Herpetological Conservation and Biology 8(2):276−287.
Submitted: 18 January 2012; Accepted: 13 May 2013; Published: 15 September 2013.

Investigating Behavioral Shifts in Aggression Between a Naturalized and Native Salamander Species of the Genus *Plethodon*

***Heather R. Cunningham*[1, 2] *and Leslie J. Rissler*[1]**

[1]*Department of Biological Sciences, Box 870345 MHB Hall, University of Alabama, Tuscaloosa, Alabama 35487, USA*
[2]*Present address: Natural History Society of Maryland, P.O. Box 18750, 6908 Belair Road, Baltimore, Maryland 21206, USA, e-mail: hcunningham@marylandnature.org*

Abstract.—**Species introductions can be devastating to native communities, but not all introductions are detrimental. Understanding what factors influence the degree of insult a community experiences from non-native species may help predict whether introduced species become truly "invasive" vs. "naturalized." Approximately 75 years ago at Mountain Lake Biological Station (MLBS) in Giles County, Virginia, USA, *Plethodon montanus* (Northern Gray-cheeked Salamander) was introduced. Since its introduction, this species naturalized with little discernible impact on the native salamander community. *Plethodon montanus* is ecologically similar in terms of body size and habitat to the native *Plethodon glutinosus* (Northern Slimy Salamander) and is broadly sympatric with this species across its range. At MLBS, the species are syntopically distributed. We examined whether familiarity influenced levels of aggressive behavior between *P. montanus* (non-indigenous but naturalized species) and *P. glutinosus* (native and resident species) at MLBS. We asked if behavior displayed by these salamanders during heterospecific encounters varied depending on whether the interacting animals were from the MLBS community ("familiar") or areas where the species are allopatric ("naïve"). Overt aggression was not observed, but more passive aggressive behavior was exhibited by *P. glutinosus* to heterospecifics from MLBS than those from naturally naïve/allopatric areas. Both species avoided heterospecifics from MLBS more than heterospecifics from naïve/allopatric areas. Our results suggest that the two species display very little aggressive behavior towards each other, but do seem to show heightened responses to salamanders from the invaded community. This lack of strong negative interactions between the species has likely facilitated the spread of the invasive *P. montanus* into the MLBS salamander community.**

Key Words.—aggression; amphibian; behavior; naturalized; *Plethodon*; salamander

Introduction

Species introductions are common and can be devastating to native communities that have evolved in the absence of the invader. Native communities vary in response (behaviorally, ecologically, and genetically) to the arrival of an invader (Porter and Savignano 1990; Petren and Case 1996; Carlton et al. 1999; Davis 2003; Suarez and Tsutsui 2008). Not all introductions are detrimental to the community, and understanding what factors influence the degree of insult a community experiences from non-native species may help predict whether introduced species become truly "invasive" vs. "naturalized." Nevertheless, predicting the ecological impacts of an introduction may be complicated due to inherent behaviors of the resident species that lessen negative impacts of an invader (Holway and Suarez 1999). For example, interspecific competition mediated by underlying territorial behavior can influence local scale distribution patterns and population sizes (Krebs and Davies 1997; López-Sepulcre and Kokko 2005). If resource availability is more or less continuous, territoriality can lead to smaller population sizes in comparison to a non-territorial assemblage (López-Sepulcre and Kokko 2005). Circumstances that promote or suppress territorial behavior have the potential to influence the fates of the introduced and native species in communities (reviewed in Caro 1998).

Salamanders of the genus *Plethodon* have often been the focus of studies investigating species' interactions and their role in structuring communities. In fact, interspecific competition is a principal organizing force in assemblages of plethodontid salamanders (Adams 2007). Territorial behavior often mediates inter- and intraspecific competition within assemblages, although the limiting resources that elicit interspecific competition is not always known (Nishikawa 1990; reviewed in Wells 2007). Often, interspecific competition may take on the form of aggressive interference rather than classic exploitative competition (Nishikawa 1990). However, in particular experimental manipulations, aggression declined when salamanders interacted with familiar versus unfamiliar individuals (Jaeger 1981; Jaeger and Peterson 2002; reviewed in Wells 2007). Salamanders within the genus *Plethodon* can distinguish between individuals, sexes, species, and populations based on chemical cues (reviewed in Wells 2007). Furthermore, chemical cues vary geographically within a species (Rollmann et al. 2000; Watts et al. 2004). The objective of our study was to use a unique situation (a population where a salamander species was introduced into a new community over 75 years ago) to investigate the impact of familiarity on the territorial behavior of two interacting salamander species.

At Mountain Lake Biological Station (MLBS), Giles County, Virginia, USA, individuals of *Plethodon montanus* (Northern Gray-cheeked Salamander, formerly *P. jordani*; Highton and Peabody 2000), a member of the *P. jordani* species complex, were released between 1935 and 1945 (Henry Wilbur, pers. comm.). Despite being native to the eastern United States, including parts of Virginia, *P. montanus* was not a native member of the salamander community at MLBS (Fig. 1; Rissler et al. 2000). Introduced individuals originated from Whitetop Mountain approximately 161 km south of MLBS. *Plethodon montanus* is closely related and ecologically similar to native *Plethodon glutinosus* (Northern Slimy Salamander), a member of the *P. glutinosus* species complex (Fig. 1). Since its introduction, *P. montanus* has established a viable population with little discernable impact on the resident *P. glutinosus* (Rissler et al. 2000). At MLBS the two species are syntopic (Fig. 2). Previous work on this system found no differences in microhabitat variables where they co-occur at MLBS (Rissler et al. 2000). Laboratory studies found *P. glutinosus* (the native) obtained burrows more often than *P. montanus* (the non-native) when this resource was limited but, even so, species cohabitated a burrow 50% of the time during

Figure 1. Geographic distributions of *Plethodon glutinosus* and *P. montanus*. Distributions based on Lannoo (2005). The location of Mountain Lake Biological Station is shown with a solid triangle.

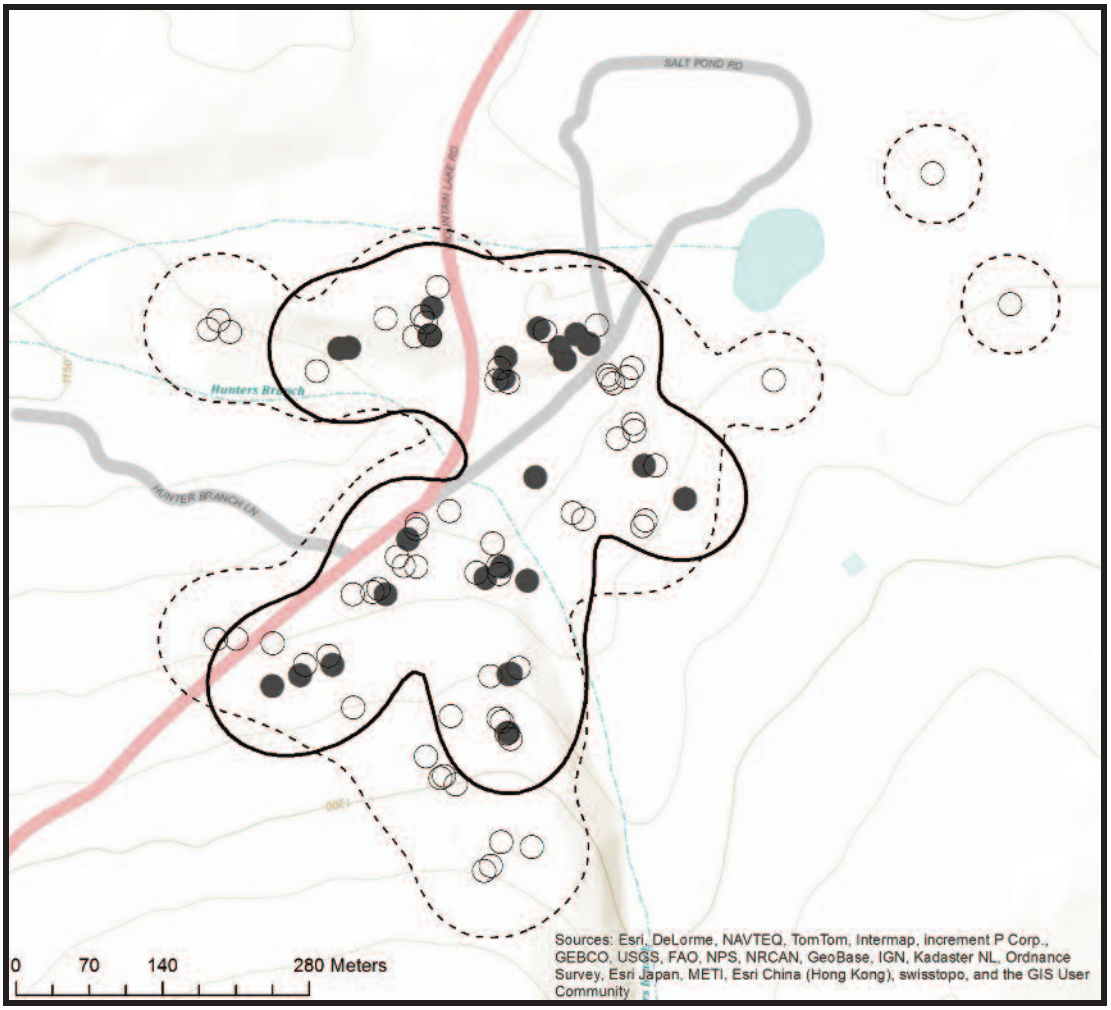

FIGURE 2. Distribution of *Plethodon montanus* at Mountain Lake Biological Station (MLBS), Virginia, USA, based on 2007 survey data. Surveys were conducted where the two species co-occur at MLBS. Map generated using ArcGIS version 10.1(Esri, Redlands, CA, USA) *Plethodon glutinosus* localities shown with open circles and *P. montanus* localities shown with solid circles. The estimated range of *P. glutinosus* is outlined by dashed line and for *P. montanus* by solid line. *Plethodon montanus* occupies an area encompassing approximately 155,711 m^2.

experiments in outdoor enclosures, and both species are often found under the same cover object in the field (Rissler et al. 2000). These findings were unexpected because territorial behavior appears to be widespread in the genus *Plethodon* including the *P. glutinosus* and *P. jordani* species complexes (reviewed in Wells 2007). Other work indicates that individuals of *P. glutinosus* exhibit territorial behavior in interspecific and intraspecific encounters (Marvin 1998; reviewed in Wells 2007). However, the impact of familiarity on aggressive behavior has not been addressed in *P. glutinosus* and *P. montanus* at MLBS.

We ask if behavior displayed by *P. glutinosus* and *P. montanus* during heterospecific encounters varies depending on whether the interacting animals are from the MLBS community or communities where the species are allopatric (and hence have never before come into contact). For the purposes of this study, we define individuals of *P. glutinosus* and *P. montanus,* from communities where the species are allopatrically distributed, as “naïve,” with respect to interactions with each other. We define individuals of *P. glutinosus* and *P. montanus* from MLBS as “familiar.” Specifically, we ask, do familiar and naïve *P. glutinosus* behave differently during encounters with familiar and naïve *P. montanus*? And, do familiar and naïve *P. montanus* behave differently during encounters with familiar and naïve *P. glutinosus*? If familiarity has affected the behavior of *P. glutinosus* and *P. montanus* at MLBS, then the levels of aggression exhibited during interspecific encounters may differ between experienced and naïve conspecifics.

Methods

Populations.—We collected twenty adult *P. glutinosus* and 20 adult *P. montanus* from MLBS where they are syntopically distributed. Individuals have spread approximately 150 meters from the point of introduction at MLBS (Henry Wilbur, pers. comm.). In 2007, we conducted a survey to determine the distribution of *P. montanus* at MLBS. We used a transect design with Hunters Branch, the site where *P. montanus* was introduced, as the center and sampled along east-west transects that were approximately 800 m in length. We then sampled North-South transects that were 570 m in length; this distance was determined through pilot transects that identified the extent of the distribution of *P. montanus*. We sampled for three days from 0700 to 1700; 24 unique *P. montanus* and 64 *P. glutinosus* occurrence localities were recorded. Using Hawth's Tools version 3.26 (Beyer 2004) 95% fixed kernel density estimator, we estimated the range area for each species (Fig. 2). The current distribution of *P. montanus* at MLBS is completely encompassed within the distribution of *P. glutinosus*; thus, we felt adult *P. glutinosus* collected from within this area had likely interacted with individuals of *P. montanus*.

To simulate the behavior of individuals of these two species at the beginning of the introduction (when both species would have been naïve), we collected *P. glutinosus* and *P. montanus* from areas where the two species are not in contact. Twenty adult *P. montanus* were collected from the summit of Whitetop Mountain, Virginia; *P. glutinosus* is not present at the summit of Whitetop Mountain (James Organ, pers. comm.), and this site is the presumed origin of the original *P. montanus* that were released at MLBS. In our study, we defined these individuals as naïve. We collected twenty adult *P. glutinosus* from a population 6 km north of MLBS (hereafter referred to as naïve) where *P. montanus* is not present. We felt the behavior exhibited by individuals from this population would be similar to the behavior that was exhibited by MLBS *P. glutinosus* at the time of introduction. Due to small population sizes, the Virginia Department of Game and Inland Fisheries and MLBS restricted the number of adult salamanders we were allowed to collect to 20 individuals of each species from each population.

We collected animals on 26 and 27 May 2006. After collection, we transported salamanders to the University of Alabama for study. Salamanders were housed individually in plastic Petri dishes lined with moist unbleached paper towels in an environmental chamber at 15° C under a 12L:12D photoperiod.

Staged laboratory encounters.—Behavioral trials were conducted August through December 2006. Previous work found that duration in captivity does not affect aggressive and territorial behaviors of salamanders (Nishikawa 1987; Selby et al. 1996; Camp 1999). We used eight treatments, each replicated 20 times, in this study (Table 1). Our methods, specifically the lack of an intraspecific aggression treatment, were similar to others studies that have investigated interspecific aggression among heterospecifics from allopatric and sympatric populations (Jaeger et al. 2002; Deitloff et al. 2009). Further, Rissler et al. (2000) found that *P. glutinosus* and *P. montanus*, from MLBS, did not discriminate between conspecifics and heterospecifics, from MLBS, but treated all intruders the same.

As *P. glutinosus* was the resident species at MLBS at the time of introduction, we designated individuals of *P. glutinosus* as the resident in all trials (Table 2). Each adult salamander encountered a naïve heterospecific, a MLBS heterospecific, and a surrogate. For surrogate treatments, we created a 'surrogate salamander' by rolling a moist, dark-colored paper towel into the approximate shape and size of a salamander. This 'surrogate salamander' was used during surrogate treatments; this is a commonly used control in behavioral studies of terrestrial salamanders (Gabor and Jaegar 1995; Griffis and Jaeger 1998; Jaeger et al. 2002; Kohn et al. 2005). We randomized the treatment order for each salamander with each individual used only once in each treatment. A minimum of five days elapsed between trials for each individual. To control for potential size effects, we paired salamanders based on snout-vent length (SVL);

Table 1. Staged laboratory encounter treatments. The resident, intruder, and source population of each treatment are defined. For additional details about the treatments please see text.

Treatment Number	Resident	Intruder
1	Familiar *P. glutinosus*	Familiar P. montanus
2	Familiar *P. glutinosus*	Naïve P. montanus
3	Familiar *P. glutinosus*	Surrogate
4	Naïve *P. glutinosus*	Familiar P. montanus
5	Naïve *P. glutinosus*	Naïve P. montanus
6	Naïve *P. glutinosus*	Surrogate
7	Surrogate	Familiar P. montanus
8	Surrogate	Naïve P. montanus

paired salamanders were ≤ 1.7 cm different in size. Neither species was larger in all encounters. Due to collection restrictions and the lack of salamander surface activity at the time we were collecting, we were unable to obtain a sufficient number of salamanders to pair salamanders based on sex; therefore, pairings were a combination of male/male, female/female, and male/female.

Staged encounters occurred in experimental units that consisted of a clear plastic box (33 × 19.05 × 10.8 cm) containing moist soil, a burrow, food dish, and an alternative refuge (Fig. 3). Within each experimental unit, we used a polyvinyl chloride (PVC) pipe "T" (1.95 cm inside diameter) as a burrow, which was partially buried at one end of the box. We placed the food dish (small Petri dish) and the alternative refuge at opposite ends of the box. The alternative refuge was a 7.62 cm piece of PVC pipe inserted through the experimental unit with the opening resting on top of the soil and the other opening capped, outside the unit. The purpose of the alternative refuge was to provide an area to which a *P. montanus* could retreat during a trial. The layout of the experimental units, specifically the distance between the food dish and burrow,

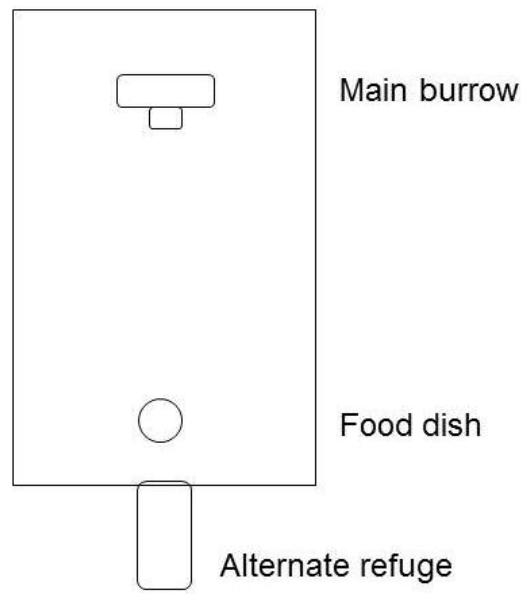

Figure 3. Diagram of the experimental unit.

Table 2. Design of staged laboratory encounters for each species from populations designated as "familiar" and "naïve." In all encounters, Plethodon glutinosus was the resident and P. montanus the intruder. Each P. glutinosus faced a familiar and naïve P. montanus (from different populations) and a surrogate. Each P. montanus faced a familiar and naïve P. glutinosus (from different populations) and surrogate. Each salamander participated in three staged encounters.

Species	Familiar Treatment	Naïve Treatment	Surrogate
Plethodon glutinosus			
"Familiar" (MLBS)	*P. montanus* (MLBS)	*P. montanus* (White Top)	Surrogate
"Naïve" (White Rock)	*P. montanus* (MLBS)	*P. montanus* (White Top)	Surrogate
Plethodon montanus			
"Familiar" (MLBS)	*P. glutinosus* (MLBS)	*P. glutinosus* (White Rock)	Surrogate
"Naïve" (White Top)	*P. glutinosus* (MLBS)	*P. glutinosus* (White Rock)	Surrogate

encouraged an individual salamander to establish the entire interior as a territory rather than just the burrow itself.

Five days prior to a trial we placed an individual *P. glutinosus* in the chamber and fed 10 small mealworms a day to permit establishment of a territory (Nunes and Jaeger 1989). We fed salamanders between 1900–2100 each day leading up to a trial. During the five days prior to the trial, we closed the interior entrance to the alternative refuge to prevent the resident salamander (*P. glutinosus*) from entering. The purpose of barring access to the refuge was to make the refuge an unmarked retreat. Five days prior to a trial we placed an individual *P. montanus* in an identical chamber and treated it with the same protocol with the exception that the alternative refuge was open, allowing the salamander to enter the tube. The rationale for this design was that individuals of *P. montanus* would become acclimated to the alternative refuge and familiar with entering and exiting the refuge prior to the trial, during which the refuge would be open allowing access.

On the day of a trial, we picked up an individual *P. montanus*, the designated intruder, in its burrow and transported it to the resident's (*P. glutinosus*) chamber. We tapped the salamander gently on the tail to encourage it to exit onto the substrate of the resident's (*P. glutinosus*) chamber. We placed an inverted Petri dish over the individual *P. montanus* to allow for acclimation. We moved the resident *P. glutinosus* to the center of its own chamber and placed an inverted Petri dish over the individual. Both the resident and intruder were acclimated for 15 min. During the acclimation period, the additional refuge was opened; this provided an area outside the territory of the resident *P. glutinosus* in which the intruder could retreat. After the acclimation period, we removed the petri dishes and recorded the behavior of the resident and intruder salamanders for 15 min.

We recorded the number of individual aggressive, avoidance, and sensory behaviors exhibited by both the resident and intruder. Previous work on plethodontid salamanders has yielded operational definitions of specific behaviors. These behaviors are categorized as overt aggressive, passive aggressive, avoidance, or sensory behavior (Table 3). Three levels of territorial defense in plethodontid salamanders have been classified (Jaeger 1986; Mathis et al. 1995). First, salamander use pheromones to mark areas and advertise territory ownership; thus, facilitating spacing in terrestrial assemblages (reviewed in Wells 2007). Second, salamanders use visual agonistic or passive aggressive postures as a second level of territory defense. Third, overt aggressive behavior or injury causing behavior may occur if an intruding salamander is not deterred (Jaeger 1986; Walls and Semlitsch 1991; Mathis et al. 1995, 2000; Wiltenmuth 1997). We also recorded the time for individuals of *P. glutinosus* to return to their burrows during trials and for *P. montanus* to enter the alternative refuge. Due to the nocturnal nature of the focal species, we conducted trials between 1900–2300 under low illumination. After each trial, we replaced the soil in each chamber and the burrow, food dish, and cleaned the additional refuge with a mild

Table 3. Salamander behavioral patterns (adapted from Nishikawa 1985 and Wiltenmuth and Nishikawa 1998).

<u>Overt aggressive behavior</u>
Bite: a salamander contacts another with an open mouth.
Chase: a salamander moves rapidly in the direction of a retreating salamander.
<u>Passive aggressive behavior</u>
Approach: a salamander moves directly towards another individual or surrogate from any direction.
Turn head towards: a salamander turns it head directly towards an individual or surrogate.
Contact: touching another salamander or surrogate with any part of its body, except the mouth, and in a relatively slow manner; includes stepping on another individual or surrogate.
<u>Avoidance behavior</u>
Retreat: a salamander moves rapidly away from a rapidly approaching individual; this is a response to overt aggressive behavior.
Avoid: moving away from the other salamander or surrogate.
<u>Sensory</u>
Nose-tap: touching the nasolabial cirri against the substrate or sides of experimental chamber.

soap and water.

Statistical analysis of laboratory behavioral trials.—The numbers of overt aggressive, passive aggressive, avoidance, and sensory behaviors exhibited during each trial were summed for each behavioral category similar to methodology of Mathis et al. (2000; Table 3). This procedure yielded total counts of behaviors representing the frequency of overt and passive aggressive, avoidance, and sensory behaviors observed during the 15 min trials.

We used generalized linear mixed effects models (GLMM) with a Poisson distribution and log link function in R version 2.15.2 (R Development Core Team 2012) using the lme4 package (Bates et al. 2012). The identification of each salamander is the random variable in all analyses because each salamander was in three trials. For each behavioral category (e.g., passive aggressive, sensory, avoidance), GLMMs were run to assess the importance of the familiarity of the species and all interaction effects. We used step-wise removal of fixed effects to determine the best model by the Akaike Information Criterion correction (AICc) score. We also put in a "null" model that had no fixed effects to make sure that including an explanatory variable in the model was better than the null based on AICc. For each response variable, we present the best model chosen by lowest AICc score and discuss results.

To compare the time adult *P. glutinosus* remained outside their burrows during the trials we used a Kaplan-Meier Survival Analysis. In our study *P. montanus* did not use the alternative refuge during the trials. We conducted survival analyses in STATISTICA version 6 for Windows (StatSoft, Tulsa, OK, USA).

Results

Question 1.—Do individuals of familiar and naïve *P. glutinosus* behave differently during encounters with familiar and naïve *P. montanus*?

Adult *P. glutinosus* exhibited passive aggressive, avoidance, and sensory behavior during trials. Salamanders did not display overt aggressive behavior during trials. Passive aggressive behavior of familiar and naïve *P. glutinosus* differed across treatments (Table 4). For this behavior, the top ranked GLMM, according to AICc, included the following significant fixed effects: familiarity of *P. montanus* (familiar vs. naïve; $Z = 5.478$, $P < 0.001$) and the interaction effect (familiar/naïve *P. glutinosus* × familiar/naïve *P. montanus*;$Z = -2.440$, $P = 0.0147$). *Plethodon glutinosus* displayed more passive aggressive behavior to familiar *P. montanus* from MLBS than to *P.*

Table 4. Frequency of behaviors exhibited by familiar and naïve *Plethodon glutinosus* toward intruders. Behavioral categories in which a statistically significant difference are denoted; *P. montanus* ($^{+}$); *P. glutinosus* ×. *P. montanus* (^{X}).

	***P. glutinosus* population**			
Behavior/Competior	**Familiar Mean**	**Standard Error**	**Naïve Mean**	**Standard Error**
Passive Aggressive^{+X}	3.77	0.57	3.10	0.52
Familiar *P. montanus*	4.26	0.88	4.95	1.23
Naïve *P. montanus*	3.38	1.32	2.45	0.63
Surrogate	3.65	0.78	1.90	0.62
Sensory	1.96	0.43	1.16	0.28
Familiar *P. montanus*	1.63	0.45	1.45	0.36
Naïve *P. montanus*	2.00	0.67	1.10	0.58
Surrogate	2.25	0.14	0.95	0.34
Avoidance^{+}	0.28	0.11	0.18	0.05
Familiar *P. montanus*	0.47	0.31	0.30	0.12
Naïve *P. montanus*	0.22	0.12	0.10	0.06
Surrogate	0.15	0.08	0.15	0.08

montanus from the naïve/allopatric population. In addition, naïve *P. glutinosus* displayed more passive aggressive behavior during encounters with familiar heterospecifics than conspecifics.

The model also included the significant effects of the response of *P. glutinosus* during encounters with naïve *P. montanus* and the surrogate (Z = 3.035, P = 0.002) and the interaction effect (familiar/naïve *P. glutinosus* × naïve *P. montanus*/surrogate; Z = -2.089, P = 0.036). *Plethodon glutino*sus displayed more passive aggressive behavior during the surrogate treatment than during interactions with naïve *P. montanus*. In addition, familiar *P. glutinosus* displayed more passive aggressive behavior during these treatments than naïve conspecifics. We found no significant differences in sensory behavior for any of the fixed effects (Table 4). Both familiar and naïve *P. glutinosus* avoided heterospecifics from MLBS significantly more than those from the naïve/allopatric area (Z = 2.232, P = 0.0256; Table 4).

The time that familiar *P. glutinosus* remained outside their burrows did not differ significantly across treatments (χ^2= 1.73, df = 2, P = 0.42). Familiar *P. glutinosus* remained outside their burrows during encounters with familiar *P. montanus* at least 2.30 min (mean = 11.28 min, SD = 4.89 min), at least 0.26 min (mean = 9.64 min, SD = 5.97 min) during encounters with naïve *P. montanus*, and at least 2.00 min (mean = 8.82 min, SD = 4.94 min) during surrogate treatments. The time naïve *P. glutinosus* remained outside their burrows differed significantly across treatments (χ^2 = 7.72, df = 2, P = 0.02). Naïve *P. glutinosus* remained outside their burrows during encounters with familiar *P. montanus* at least 0.17 min (mean = 7.75 min, SD = 6.27 min), at least 0.10 min (mean = 12.46 min, SD = 4.91 min) during encounters with naïve *P. montanus*, and at least 0.13 min (mean = 9.67 min, SD = 6.51 min) during surrogate treatments.

Question 2.—Do familiar and naïve *P. montanus* behave differently during encounters with familiar and naïve *P. glutinosus*?

Plethodon montanus adults did not display overt aggressive behavior. The only significant effect in the top ranked GLMM was the response of *P. montanus* during encounters with naïve *P. glutinosus* and the surrogate (Z = -3.097, P = 0.002); *P. montanus* actually showed more passive aggressive behavior to the surrogate than to naïve heterospecifics (Table 5). Similarly the only significant effect for sensory behavior was the increased response to the surrogate in comparison to the response towards naïve *P.*

Table 5. Frequency of behaviors exhibited by familiar and naïve *Plethodon montanus* toward intruders. Behavioral categories in which a statistically significant difference are denoted (*).

	***P. montanus* population**			
Behavior/Competior	**Familiar Mean**	**Standard Error**	**Naïve Mean**	**Standard Error**
Passive Aggressive^{+X}	2.96	0.46	2.43	0.38
Familiar *P. glutinosus*	2.94	1.02	2.05	0.58
Naïve *P. glutinosus*	2.40	0.71	2.00	0.58
Surrogate	3.57	0.66	3.26	0.79
Sensory	3.43	0.56	2.94	0.48
Familiar *P. glutinosus*	3.42	0.89	3.16	0.82
Naïve *P. glutinosus*	2.55	0.65	2.45	0.70
Surrogate	4.36	1.32	3.26	1.01
Avoidance^{+}	0.25	0.06	0.50	0.18
Familiar *P. montanus*	0.36	0.13	0.83	0.13
Naïve *P. montanus*	0.30	0.12	0.30	0.14
Surrogate	0.10	0.07	0.42	0.19

glutinosus (Z = -2.468, P = 0.014: Table 5). *Plethodon montanus* avoided *P. glutinosus* from MLBS significantly more than those from the naïve/allopatric area (Z = 1.974, P = 0.048; Table 5).

Discussion

Plethodon glutinosus adults were more discriminatory than adult *P. montanus*, but even so, we found no overt aggressive behavior between the species. Familiar and naïve *P. glutinosus* displayed passive aggressive and avoidance behavior during heterospecific encounters. Overall, *P. glutinosus* displayed more passive aggressive behavior to *P. montanus* from MLBS than those from the naïve/allopatric area, but *P. montanus* did not exhibit a similar response to heterospecifics. This type of behavior serves an important role in salamander assemblages. Passive aggressive displays are a means of conveying information about aggressive superiorities of resident salamanders (Walls and Semlitsch 1991). Salamanders may use these behaviors to avoid physical escalation during encounters (Jaeger 1986; Walls and Semlitsch 1991; Mathis et al. 1995, 2000; Wiltenmuth 1997). However, salamanders may use some passive aggressive behavior as exploratory or investigatory behaviors (e.g., look towards) (Wiltenmuth and Nishikawa 1998), which is likely why passive aggressive scores were high in surrogate treatments. *Plethodon glutinosus* also avoided heterospecifics from MLBS more than those from naïve/allopatric areas, and similarly, *P. montanus* avoided heterospecifics from MLBS more than those from naïve/allopatric areas. Therefore, we do see discrimination of familiar and naïve salamanders, for both species.

Adult *P. glutinosus* are known to aggressively defend their territories from both conspecific and heterospecific intruders (Nishikawa 1985; Marvin 1998; Price and Secki Shields 2002; Marshall et al. 2004), but in the introduced community at MLBS we see no overt aggression. Previous work on closely related *P. jordani* found that levels of behavioral aggression varied more with regard to conspecific aggression than heterospecific aggression (Hairston et al. 1987). At MLBS, *P. montanus* appears to have naturalized with little discernible impact on the native salamander assemblage. A factor that may have contributed to this is the use of non-injurious behavior to settle territorial disputes. Within many territorial species, contests are settled using passive aggressive behaviors rather than overt aggressive, injury causing behavior (Maynard Smith and Parker 1976). Rissler et al. (2000) also found that adult *P. glutinosus* and *P. montanus* did not display overt aggressive behavior during encounters.

Our results suggest familiarity may have slightly affected behavior of *P. glutinosus* and *P. montanus* at MLBS because both species show increased passive aggressive behavior to heterospecifics from the invaded community. The high level of passive aggressive behavior exhibited among familiar heterospecifics is not the pattern we expected as aggression can diminish through time (Brown and Wilson 1956; MacArthur and Levins 1967; Petren and Case 1996). For example, in dear enemy recognition, which previous work has documented in terrestrial salamanders, territorial individuals display less aggression towards familiar versus unfamiliar individuals (Wiley 1973; Brooks and Falls 1975; Jaeger 1981; Godard 1993). However, few studies have examined systems in which the introduced species appears to have integrated into native communities without dramatic impacts on the resident species (Strauss et al. 2006). Examining these types of systems may provide more insight into the roles of competition and behavior in determining the outcomes of the introduction of non-native species (Strauss et al. 2006).

Acknowledgments—We thank Henry Wilbur for allowing us to work at MLBS and providing information about the history of the introduction of *P. montanus*. We thank James Organ and Robert Jaeger for advice on this project. We thank Nicholas Caruso for assistance with the statistical analyses. We also thank Joseph Apodaca, Wally Smith, Chris Thawley, Horace Downer, and Cathy Newman for helpful comments on this manuscript. Funding was provided by MLBS and the University of Alabama Graduate School and Department of Biological Sciences. Authors used methodology in accordance with the University of Alabama IACUC protocol # 06-271-1. Salamanders were collected under Virginia Scientific Collecting Permit # 26165.

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Heather R. Cunningham is an ecologist and conservation biologist. She is currently the Statewide Coordinator of the Maryland Amphibian and Reptile Atlas. Heather completed her Ph.D. at the University of Alabama under the guidance of Dr. Leslie Rissler in 2010. Her research centers on the ecological and evolutionary factors that shape amphibian distributions with a focus on understanding the role of environmental factors and species interactions in limiting distributions. Heather's current research focuses on predicting species distributional responses to climate change, invasive species, and land-use change. (Photographed by Randy King).

Leslie J. Rissler is an evolutionary and conservation biologist. She is an Associate Professor and Curator of Herpetology at the University of Alabama. Leslie completed her Ph.D. at the University of Virginia under the guidance of Drs. Henry Wilbur and Douglas Taylor. She then went on to the University of California at Berkley with an NSF Bioinformatics fellowship where she studied with Drs. David Wake and Craig Moritz. Leslie focuses on amphibian biogeography, conservation genetics, and molecular ecology. She is currently serving a 2-year term as an NSF Program Director in Evolutionary Processes within the Division of Environmental Biology. (Photographed by Sarah Duncan).