

The Effects of Intraspecific Variation of Crayfish Behavior on Nutrient Cycling in Aquatic Environments

Undergraduate Thesis

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Abstract:

Human activities are rapidly altering species traits at a global scale. Yet, there remains a critical need to determine whether trait variation within species affects ecosystem responses to global change. In particular, intraspecific variation in feeding behavior can have strong effects on ecosystem processes, such as nutrient cycling in streams. Crayfish are dominant consumers in streams and play key roles in controlling important stream dynamics such as nutrient cycling. We hypothesized that within-population, individual variation in crayfish foraging behavior is associated with differences in nutrient excretion. The objectives of this study were to (i) to quantify individual differences in foraging behavior and boldness of crayfish using a giving up density (GUD) approach. (ii) to quantify individual differences in nutrient excretion of crayfish. (iii) to test whether foraging rate, boldness, and excretion rate are repeatable traits in the laboratory setting and (iv) to examine whether there is a relationship between individual variation in foraging rate, boldness, and excretion. These objectives were explored with both behavioral and excretion assays, and general linear and nonlinear mixed models as well as ANOVA tests. We found that behavior and excretion were repeatable, and that behavior is associated with ammonium excretion. The finding that crayfish foraging behavior is associated with differences in nutrient excretion has important implications for invasion ecology and nutrient cycling. It is known that behavioral changes occur along with invasion. These behavioral changes can significantly impact the nutrient excretion, and therefore nutrient dynamics within invaded environments.

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Introduction

Human activity is rapidly altering species traits on a global scale (Palkovacs *et al.*, 2011). The rates of the phenotypic changes that are driven by anthropogenic stressors have been shown to be higher than the rates of change driven by natural environmental variation (Palkovacs *et al.*, 2011). Changes in phenotypic traits can have significant impacts and implications for ecosystems as they can change both their dynamics and functioning (Raffard *et al.*, 2017). Many ecological models have historically ignored intraspecific trait variation, assuming that all individuals within a population are interchangeable, only using the average species phenotype (Bolnick *et al.*, 2011; Raffard *et al.* 2017). However, it has recently been shown that trait variation within a species can drive changes in critical ecosystem functions. For example, in stream ecosystems phenotypic variability, such as predation rate or leaf consumption can have significant impacts on algal production, rates of decomposition and nutrient dynamics (Raffard *et al.*, 2017). Therefore, there is a critical need to understand how trait differences occurring within a population are associated with key stream ecosystem functions and ultimately, the sustainability of freshwater ecosystems in response to changes in behavior.

Crayfish are dominant consumers in stream ecosystems and are well-known to affect important ecosystem functions. For example, as benthic grazers, crayfish have significant impacts on rates of primary production within their environments by altering periphyton biomass and increasing algal nutrient composition (Evans-White *et al.*, 2005). Crayfish can also increase the availability of N and P within aquatic systems through excretion and egestion, acting as “nutrient pumps” that regulate N and P availability to algae within the ecosystem (Evans-White *et al.*, 2005; Vanni *et al.*, 2002).

The movement of nitrogen and phosphorous from the benthos into the water column of streams and rivers can cause increases in the abundance and quality of phytoplankton. As these key nutrients are transported from the benthos into the water column as bioavailable byproducts of crayfish metabolism (Fryxell *et al.*, 2016). Through crayfish metabolism these unlocked nutrients are exported into the surrounding environment via fecal material, urine, and diffusion products. Ammonium, NH_4^+ , is the main diffusion metabolic byproduct and is excreted from the antennal gland (Raffard *et al.*, 2017; Kristneson & Hessen, 1992). Ammonium is a key nutrient within aquatic environments as it is one of the types of bioavailable nitrogen within streams. Changes in ammonium can impact ecosystem functioning through changes in nutrient availability and primary production as nutrient excretion is the most direct way animals provide nutrients to primary producers, like algae and macrophytes, and heterotrophic microbes within aquatic environments (Schmitz *et al.*, 2010; Vanni *et al.*, 2002).

Behavioral traits and intraspecific variation within populations may be linked with, and provide important insight into, ecosystem functioning and nutrient cycling. It has been shown that not only are phenotypic traits significantly impacted by changes in environmental conditions, but can in return act upon the environment reciprocally and further change it (Raffard *et al.*, 2017). For example, the trait of boldness is a response trait, being impacted by the environment, but in turn affect effect traits, such as predation rate, that impact the surrounding environment. Additionally, behavioral traits of animals are altered before other traits in response to environmental change, and differences in behavioral traits can have significant impacts on important ecosystem functions, such as nutrient cycling (Raffard *et al.*, 2017; Sih & Johnson, 2004). An example of this is can be seen in how intraspecific variation of feeding behavior in Trinidadian guppies affected both primary production and decomposition rates within an

ecosystem. Changes in phenotypes, like food selectivity, due to differences in environmental stressors, predation pressure, in turn caused changes in ammonium excretion. These changes in ammonium excretion are what impacted critical ecosystem functions (Bassar *et al.*, 2010). Also, many traits are correlated with each other, be it behavioral to behavioral or behavioral to physiological (Reale *et al.*, 2010).

Giving-up density, or GUD, is an experimental framework to quantify foraging behavior and outcomes within heterogenous environments (Bedoya-Perez *et al.*, 2013). Time, energy, and exposure to predation risk are required for foraging behaviors (Brown & Kotler, 2004). GUD enables foraging behaviors to be quantified by measuring the amount of food remaining in a depletable food within a given timeframe (Bedoya-Perez *et al.*, 2013). This enables the quantification of foraging rate as the amount of food eaten in each patch within a given time is known, allowing for an understanding of how much a forager consumes over time. The majority of studies that utilize GUD focus on terrestrial systems, but there has been some usage of it within aquatic environments, specifically with fish (Bedoya-Perez *et al.*, 2013). Specifically, it has been used to understand how mottled sculpin establish territories, forage optimally, and how GUD relates to size and population density. It also can be used as an estimation of behavioral boldness as the foragers may have differing responses to the equivalent predation risk. Further understanding of these behaviors within aquatic ecosystems could be beneficial in understanding how aquatic forager behavior relates to consumption, and therefore critical ecosystem processes.

The repeatability of behavior is important as it shows that individuals behave in a consistent manner over time, allowing for predicting future behavioral pattern. Also, if a behavior is repeatable, it shows that the data collected is not a random behavioral fluke. Additionally, behaviors that have low within-individual variance, but high among-

individual variance are considered to be more repeatable (Bell *et al.*, 2009). There have been some studies on behavioral repeatability within the context of aquatic environments and invertebrates, but the vast majority of studies that focus on repeatability of behavior focus on terrestrial organisms (Bell *et al.*, 2009). One study that has looked at the repeatability of behavior within crayfish focused on the repeatability of boldness in the noble crayfish (*Astacus astacus*). It was shown that boldness in the noble crayfish was a repeatable behavior. This consistency in levels of boldness is thought to potentially impact the trophic role and fitness of individuals, making the repeatability of this behavior ecologically significant (Vainikka *et al.*, 2011).

The rusty crayfish was (*Faxonius rusticus*) the model species for this study. The species is native to the Ohio River drainage basin but have expanded into many other parts of the USA. They have been reported in over 15 other states outside of its native range, including the Laurentian Great Lakes. Additionally, they have historically been identified as disruptors of local aquatic ecosystems when in non-native areas (Olden *et al.*, 2006). Understanding how the behavior of these crayfish may impact critical ecosystem functions may shed light on the potential impacts of rusty crayfish invasions.

The overall aim of this study is to examine if, within-population, individual variation in crayfish foraging behavior is associated with differences in nutrient excretion. Specifically, the objectives of this study are to: 1) quantify individual differences in foraging behavior and boldness of crayfish using a giving-up density approach, 2) quantify individual differences in nutrient excretion of crayfish, 3) test whether foraging rate, boldness, and excretion rate are repeatable traits in the laboratory setting, and 4) examine whether there is a relationship between individual variation in foraging rate, boldness, and excretion.

Methodology

A) Animal Collection and Housing

I collected native rusty crayfish from Flat Run, a tributary of the Licking River, between June 10th and 13th, 2019. The location sampled was within Flat Run Veterans Park in Cynthiana, Kentucky (38°23'37.5"N 84°17'22.1"W). I collected approximately ninety crayfish using a mix of small dip nets and seines. Approximately 90 individuals, with a carapace length between 19 and 31 mm, with an even male to female ratio, were kept. The crayfish were then transported to the Pintor Lab at The Ohio State University.

Within the lab, crayfish were individually held within perforated 120 mL plastic containers, labeled with individual identification numbers. These containers were kept in 10-gallon aquaria with constant filtration and aeration, as well as a pebbled bottom. The crayfish were originally fed two commercial shrimp pellets three times a week, with partial water changes at least after every feeding. For the duration of the assays the crayfish were fed three pellets twice a week with partial water changes after every feeding and at least one other time each week. The crayfish were acclimatized to the laboratory setting for a minimum of two weeks before being used in any assays.

B) Foraging Assays

To quantify differences in individual foraging rate, voracity, and GUD I conducted a laboratory behavioral assay on thirty crayfish. Individuals used within the assay ranged in size between 22-30 mm in carapace length. I used 15 males and 15 females. To standardize hunger prior to the assay, the crayfish were fed 3 shrimp pellets 18-24 hours before the assay was run. Three liters of previously aerated, dechlorinated water was put into a 16-quart rectangular plastic tub (42.5 cm x 30.2 cm x 17.8 cm). The exterior of the tub was painted black in order to

eliminate outside stimuli during the assay. In the tub there was a one-inch diameter PVC pipe, 2.5 to 3 inches in length to act as a shelter. The half of the tub that contained the shelter was then covered with thick black plastic sheeting to both further minimize the influence of outside stimuli and lower the perceived risk for the crayfish for that side due to increased cover.

Each individual crayfish was randomly assigned to an individual tank and was allowed to acclimate to the tank for 15 minutes prior to the start of the assay. After that time 2 petri dishes with 10 previously frozen chironomids, blood worms, each were placed into the tub. The chironomids were covered with layer of pea gravel in order to mimic a natural substrate as well as forcing the crayfish to put in effort to foraging. One petri dish was put in the corner opposite of the shelter on the covered side as a safe patch. The other was placed in the center of the uncovered side as the riskier patch. The crayfish were then left undisturbed for 20 minutes, after which they were put into their corresponding perforated containers for the excretion assay. The number of remaining chironomids in each of the petri dishes and the tub was counted. To estimate the repeatability of these foraging behaviors, I conducted this assay three times for each individual crayfish, with at least 48 hours in between each assay.

C) Excretion assay

To quantify excretion, crayfish were placed individually into a 250mL plastic container filled with 200mL of spring water within 10 minutes of the end of the foraging assay (described above) (Raffard *et al.*, 2017). The crayfish were then left to excrete for 3 hours, then removed from the container. The water was immediately filtered via vacuum filtration with a 0.7 μm glass microfiber filter (Whatman GE/E grade) to remove particulate matter and bacteria. The filtered water was then frozen until lab analysis. Excretion was measured after each of the three foraging

assays. The samples were sent to Stone Lab where ammonium, nitrate, nitrite, NO_2 , and phosphate excretion levels were analyzed.

D) Statistical analysis

A series of general linear and linear mixed models to test whether: 1) GUDs differed between near & far patches, and 2) whether behavior explained variation in excretion rate. Within the general linear and linear mixed models I used individual identifiers for the crayfish, each trial, GUD, patch locations, and size I also used ANOVAs in combination with the Lessells and Boag (1987) repeatability calculations to test whether foraging behavior, boldness and excretion were repeatable traits. These were performed within JMP Pro 14.

Results

It was found that the measures of GUD, and therefore behavior, and excretion were significantly repeatable. Specifically, the repeatability values were 0.3069 for GUD Near, 0.5466 for GUD Far, and 0.2504 for Ammonium excretion with standard errors of 0.1199, 0.1017, and 0.1206 respectively (Table 1). Repeatability calculations followed the formulas and guidelines outlined by Lessells and Boag (1987) which utilize the error estimates of the ANOVAs for the variables. The mean square within in the ANOVA and the mean square among in the ANOVA for the near patch GUD were 8.4184 and 19.6 respectively; for the far patch GUD the values were 2.4571 and 11.3444 respectively; and for ammonium excretion the values were 0.065342 and 0.130823 respectively. These values were used in the Lessells and Boag (1987) calculations in order to determine the repeatability (R) values.

It was found that less food was remaining in patches that were near to refuge than those that were far from refuge. The GUD was lower in the near than far patches (Estimate=0.6611, SE=0.1594, t-ratio=4.15, $p < 0.0001$; Figure 1). The mean GUD for the near patch was 7.6 with a

standard error of 0.309 and the mean GUD for the far patch was 8.922 with a standard error of 0.171.

Carapace length was shown to be negatively associated with ammonium excretion (Estimate= 0.0518, SE=0.0093, t-ratio=5.55, $p < 0.0001$; Figure 2), but not associated with foraging behavior, GUD (Estimate=0.0068, SE=0.0766, t-ratio=0.09, $p = 0.9299$). In other words, individual that were larger excreted more ammonia.

GUD, and therefore behavior, was shown to be associated with ammonium excretion (Estimate=- 0.0312, SE=0.0147, t-ratio=-2.13, $p = 0.0362$; Fig. 3). In other words, lower GUDs (e.g. consumed more food and left less behind) were associated with higher levels of ammonia excretion by the crayfish.

Discussion

My findings that GUDs were higher in the far patch than in the near patch (Figure 1) fit well with traditional GUD framework usage and findings of foraging behavior in terrestrial environments, but is relatively novel in aquatic environments. The usage of the GUD framework has been well established for studying a broad range of behaviors, including foraging behaviors, in terrestrial ecosystems with mammals (Bedoya-Perez *et al*, 2013), and there has been some established use of the framework in aquatic environments. Within terrestrial ecosystems GUD has been used to study foraging behaviors such as handling times, harvesting speed, as well as behavioral responses to predation risk in mammals like squirrels, chipmunks, gerbils, and mice (Bedoya-Perez *et al*, 2013). My findings fit well with these studies as it shows that the patch with a higher risk has the higher GUD. However, the few aquatic GUD studies primarily focus on fish and fish behavior in relation to patch quality and habitat choice (Petty & Grossman, 2010; Stenberg & Persson, 2006). Additionally, there is a lack of studies that incorporate body size into

their studies (Petty & Grossman, 2010). These findings indicate perceived risk by the crayfish and suggest that GUDs can be used as an indication of boldness. This is can be used due to the original focus of risk in relation to foraging within the GUD framework (Brown, 1988). An alternate explanation for the lower GUD in the near patch than the far patch could be that that the crayfish are simply satiated, and that the crayfish started at the near patch due to proximity. However, through personal observation, many of the crayfish were continuing to forage at the end of the foraging assays, and the rusty crayfish are known to be voracious consumers (Olden *et al.*, 2011).

The repeatability values found in this study are comparable to established values, and in the case of GUD behavior seem to be highly repeatable. Interest in the repeatability of behavior has been growing, especially in consistent individual differences in behavior. Meta-analysis of the growing pool of estimates of behavioral repeatability has shown that there is significant evidence for behavioral repeatability in organisms (Bell, 2008). The average repeatability score of these studies was 0.37. However, it was noted that besides mate choice, the repeatability of behavior in invertebrates is lower than in vertebrates (Bell, 2008). Additionally, there were no crayfish studies included in the meta-analysis, and there is limited research in the repeatability of crayfish. One of the few studies that explicitly deals with repeatability of crayfish behavior found repeatability values ranging from of $R=0.10$ to $R=0.28$, depending on the measurement (Vainikka *et al.*, 2011). These values are comparable to my measured values as they are measuring boldness of the noble crayfish. But the value for my GUD in the far patch was $R=0.5466$ which is significantly higher. This shows that there was a high consistency of variation in behavior on the individual level. This GUD measure could also be used as a proxy for the boldness of the crayfish due to the differences in perceived risk between the near and far

patches. The high level of repeatability in the GUD in the far patch indicates a high level of repeatability in the boldness of the crayfish. The repeatability of GUD and excretion is important in the validity of the results as well, as it shows that individuals are consistent over time and allows for the use of multiple trials in associating behavior and excretion. Repeatability for excretion is important as it shows that it varies consistently along with behavior. Thus, any linkages between behavior and excretion would both vary consistently and could be viewed in tandem with each other. This is important when considering how excretion impacts critical ecosystem processes and understanding how consistent variation in behavior can be linked to and likely be predictive of variation in excretion.

It was shown that both size and GUD, and therefore behavior, are both significantly associated with ammonium excretion. It has been well established that metabolism and size are linked, and as ammonium is a byproduct of crayfish metabolism and would most likely be linked to size (Armitage & Wall, 1982; McFeeters *et al.*, 2011). However, it was shown that foraging behavior was not associated with size. Therefore, even after size was accounted for foraging behavior still explained variation in ammonium excretion. This may have important implications for understanding the invasion ecology of crayfish and how invasion of the rusty crayfish into ecosystems may affect critical ecosystem processes. It has been shown that non-native populations of many species of crayfish exhibit differing behavioral traits than their native counterparts, including boldness and foraging activity, and that these intraspecific trait changes occur during invasion (Glon *et al.*, 2018; Pintor *et al.*, 2008). Thus, changes rusty crayfish behavior may significantly impact their excretion, and therefore their impact on ecosystem processes.

One of the intriguing patterns in the data was that there was a consistent decrease in the total chironomids eaten with each subsequent trial. This may suggest that the crayfish were becoming habituated and learning the assay. Although crayfish may have become habituated and less motivated to eat, individual differences in behavior were still repeatable, meaning that the individuals still varied in a consistent manner in each trial despite consuming fewer worms with each subsequent trial. Additionally, the ammonium excretions were still significantly and consistently linked to the behavior in each trial despite the decrease in total chironomids eaten.

This study showed that variation in intraspecific foraging behavior of the rusty is significantly associated with variation in nutrient excretion. This illustrates how behavioral traits of crayfish may significantly impact aquatic environments through nutrient availability. Future work could look at these relationships in both native and invasive populations of crayfish. This would enable a more holistic view of the relationship between crayfish behavior and nutrient excretion, as much of the rusty crayfish's range is nonnative, as well as illuminate changes in foraging behavior or excretion due to moving beyond the native range. Additionally, future studies could look at other behaviors and their relationship to the important trophic role that crayfish play in aquatic environments.

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Appendix A: Figures

Figure 1.

Mean GUD for each patch location with standard error bars

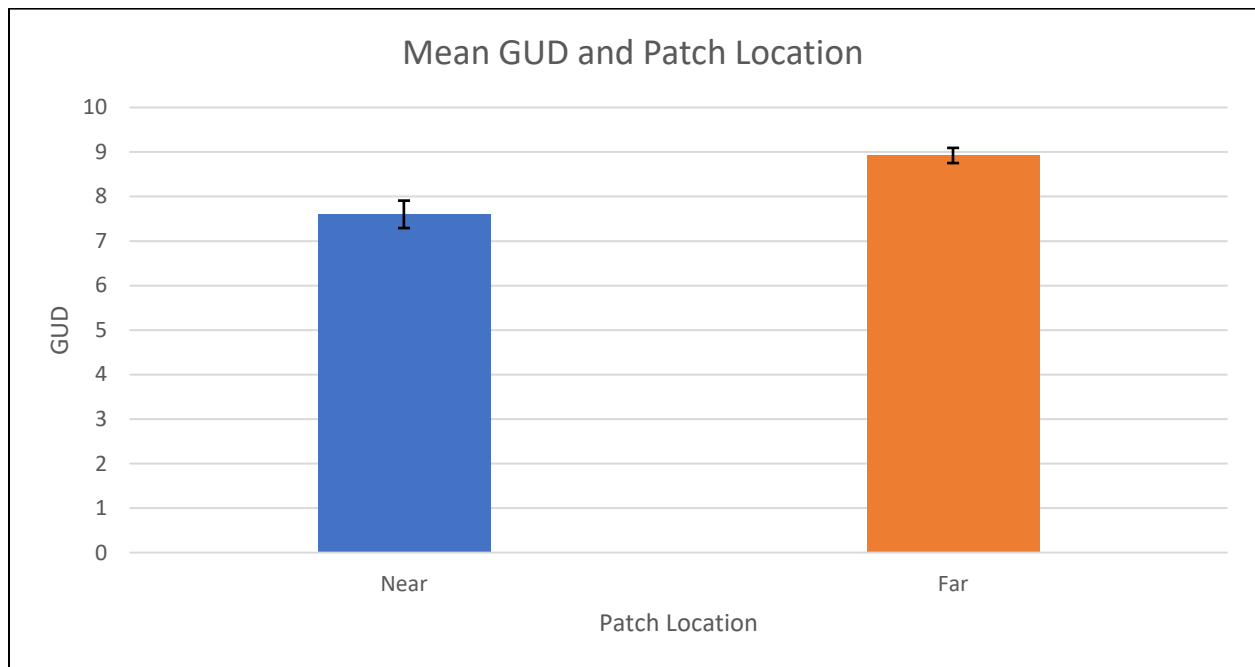


Figure 2.

Scatterplot of ammonium excretion values that have been log transformed for normality and carapace length

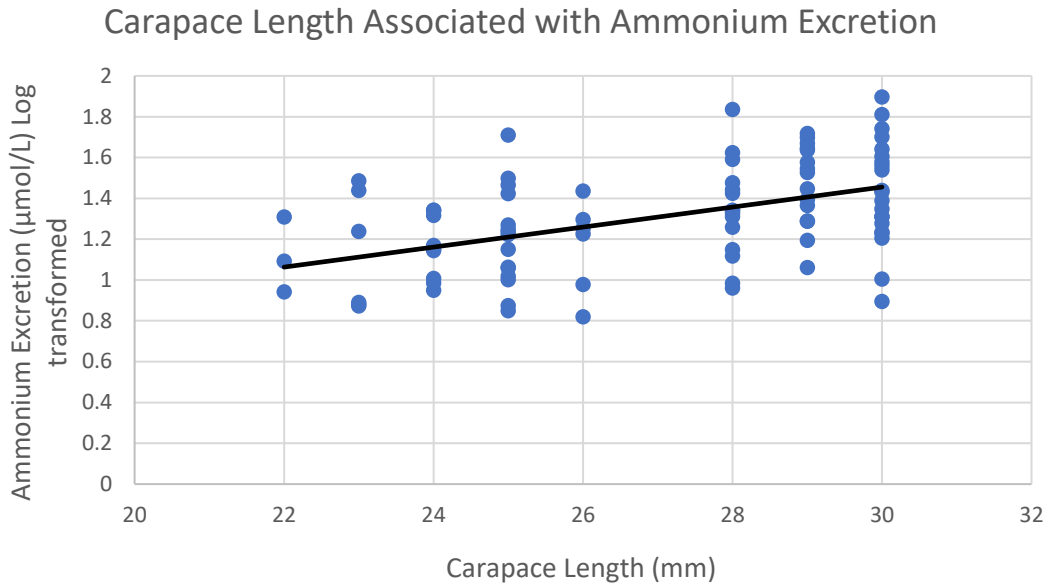
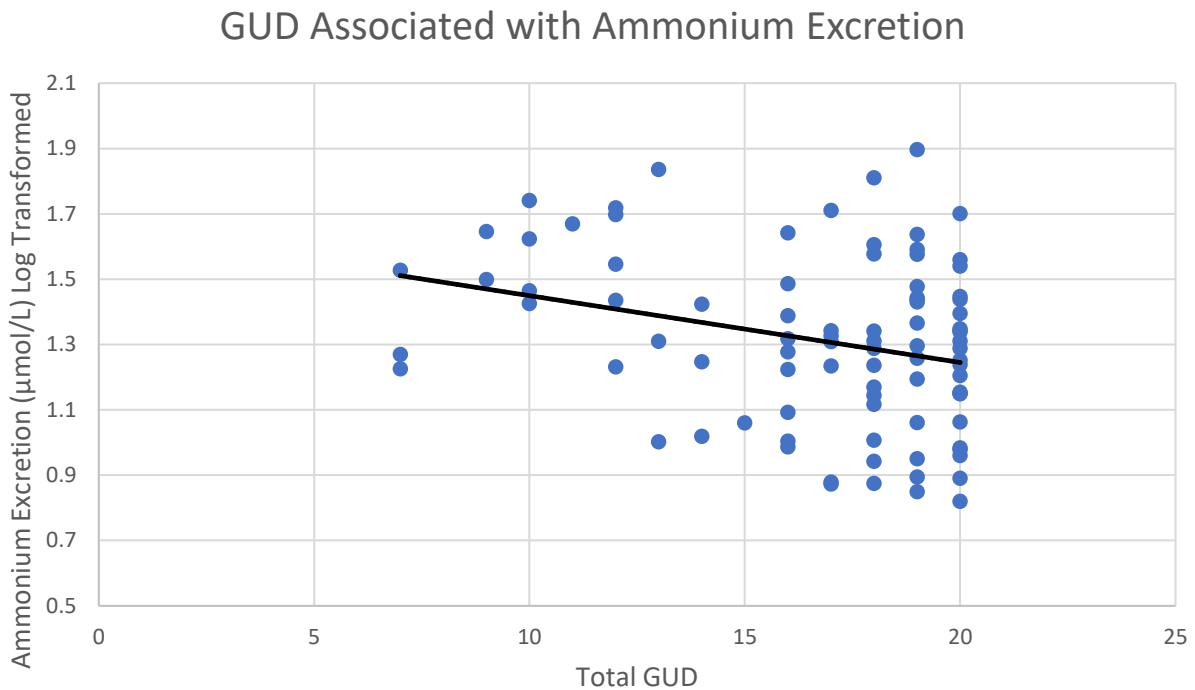


Figure 3.

Scatterplot showing ammonium excretion values that have been log transformed for normality and total GUD for each trial and individual



Appendix B: Tables**Table 1.**

The calculated repeatability scores and standard errors

Measurement	R-value	Standard Error
Worms Consumed	0.2074	0.0920
Ammonium	0.2504	0.1206
GUD Near	0.3069	0.1199
GUD Far	0.5466	0.1017