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Thermophilic Ostracod: Aquatic Metazoan with the Highest Known Temperature Tolerance

Abstract. *The upper lethal temperature for an ostracod of the genus* Potamocypris *collected from a thermal stream ranged from 49°C for incubation of more than 5 hours to 55.75°C for 1-minute incubations. Field collections were held at 35°C for less than 24 hours before experimental incubations. Calculated temperatures for 50 percent mortality for 60, 40, 20, 10, 5, and 1 minute of exposure were 50.44°, 50.96°, 51.43°, 52.03°, 52.77°, and 55.12°C, respectively.*

Ostracods of the genus *Potamocypris* occur in some hot springs of western North America (*1, 2*) where they are found actively moving over the algal-bacterial substrate at temperatures of 30° to 54°C. Field observations on thermophilic animals (*2, 3*) consistently show the temperature limit for aquatic metazoans to lie below 50°C (*4*). This species of *Potamocypris* is found at higher temperatures than reported for other aquatic metazoans. Observations of live animals at a particular temperature are not proof of continued survival at that temperature (*5, 6*), so it was of interest to experimentally document the upper limits.

Mass collections of ostracods were made from water at 46°C in one of the thermal streams of the Hunter's Hot Springs complex located in south-central Oregon (*7*). Each collection was taken to the field laboratory and was kept at 35°C in a constant temperature bath until used (all ostracods were tested within 24 hours of collection). Adult ostracods were separated by pipette from the mass collection and subjected to the incubation conditions. Every trial consisted of placing ten adults into each of three test tubes containing 20 ml of spring water preheated to the desired temperature. The temperature was maintained in a Fisher serological bath and was continuously monitored with a thermistor probe placed in a test tube (*8*); no temperature fluctuation was observed during the reported trials of 1 hour or less (trials with temperature variations were terminated). After each incubation the test tubes were immediately cooled to 40° to 45°C by immersion in an ice slurry. Then the ostracods were removed and placed onto a depression plate and examined for active movement. Those not moving were placed in vials and kept at 35°C in the constant temperature bath for 12 hours, after which they were examined. Ostracods that were active after the recuperation time were considered to have been in heat coma. Those not regaining movement were considered dead.

In a preliminary experiment 60-minute incubations were made at 11 temperatures, from 40.00° to 50.00°C at 1° intervals. After this, with another group of animals, the incubations were for 1, 5, 10, 20, 40, and 60 minutes, from 48.00° to 55.75°C at 0.25° intervals. Including the preliminary incubations, replicates, and trials with 0 and 100 percent mortalities, a total of 2820 ostracods were tested. Replicate sublethal and lethal incubations during the trials served as controls. No thermal death or heat coma occurred below 49.50°C for these incubation periods.

Table 1 shows that as the exposure time is decreased the ostracod withstands proportionately higher temperatures. During 60-minute exposures both thermal coma and death were initiated at 49.50°C, but only at 51.00°C was mortality 100 percent in all trials. Heat coma was not observed until 53.75°C during 1-minute exposures, and no death occurred until the 54.50°C incubations.

A plot of trials falling within the thermal death ranges for the different exposure times is presented in Fig. 1, where each point represents a trial with 30 ostracods. Mortality slopes were determined by the least squares method (*9*). The regressions were significant at $P < .05$ [see Fig. 1 legend and (*10*)]. The values of the lethal dose to 50 percent of the population (LD_{50}) in Table 1 also show the expected relationship between temperature and incubation time. At $P < .05$ the LD_{50} value for 1 minute was significantly higher than those for all other exposure times except for 5 minutes; no other values were different at this level of significance (*10*), although they do show the expected trend.

The data scatter shown in Fig. 1, the relatively large overlapping thermal death ranges presented in Table 1, and the general absence of significant differences between the LD_{50} values indicate the highly variable response of the ostracods to most lethal temperatures. However, the low variability and statistical significance of the 1-minute exposures probably indicate that no

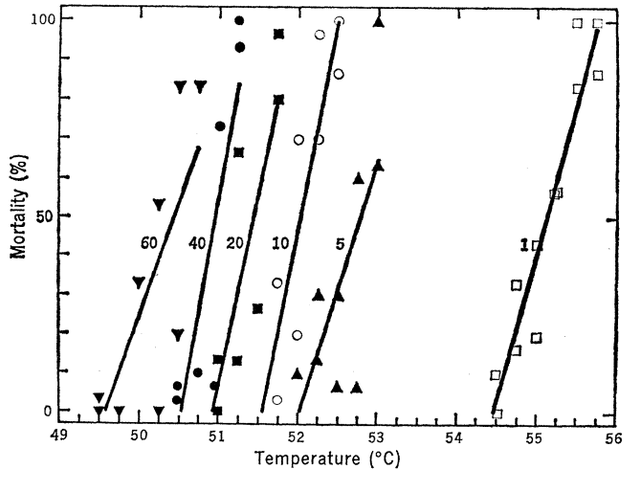

Fig. 1. Ostracod mortality within thermal death ranges. Each point represents 30 ostracods. (▼) 60-minute trials, $P < .02$; (●) 40 minute, $P < .02$; (■) 20 minute, $P < .05$; (○) 10 minute, $P < .01$; (▲) 5 minute, $P < .05$; (□) 1 minute, $P < .001$. Slope lines do not extend past the temperature above which no ostracods ever survived for that exposure period.

Table 1. Effect of temperature on the ostracod *Potamocypris* at test exposure times. Values for the initiation of heat coma are the lowest temperatures at which heat coma was observed. The thermal death range is from the lowest temperature at which death was recorded to the temperature above which no specimens survived. The LD_{50} values were calculated from slopes determined by the method of least squares (*10*).

Exposure time (min)	Initiation of heat coma (°C)	Thermal death range (°C)	Calculated LD_{50} (°C)
60	49.50	49.50–50.75	50.44
40	50.25	50.50–51.25	50.96
20	50.50	51.00–51.75	51.43
10	51.75	51.75–52.50	52.03
5	52.00	52.00–53.00	52.77
1	53.75	54.50–55.75	55.12

genetic or physiological population differences can provide tolerance to such high temperatures.

Mason (*5*) and Phelps (*11*) found ostracods in the field at 51.5°C. Mason found that *Heterocypris balnearia* did not survive longer than 4 hours at 49°C, and the *Cypridopsis* species examined by Phelps did not live more than a few hours at this temperature. The Oregon *Potamocypris* was exposed to 49°C (± 0.5°C in a heated aquarium) for 12 hours and a three-tube series was removed every hour. Under these conditions no deaths occurred until 5 hours, and there was 33 percent survival even after 12 hours. Food was not considered a source of error during this longer incubation as adults have survived at 35°C with no food for up to 10 days in the laboratory.

As a result of these findings we believe that this ostracod may have the highest temperature tolerance of any aquatic metazoan. Since the ostracod is easily cultured (reproducing populations at 40° to 45°C have been maintained in glass vessels with algae for over 2 years), it may prove to be a valuable laboratory animal for study of biochemical and physiological systems and their genetic control at the upper temperature limits to which aquatic metazoan life has evolved. In view of the production of thermal waters in the United States and other countries through the use of surface waters as coolants for power plants, the study of temperature stresses in this organism may provide valuable guidelines to the study of more economically important animal species.

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10. Methods described by R. Sokal and F. Rohlf [*Biometry* (Freeman, San Francisco, 1969), pp. 424, 446] were used to determine the significance of the regressions and the LD_{50} values and corresponding 95 percent confidence limits. The LD_{50} values were found by solving for X with Y set at 15. The 95 percent confidence intervals were: 49.14° to 52.80°C for 60 minutes; 50.16° to 51.91°C for 40 minutes; 50.48° to 52.65°C for 20 minutes; 51.40° to 52.58°C for 10 minutes; 51.45° to 55.46°C for 5 minutes; and 54.76° to 55.47°C for 1 minute.
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Synthesis of RNA–Polyadenylic Acid by Isolated Brain Nuclei

Abstract. *Nuclei, isolated from mouse brain tissue at various stages of postnatal development and incubated under cell-free conditions, synthesized RNA molecules that were associated with polyadenylic acid [poly(A)]. The RNA synthesized by these nuclei was similar to the poly(A)–associated products described for intact eukaryotic cells. The brain nuclei synthesized a similar proportion of RNA-poly(A) in the presence either of Mg^{2+} or of Mn^{2+} with $(NH_4)_2SO_4$. The RNA from neonatal brain nuclei appeared to have a greater proportion of poly(A)-containing RNA than nuclear products obtained from more mature neural tissue.*

The rate of RNA synthesis in intact mouse brain nuclei has been observed to be altered dramatically during postnatal development (*1*). Several investigators have demonstrated the presence of polyadenylic acid [poly(A)] sequences in nuclear and cytoplasmic messenger RNA (mRNA) of eukaryotic cells (*2, 3*). The presence of these poly(A) sequences in the RNA molecules has enabled the selective isolation and measurement of these molecules by a variety of techniques. In our study we have used cellulose columns, which have been shown to separate RNA-poly(A) from the bulk of cellular RNA (*4, 5*), to ascertain the presence of poly(A) sequences in the RNA synthesized by isolated mouse brain nuclei during various stages of postnatal maturation. We have sought to determine whether the RNA products synthesized by brain nuclei under cell-free conditions resembled the RNA products produced in intact eukaryotic cells (*3*).

Since previous investigators have

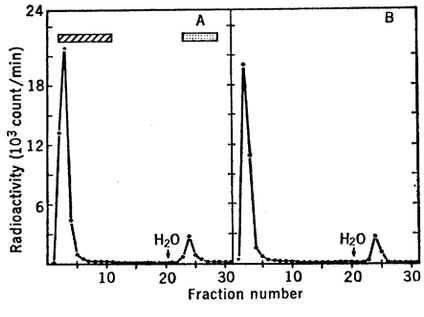

Fig. 1. Elution profiles of nuclear RNA from cellulose columns. RNA was isolated from adult mouse brain nuclei after their incubation in the presence either of Mg^{2+} (A) or of MN^{2+} with $(NH_4)_2SO_4$ (B), as described in Table 1. Elution with water is indicated by arrows. Fractions (2 ml) were collected during elution with buffer, and 1-ml fractions were collected during elution with water. In independent experiments, the elution patterns of free [^{3}H]GTP (hatched bar) and polyadenylic acid (stippled bar) were determined.