INTER- AND INTRASPECIFIC VARIATION IN ACUTE THERMAL TOLERANCE OF DAPHNIA[1]

HUGH J. MACISAAC,[2] PAUL D. N. HEBERT, AND STEVEN S. SCHWARTZ

Biology Department, University of Windsor, Windsor, Ontario N9B 3P4

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The acute thermal tolerances of six *Daphnia* species were compared at three acclimation temperatures. Of the species tested, all but one showed an increase in thermal tolerance when acclimated at higher temperature. Comparison of the regression relationships between thermal tolerances and acclimation temperature revealed no significant interspecific differences among species in their improvement of thermal tolerance caused by acclimation. However, significant intraspecific differences in thermal tolerance were noted. *Daphnia obtusa* had the highest tolerance, while *D. pulex* had the lowest. Among the other species acclimated at 10 C, thermal tolerance declined in a sequence of *D. ambigua, D. magna, D. carinata,* and *D. nivalis.* The differences in thermal tolerance noted among species were correlated with maximum temperatures of the environment from which they originated. While comparison of clones of *D. pulex* from several geographic localities revealed up to 2 C differences in acute thermal tolerance, there was no evidence of clonal variation in thermal tolerance of *D. magna.*

INTRODUCTION

The importance of temperature in regulating both the seasonal distribution and geographic distribution of aquatic ectotherms has long been recognized (Brown 1929; Moore 1939; Johnson 1952). Among such animals, those living in relatively shallow-water environments, such as ponds or tide pools, must have the ability to tolerate particularly large and often rapid shifts in temperature. While tide pool and intertidal organisms have received attention (e.g., the harpacticoid copepods studied by Kontogiannis [1973] and intertidal snails studied by Fraenkel [1968]), as have some benthic freshwater species (Sprague 1963), there have been few studies on the thermal tolerance of freshwater zooplankton.

Since many Cladocera are found in ponds in which daily and seasonal tem-

perature fluctuations may be great, and because they have a broad geographic distribution, their thermal tolerance is of particular interest. However, little work on the thermal tolerance of this group has been done since pioneering efforts over 50 yr ago. This work suggested that differences in thermal tolerance were present among closely related species and that intraspecific variation could be dramatic. For example, Banta (1939) identified a clone of *Daphnia laevis* with a thermal tolerance 9 C higher than normal for the species, while Johnson (1952) found a clone of *D. atkinsoni* with a thermal tolerance 6 C lower than normal. More recently, Craddock (1973) found evidence of variation in thermal tolerance between two *D. pulex* clones, while Goss (1980) found differences between *D. pulex* and *D. magna*. These later studies also examined the effects of acclimation temperatures on thermal tolerance (Craddock 1973; Goss 1980). Placing these results in an adaptive context is difficult because of both the limited number of strains examined and the fact that laboratory stocks of unknown geographic origin were studied. The present investigation aimed to provide more comprehensive data on the nature and extent of inter- and intraspecific variation in thermal tolerances of *Daphnia* species. Twenty-one clones representing six species and both subgenera of the

genus *Daphnia* were included. The study was designed to identify both differences in thermal tolerances and differences in the effects of acclimation on such tolerances. In addition, two species were selected for a more intensive study of geographical variation in thermal tolerance.

MATERIAL AND METHODS

A total of 21 different clones from six species of *Daphnia* were used in this study. Each of these clones was derived from a single parthenogenetic female. The clones were obtained from ponds with a depth of less than 1 m. Collection sites for each clone are listed in table 1 along with information on the mean daily maximum temperature of the warmest month of the year at the site (Atmospheric Environment Service 1941–1970; Wallen 1970; Gentilli 1971). Such temperatures give an indication of the maximum water temperature that an aquatic organism at that site must tolerate.

Each clone was acclimated in synthetic pond water (Hebert and Crease 1980) at 10 C, 16 C, and 25 C for a minimum of 2 wk. An algal suspension (primarily *Scenedesmus* and *Chroococcus*) was fed to each clone every 3 days. The thermal tolerance of a clone was tested by exposing adult individuals to temperatures in the range of 30–42 C. Temperatures were maintained with a Brown-Melsunger Thermomix circulating water bath. Survivorship was initially scored at 2 C intervals, but additional experiments at 1 C intervals were run when the original data were not sufficient for probit analysis. The 30 C test served as a control as preliminary experiments indicated that all stocks tolerated this temperature without mortality.

Temperature tolerance was assessed by taking 7–10 adult animals from each clone and placing them immediately into a 10-ml test tube containing synthetic pond water at the test temperature. The animals were left for 15 min and then removed into a 25-ml cup of synthetic pond water at room temperature. Mortality was assessed after a recovery period of 30 min. Animals which remained inactive at the bottom of the cup even when the cup was agitated were considered dead. This criterion was considered appropriate as all such inactive animals were dead when examined after 24 h. Experiments were run in triplicate, and the results of the

TABLE 1

SPECIES USED, COLLECTION SITES, AND MEAN TEMPERATURE OF THE WARMEST MONTH THERE

Species	Clone No.	Locality	Summer Temperature (C)
Daphnia ambigua	1	Neoga, Illinois	31.0
D. ambigua	2	St. Louis, Missouri	31.0
D. carinata	3	Canberra, New South Wales	28.1
D. magna	4	Cambridge, England	22.2
D. magna	5	Cambridge, England	22.2
D. magna	6	Churchill, Manitoba	16.9
D. magna	7	Helsinki, Finland	22.0
D. magna	8	Oslo, Norway	22.0
D. magna	9	Ostea Mallar Spik, Finland	22.0
D. magna	10	Tjernsrudtjern, Norway	22.0
D. nivalis	11	Launceston, Tasmania	24.8
D. obtusa	12	Urbana, Illinois	27.0
D. pulex	13	Baie du Dore, Ontario	26.1
D. pulex	14	Churchill, Manitoba	16.9
D. pulex	15	Frobisher Bay, Northwest Terr.	11.9
D. pulex	16	Helsinki, Finland	22.0
D. pulex	17	Igloolik, Northwest Terr.	7.7
D. pulex	18	Tuktoyaktuk, Northwest Terr.	14.8
D. pulex	19	Urbana, Illinois	27.0
D. pulex	20	Windsor, Ontario	27.7
D. pulex	21	Windsor, Ontario	27.7
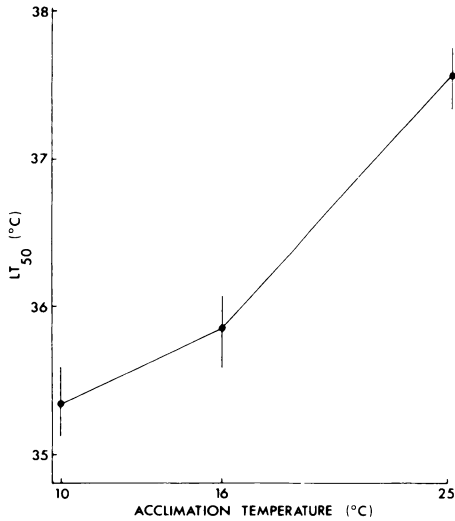
FIG. 1.—The relationship between LT_{50} (±SE) and acclimation temperature for 21 clones of *Daphnia*.

the 25 C acclimation and the two lower temperatures. *Daphnia nivalis* failed to show any evidence of thermal acclimation. However, comparison of the regression relationship between LT_{50} and acclimation temperature for each of the six species, using pooled clone data for each, revealed that there was no significant difference in the slopes of the regressions ($P > .05$), although the intercepts did vary significantly ($P < .01$, $F = 9.29$, df = 5,104) (Weisberg 1980). This latter difference indicates that species differ in their thermal tolerance but not in the degree to which acclimation affects their thermal tolerance.

At each of the three acclimation temperatures, the LT_{50} of the most sensitive clone was approximately 5 C less than that of the most resistant clone. When clones were listed in order of their LT_{50} at 10 C,

three treatments were pooled before probit analysis. The entire experiment was replicated 1 mo after the initial run in an attempt to standardize for variation in thermal tolerance that might be due to physiological changes related to nutritional status or age of adults in a specific clone. Thus, two thermal tolerance values were estimated for each clone at each of the three acclimation temperatures. Fifteen-minute-LT_{50} values were calculated for each experiment, using probit analysis. Skewness and kurtosis statistics were calculated for the 15-min-LT_{50} values recorded for clones at each of the three acclimation temperatures and also after the entire data set had been pooled. None of these statistics was significant at the 5% level, indicating that the data sets approximated normal distributions.

RESULTS

A clear relationship between acclimation temperature and thermal tolerance was evident (fig. 1) when the data for all species were pooled. The figure, however, conceals evidence of intraspecific variation (table 2). Specifically, pooled data for clones of *Daphnia pulex, D. magna,* and *D. carinata* each showed a significant shift in tolerance between each of the acclimation treatments, whereas *D. obtusa* and *D. ambigua* showed a difference only between

TABLE 2

DUNCAN'S MULTIPLE-RANGE TEST FOR THREE ACCLIMATION TEMPERATURES

Species and *N*	Acclimation Temperature (C)	Mean LT_{50} ± 1 SE (C)	Grouping
Daphnia pulex:			
14	10	33.9 ± .3	A
17	16	34.5 ± .3	B
17	25	36.3 ± .2	C
D. magna			
13	10	36.1 ± .2	A
12	16	36.9 ± .2	B
13	25	38.4 ± .2	C
D. carinata:			
2	10	35.2 ± .5	A
2	16	37.0 ± .1	B
2	25	39.0 ± .0	C
D. ambigua:			
4	10	36.9 ± .2	A
4	16	36.8 ± .1	A
4	25	38.7 ± .2	B
D. obtusa:			
2	10	37.5 ± .3	A
2	16	37.6 ± .3	A
2	25	40.1 ± .5	B
D. nivalis:			
2	10	35.2 ± .4	A
2	16	35.6 ± .4	A
2	25	36.1 ± .4	A

NOTE.—Clonal LT_{50} values for each species pooled for each acclimation temperature. Significant differences ($P = .05$) among LT_{50} values are indicated by different letters.

clear differences in temperature tolerance among species were evident (table 3). Increased acclimation temperature had little effect on the ranking of clones. Thus, *D. obtusa* had the highest temperature tolerance in all three treatments, whereas clones of *D. pulex* had the lowest tolerance. Variation in thermal tolerance among conspecific clones was limited to 2 C at each acclimation temperature.

More detailed analysis of the data in tables 2 and 3 support the following conclusions. The seven clones of *D. magna* showed no significant variation in thermal tolerance at any of the acclimation temperatures. The two clones of *D. ambigua* likewise possessed similar thermal tolerances. However, significant heterogeneity in thermal tolerance existed among the nine clones of *D. pulex* that were tested. Clones of different species isolated from a specific habitat often showed differences in thermal tolerance. *Daphnia pulex* and *D. obtusa* from Urbana showed significant differences in thermal tolerance at all three acclimation temperatures. Similarly, *D.*

magna and *D. pulex* from Churchill showed significant differences when acclimated at 10 C and 16 C. The same two species from a pond near Helsinki showed significant differences only when acclimated at 25 C.

Figure 2 reveals that there was a positive correlation ($r = .59$, $P < .01$) between the thermal tolerance of clones acclimated at 25 C and the temperature of the environment from which they were obtained. A similar correlation existed for clones acclimated at 16 C ($r = .54$, $P < .05$) and 10 C ($r = .39$, $P < .10$). Within a species, clones of *D. pulex* acclimated at 10 C ($r = .54$, $P > .05$), 16 C ($r = .73$, $P < .05$), and 25 C ($r = .80$, $P < .01$) showed a significant relationship between thermal tolerance and environmental temperature. However, the significance of this relationship was largely a consequence of the low thermal tolerance of the Igloolik stock. The seven clones of *D. magna*, while from sites geographically remote, came from habitats with similar summer temperatures, so it was not possible to determine

TABLE 3

MEAN $LT_{50} \pm 1$ SE FOR ALL CLONES AT THEIR RESPECTIVE ACCLIMATION TEMPERATURES

| | | ACCLIMATION TEMPERATURE (C) | | |
SPECIES	CLONE NO.	10	16	25
Daphnia obtusa	12	37.5 ± .3	37.6 ± .3	40.1 ± .5
D. ambigua	2	37.0 ± .1	36.9 ± .5	39.0 ± .2
D. ambigua	1	36.9 ± .3	36.7 ± .4	38.4 ± .1
D. magna	8	36.5 ± .4	36.7 ± .2	38.3 ± .2
D. magna	10	36.5 ± .6	37.5 ± .8	39.0 ± .8
D. magna	6	36.4 ± .1	37.1 ± .4	37.6 ± .6
D. magna	4	36.3 ± .2	35.8[a]	38.9 ± .3
D. magna	5	36.1 ± .3	37.4 ± .7	38.9 ± .2
D. magna	9	35.5[a]	36.1[a]	38.5[a]
D. magna	7	35.5 ± 1.1	37.2 ± .7	37.6 ± .1
D. carinata	3	35.2 ± .5	37.0 ± .1	39.0 ± .0
D. nivalis	11	35.2 ± .4	35.6 ± .4	36.1 ± .4
D. pulex	13	34.8 ± .2	35.0 ± .0	36.7 ± .4
D. pulex	16	34.8 ± .0	35.1[a]	36.3 ± .3
D. pulex	19	34.4 ± .2	35.1 ± .2	36.9 ± .0
D. pulex	20	34.2 ± .4	34.8 ± .7	36.8 ± .5
D. pulex	21	33.7 ± .0	34.7 ± .4	36.6 ± .0
D. pulex	18	32.8 ± 1.1	34.2 ± .3	36.1 ± .1
D. pulex	14	32.7 ± .5	34.3 ± .5	35.8 ± .5
D. pulex	17	NA	33.1 ± .4	34.4[a]
D. pulex	15	NA	34.6 ± .0	36.4 ± .0

NOTE.—NA, not available. Stocks are listed in descending order of their temperature tolerance after acclimation at 10 C.

[a] Not replicated.

whether a comparable pattern in thermal tolerance existed within this species.

DISCUSSION

The results of the present study indicate that *Daphnia* species vary markedly in their acute thermal tolerance. Such variation represents divergence among species in the genes controlling thermal tolerance. The interspecific differences in thermal tolerance were significantly correlated with the thermal regime of the habitats that the species occupies. Intraspecific variation in thermal tolerance was small, even among clones from distant localities, although some variation was found among clones of *D. pulex*. From this we conclude that differences in thermal tolerance of the magnitude reported by Banta (1939) and Johnson (1952) are infrequent.

Tolerance does not seem to be related to body size as species of similar size, such as *D. pulex* and *D. obtusa*, have divergent

tolerances. This lack of an association between body size and thermal tolerance has also been noted in other crustaceans, including crayfish (Spoor 1955; Claussen 1980) and fiddler crabs (Vernberg and Tashian 1959).

Past comparisons of the thermal tolerance of different *Daphnia* species have been limited. Brown (1929) found that *D. magna* had a lower thermal tolerance than *D. pulex*. Goss (1980) obtained conflicting results when populations of the two species were acclimated at 25 C or less, and our results support the conclusion that *D. magna* is more thermally tolerant than *D. pulex*. Recent research into the taxonomy of *D. pulex* in North America indicates that some previous reports of this species have involved misidentification of *D. obtusa* (Schwartz, Innes, and Hebert 1985). Brown's results may be explained by assuming that his stocks were actually the more thermally tolerant *D. obtusa*.
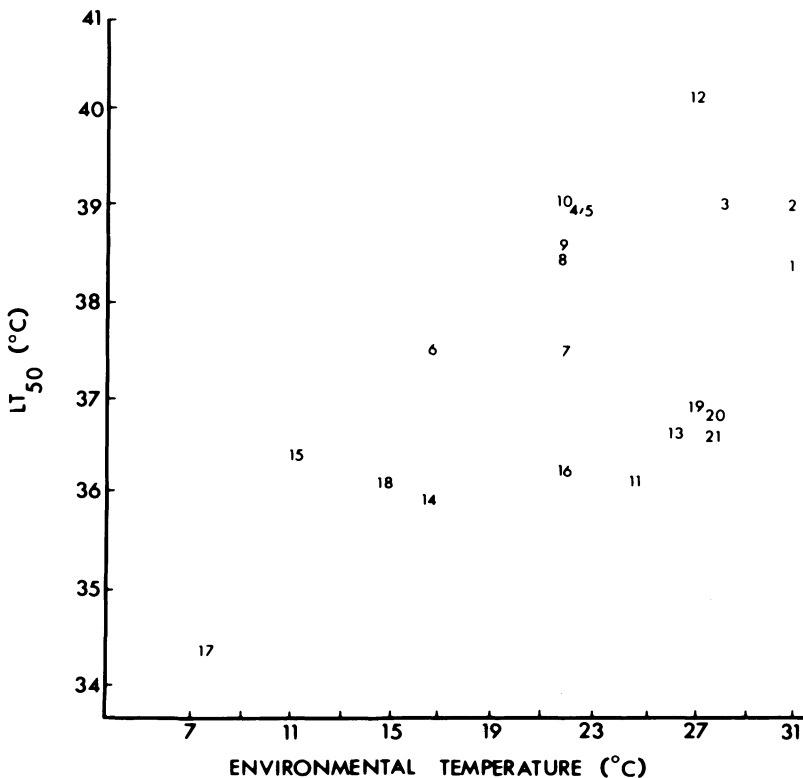
Populations of different species collected

FIG. 2.—The relationship between LT_{50} for 21 clones of *Daphnia* and mean daily maximum temperature for the warmest month of the year at their site of origin. The clones were acclimated at 25 C.

at the same site, such as *D. magna* and *D. pulex* from Churchill, Manitoba, and *D. pulex* and *D. obtusa* from Urbana, Illinois, had different thermal tolerances at each of the acclimation temperatures. The differences in LT_{50} were only 2–3 C but may be sufficient to establish seasonal trends in abundance of the species. Certainly environmental temperatures can exceed the thermal tolerance of a species.

The importance of thermal history in determining acute thermal tolerance of some *Daphnia* species has been clearly shown in this study. While the importance of the length of acclimation was not determined, it is probable that a significant

reduction in acclimation period would not affect the results. Bradley (1978) has shown that acclimation is complete in the copepod *Eurytemora affinis* in less than 24 h, while Claussen (1980) found that two crayfish species were almost fully acclimated to a 20 C temperature change in 36 h. The present study has indicated that the relative thermal tolerances of different species are not strongly affected by acclimation temperatures. However, differences in acclimation appear important enough that species should be compared at two different temperatures before decisions are made concerning their relative thermal tolerances.

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