The Effect of Bat Guano on the Forest Litter Invertebrate Food Web

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Abstract River derived nutrients can be transported over long distances into terrestrial systems by mobile predators. Insectivorous bats that feed over rivers and roost in redwood trees are one such vector. Guano associated invertebrate communities occurring in tropical caves are well documented, but the influence of guano in invertebrates in forest systems has not yet been studied. For this study, research was conducted in Northern California to test the hypothesis that nutrients deposited as bat guano in redwood tree hollows are incorporated into the terrestrial invertebrate food web. Experimental additions of bat guano were made in selected tree hollows. Invertebrates were collected in pitfall traps in and around control and experimental tree hollows for two months after guano deployment. Stable isotope analysis will be used to compare $\delta^{15}N$ values of these invertebrates. Preliminary results indicate that in the week after addition of bat guano, total number of invertebrates declines. This decline is driven by a decrease in the most abundant species of collembola, mite, and diptera. Further analysis is needed before conclusive results can be stated. This research will establish weather bat guano subsidies play a role in forest invertebrate food webs as they do in cave systems, and it may help establish a new link between river and forest food webs.

Introduction

Exchanges between river and forest food webs are one area of study that has been the focus of recent food web ecology research (Fausch *et al*. 2002). These exchanges are often made by mobile predators which feed in and around the river and then transport these marine resources upslope (Power and Rainey 2000). Insectivorous bats which feed over the river on aquatic emergent insects can translocate river derived nutrients several kilometers into the forest where they roost in rocks and large trees (Power and Rainey 2000). This study investigates the incorporation of river derived nutrients deposited via bat guano in redwood tree hollows into the terrestrial invertebrate food web.

While the influence of bat guano has not been studied in forest systems, the relationship between bat guano and invertebrate populations has been studied in caves which are used as bat roosts. Bat guano enters the food web of cave invertebrates by detritivores feeding directly on bat guano (Peck 1981). This input of nutrients supplements detritivore as well as predator populations. Predator spider populations in caves, for example, exhibit more abundance and species richness where guano inputs have increased detritivore density (Ferreira and Martins 1999).

Translocated nutrients have been shown to influence food web dynamics in several different systems. In watersheds with rivers used by salmon during spawning, marine derived nitrogen has been found in multiple trophic levels (Hocking and Reimchen 2002). On islands used as seabird roosts, beetle populations were found to be up to five times more dense than on islands which were not used as roosts (Sánchez-Piñero and Polis 2000). In this system, seabird guano fertilizes plant growth which creates more ground litter to support larger detritivore beetle populations, which in turn supplement populations of their direct predators (Sánchez-Piñero and Polis 2000). These effects may cascade along the food web influencing higher consumers such as shrub and canopy invertebrates, reptiles, small mammals, and bird populations (Hocking and Reimchen 2002). This study attempts to determine if nutrient subsidies from bat guano are significant to the forest invertebrate food web as well.

Stable isotope analysis has become a popular tool for food web research (Fausch *et al.* 2002). This type of analysis takes advantage of variability in naturally occurring stable carbon and nitrogen isotopes. The stable isotope ratios for carbon and nitrogen change predictably as they move along the food web. Compared to their food source, animals are enriched in $\delta^{15}N$ by approximately 3.4‰ and in $\delta^{13}C$ by approximately 0.5‰ (Eggers and Jones 2000). Stable isotope analysis will be used in this study to trace the enriched ^{15}N from bat guano into the invertebrate food web.

Stable isotope analysis has been used to verify the importance of supplemental nutrient inputs from outside sources in terrestrial systems. In watersheds with rivers used by salmon, marine derived nitrogen has been found in plants and small mammals (Ben-David *et al.* 1998) as well as in soil and litter invertebrates (Hocking and Reimchen 2002). Salmon are enriched in both $\delta^{15}N$ and $\delta^{13}C$ compared to terrestrial food sources in this system. Looking at both carbon and nitrogen isotope ratios is useful for determining the mechanism that these nutrients are incorporated into the terrestrial system. Both Ben-David *et al* (1998) and Hocking and Reimchen (2002) found that while enriched nitrogen isotope ratios in small mammals and invertebrates indicated the incorporation of marine derived nitrogen, the fact that carbon isotope ratios were not also enriched indicated that the nitrogen was incorporated into the system indirectly by fertilizing plant growth which the animals then ate.

Previous studies of forest soil and litter macro-invertebrates have compared ratios of stable isotopes of nitrogen, $\delta^{15}N$, and of carbon, $\delta^{13}C$, to study food web structure. Nitrogen ratios of soil macro-invertebrates have been shown to parallel the nitrogen ratios of the local forest floor litter (Ponsard and Arditi 2000). Differences in these ratios from the litter substrate to invertebrates are indicators of trophic level (Ponsard and Arditi 2000, Hocking and Riemchen 2002).

I hypothesize that the addition of bat guano in redwood tree hollows will supplement invertebrate populations of the terrestrial forest system. I will add bat guano which is naturally enriched in ^{15}N to redwood tree hollows and monitor the response in abundance of invertebrate populations. I will test to see if nutrients from bat guano are incorporated into the terrestrial invertebrate food web by conducting isotope analysis on invertebrates collected in and around redwood tree hollows. If these nutrients are assimilated by invertebrates, I expect to see enriched ^{15}N in invertebrate specimens collected where bat guano is present compared to those collected where it is not.

Methods

The study site is located in the South Fork Eel River watershed at the Angelo Nature Reserve in Northern California (Figure 1). The study plots are located 0.2 miles north of the confluence of Skunk Creek and the South Fork Eel River (Figure 2). Vegetation consists mostly of old growth coast redwood (*Sequioa sempervirens)* and douglas fir (*Pseudotsuga menziesii)*, as well as some tan oak (*Lithocarpus densiflorus)* and madrone (*Arbutus menziesii)*. Six old growth redwood trees with basal tree hollows resulting from fire scars have been selected for the study. Trees were paired by their proximity to the river, and one of each pair of trees was randomly selected for experimental manipulation.

Figure 1: map showing location of Angelo Reserve in Northern California.

Figure 2: Map of Angelo Reserve. Study site indicated with star on map.

Bat guano was brought in from an outside source for this experiment. Guano was collected from the rafters inside a barn at Pt. Reyes National Seashore in California. This sample was chosen for the experiment because it is enriched in $\delta^{15}N$ compared to the forest floor litter at the Angelo Reserve. Prior to deployment at the reserve, the guano sample was tested for sudden oak death pathogens at the Forest Pathology and Mycology Laboratory at UC Berkeley. The sample was stored in a freezer for six weeks prior to deployment at the reserve to kill any invertebrates that may have been living in the guano at the time it was collected.

Litter and soil samples were collected at the site of each pitfall location in the winter and spring. Samples were oven dried at 45°C to a constant weight. Litter depth and soil moisture were measured for each location.

Invertebrates were collected in pitfall traps located in and around each selected tree. Three pitfall traps were located inside each tree hollow. Transects radiating from each tree hollow were oriented uphill toward the ridge (east), downhill toward the river (west), and parallel to the river. The direction of the parallel transect (north or south) was determined as the direction nearest to the orientation of the tree hollow opening. Three pitfall traps were located along each transect at distances of 0.5 m, 1 m and 2 m away from the tree. Pitfall traps were also located at 2 m from the tree in either NW and NE or SW and SE directions. Each tree had a total of 14 pitfall traps (Figure 3)

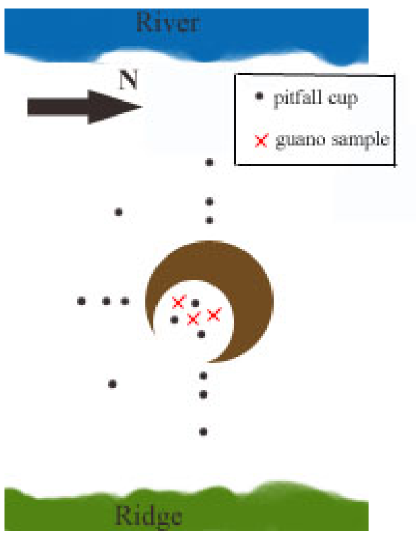


Figure 3: setup of pitfall traps and guano deposits

18 January 2004	Pre-experimental collection
25 January 2004	Pre-experimental colelction
8 February 2004	Pre-experimental collection
22 February 2004	Pre-experimental collection
8 March 2004	Pre-experimental collection
10 March 2004	Experimental collection
14 March 2004	Experimental collection
21 March 2004	Experimental collection
4 April 2004	Experimental collection
2 May 2004	Experimental collection

Table 1: pitfall trap collection dates

Pitfall traps were made from 9 cm diameter cups placed in the ground. When possible, traps were placed at a depth so that the top of the cup is level with the top of the soil layer. Where there is a large accumulation of litter and organic debris, pitfall cups were placed with the top of the cup about 15-20 cm deep into the organic layer. When not in use, cups were closed with plastic wrap secured by a rubber band to prevent invertebrates from falling into the traps. When in use, pitfall cups were filled with a few centimeters of soapy water. Soapy water reduces the surface tension of the water so that specimens drown rather than escaping or eating each other. For invertebrate collections, traps were left open for 48 hours before invertebrates were collected and immediately preserved in 70% ethanol. Five pre-experimental invertebrate collections were made. Five experimental collections were made the 48 hours after, one week after, two weeks after, four weeks after, and eight weeks after guano deployment (Table 1).

Experimental manipulation consisted of placing bat guano inside each of three selected tree hollows (Figure 3). Three different samples of bat guano were used in each experimental plot: 1) invertebrate exclusion; 2) rodent exclusion; and 3) no exclusion. The invertebrate exclusion sample was placed inside a 1mm mesh bag. The rodent exclusion sample was inside a 5cm mesh plastic cage. The no exclusion sample was placed on top of large pieces of forest litter so it could be removed and measured at the end of the experiment. Dry mass of guano was measured before deployment and at the end of the experiment. The purpose of the three exclusions was to help determine if differences in guano mass at the end of the experiment were due to decomposition over the study period or consumption by invertebrates or small mammals.

Invertebrates were identified to family using Borrer *et al.* (1992), or grouped by morphotype. For stable isotope analysis, specimens were removed from ethanol and oven dried at 45°C for at least 48 hours. Approximately 1.5 mg of each sample was submitted for stable isotope analysis. Smaller specimens were left whole for the analysis. For larger specimens, specific tissue was used (i.e. legs) to avoid the influence of undigested gut material on the isotope ratio results. Stable isotope analysis was done by the Center for Stable Isotope Biogeochemistry at the University of California at Berkeley using a PDZ Europa Scientific 20/20 Mass Spectrometer.

Results

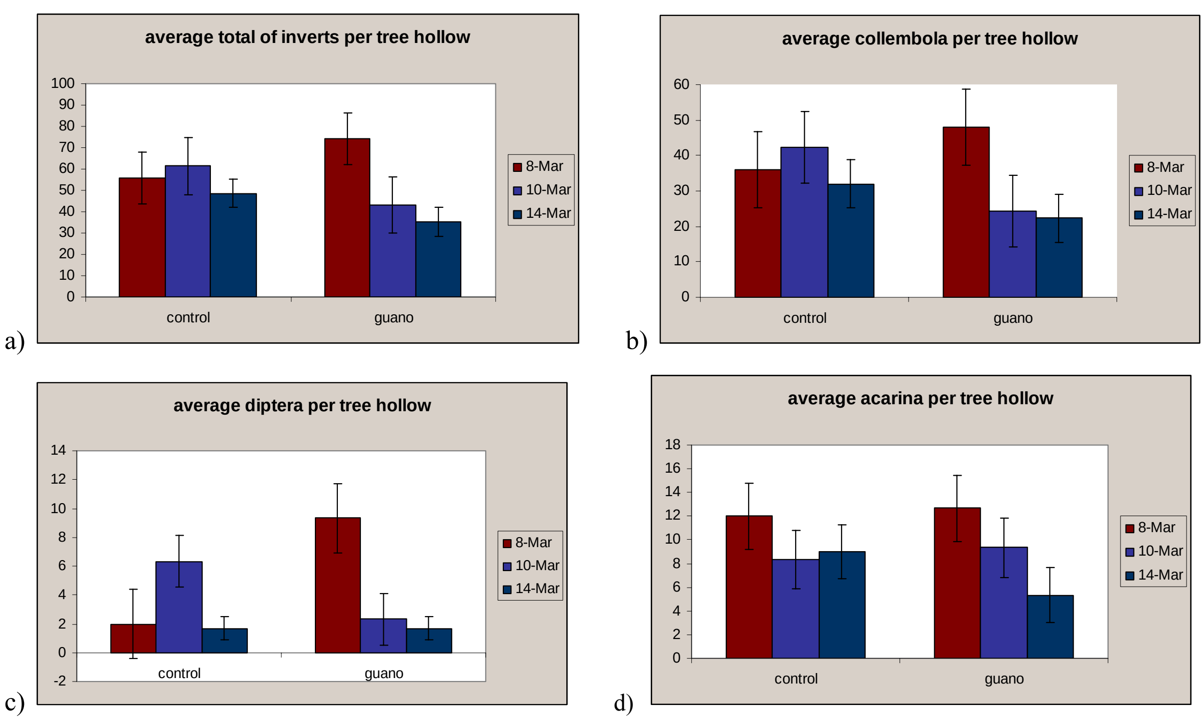
Data processing for this experiment is incomplete. Invertebrate collections from March 8 (pre-guano deployment) and March 10 (post-guano deployment) have been sorted, identified, and counted. Specimens from pitfall cups inside the tree hollows have been processed for the March 14 collection. Stable isotope analysis has not yet been completed.

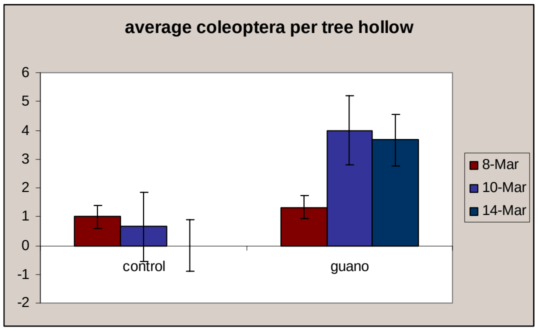
Preliminary counts of invertebrate data from the March 8^{th} and 10^{th} collections have been used to determine which invertebrates are most common and which should be used for stable isotope analysis. Springtails (Collembola), flies (Diptera), mites (Acarina), and spiders (Aranieda) have been found at every tree. Beetles (Coleoptera), millipedes (Diplopoda), and crickets (Orthoptera) have been collected in most trees. Snails, pseudoscorpions, and homoptra have also been found at at lest half the study sites. Table 2 shows a list of the most common orders found and the percentage of pitfall cups they were collected in.

	March 8 (pre-experimental)	March 10 (experimental)
Collembola	99%	98%
Acarina	86	82
Diptera	52	71
Coleoptera	35	27
Spider	25	29
Diplopoda	20	14
Orthoptera	11	12

Table 2: Occurance of common invertebrates expressed as percentage of total pitfall cups collected

Figure 4 shows graphs of tree hollow data for one pre- and two post-guano addition collections. A decreasing trend in the total number of invertebrates collected can be seen in trees with guano. This trend is not evident in control trees. The decrease in total number collected is due to decreases in Collembola, Diptera, and Acarina collected. Average Coleoptera and Diplopoda increased in guano trees but not in control trees. Other orders of invertebrates did not appear to exhibit any trend.



e) 

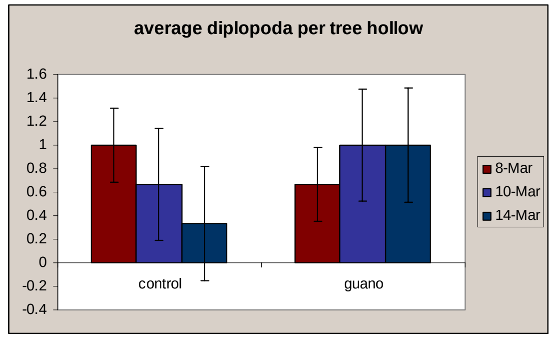
f) 

Figure 4: Change in average number collected in control and guano tree hollows for three collection dates.

Discussion

In tree hollows which serve as active bat roosts, substantial amounts of bat guano may be deposited. Guano deposition rates in giant sequoia tree hollows at two different Sierra Nevada locations average up to hundreds of milligrams per day for more active roosts (Rainey, unpublished data). This nutrient rich input may play a substantial role in subsidizing the food web. Most guano associated species in caves are supported by direct feeding on bat guano (Peck 1981), but predators are also shown to benefit from increased prey abundance where guano is present (Peck 1981, Ferreira and Martins 1998). Guano associated invertebrate communities in caves are generally made up of species that are normally forest litter inhabitants rather than specialized corophages or cave-restricted species (Peck 1981).

My results indicate that total invertebrate abundance decreased in tree hollows after guano was added (Figure 4a). This is opposite to what I expected to find based on previous studies which show that nutrient subsidies increase productivity and abundance where they are deposited. One reason for the decrease in total number of invertebrates collected could be the initial response of the system to a new input. The data analyzed only account for the short time period of one week after guano was added. Subsequent collections need to be analyzed to see if this trend continues with time after guano was added, or if invertebrate populations do become more abundant with time.

The decrease in total number of invertebrates collected in guano trees can be explained by decreases in Collembola, mites, and Diptera (Figure 4b, c, and d). Collembola and mites are found to make up a large percentage of macroscopic fauna associated with guano in caves (Chapman 1983) and both have been shown to respond to pulse inputs of bat guano in cave systems (Martin and Poulson 1976). Diptera are also commonly associated with bat guano in caves (Peck 1981, Ferreira and Martins 1999). The decrease in number of Collembola is surprising because I observed Collembola on bat guano piles as soon as 24 hours after deployment. The Collembola, mites, and Diptera collected in pitfall traps are generally very small (less than 5mm), so this decrease in abundance may not indicate a decrease in total invertebrate biomass inside tree hollows with guano added.

The results also show an increase in Coleoptera and in millipedes collected in pitfall traps inside tree hollows with guano added. This increase is not enough to offset the general decreasing trend in total number of invertebrates because larger invertebrates do not occur as frequently in pitfall traps as the smaller ones (see Table 2). Coleoptera are generally predators, and an increase in coleoptera may result in increased predation on smaller invertebrates, which could be a factor in the observed decrease in invertebrate abundance.

As more data is processed and analyzed, I expect these trends in abundance to become more clear. Stable isotope analysis will be used to determine if nutrients from guano are incorporated into the invertebrate food web, and will be useful in determining which trophic level organisms are feeding at.

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