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THE STATUS OF INVASIVE DUSKY SLUG (*ARION*) SPECIES IN THE UPPER
GREAT LAKES: A MOLECULAR APPROACH

By

Olivia Weber Hall

THESIS

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SIGNATURE APPROVAL FORM

THE STATUS OF INVASIVE DUSKY SLUG (*ARION*) SPECIES IN THE UPPER
GREAT LAKES: A MOLECULAR APPROACH

This thesis by Olivia W. Hall is recommended for approval by the student's Thesis Committee and Department Head in the Department of Biology and by the Dean of Graduate Studies and Research.

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ABSTRACT

THE STATUS OF INVASIVE DUSKY SLUG (*ARION*) SPECIES IN THE UPPER GREAT LAKES: A MOLECULAR APPROACH

By

Olivia Weber Hall

I update the distribution of two cryptic slug species, *Arion fuscus* and *Arion subfuscus*, in the Upper Great Lakes of the United States using molecular identification methods. *Arion fuscus* has not previously been reported in the literature in this region, and *Arion subfuscus* has previously been reported as abundant. However, all previous distribution studies were determined using visual identification tools, which can result in misidentification between cryptic species. To molecularly re-examine these distribution maps, I used a mitochondrial ribosomal subunit 16S PCR amplification and subsequent SspI and MfeI restriction enzyme digest to identify slug specimens. I collected slugs in 11 locations around Lake Superior, Lake Michigan, and Lake Huron using beer-baited pitfall traps from May to September of 2022. I collected slugs at one location in Marquette, Michigan biweekly, a total of eight times, throughout the summer to monitor fine-scale slug activity. I found *Arion fuscus* at 9 of the 11 locations and *Arion subfuscus* at no locations. *Arion fuscus* was found early in the spring before other slug species were active. This confirms the inaccuracy of previous distributions determined through visual identification, and calls for the widespread molecular re-examination of current slug distributions.

Keywords: *Arion fuscus*, *Arion subfuscus*, invasive species, distribution, rDNA 16S

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DEDICATION

I dedicate this thesis to my parents, Michael and Jarin Weber-Hall, and my partner, Maximilian Fowler. Thank you for your unwavering support and love.

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This thesis follows the format prescribed by *Malacologia*, to which this manuscript will be submitted. Instructions for authors can be found at:

<https://www.fieldmuseum.org/science/microsites/malacologia/about/instructions-authors>

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INTRODUCTION

There are many examples of cryptic species in invertebrates (Belyaeva et al. 2009; Barroso et al. 2010; Caputi et al. 2007; Macher et al. 2016). These are genetically different species that are difficult or impossible to separate based on physical appearances, are often reproductively isolated (Knowles 1993; Lincoln et al. 1998), and may have a history of being regarded as a single species (Bickford et al. 2007). The refugia available and geography during the Pleistocene greatly influenced many cryptic speciation events (Avice et al. 1998; Taberlet et al. 1998; Pinceel et al. 2004) because of the many allopatrically separated populations. During these speciation events, the selective pressures were on physiological or behavioral traits, like difference in resource use or environmental tolerances (Bensch et al. 2004; Damm et al. 2010; Derycke et al. 2016), which lead to cryptic species with different environmental niches, tolerances, and relationships (e.g. Macher et al. 2016).

In slugs, the genus *Arion* consists of at least 40 species, many of which belong to cryptic complexes (Pinceel et al. 2004; Barr et al. 2009; Jordaens et al. 2010; Soroka and Skujiene 2011; Hatteland et al. 2015). Even those that are not a part of a cryptic complex can easily be mistaken as other species, as the coloring and markings can differ based on many environmental conditions (Jordaens et al. 2001; Geenan et al. 2006). This has created a history of inaccurate identification and ambiguity in species distributions (Jordaens et al. 2006; Jordaens et al. 2010; Hatteland et al. 2015).

In general, the distributions of land gastropods are well documented in North America (Pilsbry 1948; Martin 2000; Forsyth 2004; Nekola 2014). Some are found as far north as the Arctic Circle (Verocai et al. 2014) and as far south as South America (Jordaens et al. 2006). However, with the recent discoveries of new species and the adoption of molecular techniques to better identify slugs, these distributions should be re-examined. For example, in Lithuania, it was widely thought that *Arion hortensis* was abundant until it was determined through molecular identifications that most of these slugs were *Arion fuscus* instead (Soroka and Skujiene 2011). The distributions of the species *Arion fasciatus*, *Arion silvaticus*, and *Arion circumscriptus* are entangled and difficult to separate due to inaccurate identifications as well (Geenan et al. 2006). The species complex *Arion fuscus* (Müller 1774) and *Arion subfuscus* (Draparnaud 1805) presents yet another example of such species (Pinceel et al. 2004; Jordaens et al. 2006; Barr et al. 2009).

Arion fuscus and *Arion subfuscus* are native to Northern Europe (Chichester and Getz 1969). Their external morphologies are identical, and they must be identified through dissection of the gonads or molecular methods (Pinceel et al. 2004). Both *A. fuscus* and *A. subfuscus* are invasive in North America, and as such, pose risks to their invaded environment. Like other invasive species, they have the potential to proliferate and upset their ecosystems when there are few natural factors limiting population growth (Elton 1958). They have been recorded harming plants, especially sensitive and endangered species (Cameron 2009; Meadley Dunphy et al. 2016). However, without the knowledge of where and when they are active, it is difficult to assess potential impacts.

In North America, it was generally accepted that *Arion subfuscus* was widely distributed (Beyer and Saari 1978; Chichester and Getz 1969; Moss and Hermanutz 2010; Gladstone 2020; Nottingham and Kuhar 2021). However, when a simple molecular technique to identify *Arion subfuscus*/*Arion fuscus* specimens was developed (Pinceel et al. 2004), it was discovered that some distributions that were thought to be *A. subfuscus*, actually belonged to the species *Arion fuscus* (L'Heureux and Angers 2018). A European study had similar findings (Jordaens et al. 2006). However, more recent studies of *Arion subfuscus* in North America have continued to use visual identification (Moss and Hermanutz 2010; Gladstone et al. 2020; Nottingham and Kuhar 2021). It is possible that some of these occurrences represent misidentification of *A. fuscus*. Although *Arion fuscus* has yet to be officially documented in the scientific literature as being present across most of North America, it was molecularly identified by former Northern Michigan University student, Rachel Sines, after finding the slug in Michigan's Upper Peninsula (R. Sines, K. Galbreath and A. Rebertus, unpublished data). Otherwise, the distribution of *Arion fuscus* throughout most of North America is unknown.

The current *A. fuscus* and *A. subfuscus* distributions in North America, including those in the Great Lakes region (Chichester and Getz 1969; Gladstone et al. 2020), are likely misconstrued due to the confusion in identification. In this study, I conducted new field collections and used molecular tools to determine: (1) where *Arion fuscus* and *Arion subfuscus* occur in the Upper Great Lakes region, and (2) the seasonal activity of *Arion fuscus* and *Arion subfuscus* populations in the Upper Great Lakes region.

MATERIALS AND METHODS

STUDY AREA

All of my collection sites are within the Upper Great Lakes region, adjacent to Lake Superior, Lake Michigan, and Lake Huron (Figure 1). Four collection sites were located in Michigan, three in Wisconsin, two in Minnesota, and two in Ontario, Canada (Table I). The dominant ecosystems transition from temperate deciduous forests in the south to northern boreal forest. This creates a zone with a unique species composition, where species from both forest types are able to thrive (Andersen 2005). The large water bodies create “lake-effect” zones that extend inland up to 80 km, where precipitation in the form of snow is enhanced and temperatures are moderated (Scott and Huff 1997). This heavier winter snowfall leads to excessive snowmelt and waterlogged soils that may persist well into spring (Schaeztl and Isard 1996; Stottlemyer and Toczydlowski 1999). The insulating effects of the lakes result in lower summer and higher winter temperatures. The minimum winter temperature is 5.5 °C higher in areas near the Great Lakes than in areas away from them (Changnon and Jones 1972; Scott and Huff 1997; Bates et al. 1999; Notaro et al. 2013).

FIELD METHODS

I collected slugs from 11 locations (Table 1; Figure 1). Seven sites had two collection periods, one in spring or early summer, and the other in late summer. These

were: Huron-Manistee National Forest-Cadillac-Manistee, Michigan; Huron-Manistee National Forest-Huron Shores, Michigan; Pictured Rocks National Lakeshore, Michigan; Firefly Lake, Wisconsin; Ledge County Park, Wisconsin; Cloquet State Forest, Minnesota; and Voyageurs National Park, Minnesota. A location in Marquette, Michigan was sampled biweekly to assess the phenology of slug activity, with 8 collections in total. These locations had two subsites, chosen for their different habitat types. The final three sites were sampled once and had one subsite to simply determine the presence or absence of *Arion fuscus*. These were: Lake Superior Provincial Park, Ontario; Michipicoten Island, Ontario; and Granite Ridge, Wisconsin. I recorded the dominant overstory tree species and litter depth at each site.

In each location and subsite, I placed ten 473-mL plastic cups in the ground with the top about 25 mm above ground level. I poured approximately 200 mL of Keystone brand beer in the cups. The cups were arranged randomly within a 5- by 5-m plot. Each location had 20 traps, 10 per subsite. In the Marquette site, I moved the subsites approximately 20 m halfway through the summer to minimize over-collection that might impact estimates of slug abundance over time. I also placed a 40- by 40-cm cardboard tile for the duration of the entire summer at each location as an alternative collection method, but this method was terminated after poor capture rates. It is unclear why this method failed, but conditions may have been too dry under the tiles because they were not placed deep enough in the leaf litter.

I placed traps, left them overnight, and removed them in the morning. For collecting traps, I decanted beer out of cups and poured all slugs out onto a flat surface. Then, I sorted slugs visually and counted them by morpho-type; *Arion subfuscus/fuscus*

complex (characterized by black vertical lines along the foot and a lack of an orange stripe under the black stripe along the tubercles) as one morpho-type, and other species found as their own morpho-types. I then took a subsample of up to seven specimens from each morpho-type, placed them in different vials, added 95% ethanol, and refrigerated them at 3° C. After 12 to 24 hours, I poured the ethanol out and replaced it with fresh ethanol. I brought the slugs collected to Northern Michigan University to perform molecular analysis to confirm the species identities.

MOLECULAR METHODS

To identify slugs from the *A. fusucus/A. subfuscus* complex, I followed the protocol of Jordaens et al. (2006). I took a small piece of tissue from the foot of the slug (approximately 1mm long), and lysed it overnight in a proteinase K solution at 56° C. I then extracted DNA from the samples following either the Qiagen Dneasy Tissue extraction protocol or the LGC Biosearch Technologies MasterPure extraction protocol. I then resuspended the extracted DNA in buffer AE. I PCR amplified the mitochondrial 16S ribosomal subunit using primers 16SAR and 16SBR. PCR reaction volumes contained 14.9 µl double-distilled water, 2 µl 10X Mg²⁺ buffer, 0.8 µl 10µM-16SAR primer, 0.8 µl 10µM-16SBR primer, 0.4 µl 10mM-dNTPs, 0.1 µl Taq DNA Polymerase, and 1 µl DNA template. PCR conditions were 3 minutes at 94° C, 35 cycles of 30 seconds at 94° C, 30 seconds at 46° C, and 1 minute at 72° C, then a final extension of 10 minutes at 72° C. Next, I ran a restriction enzyme digest on the amplified DNA using restriction enzymes SspI and MfeI. These reactions contained 9 µl double-distilled

water, 1 µl CutSmart buffer, 1 µl SSPI, 1 µl MfeI, and 5 µl PCR product. Restriction enzyme digest conditions were 2 hours at 37° C then 20 minutes at 65°C. I ran the final product on 2% agarose gel for 1 hour at 120V, soaked the gel in an ethidium bromide solution for an hour and a half, then visualized it under ultraviolet light. I used the length of the DNA fragment/site of restriction enzyme break to determine the species of the slug found (Jordaens et al. 2006).

To identify native slugs, I first visually identified all slugs using the key by Getz et al (2017). Slugs were then sorted into groups by species and by the location they were collected. I randomly chose one slug per species per location to molecularly confirm identification. To do this, I extracted DNA using the same methods described above. I PCR amplified the mitochondrial 16S ribosomal subunit using modified primers 16SAR and 16SBR for other *Arion* species, and the mitochondrial cytochrome oxidase subunit 1 using primers Pallifera-F and Pallifera-R (JR Dewaard, unpublished data) for *Pallifera* spp. I was unable to successfully amplify DNA from any *Deroceras* spp. specimens. The PCR conditions for each of these was the same as described above. These PCR products were sent to a third-party laboratory to be sequenced. I analyzed the electropherograms in Geneious, uploaded my sequences to MEGA, and used MEGA's BLAST (Tamura et al. 2021) to compare my sequences to existing sequences in the GenBank database to determine a tentative identification.

DATA ANALYSIS

To address the hypotheses that there is a seasonal variation in *A. fuscus* numbers, I generated a simple time trend regression model. For this analysis, I only used *A. fuscus*. I also ran a linearized mixed model and subsequent bootstrap (n=1000) with species as a fixed factor, time as a covariate, and location as a random factor, to compare *A. fuscus* abundance with those of the other species found (R Core Team 2023). To test whether the interaction of temperature and precipitation had an effect on slug activity, I ran a multiple linear regression model using slug count as a function of daily average temperature and the precipitation average from the window 15 days prior to collection. Temperature and precipitation data were retrieved from NOAA NOWdata.

RESULTS

Despite many purported records of *Arion subfuscus* in the Great Lakes region, I did not detect *A. subfuscus* in any of my sites. However, *Arion fuscus* was found at six of the main collection locations: Cloquet State Park, MN, Firefly Lake, WI, Marquette, MI, Pictured Rocks National Lakeshore, MI, Huron-Manistee National Forest- Manistee, MI, and Huron-Manistee National Forest-Huron Shores, MI (Figure 1). *Arion fuscus* was also found at all three supplemental collection locations: Lake Superior Provincial Park, ON, Canada, Michipicoten Island, ON, Canada, and Granite Ridge, WI. *Arion fuscus* was not found at Ledge County Park, WI, or at Voyageurs National Park, MN (Figure 1). Main sites that were less than 30 km from a Great Lake had an average catch rate of 25-85 *A. fuscus* slugs per collection through the summer, with the exception of Firefly Lake, which was approximately 80 km from Lake Superior and had an average catch rate of 28 slugs per collection. Main sites that were more than 80 km from a Great Lake had only a couple or no *A. fuscus* slugs collected there through the entire summer. *A. fuscus* was found in moist cedar forests, dry jack pine forests, temperate hardwood forests, boreal mixed wood forests, fields, disturbed areas, and even a remote, uninhabited island.

At the Marquette, MI site, slug activity was not significantly related to temperature alone ($F=0.09$, $p=0.76$), precipitation alone ($F=0.08$, $p=0.78$), or a model that also included the interaction ($F=0.40$, $p=0.75$) (Figure 2).

The Marquette, MI location was sampled biweekly to explore seasonal variation in more detail than was possible at the other locations. There, *Arion fuscus* counts

dropped significantly from spring to fall at the upland forest site ($F=33.8$, $p=0.001$), starting at 144 found in May and dropping to 0 found by September. Slugs in the moist subsite dropped more gradually from spring to fall ($F=34.34$, $p=0.001$) (Figure 3). For most other locations, I only collected samples twice, in spring-early summer and late summer, too few to assess temporal trends. Slugs at some locations declined from early to late (Firefly Lake, Huron-Manistee National Forest-Huron Shores), similar to Marquette, but at Pictured Rocks National Lakeshore, *A. fuscus* increased from early to late collections (Figure 4).

Arion fuscus was found earlier than all other species (Figure 4). At the Marquette, MI location, *A. fuscus* occurred without other species present for the first two collections on May 19th and June 2nd. The collections on June 20th were the first that detected another species. At Pictured Rocks National Lakeshore, *A. fuscus* was found within two weeks of snowmelt.

Four other species were found at various sites along with *A. fuscus*: *Pallifera ohioensis* (Sterki 1908), a native species, and *Deroceras reticulatum* (Müller 1774), *Arion fasciatus* (Nilsson 1822), and *Arion intermedius* (Normad 1852), invasive species. The MEGA BLAST search returned a 100% match for *A. fasciatus* and *Arion intermedius* specimens. *Pallifera ohioensis* and *Deroceras reticulatum* identifications should be considered tentative, since they were only identified visually. Mean counts of *Arion fuscus* were significantly different than all other species ($P<0.05$, reps=1000; Tukey-adjusted emmeans all $p<0.0001$) (Figure 4). About 300 bootstraps failed to converge, which reflects the high variation among sites and sampling periods. *Arion fuscus* counts were 20 times higher than all other species combined at the Marquette,

Michigan location, with 593 individuals of *Arion fuscus* and only 29 individuals of other species found, but this may be partially due to bait bias (see Discussion).

DISCUSSION

DISTRIBUTION AND ORIGINS

My study documents the presence of *Arion fuscus* in the Upper Great Lakes region. It is unclear whether *A. fuscus* spread into the Great Lakes region from the population in Quebec (L'Heureux and Angers 2018; Figure 1), whether they spread from the Great Lakes region to Quebec, or whether this was from a separate introduction. Gastropods disperse slowly (South 1965). If the Quebec population expanded into the Great Lakes region, it is likely a result of passive dispersal by other animals, including humans (Chichester and Getz 1969; Suarez et al. 2001; Cowie and Robinson 2003).

During the Pleistocene, *A. fuscus* was likely confined to the Balkan and the northern Alpine regions (Pinceel et al. 2005). These high latitude refugia may indicate that *A. fuscus* is well-adapted for cold environments. *Arion subfuscus*, on the other hand, is hypothesized to have originated from multiple refugia in the Iberian Peninsula, giving support to the idea that the separation of these two species may be a product of isolation in distinct Pleistocene refugia (Pinceel et al. 2004). Since then, the populations have likely expanded from their glacial refugia to their current ranges in Europe (Pinceel et al. 2004; Pinceel et al. 2005) through both long-distance locomotion (Rankin et al. 2019) and passive dispersal, such as egg dispersal by birds (Pearce et al. 2012; Shikov and Vinogradov 2013) or flooding (Rankin et al. 2019). Each population is identifiable by discrete haplotypes, such that populations from separate refugia now occur in different ranges and have slightly different genetic markers (Pinceel et al. 2004; Pinceel et al.

2005; L'Heureux and Angers 2018). Using this concept, it was determined that the invasive *A. fuscus* population in Quebec originated from two separate populations, one in northern Europe (Belgium, Denmark, Norway, and Sweden) and one in southwest Germany (L'Heureux and Angers 2018). This is evidence that there were at least two introduction events of *Arion fuscus* in North America. These introductions have likely occurred from ballast dumping (Chichester and Getz 1969; Robinson 1999) and nursery plant and produce trading at multiple ports (Chichester and Getz 1969; Zemanova et al. 2018). Other than through genetic haplotype comparisons, it is difficult to know where *Arion fuscus* introductions came from in Europe, since records of gastropods removed at ports are often classified only to genus. However, it is known that *Arion* spp. have been removed from ports along the Pacific coast of the United States and along the Great Lakes in shipments originating from France, the Netherlands, and the UK (Zemanova et al. 2018). The populations found in my study reflect such an invasion history.

Arion subfuscus was not found at any site in my study. This matches the findings from L'Heureux and Angers (2018), who found that *A. fuscus* was widespread in Quebec, Canada, but *A. subfuscus* was found at a very small percentage of sites. This may be due to different climate requirements between the two species, as *A. fuscus* may occur in areas more northern than *A. subfuscus* (Pinceel et al. 2004; Soroka and Skujiene 2011). Perhaps this region has not yet have had an *A. subfuscus* introduction, as there are no studies using dependable identification techniques that document *A. subfuscus* in the Upper Great Lakes. The discovery of abundant *A. fuscus* populations and no *A. subfuscus* populations casts doubt on the records of *A. subfuscus* in this

region that used external visual identification only (Beyer and Saari 1978; Gladstone 2020; Nottingham and Kuhar 2021).

I collected many *Arion fuscus* near Lake Superior, Lake Michigan, or Lake Huron, but collected little to no *A. fuscus* at the few sites that were distant from these lakes. Although there were only 11 locations, it appears that the range of *A. fuscus* may be limited west and south of the Great Lakes, where the temperature fluctuation increases and moisture decreases as distance to a Great Lake increases (Chagnon and Jones 1972; Scott and Huff 1997). Additionally, Pinceel et al. (2004) mapped the distribution of *A. fuscus* and *A. subfuscus* in Northern Europe using molecular methods, and found *A. fuscus* to occur more northerly (into Norway and Sweden) than *A. subfuscus* (limited to Northern France and Germany). The climate in Scandinavia is similar to the climate close to Lake Superior and other Great Lakes (Hodgkins et al., 2007). For example, the average winter temperature in the Great Lakes Basin is approximately -7°C (Hodgkins et al. 2007), and in the peak of summer, average temperatures are around 20°C (Hodgkins et al. 2007). Precipitation averages, in the forms of both rain and snow, are around 935 mm per year. Similarly, Southern Scandinavia also has cool summers and mild winters, with average winter temperatures around -1.5°C and average summer temperatures around 16°C (Björckl and Clemmensen 2004; Drobyshev et al. 2011). Annual precipitation, in the form of both rain and snow, is approximately 1000 mm (Björckl and Clemmensen 2004; Drobyshev et al. 2011). An extension of my study in the Great Lakes region with more collection sites will make the distribution clearer, and whether *A. subfuscus* is present further south.

The *Arion subfuscus*/*Arion fuscus* complex has been reported in multiple studies as present in every stand type and habitat type sampled (Chichester and Getz 1969; Beyer and Saari 1978), though these studies did not use molecular identification. It has been postulated that these species are able to disperse better than other invasive slugs in North America, because they have very flexible habitat requirements (Chichester and Getz 1969). This ability to thrive in different habitats was seen in the current study as well, as *A. fuscus* was found in highly diverse habitats, ranging from moist hardwood forests, to drier jack pine stands, to *Sphagnum* moss wetlands, to open fields. This ability could allow *A. fuscus* populations to colonize more easily than other slug species, a characteristic that is not uncommon in other invasive species (Lockwood et al. 2007; Ryser et al. 2011; Zemanova et al. 2017).

PHENOLOGY AND SEASONAL ACTIVITY

Arion fuscus was found without the presence of other species during the first two collections on May 19th and June 2nd at the Marquette, Michigan location. This pattern shows that *A. fuscus* is an early emerging species that may be able to feed on early emerging plants before other slug species become active.

It is difficult to determine the average time of year that slugs emerge, because it is dependent on an interaction of many factors, such as air currents, light, humidity, and soil and air temperature (Dainton 1943), especially as these environmental conditions change between years and study areas. However, there is evidence that slug life cycle

histories may influence when they become active in the spring. Hutchinson et al. (2016) found fully developed adults of *Arion subfuscus*/*Arion fuscus* (no molecular analysis was done to differentiate between the two species) present in all months of the year. Other *Arion* species, however, only have fully developed adults during specific windows. In the wild, two life cycle strategies are commonly seen in slugs: eggs which are laid right before winter and over-winter as eggs, and eggs which are laid mid-season, hatch before winter, and over-winter as adults (South 1989; Briner 1997). If *Arion fuscus* adults are active spring-fall (Hutchinson et al. 2016), they may be able to complete a fall hatch, emerge in the spring, and become fully active before other species that may hatch in the spring and then emerge as fully active adults later on.

The especially early emergence of *Arion fuscus* may also be a result of increased tolerance to multiple environmental factors, and may be crucial to their success in invading and dominating habitats. This behavior may be derived from its native region in northern Europe. For instance, *A. fuscus* was found to be more active than *Arion lusitanicus* during the cooler winter months, but during warm summer months, *A. lusitanicus* laid more eggs and had a higher rate of survival (Knop and Reusser 2012).

This early emergence may also give *A. fuscus* the opportunity to feed on native, early emerging plants. One example of this may be the endangered orchid *Calypso bulbosa*. *C. bulbosa* is native to the Great Lakes region and is one of the first herbaceous plants to emerge in the spring (Case 1987). Its population is rapidly declining, and *A. fuscus* may be partially to blame. A 2015 study found apparent signs of slug herbivory on the orchids (Bozic 2015), and my study confirmed that *Arion fuscus* is the only slug active when *C. bulbosa* emerges. Though this slug herbivory on *C.*

bulbosa is not confirmed, these results suggest that early emerging plants whose phenology overlaps with *A. fuscus* may be at risk. This interaction should be explored in future studies.

The change in population size from early to late collections varied by site, but the general trend seemed to be a decline across the summer months, as seen at Marquette, Huron-Manistee National Forest, and Firefly Lake. The exception to this was at Pictured Rocks National Lakeshore, where there was an increase of *A. fuscus* in summer collections. In the phenology of most slug species, including many *Arion* species, the timing, growth, and activity depend on environmental conditions like air and soil temperature, air and soil moisture, air pressure, and light (Dainton 1943; South 1989). My varied phenological results may have been the result of different environmental conditions between collections, especially at Pictured Rocks, which has direct proximity to Lake Superior and stays very cool all summer. My data do not show that slug activity was related to precipitation or temperature, but these are just a few factors that may influence slug activity (see Crawford-Sidebotham 1972; Young 1993). In addition, the weather data was from stations several km away, and may not represent the microclimatic conditions in the site the slugs were collected.

Other studies have also observed a sharp decline in *Arion fuscus/subfuscus* species in late summer (Beyer and Saari 1978), but the sharp fall decline in my study may be misleading. Despite the lack of slugs in the pit-fall traps in September, there were numerous *A. fuscus/subfuscus* complex individuals spotted throughout Marquette, MI during the same weeks (unpublished personal observation). This late-summer/early-fall time frame is also during the fall mushroom growth, and since slugs are well known

mycophagists (Buller 1922), the mismatch of observations may be due to slug preference of mushrooms over beer in the pitfall traps. The bias of using baited traps as a method for estimating fine-scale slug activity has been described in other studies as well (Young 1990).

There are other biases potentially affecting my study. There are many chemical differences between beer brands, and factors like CO₂ content and acrylic acid content can affect the attractiveness to slugs (Piechowicz et al. 2016). Although there have been no studies that compare preference of beer types across slug species, *Arion fuscus* simply may be more attracted to the beer that I used more than the other species in the area. Additionally, there may be a difference in distance of attraction to the bait between species (Bailey 1989; Hommay et al. 1998; Grimm and Schaumberger 2002), which could also lead to a biased collection.

CONSERVATION IMPLICATIONS

Arion fuscus significantly out-numbered all other species found. Invasive slugs outnumbering native slugs has been documented previously (Moss and Hermanutz 2010; Hatteland et al. 2015; Zemanova et al. 2017), but some of the other species found are invasive in the study area themselves. *Arion intermedius*, *Arion fasciatus*, and *Deroceras reticulatum* are all also invasive in North America (Getz et al. 2017). Without the history of the other species' population sizes at my study sites, it is impossible to

know if *A. fuscus*' presence has caused them to decline, and there is no evidence that *A. fuscus* is out-competing the others. Indeed, the other species' population sizes may have always been smaller. This situation may allow *A. fuscus* to easily invade and proliferate, leading to worsened ecological impacts. Assuming the capture of *A. fuscus* was not too biased by bait type, it currently far outnumbers all other species combined, which may bring with it all of the adverse impacts of low biodiversity and an unbalanced ecosystem (Elton 1958; Cameron 2009; Cardinale et al. 2012).

This study shows that *Arion fuscus* and *Arion subfuscus* do not necessarily co-occur, perhaps due to different habitat requirements. This has considerable conservation impacts. Cryptic invertebrate species have different ecological functions, different tolerances, and different habitat requirements (Chartock 1972; Macher et al. 2016; Paraskevopoulou et al. 2018). Due to these ecological differences, cryptic invasive species likely pose different threats in their respective ecosystems, and even treatments for invasive slugs may have to be adjusted accordingly between species. For example, when using molluscicides, timing the application with the peak activity of the target species can drastically increase the efficacy of the treatment (Crawford-Sidebotham 1972).

The use of visual identification for cryptic species studies is not limited to this study area (e.g. Chichester and Getz 1973; Beyer and Saari 1983; Moss and Hermanutz 2010; Hutchinson et al. 2016; Nottingham and Kuhar 2021). Some studies are even using the citizen science database, iNaturalist, to publish North American gastropod distribution studies (Gladstone et al. 2020). Although iNaturalist can be a good tool for documenting easily distinguishable species, with cryptic species it can be

problematic, since its maps rely only on visual distinction to identify species. Many more case studies using molecular methods need to be explored for cryptic invertebrate species to correct the current distribution maps recorded by studies that used only external visual identification tools. Molecular methods must become universally adopted when studying invertebrates that have a history of cryptic identification, and external features cannot be used as an identification tool for these cryptic species. Only once these types of studies are adopted will we understand the biology of these invasive species, which is needed for proper management to protect our natural ecosystems.

LITERATURE CITED

- Andersen BJ. 2005. The historical development of the tension zone concept in the Great Lakes region of North America. *The Michigan Botanist*. 44(3): 127-138.
- Avisé JC, Walker D, Johns GC. 1998. Speciation durations and Pleistocene effects on vertebrate phylogeography. *P Roy Soc Lond B Bio*. 265(1407): 1707-1712.
- Bailey SER. 1989. Foraging behaviour of terrestrial gastropods: Integrating field and laboratory studies. *J Mollus Stud*. 55: 263-272.
- Barr NB, Cook A, Elder P, Molongoski J, Prasher D, Robinson DG. 2009. Application of a DNA barcode using the 16S rRNA gene to diagnose pest *Arion* species in the USA. *J Mollus Stud*. 75(2): 187–191.
- Barroso R, Klautau M, Solé-Cava AM, Paiva PC. *Eurythoe complanata* (Polychaeta: Amphinomidae), the ‘cosmopolitan’ fireworm, consists of at least three cryptic species. *Mar Biol*. 157: 69-80.
- Bates GT, Giorgi F, Hostetler SW. 1993. Toward the simulation of the effects of the Great Lakes on regional climate. *Mon. Wea. Rev*. 121: 1373–1387.
- Belyaeva M, Taylor DJ. Cryptic species within the *Chydorus sphaericus* species complex (Crustacea: Cladocera) revealed by molecular markers and sexual stage morphology. *Mol Phylogenet Evol*. 50(3): 534-546.
- Bensch S, Perez-Tris J, Waldenstrom J, Hellgren O. 2004. Linkage between nuclear and mitochondrial DNA sequences in avian malaria parasites: Multiple cases of cryptic speciation. *Evolution*. 58(7): 1617-1621.
- Beyer WN, Saari DM. 1978. Activity and ecological distribution of the slug, *Arion subfuscus* (Draparnaud) (Stylommatophora, Arionidae). *Am Midl Nat*. 100(2): 359-367.

- Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. *Trends Ecol Evol.* 22:148–155.
- Björck S, Clemmensen LB. 2004. Aeolian sediment in raised bog deposits, Halland, SW Sweden: a new proxy record of Holocene winter storminess variation in southern Scandinavia? *Holocene.* 5: 677-688.
- Bozic AL. 2015. Habitat characteristics and demography of *Calypso bulbosa* and *Cypridium arietinum* in the Grand Sable Dunes, Michigan. Unpublished Master's Thesis. Northern Michigan University. United States—Michigan.
- Briner T. 1997. Investigations on the reproduction and the feeding behaviour of the slug *Arion lusitanicus* (Mabille). Unpublished Master's Thesis. University of Berne. Switzerland.
- Buller AH. 1922. Slugs as mycophagists. *T Brit Mycol Soc.* 7(4): 270-283.
- Cameron R. 2009. Are non-native gastropods a threat to endangered lichens? *Can Field Nat.* 123(2): 169–171.
- Caputi L, Andreakis N, Mastrototaro F, Cirino P, Vassillo M, Sordino P. 2007. Cryptic speciation in a model invertebrate chordate. *Proc Nat Acad Sci.* 104(22): 9364-9369.
- Cardinale BJ, Duffy E, Gonzalez A, Hooper DU, Perrings C, Venail P, Narwani A, Mace GM, Tilman D, Wardle DA, Kinzig AP, Daily GC, Loreau M, Grace JB, Larigauderie A, Srivastava D, Naeem S. 2012. Biodiversity loss and its impact on humanity. *Nature.* 486(7401): 59-67.
- Case FW. 1987. *Orchids of the Western Great Lakes Region.* Cranbrook Institute of Science Bulletin 48. Bloomfield Hills, MI. 251 p.

- Crawford-Sidebotham TJ. 1972. The influence of weather upon the activity of slugs. *Oecologia*. 9: 141–154
- Changnon SA and Jones DMA. 1972. Review of the influences of the Great Lakes on weather. *Water Resour Res*. 8: 360-371.
- Chartock MA. 1972. The role of detritus in a tropical marine ecosystem: Niche separation in congeneric ophiuroids, food partitioning in cryptic invertebrates, and herbivore detritus production at Eniwetok, Marshall Islands. United States—California. University of Southern California.
- Chichester LF, Getz LL. 1969. The zoogeography and ecology of arionid and limacid slugs introduced into northeastern North America. *Malacologia*. 7(2-3): 313-346.
- Cowie RH, Robinson DG. 2003. Pathways of introduction of nonindigenous land and freshwater snails and slugs. In: Ruiz GM, Carlton JT, editors. *Invasive species: vectors and management strategies*. Washington: Island Press. p. 93-122.
- Dainton B. 1943. Effect of air currents, light, humidity, and temperature on slugs. *Nature*. 151: 25.
- Damm S, Shierwater B, Hadrys H. 2010. An integrative approach to species discovery in odonates: From character-based DNA barcoding to ecology. *Mol Ecol*. 19(18): 3881-3893.
- Derycke S, De Meester N, Rigaux A, Creer S, Bik H, Thomas WK, Moens T. 2016. Coexisting cryptic species of the *Litoditis marina* complex (Nematoda) show differential resource use and have distinct microbiomes with high intraspecific variability. *Mol Ecol*. 25(9): 2093-2110.
- Drobyshev I, Niklasson M, Linderholm HW, Seftigen K, Hickler T, Eggertsson O. 2011. Reconstruction of a regional drought index in southern Sweden since AD 1750. *Holocene*. (4): 667-679.
- Elton CS. 1958. *The ecology of invasions by animals and plants*. London: Methuen. 181 p.

- Forsyth RG. 2004. Land snails of British Columbia. Royal BC Museum Handbook. Victoria (British Columbia, Canada): Royal BC Museum 188 p.
- Geenan S, Jordaens K, Backeljau T. 2006. Molecular systematics of the *Carnarion* complex (Mollusca: Gastropoda: Pulmonata): a taxonomic riddle caused by a mixed breeding system. *Biol J Linn Soc.* 89: 589-604.
- Getz LL. 1959. Notes on the ecology of slugs: *Arion circumscriptus*, *Deroceras reticulatum*, *Deroceras laeve*. *Am Midl Nat.* 61(2): 485-498.
- Getz LL, Chichester LF, Burch JB. 2017. Land mollusks of northeastern United States and southeastern Canada. *Malacological Review.* 45: 227-285
- Gladstone NS, Bordeau TA, Leppanen C, McKinney ML. 2020. Spatiotemporal patterns of non-native terrestrial gastropods in the contiguous United States. *NeoBiota.* 57: 133–152.
- Grimm B, Schaumberger K. 2002. Daily activity of the pest slug *Arion lusitanicus* under laboratory conditions. *Ann Appl Biol.* 141: 35-44.
- Hatteland BA, Solhøy T, Schander C, Skage M, Proschwitz T, Von Noble LR. 2015. Introgression and differentiation of the invasive slug *Arion vulgaris* from native *A. ater*. *Malacologia.* 58(1–2): 303–321.
- Hodgkins G, Dudley RW, Aichele SS. 2007. Historical changes in precipitation and streamflow in the US Great Lakes Basin, 1915-2004. US Geological Survey. 30 p.
- Hommay G, Lorvelec O, Jacky F. 1998. Daily activity rhythm and use of shelter in the slugs *Deroceras reticulatum* and *Arion distinctus* under laboratory conditions. *Ann appl Biol.* 132: 167-185.

- Jordaens K, Van Riel P, Geenen S, Verhagen R, Backeljau T. 2001. Food-induced body pigmentation questions the taxonomic value of colour in the self-fertilizing slug *Carnarion* spp. *J Mollus Stud.* 67: 161-167.
- Jordaens K, Pinceel J, Backeljau T. 2006. Life-history variation in selfing multilocus genotypes of the land slug *Deroceras laeve* (Pulmonata: Agriolimacidae). *J Mollus Stud.* 72: 229-233.
- Jordaens K, Pinceel J, Kriekemans H, Backeljau T. 2006. Accurate identification of cryptic slug taxa of the *Arion subfuscus/fuscus* complex by PCR-RFLP (Pulmonata: Arionidae). *J Mollus Stud.* 72(3): 323–325.
- Jordaens K, Pinceel J, Van Houtte N, Breugelmans K, Backeljau T. 2010. *Arion transsylvanus* (Mollusca, Pulmonata, Arionidae): rediscovery of a cryptic species. *Zool Scr.* 39(4): 343-362.
- Knop E, Reusser N. 2012. Jack-of-all-trades: phenotypic plasticity facilitates the invasion of an alien slug species. *Proceedings of the Royal Society B: Biological Sciences. Proc Roy Soc B-Biol Sci.* 297 (1747): 4668-4676.
- L'Heureux É, Angers B. 2018. A discreet invasion: distribution, origins, and expansion of the european slug complex *Arion subfuscus* S.l. in Quebec. *Can J Zool.* 96(4): 325–331.
- Lincoln R, Boxshall G, Clark P. 1998. A dictionary of ecology, evolution and systematics, 2nd ed. Cambridge (United Kingdom): Cambridge University Press. 361 p.
- Lockwood JL, Hoopes MF, Marchetti MP. 2007. *Invasion ecology.* Malden (MA): Blackwell Publishing. 304 p.
- Macher JN, Salis RK, Blakemore KS, Tollrian R, Matthaei CD, Leese F. 2016. Multiple-stressor effects on stream invertebrates: DNA barcoding reveals contrasting responses of cryptic mayfly species. *Ecol Indic.* 61: 159–169.

- Martin SM. 2000. Terrestrial snails and slugs (Mollusca: Gastropoda) of Maine. *Northeast Nat.* 7(1): 33-88.
- Meadley Dunphy SA, Prior KM, Frederickson ME. 2016. An invasive slug exploits an ant-seed dispersal mutualism. *Oecologia.* 181(1): 149–159.
- Moss M, Hermanutz L. 2010. Monitoring the small and slimy - Protected areas should be monitoring native and non-native slugs (Mollusca: Gastropoda). *Nat Area J.* 30(3): 322–327.
- Nekola JC. 2014. Overview of the North American terrestrial gastropod fauna. *Amer Malac Bull.* 32(2): 225-235.
- Notaro M, Holman K, Zarrin A, Fluck E, Vavrus S, Bennington V. 2013. Influence of the Laurentian Great Lakes on regional climate. *J Climate.* 26(3):789-804.
- Nottingham LB, Kuhar TP. 2021. Ambient moisture causes methomyl residues on corn plants to rapidly lose toxicity to the pest slug, *Arion subfuscus*, Müller (Gastropoda, Stylommatophora). *Crop Prot [Internet].* [cited 2023 Apr 6]; 147(2021): 105709. Available from: <https://doi.org/10.1016/j.cropro.2021.105709>
- Paraskevopoulou S, Tiedemann R, Weithoff G. 2018. Differential response to heat stress among evolutionary lineages of an aquatic invertebrate species complex. *Biology Lett [Internet].* [cited 2023 Apr 6]; 14(11): 20180498. Available from: <https://doi.org/10.1098/rsbl.2018.0498>
- Pearce TA, Mulvihill RS, Porter KA. 2012. Land slugs (Gastropoda: Pulmonata) on birds demonstrate dispersal potential. *Nautilus.* 126: 38–40.
- Piechowicz B, Watrakiewicz R, Rębisz E, Zaręba L, Balawejder M, Pieniżek M, Zwolak A, Grodzicki P, Sadło S. 2016. Beer as attractant for *Arion vulgaris*, Moquin-Tandon, 1885 (Gastropoda: Pulmonata: Arionidae). *Malacologica.* 24(3): 193–200.
- Pilsbry H. 1948. Land Mollusca of North America (North of Mexico). *Mg Acad Nat Sci Phila.* 3:1-1113.

- Pinceel J, Backeljau T, De Winter AJ, Jordaens K, Van Houtte N. 2004. Molecular and morphological data reveal cryptic taxonomic diversity in the terrestrial slug complex *Arion subfuscus/fuscus* (Mollusca, Pulmonata, Arionidae) in continental north-west Europe. *Biol J Linn Soc.* 83(1): 23–38.
- Pinceel J, Jordaens K, Pfenninger M, Backeljau T. 2005. Rangewide phylogeography of a terrestrial slug in Europe: evidence for Alpine refugia and rapid colonization after the Pleistocene glaciations. *Mol Ecol.* 14(4):1133-1150.
- R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rankin AM, Wilke T, Lucid M, Leonard W, Espindola A, Smith M, Carstens B, Sullivan J. 2019. Complex interplay of ancient vicariance and recent patterns of geographical speciation in north-western North American temperate rainforests explains the phylogeny of jumping slugs (*Hemphillia* spp.). *Biol J Linn Soc.* 127: 876-889.
- Robinson DG. 1999. Alien invasions: the effects of the global economy on non-marine gastropod introductions into the United States. *Malacologia.* 41(2): 413-438.
- Ryser S, Rindlisbacher N, Grueebler MU, Knop E. 2011. Differential survival rates in a declining and an invasive farmland gastropod species. *Agr Ecosyst Environ.* 144: 302–307.
- Schaetzl RJ, Isard SA. 1996. Regional-scale relationships between climate and strength of podzolization in the Great Lakes region, North America. *Catena.* 28(1-2): 47-69.
- Scott RW, Huff FA. 1997. Lake effects on climatic conditions in the Great Lakes Basin. MCC Research Report 97–01, Illinois State Water Survey Atmospheric Sciences Division. Champaign, IL. 617(April), 73.
- Shikov EV, Vinogradov AA. 2013. Dispersal of terrestrial gastropods by birds during the nesting period. *Folia Malacol.* 21: 105–110.

- Skujiene G, Soroka M. 2011. Species identification of slugs of genus *Arion férussac*, 1819 (Mollusca, Pulmonata) on the basis of genetics studies. *Ekologija*. 57(2): 70–80.
- South A. 1965. Biology and ecology of *Agrilolimax reticulatus* (Müller) and other slugs: spatial distribution. *J Anim Ecol*. 1: 403-417.
- South A. 1989. A comparison of the life cycles of the slugs *Deroceras reticulatum* (Muller) and *Arion intermedius* (Normand) on permanent pasture. *J Mollus Stud*. 55(1): 9-22.
- Stottlemeyer R, Toczydlowski D. 1999. Seasonal change in precipitation, snowpack, snowmelt, soil water and streamwater chemistry, northern Michigan. *Hydrol Process*. 13(14-15): 2215-2231.
- Suarez AV, Holway DA, and Case TJ. 2001. Patterns of spread in biological invasions dominated by long-distance jump dispersal: insights from Argentine ants. *Proc. Natl. Acad. Sci. U.S.A.* 98(3): 1095–1100.
- Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Mol Ecol*. 7(4): 453-464.
- Tamura K, Stecher G, and Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Mol Biol Evol*. 38:3022-3027.
- Verocai GG, Kutz SJ, Simard M, Hoberg EP. 2014. *Varestrongylus eleguneniensis* sp. n. (Nematoda: Protostrongylidae): a widespread, multi-host lungworm of wild North American ungulates, with an emended diagnosis for the genus and explorations of biogeography. *Parasite Vector*. 7(1): 1-22.
- Wilke T, Duncan N. 2004. Phylogeographical patterns in the American Pacific Northwest: lessons from the arionid slug *Prophysaon coeruleum*. *Mol Ecol*. 13(8): 2303-2315.

Young AG. 1990. Assessment of slug activity using bran-baited traps. *Crop Prot.* 9(5): 355-358.

Zemanova MA, Knop E, Heckel G. 2017. Introgressive replacement of native by invading *Arion* pest slugs. *Sci Rep-UK* [Internet]. [cited 2023 Apr 6]; 7(1): 14908. Available from: <https://doi.org/10.1038/s41598-017-14619-y>

Zemanova AM, Broennimann O, Guisan A, Knop E, Heckel G. 2018. Slimy invasion: Climatic niche and current and future biogeography of *Arion* slug invaders. *Divers Distrib.* 24: 1627-1640.

Table I: Slug trapping locations around the Upper Great Lakes region in the summer of 2022

Location	Latitude	Longitude	Stand type	Ground cover (%)	Leaf litter depth (mm)	Collections (month/day)
Main sites						
Marquette, Michigan, wetland-adjacent Moist forest site	46.4659	-87.201	<i>Acer rubrum</i>	35	6	05/19, 06/03, 06/20, 06/12, 06/29, 08/18, 09/08, 09/24
Marquette, Michigan, dry upland forest site	46.465	-87.208	<i>Tsuga canadensis</i>	70	12	05/19, 06/03, 06/20, 06/12, 06/29, 08/18, 09/08, 09/24
Pictured Rocks National Lakeshore, Michigan, hardwood forest site	46.647	-86.06	<i>Acer saccharum</i> and <i>Betula papyrifera</i>	100	12	05/15, 06/02, 06/20, 08/19
Pictured Rocks National Lakeshore, Michigan, coniferous forest site	46.649	-86.053	<i>Pinus banksiana</i> and <i>Abies balsamea</i>	100	trace	05/15, 06/02, 06/20, 08/19
Firefly Lake, Wisconsin, temperate-boreal forest site	46.003	89.63	<i>Pinus strobus</i> , <i>Acer rubrum</i> , <i>Quercus rubra</i>	80	6	06/26, 08/17

Firefly Lake, Wisconsin, lakeside forest site	46.000	-89.634	<i>Quercus rubra</i> and <i>Acer</i> <i>rubrum</i>	90	24	06/26, 08/17
Ledge County Park, Wisconsin, temperate forest site	43.4706	-88.581	<i>Quercus rubra</i> and <i>Acer</i> <i>saccharum</i>	20	trace	06/26, 08/28
Ledge County Park, Wisconsin, rocky outcrop site	43.467	-88.582	<i>Acer</i> <i>saccharum</i> and <i>Betula</i> <i>alleghaniensis</i>	100	36	06/26, 08/28
Cloquet State Forest, Minnesota, roadside temperate forest site	47.100	-92.389	<i>Pinus resinosa</i>	85	trace	06/05, 08/15
Cloquet State Forest, Minnesota, wetland adjacent forest site	47.102	-92.363	<i>Populus</i> <i>tremuloides</i>	100%	24	06/05, 08/15
Voyageurs National Park, Minnesota, rocky boreal forest site	48.433	-92.850	<i>Abies</i> <i>balsamea</i>	85	36	06/05, 08/16
Voyageurs National Park, Minnesota, boreal forest site	48.432	-92.852	<i>Abies</i> <i>balsamea</i> and <i>Acer rubrum</i>	50	12	06/05, 08/16

Huron-Manistee National Forest-Huron Shores, Michigan, temperate forest site	44.637	-83.525	<i>Abies balsamea</i>	<10	48	05/28, 08/21
Huron-Manistee National Forest-Huron Shores, Michigan, shrubland site	44.648	-83.523	Open field, adjacent to <i>Pinus strobus</i>	100	6	05/28, 08/21
Huron-Manistee National Forest-Cadillac Manistee West, Michigan, temperate forest site	44.257	-85.956	<i>Pinus resinosa</i>	50	12	05/27, 08/20
Huron-Manistee National Forest-Cadillac Manistee West, Michigan, wetland-adjacent shrubland site	44.263	-85.944	Open field, adjacent to <i>Thuja occidentalis</i>	100	trace	05/27, 08/20
Supplemental sites						
Lake Superior Provincial Park, Ontario, boreal forest site	47.320	-84.608	<i>Tsuga canadensis</i> and <i>Acer spicatum</i>	25	12	07/29

Michipicoten Island, Ontario, boreal forest site	47.73	-85.629	<i>Abies balsamea</i> and <i>Betula papyrifera</i>	N/A	N/A	07/03
Granite Ridge, Wisconsin, temperate forest site	44.145	-89.057	<i>Quercus alba</i> and <i>Pinus strobus</i>	N/A	N/A	07/29

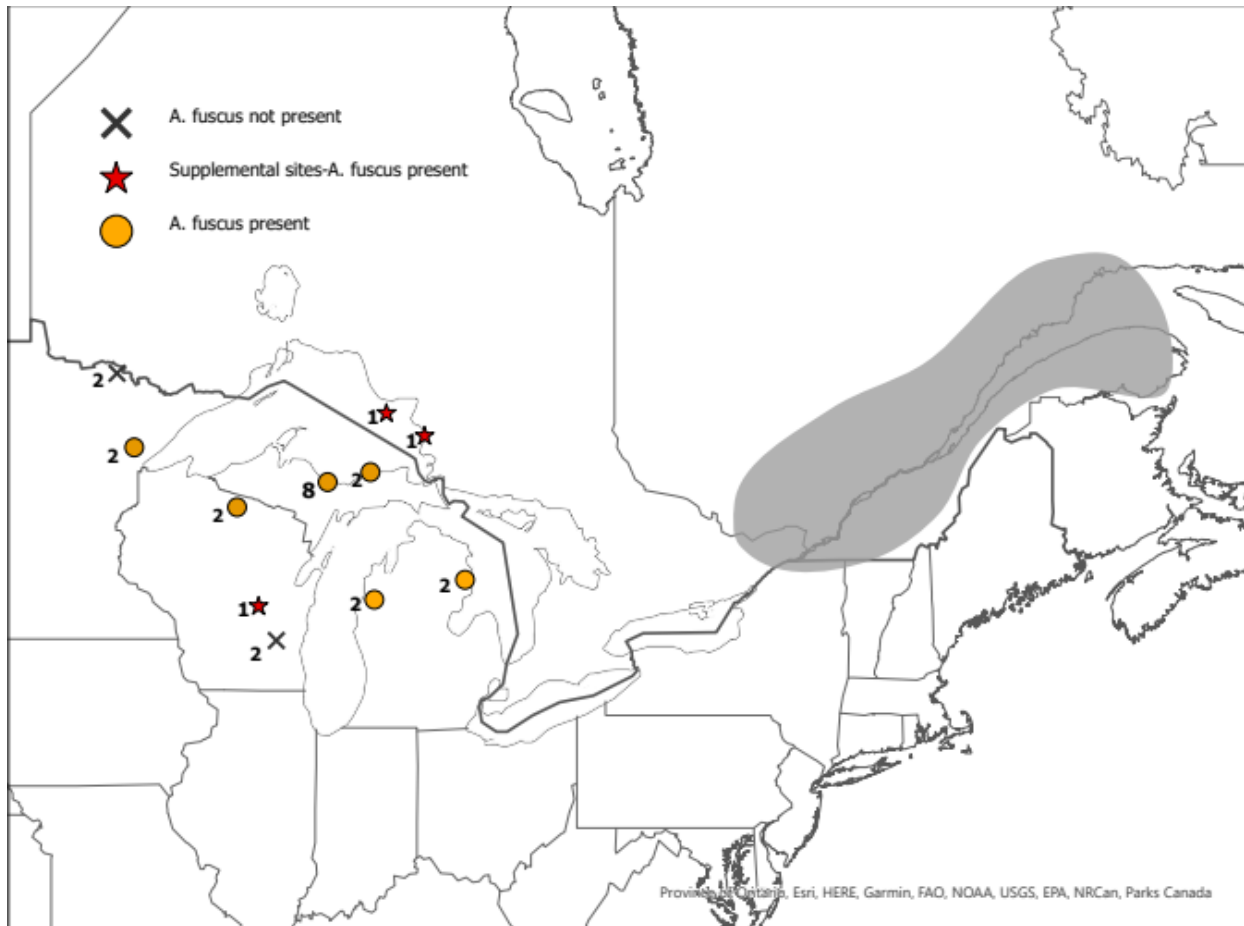


Figure I: Distribution of *Arion fuscus* in Michigan, Wisconsin, Minnesota, and Canada, May-Sept 2022. The number of collections at each location is denoted at the bottom left of its symbol. The *Arion fuscus* distribution described by L'Heureux and Angers (2018) is shaded in grey.

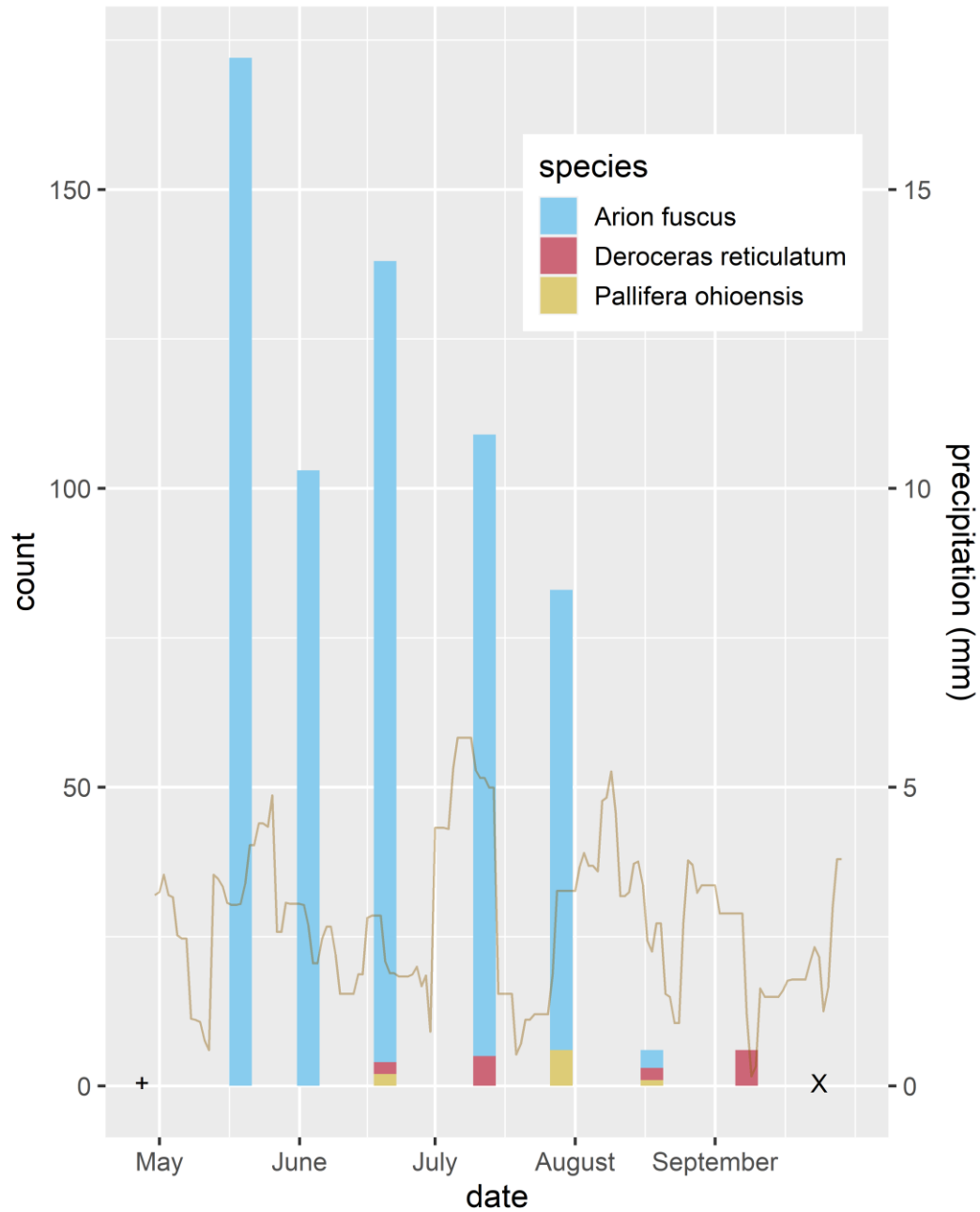


Figure II: Number of slugs of various species found in Marquette, Michigan during collections between May and September, 2022. An X is placed where no slugs were found during collection. The 15-day prior moving precipitation average is overlaid in brown. A '+' is placed on the last day of measurable snow on the ground.

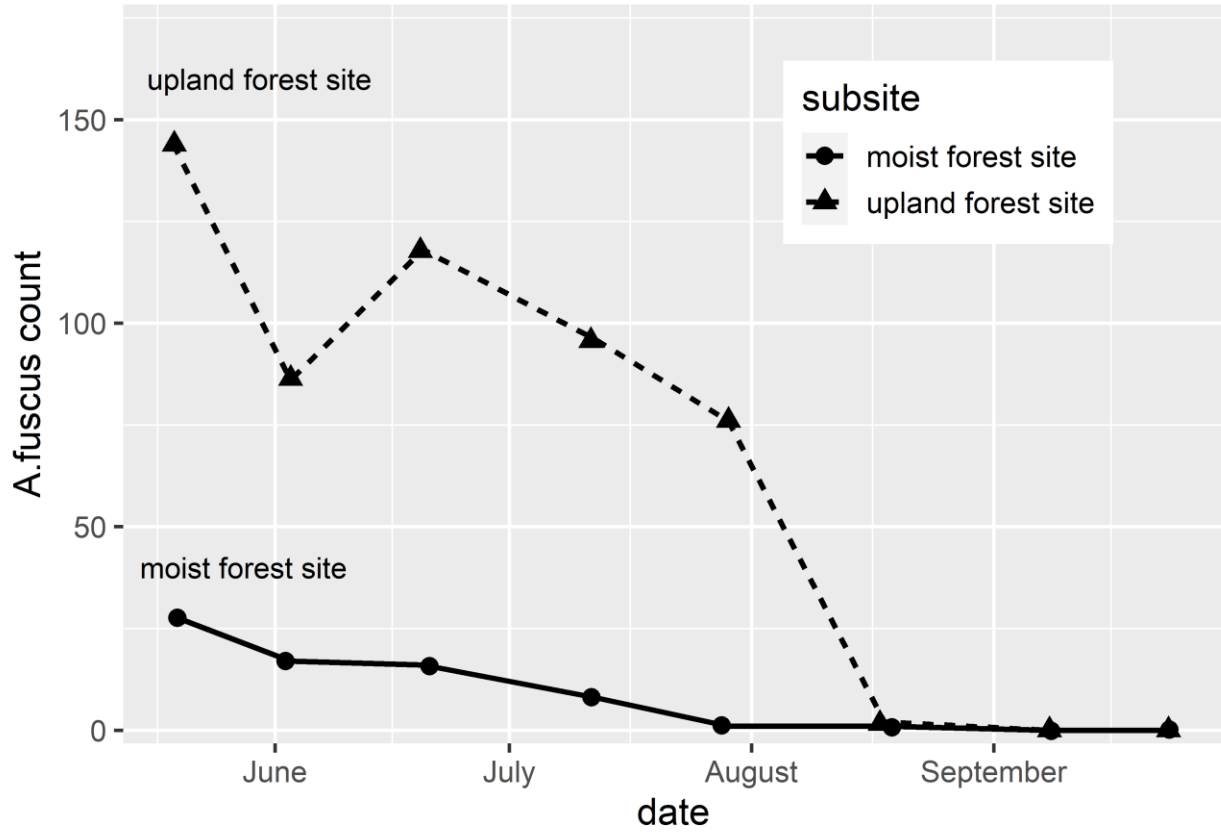


Figure III: Temporal trends in total *Arion fuscus* counts found in Marquette, Michigan by subsite during collections between May and September, 2022.

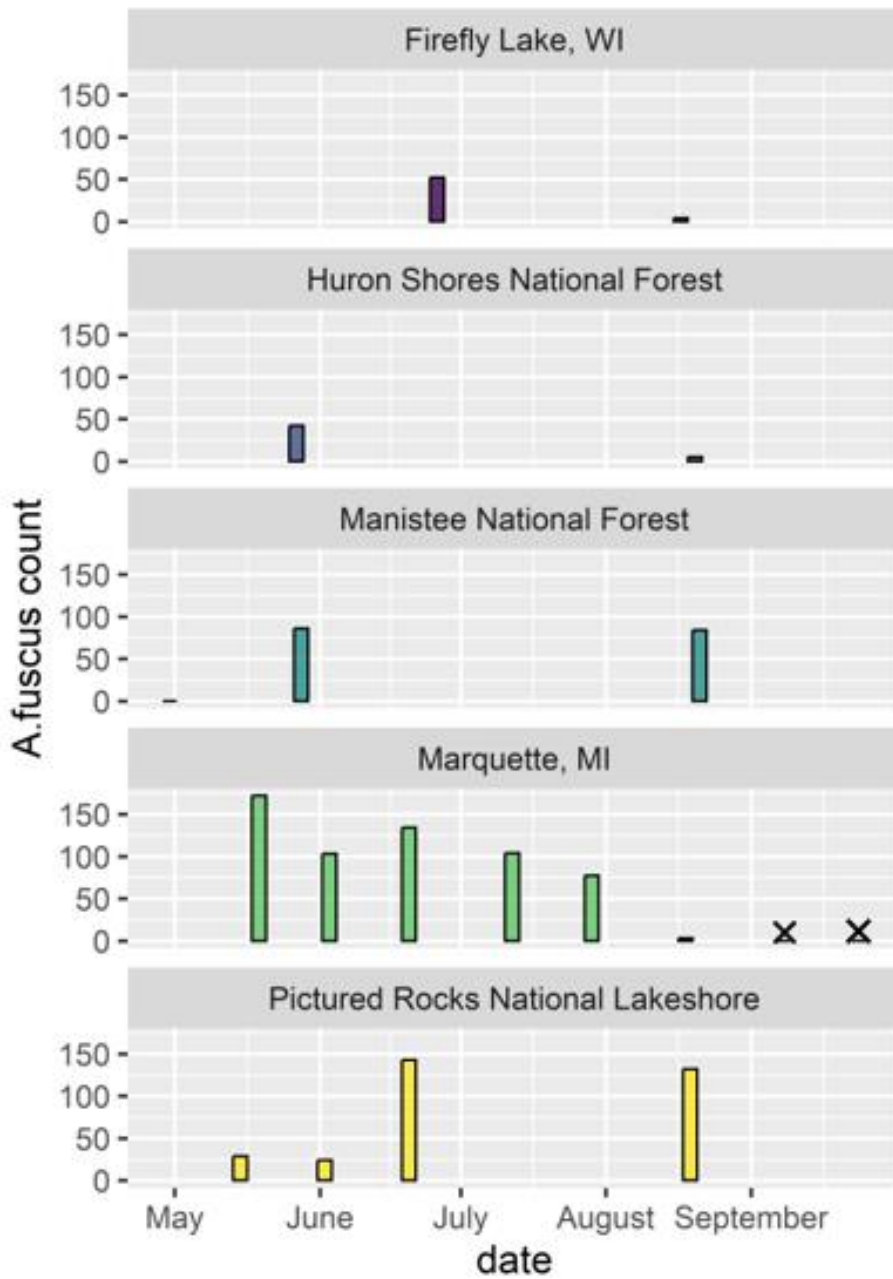


Figure IV: Number of *Arion fuscus* found at locations during collections between May and September, 2022. An X is placed where no slugs were found during collection.