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Rebound of *Adelges tsugae* spring generation following predation on overwintering generation ovisacs by the introduced predator *Laricobius nigrinus* in the eastern United States

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ABSTRACT

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), has devastated eastern hemlock (*Tsuga canadensis* [L.] Carriere) in a major portion of its native range in eastern North America. Population dynamics of HWA in the absence of predators have been studied for decades. After many years and much effort directed towards rearing and releasing biological control agents to manage HWA, one of these agents, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), is now successfully established at significant densities at sites from the southern to the mid-Atlantic states of the eastern U.S. However, high densities of HWA still persist at many locations throughout the region and spread of HWA and associated damage to hemlock continues. Population models for HWA have suggested that even upwards of 90% predation on eggs laid by the overwintering sistens generation will have minimal effect in reducing the population densities of HWA, if HWA are at high density. In this study, we tested the ability of *L. nigrinus* to reduce HWA densities, and experimentally tested these model predictions to better understand what impact, if any, *L. nigrinus* has on HWA densities. By using predator exclusion cages at field sites with well-established populations of *L. nigrinus*, we were able to record HWA densities, fecundity, overwintering mortality, and predation by *L. nigrinus*, as well as the proportion of branch tips producing new growth on study trees. Using our field-collected data, we refitted the model in ways that allowed us to predict what population densities we could expect for the following summertime progrediens generation given previous HWA density and levels of *L. nigrinus*. In both years, we found that despite high rates (greater than 80% ovisac predation) of predation by *L. nigrinus* on uncaged branches compared to caged branches, there were no significant differences in subsequent densities of the HWA spring generation between caged and uncaged treatments, as predicted by our model. In 2018, our field-collected densities of the summer progrediens generation were lower than what was predicted by the model in both predator exclusion treatments, possibly due to the model not incorporating tree health and climatic factors. Simulation models of pest insect populations based on field-collected data such as fecundity, density, overwintering mortality, and predation, could prove to be important in informing researchers and managers about the role of the biological control agent in the population dynamics of the target host.

1. Introduction

Hemlock woolly adelgid (*Adelges tsugae* Annand; Hemiptera: Adelgidae), hereafter HWA, is a major forest pest causing high mortality to eastern (*Tsuga canadensis* (L.) Carriere) and Carolina (*Tsuga caroliniana* Engelm) hemlocks in the eastern United States. HWA is native to parts of East Asia and the Pacific Northwest of North America, and DNA evidence suggest that the lineage of HWA introduced to eastern North America comes from Southern Japan (Havill et al., 2006). HWA was first discovered in eastern North America in Richmond,

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Virginia in the early 1950s and it is believed to have been brought there accidentally on infested hemlock nursery stock imported from Japan (Havill et al., 2014). Since its discovery in Richmond, Virginia, HWA has spread to 20 additional eastern states, as well as southeastern Ontario and Nova Scotia, Canada (USDA Forest Service, Northern Research Station Range Map) (Ellison et al., 2018).

HWA has two generations per year on hemlock, both of which reproduce asexually. The sistens generation is present from early summer through early spring, and it feeds from fall through spring after going through a mid-summer aestivation period (McClure, 1991, 1987). The progrediens generation develops from eggs laid by the sistens generation in late spring. These eggs hatch into crawlers, which settle at the base of hemlock needles and feed from early spring to early summer. The progrediens generation can either mature into adults on a hemlock twig or, especially at high density (McClure, 1991), develop into winged adults called sexuparae, which in Asia then seek out tigertail spruce (*Picea torano* (K. Koch) Koehne), on which they initiate a sexual generation (Havill et al., 2006). In North America, however, native spruce trees are unsuitable hosts, and therefore sexuparae fail to reproduce (McClure, 1989, 1987). HWA has no effective natural enemies that are native to eastern North America, and both hemlock species there were found to be very susceptible to infestation and subsequent decline/mortality (McClure, 1987). The loss of hemlock as a dominant forest tree prompted the USDA Forest Service to devote substantial resources to the search for natural predators, which could be released for HWA population management (Havill et al., 2014).

Several HWA predators from Asia and the Pacific Northwest were introduced to eastern North America (Onken and Reardon, 2011). To date, one species, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), has successfully established in substantial numbers (over 400,000 released from Georgia to Maine with successful establishment in each state) at field sites in the eastern U.S. (Foley et al., 2019). *Laricobius nigrinus* is active as an adult in the fall, winter, and spring. From February through April *L. nigrinus* adults lay their eggs within the sistens' ovisacs (the "woolly" wax secretions produced by females of HWA to cover the eggs). *Laricobius nigrinus* adults feed on settled sistens nymphs and adults, and as larvae they feed primarily on progrediens eggs (Zilahi-Balogh et al., 2003). After developing through four instars, *L. nigrinus* larvae finish feeding and then drop to the soil to pupate in early spring. *Laricobius nigrinus* pupates in the spring, and aestivate during summer months as adults, synchronized with sistens aestivation, resuming activity in the fall (Zilahi-Balogh et al., 2003), when the adult beetles emerge and feed on the developing HWA of the sistens generation.

Although *L. nigrinus* established and could be reliably collected at various sites in eastern North America, its impact in reducing overall HWA population densities, or on hemlock health through reduction in HWA density have not been determined (Preisser et al., 2014). However, some predator enclosure or exclosure experiments have shown *L. nigrinus* can reduce densities of HWA sistens generation in local field settings (Lamb et al., 2005; Mausel et al., 2008; Mayfield et al., 2015). To better understand population dynamics of HWA, a model was created by Elkinton et al. (2011) to see how various mortality factors affected HWA populations. Surprisingly, model simulations suggested that, even with upwards of 90% predation on eggs in the sistens ovisacs, there would be little or no reduction in the subsequent progrediens density the following spring. HWA populations exist at or near carrying capacity in eastern North America and there is strong competition for space to settle on hemlock twigs. HWA settles at the base of the hemlock needles, and when there is more than one HWA per needle, survival decreases. This is likely due to intraspecific competition for a limited food source; the carbohydrates and proteins stored in xylem ray parenchyma cells (Sussky and Elkinton, 2014; Young et al., 1995). When sistens densities are high, each female can replace herself approximately once due to habitat saturation (of settling sites), yet each adult oviposits between 40 and 200 eggs (McClure, 1991; Paradis, 2011). The vast majority of crawlers emerging from such ovisacs, therefore, die before reaching maturity because there is not enough space for them on the infested hemlock twigs. Sussky and Elkinton (2014) recorded strong density-dependent mortality, including dispersal of progrediens crawlers that die before settling and density-dependent production of winged adults (called sexuparae), which subsequently die because there is no appropriate *Picea* hosts for them in North America (McClure, 1987). This density-dependent survival may completely compensate for any effect of predation on progrediens eggs (Elkinton et al., 2011). The model left open the possibility that predation by *L. nigrinus* adults on the sistens generation of HWA in the fall might have a significant impact on HWA densities. However, if the prediction of the Elkinton et al. (2011) model is accurate, then *L. nigrinus* may have little or no significant impact in reducing HWA progrediens densities if sistens population densities are high.

In this study, we established predator exclusion experiments at field sites with populations of *L. nigrinus* to test its efficacy as a biological control agent and to test the predictions of the Elkinton et al. (2011) model. We used mesh cages to restrict access to HWA by *L. nigrinus*, and we recorded densities of HWA. The impact of *L. nigrinus* on sistens ovisacs has been reported at greater length in a companion paper (Jubb et al., 2020). The specific goal of our study was to test whether feeding by established populations of *L. nigrinus* reduces subsequent HWA progrediens densities at field sites with high sistens densities. Towards this end, we parameterized the HWA population dynamics model of Elkinton et al. (2011) using field collected data from the sistens generation. We then compared the model predictions for progrediens density to actual progrediens densities and in doing so, determine the accuracy of model predictions and whether or not *L. nigrinus* predation is having an impact on the progrediens generation. We hypothesize that densities of progrediens on branches open to predation by *L. nigrinus* would be slightly lower than those on branches that excluded *L. nigrinus*, and that density-dependent survival in the progrediens generation will at least partially compensate for predation by *L. nigrinus*.

2. Methods

2.1. Field site locations

In 2016 we selected sites in Maryland, New Jersey, North Carolina, and Virginia (Table 1). Each site had significant infestations of HWA (i.e., trees were infested with densities of around 2–3 HWA per cm or

Table 1
Field site names, GPS Coordinates, and number of sample trees in both rounds of experiment.

Site	Location	Coordinates	Number of Trees (n)	
			2016–17	2017–18
DEWA, NJ	Delaware Water Gap National Park, Walpack Township, NJ	41.12 N, −74.91 W	n = 20	n = 20
Rocky Gap, MD	Rocky Gap State Park, Gross, MD	39.70 N, −78.67 W	n = 9	n = 15
James River, VA	James River State Park, Gladstone, VA	37.64 N, −78.80 W	n = 8	n = 15
Kentland, VA	Kentland Farm, McCoy, VA	37.21 N, −80.59 W	n = 6	n = 15
Celo, NC	Celo Community, Burnsville, NC	35.82 N, −82.21 W	n = 7	n = 13

greater), as well as established populations of *L. nigrinus* (i.e., *L. nigrinus* has been recovered in multiple years following its release). These sites represent a subset of the sites used in a concurrent companion study (Jubb et al., 2020) that examined the impact of *L. nigrinus* predation on the HWA sistens generation. Before *L. nigrinus* became active in the fall, trees with branches supporting moderate to high densities of HWA were chosen as sample trees and used for subsequent experiments. The number of trees for each site in both rounds of the experiment are presented in Table 1.

2.2. Estimating adelgid density and new hemlock shoot growth

At the beginning of the experiment in mid-November of each year, following aestivation break, indicated by nymphs producing wool, we chose 20-cm sample branchlets on all 1-m long sample branches (160 branchlets total) making sure that within study branch pairs, sample branchlets had similar densities of sistens ovisacs (more details on sample branch selection and experimental establishment below in section 2.3). Densities of maturing sistens (individuals secreting wool) were measured in the field on each 20-cm branchlets (Jubb et al., 2020). On each 20-cm branchlet, we also recorded the length of 10 current-year shoots (representing new-growth from previous growing season). In April, at peak HWA egg abundance, measurements were repeated on the same branches. Overwintering mortality and the proportion of sistens ovisacs disturbed by predation were recorded on both caged and uncaged branches. Information about collection of overwintering mortality and ovisac predation data can be found Jubb et al. (2020).

In 2017, we also recorded data on the percentage of terminal branch tips with new growth on sample branches at all sites. This has been a standard technique for estimating new growth on hemlock branches in previous studies of HWA impact on hemlocks (McClure, 1991; Sussky and Elkinton, 2014). Samples obtained to estimate progrediens density in the laboratory were also used to estimate current-year growth estimates in 2017. In November 2018, branch samples were taken from the Delaware Water Gap, NJ (DEWA) field site to get an estimate of the proportion of branch tips showing some new growth in the second year. Because field collections of HWA samples were finished and the logistics of travelling to field site locations, only DEWA was sampled for proportion of twigs with new growth in 2018.

2.3. Establishment of predator exclusion cages

Pairs of 1-m-long branches that were in the lower canopy (up to 2.5-m above the ground) were marked to represent the treatments "cage," and "no cage." In sites that had fewer than 15 suitable trees, larger trees were used for multiple sets of treatments. Trees ranged from 15 to 35-cm in DBH (diameter at breast height) and included trees located in the understory and on the forest edge. From each paired branch, a 20-cm-long sample branchlet (one per 1-m-long treatment branch, total of 160 sample branchlets) was marked and the number of HWA and total length of new growth were recorded. Branchlets were selected such that HWA densities per cm of new growth on branchlets were similar within pairs. Each "cage" branch was tapped with a rod ten times to dislodge any *L. nigrinus* that may have been on the branch and was then fitted with a predator exclusion cage (1-m-long by 0.5 m wide Equinox® No-See-Um mosquito netting ~ 569 holes per square centimeter) to exclude *L. nigrinus* from having access to HWA on the branch. Self-stick vinyl foam (3.2 cm. × 48 mm.) was wrapped around the branch where the cage was attached with cable ties as padding between the cable ties and branches so as not to cut off the flow of nutrients or water.

In March of both years, coinciding with peak production of progrediens eggs by the overwintering sistens generation, the predator exclusion cages were removed and both "cage" and "no cage" treatment branches were enclosed in fine-mesh cages. The fine-mesh cages were made from silk screening (1-m-long by 0.5-m wide SeFar Basic 61/156–64 W PW) with openings (97x97 nm) smaller than first-instar HWA nymphs, thereby preventing crawler immigration and emigration between branches. By excluding *L. nigrinus* on our caged branches, we expected to have greater HWA sistens densities than on uncaged branches. These fine-mesh cages were applied to ensure that the settled progrediens resulted solely from crawlers originating on the same branch to which the predator exclusion treatment was applied.

Each spring, after the progrediens eggs had hatched, the crawlers had settled on the sample branches, and *L. nigrinus* had completed feeding, the fine-mesh cages were removed. All branches then remained uncaged for the duration of the progrediens generation (late May through June), when *L. nigrinus* was no longer active on the tree and predation by generalist insect predators is thought to be negligible (McClure, 1987).

2.4. Estimating fecundity

In the first year of data collection (2017), HWA fecundity was estimated by randomly selecting twigs (10-cm-long with at least 30 ovisacs) from each sample branch (160 twigs total) and then placing them into petri dishes sealed with parafilm, allowing sistens adults to complete oviposition in the laboratory (Tobin et al., 2013). In the second year (2018), twig samples were collected randomly from each sample branch (160 twigs total) when adult HWA had completed oviposition (as indicated by dead HWA adults in ovisacs with eggs) and these twigs

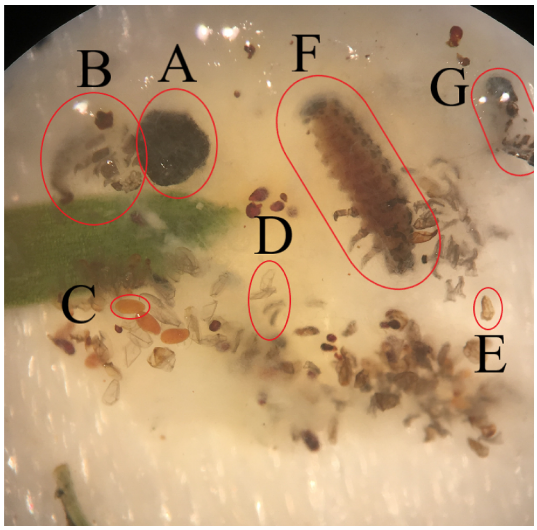

Fig. 1. View of a dissected sistens ovisac submerged in 70% ethanol to dissolve the wax. A) Adult female hemlock woolly adelgid sistens. B) *L. nigrinus* larval exuvium left over from a molt that occurred inside the ovisac. C) Healthy progrediens egg, laid by sistens adult. D) Four eggshells or "chorions." Chorions of successfully hatched eggs appear grey. E) Consumed egg, similar in appearance to a chorion, but hemolymph left inside the egg gives it a slight orange appearance. F) *L. nigrinus* larva found inside the ovisac consuming eggs. G) Sistens exuvium left over from molting. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were brought back to the laboratory for fecundity estimates. For each site three haphazardly selected ovisacs per twig sample were examined under a dissecting microscope until at least 30 ovisacs per treatment were examined. The number of chorions, unhatched dead eggs, and any eggs that were predated upon, (evidenced by hemolymph left behind in the chorion) were counted (Fig. 1). Overwintering mortality of maturing sistens was determined from the fecundity samples by counting the live sistens individuals (those that survived to the adult stage and produced eggs) and dead sistens individuals (sistens individuals that produced wool but never reached maturity and therefore did not produce eggs).

2.5. Modifying the predictive model

The sources of density-dependent mortality affecting HWA populations are (1) dispersal of progrediens crawlers, (2) decreased survival of settled progrediens, (3) sexuparae production, (4) reduction of progrediens fecundity, (5) dispersal of sistens crawlers, and (6) decreased survival of settled sistens nymphs (Sussky and Elkinton, 2014). In this experiment factors 4, 5, and 6 occurred after we collected data on the progrediens generation, leaving the first three factors as the focus of our study. As mentioned above, we used fine-mesh anti-dispersal cages on both treatments during hatch. The purpose of these cages was to remove density-dependent dispersal effects so the only density-dependent mortality factors left would be survival of settled progrediens nymphs and sexuparae production.

To determine the predicted outcomes for the Elkinton et al. (2011) model from our experimental results, we modified model parameters in the following way to mimic our experimental design. We removed mortality from progrediens dispersal, because it was prevented by the fine-mesh cages. Although the model can predict densities of the subsequent sistens generation, here we focused on what the model predicted for adult progrediens densities. For each treatment, we modified the model to start with the observed mean densities of maturing sistens nymphs and imposed the percent overwintering mortality recorded at each site as reported by Jubb et al. (2020). This yielded the observed density of sistens ovisacs in the spring. We used the observed mean proportion of disturbed ovisacs on the "no cage" treatments at each site (Jubb et al., 2020) to model the predicted impact of *L. nigrinus* predation on the subsequent density of progrediens adults.

2.6. Data analysis

All analyses were performed in R 3.5.3 (R Core Team, 2019) using RStudio, version 1.2.1335. Our statistical analyses were designed to determine if there were significant differences in adult progrediens density between treatments in the paired branches (one pair per tree) with and without predator exclusion cages. We applied these analyses to the data collected across all sites in both years to maximize the statistical power of our tests. We used a generalized linear mixed effects model (GLMM) (Package = *lme4,* Version 1.1–21) and analyzed the data with the 'glmer' function, specifying the gamma family of distributions for our density data, and we specified both site and branch pair as random effects (Bates et al., 2015). We added the value of 0.0001 to the density value on each branch to remove zeros in the data to permit analyses with the few branches with zero adelgids (Zar, 2010). We used a very similar model to analyze ovisac disturbance data, however, we used the binomial family of distributions which handles data that are proportions and no values were added to the data. These data were shared from Jubb et al. (2020) who analyzed the same data looking at the difference between treatments at the site level.

We compared the ratio of densities in the cage treatment to the corresponding no cage treatment in each cage pair to the ratio predicted by the model. We used $\log_{10}$ (ratio) for these comparisons so that the distribution of values greater than or less than 1.0 would be similar. These analyses were performed with 'lmer' function (Package = *lme4,* Version 1.1–21) assuming a Gaussian distribution. The model was fit with treatment (in this case "Predicted" and "Observed") as a fixed effect and site as a random effect and run separately in each year.

We tested for density-dependent survival in the progrediens generation in 2018, the only year in which all the relevant data needed to complete the analysis was available. Using the number of sistens ovisacs and percent sistens mortality (overwintering mortality plus *Laricobius* predation) we estimated the predicted number of sistens individuals maturing to the adult stage. The predicted number of surviving sistens adults was multiplied by the mean sistens fecundity to get the predicted number of progrediens crawlers that were produced. These numbers were expressed as densities per 20-cm branch sample. We then divided our counts of progrediens adults by the total number of progrediens crawlers to get the proportion surviving to the adult stage. We graphed the proportion of progrediens individuals surviving versus the $\log_{10}$ number of sistens adults. We analyzed these data using a logistic regression via a generalized linear model (Package = *stats,* Version 3.5.3) with a quasibinomial distribution (logit link) because the data were overdispersed (R Core Team, 2019). We ran a nearly identical logistic model to examine the effect of treatment (cage vs non-caged) on the proportion of progrediens crawlers surviving to the adult stage. All graphical data were displayed using ggplot2 (Wickham, 2009).

3. Results

3.1. Density estimates, model predictions, and density-dependent progrediens survival

Each fall of the experiment, there was no statistical difference in sistens density between treatments across sites (Table 2; Fig. 2A & 3A)

Table 2
Mean (± SE) observed densities of sistens and progrediens generations, mean model estimates of progrediens generation, and mean (± SE) proportion of ovisacs disturbed by *L. nigrinus* and results of analyses showing effect of treatment.

	Fall Densities (sistens/cm; Mean ± SE)			
Year	Cage	No Cage	z	*P*
Nov. 2016	3.2 ± 0.24	3.1 ± 0.25	−0.300	0.764
Nov. 2017	4.5 ± 0.24	4.4 ± 0.22	−0.831	0.406
	% Ovisac Disturbance (Disturbed/Total; Mean ± SE)			
	Cage	No Cage	z	*P*
Mar. 2017	8.1 ± 1.7	53.7 ± 6.8	−50.967	0.0001
Mar. 2018	4.1 ± 1.2	36.8 ± 6.5	−40.468	0.0001
	Model Prediction for Progrediens densities			
June 2017	1.3	1.18	N/A	N/A
June 2018	1.12	1.07	N/A	N/A
	Spring Densities (progrediens/cm; Mean ± SE)			
	Cage	No Cage	z	*P*
June 2017	1.2 ± 0.25	0.90 ± 0.15	−0.960	0.337
June 2018	0.14 ± 0.003	0.22 ± 0.043	0.933	0.351

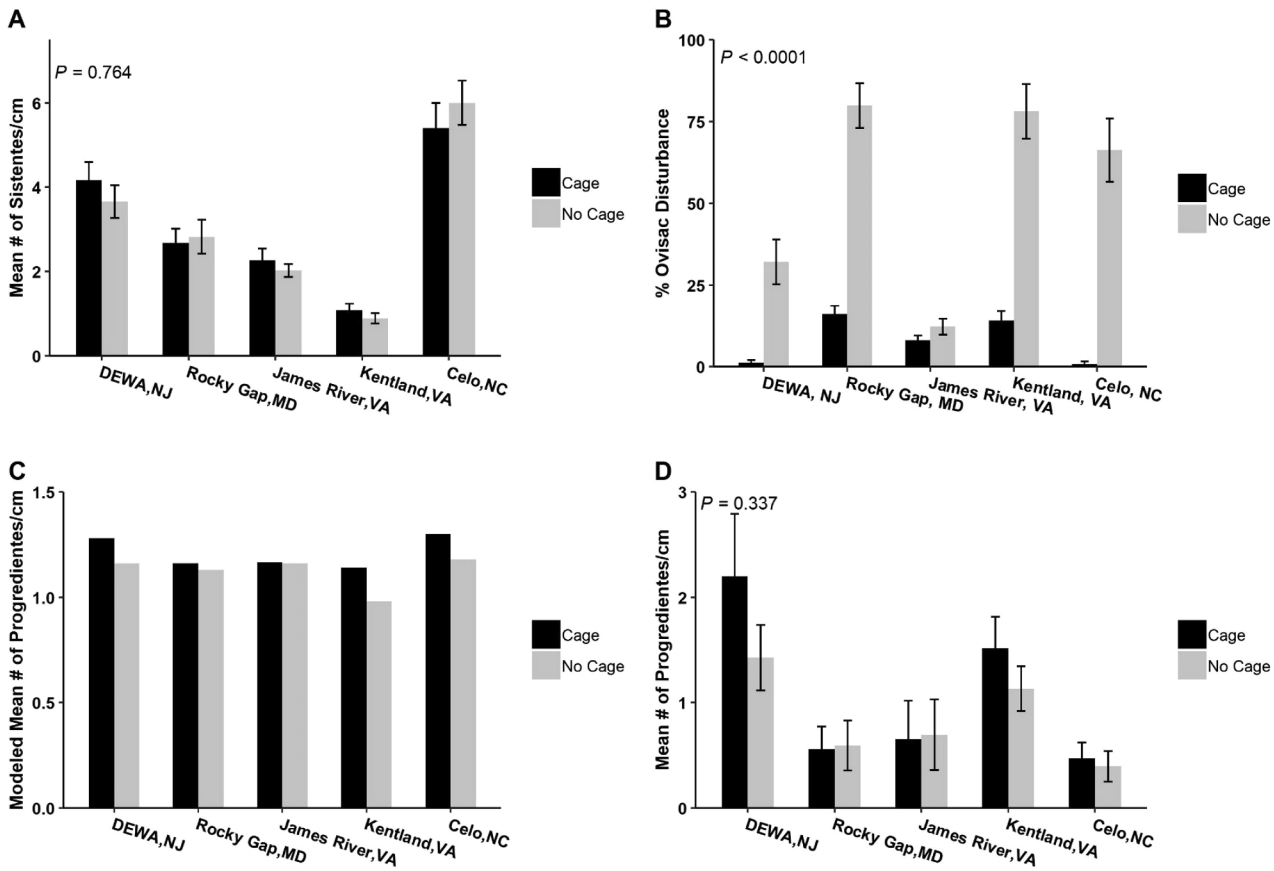

Fig. 2. Mean (± SE) observed and predicted HWA densities in 2017 and ovisac disturbance by treatment "Cage" and "No Cage." A) Density estimated for sistens generation 2016. B) Percent ovisac disturbance by *L. nigrinus*. C) Model predictions of progrediens density based on the mean sistens density, overwintering mortality, ovisac disturbance, and fecundity by treatment and site. D) Observed density of the progrediens generation 2017. "Sistentes" and "progredientes" are plural forms of "sistens" and "progrediens." *P*-values for A, B, & D are treatment (Cage/No Cage) level *P*-values across sites.

as previously reported by Jubb et al. (2020). Each spring we found highly significantly differences in ovisac disturbance between caged and uncaged branches between treatments across sites, with higher levels of ovisac disturbance on "no cage" branches that were exposed to predators (Table 2; Fig. 2B & 3B), consistent with the findings of Jubb et al. (2020) who analyzed these data by site. Model predictions for progrediens density were quite similar for both years (Table 2; Fig. 2C & 3C), even though overwintering mortality, sistens density, and ovisac disturbance values were unique to the observed values recorded for each year, and they predicted minimal difference in adult progrediens density between treatments. The average density predicted in 2017 was 1.2 HWA/cm of twig growth, and 1.1 for 2018. When we tested the observed progrediens density, we found no significant difference between treatments across sites in either year (Table 2; Fig. 2D & 3D).

There were minimal differences between the observed and predicted values of the ratios of HWA density on "no cage"/"cage" branches (Table 3). For 2017 and 2018, we found no significant difference between the observed and predicted ratios (2017: t = -0.180, df = 56.5, P = 0.8576; 2018: t = 1.872, df = 127, P = 0.0635). We determined the mean ratios by site for both years and transformed them back to the natural scale using antilog (10^x). Mean values by site and treatment can be seen in Table 3. The average observed and predicted ratio densities of "no cage"/ "cage" across sites in 2017 were 0.88 and 0.92, respectively, and for 2018 they were 1.33 and 0.99, respectively. For our logistic model, we also found a significant negative trend for progrediens survival by density of sistens per 20 cm sample branch (df = 166, pseudoR^2 = 0.49, P < 0.0001), as the density of sistens increased there was a significant drop in the proportion of progrediens surviving to adult (Fig. 4A). Our logistic model for proportion of progrediens surviving by treatment showed that there were significantly more progrediens surviving in the uncaged treatment across sites (Fig. 4B) (df = 166, t = 3.721, P < 0.001).

3.2. Estimates of sistens fecundity and new growth

In 2017, our estimates of fecundity from samples of adult HWA held in petri dishes were compromised due to fungal contamination, except for our New Jersey site (DEWA). In 2018, we were able to record fecundity for each site (Table 4), and the data indicate a sharp decline in HWA fecundity at DEWA from 2017 to 2018. We found that in both

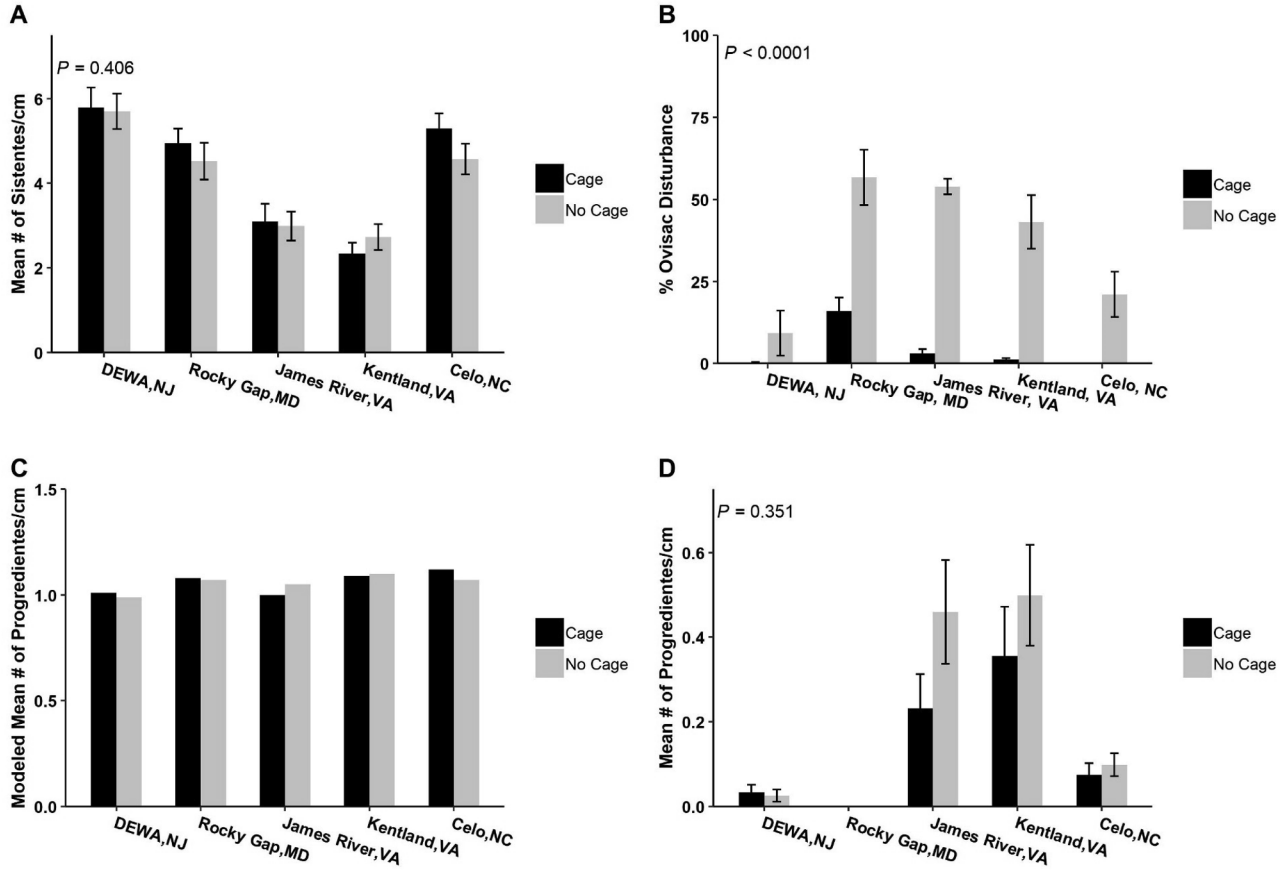

Fig. 3. Mean (± SE) observed and predicted HWA densities in 2018 and ovisac disturbance by treatment "Cage" and "No Cage." A) Density estimated for sistens generation 2017. B) Percent ovisac disturbance by *L. nigrinus*. C) Model predictions of progrediens density based on the mean sistens density, overwintering mortality, ovisac disturbance, and fecundity by treatment and site. D) Observed density of the progrediens generation 2018. "Sistentes" and "progredientes" are plural forms of "sistens" and "progrediens." *P*-values for A, B, & D are treatment (Cage/No Cage) level *P*-values across sites.

Table 3
Mean ratio of HWA densities (No Cage /Cage) for observed and predicted values in 2017 and 2018.

Site	Year	Predicted	Observed
Celo, NC	2017	0.91	1.09
DEWA, NJ	2017	0.91	0.70
James River, VA	2017	1.00	1.02
Kentland, VA	2017	0.86	0.74
Rocky Gap, MD	2017	0.97	1.07
Celo, NC	2018	0.96	1.15
DEWA, NJ	2018	0.98	1.13
James River, VA	2018	1.05	1.68
Kentland, VA	2018	1.01	1.87
Rocky Gap, MD	2018	0.99	ND

*Rocky Gap has the greatest drop in density in the progrediens generation with no HWA recorded from samples. ND = "No Data".

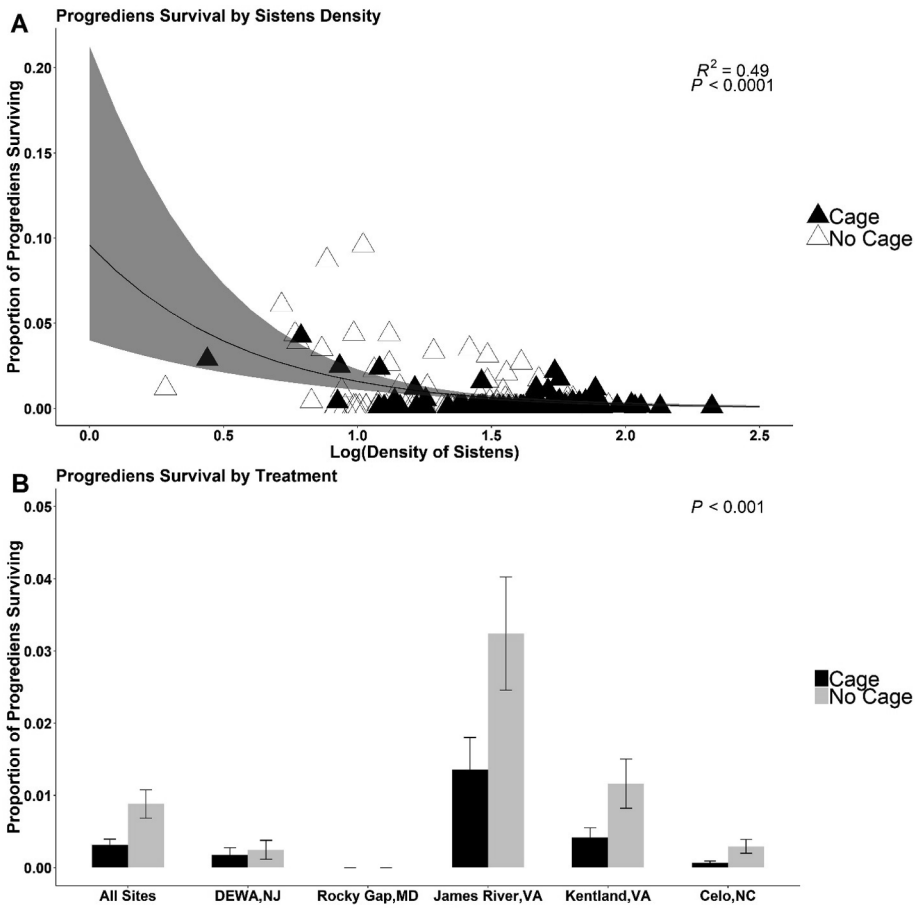

Fig. 4. A) Logistic regression of the proportion of progrediens surviving to adult versus density of sistens surviving to adult by treatment across sites in 2018. Each point represents data from a single branch, the solid line represents the fit model and the grey shading show the 95% confidence interval. B) Mean (± SE) proportion of progrediens surviving to adult by treatment, site and across sites in 2018. *P*-value represents the significant effect of treatment across sites.

Table 4
Mean (± SE) Sistens fecundity estimates for sites in 2017 and 2018.

Site	Year	Eggs per female	# Ovisacs Sampled
DEWA, NJ	2017	101.7 ± 4.45	110
DEWA, NJ	2018	22.2 ± 2.36	96
James River, VA	2018	46.7 ± 2.77	110
Rocky Gap, MD	2018	46.7 ± 2.69	117
Kentland, VA	2018	83 ± 4.35	126
Celo, NC	2018	41.7 ± 2.88	111

Table 5
Mean (± SE) percentage of hemlock twigs with new growth on sample branches across sites. Low new growth production in spring 2017 is the result of high densities from that year and effects the sistens and progrediens adults of 2018. Increasing new growth production in 2018 occur at the end of the study and would affect the sistens and progrediens adults in 2019 after our data were collected.

Site	Year	Treatment	n	New Growth %
Celo, NC	2017	Cage	13	7.21 ± 2.95
Celo, NC	2017	No Cage	14	20.86 ± 5.89
DEWA, NJ	2017	Cage	22	6.11 ± 3.46
DEWA, NJ	2017	No Cage	21	5.13 ± 3.21
James River, VA	2017	Cage	11	7.33 ± 4.60
James River, VA	2017	No Cage	15	8.33 ± 2.69
Kentland, VA	2017	Cage	15	13.80 ± 4.55
Kentland, VA	2017	No Cage	15	9.45 ± 3.58
Rocky Gap, MD	2017	Cage	15	0.00 ± 0
Rocky Gap, MD	2017	No Cage	15	0.00 ± 0
DEWA, NJ	2018	Cage	21	14 ± 6.67
DEWA, NJ	2018	No Cage	22	11 ± 4.02

years there were very low percentages of new growth on sample branches, all below 15% (Table 5).

4. Discussion

The primary objective of this study was to explain why, after over 10 years of *L. nigrinus* releases in eastern North America (Mausel et al., 2010), do HWA outbreaks persist and continue to cause hemlock mortality (Trotter et al., 2013) despite significant rates of predation by *L. nigrinus* (Jubb et al., 2020). The Elkinton et al. (2011) model helps illuminate the reason for minimal effect of predation on overall HWA densities, which is that the strong density-dependent survival affecting the progrediens generation largely compensates for predation by *L. nigrinus* on the overwintering sistens generation. A companion study (Jubb et al., 2020) in the same field sites focused on assessing the impact of *L. nigrinus* on overwintering sistens ovisacs also found that there was significantly higher rates of ovisac disturbance on uncaged treatments, which is consistent with the results of our separate statistical analysis of that same data. Each year in our study mean rates of ovisac disturbance by *L. nigrinus* differed significantly between treatments at all sites, with higher levels of ovisac disturbance on "no cage" branches that were exposed to predators (Jubb et al., 2020). As reported by Jubb et al. (2020) in 2017, the average percent ovisac disturbance across sites was 1–16% for "cage" and 12–80% for "no cage." The 2018 ovisac disturbance estimates were 0–16% for "cage", and 9–57% for "no cage." The reported differences were all highly significant. Ovisac disturbance by site and treatment can be seen in Fig. 2B and 3B. Despite the significant predation occurring on uncaged ovisacs, model predictions of minimal difference in the progrediens generation densities between

treatments (Fig. 2C & 3C) were validated by our observed progrediens densities (Fig. 2D & 3D).

Our analysis of the ratio data also showed that there was only a small difference between the exclusion treatments in the observed data and the model prediction. Elkinton et al. (2011) suggest that even with upwards of 90% predation on the progrediens eggs, there would not be a significant effect on the progrediens densities. In this study, there were two "no cage" treatments at separate sites (Rocky Gap and Kentland) in 2017 that both experienced about 80% sistens ovisac disturbance. At those two sites, the progrediens generation density showed minimal differences between treatments. These results support our hypothesis and model predictions that predation by *L. nigrinus* on sistens ovisacs will have minimal impact on the density of the subsequent progrediens generation in high-density HWA populations. Predation having a minimal impact on progrediens density is largely due to subsequent density-dependent survival factors that compensate for most of the predation on sistens ovisacs caused by *L. nigrinus*, which we documented with our analysis of progrediens survival vs. log sistens density. The data suggest a significant drop in progrediens survival with increasing density of sistens (Fig. 4A). Low rates of progrediens survival in association with high densities of sistens suggest that predation by *L. nigrinus*, even with high rates of predation seen in our study, will not have a noticeable effect on the progrediens generation densities. This is because even a low percentage progrediens surviving after *L. nigrinus* predation will fully saturate the available settling sites at the base of needles when the mother sistens generation density is high.

Under the conditions in this experiment, the model predicted that progrediens density in the uncaged treatment would be approximately 10% lower than the caged treatment. The model predicted lower progrediens density in the uncaged treatment because the high levels of predation on HWA in the uncaged treatment would only be partially compensated for by the density-dependent progrediens survival which results in a rebound in density. However, our predictions for each cage were based on the observed sistens density in the fall (Fig. 2A & 3A), the observed ovisac disturbance rate (Fig. 2B & 3B), and the observed overwintering mortality on each branch, as reported in Jubb et al., (2020). In 2018, in particular, Jubb et al. (2020) reported higher overwintering mortality inside the cages than outside at some sites. As a result of the branch to branch variation in these data, sometimes our model predicted higher progrediens densities in the "no cage" treatment than the "cage" treatment, despite the higher predation rates on the no-cage branches. That pattern explains the variation in predicted densities for the two treatments evident in (Fig. 2C & 3C). We recorded differences consistent with model predictions for density (Fig. 3D) where the "no cage" treatment had higher densities than the "cage," treatment, which is opposite of what would be expected given that *L. nigrinus* caused significant predation on the "no cage" branches. Interestingly, and for reasons that are unclear, there was significantly higher overwintering mortality in the caged branches than in uncaged branches at these sites in 2018 as reported by Jubb et al. (2020), which may be causing this difference in the model, as well as in the observed data. When establishing the experiment, branch pairs were chosen so that weather conditions experienced by HWA populations would be the same on members of the branch pair, so it is hard to know exactly what might have happened to the caged branches to result in higher winter mortality. The companion paper by Jubb et al. (2020), which collected the overwintering mortality data used to parameterize the Elkinton et al. (2011) model, speculate that microclimatic variations between branch locations or possibly the type of material used in the cages may be responsible for observed differences in winter mortality in caged branches. In another cage experiment conducted with HWA in the Pacific Northwest we found no significant difference in air temperature between cages (Crandall, R.S., Elkinton, J.S., and Lombardo, J.A., unpublished data). Additionally, Nelson and Rieske (2014) also found no difference between air temperature and humidity inside cages compared to ambient measurements for uncaged branches.

Interestingly, progrediens density in 2018 was lower than predicted by our model across both predator exclusion treatments. One possible explanation for this unexpected drop in density could be deteriorating tree health and/or unfavorable weather. Tree health is an important factor in HWA performance in that HWA prefer to settle and have better performance on healthy trees that are producing new growth (McClure, 1991; Sussky and Elkinton, 2015) and weather factors such as low or high temperatures in the winter and summer respectively would also impact HWA mortality (as reported in Sussky and Elkinton 2015; Mech, 2015). Future studies could examine the relative importance of tree health and changing weather patterns to provide a more thorough understanding of factors that influence HWA densities. Including tree health and climactic factors was beyond the scope of the Elkinton et al. (2011) model and were never its purpose.

The low proportion of new growth produced at the Delaware Water Gap, NJ (DEWA) in 2018 (Table 5) suggests that we may have had a decline in survival of HWA due to deterioration of host health (McClure, 1991). In both years, the trees produced new growth on less than 15% of terminal branchlets, which is similar to the tree growth reported by both McClure (1991) and Paradis (2011) on hemlock branches following outbreak phase of HWA densities. Similar reductions in new growth production (the number of terminal buds producing new growth on sample branches) were observed by (Sussky and Elkinton, 2014) on branches following inoculation with high densities of HWA the previous year. In our study, we suspect that the low production of new growth at the end of the 2017 sampling period was the result of host decline. The data also suggest that with the 2018 reduction in HWA fecundity and progrediens density, the trees were beginning to rebound as shown by the doubling in the percentage of tips with new growth in 2018 compared with 2017 at the DEWA site (Table 5). This new growth occurred at the end of our experiment and would have been available to the following sistens generation, not the final progrediens generation for which we measured.

In 2018 lower than average sistens fecundity was observed and, as a result, progrediens density also declined. In 2018, sistens mean fecundity across all sites was 48.1 which is much lower than reports of about 100–150 eggs per female from sistens settling on new growth of healthy hemlock (McClure, 1991; Paradis, 2011). Since only a low proportion of new growth shoots were produced by our sample trees, we would therefore expect that fecundity would also be lower due to deteriorated host health. However, because reduced fecundity was observed across all sites, we suspect that weather may also have played a role. Population densities of many forest insects fluctuate in synchrony across large regions due to shared weather impacts (the Moran effect), even though these weather events do not directly cause the fluctuations (Liebhold et al., 2004). McClure (1991) showed that population fluctuations of HWA are characterized by a two-year boom and bust cycle governed by the interaction of HWA with its hemlock host. Other recent research has documented the impact of recent cold winter events that have decimated HWA populations over the entire eastern U.S. in certain years (Cheah, 2017; Elkinton et al., 2017; McAvoy et al., 2017; Tobin et al., 2017). These events may cause the boom and bust cycle to synchronize over the region, though it is not clear if this or other weather events are the cause for the observed low densities in 2018.

The branches we selected for this experiment were heavily infested with HWA and thus near carrying capacity (two or more HWA per needle base) and the simulations we present were run at those HWA densities in the model. Previous studies by Lamb et al. (2005) suggest that *L. nigrinus* can have significant impacts on sistens and resulting progrediens generation. However, a major difference here was that the Lamb et al. (2005) experiment was a predator inclusion experiment and ours was a predator exclusion experiment which allows for a more "natural" interaction between predator and prey rather than restricting the tested *L. nigrinus* access to a single HWA infested branch. Data from a predator exclusion experiment, similar to the one reported here, in the

native range of HWA in the Pacific Northwest of the U.S. suggest that summer-active predators may be playing an important role in reducing the numbers of progrediens (Crandall, R.S., Elkinton, J.S., and Lombardo, J.A., unpublished). In that study significant differences in HWA density between caged and uncaged branches were found. This suggests that if summer-active predators, such as two adelgid-specific predator species of *Leucopis* (Diptera: Chamaemyiidae), *Leucopis argenticollis* Zetterstedt and *Leucopis piniperda* Malloch, which are abundant in the Pacific Northwest of North America (Motley et al., 2017; Kohler et al., 2016), can help reduce HWA densities well below host carrying capacity, eliminating the rebound effect documented here and effectively complimenting predation by *L. nigrinus*. Crandall, R.S., Elkinton, J.S., and Lombardo, J.A. (unpublished) data suggest that introduction of summer-active predators into eastern North America, along with *L. nigrinus*, could help make the overall biological control effort for HWA in eastern North America more effective, but overwintering establishment of these *Leucopis* species remains to be documented.

Ecological interactions are complex and may not be intuitively obvious. Using field-collected data to parameterize mathematical models allows us to explore the ecology of an organism and suggest experiments that can help us better understand these complexities. Here we used the predictions of a simulation model to design a predator exclusion experiment to test whether an introduced biological control agent can lower densities of HWA. Our results suggest that in spite of significant predation by *L. nigrinus* on HWA sistens generation ovisacs, there is no major impact on the subsequent progrediens generation densities. This is due to the strong density-dependent survival affecting the progrediens generation (Fig. 4), which largely compensates for any effect of predation by *L. nigrinus* on sistens ovisacs.

5. Conclusions

This study helps us understand the importance and potential of having predators that impact both generations of HWA. Our results help explain why high densities of HWA persist on hemlocks in many sites throughout eastern North America despite region-wide establishment of *L. nigrinus* (Mausel et al., 2010), which causes significant rates of predation on HWA ovisacs (Fig. 2B and 3B; Jubb et al., 2020). Our results support model predictions of HWA densities parameterized with field collected data from sistens generation densities, overwintering mortality, fecundity, and predation estimates. The model predicts that even with high levels of predation by *L. nigrinus* on the sistens ovisacs, there is little difference in density of adult progrediens between branches with and without ovisac predation. Our field-collected progrediens densities support the model predictions of little difference in density between treatments. The results support the hypothesis that even with high rates of *L. nigrinus* predation on the sistens generation, a low percentage of progrediens survival is sufficient to saturate the available number of settling sites on the host, counteracting a *L. nigrinus* predation effect. Nevertheless, we suggest that *L. nigrinus* could still play a significant role in a multispecies biological control complex where it works in tandem with other biological control agents that feed on the spring generation and sistens eggs, if such complimentary agents can become successfully established.

Author contribution

Study design by A.M, J.E, S.S. and T.M. Field data was collected by C.J., A.M., T.M., A.T., B.T., and R.C. Data analysis by R.C and JE. First draft was written by R.C. and following drafts were edited by J.E., C.J., A.M., S.M., and B.T.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2020.104264.

References

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67 (1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Cheah, C.A.S.-J., 2017. Predicting hemlock woolly adelgid winter mortality in Connecticut forests by climate division. Northeast. Nat. 24, B90–B118.

Elkinton, J.S., Lombardo, J.A., Roehrig, A.D., McAvoy, T.J., Mayfield, A., Whitmore, M., 2017. Induction of cold-hardiness in an invasive herbivore: the case of hemlock woolly adelgid (Hemiptera: Adelgidae). Environ. Entomol. 46, 118–124.

Elkinton, J.S., Trotter, R.T., Paradis, A.F., 2011. Simulations of population dynamics of hemlock woolly adelgid and potential impact of biological control agents. USDA Forest Service FHTET-2011-04, Morgantown, WV. 15-24pp.

Ellison, A., Orwig, D., Fitzpatrick, M., Preisser, E., Ellison, A.M., Orwig, D.A., Fitzpatrick, M.C., Preisser, E.L., 2018. The past, present, and future of the hemlock woolly adelgid *(Adelges tsugae*) and its ecological interactions with eastern hemlock *(Tsuga canadensis*) forests. Insects 9, 172. https://doi.org/10.3390/insects9040172.

Foley, J.R., Mcavoy, T.J., Dorman, S., Bekelja, K., Kring, T.J., Salom, S.M., 2019. Establishment and distribution of *Laricobius* spp. (Coleoptera: Derodontidae), a predator of hemlock woolly adelgid, within the urban environment in two localities in southwest Virginia. J. Integr. Pest Manag. 10, 30–31. https://doi.org/10.1093/jipm/pmz027.

Havill, N.P., Montgomery, M.E., Yu, G., Shiyake, S., Caccone, A., 2006. Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. Mol. Ecol. 99, 195–203.

Havill, N.P., Vieira, L.C., Salom, S.M., 2014. Biology and control of hemlock woolly adelgid. USDA Forest Service FHTET-2014-05, Morgantown, WV.

Jubb, C.S., Heminger, A.R., Mayfield III, A.E., Elkinton, J.S., Wiggins, G.J., Grant, J.F., Lombardo, J.A., McAvoy, T.J., Crandall, R.S., Salom, S.M., 2020. Impact of the introduced predator, *Laricobius nigrinus*, on ovisacs of the overwintering generation of hemlock woolly adelgid in the eastern United States. Biol. Control. 143. https://doi.org/10.1016/j.biocontrol.2019.104180.

Kohler, G.R., Wallin, K.F., Ross, D.W., 2016. Seasonal phenology and abundance of Leucopis argenticollis, Leucopis piniperda (Diptera: Chamaemyiidae), Laricobius nigrinus (Coleoptera: Deridontidae) and Adelges tsugae (Hemiptera: adelgidae) in the Pacific Northwest USA. Bull. Entomol. Res. 106, 546–550.

Lamb, A.B., Salom, S.M., Kok, L.T., 2005. Survival and reproduction of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), a predator of hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae) in field cages. Biol. Control 32, 200–207.

Liebhold, A., Koenig, W.D., Bjørnstad, O.N., 2004. Spatial synchrony in population dynamics. Annu. Rev. Ecol. Evol. Syst. 35, 467–490. https://doi.org/10.1146/annurev.ecolsys.34.011802.132516.

Mausel, D.L., S.M. Salom, L.T. Kok, and G.A.D., 2010. Establishment of the hemlock woolly adelgid predator, Laricobius nigrinus (Coleoptera: Derodontidae), in the eastern United States. Environ. Entomol. 39, 440-448. https://doi.org/10.1603/EN09088.

Mausel, D.L., Salom, S.M., Kok, L.T., Fidgen, J.C., 2008. Propagation, synchrony and impact of introduced and native *Laricobius* spp. (Coleoptera: Derodontidae) on hemlock woolly adelgid in Virginia. Environ. Entomol. 37, 1498–1507.

Mayfield III, A.E., Reynolds, B.C., Coots, C.I., Havill, N.P., Brownie, C., Tait, R., Hanula, J.L., Joseph, S.V., Galloway, A.B., 2015. Establishment, hybridization and impact of *Laricobius* predators on insecticide-treated hemlocks: Exploring integrated management of the hemlock woolly adelgid. For. Ecol. Manage. 335, 1–10.

McAvoy, T., Régnière, J., St-Amant, R., Schneeberger, N.F., Salom, S.M., 2017. Mortality and recovery of hemlock woolly adelgid (*Adelges tsugae*) in response to winter temperatures and predictions for the future. Forests 8, 497. https://doi.org/10.3390/f8120497.

McClure, M.S., 1987. Biology and control of hemlock wooly adelgid, Bulletin 851, pp. 1-9

The Connecticut Agricultural Experiment Station, New Haven, CT.
McClure, M.S., 1989. Evidence of a polymorphic life cycle in the hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). Ann. Entomol. Soc. Am. 82, 50–54. https://doi.org/10.1093/aesa/82.1.50.
McClure, M.S., 1991. Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on *Tsuga canadensis*. Environ. Entomol. 20, 258–264. https://doi.org/10.1093/ee/20.1.258.
Mech, A.M. Abiotic and biotic factors influencing eastern hemlock (Tsuga canadensis) health and hemlock woolly adelgid (Adelges tsugae) success in the Southern Appalachian Mountains. PhD Dissertation University of Georgia, Athens, GA, USA (2015).
Motley, K., Havill, N.P., Arsenault-Benoit, A.L., Mayfield, A.E., Ott, D.S., Ross, D.W., Whitmore, M.C., Wallin, K.F., 2017. Feeding by *Leucopis argenticollis* and *Leucopis piniperda* (Diptera: Chamaemyiidae) from the western USA on *Adelges tsugae* (Hemiptera: Adelgidae) in the eastern USA. Bull. Entomol. Res. 107 (5), 699–704.
Nelson, L.A., Rieske, L.K., 2014. Microclimatic variation within sleeve cages used in ecological studies. J. Insect Sci. 14, 1–5. https://doi.org/10.1093/jisesa/ieu029.
Onken, B., Reardon, R., 2011. Implementation and Status of Biological Control of the Hemlock Woolly Adelgid. USDA Forest Service FHTET-2011-04, Morgantown, WV.
Paradis, A.F., 2011. Population dynamics of the hemlock woolly adelgid (Hemiptera: Adelgidae). University of Massachusetts Amherst.
Preisser, E.L., Oten, K.L.F., Hain, F.P., 2014. Hemlock woolly adelgid in the eastern united states: what have we learned? Southeast. Nat. 13. https://doi.org/10.1656/058.013.s604.
R Core Team (2019). R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. https://www.R-project.org/.
Sussky, E.M., Elkinton, J.S., 2014. Density-dependent survival and fecundity of hemlock woolly adelgid (Hemiptera: Adelgidae). Environ. Entomol. 43, 1157–1167. https://doi.org/10.1603/EN13356.
Sussky, E.M., Elkinton, J.S., 2015. Survival and near extinction of hemlock woolly adelgid (Hemiptera: Adelgidae) during summer aestivation in a hemlock plantation. Environ. Entomol. 44, 153–159.
Tobin, P.C., Turcotte, R.M., Blackburn, L.M., Juracko, J.A., Simpson, B.T., 2017. The big chill: quantifying the effect of the 2014 North American cold wave on hemlock woolly adelgid populations in the central Appalachian Mountains. Soc. Popul. Ecol. 59, 251–258. https://doi.org/10.1007/s10144-017-0589-y.
Tobin, P.C., Turcotte, R.M., Snider, D.A., 2013. When one is not necessarily a lonely number: Initial colonization dynamics of *Adelges tsugae* on eastern hemlock, *Tsuga canadensis*. Biol. Invasions 15, 1925–1932. https://doi.org/10.1007/s10530-013-0421-3.
Trotter III, R.T., Morin, R.S., Oswalt, S.N., Liebhold, A.M., 2013. Changes in the regional abundance of hemlock associated with the invasion of Hemlock Woolly Adelgid (*Adelges tsugae* Annand). Biol. Invasions 15, 1–13.
Wickham, H., 2009. ggplot2: elegant graphics for data analysis. Springer, New York.
Young, R.F., Shields, K.S., Berlyn, G.P., 1995. Hemlock woolly adelgid (Homoptera: Adelgidae): stylet bundle insertion and feeding sites. Ann. Entomological Soc. Am. 88, 827–835.
Zar, J.H., 2010. Biostatistical Analysis. Prentice-Hall/Pearson, Upper Saddle River, N.J.
Zilahi-Balogh, G.M.G., Salom, S.M., Kok, L.T., 2003. Development and reproductive biology of *Laricobius nigrinus*, a potential biological control agent of *Adelges tsugae*. Biocontrol 48, 293–306.