A LABORATORY STUDY ON THE THERMAL TOLERANCE OF FOUR SOUTHEASTERN STREAM INSECT SPECIES (TRICHOPTERA, EPHEMEROPTERA)

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Abstract

The acute thermal tolerances of four southeastern stream insect species, *Ephemerella invaria* (Walker), *Stenonema ithaca* (Clemens and Leonard), *Symphitopsyche morosa* (Hagen), and *Brachycentrus lateralis* (Say) were determined using an artificial stream enclosure. All species were acclimated at 10°C for 72 hours prior to instantaneous immersion into heated water for 96 hours. Percent mortality was recorded and the temperature at which 50% mortality occurred determined (LT50). Data were subjected to standard statistical analysis.

Thermal tolerance values were compared between species tested and to results from previous investigations using similar methodologies. The evolution and life histories of these species were also discussed in relation to their thermal tolerance values.

Introduction

The alteration of aquatic insect communities by the heated effluents of electric power stations has been well documented over the years (Trembley, 1960, 1961; Coutant, 1962; Roback, 1965; Langford, 1971; Gibbons *et al.*, 1975). The results from these field investigations have in turn prompted numerous laboratory investigations into the thermal tolerances of individual aquatic insect species (Nebeker & Lemke, 1968; Gaufin & Hern, 1971; Heiman & Knight, 1972; Greg, 1974; Garten & Gentry, 1976). Unfortunately, these studies have been few compared to the diversity and ecological significance of this class. Furthermore, the methods employed in these studies have varied greatly, only adding to the confusion of the already difficult task of thermal tolerance determinations. Results from studies conducted in different geographical regions, even on the same species of insects, cannot be accurately compared. Thus, standardization of methodologies is necessary to provide effective comparisons between studies.

The purpose of this study was to determine the tolerance of four aquatic insect species to acute thermal shock employing the methodology established by Nebeker & Lemke (1968). Results from this study may be directly compared to results from previous and future investigations using similar methods. In addition, results here may explain the thermal tolerance variation among species from similar habitats.

Methods and materials

Laboratory Apparatus

All tests were conducted in three artificial stream enclosures similar to those used by previous authors (Nebeker & Lemke, 1968; Gaufin & Hern, 1971). Each enclosure consisted of an oval plexiglass tank (stream) immersed in an insulated stainless steel water bath. The stream, constructed from 1/8 inch clear plexiglass, measured 43 cm long, 27 cm wide, and 10 cm deep. A 25 cm long partition down the center of the stream encouraged circular water flow. Each stainless steel water bath measured 53 cm long, 46 cm wide, and 20 cm deep. The sides and bottom were insulated with 2 cm thick styrofoam.

Dechlorinated tap water was of insufficient quality to be used in a flow through system. Therefore, a closed system was developed which filtered and recycled river water obtained from the insect collection site. Approximately 3,000 liters of water were filtered per day (2 liters/minute) through activated carbon and floss. Fresh water was used for each test run. Temperatures below ambient room temperature were controlled by opposing heating and cooling units. Water temperaures in all baths were maintained to within 0.5°C of the desired temperature.

Stream flow and circulation in the water bath was pro-

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vided by constant temperature circulators which maintained a flow of approximately 2.25 liters per minute (400 stream volumes/day).

Special test cages constructed of plexiglass and fiberglass screening were used to contain the test animals yet allowed sufficient water flow. Natural substrate (small stones) placed on the bottom of each cage provided attachment sites for the insects.

The insect species selected for this study were the mayfly species *Ephemerella invaria* (Walker) and *Stenonema ithaca* (Clemens & Leonard) and the caddisfly species *Symphitopsyche morosa* (Hagen) and *Brachycentrus lateralis* (Say).

All test organisms were collected from riffle areas of the Little River, Tennessee between January and November 1978. This stream has its head waters in the Great Smoky Mountains and drains into Fort Loudon Reservoir (Tennessee River).

River water analysis included water temperature, conductivity, pH, and dissolved oxygen determinations. These analyses immediately followed each collection of organisms. Periodic analysis of the water in the artificial streams was similarly conducted. Comparisons of these analyses revealed that during each test water quality of the artificial streams did not appear to vary substantially from the water in Little River.

Experimental Methods

All tests were conducted between January and November 1978 and immediately followed collection of test organisms. Approximately 20 individuals were placed in each of three cages and allowed to acclimate for 72 hours at 10°C. After acclimation two cages were removed and placed in heated test streams of predetermined temperatures. One cage remained in the acclimation stream to serve as a control. The cages remained in the heated water for 96 hours or until all the animals died, which ever came first. After 96 hours the cages were removed and percent mortality determined. Animals were considered dead if all bodily movements had ceased. Data were subjected to standard statistical analysis and the temperature at which 50% of the organisms died (LT50) was determined for each species.

Tabel 1. Summary of Mean Lethal Temperatures (LT50), Regression Coefficients, and Range of Mortality of test species in the artificial streams with associated standard errors.

Species	Regression Coefficient	LT50 (°C)	Mortality Range
Ephemerella invaria	21.428 ± 3.280	22.9 ± 0.936	17.73°
Symphitopsyche morosa	39.643 ± 8.477	30.4 ± 0.640	12.32°
Stenomena ithaca	60.143 ± 12.074	31.8 ± 0.407	8.44°
Brachycentrus lateralis	168.000 ± 12.510	32.8 ± 0.150	3.08°

Results

Variable thermal sensitivity was apparent among the species tested (Table 1). The most sensitive species was the mayfly *Ephemerella invaria* (LT50 22.9°C) which succumbed to water temperatures 10.1 degree cooler than the most tolerant species the caddisfly, *Brachycentrus lateralis* (LT50 32.8°C). The majority of thermal tolerance values however fell within a range of 3 degrees. In general caddisflies appeared to be more tolerant of heated waters than mayflies. However, too few species were tested to give conclusive results.

Although LT50 values were all within a relatively narrow range of temperatures, significant differences still existed between these values for all species tested except *Stenonema ithaca* and *Symphitopsyche morosa*. These two species also had extensive overlap in their temporal life histories.

Those species collected and tested during summer months showed substantially higher LT50 values than species tested during winter months. Positive linear relationships were found to exist between LT50 values and river water temperature and between regression coefficients and river water temperature (See Fig. 1). This seemed to indicate that these differences in LT50 values among species was a function of river water temperature variations during the seasons in which these species grew and developed.

Significant differences were also found between the ranges over which individuals of a particular species died. These differences were reflected in the regression coefficient of the mortality versus temperature regression lines for each species (Table 1). Statistically significant differences were found to exist between the slopes for all species except those for *Ephemerella invaria* and *Symphitopsyche morosa*. The parallel regression lines for these two species suggest a similar response to temperature changes although their LT50 values were significantly different.

Discussion

Results from this investigation generally correspond with those found by previous investigators. Species of the genus

Epemerella have been found to be among the most sensitive insect species to heated water (Nebeker & Lemke, 1968; Gaufin & Hern, 1971; EPA, 1973). Thermal tolerance values from previous studies on this genus have never exceeded 22.0°C but are in general accordance with the thermal tolerance value determined by this author (22.9°C).

Since *Symphitopsyche* is a newly established genus in North America comparisons of results for this species must be made with studies on the parent genus *Hydropsyche*. *Hydropsyche* larval taxonomy prior to Schuster & Etnier (1977) was severely limited and identification to the species level difficult. Therefore, all thermal tolerance research on this genus has been conducted on organisms identified only to the generic level and presumably included species of both *Hydropsyche* and *Symphitopsyche*.

Laboratory studies on *Hydropsyche* (broad sense) have yielded a mean thermal tolerance value of 30.3°C (Gaufin & Hern, 1971; Sage, 1974). This value closely approximates the thermal tolerance value calculated for *Symphitopsyche morosa* (30.4°C). Recent ecological observations have indicated that *Symphitopsyche* species are often located in the upper stretches of medium sized mountain streams while *Hydropsyche* species are found primarily in the lower, warmer stretches of these streams. From these observations it has been concluded that *Symphitopsyche* species in general, are less tolerant of thermal extremes than *Hydropsyche* species (Schuster, in manuscript). Results from this investigation do not clearly substantiate nor disprove this conclusion. Although the thermal tolerance value for *Symphitopsyche morosa* was substantially no different from that of previously studied *Hydropsyche* (broad sense) larvae, the lack of adequate species identification of the latter makes comparisons of the values difficult.

Hydropsychid caddisflies are considered tolerant of many forms of pollution including thermal pollution. However, results from this study indicated that *Symphitopsyche morosa* was no more tolerant of heated water than the majority of other aquatic insect species tested.

Few thermal tolerance tests have been conducted on species of *Stenonema*. One previous investigation on *Stenonema ithaca* (Gregg, 1974) yielded similar thermal tolerance values (31.7°C) to that determined by this investigation (31.8°C). It is interesting to note such a close equivalence of values existing between these geographically distant populations. These findings support the idea that thermal tolerance is primarily genetically determined and that the environment only slightly modifies it.

The only other thermal tolerance study conducted on this genus was on *Stenonema tripunctatum*. It yielded a thermal tolerance value of 25.5°C (Nebeker & Lemke, 1968). This species inhabits clear, cold mountain streams and thus is likely to have a lower tolerance to heated water than *Stenonema ithaca* which inhabits warmer streams.

No thermal tolerance studies on *Brachycentrus lateralis* have been conducted prior to this investigation. Therefore, comparisons of these results must be made with those from studies on closely related species. The LT50 value for *B. lateralis* (32.8°C) was only generally consistent with values calculated for other species in this genus. All investigations on this genus have been conducted on northern stream species. Therefore, the higher LT50 values for *B. lateralis* may be attributed to adaptation or acclimitization of this species to the warmer southeastern stream temperatures.

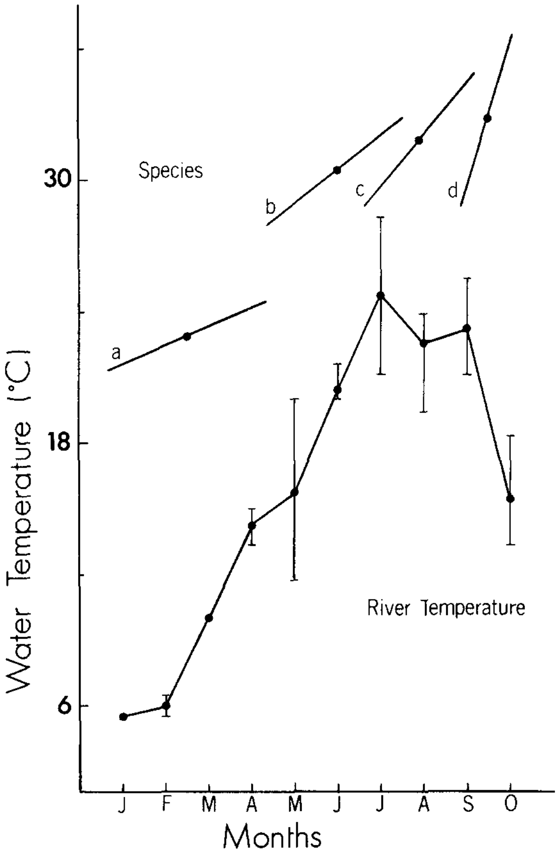

Fig. 1. Comparison of LT50 values and associated regression lines of a) *Ephemerella invaria*, b) *Symphitopsyche morosa*, c) *Stenonema ithaca*, and d) *Brachycentrus lateralis* to mean monthly temperatures of Little River at time of collection.

Successful acclimation assumes that the physiological processes were adjusted to a common baseline for all species tested. Any and all responses to a stimulus therefore reflect specific genetic differences and not differences due to previous thermal experiences of the individual organisms. Based on this assumption, the results indicated significant genetic differences in thermal tolerance for the species studied, even though they shared the same habitat. This is exemplified by the statistically different LT50 values and slopes among the species.

It has been the assumption in the past that organisms from the same or similar habitats would exhibit similar thermal tolerances. These results support this belief, provided life histories of the organisms are also taken into consideration. That is, those species with life histories which correspond to similar temperature regimes will have similar thermal tolerances. Thermal tolerance similarity of *B. lateralis, Symphitopsyche morosa,* and *Stenonema ithaca* and the dramatically different tolerance of *Ephemerella invaria* supports these conclusions. These thermal tolerance differences may be attributed to genetic adaptation to variable temperature conditions during the evolution of each species. The separate life cycles of each species exposes them to variable thermal regimes (Fig. 2). Those species exposed to only cold temperatures during their growth and development evolved low tolerances to heated waters. *Ephemerella invaria* best demonstrated this point. This species hatched during the late fall when water temperatures were cool, grew and developed during the winter months and emerged in early spring before water temperatures rose above 15°C. This species was likewise the most sensitive to heated water. Those species on the other hand which were subjected to elevated water temperatures

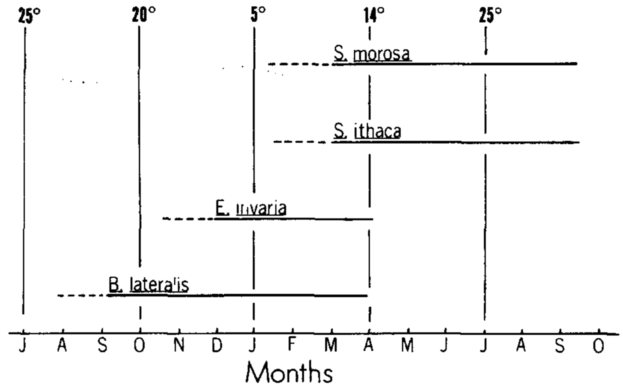

Fig. 2. Comparison of mean monthly water temperature (°C) and presence of aquatic stages of test species in Little River.

during their life histories evolved a higher tolerance to heated water. The genetic determination of thermal tolerance appears to be very specific. *Stenonema ithaca* and *Symphitopsyche morosa* were exposed to virtually identical thermal extremes (Fig. 2) and likewise there were no significant differences between their LT50 values (Table 1).

The thermal tolerance calculated for each species is only the mortality mean associated with another very important parameter, the range of temperatures over which individuals of a given species died. Differences in the regression coefficients for each species reflect the extent of this mortality range. Species with small coefficients have a wide mortality range and species with large coefficients have a narrow mortality range (Table 1). The extent of this range appeared to be dependent upon the differences between the maximum river temperature to which a species was exposed during its life history and the critical thermal maximum of most aquatic life (35°C). This is also best explained by considering the evolution of a species to the corresponding temperature regime occurring during its life cycle. This evolution is dependent upon the survival of individuals whose genetically determined thermal tolerances are compatible with the thermal extremes of their environment. Thus, only those individuals able to tolerate the maximum water temperature of their environment will survive to reproduce Insect species exposed to cool water temperatures (maximum 14°C) should have individuals within that population tolerant of temperatures over a wide range (14-15°C). However, species which have adapted to warm water conditions (maximum 25°C) should have individuals within that population tolerant of temperatures over a much narrower range of temperatures (25°C-35°C), as all individuals not tolerant of at least the maximum water temperature (25°C) have been eliminated.

Results from this study support this hypothesis. The difference between the maximum river water temperature to which *E. invaria* was exposed and 35°C was much greater (26°C) than the difference in those parameters for *B. lateralis* (10°C). Correspondingly, the range over which individuals died was much greater for *E. invaria* (17.73 degrees) than it was for *B. lateralis* (3.09 degrees). This similarity also holds true for the other two species tested. The non-significant difference in the regression coefficients of *E. invaria* and *S. morosa* is probably due to the small degrees of freedom associated with these experiments. Statistical tests conducted on the regression coefficients at a 10% significance level resulted in significant differences between these species.

If this hypothesis is true, one would also expect to find a

Table 2. Comparison of Maximum River Temperature during collection and Minimum Lethal Temperature for each species tested.

Species	Maximum River Temp.	Minimum Lethal Temp.
Ephemerella invaria	15.0	16.44
Symphitopsyche morosa	26.1	25.40
Stenonema ithaca	28.3	28.32
Brachycentrus lateralis	25.5	31.47

similarity in the minimum temperature at which individuals of a species died and maximum water temperatures to which they were exposed during their life histories. In fact, a close relationship between these values does exist for each species (Table 2).

Much has been studied and discussed on the thermal tolerance of aquatic organisms often with little agreement and much confusion. It is the hope of the authors that through a thorough systematic approach using standardized methodologies more useful tolerance information will emerge.

Summary

1. The thermal tolerances of the four species studied were significantly different, except between *Symphitopsyche morosa* and *Stenonema ithaca.* This non-significance was attributed to their sympatric life histories.

2. The regression coefficients of the mortality vs temperature regression lines for each species were significantly different, except between *Ephemerella invaria* and *Symphitopsyche morosa.*

3. Coefficients for each species were shown to be related to the temperature range over which individuals of a given species died. This range was considered dependant upon the difference between maximum river water temperature to which a species is exposed during its life history and the critical thermal maximum of most aquatic life (35°C).

4. The non-significance of coefficient values for *E. invaria* and *S. ithaca* was attributed to the small degrees of freedom associated with these experiments. A larger sample size would probably result in significant differences between the coefficients of these species.

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