Environmental Entomology, 2016, 1–8
doi: 10.1093/ee/nvw128
Research

OXFORD

Population Ecology

Emergence, Seasonality, and Hybridization of *Laricobius nigrinus* (Coleoptera: Derodontidae), an Introduced Predator of Hemlock Woolly Adelgid (Hemiptera: Adelgidae), in the Tennessee Appalachians

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Received 27 June 2016; Accepted 28 August 2016

Abstract

From 2010 through 2013, adult emergence and seasonality of *Laricobius nigrinus* Fender, an introduced predatory species native to western North America, as well as hybridization with the native species *Laricobius rubidus* (LeConte), were evaluated using emergence traps and beat-sheet sampling in areas of previous release against hemlock woolly adelgid, *Adelges tsugae* Annand. The shortest emergence period of adult *L. nigrinus* was 7 wk beginning 22 October 2010, and the longest emergence was 15 wk beginning 17 October 2012. Native *L. rubidus* also were collected from emergence traps placed on the ground surface and beat-sheet samples all 3 yr, with emergence of *L. rubidus* initiating later than *L. nigrinus* each season. Seasonality of both *Laricobius* species was similar across a 44-mo study period. Adult *L. nigrinus* were present from October through April, and larvae of *Laricobius* spp. were collected from February to May. The average number of *L. nigrinus* from emergence traps was significantly greater than the average number of beetles collected from beat-sheet samples in 2010, while the converse was observed during 2012. Hybridization between *L. nigrinus* and *L. rubidus* was documented from 10.75% of specimens collected during 2010 and 2011, indicating periodic interbreeding between the introduced and native species. These findings suggest emergence trapping may be a useful method to assess establishment, population densities, and seasonality of *Laricobius* species in areas of release to enhance their use in management of *A. tsuage*.

Key words: *Laricobius nigrinus*, *Laricobius rubidus*, hemlock woolly adelgid, *Adelges tsugae*, biological control

Eastern hemlock, *Tsuga canadensis* Carrière, is an important ecological component of forests in the southern Appalachians, as it provides unique habitats for many plant and animal species and helps regulate stream temperatures through overstory shading (Ward et al. 2004, Sackett et al. 2011). Because it provides locally stable conditions for other species and modulates and stabilizes fundamental ecosystem processes in many eastern forest ecosystems, eastern hemlock is considered a foundation species (Dayton 1972, Ellison et al. 2005). About 36,400 ha (ca. 18% of total forested area) in the Great Smoky Mountains National Park (GRSM) contain some hemlock component (NPS GRSM 2013), further illustrating its ecological importance. Currently, eastern hemlock is threatened by the introduced invasive insect hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae).

Immature and adult *A. tsugae* feed by inserting their stylet into the vascular tissue of hemlock needles, twigs, and branches and extracting nutrients from the parenchyma cells (McClure et al. 2001). Heavy infestations of *A. tsugae* can cause premature foliage drop, bud abortion, and death, which may occur within 4–10 yr after initial infestation (McClure 2000, McClure et al. 2001).

An integrated management approach, including chemical and biological controls, genetic conservation, and resistance breeding, has been employed against *A. tsugae* (Potter et al. 2012, Vose et al. 2013). Chemical insecticide treatments are effective in providing short-term suppression of *A. tsugae* on individual trees; however, to maintain long-term suppression of *A. tsugae*, chemical applications would have to be repeated indefinitely [possibly every 3–6 yr in the case of imidacloprid (Coots et al. 2013, Benton et al. 2015)]. Thus, the cost of treating infestations of *A. tsugae* across the forest landscape in this manner, coupled with concern over impacts of chemical insecticides on nontarget arthropod species, make broad-scale chemical treatment of forests infeasible in many areas (Wallace and Hain 2000, Ward et al. 2004). Efforts to conserve genetic diversity of hemlock for use in breeding programs to develop resistant varieties may ultimately result in long-term reintroduction of resistant hemlock to its former range (Potter et al. 2012). However, tree breeding and reintroduction programs require many years to develop and implement and are not designed to preserve existing trees in the landscape. Therefore, biological control, or the use of natural enemies, of *A. tsugae* has been promoted as the primary long-term, broad-scale management tactic against this invasive forest pest.

Laricobius nigrinus Fender (Coleoptera: Derodontidae) (native to western North America) is a major component of biological control programs directed against *A. tsugae* (McClure 2000, Reardon and Onken 2004). Currently, >200,000 *L. nigrinus* have been released in the eastern United States since 2004, and ca. 20,000 have been released in the GRSM (Jesse Webster, personal communication; Eisenback et al. 2010, Havill et al. 2014). *L. nigrinus* continues to be reared and released in several adelgid-infested areas.

The selection and development of *L. nigrinus* as a biological control agent for release against *A. tsugae* were based on several factors. Both larvae and adults of *L. nigrinus* are predaceous on *A. tsugae*, and prefer it over other prey species (Zilahi-Balogh et al. 2002, Reardon and Onken 2004). A single beetle can consume several *A. tsugae* (ca. 15 eggs [Zilahi-Balogh et al. 2002] or ca. five adults [Lamb et al. 2005] per day). In controlled cage studies, adelgid densities were consistently lower (ca. 28%) on branches containing *L. nigrinus* compared to those where no predators were present (Lamb et al. 2005). Additionally, the biologies of *L. nigrinus* and *A. tsugae* are synchronous (Zilahi-Balogh et al. 2003a, Mausel et al. 2008). Adult *L. nigrinus* feed on developing nymphs and adults of the sistentes generation and eggs of the progredientes generation of *A. tsugae* and lay eggs during the winter months (December through February). Larval *L. nigrinus* feed on sistentes adults and progredientes eggs of *A. tsugae* from March through early May and drop to the soil to pupate and aestivate in April and May. Pupation in the soil is completed in mid- to late-summer, and adults aestivate in the soil until emergence in the fall (Zilahi-Balogh et al. 2003b, Reardon and Onken 2004).

Much of the previous work on seasonality and synchrony between *A. tsugae* and *L. nigrinus* was conducted in Virginia (Zilahi-Balogh et al. 2003a,b), and much of the initial research on the life cycle of *A. tsugae* was conducted in the northeastern United States (McClure 1991, 2000; McClure et al. 2001; Reardon and Onken 2004). The life cycle of *A. tsugae* differs in more southern locations of the Appalachians from that reported in the northeastern United States, with both the sistentes and progredientes beginning egg production about one and two months earlier in the southeastern than the northeastern United States, respectively (Deal 2007, Grant 2008). Because these differences in the biology of the host of *L. nigrinus* exist in the southern Appalachians, concomitant differences in seasonality of *L. nigrinus* may also exist between the southeastern and northeastern United States.

Another *Laricobius* species, *L. rubidus* (LeConte), is native to the eastern United States and has been collected from eastern hemlock infested with *A. tsugae* (Montgomery and Lyon 1996, Wallace and Hain 2000, Zilahi-Balogh et al. 2005, Mausel et al. 2008, Hakeem et al. 2011). Collections of *L. rubidus* from hemlock are usually infrequent, as their primary prey is the pine bark adelgid, *Pineus strobi* Hartig (Zilahi-Balogh et al. 2005, Arsenault et al. 2015, Fischer et al. 2015). However, hybridization between *L. nigrinus* and *L. rubidus* has been documented in several locations in the eastern United States (Havill et al. 2010, 2012; Arsenault et al. 2015; Fischer et al. 2015; Mayfield et al. 2015).

Documentation of seasonal biology of *L. nigrinus* has been crucial to biological control efforts; however, the use of traps to evaluate adult emergence and seasonal biology in the field is uncommon. Limited emergence ($n =$ four adults from 1,440 larvae released) was observed in soil emergence traps, in which larval *L. nigrinus* were released to monitor their aestival survival in the soil in northern Georgia (Jones et al. 2014). However, no studies exist evaluating established populations of *L. nigrinus* using traps. Much of the research on seasonality and biology of *L. nigrinus* has involved field collections in areas of release using beat-sheets or branch clippings (Mausel et al. 2008, 2010; Hakeem et al. 2011), sleeve-cage studies in the field (Lamb et al. 2005, 2006; Mausel et al. 2008), or studies in the laboratory (Zilahi-Balogh et al. 2002, 2003a,b; Lamb et al. 2007). Trapping also may be used to passively monitor and assess populations of *L. nigrinus*. Furthermore, the use of different trap designs and placements could elucidate behavioral characteristics, such as how adults migrate from the soil to the tree canopy following emergence. Therefore, in 2010, a multiyear study was initiated to 1) assess emergence and establishment of *L. nigrinus* in areas of release using emergence traps, 2) document seasonal abundance of *L. nigrinus* in the southern Appalachians, and 3) assess hybridization of *L. nigrinus* and *L. rubidus* in and near the GRSM.

Materials and Methods

Study Area

Studies were conducted at two sites where *L. nigrinus* was released from 2006 to 2008. The first site was at Blackberry Farm, Walland, TN (bordering the GRSM; 35° 41′37″ N, 083° 52′02″ W; ca. 370 m elevation). As part of a study to evaluate the feasibility of using whole-tree canopy enclosures to establish predators of *A. tsugae* (Grant et al. 2010a, Hakeem et al. 2011), 570 *L. nigrinus* adults were released on three caged trees (190 beetles per tree) on 11 December 2007 and 11 January 2008. Periodic sampling of the caged trees demonstrated that *L. nigrinus* was reproducing, and cages were removed from trees in July 2009 to allow beetles to disperse throughout the site (Hakeem et al. 2011). Adult and larval *L. nigrinus* were collected in beat-sheet sampling beginning 2010, indicating establishment. The second site was near Elkmont Campground, GRSM (35° 39′51″ N, 083° 35′25″ W; ca. 640 m elevation), where 866 adult *L. nigrinus* were released on hemlocks bordering an open field on 14 March 2006. Establishment of *L. nigrinus* at this site was determined in 2009 (Grant et al. 2010b).

Assessment of Emergence of *L. nigrinus*

To assess emergence of *L. nigrinus* in an area of release and establishment, emergence traps were placed on the ground beneath hemlock canopies at Elkmont. Traps were constructed of a wooden frame (four-sided pyramid shape, 77.5 by 77.5 by 50 cm, ca. 0.60 m^2 area per trap) covered with antivirus screen (266 by 818 microns mesh size), which guided beetles to a 125-ml collecting head mounted to a wooden plate at the top of the trap (Fig. 1). On 3 October 2010, six traps were placed under each of four canopies (tree heights ranged from ca. 7 m to ca. 14 m) of trees growing at the edge of an open field and monitored through 3 March 2011. Under each canopy, three traps were placed with the outer-most

Fig. 1. Emergence traps deployed underneath a hemlock canopy to monitor for emergence of *Laricobius* species, Elkmont, Great Smoky Mountains National Park, TN.

edge of the trap 1 m from the trunk (i.e., inner traps), and three traps were placed with the outer-most edge of the trap 2 m from the trunk (i.e., outer traps). The following season on 6 October 2011, traps were moved to trees in the adjacent forest (within 50 m of the forest edge) to allow populations of *Laricobius* associated with previous study trees to grow and have open ground on which to drop and develop in Spring 2012. Unlike Fall 2010, three traps were placed under each of eight tree canopies, and traps were monitored until 1 March 2012. In Fall 2012, emergence cages were moved back to hemlock on the border of the field, and cages were placed in the same configuration under each of four trees as in Fall 2010. Traps were monitored from 26 September 2012 to 27 March 2013. During each sampling period during each sampling year, traps were monitored every 5–14 d.

On 3 October 2010 at the Elkmont site, funnel traps were attached to the lower boles of seven eastern hemlock trees to determine if adult *L. nigrinus* emerging outside of the ground emergence traps may reach the hemlock canopy by walking up the trunk. Trunk traps were constructed per the design of Hanula and New (1996) using a metal funnel (ca. 20 cm long, 15 cm diameter) attached to the tree with the wide opening of the funnel oriented downward. A section was cut from ca. one-quarter of the diameter of the funnel to allow the longest edge to fit on the trunk of the tree, and this cut edge was placed against the trunk and attached to the tree with roofing nails and silicon caulk. A collecting head (500 ml) with a removable lid (for ease of access during monitoring) was mounted on the small opening of the funnel. A strip of metal flashing (ca. 10 cm wide) was mounted around the circumference of the trunk not covered by the funnel. This strip was coated with Fluon (DuPont, Wilmington, DE) and served as a "drift fence" to prevent *L. nigrinus* from bypassing the funnel trap and guide them into the funnel and collecting head (Hanula and New 1996). Trunk traps were monitored every 5–14 d only for the 2010–2011 sampling season.

Voucher specimens ($n = 93$) were retained from trap collections for confirmation of visual identification in the laboratory. Vouchers then were preserved in 95% ethyl alcohol at −4 °C until molecular analysis for hybridization.

Documentation of Seasonal Abundance of *L. nigrinus*

Beginning 6 January 2010 at Blackberry Farm and 3 October 2010 at Elkmont, beat-sheet sampling was conducted every 5–14 d through June 2013. Beat-sheet sampling at Elkmont was conducted on 10 trees per sampling date throughout the study period; however, the number of trees sampled at Blackberry Farm increased incrementally over time as part of another study monitoring the dispersal of *L. nigrinus* from initial release trees. From 6 January 2010 to 15 April 2010 sampling was conducted on five trees per sampling date. From 20 April 2010 to 17 February 2011 sampling was conducted on 15 trees per sampling date. On 23 February 2011 the number of trees sampled per sampling date increased to 30 trees and was maintained at that level for the remainder of the study. On each sampling date at each site, five beat-sheet samples (striking limb 5–10 times with a wooden rod ca. 1 m long while holding a canvas beat sheet [71 by 71 cm] underneath to collect dislodged predators) were conducted on accessible limbs (up to ca. 2 m high) from each sampled tree. Numbers of adult and larval *L. nigrinus* were recorded, and voucher specimens (up to 10 individuals per collection date) were collected, placed in a vial containing 70% ethyl alcohol, and taken to the laboratory for positive identification. All collections from beat-sheet sampling were combined with emergence trap data to determine seasonality of life stages of *L. nigrinus*.

Assessment of Hybridization

All *Laricobius* voucher specimens from Blackberry Farm ($n = 48$; 13 larvae, 35 adults) and Elkmont ($n = 45$; 8 larvae, 37 adults) were sent to Nathan Havill, USDA Forest Service, Hamden, CT, for molecular genetic analysis (methods described in Havill et al. 2012). In brief, DNA was extracted from specimens and six nuclear microsatellite loci were amplified after methods described in Klein et al. (2010). Genotypes were scored using the software Genemapper 4.0 (Applied Biosystems, Foster City, CA). Early generation hybrids of *L. nigrinus* and *L. rubidus* were distinguished from parent species using the software programs Structure 2.3.2 (Pritchard et al. 2000) and NewHybrids 1.1 (Anderson and Thompson 2002).

Data Analysis

Data were analyzed as a complete randomized design. Differences in the numbers of *L. nigrinus* collected from inner and outer traps at Elkmont during the Fall 2010 and 2012 emergence periods were evaluated using one-way analysis of variance (ANOVA; PROC MIXED; SAS Institute 2011). Additionally, differences between the numbers of *L. nigrinus* collected in emergence-trap and beat-sheet collections during the emergence periods of Fall 2010 and 2012 were assessed using ANOVA. Shapiro–Wilk W test for normality and Levene's test of homogeneity of variance were used to verify that data conformed to the assumptions of ANOVA. Due to heterogeneity of group variances, all data were rank transformed. Significance for all ANOVA tests was set at $P \leq 0.05$. When significant ANOVA results were obtained, post hoc analysis to detect differences among means was determined using the least significant difference (LSD) test ($\alpha = 0.05$). Kenward–Roger method was used to adjust degrees of freedom for repeated measures (weekly sampling of traps and trees), because repeated measurements are correlated and consequently require an adjustment in the error degrees of freedom used in calculating significance tests. Original means and standard error values are reported.

Results and Discussion

Assessment of Emergence of *L. nigrinus*

The emergence period of *L. nigrinus* in the Appalachians of Tennessee is similar to that documented in the literature in other regions. Specific emergence periods of *L. nigrinus* varied across years, but followed similar trends in 2010, 2011, and 2012 (Fig. 2). In 2010, emergence spanned a 7-wk period, with adult *L. nigrinus* ($n = 63$) collected at Elkmont from 22 October to 2 December 2010. Adult *L. rubidus* ($n = 6$) were collected from traps on only one date in 2010 (3 November 2010; Fig. 2A). Emergence of *L. nigrinus* in Fall 2011 spanned 10 wk, with adults ($n = 31$) collected from 14 October to 15 December 2011. Adult *L. rubidus* ($n = 15$) were collected in traps from 2 December 2011 to 6 January 2012 (Fig. 2B). Emergence of *L. nigrinus* ($n = 92$) in Fall 2012 spanned 15 wk, with adults emerging from 17 October 2012 to 29 January 2013. Adult *L. rubidus* ($n = 26$) were found in traps on several dates from 22 November 2012 to 6 February 2013 (Fig. 2C).

Trapping can be an effective means to assess the emergence period of this predator of *A. tsugae*. Generally, *L. nigrinus* began

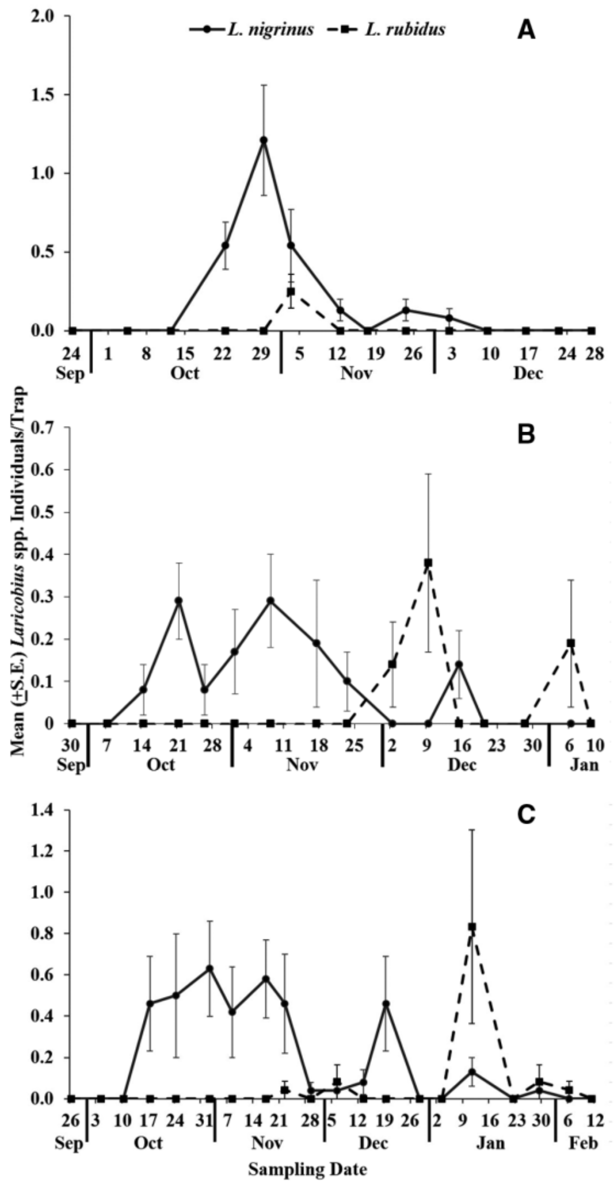

Fig. 2. Seasonal emergence of adult *L. nigrinus* and *L. rubidus* in traps at Elkmont, Great Smoky Mountains National Park, TN, (**A**) 2010, (**B**) 2011 to 2012, and (**C**) 2012 to 2013.

emerging in mid-October and completed emergence by January, with emergence of *L. rubidus* beginning 3–8 wk after the first observed *L. nigrinus* and usually continuing further into the winter months. However, the reason for the observed prolonged emergence period for *L. nigrinus* beginning in Fall 2012 is unclear. Perhaps environmental conditions (i.e., temperature and precipitation) contributed to the protracted emergence of adult *L. nigrinus*, but further investigation on the role of climatic variables is needed.

Emergence of *L. nigrinus* was statistically similar between inner and outer emergence traps at Elkmont during both 2010 and 2012. No differences were observed between numbers of *L. nigrinus* collected from inner and outer traps overall during 2010 ($\bar{x} = 0.20 \pm 0.05$, inner; $\bar{x} = 0.28 \pm 0.08$, outer; $F_{(1,\ 5.15)} = 0.001$, $P = 0.9696$) or 2012 ($\bar{x} = 0.28 \pm 0.06$, inner; $= 0.12 \pm 0.03$, outer; $F_{(1,\ 5.11)} = 1.53$, $P = 0.2695$). When the numbers of *L. nigrinus* collected on individual sampling dates from inner and outer traps were compared, no differences were observed during 2010 ($F_{(10,\ 207)} = 0.86$, $P = 0.5739$) or 2012 ($F_{(18,\ 359)} = 1.32$, $P = 0.1746$; Fig. 3). No adults of either *Laricobius* species were collected in trunk traps during the 2010–2011 sampling season.

The lack of differences observed between inner and outer emergence traps suggest that, because *L. nigrinus* is so closely associated with *A. tsugae*, adelgid populations probably were distributed relatively uniformly throughout the canopies of the trees during the study. Although previous research has shown that *L. nigrinus* may prefer adelgid infestations higher in the canopy (Mausel et al. 2010, Davis et al. 2012), emergence traps are not dependent on the distribution of *A. tsugae* within the height of the canopy, rather the concentric distribution within the canopy. Also, in the spring as *L. nigrinus* drop to the ground to pupate and aestivate over the summer, they may move small distances from where they land on the soil at the base of the tree to search for suitable pupation sites (Lamb 2005). Thus, their distribution in the canopy may not be important for collection in traps as long as traps are placed underneath the canopy. Therefore, emergence traps may be an effective tool to detect *L. nigrinus* in an area, even if beetles are restricted to the upper portion of the canopy.

The lack of collection of either *L. nigrinus* or *L. rubidus* in trunk traps may suggest that *Laricobius* adults do not walk up the trunk following emergence, but rather access hemlock foliage solely by flying. Alternatively, it is also possible that the traps were ineffective and *Laricobius* adults were able to bypass the trap by crawling over the drift fence. In longleaf pine stands, Hanula and New (1996) collected 122 different insect taxa, including numerous coleopteran genera, using the same trunk trap and drift fence design utilized here. This study was the first attempt to capture *Laricobius* spp. using this method, and further evaluation would be needed to confirm

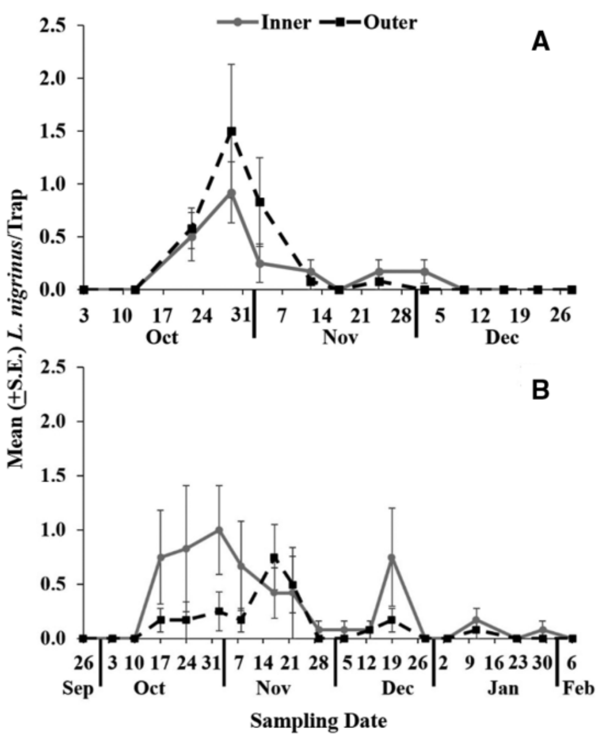

Fig. 3. Comparison of weekly trap catches in inner and outer emergence traps under hemlock canopies during (**A**) 2010 and (**B**) 2012, Elkmont, Great Smoky Mountains National Park, TN.

or deny trunk climbing by *L. nigrinus* and the efficacy of the trap for this species. If *L. nigrinus* does not use the trunk of the tree to access the tree canopy, it may warrant limited use of basal-bark sprays of systemic insecticides for *A. tsugae* control in areas where *L. nigrinus* has been released.

Documentation of Seasonal Abundance of *L. nigrinus*

When collection data from Elkmont and Blackberry Farm were compared across the 44-mo study period, seasonal trends of *Laricobius* spp. were similar (Fig. 4). Adult *L. nigrinus* were present at varying levels from October through April in all study years. *Laricobius* spp. larvae were collected from February through April during Spring 2011 and 2012. The occurrence of *Laricobius* spp. in Spring 2013 was exceptional, in that *L. nigrinus* and *L. rubidus* were both collected through late May. Additionally, the number of *L. nigrinus* collected per month dramatically increased at Elkmont during the Fall 2012 emergence when compared to previous years (Fig. 4).

The consistent recovery of *L. nigrinus* in beat-sheet samples is encouraging for biological control efforts. Although branch clipping is often conducted to acquire estimates of numbers of eggs and larvae of *L. nigrinus* (Zilahi-Balogh et al. 2003a, Mausel et al. 2010), beat-sheet sampling also can be an effective means to determine the presence of this predator of *A. tsugae*. The numbers of *L. nigrinus* collected at Elkmont increased since the study began, illustrating that *L. nigrinus* can establish and their populations increase in areas of release.

Adult *L. nigrinus* were collected in beat-sheet sampling throughout the emergence period, and when numbers of *L. nigrinus* per beat-sheet sample were compared to numbers of beetles collected per emergence trap significant differences were documented. In 2010, the average number of *L. nigrinus* collected per emergence trap over the entire emergence period ($\bar{x} = 1.43 \pm 0.40$) was significantly greater than the average number of beetles collected per beat-sheet sample ($\bar{x} = 0.30 \pm 0.12$; $F_{(1,\ 63)} = 16.10$, $P = 0.0002$). Also, greater numbers of *L. nigrinus* ($F_{(9,\ 63)} = 2.81$, $P = 0.0077$) were collected per emergence trap than per beat-sheet sample on a single sampling date (31 October 2010; Fig. 5A). While no differences were observed between emergence trap and beat-sheet sampling on individual sampling dates during the 2012 emergence period ($F_{(18,\ 96.3)} = 1.53$, $P = 0.0948$; Fig. 5B), the average number of *L. nigrinus* collected per beat-sheet sample over the entire emergence period ($\bar{x} = 1.93 \pm 0.33$) was significantly greater than the average number of beetles collected per emergence trap ($\bar{x} = 1.21 \pm 0.25$; $F_{(1,\ 37.4)} = 7.16$, $P = 0.0110$).

The similarities between beat-sheet sampling and emergence trapping, especially during 2012, indicate that populations of *L. nigrinus* can be collected multiple ways from multiple areas of infested trees. However, emergence trapping may be advantageous to beat-sheet sampling in some areas. Beat-sheet sampling is a direct sampling method and is based on a single event (or collection of events on a single date), whereas emergence traps are passive and can collect over a period of time (i.e., fewer person-hours spent field sampling). Because *L. nigrinus* may not occur on limbs accessible to beat-sheet sampling in the lower canopy (Mausel et al. 2010) or may not be present on these limbs on any specific sampling date, emergence traps may be a more reliable method of sampling during the emergence period. An added advantage to emergence trapping is that population densities may be calculated. Emergence trapping has been used to estimate populations of invasive root-feeding weevil species in northern hardwood forests (Coyle et al. 2012). By

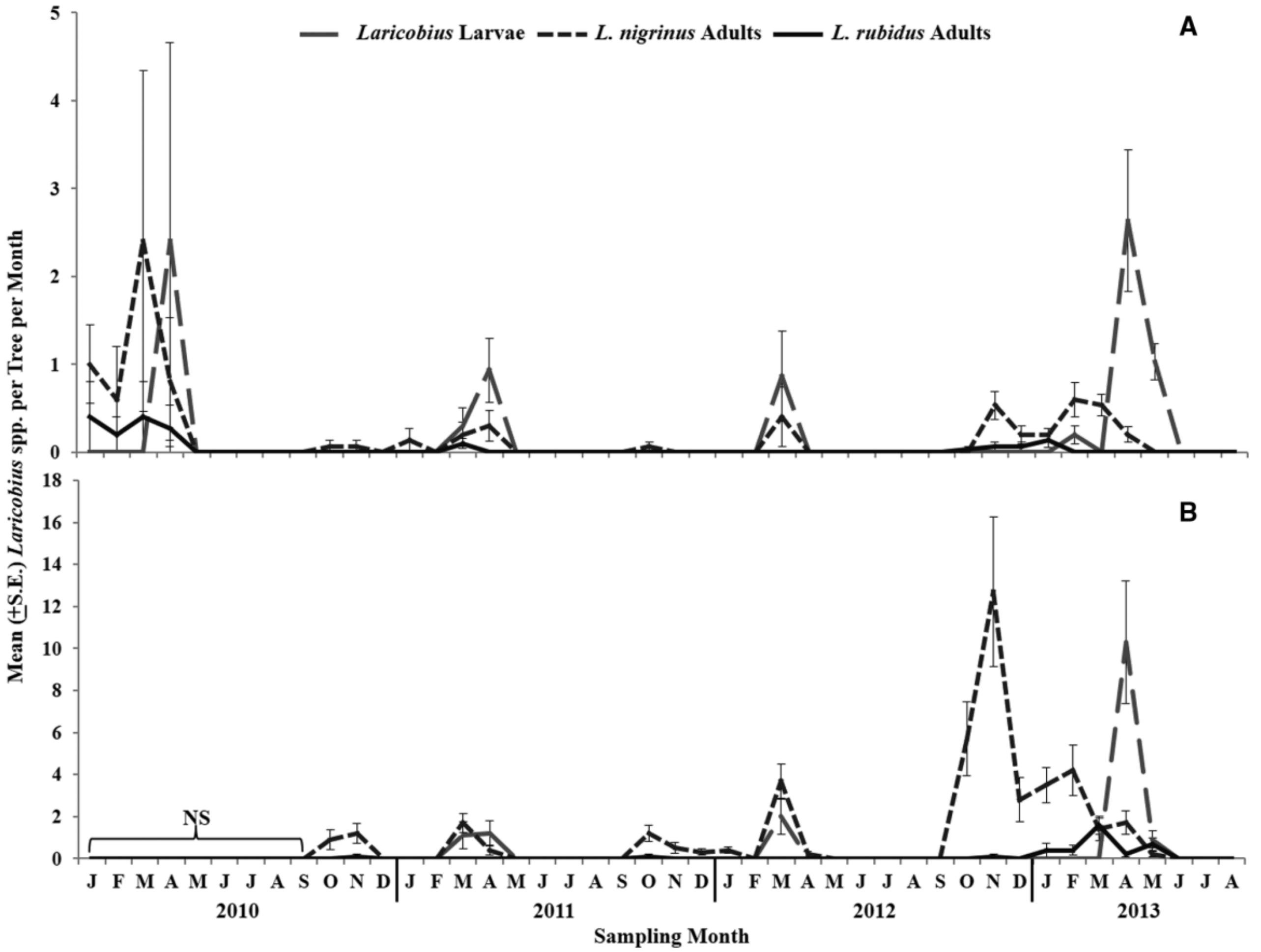

Fig. 4. Seasonality of *Laricobius* species at (**A**) Blackberry Farm and (**B**) Elkmont, 2010 to 2013 (NS = no sampling was conducted at Elkmont from January to September 2010).

employing emergence traps the exact area sampled is known, and total numbers of individuals collected in a season can be divided by this area. For example, in the current study densities of *L. nigrinus* based on emergence trap collections increased from 4.85 adults/m^2 in 2010 to 6.47 adults/m^2 in 2012 (Wiggins et al. 2016).

Assessment of Hybridization

Hybridization between *L. nigrinus* and *L. rubidus* was documented from specimens collected during 2010 and 2011 (Table 1). More hybridized individuals were collected from both Blackberry Farm and Elkmont in 2011 than in 2010. The greatest level of hybridization in a single site was 16.67% at Elkmont in 2010. The percent of hybrid individuals at Blackberry Farm increased from 2010 to 2011. The overall percent of hybridized individuals from both years and both sites combined was 10.75% (Table 1).

Hybridization of *L. nigrinus* with *L. rubidus* at both sites is not unexpected. Several studies investigating hybridization between these species on hemlock in the eastern United States have found similar (ca. 11–13%; Havill et al. 2012, Fischer et al. 2015, Mayfield et al. 2015) or greater (up to ca. 28%; Arsenault et al. 2015) levels of hybridization at locations ranging from Georgia to Pennsylvania. The fitness and impacts to prey consumption of hybrids compared to nonhybrid *L. nigrinus* individuals are unclear (Havill et al. 2012, Fischer et al. 2015). However, the introduction of *A. tsugae* into the eastern United States from Japan created a new niche for predators, and it is possible that introgression as a result of hybridization could accelerate adaptation of *L. nigrinus* to *A. tsugae* in the eastern United States (Fischer et al. 2015). Populations should continue to be monitored long-term to determine if hybridization is increasing at these sites, as well as if hybridization rates impact success of *L. nigrinus* as a biological control agent.

Assessments of establishment of *L. nigrinus* allow land managers to target on-going releases of *L. nigrinus* in areas of low establishment. Evaluating populations in areas of establishment further allows managers to conduct additional releases of *L. nigrinus* to augment populations if needed. The use of emergence traps would enhance these assessment efforts by providing a standardized method to document the emergence period, as well as estimate population densities, based on adult emergence. This information will enhance biological control programs targeting *A. tsugae*, which are an important component in preserving hemlock. Ongoing research, which has documented the continued presence of *L. nigrinus* at least 10 yrs following initial release in study areas, is encouraging for the fate of hemlock. Trees in these study areas where pesticides have not been used and *L. nigrinus* has established continue to survive and produce new growth as of Spring 2016, suggesting management of

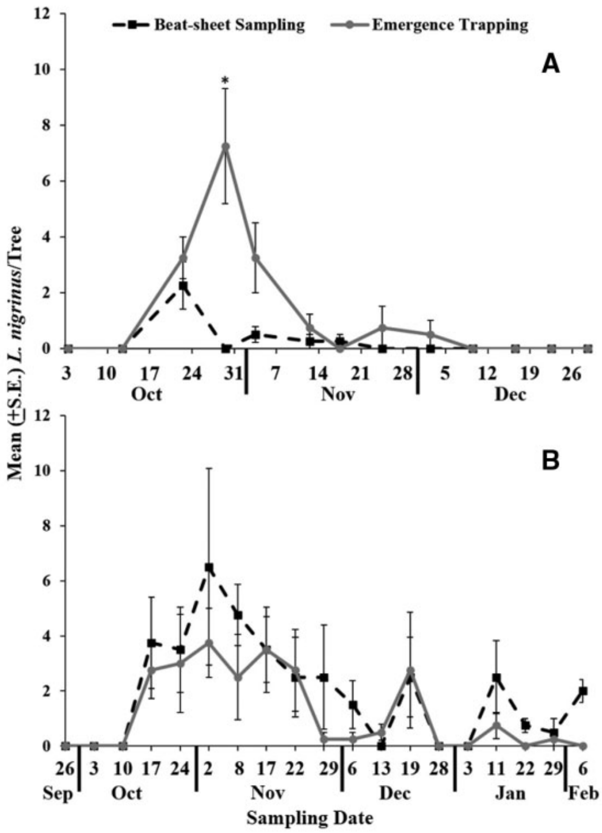

Fig. 5. Comparison of mean (±SE) number of *L. nigrinus* collected in beat-sheet sampling and emergence traps per tree for each sampling month during the emergence period in (**A**) 2010 and (**B**) 2012 to 2013 [asterisk (*) indicates significant difference determined by ANOVA and LSD, $P = 0.05$].

Table 1. Hybridization[a] of *L. nigrinus* and *L. rubidus* ($n = 93$) collected from hemlocks at Blackberry Farm, Walland, TN, and Elkmont, Great Smoky Mountains National Park, TN, 2010 and 2011

Site	Year	No. collected			% Hybrids
		L. nigrinus	*L. rubidus*	Hybrid	
Blackberry Farm	2010	22	1	1	4.17
	2011	5	16	3	12.50
Elkmont	2010	10	0	2	16.67
	2011	20	9	4	12.12
Total		57	26	10	10.75[b]

a*Laricobius* specimens assessed for hybridization by Nathan Havill, USDA Forest Service, Hamden, CT.

bAverage percent hybridization of *L. nigrinus* and *L. rubidus* collected from both study sites during both years.

A. tsugae with biological control as a central component can be sustainable. Current research to quantify the impact of *L. nigrinus* on field populations *of A. tsugae* will further assess the value of these natural enemies.

Acknowledgments

We thank Nathan Havill, USDA Forest Service, New Haven, CT, for conducting the molecular analysis. Special thanks to the Vegetation Management staff, GRSM, and Sam Beall, Blackberry Farm, for assistance with identification and establishment of study sites. Thanks to Ann Reed for assistance with statistical analyses. We also thank Bryhana Adams, Elizabeth Benton, Carla Coots, Jon Decker, Jim Dee, Renee Follum, Josh Grant, Rudi Hendrix, Monica MacCarroll, Forest Salts, and Scott Sherry for their assistance with data collection. Funding for this project was provided in part by the USDA Forest Service, Southern Region (Project 11-DG-11083150-021) and USDA Animal and Plant Health Inspection Service (Project 10-8247-0723-CA).

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