

Thermal tolerances of two stream invertebrates exposed to diurnally varying temperature

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INTRODUCTION

Abstract For two key New Zealand freshwater invertebrates (the mayfly *Deleatidium autumnale* and the snail *Potamopyrgus antipodarum*) the upper thermal tolerances were measured in the laboratory under both constant and diurnally varying temperatures. At constant temperature 50% mortality in 96 h ($_{const}LT_{50}$) occurred at $24.2 \pm 0.9^\circ\text{C}$ for mayflies and $31.0 \pm 0.6^\circ\text{C}$ for snails: values similar to previously published estimates (Quinn et al. 1994). For diurnally varying temperatures (daily amplitude 10°C) 50% mortality occurred when the daily mean temperature was $21.9 \pm 0.7^\circ\text{C}$ (mayflies) and $28.6 \pm 0.4^\circ\text{C}$ (snails) which is c. 10% ($2.5 \pm 1.3^\circ\text{C}$) lower than the $_{const}LT_{50}$. Conversely, 50% mortality occurred when the daily maximum temperature was $26.9 \pm 0.7^\circ\text{C}$ (mayflies) and $33.6 \pm 0.4^\circ\text{C}$ (snails) which is c. 10% ($2.5 \pm 1.3^\circ\text{C}$) higher than the $_{const}LT_{50}$. Many published temperature limits for stream organisms are derived from constant temperature experiments. Our results indicate that such limits should be applied to a temperature midway between the daily average and the daily maximum of a diurnal profile.

Stream invertebrates play an important role in stream ecosystems, notably by providing food for fish and helping control periphyton growth through grazing. Temperature affects the structure and function of invertebrate communities in streams (Vannote & Sweeney 1980; Ward & Stanford 1982). In New Zealand a variety of human activities have increased stream temperatures, including the discharge of heated effluents from power plants, water abstraction, and the removal of riparian vegetation (Mosley 1982; Quinn et al. 1994; Rutherford et al. 1997). Several species of mayfly and stonefly are sensitive to high temperature (Quinn et al. 1994) and are poorly represented in unshaded, pasture streams (Quinn & Hickey 1990). It has been postulated that high temperature excludes some important, but temperature sensitive, invertebrates from warm streams, thereby reducing species diversity and affecting the ability of grazers to control periphyton biomass (Quinn et al. 1994; Scarsbrook et al. in press).

Thermal tolerances have been determined for a number of benthic invertebrates. Quinn et al. (1994) used laboratory experiments conducted at constant temperature to determine and compare the upper thermal tolerances of 12 New Zealand freshwater invertebrate species. The mayfly *Deleatidium* sp. was the most sensitive to high temperature of the animals tested (50% mortality after 96 h occurred at $_{96}LT_{50} = 22.6 \pm 0.8^\circ\text{C}$) whereas the snail *Potamopyrgus antipodarum* was the least sensitive ($_{96}LT_{50} = 32.4 \pm 2.5^\circ\text{C}$). They also found that LT_{50} values declined with exposure time. Similar laboratory studies have been performed for freshwater snails in New Zealand (Winterbourn 1969) and for northern hemisphere species of caddisflies (Moulton et al. 1993), bivalve molluscs (Read & Cumming 1967), crayfish (Layne et al. 1987), dragonfly nymphs (Martin & Gentry 1974), and amphipods (Buchanan et al. 1988).

These studies provide good insight into the relative temperature tolerances of species. However, the results cannot easily be applied to the management

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of natural streams because they relate to either constant or linearly increasing temperature regimes, rather than the diurnally varying patterns found in natural streams. Knowing the LT_{50} it is standard practice to allow a safety margin (typically 3°C) and hence set the maximum acceptable temperature for protecting a particular species (Simons 1986). If the LT_{50} is derived from constant temperature experiments, the question arises whether this limit should be applied to the daily maximum or the daily mean temperature of the stream.

In this laboratory study we compared the thermal tolerance of two important New Zealand invertebrate species exposed to constant and diurnally varying temperatures. We selected the mayfly *Deleatidium* (nymphal stage) and the snail *Potamopyrgus* because Quinn et al. (1994) showed these to be amongst the most sensitive (mayfly) and least sensitive (snail) invertebrates to high temperature. This will improve the utilisation of the large body of published information on thermal tolerance derived using constant temperature experiments to help set temperature limits in natural streams.

METHODS

Experiments were conducted between March and July 1998. Medium sized *Deleatidium* nymphs (10–15 mm) were collected from the Upper Waimakariri River ($175^{\circ} 52'S$, $38^{\circ} 01'E$) and *Potamopyrgus* (2–5 mm) from the Waihou River ($175^{\circ} 49'S$, $37^{\circ} 59'E$). Both collection sites are on spring-fed streams in the Waikato region of the North Island. It is not possible to identify the species of *Deleatidium* from the nymphs used in the 1998 laboratory tests. In October 1999 mayfly nymphs were collected from the same site, reared through to adults and were found to be *Deleatidium autumnale* (Mr Brian Smith, NIWA, Hamilton pers. comm.) The Waimakariri River has a mean annual temperature of 14.2°C with a typical summer maximum of 15.7°C and a winter minimum of 12.7°C . Waihou temperature data are limited but are believed to be similar (Quinn et al. 1994). Mayflies were collected from the streambed gravel, and snails from macrophytes, using a 500 mm mesh hand net. Organisms were sorted in the field using a shallow tray containing stream water, transferred by pipette into aerated buckets containing stream water, placed in an insulated container with a small amount of ice, and transported to the laboratory within 3 h.

In the laboratory organisms were acclimated at the control temperature (c. $15\text{--}16^{\circ}\text{C}$) for 24 h in a

constant-temperature room with a 12-h light-dark cycle. Organisms were kept in aerated buckets and mayflies (but not snails) were given periphyton-coated rocks as a food source. Following acclimation, 30 individuals of each species were transferred to each of three test baths: a constant temperature treatment; a diurnally varying treatment; and a constant temperature control ($16 \pm 1^{\circ}\text{C}$). Organisms were placed in 50 ml plastic cups (two per cup) containing stream water from the acclimation buckets. Cups protruded through 5 cm diameter holes in a thin polystyrene sheet floating on the surface of the water baths into the underlying water. Cups were covered with plastic wrap to minimise evaporation. Cups were aerated through fine bore tubing (0.64 mm i.d.) connected via syringe needles (19 gauge) to an air pump. A small square of hard nylon mesh (c. 2×2 cm) was placed in the mayfly cups to provide a structure for the organisms to grip. No food source was provided during the 96-h test.

Temperatures in the heated treatments were controlled using recirculating heating and cooling units (Julabo Inc., Model VC). Heated/cooled water was pumped from the small baths of the Julabo units into the larger baths containing the test organisms. Small electric stirrers were used to mix the heated bath water and a siphon was incorporated to maintain the same water level in each bath. The diurnally varying treatment was heated and cooled according to a sinusoidal profile (programmed into the Julabo unit) with a period of 24 h, a diurnal temperature range of 10°C , and average temperatures as described below. As a check on the controller, temperatures were measured and recorded at 10-min intervals with small temperature loggers (Onset Corporation Optic StowAway Temp). All experiments were carried out under a 12-h light-dark cycle.

Organisms were checked for mortality every 24 h by gently prodding with a plastic pipette and inspecting for movement. If individual snails showed no movement after prodding they were transferred to a fresh cup of c. 16°C water to confirm survival or mortality. Live animals responded almost immediately to the cooler water and were then placed back in their test cups. Dead animals were replaced so that there were always two animals per cup. Replacement animals came from a separate container in each bath, which was subject to the same temperature regime. Any subsequent mortality of replacement animals was not included in the mortality results. Mayfly emergence was monitored c. every 12 h and emerged mayflies replaced as above. In this instance any subsequent mortality was included in the results.

Five diurnally varying temperature experiments were performed on *Deleatidium* at temperatures of 15, 20, 22.5, 25, and 30 ± 5°C, whereas seven were performed on *Potamopyrgus* at temperatures of 15, 20, 25, 27.5, 28.8, 30, and 35 ± 5°C. Four constant temperature experiments were carried out on *Deleatidium* at 20, 25, 27.5, and 30°C whereas six were carried out on *Potamopyrgus* at 20, 25, 30, 31.3, 32.5, and 35°C.

LT₅₀ values (the temperature at which 50% of the organisms were killed) and their 95% confidence intervals were calculated for each time period by probit analysis (Finney 1971) following the EPA flowchart procedure of the ToxCalc programme (Anon. 1993). For the diurnally varying experiments, two separate estimates of LT₅₀ were derived: the first (mean LT₅₀) by inputting the daily mean temperature into the ToxCalc programme, and the second (max LT₅₀) by inputting the daily maximum temperature. These two parameters were then compared with the LT₅₀ values derived from the constant temperature experiments (const LT₅₀).

RESULTS

Control mortality was below 7% in all experiments. No antagonistic behaviour was detected during any of the experiments.

Few mayflies died at either 20 or 15 ± 5°C, but significant mortality occurred at and above 25 and 22.5 ± 5°C (Fig. 1A). Over 96 h 50% mortality was exceeded for mayflies in the constant treatment at 25°C and in the diurnal treatment at 22.5 ± 5°C. At 30 and 30 ± 5°C all the mayflies died within 24 h. Mayflies were significantly more sensitive to high water temperatures than snails. Few snails died at either 30 or 25 ± 5°C, but significant mortality occurred at and above 31.3 and 28.8 ± 5°C (Fig. 1B). Over 96 h c. 80% mortality occurred for snails in the constant treatment at 31.3°C and in the diurnal treatment at 28.8 ± 5°C. At 35 and 35 ± 5°C all the snails died within 24 h.

Figure 2 shows the mean LT₅₀, const LT₅₀, and max LT₅₀ values estimated from our results plus const LT₅₀ values reported by Quinn et al. (1994). For *Deleatidium* the LT₅₀ values all decrease with exposure time which is a direct consequence of the fact that the fraction alive decreases with time (see Fig. 1). For *Potamopyrgus*, however, the LT₅₀ values after 48, 72, and 96 h exposure in our experiments are not significantly different, although each is lower than the LT₅₀ after 24 h exposure. This indicates that

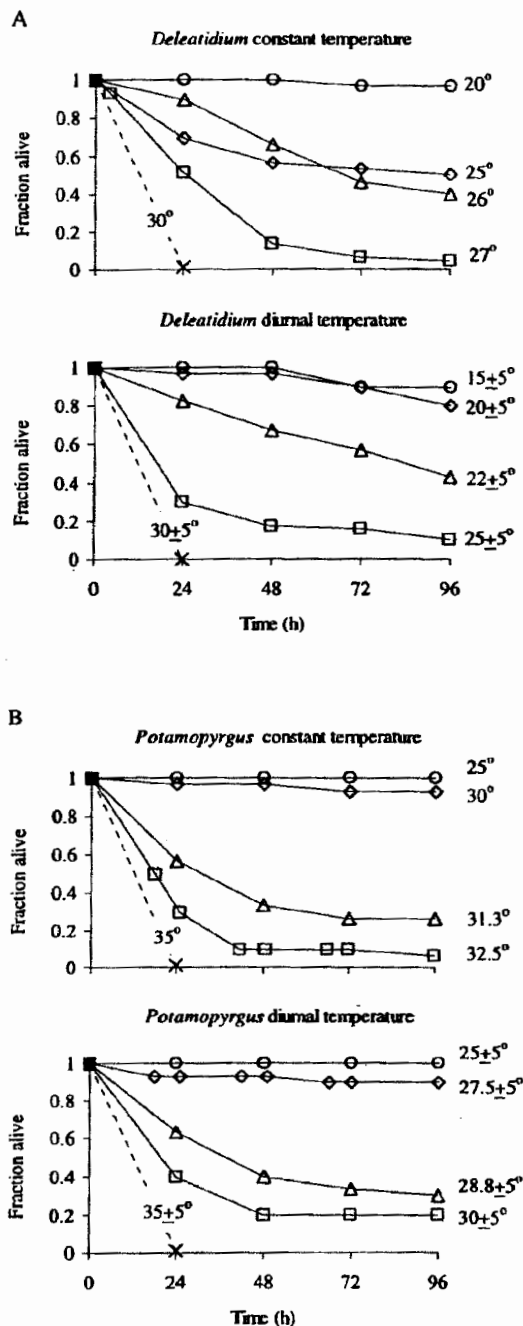


Fig. 1 A, Survival of *Deleatidium*; and B, *Potamopyrgus* exposed to constant and diurnally varying (±5°C) temperatures. All animals are acclimated at 16°C.

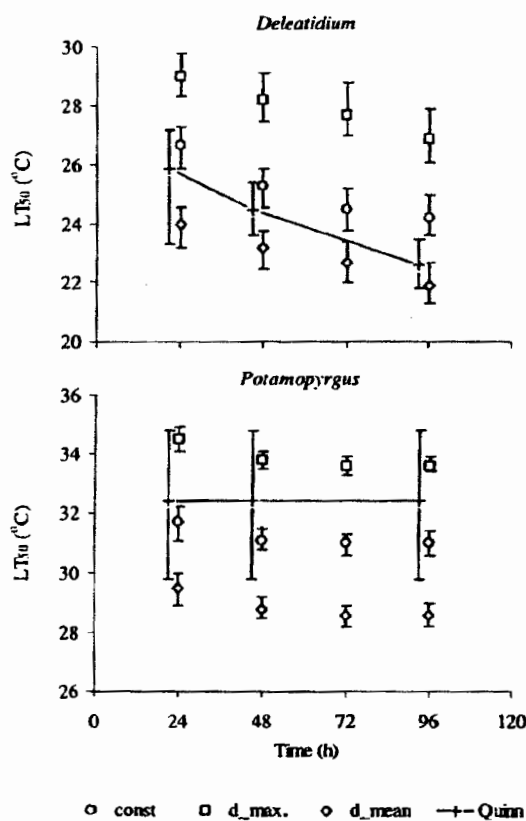


Fig. 2 Temperature causing 50% mortality (LT_{50}) versus exposure time (mean \pm 95% confidence interval). d_max and d_mean denote the daily maximum and mean temperatures at which 50% mortality occurred in the diurnally varying experiments, const denotes LT_{50} in the constant temperature experiments and Quinn denotes constant temperature LT_{50} values reported by Quinn et al. (1994).

some snails were able to acclimate to the high temperatures. Our estimates of $constLT_{50}$ are not significantly different from those reported by Quinn et al. (1994) as evidenced by the fact that the 95% confidence interval overlap. It should be noted, however, that Quinn's confidence intervals for *Potamopyrgus* are larger than ours, so that only very large differences would be detectable.

There is a significant difference (2-way ANOVA, $P < 0.01$) between our three estimates of LT_{50} . Considering mayflies, 50% mortality after 96 h occurs at a constant temperature of $24.2 \pm 0.9^\circ\text{C}$ but when the temperature varies diurnally it occurs when the daily mean is $21.9 \pm 0.7^\circ\text{C}$ and the daily maximum is $26.9 \pm 0.7^\circ\text{C}$. For snails 50% mortality occurs at a constant temperature of $31 \pm 0.3^\circ\text{C}$, a daily mean of $28.6 \pm 0.4^\circ\text{C}$, and a daily maximum of $33.6 \pm 0.4^\circ\text{C}$. Thus the 50% lethal temperatures are ranked $meanLT_{50} < constLT_{50} < maxLT_{50}$. The difference between our estimates of $maxLT_{50}$ and $meanLT_{50}$ is 5°C as expected and this provides a check on the consistency of the ToxCalc procedure.

Table 1 shows the ratios of the LT_{50} values for each time interval. The ratio $meanLT_{50}/constLT_{50}$ averages c. 0.9 which implies that when the temperature varies diurnally by $\pm 5^\circ\text{C}$, 50% mortality can be expected when the daily mean is c. 10% less than the 96-h LT_{50} measured at constant temperature. Conversely the ratio $maxLT_{50}/constLT_{50}$ averages c. 1.1 which implies that when the temperature varies diurnally by $\pm 5^\circ\text{C}$, 50% mortality can be expected when the daily maximum is c. 10% greater than the 96-h LT_{50} measured at constant temperature. These results can also be expressed as a temperature difference rather than a ratio. There is no significant difference between species or duration, nor between the differences ($constLT_{50} - meanLT_{50}$) and ($maxLT_{50} - constLT_{50}$). The overall mean difference is

Table 1 Ratio of temperatures causing 50% mortality (LT_{50}) in constant and diurnally varying temperature experiments. The 95% confidence interval is given in parentheses.

Ratio	24 h	48 h	72 h	96 h
Mayflies				
$meanLT_{50}/constLT_{50}$	0.900 (0.85–0.96)	0.917 (0.87–0.98)	0.926 (0.87–1.0)	0.905 (0.84–0.97)
$maxLT_{50}/constLT_{50}$	1.09 (1.04–1.15)	1.11 (1.06–1.18)	1.13 (1.07–1.21)	1.11 (1.04–1.23)
Snails				
$meanLT_{50}/constLT_{50}$	0.931 (0.90–0.96)	0.926 (0.90–0.94)	0.923 (0.90–0.94)	0.923 (0.90–0.94)
$maxLT_{50}/constLT_{50}$	1.09 (1.06–1.12)	1.09 (1.06–1.11)	1.08 (1.06–1.11)	1.08 (1.06–1.11)

$2.5 \pm 1.3^\circ\text{C}$ (mean \pm 95% confidence interval). Thus when the temperature varies diurnally $\pm 5^\circ\text{C}$, 50% mortality can be expected when the daily maximum is c. 2.5°C higher, or the daily mean is c. 2.5°C less, than the 96-h LT_{50} measured at constant temperature.

DISCUSSION

This study confirms the findings of Quinn et al. (1994) that the mayfly *Deleatidium autumnale* is significantly more susceptible to high temperature than the snail *Potamopyrgus antipodarum* and our estimates of LT_{50} are not significantly different from Quinn's. This study also shows that there is a significant difference between the temperature at which 50% of test animals die estimated from experiments using constant and diurnally varying temperature.

There are numerous published temperature limits for stream organisms derived from laboratory experiments in which test animals are exposed to constant, elevated temperature. Should such limits be applied to the daily mean or the daily maximum temperature when natural stream temperature varies diurnally? Our constant temperature experiments for mayflies indicate 50% mortality after 96 h at a temperature c. 25°C ($\text{const. LT}_{50} = 24.2 \pm 0.9^\circ\text{C}$). Were this limit applied to the daily mean temperature in a river where temperature varies diurnally by $\pm 5^\circ\text{C}$, the results of the $25 \pm 5^\circ\text{C}$ experiment indicate c. 90% mortality. Conversely were it applied to the daily maximum, the results of the $20 \pm 5^\circ\text{C}$ experiment indicate c. 20% mortality. In order to ensure 50% survival, the const. LT_{50} limit should be applied to a temperature midway between the daily mean and the daily maximum. This is confirmed by the $22.5 \pm 5^\circ\text{C}$ experiment which showed a c. 50% mortality.

Sweeney (1978) drew similar conclusions when investigating the response of mayfly egg development to varying temperatures. He states "...these data suggest that fluctuating (temperature) regimes are best characterised for egg hatching by a thermal value lying between average and maximum diel temperatures...". Costlow (1987) investigated the effects of temperature on survival, rates of development, and rates of larval growth for marine crabs. Results were often different under constant and diurnally varying temperatures with the best correlation often occurring at a temperature somewhere between the mean and the maximum of the diurnal regime.

Our experiments were conducted with only a single acclimation temperature (c. 16°C) and with regular diurnal temperature variations of amplitude 10°C (rather than the irregular patterns often observed in streams). We believe, however, that our main conclusions are fairly robust to such factors. Overall we believe that the results of this study will give stream managers increased confidence when applying laboratory-based temperature limits to natural streams.

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