DISTRIBUTION AND HABITAT CHARACTERISTICS OF A FRESHWATER GASTROPOD, *PLEUROCERA PROXIMA*, IN EASTERN PENNSYLVANIA

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DEDICATION

To my parents for their support and love, and to all the snails that sacrificed their lives for this project.

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ABSTRACT

DISTRIBUTION AND HABITAT CHARACTERISTICS OF A FRESHWATER GASTROPOD, *PLEUROCERA PROXIMA*, IN EASTERN PENNSYLVANIA

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Pleurocera proxima is a small, freshwater gastropod that has been recently discovered in four headwater tributaries in the Christina River watershed. The species was previously known only as far north as Virginia, so nothing is known about the species in Pennsylvania or Delaware. The purpose of this study was to determine if the species existed in additional locations in this region, and to assess the habitat where it is found. Fieldwork was conducted in Winter 2012 in the Red Clay Creek, White Clay Creek, and Brandywine Creek subwatersheds of the Christina River basin. In addition to the four streams where P. proxima was already known, 102 new streams were surveyed in this field season for presence of the species. Water chemistry and surrounding land use data were collected at all sites, and substrate characteristics and physical stream data were collected at sites with P. proxima. Only four of the 102 new sites had P. proxima. These data suggest the species is very rare in the region, and mostly populates small, headwater streams in areas with low developed land cover and high forest cover. The streams where *P. proxima* is found show lower levels of conductivity and salinity than other streams, but it is apparent further research is needed to identify the exact habitat conditions that predict *P. proxima* presence and density.

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INTRODUCTION

Pleurocera proxima is a species of gill-breathing and operculated freshwater gastropod that plays an important ecosystem role in headwater streams (Fig. 1). *P. proxima* impacts freshwater streams as a generalist grazer and shredder of detritus and periphyton (Dillon and Robinson 2009). These snails inhabit shallow freshwater environments and occur in numerous, highly discrete populations over their geographic range (Dillon 1984). Previously known from the mountains and Piedmont territory in southern Virginia to north Georgia on both sides of the continental divide (Fig. 2) (Dillon and Robinson 2011), this species has been recently found in streams in Chester County, Pennsylvania within the Christina River basin (personal communication; W. Eldridge, Stroud Water Research Center, Avondale, PA 19348).

Past studies on *Pleurocerid* snails have focused on their distribution (Dillon and Robinson 2011; Costil et al. 2001; Krieger and Burbanck 1976; Liu and Resh 1997), population dynamics (Houp 1970; Stiven and Walton 1967), genetics (Dillon and Robinson 2009; Dillon 1984; Henry et al. 2005), life history (Huryn et al. 1994), and environmental limits (Hoverman et al. 2011; Ross and Ultsch 1980; Sura and Mahon 2011). No studies have yet worked on any of these topics in the northern population found here in Pennsylvania.

Early work found *P. proxima* in four different streams in the White Clay Creek and Brandywine Creek subwatersheds of Christina River basin. The purpose of this project was to determine, to the extent possible, the extent of the distribution of *P. proxima* in streams in the Christina basin area. Studies of related snail species indicate that distribution may be affected by substrate, water depth, and current (Houp 1970).

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Determination of the ecological constraints and conditions for *P. proxima* distribution in this area was the secondary purpose of this project. This study attempts to relate distribution in Christina Basin with physicochemical parameters like land use, water chemistry, velocity, substrate, and depth.

METHODS AND ANALYSIS

Study Area

Fieldwork was conducted during January and February 2012 in the Christina River watershed. The Christina River watershed is 565 square miles and encompasses three different states (Christina Basin Clean Water Partnership, 2003). Average temperature ranges from between 25 and 40°F in the winter and between 70 and 85°F in the summer (The Weather Channel). Surveys targeted headwater streams in the two subwatersheds where snails were previously identified as well as the Red Clay Creek subwatershed, which lies between the two (Fig. 3). A list of potential stream sites was organized using GoogleEarth. From a list of headwater streams at road crossings or on public lands sites were selected for sampling in winter 2012. Sites were typically named for the nearest road.

Stream Data

Latitude and longitude coordinates were taken with a GPS (Garmin 60CSx) typically while standing on the road as close as possible to the stream. Water temperature (°C), dissolved oxygen (% and mg/L), conductivity, salinity (ppt), and pH were measured at most sites (YSI Pro Plus and pH100, Yellow Springs, Ohio, USA). Chemistry measurements were not taken at sites visited on the weekends or when the battery levels in the instruments were low. Snail surveys were conducted by walking the center of the stream for roughly 40-m, and taking sediment samples by swiping a 4" x 3" aquarium net through loose sediment at approximately 10 m intervals. *P. proxima* presence was determined by visual observation of snails and positive identification of the species.

Snails were determined to be absent when no snails were observed in a 40-m visual survey or in sediment samples in the stream. At sites where one side of the road was private property, roughly 40-m on the public side were walked.

Transect Data

Riparian habitat and stream characteristics were determined at the sites where *P*. *proxima* were present. Left bank and right bank characteristics (% cover of plant, rock, and exposed dirt) as well as classification of riparian vegetation (dominance of trees, shrubs, or grasses) were recorded. To characterize the stream, stream width (cm), maximum depth, and velocity were measured at 8-10 transects roughly 5 meters apart. Velocity was measured by the number of seconds it took for either a leaf or a sediment cloud (depending on the stream) to travel one meter. These values were later converted to meters per second. At each transect, two to three photos of the stream bottom were taken through a 30cm diameter bucket with a plastic grid attached to the bottom, for use in substrate analysis (Fig. 4). Snail density was measured at five of the eight sites where snails were observed by counting the number of snails within 6 inches (15.2cm) upstream or downstream of the transect. The snail density was calculated as the number of snails per meter of stream width.

ANALYSIS

GIS (Geospatial Analysis)

Latitude and longitude coordinates were converted into decimal coordinates, and imported into ArcGIS. Where a point was not available latitude and longitude were

estimated using GoogleMaps. Watershed was estimated as a 200-m radius circle around each site. The land use within 100-m, 200-m, and 400-m radii were examined, but ultimately a 200-m radius buffer was deemed the best. This 200-m radius buffer was combined with a land use layer of the Christina Basin watershed (Fig. 5; C. Dow, Stroud Water Research Center, Avondale, PA 19348), to estimate the land use in the watershed of each site (Fig. 6). The land use categories were simplified to four: agricultural, developed, forest, and water/wetlands. The area of land in each use category was determined using the cell count for each usage and the known cell area of 900-m² for each buffered region. These values were converted into percentages of the whole buffered area. The GIS watershed layer also helped to verify which subwatershed of Christina River Basin each site was located in.

Stream Data

Chemistry and land use data for each stream was analyzed for normality using the Shapiro-Wilk test. Given the results of this test, a nonparametric Spearman's Rho was used to test correlation between the variables. To test for influence on the presence of snails, each variable was analyzed using the nonparametric Wilcoxon rank-sums test. These tests were conducted using JUMP software.

Substrate

Substrate photos were visualized on a computer and the dominant substrate in each cell was determined. Substrate was characterized as silt, sand, gravel, or cobble. Gravel was estimated as pea to baseball sized, and cobble as larger than baseball sized rocks. Percent of each substrate class was estimated by counting the number of grid squares that was comprised of each type of substrate. These percents were then averaged across photos in the same transect. Since the area analyzed was the same for each transect and site, and the same person did the estimating, there is reasonable confidence in the accuracy of the estimates.

Transect Data

Physical variables including substrate percentages were analyzed for normality using the Shapiro-Wilk test. Spearman's nonparametric rho was used to examine correlations between the variables. A nonparametric Wilcoxon test was conducted to compare average values for each physical variable between sites. A Tukey-Kramer HSD analysis was also used in this site by site analysis to make a pairwise comparison of means. Despite requiring normality, the Tukey-Kramer was useful to identify the significant relationships. Spearman's correlations were conducted to look at the relationship between snail density and other variables at the transect level, rather than at the site level using averages.

Multivariate Regression

Lastly, to examine what combination of variables could best describe density, stream and transect level data were combined in a multivariate stepwise regression against snail density.

RESULTS

Four of the 102 new sites contained *P. proxima*, including two in the Red Clay Creek subwatershed where none were previously known. Disregarding the four sites where *P. proxima* was already known, there was about a 4% discovery rate. There are very few streams in the area that have *P. proxima*, but they do exist in at least three of the subwatersheds of Christina Basin. Two other species of snail were found during this field season in a handful of sites each, but they were never found co-occurring with *P. proxima* (Fig. 7). Only sites with *P. proxima* were considered to be sites with snails. Data for each site can be found in Appendix 1.

Stream Data

Stream Characteristics

Stream data from all sites, with and without *P. proxima*, was analyzed to assess the characteristics of streams that snails favored. All the water chemistry and land use variables except mg/L dissolved oxygen had a Shapiro-Wilk W whose p-value was small enough to reject the null hypothesis of normality (Table 1). Due to these deviations from normality, the nonparametric Spearman's test was used to assess correlations between the variables.

The observed water temperatures ranged from 2.2 to 11.5° C, as expected of the mild winter sampling season (Fig. 8a). The weather affected the stream temperature during sampling, and likely affected the other chemistry measurements. pH was generally slightly alkaline, though dipped as low as 5.41 at one site (Fig. 8b). pH and water temperature were negatively correlated (p = 0.0015)

Dissolved oxygen was above 100% in almost half the samples, reaching a maximum of 180%, indicating that the streams were highly saturated despite low winter photosynthetic levels (Fig. 8c). This meets the expectation for winter sampling, because colder temperatures increase dissolved oxygen (Allan and Castillo 2007). This negative correlation was observed at p < 0.0001.

Salinity ranged widely from 0.02 to 0.45 (Fig. 8f). As expected, salinity and conductivity were positively correlated (p < 0.0001). They both were negatively correlated with temperature ($p_s = 0.0002$ and $p_c < 0.0001$) and mg/L dissolved oxygen ($p_s = 0.0352$ and $p_c = 0.0184$) as expected, due to reduced solubility of oxygen in more saline water.

Percent developed land was significantly positively correlated with temperature (p = 0.0498), conductivity (p < 0.0001), salinity (p < 0.0001), and pH (p = 0.0147). These relationships follow expectations for the effect of developed land on water quality (Kelly et al. 2012). In contrast, percent forest cover was significantly negatively correlated with temperature (p = 0.0147), conductivity (p = 0.0034), and salinity (p = 0.0164). In general, the streams in this study were cool, slightly alkaline, saturated with dissolved oxygen, and varied widely in salinity and conductivity. Correlations of all the variables are presented in Appendix 2.

Presence of P. proxima

To determine the effect of stream chemistry and land use on the presence of *P*. *proxima*, these variables were compared between sites with and without snails. Neither stream chemistry nor land use differed between sites based on presence of *P. proxima*

(Table 2; Fig. 8, Wilcoxon Test, p>0.05). Given the large disparity between the number of sites with snails and the number of sites without snails, this result was not surprising. Stream chemistry may have differed between the sites but this study may have had low power to detect it.

The range of each variable for the sites without snails completely encompassed the range for the sites with snails so no one variable seemed to directly explain presence or absence of the species. There is some evidence for exclusion, however, from the maximum and minimum values for some of the variables. Conductivity reached a maximum of 337 uC/cm in sites with snails, compared to a maximum of 674 uC/cm in sites without snails (Fig. 8e). As expected, salinity also displayed a much reduced maximum value for sites with snails; 0.24 versus 0.45ppt (Fig. 8f).

The maximum percent of the land cover within a 200-m radius of the stream that was developed was 17% among sites with snails, whereas up to 100% of the land was developed among sites without snails (Fig. 8h). Similarly, the percent forest cover in sites with snails reached a minimum of 32%, whereas sites without snails had as low as 0% forest cover (Fig. 8i). Sites with snails reached a maximum agricultural cover of 52%, though the maximum overall was 93% cover (Fig. 8g). In summary, snails were found in sites with less than 17% developed land cover, less than 52% agricultural cover, or more than 32% forest cover.

These results give an impression of the habitat conditions that *P. proxima* require, but they do not definitely indicate what streams snails will be found in. Though the snails were only found in certain bounds of percent land cover, there were still sites with similar cover that lacked snails. Nevertheless, to determine what potential water chemistry factors may exclude snails a post hoc test was used to compare chemical variables in sites with land use percentages similar to those in which snails were found and sites with land use percentages in which snails were absent. Sites were divided by each land use category based on the percent cutoff for snails: 20% for developed, 55% for agricultural, and 35% for forest cover, and the water chemistry variables were compared using the Wilcoxon Test.

Conductivity, salinity, and pH were all significantly lower in the sites with low development (<20% developed cover) (Table 3; Fig. 9, Wilcoxon Test, p<0.05). Temperature, conductivity, and salinity were also lower among the sites with high forest cover (>35% forest cover) (Table 4; Fig. 10, Wilcoxon Test, p<0.05). No water chemistry variables differed between sites grouped by agriculture (Table 5; Fig. 11, Wilcoxon, p>0.05).

Transect Data

Reach Characteristics

Transect data from five sites with *P. proxima* was analyzed to assess the characteristics among and within streams that snails favored. Normality was tested using the Shapiro-Wilk goodness-of-fit test (Table 6). Only average gravel failed to reject Ho, and may therefore be normally distributed. Average gravel was correlated with lower max depth (p = 0.0032), higher width to depth ratio (p = 0.0013), and higher average velocity (p = 0.0535). Average silt was correlated with high maximum depth (p < 0.0001), low width to depth ratio (p = 0.0135), and low average velocity (p = 0.0004). Where the stream was wider, it was dominated by larger particles, deeper water, and

higher flow. Narrow streams were characterized by smaller substrate particles, and shallow water with low flow. These characteristics make sense because slower moving water allows time for small suspended sediments to settle to the substrate. Full transect data are in Appendix 3.

Among Site Comparisons

To determine if between site differences contributed to variation in site preference, average snail density for all transects at a site were compared (Figs. 12-20). Snail density data was available for only five of the eight sites where *P. proxima* were observed. Mill Vet and Eaten Run streams have higher snail density than the other three sites where this data was available (Fig. 12). These two sites also have lower stream width than the other sites (Fig. 13). There was no difference in percent sand or cobble among the five sites examined, but percent silt and percent gravel differed. Eaten Run and Mill Vet showed opposed substrate dominance and differed significantly in their average composition of gravel (p < 0.0001) (Fig. 19). This suggests that narrower streams had, on average, higher density of snails than wider streams. No relationship was found with substrate using this analysis. Full results of Wilcoxon Tests are presented in Appendix 4.

Within Site Comparisons

To assess the characteristics within a stream that snails favored, the correlation of physical characteristics with snail density were determined. Correlations were calculated using the data from all transects in the five sites where density was available. Snail density was negatively correlated with stream width (p < 0.0001), and with width to depth ratio (p < 0.0001). This follows the site level analysis, where it was found that sites with the highest densities of snails had smaller widths, although future work should confirm if this relationship holds with density per unit area as well as density per unit width. Density was also negatively correlated with cobble cover (p = 0.0103). This result was not found in the comparison of average values at each site, and that may be because the site by site analysis masks important intra-site environmental differences. Full correlations are presented in Appendix 5.

Multiple Regression

To assess the impact of the chemistry, land use, and physical data together on snail density, two stepwise multivariate regressions were conducted. The model with the largest adjusted r^2 value included maximum depth, temperature, dissolved oxygen (either as mg/L or %), and percent forest cover. Using either form of DO the r^2 value was 0.585489, so this combination of variables describes 60% of variation in the density of snails. Snail density was highest in streams that were deeper and cooler with high dissolved oxygen and high forest cover (Table 7, Wilcoxon Test, p<0.05).

Temperature, DO, and forest cover are correlated, however, so the next model excluded chemistry data to determine if snail density was related to the physical data. The best of these models included forest cover, maximum depth, and width. The adjusted r^2 value was 0.359583, indicating the model describes 36% of variation (Table 8, Wilcoxon Test, p<0.05). High density was related to narrow, deep streams with high

forest cover. Percent forest cover was the biggest contributing variable in each model attempted.

DISCUSSION

This study found that *P. proxima* is sporadically distributed throughout the Christina River watershed, but locally abundant. The snail seems to favor more pristine sites with higher forest cover and low levels of development. Within a stream, *P. proxima* appears to favor cooler water with low levels of conductivity. Narrow streams favor snail abundance, though it is not clear what impact substrate has on *P. proxima* local density.

New snail sites

The 4% discovery rate for *P. proxima* in the Christina Basin implies that the species is very rare in the area. Foin (1971) observed that the southern populations of *P. proxima* are typically found in aggregated populations. The results from this study indicate that this northern population similarly clusters in high densities. Although *P. proxima* has now been discovered in three of the four subwatersheds of the Christina Basin, its presence in the Christina River, the last of the four, as well as in other nearby watersheds is as yet unknown. Further survey work in the area may turn up more sites, which would greatly add to the current information about habitat preferences and trends. Future work on the other two species discovered may also add valuable insight into potential habitat partitioning between those species and *P. proxima*.

GIS

The land use within a 200-m radius buffer was used for land use analysis in this study. Given that the resolution of the land use map was only 30-m, the 100-m radius

seemed too small to get adequate data. The 400-m radius encompassed a much larger area, but in many cases a large portion of the circles of two sites overlapped. Cunningham et al. (2010) also used a 200-m buffer around stream sampling sites as an estimate of watershed. Since sampling was not conducted at an edge of the population distribution, it seemed acceptable to include area downstream of the sampling site because the population likely continued on. In the future, it would be helpful to determine how far upstream and downstream the snails are located at each site in case that would affect land use designations, and to test for environmental limits to distribution within a stream.

A 30-m resolution in the land use layer likely did not pose a problem for the analysis because riparian vegetation of less than 30-m, Cunningham et al. (2010) argues, has little effect on water quality anyway. It is not clear, however, how big of an impact land use further than 200-m away has on stream water quality. Jin et al. (2011) used GIS to define the subcatchment boundaries above each sampling site, rather than to buffer a predetermined area around each site. Originally, outside help was sought in this study to similarly define the watershed upstream of each point using hydrology and topography maps, but this effort was unsuccessful because of problems with the accuracy of the coordinates. A future reanalysis of land use using this technique would make an interesting comparison to results from this study.

Chemistry

In this study dissolved oxygen (DO) levels were high in all streams and did not differ between streams with *P. proxima* and those without. Houp (1970) suggested that

there are more *Pleurocerid* snails where there is higher dissolved oxygen. Low DO is actually an indicator of pollution in the Hilsenhoff biotic pollution tolerance index (Cunningham et al. 2010), and can cause stream stratification (Findlay and Kelly 2011). This study did not find the level of dissolved oxygen to be a limiting factor in the presence of snails, but since the surveys occurred in the winter, it could not be determined if summer DO levels would reach stressful levels. A survey of stream sites throughout the year would be helpful to examine if there are significant changes in DO across seasons, and reveal unfavorable levels of DO in streams without snails at other times of the year.

pH in the streams in this study was generally slightly alkaline. There was no observed relationship between pH and presence of snails, but since the range did not vary greatly, this is not surprising. Snail presence has been associated with harder, more alkaline water in other studies (Hoverman et al. 2011), and Houp (1970) even claims that water with a pH lower than 7.0 does not permit *Pleurocerid* life at all. Hard water may be preferable to freshwater snails because high calcium carbonate levels are beneficial for shell development, or because it is often associated with greater abundance and diversity of plant species and detrital decomposition (Hoverman et al. 2011). It is possible that since all the streams in this survey are slightly alkaline, they have adequate pH conditions to support *Pleurocerid* life and some other limiting factor is at work. A laboratory tolerance experiment of different pH levels could help verify that *P. proxima* in Pennsylvania also prefer more alkaline streams.

Stream sites with *P. proxima* had a minimum forest cover of 32%, and a maximum developed land cover of 17%. Forest cover is correlated with low temperature

and low conductivity. This makes sense because forest riparian buffer of at least 50-m has been shown to improve water quality in many ways, including reducing ion input (thereby lowering conductivity) and also shading the stream (decreasing the temperature) (Cunningham et al. 2010).

Developed land cover is correlated with high temperature, conductivity, and pH. High conductivity is typically considered a negative impact of urban development (Cunningham et al. 2010; Corsi et al. 2010; Mullaney et al. 2009). Corsi et al. (2010) found that areas with greater than 98% urban land use have the highest average maximum conductivity and that declines in biological integrity could be observed with as little as 7-12% impervious cover. Urbanization and runoff from roads, parking lots, and other paved surfaces contribute a wide variety of non-point source pollutants including ions that increase conductivity of stream water and exclude snails from areas with high urban cover (Cunningham et al. 2010).

When the stream sites were divided into two groups based on the cutoff of snail presence (greater or less than 20% developed and greater or less than 35% forest), the group with snails was associated with lower conductivity and pH in both cases. Limitation of *P. proxima* to areas with high forest cover or low urban cover could be related to a variety of environmental factors, but these data suggest that constraints by conductivity may be an important component. In fact, stream sites with snails had a maximum conductivity about half that of streams without snails.

A 2010 study on remediation of urban streams with riparian green space found that added riparian vegetation did not improve conductivity levels (Cunningham et al. 2010). This suggests that levels of conductivity might be more impacted by the wider watershed than by the character of riparian vegetation. This matches the data in this study because conductivity is related to watershed-level land use data. It appears that the land use and practices in this wider area might be important factors in stream conductivity levels, and perhaps *P. proxima* distribution.

A French study on aquatic gastropods found that high conductivity appeared unfavorable for snail populations (Costil et al. 2001). High concentrations of ions like sodium and chloride in the water column can endanger the biological integrity of aquatic systems in many ways (Findlay and Kelly 2011; Bartlett et al. 2012; Jin et al. 2011). Findlay and Kelly (2011) note that high concentrations of sodium compete with other cations for attachment to negatively charged sites on soil particles. They also recognized that ions could directly affect benthic organisms by altering their osmotic balance. Tolerance thresholds for changing osmotic conditions in the environment vary across organisms and life stages, but generally, Findlay and Kelly (2011) write, macroinvertebrate communities do worse under osmotic stress.

Salinity of freshwaters is increasing globally from both natural and anthropogenic sources, but human direct impacts are especially prominent in areas with high levels of urban development (Bartlett et al. 2012). Human influences on ion levels in streams can be attributed to leaching from landfills, discharge from wastewater and drinking water treatment facilities, runoff from salt storage, and dissolution of de-icing salts on paved surfaces (Mullaney et al. 2009; Jin et al. 2011). Chloride from road salt application is a major component of urban runoff and human contribution to high stream conductivity (Cunningham et al. 2010; Bartlett et al. 2012; Mullaney et al. 2009). Chloride concentration is related to the density of major roads and the percent annual runoff from saturated overland flows (Mullaney et al. 2009). Wells in urban areas produce water with concentrations of chloride higher than water from wells in both agricultural and forested areas (Mullaney et al. 2009). Given the relationship between conductivity and urban cover in this study, it appears that high levels of conductivity can be attributed to an anthropogenic source. A likely candidate is chloride from road salt dissolution.

Other studies have attempted to specifically test if elevated levels of chloride come from anthropogenic or natural sources. One study compared the levels of conductivity and chloride concentration over a 24-month period based on the assumption that anthropogenic sources of chloride are seasonal in nature whereas as natural sources of chloride and other ions are more constant throughout the year (Cunningham et al. 2010). Winter road salting is a major seasonal source that is especially relevant to streams in Northern North America. Runoff from freshly salted roads and paved areas increases in the winter and decreases in the summer and fall (Kelly et al. 2012; Corsi et al. 2010; Bartlett et al. 2012). Conductivity values that peaked in the winter months could be designated as anthropogenic (Cunningham et al. 2010). A New York state study more carefully attributed elevated levels to road salt runoff by testing for specific ions instead of merely level of conductivity (Jin et al. 2011). A study in Sweden found that salinity in streams increased in direct proportion to the rate of application of road salt (Corsi et al. 2010). Whatever the method, it is clear that road salt is a major source of chloride and thus high specific conductivity in streams in urban areas including in Christina Basin.

The environmental issues related to application of de-icing salt on highways and paved areas have been discussed since the 1960s (Corsi et al. 2010). Despite the early

recognition of the impacts of the practice, increasing levels of urbanization have necessitated ever-increasing salt use to keep the roads safe (Mullaney et al. 2009). Road salt usage is now at 18 million tons per year in the United States and shows little sign of slowing down (Bartlett et al. 2012).

Christina Basin Watershed is no exception to the trend for increasing road salt usage in the United States. Spanning Pennsylvania, Maryland, and Delaware, this 565 mi^2 suburbanizing watershed, home to close to 0.5 million people, has lost more than 15% of its open land since 1970 (CBCWP 2003). The area is known for trout fishing, mushroom farms, an active port, horse farms, and as home to a Wild and Scenic River, White Clay Creek. The Christina River watershed is currently about one third urban/suburban, one third agricultural, and one third forest, but with thriving corporations in big cities like Wilmington, DE, suburbanization is likely to continue. Agricultural and urban effluent, as well as development, are major factors affecting stream water quality that may limit the distribution of *P. proxima* in Christina Basin, which seems to favor more pristine streams.

Recent research suggests that salts like NaCl do not simply travel through soil and groundwater and get flushed downstream by spring rains, but have much longer retention rates (Findlay and Kelly 2011). Toxic effects on aquatic systems may span over a much broader area and longer time scale than previously believed (Findlay and Kelly 2011; Corsi et al. 2010; Bartlett et al. 2012). Consistently high chloride levels may persist for much of the year, and have an even greater effect on benthic communities like *P. proxima* (Bartlett et al. 2012). If salts are accumulating rather than getting flushed away,

it is possible the impacts of road salt application from the last several decades have yet to be seen.

The data in this study, despite strong associations, do not conclusively point to the impact of specific conductivity, or particularly chloride, on the distribution of *P. proxima*. These associations may instead implicate other contaminants that co-vary with conductivity and urban runoff including stream geomorphology, habitat availability, and other metals and organic compounds (Findlay and Kelly 2011).

Physical Characteristics

In this study, snail abundance was estimated by taking the snail count in a 6-inch wide area over the length of each transect. This value was divided by width to adjust to varying sizes of the streams. This measure was a rough estimate because the area observed in each transect was not the same, but depended on the transect width. Several other methods were tried to get a measure of density, but they were not successful. The first attempt was to use the substrate photos to count the number of snails in that area. This would have resulted in a snail count within the same area for each transect. This was not possible, however, because it was too difficult to see the snails accurately in a photo. Then snails were collected from a 4" by 3" net swipe of sediment and rocks. This method was attempted at Boy Scout Spring, resulting in a count of 78 snails. Hoverman et al. (2011) also used this technique, called "dipnetting." Although this method revealed many snails that were hidden in the sediment or were too small to see through the glare of the water, it was too time-consuming and therefore infeasible to conduct at each transect.

Snail counts were conducted at the last five of eight snail sites because other methods were being tested out during the first three stream surveys. Snail count was attempted post-sampling at one of these first three sites, London Grove, about a month after the rest of the data about the site was collected. This day happened to be one of the warmest in the entire season, especially compared to the freezing temperatures on the day the site was first sampled. On this warm day there were many more snails than on the first day the site was visited (personal observation; S. Windecker). It was evident that the snail count needed to be taken at the same time as the other chemistry and physical data. In addition, the snail counts were not conducted at exactly the same sites along the stream, so it would have been too difficult to correlate the density count with the other measured variables for each transect for the first three sites.

One possible explanation for the apparent increase in the number of snails on the warmer day is aestivation or hibernation. Aestivation is a dormant state of reduced energy consumption in response to unfavorable weather conditions. Many prosobranch snails aestivate to survive dryness, stressful ambient temperature, and shortage of food (Nowakowska et al. 2010). Growth retardation (Houp 1970) and high mortality (Ross and Ultsch 1980; Paukstis et al. 1997) have been observed in species of *Pleurocera* when exposed to freezing temperatures. The lower observed abundance of individuals during the first survey at London Grove might be related to hibernation by snails due to the below-freezing temperature. Laboratory experiments on the temperature preferences and tolerances of *P. proxima* may provide insight into limiting conditions for the species. In addition, field surveys throughout the year would provide data on changing abundance, perhaps supporting the theory of aestivation or hibernation by *P. proxima* in this area.

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Although the bucket substrate photos were not clear enough to estimate snail density, they were good enough to measure percent cover of substrate. Cunningham et al. (2010), in a study on water quality impact on benthic macroinvertebrates, similarly examined substrate by determining percent cover of sand, silt, and clay. Although the plastic grid on the bottom of the bucket enabled fairly accurate estimate of percent cover by each substrate type, this method did not directly establish a substrate preference by snails. Each transect had a density measure and a substrate measure, but it was not possible to determine exactly what substrate snails were using. Ross and Ultsch (1980) remedied this problem in an experiment on the habitat preferences of two other species of *Pleurocerid* snails. Ross and Ultsch (1980) collected individuals by hand and recorded the underlying substrate. This method afforded greater precision of the substrate preference compared to this study because exact substrate usage was recorded. In the future, transect surveys could be repeated using this method to determine if *P. proxima* in the Christina River Basin demonstrate a substrate preference.

In the site by site analysis each site was compared based on average values for each physical variable. The two sites with the highest average snail density had opposite substrate dominance. One was composed mainly of cobble and the other of silt. When each transect was examined separately, rather than on average by site, the overall effect was a slight negative correlation with cobble. This difference in result may be due to masked differences in the averages at each transect.

Other studies on species of *Pleurocera* have found that substrate is an important factor in their micro-habitat, and that they are rarely found on silt (Krieger and Burbanck 1976; Liu and Resh 1997; Houp 1970; Ross and Ultsch 1980; Cunningham et al. 2010).

It has been suggested that predominantly silty areas have greater turbidity, which may reduce the photosynthetic capability and growth of the snails' food source (Cunningham et al. 2010). In addition, coarse matter in the water column has been found to contribute to apical tip erosion (Houp 1970), as was found at a few streams in this survey. Rocky surfaces and clear water are better suited for periphyton growth and therefore for macroinvertebrate life. Sand, in contrast to silt, is supposed to be necessary for *Pleurocerid* development because it is used to coat the eggs during reproduction (Houp 1970). Krieger and Burbanck (1976) did a study on the distribution of a species of *Pleurocera* snail in Georgia, and found that their distribution was restricted by unstable substratum and high stream velocity. This may suggest preference for an intermediate stream velocity that is associated with substrate that is not silty, but still has low enough flow to have sand that is needed for reproduction. In the multivariate regression none of the substrate variables proved to be important factors in the density of the snails. Although this study did find that the species occupied silty substrate, it is possible that particle size classes for "silt" and "sand" were slightly different between this and other studies, and that substrate preference was too imprecise due to the methodology.

In this study, higher densities of snails were found in streams with small width, both in the analysis of averages by site and by transect. Width was positively correlated with depth; therefore, narrow, shallow streams in the Christina Basin region likely have higher densities of snails. Populations of *P. proxima* in North Carolina are typically associated with shallow streams (Stiven and Walton 1967), and Foin (1971) also found that *P. proxima* cluster in small, headwater streams. Studies of other species of *Pleurocera* also indicate preference for shallow water with slow flow (Houp 1970; Ross and Ultsch 1980).

Smaller streams may make ideal local environments for *P. proxima* because they have the right water velocity, beneficial periphyton growth conditions, fewer predators, less competition, or good canopy cover. Regional patterns in freshwater gastropod species richness and abundance have been attributed to all of these factors in the past (Hoverman et al. 2011). Smaller streams may have lower flow, reducing the risk of dislodgment in the current. Freshwater gastropods have been observed aggregating to stream edges and onto rocks during flooding for this very reason (Houp 1970). Grazing on periphyton requires that the water conditions be adequate for their growth and survival and for a standing crop to be present to support the population. Perhaps preference for stream physical characteristics has more do to with the requirements of their food source than of themselves.

Predation may be a key factor limiting *P. proxima* to small streams. Fish were never observed during sampling at sites with snails (personal observation; S. Windecker), though some have been spotted at other times. There are many species that occupy the larger streams in the area and that could be predators of *P. proxima*. Smaller, insectivorous fish in the Christina River basin include the rosyside dace, blacknose dace, longnose dace, golden shiner, tessellated darter, common shiner, and juvenile creek chubs and cutlips minnows. Larger, piscivorous predators include the brown trout, bluegill, pumpkinseed, and rock bass (personal communication; L. Borecki, SWRC, 16 May 2012). Prosobranch snails can generally resist predation by molluscivores because of their thick shells, and by shell-invading invertebrates by retreating behind their operculum (Hoverman et al. 2011), but predation may still be an important factor limiting *P. proxima* distribution. In the future, a predation experiment of the snail by different species of potential predator could provide some clarity to this question.

Two other gastropod species were found during this field season, though they were never found in the same stream as *P. proxima* (personal observation; S. Windecker). Competition among these three species may be an important factor in *P. proxima* distribution if they in fact occupy the same habitats. A study of the distribution and habitat characteristics of these other species is needed to understand if they occupy separate niches or are direct competitors.

Although benefits of canopy cover like reduction in salinity require a large forested area in the surrounding watershed, Cunningham et al. (2010) found that small forest riparian areas are still beneficial to streams by reducing total inorganic nitrogen and through shading. Greater canopy cover in small, headwater streams than in wider streams may provide some of the conditions ideal for *P. proxima* survival.

Future efforts to discern micro-habitat conditions favored by *P. proxima* should focus on full transect-level surveys at all sites, with and without snails. This was the original intention, but the method had to be adjusted because it was too time-consuming. Due to the low encounter rate of new snail sites, a shortened method was selected to allow for more ground to be covered. This enabled a wider watershed analysis, but limited the scope of the habitat analysis.

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CONCLUSION

In both final multivariable models, forest cover was the most important variable. The models also related high density of snails to cool, narrow, deep, streams with high dissolved oxygen. The results from the models match the results from other analyses. Many of these variables are correlated, so it is difficult to determine what exact influence is the most important for *P. proxima* distribution. A combination of these variables, or some other factor entirely that co-varies with them may be the solution.

Further work on the distribution of the species might look in Christina River, the last of the four subwatersheds. To better understand the habitat requirements of the species, future work in the field is needed to analyze the difference in physical variables between sites with and without snails. Future work in the lab would improve understanding of the relationship between the species and its food source, predators, and other gastropod competitors.

High forest cover and low developed land cover, and the associated lower levels of conductivity seem to be important exclusionary influences on snail presence. Since *Pleurocerid* snails have been described as "clean-water organisms" (Houp 1970), responding negatively to changes in temperature, nutrients, and chemical and physical pollutants, this is not unreasonable. Freshwater gastropods are often used as indicators of stressed systems (Bartlett et al. 2012). Many studies have shown that contaminants contribute to impoverished benthic habitats, and that benthic macroinvertebrates respond to pollutant levels in water (Houp 1970; Hoverman et al. 2011; Cunningham et al. 2010; Bartlett et al. 2012). Perhaps *P. proxima* are acting as indicators of better water quality in this area, but further research is needed to confirm this hypothesis.

TABLES & FIGURES



Figure 1. *Pleurocera proxima* (S. Windecker).

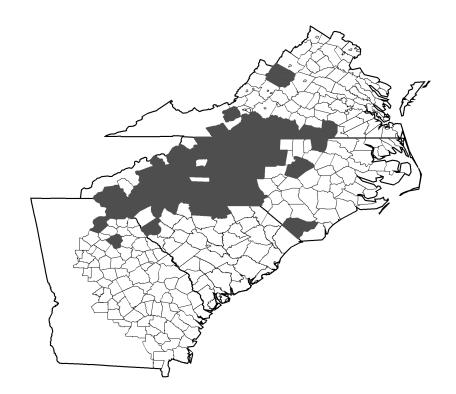


Figure 2. Previously known distribution of *P. proxima* in Southeastern United States (Dillon and Robinson 2009).

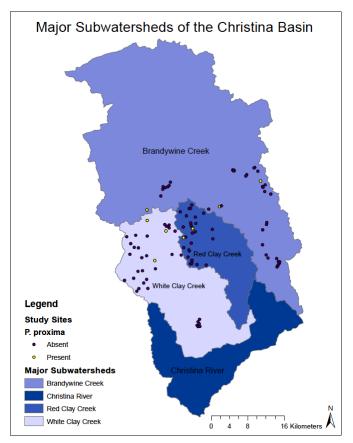


Figure 3. Major Subwatersheds of the Christina Basin (ArcGIS; S. Windecker).



Figure 4. Example of substrate analysis photo. Photos were examined to estimate % cover of silt, sand, gravel, and cobble (S. Windecker).

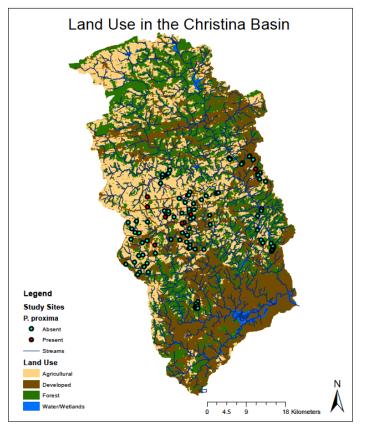


Figure 5. Land Use in the Christina Basin (ArcGIS; S. Windecker).

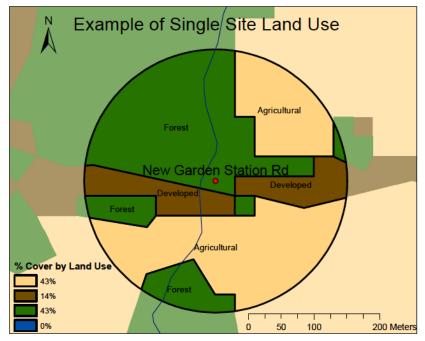


Figure 6. Sample of land use analysis for single site. Red dot is the study site and the blue line is the stream. The circle depicts the land use within a 200-m radius of the study site (ArcGIS; S. Windecker).



Figure 7. Two other species of freshwater gastropod observed during Winter 2012 field season. Never found co-occurring with *P. proxima* (S. Windecker).

Variable	W	Ν	р
Temp (C)	0.948914	80	0.0030*
DO (%)	0.616150	77	<0.0001*
DO (mg/L)	0.993716	77	0.9717
Cond	0.934881	80	0.0005*
Sal	0.946254	80	0.0021*
рН	0.896308	80	<0.0001*
%A	0.854817	106	<0.0001*
%D	0.866765	106	<0.0001*
%F	0.948247	106	<0.0004*
%W	0.579210	106	<0.0001*

Table 1. Shapiro-Wilk tests for normality of water chemistry and land use data at study sites in Christina Basin.

* significant p-values at P<0.05 reject Ho of normality.

Wilcoxon Rank	2-Sample	Test		1-way Test		
Sums Tests						
Variable	S	Ζ	р	ChiSquare	df	p
Temp (C)	323	-0.00802	0.9936	0.0003	1	0.9872
DO (%)	285.5	-0.43422	0.6641	0.1959	1	0.6581
DO (mg/L)	272	-0.65968	0.5095	0.4463	1	0.5041
Cond	303	-0.32877	0.7423	0.1134	1	0.7363
Sal	353.5	0.46566	0.6415	0.2244	1	0.6357
pH	274	-0.79398	0.4272	0.6432	1	0.4226
% A	454	0.30927	0.7571	0.0994	1	0.7525
% D	315	-0.34707	0.1780	1.8308	1	0.1760
% F	571	1.70449	0.0883	2.9257	1	0.0872
% W	409	-0.27845	0.7807	0.0818	1	0.7749

Table 2. One-way Analyses of water chemistry and land use variables by *P. proxima* presence (Wilcoxon Rank Sum Test; P>0.05 for all comparisons).

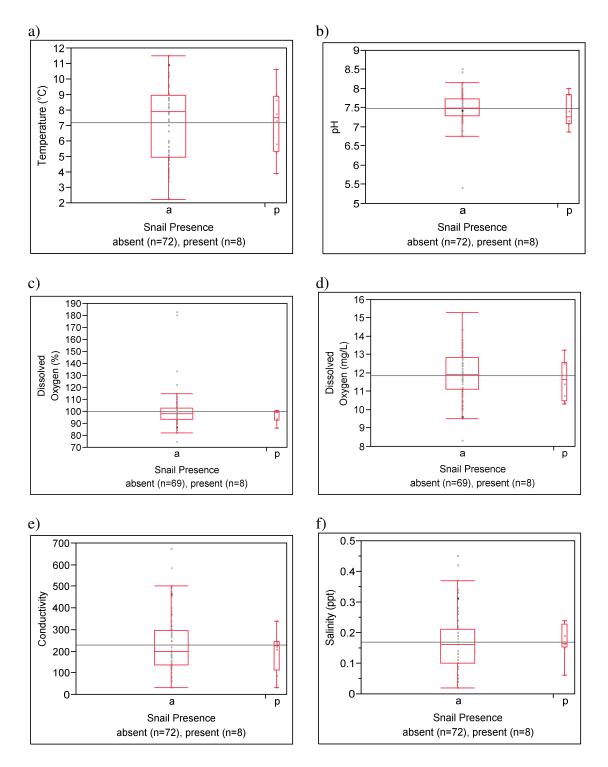


Figure 8. Box plot of water chemistry in streams with and without snails (Wilcoxon Rank Sum Test; P>0.05 for all comparisons).

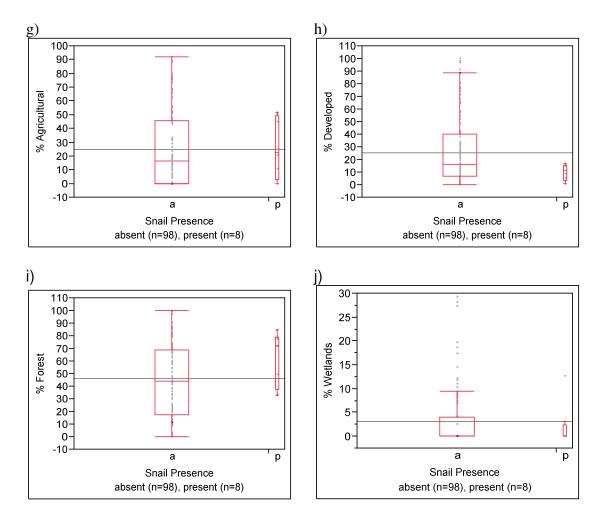


Figure 8. Box plot of water chemistry in streams with and without snails (Wilcoxon Rank Sum Test; P>0.05 for all comparisons).

Wilcoxon Rank	2-Sample	e Test		1-way Test		
Sums Tests						
Variable	S	Ζ	р	ChiSquare	df	p
Temp (C)	1657.5	1.13738	0.2554	1.3046	1	0.2534
DO (%)	1464.5	0.21419	0.8304	0.0481	1	0.8264
DO (mg/L)	1368.5	-0.75476	0.4504	0.5774	1	0.4473
Cond	1782	2.33639	0.0195*	5.4813	1	0.0192*
Sal	1778.5	2.30547	0.0211*	5.3375	1	0.0209*
рН	1836	2.85710	0.0043*	8.1906	1	0.0042*

Table 3. One-way Analyses of water chemistry variables by level of Percent Development. (Wilcoxon Rank Sum Test; < 20% N = 40 or 42; > 20% N = 37 or 38).

* significant p-values at P<0.05

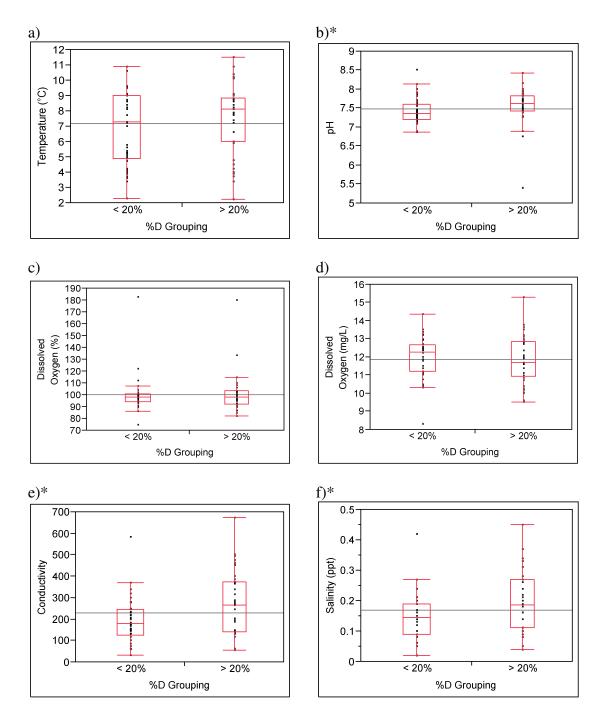


Figure 9. Box plot of water chemistry in streams with high and low percent cover of developed land. Snail sites in <20% D group (Wilcoxon Rank Sum Test; * = significant P <0.05).

Wilcoxon Rank Sums Tests	2-Sampl	e Test		1-way Test		
Variable	S	Ζ	р	ChiSquare	df	p
Temp (C)	1626	2.41963	0.0155*	5.8782	1	0.0153*
DO (%)	1310	0.23169	0.8168	0.0561	1	0.8128
DO (mg/L)	1160	-0.30262	0.1927	1.7102	1	0.1910
Cond	1671	2.85654	0.0043*	8.1876	1	0.0042*
Sal	1625	2.41176	0.0159*	5.8401	1	0.0157*
pН	1453.5	0.73980	0.4594	0.5545	1	0.4565

Table 4. One-way Analyses of water chemistry variables by level of Percent Forest. (Wilcoxon Rank Sum Test; < 35% N = 33 or 34; > 35% N = 44 or 46).

* significant p-values at P<0.05

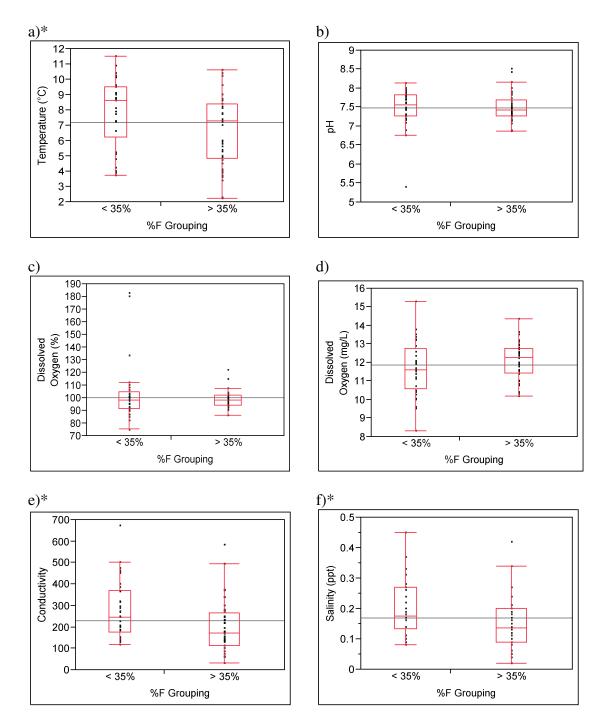


Figure 10. Box plot of water chemistry in streams with high and low percent cover of forest. Snail sites in >35% F group (Wilcoxon Rank Sum Test; * = significant P<0.05).

Table 5. One-way Analyses of water chemistry variables by level of Percent Agriculture. (Wilcoxon Rank Sum Test; P>0.05 for all comparisons; < 35% N = 33 or 34; > 35% N = 44 or 46).

Wilcoxon Rank	2-Sample	e Test		1-way Test		
Sums Tests						
Variable	S	Ζ	р	ChiSquare	df	p
Temp (C)	575.5	1.19974	0.2302	1.4556	1	0.2276
DO (%)	435.5	-0.44958	0.6530	0.2085	1	0.6480
DO (mg/L)	388.5	-1.10991	0.2670	1.2475	1	0.2640
Cond	433	-0.70740	0.4793	0.5100	1	0.4751
Sal	397.5	-1.18718	0.2352	1.4255	1	0.2325
pН	380	-1.42175	0.1551	2.0406	1	0.1532

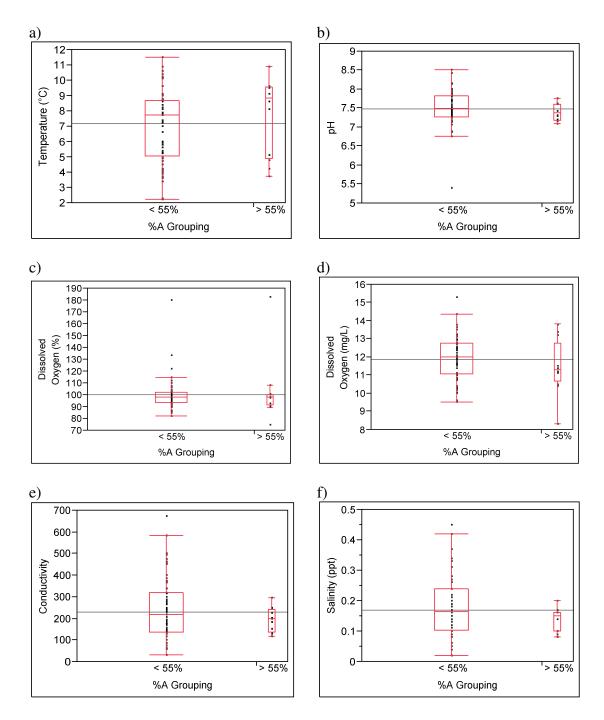


Figure 11. Box plot of water chemistry in streams with high and low percent cover of agriculture. Snail sites in <55% A group (Wilcoxon Rank Sum Test; P>0.05 for all comparisons).

Variable	W	Ν	р
Wetted Width (cm)	0.931773	78	0.0004*
Max Depth (cm)	0.952674	78	0.0057*
Width.Max Depth	0.901244	78	<0.0001*
Ave Velocity (m/s)	0.961989	75	0.0239*
Ave silt	0.929738	78	0.0003*
Ave sand	0.856293	78	<0.0001*
Ave gravel	0.974512	78	0.1193
Ave cobble	0.887136	78	<0.0001*
Density (snails/cm)	0.915700	49	0.0019*

Table 6. Shapiro-Wilk tests for normality of transect physical data at study sites in Christina Basin.

* significant p-values at P<0.05 that reject Ho of normality.

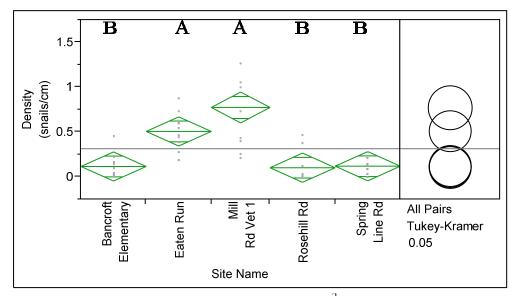


Figure 12. One-way analysis of Density (snails/cm²) by Site. Sites not connected by the same letter are significantly different.

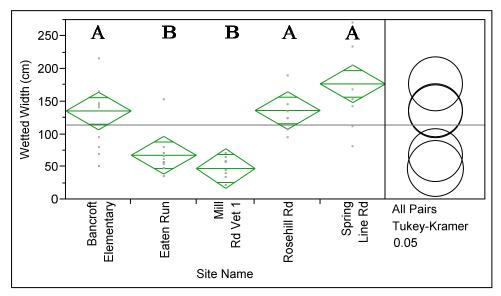


Figure 13. One-way analysis of Wetted Width (cm) by Site. Sites not connected by the same letter are significantly different.

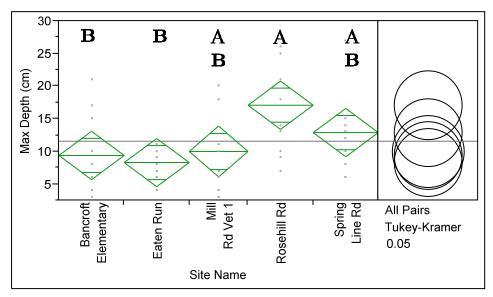


Figure 14. One-way analysis of Maximum Depth (cm) by Site. Sites not connected by the same letter are significantly different.

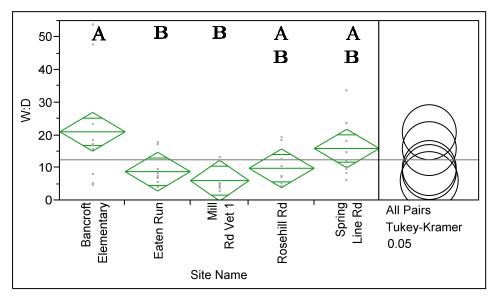


Figure 15: One-way analysis of Width to Max Depth ratio by Site. Sites not connected by the same letter are significantly different.

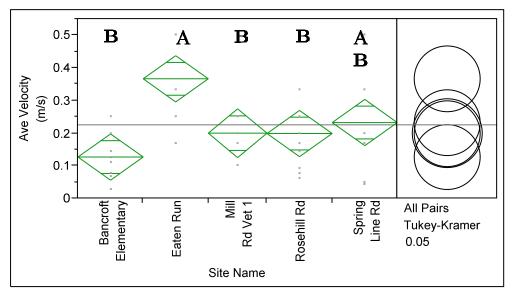


Figure 16. One-way analysis of Average Velocity (m/s) by Site. Sites not connected by the same letter are significantly different.

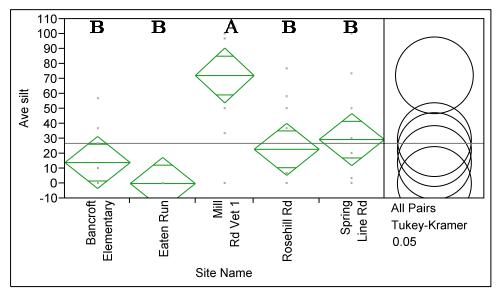


Figure 17. One-way analysis of Average Silt substrate cover by Site. Sites not connected by the same letter are significantly different.

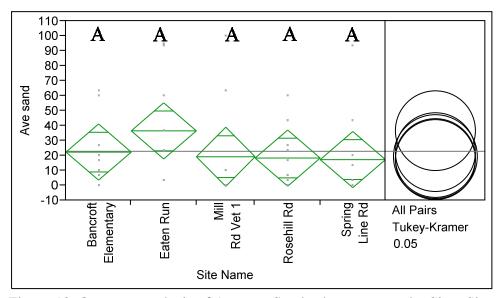


Figure 18. One-way analysis of Average Sand substrate cover by Site. Sites not connected by the same letter are significantly different. No significant differences.

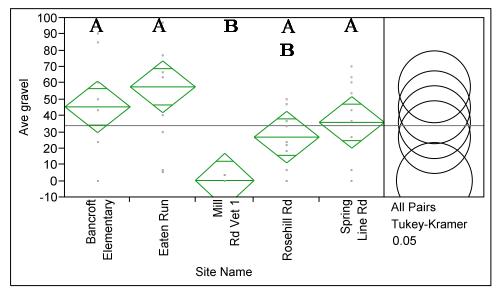


Figure 19. One-way analysis of Average Gravel substrate cover by Site. Sites not connected by the same letter are significantly different.

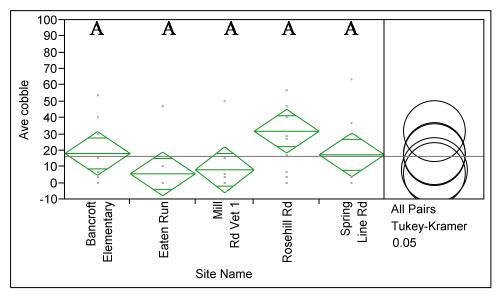


Figure 20. One-way analysis of Average Cobble substrate cover by Site. Sites not connected by the same letter are significantly different. No significant differences.

Model 1			
Term	Estimate	t Ratio	р
Max Depth (cm)	0.0176134	2.94	0.0052*
Temperature (C)	-0.17042	-2.61	0.0124*
Dissolved Oxygen (mg/L)	0.2458013	4.17	0.0001*
% Forest	0.0295196	6.53	<0.0001*
Model 2			
Term	Estimate	t Ratio	р
Max Depth (cm)	0.0175316	2.92	0.0054*
Temperature (C)	-0.242809	-4.09	0.0002*
Dissolved Oxygen (%)	0.0271649	4.17	0.0001*
% Forest	0.0292803	6.47	<0.0001*

Table 7. Results from stepwise multivariate regression of all variables against density. Both models adjusted $r^2 = 0.585498$.

* = significant at P<0.05

Term	Estimate	t Ratio	р
Wetted Width (cm)	-0.002366	-3.41	0.0014*
Max Depth (cm)	0.0192299	2.58	0.0132*
% Forest	0.0088478	3.27	0.0020*

Table 8. Results from stepwise multivariate regression of land use and physical transect variables against density. Adjusted $r^2 = 0.359583$.

* = significant at P<0.05

APPENDICES

Appendix 1. Full site data.

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	88405CC/.UC	SEC10CAL TA	CONCCOSO NA	07615CUU UC	9100811801	45 15901805	0 824901659	9 272650495	43 86554243	0	90796855 15	19.77328901	55.3865978	88 454124	0	13.74880426	62.91062432	9.180678005	31.30235514	26.90991625	75.77342693	75.3727852	100068/77.67	10/67416.00	19.3041/00/	1404001004	1662125896	0	20.57810802	28.96564871	65.85121176	43.01663237	00.96800846 77 97094873	92, 13664675	73.88412087	29.21166517	47.89011525	69.00453038	0	15.26400922	11254025.02			51 50012501	23.81761003	11 6549928	79.60506455	28.93769863	13.00572228
	25	000	0 01	11 2	10.78	689					51				8	8.1	2.9	5.6		5.4		4.7	2.0		14	1.4	48	6.2	8.6	7.8		20	8.0	26	8.9	80	6	9.4	10.4	7.4	2	2	1.1	2.0	10.4	101	9.6	8.8	8.2
	193	717	NC L	138	7 86	101					8 01					7.37	7.08	7.39		7.83		51.1	1.13	0.1	10.1	216	16	661	6.88	7.17		113	101	128	7.42	7.49	7.23	7.42	1.27	7.39	4.1	19	24.8	10	6.76	7.76	7.15	7.28	8.52
4	0.08			110	016	9000					016						0.17			0.17		0.16	11.0	01.0	11.0	71.0	110	0.04	0.2	0.13		0.12	0.24	60 0	0.14	0.1	0.16	0.16	0.34	0.27	/1.0	CT.0	270						
	747	050	V OV	178.7	9570	838					2 205					193.1	243.6	274.7		216.6		201.6	34.4	0.04	4.401	6 181	1457	56.3	286.5	173.7		1.991	9.615	132.1	201.6	115.9	227.8	226.6	494.7	368.4	232.6	C.617	4546	364	451	401.2	295.9	279.6	916
	12.21										176						10.5			12		13.8		12.0						12.6		811		115		11.7							117		646				14.37
	. 10	X		t, vr		9					5					4	4	4		96			2		80	201		33	5	4		101	200	100	80	86	10	5	-	2	6	7 9	2	1 5	22	0	1 00	5	22
	1001			1 93.5							2 00 0					2 102	1 89.4					107.7					10		-	104		=:					101						101				97.8		
() duar	22	100	501	14	101	58					53					8	8.1	5.6		5.4		4.8	2.0		14	fv	45	5.9	8.6	1			20	6	8.6	L'L	6	9.1	10.2	22	-		1.1		10.4	101	9.6	8.7	8
	188/.01-	CUPT 27	2061.01-22	2187 7817	1508 57	-75 8253	75 7476	-75 8590	-75 8354	-75.8258	75 8098	-75.7761	-75.8342	-75 8258	-75.8318	-75.8413	-75.7302	-75.7293	-75.7366	-75.7389	-75.7220	-75.8251	C/ 18. C/-	CL30 3L	7100.01-	CCV8 SL	-75 8236	-75.8453	-75.8694	-75.8515	-75.8428	C808.C/-	1401.01-	-75 7043	-75.7423	-75.7376	-75.7396	-75.7394	-75.7362	-75.7493	1051.01-	8780.01-	1537 37	76 7206	-75.7421	-75 7430	-75.7580	-75.7444	-75.7373
	8008.65	20 8663	2120.00	30 8655	30 8775	39 8885	39 8796	39 8214	39 8162	39.8193	30 8120	39.8211	39.7966	39 7835	39.7781	39.7695	39.8776	39.8681	39.8614	39.8651	39.7230	39.8490	1008.65	1040.40	002802	1128 02	1092 68	39.7656	39.7821	39.7852	2002.02	666/ 65	39.862/	39 8898	39.8908	39.8959	39.8910	39.8689	39.8536	39.8475	2098.65	9668.65	0000000	1020 02	39.8265	39 8280	39.8202	39.8172	39.8123
L'Insence																																																	
Sued	× =	1 .				• •			-				4	-		4	4	4	4	4	4	#					4 6		-	4	4	4	F	1 =	4	4	4	4	8	4	Y	A	4 6		1 4			4	4
Trate wate	19-Jan WCC	19-Ian urr	10 L- mcc	19-Ian WCC	DALTan WCC	24-Jan RWC	24-Jan BCC	26-Jan WCC	26-Tan WCC	26-Jan WCC	John mel-AC	26-Jan WCC	26-Jan WCC	26-Jan WCC	26-Jan WCC	26-Jan WCC	26-Jan RCC	26-Jan RCC	26-Jan RCC	26-Jan RCC	28-Jan WCC	31-Jan WCC	31 L-Jan WCC	21 L-Jan WCC	31 Im WCC	31 Im WCC	31-Jan WCC	31-Jan WCC	31-Jan WCC	31-Jan WCC	31-Jan WCC	31-Jan WCC	31-Jan RCC	31-Jan BWC	31-Jan RCC	31-Jan RCC	31-Jan RCC	31-Jan RCC	31-Jan RCC	31-Jan RCC	31-Jan RCC	2-Feb BWC	2-Feb RCC	Date and	2-Feb RCC	2-Feb RCC	2-Feb WCC	2-Feb RCC	2-Feb RCC
anno anno anno	1 London Grove	Chronid Farm	A WENCH PROPERTY	5 Archie's Run	6 Boar Scout Sming	7 Arcott House Creek	8 W Street Rd Forest	9 Paschall Mill Rd	10 Econillage First Pron	11 East Summit	12 Rocahill Rd	13 Avondale Center	14 South Guernsev Rd	15 Pennocks Bridge Rd	16 Hill Crest Dr	17 Hess Mill Rd	18 Wollaston Rd 1	19 Wollaston Rd 2	20 Mill Rd Vet 2	21 Mill Rd	Middle Run Valley Natural Area	23 Lloyd Rd	Walter Kd		CWING NO	Il Woodview Pd	29 Old Schoolhonse Rd	Pennbrook Dr	31 Tudor Circle	32 Kelton Pennock Bridge Rd	33 Gale Ln	34 New Garden Station Kd	35 New Bolton Center 36 Read Rd	37 Ubland Rd	38 Poplar Tree Rd (Mill Rd)	39 Unionville Baseball Fields	40 Poplar Tree Rd 2	41 Mill Rd 2	42 Line Rd	43 Pemberton Rd	44 Mull Kd Vet I	4) Eaten Kd	40 Locust Ln	10 Thomas P.J	49 Chambers Rd 1	50 Chambers Rd 2	51 Daniel Dr	52 Edgewood Dr	Portmannock Dr 1

Site # Site Name	Date Watersh	Watershed Presence	NewLat	NewLong 7	Temp (°C)	DO (%) I	DO (%) DO (mg/L)	Cond	Sal (ppt)	Hq	pHT(°C)	%Agricultural	%Developed	%Forest	%Water/Wetlands
54 Portmarnock Dr 2	2-Feb RCC	4	39.8124	-75.7370	9.6	16	11.04	176.2	0.12	7.67	9.6	14.36412809	4.113949376	81.51549641	0.00642613
55 Hartefeld Dr	2-Feb RCC		39.8058	-75.7271	8.7	102	11.9	314	0.22		8.7	0	67.8206812	32.17932677	0
56 Losito Dr	2-Feb RCC	4	39.8070	-75.7399	61			502	0.37	7.81	2.9	4.359623424	64.7813368	30.85903181	0
57 Sharp Rd	2-Feb RCC	4	39.8078	-75.7372	8.2			582	0.42	6.88	8.1	50.72124349	11.2678563	38.01091614	0
58 Chandler Mill Rd	2-Feb RCC	4	39.8051	-75.7089	8.4			127.4	0.09	7.27	8.3	4.762877005	9.333670858	85.9034601	0
	2-Feb RCC	4	39.8446	-75.6959	8.1	56	11.58	384.3	0.28		8.1	5.872622641	71.12959656	22.99778876	0
	2-Feb RCC	4	39.8593	-75.6967	8.1	92.5	10.85	371.5	0.27	-	8.1	•	41.33709526	58.6629127	0
61 Bartram Rd	2-Feb WCC	4	39.8565	-75.7703	61	86.5	10.25	366.6	0.26	7.85	61	53.17532433	26.9901513	19.83453233	0
02 T T T T T	7 P.L PUIC	9	2010/06	1961.01-	4.4	100	19.61	0101	1.0		4.4	6410/040.0/	4006060C71	C607700CC1	006/ CDC64-6
64 Laurels Forest	7-Feb BWC	4 4	39.9221	2681.21-	14	66	12.6	32.2	0.02	145	41	200000010.61	12.85751518	87.14249278	00
	7-Feb BWC	1 4	39.9234	-75.7845	49	16	124	613	0.05		4.7	0	11.7533516	79.14397581	9.102680556
	7-Feb BWC	. 4	39.9253	-75.7818	49	66	12.6	725	0.06		49	0	13.77112451	74.1121808	12.1166947
67 Laurels Gate	7-Feb BWC	4	39.9304	-75.7793	3.4	16	12.95	57	0.05	7.26	3.3	9.3992779	0	90.60073006	0
68 Laurels Wetland Forest	7-Feb BWC	4	39.9235	-75.7936	2	56	11.5	83.2	0.06	-	6.8	31.84027874	0	64.19970437	3.960024854
69 Leadline Dr	7-Feb BWC	4	39.9127	-75.5823	1.6	133.5	15.3	475.6	0.33		9.1	0	96.79992273	3.200085236	0
	7-Feb BWC	4	39.9162	75 5042	272	180	12.1	188.6	0.14		7.2	52.43408977	33.5110486	14.05486959	00
	/-Feb BWC	8	1076.65	1660.01-	10.4	76	701	5.007	21.0		10.4		2/0000000000000000000000000000000000000	4710566T./0	
73 New St Church 73 New St at Pleasant Group	7-Feb BWC		8076.65	1860.01-	88	66	107	268.2	019	145	88	6 900580446	0000096016	5.401149441	
74 College Are	7.Fab purc	1 1	30 0460	75 6074	115	110	11	VLY	50.05		11 5	0	CUPPESCE PL	10299119 SC	
	7-Feb BWC		39.9527	-75.6140	10.9	18	9.6	462.8	0.31		10.9	00	88.53748054	11.46252742	00
	7-Feb BWC	4	39.9417	-75.6294	8.6	102.5	11.9	339	0.24		8.6	0	48.73641345	51.26359451	0
77 Birmingham Rd	7-Feb BWC	4	39.9398	-75.6312	8.4	98	11.4	266.7	0.19		8.4	0	29.41104242	66:67986399	3.909101558
78 Stroud Preserve 1	7-Feb BWC	4	39.9475	-75.6542	8.4	16	11.4	129.3	0.09		8.4	21.04507345	27.90711304	42.75879884	8.289022633
79 Stroud Preserve 2	7-Feb BWC	4	39.9486	-75.6546	6	95.5	111	133.7	0.09	-	6	33.54589463	33.88623266	21.51426852	11.05361216
80	7-Feb BWC	4	39.9489	-75.6567	9.5	16	10.4	115.3	0.08		9.5	56.25970738	3.971459702	32.41237152	7.356469365
	7-Feb BWC	4	39.9486	-75.6567	6	8	10.4	145	1.0	7.33	6	52.24777978	1.657997231	35.70658721	10.38764374
	7-Feb BWC	4	39.94/3	9009.01-	9	56	911	13/2	1.0		9	24.46/31803	20.96426506	42.1294/828	11.81294629
83 Hanes Mill Rd	7-Feb BWC	4	39.8945	16/9/22-	4.1	16	11	245.5	0.18		4.1	9.993125554	22.89829921	67.1085832	00
	9-Feb BWC	4	59.8094	0060.01-	77	6 6	13.1	8.10	CO.0	78.1	77	0.5/0462004	30.34936089	11//1087.69	
85 Creek Rd 2 86 Creek Rd 3	9-Feb BWC	4 4	39.8584	0060.01-	3.1	586	120	14/./	17.0	101	5.1	32 34929671	39,69879846	00.008987166 17 56457166	0 10 38734911
87 Montchanin Rd at Twadell Mill	9-Feb BWC	4	39.8359	-75.5984	3.4	86	13	134.1	0.11	7.66	3.4	12.37458396	22.29638505	46.77793294	18.55110601
88 Montchanin Rd 2	9-Feb BWC	4	39.8203	-75.5912	3.6	97.5	12.9	148.2	0.12	7.47	3.6	18.31153957	15.87833768	65.81013072	0
	9-Feb BWC	4	39.8157	-75.5878	7.8	16	10.4	114.6	0.08		7.8	4.344692825	37.99045923	57.66485591	0
	9-Feb BWC	4	39.8022	-75.5699								0	0	97.53142652	2.468573478
	9-Feb BWC	4	39.8043	-75.5686								0	0	82.70663969	17.29336827
92 BWC East 5 93 BWC East 4	9-Feb BWC	4 4	1008.65	1222657-								00	00	2068002.08	29 20246792
	9-Feb BWC	4	39.8100	-75.5650								0	7.079030965	82.58727453	10.33370247
95 BWC West 1	9-Feb BWC	4	39.8102	-75.5670								0	766066780.0	71.74885411	28.16316286
	9-Feb BWC	4	39.8105	-75.5672								0	0.440615911	72.32086725	27.2385248
9/ DWC West 3	O P-L PCC	4	6419.45	LEIL SL								C000102002 VI	C74909C0.47	6040//07700	C6500+106.1
90 Uket Checter Fine Commany	9-Fah Buir	4 6	0150 05	151952-								10000/0/.+1	Cy1021008	CC100980 01	
	9-Feb BWC	4 1	39.9321	-75.6025	8.6	92.5	10.75	336.8	0.24	7.14		0	15.51026457	71.81540078	12.67433465
101 Middle Run Valley Natural Area	28-Jan WCC	. 4	39.7192	-75.7250								33.34748887	0	66.65251909	0
102 Middle Run Valley Natural Area	28-Jan WCC	4	39.7162	-75.7228								17.59864527	0	82.40136269	0
103 Middle Run Valley Natural Area	28-Jan WCC	4	39.7138	-75.7268								19.1621139	7.306477738	73.53140837	00
	20-Jan WCC	4	0711-60	0071-01-										100.00000	
106 Ramment Flementary Natural Area	20-Jan WCC	a \$	30 8465	017/.01-	22	8K	10.37	33	PC U	6 86	27	LCISCAIS AC	1 (090(333 (8/015/0C CL	
		2	-n1-1-20	anning -	1-1	3	Toron I	1				17107410147	7.00020012	1000101000	>

Appendix 2. Spearman's Correlations between water chemistry and land use variables at each site.

each site. Variable	by Variable	Speerman e	Prob> p
Dissolved Oxygen (%)	Temperature (∞ C)	Spearman ρ -0.1422	0.2175
Dissolved Oxygen (70) Dissolved Oxygen	Temperature (∞ C)	-0.6816	<.0001*
(mg/L)	Temperature (we)	-0.0810	<.0001*
Dissolved Oxygen	Dissolved Oxygen (%)	0.7457	<.0001*
(mg/L)	Dissolved Oxygen (70)	0.7437	<.0001
Conductivity	Temperature (∞ C)	0.5134	<.0001*
Conductivity	Dissolved Oxygen (%)	0.0578	0.6177
Conductivity	Dissolved Oxygen (mg/L)	-0.2680	0.0184*
Salinity (ppt)	Temperature (∞C)	0.4057	0.0002*
Salinity (ppt)	Dissolved Oxygen (%)	0.0180	0.8766
Salinity (ppt)	Dissolved Oxygen (mg/L)	-0.2404	0.0352*
Salinity (ppt)	Conductivity	0.9319	<.0001*
pH	Temperature (∞ C)	-0.3500	0.0015*
рН	Dissolved Oxygen (%)	0.1837	0.1097
pH	Dissolved Oxygen (mg/L)	0.3812	0.0006*
pH	Conductivity	-0.0242	0.8311
pH	Salinity (ppt)	-0.0344	0.7617
% A	Temperature (∞ C)	-0.0716	0.5278
% A	Dissolved Oxygen (%)	0.0480	0.6785
% A	Dissolved Oxygen (mg/L)	-0.0050	0.9655
% A	Conductivity	-0.1498	0.1848
% A	Salinity (ppt)	-0.1749	0.1207
% A	pH	-0.0949	0.4023
% D	Temperature (∞ C)	0.2201	0.0498*
% D	Dissolved Oxygen (%)	0.0891	0.4411
% D	Dissolved Oxygen (mg/L)	-0.0933	0.4197
% D	Conductivity	0.4363	<.0001*
% D	Salinity (ppt)	0.4224	<.0001*
% D	pH	0.2667	0.0168*
% D	% A	-0.1918	0.0489*
% F	Temperature (∞C)	-0.2718	0.0147*
% F	Dissolved Oxygen (%)	-0.0920	0.4259
% F	Dissolved Oxygen (mg/L)	0.1398	0.2251
% F	Conductivity	-0.3241	0.0034*
% F	Salinity (ppt)	-0.2677	0.0164*
% F	pH	-0.0607	0.5927
% F	% A	-0.5269	<.0001*
% F	% D	-0.5877	<.0001*
% W	Temperature (∞C)	-0.1187	0.2942
% W	Dissolved Oxygen (%)	-0.1471	0.2018
% W	Dissolved Oxygen (mg/L)	0.0000	0.9998
% W	Conductivity	-0.3263	0.0031*

Variable	by Variable	Spearman p	Prob> p
% W	Salinity (ppt)	-0.3582	0.0011*
% W	pH	0.0876	0.4397
% W	% A	-0.1890	0.0524
% W	% D	-0.2254	0.0202*
% W	% F	0.2011	0.0387*

Appendix 3. Full transect data.

Site Name	Transect	Dist to	Wetted	Max	W:D	Ave	Ave silt	Ave cand	Ave gravel	Ave cobble	#Snails/cm
Sile Name	#		Width (cm)		w.D	Velocity	Ave shi	Ave sauu	Ave glaver	Ave cooole	#Silaiis/cili
London Grove	1	Ó	103	4	25.75	-	0	13.3333	86.66667	0	
London Grove	2	5	123	6.5	18.9231	5	0	13.3333	63.33333	23.333333	
London Grove	3		123	5.5	22.3636	13	6.6667	16.6667	76.66667	0	
London Grove	4		88	10	8.8		0	20	63.33333	16.666667	
London Grove	5		110		14.6667	10.3333	0	15		16.666667	
London Grove	6		110		9.16667	10	10	53.3333	36.66667	0	
London Grove	7		125	10	12.5	20	0	21.6667	75	3.3333333	
London Grove	8		59	6	9.83333		0	6.66667	83.33333	10	
London Grove	9		178	5	35.6		0	0		65	
London Grove	10	45	72	7.5	9.6		6.6667	11.6667	78.33333	3.3333333	
Boy Scout Spring	1	0	119	7	17	5	0	26.6667	40	33.333333	
Boy Scout Spring	2	5	118	7	16.8571	9	0	56.6667	6.666667	36.666667	
Boy Scout Spring	3	10	86	6	14.3333	6	0	26.6667	20	53.333333	
Boy Scout Spring	4		158	6	26.3333	5	0	36.6667	63.33333	0	
Boy Scout Spring	5		172	7	24.5714	4	0	18.3333	60	21.666667	
Boy Scout Spring	6		140	8	17.5	7	0	30	56.66667	13.333333	
Boy Scout Spring	7		178	9	19.7778	7	0	13.3333	80	6.6666667	
Boy Scout Spring	8		160	15	10.6667	7	83.333	16.6667	0	0	
Boy Scout Spring	9		320	10	32	7.5	100	0	-	0	
Boy Scout Spring	10	45	350	8	43.75	8	66.667	33.3333	0	0	
Arscott House	1	0	172	8	21.5	2	0	0	86.66667	13.333333	
Arscott House	2	5	222	10	22.2	3	0	13.3333	73.33333	13.333333	
Arscott House	3	10	340	8	42.5	4.5	0	33.3333	60	6.6666667	
Arscott House	4		228	10	22.8	3.5	30	0		3.3333333	
Arscott House	5		280		25.4545	2	0	26.6667	23.33333	50	
Arscott House	6		143		11.9167	4.5	35	6.66667	0	58.333333	
Arscott House	7		124		9.53846	3	0	10	50	40	
Arscott House	8		214	18	11.8889	4		0		10	
Arscott House	9		193	10	19.3	3	0	0	95	5	
Arscott House	10	(none)									
Rosehill Rd	1		107	25	4.28	6	58.333	16.6667	21.66667	3.3333333	0.0280374
Rosehill Rd	2	-	135		19.2857	3	0	26.6667		40	0.0074074
Rosehill Rd	3		145	21	6.90476	6	76.667	23.3333	0	0	0.0068966
Rosehill Rd	4		124	10	12.4	5	0	60			0.0080645
Rosehill Rd	5		135			11	50	3.33333	18.33333	28.333333	
Rosehill Rd	6		123			13	6.6667	3.33333	33.33333	56.666667	
Rosehill Rd	7	_	145		10.3571	7	0	6.66667	46.66667	46.666667	0.0206897
Rosehill Rd	8		190		7.30769	16	36.667	0			0.3736842
Rosehill Rd	9		164	9	18.2222	2	0	0		93.333333	0.0121951
Rosehill Rd	10	45	95	18	5.27778	4	0	43.3333	50	6.6666667	0
Mill Rd Vet 1	1		71	6	11.8333	4	96.667	0	-	3.3333333	
Mill Rd Vet 1	2		21	4	5.25	6	0	100	0	0	1.047619
Mill Rd Vet 1	3		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Mill Rd Vet 1	4		39	3	13	6	85	10	0	5	0.2051282
Mill Rd Vet 1	5		56	20	2.8	10		0	-	0	1.6607143
Mill Rd Vet 1	6		44	10	4.4	4		0	0	50	
Mill Rd Vet 1	7		34		4.85714	4	85	62 2222	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	15	0 200625
Mill Rd Vet 1	8	35 40	64 39		3.55556	6		63.3333 0		0	0.390625
Mill Rd Vet 1 Mill Rd Vet 1	10		58		3.54545 5.27273	4	100 100	0	0	0	1.2564103 0.4310345
Ann IXA Vet I	10	4)	28	11	5.21213	,	100	0	0	0	0.4510545

Site Name	Transect		Wetted	Max	W:D	Ave	Ave silt	Ave sand	Ave gravel	Ave cobble	#Snails/cm
F . B	#			Depth (cm)	2 00000	Velocity			-		
Eaten Run	1	-		-	3.88889	4			5	0	0.6
Eaten Run	2				5.4	2			63.33333	0	0.537037
Eaten Run	3			4	17.75	2		3.33333	96.66667	0	0.1830986
Eaten Run	4			-	8.125	3	-	3.33333	96.66667	0	0.2769231
Eaten Run	5			-	7	3		23.3333	66.66667	10	0.4642857
Eaten Run	6		61	11		2		2.22222	96.66667	0	0.442623
Eaten Run	7				6.71429	3		20.0000	76.66667	0	0.7234043
Eaten Run	8			11		6			6.666667	0	0.3797468
Eaten Run	9			9	17	2		23.3333	30	46.666667	0.875817
Eaten Run	10	45	57	6	9.5	4	0	60	40	0	0.5964912
Spring Line Rd.	1	0	142	6	23.6667	2	0	0	36.66667	63.333333	0
Spring Line Rd.	2	5	153	15	10.2	6	50	20	0	30	0.124183
Spring Line Rd.	3		168	27	6.22222	20			0	0	0.1190476
Spring Line Rd.	4	15	270	8	33.75	3	20	0	43,33333	36,666667	0.0333333
Spring Line Rd.	5	20	234	10	23.4	5	3.3333	0	60	36,666667	0.0854701
Spring Line Rd.	6	25	205	14	14.6429	23	73.333	0	26,66667	0	0.2097561
Spring Line Rd.	7	30	252	14	18	6	30	3.33333	63.33333	3.3333333	0.2698413
Spring Line Rd.	8	35	112	12	9.33333	3	0	93.3333	6.666667	0	0.1428571
Spring Line Rd.	9	40	81	10	8.1	5	16.667	13.3333	70	0	0.1358025
Spring Line Rd.	10	45	154	13	11.8462	3	0	43.3333	53.33333	3.3333333	0.0844156
Bancroft Elementar	v 1	0	68	4	17	5	0	0	85	15	0.0441176
Bancroft Elementar	, -	-		3	47.6667	4	-	-	30	53.333333	0.034965
Bancroft Elementar	·			-	4,70588	15	-	6.66667	30	6.66666667	0.034905
	, -			8	18.375	8		20	43.33333	0.0000007	
Bancroft Elementary Bancroft Elementary	·			15	18.575	13		20	45.55555 50	3.3333333	0.1428571
Bancroft Elementar	, -		140		23.3333	13			50 60	3.33333333	0.0071429
Bancroft Elementar	, -			0 4	23.3333	7		10	90	50 0	0.0071429
	, .			21	7.85714	36			23.33333	3 3333333	0.0930233
Bancroft Elementar	, -			21 10	7.85714	30 9			25.55555		0.4545455
Bancroft Elementary	-				5.1 15.6667	9			43.33333	40 30	
Bancroft Elementary	y 10	45	94	0	13.000/	8	0	26.6667	45.55555	30	0.1595745

Appendix 4. Significant differ	ces between stream sites (T	Cukey-Kramer HSD P<0.05).
a) Density (Snails/cm ²)		

Stream 1	Stream 2	р
Mill Rd Vet 1	Rosehill Rd	<.0001
Mill Rd Vet 1	Bancroft Elementary	<.0001
Mill Rd Vet 1	Spring Line Rd	<.0001
Eaten Run	Rosehill Rd	0.0082
Eaten Run	Bancroft Elementary	0.0115
Eaten Run	Spring Line Rd	0.0123

b) Width (cm)

Stream 1	Stream 2	р
Spring Line Rd	Mill Rd Vet 1	< 0.0001
Spring Line Rd	Eaten Run	< 0.0001
Rosehill Rd	Mill Rd Vet 1	0.0009
Bancroft Elementary	Mill Rd Vet 1	0.0010
Rosehill Rd	Eaten Run	0.0122
Bancroft Elementary	Eaten Run	0.0136

c) Maximum Depth (cm)

Stream 1	Stream 2	р
Rosehill Rd	Eaten Run	0.0123

Rosehill Rd Bancroft Elementary	0.0373	
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d) Width to Max Depth ratio

Stream 1	Stream 2	р
Bancroft Elementary	Mill Rd Vet 1	0.0091
Bancroft Elementary	Eaten Run	0.0403

e) Average Velocity (m/s)

Stream 1	Stream 2	р
Eaten Run	Bancroft Elementary	0.0002
Eaten Run	Rosehill Rd	0.0131
Eaten Run	Mill Rd Vet 1	0.0176

f) Average Silt

Stream 1	Stream 2	р
Mill Rd Vet 1	Eaten Run	<.0001
Mill Rd Vet 1	Bancroft Elementary	0.0003
Mill Rd Vet 1	Rosehill Rd	0.0026
Mill Rd Vet 1	Spring Line Rd	0.0113

g) Average Gravel

Stream 1	Stream 2	р	
Eaten Run	Mill Rd Vet 1	<.0001	
Bancroft Elementary	Mill Rd Vet 1	0.0024	
Spring Line Rd	Mill Rd Vet 1	0.0247	

Appendix 5. Spearman's Correlations between physical variables for each transect.

11 1	s conclutions setween physica		
Variable	by Variable	Spearman p	Prob> p
Max Depth (cm)	Wetted Width (cm)	0.2086	0.0669
W:D	Wetted Width (cm)	0.6955	<.0001*
W:D	Max Depth (cm)	-0.5145	<.0001*
Ave Velocity (m/s)	Wetted Width (cm)	-0.0981	0.4025
Ave Velocity (m/s)	Max Depth (cm)	-0.2390	0.0389*
Ave Velocity (m/s)	W:D	0.1033	0.3776
Ave silt	Wetted Width (cm)	0.0638	0.5791
Ave silt	Max Depth (cm)	0.4493	<.0001*
Ave silt	W:D	-0.2785	0.0135*
Ave silt	Ave Velocity (m/s)	-0.3979	0.0004*
Ave sand	Wetted Width (cm)	-0.1832	0.1083
Ave sand	Max Depth (cm)	-0.0257	0.8236
Ave sand	W:D	-0.1411	0.2178
Ave sand	Ave Velocity (m/s)	-0.0662	0.5728
Ave sand	Ave silt	-0.4297	<.0001*
Ave gravel	Wetted Width (cm)	0.1082	0.3456
Ave gravel	Max Depth (cm)	-0.3294	0.0032*

Variable	by Variable	Spearman p	Prob> p
Ave gravel	W:D	0.3582	0.0013*
Ave gravel	Ave Velocity (m/s)	0.2239	0.0535
Ave gravel	Ave silt	-0.5701	<.0001*
Ave gravel	Ave sand	-0.0777	0.4988
Ave cobble	Wetted Width (cm)	0.2057	0.0709
Ave cobble	Max Depth (cm)	-0.1036	0.3669
Ave cobble	W:D	0.2582	0.0225*
Ave cobble	Ave Velocity (m/s)	0.1606	0.1686
Ave cobble	Ave silt	-0.2660	0.0186*
Ave cobble	Ave sand	-0.1807	0.1133
Ave cobble	Ave gravel	-0.0684	0.5517
Density (snails/cm2)	Wetted Width (cm)	-0.5406	<.0001*
Density (snails/cm2)	Max Depth (cm)	0.0370	0.8005
Density (snails/cm2)	W:D	-0.5642	<.0001*
Density (snails/cm2)	Ave Velocity (m/s)	0.0617	0.6736
Density (snails/cm2)	Ave silt	0.1582	0.2776
Density (snails/cm2)	Ave sand	0.0353	0.8099
Density (snails/cm2)	Ave gravel	-0.1843	0.2049
Density (snails/cm2)	Ave cobble	-0.3632	0.0103*

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