THE INFLUENCE OF ENERGY AVAILABILITY ON POPULATION-, COMMUNITY-, AND ECOSYSTEM-LEVEL PROCESSES IN CAVE STREAM ECOSYSTEMS

by

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ABSTRACT

Detritus from surface environments supplies the energy that shapes community structure and fuels productivity in most cave ecosystems. However, only qualitative descriptions of cave energy dynamics are available, hindering development of quantitative models describing how energy availability influences cave ecosystem processes. In contrast, the importance of detritus for surface ecosystem processes has been appreciated for decades. This dissertation begins to close this knowledge gap by exploring how energy availability shapes cave stream ecosystem processes at multiple organizational levels (ecosystem-, community-, population-level) and time scales (evolutionary vs. ecological).

Chapter Two examined potential correlations between litter breakdown rates and detrital storage, but found no such relationships among four cave streams. However, surface-adapted species dominated macroinvertebrate biomass, suggesting that surface-adapted taxa can have a significant influence on cave ecosystem processes. In Chapter Three, a whole-reach litter amendment was conducted to explore the influence of enhanced detrital inputs on cave community structure and energy flow. While the litter amendment significantly increased total consumer biomass via assimilation of amended corn-litter, the response was dictated by evolutionary history. Biomass of surface-adapted taxa increased significantly following the amendment, while biomass of obligate cave species remained unchanged. As in Chapter Two, consumer biomass was dominated by surface-adapted taxa, reinforcing their role in cave ecosystem processes relative to cave-adapted taxa, the traditional focus of cave studies.

Chapters Four and Five utilized a 5+-year mark-recapture data set on the cave-adapted crayfish *Orconectes australis* to explore how energy availability has shaped its evolutionary history and population dynamics. Time-to-maturity, age-at-first-reproduction, and longevity of *O. australis* were substantially longer than those estimated for most crayfish species, indicating evolution of a K-selected life history. Chapter Five found that biomass and secondary production of *O. australis* were positively related to resource availability. Energetic models indicated resource deficits were not present, but that nearly all prey production is necessary to support each *O. australis* population. Thus, inter- and intra-specific competition for resources within caves is likely high. Collectively, Chapters Four and Five provide the first quantitative explanation of why K-selected life histories are an evolutionary advantage to obligate cave taxa like *O. australis*.

LIST OF ABBREVIATIONS AND SYMBOLS

mg	Milligrams
g	Grams
µg	Microgram
g^{-1}	Per gram
km	Kilometer
m	Meter
m^{-2}	Per square meter
cm	Centimeter
cm^{-2}	Per square centimeter
L	Liter
L^{-1}	Per liter
mm	Millimeter
µm	Micrometer
yr(s)	Years(s)
yr^{-1}	Per year
$month^{-1}$	Per month
d	Day
d^{-1}	Per day
h	Hour
^{o}C	Degrees Celsius

n	Number
~	Approximately
c.	Approximately
%	Percent
>	Greater than
<	Less than
≥	Greater than or equal to
≤	Less than or equal to
=	Equal to
+	Plus
±	Plus minus
#	Number
x	Times or by
k	Litter breakdown rate
et. al.	And others
e.g.	For example
post hoc	Occurring after the event
sensu	In the sense of
U.S.A.	United States of America
AL	Alabama
TN	Tennessee

NC	North Carolina
IL	Illinois
ON	Ontario
U.K.	United Kingdom
VIAT	Visible implant alpha tag
VIE	Visible implant elastomer
Fig.	Figure
p.	Page
pp.	Page
S	Surface
C	Cave
SF	Size frequency
MR	Mark-recapture
VBGF	von Bertalanffy growth model
GM	Growth model
Inc	Incubation period
Temp	Temperature
BD	Below detection
OM	Organic matter
CR	Control reach
MR	Manipulation reach

OCL	Ocular carapace length
TCL	Total carapace length
AFDM	Ash free dry mass
DM	Dry mass
B	Biomass
G	Annual instantaneous growth rate
P	Annual secondary production
AE	Assimilation efficiency
NPE	Net production efficiency
P:B	Annual secondary production to Biomass ratio
C:N	Carbon to nitrogen ratio
NH_4^+-N	Nitrogen from ammonium
NO_3^--N	Nitrogen from nitrate
SRP	Soluble reactive phosphorous
DOC	Dissolved organic carbon
$\delta^{13}C$	Carbon 13 natural isotope
N	Nitrogen
P	Phosphorus
C.I.	Confidence interval
CV	Coefficient of variation
BACI	Before-after-control-impact

AIC	Akaike information criterion
K-S	Kolmogorov-Smirnov
ANOVA	Analysis of variance
ANOSIM	Analysis of similarity
nMDS	Non-metric multidimensional scaling
SIMPER	Analysis of similarity percentages
SD	Standard deviation
SE	Standard error
$\log_{10}$	Logarithm of base 10
P	Probability associated with the occurrence under the null hypothesis of a value as
W	Compute value of Wilcoxon signed-rank test
t	Computed value of t test
F	Computed value of F test
df	Degrees of freedom: number of values free to vary after certain restrictions have been placed on the data
r^2	Proportion of variability explained by statistical model
ρ	Spearman’s rho
α	Probability according to null hypothesis of a type-1 error probability

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CHAPTER 1

INTRODUCTION

Karst landscapes are formed by the dissolution of a consolidated soluble bedrock such as limestone or dolomite by groundwater (Van Brahana 2003). This process, commonly referred to as karstification, produces landscapes that are commonly identified by the presence of sinkholes, springs, sinking streams, and caves (Van Brahana 2003). Karst landscapes underlie 12% to 15% of the Earth's surface, ~15% of the United States (Juberthie 2001), and roughly 25% of the world's population relies on karst aquifers for drinking water (see Van Brahana 2003).

The cave systems that are formed in the karst landscapes throughout North America, Europe, Asia, and Africa contain more than 7700 vertebrate and invertebrate species (terrestrial and aquatic; Gibert and Culver 2005). Many of these taxa are obligate cave species, capable of surviving and reproducing only within cave systems, and are of great conservation concern given their high degree of endemism and endangerment. Given the economic and ecological importance of cave systems, and karst landscapes as a whole, it should come as no surprise that much research has been conducted within them (see Culver et al. 1995; Wilkens et al. 2001; Culver and White 2005 and references within). However, basic information regarding the factors that influence the structure and function of these unique ecosystems is still sorely lacking.

In cave ecosystems, energy (carbon) as opposed to nitrogen (N) or phosphorus (P), availability has been suggested as the primary factor influencing trophic dynamics, community structure, and evolutionary processes (Culver et al. 1995; Graening and Brown 2003). Energy availability is limited due to the absence of light, which prevents primary production (except in chemolithoautotrophy-based systems; Engel et al., 2004), and by reduced surface connectivity, which limits the inputs of allochthonous organic matter that supports biological productivity (Poulson & Lavoie, 2001). Many obligate cave species share a similar set of traits that are presumed to be adaptations to limited energy availability, including lower metabolic rates, increased starvation resistance, and more K-selected life history characteristics (e.g. long life span, slow growth rate, and reduced fecundity; see Hüppop, 2001 and 2005). However, because of the limited number of species for which there are physiological or life history data, it is unknown if these traits are common to all obligate karst species.

Ecosystem-level evidence thought to support the energy-limitation hypothesis comes largely from reports of community shifts or increased biomass following incidental inputs of organic pollutants (Sinton 1984; Smith et al. 1986; Madsen et al. 1991; Notenboom et al. 1994; Simon and Buikema 1997; Sket 1999). However, evidence for carbon limitation from such studies is confounded because organic pollution is typically a heterogeneous mixture of organic and inorganic material (i.e., organic matter, dissolved nutrients, microbes, and toxins), making it impossible to discern which component or combination of components causes changes in recipient communities. Only recently have studies found that energy availability is a predictor of consumer biomass or productivity in cave streams and groundwater ecosystems unaffected by pollution (Datry et al., 2005; Cooney & Simon, 2009; Huntsman et al., 2011a, b).

This ongoing debate has a long history. More than a century ago, Racovitza (1907) questioned whether cave systems were actually energy-limited. More recently, Simon et al. (2007) highlighted the continuing paucity of data describing organic carbon flow in cave systems. The objective of this dissertation was to explore how energy availability affects ecosystem-, community-, and population-level processes in cave stream ecosystems on both ecological- and evolutionary- time scales. In Chapter 2, the linkages among organic matter abundance (i.e. a surrogate for energy availability), the structure of litter macroinvertebrate communities, and litter breakdown rates are examined during two seasonal periods in four cave streams. The energy-limitation hypothesis is experimentally tested in Chapter 3 by tracking the changes in a single cave-stream community following a reach-scale detritus amendment. Finally, Chapters 4 and 5 utilize a 5+-year mark-recapture data set on the obligate cave crayfish *Orconectes australis* to first re-describe the species's life history characteristics (e.g., life span and time-to-maturity) and examine whether limited energy-availability has influenced a K-selected life history, and to, second, use the trophic basis of production approach (sensu Benke & Wallace, 1980) to examine how the energetic demands of *Orconectes australis* (Rhoades) compare to resource availability and turnover rates. Collectively, these chapters represent the most robust test of the energy-limitation hypothesis in cave ecosystems to date.

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CHAPTER 2

EFFECTS OF ORGANIC MATTER AND SEASON ON LEAF LITTER COLONIZATION AND BREAKDOWN IN CAVE STREAMS

Abstract

Low organic matter availability is thought to be a primary factor influencing evolutionary and ecological processes in cave ecosystems. Links among organic matter abundance, macroinvertebrate community structure and breakdown rates of red maple (*Acer rubrum*) and corn litter (*Zea mays*) in coarse- (10 × 8 mm) and fine-mesh (500-µm) litter bags over two seasonal periods were examined in four cave streams in the southeastern U.S.A. Organic matter abundance differed among cave streams, averaging from near zero to 850 g ash-free dry mass m^{-2}. Each cave system harboured a different macroinvertebrate community. However, community structure was similar among caves, with low shredder biomass (2-17% of total biomass). Corn litter breakdown rates (mean $k = 0.005\ d^{-1}$) were faster than red maple (mean $k = 0.003\ d^{-1}$). Breakdown rates in coarse-mesh bags ($k = 0.001$-$0.012\ d^{-1}$) were up to three times faster than in fine-mesh bags ($k = 0.001$-$0.004\ d^{-1}$). Neither invertebrate biomass in litter bags nor breakdown rates were correlated with the ambient abundance of organic matter. Litter breakdown rates showed no significant temporal variation. Epigean (surface-adapted) invertebrates dominated biomass in litter bags, suggesting that their effects on cave ecosystem processes may be greater than hypogean (cave-adapted) taxa, the traditional focus of cave studies. The functional diversity of cave communities and litter breakdown rates are broadly similar to those found in previous litter breakdown studies in cave streams, suggesting that the factors that control organic

matter processing (e.g. trophic structure of communities) may be broadly similar across geographically diverse areas.

Introduction

Cave streams are quintessential donor-controlled ecosystems representing one endpoint along a heterotrophic spectrum (Polis & Strong, 1996; Simon, Benfield & Macko, 2003). Lacking photoautotrophs, the productivity of most cave ecosystems (except those based on chemolithoautotrophy; Sarbu, Kane & Kinkle, 1996, Sarbu, 2001; Engel *et al*., 2004) depends on organic matter from the surface (Poulson & Lavoie, 2001). The amount and type of organic matter imported into a cave is largely determined by its connectivity to the surface (Poulson & Lavoie, 2001). Systems weakly connected to the surface typically receive fine particulate and dissolved organic matter, while systems that are well connected via cave entrances and sink holes may receive relatively high inputs of dissolved, fine and coarse particulate organic matter (e.g. leaves and woody debris, Poulson & Lavoie, 2001). Regardless of connectivity, the quantity and quality of these inputs are often low compared to surface streams due to a lack of direct riparian inputs and prior biological processing in surface and soil habitats, resulting in the characterization of cave ecosystems as being energy-limited (Culver, 1982; Culver, Kane & Fong, 1995; Poulson & Lavoie, 2001; Graening & Brown, 2003). As a consequence of differences in cave-surface connectivity, however, gradients in resource availability exist that may influence the biomass and diversity of species found within caves. These differences in community structure may ultimately affect cave ecosystem processes.

A few studies have attempted to examine the links among organic matter inputs, community structure and ecosystem processes in cave systems. Schneider, Christman & Fagan (2011) showed that organic matter quality (leaf litter vs. rat carcasses) influenced terrestrial community structure in cave “pits” (i.e. vertical cave passages with direct connections to the surface). Huntsman *et al.* (2011b) found that growth rates and production of the obligate cave salamander *Gyrinophilus palleucus* (McCrady) were positively related to amount of organic matter in cave streams, presumably because of higher prey availability. In a study of groundwater systems, Datry, Malard & Gibert (2005) reported that aquifers recharged by storm water received higher inputs of dissolved organic matter and had higher densities and diversities of invertebrates, suggesting that organic matter supply may influence groundwater community structure. Similar patterns have been observed in both phreatic aquifers and cave streams affected by organic pollution (Sinton, 1984; Smith *et al*., 1986; Madsen, Sinclair & Ghiorse, 1991; Notenboom, Plénet & Turquin, 1994; Simon & Buikema, 1997; Sket, 1999).

Fewer studies have examined how organic matter abundance affects ecosystem processes in cave streams. Cooney & Simon (2009) used a microcosm experiment to show that amendment of dissolved organic matter increased microbial productivity and enzyme activity on cave-stream rocks. The processing of particulate organic matter in caves has also received some attention. Simon & Benfield (2001) found that cave streams with direct upstream connections to the surface (e.g. cave entrances and sinkholes) had high organic matter inputs, communities dominated by epigean (surface) and stygophilic (species that reproduce in both cave and surface habitats) taxa and faster decomposition of white oak (*Quercus alba* L.) litter. In contrast, streams without large upstream openings to the surface had lower organic matter standing stocks, were dominated by hypogean (cave specialist) taxa and had slower rates of litter breakdown. Differences in litter breakdown rates among cave streams were attributed to the degree of surface connection, which controlled both organic matter inputs and the biomass of surface taxa. Relationships between organic matter abundance and litter breakdown are not simple, however.

For example, a regression analysis of data reported by Simon & Benfield (2001) revealed no significant relationship between organic matter abundance and litter breakdown rates among the five cave streams they studied (M. P. Venarsky, unpubl. analysis). Similarly, Huntsman, Venarsky & Benstead (2011a) found no correlation between organic matter abundance and carrion breakdown rates in four cave streams, suggesting that factors additional to the abundance of organic matter influence rates of carrion processing in caves.

Here, the linkages among organic matter abundance (i.e. a surrogate for resource supply), the structure of litter macroinvertebrate communities and litter breakdown rates during two seasonal periods were examined in four cave streams in northeastern Alabama and southeastern Tennessee, U.S.A. These ecosystems contained different amounts of organic matter, allowing the test of three related hypotheses. First, macroinvertebrate abundance is often higher in groundwater systems receiving high inputs of organic matter compared with those receiving low inputs (Datry *et al.*, 2005). Thus, macroinvertebrate biomass in litter packs was hypothesized to be positively correlated with ambient organic matter abundance because caves with higher quantities of organic matter are likely to support more macroinvertebrates. Second, because positive correlations between litter breakdown rates and consumer biomass have been shown for cave and surface streams (Simon & Benfield, 2001; Hieber & Gessner, 2002), litter breakdown rate was predicted to be positively correlated with consumer biomass. Third, because many environmental characteristics in cave systems vary little temporally (e.g. light, humidity and temperature; Juberthie, 2001; Culver & Pipan, 2009), consumer biomass and the rate of litter breakdown was also predicted to vary temporally. The design of this study allowed the quantification of how organic matter abundance affects cave stream macroinvertebrate communities and how variation in community structure affects rates of organic matter processing, a key ecosystem process in cave and groundwater ecosystems.

Methods

Study sites

Four streams in hydrologically separate cave systems were studied. Tony Sinks, Salt River and Jess Elliot caves are in Jackson County, Alabama, U.S.A. Big Mouth Cave is in Grundy County, Tennessee, U.S.A. Distances between caves ranged from 9 km to 76 km. These caves were chosen for the following four reasons. First, their communities appeared similar in structure upon initial inspection (i.e. they all contained cave salamanders and isopods and cave and surface crayfish). Second, organic matter abundance differed among caves due to differing connectivity to surface systems. Third, visual surveys of surface habitats in the recharge area of each cave suggested human disturbance was minimal (e.g. no clear-cuts, little residential development and few roads within the study catchments). Lastly, physical characteristics of the study reaches within and among cave streams were similar. Stream width ranged between 5 and 10 m, while depth ranged from 20 to 80 cm and the substratum was composed of a mixture of sand and gravel interspersed with exposed areas of bedrock. All study reaches were between 200 and 600 m (horizontally) from the nearest known cave entrance and ranged from 70 to 90 m in length. Cave streams did not have known direct aquatic connections (e.g. upstream cave entrances) to surface habitats during base-flow conditions.

Litter colonization and breakdown rates

Rates of litter breakdown were measured using bags containing red maple (*Acer rubrum* L.) and corn (*Zea mays* L.) litter. Choice of these two species allowed us to examine the effects of litter quality on breakdown rate; both species breakdown relatively rapidly (Webster &

Benfield, 1986; Griffiths *et al*., 2009) but their C:N ratios differ (red maple C:N = 53 [Ostrofsky, 1997], corn C:N = 24 to 28 [Griffiths *et al*., 2009]). Corn litter was also included in order to examine its potential as a litter source in a separate reach-scale detritus addition study (M. P. Venarsky, unpublished). Red maple litter was collected shortly after abscission from the Coweeta Hydrologic Laboratory, North Carolina, and Tuscaloosa County, Alabama, U.S.A. Senesced corn litter from non-Bt plants (i.e. a variety that has not been genetically modified to contain *Bacillus thuringiensis* genes) was collected from an agricultural field near Salem, Illinois, U.S.A.

Litter of both species was air-dried and ~4 g (±0.1 g) was placed into each of 14 × 12 cm fine- (500-μm) and coarse-mesh (10 × 8 mm) bags. The different mesh sizes controlled invertebrate access to the leaf litter. The fine-mesh bags allowed access by only small-bodied invertebrates (e.g. microcrustaceans, Chironomidae, and Oligochaeta), while coarse-mesh bags allowed access by both small- and large-bodied taxa (e.g. crayfish and late instar Ephemeroptera). Litter bags (*n* = 100) were deployed among five stations (fiver litter bags per litter species per mesh size per station) in each stream. Stations were separated by 5 to 10 m, except in Jess Elliot Cave where two stations were separated by 85 m. Litter bags were tethered to nylon string at 0.25-m intervals and anchored in the stream by a sandbag. To assess handling loss, three litter bags of each litter species and mesh size were retrieved on the day of deployment. On each of 10 sampling dates, one bag of each litter species and mesh size was retrieved from each station in each of the four caves (*n* = 5 of each combination). Litter bags were stored on ice from the time of collection to processing (*c*. 24 hrs). To assess temporal variation in litter breakdown rates, the litter-bag study was conducted twice, from January to July 2008 and from July to December 2008 (to January 2009 for Tony Sinks). Red maple litter from the different source locations was not mixed between experimental periods; litter from Macon Co., North Carolina was used during the first experimental period and litter from Tuscaloosa Co., Alabama for the second period. No effects of different maple litter sources were detected (see *Results*).

Water temperature and depth were recorded in each stream every 30 min using a Solinst Barologger model 3001 data logger (Solinst, Georgetown, Ontario, Canada). The coefficient of variation (CV) around average daily water levels was calculated in each cave to quantify flow variability; a higher CV indicates more variable hydrology. CV around water depths could not be calculated for the first deployment date because of instrument failure.

Water samples were collected from each stream on each sampling date and analysed for NH_4^+-N, NO_3^--N and soluble reactive phosphorus (SRP). All water samples were filtered through glass-fibre filters (0.7-µm nominal pore size). Water samples were transported to the laboratory on ice and frozen until analysis. Temporal trends in organic matter abundance were examined by quantifying organic matter on eight dates (four per incubation period) using a 490-cm^2 corer placed at two random points within 4 m of each litter-bag station. The corer was inserted into the stream bottom, large organic matter was removed, and the substratum disturbed to a maximum depth of 4 cm. Remaining suspended organic matter was removed from the water column of the corer via 10 sweeps of the water column with a 250-µm mesh net.

Sample processing

Contents of litter bags were placed into a 3.8-L bucket containing *c*. 2 L of water. The contents were agitated to dislodge macroinvertebrates from litter, which was removed by hand. The water was then sieved (250-µm), additional leaf material was removed and invertebrates retained on the sieve preserved in 5% formalin. Organic matter from both the litter bags and cores was processed identically. Organic matter was dried at 60˚C to constant mass and combusted at 500˚C for 6 h. Combusted material was wetted, re-dried and weighed. The difference between oven-dried mass and ash mass was used to estimate the ash-free dry mass (AFDM) of organic matter. For water samples, concentrations of NH_4^+-N were measured using the orthophthaldialdehyde fluorometric method (Holmes *et al*., 1999 as modified by Taylor *et al*., 2007). Concentrations of NO_3^--N were measured using ion chromatography (Dionex ICS 2000 Ion Chromatograph; APHA, 1998). Soluble reactive phosphorus (SRP) concentrations were quantified using the ascorbic acid method (Murphy & Riley, 1962).

Macroinvertebrates were identified to the lowest possible taxonomic level (usually genus for insects and molluscs, class or order for microcrustaceans). Because species-level identifications were not made, no all specimens were classified definitively into hypogean (cave-adapted species) or epigean (surface species) groups. Specimens were identified as hypogean when: i) they could be identified to genus, ii) they lacked eye structures, and iii) the genus was known to contain hypogean species. The insect taxa (Table 1) identified in this study have previously been reported from caves (e.g. Brussock, Willis & Brown, 1988; Simon & Benfield, 2001), but are probably epigean species able to survive in cave streams (Romero, 2009). Body lengths of macroinvertebrates were measured to the nearest mm. Biomass was estimated using published length-mass relationships (Calow, 1975; Culver *et al*., 1985; Leeper & Taylor, 1998; Benke *et al*., 1999; Doroszuk *et al*., 2007; Lemke & Benke, 2009; Huntsman *et al.* 2011a). Taxa were categorized into functional feeding groups after Merritt, Cummins & Berg (2008).

Data analysis

Differences in benthic organic matter abundance among caves and sampling dates were assessed using two-way ANOVA. Litter breakdown rate (-*k*) was calculated by linear regression of natural-log transformed data (% AFDM remaining) against elapsed days. A *k*-coefficient was estimated for each litter species and mesh type at each station. A paired *t*-test was used to compare breakdown rates (*k*-coefficients) of corn and maple litter. Differences in breakdown rates among mesh sizes, incubation periods and caves within each litter type were assessed using three-way ANOVA. Three-way ANOVAs were run separately for the two litter species. Following all ANOVAs, a Bonferroni pair-wise *post hoc* comparison was conducted. Within each litter species, mesh size and incubation period, simple linear regression was used to assess the effect of i) organic matter abundance on macroinvertebrate biomass and breakdown rate, and ii) macroinvertebrate biomass on breakdown rate. Data were transformed ($\log_{10}$+1) as needed to meet the assumptions of statistical analyses. All parametric analyses were conducted using Data Desk version 6.1 (Data Description Inc., 1996).

Three multivariate techniques in PRIMER (Version 6, Plymouth Marine Labs, Plymouth UK; Clarke & Warwick, 2001) were used to compare macroinvertebrate community structure within and among caves: analysis of similarities (ANOSIM), non-metric multidimensional scaling (nMDS) ordinations and similarity percentages (SIMPER). Similarity matrices were first computed using the Bray-Curtis coefficient on $\log_{10}$-transformed biomass data. A series of two-way nested ANOSIMs was then conducted in order to reduce the number of factors in the original data set, with each single factor (litter type, mesh size, incubation period, sampling date and station identity) nested within the cave factor. Ultimately, none of these five factors significantly affected community structure within each cave (see *Results*). Consequently, for each cave the biomass of each taxon was averaged from each station (i.e. across litter types, mesh sizes and incubation periods) and compared this reduced data set to the original using the RELATE routine in PRIMER. The station-level mean taxon biomass data was used in all subsequent analyses of community structure (i.e. as five replicates within each cave). First, nMDS was used to generate graphical summaries of the relationships in community structure among caves. Samples with higher similarity are plotted closer to one another; a stress value indicates how accurately the plot represents the actual multivariate relationships (values <0.1 are optimal, values >0.2 are less useful; Clarke & Warwick, 2001). Bubble plots were incorporated into the nMDS plots to illustrate trends in community composition among caves. Second, one-way ANOSIMs were performed on the mean taxon biomass data to test for an effect of cave identity on community structure. Finally, the SIMPER routine was used to identify those taxa that contributed disproportionately to the overall dissimilarity in community structure among caves. Two values were calculated in this analysis: i) the average contribution of an individual taxon to overall dissimilarity and ii) its standard deviation across samples. The ratio of these two values indicates how consistently the taxon discriminates among caves.

Results

Mean daily water temperature and NH_4^+-N, NO_3^--N, and SRP concentrations varied little among caves or incubation period (Table 2). The coefficients of variation (CV) for the daily water-level profiles in Tony Sinks, Salt River, Jess Elliot and Big Mouth caves were 0.13, 0.15, 0.02 and 0.13, respectively. The CV for Big Mouth Cave was driven by three dates near the end of the study. Removing these dates decreased the CV to 0.02. Tony Sinks Cave (807 g m^{-2}) had the highest amount of organic matter and Jess Elliot Cave (1 g m^{-2}) the lowest. Organic matter abundance was significantly different among caves ($F_{3,288} = 30.6$, $P < 0.001$; Fig. 1a). Organic matter did not vary significantly among sampling dates within each cave ($F_{9,288} = 1.7$, $P = 0.10$; Fig. 1b) and the cave × date interaction was not significant ($F_{19,288} = 0.8$, $P = 0.75$). The July peak in organic matter in Big Mouth Cave was driven by a single sample, rather than an increase in organic matter in all samples.

Macroinvertebrate community structure

Mean total macroinvertebrate biomass per bag for corn litter ranged from 0 mg DM in Big Mouth Cave to 31.4 mg DM in Tony Sinks Cave, and from 0 mg DM in Salt River Cave and 17.1 mg DM in Tony Sinks Cave for maple litter. With the exception of corn litter in coarse-mesh bags, litter bags in Tony Sinks Cave contained two to 20 times more macroinvertebrate biomass than the other caves by the end of each incubation period (Fig. 2 a-d).

Thirty taxa were identified among the four caves (Table 1). Only three taxa were characterized as hypogean (*Caecidotea*, *Stygobromus* and *Crangonyx*). The mean biomass of hypogean taxa, which were dominated by *Caecidotea*, ranged from 0 to 3.1 mg DM per litter bag. The average contribution of hypogean taxa to total biomass per litter bag was 16% in Big Mouth Cave, 9% in Jess Elliot Cave, 14% in Salt River Cave and 10% in Tony Sinks Cave.

Several taxonomic groups (Chydoridae, Ostracoda, Cyclopoida, Harpacticoida and Oligochaeta) are known to contain both epigean and hypogean species (Romero, 2009). Thus, specimens belonging to these groups could not be confidently categorized as hypogean or epigean. Collectively, mean biomass per litter bag of these taxonomic groups ranged from 0 to 14.3 mg DM. On average, these taxa accounted for 22% of total biomass per litter bag in Big Mouth Cave, 12% in Jess Elliot Cave, 17% in Salt River Cave and 22% in Tony Sinks Cave.

Five taxa were identified as potential leaf shredders (*Allocapnia*, *Polypedilum*, *Chironomus*, *Stygobromus* and *Crangonyx*), of which the latter two are hypogean. Potential shredder biomass was dominated by *Polypedilum* and averaged from 0 to 18.7 mg DM per litter bag. On average, potential shredder biomass accounted for 5% of total biomass per litter bag in

Big Mouth Cave, 2% in Jess Elliot Cave, 9% in Salt River Cave and 17% in Tony Sinks Cave (Table 1).

The nested two-way ANOSIMs (factors nested within each cave) of the entire biomass data set ($n = 800$) showed that the following five variables did not significantly affect community structure within each cave ($P > 0.05$): incubation period (R-statistic = 0.02), mesh size (R-statistic = 0.04), station identity (five per cave, R-statistic = 0.01), litter species (R-statistic = 0.01) and date within incubation period (R-statistic = 0.11). The reduced data set for each cave obtained by averaging the biomass of each taxon from both litter species, mesh sizes and incubation periods across each station was very similar to the original data set (RELATE, Spearman's rho = 0.70, $P < 0.05$).

Animal community structure differed significantly among caves (R-statistic = 0.65, $P = 0.001$), with pair-wise ANOSIM (R-statistics = 0.53 to 0.90, $P = 0.008$ to 0.016) and nMDS both indicating significant differences in community structure (Fig. 3). Six taxa accounted for 79 to 87% of the overall dissimilarity among all pair-wise cave comparisons (Fig. 4). *Lirceus* (Isopoda) occurred only in Tony Sinks Cave, which also contained an unusually high biomass of macroinvertebrates, particularly Oligochaeta and *Polypedilum* (Fig. 4). Among the remaining caves, Salt River Cave had a high biomass of Tanypodinae genus A and *Polypedilum*, while Tanypodinae genus B was the dominant taxon in Jess Elliot Cave. Macroinvertebrate biomass in Big Mouth Cave was dominated by *Caecidotea* (Fig. 4). The biomass of *Caecidotea* (Isopoda), the only member of this group of taxa classified as hypogean, was similar among caves (Fig. 4).

Litter breakdown rates

The breakdown rate of corn litter in coarse mesh bags (mean 0.007 d^{-1}, range 0.004 to 0.012) was, on average, double that in fine mesh bags (mean 0.003 d^{-1}, range 0.002 to 0.004;

Figs. 5a, b). The breakdown rate of red maple litter in coarse mesh bags (mean 0.004 d^{-1}, range 0.001 to 0.012) was, on average, four times faster than that in fine mesh bags (mean 0.001 d^{-1}, range 0.001 to 0.003; Figs. 5a, b).

Breakdown rates within each mesh size were similar between incubation periods in each cave (three-way ANOVA *post-hoc* pair-wise comparisons, $P > 0.05$), allowing incubation periods within each mesh size and cave to be pooled. Two-way ANOVAs conducted on cave and mesh size gave similar results for both corn and red maple litter: cave ($F_{3,72} = 13.7$, $P < 0.001$ for both litter types), mesh size ($F_{1,72} = 349$ and 100, corn and maple, respectively, $P < 0.001$), and cave × mesh-size interaction ($F_{3,72} = 4$, $P < 0.02$ for both litter types). Breakdown rates of leaf litter in fine-mesh bags were slower than those in coarse-mesh bags (Fig. 5). The average corn litter breakdown rate ($k = 0.005$ d^{-1}) was faster than that of red maple ($k = 0.003$ d^{-1}; Fig. 5 [paired *t*-test (79) = 14.1, $P < 0.001$]).

Corn litter breakdown rate was similar among caves for each mesh size ($P > 0.05$, Fig. 5a), except for fine-mesh bags in Big Mouth Cave ($P = 0.03$). While the fine-mesh bags containing corn litter in Big Mouth Cave had a statistically slower breakdown rate, the size of the difference (~0.001 day^{-1}) was small (Fig. 5a). Maple litter breakdown rate was less consistent than that of corn litter (Fig. 5b). Maple in fine-mesh bags had a similar rate among caves ($P > 0.05$), and the rate was also similar to that in coarse-mesh bags in Big Mouth and Jess Elliot caves ($P > 0.05$). Breakdown of maple litter in coarse-mesh bags was fastest in Salt River and Tony Sinks caves and slowest in Big Mouth and Jess Elliot caves ($P > 0.05$). Mean organic matter abundance per cave was not a significant predictor of breakdown rates of either litter type or mesh size ($F_{1,2} = 0.05$ to 4.93, $r^2 = 2$ to 71%, $P = 0.2$ to 0.9). Furthermore, mean macroinvertebrate biomass was not a significant predictor of breakdown rates of either litter type in coarse- or fine-mesh bags ($F_{1,2}$ = 0.06 to 8.10, r^2 = 3 to 80%, P = 0.1 to 0.8).

Discussion

A large range (~0-850 g AFDM m^{-2}) in the abundance of organic matter among the cave streams was found in this study. A few previous studies have reported the abundance of organic matter in cave streams from *c.* 30 to 130 g AFDM m^{-2} (Simon & Benfield, 2001, 2002; Simon, Pipan & Culver, 2007). The large range in organic matter abundance shown in this study illustrates how resource supply can both vary greatly among caves within a limited geographic area and in some cases can be similar to that in many surface stream types (e.g. arid/semi-arid, deciduous and boreal streams; Jones, 1997). Factors that contribute to this variability in organic matter abundance among cave ecosystems are unknown, but are likely to be a combination of landscape features (e.g. topology and plant community structure), cave structure (e.g. depth of cave and size of voids and fractures in the surrounding bedrock) and climate (e.g. precipitation and hydrology). For example, the large quantities of organic matter in Tony Sinks Cave were probably the result of strong surface connections. This cave contained several entrances, both vertical and horizontal, that intersected intermittent stream channels. Additionally, large ceiling fissures were present in portions of the cave, below which large deposits of organic matter were observed.

In surface streams, the quantity of organic matter is known to influence macroinvertebrate biomass (Dobson & Hildrew, 1992; Wallace *et al*., 1999; Negishi & Richardson, 2003). In this study, however, no significant correlation was found between ambient organic matter biomass and macroinvertebrate biomass in litter bags. Nevertheless, Tony Sinks Cave had both the largest amount of organic matter and the highest macroinvertebrate biomass per litter bag. Similarly, Huntsman *et al.* (2011b) reported that benthic macroinvertebrate biomass was higher in cave streams with higher quantities of organic matter. Together, these studies show that organic matter abundance can influence macroinvertebrate biomass in cave streams.

Epigean taxa dominated the communities in all caves in this study, while potentially shredding taxa contributed little to total biomass. Other litter breakdown studies in cave streams have also reported a relatively high biomass of epigean taxa, with shredding taxa composing a small percentage of the overall community (Galas *et al.,* 1996; Brussock *et al.,* 1988). The absence of shredding taxa in cave systems is probably due to the combined limitation in both habitat access and resource availability (e.g. coarse particulate organic matter). Active colonization by shredding insect taxa via oviposition is reduced in cave systems because of the limited number of openings to surface habitats. Additionally, perennial aquatic surface habitats are not always present in recharge areas, thereby limiting the possibility of cave streams being actively (e.g. via drift) or passively (e.g. displacement due to floods) colonized by both insect and crustacean (e.g. amphipods) shredding taxa. Simon & Benfield’s (2001) study illustrates how connectivity and resource availability interact to structure cave stream communities. Cave streams with direct upstream surface connections received higher inputs of coarse particulate organic matter and had higher shredder abundances (epigean and stygophilic taxa) than disconnected cave streams that only received fine particulate organic matter. Similar to Simon & Benfield (2001), the highest shredder biomass was found in Tony Sinks Cave, which had both the highest organic matter abundance and strongest apparent surface connection.

The breakdown of organic matter in cave and surface streams is mediated by the same processes, including leaching, microbial processing, consumption, and physical fragmentation due to high water velocities (Webster & Benfield, 1986; Simon & Benfield, 2001). Despite differences among caves in organic matter abundance and total macroinvertebrate biomass per litter bag, breakdown rates within each litter type and mesh size were similar except for red maple in coarse-mesh bags in Salt River and Tony Sinks caves. An absence of shredders can affect litter breakdown rates in surface systems (Wallace *et al.,* 1986; Hieber & Gessner, 2002; Eggert & Wallace, 2003) and previous studies in cave systems have attributed slow leaf litter breakdown rates to the absence of large shredding taxa (Brussock *et al*., 1988; Galas *et al*., 1996; Simon & Benfield, 2001). The generally low shredder biomass found in the present study was likely to be a significant factor driving similarities in breakdown rates among cave streams.

Breakdown rates of corn and red maple litter spanned a wide range of values ($k = 0.001$ to $0.012\ d^{-1}$). Mesh size significantly affected breakdown rate, which was generally faster and more variable in coarse- than in fine-mesh litter bags. The difference in breakdown rate among mesh sizes was unlikely to have been due to shredder exclusion because: i) shredder biomass was universally low (2 -17% of total biomass per litter bag) among all cave stream communities, ii) large-bodied shredders (e.g. crayfish, plecopterans, trichopterans, amphipods) were not well represented in the coarse-mesh litter bags, and iii) the dominant potential shredder was a small-bodied dipteran (*Polypedilum*) capable of colonizing both coarse and fine mesh litter bags (Table 1). Slower litter breakdown in fine mesh bags have been attributed to anaerobic conditions due to reduced gas exchange (Cummins *et al.*, 1980, Webster & Benfield, 1986). Compaction of leaf litter occurred within the litter bags from this study, which potentially generated anaerobic conditions in the centre of fine-mesh bags. However, macroinvertebrate biomass in fine mesh bags was similar to or higher than in coarse-mesh bags and there was no evidence of anaerobic conditions (e.g. blackened litter). A more probable cause of the slower breakdown in fine-mesh bags was the greater retention of litter fragments from physical and invertebrate processing. The relatively high breakdown rates of litter in coarse-mesh bags in Tony Sinks and Salt River were probably due to the effects of flooding. The large coefficient of variation (CV) for discharge in these systems indicates that floods occurred frequently, potentially accelerating coarse-mesh breakdown rates via fragmentation and abrasion due to high water velocities (Canton & Martinson, 1990).

Mean corn litter breakdown rates estimated in this study were two to 20× lower than past estimates for surface streams (Table 3). Not surprisingly, the available surface studies of corn litter breakdown have been in agricultural streams (Rosi-Marshall *et al.*, 2007; Griffiths *et al.*, 2009; Swan *et al.*, 2009), with higher nutrient concentrations (e.g. N and P) and shredder abundances than the cave streams used in this study. The oligotrophic state of the cave streams in this study, coupled with low shredder biomass, probably contributed to the relatively low breakdown rates of corn litter measured in this study.

Mean breakdown rates for maple litter in the study streams in this study were either similar to or lower than estimates made using similar methods in surface systems (Table 3) with comparable nutrient concentrations. Shredder abundances were higher in the surface stream studies, which is likely to have contributed to faster litter breakdown. Litter breakdown estimates from this studies fine-mesh bags were two to five times slower than estimates reported in a study by Gulis & Suberkropp (2003) that used 1-mm mesh bags to minimize macroinvertebrate colonization. While the smaller mesh size used in this study may have contributed to slower breakdown via increased fragment retention, limited microbial colonization may have also played a role. Microbial abundance and diversity have been reported to be lower in both hyporheic habitats and aquifers than in surface streams (Ellis, Stanford & Ward, 1998;

Crenshaw, Valett & Tank, 2002; Krauss *et al*., 2003). Simon & Benfield (2001) also found that fungal biomass was lower on leaves in cave streams without direct upstream surface connections. Thus, the relatively low rates of litter breakdown in fine-mesh bags in this study may have been caused by low microbial activity.

Environmental variables (e.g. light and temperature) within cave ecosystems have traditionally been viewed as stable compared to surface systems (Juberthie, 2001), which should reduce seasonal patterns of ecosystem processes. In cave systems, temporal stability in the structure of macroinvertebrate communities and the ecological processes they mediate is probably affected substantially by reduced seasonality in environmental cues (e.g. light and temperature), continuous resource availability (which may be small or large) and reduced immigration of epigean taxa due to limited surface connectivity. Accordingly, organic matter abundance, macroinvertebrate community structure and litter breakdown rates did not differ between incubation periods in this study. Unlike most cave streams, many temperate surface systems receive seasonal pulses of detritus following plant senescence (e.g. forest headwater streams). Seasonal changes in temperature, light, hydrology and resource availability have been linked to temporal patterns in community structure, which have in turn been shown to affect organic matter processing in surface systems (e.g. Benstead & Huryn, 2011). The macroinvertebrate communities of cave streams, which contain few shredders (Brussock *et al*., 1988; Galas *et al*., 1996; Simon & Benfield, 2001), appear to process organic matter at a roughly constant rate year-round. To date, no other studies have been published that have examined temporal patterns in groundwater communities. Similar to this study, Farnleitner *et al*. (2005) showed that microbial communities in two karst springs showed little to no temporal variability in structure, suggesting that their ecological function may also have been stable through time.

Historically, limited resource inputs have been considered to be the primary factor influencing the ecology and evolution of hypogean communities. The results from this study offered mixed support for this hypothesis. Organic matter abundance was correlated neither with consumer biomass in litter bags nor litter breakdown rates. However, Tony Sinks Cave had the highest organic matter abundance and consumer biomass, suggesting that resource inputs can influence cave community biomass without corresponding effects on function (e.g. inclusion of shredding taxa). Organic matter abundance does not appear to be the primary factor influencing the presence or absence of taxa among caves in this study because Jess Elliot, Salt River, and Big Mouth caves all contained different communities despite marginal differences in organic matter abundance. However, the distinct community found in Tony Sinks Cave (e.g. indicated by its distinct grouping in the nMDS plots, Fig. 3) does suggest that organic matter abundance may play some role. Thus, in addition to organic matter abundance, other cave (e.g. surface connectivity, cave morphology and recharge area) and surface (e.g. topography and vegetation type) characteristics should be quantified to understand better the factors controlling cave community composition.

Hypogean taxa have traditionally been the focus of cave studies. Nevertheless, this study suggests that their role in energy flow, nutrient cycling and food web dynamics might be relatively small in many cave systems. Hypogean taxa represented only a small proportion of the consumer biomass found in this studies litter bags. In another recent study, Huntsman *et al.* (2011b) showed that epigean prey contributed *c.* 50 to 100% of the production of the obligate cave salamander *Gyrinophilus palleucus* (McCrady) in two southeastern U.S.A. caves. Thus, the inclusion of the entire community, epigean and hypogean, in studies of cave ecosystems will be necessary to understand the ecological processes that occur within them.

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Table 1. Mean (1 S.D.) macroinvertebrate biomass (mg dry mass) in leaf packs incubated in four cave streams in northeastern Alabama and southeastern Tennessee, U.S.A. Note: *** indicates hypogean (i.e. cave-adapted) taxa and ^^^ indicates potential shredder taxa.

	Big Mouth	Jess Elliot	Salt River	Tony Sinks
Nematoda	< 0.001 (<0.001)	< 0.001 (<0.001)	< 0.001 (<0.001)	< 0.001 (<0.001)
Mollusca				
Bivalvia				0.001 (0.011)
Gastropoda				< 0.001 (0.002)
Oligochaeta	0.002 (0.013)	< 0.001 (0.002)	0.007 (0.066)	0.953 (5.792)
Ostracoda	< 0.001 (0.002)			0.042 (0.157)
Cladocera				
Chydoridae	0.001 (0.008)		< 0.001 (0.001)	0.001 (0.005)
Non-chydorid Cladocera				< 0.001 (0.001)
Copepoda				
Cyclopoida	0.008 (0.021)	0.006 (0.016)	0.013 (0.032)	0.024 (0.049)
Harpacticoida	< 0.001 (0.001)	< 0.001 (0.001)	< 0.001 (<0.001)	< 0.001 (0.002)
Malacostraca				
Isopoda				
*Caecidotea****	0.209 (0.808)	0.229 (0.953)	0.372 (1.176)	0.298 (1.195)
Lirceus				0.273 (1.102)
Amphipoda				0.036 (0.473)
*Crangonyx****^^^		0.011 (0.121)		0.006 (0.073)
*Stygobromus****^^^		0.008 (0.112)		

Insecta				
Plecoptera				
Allocapnia^^^	0.002 (0.032)		0.003 (0.046)	
Ephemeroptera				
Eurylophella	0.002 (0.023)	0.002 (0.023)	0.016 (0.120)	0.028 (0.230)
Paraleptophlebia	0.002 (0.029)		0.005 (0.071)	0.083 (0.421)
Diptera				
Dixidae	< 0.001 (0.002)			
Ceratopogonidae				0.001 (0.015)
Chironomidae				
Tanypodinae				
Genus A	0.003 (0.034)	0.044 (0.137)	0.107 (0.335)	0.120 (0.363)
Genus B	0.008 (0.101)	0.306 (0.880)	0.017 (0.089)	0.297 (0.671)
Tanytarsini				
Micropsectra	0.003 (0.013)		0.001 (0.007)	0.003 (0.018)
Chironomini				
Polypedilum^^^	0.013 (0.062)	0.001 (0.010)	0.131 (0.590)	1.683 (5.157)
Paratendipes	0.008 (0.038)		0.002 (0.023)	0.009 (0.078)
Chironomus^^^	0.001 (0.011)		0.010 (0.085)	0.006 (0.063)
Dicrotendipes	< 0.001 (0.006)			
Orthocladiinae				
Corynoneura	< 0.001 (0.005)			< 0.001 (0.003)
Parametriocnemus	0.005 (0.038)	0.017 (0.073)	< 0.001 (0.003)	0.023 (0.110)
Heleniella	0.001 (0.005)			0.005 (0.039)
Rheocricotopus			0.003 (0.025)	0.003 (0.026)
Diplocladius	0.005 (0.042)	< 0.001 (0.002)	0.005 (0.039)	
Eukiefferiella	0.001 (0.008)	0.002 (0.013)		

Table 2. Mean (±1 S.D.) daily temperature and NH_4^+-N, NO_3^--N, and soluble reactive phosphorus (SRP) concentrations in four cave streams in northeastern Alabama and southeastern Tennessee, U.S.A. during two incubation periods. Inc = incubation period, Temp = temperature, BD = below detection.

Cave	Inc	Temp (^oC)	NH_4^+-N ($\mu g\ L^{-1}$)	NO_3^--N ($\mu g\ L^{-1}$)	SRP ($\mu g\ L^{-1}$)
Big Mouth	1	13 ± 1	6 ± 5	367 ± 139	4 ± 5
	2	13 ± 1	11 ± 18	207 ± 117	2 ± 2
Jess Elliot	1	13 ± 0	BD	242 ± 50	5 ± 6
	2	14 ± 0	BD	231 ± 63	2 ± 3
Salt River	1	12 ± 1	3 ± 2	286 ± 83	5 ± 2
	2	13 ± 1	4 ± 2	265 ± 27	4 ± 3
Tony Sinks	1	14 ± 1	4 ± 3	259 ± 34	2 ± 3
	2	14 ± 1	7 ± 4	284 ± 39	1 ± 2

Table 3. Selected published breakdown rates (k, d^{-1}) for corn (*Zea mays*) and red maple (*Acer rubrum*) from surface and cave streams. Note that in Simon & Benfield's (2001) study, breakdown rates were examined in caves streams that had either "direct" (i.e. upstream cave entrances or sinkholes) or "indirect" (i.e. small cracks and fissures) connections to surface environments.

Litter type	k (d^{-1})	Habitat	Mesh size (mm)	Study
Non-BT corn (*Zea mays*)	0.015	Surface	10 x 10	Rosi-Marshall *et al.* (2007)
	0.015	Surface	10 x 2.5	Griffiths *et al.* (2009)
	0.015 - 0.07	Surface	7 x 11	Swan *et al.* (2009)
	0.004 - 0.012	Cave	10 x 8	This study
	0.002 - 0.004	Cave	0.5 x 0.5	This study
Red Maple (*Acer rubrum*)	0.007 - 0.014	Surface	5 x 5	Benfield *et al.* (2001) and citations therein
	0.008 - 0.009	Surface	5 x 5	Eggert & Wallace (2003)
	0.005	Surface	1 x 1	Gulis & Suberkropp (2003)
	0.005 - 0.025	Surface	5 x 5	Greenwood *et al.* (2007)
	0.007 - .015	Surface	5 x 5	Webster *et al.* (2001) and citations therein
	0.001 - 0.012	Cave	10 x 8	This study
	0.001 - 0.003	Cave	0.5 x 0.5	This study

Figure 1. (a) Box and whisker plot of organic matter (OM) abundance in four cave streams in northeastern Alabama and southeastern Tennessee, U.S.A. Grey boxes are the 25th and 75th percentile and whiskers are 5th and 95th percentiles. The dashed and solid lines within each box are the mean and median, respectively. Significant differences (*P* < 0.05) among caves are indicated by different letters. (b) Time series of OM abundance in each cave. Vertical dashed line separates incubation periods: left = incubation period 1 (Jan to Jul 2008), right = incubation period 2 (Jul to Dec 2008, or to Jan 2009 for Tony Sinks Cave).

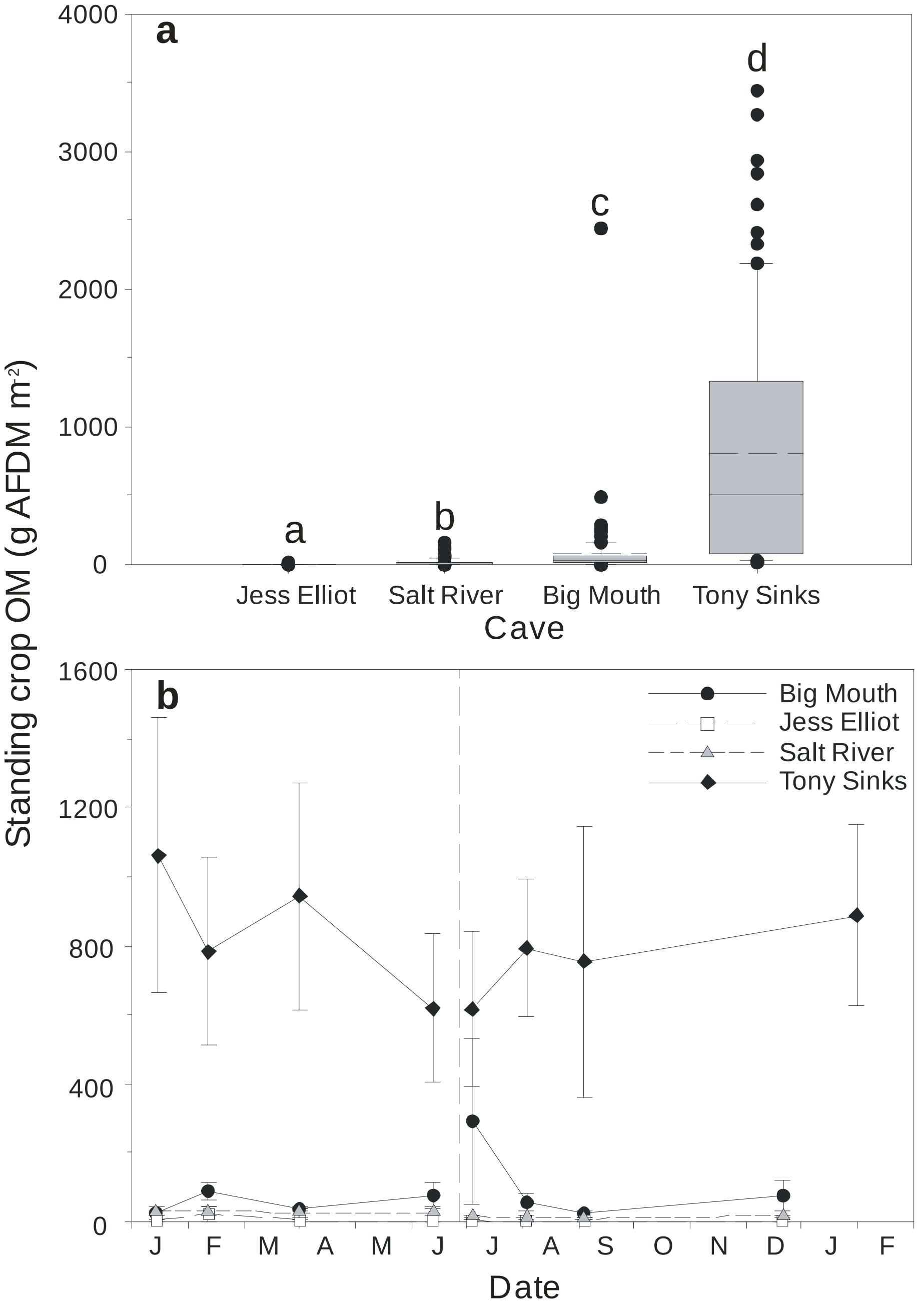


Figure 2. Mean (±1 SE) macroinvertebrate biomass per g ash-free dry mass (AFDM) of leaf litter remaining in litter bags placed in four cave streams in northeastern Alabama and southeastern Tennessee, U.S.A. (a, b) Coarse-mesh litter bags containing maple and corn litter, respectively. (c, d) Fine-mesh litter bags containing maple and corn litter, respectively. Vertical dashed line separates incubation periods: left = incubation period 1 (Jan to Jul 2008), right = incubation period 2 (Jul to Dec 2008, or to Jan 2009 for Tony Sinks Cave).

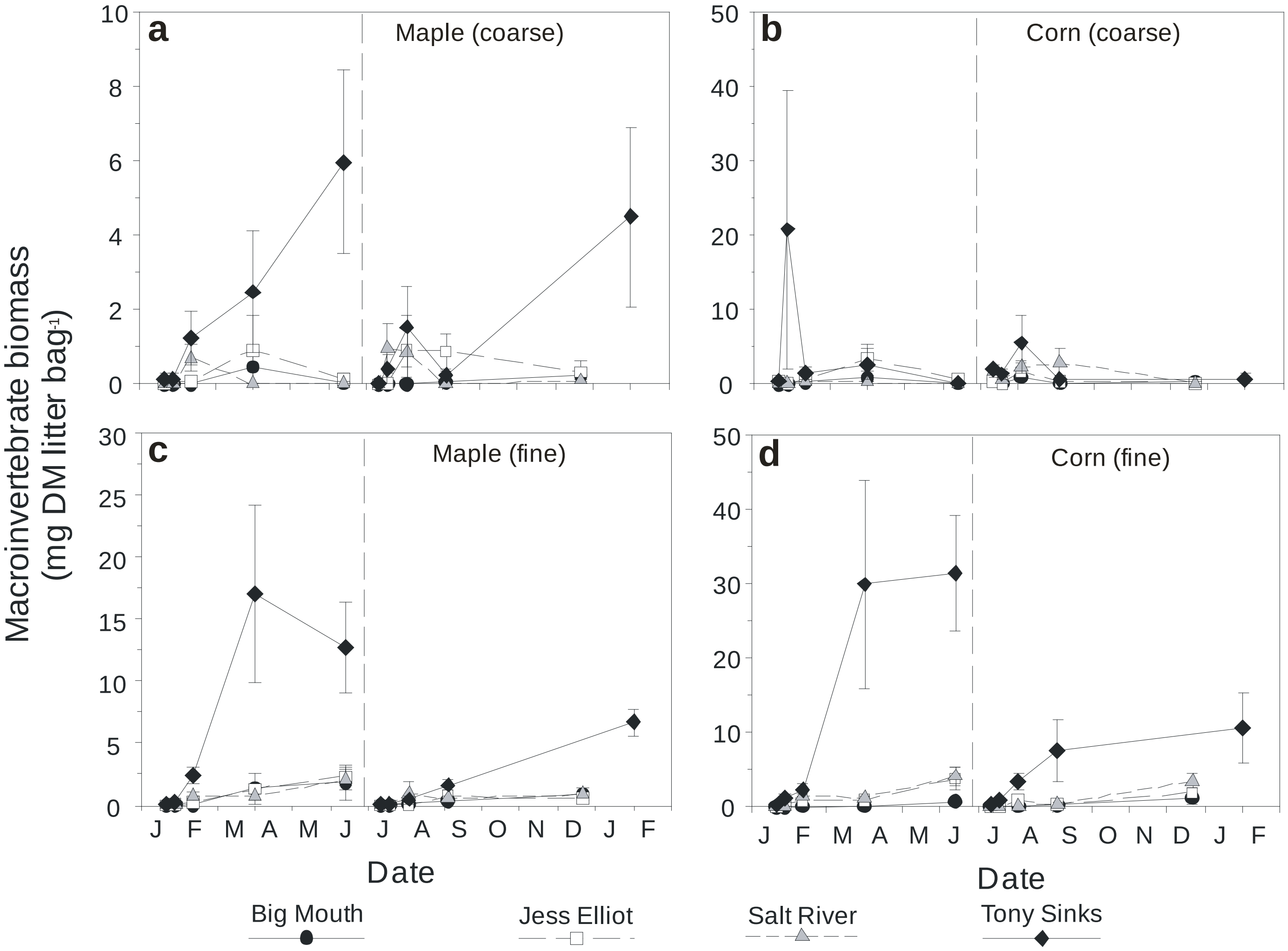


Figure 3. Non-metric multidimensional scaling ordination plot of macroinvertebrate litter bag communities from four cave streams in northeastern Alabama and southeastern Tennessee, U.S.A. Data points are based on $\log_{10}$-transformed macroinvertebrate biomass data that were averaged across stations within each cave (see text).

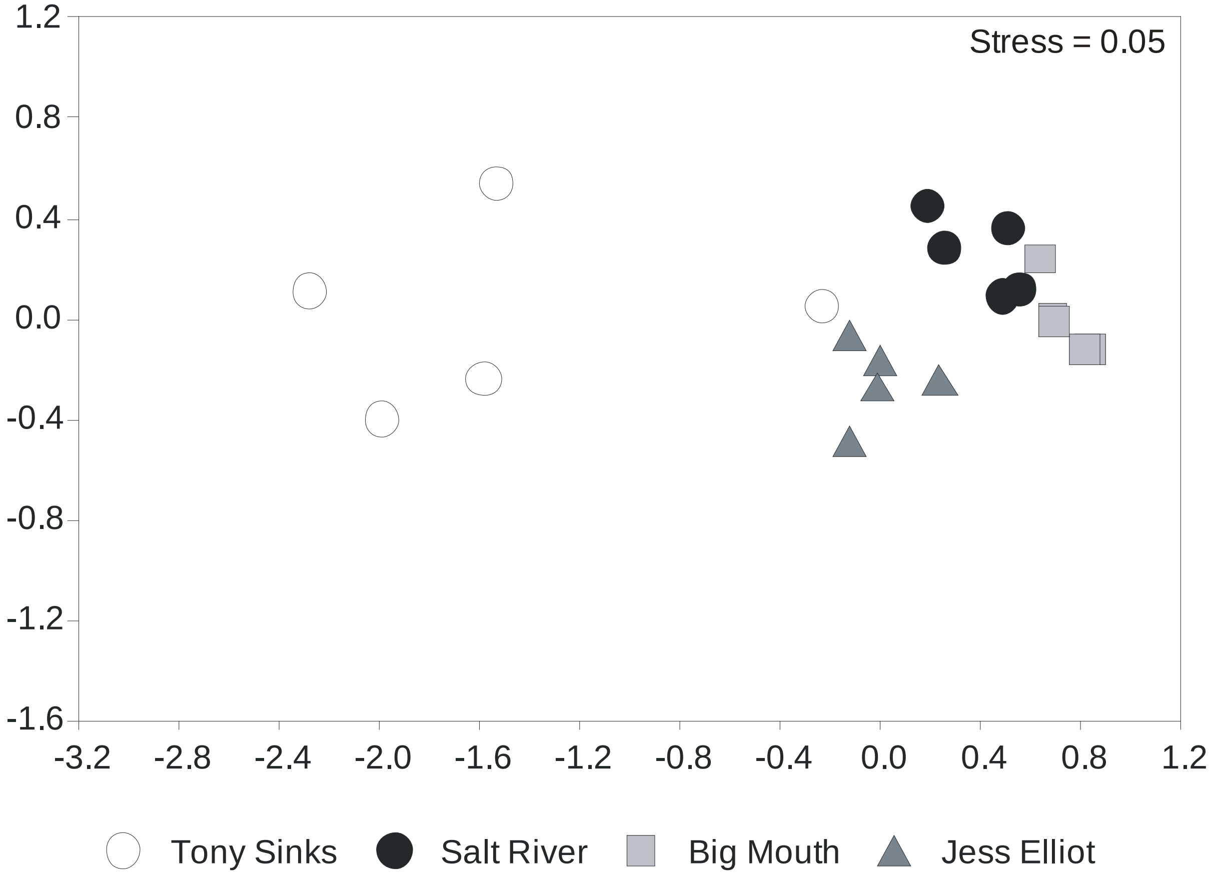


Figure 4. Non-metric multidimensional scaling ordination plots based on $\log_{10}$-transformed biomass of six taxa that contributed 79 to 87% to the overall dissimilarity among all pair-wise cave comparisons between cave sites. The diameter of each circle is proportional to the taxon's biomass in each cave. (a) Oligochaeta, (b) Tanypod genus A, (c) *Caecidotea*, (d) Tanypod genus B, (e) *Lirceus* and (f) *Polypedilum*. Stress = 0.05 for all figures.

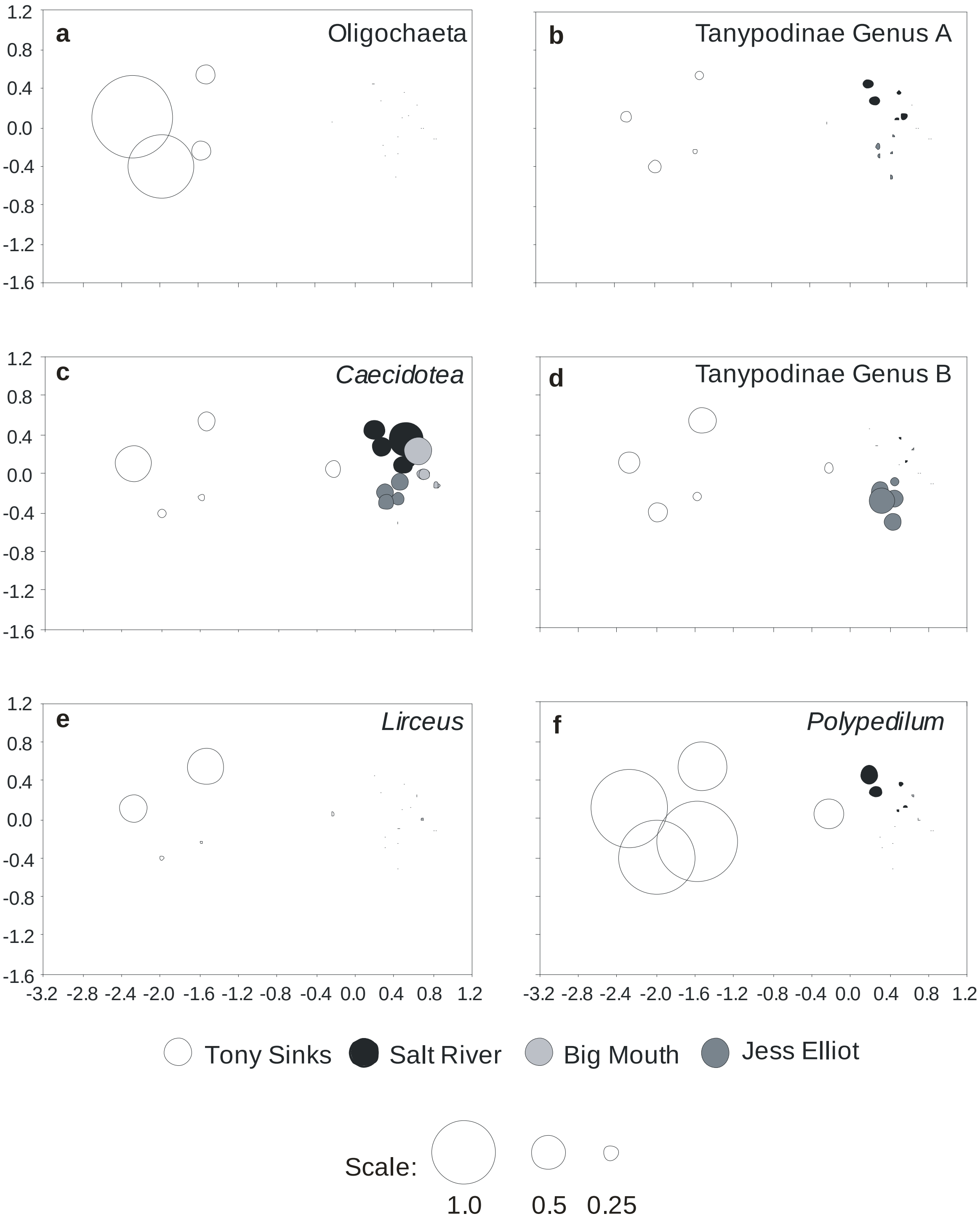
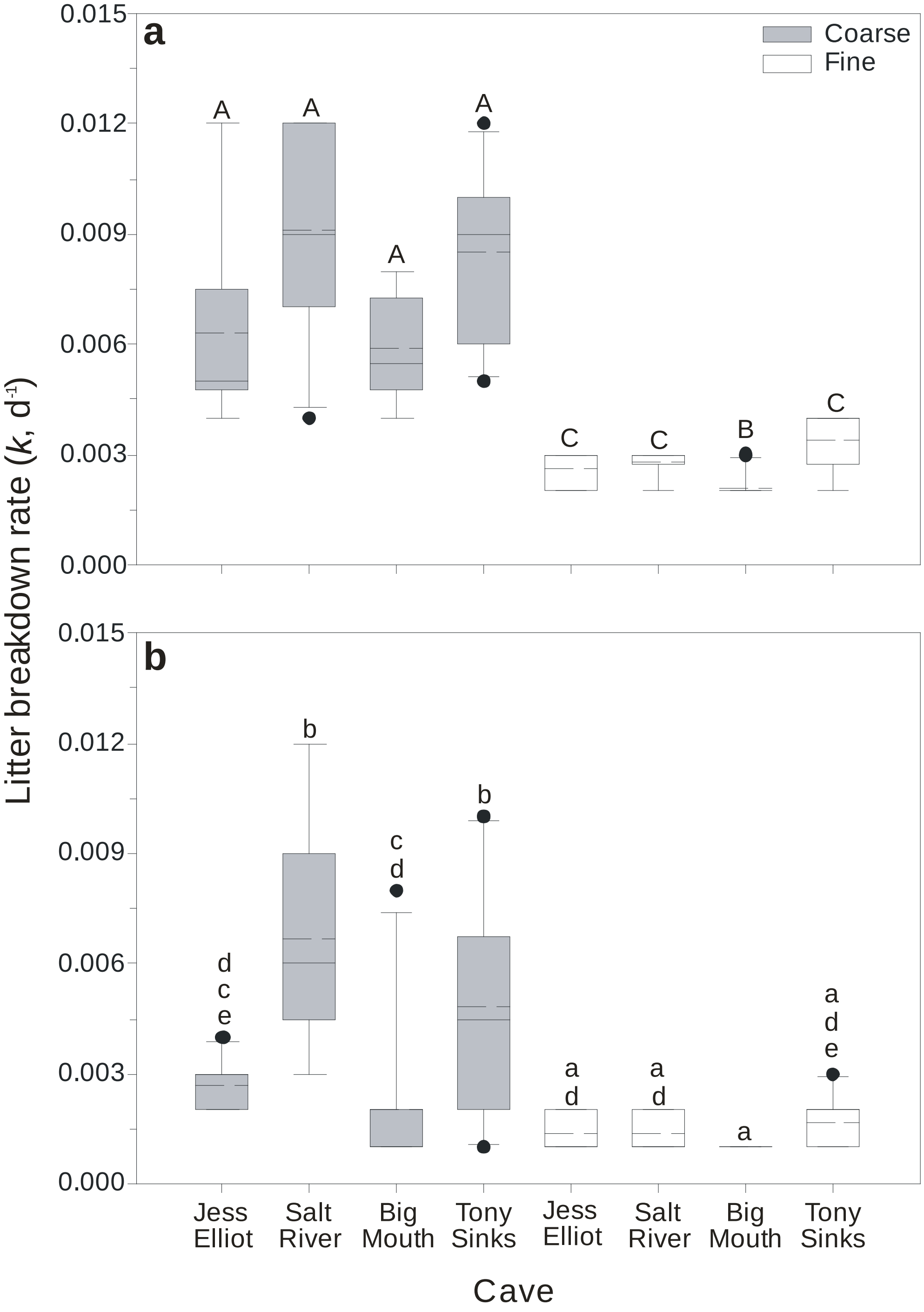


Figure 5. Box and whisker plot of litter breakdown rate (*k*, d^{-1}) of corn (a) and red maple (b) in coarse- and fine-mesh bags in four cave streams in northeastern Alabama and southeastern Tennessee, U.S.A. Boxes are the 25th and 75th percentile and whiskers are 5th and 95th percentiles. The dashed and solid line within each box is the mean and median, respectively. Significant differences ($P < 0.05$) among caves and mesh sizes are indicated by different letters. Note that different case letters do not indicate significant differences between litter types.



CHAPTER 3

TESTING ENERGY LIMITATION OF A CAVE STREAM ECOSYSTEM USING A WHOLE-REACH DETRITUS AMENDMENT

Abstract

Energy limitation has long been considered the primary factor influencing *in situ* evolutionary and ecosystem processes in cave ecosystems. Few studies, however, have provided adequate data to test this hypothesis, either because they have focused on specific ecosystem processes or trophic levels, or because they involved factors (e.g., heterogeneous organic pollution) that confound data interpretation. In this study, the energy-limitation hypothesis in cave ecosystems was tested explicitly using a detrital manipulation experiment. From February 2010 to February 2011, a 100-m reach of a carbon-poor cave stream was amended with corn-litter and the response in consumer biomass was followed relative to that of an upstream reference reach. During one year of pre-manipulation (February 2009 to January 2010), mean standing crop organic matter was 19 to 34 g ash-free dry mass [AFDM] m^{-2}. The corn litter amendment significantly increased mean standing crop organic matter in the manipulation reach to 423 g AFDM m^{-2}. Total macroinvertebrate biomass increased by more than 5 times following the litter amendment. Stable isotope analyses indicated that corn-derived carbon represented 16-73% of macroinvertebrate biomass depending on taxon, indicating that increases in consumer biomass were driven by assimilation of corn-derived carbon. However, biomass of facultative surface species significantly increased following the amendment, while the biomass of obligate cave species remained unchanged. Facultative species are adapted to energy-rich surface

streams, while the obligate-cave species are adapted to survive in the energy-poor cave environment. These differences in evolutionary history likely contributed to the differential response to the corn litter amendment. While cave communities *per se* have the ability to exploit short-term increases in energy availability, species-specific responses are dictated by differing selective pressures and resulting life-history traits.

Introduction

Community ecologists have identified several mechanisms that influence the structure and function of ecosystems, including predator-prey interactions, disturbance regime, and resource quantity and quality (Morin 2011). Likewise, evolutionary biologists have identified many of the same mechanisms as important drivers in both morphological and life history evolution (see Stearns 2000, Reznick et al. 2001). Thus, an understanding of evolutionary history can provide insight into the factors that influence species abundance, diversity, or composition within communities. This study illustrates how cave ecosystems are an ideal setting to examine the interplay between evolutionary history and contemporary community structure.

In cave ecosystems, there is no photosynthetic primary production and chemolithoautotrophy appears to be limited to relatively few systems (Sarbu et al. 1996; Engel et al. 2004), making most communities entirely reliant on allochthonous sources of organic matter (100% donor-control; Polis and Strong 1996). Limited surface connectivity reduces the quantity of detrital inputs, while their quality is diminished by prior biologic processing in soil horizons and transport flow paths. Thus, limitation mediated by the availability of organic carbon, as opposed to via nitrogen (N) or phosphorus (P) supply, has historically been considered the primary factor influencing both ecological and evolutionary processes in cave ecosystems (Culver et al. 1995; Graening and Brown 2003; but see Schneider et al, 2010).

Regardless of strong selection pressures, cave systems can harbor a diverse array of vertebrate and invertebrate taxa that can be categorized into two groups, each with a distinct evolutionary history. One subset of the cave community is represented by obligate cave species, which share a similar set of characteristics (termed troglomorphic traits) that are presumed to be adaptations to the low-energy cave environment. These traits include K-selected life history characteristics (e.g. reduced growth rate and fecundity) and relatively low metabolic rates (Poulson & Lavoie, 2001; Hüppop, 2001, 2005). Another subset of the cave community consists of facultative cave species (e.g. surface species) that enter caves either actively (e.g., migratory or foraging movements) or passively (e.g., washed in during flood events). Some facultative species can survive and reproduce in both cave and surface environments, while others are "transients," which subsequently either exit the cave or enter its food web as prey or carrion (Poulson & Lavoie, 2001; Hüppop, 2001, 2005).

Under conditions of resource limitation, obligate cave species are assumed to have a competitive advantage due to their troglomorphic traits. However, when resource levels increase, facultative species may out-compete obligate cave species because of their ability to exploit available resources at higher rates (e.g. via higher growth rates and fecundities). Support for this hypothesis comes from reports of community shifts following incidental inputs of organic pollutants (Sinton 1984; Smith et al. 1986; Madsen et al. 1991; Notenboom et al. 1994; Simon and Buikema 1997; Sket 1999, 2005; Wood et al. 2002; Culver and Pipan 2009). Results from these studies, however, are confounded because organic pollution is typically a mixture of organic and inorganic material (i.e., organic matter, dissolved nutrients, microbes, and toxins), making it impossible to discern which component or combination of components causes changes in recipient communities.

More recent observational and experimental studies also support an energy-limitation hypothesis (see Datry et al. 2005; Cooney and Simon 2009; Schneider et al. 2011; Huntsman et al. 2011b). However, these studies only examined a portion of the community (e.g. only microbes or metazoan consumers).

In this study we tested the energy-limitation hypothesis directly by amending a cave stream with detritus and then assessing the response of the entire cave stream food web, from microbes to top predators. We hypothesized that the detritus amendment would reduce energy limitation, resulting in variable population-level responses depending on different life history strategies (e.g. obligate cave versus facultative species). Because of the competitive differences within the cave community, we predicted that the carbon addition would increase biomass of both obligate and facultative cave species, but responses of facultative species would be of greater magnitude.

Materials and Methods

Study site

This study was conducted in Bluff River Cave, Jackson Co., Alabama, U.S.A. The recharge area is mostly forested (mixed hardwoods) with very little urbanization; anthropogenic impacts on water quality therefore were assumed to be minimal. Bluff River Cave has ~1200 m of large passage (height 20 m, width 10 m), of which 1000 m is stream channel with a depth ranging from ~2 cm to 1.75 m during base-flow. Water depth can exceed 3 to 9 m during spates, however.

Bluff River Cave contains a diverse assemblage of obligate cave and facultative species, including copepods, amphipods, isopods, larval insects (Chironomidae, Ephemeroptera and Plecoptera), the obligate cave crayfish *Cambarus hamulatus*, the facultative cave crayfish

Cambarus tenebrosus, the obligate cave salamander *Gyrinophilus palleucus*, and a facultative salamander, *Eurycea cirrigera*. This level of taxonomic and trophic diversity allowed us to test predictions about how a food web containing both obligate and facultative cave species would respond to increased energy supply.

Experimental design

A before-after control-impact (BACI) sampling design was used for this study. Two 100-m stream reaches, separated by 25 m, were chosen: an upper control reach and a lower manipulation reach. These stream reaches were located ~400m stream distance from the nearest cave entrance. Wetted channel width ranged from 0.5 to 4 m and depth ranged from 0.2 to 0.8 m.

Following 1 yr of pre-treatment sampling in both reaches (Feb 2009 to Feb 2010), the manipulation reach was amended with corn stover (leaves, stalks, and husks left over from corn harvest) so that standing crop organic matter was maintained at ~500 g dry mass (DM) m^{-2} for 1 yr (Feb 2010 to Feb 2011). Corn stover was chosen as the carbon source because it was easily obtained, readily utilized by stream microbes and macroinvertebrates (Griffiths et al. 2009; Venarsky et al. 2012), and has a distinctive C4 $\delta^{13}C$ signature (-11 ‰; Fry 2006) that allows it to be traced through the recipient food web using stable isotope analysis (Bender 1968). The corn variety chosen for the manipulation (variety W5280: Wyffels Hybrids Inc., Geneseo, Illinois, U.S.A) did not contain the *Bacillus thuringiensis* toxin, which could have affected invertebrate consumers in the manipulation reach (Rosi-Marshall et al. 2007).

Immediately following the first addition of corn litter (Feb 2010), 20 mesh barriers were constructed at 5-m intervals to increase litter retention in the manipulation reach. Mesh barriers were positioned perpendicular to the stream channel and were constructed using a 1×0.5-m piece of netting (mesh size 2.5×1.5-cm) attached between two sandbags filled with stream substrate. Fresh corn litter was occasionally added as needed to maintain the target addition level.

Environmental characteristics

Water temperature was recorded every 30 min using a HOBO Water Level Logger model U20-001-04 data logger (Onset Computer Corp., Pocasset, Massachusetts, USA). Water samples were collected from each reach on each sampling date and analyzed for NH_4^+-N, NO_3^--N, soluble reactive phosphorus (SRP). All water samples were filtered through glass-fiber filters (0.7-µm nominal pore size). Water samples were transported to the laboratory on ice and frozen until analysis. Concentrations of NH_4^+-N were measured using the orthophthaldialdehyde fluorometric method (Holmes et al., 1999; Taylor et al., 2007). Concentrations of NO_3^--N were measured using ion chromatography (Dionex ICS 2000 Ion Chromatograph; APHA, 1998). Soluble reactive phosphorus (SRP) concentrations were quantified using the ascorbic acid method (Murphy & Riley, 1962).

Meio-, macro-fauna, and organic matter sampling

Sampling occurred on nine dates prior to (Feb 2009 to Jan 2010) and eleven dates following (Mar 2010 to Feb 2011) the initiation of the litter amendment. On each date, the density of small macrofauna (amphipods, isopods, and insects), meiofauna (copepods and cladocerans), and standing crop organic matter was estimated from 10 random samples taken within each study reach. Samples were collected using a stove-pipe corer (22.5 cm diameter) to a depth of 30 cm or bedrock, whichever came first. Samples were preserved in 5% formalin. Densities of large macrofauna (salamanders and crayfish) were estimated by placing block nets at the ends of each study reach and making three collection passes. Individuals were measured

(±1 mm; snout-vent length for salamanders and ocular carapace length for crayfish) and released after sampling was completed.

In the laboratory, invertebrates were removed from the core-samples, identified to the lowest practical taxonomic level and measured to the nearest 1-mm. Biomass was estimated using length-mass relationships (Calow, 1975; Culver et al., 1985; Leeper & Taylor, 1998; Benke et al., 1999; Doroszuk et al., 2007; Lemke & Benke, 2009; Huntsman et al. 2011a and b). Taxa were categorized into functional feeding groups after Merritt et al. (2008). Taxa were also assigned to either an obligate-cave or facultative-cave life history categories. Obligate cave species were identified by their lack of eyes and pigment. Some taxa could not be placed into either category (e.g. oligochaetes, cyclopoids, harpacticoids, ostracods, nematodes, and cladocerans), however. Organic matter from each sample was dried at 60˚C to constant mass, weighed and combusted at 500˚C for 6 h. Combusted material was wetted, re-dried and weighed. The difference between oven-dried mass and ash mass was used to estimate the ash-free dry mass (AFDM) of organic matter.

Food web analysis

For stable isotope analyses, samples of particulate detritus and consumer tissues were collected from both study reaches every 1-2 months following the litter amendment. Samples were transported to the laboratory on ice. Invertebrates were sorted the following day, lyophilized, and stored at -20˚C until analysis for stable isotope composition (Marine Biological Laboratory, Stable Isotope Laboratory, Woods Hole, MA, USA and Analytical Chemistry Laboratory, University of Georgia, Athens, U.S.A.). Data for each consumer group from the manipulated reach was analyzed using a standard linear mixing model (two-source, single-isotope) with the $\delta^{13}C$ of corn litter and the $\delta^{13}C$ of the same consumer in the reference reach as the two end-members. This conservative approach to calculating contribution of novel carbon to consumer biomass has been used successfully in similar additions of C4-plant carbon (e.g., Wilcox et al. 2005).

Data analyses

Changes in both standing crop organic matter and organismal biomass before and after the litter amendment were assessed using an unreplicated BACI analysis (Stewart-Oaten et al. 1986; Schroeter et al. 1993). For each parameter of interest, the mean monthly value from the control reach was subtracted from the corresponding monthly manipulation reach value (e.g. monthly effect size). Then, either a Student's paired *t*-test or a Wilcoxon signed-rank test (for non-normally distributed data) was used to compare the mean monthly effect sizes before and after the litter amendment.

Three multivariate techniques were used to compare macroinvertebrate community structure from the core samples among study reaches before and after the litter addition: analysis of similarities (ANOSIM), non-metric multidimensional scaling (nMDS) ordination and similarity percentages (SIMPER). Similarity matrices were first computed using the Bray-Curtis coefficient on untransformed biomass data. The original data set ($n = 400$) was reduced by averaging samples within each month and study reach ($n = 40$). First, we used nMDS to generate graphical summaries of the relationships in community structure between reaches. Bubble plots were incorporated into the nMDS ordinations to illustrate trends in community composition and taxon biomass between reaches. Second, we performed one-way ANOSIMs to test for an effect of reach identity on community structure. Finally, we used the SIMPER routine to identify those taxa that contributed disproportionately to the overall dissimilarity in community structure between reaches. All multivariate analyses were conducted in PRIMER (Version 6, Plymouth Marine Labs, Plymouth UK; Clarke & Warwick, 2001).

The results from this study were combined with studies from three other cave streams in northeastern Alabama (Huntsman et al. 2011, Chapter 5) and long-term data from forested headwater streams at Coweeta Hydrologic Laboratory Southern Research Station (C53, C54, C55) with- and without-litter exclusion experiment (Wallace et al. 1999, Cross et al. 2006, Meyer et al., 1998) to examine for correlations between organic matter storage and macroinvertebrate biomass. The results from the surface and cave stream studies are comparable because of their similarities in: i) key environmental characteristics (e.g., low amount of primary production in the forested headwater streams Webster et al. 1983, Lowe et al. 1986, Mulholland et al. 1997; Wallace et al. 1999), ii) sampling regimes, and iii) biogeographic regions, which indicates that the the pool of surface species that colonized the streams come from a similar species-pools. This data set was analyzed in Data Desk version 6.1 (Data Description Inc., 1996) using simple linear regression. Data were transformed as needed.

Results

Water temperature and nutrients

Average daily water temperature during the study was 13.5°C and varied ±2°C. Soluble reactive phosphorus (8-10 µg L^{-1}) and NH_4^+-N (4-5 µg L^{-1}) were similar and low in both study reaches pre- and post-litter amendment (Table 1). Average NO_3^--N was similar between reaches pre-litter amendment (258-268 µg L^{-1}), but was lower in the manipulation reach following the litter amendment (193 µg L^{-1}).

Organic matter

Mean monthly standing crop organic matter was low in both study reaches prior to the litter amendment, ranging from 9 to 136 g AFDM m^{-2}. Following the litter amendment, standing crop organic matter significantly increased in the manipulation reach (159-866 g AFDM m^{-2}), while levels in the control reach remained low (9-75 g AFDM m^{-2}; *t*-test: df = 8, $t = -4.3$, $P = 0.003$; Fig. 1a).

Macroinvertebrates

Forty-five different invertebrate taxa were identified from the core samples (Table 2). Three taxa were identified as obligate cave species*: Crangonyx*, *Stygobromus,* and *Caecidotea*. Six taxa could not be placed with confidence into either category: Cyclopoida, Harpacticoida, Ostracoda, Oligochaeta, Chydoridae, and Nematoda. The remaining taxa were classified as facultative cave species. Prior to the litter amendment, 23 and 22 taxa were documented from the control and manipulation reaches, respectively. Richness increased in both reaches following the litter amendment, with 31 taxa documented in the control reach and 37 taxa documented in the manipulation reach. The increases in species richness following the litter amendment was driven by increases in Diptera, especially of Chironomidae, and by Plecoptera taxa (Table 2).

Total macroinvertebrate biomass from core samples mirrored the patterns in standing crop organic matter (Fig. 1b). Before the litter amendment, total macroinvertebrate biomass in both study reaches was low but increased significantly (~5 times) in the manipulation reach following the litter amendment (Wilcoxon signed rank test: $n = 20$, $W = 44$, $P = 0.008$). Facultative species, rather than obligates, generally dominated total macroinvertebrate biomass in both study reaches before and after the litter amendment. Facultative species accounted for 14-77% of total macroinvertebrate biomass by date, while obligate cave species accounted for 0-52% of total macroinvertebrate biomass. The lack of a significant biomass response to detritus amendment by obligate cave taxa (*t*-test: df = 8, $t = 1.1$, $P = 0.31$) contrasted with the strong, significant increase in the biomass of facultative species (Wilcoxon signed rank test: $n = 20$, $W = 44$, $P = 0.008$; Fig. 1c, d).

Macroinvertebrate community structure changed significantly following the litter amendment (R-statistic = 0.50, $P = 0.001$; Fig. 2). Pairwise ANOSIMs indicated that community structure did not differ among study reaches prior to the litter amendment (Pre-CR vs. Pre-MR; R-statistic = 0.10, $P = 0.058$) but diverged dramatically following the litter amendment (Post-CR vs. Post-MR; R-statistic = 0.62, $P = 0.001$; Fig. 2). Pre-litter amendment community structure within each reach differed from post-litter amendment structure within each reach (Pre-CR vs. Post-CR and Pre-MR vs. Post-MR; R-statistic = 0.53 – 0.70, $P = 0.002$; Fig. 2). Seven taxa accounted for 82-91% of the overall dissimilarity among all pair-wise comparisons (Fig. 3). Three taxa, *Polypedilum*, Oligochaeta, and Ephemeroptera, increased in biomass in both study reaches following the litter amendment, but the strongest increase occurred in the manipulation reach. A fourth taxon, *Paraphaenocladius*, increased similarly in both study reaches following the amendment, while biomass of two taxa, Tanypodinae genus A and B, increased only in the manipulation reach following the amendment. The biomass of a final taxon—the obligate cave isopod *Caecidotea*—was similar between both study reaches before and after the amendment.

Organic matter storage explained a large and significant amount of the variation in macroinvertebrate biomass within the combined cave and surface stream data sets (Fig. 5; $F_{30} = 268$, $R^2 = 0.90$, $P < 0.001$). Annual mean organic matter storage and macroinvertebrate biomass were generally lowest in the cave streams and highest in the forested headwater surface streams without experimental litter-exclusion. The litter exclusion experiment by Wallace et al. (1999) reduced both organic matter storage and macroinvertebrate biomass to levels similar to that of high-detritus cave streams, while the litter amendment experiment in this study increased organic matter storage and macroinvertebrate biomass to levels similar to the forested headwater surface stream with litter exclusion (Wallace et al. 1999).

Crayfish and salamanders

Larger consumers in the food web (crayfish and salamanders) followed the same general patterns as those of macroinvertebrates (Fig. 1e, f, g, h). The biomass of the obligate cave crayfish *C. hamulatus* did not respond to the litter amendment (*t*-test: df = 8, $t = 0.4$, $P = 0.71$), while the biomass of the facultative crayfish *C. tenebrosus* (Wilcoxon signed rank test: $n = 20$, $W = 33$, $P = 0.04$) and salamander *Eurycea* sp. (Wilcoxon signed rank test: $n = 20$, $W = 36$, $P = 0.01$) showed a significant positive response. Patterns could not be discerned for the obligate cave salamander *G. palleucus* due to low capture rates.

Carbon flow

Three species, five groups that include multiple species (e.g. Ephemeroptera or Oligochaeta), and four types of organic matter were included in the stable isotope analyses (Table 3). Within the control reach, δ^{13}C values for all types of organic matter ranged from -27 to -28‰, indicating a C3 plant origin. The δ^{13}C values of wood and coarse particulate organic matter (CPOM; e.g., leaves from ambient organic matter) in the manipulation reach also indicated a C3 plant origin, while the corn litter δ^{13}C value was characteristic of C4 plants (~-11‰; Fry 2006). Fine particulate organic matter (FPOM) in the manipulation reach appeared to be partially composed of corn litter due to its higher δ^{13}C value compared with FPOM from the control reach. With the exception of *C. tenebrosus*, the δ^{13}C values for consumers within the control reach (-25 to -27‰) were similar to those of organic matter, indicating that growth of consumers within the control reach was supported by carbon from C3 plant detritus. All consumers within the manipulation reach, however, were partially supported by carbon from the corn litter, because the δ^{13}C values of their tissues were higher and more similar to the δ^{13}C values of corn litter than to C3 plants. Using the consumers in the control reach and corn litter as end-members, the mixing models indicated that 16-73% of consumer carbon in the manipulation reach was derived from corn litter.

Discussion

The energy-limitation hypothesis

The results of this study provide robust support for the energy-limitation hypothesis in cave ecosystems. Following the litter amendment, the biomass of macroinvertebrates, *C. tenebrosus*, and *Eurycea* sp. more than doubled in the manipulated reach, illustrating that cave communities are capable of responding quickly to changes in energy availability. Stable isotope analyses indicated that the increase in biomass following the litter amendment was partially supported by the consumption and assimilation of carbon from the added corn litter.

Consistent with the predictions for this study, evolutionary history influenced the response of the cave community to the litter amendment. The biomass of facultative species increased significantly following the litter amendment, which was predicted because of their adaptations (e.g. higher growth rates and fecundities) for survival in surface ecosystems. In contrast, the obligate cave species, whose troglomorphic traits (e.g. reduced growth rate and fecundity) presumably are adaptations to a low-energy environments, did not respond (e.g. increase in biomass) to the increased availability of energy resources following the litter amendment.

Organic matter storage

Prior to litter amendment, the mean organic matter biomass within both study reaches (19-34 g AFDM m^{-2}) was within the lower range reported from both cave (0-850 g AFDM m^{-2}; Simon & Benfield, 2001, 2002; Huntsman *et al.*, 2011 a, b; Venarsky *et al.*, 2012; see Chapter 5) and surface streams (20-35,000 g m^{-2}; Jones, 1997), indicating that Bluff River Cave was a cave stream ecosystem that received minimal allochthonous inputs of organic matter. While the litter amendment significantly increased mean organic matter storage within the manipulation reach (423 g AFDM m^{-2}), the level was not higher than those reported from other cave streams within close geographic proximity (~30 km) to Bluff River Cave (see Huntsman *et al.*, 2011 a, b; Venarsky *et al.*, 2012; see Chapter 5). Thus, the litter amendment did not increase energy levels beyond the range of that experienced by communities in nearby cave streams.

Facultative species

The significant increase in consumer biomass following the litter amendment was driven by facultative species, which generally dominated consumer biomass both before and after the litter amendment. The strong response by facultative species was likely driven by a combination of dispersal, reproduction, and growth, depending on taxon. While the dispersal patterns of facultative species in cave streams are not generally known, their dispersal abilities in surface streams have been well documented. Many macroinvertebrate insects and crustaceans (e.g. amphipods) in surface streams are capable of dispersing both upstream and downstream several meters per day due to both density-dependent and -independent factors (Turner and Williams 2000; Elliott 2003). A study by Weingartner (1977) found that the facultative cave crayfish *Cambarus laevis* had a wide range of movement patterns in an Indiana cave stream, ranging from <10 m to >65 m day^{-1}. Surface species of *Eurycea* also tend to drift downstream after hatching (see Stoneburner 1978; Bruce 1986) and the number of individuals drifting from a stream reach can be influenced by prey availability (Johnson and Wallace 2005). Thus, the colonization of the manipulation reach by facultative species likely occurred from habitats both upstream and downstream of the study reaches. Additionally, the biomass of surface stream macroinvertebrate insects and larval salamanders have been correlated with resource availability (Dobson and Hildrew 1992; Wallace et al. 1999; Negishi & Richardson, 2003; Johnson and Wallace 2005), which suggests that the facultative species remained in the manipulation following the litter amendment because of the higher availability of resources (e.g. organic matter and prey).

In addition to in-stream dispersal, oviposition by aerial adult insects also likely contributed to the higher biomass of facultative macroinvertebrates in the manipulation reach. Aerial adult dipterans have been recorded in six cave systems near (≤ 40 km) Bluff River Cave (M.P. Venarsky, personal observation). Prior to and following the litter amendment, aerial adult dipterans were observed near the Bluff River Cave entrance and in both study reaches. Adult Ephemeroptera were also observed emerging from the manipulation reach during one sampling period following the litter amendment. Thus, cave streams can potentially be both actively (e.g. migration or oviposition) and passively (e.g. dispersal during flooding) colonized by facultative macroinvertebrate species.

Lastly, the increase in facultative biomass within the manipulation reach can also be attributed to the growth of individuals. The $\delta^{13}C$ composition of both facultative and obligate consumers within the manipulation reach indicated that corn-carbon was assimilated into animal tissue. In some taxa, such as Ephemeroptera and Chironomidae, a majority of the carbon within body tissues was acquired from the corn-litter, while the biomass of larger consumers like *C. tenebrosus* appeared to be composed of a lower portion of corn-derived carbon. The discrepancies in $\delta^{13}C$ composition among taxa can be attributed to several factors, including i) body size and growth rates, which influence tissue turnover (e.g. crayfish vs. Chironomidae), ii) time spent feeding in the manipulation reach prior to sampling, and iii) species mobility, which would allow a species to feed within both study reaches (e.g. mobile crayfish). Regardless of these factors, the stable isotope analyses indicate that the corn litter addition supported the growth of individuals and that the increase in facultative biomass was not solely the result of habitat or hydrological modifications due to the presence of large quantities of CPOM within the stream channel.

Obligate cave species

The biomass of obligate cave species did not change in the manipulation reach following the litter amendment, which was likely caused by several factors. The reduced growth rates and lower fecundities that are characteristic of many obligate cave species (Hüppop 2001, 2005; Chapters 4, 5), coupled with the relatively short time frame of this study, could have prevented biomass from increasing. Additionally, physiological and behavioral adaptations, such as lower metabolic and activity rates (Hüppop 2001, 2005), may reduce the amount of energy that obligate cave species require for growth and survival, thereby allowing more efficient utilization of resources in a smaller area and reducing the need to disperse over large distances in search of food resources.

The lack of a significant increase in biomass following the litter-amendment indicates that an evolutionary trade-off has likely occurred within obligate-cave species. While obligate-cave species are capable of surviving and reproducing within the low-energy cave environment, they do not have the ability to exploit efficiently (e.g. growth and reproduction) large quantities of resources over short time periods. However, previous studies in un-manipulated cave streams have reported that the biomass and production of obligate cave species are positively correlated with energy availability (Huntsman et al. 2011b and Chapter 5), which indicates that obligate cave species can respond (e.g. increased biomass or productivity) to changes in energy availability over long time periods.

Studies that have examined community structure in cave and groundwater ecosystems following organic pollution events commonly cite competitive exclusion (e.g., due to differential life histories and physiologies; see above discussion) by facultative species as a possible mechanism behind the extirpation of obligate-cave species (e.g., Sinton 1984, Notenboom et al. 1994, Sket 1999, Sket 2005; Culver and Pipan 2009). Both the continued presence of obligate-cave species within the manipulation reach following the litter addition (e.g., short-term population response) and the positive correlations between energy availability and obligate-cave species productivity reported in other cave studies (e.g., long-term population response; Huntsman et al. 2011b and Chapter 5), indicate that increased energy-availability, at least within the bounds of this and previous studies (Fig. 4), does not necessarily cause the extirpation of obligate cave species through competitive interactions with facultative species. Thus, the changes in cave community structure following pollution episodes are more likely due to changes in water quality (e.g., toxic chemicals, heavy metals, anoxic conditions) rather than changes to interspecific competitive interactions due to modifications in cave ecosystem energy dynamics (e.g., increase in highly labile organic matter).

Carbon flow through cave food webs

In surface streams, microorganisms (e.g. bacteria and fungi) serve as important intermediates in the transfer of carbon from particulate and dissolved organic matter to consumers at higher trophic levels (Suberkropp & Klug 1976, Bärlocher & Kendrick 1981; Hall & Meyer 1998). Carbon flow through cave stream food webs flows through similar pathways. Simon et al. (2003) found that dissolved organic carbon was available to primary consumers and predators following immobilization through epilithic biofilms. Additionally, Simon & Benfield (2001) reported fungal colonization patterns of CPOM and wood were similar in cave and surface streams. Thus, it is likely that uptake of leached dissolved organic carbon microbial biofilms was instrumental in the transfer of corn-litter carbon to the macroinvertebrate, crayfish, and salamanders in the manipulation reach. Throughout the litter amendment experiment conducted in this study, samples were collected to analyze microbial $\delta^{13}C$ composition via compound-specific stable isotope analysis of phospholipid fatty acids. These analyses will be complete in the near future and will help elucidate the role of microbes in the flow of carbon through the food web in Bluff River Cave.

Cave streams vs. surface streams

Facultative species, as opposed to obligate-cave species, dominated macroinvertebrate biomass both before and after the litter amendment and were also the component of the cave community responsible for the large increase in total macroinvertebrate biomass following the litter amendment. Previous litter-breakdown studies in cave streams have also reported that facultative species accounted for the majority of macroinvertebrate biomass in litter-bags (Brussock et al., 1988; Galas et al., 1996; Venarsky et al. 2012). Given the dominance of facultative species in this and other cave studies, it appears that some cave streams have much more in common with surface streams than previously appreciated. In addition to community structure, several of the characteristics that define the environment of cave ecosystems, such as the lack of light and dependence on allochthonous sources of energy, are also similar to some surface stream ecosystems. For example, forested headwater surface streams can have dense canopies throughout the year, which cause both continuously low rates of primary production and a consequently heavy reliance on allochthonous inputs of organic matter from the surrounding watershed to support *in situ* productivity (see Webster et al. 1983, Lowe et al. 1986, Mulholland et al. 1997; Wallace et al. 1999).

If some cave and surface stream ecosystems share similar community structures and environmental characteristics, do they also have a similar response to changes in allochthonous inputs of detritus? A study by Wallace et al. (1999) reduced allochthonous inputs of detritus (e.g. leaf-litter and woody debris) to a forested headwater stream by 95% over a 4-year period. The exclusion experiment reduced organic biomass within the stream by ~77%, which subsequently reduced macroinvertebrate biomass by ~78%. Comparing the results of Wallace et al. (1999) with those from this and two previous cave stream studies shows that Wallace et al. (1999) essentially transformed a high-energy surface stream into a high-energy cave stream, while the experiment conducted in this study converted a low-energy cave stream into a low-energy forested headwater surface stream (Fig. 4). Thus, some cave and surface streams are fundamentally similar and appear to be linked to one another along a detrital subsidy spectrum that includes both high (e.g. surface) and low (e.g. cave) rates of detrital inputs.

While cave and forested headwater streams appear to be linked along a detrital subsidy spectrum, they are substantially separated along a time continuum. The results of this study and those of Wallace et al. (1999) illustrate the importance of detrital inputs on short-term ecological time scales, while unmanipulated cave ecosystems exemplify how detrital exclusion on evolutionary time scales influences the evolution of specialized life histories and physiologies that prevent obligate cave species from responding to short-term increases of energy resources.

Conclusions

Detrital inputs are the primary source of energy fueling biological productivity within many cave ecosystems and are also important in structuring their communities. However, little attention has been given to quantifying energy dynamics in cave ecosystems. This is in stark contrast to aquatic surface ecosystems, in which the importance of detrital pathways and resource subsides to ecosystem dynamics have been appreciated for many years. Numerous studies within aquatic surface ecosystems have explored how the quantity, quality, and type of detrital inputs influence ecosystem processes and the structure of microbial-, meio-, and macro-faunal communities (see Moore et al. 2004). This study begins to close this knowledge gap by providing the first rigorous experimental test of the energy-limitation hypothesis in cave ecosystems. Similar to several recent studies (Datry et al., 2005; Cooney & Simon, 2009; Huntsman et al., 2011b; Chapter 5), the results of this study provide robust support for the energy-limitation hypothesis in cave ecosystems, finding that detrital inputs are important in structuring aquatic cave communities. However, the distinct evolutionary histories (e.g. facultative vs. obligate) dictated the species-level responses to short-term increases in energy availability. Additionally, this study illustrates how cave and surface stream ecosystems have much more in common than previously thought due to their similarities in community structure and responses to fluctuations in energy inputs. Lastly, several recent studies have illustrated that energy inputs can vary greatly among caves, indicating that energy limitation varies among cave ecosystems (Simon & Benfield, 2001, 2002; Huntsman *et al.,* 2011 a, b; Venarsky *et al.,* 2012; see Chapter 5). Thus, additional experimental and natural-gradient studies conducted over longer time-periods and including wider ranges of energy availabilities are required to develop a more complete understanding of cave ecosystem energy dynamics.

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Table 1. Mean (range) of soluble reactive phosphorus (SRP), NH_4^+-N, and NO_3^--N concentrations in control (CR) and manipulation (MR) reaches in Bluff River Cave, AL, U.S.A. BD = below detection.

		SRP (µg L^{-1})	NH_4^+-N (µg L^{-1})	NO_3^--N (µg L^{-1})
Pre-addition	CR	8 (5-13)	4 (2-14)	268 (186-456)
	MR	10 (7-13)	4 (BD-12)	258 (170-461)
Post-addition	CR	9 (BD-20)	5 (2-12)	236 (180-365)
	MR	9 (4-14)	5 (1-18)	193 (97-275)

Table 2. Mean (±1 standard deviation) macroinvertebrate biomass (mg dry mass m^{-2}) from core samples pre- and post-litter amendment in the control and manipulation reaches in Bluff River Cave stream.

	Control reach		Manipulation reach	
	Pre addition	Post addition	Pre addition	Post addition
Nematoda	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)
Mollusca				
Sphaerium	0.01 (0.05)	0.10 (0.43)		0.12 (0.67)
Gastropoda				
Helical shell	0.15 (1.46)	0.03 (0.24)		0.60 (4.63)
Spiral shell			0.04 (0.26)	0.14 (0.75)
Oligochaetes	12.58 (26.06)	50.43 (79.06)	14.16 (34.97)	168.09 (467.79)
Ostracoda	0.14 (0.59)	0.09 (0.35)	0.19 (1.46)	2.29 (6.29)
Cladocera				
Chydoridae				0.85 (3.05)
Copepoda				
Cyclopoida	0.26 (0.46)	0.47 (0.76)	0.11 (0.27)	18.42 (45.69)
Harpacticoida	0.01 (0.03)	0.02 (0.06)	0.01 (0.03)	0.14 (0.34)
Malacostraca				
Isopoda				
*Caecidotea******	24.29 (55.59)	11.86 (19.48)	8.52 (16.99)	12.44 (50.76)
Amphipoda				
Crangonyctidae	0.02 (0.19)	0.01 (0.13)		
*Crangonyx******	0.02 (0.15)	0.24 (2.02)	0.15 (1.34)	0.03 (0.33)
*Stygobromus******	1.09 (3.62)	1.04 (6.88)	0.30 (2.35)	0.15 (1.29)
Insecta				
Plecoptera				
Capniidae		0.06 (0.58)		
Perlidae	0.12 (1.14)			
Leuctridae		0.23 (1.36)	0.05 (0.48)	0.17 (1.56)

Table 2. Continued

Leuctra				0.04 (0.43)
Perlodidae		1.58 (8.64)		0.86 (6.03)
Cultus		2.89 (15.82)		1.98 (16.37)
Taeniopterygidae		0.12 (1.25)	0.06 (0.57)	0.36 (2.33)
Strophopteryx		0.12 (1.23)		
Chloroperlidae		0.65 (3.61)		
Alloperla	0.42 (4.00)	0.16 (1.65)		0.63 (6.64)
Ultaperla	0.58 (5.51)			
Ephemeroptera				
Baetidae				0.23 (2.18)
Heptageniidae		0.21 (1.19)	0.12 (0.78)	
Ephemerellidae	0.04 (0.24)		0.06 (0.34)	0.08 (0.81)
Eurylophella	0.73 (3.26)	0.57 (1.60)	0.78 (2.55)	27.10 (112.18)
Leptophlebiidae	0.09 (0.44)	0.11 (0.51)	0.30 (2.42)	0.64 (3.12)
Paraleptophlebia	0.24 (1.53)	0.07 (0.41)	0.07 (0.39)	3.70 (14.57)
Hydroptilidae				0.02 (0.22)
Potamyia			4.87 (46.17)	
Diptera				
Psychodidae				0.02 (0.15)
Ceratopogonidae		0.06 (0.59)	0.01 (0.08)	
Dolichopodidae		0.01 (0.15)		0.05 (0.32)
Tipulidae				0.01 (0.11)
Pilaria		1.21 (8.59)	0.05 (0.46)	1.20 (6.30)
Chironomidae				
Tanypodinae	0.04 (0.28)	0.03 (0.36)	0.01 (0.11)	0.08 (0.47)
Genus A	4.66 (7.41)	3.61 (5.80)	1.21 (3.81)	16.18 (39.78)
Genus B	2.04 (4.23)	2.07 (4.89)	0.87 (3.51)	11.11 (23.88)

Table 2. Continued

Chironomini				
Paratendipes		<0.01 (0.02)		1.60 (8.60)
Polypedilum	30.77 (175.57)	7.22 (23.69)	8.45 (57.90)	169.23 (272.25)
Orthocladiinae				
Corynoneura	0.02 (0.14)	0.04 (0.36)		0.05 (0.25)
Diplocladius				0.21 (1.74)
Eukiefferiella	0.01 (0.08)	0.08 (0.45)	0.02 (0.18)	0.34 (1.22)
Heleniella	0.15 (0.69)	0.52 (3.87)	0.11 (0.63)	0.41 (1.58)
Limnophyes				0.13 (0.75)
Mesosmittia				0.04 (0.42)
Orthocladius		0.01 (0.13)		0.92 (8.03)
Paraphaenocladius	8.61 (14.24)	19.39 (23.77)	5.37 (12.31)	23.64 (47.00)
Rheocricotopus				0.06 (0.44)
Rheosmittia		0.02 (0.16)		
Stilocladius				0.03 (0.20)
Thienemanniella				0.04 (0.19)
Tanytarsini				
Micropsectra	<0.01 (0.01)	0.01 (0.12)	<0.01 (0.02)	0.09 (0.63)

Table 3. Average (± standard error) of $\delta^{13}C$ (‰) values for consumers and organic matter in the control and manipulation reaches post-litter addition in Bluff River Cave. Percent of consumer biomass composed of carbon from the amended corn litter as estimated from a standard linear mixing model (two-source, single-isotope). FPOM = fine particulate organic matter (≥250 to 500 µm). CPOM: C3 plants = coarse particulate organic matter (≥500 µm) that is only of C3 plant origin.

	Control	Manipulation	% carbon from corn litter
C. tenebrosus	-21 (1)	-17 (0.7)	16
Eurycea sp.	-25 (0.2)	-16 (0.8)	56
Caecidotea	-25 (0.2)	-17 (1.1)	50
Ephemeroptera	-25 (0.6)	-13 (0.3)	73
Plecoptera	-25 (0.3)	-19 (1.6)	36
Chironomidae: Non-Tanypodinae	-27 (0.3)	-15 (0.4)	70
Chironomidae: Tanypodinae	-26 (0.3)	-15 (0.7)	61
Oligochaeta	-26 (0.2)	-17 (0.7)	48
Wood	-27 (0.3)	-27 (0.3)	
FPOM	-27 (0.2)	-25 (0.8)	
CPOM: C3 plants	-29 (0.2)	-28 (0.3)	
Corn litter		-11 (0.1)	

Figure 1. Mean (bars are standard errors) in control (black circles) and manipulation (white squares) reaches in Bluff River Cave, Jackson Co., AL, U.S.A. of (a) organic matter, (b) total macroinvertebrate biomass, (c) obligate cave macroinvertebrate biomass, (d) facultative macroinvertebrate biomass, (e) *Cambarus hamulatus*, (f) *Cambarus tenebrosus*, (g) *Gyrinophilus palleucus*, and (h) *Eurycea* sp. Vertical dashed line indicates beginning of litter amendment.

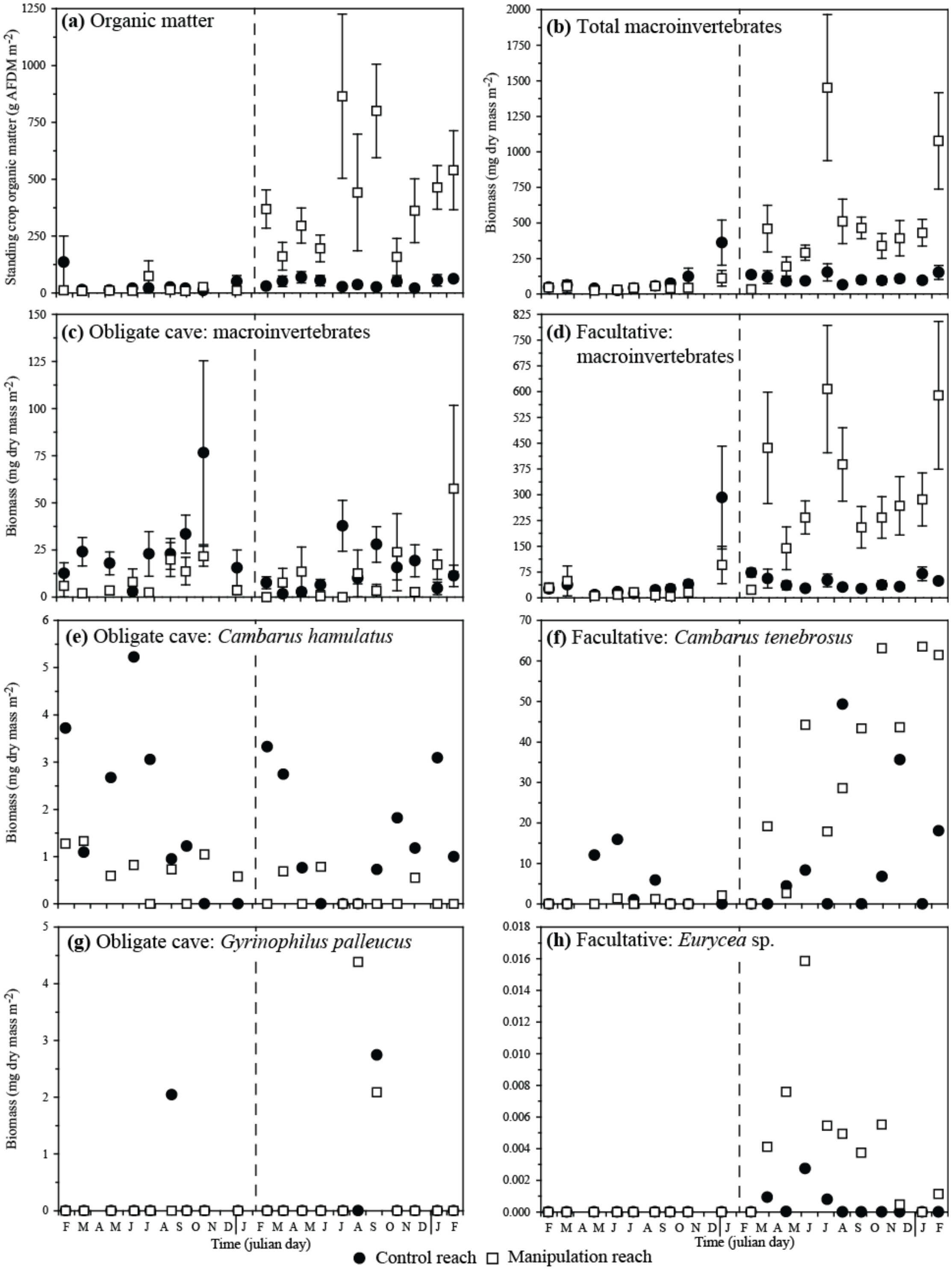


Figure 2. Non-metric multidimensional scaling ordination plot of the macroinvertebrate communities in the control (black symbols) and manipulation (white symbols) reaches pre- (circles) and post-addition (triangles) from Bluff River Cave, Jackson Co., AL, U.S.A. Data points are based on untransformed macroinvertebrate biomass data that were averaged within reaches each sampling month (see text).

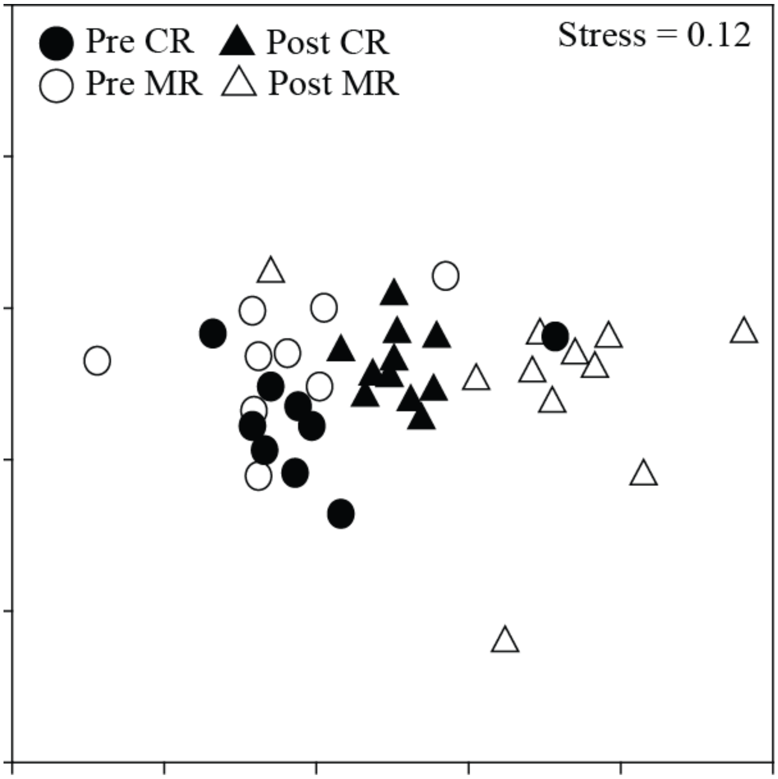


Figure 3. Non-metric multidimensional scaling ordination plots based on untransformed biomass of seven taxa that contributed 82-91% to the overall dissimilarity among all pair-wise cave comparisons between the control and manipulation reaches pre- and post-addition. The diameter of each circle is proportional to taxon biomass (mg dry mass m^{-2}). (a) *Caecidotea*, (b) Oligochaeta, (c) Ephemeroptera, (d) *Polypedilum*, (e) Tanypod genus A, (f) Tanypod genus B, (g) *Paraphaenocladius*. Stress = 0.12 for all figures.

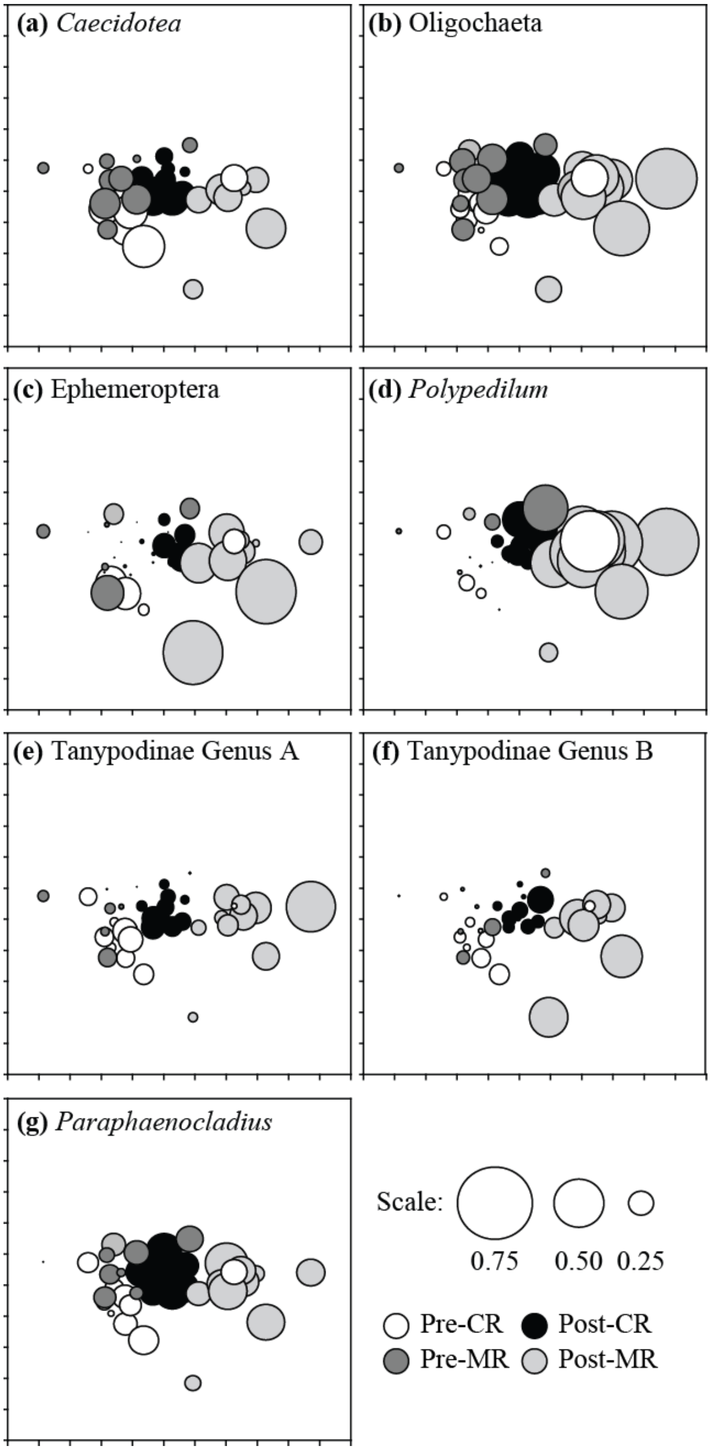
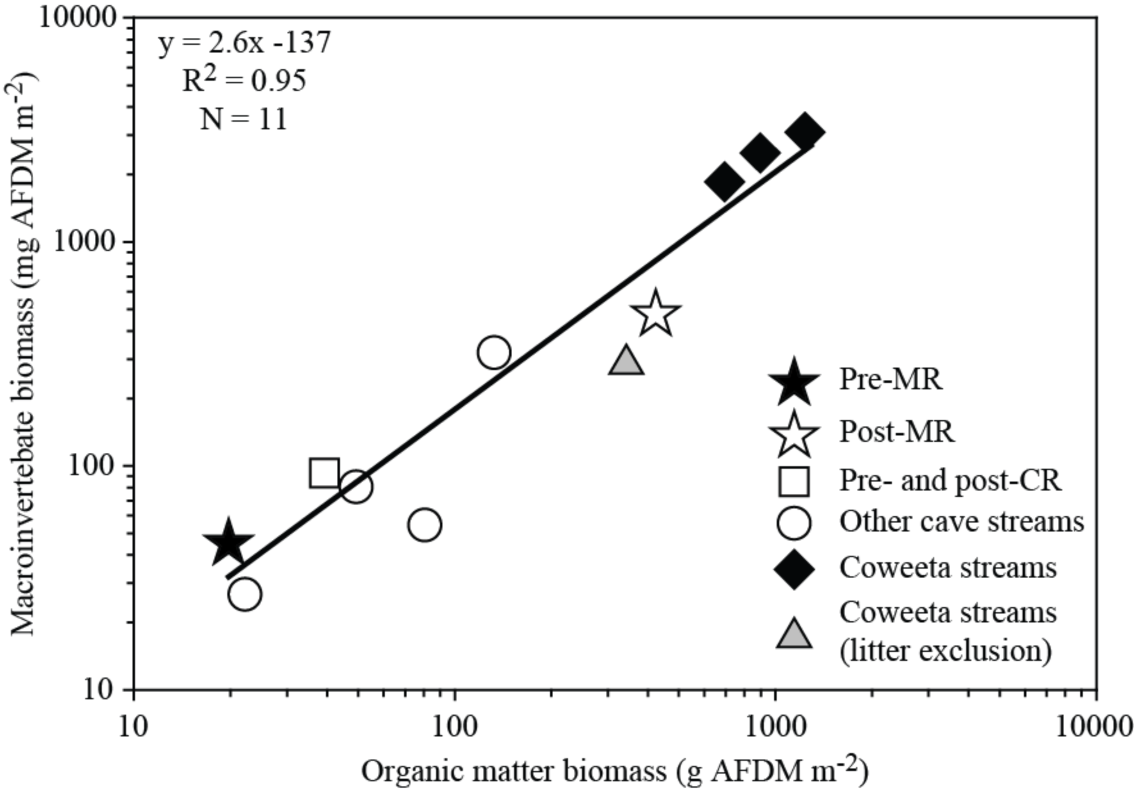


Figure 4. Relationship between organic matter biomass and macroinvertebrate biomass in cave and forested headwater surface streams. Included in the graph are data from the control reach (CR) and manipulation reach (MR) pre-and post-litter amendment in Bluff River Cave stream, three other cave streams in northeastern Alabama (Huntsman et al. 2001, Chapter 5), and forested headwater streams at Coweeta Hydrologic Laboratory Southern Research Station (C53, C54, C55) with (grey triangles) and without (black diamonds) litter exclusion (Wallace et al. 1999, Cross et al. 2006, Meyer et al. 1998). AFDM = ash-free dry mass.



CHAPTER 4

REXAMINING EXTREME LONGEIVTY OF THE CAVE CRAYFISH *ORCONECTES AUSTRALIS* USING NEW MARK-RECAPTURE DATA: A LESSON ON THE LIMITATATIONS OF ITERATIVE SIZE-AT-AGE MODELS

Abstract

Centenarian species, defined as those taxa with life spans that frequently exceed 100 years, have long been of interest to ecologists because they represent an extreme end point in a continuum of life history strategies. One frequently reported example of a freshwater centenarian is the obligate cave crayfish *Orconectes australis*, with a maximum longevity reported to exceed 176 years. As a consequence of its reported longevity, *O. australis* has been used as a textbook example of life-history adaptation to the organic-carbon limitation that characterizes many cave-stream food webs. Despite being widely reported, uncertainties surround the original estimates of longevity for *O. australis*, which were based on a single study dating from the mid-1970s. In the present study, the growth rate, time-to-maturity, female age-at-first-reproduction and longevity of *O. australis* was reevaluated using a mark-recapture study of more than 5 years based upon more than 3,800 free-ranging individuals from three isolated cave streams in the southeastern United States. The results of this study indicate that accurate estimates of the longevity of *O. australis* are $\leq$22 years, with only a small proportion of individuals (<5%) exceeding this age. Estimates for female time-to-maturity (4 to 5 years) and age-at-first-reproduction (5 to 6 years) are also substantially lower than earlier estimates. These new data indicate that the age thresholds for life history events of *O. australis* are comparable to other estimates for a modest

assemblage of cave and surface species of crayfish for which credible age estimate exist, suggesting that a cave environment *per se* is not required for the evolution of extreme longevity in crayfish.

Introduction

A select group of aquatic animals exhibit remarkable longevity. The ocean quahog clam (*Arctica islandica* [L.]), for example, has been reported to live for more than 400 years (Wanamaker *et al.*, 2008), while marine tubeworms surrounding hydrocarbon seeps can live for over 200 years (Bergquiest *et al.*, 2000). Among vertebrates, several species of deep-sea fishes have life spans in excess of 100 years (see Koslow *et al.*, 2000; Finch, 2009). Such "centenarian taxa" have long been of interest to biologists because they represent an extreme end point on the life history continuum and provide insights into the physiological processes (e.g. genome maintenance and reduced oxidative stress) that produce exceptionally long life spans (Bodnar, 2009; Voituron *et al.*, 2011). Not surprisingly, the population dynamics of such long-lived species also pose unique challenges for management and conservation.

Among freshwater taxa, cave-adapted organisms offer some excellent examples of extreme longevity. In cave ecosystems, resources are limited due to the absence of light, which prevents primary production (except in chemolithoautotrophy-based systems; Engel *et al.*, 2004), and by reduced surface connectivity, which limits inputs of allochthonous organic matter (Poulson & Lavoie, 2001). In response, many obligate cave species have evolved K-selected life history characteristics, such as long life span, slow growth rate and reduced fecundity (Hüppop, 2001). One frequently reported example of longevity in a cave organism is the extreme life span estimated for the obligate cave crayfish *Orconectes australis* (Rhoades). Cooper (1975) used a mark-recapture approach to study the population size, age structure,and growth of *O. australis* in Shelta Cave, Alabama, USA. Using models based on growth rates from free-roaming individuals, he predicted that it would take 37 to 176 years for *O. australis* to reach a carapace length of 47 mm. Using Cooper's (1975) data, Culver (1982) further estimated female time-to-maturity to range from 16 to 35 years, while female age-at-first-reproduction was predicted to range from 29 to 105 years.

Given the astonishing longevity suggested by his data, Cooper (1975; p. 314) expressed some doubts about his analytical approach, stating "This apparently extraordinary finding requires further comment. Two alternative approaches are open: (1) consideration of factors which could actually confer "immortality" on these populations, and (2) further search for the flies which are undoubtedly lurking in the ointment of growth records (based on carapace lengths) and rates inferred from them." Despite this plea for caution, these longevity estimates have been referred to in comparative life history studies (Hobbs, Hobbs & Daniel, 1977; Weingartner, 1977; Streever, 1996; Cooper & Cooper, 2004; Vogt, 2012) and toxicology studies (Dickson, Briese & Giesy, 1979). References to these estimates can also be found in books, popular magazines, journal newsletters and websites which discuss life history evolution in cave species, cave conservation issues, and the uniqueness of cave ecosystems (Culver, 1982; Anonymous, 1999; Hüppop, 2001; Krajick, 2001; Poulson & Lavoie, 2001; Culver, 2005; Krajick, 2007; web search: *Orconectes australis* life span; 100 year-old cave crayfish, 17 July 2011).

If Cooper's (1975) models are correct, *O. australis* belongs to a small group of animals known to have 100+-year life spans (see Ziuganov *et al.*, 2000; Finch, 2009). However, extraordinary life span estimates must be informed by extraordinary data. In light of Cooper's (1975) uncertainties regarding his longevity models, this study sought to reevaluate the life history of *O. australis*. Access to Shelta Cave is now restricted and the site has also been affected by groundwater pollution (Burnett *et al.*, 2003). Consequently, growth rate, time-to-maturity, female age-at-first-reproduction and maximum longevity were measured for *O. australis* inhabiting three hydrologically isolated cave systems in Alabama, U.S.A. that are close to Shelta Cave and that share genetically similar populations.

Methods

Study sites

Hering, Limrock and Tony Sinks caves

Three caves containing streams in northeastern Alabama (Jackson County, U.S.A.) were chosen for study: Hering, Limrock, and Tony Sinks caves. These caves contained large populations of *O. australis* and had similar macrofaunal assemblages, which included the facultative cave crayfish *Cambarus tenebrosus* Hay and the obligate cave salamander *Gyrinophilus palleucus* (McCrady). The southern cavefish (*Typhlichthys subterraneus* [Girard]) and the mottled sculpin (*Cottus bairdi* [Girard]) also occurred in Limrock and Hering caves.

Shelta Cave

Cooper's (1975) study site, Shelta Cave, is located 20 to 42 km west of this studies sites. Shelta Cave consists of three large rooms measuring about 610 m long (Cooper, 1975, p. 22, Fig. 3), with only a small portion of the cave containing water perennially (~150 m; Cooper, 1975, p. 106, Fig. 19). Macrofaunal assemblages in Shelta Cave during Cooper's (1975, p. 57) study were similar to those at sites from this study and consisted of three species of cave crayfish (including *O. australis*) in addition to *C. tenebrosus*, *G. palleucus* and *T. subterraneus*. Water temperature in Shelta Cave averaged 15.5˚C (Cooper, 1975, p. 115). Since Cooper's (1975) study, the population of crayfish has decreased to less than 10% of its initial size due to decreased water quality and possibly the loss of a resident bat colony (Elliott, 2001).

A phylogeographic study by Buhay & Crandall (2005) showed that the populations of *O. australis* in Limrock, Hering and Shelta caves shared common mtDNA 16S haplotypes, indicating that at least these three populations share a common evolutionary history. While Tony Sinks Cave was not included in Buhay and Crandall's study, its close geographic proximity to the other caves (10-42 km) suggests that its crayfish population falls within the same well-defined clade, supporting their recognition as a single species.

Life history sampling and growth modeling in the present study

A study reach ranging from 327 to 1202 m containing a series of riffle and pool habitats with sand, gravel and bedrock substrata was designated in each cave. Sampling began in November 2005 in Hering, January 2006 in Limrock and July 2006 in Tony Sinks caves and was conducted semi-monthly (conditions permitting) to August 2011. On each visit, study reaches were surveyed on foot and all crayfish encountered were collected using dip-nets. Captured crayfish were marked using both internal tags [Visible Implant Alpha Tags (VIAT), Northwest Marine Technology, Shaw Island, WA, USA] and Visible Implant Elastomer (VIE; Northwest Marine Technology). VIATs are small (1.0 × 2.5 mm), fluorescent, uniquely numbered tags that were placed beneath the abdominal cuticle. The VIE was injected directly posterior to the VIATs and was used to assess tag loss, which was infrequent. Once marked, the total carapace length (TCL) and ocular carapace length (OCL; posterior margin of ocular cavity to posterior center-margin of carapace) of each crayfish was measured (±0.1 mm) with dial calipers, its reproductive status (Form I or II for males; presence of cement glands, ova, or young for females) was recorded, and it was released at the point of capture. OCL was used rather than TCL to avoid errors due to damage to the acumen following release. Water temperature was recorded every 30 min using a Solinst Barologger model 3001 (Solinst, Georgetown, Ontario, Canada) from June

2007 to July 2011. Temperature data are not available for Tony Sinks Cave from March 2009 to January 2010 due to instrument loss.

Crayfish growth rates were estimated as the difference between OCL at initial marking and the OCL upon recapture divided by days elapsed. This rate was then multiplied by 365 to acquire an annual growth increment. Since growth increments are “episodic” due to the molting cycle, annual growth increments were only calculated for individuals recaptured over intervals of 350 days or longer. Negative annual growth-increments were attributed to measuring error and were excluded from analyses. For crayfish recaptured multiple times, the annual growth increment was calculated using the recapture date closest to the 350-day minimum. Annual growth increments were regressed against average OCL to estimate the size-specific annual growth rate. To estimate size-at-age, the size-specific annual growth rate was first seeded with a 3 mm OCL individual, the size of juveniles attached to the pleopods of a single female collected from Hering Cave. This process was then iterated at annual intervals and growth trajectories bounded by 95% confidence limits were then constructed using a bootstrap technique (Whitmore & Huryn, 1999). A significant difference in growth trajectories among caves was assumed when 95% confidence intervals did not overlap.

Separate growth trajectories were produced for male and female crayfish to assess sex-specific patterns. Time-to-maturity was estimated by plotting the smallest reproductive (Form I) male and female (using presence of cement glands) onto the resulting growth trajectories. The largest male and female and the smallest female with ova or young were used to estimate minimum life span and age-at-first-reproduction, respectively. Ages constrained by 95% confidence limits represented the estimated range. Cumulative size-frequency distributions were constructed for each cave, including Shelta Cave (see below), and then compared to examine for differences in size structure among populations using pairwise Kolmogorov-Smirnov (K-S) tests. K-S tests were performed in program R version 2.14.0 (R Development Core Team, 2008).

Cooper's (1975) growth models

Growth rates reported by Cooper (1975) were estimated as the difference between TCL at initial marking and upon recapture divided by months elapsed. Cooper (1975) calculated monthly growth rates for a total of 56 individuals (Cooper, 1975, pp. 273-280, Tables 36 and 37) ranging in size from 10 to 47 mm TCL. Only 10 of these were less than 23 mm TCL, however. Cooper (1975) constructed his mean and maximum growth models by placing the 56 individuals for which he calculated monthly growth rates into six size-classes. The mean and maximum monthly growth rate for each size class was then used to estimate the number of months required for an individual to grow through each size-class. Essentially, Cooper (1975) constructed a series of linear growth models that approximated an exponential growth model (see results below).

To compare the results of this study with those of Cooper (1975), first Cooper's (1975) original growth models were reconstructed (Cooper, 1975, p. 312, Table 43). Annual growth rates were then calculated from the crayfish that Cooper (1975) had marked for at least 11 months (n = 26; Cooper, 1975, pp. 273-280, Tables 36 and 37), and growth models were constructed using the methods described in this study (see above). A subset of Cooper' (1975) data was used because it better reflected the rationale (e.g. crayfish molt cycle) and methods (e.g. annual growth rates) used to construct growth models for the three study populations from this study. A size-frequency histogram for the Shelta Cave population was also recreated (Cooper, 1975, p. 158, Fig. 26) by digitizing the original figure (DigitizeIt version 1.5.8b; Bormann, 2010). Cooper's (1975) TCL was converted to OCL using a conversion factor that allowed size-classes and cumulative size-frequency distributions to be compared among studies. Crayfish from Hering

Cave were used to generate the conversion factor by regressing OCL against TCL (n = 925, r^2 = 0.98; OCL = 0.79(TCL) – 0.40).

Results

Hering, Limrock and Tony Sinks caves

A total of 3812 crayfish were marked in Hering (919 individuals), Limrock (943) and Tony Sinks (1950) caves. The longest period between a mark and recapture date for a single crayfish was more than 5 years (1920 days). Growth models were constructed using 78 (37 males and 41 females) crayfish in Hering Cave, 112 (47 males and 65 females) in Limrock Cave and 97 (46 males and 51 females) in Tony Sinks Cave (Fig. 1). The 95% confidence intervals for male and female growth models overlapped within each cave, indicating sex-specific growth rates were similar (data not shown). Growth trajectories were generally similar among caves, with the 95% confidence intervals estimated for the Tony Sinks Cave overlapping those estimated for Hering and Limrock caves (Fig. 2). The models for Hering and Limrock caves overlapped little during model years 1 to 4, suggesting growth rates were only marginally similar during this peak growth period. Average daily water temperature in all caves was approximately 13.0˚C and showed little annual variation (standard deviation of average daily water temperature: ±1˚C).

The smallest mature male (Form I; 12-13 mm OCL), female (obvious cement glands; ~16 mm OCL), and ovigerous female (ova or young present; 19-21 mm OCL) were similar in size among caves (Fig. 3). Males in Tony Sinks Cave reached maturity earlier (~2.5 years) than those of Hering and Limrock caves (~3.5 years; Fig. 3, Table 1). Females matured earliest in Hering Cave (3.5 years) and latest in Limrock Cave (5.1 years). Age-at-first-reproduction was also earliest in Hering (4.6 years) and latest in Limrock (6.4 years). The largest male (35.7 mm OCL) and female (33.3 mm OCL) crayfish were collected from Hering Cave, while the largest males and females in Tony Sinks Cave were 25 and 29 mm OCL, respectively, and were 33 and 29 mm OCL in Limrock Cave. Due to the asymptote produced by the growth models, only minimum life span could be estimated (Fig. 3, Table 1). Minimum male life span was shortest in Tony Sinks Cave (11+ years) and longest in Hering Cave (22+ years). Minimum female life span was shortest in Limrock Cave (15.5 years) and longest in Hering and Tony Sinks caves (22+ years). Minimum life span could not be confidently estimated in all caves because the growth models reached an asymptote before they intersected with the size of the largest male and female.

Size-frequency distributions were constructed for crayfish populations in each cave using data pooled from 2005-2011. Pairwise comparisons revealed no significant differences in size-structure among Hering, Limrock and Tony Sinks caves (K-S test, $P > 0.05$; differences between Hering and Tony Sinks caves were marginally significant, $P = 0.05$, Fig. 3). More than 70% of males and 50% of females from all caves were mature (Fig. 3, Table 1). The male:female ratio indicated a positive bias toward females in all caves (Table 1; male:female = 0.76 to 0.89). Ninety-five percent of the individuals comprising populations in Hering and Limrock caves were estimated to be ≤13 years old. In Tony Sinks Cave, 95% of the population was ≤8 years old (Fig. 3). Twenty-five percent of the population was ≤4 years old in Tony Sinks Cave, ≤6 years old in Limrock Cave, and ≤4 years old in Hering Cave.

Analysis of Cooper's (1975) data from Shelta Cave

When the modeling technique used in this study was applied to Cooper's (1975) data, it produced similar results to those of his original mean and maximum growth models (Fig. 4). The smallest mature male (Form I) reported by Cooper (1975) in Shelta Cave had a TCL of 21 mm (Cooper, 1975, pp. 157 and 205). Time-to-maturity for this individual was estimated at 4 to 8 years using Cooper's models and 6 to 21 years using this study's models (Fig. 4). The smallest mature (cement glands) and ovigerous (presence of ova or young) females reported in Cooper (1975) were 31 mm (Cooper, 1975, p. 236) and 37 mm (Cooper, 1975, p. 244) TCL, respectively. Time-to-maturity estimated using Cooper's models for a 31 mm TCL female was 11 to 19 years, while estimates using this study's models ranged from 11 to 34 years (Fig. 4). Age-at-first-reproduction estimated using Cooper's models for a 37 mm TCL female was 14 to 31 years, while an age of 16+ years was estimated using this study's models (Fig. 4). The largest specimen of *O. australis* reported for Shelta Cave (Cooper, 1975, p. 157) was 47 mm TCL. While this specimen exceeded the upper 95% confidence limit of this study's models, Cooper's (1975) models estimated an age of 38+ years (Fig. 4).

The majority of individuals that Cooper (1975) used to estimate monthly growth rates were substantially larger than those from Limrock, Hering and Tony Sinks caves (Fig. 1). The size-frequency distribution in Shelta Cave was similar to Hering (K-S test, $P > 0.05$), but different from Limrock and Tony Sinks (K-S test, $P = 0.03$). This was probably caused by the disproportionate representation of larger size classes in Shelta Cave (Figs. 3 and 4). The modal TCL of *O. australis* in Shelta Cave was 38 mm (Fig. 4), while the modal TCL for Limrock, Hering and Tony Sinks caves ranged from 21 to 26 mm (16 to 20 mm OCL, Fig. 3). Ninety-five percent of the population in Shelta Cave was estimated to be ≤32 years old using Cooper's (1975) mean model and ≤24 years in age using Cooper's (1975) maximum model and the upper 95% confidence interval this study's models (Fig. 4). Cooper's (1975) models predicted that 25% of the population was ≤10 years in age, while this study's models predicted that 25% of the population would be ≤20 years in age.

Discussion

A reevaluation of the life history of O. australis

Estimates of life span for *O. australis* from this study are substantially lower than Cooper's (1975) estimate of 37 to 176 years, indicating that his trepidation regarding these estimates was warranted. The results of this study suggest a more accurate estimate of the life span for *O. australis* is ≤22 years, with only a few percent of the individuals of a given population exceeding this age. Estimates for female time-to-maturity (4 to 5 years) and age-at-first-reproduction (5 to 6 years) from this study are also substantially lower than the estimates of 16 to 35 years and 29 to 105 years, respectively, reported by Culver (1982) using Cooper's (1975) data.

The reevaluation of the longevity of *O. australis* indicates that it is comparable to other estimates for both cave and surface species of crayfish (Table 2). Weingartner (1977), for example, reported that the life span and time-to-maturity for the obligate cave crayfish *Orconectes inermis* (Cope) ranged from 9 to 10 years and 2 to 3 years, respectively, while Streever (1996) estimated a 16+-year life span for the obligate cave crayfish *Procambarus erythrops* (Relyea & Sutton). Hobbs & Lodge (2010) recently suggested *Orconectes inermis* has a 60-year life span. This estimate, however, is not supported by the citations provided by the authors (e.g. Cooper and Cooper, 1978; Hobbs, 1978; Streever, 1996), indicating that Weingartner's (1977) estimates for *O. inermis* are the most credible for this species. Estimates of life span and time-to-maturity in surface crayfish also vary widely, ranging from 1 to 60 years and 6 months to 14 years, respectively (see Vogt 2012). In other species of *Orconectes*, life span ranges from 1 to 5 years and time-to-maturity from 6 months to 2 years (Table 2). Regardless of the shorter life span estimate for *O. australis* from this study, its estimated longevity (~22 years) is relatively great compared with surface species in the same genus, indicating a K-selected life history and a high degree of specialization to cave habitats (Table 2).

What explains the large discrepancy between the estimates of Cooper (1975), Culver (1982) and this study? Since the general approach and methods in the two studies were similar, the differences between conclusions must be related to the data used to drive the size-at-age models. Below potential factors related to the data are discussed, particularly with respect to: (1) the size distribution of crayfish used to estimate growth rates, (2) the size thresholds used to define life-history stages (e.g. size-at-maturity, age-at-first-reproduction) and (3) the general limitations of using iterative growth models to estimate size-at-age.

Differences in the size distribution of crayfish used to estimate growth rates

The morphology of a growth model is influenced by the distribution of size-classes included in its construction. In many species, smaller size-classes show greater mass-specific growth per unit time than larger size-classes. Growth models that include a wide range of size-classes will typically show an early period of exponential growth, followed by an abrupt plateau. However, growth models become more protracted and almost linear when smaller size-classes are underrepresented, which can ultimately cause inaccurate estimates (i.e. overestimates) of life span, time-to-maturity and age-at-first-reproduction.

In Cooper’s (1975) original size-at-age models, only 12 of the 56 individuals were less than 30 mm TCL, and only two of the 26 individuals used to produce size-at-age models based on Cooper’s (1975) data were less than 30 mm TCL (23 mm OCL). When comparing the distribution of individuals used to estimate growth rates (Fig. 1) with the actual size distribution of *O. australis* sampled from Shelta Cave (Fig. 4), it is clear that there was a strong bias to large individuals in Cooper’s full set of recapture data, even though smaller individuals (e.g. 12 to 30 mm TCL) were well represented in the population. Unlike the growth models for Shelta Cave, all available size-classes were well represented in this study's models from the three new Alabama cave sites, which produced distinct periods of exponential growth in each model.

Differences in size thresholds used to define life-history stages

While methods used to distinguish ovigerous females were consistent among studies (presence of ova or attached young), those used to set thresholds for female size-at-maturity differed significantly. Cooper (1975, p. 202) conservatively identified mature female crayfish as those displaying "late stage (3-4) oocytes and, usually, cement glands". Mature females were identified in this study using the presence of cement glands alone, which is a reliable indicator of maturity in female surface crayfish (see Reynolds, 2002). Applying the definition from this study to Cooper's (1975) data for Shelta Cave resulted in a reduction of female time-to-maturity by 5 to 16 years.

Limitations of iterative growth models

The asymptotic relationship between size and age is an inherent limitation to size-at-age estimates made using iterative growth models. If the models are interpreted literally, the largest individuals in a population are not significantly different from an infinitely old crayfish, such as in the model for Hering Cave (Fig. 3). In such cases, size is no longer an accurate predictor of age because annual growth increments become vanishingly small or stop altogether. Additionally, iterative models do not account for anomalies, such as individuals that are much larger than average at birth or those that have exceptionally fast growth rates (Weingartner, 1977, p. 208). Presumably, it is factors such as these that are the "flies lurking in the ointment of growth records" to which Cooper was referring (1975, p. 314). To avoid such drawbacks, iterative models must be interpreted within the context of population structure and dynamics. For example, Hering Cave's growth model cannot estimate the age of the largest male or female.

When examining the frequency histogram for Hering Cave, however, it is immediately apparent that the largest size-classes account for a very small percentage (~5%) of the total population. Thus, both from an ecological and conservation perspective, the majority of population dynamics (e.g. production, reproduction and mortality) occurred within the smaller size-classes and on much shorter time scales than would be expected from only the largest size-classes (Fig. 3).

The longevity of O. australis*: a new perspective*

Single species are often used as benchmarks to illustrate biological theories or define a particular ecosystem. Examples include the use of Galápagos finches to illustrate evolution via natural selection or the status of the red-eyed tree frog (*Agalychnis callidryas* [Cope]) as a "poster-species" for conservation of Central American rainforests. Previously, the 100+-year life span of *O. australis* was used to illustrate how evolution can shape extreme life histories in obligate cave species and to focus conservation efforts on cave ecosystems. However, the reexamination of Cooper's (1975) data, coupled with new life-history information, strongly suggest that *O. australis* is not a "centenarian" species. While the new estimates from this study are substantially lower than Cooper's (1975), they remain impressive, however. The life span of *O. australis* is 4 to 20× longer than any other crayfish within the same genus, which continues to provide an excellent example of potential K-selected life-history evolution in an obligate cave species. This study also emphasizes the importance of interpreting the life-history information of long-lived species within the context of both population structure and methodological limitations. Using a minority (e.g. the largest or oldest individuals) to interpret the dynamics of an entire population will misrepresent the time-scale over which important life history events (e.g. reproduction) occur and potentially influence the development of species-specific management strategies.

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Table 1. Estimated minimum life span (years), mean time-to-maturity (years) and age-at-first-reproduction (years), proportions of males and females that were mature, and male:female ratio for *Orconectes australis* in Hering, Limrock and Tony Sinks caves. Ranges are in parentheses.

	Life span		Time-to-maturity		Age-at-first-reproduction	% of gender mature		
	Male	Female	Male	Female	(ovigerous female)	Male	Female	Male:Female
Hering	22+	22+	3.3 (2.75 – 3.75)	3.5 (3 - 4)	4.6 (3.75 – 5.5)	72	63	0.78
Limrock	22+	15.5+	3.9 (3 - 4.75)	5.1 (4 - 6.25)	6.4 (5 - 7.75)	88	58	0.76
Tony Sinks	11+	22+	2.5 (1.75 - 3.25)	3.9 (2.75 - 5)	5.5 (4 - 7)	83	50	0.89

Table 2. Estimated life span (years, shortest to longest) and time-to-maturity (years) of selected surface (S) and cave (**C**) crayfish with the method utilized for approximation. SF = size-frequency, MR = mark-recapture, GM = growth model, VBGF = von Bertalanffy growth function.

Habitat	Species	Life span	Time-to-maturity	Method	Author
S	*Procambarus clarkii* Girard	1 - 12		SF, VBGF	Scalici *et al*., 2010; Fidalgo, Carvalho, & Santos, 2001
S	*Orconectes* spp.	1 - 5	1 - 2	MR, SF, GM	Momot, 1984; Payne & Payne, 1984; Muck, Rabeni, & Distefano, 2002
S	*Cambarus halli* (Hobbs)	2+		SF	Dennard, Peterson & Hawthorne, 2009
S	*Fallicambarus gordoni* Fitzpatrick	2 - 3		SF	Johnston & Figiel, 1997
S	*Cambarus hubbsi* Creaser	3		SF	Larson & Magoulick, 2011
S	*Procambarus suttkusi* Hobbs	3		SF	Baker, Stewart & Simon, 2008
S	*Paranephrops planifrons* (White)	3 - 4	1 - 2	MR, SF	Parkyn, Collier & Hicks, 2002
S	*Cambarus elkensis* Jezerinac and Stocker	5	2.5 - 3	SF	Jones & Eversole, 2011
S	*Fallicambarus fodiens* (Cottle)	6		MR, SF	Norrocky, 1991
S	*Cambarus dubius* Faxon	7	1	SF, GM	Loughman, 2010
S	*Astacus leptodactylus* (Eschscholtz)	7		SF, VBGF	Deval *et al*., 2007
C	*Orconectes inermis* Cope	9-10	2 - 3	MR, GM	Weingartner, 1977
S	*Parastacoides tasmanicus tasmanicus* Clark	10	3 - 5	MR	Hamr & Richardson, 1994
S	*Cambaroides japonicus* (Haan)	10 -11	5 - 6	SF, VBGF	Kawai, Hamano, Matsuura, 1997
S	*Pacifastacus leniusculus* (Dana)	11	3 - 4	MR, GM	Flint, 1975
S	*Cambarus bartonii* (Fabricius)	13	5	MR, GM	Huryn & Wallace, 1987
S	*Paranephrops zealandicus* (White)	16+	6	MR, GM	Whitmore & Huryn, 1999
C	*Procambarus erythrops* Relyea & Sutton	16+		MR	Streever, 1996
S	*Astacoides betsileoensis* Petit	20		MR, VBGF	Jones *et al*., 2007

Table 2 Continued

C	*Orconectes australis* Rhoades	22+	4 - 6	MR, GM	This study[1]
S	*Astacoides granulimanus* Monod and Petit	25+	7	MR, VBGF	Jones & Coulson, 2006

[1] Vogt (2012) contains a reference to a preliminary agency report by Huryn, Venarsky & Kuhjada (2008) that suggests that the life span of *O. australis* may approach 50 years. While the growth models used to estimate longevity in both Huryn, Venarsky & Kuhjada (2008) and this study are methodologically identical and share some data (1,650 marked crayfish that were recaptured during 2005 to 2008), the data conclusions presented in this study are more robust due to a much larger data set (3,812 marked crayfish that were recaptured during 2005-2011).

Figure 1. Annual growth increment (mm y^{-1}) vs. mean ocular carapace length (mm) for *Orconectes australis* crayfish in Hering, Limrock, Tony Sinks and Shelta (Cooper, 1975) caves. Dashed lines are results of least squared regression. For the Shelta Cave data, only crayfish that were marked for ≥11 months were included in the reconstruction of Cooper's growth models [see *Methods: Cooper's (1975) growth models*] and in the least squared regression line.

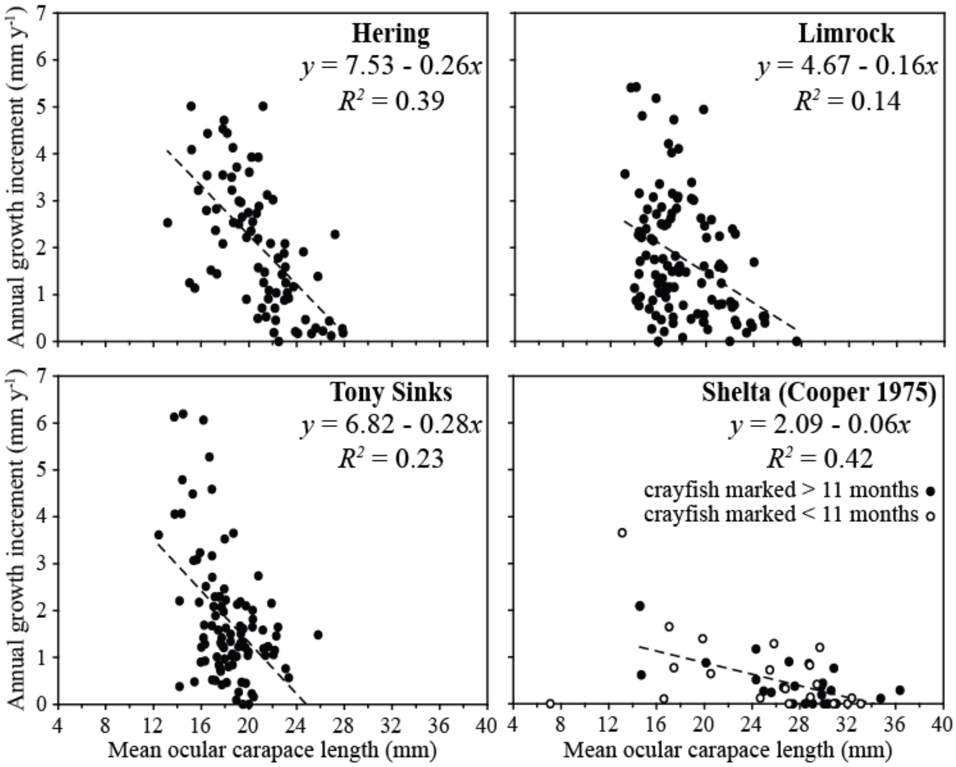


Figure 2. Growth models for *Orconectes australis* from Hering, Limrock and Tony Sinks caves. Lines indicate the upper and lower 95% confidence intervals for each model.

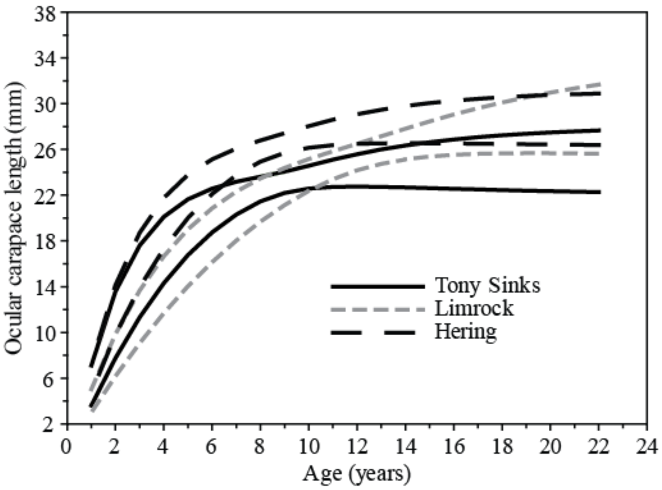


Figure 3. Growth models for *Orconectes australis* from Hering, Limrock and Tony Sinks caves. Dotted lines are upper and lower 95% confidence intervals. The pooled size-frequency distribution for each population is plotted to the right of each growth model. To the left of the size-frequency distribution are box and whisker plots. The boxes are the 25th and 75th percentile containing the mean (dashed line) and median (solid line); whiskers are error bars; dots are the 5th and 95th percentiles.

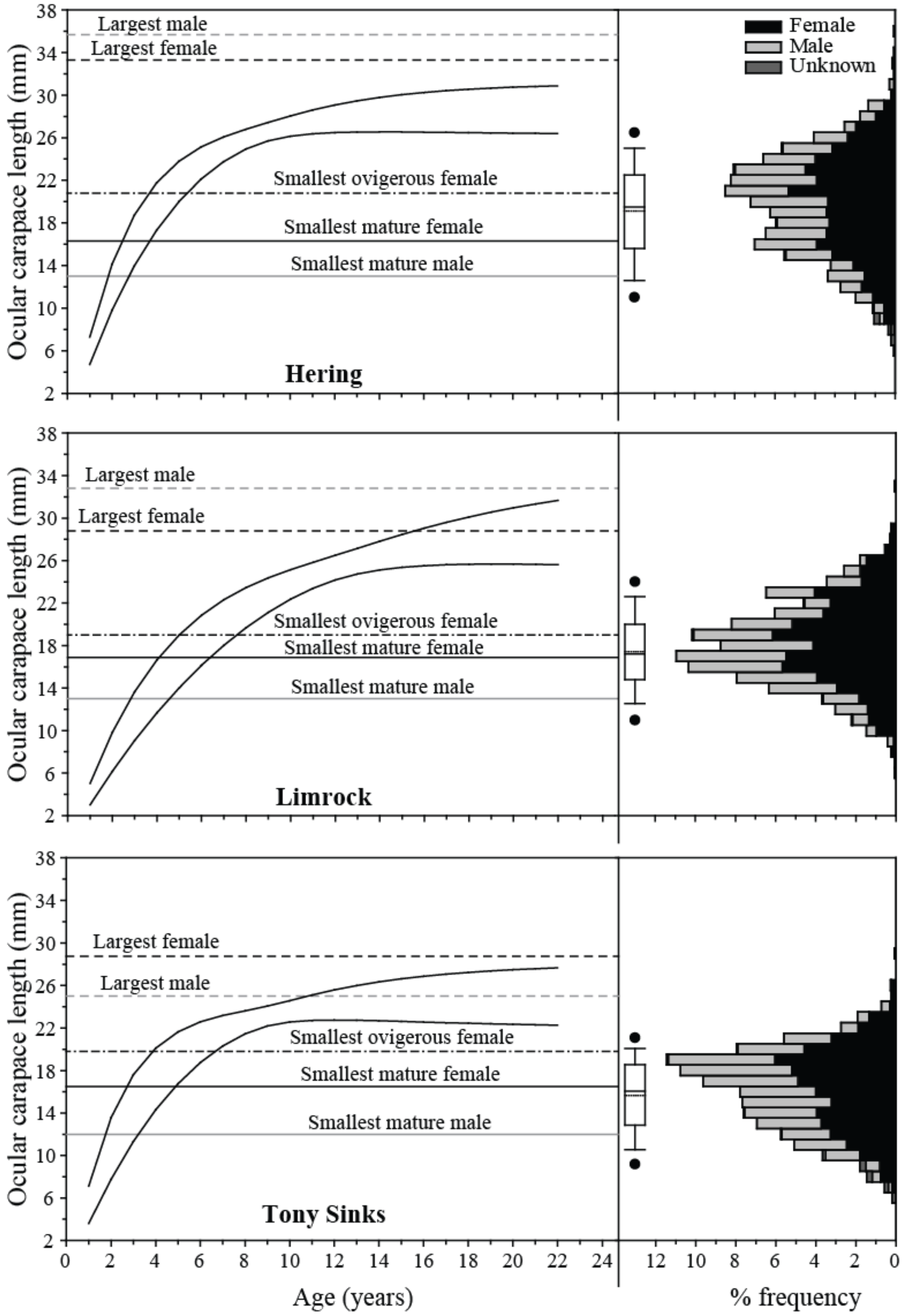
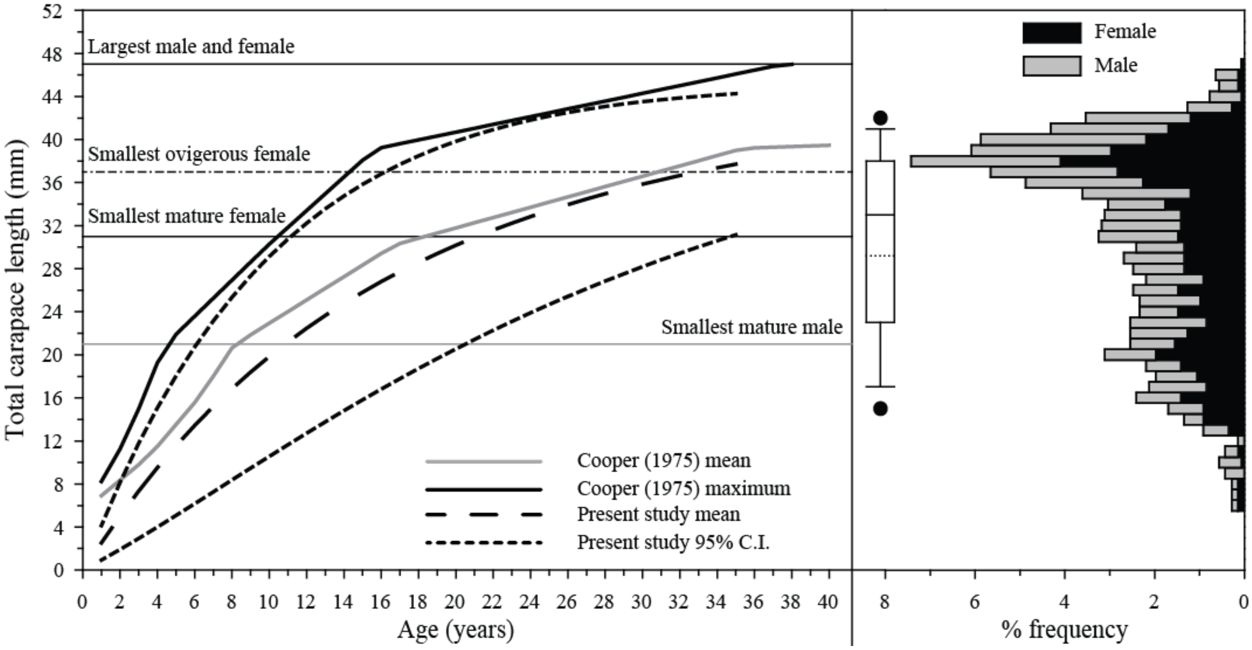


Figure 4. Growth models for *Orconectes australis* from Shelta Cave. Black and grey solid lines are a reconstruction of Cooper's (1975) maximum and mean growth models. The black dotted lines are the upper and lower 95% confidence intervals and the black dashed line is the mean for the growth model that was constructed using a subset of Cooper's (1975) data and this study's modeling technique [see *Methods: Cooper's (1975) growth models*]. The pooled size-frequency distribution for the population is plotted to the right of the growth model. To the left of the size-frequency distribution is a box and whisker plot. The box represents the 25th and 75th percentile with the mean (dashed line) and median (solid line); whiskers are error bars; dots are the 5th and 95th percentiles.



CHAPTER 5

CONSUMER-RESOURCE DYNAMICS IN A CAVE STREAM ECOSYSTEM

Abstract

In cave ecosystems, energy limitation is hypothesized to be the primary factor influencing evolutionary and ecological processes. In this study, the energy-limitation hypothesis was tested by comparing the energetic demands of the obligate cave crayfish *Orconectes australis* to resource supply rates in three separate cave systems with varying quantities of food resources (i.e. organic matter and macroinvertebrate prey). Crayfish population size, biomass, and secondary production were estimated using a 5+-year mark-recapture data set from each cave. Crayfish demand was compared to resource supply rates using the trophic basis of production approach. Several different energetic scenarios were modeled that included different crayfish diets and resource supply rates. Detrital storage and macroinvertebrate biomass covaried among caves, ranging from 22 to 132 g AFDM m^{-2} and 27 to 320 mg AFDM m^{-2}, respectively. Crayfish population sizes (1311 to 5044 individuals), biomass (80 to 862 mg AFDM m^{-2}) and secondary production (21 to 335 mg AFDM $m^{-2}yr^{-1}$) mirrored the patterns in resource availability among caves, providing further support for the energy-limitation hypothesis. Energetic models constructed using a crayfish diet based on plant detritus only indicated that crayfish demand would be lower than supply rates. However, models based on a macroinvertebrate prey diet indicated that the crayfish populations would consume nearly all macroinvertebrate prey production within each cave. Collectively, the results from this study provide robust support for the energy-limitation hypothesis, but also show that cave ecosystems

are potentially capable of supporting relatively large populations of obligate cave species. Additionally, the energetic budgets provide the first quantitative explanation of why K-selected life-history characteristics and highly efficient physiologies are an evolutionary advantage to obligate cave species.

Introduction

The movement of resources among ecosystems, commonly referred to as resource subsidies (*sensu* Polis & Strong, 1996), is an ever-present feature within the ecological landscape. The roles that such subsidies play in structuring recipient ecosystems can vary widely as a function of resource quantity, quality (e.g., C:N:P ratios), and type (e.g., nutrient vs. organic matter vs. prey; see Cebrian, 1999; Moore *et al.*, 2004; Marczak *et al.*, 2007). Cave ecosystems, however, appear to represent an extreme endpoint along the resource subsidy spectrum because the lack of light prevents primary production, which causes nearly all caves (except those systems based on chemolithoautotrophy; see Engel *et al.*, 2004) to be reliant on inputs of organic matter from surface environments to support biological productivity (Poulson & Lavoie, 2001; Simon et al. 2007). However, reduced surface connectivity typically limits allochthonous inputs, which has caused caves to be characterized as energy-limited ecosystems (Hüppop, 2001 & 2005).

One line of evidence that indicates cave ecosystems are energy-limited environments is the remarkable set of traits that are shared among many obligate cave species, such as lower metabolic rates, increased starvation resistance, and more K-selected life-history characteristics (e.g. long life span, slow growth rate, and reduced fecundity; see Hüppop, 2001 & 2005; Venarsky et al., 2012b). Energy limitation also appears to be an important factor structuring entire cave communities, as several studies have reported community shifts or increased biomass following incidental inputs of organic pollutants (Sinton, 1984; Smith *et al.,* 1986; Madsen *et al.,* 1991; Notenboom *et al.,* 1994; Simon & Buikema, 1997; Sket, 1999). However, evidence from such studies is confounded because organic pollution is typically a heterogeneous mixture of organic and inorganic material (i.e., organic matter, dissolved nutrients, microbes, and toxins), making it impossible to discern which component or combination of components causes changes in recipient communities. More recent studies have found that consumer biomass and productivity are positively related to energy availability in cave and groundwater ecosystems unaffected by pollution (Datry *et al.,* 2005; Cooney & Simon, 2009; Huntsman *et al.,* 2011b).

While these previous physiological and ecological studies have provided a general test of the energy-limitation hypothesis, they have not placed the demands (e.g. consumption and growth) of the cave community within the context of energy dynamics (e.g. resource supply rates). Consequently, it is not known if cave communities are actually energy-limited in the sense that they are consuming all or a large proportion of the available resources, which has been shown in a number of surface aquatic systems (*sensu* the Allen Paradox; see Huryn, 1996; Huryn & Wallace, 2000).

In this study, the energetic demands of the obligate cave crayfish *Orconectes australis* were compared to resource availability in its cave stream habitat. This study was conducted in three separate cave systems with varying quantities of resources (e.g. organic matter and macroinvertebrates) to test two hypotheses. First, the energy-limitation hypothesis predicts that consumer productivity is limited by energy availability. Consequently we tested the hypothesis that secondary production of *O. australis* is positively correlated with standing crop organic matter and macroinvertebrate prey biomass using a 5+-year mark-recapture data set. Second, since *O. australis* is the dominant macroconsumer by biomass in these cave systems, we predicted the energetic demands of *O. australis* would be roughly equivalent to the resources available for consumption in each cave. This prediction was tested using the trophic basis of production approach (Benke & Wallace, 1980) and different energetic scenarios that included potential resource supply rates within the cave streams, combined with potential crayfish diets (e.g. detritivory vs. strict predation).

Materials and Methods

Study sites

Three caves containing streams in northeastern Alabama (Jackson County, U.S.A.) were chosen for study: Hering, Limrock, and Tony Sinks. These caves contained large populations of *O. australis* and had similar macrofaunal assemblages, which included the facultative cave crayfish *Cambarus tenebrosus* and the obligate cave salamander *Gyrinophilus palleucus*. The southern cavefish (*Typhlichthys subterraneus*) and the mottled sculpin (*Cottus bairdi*) also occurred in Limrock and Hering caves. During this 5+-year study, *O. australis* was by far the most abundant of these species and generally represented >95% of the total number of individuals encountered for all species on each sampling date. This suggests *O. australis* was also the largest consumer of resources within the cave streams. A study reach ranging from 327 to 1202 m in length (1298 to 5323 m^2), containing a series of riffle and pool habitats with sand, gravel, and bedrock substrates, was established in each cave. Stream area was estimated in September 2010 by measuring stream width at 10-m intervals.

Water temperature

Water temperature was recorded in each stream every 30 minutes using a Solinst Barologger model 3001 (Solinst, Georgetown, Ontario, Canada) from June 2007 to July 2011.

Temperature data were not available for Tony Sinks from March 2009 to January 2010 due to instrument loss.

Standing crop organic matter and macroinvertebrates

On each of four dates (March, July and November 2009 and February 2010) fifteen random samples were collected from each cave using a 22.5-cm diameter corer to quantify benthic organic matter and macroinvertebrate biomass. The corer was inserted into the stream bottom, large organic matter was removed, and the substrate was disturbed to a maximum depth of 4 cm. Remaining suspended organic matter was removed from the corer via ten sweeps of the water column with a 250-µm mesh net. Samples were returned to the laboratory on ice and processed within 48 hours.

Once returned to the laboratory, samples were processed through a 250-µm sieve and all macroinvertebrates were removed and preserved in 5% formalin. The remaining organic matter was dried at 60°C for ~2 weeks, weighed, combusted at 500°C for 6 h, and then weighed again to estimate ash-free dry mass (AFDM). Macroinvertebrates were identified to the following levels: family/tribe for insects, class or order for microcrustaceans, class for annelids, and phylum for nematodes. Dry mass was estimated using published length-mass relationships (Calow, 1975; Culver *et al.,* 1985; Leeper & Taylor, 1998; Benke *et al.,* 1999; Lemke & Benke, 2004; Doroszuk *et al.,* 2007). Macroinvertebrate dry mass was converted to AFDM assuming AFDM is 93% of dry mass (Benke & Wallace, 1980). Using Data Desk version 6.1 (Data Description Inc., 1996), a two-way analysis of variance (factors – cave and sampling date) was conducted to examine differences in standing crop organic matter and macroinvertebrate biomass among caves.

Crayfish sampling

Sampling for crayfish began in November 2005 in Hering, January 2006 in Limrock, and July 2006 in Tony Sinks caves and was conducted semi-monthly (conditions permitting) until August 2011. On each visit, study reaches were visually surveyed by two observers on foot and all crayfish encountered were collected using dip-nets. Captured crayfish were marked using both internal tags [Visible Implant Alpha Tags (VIAT), Northwest Marine Technology, Shaw Island, WA, USA] and Visible Implant Elastomer (VIE; Northwest Marine Technology). VIATs are small (1.0 × 2.5 mm), fluorescent, uniquely numbered tags that were placed beneath the abdominal cuticle. The VIE was injected directly posterior to the VIATs and was used to assess tag loss, which was infrequent. Once marked, the ocular carapace length (OCL; posterior margin of ocular cavity to posterior center-margin of carapace) of each crayfish was measured (±0.1 mm) with dial calipers and was then released near the point of capture. OCL was used rather than TCL to avoid errors due to damage to the acumen following release (Venarsky *et al.*, 2012b).

Growth

Annual crayfish growth rates (*G*) were estimated as:

$$G = \frac{ln(W_{fn}/W_{in})}{yr},$$

where W_{fn} is g AFDM upon recapture, W_{in} is g AFDM at initial marking, and *yr* is years elapsed. Length-AFDM equations for *O. australis* were acquired from Huntsman *et al.* (2011a). Since growth increments are "episodic" due to the molting cycle, annual growth increments were only calculated for individuals recaptured over intervals of 350 days or longer to ensure that molting occurred between recapture events. Negative annual growth-increments were attributed to measuring error and were excluded from analyses. For crayfish recaptured multiple times, the annual growth increment was calculated using the recapture date closest to the 350-day minimum (Venarsky *et al.,* 2012b). Annual growth increments were regressed against average crayfish biomass (g AFDM) to estimate the size-specific annual growth rate.

Abundance and biomass

The abundance and biomass of *O. australis* in each cave was estimated from June 2007 to May 2011 (~46 months or 3.8 years) with sampling occurring at monthly or bimonthly intervals. Crayfish abundance was estimated using the mark-recapture data (described in "Growth" section above) and Program MARK (White & Burnham, 1999). A "Closed Capture" model in Program MARK was used, which assumes that no births, deaths, immigration or emigration occurs. Because the data set spanned multiple years, some assumptions were probably violated. However, the severity of these violations were likely minimized because: i) *O. australis* is long-lived (≤ 22 years; Venarsky *et al.*, 2012b) suggesting low mortality rates, ii) only 8 ovigerous females were found among the 3 caves, indicating recruitment was minimal, and iii) size-frequency histograms were not significantly different among years within each cave, suggesting a stable population structure (see *Crayfish production* section in Results). The most severe violations were likely those regarding immigration and emigration. However, immigration and emigration were likely minimal within the greater groundwater recharge area (e.g. analogous to surface stream watersheds) of the caves. Each model produced during the Program MARK analysis was ranked based on Akaike's information criterion (AIC); the lowest AIC value represents the best fit model for the data (Akaike, 1973; Burnham & Anderson, 2002).

Crayfish abundance was converted to biomass by first distributing the total population size acquired from Program MARK among the observed size-classes in a cumulative size-frequency distribution of carapace lengths for each cave. Biomass was then calculated using the geometric mean of each size-class. Estimates of abundance and biomass were standardized to area using wetted stream area estimates. For each cave, yearly size-frequency distributions were constructed and compared for annual variability using pairwise Kolmogorov-Smirnov (K-S) tests. K-S tests were performed in program R version 2.14.0 (R Development Core Team, 2008).

Secondary production

Annual crayfish production for each size-class was estimated using the following formula:

$$P = G \text{ x } \bar{B}$$

where G = annual instantaneous growth rate (g g^{-1} AFDM yr^{-1}) and $\bar{B}$ = mean biomass (g AFDM m^{-2}; Huryn & Wallace, 1987a). Variation in growth rates and biomass was accounted for in these analyses using a bootstrapping technique (see Efron & Tibshirani, 1993; Whitmore & Huryn, 1999). For G, the annual growth rates were randomly resampled with replacement and a regression equation was then calculated for the size-specific annual growth rate of the randomly generated data. This procedure was replicated 1000 times.

For $\bar{B}$, the estimated variance for the population sizes provided by Program MARK was entered into the Normal Distribution function in Microsoft Excel 2010 and 1000 random population sizes were generated. Each randomly generated population was then distributed among size-classes using the cumulative size-frequency distribution. This approach allowed the calculation of annual production for different year-classes, which were then summed to estimate total annual production. Estimates of P and $\bar{B}$ were compared among caves using pairwise two-sample randomization tests (Manly, 1991).

Trophic basis of production

Because gut content analyses were not conducted during this study, upper and lower limits of resource demand for *O. australis* were estimated by assuming that they consumed either

100% organic matter (i.e., maximum amount of organic carbon directly consumed) or 100% macroinvertebrates (i.e., minimum amount of organic carbon directly consumed). Demand was estimated by dividing the annual production of *O. australis* by the product of diet-specific (e.g. organic matter or macroinvertebrate) assimilation efficiency (AE) and net production efficiency (NPE; Benke & Wallace, 1980). Estimates of AE and NPE were acquired from the literature for both surface and cave crayfish fed diets similar to those found in natural habitats (e.g. organic matter or macroinvertebrates; Table 2).

Resource supply rate (g m^{-2} yr^{-1}) was estimated assuming that i) the standing crops of organic matter and macroinvertebrate biomass did not vary through time and ii) that the resources incorporated (e.g., consumption and assimilation) into consumer biomass or lost through respiration or downstream export are replaced (i.e., steady state conditions). These assumptions appear to be valid given the low amount of variation in monthly estimates of organic matter and macroinvertebrate biomass reported for three cave sites in Venarsky *et al.* (2012a) and Chapter 3 of this dissertation.

During the processing of core samples, standing crop organic matter was not partitioned into different categories (e.g. wood or leaves). Thus, minimum and maximum annual organic matter supply rates were estimated by assuming that all organic matter was either leaf-litter (e.g. maximum supply rate) or wood (e.g. minimum supply rate). Organic matter supply rate was then estimated as the product of mean annual organic matter biomass and average annual breakdown rate of leaf-litter or wood. Breakdown rates of leaves and wood were acquired from the literature (Table 1). Breakdown rates for leaves were from cave streams (Table 1) and wood rates were from surface and cave streams. Annual secondary production of macroinvertebrates was estimated by multiplying biomass by a conservative production:biomass relationship of 5 (Benke & Huryn, 2007).

Variability in estimates of NPE, mean annual standing crop organic matter, mean annual macroinvertebrate biomass, and mean organic matter breakdown rates were accounted for by calculating 1000 means using the bootstrapping procedure described above for crayfish growth. Diet-specific crayfish demand was then compared to organic matter supply rates and macroinvertebrate secondary production using a two-sample randomization test (Manly, 1991). To determine whether resource deficits or surpluses existed, the bootstrapped data sets for diet-specific crayfish demand were subtracted from the bootstrapped data sets for organic matter supply rates and macroinvertebrate secondary production.

Results

Water temperature

Average daily water temperature in all caves was approximately 13˚C and showed little annual variation (standard deviation of average daily water temperature: ±1˚C).

Standing crop organic matter and macroinvertebrate biomass

Standing crop organic matter was highest in Tony Sinks (132 g AFDM m^{-2}) and lowest in Hering (22 g AFDM m^{-2}), but differed only between Tony Sinks and Hering ($F_{2,\,168} = 3.42$, $P = 0.03$; Fig. 1A). Standing crop organic matter did not vary among sampling dates ($F_{3,168} = 0.43$, $P = 0.73$) and the cave × date interaction was not significant ($F_{6,168} = 1.30$, $P = 0.26$). As mean quantity of resources increased among caves, resource aggregations became larger and patchier, which increased the spatial variability in resource availability (Fig. 1A). Macroinvertebrate biomass was highest in Tony Sinks (320 mg AFDM m^{-2}) and lowest in Hering (27 mg AFDM m^{-2}) and was significantly different among all caves ($F_{2,\,166} = 39.55$, $P < 0.001$; Fig. 1A; Table 3).

Macroinvertebrate biomass varied significantly among sampling dates ($F_{3,166} = 6.80$, $P < 0.001$) but did not vary within each cave (cave × date: $F_{6,166} = 1.12$, $P = 0.35$).

Crayfish production

A total of 3812 crayfish were marked in Hering (919 individuals), Limrock (943), and Tony Sinks (1950) caves over the 5+-year study. Growth models were constructed using 78 (37 males and 41 females) crayfish in Hering Cave, 112 (47 males and 65 females) crayfish in Limrock Cave, and 97 (46 males and 51 females) crayfish in Tony Sinks Cave. Monthly capture rates of crayfish were highly variable within each cave and on average were lowest in Hering (35 individuals) and highest in Tony Sinks (71 individuals; Table 4). The mean percent of recaptures each month was also highly variable, being lowest in Tony Sinks (20%) and highest in Limrock (39%; Table 4). The reduced mark-recapture data set (e.g., ~46 months or 3.8 years) used in the Program MARK analyses included a total of 3600 crayfish: Hering (775), Limrock (910), Tony Sinks (1915). Crayfish population size estimates using Program MARK were lowest in Hering (1311) and highest in Tony Sinks (5044; Table 4). Crayfish biomass was significantly different among all caves and was lowest in Limrock (80 mg AFDM m^{-2}) and highest in Tony Sinks (862 mg AFDM m^{-2}; Fig. 1B; Table 4). Crayfish production was also significantly different among all caves and was lowest in Limrock (21 mg AFDM $m^{-2}yr^{-1}$) and highest in Tony Sinks (335 mg AFDM $m^{-2}yr^{-1}$; Fig. 1B; Table 4). Crayfish biomass turnover (e.g., *P:B*) was lower in Hering (0.28) and Limrock (0.27) caves than in Tony Sinks (0.39; Table 4). Size-frequency distributions among years within each cave were similar (K-S test, $P > 0.05$), indicating that population structure was similar among sampling years.

Resource supply

Mean breakdown rates used to estimate organic matter supply rates were 2.2 yr^{-1} (1.3-3 yr^{-1}; 95% confidence interval) and 0.6 yr^{-1} (0.4-0.8 yr^{-1}; 95% confidence interval; Table 1) for leaf-litter and wood, respectively. Using leaf-litter breakdown rates, mean organic matter supply rates were lowest in Hering (47 g AFDM m^{-2} yr^{-1}) and highest in Tony Sinks (284 g AFDM m^{-2} yr^{-1}; Fig. 2A; Table 5). Using the wood breakdown rates, mean organic matter supply rates were lowest in Hering (13 g AFDM m^{-2} yr^{-1}) and highest in Tony Sinks (80 g AFDM m^{-2} yr^{-1}; Fig. 2A; Table 5). Using a P:B ratio of 5, mean macroinvertebrate production was lowest in Hering (0.13 g AFDM m^{-2} yr^{-1}) and highest in Tony Sinks (1.61 g AFDM m^{-2} yr^{-1}; Fig. 2B; Table 5). The large amount of variability in resource standing stocks translated into wide confidence intervals in the resource supply rates (Fig. 2A, B).

Trophic basis of production

Mean net production efficiency used to estimate energetic demands for *O. australis* was 0.46 (0.38-0.54; 95% confidence interval; Table 2). Assuming a diet composed only of organic matter, demand by *O. australis* was lowest in Limrock (0.47 g AFDM m^{-2} yr^{-1}) and highest in Tony Sinks (7.4 g AFDM m^{-2} yr^{-1}; Fig. 2A; Table 5). Crayfish demand was significantly lower than wood and leaf supply rates in all caves (Fig. 2A,C). Assuming *O. australis* consumed only macroinvertebrates, mean demand was lowest in Limrock (0.06 g AFDM m^{-2} yr^{-1}) and highest in Tony Sinks (0.9 g AFDM m^{-2} yr^{-1}; Fig. 2B, Table 5). Crayfish demand was similar to prey supply in Hering and Tony Sinks and was significantly lower than prey supply in Limrock (Fig. 2B, D). The inclusion of zero in the 95% confidence intervals for the macroinvertebrate surpluses in Hering and Tony Sinks suggests that the demand by *O. australis* is not significantly different from supply and does not verify the presence of a resource deficit (Fig. 2D).

Discussion

The energy-limitation hypothesis

The results of this study provided general support for the energy-limitation hypothesis in cave ecosystems. First, the patterns in the biomass and secondary production of *O. australis* mirrored the gradients in standing crop organic matter and macroinvertebrate biomass among caves (Fig. 1A, B). Second, the comparisons of resource supply rates and crayfish demand indicate that nearly all macroinvertebrate production would need to be consumed to support the populations, which suggests these populations of *O. australis* are limited by prey availability.

Resource availability

The range in standing crop organic matter (22-133 g AFDM m^{-2}) within the cave streams in this study falls within the lower range of reports from other cave (0-850 g AFDM m^{-2}; Simon & Benfield 2001 & 2002; Venarsky *et al.*, 2012a; Huntsman *et al.*, 2011 a, b; see Chapter 3) and surface streams (20-35,000 g m^{-2}; Jones, 1997). The large range in organic matter biomass among the cave streams in this study illustrates that, similar to surface streams, resource inputs can vary greatly. One factor likely contributing to the large variability in organic matter biomass among these cave streams is cave structure, such as the depth of cave and size of voids and fractures in the surrounding bedrock. For example, the higher quantities of organic matter in Tony Sinks Cave were probably the result of strong surface connections. This cave contained several entrances, both vertical and horizontal, that intersected intermittent stream channels. Additionally, large ceiling fissures were present in portions of the cave, below which large deposits of organic matter were observed.

Macroinvertebrate biomass mirrored the patterns in standing crop organic matter (Fig. 1). Similar to standing crop organic matter, macroinvertebrate biomass (29 to 346 mg dry mass m^{-2}) in the cave streams from this study fall within the lower range of estimates from surface streams not impacted by anthropogenic pollution (156 to 20,206 mg dry mass m^{-2}; Benke, 1993). Only two other studies to date have quantified macroinvertebrate biomass in cave streams, both of which fall within the range of estimates from this study (10 to 300 mg dry mass m^{-2}; Huntsman *et al.*, 2011 b; Chapter 3 this document). Thus, given that both organic matter and macroinvertebrate biomass were within the lower ranges of reports from surface streams, the cave streams in this study appear to fit the generalized characterization of energy-limited cave ecosystems.

Population size estimates

Estimating the population size of crayfish is challenging because they are potentially very mobile species and the complexity of their habitat is often high, particularly in surface streams (i.e., because of the presence of macrophytes and large woody debris). While habitat complexity is relatively diminished within many cave streams (i.e., no or little biogenic structure, prevalence of bedrock structure), cave crayfish still reside in areas that are inaccessible to humans, such as bedrock fissures or under large boulders. Thus, visuals surveys and other count-based methods will likely misrepresent population sizes of both cave and surface crayfish because a majority of the population may be unavailable for direct sampling. Rabeni *et al.* (1997) evaluated various methods (i.e., quadrat sampling, visual surveys, and mark-recapture) for estimating population sizes of crayfish in surface streams and found that visual surveys significantly underestimated population size when compared to mark-recapture methods using multiple sampling events. Monthly capture rates of *O. australis* within each cave varied 5 to 10 times and the majority of crayfish captured in most months were unmarked, both of which indicate that a much larger population of crayfish was present than monthly capture rates would have shown. Thus, on any given sampling date many of the crayfish were likely in inaccessible habitats, such as in fissures or under large slabs of bedrock. Any such pattern or habitat use has clear implications for our whole-system energy budgets (see below).

Molecular-based methods also suggest that visual based surveys, which are analogous to the monthly capture rates in this study, do not accurately estimate the population size of obligate cave species. Buhay and Crandall (2005) examined mitochondrial-DNA haplotypes among 43 populations of *O. australis* occurring throughout northeastern Alabama and eastern Tennessee, including both the Hering and Limrock populations. They found both high genetic diversity and large effective population sizes, which suggest that the population sizes of *O. australis* throughout its range are likely large. Thus, using visual surveys will grossly underestimate population sizes for large mobile obligate cave species like *O. australis*, which can lead to inaccurate estimations of ecosystem processes (e.g. secondary production and energetic demands) and conservation assessments (e.g. threatened or endangered status).

Crayfish secondary production

O. australis is a slow-growing species, reaching maturity in ~5 years and capable of living for ~22 years (Venarsky *et al.*, 2012b). The slow growth of *O. australis* translated into estimates of secondary production and P:B values that are among the lowest recorded for either cave or surface species of crayfish (Table 6). However, the production estimates from the Tony Sinks population of *O. australis* are higher than those for some surface species of crayfish. Thus, while cave ecosystems do appear to be energy-limited when compared to some surface ecosystems, they are capable of supporting similar rates of crayfish production.

Energy budgets

Several different energetic scenarios were modeled in this study using potential crayfish diets and resource supply rates so that the energy-limitation hypothesis could be explored from an energy-dynamics perspective. Collectively, the energy budgets constructed for *O. australis* do not show that crayfish demand is higher than resource supply rates, which indicates that resource deficits do not exist and that these populations do not require more resources than are present within the cave stream channels.

In this study, the energetic demands of crayfish populations were bracketed between strict detritivory and strict predation. While leaf and wood supply rates were large enough in all caves to fulfill crayfish demand, it is unlikely that *O. australis* feeds strictly on detritus because: i) the majority of growth in juvenile surface crayfish taxa has been attributed to the consumption of animal material (Whitledge & Rabeni 1997), ii) both surface and cave crayfish are known to be omnivorous (Weingartner, 1977; Nyström, 2002; Parkyn *et al.*, 2001), and iii) *O. australis* maintained in the laboratory does not consume conditioned leaf litter (M.P. Venarsky, personal observation). Thus, these populations of *O. australis* were most likely supported by macroinvertebrate production, rather than organic matter.

The energetic models based on a macroinvertebrate diet suggest that the Hering and Tony Sinks populations of *O. australis* consumed all of the macroinvertebrate production, while a very small surplus of macroinvertebrate production was present in Limrock. These results suggest *O. australis* is likely limited by macroinvertebrate production, which is not a feature unique to cave ecosystems. The near complete consumption of prey production by predators has been observed in several surface streams (see Huryn & Wallace, 2000). Because of the limited potential for prey surpluses, competition for resources among *O. australis* and other predators in these cave systems is likely high, especially in Tony Sinks and Limrock where the large variability in macroinvertebrate biomass indicates that large quantities of resources are concentrated into small areas. Thus, the energetic budgets constructed for *O. australis* in this study provide the first quantitative explanation of why K-selected life history characteristics, highly efficient physiologies, and enhanced sensory systems for food acquisition are an evolutionary advantage to obligate cave predators.

Conclusions

Support for the energy-limitation hypothesis has historically come from either laboratory-based studies focused on individual physiological characteristics (e.g. metabolic rates) of obligate cave species or field based population- or community-level studies looking for correlations between resource availability and species biomass or productivity. While each approach has its strengths, neither typically places its results within the context of energy dynamics of actual cave ecosystems (e.g. resource supply rates vs. consumption and growth). The trophic basis of production approach (sensu Benke & Wallace, 1980) used in this study incorporated all aspects of the physiology and life history of *O. australis* and placed them within the context of potential resource supply rates. Collectively, the results from this study provide robust support for the energy-limitation hypothesis and add to the body of evidence supporting the energy-limitation hypothesis in cave and groundwater ecosystems (e.g., Datry *et al.*, 2005; Cooney & Simon, 2009; Huntsman *et al.*, 2011b; Chapters 3). Additionally, this study shows that populations sizes of obligate cave species can be much larger than visual surveys would suggest and that cave ecosystems are capable of supporting larger populations of obligate cave species than previously appreciated.

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Table 1. Leaf and wood breakdown rates (k, yr^{-1}) utilized to estimate organic matter supply rates within Hering, Limrock, and Tony Sinks caves.

Litter type	Species	k (yr^{-1})	Reference
Leaves	Post oak (*Quercus stellata*)	7.3	Brown & Schram, 1982
	Sorb (*Sorbus aucuparia*)	0.4	Galas *et al.*, 1996
	Alder (*Alnus incana*)	0.7	Galas *et al.*, 1996
	Moss (*Polytrichum* sp.)	1.1	Galas *et al.*, 1996
	White oak (*Quercus alba*)	2.9, 3.3	Brussock *et al.*, 1998
	White oak (*Quercus alba*)	1.1 - 9.5	Simon & Benfield, 2001
	Red maple (*Acer rubrum*)	0.4 - 3.1	Venarsky *et al.*, 2012a
Wood	White oak (*Quercus alba*)	1.5 - 2.4	Simon & Benfield, 2001
	Various (23 species)	0.02 - 3.1	Spanhoff & Meyer, 2004

Table 2. Assimilation efficiencies (AE) and net production efficiencies (NPE) used to estimate resource demand by *Orconectes australis* in Hering, Limrock, and Tony Sinks caves.

Habitat	Species	AE	NPE	Reference
Cave	*Orconectes inermis*	Leaf material - 0.10 Chironomid - 0.85		Weingartner, 1977
Surface	*Panulirus homarus* (spiny lobster)		0.43-0.66	Rathinam *et al.*, 2009 (Table 3)**
	Procambarus clarkii		0.39-0.74	Gutierrez-Yurrita & Montes, 2001 (Table 2)**
	Cherax tenuimanus		0.54-0.81	Villarreal, 1991 (Table 4)**
	Various (8 crayfish species)		0.09-0.75	Momot, 1995 (Table 3)

**NPE calculated following Benke & Wallace (1980)

Table 3. Mean (standard error) macroinvertebrate biomass (mg dry mass m^{-2}) from core samples in Hering, Limrock, and Tony Sinks caves.

	Hering	Limrock	Tony Sinks
Nematoda	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)
Oligochatete	0.036 (0.011)	0.02 (0.011)	0.26 (0.108)
Mollusca			
Bivalvia			0.1 (0.056)
Gastropoda			<0.001 (<0.001)
Ostracoda	0.001 (0.001)	0.002 (0.001)	0.046 (0.012)
Cladocera			<0.001 (<0.001)
Chydoridae	<0.001 (<0.001)		
Copepoda			
Cyclopoida	0.004 (0.002)	0.008 (0.003)	0.014 (0.003)
Harpacticoida		<0.001 (<0.001)	0.005 (0.001)
Malacostraca			
Amphipoda		0.013 (0.013)	0.001 (0.001)
Gammarus			0.798 (0.407)
Isopoda	0.024 (0.016)	0.027 (0.014)	
Caecidotea	0.148 (0.072)	0.468 (0.128)	0.121 (0.087)
Lirceus			1.666 (0.867)
Insecta			
Coleoptera			0.003
Elmidae			<0.001
Ephemeroptera	0.031 (0.017)	0.043 (0.012)	
Ephemerellidae	0.129 (0.051)	0.052 (0.02)	0.581 (0.201)
Leptophlebiidae			0.248 (0.091)
Plectoptera	0.001 (0.001)	0.011 (0.009)	0.001 (0.001)
Allocapnia			1.119 (1.09)
Capniidae			0.082
Chloroperlidae			1.697 (0.264)
Leuctridae			0.483
Peltoperlidae			1.787
Perlodidae			0.055 (0.026)
Tricoptera		<0.001 (<0.001)	
Hydroptilidae		0.011 (0.011)	
Diptera			
Empididae			<0.001
Simulidae		0.001 (0.001)	
Tipulidae			0.027 (0.015)
Chironomidae	0.033 (0.018)	0.012 (0.008)	

Table 3. Continued

Tanypodinae	0.039 (0.019)	0.32 (0.09)	0.637 (0.117)
Non-Tanypodinae	1.311 (0.532)	1.935 (1.07)	13.173 (3.638)

Table 4. Mean capture rate (range), % crayfish recaptured (range), population size (error) from Program MARK, biomass (95% confidence interval), production (95% confidence interval), and production:biomass (95% confidence interval) of *Orconectes australis* within Hering, Limrock, and Tony Sinks caves. Significant differences among caves are indicated by different letters. AFDM = ash-free dry mass.

	Hering	Limrock	Tony Sinks
Capture rate (individuals month^{-1})	35 (13-67)	43 (9-90)	71 (28-126)
% crayfish recaptured (month^{-1})	35 (11-60)	39 (8-62)	20 (3-43)
Population size (individuals)	1311 (50)	1380 (41)	5044 (196)
Biomass (mg AFDM m^{-2})	132 (141 - 123)^{A}	79.5 (84 - 75)^{B}	862 (929 - 795)^{C}
Production (mg AFDM m^{-2} yr^{-1})	37 (42 - 32)^{a}	21 (24 - 18)^{b}	335 (402 - 267)^{c}
Production:biomass ratio	0.28 (0.31 - 0.25)^{Y}	0.27 (0.30 - 0.24)^{Y}	0.39 (0.46 - 0.32)^{Z}

Table 5. Estimates of mean wood and leaf supply rates, macroinvertebrate production, and crayfish demand in Hering, Limrock, and Tony Sinks caves. All values are g ash free dry mass m^{-2} yr^{-1}. Numbers in parentheses are 95% confidence intervals.

Cave	Wood supply rate	Leaf supply rate	Macroinvertebrate production	Crayfish demand (organic matter)	Crayfish demand (macroinvertebrates)
Hering	13 (7-19)	47 (22-73)	0.13 (0.05-0.21)	0.82 (0.63-1)	0.1 (0.08-0.12)
Limrock	48 (5-91)	172 (9-334)	0.27 (0.07-0.46)	0.47 (0.37-0.57)	0.06 (0.04-0.07)
Tony Sinks	80 (28-131)	284 (85-483)	1.61 (0.9-2.32)	7.4 (5.36-9.44)	0.9 (0.63-1.11)

Table 6. Estimates of biomass (g ash -ree dry mass [AFDM] m^{-2}), production (g AFDM m^{-2} yr^{-1}) and production:biomass ratios (P:B) from surface and cave crayfish populations. Dry weight was assumed to be 30% of wet weight and AFDM as 64% of dry mass. The AFDM conversion factor was estimated using data from *Orconectes australis*.

Habitat	Species	Biomass	Production	P:B	Reference
Cave	*Orconectes australis*	0.080-0.862	0.021-0.335	0.27-0.39	This study
	Orconectes inermis	0.052	0.027	0.53	Weingartner, 1977
Surface	*Orconectes virilis*	0.129	0.155	1.1	Mitchell & Smock, 1991
	Orconectes punctimanus	0.129	0.384		Brewer *et al.*, 2009
	Cambarus bartonii	0.706-1.7	0.028-0.961	0.58	Huryn & Wallace, 1987b; Mitchell & Smock, 1991; Roell & Orth, 1992; Griffith *et al.*, 1996
	Paranephrops planifrons	0.429-2.3	0.512-2.18	0.95-1.2	Parkyn *et al.*, 2002
	Orconectes ozarkae	0.211	1.15		Brewer *et al.*, 2009
	Cambarus immunis		1-6		Lydell, 1938
	Pacifastacus leniusculus		2.56		Mason, 1975
	Orconectes luteus	0.949	4.2		Brewer *et al.*, 2009
	Paranephrops zealandicus	4-33	2-11	0.33-0.43	Whitmore & Huryn, 1999
	Various (8 species)	0.106-70	0.384-177	0.3-5.9	Momot, 1984

Figure 1. (A) Box and whisker plot of organic matter (left *y*-axis) and macroinvertebrate biomass (right *y*-axis) in Hering, Limrock, and Tony Sinks caves. Grey boxes are the 25th and 75th percentile and whiskers are the 5th and 95th percentiles. The dashed and solid lines within each box are the mean and median, respectively. (B) Biomass (left *y*-axis) and secondary production (right *y*-axis) estimates for *Orconectes australis* in Hering, Limrock, and Tony Sinks caves. Bars are 95% confidence intervals. AFDM = ash-free dry mass. Significant differences ($P < 0.05$) indicated by different letters. Note that different case letters do not indicate significant differences.

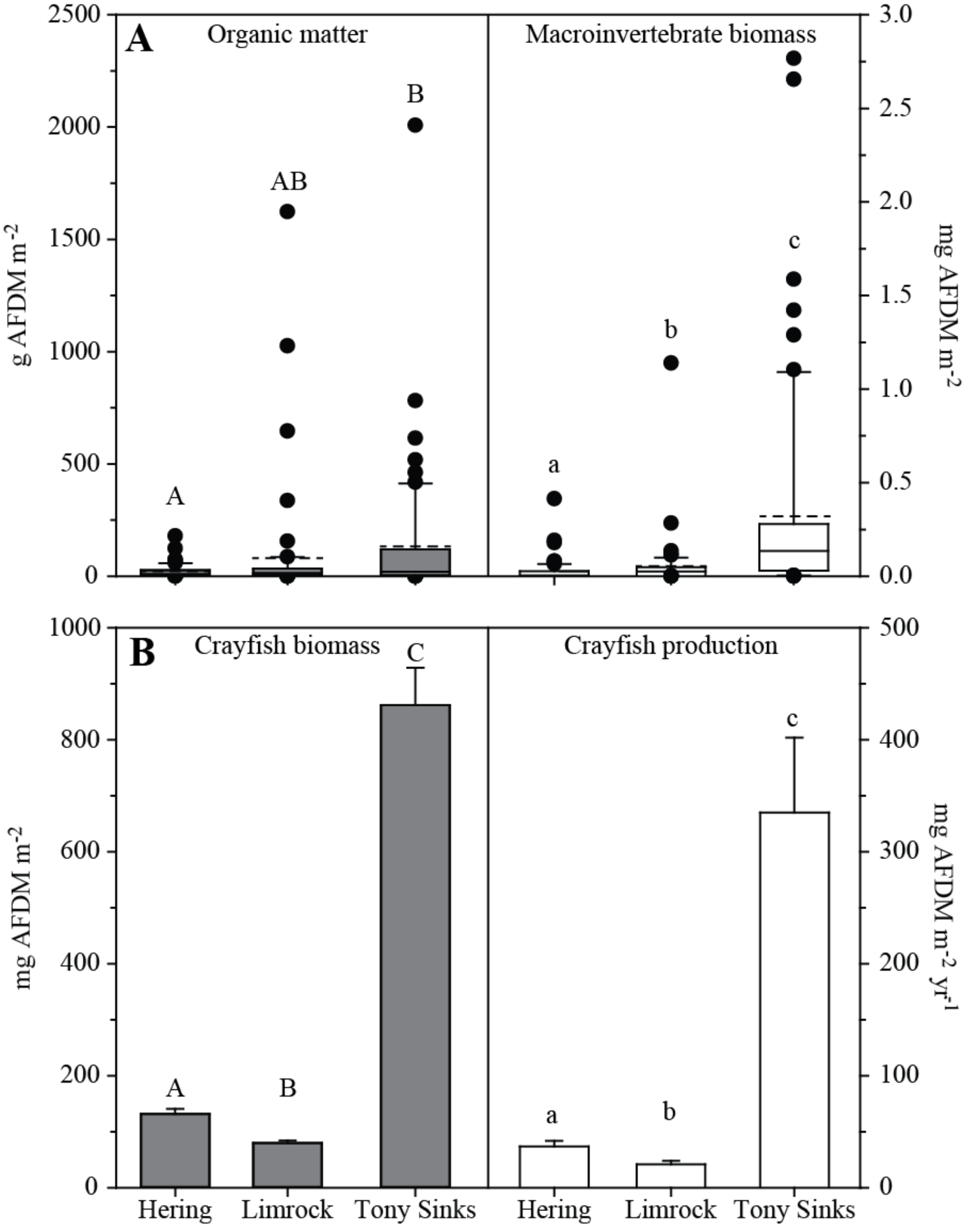
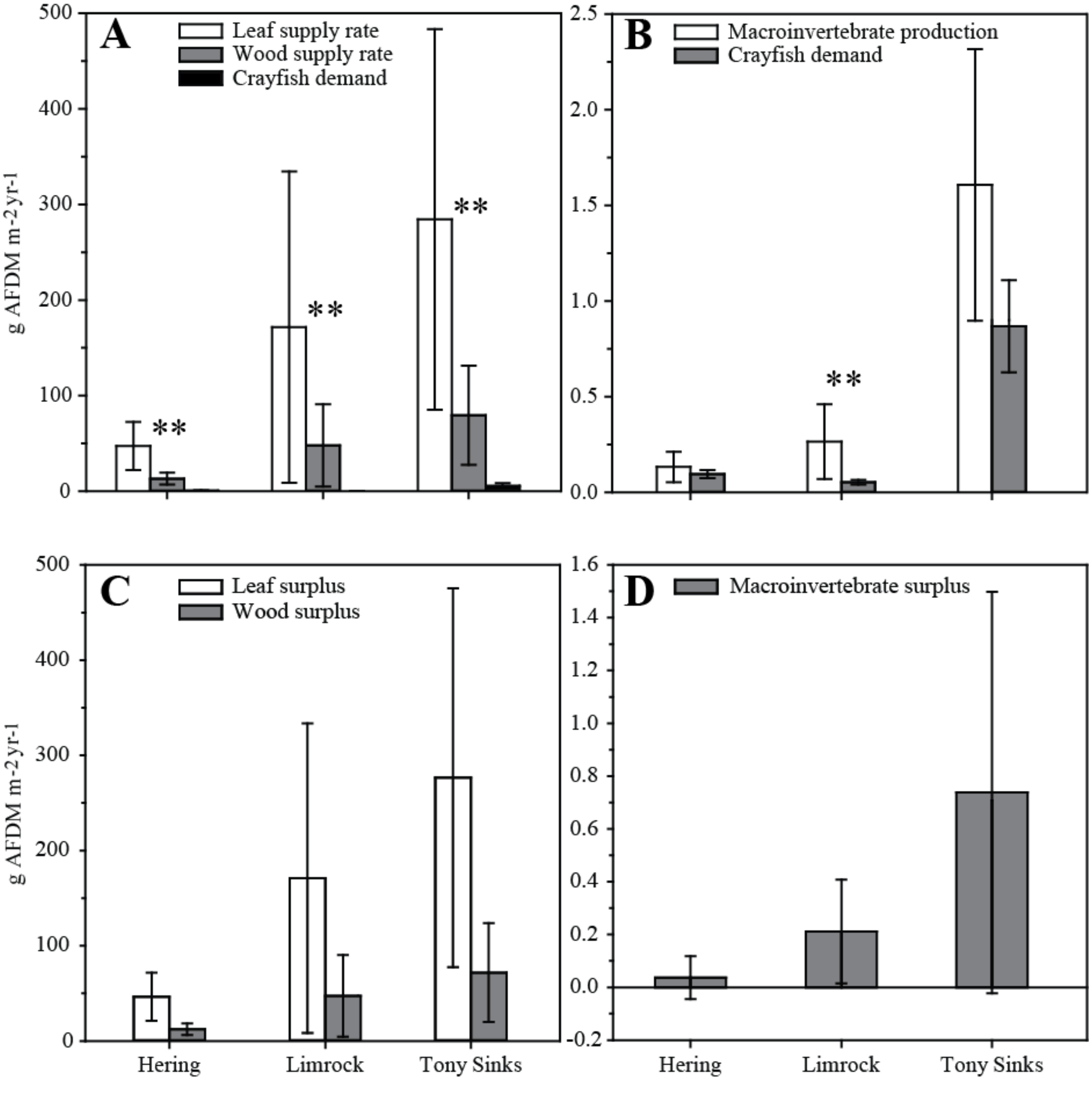


Figure 2. (A) Organic matter supply rate and (B) macroinvertebrate production in Hering, Limrock, and Tony Sinks caves with crayfish demand based on diets of organic matter and macroinvertebrates, respectively. (C) Organic matter and (D) macroinvertebrate surpluses in Hering, Limrock, and Tony Sinks caves. AFDM = ash-free dry mass. Asterisks indicate significant differences between resource supply rates and macroinvertebrate production and crayfish demand (e.g. non-overlapping 95% confidence intervals).



CHAPTER 6

OVERALL CONCLUSIONS

Due to the lack of photosynthetic primary production and limited surface connectivity that reduces the quantity and quality of detrital inputs (e.g. leaves, wood, and dissolved organic carbon), cave ecosystems have been considered energy-limited. The perception of energy limitation is at the very core of conceptual models describing the trophic dynamics, community structure, and evolutionary processes in cave ecosystems (Culver et al. 1995; Graening and Brown 2003; Simon et al. 2007). However, the energy dynamics within and among cave ecosystems have traditionally been described qualitatively and have rarely been quantified and correlated with population-, community-, or ecosystem-level processes (but see Simon & Benfield 2001, 2002; Cooney & Simon 2009; Huntsman et al., 2011a, b), which has hindered the development of quantitative models describing how energy availability influences cave ecosystem processes. This limited body of knowledge on cave ecosystem energy dynamics is in stark contrast to that of surface ecosystems, in which numerous studies have described how the quantity, quality, and type of detrital inputs influence both ecosystem processes and the structure of micro-, meio-, and macro-faunal communities (see Moore et al. 2004). This dissertation begins to close this knowledge gap by exploring how energy availability influences cave ecosystem processes at multiple organizational levels (e.g., ecosystem-, community-, and population-level) and time scales (e.g., ecological vs. evolutionary).

Chapter Two explored the relationships among organic matter biomass, macroinvertebrate community structure and litter breakdown rates. Organic matter biomass differed greatly among the four cave streams in this study, ranging from near zero to 850 g ash-free dry mass (AFDM) m^{-2}, which illustrates that the degree of energy-limitation can vary among cave systems within close geographic proximity. Despite the large differences in organic matter biomass, neither macroinvertebrate biomass in litter bags nor litter breakdown rates were correlated with ambient organic matter biomass. The similarity in litter breakdown rates appears to have been driven by a functional similarity among the cave communities. Potential leaf shredding macroinvertebrates were nearly absent in all caves and only contributed 2 to 17% of total macroinvertebrate biomass. Surface-adapted species dominated the biomass in litter bags in this study, suggesting that surface-adapted species have a greater effect on cave ecosystem processes than the cave-adapted taxa that have been the traditional focus of cave studies. The litter breakdown rates, community diversity within each cave (e.g., lack of leaf shredders and dominance of surface-adapted species), and the lack of correlation between litter breakdown rate and organic matter biomass found in this study are broadly similar to those found in previous litter breakdown studies in cave streams. These broad-scale similarities suggest that the factors that control litter breakdown and community structure within caves may thus be generally similar across geographically diverse areas.

In Chapter Three, a short-term (one year) litter amendment experiment was conducted to examine the relationship between cave community structure and organic matter availability. Non-transgenic corn (*Zea mays*) litter was added to a 100-m reach of cave stream and the response in consumer biomass and carbon source was followed relative to that of an upstream reference reach. Following the litter amendment the biomass of surface-adapted species significantly increased, while the biomass of obligate-cave species remained unchanged. This response appears to be related to the evolutionary history of the species. The suite of characteristics (e.g., higher growth rates and fecundity) that allow surface-adapted species to survive in energy-rich surface streams also likely allowed them to exploit the large quantities of additional resources present within the cave stream following the litter amendment. In contrast, obligate-cave species are adapted (e.g., reduced growth rates and fecundities) to survive in the energy-poor cave environment, which likely prevented a large biomass response to the short-term increase of resources following the amendment. These differences in evolutionary history also likely explain the dominance of surface-adapted species in the litter breakdown experiments conducted in Chapter 2, because the litter bags utilized in the experiments were essentially small resource islands that were analogous to the manipulation reach in Chapter 3. Thus, while cave communities have the ability exploit short-term increases in energy availability, species-specific responses are dictated by their evolutionary history.

A commonly cited convergent trait that many obligate cave species have evolved in the energy-limited cave environment is K-selected life history characteristics, which are characterized by longer life spans and slower growth rates. One species that has been used as a textbook example to illustrate K-selected evolution in obligate cave species is *Orconectes australis*, whose time to maturity and longevity were estimated at 35 and 176 years, respectively (Cooper 1975). However, uncertainties surrounded these extraordinary estimates. Chapter 4 used a 5+-year mark-recapture data set to re-examine the time-to-maturity, age-at-first-reproduction, and longevity of three populations of *O. australis*. The results from Chapter 4 indicate that accurate estimates of the longevity of *O. australis* are <22 years, with only a small proportion of individuals (<5%) exceeding this age. The estimates for female time-to-maturity (4 to 5 years) and age-at-first-reproduction (5 to 6 years) from this study are also substantially lower than earlier estimates. These new data indicate that the age thresholds for life history events of *O.*

australis are comparable to other estimates for a modest assemblage of both cave and surface species of crayfish for which credible age estimate exist. Regardless, these shorter longevity and time-to-maturity estimates for *O. australis* are still relatively great compared with surface species in the same genus, indicating that this species has evolved K-selected life history traits and has a high degree of specialization to cave habitats.

Support for the energy-limitation hypothesis in cave ecosystems has historically come from either laboratory-based studies focused on individual physiological characteristics (e.g. metabolic rates) of obligate cave species or field-based population- or community-level studies examining for correlations between resource availability and species biomass or productivity. While each approach has its strengths, neither places its results within the context of energy dynamics of actual cave ecosystems (e.g. resource supply rates vs. consumption and growth). In Chapter Five, the mark-recapture data set for *O. australis* from Chapter Four was combined with both the trophic basis of production approach (sensu Benke & Wallace, 1980) and estimates of resource supply rates (e.g. organic matter and macroinvertebrate prey) to place the energetic demands of *O. australis* within the context of cave energy dynamics. Similar to the results of Chapter 3, macroinvertebrate biomass increased with organic matter standing stock among the three cave streams. Both the biomass and secondary production of *O. australis* were positively related to resource standing stocks. The energy budgets showed no indication of resource deficits. The energetic models, however, indicated that nearly all prey production is necessary to support the populations of *O. australis*, which suggests that inter- and intra-specific competition for resources within these caves is likely high. Thus, the energetic budgets constructed for *O. australis* in this study provide the first quantitative explanation of why K-selected life history characteristics, highly efficient physiologies, and enhanced sensory systems for food acquisition are an evolutionary advantage to obligate cave species.

Collectively, this dissertation represents the most robust examination to date of how energy availability shapes the structure and function of cave communities. On evolutionary time scales the low quantities of energy inputs appear to have influenced the evolution of K-selected life history characteristics (see Chapters 4 and 5). These adaptations likely allow obligate cave species to respond (e.g. increased population size) to long-term (see Chapter 5) rather than short-term (see Chapter 3) increases in energy availability. Surface-adapted taxa dominated the biomass of cave communities (see Chapters 2 and 3), suggesting that their effects on cave ecosystem processes may be greater than those of cave-adapted taxa, which have been the traditional focus of cave studies. Lastly, this dissertation has demonstrated that some cave and surface (e.g., forested headwater streams) ecosystems are fundamentally similar and appear to be linked to one another along a detrital subsidy spectrum that includes both high (e.g. surface) and low (e.g. cave) rates of detrital inputs (Chapter 3).

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