To the Graduate Council:

I am submitting herewith a thesis written by Steven J. deKozlowski entitled "A Laboratory Study on the Thermal Tolerance of Four Southeastern Stream Insect Species (Trichoptera, Ephemeroptera)." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology.

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We have read this thesis and recommend its acceptance:

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A

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

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Steven J. deKozlowski
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ABSTRACT

The acute, thermal tolerance was determined for four southeastern stream insect species, *Ephemerella invaria* (Walker), *Stenenoma ithaca* (Clemens and Leonard), *Symphitopsyche morosa* (Hagan), and *Brachycentrus lateralis* (Say), using an artificial stream enclosure. All test species were collected from the Little River and acclimated at 10°C for 72 hours. Animals were then instantaneously immersed into heated water for 96 hours, after which percent mortality was recorded and the temperature at which 50% mortality occurred determined (LT50). Data was subjected to standard statistical analysis.

LT50 values and regression coefficients were found to be significantly different for most of the species tested. Regression coefficients were shown to be related to the temperature range over which individuals of a given species died. This range was considered dependent upon the difference between maximum river water temperature to which a species was exposed during it's life history and the critical thermal maximum of most aquatic life (35°C). Nonsignificance of LT50 values and regression coefficients were attributed to the small degrees of freedom associated with these experiments and exposure to similar thermal regimes.

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I. INTRODUCTION

General

The greatest nonconsumptive use of water in the United States is for cooling purposes. The greatest demand for cooling water is by the electric generating industries (Milhursky and Kennedy, 1967). These industries have a low thermal efficiency and a large part of the heat produced is released through cooling systems into aquatic and atmospheric environments (Novotny and Krenkel, 1973). By the year 2000 production of electric energy will be 9 times that of 1970, requiring an even greater amount of cooling water for the dissipation of heat. Electric generating industries alone are responsible for increasing the cooling water temperatures by an average of 8-9°C (EPA, 1974). These increased temperatures in the receiving water may have deleterious effects on the biota. Trembley (1960, 1961) and Coutant (1962) noted sharp decreases in species diversity and numbers in the heated zone of the Delaware River during the summer months.

Alteration of aquatic ecosystems may have long-lasting effects upon the biota. Therefore, determination of the organisms' environmental requirements must be made in order to set realistic water quality standards. A primary step in the determination of tolerance to heated water is by acute bioassay. The results of such a test serve as extreme upper limits for heated effluents as the property measured is extreme—death. Results of thermal tolerance studies are not comparable unless similar test procedures are conducted. Unfortunately standardized methodology for testing stream organisms has only recently been devised and accepted. Nebeker and Lemke (1968) devised these standard procedures and employed them to determine thermal tolerances of several northern insect species. Gaufin and Hern (1971) used them to test several more insect species from Montana. Since these studies, no further work has been conducted using this methodology.

The purpose of this study was to determine thermal tolerances to acute temperature shock of four aquatic insect species. This was the first time a standardized metholology has been used to test southeastern stream species. The results will permit comparisons with similarly studied species and will hopefully aid in the arduous task of determining water quality standards.

Experimental Organisms

Aquatic insects were selected as the test organisms for three reasons: (1) their predominance in the stream community, (2) their importance in the aquatic ecosystem, and (3) their value as indicators of water quality.

Aquatic insects although not the most numerous are the predominant invertebrates in the stream community. Out of the 30 to 35 recognized orders of insects, 11 contain species that are aquatic or semi-aquatic. These species have invaded nearly all types of freshwater habitats (Pennack, 1978). Aquatic insects are an important component of freshwater ecosystems. They serve as a vital link in aquatic food-chains situated between primary producers and the higher trophic level carnivores. Numerous investigators have found them to be the major diet of stream fishes (Jensen, 1969). Due to their varied diets (herbivores, carnivores, omnivores, and detritivores) and feeding strategies (grazers, filter feeders, predators) they are active in the transfer of energy and nutrient cycling in aquatic ecosystems. Successful survival of aquatic insects is therefore essential for the preservation of healthy aquatic environments. In fact the presence or absence of certain aquatic insects has been used as a general indicator of environmental quality (Hynes, 1965; Goodnight, 1973).

In the past, identification of indicator organisms has generally been only to the generic level. Resh (1975) warned that the use of generic identifications are of dubious value for water quality assessment. He stated that generic identification tells no more about ecological indicators or water quality than identification to the family level. Therefore, identification must be made to the species level if results are to have any meaning.

During this study careful attention was made to identify all test organisms to species. Insect species were selected for their importance in the stream community, ease of acquiring large numbers, and adaptation to laboratory conditions. Tests were conducted on two caddisfly (Trichoptera) species *Symphitopsyche morosa* (Hagen) and *Brachycentrus lateralis* (Say), and two mayfly species *Ephemerella invaria* (Walker) and *Stenonema ithaca* (Clemens and Leonard). Wiggins (1976) emphasized the significance of Trichoptera as indicator organisms and their importance in the aquatic ecosystem as transferers of energy and essential links in the food chain. Second only to the diptera, Trichoptera are the most abundant insect order in lotic environments. Caddisflies have been found to make up roughly 10% of stream faunas (Roback, 1965).

Symphitopsyche is an African genus of some antiquity with which the North American *bifida* group, formerly of *Hydropsyche*, has been synonymized (Ross and Unzicker, 1977). It includes about 14 known species in eastern and central North America (Schuster and Etnier, 1978), nine of which are found in Tennessee (Etnier and Schuster, 1979). Hydropsychid larvae are frequently encountered and often the most abundant caddisfly found in streams (Schuster and Etnier, 1978).

Roback (1965) noted after investigating nine rivers that 80% of the caddisfly fauna were hydropsychids.

Symphitopsyche morosa (Hagen) is widespread from northeastern U.S. and southern Canada south to the southeastern states roughly following the Appalachian Mountains. This species prefers large riffle areas of medium sized streams. The larvae are found in retreats under medium sized stones. Although precise life history studies have not been done, it is thought to have a univoltine life cycle and emerges from April through September (Schuster and Etnier, 1978). Identification in the field was made easily by the insect's characteristic light checkerboard pattern on the frontocypeus.

Hydropsychids have been used extensively as laboratory test organisms (Marlier, 1949). However, S. morosa has had little use in the laboratory (Fedeen, 1972).

Brachycentrus lateralis (Say) reaches a length of approximately 12 mm. Larvae are easily identified in the field by case structure and head color pattern. The case is a four sided, "log cabin" type, constructed of narrow pieces of plant material. The head is yellow with three black lines on the dorsum.

There is little information available on the ecology of this species. It appears to prefer rapids of medium to large streams and is found attached to permanent rocks and plants.

Numerous individuals were found attached to Podostemum ceratophylum. It has a univoltine life cycle as do other members of the genus. In the Little River (Tennessee) larvae hatch during August, grow and develop through the winter, and adults emerge in April. Distribution is restricted to Michigan, Illinois, Kentucky and Tennessee (Ross, 1944). It has also been found in the St. Lawrence River near Montreal, Canada (Corbet et al., 1966). Studies on members of the genus Brachycentrus have been conducted by Gallepp (1977), however, B. lateralis has rarely been used in laboratory studies.

Ephemeroptera usually comprise the third most numerous order in streams. Britt (1975) stressed the importance of mayflies in the stream community. He noted that they are important to scientific study for four reasons: (1) they become abundant under favorable conditions, (2) they are a major source of food for fish and other aquatic and terrestrial animals, (3) they are adapted to a large range of habitats, from streams to ponds, and (4) species are habitat specific, thereby serving as useful indicators of environmental quality. Leonard (1965) briefly reviewed some environmental requirements of mayflies, concluding that there was an inadequate amount of knowledge in this area.

Ephemerella invaria (Walker) and Stenonema ithaca (Clemens and Leonard) were selected to represent the mayfly fauna due to their abundance in Little River. These species also represent the two largest genera of the Order Ephemeroptera.

Ephemerella invaria nymphs range in length from 7.0-9.5 mm. Body color is highly variable. Pigmentation ranges from light brown with indistinct markings to extremely dark brown with distinct light markings. The species was not readily identifiable in the field beyond genus. However, identification was possible in the laboratory by the following characteristics: gills on abdominal segments 3-7, caudal filaments with heavy intersegmental setae, minute paired submedian tubercles on abdominal tergal segments 3-8, and tarsal claws possessing 5-7 denticles (Allen and Edmunds, 1965).

Ephemerella invaria is found in the northern U.S. from Wisconsin to Maine and its range extends south including all of Tennessee and northern portions of Alabama, Georgia, Mississippi, and Arkansas (Allen and Edmunds, 1965). This species inhabits the rapids of small to medium sized streams and attaches to rocks and vegetation. It was usually found attached to vegetation.

Although it has an extensive range, use of this species in the laboratory is nonexistent. This may be due to its very short univoltine life cycle. Nymphs are present only during the winter and emerge by April. Winter conditions thus hamper collection of the insect.

Stenonema ithaca is a member of the pullchellum group characterized by truncated gills on segments 1-6 and a finger like 7th gill. Mature nymphs range in length from 10-11 mm. Identification in the field was based on body shape and a distinct "V" shaped marking on sternites 4-8. By close examination of mouthparts this species can be identified by the 4-6 pectinate spines and lack of setae on the crown of the maxilla. The mandibles each have 6-7 teeth on the inner margin of the outer canine. The claws are not pectinate and there are posterolateral spines on abdominal segments 7-9.

Lewis (1974) stated that S. ithaca prefers riffle areas of medium to large streams. According to Lewis (1974) distribution of this species is restricted to the Susquehanna watershed of Central New York. Since then specimens have been identified from Tennessee, North Carolina, South Carolina, Georgia, and Alabama (Berner, 1977).

Organic pollution tolerance studies on Stenonema are numerous (Gaufin, 1958; Gaufin and Tarzwell, 1952; Beck, 1954 and 1969; and Leonard, 1965). However, tolerance of Stenonema nymphs to industrial wastes, pesticides, and heated water are largely unknown. The genus is absent from heavily industrialized stretches of streams and areas affected by acid mine drainage (Leonard, 1965; Parsons, 1968). Lewis (1974) catagorized Stenonema ithaca as facultative 1, able to withstand some organic enrichment if the dissolved oxygen concentration remains above 5.0 mg/l and pH and water temperature are unaffected.

II. LITERATURE REVIEW

Literature concerning the effects of heated effluents on aquatic life is fairly extensive. Comprehensive bibliographies have been compiled of thermal tolerance studies primarily on fishes with some references to invertebrate studies (Kennedy and Milursky, 1967; Raney and Menzel, 1967; and Tarzwell, 1968). Invertebrate studies have been extensively reviewed with specific references made to aquatic insects (Coutant, 1968, 1969, 1970, 1971; Coutant and Goodyear, 1972; Coutant and Pfuderer, 1973, 1974; Coutant and Talmage, 1975, 1976, 1977, 1978). In depth reviews on the effects of heated effluents on the physiology, biochemistry, reproduction, development, and population dynamics of aquatic insects are also available (Jenson, 1968).

Numerous field studies concerning temperature requirements of aquatic insects have been conducted over the years the results of which indicated a decrease in species diversity and numbers in areas receiving heated water (Coutant, 1962; Roback, 1965; Langford, 1971; Mecom, 1972; Gibbons et al., 1975). Community studies such as these have encouraged researchers to take a closer look at tolerances of individual species. A popular approach to individual species tolerance is short-term bioassays in which insects are subjected to heated water by instantaneous immersion. Acute, thermal tolerance studies of this type on aquatic insects prior to 1968 are few and comparisons of results difficult due to variations in experimental design.

Two major variations in experimental design have been the use of static (Stickney, 1922; Whitney, 1939; and Marlier, 1949) versus flowing water systems (Nebeker and Lemke, 1968; Gaufin and Hern, 1971; Heiman and Knight, 1972; and Gregg, 1974). Results from experiments implementing static and flowing water systems have yielded higher thermal tolerance values for the latter (Heiman and Knight, 1972). It was determined from these studies that flowing water systems yielded more realistic thermal tolerance values for stream organisms as they removed the respiratory stress factor.

The use of mayflies and caddisflies as test organisms is not extensive. This may be due to their obscure existence which has had little direct impact upon man. Whitney (1939) tested six mayfly species from various habitats. Animals were acclimated at 10°C for 24 hours and then transferred to vessels at various test temperatures for 24 hours. Whitney found that (1) nymphs from slow or still waters had greater resistance to high temperatures than those from swift water habitats, (2) for each species a greater percentage died at the higher temperatures, and (3) interspecific thermal tolerance differences could not be attributed to size differences or oxygen uptake efficiencies.

Marlier (1949) tested two European species of caddisfly, Hydropsyche anguistipenis and Hydropsyche instabilis. He found mean thermal tolerances after 24 hours of exposure (TLm24) of approximately 31°C for H. anguistipenis and 22°C for H. instabilis. Marlier attributed this interspecific variation to habitat differences. Stream temperatures rarely exceeded 27°C and 18°C respectively for the two species. He also noted that lethal temperatures varied seasonally.

Nebeker and Lemke (1968) tested twelve aquatic insect species including two mayflies and one caddisfly. Test organisms were acclimated for one week at 10°C before being subjected to elevated temperatures. Insects were subjected to elevated temperatures for 96 hours. Thermal tolerances were calculated as the temperature at which 50% survived (TLm96). Results indicated that the mayflies were less tolerant of heated water than the caddisfly. The mayflies studied were Ephemerella subvaria and Stenonema tripunctatum, which had mean thermal tolerance values of 21.5°C and 25.5°C respectively. They also concluded that the most sensitive species were those typically not exposed to great variations in temperature or warm water during their life histories.

Gaufin and Hern (1971) determined thermal tolerances of 6 species of caddisflies, using similar methods as Nebeker and Lemke (1968). The most sensitive species tested was the mayfly Cigmulia par (TLm96 = 11.7°C) and one of the most tolerant species was the caddisfly *Hydropsyche* sp. (30.05°C). They also found that in general caddisflies were more tolerant of heated water than mayfly species. The authors attributed species differences to varying ecological distributions and habitat requirements.

Gregg (1974) utilized instantaneous immersion tests to determine the mean lethal time (LT50) per test temperature for two mayfly species, *Stenonema ithaca* and *Isonychia* sp. He acclimated the insects at 8°C, 14°C, and 23°C then subjected them to test temperatures. He noted that there was comparable sensitivity to heat shock for these species after acclimation to 8°C. A mean thermal tolerance for 96 hours of 31.7°C can be calculated from his time to death regression equation. Sage (1974) conducted thermal tolerance studies of two populations of the caddisfly *Hydropsyche sp*. above and below a heated water outflow. She found that the population below the heated effluent had a slightly greater tolerance (30.6°C) than that above (30.4°C).

A critical component of all thermal tolerance bioassays is the duration and temperature of acclimation. Greater resistance to elevated temperatures at higher acclimation temperatures have been noted by several authors (Gregg, 1974; Heiman and Knight, 1972). Comparison of results from different authors is difficult as acclimation temperature time varies. Acclimation temperatures are generally 10°C

(Whitney, 1939; Nebeker and Lemke, 1968; Gaufin and Hern, 1971; and Heiman and Knight, 1972) or 20°C (Stickney, 1922; and Heiman and Knight, 1972) but temperatures as low as 8°C (Gregg, 1974) and as high as 30°C (Heiman and Knight, 1972) have also been used. Acclimation time has also been highly variable. Acclimation times of only 24 hours (Whitney, 1939) are not thought to be sufficient for complete readjustment of all the insect's physiological and enzymatic systems. Acceptable acclimation times range from three days to two weeks and are species dependent. Some species require longer acclimation times than others. There is disagreement on reacclimation to lower temperatures. Heiman and Knight (1972) concluded that acclimation to higher temperatures was rapid and increased insect resistance to higher test temperatures. However, acclimation to lower temperatures was extremely slow, thus reacclimation to 10°C in the summer was considered ecologically meaningless. Gregg (1974) contradicts these conclusions as he noted an increased sensitivity to higher test temperatures after reacclimation to lower temperatures for only seven days.

III. METHODS AND MATERIALS

Laboratory Apparatus

All tests were conducted in three artificial stream enclosures similar to those used by previous authors (Nebeker and Lemke, 1968; Gaufin and Hern, 1971). Figure 1 shows a diagram of the enclosure. Each enclosure consisted of an oval plexiglass tank (stream) immersed in an insulated stainless steel water bath. The stream measured 43 cm long, 27 cm wide, and 10 cm deep. It was constructed of 1/8 inch clear plexiglass. A 25 cm long partition down the center of the stream encouraged circular water flow. Four 6 mm holes were located 15 mm from the top to provide constant water depth within the stream. The stream was held off the bottom of the bath by inverted babyfood jars. This provided uniform temperature distribution around the stream. 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 brought back from the collection site. Eureka Power Filters were used to filter approximately 3,000 liters per day (2 liters/minute) through activated carbon and floss.

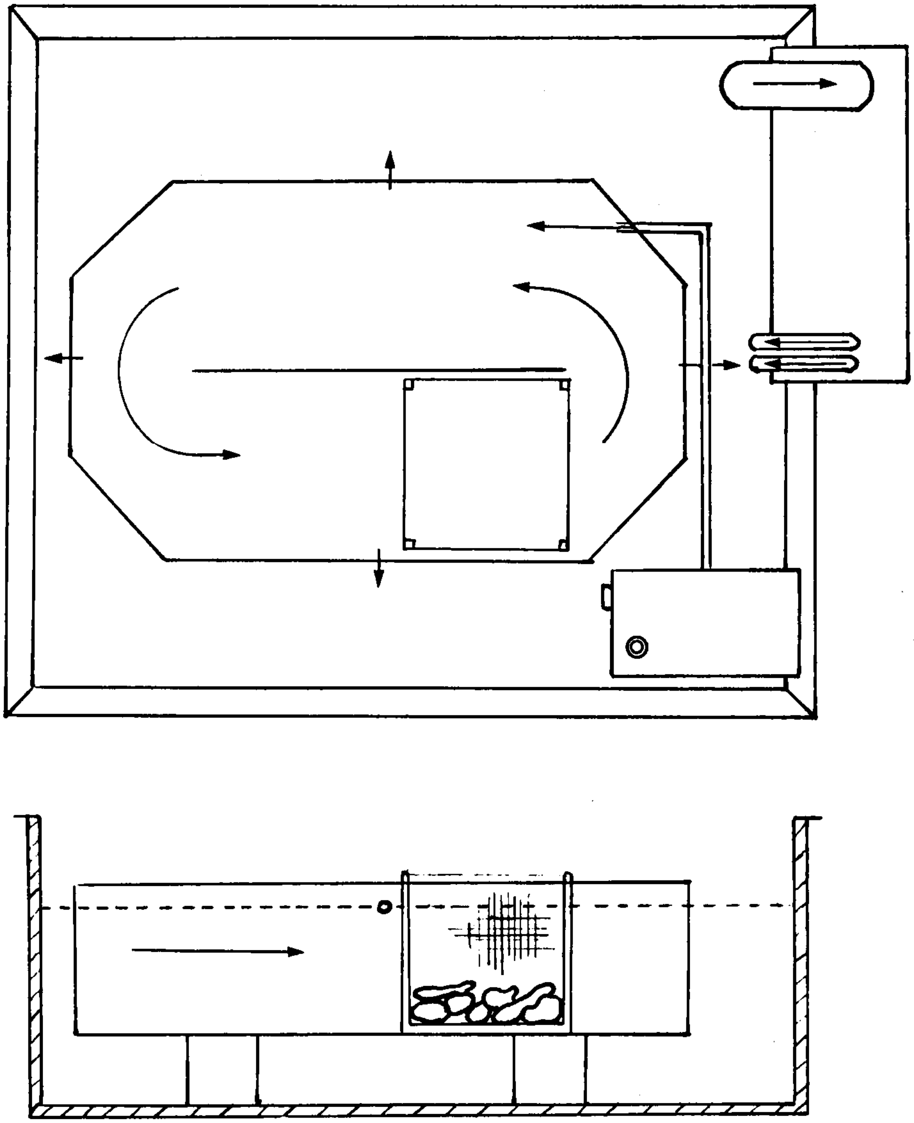

Figure 1. Diagram of artificial stream enclosure.

Freshwater was used for each test run. Water temperature and stream flow were controlled by Haake E52 Constant Temperature Circulators or Techne Tempunits. Water temperatures above ambient room temperature were controlled by Techne Tempunits to within 0.5°C of the desired temperature. Temperatures below ambient room temperature were controlled by opposing heating and cooling units.

The cooling system consisted of a Haake C11 Frigid Midget cooling unit, a Masterflex pump, and a stainless steel cooling coil which lined the bottom of the water bath. Tygon tubing connected these three parts. A schematic diagram of the cooling system is illustrated in Figure 2. Refrigerated antifreeze was pumped through the tubing from the cooling unit to the cooling coil in the water bath then back to the cooling unit for refrigeration. This system was able to maintain water temperatures at 10°C for prolonged periods of time without any apparent stress to the equipment. Haake E52 Constant Temperature Circulators were used to raise water temperatures to 0.5°C of the desired temperature.

The constant temperature circulators also provided stream flow and circulation in the water bath. A "Y" joint split water flow from the circulators so approximately half entered the stream to provide current and half circulated the bath water. An adjustable hose clamp was used to regulate stream flow. Stream flow was maintained at approximately 2.25 liters per minute (400 stream volumes/day).

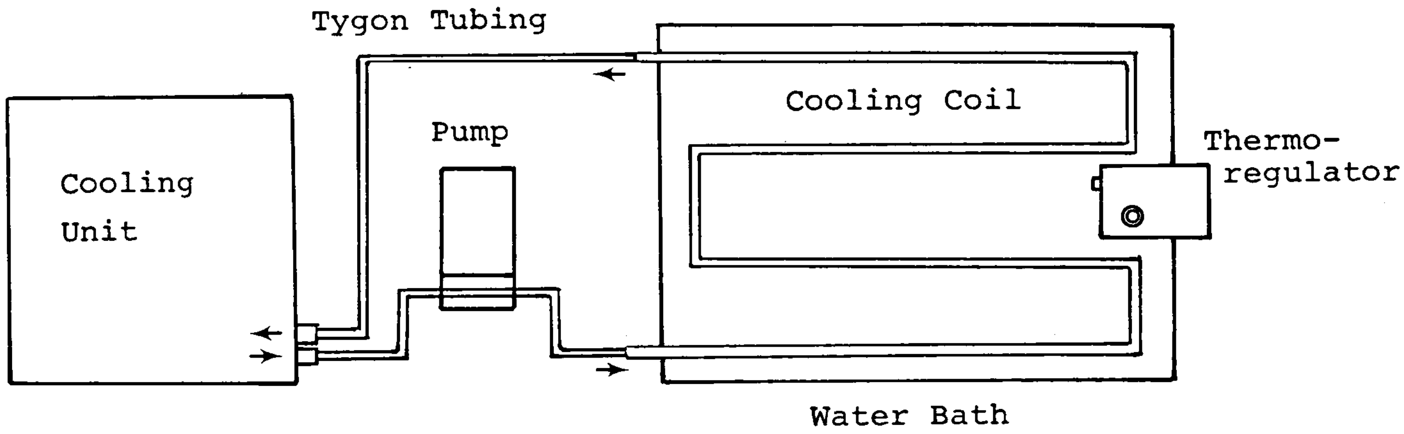

Figure 2. Schematic diagram of water bath temperature control system.

Special test cages were designed to contain the animals and yet allow sufficient water flow. The cages were constructed of 1/8 inch plexiglass bottom and corner supports with fiberglass screening on the sides. Cage dimensions were 12 cm x 12 cm x 10.5 cm and designed to fit precisely into the stream. Figure 1 shows the test cage in relation to the stream. Natural substrate composed of small stones was placed on the bottom of each cage to provide attachment sites for the insects. The cages could be easily removed from one stream and placed in another while still containing the animals. A short exposure time to air during the transfer was the primary disadvantage, however, it was assumed that the results were not significantly affected.

Organism Collection

All test organisms were collected from the Little River, which has its head waters in the Great Smoky Mountains. It flows west for approximately 50 miles (80.5 km) through Blount County, Tennessee, where it drains into Fort Loudon Reservoir (Tennessee River). River flow ranged from 57 CFS to 8910 CFS during the testing period (kindly provided by USGS).

All insects were collected from two sites along the river, river mile 21.56 and river mile 23.75. The river at both sites was approximately 15 meters wide and ranged in depth from 15 cm to 60 cm. Rapids and riffles extended the width of the river. Substrate composition consisted of small to large sized rocks with areas of vegetation covered bedrock. All the animals were collected in the riffle areas from small to medium sized rocks and approximate water depth of 25 cm.

Ephemerella invaria was collected by disturbing the substrate upstream of a hand-held screen. Dislodged insects were caught on the screen then removed by forceps and placed in collecting jars. All other insect species were collected by carefully removing individuals from rocks with forceps and placing them in collecting jars. Although 60 individuals were required for each test about 70 individual insects were collected to allow for death during transportation and handling. Approximately 10 individuals were placed in each collecting jar. The bottoms of the collecting jars were lined with small stones to provide attachment sites for the insects. The jars were stored in a styrofoam cooler filled with river water to maintain a constant temperature. Zhis water was periodically changed during the collections to prevent excessive heating and dissolved oxygen depletion.

Preliminary insect identification was made in the field using prominent morphological characteristics. Final species identification was made in the laboratory using a binocular microscope and several taxonomic keys (Lewis, 1974; Schuster and Etnier, 1978; Allen and Edmunds, 1965; Ross, 1944; and Edmonds et al., 1976).

Water Analysis

River water analysis included water temperature, conductivity, pH, and dissolved oxygen determinations. These analyses immediately followed the collection of organisms. Dissolved oxygen was determined using the Haake method. Conductivity was determined using a YSI 33 conductivity meter and a YSI 311 probe with a cell constant of 5.00 +/- 0.1. Water temperature was determined using a hand-held thermometer placed in the stream for at least 30 seconds prior to reading. Water samples were brought back to the laboratory to determine pH. These measurements were made using an Accumet 220 pH meter and a combination electrode. Water in the artificial stream was analyzed periodically for the same parameters as the river water. This insured a relatively stable chemical environment and provided quantitative comparisons with natural river water quality fluctuations. Results of water analysis are listed in Table 1. From these results one can see that water quality of the artificial streams did not vary substantially from the water in Little River.

TABLE 1. WATER ANALYSIS FOR LABORATORY (ARTIFICIAL STREAM) AND LITTLE RIVER WITH MEAN AND RANGE VALUES.

Species	Source	Temperature (°C)	O_2 (mg/liter)[a]	pH	Conductivity (siemens)
Ephemerella invaria	Laboratory	22.6 (10.0-35.0)	10.18 (7.93-11.86)	7.04 (6.30-7.60)	62.0 (44-86)
	River	9.9 (5.5-15.0)	11.61 (10.82-12.85)	7.00 (6.65-7.60)	49.7 (37-62)
Symphitopsyche morosa	Laboratory	26.1 (10.0-34.0)	9.21 (7.04-11.45)	7.19 (6.49-7.55)	68.5 (49-94)
	River	19.4 (13.3-26.1)	9.70 (8.14-11.17)	7.48 (6.65-8.30)	58.4 (36-76)
Stenonema ithaca	Laboratory	31.0 (10.0-38.0)	8.84 (6.49-11.33)	7.48 (7.20-8.09)	73.8 (50-93)
	River	23.4 (19.4-28.3)	8.70 (7.57-9.43)	7.41 (6.60-8.65)	64.4 (48-85)
Brachycenteus lateralis	Laboratory	29.2 (10.0-35.0)	8.86 (6.80-11.45)	7.48 (7.10-7.91)	88.2 (77-101)
	River	20.8 (13.3-25.5)	9.51 (7.96-11.43)	7.81 (7.50-8.26)	73.5 (56-89)

a = all values above 87% saturation.

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. At the end of the acclimation period 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. Corrections for mortality in the controls were made using Abbott's formula (Finney, 1962). Percent mortality for each species was plotted against temperature using a probit-log transformation to linearize the data (Sokal and Rohlf, 1969). Transformed mortality data was then analyzed by single classification analysis of variance to determine whether mortality was a function of temperature. Regression lines were fitted to these data and the lethal temperature at which 50% of the organisms died after 96 hours (LT50) was calculated for each species. Differences in LT50 values were determined by employing a standard T-test (Stanley, 1963). Regression data was analyzed by analysis of covariance to test for a common line.

Parallelism of regression lines was determined by subjecting regression coefficients to the Simultaneous Test Procedure (Sokal and Rohlf, 1969). All statistical analyses were tested at the 5% significance level.

IV. RESULTS

Variable thermal sensitivity was apparent between the species tested. Mean lethal temperatures (LT50) after 96 hours of exposure to heated water are listed in Table 2 for each species. The most sensitive species was the mayfly *Ephemerella invaria* (LT50 22.9°C) which succumbed to water temperatures 10.1 degrees cooler than the most tolerant species, the caddisfly, *Brachycentrus lateralis* (LT50 32.8°C). *Stenonema ithaca*, *Symphitopsyche morosa*, and *B. lateralis* had LT50 values 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.

The t-test employed to determine significant differences between LT50 values indicated significant differences between LT50 values for all species except *S. morosa* and *S. ithaca*. These values are listed in Table 3.

Those species collected and tested during the summer months showed substantially higher LT50 values than the species tested during the winter months. It is presumed that these differences were the result of river water temperature variations during these seasons. Figure 3 graphically illustrates river thermal variations throughout the year and LT50 relationships. As one can see, a positive

TABLE 2. SUMMARY OF MEAN LETHAL TEMPERATURES (LT50) AND REGRESSION COEFFICIENTS WITH ASSOCIATED STANDARD ERRORS

Species	Regression Coefficient	LT50 (°C)
Ephemerella invaria	21.428 ± 3.282	22.9 ±0.936
Symphitopsyche morosa	39.643 ± 8.477	30.4 ±0.640
Stenonema ithaca	60.143 ±12.074	31.8 ±0.407
Brachycentrus lateralis	168.000 ±12.510	32.8 ±0.150

TABLE 3. RESULTS OF T-TEST FOR SIGNIFICANCE BETWEEN LT50 VALUES

Species	t-ratio
E. invaria and *S. morosa*	6.160*
E. invaria and *S. ithaca*	7.689*
E. invaria and *B. lateralis*	8.735*
S. morosa and *S. ithaca*	1.870[NS]
S. morosa and *B. lateralis*	3.528*
S. ithaca and *B. lateralis*	2.207*

* = Significance at .05.

NS = Not significant.

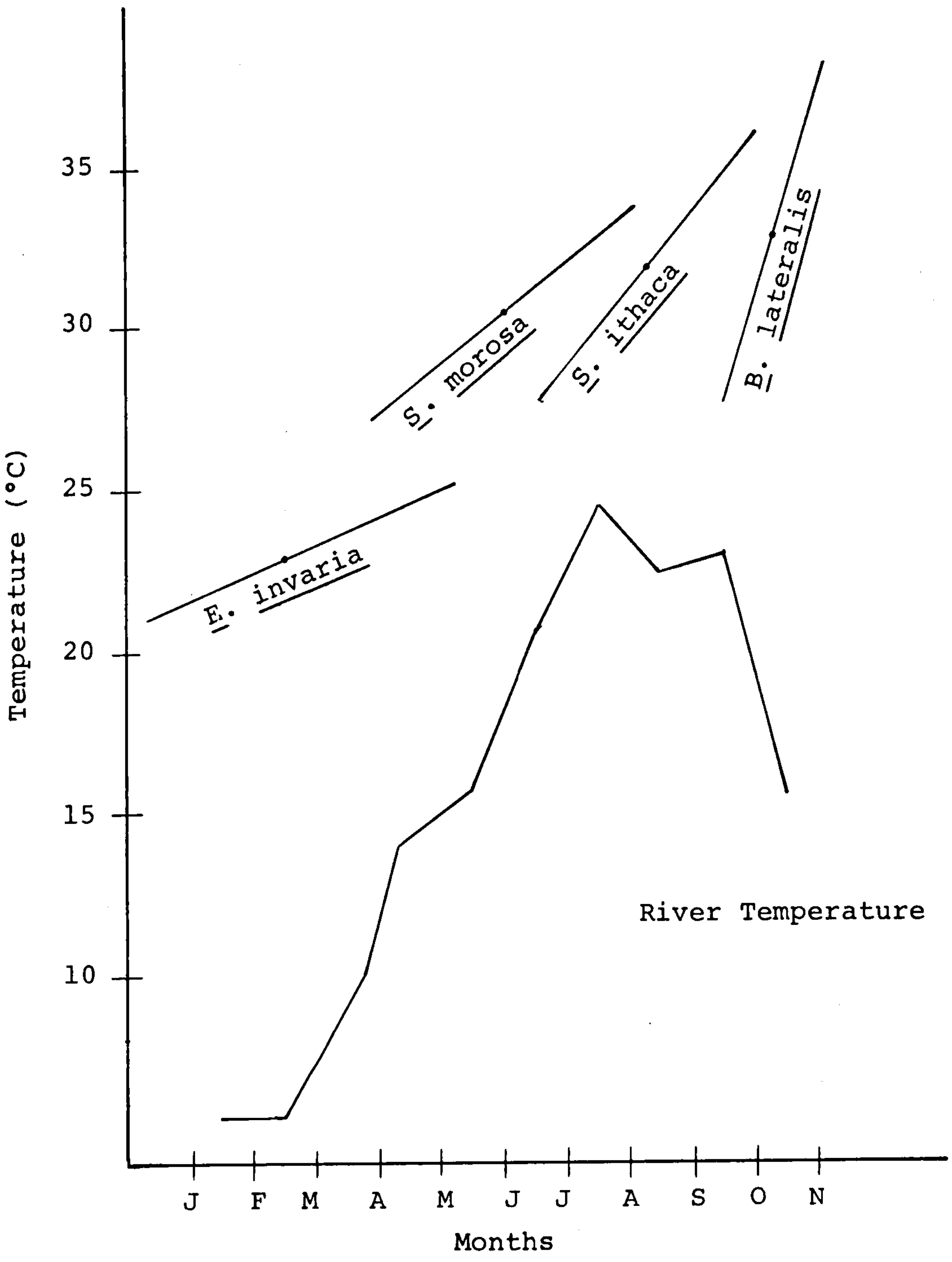

Figure 3. Comparison of LT50 values and associated Regression Lines to Mean Monthly Temperatures of Little River.

linear relationship exists between LT50 values and river water temperature.

Results of the analysis of variance tests showed that a significant amount of insect mortality could be explained by temperature. These results are presented in Table 4. There was a positive linear regression of mortality versus temperature. The regression lines fitted to these data are graphically presented for each species in Figures 4, 5, 6, and 7. A composite graph of regression lines for all species is presented in Figure 8. From this figure one can see the relative slope relationships of all the species. Analysis of covariance tests on these regression data indicated that individual regression lines for each species were a better fit for the data than a common regression line for all species. Results of the analysis of covariance are presented in Table 5.

Results from the multiple range test are listed in Table 6 and Figure 8 graphically illustrates these slope relationships. These results indicate that significant differences exist between regression coefficients for all species except those for *E. invaria* and *S. morosa*. The parallel regression lines for these species suggests a similar response to temperature changes although, their LT50 values were significantly different.

A positive linear relationship existed between regression coefficients and river water temperature similar to that

TABLE 4. RESULTS OF ANALYSIS OF VARIANCE APPLIED TO PERCENT MORTALITY OF EACH SPECIES AT VARYING WATER TEMPERATURES

Species	Source	Sum of Square	Degrees Freedom	Mean Square	F-Ratio
Ephemerella invaria					
	Between	41.785	1	41.785	42.638*
	Within	4.898	5	0.980	
	Total	46.683	6		
Symphitopsyche morosa					
	Between	22.002	1	22.002	21.871*
	Within	6.037	6	1.006	
	Total	28.039	7		
Stenonema ithaca					
	Between	50.640	1	50.540	24.811*
	Within	16.329	8	2.041	
	Total	66.969	9		
Brachycentrus lateralis					
	Between	56.448	1	56.448	180.345*
	Within	2.819	7	0.313	
	Total	59.267	8		

* = Significance at $\alpha \leq .05$.

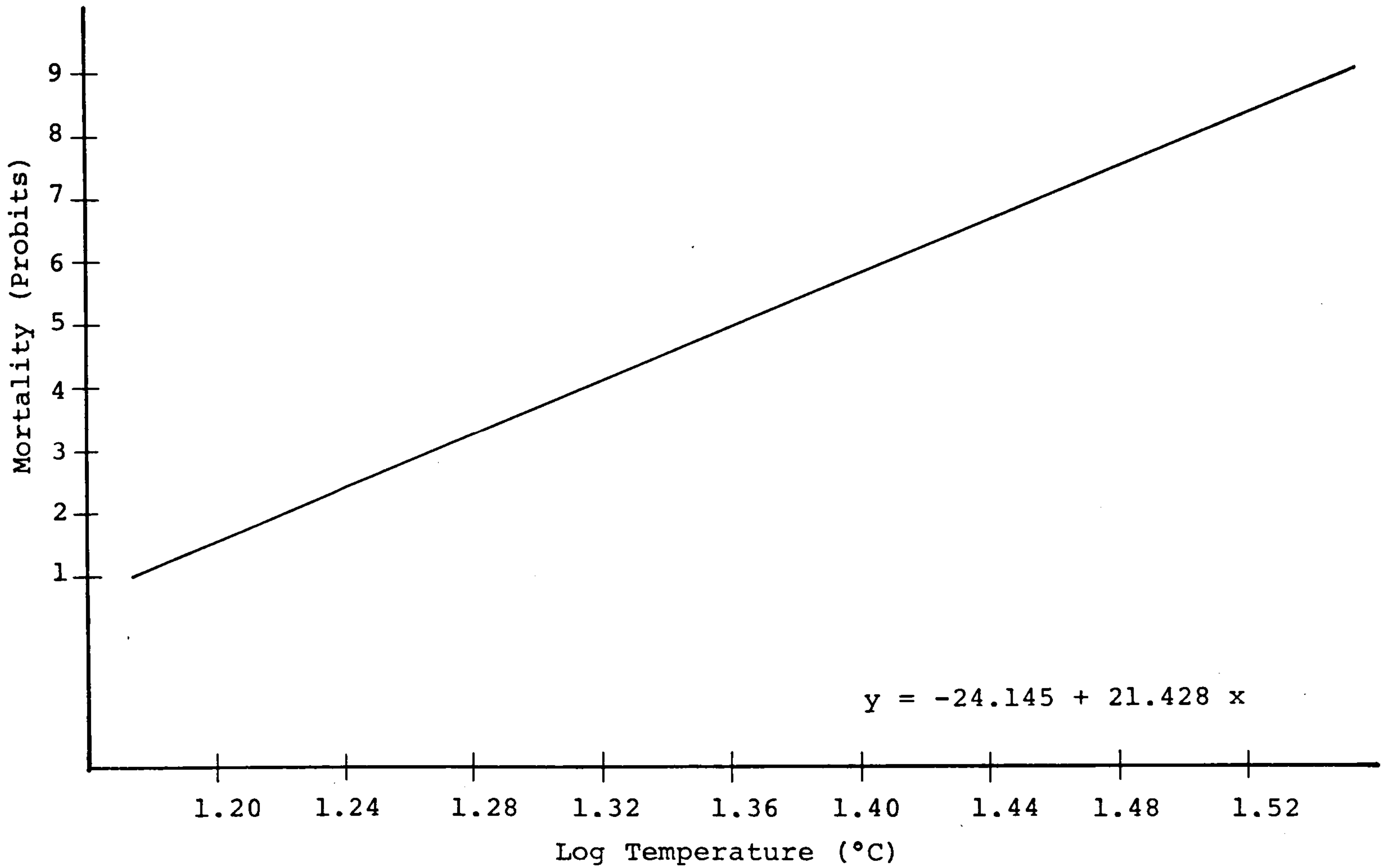

Figure 4. Mortality of *Ephemerella invaria* at varying water temperatures.

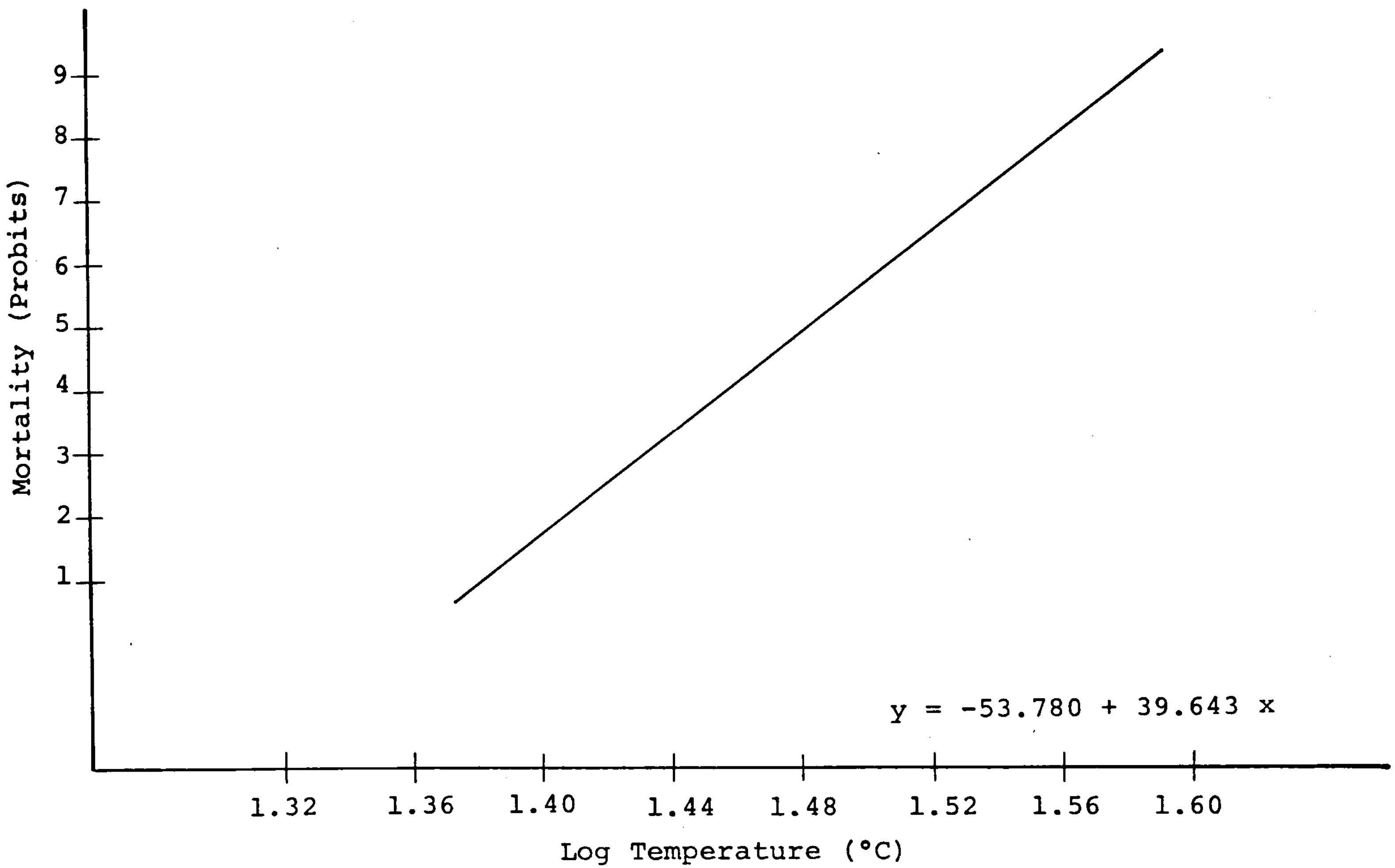

Figure 5. Mortality of *Symphitopsyche morosa* at varying water temperatures.

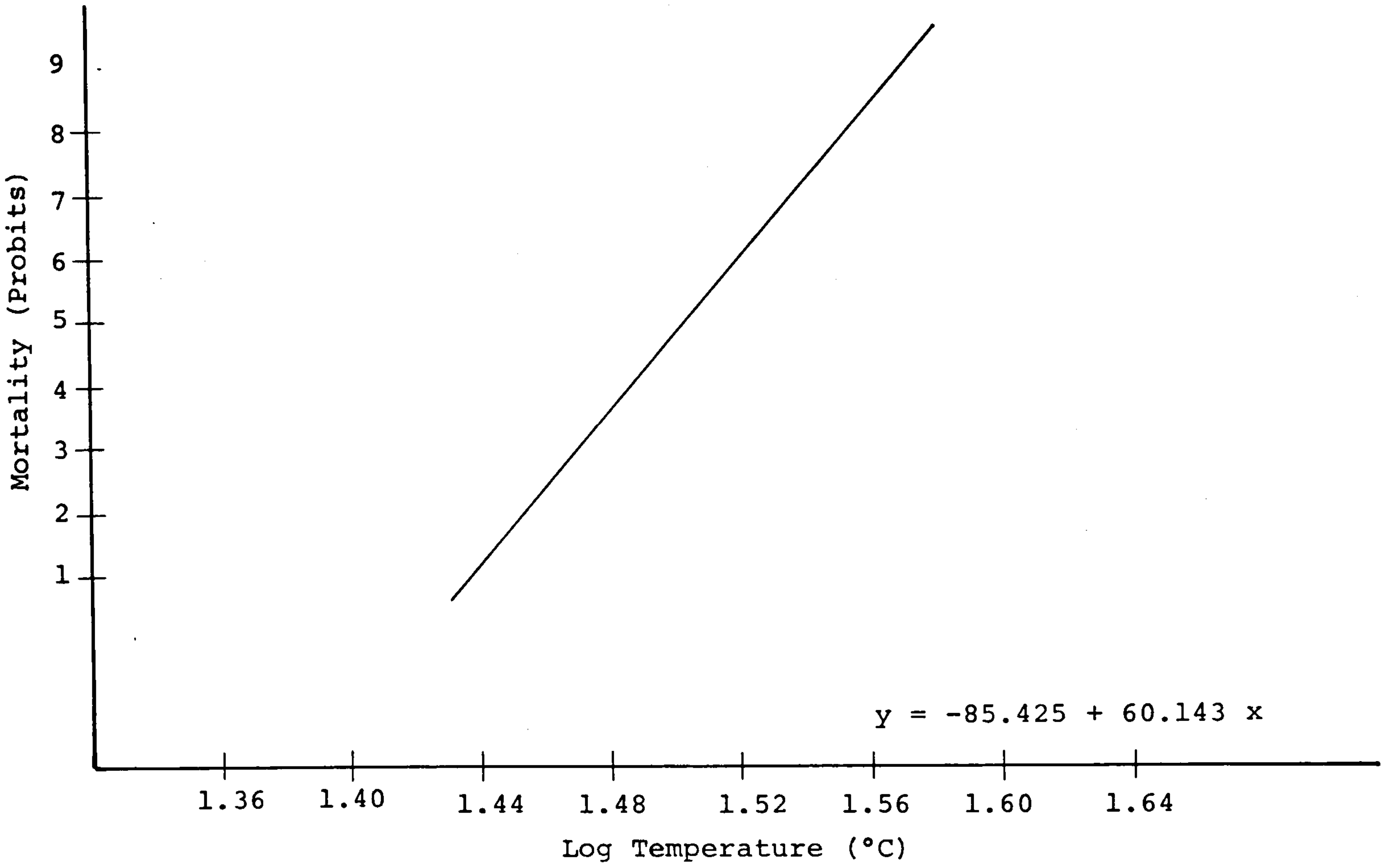

Figure 6. Mortality of <u>Stenonema ithaca</u> at varying water temperatures.

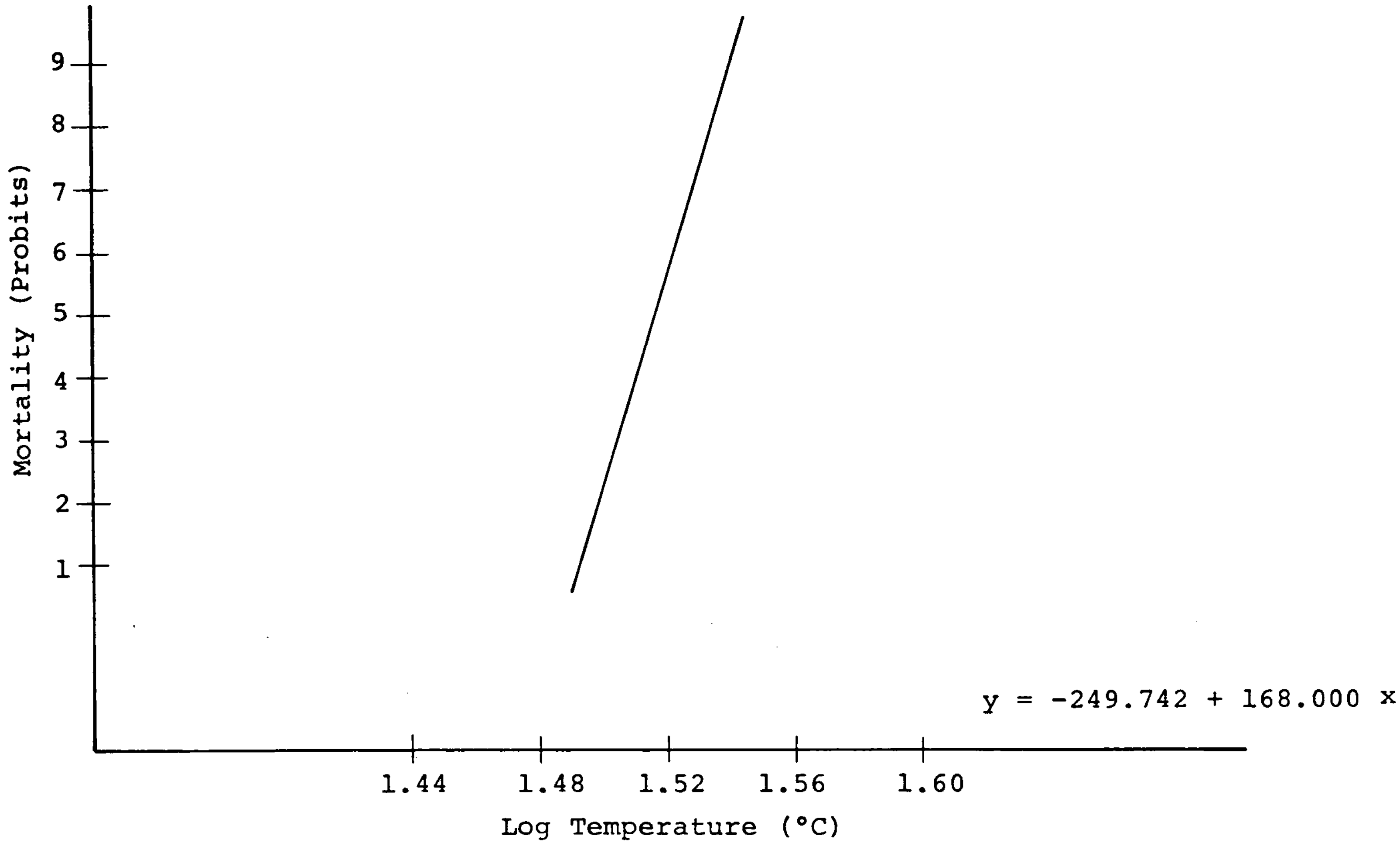

Figure 7. Mortality of *Brachycentrus lateralis* at varying water temperatures.

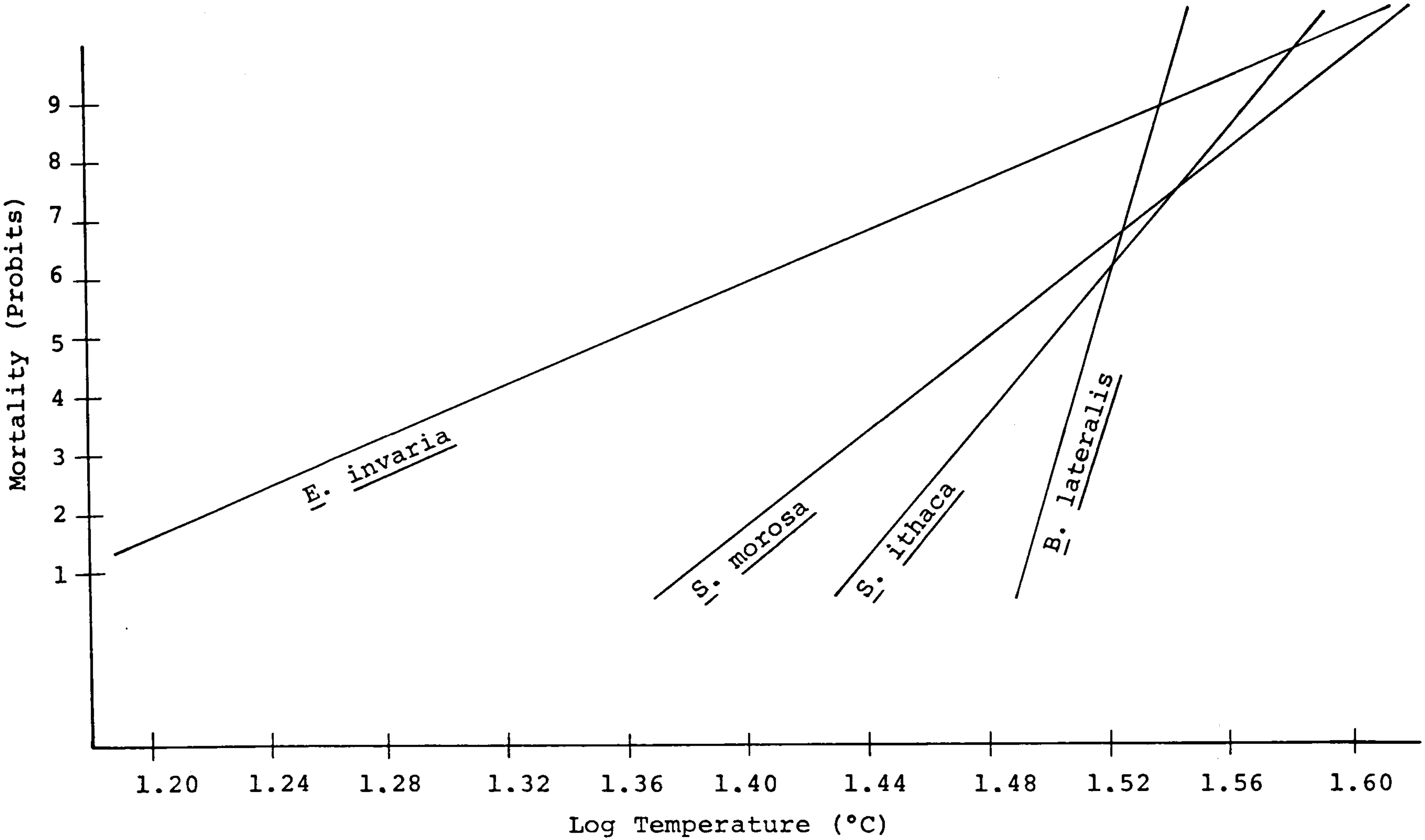

Figure 8. Mortality at varying water temperatures for all species tested.

TABLE 5. RESULTS OF ANALYSIS OF COVARIANCE TEST FOR COMMON LINE APPLIED TO REGRESSION DATA FOR ALL SPECIES TESTED

Source	Sum of Squares	Degrees Freedom	Mean Squares	F-Ratio
Due to common regression line	109.415	6	18.232	15.761*
Deviations from individual regression lines (error)	30.083	26	1.157	
Deviations from common regression line	139.498	32	4.359	

* = Significance at $\alpha \leq .05$.

TABLE 6. RESULTS OF SIMULTANEOUS TEST PROCEDURE ON REGRESSION COEFFICIENTS OF SPECIES TESTED

Species	Sum of Squares
E. invaria S. morosa S. ithaca B. lateralis	58.77*
E. invaria S. morosa S. ithaca	20.29*
S. morosa S. ithaca B. lateralis	28.98*
E. invaria S. morosa	4.02^{NS}
S. morosa S. ithaca	72.61*
S. ithaca B. lateralis	20.36*

* = Significance at .05.

NS = Not significant.

$SS_{critical}$ = 10.03.

found between LT50 values and river water temperature. This relationship can be seen in Figure 3, p. 27.

In addition to the quantitatively measured responses of the insects to heated water several qualitative responses were also noted. It was observed that a majority of deaths occurred while the insects were in the process of molting or emerging. These periods are commonly known to be the most vulnerable time for many invertebrates (Bursell, 1974). This was especially apparent with the mayflies *E. invaria* and *S. ithaca*. The dead of these species were often found with ruptured cuticles along the ecdysial lines.

Elevated temperatures also promoted rapid metamorphosis. *Symphitopsyche morosa* was encouraged to pupate early when exposed to heated water. This rarely resulted in death to the individuals. However, individuals did not construct pupal cases as is typical in the field. These pupae were thus exposed and unprotected from predation and the abiotic environment.

Early emergence due to accelerated metamorphosis at elevated temperatures was observed for *S. ithaca*. This was a major cause of death at 33°C and 34°C for this species. The majority of individuals died during the rigorous ordeal of escaping the nymphal exuvia. Those able to escape rarely survived the subimago stage to become adults. Early emergence was not observed for *E. invaria* although they too were late instar mayflies.

Behavioral aberrations were observed for all species tested when they were exposed to elevated temperatures. Drifting was noted for both mayfly species at the momemt of submergence into heated waters. Degree of drifting appeared to be a positive linear function of water temperature. Abdominal undulations of S. morosa were noted to increase at elevated temperatures. This phenomenon has been observed in other species of caddisflies and is probably a response to low dissolved oxygen concentrations at the warmer temperature. The filtering behavior of B. lateralis ceased and the animals withdrew into their cases when exposed to water temperatures above 32°C. Control groups exhibited normal filtering activity. Gallepp (1977) found similar behavior changes with the closely related species B. americanus and B. occidentalis.

V. DISCUSSION

Results from this investigation generally correspond with those found by previous investigators. Species of the genus *Ephemerella* have generally been found to be among the most sensitive insect species to heated water (Nebeker and Lemke, 1968; Gaufin and Hern, 1971; EPA, 1973). Thermal tolerance values from previous studies on this genus have never exceeded 22.0°C while the LT50 determined for *E. invaria* was 22.9°C. Several factors may account for this discrepancy. Slight variations in experimental design from previous investigations may yield slightly different thermal tolerance values. Acclimation temperature and times varied for all these studies. This has been shown to affect thermal tolerance values by several degrees. Ecological factors are also important. All previous investigations were performed on species from northern streams. Since these streams have lower mean annual temperatures than southeastern streams, adaptation by northern species to the colder stream temperatures may account for the differences in thermal tolerance values. Interspecific variation may also explain these differences.

Although laboratory studies to date have determined low thermal tolerance values for species of *Ephemerella*, a field study by Trembley (1961) contradicts those findings. He found nymphs inhabiting an area of the Delaware River where water temperatures reached 30°C. Unfortunately, no species identifications were made. By conducting thermal tolerance investigations on more species of *Ephemerella*, it may be determined that the genus has a broader thermal tolerance range than is now apparent.

Since *Symphitopsyche* is a newly established genus in North America, comparisons of results must be made with studies of the parent genus *Hydropsyche*. *Hydropsyche* larval taxonomy prior to Schuster and Etnier (1978) was severely limited and identification to species 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*. This investigation provided the first thermal tolerance values for *Symphitopsyche* species.

In general, Hydropsychid caddisflies are considered tolerant of many forms of pollution including thermal pollution. *Hydropsyche* have been collected from rivers where water temperatures were extremely high, reaching maximum temperatures of 41°C (Roback, 1965; Trembley, 1961). It is unknown, however, whether these species were permanent inhabitants of these waters.

Laboratory studies on *Hydropsyche* (broad sense) have yielded a mean thermal tolerance value of 30.3°C (Gaufin and

Hern, 1971; Sage, 1974). This value closely approximates the thermal tolerance value calculated for S. 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 prove nor disprove this conclusion. Although the thermal tolerance value for Symphitopsyche morosa was essentially no different from that of previously studied Hydropsyche (broad sense) larvae, no species identifications were made. Therefore, comparisons of the values are meaningless. Additional thermal tolerance studies must be conducted on these two genera using identical methodologies, to prove that their thermal tolerances are significantly different.

Results from this study also indicated that this hydropsychid caddisfly is no more tolerant of heated water than the majority of other aquatic insects tested.

The mayfly, Stenonema ithaca had a tolerance similar to that of S. morosa. A previous study on S. ithaca yielded a similar thermal tolerance vlaue to that determined by this investigation (Gregg, 1974). This is the only species tested for which previous thermal tolerance values have been determined. It is interesting that such a close equivalence of values exists between these geographically distant populations. These findings support the idea that thermal tolerance is primarily genetically determined and environment only slightly modifies it. An interesting study not yet conducted would be to determine the thermal tolerances of several populations of a species over its entire geographic range. It would be mandatory that identical methods be used and identification to the species level be accurate. Results of such a study could determine the extent of the association between genetically determined thermal tolerance and local environmental conditions. Suitable organisms from this study which are easily identified and have extensive latitudinal distributions are *Stenonema ithaca* and *Brachycentrus lateralis*.

The only other thermal tolerance study conducted on this genus was on *Stenonema tripunctatum*. It yielded a thermal tolerance of 25.5°C (Nebeker and Lemke, 1968). This species inhabits clear, cold mountain streams, and thus is likely to have a lower tolerance to heated water than *S. 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 closely related species have been conducted on northern stream species. Therefore, the higher LT50 values for *B. lateralis* may be attributed to adaptation or acclimatization of this species to the warmer southeastern stream temperatures.

Successful acclimation assumes that the physiological processes (i.e. enzyme systems) were adjusted to a common baseline for all species tested. Any and all responses to a stimulus thus reflect specific genetic differences and not differences due to previous experiences of the individuals (i.e. temperature exposure). Based on this assumption, the results indicate significant genetic differences in thermal tolerance for the species studied even though they share the same habitat. This is exemplified by the statistically different LT50 values and regression coefficients between 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, S. morosa, and S. ithaca and the dramatically different tolerance of E. 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 different thermal regimes (Figure 9). Those species exposed to only cold temperatures during their growth and development evolved low thermal tolerances. Ephemerella invaria best demonstrated this point. This species hatched during late fall when water temperatures were cool. It 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, succumbing to temperatures 7.5 degrees below the next most sensitive species (See Table 2, p. 26). Those species on the other hand which were subjected to elevated water temperatures during their life histories evolved a higher tolerance to heated water. Stenonema ithaca, Symphitopsyche morosa, and Brachycentrus lateralis were exposed to water temperatures of up to 28°C during their life cycles and their considerably greater thermal tolerance over E. invaria reflects that fact. The genetic determination of thermal tolerance appears to be very specific. Stenonema ithaca and Symphitopsyche morosa were sympatric species

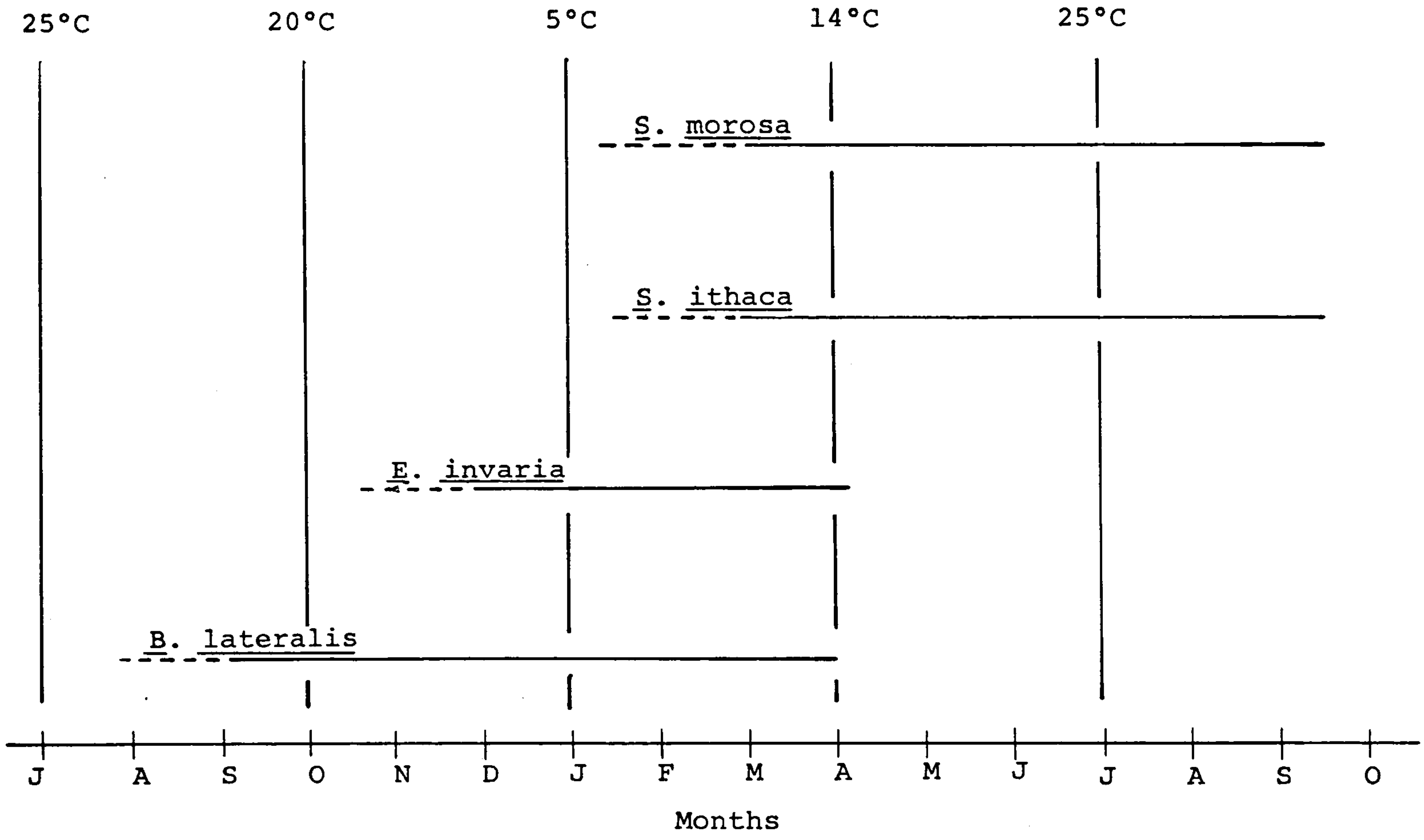

Figure 9. Comparison of mean monthly water temperatures and presence of aquatic stages of test organisms in Little River.

exposed to virtually identical thermal extremes (See Figure 9) and likewise there is no significant difference between their LT50 values (See Table 3, p. 26).

The thermal tolerance calculated for each species is only the mean mortality temperature associated with another very important parameter, the range of temperatures over which individuals of a given species died. The magnitude of the regression coefficients associated to each species' regression line reflects 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 (See Table 7). The extent of this range appeared to be dependent upon the difference 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 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. All aquatic organisms have a range of temperatures within which they can live and reproduce. For many aquatic insects this range extends from 0°C to a critical thermal maximum of approximately 35°C. Hawkes (1969) stated that most enzymes vital to metabolic

TABLE 7. RELATIONSHIP OF REGRESSION COEFFICIENT AND RANGE OF TEMPERATURES OVER WHICH MORTALITY OCCURRED FOR SPECIES TESTED

Species	Regression Coefficient	Range of Temperatures	Change in Temperature
E. invaria	21.428	16.44°C-34.17°C	17.73°
S. morosa	39.643	25.40°C-37.72°C	12.32°
S. ithaca	60.143	28.32°C-36.76°C	8.44°
B. lateralis	168.000	31.47°C-34.55°C	3.08°

respiration are inactivated by temperatures above 35°C. Roback (1965) records no species living in waters above 35°C. This investigator also found no species to survive at 35°C for more than a few hours.

Cold stress does not appear to be a major factor to the thermal tolerance range of a species since all individuals of all species studied appeared tolerant of temperatures approaching 0°C. However, the tolerance of individuals within a species to heat stress is variable and the range of this variation is different between species (Table 7). Only those individuals with genetically determined thermal tolerances compatible with the extremes of their environment will survive to reproduce. Therefore insect species exposed to cool water temperatures (maximum 14°C) should have individuals within that population tolerant of temperatures over a wide range (14° to 35°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 narrow range (25° to 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 (21°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 expect to find a 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. These values are listed in Table 8. Although statistical analysis was not performed on these data, one can see that a close relationship between these values appears to exist for each species. The only exception are those values for B. lateralis.

TABLE 8. 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

VI. CONCLUSIONS

The thermal tolerances of the four species studied were significantly different except between _S. morosa_ and _S. ithaca_. This non-significance was attributed to their exposure to similar thermal regimes. The regression coefficients of the regression lines for each species were also significantly different except between _E. invaria_ and _S. morosa_. 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 dependent upon the difference between maximum river water temperature to which a species was exposed during its life history and the critical thermal maximum of most aquatic life (35°C). The non-significance of coefficient values for _E. invaria_ and _S. morosa_ 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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