

AN ABSTRACT OF THE THESIS OF

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Title: Top-down and Bottom-up Controls of *Adelges tsugae* in the Pacific Northwest Inform Its Biological Control in Eastern North America.

Abstract approved:

Darrell W. Ross

The hemlock woolly adelgid (Hemiptera: Adelgidae: *Adelges tsugae*; HWA) is an invasive and damaging pest on hemlock in eastern North America. HWA is native to the Pacific Northwest, where it does not damage hemlock. In support of classical biological control of HWA in eastern North America, abundances of native predators on HWA in the Pacific Northwest were assessed in an observational study. HWA and its predators were counted and identified on branch samples from western hemlock (Pinaceae: *Tsuga heterophylla*) in the Puget Sound area. Species densities were calculated on a per-centimeter basis. Two species of cryptic congeners, silver flies (Diptera: Chamaemyiidae: *Leucopis argenticollis* and *Le. piniperda*), were innovatively identified using DNA sequencing. More individuals of the two *Leucopis* spp. (120 in total) combined were found than the next most abundant predator (52 in total), a beetle (Coleoptera: Derodontidae: *Laricobius nigrinus*). *Laricobius nigrinus* has already been introduced by the hundreds of thousands and established on hemlock as part of HWA biological control efforts in eastern North America. However, *La. nigrinus* has not succeeded in perceptibly reducing hemlock decline or mortality there. *Leucopis argenticollis* was more abundant than *Le. piniperda*, with 55 flies identified as *Le. argenticollis* and 25 identified as *Le. piniperda*. Since ~67% of the flies were successfully identified to species, it was inferred using relative proportions that the most abundant predator collected was *Le. argenticollis*, with ~83 individuals,

followed by *La. nigrinus*, and then *Le. piniperda*, with ~38 individuals. The occurrence of the *Leucopis* spp. on both seasonal generations of HWA, while *La. nigrinus* was found only on the early generation, confirmed a natural history difference between the flies and the beetle; *Leucopis* occurs and feeds on both generations of HWA, while *La. nigrinus* pupates underground during the late HWA generation. This implies that *Leucopis* could complement *La. nigrinus* in control of HWA. Because beetle pupae were not collected (whereas fly puparia were) due to this natural history difference, puparia were excluded for additional comparison. *Leucopis* spp. larvae and adults totaled 69 compared to 52 *La. nigrinus*. Since site was found to be non-significant as a factor in explaining mean cumulative species densities in three one-way ANOVAs (HWA: F-value = 1.88; d.f. = 3, 21, p -values = 0.17; *Leucopis*: F-value = 1.95; d.f. = 3, 21, p -values = 0.15; *La. nigrinus*: F-value = 0.4; d.f. = 3, 21, p -values = 0.75), densities were related across sites in further analysis. Pearson's product-moment correlations of HWA and *La. nigrinus* densities were non-significant, while HWA and *Leucopis* spp. densities were positively correlated (e.g., for larval *Leucopis* densities, t -value = 2.64; d.f. = 23; p -value = < 0.01; 95% CI = 0.17 to 1; estimated correlation = 0.48). Combined larval and adult *Leucopis* spp. and *La. nigrinus* densities were moderately negatively correlated (t -value = -1.81; d.f. = 23; p -value = 0.04; 95% CI = -1 to 0.02; estimated correlation = -0.35). *Leucopis argenticollis* and *Le. piniperda* densities were negatively, though non-significantly, correlated (Spearman's rank correlation coefficient: ρ = -0.28; d.f. = 23; p -value = 0.09). In a companion project, HWA abundance levels were assessed on western hemlock clones of known genetics at a commercial seed orchard, allowing for consideration of spatial versus host resistance explanations for the distribution of HWA there. The results were not strongly supportive of potential resistance to HWA by western hemlock. Hence, evidence for bottom-up control of HWA in the Pacific Northwest was weak from this limited dataset, while the data on predators contributes to knowledge of top-down control of HWA in the Pacific Northwest. These results recommend further study of the *Leucopis* spp. for potential introduction as biocontrols of HWA in eastern North America.

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Top-down and Bottom-up Controls of *Adelges tsugae* in the Pacific Northwest Inform
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APPROVED:

Major Professor, representing Forest Ecosystems and Society

Head of the Department of Forest Ecosystems and Society

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Alexander Rose, Author

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Chapter 1: General Introduction and Literature Review

Background: Invasive Species Hypotheses and Control

Since Hairston, Smith, and Slobodkin (1960) seminally postulated that the apparent abundance of plants vulnerable to herbivory is due to top-down control by natural enemies of herbivores, the relative importance of top-down versus bottom-up controls of primary consumers has been an important debate in ecological theory. Under the enemy release hypothesis, biological invasions, which cause major biodiversity loss (Davis 2003) and economic damage (Pimental *et al.* 2005), succeed because invasive species are released from control by their natural enemies, such as predators, in their non-native ranges (Heger and Jeschke 2014). Successful biological control, which uses natural enemies against invasive species or other pests with fewer environmental, health, and financial costs than alternative control methods (Menzler-Hokkanen 2007), can require a complex of natural enemies (Van Driesche *et al.* 2010). However, invasive species' success may be due to bottom-up effects as well. Certain habitats and communities can be considered more "invasible," meaning more readily invaded, than others (Radosovich *et al.* 2007). Under growing global change, including climate change and biotic invasions, novel ecosystems with unprecedented compositional and functional states may be increasingly likely, which calls into question traditional conservation efforts to restore ecosystems to historical baselines (Seastedt 2008). Classical biological control, the introduction and establishment of natural enemies of problematic or invasive species into habitats invaded by the latter, may be more justified now given novel ecosystems than previously given assumptions of stable ecosystem states. Human-mediated global changes, including the creation of novel species assemblages in part via species introductions, threaten the balance and functionality of ecosystems. Classical biological control could help remedy this situation in some cases by dampening the explosive populations of invasive species in their non-native ranges through top-down control by co-evolved natural enemies.

If non-native, invasive species are successful due to release from natural enemies, then classical biological control (biocontrol) could help restore balance to invaded ecosystems by

controlling invasive species. However, there can be unintended consequences of species introduced as biocontrol agents. There are potential direct and indirect non-target effects of biocontrol. Direct non-target effects include consumption of native species by introduced natural enemies. Another direct effect is hybridization of introduced species with native species. Consumption of an abundant invasive species by a predator or other natural enemy can lead to a positive numerical response by the natural enemy population, which can mediate indirect negative effects on native species. For instance, if populations of natural enemies introduced as biocontrol agents increase in response to abundant invasive prey species, then there can be increased predation on native prey species. There can also be competitive interactions between introduced natural enemies and native intraguild species (Noonburg and Byers 2005), interactions which can be both direct and indirect.

Hypothetically, intraguild species may compete interspecifically for resources or become partitioned into resource niches. Indirect competition includes exploitative or scramble competition, mediated by a shared resource that one species consumes more efficiently or effectively than competitor species. Predator-prey models suggest that only one of multiple enemies will persist once lowest prey equilibrium densities are reached (Mills 2006). Direct competition includes interference or contest competition, in which competitors limit each other's access to a resource, such as by fighting or killing one another (Mills 2006). Apparent competition can occur when competitors have a natural enemy in common (Mills 2006). Intraguild predators can coexist by partitioning the shared niche in space and time (Amarasekare 2008). However, does competition necessarily lead to partitioning? Investigating a complex of predators for biocontrol involves consideration of this question. Darwin argued that interspecific resource competition can limit populations and that specialization reduces this effect (Darwin 1859). Successful biological control can require a complex of natural enemies (Van Driesche *et al.* 2010), but program effectiveness could be reduced if those enemies compete with each other.

The Hemlock Woolly Adelgid, an Invasive Forest Insect Pest

The hemlock woolly adelgid (Hemiptera: Adelgidae: *Adelges tsugae*; HWA) was first collected in the eastern United States in 1951 in Richmond, Virginia and was probably introduced from southern Japan (Havill *et al.* 2006; Stoetzel 2002). HWA substantially alters ecosystem function and structure in eastern North America by killing eastern hemlock (Pinaceae: *Tsuga canadensis*), a foundational species (Martin and Goebel 2013). Feeding at the bases of needles, individuals insert their stylets into xylem ray parenchyma cells to access stored nutrients, depleting nutrients, and causing water stress and defoliation (Havill *et al.* 2014). HWA feeding may cause a hypersensitive response that restricts water transport (Havill *et al.* 2014). The insect kills trees of all ages in as little four years, but can take up to 15 years. Mortality may reach 95% in some areas (Havill *et al.* 2014). Because low winter temperatures currently limit northward movement, climate change will likely facilitate the northward expansion of HWA (Paradis *et al.* 2007). In the Pacific Northwest where it is native (Havill *et al.* 2016a; Havill *et al.* 2006), HWA feeds and develops on western (*Tsuga heterophylla*) and mountain hemlocks (*Tsuga mertensiana*) with little defoliation possibly because of host resistance and/or native predators (Kohler 2007).

The Hemlock Woolly Adelgid Life Cycle and Lineages

HWA may not cause significant tree mortality in its native ranges because of adaptations by host trees and natural enemies that could have arisen from HWA's being present in the Pacific Northwest for a relatively long period of time. Haplotype mtDNA evidence indicates that HWA populations in the Pacific Northwest and eastern North America are both more closely related to Japanese populations than they are to each other, and that HWA was not recently introduced to the Pacific Northwest (Havill *et al.* 2016a; Havill *et al.* 2006). This genetic evidence shows that HWA is native to the Pacific Northwest.

The life cycle of HWA is complex (Fig. 1). Ancestrally and in Asia, winged adults (the sexuparae) from the sistens generation fly from the alternate host (hemlock) to the primary host

(spruce) where they bear the sexual generation (the sexuales). The sexual generation produces parthenogenic females (the fundatrices) that feed on spruce and produce a generation that lives in galls (the gallicolae). The gallicolae mature as winged adults and fly back to hemlock to produce the progrediens generation. The progrediens generation on hemlock produces the sistens generation, which aestivates for a few months before maturing. The sistens generation apparently never produces winged sexuparae in the Pacific Northwest, where populations are derived from ancestrally parthenogenic lineages in Japan (Havill *et al.* 2016a). Some of the sistens generation in eastern North America do become winged sexuparae and produce sexuales on spruce, but these die and fail to reproduce, apparently because of inappropriate spruce species as primary hosts (Havill *et al.* 2016a; McClure 1989). After hatching from eggs, HWA passes through four nymphal instars before adulthood. The first instar is the mobile “crawler” stage. Once crawlers settle and insert their stylets to begin to feed, they are sessile and become more convex and sclerotized in the second instar, increasing in width throughout the later instars to the point where their legs do not extend beyond the lateral margins of the thorax (McClure 1989).

Figure 1.

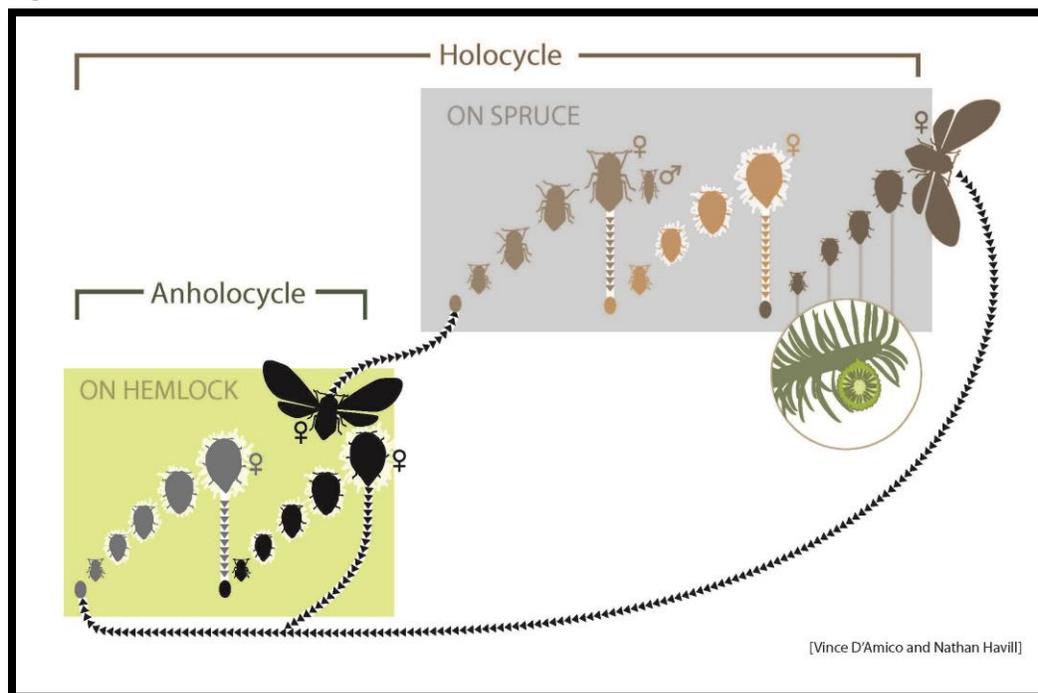


Figure 1. Life cycle of the hemlock woolly adelgid (Havill *et al.* 2016a).

Ecosystem and Economic Effects

HWA kills native eastern hemlock and Carolina hemlock (*Tsuga caroliniana*, a rare Appalachian endemic) in its invaded range of eastern North America, causing economic loss (Li *et al.*, 2014; Holmes *et al.*, 2010) and altering the ecosystem (Adkins and Rieske 2013; Martin and Goebel 2013; Lemos and Finzi 2011; Tingley *et al.* 2002; Brooks 2001). Eastern hemlock was recently listed as near-threatened largely due to HWA-caused decline (Farjon 2013a). Carolina hemlock is considered near-threatened for the same reason (Farjon 2013b; McClure 2001).

Since eastern hemlock is considered a foundational species, HWA substantially alters ecosystem function and structure in eastern North America (Martin and Goebel 2013). As a foundational species, eastern hemlock is abundant and dominant in the overstory, particularly in riparian areas, defining ecosystem structure and functions such as species diversity, light availability, and productivity through strong ecosystem interactions (Martin and Goebel 2013). Evans *et al.* (2011) found HWA to be present at 25 of 49 randomly and systematically selected hemlock-dominated, riparian stands spread across eastern hemlock's range in eastern North America, from Alabama to Maine. All of the stands with HWA present had some degree of hemlock decline (Evans *et al.* 2011). HWA-induced mortality of eastern hemlock and preemptive logging of hemlock in response to the spread of HWA result in replacement of hemlock by hardwoods such as birch and maple (Farnsworth *et al.* 2012), which alters stream shading in winter when those deciduous trees provide less shade than evergreen hemlock. This shift in forest composition can also alter soil and water chemistry (Martin and Goebel 2013; Stadler *et al.* 2005). It is thought that hardwoods will likely succeed declining hemlock in the overstory of most erstwhile hemlock-dominated stands because of a lack of other conifers and an abundance of hardwoods in those stands (Evans *et al.* 2011), resulting in less stream shading in riparian areas in winter and less acidic input from needles to soils. As hardwoods succeed hemlock as the dominant tree species in many Appalachian riparian areas due to the spread of HWA, it has been inferred that arthropod communities will change in abundance, composition, and diversity (Adkins and Rieske 2013). Adkins and Rieske (2013) inferred arthropod community changes by comparing samples from riparian areas dominated by hardwoods and hemlock. For another

example of ecosystem effects, hemlock mortality from HWA is associated with changes in avian species composition (Tingley *et al.* 2002). A three-year study of the effects of HWA-induced hemlock decline and mortality simulated via logging found only a brief decline of eastern redback salamanders before a rebound (Brooks 2001). Experimental simulation of HWA-induced mortality of eastern hemlock (by girdling) and removal of hemlock by logging in the Harvard Forest Hemlock Removal Experiment demonstrated that herbaceous biodiversity increased because of the paucity of the herbaceous layer in hemlock stands (Ellison *et al.* 2015). Despite the local increase in biodiversity, HWA-induced mortality of foundational hemlock will diminish between-site and regional biodiversity (Ellison *et al.* 2015). The relatively species poor, herbaceous undergrowth of hemlock stands may be more typical of second growth hemlock forests than the few remaining old-growth hemlock stands (Ellison *et al.* 2015), which are also threatened by HWA.

The destruction of hemlock forests by HWA is a continuing economic problem. Along with the spruce beetle, the gypsy moth, and southern, mountain, and other pine beetles, the hemlock woolly adelgid is listed as one of the top few most damaging insect pests in the United States in terms of spread and economic effects by Dale *et al.* (2001) in their review of the interactions of forest disturbances and climate changes. In addition to altering ecosystems, hemlock decline due to HWA causes economic damages, including loss of nursery stock and timber, and decreased recreation opportunities (Holmes *et al.* 2010), as well as decreased property values, such as a \$24.6 million estimated loss in property values in Connecticut and Massachusetts (Li *et al.* 2014).

Climate Change as a Factor in the Invasion

Given the dispersal and reproductive abilities of HWA, the range of susceptible hemlock, and climate change, HWA will likely spread farther and affect more hemlock forests in the future. Although first instar crawlers are the typical dispersing stage of HWA, experimental inoculation demonstrates the reproductive potential of HWA. Parthenogenic HWA can successfully establish on a new host tree after the introduction of only one progrediens ovisac; in

an experiment using different introduction densities of HWA, about 39% of host trees initially inoculated with one ovisac held an established progrediens generation, which then initiated the second (sistens) generation (Tobin *et al.* 2013). Wind has been shown to carry adelgids up to about 30 miles (48 km) when convection currents lifted them to high altitudes, but dispersal by wind is usually not this far (McClure 1990; Lowe 1966). The eggs and crawlers (the only mobile life stage besides the sexuparae) of HWA are dispersed by birds, including potentially long distances by migratory birds (Russo *et al.* 2016; McClure 1990). Already occupying more than half of eastern hemlock's natural range (primarily in the Southeast, the Atlantic states, and New England), HWA has the potential to spread farther throughout the range of eastern hemlock, which extends from Alabama and northern Georgia in the south to the Canadian Maritime Provinces and the Great Lakes region in the north (Havill *et al.* 2014). Although low winter temperatures in colder climates may have slowed HWA's expansion northward (Havill *et al.* 2014; Paradis *et al.* 2007), climate change could already be facilitating the spread of the insect northward in latitude and upward in elevation (Paradis *et al.* 2007). Paradis *et al.* (2007) expanded upon experiments showing that HWA suffers nearly complete mortality at -20 to -30 degrees Celsius and below.

Bottom-up Control of the Hemlock Woolly Adelgid

There is the possibility of bottom-up control of HWA via host tree resistance. Chinese hemlock (*Tsuga chinensis*) and northern Japanese hemlock (*Tsuga diversifolia*) are considered most resistant to HWA, while western hemlock is considered moderately resistant (Vose *et al.* 2013). Eastern hemlock and Carolina hemlock are considered highly susceptible (Vose *et al.* 2013). Individual eastern and Carolina hemlocks vary in their apparent resistance to HWA (Vose *et al.* 2013). Hybridization between Asian and North American hemlock species has been investigated as a potential way to introduce resistance genes (Bentz *et al.* 2002). However, eastern hemlock was found to be incompatible in crosses with other hemlock species (Bentz *et al.* 2002).

Chemical Control of the Hemlock Woolly Adelgid

While insecticides are considered impractical for control of HWA at the scale of forests (Onken and Reardon 2011), there is some promise for insecticides at the scale of high-valued individual trees and stands. However, there are environmental pollution concerns with insecticides. Single applications of the systemic neonicotinoids clothianidin and imidacloprid are effective at reducing HWA abundance on individual trees for years (Joseph *et al.* 2011a). The persistence of these insecticides is worrisome, though. For example, after two years (the end of the experiment), imidacloprid levels in trees were only slightly lower than levels at the time of application (Dilling *et al.* 2010). Concentrations of imidacloprid following one application were still detectable in trees after eight years (Cowles and Lagalante 2009). Such persistence could result in unanticipated non-target effects and pollution. Imidacloprid, in combination with *Laricobius nigrinus* (an introduced biocontrol agent, see below), its native congener *Laricobius rubidus*, and hybrids of the two, reduced HWA abundance compared to controls in a stand of trees, suggesting the potential for integrated management of HWA (Mayfield *et al.* 2015).

Top-down Control of the Hemlock Woolly Adelgid

HWA does not kill western hemlock and mountain hemlock (*Tsuga mertensiana*) in the Pacific Northwest, which may be attributable to bottom-up control, top-down control by native predators, or both. After unsuccessful attempts to use various predators from China and Japan, the Pacific Northwest native predator *Laricobius nigrinus* (Coleoptera: Derodontidae) became the focus of HWA biocontrol (Onken and Reardon 2011). *Laricobius nigrinus* was first introduced to the invaded range in 2003. Several hundred thousand *La. nigrinus* eggs and adults have been released and become established throughout the invaded range of HWA (Havill *et al.* 2014; Mausel *et al.* 2010). However, there has been no perceptible slowing of hemlock decline or mortality at sites with *La. nigrinus* compared to sites without *La. nigrinus* (Mausel *et al.* 2011; Onken and Reardon 2011). Introduced *La. nigrinus* has hybridized with a congener native to

eastern North America, *La. rubidus*, and introgression has been demonstrated (Havill *et al.* 2012). There are no known parasitoids of adelgids (Cheah *et al.* 2004).

The continuing decline of hemlock in the invaded region justifies surveying, identifying, and testing additional potential predators of HWA.

Kohler (2007) found three native species to be the most abundant predators on HWA-infested western hemlock in the Pacific Northwest; *La. nigrinus* was most abundant, while two species of *Leucopis* flies, *Le. argenticollis* and *Le. piniperda* (Diptera: Chamaemyiidae, silver flies), were the next most abundant. Both chamaemyiid and derodontid larvae and adults were modeled as having a strong positive correlation with HWA population density (Kohler 2007). However, Kohler's sampling technique was apparently less effective in collecting *Leucopis* adults than *Laricobius* adults and underestimated the flies' relative abundance (Ross *et al.* 2011). Kohler (2007) found chamaemyiid larvae to be the most abundant holometabolous predators in the early summer, which he speculated implies that they are the most important predators on HWA sistens eggs and nymphs. Indeed, a different sampling technique involving clipping branches for inspection in the laboratory found more than twice as many individuals of all life stages of the *Leucopis* species than of *La. nigrinus* and the *Leucopis* spp. were present for a much longer period of time (Kohler *et al.* 2016).

The *Leucopis* spp. are promising as potential additional biocontrols of HWA. As evidence of the general potential for the silver fly family for biocontrol, chamaemyiids have been successfully used against several invasive adelgid species in Chile, Hawaii, and New Zealand (citations in Ross *et al.* 2011). For instance, in Hawaii, the densities of the introduced adelgid *Pineus pini* were strongly correlated with the chamaemyiid *Neoleucopis tapiae* over a two-year period only three to five years after introduction of the predator (Greathead 1995; Culliney *et al.* 1988). The *Leucopis* spp. found with HWA in the Pacific Northwest have been shown to feed and survive to their adult stage on other adelgid species in the laboratory, surviving best on HWA (Grubin *et al.* 2011). Their ability to survive on non-target adelgids might allow them to survive periods when HWA populations are at low levels provided they have the searching ability to locate them in the wild (Grubin *et al.* 2011). Kohler *et al.* (2008) and Grubin *et al.* (2011) showed that the flies are well-synchronized with HWA. A field release study conducted

in 2015 and 2016 demonstrated survival and successful reproduction by the *Leucopis* spp. on HWA infesting eastern hemlock in New York and Tennessee (Motley *et al.* 2017). *Laricobius nigrinus* completes development and is relatively specialized on HWA (Zilahi-Balogh *et al.* 2002), although *La. nigrinus* has been found in association with other adelgid species in the Pacific Northwest (Mausel *et al.* 2011). However, sampling by clipping terminal twigs found *La. nigrinus* larvae only on progrediens HWA, whereas *Leucopis* larvae were found on both progrediens and sistens HWA (Kohler *et al.* 2016). Since the *Leucopis* spp. feed on both the progrediens and sistens generations of HWA, and *La. nigrinus* only feeds on the progrediens generation and aestivates during the summer (Zilahi-Balogh *et al.* 2002), the flies could complement *La. nigrinus* in biocontrol of HWA (Grubin *et al.* 2011).

This study built upon the work with *Leucopis* and *La. nigrinus* in part by distinguishing *Le. argenticollis* and *Le. piniperda* larvae. In previous studies, it was not possible to distinguish the larvae by species without rearing them to adults (Motley *et al.* 2017; Grubin *et al.* 2011; Kohler *et al.* 2008). The larvae are the predatory stage on HWA. In his beat sampling, Kohler (2007) found *Le. argenticollis* adults to be more common than *Le. piniperda* adults. Identifying *Leucopis* spp. in the larval stage allowed us to compare the abundance and distribution of *Leucopis* relative to one another and *La. nigrinus* more easily than was possible in the past. We also sampled a wider range of sites to compare the relative abundance of the three specialist predators in their native environment.

The HWA biocontrol program requires additional predators because, there are no known parasitoids of adelgids, HWA has a complex polymorphic life cycle, and only Anthocoridae (Hemiptera) and Chamaemyiidae (Diptera) have ever succeeded as biocontrol agents against adelgids (citations in Kohler *et al.* 2008; Zilahi-Balogh *et al.* 2002, cited in Kohler 2007).

Chapter 2: Quantifying the relative abundance of three specialist predators of the hemlock woolly adelgid in the Pacific Northwest

Introduction: Study Objectives

The primary objective of this study was to determine the densities and relative abundances of the three specialist predators found associated with HWA in the Pacific Northwest. An earlier study that used a beat sampling method found that *La. nigrinus* was about 2.5 times more abundant than the two species of *Leucopis* combined (Kohler *et al.* 2008). However, there is nothing known about the efficacy of beat sampling for the different species of predators and it has been suggested that the method overestimates the abundance of *La. nigrinus* relative to the *Leucopis* spp. (Ross *et al.* 2011). More recently, a study that compared predator densities on clipped twigs from 13 trees at two sites found that the two *Leucopis* spp. combined were 2.3-3.5 times more abundant than *La. nigrinus* (Kohler *et al.* 2016). The present study used the clipped twig sampling method at more locations to see if the results of Kohler *et al.* (2016) are representative of predator densities across a wider range of HWA in the Pacific Northwest. This study, unlike earlier ones, was also able to distinguish larval *Le. argenticollis* and *Le. piniperda*, using newly developed molecular techniques.

Because of possible niche partitioning, site effects, and distribution differences, I hypothesized that the three predators would differ in relative abundance and density. I predicted the fly larvae would be more abundant than the beetle larvae because of the underestimation of the flies by Kohler (Ross *et al.* 2011) and the fact that the flies feed on both generations of HWA, whereas the beetle only feeds on the progrediens generation. I predicted *Le. argenticollis* larvae would be more abundant than *Le. piniperda* larvae based on Kohler's (2007) finding of more adults of the former than the latter. I predicted relative densities of the predators would vary by site. I predicted predator abundance and density would be positively correlated with prey abundance density because populations of these specialist predators should track the HWA population. I predicted abundances of the two, presumably competing, *Leucopis* spp. would be negatively correlated and that a geographic distribution pattern would segregate these two

congeners. I hypothesized that predator densities relative to HWA density would vary by predator species, predicting that predator densities, especially *Leucopis* densities, would positively correlate with HWA density. Specifically, I predicted that the densities of the *Leucopis* spp. would exhibit stronger positive correlations with the prey than would that of *L. nigrinus*.

Materials and Methods

Hemlock woolly adelgid and associated adelgid specific predator densities were quantified from samples collected at field sites in western Oregon and Washington. Based on previous research, the predators of interest were *La. nigrinus*, *Le. argenticollis*, and *Le. piniperda* (Kohler *et al.* 2008). Field sites were chosen for their high HWA abundance on western hemlock.

The sampling locations and number of trees sampled at each location were: 13 trees at a Sierra Pacific Industries seed orchard on Whidbey Island (Google Maps: 48.201017, -122.630691); six trees in Point Defiance Park in Tacoma (Google Maps: 47.305168, -122.515241); four trees on Vashon Island; and two trees in front of an apartment complex, “Tanara Villa Apartments,” 6322 N 26th St., Tacoma (Google Maps: 47.271998, -122.523851). The Vashon Island site included a tree on Gorsuch Rd. (Google Maps: 47.455584, -122.449715), two trees near the intersection of SW Dilworth Rd. and Shanahan-Glen Acres Rd. (Google Maps: 47.464854, -122.436530), and one tree on SW Van Olinda Rd. (Google Maps: 47.468987, -122.445693). Additional sites were opportunistically sampled only once or twice since they supported low HWA populations. However, data from collections at these sites were combined with the data from the four main sampling locations. These included two trees each sampled once in W.B. Nelson State Park on HWY 34 east of Waldport, Oregon (Google Maps: 44.415215, -124.034968), one tree sampled twice in Grant Park in Portland, Oregon (Google Maps: 45.540730, -122.628954), and one tree sampled once in a parking lot at NW Walnut St. and 9th St. in Corvallis, Oregon (Google Maps: 44.594563, -123.251950). A total of 29 HWA-infested western hemlock trees were sampled, of which 25 were at the four main sites.

Terminal twigs showing HWA infestation by the white flocculence or “wool” made by egg-laying adults were collected from sample trees. Two to five haphazardly-chosen, 5-10 cm branch tips at least 0.5 m from each other were collected on each sampling date. At the four main sites in Washington, the same trees were repeatedly sampled at roughly two-week intervals April 3 to July 23, 2016. All twigs from each tree were pooled. In the lab, the numbers of HWA nymphs and adults with eggs were counted. Although eggs were not counted, adults were always present with eggs. *Laricobius nigrinus* were identified based on morphology. DNA sequencing was used to identify *Leucopis* eggs, larvae, and puparia as either *Le. argenticollis* or *Le. piniperda*. This involved extracting DNA using the Mag-Bind Blood and Tissue Kit (Omega Bio-Tek, NorCross, Georgia), cutting a small slit in the side of specimens, incubating the specimens with proteinase for at least 1 h in a microcentrifuge tube, spinning at 14,000 rpm to squeeze body contents into solution, and then amplifying and sequencing the 658 bp portion of the mitochondrial cytochrome oxidase I gene used for barcoding animals. The adult *Leucopis* flies were identified based on the relative number of pronotal hairs (N. P. Havill, personal communication). The numbers of *Leucopis* larvae, puparia, and adults of the two species were counted. Eclosed, empty *Leucopis* puparial cases were counted as puparia because there was no way to determine whether an adult *Leucopis* spp. or adult parasitoid had emerged from them. Consequently, we could not assume that an adult fly had successfully developed in the puparial case. However, except for one identified using DNA sequencing, these puparial cases could not be identified to species by sight or DNA sequencing. *Laricobius nigrinus* were identified based on morphology (Havill *et al.* 2016b; Zilahi-Baloghi *et al.* 2002). The numbers of *La. nigrinus* larvae and adults were counted. Since *La. nigrinus* pupates underground (Zilahi-Balogh *et al.* 2002), pupae of this species were not found on branches. Other predatory species besides *Leucopis* and *Laricobius* were counted and identified to family or genus opportunistically. The total length of sample twigs, including the length of side branches, was measured to express HWA and predator densities on a per-centimeter-of-stem basis.

Data Analyses

Individual predator species' and HWA mean densities were calculated for all trees at each sample location and graphed over time. Graphs were prepared for each of the four main sites as well as over all four main sites combined. When two collection dates were only one day apart, the dates were consolidated to make them the later of the two dates for clearer graphical display. Mean cumulative densities were calculated by taking the means of the summed densities on each tree over time. Therefore, each data point for mean cumulative densities represented the mean density of repeated measures of a species on a particular tree over all sampling events. These mean cumulative densities of the combined *Leucopis* spp. and *La. nigrinus* were found to be normally distributed and nearly normally distributed, respectively. Mean cumulative densities of each of the *Leucopis* spp., *Le. argenticollis* and *Le. piniperda*, were found to be highly right-skewed and not normally distributed. Transformations could not consistently normalize these data because of the many zero values. Therefore, tests assuming normality were not applied to the mean cumulative densities of *Le. argenticollis* and *Le. piniperda*. Mean cumulative densities were used for these tests. Mean cumulative densities of each of the predator species and HWA were subjected to one-way ANOVAs to assess the effect of site as a factor on cumulative density. These analyses informed further analysis by allowing site to be ignored if it was not significant in explaining density. Pearson's product-moment correlation was used to correlate mean cumulative densities of HWA, *La. nigrinus*, and the combined *Leucopis* spp. Spearman's rank correlation coefficient was used to correlate the mean cumulative densities of *Le. argenticollis* and *Le. piniperda*, which were not normally distributed but did satisfy the Spearman's rank correlation coefficient test's assumptions of ordinal or continuous, and monotonically related, data. A *p*-value of 0.05 was considered significant for all tests.

Results

The *Leucopis* spp. were more abundant than other predators; there were 120 total *Leucopis*, 52 total *La. nigrinus*, 50 spiders, 19 hemerobiid larvae (Neuroptera), 10 coccinellid

(Coleoptera) larvae, and fewer others across all of the sites (Fig. 1; Table 1). The *Leucopis* spp. were consistently more abundant than other species across all sites and at each site, except Point Defiance, where there was one more *La. nigrinus* collected than *Leucopis* spp. (Figs. 1 and 2; Table 1).

Leucopis argenticollis was more abundant than *Le. piniperda*. Three of the five identified adult *Leucopis* were *Le. argenticollis*, while two were *Le. piniperda* (Fig. 1). From the DNA analysis, a total of 55 *Le. argenticollis* larvae and puparia were identified compared to a total of 25 *Le. piniperda* larvae and puparia (Fig. 1). The densities of *Le. argenticollis* and *Le. piniperda* followed similar patterns over time increasing from April to early May and then declining gradually until the end of July (Fig. 3).

Figure 1.

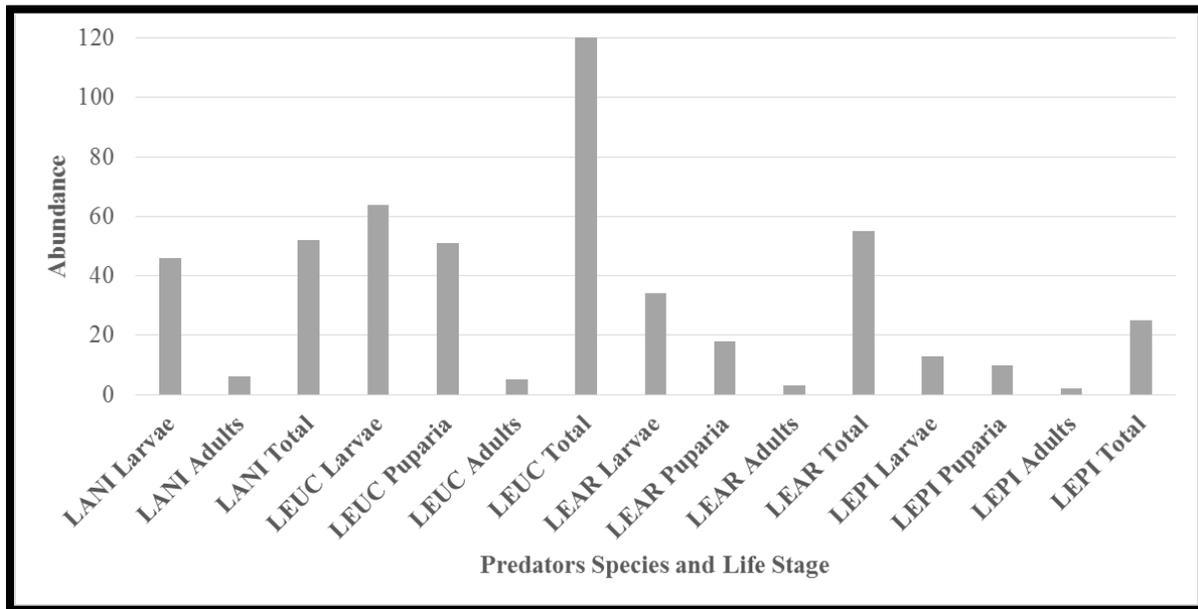


Figure 1. Total numbers of adelgid specific predators collected by life stage across all sites (including the opportunistically sampled sites). “LANI” stands for *La. nigrinus*, “LEUC” stands for both the *Leucopis* spp. combined, “LEAR” stands for *Le. argenticollis*, and “LEPI” stands for *Le. piniperda*.

Figure 2.

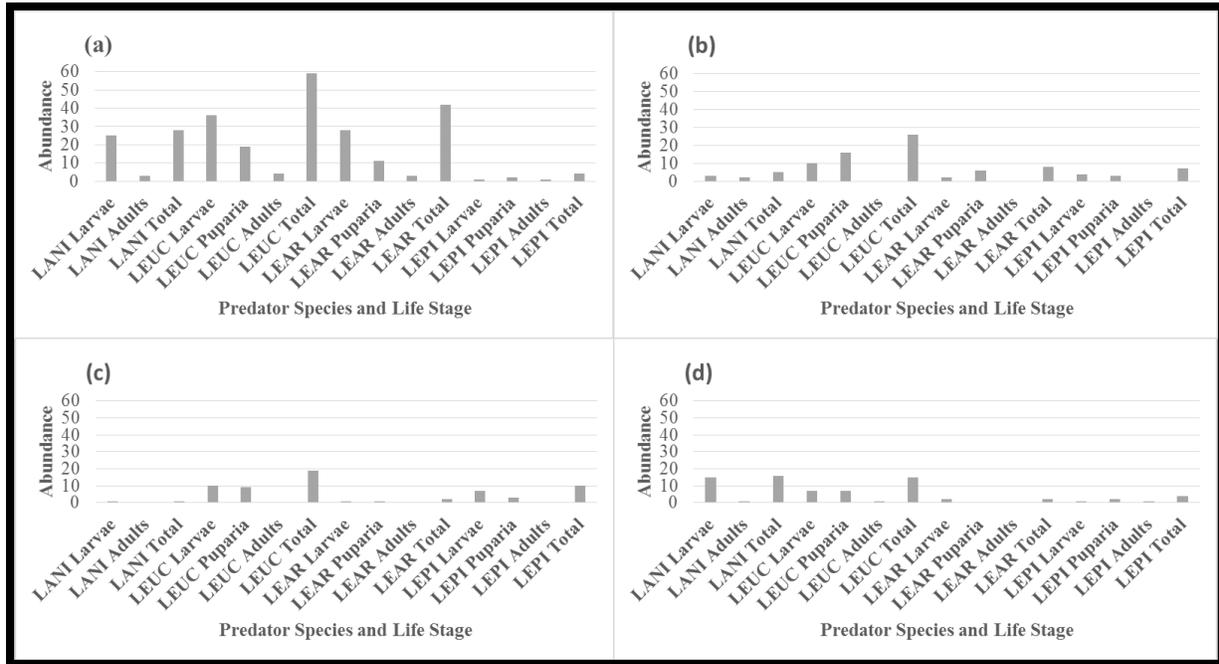


Figure 2. Total numbers of adelgid specific predators collected by life stage at each of the four main sites: Whidbey Island (a), Vashon Island (b), Tacoma-Tanara Villa (c), and Point Defiance (d). “LANI” stands for *La. nigrinus*, “LEUC” stands for both the *Leucopis* spp. combined, “LEAR” stands for *Le. argenticollis*, and “LEPI” stands for *Le. piniperda*.

Table 1. Total numbers of other, incidental predators collected across all sites (including the opportunistically sampled sites).

Total Numbers of Other Predators				
Total Other Predators	Spiders	Hemerobiidae larvae	Total Coccinellidae larvae	Coccinellidae: <i>Scymnus</i> larvae
96	50	19	10	5
Total Syrphidae larvae	Syrphidae: <i>Eupeodes</i> larvae	Chrysopidae larvae	Anthocoridae	
9	8	5	2	

Figure 3.

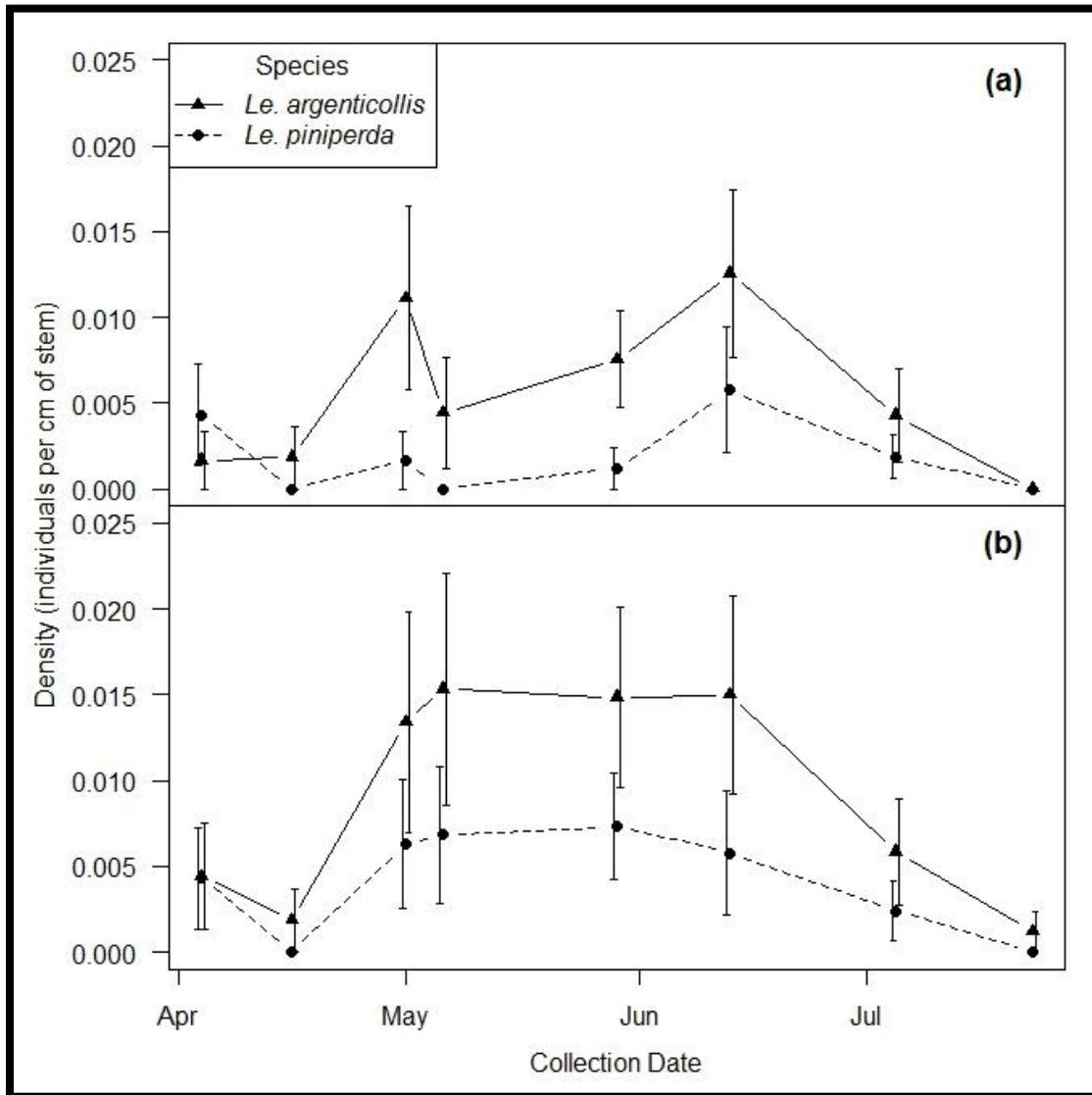


Figure 3. Mean densities of all identified *Le. argenticollis* and *Le. piniperda* larvae (a) and combined larvae, puparia, and adults (b) across the four main sites from April 3 to July 23, 2016. Error bars are standard errors of the mean.

When puparia are excluded from the totals, *Leucopis* spp. larvae and adults totaled 69 compared to 52 *La. nigrinus*. To use the most important life stage relative to predation for

Leucopis spp. in samples was 0.011 larvae/cm of stem (n = 178 samples; s.d. = 0.022) and the mean density of *La. nigrinus* larvae was 0.008 larvae/cm of stem (n = 178 samples; s.d. = 0.028) across all sites. Also across all sites, there was a total number of 70 samples with *Leucopis* spp. present. The total number of samples with *La. nigrinus* present was 24.

The relationship of temporal distribution of the *Leucopis* spp. and *La. nigrinus* suggest that the densities of the flies are more consistent at the sites throughout the season than the beetles, which had highest densities early in the season before declining. Specifically, the overall density of larval and adult *La. nigrinus* was highest in April, declined by early May, and remained at that low density until sampling ended in late July (Fig. 4). The overall density of larval and adult *Leucopis* spp. increased in May, was highest in June, and declined in late July (Fig. 4). Similar patterns were observed for larvae and adults of the predators when graphed separately, although larval densities of *Leucopis* spp. tended to be more constant throughout the sampling period (Fig. 4). Densities of *Leucopis* puparia peaked in May (Fig. 4).

Figure 4.

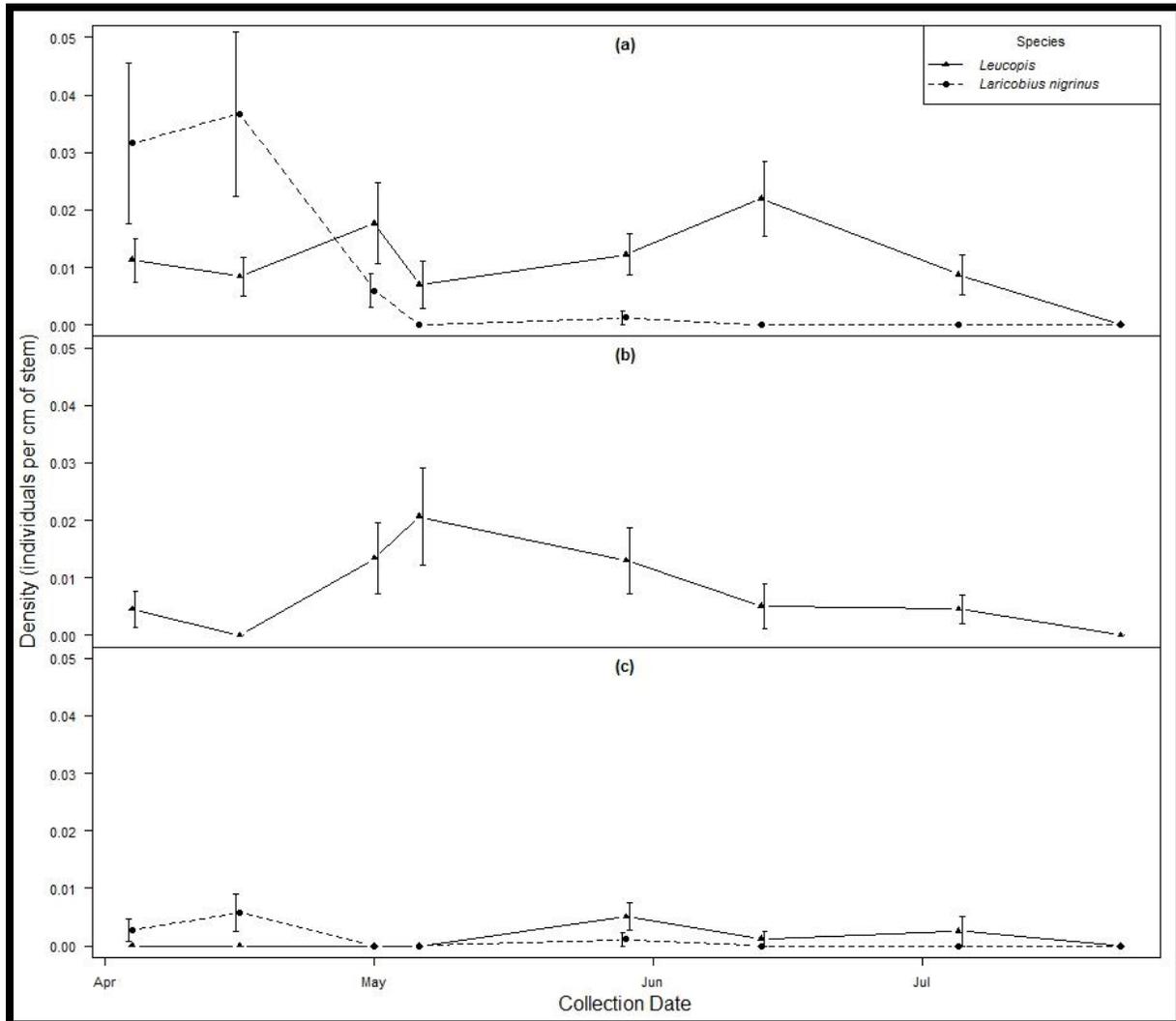


Figure 4. Mean densities of larval (a) and adult (c) *Leucopis* and *La. nigrinus* across all sites, as well as of pupal *Leucopis* (b), from April 3 to July 23, 2016. Points represent collection events. Error bars are standard errors of the mean.

The density of HWA nymphs generally increased until after May 6 (Fig. 5). The pattern of this increase in nymphs followed by an increase in adults from May 29 through June 13 was inferred to represent the start of the sistens generation.

Figure 5.

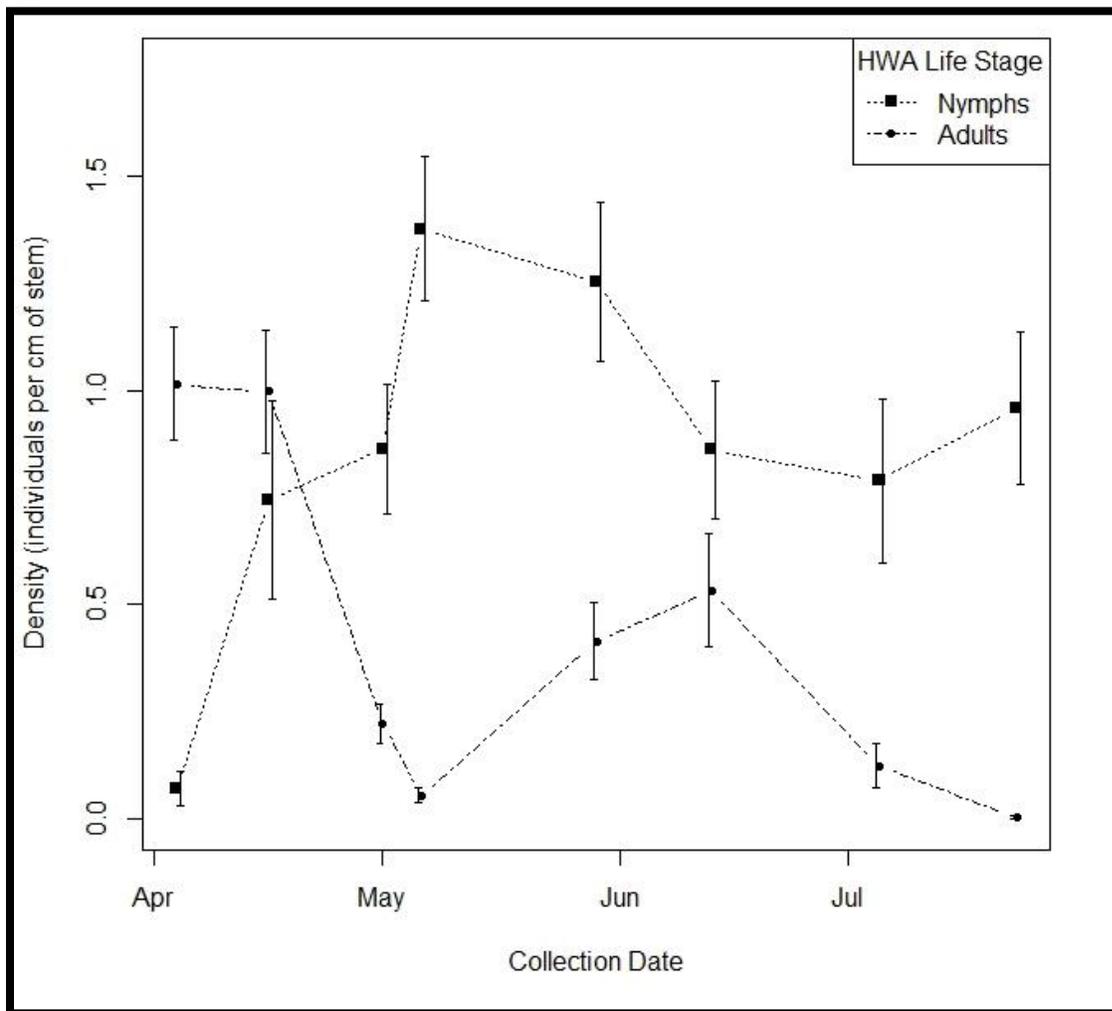


Figure 5. Mean densities of HWA nymphs and adults across all sites from April 3 to July 23, 2016. Points represent collection events. Error bars are standard errors of the mean.

The phenological patterns of the mean predator densities appear to perhaps vary by site over time (Figs. 6-9). However, regardless of phenology, there was no significant effect of site

on HWA (combined nymphs and adults) or predator (combined larval, puparial, and adult *Leucopis* spp., and combined larval and adult *La. nigrinus*) mean cumulative densities in three one-way ANOVAs (HWA: F-value = 1.88, d.f. = 3, 21, p -values = 0.17; *Leucopis*: F-value = 1.95, d.f. = 3, 21, p -values = 0.15; *La. nigrinus*: F-value = 0.4, d.f. = 3, 21, p -values = 0.75). Variation in time was not applicable for mean cumulative densities and thus also not a factor in these ANOVAs.

Figure 6.

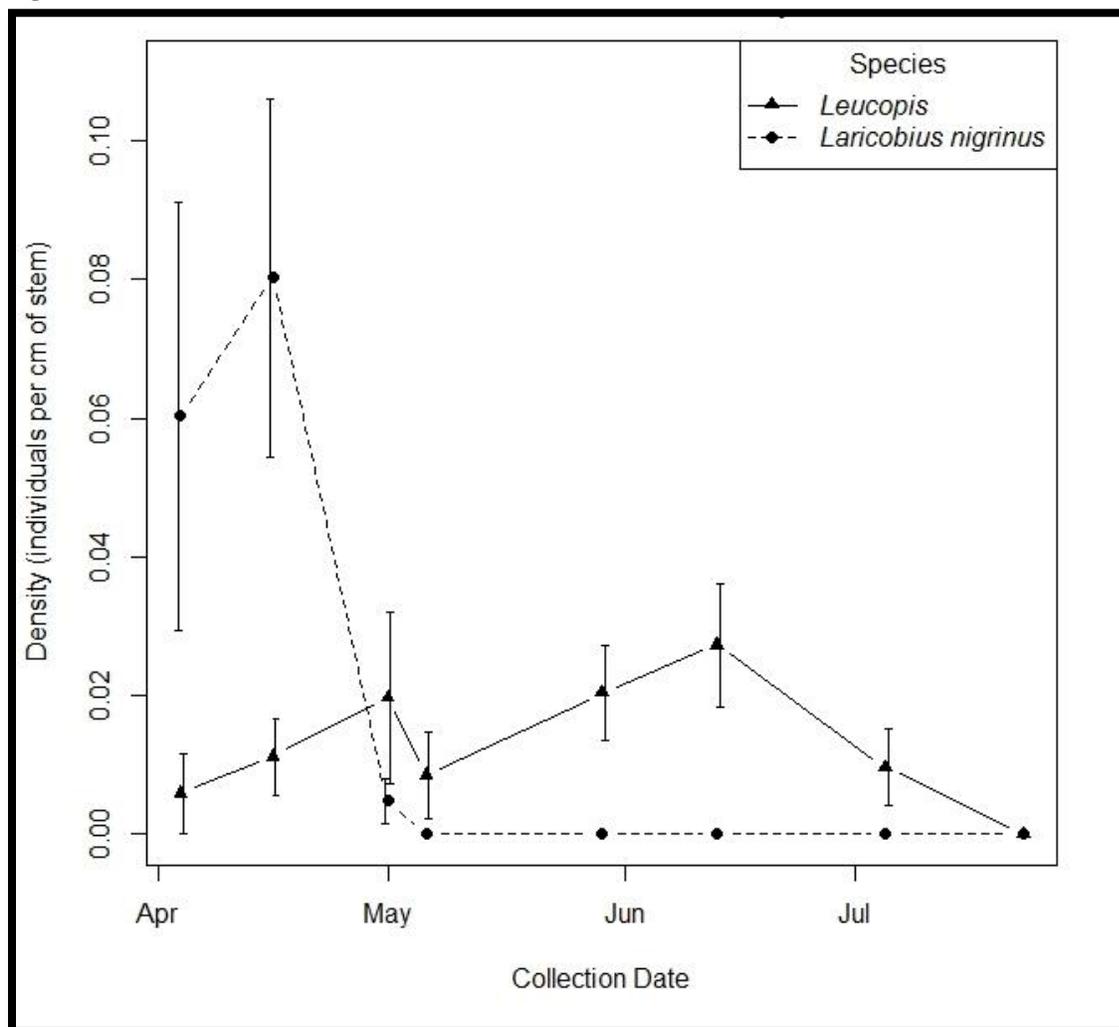


Figure 6. Mean densities of larval and adult *Leucopis* spp. and *La. nigrinus* on Whidbey Island from April 4 to July 23, 2016. Points represent collection events. Error bars are standard errors of the mean.

Figure 7.

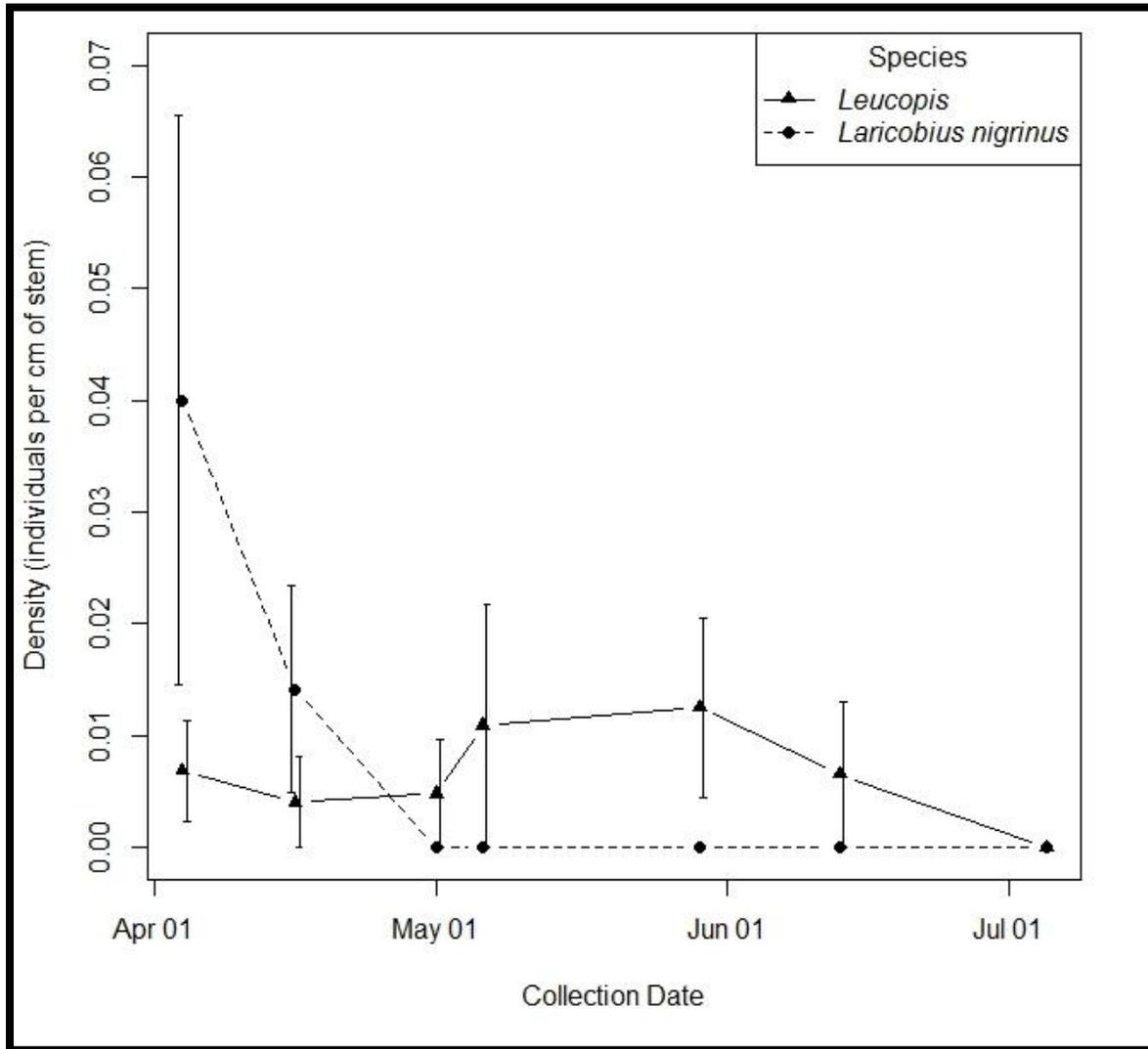


Figure 7. Mean densities larval and adult *Leucopis* spp. and *La. nigrinus* at Point Defiance from April 3 to July 5, 2016. Points represent collection events. Error bars are standard errors of the mean.

Figure 8.

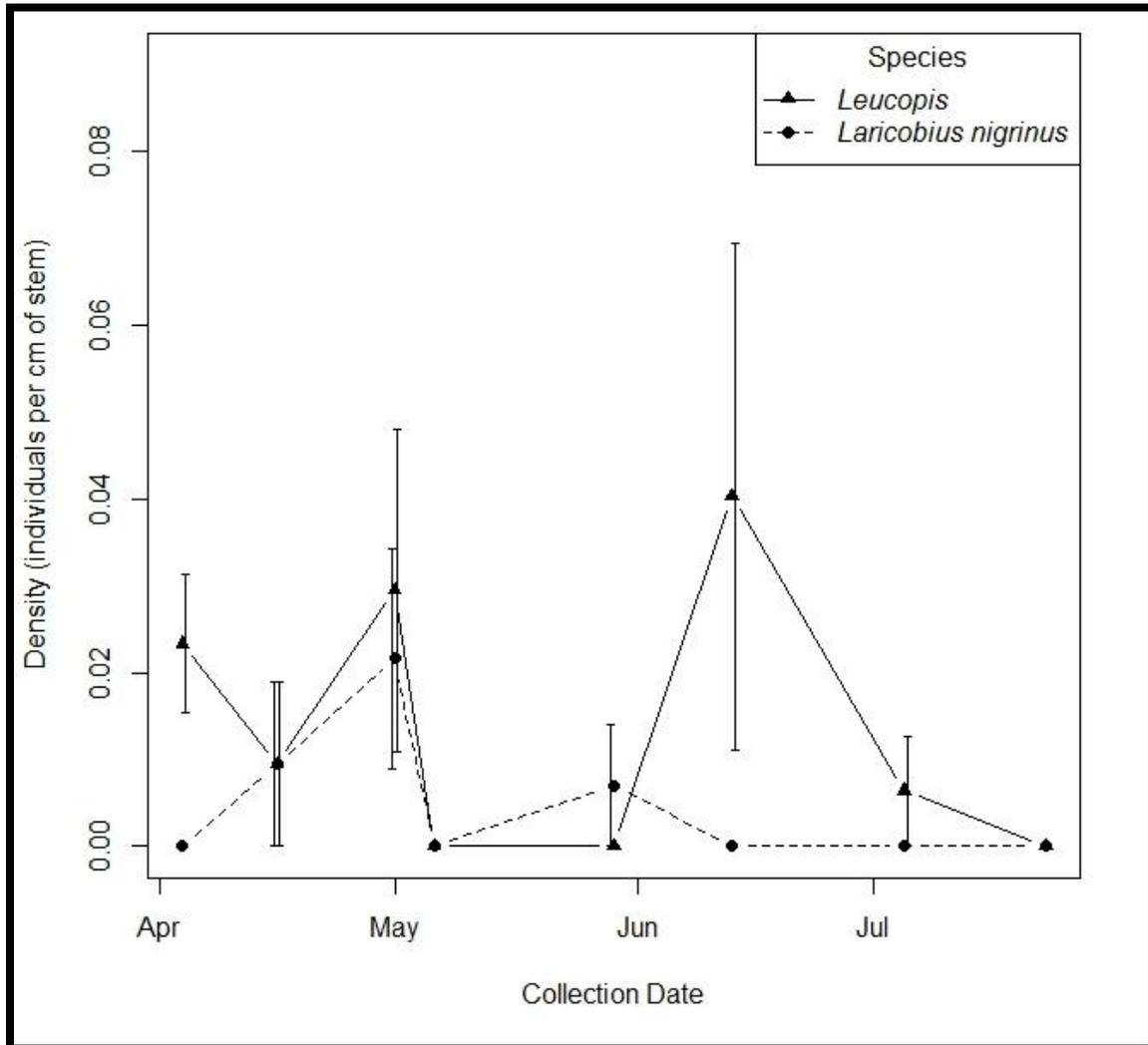


Figure 8. Mean densities of larval and adult *Leucopis* spp. and *La. nigrinus* on Vashon from April 3 to July 23, 2016. Points represent collection events. Error bars are standard errors of the mean.

Figure 9.

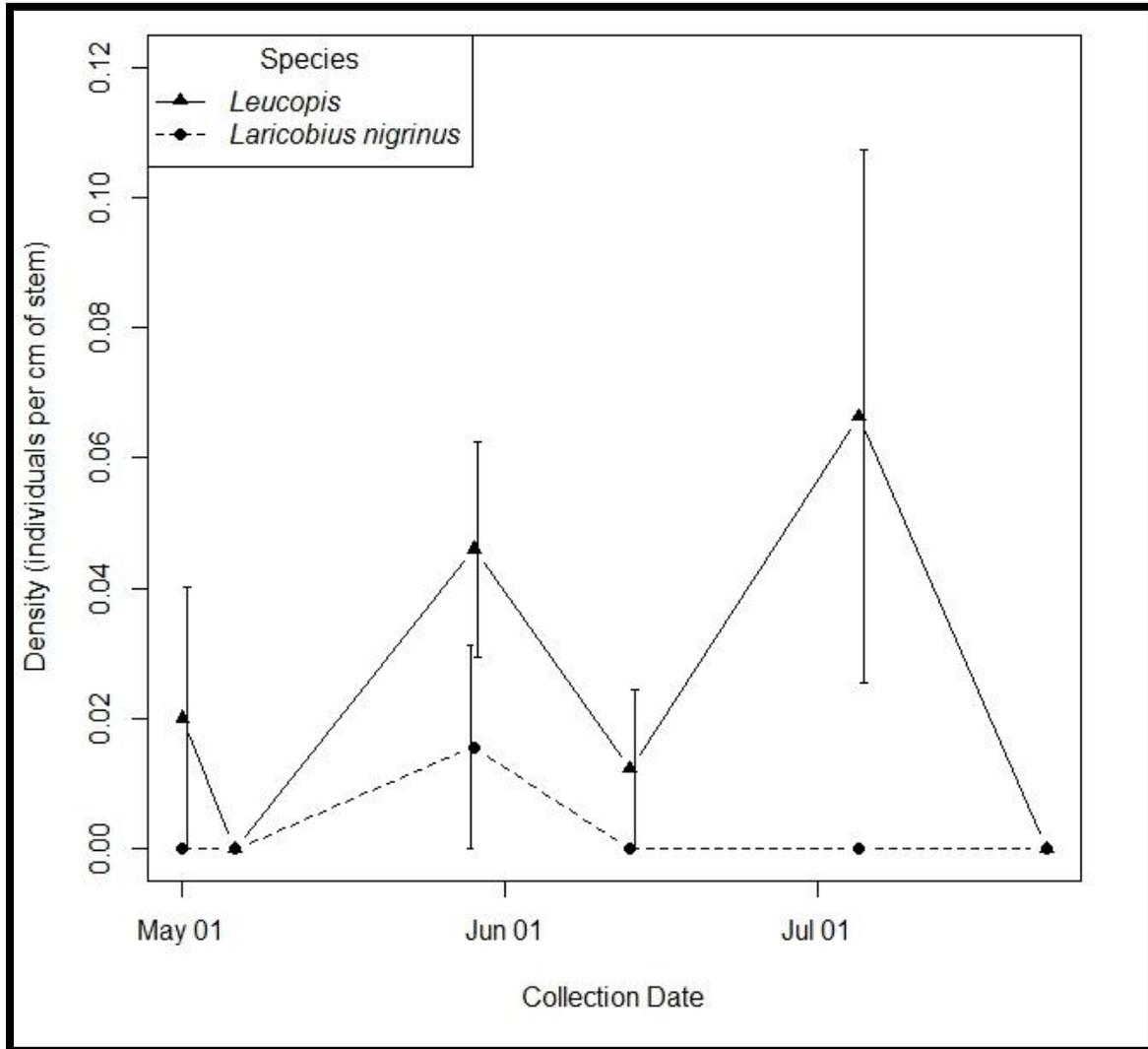


Figure 9. Mean densities of larval and adult *Leucopis* spp. and *La. nigrinus* in Tacoma from May 1 to July 23, 2016. Points represent collection events. Error bars are standard errors of the mean.

The Pearson's product-moment correlation of HWA and combined larval and adult *La. nigrinus* was found to be non-significant, with an alternative hypothesis of positive correlation (t -value = -0.34; d.f. = 23; p -value = 0.63; 95% CI = -0.40 to 1; estimated correlation = -0.07). The Pearson's product-moment correlation of HWA and larval *La. nigrinus* was likewise found to be

non-significant, with an alternative hypothesis of positive correlation (t-value = -0.63; d.f. = 23; p -value = 0.73; 95% CI = -0.45 to 1; estimated correlation = -0.13). Combined larval and adult *La. nigrinus* and *Leucopis* spp. were significantly, moderately, negatively correlated, with an alternative hypothesis of negative correlation (t-value = -1.81; d.f. = 23; p -value = 0.04; 95% CI = -1 to -0.02; estimated correlation = -0.35). Larval *La. nigrinus* and *Leucopis* were significantly, moderately, negatively correlated, with an alternative hypothesis of negative correlation (t-value = -1.92; d.f. = 23; p -value = 0.03; 95% CI = -1 to -0.04; estimated correlation = -0.37). HWA correlated significantly, moderately, and positively with both larval and combined larval, puparial, and adult *Leucopis*, tests with an alternative hypothesis of positive correlation: HWA and larval *Leucopis* (t-value = 2.64; d.f. = 23; p -value < 0.01; 95% CI = 0.17 to 1; estimated correlation = 0.48); and HWA and combined *Leucopis* (t-value = 1.98; d.f. = 23; p -value = 0.03; 95% CI = 0.05 to 1; estimated correlation = 0.38). *Leucopis argenticollis* and *Le. piniperda* were non-significantly, negatively correlated using a non-parametric test, Spearman's rank correlation coefficient, with an alternative hypothesis of negative correlation (Fig. 10; ρ = -0.28; d.f. = 23; p -value = 0.09).

Figure 10.

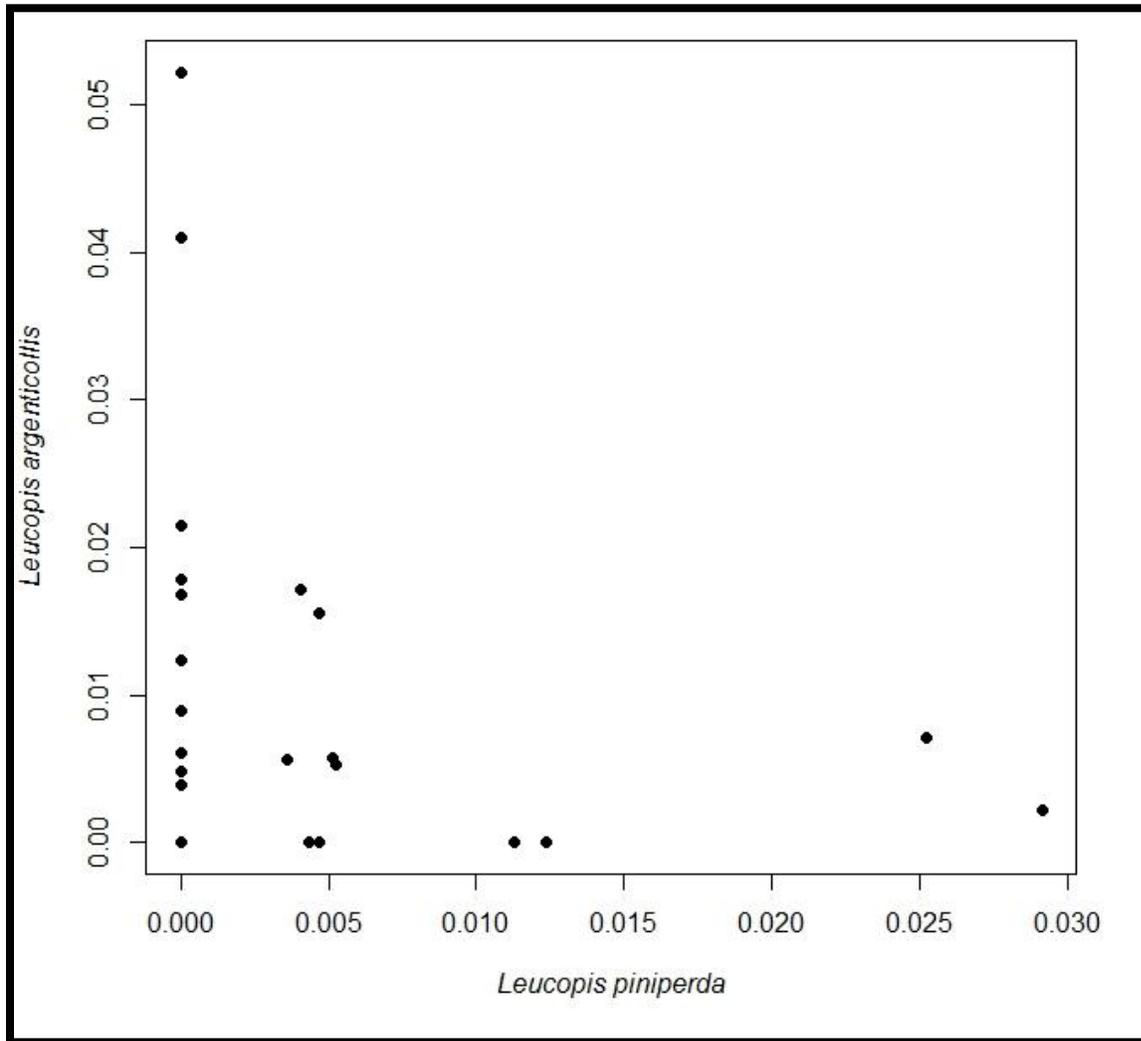


Figure 10. Combined mean cumulative densities of *Le. argenticollis* and *Le. piniperda* are related to each other. The relationship is apparently monotonic and negative, though this relationship was not significant ($\rho = -0.28$; d.f. = 23; p -value = 0.09).

Discussion

The *Leucopis* spp. were more abundant than *La. nigrinus* overall, and particularly more abundant later in the season from early May onward, through July and the end of sampling, corresponding with the sistens HWA generation (Figs. 1 and 4). These results agree with Kohler *et al.* (2016) that *Leucopis* spp. associate with and feed on both HWA generations, while *La. nigrinus* feeds only on the progrediens generation.

Figure 1 may appear to indicate that there were almost as many *La. nigrinus* (52 in total) as *Le. argenticollis* (55 in total) and many more than *Le. piniperda* (25 in total). However, not all of the *Leucopis* larvae and puparia were successfully identified using DNA sequencing. In particular, with the exception of one identified with DNA sequencing, empty puparial cases that were counted as puparia could not be identified to species. About two-thirds of the *Leucopis* were identified to species (67% across all sites). Because of this, the finding of a similar number of *La. nigrinus* to *Le. argenticollis* presented is misleading. If the ratio of *Le. argenticollis*:*Le. piniperda* (2.2:1) were to hold for the remaining *Leucopis* not identified to species, then the total for *Le. argenticollis* would be ~83 and the total for *Le. piniperda* would be ~38. This means that this study arguably found *Le. argenticollis* to be by far the most abundant predator, followed by *La. nigrinus*, and then *Le. piniperda*.

The densities of the two *Leucopis* spp. paralleled each other over time and *Le. argenticollis* tended to occur at slightly higher densities than *Le. piniperda* (Fig. 3). There were more *Leucopis* identified as *Le. argenticollis* than *Le. piniperda* (Fig. 1). The finding of more *Le. argenticollis* than *Le. piniperda* is consistent with the higher abundances of adults of the former found by Kohler *et al.* (2008).

Seventy-six percent of the identified *Le. argenticollis* specimens came from Whidbey Island (42 out of 55, Fig. 2), while *Le. piniperda* were more evenly distributed across the four main sites (Fig. 2). The two *Leucopis* spp. may be segregated geographically, but the data here are insufficient to demonstrate this, especially considering that this study involved only one season of sampling and some of the *Leucopis* specimens were not successfully identified to

species. The parallel densities of these two congeneric species combined across all sites over time (Fig. 3) indicates a phenological similarity, perhaps not supporting partitioning in time. Additionally, there could be other scales of partitioning or sharing the resource, such as within trees or along branches.

The lack of an effect of site on combined *Leucopis* spp., *La. nigrinus*, or HWA densities contradicted the prediction of variation by site, partially falsifying the hypothesis of site effect and distribution differences among the predators. This finding also helped to legitimize comparing species densities across all sites and using linear correlations to test their interspecific associations. This is because mean cumulative densities ignored site as a variable by using the mean densities over all sampling events for each tree as data points. The prediction of positive correlation between densities of HWA and the specialist predator *La. nigrinus* was not supported. The densities of HWA and the *Leucopis* spp. were positively correlated, as predicted. The intraguild predators were negatively correlated. Both larval densities, and larval and adult combined densities, of *La. nigrinus* and the *Leucopis* spp. were negatively correlated. The densities of *Le. argenticollis* and *Le. piniperda* were not significantly correlated, but the direction of the correlation was negative as predicted from hypothesizing that the two, presumably competing, intraguild predators would somehow be segregated (Fig. 10).

In addition to the higher abundance of *Leucopis*, the fact that *La. nigrinus* densities were not correlated with HWA, while *Leucopis* spp. were positively correlated with HWA, argues for the *Leucopis* spp. as being the more important predators for controlling HWA, at least in the scope of this seasonal window at these particular sites. Grubin *et al.* (2011) also found positive correlation between *Leucopis* spp. and HWA. Kohler *et al.* (2008) found that the abundances of larval *Leucopis* spp. and *La. nigrinus* were positively correlated with HWA abundance score. *Leucopis* spp. and *La. nigrinus* were the most abundant predators in the Kohler *et al.* (2008) survey, too.

The negative interspecific correlations between these predators may indicate possible competition among these intraguild predators. Since the predators utilize the same resource (HWA) in a similar way (as active predators on all life stages), they fit a definition of being in the same guild (Simberloff and Dayan 1991; Root 1967). Traditionally, the guild concept was

formulated as a way to look for and contrast competition with niche partitioning (Simberloff and Dayan 1991). These intraguild predators are presumably competing for the shared resource of HWA on hemlock, a supposition that is supported by the negative correlations between them and their possible geographic segregation. If these species are negatively correlated based on mean cumulative densities, then some kind of niche partitioning that ameliorates competition may be expected. The mismatch of the abundances of the two *Leucopis* spp. at the different main sites supports the prediction of a pattern of geographic segregation by these species and suggests niche partitioning in space (Fig. 2), if not time (Fig. 3). The majority of identified *Le. argenticollis* specimens were found on Whidbey Island (42 out of 55 total), while *Le. piniperda* was more evenly distributed across sites.

Competition could occur between these three predator species, given their shared niche, and would be worthwhile to investigate, especially interference competition in consideration of their use as biocontrol agents. In two species of aphid-feeding ladybird beetles, rates of intraguild predation were demonstrated to be one-sided, with implications for the dominance of the more aggressive species (Yasudo *et al.* 2001). If, hypothetically, significant intraguild predation were to occur between the two *Leucopis* spp., then this could hamper their effectiveness as biocontrols if introduced at the same sites. Similarly, this could also be a concern with *La. nigrinus* and the *Leucopis* spp. It is not difficult to imagine the predatory adults of *La. nigrinus* occasionally feeding upon relatively small, early instar *Leucopis* larvae when encountered. High predator densities with limited prey could increase these encounters and thus this hypothetical interference competition. The adult *Leucopis* are not predatory on HWA. Of course, intraguild predation or other forms of interference competition were not observed in this study. In addition, invasive HWA population levels are so high as to likely preclude exploitative competition as a possibility; HWA abundance is probably not limiting for *La. nigrinus* in eastern North America. Instead, winter minimum temperatures and sizes of introductions of *La. nigrinus* have been shown to positively correlate with and determine the establishment and persistence of *La. nigrinus* in eastern North America, where HWA populations occur at outbreak levels (Havill *et al.* 2016b; Sumpter 2016). Still, generally, intraguild predation can disrupt biological control and be pervasive among communities of biocontrol agents controlling arthropods (Rosenheim *et al.*

1995). The existence of such competition and its circumstances should therefore be investigated among *La. nigrinus* and the two *Leucopis* spp.

Because *La. nigrinus* feeds on the progrediens, not the sistens, generation of HWA and aestivates as pupae in soil in mid-summer, the sampling period of this study might be considered biased to favor a finding of relatively high *Leucopis* density. Indeed, Kohler (2007) collected *La. nigrinus* adults throughout the year except during the summer when the beetle aestivates as pupae in the soil. Although this study's sampling dates might be inadequate for capturing the *La. nigrinus* life cycle as observed in eastern North America, the sampling period of this study is appropriate based on the Northwest surveys described in Kohler (2007) and Kohler *et al.* (2016). The graphs of *La. nigrinus* compared to *Leucopis* spp. in these two papers support starting sampling in early April in the Pacific Northwest as was done in this study. During one year beginning in fall 2005, Kohler (2007) found larval *La. nigrinus* abundance to be highest in April-May, while larval *Leucopis* spp. abundance was highest in May-July in the Salem, Oregon area. During the same year, larval *La. nigrinus* abundance was also highest in April-May in the Portland, Oregon area, while larval *Leucopis* spp. abundance was highest in April-July. In western Washington, at sites of closest geographic proximity to the current study's sites, larval *La. nigrinus* abundance was highest in June, while larval *Leucopis* spp. abundance was highest in February-March and June-July. Based on these results from sites obviously similar in location, climate, and presumably other environmental variables, to those of this study, it is therefore clear that a sampling period from April through July was probably appropriate for capturing peak abundance of the prey and predator species.

Although the predator density results from Kohler's thesis (2007) are not comparable to this study's due to methodological differences, Kohler *et al.* (2016) used more comparable methods. Unlike this study, Kohler (2007) used beat sheeting, combining three beat sheet samples into a 0.5 square meter area for calculating density. This led to a different metric of density from that of this study. Unlike this study, in which only terminal twigs were sampled, Kohler (2007) sampled a farther distance back from the branch tip than the 5-10 cm that was sampled in the present study, although, similarly to this study, he did sample in the lower canopy, <2.5 m. Kohler *et al.* (2016) employed more comparable methods; two randomly-

selected, terminal twigs were sampled per tree and the lengths were comparable, at 3-10 cm/twig. The sites sampled in Kohler *et al.* (2016) were St. Paul, Oregon and Olympia, Washington.

It is worthwhile to compare the similar, more recent study and this one. Kohler *et al.* (2016) found the *Leucopis* spp. to be 3.5 and 2.3 times more abundant than *La. nigrinus* at the St. Paul and Olympia field sites, respectively. In this study, *Leucopis* spp. were ~2.3 times more abundant than *La. nigrinus* overall, a finding similar to the ratios of Kohler *et al.* (2016). The ratio of the *Leucopis* spp. to *La. nigrinus* was 0.9 at Point Defiance, while on Whidbey Island it was ~2.29. The *Leucopis* spp. were predominantly abundant at Tacoma-Tanara Villa and Vashon Island, with there being 16 times more *Leucopis* than *La. nigrinus* at Tacoma-Tanara Villa and 7.25 times more on Vashon Island (Table 1).

The density graphs presented by Kohler *et al.* (2016) show *Leucopis* spp. and *La. nigrinus* densities at St. Paul ranging from about 0-0.06 and 0-0.75, respectively, while their respective densities at Olympia ranged from about 0-0.2 and 0-0.02. Except for the high density of *La. nigrinus* at St. Paul, these ranges are similar to those of this study.

The results of this study could be biased by the methods used to collect samples. In contrast to beat sheet sampling, the chance of winged adults' escaping was reduced by placing bags over branches while clipping. Although some winged predators could have still slipped past (especially the more agile *Leucopis*), this was never observed. Sampling in this study, which involved taking terminal twigs from the lower part of trees, was limited within the architecture of the trees, possibly biasing the results in some unknown way. In one study, HWA ovisacs (adult females with eggs covered by flocculence) were densest at branch tips and mostly did not significantly differ in density by vertical location in trees (Joseph *et al.* 2011b). HWA is thought to prefer new to old hemlock shoots (Davis *et al.* 2012). This information provides *post-hoc* justification for this study's haphazard sampling of branch tips within arm's reach. Samples were placed on ice in a cooler for transport and then refrigerated in the lab before being processed soon thereafter, so intraguild predation occurring post sampling was minimized, but still could have occurred.

These findings add to the body of work supporting the silver flies as promising additional biocontrol agents of HWA. *Leucopis* has been shown to be able to establish and reproduce for at least one generation in caged releases onto HWA-infested eastern hemlock in New York and Tennessee (Motley *et al.* 2017). The combined *Leucopis* spp. in this study were more abundant overall than *La. nigrinus*. The chamaemyiid flies occurred on both HWA generations, unlike the beetles. The *Leucopis* spp. were significantly positively correlated with HWA, while *La. nigrinus* was not.

Chapter 3: An Exploration of Possible Bottom-up Control of the Hemlock Woolly Adelgid in the Pacific Northwest

Introduction

In accordance with the enemy release hypothesis and the paradigm of classical biological control, invasive HWA in eastern North America is at pest levels because it is not being controlled by natural enemies, specifically predators. Therefore, predators from the native range of HWA have been introduced as biological control agents. However, these agents have thus far not succeeded in perceptibly reducing HWA populations or slowing hemlock decline or mortality (Onken and Readon 2011; Mausel *et al.* 2011). The major component (Chapter 2) of this thesis recommends additional predators be considered for introduction as prospective biocontrol agents of HWA. However, those predators would not face an environment and host situation identical to what they experience in their native Pacific Northwest. For one thing, there are different climates in these two regions and possible ramifications of this are discussed in the conclusion (Chapter 4) of this thesis. For another, HWA populations in the East and the West are phylogenetically divergent, the former having been introduced separately and much more recently from Japan than those of the latter, which have been in the Pacific Northwest for millennia, making them effectively native (Havill *et al.* 2016a). This means that hemlock hosts and predators of HWA in the Pacific Northwest may be adapted to this native insect. Eastern and

Carolina hemlocks are obviously not adapted to invasive HWA in eastern North America, being neither resistant to nor tolerant of it. In contrast, western hemlock apparently is not much affected by HWA because it does not sustain outbreak levels of infestation. However, the extent that this contrast with the situation in eastern North America, where trees are heavily infested and killed by HWA, is due to top-down control by native predators in the Pacific Northwest, bottom-up control by hemlock tolerance or resistance, or other differences such as climate, is unclear. It is possible that HWA is not a pest, i.e., it does not reach economically damaging or outbreak levels, in the Pacific Northwest primarily because hemlock there is less susceptible or more resistant to HWA than eastern hemlock is to invasive HWA in the East. Such bottom-up control is plausible and, if more important than top-down control by native predators, it also might cast doubt upon introducing predators to eastern North America for biological control of invasive HWA there.

Possible bottom-up control of HWA was assessed in a clonal western hemlock seed orchard in the Pacific Northwest. Two hypotheses structured the assessment.

Alternative Hypothesis 1: There is a spatial explanation for the distribution of HWA among hemlock tree clones in the seed orchard. For example, there are two possible, non-mutually exclusive, spatial histories of HWA. HWA may have a clumped distribution among the trees due to colonization of trees from infested trees in close proximity, or HWA has a pattern of distribution reflecting its spatial spread from a founding infestation in one area of the seed orchard. Another alternative hypothesis would be that HWA has a dispersed distribution in space, though this spatial pattern seems unlikely for a non-territorial, sedentary herbivore living in dense aggregations and was not tested here. The null hypothesis for the alternative hypothesis of a clumped distribution is that the distribution of HWA in the orchard is not obviously spatial and would be shown by random spatial distribution of HWA.

Alternative Hypothesis 2: There is a genetic explanation for the distribution of HWA among hemlocks in the seed orchard. Trees of certain known clones in the seed orchard may host more HWA than others because of genetic differences in susceptibility and/or resistance to HWA. If genets (genetic individuals) comprising cloned replicates (physically individual trees, ramets) vary in levels of HWA in the orchard, then this hypothesis would be supported, whereas

if genets do not vary, then the null hypothesis of independence of HWA infestation level and genetics would be supported.

These two hypotheses were not considered mutually exclusive; both spatial and host genetic variables could account for the distribution of HWA levels in the orchard. Additionally, it is possible that other confounding variables might have been involved such as differential distribution of predators in the orchard providing top-down control of HWA. This leads to another alternative hypothesis, that HWA distribution is based on stage or phase differences in the predator-prey cycles. The common environment experienced by the similar hemlock trees in the orchard controls for most other imaginable confounding variables, such as weather.

Methods

We surveyed western hemlock trees in a commercial seed orchard on Whidbey Island, Washington. All trees were subjected to the same management history and therefore considered grown in a homogeneous environment. Trees were the same age. Trees were grouped into two similar “blocks,” a northern and a southern block, in the orchard separated by an open, grassy area about 50m in width. The northern block was approximately 1.1 ha and the southern block was approximately 1.2 ha.

The trees were grafted clones of different parentages (hereafter, genets) representing known, but proprietary genetics. Therefore, proprietary genet numbers have been changed for publication. Numbers of replicates (i.e. clones or, hereafter, ramets) of different genets ranged from one to seven individual trees.

On July 5, 2016, 452 trees were assessed for their HWA abundance by categorizing infestations levels as absent (no HWA flocculence, “wool,” observed), low, or high. The subjectivity of this assessment was reduced by verbal consensus between two experienced observers looking at each tree from opposite sides and by consistently ranking each tree based on pre-determined standards.

Data Analyses

Join-count statistics were used to test for spatial autocorrelation among the nominal HWA data. The HWA abundance level data were consolidated into presence/absence. These tests were run separately for the two blocks because the blocks were composed of exclusively different hemlock genets. Ramets were randomly located within each block. The Z-distribution was used for reference and this theoretical distribution was considered dependent instead of independent. That is, a distribution assuming non-free sampling without replacement was used instead of one assuming free sampling with replacement; the probability of occurrence for this distribution was determined from the sample area, which was not assumed to represent a larger area; and this probability was not known *a priori* independent of sampling. In the calculations for the join-count statistics, the numbers of neighbors with different categorical attributes were counted for each sampling unit quadrat in space. In this case, sampling quadrats were trees and the categorical attribute was presence/absence (occurrence) of HWA. The “queen” neighboring scheme was used, meaning that neighbors of a focal tree were quadrats containing trees potentially in eight directions from the quadrat representing the focal tree. These eight directions were those diagonally neighboring the quadrat to the northwest, northeast, southeast, and southwest, as well as those neighboring straight-on to the west, north, east, and south. [Only the latter four directions would be those used for the alternative, “rook” neighboring scheme.] A bilateral Z-test was used with an alpha value of $p = 0.05$ for the number of joins of presence and absence quadrats.

Chi-square analysis was used to assess the significance of differences in frequency of HWA levels on different genets of hemlock. For the Chi-square analysis, hemlock genets with certain numbers of ramets in the orchard were used. The two blocks were not separated in this analysis because the hypothesis being addressed was not spatial. To reduce the risk of Type I error stemming from multiple comparisons, the Bonferroni correction was used for adjusting the alpha value for evaluating the p -values from these Chi-square tests. Since five tests were conducted, a standard alpha value of $p = 0.05$ was adjusted to 0.01, which was considered significant.

Results

A clumped spatial distribution of HWA on the hemlock tree clones was not supported. Z-tests of the two join-count statistics for both the northern and southern blocks were non-significant ($Z_{\text{observed}} < Z_{\text{critical}}$). Thus, the null hypothesis of a random distribution of presence and absence of HWA among the trees could not be rejected. In other words, the observed distribution was not significantly different from random.

The proportions of ramets with HWA levels of absent, low, and high for genets comprising four or more ramets were determined (Table 1). Most of the genets with four or more ramets had no ramets lacking HWA (21 out of 34 in total; **bolded** in Table 1). None of the remaining 13 genets with four or more ramets, some of which lacked HWA, was exclusively HWA-free. In summary, all genets with four or more ramets had some ramets hosting HWA.

Table 1. The proportions of ramets with HWA absent, low, and high are displayed for genets comprising four or more ramets. Those genets that had no ramets free of HWA are **bolded**.

Northern Block				
Generic genet number	Number of ramets (i.e. clones or replicates)	Proportion HWA Absent	Proportion HWA Low	Proportion HWA High
37	5	0.8	0.2	0
39	7	0.14	0.71	0.14
40	6	0.17	.17	.17
42	4	0	0.75	0.25
43	4	0	1	0
44	4	0.25	0.75	0
45	6	0	0.67	0.33
46	4	0.25	0.5	0.25
47	4	0.25	0.75	0
48	4	0.25	0.75	0
51	5	0	0.6	0.4
52	4	0	0.75	0.25
53	4	0	1	0
68	4	0	1	0
Southern Block				
Generic genet number	Number of ramets (i.e. clones or replicates)	Proportion HWA Absent	Proportion HWA Low	Proportion HWA High
75	5	0	0.4	0.6
76	4	0	0.25	0.75
78	5	0	0.8	0.8
79	6	0	0.17	0.83
80	5	0	0.4	0.8
81	6	0.17	0.17	0.67
82	5	0	0.2	0.8
83	5	0	0.4	0.6
84	5	0	0.2	0.8
87	6	0.2	0.2	0.6
88	4	0.25	0.5	0.25
114	4	0	1	0
115	5	0	0.8	0.2
116	5	0.2	0.8	0
117	4	0	0.5	0.5
118	4	0	0.5	0.5
119	4	0.25	0.75	0
120	4	0	1	0
121	4	0	1	0
125	5	0.2	0.8	0

The first Pearson's Chi-square test used genets with four or more ramets each and HWA levels of absent, low, and high. The frequencies of these levels significantly varied by genet ($X^2 = 107.3$; d.f. = 66; p -value = <0.001). The second test again used genets with four or more ramets each, but HWA levels of absent and present (combined low and high) were used. The frequencies of HWA presence/absence did not significantly vary by family ($X^2 = 48.7$; d.f. = 33; p -value = 0.04). The third test used genets with five or more ramets only and HWA levels of absent, low, and high. The result was significant variation in HWA level by family ($X^2 = 61.7$; d.f. = 32; p -value = <0.01). The fourth test also used genets with five or more ramets, but HWA levels of absent and present. For this test, frequencies of HWA presence/absence significantly varied by family (data in Table 2; $X^2 = 35.8$; d.f. = 16; p -value = <0.01). Finally, the fifth test only used genets with six or more ramets only and HWA levels of absent and present. The result of this test was non-significant ($X^2 = 2.1$; d.f. = 4; p -value = 0.71).

Table 2. The numbers of ramets with HWA absent and present for genets comprising five or more ramets are shown. These are the data that were used for the fourth Chi-square test ($X^2 = 35.8$; d.f. = 16; p -value = <0.01).

Generic genet number	Number of ramets (i.e. clones or replicates) with HWA absent	Number of ramets (i.e. clones or replicates) with HWA present
37	4	1
39	1	6
40	1	5
45	0	6
51	0	5
75	0	5
78	0	5
79	0	6
80	0	5
81	1	5
82	0	5
83	0	5
84	0	5
87	0	5
115	0	5
116	0	5
125	0	5

Discussion

HWA distribution among the hemlock trees was indistinguishable from random. The hypothesis of spatial clumping of HWA in the seed orchard could be rejected based on the non-significant Z-tests of the join-count statistics. This helped to justify pooling all of the genets across the two blocks for the Chi-square analysis because spatial configuration could be tentatively eliminated as an explanatory variable for HWA levels on the different trees. There was no apparent local uniqueness in space experienced by the hemlock trees that would affect the spatial distribution of HWA.

However, spatial variation could occur at scales other than trees and in patterns other than clumped or random distribution. This variation could also be due to factors other than colonization and dispersal. Local uniqueness contributes to spatial heterogeneity (Levins 1976). Phase differences in predator-prey cycling could result in spatial variation. Spatial variation could also exist within trees, such in terms of microclimate or fine-scale habitat resources (e.g. availability of feeding locations), though this study's scale was at the level of trees. Each tree seems a relatively stable habitat for HWA until it is removed as part of management. If recurrent disturbance can prevent a "system from ever attaining a monotonous homogenous steady state" and if disturbance at local scales can be asynchronous at more regional scales, adding microvariation to a system due to phase or maturity differences (Levins 1976), then there may not be an overall steady state of HWA levels on hemlock trees in this seed orchard. However, the trees are removed randomly in regard to HWA level.

The Chi-square analysis shows that there probably is a genetic basis for different levels of HWA on western hemlock trees in the seed orchard, but there are limitations to this interpretation. All of the tests using HWA levels of absent, low, and high were significant, meaning that frequencies of ramets with HWA levels of absent, low, and high varied by genet. However, this might not say much about resistance to HWA if most of the variation in frequency is due to low and high levels, which were subjectively, but not absolutely, different from each other and both represent an HWA infestation. The fact that the vast majority of genets with four or more ramets had no trees lacking HWA (21 out of 34 in total; **bolded** in Table 1) and none of

these genets was exclusively HWA-free (Table 1), implies little or no strong genetic resistance to HWA. This is supported by the non-significant result of the Chi-square test of HWA presence/absence frequencies among genets with four or more ramets. However, there may be genetic variation in HWA susceptibility or tolerance because some genets tended to host more HWA than others.

The small numbers of ramets in the different genets reduced the degrees of freedom for the Chi-square tests and thus the ability to infer genetic differences in frequencies of HWA levels. For example, only five genets fit the criteria of having six or more ramets (i.e., only six or seven ramets because no genets had more than seven ramets) for the last test, which was non-significant due to the low degrees of freedom. If more of the genets had been composed of more ramets, then more of the data could have been used. There was significant variation by genet in presence/absence of HWA for genets with five or more ramets, though. The genets used for this test are worth examining more closely for possible resistance to HWA because of this significance and their relatively large number of ramets (Table 2). The genet with the generic number 37 uniquely had HWA absent on four out of five ramets, whereas all of the other genets had HWA present on the majority of ramets and most of these genets had no ramets lacking HWA. Genet number 37 may be worth further investigating for possible resistance to HWA.

In conclusion, a spatial pattern of clumping did not explain the distribution of HWA in the seed orchard and there was some evidence of genetic variation in hemlock in terms of HWA.

Chapter 4: General Conclusion

The *Leucopis* flies appear to be important predators of HWA in the Pacific Northwest. They may control HWA populations and keep them from reaching damaging levels. The results of this study suggest that the *Leucopis* spp. from the Pacific Northwest are deserving of further study as biological control agents for HWA in eastern North America.

There is increasing evidence to suggest that the *Leucopis* flies may be effective biocontrols of HWA. They are the most, or some of the most, abundant predators associated with HWA at many sites in the Pacific Northwest (Kohler *et al.* 2016; Grubin *et al.* 2011; Kohler *et al.* 2008). They are relative specialists of HWA (Grubin *et al.* 2011). They will feed, survive, develop, and reproduce on HWA in eastern North America (Motley *et al.* 2017).

Questions that should be considered before proceeding with introduction of *Leucopis* into eastern North America as biocontrols are as follows.

Will introduction sites suit *Leucopis* climatically?

Where will the source populations for *Leucopis* be located in the Pacific Northwest and what considerations will go into determining these sources? One consideration may be climate. Will the *Leucopis* survive and reproduce for the long term at release sites in eastern North America? This question has been somewhat addressed in the affirmative by a field release study in New York and Tennessee, the respective northern and southern limits of the range of eastern HWA, demonstrating survival, reproduction, and recovery of *Leucopis* after four weeks (Motley *et al.* 2017).

Establishment of *Leucopis* may face similar challenges to those faced by HWA and *La. nigrinus* in eastern North America. In general, climate is drier and colder in the East than it is in the Pacific Northwest. Climate in eastern North America has been shown to affect HWA's spread, with lower temperatures having possibly slowed its northward expansion (Havill *et al.* 2014) and persistence at sites, which has been shown to be positively correlated with winter minimum temperatures (Sumpter 2016). Climate has appeared to affect establishment and persistence of *La. nigrinus*; beetles collected from coastal sites in the Pacific Northwest seem to establish better in warmer climates in the Southeast, while beetles from inland Idaho and Montana may be more cold adapted and may fare better in colder climates in the Northeast (Havill *et al.* 2016b). In potential future biocontrol efforts using *Leucopis*, source sites and

introduction sites should ideally be climatically matched, such as by using USDA plant hardiness zones, as is now being done with *La. nigrinus* (Havill *et al.* 2016b).

Will *Leucopis* and *Laricobius nigrinus* compete, and would that competition affect the success of biocontrol?

Should sites with already established *La. nigrinus* be avoided due to possible competition? If competition would occur, should only one or neither of the *Leucopis* spp. be introduced to avoid competition?

Introduced *Leucopis* will share eastern HWA with *La. nigrinus*, but interspecific competition may not necessarily reduce the effectiveness of biocontrol. *Laricobius nigrinus* is now found almost everywhere in the East where HWA occurs, so avoiding beetle populations might be an infeasible and impractical objective of *Leucopis* introduction. Instead of being a "silver bullet" for controlling HWA on their own, the *Leucopis* may control HWA in conjunction with and complementary to *La. nigrinus*. Furthermore, effectiveness of top-down control may be increased with multiple predators that complement instead of compete with each other. Multiple predators may be necessary to control herbivores (Van Driesche *et al.* 2010). Since *Le. argenticollis*, *Le. piniperda*, and *La. nigrinus* are sympatric in the Pacific Northwest, and the *Leucopis* spp. feed on both generations of HWA while *La. nigrinus* does not, perhaps the species complement more than they compete with each other on HWA. Competition may actually promote coexistence under certain circumstances. If intraspecific interference competition is greater than interspecific interference competition and outweighs the effects of exploitative competitive superiority, then coexistence can be promoted (Mills 2006).

Competition could reduce the effectiveness of biocontrol using multiple enemies. Phenological differences can result in competitive exclusion if the earlier species affects the shared resource negatively for the latecomer (Mills 2006). Denoth *et al.* (2002) found that natural enemy establishment in 108 biocontrol projects was significantly greater for single rather than multiple introductions, suggesting the importance of interspecific competition, though success rate was not related to the number of enemies introduced. If the higher abundances of *La.*

nigrinus than the *Leucopis* spp. during the progreiens generation resulted in reduction in the quantity or quality of the HWA resource for *Leucopis* later in the season, then the complementarity of *La. nigrinus* and *Leucopis* as predators could be reduced due to exploitative competition.

Ecosystems are complex and species at lower trophic levels may be kept from reaching outbreaks levels because of the plethora of other species at higher levels consuming them. The enemy release hypothesis and thus the rationale of biocontrol fit this conception of top-down control in ecosystems. Indeed, in surveys of natural enemies associated with native HWA on hemlock in China, more than 60 species of predaceous insects in seven families were collected (Wang *et al.* 1998). Kohler (2007) found a total of 55 species of predators in 14 families on western hemlock infested with HWA at 16 locations in Oregon and Washington. Top-down control that possibly keeps HWA from reaching outbreak levels where it is native may result from the combined forces of such large assemblages of predators, some of which may be specialists of HWA, but many of which may also be generalists. Will diverse, native, generalist predators in eastern North America contribute to control of invasive HWA? If the *Leucopis* spp. become established in the East and apparently succeed as biocontrols, will part of that success be attributable to an entire assemblage of native predators? Without such an assemblage of native predators on HWA in eastern North America, which is a reasonable supposition for an invasive species, will three specialist predators provide enough top-down control?

Considering the weak evidence for competition among the *Leucopis* spp. and *La. nigrinus*, as well as the argument for multiple enemies, introducing additional predators of HWA into eastern North America seems advisable.

How will differences in the hosts of HWA affect the novel predator-prey interaction?

Will *Leucopis* recognize eastern hemlock as a host of its prey and successfully locate hemlock trees and HWA? The predatory-prey-plant host interaction will be novel because HWA populations in eastern North America are phylogenetically distinct from those in the Pacific

Northwest and differ in their host tree species (Havill *et al.* 2016a). In the lab, feeding HWA from Connecticut and Washington to *Leucopis* resulted in no significant differences in survival or development times of *Leucopis* larvae (Motley *et al.* 2017). Although it is yet unknown if the introduced *Leucopis* will be able to locate eastern HWA on eastern hemlock, their ability to survive on the novel host has been confirmed.

Will biocontrol not succeed because bottom-up factors are more important for controlling HWA than natural enemies?

If one or both *Leucopis* spp. were successfully established for biocontrol of HWA in eastern North America, the flies still might not succeed in slowing or stopping the damage caused to hemlock by invasive HWA there because of the relative importance of bottom-up versus top-down control. As tentatively explored in Chapter 3 of this thesis using a small, observational dataset, genetic variation in susceptibility or resistance to HWA by western hemlock may partially explain its apparent resistance to HWA in addition to control of HWA by natural enemies in the Pacific Northwest. However, the limited sample size and equivocal results dampen the strength of the findings in Chapter 3. Additionally, there are mature eastern hemlocks growing side-by-side western hemlocks in Portland and Seattle that show no signs of damage by HWA. Most likely, both bottom-up and top-down controls of HWA in the Pacific Northwest help to check its numbers, but top-down control is more important. McEvoy (2002) cites Lawton and McNeill (1979) in pointing out that, as is typical in ecology, “it is not a matter of ‘either-or’ but ‘both’; it was long ago recognized that both top-down and bottom-up forces might be operating on herbivores in any given system (Lawton and McNeill 1979).”

The ancestral life cycle of HWA provides clues as to the importance of bottom-up control and begs questions about the innocuous nature of HWA in the Pacific Northwest. Evolution of a holocycle involving alternating between two host species has been hypothesized to be reinforced by negative density dependent selection against exhausting host resources (Havill and Footitt 2007). The hypersensitive, induced responses of host trees to adelgid feeding can reduce growth and thus resources available to adelgids. Host-switching from year-to-year could give hosts time

to recover (Havill and Footitt 2007). If HWA in eastern North America lacks suitable spruce species as primary hosts to complete the holocycle and, therefore, hemlock never escapes from HWA feeding, then could this help explain the invasiveness of HWA? HWA populations in eastern North America do produce sexuparae, which would fly to spruce ancestrally (McClure 1989). However, native HWA is also trapped on hemlock in the Pacific Northwest, where sexuparae are not produced (Havill *et al.* 2016a). It has been observed that populations of HWA in eastern North America increase rapidly upon initially infesting a healthy tree, which reduces new growth the following year, causing the population to feed on less nutritious old growth and then crash in a kind of density dependence (McClure 1991). Perhaps such host-related density dependence regulates HWA in the Pacific Northwest, though populations do not apparently affect the quality of host trees the way they do in the East, making this explanation questionable.

Other concerns with introducing *Leucopis*

Are there any close relatives of the *Leucopis* spp. in eastern North America that may be at-risk of hybridization with introduced *Leucopis*, such as occurred with *La. nigrinus* and *La. rubidus*? Hybridization with native species is a potentially valid concern with any introduced biocontrol agent. *Leucopis obscura*, which was originally introduced from Europe for biocontrol of the invasive balsam woolly adelgid (*Adelges piceae*) on fir, feeds and occurs on the pine adelgid (*Pineus strobi*) in the East, but has not been observed on HWA (Montgomery and Lyon 1996, cited in Ross *et al.* 2011). Wallace and Hain (2000, cited in Ross *et al.* 2011) found low numbers of an unknown *Leucopis* sp. on invasive HWA at two sites using twig sampling and beat sheeting in only one of two survey years; the species comprised 2 and 6.6 percent of all predators at each site, respectively. Should the range of invasive HWA in eastern North America be well covered or represented by initial *Leucopis* introductions? This could increase the chances of establishment and control success, but also amplify the risks of unforeseen consequences. Should only adult flies be introduced to avoid parasitoids, as recommended by Ross *et al.* (2011)? Parasitoids could hamper the effectiveness of the *Leucopis* by increasing their mortality and decreasing their population growth, so the answer to this last question is probably yes.

Future work

An ideal experiment to account for both bottom-up and top-down controls of HWA in consideration of biocontrol would involve eastern and western hemlocks infested with controlled levels of HWA in the same environment in eastern North America and a full factorial design involving different combinations of the *La. nigrinus*, *Le. argenticollis*, and *Le. piniperda*.

Often overlooked considerations of choosing biocontrol agents include adaptation to the target host and genetic diversity of the biocontrol source populations. If potential for adaptation to the target host is desirable in a biocontrol agent, then source populations that are genetically diverse should be selected to enable potential evolution of adaptation (Roderick and Navajas 2003). This leads to the recommendation of sourcing *Leucopis* for introduction from multiple sites to maximize and well represent genetic diversity, and thus adaptability, of the species. Larger introduction sizes and multiple source populations could reduce the loss of potentially adaptive alleles by founder effects. This is especially important for any introduced population because typical population assumptions about dispersal and gene flow being in equilibrium with genetic drift do not hold when limited introduced populations are far removed from larger source populations (Roderick and Navajas 2003). Potential local adaptations of *Leucopis* populations to environmental variables are also worth considering and this could inform matching source populations with introduction sites. Sites with similar surrounding forest habitat, latitude, elevation, and distance from the ocean could be matched, for example. This is, however, not very practical considering, for instance, the urgency of the problem of HWA-driven hemlock decline in eastern North America.

Why should there be skepticism about the future success of HWA biocontrol with *La. nigrinus*?

Laricobius nigrinus has been introduced since 2003. Over 200,000 beetles have been released at over 20 release sites since then (Havill *et al.* 2016b). Although geographically limited in early years of the program, source populations have come from Vancouver, British Columbia,

the Seattle, Washington area, Montana, and Idaho (Havill *et al.* 2016b). The success of *La. nigrinus* as a biocontrol agent should not be much hampered by loss of adaptive diversity due to founder effects or genetic drift given the sheer numbers of beetles introduced and the diversity of source populations over a more than 15 year-long period of introductions. Nor should its success be adversely affected by lack of establishment due to maladaptation to eastern environments, including climate, because *La. nigrinus* populations have been established from the southern Appalachian Mountains to New England, the entire invaded range of HWA (Mausel *et al.* 2011). However, as previously stated, *La. nigrinus* has not succeeded in controlling HWA. Indeed, “the ratio of *Laricobius* spp. to HWA remains much lower at the release sites than observed in their native range” (Mausel *et al.* 2011).

Final conclusion

The *Leucopis* spp. hold promise as biocontrols that will slow hemlock decline and mortality by controlling the invasive adelgid. *Laricobius nigrinus* has not succeeded as a biocontrol agent of HWA and bottom-up control of HWA, though uncertain, is probably less important than top-down control. In Chapter 2, it was shown that the *Leucopis* spp. were together more abundant than *La. nigrinus* and that their densities were more strongly correlated with HWA’s densities. *Leucopis argenticollis* was found to be more abundant than *Le. piniperda*, as was also found by Kohler *et al.* (2008). As confirmed by this study, the *Leucopis* spp. feed on both generations of HWA, while *La. nigrinus* only feeds on the early (progreddiens) generation (Kohler *et al.* 2016). This implies that *Leucopis* could complement *La. nigrinus* in biocontrol of HWA (Grubin *et al.* 2011) and that the flies are superior to the beetle for controlling HWA throughout the season. Chamaemyiid flies have successfully controlled other adelgids (see citations in Ross *et al.* 2011). Besides minute pirate bugs (Anthocoridae), Chamaemyiidae is the only insect family to have ever done so, though this was in controlling *Pineus* spp., a different adelgid genus from *Adelges* (Zilahi-Balogh *et al.* 2002, cited in Kohler 2007). The silver flies have been shown to survive and reproduce on eastern hemlock at sites in New York and Tennessee (Motley *et al.* 2017). Motley *et al.* (2017) also found no significant differences in

development, survival, or percent parasitism between *Leucopis* fed HWA from either Connecticut or Washington, respectively, on eastern hemlock or western hemlock. This thesis adds information to the body of knowledge on the Pacific Northwest natives *Le. argenticollis* and *piniperda*.

Bibliography

- Adkins, J. K., and L. K. Rieske. 2013. Loss of a foundation species due to an exotic invader impacts terrestrial arthropod communities. *Forest Ecology and Management* 295: 126-135.
- Amarasekare, P. 2008. Coexistence of intraguild predators and prey in resource-rich environments. *Ecology* 89: 2786–2797.
- Bentz, S. E., Riedel, L. G. H., Pooler, M. R., and A. M. Townsend. 2002. Hybridization and self-compatibility in controlled pollinations of eastern North American and Asian hemlock (*Tsuga*) species. *Journal of Arboriculture* 28: 200-205.
- Brooks, R. T. 2000. Effects of the removal of overstory hemlock from hemlock-dominated forests on eastern redback salamanders. *Forest Ecology and Management* 149: 197-204.
- Cheah, C., Montgomery, M., Salem, S., Parker, B., Skinner, M., and S. Costa. 2004. Biological control of hemlock woolly adelgid, pp. 89-102. In FHTET-2004-04. U.S. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV, US.
- Cowles, R. and A. Lagalante. 2009. Activity and persistence of systemic insecticides for managing hemlock woolly adelgids, pp. 17-18. In K. McManus and K. W. Gottschalk (eds.), Proceedings, 20th U.S. Department of Agriculture interagency research forum on invasive species, 13-16 January 2009, Annapolis, MD. Gen. Tech. Rep. NRS- P- 51, U.S. Department of Agriculture, Forest Service, Northern Research Station, Newtown Square, PA.
- Culliney, T. W., Beardsley, Jr., J. W., and J. J. Drea. 1988. Population regulation of the Eurasian pine adelgid (Homoptera: Adelgidae) in Hawaii. *Journal of Economic Entomology* 81: 142-147.
- Dale, V. H., Joyce, L. A., McNulty, S., Neilson, R. P., Ayres, M. P., Flannigan, M. D., Hanson, P. J., Irland, L. C., Lugo, A. E., Peterson, C. J., Simberloff, D., Swanson, F. J., Stocks, B.

- J., and B. M. Wotton. 2001. Climate Change and Forest Disturbances. *Bioscience* 51: 723-734.
- Darwin, C. 1859. On the origin of species by means of natural selection, or, the preservation of favoured races in the struggle for life. J. Murray, London.
- Davis, M. 2003. Biotic globalization: Does competition from introduced species threaten biodiversity? *Bioscience* 53: 481–489.
- Davis, G. A., Salom, S. M., Brewster, C. C., Onken, B. P., and L. T. Kok. 2012. Spatiotemporal distribution of the hemlock woolly adelgid predator *Laricobius nigrinus* after release in eastern hemlock forests. *Agricultural and Forest Entomology* 14: 408-418.
- Dilling, C, Lambdin, P., Grant, J., and R. Rhea. 2010. Spatial and temporal distribution of Imidacloprid in Eastern Hemlock in the Southern Appalachians. *Journal of Economic Entomology* 103(2): 368- 373.
- Evans, D. M., Aust, M. W., Dolloff, C. A., Templeton, B. S., and J. A. Peterson. 2011. Eastern hemlock decline in riparian areas from Maine to Alabama. *Northern Journal of Applied Forestry* 28(2): 97-104.
- Ellison, A. M., Plotkin, A. A. B, and S. Khalid. 2015. Foundation Species Loss and Biodiversity of the Herbaceous Layer in New England Forests. *Forests* 7(1): 9.
- Denoth, M., Frid, L., and J. H. Meyers. 2002. Multiple agents in biological control: improving the odds?. *Biological Control* 24: 20-30.
- Farjon, A. 2013a. *Tsuga canadensis*. IUCN Red List Threat. Species 2013. at <http://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T42431A2979676.en>
- Farjon, A. 2013b. *Tsuga caroliniana*. IUCN Red List Threat. Species 2013. at <http://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T34200A2850654.en>
- Farnsworth, E. J., Plotkin, A. A., and A. Ellison. 2012. The relative contributions of seed bank, seed rain, and understory vegetation dynamics to the reorganization of *Tsuga canadensis* forests after loss due to logging or simulated attack by *Adelges tsuga*. *Canadian Journal of Forest Research* 42: 2090-2105.
- Greathead, D. J. 1995. The *Leucopis* spp. (Diptera: Chamaemyiidae) introduced for biological control of *Pineus* sp. (Homoptera: Adelgidae) in Hawaii: implications for biological control of *Pineus boernerii* in Africa. *The Entomologist* 114: 83-90.
- Grubin, S. M., Ross, D. W., and K. F. Wallin. 2011. Prey suitability and phenology of *Leucopis* spp. (Diptera: Chamaemyiidae) associated with hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. *Environmental Entomology* 40: 1410–1416.
- Hairston, N.G., Smith, F.E., and Slobodkin L. 1960. Community structure, population control and competition. *American Naturalist* 94: 421-425.

- Havill, N. P., and R. G. Footitt. 2007. Biology and evolution of Adelgidae. *Annual Review of Entomology* 52: 325-349.
- Havill, N. P., Montgomery, M. E., Yu, G., Shiyake, S., and A. Caccone. 2006. Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the sources of the introduction to eastern North America. *Annals of the Entomological Society of America* 99: 195-203.
- Havill, N. P., Davis, G., Mausel, D. L., Klein, J., McDonald, R., Jones, C., Fischer, M., Salom, S., and A. Caccone. 2012. Hybridization between a native and introduced predator of Adelgidae: An unintended result of classical biological control. *Biological Control* 63: 359-369.
- Havill, N. P., Vieira, L. C., and S. M. Salom. 2014. Hemlock woolly adelgid: A threat to eastern forests. *National Woodlands Spring* 37(2): 14-15
- Havill, N. P., Shiyake, G., Lamb, G. A., Footitt, R. G., Yu, G., Paradis, A., Elkinton, J., Montgomery, M. E., Sano, M., and A. Caccone. 2016a. Ancient and modern colonization of North America by hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), an invasive insect from East Asia. *Molecular Ecology* 25(9): 2065-2080.
- Havill, N. P., Vieira, L. C., and S. M. Salom. 2016b. Revised. Biology and control of the hemlock woolly adelgid. FHTET-2014-05. U.S. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team.
- Heger, T., and J. Jeschke. 2014. The enemy release hypothesis as a hierarchy of hypotheses. *Oikos* 123, 741–750.
- Holmes, T. P., Liebhold, A. M., Kovacs, K. F., and B. V. Holle. 2010. A spatial-dynamic value transfer model of economic losses from a biological invasion. *Ecological Economics* 70, 86–95.
- Joseph, S. V., Kristine, B. S., Quick, J., and J. L. Hanula. 2011a. The range and response of neonicotinoids on hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae). *Journal of Environmental Horticulture* 29(4): 197–204.
- Joseph, S. V., Hanula, J. L., and S. K. Braman. 2011b. Distribution and abundance of *Adelges tsugae* (Hemiptera: Adelgidae) within hemlock trees. *Journal of Economic Entomology* 104(6): 1918-1927.
- Kohler, G. R. 2007. Predators associated with hemlock woolly adelgid infested western hemlock in the Pacific Northwest. M.S. thesis, Oregon State University, Corvallis, Oregon.
- Kohler, G. R., Stiefel, V. L., Wallin, K. F., and D. W. Ross. 2008. Predators associated with the hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. *Environmental Entomology* 37(2): 494-504.

- Kohler, G. R., Wallin, K. F., and D. W. Ross. 2016. Seasonal phenology and abundance of *Leucopis argenticollis*, *Leucopis piniperda* (Diptera: Chamaemyiidae), *Laricobius nigrinus* (Coleoptera: Derodontidae) and *Adelges tsugae* (Homoptera: Adelgidae) in the Pacific Northwest USA. *Bulletin of Entomological Research* 106(4): 546-550.
- Lemos, P. and A. Finzi. 2011. The decline of a northeastern foundation species (*Tsuga canadensis*) and its implications for forest carbon storage. In *Proceedings, 96th Annual Meeting of the Ecological Society of America, 7-12 August, 2011, Austin, TX.*
- Levin, S. 1976. Population dynamic models in heterogeneous environments. *Annual Review of Ecology and Systematics* 7: 287-310.
- Li, X., Preisser, K. L., Boyle, K. J., and T. P. Holmes. 2014. Potential social and economic impacts of the hemlock woolly adelgid in southern New England. *Southeastern Naturalist* 13, 130–146.
- Lowe, J. H. 1966. Biology and dispersal of *Pineus pinifoliae* (Fitch). Ph.D. dissertation. Yale University, New Haven, Connecticut. Yale.
- Noonburg, E., and J. Byers. 2005. More harm than good: when invader vulnerability to predators enhances impact on native species. *Ecology* 86(10): 2555–2560.
- Martin, K. L., and P. C. Goebel. 2013. The foundation species influence of eastern hemlock on biodiversity and ecosystem function on the Unglaciaded Allegheny Plateau. *Forest Ecology and Management* 289, 143–152.
- Mausel, D. L., Salom, S. M., Kok, L. T., and G. A. Davis. 2010. Establishment of the hemlock woolly adelgid predator, *Laricobius nigrinus* (Coleoptera: Derodontidae), in the eastern United States. *Environmental Entomology* 39(2): 440-448.
- Mausel, D. L., Davis, G. A., Lamb, A. S., Zilahi-Balogh, G. M. G., Kok, L. T., and S. M. Salom. 2011. Chapter 6: *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), pp. 90-102. In B. Onken and R. Reardon (eds.), *Implementation and status of biological control of the hemlock woolly adelgid*. FHTET-2011-04. U.S. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.
- Mayfield, A. III, Reynolds, B. C., Coots, C. I., Havill, N. P., Brownie, C., Tait, A. R., Hanula, J. L., Joseph, S. V., and A. B. Galloway. 2015. Establishment, hybridization and impact of *Laricobius* predators on insecticide-treated hemlocks: Exploring integrated management of the hemlock woolly adelgid. *Forest Ecology and Management* 33: 1-10.
- McClure, M. 1989. Evidence of a polymorphic life cycle in the hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). *Annals of the Entomological Society of America* 82(1): 50-54.
- McClure, M. 1990. Role of wind, birds, deer, and humans in the dispersal of hemlock woolly adelgid (Homoptera: Adelgidae). *Environmental Entomology* 19(1): 36-43.

- McClure, M. 1991. Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on *Tsuga canadensis*. *Environmental Entomology* 20(1): 258-264.
- McClure, M. 2001. Biological control of hemlock woolly adelgid in the eastern United States. FHTET-2000-08. U.S. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team.
- McEvoy, P. B. 2002. Insect-plant interaction on a planet of weeds. *Entomologia Experimentalis et Applicata* 104(1): 165-179.
- Menzler-Hokkanen, I. 2007. Chapter 2: Socioeconomic Significant of Biological Control, pp. 13-25. In J. Eilenberg and H. M. T. Hokkanen (eds.), *An ecological and societal approach to biological control*. Dordrecht, The Netherlands, Springer.
- Mills, N. 2006. Interspecific competition among natural enemies and single versus multiple introduction in biological control, pp. 191-220. In J. Brodeur and G. Boivin (eds.), *Trophic and guild interactions in biological control, progress in biological control vol. 3*. Dordrecht, The Netherlands: Springer: 191-220.
- Montgomery, M. and S. Lyon. 1996. Natural enemies of adelgid in North America: their prospect for biological control of *Adelges tsugae* (Homoptera: Adelgidae), pp. 89-102. In S. Salom, T. Tigner and R. Reardon (eds.), *Proceedings: First Hemlock Woolly Adelgid Review*, 12 October, 1995, Charlottesville, VA. FGTET-96-10. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.
- Motley, K., Havill, N. P., Arsenault-Benoit, A. L., Mayfield, A. E., Ott, D. S., Ross, D. W., Whitmore, M. C., and K. F. Wallin. 2017. Feeding by *Leucopis argenticollis* and *Leucopis piniperda* (Diptera: Chamaemyiidae) from the western USA on *Adelges tsugae* (Homoptera: Adelgidae) in the eastern USA. *Bulletin of Entomological Research*. Published online 14 March 2017.
- Onken, B., and R. Reardon. 2011. Chapter 22: An overview and outlooks for biological control of hemlock woolly adelgid, pp. 230-236. In B. Onken and R. Reardon (eds.), *Implementation and status of biological control of the hemlock woolly adelgid*. FHTET-2011-04. U.S. Department of Agriculture, Forest Health Technology Enterprise Team, Morgantown, WV.
- Paradis, A., Elkinton, J., Hayhoe, K., and J. Buonaccorsi. 2007. Role of winter temperature and climate change on the survival and future range expansion of the hemlock woolly adelgid (*Adelges tsugae*) in eastern North America. *Mitigation and Adaptation Strategies for Global Change* 13(5): 541-554.

- Pimentel, D., Zuniga, R. and Morrison, D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52(3): 273–288.
- Radosevich, S., Holt, J., and C. Ghera. 2007. Ecology of weeds and invasive plants: Relationship to agriculture and natural resource management: Chapter 3: Invasibility of agricultural and natural ecosystems. 3rd ed. John Wiley and Sons, Inc., Hoboken, NJ.
- Roderick, G. K., and M. Navajas. 2003. Genes in new environments: Genetics and evolution in biological control. *Nature Reviews: Genetics* 4: 889-899.
- Rosenheim, J. A., Kaya, H. K., Ehler, L. E., Marois, J. J., and B. A. Jaffee. 1995. Intraguild predation among biological control agents: Theory and evidence. *Biological Control* 5(3): 303-335.
- Root, R. B. 1967. The niche exploitation pattern of the blue-grey gnatcatcher. *Ecological Monographs* 37: 317-350.
- Ross, D. W., Gaimari, S. D., Kohler, G. R., Wallin, K. F., and S. M. Grubin. 2011. Chapter 8: Chamaemyiid predators of the hemlock woolly adelgid from the Pacific Northwest, pp. 97-106. In B. Onken and R. Reardon (eds.), *Implementation and status of biological control of the hemlock woolly adelgid*. FHTET-2011-04. U.S. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.
- Russo, N., Cheah, C., and M. Tingley. 2016. Experimental evidence for branch-to-bird transfer as a mechanism for avian dispersal of the hemlock woolly adelgid (Hemiptera: Adelgidae). *Environmental Entomology* 45(5): 1107-1114.
- Seastedt, T., Hobbs, R., and K. Suding. 2008. Management of novel ecosystems: are novel approaches required?. *Frontiers in Ecology and the Environment* 6(10): 547-553.
- Simberloff, D. and T. Dayan. 1991. The guild concept and the structure of ecological communities. *Annual Review of Ecology and Systematics* 22: 115-143.
- Sumpter, K. 2016. Evaluating a potential area-wide IPM strategy for managing hemlock woolly adelgid in the eastern United States. M.S. thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA.
- Stadler, B., Mueller, T., Orwig, D., and R. Cobb. 2005. Hemlock woolly adelgid in New England forests: canopy impacts transforming ecosystem processes and landscapes. *Ecosystems* 8(3): 233-247.
- Stoetzel, M. 2002. History of the introduction of *Adelges tsugae* based on voucher specimens in the Smithsonian Institute National Collection of Insects, pp. 12. In B. Onken, R. Reardon, and J. Lashomb (eds.), *Proceedings, Hemlock woolly adelgid in the eastern United States*

- symposium, 5-7 February, 2002. New Jersey Agricultural Experimental Station, Rutgers University, East Brunswick, NJ
- Tingley, M. W., Orwig, D. A., Field, R., and G. Motzkin. 2002. Avian response to removal of a forest dominant: Consequences of hemlock woolly adelgid infestations. *Journal of Biogeography* 29(10/11): 1505-1516.
- Tobin, P. C., Turcotte, R. M., and D. A. Snider. 2013. When one is not necessarily a lonely number: initial colonization dynamics of *Adelges tsugae* on eastern hemlock, *Tsuga canadensis*. 15(9): 1925-1932.
- Van Driesche, R. G., Carruthers, R. I., Center, T., Hoddle, M. S., Hough-Goldstein, J., Morin, L., Smith, L., Wagner, D. L., Blossey, B., Brancatini, V., Casagrande, R., Causton, C. E., Coetzee, J. A., Cuda, J., Ding, J., Fowler, S. V., Frank, J. H., Fuester, R., Goolsby, J., Grodowitz, M., Heard, T. A., Hill, M. P., Hoffmann, J. H., Huber, J., Julien, M., Kairo, M. T. K., Kenis, M., Mason, P., Medal, J., Messing, R., Miller, R., Moore, A., Neunschwander, P., Newman, R., Norambuena, H., Palmer, W. A., Pemberton, R., Panduro, A. P., Pratt, P. D., Rayamajhi, M., Salom, S., Sands, D., Schooler, S., Schwarzlaender, M., Sheppard, A., Shaw, R., Tipping, P. W., and R. D. van Klinken. 2010. Classical biological control for the protection of natural ecosystems. *Biological Control* 54(1), S2–S33.
- Vose, J. M., Wear, D. N., Mayfield, A. E. III, and N. C. Dana. 2013. Hemlock woolly adelgid in the southern Appalachians: Control strategies and potential management responses. *Forest Ecology and Management* 291: 209-219.
- Wallace, M. S. and F. P. Hain. 2000. Field survey and evaluation of native and established predators of the hemlock woolly adelgid (Homoptera: Adelgidae) in the southeastern United States. *Environmental Entomology* 29(3): 638-644.
- Wang, H., Yao, G., Li, C., Zhang, C., Li, L., Guo, H., and D. Zhoo. 1998. Investigation on hemlock woolly adelges and its natural enemies in China, pp. 41-46. In *Proceedings, Resource technology 1997: Beijing international symposium proceedings*. China Forestry Publishing House, Beijing.
- Yasuda, H., Kikuchi, T., Kindlmann, P. and S. Sato. 2001. Relationships between attack and escape rates, cannibalism, and intraguild predation in larvae of two predatory ladybirds. *Journal of Insect Behavior* 14(3): 373-384.
- Zilahi-Balogh, G. M. G., Kok, L. T., and S. M. Salom. 2002. Host specificity of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), a potential biological control agent of the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). *Biological Control* 24(2), 192-198.