

## Upper thermal tolerances of twelve New Zealand stream invertebrate species

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**Abstract** The upper thermal tolerances of 12 New Zealand freshwater invertebrate taxa were compared using a laboratory lethality testing protocol. Temperatures that were lethal to 50% of the test organisms (LT<sub>50</sub>s), following acclimation to 15°C, generally declined over the 4 day period of the tests. LT<sub>50</sub> values after 48 and 96 h exposure ranged from 24.5 to > 34°C and 22.6 to 32.6°C, respectively, indicating that temperatures that occur in summer in many NZ streams and rivers may limit the distribution and abundance of some of these invertebrate species. Larval insects included both the most sensitive species (a plecopteran and two Ephemeroptera) and the most tolerant (the larvae of the elmid beetle *Hydora* sp.) in this study. The two molluscs studied (*Potamopyrgus antipodarum* (Prosobranchia: Hydrobiidae) and *Sphaerium novaezelandiae* (Bivalvia: Sphaeriidae)) showed high thermal tolerance, whilst two crustaceans (*Paratya curvirostris* (Athyidae) and *Paracalliope fluviatilis* (Eusiridae)) were moderately tolerant. The effect of a higher acclimation temperature (20°C) on thermal tolerance was also investigated for the three most sensitive species. This appeared to increase the thermal tolerance of the two Ephemeroptera (*Deleatidium* spp. and

*Zephlebia dentata* (both Leptophlebiidae)) but to reduce the tolerance of the plecopteran *Zelandobius furcillatus* (Gripopterygidae).

**Keywords** benthic invertebrates; streams; temperature tolerance; lethality; thermal; laboratory tests

### INTRODUCTION

Temperature is one of the primary factors influencing growth, metabolism, and survivorship of stream invertebrates (Hynes 1970; Sweeney 1978; Sweeney & Vannote 1986; Rempel & Carter 1987; Brittain 1991). A variety of human activities can affect thermal regimes in streams and rivers, including discharge of heated effluents from industry and thermal/geothermal power plants; impoundment discharges (Brooker 1981); abstraction (Dymond 1984); and removal of riparian shade (Beschta & Taylor 1988). Information on the effects of temperature on stream invertebrates is required for management of these activities and to understand the role of this prime forcing factor in determining invertebrate community structure and function.

The limited data available on thermal tolerances of New Zealand freshwater invertebrate species indicate a wide range of tolerances. Studies of the faunas of thermal and warm springs (Winterbourn 1968; Stark et al. 1976; James 1985) showed that some Coleoptera and Diptera can tolerate temperatures of 40–45°C and some other Diptera, Hemiptera, Odonata, Ostracoda and Pulmonata tolerate temperatures around 35°C. However, Plecoptera appear to be largely restricted to rivers with summer temperatures typically below 19°C (Quinn & Hickey 1990). Notably, the thermal tolerances of the vast majority of the common taxa in New Zealand streams and rivers are unknown.

Recently, Hickey & Vickers (1992) developed a standard 96 h LC<sub>50</sub> test for measuring the toxicity of substances to the mayfly *Deleatidium* spp. This

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technique also has potential for screening thermal tolerances of running water invertebrates. In contrast to the elaborate artificial stream systems used for testing thermal tolerances of stream invertebrates by several previous workers (e.g. Nebeker & Lemke 1968; Gauvin & Hern 1971; de Kowzowski & Bunting 1981), the method of Hickey & Vickers (1992) has low establishment costs and allows ample replication and concurrent, parallel studies of a number of species. This study aimed to evaluate the applicability of these methods to measurement of thermal tolerances of stream biota from a range of taxonomic groups (oligochaetes, molluscs, crustaceans and insects) and to provide information on the tolerances of a range of the "common core" taxa that are found in "unmodified" streams and rivers throughout New Zealand.

## MATERIALS AND METHODS

Most of the organisms were collected from a site on the Waihou River (175° 49'E, 38° 1'S) in the Waikato region of the North Island between July and October 1992 by sampling the sediment and macrophytes using a 500 µm mesh hand net. Information on this spring-fed river's thermal regime is limited, but the similar Waimakariri River (5 km east) 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 (NIWA database, Christchurch). Water temperature of the Waihou River varied between 12.1°C and 14.3°C on the sampling occasions. The thermal stability of the sampling site allowed us to collect animals exposed to similar thermal acclimation temperatures over the four month period of the study.

The Waihou River originates in an area of native and *Pinus* plantation forest on the Mamaku Plateau but the unshaded sampling site is in an area of pastoral agriculture. This site provides a wide range of microhabitats, from gravel riffles to dense beds of the macrophytes *Ranunculus fluitans* and *Nasturtium officinale*, and has a highly diverse and abundant invertebrate fauna. *Deleatidium* spp. (Ephemeroptera: Leptophlebiidae), *Pycnocentroides aureola* (Trichoptera: Conoesucidae), *Aoteapsyche colonica* (Trichoptera: Hydropsychidae), and *Hydora* sp. (Coleoptera: Elmidae) were common in the gravel riffle areas whereas *Potamopyrgus antipodarum* (Prosobranchia: Hydrobiidae) and *Lumbriculus variegatus* (Oligochaeta: Lumbriculidae) were abundant in detritus-rich riffles

downstream of macrophyte beds. *Pycnocentria evecta* (Trichoptera: Conoesucidae), *Zephlebia dentata* (Ephemeroptera: Leptophlebiidae), and *Zelandobius furcillatus* (Plecoptera: Gripopterygidae) were common on the macrophytes. *Sphaerium novaezealandiae* (Bivalvia: Sphaeriidae) were collected from near the outlet of Lake Rotoiti (176° 21'E, 38° 1'S) using a bottom dredge in October 1992, when an average water temperature of approximately 15°C was expected (pers comm. M. Gibbs). This species is widely distributed in sandy areas of streams and rivers. The two crustacean species (*Paracalliope fluviatilis* (Amphipoda: Eusiridae), and *Paratya curvirostris* (Decapoda: Atyidae)) were collected from a site on the lower Waikato River opposite the power station at Meremere (175° 4'E, 37° 18'S) in October 1992, when the water temperature was 14.7°C.

Middle to late instar specimens of the larval insects and crustaceans, and medium to large molluscs, were selected from the hand-net collections, using wide-mouthed pipettes, and transported back to the laboratory in continuously aerated river water in 4 litre PVC pails. The river water was replaced with preconditioned tap water (treated Waikato River water) on arrival at the laboratory. Initially, preconditioning of the water involved aeration for at least 24 h, but later tests were performed using a single large batch of water that had been filtered through activated carbon and aerated for one week. This water had alkalinity of 38 g m<sup>-3</sup> (as Ca CO<sub>3</sub>), hardness 41 g m<sup>-3</sup> (as Ca CO<sub>3</sub>), conductivity 20 mS m<sup>-1</sup> and pH of 7.9. Organisms were transferred to the preconditioned tap water and kept under these conditions for 24 h to allow gut clearance, which minimised fouling of sample vessels, and to ensure acclimation to the control temperature of 15°C, which was very close to the stream temperature at the collection sites. The organisms were then transferred to the experimental apparatus in a laboratory with a natural diurnal light regime provided by external windows.

Two designs of incubation apparatus were used in these experiments. In the first two experiments five organisms were placed in 60 ml plastic cups filled with 40 ml of preconditioned tap water at 15°C. These cups (five replicates per temperature) were mounted in 5 cm diameter holes in polystyrene sheeting (0.25 × 0.25 m) covered with Perspex™ sheeting and floating on the surface of waterbaths. Aeration and current was supplied to each cup via fine bore (0.6 mm ID × 50 mm) tubing through

holes in the Perspex™ sheeting and connected via syringe needles (23 gauge) to larger tubing from an air pump.

This design was satisfactory in the first experiment which investigated three species of collector/browsers (*Deleatidium* spp., *Pycnocentroides aureola* and *Potamopyrgus antipodarum*). Mortality was less than 10% in the controls and the organisms showed no signs of antagonistic behaviour. However, in the second experiment, involving the free-living caddisfly *Aoteapsyche colonica*, individual cups were used to isolate each individual organism and prevent the antagonistic and cannibalistic interactions which would normally be observed. In subsequent experiments twenty or thirty replicates in individual cups were used for all species at each temperature.

Five temperatures (c. 15, 20, 25, 30 and 35°C) were used to determine the thermal tolerance of each species. These were achieved by manually raising the temperature from 15°C by 2 to 4°C every hour until the desired temperature was reached. This procedure avoids inducing thermal shock without allowing time for acclimation to raised temperatures (Gaufin & Hern 1971). The water temperature was maintained at the desired temperature ( $\pm 0.2^\circ\text{C}$ ) using a proportional temperature controller (Yellow Springs Instrument model 72). Temperature of the baths was evenly distributed using a mechanical stirrer and recorded continuously. Half of the incubation water was removed and replaced with preconditioned tap water at the appropriate temperature every 24 h, for the first experiment and for the large shrimp *Paratya curvirostris*, and every 48 h in the other experiments.

Survival was recorded at 24, 48 and 96 h after the target temperature was reached. Organisms showing no obvious movement, or gaping bivalves, were gently prodded or “jetted” with water from a pipette to check for life. Mollusc mortality was confirmed by checking for movement after one hour in 15°C water. All organisms were preserved in 70% isopropyl alcohol and their identities confirmed after each experiment.

If 90% control survival was obtained,  $LT_{50}$  values (the temperature at which 50% of the organisms were killed) and 95% fiducial limits were calculated for each time period by probit analysis (Finney 1971) following the EPA flowchart procedure of the ToxCalc programme (Anon 1993). A trimmed Spearman-Kärber analysis was used to estimate the  $LT_{50}$  values in cases where the data

did not fit the probit model, except when 100% survival and 100% mortality occurred at consecutive temperatures in which case the  $LT_{50}$  values were set at the geometric mean of the two bracketing temperatures.

After initial estimates of  $LT_{50}$  values had been obtained, experiments were run to obtain more precise estimates for the more sensitive taxa whose  $LT_{50}$  values indicated the greatest potential for temperature to significantly restrict distributions in New Zealand streams. This was achieved using a narrower range of temperatures, especially around the initial  $LT_{50}$  value.

Three species (*Zelandobius furcillatus*, *Deleatidium* spp. and *Zephlebia dentata*) were used to investigate how acclimation to higher temperatures affected thermal tolerance. Survival at 26°C (near the 48 h  $LT_{50}$  for 15°C acclimated organisms of these species) was compared for organisms held in the laboratory for 1 week at  $15.4 \pm 0.4^\circ\text{C}$  or  $20.0 \pm 0.4^\circ\text{C}$  in aquaria ( $0.3 \times 0.4 \times 0.3$  m) containing Waihou River water, stones with epilithon attached, detritus and macrophytes. During acclimation, aeration stones located behind a baffle at the end of the aquarium provided a gentle current over the bottom of the aquaria. After acclimation, the test organisms were held at their acclimation temperature in food-free water for 24 h and then transferred to the experimental apparatus where lethality was recorded after 24, 48 and 96 h at 26°C.

## RESULTS

The  $LT_{50}$  values and 95% confidence limits for all species tested are summarised in Table 1. The  $LT_{50}$  progressively decreased with time for most species, with the 96 h  $LT_{50}$  values ranging from 22.6°C to 32.6°C.

The insect species varied widely in their upper thermal tolerances. Larvae of the elmid *Hydora* sp. and the mayfly *Deleatidium* spp. yielded the highest and lowest median lethal temperatures at 96 h, respectively. The  $LT_{50}$  values declined with the period of exposure for both *Deleatidium* spp. and *Z. dentata*, indicating short-term tolerance of higher temperatures. The stonefly *Z. furcillatus* had a similar high level of temperature sensitivity to the two mayflies. Repeated measurements for these three species gave similar  $LT_{50}$  estimates.

Of the caddisfly larvae tested, *Pycnocentroides aureola* had the highest  $LT_{50}$  at 96 h, whereas the other two species, *Pycnocentria evecta* and *Aoteapsyche colonica*, had much lower values.

The snail *Potamopyrgus antipodarum* and the clam *Sphaerium novaezelandiae* had similar, relatively high, upper thermal tolerance. The two crustaceans tested had similar thermal tolerances to each other, that declined only slightly with time. In contrast, the  $LT_{50}$  of the oligochaete *Lumbriculus variegatus* declined by 5°C between 48 and 96 h.

The thermal acclimation results are shown in Fig. 1. These indicate that individuals of the two mayfly species (*Z. dentata* and *Deleatidium* spp.) that were acclimated at 20.4°C had higher tolerance to elevated temperature than those acclimated at 15.4°C. However, the stonefly *Z. furcillatus* showed lower thermal tolerance after acclimation at 20°C.

**DISCUSSION**

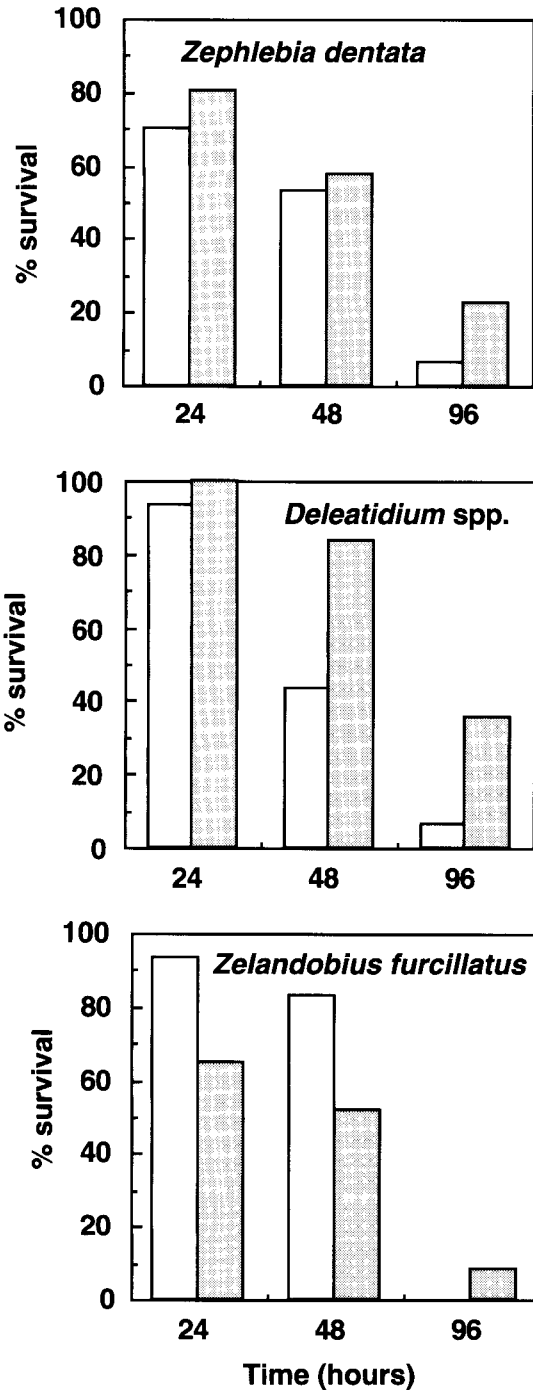
The apparatus used in these experiments allows ample replication and provides a convenient means of testing thermal tolerance of aquatic invertebrates from a variety of taxonomic groups including Ephemeroptera, Trichoptera, Plecoptera, Crustacea, Mollusca and Oligochaeta. Separate containers for

housing individual organisms were found necessary to avoid stress effects due to antagonistic interactions between some species. These also avoid the possible effects on water quality of rapid degradation following death of “companion” animals in shared containers at high temperatures.

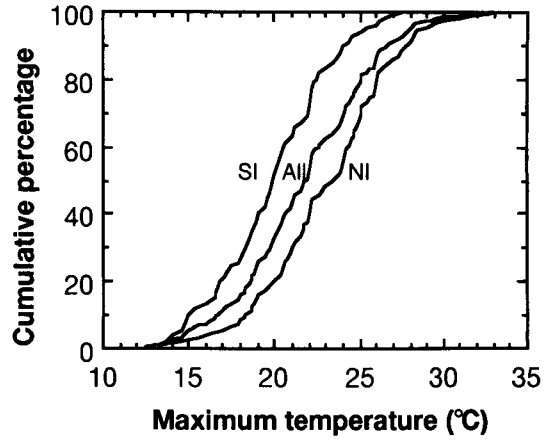
We observed a wide range of upper thermal tolerances amongst stream invertebrates. The general pattern of tolerances amongst families was consistent with observed distributions of invertebrates in New Zealand thermal and warm spring waters, which also indicate that Coleoptera are particularly tolerant (observed at temperatures of 34 to 45°C), Prosobranchia (*Potamopyrgus antipodarum*) moderately tolerant (observed at up to 28°C) and Lumbriculidae were relatively sensitive (observed at up to 24°C) (Winterbourn 1968). Notably, the upper limit of the natural distribution of *P. antipodarum* in the field (Winterbourn 1968; James 1985) is approximately 4°C lower than the upper lethal limit observed in this study and by Winterbourn (1969). This was explained by Winterbourn’s (1969) finding that

**Table 1**  $LT_{50}$  values with associated 95% fiducial limits (where given by probit analysis) for freshwater invertebrates at 24, 48 and 96 hours. § = no data due to power failure. † =  $LT_{50}$  calculated as geometric mean of lower temperature (0% lethality) and upper temperature (100% lethality). \* = confidence limits provided by lower temperature (0% lethality) and upper temperature (100% lethality). ‡ = data excluded due to 15% mortality in controls.

Taxa	24 hour		48 hour		96 hour	
<b>Insecta</b>						
<i>Deleatidium</i> spp.	(run 1)	26.8 (25.9–28.2)	24.5	(23.6–25.4)	22.6	(21.7–23.4)
	(run 2)	25.9 (24.6–28.5)	§		§	
<i>Sephlebia dentata</i>	(run 1)	26.9 (25.9–28.4)	25.3	(24.4–26.3)	23.6	(22.7–24.4)
	(run 2)	26.8 (25.4–30.4)	§		§	
<i>Zelandobius furcillatus</i>	(run 1)	26.0 (19.8–29.1)*	25.5	(24.0–27.3)	‡	‡
	(run 2)	c.28	26.5	(25.5–27.5)	‡	‡
<i>Aoteapsyche colonica</i>		27.8 (26.0–33.6)*	27.0	(21.0–29.2)*	25.9	(24.1–27.1)
<i>Pycnocentroides aureola</i>		32.4† (30–35)*	32.4†	(30–35)*	32.4†	(30–35)*
<i>Pyconocentria evecta</i>		30.4 (24.5–33.5)*	26.8	(24.5–29.1)*	25.0	(20–29.1)*
<i>Hydora</i> sp		>34	>34		32.6†	(31.4–34)*
<b>Mollusca</b>						
<i>Potamopyrgus antipodarum</i>		32.4† (30–35)*	32.4†	(30–35)*	32.4†	(30–35)*
<i>Sphaerium novaezelandiae</i>		32.8 (31.7–34)*	31.8	(28.3–34)*	30.5	(28.3–34)
<b>Crustacea</b>						
<i>Paratya curvirostris</i>		27.4 (24.8–31.4)	26.5	(25.6–27.5)	25.7	(24.6–26.8)
<i>Paracalliope fluviatilis</i>		27.5 (23.6–33.5)	26.3	(25.4–27.2)	24.1	(23.0–25.1)
<b>Oligochaeta</b>						
<i>Lumbriculus variegatus</i>		30.1 (28.9–31.8)	28.9	(27.1–31.7)	26.7	(24.9–28.7)



**Fig. 1** Comparison of survival of three invertebrate taxa at 26°C after acclimation at 15.4°C (open bars) and 20.4°C (shaded bars) for 1 week.



**Fig. 2** Cumulative frequency curves for maximum temperatures recorded at 106 and 149 river sites throughout the South Island (SI) and North Island (NI) of New Zealand, respectively, and both islands combined (All)(from data in Mosley (1982)).

the snail’s activity began to decline above 28°C. If this response is typical of other species, it suggests that 96 h LT<sub>50</sub> temperatures may be a few degrees higher than the temperatures limiting distribution in the field.

Selective pressures could result in greater thermal tolerance amongst populations of invertebrate species from streams with high summer temperatures than the same species in the relatively cool Waihou River, where most of the experimental animals were collected. The aerial dispersion of adult insects would be expected to reduce the likelihood of such local variations developing, but further work to examine this issue is warranted.

Comparison of the LT<sub>50</sub> data in Table 1 with the maximum temperatures recorded at 255 New Zealand river sites (Mosely 1982) indicates that temperature stress may be important in determining the distributions of several of the species investigated (Fig. 2). In particular, the maximum recorded temperatures exceeded the 96 h LT<sub>50</sub> values of the mayflies *Deleatidium* spp. and *Z. dentata* at 39%, and 35% of these sites. High temperature is more likely to be an important factor limiting lotic invertebrates in the North Island than in the South Island sites (Fig. 2).

Thermal stress is expected to affect distributions of invertebrates most commonly in lowland reaches of rivers, because maximum temperatures typically

increase progressively from headwaters to the mouths of rivers (Ward 1985). However, due to their shallow depth, headwater streams are particularly prone to heating if riparian vegetation is removed (Brown & Krygier 1970; Quinn et al. 1992), so that temperature effects on distribution are also possible in these situations.

The relatively low 96 h  $LT_{50}$  values recorded for the stonefly and mayfly taxa are consistent with the finding of Quinn & Hickey (1990) that Plecoptera and Ephemeroptera were much less abundant in rivers where typical summer temperatures exceeded 19°C and 21°C, respectively. The 96 h  $LT_{50}$  values of two of the New Zealand trichopteran species tested in this study were within the range of 21.8 to 30.1°C observed for six North American trichopteran species (Gaufin & Hern 1971), whereas *Pycnocentroides aureola* was more tolerant.

Acclimation to temperatures higher than 15°C may increase  $LT_{50}$  values somewhat (Cossins & Bowler 1987). When acclimation temperature was increased from 10 to 20°C, upper lethal temperatures of various freshwater Crustacea increased by up to 1.9°C (Spoor 1955; Sprague 1963) and 0.75°C for the stonefly, *Paragnetina media* (Heiman & Knight 1972). The increases we observed in survival of *Zephlebia dentata* and *Deleatidium* spp. at 26°C after acclimation at 20°C rather than 15.4°C also indicate relatively small acclimation effects on upper lethal temperatures. On the other hand, the lower 24- and 48-h survival of *Zelandobius furcillatus* acclimated at the higher temperature indicates that continued exposure of this species to temperatures approaching the incipient lethal temperature increased its sensitivity to high temperature. If the inability of this stonefly to acclimate to higher temperatures is typical for other New Zealand stonefly species, it may explain the more restricted distribution of stoneflies than mayflies in relation to maximum seasonal temperature in New Zealand rivers (Quinn & Hickey 1990), despite the similar 48 h  $LT_{50}$  values of the mayfly and stonefly species in this study (Table 1). However, other factors, such as differences in the thermal sensitivities of the eggs and sublethal effects could also be involved.

The results of this study provide both a ranking of a variety of common stream invertebrates in terms of their upper thermal tolerance and information on the upper bound of their tolerance to constant temperatures. In natural streams, however, temperature usually has diurnal and

annual fluctuations, so that comparisons of the effects of exposure to constant and diurnally varying temperatures would help application of the results to nature. Sublethal temperature increases can also affect the length of invertebrate life histories and the size and fecundity of emerging adult insects, which affects their reproductive output (e.g., Sweeney & Vannote 1986; Brittain 1991; Sweeney 1993). Longer-term studies on such sublethal effects would provide a better understanding of the effects of temperature increases and hence a sounder basis for temperature management. Nevertheless, the present methodologies and findings provide a useful starting point for such studies.

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