of imidacloprid can be more pronounced over longer exposure times [13,40].

While surface water samples provide short-term information about water quality conditions, aquatic macroinvertebrate assessments provide both short- and long-term perspectives on water quality conditions [41]. The presence of a taxon in a stream indicates that water quality conditions have been conducive to support the survival of that taxon. The effects of a short-term pollution event on a taxon can be observed on a long-term basis (approximately 1 yr), until the next generation of that taxon is present [41].

The present study is part of a larger project to conduct a retrospective assessment of the hemlock woolly adelgid integrated pest management program of Great Smoky Mountains National Park. The purpose of the present study is to assess whether imidacloprid use for the suppression of hemlock woolly adelgid has negative effects on macroinvertebrate communities in streams. One other study has assessed both imidacloprid concentrations and macroinvertebrate communities in forest streams for the first 2 yr after imidacloprid application. Although no negative effects to aquatic

Table 1. Imidacloprid treatment history and water sample results in streams in Great Smoky Mountains National Park

Stream	Imidacloprid concentration[a]	Treated hectares	Total kg a.i.[b]	Treatment cycles	No. of months between: Last treatment, water sample	No. of months between: Last treatment, EPT sample	No. of months between: Water sample, EPT sample
Alum Creek	28.5 ± 3.8	19.0	14.8	5	10	11	1
Camel Hump Creek[c]	<LOD[d]	N/A	N/A	N/A	N/A	N/A	3
Cane Creek	<LOD	14.5	6.3	3	28	20	8[e]
Chasteen Creek	36.8 ± 3.4	42.6	16.8	4	42	37	5[e]
Dunn Creek	379.1 ± 7.9	47.1	114.0	6	21	22	1
Indian Creek	31.2 ± 1.5	47.2	38.3	5	14	14	0
Kingfisher Creek[f]	33.6 ± 6.6	29.4	20.9	4	0	1	4[e]
Panther Creek	<LOD	26.6	1.8	1	4	5	1
Shop Creek	82.2 ± 25.8	23.3	7.6	1	15	14	1[e]

[a]Means (± standard deviation) are an average of the concentrations of 3 samples collected at each sample location.
[b]kg active ingredient (a.i.) applied in the treatment area.
[c]Treatment history data were not available for Camel Hump Creek.
[d]Imidacloprid concentration was below the limit of detection (LOD; 20 ng/L).
[e]Water samples were collected after macroinvertebrate community samples were collected.
[f]A treatment occurred between macroinvertebrate community sample and water sample collections.
EPT = Ephemeroptera, Plecoptera, Trichoptera.

macroinvertebrates were observed, imidacloprid was detected in only 1 water sample at the very end of the 2-yr sampling time [42]. Thus, risks to macroinvertebrates in the known presence of imidacloprid were not assessed [42]. Imidacloprid has been detected in the majority of streams assessed in the present study during ambient conditions [25], so the present study provides assessments of aquatic macroinvertebrate communities in the known presence of imidacloprid in streams.

MATERIALS AND METHODS

Stream sites

Aquatic macroinvertebrate multihabitat bioassessments were conducted in 9 streams that flowed through hemlock forest conservation areas. Imidacloprid has been detected previously in 6 of the 9 streams sampled in the present study [25]. The bioassessments were conducted to determine whether imidacloprid use impacted stream water quality and biota in Great Smoky Mountains National Park. Two sites were assessed in each stream: a site downstream from the imidacloprid-treated conservation area and a control site upstream of the conservation area, hereafter referred to as downstream and upstream sites, respectively (Figure 1). No paved roads or developed campgrounds were located near the stream sites, with the exception of the Alum Creek downstream site, which was located near a hiking trail parking lot. Access to most sites required hiking, often to locations with no trail access. Each site consisted of a 100-m stream reach. Downstream sites

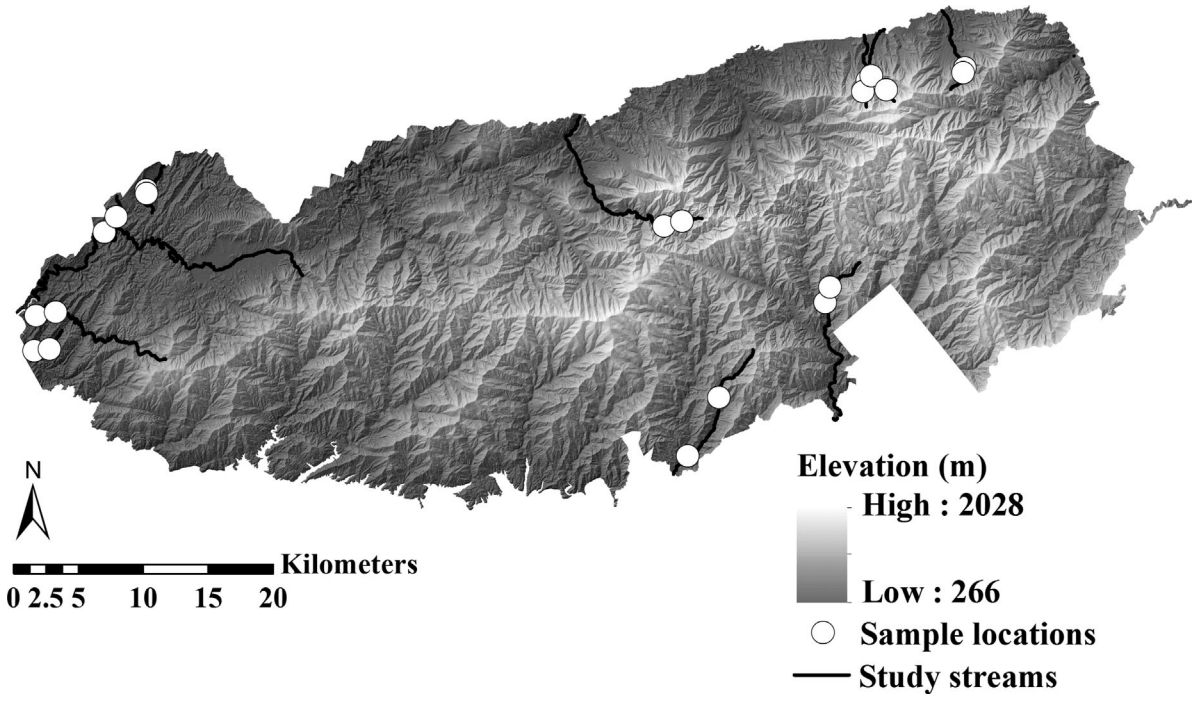

Figure 1. Downstream and upstream sites in Great Smoky Mountains National Park, United States.

were selected where the stream flowed out of the conservation area, and upstream sites were a minimum of 50 m upstream from the conservation area. Water flowing through the downstream sites flowed through the entire imidacloprid-treated conservation area, and thus, communities located at downstream sites had the highest chance of experiencing negative nontarget effects of imidacloprid pollution. Hemlock conservation areas were located in wilderness areas with contiguous natural forests.

Conservation areas ranged from 14.5 to 47.2 ha, and contained between 100 and 1000 hemlocks that had received soil drench treatments, which were initiated 1 mo to 8 yr before stream sampling. Between 1.8 and 114.0 kg imidacloprid active ingredient were applied to the conservation areas. Hemlocks in some conservation areas received imidacloprid soil drench treatments multiple times. A treatment cycle refers to either treatment of an entire conservation area or when additional acreage was added to an existing conservation area (see Table 1 and Benton et al. [25] for additional details). All applications were made according to the product label [43], and the label limit of 0.45 kg active ingredient/ha/yr was not exceeded. Imidacloprid applications were often applied in riparian areas; however, applications were not applied within 3 m of stream banks.

Historical aquatic macroinvertebrate presence data were available from previous water quality assessments conducted between 1994 and 1997 at all downstream sites, hereafter referred to as baseline samples. These historical data serve as a baseline of species presence in streams before any environmental impacts of hemlock woolly adelgid infestations or imidacloprid use in Great Smoky Mountains National Park. Downstream and upstream sampling was conducted within 2 wk of the dates (day and month) that baseline samples were collected to reduce changes in macroinvertebrate communities because of seasonality. All downstream and upstream samples were collected from June to September 2012.

Sample collection and identification

Rapid Bioassessment Methods developed by the North Carolina Department of Environment and Natural Resources (NCDENR) have been used by Great Smoky Mountains National Park personnel to conduct bioassessments for more than 20 yr [41,44]. Baseline samples were collected according to a previous, but similar, version of the current Great Smoky Mountains National Park protocol [45]. The Rapid Bioassessment is a standardized sampling technique used by many regulatory agencies to determine water quality [46]. Downstream and upstream samples were collected according to current Great Smoky Mountains National Park protocols [44].

Six sampling methods (kicknet, D-net, leaf pack, rock wash, sand samples, and visual samples) were employed at each downstream and upstream site to collect from multiple stream habitats within the stream reach. Each method was repeated 4 times within the reach for a total of 24 individual samples collected at each site. Methods were standardized by time or area to ensure equal sampling effort between upstream and downstream sites and among the 9 study streams. Kicknet (1-m^2) samples were collected by disturbing the substrate in riffles upstream from kicknet placement for 2 min. The substrate was disturbed either by kicking or moving rocks by hand, depending on the gradient of the stream. Dislodged insects flowed into the kicknet. D-net (30-cm) samples were collected in low-velocity flow areas of the stream for 1 min. Stream banks were repeatedly disturbed with the D-net, or soft substrates were disturbed by kicking. The D-net was then swept through the recently disturbed water column to collect specimens. Leaf pack samples consisted of approximately 10-cm^3 leaf packs collected from many habitats within the stream. Leaf packs were submerged in a bucket (19 L) of stream water. Leaves were agitated in the water to dislodge specimens and then removed from the bucket. The contents of the bucket were poured through a filter to collect specimens. Ten rocks were collected evenly from areas of high- and low-velocity flow within the stream for rock wash samples. Rocks were rinsed in a bucket of stream water, and dislodged specimens were collected by pouring water into the bucket through a sieve. Sand samples were collected by placing a triangle aquatic net (20 cm) downstream from an approximately 30-cm^2 area of sandy or fine gravel substrate. The substrate was disturbed by hand, and specimens dislodged from the substrate were collected in the net. Contents of the net were dislodged in a bucket of stream water. The water in the bucket was poured through a filter to collect specimens. Hand collection (visual) samples were collected for 5 min. Emphasis was put on hand-collecting specimens in habitats that may have been missed by other collection methods.

Samples were placed in 250- or 500-mL Nalgene jars and preserved in 95% ethanol in the field. Most liquid was decanted from the samples with high organic matter content and replaced with ethanol 2 to 3 d after sample collection to ensure specimen integrity during storage. Samples were stored in the laboratory at 21 °C before sample processing and specimen identification.

Samples were initially processed by removing aquatic macroinvertebrate specimens from debris in the sample and sorting to order. Because of sample volume and time constraints, 3 of the 4 repetitions of each sampling method were randomly selected for rough sorting and specimen identification. Mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera), henceforth referred to as EPT, are sensitive aquatic taxa. The EPT specimens were identified to the lowest taxonomic unit given the maturity of the specimens and taxonomic key availability. The following resources were used to identify specimens: *Aquatic Insects and Oligochaetes of North and South Carolina* [47], *An Introduction to the Aquatic Insects of North America* [48], and a draft caddisfly key (J. Morse, Clemson University, Clemson, South Carolina, USA personal communication). Data from specimens identified to the lowest taxonomic unit for each taxon were analyzed, with a few exceptions because of key availability or taxonomic difficulty. Representatives of each identified taxon were verified by a specialist. Specimens that were only mature enough to identify to order or family were excluded from the analyses, with a few family-level exceptions.

Data analyses

All statistical tests were considered significant at $p < 0.05$. Data were stored using an Excel file. Sample data from the 6 collection methods were composited and entered into a site by taxon data matrix. Tolerance values, obtained from NCDENR and Tennessee Department of Environment and Conservation (TDEC) standard operating procedures, were designated for each taxon [41,49]. In downstream–upstream comparisons, tolerance values were weighted by the abundance of each taxon.

$$TV_{\text{mean}} = \frac{\Sigma TV_{\text{taxon}} \times n}{N}$$

where TV_{mean} is mean tolerance value for a site, TV_{taxon} is the tolerance value for a particular taxon, n is the abundance of a

particular taxon at a stream site, and N is the total number of specimens collected at a stream site. However, in downstream–baseline comparisons, tolerance values were not weighted by abundance data, because only species lists were available for baseline samples. In addition, functional feeding groups and life habit categories were designated for each taxon. Category assignments were determined using standard operating procedures from NCDENR [41], TDEC [49], and Merritt et al. [48]. Functional feeding groups included collector–filterers, predators, generalists, scrapers, and shredders. Taxa with 2 or more functional feeding group designations were placed in the generalist category. Life habit categories were burrowers, clingers, generalists, and sprawlers. Generalists included all taxa that fit into more than 1 life habit category. The Palaeontological Statistics Program (PAST) was used for all data analyses [50].

For overall downstream–upstream and downstream–baseline comparisons, data were tested for normality using a Shapiro–Wilks test. Data that were not normal were log transformed and tested for normality again. Raw and log transformed data were analyzed by t tests ($p < 0.05$). If log transformation did not produce a normal distribution, then data were rank transformed and analyzed by a nonparametric Mann–Whitney U test ($p < 0.05$).

Abundance, richness, dominance, Shannon diversity, Buzas and Gibson's evenness, and mean tolerance value were used as community measures to compare all downstream and all upstream sites. These analyses provided overall comparisons between control sites (upstream) and downstream sites. Linear regressions were used to determine whether a relationship ($p < 0.05$) between both abundance and richness and imidacloprid concentrations at each downstream site existed. Imidacloprid concentration was the predictor variable, and richness and abundance were response variables. Imidacloprid concentrations were previously determined [25].

Dominance, Shannon diversity, and evenness comparisons were made between paired downstream and upstream sites (i.e., the upstream and downstream site for each sampled stream). Permutations ($n = 9999$) from data from each downstream/upstream pair were generated to create a normal distribution [50]. The distribution was based on differences in each community measure between downstream and upstream sites. Dominance, Shannon diversity, and evenness distributions were generated for each stream pair to facilitate pairwise comparisons of streams. If the observed difference of a community measure for a stream pair was below the 2.5th percentile or above the 97.5th percentile, then the observed difference was significantly different from what would be expected from a random comparison ($p < 0.05$). Abundance, richness, and mean tolerance value comparisons were not made, as permutations could not be generated from a single value for each site.

Macroinvertebrate functional feeding groups and life habits in community analyses can indicate whether the trophic composition and certain life habits of communities differ between downstream and control sites [48,51]. Assessed categories include collector–filterers, generalists, predators, scrapers, or shredders. Collector–gatherers were not assessed as a separate group, as all collector–gatherer taxa exhibited at least 2 functional feeding group designations. Thus, all collector–gatherers are included within the generalist category. Life habits assessed include burrowers, clingers, generalists, and sprawlers. All swimmer and climber taxa exhibited more than 1 life habit, and were included in the generalist category.

Functional feeding groups and life habits were compared between downstream and upstream sites. Three functional feeding group and life habit comparisons were made: abundance, richness, and proportion. Abundance was determined by the total number of individuals in each functional feeding group and each life habit category at each site. Richness was determined by the total number of taxa in each functional feeding group and each life habit category at each site. Proportion was considered as the relative percentage composition, based on abundance, of each functional feeding group and life habit category at each stream site.

Because abundance data were not available for baseline data, comparisons between baseline samples and downstream sites were limited. Community measure comparisons included mean tolerance value, richness, and richness of functional feeding groups and life habits.

RESULTS AND DISCUSSION

Comparisons of overall downstream–upstream community measures

During the present study 15 028 EPT were collected and identified. Of the specimens collected 9019 were mature enough to be identified to genus or species level. Data from genus and species-level identifications were used in data analyses. However, exceptions were made to include the families Hydroptilidae, Leptoceridae, and Baetidae in the analyses. Mayfly data included 3071 individuals; 21 distinct taxa from 8 families were identified. Most mayfly identifications were to the genus level. Caddisfly data included 2123 individuals from 19 families; 50 distinct taxa were identified, 35 of which were species-level identifications. Stonefly data included 3825 individuals; 16 distinct stonefly taxa from 7 families were identified. However, because of immaturity of specimens or lack of species level keys, only 3 stonefly taxa were identified to species level.

Abundance ranged from 108 to 915 individuals collected from each site (Table 2). Abundance of EPT at each downstream site was not related to imidacloprid concentrations detected at downstream sites ($p = 0.618$, $R^2 = 0.04$) [25] (Figure 2). Streams where higher concentrations of imidacloprid were detected at a one-time sampling event did not have lower abundance. Mean abundance for all downstream and all upstream sites was 552.670 and 445.110, respectively. Abundance was not significantly different between downstream and upstream sites ($p = 0.412$; Table 3).

Taxa richness at stream sites ranged from 10 to 36 (Table 2). Richness at downstream sites also was not related to observed imidacloprid concentrations in the sampled streams ($p = 0.815$, $R^2 = 0.01$) (Figure 3). Detected concentrations of imidacloprid, which were below USEPA thresholds, did not result in low species richness where higher concentrations were present. The highest concentration observed in Great Smoky Mountains National Park was 0.379 μg/L from Dunn Creek. Although the imidacloprid concentration observed at Dunn Creek is an accurate measurement influenced by the amount of imidacloprid applied in the watershed, it is higher than the other observed imidacloprid concentrations [25]. A regression analysis for both richness and abundance was performed excluding the Dunn Creek data. Richness and abundance were not related to observed imidacloprid concentrations in the additional analyses ($p = 0.197$, $R^2 = 0.26$; $p = 0.299$, $R^2 = 0.18$, respectively). Thus, there was no statistically significant relationship between

Table 2. Ephemeroptera, Plecoptera, and Trichoptera richness and abundance in streams in Great Smoky Mountains National Park, 2012

	Richness[a]			Abundance[b]	
Stream	Downstream[c]	Upstream[d]	Baseline[e]	Downstream	Upstream
Alum Creek	26	10	13	583	322
Camel Hump Creek	27	25	21	814	592
Cane Creek	35	24	26	465	392
Chasteen Creek	36	31	18	705	878
Dunn Creek	30	22	21	754	176
Indian Creek	28	31	25	915	856
Kingfisher Creek	20	23	18	350	334
Panther Creek	23	28	21	280	308
Shop Creek	17	19	16	108	148

[a]Richness is the total number of taxa collected at each site.
[b]Abundance is the total number of individuals collected at each site.
[c]Sites downstream from imidacloprid-treated conservation areas.
[d]Sites upstream from conservation areas.
[e]Baseline data collected from each downstream location between 1994 and 1997.

imidacloprid concentrations and both richness and abundance whether or not the Dunn Creek data point was included in the analyses. Because invertebrate communities were only affected by imidacloprid concentrations in excess of 1 μg/L in a mesocosm study [52], it was not surprising that abundance and richness were not related to imidacloprid concentrations observed in streams.

Dunn Creek had an EPT richness of 30. Mean downstream and upstream richness values were 22.889 and 23.667, respectively. Richness did not significantly differ between downstream and upstream sites ($p = 0.303$; Table 3).

Higher dominance values indicate a community that is dominated by a single taxon, which is typically not characteristic of a diverse community. However, higher evenness values indicate a community with more even abundance distribution among numerous taxa. High Shannon diversity is characteristic of a more diverse community [53]. Thus, a more diverse community would have lower dominance, higher evenness, and higher Shannon diversity.

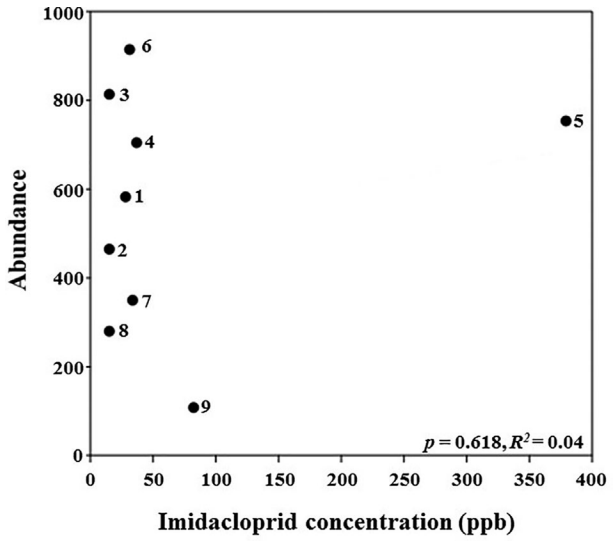

Figure 2. Relationship between imidacloprid concentrations and Ephemeroptera, Plecoptera, and Trichoptera abundance, linear regression ($p < 0.05$). 1 = Alum Creek; 2 = Camel Hump Creek; 3 = Cane Creek; 4 = Chasteen Creek; 5 = Dunn Creek; 6 = Indian Creek; 7 = Kingfisher Creek; 8 = Panther Creek; 9 = Shop Creek.

Dominance, evenness, and Shannon diversity community measures indicated similar communities at downstream compared with upstream sites. Dominance was 0.156 and 0.177 for downstream and upstream sites, respectively (Table 3), indicating that communities were not dominated by a single taxon. Evenness, 0.397 and 0.416 for downstream and upstream sites, respectively (Table 3), indicated that these communities have moderately even abundance distributions among taxa. Downstream and upstream sites did not differ in dominance ($p = 0.581$) or evenness ($p = 0.743$; Table 3). Shannon diversity, which was 2.307 and 2.135 at downstream and upstream sites, respectively, was not significantly different between downstream and upstream sites ($p = 0.401$).

Tolerance values, which are scaled from 0 to 10, indicate the ability of a taxon to survive in stressful water quality conditions. Lower values indicate an intolerant taxon that requires pristine water quality for survival, and higher values indicate a tolerant taxon that can survive in poor water quality. Low mean tolerance values at downstream (1.713) and upstream (1.856) sites demonstrate that the EPT communities are comprised of taxa that are intolerant to poor water quality conditions (Table 3). Mean tolerance values did not differ between downstream and upstream sites ($p = 0.608$). Presence of imidacloprid concentrations harmful to aquatic communities would prohibit the survival of intolerant indicator taxa. Only 4 taxa had tolerance values above 5.0. Hydroptilidae (Trichoptera: Hydroptilidae; $n = 10$) has a tolerance value of 6.5 and was only collected from 2 sites. As hydroptilid specimens were only identified to family, the highest tolerance value assigned to a genus in the family was used in analyses as a conservative approach. *Cheumatopsyche* spp. (Trichoptera: Hydropsychidae; $n = 143$) has a tolerance value of 6.6 and was only collected at 3 downstream and 2 upstream sites. The mayfly genera *Caenis* (Ephemeroptera: Caenidae; $n = 24$) and *Stenonema* (Ephemeroptera: Heptageniidae; $n = 2$) have tolerance values of 6.8 and 6.9, respectively. These mayflies were only present at a few stream sites. However, *Tallaperla* spp. (Plecoptera: Peltoperlidae), the most abundant taxon ($n = 2171$), has a tolerance value of 1.3 and was collected at every site. If taxa at downstream sites were affected by poor water quality, it would be expected that mean tolerance values of taxa at downstream sites would be higher than those at upstream control sites. Taxa with higher tolerance values at downstream sites have not been observed. Predominance of tolerant taxa is a sign of compromised water

Table 3. Comparisons of Ephemeroptera, Plecoptera, and Trichoptera community measures between all downstream and all upstream sampling sites, Great Smoky Mountains National Park, 2012

	Downstream		Upstream		
Community measure	Mean[a]	95% confidence interval	Mean[b]	95% confidence interval	*p* value[c]
Abundance	552.670[d]	344.790–760.541	445.110	236.790–653.430	0.412
Richness	22.889	22.021–31.757	23.667	18.656–28.678	0.303
Dominance[e]	0.156	0.115–0.212	0.177	0.119–0.262	0.581
Evenness	0.397	0.332–0.463	0.416	0.308–0.521	0.743
Shannon diversity[e]	2.307	2.093–2.542	2.135	1.780–2.561	0.401
Tolerance value	1.713	1.273–2.153	1.856	1.408–2.303	0.608

[a]Mean of each community measure from all downstream sites.
[b]Mean of each community measure from all upstream sites.
[c]Significant at $p < 0.05$, *t* test.
[d]There were no significant differences in community measures of downstream and upstream sites.
[e]Log-transformed. Means and confidence intervals displayed are back-transformed from log-transformed means and confidence intervals used in the statistical analysis.

quality [20]. However, few tolerant taxa were collected, and the most abundant taxon has a low tolerance value.

Comparisons of pairwise downstream–upstream community measures

Pairwise comparisons of dominance, evenness, and Shannon diversity were made between downstream and upstream sites of each stream to assess potential imidacloprid impacts in individual streams. Camel Hump Creek and Shop Creek had similar dominance, evenness, and Shannon diversity between downstream and upstream sites ($p > 0.05$; Table 4).

Alum Creek, Cane Creek, Dunn Creek, and Indian Creek community measure analyses reveal higher diversity communities at the downstream sites. Dominance was lower and Shannon diversity was higher at Alum Creek downstream site ($p < 0.001$; Table 4). Dominance was lower and Shannon diversity was higher at the Cane Creek downstream site ($p < 0.001$). Dunn Creek, the site with the highest recorded imidacloprid concentration [25], had lower dominance at the downstream site ($p = 0.022$). At Indian Creek, dominance was lower, evenness was higher, and Shannon diversity was higher at the downstream site ($p < 0.001$).

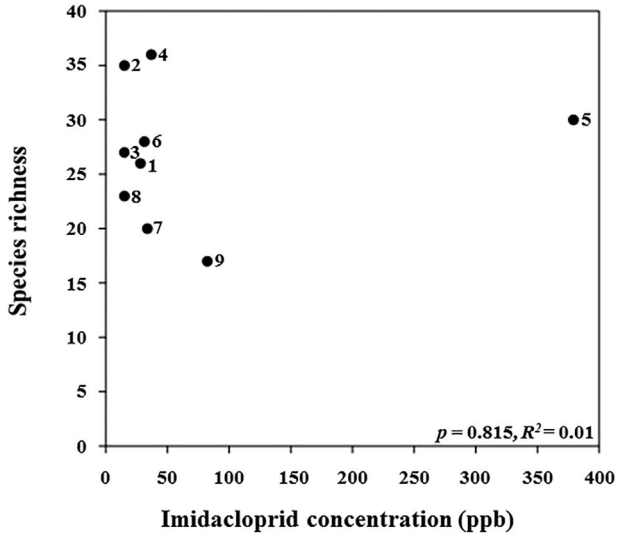

Figure 3. Relationship between imidacloprid concentrations and Ephemeroptera, Plecoptera, and Trichoptera species richness, linear regression ($p < 0.05$). 1 = Alum Creek; 2 = Camel Hump Creek; 3 = Cane Creek; 4 = Chasteen Creek; 5 = Dunn Creek; 6 = Indian Creek; 7 = Kingfisher Creek; 8 = Panther Creek; 9 = Shop Creek.

Chasteen Creek, Kingfisher Creek, and Panther Creek community measures indicate higher diversity communities at upstream sites. Dominance was lower at the Chasteen Creek upstream site ($p = 0.004$). Dominance was lower, evenness was higher, and Shannon diversity was higher at Kingfisher Creek upstream site ($p < 0.001$, $p = 0.020$, and $p < 0.001$, respectively; Table 4). Panther Creek upstream site had lower dominance ($p < 0.001$), whereas evenness and Shannon diversity were higher ($p < 0.001$).

Differences in community measures between downstream and upstream sites are not completely unexpected. Downstream and upstream pairs were separated by approximately 350 to 8700 m of stream length flowing through conservation areas. Streams flow through the diverse forests of Great Smoky Mountains National Park, with a variety of ecosystem inputs between downstream and upstream sites. While some differences in paired sites of individual streams are expected, because of stochastic effects in the environment, overall trends of lower community diversity in downstream sites would be concerning. However, the results of pairwise community analyses did not show this effect. Pairwise comparisons showed a mix of both upstream and downstream sites with higher diversity community measures, as well as 2 streams with no difference between upstream and downstream pairs. Detrimental effects in downstream sites that could be attributed to imidacloprid contamination were not observed.

Comparisons of downstream–upstream functional feeding groups

Similar abundance of collector–filterers, generalists, predators, scrapers, and shredders was observed between upstream and downstream sites ($p = 0.275$, $p = 0.925$, $p = 0.677$, $p = 0.167$, and $p = 0.649$, respectively; Table 5). Communities in both downstream and upstream sites had high generalist abundance and low predator abundance. Collector–filterers, scrapers, and shredders were moderately abundant in comparison. Taxa richness of collector–filterers, generalists, predators, scrapers, and shredders did not vary between downstream and upstream sites ($p = 0.165$, $p = 0.500$, $p = 0.759$, $p = 0.718$, and $p = 0.288$, respectively; Table 5). In addition, downstream and upstream sites had similar proportions of collector–filterers, scrapers, and shredders ($p = 0.497$, $p = 0.246$, and $p = 0.641$, respectively; Table 5). Proportions of generalists and predators were rank transformed and analyzed by a Mann–Whitney *U* test (Table 6), and similar proportions were found in upstream and

Table 4. Pairwise comparisons of Ephemeroptera, Plecoptera, and Trichoptera community measures between downstream and upstream sampling locations

	Downstream		Upstream		
Stream and community measure	Value[a]	95% confidence interval[b]	Value	95% confidence interval	p value[c]
Alum Creek					
Dominance	0.122 A[d]	0.111–0.133	0.258 B	0.235–0.283	<0.001
Evenness	0.440 A	0.408–0.480	0.484 A	0.442–0.531	0.402
Shannon diversity	2.438 A	2.362–2.523	1.576 B	1.487–1.669	<0.001
Camel Hump Creek					
Dominance	0.109 A	0.101–0.119	0.108 A	0.099–0.119	0.812
Evenness	0.461 A	0.434–0.492	0.508 A	0.469–0.545	0.303
Shannon diversity	2.522 A	2.462–2.587	2.541 A	2.462–2.612	0.735
Cane Creek					
Dominance	0.149 A	0.125–0.164	0.222 B	0.185–0.251	<0.001
Evenness	0.333 A	0.311–0.385	0.321 A	0.294–0.371	0.723
Shannon diversity	2.457 A	2.386–2.601	2.040 B	1.954–2.186	<0.001
Chasteen Creek					
Dominance	0.152 A	0.134–0.165	0.126 B	0.116–0.134	0.004
Evenness	0.314 A	0.293–0.350	0.363 A	0.345–0.394	0.149
Shannon diversity	2.424 A	2.355–2.534	2.420 A	2.370–2.504	0.950
Dunn Creek					
Dominance	0.117 A	0.106–0.128	0.150 B	0.115–0.179	0.022
Evenness	0.424 A	0.396–0.459	0.478 A	0.430–0.573	0.984
Shannon diversity	2.542 A	2.474–2.622	2.535 A	2.246–2.535	0.123
Indian Creek					
Dominance	0.127 A	0.117–0.137	0.481 B	0.443–0.507	<0.001
Evenness	0.405 A	0.383–0.437	0.127 B	0.119–0.146	<0.001
Shannon diversity	2.428 A	2.373–2.504	1.367 B	1.304–1.511	<0.001
Kingfisher Creek					
Dominance	0.392	0.332–0.449	0.189 B	0.160–0.208	<0.001
Evenness	0.274	0.233–0.322	0.360 B	0.330–0.417	0.020
Shannon diversity	1.702	1.539–1.863	2.114 B	2.027–2.261	<0.001
Panther Creek					
Dominance	0.213 A	0.178–0.255	0.086 B	0.075–0.102	<0.001
Evenness	0.370 A	0.315–0.426	0.594 B	0.527–0.645	<0.001
Shannon diversity	2.142 A	1.979–2.281	2.811 B	2.692–2.894	<0.001
Shop Creek					
Dominance	0.149 A	0.118–0.178	0.186 A	0.141–0.247	0.194
Evenness	0.554 A	0.484–0.659	0.507 A	0.408–0.591	0.482
Shannon diversity	2.243 A	2.107–2.416	2.265 A	2.049–2.419	0.890

[a]Value of each community measure for each stream site.
[b]95% confidence interval of each community measure generated by a permutation process ($n = 9999$).
[c]$p < 0.05$, based on difference between downstream and upstream community measures compared with random distribution of differences generated by a permutation process.
[d]Means within a row followed by the same capital letters are not significantly different.

downstream sites ($p = 0.860$ and $p = 0.627$, respectively). Means and confidence intervals for all functional feeding groups are presented (Table 5). Results of all downstream–upstream comparisons where data were rank transformed and analyzed by a Mann–Whitney U tests are listed in Table 6. Given that abundance, richness, and proportion of functional feeding groups do not differ between downstream and upstream sites, imidacloprid use in Great Smoky Mountains National Park is not affecting trophic composition of EPT communities.

Comparisons of downstream–upstream life habits

Clinger and generalist abundance was high, whereas burrower and sprawler abundance was much lower. No differences in the abundance of burrowers, clingers, generalists, and sprawlers between downstream and upstream sites were detected ($p = 1.000$, $p = 0.175$, $p = 0.934$, and $p = 0.278$, respectively; Tables 6 and 7). Burrower, clinger, and generalist richness did not vary between downstream and upstream sites ($p = 0.951$, $p = 0.486$, and $p = 0.521$, respectively; Tables 6 and 7). The mean rank of sprawler richness was higher at downstream sites, indicating a higher sprawler richness at downstream sites ($p = 0.044$; Table 6). Clingers and generalists made up the highest proportions of downstream and upstream communities. The proportion of burrowers, clingers, generalists, and sprawlers did not differ between downstream and upstream sites ($p = 0.952$, $p = 0.221$, $p = 0.138$, and $p = 0.192$, respectively; Tables 6 and 7). Overall, abundance, richness, and proportion of taxa with different life habits from downstream and upstream communities were similar, with the exception of sprawler richness. Imidacloprid use did not negatively affect the abundance, richness, or proportion of taxa with different life habits at downstream sites.

Comparisons of downstream–baseline communities

Because baseline data were limited to presence/absence data, analyses were limited to richness and mean tolerance value comparisons. Mean downstream and baseline richness, 24.000 and 23.111, respectively, were not significantly different ($p = 0.738$; Table 8). Baseline mean tolerance value was low, at 1.869, indicating that baseline EPT communities were comprised of taxa that were intolerant to poor water quality. The nonweighted downstream mean tolerance value

Table 5. Comparisons of Ephemeroptera, Plecoptera, and Trichoptera functional feeding groups between all downstream and all upstream sampling sites, Great Smoky Mountains National Park, 2012

	Downstream		Upstream		
Community measure	Mean[a]	95% confidence interval	Mean[b]	95% confidence interval	*p* value[c]
Abundance					
Collector–filterers	49.889[d]	27.520–72.258	33.889	10.137–57.641	0.275
Generalists[e]	223.821	107.201–467.304	215.427	118.741–390.211	0.925
Predators	11.667	5.043–18.290	9.889	2.850–16.928	0.677
Scrapers[e]	54.853	29.322–102.612	33.558	21.023–53.023	0.167
Shredders	75.667	15.425–135.910	61.000	20.080–101.920	0.649
Richness					
Collector–filterers	4.333	3.317–5.350	3.333	2.118–4.549	0.165
Generalists	10.667	8.670–12.664	9.778	7.577–11.978	0.500
Predators[e]	2.095	0.840–5.223	2.395	1.649–3.479	0.759
Scrapers	6.444	5.107–7.782	6.111	4.506–7.716	0.718
Shredders	2.778	1.639–3.916	2.111	1.301–2.921	0.288
Proportion					
Collector–filterers	0.119	0.063–0.174	0.093	0.029–0.157	0.497
Generalists[f]	0.564	40.873–71.986	0.613	47.554–74.985	–
Predators[f]	0.026	0.013–0.039	0.026	0.007–0.045	–
Scrapers[e]	0.124	0.089–0.173	0.091	0.055–0.149	0.246
Shredders[e]	0.076	0.006–0.237	0.102	0.042–0.246	0.641

[a]Mean of each community measure from all downstream sites.
[b]Mean of each community measure from all upstream sites.
[c]Significant at $p < 0.05$, *t* test.
[d]There were no significant differences in functional feeding groups between upstream and downstream sites.
[e]Log-transformed. Means and confidence intervals displayed are back-transformed from log transformed means and confidence intervals used in the statistical analysis.
[f]Rank-transformed and analyzed by a Mann–Whitney *U* test. Results of Mann–Whitney *U* tests are provided in Table 6.

was 1.982, which was not significantly different from baseline ($p = 0.554$).

Functional feeding groups were similar between downstream and baseline samples, with the exception of predators. Collector–filterer, generalist, scraper, and shredder richness values were not significantly different between downstream and baseline samples ($p = 0.365$, $p = 0.075$, $p = 0.147$ and $p = 0.116$, respectively; Tables 8 and 9). Results of all downstream baseline comparisons where data was rank transformed and analyzed by a Mann–Whitney *U* test are listed in Table 8. Baseline predator richness (4.556) was significantly higher than downstream predator richness (2.889; $p = 0.042$; Table 8). Life habits were similar between downstream and baseline communities, with the exception of generalists. Burrower, clinger, and sprawler richness values were similar between downstream and baseline stream samples ($p = 0.951$, $p = 1.000$, and $p = 0.427$, respectively; Tables 8 and 9). Generalist richness was higher in downstream samples ($p = 0.025$). Because predator richness was higher in baseline samples, while generalist life habit richness was higher in downstream samples, richness differences do not clearly indicate impaired communities at downstream sites. The similarity in richness, tolerance values, functional feeding groups, and life habits of taxa between downstream and baseline

Table 6. Mann–Whitney *U* comparisons of Ephemeroptera, Plecoptera, and Trichoptera community measures between all downstream and all upstream sampling locations

Classification	Downstream mean rank[a]	Upstream mean rank[b]	Mann–Whitney *U*	*z* score	*p* value[d]
Functional feeding group					
Proportion					
Generalists	4.611 A[c]	4.889 A	38.0	−0.177	0.860
Predators	5.083 A	4.417 A	34.5	−0.486	0.627
Life habit					
Abundance					
Burrowers	4.722 A	4.778 A	40.0	0	1.000
Sprawlers	5.444 A	4.056 A	28	−1.085	0.278
Richness					
Burrowers	4.750 A	4.750 A	40.5	0.061	0.951
Sprawlers	5.972 A	3.528 B	18.5	−2.018	0.044
Proportion					
Burrowers	4.694 A	4.806 A	39.5	−0.061	0.952
Sprawlers	5.583 A	3.917 A	25.5	−1.304	0.192

[a]Mean rank of each community measure from all downstream sites.
[b]Mean rank of each community measure from all downstream sites.
[c]Means within a row followed by the same capital letters are not significantly different.
[d]Significant at $p < 0.05$, Mann-Whitney *U* Test.

Table 7. Comparisons of Ephemeroptera, Plecoptera, and Trichoptera life habits between all downstream and all upstream sampling sites, Great Smoky Mountains National Park, 2012

	Downstream		Upstream		
Community measure	Mean[a]	95% confidence interval	Mean[b]	95% confidence interval	*p* value[c]
Abundance					
Burrowers[d]	0.333	−0.210–0.877	0.444	−0.335–1.224	–
Clingers	169.330 A[e]	93.417–245.250	109.780 A	49.956–169.600	0.175
Generalists	323.220 A	202.090–444.360	315.890 A	155.360–476.42	0.934
Sprawlers[d]	2.556	1.164–3.947	1.889	−0.197–3.974	–
Richness					
Burrowers[d]	0.222	−0.117–0.561	0.222	−0.117–0.561	–
Clingers	14.566 A	11.890–17.221	13.222 A	9.83–16.612	0.486
Generalists	10.667 A	8.527–12.807	9.889 A	8.194–11.584	0.521
Sprawlers[d]	1.333	0.790–1.877	0.556	−0.003–1.114	–
Proportion					
Burrowers[d]	0.076	−0.064–0.216	0.025	−0.262–0.769	–
Clingers	0.335 A	0.270–0.401	0.271 A	0.173–0.368	0.221
Generalists	0.642 A	0.588–0.695	0.719 A	0.618–0.819	0.138
Sprawlers[d]	0.022	−0.012–0.057	0.008	−0.004–0.020	–

[a]Mean of each community measure from all downstream sites.
[b]Mean of each community measure from all upstream sites.
[c]Significant at $p < 0.05$, *t* test.
[d]Rank-transformed and analyzed using a Mann–Whitney *U* test. Results of Mann–Whitney *U* test are in Table 6.
[e]Means within a row followed by the same capital letters are not significantly different.

Table 8. Comparisons of Ephemeroptera, Plecoptera, and Trichoptera community measures between all downstream sampling locations and baseline data

	Downstream		Baseline		
Community measure	Mean[a]	95% confidence interval	Mean[b]	95% confidence interval	*p* value[c]
Richness	24.000 A[d]	19.436–28.564	23.111 A	19.183–27.039	0.738
Tolerance value	1.982 A	1.642–2.322	1.869 A	1.602–2.135	0.554
Functional feeding group					
Richness					
Collector–filterers	4.222 A	3.98–5.146	3.667 A	2.650–4.684	0.365
Generalists	10.556 A	8.518–12.593	8.444 A	6.902–9.987	0.075
Predators	2.889 A	1.647–4.131	4.556 B	3.334–5.778	0.042
Scrapers[e]	6.444	5.107–7.782	5.222	4.150–6.294	–
Shredders[e]	2.778	1.639–3.916	1.778	1.137–2.418	–
Life habit					
Richness					
Burrowers[e]	0.222	−0.117–0.561	0.222	−0.0117–0.561	–
Clingers	14.444 A	11.779–17.110	14.444 A	11.249–17.639	1.000
Generalists	10.778 A	8.611–12.944	7.889 B	6.284–9.494	0.025
Sprawlers[e]	1.333	0.70–1.877	1.111	0.214–2.008	–

[a]Mean of each community measure from all downstream sites.
[b]Mean of each community measure from all baseline sites.
[c]Significant at $p < 0.05$, *t* test.
[d]Means within a row followed by the same capital letters are not significantly different.
[e]Rank transformed and analyzed using a Mann–Whitney *U* test. Results of Mann–Whitney *U* test are in Table 9.

Table 9. Mann–Whitney *U* comparisons of Ephemeroptera, Plecoptera, and Trichoptera community measures between downstream sampling locations and baseline data

Classification	Downstream mean rank[a]	Baseline mean rank[b]	Mann–Whitney *U*	*z* score	*p* value[c]
Functional feeding group					
Richness					
Scrapers	5.667[d]	3.833	24.0	−1.451	0.147
Shredders	5.772	3.778	23.0	−1.573	0.116
Life habit					
Richness					
Burrowers	4.750	4.750	40.5	0.061	0.951
Sprawlers	5.250	6.250	31.5	−0.795	0.427

[a]Mean rank of each community measure from all downstream sites.
[b]Mean rank of each community measure from all baseline samples.
[c]Significant at $p < 0.05$, Mann-Whitney *U* Test.
[d]There were no significant differences between downstream and baseline samples.

samples indicates that water quality conditions did not vary dramatically in the time between baseline sample collection in the mid-1990s and downstream collections in 2012 after implementation of the hemlock woolly adelgid integrated pest management program.

CONCLUSIONS

Given global concerns about nontarget impacts of neonicotinoids, it is important that pest management programs do not cause undue risks to ecosystems. Concern for reduction of nontarget impacts is especially relevant for management programs, such as the hemlock woolly adelgid integrated pest management program at Great Smoky Mountains National Park, operating with the objective of ecosystem preservation. Imidacloprid is used to preserve systems such as these, where pest suppression often can be maintained for multiple years using 1 treatment [35,54,55].

The use of imidacloprid in hemlock systems is inherently different from pesticide use in agricultural systems. Applications in this forest setting involve only soil and trunk applications of a single insecticide class on isolated acreage with numerous years between treatments. In agricultural settings pesticides can be applied by numerous methods (e.g., soil, foliar, aerial, and broadcast). The diversity of application methods means possible pesticide contamination of aquatic systems by multiple routes, such as runoff, leaching, and spray drift. In addition, pesticides are applied to a larger proportion of the landscape. Often numerous pesticides are used within a season and can be applied year after year. As the number of active ingredients and frequency of applications can be higher in agricultural settings, the possibility of nontarget effects for pesticide use in general will be higher than in hemlock systems.

The present research is the first study to assess potential effects of imidacloprid on stream aquatic macroinvertebrate communities in natural settings, in the known presence of imidacloprid, where other insecticidal pollutants were not confounding factors. The assessment of the Great Smoky Mountains National Park hemlock woolly adelgid integrated pest management program demonstrates that EPT communities downstream from hemlock conservation areas are similar to baseline conditions and upstream controls. Imidacloprid concentrations detected in surface waters in Great Smoky Mountains National Park did not exceed USEPA environmental standards; however, detected concentrations did exceed guidelines set by the Netherlands and Canada. Sites downstream from conservation areas are typified by rich and diverse EPT communities, with taxa that are intolerant to poor water quality conditions. Thus, the use of imidacloprid for hemlock woolly adelgid suppression in a forest setting at Great Smoky Mountains National Park has not had a negative effect on EPT communities. The results of the present study and those of Benton et al. [25] demonstrate that imidacloprid, when used within the limits of USEPA federal regulations, has not had detrimental impacts on aquatic macroinvertebrates.

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Data Availability—Data can be accessed at the following National Park Service link: https://irma.nps.gov/DataStore/Reference/Profile/2236558. Data used in this article are contained in the following files: Baseline data: GRSM historical data_baseline.xlsx; downstream and upstream data: GRSM Macroinvertebrate EPT raw data.xlsx

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