Post Release Recovery of Hemlock Woolly Adelgid Predators in the North Georgia Mountains[1]

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Abstract Eastern hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelmann, provide unique habitat that is threatened by the invasive hemlock woolly adelgid, *Adelges tsugae* Annand, which arrived in Georgia in 2003. In an attempt to conserve a portion of the mature hemlocks in north Georgia, the USDA Forest Service created over 100 Hemlock Conservation Areas throughout the Chattahoochee National Forest and designated them to receive chemical and/or biological control. *Sasajiscymnus tsugae* Sasaji and McClure, *Laricobius nigrinus* Fender, and *Scymnus sinunodulus* Yu and Yao are predatory beetles reared in the laboratory and released in these areas. To determine establishment of these predators, infested hemlock trees were sampled during spring 2010 - 2012 at some of these release sites. Additionally, nonrelease sites, 0.4 - 1.6 km from release areas, were sampled in 2012 to evaluate predator spread from release trees. Five hundred ninety-two *S. tsugae*, 232 *L. nigrinus*, 262 native *L. rubidus,* and 58 *Laricobius* hybrids were recovered at multiple sites over those years. *Sasajiscymnus tsugae* was found at 3 sites, 3 years after release and at 2 other sites, 2 years after release. *Laricobius nigrinus* was found at 1 site, 3 years after release and at 2 sites, 2 years after release. *Scymnus sinunodulus* was never recovered. Our results demonstrate that *S. tsugae* and *L. nigrinus* are established in north Georgia, and that the native *L. rubidus* is commonly associated with *A. tsugae* and is hybridizing with *L. nigrinus*; however, the population sizes, efficacy, and survival rates of all these predators are still unknown.

Key Words *Adelges tsugae*, *Laricobius nigrinus*, *Laricobius rubidus*, *Sasajiscymnus tsugae*, biological control

Eastern (Canadian) hemlock, *Tsuga canadensis* (L.) Carrière, is one of the most shade tolerant and long-lived tree species in eastern North America (Ward et al. 2004), where it can be found ranging from Georgia into Canada. It also occurs in Michigan and Wisconsin along with isolated pockets in Alabama and Indiana. The dense canopy and location of these trees provides a unique habitat for many plant and animal species (Ward et al. 2004). In contrast, Carolina hemlocks (*Tsuga caroliniana* Engelmann) are limited to a small area of western North Carolina and patches in the surrounding states. Both species are threatened by the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), and the loss of this unique habitat would alter forest community and ecosystem dynamics (Ellison et al. 2005).

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Hemlock woolly adelgid is an invasive, aphid-like insect that, whereas currently described as a single species, contains several divergent lineages endemic to different regions in Asia and western North America (Havill et al. 2006). *Adelges tsugae* feeds on the xylem ray parenchyma cells of hemlock trees (Young et al. 1995). Hemlock species in their native ranges are resistant or tolerant to *A. tsugae*, and native predators prevent it from damaging the trees (Montgomery et al. 2009). The parthenogenetic *A. tsugae* has 2 generations a year, with a longer sistens generation (summer – early spring generation) that aestivates as first-instar nymphs, becoming active in late fall when they produce cottony ovisacs that give rise to the short progrediens generation (spring generation) (McClure 1989).

The first east coast record of *A. tsugae* was in 1951 in Richmond, VA (McClure 1989), most likely imported on ornamental hemlocks from Japan (Havill et al. 2006). Both eastern and Carolina hemlock show no resistance to *A. tsugae*, and no specialized predators are found on the East Coast (Montgomery and Lyon 1996, Wallace and Hain 2000). This lack of population control, along with the adelgids bivoltine asexual lifecycle and mobile crawler stage, has allowed them to spread rapidly with the help of wind, migratory birds, and mammals (McClure 1990). As of 2013, *A. tsugae* was found as far north as Maine, south to Georgia, and west into Tennessee and Kentucky. Hemlock mortality can occur in 4 - 6 years (Mayer et al. 2002, McClure 1991) and faster in the southern range due to other factors such as drought stress (Ward et al. 2004) and mild winters that are positively related to *A. tsugae* survival (Trotter and Shields 2009).

Hemlock occupies only about 4% of approximately 277,210 ha in the Blue Ridge and Mountain-Piedmont transition zone in Georgia with two-thirds of hemlocks being a minority species in hardwood and white pine (*Pinus strobus* L.) mixed composition stands (Meyer 2005). *Adelges tsugae* was first discovered in Georgia in 2003 in Rabun Co. Since then, it has spread west and now, in 2014, the infestation covers the entire range of hemlock in Georgia.

In response to *A. tsugae* arrival in north Georgia, the U.S. Forest Service created 144 Hemlock Conservation Areas (HCAs) within the Chattahoochee National Forest to be treated either with biological controls and/or insecticides to try to save a portion of the mature hemlock trees in this region (Meyer 2005) . The HCAs range between 3 - 447 ha, typically containing multiple beetle release sites that are generally separate from the insecticide (imidacloprid soil drench or injection) treatments (see Joseph et al. 2011). Within each release site, multiple trees will have beetle releases, though not every release tree is always individually marked. The 3 predators released in Georgia and other eastern states are: *Sasajiscymnus tsugae* Sasaji and McClure (Coleoptera: Coccinellidae), *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), and *Scymnus sinuanodulus* Yu and Yao (Coleoptera: Coccinellidae).

Sasajiscymnus tsugae, a small black beetle native to Japan, has the longest history of releases in the eastern U.S. Beetles were first released in Connecticut in 1997 (Cheah 2011) and have since been released repeatedly throughout the introduced range of *A. tsugae*. *Sasajiscymnus tsugae* was first released in Georgia in 2004 (Asaro et al. 2005). They are active from March to November and have 2 generations per year with eggs of *S. tsugae* found in the spring starting when daytime temperatures average 15°C (Cheah 2011). Both larvae and adults feed on all stages of *A. tsugae* (Cheah and McClure 1998). In Georgia, adult *S. tsugae* are typically released from March to June.

Laricobius nigrinus is a small black beetle native to the Pacific Northwest. Adults are active during late September to May, with eggs being found between late January to May and larvae from March to early June (Mausel et al. 2011, Zilahi-Balogh et al.

2003a). *Laricobius nigrinus* spends the summer underground as pupae. They have one generation per year and need *A. tsugae* eggs to mature into adults which can then feed on all life stages (Zilahi-Balogh et al. 2002). In Georgia, the typical release method for *L. nigrinus* involves allowing females to oviposit on adelgid infested branches in the laboratory, and these twigs with beetle eggs are placed in the field. Some adult releases of laboratory reared beetles also have occurred. Relatively few adults are released, however, due to high pupal mortality in the laboratory and asynchronous emergence times following summer aestivation (Salom et al. 2012). Currently adult releases are more likely to include wild caught adults from either North Carolina or Washington. Adults are released from fall to late spring.

Scymnus sinuanodulus is a small beetle from China that is red-brown in color with one black spot on each elytron. Adult activity begins in spring, and they have one generation per year. Larvae will feed on all life stages of *A. tsugae* but they survive significantly better on *A. tsugae* eggs (Lu and Montgomery 2001, Lu et al. 2002). In Georgia, the typical release methods for *S. sinuanodulus* were either as eggs laid on twigs or release of adults at the end of the rearing season. However, rearing efforts have halted for this predator because data suggest *S. sinuanodulus* was not able to survive in Tennessee following release in whole tree cages (Hakeem et al. 2011) or field releases in North Carolina (McDonald et al. 2008).

Beetle releases started in eastern Georgia in 2004 (Asaro et al. 2005) where *A. tsugae* was initially discovered, and the releases then moved west following the spread of the infestation. By the end of the 2011 release season, roughly 1,300,000 predator beetles had been released in the north Georgia mountains with *S. tsugae* totaling 998,531 (871,869 adults, 126,662 eggs), *L. nigrinus* totaling 263,018 (24,591 adults, 238,427 eggs), and *S. sinuanodulus* totaling 47,461 (3,333 adults, 44,128 eggs) (Jones 2013). Most areas receive predator releases over multiple-years if the hemlocks remain healthy enough to support the predatory beetles. As hemlock trees succumb to *A. tsugae* infestation, visible decline can be seen as lack of new growth, followed by tip die-back and thinning of the crown. This decline in tree health also leads to declining *A. tsugae* populations to the point where no new ovisacs can be found as there are no suitable feeding sites. However, if the hemlocks start to produce new growth (often the year after *A. tsugae* is no longer observed on the tree) additional predatory beetle releases have been made.

Although beetle releases began in Georgia as early as 2004 (Asaro et al. 2005, Mausel et al. 2010) the sites being monitored for beetle establishment had predators released from 2007 - 2012. This has given the predators up to 6 years to establish and grow in these areas. In addition, *Laricobius rubidus* (LeConte), a native predator of pine bark adelgid, *Pineus strobi* (Hartig), are also found on *A. tsugae* infested hemlocks where it can complete development (Mausel et al. 2008, Zilahi-Balogh et al. 2005) and interbred with *L. nigrinus* (Havill et al. 2012).

With effective biological control agents characterized in part as those that easily establish (Clausen 1951), our objectives were to determine which of the three predator beetle species released in Georgia are established in the forest and if they are spreading from the points of release.

Materials and Methods

Selection of release sites and trees. GPS coordinates, provided by the 4 laboratories making beetle releases in North Georgia, were used to locate release areas

and trees on which beetles were released and were further identified by metal tags and/or flagging when possible. If *A. tsugae* could be found on the release tree, then it was selected for sampling. However, if no infestation was found on the tree, then surrounding trees were examined until *A. tsugae* was located. Therefore, sampling was sometimes conducted on other nearby trees.

In 2010, 13 sites in 11 Hemlock Conservation Areas (HCAs) were selected for sampling based on having prior beetle releases and no signs of severe hemlock decline (Fig. 1, Table 1). In 2011, 14 sites in 11 HCAs were sampled. Four of the original sites from 2010 were excluded due to road closure, a tornado, or because additional beetles were released in 2011 which would have confounded sampling results. Five additional sites were selected (Fig. 1, Table 1). In 2012, 17 sites in 14 HCAs were sampled including 7 sites that were sampled the previous 2 years, 5 sites that were sampled the prior year, 2 sites that were sampled in 2010 but not 2011, and 3 new sites where beetles had been released 2 - 3 years previously with no subsequent releases (Fig. 1, Table 1).

Determination of establishment. To determine establishment of the predator beetles, branch clippings were collected using the sampling methods of Mausel et al. (2010) which are an effective method for collecting larvae. Because our goal was to sample all predators at the same time, this method was used to sample for eggs

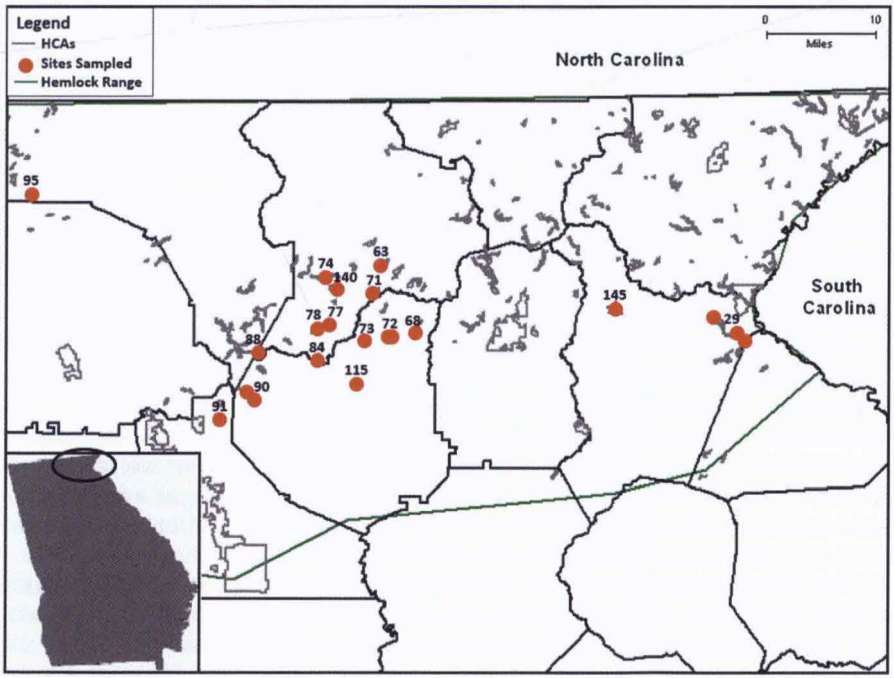

Fig. 1. Hemlock conservation areas (HCAs) in the Chattahoochee National Forest in Georgia. The HCAs are outlined in grey and those sampled for predatory beetles are represented by red dots. The numbers listed above the dots correspond to site location in Table 1.

Table 1. Total number of twigs collected and trees sampled for recovery at each sampling site from 2010 - 2012. In 2010, all sites had 10 trees sampled for a total of 150 twigs/site so only the date sampled is listed.

					2010	2011			2012		
HCA #	Site	Altitude (m)	Latitude	Longitude	Date Sampled	Date Sampled	# twigs collected	# trees sampled	Date Sampled	# twigs collected	# trees sampled
29	Lower Panther A	225	34.66906	–83.36742					3/27/2012	150	10
29	Lower Panther B	261	34.67403	–83.37290	4/21/2010				3/27/2012	150	10
29	Upper Panther	449	34.69642	–83.41708	4/21/2010				3/27/2012	150	10
145	Soque River	457	34.71582	–83.57611	4/12/2010	4/23/2011	23	8	3/22/2012	150	10
115	Yahoola Creek	495	34.61288	–83.98640	4/7/2010	4/26/2011	18	6	4/4/2012	150	10
90	Jones Creek A	513	34.60015	–84.13520		4/13/2011	150	10	4/3/2012	6	1
68	Boggs Creek	517	34.68780	–83.88995	4/7/2010			Tornado: area closed			
90	Jones Creek B	522	34.60261	–84.15091	4/14/2010	4/13/2011	150	10	4/3/2012	16	4

Table 1. Continued.

HCA #	Site	Altitude (m)	Latitude	Longitude	2010	2011			2012		
					Date Sampled	Date Sampled	# twigs collected	# trees sampled	Date Sampled	# twigs collected	# trees sampled
72	Waters Creek A	526	34.67639	–83.94282	4/15/2010	4/19/2011	150	10			
72	Waters Creek B	542	34.67708	–83.93812	4/15/2010	4/19/2011	20	4			
91	Cochran Creek	544	34.56968	–84.21544	4/14/2010			Access road closed			
78	Canada Creek 1	718	34.68727	–84.05750					4/11/2012	150	10
74	Coopers Creek	724	34.74991	–84.03668		4/22/2011	14	6	4/19/2012	150	10
73	Dockery Lake	731	34.67544	–83.97596	4/9/2010	4/22/2011	46	4	4/5/2012	150	10
77	Canada Creek 2	739	34.68952	–84.04485	4/23/2010	4/22/2011	24	3	4/11/2012	150	10
140	Mart Helton	775	34.74240	–84.02956		4/22/2011	18	6	4/19/2012	150	10

Table 1. Continued.

					2010	2011			2012		
HCA #	Site	Altitude (m)	Latitude	Longitude	Date Sampled	Date Sampled	# twigs collected	# trees sampled	Date Sampled	# twigs collected	# trees sampled
88	Noontootla	841	34.64995	–84.17449					4/11/2012	150	10
95	Jacks River	852	34.86093	–84.51434	4/22/2010	4/26/2011	19	10	4/20/2012	2	1
84	Blackwell Creek	856	34.64338	–84.04360	4/23/2010	4/11/2011	150	10	4/5/2012	150	10
71	Slaughter Creek	974	34.74132	–83.95564		4/22/2011	19	6	3/22/2012	150	10
63	Wolf Pen Gap	1024	34.76447	–83.95298		4/22/2011	48	18	4/13/2012	150	10

and larvae when average daytime temperatures were approx. 15°C as *L. nigrinus* (Zilahi-Balogh et al. 2003b), *S. tsugae* (Cheah 2011), and *S. sinuanodulus* (Lu and Montgomery 2001) oviposit at this temperature.

In 2010, 10 trees at each site were sampled using a pole pruner (6 m long) to remove 2 or 3, 1 m long adelgid-infested limbs per tree (Table 1). Each limb was placed on a white tray while being processed to capture larvae or adults that were dislodged. From those limbs, 15 adelgid infested twigs (approx. 15 - 20 cm long) were clipped from each tree and placed immediately into 7.6 L zip lock bags. The remaining branches were beaten over a sheet of white cloth to dislodge any additional larvae or adults. If beetles were found, they were placed into Petri dishes, returned to the laboratory, and identified. A total of 150 twigs were collected per site. These twigs were placed into floral foam blocks and kept in separate BugDorm Insect Tents (60 × 60 × 60 cm, model BD2120, MegaView Science Co., Ltd, Taichung, Taiwan). Branches in the rearing tents were elevated by placing them on hardware cloth (1.27 cm mesh) so that *L. nigrinus* larvae, which drop from the branches to pupate, could be collected more easily. Foam blocks were wrapped in plastic wrap except the top which had a fine screen cover. This allowed for easy weekly watering through the screen top but prevented larvae from being lost in the block. Twigs were held at 20°C, 14L:10D, and 65%RH. Supplemental adelgid-infested hemlock twigs collected from nonrelease areas were added each week to provide additional food for the predators during 2010. However, since 2011 when *L. rubidus* was found in the area where infested hemlocks were harvested for the laboratory colony, supplemental foliage was not added the following years. During this time emerging larvae or adult beetles were collected and placed in 95% EtOH and stored in a freezer until voucher specimens were prepared and/or DNA analysis was conducted. Vouchers were deposited at Yale University's Peabody Museum of Natural History. For *Laricobius* samples, the identity was determined using the methods described in Havill et al. (2012). Briefly, individual genotypes were scored using 6 microsatellite markers and were compared with known reference samples using the software Structure 2.3.2 (Pritchard et al. 2000) and NewHybrids 1.1 (Anderson and Thompson 2002) to determine whether a beetle was *L. nigrinus, L. rubidus,* or a hybrid. After 9 weeks the tents were emptied and each branch carefully examined for any remaining beetles.

In 2011, four sites (3 HCAs) were sampled using the 2010 methods described above. The remaining 10 sites were sampled using pole pruners or hand shears to collect branches from nearby trees due to declines in *A. tsugae* populations. If 150 adelgid-infested twigs were not found, the number of twigs collected and trees sampled was recorded (Table 1). Beetles were reared from the twigs using the rearing tents as in 2010 or if less than 30 twigs were available, in smaller 64.4 L clear plastic container with locking lid (68 × 45 × 30 cm) that were modified by cutting 38 × 23 cm holes in the longer sides and lid and hot gluing no-see-um netting (Mosquito Curtains, Inc., Atlanta, GA) to cover the hole. In 2012, the modified sampling techniques of 2011 were used again due to lack of adelgid-infested branches to sample (Table 1).

Determination of spread from release areas. To evaluate spread, we sampled 0.4 - 1.6 km from 13 release sites in 10 HCAs and 1 site between release sites in 2 HCAs (Table 2). Sites were selected by entering all release site coordinates into Arc MAP and creating radii of 0.4, 0.8, and 1.6 km. Locations with no overlap with other sites and with sufficient hemlock (i.e., along rivers) were selected for sampling. If roads allowed for easy access in a different direction, these areas also were examined for hemlocks as well as *A. tsugae*. Sampling of the nonrelease sites were conducted in the same manner as release sites using pole-pruners to sample 150 twigs, when possible, from 10 trees.

Table 2. Recovery of predatory beetles from nonrelease sites. Locations of sites are indicated by distance (km) from their relative release site.

HCA #	Site	Distance (km) from Release	Date Sampled	# twigs collected	# trees sampled	Beetles Recovered			
						St	Ln	LnxLr	Lr
29	Lower Panther B	0.4	3/27/2012	150	10	0	0	0	0
145	Soque River A	0.4	3/22/2012	150	10	0	0	**1**	**16**
145	Soque River B	1.6	3/22/2012	150	10	0	0	0	**1**
90	Jones Creek B	0.4 - 1.2	4/3/2012	150	10	0	0	0	**8**
78	Canada Creek 1	0.4	4/11/2012	150	10	**25**	**0**	**0**	**0**
74	Coopers Creek	0.4	4/19/2012	150	10	0	0	0	**8**
140	Mart Helton								
73	Dockery Lake	0.4	4/5/2012	45	9	0	0	0	0
77	Canada Creek 2	0.4	4/11/2012	18	3	0	0	0	**3**
88	Noontootla	0.4 - 1.6	4/11/2012	150	10	0	0	0	**1**
84	Blackwell Creek	0.4 - 0.8	4/5/2012	150	10	0	0	0	**4**
71	Slaughter Creek A	0.4	3/22/2012	45	3	**1**	**0**	**0**	**4**
71	Slaughter Creek B	0.4	3/22/2012	150	10	0	**1**	0	**10**
63	Wolf Pen Gap	0.4	4/13/2012	150	10	**1**	**2**	**1**	**4**
					total	**27**	**3**	**2**	**56**

Statistical analyses. Recovery efforts in 2012 had the potential of sampling up to 3 years after releases which would indicate establishment. Therefore, 2012 data were analyzed using Fisher's exact test (PROC FREQ, SAS Institute 2000) to determine whether recovery of *S. tsugae*, *L. nigrinus*, or *Laricobius* hybrids were independent of the year of the last predator releases ($P \leq 0.05$). *Laricobius* hybrid recovery was compared on the basis of the last year of *L. nigrinus* releases.

Results

Recovery efforts. *Sasajiscymnus tsugae* was recovered from 6 sites (153 beetles) in 2010, 7 sites (100 beetles) in 2011, and 9 sites (312 beetles) in 2012, respectively (Table 3). *Laricobius nigrinus* was recovered from 4 sites (25 beetles) in 2010, 3 sites (58 beetles) in 2011, and 6 sites (146 beetles) in 2012 (Table 4). *Laricobius nigrinus* x *rubidus* hybrids were found at 2 sites (20 beetles) in 2010, 3 sites (15 beetles) in 2011, and 4 sites (19 beetles) in 2012 (Table 4). In addition to recovery of introduced predators, native *L. rubidus* were recovered from 4 sites (21 beetles) in 2010, 3 sites (15 beetles) in 2011, and 4 sites (19 beetles) in 2012 (Table 5). *Scymnus sinuanodulus* was never recovered during the study. Using the Fisher's exact test, 2012 recovery data were compared in regards to year of their last release (2009, 2010, or 2011). There were no significant differences in *S. tsugae* ($P = 1.0000$), *L. nigrinus* ($P = 0.7580$), or hybrids ($P = 0.5036$) recovered based on year they were last released. Whereas there was no significant difference, more sites were positive for *L. nigrinus* and hybrid recovery when sampled 1 year after release (3 of 9 sites) than 2 years after release (1 of 4 sites for *L. nigrinus*, 2 of 4 sites for hybrids) and 3 years post release (0 of 4 sites). *Sasajiscymnus tsugae* did not vary on whether a site was positive for recovery 1 year after release (4 of 8 sites), 2 years after release (2 of 3 sites), and 3 years after release (3 of 6 sites).

Determination of establishment. Beetles were considered to be established if they could be found at least 3 generations after their last release. Due to continual releases at the sampling sites by multiple laboratories, only 4 sites did not have beetles released in the 3 years prior to 2012. A single site, HCA# 78 Canada Creek 1, had both *S. tsugae* and *L. nigrinus* established together (Tables 3, 4). *Sasajiscymnus tsugae* also was recovered 3 years after release at HCAs # 63 Wolf Pen Gap and # 71 Slaughter Creek, so this beetle can be considered established at these sites as well. *Sasajiscymnus tsugae* was recovered 2 years after release at HCA sites # 73 Dockery Lake and # 88 Noontootla (Table 3). Because *S. tsugae* has 2 generations per year the recovered beetles should belong to the fourth generation produced in the field. In addition, *L. nigrinus* was recovered 2 years after release at HCA sites # 73 Dockery Lake and # 88 Noontootla, and *Laricobius* hybrids were recovered from HCA# 71 Slaughter Creek 4 years after *L. nigrinus* were released at that site (Table 4).

Determination of spread from release areas. Of the 13 nonrelease sites sampled within 0.4 - 1.6 km of release sites, 11 of the sites were positive for recovery. Twenty-seven *S. tsugae* were recovered from 3 sites, 3 *L. nigrinus* from 3 sites, 2 *Laricobius* hybrids from 2 sites, and 56 native *L. rubidus* from 10 sites (Table 2).

Discussion

Sasajiscymnus tsugae and *L. nigrinus* were consistently recovered at 3 HCAs every year, and *S. tsugae* was consistently found at a fourth as well. It is uncertain as to

Table 3. Recovery of *Sasajiscymnus tsugae* from predatory beetle release sites sampled during 2010 - 2012. Year of release indicates the year *S. tsugae* was released in the area with releases during the sampling year occurring after the area was sampled. Years (Yrs.) post release indicates how long it has been since the last *S. tsugae* release in the area prior to sampling with (-) for no prior releases.

		No. of *S. tsugae* Recovered				Yrs. Post Release		
HCA #	Site	2010	2011	2012	Year of *S. tsugae* Release	2010	2011	2012
29	Lower Panther A			0	2008, 2009, 2010, 2011			1
29	Lower Panther B	**1**		**1**	2010, 2011	-		1
29	Upper Panther	**2**		**1**	2009, 2010	1		1
145	Soque River	0	0	**3**	2009, 2010, 2011	1	1	1
115	Yahoola Creek	0	0	0	2008, 2009, 2010, 2011	1	1	1
90	Jones Creek A	0	**1**	0	2009, 2010		1	2
68	Boggs Creek	0	tornado: closed		2008, 2009, 2010	1		
90	Jones Creek B	**1**	**1**	0	2009, 2010, 2011	1	1	1
72	Waters Creek A	0	**4**		2008, 2009, 2010, 2011	1	1	
72	Waters Creek B	0	0		2009, 2011	1	2	
91	Cochran Creek	**2**	road closed		2009, 2010, 2011	1		
78	Canada Creek 1			**20**	2008, 2009			**3**
74	Coopers Creek		0	0	2008, 2009		2	**3**
73	Dockery Lake	**139**	**32**	**19**	2008, 2009, 2010	1	1	2
77	Canada Creek 2	**8**	0	0	2008, 2009	1	2	**3**

Table 3. Continued.

HCA #	Site	No. of *S. tsugae* Recovered 2010	2011	2012	Year of *S. tsugae* Release	Yrs. Post Release 2010	2011	2012
140	Mart Helton		0	0	2008, 2009		2	**3**
88	Noontootla			**138**	2009, 2010			2
95	Jacks River	0	0	0	2009, 2010, 2011	1	1	1
84	Blackwell Creek	0	**27**	**27**	2010, 2011	-	1	1
71	Slaughter Creek		**8**	**50**	2006, 2007, 2008, 2009		2	**3**
63	Wolf Pen Gap		**27**	**53**	2008, 2009		2	**3**
	Total	**153**	**100**	**312**				

Table 4. Recovery of Laricobius nigrinus and L. nigrinus x L. rubidus hybrids during 2010 - 2012. Year of release indicates when L. nigrinus was released in the HCA. Years (Yrs.) post release indicates how long it has been since the last L. nigrinus release in the area prior to sampling with (-) for no prior releases and 0 if a release was made prior to sampling.

		No. of *L. nigrinus* and hybrids Recovered									
		2010		2011		2012			Yrs. Post Release		
HCA #	Site	Ln	Ln x Lr	Ln	Ln x Lr	Ln	Ln x Lr	*L. nigrinus* Releases	2010	2011	2012
29	Lower Panther A					0	0	2008, 2009, 2010*			1
29	Lower Panther B	0	0			0	0	2008, 2010, 2011	2		1
29	Upper Panther	**9**	**2**			0	0	2009, 2010*	2		1
145	Soque River	0	0	0t	0	**1**	0	2007, 2008, 2010*, 2011	2	0	1
115	Yahoola Creek	**1**	0	0	0	0	0	2009, 2010	1	1	2
90	Jones Creek A	0	0	0	0	0	0	2009, 2010, 2011		1	2
68	Boggs Creek	0t	0		tornado: closed			2008, 2009, 2010	0		
90	Jones Creek B	0	0	0	0	0	0	2009, 2010, 2011	1	1	1
72	Waters Creek A	0	0	0	**1**				-	-	
72	Waters Creek B	0	0	0	0			2010	-	1	
91	Cochran Creek	0	0		road closed			2009, 2010, 2011	1		
78	Canada Creek 1					**1**	0	2008, 2009			**3**
74	Coopers Creek			0	0	0	0	2009		2	**3**
73	Dockery Lake	**12t**	**20**	**4**	0	**5**	0	2008, 2009, 2010	0	1	2

Table 4. Continued.

		No. of *L. nigrinus* and hybrids Recovered									
		2010		2011		2012			Yrs. Post Release		
HCA #	Site	Ln	Ln x Lr	Ln	Ln x Lr	Ln	Ln x Lr	*L. nigrinus* Releases	2010	2011	2012
77	Canada Creek 2	0	0	0	0	0	0	2008	2	**3**	**4**
140	Mart Helton			0t	0	0	0	2009, 2010*		0	1
88	Noontootla					**44**	**11**	2009, 2010			2
95	Jacks River	0	0	0	0	0	0	2010	-	1	2
84	Blackwell Creek	**3**	0	**13t**	**7**	**78**	**2**	2008, 2009, 2010*	1	0	1
71	Slaughter Creek			0	0	0	**5**	2007, 2008		**3**	**4**
63	Wolf Pen Gap			**41t**	**7**	**17**	**1**	2008, 2010*, 2011		0	1
	Total	**25**	**22**	**58**	**15**	**146**	**19**				

t indicates releases occurred in the area prior to sampling.

* indicates winter releases which would be a part of the following year release season.

Table 5. *Laricobius rubidus* collected in predatory beetle release sites during 2010 - 2012.

		No. of *L. rubidus*		
HCA #	Site	2010	2011	2012
29	Lower Panther A			0
29	Lower Panther B	0		**1**
29	Upper Panther	**1**		0
145	Soque River	**2**	0	**6**
115	Yahoola Creek	0	0	0
90	Jones Creek A	0	**3**	0
68	Boggs Creek	0		
90	Jones Creek B	0	0	0
72	Waters Creek A	**1**	**22**	
72	Waters Creek B	0	**2**	
91	Cochran Creek	0		
78	Canada Creek 1			**8**
74	Coopers Creek		0	0
73	Dockery Lake	**16**	**35**	**25**
77	Canada Creek 2	0	0	0
140	Mart Helton		**7**	0
88	Noontootla			0
95	Jacks River	0	0	0
84	Blackwell Creek	0	**27**	0
71	Slaughter Creek		**1**	**15**
63	Wolf Pen Gap		**24**	**10**
	Total	**20**	**121**	**65**

what makes these sites suitable locations, but it could be due to higher elevations (above 700 m) and that the tree health and *A. tsugae* populations were rebounding in those areas. It is clear that both *S. tsugae* and *L. nigrinus* can establish in north Georgia; however, their population sizes, efficacy, and survival rates are still unknown. Because *S. sinuanodulus* was never recovered it appears that either this predator is not suitable as a biological control agent in this region, or not enough beetles were released for them to establish.

Documented recovery of *S. tsugae* is minimal (Cheah et al. 2005), and to our knowledge, only one other study has kept voucher specimens of recovered beetles

(Hakeem et al. 2010). With a total of 565 *S. tsugae* recovered at roughly 50% of the sites for 3 years, these results document not only the largest recovery of *S. tsugae* recorded, but also at a high success rate. Hakeem et al. (2010) were able to recover beetles at 21% of their sites using beat sheets, whereas Cheah et al. (2005) recovered beetles at 65% of the sites 1 year after release.

The use of beat sheets limit sampling efforts to the lower canopy, and the presence of predators can be underestimated when *A. tsugae* is present throughout the trees. Research has shown that the predators can be found high in the canopy for *S. tsugae* (4 - 20 m, Cheah et al. 2005, Cheah and McClure 2002) and *L. nigrinus* (above 15 m, Davis et al. 2012). However, *L. nigrinus* were recovered below 7 m when *A. tsugae* densities were highest there compared with the rest of the tree strata (Davis et al. 2012).

Collections at nonrelease sites showed that the native *L. rubidus* is a common predator associated with *A. tsugae*, but its effect on populations is unknown. Because it is so common, its presence raises concerns of bringing *L. rubidus* into the rearing laboratory on infested foliage intended to feed beetle colonies which could result in competition and hybridization making the colony no longer "pure" *L. nigrinus*.

With hundreds of thousands of predatory beetles released throughout north Georgia, continued recovery efforts would greatly improve our knowledge of establishment and dispersal. Additional methods can be used, such as using a sweep net that can reach high into the canopy (Shiyake et al. 2008) or tree climbing (Davis et al. 2012), as well as increased frequency of sampling to determine when life stages are found in Georgia.

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