BRIGHAM, JOSHUA S., M.S. Linking Methane Concentration and Stable Isotope Distribution to Methane Derived Carbon in Food Webs in North Carolina Piedmont Streams. (2018)
Directed by Dr. Anne E. Hershey 42pp.

In streams, a portion of methane produced by methanogens can be oxidized by MOB. When consumers eat these bacteria, MDC is routed into the aquatic food web. The goal of this study was to determine the distribution and stable isotope signature of methane, and determine the influence of MDC on the diets of *Corbicula fluminea* and hydropsychid caddisflies in North Carolina piedmont streams. Sampling of twelve streams occurred during summer 2014, winter 2015, and summer 2015. Analysis of methane concentrations found that porewater concentrations (3.19 to 761.72 umol/l) were significantly higher than streamwater concentrations (0.37 to 6.41 umol/l) during all seasons, with porewater methane being highest during summer 2014. Analysis of $\delta^{13}C$-CH_4 found that streamwater samples (-34.04 to -63.33 ppt) were higher in $\delta^{13}C$-CH_4 than porewater samples(-34.4 to -74.84 ppt). Porewater samples were not significantly different between seasons, but streamwater samples were significantly higher in $\delta^{13}C$-CH_4 during winter 2015. Examination of spatial relationships between stream and porewater, and consumer $\delta^{13}C$ values suggested that methane concentration was not a good predictor of consumer $\delta^{13}C$. Stable isotope analysis demonstrated that hydropsychids were significantly lower in $\delta^{13}C$ than seston during summer 2014 (1ppt difference), winter 2015 (3.5ppt difference), and summer 2015 (1.5ppt difference). *Corbicula* $\delta^{13}C$ values were significantly lower than their diet sources during both summer seasons but were not significantly different from them during the winter, with

Corbicula having a value of -30 ppt for summer 2014 and -29.5 ppt for summer 2015. Comparisons of the $\delta^{13}C$ of two genera of hydropsychids demonstrated that *Hydropsyche* were significantly depleted than seston (approximately 1.5ppt). Mixing model analysis demonstrated usage of MOB in the range of 4-11% of diet by consumers, although differences between genera and seasonal differences were found. Analysis demonstrated that hydropsychids were utilizing MOB to a greater extent than *Corbicula* during the winter. The researched streams have higher concentrations of methane in porewaters that are also lower in $\delta^{13}C$-CH_4. The study also demonstrated that both consumers are utilizing MOB as part of their diet.

LINKING METHANE CONCENTRATION AND STABLE ISOTOPE

DISTRIBUTION TO METHANE DERIVED CARBON IN FOOD

WEBS IN NORTH CAROLINA PIEDMONT STREAMS

by

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CHAPTER I

INTRODUCTION

Methanogens utilize reactants found as end products or byproducts of decomposition (acetate or CO_2 and H_2) to carry out metabolic processes, while also producing methane as a product (Zinder 1993). When methane is present in the porewaters of stream sediments, it can travel upwards and eventually leave the stream surface through evasion or ebullition (Spawn et al., 2015; Stanley et al., 2016). This is problematic because methane is a potent greenhouse gas in the atmosphere (Billet and Moore, 2007, Whalen, 2005). Methane that is not lost to the atmosphere can be utilized by methane oxidizing bacteria (MOB) (Jones and Grey, 2011). MOB metabolize methane, using it as a source of carbon and energy (Trimmer et al., 2015). While MOB play an important role in diminishing a potent greenhouse gas (Borrel et al., 2011), recent research has demonstrated that they also can be important in the diet of aquatic consumers (Hershey et al., 2015; Trimmer et al., 2015). When consumers eat MOB, methane-derived carbon (MDC) is entrained into the food web (Trimmer et al., 2009). While various studies have demonstrated this phenomenon in streams and lakes in a variety of consumers (Jones and Grey, 2011, Kankaala et al., 2006, Kohzu et al., 2004; Trimmer et al., 2009), more research is needed to determine which consumers are utilizing this source of carbon, as well as how important it is quantitatively.

Hydropsychid caddisflies and the invasive clam *Corbicula fluminea* are two consumers found ubiquitously in the piedmont streams of Guilford County, NC. Both hydropsychid caddisflies and *Corbicula* consume and process organic matter in streams, providing a vital role in transferring energy to higher trophic levels and preventing energy loss to downstream reaches (Wallace et al., 1977, McCullough et al., 1979, Sousa et al., 2008). Hydropsychid caddisflies build retreats on the tops, sides, and bottoms of rocks located in riffles (Randall and Mackay, 1980). Silken nets are then constructed at the entrance of these retreats and utilized for collecting organic particles from the seston (Benke and Wallace, 1980). *Corbicula* utilize pedal feeding to consume organic material found in sediments, as well as filter feeding to consume seston (Cohen et al., 1984, Foe and Knight, 1986). In addition to consuming organic material derived from seston, hydropsychids also consume MOB that are present on their nets and retreats (Monteverde 2015). Likewise, *Corbicula* could be consuming MOB, as clams reside in surficial sediments where MOB can be prevalent (Smith, 2013). Both *Corbicula* and hydropsychids may also filter MOB out of streamwaters where the bacteria are also present (Whiticar and Faber, 1985).

Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) can provide a useful tool for determining dietary sources for a consumer as well as trophic relationships between different organisms (Ben-David and Flaherty, 2012). Typically, with $\delta^{13}C$ we see an approximately 1ppt enrichment between a consumer and its food source due to respiration of ^{12}C in CO_2 and excretion through waste (DeNiro and Epstein, 1978). For $\delta^{15}N$, an enrichment of approximately 3 ppt is also found between trophic levels of studied species due to loss of

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[14]N in waste, while ^{15}N is retained in tissues (Deniro and Epstein, 1978). In combination, these tools can be helpful for describing dietary sources for a consumer and trophic relationships among consumers (Ben-David and Flaherty, 2012; Phillips, 2012)

Stable isotope analysis can also be a helpful tool for tracing usage of MDC by consumers in streams. Organisms that consume MDC will have $\delta^{13}C$ values indicative of usage, possessing $\delta^{13}C$ values that are lower than photosynthetic food sources (Trimmer et al., 2009). Methane that is produced in aquatic sediments will possess $\delta^{13}C$-CH_4 values ranging from as low as -110 ppt to -50 ppt due to the large isotopic fractionation that occurs during methanogenesis (Whiticar, 1999). This is much lower than photosynthetic food sources for consumers which fall in the range of -28ppt to -12ppt (Whiticar, 1999).

The consumption of methane by MOB (methanotrophs) is a significant sink of carbon in aquatic ecosystems (Whiticar, 1999). In fact, the majority of methane produced in aquatic ecosystems is consumed by methanotrophs (Whiticar, 1999). Much like the fractionation that occurs during methanogenesis, methane oxidation is also associated with high isotope fractionation that results in the remaining methane being more enriched in the heavier isotope (Whiticar, 1999). The oxidation of methane in aquatic ecosystems by methanotrophs results in methane that is enriched in $\delta^{13}C$ by as much as 20 ppt (Jones and Grey, 2011). Several studies have demonstrated that methanotrophs preferentially consume the isotopically lighter ^{12}C-CH_4 when methane is oxidized, leaving the heavier ^{13}C-CH_4 isotope behind (Whiticar, 1999) This enrichment signifies preferential use of the lighter carbon isotope by methanotrophs and subsequently results in the remaining methane being more enriched in the heavier isotope (Barker and Fritz, 1981, Whiticar,

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1999). The δ^{13}C of MOB can be estimated in a stream by finding the δ^{13}C-CH4 and then applying a fractionation factor for MOB (Trimmer et al., 2009). Fractionation factors, which are differences in the isotopic composition between products and reactants (Smith et al., 2013), can be determined from literature values (Whiticar 1999), or direct field measurements (Trimmer et al., 2009)

MOB that metabolize ^{13}C depleted methane will also have depleted δ^{13}C values, with stream studies giving estimates between -83.8 ppt and -67.8 ppt (Jones and Grey, 2011, Summons et al., 1994, Trimmer et al. 2009). Consumers that subsequently consume MOB have characteristically depleted δ^{13}C values compared to their photosynthetically-derived food sources (Trimmer et al., 2009). Jones and Grey (2011) demonstrated that chironomid larvae found in a lake utilized MOB as part of their diet, with MOB sometimes making up as much as 60% of chironomid carbon biomass. Hershey (2015) recently demonstrated that integration of MDC into the diet of chironomid larvae in arctic lakes varied from as much as 0 to 40% of total C and was highly dependent on rates of methane oxidation. Trimmer (2009) found that some caddisfly species found in a chalk stream also utilized MOB, with some species having MDC make up 30% of their carbon biomass. However research in streams is more limited and deserves further investigation into the nature of how consumers are using MDC (Jones and Grey, 2011).

Mixing models are an essential tool for determining the diet contributions of food sources to a consumer, and have been used in various forms in a variety of studies (Phillips, 2012). Algebraic mixing models involve a consumer δ^{13}C that is equal to the

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proportion times the $\delta^{13}C$ of the first diet source plus the proportion times the $\delta^{13}C$ of any other food sources (Phillips, 2012). Basic algebraic mixing models have been utilized in several studies to determine the contributions of two or more food sources to the diet of a consumer (Trimmer et al., 2009, Hershey et al., 2015). Usage of these models allows for the determination of proportions of food sources used by a consumer of interest by inputting the $\delta^{13}C$ values of the consumer and food sources into the mixing model (Phillips, 2012). After estimation of the $\delta^{13}C$ of MOB these models can even be applied to consumers that ingest MOB to determine the contribution of MOB to their diet (Hershey et al., 2015).

More research is needed to determine the extent that consumers utilize MDC as part of their diet in streams. This study utilized surveys over the course of three seasons to evaluate methane concentration and $\delta^{13}C\text{-}CH_4$, in addition to utilizing mixing models to determine usage of MDC of hydropsychid caddisflies and *Corbicula fluminea*. The goal of this study was to determine the distribution and stable isotope signature of methane, and determine the influence of methane-derived carbon on the diets of *Corbicula fluminea* and hydropsychid caddisflies. The first aim of this study was to characterize the methane concentrations and $\delta^{13}C\text{-}CH_4$ of the stream and porewaters of Guilford County Piedmont streams. The hypotheses for this aim were that (a) porewaters possess higher methane concentrations than streamwaters and that (b) porewaters possess lower $\delta^{13}C\text{-}CH_4$ values than streamwaters. The second aim of this study was to determine the importance of methane-derived carbon for dominant aquatic consumers in Piedmont streams and how it varies spatially with stream and porewater methane concentrations.

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The hypotheses for this aim were that (a) the importance of methane-derived carbon to dominant aquatic consumers in Piedmont streams varies spatially with stream and porewater methane concentrations, that (b) feeding mode determines the degree to which a consumer utilizes methane derived carbon, and that (c) differences between usages of methane derived carbon exist between two separate genera of hydropsychids; *Hydropsyche* and *Cheumatopsyche*.

CHAPTER II

MATERIALS AND METHODS

Sampling commenced during the summer of 2014. Twelve stream reaches located in Guilford County, NC, were selected for sampling (Fig. 1).
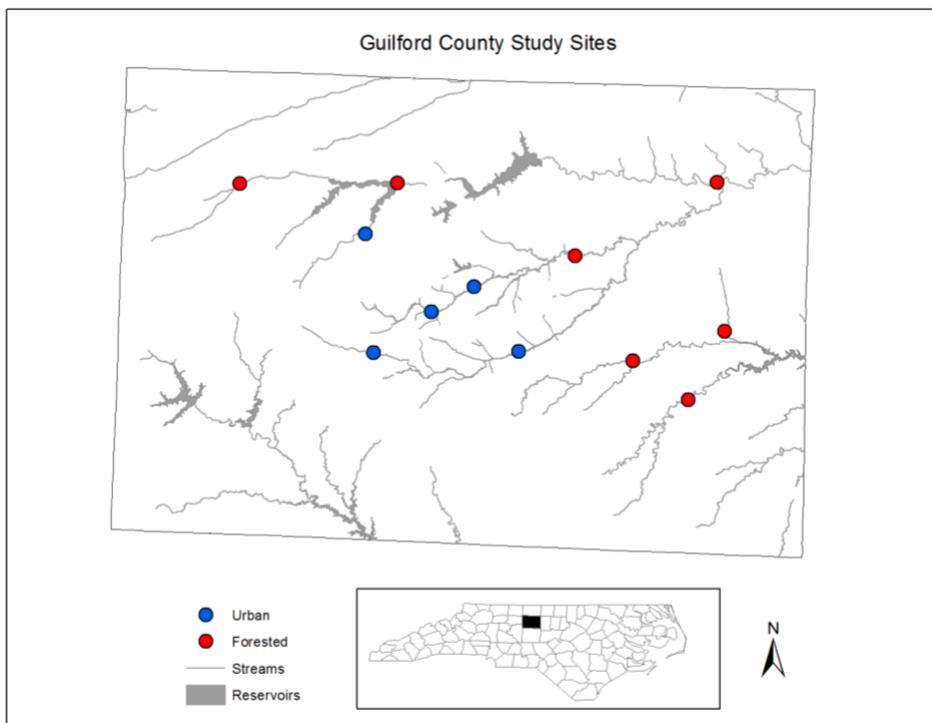

Figure 1. Map of Stream Reaches Sampled in Guilford County, NC.

These twelve sites were spread across 7 streams and represented urban and forested land uses. Streams ranged from third to fourth order. Substrate type ranged from sandy to gravel sediment. Surrounding vegetation ranged from grass to forested riparian zones.

Sampling only occurred during base flow. Prior to each sampling event, discharge was checked utilizing real-time USGS monitoring data. Six porewater and three streamwater samples for methane were collected using a 10 ml syringe. For streamwaters, 10 ml of water was collected, and 5 ml was injected into a serum vial using a 22-gauge needle. 3 ml of water from the same syringe was then injected into a paired 12 ml Exetainer. Porewater samples were collected by using a 10 ml syringe with an attached 15.2 cm 16-gauge needle. The needle was placed 12.7 cm into stream sediment and 10 ml of porewater was extracted. 5 ml of this sample was injected into a serum vial, and 3 ml was injected into an exetainer. Serum vials (25 ml) used for sampling methane concentration were acid-cleaned, sealed with rubber stoppers and metal crimp seals, evacuated, filled with nitrogen to 1 atm, and then acidified with 0.1 ml of 10% HCl. Exetainers (12 ml) used for δ^{13}C-CH4 were similarly purged, filled with nitrogen, and acidified. For each site, at least ten *Corbicula* were collected by hand from surficial sediments and placed in a bottle containing streamwater. Between 10 and 20 hydropsychid caddisflies were collected from rocks located within riffles. Hydropsychids were captured using forceps and placed in a bottle containing streamwater. Surficial sediments were collected using glass scintillation vials to gently scoop the topmost layer of sediment. Samples for seston were collected by filling three 1 l Nalgene bottles with streamwater.

Upon return to the lab, samples were immediately processed. Serum vials were stored upside-down to minimize exchange of gases with the atmosphere until they were analyzed for headspace methane concentration utilizing a gas chromatograph with a

flame ionization detector (GC-FID). Exetainers were also stored upside-down until being sent to the University of California Davis Stable Isotope Facility for $\delta^{13}C$-CH_4 analysis. Surficial sediment samples were dried to 65 °C in a drying oven. Seston samples were filtered onto glass fiber filters and dried to 65 °C in a drying oven. The soft tissues of *Corbicula* were removed from their shells and dried to 65 °C in a drying oven. Hydropsychid caddisflies were cleaned with deionized water, put in a petri dish filled with deionized water, and placed in a refrigerator overnight to evacuate gut contents. Afterwards, they were dried to 65 °C in a drying oven. Dried samples were prepared for stable isotope analysis by grinding with an acid-washed mortar and pestle, weighed and placed in foil capsules. Solid samples were sent to the University of California Santa Cruz stable isotope facility for analysis.

Sampling was repeated during the winter of 2015. Only 10 of the original 12 sites were sampled due to adverse weather conditions causing two streams to be frozen and filled with mud and therefore unsafe for sampling. The same methods described previously were utilized for sampling. Processing and analysis of samples were carried out using the same methods described previously.

Sampling was repeated during the summer of 2015. The same 12 sites were sampled, and the same methods for collection of water, sediment, seston, and *Corbicula* were utilized. However, upon collection of hydropsychid caddisflies, specimens were separated into the genera *Hydropsyche* and *Cheumatopsyche*. These were the two most commonly found genera during previous seasons.

Upon return to the lab, water, sediment, seston, and *Corbicula* samples were processed following previously used methods. The genera of hydropsychid caddisflies were confirmed utilizing a stereomicroscope. Samples were prepared using the same methods as previous seasons. Solid samples were again sent to the University of California Santa Cruz stable isotope facility for stable isotope analysis. Exetainers were sent to the University of California Davis for δ^{13}C-CH$_4$ analysis.

Data Analyses

Data were analyzed using the statistical program R. This program was used to conduct t-tests, one and two-way ANOVAs, blocked two-way ANOVAs (summarized in table 1) and simple linear regressions. All tests utilized outlier analysis (Cook's distance, Studentized residuals, Bonferroni test, and hat values), as well as tests for variance (Levene's test) and normality (Shapiro-Wilk test). Log transformations were used for some data sets to improve assumptions of equal variance and normality.

Stream and porewater methane concentration and δ^{13}C-CH$_4$ data were analyzed utilizing two-way ANOVAs followed by Tukey-Kramer analysis to determine significant differences between type of sample (stream or porewater) and seasons. Water methane concentration was log transformed. Six outliers were removed from a total of 72 δ^{13}C-CH$_4$ data points. To determine significant differences between seasons by water type, data was then analyzed using separate one-way ANOVAs followed by Tukey-Kramer analysis.

$\delta^{13}C$ of consumers and their respective diet sources (sediment and seston for *Corbicula* and seston for hydropsychids) were analyzed using paired t-tests for hydropsychids and blocked two-way ANOVAs for *Corbicula*, with site serving as the blocking factor. These tests were performed in order to determine if consumers were significantly lower in $\delta^{13}C$ than their food sources, implying use of MDC (Trimmer, 2009). Paired t-tests for hydropsychids were 1-tailed as it was expected that hydropsychids would be lower in $\delta^{13}C$ than seston. For tests of hydropsychids, both genera were pooled for summer 2015 by averaging both genera together by site. For tests of *Corbicula* and diet, summer 2014 data was log transformed and two outliers were removed out of 36 data points. For winter 2015, data were log transformed and two outliers were removed out of 30 data points. For summer 2015, data were log transformed and three outliers were removed out of 36 data points. The $\delta^{13}C$ of the two genera of hydropsychids and seston were analyzed using a two-way ANOVA blocked by site in order to determine if consumers were more depleted than their food source. Data were log transformed and two outliers were removed out of 32 data points.

A two-source algebraic mixing model was used to estimate energy source contributions to the diets of consumers. Two separate mixing models were applied to the diet of *Corbicula*, one using seston and MOB as diet sources, and one using sediment and MOB as diet sources. A three way mixing model was not able to be performed on *Corbicula* in this study because this would have required data for a second isotope (Phillips, 2012). $\delta^{13}C$ of MOB was determined by subtracting streamwater $\delta^{13}C$-CH_4 from porewater $\delta^{13}C$-CH_4 as an estimate of MOB fractionation and then adding this value to

porewater δ^{13}C-CH$_4$ to obtain a δ^{13}C estimate for MOB. A two-source algebraic mixing

model (formula 1) was then used as described in Phillips (2012) to determine %

contributions of each food source to the consumers.

$$\delta^{13}C_{consumer} = f_1 \, \delta^{13}C_{seston} + f_2 \, \delta^{13}C_{MOB}$$

$$f_1 + f_2 = 1$$

(1)

This model was used to determine percent contribution of seston and MOB to the diet of

each consumer for each season, where $\delta^{13}C_{consumer}$ is the δ^{13}C value of the consumer,

$\delta^{13}C_{seston}$ is the δ^{13}C value of seston, $\delta^{13}C_{MOB}$ is the δ^{13}C value of MOB, f_1 is the diet

percentage of seston, and f_2 is the diet percentage of MOB. Mixing model analysis was

followed by sensitivity analysis for $\delta^{13}C_{MOB}$ because this input parameter was derived

and not directly measured, and hence was more uncertain. This was performed by

dividing the standard error by the mean for $\delta^{13}C_{MOB}$ to estimate uncertainty and then re-

running the models using the upper and lower bounds on the input values for $\delta^{13}C_{MOB}$.

Significant differences between hydropsychid and *Corbicula* usage of MOB by

season were determined utilizing paired two tailed t-tests. Testing for significant

differences between *Hydropsyche* and *Cheumatopsyche* usage of MOB for summer 2015

was also determined using a paired two tailed t-test. Two-way blocked ANOVAs were

used to compare *Corbicula* by season, with site serving as the blocking factor. The

Corbicula data were log transformed. A separate two-way blocked ANOVA was used to

compare hydropsychids by season, with site serving as the blocking factor.

Hydropsychid data had two outliers removed out of 26 data points.

Spatial correlation between stream and porewater methane concentration and $\delta^{13}C$ of consumers was carried out using simple linear regressions. For these regressions there were three possible outcomes: 1) no correlation 2) a negative correlation and 3) a positive correlation. No correlation would imply that methane is not a significant driver for the $\delta^{13}C$ of consumers. A negative correlation with increasing methane and decreasing $\delta^{13}C$ would imply that there is more methane available for methane oxidation, and that subsequent methane oxidation will result in MOB being higher in ^{12}C, subsequently resulting in consumers being higher in ^{12}C and lower in ^{13}C. A positive correlation with increasing methane and increasing $\delta^{13}C$ would mean that correlation is due to some factor other than methane concentration. For summer 2014 comparisons, one outlier was removed for each comparison out of a total of 12 data points. For winter 2015 comparisons, one outlier was removed for the comparison of streamwater methane to $\delta^{13}C$ of hydropsychids out of 10 data points. For summer 2015 comparisons, one outlier was removed for the comparison of streamwater methane to $\delta^{13}C$ of *Hydropsyche*, and for the comparison of porewater to $\delta^{13}C$ of *Hydropsyche* out of 12 data points. One outlier was removed for the comparison of streamwater to *Cheumatopsyche*, and for the comparison of porewater to *Cheumatopsyche* out of 8 data points. One outlier was removed for the comparison of porewater methane to *Corbicula* out of 12 data points. For the pooled regressions one outlier was removed for the comparison between surfacewater methane and hydropsychids out of 34 data points. Two outliers were removed for the comparison between surfacewater methane and *Corbicula*, and also for the comparison between porewater and *Corbicula* out of 33 data points.

CHAPTER III

RESULTS

Analysis of stream and porewater methane samples through two-way ANOVA ($F_{5,62}$=14.13, p<0.001) followed by Tukey Kramer analysis demonstrated that porewater methane concentrations were significantly higher than streamwater concentrations (df=1, p<0.001) and that winter 2015 samples were significantly lower than summer 2014 samples (df=1, p<0.01), whereas there was no significant difference between both summer seasons (df=1, p=0.218) or winter 2015 and summer 2015 (df=1, p=0.222). There was a significant interaction effect ($F_{2,62}$=3.146, p=0.0499), in addition to significant season ($F_{2,62}$=5.395, p=0.0069) and sample type ($F_{1,62}$=53.589, p<0.001) effects. Subsequent one-way ANOVA found that differences in streamwater methane concentration was not statistically significant between seasons ($F_{2,31}$=1.454, p=0.249). One-way ANOVA for differences in porewater between seasons were significant ($F_{2,31}$=4.844, p=0.0148). This was followed by Tukey-Kramer for porewater by season that found that summer 2014 samples were significantly higher in methane concentration than winter 2015 (df=1, p=0.028) and summer 2015 (df=1, p=0.033). There was no significant difference between summer 2015 and winter 2015 (df=1, p=0.98) (Fig. 2).
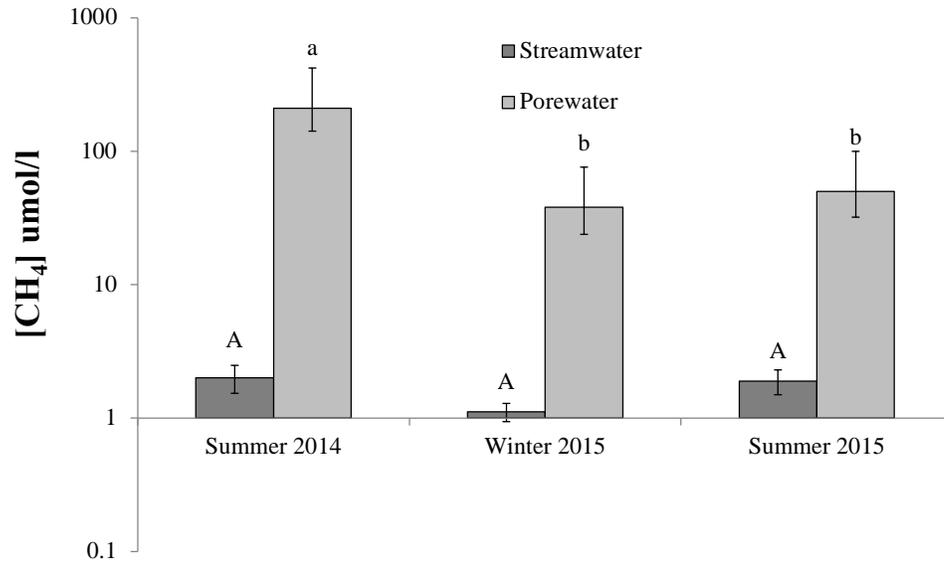
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Figure 2. Methane Concentrations by Season

Porewater and streamwater CH_4 concentration (mean $\pm$ SE) for all seasons (capital letters are comparing streamwater samples by season and lowercase letters are comparing porewater samples by season). Streamwater methane concentration was not significantly different between seasons. Porewater methane samples were significantly higher in summer 2014 compared to winter 2015 and summer 2015.

Two-way ANOVA ($F_{5,56}$=15.66, p<0.001) followed by Tukey Kramer analysis of $\delta^{13}C$-CH_4 samples for stream and porewaters demonstrated that porewater $\delta^{13}C$-CH_4 was significantly lower than streamwater $\delta^{13}C$-CH_4 (df=1, p<0.001) and that $\delta^{13}C$-CH_4 samples were lower in summer 2014 than winter 2015 (df=1 p<0.01) (Fig. 3). There was no significant interaction effect ($F_{2,56}$=2.549, p=0.087), but there were significant season ($F_{2,56}$=5.973, p=0.0044) and water type ($F_{1,56}$=61,249 p<0.001) effects. Subsequent one-way ANOVA found significant differences between streamwater samples ($F_{2,31}$=7.613, p=0.002) with Tukey-Kramer analysis demonstrating that winter 2015 samples were significantly lower than summer 2014 (df=1, p=0.002) and summer 2015 (df=1,

p=0.011). There was no significant difference between summer seasons (df=1, p=0.8). A one-way ANOVA comparing porewater by season found no significant difference between seasons ($F_{2,31}=0.143$ p=0.868).
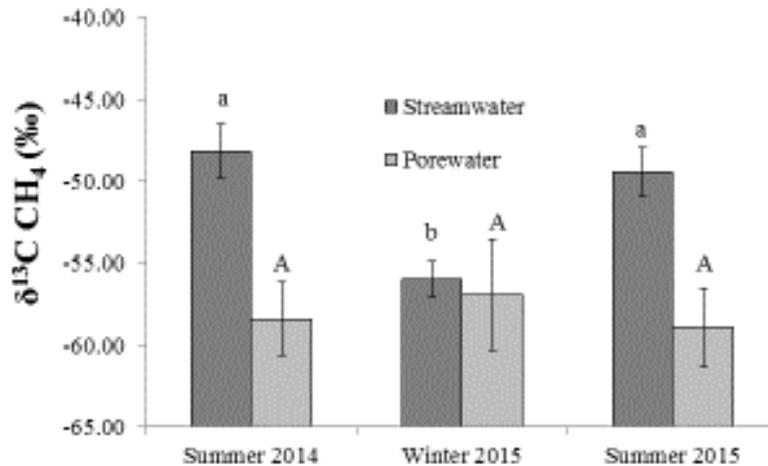

Figure 3. δ^{13}C-CH$_4$ by Season

Porewater and streamwater δ^{13}C-CH$_4$ (mean $\pm$ SE) for all seasons (capital letters are comparing streamwater samples by season and lowercase letters are comparing porewater samples by season). Streamwater samples were significantly lower than both summer seasons during the winter. For porewater samples, there was no significant difference between seasons.

Analysis of δ^{13}C data for *Corbicula*, sediment, and seston demonstrated that significant differences between diet components and consumer occurred during summer 2014 (blocked two-way ANOVA, $F_{12,19}=9.44$, p<0.001), with post hoc Tukey-Kramer demonstrating that sediment δ^{13}C was significantly higher than clam δ^{13}C (df=1, p<0.001), seston δ^{13}C was significantly higher than clam δ^{13}C (df=1, p=0.048), and sediment δ^{13}C was significantly higher than seston δ^{13}C (df=1, p<0.001) (Fig 4a). Analysis of winter 2015 data demonstrated that no δ^{13}C values were significantly

different from one another (blocked two-way ANOVA, $F_{10,15}=0.8827$, $p=0.568$) (Fig. 4b).

Significant differences between $\delta^{13}C$ values occurred during summer 2015 (blocked two-way ANOVA, $F_{13,19}=7.697$, $p<0.001$), with post hoc Tukey-Kramer demonstrating that sediment $\delta^{13}C$ was significantly higher than clam $\delta^{13}C$ (df=1, $p<0.001$), seston $\delta^{13}C$ was significantly higher than clam $\delta^{13}C$ (df=1, $p<0.01$), and sediment $\delta^{13}C$ was significantly higher than seston $\delta^{13}C$ (df=1, $p<0.01$) (Fig. 4c).
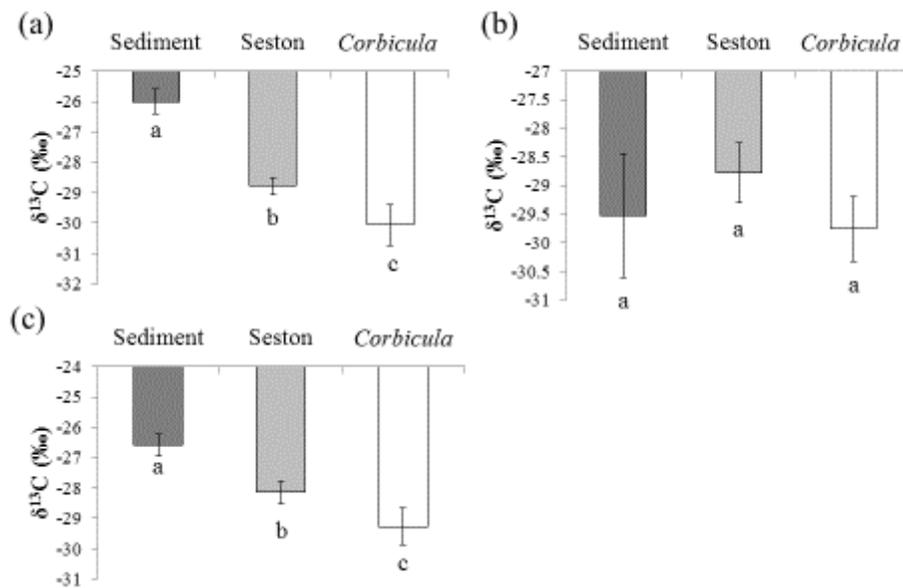

Figure 4. $\delta^{13}C$ of *Corbicula* and Diet by Season

$\delta^{13}C$ values (mean $\pm$ SE) for *Corbicula*, seston, and sediment for all seasons. During summer 2014, clams had significantly lower $\delta^{13}C$ than seston and sediment (a). During winter 2015, there was no difference in $\delta^{13}C$ between samples (b). During summer 2015, clams had significantly lower $\delta^{13}C$ than seston and sediment (c).

Analysis of $\delta^{13}C$ data for hydropsychids and seston demonstrated that the consumer had significantly lower $\delta^{13}C$ than seston during summer 2014 (paired one-tailed t-test, t=2.2502, df=10, p=0.024) (Fig. 5a) and winter 2015 (paired one-tailed t-test,

t=5.8767, df=8, p<0.001) (Fig. 5b). Analysis of $\delta^{13}C$ data for pooled hydropsychids and

seston demonstrated that the consumer had a significantly lower $\delta^{13}C$ value than seston

during summer 2015 (paired one-tailed t-test, t=1.9503, df=11 p=0.0413) (Fig. 5c).

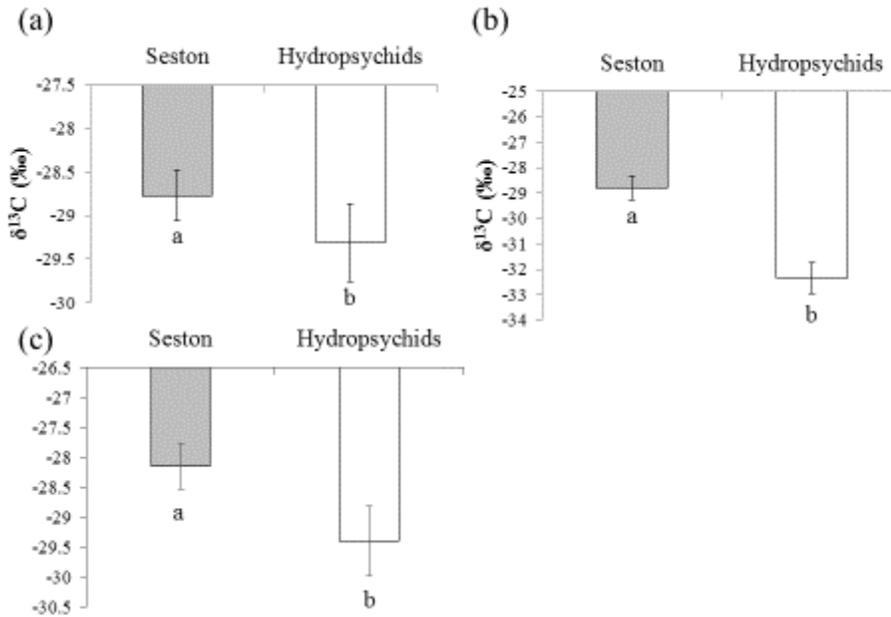Figure 5. $\delta^{13}C$ of Hydropsychids and Diet by Season

$\delta^{13}C$ values (mean $\pm$ SE) for hydropsychids and seston. Hydropsychidae had significantly lower $\delta^{13}C$ than seston during summer 2014 (a) and winter 2015 (b). Pooled hydropsychids were significantly different than seston during summer 2015 (c).

Analysis of $\delta^{13}C$ data for seston and hydropsychids (*Hydropsyche* and

Cheumatopsyche) demonstrated that significant differences existed between seston and

the two genera (blocked two-way ANOVA, $F_{13,16}$=6.374, p<0.001) (Fig. 6). A significant

block effect was found ($F_{11,16}$=6.6827, p<0.001). Further post hoc Tukey-Kramer analysis

demonstrated that seston had significantly higher $\delta^{13}C$ than *Hydropsyche* (df=1,

p=0.029), but that *Hydropsyche* and *Cheumatopsyche* were not significantly different in

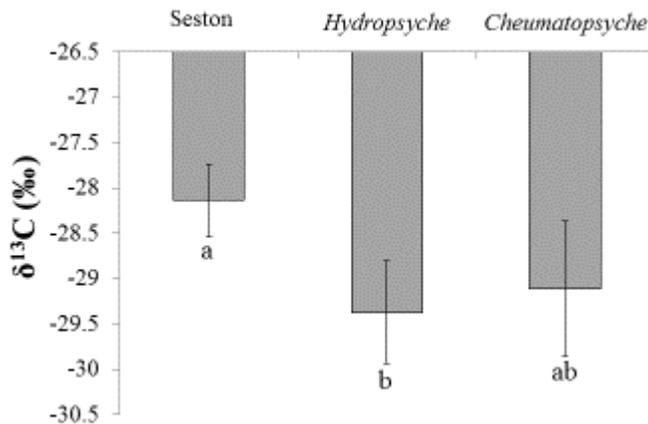$\delta^{13}C$ (df=1, p=0.8356), nor were seston and *Cheumatopsyche* (df=1, p=0.14).

Figure 6. $\delta^{13}C$ of Hydropsychid Genera and Diet by Season

$\delta^{13}C$ values (mean $\pm$ SE) for hydropsychids and seston during summer 2015. Seston had significantly higher $\delta^{13}C$ than *Hydropsyche*. There was no significant difference in $\delta^{13}C$ between seston and *Hydropsyche*, or *Hydropsyche* and *Cheumatopsyche*.

Analysis of mixing model data for hydropsychids and *Corbicula* for summer 2014 demonstrated that MOB usage was not significantly different between consumers, with MOB making up 9% of the diet of *Corbicula* and 4% of the diet of hydropsychids (paired two-tailed t-test, t=1.0099, df=5, p=0.36) (Fig. 7a). Analysis of mixing model data for hydropsychids and *Corbicula* for winter 2015 demonstrated that MOB usage was significantly higher by hydropsychids, with MOB making up 4% of the diet of *Corbicula* and 11% of the diet of hydropsychids (paired two-tailed t-test, t=-3.0179, df=5, p=0.029) (Fig. 7b). Analysis of mixing model data for hydropsychids and *Corbicula* for summer 2015 demonstrated that MOB usage was not significantly different between consumers,

with MOB making up 5% of the diet of *Corbicula* and 6% of the diet of hydropsychids

(paired two-tailed t-test, t=-0.089, df=8, p=0.9312) (Fig. 7c).
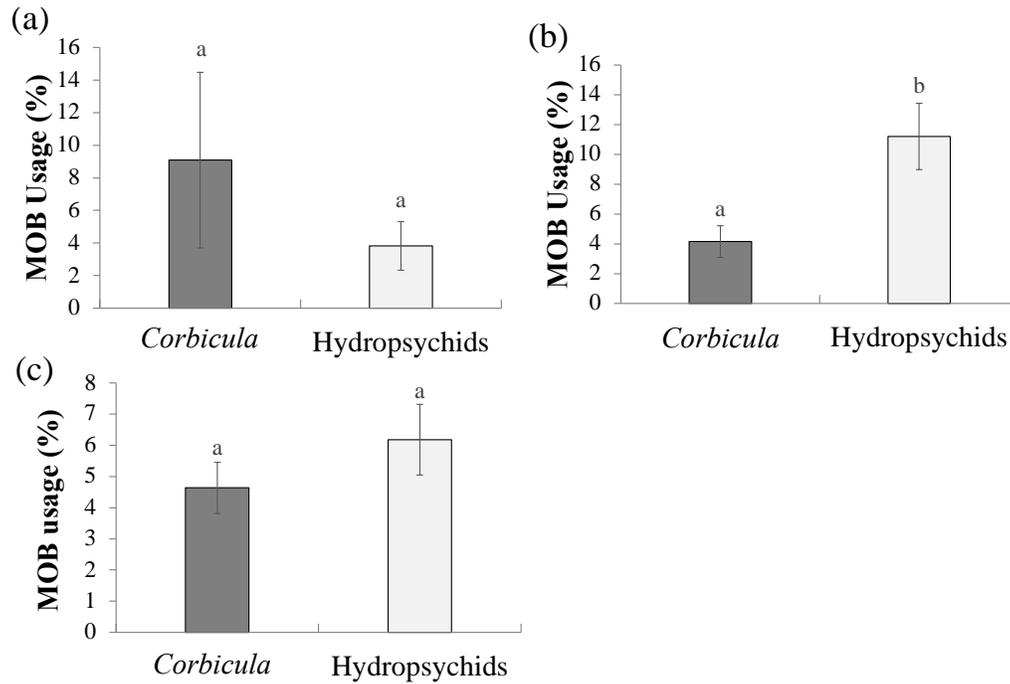
Figure 7. MOB Usage for *Corbicula* and Hydropsychids

Hydropsychid and *Corbicula* methane oxidizing bacteria (MOB) usage (mean $\pm$ SE) for all season. MOB usage was not significantly different between consumers during summer 2014 (a) or summer 2015 (c). MOB usage was significantly higher for hydropsychids during winter 2015 (b).

Analysis of mixing model data for hydropsychids by genera for summer 2015 demonstrated that MOB usage was not significantly different between genera (paired two-tailed t-test, t=-0.93787, df=5, p=0.39) (Fig. 8)
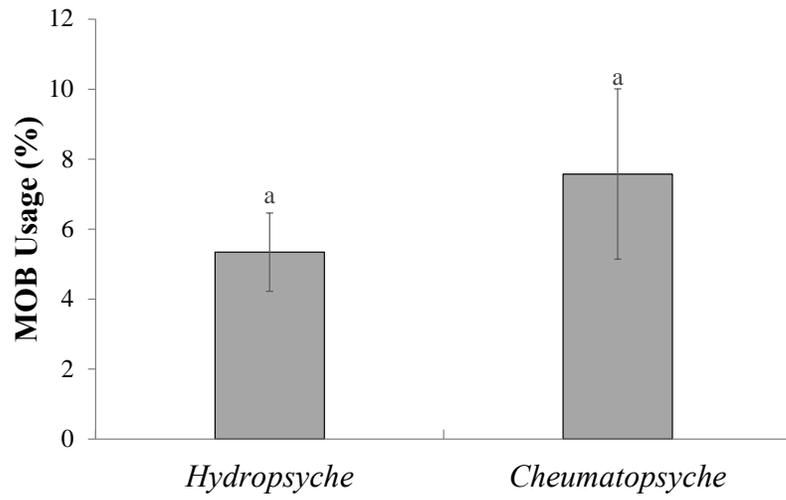
Figure 8. MOB Usage for Hydropsychid Genera

Hydropsyche and *Cheumatopsyche* methane oxidizing bacteria (MOB) usage (mean $\pm$ SE) for Summer 2015. MOB usage was not significantly different between genera.

Analysis of mixing model data for hydropsychids demonstrated that significant differences in %MOB usage existed between seasons (blocked 2-way ANOVA, $F_{11,12}=3.55$, p=0.019) (Fig. 9). Tukey-Kramer demonstrated that usage of MOB was significantly higher in winter 2015 than summer 2015 (df=1, p=0.016). Significant difference did not occur between summer 2014 and summer 2015 (df=1, p=0.701), or summer 2014 and winter 2015 (df=1, p=0.098).
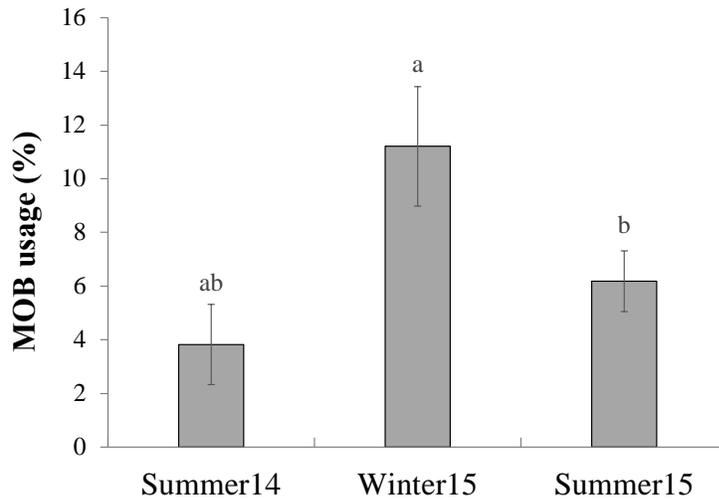
Figure 9. Hydropsychid MOB Usage by Season

Hydropsychid methane oxidizing bacteria (MOB) usage (mean $\pm$ SE) for all seasons. MOB usage was significantly higher during winter 2015 compared to summer 2015. There was no significant difference in MOB usage between summer 2014 and winter 2015, and summer 2014 and summer 2015.

Mixing model analysis of MOB usage for *Corbicula* by season demonstrated that there were no significant differences between seasons (blocked two-way ANOVA, $F_{13,8}$=1.492, p=0.2903) (Fig. 10).
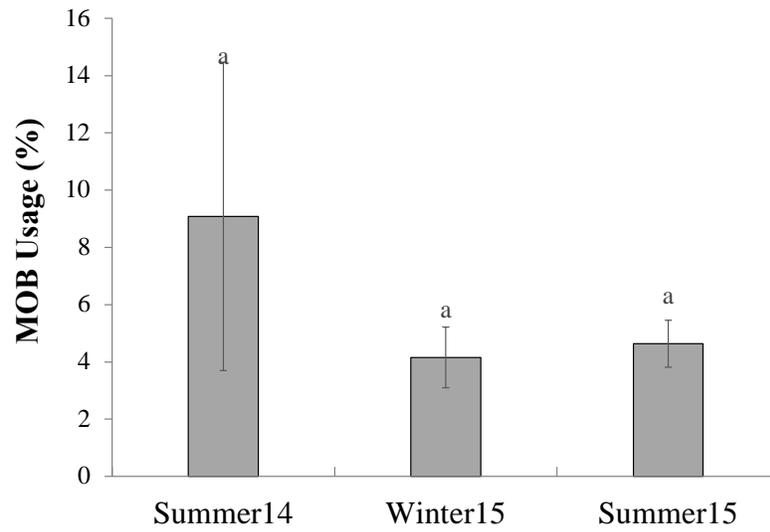
Figure 10. *Corbicula* MOB Usage by Season

Corbicula methane oxidizing bacteria (MOB) usage (mean $\pm$ SE) for all seasons. Mixing model analysis of MOB usage for *Corbicula* by season demonstrated that there were no significant differences between seasons.

Table 1. Summary of Blocked ANOVA Analysis

Summary of blocked 2-way ANOVA results for δ^{13}C comparisons of *Corbicula* to food sources, *Corbicula* methane derived carbon (MDC) usage by season, Hydropsychid MDC usage by season and δ^{13}C comparisons of *Hydropsyche, Cheumatopsyche*, and diet.

Analysis	F value	n	df	P	Sig. pairwise contrasts
Diet analyses					
Corbicula Summer 2014	9.44	34	12,19	<0.001	
Site effect	3.237	12	10	0.0133	
Diet effect	40.457	3	2	<0.001	Sed.>Seston>Corbicula
Corbicula Summer 2015	7.697	36	13,19	<0.001	
Site effect	4.4934	12	11	0.002	
Diet effect	25.3171	3	2	<0.001	Sed.>Seston>Corbicula
Corbicula Winter 2015	0.8827	28	10,15	0.5682	
Site effect	0.9510	10	8	0.5062	
Diet effect	0.6097	3	2	0.5565	
Hydropsychid genera diet	6.374	32	13,16	<0.001	
Site effect	6.6827	12	11	<0.001	
Diet effect	4.6737	3	2	0.025	seston>Hydropsyche
Seasonal analyses					
Hydropsychid MOB usage	3.55	26	11,12	0.0195	
Site effect	2.930	12	9	0.0430	
Season effect	6.341	3	2	0.0132	win. 2015>sum. 2015
Corbicula MOB usage	1.492	25	13,8	0.2903	
Site effect	1.3958	12	11	0.3250	
Season effect	2.0228	3	2	0.1946	

Mixing model analysis of consumers and their respective diet sources, seston for hydropsychids, and both seston and sediment for *Corbicula*, demonstrated that each consumer was utilizing MOB as a food source during all seasons (Table 2).

Table 2. Summary of Mixing Model Results

Summary of mixing model results for all seasons and consumers (mean $\pm$ SE).

Season	Consumer	% Seston	%Methane oxidizing bacteria
Summer 2014	*Corbicula*	91+4.8	9+4.8
Winter 2015	*Corbicula*	96+0.9	4+0.9
Summer 2015	*Corbicula*	95+0.9	5+0.9
Summer 2014	Hydropsychid	96+1.2	4+1.2
Winter 2015	Hydropsychid	89+1.9	11+1.9
Summer 2015	*Hydropsyche*	95+1	5+1
Summer 2015	*Cheumatopsyche*	92+2.1	8+2.1
Season	**Consumer**	**% Sediment**	**%Methane oxidizing bacteria**
Summer 2014	*Corbicula*	86+4.8	14+4.8
Winter 2015	*Corbicula*	89+10	11+10
Summer 2015	*Corbicula*	93+1.0	7+1.0

Results for sensitivity analysis due to uncertainty in values for $\delta^{13}C_{MOB}$ were within the confidence limits of the results for % MOB usage in Table 2. For instance, for the model looking at sediment and MOB contribution to the diet of *Corbicula* during summer 2015, the original diet percentages for sediment and MOB were 93$\pm$1 for sediment usage and 7$\pm$1 for MOB usage. The sensitivity analysis gave outputs of 92% for sediment and 8% for MOB for the upper bound, and outputs of 94% for sediment and 6% for MOB for the lower bound.

Simple linear regression analyses for summer 2014 found no significant relationship between streamwater and hydropsychid $\delta^{13}C$ or porewater and hydropsychid $\delta^{13}C$ (Fig. 11a, 11b). Analysis of data also found no relationship between streamwater and *Corbicula* $\delta^{13}C$ or porewater and *Corbicula* $\delta^{13}C$ (Fig. 11c, 11d).
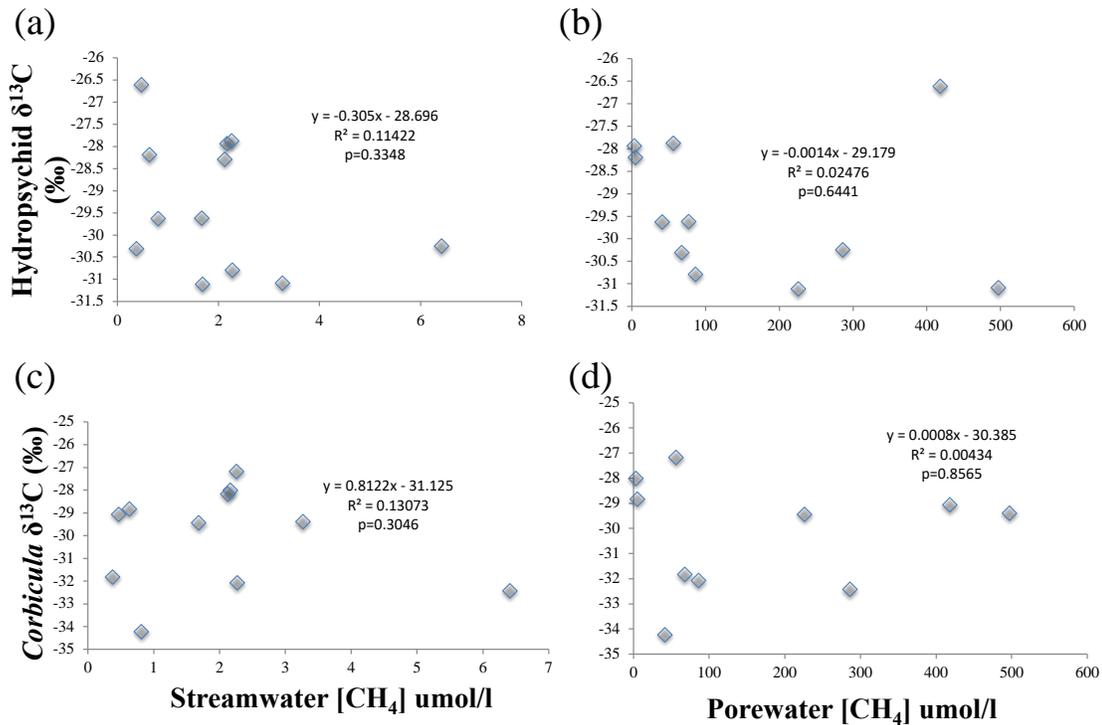
Figure 11. Summer 2014 Spatial Regressions

Simple linear regressions comparing stream and porewaters to $\delta^{13}C$ of hydropsychids and *Corbicula*. Analysis found no significant relationship between streamwater and hydropsychid $\delta^{13}C$ or porewater and hydropsychid $\delta^{13}C$ (a, b). Analysis also found no relationship between streamwater and *Corbicula* $\delta^{13}C$ or porewater and *Corbicula* $\delta^{13}C$ (c, d)

Regression analysis of winter 2015 samples found that streamwater methane concentration was significantly related to hydropsychid $\delta^{13}C$ ($F_{1,7}=12.24$, p=0.01), with increasing streamwater methane concentrations leading to increasing hydropsychid $\delta^{13}C$ (Fig. 12a). No significant relationship was found between porewater methane concentration and hydropsychid $\delta^{13}C$ (Fig. 12b). Regression analysis of data also found no relationship between streamwater and *Corbicula* $\delta^{13}C$ or porewater and *Corbicula* $\delta^{13}C$ (Fig. 12c, d).

(a)

y = 2.4582x - 35.093
R² = 0.44565
p=0.01

(b)

y = 0.0111x - 32.771
R² = 0.06114
p=0.491

(c)

y = 1.1457x - 31.041
R² = 0.12335
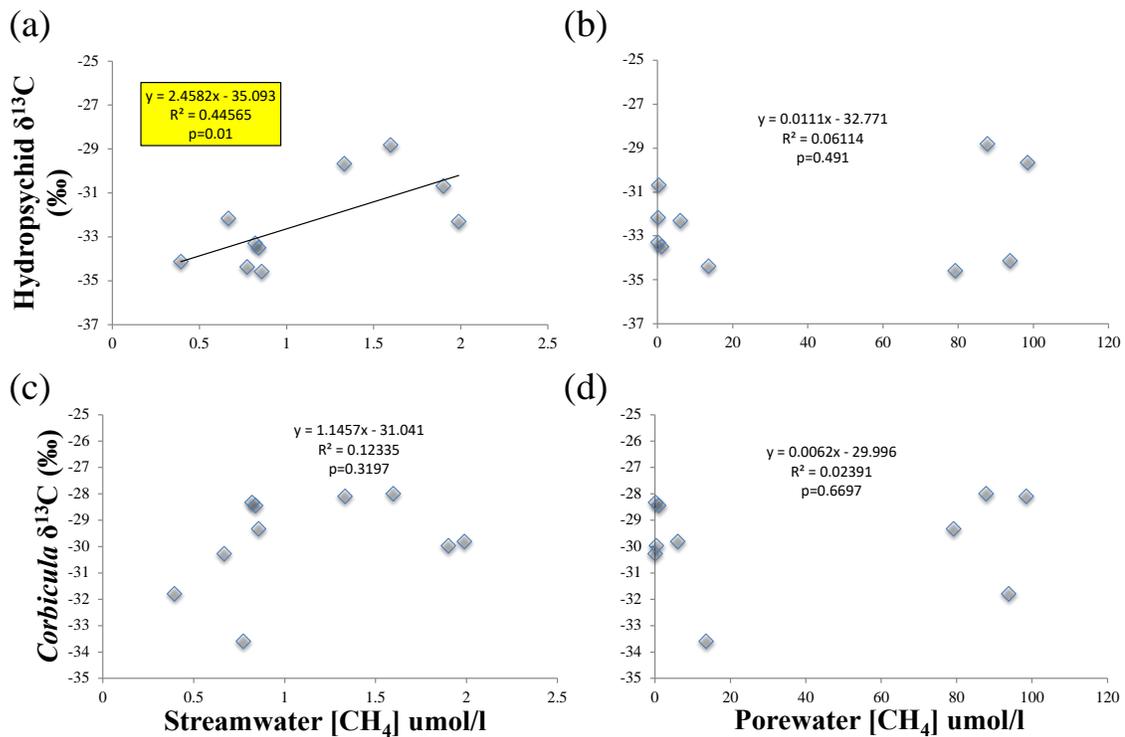p=0.3197

(d)

y = 0.0062x - 29.996
R² = 0.02391
p=0.6697

Figure 12. Winter 2015 Spatial Regressions

Simple linear regressions comparing stream and porewaters to $\delta^{13}C$ of hydropsychids and *Corbicula*. Streamwater methane concentration was significantly related to hydropsychid $\delta^{13}C$ (a)(p=0.01). No significant relationship was found between porewater methane concentration and hydropsychid $\delta^{13}C$ (b). Analysis of data also found no relationship between streamwater and *Corbicula* $\delta^{13}C$ or porewater and *Corbicula* $\delta^{13}C$ (c, d)

Regression analysis of summer 2015 samples found no significant relationship between streamwater methane concentration and *Hydropsyche* $\delta^{13}C$ (Fig. 13a). Regression analysis of summer 2015 samples found that porewater methane concentration was significantly related to *Hydropsyche* $\delta^{13}C$ ($F_{1,9}=8.11$, p=0.019), with increasing porewater methane concentrations leading to decreasing *Hydropsyche* $\delta^{13}C$ (Fig 13b). Regression analysis also found no relationship between streamwater and *Cheumatopsyche* $\delta^{13}C$ or porewater and *Cheumatopsyche* $\delta^{13}C$ (Fig. 13c, d).

Figure 13. Summer 2015 Hydropsychid Spatial Regressions

Simple linear regressions comparing stream and porewaters to $\delta^{13}C$ of *Hydropsyche* and *Cheumatopsyche*. Analysis of summer 2015 samples found no significant relationship between streamwater methane concentration and *Hydropsyche* $\delta^{13}C$ (a). Porewater methane concentration was significantly related to *Hydropsyche* $\delta^{13}C$ (p=0.019) (b). Analysis of data also found no relationship between streamwater and *Cheumatopsyche* $\delta^{13}C$ or porewater and *Cheumatopsyche* $\delta^{13}C$ (c, d).

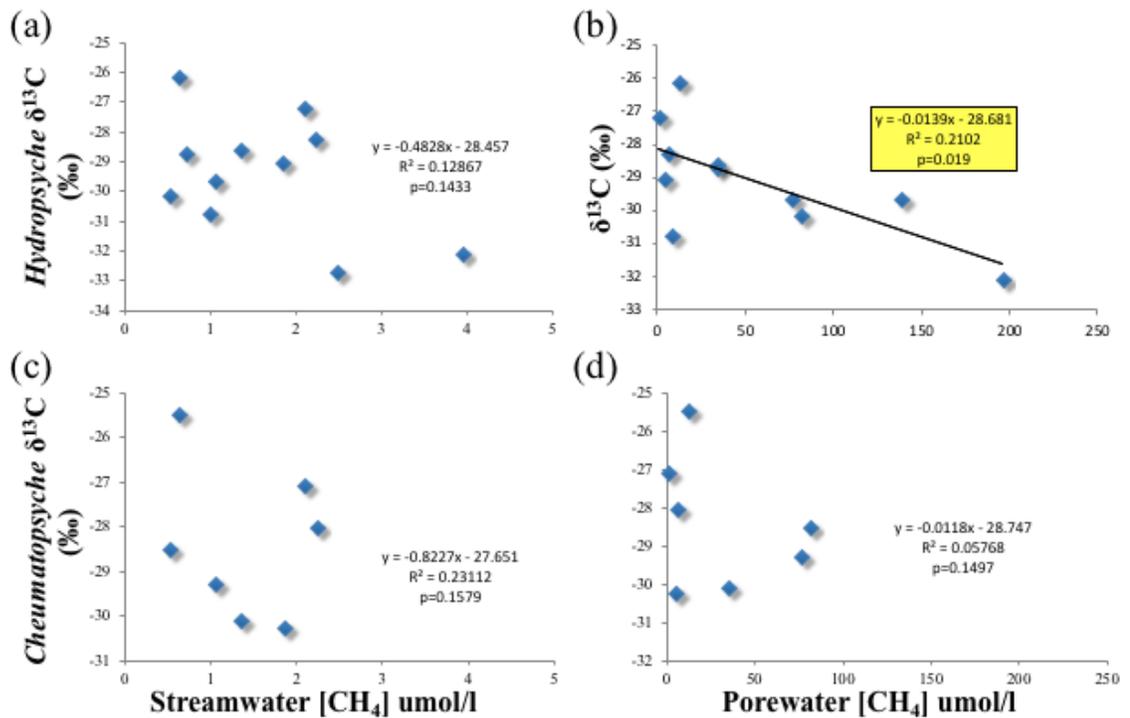Regression analysis of summer 2015 data for spatial relationship of *Corbicula* $\delta^{13}C$ with stream and porewater methane concentration found no relationship between streamwater and *Corbicula* $\delta^{13}C$ or porewater and *Corbicula* $\delta^{13}C$ (Fig. 14a, b)

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(a)

(b)
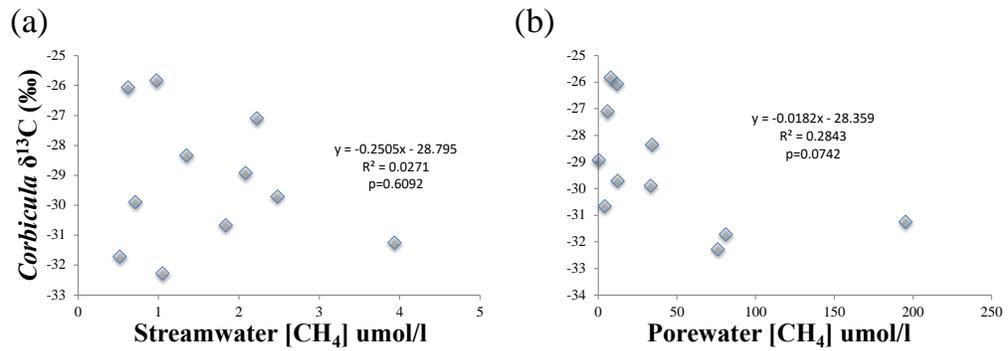
Figure 14. Summer 2015 *Corbicula* Spatial Regressions

Simple linear regressions comparing stream and porewaters to δ^{13}C of *Corbicula*. No relationship was found between streamwater and *Corbicula* δ^{13}C or porewater and Corbicula δ^{13}C (a, b)

Simple linear regressions of data pooled for all seasons found no significant relationship between streamwater and hydropsychid δ^{13}C or porewater and hydropsychid δ^{13}C (Fig. 15a, b). Regression analysis of data also found no relationship between streamwater and *Corbicula* δ^{13}C or porewater and *Corbicula* δ^{13}C (Fig. 15c, d)

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(a)

Hydropsychid δ¹³C (‰)

y = 0.339x - 31.234
R² = 0.0379
p=0.1865

(b)

y = 0.004x - 31.224
R² = 0.1183
p=0.117

(c)

Corbicula δ¹³C (‰)

y = -0.1115x - 29.49
R² = 0.0056
p=0.2724

Streamwater [CH₄] umol/l

(d)

y = -4E-06x - 29.681
R² = 1E-07
p=0.6879

Porewater [CH₄] umol/l
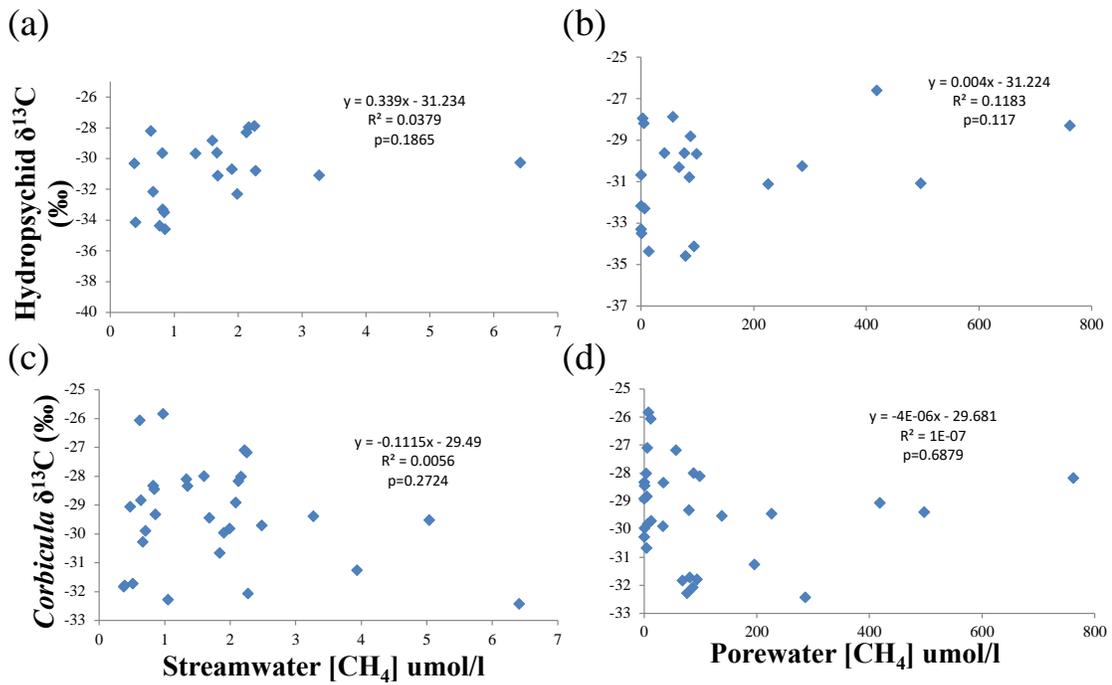
Figure 15. Spatial Regressions Pooled by Season

Simple linear regressions for comparing stream and porewaters to δ¹³C of hydropsychids and *Corbicula* pooled for all seasons. No significant relationship was found between streamwater and hydropsychid δ¹³C or porewater and hydropsychid δ¹³C (Fig. 8a, 8b). Analysis of data also found no relationship between streamwater and *Corbicula* δ¹³C or porewater and *Corbicula* δ¹³C (Fig. 8c, 8d).

CHAPTER IV

DISCUSSION

Data collected for methane concentration supported the hypothesis that methane concentration is higher in porewaters than streamwaters in the studied Piedmont streams. Data collected during the summer of 2014, winter of 2015, and summer 2015 demonstrated that porewater methane concentrations were significantly higher than streamwater samples during all seasons (Fig. 2), with site averages ranging from 0.37 to 6.41 umol/l for streamwater and porewater values ranging from 3.19 to 761.72 umol/l. These results are consistent with other studies that have found high concentrations of methane in both porewaters and streamwaters, with porewater usually representing a much higher concentration than streamwater (Mbaka et al., 2014; Rulik et al., 2000; Schindler et al., 1998; Stanley et al., 2016). For instance, Mbaka (2014) found porewater samples to be significantly higher than streamwater samples in stream impoundments, with porewater samples ranging from 0.3 to 1657.5 umol/l and streamwater samples ranging from 0.07 to 0.7 umol/l. Stanley (2016) created a database of methane concentrations of numerous methane studies, and found that the gas tends to be saturated in lotic systems, in one case even causing the stream to have a fizzy appearance due to high ebullition. It has been documented that methanogens thrive in anoxic microsites located within stream sediments (Crawford et al., 2014), as opposed to the oxic stream water (Stanley et al., 2016). In fact, research has demonstrated that methane can be

formed within these anoxic stream porewaters (Crawford et al., 2014). Another contributing factor to higher porewater methane concentrations could be subsurface (lateral) inputs of methane from the surrounding landscape (Jones and Mulholland, 1998). These individual processes, or most likely, a combination of both, contributed to the observed porewater methane concentrations in the sampled streams.

δ^{13}C-CH$_4$ data collected for stream and porewaters (Fig. 3) supported the hypothesis that porewater δ^{13}C-CH$_4$ would be lower than streamwater δ^{13}C-CH$_4$. Streamwater δ^{13}C-CH$_4$ values ranged from -34.04 to -63.33 ppt, whereas porewater values ranged from -34.4 to -74.84 ppt, representing an average difference of -7.24 ppt between stream and porewater across all seasons. The higher values in streamwater compared to sediment porewater are a result of the isotopic shift that occurs when methane is oxidized in the sediments, a process that has been demonstrated in sediment cores (Whiticar, 1999) as well as in sediment profiles of peatlands (Dorodnikov et all, 2013). The results from this study were within the range observed in terms of porewater δ^{13}C-CH$_4$ to the work of Dorodikov (2013) where porewater samples taken from a peatland had a marked shift in δ^{13}C-CH$_4$, with deeper peat layers having δ^{13}C-CH$_4$ values up to -71 ppt. The results observed in this study are most likely a result of methane oxidation in the sediments and streamwater, which subsequently causes streamwaters to have a higher δ^{13}C-CH$_4$ due to the lighter isotope being used preferentially by MOB (Mahieu et al., 2008). The lack of significant difference between δ^{13}C-CH$_4$ of water samples in the winter is suggestive of lower rates of methane oxidation during this season. This could potentially be explained by lower rates of methane oxidation during

lower temperature conditions, as higher temperatures have been shown to increase rates of methane oxidation in streams (Shelley et al., 2015).

The use of MDC by consumers has been demonstrated in a variety of aquatic environments, including lakes (Jones and Grey, 2011) and streams (Trimmer et al., 2009), as well as microcosm studies (Deines et al., 2007). Regardless, there is still work to be done in determining what other consumers are utilizing methane as a source of carbon in streams and rivers (Trimmer et al., 2015). Hydropsychid and *Corbicula* usage of MOB has not been widely explored (but see Monteverde 2015). Stable isotope analysis of consumers and their respective diet sources suggested the use of MOB by both consumers (Figures 4 and 5). Assimilation of photosynthetically-derived food sources results in consumer $\delta^{13}C$ values higher than their food source (Ben-David et al. 2012), whereas consumers that have utilized MDC possess lower $\delta^{13}C$ values compared to photosynthetically-derived food sources (Deines et al., 2007; Kankaala et al., 2006). *Corbicula* were significantly lower in $\delta^{13}C$ than seston and sediments during both summer seasons, suggesting that MDC could be a source of energy during this season (Fig 4a, 4c). There were no significant differences in $\delta^{13}C$ between seston, sediment, and *Corbicula* during the winter, suggesting that MDC is not an important subsidy during this season (Fig. 4b). This was reflected in the mixing model results for both seston and sediment as diet sources. For the model using seston and MOB as diet sources for *Corbicula*, the summer seasons had larger usage of MOB with $9\pm4.8\%$ for summer 2014 and $5\pm0.9\%$ for summer 2015, which was larger than the winter ($4\pm0.9\%$ MOB usage) (Table 2). This could be due to a decrease in methane oxidation due to lower

temperatures (Shelley et al., 2015), resulting in the formation of less MDC. For the model using sediment and MOB as diet sources for *Corbicula*, summer 2014 had the highest usage of MOB (14 ± 4.8), followed by winter 2015 (11 ± 10), and lastly by summer 2015 7 ± 1.0.

Hydropsychids had lower $\delta^{13}C$ than seston during summer 2014 and winter 2015, suggesting that MDC could be an important subsidy for them during these seasons (Fig. 5a,b). Pooled hydropsychids were also significantly lower in $\delta^{13}C$ than seston during summer 2015 (Fig. 5c). This was also reflected in mixing model results that showed that hydropsychids had MOB usage ranging from $4 \pm 1.2\%$ in summer 2014 to $11 \pm 1.9\%$ during the winter. Previous research by Monteverde (2015) demonstrated that MOB was present in the nets and retreats of hydropsychids through passive capture from the seston and growth on retreats and nets in response to methane in the water column. Furthermore, Monteverde (2015) also found that these MOB were actively harvested by hydropsychids. It appears from this current study that hydropsychids were utilizing these MOB, resulting in lower $\delta^{13}C$ values than seston. Interestingly, Trimmer (2009) demonstrated that two caddisfly species also had depleted $\delta^{13}C$ values compared to their normal food sources, indicating usage of MDC. These caddisflies represented species from a different family (Glossosomatidae) that build cases instead of retreats, and had more depleted $\delta^{13}C$ values than the caddisflies studied in this survey. The caddisflies studied by Trimmer (2009) also utilized a much larger percentage of MOB (30%), although this could possibly be to the fact that these genera grazed biofilm, unlike the genera studied in this survey which were assumed to gain all of their MOB from seston

and from MOB located within their nets and retreats. Stream type could have also explained these large usages of MOB, as the chalk streams studied provided an ideal habitat for methanogens and had supersaturation of streamwater exceeding 50 times atmospheric levels. This far exceeds the streamwater concentrations found in this study. In contrast, the two consumers studied in NC Piedmont streams seemed to be using a very low percentage of MOB as part of their diet (Table 2).

The hypothesis that the two genera of hydropsychids studied would have different usage of MDC due to niche separation was not supported (Figures 6 and 8). This lack of significance suggests that niche separation does not impact their usage of MDC. This was reflected in the lack of significant differences between the two consumers in terms of $\delta^{13}C$ and percent usage of MOB.

Results comparing the usage of MOB between consumers did not support the hypothesis that feeding mode determines the degree to which a consumer utilizes MDC, as in the majority of seasons, there was no difference in usage between consumers (Figure 7). Mixing model analysis for consumers and seston estimated that all consumers were using MOB as part of their diet during all seasons (Table 2). Hydropsychids utilized MOB to a greater extent during the winter, corresponding to their lower $\delta^{13}C$ value during this season (Figure 9). This was the only time in which the consumers varied significantly in their usage of MOB. Monteverde (2005) demonstrated that MOB are present within the nets and retreats of hydropsychids. However, it is unknown at this time why hydropsychids would be using MDC to a greater extent during the winter. As for *Corbicula*, their usage of MOB did not vary between seasons (Figure 10).

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Regardless of these results, the limitations of the utilized mixing model must be acknowledged. First, the model assumed that consumers were only utilizing seston as part of their diet. However, it is known that sediment carbon can contribute to the diet of *Corbicula* in addition to seston (Cohen et al., 1984; Foe and Knight, 1986). In this study however, sediment appeared to not make up a significant contributing factor to the diet of the studied *Corbicula* during the majority of seasons, with *Corbicula having* $\delta^{13}C$ values approximately 4 ppt lower than sediment during summer 2014 and 3ppt lower than sediment during the summer 2015. Regardless, separate mixing models utilizing sediment and MOB as food sources for *Corbicula* were performed for all seasons. Separate mixing models using sediment and MOB as diet sources demonstrated that sediment made up a similar contribution to the diet of clams (86 ± 4.8 to $93\pm1\%$) compared to mixing models using seston, and also resulted in similar contributions of MOB to diet ranging from 7 ± 1.0 to $14\pm4.8\%$ (Table 2). While a three way mixing model was not able to be performed, clearly all mixing models indicated that MOB makes a contribution to the diets of both consumers.

The second limitation was that the fractionation effect on methane was estimated by finding the differences between direct field measurements of $\delta^{13}C$-CH_4 porewater and $\delta^{13}C$-CH_4 streamwater, knowing that methane will become higher in ^{13}C as it travels upwards in a stream because MOB will preferentially utilize the lighter isotope, leaving the heavier behind (Kohzu, et al., 2004, Stanley et al., 2016). Adding this value back to $\delta^{13}C$-CH_4 of porewater gave an estimate of $\delta^{13}C$ of MOB. This method gave an average methane fractionation value of 6.75. Published values of methane fractionation are

approximately 4-30 (Whiticar, 1999), however these values can be variable under different experimental conditions (Coleman, 1981). In the case of this study, $\delta^{13}C$ of MOB was derived from direct field measurements of streamwater and porewater $\delta^{13}C$-CH_4. To investigate this limitation, the mixing models were followed by sensitivity analysis for $\delta^{13}C_{MOB}$ as this was a derived input parameter and was likely more uncertain than the other input parameters that were directly measured. Results from most of these sensitivity analyses on $\delta^{13}C_{MOB}$ were comparable to those from the original mixing models, providing values that were consistent with the values and confidence limits of usage of seston, sediment, and MOB present in Table 2.

The third limitation was that only a simple algebraic mixing model was utilized to determine usage of various diet sources for consumers. More complex modeling systems that involved fitting data to Bayesian models such as Stable Isotope Analysis in R (SIAR) were originally considered. However due to criticisms in the literature focused on the fact that this model uses distributions rather than averages and can often overlook evaluation of the model before it is run (Smith et al., 2013), it was deemed appropriate to utilize algebraic mixing models. The utilization of an algebraic mixing model allows for the diet calculation of consumer diets using stable isotope data without fitting them to probability distributions, which gives a single value for consumer $\delta^{13}C$ instead of a polygon within the consumer must lie (Smith et al., 2013).

Spatial analyses comparing relationships between stream and porewater methane and $\delta^{13}C$ of consumers yielded little support for the spatial correlation hypothesis (Figures 11-15). Similar results were found by Mbaka (2014) where no significant

relationships between stream and porewater methane concentration and δ^{13}C of chironomid larvae were found. An explanation for the lack of strong correlations in this study could potentially be due to the low use of MDC by consumers found in this study.

It is clear from this survey that the streams studied possess high porewater methane concentrations ranging from 3.19 to 761.72 umol/l. This is consistent with other studies, with Mbaka (2014) finding stream porewater concentrations in the range of 0.3 umol/l to 1657.5 umol/l. The studied streams also possess δ^{13}C-CH$_4$ ranging from -34.4 to -74.84 ppt, giving an average enrichment of -7.24 ppt. On the other hand, Jones and Grey (2011) found methane enrichment that was approximately 20ppt. However these values have been shown to be variable under differing experimental conditions (Coleman, 1981). The two consumers studied are utilizing MDC as part of their diets (Table 2), though to a smaller degree than photosynthetically-derived sources like organic material in the seston and sediment. While the usage of MDC by consumers has been investigated in various aquatic environments (see Trimmer et al., 2009; Kankaala et al., 2006), this study provides new evidence for the usage of MDC by two consumers that have received little to no previous investigation in their usage of MDC (however see Monteverde, 2015). This study provides evidence for a seasonally variable but low level of usage of MDC by hydropsychid caddisflies and *Corbicula* in Piedmont streams. More research is necessary to understand how methane is impacting the diets of other consumers in lotic systems, and the conditions that lead to variation in use of MDC across lotic ecosystems.

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