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CORBICULA FLUMINEA FOOD WEB ECOLOGY: AN EXPERIMENTAL TRANSPLANT APPROACH IN A KARST RIVERINE SYSTEM

A Capstone Experience/Thesis Project

Presented in Partial Fulfillment of the Requirements for

the Degree Bachelor of Science with

Honors College Graduate Distinction at Western Kentucky University

By:

Autumn R. Smith

*****

Western Kentucky University
2015

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ABSTRACT

The Asiatic clam *Corbicula fluminea* is a filter-feeding habitat generalist. Carbon isotopic composition ($\delta^{13}C$) of *C. fluminea* body tissue was compared between reaches of Kentucky's upper Green River that differed in *Cladophora* growth. *Corbicula fluminea* from an upstream reach with little *Cladophora* was translocated to a downstream reach with high *Cladophora* levels. Individuals from both reaches were placed in mussel silos in the same downstream reach in autumn 2012 and again in 2013 for 77 and 119 days, respectively. Flow during 2012 consisted of no high flow events until late autumn. Flow patterns in 2013 were consistently higher and more variable. In 2012 the upstream *C. fluminea* were ^{13}C-depleted over time compared to no temporal change in the downstream *C. fluminea.* The trend was opposite in 2013. Upstream *C. fluminea* were more ^{13}C-enriched over time whereas, again, there was no temporal change in the downstream *C. fluminea*. Estimated dietary contributions of basal resources using IsoSource found between-year trends that suggested that *Cladophora* fragments may represent an important food component during years with low flows and dense macroalgal growth.

Keywords: *Corbicula fluminea*, food web ecology, *Cladophora*, Green River, Karst riverine system, transplant approach

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CHAPTER 1

INTRODUCTION

Karst geology

Karst landscapes are formed by the dissolution of soluble rocks, rather than from physical abrasion by water or wind (Ford & Williams 2007). Karst bedrock is soluble by weak acids, usually carbonic acid, which is formed when carbon dioxide (CO_2) reacts with water (Yuan 1988). Most karst bedrock is limestone, which is mainly comprised of calcite, but there are some karst landscapes that are composed of other types of rock (Palmer 2007). Carbonate rocks weather faster than silicate rocks, so they have a larger impact on the amount of dissolved inorganic carbon (DIC) and calcium in riverine systems (Roy et al. 1999). DIC is the most common form of carbon in rivers (Brunet et al. 2009). Weathering and dissolution of limestone bedrock also increases the amount of DIC in groundwater. These groundwater inputs have a major affect on DIC concentrations in riverine systems (Redfield 1958; Kling et al. 1992; Cox et al. 2000; Friedlingstein et al. 2001; Hope et al. 2001; Doctor et al. 2008; Schulte et al. 2011).

Carbon dioxide, bicarbonate, and carbonate are the three interchangeable forms of inorganic carbon that exist in water. The most common form of inorganic carbon in acidic water is CO_2 (Hutchinson 1957). The vast majority of DIC in Kentucky's Upper Green River is in the form of bicarbonate. Karst groundwater inputs raise the pH of riverine systems, which in turn causes bicarbonate to be the most common form of DIC

in the streams of karst landscapes.

The abundance of bicarbonate is the main available form of DIC for primary consumers, but they need high CO_2 levels for photosynthetic pathways. This results in primary producers being carbon-limited when the pH is alkaline and the most common DIC form is bicarbonate (Raven et al. 1985; Finlay 2003). The primary producers have to use carbon concentrating mechanisms (CCMs) to convert bicarbonate to carbon dioxide. These are very costly processes, but primary producers up regulate CCM activity when carbon dioxide levels are low because the benefit of converting bicarbonate is greater than the energy cost associated with the CCMs (Giordano et al. 2005). Karst regions may create environments that favor species that have CCMs because of the high amount of bicarbonate present in the alkaline riverine systems (Raven et al. 1985; Finlay 2003). Carbon limitation is unlikely for most species in karst areas though because of the high DIC inputs from groundwater.

Cladophora biology

Cladophora is a filamentous macroalga that is present worldwide in a variety of marine and freshwater habitats (Dodds & Gudder 1992). *Cladophora* is composed of green single cells that form long branching chains that appear as green thread or hair when viewed macroscopically (AquaScaping World 2012).

Cladophora is a C_3 plant. Photosynthesis by aquatic producers involves the uptake of CO_2 with an associated depletion of ^{13}C. This depletion is due, in part, to diffusion of carbon into the plant and carbon fixation. As a result, ^{13}C is depleted by 5–25‰ (Vogel et al. 1993).

Cladophora is sometimes considered a nuisance species, and during warm weather, can grow in thick mats that cover the entirety of a river bottom (Penick et al. 2012). The accumulation and growth of *Cladophora* has a positive correlation with high nutrient levels (Dodds & Gudder 1992, Penick et al. 2012). A recent study shows that *Cladophora* cover, however, may be more correlated to water velocity than nutrient levels (King et al. 2014). High water levels can easily scour *Cladophora*, and its growth increased in seasonal low-flow periods (Power 1990; Ensmingner et al. 2000; Power et al. 2009). Karst flow regimes often have pulses and varying flow, so *Cladophora* mats are commonly dislodged in these riverine systems (Penick et al. 2012). Since Kentucky's upper Green River is a karst riverine system, it commonly has flow variance that affects the *Cladophora* levels.

Cladophora is considered "grazer resistant," but some macroinvertebrates use it directly as a food resource (Rhame & Stewart 1976, Tinsley 2012). *Cladophora* can also be used as an indirect food source by hosting epiphytes, most commonly diatoms, which are eaten by consumers (Dodds 1991). Epiphytes do not compete with *Cladophora* for space, but instead, lower drag on *Cladophora* in high velocity areas, which leads to less dislodgment of the macroalga (Dodds 1991). The epiphytes do block photosynthesis under high density conditions, but this usually isn't a significant problem because primary consumers intake up to 75% of the epiphytes residing in *Cladophora* tufts (Dodds 1991). Algal blooms of *Cladophora* may cause generalist consumers to feed primarily on *Cladophora* due to its abundance, which could lead to an increased niche breadth for several primary consumers (Feinsinger et al. 1981).

Corbicula fluminea biology

The invasive Asiatic clam *Corbicula fluminea* is a small mollusc that was first introduced in North America in 1920 to serve as a food resource for humans (Suriani et al. 2007). *Corbicula fluminea* resides in a broad variety of aquatic habitats, including stream, rivers, and ponds. *Corbicula fluminea* has a short life cycle, early sexual maturity, and fast population growth (Sousa et al. 2008). Because of these characteristics, *C. fluminea* can easily colonize a wide variety of habitats and have an increased potential to be an invasive species (Sousa et al. 2008). The introduction of *C. fluminea* has been associated with a reduction of diversity in some biological communities (Suriani et al. 2007).

Corbicula fluminea has great adaptation abilities; it can accumulate various pollutants, and it can be a good biological monitoring species (Doherty 1990). *Corbicula fluminea* is currently used by the national French program ECODYN as a biomarker for water contamination (Legeay et al. 2005).

Corbicula fluminea are both filtering- and deposit-feeders. Depending on the habitat, this species feeds on a combination of microscopic algae and detrital materials. In riverine systems underlain by fine sediments they have been shown to feed on unicellular algae (Rosa et al. 2011).

Stable isotopes

Stable isotopes are measured as the ratio of a heavy to a light isotope. The two most abundant isotopes are typically quantified. When quantifying isotopic ratios, expressed as δ, the ratio is compared to a known standard and expressed as:

$$\delta^{13}C_{sample} = \frac{m(^{13}C/^{12}C)_{sample} - m(^{13}C/^{12}C)_{reference}}{m(^{13}C/^{12}C)_{reference}}$$

Different standards are used depending on the isotopes quantified. The original carbonate standard was Pee Dee Belemnite (PDB). Before this original standard was exhausted, it was used to calibrate a sample of white marble known as NBS-19 (Friedman et al. 1982). The new standard, regarded as identical to the original, and based off the calibration of NBS-19 is the Vienna Pee Dee Belemnite (VPDB).

Stable isotopes do not undergo radioactive decay but instead fractionate (Urey 1948). Less than 10% of all known isotopes are stable (Fry 2006). Fractionation is the process whereby one isotope of an element is preferentially used in a process over another isotope. Because fractionation is mass dependent, the relative difference in mass between the isotopes must be large enough to be detected.

Stable isotopes are now commonly employed as an inexpensive and accurate method to study dietary habits and food webs (Fincel et al. 2012). Carbon and nitrogen are two of the most frequently used elements in food web studies. Traditionally, stomach-content analysis and feeding observations have been used to characterize consumed food resources. These techniques, however, do not provide an accurate picture of energy and nutrient flow due to food resources that are differentially assimilated (Rounick & Winterbourn 1986). While gut-content analysis may provide a quick assessment of resources ingested, stable isotope analysis reflects a consumer's assimilated diet (Phillips 2012). Stable isotope analysis can also identify more complex interactions (e.g. omnivory) that are common in many ecosystems (Post 2002).

The isotopic composition of a consumer's tissue is a function of heavy to light isotopic ratios of the food resources they use and assimilate (Ben-David & Flaherty 2012). Small differences in isotopic ratios found in nature are used in natural abundance studies (Boschker & Middelburg 2002). Differences in natural abundance of ^{13}C and ^{12}C can be traced as they move relatively unaltered through food webs and can indicate which of several distinct types of food resources, derived mainly from primary producers, are the original sources of dietary carbon (Rounick & Winterbourn 1986; Peterson & Fry 1987).

Study purpose and questions

This research compared *C. fluminea* diets using stable isotopes as evidence of food items integrated into body tissue. This study used an experimental transplant approach to compare stable isotopic carbon signatures of whole *C. fluminea* body tissue in Kentucky's upper Green River (Fig. 1) during autumn in two riverine reaches ("upstream" and "downstream"). The upstream and downstream reaches differed in underlying karst development and *Cladophora* standing stocks. The diet of *C. fluminea* was assessed during autumn in consecutive years that differed in flow regimes. The dietary study period occurred when growth and accrual of *Cladophora* would be maximized during low-flow periods, provided that flow conditions were stable to not induce scouring (Fig. 4).

Two questions were addressed. First, how do stable carbon isotopic ratios of *C. fluminea* body tissue compare between the two river reaches with basal food resources differing in $\delta^{13}C$ isotopic signatures? This comparison was based on burrowed, *in-situ C.*

fluminea collected from each reach. Second, does the body tissue of upstream *C. fluminea* change when transplanted to the downstream reach? This was done to assess if transplanted *C. fluminea* were able to assimilate to local-scale food resources. This comparison was based on placing *C. fluminea* individuals in experimental mussel silos.

CHAPTER 2

METHODS

Study reach descriptions

This research occurred in two reaches in the upper Green River between the Green River Lake (GRL) and Mammoth Cave National Park, Kentucky (Fig. 1). The upper Green River flows in a westerly direction through the Interior Low Plateau region. This region has a landscape characterized with the most well-developed karst system in the U.S. (Fenneman 1938, Palmer & Palmer 2009). Soils in this region are derived from weathering of bedrock, and land is predominantly used for agriculture with some forest (Woods et al. 2002).

The upper Green River flows over surficial geology that transitions from a mixed carbonate-siliciclastic landscape upstream to a highly-karstified carbonate-dominated lithology downstream. The upstream reach was 43 km downstream of GRL and drains a 1,919 km^2 basin of heterogeneous surficial lithologies, mainly Devonian shales (38%) and Mississippian limestones (51%). Other lithologies in this basin include Ordovician dolostones (1.3%), Mississippian sandstones (1.1%), and Pleistocene and Holocene sands (6.4%) (Osterhoudt 2014). The downstream reach was 155 km downstream of GRL, draining a 4,489 km^2 basin dominated by Mississippian carbonates (77%) (Palmer & Palmer 2009). Relatively small areas within this basin also include siliciclastic bedrocks such as shale (16.2%), sand (3.7%), sandstone (1.3%), and siltstone (0.2%)

(Osterhoudt 2014). Surface stream density is low in this region (Woods et al. 2002).

The upper Green River is a 6^{th}- (upstream reach) to 7^{th}-order stream (downstream reach) with well-defined banks. Both study reaches were located in a shallow riffle comparable with gravel and cobble substrates. Riparian edges are forested predominantly with Silver maple (*Acer saccharinum* L.*),* Box elder (*Acer negundo* L.*),* and Sycamore (*Platanus occidentalis* L.*).* The upper Green River is also eutrophic. Nitrogen to phosphorous ratios are >20 in these reaches (Penick et al. 2012).

Macrophyte and macroalga are present in shallow riffles of this river. Large patches of the riverweed *Podostemum ceratophyllum* (Michx.) were present in all four sites. *Cladophora* abundance is very low during winter and spring, but can be increasingly abundant during summer and autumn if water levels are low. Upstream reaches typically display little *Cladophora* growth, characterized by small patches of filaments that were typically under a foot long. Downstream reaches had dramatically higher *Cladophora* growth, with dense mats nearly covering the entire river channel by mid-June during low-flow periods (Penick et al. 2012, Malloy 2014).

Field and laboratory methods

Corbicula fluminea individuals were collected from each reach with a D-frame net equipped with a 500-µm mesh net. At the onset of each study period a grid network of 32 (8 * 4) experimental mussel silos (Figs. 2–3) was setup in the downstream reach. The designation of a silo to hold upstream or downstream *C. fluminea* was determined at random. Mean depth ($t = 1.82$, $p = 0.08$) and velocity ($t = 0.58$, $p = 0.57$) immediately upstream of the silos housing upstream or downstream *C. fluminea* were not significantly

different at the onset of the study period in 2012 (Table 1). Mean depth ($t = 0.73$, $p = 0.47$) was also not significantly different in 2013 (Table 1).

Each silo held four individuals of approximately the same size. A subset of individuals (n = 4 samples, 4 individuals/sample) from both reaches were set aside for initial stable isotope analyses (SIA). Four silos per treatment were selected at random at intervals determined, in part, according to flow conditions that permitted wading (Fig. 4). *In-situ C. fluminea* were also collected from each reach either on the same day or within one day of silo retrieval.

Once in the laboratory all *C. fluminea* individuals were maintained in a small, aerated tank for a minimum of two days to expel feces and pseudofeces. Individuals were placed in boiling water for a few seconds to induce death and the soft inner tissue was separated from the shell. Only the soft tissue was used for SIA.

Several potential food resources were collected from each reach. These included a combination of live or decaying *Cladophora* tissue, epilithic biofilm, transported organic matter (TOM) and benthic organic matter (BOM). Fresh *Cladophora* tissue was collected from the river bottom. In the upstream reach where *Cladophora* patches were generally very small, often a whole mat was collected. Previous research in this river indicated that isotopic carbon ratios of decaying *Cladophora* also changes little over time. Hence, for this study we assumed that fresh *Cladophora* was likely representative of decaying *Cladophora* tissue (Grubbs, unpublished data) and vice versa. *Cladophora* was first rinsed thoroughly in deionized water to remove dirt and large impurities, inspected under a dissection scope (7-10x), and any remaining detritus and consumers were removed before drying for at least 48 hours at 70^0 C.

The TOM samples were obtained by collecting 60 L of surface river water from mid-channel. In the laboratory, TOM was first partitioned into a 1000–100 µm fine (FTOM) fraction using a sieve. The filtrate was vacuum-filtered through a 1-µm Gelman glass fiber filter (GFF) to obtain an ultrafine (UTOM) fraction. Because seston levels were typically very low, the FTOM and UTOM fractions were usually combined for a composite (CTOM) fraction. Dissolved fraction (DTOM) was obtained by evaporating river water after it had passed through a 1-µm glass fiber filter. Filtered water was heated, but not boiled, to facilitate evaporation. Sulfuric acid was added to the DOM water samples until water reached a pH of 2.0 to discourage microbe growth during filtering and processing.

Epilithic biofilm was obtained from rocks in shallow habitats (< 0.5 m). Rocks with little silt were preferentially selected and scrubbed in a bucket of river water with a toothbrush to remove epilithon. Biofilm was concentrated by vacuum-filtering samples through a 1-µm GFF. Both TOM and biofilm were partitioned into separate algal and detrital fractions using a colloidal silica separation technique (Hamilton & Lewis 1992). Filtered samples were placed in 30mL of a 70% Ludox solution and centrifuged at 1200 rpm for 15 min to separate algal and detrital fractions. Samples were then observed and centrifuged again if the separation was incomplete. The resulting algal and detrital fractions were placed into separate centrifuge tubes with 30 mL of 70% Ludox and centrifuged again at the same rpm for 15 min to complete the separation process. Samples were rinsed with ample deionized water a final time and filtered through a 1-µm GFF to separate the sample from the Ludox solution.

Benthic organic detritus was (BOM) collected with a coring device comprised of a PVC pipe with a 5-cm diameter opening. The BOM sample was partitioned into two size fractions using 1 mm (coarse, CBOM, >1000 µm) and 100 µm (fine, FBOM, 1000–100 µm) sieves. The filtrate was vacuum-filtered through a 1-µm Gelman glass fiber filter (GFF) to obtain an ultrafine (UBOM) fraction. A dissolved fraction (DBOM) was prepared and collected in identical fashion to DTOM.

C. fluminea and all resource samples were prepared for SIA identically. Dried samples were pulverized to a fine powder using a Wig-L-Bug®, measured out into separate sizes for resources (4.5 mg) and consumers (1.5 mg), and placed into 5x9 mm tin capsules. Carbon and nitrogen stable isotopic ratios were analyzed on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the UC Davis Stable Isotope Facility, University of Davis, California, USA. Stable isotope ratios were calculated as $\delta^{13}C$ or $\delta^{15}N$ (per mil) = $([R_{sample}/R_{standard}]-1) * 1000$, where R is the ^{13}C:^{12}C (=$\delta^{13}C$) or ^{15}N:^{14}N (=$\delta^{15}N$) ratio. Pee Dee Belemnite and atmospheric nitrogen (AIR) were used as standards for carbon and nitrogen analysis, respectively. Machine error for $\delta^{13}C$ was 0.2‰, and machine error for $\delta^{15}N$ was 0.3‰.

The linear mixing model IsoSource (Phillips & Gregg 2003) was used to estimate dietary contributions of basal food resources to the assimilated diet of *C. fluminea*. Trophic enrichment factors of 0.4 for $\delta^{13}C$ and 3.4 for $\delta^{15}N$ were added to all food resources prior to analyses to account for fractionation during digestion and assimilation (Phillips & Gregg 2003). Increment and tolerance levels were initially set at 1% and 0.2% for each analysis, respectively. When consumer isotopic values fell outside of mixing

polygons, tolerance levels were increased by increments of 0.1% until either IsoSource produced feasible results or the tolerance level reached 0.5%.

The choice of basal resources was based on which combination of live or decaying *Cladophora* tissue, epilithic biofilm, TOM, and DOM were collected and available for analyses. IsoSource results were displayed as ranges of resource contributions (1-99th %tiles of all feasible results).

CHAPTER 3

RESULTS

Flow conditions

River flow conditions were different during the two years. During the study period in 2012, flow conditions were typically low enough to permit easy access to sample in-situ *C. fluminea* from both reaches and for retrieval of silos from the downstream reach (Fig. 4), but only during the first 77 d. During 2013, however, there were small peak-flow events that made sampling and silo retrieval more problematic (Fig. 4). The higher flow regimes during the second year also caused silos to be partly buried, and silo inserts to be partly clogged, with sand. Silo burial and insert clogging did not occur during 2012.

Survivorship

In 2012, only two sets of silos were retrieved due to flows that were too high after 77 d in-stream (Table 2). There was 100% *C. fluminea* survivorship in all silos. In contrast, the *C. fluminea* survivorship in 2013 was consistently less than 60%. The survivorship was 50% at a maximum during the first two retrieval dates. There was a maximum survivorship rate of only 56% for the upstream *C. fluminea* in the last two retrieval dates. Survivorship was consistently markedly less in 2013 than in 2012 (Table 2).

$\delta^{13}C$ trends

2012 *in-situ* trends

During 2012, the upstream *C. fluminea* had a mean $\delta^{13}C$ value of -30.22 after 0 d, -30.75 after 48 d, and -31.62 after 77 d. The downstream *C. fluminea* had a mean $\delta^{13}C$ value of -30.66 after 0 d, -30.91 after 48 d, and -30.89 after 77 d. The upstream *C. fluminea* were initially more ^{13}C-enriched than downstream individuals, but became markedly ^{13}C-depleted over the 77 d. Comparatively, *C. fluminea* from the downstream reach became only slightly ^{13}C-depleted after 77 d (Fig. 5).

2012 mussel silo trends

During 2012, the transplanted upstream *C. fluminea* had mean $\delta^{13}C$ values -30.52 after 48 d and -30.63 after 77 d. The downstream *C. fluminea* had a mean $\delta^{13}C$ value of -30.78 after 48 d and -30.60 after 77 d. The upstream *C. fluminea* that were transplanted to the downstream reach became more ^{13}C-enriched than the *in-situ* individuals. The transplanted upstream *C. fluminea* were minimally ^{13}C-enriched showed little change after 77 d. Similarly, the downstream *C. fluminea* displayed only nominal changes after 77 d. The upstream and downstream *C. fluminea* had essentially analogous $\delta^{13}C$ values for both retrieval dates (Fig 5).

2013 *in-situ* trends

During 2013, the upstream *C. fluminea* had a mean $\delta^{13}C$ value of -31.09 after 57 d and -30.74 after 83 d. The downstream *C. fluminea* had a mean $\delta^{13}C$ value of -30.73 after 0 d, -29.34 after 57 d, -29.50 after 83 d, and -30.42 after 119 d. The upstream *C. fluminea*

were more ^{13}C-depleted than downstream individuals. Downstream *C. fluminea* were highly ^{13}C-depleted after 0 d and 119 d. There was no data for the upstream *C. fluminea* for these retrieval dates, but these highly ^{13}C-depleted values are considered inconsistent with the other data (Fig. 6).

2013 mussel silo trends

During 2013, the transplanted upstream *C. fluminea* had a mean δ^{13}C values of -31.00 after 36 d, -30.87 after 57 d, -29.90 after 83 d, and -29.77 after 119 d. The downstream *C. fluminea* had mean δ^{13}C values of -29.74 after 36 d, -29.69 after 57 d, -29.75 after 83 d, and -29.46 after 119 d. The upstream *C. fluminea* that were transplanted to the downstream reach were more ^{13}C-enriched over time, and after 83 d, were markedly more ^{13}C-enriched than the *in-situ* upstream *C. fluminea*. The downstream *C. fluminea* were relatively constant and displayed only nominal changes over the 119 d (Fig. 6).

2012 and 2013 trend comparison

In 2012, the upstream *C. fluminea* transplanted to the downstream reach were more ^{13}C-depleted over time. In contrast, in 2013 the transplanted upstream *C. fluminea* to the downstream reach were more ^{13}C-enriched over time. Even though the trends are opposite for each year, the *C. fluminea* that were transplanted upstream appear to be assimilating to the downstream food sources, only in opposite directions each year (Fig. 5-6).

Percent contribution to diet

In 2012, there were feasible results from IsoSource for the silo *C. fluminea* and the *in-situ* downstream *C. fluminea*, but there was no solution for the upstream *in-situ C. fluminea*. For the three categories of *C. fluminea* (upstream silo, downstream silo, and downstream *in-situ*), *Cladophora* comprised the largest estimated contribution to their assimilated diets, and over the entire data set, ranging between a minimum of 55% and maximum of 86% (Table 3). The only other resources with substantial contributions to their diet were sestonic dissolved organic matter (SDOM) and ultrafine benthic organic matter (UBOM). SDOM comprised 21-29% of the downstream *in-situ C. fluminea* for the retrieval date June 6 (Table 3). UBOM comprised 24-41%, 9-37%, 19-38%, and 14-37% of the diets of downstream *in-situ C. fluminea* for the retrieval date October 30, downstream silo *C. fluminea* for the retrieval date October 30, downstream *in-situ C. fluminea* for the retrieval date November 28, and downstream silo *C. fluminea* for the retrieval date November 28, respectively (Table 3). In the upstream silo *C. fluminea*, the only food resource that had a significant contribution was *Cladophora*, which comprised around 80% of their diet (Table 3). All other food resources had a marginal contribution to both the upstream and downstream *C. fluminea* diets.

In 2013, there were feasible results from IsoSource for all *C. fluminea* expect the December 3 downstream *in-situ C. fluminea.* In the upstream *in-situ C. fluminea*, fresh *Cladophora* tissue comprised the majority of their diet with estimated contributions of 48-57% and 42-51% for the retrieval dates of October 2 and November 4, respectively (Table 4). Composite transported organic matter (CTOM) comprised the next largest portion with estimated contributions of 8-43% and 15-50% for the same retrieval dates

(Table 4). All other food resources analyzed contributed markedly less. For all downstream *in-situ C. fluminea,* UBOM comprised the majority of their diet with estimated contributions of 50-59%, 69-76%, and 31-70% for the retrieval dates August 6, October 2, and November 4 respectively. Fresh *Cladophora* contributed the second largest portion to the diet of the downstream *in-situ C. fluminea* with estimated dietary contributions of 40-44%, 24-27%, and 27-39% for the retrieval dates August 6, October 2, and November 4 respectively. All other food resources had a marginal contribution to the diet of the downstream *in-situ C. fluminea.*

CHAPTER 4

DISCUSSION

From this transplant experiment, the two study questions were answered. The results of 2012 and 2013 were very different with regard to flow conditions, survivorship, and $\delta^{13}C$ trends. In 2012, the river was characterized by relatively constant baseflow (Fig. 4). During 2013, however, there were small peak flow events in July, September, and late October (Fig. 4). Variability in flow is common within karst riverine systems, such as the upper Green River (Penick et al. 2012). With these flow events, the silo inserts became clogged with sand, and the silos themselves were partially buried. The flow obstruction within the silos was most likely the cause of *C. fluminea* mortality in 2013. Flow was consistently low during 2012 when there was little sand deposition in the silos and 0% mortality (Table 2).

Benthic organic matter (BOM) may explain how the stable carbon isotopic ratios of *C. fluminea* body tissue compared between the two river reaches. Most *in situ C. fluminea* obtained in this study were found buried or partially buried in the gravel sediment. In 2012 particularly, the diet of *in situ C. fluminea* consisted of a considerable amount of BOM (Malloy 2014). This BOM appeared to be mostly comprised of terrestrial sources, namely from decaying leaves from the trees bordering the upper Green River (Malloy 2014). The $\delta^{13}C$ values in leaves are relatively constant for the entire

length of the river because leaves obtain most of the carbon in their tissues from atmospheric CO_2, with only a small influence from bicarbonate in groundwater, which is the most available form of DIC in karst rivers (Raven et al. 1985; Finlay 2003). Due to this consistency, the $\delta^{13}C$ values of the *in situ C. fluminea* in the upstream and downstream reaches wasn't drastically different. In both years, the upstream reach was more ^{13}C-depleted. The reason for this is unclear because the carbon signal from the Green River Lake is unknown. This carbon input from the lake may be contributing to the upstream ^{13}C depletion. Thus, the stable carbon isotopic ratios of *C. fluminea* body tissue were more ^{13}C-depleted in the upstream river reach.

The peaks in discharge rate mentioned earlier also affected the basal food resource content in the water column of the downstream portion of the river. With such large volumes of water being quickly transported down the river, the division between the upstream and downstream river environments likely became less distinguishable. With high pulses in river velocity, *Cladophora,* epilithic biofilm, and other food resources can be scoured and dislodged from rocks or the river bottom (Power 1990; Ensmingner et al. 2000; Power et al. 2009). The availability of basal food resources, which correlates with the $\delta^{13}C$ values, of the downstream reach may have become similar to that of the upstream reach. This is the most likely the explanation for the inconsistent downstream *C. fluminea* $\delta^{13}C$ values for day 0 and day 119. These values were highly ^{13}C-depleted compared to the 57 d and 83 d downstream *C. fluminea* $\delta^{13}C$ values, but were analogous to the consistently ^{13}C-depleted upstream *C. fluminea*. This suggests that the upstream and downstream reach environments were similar during these high flow conditions of 2013.

Because *C. fluminea* are deposit- and filter-feeding organisms, they feed on a combination of microscopic algae and detrital materials, depending on which basal food resources are available for consumption (Rosa et al. 2011) at a given time. Since *C. fluminea* can reside in a variety of habitats (Sousa et al. 2008), it would be expected for the $\delta^{13}C$ body tissue values of transplanted *C. fluminea* to be similar to the food resources available in their new habitat. According to the data, the body tissue of upstream *C. fluminea* does change when transplanted to the downstream reach. In both 2012 and 2013, it was evident by the $\delta^{13}C$ trends that the transplanted upstream *C. fluminea* were assimilating to the local food resources in the downstream environment. In 2012, the upstream *C. fluminea* transplanted to the downstream reach were more ^{13}C-depleted over time, and in 2013, the transplanted upstream *C. fluminea* became more ^{13}C-enriched over time (Fig. 5-6). In both years, even though the trends are opposite, the transplanted upstream *C. fluminea* $\delta^{13}C$ values are becoming equivalent to the downstream *C. fluminea* $\delta^{13}C$ values over time, which is evidence that they are consuming the local downstream food resources. This illustrates a great ability of habitat adaptation in *C. fluminea*.

Why the assimilation trends were opposite between years is not fully understood. A possible explanation is that the available basal food resources differed in proportions in each year. The downstream reach in 2012 likely consisted of highly ^{13}C-depleted food resources, which were reflected in the $\delta^{13}C$ values of both the downstream *C. fluminea* and the transplanted upstream *C. fluminea*. The downstream reach in 2013 probably consisted of food resources that were more ^{13}C-enriched, which is consistent with the $\delta^{13}C$ values of both the downstream *C. fluminea* and the transplanted upstream *C.*

fluminea. In 2013, *Cladophora* was less available because of the varying pulses in flow that tended to scour and uproot the *Cladophora* standing stock. The thick mats of *Cladophora* that covered the bottom of the Green River in 2012 were absent in 2013. The estimated percent source contributions to diet in 2012 and 2013 conveyed this pattern. The percentage that *Cladophora* comprised of the *C. fluminea* diet was markedly decreased between 2012 and 2013 from approximately 70% of the *C. fluminea* diet to approximately 40% (Table 3 and 4). This decrease in *Cladophora* availability is most likely the reason that the *C. fluminea* still assimilated, but in opposite trends between 2012 and 2013.

Contradictory to what I anticipated, the upstream *C. fluminea* in 2012 and 2013 had *Cladophora* as the main food resource. Typically, *Cladophora* grows in thick mats in the downstream reach and is in less abundance in the upstream reach, so I expected the outcome that *Cladophora* was the main food resource for the downstream *C. fluminea*, not upstream *C. fluminea*. In 2013, the downstream *in-situ C. fluminea* relied on UBOM as their main food resource. Also, in 2013, mats of the *Cladophora* were commonly dislodged due to the high and varying flow (Penick et al. 2012). *Cladophora* consistently contributing highly to the upstream *C. fluminea* diets was either a coincidence or *Cladophora* was more prevalent and accessible than other food resources. This would lead us to reassess and conduct studies against the common assumption that *Cladophora* cover is thicker in downstream reaches.

As expected, the stable carbon isotopic ratios of *C. fluminea* body tissue are dependent upon the food resources available in the river reach. There was no consistent pattern in upstream or downstream $\delta^{13}C$ values; they were opposite in 2012 and 2013. In

2012, *Cladophora* was the main contributor to the diet of all *C. fluminea*, regardless of location. UBOM was the main contributor to downstream *in-situ C. fluminea* in 2013, but *Cladophora* was the main contributor to the upstream *in-situ C. fluminea* diet. As was hypothesized, *C. fluminea* were able to assimilate to local-scale food resources, which is significant to food web ecology and can be used to further study how transplantation affects the diets of freshwater invertebrates.

Table 1. Mean (±1 S.E.) depth (cm) and velocity (m/s) immediately upstream of the experimental mussel silos at the onset of each study year. Velocity was not measured in 2013.

	Year			
	2012		2013	
	U	D	U	D
d	56.1 (± 1.4)	59.7 (± 1.4)	68.0 (± 1.2)	66.9 (±1.0)
v	0.53 (± 0.02)	0.55 (± 0.02)	n.d.	n.d.

U = upstream source of *C. fluminea*, D = downstream source of *C. fluminea*, d = depth, v = velocity, n.d. = no data.

Table 2. Mean survivorship (%) of *Corbicula fluminea* in experimental mussel silos. Four *C. fluminea* per silo and four silos were retrieved each sampling date.

	Year				
	2012			2013	
Days in-stream	U	D	Days in-stream	U	D
48	100	100	36	50.0	50.0
77	100	100	57	43.8	50.0
n.d.	n.d.	n.d.	83	56.2	43.8
n.d.	n.d.	n.d.	119	56.2	25.0

U = upstream source of *C. fluminea*, D = downstream source of *C. fluminea*, n.d. = no data due to flow conditions too high to retrieve silos.

Table 3. The 2012 estimated dietary source contributions (1-99%) calculated using IsoSource 1.3. There was no solution for all upstream *in-situ C. fluminea*.

Date	***C. fluminea***	**Resource**	**FC or DC**	**EBC**	**CTOM**	**UBOM**	**SDOM**	**DDOM**
June	DI	D	67-73	0-10*	0-1	n/a	21-28	n/a
Oct	DI	D	58-66	0-9	0-4	24-41	0-3	0-7
Nov	DI	D	58-69	0-4	0-6	19-38	0-7	0-13
Oct	DS	D	59-72	0-16	0-7	9-37	0-5	0-12
Nov	DS	D	55-68	0-6	0-8	14-37	0-10	0-17
Oct	US	D	71-81	0-2	0-27	0-2	0-20	0-3
Nov	US	D	74-86	0-7	0-17	0-9	0-17	0-8

D=downstream, U=upstream, I=*in-situ,* FC=fresh or DC=decaying *Cladophora,* EBC= epilithic biofilm-composite, CTOM=composite transported organic matter, UBOM= ultra fine benthic organic matter (500-100 μm), SDOM= sestonic dissolved organic matter, DDOM=detrital dissolved organic matter, *=epilithic biofiolm-algal.

Table 4. The 2013 estimated dietary source contributions (1-99%) calculated using IsoSource 1.3. There was no solution for the downstream *in-situ C. fluminea* that were collected on 3-Dec.

Date	***C. fluminea***	**Resource**	**FC**	**EBC**	**EBA**	**EBD**	**CTOM**	**UBOM**	**SDOM**
Aug	DI	D	40-44	0-3	n/a	n/a	0-7	50-59	0-0
Oct	DI	D	24-27	0-2	n/a	n/a	0-6	69-76	0-0
Nov	DI	D	27-39	0-15	n/a	n/a	0-32	31-70	0-0
Oct	UI	U	48-57	n/a	0-13	0-10	8-43	0-33	0-15
Nov	UI	U	42-51	n/a	0-13	0-10	15-50	0-32	0-14

D=downstream, U=upstream, I=*in-situ,* FC=fresh *Cladophora*, EBC= epilithic biofilm-composite, EBA=epilithic biofilm-algal, EBD=epilithic biofilm-detrital, CTOM=composite transported organic matter, UBOM= ultra fine benthic organic matter (500-100 μm), SDOM= sestonic dissolved organic matter.

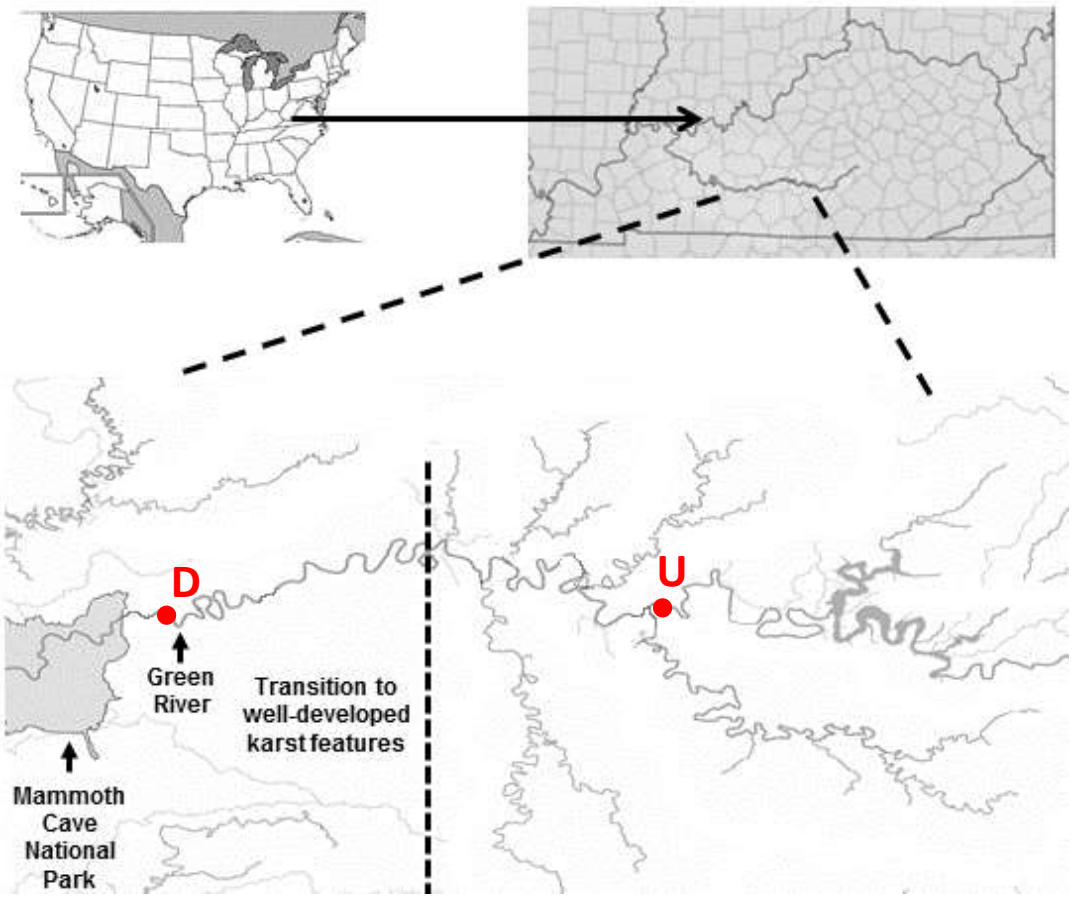

Figure 1. Map depicting the location of the Green River in Kentucky, including the location of the upstream (U) and downstream (D) study reaches marked by the solid circles.

Figure 2. Cartoon and photograph of an experimental mussel silo and photograph of the insert.

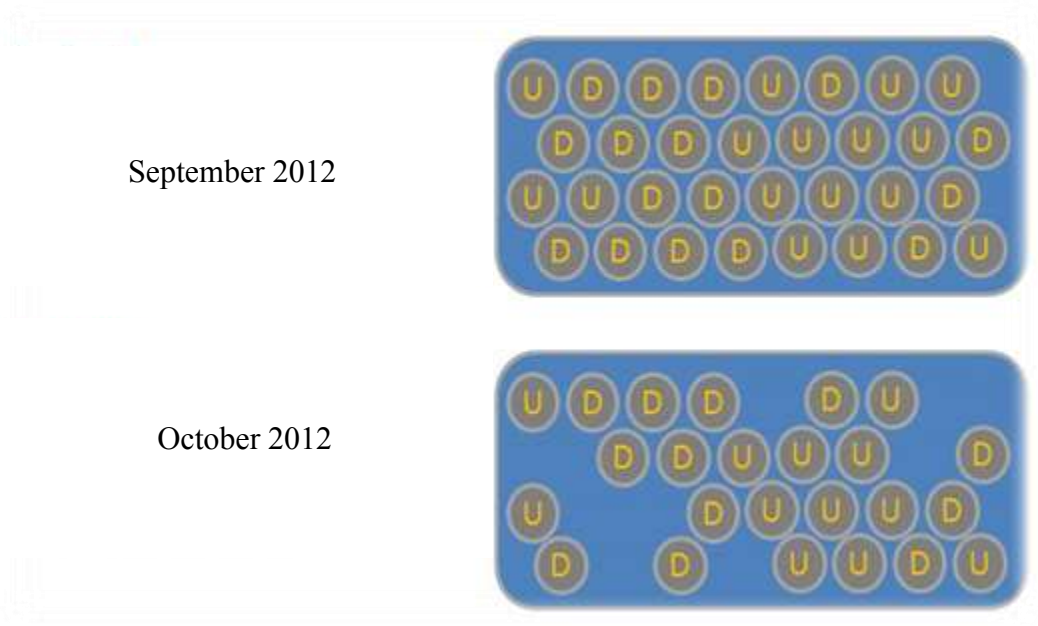

Figure 3. Grid network of experimental silos deployed in the downstream reach for the initial setup and first sampling period in autumn 2012. The design was identical in 2013 except silo designation was in a different random array. U = upstream *C. fluminea* , D = downstream *C. fluminea*. Each silo holds four individuals.

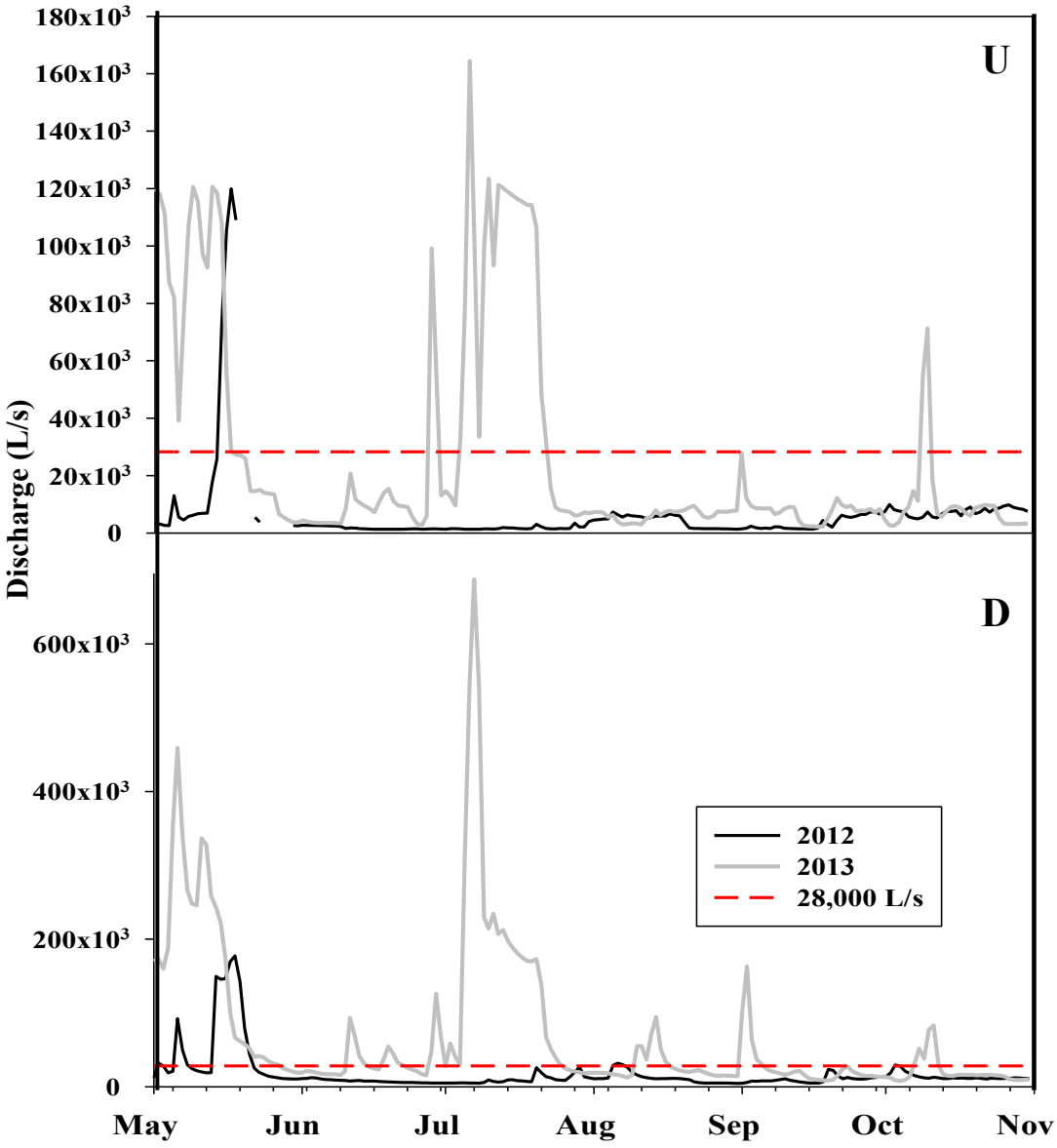

Figure 4. Discharge rates at two reaches in the upper Green River. The red dashed line denotes maximum wadable discharge rate. Data from USGS station Louisville, KY. Greensburg discharge rate was calculated from gauge height using formula from Osterhoudt (2014). D = downstream reach, U = upstream reach. The downward arrows refer to setup (dashed line) and sampling (solid line) dates.

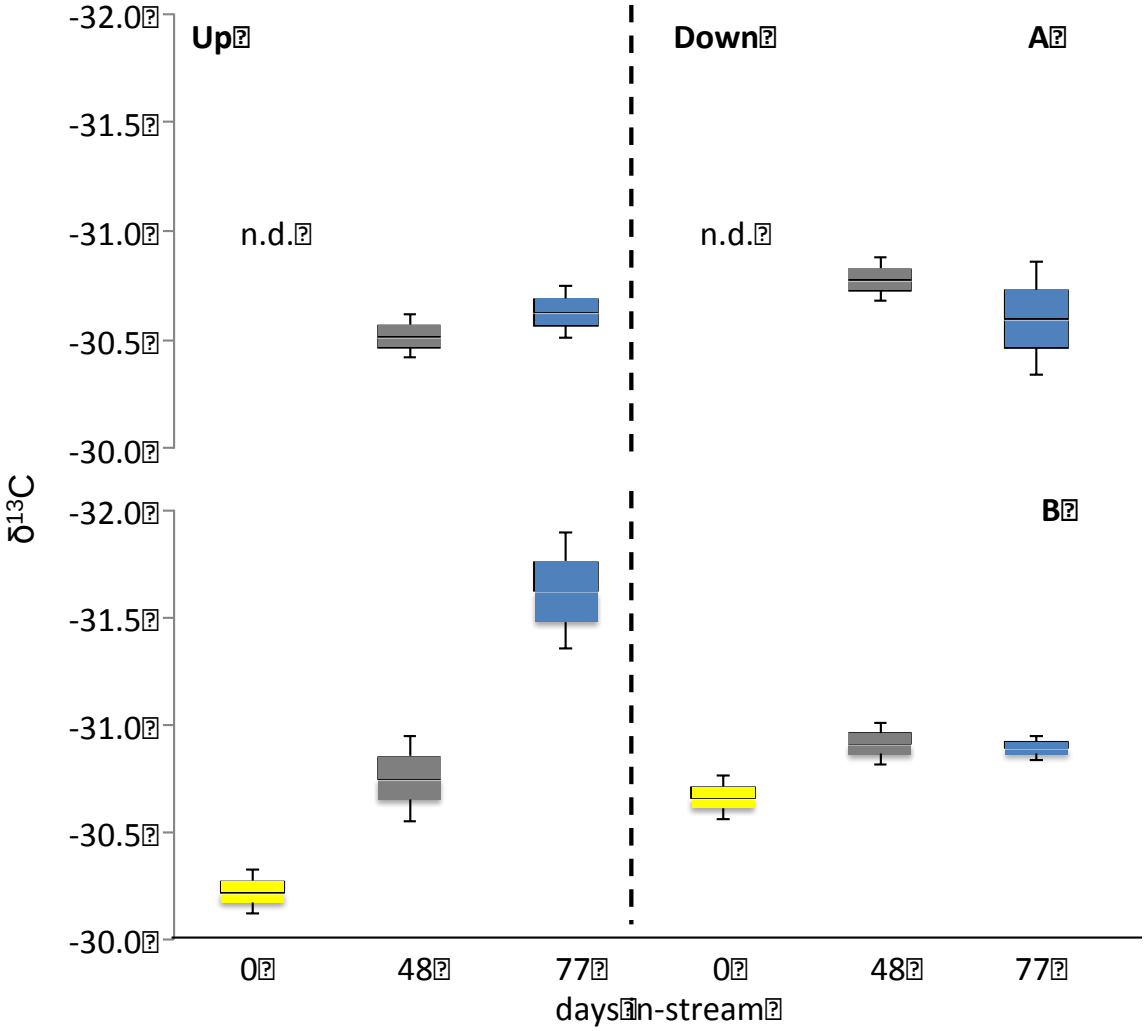

Figure 5. 2012 $\delta^{13}C$ data of *C. fluminea* body tissue between *in-situ* and siloed individuals in the upstream and downstream study reaches. A=silo, B=*in-situ,* n.d.=no data

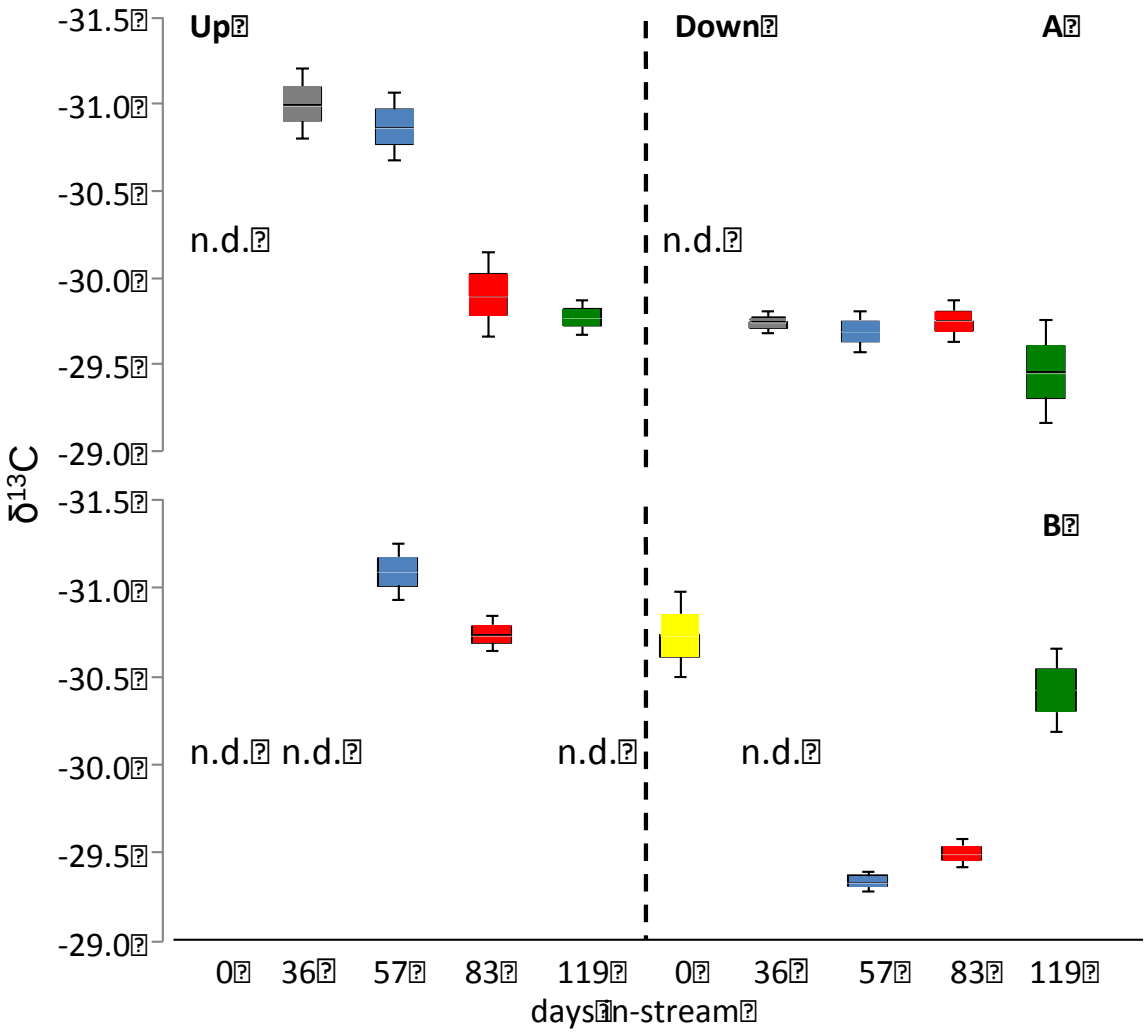

Figure 6. 2013 $\delta^{13}C$ data of *C. flumineus* body tissue between *in-situ* and silo individuals in the upstream and downstream study reaches. A=silo, B=*in-situ*, n.d.=no data

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