BEHAVIORAL AND MORPHOLOGICAL VARIATION IN BRACHYCENTRIDS (BRACHYCENTRIDAE *BRACHYCENTRUS* SPP.) OF TWO NORTHERN MICHIGAN STREAMS

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Biology 390 Natural History and Evolution

13 August 2009

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ABSTRACT

Caddisflies of order Trichoptera are an important indicator species for many aquatic habitats. These organisms may be useful in analyzing the effects of global climate change. We observed morphological and behavioral differences of Trichoptera (Brachycentridae *Brachycentrus* spp.) between Carp Creek and Maple River, two stream habitats of differing temperature and dissolved oxygen levels. We collected samples of larvae using D-nets and measured length and width of larvae and casings. We expected there to be little differences between morphology and behavior of Brachycentrids in these two streams. Unexpected results showed that Brachycentrids collected in the Maple River were pupating, and some cases contained adult flies. Consequently, the differences in the two sites may be the result of different evolutionary tactics in Brachycentrids.

Keywords: Brachycentridae *Brachycentrus*, caddisfly, temperature, dissolved oxygen level, behavioral difference, morphological difference.

INTRODUCTION

The order Trichoptera (caddisflies) encompasses over 12,000 species in North America, which, as larvae, live in rivers and streams. Within this order, there are many different families with dissimilar methods of larval food collection: some build protective cases and are filter feeders or scrapers, others are predatory and build nets or burrow. These methods are mutually exclusive and within the case builders, differences in cases are used to classify families (Merritt and Cummins 1996).

We found the tube-case-building family Brachycentridae common to both our experimental sites, and therefore decided to examine members of this family for differences in behavior and morphology. Brachycentridae larvae typically live in a tapered, square wooden case formed from a transverse arrangement of twigs, pine needles and other available organic matter (Gallepp 1975). The larvae construct the cases for protection against predation, environmental stresses, and to serve as a cocoon during pupation stages (Otto 1980). While clinging to logs, the larvae breathe using the oxygen circulating through their gills in the current of the stream (Merritt and Cummins 1996). Larvae of the genus *Brachycentrus* filter feed detritus by holding out their legs as the current flows by—a feeding technique unique to *Brachycentrus* among the tube-case making families (Gallepp 1974; Merritt 1996).

Caddisflies prefer cool, flowing water, where they live from one to two years in their larval stage, progressing through 5 instars. (Hereafter, when we refer to ‘caddisfly’ we will be referring to Brachycentridae *Brachycentrus* – the family and genus of the caddisflies studied). Their pupal stage lasts 2 to 3 weeks during which they fasten to a log and seal the front end of their case. After pupation is complete, the adult fly emerges from its case. The adult fly lives for no more than one month within the immediate area. (Merritt and Cummins 1996)

In a 1977 study, Gallepp demonstrated that water temperatures affect Trichoptera behavior. As temperatures increase, respiration rates increase, and as temperatures decrease, respiration rates decrease (Gallepp 1977). Changes in respiration rates often influence visible changes in behavior of the Trichoptera larvae; this may be evident because larvae circulate oxygen better when withdrawn in their cases than when actively filter feeding (Merritt and Cummins 1996). As Gallepp (1977) noted, larvae withdraw into their cases more often when temperature levels rise, and larvae emerge from their cases and feed more freely when temperature levels decrease.

When water temperatures increase, dissolved oxygen levels reach saturation more quickly. Consequently, warmer waters hold less dissolved oxygen since they reach saturation sooner. Colder water can hold greater amounts of dissolved oxygen without reaching the level of saturation (Cox 2009). These factors may explain the increased respiration levels of *Brachycentrus* genera observed in the Gallepp (1977) study. Lower oxygen levels, which correlate with increased water temperatures, force the larvae inside their cases in order for them to better circulate water through their gills.

Since the water in Maple River was warmer than the water in Carp Creek, we expected higher dissolved oxygen levels at Carp Creek than at Maple River. Therefore, we expected to see more *Brachycentrus* larvae outside their cases and actively filtering along Carp Creek than at Maple River sites. Due to the fact that there were no observed behavioral differences between larvae at either site, most larvae were able to withstand higher water temperatures and lower dissolved oxygen levels; they may have already evolved the adaptations to survive in both aquatic habitats. We did not expect to measure any morphological differences between *Brachycentrus* larvae at either field site.

Brachycentrus is of interest to the scientific community because it is an indicator species useful in assessing the quality of aquatic stream habitat (McCabe 2003; Merritt and Cummins 1996). Since it is an indicator species, it is necessary to understand how this organism adapts to environmental stresses. *Brachycentrus* will be an important determining factor in analyzing the effects of global climate change. Our goal was to determine whether *Brachycentrus* larvae have evolved different behaviors and morphologies between two different natural environments in northern Michigan. We hope our results will enable the scientific community to better predict how well Brachycentridae and other Trichoptera will survive as the effects of global climate change become more severe.

MATERIALS AND METHODS

We conducted our research in the northern lower peninsula of Michigan in the Douglas Lake watershed. The sites of interest were two tributaries of Douglas Lake: the Maple River, which receives surface water from the lake, and Carp Creek, which receives groundwater by infiltration from Douglas Lake.

Carp Creek — This stream flows through a cedar swamp with considerable woody debris, including beaver dams. On average, Carp Creek is narrower than the Maple River, with approximately 80% of its banks covered in vegetation consisting primarily of trees and shrubs. The stream is subject to intermittent sun and shade. It receives greater shade coverage than Maple River throughout the day. Substrate consists of 70% sand with few cobbles.

Maple River — Maple River has sparse woody debris and is wider than Carp Creek. Vegetation, primarily grasses and trees, covered approximately 60% of the banks with erosion evident. Due to its greater width and sparser vegetation, Maple River has greater exposure to sunlight with fewer shaded areas. Substrate consists of approximately 60% sand and 40% cobble. Small fish (< 1 inch in length) are abundant and are predators of the caddisflies (Merritt and Cummins 1996).

Two sites along Maple River and two sites along Carp Creek were sampled for *Brachycentrus* larvae on July 15th and August 2nd. These samples were within distances ranging from 5 m to 100 m downstream of road crossings.

We recorded water chemistry at each site using an Oaklon CON 10 series total dissolved solids meter and YSI Model 55/50 ft dissolved oxygen meter. We recorded temperature (TEMP, °Celsius) and dissolved oxygen levels (DO, mg/L). In addition, within five random 1 m^2 quadrats at each of the four sites we assessed substrate and suitable habitat, recorded average water depth, and, where applicable, gathered Trichoptera samples. Prior to gathering the Trichoptera for study, we noted the behavior of the organisms: 1) number of Trichoptera present, 2) type of organic substrate on which larvae were found, 3) which direction the larvae were facing in the stream, 4) depth at which the larvae were located, and 5) if the larvae were outside their cases, feeding or otherwise. Lastly, we took benthic samples using D-nets dragged upstream for 10 seconds in one randomly chosen quadrat at each site.

After collection, we identified the larvae under a microscope as *Brachycentrus*, then we preserved them in 70% ethanol. We measured case length and width (in mm) and larvae length and width (in mm) using Mitutoyo Absolute Digimatic calipers.

Statistical analysis was conducted using SPSS software. In order to analyze our results concerning morphological differences, independent samples *t-tests* were performed. We also used chi-square analysis to compare the occurrence of sealed and unsealed cases at either site.

RESULTS

The Maple River had an average temperature of 16.275^{o}C, a higher temperature than Carp Creek, which had an average temperature of 11.75^{o}C (Figure 1). Using an independent samples *t-test* with $N = 8$, we observed a significant difference in these temperatures ($p < 0.001$). As expected from the inverse relationship between temperature and dissolved oxygen, Maple River had lower dissolved oxygen levels (7.37 mg/L) than Carp Creek (8.79 mg/L) (Figure 2). This difference was also statistically significant (independent samples *t-test*; $N = 4$; $p = 0.023$). We observed very few morphological differences between unsealed cases at Maple River and Carp Creek (Figure 3). These observations were confirmed by independent samples *t-tests* for the case length ($N = 225$; $p = 0.139$) and for case width ($N = 225$; $p = 0.952$). Likewise, no statistically significant difference was found between larvae width at either site (independent samples *t-test*; $N = 183$; *p-value* = 0.150). There was, however, a statistically significant difference between larvae length (independent samples *t-test*; $N = 183$; *p-value* = 0.008).

A chi-square test demonstrated that there were important differences in the occurrence of sealed cases between Carp Creek and Maple River ($\chi 2 = 121$; $df = 1$; $p < 0.001$). Another chi-square test showed there were also significant differences between the unsealed cases ($\chi 2 = 32.11$; $df = 1$; $p < 0.001$) At Carp Creek only 15% of Brachycentrids within our 10 quadrats were withdrawn into their cases while at Maple River 96% of Brachycentrids within our 10 quadrats were withdrawn into their cases (Figure 4).

Density measurements were quantitatively analyzed using an independent samples *t-test,* which illustrated that there was no statistically significant difference between densities of larvae at the four experimental sites ($N = 10$; $p = 0.198$).

At Carp Creek site 1, we found 100% of the Brachycentrids on logs, while at Carp Creek site 2, 83% of the Brachycentrids were found on logs and 17% were found clinging onto aquatic plants. At Maple River site 1, there were no caddisflies of the *Brachycentrus* genera. In contrast, at Maple River site 2, we found 96% of larvae clinging to logs while we found only 4% on aquatic plants (Figure 5).

All of the Brachycentrids in our Carp Creek samples were facing upstream on their substrates, as were 88% of the Brachycentrids found at our Maple River samples. The remaining 12% were facing in various directions in clusters, though none positioned themselves directly downstream.

We never found the Brachycentrids of all quadrat samples less than 12 cm from the bottom of either stream. However, the larvae found at Maple River site 1 were notably lower in the water column due to the deeper depth of Maple River.

An unexpected result was that 66% of Brachycentrids were in pupation stages in Maple River. Remarkably, there was the discovery of two adult flies in sealed cases at the same site. In comparison, at Carp Creek there were no caddisflies in pupation; they were all in larval stages. Of the larvae successfully extracted from their cases while pupating, their average body length was 10.56 mm (Figure

3). This average is significantly larger than the average length of non-pupating larvae, which was 8.77 mm (independent samples *t-test*; N = 42; *p-value* < 0.001).

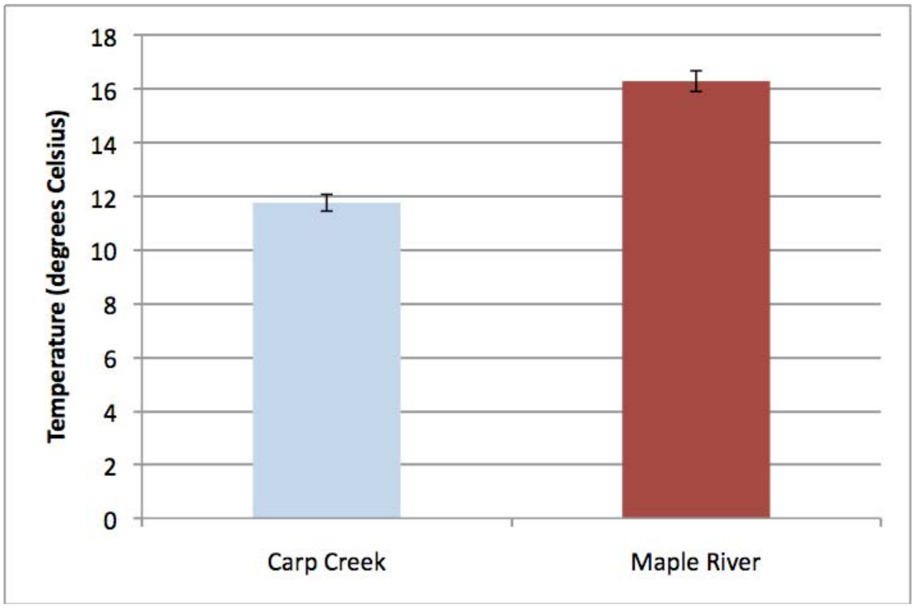

Figure 1. Average temperature at Carp Creek sites (N=2) and Maple River sites (N=2).

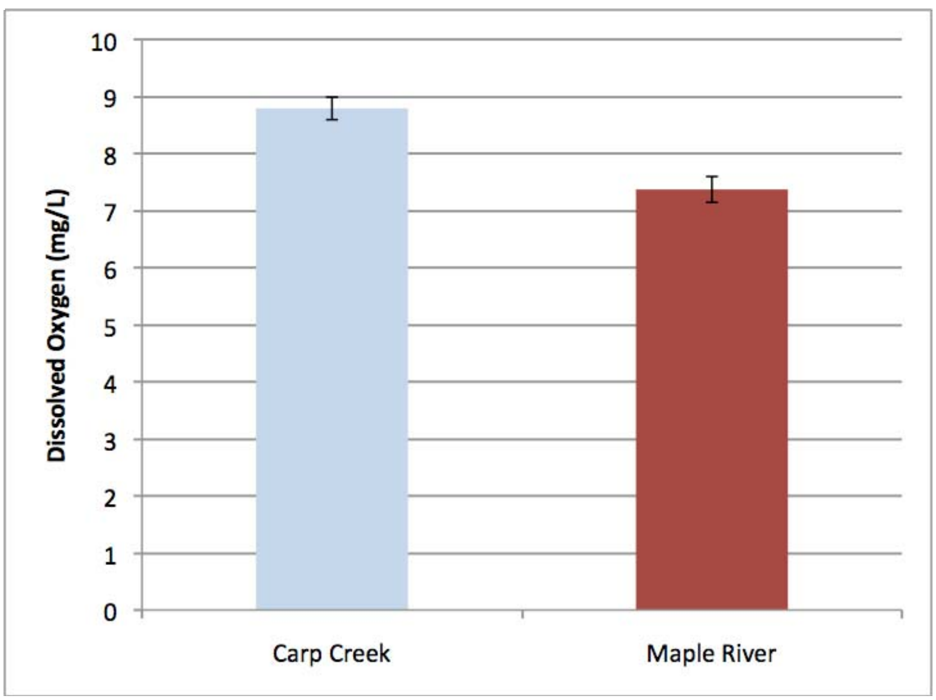

Figure 2. Average dissolved oxygen level at Carp Creek sites (N=2) and Maple River sites (N=2).

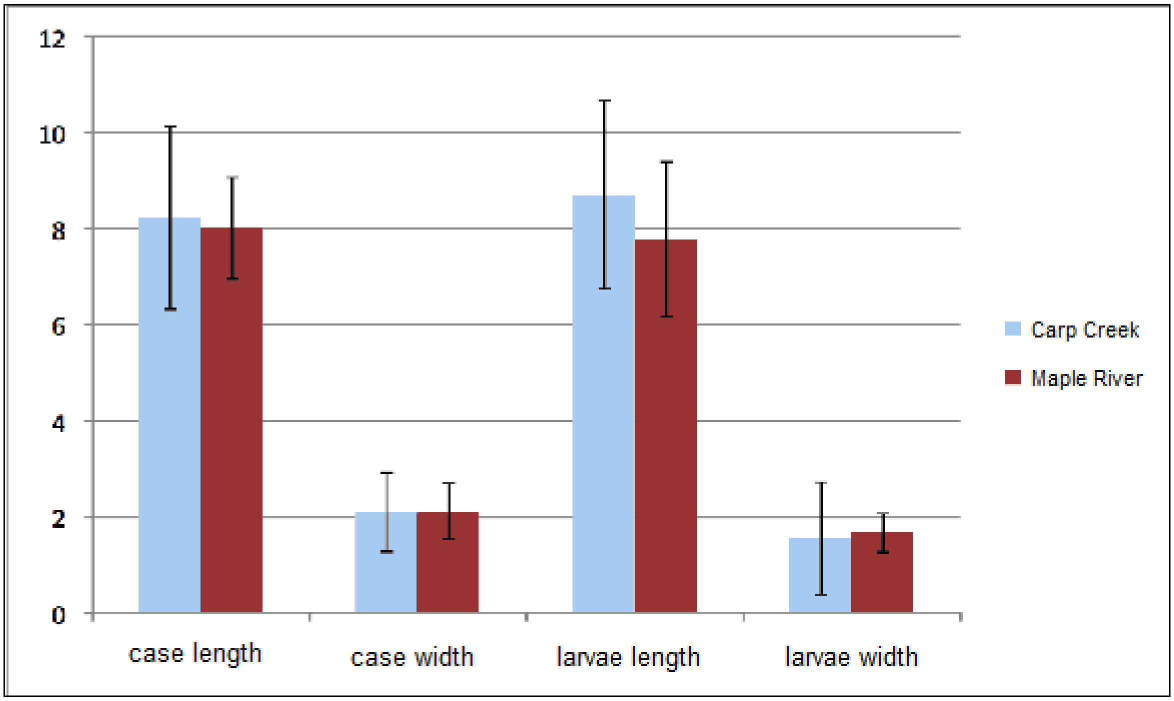

Figure 3. Average measurements of unsealed case length and unsealed case width (N=225) as well as larvae length and larvae width at Carp Creek and Maple River (N=183).

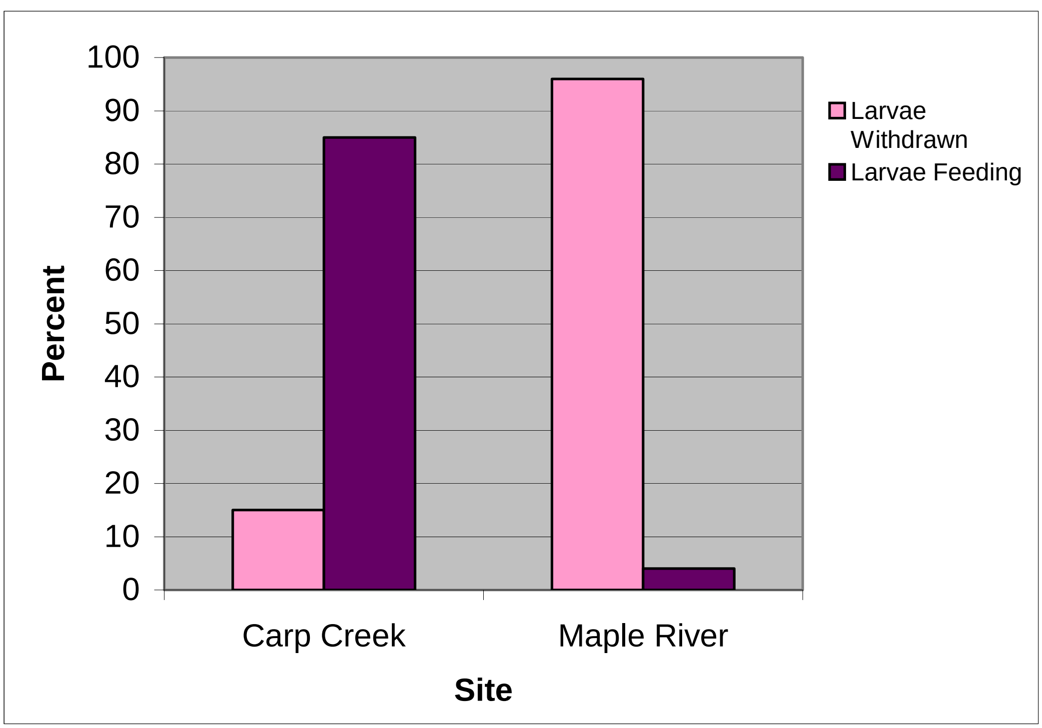

Figure 4. Percentage of larvae withdrawn and feeding at Carp Creek (N = 332) and Maple River (N = 125).

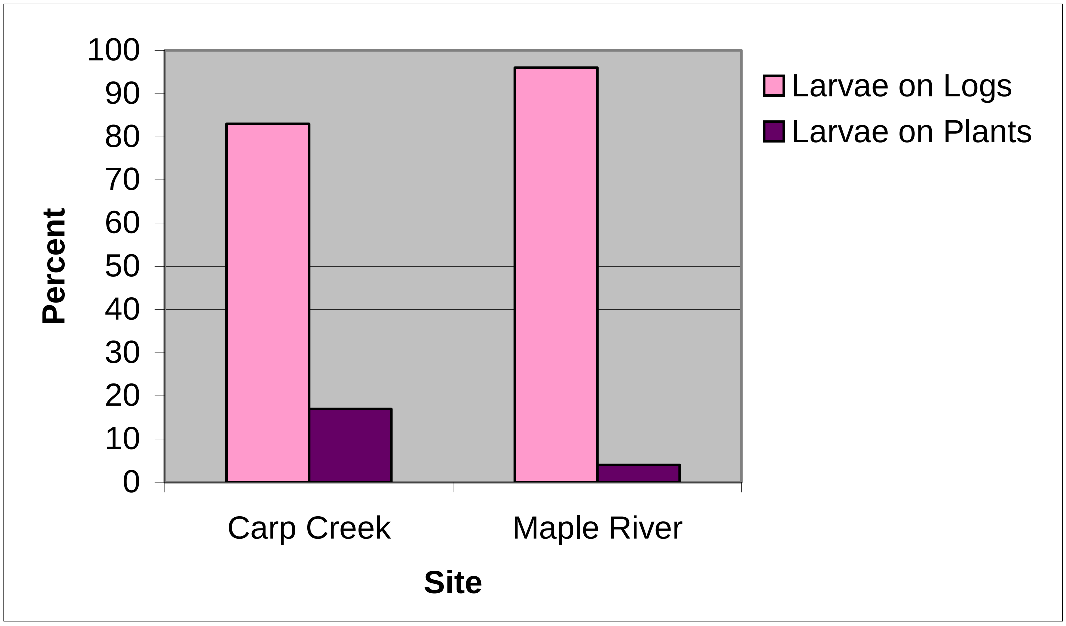

Figure 5. Percentage of larvae on logs and plants at Carp Creek (*N* = 332) and Maple River (*N* = 125).

DISCUSSION

In support of our hypothesis, the water at Maple River was warmer with lower dissolved oxygen levels than the water at Carp Creek. In addition, we saw more *Brachycentrus* larvae outside of their cases filtering along Carp Creek than at Maple River. These were the only behavioral differences observed. There were no significant variations in population density, substrate usage, or the maximum depth occupied in any of our quadrants between the two streams. We did not expect to see any differences in the previous factors because *Brachycentrus* have strict habitat requirements; therefore they were restricted to similar habitats in both streams.

Previous studies have shown that environmental factors affect caddisfly development (Jannot 2008). Of these factors, water temperature and dissolved oxygen appear to have a significant impact (Mackay 1979). Other variables such as population density, resource limitation, and predation are also determinants of the timing of the life cycles of Trichoptera (Mendez 2008). The significant differences in temperature and dissolved oxygen levels between the two experimental sites may account for the differences in developmental stages of the Brachycentrids sampled.

There were no significant morphological differences in case length, case width, or larvae width between the Brachycentrids of Carp Creek and Maple River. However, there was a significant difference in larvae length. The larvae at Maple River had a larger average length than the larvae at Carp Creek (Figure 3). This result was unexpected, but one explanation is that the Brachycentrids of Maple River were in more advanced stages of their life cycle. Although the caddisfly larvae included in these results were not in pupation at Maple River, it is possible that they were at a later instar stage. This may have contributed to the additional fact that the number of Brachycentrids in sealed cases occurred at a much higher frequency in Maple River than in Carp Creek, as shown by the chi-squared test.

At Carp Creek all of the Brachycentrids found within our quadrats faced upstream whereas only 88% of Brachycentrids found in Maple River faced upstream. This observation could be an additional consequence arising from the fact that the Brachycentrids were at different life stages at each site. Since the caddisflies need water currents in order to filter their food, they produce silk in order to attach the anterior end of their cases to their substrate. Then, they extend their legs while facing upstream into the current in order capture food particles (Wallace 1980). Since 96% of the samples found in Carp Creek were withdrawn into their cases and pupating, they were not filter feeding. Therefore, it was not necessary for them to face upstream.

Some possible sources of error were that there were a significant number of uncontrolled variables (nutrient levels, predators) between the two streams. For better results, the experiments should be conducted in a controlled laboratory in addition to field observations of the *Brachycentrus* in their natural habitat. A study conducted by Mackay (1979) showed that water temperatures are not independent of stream width. For instance, in smaller streams, temperatures are less important in determining the caddisfly population size; therefore our results for Carp Creek could be affected by this fact (Haidekker and Hering 2008). To control for this variable, streams should be chosen that are more similar in size. Also, better techniques could be developed to extract the *Brachycentrus* larvae from their cases during pupation. Many of our Brachycentrids were irremovable from their sealed cases because their bodies soften during this part of the life cycle, and therefore they were damaged during the process. In order to have a large sample size more representative of the population, more quadrants could be taken at each field site.

Since there were observed differences in the timing of the Brachycentrids' life cycle between the two streams sampled, further analysis needs to be conducted. Gallepp (1977) discovered that Brachycentrids were more carnivorous during the last instar stage that is completed before pupation. In general, the larvae consume different food particles at different instars. Analysis of the gut contents of the larvae at each field site could help verify the advancement of their development. Further research could determine whether or not the caddisflies begin their life cycles earlier in the season or whether or not their life stages are shorter in duration at Maple River. In addition, other aquatic invertebrates could be studied at each site to determine whether their development is more advanced at Maple River than Carp Creek.

Many invertebrates are dependent upon seasonal temperature fluctuations to control the timing of their life cycles (Talmage and Coutant 1978). Changes in season can affect water temperature. Increases in water temperature can induce thermal shock in the larvae of aquatic invertebrates, which can negatively affect their growth patterns (Coutant and Talmadge 1977). The warmer water of Maple River could account for the advanced life stages of the Brachycentrids found there. Although lower dissolved oxygen levels were observed, they are a consequence of the warmer water; therefore the water temperature is the ultimate determining factor. Warmer water temperatures and the effects on the life cycle of caddisflies are a cause of concern due to the predicted effects of global climate change. In some areas, atmospheric temperatures are expected to rise, which will correspondingly increase water temperatures. In addition, warming can affect seasonal cycles that the Brachycentrids depend upon to determine their life stages. It remains to be seen by further research whether Brachycentrids, and other families of the Trichoptera order, have the capabilities to adapt to the severe stresses that will be induced by global climate change.

Acknowledgements – We would like to express our appreciation to Jordan Price and Jennifer Rowe for their supportive input and assistance in the field.

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