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PHYSIOLOGICAL TOLERANCE RANGES OF LARVAL *CAENIS LATIPENNIS* (EPHEMEROPTERA: CAENIDAE) IN RESPONSE TO FLUCTUATIONS IN DISSOLVED OXYGEN CONCENTRATION, PH AND TEMPERATURE

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Abstract.—Laboratory experiments were conducted to investigate the physiological tolerance ranges of the mayfly *Caenis latipennis* (Ephemeroptera: Caenidae) from Tanyard Branch Creek in Walker County, Texas in response to stepwise fluctuations in dissolved oxygen concentrations, temperature and pH. Survivorship decreased slightly at a dissolved oxygen concentration of 7.0 mg/L, while trial groups suffered a dramatic decrease in survivorship at a dissolved oxygen concentration of 4.5 mg/L. Mean CTMax (Critical Thermal Maximum) for 10 individuals was 37.8°C with a range from 36.7°C to 38.5°C. Mean critical lower pH for three trials of 10 individuals was 2.56 and mean critical upper pH for three trials of 10 individuals was 12.5.

Assessments of benthic macroinvertebrate communities provide general information regarding the water quality of the streams that support them once baseline information regarding specific streams has been gathered (Edmunds et al. 1976; Hilsenhoff 1977; Barbour et al. 1999; Rabeni et al. 1999; Lydy et al. 2000). However, the ultimate goal of managing stream quality through the practice of bioassessment is the ability to make stream management decisions based on reference data (chemical, physical and biological). These data are typically gathered from a specific region to bypass the expense and time of developing baseline information from each regional stream (Barbour et al. 1999). The cost effectiveness of stream bioassessment versus physical/chemical monitoring is realized only after this baseline information is gathered (Barbour et al. 1999).

A critical requirement of a regionally specific bioassessment program is an understanding of the physiological tolerance ranges of the species comprising the resident benthic macroinvertebrate community. While information exists regarding species specific tolerance ranges, this information is typically anecdotal and not empirically derived (Hilsenhoff 1977; 1982). In addition, many species have large geographical ranges raising the possibility that a continuum of intraspecific physiological tolerance ranges occur. This stresses the necessity for determining regionally specific species tolerance ranges.

Caenis latipennis occurs throughout North America north of Mexico, including south central Canada to extreme southern Texas with a disjunct population in southern Mexico (Provonsha 1990). In a previous study, streams from two neighboring counties in southeast Texas (Walker and San Jacinto counties) were monitored monthly for a period of one year regarding their ephemeropteran community diversity responses to fluctuating physical/chemical parameters. Regression analysis of mayfly diversity against fluctuation of stream quality values indicated that of the eight parameters sampled throughout the period, dissolved oxygen, temperature and pH show the greatest correlation with fluctuating mayfly diversity (Puckett 2003).

The goal of this study was to determine the range of dissolved oxygen, temperature and pH that *C. latipennis* can tolerate with the hope that this information can be used in stream bioassessment practices specific to Walker County streams. The techniques used here may provide a model for further investigations into species specific tolerance ranges. Although this is not an investigation into the potential intra-specific geographical physiological tolerance gradient mentioned above, the data presented here could serve for comparison to similar values obtained for *C. latipennis* in other areas of its distribution.

MATERIALS AND METHODS

Caenis latipennis larvae were collected from Tanyard Branch Creek, taken to the laboratory at Sam Houston State University and allowed to acclimate to laboratory conditions over a period of approximately one week. Mayflies were collected using a standard 0.8 m by 0.8 m kick screen and were transferred to the laboratory in 4 dram vials containing stream water. Larvae were housed in mesh bottomed containers that were submerged in water from the stream in which they were collected. Of the thirty individuals housed in each container, twenty were selected (10 per trial and 10 per control) for both dissolved oxygen and pH experiments. Individuals were selected from the remaining laboratory population for critical thermal maximum (CTMax) experiments.

Dissolved Oxygen Tolerance.—A 2 liter beaker was capped with a 1.5 cm styrofoam disk that was cut to precisely fit the beaker mouth. Holes were then cut in the disk to accommodate the container that housed the mayflies, the connector hose from a N_2 cylinder and dissolved oxygen meter (YSI® Dissolved Oxygen Meter-Model 55/12FT).

The containers that housed the mayflies during the trials were made by first removing the bottoms of two 100 mL plastic cups. A 7.6 cm by 7.6 cm piece of fine mesh was then stretched around the bottom opening of one cup and forced into the second cup. Once taut, this mesh provided an artificial substrate and allowed for a homogenous mixing of water inside and outside of the container. The conical shape of the cups also allowed for a tight fit into the hole in the styrofoam disk which diminished the amount of diffusion of atmospheric oxygen. A plunger to seal off the original opening of this container was built by attaching a 12 cm section of Pyrex® glass cylinder to the center of the removed cup bottom. During trials this plunger was placed into the cup so that it fit snugly beneath the water line, again with the goal of reducing atmospheric oxygen diffusion into the trial beaker. The entire apparatus was placed on a Corning® stirrer/hot-plate. During trials the stir bar revolved at approximately 68 rpm. Stirring the water during trials was essential for the operation of the dissolved oxygen meter.

De-ionized water was used in all trials. Mayflies were placed in DI water three hours before the start of each trial. During the trials dissolved oxygen was removed by purging the water slowly with gaseous nitrogen to lower oxygen levels by 0.5 mg/L increments. Each O_2 level was held for 45 min. until lethal O_2 levels were met. The time interval of 45 min. was determined after subjecting a pre-trial group of ten individuals directly to a dissolved oxygen concentration of 0.5 mg/L. After 40 min. all individuals were dead. Control groups were setup in an identical fashion excluding only the N_2 purge. Ten individuals each in trial and control groups were monitored. All other water parameters remained constant during trials.

Thermal Tolerance.—Determination of lethal maximum temperature levels was carried out in a similar apparatus as that described for dissolved oxygen trials. However, in the temperature trials an aquarium heater and oxygen pump/bubbler were added to the apparatus and the nitrogen component removed. Additionally, the plunger described in the dissolved oxygen trials was removed. Critical thermal maximum (CTMax) trials rely on the observation of a trial endpoint that is specific to the organism being studied (Lutterschmidt & Hutchison 1997). For

Caenis latipennis, observation of lack of righting response followed by the mayfly's inability to cling to the artificial substrate was always followed immediately by death. Inability to cling to the artificial substrate was used as an endpoint in these trials.

Temperature was raised 1.5°C/min. until the endpoint was observed. A total of ten individuals were subjected to these trials. Each trial was performed on one individual per trial while controls were simultaneously run and held at room temperature. As in dissolved oxygen trials, de-ionized water was used. Trial and control individuals were allowed the same acclimation period of approximately 3 hours. All other water parameters remained constant during trials.

pH Tolerance.—pH trials were also carried out in closed beakers. However, in these trials 1 liter beakers were used to minimize chemicals necessary to accomplish stepwise manipulation of pH. Mayflies were housed as described above.

Three trials were run in which a group of 10 individuals were subjected to stepwise fluctuations of pH (both up and down) starting at a pH value of 8.0. Separate trial groups were used for each trial. pH levels were manipulated by titration with 2<u>N</u>HCl (pH decrease) and 2<u>N</u>NaOH (pH increase). Levels were raised or lowered by half a pH unit per hour. The time period of one hour was decided upon after subjecting a pre-trial group of 10 individuals to water with a pH value of 2. In just under an hour all individuals were dead. VWR Scientific Products® benchtop pH meters (Model SB21) were used to monitor pH levels during trials. Stream water was used in these trials rather than de-ionized water as a result of discrepancy between the pH levels of stream and de-ionized water. Death was signaled by individuals bending at the first abdominal segment accompanied by an inability to remain attached to the artificial substrate. Control groups of ten individuals were run simultaneously. All other water parameters remained constant during trials.

RESULTS

Dissolved Oxygen Tolerance.—When exposed to stepwise reduction of dissolved oxygen, survivorship of *Caenis latipennis* showed a subtle decrease once a dissolved oxygen concentration of 7.0 mg/L was reached. However, a dramatic decrease in survivorship was observed after dissolved oxygen concentration levels were reduced to 4.5 mg/L (Fig. 1a). Mortality continued to increase with relative dissolved

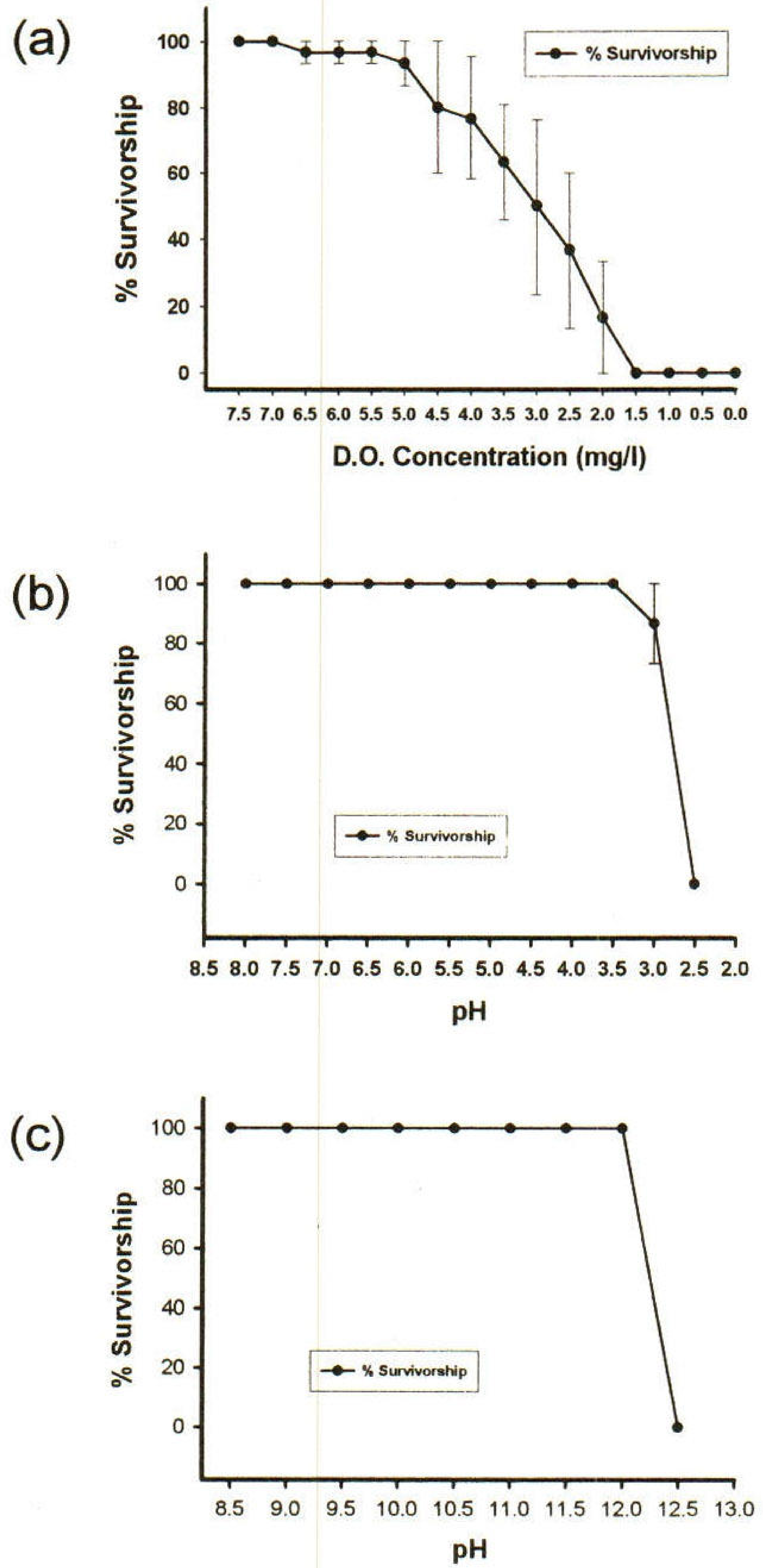

Figure 1. Survivorship of three *Caenis latipennis* (a) dissolved oxygen tolerance threshold trials, (b) pH decrease trials (One-way *ANOVA* on ranks [$P=0.795$]) and (c) pH increase trials (One-way *ANOVA* on ranks [$P=1.000$]).

oxygen reduction with no individuals surviving below 1.5 mg/L. Percent survivorship of the control groups during trials 1, 2 and 3 were 80%, 90% and 100% respectively.

Thermal Tolerance.—CTMax trials show that the average upper critical temperature for *Caenis latipennis* is 37.8°C. All ten individuals subjected to CTMax trials died between 36.7°C and 38.5°C. The critical thermal maximum temperature of individuals in these trials was well above the maximum temperature value recorded in the stream during the monitoring period (22.3°C). Controls were run simultaneously at a temperature of 24.5°C with no mortality.

pH Trials.—The critical lower pH level under which *Caenis latipennis* could not survive was 2.5 (Fig. 1b). In two of the three trials all individuals were alive after being exposed to stepwise decrease of pH to a level of 3.0 with 100% mortality after exposure to the same water at a pH of 2.5. During the third trial 40% of the individuals died at pH of 3.0 with the remaining individuals dying at a pH of 2.5. The lowest pH value recorded from a stream during the monitoring period was 7.7. Controls groups were run during the trials in a sample of the same water that was used for trial groups. This water maintained a pH of 8.2 from collection through the end of trials. No mortality was recorded in the control groups.

The critical upper pH level above which *Caenis latipennis* could not survive was 12.5 (Fig. 1c). All individuals in each of three trials were alive after being exposed to stepwise increase of pH to a level of 12.0, after which at a pH value of 12.5 all three groups experienced 100% mortality. The highest pH value recorded during the monitoring period was 8.6. Control groups were run during the trials at a pH of 8.2 in which no mortality was recorded.

DISCUSSION

Caenis latipennis can cope with dramatic fluctuations in pH, dissolved oxygen, and temperature. It is very unlikely that under natural conditions *C. latipennis* larvae would be exposed to water quality parameter values that would fall outside of the tolerance values determined in the laboratory. This suggests a species that should be considered extremely tolerant of a wide range of values pertaining to the water quality parameters investigated in this study. This information is in agreement with previously published pollution tolerance values regarding *C. latipennis* by Hilsenhoff (1987).

The unlikelihood that the values of the parameters investigated here should, in natural systems, fall outside of this species range of tolerance suggests that the utility of *C. latipennis* as an indicator of stream quality is limited. However, when found in systems of low mayfly diversity this species and others found to be similarly tolerant could serve as valuable predictors of acute stream perturbation. At best, *C. latipennis* should be assigned little weight when included in stream assessments based on some biological index such as Hilsenhoffs Biotic Index.

The relative ease with which the range of tolerance values regarding the parameters investigated were obtained suggests that empirically derived tolerance ranges for most Ephemeropteran species can be determined. Due to general similarities in morphology, life history, and ecological requirements, it is likely that these laboratory methods could also be used to gather data regarding physiological requirements of other stream macroinvertebrates such as the orders Plecoptera and Trichoptera. With specific data regarding true tolerance ranges of these insects and other stream invertebrates, bioassessment practices can be approached and interpreted with greater accuracy and relied upon with greater confidence.

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