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Habitat-Specific Diet of the Mottled Sculpin and Its Impact on Benthic Invertebrate Community Structure

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HABITAT-SPECIFIC DIET OF THE MOTTLED SCULPIN AND ITS IMPACT ON BENTHIC INVERTEBRATE COMMUNITY STRUCTURE

by

Brendan M. Earl

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The relationships between predators and their prey are important and influential components in the structure of ecological communities. These interactions not only impact the species involved, but can also have a wide range of direct and indirect effects that resonate throughout the community. In Michigan trout streams, one of the main predators of benthic invertebrates is the mottled sculpin (*Cottus bairdi*) whose diet and selective predation may influence benthic invertebrate community structure. Using gastric lavage, the stomach contents of sculpins from both erosional and depositional habitats were collected and analyzed to determine the main prey types, prey preference, and habitat effects in the diet. Prey preference was determined using Chesson’s $\alpha$ to calculate prey selectivity. The predatory effects on the benthic invertebrate community were examined using caging experiments. Habitat did significantly affect sculpin diet with the top three prey types being *Ephemera*, *Chironomidae*, and *Hydropsyche*. The selectivity index showed that sculpins are generalist feeders, although they did significantly avoid prey types with low movement rates, case-building ability, and small size. The predatory effects of the sculpin diet did not significantly affect the benthic invertebrate community.
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INTRODUCTION

The structure of stream communities is a complex and intricate topic that has been the focus of several studies (Flecker 1984, Kohler 1992). Competition (Hart 1983, Kohler 1992), predation (Wooster 1994, Forrester 1994, Dahl 1998), disease (Kohler and Wiley 1997, Kohler and Hoiland 2001), and disturbance (e.g. extreme flow fluctuations due to floods or droughts) (Boulton 2003, Thomson 2002, Hax and Golladay 1998) are just a few of the processes that have been investigated to attain a better understanding of the mechanisms structuring stream communities. The thorough examination of all these different aspects of stream communities should result in a clearer, more accurate depiction of its structure.

One important and influential interaction in any ecological community is the relationships between its predators and their prey. This type of interaction not only impacts the organisms involved, but also has a wide range of effects that resonate throughout the community. Predators play prominent roles in many systems and they affect biological communities directly and indirectly (Kerfoot and Sih 1987). A direct effect is the reduction of prey abundance or biomass. Indirect effects include non-fatal but nevertheless significant alterations in spatial and temporal patterns of prey activity and distribution, the life histories of prey species, and the entire dynamics of a population (Allan 1995). In addition, predation from a top predator can cascade down the food web. For example when a predator reduces the abundance of an herbivore, the abundance of the herbivore’s prey may increase. This dynamical relationship between predator and prey is believed to be one of the main processes structuring populations and communities.
Accordingly, countless studies have been done exploring different predator-prey relationships in numerous ecosystems, however, very little of the available literature deals with the issue of predation and its effects on the stream community. The sculpin, in particular, is one predator whose role in the stream community is relatively unknown. It is a common member of coldwater trout streams reaching maximum lengths of 110 mm and weighing up to 18.22 grams. Being a bottom dwelling fish, the sculpin feeds almost exclusively on benthic invertebrates and may play a major role in shaping the benthic invertebrate community. This fish species has been found to have a greater impact than the trout on the benthic invertebrate community due to the fact trout may include large amounts of terrestrial animals in their diet (Dahl, 1998). In addition, sculpins are usually very abundant in coldwater streams, so much so that their biomass (kg/ha) and production is often similar to that of trout (Petrosky and Waters 1975, LeCren 1969). Sculpins may shape the benthic invertebrate community not only through direct predation but also by changing the behavior of invertebrate predators or primary consumers. Just the presence of sculpin has been known to change the foraging behavior and amount of prey eaten by invertebrate predators like the stonefly *Agneta capitata* (Soluk and Collins 1988) and primary consumers such as *Baetis* (Kohler and McPeek 1989).

Since the sculpin is a predator that feeds in a variety of habitats, it will come in contact with and feed on an assortment of invertebrate prey. This includes the two main habitats in coldwater trout stream; erosional areas with fast-flowing water and a hard, rock substrate and depositional habitat with a much slower current a silt and sand substrate. These are drastically different stream habitat and will consequently contain
very different types and abundances of benthic invertebrates. Commonly known to forage in the stony-bottomed erosional areas of the stream, the sculpin has also been found in the depositional habitat (Figure 1).

Figure 1: Habitat Use by the Mottled Sculpin in Seven Mile Creek

Note: These graphs show the distribution of sculpin in Seven Mile Creek in regards to the proportion of biomass and the proportion of the population found in depositional habitat relative to the availability of depositional habitat in 10, 30-meter sections of stream for 1994-1996. Source: Kohler, personal communications

In Seven Mile Creek from 1994-1996, the depositional habitat-use of sculpin was examined. With increasing availability of depositional habitat, a larger proportion of sculpin biomass and the general population are found residing in this type of habitat in Seven Mile Creek. These results suggest that sculpin do spend a significant amount of time in depositional habitat; whether they forage in this habitat and impact the benthic invertebrates found here are topics my study has investigated.

Knowing the diet of this fish and how it changes with different habitats can help to predict and explain the effects this fish has on the benthic invertebrate community. There is a strong connection between the diet, habitat, and predator impact of the sculpin that has yet to be fully explored. The habitat-specific diet, prey preference, and predatory effects of sculpin are still relatively unknown due to conflicting studies and lack of
research. This study provides an extensive look into the diet and benthic invertebrate impact of the mottled sculpin (*Cottus bairdi*), the most common member of Michigan’s coldwater trout systems. Using gastric lavage and caging experiments, the diet and predatory effects of the mottled sculpin were analyzed in the two main habitats of the stream, soft-bottomed depositional sites of the stream and the hard-bottomed, cobble-filled erosional streambed in the main channel of the stream. This information can be used in conjunction with data on sculpin habitat use to predict the habitat-specific effects of sculpin on benthic invertebrates. The link between diet and predator impact needs to be known in order to better understand the sculpin and ultimately the stream community as a whole. This study helps further the understanding of sculpin and its role in the stream community by:

1. Examining the diet of the sculpin and whether it varies between different habitats and locations in the stream.

2. Determining whether sculpin predation affects the structure of the benthic invertebrate community.

3. Determining whether effects of sculpin predation differ among the dominant stream habitats.
CHAPTER I

EXPLORING PREY PREFERENCE AND SELECTIVITY IN THE DIET OF THE MOTTLED SCULPIN

Introduction

The sculpin is a fish species in trout stream systems that feeds almost exclusively on benthic invertebrates and forages in a variety of habitats. Since its diet consists of benthic invertebrates, the sculpin could be a major force in structuring the benthic invertebrate community by the direct effect of determining the population sizes of certain prey species, but also indirect effects including modifications in a prey item’s activity, distribution, and life history. With its foraging habits, sculpins could seek their prey in different habitats including the sedimentary depositional areas and the cobble-filled erosional habitat, the two examined in this study. It may, consequently, come in contact with a variety of potential prey items and being a generalist feed on many of them. The direct and indirect effects of sculpin predation may impact the entire benthic invertebrate community encompassing multiple habitats and numerous prey species. These aspects of the sculpin diet make this fish an important component of many stream communities. In order to better understand trout stream communities, it is imperative to know the diet of the sculpin and how it changes between habitats.

Diet Studies

The diet of specific sculpin species has occasionally been studied in their native stream habitats. Bailey (1952) studied the feeding habits and life history of the Rocky
Mountain mottled sculpin (Cottus bairdi punctulatus). He reported that bottom dwelling insects made up 99.7% of the total number of all food items found in the stomachs of sculpin. The other 0.3% was composed of items such as snails (Physa), fingernail clams (Pisidium), water mites (Hydrachnidae), sculpin eggs, and fish. The only terrestrial insect eaten was an adult ground beetle. The most abundant order in the diet of the Rocky Mountain mottled sculpin was Diptera with 95.6% of them being chironomids. Caddiesflies (Trichoptera) and mayflies (Ephemeroptera) were the second and third most abundant insects found in the stomach of this sculpin species. Similar results were attained when the diet of the Utah sculpin (Cottus bairdi semiscaber) was studied (Zarbock 1951). Diptera, Ephemeroptera, Plecoptera, and Trichoptera, in order of decreasing abundance, contributed the bulk of the diet of the Utah sculpin. These same insects appeared in the diet of the black sculpin (Cottus baileyi), especially when the insects were in immature stages (Novak and Estes 1974). Diptera, Ephemeroptera, Trichoptera, Coleoptera, and Plecoptera comprised 98.8% of the total number of food items in the black sculpin diet. Ephemeroptera contributed 46.8% of the total number of organisms consumed with Baetis and Pseudocloeon making up 30% of it. Hydropsyche, Cheumatopsyche, and Glossosoma were the most common Trichopterans eaten. Plecopterans were relatively unimportant in the diet of the black sculpin composing less than 1% on the total. Again, chironomids were the most important item in the diet of the sculpin making up 26.6% of the total diet. The second most abundant Dipteran was Antocha with 5.3%. The diet of the northern mottled sculpin (Cottus bairdi bairdi) varied with the body size of the fish (Daiber 1956). When comparing the diets of the
northern mottled sculpin and the barred fantail darter, it was found that these two fish, which occupy the same habitat, share many of the same prey species. The six prey items that are of great importance to both species include *Stenonema, Arthroplea* (Ephemeroptera), *Brachyptera* (Plecoptera), *Psilotreta frontalis* (Trichoptera), Chironomidae, and *Prosimulium hirtipes* (Diptera). They constitute 76% of the diet of both fishes by number and 72% and 56% by weight for the darter and sculpin, respectively. The small sculpins (16-31mm) fed mainly on *Stenonema, Brachyptera*, and the Chironomidae, which comprised 78.5% of the organisms found in their stomachs. *Stenonema, Brachyptera, Psilotreta, Prosimulium*, and the Chironomidae made up 87.2% of the stomach contents of medium sized sculpin (32-51mm). Larger sculpins (52-72 mm) fed mainly on *Stenonema, Acroneuria lycorias, Psilotreta, Helicopsyche borealis, Ophiogomphus*, and the Chironomidae, which made up 60.5% of their stomach contents.

Dineen (1951) investigated the diet of the northern mottled sculpin (*Cottus bairdi bairdi*) in Minnesota. While comparing the diets of the brook trout, brown trout, and sculpin, Dineen (1951) found that the sculpin’s five major food items were the amphipod *Gammarus* and the immature stages of mayflies, caddisflies, stoneflies, and dipterans. Although the previously mentioned studies do share many of the same results and conclusions, there are some studies that show different assessments of the sculpin diet and include different prey species.

One such study was performed on the mottled sculpin (*Cottus bairdi*) in a creek in Illinois. Anderson (1975) found that the foremost food item in the stomachs of these sculpin was the aquatic sowbug *Asellus militaris*. This isopod was dominant both
numerically and by volume, even though it was not the most abundant invertebrate at the sampling site. Another study by Ebert and Summerfelt (1969) reported that ostracods were found in the stomachs of 50% of the piute sculpin (*Cottus beldingii*) sampled, while chironomids had only about a 35% occurrence. Gilson and Benson (1979) compared the prey preference of two different size classes of the mottled sculpin (*Cottus bairdi bairdi*). Prey preference refers to the relationship between the relative frequency of a prey item in a predator’s diet and its relative frequency in the environment and whether the predator is consuming the prey item in quantities greater than expected considering its abundance in the environment. An experimental riffle was constructed in a West Virginia stream and prey selection was determined by comparing sculpin stomach contents to prey abundance in the benthos. The large sculpins preferred *Isogenus hastatus* naiads and *Pycnopsyche* larvae and were an important part of the diet in terms of number and biomass. Hydropsychid larvae were the principal prey of small sculpin. Both sculpin groups demonstrated selection for *Neophylax concinnus* by ingesting larva in excess of its relative abundance. This information does not seem to stray from the results of the other studies except for one important aspect. It is not the invertebrate species eaten that is unusual, it is the ones that are not. Chironomid larvae were not important prey of either sculpin size group, despite their numerical dominance in control baskets. They made up 54% of total number of benthic invertebrates in the control. However, they only made up 3% and 0% of the total number of invertebrates eaten by the large and small sculpin groups, respectively. Englund and Evander (1999) had similar findings when studying the diet of *Cottus gobio*. The prey taxa Chironominae had a relative abundance of only
0.16% in the stomachs of sculpins despite having a relative abundance of 39.6% in the benthos. Another chironomid sub-family, Orthocladiinae, was also abundant in the benthos (14.8%), but was rarely seen in the gut contents of the sculpin (0.32%). The species with the highest relative abundance in the sculpin stomach was the caddisfly larvae *Neureclipsis bimaculata* with 10.3%, while having the relative abundance in the benthos of 12.5%. Although the previous two articles examine the feeding selectivity of sculpin, many older sculpin diet studies do not explore the relationship between the relative abundance of prey consumed and the prey abundance in the environment.

*Habitat-Diet Studies*

One particularly interesting study examined the influence of substrate composition and suspended sediment on the diet and predator effects of the torrent sculpin (*Cottus rhotheus*) (Brusven and Rose 1981). This study, conducted in a laboratory stream, showed that torrent sculpin congregated in regions of limited cover when exposed to a sand substrate. When cobbles were added, their distribution was more uniform. The various combinations of sand, pebbles, and cobbles significantly influenced sculpin predation on the stonefly *Hesperoperla pacifica*, the mayfly *Ephemerella grandis*, and the caddisfly *Rhyacophila vaccua*. On the sand substrate, each insect species experienced 95-100% predation, however, when different combinations of cobbles and pebbles were added predation was appreciably reduced. When testing the influence of suspended sediment levels on predation, it was found that only one of nine comparisons showed significant differences. For the conditions tested in the study,
substrate condition rather than suspended sediment had the greatest influence on
depressing predation.

My study of the diet of mottled sculpin differs from those mentioned above for
two main reasons. First, I examined the diet of the sculpin in the two main habitats of
coldwater trout systems, the hard-bottomed erosional areas and the soft-bottomed
depositional habitat. Differences in the diet of sculpin between these two habitats have
not been studied by other investigators. Second, my study also examines prey preference
and selectivity of the mottled sculpin in erosional habitats. Many of the diet studies on
the sculpin (Dineen 1951, Bailey 1952, Daiber 1956) were performed several decades
ago and did not quantitatively assess prey selectivity.

Methods

Site Description

The study site was Seven Mile Creek, a second order stream located east of
Kalamazoo, MI. Seven Mile Creek is part of the Kalamazoo River drainage basin with a
drainage area of approximately 36 km² at the study site. It mainly drains wetlands and
woodlands with little urban or agricultural development. This coldwater stream’s
maximum summer temperature is 22°C and it receives extensive groundwater inputs,
which contributes to its highly stable flow regimes. Trout are present (largely brown
tROUT) but not abundant and other large predatory fish are uncommon, which makes this
creek an excellent site for a study on the effects of sculpin predation. The population size
of sculpin in Seven Mile Creek (Table 1) is similar to that of other trout streams in this region.

Table 1: Sculpin Population Size in Seven Mile Creek

<table>
<thead>
<tr>
<th>Stream</th>
<th>Area (m²)</th>
<th>Density (#/100 m²)</th>
<th>Biomass (g/100 m²)</th>
<th>% YOY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seven Mile Ck. 1993</td>
<td>807</td>
<td>29.23</td>
<td>117.11</td>
<td>12.77</td>
</tr>
<tr>
<td>1994</td>
<td>823</td>
<td>110.15</td>
<td>108.99</td>
<td>83.7</td>
</tr>
<tr>
<td>1995</td>
<td>778</td>
<td>51.52</td>
<td>121.66</td>
<td>23.14</td>
</tr>
<tr>
<td>1996</td>
<td>293</td>
<td>28.64</td>
<td>64.84</td>
<td>46.05</td>
</tr>
</tbody>
</table>

Note: YOY = Young of Year, fish less than one year old. Source: Kohler, personal communications

This chart depicts the population size of sculpin in Seven Mile Creek with both density and biomass estimates. It shows that while the density does fluctuate, the biomass is relatively stable. In addition to a relatively stable sculpin population, Seven Mile Creek has riffle and run substrates largely consisting of gravels and cobbles over sand. There are several different habitats along the stream, like areas of erosion and deposition, which is typical of stony-bottomed coldwater streams in the Great Lakes region (Kohler and Wiley 1997), therefore the results of this study should provide insight into similar systems.

Diet Study Design

The diet of the sculpin was examined in fish collected on four dates: 1 June 2004, before the caging experiment began; 29 July 2004, between experimental runs of the caging experiment; 4 October 2004, after the caging experiment had concluded; and 6 May 2005. Past studies collected samples and studied the diet of mottled sculpin in the afternoon hours (Daiber 1956, Bailey 1952), which agrees with the findings of Kohler and McPeek (1989) who found that mottled sculpin feed largely during the daytime.
Accordingly, I collected fish in the late morning to early afternoon. On each date I attempted to collect at least 20 fish from each habitat type. The fish were collected using a backpack electroshocker to stun the sculpin allowing them to be scooped up using a net. The electroshocker was set at a peak voltage of 200, the duty was set at 10.0, the rate at 50, and the quadrapulse was off. According to past studies (Barrett and Grossman 1988), electrofishing has no long-term negative effects on the sculpin and does not affect mortality rates. The fish were separated based on the habitat they were found in, either in the soft-bottomed depositional areas or the fast-flowing erosional main channel. The fish were anesthetized using MS-222 (Tricaine-S, a brand of Tricaine Methanesulfonate) at 80 mg/L and their stomach contents were removed using gastric lavage (Culp et al. 1988, Foster 1977, Light et al. 1983). Fish under 50 mm were deemed too small to undergo gastric lavage and were sacrificed. They were given an overdose of anesthesia, preserved in 10% buffered formalin, and their gut contents were removed by dissection by at the lab.

**Gastric Lavage**

The gastric lavage apparatus (Culp et al. 1988) consisted of a flat piece of Plexiglas with two Plexiglas strips to hold the anesthetized fish during the procedure and guide the water and gut contents exiting the fish’s mouth. This Plexiglas structure was mounted to two downward sloping, intersecting pieces of wood. This allowed for the proper drainage from the fish’s mouth into the collecting vial. Attached to the apparatus was a 20 mL syringe filled with water with a 20 gauge, 1 ½ inch long needle. Over the needle was a length of polyethylene tubing (inside x outside diameter of 0.86 mm x 1.270
mm), which was inserted into the fish's stomach through the mouth. By compressing the syringe, 20 mL of water flowed through the microtube and into the fish’s stomach flushing out its gut contents. The gut contents were usually scattered parts of invertebrates including heads, legs, and parts of the abdomen, although there were some whole or nearly intact invertebrates. After being flushed out of the mouth, the gut contents drained down the Plexiglas through a small funnel into a 20 mL plastic scintillation vial. The gut contents were collected on a 0.10 mm sieve to remove any water, preserved with 4% formalin, and taken back to the lab for examination. Through the careful inspection of various body parts of different invertebrates in different stages of digestion, prey items could be counted and identified at least to genus, often to species, under a dissecting microscope.

*Statistical Analysis*

The gut contents from each fish captured in the erosional and depositional habitats were analyzed and the results separated into the proportions of prey items comprised of erosional invertebrates, depositional invertebrates, and invertebrates found in both habitats. Erosional invertebrates included *Glossosoma, Neophylax sp.*, *Goera, Brachycentrus, Hydropsyche sp.*, *Cheumatopsyche, Chimarra, Hydroptila, Rhycophila, Agapetus, Psychomyia, Amphinemura, Isoperla, Baetis sp.*, *Stenonema, Paraleptophlebia, Isonychia, Drunella lata, Antocha, Simulium, Chelifera, Hemerodromia, Optioservus*, and *Nigronia*. Depositional invertebrates consisted of *Triaenodes, Caenis, Gammarus*, Copepoda, Ostracoda, and Isopoda. The invertebrates that occur in both habitats are *Ephemerella sp.*, Chironomidae, Mites, Snails, and Clams.
Data from previous sampling and published literature (Merritt and Cummins 1996) was the basis for determining whether an invertebrate was classified as residing in erosional, depositional, or both habitats.

To detect any significant difference in the proportion of erosional invertebrates found in the stomachs of fish captured in the two distinct habitats, the data was analyzed using PROC MIXED in SAS. A 3-way univariate repeated measures ANOVA design was applied with three fixed effects variables. The dependent variable was proportion of erosional invertebrates eaten. One fixed effect was habitat type (hab) either erosional or depositional depending on where the fish was found. Another fixed effect treatment factor was fish size. Sculpins were separated into two size classes with those less than 65 mm being considered under two years of age and fish with lengths greater than or equal to 65 mm were treated as being 2 years or older. The repeated measures variable in the model was date, which had values of 1, 2, or 3 corresponding to the three collection dates. The data from October collection date was not included due to the low number of depositional fish collected. The subject in SAS’s Mixed Procedure design was the individual fish, which was nested within the habitat treatments and size of fish (ind(hab*size)). The subject is the sampling unit and the focus of the R matrix, the R correlation matrix, and the covariance parameter estimate. In this design, the covariance structure that fit best was autoregressive (order 1) because it had the lowest AIC (Akaike’s Information Criterion) and BIC (Schwarz’s Bayesian Index Criterion) fit statistics and the estimation method was REML. The degrees of freedom were calculated using Satterthwaite’s method. Using this 3-way univariate repeated measures ANOVA
design and the PROC MIXED in SAS, I tested whether sculpin diet composition varied by habitat, date, and fish size.

Continuing to investigate the relationship between diet and habitat, an ANCOVA was designed with habitat (hab) and the repeated measure of time (date) as the treatments and length as the covariate. Length was used as a covariate and surrogate for age in order to correct for selectivity preference due to age and isolate selectivity preference based on habitat. This permits us to examine the effects of habitat on the diet of sculpin (proportion of erosional invertebrates in the gut) without any size artifacts. Additionally, it will determine whether certain interactions (length*hab*date) are primarily due to the effects length has on the diet of mottled sculpin. This length by habitat by date interaction was examined further using Minitab to perform regressions and plot the effects of length for all dates and habitats. Besides analyzing the normal proportion of erosional invertebrates, the angular transformed data (proportional data such as this is often angularly transformed) was also examined using this ANCOVA design and the previously mentioned ANOVA. The angular transformed data did not produce results significantly different from the normal data, therefore transformation was unnecessary and the results from the normal data were reported. Applying regressions, analyses of variance, and analyses of covariance to the stomach content data, provides an accurate description of the use of habitat by the sculpin when it comes to foraging and whether this changes with age.

To attain the relative abundances of invertebrates needed to calculate measurements of selectivity, the erosional substrate was estimated from samples of
individual rocks using methods similar to Kohler and Wiley (1997). Substrates were carefully lifted from the stream bottom with a mesh net held immediately downstream to capture any dislodged animal and the substrate and net contents were preserved in 4% formalin. In the lab, invertebrates and organic material were washed from the rocks and poured through a stacked series of sieves (0.25, 2.0, 4.0, 8.0 mm) and rinsed thoroughly with water. The rocks were then digitally photographed and their projected surface area measured using an imaging software package called Image J. Combining these areas with the data from the samples, an overall density and densities for each species of invertebrate could be calculated. The rocks were later returned to the stream after the experiment had concluded. The material washed off the rocks onto the 2.0, 4.0, and 8.0 mm sieves, the coarse fraction of the sample, was transferred to a Petri dish and using a dissecting microscope all organisms were sorted, identified, and counted. The material collected on the 0.25 mm sieve, the fine fraction, was transferred to a 1000 mL Erlenmeyer flask and water elutriated by swirling the flask and pouring off the organic material into a 0.25 mm sieve. The procedure was repeated until the organic material was completely separated from the inorganic material. The remaining inorganic sediments were transferred to a Petri dish and inspected for organisms, if organisms were found they were transferred to the sieve containing the organic material. If the fine fraction of the sample contained more than 600 organisms, the sample had to be split, so the elutriated organic material was then washed into a graduated 1000 mL beaker and brought up to approximately 400 mL with water. Next, the beaker contents were transferred to a Folsom plankton splitter and divided as many times as necessary to obtain
200-300 organisms. The organisms in the entire fine sample or split fine sample were sorted, identified, and counted using a dissecting microscope. The data collected was combined with that of the coarse fraction of the sample and the area sampled (usually between 229-879 cm$^2$) to produce densities.

Using the gut content data and the relative abundances of invertebrates in the environment obtained by substrate sampling, the prey preference of the mottled sculpin was measured using Chesson’s $\alpha$. In this case, this measurement is appropriate because food consumption does not significantly reduce the abundances of each food types so $\alpha_i$ is simply estimated as the ratio of the proportion of food type $i$ in the diet ($r_i$) to the proportion of food type $i$ in the environment ($n_i$). The resulting values are scaled so that the $\alpha_i$’s sum to 1 by dividing by sum total of all the selectivity values ($r_j / n_j$) (equation 1) (Chesson 1978, Chesson 1983).

$$\alpha_i = \frac{r_i}{n_i}, \quad i = 1, 2, \ldots, m \quad (\text{Chesson 1978}) \quad (1)$$

$$\sum_{j=1}^{m} \frac{r_j}{n_j}$$

These $\alpha_i$’s are relative measures that indicate the preference of a consumer for a food type relative to the other food types present. Using standard t-tests, $\alpha_i$’s were tested to see if the preference for a particular prey item was significant or if the sculpin was randomly foraging and eating prey in proportion to their availability. Equation 2 was used to calculate the t statistic comparing the $\alpha_i$’s to the $\alpha$ for random foraging ($1/m$) where $m$ is the number of prey types found in sculpin stomachs on a given date, $\alpha_i$ is the sample mean, and $s^2$ is the sample variance of the K estimates of $\alpha_i$. 

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Despite sculpin feeding in the depositional habitat, Chesson’s $\alpha$ could only be calculated for fish in the erosional habitat due to the large amounts of erosional invertebrates found in the depositional fish’s stomachs but not in their environment. Also, quantitative estimates of depositional invertebrate abundance were not taken on some dates, which in retrospect was a critical oversight.

**Results**

*Gastric Lavage Procedure*

The gastric lavage apparatus and the technique used in this study seemed to work extremely well. After the first collection, 12 sculpins whose stomach contents had been removed were sacrificed and taken back to the lab for dissection. Upon examination of their stomachs, there were no remaining prey contents left and all stomachs were empty. Of 196 sculpins sampled only 8 had empty stomachs (4.1%), which is evidence not only that sculpin are continuously feeding but also the gastric lavage technique used in this study worked successfully. Besides being efficient, there were no casualties while performing gastric lavage and there seemed to be no harmful side effects. Frequently the prey items were whole and intact and readily identifiable body parts like heads, legs, and scelerites were often present.
Prey Items in the Diet of the Sculpin

Gut content samples were taken from 196 sculpins over four collection dates and the caging experiment. One hundred and eight fish were found in the erosional habitat and 88 sculpins were captured in the depositional habitat. The first collection date 40 sculpin stomachs were pumped, 58 the second collection date, 25 the shortened third collection, 63 the final collection, and 10 from the caging experiment. The size distribution of the sculpin captured was normal (Figure 2).

![Figure 2: Sculpin Size Histogram](image)

Note: The sizes of the sculpin collected ranged from 24 mm to 93 mm with a mean length of 58.4 mm.

The diet of the sculpin seems very general and includes numerous prey species. In all, 37 different prey types representing 2118 total invertebrates were found in the gut contents of the 196 sculpins sampled with an average of 10.8 invertebrates per sculpin stomach. The entire range of prey types is depicted on Table 3 along with each prey types’ contribution to the sculpin diet (percent of all prey items by number pooled over
all collection dates and both habitats) and the occurrence rate (percentage of stomachs that prey type appeared in).

Table 2: Composition of Sculpin Diet by Prey Type

<table>
<thead>
<tr>
<th>Prey Item</th>
<th>% Composition of Diet by Number</th>
<th>Frequency of Occurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ephemerella sp.</td>
<td>27.76</td>
<td>41.84</td>
</tr>
<tr>
<td>Chironomidae</td>
<td>15.58</td>
<td>59.18</td>
</tr>
<tr>
<td>Hydropsyche sp.</td>
<td>9.02</td>
<td>44.39</td>
</tr>
<tr>
<td>Antocha</td>
<td>8.69</td>
<td>36.22</td>
</tr>
<tr>
<td>Drunella</td>
<td>6.80</td>
<td>31.12</td>
</tr>
<tr>
<td>Simulium</td>
<td>5.57</td>
<td>12.76</td>
</tr>
<tr>
<td>Caenis</td>
<td>4.34</td>
<td>18.37</td>
</tr>
<tr>
<td>Stenonema</td>
<td>4.20</td>
<td>25.51</td>
</tr>
<tr>
<td>Baetis sp.</td>
<td>4.06</td>
<td>26.02</td>
</tr>
<tr>
<td>Amphinemura</td>
<td>3.87</td>
<td>11.22</td>
</tr>
<tr>
<td>Cheumatopsyche</td>
<td>2.69</td>
<td>17.35</td>
</tr>
<tr>
<td>Glossosoma</td>
<td>1.79</td>
<td>13.27</td>
</tr>
<tr>
<td>Isonychia</td>
<td>0.99</td>
<td>7.14</td>
</tr>
<tr>
<td>Gammarus</td>
<td>0.76</td>
<td>4.59</td>
</tr>
<tr>
<td>Psychomyia</td>
<td>0.42</td>
<td>3.57</td>
</tr>
<tr>
<td>Hydroptila</td>
<td>0.38</td>
<td>4.08</td>
</tr>
<tr>
<td>Snail</td>
<td>0.38</td>
<td>3.57</td>
</tr>
<tr>
<td>Neophylax sp.</td>
<td>0.33</td>
<td>2.55</td>
</tr>
<tr>
<td>Goera</td>
<td>0.28</td>
<td>3.06</td>
</tr>
<tr>
<td>Paraleptophlebia</td>
<td>0.28</td>
<td>3.06</td>
</tr>
<tr>
<td>Optioservus</td>
<td>0.28</td>
<td>3.06</td>
</tr>
<tr>
<td>Copepoda</td>
<td>0.28</td>
<td>2.55</td>
</tr>
<tr>
<td>Agapetus</td>
<td>0.14</td>
<td>1.53</td>
</tr>
<tr>
<td>Chelifera</td>
<td>0.14</td>
<td>1.53</td>
</tr>
<tr>
<td>Terrestrial Beetle</td>
<td>0.14</td>
<td>1.53</td>
</tr>
<tr>
<td>Ostracoda</td>
<td>0.14</td>
<td>0.51</td>
</tr>
<tr>
<td>Chimarra</td>
<td>0.09</td>
<td>0.51</td>
</tr>
<tr>
<td>Mites</td>
<td>0.09</td>
<td>1.02</td>
</tr>
<tr>
<td>Crayfish</td>
<td>0.09</td>
<td>1.02</td>
</tr>
<tr>
<td>Brachycentrus</td>
<td>0.05</td>
<td>0.51</td>
</tr>
<tr>
<td>Rhychohila</td>
<td>0.05</td>
<td>0.51</td>
</tr>
<tr>
<td>Triaenodes</td>
<td>0.05</td>
<td>0.51</td>
</tr>
<tr>
<td>Isoperla</td>
<td>0.05</td>
<td>0.51</td>
</tr>
<tr>
<td>Hemerodromia</td>
<td>0.05</td>
<td>0.51</td>
</tr>
<tr>
<td>Clam</td>
<td>0.05</td>
<td>0.51</td>
</tr>
<tr>
<td>Nigronia</td>
<td>0.05</td>
<td>0.51</td>
</tr>
<tr>
<td>Isopoda</td>
<td>0.05</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Note: Prey types arranged in descending order of diet composition

The top five prey types in both number and occurrence are *Ephemerella sp.*, *Chironomidae*, *Hydropsyche sp.*, *Antocha*, and *Drunella lata*. The mayfly *Ephemerella*
make up the largest portion of the sculpin diet and has the third highest occurrence in the stomach. The lower frequency in the gut is most likely due to the lifecycle of this Ephemeropteran and its unavailability as a prey item the entire year. This is the case for many of the mayfly species in the sculpin diet, like *Drunella, Caenis, Baetis,* and *Stenonema,* which are eaten in large numbers only at certain times of year. Although five of the top ten prey items are mayfly taxa, other invertebrates are also an important food source for sculpin. The second most common food source for sculpin is the Dipteran family Chironomidae. This prey type is available year round thus having the highest occurrence in the sculpin stomach. At an occurrence rate of 59.18%, over half of all the sculpins sampled had this prey type in their stomachs. Along with chironomids, *Simulium* and *Antocha* were two other Dipterans that were an important part of the sculpin diet. Two caddisfly taxa were a crucial part of the sculpin diet. *Hydropsyche* was the third most common food type and occurred in the second most gut content samples. Along with *Cheumatopsyche,* these caddisfly taxa seemed to show up in the gut contents year round and had much higher occurrences than expected in comparison to their contributions to the overall diet. Besides the previously mentioned mayflies, Dipterans, and caddisflies, the only other significant component to the diet of sculpin is the stonefly *Amphinemura.*

*Foraging in Erosional and Depositional Habitats*

The ANOVA testing the fixed effects of habitat, time, and fish size on the normal proportion of erosional invertebrates consumed by sculpin produced several significant
results. All three effects in the model were significant as well as several interactions (Table 4).

Table 3: Variables Affecting Proportion of Erosional Invertebrates in Gut Contents

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num D.F.</th>
<th>Den D.F.</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hab</td>
<td>1</td>
<td>68.4</td>
<td>50.85</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>size</td>
<td>1</td>
<td>68.4</td>
<td>4.77</td>
<td>0.0324</td>
</tr>
<tr>
<td>hab*size</td>
<td>1</td>
<td>68.4</td>
<td>0.34</td>
<td>0.5635</td>
</tr>
<tr>
<td>date</td>
<td>2</td>
<td>102</td>
<td>25.36</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>hab*date</td>
<td>2</td>
<td>102</td>
<td>11.33</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>date*size</td>
<td>2</td>
<td>102</td>
<td>1.41</td>
<td>0.2485</td>
</tr>
<tr>
<td>hab<em>date</em>size</td>
<td>2</td>
<td>102</td>
<td>7.33</td>
<td>0.0011</td>
</tr>
</tbody>
</table>

Note: Num D.F. = Numerator Degrees of Freedom, Den D.F. = Denominator Degrees of Freedom

First, there is a significant habitat effect suggesting sculpins foraging in erosional and depositional habitats have significantly different diets ($F_{1,68.4} = 50.85$, $P < 0.0001$).

The sculpins found in the erosional habitat have consistently higher mean proportion of erosional invertebrates in their stomachs across most dates and in both size classes (Figure 3).

Figure 3: Proportion of Erosional Prey in the Diet by Habitat, Date, and Class Size
This provides evidence that the foraging habitat plays an important role in the diet of sculpin. However, the two interactions involving habitat are also significant meaning it is not really possible to interpret the habitat main effect in a straightforward way and the interactions must also be carefully examined.

The diet of the mottled sculpin, in terms of proportion of erosional invertebrates in the gut, was also significantly different between size classes indicating that sculpin foraging activities could vary with age ($F_{1,68.4} = 4.77$, $P = 0.0324$). Fish less than 2 years of age have a significantly different diet than sculpin two years and up. The collection periods were also a source of significant variation and shows that the sculpin diet changes over time ($F_{2,102} = 25.36$, $P < 0.0001$). Over the course of a year and three collection periods the diet of sculpin showed vast disparities. Again, both of the size and date fixed effects were involved in significant interactions so they, like habitat, do not have a straightforward interpretation. Looking at the double interactions, the habitat by size interaction is not a significant source of variation ($F_{2,102} = 0.34$, $P = 0.5635$) and the relationship between habitat and date suggests that the habitat effects on the proportion of erosional invertebrates consumed changes over time ($F_{2,102} = 11.33$, $P < 0.0001$). Since this interaction is included in the significant triple interaction, the only interpretation that can clearly be made from this ANOVA is that this triple interaction of habitat by date by size was significant. As shown in Figure 3, there are significant habitat effects, however, they are dependent on both the size of the sculpin and the time of year. Size was also significant for the July depositional collection and the May erosional collection, but these
were on two different dates in two different habitats. Because habitat, size, and date were
so closely associated and produced a significant interaction, the relationship between
them was examined further using an ANCOVA design.

Length by Habitat by Date Interaction

The length of a sculpin is a good indicator of its age and whether age affects the
diet of sculpin. Consequently, exploring the relationship between length and the
proportion of erosional prey items found in the stomach will provide insight on how the
foraging habits and habitat-use of a sculpin changes with age. To do this, an ANCOVA
was performed using SAS with length of fish as a covariate, habitat (hab) and time (date)
as independent variables, and proportion of erosional organisms found in gut as the
response variable. The length by habitat by date interaction proved significant ($F_{6,82} =
6.24, P < 0.001$) (Table 4), indicating that the slopes of the proportion of erosional prey
as a function of sculpin length were significantly different from 0. I then tested whether
the slopes were equal over all treatment combinations by including length and its
interaction with habitat and date in the model. The significant 3-way interaction in this
model ($F_{5,82} = 3.22, P = 0.0106$) (Table 5) indicated that the slopes were not equal.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num D.F.</th>
<th>Den D.F.</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>length<em>hab</em>date</td>
<td>6</td>
<td>82</td>
<td>6.24</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>hab</td>
<td>1</td>
<td>61</td>
<td>2.62</td>
<td>0.1107</td>
</tr>
<tr>
<td>date</td>
<td>2</td>
<td>82</td>
<td>1.36</td>
<td>0.2636</td>
</tr>
<tr>
<td>hab*date</td>
<td>2</td>
<td>82</td>
<td>3.6</td>
<td>0.0317</td>
</tr>
</tbody>
</table>

Note: Num D.F. = Numerator Degrees of Freedom, Den D.F. = Denominator Degrees of Freedom
Table 5: ANCOVA Results Exploring Interaction Including Length as a Fixed Effect

<table>
<thead>
<tr>
<th>Effect</th>
<th>Num D.F.</th>
<th>Den D.F.</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>length</td>
<td>1</td>
<td>82</td>
<td>4.63</td>
<td>0.0343</td>
</tr>
<tr>
<td>length<em>hab</em>date</td>
<td>5</td>
<td>82</td>
<td>3.22</td>
<td>0.0106</td>
</tr>
<tr>
<td>hab</td>
<td>1</td>
<td>61</td>
<td>2.62</td>
<td>0.1107</td>
</tr>
<tr>
<td>date</td>
<td>2</td>
<td>82</td>
<td>1.36</td>
<td>0.2636</td>
</tr>
<tr>
<td>hab*date</td>
<td>2</td>
<td>82</td>
<td>3.6</td>
<td>0.0317</td>
</tr>
</tbody>
</table>

Note: Num D.F. = Numerator Degrees of Freedom, Den D.F. = Denominator Degrees of Freedom

Therefore, regressions were applied exploring the relationship between erosional invertebrates in the diet and length for each habitat-date combination. Figure 4 graphically illustrates the length-diet relationship in both the erosional and depositional habitats for the June, July, and May collections. All the regressions have equations with positive slopes, except for the July collection in the erosional habitat and only two of the regressions produced significant results; the July collection in the depositional habitat ($F_{1,24} = 29.90, P < 0.001$) and the May collection in the erosional habitat ($F_{1,30} = 5.19, P = 0.030$). The two significant regressions occurred in different habitats during two separate collections. The overall significant length effect on the diet of sculpin in both the ANOVA and ANCOVA statistical tests was a result of these collections. Therefore, length is positively correlated with foraging in the rocky-bottomed, erosional area and consuming invertebrates existing in this environment, but only in certain patches of habitat on particular dates.
Figure 4: Length-Diet Relationship in Both Habitats for 3 Collection Dates

June 2004 - Erosional Habitat
PEI = 0.676977 + 0.0038988 Length
S = 0.228147 R-Sq = 0.7 % R-Sq(adj) = 0.0 %

June 2004 - Depositional Habitat
PEI = 0.177245 + 0.0007554 Length
S = 0.181990 R-Sq = 0.1 % R-Sq(adj) = 0.0 %

July 2004 - Erosional Habitat
PEI = 0.791129 - 0.0000287 Length
S = 0.262138 R-Sq = 0.0 %

July 2004 - Depositional Habitat
PEI = -0.258421 + 0.0143159 Length
S = 0.229434 R-Sq = 65.5 % R-Sq(adj) = 53.6 %

May 2005 - Erosional Habitat
PEI = -0.0529079 + 0.0088039 Length
S = 0.206183 R-Sq = 14.7 % R-Sq(adj) = 11.9 %

May 2005 - Depositional Habitat
PEI = -0.0442285 + 0.0055664 Length
S = 0.198803 R-Sq = 5.1 % R-Sq(adj) = 1.8 %

Note: PEI = Proportion of Erosional Invertebrates
Prey Selectivity in the Diet of the Sculpin

To determine prey preference, the selectivity of each prey type must be calculated. Prey selectivity includes the relative abundances of invertebrates in the environment. Since depositional fish had mostly erosional invertebrates in their guts, none of these invertebrates were present in the depositional habitat so it was impossible to accurately determine the prey selectivity for these fish. However, the preferences for the erosional fish were evaluated according to the collection date due to the fact the number of potential prey species present and their abundances fluctuate over time. The first collection date, June 1, 2004, 22 erosional fish were sampled and 12 different prey items were found in both the gut contents and at detectable levels in the environment. The prey selectivity of these 12 food items was measured using Chesson’s $\alpha$. One-sample 2-tailed t-tests using Minitab were then applied comparing the selectivity of each prey type to what it would be if sculpin showed no preference in selecting prey, in this case 1 divided by 12. Figure 5 illustrates the prey selectivity of the 12 prey items for this collection period and their associated p-values depicting the significance of the selectivity. The mayfly *Stenonema* is the only invertebrate that sculpin consumed in a number significantly greater than its relative abundance. Conversely, there are several food sources that sculpin do not prefer and feed on them in quantities less than their relative abundances. These species include *Agapetus, Amphinemura*, Chironomidae, and *Antocha*. The mayflies *Caenis* and *Drunella* were a food source for sculpin collected in erosional areas, however, their abundances in the environment were so diminutive that they were undetectable and an accurate $\alpha$ could not be determined.
Note: The dashed line at 0.0833 represents selectivity if random foraging were taking place.

In the July collection (29 sculpin analyzed), there were numerous prey preferences that were significant from the 15 species present (Figure 6). Two caddisfly genera, *Hydropsyche* and *Cheumatopsyche*, had $\alpha$ values that were much higher than was expected if the sculpin were randomly foraging, while several prey species had measurements of selectivity lower than expected. These include the caddisflies *Glossosoma* and *Psychomyia*, the mayflies *Paraleptophlebia* and *Isonychia, Optioservus*, Mites, and Snails. Again *Drunella* was found in the gut contents but were not detected in the erosional habitat along with *Simulium* and *Nigronia*. Interestingly, none of the prey sources that had significant selectivity in the second collection were significant in the first collection.
Figure 6: July 2004 Prey Selectivity and Associated P-values

Note: The dashed line at 0.0667 represents selectivity if random foraging were taking place.

The third collection on October 4, 2004 totaled 22 sculpin and the measurements of selectivity were calculated for 10 prey types. No prey type was significantly positively selected by sculpin (Figure 7). There were, however, two prey species (Paraleptophlebia and Antocha) that were significantly under consumed by the sculpin while foraging and both displayed the same pattern in the second collection. Caenis and Snails were two prey types in the stomachs of the sculpin but not found in substrate samples of the channel environment.
Figure 7: October 2004 Prey Selectivity and Associated P-values

Note: The dashed line at 0.01 represents selectivity if random foraging were taking place.

The fourth and final collection occurred May 6, 2005 where 32 sculpins were sampled and the α’s for 11 prey types were measured and tested. However, numerous food sources found in the gut contents of sculpin were not found in measurable numbers in the stream substrate including Drunella, Hydroptila, Chimarra, Goera, Isonychia, Psychomyia, Cheumatopsyche, and Brachycentrus. This was the third time Drunella has appeared in the sculpin diet but not in the environment and on this occasion they were plentiful in the gut contents. Figure 8 illustrates the results from this collection and 7 different prey types proved to have significant selectivity values. One prey type, Ephemerella, had an extremely significant selectivity and the highest of any in this study. Other invertebrates’ selectivity values may have decreased in response to the sculpin’s significant prey preference towards Ephemerella. Six insects had significantly lower
selectivity values, *Glossosoma*, *Neophylax*, *Baetis*, *Simulium*, *Antocha*, and *Chelifera*. Only two of these organisms had significantly low selectivity measurements on other dates.

**Figure 8: May 2005 Prey Selectivity and Associated P-values**

![Graph showing selectivity values for different organisms](image)

Note: The dashed line at 0.0909 represents selectivity if random foraging were taking place.

**Discussion**

*Diet of the Mottled Sculpin*

The diet of the mottled sculpin in Seven Mile Creek seems to be very generalized with numerous prey items. In all, 37 different prey types were found in the stomachs of these fish; however, some were more common than others. The five insects that were most abundant in the diet of sculpin were *Ephemerella sp.*, *Chironomidae*, *Hydropsyche sp.*, *Antocha*, and *Drunella lata*. Other important food sources were *Caenis*, *Baetis*,
Stenonema, Antocha, Simulium, and Cheumatopsyche. The mayfly Ephemerella, the most abundant type in the sculpin diet, had only the second highest occurrence in gut content samples. This is presumably due to the lifecycle of this Ephemeropteran. After the larger mature individuals emerge, the population consists of smaller individuals, which may not be as suitable a prey item to sculpin. These larger individuals are not available as a prey item the entire year, as is the case for many of the mayfly species in the sculpin diet, like Drunella, Caenis, Baetis, and Stenonema. The lower occurrence rates for mayfly species indicates that sculpin may show size selectivity; preferring larger, more mature insects. The second most abundant insect in the sculpin diet was chironomids, which had the highest occurrence rate. The species of invertebrates that composed the diet of the mottled sculpin in this study is similar to the results of studies by Bailey (1952), Zarbock (1951), and Novak and Estes (1974). The results of these past studies contended that Dipterans, Ephemeropterans, and Trichopterans were the three most common prey items and this study only provides further evidence to support this theory. Novak and Estes’s (1974) study on the black sculpin produced very similar results as this study. In both studies the most common Trichopterans were Hydropsyche, Cheumatopsyche, and Glossosoma. Similarly the most common Dipterans in the diet were Chironomidae and Antocha and Ephemeropterans made up almost half of the diet. Although these studies on different species of sculpin produced similar results, curiously, several studies on subspecies of Cottus bairdi yielded different outcomes.

A diet study on the northern mottled sculpin (Daiber 1956) had only one prey species in common with this study and it was the mayfly Stenonema. Some of the top
prey species in this study, *Helicopsyche borealis* and *Ophiogomphus*, were present in Seven Mile Creek, but not in the stomachs of sculpin. Studying this same subspecies of sculpin, Dineen (1951) found that the main food type was the amphipod *Gammarus*, which sculpin rarely consumed in this study. The mottled sculpin was studied again in Illinois (Anderson 1975) and the aquatic sowbug *Asellus militaris* was the favorite food item. Finally, Gilson and Benson (1979) found that the sculpin diet consisted primarily of *Isogenus hastatus* naiads and *Pycnopsyche* larvae and *Neophylax* was ingested in excess of its relative abundance. *Pycnopsyche*, quite abundant in Seven Mile Creek, was never found in the gut contents of the sculpin in this study and *Neophylax* was rarely preyed upon. The diet of the mottled sculpin identified by this study, although differing from several performed on mottled sculpin subspecies, does coincide with most of the published literature. Mottled sculpin seem to feed on a variety of invertebrates, while generally consuming a great deal of Ephemeropterans, Dipterans, and Trichopterans.

**Habitat-specific Foraging**

Invertebrates that strictly reside in either the fast-flowing erosional habitat or the sedimentary, depositional areas were both found in the stomach contents of mottled sculpin in Seven Mile Creek. This suggests that mottled sculpin forage in these two main habitats of coldwater streams and may directly or indirectly affect these benthic invertebrate communities. Fish captured in the erosional habitat when compared to fish found in depositional areas had considerably different proportions of erosional invertebrates in their stomach contents. The depositional fish had significantly less erosional invertebrates in their stomachs and many more depositional organisms. This
difference is most likely a product of the location they were foraging in at the time of capture, providing evidence that sculpin do hunt prey in both habitats. Despite foraging in both habitats, sculpin seem to prey on invertebrates in the erosional habitat more frequently. A greater proportion of the sculpin’s diet consisted of strictly erosional prey items as compared to depositional invertebrates on all dates and size classes. These findings, although not surprising considering erosional habitat does generally comprise more than 60% of the available area in most reaches of Seven Mile Creek, may imply that sculpin either use this erosional habitat more often or more efficiently. The greater quantity of erosional invertebrates in the diet could be a consequence of several different foraging activities, such as, time spent in the erosional habitat, better foraging success in that environment, or preference towards erosional invertebrates.

The sculpin’s diet and foraging habits change seasonally throughout the year probably based on the availability and abundances of specific species of invertebrates at different times. This is supported by the significant difference between the three collections dates in the proportions of erosional invertebrates in the diet. More often than not, the three collections displayed vast disparities. The presence, availability, and abundances of different potential prey items regularly varies and the diet and foraging activities of sculpin have to adjust. One possible impact on the habitat-use of sculpin during foraging could be temporal. Throughout the year, the benthic invertebrate community in both habitats is changing and this may force sculpin habitat-use to fluctuate also. Furthermore, the significant relationship between habitat and date suggests that the habitat effects on the proportion of erosional invertebrates consumed
can change with the seasons. So while the sculpin diet is determined by the foraging habitat and season, the habitat effects are continuously changing over time. The presence, availability, and abundances of different potential prey items regularly vary and the diet, habitat-use and foraging activities of sculpin have to adjust. Another factor that may contribute to the diet and habitat-use of the mottled sculpin is length.

Length of a sculpin has a significant affect on the diet and the interaction between length, habitat, and date also showed significance. The variation in the proportion of erosional invertebrates eaten caused by this interaction was not primarily due to length effect. When this relationship between age and diet is broken down by habitat type and date, only two of the regressions, on different dates and in different habitats, proved significant. The interaction suggests that the habitat effects on the diet of sculpin during different times of the year can change with age. Length and habitat-use are only two of numerous aspects of the foraging habits of the mottled sculpin.

**Prey Preference**

After careful examination of sculpin prey preference using Chesson's $\alpha$ over four separate collection dates, it seems clear that sculpin do not constantly select for only one or two prey types. They have a very generalized diet; very few prey types had selectivity values higher than what would be expected with random foraging. When prey taxa did show this elevated selectivity, it was only for one collection date and was a prey item that composed a large portion of the overall diet of the sculpin. For example, *Stenonema* was significantly preferred by sculpin in only the June collection and comprised 4.2% of the overall diet. In the July collection, both *Hydropsyche* and *Cheumatopsyche* had
significant α values and they constituted 9.02% and 2.69% of the total diet, respectively. The May collection had *Ephemerella* significant for the lone time and this genus composed the largest portion of all invertebrates eaten at 27.76%.

These preferred prey types have several characteristics in common that make them the focus of sculpin foraging. These caddisfly and mayfly species are larger benthic invertebrates and some fish, including sculpin, are known to be size selective feeders (Newman and Water 1984). Sculpin seemed to prefer *Stenonema, Ephemerella, Hydropsyche,* and *Cheumatopsyche,* in part, because of their larger size. The opposite can be said about the sculpin’s preference toward Dipterans and other smaller potential food items. Dipterans like *Antocha,* *Chironomidae,* *Chelifera,* and *Simulium* and other smaller prey types like mites had significantly low measurements of selectivity. Sculpins may show size selectivity for larger arthropods due increased detection. While waiting to ambush prey, sculpins may be able to detect large invertebrates like mayflies more successfully than smaller midges.

Also, the lifestyles and activity levels of these four species may cause them to be preferred by sculpin. *Stenonema, Ephemerella, Hydropsyche,* and *Cheumatopsyche* all actively forage for food along the streambed and are rather mobile benthic invertebrates. A study on slimy sculpin (*Cottus cognatus*) predation indicated a preference for larger, more motile prey (Cuker et al. 1992). Sculpins are considered to be ambush predators (Kohler and McPeek 1989, Brusven and Rose 1981) and mobility may make certain prey types more vulnerable to sculpin predation. Being an ambush predator, sculpins wait for mobile prey to come to them, so active foraging invertebrates such as *Ephemerella* and
Stenonema are more likely to be preyed upon. Less mobile prey types, like Agapetus and Glossosoma, have lower encounter rates with sculpin and are less likely to be consumed.

Besides being more mobile than most Trichopterans, Hydropsyche and Cheumatopsyche do not build cases or live in tubes making them more accessible as a prey source. Sculpins often avoided prey species that build cases or live in tubes. Many caddisfly species like Glossosoma, Agapetus, and Neophylax have prey defenses, which include building hard cases out of stone. This type of prey defense makes these invertebrates harder to handle and feed on. Sculpins have a similar aversion towards hard-bodied invertebrates like snails and Optioservus and insects living in tubes like Psychomyia, Antocha, and many Chironomidae. These traits make these prey species less appealing and more inaccessible to foraging sculpins. The four prey types that were significantly preferred all have the characteristics of mobility, larger size, and no hard external coverings in common making preferred prey for the mottled sculpin.

Since this selectivity index depended on the relative abundance of prey types in the environment, invertebrates with significant selectivity values usually fell into two categories; invertebrates rare in the environment but eaten and prey abundant in the environment but not often consumed. When Stenonema and Cheumatopsyche were significantly preferred, they were rare in the erosional habitat, however, due to their size, mobility, and lack of defense they encountered sculpins and were easily detected and captured. Antocha, Simulium, Chironomidae, Glossosoma, Mites, Optioservus, and Agapetus were significantly under consumed despite being very abundant in the environment. These prey types possessed defenses to combat sculpin predation like
living in cases and tubes or have low mobility and a smaller body size that discourages predation.

After analyzing the selectivity of numerous prey types of the mottled sculpin, it seems that this fish demonstrates low prey specificity and selectivity when it comes to foraging. No food source had significantly high selectivity values for more than one collection. The large amounts of potential prey types could account for this trend with 37 different invertebrates found in the gut contents. However by evaluating a few prey species that were preferred and the several that were avoided, the characteristics that sculpin prefer in a prey source can be determined. Sculpin seem to prefer larger, mobile prey that lack hard external coverings like shells and cases due to their ambush predatory strategy. They show preference towards large foraging insects like mayflies and caddisflies rather than small, non-mobile reclusive Trichopterans or Diptera living in tubes or cases. This prey preference of the mottled sculpin may impact benthic invertebrate community structure both directly and indirectly.
CHAPTER II

EFFECTS OF MOTTLED SCULPIN PREDATION ON BENTHIC INVERTEBRATE COMMUNITY STRUCTURE

Introduction

The interaction between predators and prey is one of the fundamental relationships structuring most ecological systems. Predation not only affects the species directly involved, but also indirectly influences the entire community. While a direct effect is the reduction of prey abundance or biomass, indirect effects include changes in the activities, distributions, and life histories of prey species. In stream systems, this predator-prey relationship is believed to be one of the main processes structuring the benthic invertebrate community. The mottled sculpin (*Cottus bairdi*) is one of the main predators of benthic invertebrates in coldwater trout systems. Accordingly, its predatory effects could impact benthic invertebrate community structure and was examined in this study using caging experiments.

One of the main ways to study predation has been the experimental manipulation of predator density in the field. Field experiments where predator or competitor densities are manipulated within a small portion of the habitat are one of the most important techniques in community and stream ecology (Englund and Olsson 1996). With caging experiments, important processes like predation rate (Gilliam et al. 1989), prey movement (Dahl and Greenberg 1999), and predator-prey interactions (Englund and Evander 1999) can be more accurately studied and their results analyzed to determine a
predator's role in a natural community. Although spatial scale (Englund 1997) and cage artifacts (Englund and Olsson 1996) can affect the results of predator caging experiments, it is still a valuable technique to study predator-prey relationships in a stream community.

Most stream caging experiments examine the effects of an invertebrate (Peckarsky 1985, Lancaster 1990) or vertebrate (Gilliam et al. 1989, Flecker 1984) predator on invertebrate prey. When it comes to the predator-prey relationship between fish and aquatic insects, many researchers have examined the predatory behavior and tendencies of the trout (Dahl 1998, De Crespin De Billy and Usseglio-Polatera 2002) due to the fact that it is often the largest predator (Forrester 1994) and holds great importance in stream communities. While salmonids have received most of the attention, there have been studies on the predatory effects of other stream fish such as creek chub (Gilliam et al. 1989) and sculpin (Flecker 1984). Only a few, however, have used the sculpin as the predator and none have examined habitat-specific effects of predators in streams. This literature does give insight, though, on the possible diet of the sculpin and its effect on the composition of the benthic invertebrate community.

*Predator-Prey Caging Experiments in Stream Habitats*

A small portion of the literature available on caging experiments in streams has to do with testing their validity. These studies investigate how spatial scale and prey movements affect the results of caging experiments. Englund (1997) found that the smaller the enclosure the more the results will reflect prey dispersal. Dahl and Greenburg (1999) also found that prey movement can cause seemingly large predator effects when they manipulated the mesh size of their enclosures.
Several caging experiments have focused on one or two species of fish and their effects on the invertebrate community. A study by Forrester (1994) examined how the brook charr affected the abundance and drift propensity of five species of mayflies. Studies similar to this have been performed on several species of fish and invertebrate predators to examine their effects on prey abundance. Predators like trout (Dahl 1998), creek chubs (Gilliam et al. 1989), stoneflies (Peckarsky 1985), and caddisflies (Lancaster et al. 1990) have all been the subject of caging experiments. The one major flaw in the available literature on caging experiments is the variability among studies in how manipulations were performed. Different studies use different subjects, mesh sizes (between 1.0-13.0 mm), enclosure sizes (0.0045-20.0 m²), and durations (3-570 days). These factors may greatly influence the results by affecting prey movement and creating different cage artifacts, which make comparisons among studies difficult.

Caging Experiments Focusing on Benthic Invertebrates

A few of the studies mentioned above and several others concentrate on the effects of predation on the benthic invertebrate community. Gilliam et al. (1989) used screened wooden channels to test whether creek chub predation influences benthic invertebrate abundance. They found that the total volume of invertebrates was significantly reduced by the presence of fish with substantial reductions in the densities of isopods and oligochaetes. Dahl and Greenberg (1996) came a different conclusion when they explored the impact on benthic prey by benthivorous predators. This meta-analysis suggested that on average benthic feeding predators only have moderate negative effects on the density of benthic prey. Wooster (1994) had analogous findings in his
meta-analysis studying vertebrate and invertebrate predator impacts on benthic invertebrate prey. He reported that overall predators had a small to moderate effect on prey density. Although benthic invertebrates were the prey in these and other studies, different vertebrate and invertebrate predators were used. These predators would have unique prey items and prey preferences so their influences on the benthic fauna, both direct and indirect, would be different compared to the fish of interest in this study, the mottled sculpin.

Sculpin Studies and Their Use in Caging Experiments

The relationship between sculpin and its invertebrate prey has been studied using caging experiments, however, the impact sculpin have on these benthic macroinvertebrates is unclear. While some studies show a strong effect of sculpin on prey density, others show little or no effect. Flecker (1984) studied the relationship between two species of sculpin (Cottus bairdi and Cottus girargi) and their invertebrate prey in a creek in West Virginia. The experimental design consisted of five treatments, which included exclusion of all fish, enclosures containing 3, 6, or 12 sculpin, and open cages that allowed access to all vertebrate predators. Twenty cages were built representing 4 replicates of each treatment and the sculpin were left in these cages for 13 days. It was found sculpin caused no significant reductions in prey abundance. There was, however, a very weak overall fish effect reducing the abundances of only a few invertebrate taxa including the stonefly Leuctra and Chironomidae. Since Chironomidae comprised the majority of the invertebrates present, fish predation influenced total invertebrate abundance. A study on the slimy sculpin (Cottus cognatus) by Ruetz et al.
(2004) used 1 m² cages within 6 riffles in Valley Creek, Minnesota to manipulate fish density using 3 different treatments; enclosures with 3 sculpins, fishless enclosures, and an open control cage. No significant sculpin or overall fish effect on benthic invertebrates inhabiting gravel substrate was detected suggesting that the strength of top-down control by slimy sculpins on invertebrates was weak. The weak effect of sculpin on benthic invertebrate community structure does contrasts with other studies finding significant sculpin predatory effects.

Dahl (1998) studied the sculpin *Cottus gobio* in a Swedish stream. Using roughly the same enclosure and mesh size as Flecker, Dahl had 4 treatments with 4 replicates each. However, he included brown trout in his experiment so the treatments were exclusion of all fish, 2 brown trout only, 2 sculpins only, and one trout and one sculpin. In this study, sculpins significantly reduced the abundance of mayfly nymphs, amphipods, crayfish, caddis larvae, and *Leuctra* stonefly nymphs. Englund and Olsson (1996) also performed experiments using the species *Cottus gobio* in a Swedish stream that did not contain sculpin. Their three month long experiment consisted of twenty cages with predator-treatment cages and fishless controls placed alternately in a downstream direction. One small and one large sculpin were placed in each predator cage. In this study, the total number of individuals and the abundances of the five most abundant taxa were reduced by more than 50% in the presence of fish. Individual taxa that were affected included net-spinning caddis larvae and three chironomid taxa. However, Englund and Evander (1999) later discovered that the reduction in the chironomids was due to the positive effect of caddis larval nets on chironomid densities.
Consequently, the sculpin only directly affected the densities of three species of caddisflies.

Sculpins have been used in several caging experiments (Flecker 1984, Dahl 1998, Englund and Olsson 1996), some of which have shown significant negative impacts of sculpin on at least some members of the benthic invertebrate community. However, none addressed how predatory effects vary among habitats. These studies only observed sculpin impact in hard-bottomed erosional areas. My main objective was to examine the predatory impact of sculpin in both erosional and depositional areas, unlike any previous study to my knowledge. Using caging experiments, the predatory impact of sculpin on the benthic invertebrate were analyzed in the erosional and depositional habitats along with changes in predatory effects between these two habitats.

Methods

Site Description

This experiment was conducted in Seven Mile Creek, a second order stream located east of Kalamazoo, MI. Seven Mile Creek is part of the Kalamazoo River drainage basin and its drainage area at the study site is approximately 36 km². It mainly drains wetlands and woodlands with little urban or agricultural development. This coldwater stream’s maximum summer temperature is 22°C and receives extensive groundwater inputs, which contributes to it highly stable flow regimes. Trout are present (largely brown trout) but not abundant and other large predatory fish are uncommon, which makes this creek an excellent site for a study on the effects of sculpin predation.
The population size of sculpin in Seven Mile Creek (Table 1) is similar to that of other trout streams in this region.

Table 1: Sculpin Population Size in Seven Mile Creek

<table>
<thead>
<tr>
<th>Stream</th>
<th>Year</th>
<th>Area (m²)</th>
<th>Density (#/100 m²) Estimate</th>
<th>SE</th>
<th>Biomass (g/100 m²)</th>
<th>% YOY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seven Mile Ck.</td>
<td>1993</td>
<td>807</td>
<td>29.23</td>
<td>2.35</td>
<td>117.11</td>
<td>12.77</td>
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<td></td>
<td>1994</td>
<td>823</td>
<td>110.15</td>
<td>7.36</td>
<td>108.99</td>
<td>83.7</td>
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<tr>
<td></td>
<td>1995</td>
<td>778</td>
<td>51.52</td>
<td>1.47</td>
<td>121.66</td>
<td>23.14</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>293</td>
<td>28.64</td>
<td>1.37</td>
<td>64.84</td>
<td>46.05</td>
</tr>
</tbody>
</table>

Note: YOY = Young of Year, fish less than one year old. Source: Kohler, personal communications

This chart depicts the population size of sculpin in Seven Mile Creek with both density and biomass estimates. It shows that while the density does fluctuate, the biomass is relatively stable. In addition to a relatively stable sculpin population, Seven Mile Creek has riffle and run substrates largely consisting of gravels and cobbles over sand. There are several different habitats along the stream, including erosional and depositional areas. Different habitats may change the foraging behavior of the sculpin, which leads to a change in diet. All in all, Seven Mile Creek is typical of a stony-bottomed coldwater stream in the Great Lakes region (Kohler and Wiley 1997), therefore the results of this study should provide insight into similar systems.

Experimental Design

The experimental design consisted of comparing invertebrate abundances over a 3-week period among 3 predator treatments and 2 different habitats with 2 replicates of each treatment combination. A total of 12 cages were placed into Seven Mile Creek in two different locations of the stream. Each block of six cages consisted of 3 different treatments in each of the two habitats. Cages were placed in two different habitats: soft-
bottomed depositional sites and hard-bottomed, cobble-filled erosional areas of the stream. The predator treatments were exclusion of all fish, enclosures that include sculpin proportional to that of the natural stream (2 per cage), and open cages that allowed access to all vertebrate predators and crayfish. The sculpin placed in the enclosure cages had a size range of 58-65 mm with a mean length of 60.5 mm and standard deviation of 2.42 mm. The fish weighed between 2.4 and 3.5 g with a mean weight of 2.89 g and a standard deviation of 0.38 grams. The cages were placed in the creek in early July and four days later the sculpin were added and invertebrate colonization occurred until the experiment was terminated 3 weeks later. Throughout this period debris was routinely removed from the outside of the cages to promote water flow. Stream substrate was sampled 3 times during the experiment: 7, 14, and 24 days after sculpin were added. Rocks were collected from the erosional cages when sampling the substrate and sediment cores were taken from the depositional cages. After the third sampling period, the sculpin were collected, their stomach contents removed using gastric lavage, and released. This concluded the first run of the experiment and produced 2 replicates of data. The front and back mesh of the cages was removed and the cages remained dormant in the stream for approximately two weeks. In mid-August, new sculpins were collected and the cages reassembled to run this experiment a second time, using the same procedure. Treatments were assigned at random to cages in each block. Two experimental runs using this design produced a total of four replicate sets of data.
Cage Design

The cages were approximately 0.5 m x 2 m x 0.5 m and were built using ½ inch PVC pipe for the frame. A piece of ¼ inch mesh covered all sides, including the top, and extended out 3-4 inches from the bottom of the cage. This bottom flange was buried to prevent any sculpin from exiting or entering under the cage. Open cages were identical to other cages except they lacked the mesh covering on the downstream side. The mesh was secured to the PVC pipe using cable ties. The cages were anchored in the stream by attaching them with cable ties to steel rebar that had been hammered into the streambed. Rebar and cable ties were also used to fashion and secure a V-shaped piece of ½ inch mesh in front of the cage to deflect large organic material and help prevent clogging of the cage mesh. Along the side of the cage, grid marks were made to divide the cage into 76 quadrats (10 cm x 10 cm) to allow for random sampling with a 5 cm buffer zone on all sides.

Sampling Procedure

For the stony-bottomed erosional habitat, three rocks were collected from each cage for the first two sampling periods of the experimental run and five rocks for the third sample. The total surface area of these rock samples ranged between 85 and 312 cm². Each particular rock was collected using a random number generator to select a grid square (1-76) within the cage and the rock was selected from that area with a mesh net held immediately downstream to capture any dislodged animal. The rocks and net contents were preserved in 4% formalin and taken back to the lab to be processed. In the lab, invertebrates and organic material were washed from the rocks and poured through a
stacked series of sieves (0.25, 2.0, 4.0, 8.0 mm) and rinsed thoroughly with water. The rocks were then digitally photographed and their projected surface area measured using an imaging software package called Image J. Combining these areas with the data from the samples, an overall density and densities for each species of invertebrate could be calculated for each cage and treatment. The rocks were later returned to the stream after the experiment had concluded. The material washed off the rocks onto the 2.0, 4.0, and 8.0 mm sieves, the coarse fraction of the sample, was transferred to a Petri dish and using a dissecting microscope all organisms were sorted, identified, and counted. The material collected on the 0.25 mm sieve, the fine fraction, was transferred to a 1000 mL Erlenmeyer flask and water elutriated by swirling the flask and pouring off the organic material into a 0.25 mm sieve. The procedure was repeated until the organic material was completely separated from the inorganic material. The remaining inorganic sediments were transferred to a Petri dish and inspected for organisms, if organisms were found they were transferred to the sieve containing the organic material. If the fine fraction of the sample contained more than 600 organisms, the sample had to be split, so the elutriated organic material was then washed into a graduated 1000 mL beaker and brought up to approximately 400 mL with water. Next, the beaker contents were transferred to a Folsom plankton splitter and divided as many times as necessary to obtain 200-300 organisms, usually only once. The organisms in the entire fine sample or split fine sample were sorted, identified, and counted using a dissecting microscope. The data collected was combined with that of the coarse fraction of the sample and the area sampled (usually between 85-350 cm$^2$) to produce densities.
Sampling in the soft-bottomed, depositional areas was accomplished by using a core tube (area = 6.16 cm$^2$) driven approximately 8 cm into the soft sediments. Three samples were taken at random from each depositional cage on the first two sampling dates and five were taken on the third date. These cores were preserved with 4% formalin and taken back to the lab for processing. In the lab, the collected material was transferred to a 1000 mL Erlenmeyer flask, elutriated, and the organic material was poured through a 0.10 mm sieve. The procedure was repeated until the organic material was separated from the inorganic material. The remaining inorganic sediments were transferred to a Petri dish and inspected for organisms, if organisms were found they were transferred to the sieve containing the organic material. The organic material in the 0.10 mm sieve was washed to remove any remain silt or sand and transferred into a tallform beaker using Ludox. Ludox is a colloidal silica solution with properties that make it ideal for the extraction of meiofauna. It comes in a range of grades but Ludox TM was used in this study, which has a specific gravity of 1.40. The specific gravity needed to extract meiofauna is 1.15, so a dilution of two parts Ludox to three parts freshwater produced the correct density. Ludox flotation was the technique used in this study to separate the invertebrates from the sediment components. The object of Ludox flotation is to suspend the invertebrates in the Ludox, which has a specific gravity very close to that of the invertebrates themselves, while the other organic material will not remain buoyant and slowly sink. After the organic material is washed into the tallform beaker using Ludox, more Ludox is added so that it is at least 10 times of the sample volume. The mixture is stirred to evenly distribute the sample throughout the volume and
left to settle for at least 40 minutes. Once good separation has occurred, the surface film containing the invertebrates floating on top of the Ludox was removed using suction and poured through a 0.10 mm sieve where it was washed with freshwater and transferred to a Petri plate where the invertebrates were sorted, identified, and counted using a dissecting microscope. As for the sediment at the bottom of the beaker, more Ludox was added to bring the beaker up to its original volume making sure the specific gravity stays at 1.15. The whole flotation process was repeated three more times, so that each sample went through 4 rounds of Ludox flotation. For the first several samples processed, the remaining sediment material was inspected for invertebrates not successfully extracted by the flotation procedure to determine the efficiency of this technique. This specific flotation procedure left extremely few invertebrates in the remaining sediment and was very effective.

Statistical Analysis

The information collected from the caging experiment was analyzed using PROC MIXED in SAS. The analysis followed a 3-way univariate repeated measures ANOVA design and included both temporal (exp) and spatial (block) blocking factors to account for replication of the experiment at two different times during the year and at two different locations or spatial blocks in the stream. One variable in the ANOVA was the fish treatment assigned to each of the cages (fish), which had the values of enclosure, open, or exclosure. Another fixed effect was the habitat the cage was in (hab), so it was either a depositional or erosional cage. The final variable in the model was the repeated measure of week and had the values or 1, 2, or 3 corresponding to the three sampling
periods. The ANOVA was first run with the log total density of invertebrates as the dependent variable then several more times using the log densities of specific invertebrate species. The p-values of these three fixed effects and all their interactions is what indicate whether fish, sculpin, habitat, and time have significant effects on the benthic invertebrate community. In SAS's Mixed Procedure design, the subject was the cage itself, which has 12 levels because there were 12 cages in the stream, however, it was nested within the fish treatments (cage(fish)). The subject is the sampling unit and the focus of the R matrix, the R correlation matrix, and the covariance parameter estimate. In this design, the covariance structure used was autoregressive (order 1) because it had the lowest AIC (Akaike's Information Criterion) and BIC (Schwarz's Bayesian Index Criterion) fit statistics and the estimation method was REML. Using this ANOVA design and the Mixed Procedure in SAS, I tested an overall fish effect on the abundance of benthic invertebrates, whether this fish effect was due largely to the effects of sculpin, and how these effects differed between habitats.

Results

Main Effect Interactions

Very few main effect interactions were statistically significant (Table 6). All the interactions that involved the fish treatments (e.g., fish treatment by habitat, fish treatment by week, and fish treatment by habitat by week) were insignificant. Because of this, the fish treatment main effect can be interpreted on its own.
Table 6: P-values for Log Total Density and Log Species Densities

<table>
<thead>
<tr>
<th></th>
<th>exp</th>
<th>block</th>
<th>fish</th>
<th>hab</th>
<th>week</th>
<th>fish*hab</th>
<th>hab*week</th>
<th>fish*week</th>
<th>fish<em>hab</em>week</th>
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<tr>
<td>D.F. (Num, Den)</td>
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<td>2, 36</td>
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<td>2, 36</td>
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</tr>
<tr>
<td>Total Density</td>
<td>0.8912</td>
<td>0.9263</td>
<td>0.4466</td>
<td><strong>0.0362</strong></td>
<td><strong>0.0036</strong></td>
<td>0.0596</td>
<td>0.7674</td>
<td>0.0889</td>
<td>0.1141</td>
</tr>
<tr>
<td>Chironomidae</td>
<td><strong>0.0260</strong></td>
<td>0.7588</td>
<td>0.6471</td>
<td>&lt;.0001</td>
<td>0.0596</td>
<td>0.7674</td>
<td>0.0889</td>
<td>0.1141</td>
<td>0.1130</td>
</tr>
<tr>
<td>Hydropsyche sp.</td>
<td>0.1181</td>
<td>0.5965</td>
<td>0.7464</td>
<td>&lt;.0001</td>
<td>0.1516</td>
<td>0.7464</td>
<td>0.1516</td>
<td>0.4047</td>
<td>0.4047</td>
</tr>
<tr>
<td>Antocha</td>
<td>0.2740</td>
<td>0.5069</td>
<td>0.7858</td>
<td>&lt;.0001</td>
<td>0.1087</td>
<td>0.7858</td>
<td>0.1087</td>
<td>0.8041</td>
<td>0.8041</td>
</tr>
<tr>
<td>Simulium</td>
<td>0.3830</td>
<td>0.7878</td>
<td>0.2879</td>
<td><strong>0.0023</strong></td>
<td>0.4477</td>
<td>0.6343</td>
<td>0.2236</td>
<td>0.9299</td>
<td>0.4451</td>
</tr>
<tr>
<td>Caenis</td>
<td><strong>0.0476</strong></td>
<td>0.6085</td>
<td>0.4549</td>
<td><strong>0.0002</strong></td>
<td>0.0797</td>
<td>0.7051</td>
<td>0.7486</td>
<td>0.8575</td>
<td>0.4561</td>
</tr>
<tr>
<td>Baetis sp.</td>
<td>0.9115</td>
<td>0.3556</td>
<td>0.5956</td>
<td>&lt;.0001</td>
<td>0.0795</td>
<td>0.7180</td>
<td>0.1728</td>
<td>0.3419</td>
<td>0.8690</td>
</tr>
<tr>
<td>Ephemereella sp.</td>
<td><strong>0.0173</strong></td>
<td>0.3087</td>
<td>0.9084</td>
<td>&lt;.0001</td>
<td>0.2088</td>
<td>0.8912</td>
<td><strong>0.0404</strong></td>
<td>0.6792</td>
<td>0.8641</td>
</tr>
<tr>
<td>Stenonema</td>
<td><strong>0.1102</strong></td>
<td>0.5838</td>
<td>0.3604</td>
<td>&lt;.0001</td>
<td><strong>0.0476</strong></td>
<td>0.3604</td>
<td><strong>0.0476</strong></td>
<td>0.3093</td>
<td>0.3093</td>
</tr>
<tr>
<td>Copepoda</td>
<td>0.3331</td>
<td>0.9454</td>
<td>0.3671</td>
<td>&lt;.0001</td>
<td><strong>0.0044</strong></td>
<td>0.3671</td>
<td><strong>0.0044</strong></td>
<td>0.5316</td>
<td>0.5316</td>
</tr>
<tr>
<td>Oligochaetiae</td>
<td><strong>0.0163</strong></td>
<td>0.7791</td>
<td>0.7476</td>
<td>&lt;.0001</td>
<td>0.3304</td>
<td>0.6751</td>
<td>0.2674</td>
<td>0.3971</td>
<td>0.6779</td>
</tr>
</tbody>
</table>

Note: D.F. = Degrees of Freedom, Num = Numerator D.F., Den = Denominator D.F.
Significance (α = 0.05) is indicated by **bold**

The one interaction that showed some significance was the habitat by week interaction (hab*week). The p-values for Copepoda density, *Ephemereella* density, and Stenonema density were all significant meaning the habitat effects on these species densities vary over time.

*Fish Effects*

Unfortunately, not all the fish placed into the cage were recovered. During the first run of the experiment, the two sculpin placed into one of the erosional enclosure cages were not retrieved, despite electrofishing and kick seining the cage several times at the conclusion of the experiment. No breaches were found in the cage when it was inspected daily, but both blue and green herons were seen in the vicinity before the first collection. Since those sculpin seemed to be lost to predation, in the second run of the experiment tops made from ½ or ¼ inch mesh were attached to all cages. After the second run of the caging experiment was over, four sculpin were not recovered, two from an erosional cage and two from a depositional cage. In this case, breaches were found in...
the cages during daily inspections shortly after the second sampling date. These breaches were along the bottom of the cage where fast current, due to flooding caused by repeated thunderstorms, blew away substrate and exposed the underside of the cage. Knowing the approximate time period when those three cages lost the sculpin, the data from those samples (three weeks from the first cage and one each from the second and third cages) could be excluded when the ANOVA was applied. However, the results were not significantly different from those produced by the original data, despite the correction for fish loss. The original results and the results excluding these observations were reported.

The manipulation of fish density had seemingly no effects on the prey community in the cages. The ANOVA was applied to both the untransformed and log-transformed data, however, there was no differences in the results produced. Since density data is commonly log-transformed for ANOVA, I have reported the results from the analyses performed on log-transformed. Results for untransformed data are shown in Appendix A. Presented in Table 6 are the p-values of all the main effects and interactions for the log total density of invertebrates and the log species densities for the six most abundant invertebrates in the cages (in order Chironomidae, Ephemerella sp., Oligochaetae, Copepoda, Optioservus, and Antocha) and the prey species most commonly found in the gut (in order Ephemerella sp., Chironomidae, Antocha, Hydropsyche sp., Simulium, Caenis, Stenonema, and Baetis sp.). One common prey item, the mayfly Drunella, was not included in the table due to its rarity in the environment, which made its population density impossible to accurately measure.
Examining the p-values for the fish treatments (fish) it is evident that manipulating fish density had no significant effect on the total density of invertebrates or that of any individual species. None of the p-values were close to 0.05 with the lowest being that for Simulium at 0.2879. Excluding the observations where sculpin may have escaped the cages does affect the p-values and their significance. The reduction in the number of significant effects is a result of a smaller size as seen in Table 7.

Table 7: P-values for Log Densities Excluding Observations

<table>
<thead>
<tr>
<th></th>
<th>exp</th>
<th>block</th>
<th>fish</th>
<th>hab</th>
<th>week</th>
<th>fish*hab</th>
<th>hab*week</th>
<th>fish*week</th>
<th>fish<em>hab</em>week</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.F. (Num, Den)</td>
<td>1,15</td>
<td>1,15</td>
<td>2,15</td>
<td>1,15</td>
<td>2,32</td>
<td>2,15</td>
<td>2,32</td>
<td>4,32</td>
<td>4,32</td>
</tr>
<tr>
<td>Total Density</td>
<td>0.8741</td>
<td>0.7102</td>
<td>0.6594</td>
<td>0.0395</td>
<td>0.0078</td>
<td>0.5454</td>
<td>0.1215</td>
<td>0.1915</td>
<td>0.7558</td>
</tr>
<tr>
<td>Chironomidae</td>
<td>0.0260</td>
<td>0.6182</td>
<td>0.6701</td>
<td>&lt;0.001</td>
<td>0.829</td>
<td>0.7859</td>
<td>0.1246</td>
<td>0.1735</td>
<td>0.1571</td>
</tr>
<tr>
<td>Hydropsychae sp.</td>
<td>0.1993</td>
<td>0.8530</td>
<td>0.7395</td>
<td>&lt;0.001</td>
<td>0.2609</td>
<td>0.7218</td>
<td>0.2867</td>
<td>0.4721</td>
<td>0.4830</td>
</tr>
<tr>
<td>Antocha</td>
<td>0.1206</td>
<td>0.2581</td>
<td>0.8619</td>
<td>&lt;0.001</td>
<td>0.1240</td>
<td>0.8601</td>
<td>0.1132</td>
<td>0.9282</td>
<td>0.8869</td>
</tr>
<tr>
<td>Simulium</td>
<td>0.2942</td>
<td>0.6444</td>
<td>0.6633</td>
<td>&lt;0.008</td>
<td>0.2114</td>
<td>0.9897</td>
<td>0.0836</td>
<td>0.9966</td>
<td>0.9030</td>
</tr>
<tr>
<td>Caenis</td>
<td>0.0090</td>
<td>0.5990</td>
<td>0.5740</td>
<td>&lt;0.003</td>
<td>0.2036</td>
<td>0.7176</td>
<td>0.7212</td>
<td>0.8519</td>
<td>0.7623</td>
</tr>
<tr>
<td>Baetis sp.</td>
<td>0.9362</td>
<td>0.3901</td>
<td>0.6127</td>
<td>&lt;0.001</td>
<td>0.1474</td>
<td>0.8015</td>
<td>0.2547</td>
<td>0.3964</td>
<td>0.9298</td>
</tr>
<tr>
<td>Ephemereida sp.</td>
<td>0.0321</td>
<td>0.4132</td>
<td>0.9894</td>
<td>&lt;0.001</td>
<td>0.4222</td>
<td>0.7607</td>
<td>0.1207</td>
<td>0.6577</td>
<td>0.6239</td>
</tr>
<tr>
<td>Stenonema</td>
<td>0.1002</td>
<td>0.4891</td>
<td>0.4965</td>
<td>&lt;0.001</td>
<td>0.0687</td>
<td>0.5338</td>
<td>0.0724</td>
<td>0.4392</td>
<td>0.3954</td>
</tr>
<tr>
<td>Copepoda</td>
<td>0.2944</td>
<td>0.8512</td>
<td>0.5406</td>
<td>&lt;0.001</td>
<td>0.0043</td>
<td>0.6075</td>
<td>0.0047</td>
<td>0.4451</td>
<td>0.4615</td>
</tr>
<tr>
<td>Oligochaetidae</td>
<td>0.0696</td>
<td>0.7954</td>
<td>0.8176</td>
<td>&lt;0.001</td>
<td>0.5682</td>
<td>0.6086</td>
<td>0.3626</td>
<td>0.4060</td>
<td>0.6649</td>
</tr>
</tbody>
</table>

Note: D.F. = Degrees of Freedom, Num = Numerator D.F., Den = Denominator D.F. Significance (α = 0.05) is indicated by bold

Habitat Effects

Between the two habitats studied in this experiment, the hard-bottomed, fast-flowing erosional sites and the soft-bottomed, depositional areas; there is an extremely significant difference in the total density of invertebrate. There are also significant habitat effects on most prey species, except those whose habitat main effects are confounded by on the significance of their habitat by time interactions and cannot be interpreted. In Table 8, the p-value for the total density is significant (hab) and all the p-values for the species densities are significant (most being < 0.0001). The depositional cages had a significantly higher total density of invertebrates and higher density for
species such as Copepoda and Oligochaetae. The erosional cages had significantly more
Chironomidae, *Ephemerella sp.*, Optioservus, Antocha, Hydropsyche sp., Caenis,
*Stenonema*, *Baetis sp.* and Simulium. Clearly, these two distinct habitats contain different
species of invertebrates in different abundances.

Table 8: Density Descriptive Statistics for Erosional and Depositional Cages

<table>
<thead>
<tr>
<th></th>
<th>Erosional Mean</th>
<th>S.E.</th>
<th>Depositional Mean</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Density</td>
<td>19356</td>
<td>1412</td>
<td>29771</td>
<td>2582</td>
</tr>
<tr>
<td>Chironomidae</td>
<td>6355</td>
<td>461</td>
<td>1239</td>
<td>173</td>
</tr>
<tr>
<td>Hydropsyche sp.</td>
<td>990</td>
<td>333</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Antocha</td>
<td>1018</td>
<td>161</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Simulium</td>
<td>97.7</td>
<td>38.7</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Caenis</td>
<td>113.5</td>
<td>25.8</td>
<td>18</td>
<td>12.8</td>
</tr>
<tr>
<td>Baetis sp.</td>
<td>1023</td>
<td>124</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Ephemeralia sp.</td>
<td>2346</td>
<td>354</td>
<td>24</td>
<td>17</td>
</tr>
<tr>
<td>Stenonema</td>
<td>550.9</td>
<td>88.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Copepoda</td>
<td>0</td>
<td>0</td>
<td>17078</td>
<td>1935</td>
</tr>
<tr>
<td>Oligochaetae</td>
<td>908</td>
<td>131</td>
<td>9137</td>
<td>899</td>
</tr>
<tr>
<td>Optioservus</td>
<td>1265</td>
<td>273</td>
<td>223</td>
<td>99</td>
</tr>
</tbody>
</table>

Note: Densities in # of Individuals / m², S.E. = Standard Error

**Week Effects**

For the log transformed data, the time main effect for the total density is
significantly (week). The 3-way ANOVA using the Mixed procedure in SAS also showed
significance in the species densities for copepods and the mayfly *Stenonema*. When the
time effect is broken down by habitat, there are large reductions in total density of
invertebrates in the depositional habitat across all weeks (Figure 9). The mean total
density of invertebrates in the depositional habitat is significantly reduced over time
resulting in a significant habitat by date interaction. In the erosional habitat, total density
actually increases from week 1 to week 2 and decreases in week 3 indicating different
habitat-specific effects over time on the total density of invertebrates. Besides the total
density, some species densities changed significantly over the weeks. The 3-way ANOVA using the Mixed procedure in SAS also showed significance in the species densities for copepods and the mayfly *Stenonema*.

**Figure 9: Habitat Effects Over Time**

![Graph showing habitat effects over time](image)

**Blocking Factors**

The ANOVA design incorporated two blocking factors, a temporal blocking factor that took into account the two different times of year the caging experiment was run and a spatial blocking factor that represented the two locations in the stream where the cages were placed. The spatial blocking factor (block) was an insignificant source of variation for log total density and all the log species densities. The temporal blocking factor (exp) was an insignificant source of variation for the log density, and for most of the log species densities. However, there were some species that showed that the time
the caging experiment was performed was a significant source of variation. Most of these species are mayflies (*Caenis* and *Ephemerella sp.*) and the significant p-values can be explained by their lifecycle.

Discussion

**Sculpin Predatory Effects**

Since the fish treatments included an open control cage that allowed access to all vertebrate predators and large invertebrate predators such as crayfish, an exclosure cage that excluded all fish (and large crayfish), and an enclosure cage that contained only sculpin, the lack of effect of cage treatment has many possible explanations. First, the results suggest that sculpin predatory effects have no significant effect on several species of invertebrates and the benthic community as a whole due to the fact that there was no difference between the open cages and the enclosure cages. Also, there seems to be no overall fish effect on the abundance of benthic invertebrates in Seven Mile Creek since there is no significant difference in the open and exclosure cages.

However, this study failing to display significant sculpin predatory effect on the benthic invertebrate community is not altogether surprising when taking into account past research. Wooster’s (1994) meta-analysis of the overall effect of predation determined that on average predators only have a small to moderate impact on prey density. Also, Flecker (1984) argued that fish had little influence on the diversity and abundance of most insect species and sculpin alone did not significantly reduce invertebrate abundance. Ruetz et al. (2004) using a similar cage and experimental design (creating a more natural
stream mesocosm than most studies) also found no significant sculpin or overall fish effect. The most likely explanation for the lack of predatory effects on the benthic invertebrate community is that the cascading effects of sculpin and other large predators are relatively weak in Seven Mile Creek. Sculpin having truly insignificant effects on benthic invertebrate community structure would not be altogether surprising considering their generalized diet, numerous prey items, and weak prey preference. Sculpin predation may not considerably reduce abundances of benthic invertebrates either directly or indirectly.

Although it is not unusual that this study produced no significant sculpin effect or overall fish effect, there are several plausible reasons why the expected predatory effects were not detected. The mesh size used for the cage may be one reason that the fish treatment was insignificant. A distinguishing feature of streams is the unidirectional flow of running water that results in high dispersal and rapid recolonization by aquatic organisms (Flecker 1984). Several previous studies have reported that the strongest effects of predators on invertebrates would occur in enclosures with small mesh size because it restricts immigration and emigration. In cages with smaller mesh and lower immigration, fish should eventually reduce densities of prey. This is unlike enclosures with larger mesh, where higher immigration rates may compensate for predation and reduce the likelihood of detecting fish effects (Cooper et al. 1990, Lancaster et al 1991, Dahl and Greenberg 1999). Conversely, mesh sizes too small may eliminate or restrict sources of recruitment and loss and will provide distorted views on how predators affect the abundance of prey. In this stream experiment, mesh size should not have been a
concern since the importance of mesh size declines with increasing cage size and the
cages used in this experiment were relatively large. Still, the size of the mesh may have
been too large to generate significant findings seen in experiments using smaller mesh
size. The lack of significant sculpin and overall fish effect could be due to the rapid
recolonization by stream insects that results in the inability of predators to regulate
abundances of benthic prey taxa. Larger mesh may have been an important cage artifact
that might have potentially affected the outcome of this predation study. Enclosure size
might also influence the impact sculpin have on prey density. First, the number of
microhabitats will vary with the size of the enclosure. Small enclosures may approximate
the size of a single microhabitat, such as a single stone or leaf pack. Larger enclosures,
however, will contain a variety or microhabitats and most likely several of the same type
of microhabitats, like multiple large stones or leaf packs. Generally, in smaller cages the
impact predators have on prey density might be a function of the impact they have on the
microdistribution of prey. However if predators simply cause prey to move from one
microhabitat to another, large enclosures might show little impact of predator presence if
prey movement occurs within a single enclosure (Wooster 1994). Compared to studies
done in the past, the cage size in this experiment was large in order to avoid caging
effects and better simulate the natural system. Although replicating a more realistic
mesocosm than other studies examining system-wide effects of fish predators, the larger
cages may have overlooked any fish effects due to prey movement from one microhabitat
to another. Studies using smaller enclosures, although less realistic and reflective of the
natural stream systems, often produce significant predator effects due to the restriction of prey movement within the cage.

Normally, it was necessary to clean off the debris that had collected on the mesh of the cages twice a day. However when a great deal of rain fell causing the water level to rise and the current to increase, the mesh was constantly monitored, otherwise organic material, sand, and silt would collect in the cages. When the stream returned to normal, most of the debris that had been deposited into the cages exited, except for some sand and silt. Because of the unusually wet summer, this deposited sediment slowly built up. This covered rocks and eliminated suitable habitat for many benthic invertebrates in erosional cages. It also affected depositional cages by slowly filling them in and reducing water depth. The loss of habitat most likely caused benthic invertebrate to decline swamping any negative effects of predation by sculpin, other fish, or crayfish. This sediment build-up along with the reduced flow of water coming through the mesh are possible cage artifacts that may have affected my ability to detect treatment effects in the cages.

Another explanation for the absence of fish effects deals with design of the experiment. Only two sculpins were placed in a cage with a 1 m² area. The sculpin density in the cages may have been too low. Although the sculpin density used was a realistic estimate, it may not have been high enough to produce any significant predatory effects. The low statistical power of the cage data as a consequence of too few replicates of the treatments could also have prevented any significant results. If the experimental design had included more replicates a significant predatory impact may have been detected. These two imperfections in the experimental design of the caging experiment
magnified by the loss of several sculpins during experimental runs (which only further
decreases statistical power) could have resulted in diminished fish effects.

_Benthic Invertebrate Habitats_

It is commonly known that different benthic invertebrates exist in different stream
habitats, and this concept was definitely supported by the results of this study. The
abundance and species of benthic invertebrates found living in the fast-flowing erosional
sites were drastically different from those in the depositional areas. The erosional habitat
had significantly more Chironomidae, _Ephemerella sp._, _Optioservus, Antocha, Hydropsyche sp._, _Caenis, Stenonema, Baetis sp._, and _Simulium_. The depositional habitat
had a significantly higher total density because it contained many more copepods and
oligochaetes. It was surprising that none of the taxa coexisted in both habitats in the
same abundances. If there is any inaccuracy in this data, it could originate from the
sampling procedure. When the substrate was sampled, only rocks and sediment cores
were taken. Invertebrates that typically spend a majority of their time on organic material
like submerged logs or driftwood, for example mayflies, would not have been accurately
sampled and counted. Accordingly, the densities for some invertebrates like the mayfly
_Caenis_, which are present in sedimentary habitat on submerged wood, may not be
accurate. However since the sampling objective was to quantify the abundances of
organisms living in or on soft sediments not on solid substrates in soft sediments, the
sampling protocol followed in this study should have been efficient and effective.
Densities Over Time

Although the repeated measure of time was significant with the total density decreasing week to week and there being a significant difference between week 1 and week 3, it could not have been due to sculpin predatory activities or the overall fish effect considering the fish by time interaction was not significant. The most plausible explanation for the log total density and several log species densities being significant in this manner is the natural changes in invertebrate abundances that occur during throughout the year. The collections took place in four different months over a two-year period leaving plenty of time for the densities of these invertebrates to change because of natural occurrences like weather, temperature, and lifecycle events like emergence into adulthood.

Another reason why the total density and some species densities decreased over time may have been caging effects. The emergence of benthic invertebrates or other natural occurrences such as floods could be a reason. However, they would have to occur in each temporal block to get a consistent time effect so caging effects seem to be the more likely culprit. Since there were no fish effects, it could be reasoned that the slow build-up of sand and silt in the cages eliminated suitable habitat for invertebrates. The mesh of the cages would slow the current enough for sand and silt to be deposited inside the cages and this phenomenon would be intensified whenever the water level would rise as a result of precipitation. This deposited sand covered rocks and microhabitats where many of the invertebrates thrive making the cage environment less hospitable causing some species densities and the overall density of invertebrates to decrease. This cage
artifact explains why some of the time main effects (week) and the habitat by date
interactions (hab*week) were significant. The significant habitat by time interaction was
primarily due to large reductions in total density over time in the depositional habitat.
Unlike the erosional habitat whose total density only decreased between week 2 and
week 3, the depositional habitat experienced large drops in density from week to week
providing evidence that cage artifacts greatly influenced cages in the depositional habitat.

Temporal and Spatial Factors

Two sources of variation in the caging experiment that had the potential to be
significant were a temporal factor, the experimental runs were at different times of the
year, and a spatial factor, the blocks of cages were at different locations in the stream.
Because these two factors were a concern, they were assigned as blocking factors in the
statistical design. The temporal blocking factor (exp) did not significantly affect the log
total density and most log species densities. It was, nonetheless, significant for the
densities of some of the mayfly species like Caenis, Ephemerella sp., and Stenonema.
This can be explained by the lifecycle of these specific species of Ephemeroptera.
During the first run the cage experiment in July, these species of mayfly were late in the
larval stage and were ready to emerge into adulthood. By the time the second run took
place, they had already emerged and were not present in the stream in large numbers.
The second blocking factor was spatial (block) and was insignificant for all the densities
indicating that the location of the cages in the stream had no effect on the outcome of the
study. When choosing the two locations for the cage blocks, it was essential that both
locations were similar in the types of substrate, amount of both erosional and depositional
habitat, and were a typical representation of the stream ecosystem found at Seven Mile Creek. By designing the experiment this way, it helped to ensure that location would not be a significant source of variation.

**Experimental Design**

The experimental design was very similar to that of previous studies. The enclosure size of 1 m² is comparable to that of Flecker (1984) (0.86 m²), Gilliam et al. (1989) (0.50 m²), and Ruetz et al. (2004). The ¼ inch (6.35 mm) mesh size used in this study was precisely the same as Flecker (1984) and the experimental duration of 24 days was again similar to Flecker (1984) (21 days) and Lancaster et al. (1990) (21 days). The locations for each cage were a good and common representation of both types of habitats. The cages were well built and sturdy and were able to easily handle any weather or high water. They were anchored firmly into the streambed and never drifted or became loose. The substrate of cobbles and sediment were easily sampled and processed in this study had been utilized before in caging experiments (Flecker 1984, Gilliam et al. 1989). There are no obvious intrinsic flaws in the cage design, experimental design, or sampling procedure. This experiment was well planned, performed, and analyzed and no significance was detected it may be due to uncontrollable and unexpected cage artifacts or there may be that sculpin and all fish have no significant affect on the benthic invertebrate community in Seven Mile Creek.
CONCLUSION

The diet of the mottled sculpin in Seven Mile Creek is a complex and intricate issue that has multiple dimensions. Prey items, habitat-use, and prey preference, which were covered in this study, are just a few of the possible topics of research when dealing with such a complex system. Combined with the caging experiment data exploring the predatory impact of the sculpin on the benthic invertebrate community, this study provides great insight and a better understanding of the role of sculpin in structuring the stream community.

Numerous prey items and weak prey preference characterize the diet of the mottled sculpin. However, whether a food source was significantly preference or avoided depended on certain characteristics. Large mayflies and caddisflies, were consumed in excess of their relative abundances due to their higher detection rates. This size selectivity of the sculpin resulted in smaller invertebrates, like several Dipterans, to be under-consumed relative to their density in the environment. Prey types that were significantly avoided were many Trichopterans and other invertebrates that build cases, live in tubes, or have shells. These effective prey defense strategies provided protection and made these prey types less accessible. The final characteristic that sculpin prefer is mobility, which increases encounter rates. Prey types with limited mobility are often not encountered or detected, therefore, are not significantly preyed upon by sculpin.

The sculpin diet included invertebrates that reside in both erosional and depositional habitats indicating that these fish do forage in both habitats. The habitat where sculpin forage does significantly affect their diet. However, this habitat effect
often changes with variables like time of year and the age of the fish. This habitat-specific diet of the mottled sculpin produced no significant impact on the benthic invertebrates in erosional and depositional stream habitats. The absence a predatory impact effect suggests that sculpin have weak direct and indirect effects on benthic invertebrate community structure.

Knowing the different prey items, their associated selectivity values, and the habitat-specific effects that make up the sculpin diet provides the ability to better predict effects on sculpin and on the stream community in general which could be important if any of these invertebrate populations were decimated by disease or an introduced species. For example in the late 1980s, Seven Mile Creek endured a *Glossosoma* population collapse due to a pathogen outbreak (Kohler and Wiley 1997). The results from this diet study can be used to identify possible impacts on sculpin population from the loss of a food source and in turn how the impact of sculpin on its other prey types may change. In this case, it probably did not hurt the sculpin population since *Glossosoma* are not a large portion of the sculpin diet and they show low selectivity toward this specific food type. It may in fact have benefited sculpin by releasing more suitable prey from competition thereby increasing their numbers. The sculpin diet and its associated predatory impact is important to understand especially because of foreign species invading this region. The round goby, a destructive non-native fish species slowly invading Great Lakes stream systems, shares the same food sources and habitats with mottled sculpin (French and Jude 2001). If this introduced species out-competed and displaced the sculpin, the results from this study may be useful in predicting its effects on the benthic invertebrate community.
Although this study does provide insight on the diet, habitat-use, and predatory effects of the sculpin further research is needed to better assess the role of the sculpin in stream communities.

One area that is in need of further study is the use of habitat by the sculpin. Evidence was provided by this study that sculpin feed in both the erosional and depositional environment, but what percentage of time is spent in both habitats is still unknown. Another possible study could be the manipulation of prey types in a controlled environment, to see if the $\alpha$ values from this study can be used to accurately predict the loss or addition of a prey type. Finally, long-term caging experiments need to be performed to reexamine the predatory efforts of the sculpin. It is recommended that the experimental run time is longer and the experiment is performed with more replicates.

Through gastric lavage and caging experiments, this analysis investigated the food sources, habitats, and prey preferences associated with the diet of the mottled sculpin in Seven Mile Creek along with the resulting predatory impact on the benthic invertebrate community. Although this study demonstrated that sculpin have relatively low prey preference and no significant predatory effects, it does supply useful information on the prey items, habitat-use, and foraging habits involved in the diet of the mottled sculpin.
APPENDIX A

Caging Experiment Results Using Normal Data
### Table 11: P-values for Total Density and Species Densities in Caging Experiment

<table>
<thead>
<tr>
<th></th>
<th>exp</th>
<th>block</th>
<th>fish</th>
<th>hab</th>
<th>week</th>
<th>fish*hab</th>
<th>hab*week</th>
<th>fish*week</th>
<th>fish<em>hab</em>week</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D.F. (Num, Den)</strong></td>
<td>1, 16</td>
<td>1, 16</td>
<td>2, 16</td>
<td>1, 16</td>
<td>2, 16</td>
<td>2, 16</td>
<td>2, 16</td>
<td>4, 36</td>
<td>4, 36</td>
</tr>
<tr>
<td><strong>Total Density</strong></td>
<td>0.4929</td>
<td>0.9932</td>
<td>0.4365</td>
<td>0.0143</td>
<td>0.0012</td>
<td>0.5542</td>
<td>0.0375</td>
<td>0.1092</td>
<td>0.7516</td>
</tr>
<tr>
<td><strong>Chironomidae</strong></td>
<td>0.9471</td>
<td>0.6836</td>
<td>0.8030</td>
<td>&lt;.0001</td>
<td>0.0150</td>
<td>0.8781</td>
<td>0.2316</td>
<td>0.5414</td>
<td>0.5234</td>
</tr>
<tr>
<td><strong>Hydropsyche</strong></td>
<td>0.3394</td>
<td>0.5712</td>
<td>0.3895</td>
<td>0.0064</td>
<td>0.1977</td>
<td>0.3895</td>
<td>0.1977</td>
<td>0.6163</td>
<td>0.6163</td>
</tr>
<tr>
<td><strong>Antocha</strong></td>
<td>0.8362</td>
<td>0.8710</td>
<td>0.6820</td>
<td>0.0008</td>
<td>0.1773</td>
<td>0.6820</td>
<td>0.1773</td>
<td>0.7783</td>
<td>0.7783</td>
</tr>
<tr>
<td><strong>Simulium</strong></td>
<td>0.6041</td>
<td>0.5418</td>
<td>0.2955</td>
<td>0.0658</td>
<td>0.7512</td>
<td>0.5721</td>
<td>0.2213</td>
<td>0.2748</td>
<td>0.1511</td>
</tr>
<tr>
<td><strong>Caenis</strong></td>
<td>0.0251</td>
<td>0.6755</td>
<td>0.4550</td>
<td>0.0026</td>
<td>0.2872</td>
<td>0.3206</td>
<td>0.6813</td>
<td>0.8997</td>
<td>0.5800</td>
</tr>
<tr>
<td><strong>Baetis sp.</strong></td>
<td>0.7353</td>
<td>0.5270</td>
<td>0.9014</td>
<td>&lt;.0001</td>
<td>0.0555</td>
<td>0.9247</td>
<td>0.0578</td>
<td>0.7421</td>
<td>0.8194</td>
</tr>
<tr>
<td><strong>Ephemerella sp.</strong></td>
<td>0.0027</td>
<td>0.8959</td>
<td>0.8709</td>
<td>&lt;.0001</td>
<td>0.5063</td>
<td>0.8632</td>
<td>0.4178</td>
<td>0.4472</td>
<td>0.5755</td>
</tr>
<tr>
<td><strong>Stenonema</strong></td>
<td>0.0415</td>
<td>0.9807</td>
<td>0.1695</td>
<td>&lt;.0001</td>
<td>0.0732</td>
<td>0.1695</td>
<td>0.0732</td>
<td>0.2501</td>
<td>0.2501</td>
</tr>
<tr>
<td><strong>Copepoda</strong></td>
<td>0.3442</td>
<td>0.8948</td>
<td>0.3582</td>
<td>&lt;.0001</td>
<td>0.0027</td>
<td>0.3582</td>
<td>0.0027</td>
<td>0.3610</td>
<td>0.3610</td>
</tr>
<tr>
<td><strong>Oligochaetae</strong></td>
<td>0.0566</td>
<td>0.6839</td>
<td>0.3669</td>
<td>&lt;.0001</td>
<td>0.1741</td>
<td>0.4110</td>
<td>0.1613</td>
<td>0.0860</td>
<td>0.1278</td>
</tr>
</tbody>
</table>

Note: D.F. = Degrees of Freedom, Num = Numerator D.F., Den = Denominator D.F.  
Significance (α = 0.05) is indicated by **bold**

### Table 12: P-values for Total Densities Excluding Observations

<table>
<thead>
<tr>
<th></th>
<th>exp</th>
<th>block</th>
<th>fish</th>
<th>hab</th>
<th>week</th>
<th>fish*hab</th>
<th>hab*week</th>
<th>fish*week</th>
<th>fish<em>hab</em>week</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D.F. (Num, Den)</strong></td>
<td>1, 15</td>
<td>1, 15</td>
<td>2, 15</td>
<td>1, 15</td>
<td>2, 15</td>
<td>2, 15</td>
<td>2, 15</td>
<td>4, 32</td>
<td>4, 32</td>
</tr>
<tr>
<td><strong>Total Density</strong></td>
<td>0.6455</td>
<td>0.8818</td>
<td>0.6215</td>
<td>0.0296</td>
<td>0.0023</td>
<td>0.6455</td>
<td>0.0389</td>
<td>0.1023</td>
<td>0.7305</td>
</tr>
<tr>
<td><strong>Chironomidae</strong></td>
<td>0.8948</td>
<td>0.5700</td>
<td>0.7998</td>
<td>&lt;.0001</td>
<td>0.0325</td>
<td>0.8338</td>
<td>0.3039</td>
<td>0.6036</td>
<td>0.5924</td>
</tr>
<tr>
<td><strong>Hydropsyche</strong></td>
<td>0.3193</td>
<td>0.5187</td>
<td>0.4691</td>
<td>0.0142</td>
<td>0.2707</td>
<td>0.4404</td>
<td>0.2469</td>
<td>0.6697</td>
<td>0.6832</td>
</tr>
<tr>
<td><strong>Antocha</strong></td>
<td>0.2334</td>
<td>0.4592</td>
<td>0.5128</td>
<td>0.0005</td>
<td>0.3149</td>
<td>0.5014</td>
<td>0.2821</td>
<td>0.4811</td>
<td>0.4768</td>
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<tr>
<td><strong>Simulium</strong></td>
<td>0.5533</td>
<td>0.5024</td>
<td>0.4928</td>
<td>0.0630</td>
<td>0.7437</td>
<td>0.7979</td>
<td>0.2399</td>
<td>0.3205</td>
<td>0.2024</td>
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<tr>
<td><strong>Caenis</strong></td>
<td>0.0469</td>
<td>0.6711</td>
<td>0.4860</td>
<td>0.0051</td>
<td>0.3626</td>
<td>0.3473</td>
<td>0.5639</td>
<td>0.8733</td>
<td>0.7445</td>
</tr>
<tr>
<td><strong>Baetis sp.</strong></td>
<td>0.8138</td>
<td>0.6201</td>
<td>0.9303</td>
<td>&lt;.0001</td>
<td>0.1423</td>
<td>0.9815</td>
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<tr>
<td><strong>Ephemerella sp.</strong></td>
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<td>0.6178</td>
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<td>0.3636</td>
</tr>
<tr>
<td><strong>Stenonema</strong></td>
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<td>0.8497</td>
<td>0.1797</td>
<td>0.0001</td>
<td>0.1033</td>
<td>0.1714</td>
<td>0.0911</td>
<td>0.3259</td>
<td>0.3226</td>
</tr>
<tr>
<td><strong>Copepoda</strong></td>
<td>0.3084</td>
<td>0.8587</td>
<td>0.5807</td>
<td>&lt;.0001</td>
<td>0.0022</td>
<td>0.6725</td>
<td>0.0024</td>
<td>0.2369</td>
<td>0.2525</td>
</tr>
<tr>
<td><strong>Oligochaetae</strong></td>
<td>0.0911</td>
<td>0.7773</td>
<td>0.4028</td>
<td>&lt;.0001</td>
<td>0.3410</td>
<td>0.4326</td>
<td>0.3223</td>
<td>0.1214</td>
<td>0.1528</td>
</tr>
</tbody>
</table>

Note: D.F. = Degrees of Freedom, Num = Numerator D.F., Den = Denominator D.F.  
Significance (α = 0.05) is indicated by **bold**
APPENDIX B

Approval Letter From the Institutional Animal Care and Use Committee
Date: May 18, 2004

To: Steve Kohler, Principal Investigator
    Brendan Earl, Student Investigator

From: Robert Eversole, Chair

Re: IACUC Protocol No. 04-03-02

Your protocol entitled "Habitat-Specific Diet of the Mottled Sculpin and Its Impact on Benthic Invertebrate Community Structure" has received approval from the Institutional Animal Care and Use Committee. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: May 18, 2005


