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"Avian Dispersal of the Hemlock Woolly Adelgid (Adelges tsugae Annand)"

Nicholas Russo
University of Connecticut - Storrs

Avian Dispersal of the Hemlock Woolly Adelgid

(*Adelges tsugae* Annand)

Nicholas J. Russo[1], Carole Cheah[2], and Morgan W. Tingley[1]

1. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269

2. Valley Laboratory, Connecticut Agricultural Experiment Station, Windsor, CT, 06095

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Abstract

In light of evidence that birds are agents of hemlock woolly adelgid (*Adelges tsugae* Annand) dispersal, this study provides evidence that potential for avian dispersal of the hemlock woolly adelgid (HWA) varies according to HWA phenology. From May 14 to July 20, 2015, taxidermy bird mounts were placed on HWA-infested eastern hemlock branches at the Connecticut Agricultural Experiment Station's Valley Laboratory research farm in order to explore a variety of mechanistic hypotheses about how transfer of HWA mobile first instars, or crawlers, occurs between infested hemlocks and birds. Additionally, a separate, concurrent experiment investigated whether birds carrying HWA crawlers may cause new infestations if they perch on uninfested branches. In the first set of experiments, across all trials, the mean number of crawlers found on perched experimental birds, regardless of trial duration, was 0.68±1.37. The abundance of crawlers on experimental birds peaked during the first week of trials, coinciding with avian spring migration, and the emergence of progrediens generation crawlers from overwintering sistens adults. Crawler abundance spiked again during the week of June 14, when the progrediens generation matured and sistens generation crawlers began to emerge. Crawler counts for individual trials did not differ according to duration of experimental perch, but they did show a positive linear relationship with the number of ovisacs counted within 6 cm of the perching point. Although passive perching experiments did result in crawlers actively moving onto perched birds, significantly more crawlers were found on birds actively brushed against the same branch. In the final experiment, which examined the role of HWA-contaminated birds in passive HWA dispersal, 5 of 17 experimental branches developed a light infestation at least two weeks after the introduction of a perched bird carrying 5, 10, or 20 HWA crawlers, suggesting that HWA-contaminated birds are capable of causing new infestations on uninfested hemlocks.

Introduction

Each spring, millions of migratory birds return to their breeding grounds throughout New England, and some may be carrying the hemlock woolly adelgid (*Adelges tsugae* Annand), (Hemiptera: Adelgidae). This hemipteran hitchhiked to a Virginia landscape company in the early 1950s via imported trees (Souto et al., 1996), and with no immediate predators, the hemlock woolly adelgid (HWA) has invaded forests of the eastern U.S. and proliferated amongst populations of its ecologically naïve hosts, the eastern hemlock [*Tsuga canadensis* (L.) Carrière] and Carolina hemlock [*Tsuga caroliniana* Engelm. (Pinaceae)]. Adelgids pierce the base of hemlock needles and feed on nutrients stored in the parenchyma cells of the xylem rays, stunting or preventing annual needle growth as they develop through four instars into adulthood (McClure, 1989a and 2001). The ensuing damage to a hemlock's vascular system leads it to defoliate and die after at least four years of infestation, leading to a cascade of habitat and community changes triggered by the removal of this ecological foundation species (Tingley et al., 2002; Ellison, 2014).

Infestations that last several years provide a large window of time for further dispersal. With such a slow rate of devastation and an increasing tolerance of cold winter temperatures (Butin et al., 2005), the invasive HWA has spread to more northerly New England forests in the past decade (Paradis et al., 2007), and continues to be dispersed by multiple vectors, including wind, humans, mammals, and birds (McClure, 1990). The HWA reproduces asexually on its host in two generations per year; the progrediens generation, which hatches from late April through mid-June, and the sistens generation, which hatches from mid-June through July and aestivates until October, when development resumes throughout the winter. Emergence of progrediens HWA crawlers from overwintering sistens adults peaks during the first three weeks of May

(McClure, 1989a), coinciding with avian spring migration. Despite the presumed importance of this occurrence in the northward spread of HWA, only one study, McClure (1990), which was performed over two decades ago and after peak progrediens crawler activity, investigated the role of birds in HWA dispersal. McClure's study consisted of two sampling sites in Connecticut and 33 birds captured in mist nets, and simply concluded that HWA eggs and crawlers can be found on birds. Subsequent studies only acknowledge that birds are contributing to the expansion of this invasive insect (McClure & Cheah, 1999; Turner et al., 2011; Orwig et al., 2012), and no further exploration of this mechanism has occurred since 1990.

The purpose of this study is to understand the mechanisms of potential avian dispersal of the hemlock woolly adelgid by testing the processes by which birds may acquire adelgids from infested hemlocks during and beyond the period of songbird migration, and to determine whether birds carrying HWA crawlers may cause new infestations after visiting healthy hemlocks. We investigated five key questions: 1) Do crawlers of both the progrediens and sistens generation initiate their transport by crawling onto a bird, or must they be removed by a bird brushing against a branch? 2) How does the number of crawlers found on perched birds (hereafter, "crawler count") change with perch duration? 3) How does crawler transfer from branch to bird vary temporally from bird migration to breeding season? 4) Does HWA ovisac population density surrounding the perching point of a bird influence its likelihood of acquiring HWA crawlers? 5) Could a bird carrying HWA crawlers cause a new infestation if it visits an uninfested branch? Collecting and quantifying adelgids from perched birds in the transition from spring to summer may also provide information that will be useful to the nature and timing of biological control of the hemlock woolly adelgid, and lead to further research concerning the dispersal of the hemlock woolly adelgid and invasive species with similar behaviors and life cycles, such as the elongate hemlock scale (*Fiorinia externa* Ferris; McClure 1989b) and balsam woolly adelgid (*Adelges piceae* Ratzeburg; Lass et al., 2013).

Materials and Methods

Study Site

All experiments were conducted at the Valley Laboratory, a research component of the Connecticut Agricultural Experiment Station in Windsor, CT. All perching experiments were conducted within an isolated stand of eastern hemlocks on the eastern edge of the property. This specific plot was chosen because its mean HWA mortality for the winter of 2015 reached only 82%, which was among the lowest rates in the state (C. Cheah, pers. comm.). The 100 m^2 stand consisted of 60 eastern hemlocks spaced 2.5 m apart, and was planted about 30 years ago for HWA biological control studies. The stand is heavily infested with HWA on its eastern and southwestern corners, and lightly to moderately infested on its southern and western sides. The northern side of the plot suffered severe foliage dieback from previous infestations, so branches were randomly selected for experimentation according to infestation density from all other cardinal directions of the stand throughout the study.

Hemlock-to-Perching Bird Transfer Experiment

A single experimental setup was used to investigate questions 1-4. From May to July, 2015, one researcher (N.Russo) perched birds on eastern hemlock branches and counted how many HWA crawlers transferred from branches to perched birds. Fully articulated taxidermy bird mounts supplied by UConn vertebrate collections were chosen at random to be perched on selected branches. All birds used were members of the family *Parulidae*, ranging in length from 12 to 15 cm. Before experimentation, all birds chosen for trials were blown thoroughly with compressed air (see methods in next section), and all material collected from the birds was examined under a dissecting microscope. The exterior of each bird was also examined under a dissecting microscope so any stray material on the birds' feathers could be removed prior to experimental initiation.

Many of the birds used in this experiment were preserved in a perching position, and could be perched on branches without support. For some birds, however, a 13 x 20 cm "hammock" of 1 cm-gauge hardware cloth bent loosely over a plastic bin covered with a second section of hardware cloth of the same dimensions was used to keep each perching bird in place and hold the protruding feet curled over a branch. By keeping each branch straight and enabling each bird to be lowered to its perching point, this design was meant to re-create the normal perching position of a live bird (Figure 1).

Experimental hemlock branch tips within the plot were selected for this experiment according to a balanced, random design with respect to HWA infestation severity. In the interest of minimizing disturbance to natural HWA populations on experimental branches, infestation density was measured by counting all ovisacs within a 6 cm radius of the perching point, rather than using an areal density measurement. Branches were classified as having a low (1 to 9 ovisacs), medium (10 to 19 ovisacs) or high density infestation (> 19 ovisacs.) Only full, bulging ovisacs were counted towards classification, because smaller, denser ovisacs signified winter die-off and thus low fecundity or no oviposition (Parker et al., 1998). An inventory of sistens generation ovisacs both counted toward classification and considered nonviable was conducted on June 3 in order to justify classification selections. The mean diameter of 50 ovisacs considered viable for sistens ovisac count records was 2.23±0.28 mm, which is about twice as large as the mean diameter of 1.01±0.21 mm for 50 sistens ovisacs not considered for ovisac counts.

By June 18, many progrediens crawlers had matured and oviposited, and sistens crawlers had begun hatching out. On this date, a mixture of sistens and progrediens ovisacs was counted towards density classification for a single trial. Beyond this date and until the final trial on July 20, only progrediens ovisacs were counted toward density classification, and were distinguished from sistens ovisacs by their bulging, brilliant white appearance. By June 18, sistens ovisacs, if any remained on a branch, were limp, dull, and frayed.

Branch tips were flagged according to infestation density, and a unique, two-category identifier was given to each branch. Each perching point was no more than 6 cm away from the newest annual growth, which HWA crawlers prefer to settle on. The presence of new growth also indicates that the HWA population has not outstripped its resources on that branch, so the population of emerging crawlers is expected to fall within its normal range of 48.6 ±5.8 crawlers per sistens ovisac, and 21.7 ± 3.6 per progrediens ovisac (McClure, 1989a).

Since suitable branches for the hemlock-to-perching bird transfer experiment were of limited supply at the study site, approximately half of all branches used from May 22 to June 18 were re-used branches from previous trials of the same duration. Re-usage became unnecessary after June 18 because as sistens adults died and fell off branches, and individuals of the progrediens generation fully matured and began to oviposit, the number of ovisacs present on branches used before this date changed, and many developed an infestation to a different degree, thus falling outside the range of their original density classification.

For each trial, a bird was perched on a branch for 1, 5, 15, or 30 minutes. The alternating sequence of perching times over two days of experimentation was kept nearly constant, with only minor adjustments to account for errors in the field (such as a restarted trial,) so that a perching trial was performed for each of the three density classes (High, Med, and Low), and for each of the four time intervals every day, for a total of twelve trials per day. From June 4 until the end of the study, hemlock branches without any HWA ovisacs were used as zero controls weekly or bi-weekly for perching trials at all four time durations. The date, time, and perching height of every trial performed was recorded.

Collecting Process

After perching for its designated duration, each bird was transported directly to the collecting station, which consisted of a white collecting tray on top of a white 56 x 71 cm poster board. The tray and poster board were blown with compressed air and wiped with a paper towel before each trial so any crawlers carried by the wind would be removed from the station before each new collection.

At the collecting station, each bird was held above the collecting tray and compressed air was used to blow up and down its breast, belly, flanks, and legs, then up and down its head, back, and rectrices, and the process was repeated until no new arthropods were observed falling into the collecting tray, as recommended by Clayton and Walther (1997). The birds were not subject to more invasive collection procedures for dead birds, such as body washing methods outlined in Clayton and Walther (1997), because the birds were intended to be reused, and HWA crawlers are expected to be found on or toward the surface of a bird's feathers, and thus easily removed.

Once it was determined that no new material was falling into the tray or onto the surrounding poster board after a trial, all crawlers found at the collecting station were collected using a small paintbrush dipped in 70% ethanol, and preserved in ethanol-filled vials for preservation and viewing under a dissecting microscope. Although HWA crawlers are identifiable without magnification by their oval shape, dark brown to reddish brown appearance, and slow-paced crawling, any other organisms or specks of material the same size as an adelgid crawler were also collected and viewed under a dissecting microscope.

Under the microscope, HWA crawlers were identified by their size (44-µm long by 27-µm wide), dark amber to brown coloration, and slim body margins relative to leg length (McClure, 1989a). Mobile first instars, or crawlers, were distinguished from later instars mainly by lack of a stylet bundle, which indicates settlement, and therefore inability to settle elsewhere if transported (McClure and Cheah, 2002). Accordingly, the crawler stage was the only developmental stage of interest, but all other specimens collected were noted. Dead HWA crawlers, which have a wrinkled, folded appearance when recently dead and a black, shriveled appearance when long dead (Figure 2), were noted but not counted towards the final crawler count for a trial.

Hemlock-to-Moving Bird Transfer Experiment

Each week from May 20 to July 14, three branches (one of each density class,) used previously for 30-minute perching trials in the same day were selected for the hemlock-to-moving bird transfer experiment. For each trial, a single bird mount was brushed five times against the upper side of a hemlock branch, then five times on the underside. All brushes were applied to the bird's breast, belly, sides, and flanks. Adelgids were collected from the bird and observed after each trial using the collection procedure outlined for the hemlock-to-perching bird transfer experiment.

Bird-to-Hemlock Transfer Experiment

From June 19 to July 6, one researcher (N.Russo) additionally conducted a bird-to-hemlock transfer experiment in which birds carrying a known number of HWA crawlers were perched on healthy branches to further examine the processes by which birds may disperse HWA. This experiment required uninfested branches from multiple sites for both *in situ* and *ex* *situ* trials. For *in situ* trials, a total of 12 uninfested branches were selected from four different eastern hemlocks located at the Valley Laboratory in areas separate from the main hemlock stand. For *ex situ* trials, 9 branches approximately 55 cm in length were clipped and removed from eastern hemlocks located near the western edge of Old Man McMullen Pond in Great Mountain Forest in Norfolk, CT. Clipped branches were kept in water and transported to the Valley Laboratory on the same day for experimentation. A total of 21 branches were used for the bird-to-hemlock transfer experiment.

Hundreds of HWA crawlers were collected from hemlock branches within the main study area in a white tray. A thin, dry, white paintbrush was used to transfer live crawlers from the tray to the legs, belly, flanks, and tarsal feathers of each bird used in the experiment. A total of 5, 10, or 20 crawlers was placed on each bird, which was then perched on an unsettled 40-55 cm branch section for 10 minutes, covered by a nylon sleeve cage. After 10 minutes, the bird was removed, and a sleeve cage was immediately tied around each branch to ensure that no crawlers could be transported onto the branch by any other means. The 9 branch samples from Great Mountain Forest were each kept in a cup of water, held upright with 17 ½ x 30 cm plastic graft piping and kept outdoors in the same conditions as the 12 *in situ* branches. In all other ways, *in situ* and *ex situ* branches were treated experimentally the same.

In order to provide ample time for the introduced crawlers to settle, the sleeve cages were removed from each branch after 16 to 40 days and settled first instar adelgids were counted using 1.5 power magni-focusers. All settlement counts were verified using a dissecting microscope.

Analysis

The statistical software R was used to analyze the results of each experiment. A one-way ANOVA was used to test for relationships between categorical data for each grouping (e.g., duration, density class, temporal groupings) and crawler count. All significant ANOVA results were treated with Tukey's HSD test. Simple linear regression was used to investigate the effect of continuous variables on crawler count. Due to the areal nature of the raw measurement (0 to 70), ovisac counts were root square transformed prior to analysis.

Results

Hemlock-to-Perching Bird Transfer Experiment

Hemlock-to-perching bird transfer trials were performed on 28 different days from May 14 to July 20, 2015, two to four times per week, resulting in 345 unique trials used for analysis. 223 total crawlers were collected in 119 of these 345 trials, and of these occasions, the mean number of crawlers found was 1.96, and the mode was 1. The number of crawlers collected from individual perched birds (i.e., "crawler count") ranged from 0 to 11. Throughout the 345 trials, a total of 90 specimens not considered for analysis were also collected, including 34 mature or developing settled adelgids, 15 crawlers dead before collection, 32 organisms of other species, and at least 19 HWA eggs.

We expected density classification to be a strong determinant of crawler count. To begin, all trials performed on branches of the Zero class yielded zero crawlers. Excluding these Zero class branches, the crawler counts among the High, Med, and Low classifications were only marginally insignificantly different (ANOVA: $F_{2,320}=2.932$, $p=0.055$; Figure 3). However, the root-transformed ovisac count was positively and significantly related to the number of crawlers collected from an experimental bird (linear regression: $p=0.002$, *slope*=0.13; Figure 4).

We also expected that crawler counts would increase with experimental duration, assuming a constant transfer rate. However, neither crawler count nor nonzero crawler count (i.e., only trials where > 0 crawlers were found,) differed significantly in relation to perch durations of 1, 5, 15, or 30 minutes (For all data, ANOVA: $F_{3,343}$=0.696, *p*=0.555; Figure 5, For nonzero crawler count, ANOVA: $F_{3,115}$=0.357; *p*=0.784).

Of main interest was how crawler counts changed over the course of the season, from May to July. Crawler count differed significantly by experimental week (ANOVA: $F_{10,334}$= 8.954, *p*<0.001) and biweekly intervals (ANOVA: $F_{5,339}$=9.776, *p*<0.001; Figure 6), and a Tukey's HSD test revealed that significantly higher counts were recorded during the earliest emergence of the progrediens crawlers (interval May 10-23) than during all other biweekly intervals except June 21-July 4, when sistens generation crawlers began to hatch (*p*<0.01 for all comparisons). Crawler counts during the interval June 21-July 4 were only significantly higher, however, than the interval May 24-June 7 (*p*=0.003). A smoothed loess line (Figure 7) clearly illustrates shifts in crawler abundance from its peak at the beginning of experimentation in May, followed by the gradual settlement of the progrediens generation through mid-June, the emergence of sistens crawlers causing a less dramatic rise in mid- to late June, and a steady decrease in abundance as the sistens crawlers settled to aestivate for the summer.

Although unrelated to the initial questions, the effect of a second experimental branch use on crawler count data was of concern once suitable experimental branches had to be re-used. A Welch's two-sample t-test was applied to crawler count data in relation to new versus re-used branches for each experimental week in which re-usage occurred (May 22 to June 18). For each of the five weeks in question, no significant difference was found between new and re-used branches in relation to crawler count (*p*-values between 0.333 and 0.976).

Two additional factors were considered in this experiment but not hypothesized as having a significant effect on crawler count. Time of day was recorded for each perching trial, but did not influence crawler count (linear regression: *p*= 0.880). Height of perch was also recorded for each trial, but not found to have a significant effect on crawler count (linear regression: p= 0.422).

Hemlock-to-Moving Bird Transfer Experiment

Hemlock-to-moving bird transfer trials were performed on nine different experimental days from May 20 to July 13, for a total of 27 trials. Of these 27 trials, 15 yielded crawlers (56%) and a total of 41 crawlers were collected. With the exception of one comparison of trials, all hemlock-to-moving bird trials yielded more crawlers than the 30-minute hemlock-to-perching bird trials performed on the same day (Table 1). Crawler counts differed significantly by experimental day ($F_{8,18}$=5.383, p=0.001), with the experiments performed on May 20 (during the period of peak crawler abundance) yielding significantly more crawlers than all other experimental days through July 1 (p-values ranging from 0.002 to 0.011). Crawler counts for the hemlock-to-moving bird experiment did not show a significant rise in mid-June as the hemlock-to-perching bird transfer experiment data did, and the crawler abundance on July 13 was smaller than the May 20 abundance with only a marginal insignificance (p=0.056). It must be noted that one trial was performed on a branch of the high density class rather than the medium class on May 20, although the influence of density classes High, Med, and Low on crawler count were insignificant for the brush trials (ANOVA: $F_{2,24}$ = 1.425, p=0.26), as was ovisac count within a 6 cm radius (linear regression: p=0.332).

Bird-to-Hemlock Transfer Experiment

Of the 21 branches used in the bird-to-hemlock transfer experiment, 9 showed crawler settlement after 16 to 40 days (43%). Of these 9 branches, 4 showed settlement far beyond the number of crawlers applied, and were discarded from further analysis due to presumed deficiencies in prior examination. Of the 5 remaining branches on which settlement occurred, the percentage of crawlers settled on the experimental hemlock branches in relation to number of crawlers applied to the perched bird ranged from 20 to 110%. Settlement occurred on both *in situ* and *ex situ* branches (Table 2).

Discussion

The high incidence of both progrediens and sistens generation HWA crawlers collected from perched birds in the hemlock-to-perching bird transfer experiment strongly implicates a more active role of HWA crawlers in their own dispersal between hemlocks than previously thought. Prior studies on HWA dispersal claim that crawlers are spread passively by wind, humans, deer, and birds (McClure, 1990; Turner et al., 2011). Although instances of HWA crawlers crawling onto perched birds may not necessarily be intentional, crawlers are initiating their transport on potential dispersers. This event, in which crawlers actively move onto birds, appears to be more common during periods of peak crawler abundance.

One researcher (N. Russo) observed crawlers actively moving onto a perched plastic bird under a dissecting microscope twice on May 6, on an occasion separate from this study. The ornamental bird was placed on a densely infested branch tip, directly alongside two active progrediens crawlers. Directly following the introduction of the bird, the first crawler waved its antennae and forelimbs for 3-5 seconds and crawled directly onto the plastic bird's toe, and then straight up the foot. The second crawler turned around to face the foot, and repeated the same behavior. Care was taken not to coax or prod the crawlers. Crawlers would not move onto the bird foot when it was placed > 0.5 mm away, however, or if they were not actively crawling. When this procedure was repeated for ten sistens crawlers on July 15, all crawlers stopped forward taxis and changed direction to crawl away from the bird's foot. These anecdotal observations, when considered with the results of the hemlock-to-perching-bird transfer experiment, suggest that further investigations of the mechanics of HWA dispersal should include quantitative studies on HWA crawler behavior, particularly in response to disturbance.

The greater abundance of crawlers found on a bird brushed against a branch versus a bird perched on the same branch suggests that birds acquire more HWA crawlers through repeated body contact with infested branches than by simply perching on them. Contact between active birds and HWA-infested branches may occur as a result of repeated entrances to and exits from an infested hemlock, and perhaps foliage-gleaning behaviors typical of some hemlock-associated songbirds (MacArthur, 1958; Tingley et al., 2002). The occurrence of HWA individuals as eggs, sessile adults and instars, and dead crawlers on birds used for the hemlock-to-perching bird experiment suggests that on some occasions, adelgids were dislodged as birds were mounted or removed from branches. McClure found up to 15 HWA eggs and live crawlers on wild birds (McClure, 1990), so both types of contact between bird and infested hemlock are likely modes of HWA transfer.

Further hemlock-to-moving bird transfer experiment results did not match the trends of the hemlock-to-perching bird transfer experiment data, but since the former dataset is much smaller (i.e., 27 trials compared to 345,) the hemlock-to-perching bird results are more reliable in the analysis of crawler abundance in relation to multiple variables. The hemlock-to-moving bird results did show an expected peak in crawler abundance on May 20, but the absence of both a second rise in abundance in June and a clear relation between ovisac count and crawler count may be attributed to the scarcity of trials. Nevertheless, the main purpose of the hemlock-to-moving bird transfer experiment was simply to investigate the mechanics of HWA transfer as it relates to bird behavior, so only the results of the hemlock-to-perching bird transfer experiment are considered further.

The temporal shifts in HWA crawler abundance throughout our hemlock-to-perching bird experiments match the expected shifts in abundance according to seasonal HWA phenology (McClure, 1989a). The peak of crawler abundance during the interval May 10-23 also coincides with the final stages of avian spring migration in Connecticut. McClure (1990) suggested a study of crawler abundance on migratory birds because HWA crawlers are more abundant in early May than during the regular passerine breeding season, which normally starts for Connecticut forest birds shortly before the first week of June. The findings of this study provide further evidence that birds may play a role in long-distance dispersal of HWA during migration. Since crawlers emerging from overwintering sistens are more abundant in years of lower winter mortality, and progrediens crawlers are generally more abundant during the first three weeks of May (McClure, 1989a; Butin et al., 2007), the hemlock-to-perching bird transfer experiment might show a higher peak of abundance if performed during a year of lower mortality, or if performed from the beginning of the progrediens hatch period, which typically occurs in late April and continues through mid-June.

Since no significant differences in crawler count resulted from varying perch duration, the results of this study do not point toward a constant transfer rate with time, suggesting instead that hemlock-to-perching bird crawler transfer may be incidental or influenced by other factors not considered during this study. Such factors include scattered hatch times amongst ovisacs on a given branch, or an uneven abundance of active crawlers immediately surrounding the perching point, for which ovisac count was a presumed indicator. Unknown information that may be useful in this study and future studies on HWA population and dispersal dynamics include the rate at which crawlers from either generation travel, and the average displacement from their point of emergence to point of settlement in relation to infestation density. Such information would allow for more useful measurements of ovisac density and presumed crawler activity surrounding the perching point. Taking these unknowns into consideration, the perch duration component of this experiment suggests that a bird may perch for only a minute before several HWA crawlers move onto it, according to temporal HWA crawler abundance.

The positive linear relationship between crawler count and ovisac count within 6 cm of the perching point suggests that the number of HWA crawlers a bird is likely to acquire and disperse increases according to the severity of infestation surrounding the perching point. Since crawler counts did not rise in proportion to ovisac density, however, these results implicate the role of density-dependent feedback in crawler abundance. As a population of HWA on a branch burgeons, resources become limited, and overall survival and fecundity decreases (McClure, 1991; Sussky and Elkinton, 2014). Although more ovisacs, and thus more HWA adults were present on branches of the high density class, individuals will lay fewer eggs if their branch of settlement is densely populated, so there were likely fewer eggs per ovisac on high density branches versus medium and low density branches. As ovisac density rose, the number of crawlers potentially emerging from ovisacs likely decreased, thereby generating a more gradual rise in crawler abundance (Figure 4).

The absence of crawlers on all birds perched on branches without a detected infestation indicates that HWA adults and ovisacs must be present for crawler transfer to be likely. Birds may still be able to acquire HWA crawlers from branches without a detected infestation on rare occasions, however, because viable HWA eggs can be found on non-hemlock species in infested forests (McClure, 1990). Excluding zero class branches, the relative insignificance of density class as an indicator of potential crawler count, along with the positive linear relationship between ovisac count and radial crawler count suggest that density classifications used in this experiment must be calibrated to a more appropriate standard. Possible solutions include expanding the field of measurements under the medium classification to condense the field of high density measurements, which ranged from 20 to 70 ovisacs per perch radius.

The instances of settlement on 5 of 17 branches used in the bird-to-hemlock transfer experiment suggest that birds are capable of causing new infestations by depositing HWA crawlers onto uninfested branches. Three *in situ* trials of the bird-to-hemlock transfer experiment were performed during a period of greater sistens crawler abundance (June 19), and two of these three branches were the only *in situ* branches to show crawler settlement. Unfortunately, too few experiments were performed to draw conclusions about the likelihood of settlement in relation to time of season, and the *ex situ* trials, which all showed crawler settlement, are difficult to compare to the *in situ* trials in any capacity due to differences in experimental conditions. The possibility of settlement and production is governed by multiple biotic and abiotic factors such as wind and weather (McClure, 1989b; McClure, 1990; Turner et al., 2011), and predation by specialist predators such as *Pseudoscymnus tsugae* (McClure and Cheah, 1999), and generalist predators such as arachnids (McClure and Cheah, 2002), both of which occur in the main hemlock study area. Further research should include an evaluation of settlement success after avian dispersal in relation to seasonal fluctuations of crawler abundance, since early-emerging crawlers for both generations are also more likely to settle than those emerging later in the hatch period (Butin et al., 2007). Fumigation treatment would ensure that no living HWA crawlers or predators were present on the branch prior to experimentation, and smaller branch tips (~20 cm), would make examination for the presence of settlement more manageable for future study.

One source of heterogeneity within our data may be the prospect of collecting "stray crawlers" from previous trials. On June 1, all seven birds used for the first eight days of experiments were examined under a dissecting microscope for potential remnant biological material. Additionally, the stray debris at the bottom of the research specimen container was collected and examined under a microscope. A total of two dead crawlers were found at the bottom of the container, and none were found on the birds. This process was repeated for the six new bird mounts used for the last 22 days of experiments. Ten dead crawlers were found in the stray debris at the bottom of the second container, and no crawlers were found on these six birds. With an average of 0.4 stray crawlers found per day of experimentation, it is possible but unlikely that uncollected crawlers from previous trials were accidentally counted toward future trials.

Birds have been found to carry by ectozoochory several types of organisms, such as aquatic plants and invertebrates (Reynolds et al., 2015), parasitic mites (Dietsch, 2005), and bryophyte diaspores (Lewis et al., 2014). In at least one case, this transfer has resulted in the dispersal of organisms from one geographic pole to another (Lewis et al., 2014). While birds have been documented carrying hemlock woolly adelgid crawlers and eggs previously, (McClure, 1990), this study highlights the importance of HWA phenology as it relates to crawler abundance, and thus the potential of birds to disperse HWA while migrating. Studies of this process on wild bird populations are needed to determine whether these seasonal dispersal dynamics occur in nature. Further advances in the study of HWA dispersal dynamics may aid in the control of HWA as climate change allows this insect to spread to untouched forests farther north.

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Table 1: All hemlock-to-moving bird trials (Brush Crawler Count) yielded more crawlers than the 30-minute hemlock-to-perching bird trials (Perch Crawler Count) performed on the same branch earlier the same day, except in the case of trials performed on branch H98.*

Date of Experiment	Branch	Density Class	Perch Crawler Count	Brush Crawler Count
5/20/2015	M14	Med	1	4
5/20/2015	H6	High	2	9
5/20/2015	H15	High	0	6
6/1/2015	L25	Low	0	0
6/1/2015	M27	Med	0	0
6/1/2015	H25	High	0	0
6/5/2015	L15	Low	0	0
6/5/2015	H41	High	0	1
6/5/2015	M41	Med	0	0
6/10/2015	L49	Low	0	0
6/10/2015	H31	High	0	0
6/10/2015	M51	Med	0	0
6/19/2015	M63	Med	0	0
6/19/2015	L62	Low	0	1
6/19/2015	H63	High	0	0
6/26/2015	M73	Med	1	1
6/26/2015	L73	Low	0	0
6/26/2015	H75	High	1	1
7/1/2015	M87	Med	1	1
7/1/2015	H86	High	0	0
7/1/2015	L88	Low	0	2
7/8/2015	L91	Low	1	1
7/8/2015	M90	Med	0	1
7/8/2015	H92	High	0	7
7/13/2015	M100	Med	1	2
7/13/2015	H98	High	2	1*
7/13/2015	L100	Low	0	3

Table 2: Two in situ *experiments showed settlement (20% and 40%) and all three* ex situ *branches showed settlement (40%, 60%, and 110%)*

Condition	Date of Experiment	Crawlers Applied	Crawlers Settled
in situ	6/19/2015	5	0
in situ	6/19/2015	10	2
in situ	6/19/2015	20	0
in situ	7/6/2015	5	2
in situ	7/6/2015	10	0
in situ	7/6/2015	20	0
in situ	7/6/2015	0	0
in situ	6/29/2015	5	0
in situ	6/29/2015	10	0
in situ	6/29/2015	20	0
in situ	7/6/2015	5	0
in situ	7/6/2015	10	0
in situ	7/6/2015	20	0
in situ	7/6/2015	0	0
ex situ	7/6/2015	5	2
ex situ	7/6/2015	10	11*
ex situ	7/6/2015	20	8
	Total	175	25

**An eleventh crawler may have been applied to the bird perched for this trial, or a small number of crawlers may have already been present and overlooked on the branch used for this trial.*

Figure 1: Perching apparatus used in hemlock-to-perching bird transfer experiment

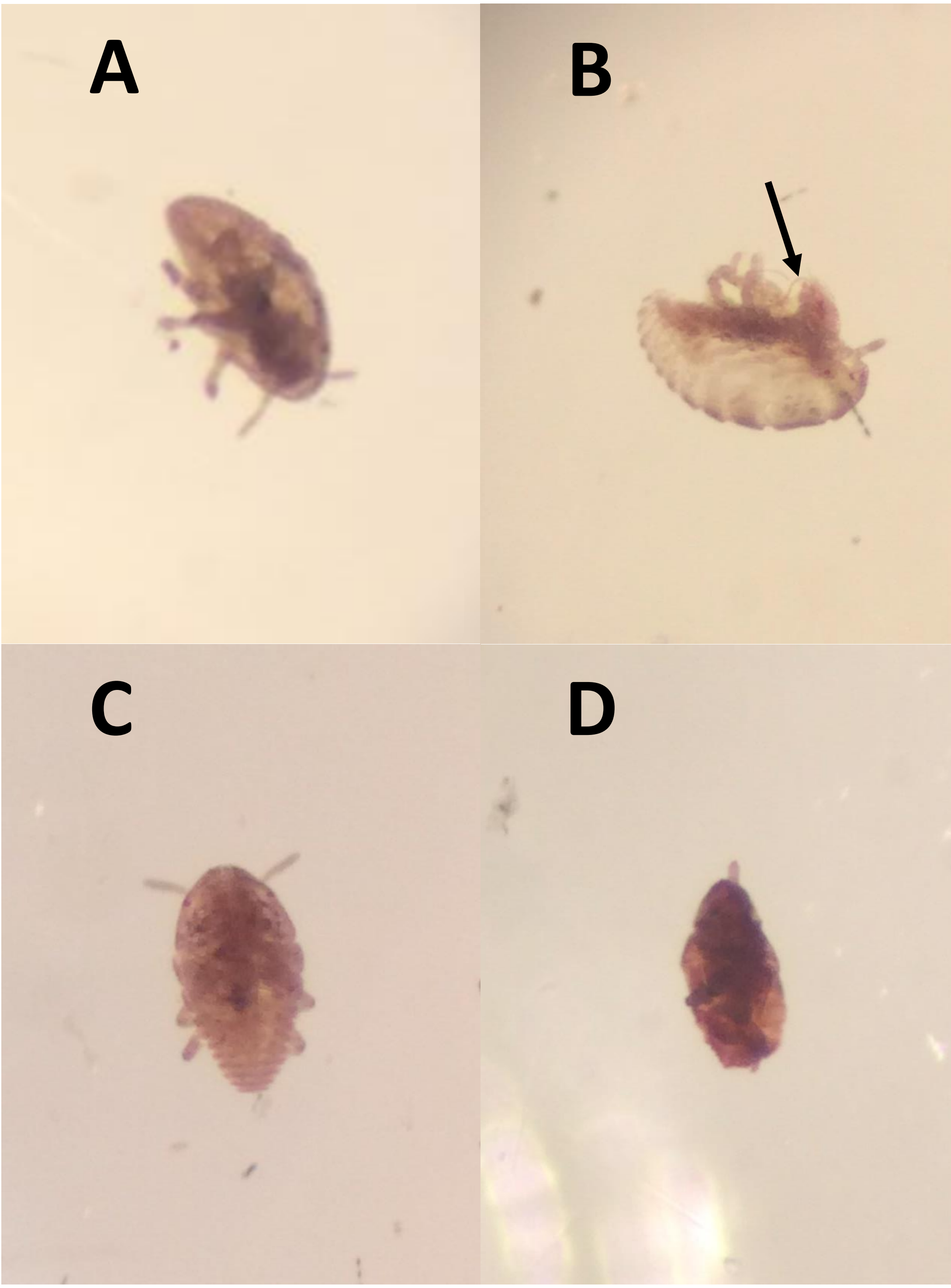

Figure 2: Examples of HWA specimens collected from birds including a) a crawler that lacks a stylet bundle, and was thus counted towards crawler abundance measurements, b) an individual that has settled and developed beyond the first instar, as identified by the presence of a stylet bundle (arrow) and thus recorded as a miscellaneous specimen, c) an example of a living crawler counted toward crawler abundance analysis, and d) a dead crawler, identifiable by its wrinkled, darkened appearance.

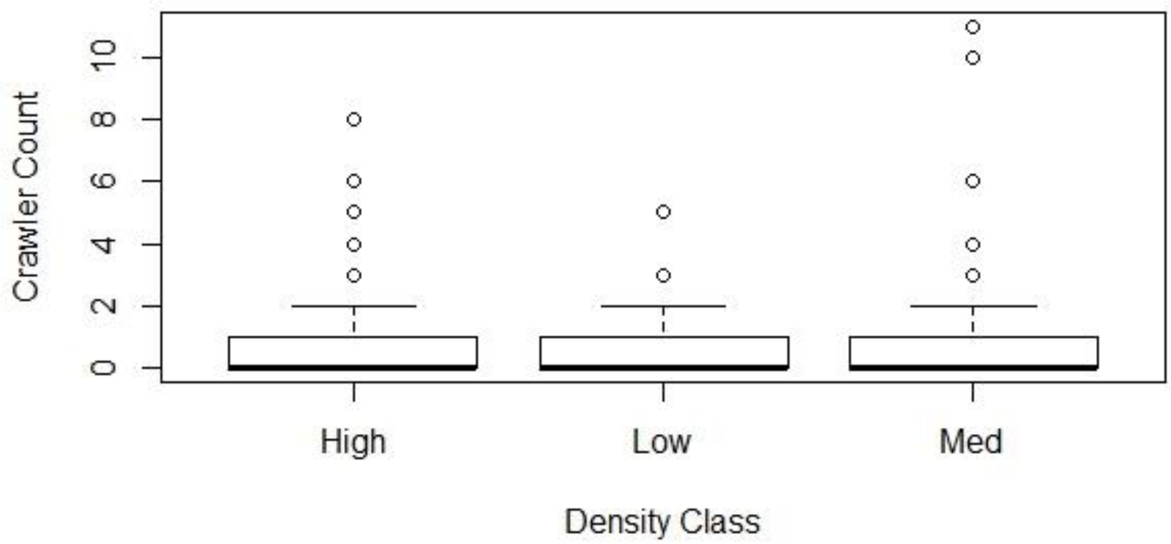

Figure 3: When the Zero class is removed, density classification is a marginally insignificant indicator of crawler count ($F_{2,320}$=2.932, p=0.055)

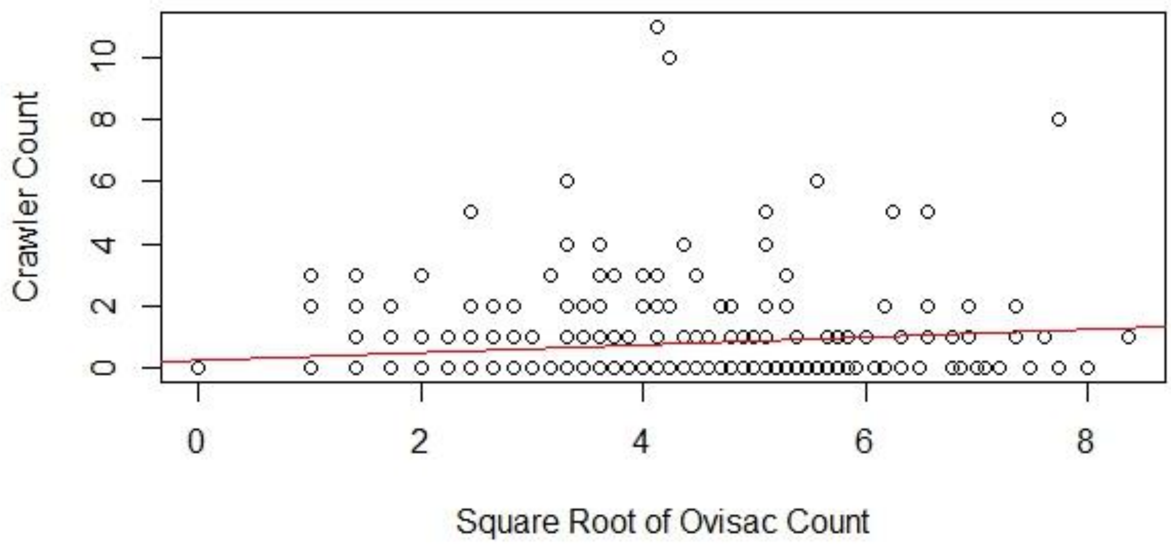

Figure 4: A positive linear relationship exists between crawler count and square root of ovisac count (p=0.0019, slope=0.13)

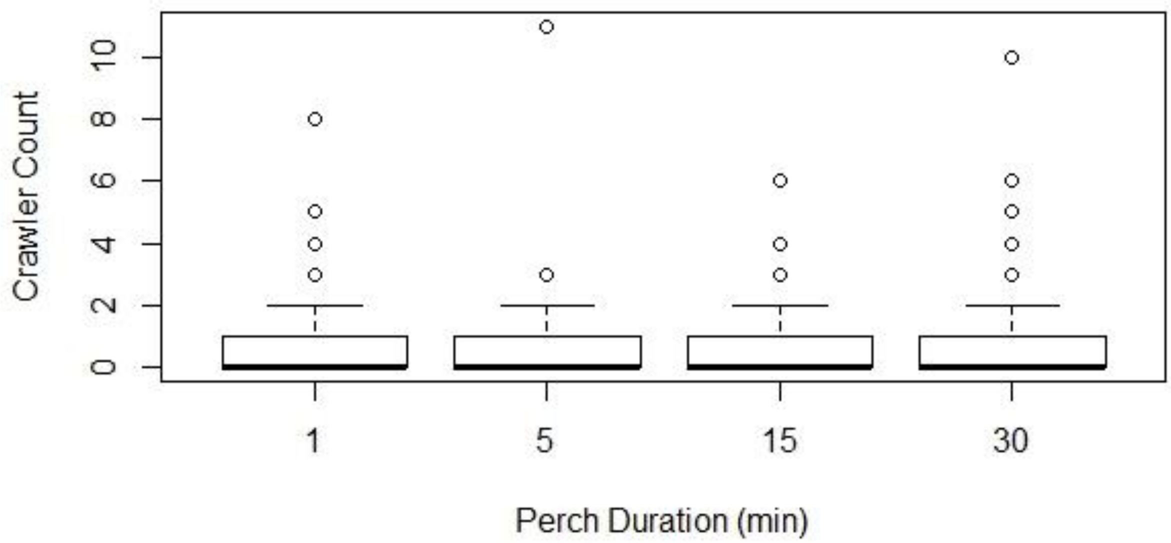

Figure 5: The number of crawlers collected from a perching bird was not influenced by perch duration ($F_{3,343}$=0.696, p*=0.555).*

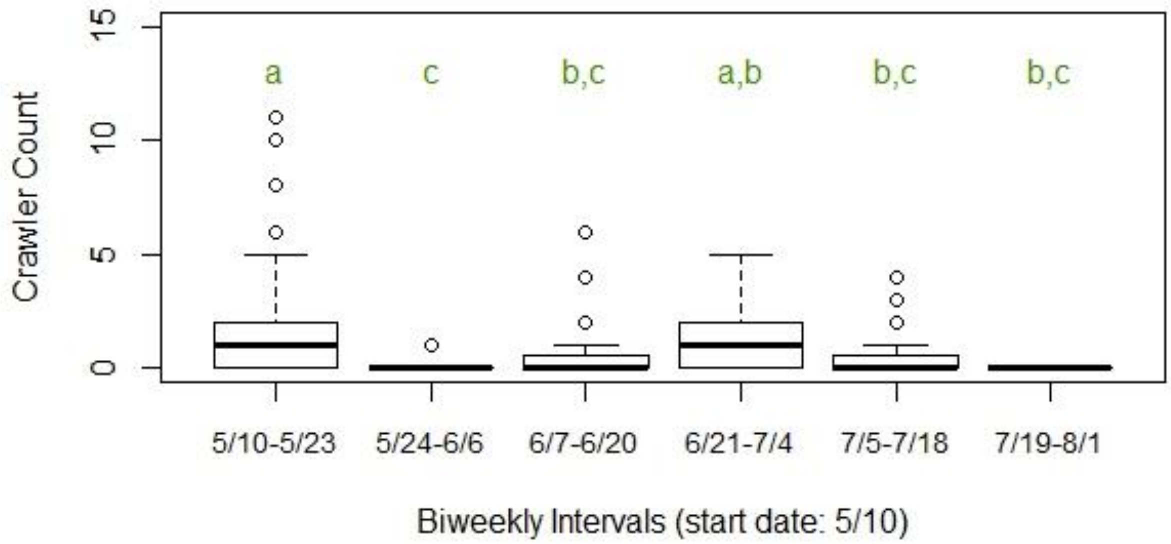

Figure 6: Crawler abundance in biweekly intervals over the collection period May 14 to July 20. Intervals with the same lowercase letter above them are not significantly different (Tukey's HSD, p<0.05).

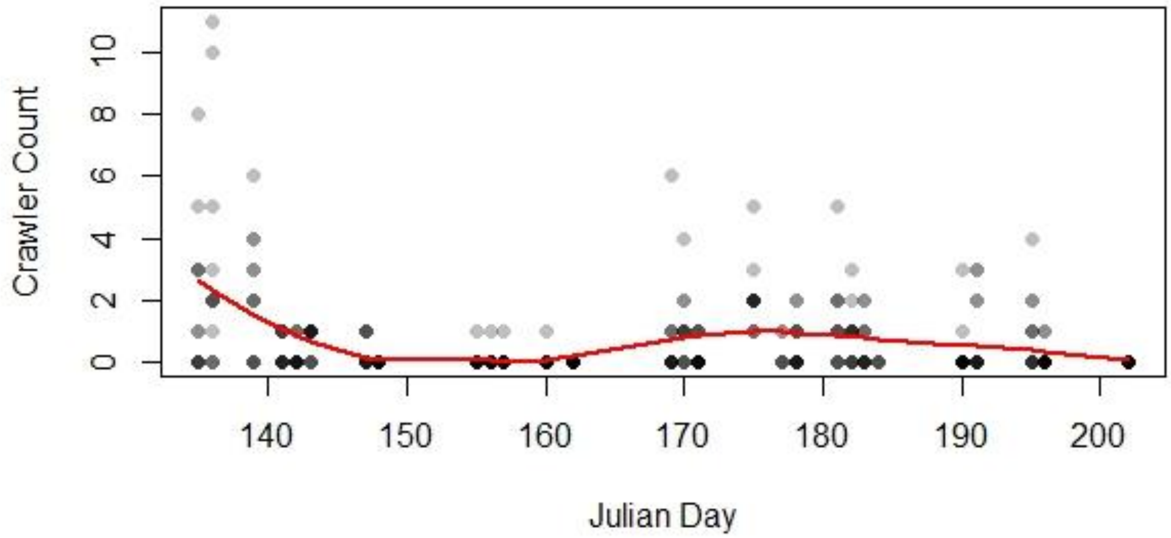

Figure 7: Crawler abundance for each Julian day over the collection period with fitted loess line (span = 0.5) to represent temporal shifts in crawler abundance. Plot points are darkened according to crawler count frequency.