ABSTRACT

Title of Document: EVALUATING COMPETITION BETWEEN THE NON-NATIVE SLUG *ARION SUBFUSCUS* AND THE NATIVE SLUG *PHILOMYCUS CAROLINIANUS*

Megan Elisabeth Paustian, Ph.D., 2010

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The degree to which invasive species have altered the demography, ranges, and microhabitat occupation of native species is poorly known. Yet, the competition-mediated decline of native populations, in concert with other factors such as habitat degradation, can place native species at risk of extirpation. Understanding whether competition between native and non-native species can take place under ordinary environmental conditions can allow us to extrapolate whether native species are likely to have experienced harm in the past and/or if they are likely to do so in the future. The native slug *Philomycus carolinianus* is likely to compete for resources with the aggressive non-native slug *Arion subfuscus* in central Maryland forests. In order to establish whether competition occurs between these two species, I tested for the following criteria: the existence of competitive displacement in the field, overlap in the use of limited resources (shelter and food), a decline in the fitness of *P. carolinianus* in the presence of *A. subfuscus*, and the action of competition mechanisms (interference and exploitation) between them. Field surveys showed that displacement between *A. subfuscus* and *P. carolinianus* does not apparently occur within mixed natural

populations. Resource use of the two slugs overlapped, with part of the diet (i.e. fungus) and a large proportion of the microhabitats occupied (i.e. coarse woody debris) in common. A lab experiment established that low natural levels of food (fungus) can limit the fitness of each slug species, while shelter (coarse woody debris) was not limiting. When sharing a low-resource lab cage with either *A. subfuscus* or conspecifics, *P. carolinianus* experienced a similar decline in fitness, suggesting that exploitative resource competition was no greater between heterospecifics than between conspecifics. No evidence of heterospecific interference (competition independent of resource levels) was found. Given the limited support for the criteria of competition, *A. subfuscus* was not shown to be an immediate threat to the persistence of *P. carolinianus*.

EVALUATING COMPETITION BETWEEN THE NON-NATIVE SLUG *ARION SUBFUSCUS* AND THE NATIVE SLUG *PHILOMYCUS CAROLINIANUS*

By

Megan Elisabeth Paustian

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Membership in the Barbosa Lab, who during my tenure has included Astrid Caldas, John Kemper, Eric Lind, Raul Medina, Carlo Moreno, and Gwen Shlichta, has been a boon to my research. While light-trapping moths at Patuxent National Wildlife Refuge, my lab-mates first observed that “big slugs” were crawling on the trees at night. Thus, during my first summer of graduate school, they introduced me to the fascinating creatures known as philomycid slugs. My fellow Barbosa-ans showed me potential field sites and offered advice in field and lab logistics. During lab meetings, they consistently impressed me with their excellent insights in designing experiments and presenting results for scientific meetings. They have been a model of ambitious, exacting research and good company besides.

My dissertation committee, consisting of Galen Dively, David Inouye, Tim Pearce, and Mike Raupp, helped to craft and refine my research plans to make them

much stronger. In particular, Tim considered project feasibility from the perspective of a landsnail expert, and our discussions provided me with many great ideas throughout the years I was working on this project. He also contributed much to the editing of the final manuscript.

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Finally, I would like to thank my family and David Powers, who endured much fretting, musing, and obsessing from my end as my research project gradually took shape. My parents allowed me keep my captive slug colony at their house during the winter holidays, and David cared for my slugs in my absence. All warmly humored and appreciated my enthusiasm for a quirky research subject and an unconventional career path.

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CHAPTER 1

The Abundance and Distribution of Co-Occurring Slug Species

INTRODUCTION

Non-native species are one of the major threats to the persistence of biodiversity today (Simberloff 1997). The harm posed by non-native species as predators and pathogens of native species and as ecosystem engineers is well recognized (Gurevitch and Padilla 2004). Less well known, and considered of lesser importance, is the degree to which non-native species compete with native species. Evidence of interspecific competition is difficult to establish (Schoener 1983; Simberloff 1997; Byers 2000) and requires a series of field surveys and experimental manipulations of the two species (Wiens 1989; Chapter 3). Among these pieces of evidence, field observations that indicate population displacement of a native species at a habitat or microhabitat scale are crucial to demonstrating that the non-native competitor harms the native species in a natural setting (Cross and Benke 2002).

Most systems in which native and non-native species compete are difficult to study, because the history of the invasion process and the current extent of sympatry among the species are rarely well known (Eastwood et al. 2007). Ideally, studies of competition between a native and a non-native species would track the progressing invasion front of the non-native species (Denno et al. 1995). The harm perpetrated by a non-native competitor can be measured as a population decline, shift in niche usage, or extirpation of the natives as the invasion front moves forward (Bøhn et al. 2008; Cheng et al. 2009). Systems in which the competitive interactions between native and non-native

species are well-known tend to be those with an invasion still in progress, such as the Argentine ant, the rusty crayfish, the New Zealand mudsnail, and the Asian tiger mosquito (Cope and Winterbourn 2004; Juliano and Lounibos 2005; Buczkowski and Bennett 2008a; Pintor et al. 2008). The relative ease of following an invasion front – a convenient natural experiment – may promote their study. However, catching competitive displacement in the act is rare (Reitz and Trumble 2002), and not all invasions that pass through result in the obvious habitat/microhabitat displacement of a native ecological analog. The original population sizes or distributions of native species are usually unknown (Parker et al. 1999), especially if the native species are not considered ecologically or economically important. Nor are the current ranges of most non-native species well known (Strayer 2009). Further, often, the non-native competitor is not noticed until many years after its introduction and population build-up (Byers and Goldwasser 2001).

Even if a non-native species were introduced and established its range in the distant past, competitive interactions between non-native and native species that are combined experimentally or that co-occur on the same sites may still be measurable. In post-invasion studies of competition between non-native and native species, researchers typically begin by noting an apparent displacement of the native species on a micro- or macrohabitat scale in the field, and they test for harm to the native species or for a mechanism of displacement when the two species are combined experimentally in the lab or field (e.g., Petren and Case 1996; Cope and Winterbourn 2004; Eastwood et al. 2007; Van Riel et al. 2007; Krassoi et al. 2008; Shinen and Morgan 2009; Stokes et al. 2009; Strubbe and Matthysen 2009). An alternative approach is to perform detailed surveys of

field populations to determine whether a native species and an established non-native species compete. Careful field surveys may capture subtle or small-scale spatial displacement that may suggest territorialism, avoidance, or differential fitness between sympatric non-native and native species (e.g., Wauters et al. 2002; Harris et al. 2006). Field surveys or long-term field experiments are critical, because competitive interactions observed in the lab may be insignificant when allowed to play out in a natural setting (Cross and Benke 2002).

This research aims to determine whether the native slug species *Philomycus carolinianus* (Bosc) and *Megapallifera mutabilis* (Hubricht) compete with the non-native Eurasian slug *Arion subfuscus* (Draparnaud) in central Maryland forests. *A. subfuscus* is a relatively aggressive slug (Rollo and Wellington 1979; Fernandez 1990) that often forms dense populations (up to 10 slugs/m^2, pers. obs.) and occurs widely in North American forests (Chichester and Getz 1969; Getz 1974; J.B. Burch pers. comm.). The native philomycid slugs *P. carolinianus* and *M. mutabilis* co-occur with the non-native species *A. subfuscus* at many forested sites. Although widespread extirpations of the native philomycid slugs apparently have not happened, it is unknown whether they interact competitively with *A. subfuscus* in the field or if their populations have declined since the introduction of *A. subfuscus* (Chichester and Getz 1968). Due to the lack of historical records, the slug fauna of North America pose a challenge to investigating the effects of non-native competitors on native species. Binney first recorded the presence of *A. subfuscus* in New England in 1842 (Chichester and Getz 1969), but European slugs could have been introduced as early as the 18th century (Getz and Chichester 1971). Early malacologists (and even recent researchers) overlooked the slug fauna and placed a

greater focus on other molluscs such as snails (Getz and Chichester 1971; Hubricht 1985). Regrettably, we do not know how quickly *A. subfuscus* has spread or how the native slug fauna may have responded. The eastern North American slug fauna is still changing and new introductions are occurring (Chichester and Getz 1968), including additional biotypes of *A. subfuscus* (Pinceel et al. 2005). However, field observations may be effectively used to understand competitive interactions of natural populations of gastropods. Surveying grids or nearest-neighbor distances in the field is a well-established approach to studying behavior, demography, and movement within gastropod populations (South 1965; Hunter 1966; Jennings and Barkham 1975; Baur 1986; Kleewein 1999). Therefore, field surveys may be used as part of the evidence to determine whether *A. subfuscus* and the native philomycid slugs compete.

In conjunction with laboratory experiments to test the fitness effects and mechanisms of competition between *P. carolinianus* and *A. subfuscus*, the spatial displacement of the native philomycid slugs *P. carolinianus* and *M. mutabilis* and the non-native *A. subfuscus* was assessed in the field through a series of surveys on a local scale (5 x 5 m cells) and on a microhabitat scale (< 50 cm^2). The daily interactions among individuals are encompassed on these scales, and slug home ranges would generally fit easily within 5 x 5 m cells (Cook and Radford 1988; Pearce and Örstan 2006). *A. subfuscus* may affect native slug microhabitat use, arrangement of home territories, and/or fitness, resulting in small-scale changes in the distributions of philomycid populations. To encompass spatial and temporal variation in competition, I surveyed slug populations across two years in three forest habitats characterized by different flora.

Distribution patterns of *P. carolinianus* and *M. mutabilis* may suggest spatial displacement by, and hence competition with, *A. subfuscus*. In order to resolve the various alternative scenarios the following questions need to be addressed: Is the non-native slug *A. subfuscus* more often spatially disassociated with the native slugs *P. carolinianus* and *M. mutabilis* than the two native slugs are with each other? On a small scale (< 50 cm^2), do *A. subfuscus* and *P. carolinianus* occur farther away from each other than from conspecifics? Spatial patterns of overdispersed individuals within a species may suggest antagonistic behavior (Rollo and Wellington 1979). On a small scale, does *A. subfuscus* less often occur in groups with other slugs (including either or both heterospecifics and conspecifics) than the two philomycids?

METHODS

Study Site

Slug abundances were tallied in mesic lowland beech-oak forests in the Central Tract of Patuxent Research Refuge (PRR), Laurel, Maryland, USA. I chose three sites having measurable numbers of all three slug species of interest. The three sites also differed in tree species composition, amount of underbrush, and level of light. Site A (N 39º03’07.6”, W 076º49’12.0”) occurred on a slope in a mixed deciduous forest dominated by American beech (*Fagus grandifolia*), with smaller numbers of tulip poplar (*Liriodendron tulipifera*), black gum (*Nyssa sylvatica*), and white oak (*Quercus alba*). Some undergrowth occurred towards one end of the site. Site B (N 39º03’19.7”, W 076º48’47.6”) was a beech-maple (*Acer*) forest with a diverse composition of

subdominant tree species, including tulip poplar, sweet gum (*Liquidambar styraciflua*), and pawpaw (*Asimina triloba*). The canopy was mostly open, much undergrowth occurred through most of the site, and the ground was prone to infrequent flooding. At site C (N 39º03'24.4", W 076º49'23.9"), beech was dominant with white oak and red oak (*Quercus rubra* or *Q. falcata*) subdominant. This site was shady and encompassed many dead fallen conifer trunks and little undergrowth. The geographic coordinates were taken from the approximate center point of each site. Sites were within 0.5-1 km of each other, were undisturbed second-growth forest, and occurred entirely within 100 m of the forest edge. Sites were 200-1000 m from residential developments.

Prior to performing systematic field surveys, I identified the slug species occurring at these sites through examination of their external and internal anatomy (see Pilsbry 1948, Webb 1950, Fairbanks 1990, Barker 1999). Voucher specimens of *P. carolinianus* (USNM 1125375, USNM 1125378) and *M. mutabilis* (USNM 1125376 USNM 1125377) were deposited at the National Museum of Natural History, Smithsonian Institution, Washington, DC.

Field Survey Methods

Slug identity and abundance was surveyed at several sites and through time to allow for the possibility that displacement is only clearly manifest at some sites (e.g. if resource amounts vary among sites) or only during some times of the year (e.g. during periods of food or shelter shortage). I set up three grids in each of the three field sites (Fig. 1.1). Each grid was a 25 x 25 m square with an arrangement of 5 x 5 cells (i.e., a total of 25, 5 x 5 m cells) (see Bohan et al. 2000). The three grids per site were aligned in a row and

shared some edges in common. Flags were planted at the corners of each cell and string demarcated the edges of each cell. I performed surveys of slugs occurring on the grids sometime between dusk and midnight (18:45-23:30) when slugs were active, between early June and late September. I undertook six surveys in 2007 (during June 11-19, July 9-11, July 23-25, August 14-16, September 4-6, and September 23-25) and three surveys in 2008 (June 9-12, August 4-9, and September 27-29). At least one evening was required to survey each site. Sites were always surveyed in the order A, C, and B. I identified and counted the slugs visible on live and dead tree trunks within my reach (i.e. below 2.25 m in height), on and beneath dead wood on the forest floor, and on vegetation. A maximum of five minutes survey was allowed per cell. The main goal was to obtain accurate measures of relative slug abundances across sites, so the bias inherent in these methods should be acceptable as long as it remained consistent across sampling units.

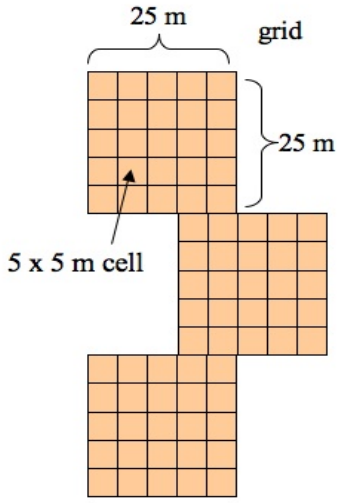


Fig. 1.1 A set of three grids on one field site. Each cell is 5 x 5 m, and each grid is a square consisting of a 5 x 5 arrangement of these cells. The upper and lower grids touch the middle grid along two edges to allow grids to be combined for analysis.

After slug population surveys were performed, I determined that the habitat factor of coarse woody debris (CWD) needed to be accounted for prior to analyzing population associations. A spatial analysis using the program SADIE (Perry 1998) indicated that pairs of the slug species were frequently positively associated (Fig. 1.2; see Appendix A, Section III for details of methods and results). Personal observations and measurements of the forest floor immediately around slugs suggested that all species were aggregating in areas rich in CWD. Measurements of the 1.0 m^2 microsites surrounding slugs (Chapter 3) showed that slugs occupied microhabitats with an order of magnitude more coarse woody debris than randomly-chosen 1.0 m^2 patches of forest (average: 15970 cm^3 vs. 1497 cm^3). Microhabitat requirements, such as CWD volume, can contribute greatly to aggregation (South 1965; Kappes 2005). South (1965) suggested that the availability of shelter, as a site of oviposition and its importance in limiting mortality, are the main factors controlling slug distributions. To account for this factor, the total volume of CWD at least 4.5 cm in diameter and at a moderate to advanced level of decay (stage three to five in decay in Stokland and Kauserud 2004) was calculated for each cell. For each piece of CWD, at least two diameters and the length was recorded. The volume of each piece was calculated as $v = (l \pi d^2)/4$, where v is volume, l is length, and d is average diameter.

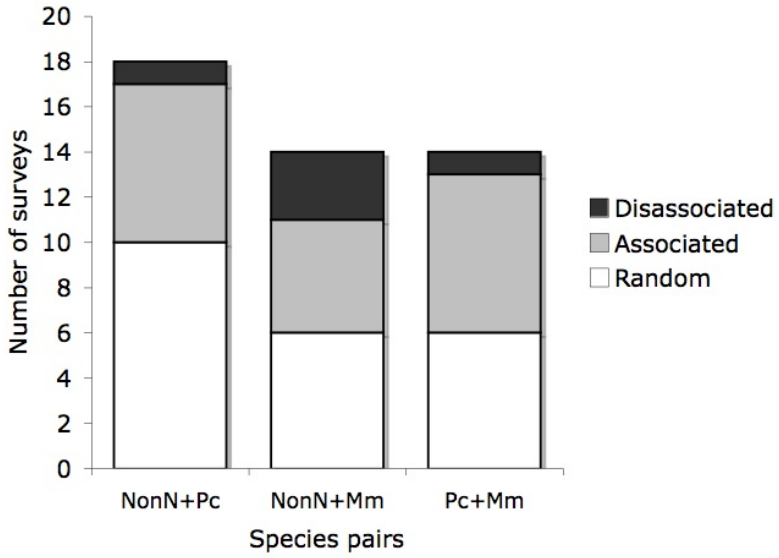


Fig. 1.2 The number of surveys that demonstrated significant disassociation, significant association, or neither between each species pair as determined through the spatial analysis program SADIE (see Appendix A, Section III for details of methods and results). NonN are non-native slug species (including mostly *A. subfuscus*), Pc are *P. carolinianus*, and Mm are *M. mutabilis*.

Additionally, at PRR, I surveyed the distances between individual pairs of slugs separated by less than 50 cm. The distance of 50 cm is an arbitrary length chosen to encompass the scale of interaction of slugs. Distances were measured for 63 *P. carolinianus – P. carolinianus* pairs, 35 *P. carolinianus – A. subfuscus* pairs, and 149 *A. subfuscus – A. subfuscus* pairs between May to September 2008 and April to May 2009. Distances between *M. mutabilis* and other slug species were not analyzed because too few individual of *M. mutabilis* were found. For each species, the proportion of slugs found in "groups" with other slugs (< 50 cm apart from other slugs) was also calculated. I surveyed 198 slugs total to determine the proportions occurring in groups.

Statistical Analysis

To determine whether pairs of slug species were (dis)associated on field grids, the relationship of each pair of co-occurring slugs was evaluated with CWD treated as a covariate. I analyzed population abundance datasets without spatial autocorrelation and datasets rescaled to eliminate the factor of spatial autocorrelation. I compared pairs of species abundance data for each survey session and site to determine if any pairs were spatially disassociated at any site or point in time.

A VARIOGRAM analysis in SAS was applied to each dataset combination of species, survey, and site to determine at what scales slug populations exhibited spatial autocorrelation (SAS Institute Inc. 2008). The distance interval between analyzed units is termed a spatial lag, which can be rescaled such that there are larger units that each include more points, or smaller units that each include fewer points (Fortin and Dale 2005). Spatial lags may be rescaled to eliminate the factor of spatial autocorrelation. I set the initial lag size at one cell length and the maximum number of lags at 10 cell lengths to match approximately the original scale of the grids. If a dataset did not produce a significant value for Moran's I, a coefficient of autocorrelation (Moran 1950), the dataset was considered spatially random at the original scale. If a dataset had a significant Moran's I, lag size was increased to 2 and to 5 and the VARIOGRAM analysis was repeated. (A semivariogram produced through VARIOGRAM was also examined to suggest at what lag size spatial autocorrelation leveled off.) Datasets examined at lag 2 (two cell lengths) were rescaled such that each unit was composed of a square of four cells. The fifth row and fifth column of each grid of cells were dropped because the cell count was an odd number. The resulting set of grids per site had 12 cells instead of the

original 75 cells. The lag 2 analysis was also attempted by splitting each 5 x 5 grid into four units of 2.5 cells per side and dividing up the counts of split cells among the new units. However, units of 2 x 2 cells and units of 2.5 x 2.5 cells produced the same outcome in the partial Mantel test, so only the former results are presented. Datasets of lag 5 (five cell lengths) were rescaled to include all 25 cells in a single grid of 5 x 5 cells as a new unit. However, because the resulting dataset was very small (three units), all of the lag 5 datasets across surveys and sites were combined for each species pair, making three lag 5 datasets. If a rescaled dataset did not have a significant Moran's I at lag 2, this scale of dataset was used in analyses. If a rescaled dataset had a significant Moran's I at lag 2 but not at lag 5, the dataset was analyzed at lag 5. If a dataset was autocorrelated at all scales, it was not used. See Appendix A, Section IV for a summary of the datasets that were rescaled, remained unchanged, or were unusable.

The association of the spatial arrangement between a pair of species at each site and survey was analyzed through a partial Mantel test. The measure of association is the Mantel statistic Z,

$$Z = \sum_{ij} X_{ij} Y_{ij}$$

where i and j are points, X_{ij} and Y_{ij} are the distances between each pair of points, and X and Y are distance matrices. (For my study, points i and j are grid cells, distances X_{ij} and Y_{ij} are differences in the count of slugs of one species between cells i and j, and matrices X and Y each contain all the "distances" among cells for a single slug species.) The value Z is the total of products of the corresponding values within the two matrices (Mantel 1967; Rosenberg 2001). The factor of CWD density was treated as a covariate to each

pair of slug abundance datasets through a partial Mantel test. The matrices X and Y were regressed against a third distance matrix (here, the distance matrix of CWD), and the resulting residuals were applied to the Mantel test formula (Smouse et al. 1986). To test the significance of the Z statistic, 10000 permutations of the original datasets were performed, in which the values of one dataset were shuffled while the other was maintained, and the partial Mantel test were performed on the permuted datasets. The size of the observed Z statistic was compared with the Z statistic of the randomized datasets (Rosenberg 2001). Two datasets are significantly positively associated if fewer than 500 of the 10000 permutations produces a higher Z statistic (i.e. right-tailed $P < 0.05$), and two datasets are significantly disassociated if fewer than 500 of the 10000 permutations produces a lower Z statistic (i.e. left-tailed $P < 0.05$). A normalized Mantel coefficient (r), the correlation of corresponding values in X and Y, was determined for each Z statistic. The partial Mantel tests were analyzed through the software package PASSaGE (Rosenberg 2001).

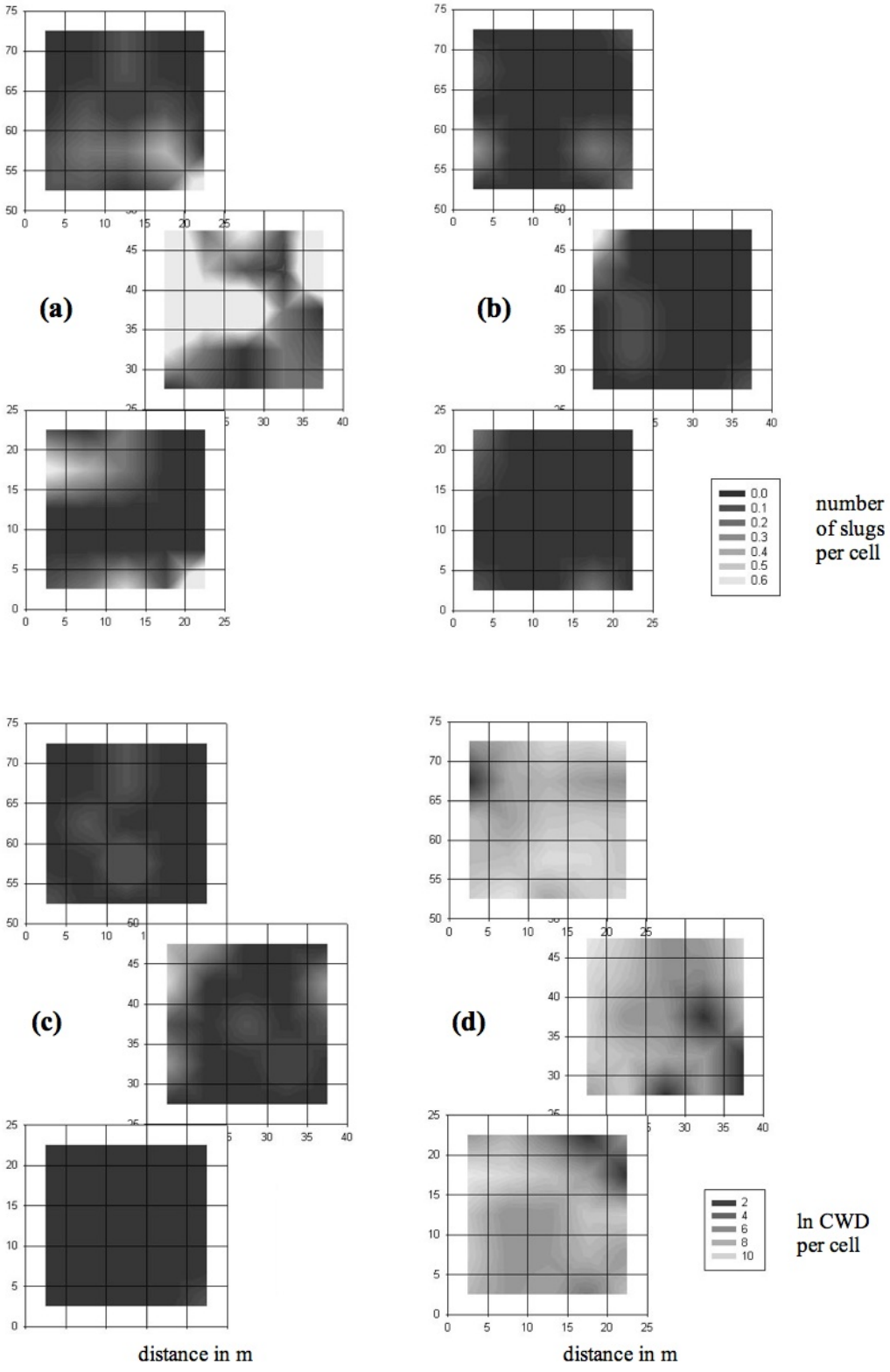
A one-way ANOVA was used to compare the distances among slug species pairings, with species pair as the factor. A chi-square test was used to determine if slug species occurred in similar frequencies in groups versus as individuals. SAS software version 9.2 was used to perform the ANOVA and the chi-square test (SAS Institute Inc. 2008).

RESULTS

The number of slugs of each species was counted per grid cell (e.g. Fig. 1.3). *A. subfuscus* was the most abundant species at each site (average of 0.22 slugs per cell) (Fig.

1.4). However, *P. carolinianus* was similarly abundant to *A. subfuscus* within site B (averages of 0.12 and 0.13 slugs per cell, respectively) and within site C (averages of 0.23 and 0.28 slugs per cell, respectively). *M. mutabilis* was the least common slug at all sites (average of 0.05 per cell).

Fig. 1.3 An example of slug population and CWD distributions at Site A. The average slug count per cell across surveys is shown for (a) *A. subfuscus*, (b) *M. mutabilis*, and (c) *P. carolinianus*. (d) The natural log of CWD volume is shown. Cells are small 5 x 5 m boxes within each grid. Graphs were produced through SigmaPlot version 8.0 (Systat Software, Inc. 2002).


(a)
(b)
(c)
(d)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
number of slugs per cell
2
4
6
8
10
ln CWD per cell
distance in m
distance in m

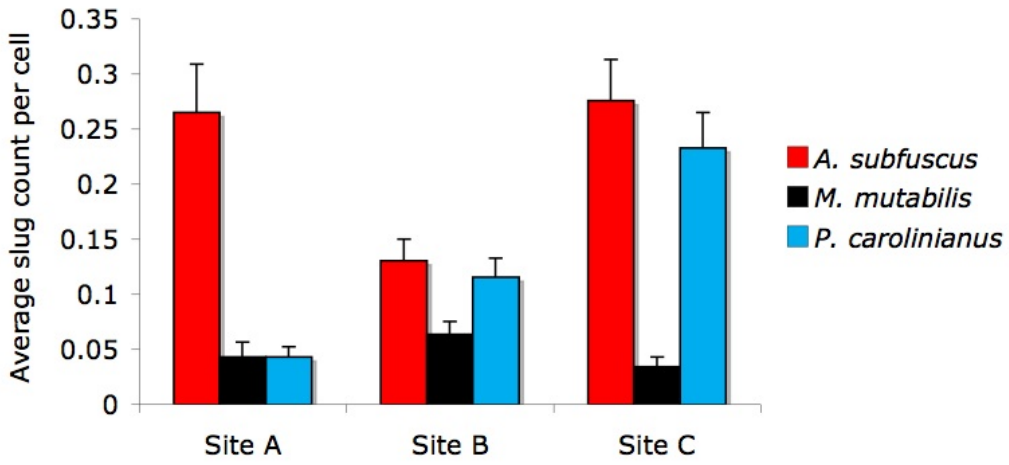


Fig. 1.4 Average abundance per cell of each slug species at each site. Slug numbers are averaged over all nine surveys. Error bars are +SE.

Slug numbers observed declined precipitously between the first and third surveys in 2007 and did not recover the next year (Fig. 1.5; also see Appendix A, Section II), resulting in many datasets of slug abundances that were too small to use in analysis in both years. Of the original 81 datasets (representing all combinations of three species, three sites, and nine surveys), 33 were unusable. One or fewer slugs were found in 22 datasets, five were spatially autocorrelated at all possible scales, and six had no other remaining dataset with which to be paired (given that all other datasets associated with that site and survey were unusable).

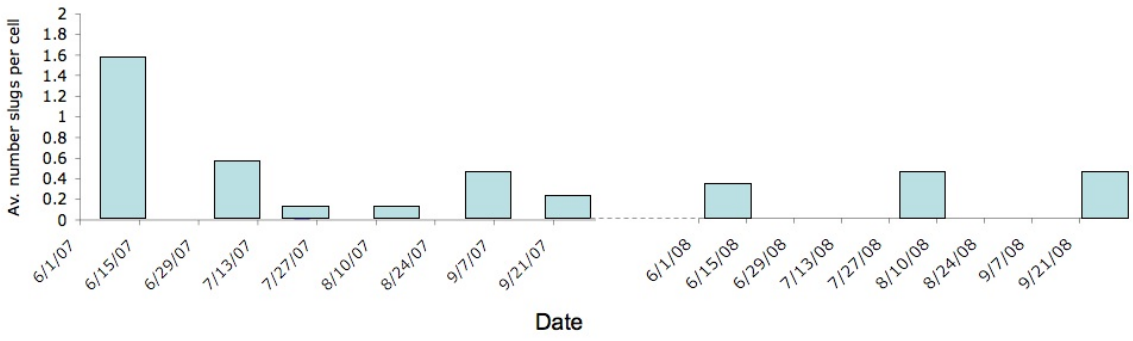


Fig. 1.5 Average abundance of all slug species through time. The average number of slugs per cell for each date is shown.

Even though CWD amount was treated as a covariate in the partial Mantel analysis of species pair abundances, no species had a significant disassociation with any other species at any site or during any survey (see Appendix A, Section V). Rather, positive associations (in which fewer than 500 out of 10000 simulations were equal to or more associated than the observed populations, determined by the Z-statistic, or right-tailed $P < 0.05$) were shared between *A. subfuscus* and *P. carolinianus* populations in 23% of the 13 surveys, between *A. subfuscus* and *M. mutabilis* populations in 12.5% of the eight surveys, and between *P. carolinianus* and *M. mutabilis* in 30% of the 10 surveys (Fig. 1.6a). Also, pairs of populations were significantly positively associated in 43% of the seven surveys at site A, in none of the five surveys at site B, and in 21% of the fourteen surveys at site C (Fig. 1.6b). Population pairs with negative correlation coefficients ($r < 0$; none significant) were scattered throughout the surveying seasons. However, fewer slugs per cell were found during the four surveys with the top percentages of negative correlations between populations than during the four surveys with the bottom percentage negative correlations (average slugs per cell: 0.218 in the

surveys with a higher percentage of negative correlations, 0.665 in the surveys with a lower percentage of negative correlations) (Fig. 1.7).

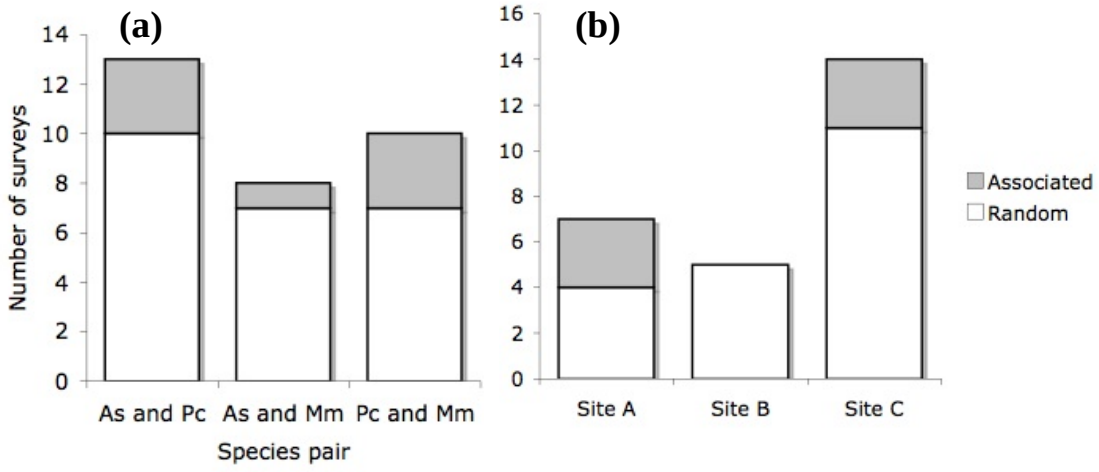


Fig. 1.6 Counts of surveys that show randomness or association between pairs of species distributions, obtained through partial mantel tests with CWD amount held constant. (**a**) Species pairings include all possible combinations of As (*A. subfuscus*), Pc (*P. carolinianus*), and Mm (*M. mutabilis*). (**b**) Counts of randomness and association are divided by field site.

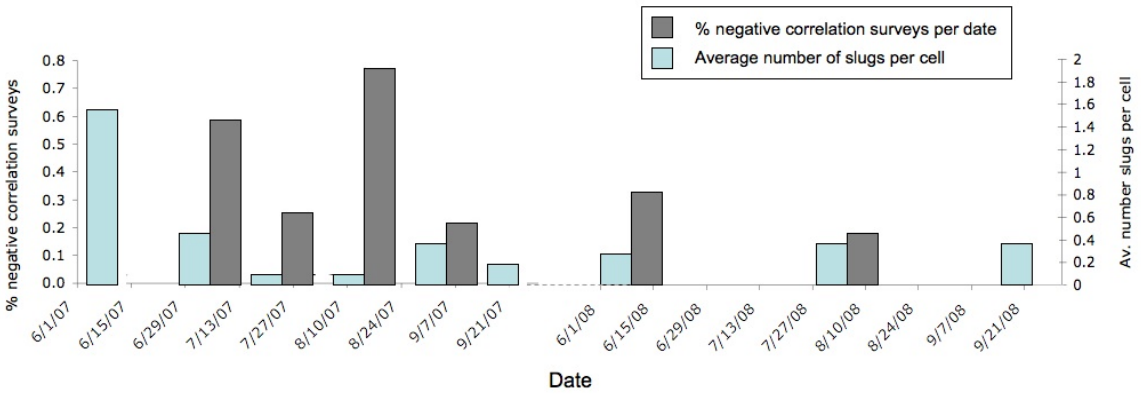


Fig. 1.7 Percentage of species population pairs whose spatial distributions were negatively correlated ($r < 0$) for each survey date. The average number of slugs per cell for each date is also shown.

Distances between pairs of slugs in the field did not differ significantly regardless of the identity of the slug species (ANOVA: $F_{2,193} = 0.26$, $P = 0.768$) (Fig. 1.8).

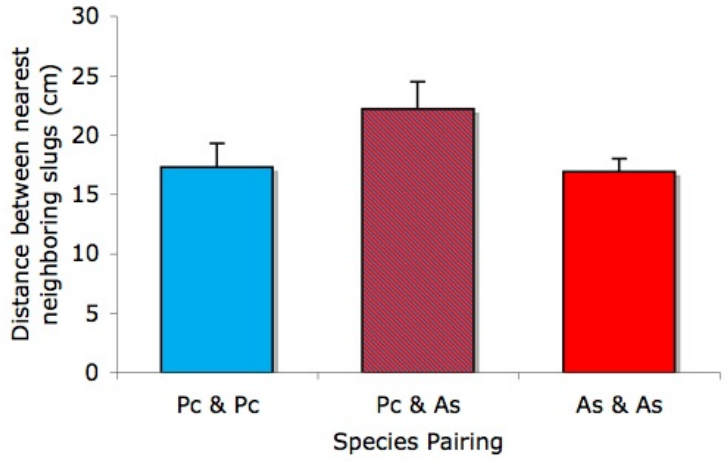


Fig. 1.8 Effect of species identity on the distances between pairs of slugs in the field. Pc is *P. carolinianus* and As is *A. subfuscus*. Error bar is +SE.

Among active slugs observed in the field, different proportions of each species occur in groups (less than 50 cm apart from other slugs of their species) ($\chi^2 = 15.7$, $P = 0.0004$) (Fig. 1.9). This difference is due to *M. mutabilis*, which is significantly less likely to occur in groups than *P. carolinianus* and *A. subfuscus*. If only *A. subfuscus* and *P. carolinianus* are analyzed, both species occur in groups at approximately the same frequency ($\chi^2 = 2.86$, $P = 0.0908$).

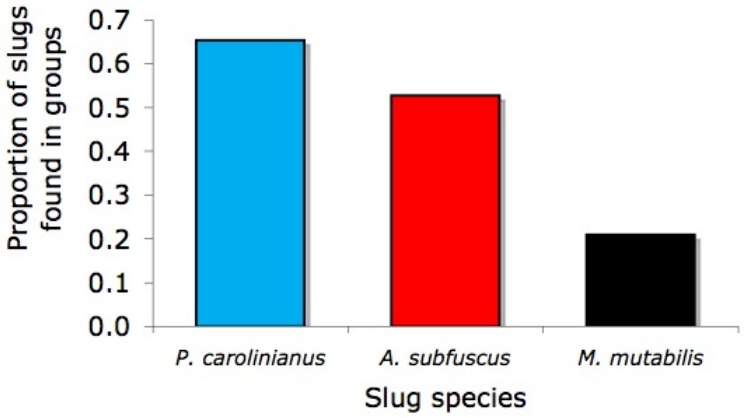


Fig. 1.9 Proportion of individuals of each species occurring in groups (< 50 cm from other slugs).

DISCUSSION

This series of surveys examined whether the native slug species *M. mutabilis* and *P. carolinianus* are dissociated in the field from a potential competitor, the non-native slug *A. subfuscus*. Populations were found to be aggregated, or more often, randomly distributed with respect to one another on a 5 x 5 m cell grid. On a smaller scale (< 50 cm), the proximity of *A. subfuscus* and *P. carolinianus* to other slugs was similar and

unaffected by the species identity of nearby slugs. Thus, *A. subfuscus* does not appear to be displacing the two philomycids on either scale investigated.

In this study, most surveys of slugs on a 5 x 5 m grid and all smaller-scale surveys determined that heterospecific species were randomly distributed with respect to one another. On a scale of 50 cm, *A. subfuscus* and *P. carolinianus* occurred at similar mean distances from heterospecific or conspecific individuals, and thus, did not appear to respond to each others' species identity. When active, *A. subfuscus* and *P. carolinianus* both occurred near other slugs (within 50 cm^2) at similar frequencies, and *M. mutabilis* occurred less often near other slugs. Thus, under the field conditions investigated, *A. subfuscus* does not show an inherent tendency to deter other slugs, i.e. to exhibit territoriality or other forms of interference, which would tend to create over-dispersion (Rollo and Wellington 1979). These results accord with studies of heterospecific distribution patterns in other gastropods (Bohan et al. 2000; Cross and Benke 2002). In contrast to the many examples of aggregation within gastropod species, studies have usually shown that a potential competitor does not affect spatial dispersion patterns between gastropod species. The slugs *Deroceras reticulatum* and *Arion intermedius* were usually randomly distributed in relation to one another in meadow sites (Bohan et al. 2000). Two freshwater snails (*Elimia cahawbensis* and *E. carinifera*) exhibited random spatial distributions with respect to one another in the field (Cross and Benke 2002). Intraspecific competition within these snail species was greater than interspecific competition between these snails (Cross and Benke 2002), as was demonstrated for *P. carolinianus* and *A. subfuscus* (Chapters 3 and 4) and predicted by competition theory

(Gause 1934). The absence of a spatial relationship may be expected for species pairs exhibiting limited interspecific competition.

Slug species were never disassociated with other species in the field on the scale of 5 x 5 m cells, and in fact, different species seem to be attracted to the same microhabitats. Despite my use of a partial Mantel test to correct for the factor of CWD volume, a predicted cause of aggregation among slug species, pairs of slug species were often positively associated at different times through the surveying season. Aggregation is very common within terrestrial gastropod populations and may be the norm for some species (South 1965; Hunter 1966; Baur 1986; Kleewein 1999; Glen and Moens 2002). Other studies that found spatial associations among slugs have suggested that habitat requirements are causing species distributions (Bohan *et al.* 2000). I corrected for the factor of CWD amount, but slug populations may be structured by additional microhabitat factors such as degree of food availability, soil moisture, and/or soil chemistry, in addition to some behavioral factors. For example, the slug *Limax maximus* seeks shelter closest to food (Rollo and Wellington 1979). A principal component analysis of factors relevant to land snail abundance found that soil pH, density of deciduous trees, and ground moisture were most important (Hylander et al. 2005). However, these microhabitat factors are not consistently important. Bohan et al. (2000) found that plants and soil moisture were unrelated to distributions of slugs in fields. Recently-hatched gastropods tend to be highly aggregated, because they emerge from egg masses (Hunter 1966; South 1992; Bohan et al. 2000b), which may themselves be clustered under refuges. Slugs become less clustered as they age (South 1965; Conner et al. 2008), although densities may persist around favorable ovipositing sites. Aggregations

within the habitat may result randomly from gastropods' limited dispersal tendencies (Pearce and Örstan 2006). Behavior such as mating (Kleewein 1999) and a tendency to slow activity when contacting mucus (Williamson et al. 1976) may also cause slugs to aggregate. Any number of these environmental and behavioral factors may be responsible for persistent aggregation among field populations of *A. subfuscus*, *P. carolinianus*, and *M. mutabilis*.

Interestingly, the frequency of population association varied among sites, with the highest number of associations at site A and the fewest at site B. An environmental factor such as resource availability is likely to cause slugs to behave differently at each site. Perhaps site B promotes a lower localized density of slugs because it provides the most live trees as habitat, while site A has the fewest (total diameter at breast height of live trees 40 cm and 27 cm per cell, respectively). Site B also has the most CWD and site A the least (155200 cm^3 and 47313 cm^3 CWD per cell, respectively). Although the partial Mantel test treated CWD as a covariate and removed its effects from analysis within each site, a greater availability of CWD may have enabled a denser growth of slug foods and a greater availability of favorable microhabitats across the whole of site B. The influence of other potential environmental factors on the frequency of aggregation is unknown.

Negative correlations among populations tended to occur when few slugs were observed, i.e. during periods of drought (Appendix A, Section II). These negative associations among populations suggest that observed aggregations might have been breaking up during periods of low activity. In contrast, most studies of gastropod field populations have found that animals aggregate during unfavorable weather in shared refuges (South 1992), both during the height of drought in summer (Baur 1986) and

during winter (Rollo and Wellington 1979). The cause of this discrepancy between current and previous results is unknown. Different species may have been selecting different kinds of shelter, or unlike other gastropod systems, may have been avoiding one another during periods of drought. Across taxa, drought tends to be associated with the appearance of competition (Schoener 1983). Alternatively, the scale of the study (5 x 5 m cells) may have hidden smaller aggregations. Associations within a slug species tend to coalesce and break up across different spatial scales of analysis due to the actions of different spatially-influenced factors (South 1965; Bohan et al. 2000).

This study does not support the theory that *A. subfuscus* is spatially displacing the two native philomycids from substrates. Despite shared resources and habitats, species often coexist without affecting each other's population sizes and distributions. Understanding how coexistence happens can be difficult (Birch 1979), and possible explanations, including environmental factors that suppress populations far below carrying capacity, subtle differences in niche usage, and temporal displacement, are manifold. Predators, food quality, and climate can be major factors influencing population abundance and distribution (Baur and Baur 1990; Loreau 1992; Ferrenberg and Denno 2003), keeping populations below their carrying capacity and limiting competition (Birch 1979). Interspecific competition for resources between *A. subfuscus* and *P. carolinianus* is not strong (Chapter 3). The relative strengths and importance of other population-regulating factors among these species are unknown. Reproduction is temporally displaced between *A. subfuscus* and the two philomycid species. In central Maryland, *A. subfuscus* mostly lays its eggs between mid-September and mid-October, whereas both *P. carolinianus* and *M. mutabilis* lay their eggs in the late spring and

throughout the summer (pers. obs.). This temporal difference in life cycles suggests that there many be minimal competition among heterospecific juveniles or among heterospecific reproducing adults. Although I found substantial overlap in substrate choice by these three slug species (Chapter 2), I may have been unable to discern subtle qualitative differences within microhabitats, such as interior levels of moisture, preferred by different species. Other temporal variables, such as periods of drought, can affect competition level (Schoener 1983). In general, both native and non-native species are able to establish and persist in an area because of spatial and temporal heterogeneity that allows the propagation of a poorer competitor (Melbourne et al. 2007).

Despite the absence of apparent displacement under the spatial scales and timeframes considered, competitive exclusion of the two native philomycids from habitats shared with *A. subfuscus* might conceivably happen over a long period of time (Mooney and Cleland 2001). The red squirrels (*Sciurus vulgaris*) and non-native gray squirrels (*Sciurus carolinensis*) of Europe exhibit such a pattern, in which habitat-wide displacement of the red squirrel is occurring without small-scale alterations in their territory size or distribution (Wauters et al. 2002). Gradual competitive displacement has been documented for some gastropod systems. Landsnail *Cepaea hortensis* replaced *Cepaea nemoralis* in some locations but only after a 20-year span (however, vegetation shifts may have been responsible, Cowie and Jones 1987). There were 50-year time lags between the introduction of a non-native freshwater snail (*Batillaria attramentaria*) and the extirpation of a native snail (*Cerithidea californica*), in both real life and in population simulations (Byers and Goldwasser 2001). Researchers did not identify that *B. attramentaria* was a threat to *C. californica* until many years after the introduction of *B.*

attramentaria, when the native population density finally declined after years of reduced fecundity; Byers and Goldwasser (2001) term this lag an "extinction debt." Within the *A. subfuscus*-philomycid system, any displacement of the philomycid slugs would likely be very slow, in part because they are not exact ecological analogues or strong competitors (Chapters 2 and 3).

Perhaps, a likelier outcome is that the complete displacement of *M. mutabilis* and *P. carolinianus* will never happen on a large scale. Even though many European slug species share similar niches with each other, these species typically coexist within the same habitats (Jennings and Barkham 1975). As this study determined, *A. subfuscus* apparently shared habitats and microhabitats with the two philomycid species without affecting their spatial distributions. Data on various taxa indicate that non-native competitors have very rarely caused native extinctions (Sax et al. 2002; Gurevitch and Padilla 2004). Usually, non-native species enter vacant niches within ecosystems (Mooney and Cleland 2001). Extinctions of native species are far more often attributed to predation and anthropogenic habitat destruction than competition (Gurevitch and Padilla 2004). As a weak force, competition would operate over a long time scale, probably enabling the interruption of competition asymmetry and possibly the evolutionary adaptation of natives (Davis 2003). A disadvantaged species could be replenished by influxes of migrants or stochastic events that kill their competitors.

Although *A. subfuscus* does not appear to threaten these two native philomycid species, *A. subfuscus* may be a substantially disruptive force in forest ecosystems and deserves more attention for this reason. *A. subfuscus* forms relatively dense populations in forests where only low-density populations of native slugs existed before (Chichester

and Getz 1968, 1969). Ecologists are concerned that earthworms introduced from Eurasia are altering geochemical cycling, soil microorganisms, and plant diversity in North American forests through their differential feeding habits and affect on decomposition processes (Bohlen et al. 2004; Hendrix et al. 2008; Nuzzo et al. 2009). Gastropods differentially feed on preferred species of leaf litter and fungus (Mason 1970a; Richter 1979) and thus, like earthworms, can be a significant force in structuring decomposition. Mason (1970b) estimated that up to 16% of annual leaf litter could be removed from a European beech forest by slugs. Banana slug *Ariolimax columbianus* contributes 24.75 kg/ha of feces in Pacific Northwest forests (Richter 1979). Thus, dense populations of *A. subfuscus* also have a potential to alter decomposition processes and their associated biota in North American forests.

CHAPTER 2

Comparison of Food and Microhabitat Preferences among Slugs in Mid-Atlantic Forests

INTRODUCTION

The niche is a long-established concept in ecology that remains useful as a means to estimate where a species can occur in the environment and thus how it interacts with other species, particularly as the environment and the composition of the species in the community changes (Kearney and Porter 2009). The "competitive displacement principle" indicates that species that share the same niche cannot coexist over the long term (DeBach 1966), and the amount of niche overlap between two species is positively related to the strength of competition between them (Schoener 1983). A non-native species may enter a system in which another species belonging to the same trophic level and using similar resources already exists. In these cases, competition tends to be greater between the non-native and native species than between the native and any of the native competitors with which it coevolved (Schoener 1983). If competition is asymmetric and favors the non-native species, the native species may experience a population decline and/or a displacement from its habitat (Holway 1999; Krassoi et al. 2008; Shucksmith et al. 2009). A critical first step in determining whether native species are likely to be harmed by an introduced species is to investigate dimensions and degree of niche overlap among them in comparison to other native species (Gutierrez et al. 2007; Desbiez et al. 2009).

I investigated the similarity in the food and microhabitat preferences of the non-native slug species *Arion subfuscus* (Draparnaud) and the native slug species *Philomycus carolinianus* (Bosc) and *Megapallifera mutabilis* (Hubricht) in order to determine whether these species are likely to compete with one another for resources. *P. carolinianus* and *M. mutabilis* are philomycid slugs that are native to eastern North America (Hubricht 1985), while *A. subfuscus* is a Eurasian arionid slug that was first introduced to the region more than 150 years ago (Binney 1842, cited in Chichester and Getz 1969). All three species are common in central Maryland forests (Hubricht 1985; Chichester and Getz 1973; Getz 1974) where populations are often sympatric.

Terrestrial slugs are likely to be strong competitors because they share many resources. Differences in niches among terrestrial gastropods are difficult to demonstrate (Cameron 1978). Major dietary overlap occurs among many slug species (Jennings and Barkham 1975), and adults of larger species may enter into interspecific competition because few refuges of suitable size tend to be available (Cook 1992). *P. carolinianus, M. mutabilis,* and *A. subfuscus* occur on coarse woody debris (CWD) and live trees (Pilsbry 1948; Kappes 2008; Aydin Örstan, pers. comm.), and pairs of these species occasionally co-occur in close proximity (e.g. < 50 cm apart) (Chapter 1). *A. subfuscus* has been observed to consume diverse foods, including dead and senescent plants, algae, dead animals, feces of other animals, and especially fungus (Graham 1955; Chichester and Getz 1973; Jennings and Barkham 1975; Beyer and Saari 1978). *P. carolinianus* is known to consume mainly fungus (Pilsbry 1948; Chichester and Getz 1973; Branson 1980), whereas *M. mutabilis* appears to feed mostly on algae (Aydin Örstan, pers.

comm.). Both *A. subfuscus* and *P. carolinianus* readily consume cultivated mushrooms used in lab experiments (pers. obs.).

Through a series of observations of food preferences and microhabitat use by these three slug species, I answered the following questions: to what degree do the food preferences of the three species overlap? To what degree do their microhabitat preferences overlap? Is overlap greater between pairs of non-native and native species than between the two native species? I hypothesized that *P. carolinianus* and *M. mutabilis* would exhibit greater resource overlap with *A. subfuscus* than they would with each other. By investigating shared resource use among these species, I sought to bring evidence to bear on the broader question of whether *P. carolinianus* and *A. subfuscus* compete.

METHODS

Microhabitat Choice Surveys

In 2006, one 20 x 20 m plot was established at each of four field sites as a pilot survey of slug abundances, microhabitats, and food types. Three sites (two in the central tract and one in the north tract) were located in Patuxent Research Refuge (PRR), Laurel, MD (N 39° 04,' W 076° 46'), and one site was located in Greenbelt Township, MD (N 39° 00' 40," W 076° 53' 27"). All sites were moist lowland deciduous forests. Prior to surveying slugs occurring at these sites, I identified slug species by examining their internal and external anatomy (see Pilsbry 1948, Webb 1950, Fairbanks 1990, Barker 1999). Between June 19 and July 12, 2006, I surveyed slugs during or soon after rain or at night, and I

visited each site one to three times. I identified and counted the slugs visible within my reach and underneath dead fallen wood, and I noted the substrate types (microhabitats) on which these slugs were crawling or sheltered. I recognized six categories of microhabitat: standing dead tree, live tree, CWD, leaf litter, vegetation (including live leaves on trees, living forbs, and grasses), and other (such as soil or rock).

During field surveys of slugs at PRR in 2007 and 2008 (see Chapter 1), the microhabitats occupied by individual slugs on a subset of predetermined grid cells were recorded. Surveys were conducted between dusk and midnight (18:45-23:30) from early June to late September in 2007 (6 surveys) and 2008 (3 surveys). In total, 501 individual microhabitat choices were observed for the slug species *A. subfuscus*, *M. mutabilis*, and *P. carolinianus*.

I sought to compare microhabitat choice with microhabitat availability. For 56 slugs observed during the first survey session in mid June, 2007, the microhabitat types on which they were found were recorded. For each grid cell, diameters of live trees at breast height were quantified, and volumes of CWD and dead trees were estimated using the formula for the volume of a cylinder, $v = (l \pi d^2)/4$, where v is volume, l is length, and d is average diameter.

Food Choice Surveys

During the 2006 pilot field survey (see Microhabitat Choice Surveys, above), slugs were collected from the four field sites and brought to the lab. These slugs originated from habitats where all three species were present. In the lab, individual slugs or groups of slugs (belonging to the same species and occurring in the same microhabitat) were kept

without food in separate plastic deli containers for one to two days. I placed their voided feces in 70% ethanol. In total, I collected the feces of 64 *A. subfuscus*, 45 *M. mutabilis*, and 53 *P. carolinianus*.

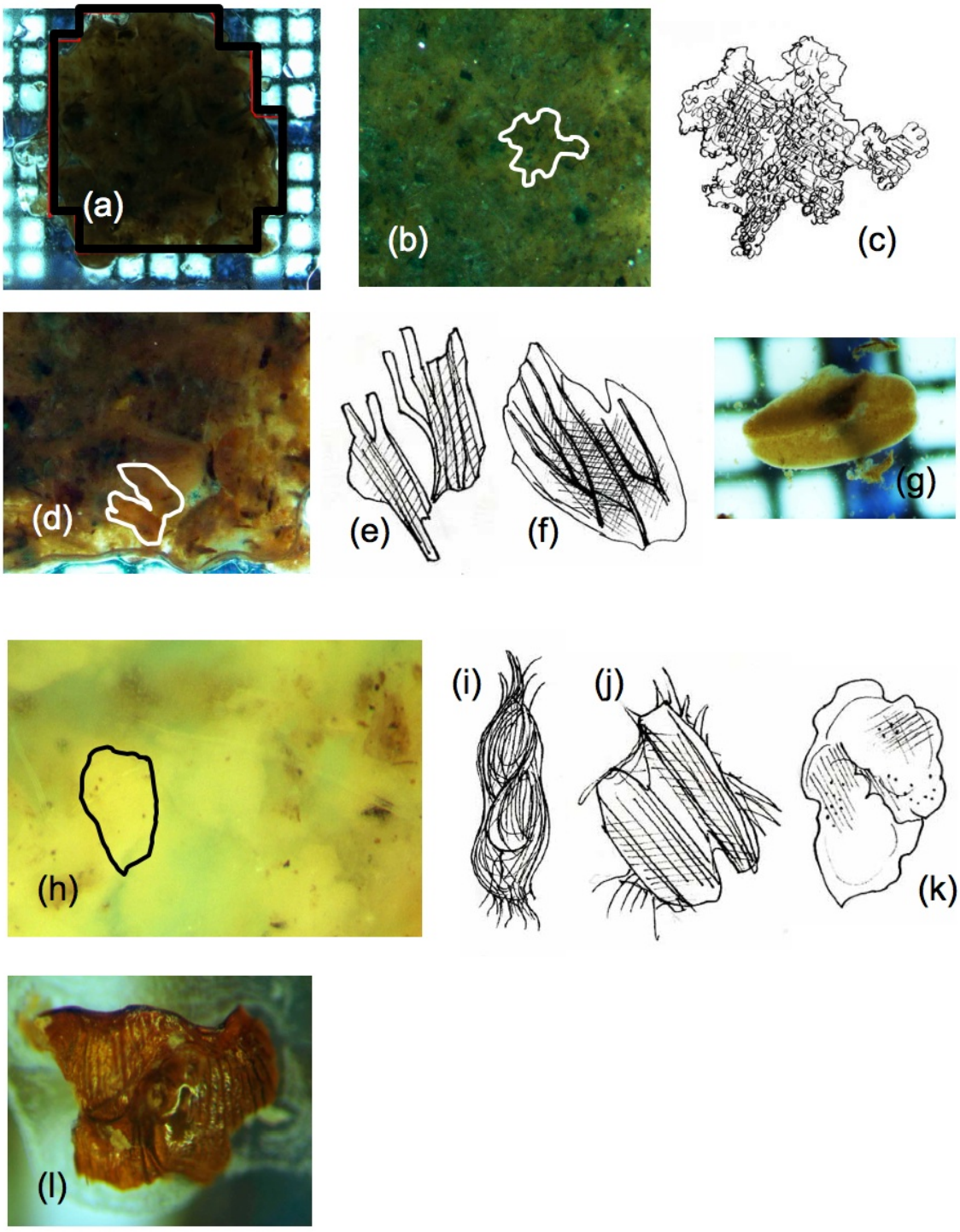
I fed single-food item diets to individuals of the slug *Deroceras laeve* (Müller) from lab colonies to obtain a reference collection of the food items that might appear in fecal samples. *D. laeve* were used because their feeding preferences are broad, and excreted material did not differ qualitatively among *D. laeve* and the other species (pers. obs.). Individuals were fed dead leaves (of trees, grasses, or forbs), live leaves (of trees, grasses, forbs, or lettuce), dried wild mushrooms, cultivated mushrooms, and earthworms to cover the likely scope of materials, fresh and dead, that would be in the feces of field collected slugs. I also studied undigested samples of the various food types under a compound microscope (Fig. 2.1). In fecal pellets, algae appeared as an amorphous mass of single green cells (Fig. 2.1b, 2.1c), often with bits of dead wood from which it was probably scraped. The chlorophyll colored the ethanol green. Plant tissue was characterized by texturally tough masses of relatively thick, ragged-ended fibers with long, evenly-spaced rows of cells (Fig. 2.1d, 2.1e). Wood appeared as very evenly-spaced three-dimensional tracheids (and little else), that resisted compression with forceps. Live plant material contained visible green chlorophyll. Leaves (alive or dead) tended to be flat and contained veins (Fig. 2.1f). Fungus varied greatly in form from amorphous aggregates of cells containing dark, scattered nuclei (Fig. 2.1h, 2.1k) to thin, hair-like loose or connected fibers (Fig. 2.1i, 2.1j) and star-shaped branching hyphae. Fungus was spongy to occasionally tough in texture. The hyphae were usually white (Fig. 2.1h) but also ranged in color from grey, orange, to occasionally black. Spores were infrequently

visible, and these were helpful in identifying material as fungus. Crystalline or glossy minerals probably derived from soil were distinct and easily recognizable. Pieces of chitinous insect exoskeleton were occasionally found (Fig. 2.1l), including whole mites (which may have been consumed unintentionally or had been feeding on the feces).

I examined the feces of wild-caught animals under a light microscope (Pallant 1972; Jennings and Barkham 1975; Chatfield 1976; Speiser and Rowell-Rahier 1991; Hatziioannou et al. 1994; Hägele and Rahier 2001), separated distinguishable food types, and compressed the separated feces between two microscope slides in order to obtain a relative volume of each food type in the feces (Fig. 2.1a). I applied a standardized treatment to all the samples, letting the weight of the slide on top compress the material. The feces were resistant to changing in area with additional compression. The area covered by each food type was determined by placing the slide over a grid composed of 1.0 x 1.0 mm squares (Cook and Radford 1988). Categories of food types were algae, fungus, plant tissue, wood, minerals from soil, insect exoskeleton, and unidentifiable "other" materials, the lattermost typically being less than 5% of food volume for all species.

Fecal samples per species were too few to compare feeding preferences among sites.

Fig. 2.1 Identification of food types in the fecal material of *A. subfuscus, P. carolinianus,* and *M. mutabilis*. Each food type was compressed between two glass slides and quantified on (**a**) a microgrid, where grid scale equals 1 mm per box side, and quantification is represented by the black line drawn around the food mass. The main food types identified were (**b,c**) algae, (**d,e,f,g**) plant, and (**h,i,j,k**) fungus. Plant recognition was often aided by the presence of distinct structures such as (**f**) leaf veins and (**g**) pollen grains, and fungus was recognizable by the presence of (**i,j**) thin, hairlike mycelia. (**l**) Insect chitin was sometimes seen. (Outline of algal clump (**c**) is shown on algae feces (**b**), plant fibers (**e**) are shown on plant feces (**d**), and fungus clump (**k**) is shown on fungus feces (**h**).)


(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)

Statistical Analysis

Pianka's index of niche overlap O is a means to estimate the degree to which two species make use of the same "types" of a given resource (e.g. food or microhabitat) that includes many categories or types. O compares the relative proportions of resource types for a given resource used by a pair of species (Pianka 1973). I calculated O separately for each of two resources: microhabitat and food. Relative counts of microhabitat types were compared between species pairs, and relative volumes of food types found in the feces were compared between species pairs.

$$O_{jk} = \sum_{i=1}^{n} (p_{ij}\, p_{ik}) \,/\, \sqrt{(\sum_{i=1}^{n} p_{ij}^2 \sum_{i=1}^{n} p_{ik}^2)}$$

For species j and k, n is the number of resource types in a single given resource, and i is each type. Within a species, counts or volumes of each resource type are averaged across individuals, and a single proportion is estimated for each resource type out of all types used by that species. The symbol p_{ij} is the proportion of resource i out of all resources used by species j, and p_{ik} is the proportion of resource i out of all resources used by species k (Pianka 1973). The software program EcoSim was used to calculate O between each species pair and the mean O of all species pairs (Gotelli and Entsminger 2009). A simulation that randomized resource amounts across species but retained niche breadth (i.e., the number of resource types used per species) within species was run 10,000 times. The observed Pianka's index value was compared against the range of simulated index values. If the observed O was less than 5% of the simulated values, then the overlap in resources between species was significantly less than expected by chance, whereas an O

greater than 95% of simulated values indicated that species overlap significantly more than by chance (Gotelli and Entsminger 2009).

An overall niche overlap is estimated as the product of all indices of niche overlap calculated for a pair of species (Pianka 1973). I multiplied the two indices of food and microhabitat overlap to estimate the overall niche overlap between each pair of species.

For each slug species, I compared substrate availability per grid cell with microhabitat choice. The two microhabitat categories of slugs were individuals found on live trees and individuals found on dead wood. ("Dead wood" comprises dead trees and CWD. These microhabitat types were combined into one category for analysis because they are qualitatively very similar.) A t-test was used to compare the dead wood volume per grid cell between the two microhabitat categories of slugs, and a separate t-test was used to compare the sum of diameters at breast height (DBH) of live trees per grid cell between the two microhabitat categories of slugs. Unequal variance, unpaired t-tests were used. Volume was used to approximate dead wood availability because slugs tend to occupy interior crevices (pers. obs.), and measurements of entire dead logs was possible. Sum of DBH was used to approximate live tree availability because it is a simple estimate of tree biomass commonly used in forestry. Slug use of leaf litter and vegetation in relation to their availability in the field was not analyzed, because neither philomycid slug used these substrates (Fig. 2.2).

RESULTS

Microhabitat Overlap

CWD was the most common microhabitat for *A. subfuscus* and *P. carolinianus*, given that 39.5 and 47.8%, respectively, of the individuals of each species were found on this microhabitat type. Live trees such as American beech (*Fagus grandifolia*) were the most common microhabitat for *M. mutabilis*, in that 78.7% of individuals were found on live trees (Fig. 2.2). *A. subfuscus* was also common on live trees (i.e., 32.6% of *A. subfuscus* individuals observed were on live trees) and it was the only slug observed on vegetation (8.4% of *A. subfuscus* individuals observed).

A. subfuscus and *P. carolinianus* overlapped the greatest in microhabitat use (O = 0.778), followed by *A. subfuscus* and *M. mutabilis* (O = 0.689). *P. carolinianus* and *M. mutabilis* experienced a much smaller overlap in microhabitat use than the other species pairs (O = 0.262). Across all species, the observed mean microhabitat overlap is not significantly greater than expected by chance (*P* = 0.8826).

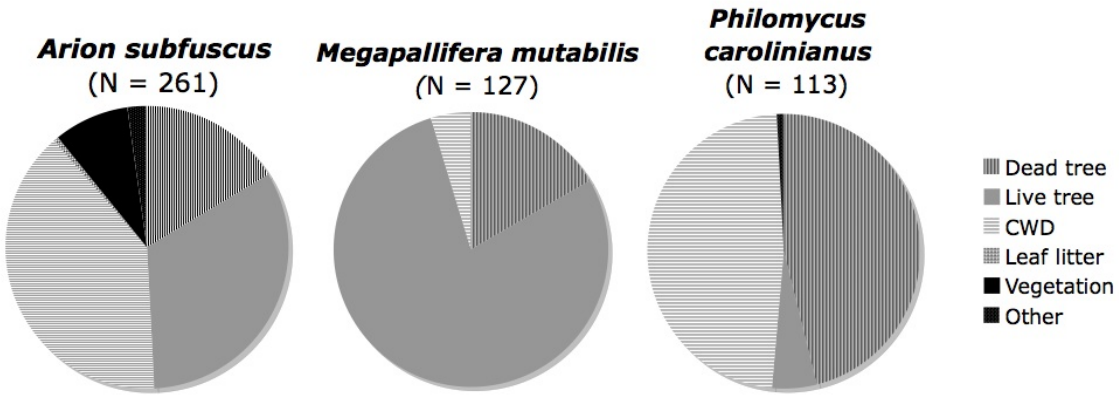


Fig. 2.2 Proportions of microhabitat types on which individuals of each species were found.

Choice of microhabitat type was related to local substrate availability. *A. subfuscus* and *P. carolinianus* that had chosen dead wood as microhabitat also occupied cells with a significantly higher amount of dead wood than slugs that had chosen live trees as microhabitats (t-test, *A. subfuscus*: $t = 1.750$, $df = 20.0$, $P = 0.0478$; t-test, *P. carolinianus*: $t = 1.979$, $df = 7.7$, $P = 0.0423$) (Fig. 2.3a). Similarly, slugs found on live trees occupied cells with a higher total diameter at breast height of live trees than slugs found on dead wood (t-test, *A. subfuscus*: $t = -3.190$, $df = 12.6$, $P = 0.0037$; t-test, *P. carolinianus:* $t = -3.826$, $df = 4.6$, $P = 0.0073$) (Fig. 2.3b). However, neither dead wood nor live tree measurements differed significantly between *M. mutabilis* that had chosen to occupy dead wood or live trees (t-test, dead wood: $t = 0.640$, $df = 8.4$, $P = 0.2697$; t-test, live trees: $t = -0.724$, $df = 7.4$, $P = 0.2458$).

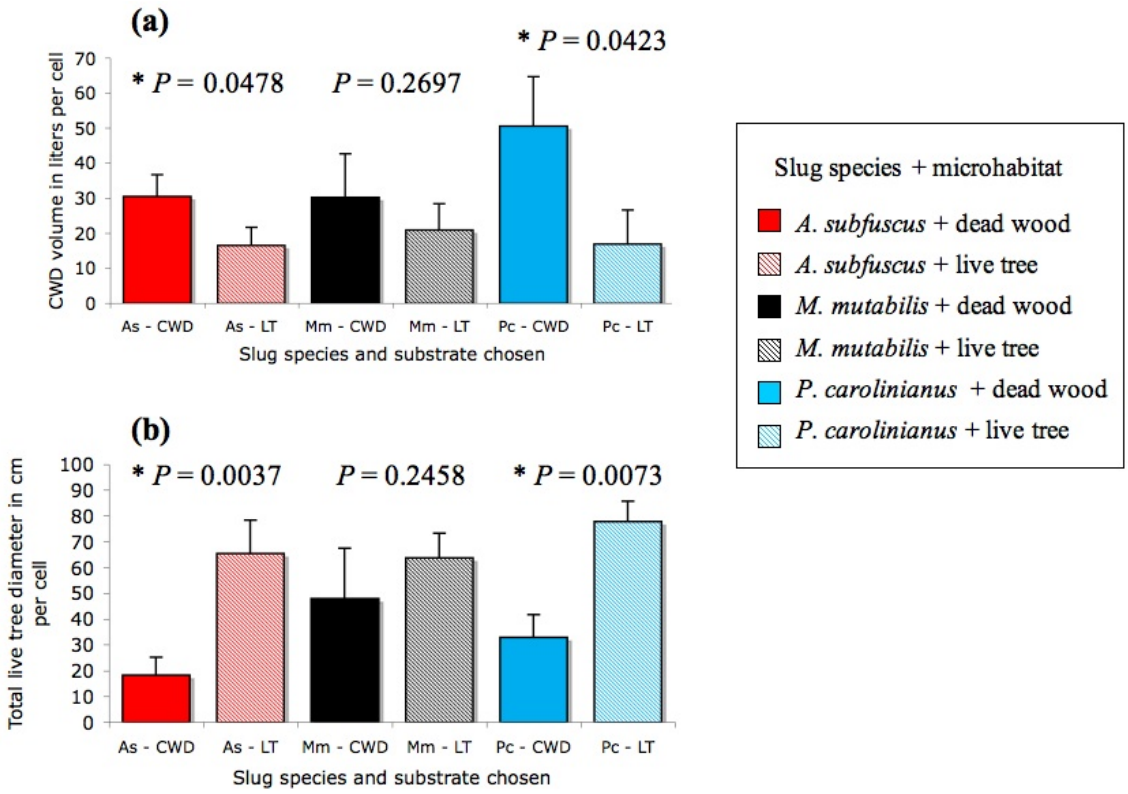


Fig. 2.3 (**a**) Volume of dead wood in cells occupied by slugs that had chosen dead wood or live trees as habitat. (**b**) Sum of diameter at breast height of live trees in cells occupied by slugs. (* indicates significantly different means within a species. Error bar is +SE.)

Food Type Overlap

One food type was consumed in high volumes (≥69%) by each species (Fig. 2.4). *A. subfuscus* favored plants (69.0%), *M. mutabilis* favored algae (78.7%), and *P. carolinianus* favored fungus (81.1%). The second-most favored food was fungus for both *A. subfuscus* (13.8%) and *M. mutabilis* (13.3%).

Foods consumed overlapped the least between *A. subfuscus* and *M. mutabilis* (O = 0.119), with *P. carolinianus* and *M. mutabilis* (O = 0.217) and *A. subfuscus* and *P. carolinianus* (O = 0.248) sharing moderately more food types by volume. Slugs did not

show a significant difference in diet but rather exhibited random food use overlap among species pairs (P = 0.6548).

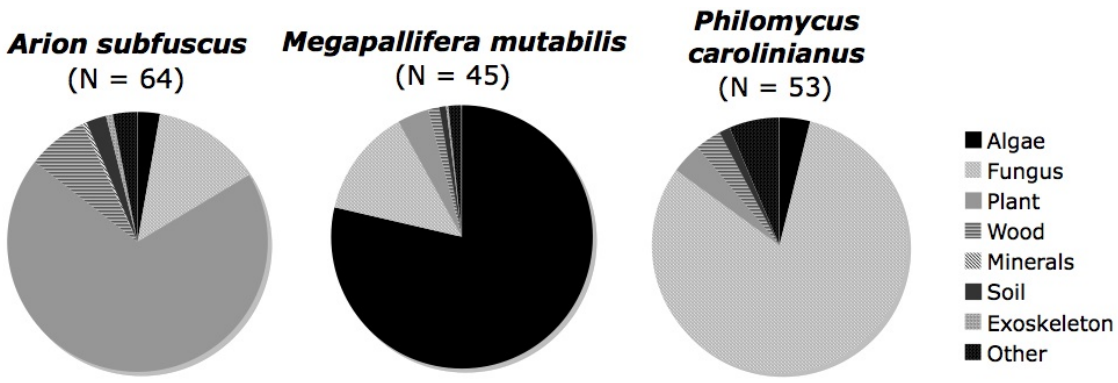


Fig. 2.4 Average proportions of food types by volume consumed by each species of slug.

Overall niche overlap (microhabitat O * food O) was greatest between *A. subfuscus* and *P. carolinianus* (O = 0.193), followed by *A. subfuscus* and *M. mutabilis* overlap (O = 0.082) and *P. carolinianus* and *M. mutabilis* overlap (O = 0.057).

DISCUSSION

The overlap in substrate and food resources was consistently greater between *A. subfuscus* and *P. carolinianus* than between the two native species. Thus, the potential for competition is highest between *A. subfuscus* and *P. carolinianus*. However, their small overlap in food use may be enough to minimize competition (Pianka 1973), even if they share microhabitats.

P. carolinianus and *M. mutabilis* exhibited little overlap in resource use. As cohabitants of northeastern forests, it is possible that niche displacement between the two native species has already occurred through resource partitioning (Connell 1980). In

contrast, *A. subfuscus* has been present in North America for fewer than 200 years, which may have been insufficient for co-habiting slug species to evolve unique microhabitat preferences to the degree that *P. carolinianus* and *M. mutabilis* have. Species that have evolved in allopatry might compete to a greater degree when brought together than species that evolved for a long period in sympatry (Goodyear 1992), because species that evolve in sympatry tend to develop mechanisms that allow them to coexist (e.g. niche displacement) (Hairston 1980) or else go extinct.

In total, food overlap was modest and similar among all species pairs. Under ordinary conditions of resource availability, competition for food may be weak. Between early May and mid July, when feces were collected, proportions of food types chosen by any one species were unchanged. This was true for all slug species (see Appendix B), suggesting that a species' food niche does not abruptly shift through the seasons. However, I did not collect feces during excessive drought or cold weather. Under such conditions, slugs may be confined to smaller areas of habitat, or fewer food types may be available (Mason 1970a; Butler 1976; Baur and Baur 1990), forcing them into greater competition (Wiens 1989; Davis 2003). In some gastropod systems, degree of diet overlap within species pairs can vary through the year (Hatziioannou et al. 1994).

The diets of the two philomycid slugs resembled the species' food preferences as described in the literature, while *A. subfuscus's* diet contained a greater proportion of plant material than expected. Previous studies have noted that *A. subfuscus* favors fungus as food (Graham 1955; Chichester and Getz 1973; Jennings and Barkham 1975; Beyer and Saari 1978), whereas I observed that field-collected *A. subfuscus* are mainly herbivores (or detritivores, given that most plant material consumed appeared dead and

brown, rather than fresh and green). Observed *P. carolinianus's* diet selections were in accord with previous studies (Chichester and Getz 1973; Branson 1980). Given that *P. carolinianus* occupying the same field sites were clearly finding and consuming fungus, and *A. subfuscus* from these populations readily consumed cultivated mushrooms in the lab, the observed natural diet of *A. subfuscus* is unexpectedly richer in plant material rather than fungus. Perhaps *A. subfuscus*'s preferred diet has shifted to incorporate more plant material in the presence of the fungivorous slugs, i.e. *A. subfuscus* may actually be displaced from fungus by *P. carolinianus*. However, diet-shifting may not always happen in the presence of a competitor. For example, the non-native mudsnail *Batillaria attramentaria* and the native mudsnail *Cerithidea californica* did not alter their diatom size feeding preferences in sympatry (Whitlatch and Obrebski 1980).

The local availability of substrate affected *A. subfuscus* and *P. carolinianus*'s but not *M. mutabilis*'s choice of microhabitat. Dead wood amount was higher in cells in which *A. subfuscus* and *P. carolinianus* occupied dead wood rather than live trees, and more (or higher diameter) live trees occurred in cells in which these slugs were found on live trees instead of dead wood. The correlation of substrate choice with substrate amount suggests that these slugs respond somewhat passively to their environment. This result also suggests that *P. carolinianus* is flexible in microhabitat choice, which may help to lessen the occurrence of competition or any potential detrimental outcomes of competition with *A. subfuscus* for microhabitats. I am not aware of other studies of gastropods that compare substrate choice to its availability. Food choice is known to correlate with local food availability (Cook and Radford 1988; Speiser and Rowell-Rahier 1991; Haegele and Rahier 2001), although *Deroceras reticulatum* was shown to

feed preferentially on a plant species when it was rare (Cottam 1985). In contrast to the other two slugs, *M. mutabilis* preferred live trees (especially beech) as substrates regardless of the amount of dead wood in their habitats. Thus, this species is relatively specialized, while the other two species exhibit flexibility in microhabitat choice. *M. mutabilis* was found to consume mostly algae, which grows upon the trunks of live trees such as American beech (*Fagus grandifolia*). Its use of live trees as microhabitats may influence its food preferences (and vice versa).

Additional studies that I performed suggest that the natural availability of resources counteracts competition promoted by resource overlap among these slugs. Naturally low levels of fungus, typical of forest habitats occupied by these slugs, were shown to be a limiting resource to both *A. subfuscus* and *P. carolinianus* in the lab, while natural amounts of shelter were generally not limiting (no data are available for *M. mutabilis*; see Chapter 3). Thus, the resource (food) determined in the present study to overlap little among species was naturally limiting, while the resource (shelter) that overlaps for all three species was not a limiting resource.

My simple estimates of niche overlap through measurements of food and shelter use should be presented with a caveat: approximation of niche dimensions requires much more information (Hutchinson 1957; Kearney and Porter 2009). I attempted a broad parsing of resources into food and shelter categories. Perhaps there are subcategories of resources that I did not distinguish but that these slugs recognize and actively select (e.g. Maraun et al. 2003). Other niche axes that were not investigated, such as soil moisture, vapor pressure deficit, temperature, or fungus food species (Rising and Armitage 1969; Thompson et al. 2006), might offset or contribute to interactions among these species.

Also, niche overlap does not take into account the value of resources that are sought in small quantities. Infrequently-used resources may be very important if uncommon and highly nutritious or limiting, such as dead animals (Speiser 2001). Additionally, slugs were hidden at unknown frequencies within logs or soil cracks, preventing me from tallying these microhabitat choices. These niche overlap estimates thus only roughly suggest the environmental dimensions likely to be the resource serving as the basis for competition among species.

In conclusion, because the native slugs *M. mutabilis* and *P. carolinianus* are more unlike each other than the native-non-native species pair of *P. carolinianus* and *A. subfuscus*, it is likely that *P. carolinianus* and *A. subfuscus* are competing more strongly than *P. carolinianus* and *M. mutabilis*. However, I do not know whether the niche difference between these two slugs is "small enough" to produce appreciable competition, or conversely, "large enough" to enable permanent coexistence (MacDougall *et al.* 2009). Only through experimental manipulations of slug populations, such as by measuring the fitness of *P. carolinianus* and *A. subfuscus* combinations in the lab, will the extent of competition latent in the degree of niche overlap be manifest (see Chapter 3).

CHAPTER 3

Evaluating the Presence of Competition between a Native and a Non-native Slug Species in Captivity

INTRODUCTION

Many non-native species became established in the distant past, and no records exist of the original state of the ecosystems or native populations with which they came into contact (Eastwood et al. 2007). These non-native species contribute a substantial proportion of the species diversity, abundance, and biomass of many ecosystems (Windham 2001; Hall et al. 2003; Strayer et al. 2009). Most established invaders appear to have minimal impacts on their environment and seem to coexist with native competitors (Sax et al. 2002; Ricciardi and Cohen 2007). However, we usually do not know if and how the behavior, population size, demography, or the range of native species was historically altered, much less whether the populations of native species are now in decline. The non-native species whose interactions with native species are most thoroughly understood are still in the process of invading, have decimated populations of native species on a regional or global scale, or are economically or environmentally significant, such as the Argentine ant, rusty crayfish, New Zealand mudsnail, and Asian tiger mosquito (Cope and Winterbourn 2004, Juliano and Lounibos 2005, Buczkowski and Bennett 2008a, Pintor et al. 2008). These were systems studied under ideal research circumstances, i.e., an invasion front allowed researchers to measure native population size prior to and after the introduction of the non-native species (Bohn et al. 2008). However, systems that are not subject to a spectacular current invasion process should

not be ignored. If native and non-native species compete, native populations may be subject to continued, if gradual, displacement on a microhabitat or habitat scale (Byers and Goldwasser 2001). Continued population declines in addition to historical population losses place endemic species at a greater risk of extirpation (e.g. Pimm et al. 1988).

Non-native Eurasian slugs have colonized and spread into temperate regions worldwide, including many relatively undisturbed habitats (Chichester and Getz 1969; Getz and Chichester 1971). Eurasian slugs began entering North America about 200 years ago and are now established throughout the eastern seaboard (Binney 1842 cited in Chichester and Getz 1969; Getz 1974). The original population sizes and distributions of the native slug fauna in eastern North America prior to non-native slug colonization are completely unknown (Chichester and Getz 1968), as is typical for molluscs (Cope and Winterbourn 2004). Thus, there is no direct evidence of past impacts of non-native slug competitors on native slugs and few clues to future impacts. Indications of past population displacements would suggest that native populations remain at risk of extirpation, and evidence that part of the biomass once composed of native species has been co-opted by non-natives would suggest shifts in ecosystem processes (e.g. types of foods consumed by slugs).

Despite these knowledge limitations, the experimental establishment of competition in the present day can suggest past and future interactions between native and non-native species. Wiens (1989) suggested a series of criteria that collectively should be determined to confirm the occurrence of competition between species: (1) apparent spatial or temporal competitive displacement from a habitat, microhabitat, or niche (Wiens 1989; Denno *et al.* 1995), (2) overlap in the use of a scarce resource (Birch

1957; Colwell and Futuyma 1971) (3) intraspecific competition, indicating a potential to compete with heterospecifics, (4) exploitation or interference competition mechanisms that reduce resource availability, and (5) harm to a species in response to resource loss (Wiens 1989). Although species may compete by means other than by contesting resources, such as through apparent competition (propagation of shared predators and diseases) (DeBach 1966; Reitz and Trumble 2002; Davis 2003), Wiens's narrow focus provides exacting guidelines for studying cases of competition for limited resources.

Slugs are appropriate candidate organisms for studies of resource competition, because their dispersal abilities are limited (Burch and Pearce 1989; South 1992), slugs aggregate in favorable microhabitats (Pearce and Örstan 2006), and the niches of species overlap appreciably (Jennings and Barkham 1975; Cameron 1978). Slugs share major dietary components (Jennings and Barkham 1975) such as macrofungus (Ingram 1949; Chatfield 1976; Speiser 2001), an ephemeral and scattered resource. Many authors speculate that shelter is a major source of competition among slug species (Rollo 1983a; Pearce 1997). This may particularly be true of coarse woody debris (CWD), a microhabitat that serves as a moisture reservoir, food source, and ovipositing site for many gastropod species (Rollo and Wellington 1979; Kappes 2005). Rollo and Wellington (1979) demonstrated the potential for strong competitive exclusion between the non-native slug *Limax maximus* and the Pacific northwest endemic *Ariolimax columbianus*. Through aggression, *L. maximus* caused lowered feeding rates, growth, fecundity, and eventually extinctions of *Arion ater* and *A. columbianus* populations in field cages that these slugs shared (Rollo 1983a, 1983b). Despite the potential of non-native slugs to cause harm, extremely little is known about how non-native slugs interact

with native slugs in general and in particular with the eastern North American endemic philomycid slugs.

I am investigating whether the Eurasian non-native slug *Arion subfuscus* (Draparnaud) competes with the native slug *Philomycus carolinianus* (Bosc). *P. carolinianus* is common throughout eastern North American forests (Grimm 1971; Chichester and Getz 1973; Hubricht 1985). *A. subfuscus* was probably introduced through trade to New England cities more than 150 years ago (Binney 1842, cited in Chichester and Getz 1969), and they have since become abundant (up to 10 slugs/m^2, pers. obs.), and wide-spread in North American forests (Chichester and Getz 1969; Getz 1974; J.B. Burch pers. comm.). *A. subfuscus* may harm the fitness of *P. carolinianus* because *A. subfuscus* is relatively aggressive while *P. carolinianus* is not (Webb 1950; Rollo and Wellington 1979; Fernandez 1990), and the two species occur in the same forests (Chichester and Getz 1969; Getz 1974), are similar in size (both up to 7 cm in length in central Maryland; pers. obs.) and share resources. A fecal study indicated that *P. carolinianus* mostly eats fungus and that *A. subfuscus* has a varied diet including a substantial amount of fungus (see Chapter 2). Both species favor CWD as microhabitats (see Chapter 2).

P. carolinianus and *A. subfuscus* may be subject to weather-related or age-specific competition. However, these factors are commonly overlooked in studies of competition between native and non-native species. Seasonal changes or extreme weather conditions might force competition, or conversely, might ameliorate it (Holway et al. 2003; Alcaraz et al. 2008; Rwomushana et al. 2009). For example, dry weather increases gastropods' reliance on shelter (Hunter 1978; Rollo and Wellington 1981), which causes

gastropods to aggregate in refuges (South 1992) and presumably to interact more often with one another. The negative effects of competition may vary among life stages, in part because size differences among individuals affect the strength of competition (Schoener 1983). Particularly in studies of gastropod competition, the factor of age has been acknowledged, and young individuals are commonly used as experimental subjects because of age-related differences in the consequences of competition (e.g. Baur and Baur 1990; Foster and Stiven 1996; Pearce 1997; Conner et al. 2008). For example, competition often affects juvenile gastropod fitness more than adult fitness in gastropods (Tattersfield 1981; Cook 1989; Conner et al. 2008), and juvenile survival can be key to population regulation (Wolda and Kreulen 1973; Hunter 1978). In central Maryland, *P. carolinianus* juveniles hatch in the summer when *A. subfuscus* are adults (pers. obs.). The young *P. carolinianus* may be competing with heterospecific adults for food prior to egg-laying during a period when *A. subfuscus* are known to be especially aggressive (Rollo and Wellington 1979). In my study of competition between these two slug species, I tested whether different moisture levels affected competition level and whether interspecific competition existed within each of two life stages (adult and juvenile) of *P. carolinianus*.

With respect to Wiens's (1989) criteria for competition, I determined whether the native slug *P. carolinianus* and the non-native slug *A. subfuscus* compete by conducting a series of field population surveys and laboratory experimental manipulations. I conducted experiments to test whether resources used by these species are limiting (Wien's criterion 2) and whether *P. carolinianus* is harmed in the presence of *A. subfuscus* (criterion 5). I posed the following questions: Are natural levels of shelter or food sufficiently low to

limit slug fitness? Is *P. carolinianus* fitness different when they are grouped with *A. subfuscus* than when they are grouped with conspecifics? Does competition level vary among different environmental regimes (dry vs. moist conditions)? Do juvenile *P. carolinianus* experience competition with *A. subfuscus*?

METHODS

Experiment 1: Limiting Resources

The purpose of this work was to determine whether natural levels of resource availability can be sufficiently low to reduce the fitness of slugs. In order to determine the amount of resources to be used in the lab experiments, the range in unit area covered by shelter (CWD) and food (fungus) naturally occurring in 1.0 m^2 microsites inhabited by a slug of any species was estimated. For a separate study (see Chapter 1), slug abundances were assessed in mesic lowland beech-oak forests at Patuxent Research Refuge, Laurel, Maryland. Population surveys took place on grids of 75, 5 x 5 m cells at each of three field sites. In a random subset of 90 of these cells, the locations of individual slugs were marked once with flags during the hours of 19:45 to 23:30, June 19th to 26th, 2007. One 1 x 1 m microsite was located at the center of each of 44 cells where slugs had not been found, while in 46 cells where slugs were found and flagged, a microsite was centered on each flag, and several habitat measurements (explained below) on each microsite were then averaged for each cell (Fig. 3.1). The volume of wood pieces or trees $\geq$ 5 cm in diameter (including CWD and fine woody debris as defined by Travaglini et al. 2006) within each microsite was estimated from their length and diameter measurements. In

order to estimate macrofungus mass per microsite, one transect was drawn on the longest dimension of each piece of wood and two transects were drawn on each tree trunk (to 1 m in height). The wood surface area covered by fungus was estimated as the transect lengths that intersect with fungus. Fungus mass per unit area of wood surface was estimated by weighing the mass of dry fungus scraped from each of five pieces of bark covered with a continuous patch of fungus. These samples produced an average fungal mass of 0.056 g per cm^2 of bark. Although slugs consume fungal mycelia inside dead wood as well as fungus growing on the surface of the wood, slugs appear to favor the external fruiting bodies, which they emerge to feed upon in favorable weather (pers. obs.).

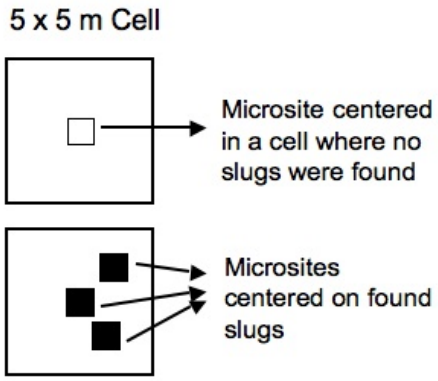


Fig. 3.1 Field microsites in which natural resource levels (CWD and fungus) were measured. A 1.0 m^2 microsite was placed at the center of the top cell, in which no slugs had been observed. In the bottom cell where three slugs had been observed, microsites were centered on the location of each slug, and the natural resource amounts were averaged across the three microsites.

A lab experiment evaluated separately for each species whether different amounts of shelter and food affected individual fitness. Adult *P. carolinianus* and *A. subfuscus* were collected from Patuxent Research Refuge (PRR) during June and July of 2007. Slug species were identified based on their internal and external anatomy (see Pilsbry 1948, Webb 1950, Barker 1999). Adults were distinguished from hatchling or juvenile slugs by their size. (Reproductive maturity appeared to correlate strongly with the size of *P. carolinianus* but was inconsistently correlated with the size of *A. subfuscus* (pers. obs.).) Leaf litter and topsoil were also collected and incorporated into each experimental habitat, i.e., a plastic tub mesocosm (12.5 cm wide, 32.5 cm long, and 17.5 cm tall; 7100 cm^3 volume and about 400 cm^2 bottom area). Slugs from each species were divided approximately into a group of larger individuals and a group of smaller individuals, and one slug from each group was randomly paired together. Each pair of slugs was placed in a replicate mesocosm. For each slug, size and placement of spots (for *P. carolinianus*) or sharpness of stripes (for *A. subfuscus*) and mantle coloration were recorded in order to help to distinguish the two slugs per mesocosm throughout the experiment. (Spot and stripe patterns did not change through time (pers. obs.).) Slug density within mesocosms was similar to high-density levels observed in the field, i.e. up to 10 slugs in a 0.25m^2 patch.

The experiment was designed as a 3 x 3 factorial with three levels each of shelter and food. Shelter volumes were calculated to replicate in the 400 cm^2 mesocosm bottom the volume of shelter per unit ground area found in the field (Table 3.1). The volume of shelter chosen to represent low shelter availability was 60 cm^3 (a substandard shelter amount, or the average volume per 400 cm^2 ground area for microsites unoccupied by

slugs). For medium shelter availability, I used 600 cm^3 (the average volume for occupied microsites), and for high shelter availability I used 1500 cm^3 (a greater than average shelter amount, and one fourth of the greatest volume occurring in occupied microsites). Hardwood branches (CWD) between 5 and 10 cm in diameter and at a moderate level of decay (stage three of five stages of decay; see Stokland and Kauserud 2004) were selected for shelter. The branches were sawed into one to three pieces to fit the mesocosms. Any superficial growth of algae or fungus was scraped off. Because fungus was sparsely distributed in the field and the minimum or average fungal mass per unit ground area would likely starve slugs confined to the mesocosms, I chose fungus levels close to the natural maximum. The increments of fungal mass to be used as food were 0.20 g for low shelter (ten times the average mass per 400 cm^2 ground area in microsites occupied by slugs) 3.0 g for high shelter (the maximum in microsites occupied by slugs), and 1.5 g for medium shelter (the midpoint between low and high). Half of the mass of fungus provided as food consisted of dried wild fungus, and the other half was commercially-available white mushrooms (*Agaricus bisporus*). There were five replicates per treatment. The experimental habitats were lined with soil 2 cm deep and leaf litter 5 cm deep. Mesocosms were sealed with lids into which 12 holes 0.5 cm in diameter were drilled to promote air flow, and lids were taped down to prevent slugs from escaping through cracks.

Table 3.1 Average and maximum levels of CWD and fungus found in 1.0 m^2 field microsites that were unoccupied or occupied by slugs. CWD is measured in units cm^3 per cm^2 of forest floor, and fungus is measured in cm of epiphytic fungus covering each cm of linear transect on the surface of CWD.

	Microsite status	Unoccupied	Occupied
CWD levels	Average	0.15	1.60
	Maximum	2.75	15.93
Fungus levels	Average	0.002	0.023
	Maximum	0.043	0.365

Slug mass, fecundity, and survival were recorded every ten days (+/-4 days) as indicators of slug fitness between July 26th and November 4th, 2007. Although the reproductive fitness of an individual is ideally measured as the fecundity of its own offspring (Fisher 1958), alternative measures of fitness that strongly contribute to long-term reproductive success are usually sought. Besides fecundity and mortality, I used mass as a metric of fitness because it is well-documented to correlate with clutch size, clutch frequency, and lifetime fecundity in gastropods (Wolda and Kreulen 1973; Carter and Ashdown 1984; Goodfriend 1986; Bengtsson and Baur 1993). Egg masses were collected from mesocosms every ten days while the soil and leaf litter were being changed. I assumed that eggs had been laid by the slug that had lost the greater weight since the previous measuring session. Both slug species laid eggs in clusters. *P. carolinianus* eggs were about 4 mm in diameter, translucent white, and with an average count of 23 per clutch, while *A. subfuscus* eggs were about 2.5 mm in diameter, opaque

white with a yellowish tinge, and with an average count of 35 per clutch. *A. subfuscus* did not lay enough eggs to perform statistical analyses. During each measurement session, the branches and mesocosm sides were washed to limit the growth of pathogens, and food and leaves were replaced. Individual slugs that died (or that could not be found) after two weeks were replaced with adult slugs of similar size also collected from PRR in order to maintain slug density per mesocosm. Slugs could not be found when dead individuals decayed too quickly for their remains to be discovered or because they escaped from their mesocosms. If a slug died or disappeared before week four, the fitness measurements of its replacement slug were used in statistical analysis (e.g. Petren and Case 1996), because the replacement was subject to the treatment for a longer period of time than the original slug. (The number of replacement slugs whose measurements were used was similar across treatments and did not exceed 20% per treatment.) If a slug died at or after week four, its own fitness measurements were used.

Experiment 2: Mixed Species

A 2 x 2 x 2 factorial design was used to evaluate whether the factors of *A. subfuscus* (presence or absence), shelter amount (low or high), and food abundance (low or high) affect the fitness of *P. carolinianus*. Unless otherwise stated, methods were identical to Experiment 1. The *A. subfuscus* absence mesocosms contained four *P. carolinianus* adults, and the *A. subfuscus* presence mesocosms contained two *P. carolinianus* and two *A. subfuscus* adults. Unlike experiment 1, medium levels of food and shelter were not used in order to simplify the experimental design. Low and high shelter were the same amounts used in Experiment 1. Double the mass of food in Experiment 1 was used

because twice the number of slugs was present. Low food treatments consisted of 0.54 g fungus and high food treatments were 4.0 g fungus. An unbalanced design was employed to maximize the number of mixed-species replicates, the main focus of the experiment, in virtue of a limited supply of *P. carolinianus* specimens. There were five replicates per conspecific treatment and nine replicates per mixed species treatment. A digital photograph was taken of each slug's mantle in order to allow individuals to be identified during each fitness measuring session. A slug's mantle patterns could almost always be distinguished from those of its mesocosm-mates. If not, slugs were assumed to be the individual whose weight the previous week they most closely matched.

Fitness measurements were taken every seven days (+/-1 day) between July 10th and September 9th, 2008 for a total of eight measurements. Mass, fecundity, and mortality were recorded, and distances between pairs of slugs were measured. To attribute a set of eggs to individual slugs in a mesocosm, I considered the mass change of each slug since the previous week. Eggs were divided into equal portions among slugs that lost more than 0.10 g in a week (about 5-10% body mass), and slugs that lost at least twice the weight of other slugs were assigned twice the number of eggs. Distances (in cm) between each pair of slugs were measured before the mesocosm contents were thoroughly disturbed. (Wide distances between slugs or displacement from habitat may indicate antagonism (Rollo and Wellington 1979; Poling and Hayslette 2006; Shucksmith et al. 2009).) Otherwise, mesocosm maintenance and weekly fitness measurements followed the methods of Experiment 1. Dead or missing slugs were replaced as per Experiment 1, and the number of the original four slugs that had died was counted per mesocosm.

Experiment 3: Shelter and Moisture

This experiment investigated the influences of differences in ambient moisture levels and shelter amount on slug fitness. Because the soil in the experimental mesocosms was kept wet throughout Experiment 1, the shelter logs may not have had a chance to exert their natural role in slug survival. Shelter amount did not affect slug fitness in either prior experiment (see Results of Experiments 1 and 2).

A 2 x 2 x 3 factorial experiment was designed to test the combined effects of moisture level (dry with a nylon fine mesh top on the mesocosm or wet with a plastic lid), shelter amount (low or high), and presence of heterospecifics (either four *P. carolinianus*, two *P. carolinianus* and two *A. subfuscus*, or four *A. subfuscus*) on slug fitness. After a one-week trial run of wet mesocosms, mesh topped mesocosms were about 60% relative humidity while lidded mesocosms were about 90% relative humidity. There were four replicates per treatment. *A. subfuscus* and *P. carolinianus* fitnesses were analyzed separately. Unless otherwise stated, methods were identical to the other two experiments. Low and high shelter were the same amounts used in Experiment 1. All treatments received the same amount of fungus food, 4.0 g, which represents the high food level used in Experiment 2. Slug mass, fecundity, and mortality were measured every seven days (+/-1 day) between May 7th and July 23rd, 2009 for a total of eight measuring sessions. Distances between each pair of slugs was measured per session and averaged for each mesocosm.

Experiment 4: Juveniles

The presence of *A. subfuscus* did not have a significant effect on most *P. carolinianus* fitness variables (see Results of either Experiments 2 or 3). Juvenile gastropods often respond more strongly than adults to competition (Pearce 1997; Conner et al. 2008). Thus, I applied a similar design as in Experiment 2 to *P. carolinianus* juveniles.

A 2 x 2 x 4 factorial experiment was designed to test the effects of food amount (low or high), shelter amount (low or high), and cohabitants (either four *P. carolinianus* juveniles, eight *P. carolinianus* juveniles, four *P. carolinianus* juveniles plus one adult, or four *P. carolinianus* juveniles plus one adult *A. subfuscus*) on the fitness of juvenile *P. carolinianus*. There were five replicates per treatment. Juveniles were raised in the lab from eggs laid by captive adults during the late spring and summer of 2009. Juveniles were randomly assorted such that siblings would be distributed across treatments. Each juvenile entering the experiment was at least one week old and had an average initial mass of 0.03 g. Each replicate was housed in a 240 mL cylindrical plastic deli container (11 cm diameter) and sealed with a nylon mesh top. The low food amount was 0.54 g (the same as Experiment 2), and the high food amount was 2.0 g (the same as the Experiment 1). Shelter amounts were calculated to represent the same volume of wood to unit ground area of the deli containers as the mesocosms used in all previous experiments. Low shelter was 5 cm^3 and High shelter was 130 $cm.^3$ Because individual juveniles cannot be visually distinguished, all juveniles per replicate were weighed en masse every eight days (+/-3 days) between June 29th and October 28th, 2009. The average mass per juvenile slug was estimated for each replicate.

Statistical Analysis

SAS software version 9.2 was used to evaluate the results of each experiment through a factorial, mixed-model ANCOVA (SAS Institute Inc 2008). Denominator degrees of freedom were determined by the Satterthwaite procedure (Satterthwaite 1946). The initial mass of each slug (or of all target slugs per container, in experiment 4) was a covariate in all experiments. For experiments 1-3, mesocosm was a replicate in which each slug was treated as a subsample. For experiment 4, container was a replicate represented by a single average value per fitness variable. Independent variables were shelter and food in Experiment 1; shelter, food, and heterospecific presence in Experiment 2; shelter, moisture, and heterospecific presence in Experiment 3; and shelter, food, and cohabitant identity in Experiment 4.

Response variables were assessed separately for each species. Response variables for *P. carolinianus* adults were average mass change per day, final mass, final mass plus mass lost due to egg laying, and total number of eggs laid. To estimate the average mass change per day per slug, the change in a slug's weight for each measurement session was divided by the number of days between measurement sessions to obtain an estimate of its mass change for each day between sessions, and the slug's daily mass changes were averaged across the entire period of the experiment. Lost body mass due to egg laying was estimated at 0.012 g per egg laid (pers. obs.). Lost egg mass was added to the final mass to calculate the response variable final mass plus egg laying mass lost. Slug mass was always measured as wet mass, rather than dry mass, because slugs were kept alive to be measured at regular intervals and to be used in additional experiments. However, in experiments 1, 2, and 4, slugs' masses were unlikely to be influenced by moisture,

because the lidded and sealed mesocosms maintained an environment with 90% relative humidity (see above). Count of slugs dead per mesocosm was analyzed in Experiments 2 and 3 with each whole mesocosm treated as a replicate. Egg hatching rate was analyzed as a fitness variable, but in no case did the proportion of eggs hatched respond to treatment and so is not reported here. (For example, in experiment 2, the proportion of eggs hatched out of total eggs laid ranged from 0.905 +/-0.066 SE in the high food, high shelter treatment to 0.752 +/-0.069 SE in the high shelter, low food treatment (P = 0.1183).) Response variables for *A. subfuscus* adults were average mass change per day and final mass (Experiments 1 and 3). *A. subfuscus* did not lay enough eggs to evaluate statistically. Response variables for *P. carolinianus* juveniles were average mass change per week and final mass, averaged for each mesocosm (Experiment 4). The distances between *P. carolinianus* and *A. subfuscus* slug pairs were response variables in mixed species mesocosms (Experiments 2 and 3). Each mesocosm was a replicate with a single value of *Pc-Pc* distances, *Pc-As* distances, and *As-As* distances averaged across all weeks (where Pc is *P. carolinianus* and As is *A. subfuscus*). The midpoint of *Pc-Pc* distances and *As-As* distances for each replicate served as its covariate; this midpoint represented an "expected" value for *Pc-As* distances if their combined interactions were simply intermediate between *Pc-Pc* distances and *As-As* distances.

Competition intensity may be affected by the availability of resources (Duyck et al. 2004; Poling and Hayslette 2006), and thus, competition may only be apparent under circumstances of low resources. For Experiment 2, planned comparison tests were used to compare *P. carolinianus* fitness between single-species and mixed species mesocosms within low resource treatments (low shelter and low food) and within high resource

treatments (high shelter and high food). These treatment comparisons were the differences of least squares means (t-tests) as obtained through ANCOVA. Because these tests were planned *a priori*, they did not require alpha adjustment for multiple comparisons. For experiment 4, least squares means were adjusted through a Tukey test so that I could compare means *a posteriori*.

Data were sufficiently normal so that transformation was not needed. Because a few juveniles gained weight at an exceptionally high rate in Experiment 4 and caused a strong skew in the response variables, I eliminated 4 outliers of the variable average mass change and 3 outliers of the variable final mass (i.e. 20% of the sample size of 5 was eliminated for a few treatments).

RESULTS

Experiment 1: Limiting Resources

Food amount significantly affected every fitness variable measured for both species (Fig. 3.2): the average mass change per day ($P < 0.0001$), final mass ($P = 0.0001$), final mass plus egg laying mass lost ($P = 0.0001$), and total number of eggs laid ($P = 0.0001$), of *P. carolinianus*, and the average mass change per day ($P = 0.0008$) and final mass ($P = 0.0001$) of *A. subfuscus*. Weight loss was consistently least in the high food treatment and highest in the low food treatment. (Note that degrowth is a common phenomenon in mature slugs that increases with egg-laying (Rollo and Shibata 1991).) For other details of the analyses (e.g., *F*-statistics and *df* values) see Appendix C. No measure of fitness responded to shelter, in contrast to food amount. Interactions were not significant.

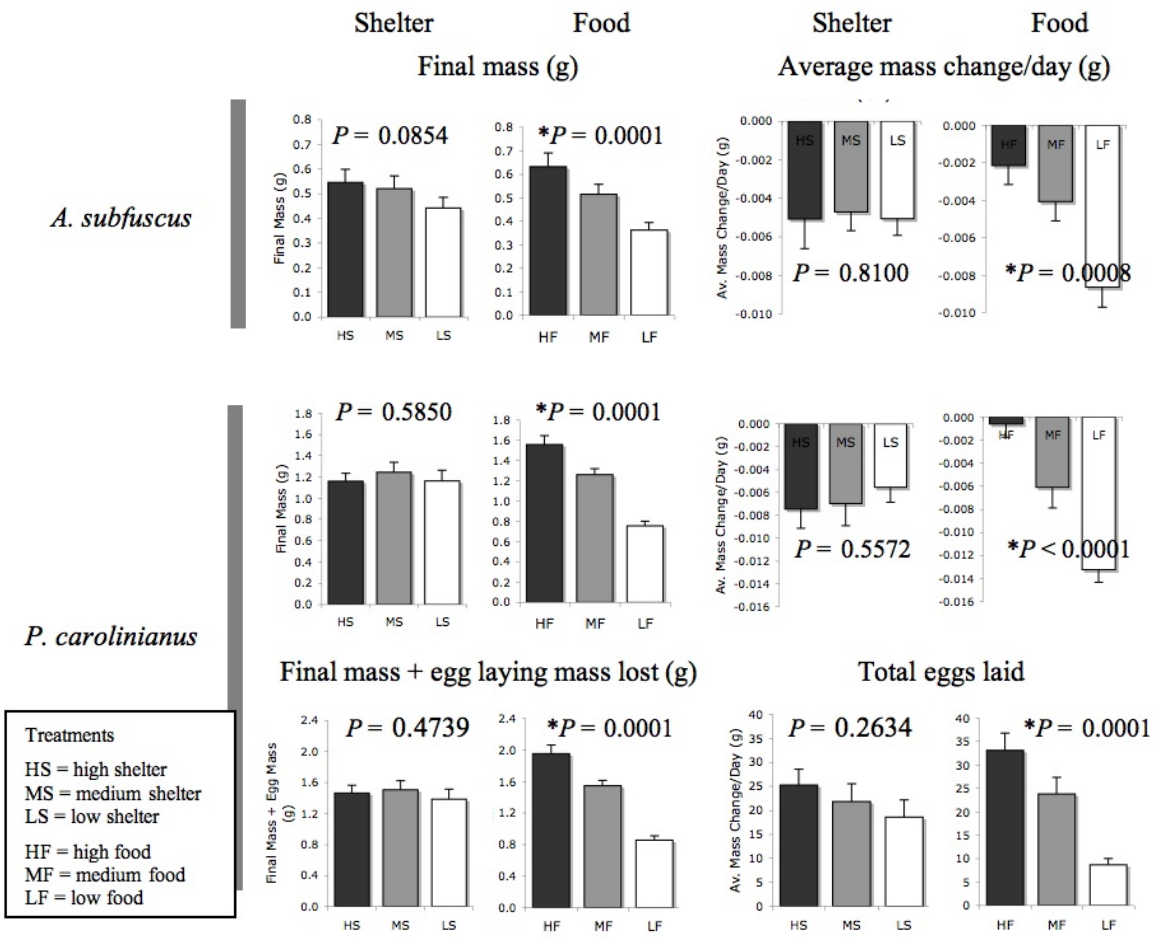


Fig. 3.2 Fitness of *P. carolinianus* and *A. subfuscus* slugs under various levels of food and shelter. Error bar indicates +SE. (* indicates significant *P*-value. See *F*-statistics and *df* values in Appendix C.)

Experiment 2: Mixed Species

A. subfuscus presence did not affect the fitness of *P. carolinianus* except for its final mass plus egg laying mass lost; final mass plus egg laying mass lost was greater in mixed-species treatments (1.190 +/-0.034 g) than in single-species treatments (1.083 +/-0.038 g)

(ANCOVA: $F_{1,42.2} = 4.32$, $P = 0.0439$). The main effects generally support the results of Experiment 1 (Fig. 3.3). That is, food amount exerted a significant effect on all fitness measures of *P. carolinianus*, while *Pc-As* distances were unaffected by any factor. Food amount affected the average mass change per day ($P < 0.0001$), final mass ($P < 0.0001$), final mass plus egg laying mass lost ($P < 0.0001$), and total number of eggs laid ($P = 0.0003$) of *P. carolinianus*. The only fitness response to shelter amount was the number of eggs that *P. carolinianus* laid. More eggs were laid in high shelter conditions (19.9 +/-1.5 eggs) than in low shelter conditions (14.1 +/-1.5 eggs) (ANCOVA: $F_{1,33.7} = 7.52$, $P = 0.0097$). An interaction of food and shelter amount resulted in the greatest percentage of slug deaths in the low shelter, low food treatments (39.4% +/-7.8%) and the fewest deaths in the low shelter, high food treatments (7.8% +/-7.8%) (ANCOVA: $F_{1,48} = 4.10$, $P = 0.0484$).

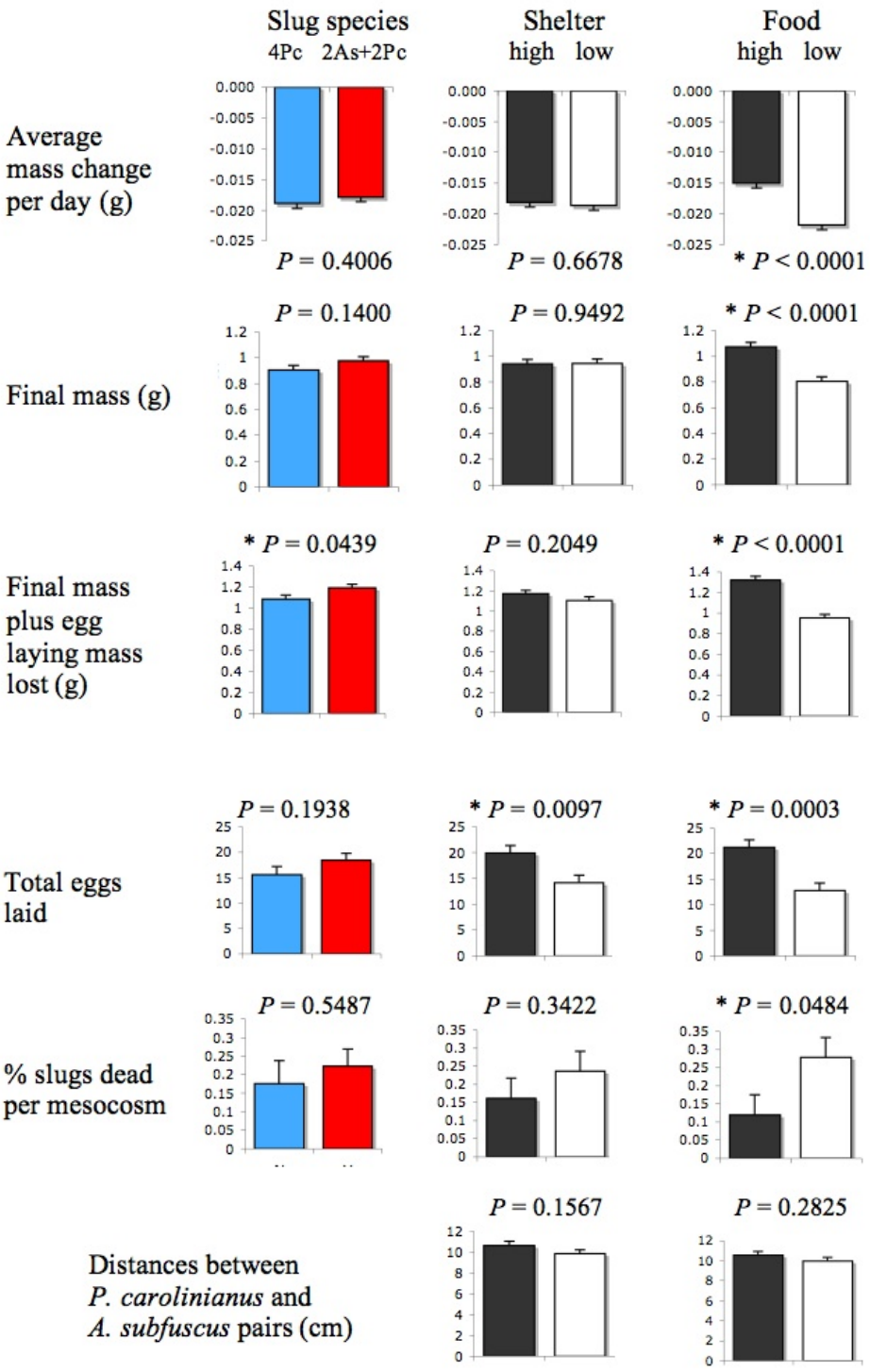
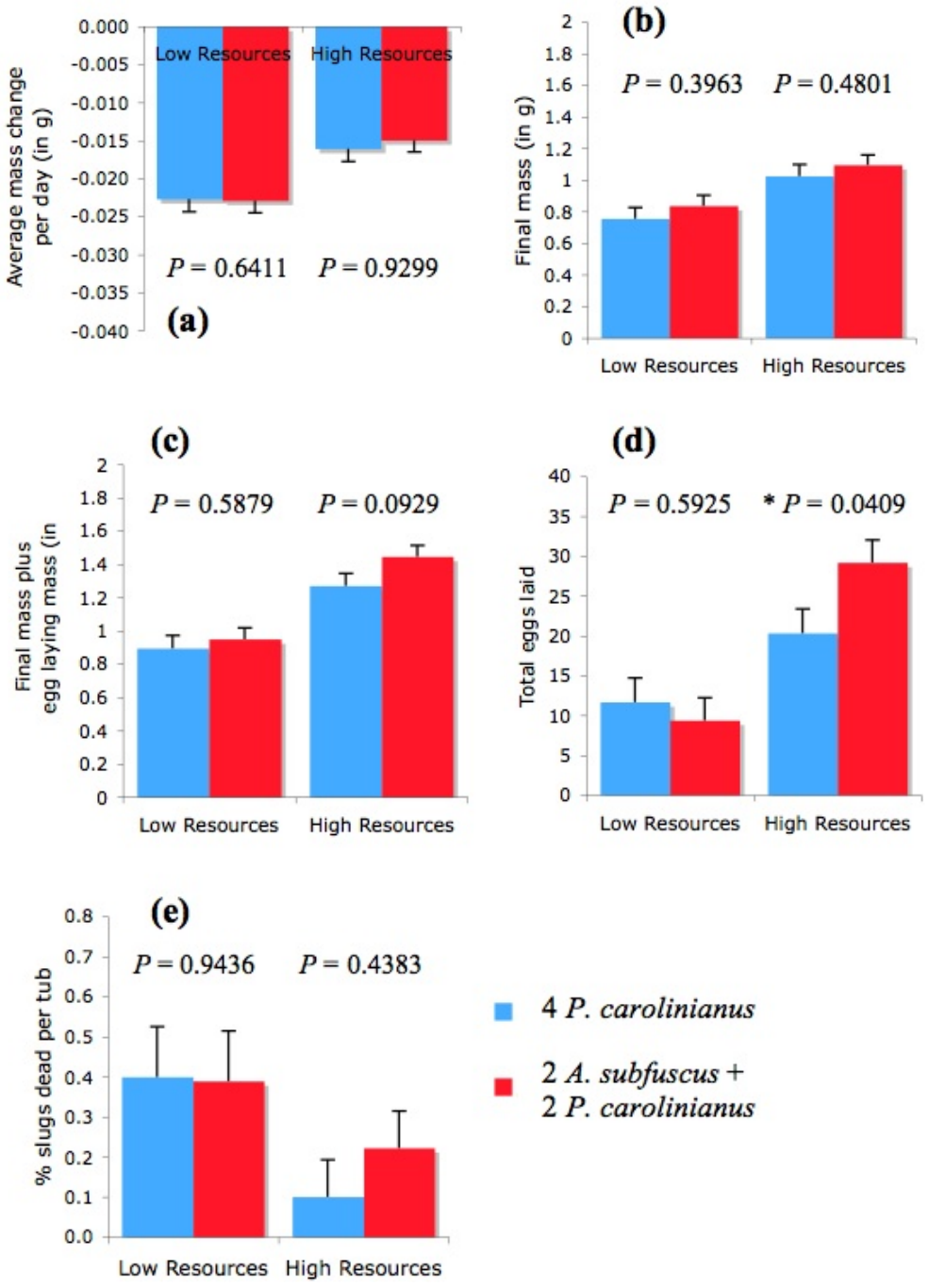


Fig. 3.3 Fitness of *P. carolinianus* under the factors of food, shelter, and heterospecific presence. Pc is *P. carolinianus* and As is *A. subfuscus*. Error bar indicates +SE. (* indicates significant *P*-value. See *F*-statistics and *df* values in Appendix C.)

In neither low resource nor high resource treatments was *P. carolinianus* fitness (except for eggs laid overall) significantly affected by *A. subfuscus* presence (Fig. 3.4). Under high resource conditions, more eggs were laid in mixed-species treatments (29.2 +/-3.1 eggs) than in single-species treatments (20.3 +/-2.8 eggs) (t-test: t = -2.13, df = 33.7, P = 0.0409).

Fig. 3.4 Fitness of *P. carolinianus* slugs in the absence or presence of *A. subfuscus*. Planned comparisons are **(a)** average mass change per day, **(b)** final mass, **(c)** final mass plus egg laying mass lost, **(d)** total eggs laid, and **(e)** percent *P. carolinianus* dead per mesocosm. High and low resource mesocosms were analyzed separately but are depicted together, grouped by response variable. Low resources are low shelter and low food, and high resources are high shelter and high food. Error bar indicates +SE. (* indicates significant *P*-value.)

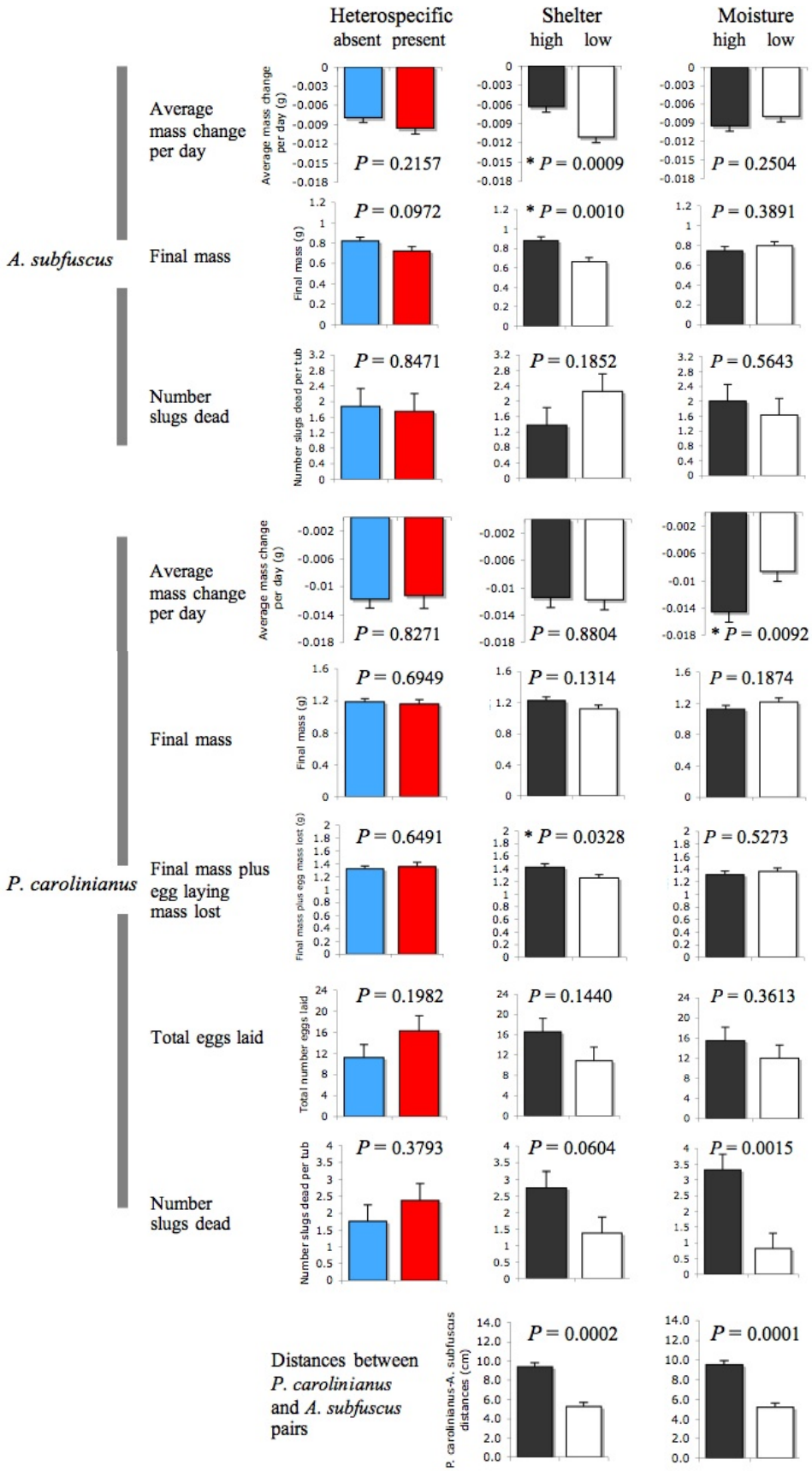

Average mass change per day (in g)
0.000
-0.005
-0.010
-0.015
-0.020
-0.025
-0.030
-0.035
-0.040
Low Resources
High Resources
P = 0.6411
P = 0.9299
(a)
(b)
Final mass (in g)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
P = 0.3963
P = 0.4801
Low Resources
High Resources
(c)
Final mass plus egg laying mass (in
P = 0.5879
P = 0.0929
Low Resources
High Resources
(d)
Total eggs laid
40
35
30
25
20
15
10
5
0
P = 0.5925
* P = 0.0409
Low Resources
High Resources
(e)
% slugs dead per tub
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
P = 0.9436
P = 0.4383
Low Resources
High Resources
4 P. carolinianus
2 A. subfuscus +
2 P. carolinianus

Experiment 3: Shelter and Moisture

Shelter and moisture levels each only affected a few fitness variables for *P. carolinianus* (Fig. 3.5). The species identity of mesocosm mates did not have a significant effect on the fitness of either species, and there was no interaction of species identity with moisture level. The average mass loss per day for *P. carolinianus* was smaller in high moisture treatments (-0.0086 +/-0.0015 g) than in low moisture treatments (-0.0146 +/-0.0015 g) (ANCOVA: $F_{1,31.9} = 7.68$, $P = 0.0092$). Shelter amount was associated with an increase in the final mass plus egg laying mass lost (1.42 +/- 0.05 vs. 1.25 +/-0.05 g) (ANCOVA: $F_{1,27.9} = 5.04$, $P = 0.0328$) as a combined result of more although not significantly different number of eggs laid (17.0 +/-2.6 eggs vs. 11.4 +/-2.7 eggs) and greater but not significantly different final mass (1.22 +/-0.05 g vs. 1.12 +/-0.05 g) in higher shelter mesocosms. Shelter and moisture amounts interacted to affect the number of *P. carolinianus* dead per mesocosm. More died in the high shelter, high moisture treatment (4.8 +/-0.7 dead) than in each of the other treatment combinations (with an average of 1.2 dead) (ANCOVA: $F_{1,24} = 4.62$, $P = 0.0419$). In contrast to Experiment 1, *A. subfuscus* had a lower final mass (0.6640 +/-0.0410 g vs. 0.8780 +/-0.0410 g) and greater average mass loss per day (-0.0111 +/-0.0009 g vs. -0.0063 +/-0.0009 g) in low shelter conditions than in high shelter conditions (ANCOVA: $F_{1,26.3} = 13.61$, $P = 0.0010$; $F_{1,26.8} = 13.80$, $P = 0.0009$). This would suggest that *A. subfuscus* fitness was affected by shelter amount during this experiment but not during Experiment 1. Moisture level had no bearing on *A. subfuscus* fitness. Distances between *P. carolinianus* and *A. subfuscus* were affected by an interaction of shelter and moisture, such that slugs were much closer to one another in

low shelter, low moisture conditions (2.5 +/-0.9 cm) than in the other treatments (mean: 8.9 cm) (ANCOVA: $F_{1,11} = 5.69$, $P = 0.0361$).

Fig. 3.5 Effects of shelter, moisture, and heterospecific presence or absence on the fitness of *P. carolinianus* and *A. subfuscus*. "Heterospecific presence" for either species means that two *A. subfuscus* and two *P. carolinianus* were present per mesocosm, while "heterospecific absence" means that four conspecifics were present. Error bar indicates +SE. (* indicates significant *P*-value in the absence of interactions between factors. See *F*-statistics and *df* values in Appendix C.)


Heterospecific
absent present
Shelter
high low
Moisture
high low
A. subfuscus
Average mass change per day
Final mass
Number slugs dead
P. carolinianus
Average mass change per day
Final mass
Final mass plus egg laying mass lost
Total eggs laid
Number slugs dead
Distances between P. carolinianus and A. subfuscus pairs
Average mass change per day (g)
Final mass (g)
Number slugs dead per tub
Final mass plus egg mass lost (g)
Total number eggs laid
P. carolinianus-A. subfuscus distances (cm)
P = 0.2157
* P = 0.0009
P = 0.2504
P = 0.0972
* P = 0.0010
P = 0.3891
P = 0.8471
P = 0.1852
P = 0.5643
P = 0.8271
P = 0.8804
* P = 0.0092
P = 0.6949
P = 0.1314
P = 0.1874
P = 0.6491
* P = 0.0328
P = 0.5273
P = 0.1982
P = 0.1440
P = 0.3613
P = 0.3793
P = 0.0604
P = 0.0015
P = 0.0002
P = 0.0001

Experiment 4: Juveniles

Juvenile final mass and average mass change per day were affected by the identity of their cohabitants (i.e., other juveniles, adult *P. carolinianus*, or adult *A. subfuscus*) (Fig. 3.6). Juvenile mass increased more in the presence of other juveniles than with either a conspecific adult or a heterospecific adult (average mass change per day: 0.0019 +/- 0.0002 g with juveniles vs. 0.0009 +/-0.0003 g with adults; ANCOVA: $F_{3,58} = 4.35$, $P = 0.0078$) (final mass: 0.1195 +/- 0.0111 g with juveniles vs. 0.0695 +/-0.0088 g with adults; ANCOVA: $F_{3,59} = 6.57$, $P = 0.0007$). Final mass was greater in high food treatments (0.112 +/-0.008 g) than in low food treatments (0.078 +/-0.007 g) (ANCOVA: $F_{1,59} = 9.84$, $P = 0.0027$), while differences in average mass change per day were not significant (0.00167 +/-0.0002 g in high food vs. 0.00117 +/-0.0002 g in low food) (ANCOVA: $F_{1,58} = 2.90$, $P = 0.0939$). Shelter amount had no influence on juvenile fitness.

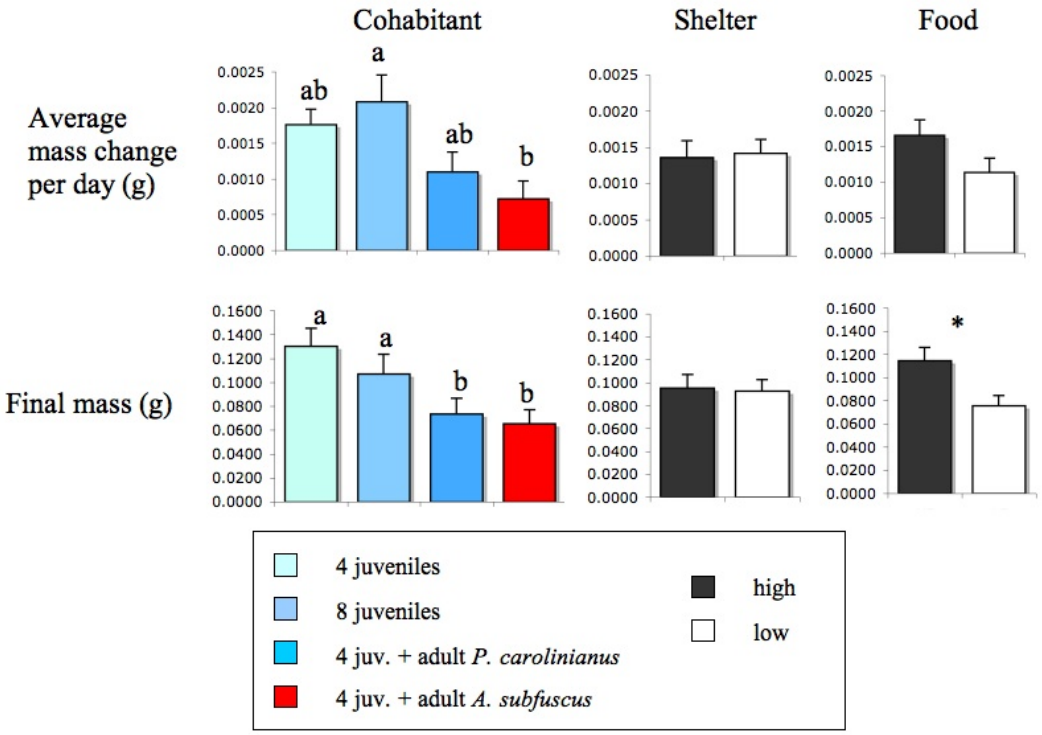


Fig. 3.6 Fitness per *P. carolinianus* juvenile among different cohabitant treatments, shelter levels, and food levels. Averages represented by the same letter are not significantly different. Error bar indicates +SE. (* indicates significant *P*-value, and different letter indicates a significant difference between treatments. See *F*-statistics and *df* values in Appendix C.)

DISCUSSION

This study tested and ultimately rejected the hypothesis that the native slug *P. carolinianus* and the non-native slug *A. subfuscus* compete. I investigated whether natural levels of resources were limiting to both *A. subfuscus* and *P. carolinianus*, whether the fitness of *P. carolinianus* adults or juveniles decline when they share a habitat with *A. subfuscus*, and whether moisture level influences whether shelter is a limiting resource. Food was found to be a limiting resource for all experimental subjects, and shelter can be a limiting resource for *A. subfuscus* and for ovipositing individuals of *P. carolinianus*.

However, interspecific competition between the species was never greater than intraspecific competition within *P. carolinianus.*

Food was a limiting resource at naturally low levels, affecting most fitness variables for both species and for both adult and juvenile *P. carolinianus* (Experiments 1, 2, and 4). Field levels of food have been shown to be limiting for other gastropods. Stream algae density exists below the level that allows maximal population growth in several freshwater gastropods (Eisenberg 1970; Cross and Benke 2002), and natural levels of food on the forest floor limit growth in some landsnails (Pearce 1997). Shelter was not generally a limiting resource for either slug species (Expt. 1) or for juvenile *P. carolinianus* (Expt. 4). Although shelter is suspected to be a limiting resource for slugs (Rollo and Wellington 1979), many slug species, including philomycids, commonly aggregate in multispecies groups in shelters and appear to be tolerant of each others' presence (Webb 1950; Cook 1981). *P. carolinianus* were found to distribute themselves randomly under provided shelter, regardless of the number of conspecifics already present (Tim Pearce and Cagin Unal, unpub. results). In low shelter treatments, *P. carolinianus* laid fewer eggs (Expt. 2) and had a lower final mass plus egg laying mass lost (Expt. 3). Thus, shelter amount can be a limiting resource for ovipositing by *P. carolinianus*. Slugs lay almost all of their eggs under shelter (Rollo and Wellington 1979), and competition for favorable egg-laying sites can result in lower fecundity per snail in high-density gastropod populations (Carter and Ashdown 1984). Also, *A. subfuscus* lost more mass in low shelter treatments in one case (Expt. 3), suggesting that shelter can occasionally be important to *A. subfuscus* fitness. *A. subfuscus* may have

experienced greater desiccation or expended more energy in the pursuit of shelter in low-shelter conditions.

The presence of *A. subfuscus* did not affect the fitness of *P. carolinianus* except for egg-laying. In a similar study, *Philomycus* cf *flexuolaris*'s use of shelter was unaffected by the presence of *Limax maximus* in a lab setting (Tim Pearce and Paul Robb, unpub. results). For gastropods, the frequency at which interspecific competition exceeds intraspecific competition strength (Cameron and Carter 1979; Brown 1982; Tilling 1985; Riley et al. 2008) is counterbalanced by systems in which intraspecific competition is greater than or equal to interspecific competition strength (Fenchel and Kofoed 1976; Tattersfield 1981; Baur 1990; Cross and Benke 2002; Cope and Winterbourn 2004). From the perspective of *P. carolinianus*, the system of *P. carolininaus* and *A. subfuscus* fall into the latter category. Surprisingly, the fecundity of individual *P. carolinianus* increased in heterospecific, high-shelter mesocosms (Expts. 2 and 3). *P. carolinianus* must be experiencing greater intraspecific than interspecific competition for egg-laying sites. Few *A. subfuscus* were laying eggs at the time of the experiment, which, in high shelter mesocosms, may have maximized the availability of suitable ovipositing spaces for their cohabiting *P. carolinianus*. If the seasonal timing of reproduction in the field were consistent with these lab results, shelter as an ovipositing site may not be a contested resource between these species.

Distances between heterospecific pairs of slugs were unaffected by treatment, suggesting that any inherent antagonism was unaffected by resource level. In fact, *P. carolinianus* and *A. subfuscus* pairs were closer together in low moisture, low shelter treatments than in other treatments (Expt. 3). They appeared to be tracking the same

moist soil under the single piece of shelter, conserving moisture by huddling together, or both. Slugs adeptly manage water loss by moving into moist shelter (Luchtel and Deyrup-Olsen 2001), which was a single piece of CWD in the present experiment. Under excessively dry conditions, slugs such as *Limax pseudoflavus* also huddle together to limit water loss (Cook 1981).

Unexpectedly, shelter amount interacts with food or moisture factor levels in affecting mortality, resulting in alternately positive and negative significant associations of *P. carolinianus* mortality with shelter level. The highest number of *P. carolinianus* died in low shelter, low food treatments and the fewest died in low shelter, high food treatments (Expt. 2). Significantly more *P. carolinianus* (but not *A. subfuscus*) died in high shelter, high moisture treatments in Expt. 3 than in other treatments. Also, under high moisture conditions, *P. carolinianus* lost more mass per day (Expt. 3). Perhaps high shelter amounts under moist conditions may have promoted pathogen growth, overriding any positive affects of shelter except under low shelter, low food conditions (Expt. 2).

Juvenile *P. carolinianus* mass gain was greater in the presence of other juveniles than in the presence of a conspecific adult or *A. subfuscus* adult (Expt. 4). This result is in accord with other studies of young gastropods (Cook 1989; Baur and Baur 1990; Conner et al. 2008). For example, juvenile *Mesodon thyroidus* and *Neohelix albolabris* snails did not grow as much in the presence of conspecific adults in the lab (Pearce 1997). Juveniles can experience greater exploitative competition for resources from adults than from other juveniles (Pearce 1997), perhaps due to simple biomass differences. In this experiment, there was about a tenfold difference in the mass of four juveniles (0.13 +/-0.01 g) to an adult *P. carolinianus* (1.55 +/-0.09 g) or an adult *A. subfuscus* (1.49 +/-0.09 g). The

mucus of adults or other interactions with adults may also inhibit the growth and activity of young animals (Conner et al. 2008; Foster and Stiven 1996) or may cause juveniles to "purposefully" suppress their own growth to maximize their resource-use efficiency in a high density population (Tattersfield 1981). (Although, interestingly, three of the four "outlier" replicates, in which the mass of one juvenile was very high, were treatments with eight rather than four juveniles, hinting that juvenile density may spur growth in a few individuals.) The species identity of the adult did not affect juvenile *P. carolinianus* fitness, suggesting that competition strength does not differ between *A. subfuscus* and *P. carolinianus*. Conner et al. (2008) found that the survival rate of juvenile *Pomacea paludosa* was greater with a conspecific adult than with a *Pomacea canaliculata* adult. However, in my system, the strength of competition between juvenile *P. carolinianus* and adult *A. subfuscus* and *P. carolinianus* (as measured by the negative effect on juvenile fitness) simply parallels the competition strength between adult *P. carolinianus* and their heterospecifics or conspecifics.

This study did not and cannot address all potential sources of competition, such as apparent competition through disease and predation (DeBach 1966; Davis 2003). *P. carolinianus* died often in the high humidity, crowded conditions of high shelter, high moisture mesocosms, while *A. subfuscus* mortality was unaffected by treatment (Expt. 3). Although mesocosms are artificially confining and do not replicate natural weather conditions, this suggests that *A. subfuscus* may experience a fitness advantage during periods of excessive precipitation and resulting pathogen growth in the field. Although very little is known about relative efficacy of native slug predators, apparent competition through predators is also possible. For example, salamanders readily consume hatchling

A. subfuscus (John Maerz, unpub. results). If these salamanders are predators of *P. carolinianus* as well, growth of the salamander population in response to *A. subfuscus* abundance could result in a local decline of *P. carolinianus*.

Pairs of non-native and native species that appear to be likely competitors are often not, regardless of shared resource use and the ubiquity of the non-native competitor. Although the diets of the mourning dove and the non-native Eurasian collared dove are very similar, the mourning dove was found to be competitively dominant to, and so not apparently endangered, by the collared dove (Poling and Hayslette 2006). No evidence for spatial displacement was found between black rats and native Galapagos rice rats in a remaining patch of habitat, despite evidence of past rice rat extirpations and a current diet overlap with black rats (Harris et al. 2006). On a numerically-equivalent basis, interspecific competition between the two slugs was never greater than intraspecific competition within *P. carolinianus*. Pairs of competing species that exhibit population-wide detriment or habitat displacement to one species tend to experience asymmetrical competition in one-on-one interactions (e.g. Krassoi et al. 2008; Riley et al. 2008).

Researchers are increasingly discovering that the invasiveness and ubiquity of a non-native species does not correlate with its impact on ecosystems (Ricciardi and Cohen 2007), and the interactions of *A. subfuscus* and *P. carolinianus* fit this pattern. The densities of *P. carolinianus* and *A. subfuscus* in these experimental mesocosms are similar to their densities on coarse woody debris in the field, so they are likely to experience similarly weak competition in the field. Perhaps, a temporary-to-permanent stable coexistence between non-native and native competitors is very common in nature, even if the non-native species spreads widely, becomes highly abundant, and appears to

interact regularly with natives. Indeed, one of the fundamental patterns in ecology is the highly skewed nature of species relative abundance distributions: when counting species sharing a resource base, a few species are numerically dominant, representing a great fraction of the individuals encountered, while most species in the assemblage are scarce (Fisher et al. 1943; Preston 1948; McGill et al. 2007).

Other studies corroborate that competition between *A. subfuscus* and *P. carolinianus* is not likely to be great. Resources are shared to a greater extent between *A. subfuscus* and *P. carolinianus* than between either species and the native philomycid slug *Megapallifera mutabilis* (Chapter 2), and exploitation competition between *A. subfuscus* and *P. carolinianus* exists (Chapter 4). However, populations are often associated and never dissociated in the field (Chapter 1), suggesting that spatial displacement does not occur. Intra-specific interference seems to affect *P. carolinianus* fitness more than inter-specific interference, and mucus of either species does not function as a competition mechanism for *P. carolinianus* (Chapter 4). In conclusion, individuals of *A. subfuscus* may present no greater threat to the fitness of *P. carolinianus* than members of their own species.

CHAPTER 4

Mechanisms of Competition between a Non-native Eurasian Slug Species *Arion subfuscus* (Draparnaud) and the Native Slug Species *Philomycus carolinianus* (Bosc)

INTRODUCTION

The degree to which non-native species have altered the demography, ranges, and microhabitat occupation of native species is poorly known (Parker et al. 1999), especially when populations of non-native and native species co-occur. Yet, the competition-mediated decline of native populations, in concert with other factors such as habitat degradation, can place native species at risk of extirpation (Van Riel et al. 2007; Kandori et al. 2009). Critically, understanding whether competition between native and non-native species can take place under ordinary environmental conditions can allow us to extrapolate whether native species are likely to have experienced harm in the past and/or if they are likely to do so in the future.

Determining whether two species compete requires evaluating a series of criteria (Wiens 1989; see Chapter 3). A non-native competitor can lower the growth or fecundity of individuals of a second species, cause shifts in its microhabitat or habitat use, and/or alter the size, growth, and demography of the native species' populations (Parker *et al.* 1999). Except for a few sets of experiments that exhaustively investigated species of major ecological or economic importance, such as Argentine ants, the rusty crayfish, the New Zealand mudsnail, and Asian tiger mosquitoes (Cope and Winterbourn 2004; Juliano and Lounibos 2005; Buczkowski and Bennett 2008a; Pintor et al. 2008), studies of competition between non-native and native species typically only evaluate a subset of

these demographic and fitness effects and therefore potentially misinterpret the overall impact of competition. For example, studies often test in isolation whether the non-native species harms the fitness of individuals or the growth of subpopulations of the native species (Shinen and Morgan 2009), or whether a likely competition mechanism such as territoriality or contest competition is disproportionately exhibited by the non-native species against the native species. Although highly suggestive, the operation of a competition mechanism between two species does not by itself indicate that the "losing" species is experiencing a fitness decline. Also, population declines in the field cannot be attributed to competition unless they are accompanied by other evidence (Gurevitch and Padilla 2004). From a practical perspective, if the competition mechanism is known, recognition of a fitness decline of a native species in the presence of the non-native species can contribute more to predicting competition outcomes (Schmitt 1996) or designing conservation efforts. I investigated whether the native North American slug *Philomycus carolinianus* (Bosc) competes with the non-native Eurasian slug *Arion subfuscus* (Draparnaud) as reflected in a series of criteria that are reported in Chapter 3.

A. subfuscus was probably introduced to port cities in New England more than 150 years ago (Binney 1842, cited in Chichester and Getz 1969). *A. subfuscus* and *P. carolinianus* have had ample opportunity to interact in natural habitats in eastern North America. They are widespread and common in mesic forests (Chichester and Getz 1969 Getz 1974), and they frequently co-occur on fallen dead logs. Both species attain a maximum length of 7.0 cm in central Maryland (pers. obs). *A. subfuscus* is a relatively aggressive slug that is known to bite conspecifics and heterospecifics (Rollo and Wellington 1979; Fernandez 1990). Previous studies tested whether the two species

overlap in resource use (Chapter 2), whether individuals and subpopulations are disassociated with one another in the field (Chapter 1), whether resources exist at limiting levels (capable of inducing resource competition) in the field (chapter 3), and whether the fitness of *P. carolinianus* declines in the presence of *A. subfuscus* under natural resource levels reproduced in the laboratory (Chapter 3).

For the present study, I determined whether *A. subfuscus* interacts with *P. carolinianus* through the competition mechanisms of exploitation or interference. Interference is competition perpetrated through aggression, allelochemistry, and other direct interactions between individuals, while exploitation is an indirect form of competition in which resources are used up before they can be accessed by competitors (Schoener 1983). These mechanisms are well known to influence gastropod body size, growth rate, fecundity, mortality, and activity level (Tilling 1985; Baur 1988; Baur and Baur 1990). Gastropods compete for shelter through the mechanisms of mucus interference and aggression (Rollo and Wellington 1979; Dan and Bailey 1982; Tilling 1985; Pearce 1997), whereas competition for food can occur through exploitation competition (resource pre-emption) and/or mucus interference and aggression (Cameron and Carter 1979; Rollo 1983b; Cook 1989; Pearce 1997). Gastropod mucus can inhibit the growth and activity of heterospecifics and conspecifics (Williamson *et al.* 1976; Baur 1988; Conner et al. 2008). However, heterospecific mucus does not always deter slugs (Jordaens *et al*. 2003), and the negative effects tend to be greatest within species and to diminish with increasing taxonomic distance between two species (Cameron and Carter 1979; Dan and Bailey 1982). A likelier mechanism of interspecies competition is aggression. A good example is the slug *Limax maximus*, which often bites, pursues,

attacks, and kills other slugs. *L. maximus* kills heterospecifics, whereas conspecific encounters are rarely fatal (Rollo and Wellington 1979). Even coexistence with a mildly aggressive species can ultimately limit fecundity and survival (Rollo 1983a). In contrast to interference, exploitation competition has not received much study in gastropods. With the exception of a few studies (Pearce 1997; Riley et al. 2008), exploitation is not explicitly tested for (but is often the assumed mechanism (Cook 1989)) in gastropod studies. Fecundity and growth rate of snails can be greatly enhanced by adding food to a natural system, suggesting that exploitation competition for food is occurring (Eisenberg 1970; Pearce 1997). Shelter might be a source of exploitation competition for ovipositing gastropods when favorable egg-laying spots are exhausted in dense populations (Carter and Ashdown 1984).

Through a series of lab experiments, I addressed the following questions: does *P. carolinianus* engage in exploitation or interference competition for food or shelter with *A. subfuscus*? Does the mucus of *A. subfuscus* act as a competition mechanism with *P. carolinianus*? I hypothesized that (1) *P. carolinianus* and *A. subfuscus* engage in exploitation, i.e., when in a heterospecific treatment, *P. carolinianus* has greater fitness under high than low resources. (2) Interference competition between *P. carolinianus* and *A. subfuscus* is greater than interference competition within *P. carolinianus,* i.e., if resources are high (inexhaustible) *P. carolinianus* has a higher fitness when paired with conspecifics than when paired with *A. subfuscus*. (3) *P. carolinianus* is deterred by *A. subfuscus* mucus relative to *P. carolinianus* mucus on food and on shelter.

METHODS

Experiment 1: Exploitation Versus Interference

The potential for exploitation and interference to act as the mechanisms of competition was evaluated through a series of laboratory comparisons. A 2 x 2 x 2 factorial design was established to evaluate the influence of shelter amount (low or high), food abundance (low or high), and *A. subfuscus* (present or absent) on the fitness of *P. carolinianus* (Table 4.1a). Four slugs, including either four *P. carolinianus* (representing the absence of *A. subfuscus*) or two *P. carolinianus* and two *A. subfuscus* (representing the presence of *A. subfuscus*) were placed in each replicate mesocosm. There were five replicates per conspecific treatment and nine replicates per mixed species treatment. Shelter and food abundances approximated levels of resources occurring naturally in the field. Fitness response variables, including eggs laid per slug, slug mass, and slug mortality, were recorded every seven days (+/-1 day) for eight sampling periods between July 10th and September 9th, 2008 (a total of eight values per response variable). A detailed description of additional methods can be found in Chapter 3, Experiment 2.

When two competing species co-exist, we must account for multiple concurrent interactions: exploitation and interference competition, both within and between species. Planned (*a priori*) comparisons among treatments were used to separate these interactions as much as possible in order to evaluate whether exploitation or interference competition was occurring between species.

Experiment 1.A: Exploitation Competition

Exploitation competition can be manifest when resource levels are below optimum (Duyck *et al*. 2004). Exploitation competition between *P. carolinianus* and *A. subfuscus* may be shown if, when in a heterospecific treatment, *P. carolinianus* has greater fitness under high than low resources. A previous study suggested that antagonistic behaviors (as indicated by distances between heterospecific pairs of slugs) was unaffected by resource level (see Chapter 3). So, interference via aggression can be ignored when testing for exploitation competition between resource levels. However, the influence of resource level on mucus interference (if present) was unknown. The effect of exploitation competition on *P. carolinianus* in mixed-species groups was measured by comparing *P. carolinianus* fitness between high and low resource tubs containing *A. subfuscus* (Table 4.1b). The analysis was performed separately for food and for shelter.

Experiment 1.B: Interference Competition

Exploitation competition can be eliminated from a system by making resource levels superabundant. Interference competition by *A. subfuscus* on *P. carolinianus* would be indicated if resource levels were high and effectively inexhaustible, but *P. carolinianus* still had greater fitness under *A. subfuscus* absence treatments than under *A. subfuscus* presence treatments. I tested whether interspecific interference (between *P. carolinianus* and *A. subfuscus*) affected *P. carolinianus* fitness differently than intraspecific interference (among *P. carolinianus*) by comparing *P. carolinianus* fitness between conspecific and heterospecific tubs with high levels of resource (Table 4.1b). I analyzed

the high food tubs and high shelter tubs separately. This test for interference does not distinguish between aggression and mucus interference as competition mechanisms.

Experiment 1: Statistical Analysis

SAS software version 9.2 (SAS Institute Inc. 2008) was used to evaluate the planned comparisons through the least squares means (t-tests) procedure obtained during a factorial, mixed-model ANCOVA. The covariate was the initial mass of each slug, the replicate was the mesocosm, and each slug was treated as a subsample in each mesocosm. The fitness (response) variables for each slug were average mass change per day, final mass, eggs laid overall, and final mass plus egg laying mass lost, and the percent slugs dead per mesocosm were calculated as an overall fitness variable for each mesocosm (see Chapter 3). Denominator degrees of freedom were determined by the Satterthwaite procedure (Satterthwaite 1946). Independent variables were shelter, food, and heterospecific presence. Planned comparison tests were used to compare *P. carolinianus* fitness between high and low resource levels within mixed-species tubs (exploitation competition) and to compare their fitness between single-species and mixed species tubs within high resource treatments (interference competition). Food and shelter were evaluated separately as factors. These tests were planned *a priori* and did not require alpha adjustment for multiple comparisons.

Table 4.1 Description of treatments used in the experiments testing for exploitation and interference, including (a) *A. subfuscus*, food, and shelter as the three factors with two levels each. *A. subfuscus* present treatments contained two *A. subfuscus* and two *P. carolinianus* each, while *A. subfuscus* absent treatments contained only four *P. carolinianus*. (b) Paired comparisons of subsets of treatments were used to test both the hypotheses of exploitation and interference, with food and shelter effects evaluated separately.

(a) Factors	Factor levels	Abbreviations
A. subfuscus	absent	4Pc
	present	2As2Pc
Food	low	LF
	high	HF
Shelter	low	LS
	high	HS
(b) Hypothesis tested	Paired comparisons of treatments	
	Between food levels	Between shelter levels
Exploitation	2As2Pc_LF vs. 2As2Pc_HF	2As2Pc_LS vs. 2As2Pc_HS
Interference	4Pc_HF vs. 2As2Pc_HF	4Pc_HS vs. 2As2Pc_HS

Experiment 2: Interference via Mucus

I tested the effect of mucus – whether present, or whether belonging to *A. subfuscus* or *P. carolinianus* - on *P. carolinianus* preferences for food and shelter.

Experiment 2.A: Interference via Mucus-on-Food

Each of twenty *P. carolinianus* was placed in a separate large mesocosm (12.5 cm wide, 32.5 cm long, and 17.5 cm tall) and given a choice of three food pieces: food without mucus, with a conspecific's mucus, and with *A. subfuscus*'s mucus (e.g., Pearce 1997). Sixty wedges of commercially-grown white mushrooms (*Agaricus bisporus*) weighing 0.50g (+/- 0.005g) were used. The wedges were laid flat in one layer, and one of three mucus treatments was applied: no mucus, *A. subfuscus* mucus, and *P. carolinianus* mucus. In order to coat the mushroom wedges with mucus, twelve *A. subfuscus* were provided 20 mushroom pieces lacking mucus and allowed to move and feed freely for about 30 minutes, although I occasionally relocated individuals to pieces that lacked mucus. Eight *P. carolinianus* crawled upon 20 mushroom pieces for the *P. carolinianus* mucus treatment. After 30 minutes, mucus was fairly evenly distributed across all mucus-treated mushrooms. Food pieces were each set on a labeled plastic container lid that was about 3 cm in diameter.

After the mucus application, I tested which of the three mucus types individuals of *P. carolinianus* accepted based on the relative amounts of each mushroom treatment consumed. Each of 20 plastic (32.5 x 12.5 x 17.5 cm) tubs was lined with a strip of moist paper towel. Twenty sectioned branches that were 5-6 cm in diameter, 3-4 cm long, and in stage three of decay (Stokland and Kauserud 2004) were soaked in water for one hour. A branch section was placed in the center of each mesocosm as shelter for the slug. One of each of the 3 food types was placed 2 cm from the shelter (Fig. 4.1a). (The food lids were arranged so that they would be as close to equidistant from each other as possible while still fitting in the oblong mesocosm.) The food dishes were rotated such that

treatments were in each location in the mesocosm for the same number of replicates (Fig. 4.1b). Twenty *P. carolinianus* slugs had been deprived of food for 24 hours. Each slug was set on the top of the shelter, consistently facing the same direction in each mesocosm. Lids were placed on the tubs. To estimate natural water loss from mushroom, six wedges of mushroom identical to those used in the experiment were placed in a mesocosm without slugs as a control and weighed at the end of the experiment.

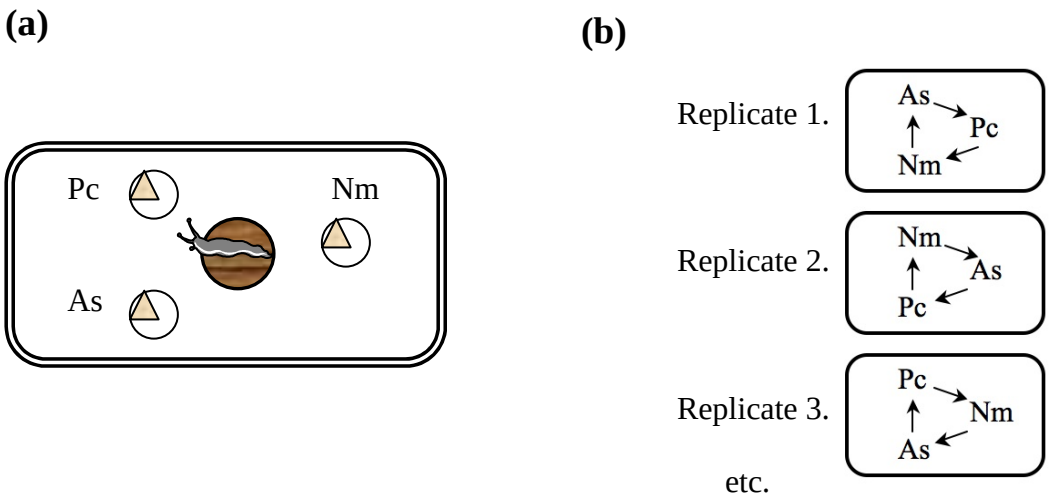


Fig. 4.1 Arrangement of the contents of a mucus-on-food mesocosm. (**a**) For each mesocosm, mushroom pieces (triangles) with *P. carolinianus* mucus (Pc), *A. subfuscus* mucus (As), and no mucus (Nm) were arranged around a central shelter on which a single *P. carolinianus* was first placed. (**b**) The location of the treated mushrooms was shifted clockwise around the mesocosm for each successive replicate mesocosm.

The experiment began at 22:45 on November 13th, 2007, and ended 36 hours later. At that time, many mushrooms appeared to be at least half-consumed. All mushrooms were weighed, placed in a drying oven for 24 hours, and weighed again. Wet

masses of mushrooms were multiplied times a correction factor of 1.40 to account for water loss due to drying. (The correction factor was calculated from the control mushrooms as the initial mass divided by the final mass.) The results for two slugs that died during the experiment were excluded from the analysis.

A one-way ANOVA tested whether slugs consumed different amounts of mushroom depending on the type of mucus applied to its surface. Mucus type was the independent variable, final mass of the mushroom was the response variable, and the mass of the slug was the covariate. Wet mass and dry mass of the mushroom food were analyzed separately. An initial model treated the identity of the individual slug as a block; this factor was non-significant and so was removed from the model.

Experiment 2.B: Interference via Mucus-on-Shelter

An experiment of parallel design to the mucus-on-food experiment tested whether *P. carolinianus* preferentially responded to the species-specific identity of mucus on shelter. Seventy-two sectioned branch pieces 5-7.5 cm diameter by 1.5-3 cm in length (in stage three of decay) were scrubbed to remove any traces of mucus and soaked in water for one hour. Wood pieces were treated with mucus for 30 minutes as per the Mucus-on-Food experiment, resulting in shelter with *P. carolinianus* mucus, *A. subfuscus* mucus, or no mucus. Trios of shelter pieces from the same log were maintained within each of 24 mesocosm replicates (Fig. 4.2). A dish of 0.50 g of mushroom was placed in the center of each mesocosm on a single layer of paper towel, and shelters were set in a triangle, 2 cm from the central food dish. *P. carolinianus* were set facing a single direction between two shelters. As before, shelters were arranged randomly (Fig. 4.1b), and the treatment of

each shelter was marked on the mesocosm lid. At 3, 6, 9, 12, 18, and 24 hours following the start of the experiment, I recorded the shelter with which each slug was in contact.

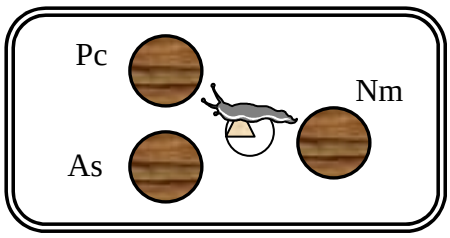


Fig. 4.2 Arrangement of the contents of a mucus-on-shelter mesocosm. Shelter with *P. carolinianus* mucus (Pc), shelter with *A. subfuscus* mucus (As), and shelter with no mucus (Nm) surrounded a central food dish beside which a single *P. carolinianus* was placed. (Order of mucus treatments varied among mesocosms.)

A Chi-square test was performed to determine whether equal numbers of slugs chose each shelter treatment as their first shelter. The shelter type occupied by each slug was not independent across observations, because slugs tended to remain for multiple observation sessions on the same shelter. Thus, a metric m was devised to take into account multiple sessions:

$$m_i = (n_{iPc} - n_{iAs}) / n_i$$

where n_i is the total number of sessions for which each slug i occupied shelter (including the no mucus shelter), and n_{iPc} and n_{iAs} are the number of those sessions for which *P carolinianus*- and *A. subfuscus*-mucus shelters respectively were occupied by slug i. A one-tailed t-test of m was performed to determine whether the mean differed from 0,

meaning equal time spent between *P. carolinianus*- and *A. subfuscus*-mucus treatments. The observations of one slug that died were excluded from the analysis.

RESULTS

Experiment 1.A: Exploitation Competition

Exploitation competition is manifested as greater fitness in high-resource treatments than in low-resource treatments. This hypothesis was supported for food and, with respect to eggs laid overall, for shelter as resources. All fitness metrics except for percent slugs dead were significantly higher for treatments with high food than low food (Fig. 4.3). In high food conditions, slugs lost less mass per day ($P < 0.0001$), had a higher final mass ($P = 0.0001$) and final mass plus egg mass lost ($P < 0.0001$), and laid more eggs ($P = 0.0008$). The number of eggs laid per slug was greater in high shelter conditions than low shelter conditions (eggs laid overall: $t = 3.50$, $df = 55.9$, $P < 0.001$; 22.8 +/-2.0 eggs vs. 12.9 +/-2.0 eggs, respectively), although other fitness measures were unaffected by shelter amount. Note that slugs lost weight during the course of the experiment (average: -0.019 g per day). Degrowth is a common phenomenon in mature slugs that increases with egg-laying (Rollo and Shibata 1991).

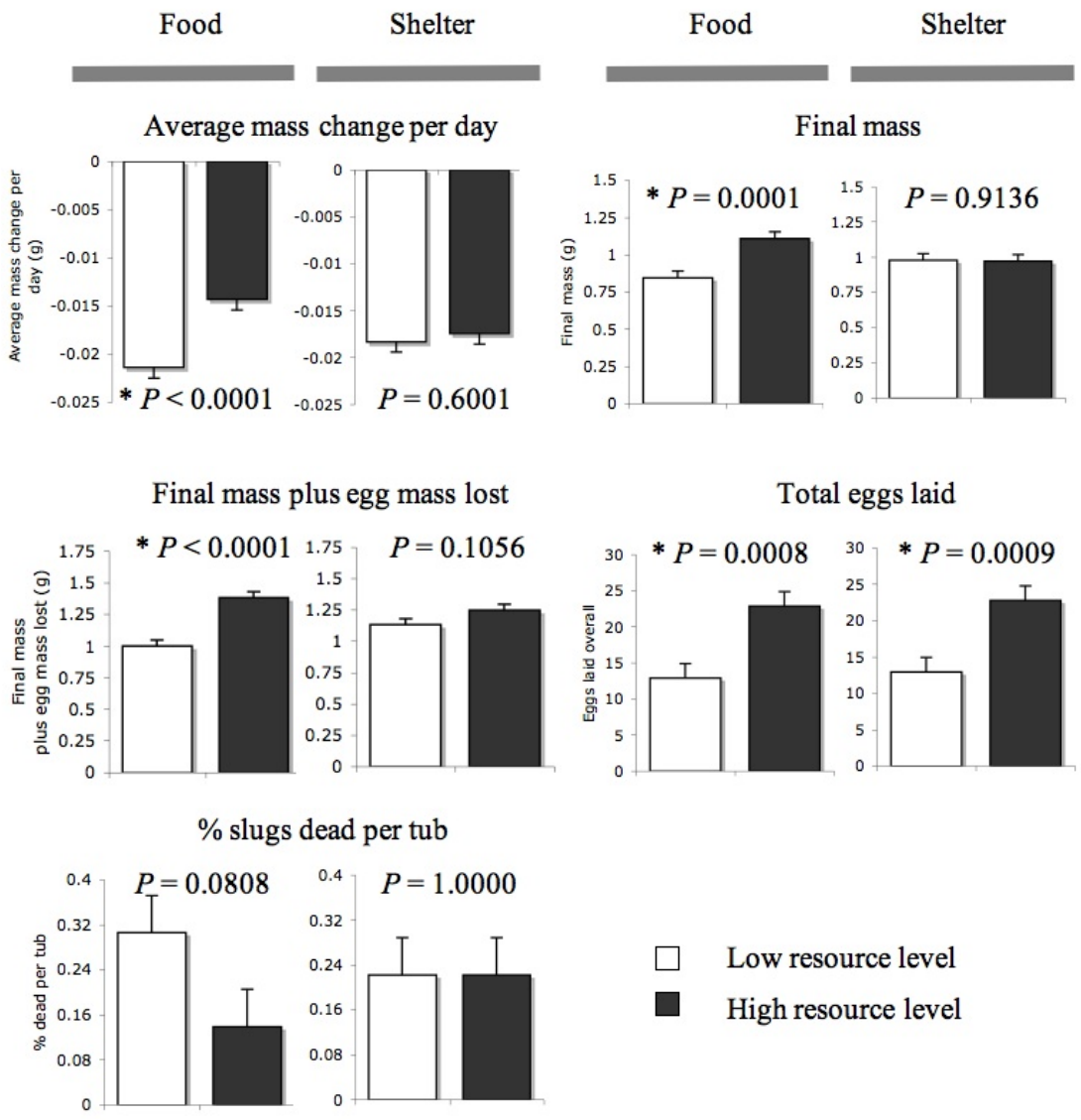


Fig. 4.3 Exploitation competition, tested through paired comparisons of the fitness of *P. carolinianus* slugs under high vs. low food and high vs. low shelter conditions. Mixed-species tubs were used, with two *A. subfuscus* and two *P. carolinianus* per mesocosm. % slugs dead only represents *P. carolinianus*. Error bar is +SE. (* indicates significant *P*-value. See *t*-statistics and *df* values in Appendix D, Section I.)

Experiment 1.B: Interference Competition

Under high resource conditions, exploitation competition is not as likely to be manifest, leaving the effect of interference competition on fitness. This experiment tested the hypothesis that interference competition between *A. subfuscus* and *P. carolinianus* is greater than interference competition within *P. carolinianus*. The hypothesis was rejected for both high food and high shelter conditions. *P. carolinianus* did not perform significantly differently under high food conditions (Fig. 4.4). Under high shelter conditions, *P. carolinianus* paired with *A. subfuscus* laid more eggs than slugs in conspecific treatments (overall mass change plus egg mass lost: $t = -2.10$, $df = 42.2$, $P = 0.0420$; eggs laid overall: $t = -2.33$, $df = 33.7$, $P = 0.0260$). Other fitness measurements under high shelter were similar between conspecific and heterospecific treatments.

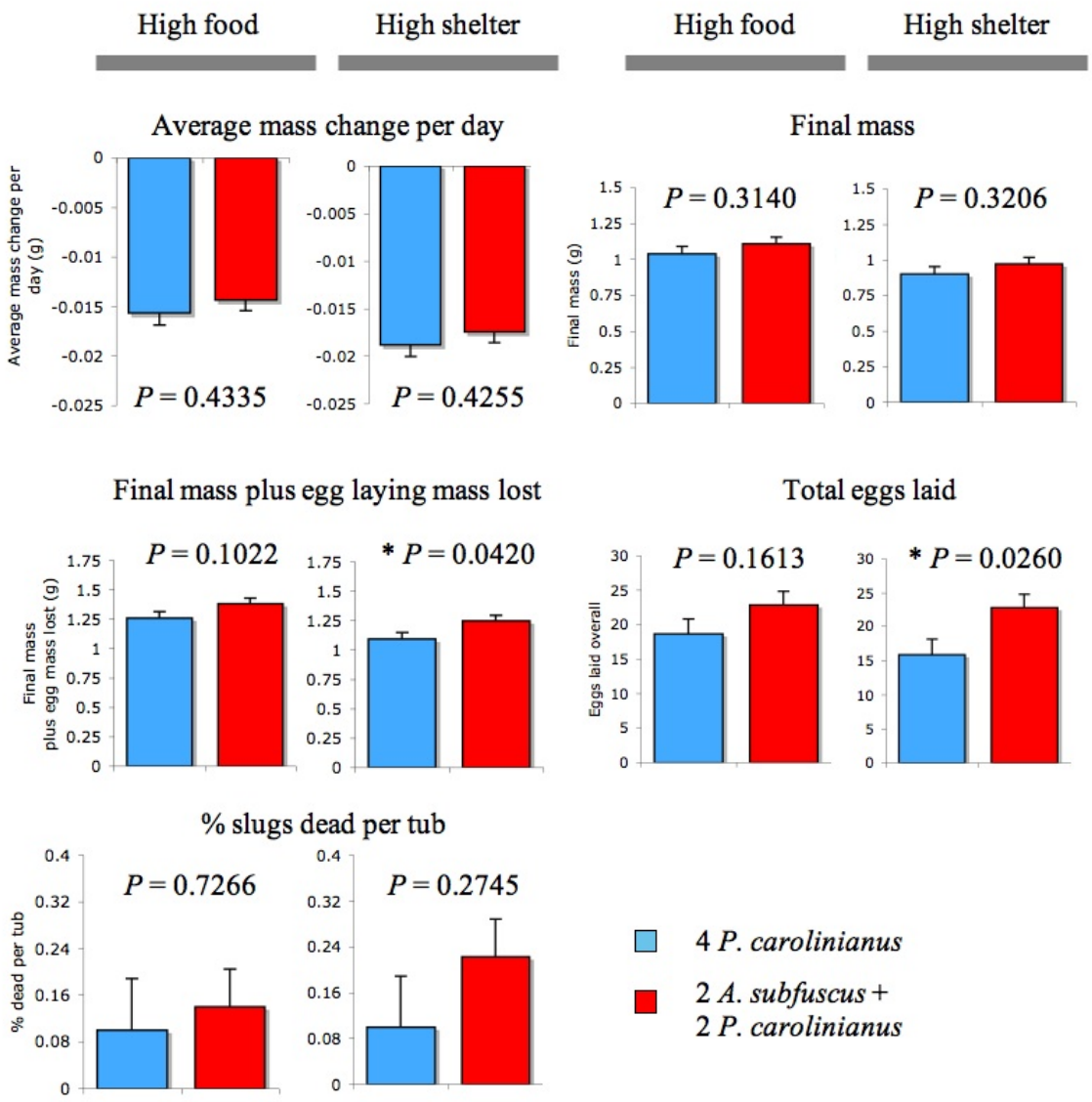


Fig. 4.4 Fitness of *P. carolinianus* slugs in *A. subfuscus* absence (four *P. carolinianus*) vs. *A. subfuscus* presence (two *A. subfuscus* and two *P. carolinianus*) treatments, under high food or high shelter conditions. % slugs dead only represents *P. carolinianus*. Error bar is +SE. (* indicates significant *P*-value. See *t*-statistics and *df* values in Appendix D, Section I.)

Experiment 2: Mucus-on-Food and Mucus-on-Shelter Competition

P. carolinianus did not feed preferentially in response to the species identity of mucus or to the presence or absence of mucus (1-way ANOVA: $F_{2,50} = 0.56$, $P = 0.5772$ for dry mass mushrooms; 1-way ANOVA: $F_{2,50} = 0.20$, $P = 0.8198$ for wet mass mushrooms) (Fig. 4.5a and 4.5b). Their first choice of shelter was unaffected by mucus (Chi-square: $\chi^2 = 2.174$, $P = 0.3372$) (Fig. 4.5c), and they did not prefer to spend more time in contact with shelter with *P. carolinianus*-mucus than *A. subfuscus*-mucus (one-tailed t-test: $t = 0.0436$, $df = 22$, $P = 0.483$) (Fig. 4.5d). However, *P. carolinianus* was observed disproportionately frequently on log pieces lacking any mucus than on logs with mucus produced by either species. (i.e., of 135 slugs seen on shelter across observation sessions 54 slugs were on no-mucus logs, which is greater than the expected count of 45 (one third of 135).

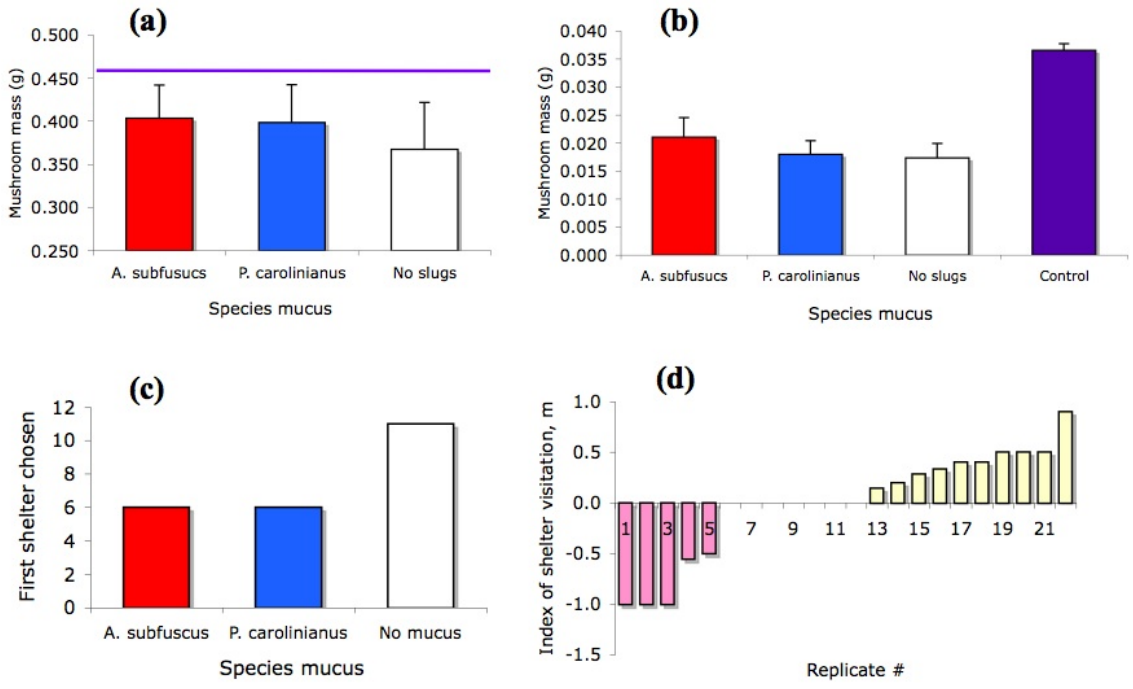


Fig. 4.5 Mucus-on-food and mucus-on-shelter experimental results. (**a**) Dry mass of mushrooms remaining after *P. carolinianus* feeding. The purple line indicates the controls' average dry mass. (**b**) Wet mass of mushrooms remaining plus water weight lost by treatment. Bar is +SE. (**c**) First choice of shelter by treatment. (**d**) Index "m" of contacts per shelter type, where m < 0 indicates *P. carolinianus* individuals that spent more sessions on *A. subfuscus*-mucus shelters and m > 0 indicates slugs that spent more sessions on *P. carolinianus*-mucus shelters. (No results were significant.)

DISCUSSION

In accord with other experiments, which did not provide strong support for competition, this study showed that competition mechanisms play a limited role in interactions between the native slug *P. carolinianus* and the non-native slug *A. subfuscus*. Exploitation competition occurred between the species, with *P. carolinianus* fitness

harmed by competition with *A. subfuscus*. Interference competition was minimal, although competition within *P. carolinianus* was greater than competition between the species for egg-laying sites.

Resource exploitation, or direct resource use, lowers fitness when resource levels are low (Duyck et al. 2004). Tests for exploitation compared *P. carolinianus* fitness between high and low resource conditions in a mixed species treatment. Exploitation competition was shown to occur for both food and shelter. *P carolinianus* exhibited generally higher fitness under high food conditions and laid more eggs in high shelter treatments. Several gastropod systems have been shown to exhibit exploitation competition for resources. Exploitation occurred for food and moisture in juvenile *Mesodon thyroidus*, which experienced lower growth rates when adults were present but not with augmented food and water (Pearce 1997). The marine snails *Tegula aureotincta* and *Tegula eiseni* competed for food, with increasing snail density resulting in a decline of algae (Schmitt 1996), and the freshwater snails *Elimia cahawbensis* and *E. carinifera* competed for stream algae, which was shown to be a limiting resource at natural levels (Cross and Benke 2002).

Interference competition can lower fitness independently of resource levels. I assessed the occurrence of interference competition by determining whether, given high (inexhaustible) resources, *P. carolinianus* had lower fitness when combined with another species than with conspecifics (e.g., Pearce 1997). For most fitness measures, there was no difference whether individuals were in the presence of an equal number of conspecifics or heterospecifics, indicating that interspecific and intraspecific competition were of similar strength. However, more eggs were laid per *P. carolinianus* in the

heterospecific treatment than in the conspecific treatment (also see Chapter 3). When ovipositing, *P. carolinianus* may experience less interspecific competition with *A. subfuscus* than intraspecific competition for shelter with conspecifics that are also laying eggs.

Secure, moist shelter is essential for the survival of both eggs and juveniles (Hunter 1978; Kappes 2005) and is likely to play a major role in competition among slugs (Rollo and Wellington 1979). For example, *Cepaea nemoralis* laid fewer egg clutches at higher densities, perhaps because there were not enough "favorable" spots in the soil for the snails to lay their eggs (Carter and Ashdown 1984). However, the present study may be the first that has demonstrated greater egg-laying with increased shelter amount in gastropods, i.e., exploitation competition for egg-laying sites. Several mechanisms could enable competition for ovipositing sites among gastropods. Perhaps, egg-laying sites are limited in low shelter conditions because mothers avoid putting their eggs near other clusters of eggs. Egg cannibalism by hatchlings is a common strategy in gastropods, and it may increase in denser populations (Baur 1988). Adult mucus density or egg allelochemicals might also inhibit laying, although I am not aware of studies that have specifically tested for these mechanisms.

Despite the likely importance of competition for oviposition sites within *P. carolinianus* populations, the native slug is unlikely to compete for this resource with *A. subfuscus.* In central Maryland, *P. carolinianus* lays eggs in the late spring through the summer, whereas *A. subfuscus* lays most eggs between mid-September to mid-October (pers. obs.). This temporal disjunction in egg-laying makes it unlikely that interspecific competition for oviposition sites happens in this region. However, conflicts over

ovipositing sites may occur between *P. carolinianus* and *A. subfuscus* in colder regions with shorter summers. For example, *A. subfuscus* lay eggs in July to early September rather than the fall in Nova Scotia (Pelluet and Watts 1951), which is the northern end of the range of many philomycids (Grimm 1996).

A. subfuscus mucus did not deter *P. carolinianus* from fungus and shelter (see Appendix D, Section II). Thus, *A. subfuscus* did not exhibit any mucus-based interference competition with *P. carolinianus*. However, it would be worth investigating whether *any* mucus on shelter will deter *P. carolinianus* relative to shelters with no mucus; a more powerful test in which only two shelter options are provided (mucus and no mucus) may show that mucus in general can attract or deter *P. carolinianus* from selecting a shelter. The slug *Deroceras laeve* prefers surfaces with the mucus of either conspecifics or heterospecifics to surfaces with no mucus (Jordaens et al. 2006). The fitness of some gastropods is affected negatively by mucus (Williamson *et al.* 1976; Baur 1988; Jordaens *et al*. 2003; Conner et al 2008). A wider taxonomic distance between two species can limit the negative effects of mucus (Dan and Bailey 1982), perhaps because niche overlap is greatest within a species (Wiens 1989) and so gastropods have reason to avoid their own species. However, in this study, no evidence was found that intraspecific competition through mucus occurs for *P. carolinianus* as it occurs in *Helix aspersa* (Cameron and Carter 1979) and other species (Kawata and Ishigami 1992; Bull et al. 1992; Schmitt 1996). It would be interesting to compare the strength of competition within and between species of gastropods with their use of mucus as an interference mechanism, to suggest in which gastropod systems (e.g. freshwater or terrestrial, at naturally low density or high density) mucus has evolved to be an interference

mechanism. For example, *P. carolinianus* occur at relatively low densities (Chichester and Getz 1969), a trait which may correlate with low competition and limited use of mucus as an interference mechanism across gastropod taxa.

Non-native species whose competition mechanisms are clearly more effective than those of their native competitors can cause local extinctions of the natives (Holway 1999; Cole et al. 2005). The non-native Argentine ant (*Linepithema humile*), which alters the composition of, and causes local extinctions in, the native ant fauna, exhibits more effective exploitation (higher rate of discovery and faster recruitment to food sources (Holway 1999; Buczkowski and Bennett 2008b)) and interference competition mechanisms (greater success in contests over food sources (Carpintero and Reyes-Lopez 2008), faster recruitment of ants to engage in colony battles (Buczkowski and Bennett 2008a), and lower mortality (Buczkowski and Bennett 2008b)) than native ants. The house gecko (*Hemidactylus frenatus*) is more effective at exploiting food (lowering the local insect density (Petren and Case 1996)) and displacing other geckos from refugia through aggression (Cole et al. 2005) than native gecko species, resulting in the extirpations of other geckos from islands to which it was introduced (Cole et al. 2005). Exploitation competition for food did occur between *P. carolinianus* and *A. subfuscus*. However, in contrast to these pairs of native and non-native species, *P. carolinianus* and *A. subfuscus* consistently showed limited evidence of interspecific competition (Chapter 3), and *P. carolinianus* were more affected by intraspecific competition than interspecific competition for ovipositing sites. Mechanisms of competition, which are key factors in the displacement of a native competitor when they asymmetrically favor the non-native

species (Holway 1999), do not provide an advantage to *A. subfuscus* and, by themselves, do not suggest that *A. subfuscus* is likely to displace *P. carolinianus*.

Multiple lines of evidence, including both mechanisms of competition and consequences of competition (i.e., reduced fitness or spatial displacement), are essential to demonstrate the presence or absence of competition (Wiens 1989). Previous studies have shown that *P. carolinianus* does not experience displacement due to *A. subfuscus* in the field (Chapter 1), overlap in niche dimensions between the two species is limited (Chapter 2), and *P. carolinianus* fitness increases when placed with *A. subfuscus* relative to conspecifics (Chapter 3). In addition to these lines of evidence, the present study supports that competition between *A. subfuscus* and *P. carolinianus* is not strong. The persistence of *P. carolinianus* in sympatry with populations of *A. subfuscus* does not appear to be under immediate threat (see Chapter 1). When the future of a native species is of major concern, its interactions with a likely non-native competitor should receive a thorough evaluation. Such efforts are needed to determine whether competition is indeed occurring, whether alternate factors are at play in the decline of a native (e.g., habitat destruction occurring concomitantly with the range spread of an non-native species), and which aspects of the interaction might be targeted in conservation efforts to limit competition, such as providing additional shelters if exploitation competition for refuges is occurring.

APPENDIX A

Section A.I.:

Daytime field surveys methodology

Daytime field surveys using shelter traps were attempted as a complementary method to visual night surveys. The fact that this method was ineffectual in temperate forest settings may be of interest to other researchers intending to survey forest populations of slugs.

I had sought to perform surveys in the field by laying out artificial shelters that could be checked for slugs during the day (South 1965, 1989; Schrim and Byers 1980). For each cell of the field grids, three pieces of cardboard (about 75 x 30 cm) were wrapped with twine around the trunks of the largest live trees and fallen logs. Four particleboard tiles (30 cm x 30 cm) were placed on the ground. Slugs did not use the artificial shelters during dry weather, rendering the shelters generally ineffectual for daytime surveys. Soil beneath the tiles did not remain moist, which would have encouraged slugs to remain beneath. I suspect that this methodological failing may be a result of the environment: artificial slug shelters have been used predominantly in agricultural settings (South 1964; Byers et al. 1989). The forests of central Maryland may be too dry, or they may provide more attractive natural shelters to slugs. Researchers considering using shelter traps in a forest setting might encourage slug usage by distributing the traps during wet weather or by artificially moistening the traps, neither of which I attempted.

Section A.II.:

Response of slug numbers to rainfall

The effect of rainfall on slug activity and abundance is presented as an important consideration in surveying slugs and may be of interest to future researchers.

INTRODUCTION

Attempts to model activity levels and population dynamics of gastropods have revealed that environmental moisture levels tend to correlate with observed slug numbers (Cook 2001). Moisture availability affects the survival of slugs, in particular juveniles (Hunter 1978; South 1989; Choi et al. 2004), and rainfall induces egg laying in gastropods (Wolda 1973; Rollo and Shibata 1991). In addition to affecting slug population persistence, rainfall is a major factor constraining slug activity. Most slugs forage and mate at night or else during or after rain (Judge 1972), when the vapor pressure deficit is low (Crawford-Sidebotham 1972).

During my field surveys of slug populations at Patuxent Research Refuge (PRR), Laurel, Maryland in the late spring through early fall of 2007 and 2008 (Chapter 1), numbers of observed slugs varied greatly across the nine field surveys and over the two years (Figs. 3 and 4, Chapter 1). I suspect that the decline of *A. subfuscus, M. mutabilis,* and *P. carolinianus* during 2007 was a response to the onset of drought that summer. Thus, I performed a simple analysis on my observations to answer the question, did the abundance of slugs observed during field surveys at PRR correspond to recent levels of rainfall?

METHODS

Populations of the slug species *A. subfuscus*, *M. mutabilis*, and *P. carolinianus* were surveyed between the late spring through early fall of 2007 and 2008 at Patuxent National Wildlife Refuge, Laurel, Maryland. For further details, see Chapter 1. Nine surveys took place. For each survey, the average number of slugs for each species per 5 x 5 m cell was calculated across a total of 225 cells on all field grids. Using local daily rainfall records (Laurel 3W weather station: http://www.ncdc.noaa.gov/oa/mpp/freedata.html), I calculated the total amount of rainfall of the previous one and two weeks prior to each day of a survey. Because surveys spanned at least three days, these rainfall totals were averaged to obtain a single value of "one week prior" and of "two weeks prior" for each survey.

I performed a regression analysis of average slug numbers per cell against the total rainfall, with "one week prior" and "two weeks prior" analyzed separately. I employed the regression program GENMOD in SAS with a Poisson distribution model, because the slug count data were non-normal (SAS Institute Inc 2008).

RESULTS

P. carolinianus and *M. mutabilis* counts were not associated with rainfall in the previous one week (*P. carolinianus*, Poisson regression: $Z = 1.65$, $P = 0.0990$; *M. mutabilis*, Poisson regression: $Z = 0.33$, $P = 0.7384$) (Fig. A.1a). However, both *P. carolinianus* and *M. mutabilis* numbers were associated with the previous two weeks of rainfall (*P. carolinianus*, Poisson regression: $Z = 2.18$, $P = 0.0292$; *M. mutabilis*, Poisson regression: $Z = 2.40$, $P = 0.0166$) (Fig. A.1b).

There was no association of *A. subfuscus* counts with either the previous one week of rainfall (Poisson regression: Z = -0.60, P = 0.5501) or the previous two weeks of rainfall (Poisson regression: Z = 0.90, P = 0.3698).

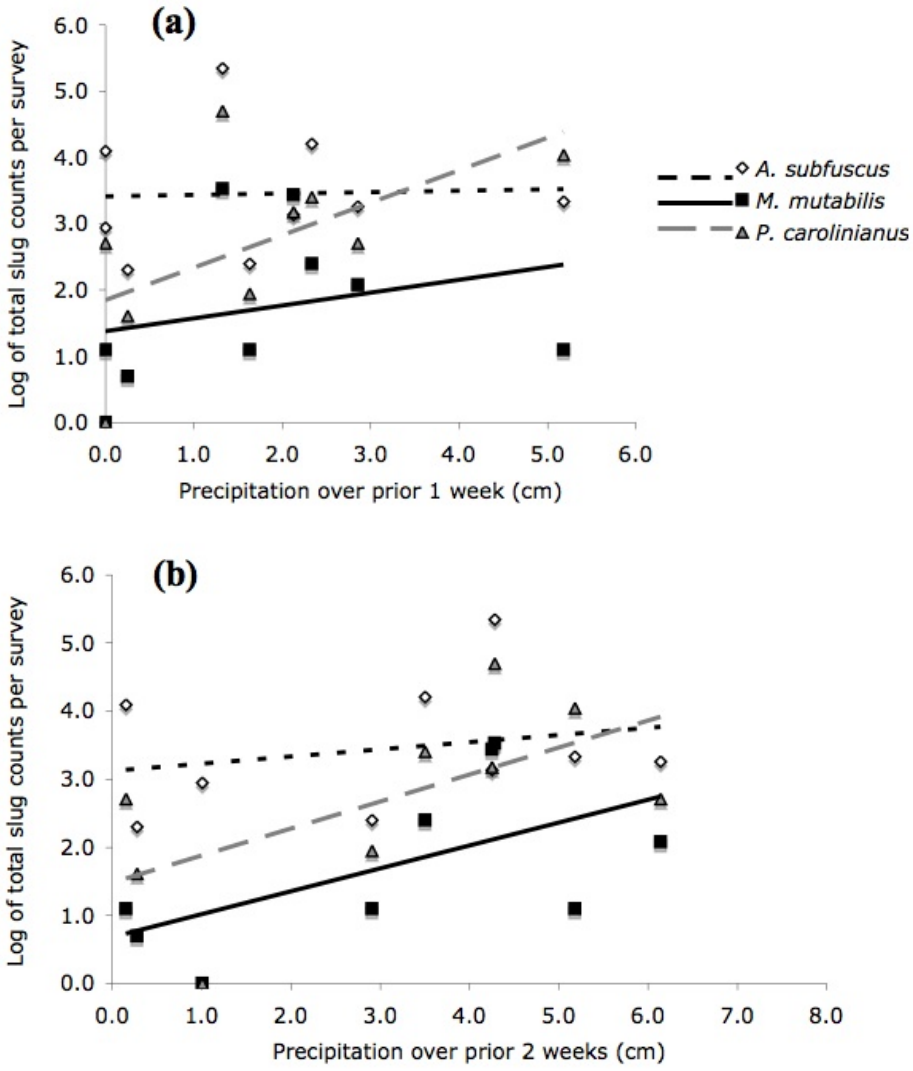


Fig. A.1 Natural log of total counts per slug species for each survey, versus the total rainfall over (**a**) the previous one week and (**b**) the previous two weeks. For each slug, each point represents one of nine survey sessions.

DISCUSSION

The previous two weeks of rainfall were positively correlated with numbers of *M. mutabilis* and *P. carolinianus*, whereas *A. subfuscus* numbers did not correlate with either span of rainfall. Rainfall may have induced activity in *M. mutabilis* and *P. carolinianus*. Jaremovic and Rollo (1979) found that most individuals of *Cepaea nemoralis* did not become active on a given day unless there was rain, and among environmental factors, rain was most strongly correlated with *C. nemoralis* activity. *M. mutabilis* and *P. carolinianus* numbers may also reflect the response of population size to rainfall, if individuals died during long-term droughts. Models used by Choi et al. (2004) suggest that rainfall contributes the most to juvenile recruitment (egg survival) and adult mortality in the slug *Deroceras reticulatum*. However, relative to two limacid slug species, *P. carolinianus* experiences slower dehydration and greater tolerance of water loss, suggesting that this species resists mortality from drought (Thompson et al. 2006). (No similar comparative data exist for *A. subfuscus* or *M. mutabilis*.)

M. mutabilis and *P. carolinianus* activity may have responded in part to the degree of substrate saturation resulting from long-term rainfall. Activity exposes more of the mantle to the air (Cook 1981), and gastropod locomotion requires mucus to be released (Machin 1978; Denny 1981). Having a wet substrate, such as water-saturated logs after a long or heavy period of rainfall, limits the expense of mobility and helps to prevent slugs from drying out when active (Barnes and Weil 1944, 1945). For example, Jaremovic and Rollo (1979) found that *C. nemoralis* on the ground became active more often than snails on bushes, which were relatively dry and exposed.

In comparison to the two philomycid species, *A. subfuscus* numbers correlated poorly (although positively) with recent rainfall levels, which may be a trait that makes them successful invaders. *A. subfuscus* may not respond as readily to rainfall and substrate moisture as the two philomycid species. One possible reason for their relative detachment from weather conditions is that *A. subfuscus* may be able to attain acceptable bodily moisture content through behavioral adaptations. Given a choice, slugs tend to take shelter within moist cracks in coarse woody debris, leaf litter, soil, and rocks (Luchtel and Deyrup-Olsen 2001). Perhaps *A. subfuscus* is more adept than the philomycid species at maintaining body moisture by moving back and forth between moist and less moist microhabitats (Lyth 1983; Cook 2001). Alternatively, *A. subfuscus* response to moisture may be attenuated by a sensitivity to other environmental factors, such as wind and temperature (Dainton 1954a, 1954b; Crawford-Sidebotham 1972), such that they don't readily emerge even if rainfall amounts have been high.

Even after rain in the late summer of 2007, slug numbers did not increase during the rest of 2007 or 2008. The water deficit may have been great enough that the environment was still too dry for slug activity, or else a significant portion of slugs may have died directly from the drought. It is unknown whether activity levels or mortality was responsible for the change in slug numbers observed. However, at least part of the response was probably mortality: numbers never returned to the levels observed during the first survey.

Section A.III.:

Methods and results of spatial analysis through SADIE

In my first attempt to determine spatial disassociation between native and non-native slugs (Chapter 1), I identified patterns of slug population spatial distribution within each field grid by using the Spatial Analysis by Distribution IndicEs (SADIE) program (Perry 1998). The methods and datasets produced during this analysis are presented here as a demonstration of an alternative method of spatial analysis to a partial Mantel test. These initial results also showed that slug populations were often spatially associated. In response, I attempted to eliminate the factor of coarse woody debris (CWD) that may have caused aggregations of slugs in order to study underlying patterns of interaction between slug populations (Chapter 1).

Through SADIE, pairs of species abundance datasets were compared individually for each survey and field site to determine if any pairs are spatially disassociated, at any site or point in time. I used counts of *M. mutabilis, P. carolinianus,* and all non-native species per cell; I originally chose to add together all non-native species (which are mostly *A. subfuscus*) because my goal had been to compare native slug abundance against all non-native slugs in general. (Given the current degree of specificity of my research, which restricts all lab experiments to *A. subfuscus* and *P. carolinianus*, and given the relatively low abundance of other slug species within forest sites, I used *A. subfuscus* numbers rather than total non-native numbers to analyze patterns of field association in subsequent spatial analysis.)

SADIE is a non-parametric method to determine the degree of spatial association between two count datasets. For a given dataset, SADIE calculates the minimum distance that individuals in a grid must move to reach regularity, i.e. the same number of individuals in each grid cell (Perry 1995, 1998). For each cell on a grid, SADIE calculates its cluster index, *z*, as the average inflow/outflow distance of individuals from that cell to reach overall regularity on the grid (Perry et al. 1999). The association χ_k between two sets of co-occurring populations on one grid cell, k, is measured as:

$$\chi_k = N\,(z_{k1} - q_1)(z_{k2} - q_2) \,/\, [\Sigma_k(z_{k1} - q_1)^2 \Sigma_k(z_{k2} - q_2)^2]^{1/2}$$

where z_{k1} is the cluster index of population 1 at cell k, z_{k2} is the cluster index of population 2 at cell k, $q_1$is the mean *z* of population 1, q_2 is the mean *z* of population 2, and *N* is the number of cells. The total spatial association for a grid, $X = \Sigma_k\chi_k/N$, of χ_k, is the average of all cells' individual standardized χ_k values. Significance of *X* is calculated by comparing the observed value of *X* against a distribution of random permutations of the z_k value among cells of the grid (Winder et al. 2001).

For most surveys, pairs of populations were neither associated nor disassociated but were randomly distributed with respect to one another (22 of 46 surveys total) (Table A.1). A positive association between species pairs was exhibited in a slightly smaller number of surveys (19 of 46 surveys). Species pairs were disassociated in only five surveys, three of which were between non-native slugs and *M. mutabilis*. Otherwise, similar proportions of each species pairing were randomly distributed or associated.

Given that more than half of slug populations were randomly associated, I can conclude that most slug populations did not have a distributional influence on each other. The high number of positive associations suggests that slugs are using the same resources, such as coarse woody debris or food (Chapter 2; Bohan *et al.* 2000). I attempted to eliminate the likeliest habitat factor responsible for slug population aggregations in order to retest for spatial (dis)associations between slug species pairs. My observations suggested that slugs were aggregated on CWD. In response, partial Mantel tests were conducted in the program, PASSaGE (Rosenberg 2001) to eliminate the factor of CWD by treating it as a covariate while reanalyzing associations between populations (Chapter 1).

Note that SADIE and PASSaGE results should not be compared directly. The PASSaGE analysis takes into account CWD as a factor, several datasets were eliminated because autocorrelation could not be removed, and other adjustments were made to the underlying datasets.

Table A.1 Overall association *X* of slug species pairs at each site. Light gray boxes indicate a significant positive association ($P < 0.025$) and dark gray boxes indicate a significant negative association ($P > 0.975$) under a two-tailed distribution. NN are non-native slugs, Pc are *P. carolinianus*, and Mm are *M. mutabilis*. Blank cells represent surveys for which population sizes were too small to conduct statistical analyses.

	Site A			Site B			Site C		
Species Pairs	NN + Pc	NN + Mm	Pc + Mm	NN + Pc	NN + Mm	Pc + Mm	NN + Pc	NN + Mm	Pc + Mm
	Survey 1			Survey 1			Survey 1		
X	0.4947	0.4582	0.3831	0.3168	0.4954	0.413	0.1954	0.2694	0.1275
P	<0.0001	<0.0001	0.0014	0.004	<0.0001	0.0005	0.0513	0.0134	0.1385
	Survey 2			Survey 2			Survey 2		
X	0.4658	-0.2878	-0.4015	0.0335	-0.5671	0.246	0.1836	-0.0916	0.1598
P	0.0001	0.989	0.9997	0.3898	>.9999	0.0165	0.0703	0.7416	0.1644
	Survey 3-6			Survey 3-6			Survey 3-6		
X	0.2855			0.2633	0.1863	0.6528	0.1092	0.0886	0.7165
P	0.0379			0.0133	0.0608	<0.0001	0.211	0.2508	<0.0001
	Survey 7			Survey 7			Survey 7		
X	0.2372	0.1672	0.1004	0.3729	0.1395	0.2852	-0.081		
P	0.0813	0.0792	0.1932	0.0015	0.1582	0.02	0.7469		
	Survey 8			Survey 8			Survey 8		
X	0.1600	0.3217	0.0351	0.3118	0.3956	0.0776	-0.4006	-0.2999	0.1065
P	0.0971	0.0079	0.3792	0.0107	0.0002	0.2699	0.9993	0.9903	0.1934
	Survey 9			Survey 9			Survey 9		
X	-0.1773			0.2085	-0.26	0.2555	0.3123		
P	0.9338			0.0381	0.9745	0.0167	0.0067		

Section A.IV.:

Field survey datasets used in spatial analysis through PASSaGE

Table A.2 This table details the original field survey datasets and whether they were kept in their original form, rescaled, or dropped from analysis. For explanations of these dataset types, see Chapter 1 or Appendix B, Section V.

O = original dataset used

2 = rescaled to lag 2

5 = rescaled to lag 5

X = dataset unusable.

Survey number	Site	*A. subfuscus*	*M. mutabilis*	*P. carolinianus*
1	A	X	2	2
1	B	X	X	X
1	C	X	2	2
2	A	O	O	O
2	B	5	5	5
2	C	O	O	O
3	A	5	X	5
3	B	X	X	X
3	C	O	O	O
4	A	X	X	X
4	B	O	O	O
4	C	X	X	X
5	A	O	X	O
5	B	2	X	2
5	C	O	O	O
6	A	X	X	X
6	B	X	X	X
6	C	X	X	X
7	A	O	X	O
7	B	5	5	5
7	C	2	X	2
8	A	O	O	O
8	B	5	5	5
8	C	O	O	O
9	A	X	X	X
9	B	2	X	2
9	C	X	X	X

Section A.V.:

Spatial associations of species pairs obtained through PASSaGE

Table A.3 Statistical details are shown of the partial Mantel tests of the association between species pairs' abundances, with coarse woody debris treated as a covariate. Most datasets were analyzed at their original scale (75 cells; lag =1 cells), while others exhibiting spatial autocorrelation were rescaled to a coarser grain of 12 cells (lag = 2 cells), or rescaled to an even coarser grain of three cells (lag = 5 cells) and combined across sites and surveys. A left-tailed $P < 0.05$ and $r < 0$ indicates a significant negative association, while a right-tailed $P < 0.05$ and $r > 0$ indicates a significant positive association between the abundance datasets. (* shows a significant *P*.)

Survey number	Site	Number of cells	Species pair	correlation *r*	left-tailed *P*	right-tailed *P*
2	A	75	As and Pc	0.1735	0.91111	0.08899
5	A	75	As and Pc	0.0214	0.88871	0.11139
7	A	75	As and Pc	1.0000	1.00000	*0.00010
8	A	75	As and Pc	0.0869	0.76592	0.23418
4	B	75	As and Pc	-0.0540	0.14459	0.85551
5	B	12	As and Pc	-0.1807	0.09249	0.90761
9	B	12	As and Pc	0.2772	0.90041	0.09969
2	C	75	As and Pc	0.2580	0.98340	*0.01670
3	C	75	As and Pc	0.3827	0.96500	*0.03510
5	C	75	As and Pc	0.1084	0.87861	0.12149
7	C	12	As and Pc	-0.1131	0.38336	0.61674
8	C	75	As and Pc	-0.0534	0.20588	0.79422
	all	12	As and Pc	0.5321	0.99490	*0.00520
2	A	75	As and Mm	-0.0452	0.55024	0.44986
8	A	75	As and Mm	0.3450	0.98580	*0.01430
4	B	75	As and Mm	-0.0349	0.37186	0.62824

2	C	75	As and Mm	-0.0628	0.25097	0.74913
3	C	75	As and Mm	-0.0278	0.37506	0.62504
5	C	75	As and Mm	0.0948	0.88671	0.11339
8	C	75	As and Mm	0.0362	0.90431	0.09579
	all	9	As and Mm	-0.1089	0.23708	0.76302
1	A	12	Mm and Pc	0.2361	0.85691	0.14319
2	A	75	Mm and Pc	-0.0354	0.63924	0.36086
8	A	75	Mm and Pc	0.4920	0.98240	*0.01770
4	B	75	Mm and Pc	-0.0301	0.63404	0.36606
1	C	12	Mm and Pc	0.2431	0.84972	0.15038
2	C	75	Mm and Pc	-0.0179	0.64224	0.35786
3	C	75	Mm and Pc	0.4718	0.98880	*0.01130
5	C	75	Mm and Pc	0.5095	0.99570	*0.00440
8	C	75	Mm and Pc	0.0996	0.88531	0.11479
	all	12	Mm and Pc	-0.0175	0.60484	0.39526

APPENDIX B

Diets of *A. subfuscus, M. mutabilis,* and *P. carolinianus* through the year

In this section, the diet of slugs is compared between two periods of the year to test whether season was likely to have altered slug feeding habits and therefore diet overlap between slug species pairs. This is a supplementary test to provide some basis for the assumptions in Chapter 2 that diet is fairly consistent through time and therefore that the calculated overlap statistics are meaningful.

Gastropod diets often vary during the course of a year. Changes in food quality, food availability, and gastropod nutritional requirements cause seasonal variation in foods consumed (Speiser and Rowell-Rahier 1991). Jennings and Barkham (1975) observed seasonal changes in the proportions of fungus and animal matter consumed by some slug species, possibly in response to availability of food (Beyer and Saari 1978). Herbivorous gastropods often shift between live and senescent plant material, or among plant species, depending on the allelochemical content of their food plants (Chatfield 1976; Richter 1976; Hatziioannou et al. 1994; Hägele and Rahier 2001). Unfavorable weather may force slugs to remain close to the ground when feeding and to forgo their preferred foods (Jennings and Barkham 1975). However, Hägele and Rahier (2001) noted that *Arianta arbustorum* consumed a consistent proportion of senescent plants across their field season. For my dataset, I sought to answer the question, do proportions of food consumed by the slugs *A. subfuscus, M. mutabilis,* and *P. carolinianus* vary between the late spring and early summer? A shift would indicate that feeding niche dimensions for these slugs vary during the course of the year.

In order to determine whether the time of year affects the diets of slugs, I conducted a regression analysis to compare relative volumes of each food type between fecal surveys taken during the two periods of time. See Chapter 2 for methods in quantifying fecal material types on a grid. A regression analysis in SAS treated each food type (algae, fungus, plant, wood, minerals, soil, exoskeleton, and "others") as one data point, with the average fecal volume per slug at period 1 (May 4^{th} – June 5^{th}) on the x-axis and the volume at time 2 (June 12^{th} – July 12^{th}) on the y-axis. The volumes were non-normally distributed, and so they were log-transformed. A separate regression was performed for each species.

Amounts were proportionately similar among food types between the two time periods. *A. subfuscus* and *M. mutabilis* each showed a significant association between food type proportions (*A. subfuscus*, regression: $F = 9.01$, $P = 0.0239$, $R^2 = 0.6003$; *M. mutabilis*, regression: $F = 6.35$, $P = 0.0452$, $R^2 = 0.5143$) (Fig. B.1). Food types consumed by *P. carolinianus* were not quite significantly proportionate between time periods (regression: $F = 3.77$, $P = 0.1002$; $R^2 = 0.3859$). *P. carolinianus* feces mostly consisted of fungus, of which amounts were similar (174 vs. 193 boxes per grid) between the time periods 1 and 2, while the amount of "other" foods (19 vs. 0 boxes) and algae (10 vs. 1) were greater in time period 1 than 2.

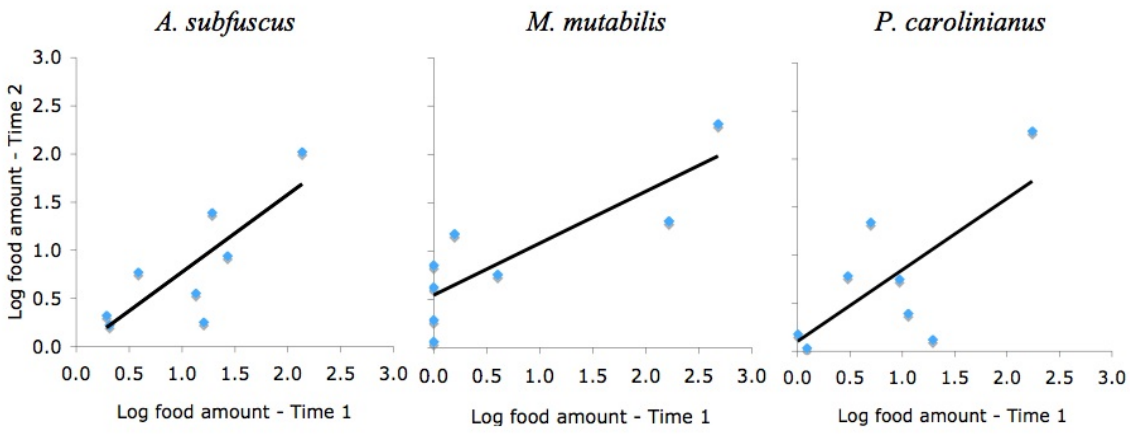


Fig. B.1 Amounts of each food type in feces collected during time period 1 (May 4th-June 5th) vs. time period 2 (June 12th – July 12th) for each slug species, to determine if foods consumed change through time. Food amounts are log-transformed volume units on a microgrid (see Chapter 2). Each point represents one of eight food types, i.e. algae, fungus, plant, wood, minerals, soil, exoskeleton, and other.

Thus, dietary proportions did not differ greatly for these slug species between the two time periods, even though they encompass a growing period during which plant and fungus availability may differ. Only *P. carolinianus* showed a marginally non-significant association of food amounts between time periods. (Given that the main diet component, fungus, remained in similar proportions in the feces and thus probably was not lacking in availability, *P. carolinianus* may have consumed more algae and "other" foods earlier in the year in order to obtain micronutrients (Speiser and Rowell-Rahier 1991).) These results contrast with evidence of seasonal variation in feeding behavior in other gastropods (Jennings and Barkham 1975; Beyer and Saari 1978; Speiser and Rowell-Rahier 1991), but they are in accord with data indicating that there is a seasonal consistency in senescent plant consumption by *Arianta arbustorum* (Hägele and Rahier

2001). Slugs might consume significantly different foods at other times of year not investigated, e.g. in the fall, if food availability and quality changes drastically. However, many gastropods exhibit enduring preferences among food species (Cates and Orians 1975, Molgaard 1986, Speiser 2001). Perhaps the feeding preferences of *A. subfuscus*, *M. mutabilis*, and *P. carolinianus* drive them to consistency.

APPENDIX C

Tables of ANCOVA statistics for each experiment manipulating species presence and resource level

Experiment 1: Limiting Resources

Table C.1 ANCOVA analysis of fitness of *P. carolinianus* and *A. subfuscus* slugs under various levels of food and shelter. Treatments were single-species. Interactions were not significant and so are not shown. Num *df* is numerator degrees of freedom, and Den *df* is denominator degrees of freedom. (* indicates significant *P*-value.)

Fitness measures	Independent factor	Num *df*	Den *df*	*F*	*P*
P. carolinianus					
Average mass change per day	Food	2	80	27.22	*<0.0001
	Shelter	2	80	0.59	0.5572
Final mass	Food	2	80	70.71	*0.0001
	Shelter	2	80	0.54	0.5850
Final mass plus egg laying mass lost	Food	2	80	101.76	*0.0001
	Shelter	2	80	0.75	0.4739
Total eggs laid	Food	2	80	18.78	*0.0001
	Shelter	2	80	1.36	0.2634
A. subfuscus					
Average mass change per day	Food	2	35.6	8.82	*0.0008
	Shelter	2	35.4	0.21	0.8100
Final mass	Food	2	80	13.26	*0.0001
	Shelter	2	80	2.54	0.0854

Experiment 2: Mixed Species

Table C.2 ANCOVA analysis of the main effects (food, shelter, and mixed species) of Experiment 2 on *P. carolinianus* fitness. (* indicates significant *P*-value. The only significant interaction is shown. In this case, a main effects factor with a $P < 0.05$ was not marked as significant.)

Fitness measures	Independent factor	Num *df*	Den *df*	*F*	*P*
Average mass change per day	Food	1	41.4	31.78	*<0.0001
	Shelter	1	41.4	0.19	0.6678
	Mixed Species	1	41.3	0.72	0.4006
Final mass	Food	1	44.8	30.25	*<0.0001
	Shelter	1	44.8	0.00	0.9492
	Mixed Species	1	44.7	2.26	0.1400
Final mass plus egg laying mass lost	Food	1	42.3	52.01	*<0.0001
	Shelter	1	42.3	1.66	0.2049
	Mixed Species	1	42.2	4.32	*0.0439
Total eggs laid	Food	1	33.8	16.43	*0.0003
	Shelter	1	33.7	7.52	*0.0097
	Mixed Species	1	33.7	1.76	0.1938
Count of slugs dead	Food	1	48	4.10	0.0484
	Shelter	1	48	0.92	0.3422
	Mixed Species	1	48	0.36	0.5487
	Shelter x Food	1	48	4.10	*0.0484
Distances between Pc and As	Food	1	31	1.20	0.2825
	Shelter	1	31	2.11	0.1567

Experiment 3: Shelter and Moisture

Table C.3 ANCOVA analysis of the main effects (shelter, moisture, and mixed species) of the shelter moisture experiment on *P. carolinianus* and *A. subfuscus* fitness. (* indicates significant *P*-value. No interactions were significant except for the two shown. In these cases of interaction, main effects factors for which $P < 0.05$ were not marked as significant.)

Fitness measures	Independent factor	Num *df*	Den *df*	*F*	*P*
P. carolinianus					
Average mass change per day	Shelter	1	31.9	0.02	0.8804
	Moisture	1	31.9	7.68	*0.0092
	Mixed Species	1	31.2	0.05	0.8271
Final mass	Shelter	1	27.2	2.42	0.1314
	Moisture	1	27.2	1.83	0.1874
	Mixed Species	1	26.5	0.16	0.6949
Final mass plus egg laying mass lost	Shelter	1	27.9	5.04	*0.0328
	Moisture	1	27.8	0.41	0.5273
	Mixed Species	1	27.2	0.21	0.6491
Total eggs laid	Shelter	1	24.6	2.28	0.1440
	Moisture	1	24.6	0.87	0.3613
	Mixed Species	1	24.2	1.74	0.1982
Count of slugs dead	Shelter	1	24	3.88	0.0604
	Moisture	1	24	12.83	0.0015
	Mixed Species	1	24	0.80	0.3793

	Shelter x Moisture	1	24	4.62	*0.0419
A. subfuscus					
Average mass change per day	Shelter	1	26.8	13.80	*0.0009
	Moisture	1	26.8	1.38	0.2504
	Mixed Species	1	26.8	1.61	0.2157
Final mass	Shelter	1	26.3	13.61	*0.0010
	Moisture	1	26.3	0.77	0.3891
	Mixed Species	1	26.3	2.96	0.0972
Count of slugs dead	Shelter	1	24	1.86	0.1852
	Moisture	1	24	0.34	0.5643
	Mixed Species	1	24	0.04	0.8471
Distances between Pc and As	Shelter	1	11	29.11	0.0002
	Moisture	1	11	33.41	0.0001
	Shelter x Moisture	1	11	5.69	*0.0361

Experiment 4: Juveniles

Table C.4 ANCOVA analysis of the fitness of *P. carolinianus* juveniles occurring with different cohabitants, food levels, and shelter levels. (No interactions were significant. * indicates significant *P*-value.)

Fitness measures	Num *df*	Den *df*	*F*	*P*
Average mass change per day				
Food	1	58	2.90	0.0939
Shelter	1	58	0.03	0.8589
Cohabitants	3	58	4.35	*0.0078
Final mass				
Food	1	59	9.84	*0.0027
Shelter	1	59	0.00	0.9562
Cohabitants	3	59	6.57	*0.0007

APPENDIX D

Section D.I.:

Tables of t-statistics for exploitation and interference planned comparisons

Table D.1 Exploitation competition, tested through paired comparisons of the fitness of *P. carolinianus* slugs under high vs. low food and high vs. low shelter conditions. Mixed-species mesocosms containing two *P. carolinianus* and two *A. subfuscus* each were used. (* indicates significant *P*-value.)

Fitness measures	**Food: high vs. low**			**Shelter: high vs. low**		
	df	***t***	***P***	***df***	***t***	***P***
Average mass change per day	69.3	4.32	*<0.0001	69.3	0.53	0.6001
Final mass	68.7	4.06	*0.0001	68.7	-0.11	0.9136
Final mass plus egg laying mass lost	62.9	5.66	*<0.0001	62.9	1.64	0.1056
Total eggs laid	55.9	3.53	*0.0008	55.9	3.50	*0.0009
% Slugs dead	48	-1.78	0.0808	48	0.00	1.0000

Table D.2 Interference competition, measured as fitness of *P. carolinianus* slugs in *A. subfuscus* absence vs. *A. subfuscus* presence treatments, under high food or high shelter conditions. (* indicates significant *P*-value.)

Fitness measures	***A. subfuscus* presence vs. absence (high food)**			***A. subfuscus* presence vs. absence (high shelter)**		
	df	***t***	***P***	***df***	***t***	***P***
Average mass change per day	41.3	-0.79	0.4335	41.3	-0.80	0.4255
Final mass	44.7	-1.02	0.3140	44.7	-1.00	0.3206
Final mass plus egg laying mass lost	42.2	-1.67	0.1022	42.2	-2.10	*0.0420
Total eggs laid	33.7	-1.43	0.1613	33.7	-2.33	*0.0260
% Slugs dead	48	-0.35	0.7266	48	-1.11	0.2745

Section D.II.:

Response of slugs to the species identity of and food consumed by a mucus-producer

This experiment was a side project to determine whether *P. carolinianus* response (or non-response) to mucus depended on the species producing the mucus or the food it consumes. Although *P. carolinianus* remained consistently unaffected by mucus quality, these methods may be of interest to researchers investigating these phenomena in gastropod species that do respond to mucus.

INTRODUCTION

Gastropods depend on mucus to navigate through their environment and to communicate with other gastropods. Their locomotion is energetically expensive and causes major water loss (Machin 1978; Denny 1980). Perhaps to minimize the costs of locomotion, gastropods respond readily to environmental cues including mucus trails left by other gastropods (Chelazzi et al. 1988; Cook 1992). Slugs can save energy by following other slugs (Rollo and Wellington 1981) rather than by discovering and forging a new trail. Mucus also has roles in defense, competition, mating, homing, and other behaviors. *Deroceras laeve* avoid places smeared with the mucus of stressed conspecifics (Jordaens et al. 2003). Mucus serves as a competition mechanism that interferes with growth and activity within and among many gastropod species (Cameron and Carter 1979; Carter and Ashdown 1984; Pearce 1997). Many slug species exhibit a phase of courtship in which a slug follows a prospective partner's mucus trail (Reise 2007). Gastropods follow slime trails and orient toward chemical "beacons" deposited in shelters while they are homing

towards shelters (Peake 1978; Cook 1979). Large slugs tend to have daytime shelters that they recognize chemically and which groups of slugs share (Cook 1992).

Given that individuals most closely share the niche requirements of their own species (DeBach 1966; Reitz & Trumble 2002; Duyck *et al*. 2004), slugs would benefit from recognizing and most strongly responding to the mucus of conspecifics in order to follow them to food sources and to appropriate shelter. Presumably, those species that engage in trail following during courtship (Reise 2007) or that differentially respond to the mucus of conspecifics or heterospecifics during competitive interactions (Cameron & Carter 1979; Lee and Silliman 2006) recognize the species identity of the mucus-producer. However, the effect of food consumption on the chemical qualities of slug mucus is unknown. Perhaps, chemical traces of foods exuded in mucus would be an indicator of favorable microhabitats, i.e., shelter near preferred foods, regardless of the species identity of the mucus producer. However, previous studies did not evaluate whether the stimulus is an endogenous chemical produced specifically by each slug species or a chemical trace of attractive food consumed and exuded in the mucus.

Slugs may respond to the species producing the mucus, the food consumed by the mucus producer, or both. To determine whether *P. carolinianus*'s response to mucus depends on the food of the mucus-producer, I fed both *A. subfuscus* and *P. carolinianus* different foods and measured the amount of time *P. carolinianus* lingered in the presence of their mucus.

METHODS

In October, 2009, four *A. subfuscus* and four *P. carolinianus* adults were selected. Each slug was placed in its own 240 ml deli container with commercial white mushroom (two *P. carolinianus* and two *A. subfuscus*), dried wild fungus (two *P. carolinianus*), or lettuce (two *A. subfuscus*). Slugs were allowed to feed for at least 48 hours.

A circle of 5.0 cm diameter was drawn on the center of small Petri dishes about 9 cm in diameter. The food treatment slugs were allowed to crawl on the central circle of the dishes for 30 minutes, and afterwards, the deposited mucus was spread over the entire circle with a small spatula. The five treatments to which individual dishes were subjected were no mucus, mucus of *A. subfuscus* with lettuce food, mucus of *A. subfuscus* with fresh mushroom, mucus of *P. carolinianus* with dried mushroom, and mucus of *P. carolinianus* with fresh mushroom. Partly-grown *P. carolinianus* juveniles that had been raised from eggs were the subjects to be exposed to the mucus. All had fed on a diet of store-bought white mushrooms for at least 48 hours before the experiment began. A total of 32 mucus-exposure trials per treatment took place over two separate days. Each juvenile subject was placed in the center of a circle, and 600 seconds were allowed to pass. I recorded for each slug when its tentacles protruded from the circle ("head out") and when the tail tip left the circle ("tail out"). Subjects underwent several trials with dish treatment assigned randomly. Each dish was reused four times, which did not affect the outcome of the experiment. (The results were the same if the first or all trials were considered.)

A similar experiment was attempted for *A. subfuscus*, but subjects became immobile after a few sessions and no more trials could take place. *A. subfuscus* responded

to repeated contact with forceps by compressing their bodies to the substrate, a defensive posture (Rollo and Wellington 1979), while *P. carolinianus* seemed to react less to the same contact.

Two one-way ANOVAs were performed in SAS (SAS Institute Inc. 2008). For both ANOVAs, mucus treatment was the independent variable, and the time until "head out" or "tail out" was the dependent variable. Slugs that did not move within 600 seconds were excluded from the analysis.

RESULTS

The average time for "heads out" did not differ among treatments (1-way ANOVA: $F_{4,71} = 0.136$, $P = 0.968$) (Fig. D.1). Also, the average time for "tails out" did not vary among treatments (1-way ANOVA: $F_{4,68} = 1.184$, $P = 0.3265$).

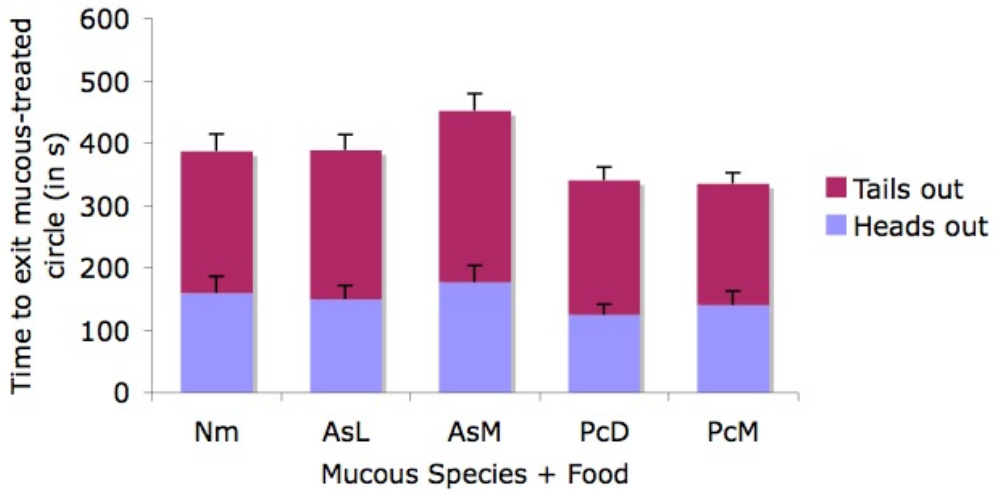


Fig. D.1 The average time for each juvenile *P. carolinianus* slug's tentacles and tail tip to leave a mucus-treated circle. Treatments were no mucus (Nm), *A. subfuscus* with lettuce food (AsL), *A. subfuscus* with fresh mushroom (AsM), *P. carolinianus* with dried mushroom (PcD), and *P. carolinianus* with fresh mushroom (PcM). Bars indicate average time +SE.

DISCUSSION

P. carolinianus did not differ significantly in their responses to the identity of the mucus-producer or the foods consumed by the mucus-producer. However, the time for "heads out" and "tails out" was highly variable among individual slugs, which may have masked slight trends in mucus preferences.

Perhaps *P. carolinianus* does not respond to mucus of any type, treatments were not composed of equivalent offerings, or smearing the mucus may have altered the qualities of the mucus. Mucus contains chemicals that can act as social cues, enabling other gastropods to find partners for mating, to find home shelters, to avoid predators, and to engage in territoriality or growth suppression (Cameron and Carter 1979; Carter

and Ashdown 1984; Pearce 1997; Lee and Silliman 2006; Reise 2007). Unlike slugs such as *Lehmannia valentiana* and several *Limax* species (Cook 1981; South 1992), *P. carolinianus* is not known to seek out huddles of other slugs (Thompson et al. 2006), although they do aggregate in moist crannies in dead wood. *P. carolinianus* does not appear to notice the presence of other slugs, but rather, randomly selects pieces of shelter wood regardless of the number of conspecifics occupying it (Tim Pearce and Cagin Unal, unpub. results). Mucus was not shown to be an interference mechanism within *P. carolinianus* or between *P. carolinianus* and *A. subfuscus* (Chapter 4). No one has investigated mucus as a homing mechanism or as a part of the courtship process in philomycid slugs. The social aspects of mucus may not be as important to *P. carolinianus* as to other species. A similar experiment should be attempted for another species, e.g. *Limax maximus* or *D. reticulatum*, for which several social functions of mucus have been discovered, to test whether the species of the mucus-producer, its food consumed, or both determine slug response to mucus.

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