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The influence of climate change scenarios on populations of the mayfly *Cloeon dipterum*

Dermot McKee & David Atkinson
School of Biological Sciences, The University of Liverpool, Liverpool L69 3BX, U.K.
E-mail: dmckee@liv.ac.uk

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Abstract

Populations of the mayfly *Cloeon dipterum* from 48 ponds (3000 l fibre-glass tanks of 1 m depth) were monitored over the course of 1 year. To simulate possible patterns of climatic change, the ponds were subject to three temperature treatments: continuous heating to 3 °C above ambient; heating to 3 °C above ambient during the summer only; and no heating. Further experimental complexity included enhanced nutrient input into the ponds and the presence or absence of fish, giving a factorial combination of 3 temperature regimes × 2 nutrient levels × presence/absence of fish predation.

Few nymphs were found in the presence of fish. Where fish were absent, the temperature treatments did not significantly affect nymph abundances, and only marginally influenced mean nymph body-lengths. In contrast, the nutrient treatment had significant effects on both nymph abundance and size, with greater numbers of generally larger nymphs occurring in those fish-free ponds receiving additional nutrients. Adult emergence began earlier in the year from the heated ponds, particularly those ponds receiving additional nutrients. Adult body-length differed between temperature treatments, but consistent patterns were difficult to ascertain because of interactions with nutrient treatment and seasonal effects.

Our results show that during the short term at least, elevated temperature as a simulation of climate change does not have an overwhelming influence on either mayfly abundance or size. The influence of temperature is subtle and subject to complex interaction with other habitat variables. We therefore suggest that the direct consequences of small changes in temperature will likely be of little significance to *C. dipterum*, relative to indirect effects operating through interactions with predation and nutrient input.

Introduction

Thermal conditions strongly influence life-history strategies and population characteristics of aquatic insects. The major role of temperature in regulating growth, maturation and adult emergence is well known (e.g. Sweeney & Vannote, 1986; Söderström, 1988; Fuller & Fry, 1991; Giberson & Rosenberg, 1992; Watanabe et al., 1999). Indeed, temperature may be one of the main factors controlling the geographic distribution of aquatic insect populations (Vannote & Sweeney, 1980; Ward & Stanford, 1982; Ward, 1992).

Recent climate models suggest a significant and sustained increase in mean global air temperatures (Houghton et al., 1996; Conway, 1998). Whilst debate continues as to the precise magnitude and variability of this climatic change, particularly on a regional basis, one thing is certain: many aquatic ecosystems will become warmer, and warming will occur with unprecedented rapidity. Furthermore, we might anticipate that organisms found in those habitats with little capacity to buffer physical change may be particularly susceptible to this warming effect. Aquatic insects living in shallow bodies of freshwater fall into this category, and it is possible that the demography and distribution of many species will be affected.

Here, we examine the influence of a 3 °C rise in temperature on natural populations of the mayfly

Cloeon dipterum living in small, shallow, experimental ponds. A 3 °C rise in temperature, as used in this experiment, is in line with current predictions about climate change during the next century in the U.K. (Conway, 1998). We test the following prediction: elevated temperature should result in smaller populations of smaller nymphs, and smaller earlier emerging adults, particularly under conditions of resource limitation. Increasing temperature increases the metabolic rate of aquatic ectotherms leading to disproportionately high respiration relative to assimilation. As a result, faster growth rates and decreased body-sizes at higher temperatures are often observed (Atkinson, 1994). When resources are limiting, these effects are exacerbated, and increased competition, starvation and mortality may be more likely to occur (Odum, 1985).

Natural history and background

Cloeon dipterum (Ephemeroptera: Baetidae) occurs throughout Europe in bodies of still and slow moving water. Depending on geographical location, populations may be bivoltine or multivoltine, with animals overwintering as nymphs and adults flying from spring through to early-autumn (Elliott & Humpesch, 1983; Elliott et al., 1988). Nymphs are classified as swimming and climbing collector-gatherers, feeding mainly on filamentous algae, periphyton and organic detritus (Brown, 1961; Cianciara, 1980; Elliott et al., 1988). The number of nymphal instars is variable, and growth increments are not necessarily constant either within or between generations. Nevertheless, there is a strong log-linear correlation between nymph body-length and weight (Cianciara, 1980). On emergence, adults mate, and then the females rest in vegetation for some period of time before ovipositing into the water column where the eggs immediately hatch (Brittain, 1982; Elliott et al., 1988). However, eggs accidentally released from last instar nymphs and from subimagos can be viable, and parthenogenesis is thought to play a role in the reproduction of some, if not all, populations (Harker, 1997).

The animals examined here came from 48 ponds located at Ness Botanic Gardens near Liverpool, U.K. (53° 16′ N, 3° 02′ W). The ponds (3000 l fibreglass tanks of depth 1 m and diameter 2 m) were created during the spring and summer of 1998 and contain a layer of sediment, well established stands of macrophytes (*Elodea nuttallii*, *Lagarosiphon major*, *Potamogeton natans*), filamentous algae and diverse communities of invertebrates. *C. dipterum* colonised the ponds naturally. From October 1998, the ponds received a factorial series of experimental treatments (3 heating × 2 nutrient × 2 fish), giving four replicate ponds at each treatment level using a random block design. Sixteen ponds were continuously heated to 3 °C above ambient; 16 were heated to 3 °C above ambient during the ‘summer’ only (1st April to 30th September); and 16 were left unheated. Half the ponds received additions of a nutrient solution; and half contained populations of three-spined sticklebacks (*Gasterosteus aculeatus*).

Heating was achieved by pumping hot water from a boiler around the experimental site, and then through control valves and an element resting on the sediment of each pond. A sensor mounted in each pond provided a computer with constant information about water temperatures. Using unheated ponds as ambient temperature references, the computer regulated the flow rate of hot water through the elements of the heated ponds (by adjusting the control valves) so that a 3 °C temperature differential was continuously maintained. The system was highly reliable and operated at a gross sensitivity of ± 0.25 °C.

Nutrients were added to the relevant ponds every 3 weeks during the ‘winter’ (1st October to 31st March) and every 2 weeks during the summer. A solution of sodium nitrate ($NaNO_3$) and di-Sodium hydrogen orthophosphate (Na_2HPO_4) was used to give instantaneous concentrations of 0.5 mg nitrogen l^{-1} and 0.05 mg phosphorus l^{-1} during winter, and 0.17 mg nitrogen l^{-1} and 0.017 mg phosphorus l^{-1} during summer (assuming that each pond contained 2890 l of water).

Materials and methods

The ponds were sampled every 3 weeks during the winter (10 samples) and every 2 weeks during the summer (12 samples). A length of 64 mm diameter plastic drain-pipe was used to take three vertical columns of water (9 l) from each pond on each sampling date. To do this, the pipe was lowered to a position just above the sediment at randomly chosen locations in each pond (always including one column taken down the wall of the pond). The water was then mixed and filtered through a 50 μm nylon mesh screen. All nymphs thus retained were

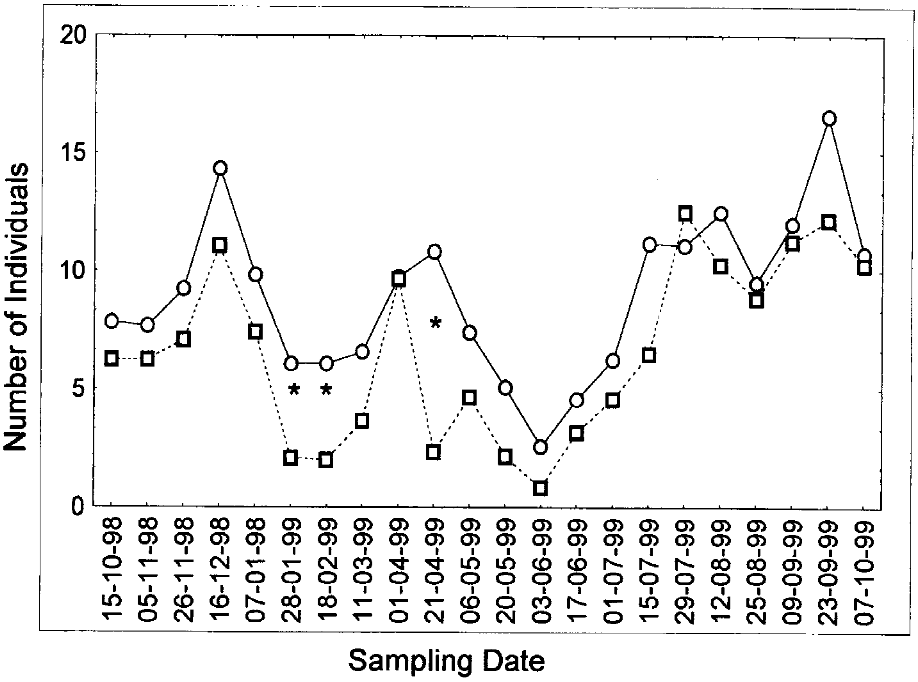

Figure 1. Variation in mean numbers of nymphs between nutrient treatments in fish-free ponds. Circles represent animals from ponds receiving additional nutrients; squares represent animals from ponds not receiving additional nutrients; asterisks indicate sampling dates where LSD tests showed statistically significant differences between pairs of means ($p < 0.05$).

preserved in 30% ethanol for immediate counting and measurement, before further storage in 80% ethanol.

Adults that had overwintered as nymphs were trapped as they emerged from the ponds during the spring (21-04-99–22-06-99). A second trapping session (18-08-99–07-10-99) caught summer generation adults. The traps were constructed using fine nylon mesh (domestic curtain material) attached to wooden supporting struts to give a pyramidal tent, covering 0.6 m^2 (20%) of the surface area of each pond. On a daily basis, freshly emerged sub-imagoes were removed from the traps and preserved in ethanol for immediate counting and measurement. Trapping was continuous during the two periods unless more than 100 males and 100 females were obtained from the ponds within a particular treatment. If and when this point was reached, the traps were removed from those ponds.

Body-length measurements of both nymphs and adults (i.e. sub-imagoes) were taken dorsally from the front of the head to the end of the last abdominal segment, using a dissecting microscope fitted with an eye-piece graticule. All data were log-transformed for analysis of variance (ANOVA).

Table 1. Mean number of nymphs caught per sample, followed by standard deviation and total number of individuals obtained over the year, from the ponds within each experimental treatment

Treatment	Mean	SD	*n*
Continuous heating + nutrients + fish	1.0	0.5	90
Continuous heating + fish	1.0	0.3	89
Summer heating + nutrients + fish	1.9	1.4	170
Summer heating + fish	0.9	0.6	79
No heating + nutrients + fish	1.5	0.9	134
No heating + fish	1.6	1.2	138
Continuous heating + nutrients	9.1	3.1	799
Continuous heating	5.7	3.1	492
Summer heating + nutrients	6.9	2.8	599
Summer heating	6.5	2.2	592
No heating + nutrients	11.0	2.1	970
No heating	7.6	4.0	681

Results

The ponds containing stickleback populations yielded few nymphs compared with the fish-free ponds (Table 1). Consequently, further analysis focuses only on data obtained from the fish-free ponds.

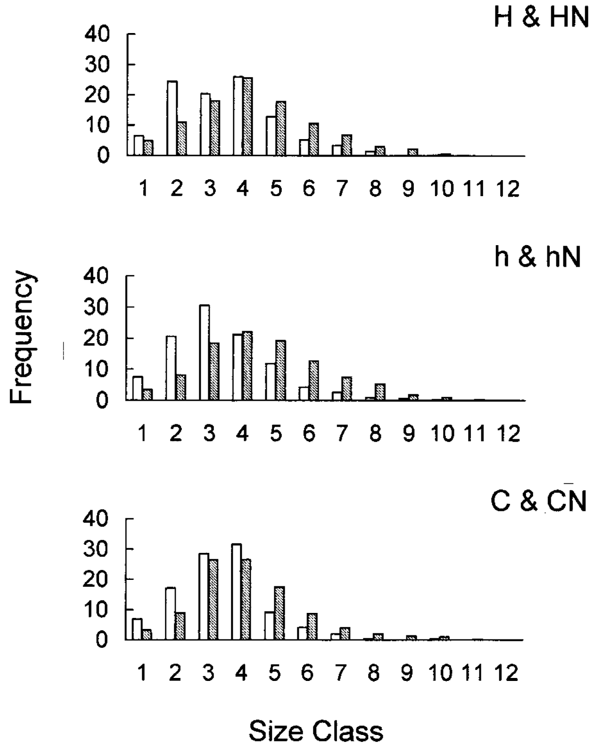

Figure 2. Body-length distributions of nymphs. Size class 1 represents animals up to 1 mm in length, size class 2 represents animals between 1 mm and 2 mm, and so on up to size class 12 which represents animals between 11 mm and 12 mm. Shaded bars are animals from ponds receiving additional nutrient input. H = continuously heated ponds; h = summer heated ponds; C = unheated ponds; N = additional nutrient input.

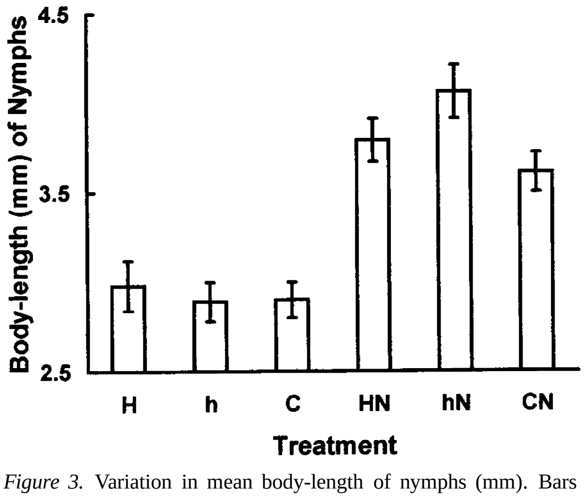

Figure 3. Variation in mean body-length of nymphs (mm). Bars are 95% confidence intervals. H = continuously heated ponds; h = summer heated ponds; C = unheated ponds; N = additional nutrient input.

Table 2. Results of repeated measures ANOVA of nymph numbers in the fish-free ponds

Effect	*F*	*p*-level
Temperature	$F_{2,18} = 0.60$	0.5999
Nutrients	$F_{1,18} = 5.71$	0.0280*
Repeated-measures factor (time)	$F_{21,378} = 6.95$	0.0001***
Temperature × nutrients	$F_{2,18} = 1.08$	0.3622
Temperature × time	$F_{42,378} = 1.20$	0.1899
Nutrients × time	$F_{21,378} = 0.72$	0.8098
Temperature × nutrients × time	$F_{42,378} = 1.14$	0.2674

Repeated measures ANOVA indicated that nymph numbers varied significantly with time and with nutrient treatment, but were not significantly influenced by temperature treatment (Table 2). Populations peaked during the late summer, and tended to be larger in the ponds that received additional nutrients (Fig. 1), although LSD tests showed this trend to be statistically significant on only three occasions during the winter. This observation was confirmed when the data were split into the winter and summer sampling periods, and reanalysed using repeated-measures ANOVA as before. Again, nymph numbers varied significantly with time, but were only significantly affected by the nutrient treatment during the winter period ($F_{1,18} = 4.64$, $p = 0.0449$). No other terms in the models were significant.

Body-length distributions for nymphs from each fish-free experimental treatment are shown in Fig. 2. The majority of nymphs throughout the year were in the size range 1–6 mm. Newly-hatched nymphs (size class <1 mm) represented 3–7% of the total number of individuals examined and appeared only for short periods of time corresponding to adult egg-laying during the summer. Nymphs >6 mm were relatively rare, occurring with greatest frequency in the ponds that received additional nutrients. Distributions from the ponds that did not receive additional nutrients were more skewed towards the smaller size classes of nymphs. Indeed, one-way ANOVA suggested that there were significant differences in overall mean nymph body-lengths between treatments ($F_{5,4127} = 58.26$, $p < 0.0001$). Subsequent comparison of the ANOVA means (Tukey HSD for unequal sample sizes) showed that the data split into two main groups depending on nutrient treatment. Nymphs from the ponds that did not receive additional nutrients were significantly smaller than their counterparts from the ponds that did receive additional nutrients ($p < 0.0001$ for all comparisons). The only apparent significant effect of the temperature treatments on nymph lengths occurred in the ponds that received additional nutrients, where mean length was significantly greater in the summer only heated ponds compared with the unheated ponds ($p = 0.0027$), but not with the continuously heated ponds ($p = 0.0942$). Figure 3 illustrates these patterns.

The first adults of the season emerged on the 21st April, and the last on the 27th September. Emergence from the heated ponds began upto 2 weeks earlier than emergence from the unheated ponds (Fig. 4). Over the two sampling periods, greater numbers of individuals emerged from the ponds that received additional nutrients (Fig. 4). Emergence from the unheated ponds that did not receive additional nutrients was particularly poor.

Adult females were consistently larger than adult males and, for both sexes, individuals from the over-winter generation were consistently larger than those from the summer generation (Fig. 5). Treatment effects on body-length were more apparent for individuals from the over-winter generation. Here, the largest individuals emerged where additional nutrients were supplied to the summer only heated ponds and the unheated ponds. The smallest individuals came from the summer only heated ponds and the unheated ponds that did not receive additional nutrients. Individuals from the continuously heated ponds were intermediate in size. For the summer generation, individuals from the summer only heated ponds were consistently the smallest; individuals from the other ponds were larger and generally similar in size.

Discussion

Even though the temperature changes associated with climate change are relatively small, they may have major consequences for some aquatic species (Schindler, 1997). Inability to survive in a particular warmed habitat may be a response to general mean temperatures, or may be a response to extreme events occurring at certain times of the year. For example, unusually hot days during the summer may push an organism past its upper temperature tolerance limits, or milder conditions during the winter may disrupt diapausing life-history stages. On the other hand, non-lethal effects, such as the promotion of faster nymphal growth and smaller size at maturity, are perhaps more likely to be observed in natural populations subject to gradual shifts in environmental temperature (Hogg & Williams, 1996). Changes in life-history parameters, particularly with regard to maturation, may affect a species population characteristics because body-size and timing of reproduction are correlated with fitness (Stearns, 1992).

Here, our temperature treatments did not apparently affect the survivorship of *C. dipterum* nymphs throughout the year, and had no significant influence on population sizes. The continuous heating treatment began in the autumn after the point at which nymphal recruitment stopped for the season. Loss of nymphs from the populations could not have been compensated for over the winter, but variation in mortality between ponds based on temperature differences was not observed. During the summer, temperatures in the heated ponds clearly did not exceed lethal limits, and population sizes were high and similar to those in the unheated ponds. Over this period, we infer from the occurrence of newly-hatched nymphs that adult females did not express a choice for their ovipositing sites based on water temperatures alone, as similar numbers of newly-hatched nymphs were recruited into the populations across the temperature treatments. A reduction in total densities of stream invertebrates following commencement of a global-warming thermal regime has been recorded by other workers (Hogg & Williams, 1996), although species richness and community biomass did not change, and individualistic population responses by species are probably to be expected.

We found a general lack of temperature effects on mean nymph body-lengths, even though many laboratory studies show temperature to be one of the primary factors influencing the size and growth of aquatic insect nymphs (e.g. Humpesch, 1981; Giberson & Rosenberg, 1992). Our null hypothesis anticipated that nymphs from the continuously heated ponds would be significantly smaller than nymphs from the unheated ponds, with those from the summer only heated ponds being intermediate in size. In fact, nymphs from the summer only heated ponds receiving additional nutrients showed a tendency to be the largest, leading us to speculate that rapidly rising temperatures during the spring (linked to the heating being switched on in these ponds at the beginning of April) might promote significantly increased assimilation and growth rates in over wintering individuals. However, no specific season could be linked to these larger sizes after breakdown of the data.

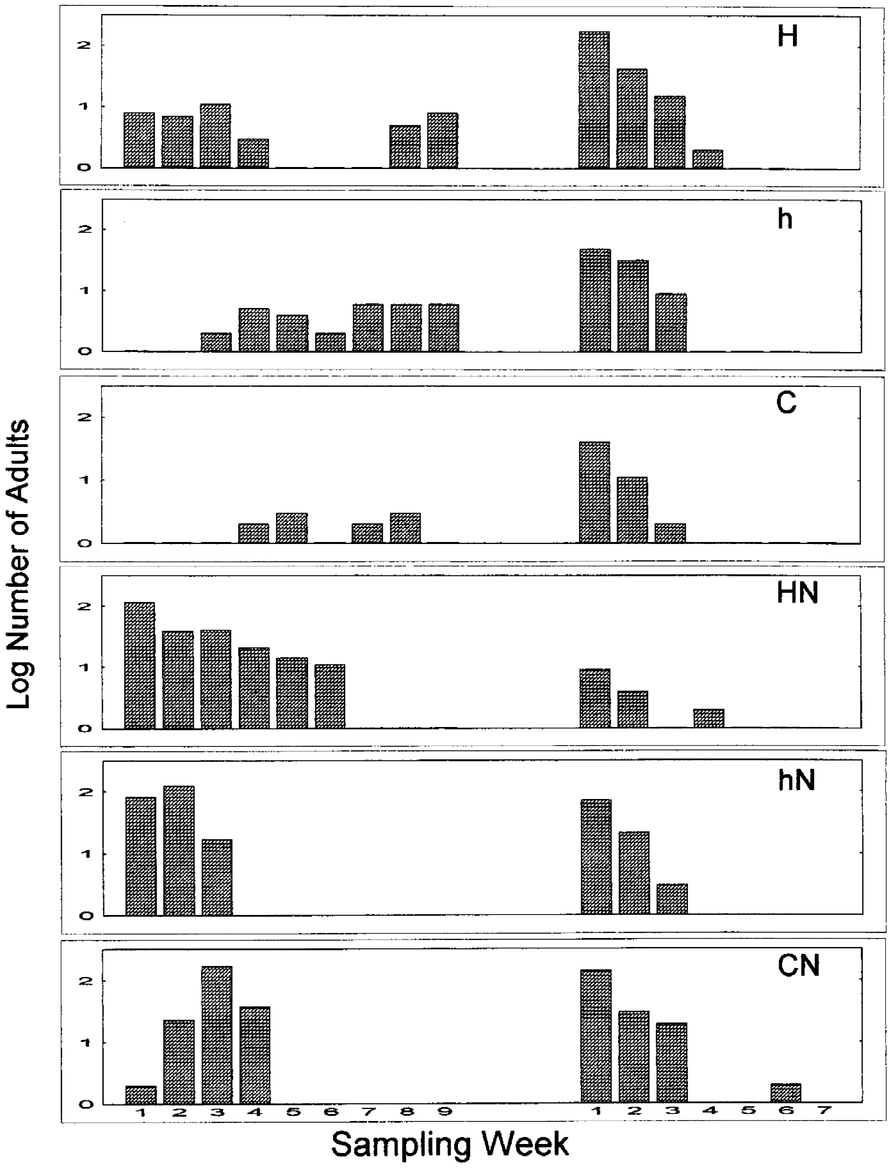

Figure 4. Numbers of adults trapped (log-scale) over consecutive weeks of sampling periods (beginning 21-04-99 and 18-08-99, respectively). H = continuously heated ponds; h = summer heated ponds; C = unheated ponds; N = additional nutrient input.

In contrast to the temperature treatments, the general effects of fish predation and enhanced nutrient levels were strong and clear cut. When sticklebacks were present, nymph population sizes were dramatically reduced. This was due to the direct predatory activities of the fish, although other potential predators (e.g. odonate nymphs and notonectids) also occurred in the ponds, even when sticklebacks were present. Interestingly, though survival chances were low, small nymphs did appear in the ponds containing fish, again posing questions about female choice of ovipositing sites. Adult egg-laying females were either unable to detect the presence of sticklebacks or other criteria outweighed this factor, although avoidance of oviposition sites where fish kairomones are present has been demonstrated for other pond-dwelling insects whose larvae are susceptible to fish predation (Berendonk, 1999). When there was additional nutrient input, nymph populations tended to be larger and mean individual body-lengths greater. Increased levels of phosphate and nitrate probably led to increased quantities and/or qualities of food for the nymphs. Im-

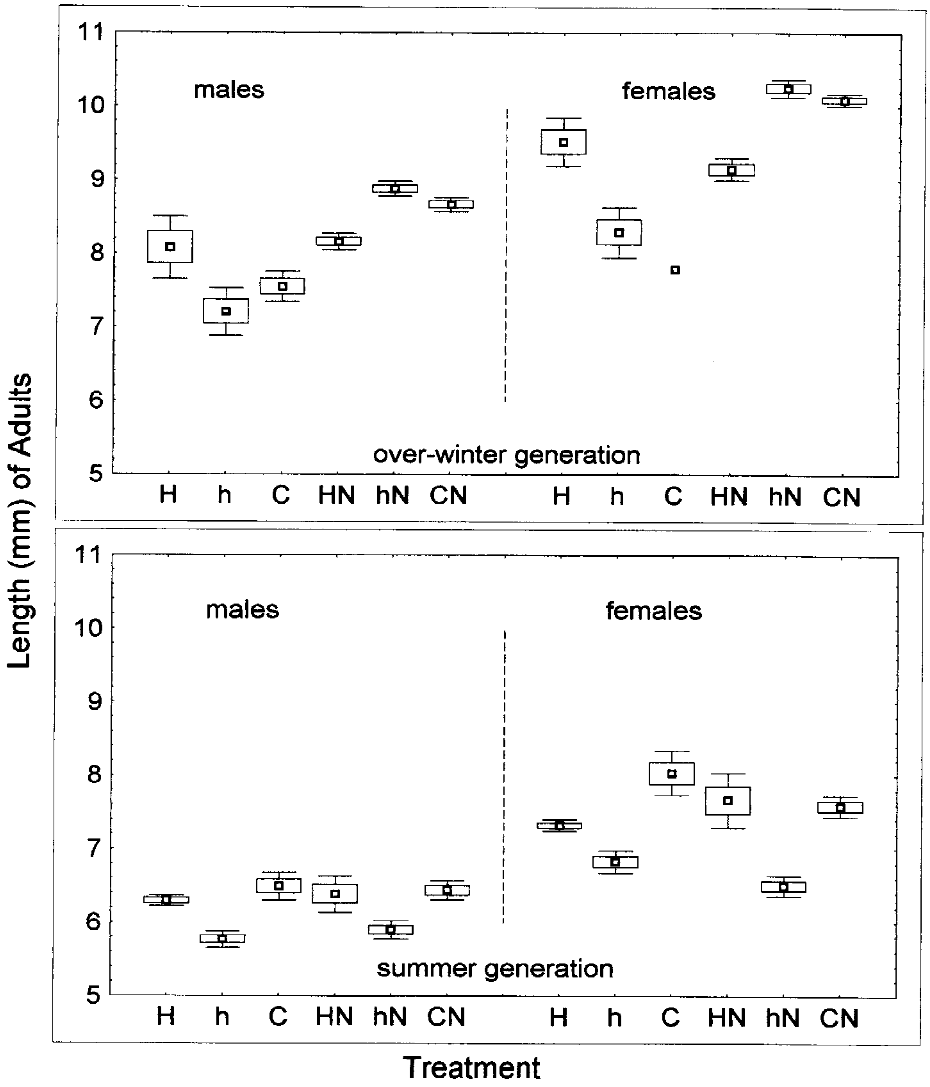

Figure 5. Adult body-lengths (mm). H = continuously heated ponds; h = summer heated ponds; C = unheated ponds; N = additional nutrient input. Bars are 95% confidence intervals.

proved food resources can reduce density dependent mortality, and can lead to higher individual growth rates and body-sizes (Söderström, 1988; Giberson & Rosenberg, 1992).

Adult emergence from the heated ponds upto 2 weeks early is a similar result to those obtained from a study of an experimentally heated natural stream channel (Hogg & Williams, 1996) and from a thermally polluted river below a power station (Langford, 1975). Adult body-length was variable, depending on sex, time of emergence, and heating and nutrient treatments. Female mayflies are generally larger than males, and in those species with a protracted emergence period a negative relationship between emergence date and body-size is often apparent (Brittain, 1982; Hogg & Williams, 1996; Corkum et al., 1997). Furthermore, larger females tend to produce greater numbers of eggs (Brittain, 1982). We recorded complex and difficult to explain body-length patterns across the experimental treatments. Anticipated negative relationships between adult size and temperature were not consistent, although such trends have been demonstrated by other workers (e.g. Sweeney & Vannote, 1984; Söderström, 1988; Hogg & Williams, 1996). Moreover, even though on average nymphs were significantly larger in the ponds receiving additional nutrients, this pattern was not strongly reflected in the adults. Disparity in body-lengths was least for adults from the summer generation, suggesting that faster growth under naturally more conducive conditions relative to winter (i.e. overall warmer temperatures and more abundant food resources) minimised the impact of the experimental treatments.

Few elements of our initial prediction about how *C. dipterum* populations in the ponds would respond to our experimental treatments were strongly supported. However, before firm and general conclusions can be made about potential responses to climatic warming, further study is needed. In particular, we recognise the possible limitations of studies where both the aquatic juvenile stage and terrestrial adult stage are not both exposed to elevated temperatures. Temperature effects on adult behaviour, and on egg size and development during the adult stage, could easily influence population and community processes, especially over the long term. Nevertheless, we surmise that plasticity in growth rate and developmental time will enable *C. dipterum* to respond to small changes in environmental temperature with minimal overall effects on abundance and size of individuals.

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