

Differences in the Critical Thermal Maximum Between Two Size Classes of *Stenonema femoratum* (Ephemeroptera: Heptageniidae)

Jaiden Frantz

David Houghton

Follow this and additional works at: <https://scholar.valpo.edu/tgle>



Part of the [Entomology Commons](#)

This Peer-Review Article is brought to you for free and open access by the Department of Biology at ValpoScholar. It has been accepted for inclusion in The Great Lakes Entomologist by an authorized administrator of ValpoScholar. For more information, please contact a ValpoScholar staff member at scholar@valpo.edu.

Differences in the Critical Thermal Maximum Between Two Size Classes of *Stenonema femoratum* (Ephemeroptera: Heptageniidae)

Jaiden K. Frantz and David C. Houghton*

Department of Biology, Hillsdale College, 33 East College Street, Hillsdale, MI 49242

*Corresponding author: (e-mail: dhoughton@hillsdale.edu)

Abstract

Temperature is an important variable affecting the behavior and survival of aquatic organisms; however, little is known about the effects of size and corresponding developmental differences on aquatic insect temperature tolerance. We tested the critical thermal maximum (CTM) of large (head capsule width mean = 3.5 mm) and small (1.9 mm) specimens of *Stenonema femoratum* (Say) by raising the experimental temperature by 0.1, 0.3, and 0.5 °C per minute during laboratory CTM trials. Size class and temperature increase rate combinations were randomized, and each combination was tested over four trials, for a total of 24 trials of four specimens each. Two-way Analysis of Variance determined CTM was significantly higher for small specimens (35.2 °C) compared to large specimens (33.0 °C) regardless of temperature increase rate. There was no significant difference in CTM based on temperature increase rate, or interactions between size class and temperature increase rate. This result demonstrates that the highest thermal tolerance of a species is not necessarily in its largest specimens, and that determining a single CTM for a species based on multiple size classes would likely lead to erroneous results.

Keywords: critical thermal maximum, mayfly, temperature, Michigan

Temperature is an important variable affecting the behavior and survival of aquatic organisms (Reanault et al. 2005). Since nearly all aquatic organisms, being ectothermic, rely heavily on the specific temperature of their surroundings to regulate their internal bodily processes (Bawa et al. 2021), minor temperature changes could have a large impact on the organisms of aquatic ecosystems. As anthropogenic disturbances continue to increase the temperature of aquatic ecosystems, the impact that temperature has on organismal survival is important to understand (DeKozlowski et al. 1981, Dallas and Rivers-Moore 2012).

The critical thermal maximum (CTM) is a commonly used measurement for quantifying temperature limits in organisms (Lutterschmidt and Hutchison 1997). This value is determined through a process in which a particular behavior indicates an organism's sublethal thermal limit as the temperature of its enclosure is increased at a prescribed rate. For aquatic insects, CTM is usually indicated by a test specimen releasing its grip on a substrate within the test enclosure (Dallas and Rivers-Moore 2012). Several different variables have been tested for their effects on CTM, for example: acclimation temperature, season, external stressors such as predation or pollution, and

differences in the natural environmental conditions of collection sites (Ernst et al. 1984, Rowe et al. 1988, Renault et al. 2005, O'Leary et al. 2014).

Little research has been conducted on the importance of specimen size in affecting CTM within a single aquatic insect species. Size is an important variable since it usually relates directly to a specimen's developmental state and instar (Resh and Cardé 2009). Thus, CTM testing may reveal life stages that are particularly vulnerable to thermal stress. Larger specimens may also have an artificially higher CTM under experimental conditions simply because they take longer to heat up (Ribeiro et al. 2012). This problem could be addressed by utilizing different temperature increase rates for CTM trials, because slower increase rates should mitigate the difference in warming speed between larger and smaller specimens over the short duration of the CTM trial.

Stenonema femoratum (Say) (Ephemeroptera: Heptageniidae) occupies a wide variety of habitats and river types across the United States and Canada (Bednarik and McCafferty 1979). In a study of a southern Ontario stream, the species was primarily univoltine with a large adult emergence in May. The presence of large nymphs later in

Table 1. Stream temperature of the Little Manistee River on each collection date in 2022. Temperature readings were taken at a depth of ~15 cm in the middle of the stream, about 1 m deep and 5 m from either shoreline.

Date of collection	Time of collection	Temperature (°C)
11 May	11:00 AM	16.2
13 May	10:15 AM	15.4
15 May	1:00 PM	16.5
17 May	11:30 AM	14.2
19 May	12:30 PM	15.3
23 May	12:15 PM	15.1
24 May	10:00 AM	14.3
30 May	12:30 PM	19.1
31 May	1:00 PM	18.9
04 June	10:00 AM	14.9
Mean temperature:		16.0

the summer and a secondary peak of adults in mid-August, however, suggested either cohort spreading or else a second generation (Rowe and Berrill 1989). The species was more clearly bivoltine in several Missouri populations (Ball 2002).

Due to their abundance in the early summer months and their predictable CTM endpoint, nymphs of *S. femoratum* have been utilized to address effects of temperature in various capacities, including seasonal changes in CTM (Lyman 1955, Houghton and Shoup 2014). Nothing, however, is known about the impact of specimen size on the CTM of the species. The purpose of this study, therefore, was to measure thermal tolerance in two distinct size classes of *S. femoratum* nymphs using different temperature increase rates. We hypothesized the larger size class would have a higher CTM due to their larger volume, especially when using faster temperature increase rates.

Materials and Methods

Specimens of immature *S. femoratum* were collected on ten occasions from early May to early June 2022 from a 50 m stretch of Little Manistee River (44°02'02" N, 85°43'29" W), a second-order woodland stream located near Luther, Michigan. A detailed description of this site can be found in Houghton and Wasson (2013). This site was chosen for its abundance of the woody and rocky substrates preferred by *S. femoratum* (Johnson et al. 2018).

To determine an appropriate temperature at which to acclimate our specimens in the laboratory, river temperature was measured after each collection using a YSI-55 dissolved oxygen probe at a water depth of ~15 cm. Temperature readings were taken in the middle of the stream near specimen

collection, which was about 1 m deep and 5 m from either shoreline. All temperature measurements were taken between 10:00 am and 1:00 pm. Over the course of the study, the temperature of the river averaged around 16 °C (Table 1).

Specimens were located by inspecting river rocks by hand and gently removing identified nymphs of *S. femoratum* using wide featherweight forceps. Specimens were then placed in a Styrofoam container filled with water collected from the site and transported back to the laboratory. Each collection lasted 1–2 h and typically yielded 15–45 specimens of varying size.

All specimens were confirmed as *S. femoratum* in the laboratory using Waltz and Burian (2008) and separated into 'large' (3.2–3.8 mm) and 'small' (1.7–2.2 mm) size classes based on the width measured at the widest part of each specimen's head capsule using a small ruler (Fig. 1). These size classes were chosen due to the distinct difference between them and for the abundance of specimens of both classes in the river during our study period. The large specimens represented near terminal instar, whereas the small specimens represented instars about midway through their nymphal development. Individuals that did not fit into these classes were discarded. Specimens of each size class were transferred in groups of 10–20 to a 20 cm × 17 cm × 15 cm flow-through container containing a 12 cm × 12 cm × 8 cm rock from the collection site to act as a substrate for specimens. These containers were then placed into a Frigid Units Living Stream™ environment (www.frigidunits.com), which simulated current and ensured that all specimens were exposed to the same acclimation temperature. Any nymph that molted during acclimation was discarded. All specimens were acclimated for 72 h at

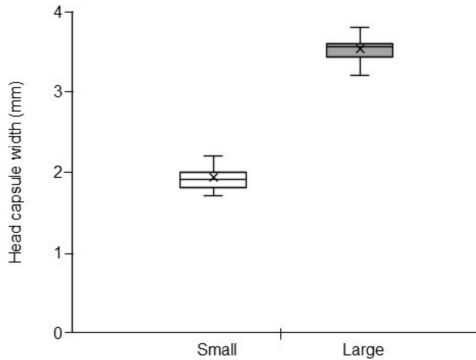


Figure 1. Box plots of the head capsule widths of the large and small size classes of *S. femoratum* specimens collected and tested during this study. $n = 48$ for both size classes.

16.1 (± 0.4) °C and ambient photoperiod using artificial (non-solar) indoor lighting. This acclimation temperature was chosen to approximate the mean temperature of the collection site (Table 1).

CTM trials were conducted using a Julabo MB-13 circulating water bath (www.julabo.com) set to 40% external and 60% internal circulation (Houghton et al. 2014), which produced a circulating velocity of around 0.3 meters per second as measured by a FloWatch FW 450 velocimeter (www.inspectusa.com). The interior of the water bath was lined with 1×1 mm mesh and included a 12 cm × 12 cm × 8 cm rock from the collection site in its center. The same rock was used

for all trials. The water bath was linked to a computer using Julabo EasyTemp™ software, thereby allowing for precise (± 0.02 °C) temperature increase rates, specifically 0.1 °C, 0.3 °C, and 0.5 °C per minute (Houghton et al. 2014). Four trials were conducted at each temperature increase rate and size class combination for a total of 24 trials. A random number generator was used to determine trial order.

During each trial, four specimens of the same size class were placed on the rock substrate of the water bath. They were given one minute to orient themselves to the current before the temperature was raised at the prescribed rate from 16.1 °C until all specimens reached their CTM. CTM was indicated by a release from the rock substrate and an inability to re-attach. Once the trial ended, specimens were placed in a 400 mL bowl containing water from the water bath and floated in the Living Stream for 1 h to reacclimate back to 16.1 °C (O’Leary et al. 2014). The specimens were then returned to their flow-through containers in the Living Stream and their survival assessed every 12 h for 48 h to confirm the non-lethality of the trial.

A Two-way Analysis of Variance was conducted using Excel for Windows with the Real Statistics add-in (www.real-statistics.com) to assess differences in CTM between large and small specimens, the different rates of temperature increase, and any interaction between the two variables. Data were tested to ensure that they met parametric assumptions before analysis. We assessed

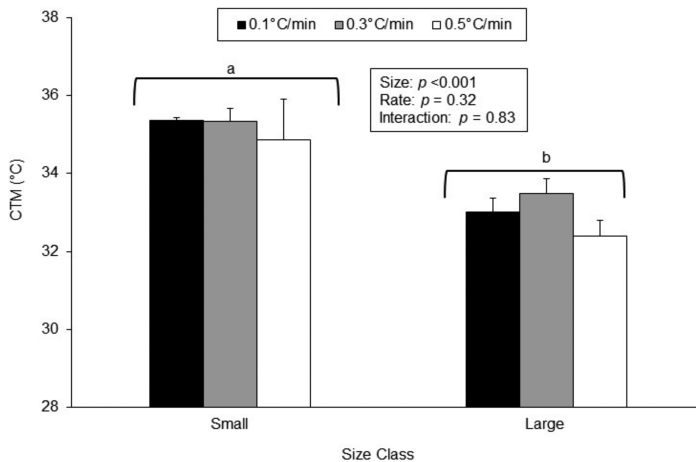


Figure 2. Mean (+SE) CTM values for *S. femoratum* based on size class and temperature increase rates. P -values determined by 2-way Analysis of Variance, $F = 26.5, 1.204, 0.192$; $df = 1, 2, 2$. Superscript letters denote statistically distinct groups based on a Tukey HSD test. $n = 4$ trials for each bar.

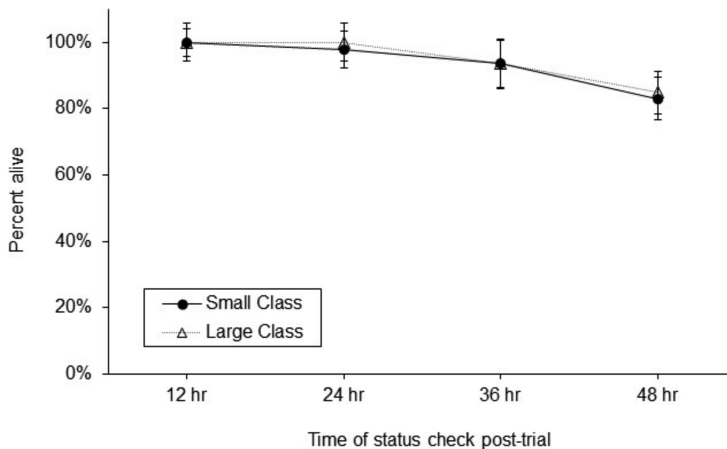


Figure 3. Mean (\pm SE) rate of survival for small and large size class specimens of *S. femoratum* utilized in our experiment trials, measured at 12-h intervals for 48 h after trials. $n = 48$ for the small size class and 46 for the large size class.

significance using a standard alpha level of 0.05.

Results

The large specimens had head capsules almost twice as wide as those of the small, with no overlap between them (Fig. 1). The CTM was significantly higher for small specimens (35.2 °C) than large specimens (33.0 °C) regardless of temperature increase rate ($P < 0.001$) (Fig. 2). There was no significant difference in CTM based on temperature increase rate ($P = 0.32$), or interactions between size class and temperature increase rate ($P = 0.83$). The 24-h post-trial survival was 100% for the large specimens and 98% for the small specimens (Fig. 3). There was an increase in mortality during the second 24 h period, with 83% of small and 85% of large specimens surviving the full 48 h after testing. Two large specimens molted to subimago during the post-trial assessment period.

Discussion

Our results demonstrated that large nymphs of *S. femoratum* had lower thermal tolerance than did medium-sized ones. Few studies of aquatic insects have directly addressed this topic, with conflicting results among those that have. Heiman and Knight (1972) observed a higher upper lethal temperature in larger specimens of the stonefly *Paragnetina media* (Walker) (Plecoptera: Perlidae) than in smaller ones. They attributed this difference to a higher physiological robustness in female specimens, with size covarying with sex. Garten and

Gentry (1976) found that larger species of dragonfly (Odonata) had higher CTMs than did smaller species, but that such differences were “trivial” compared to CTM differences between different acclimation temperatures. Ernst et al. (1984) found no difference in the CTM of any of three tested species of stonefly based on age, size, or sex, including those that were nearing or at their terminal instar. After testing many species, Dallas and Rivers-Moore (2012) generally determined no difference in CTMs between larger and smaller specimens of the same family, except for an increased CTM in larger specimens of telagodontid mayflies (Ephemeroptera) relative to smaller specimens. Since none of these studies tested specimen size as a primary variable, it is possible that size effects were masked by more distinct differences in CTM between different species, different sexes, different acclimation temperature or other differences in experimental set-ups, or different collection seasons or habitats.

Our multiple temperature increase rates further supported the physiological thermal tolerance differences of specimens of different size. Since smaller specimens should physically heat up faster than larger ones (Ribeiro et al. 2012), we expected to see a higher CTM in larger specimens at faster temperature increase rates, whereas this heating rate difference would be less pronounced with slower rates since all specimens would have a greater time to equalize in temperature. Instead we observed no differences in CTM among temperature increase rates, or their interactions with size. This observation suggests that the rate at

which specimens heat up under laboratory conditions is less important than their inherent thermal tolerance, although it is also possible that all specimens were small enough that heating rate differences between them were negligible. The study most similar to ours (Houghton et al. 2014), found that CTM of the caddisfly *Pycnopsyche guttifera* (Walker) (Trichoptera: Limnephilidae) decreased with a 0.1 °C per minute increase rate relative to that of 0.3, 0.5, and 0.7 °C increase rates. This study did not address different sizes or stages of specimens, however, since only 5th instar larvae were used of this highly synchronous species. Several studies of fish and terrestrial insects have returned contradictory results as well, with some species exhibiting lower CTM, some unaffected by temperature increase rate, and others even exhibiting a higher CTM as temperature increase rates decreased (Becker and Genoway 1979, Elliot and Elliot 1995, Mora and Maya 2006, Ribeiro et al. 2012). These differing results between studies may be due to the different species used and subtle differences in experimental design.

The specific reason for higher thermal tolerance in smaller specimens is not clear. It is likely that the impending incomplete metamorphosis from nymph to subimago could cause a lower tolerance to any type of disturbance, including temperature (Comte and Olden 2017). Barnes et al. (2019) hypothesized that the higher mobility and subsequent exposure to greater thermal extremes of juvenile widow spiders (*Latrodectus*) (Araneae: Theridiidae) relative to the larger adults may lead to the observed higher CTM in the juveniles. A similar phenomenon may occur in smaller specimens of *S. femoratum*. It is also possible that size and thermal tolerance were both affected by the specific temperature profiles that specimens were exposed to during development (Bouchard and Ferrington 2009, Bonacina et al. 2023). Smaller lizard specimens have been demonstrated to more effectively obtain oxygen at higher temperatures than larger specimens, therefore maintaining metabolic stability and tolerating higher temperatures (Claunch et al. 2020). While we did not measure oxygen absorption or utilization in this study, the inherently low dissolved oxygen in warm water certainly suggests asphyxiation as a potential causative mechanism of CTM (Portner and Knust 2007, Houghton et al. 2014).

With a determined CTM of 33.5 °C, based on 16 °C acclimation and a 0.3 °C per minute increase rate, our large specimens would rate as 'moderately sensitive' relative to other aquatic insects, most of which were acclimated to 17 °C and raised at 0.33 °C per minute (Dallas and Rivers-Moore 2012). In

comparison, a population of *S. femoratum* from a small, thermally unstable stream 5 km west of our study site had CTM values ranging from 35.0–36.1 based on acclimation temperatures of 15 °C and 18 °C, and a 0.33 °C per minute rate of experimental increase (Houghton and Shoup 2014). The lower CTM of *S. femoratum* in our study may be due to the cold and stable nature of the Little Manistee River rarely exposing specimens to temperatures above 20 °C (Tonello 2008, Shoup and Houghton 2013).

Several sources of experimental error may have affected our results. Smaller specimens present a smaller profile to the current, which may allow them to withstand such current for a longer period, thus artificially raising their CTM relative to that of larger specimens (Vaughn 1985). The ~15% mortality across our 36–48 h post-trial observation period raises the possibility of some lethality in our CTM protocols (Fig. 3). The consistency of deaths across both size classes, however, suggests that even if deaths were partially caused by our experiment, they likely did not influence the overall conclusions. The two large specimens that molted to their subimago stage during this period made their post-trial mortality impossible to determine and lowered the sample size of large specimens. Lastly, mayfly nymphs are notoriously fragile, and several of our trial specimens dropped their limbs even with careful handling. While specimens with missing limbs appeared unharmed (Díaz-Ricaurte et al. 2022), and no specimens missing >1 leg from either side were included in experimental trials, it is possible that 1–2 total missing legs may have artificially lowered the CTM of particular specimens.

Further research is needed to clarify our conclusions. In particular, testing the CTM of additional size classes would determine if CTM differences follow a linear, polynomial, or other pattern. Very small specimens may have an even lower CTM than large ones. While we don't know the voltinism of *S. femoratum* at our site, the possibility of bivoltinism (Rowe and Berrill 1989) with corresponding winter and summer cohorts suggests the possibility of some thermal tolerance differences related to life cycle. The noted variation in CTM within populations of *S. femoratum* of close proximity (Houghton and Shoup 2014) suggests wide physiological variation in this widespread species; thus, other populations should be tested to see if our conclusions are consistent. Differences between male and female specimens may also affect CTM. Moreover, given the frequently confounding results presented by different studies, additional CTM trials of various organisms need to be conducted

with a greater laboratory standardization (Bonacina et al. 2023).

Our results demonstrate that determining a single CTM for a species based on multiple size classes would likely lead to erroneous results. Fortunately, testing large specimens is already typical for CTM studies due to the ease of collecting and experimenting on the larger specimens (Dallas and Rivers-Moore 2012). Ultimately, our data reinforce the validity of testing thermal tolerance at or near the terminal instar of a species.

Acknowledgments

Funding for this research was supported by a Hillsdale College LAUREATES grant to JKF, and by the Hillsdale College Biology Department. We thank Faith Linton, Shannon Spahr, Dan Votel, and the rest of the 2022 'Skookum Squad' for field assistance, and two anonymous reviewers for improving earlier version of the manuscript. This paper won the best student presentation award at the 68th Annual Meeting of the Michigan Entomological Society. It is paper #36 of the GH Gordon BioStation Research Series.

Literature Cited

- Barnes, C. L., N. W. Blay, and S. M. Wilder. 2019. Upper thermal tolerances of different life stages, sexes, and species of widow spiders (Araneae, Theridiidae). *Journal of Insect Physiology* 114: 10–14.
- Ball, S.L. 2002. Population variation and ecological correlates of tycho-parthenogenesis in the mayfly, *Stenonema femoratum*. *Biological Journal of the Linnean Society* 75: 101–123.
- Bawa, S. A., P. C. Gregg, A. P. Del Soccoro, C. Miller, and N. R. Andrew. 2021. Estimating the differences in critical thermal maximum and metabolic rate of *Helicoverpa punctigera* across life stages. *PeerJ*: 9:e12479. Available from <https://doi.org/10.7717/peerj.12479>.
- Becker, C. D., and R. G. Genoway. 1979. Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environmental Biology and Fisheries* 4: 245–256.
- Bednarik, A. F., and W. P. McCafferty. 1979. Biosystematic revision of the genus *Stenonema* (Ephemeroptera: Heptageniidae). *Canadian Bulletin of Fisheries and Aquatic Sciences* 201.
- Bonacina, L, F. Fasano, V. Mezzanotte, and R. Fornaroli. 2023. Effects of water temperature on freshwater macroinvertebrates: a systematic review. *Biological Reviews* 98: 194–221.
- Bouchard, RW, Ferrington, LC, Jr. 2009. Winter growth, development, and emergence of *Diamesa mendotae* (Diptera: Chironomidae) in Minnesota streams. *Environmental Entomology* 38: 250–259.
- Claunch, N. M., E. Nix, A. E. Royal, L. P. Burgos, M. Corn, P. M. DuBois, K. N. Ivey, E. C. King, K. A. Rucker, T. K. Shea, J. Stepanek, S. Vansdadia, and E. N. Taylor. 2020. Body size impacts critical thermal maximum measurements in lizards. *Journal of Experimental Zoology* 335: 96–107.
- Comte, L., and J. D. Olden. 2017. Evolutionary and environmental determinants of freshwater fish thermal tolerance and plasticity. *Global Change Biology* 23: 728–736.
- Dallas, H. F., and N. A. Rivers-Moore. 2012. Critical thermal maxima of aquatic macroinvertebrates: towards identifying bioindicators of thermal alteration. *Hydrobiologia* 679: 61–76.
- DeKozlowski, S. J., and D. L. Bunting, II 1981. A laboratory study on the thermal tolerance of four southeastern stream insect species. *Hydrobiologia* 79: 141–145.
- Díaz-Ricaurte, J. C., E. C. Guevara-Molina, J. M. Alves-Nunes, F. C. Serrano, and M. Hrnecir. 2022. Linking body condition and thermal physiology in limping crickets: Does limb autotomy incur costs concerning behavioral thermal tolerance? *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* 337: 393–402.
- Elliot, J. M., and J. A. Elliot. 1995. The effect of the rate of temperature increase on the critical thermal maximum for parr of Atlantic salmon and brown trout. *Journal of Fisheries Biology* 47: 917–919.
- Ernst, M. R., T. L. Beitingger, and K. W. Stewart. 1984. Critical thermal maxima of nymphs of three Plecoptera species from an Ozark foothill stream. *Freshwater Invertebrate Biology* 3: 80–85.
- Garten, C. T., and J. B. Gentry. 1976. Thermal tolerance of dragonfly nymphs. II. Comparison of nymphs from control and thermally altered environments. *Physiological Zoology* 49: 206–213.
- Heiman, D. R., and A. W. Knight. 1972. Upper lethal temperature relations of the nymphs of the stonefly, *Paragnetina media*. *Hydrobiologica* 39: 479–493.
- Houghton, D.C., A. C. Logan, and A. J. Pytel. 2014. Validation of CT_{max} protocols using cased and uncased *Pycnopsyche guttifera*. *The Great Lakes Entomologist* 47: 1–8.
- Houghton, D.C., and L. Shoup. 2014. Seasonal changes in the critical thermal maxima of

- four species of aquatic insects. *Environmental Entomology* 43: 1059–1066.
- Houghton, D.C., and J. L. Wasson. 2013.** Abrupt biological discontinuity in a small Michigan stream due to historical riparian canopy loss. *Journal of Freshwater Ecology* 28: 293–306.
- Johnson, K. S., E. Rankin, J. Bowman, J. Deeds, and N. Kruse. 2018.** Predicting mayfly recovery in acid mine-impaired streams using logistic regression models of in-stream habitat and water chemistry. *Environmental Monitoring and Assessment* 190: 1–16.
- Lutterschmidt, W. I., and V. H. Hutchison. 1997.** The critical thermal maximum: history and critique. *Canadian Journal of Zoology* 75: 1561–1574.
- Lyman, F. E. 1955.** Seasonal distribution and life cycles of Ephemeroptera. *Annals of the Entomological Society of America* 48: 380–391.
- Mora, C., and M. F. Maya. 2006.** Effect of the rate of temperature increase of the dynamic method on the heat tolerance of fishes. *Journal of Thermal Biology* 31: 337–341.
- O’Leary, B.C., D. C. Houghton, and J. Van Zant, 2014.** Differences in thermal tolerance between two thermally isolated and genetically indistinct populations of *Paragnetina media* (Plecoptera: Perlidae). *The Great Lakes Entomologist* 47: 101–113.
- Portner, H. O., and R. Knust. 2007.** Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* 315: 95–97.
- Renault, D., P. Vernon, and G. Vannier. 2005.** Critical thermal maximum and body water loss in first instar larvae of three Cetoniidae species. *Journal of Thermal Biology* 30: 611–617.
- Resh, V. H., and R. T. Cardé. 2009.** *Encyclopedia of Insects* (2nd ed.). Academic Press, Cambridge, MA.
- Ribeiro, P. L., A. Camancho, and C. A. Navas. 2012.** Considerations for assessing maximum critical temperatures in small ectothermic animals: insights from leaf-cutting ants. *PLoS ONE* 7(2): e32083.
- Rowe, L., and M. Berrill. 1989.** The life cycles of five closely related mayfly species (Ephemeroptera: Heptageniidae) coexisting in a small southern Ontario stream pool. *Aquatic Insects* 11: 73–80.
- Rowe, L., M. Berrill, and L. Hollett. 1988.** The influence of season and pH on mortality, molting, and whole-body ion concentrations in nymphs of the mayfly *Stenonema femoratum*. *Comparative Biochemistry and Physiology* 90: 405–408.
- Shoup, L., and D. C. Houghton. 2013.** The effect of acclimation temperature on the critical thermal maximum of a cold-water population of *Pteronarcys dorsata* (Say) (Plecoptera: Pteronarcyidae). *The Great Lakes Entomologist* 46: 165–173.
- Tonello, M. A. 2008.** Little Manistee River. Michigan Department of Natural Resources, Status of the Fishery Resource Report. Available from <https://www2.dnr.state.mi.us/publications/pdfs/ifr/ifrlibra/Status/Waterbody/2005-8.doc>. Accessed 15 February 2023.
- Vaughn, C.C. 1985.** Evolutionary ecology of case architecture in the snailcase caddisfly, *Heleopsyche borealis*. *Freshwater Invertebrate Biology* 4: 178–186.
- Waltz, R. W., and S. K. Burian. 2008.** Ephemeroptera, pp. 181–236. *In:* Merritt et al. [eds] *Introduction to the Aquatic Insects of North America*. Kendall Hunt, Dubuque, IA.