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UPPER LETHAL TEMPERATURES AND EFFECTS OF ACCLIMATION IN NAIADS OF THE DRAGONFLY, PLATHEMIS LYDIA (ODONATA : LIBELLULIDAE)

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ABSTRACT

The upper lethal temperatures of the dragonfly naiad *Plathemis lydia* (Drury) were measured. Naiads acclimated to temperatures of 5, 20 ± 4, and 36 ± 2 deg Centigrade, were determined to have an LT_{50} of 41.7 deg, and 43.2 deg respectively. Mortality differences at 42 deg C and 43 deg C among acclimated groups were significant at the 1% and 0.1% levels.

The mechanism of temperature relations in ectotherms have been reviewed by Prosser (1973) and Wieser (1973). Thermal acclimation in ectothermic animals is now considered to be characterized by an increased capacity to carry on steady state physiological processes at modified temperatures. Temperature compensation and concomitant metabolic shifts are accomplished by the selective induction of isozymes, changes in ion distribution, and membrane alterations. Isozyme induction consists of the activation and serial synthesis of multiple forms of a particular enzyme, each enzymatic form having a characteristic K_M value with an optimum activity at a determinant temperature (Hochachka, 1973). A plot of K_M versus temperature for an acclimated ectotherm goes through a minimum (maximum substrate affinity) at a point that correlates with the acclimation temperature, and reflects the mobilization of a specific isozyme (Baldwin and Hochachka, 1970; Newell, 1973). The mechanism thus compensates for the normal Q_{10} effect on kinetic activity, and over certain ranges the Q_{10}

may approach 1.0 (Prosser, 1973).

Compensation to temperature extremes can involve short term changes which result in an increasing tolerance for previously lethal temperatures. The shift in lethal thresholds is referred to as resistance acclimation by Prosser (1967, 1973) and has been readily shown for several species of fish (Fry and Hochachka, 1970). Information on the thermal responses of many vertebrates and invertebrates has been published (Whittow, 1970), and it is apparent that research on aquatic ectotherms, with the exception of fish and crustaceans, has concentrated on littoral animals. Fresh water invertebrates have received less attention, although in temperate climates these animals are typically exposed to wide fluctuations in temperature. Larval stages of many insects, for example, may spend years in small ponds and streams which are characterized by considerable variations in temperature. The biology of these animals can be better understood if the mechanisms that allow temperature adaptation are known. The purpose of the present study was to ascertain the upper lethal temperature for naiads of the dragonfly, *Plathemis lydia* (Drury), an aquatic species that is locally abundant in Illinois and for which no previous temperature data exist. The effects of warm and cold acclimation on the upper lethal temperature were also investigated.

MATERIALS AND METHODS

Dragonfly naiads were collected during September, October and November, 1975, from a small farm pond 6 km south of Carlinville, Illinois (Macoupin County). The pond is located in a forested area and is partially shaded most of the day. Insects were collected by dredging the bottom sediment with a dip net and rinsing the mud from the detritus. The highest naiad density was found two to three meters from shore in water one-half to one meter deep. The water temperature at these spots was 23 - 24 deg C in early September, 16 deg C in early November, and 4 deg C by December 1st, 1975. Approximately 500 insects were collected and sorted at the laboratory in porcelain trays. Only the naiads of *P. lydia* were retained for study. This species is very common and widely distributed in the United States and Canada (Needham, 1955). The specimens ranged between 8 and 27 mm in length, measured from the labial crenulations to the apex of the inferior caudal appendages. However, only insects between 10 and 18 mm long were used for upper lethal temperature determinations.

The naiads were kept in plastic containers of pond water placed in constant temperature cabinets

(Hythermco, Model 555210) adjusted to the desired temperature. One group of animals was maintained at 20 deg C, which reflects the water temperature at which they were found. A second group was cooled slowly to 5 deg C and maintained at this temperature for 8 days before testing. A third group was kept at 36 deg C for 3½ days and then tested.

The apparatus used to determine upper lethal temperatures consisted of large glass bowls containing pond water which were placed in constant temperature water baths (Fisher, Model 131, and P/S Co., Model 83). Transparent plastic plates covered the bowls and mercury thermometers were inserted through holes drilled in the plates. Once the temperature of the pond water stabilized, it could be kept within plus or minus 0.2 deg C. The bottom of each bowl was provided with a filter paper substrate to which the naiads could cling.

Insects were tested for thermal resistance at a range of temperatures established in preliminary tests as including the upper lethal point. Time of exposure was one hour. Ten individuals were selected at random from one of the groups and placed directly into a previously prepared water bath. Exposing organisms to experimental temperatures in this way characterizes a step function (Clarke, 1967), and is distinguished from a ramp function characterized by a gradual rise in temperature. After 60 minutes all animals were transferred to clean bowls of pond water at 20 deg C for recovery, and were tested for loss of irritability by touching the anal aperture with a probe. This method was determined to be the most reliable way of confirming death in individual specimens. At 12 and 48 hours after treatment, the naiads were inspected again. A recovery period of 48 hours was chosen because animals stressed by the experimental conditions would either die or recover completely within two days. Control groups of untreated naiads showed no mortality during this period.

The naiads used in this study included several different larval instars. The naiads selected for use, however, were chosen over a range of sizes representing a discrete age class. The insects were denied food throughout the testing period as feeding has been shown to affect lethal temperature determinations (Cloudsley-Thompson, 1970; Wieser, 1973). The temperature cabinets were kept dark to reduce the effects of photoperiod which has been shown to affect the metabolic activity of _P. lydia_ (Shepard and Lutz, 1976).

RESULTS AND DISCUSSION

Upper lethal temperature determinations are summarized in Table 1. The results show a fairly narrow thermal range in which heat deaths occur, particularly for those insects acclimated to warmer temperatures. Naiads could tolerate exposure to temperatures of 41 or 42 deg C for one hour but could not withstand a temperature of 44 deg C. The increase in percentage mortality is particularly sharp between 42.5 and 43 deg C for naiads acclimated to 20 deg C, and between 43 and 43.5 deg C for naiads acclimated to 36 deg C (Fig. 1). Similar results have been shown for other insects, including Tenebrio molitor and Periplaneta americana (Richards, 1956, 1964) as well as for other invertebrates. Spoor (1955) found that the crayfish, Orconectes rusticus , could survive at least 10 days at 35 deg C but only 24 hours at 35.6 deg C. Orr (1955), working with several species of marine ectotherms, has also found a sharply delineated time of exposure beyond which recovery from heat coma would not occur. With regard to the thermal death points of annelids and molluscs, Bullard (1964) has stated that metabolic reaction rates in adapted animals can fall steeply at temperatures close to the upper and lower lethal limits of the organisms. These observations on P. lydia and other ectotherms may reflect the sudden breakdown of an important physiological process at a certain temperature with a subsequent lethal interuption of body functions. The breakdown would occur when rapid adaptive mechanisms are no longer able to cope with changing temperatures.

The thermal death curves are shown in Figure 1. Percentage mortality values from Table 1 are plotted against the experimental temperatures for all three acclimation groups. It should be noted that the curves from which the LT_{50} values (Cloudsley-Thompson, 1970) have been taken are determined by only two points. The values, 41.7, 42.7 and 43.2 deg C for naiads acclimated to 5, 20 and 36 deg C respectively, suggest a slight change in temperature tolerance after acclimation. The difference is not great, however, and would be of limited value to an organism under stress. Experiments by Whitney (1939) on the mayfly naiad, Baetis rhoda, produced similar results. Exposure of mayflies to high temperatures for 40 hours did not result in a significantly higher heat tolerance for this species. Spoor (1955) found a gain of only one degree in the LT_{50} of crayfish acclimated at 30 deg C for one week. In contrast, work collected by Prosser (1973) on various aquatic ectotherms indicates that resistance adaptation can produce a lethal threshold shift of several degrees

Table 1. Survivorship of <u>Plathemis</u> <u>lydia</u> naiads at high temperatures. Values in parentheses indicate the results of individual trials.

Acclimation Temperature (deg C)	Test Temperature (deg C)	Number of Tests	Number of Naiads	Survivorship	
				Number Surviving	Percent Mortality
	41	1	10	10	0
20	41.5	1	10	9	10
	42	4	40	37	7.5
	42.5	3	30	24 (5,9,10)	20
	43	3	30	4 (0,0,4)	86.7
	44	2	20	0	100
	41	3	30	22 (9,6,7)	26.7
5	42	3	30	12 (6,3,3)	61.3
	43	2	20	0	100
	42	2	20	20	0
	43	3	30	20 (6,6,8)	33.3
36	43.5	2	20	2 (2,0)	90
	44	2	20	0	100
	45	1	10	0	100

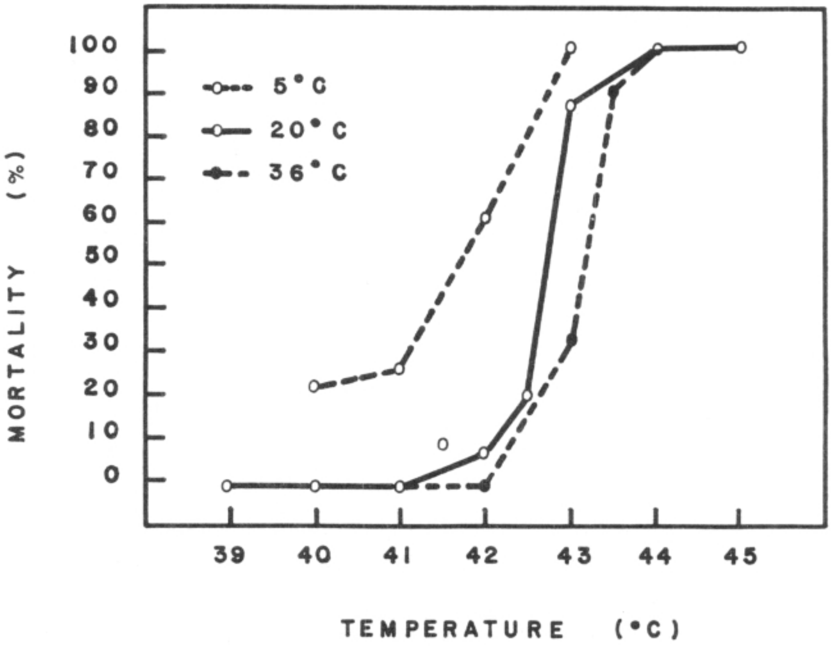

Fig. 1. Mortality of Plathemis lydia naiads acclimated to 20, 5 and 36 deg C, at various temperatures.

in some animals, particularly fish and intertidal gastropods. Grant (1953), has also found a 0.3 deg C gain of heat tolerance in the earthworm Pheretime hupeiensis for every one deg C rise in acclimation temperature.

Chi square analysis of the mortality figures from Table 1 indicate that the difference in temperature tolerance for acclimated groups was most significant at 42 and 43 deg C (Table 2). The lethal temperature point of the group acclimated at 20 deg C lies between these two values.

Table 2. Chi square values of thermal death mortality in Plathemis lydia. The acclimated groups are contrasted at three temperatures. Temperatures are given in deg C.

20 deg vs. 5 deg		20 deg vs. 36 deg		5 deg vs. 36 deg	
T^o	X^2	T^o	X^2	T^o	X^2
41	2.13	41	0.00	41	2.13
42	9.70*	42	0.12	42	9.82*
43	1.33	43	64.00**	43	66.66**

* Significant at the $p < 0.01$

** Significant at the $p < 0.001$

The temperatures to which the P. lydia naiads were exposed do not duplicate conditions expected in their environment. It is unlikely that the pond in which they were found warms beyond 25 deg C. And when heated, naiads would probably seek the cooler areas of the pond. Moore (1955), however, has collected fairy shrimp in ditch water at 42 deg C, a temperature close to the upper lethal point of 44.5 deg C. Organisms living in shallow stationary pools must undoubtedly be exposed to considerable warming during summer months, and those capable of tolerating extreme temperatures have a selective advantage. Under laboratory conditions, P. lydia naiads surrived at least two weeks at 35 deg C with no apparent deleterious effects. As these insects must overwinter in water close to freezing, they are capable of a wide range of thermal tolerance. Tolerance of high temperatures in the laboratory, however, should not imply adaptation to these regimes in a natural environment (Reid, 1967). It is the capacity to compensate for temperatures that an organism may actually experience that has survival value. It has not been established that acclimation effects compensation to temperature extremes although they may occur together (Precht, 1958). However, acclimation need not alter vulnerability to extreme temperatures if the normal compensation range is wide. Data from this experiment indicate that the aquatic stage of Plathemis lydia can tolerate a wide range of temperatures (Table 1) and can show a small, but significant shift in the upper lethal temperature under thermal stress. The magnitude of this shift, however, does not approach the 4 to 5 degree C shifts in upper lethal temperatures that have been reported for other aquatic arthropods as a result of warm and cold acclimation (Altman and Dittmer, 1973; Barkes, 1959; Newell, 1966, 1973; Prosser, 1973; Vernberg and Vernberg, 1970; Wieser, 1973). This suggests that Plathemis naiads may utilize other thermal compensatory mechanisms such as supercooling (Cloudsley-Thompson, 1970) in addition to thermal acclimation in order to adjust to changing environmental regimes.

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