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The Ecological Roles Of Podostemum ceratophyllum and Cladophora in the Habitat and Dietary Preferences of the Riverine Caddisfly Hydropsyche Simulans

Brenna Tinsley
Western Kentucky University, brenna.tinsley702@topper.wku.edu

THE ECOLOGICAL ROLES OF *Podostemum ceratophyllum* AND *Cladophora* IN THE HABITAT AND DIETARY PREFERENCES OF THE RIVERINE CADDISFLY *Hydropsyche simulans*

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

Brenna E. Tinsley

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Western Kentucky University

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CE/T Committee:

Professor Scott Grubbs, Advisor

Professor Albert Meier

Professor Ingrid Cartwright

Approved By

_____

Advisor

Department of Biology

ABSTRACT

The net-spinning caddisfly *Hydropsyche simulans* can be a common inhabitant of shallow reaches in riverine systems, and is easily the most common hydropsychid in the upper Green River, Kentucky. This study was performed in summer 2011 and focused on two main questions: 1. Do the larvae of the riverine caddisflies *H. simulans* and *Cheumatopsyche* preferentially inhabit dense patches of *P. ceratophyllum* compared to bare substrates in the upper Green River?, and 2. Do larvae of *H. simulans* and *Cheumatopsyche* consume the filamentous alga *Cladophora* during the annual late summer algal bloom in the upper Green River? Densities of both hydropsychid taxa were significantly higher in very high (> 75% areal coverage) *P. ceratophyllum* habitat. A multi-source mixing model (IsoSource) using both $\delta^{13}C$ and $\delta^{15}N$ stable isotope data revealed that *Cladophora* was a prominent assimilated dietary item during August and September, indicating that both taxa can preferentially graze the filamentous alga during seasonal blooms. There appears to be a clear habitat preference for *P. ceratophyllum* for net-spinning caddisfly larvae, as well as the implication of behavior to switch from grazing off the nets to grazing directly on *Cladophora* sp. when the resource is abundant during late summer and into early autumn.

List of Keywords: *Cladophora*, Green River, *Hydropsyche simulans*, caddisfly, *Podostemum ceratophyllum*, filamentous algae, *Cheumatopsyche*

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VITA

April 3, 1990	Born – Glasgow, Kentucky
2008	Glasgow High School, Glasgow, Kentucky
2010	Research Experience: Lab of Dr. Scott Grubbs, Lab/Research Assistant; Lab of Dr. Albert Meier, Lab/Research Assistant
2010	Presented at Kentucky Academy of Science (KAS) Conference: Influence of Filamentous Algae on Nutrient and Sestonic Algal Levels in the Green River, Kentucky (3rd place in section), and Abiotic Influences on the Calling Activity of Four Anuran Species (1st place in section)
2011	Presented at Kentucky Academy of Science (KAS) Conference: The role of *Podostemum ceratophyllum* in providing habitat for net-spinning caddisflies (2nd place in section)
2012	Presented at WKU Student Research Conference: The ecological roles of *Podostemum ceratophyllum* and *Cladophora* in the habitat and dietary preferences of the riverine caddisfly *Hydropsyche simulans* (1st place in session)

FIELDS OF STUDY

Major Field: Biology - Ecology and Behavioral Studies
Minor Field: Sustainability

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CHAPTER 1

INTRODUCTION

A mosaic of heterogeneous patches, structurally ranging from lotic to lentic, characterizes river and floodplain systems (Delong and Thorp, 2006; Delong, 2010). Aquatic communities are influenced by biotic and abiotic components of the system in which they reside. In riverine systems, geomorphic, soil composition, lithologic, shading, and land-use characteristics are important abiotic factors (Penick, 2010). Many of these abiotic factors contribute to which nutrients are available and which are limiting. Primary productivity and algal biomass accrual are influenced by nutrient availability (Lohman et al., 1991; Dodds et al., 2002). Primary producers in lotic systems are either suspended in the water column (i.e., sestonic) or attached to substrates (i.e., benthic) (Penick, 2010).

Different primary producers may be limited by different nutrients (Hecky and Kilham, 1988; Borchardt, 1996). Limiting nutrients are those which are unavailable at the levels required by cellular growth (Dodds et. al. 2002). Phosphorus and nitrogen are typically found to be the main limiting macronutrients in aquatic communities, including stream systems (Lohman et al., 1991). Even the terrestrial plants that live in the floodplain may contribute to the riverine community's structuring by providing shade,

inhibiting some growth while encouraging others, and by contributing organic matter which can shift the nutrient composition to favor a change in dominant primary producers (Tank and Dodds, 2003; Veldbloom and Haro, 2011).

Aquatic macrophytes in riverine systems can serve as habitat for the biota as well as stable substrate for the growth of macroalga, such as *Cladophora* sp. (Delong, 2010). *Podostemum ceratophyllum* Michx. is an aquatic macrophyte that can be highly productive in shallow, swift-flowing, riffle areas on bedrock substrate in the eastern United States (Hill & Webster, 1984; Hutchens et al., 2004) and is distributed in tropical to temperate rivers in the Nearctic region (Hill & Webster, 1984). The Podostemaceae is a family of herbaceous annual and perennial aquatic macrophytes that tend to resemble aquatic bryophytes or freshwater algae in physical appearance (Philbrick, 1984). Highly productive *P. ceratophyllum* is indicative of high quality, well-oxygenated rivers in the southeastern U.S. (Hill & Webster, 1984) and provides stable habitat for macroinvertebrate communities (Hutchens et al., 2004). This small aquatic plant commonly acts to increase the stability and anchoring of benthic substrates due to the spreading configuration of the root-like holdfast structures (Hill & Webster, 1984).

During summer, *P. ceratophyllum* can form dense mats over stable substrate and can grow stems exceeding 15 cm in length (Hutchens et al., 2004). These characteristics allow it to provide a habitat where macroinvertebrate species can hide and hunt, much like a dense terrestrial jungle, by increasing the surface area on which they can live (Hutchens et al., 2004). Without the cover this macrophyte provides, macroinvertebrate diversity abundance and biomass are markedly lower (Hutchens et al., 2004).

Podostemum ceratophyllum also provides stable substrate for attachment by filamentous macroalgae, specifically *Cladophora* sp. in the Kentucky's upper Green River, which may act as a food resource for macroinvertebrates during the species' periods of rapid summer growth.

Cladophora is a genus of filamentous algae that is common to riverine systems with high nutrient inputs. Members of this genus can be found in temperate and tropical waters, in lentic and lotic systems, and inhabits a broad variety of marine, brackish, or freshwater environments (Dodds & Gudder, 1992). Species of this genus are typically made up of green single cells that can form long branching chains, or filaments, that look like green thread or hair to the naked eye (AquaScaping World, 2012). The filaments have a tendency to grow on nearly any submerged, stable surface and, as they grow, collect debris washed over them by the current (AquaScaping World, 2012). *Cladophora* sp. can cover large areas on the bottom surfaces of shallow riverine habitats and potentially outcompete other riverine primary producers, including sestonic (i.e., suspended in water column) and periphytic (i.e., microscopic forms attached to rocks) algae, for light and nutrients.

Cladophora sp. can dominate primary production in nutrient-enriched systems (Dodds & Gudder, 1992). This often occurs during rapid growth periods in riverine systems that experience low-flow conditions during the summer and early autumn (Power et al., 2009; Penick, 2010). The upper Green River experiences such events and the blooms that occur during this time grow with such rapidity that a visible difference in cover can be seen within a short time period (i.e., a few weeks), even over a large area.

This macroalga was also recently demonstrated to play host to epiphytic diatoms containing nitrogen-fixing cyanobacteria, which may allow the algae to survive in low-nitrogen environments (Dodds & Gudder, 1992; Bratt, 2011).

In freshwater streams, it is typical that the growth of larval aquatic insects, such as the hydropsyche, is strongly influenced by food quality and availability, temperature, and population density (Veldbloom and Haro, 2011). Net-spinning caddisflies of the family Hydropsychidae were the consumers focused upon during this study, namely *Hydropsyche simulans* Ross and the *Cheumatopsyche* sp. Members of this family often make up an integral portion of filter-feeding fauna in riverine systems they inhabit (Georgian and Wallace, 1981). Hydropsychid larvae characteristically spin a net, built not unlike a square grid, constructed perpendicular to the current (Georgian and Wallace, 1981). Hydropsychid larvae have been shown to be more prevalent in microhabitats containing large, stable substrates, typically rocky substrata and aquatic macrophytes, with high flow velocity (Georgian and Thorp, 1992). Multiple species of hydropsychids often coexist in these lotic ecosystems (Benke and Wallace, 1980).

The larval stages of hydropsychids construct a fixed retreat below the surface of the water that includes a net that retains food materials in transport in the river current. The larval stage of *H. simulans* grazes opportunistically, namely on algae, detritus, and microarthropods (Rhame and Stewart, 1976; Wiggins, 1998). This species has also been shown, however, to graze directly on *Cladophora* sp. during periods of high filamentous growth (Rhame and Stewart, 1976). One of the primary purposes of this project is to investigate the influence of *Cladophora* sp. on the algal-feeding habits of the riverine

caddisfly *H. simulans*. The study species is common in the shallow area of large streams, such as riffles, and is widespread throughout eastern North America.

There are several methods of determining the constituents of the diets of organisms, such as direct observation of feeding, analyses of gut contents, and analyses of stable isotopes and/or nutrients within the organism's tissues and that of potential foods (Raikow and Hamilton, 2001). Based on the delicate nature and size of the study organism in this project, analyses of stoichiometry and stable isotopes were the best methods to determine the makeup of the diet of *H. simulans*. Composition stoichometry can be used to determine elemental makeup of materials and the amount (mass) of each elemental component, such as the carbon to nitrogen (C:N) ratio in a substance. C:N ratios indicate which food sources are most useful, as typically the higher the ratio is, the less suitable the food source is to potential consumers.

The use of stable isotopes as an inexpensive yet accurate method to study aquatic food webs is becoming increasingly common (Raikow and Hamilton 2001, Atkinson et al. 2010). Stable isotopes are variants of a chemical element that either do not degrade or have half-lives too long to be measured, and have the same number of protons but a differing numbers of neutrons. The stable isotope portion of this study is classified as a natural isotope abundance study, which have been used increasingly as a means of determining the relative frequency of consumption of various food sources (Raikow and Hamilton, 2001). The distinctive carbon and nitrogen isotope signatures, ^{13}C and ^{15}N, can illustrate the larger process of nutrient cycling through a system and indicate what food sources have been major contributors in an animal's diet (Phillips and Gregg, 2003).

These distinctive isotopes can be compared with the more common isotopes of these elements, ^{12}C and ^{14}N, providing a unique ratio that can be used to identify the source(s). The ratio of "heavy" (^{13}C) to "abundant" carbon (^{12}C) provides insight into which food resources derived mainly from primary producers (floodplain tree species, *Cladophora*, periphytic algae, sestonic algae, and organic matter in transport within the water column) become assimilated into the body tissues by primary consumers and detritivores (Peterson and Fry 1987). The ratio of "heavy" (^{15}N) to "abundant" (^{14}N) nitrogen provides a method of assessing trophic position (e.g., primary or secondary consumer) within a food web (Cabana and Rasmussen 1994).

Several researchers (Delong and Thorp, 2006) have shown that living (e.g., algae, animal) and nonliving (e.g., detritus) components of a riverine food web produce distinct isotopic carbon and nitrogen signatures, making it possible to understand the relationship between resource availability to a consumer and what is actually consumed and assimilated into body tissue. Isotopic ratios of benthic invertebrates can vary between not only families, but species, due to the fact that one food resource that is considered high-quality by one species may be passed over by another (Veldbloom and Haro, 2011). Stable isotope analyses of carbon and nitrogen were performed during the peak of an algal bloom and after the first scour event of the season to assess the linkages between the diet of *H. simulans* and the *Cheumatopsyche* sp. and several potential food resource items, particularly *Cladophora* sp.

Two main questions were addressed in this study:

1. Do the larvae of the riverine caddisfly *H. simulans* and *Cheumatopsyche* sp. preferentially inhabitat dense patches of *P. ceratophyllum* compared to bare substrates in the Green River?, and
2. Do the larvae of *H. simulans* and *Cheumatopsyche* sp. consume *Cladophora* sp. during the annual late summer algal bloom in the Green River?

My first hypothesis for this study is that the larvae of the riverine caddisflies *H. simulans* and the *Cheumatopsyche* sp. are found in a habitat of *P. ceratophyllum* significantly more often than a habitat of bare substrate in the upper Green River, based on the growth habit of the plant and the observation of many caddisfly nets on them during the summer months. I anticipated that *H. simulans* and the *Cheumatopsyche* sp. larvae would actively choose to build their fixed retreats on and in the tangled mats of *P. ceratophyllum* readily available on the river's bed. I expected them to choose this over loose substrate or bare rock, which are also abundant during the study period, where there wouldn't be as much surface area to work with when spinning a net.

My second hypothesis is that the seasonal availability of the filamentous species of the algae *Cladophora* would have a significant effect on the dietary habits of the larvae of *H. simulans* and the *Cheumatopsyche* sp. in the upper Green River. I anticipated that increased availability of *Cladophora* sp. during summer and early fall will result in reduced levels of sestonic algae, which will in turn be reflected in the diet of the caddisfly. I predicted that when both *Cladophora* sp. and sestonic algal levels were low, such as after a scour event, stable isotope analyses would show that the main dietary

component of the caddisfly was detritus. Oppositely, during a *Cladophora* sp. bloom, the levels of sestonic algae in the caddisfly diet will decrease and *Cladophora* sp. will be consumed and comprise the majority of the diet.

CHAPTER 2

METHODS

Study Reach Description

The field research took place during June–October 2011 in a 7th-order reach of the upper Green River (37.2431, -86.0027) located in central Kentucky, U.S.A. at the Western Kentucky University Upper Green River Biological Preserve (WKU-UGRBP). The Green River originates in Lincoln County, Kentucky, and flows ca. 600 km west before emptying into the Ohio River. The Green River Basin is the largest of Kentucky's twelve primary river basins, draining nearly 24,000 km^2 of the Interior Plateau (71) and Interior Valley and Hills (72) Level III Ecoregions, nearly 23% of the commonwealth (Woods et al., 2002).

The study reach is located at the downstream edge of the WKU-UGRBP (Figure 1.). A woody riparian corridor lines the entire portion of the WKU-UGRBP, comprised mainly by American sycamore (*Platanus occidentalis* L.), silver maple (*Acer saccharinum* L.), and box elder (*A. negundo* L.). The study reach is ca. 50 m wide, characterized by an open canopy and shallow run habitats underlain by small cobbles and gravel substrates, and is positioned within the Crawford-Mammoth Cave Upland Level IV Ecoregion. This ecoregion is underlain by Mississippian-age limestone and Chesterian-age fractured bedrock formations with low surface stream density and nitrogen-rich groundwater (Woods et al., 2002). Karst systems, especially those that are

limestone, are unique in that nutrient inputs can come both from surface runoff during precipitation events and directly from the dissolution of the parent rock (Holloway et al., 1998; Morford et al., 2011). Previous research conducted at the study reach revealed that during base-flow conditions in summer and autumn that nitrogen levels are high, particularly for nitrate (mean: 1.1–1.4 mg/L) but less so for ammonia (mean: 0.02–0.09 mg/L) (Penick, 2010). Base-flow phosphorous levels are also moderately high (soluble reactive phosphorous, mean: 0.08–0.10 mg/L; total phosphorous, mean: 0.10-0.21 mg/L).

There are several aquatic macroproducers present at the study reach, including a productive and dense bed of the vascular plant *Podostemum ceratophyllum* (Michx). During late summer, a dense bloom of *Cladophora* sp. rapidly proliferates and reaches maximum standing stocks in stable flow conditions prior to high-flow scouring events in autumn. *Fontinalis* sp., *Potamogeton* sp. and *Spirogyra* sp. are also present, but markedly less abundant. Dense beds of *Justicia* sp. are present along exposed gravel bars immediately above the base flow channel.

Over 30 species of fish have been collected from shallow (< 1 m) habitats along the study reach (Wilsey, 2008; Grubbs, unpublished data). Several species are particularly abundant, namely *Campostoma oligolepis* (Hubbs & Green) (largescale stoneroller), *Cottus carolinae* (Gill) (banded sculpin), *Etheostoma blenniodes* (Rafinesque) (greenside darter), *E. zonale* (Cope) (banded darter), *Hypentelium nigricans* (Lesueur) (northern hog sucker), *Lepomis macrochirus* (Rafinesque) (bluegill), *Micropterus punctulatus* (Rafinesque) (spotted bass), *Moxostoma* spp. (redhorses), *Notropis atherinoides* (Rafinesque) (emerald shiner), *N. telescopus* (Cope) (telescope shiner), *Noturus elegans* (Taylor) (elegant madtom), and *Pimephales notatus* (Rafinesque) (bluntnose minnow).

There are several common macroinvertebrate taxa that have been obtained from coarse substrate habitats along the study reach. Abundant molluscan taxa include a gastropod snail *Leptotoxis praerosa* (Say), the introduced Asiatic clam *Corbicula fluminea* (Müller), and a productive and diverse assemblage of unionid mussels (Cicerello, 1999). Several unionids are abundant, especially *Actinonaias ligamentina* (Lamarck) (mucket), *Amblema plicata* (Say) (threeridge), *Cyclonaias tuberculata* (Rafinesque) (purple wartyback), *Elliptio dilatata* (Rafinesque) (spike), *Megalonaias nervosa* (Rafinesque) (washboard), *Obliquaria reflexa* (Rafinesque) (threehorn wartyback), *Quadrula quadrula* (Rafinesque) (mapleleaf), and *Tritogonia verrucosa* (Rafinesque) (pistolgrip) (Cicerello, 1999).

In addition to the study species *Hydropsyche simulans* (Ross) and *Cheumatopsyche* spp., abundant aquatic insect taxa include ephemeropterans *Baetis* sp., *Caenis* sp., *Maccaffertium mediopunctatum* (McDunnough), *Serratella deficiens* (Morgan), and *Tricorythodes* sp., the giant stonefly *Pteronarcys dorsata* (Say), aquatic beetles *Dineutus* sp., *Stenelmis crenata* group, and *Psephenus herricki* (Dekay), the megalopteran *Corydalus cornutus* (L.), caddisflies *Cheumatopsyche* sp., *Hydropsyche simulans* (Ross), and *Oecetis* sp., and the dipteran blackfly *Simulium* sp. (Grubbs, unpublished data). Chironomid dipteran larvae are very abundant but have yet to be identified below the family level.

Field and Laboratory Methods

Environmental variables

Nutrient content, dissolved oxygen levels and pH were quantified monthly. Samples for nutrient analyses (n = 4) were obtained at midstream in acid-washed 275-mL bottles

during low water conditions at flow ca. < 11,000 L/s (< ca. 400 cfs). Total phosphorus (acid persulfate digestion), soluble reactive phosphorus (ascorbic acid method), nitrate (cadmium reduction method), ammonia (salicylate method), and total nitrogen (persulfate digestion method) levels were determined spectrophotometrically. Dissolved oxygen and pH readings were taken during mid-day with a Hach HQ40d digital meter. Discharge data were obtained from a USGS streamflow monitoring station at Munfordville (Station number 03308500; 37.2667, -85.8872), located ca. 11.5 km NW upstream of the study reach. Due to the paucity of surface tributaries between Munfordville and the study reach it was assumed that flow conditions would be representative of the USGS station. Temperature data were obtained with a HOBO Water Temp Pro v2 data logger configured at 1-hr intervals.

Algal biomass

Biomass levels of sestonic and filamentous algae were measured monthly during low-flow conditions. Sestonic algal samples (n = 4) were obtained at midstream in acid-washed 275-mL bottles coincident with the collection of nutrient samples. The bottles were then immediately placed inside a covered cooler until the samples were able to be filtered in order to prevent degradation of chlorophyll. Sestonic samples were vacuum-filtered on 47-mm diameter, 0.7-μm pore size Whatman GF glass fiber filters, placed in individual Petri dishes, wrapped in foil, and kept refrigerated in the dark for up to 14 days. The algal samples were analyzed for chlorophyll-α concentrations using USGS methods (Yin, 2005). Each filtered sample was placed in a 50 mL centrifuge tube with 3–6 glass beads and 10 mL of a 50:50 dimethyl sulfoxide (DMSO) and acetone solution. The tube was vortexed for 30 seconds, stored overnight in the dark at 4°C, centrifuged 10

min at 4300 rpm, and the supernatant transferred to a new tube. A second 10 mL of DMSO:acetone was added to the original sample and the process was repeated. The two supernatant liquids were combined. A final five mL of DMSO:acetone was added to the original sample and the process repeated. The resulting supernatant was centrifuged and 5 mL of the liquid was analyzed with a Shimadzu RF-5301 PC spectrofluorometer.

Samples were measured against set chlorophyll standards produced from an initial 239 ppb solution and 20% serial dilutions of 47.60 ppb, 9.52 ppb, 1.90 ppb, and 0.38 ppb. A linear regression of intensity vs. concentration of the standards established a standard curve and was used to calculate chlorophyll-α concentrations (mg/L) for the sestonic samples.

Percent areal coverage of *Cladophora* sp. and *P. ceratophyllum* were calculated in July and August along longitudinally positioned cross-stream transects (n = 10). Standing stocks of *Cladophora* sp. and *P. ceratophyllum* were quantified in August, September, and October. Different transect sets were selected each month (n = 5) to avoid resampling. Along a transect, 1-m intervals were established and four points were randomly selected. At each point a Hess sampler (0.09 m^2 sampling area) was placed at the center of each point and all *Cladophora* sp. and *P. ceratophyllum* was removed by hand, placed into individually-labeled plastic bags, and transported to the laboratory in a cooler. Samples were placed into a drying oven for 48 h at 80^{o}C, weighed to the nearest 0.01g to quantify dry mass (DM), crushed with a mortar and pestle, transferred to pre-weight porcelain crucibles, and placed in a muffle furnace for 4 h at 550^{o}C. The remaining ashed, inorganic materials were weighed to the nearest 0.01g. Ash-free dry

mass (AFDM; mg/m^2) for each sample was determined by subtracting the mass of the ashed materials from DM.

Macroinvertebrate sampling

To quantify benthic densities (no./m^2) of the two hydropsychid taxa, sampling was conducted by randomly choosing areas of substrate in the study reach in which there was either very high or very low *P. ceratophyllum* growth present. Those areas considered as very low growth consisted < 5% areal coverage, while those considered very high contained >75% coverage. Five samples were taken from each habitat type in August (= mid-summer) and September of 2011(= late summer) using a Hess sampler (0.09 m^2 sampling area). If the sampling area contained any cobble-sized substrates with filamentous algal or *P. ceratophyllum* growth present, it was either cleaned of the growth or, if it was small enough to completely fit within the sampling area, it was taken as part of the sample. Everything contained in the catch-net was then washed into a bucket where any large stones were either removed, or cleaned, and then removed. The remaining portion of the sample was rinsed through a 500-µm sieve, placed in a Nalgene container, and preserved in 95% ETOH. In the laboratory, samples were re-rinsed through a 500-um sieve and then full-sorted under a dissecting microscope to partition out all *H. simulans* and *Cheumatopsyche* sp. larvae.

Food resources

Potential dietary components for *H. simulans* and the *Cheumatopsyche* sp. included *Cladophora* sp., *P. ceratophyllum*, floodplain tree leaves, epiphytic biofilm, sestonic algae, and transported organic matter (TOM). Samples for all resources were obtained in

mid and late summer. Fresh specimens of *H. simulans* were obtained at the same time in both months while the *Cheumatopsyche* sp. was collected only in September.

Cladophora sp. samples were obtained by cutting filaments near the tip, placed in individual plastic bags, and held on ice. *Cladophora* sp. samples were inspected under a dissecting microscope (7–10X) and any visible detrital material was removed. Samples of *P. ceratophyllum* were obtained by cutting plant tissue above the roots, placed in individual plastic bags, and held on ice. The *P. ceratophyllum* samples were inspected under a dissecting microscope (7–10X) to remove detritus and *Cladophora* sp. Composite samples of American sycamore, box elder, and silver maple leaves were collected adjacent to the stream reach, placed in individual plastic bags, and held on ice. Rocks with epiphytic biofilm development were scrubbed with a tooth brush into a bucket containing river water, transferred to an acid-washed 275-mL bottle and kept in a refrigerator. Surface samples of TOM were obtained at two near-shore and two mid-channel points. Individual samples (20 L each) for each transect were pooled into one 80-L composite sample to provide a cross-channel representation for the water column.

The processed samples of filamentous algae, *P. ceratophyllum*, and leaf litter were dried at 80^{o}C for 24 h. Dried samples were pulverized to a fine powder using a Wig-L-Bug. The epilithic biofilm samples were initially vacuum-filtered through a 1-µm Gelman glass fiber filter. The composite TOM samples were first passed through two sieves to partition into coarse (CTOM; > 1mm diameter) and fine (FTOM; > 100-1,000 µm diameter) particle size classes. Subsequently, there was insufficient CTOM material to process further and this fraction was discarded. Water that passed through the 100-µm sieve was retained and vacuum-filtered through a 1-µm Gelman glass fiber filter to obtain

an ultrafine (UTOM; 1-100μm diameter) fraction. The resulting filtrate was processed to obtain a colloidal dissolved organic matter (cDOM) sample was according to Delong and Thorp (2006). The cDOM sample was adjusted to a pH of 4.2–4.3, oxygenated with aquarium bubblers, and evaporated at 60-65^{o}C to a particulate residue. The latter was pulverized with the Wig-L-Bug.

Epilithic biofilm, FTOM, and UTOM samples were processed using a colloidal silica technique to separate algal and detrital components (Hamilton et al., 1992; Delong & Thorp, 2006). Glass fiber filters were placed in 50-mL conical centrifuge tube with a 76% solution of Ludox TM-50 (50 wt. % suspension) colloidal silica and centrifuged at 1200 rpm for 15 min. The living algal fraction remaining in the top aqueous layer was separated from the detrital fraction present at the bottom of the centrifuge tube and the separation process was repeated. The separate fractions were vacuum-filtered through separate 1-μm Gelman glass fiber filters and washed with distilled water to remove excess silica. Material was carefully peeled - off of the filters and dried at60^{o}C for 48 h.

Total C content, total N content, and C and N stable isotopic ratios were quantified with a Thermo Electron LTQ-Orbitrap Hybrid MS (http://cmsf.ucdavis.edu/instrumentation.html) mass spectrometer in the University of California Davis Campus Mass Spectrometry Facilities (http://cmsf.ucdavis.edu/). Isotopic ratios were expressed as:

$$\delta^{13}C \text{ or } \delta^{15}N \text{ (per mil)} = ([Rsample/Rstandard]-1) * 1000$$

where R is the ^{13}C:^{12}C (=$\delta^{13}C$) or ^{15}N:^{14}N (=$\delta^{15}N$) ratio. A bovine protein (peptone) laboratory standard was referenced against an international standard. Stable N and C

isotopic analyses were performed on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at UC Davis Stable Isotope Facility, University of Davis, California, USA. Stable isotope ratios were calculated as dX(%) = (RSample/RStandard - 1) * 1000 where X is either 13C with the corresponding ratio, R, 13C/12Cor 15Nwith corresponding R, 15N/14N. Pee Dee Belemnite and atmospheric nitrogen (AIR) were used as standards for carbon and nitrogen analysis, respectively (Boll et al., 2012).

Statistical Methods

A two-way analysis of variance (ANOVA) was used to assess the importance of the *P. ceratophyllum* treatment (very low vs. very high) and *Cladophora* sp. levels (before [July] and after a partial scour [September]) on hydropsychid densities. The ANOVAs were performed separately for *H. simulans* and the *Cheumatopsyche* sp. SPSS 19.0 (IBM Corporation) was employed for the ANOVA's.

Stoichiometry was used to quantify and compare molar C:N ratios in the body tissue of consumer larvae (without their gut) and the basal food resources as a means of assessing which resource was most likely being consumed and assimilated. A multisource mixing model, IsoSource, was employed to model the contribution of different food resources (Phillips and Gregg, 2003) to the diets of *H. simulans* and the *Cheumatopsyche* sp. IsoSource creates all possible iterative combinations of resource proportions (each combination = 100%) at set increments (1%, tolerance at 0.05) to established a predicted source mixture. The predicted multisource signature was then compared to the observed $\delta^{13}C$ and $\delta^{15}N$ values of the consumer. All basal food resources (*Cladophora* sp., *P. ceratophyllum*, tree leaves, biofilm-algal, biofilm-detrital, TOM-algal, and TOM-detrital)

were used for assessing potential mixing contributions to the diet of both consumers. To correct for anticipated fractionation that results from trophic transfer, $\delta^{13}C$ and $\delta^{15}N$ were adjusted by +0.4‰ and +3.4‰, respectively (Post, 2002; Delong and Thorp, 2006). Because IsoSource works only on one temporal dataset at a time, mixing models were employed separately on *H. simulans* in August and September. IsoSource was applied to the *Cheumatopsyche* sp. only in September. Fresh material of the latter taxon was not obtained in July.

CHAPTER 3

RESULTS

Environmental variables

Mean pH in the study ready was 7.92 and varied little across the study period (range: 7.72 – 8.10). Dissolved oxygen levels rarely exceeded 9.0 mg/L and exhibited an overall mean of 8.3 mg/L. Temperature data was not obtained in June. In-stream temperatures were warmest during July, followed closely by August (Table 1). Temperatures decreased markedly during September and October. River discharge was variable throughout the study period, but with the exception of the first half of June the study reach was generally accessible for in-stream sampling via wading. June had easily the highest mean discharge levels (61517.9 L/s; Table 1). There were also short-term summer peak-flow events in July, leading to this month producing the second highest mean discharge level (18009.5 L/s; Table 1), and again in early September (ca. 48400 L/s) prior to the second benthic sampling event. Discharge levels were relatively stable in August and October.

Nitrogen and phosphorous levels in the upper Green River at the study reach were high, particularly for the nitrate and soluble reactive phosphorous components (Table 2). The high values for these two nutrients were likewise reflected in high total nitrogen and total phosphorous levels. Ammonia levels are comparatively lower and remained relatively constant during summer and autumn. The TN:TP ratios were highest in early

summer, decreased nearly two-fold by late summer, and remained stable through October.

Primary producer standing stocks and percent cover

Mean sestonic algae levels increased from July to August, but peaking only at 10.5 µg/L chlorophyll-α (Table 3) and then decreased nearly 5-fold by September with only a marginal increase in October. *Cladophora* sp. coverage of the stream bed increased quickly during summer, from 15.6% to nearly 75% areal coverage in August (Table 3). *Cladophora* sp. standing stocks were similarly highest in August. The scouring event in early September resulted in moderately-decreased standing stocks. Both standing stocks and percent areal coverage of the streambed by *P. ceratophyllum* remained stable across the study period, increasing only from 48% (August) to 57% (October; Table 3).

Hydropsychidae community

Hydropsyche simulans and *Cheumatopsyche* sp. larvae were the only hydropsychid taxa obtained from the study reach. Because *Cheumatopsyche* larvae are difficult to identify to species (Wiggins 1998, Burrington 2011), multiple taxa may have been present. Densities of both hydropsychid taxa were greatest in the very high *P. ceratophyllum* areal coverage habitat, yet varied with regard to month (Table 4). The high *H. simulans* density coincided with rapidly-increasing *Cladophora* sp. standing stocks. Density of the *Cheumatopsyche* sp. larvae remained high during *Cladophora* sp. proliferation and following the modest scour event in early September (Table 3). Density of *H. simulans* was significantly higher in the very high *P. ceratophyllum* habitat ($F = 38.3$, $p < 0.001$), with a ca. 24-fold higher density in July and also nine-fold greater in September.

Hydropsyche simulans density was also higher in July ($F = 10.6$, $p = 0.005$), but the interaction effect of treatment and month was likewise significant ($F = 10.3$, $p = 0.006$). Similar to *H. simulans*, the density of the *Cheumatopsyche* sp. was significantly higher in the very high *P. ceratophyllum* habitat ($F = 22.2$, $p < 0.001$), but neither month ($F < 0.1$, $p = 0.767$) nor the treatment-month interaction term ($F = 0.1$, $p = 0.733$) were significant.

Food resources

Mean stoichiometric C:N ratios and stable isotopic values varied considerably across the basal food resources (Tables 5–6). Mid-summer C:N ratios ranged from 5.3 (detrital component of epilithic biofilm) to 21.2 (algal UFTOM) and 24.2 (detrital FTOM), while late summer C:N ratios ranged from 4.0 (cDOM) to 23.4 (floodplain tree leaves) (Table 5). The mid-summer $\delta^{13}C$ values ranged from -34.2 (*Cladophora* sp., most ^{13}C-depleted) to -28.8‰ (detrital UFTOM, most ^{13}C-enriched), while the late summer $\delta^{13}C$ values ranged from -39.7 (*P. ceratophyllum,* most ^{13}C-depleted) to -24.9‰ (FTOM algal, most ^{13}C-enriched) (Table 6). The mid-summer C:N ratio value for *H. simulans* (5.0) most closely aligned to detrital epilithon (5.3) and possibly *Cladophora* sp.(8.9), while in late summer the C:N ratio values for *H. simulans* (4.8) and the *Cheumatopsyche* sp. (5.5) were most similar to cDOM (4.0) and possibly *Cladophora* sp.(9.8). The mid-summer $\delta^{13}C$ value for *H. simulans* (-34.0) was nearly identical with *Cladophora* sp.(34.2) and somewhat close to detrital epilithon (-31.9), while in late summer the $\delta^{13}C$ value for *H. simulans* (-32.7) and the *Cheumatopsyche* sp. (-31.5) were most similar to leaves (-30.6) and algal epilithon (-29.9) (Table 6). The IsoSource model indicated that during mid-summer that *Cladophora* sp.(84–86%) was likely the main food source of *H. simulans*, with *P. ceratophyllum* (10–11%) estimated as a distant second. The maximum

contribution for any epilithic or TOM source was only 3% (Table 7). Similarly, in late summer the IsoSource model analysis indicated *Cladophora* sp. as the main food source of both *H. simulans* and the *Cheumatopsyche* sp. (66–72% and 56–60%, respectively), with tree leaf material a distant second for *H. simulans* (3–14% and 2–13%, respectively) (Table 7). Each of the remaining resource types was estimated to contribute only a small fraction to the late summer diet of *H. simulans*. In contrast, both TOM components were also likely important dietary items to the *Cheumatopsyche* sp. during late summer (Table 7).

CHAPTER 4

DISCUSSION

Hydropsychid larvae are found in a wide range of lotic environments (Wiggins 1998), typically in areas of higher current (Fuller and Mackay 1980) and associated with coarse substrates (e.g. cobbles or wood; Wallace 1975, Oswood 1979, Cudney and Wallace 1980). Fairchild and Holomuzki (2002) found that the distribution of the net-spinning caddisfly *Hydropsyche betteni* was positively correlated with the amount of habitable, rocky substrates. The first hypothesis for this study, that larvae of the two riverine hydropsychid caddisflies *H. simulans* and the *Cheumatopsyche* sp., are found preferentially in dense *P. ceratophyllum* habitat in the upper Green River was strongly supported.

Densities of both *H. simulans* and the *Cheumatopsyche* sp. were markedly and significantly higher in the very high *P. ceratophyllum* habitat compared to the very low *P. ceratophyllum* habitats during both July and September. There was a significant effect of time between the sampling periods for *H. simulans*, a difference that wasn't mirrored with the *Cheumatopsyche* sp. The difference for *H. simulans* could have been an effect of life cycle rather than a result of a small decrease in *Cladophora* sp. standing stocks following the small spate-scouring event. The nearly three-fold decrease of larval *H. simulans* density may have resulted from adult emergence prior to the September sampling event. Rhame and Stewart (1976) observed *H. simulan*s emergence during July

and August during their year-long study on the Brazos River in Texas. The peak density (2002 individuals/m^2) of *H. simulans* in their study occurred in mid-summer, followed by a sharp decrease one month later to 610 individuals /m^2, was very similar to the 1862 to 636 individuals/m^2 drop in density that occurred in this study.

The observed result from the *Cheumatopsyche* sp. could have been due to there being multiple species in the samples with possibly different, or very similar, species-specific life cycle timing. Several hydropsychid life cycle studies have reported two or more *Cheumatopsyche* species (e.g., Rhame and Stewart 1976, Sanchez and Hendricks 1997). Even if only one species was present, there may have been multiple generations present during the summer months. For example, Alexander and Smock (2005) reported considerable size variation and likely multiple generations of *C. analis* in a Virginia stream.

Podostemum ceratophyllum provides important habitat for riverine macroinvertebrates (Grubaugh et al. 1997, Hutchens et al. 2004) and possibly also for riverine fishes (Connelly et al. 1999, Neely et al. 2003, Argentina et al. 2010a). Although the mean monthly standing stocks reported in this study (48.5–56.7 AFDM g/m^2; Table 3) during summer and autumn were ca. 2–5 fold lower than the maximum values (101.3–244.8 AFDM g/m^2) reported by Hill and Webster (1980) in the New River, Virginia, dense patches of this macrophyte covered ca. 50% of the benthic substrata within the study reach (Table 3). This value would be higher if portions of the stream reach lacking coarse substrates were not included in this. Similarly, Argentina et al. (2010a) reported pre-manipulation percent cover values of 32.8 and 60.6% in their work in Conasauga

River, Georgia and Tennessee and Argentina et al. (2010b) documented a maximum percent cover of 55% from 20 shoals from the same river system.

Hutchens et. al. (2004) found that a complete removal of *P. ceratophyllum* in the Little Tennessee River greatly reduced total macroinvertebrate abundance and biomass and showed a strong positive relationship between the macrophyte surface area and total macroinvertebrate abundance and biomass. They estimated that *P. ceratophyllum* increased surface area by 3–4 fold compared to bare bedrock habitat, revealing that *P. ceratophyllum* provides important habitat and promotes riverine benthic macroinvertebrate productivity. Hutchens et. al. (2004) also suggested that the ability of *P. ceratophyllum* to facilitate high macroinvertebrate productivity is similar to the ecological roles played by bryophytes and the filamentous alga *Cladophora* sp., citing specifically the findings of Dodds and Gudder (1992) that *Cladophora* sp. mats have the ability to support a dense macroinvertebrate community.

Grubaugh et al. (1997) similarly found in their study in an Appalachian river continuum that macroinvertebrate secondary production was highest on *P. ceratophyllum.* They attributed this to the macrophyte's ability to stabilize the gravel and small cobble substrates. Secondary production estimates on bedrock habitats containing *P. ceratophyllum* were also higher than those containing bryophyte mosses and other habitats with different substrates that lacked *P. ceratophyllum*. Unlike the steady standing stock of *P. ceratophyllum* that was observed in this study, Hill and Webster (1984) recorded an increase in standing stock of *P. ceratophyllum* from mid-May until late August before starting to decline in their study area on the New River of Appalachia.

The second hypothesis, that the seasonal availability of the filamentous algae *Cladophora* would have a significant effect on the dietary habits of the larvae of the riverine caddisfly *H. simulans* in the upper Green River, was also supported. The high density of *H. simulans* and the *Cheumatopsyche* sp. coincided, in part, with high *Cladophora* sp. standing stocks. This positive correlation between larval density and *Cladophora* sp. is similar to Fairchild and Holomuzki (2002), who found that the presence of filamentous algae on substrates significantly increased larval density of hydropsychid caddisflies. They also observed that hydropsychids will use *Cladophora* sp. to construct their retreats, mirroring the previously mentioned statement on the supportive role these algae can play for macroinvertebrate communities of Dodds and Gudder (1992).

Hydropsychid larvae are functionally defined as filtering-collectors (e.g. Wallace and Merritt 1980, Merritt and Cummins 2006) but are opportunistic in their feeding habits, with substantial dietary variation that can include a combination of detrital, algal, and animal tissues (e.g. Coffman et al. 1971, Rhame and Stewart 1976, Benke and Wallace 2000) depending in season and availability of potential food items. The mean stoichiometric C:N ratios of *Cladophora* sp.(8.9) were similar to *H. simulans* in August (5.0) and September (4.8) and the *Cheumatopsyche* sp. in September (5.5). Although the epilithic detrital C:N ratio (5.3) was nested in the range of each of the hydropsychid consumer taxa, the IsoSource modeling results showed this basal resource was at most only a minor dietary item. The C:N ratio of cDOM (4.0) was likewise similar to each of the hydropsychid consumers, but during preliminary IsoSource modeling the contribution of this resource never exceeded 1% and was subsequently eliminated as a potential food

source in the final dietary analyses. Veldbloom and Haro (2011) observed that the elemental composition of an organism can vary temporally, indicated in their study by the C:N ratio of their study species of suspension-feeding caddisfly *Brachycentrus occidentalis* showing large differences seasonally. Their findings were similar in that C:N ratios of several basal shifted changed between the two sampling periods. The most dramatic example being the C:N ratio of detrital epilithon increasing from 5.3 –11.5 between mid- and late summer.

The comparison of stable isotopic $\delta^{13}C$ values between *Cladophora* and mid-summer *H. simulans* body tissue, and more importantly, the evaluation of the IsoSource contribution models, strongly suggested that the filamentous alga was a prominent assimilated dietary item during mid-summer. This result mirrors that of Rhame and Stewart (1976), who had found that the proportion (by volume) of gut contents of *H. simulans* in the Brazos River, Texas, composed of filamentous green algae increased from <1 – 98% between July and December during a rapid growth period. Positive interactions between *Cladophora*-induced habitat availability and *Hydropsyche* communities have been previously demonstrated (Dudley et al. 1986). Delong and Thorp (2006), however, reported that sestonic algae were the predominant dietary item for *H. orris* in the upper Mississippi River. Sestonic detritus, macrophytes, and benthic algae each contributed markedly less. Delong and Thorp (2006) indicated that since their benthic algal samples included both microscopic and macroscopic forms, that their respective $\delta^{13}C$ signals may be distinct.

Although easily a secondary dietary item, the modeled presence of *P. ceratophyllum* to *H. simulans* was a surprise, and not repeated with either the late summer *H. simulans*

or the *Cheumatopsyche* sp. Detrital material of *P. ceratophyllum* decays at a rate faster than leaves of most tree species (Hill 1982), suggesting that the leafy material of this macrophyte may be of sufficient dietary quality for some generalist consumers. For example, the crayfish *Procambarus spiculifer* has been shown to selectively feed on *P. ceratophyllum* (Parker et al. 2007). Ironically, however, although the C:N ratios reported in this study decreased from 14.7 to 11.2 this potential basal food source was only meagerly used as a food source by late summer.

The late summer $\delta^{13}C$ values for *H. simulans* suggested a dietary shift toward a larger proportion of floodplain tree leaf material and slightly higher amounts of both epilithic and sestonic materials. The modest decrease in the modeled contribution of *Cladophora* sp., with reduced standing stocks of nearly three-fold between August and September, and the concomitant increase in each of the other resource categories (except *P. ceratophyllum*), coincided with the partial scour event that occurred prior to the second benthic sampling event. Why the contribution of *P. ceratophyllum* as a dietary item decreased in light of the combination of slightly higher standing stocks and lessened *Cladophora* sp. availability is unknown.

The late summer diet of the *Cheumatopsyche* sp. was similarly dominated by *Cladophora* sp. and leaf material, but unlike *H. simulans*, also included a comparatively high proportion of both TOM components. The presence of multiple food items in the diet of hydropsychid larvae has been demonstrated several times (e.g. Benke and Wallace 1980), yet in most studies one food item tends to easily be the most important. Similar to *H. simulans* in the Brazos River, Rhame and Stewart (1976) found that the *Cheumatopsyche* sp. larvae consumed the rapidly-proliferating *Cladophora* sp. when

available. Proportion by volume increased from 37 to 93% between July and October, and was still 65% by December.

Other caddisflies can consume *Cladophora*. McNeely and Power (2007) went so far as to suggest that the larger populations of armored grazing species of caddisflies (Leptoceridae) supported by the higher productivity during early summer in their study might affect the abundance of algae due to their high numbers and very focused consumption.

There is also ample evidence that hydropsychid larvae, including *Hydropsyche* species, can rely heavily upon animal tissue as a preferred and/or easily assimilated dietary item. This is particularly true for Arctopsychinae (*Arctopsyche* and *Parapsyche*) (e.g. Benke and Wallace 1980, Ross and Wallace 1983), yet also for some species of *Hydropsyche*. Benke and Wallace (1980) showed that animal material contributed >50% to annual production of *H. macleodi* and *H. sparna* in a southern Appalachian river.

In conclusion, the densities of both *H. simulans* and the *Cheumatopsyche* sp. are significantly higher in very high *P. ceratophyllum* habitat compared to very low habitats during both sampling periods. There was also shown to be a significant effect of time as well as an interaction between time and habitat for *H. simulans*, while there was a significant effect of habitat for both *H. simulans* and the *Cheumatopsyche* sp. These results support the hypothesis that these hydropsychid larvae preferentially inhabit *P. ceratophyllum* as opposed to bare substrate.

With regard to the question of *Cladophora* sp. making up a significant portion of the hydropsychid's diet, the stoichiometry suggested *Cladophora* sp. as a highly beneficial food source during both sampling periods. In accordance with this, the stable isotopic

values of *H. simulans* and the potential food resources sampled for August suggested that *Cladophora* sp. are the main food source. IsoSource also strongly indicated *Cladophora* sp. as the main portion of diet of *H. simulans* in August and both hydropsychids in September. All of these results suggest that these caddisflies do, in fact, take advantage of the annual summer *Cladophora* sp. bloom.

TABLE 3.1 Mean monthly temperature (°C) and discharge (L/s) data during the study period June–October 2011 in the upper Green River, Kentucky.

	Month					
Variable	Jun	Jul	Aug	Sept	Oct	Overall
Temperature	NA	25.1	24.2	19.7	15.0	21.0
Discharge	61517.9	18009.5	7453.0	13655.5	8752.0	21671.7

NA = data not collected

TABLE 3.2 Mean (n = 4) monthly nutrient levels (mg/L) during the study period June–October 2011 in the upper Green River, Kentucky.

	Month					
Nutrient	Jun	Jul	Aug	Sept	Oct	Overall
Nitrate	0.95	1.25	0.88	0.80	0.68	0.77
Ammonia	0.02	<0.01	0.02	0.02	0.03	0.02
TN	2.75	2.13	1.90	0.80	1.37	1.79
TP	0.10	0.15	0.18	0.12	0.10	0.13
SRP	0.09	0.11	0.12	0.06	0.09	0.09
TN:TP	31.43	20.24	15.51	14.55	15.22	19.39

TN = total nitrogen. TP = total phosphorous, SRP = soluble reactive phosphorous

TABLE 3.3 Mean standing stocks and percent cover of primary producers during the study period June–October 2011 in the upper Green River, Kentucky.

Primary producer	Month Jun	Jul	Aug	Sept	Oct
Sestonic algae (Chl-α, µg/L)	NA	6.09	10.48	2.08	2.96
Cladophora (AFDM, mg/m^2)	NA	NA	19.76	7.74	9.09
Cladophora (% cover)	NA	15.61	74.52	NA	NA
P. ceratophyllum (AFDM, mg/m^2)	NA	NA	48.46	48.93	56.73
P. ceratophyllum (% cover)	NA	52.35	51.04	NA	NA

Chl-α = chlorophyll-α, AFDM = ash-free dry mass, NA = data not collected

TABLE 3.4 Mean (± 1 S.E.) densities (no./m^2) of *Hydropsyche simulans* and *Cheumatopsyche* in very high (VH, > 75%) and very low (VL, < 5%) areal coverage of *Podostemum ceratophyllum* during August and September 2011 in the upper Green River, Kentucky.

Taxon	Treatment	Month Aug		Sept	
H. simulans	VH	1862.2	± 329.9	635.6	± 185.6
	VL	77.8	± 24.1	68.9	± 23.7
Cheumatopsyche	VH	3095.6	± 849.7	3537.8	± 1065.9
	VL	124.4	± 31.1	93.3	± 30.3

TABLE 3.5 Comparison of mean (± 1 S.E., if applicable) stoichiometric C:N ratios between the two hydropsychid consumers and basal food resources during August and September 2011 in the upper Green River, Kentucky. Resource C:N ratio values in bold type are those speculated to be prominent assimilated dietary components.

	Month					
Consumer or resource	Aug	± 1 S.E.	n	Sept	± 1 S.E.	n
Consumer						
Hydropsyche simulans	5.0	± 0.08	2	4.8	NA	1
Cheumatopsyche	NA	NA	NA	5.5	NA	1
Basal resource						
Cladophora	**8.9**	± 0.17	5	**9.8**	0.30	5
Podostemum ceratophyllum	14.7	± 0.58	5	11.2	0.41	5
Leaves	18.0	± 0.39	5	23.4	0.86	5
Epilithic - algal	14.8	NA	1	11.2	1.22	3
Epilithic - detrital	**5.3**	NA	1	11.5	1.13	2
FTOM - algal	17.9	NA	1	11.7	NA	1
FTOM - detrital	24.1	NA	1	15.1	5.03	2
UFTOM - algal	21.2	NA	1	NA	NA	NA
UFTOM - detrital	14.0	NA	1	NA	NA	NA
cDOM	NA	NA	NA	**4.0**	0.45	5

FTOM = fine transported organic matter (1000 – 100 µm), UFTOM = ultrafine transported organic matter (100 – 1 µm), cDOM = colloidal dissolved organic matter (< 1 µm), NA = data not collected or applicable.

TABLE 3.6 Comparison of mean (± 1 S.E., if applicable) stable isotopic signatures ($\delta^{13}C$, $\delta^{15}N$) between the two hydropsychid consumers and basal food resources during August and September 2011 in the upper Green River, Kentucky. Signatures in bold type are those speculated to be prominent assimilated dietary components.

	Month									
	Aug					Sept				
Consumer or resource	$\delta^{13}C$	± 1 S.E.	$\delta^{15}N$	± 1 S.E.	n	$\delta^{13}C$	± 1 S.E.	$\delta^{15}N$	± 1 S.E.	n
Consumer										
Hydropsyche simulans	-34.0	0.17	7.5	< 0.01	2	-32.7	NA	7.6	NA	1
Cheumatopsyche	NA	NA	NA	NA	NA	-31.5	NA	7.3	NA	1
Basal resource										
Cladophora	**-34.2**	0.64	3.6	1.11	5	-36.3	0.14	5.6	0.12	5
Podostemum ceratophyllum	-37.7	0.21	8.1	0.16	5	-39.7	0.10	7.2	0.16	5
Leaves	-30.9	0.11	4.3	0.10	5	**-30.6**	0.19	4.6	0.12	5
Epilithic - algal	-28.4	NA	6.1	NA	1	**-29.9**	0.10	5.2	0.53	3
Epilithic - detrital	**-31.9**	NA	7.7	NA	1	-28.6	0.23	4.9	0.14	2
FTOM - algal	-28.3	NA	4.5	NA	1	-24.9	NA	5.3	NA	1
FTOM - detrital	-27.9	NA	4.6	NA	1	-27.6	0.82	4.8	0.14	2
UFTOM - algal	-28.9	NA	14.4	NA	1	NA	NA	NA	NA	NA
UFTOM - detrital	-28.8	NA	14.4	NA	1	NA	NA	NA	NA	NA
cDOM	NA	NA	NA	NA	NA	-25.1	0.18	9.0	0.36	5

FTOM = fine transported organic matter (1000 – 100 µm), UFTOM = ultrafine transported organic matter (100 – 1 µm), cDOM = colloidal dissolved organic matter (< 1 µm), NA = data not collected or applicable.

TABLE 3.7 Estimated range (25–75th percentiles) of the contribution of basal food resources to the assimilated diet of hydropsychid consumers during August and September 2011 in the upper Green River, Kentucky.

Basal resources	Consumer		
	H. simulans (Aug)	*H. simulans* (Sept)	*Cheumatopsyche* (Sept)
Cladophora	84–86	66–72	56–60
P. ceratophyllum	10–11	2–6	0–0
Leaves	0–3	3–14	2–13
epilithic - algal	0–1	1–6	0–1
epilithic - detrital	0–2	1–6	0–0
TOM - algal, 1000-1 µm	0–1	2–8	10–27
TOM - detrital, 1000-1 µm	0–1	1–7	6–22

Percentiles were calculated using a seven-source mixing model in the IsoSource program (Phillips and Gregg 2003). TOM = transported organic matter.

Figure 2.1 Location of the study reach (solid red circle).

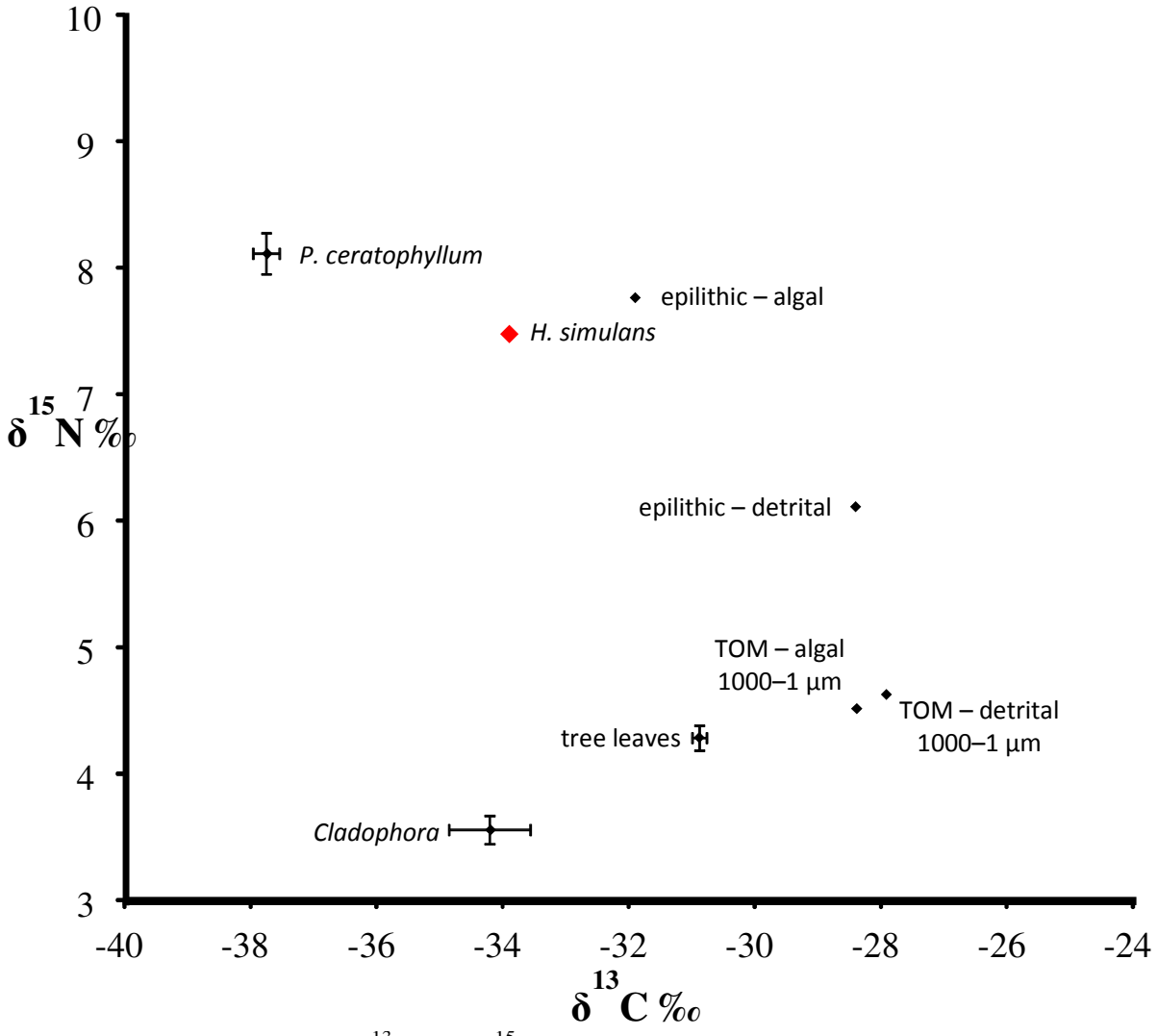

Figure 3.1 Mean $\delta^{13}C$ and $\delta^{15}N$ (± 1 S.E., if n > 1) values of basal food sources and *H. simulans* in the upper Green River, July 2011. TOM = transported organic matter.

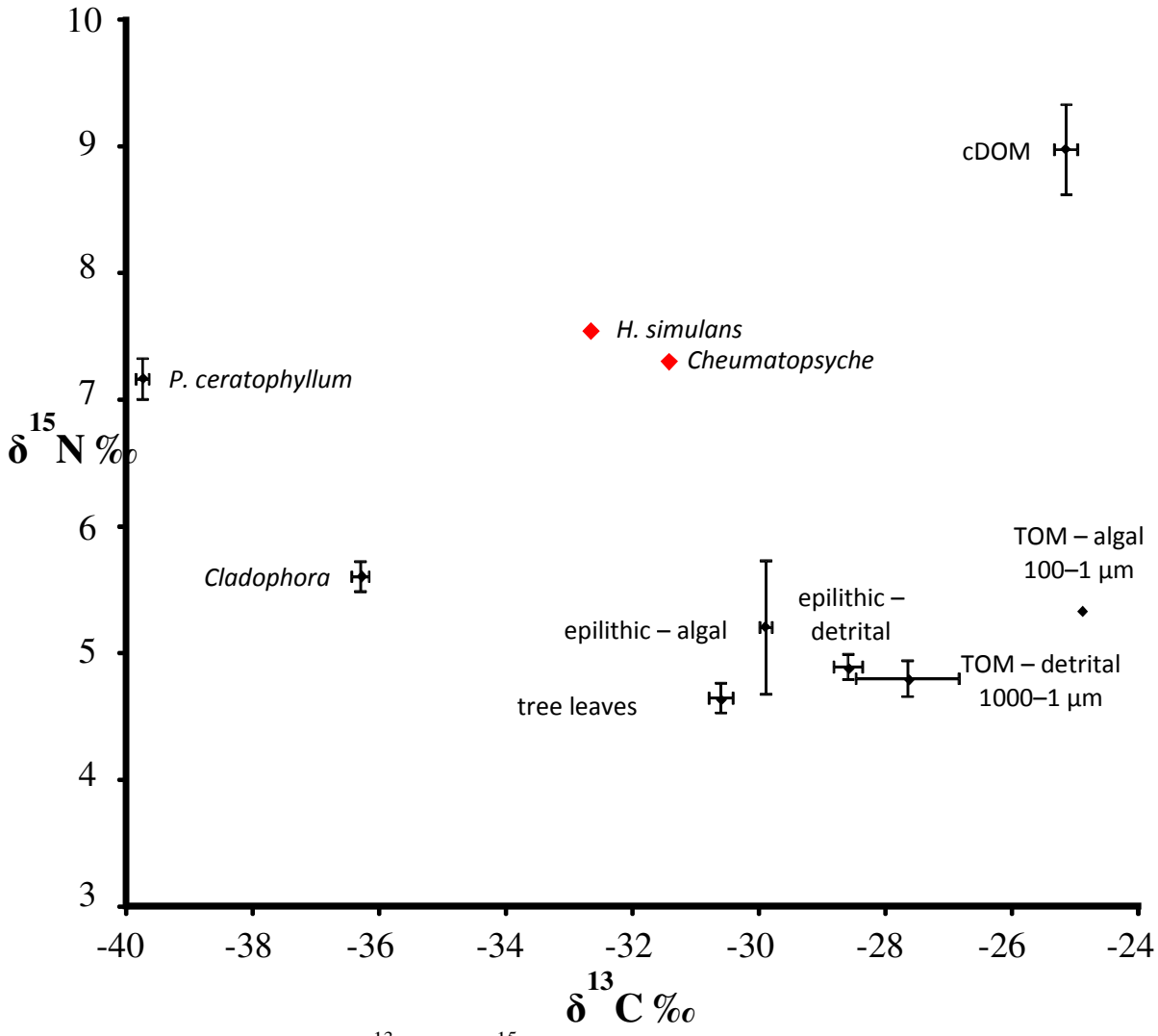

Figure 3.2 Mean $\delta^{13}C$ and $\delta^{15}N$ (± 1 S.E., if n > 1) values of basal food sources and both hydropsychid consumers in the upper Green River, September 2011. cDOM = colloidal dissolved organic matter, TOM = transported organic matter.

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