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RMBL Paper

Nutrient excretion rates of common aquatic taxa in high elevation ponds at the Mexican Cut
Nature Preserve

Abstract

Nutrient cycling is crucial for proper functioning of aquatic ecosystems. Animals play an integral part of this process, especially in nutrient poor ecosystems such as the high elevation ponds at the Mexican Cut Nature Preserve. Nutrients such as nitrogen (N) and phosphorus (P) are both limiting nutrients, and animal excretion increases the availability of these nutrients. We measured the excretion rates of common aquatic macroinvertebrates and the demand for nutrients in these pond ecosystems. We found excretion rates in aquatic macroinvertebrate taxa varied over several orders of magnitude for both nitrogen and phosphorus. It appeared that smaller taxa had higher mass-specific excretion rates, but we did not test these differences statistically. We compared nutrient demand between permanent and semi-permanent pond types, but did not observe any significant difference between the two. The macroinvertebrate community contributed significantly to pond N and P demand. Phosphorus contribution ranged from 25.5% to 67.7%, while macroinvertebrates contributed over 100% of N demand in all but one pond and as much as 352% of demand in one case. These results demonstrate the importance of these macroinvertebrate communities to nutrient cycling, and the potential consequences that climate driven changes in community composition could have on ecosystem functioning in these ponds.

Introduction

Nutrient cycling, defined as the transformation of nutrients from one chemical form to another, and/or the flux of nutrients between organisms, habitats, or ecosystems, plays a crucial role within aquatic ecosystems. Many studies focus on nitrogen (N), phosphorus (P), and the nitrogen-phosphorus ratio (N:P) because these nutrients often limit primary production (*e.g.*, Vanni 2002). Primary producers form the base of aquatic food chains and therefore understanding nutrient cycling aids in understanding overall ecosystem functioning (Vanni 2002).

Many studies have examined the roles that animals play in nutrient cycling in aquatic ecosystems (Grimm 1988, Hall et al. 2003, Hall et al. 2007, McIntyre et al. 2008, Balik et al. *in prep*, Vanni 2002). Organisms can affect nutrient cycling directly, through the transformation of nutrients within their bodies, and indirectly, through the impacts organisms have on their prey and/or physical habitat structure (Vanni 2002). Grimm (1988) found that up to 70% of nitrogen retained by a desert stream ecosystem was recycled through aquatic insect excretion. Additionally, Hall (2003) found snails consumed 75% of gross primary production and their excretion accounted for 2/3 of ammonium demand in a stream ecosystem. In this study, we focus on the direct effects (such as excretion) of aquatic animals because they are much easier to quantify than indirect effects. Further, most animals excrete inorganic N and P in forms that are easily assimilated by primary producers. Thus, the excretion rates of N, P, and N:P of different animals are important to understanding nutrient cycling within aquatic ecosystems (Vanni 2002).

A closely related guild of caddisflies exhibited large interspecific variation in excretion of N, P, and N:P, however, species-specific excretion rates did not vary significantly across habitats or time of day (Balik et al. *in prep*). Due to this consistency within species, excretion rates could

be used to predict ecosystem consequences of changes in community assemblages. Assuming this holds true for other aquatic macroinvertebrates, understanding other taxon-specific excretion rates would expand our predictive power of how changes in aquatic macroinvertebrate communities could impact nutrient cycling in aquatic ecosystems.

The high elevation ponds of the Mexican Cut Nature Preserve (Figure 10) are extremely oligotrophic, meaning both N and P are limiting nutrients (Wissinger et al. 1999, Elser et al. 2009). Because N & P are limiting nutrients in these high-elevation ponds, it is likely that animal-driven nutrient cycling is crucial for supporting ecosystem functioning (Vanni 2002) and thus is an important topic for study as these ecosystems are sentinels for climate change (Williamson et al. 2008). Additionally, despite the potential importance of understanding whole ecosystem nutrient cycling, few studies have examined nutrient uptake in ponds (Stanley and Ward 1997), and measuring uptake in three different permanence types will provide interesting comparisons of the supply and demand of nutrients in different ecosystems.

We expanded on Balik et al.'s initial results and determined the extent to which the aquatic animal community drives N and P cycling in high elevation ponds. To do this, we (1) measured taxon-specific N, P, and N:P excretion rates, and (2) uptake rates of nutrients in the two dominant habitats in these ecosystems. We used these data to ask what percentage of nutrient demand is supplied by the aquatic animals, such as invertebrates? What is the relative importance of nutrient supply by each taxon or specific groups, such as the guild of detritivorous caddisflies? How might the supply rates of limiting nutrients from these groups be altered by factors such as fluctuations in salamander predation, pond permanence, or climate change?

We expected that the biomass dominant taxon (*eg.* caddisflies) would contribute the most N and P to the ecosystem due to abundance, however, there might also be a less abundant taxon

that has high mass-specific excretion and provides the most nutrients to the system. It is also possible that permanent ponds rely more heavily on animal nutrient cycling due to the fact that animals are present longer in these systems. Additionally, many of the animals in temporary ponds have faster growth rates and thus might have lower mass-specific excretion than the taxa in permanent ponds (Wissinger et al. 1999, 2003, Vanni and McIntyre 2016).

Methods

In order to measure nutrient excretion we followed the protocol established by Balik et al. (2018). Individuals were placed in bags containing filtered water from the site. The volume of water and number of individuals per bag varied depending on the body size of individuals within each taxon. In all but a few cases we filtered 200 mL of water for the macroinvertebrate samples. The number of replicates (bags) per taxa varied from two to five based on abundance and ease of capture. Each bag contained organisms of approximately the same size, while a gradient of sizes were collected among the replicates. Control samples did not contain macroinvertebrates and provided a baseline measure of nutrient content. The bags were placed in the water, to maintain constant temperature, and organisms were allowed to incubate for one hour. This one hour interval allowed the organisms to settle from handling stress and ends before they become stressed from other factors such as hunger (Whiles et al. 2009). Then, water samples from the bags were collected using a filtered syringe. In addition to macroinvertebrates, we measured the excretion rates of the tiger salamanders (*Ambystoma tigrinum nebulosum*) commonly found in these ponds (Whiteman et al. 1994) with the help of Dr. Howard Whiteman. He and his lab handled the salamanders while we collected the water samples after the salamander had been removed from the bags. For each bag, 20mL of filtered water was placed in a glass tube for

phosphorus analysis, and 20mL in four replicate amber Nalgene bottles to measure ammonium. Samples were placed on ice in the field. Upon return to the lab, samples were refrigerated and analyzed the following day to prevent nutrient degradation.

We analyzed soluble reactive phosphorus (SRP), a measure of inorganic phosphate that is readily available and often limits microorganisms growth, using the colorimetric method (Ostrofsky and Rigler 1987) with a Thermo Scientific GENESYS 10S UV-VIS Spectrophotometer (Waltham, Massachusetts, USA). Ammonium, a form of N that is readily available to both autotrophs and heterotrophs and often limits microorganism growth, was analyzed using a fluorometric method with a Turner Designs Trilogy Fluorometer (Sunnyvale, California, USA; Taylor et al. 2007). Mass-specific excretion rates ($\text{g mg}^{-1} \text{d}^{-1}$) for each N and P were calculated as the difference between final and initial concentrations (g/L) of nutrient in the bags divided by total macroinvertebrate dry mass in the bag (mg) and incubation time (h), and multiplied by 24h and the volume of water (L) in the bag (Balik et al. 2018).

We identified insects to genus (except in midges and mites) using a dissecting scope and a number of taxonomic keys (Peckarsky et al. 1990, Thorp and Covich 1990, Merritt et al. 2008). We then counted and placed all of the organisms in each sample in pre-weighed weighing boats (one for each sample). Next, we placed them in a drying oven at 60 degrees Celsius for at least 48 hours. Finally, we weighed the dried samples and calculated total macroinvertebrate dry mass by subtracting the boat weight from the boat and insect weight.

To measure pond nutrient uptake by autotrophic and heterotrophic microorganisms in the water column and on the pond bottom, we followed the methodology outlined by Stanley (1997). We placed thin-walled (1 mm) clear plastic cylinders (12.5 cm in diameter) at representative locations around the ponds in pairs, one for each nitrogen and phosphorus. Ammonium or

phosphate were added at known concentrations depending on water depth to each cylinder, and then water samples were taken at 0, 15, 30, 60, and 120 minutes to quantify the decline in nutrient concentrations. Uptake rates were estimated by fitting a log-linear or non-linear exponential decay model to the relationship between measured nutrient concentrations as a function of time. The slope of this relationship was the nutrient uptake rate per unit time in $\mu\text{g min}^{-1}$. To express uptake rates per unit time per area (m^2) or water volume (L), we converted to and then divided $\mu\text{g d}^{-1}$ by either area or water volume (estimated by multiplying water depth in the cylinder by its area). Within each pond, we measured uptake at three locations within each of the two major habitat types in these ponds: sedge detritus (*Carex* spp.), and no visible sedge detritus for a total of six measurements in each pond. We repeated these measurements in three ponds for each permanent and semi-permanent, and one temporary pond (due to the rapidity of drying rates) this year for a total of seven ponds in June and July.

Aquatic macroinvertebrate and salamander abundance and biomass data for the ponds at Mexican Cut have been well documented by Dr. Scott Wissinger and Dr. Howard Whiteman respectively. With these data, and our calculations of mass-specific excretion rates, we estimated the population-level excretion rate for each taxon. Additionally, by summing across taxa we approximated the animal community-wide excretion rate for the pond ecosystem. Finally, with nutrient uptake data for the ponds we estimated the percent nutrient contribution of each taxon, or group, to the whole ecosystem.

We did not receive recent population data in time to analyze for this draft, however, we was able to simulate population estimates using a data set from 2002 (provided by Jared Balik and taken by the Wissinger lab). These data set contains ash-free dry mass (AFDM) per square meter (g m^{-2}) estimations of macroinvertebrates at the taxonomic level of order for nine ponds.

The ponds are either permanent or semi-permanent, therefore population level excretion rates could not be estimated for temporary ponds. We estimated total mass of each species for caddisflies, and total mass of each genus for the other macroinvertebrates by dividing the Order AFDM by the number of taxa that fell under that order. The orders included Trichoptera, Coleoptera, Hemiptera, Odonata, Diptera, and Miscellaneous. We multiplied the mass-specific excretion rate by the estimated biomass (converted to ug) to obtain the population excretion rate per square meter ($\text{gP d}^{-1} \text{m}^{-2}$ and $\text{gN d}^{-1} \text{m}^{-2}$). We then calculated the percent contribution of each population to overall macroinvertebrate excretion by dividing the population excretion rate by the overall macroinvertebrate excretion within each pond. Next we averaged percent contribution across all of the ponds for which we had both uptake and population estimates.

To estimate percent contribution of each taxon to pond nutrient uptake we first divided per day pond uptake by the area within the cylinders to estimate per day pond uptake per square meter ($\text{gP d}^{-1} \text{m}^{-2}$ and $\text{gN d}^{-1} \text{m}^{-2}$). Then, we divided the population excretion estimation by the pond uptake estimation. These percent contribution values were then averaged across all of the ponds as described above.

Results

Similar to Balik et al.'s results, we found that mass-specific excretion of N, P, and N:P differed greatly between the different genera, over several orders of magnitude. Additionally, the taxonomic rankings of mass-specific excretion of N, P, and N:P differed (Figure 1-3). For P mass-specific excretion, it appears that smaller taxa (such as copepods and midge larvae) have the highest mass-specific excretion. This pattern is not perpetuated in mass-specific excretion of N nor in mass-specific N:P excretion ratio. The N:P excretion ratio was highest in predatory taxa such as ditiscids, aeshnids, and a genus of leeches (Figures 1-3). This is likely because these taxa

consume more protein than non-predators, and therefore have a more N rich diet. It follows logically that the more nitrogen an organism consumes, the more nitrogen it excretes.

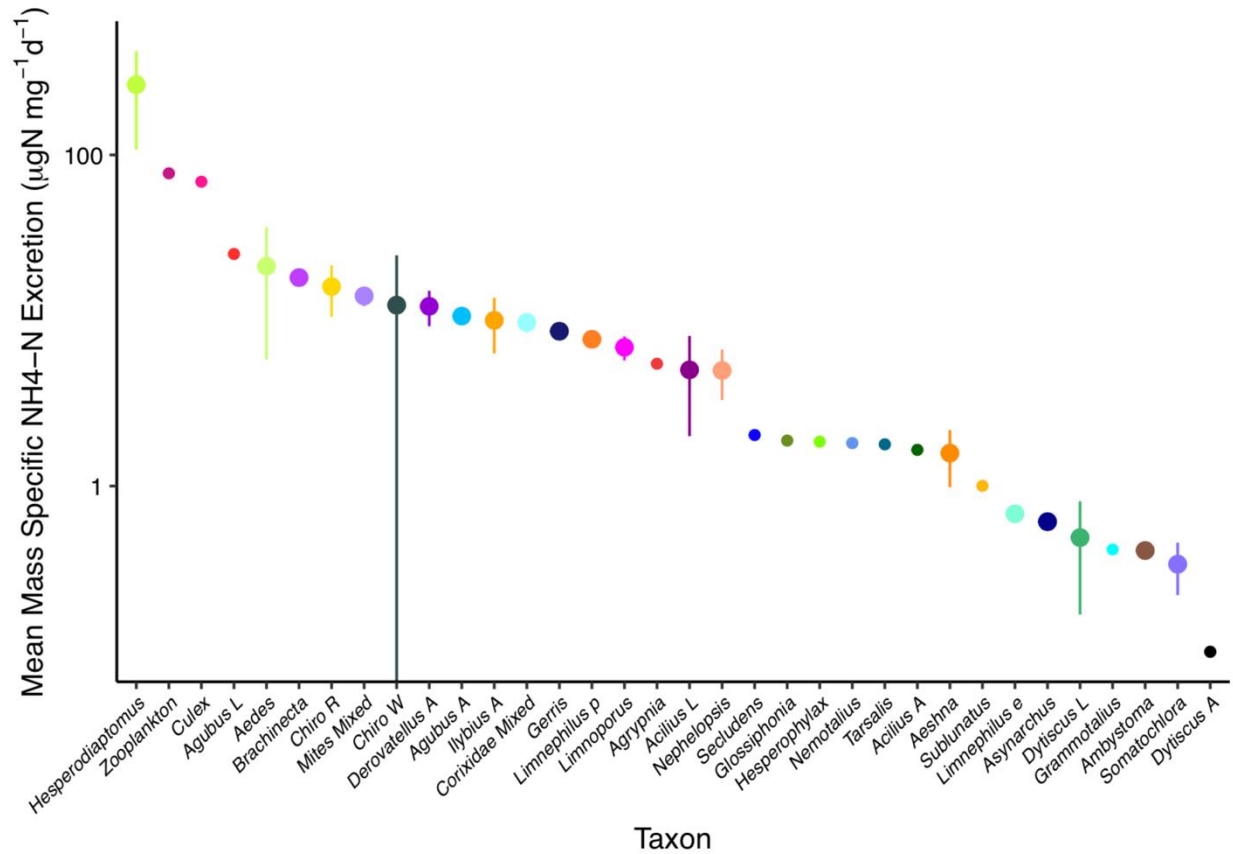


Figure 1. Mean mass-specific N excretion by taxon. The sizes and lines represent one standard error around the mean. A small dot indicates that there was only one observation for that taxon. The y-axis is log₁₀ scaled. Mixed indicates that multiple genera were present in a single sample due to difficulty of identification in the field.

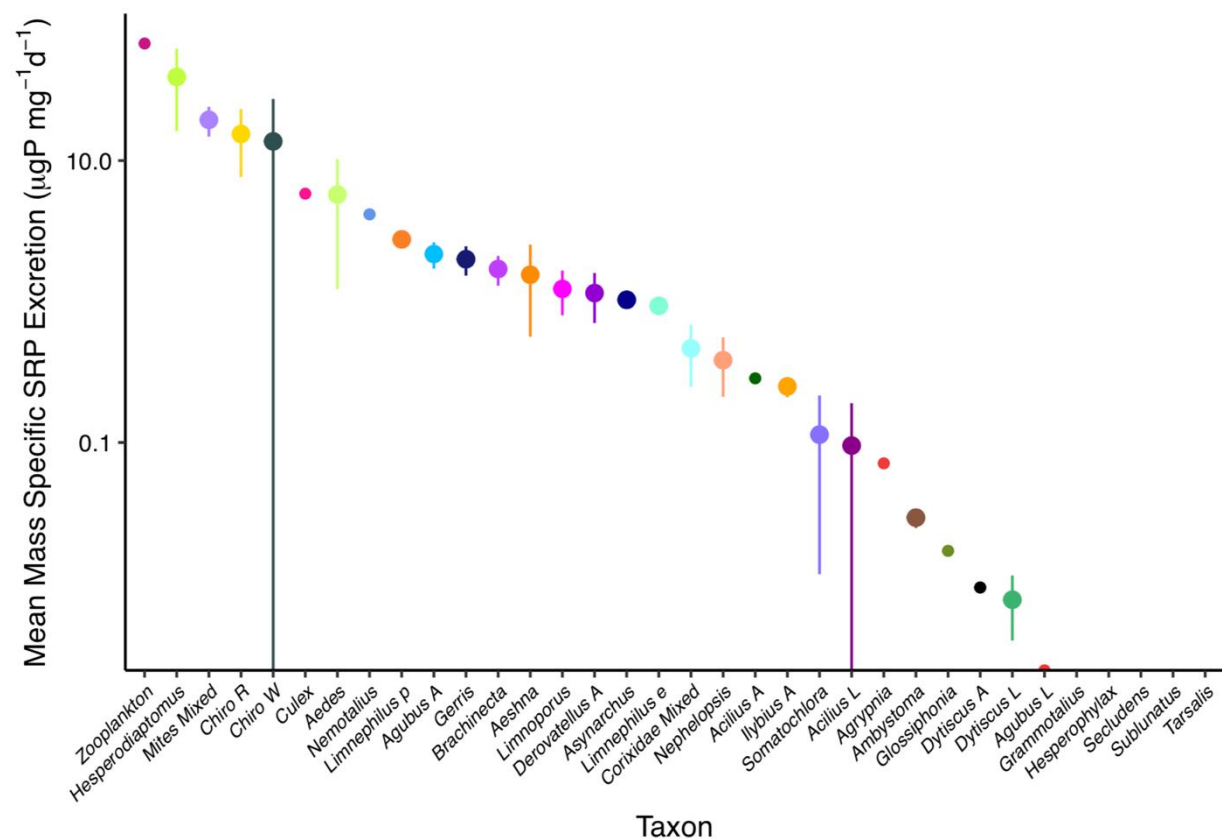


Figure 2. Mean mass-specific P excretion by taxon. The sizes and lines represent one standard error around the mean. A small dot indicates that there was only one observation for that taxon. The y-axis is log₁₀ scaled. Mixed indicates that multiple genera were present in a single sample due to difficulty of identification in the field.

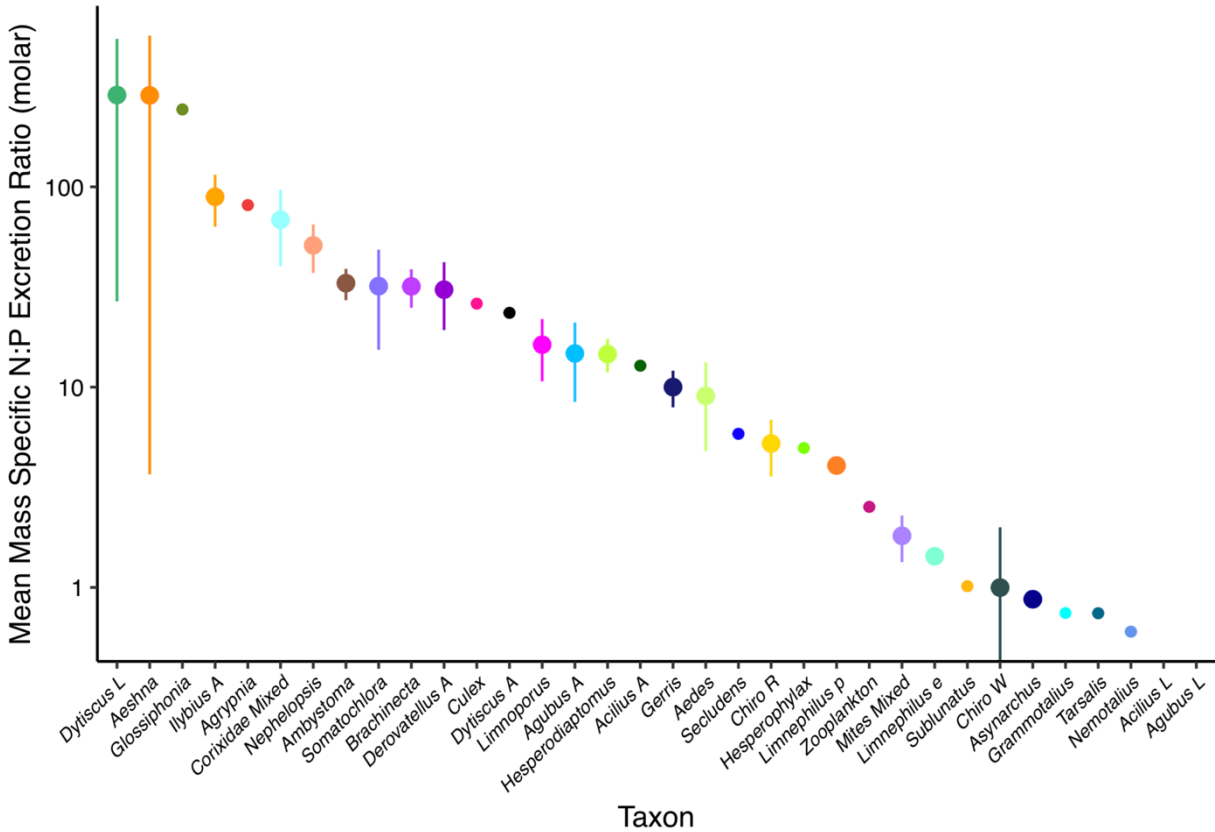


Figure 3. Mean mass-specific N:P excretion ratio by taxon. The sizes and lines represent +/- one standard error around the mean. A small dot indicates that there was only one observation for that taxon. The y-axis is log10 scaled. Mixed indicates that multiple genera were present in a single sample due to difficulty of identification in the field.

The average P uptake for permanent ponds was $64.8 \text{ } \mu\text{gP d}^{-1} \text{ m}^{-2}$, $44.2 \text{ } \mu\text{gP d}^{-1} \text{ m}^{-2}$ for semi-permanent ponds. The average N uptake for permanent ponds was $1183.2 \text{ } \mu\text{gN d}^{-1} \text{ m}^{-2}$, $847.5 \text{ } \mu\text{gN d}^{-1} \text{ m}^{-2}$ for the semi-permanent ponds. There does not appear to be a significant difference between the two pond permanence types in either N or P uptake (Figures 4 and 5). Nitrogen uptake is slightly higher than phosphorus uptake, but without running statistical tests we cannot say whether this difference is significant.

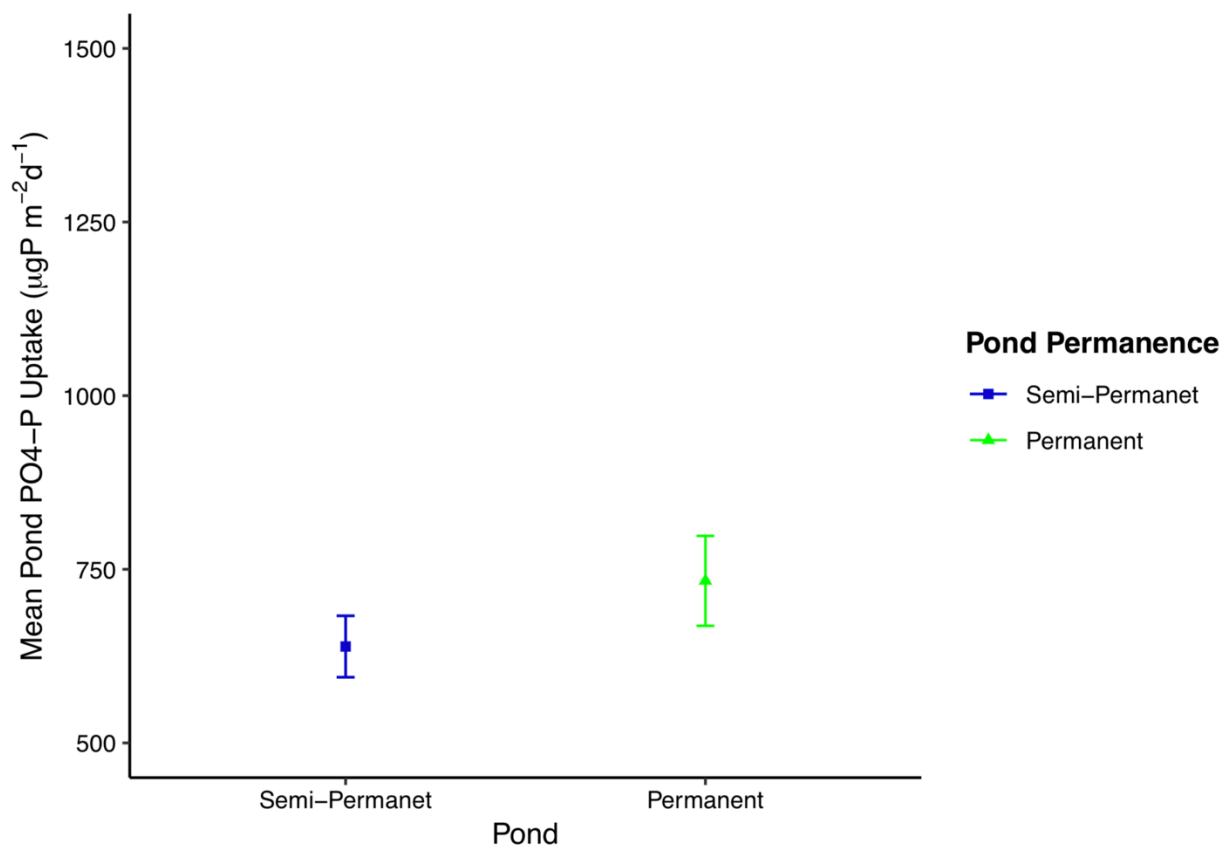


Figure 4. Mean P uptake by pond type. The bars indicate +/- one standard error around the mean.

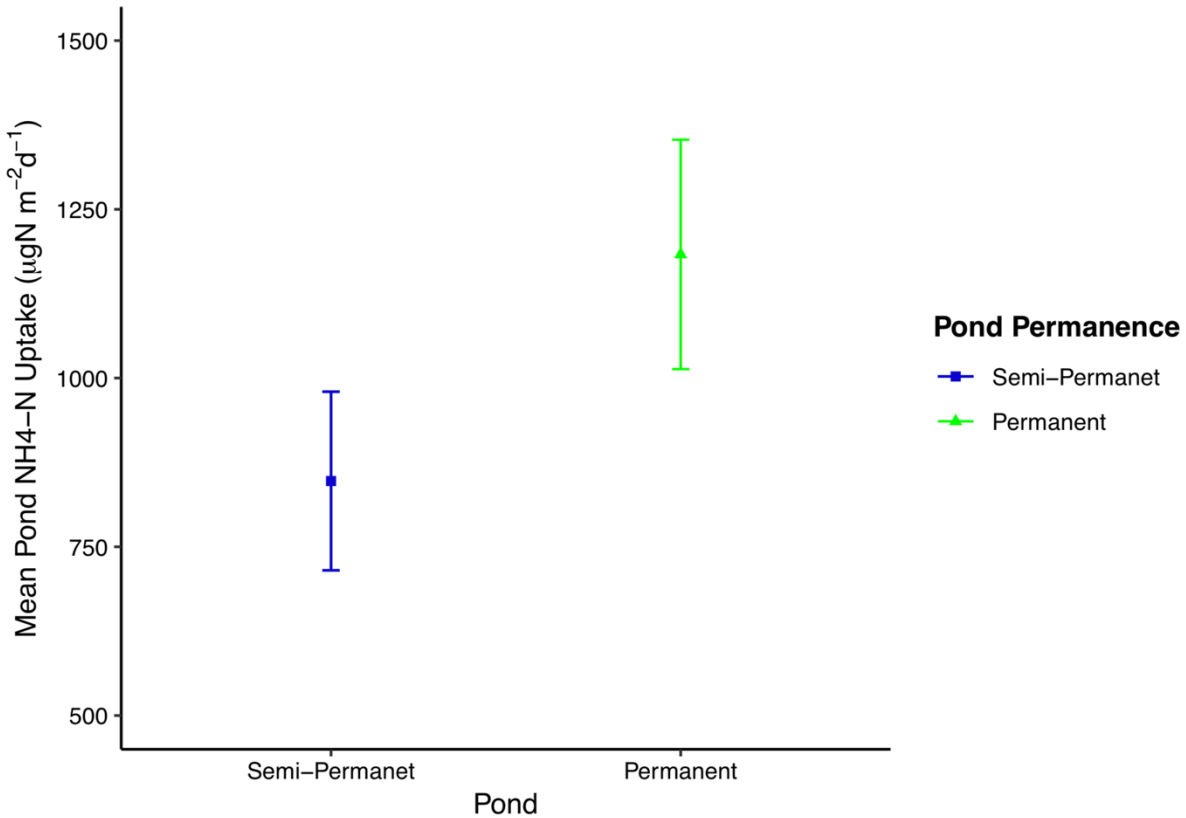


Figure 5. Mean N uptake by pond type. The bars indicate +/- one standard error around the mean.

The copepods (genus *Hesperodiaptomus*) contribute the most P and N to overall macroinvertebrate excretion (21.20% of P and 33.80% of N). For phosphorus, smaller taxa, like midge larvae and mites, appeared to contribute the most to overall community excretion. This does not hold true, however, for N excretion, which appears to be more dominated by Dytiscids and *agrypnia* (a species of caddisfly) (Figures 6 and 7). Unlike most contribution analyses, it appears that there is a large number of taxa that fall in the middle ground of the contribution spectrum. In most cases there are one or two keystone species that contribute most of the nutrients to community-wide excretion. Here, however, a fair number of taxa contribute between one and ten percent of community-wide excretion for both N and P (Figures 6 and 7).

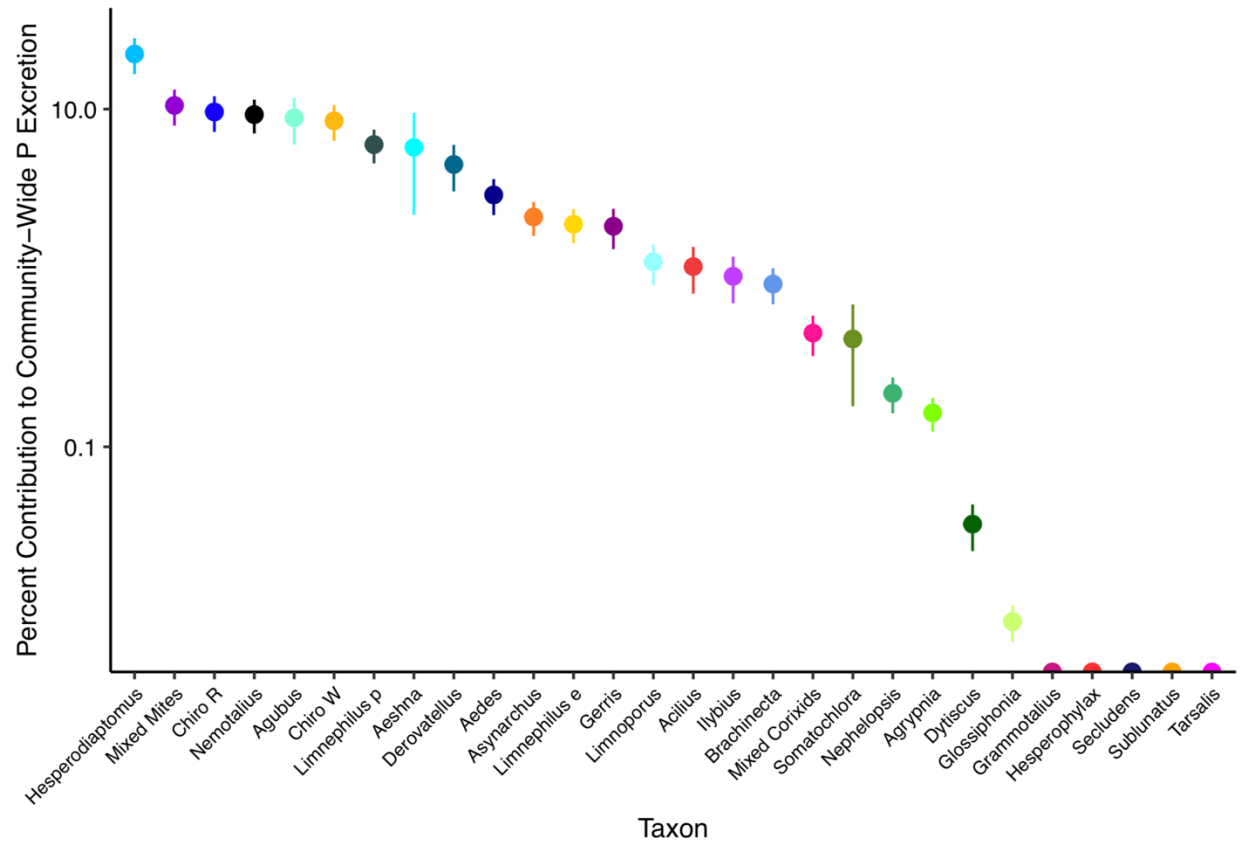


Figure 6. Percent contribution of each taxon to community-wide P excretion. Colors indicate different taxa, and the bars indicate +/- one standard error around the mean. The y-axis is log10 scale.

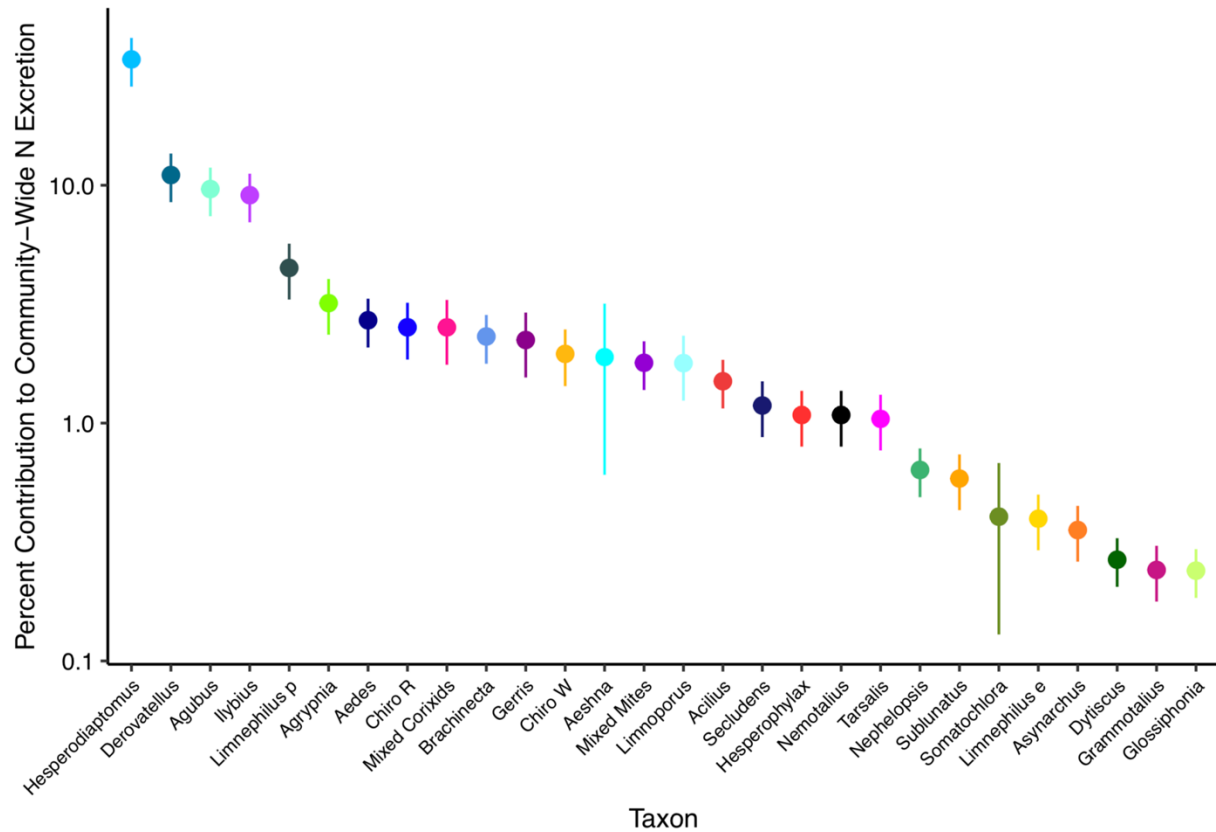


Figure 7. Percent contribution of each taxon to community-wide P excretion. Colors indicate different taxa, and the bars indicate +/- one standard error around the mean. The y-axis is log10 scale.

Macroinvertebrates contributed a highly significant amount of nutrients to pond uptake (demand). Their contribution ranged between 25.5% and 67.7% of phosphorus demand. The macroinvertebrates in the semi-permanent ponds appear to contribute slightly less to P uptake than those in permanent ponds (Figure 8). The contribution to N demand is even more significant. In all ponds except pond 1, which had double the demand for nitrogen than all other ponds ($1721.01 \text{ gN d}^{-1} \text{ m}^{-2}$), macroinvertebrates contributed over 100% on nitrogen demand. In pond 12 macroinvertebrates supplied 352.5% of pond nitrogen uptake.

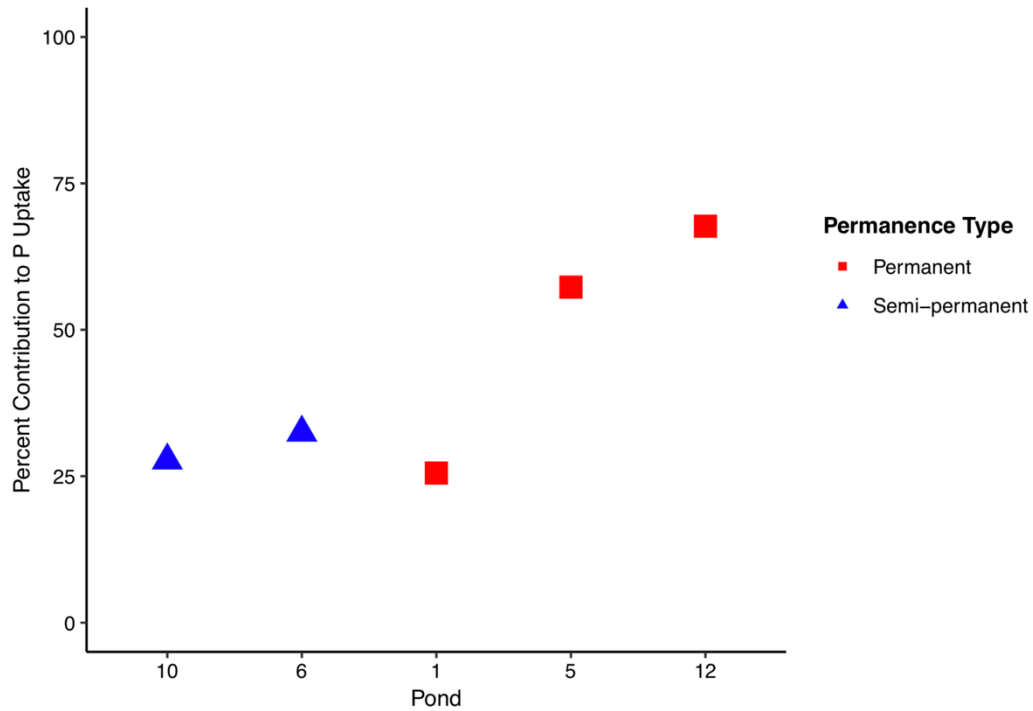


Figure 8. Percent contribution of the macroinvertebrate community to phosphorus uptake within each pond. Color and shape distinguishes semi-permanent from permanent ponds.

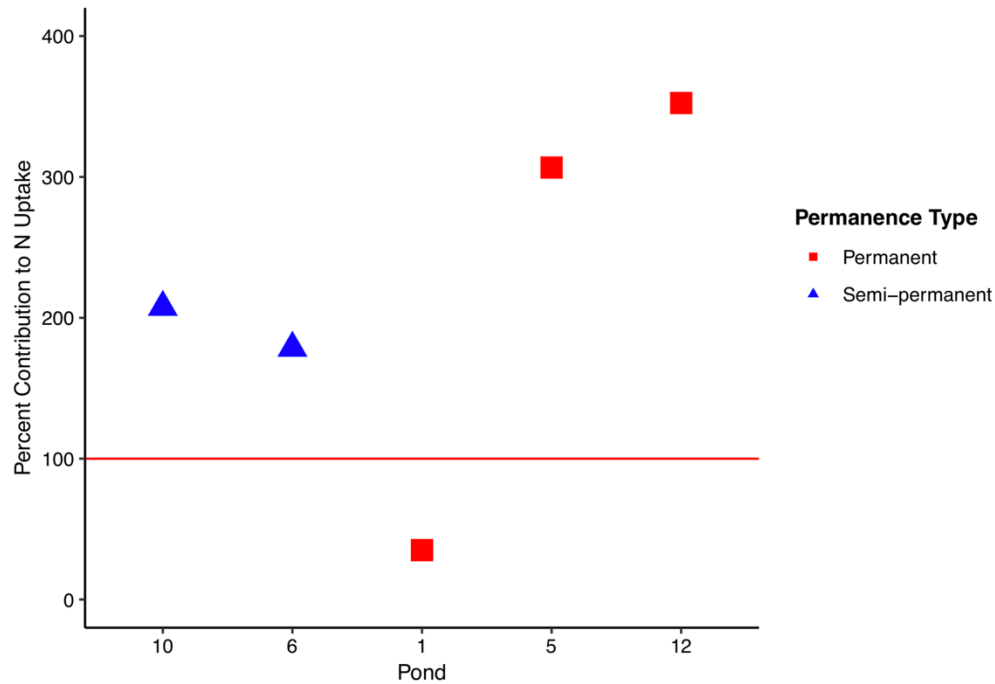


Figure 9. Percent contribution of the macroinvertebrate community to nitrogen uptake within each pond. Color and shape distinguishes semi-permanent from permanent ponds. The red line indicates 100% of contribution to N demand

Discussion

Our results seem to support the hypothesis that smaller organisms have a higher mass-specific excretion rate. The copepods and chironomids generally exhibited a higher mass-specific excretion rate than other, larger organisms. We have not yet analyzed these differences statistically, however, so no concrete conclusions can be drawn. These same taxa, however, also tended to be more variable in their excretion rates. This could be a result of not having enough individuals in some sample to detect their excretion. The shift in taxonomic ranking between the excretion rates of N and P, and the N:P ratio of excretion could be a result of differences in diets between trophic levels. Future analyses should be conducted to determine if there are significant differences between trophic levels or guilds. We also hope to perform trait-based analyses in the future to see if traits such as tissue stoichiometry or body size significantly impact excretion rates in these macroinvertebrates.

The estimations for population excretion and population contribution provide interesting preliminary observations, but may be slightly inaccurate. The dry mass we measured and the AFDM provided in the data set used are different numbers, AFDM being slightly less than regular dry mass. This means that my estimation for population excretion could be slightly underestimating actual excretion rates. The way we calculated the mass of a given taxa could also hide important differences between ponds and pond types. It is highly unlikely that the biomass within an order is evenly distributed among the genera of that order. Therefore, in future research it will be important to have more accurate and current biomass data.

Macroinvertebrates in these pond ecosystems contributed significantly to pond nutrient demand. This demonstrates the importance of these communities to ecosystem functioning in

these ponds. The copepods (*Hesperodiaptomus shoshone*) contributed the most to community-wide excretion, however, many other taxa contributed significantly as well. This indicates that changes in community composition could have consequences for nutrient cycling in these ponds and broader ecosystem functioning. Over the past years scientists studying these ponds have noticed a change in macroinvertebrate communities in response to climate change as taxa from lower elevations move upslope (Wissinger, *unpublished data*). Additionally, early snow melt and pond drying dates could affect these communities as they are highly dependent on hydroperiod (Wissinger et al. 1999, 2003). Due to the rapidly changing climate, it is important to understand how ecosystems will respond to these alterations. Looking at macroinvertebrate communities and their significance is one way to accomplish this in aquatic ecosystems.

Ponds at the Mexican Cut Nature Preserve

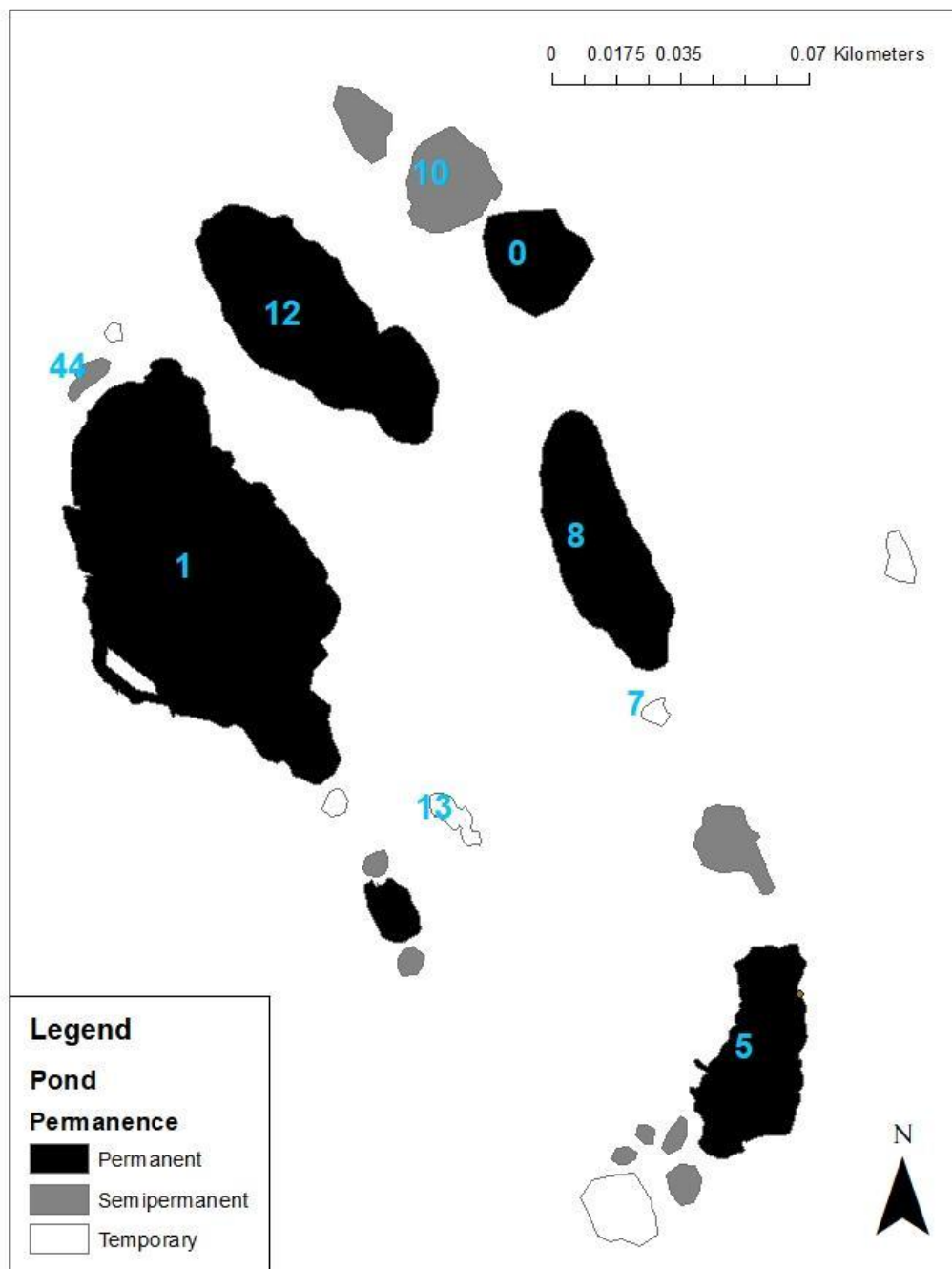


Figure 10. Map of the ponds at the Mexican Cut Nature Preserve, Gothic Colorado. Pond shapefiles provided by Dr. Scott Wissinger

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