

Prior occupation by scirtid beetles does not affect mosquito and midge populations in treeholes

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The primary resource of temperate forest treeholes is leaf litter, which exists along a continuum of decay states, with different insects specializing on particular states. Scirtid beetles (*Helodes pulchella*) take part in a processing chain interaction by shredding and processing leaf litter, thereby creating material for consumption by other detritivores. Variation in scirtid density and resource availability could thereby influence the distribution of resources and thus the abundance of the dominant eastern treehole mosquito *Aedes triseriatus*. In addition, pupated or dead scirtid beetles typically leave behind processed or semi-processed leaf litter, particularly if the numbers of beetles is large, which potentially impacts insect communities after the beetles are gone. We tested the hypothesis that prior presence of scirtids has observable effects on the abundance of treehole mosquito populations. We used a two-factor fully crossed design (3 leaf litter levels × 3 scirtid densities) with 4 replicate mesocosms per treatment to monitor larval insect abundance from April 2004 to June 2005. Scirtids did not persist in mesocosms to the first census of 2005. Abundance of *A. triseriatus* was unaffected in 2005 by either initial scirtid density or leaf litter availability. In addition, we detected no statistically significant effects of scirtids on the rate of leaf litter decay.

Introduction

Allochthonous plant material represents the major input of energetic resources for many freshwater detritus-based habitats (Petersen & Cummins 1974, Fish & Carpenter 1982, Walker *et al.* 1997, Wallace *et al.* 1997). In the case of water-filled treeholes, energetic inputs can be highly heterogeneous (Kitching 2000, Paradise

2004), and availability of resources to consumers may be dependent upon processing chain interactions (Paradise & Dunson 1997, Paradise 1999, Daugherty & Juliano 2002). Processing chains comprise at least two species, one of which modifies a resource for another. Facilitative effects, such as processing chains, may play a large role in structuring communities and have been hypothesized to affect diversity and

individual populations (Hacker & Gaines 1997, Jones *et al.* 1997, Bruno *et al.* 2003).

The distribution of resources can be altered by chemical, physical, or biological processing mechanisms (Heard 1994a). This alteration may benefit consumers that specialize on a more advanced decay state (Heard 1994a). As leaf litter decomposes, the size distribution of particulate organic matter changes. The scirtid beetle *Helodes pulchella* (Guerin) is a common resident of treehole communities in the southeastern U.S. and is a leaf shredder (Barrera 1996, Paradise & Dunson 1997, Paradise 2004). When processing leaf litter, shredders often do not consume all they shred, and this can increase particulate and dissolved organic matter (Daugherty & Julian 2002). Commensalism results as smaller particles are made available to microorganisms, filter-feeders, and browsers through the actions of the processor (Heard 1994b, Paradise & Dunson 1997, Paradise 1999, Daugherty & Juliano 2002). Changes in resources could remain long after processors have left the habitat. Thus, the activity of scirtid beetles potentially increases abundance of larval populations over the long-term by increasing resource heterogeneity, in terms of particle sizes and microorganism abundance (Naeem 1990). Fecal production by scirtids or decaying scirtid carcasses also could increase resources for other consumers (Daugherty *et al.* 2000, Daugherty & Juliano 2003). Processing chain interactions have been reported in many aquatic habitats, including streams, pitcher plants, *Heliconia* bracts, carcasses, treeholes, and bromeliads (Seifert & Seifert 1979, Bradshaw 1983, Naeem 1990, Heard 1994a, 1994b, Dieterich *et al.* 1997, Paradise & Dunson 1997, Paradise 1999, Jonsson & Malmqvist 2005, Srivastava 2006). The effects of processors on consumer populations after processors are absent may be extensive, yet have not been examined.

Southeastern U.S. treeholes are dominated numerically by larvae of the eastern treehole mosquito *Aedes triseriatus* (Say) (Lounibos 1983, Harlan & Paradise 2006), a filter-feeder and browser that consumes small particulate matter and microorganisms (Merritt *et al.* 1992). Other common detritivores include the ceratopogonid midge *Culicoides guttipennis* (Coquillett), the syrphid fly *Mallota posticata* (Fabricius),

and the psychodid fly *Telmatoctopus albipunctatus* (Williston). In the laboratory, scirtid beetles facilitate both *A. triseriatus* and *C. guttipennis* when resources are limiting (Paradise & Dunson 1997, Paradise 1999, Paradise 2000). Although these experiments showed that scirtids increase the growth, mass, or survival of consumers, no studies have examined the long term effects of scirtid absence on consumer populations.

We hypothesized that the effects of scirtid processing would continue in the absence of scirtids, and would result in increases in abundance of the two common larval species *A. triseriatus* and *C. guttipennis*. We specifically hypothesized a greater effect on these consumers when resources were limiting and scirtids densities had been low as compared with when scirtids were never present or very abundant. High densities of scirtids would reduce the resources available for other consumers (Heard 1994a). We further hypothesized that these effects would remain even after scirtids were gone if resources were not renewed. To test hypotheses about processing chain interactions, we conducted a field mesocosm experiment in which leaf litter and scirtid beetle densities were independently varied. Artificial mesocosms were stocked with different densities of scirtid beetles and monitored for two years. During the first year, mesocosms with low densities of scirtids produced larger mosquito pupae and had higher species richness during part of the year. The abundance of the midge *C. guttipennis* was lower in the presence of scirtids (Burkhart *et al.* 2007). Mesocosms remained open to colonization for two field seasons; the second season allowed us to determine the effects of scirtid absence on the two most numerically abundant consumers, *A. triseriatus* and *C. guttipennis*.

Materials and methods

We cut 7.62 cm internal diameter PVC pipe into 11 cm lengths to create mesocosms with a total capacity of approximately 540 ml. Fiberglass window screening was affixed to the pipe inside by overlapping it beyond the two ends. The screen created a textured inner surface and darkened the interior to promote insect oviposi-

tion and allow scirtids to crawl to the top for air. We sealed the end caps that held screens with silicon caulk. Half of a PVC coupling was placed over the top and caulked to seal the overlapping screen. We attached mesocosms in pairs to a frame with expandable polyurethane foam (Great Stuff, Dow Chemical Co.). Frames were approximately 50 cm wide by 35 cm high by 25 cm deep and were constructed of 1.5 cm PVC pipe. We flushed mesocosms with distilled water several times in the laboratory over three weeks to remove any volatile chemicals.

The frames were tied to trees approximately one meter off the ground in a hardwood forest on the Davidson College Ecological Preserve (DCEP, Davidson, North Carolina; 35°30'37''N, 80°49'48''W) in September 2003. Fiberglass window screen (2 mm mesh) was glued to the top of each frame to reduce the amount and size of debris that entered mesocosms. Finally, each frame and the trunk of its tree were wrapped in 2.5 cm mesh chicken wire to exclude most vertebrates, and a hinged door was cut into the cage to allow access (Paradise 2006).

We randomly assigned one of ten treatment combinations to each mesocosm. We used three levels of leaf litter crossed with three levels of scirtid density, all of which were within the ranges found in treeholes (Paradise 2004, Paradise, unpublished data), with four replicates of each treatment combination. Leaf litter levels were 1, 5, and 10 g of dried red oak (*Quercus rubra* L.) leaves l⁻¹ (hereafter low, intermediate, and high, respectively). We collected medium-sized scirtid larvae (mostly 2nd instar) from a large basal treehole present on the DCEP and added them at three densities: 0, 26, and 100 individuals l⁻¹ (hereafter none, low, and high, respectively). The tenth treatment consisted of four mesocosms supplied only with water, giving a total of 40 mesocosms. The latter treatment was used to estimate debris accumulation, which was limited to dust and small particles that could fit through the 2 mm mesh covering the cages.

We collected senescent leaves of red oak (*Q. rubra*) from the forest floor in September 2003. They were brought back to the laboratory, dried at 60 °C, and weighed as whole or nearly whole leaves to achieve the mass necessary for each treatment. Leaves were added to mesocosms in

early October 2003, along with a small aliquot of filtered treehole water and ~500 ml of distilled water. Mesocosms were then covered with plastic sheeting held in place with cable ties to prevent oviposition. In late October 2003, scirtids were added at the appropriate densities. Mesocosms were covered with no-see-um netting (0.5 mm mesh), which allowed for gas exchange between the water column and the atmosphere but prevented colonization by insects. The mesocosms remained in this state until early March 2004, when they were opened for colonization.

In early April 2004 we began monitoring colonization of insect larvae. Mesocosms were sampled every two to three weeks to ascertain scirtid survival, maintain constant water levels, and eliminate any larvae of the predator *Toxorhynchites rutilus*, as we were interested only in bottom-up effects. We performed three complete censuses in 2004 (see Burkhart et al. 2007, for complete 2004 results) and in 2005, which involved removing all water and leaf litter from each mesocosm using basters and forceps. All leaves were individually examined for insect larvae. The water was poured into large enamel pans, and carefully examined for insect larvae. All larvae were identified to the lowest taxonomic level possible and counted. All other material, except for *T. rutilus* larvae, was returned to its original mesocosm.

We terminated the experiment in June 2005 after the final census was completed. Because of an early warm spell, this last census was late enough in the field season to capture the first generation of mosquito and midge larvae. We brought all leaf litter and coarse particulate matter back to the laboratory. Unable to determine decay of leaves during the experiment, we used this method to assess leaf litter condition. Debris that had fallen into mesocosms during the experiment was < 1 mm in size, as determined by analysis of material that collected in the four mesocosms without leaf litter. We rinsed leaf litter from other mesocosms through a #35 sieve (0.5 mm mesh size), which allowed us to estimate the loss of mass from the initial stock.

We determined percent dominance and density of the two most common insect larvae (the mosquito *A. triseriatus* and the midge *C. guttipennis*). Percent dominance was calculated as

abundance of most common species divided by total insect abundance, multiplied by 100. We used profile analysis (von Ende 2001) to test the hypothesis that prior presence of scirtids affects treehole insect communities. Profile analysis is a statistical repeated-measures technique in which differences and averages at consecutive time points become transformed variables in two-way multivariate analyses of variance (MANOVAs). The differences are used to test for main effect by time interactions, and averages are used to test for main effects. Profile analysis allowed us to compare responses of any one variable over time without the variance problems associated with repeated measures ANOVAs that use time as a factor (von Ende 2001). The log transformed densities of mosquito and *C. guttipennis* larvae and percent dominance were our response variables, and leaf litter level and initial scirtid densities were fixed effects. Tests for interactions between time and other factors were done by comparing differences at sequential sampling points, while tests for main effects were performed by using the averages of successive time points. We adjusted α to 0.0167 for the profile analyses (experiment-wise α of 0.05/3 profile analyses) to reduce the probability of Type 1 errors. All data were tested for univariate normality and homoscedasticity, and densities were log-transformed prior to transforming consecutive averages and differences to achieve normality.

Results

By September 2004, scirtids had declined in most mesocosms due to pupation or mortality (Burkhart *et al.* 2007). Decline in scirtid densities was highest at low resources and high scirtid densities (100 l^{-1}). By February 2005, only a few scirtids remained in some high scirtid-high leaf litter mesocosms, but none remained in any other treatment combination. This high mortality in remaining scirtid populations may have been caused by a solid freeze in early January. By April 2005, scirtids were absent from all mesocosms.

Cumulatively, we found larvae or pupae of eight dipteran species. Species observed

belonged to the families Culicidae (mosquitoes), Ceratopogonidae (biting midges), Psychodidae (moth flies), Syrphidae (hover flies), and Dolichopodidae (long-legged flies). Mosquito species included *A. triseriatus*, *A. albopictus*, and *T. rutilus*. To determine the density of non-predatory mosquitoes, we counted mosquitoes as a single genus (*Aedes*) unless we positively identified more than one species (e.g., early instar *A. triseriatus* and *A. albopictus* are difficult to distinguish). However, over 90% of mosquito pupae and adults collected the previous year from these mesocosms and from natural treeholes in the DCEP were positively identified as *A. triseriatus*.

We found no evidence of any effects of scirtid carryover or leaf litter effects on mosquito densities or dominance (Table 1 and Fig. 1a–c). In 2005, 94.9% of all larvae consisted of mosquitoes, thus dominating the insect communities across all treatment combinations. Mosquito larvae (not counting *T. rutilus*) were found in 93.5% of all counts, including early first and second instar larvae observed in late February 2005, which was caused by a large, synchronous hatch of eggs during a warm period in February.

We found no effects of past scirtid presence or leaf litter on midge densities in 2005 censuses (Table 1 and Fig. 1d–f). *Culicoides guttipennis* passes through at least two generations per year in North Carolina and the final generation of the season overwinters as larvae (Paradise pers. obs.). We found *C. guttipennis* in 17.6% of 2005 counts, which was much lower than the 86.7% of the previous year, indicating that the harsh winter may have affected this species along with scirtids. This midge made up 9.8% of all larvae as compared with 28.7% the previous year (Burkhart *et al.* 2007).

Mesocosms typically contained between one and four insect species at any time; we never observed any mesocosm that contained more than 40% of the regional species pool (Harlan & Paradise 2006). The median local species richness was 1, and 85.2% of the time mesocosms contained one or two species. Treatment effects on other species also were not evident, although statistics were not performed on individual species due to low densities. The psychodid *T. albipunctatus* and the syrphid *M. posticata* were

found in 11.1% and 2.8% of counts, respectively, and neither appeared until the final census in June. Densities of these larvae in June were low (1.4 ± 0.6 (SE) l^{-1} for *T. albipunctatus* and 0.5 ± 0.4 (SE) l^{-1} for *M. posticata*). There was no discernible pattern of occurrence across scirtid densities. Other larvae belonging to the families Dolichopodidae (*Systemus* sp.) and Syrphidae (unknown species) also were observed, all in less than 5% of counts.

Discussion

The disappearance of scirtids allowed us to examine the effects of prior scirtid presence on treehole communities. This is an important consideration because the dynamic nature of treehole metacommunities suggests that larval populations in a local community may not persist for long periods of time (Kitching & Beaver 1990, Paradise 2004). Ecological theory suggests that processing chains may play a large role in structuring communities (Heard 1994a, Bruno et al. 2003). As leaf litter decays in treeholes, it changes from coarse to fine particles. Shredders can facilitate this change (Daugherty & Juliano 2002, 2003), making resources available to other detritivores. Strong positive effects on

other consumers are likely when resources are limiting and scirtids have been processing leaf litter resources (Heard 1994a, Paradise 1999). The foraging activity of scirtids could decrease dominance and increase consumer diversity by increasing the heterogeneity of resources (Naeem 1990, Heard 1994b), and this heterogeneity may persist even after a shredder population is gone. We could not determine the fate of the scirtids, but their presence in mesocosms for almost one year suggests that they may have had an effect on resource decay. While the loss of scirtids led to no scirtid density effect on the proportion of leaf mass lost (Burkhart et al. 2007), it is likely that their activity facilitated growth of microbes, on which other consumers, especially mosquitoes, feed (Kaufman et al. 2001). Based on the disappearance of late instar scirtid larvae in fall 2004 and the absence of observed carcasses, we suspect that most scirtids did, in fact, leave mesocosms to pupate. This indicates that scirtid carcasses were not available to decay and provide resources to the community.

We predicted positive, facilitative impacts of scirtid beetles on treehole consumer populations to last beyond the time when scirtids were present. Dominance was predicted to decrease with either increasing resources or facilitative effects of a processor (Hacker & Gaines 1997).

Table 1. Statistical results of profile analysis MANOVAs on censuses from 2004. Densities were log-transformed. Scirtid = scirtid density.

Response variable	Source of variation	Wilk's λ	df	p
Log mosquito density	Leaf litter	0.85	4, 52	0.38
	Scirtid	0.78	4, 52	0.16
	Leaf litter \times Scirtid	0.65	8, 52	0.16
	Leaf litter \times Date	0.92	4, 52	0.73
	Scirtid \times Date	0.82	4, 52	0.25
	Leaf litter density \times Scirtid \times Date	0.82	8, 52	0.70
Dominance	Leaf litter	0.81	4, 52	0.25
	Scirtid	0.76	4, 52	0.12
	Leaf litter \times Scirtid	0.64	8, 52	0.15
	Leaf litter \times Date	0.98	4, 52	0.98
	Scirtid \times Date	0.83	4, 52	0.31
	LL \times Scirtid \times Date	0.77	8, 52	0.51
Log <i>C. guttipennis</i> density	Leaf litter	0.80	4, 52	0.21
	Scirtid	0.81	4, 52	0.24
	Leaf litter \times Scirtid	0.66	8, 52	0.19
	Leaf litter \times Date	0.77	4, 52	0.14
	Scirtid \times Date	0.89	4, 52	0.55
	Leaf litter density \times Scirtid \times Date	0.83	8, 52	0.74

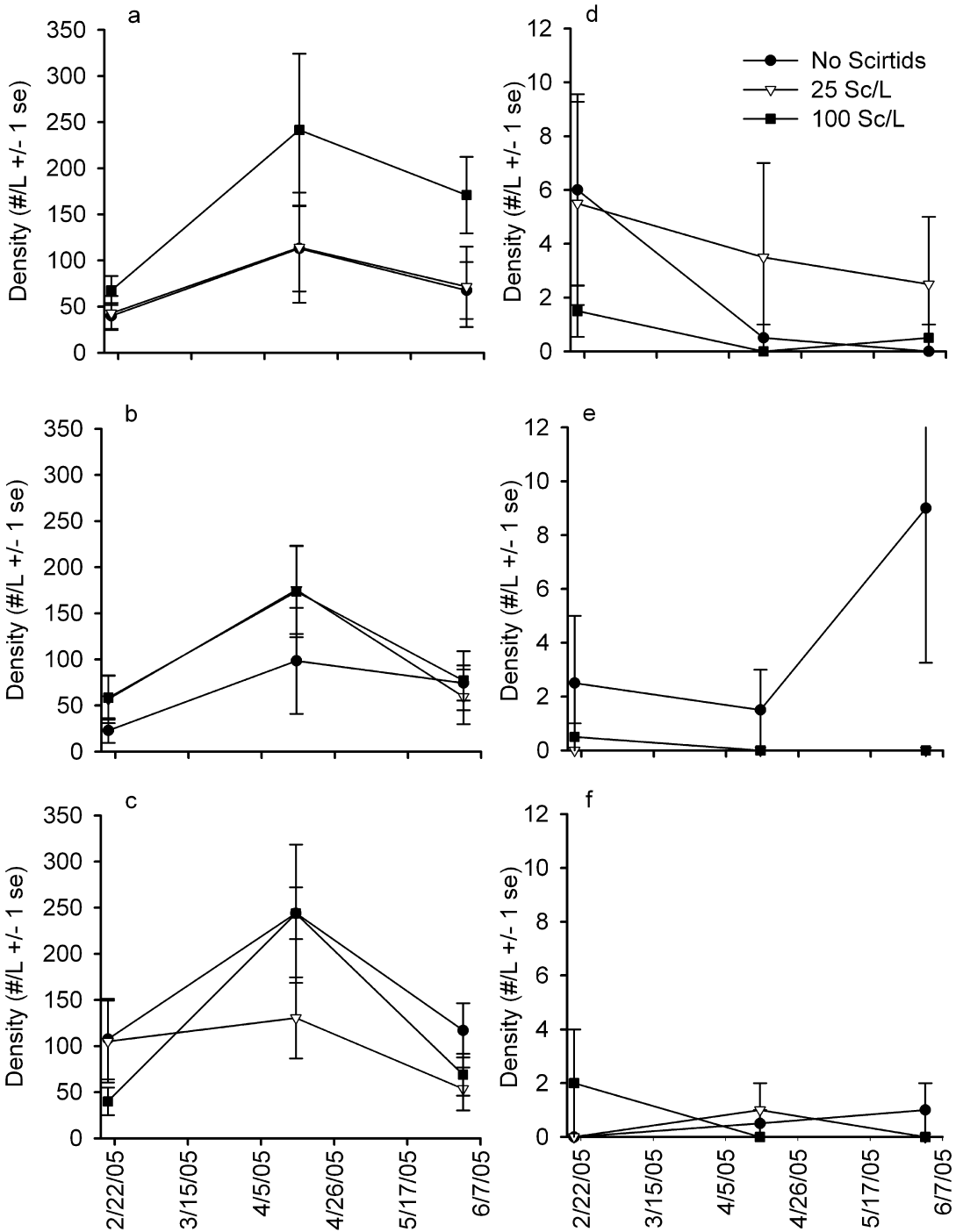


Fig. 1. Mosquito (*A. triseriatus* and *A. albopictus*) and midge (*C. guttipennis*) densities over time in each of nine treatment combinations. Because *A. triseriatus* and *A. albopictus* are difficult to identify to species in early instars they were pooled. — **a:** Mosquito densities in low leaf litter mesocosms. — **b:** Mosquito densities in intermediate leaf litter mesocosms. — **c:** Mosquito densities in high leaf litter mesocosms. — **d:** Midge densities in low leaf litter mesocosms. — **e:** Midge densities in intermediate leaf litter mesocosms. — **f:** Midge densities in high leaf litter mesocosms. Sample size equals 4 for each mean at each time point.

Facilitative impacts should be stronger under conditions of low resources and low processor density — facilitation is most likely to occur when resources are limiting and efficiency of processing is low due to low density of shredders (Heard 1994a, 1994b, Hacker & Gaines 1997). We demonstrate that there were no effects of past occupation by scirtids on densities or dominance of common insects inhabiting treehole analogs, suggesting that benefits of scirtid presence do not last even into the following spring.

During the previous year, we demonstrated density-dependent scirtid effects on the communities, while scirtids were still near their initial densities (Burkhart *et al.* 2007). The likely mechanism of effect here is that processing occurred through winter and spring 2004 and facilitated microorganism growth, which then, during the short-term, facilitated growth of individual mosquitoes and inhibited densities of *C. guttipennis* densities (Burkhart *et al.* 2007). It should be noted that the mosquito and midge larvae counted in 2005 hatched from eggs laid in 2004, while many scirtids were still present in mesocosms. This may indicate that there is no effect of scirtid presence or density on oviposition behavior of mosquitoes and midges. It suggests also that after scirtid populations are gone from these communities, there is no effect of past densities on survival of other larvae.

Research indicates that scirtid beetles can have a significant positive impact on populations of detritivores within the communities of which they are a part (Paradise & Dunson 1997, Paradise 1999, Burkhart *et al.* 2007). We demonstrate here that the positive effects of scirtid beetles in naturally colonized container communities disappear when scirtid populations are depleted. In our mesocosm experiment, scirtid beetles positively affected species richness and mass of pupating mosquitoes, and negatively affected abundance of the midge, *C. guttipennis*, during the first field season, when scirtids remained near initial densities in communities (Burkhart *et al.* 2007). Thus, scirtid processors do affect treehole communities, but effects on the most abundant consumers do not persist after scirtids are no longer present in communities.

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