Influence of Parasitism on Consumer Driven Nutrient Recycling by Aquatic Insects

Student: Matthew Ealy
Mentors: Brad Taylor and Andrew Sanders

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Abstract

Parasites have only recently gained the attention they deserve in studies involving their role in ecosystems. Using ecological stoichiometry will allow parasite roles to be established. I attempted to establish parasite effects on hosts by measuring the excretion Nitrogen:Phosphorus of infected *Megarcys signata*, infected *Baetis bicaudatus*, and their parasite free counterparts. I had hypothesized that the excretion N:P of infected hosts would be greater than the excretion N:P of parasite free hosts. However, the data is proving that the excretion N:P does not shift for *Megarcys signata* of increasing infection level. The shift in excretion occurs from parasite free to parasitized; the N:P's of excretion significantly differ. The *Megarcys signata* mass specific excretion rates of ammonium(N) and phosphate(P) do increase, but N increased at nearly twice the rate of P. My interpretation of these results is that the change in excretion is not because of a functional change in the *Megarcys*, but simply because of the addition of mites to the overall organism excretion equation. The increase of N and P excretion reflects the low body N:P of the water mite parasites. The mass specific excretion of phosphorous and nitrogen from *Baetis bicaudatus* were not found to be significantly different as the increased. Since nitrogen and phosphorus excretion did not change, this implies that the N:P of excretion did not change. The difference between the effects of mites and *Mermithids* is proposed to be because of their location and how they feed on the host.

Introduction

Insect larvae make up a major proportion of the consumer biomass within the streams surrounding Rocky Mountain Biological Laboratory. As consumers, insect larvae eat algae, detritus, and other invertebrates. Consumer-driven nutrient recycling is the process by which an organism consumes another organism and recycles some of the nutrients locked within back to the environment. After assimilating the fixed nutrients contained in their food resources, the consumer then excretes some as waste in highly labile inorganic forms, which are easily taken up by osmotrophs such as plants, algae and heterotrophic microbes, at the bottom of the food chain (Grimm, 1988). The insect larvae thus play an important role in consumer driven nutrient recycling (CDNR). Without consumers like the insect larvae, the nutrients locked within living matter and

detritus would not be available to autotrophs and microbes except through decomposition (Elser, 1999).

CDNR follows ecological stoichiometry; if a consumer is physiologically unchanged, but their diet varies, then a change in waste excretion will be observed (Olsen et al. 1986). If a consumer changes physiologically and their diet remains relatively constant, then a change in waste excretion is observed (Elser, 1999). CDNR can be thought of in terms of the concept of conservation of mass. The concept follows the simple formula of conservation of mass: consumption – growth - egestion = excretion. If we were to measure the exact mass of the nutrients that enter and exit an organism, we could then calculate the exact mass of the nutrients it assimilated(Elser, 1999). What leaves the system is usually not in the same form as it entered, but the overall mass is conserved. Adding parasites into the mass conservation equation changes the value of the growth term in the above equation and consequently changes the exit mass term(excretion), resulting in less nutrients allowed back into the cycle. To improve the CDNR model, the inclusion of pathogens and parasites is starting to be explored. This is because parasites may represent a physiological change in the host by such ways as causing an immune response. Stream insect larvae can have a number of parasites, and there is evidence that infection with a parasite can alter rates at which consumers recycle nutrients. This has been shown with snails, which when infected with trematode worms excreted at higher N:P levels than uninfected snails (Bernot, 2013).

Parasites are small compared to their hosts, thustheir bodies are low N:P, meaning they require resources that are P rich.(Elser, 1999) Parasites develop quickly, building high %P organelles and molecules such as ribosomes and mRNA. At normal population levels of hosts and parasites, the growth of any particular species could be limited by the availability of nitrogen because the parasite infection is not changing the nitrogen to phosphorus ratio.. When a parasite epidemic occurs, the growth limiting nutrient can be shifted toward phosphorous as the increasing biomass of parasites continously decreaseshost excretion N:P levels. By the principle of conservation of mass described above, the introduction of parasites now changes the assimilation term in the formula. What exits the infected hosts is now less than what an uninfected host would excrete. This effect is amplified as the parasite biomass grows. Parasites can have a negative

feedback as the parasitized hosts now excrete less P, resulting in other consumers becoming limited by P and excreting high N:P waste as well. Effectively reducing the P availability within the entire cycle, limiting growth overall (Bernot, 2013). Past Researchhas shown that by shifting the growth limiting nutrient from phosphorous to silicate the dominant algae shifted to a different species (Tilman, 1977). Parasite infections and the degree to which they occur can affect the overall excretion rate of the host species and the growth of the other members of of the community involved in nutrient recycling.

Studying the effects of parasites on stream insect excretion helps us understand the role of parasites in CDNR. How parasites affect the excretion of stream insect hosts and how excretion varies with the degree of infection are key questions we looked at in this study. By collecting unparasitized and parasitized stream insects, this study provides some insight into how parasites affect the host's excretion N:P levels. I hypothesized that the excretion of parasitized hosts will differ from the excretion of an unparasitized host because of the physiological change that parasite infections cause. Specifically, not only would the excretion N:P of parasitized insect larvae be higher than unparasitized host insect larvae, but as the number of parasites increases so will the N:P of the host excretions.

Methods

Megarcys signata/water mite larvae (Acari: Hydrachnida)

Individuals of the stonefly *Megarcys signata* (Plecoptera: Perlodidae) were collected from different locations within the East River Valley. One site slightly lower than the RMBL town site (Map 3), one within the town site (Map 2), and one site at the outlet of Emerald Lake (Map 1). One location along Copper Creek within the RMBL town site was also sampled. We used a 500µm kick net and collected about 200 *Megarcys signata* individuals in total. The stoneflies were separated into different containers based on whether or not they were infected with mites. For each sample, three individuals were taken from one of the containers and placed into a 710mL whirlpak

with 120mL of stream water filtered with a 1 micrometer glass fiber filter. Each sample was placed into a temperature controlled cooler for one and a half hours, to allow sufficient excretion concentrations to develop. The temperature and time were recorded when the samples were placed in the cooler as well as when they exited. Phosphorous and Nitrogen samples were taken with a 60mL syringe. Phosphorous samples were kept in standard glass test tubes, while nitrogen was kept in 60mL Nalgene amber bottles to prevent light degeneration. Using the syringe, we extracted 60mL from each bag. A 1 μm glass fiber filter manifold was placed on the tip of the syringe. 10mL of sample was dispensed into both the tube and the bottle to serve as a rinse. After the rinse, 20mL was placed into each. The bags containing the insects were then sealed and frozen once we returned to the lab.

Baetis Bicaudatus/Mermithid

Four-hundred individuals of the mayfly *Baetis bicaudatus* were collected from two locations within the East River valley: Upper Rock Creek and Marmot Creek. Marmot Creek has a high prevalence of parasitized *B. bicaudatus*, while Upper Rock Creek has a low prevalence. Ten individuals were randomly sorted from both locations into mesocosms. Three rocks from the East river were placed into each mesocosms. These rocks provided the necessary algae to feed the mayfly larvae. The mayflies were allowed to graze on the rocks for three days to homogenize the diets between the two collection locations. After three days, the *Baetis* from each mesocasm were collected and placed into whirlpaks labeled with numbers corresponding to the mesocosms. One-hundred mL of stream water filtered with a 1.0 μm glass fiber filter was placed into each whirlpak. The whirlpaks were placed into a cooler filled with stream water. After letting the *Baetis* sit for at least one hour, we removed them and took samples for phosphorus and nitrogen testing using the same method used for *Megarcys signata* excretion samples. The bags containing the insects were sealed and frozen upon returning to the lab.

General Sample Analysis and Insect Processing

We used two methods to process excretion samples: PO_4-P was analyzed using the molybdate blue method (APHA 1995); NH_4-N was analyzed using the flourometric method as modified by Taylor et al. (2007).

Once thawed, the insects were thoroughly checked for parasites, and any parasites present were removed and counted. The insects were then placed into pre-weighed scint vials assigned to each bag which were then placed into an oven to dry. Parasites were placed into pre-weighed aluminum tins assigned to each bag, and also placed into the drying oven. Both the parasite(s) and the insects were allowed to dry for at least 24 hours. After drying, both were placed in a container with desiccant to cool and prevent condensation. Once room temperature, the vials and tins were reweighed. The vials were then sealed and saved for future analysis of body stoichiometry.

Data Analysis

Data were analyzed using JMP(SAS institute). The t-test was performed with the response being excretion N:P, and the predictor being infected or uninfected. For *Megarcys signata* specifically we regressed mass-specific excretion rates of N and P against the number of mites on each stonefly, including the excretion values for the uninfected insects (0 mites level). This analysis was used to determine the effects of ectoparasitic load (i.e., the number of mites) on stonefly nutrient excretion. For *Baetis Bicaudatus* we regressed mass specific nitrogen, phosphorous excretions, and the N:P of excretions against the prevalence of infected *Baetis.* The regression was used to determine the effects of endoparasitic load on mayfly nutrient excretion.

Results

Megarcys signata/water mite larvae (Acari: Hydrachnida)

Megarcys signata mass specific nitrogen excretion rates were found to increase as the number of mites per insect increased as shown in figure 1(R^2 = 0.433642, p <0.0001, F ratrio = 24.5, dF = 33). Mass specific P(Phosphorous) excretion rates were also found to increase as the number of mites per insect increased as shown in figure 2((R^2 = 0.574669, p < 0.0001, F ratio = 43.24, dF = 33). The ratio of N:P did not significantly change as the number of mites per insect

increased as shown in figure 3. We performed a t-test on the N:P of excretions between parasitized and un-parasitized *M. signata* and found a significant difference in excretion stoichiometry between the two treatments, as shown in figure 4 ($p = 0.0175$, t ratio = -2.5, dF = 30.35). The mass of *Megarcys* signata was not found to significantly differ as the number of mites per insect increased ($R^2 = 0.003939$, $p = 0.7244$, F ratio = 0.13, dF = 33).

Baetis Bicaudatus/Mermithid

Baetis bicaudatus mass specific nitrogen excretion ($R^2 = 0.009159$ $p = 0.4789$) and phosphorous excretion ($R^2 = 0.0504$, $p = 0.0933$) were found not to differ as the prevalence per bag increased. No relationship between N:P ratio and prevalence was found ($R^2 = 0.011528$, $p = 0.4309$).

Discussion

Megarcys signata/water mite larvae (Acari: Hydrachnida)

As the number of mites per insect increased the mass specific N & P also increased. (Figures 1 & 2). There was no significant change in the N:P of excretion, but there is a significant difference between parasitized and unparasitized insect excretion (Figures 3 & 4). Excretion changed no matter the infection level: once a *Megarcys* is parasitized, the overall excretions start to increase above that of an uninfected *Megarcys*. As that infection increases so does the excretion rates of both nitrogen and phosphorus. To obtain excretion data for *Megarcys* with over 20 mites, we will have to collect more samples in the future. The gap in *Megarcys* data must be filled with future samples to complete the story.

The data suggests that maybe the mites are taking nutrients from the insects that were not intended to be excreted by feeding on the hemolymph through the softer section of the exoskeleton. Without changing the excretion of the *Megarcys*. If the mites remove nutrients from their host, assimilate, and then excrete them, the overall excretions would increase. The slope of the mass

specific P excretion is nearly half (m = 0.0001) the slope for mass specific N excretion (m = 0.0003). This suggests that the N excretion increases nearly twice as the rate of P excretion as the number of mites per insect increases. This further provides evidence that the increase of excretions is coming from the mites because of the assumed low body N:P. Their low body N:P results in their excretion having high N:P as they retain more of the phosphorus they consume (Elser, 1996). The addition of mites increases the overall excretion of the host and follows the assumed low body N:P of the mites. The mites feeding on *Megarcys* are doing what grazer invertebrates do with algae, recycling nutrients that would have otherwise remained locked up for an extended period of time.

Our results show that *Megarcys* P excretions significantly change as the mass increases, but N excretions do not. If the *Megarcys* excretions are indeed remaining relatively constant as the infection number of mites increases, it suggests that the *Megarcys* is either consuming more invertebrates to make up for the stolen nutrients or their digestion system is absorbing more nutrients from the same number of invertebrates. I hypothesize that the *Megarcys signata* are increasing the number of invertebrates they consume to make up for the lost nutrients through infection. My hypothesis came from the data indicating that mites are having no effect on the mass of *Megarcys* signata (Figure 5). Also, I think that the *Megarcys* are already absorbing as much as they can from the invertebrates they consume. I hypothesize that the total egestion would be a good place to start looking for answers. By placing *Megarcys* with no mites and others with varying infection levels, each in their own containers with smaller invertebrates, the water can be sampled at intervals of two hours and the total levels of Egestion, N, and P can be compared. Egestion may be more visible than the excretion studied here. Collection of visible egestion and comparing them could also help with a preliminary study. I am also interested in determining the body C:N:P of the mites and *Megarcys signata* to better understand the proportion of nutrients assimilated. Body C:N:P can help distinguish the excretions from the host and the parasite.

Baetis Bicaudatus/Mermithid

The *Baetis bicaudatus* data did not support my hypothesis. The excretion, both N & P, did not differ as the prevalence per bag increased (Figures 6 & 7). Unsurprisingly, knowing the previous results (Figures 6 & 7), the N:P of excretion did not differ as prevalence increased. A t-

test could not be performed on the N:P of excretions as we did with *Megarcys signata* because there was not a clear way to determine whether a bag of *Baetis* should be considered parasitized or not. The *Mermithid* that infects *Baetis* is an endoparasite and only one infects each *Baetis*. Data suggests that the endoparasite, living inside the host, has characteristics that explain why a change in excretion was not found (Figure 8). Characteristics different than the water mites, such as: Investing less nitrogen into a sturdy exoskeleton and the nutrients required to survive within a host. Endoparasites infect their hosts differently than the ectoparasitic mites of *Megarcys* which may contribute to the difference in how the parasites affect their hosts.

Mermithids consume nutrients from inside the host. Most infected *Baetis* were discovered not to have intestines or their intestines were empty while under a dissecting scope, their adipose tissue was also scarce. The reduction in adipose tissue suggests that the Mermithid is receiving the majority of the nutrients. The empty intestines suggest that the *Mermithids* were acting as a pseudo digestion system. Infected *Baetis* are indeed smaller than their uninfected counterparts (Figure 9). I propose that the *Baetis* are smaller in mass because the *Mermithid* is stealing the majority of the nutrients, perhaps allowing enough to pass to Baetis for it to survive. The change in nutrients being assimilated by *Baetis* could explain why the excretions do not change. The *Mermithids* are now the main recipient of the nutrients the *Baetis* consumes and excretions are also determined by the *Mermithid*. In order for the excretions to not be significantly different as prevalence increases, the body N:P of the *Mermithid* must be nearly the same as the body N:P of *Baetis bicaudatus*. If it were not, the excretions would have indeed changed as the *Mermithid* would have retained a different proportion of the nutrients. Some Mermithids accounted for 40% of the mass in some bags without a change in excretion. This suggests that the body N:P of the Mermithid and Baetis are the same as well. We are attempting to obtain a sample large enough to analyze the body C:N:P of the *Mermithid* and *Baetis bicaudatus.*

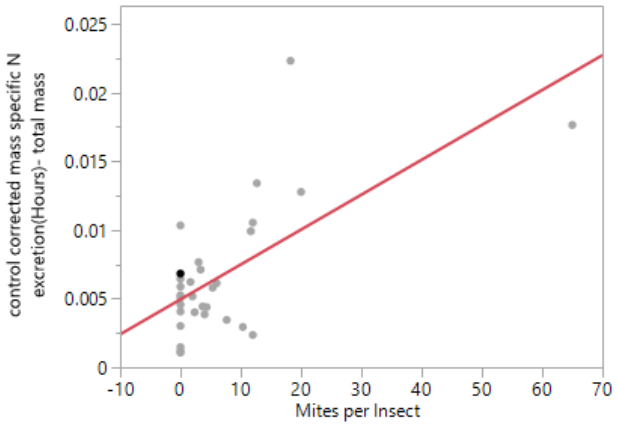
Conclusion

Megarcys signata is parasitized by the ectoparasite, the water mites. *Baetis bicaudatus* is parasitized by the endoparasite the Mermithid worm. Both parasites live in completely different environments, requiring different nutrients and behaviors to survive. *Mermithid* does not have to invest as much nitrogen in its outer layer like the water mites. Water mites must invest in an

exoskeleton to protect them from the surrounding environment. By comparing and understanding the differences between these two types of parasites, I think a better understanding of how parasites affect their hosts will be found. How parasites affect the excretions of their hosts is important to understanding how parasite epidemics can shift nutrient limitations within ecosystems. Changes in nutrient limitations can shift dominance from one species to another or solidify the dominance of a species (Tilman, 1977). Perhaps ectoparasites are the only type that actually change host excretion. Future research will include two more host and parasite combinations to see if the data shows ectoparasites affected excretion more than endoparasites. As caretakers of Earth, we must not let our activities affect the delicate balance between nutrients in our water sources. Human activity is currently wreaking havoc on our environment. Our actions have the potential of affecting species in unforeseen ways, if our actions cause a parasite population to explode it is crucial to understand how that parasite's role changes in consumer driven nutrient recycling as they become epidemic.

Figures & Maps

Figure 1:



Linear Fit

control corrected mass specific N excretion(Hours)- total mass = 0.0049465 + 0.0002538*Mites per Insect

Summary of Fit

RSquare	0.433642
RSquare Adj	0.415944
Root Mean Square Error	0.003483
Mean of Response	0.006479
Observations (or Sum Wgts)	34

Analysis of Variance

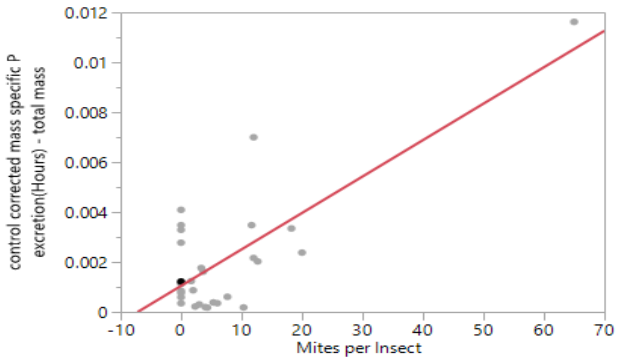
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.00029726	0.000297	24.5014
Error	32	0.00038823	0.000012	Prob > F
C. Total	33	0.00068549		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.0049465	0.000673	7.35	<.0001*
Mites per Insect	0.0002538	5.128e-5	4.95	<.0001*

Figure 1: Mass specific nitrogen excretions as a function of the number of mite per insect. The excretion of nitrogen per milligram of *Megarcys signata* increased as the number of mites per insect increased.

Figure 2:



Linear Fit

control corrected mass specific P excretion(Hours) - total mass = 0.0010492 + 0.0001459*Mites per Insect

Summary of Fit

RSquare	0.574669
RSquare Adj	0.561377
Root Mean Square Error	0.001508
Mean of Response	0.00193
Observations (or Sum Wgts)	34

Analysis of Variance

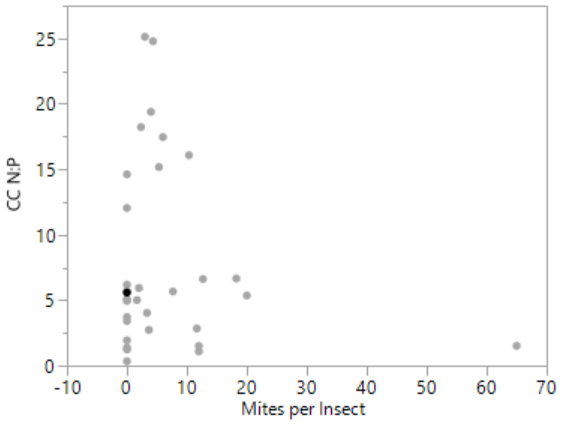
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.00009828	0.000098	43.2354
Error	32	0.00007274	2.273e-6	Prob > F
C. Total	33	0.00017102		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.0010492	0.000291	3.60	0.0011*
Mites per Insect	0.0001459	2.22e-5	6.58	<.0001*

Figure 2: Mass specific phosphorus excretion as a function of the number of mites per insect. The excretion of phosphorus per milligram of *Megarcys signata* increased as the number of mites per insect increased.

Figure 3:



Linear Fit

CC N:P = 7.990811 - 0.072161*Mites per Insect

Summary of Fit

RSquare	0.01479
RSquare Adj	-0.016
Root Mean Square Error	7.072067
Mean of Response	7.555192
Observations (or Sum Wgts)	34

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	24.0267	24.0267	0.4804
Error	32	1600.4522	50.0141	Prob > F
C. Total	33	1624.4789		0.4932

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	7.990811	1.366022	5.85	<.0001*
Mites per Insect	-0.072161	0.104112	-0.69	0.4932

Figure 3: Excretion ratio of moles of nitrogen over moles of phosphorus as a function the number of mites per *Megarcys signata*. No significant change was found in the excretion N:P as the number of mites increases.

Figure 4:

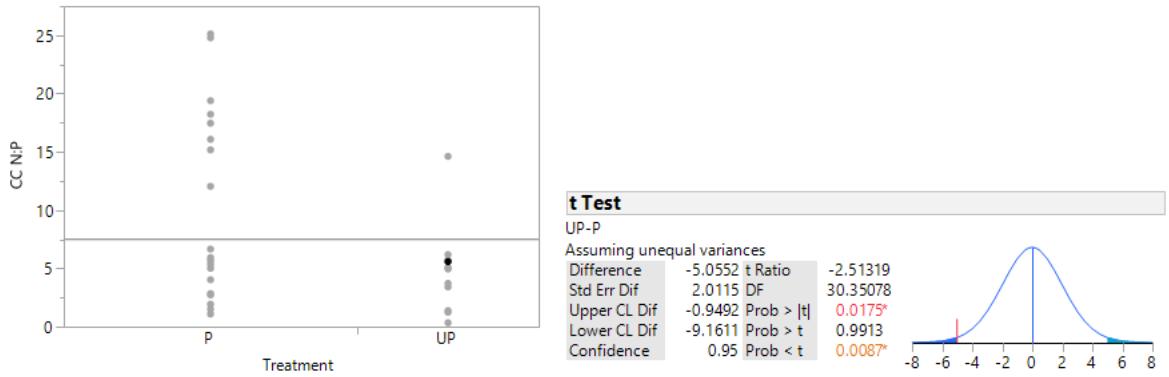


Figure 4: T-test with N:P of excretions as the response and the predictor is whether the *Megarcys* were parasitized(P) or not(UP). The N:P of excretions were shown to be significantly different between treatments. Levene's test was performed to determine unequal variance.

Figure 5

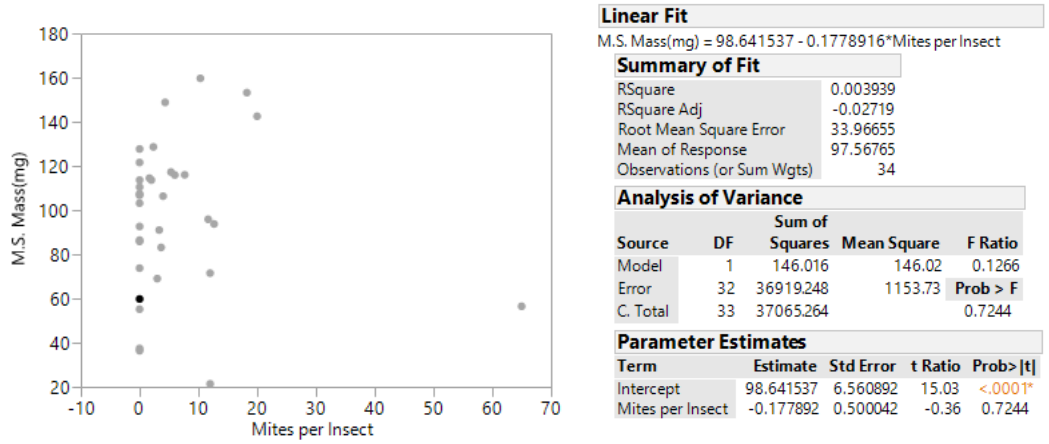
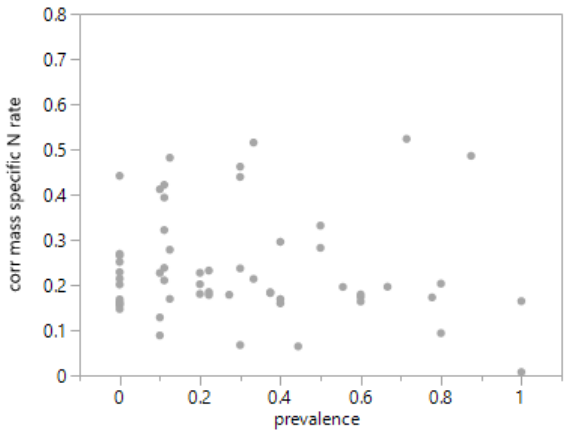


Figure 5: Mass of *Megarcys signata* as a function of the number of mites each were infected with. Data indicates there is no effect on the growth of *Megarcys* as the infection level increases.

Figure 6:



Linear Fit

corr mass specific N rate = 0.2503141 - 0.0406502*prevalence

Summary of Fit

RSquare	0.009159
RSquare Adj	-0.00886
Root Mean Square Error	0.119368
Mean of Response	0.238104
Observations (or Sum Wgts)	57

Analysis of Variance

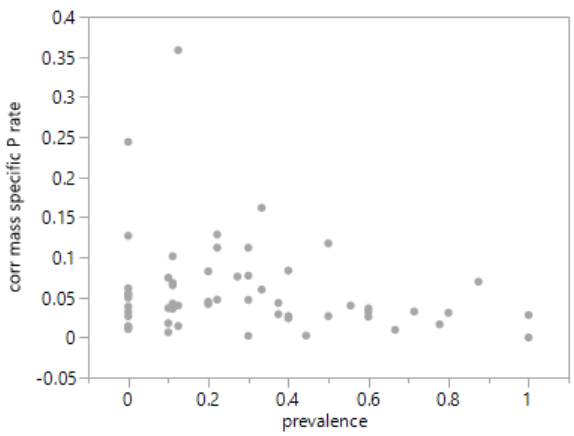
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.00724392	0.007244	0.5084
Error	55	0.78368485	0.014249	Prob > F
C. Total	56	0.79092877		0.4789

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.2503141	0.023307	10.74	<.0001*
prevalence	-0.04065	0.057012	-0.71	0.4789

Figure 6: Control corrected mass specific nitrogen excretion rate as a function of the prevalence (#of Worms/#of *Baetis*) of the parasitic worm in each bag of *Baetis*. No significant difference was found in the mass specific N excretion rate as prevalence increased.

Figure 7:



Linear Fit

corr mass specific P rate = 0.0719447 - 0.0478378*prevalence

Summary of Fit

RSquare	0.050376
RSquare Adj	0.03311
Root Mean Square Error	0.058638
Mean of Response	0.057576
Observations (or Sum Wgts)	57

Analysis of Variance

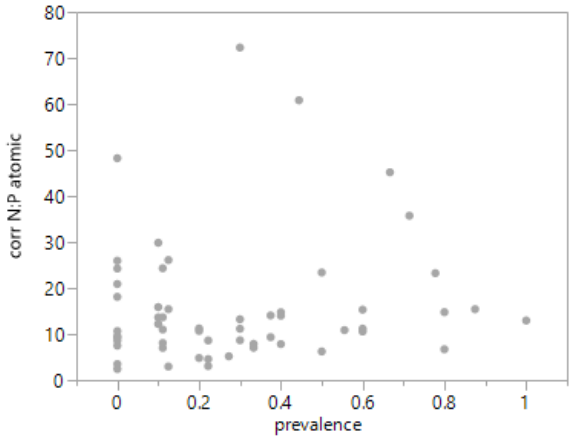
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.01003206	0.010032	2.9177
Error	55	0.18911058	0.003438	Prob > F
C. Total	56	0.19914264		0.0933

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.0719447	0.011449	6.28	<.0001*
prevalence	-0.047838	0.028006	-1.71	0.0933

Figure 7: Control corrected mass specific phosphorous excretion rates as a function of the prevalence of worms in each bag of *Baetis bicaudatus*. No significant difference was found in the mass specific excretions of phosphorus as prevalence increased.

Figure 8:



Linear Fit

corr N:P atomic = 14.307096 + 5.5377686*prevalence

Summary of Fit

RSquare	0.011528
RSquare Adj	-0.00678
Root Mean Square Error	13.75514
Mean of Response	15.90122
Observations (or Sum Wgts)	56

Analysis of Variance

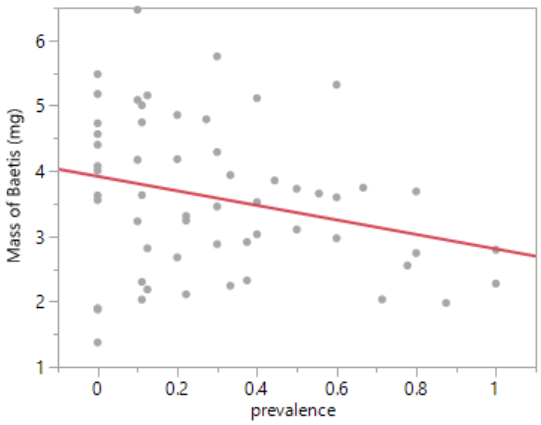
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	119.157	119.157	0.6298
Error	54	10217.013	189.204	Prob > F
C. Total	55	10336.170		0.4309

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	14.307096	2.722815	5.25	<.0001*
prevalence	5.5377686	6.978143	0.79	0.4309

Figure 8: Control corrected atomic N:P of excretions as a function of the prevalence of parasitic worms in each bag of *Baetis bicaudatus*. No significant difference was found in the N:P of excretions as prevalence increased.

Figure 9:



Linear Fit

Mass of Baetis (mg) = 3.91723 - 1.1114325*prevalence

Summary of Fit

RSquare	0.07175
RSquare Adj	0.054873
Root Mean Square Error	1.128618
Mean of Response	3.583404
Observations (or Sum Wgts)	57

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	5.415198	5.41520	4.2513
Error	55	70.057857	1.27378	Prob > F
C. Total	56	75.473054		0.0440*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	3.91723	0.220364	17.78	<.0001*
prevalence	-1.111433	0.539043	-2.06	0.0440*

Figure 9: Mass of *Baetis bicaudatus* as a function of prevalence of infected *Baetis* per bag. The mass of *Baetis* per bag decreased as the prevalence increased.

Map 1:

Map 1: Satellite image of the Emerald lake outlet where *Megarcys signata* were sampled.

Map 2:

Map 2: Satellite image of Copper Creek before merging into the East River. *Megarcys signata* were sampled here.

Map 3:

Map 3: Satellite image of the East River. Considered the Lower East River because it is south of the RMBL town site. *Megarcys signata* was sampled here.

Cited Literature

1. Allan, J. David. "The Effects of Reduction in Trout Density on the Invertebrate Community of a Mountain Stream." *Ecology* 63.5 (1982): 1444-455. Web
2. APHA, AWWA, and WEF. 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington DC.
3. Bernot, R.J. (2013) Parasite–host elemental content and the effects of a parasite on host-consumer-driven nutrient recycling. *Freshwater Science* 32, 299-308.
4. Elser, J.J., Elser, M.M., Mackay, N.A., and Carpenter, S.R. (1988) Zooplankton-Mediated Transitions between N-Limited and P-Limited Algal Growth. *Limnology and Oceanography* 33, 1-14.
5. Elser, James J., and Jotaro Urabe. "The Stoichiometry of Consumer-Driven Nutrient Recycling: Theory, Observations, and Consequences." *Ecology*80.3 (1999): 735. Web.
6. Grimm, N.B. (1988) Role of Macroinvertebrates in Nitrogen Dynamics of a Desert Stream. *Ecology* 69, 1884-1893.
7. HERNANDEZ, A. D., AND M. V. K. SUKHDEO. 2008. Parasite effects on isopod feeding rates can alter the host's functional role in a natural stream ecosystem. International Journal of Parasitology 38:683–690.
8. Kuris, A. M., R. F. Hechinger,J.C.Shaw,K.Whitney,L. Aguirre-Macedo,C.Boch, A.Dobson, E.J. Dunham, B. L. Fredensborg, T.C. Huspeni, J.Lorda, L. Mababa,F. Mancini, A.Mora,M.Pickering, N.Talhouk,M.E. Torchin, And K. D. Lafferty. 2008. Ecosystem Energetic Implications Of Parasite And Free-Living Biomass In Three Estuaries. Nature 454:515–518.
9. Olsen, Y., A. Jensen, H. Reinertsen, K. Y. Borsheim, M. Heldal, and A. Langeland. 1986. Dependence of the Rate of Release of Phosphorus by Zooplankton on the P-C Ratio in the Food-Supply, as Calculated by a Recycling Model. Limnology and Oceanography **31**:34-44.
10. "Statistics - Data Analysis - DOE - Six Sigma." Statistics. SAS Institute, n.d. Web. 15 Aug. 2016.

11. Taylor, B. W., C. F. Keep, R. O. Hall, B. J. Koch, L. M. Tronstad, A. S. Flecker, and A. J. Ulseth. 2007. Improving the fluorometric ammonium method: matrix effects, background fluorescence, and standard additions. Journal of the North American Benthological Society **26**:167-177.

12. Tilman, David. "Resource Competition between Plankton Algae: An Experimental and Theoretical Approach." *Ecology* 58.2 (1977): 338-48. Web.