Trophic Dynamics of Smallmouth Bass Invading Coldwater Ecosystems

What impact will invasive smallmouth bass have on the trophic dynamics of coldwater ecosystems?

Virginia Department of Game and Inland Fisheries

Environmental Studies Capstone

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EXECUTIVE SUMMARY:

Smallmouth bass (*Micropterus dolomieu*) are a freshwater fish species that was introduced into Virginia in the late 1800's. The range of smallmouth bass is expected to expand, with climate change being a primary-driver of this projected range increase. The expansion of the range of smallmouth bass in Virginia poses a threat to coldwater ecosystems and will likely cause increased encroachment on native brook trout (*Salvelinus fontinalis*). The trophic ecology of smallmouth bass in rivers is poorly studied so the effects of this invasion remain unclear. Although smallmouth bass are currently not likely to have a major negative impact on brook trout since the present area of overlap of these two species is limited, it is important to be proactive in gaining an understanding of what future impacts the invasion of smallmouth bass will have on brook trout and coldwater ecosystems as a whole.

In order to understand the impact of smallmouth bass on coldwater ecosystems, I assessed the trophic ecology of smallmouth bass across a hierarchical river network (James River Basin, VA) using both stable isotope analysis and gut content analysis. It was hypothesized that the opportunistic diet of smallmouth bass would follow prey availability based on the River Continuum Concept which states that as the size of a watershed decreases there is a corresponding decrease in the diversity of fish species and an increase in the diversity of macroinvertebrates (Vannote et al., 1980). A shift towards reduced trophic positions and an increased incidence of drift feeding by smallmouth bass was expected to occur in smaller watershed area sample sites.

In order to increase the ability to detect a change in trophic position as a function of watershed size, twelve sample sites throughout the James River watershed were chosen based on

maximizing differences in watershed size among sample sites. Stable isotope analysis was used in this project to calculate smallmouth bass trophic position at sample sites following Post 2002. Stable nitrogen isotopes fractionate at each trophic level exchange to become enriched in $\delta^{15}N$ relative to prey, allowing for trophic position estimates of consumers (Post 2002). Carbon isotopes undergo biologically insignificant levels of fractionation and were used in this project as tracers to understand carbon source and energy flows within food webs. Because there is significant isotopic variation among sample sites, it was necessary to use a baseline organism to normalize isotope measurements which allows for comparison of isotope values among sample sites (Anderson & Cabana, 2007). Gut content analysis was used in conjunction with stable isotope analysis as a complement to trophic position estimates derived from the stable isotope methods.

Preliminary trophic positon estimates, calculated using stable isotope analysis, show that there is no significant difference in trophic position among sample sites ($P>0.05$). Gut content analysis also did not demonstrate a significant change among the different stream size classes ($P>0.05$). These results indicate that smallmouth bass remain at a consistent trophic position and express similar levels of piscivory[1] throughout the watershed, both in the headwaters and in the mainstem of the James River. Further, the diet of smallmouth bass was not found to follow prey availability according to the River Continuum Concept and instead the smallmouth bass diet remained consistent throughout the watershed. This result was reflected by consistent trophic position estimates from stable isotope analysis and a consistent frequency of fish prey found in gut contents. This finding represents a notable threat to coldwater ecosystems because it reveals high levels of predation on fish throughout the watershed, including in the headwaters where

[1] Consumption of fish

direct predation upon brook trout could occur. This data indicates that smallmouth bass invasions are likely to impact brook trout populations due to predation, although competition between brook trout and smallmouth bass is also a possible consequence and indirect food web effects are likely.

The results of this research indicate the diet of smallmouth bass contains a large portion of fish at all locations in the watershed and this is likely to have a direct effect on native brook trout; however, a future behavioral study that examines the predatory or competitive interactions between brook trout and smallmouth bass is needed to better quantify the effect of invasive smallmouth bass on coldwater ecosystems. Conducting a behavioral study would document areas in Virginia where these two species occur in sympatry[2] as well as areas where these two species could potentially overlap in the future. If these two species are expected to come into contact with one another in sympatry or overlap it will be necessary to better understand the interactions, and the extent of these interactions, that occur between these two species.

Although smallmouth bass appear to be a continued threat to brook trout in coldwater ecosystems, management solutions are currently limited. Both species are important sportfish in Virginia and managing one at the expense of the other does not maximize stakeholders' interests. Direct removal of smallmouth bass would be extremely costly and challenging, with a low likelihood of success. Restrictions on expansion of the range of smallmouth bass, either through physical or electrical barriers, to areas currently inhabited by brook trout would likely be the best method to effectively mitigate the impacts of smallmouth bass invasions into coldwater ecosystems. It is vital to anticipate the future potential trophic impacts of smallmouth bass on coldwater ecosystems as this will allow for increased proactive management options.

[2] Overlap of species

INTRODUCTION:

Smallmouth bass (*Micropterus dolomieu*) are a freshwater fish species that are native to the upper Mississippi River Basin and the Great Lakes region (Brown et al. 2009). Smallmouth bass were introduced into Virginia in the late 1800's and have since become well-established in a variety of aquatic systems in the state and are also a popular sportfish. Smallmouth bass are classified as a hardy cool/warmwater species, capable of inhabiting a range of conditions (Brown et al. 2009). Smallmouth bass are known predators and competitors with native species and are considered to be strong invaders (Gard 2004). Smallmouth bass have the potential to be considered an invasive species in Virginia if they have quantifiable direct negative effects on ecosystems that they have been introduced to. Elements supporting their threat as a capable invasive species include their small size at the ontogeny of piscivory (40-100 mm TL), juvenile usage of protective cover, low overlapping distribution with other predators, their high fecundity rate, and disposition to parental care (Brown et al. 2009, Mittelbach and Persson 1998). Smallmouth bass have become invasive in a variety of other systems where they have had a quantifiable deleterious effect on native fish species, including salmonids. For example, in the Northeastern U.S. smallmouth bass introduced into lakes were found to be strong predators on native fishes, including brook trout (*Salvelinus fontinalis*), which led to reduced population abundances (Whittier and Kincaid 1999).

Smallmouth bass are expected to increase their range northward in response to climate change (Jackson and Mandrak 2002, Shuter and Post 1990). Expansion of their range up elevation and thermal gradients into mountain headwater streams can be considered an analog for expansion northward allowing smallmouth bass to expand their range in Virginia into mountain headwater streams where they had been previously thermally restricted prior to climate change. The coldwater communities that currently exist in the headwater streams will either have to slowly adapt to the warming temperatures or face extirpation from these areas. New research suggests that certain fish species can undergo thermal plasticity, or a capacity to compensate for slightly shifted thermal regimes, which could make coldwater fish species more resilient to climate change effects (Farrell and Franklin 2016). Proposed thermal plasticity of coldwater fish species would lead to increased sympatry of brook trout and invasive smallmouth bass and increased interactions between these two species.

Brook trout are Virginia's only native "trout" species and are also a very popular recreational gamefish with considerable intrinsic value as the state freshwater fish of Virginia. Brook trout habitat requirements include cold, clean, and well-oxygenated water, which in Virginia primarily occurs in the state's mountain headwater streams (Martin and Petty 2009, EBTJV 2006). Stream temperatures and drainage network topology influence the overlap of these two species, with minimal current overlap occurring between these two species and generally only occurring temporarily where these two species' thermal regimes occur in close geographic proximity (Martin and Petty 2009). Brook trout populations exhibiting thermal plasticity would allow for increased overlap between these two species in response to climate change and the management question must be asked: "can we handle smallmouth bass in our trout streams?" With this overlap due to the future implications of climate change, it is important to proactively address what impact smallmouth bass invasions into previously thermally restricted areas will have on coldwater ecosystems. Invasive smallmouth bass in other systems, such as the Columbia River basin in the Pacific Northwest, were noted to be significant predators of juvenile salmon species (Lawrence et al. 2014, Petersen and Kitchell 2001). In streams that were thermally impaired due to the loss of riparian vegetation, allowing increased sunlight penetration and warming, the overlap of smallmouth bass and salmon species was greater (Lawrence et al. 2014, Petersen and Kitchell 2001). Therefore, it is likely the case that for areas in Virginia that have been thermally impacted, either through removal of riparian vegetation or through climate change, there would be an increased overlap of smallmouth bass and brook trout and thus greater interactions between the two species. Brook trout are already facing a myriad of other preexisting stressors, including water quality and land-use changes, stream acidification, sedimentation, increased summer temperatures and low flows which will make them more vulnerable to the effects of invasion by an introduced piscivore (EBTJV 2006). In order to effectively manage brook trout populations it is vital to also investigate the current and potential future impacts that smallmouth bass could have on the trophic dynamics of coldwater ecosystems. Special considerations must be given to the effect on brook trout, either through predation or competition.

The trophic dynamics of smallmouth bass river systems are poorly represented in the scientific literature and are better studied in lake systems. With the goal of better understanding the trophic dynamics of smallmouth bass in river systems and their effect on coldwater ecosystems, I developed an experimental design using both stable isotope analysis methods and gut content analysis on smallmouth bass samples collected throughout a hierarchical river network (James River Basin, VA, Figure 1). These methods allowed for an estimation of the trophic positions of smallmouth bass populations at different locations throughout the watershed, which provided an insight into the level of piscivory occurring at each sample site. Greater insight into the diet of smallmouth bass in different locations in the watershed improves understanding into the level of predation and/or competition that occurs between smallmouth bass and other native fish species, such as brook trout, allows for more quantitative estimate into the impact of invasive smallmouth bass on coldwater ecosystems.

According to the River Continuum Concept, which describes the changes a river experiences as it flows from its headwaters to its mouth, there is an increased diversity of macroinvertebrates and decreased diversity of fish species in headwater streams with the opposite trend occurring lower in the watershed (Vannote et al. 1980). As opportunistic predators that are likely to reflect prey availability, smallmouth bass could exhibit reduced levels of piscivory and increased incidence of drift feeding in the headwaters than they do in the mainstem of the James River. This would cause smallmouth bass to undergo a diet shift and sit at a lower trophic position in the smaller streams which some published data suggests is possible (Johnson and Dropkin 1995, 1993). Results from this study provide an insight into the research question of whether smallmouth bass undergo a trophic position shift as they move up the watershed. If a shift to a lower trophic position does occur and smallmouth bass display reduced rates of piscivory in headwater streams, where they could occur in sympatry with brook trout, then the effects of direct predation by smallmouth bass on brook trout would likely be limited. However, if smallmouth bass remain highly piscivorous throughout the watershed then they are likely to be harmful to native brook trout in Virginia as predation on brook trout would occur in areas where these two species overlap.

It is hypothesized that the diet of smallmouth bass will reflect prey availability following the River Continuum Concept and there would be reduced piscivory and increased insectivory in smaller streams with an increased occurrence of drift feeding (Vannote et al. 1980). These results would be reflected in decreased trophic position estimates calculated with stable isotope analysis and also a decreased frequency of fish prey in gut contents in the smaller watershed sample sites.

If smallmouth bass are found to be highly piscivorous in headwater streams where they could occur in sympatry with brook trout it is likely they are having a deleterious effect on brook trout and management changes should be urgently considered. Although this project will not arrive at a direct conclusion of management recommendations it will provide a proactive insight into the impacts invasive smallmouth bass will have on Virginia's coldwater ecosystems. From this analysis of trophic position of smallmouth bass across the different sample sites, a model can be developed to help predict smallmouth bass trophic position as a function of watershed area. This model can be applied throughout Virginia's coldwater streams and also in other systems where smallmouth bass have become invasive and pose a threat to native brook trout.

Available Evidence:

There is currently minimal published data existing in the scientific literature on the impact of smallmouth bass invasion on coldwater salmonids. It is accepted that smallmouth bass generally reduce the abundance and biodiversity of native small-bodied fishes following invasion of a waterbody by direct predation or through competition with native piscivores (Jackson and Mandrak 2002). Smallmouth bass invasions are also known to cause cascading effects on stream trophic dynamics, indirectly influencing benthic and algal assemblages either positively or negatively (Rudman et al. 2016, Jackson and Mandrak 2002). In the 1990's a study conducted in the northeast from 203 lakes showed that introduced predators, including smallmouth bass, had a negative effect of native species richness through predation or competition (Whittier and Kincaid, 1998). The most relevant study relating to smallmouth bass impacts on native salmonids was conducted on the Columbia River in the Pacific Northwest and showed that a thermal increase lead to increased consumption of out-migrating native pacific salmon smolt (*Oncorhynchus spp*) (Petersen and Kitchell 2001).

RESEARCH METHODS:

Examination of the trophic ecology of invasive smallmouth bass throughout an entire watershed provided insights into the impacts smallmouth bass have in different locations in the watershed. Of special concern was the impact of smallmouth bass on coldwater ecosystems, specifically their effect on native brook trout either through competition or direct predation. To investigate the trophic dynamics of smallmouth bass stable isotope analysis methods were used in conjunction with gut content analysis to assign smallmouth bass populations trophic positions at different locations within the James River watershed (Figure 1). These techniques were used to determine if smallmouth bass remain highly piscivorous in higher gradient headwater streams, where they could occur in sympatry with brook trout, or if smallmouth bass switch to a more benthic macroinvertebrate dominated diet in these smaller streams in accordance with the River Continuum Concept (Vannotte et al. 1980). Although it is generally assumed that invasion by smallmouth bass into waters inhabited by brook trout will cause increased stress to these native coldwater fish, without further investigation into the trophic dynamics that occur between these two species a quantitative assessment of the impact of smallmouth bass invasion cannot be accurately made.

Sample site locations were chosen in the James River watershed with the goal of maximizing the size range of watersheds represented (39-15132km^2, Figure 1). Having the greatest possible range of watershed areas better allowed for detection of any possible shift in trophic position from the mainstem of the James River upstream of Richmond to its headwater streams in the mountains in the western part of the state. As a hierarchical river system, the James River watershed branches in a dendritic fashion at the confluence of its various tributaries with the mainstem. This branched system allowed for a wide range of sample sites that compose a mix of land-uses and habitat characteristics and also provided a wide distribution of thermal gradients and stream topography. Sample sites were also chosen with consideration regarding the movement of smallmouth bass within the watershed. Sample sites located on a branch off the mainstem of the river were chosen a minimum of five miles upstream from the confluence in order to limit movement of smallmouth bass from the mainstem of the river up into the sample sites which would skew trophic position estimates. Samples sites were also chosen in terms of practicality of sampling efforts and access at both public locations or through permission from private landowners. The sampling sites covered a broad range of thermal regimes and ecosystems where the nonnative smallmouth bass coexisted with different aquatic communities. Some of the headwater sampling locations occurred in areas where sympatry, at least temporally, with brook trout was possible to occasionally occur; however, this was not a major criteria in the decision making of sample site locations, as areas where these two species occur in sympatry are uncommon and difficult to locate.

In July and June 2015, I collected fifteen to twenty smallmouth bass per sample site, providing a total sample size of 226 smallmouth bass. Sampling was completed in a single season in order to limit seasonal variation of isotopic values within the system. All samples were collected using electrofishing and hook-and-line methods. The size range of smallmouth bass was restricted from 100-300 mm total length in order to limit the effects of fish size on isotopic values. The minimum size of 100 mm was chosen because by the time smallmouth bass have reached this length they are piscivorous (Mittlebach & Persson 1998). The trophic ecology for smallmouth bass throughout the distribution of these sample sites was examined using stable isotope analysis methods and gut content analysis. White muscle tissue biopsies were collected from smallmouth bass samples and were dried at 60˚C for 48 hours. Died tissue samples were ground to a fine powder for isotopic analysis in the Washington and Lee Stable Isotope Ratio Mass Spectrometry Lab. In addition to smallmouth bass samples, baseline organisms were also collected along with crayfish and a variety of predator macroinvertebrates to be used in isotopic analysis to give a more complete understanding of the trophic ecology at each sample site. Smallmouth bass gut contents were analyzed for frequency of occurrence and abundance were cataloged at the lowest taxonomic level identified.

Stable nitrogen isotopes are a powerful ecology tool and can be used to estimate the trophic positions of aquatic consumers which provides an insight to their level of piscivory at different sample sites (Zanden et al. 1997). An isotope is an atom of the same element that contains a differing number of neutrons and therefore has a different atomic mass (Michener and Lajtha 2007). The ratio of different isotopes in a sample can be analyzed with a stable isotope ratio mass spectrometer which uses the differing atomic mass and charge of isotopes to measure their relative abundances. The ratio of isotopes of an element are expressed in delta notation: $\delta^{15}N = (^{15}N/^{14}N_{sample} - ^{15}N/^{14}N_{reference}/^{15}N/^{14}N_{sample}) \times 1000$ (Michener and Lajtha 2007). At each trophic level exchange, the relative abundance of the heavier ^{15}N isotope increases slightly compared to the lighter isotope ^{14}N (Post 2002). This increase in the ratio of heavy to light nitrogen isotopes at each trophic level exchange is due to the heavier isotope reacting marginally slower than the lighter isotope in metabolic processes, causing consumers to be enriched somewhat in ^{15}N relative to their prey in a process known as isotope fractionation (Michener and Lajtha 2007). Specifically, aquatic consumers are found to be enriched in ^{15}N relative to their prey by 3.4 ‰ (Post 2002). This bioaccumulation of ^{15}N relative to ^{14}N in consumers provides a distinct isotopic signature and allows for estimates of trophic level.

However, for estimates of trophic position to be assigned to consumers at each sample site, it was necessary to establish a baseline $\delta^{15}N$ value for each sample site to correct for spatial variation of isotopic values among sample sites (Anderson and Cabana 2007). Benthic macroinvertebrate primary consumers which consume solely autochthonous production, specifically scrapers which show the lowest $\delta^{15}N$ values and generally are widely distributed, were used as baseline organisms to establish the baseline $\delta^{15}N$ values in a system (Anderson and Cabana 2007). The scraper benthic macroinvertebrates collected for use as baseline organisms were water-pennies (***Psephenidae***) and aquatic snails (*Hydrobiidae, Phyisdae,* & *Pleuroceridae*). Snails were found to be slightly more enriched in ^{15}N compared to water-pennies which is likely caused by a longer tissue turnover time in the snails. Picking only one of these two organisms to serve as a baseline would cause trophic position estimates of smallmouth bass to be either artificially high or low; however, during the data analysis process it was found that the average of these two organisms $\delta^{15}N$ provided a very good baseline value to accurately estimate trophic positions of smallmouth bass using a fractionation value of 3.4‰ (Post 2002). If only one of these two baselines was collected from a sample site, a linear regression was performed in order to provide an average baseline value to be used in trophic position estimations. Once a baseline $\delta^{15}N$ value for each system was established, the trophic position of aquatic organisms could be estimated using the following equation where f is the mean fractionation value of 3.4‰ (Post 2002).

$$\text{Trophic Position}_{(consumer)} = ([\delta^{15}N_{consumer} - \delta^{15}N_{baseline}]/f) + 2$$

Unlike stable nitrogen isotopes, stable carbon isotopes do not undergo biologically significant levels of isotopic fractionation (Fry & Sherr 1984). Because carbon isotope values change little as they move through food webs they can be used as biological traces to determine carbon bases and energy sources as they flow through food webs (France and Peters 1997). These methods of using stable carbon isotopes as energy source tracers and nitrogen stable isotope fractionation to measure trophic level can be seen in a mock carbon:nitrogen isotope biplot created for visualization purposes (Figure 2).

Gut content analysis was used to supplement trophic position estimates calculated from stable nitrogen isotope data. Prey item data was cataloged for both numerical abundance[3] and frequency of occurrence[4] using the lowest taxonomic level identified.

RESULTS:

Preliminary analysis of smallmouth bass trophic position estimates for each sample site using $\delta^{15}N$ isotopes shows that trophic position does not significantly change with watershed size ($P>0.05$, Figure 3). Since trophic position estimates calculated with stable isotopes indicate that trophic position remains relatively consistent throughout the watershed, this result can be inferred to mean that the diet and rate of piscivory of smallmouth bass also stays relatively consistent throughout the entire watershed. Although it was predicted that larger bodied smallmouth bass would display a greater degree of piscivory, there was minimal influence of fish size on trophic position estimates ($R=0.0724$, Figure 4).

Analysis of gut contents as a supplement to stable isotope trophic position estimates also indicated that the diet of smallmouth bass was relatively consistent throughout the watershed. Sample sites were grouped into four stream size classes, with three samples sites per class, based on watershed sizes. No significant effect of stream size class was detected on occurrence of prey in the guts of smallmouth bass ($P>0.05$. Figure 5). Analysis for numerical abundance also indicated similar consistent results of prey items in guts among stream size classes ($P>0.05$, Not

[3] Number of specific prey item divided by total number of prey items
[4] Number of fish containing specific prey item divided by number of fish with any prey item in their gut

shown). Stream size class 1 expressed artificially high levels of piscivory because one of the sample sites in this group had a small sample size of smallmouth bass with prey items in their guts and was likely overrepresented in fish prey, skewing the stream size class rate of piscivory (Figure 5).

Because there was no statistical difference in prey items in smallmouth bass gut contents among the different sample site size classes, prey items were then analyzed for occurrence rates in smallmouth bass guts for the entire sample size of collected smallmouth bass. Crayfish were the most dominate prey item in smallmouth bass guts (33%) followed closely by primary consumer macroinvertebrates (28%), other ray-finned fishes (24%), and secondary consumer macroinvertebrates (14%) for the entire sample (Figure 6). Along with being the most dominate prey item in gut contents for the entire sample size, crayfish were also represented a consistently high percentage of gut contents at all stream size classes (Figure 5).

Crayfish and predatory macroinvertebrates were also isotopically analyzed and a carbon:nitrogen isotope biplot for all organisms in this study was created to allow for insight into both trophic position estimates using nitrogen isotope data and energy flows using carbon isotope data for the entire food web in these systems. (Figure 7).

DISCUSSION:

We must reject the hypothesis that the trophic position of smallmouth bass changes as a function of watershed size and that the diet consumed by smallmouth bass is directly influenced by the availability of prey items. Smallmouth bass expressed consistent levels of piscivory in all sample sites, encompassing a range of watershed sizes and habitats. While this is a statistically null difference across sample sites, this is a very important biological result as it shows smallmouth bass have a similar trophic position, and therefore similar rates of piscivory in both mainstem of the James River and in very small mountain headwater streams.

Further, the average estimated trophic position of smallmouth bass for all sample sites in this study is 3.7 ± 0.14 and this is comparable to the smallmouth bass literature trophic position, based on previous diet studies, of 3.6 ± 0.2 which demonstrates that the trophic position of smallmouth bass not only remain consistent in different locations throughout the same watershed but also remains consistent across entirely different systems. (Scott & Crossman 1973). The smallmouth bass literature mean trophic position of 3.6 ± 0.2 and the average trophic position of smallmouth bass in the James River Basin of 3.7 ± 0.14 depict smallmouth bass as sitting in between tertiary and secondary consumers. Because food web dynamics are much more complicated and interwoven than standard simplified food chains, this intermediate trophic position of 3.7 is due to the prey of smallmouth bass existing on multiple trophic levels and represents a degree of both piscivory and insectivory expressed by smallmouth bass.

There is a widely accepted assumption in fisheries ecology that trophic position experiences a positive relationship with increased fish size (Romanuk et al. 2010). In this study, it was found that this positive relationship between total length and trophic position was expressed only weakly and was not a biologically significant trend (Figure 4). However, the presence of this weak relationship indicates that trophic position does increase slightly with the total length of smallmouth bass and this is an important result to acknowledge because it validates stable isotope analysis as a successful method to estimate trophic position since this slight trend was detected. Thus, if a difference in trophic position estimates among sample sites was present, it would likely have been detected using these methods. Even a weak relationship between size and trophic position estimates demonstrates the importance of restricting the size range of collected smallmouth bass to minimize much of the influence of size on trophic position.

Gut content results indicate a consistent frequency of occurrence and numerical abundance of prey items in smallmouth bass guts among stream size classes (Figure 5). This result is in agreement with consistent levels of piscivory shown throughout the watershed, estimated through stable isotope analysis. It is important to use gut content analysis to backup estimates of trophic position as calculated with stable isotope analysis. Using these two methods in conjunction with each other gives better insight into diet and trophic position than if they were used separately. However, stable isotope analysis does have inherent benefits over gut content analysis in a diet study since stable isotopes provide a much greater temporal insight into diet whereas gut contents only provide an instantaneous snapshot of whatever prey is in the guts when the sample is collected. Therefore, stable isotopes can provide a much longer signature of what is more representative of the overall diet and does not contain the outlier prey items that may be present in gut content analysis. Further, every sample collected can be used for stable isotope analysis through a muscle tissue biopsy and a major drawback of using gut content analysis is that not all fish contain prey items in their guts (only roughly a third of smallmouth bass collected in this study contained prey items) which gives a much smaller sample size and reduces statistical power. It is also important to note that gut content studies have inherent flaws in their ability to assign trophic position to consumers and accurately determine their diet. Drawbacks of gut content analysis include a differing dissolution rate of prey species which varies greatly leading to a bias, and observed gut contents can greatly differ from the primary diet consumed, making stable nitrogen isotope methods an improved biological tracer for diet studies (Zanden et al. 1997). Therefore, gut content analysis only served as supplemental data in this study.

The dominance of crayfish in the diet of smallmouth bass at all stream size classes yields the potential caveat to the result of consistent trophic position estimates from stable isotope analysis throughout the watershed because crayfish could potentially be obscuring differences in piscivory among sample sites. Classified as detritivores and omnivores, crayfish consume a large portion of their diet from dead organic matter (Whitledge and Rabeni 1997). As a result of consuming dead organic matter crayfish incorporate a nitrogen isotope signature that is enriched in $\delta^{15}N$ due to further fractionation relative to their diet (Whitledge and Rabeni 1997). Because crayfish are enriched in $\delta^{15}N$, trophic position estimates following Post 2002 will give crayfish a higher trophic position than the one the one they actually occupy. Examination of the carbon:nitrogen isotope biplot shows the enriched $\delta^{15}N$ value of crayfish and a lower than expected $\delta^{15}N$ value for hellgrammites due to some consumption of vegetation (Anderson and Cabana 2007, Figure 7).

Since crayfish represent such a large portion of the smallmouth bass diet (33% in total) and make up a relatively consistent portion of their diet at all sample sites, crayfish could potentially be causing the $\delta^{15}N$ signature of smallmouth bass to become consistent across sample sites due to skewed trophic position estimates based on enriched $\delta^{15}N$ values. This could cause differences in trophic position estimates to be obscured and lead to a failure to detect a change in piscivory that occurs across the watershed. Carbon isotope data was analyzed to assess possible effect of crayfish on smallmouth bass. Crayfish, as omnivores and detritivores, consume a mix of terrestrial and aquatic vegetation along with dead organic matter from multiple trophic levels and thus have a different carbon source than do other macroinvertebrates, which primarily consume periphyton[5] (Whitledge and Rabeni 1997). Crayfish were found to be more enriched in $\delta^{13}C$ than hellgrammites and other macroinvertebrates and this likely represent a different carbon source into the food web (Figure 7). A likely carbon source for crayfish that explains their enriched $\delta^{13}C$ signature could be terrestrial inputs (Post 2002). Smallmouth bass display a mean carbon isotopic values between crayfish and other, less enriched, macroinvertebrates, and a thus likely incorporated carbon from both sources (Figure 7).

The ultimate effect of crayfish on smallmouth bass trophic position estimates is still unclear. Crayfish do represent a large portion of the diet of smallmouth bass at all stream sizes classes and appear to incorporate some of their isotopic signatures. However, there is no available literature that supports the idea that the enriched $\delta^{15}N$ values of crayfish could influence trophic position estimates in predatory fish. Perhaps the strongest argument against crayfish obscuring trophic position estimates is that the occurrence and abundance of fish prey in gut contents remained relatively consistent among stream size classes (Figure 5). If crayfish were obscuring a change in trophic position at different locations within the watershed then the gut content data would still be able to detect a change in rate of piscivory. This fact further demonstrates the importance of collecting gut content data in conjunction with stable isotope analysis to help back up stable isotope derived trophic position estimates.

If possible influence of crayfish on smallmouth bass trophic position estimates is dismissed as unlikely, then what is left is a statistically null difference of trophic estimates among sample sites. This result represents an important finding for assessing the impacts of smallmouth bass on native brook trout as it shows that smallmouth bass will remain highly piscivorous throughout the entire watershed, including areas where they can occur in sympatry with brook trout. The question going forward is no longer what effect smallmouth bass invasions

[5] Mixture of algae attached to submerged substrate

will have on coldwater ecosystems, but rather a question of where these effects will take place. This becomes a function of the areas that smallmouth bass can potentially invade and establish viable populations. Currently, the potential range of smallmouth bass is primarily restricted by an instream thermal gradient. Climate change will likely expand the range of smallmouth bass both northward and further upstream up the thermal gradient (Jackson and Mandrak 2002, Shuter and Post 1990). Expansion of their range will lead to increased overlap of smallmouth bass and brook trout and would result in increased interactions between the two species. Based on these results, the diet of smallmouth bass in areas where they overlap with brook trout will likely contain a large portion of fish, indicating probable predation on brook trout, especially juveniles. Smallmouth bass that have been introduced into native brook trout range in the Adirondacks have caused direct negative impacts on brook trout (Whittier and Kincaid 1999). Brook trout catch rates increased in a northeastern lake following removal efforts of smallmouth bass, indicating that invasive smallmouth bass had reduced brook trout populations in the lake (Whittier and Kincaid 1999). Addition of an invasive piscivore like smallmouth bass into a system could lead to a top-down trophic cascade where the reduction of prey populations releases control of primary consumers at lower trophic levels (Rudman et al. 2016).

Although it is unlikely smallmouth bass invasions into coldwater ecosystems in Virginia would lead to complete collapse of brook trout populations, they would likely add an increased stressor onto native brook trout populations which are already suffering due to habitat loss, stream acidification, and warming temperatures (EBTJV 2006). These factors make brook trout populations increasingly vulnerable to the addition of an introduced piscivore which could potentially reduce the range and lower the population abundances of brook trout. This would have direct implications for the popular brook trout recreational fishery and would also reduce freshwater fishing license sales and other angling related expenditures in Virginia.

Regardless of the results of this study, both species have value to their respective stakeholders in Virginia and aggressive management to promote the well-being of one of these species at the expense of the other would undoubtedly cause issues with the general public. Both species have become important recreational gamefish in Virginia and contribute to a portion of the $1.3 billion in annual economic impact to the commonwealth (VDGIF 2015). It is also very important to note that targeted removal of an already established invasive piscivore has been found to be very labor and cost intensive, and often times is not successful (Tyus and Saunders 2000). As such, there are currently limited management solutions to successfully mitigate the impact of smallmouth bass invasions into coldwater ecosystems. Restricting the further expansion of smallmouth bass, either through physical or electrical barriers, should take precedent over removal efforts from areas they have already established self-sustaining populations. This study demonstrates the importance of being proactive in understanding the full-range of impacts posed by invasive species before they occur as this will allow for preparedness for the ecological impacts and offer further opportunities for mitigation of these impacts. The invasion of smallmouth bass is directly implicated to climate change and it is important to understand what impacts the shifting of thermal regimes in aquatic systems will have.

CONCLUSION:

This study provides insights into the diet of smallmouth bass throughout the James River watershed. Through trophic position estimates from stable isotope methods and gut content analysis it was shown that the diet of smallmouth bass does not reflect prey availability and that smallmouth bass remain highly piscivorous in all sample sites. Smallmouth bass also appear to be primarily ambush predators of fish in all sample sites and do not make a significant shift to drift feeding of macroinvertebrates in smaller streams. As the range of smallmouth bass expands due to global climate change the results of this study will have important implications in determining the extent of what the impacts of smallmouth bass will be on coldwater ecosystems. However, this study only provides one source of evidence into the impacts of smallmouth bass invasions and it will be necessary to consider other forms of information to proactively address the full impacts of invasion and to give an accurate and achievable policy recommendation. Specifically, addressing the spatial extent of areas of possible overlap between these two species in response to climate change is vital. A thorough understanding of the interactions that occur between these two species is another factor that must be considered when quantifying the total impact of smallmouth bass invasions.

At this point it is not feasible to advise concrete policy recommendations to mitigate the effects of smallmouth invasions in Virginia. Instead, these results indicate that smallmouth bass will have a negative effect in the coldwater ecosystems that they are introduced to since their rates of piscivory remain high in all sample sites. As part of a proactive response to smallmouth bass invasions it is imperative to restrict their further expansion in order to limit areas of overlap between smallmouth bass and brook trout. Physical and electrical barriers could be effective in limiting range expansion, although maintaining currently established thermal gradients to prevent the spread of smallmouth bass would be most effective. While it will be difficult to control warming temperatures on a regional level, actions on the local scale, such as preventing removal of riparian overhead vegetation, can be taken to limit thermal pollution in streams and control further expansion of smallmouth bass. Smallmouth bass are not currently a major problem in Virginia, but based on these results they do pose a serious threat to coldwater ecosystems. The full effects of the potential impacts of smallmouth bass invasions into these areas must be addressed proactively if we hope to successfully mitigate some of their future impacts.

APPENDIX:

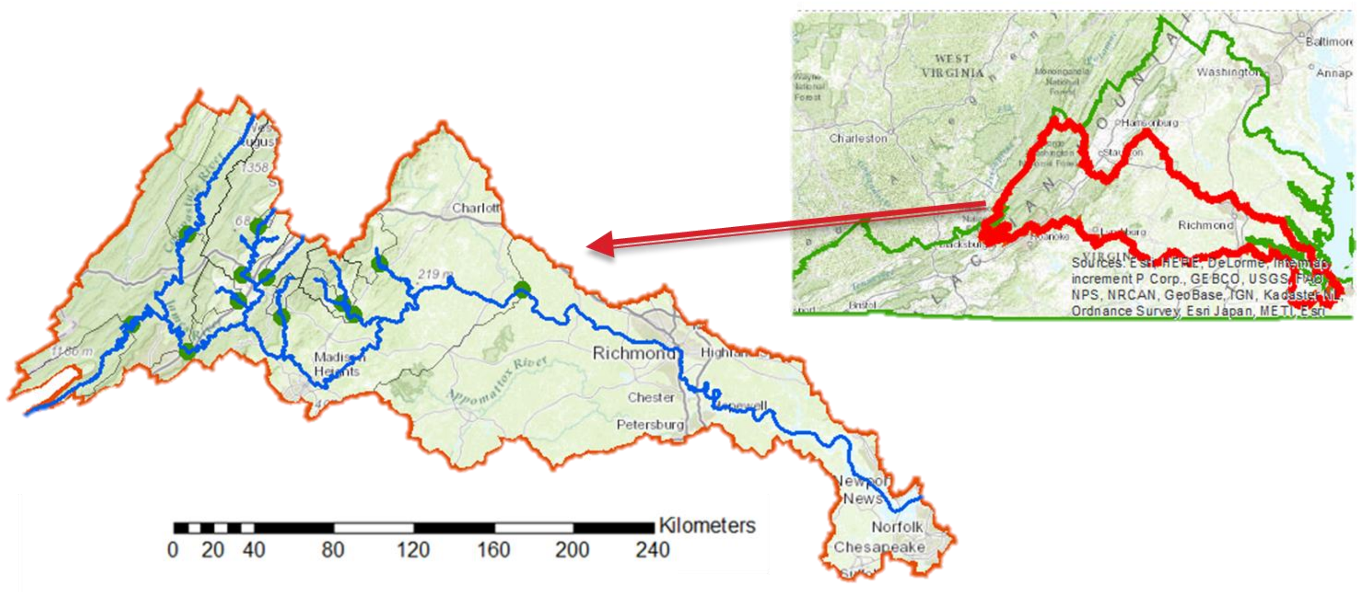

Figure 1. James River Basin watershed, Virginia. Sample sites and their respective watersheds are depicted.

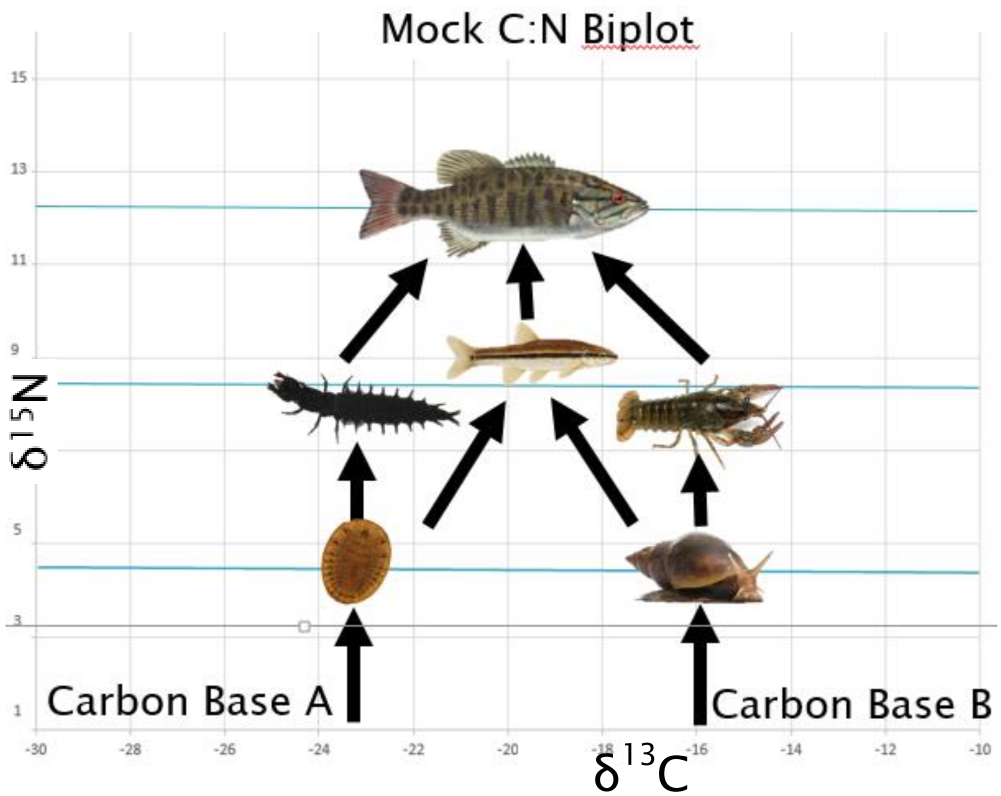

Figure 2. Mock C:N isotope biplot for visualization of the stable isotope methods employed in this Capstone.

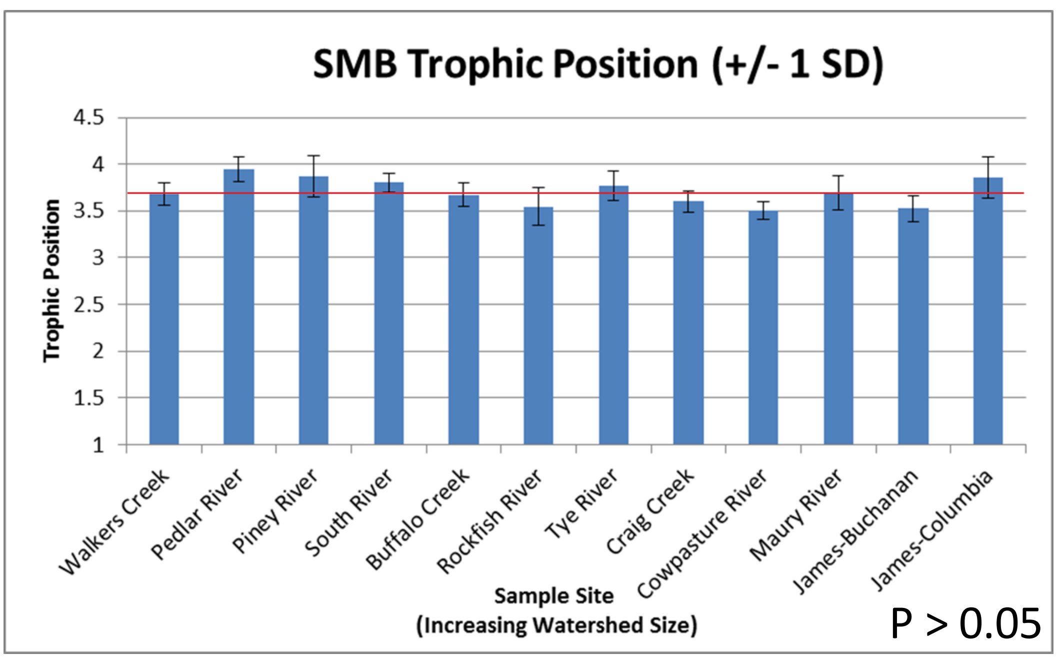

Figure 3. Mean trophic position estimates for each sample site calculated from $\delta^{15}N$ values. The average trophic position for sample site of 3.7 is depicted with the red line

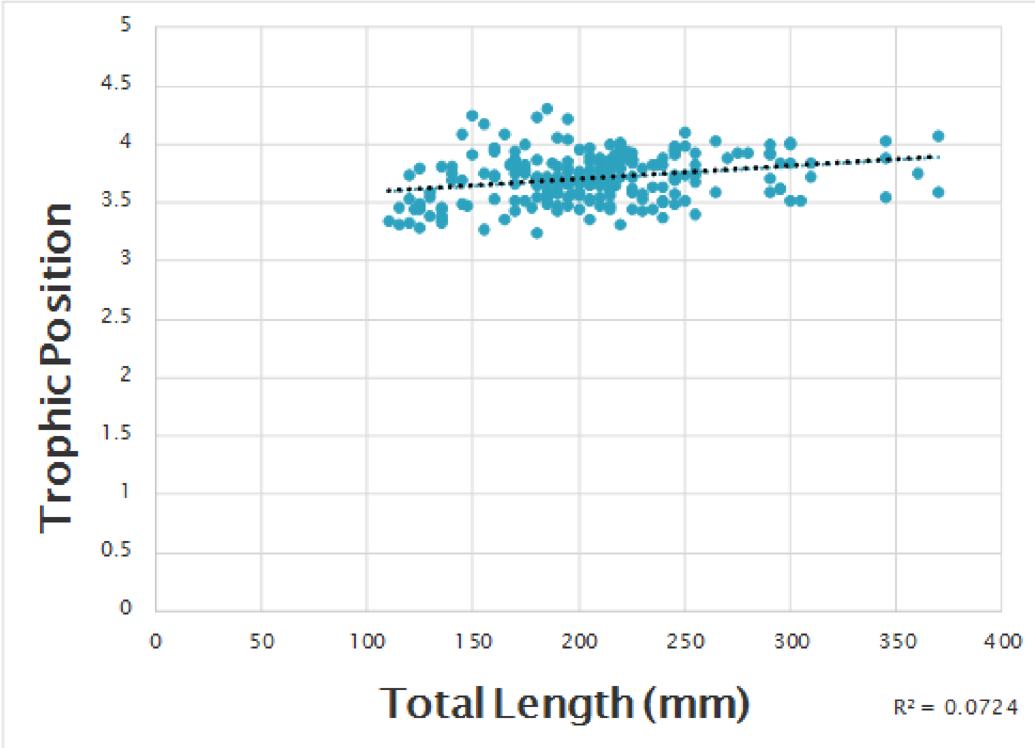

Figure 4. Trophic position estimates of smallmouth bass as a function of fish total length (mm).

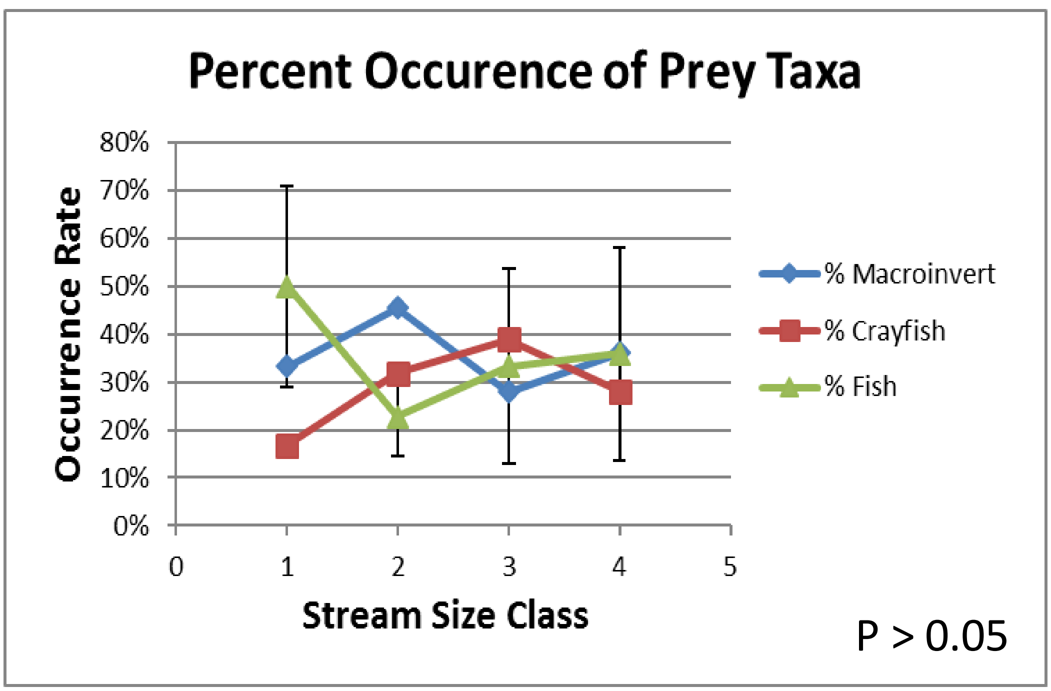

Figure 5. Percent occurrence of prey taxa. Note no statistical difference in fish prey among stream size classes.

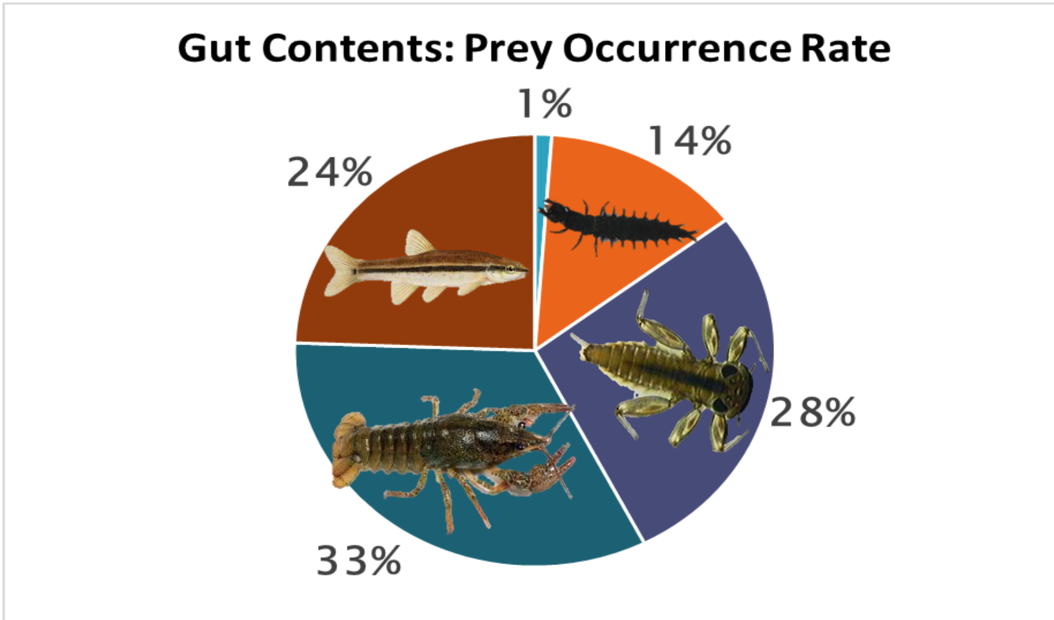

Figure 6. Frequency of occurrence of prey items in smallmouth bass total sample size.

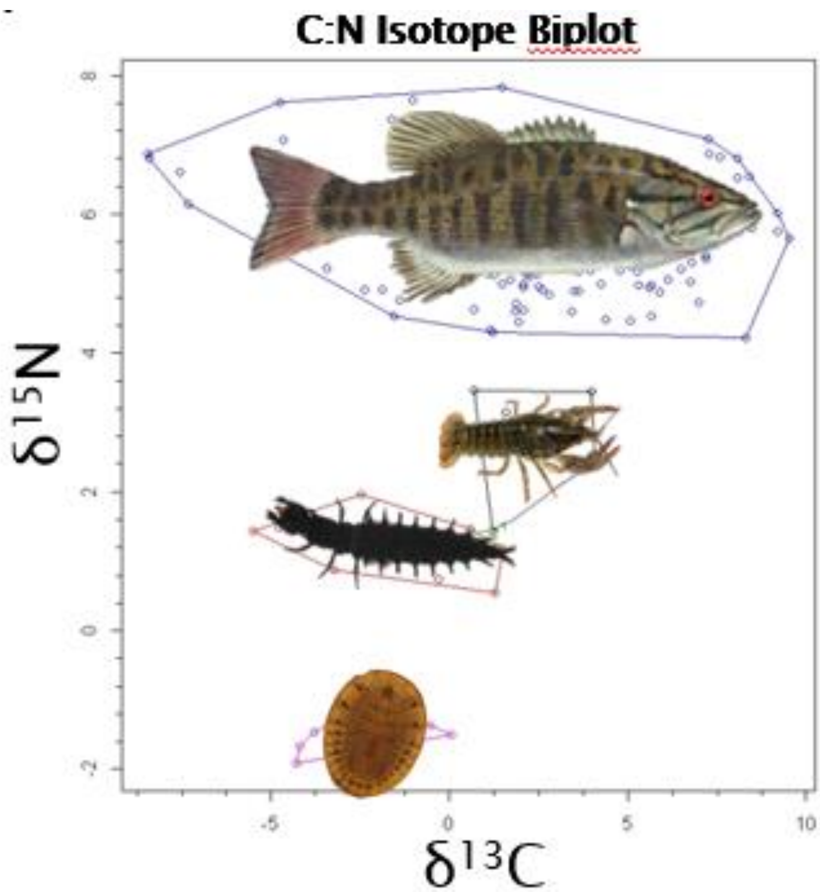

Figure 7. Carbon and nitrogen isotope biplot of isotope data from organisms collected at all sample sites. Illustrates isotopic fractionation of ^{15}N at each trophic level and the different carbon energy pathways assimilated by smallmouth bass

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