ABUNDANCE AND DISTRIBUTION OF *DESMOGNATHUS FUSCUS* ALONG A STREAM GRADIENT

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Abstract. To maintain the integrity of a headwater system subject to anthropogenic disturbance, one must first be familiar with how that system works in its natural state. Much focus has been given to studying salamanders within the context of riparian management and the effectiveness of buffers because they act as ecological indicators. In this study, I attempt to identify influential microhabitat components affecting the occurrence of the Northern Dusky Salamander (*Desmognathus fuscus*). I explored whether the gradual change in water chemistry and nutrient concentrations from the headwaters to farther downstream, a natural gradient, influences microhabitat selection of the *D. fuscus.* I also examined the influence of substrate composition and the occurrence of seeps. I hypothesized that salamander distribution could be explained by water chemistry, water nutrient levels, substrate composition, seep surface area, or a combination of these factors. I sampled nine locations along Norris Brook in Hubbard Brook Experimental Forest, New Hampshire, USA from June to July 2009.At each site, I estimated relative salamander abundance and recorded water chemistry characteristics including temperature, pH, acid-neutralizing capacity (ANC) and physical habitat characteristics including covertype, substrate composition, and surface area of seeps. A total of 147 northern dusky salamanders were found. There was a significant positive correlation between pH and ANC, lending confidence to the sampling design. There was no significant relationship between relative salamander abundance any of the water parameters tested, though this may be because the range sampled was not wide enough to reach the biological threshold of *D. fuscus*. There was also no significant relationship between salamander abundance and any of the physical habitat features tested. My findings imply that salamanders are fairly flexible in their microhabitat selection.

INTRODUCTION

The ecological interactions of headwater streams are especially important in a riparian system because they affect everything downstream (Lowe and Likens 2005). Thus, it is important to protect these sensitive waterways. To maintain the integrity of a headwater system, one must first be familiar with how that system works in its natural state. Lowe and Likens (2005) identified the study and comparison of degraded headwaters and pristine headwaters as well as spatial population dynamics of species in headwater systems to be research priorities. Much focus has been given to studying salamanders within the context of riparian management. Amphibians are more sensitive to their surroundings than other vertebrates because of their permeable skin. This trait makes amphibians a useful biological indicator of ecosystem health because they will be affected by compromised environmental conditions more quickly than other vertebrates. Stream salamanders are closely tied to riparian habitat both physically and behaviorally, making them an excellent indicator of riparian health (Lowe and Bolger 2002, Perkins and Hunter 2006, Ward et al. 2008). Stream salamanders are sensitive to watershed disturbance, even if there is a buffer left to protect the stream (Wilson and Dorcas 2003). However, recent literature challenges the validity of utilizing stream salamanders as indicators. Kerby et al (2010) argue that stream salamanders are not particularly sensitive to chemical contamination.

A variety of chemical and physical factors may affect microhabitat selection of stream salamanders. Grant et al (2005) suggest that stream salamanders may be sensitive to acid-neutralizing capacity (ANC), but based this suggestion on data inferred from pH and bedrock type, rather than direct measurements. Roudebush (1988) demonstrates that low pH levels inhibit the feeding behavior of two species of desmognathine salamander larvae. Smith and Grossman (2003) show that larval Southern Two-lined Salamanders (*Eurycea cirrigera*) display microhabitat selection, favoring areas with substrata that provide cover and emphasize the importance of habitat heterogeneity. Likewise, Davic and Orr (1987) demonstrate a positive relationship between rock density and population density of Black-bellied Salamanders (*Desmognathus quadramaculatus*).

In this study, I attempt to identify influential microhabitat components affecting the occurrence of the Northern Dusky Salamander (*Desmognathus fuscus*). I explored whether the gradual change in water chemistry and nutrient concentrations from the headwaters to farther downstream, a natural gradient, influences microhabitat selection of *D. fuscus*. I also examined the influence of substrate composition and the occurrence of seeps. I tested the assumption that salamanders are not evenly distributed along headwater streams, thereby exhibiting a form of microhabitat selection. I hypothesized that salamanders are more abundant in lower sections of headwater streams than upper sections. Water chemistry in upper stream sections is closer in nature to groundwater and will have a lower pH (Likens and Buso 2006). I felt the more basic water downstream would provide better habitat. I also hypothesized that relative abundance of *D. fuscus*, as measured by the number of adults found per survey, is related to water chemistry, water nutrient levels, substrate composition, seepage surface area, or any combination of these factors. Identifying the range of change for different chemical and physical features of a headwater system and comparing that with salamander abundance may be useful in determining limiting factors of suitable habitat for stream salamanders. Should important habitat components be identified, it would add to our knowledge base of specific site characteristics favored by *D. fuscus* and perhaps aid riparian management efforts to preserve the integrity of sensitive waterways.

Study Area

Hubbard Brook Experimental Forest (HBEF) in the White Mountain National Forest of New Hampshire offers the ideal opportunity to study a Northern Hardwood riparian system in its natural state. Perhaps most famous for its experimental manipulation of watersheds, HBEF has been a center for long-term ecological research on northeastern forests (Burton 1973; Likens and Buso 2006). Topography within the forest is hilly, occasionally quite steep. Soils tend to be acidic, coarse, and glacially-derived. Forests are predominantly of the Northern Hardwood covertype, with Spruce-Fir dominating the upper altitudes. The climate is cool, with long winters and mild summers (Hubbard Brook Ecosystem Study). Previous research conducted at HBEF has shown that water chemistry varies throughout the summer season and that water chemistry may have a direct effect on biological conditions (Likens and Buso 2006).

Norris Brook is a south-facing watershed within HBEF. It is not an experimental watershed, but acid addition experiments have been undertaken in the past (Hall et al. 1980). Norris Brook was selected as a study site because it had a higher pH than other north-facing slopes in the valley (Likens and Buso 2006). In addition, the turning of cover was not permitted in the experimental watersheds, so Norris Brook provided a better setting for salamander surveys. My study area encompassed a section of Norris Brook occurring between 250 to 400m in elevation.

Study Species

Three species of stream salamanders occur in HBEF: Northern Two-lined Salamander (*Eurycea bislineata*), Northern Dusky Salamander (*Desmognathus fuscus*), and Spring Salamander (*Gyrinophilus porphyriticus*) (Figure 1). This study focused on *D. fuscus*, a small to mid-sized darkly colored salamander with adults reaching a snout-vent length of 4 to 8 cm (Gibbs et al. 2007). It is the most terrestrial of the three stream salamanders, preferring seeps and undisturbed stream banks, but is usually found within 1-2 meters of water (J. Andrews, personal communication; Gibbs et al. 2007).

D. fuscus displays strong fidelity to a home range. Home range size varies on geographic location and resource availability, but is between 1 to 2m^2 under favorable conditions (Ashton 1975; Barthalmus and Bellis 1972). During times of stress (e.g. drought), *D. fuscus* may shift their home range to areas with less stressful conditions (Barbour et al 1969). During the winter, avoidance of freezing temperatures is an important component of microhabitat selection. *D. fuscus* may remain active overwinter in springs and seepage areas where the temperature remains above 3°C (Hamilton Jr. 1943, Burton 1973). Thus, the presence of seeps may play an important role in habitat selection of *D. fuscus*, though it is not clear if that influence is seasonal. Studies in Ohio show that *D. fuscus* principally nests in rocks along streambanks (Jones 1986; Orr and Maple 1978), but they will often lay their eggs in under moss on top of rocks and under logs near water (J. Andrews, unpublished data). Females lay their eggs during the summer (late June through early August) and development requires 46-61 days (Jones 1986; Juterbock 1986). Females will guard their eggs and feed sporadically during this time (Juterbock 1986, 1987). Once hatching occurs, the larval period lasts about 1 year (Juterbock 1990).

METHODS

Water Sample and Field Chemistry Collection: All water sampling and salamander surveys took place between 23 June 2009 and 29 July 2009. I chose nine sample locations to represent a gradient between the upper headwaters and further downstream (Fig. 2). I selectively chose waterfalls as sample sites to minimize contamination from scooping sediment and because these were sites thought to have flowing water in them throughout the whole summer. I calibrated the pH probe in the field using pH buffer 7.0 and 4.01 at the start of each field day. I cooled buffer solutions to stream temperature before calibration. The probe was allowed to adjust in the stream for about 5 minutes before the temperature was recorded or the pH was measured. I measured temperature in the stream so as to be the most accurate and recorded it to the nearest tenth. I measured pH with a small sample of water in a 300mL beaker in order to obtain a stable reading with the probe. When a site was sampled, one 500mL sample and one 60mL sample was collected in acid-washed bottles. The bottles were rinsed three times in stream water then filled with falling water. Samples were refrigerated until chemistry analysis could be done.

Laboratory analysis

The 500mL samples were brought to room temperature by sitting unrefrigerated overnight or by heating in a warm water bath. I analyzed ANC samples using an automatic titrator (Orion 960) according to the methods outlined in Buso et al. (2000). The 60mL samples were sent to the USDA Forest Service Northern Research Station's Durham lab for analysis of water nutrient levels. Analysis was conducted according to the lab's protocols and procedures. Concentrations of aluminum, calcium, iron, potassium, magnesium, manganese, sodium, phosphorous, sulfur, silicon, strontium and zinc were obtained.

Salamander Surveys

Salamander surveys began immediately after each water sampling event. I marked every 25m interval of the stream as a visual aid to estimate distance. Surveys began 50m downstream of each sample site and continued until 75 pieces of cover had been turned in the stream and within 2m of the stream edge. Cover was defined as cobble sized rocks (or several smaller rocks that added to an equivalent size), logs, and leaves. Logs tended to be about 10cm in diameter and 45cm long and counted as two pieces of cover if they were significantly larger. I recorded the duration of each survey and distance traveled was estimated using the 25m markers and recorded. Salamanders were captured using an aquarium dip net. I recorded the number of salamanders found by species. Unidentifiable larvae were grouped into the *E. bislineata/D. fuscus* larvae group. Salamanders that escaped before being identified were not recorded in counts. I calculated salamander abundance for each sampling event as the number of salamanders found per survey normalized by the distance traveled. I calculated mean relative salamander abundance for each sampling location and extrapolated per 100m.

Mapping

I visually determined covertype and substrate composition at each 25m interval. I determined covertype using dominant canopy cover at the stream edge and appraised it for approximately 30m distance from the stream edge. I determined substrate composition of the stream channel to bankfull for approximately 10m and evaluated it by percent cover. Substrate was classified into one of five categories: bedrock/boulders, pebbles/cobbles, sand/sediment, leaf litter, and woody debris. Using the average distance traveled for each salamander survey and the known starting location, I identified the area covered by each survey. The average substrate composition at each survey location was calculated within that area.

I measured the location of each using the 25m markers. I estimated approximate surface area by measuring the length and width of each seep. Seeps were only mapped if they occurred within 10m of the stream edge. I calculated total seep surface area for each survey area.

Statistical Analysis

To see if there was a difference between the abundance of salamanders in the upper section of the stream and the lower section of the stream, sample sites were classified into "upper" and "lower" sections based on the elevational gradient of the study area. Sample sites below 300m in elevation (NS1-NS3 and NS6) were defined as the "lower" section of Norris Brook and sites falling above 300m in elevation (NS4, NS5, and NS7-NS9) were defined as the "upper" section of Norris Brook. Criteria for normal distribution were met. I performed an independent t-test to assess if there was a difference between average number of salamanders found in the upper section of the stream or the lower section of the stream.

Since sampling was repeated at the nine locations, using all data points would have violated the assumption of independence. Thus, I used means in all statistical analyses. The statistical software JMP 8 (©2008 SAS Institute Inc.) was used to test for a multivarate correlation between the relative abundance of salamanders and water chemistry levels, water nutrient concentrations, substrate composition, and the number of seeps. Criteria for normal distribution were not met, so nonparametric analyses were used. Spearman's ρ was calculated for all correlations.

RESULTS

Relative Abundance

All three species of salamanders were found in both larval and adult forms. A total of 147 adult *D. fuscus* were found (Table 1). Individuals were not individually marked, so it is unknown what percentage of these were recaptures. The highest relative abundance of *D. fuscus* occurred at sample location NS2 and the lowest occurred at NS9(Table 2), but there was no significant difference between the number of salamanders found in the upper section of the stream and the lower section of the stream (Fig 3).

Water Chemistry

The average recorded temperature for each sampling location ranged from 13.1ºC to 15 ºC. Average pH ranged from 5.5 to 6.4, and average ANC ranged from 7.3 μeq to 36.4μeq (Table 1). As one would expect, pH and ANC were very strongly directly correlated (Spearman's $\rho = 0.8768$, $p < 0.05$) (Fig 4). Though all three parameters varied between sampling locations, they were fairly stable throughout the study period.

Water Nutrients

Table 4 shows average nutrient concentrations at each sampling location. Nutrient concentrations varied greatly between sampling locations and throughout the study period.

Physical Characteristics

Northern Hardwood was the dominant covertype throughout the study area, but there were substantial sections of the Eastern Hemlock covertype in the lower portion of the stream. Covertype was very patchy and so was not included in any statistical analysis (Fig 5). Substrate composition was highly variable throughout the study area, with no clear transition or differences between the upper and lower section of the stream. Cobbles/pebbles and sand/sediment were the two most dominant substrate types (Table 5). There were 24 seeps in the study area totaling 466.6 m^2 in surface area. There were 9 seeps totaling 82.1 m^2 in surface area located within a salamander survey area.

Statistical Analyses

The relative abundance of *D. fuscus* was not correlated with any water chemistry parameters (Fig. 6), water nutrient levels (Fig. 7), substrate type or seep surface area (Fig. 8). Surface area of seeps was the closest variable to being significant (Table 6).

DISCUSSION

The strong correlation between pH and ANC is a known relationship and is to be expected. The detection of such a relationship provides a certain validation for my sampling design as it indicates that a large enough range was sampled to detect a relationship between these two variables, so other relationships may be detectable within this range as well.

These data would suggest that natural variability in water chemistry and nutrients do not affect salamander microhabitat selection. This implies that salamanders are fairly tolerant of a wide range of chemical conditions. These findings support the claim made by Kerby et al. (2010) that salamanders may not be as sensitive to chemical contamination as we once thought. They are also consistent with the findings of Orser and Shure (1972) who claim that the chemical components they examine in their study are not limiting factors to salamander abundance. Alternatively, it may be that salamanders are adaptable to a wide range of conditions so long as those conditions are stable. Headwater systems with their constant input from groundwater may provide more stability than lower sections of streams with a high degree of variability in their chemical conditions. More research on how this affects salamander distribution is warranted.

These data would also suggest that natural variability in substrate composition and the location of seeps do not affect salamander microhabitat selection. This implies that salamanders are flexible in their microhabitat selection regarding these physical characteristics. Embeddedness is one factor not examined in this study that is probably essential to salamander habitat usage. Quantifying embeddedness is a challenge, but has been done in studies of stream-dwelling invertebrates (Jacobson 2005). I believe that research on the importance of embeddedness to stream salamander habitat selection is critical to improving our understanding of how disturbance affects stream salamanders.

The lack of any significant correlations in my study does not conclusively prove the absence of any relationships. It must be kept in mind that this project is a case study for Norris Brook in HBEF. Comparing habitat selection by salamanders across several different streams may reveal a relationship not present in a single stream. The sample size was very small and therefore any statistical findings are suspect. It may be that the range sampled was not wide enough to reach the biological threshold of *D. fuscus*. Another drawback may be the imperfect detection of salamanders, especially with changing water levels due to rain. Since females are nest-guarding during this season, they have limited movements. I did not find any nesting females despite tendency for females to move as far upstream as possible before selecting nest sites (Snodgrass et al. 2007). Their absence may incorrectly deflate my results of salamander abundance. This may be further complicated if females select home ranges based on availability of suitable nesting habitat.

Certain aspects of habitat use by salamanders could have been explored more, but were not due to time and resource constraints. The presence of fish predators likely affects the behavior of stream salamanders. Norris Brook contains brook trout (*Salvelinus fontinalis*) and is also the only tributary in the Hubbard Brook valley to contain slimy sculpin (*Cottus cognatus*) (Warren et al. 2008). Brook trout may limit salamander distribution in streams (Lowe and Bolger 2002). The uppermost regions of headwater systems may provide a predator-free refuge for adult salamanders, their eggs, and larvae (Snodgrass et al. 2007). Food supply may also be a critical factor in microhabitat selection. *D. fuscus* eats a variety of aquatic and terrestrial organisms (Burton 1973). The diversity of their diet may allow them to be less specific in their habitat selection. Additionally, the presence or absence of moss was not examined in relation to salamander abundance. *D. fuscus* is often found in mossy areas (Jim Andrews, personal communication). Moss may be a critical component of *D. fuscus* habitat, providing moist areas for nesting or foraging.

Burton (1973) reported that *D. fuscus* larvae were not found after 29 June. The latest date I found larvae in the *E. bislineata*/*D. fuscus* group was 15 July. Since E. bislineata was not commonly found in the study area (10 adults total for the duration of the sampling period) it may be of interest to sample for salamander larvae to see if development cycles have changed in the last 40 years. This may be particularly interesting with regards to the effects of climate change on the reproductive cycles of fauna.

Salamanders were found at every site sampled, demonstrating that they utilize all areas of the headwater system of Norris Brook. While all salamanders were searched for, only *D. fuscus* was found in substantial numbers. It would be interesting to compare this case study to other streams in HBEF to see of this trend is restricted to Norris Brook. If *D. fuscus* are unusually abundant in Norris Brook when compared with other streams in HBEF, further investigations would be required to determine what makes it such ideal habitat as my own results have proven inconclusive.

ACKNOWLEDGEMENTS

This material is based upon work supported by the National Science Foundation under Grant No. DBI 0552871.

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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APPENDIX

FIGURE 1. From left to right: Northern Two-lined Salamander (*Eurycea bislineata*), Northern Dusky Salamander (*Desmognathus fuscus*), and Spring Salamander (*Gyrinophilus porphyriticus*).

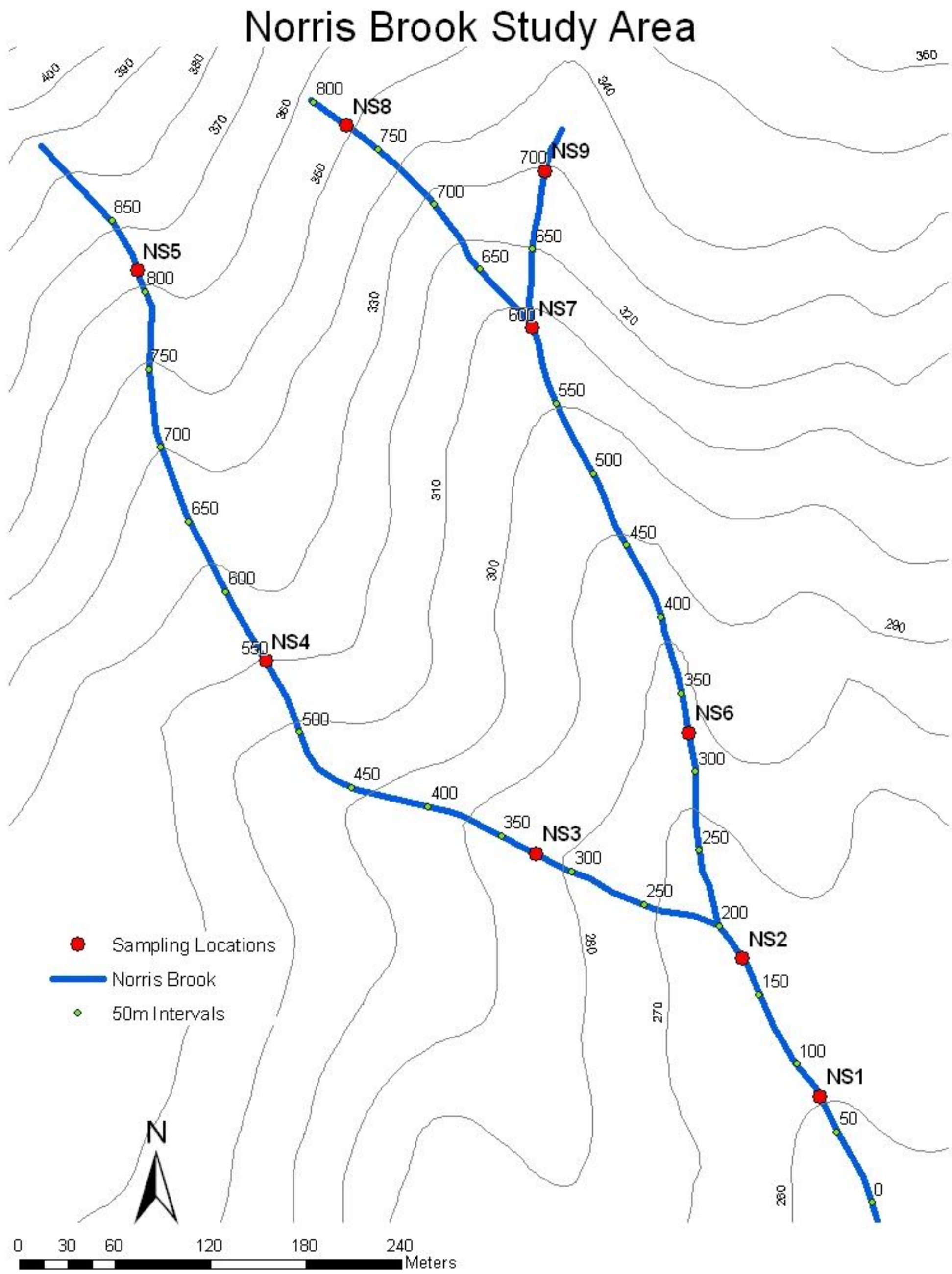


FIGURE 2. Sampling locations of a salamander study in Norris Brook, New Hampshire, USA between 23 June 2009 and 29 July 2009. Only every 50 meter interval is displayed, but every 25 meter interval was marked.

TABLE 1. A summary of the number of stream salamanders found along an elevational gradient in Norris Brook, Hubbard Brook Experimental Forest, NH, USA between 23 June 2009 and 29 July 2009. The Northern Two-lined Salamander (*Eurycea bislineata*), Northern Dusky Salamander (*Desmosgnathus fuscus*), and Spring Salamander (*Gyrinophilus porphyricticus*) were found. Salamander surveys continued until 75 pieces of cover had been turned. I could not differentiate between *E. bislineata* and *D. fuscus* larvae, so larvae of both species were grouped under one category. Each location was sampled five times.

Sample Location	Elevation (m)	Average Survey Distance (m)	*E. bislineata*	*D. fuscus*	*G. porphyriticus*	*E. bislineata/D. fuscus* Larvae	*G. porphyriticus* Larvae
NS1	261	81	2	14	1	5	0
NS2	266	66	2	28	1	6	0
NS3	284	75	1	18	0	0	1
NS4	320	64	0	11	0	0	0
NS5	365	71	0	22	0	0	3
NS6	276	67	0	18	1	7	0
NS7	307	66	2	15	3	4	0
NS8	352	76	0	14	1	0	1
NS9	330	74	3	7	1	1	0
Total			10	147	8	23	5

TABLE 2. Relative abundance of *Desmognathus fuscus* along an elevational gradient in Norris Brook, Hubbard Brook Experimental Forest, NH, USA between 23 June 2009 and 29 July 2009. All sites greater than 300m in elevation were defined as being in the "upper" part of the stream while all sites less than 300m in elevation were defined as being in the "lower" part of the stream. Each site was sampled five times.

Sample Location	Elevation (m)	Section	Total # of *D. fuscus*	Mean # of *D. fuscus* ± *SD*	Relative Abundance (# *D. fuscus*/100m)
NS1	261	Lower	14	2.8 ± 1.8	3.7
NS2	266	Lower	28	5.6 ± 1.5	8.7
NS3	284	Lower	18	3.6 ± 2.2	5.9
NS4	320	Upper	11	2.2 ± 1.3	3.4
NS5	365	Upper	22	4.4 ± 0.9	6.2
NS6	276	Lower	18	3.6 ± 1.7	5.3
NS7	307	Upper	15	3.0 ± 1.7	4.8
NS8	352	Upper	14	2.8 ± 1.3	6.3
NS9	330	Upper	7	1.4 ± 1.5	2.0

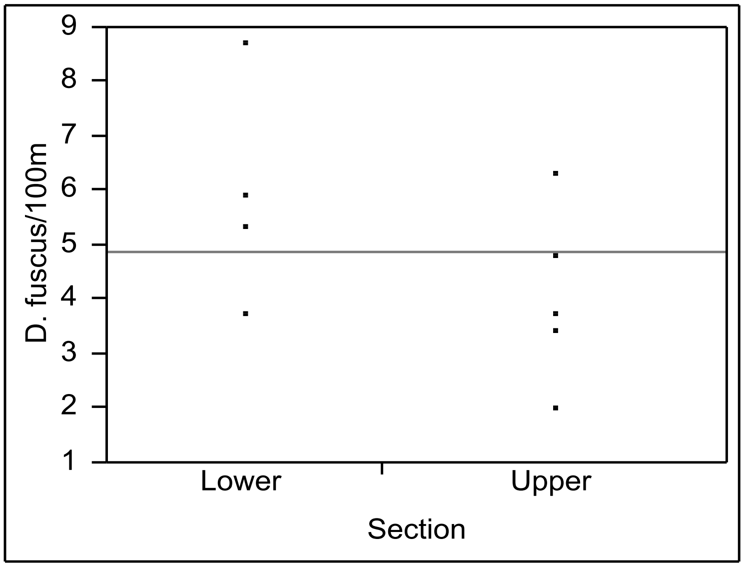


FIGURE 3. T-test results comparing the relative abundance of *Desmognathus fuscus* in the upper and lower section of a study area in Norris Brook, Hubbard Brook Experimental Forest, NH, USA between 23 June 2009 and 29 July 2009. All sites greater than 300m in elevation were defined as being in the "upper" part of the stream while all sites less than 300m in elevation were defined as being in the "lower" part of the stream. No significant difference was found ($t_{(6)} = -1.468$, $p > 0.05$).

TABLE 3. The average water chemistry concentrations and their standard deviation at nine sampling locations in Norris Brook, NH, USA between 23 June 2009 and 29 July 2009. Each site was sampled five times.

Sample	pH		ANC (μeq)		Water Temp (°C)	
Location	mean	±	mean	±	mean	±
NS1	6.3	0.09	25.1	4.1	13.8	1.0
NS2	6.2	0.04	26.2	1.5	14.3	1.2
NS3	6.0	0.07	21.1	1.8	14.5	1.2
NS4	5.9	0.08	13.6	3.4	15.0	1.2
NS5	5.5	0.06	7.3	2.7	14.3	1.0
NS6	6.2	0.17	29.0	4.6	13.7	1.5
NS7	6.4	0.10	36.4	4.9	13.4	1.3
NS8	6.0	0.06	23.7	2.4	13.6	1.4
NS9	6.2	0.09	28.2	4.9	13.1	1.2

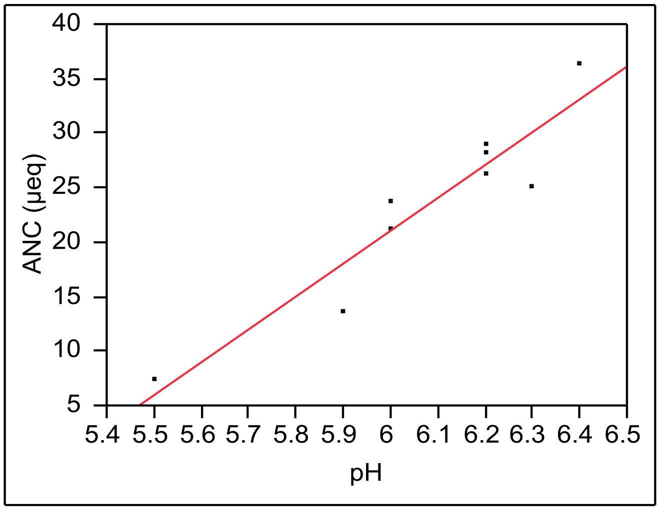


FIGURE 4. The positive correlation between pH and acid neutralizing capacity (ANC) at nine sampling locations in Norris Brook, NH, USA between 23 June 2009 and 29 July 2009. Each site was sampled five times.

TABLE 4. The average water chemistry concentrations and their standard deviation at nine sampling locations in Norris Brook, NH, USA between 23 June 2009 and 29 July 2009. Each site was sampled five times.

	Al (mg/L)		Ca (mg/L)		Fe (mg/L)		K (mg/L)		Mg (mg/L)		Mn (mg/L)	
Sample Location	mean	±	mean	±	mean	±	mean	±	mean	±	mean	±
NS1	0.0519	0.0199	1.1247	0.0729	0.0051	0.0028	0.1007	0.0263	0.2666	0.0219	0.0003	0.0004
NS3	0.0399	0.0136	1.0806	0.0500	0.0097	0.0037	0.0775	0.0437	0.2363	0.0131	0.0001	0.0001
NS4	0.0472	0.0184	0.9484	0.0961	0.0033	0.0015	0.0345	0.0208	0.1931	0.0173	0.0044	0.0033
NS5	0.0608	0.0108	0.8623	0.0730	0.0017	0.0011	0.0329	0.0221	0.1825	0.0196	0.0037	0.0014
NS6	0.0479	0.0113	1.1970	0.1345	0.0038	0.0011	0.1394	0.0595	0.2827	0.0357	0.0005	0.0004
NS7	0.0620	0.0158	1.3577	0.1259	0.0075	0.0022	0.1539	0.0745	0.3051	0.0357	0.0004	0.0004
NS8	0.0398	0.0114	1.1135	0.1099	0.0061	0.0005	0.0488	0.0325	0.2285	0.0288	0.0002	0.0002
NS9	0.0597	0.0191	1.2441	0.1353	0.0105	0.0051	0.1172	0.0339	0.2692	0.0344	0.0003	0.0004

	Na (mg/L)		P (mg/L)		S (mg/L)		Si (mg/L)		Sr (mg/L)		Zn (mg/L)	
Sample Location	mean	±	mean	±	mean	±	mean	±	mean	±	mean	±
NS1	0.9739	0.0458	0.0052	0.0027	1.6395	0.1185	3.1116	0.2191	0.0094	0.0007	0.0044	0.0003
NS2	1.0502	0.0828	0.0079	0.0049	1.7004	0.0953	3.3414	0.1772	0.0097	0.0003	0.0062	0.0031
NS3	0.8520	0.0405	0.0077	0.0037	1.5271	0.0498	2.7814	0.1641	0.0086	0.0004	0.0061	0.0011
NS4	0.6986	0.0643	0.0097	0.0052	1.4933	0.1471	2.2734	0.3666	0.0074	0.0006	0.0070	0.0013
NS5	0.6924	0.0802	0.0074	0.0034	1.5208	0.1231	2.2076	0.2513	0.0070	0.0007	0.0085	0.0008
NS6	1.0756	0.1040	0.0071	0.0030	1.7651	0.1841	3.4203	0.4374	0.0099	0.0011	0.0041	0.0006
NS7	1.2131	0.1296	0.0062	0.0031	1.8845	0.1016	3.9387	0.2990	0.0113	0.0008	0.0046	0.0009
NS8	1.0272	0.1178	0.0035	0.0022	1.6443	0.1653	3.3711	0.3895	0.0095	0.0010	0.0033	0.0005
NS9	1.0806	0.0714	0.0058	0.0011	1.7879	0.1341	3.6060	0.3506	0.0107	0.0013	0.0048	0.0002

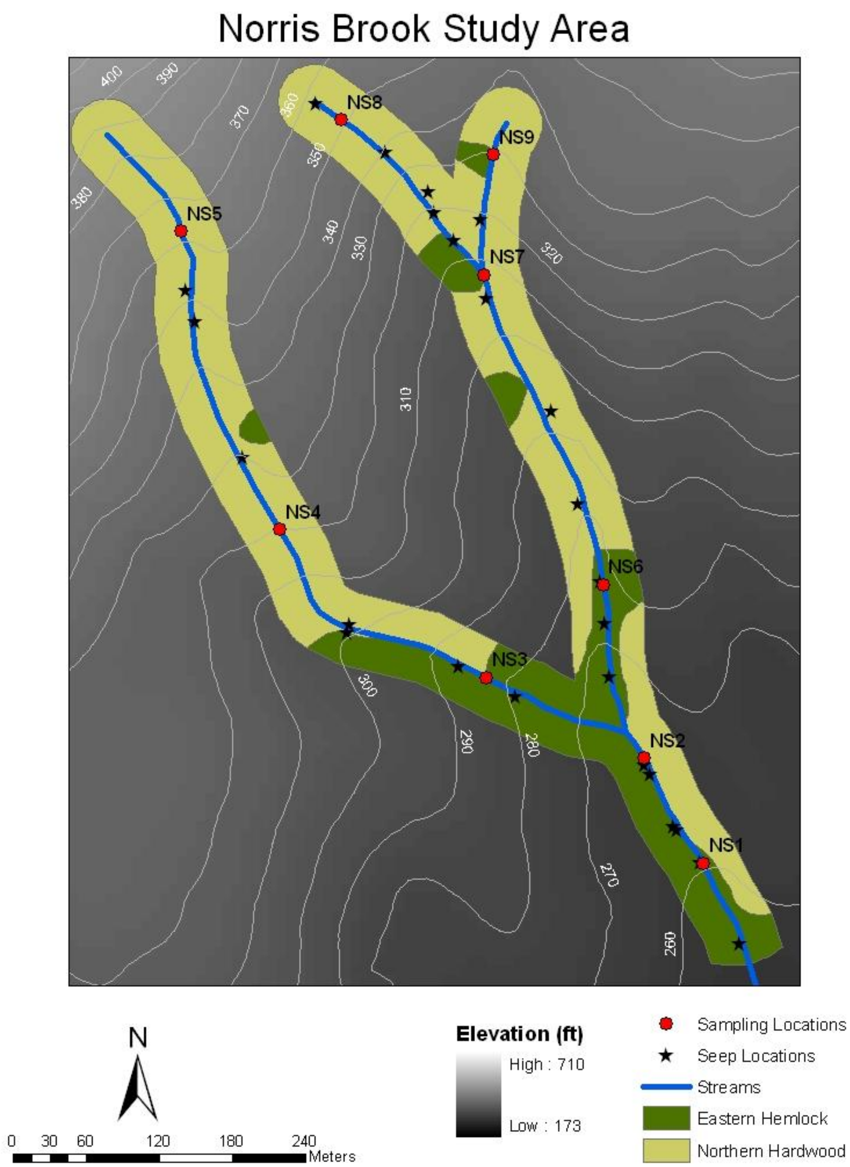


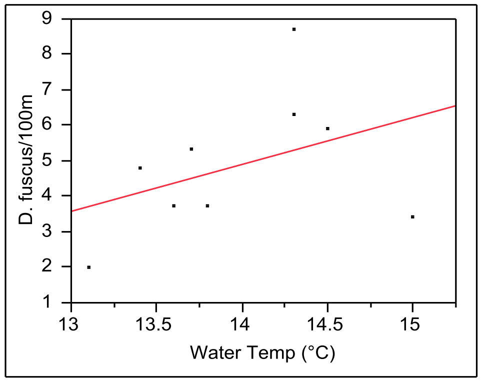
FIGURE 5: Covertype, locations of seeps, and sampling locations within the study area of a salamander study in Norris Brook, New Hampshire, USA between 23 June 2009 and 29 July 2009.

TABLE 5. Percent substrate composition and surface area of seeps for nine sampling transects for a salamander study in Norris Brook, New Hampshire, USA between 23 June 2009 and 29 July 2009.

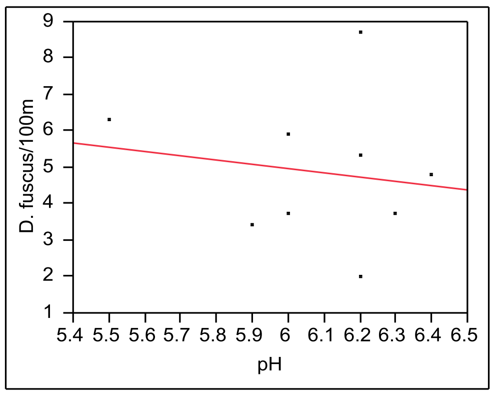
Sample Location	Average Survey Distance (m)	Bedrock/ Boulders (%)	Cobbles/ Pebbles (%)	Sand/ Sediment (%)	Woody Debris (%)	Leaf Litter (%)	Surface Area of Seeps (m^2)
N1	81	2	56	29	9	4	1.5
N2	66	19	58	9	10	4	15.6
N3	75	40	7	40	5	8	8.85
N4	64	30	28	23	7	12	0
N5	71	6	34	27	20	13	0
N6	67	6	41	36	8	9	46.2
N7	66	13	15	55	5	12	2.4
N8	76	5	35	30	18	13	7.5
N9	74	12	5	12	13	58	0

TABLE 6. Statistical results of a salamander study in Norris Brook, New Hampshire, USA between 23 June 2009 and 29 July 2009. Multivariate correlations between the relative abundance of *Desmognathus fuscus* and all variables based on data collected five times from nine sampling locations.

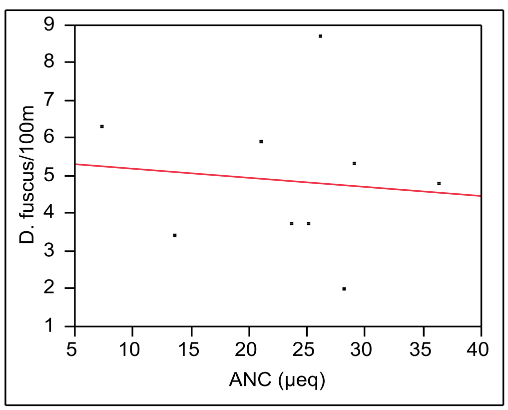
Variable	Spearman ρ	Prob>|ρ|
Water Chemistry		
Water Temp (°C)	0.378	0.316
Field pH	-0.124	0.751
ANC (Meq)	-0.109	0.781
Water Nutrients		
Al	-0.092	0.814
Ca	-0.193	0.620
Fe	-0.251	0.515
K	0.201	0.604
Mg	0.084	0.831
Mn	-0.184	0.635
Na	-0.193	0.620
P	0.427	0.252
S	-0.092	0.814
Si	-0.285	0.458
Sr	-0.193	0.620
Zn	0.318	0.404
Physical Characteristics		
Bedrock/Boulders (%)	0.193	0.618
Cobbles/Pebbles (%)	0.483	0.188
Sand/Sediment (%)	0.050	0.898
Woody Debris (%)	-0.050	0.898
Leaf Litter (%)	-0.466	0.206
Surface Area of Seep (m^2)	0.545	0.129



Rsquare = 0.163



Rsquare = 0.025



Rsquare = 0.012

FIGURE 6. The correlations between average water chemistry concentrations and the relative abundance of *Desmognathus fuscus* at nine sampling locations in Norris Brook, NH, USA between 23 June 2009 and 29 July 2009. Each site was sampled five times. There were no significant relationships detected.

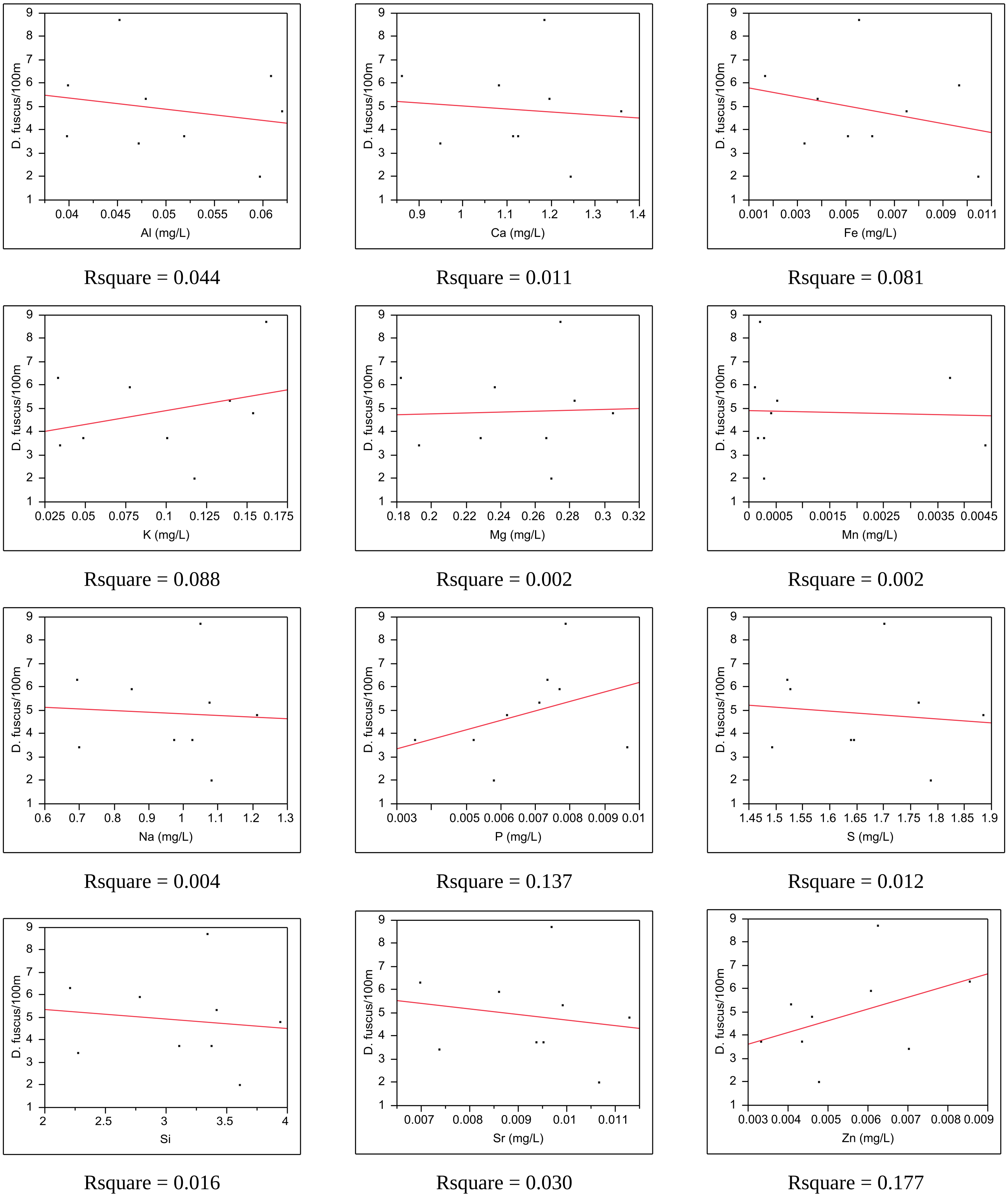


FIGURE 7. The correlations between average water nutrient concentrations and the relative abundance of *Desmognathus fuscus* at nine sampling locations in Norris Brook, NH, USA between 23 June 2009 and 29 July 2009. Each site was sampled five times. There were no significant relationships detected

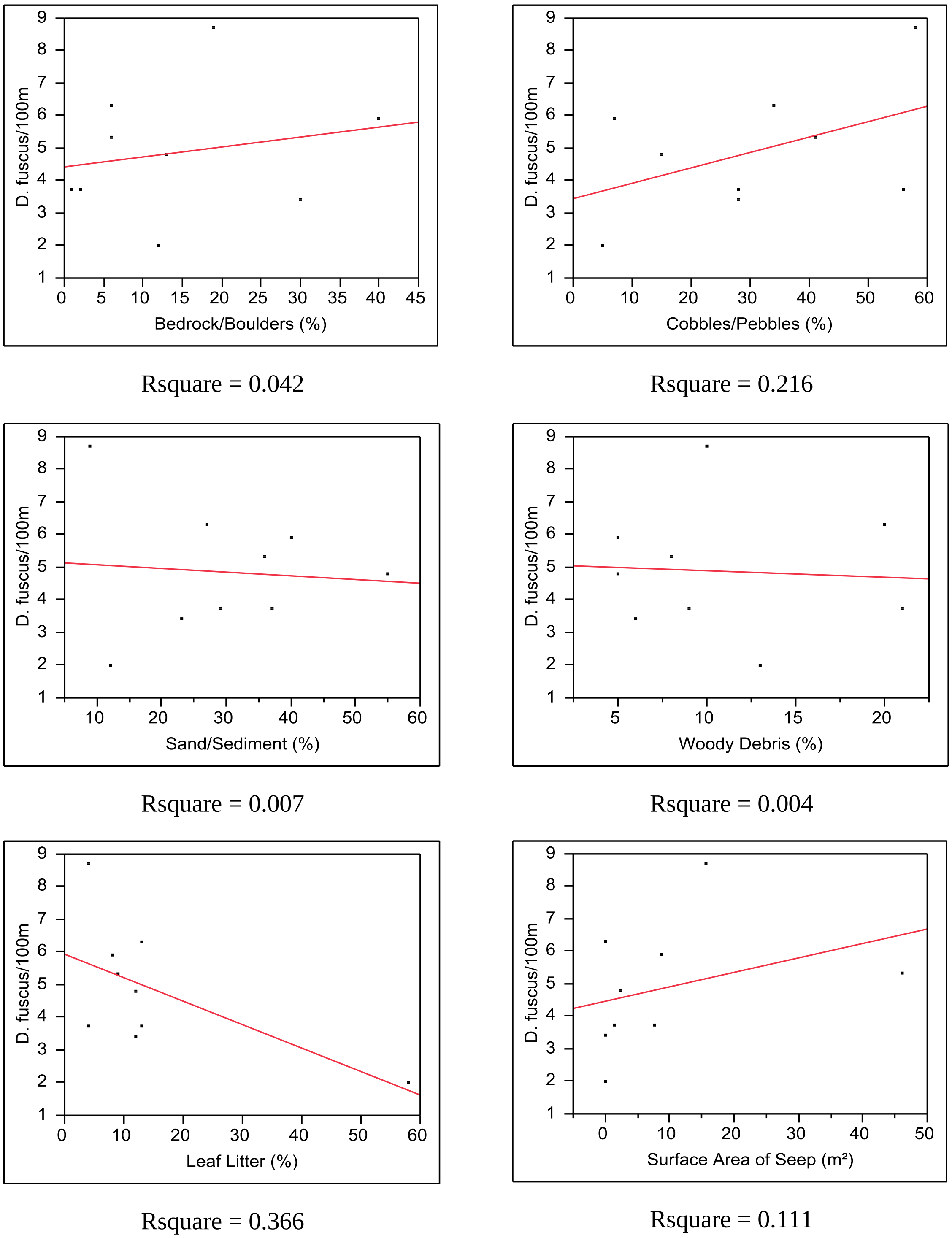


FIGURE 8. The correlations between substrate composition and the relative abundance of *Desmognathus fuscus* and the surface area of seeps and the relative abundance of *Desmognathus fuscus* at nine sampling locations in Norris Brook, NH, USA between 23 June 2009 and 29 July 2009. Each site was sampled five times. There were no significant relationships detected.

TABLE 7. Results of salamanders surveys of along an elevational gradient in Norris Brook, Hubbard Brook Experimental Forest, NH, USA between 23 June 2009 and 29 July 2009. Northern Two-lined Salamander (*Eurycea bislineata*), Northern Dusky Salamander (*Desmosgnathus fuscus*), and Spring Salamander (*Gyrinophilus porphyricticus*) were found (For complete data on adult *D. fuscus*, see Appendix II). Salamander surveys continued until 75 pieces of cover had been turned. I could not differentiate between *E. bislineata* and *D. fuscus* larvae, so larvae of both species were grouped under one category.

Sample Location	Replicate #	Sample Date	Survey Distance (m)	*E. bislineata*	*G. porphyriticus*	*E. bislineata*/ *D.fuscus* Larvae	*G. porphyriticus* Larvae
NS1	1	23-Jun-09	105	0	0	2	0
	2	2-Jul-09	75	0	0	0	0
	3	10-Jul-09	75	0	0	0	0
	4	15-Jul-09	75	1	0	3	0
	5	28-Jul-09	75	1	1	0	0
NS2	1	25-Jun-09	55	1	1	0	0
	2	2-Jul-09	75	0	0	0	0
	3	10-Jul-09	65	0	0	2	0
	4	15-Jul-09	68	1	0	4	0
	5	28-Jul-09	65	0	0	0	0
NS3	1	26-Jun-09	75	0	0	0	0
	2	2-Jul-09	xx	0	0	0	1
	3	10-Jul-09	80	0	0	0	0
	4	15-Jul-09	65	0	0	0	0
	5	28-Jul-09	80	1	0	0	0
NS4	1	26-Jun-09	60	0	0	0	0
	2	3-Jul-09	65	0	0	0	0
	3	10-Jul-09	65	0	0	0	0
	4	15-Jul-09	65	0	0	0	0
	5	28-Jul-09	65	0	0	0	0
NS5	1	26-Jun-09	75	0	0	0	0
	2	3-Jul-09	75	0	0	0	0
	3	10-Jul-09	65	0	0	0	2
	4	15-Jul-09	65	0	0	0	1
	5	28-Jul-09	75	0	0	0	0
NS6	1	23-Jun-09	75	0	1	3	0
	2	1-Jul-09	80	0	0	1	0
	3	9-Jul-09	62	0	0	2	0
	4	14-Jul-09	55	0	0	1	0
	5	28-Jul-09	65	0	0	0	0
NS7	1	24-Jun-09	65	1	0	1	0
	2	1-Jul-09	75	0	2	0	0
	3	9-Jul-09	53	0	0	0	0
	4	14-Jul-09	75	0	1	3	0
	5	29-Jul-09	60	1	0	0	0
NS8	1	23-Jun-09	60	0	0	0	1
	2	1-Jul-09	125	0	0	0	0
	3	9-Jul-09	60	0	0	0	0
	4	14-Jul-09	75	0	0	0	0
	5	29-Jul-09	62	0	1	0	0
NS9	1	23-Jun-09	100	1	0	0	0
	2	1-Jul-09	75	0	0	0	0
	3	9-Jul-09	60	2	0	1	0
	4	14-Jul-09	65	0	0	0	0
	5	29-Jul-09	70	0	1	0	0

TABLE 8. The number and relative abundance of adult Northern Dusky Salamanders (*Desmosgnathus fuscus*) found along an elevational gradient in Norris Brook, Hubbard Brook Experimental Forest, NH, USA between 23 June 2009 and 29 July 2009. (For complete data on additional salamanders found, see Appendix I). Salamander surveys continued until 75 pieces of cover had been turned.

Sample Location	Replicate #	Sample Date	*D. fuscus*	Survey Distance (m)	Relative Abundance (# of *D. fuscus*/100m)
NS1	1	23-Jun-09	1	105	1
	2	2-Jul-09	1	75	1.3
	3	10-Jul-09	4	75	5.3
	4	15-Jul-09	5	75	6.7
	5	28-Jul-09	3	75	4
Mean			**2.8**	**81**	**3.6**
NS2	1	25-Jun-09	7	55	12.7
	2	2-Jul-09	4	75	5.3
	3	10-Jul-09	6	65	9.2
	4	15-Jul-09	7	68	10.3
	5	28-Jul-09	4	65	6.2
Mean			**5.6**	**66**	**8.7**
NS3	1	26-Jun-09	4	75	5.3
	2	2-Jul-09	1	xx	xx
	3	10-Jul-09	3	80	3.8
	4	15-Jul-09	7	65	10.8
	5	28-Jul-09	3	80	3.8
Mean			**3.6**	**75**	**5.9**
NS4	1	26-Jun-09	2	60	3.3
	2	3-Jul-09	1	65	1.5
	3	10-Jul-09	4	65	6.2
	4	15-Jul-09	1	65	1.5
	5	28-Jul-09	3	65	4.6
Mean			**2.2**	**64**	**3.4**
NS5	1	26-Jun-09	5	75	6.7
	2	3-Jul-09	3	75	4
	3	10-Jul-09	5	65	7.7
	4	15-Jul-09	4	65	6.2
	5	28-Jul-09	5	75	6.7
Mean			**4.4**	**71**	**6.3**
NS6	1	23-Jun-09	4	75	5.3
	2	1-Jul-09	5	80	6.3
	3	9-Jul-09	5	62	8.1
	4	14-Jul-09	3	55	5.5
	5	28-Jul-09	1	65	1.5
Mean			**3.6**	**67**	**5.3**
NS7	1	24-Jun-09	4	65	6.2
	2	1-Jul-09	0	75	0
	3	9-Jul-09	3	53	5.7
	4	14-Jul-09	4	75	5.3
	5	29-Jul-09	4	60	6.7
Mean			**3**	**66**	**4.8**
NS8	1	23-Jun-09	1	60	1.7
	2	1-Jul-09	4	125	3.2
	3	9-Jul-09	2	60	3.3
	4	14-Jul-09	3	75	4
	5	29-Jul-09	4	62	6.5
Mean			**2.8**	**76**	**3.7**
NS9	1	23-Jun-09	1	100	1
	2	1-Jul-09	1	75	1.3
	3	9-Jul-09	0	60	0
	4	14-Jul-09	4	65	6.2
	5	29-Jul-09	1	70	1.4
Mean			**1.4**	**74**	**2.0**

TABLE 9. The water chemistry concentrations at nine sampling locations in Norris Brook, NH, USA between 23 June 2009 and 29 July 2009. "xx" indicates no data due to equipment failure.

Sample	Replicate #	Sample Date	Water Temp (°C)	pH	ANC (μeq)
NS1	1	23-Jun-09	13.6	6.3	19.5
	2	2-Jul-09	13.8	6.2	25.1
	3	10-Jul-09	13.3	6.2	23.7
	4	15-Jul-09	12.8	6.2	26.3
	5	28-Jul-09	15.4	6.4	30.7
NS2	1	25-Jun-09	15.5	6.2	26.1
	2	2-Jul-09	13.8	6.2	26.8
	3	10-Jul-09	13.5	6.2	24.1
	4	15-Jul-09	12.9	6.2	25.9
	5	28-Jul-09	15.7	6.3	28.1
NS3	1	26-Jun-09	15.5	5.9	23.2
	2	2-Jul-09	13.9	6	19.3
	3	10-Jul-09	13.8	6	19.0
	4	15-Jul-09	13.4	6	21.9
	5	28-Jul-09	16	6.1	21.9
NS4	1	26-Jun-09	15.5	5.8	15.2
	2	3-Jul-09	xx	xx	8.0
	3	10-Jul-09	14.1	6	14.3
	4	15-Jul-09	13.8	5.9	13.2
	5	28-Jul-09	16.4	5.9	17.1
NS5	1	26-Jun-09	14.2	5.5	10.3
	2	3-Jul-09	xx	xx	3.6
	3	10-Jul-09	13.5	5.6	6.7
	4	15-Jul-09	13.6	5.5	6.5
	5	28-Jul-09	15.7	5.6	9.4
NS6	1	23-Jun-09	13.5	6.5	29.3
	2	1-Jul-09	13.9	6.1	32.6
	3	9-Jul-09	12.2	xx	23.6
	4	14-Jul-09	12.9	6.2	25.1
	5	28-Jul-09	16.2	6.2	34.3
NS7	1	24-Jun-09	13.3	6.5	36.9
	2	1-Jul-09	13.7	6.4	40.9
	3	9-Jul-09	11.8	xx	31.1
	4	14-Jul-09	12.8	6.3	31.6
	5	29-Jul-09	15.4	6.3	41.5
NS8	1	23-Jun-09	13.7	6.1	24.7
	2	1-Jul-09	13.6	6	26.5
	3	9-Jul-09	11.9	xx	21.0
	4	14-Jul-09	12.9	6.1	21.5
	5	29-Jul-09	15.7	6	24.9
NS9	1	23-Jun-09	12.8	6.3	28.8
	2	1-Jul-09	13.3	6.2	35.1
	3	9-Jul-09	11.7	xx	25.2
	4	14-Jul-09	12.5	6.1	22.2
	5	29-Jul-09	15	6.1	29.6

TABLE 10. Percent substrate composition at 25m intervals within the salamander study area ranging in elevation from 250m to 400m in Norris Brook, New Hampshire, USA between 23 June 2009 and 29 July 2009. Branch 1 is the western branch of the study area, branch 2 is the western fork of the eastern branch, and branch three is the eastern fork of the eastern branch. The 25m intervals increase going upstream.

Branch	Location(m)	Bedrock/Boulders(%)	Cobbles/Pebbles(%)	Sand/Sediment(%)	Woody Debris(%)	Leaf Litter(%)
1	0	5	40	50	4	1
	25	1	79	10	5	5
	50	1	68	30	1	0
	75	3	17	52	22	6
	100	2	60	25	8	5
	125	1	80	8	6	5
	150	2	72	20	5	1
	175	0	65	10	15	10
	200	70	20	1	5	4
	225	70	14	4	5	7
	250	55	15	15	10	5
	275	90	1	5	2	2
	300	25	0	70	2	3
	325	5	20	45	10	20
	350	5	15	55	15	10
	375	5	10	55	20	10
	400	5	5	75	5	10
	425	5	25	50	15	5
	450	0	40	45	10	5
	475	30	25	10	15	20
	500	30	30	15	10	15
	525	50	35	5	5	5
	550	10	20	50	5	15
	575	15	20	50	5	10
	600	15	5	65	5	10
	625	10	25	55	5	5
	650	0	5	65	10	20
	675	0	15	25	20	40
	700	5	40	35	10	10
	725	1	35	40	14	10
	750	2	23	35	30	10
	775	1	25	35	19	20
	800	15	55	10	10	10
	825	8	57	17	11	7
2	200	3	75	5	15	2
	225	15	15	55	10	5
	250	5	45	20	15	15
	275	3	48	38	8	3
	300	5	20	50	10	15
	325	10	55	20	5	10
	350	10	10	60	10	10
	375	3	65	27	1	4
	400	30	20	15	20	15
	425	0	55	20	10	15
	450	15	45	20	15	5
	475	5	40	30	20	5
	500	5	50	15	15	15
	525	25	5	60	5	5
	550	70	15	5	5	5
	575	45	25	10	10	10
	600	3	25	50	5	17
	625	25	5	20	30	20
	650	20	10	40	15	15
	675	15	5	35	5	40
	700	10	10	25	30	25
	725	5	10	20	40	25
	750	1	4	30	40	25
	775	1	50	24	5	20
	800	3	2	15	20	60
3	625	10	35	15	10	30
	650	10	5	10	5	70
	675	15	5	10	30	40
	700	10	5	15	5	65
	725	5	2	33	30	30

TABLE 11. The water chemistry concentrations at nine sampling locations in Norris Brook, NH, USA between 23 June 2009 and 29 July 2009. “xx” indicates no data due to human error.

Sample Location	Replicate #	Sample Date	Al mg/L	Ca mg/L	Fe mg/L	K mg/L	Mg mg/L	Mn mg/L	Na mg/L	P mg/L	S mg/L	Si mg/L	Sr mg/L	Zn mg/L
NS1	1	23-Jun-09	0.052	1.141	0.004	0.104	0.275	0.000	0.973	0.007	1.801	3.135	0.010	0.004
	2	2-Jul-09	0.086	1.207	0.010	0.145	0.286	0.000	1.018	0.009	1.587	3.212	0.010	0.005
	3	10-Jul-09	0.038	1.078	0.003	0.081	0.255	0.000	0.919	0.004	1.609	3.011	0.009	0.004
	4	15-Jul-09	0.040	1.172	0.004	0.088	0.283	0.000	1.021	0.002	1.708	3.392	0.010	0.004
	5	28-Jul-09	0.043	1.026	0.005	0.086	0.234	0.001	0.939	0.004	1.492	2.807	0.008	0.004
NS2	1	25-Jun-09	0.041	1.221	0.005	0.332	0.273	0.000	1.190	0.009	1.768	3.313	0.010	0.012
	2	2-Jul-09	0.070	1.194	0.009	0.213	0.283	0.000	1.035	0.005	1.558	3.188	0.010	0.006
	3	10-Jul-09	0.045	1.157	0.002	0.085	0.272	0.000	0.982	0.004	1.696	3.193	0.010	0.004
	4	15-Jul-09	0.025	1.127	0.004	0.083	0.267	0.000	0.995	0.006	1.675	3.393	0.009	0.004
	5	28-Jul-09	0.045	1.228	0.007	0.095	0.278	0.001	1.048	0.016	1.804	3.619	0.010	0.005
NS3	1	26-Jun-09	0.030	1.001	0.007	0.060	0.216	0.000	0.804	0.008	1.453	2.526	0.008	0.005
	2	2-Jul-09	0.062	1.112	0.013	0.149	0.246	0.000	0.895	0.005	1.499	2.817	0.009	0.007
	3	10-Jul-09	0.031	1.063	0.005	0.044	0.234	0.000	0.814	0.005	1.556	2.737	0.009	0.005
	4	15-Jul-09	0.043	1.125	0.011	0.047	0.249	0.000	0.871	0.007	1.571	2.867	0.009	0.005
	5	28-Jul-09	0.034	1.102	0.013	0.087	0.237	0.000	0.877	0.014	1.557	2.960	0.008	0.008
NS4	1	26-Jun-09	0.066	0.928	0.003	0.028	0.187	0.005	0.663	0.009	1.357	2.040	0.007	0.008
	2	3-Jul-09	0.067	0.930	0.005	0.070	0.197	0.009	0.693	0.015	1.528	2.178	0.007	0.007
	3	10-Jul-09	0.044	0.945	0.002	0.024	0.195	0.004	0.696	0.003	1.472	2.258	0.007	0.006
	4	15-Jul-09	0.031	0.837	0.002	0.017	0.169	0.004	0.637	0.007	1.384	1.990	0.006	0.006
	5	28-Jul-09	0.028	1.102	0.005	0.035	0.217	0.000	0.805	0.014	1.726	2.901	0.008	0.009
NS5	1	26-Jun-09	0.060	0.952	0.001	0.033	0.214	0.005	0.798	0.009	1.667	2.476	0.008	0.008
	2	3-Jul-09	0.076	0.782	0.003	0.034	0.165	0.005	0.585	0.008	1.347	1.879	0.006	0.008
	3	10-Jul-09	0.063	0.888	0.001	0.017	0.188	0.002	0.689	0.005	1.539	2.265	0.007	0.008
	4	15-Jul-09	0.045	0.792	0.001	0.012	0.168	0.004	0.656	0.003	1.459	2.023	0.006	0.009
	5	28-Jul-09	0.060	0.897	0.003	0.068	0.178	0.003	0.733	0.012	1.592	2.396	0.007	0.010
NS6	1	23-Jun-09	0.037	1.343	0.005	0.180	0.318	0.000	1.183	0.008	2.011	4.065	0.011	0.005
	2	1-Jul-09	0.064	1.331	0.003	0.225	0.318	0.001	1.194	0.008	1.886	3.611	0.011	0.004
	3	9-Jul-09	0.046	1.155	0.004	0.103	0.275	0.001	1.021	0.011	1.736	3.305	0.010	0.004
	4	14-Jul-09	0.055	1.118	0.003	0.096	0.268	0.000	1.000	0.004	1.636	3.204	0.009	0.003
	5	28-Jul-09	0.039	1.038	0.003	0.094	0.235	0.001	0.980	0.005	1.557	2.917	0.009	0.004
NS7	1	24-Jun-09	0.042	1.257	0.006	0.052	0.254	0.000	1.142	0.008	1.930	3.841	0.011	0.004
	2	1-Jul-09	0.072	1.446	0.010	0.258	0.340	0.001	1.309	0.005	1.861	4.155	0.012	0.005
	3	9-Jul-09	0.066	1.264	0.007	0.138	0.292	0.000	1.104	0.009	1.771	3.562	0.011	0.004
	4	14-Jul-09	0.049	1.286	0.005	0.143	0.301	0.000	1.118	0.001	1.826	3.816	0.011	0.004
	5	29-Jul-09	0.080	1.535	0.010	0.179	0.338	0.001	1.392	0.007	2.034	4.319	0.012	0.006
NS8	1	23-Jun-09	0.052	1.073	0.006	0.103	0.230	0.000	0.883	0.006	1.592	3.008	0.009	0.004
	2	1-Jul-09	0.049	1.155	0.007	0.052	0.241	0.000	1.075	0.004	1.610	3.462	0.010	0.003
	3	9-Jul-09	0.038	1.244	0.006	0.040	0.257	0.000	1.180	0.004	1.904	3.887	0.011	0.003
	4	14-Jul-09	0.035	1.146	0.006	0.030	0.235	0.000	1.061	0.000	1.665	3.543	0.010	0.003
	5	29-Jul-09	0.024	0.950	0.006	0.020	0.180	0.000	0.937	0.003	1.450	2.957	0.008	0.003
NS9	1	23-Jun-09	0.036	1.202	0.003	0.127	0.279	0.000	1.045	0.007	1.819	3.391	0.010	0.005
	2	1-Jul-09	0.079	1.390	0.014	0.160	0.299	0.000	1.175	0.006	1.819	3.979	0.012	0.005
	3	9-Jul-09	0.071	1.307	0.011	0.096	0.279	0.000	1.092	0.005	1.915	3.819	0.012	0.005
	4	14-Jul-09	0.053	1.077	0.014	0.085	0.219	0.001	1.011	0.005	1.598	3.235	0.009	0.005
	5	29-Jul-09	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx

TABLE 12. Seeps located within the salamander study area ranging in elevation from 250m to 400m in Norris Brook, New Hampshire, USA between 23 June 2009 and 29 July 2009. Branch 1 is the western branch of the study area, branch 2 is the western fork of the eastern branch, and branch three is the eastern fork of the eastern branch. The location is based on 25m intervals that increase going upstream. If the seep is located within a salamander survey area, the sample transect is identified. "x" indicates that the seep is located outside of a salamander survey area.

Branch	Location (m)	Length (m)	Width (m)	Surface Area (m²)	Sample Transect
1	0	13	0.9	11.7	x
	103	1.5	1	1.5	NS1
	161	2.6	1	2.6	NS2
	170	6.5	2	13	NS2
	297	2.3	2	4.6	NS3
	350	2.5	1.7	4.25	NS3
	442	5.5	1.3	7.15	x
	442	10	3	30	x
	449	4	2.2	8.8	x
	617	1.5	1.5	2.25	x
	737	5.8	1.1	6.38	x
	762	7.7	1.5	11.55	x
2	248	2.9	1.4	4.06	x
	293	11	2.6	28.6	NS6
	328	4.4	4	17.6	NS6
	394	3.7	1.1	4.07	x
	475	8.5	3.6	30.6	x
	581	4	0.6	2.4	NS7
	636	20	9	180	x
	667	14	4	56	x
	682	6	3	18	x
	730	5	0.9	4.5	NS8
	799	5	0.6	3	NS8
3	645	7	2	14	x