

Physiological Responses to Temperature and Desiccation in the Endemic New Mexico Plethodontids, *Plethodon neomexicanus* and *Aneides hardii*

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Measurements of the critical thermal maximum (CTM), oxygen consumption, and heart rate of *Plethodon neomexicanus* and *Aneides hardii* were made for comparison with similar data for other members of the family Plethodontidae. At an acclimation temperature of 20° C, the mean CTM for *A. hardii* was 33.3° C, and for *P. neomexicanus*, 33.5° C. Mean oxygen consumption increased from 30.9 $\mu\text{l/g/hr}$ at 5° C to 93.9 $\mu\text{l/g/hr}$ at 25° C in *A. hardii* and from 35.4 $\mu\text{l/g/hr}$ at 5° C to 60.9 $\mu\text{l/g/hr}$ at 25° C in *P. neomexicanus*. The metabolism-temperature (M-T) curve for *P. neomexicanus* was similar to that of *Desmognathus quadramaculatus*, an eastern plethodontid, but the M-T curve of *A. hardii* was more comparable to that of a lunged salamander. Heart rate increased directly with temperature in both species. The mean heart rate increased from 22/min at 10° C to 64/min at 25° C in *A. hardii* and from 24/min at 10° C to 73/min at 25° C in *P. neomexicanus*. The low CTM recorded for these species may be related to the low temperatures characteristic of their microhabitat. The difference in M-T curves in these species may reflect differences in surface area-mass relationships resulting in differences in cutaneous gas exchange.

INTRODUCTION

THE two relict species of plethodontid salamanders in New Mexico, *Plethodon neomexicanus* and *Aneides hardii*, are of considerable importance in considerations of the evolution and dispersal of the Plethodontidae. Since these species were discovered fairly recently (Stebbins and Riemer, 1950; Taylor, 1941), and inhabit restricted areas, little is known of their biology. The life history of *A. hardii* is fairly well known (Johnston and Schad, 1959) but almost nothing is known of the life history and ecology of *P. neomexicanus*. Consequently any studies dealing with the biology of these species could contribute to our understanding of the relationships between plethodontids of the eastern and western United States.

Measurements of selected physiological parameters such as oxygen consumption (Whitford and Hutchison, 1965), limits of desiccation (Ray, 1958), and critical thermal maxima (Hutchison, 1961) have proved valuable in interpreting ecological relationships and geographical distribution of species of amphibians. Therefore measurements of oxygen consumption and heart rate at different temperatures, critical thermal maxima,

and vital limits of desiccation were made on *A. hardii* and *P. neomexicanus* to obtain comparisons with similar measurements on related species of eastern and western plethodontids.

METHODS AND MATERIALS

A total of 58 *P. neomexicanus* were collected at Del Norte Pass (elev. 9200 ft) in the Jemez Mountains, Los Alamos Co. New Mexico, 6 and 7 August 1966.

Several collections of *A. hardii* were made in the vicinity of Cloudcroft (elev. 8000+ ft) Lincoln Co., N. M. in July and August 1966. Both *A. hardii* and *P. neomexicanus* were placed in large glass finger bowls half filled with rotted wood and humus and kept at 15° C and a 12 hr photoperiod in a controlled environment chamber until conditions were changed for acclimation at the desired temperature. The animals were acclimated to the desired study temperatures for a minimum of one week prior to any experiments. The salamanders were fed small red worms and fruit flies during the storage and acclimation periods.

Measurements of oxygen consumption were made in a Gilson Microrespirometer. The

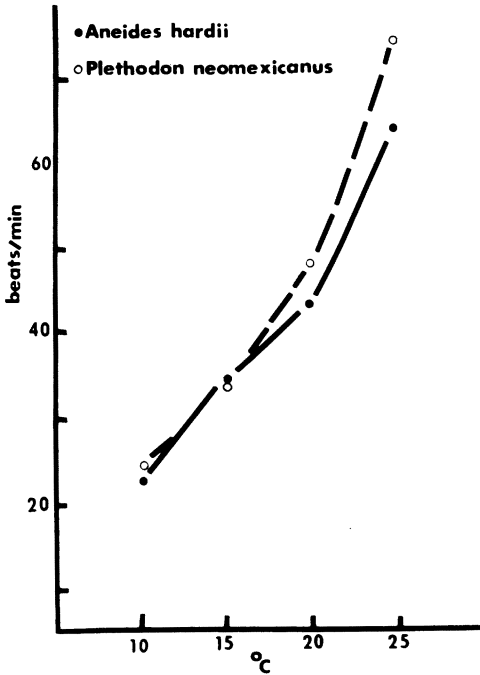


Fig. 1. The relationship of heart rate (beats/min) and temperature ($^{\circ}\text{C}$) in *Aneides hardii* and *Plethodon neomexicanus*.

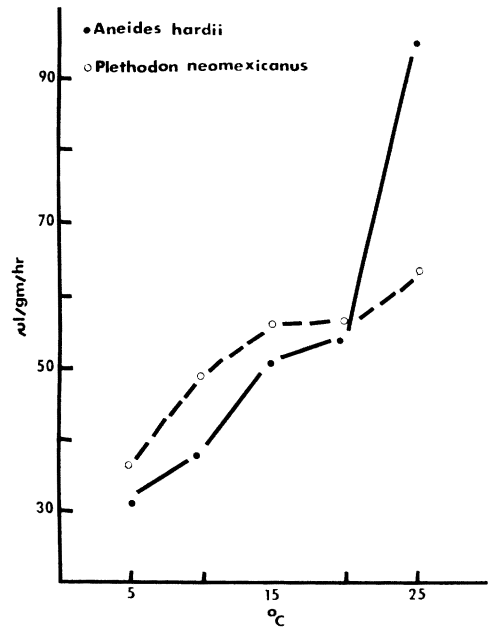


Fig. 2. The relationship of oxygen consumption ($\mu\text{l/gm/hr}$) and temperature ($^{\circ}\text{C}$) in *Aneides hardii* and *Plethodon neomexicanus*.

oxygen consumption of seven individuals of each species was measured at the same time for at least three consecutive hr at each acclimation temperature. All gas volumes were corrected to STP.

Critical thermal maxima (CTM) for ten individuals of each species acclimated at 20°C was determined by the methods outlined by Hutchison (1961). Heart rates were measured by inserting fine needle electrodes into the thoracic cavity on either side of the heart. The leads from the electrodes were connected to high gain preamplifier and physiograph recorder. The animals were returned to the acclimation chamber after connection of electrodes for a period of 20–30 min to allow the salamanders' body temperature to return to the desired level and for adjustment to the presence of the electrodes. ECG recordings were made on four or five individuals of each species at all acclimation temperatures except 5°C .

Vital limits of desiccation were determined by the methods of Ray (1958). Dried air was drawn through a cylindrical chamber at 600 cc/min. The salamander was placed on a plastic hardware screen platform sus-

pending in the center of the cylinder allowing passage of dried air over all parts of the animal. The critical activity point (CAP) was determined by the loss of righting response. After the CAP was reached most of the animals were killed by a sharp blow on the head, and placed in a drying oven at 80°C for a period of 24 hr to obtain dry weight and body water content.

Body surface area measurements were made by skinning the animal and tracing the outline of the skin with a polar planimeter as described by Whitford and Hutchison (1967).

RESULTS

The mean CTM \pm 95% confidence interval was $33.25^{\circ}\text{C} \pm 0.07$ for *A. hardii* and $33.51^{\circ}\text{C} \pm 0.45$ for *P. neomexicanus*. A comparison of the 95% confidence intervals indicates the CTM in these species was significantly lower than that determined for terrestrial eastern plethodontids by Hutchison (1961) and 1.0°C lower than that of *Aneides lugubris* (Rosenthal, 1957).

There was a direct and nearly linear increase in heart rate with temperature in both species (Fig. 1). However, there was a con-

TABLE 1. Q_{10} VALUES FOR HEART RATE AND OXYGEN CONSUMPTION IN *Aneides hardii* AND *Plethodon neomexicanus* AT DIFFERENT TEMPERATURES.

Temp. °C	<i>A. hardii</i>		<i>P. neomexicanus</i>	
	O ₂ Consumption	Heart Rate	O ₂ Consumption	Heart Rate
5-10	1.41		1.88	
10-15	1.88	2.88	1.30	2.02
15-20	1.10	1.59	1.00	1.99
20-25	3.17	2.22	1.21	2.37

siderable difference in the metabolism-temperature (M-T) curves of *A. hardii* and *P. neomexicanus* (Fig. 2). In *A. hardii* oxygen consumption increased directly with temperature with a sharp increase in rate between 20° and 25° C. The M-T curve of *P. neomexicanus* exhibited a reduction in rate of increase at temperatures above 10° C as indicated by lower Q_{10} values (Table 1). A comparison of Q_{10} values for heart rate and oxygen consumption (Table 1) shows a marked difference in the response of these parameters to temperature in these salamanders. In addition there was a reduction in rate of increase of both the M-T curves and heart rate curves in these species between 15° C and 20° C as indicated by low Q_{10} values between these temperatures (Table 1).

The measurement of both heart rate and oxygen consumption allows partial evaluation of the mechanisms by which these species meet the increased oxygen demand of their tissues at different temperatures (Dawson and Templeton, 1966). Using oxygen consumption and heart rate, the oxygen consumption per heartbeat, or oxygen pulse, can be calculated. Values for the oxygen pulse of *A. hardii* and *P. neomexicanus* are indicated in Table 2. The oxygen pulse of

P. neomexicanus exhibited a steady decrease at higher temperatures while that of *A. hardii* varied little at different temperatures.

The relationship between body weight and surface area in *P. neomexicanus* and *A. hardii* was the same as that for other salamanders when plotted on the line given by $S = 8.42 W^{0.694}$ (Whitford and Hutchison, 1967). However, the average weight of the *A. hardii* (0.97 g) used in the metabolism studies was 1.5 g less than that of *P. neomexicanus* (2.47 g). Thus *A. hardii* had more surface area per unit weight exposed to the environment for gas exchange and water loss than *P. neomexicanus* (Table 3).

The data on vital limits of desiccation are summarized in Table 4. *P. neomexicanus* lost an average of 32.5% of its initial body weight at the critical activity point. *A. hardii* lost an average 29.6% of its body weight at the CAP. Approximately 50% of both species survived dehydration to the CAP. There was an inverse relationship between body size and dehydration rate. The mean rate of dehydration for *P. neomexicanus* was 0.345 g/hr and for *A. hardii*, 0.240 g/hr. Although *A. hardii* had more surface area per unit weight exposed to the drying environment than *P. neomexicanus*, it lost water at a lower rate. The vital limits of water loss in *P. neomexicanus* was similar to that of the western *Plethodon elongatus* but higher than that of the eastern *P. cinereus* and *P. glutinosus* (Littleford *et al.*, 1947). The vital limits of desiccation in *A. hardii* was lower than that of the California *Aneides* (Ray, 1958).

DISCUSSION

The CTM of the endemic New Mexican plethodontids is comparable to that of *Desmognathus quadramaculatus*, which inhabits cold mountain streams in the southern Appalachians (Hutchison, 1961). The

TABLE 2. OXYGEN CONSUMPTION PER HEART BEAT (OXYGEN PULSE) IN *Plethodon neomexicanus* AND *Aneides hardii* AT DIFFERENT TEMPERATURES.

Temp. °C	<i>P. neomexicanus</i>			<i>A. hardii</i>		
	O ₂ Consumption cc/g/hr	Heart Rate beats/hr	O ₂ Pulse cc/beat/g	O ₂ Consumption cc/g/hr	Heart Rate beats/hr	O ₂ Pulse cc/beat/g
10	0.048	1440	3.33×10^{-5}	0.037	1350	2.74×10^{-5}
15	0.055	2040	2.70×10^{-5}	0.050	2040	2.45×10^{-5}
20	0.057	2880	1.98×10^{-5}	0.053	2580	2.05×10^{-5}
25	0.061	4440	1.37×10^{-5}	0.094	3840	2.45×10^{-5}

TABLE 3. THE RELATIONSHIP OF BODY SURFACE AREA TO WEIGHT IN *Plethodon neomexicanus* AND *Aneides hardii*.

	Wt g	Surface Area cm ²	Surface Area/Unit Wt cm ² /g
<i>P. neomexicanus</i>	2.77	21.03	7.59
	1.59	12.90	8.11
<i>A. hardii</i>	1.34	11.90	8.80
	0.27	5.41	20.03

microhabitats of both *A. hardii* and *P. neomexicanus* are characterized by low temperatures. Both species are inhabitants of the spruce-fir zone at elevations greater than 8000 ft and are found on the ground surface only during the warmest months of summer. When active on the surface, these species are usually found in well rotted logs which are saturated with water. Field temperatures recorded with a Schultheis quick reading thermometer in rotted logs where salamanders were taken, varied from 12.5 to 13.0° C. Thus the low CTM recorded for these species probably reflects adaptations to the low temperatures characteristic of the microclimate of their habitat.

The difference in M-T curves in *A. hardii* and *P. neomexicanus* is probably related to differences in respiratory surface area and consequently the ability to obtain oxygen. This is supported by the difference in surface area/mass ratio of the two species and by changes in oxygen pulse with temperature. *P. neomexicanus* had an M-T curve similar to that of *D. quadramaculatus* (studied by Whitford and Hutchison, 1967) while the M-T curve of *A. hardii* was similar to a lunged salamander. Whitford and Hutchison (1967) presented evidence demonstrating the importance of respiratory surface area in limiting metabolic rate in salamanders which appears to apply to the M-T difference in these species. Since the oxygen pulse of *A. hardii* varied little with temperature, the increased oxygen demand of its tissues was met by increased heart rate and thus increased peripheral blood flow resulting in more rapid diffusion of oxygen through the skin. Cutaneous gas exchange has been shown to account for between 75% and 90% of the total oxygen consumption of plethodontids (Whitford and Hutchison, 1965). Consequently a larger body surface area to

TABLE 4. VITAL LIMITS OF DESICCATION IN *Plethodon neomexicanus* AND *Aneides hardii* AT 20° C.

Animal No.	Original Wt	Wt at CAP	% Body Wt Lost	Oven Dried Wt	Wt Body Water	% Body Water Lost at CAP
<i>P. neomexicanus</i>						
1	2.96	2.15	27.4	0.77	2.19	40.0
2	1.58	1.15	27.2	0.35	1.23	35.0
3	2.35	1.76	25.1	0.50	1.85	32.0
4	2.64	1.75	33.7	survived—not sacrificed		
5	1.12	0.67	40.2	0.21	0.91	49.4
6	1.81	1.30	28.2	survived—not sacrificed		
7	2.77	1.81	34.6	survived—not sacrificed		
8	1.01	0.65	35.6	0.20	0.81	44.4
9	1.96	1.17	40.3	0.41	1.55	50.9
			$\bar{x} = 32.5$			$\bar{x} = 42.0$
<i>A. hardii</i>						
1	0.57	0.39	31.5	0.12	0.45	40.0
2	0.61	0.44	27.8	0.13	0.48	35.4
3	0.32	0.24	25.0	0.05	0.27	29.6
4	1.24	0.87	29.8	0.33	0.91	40.6
5	1.15	0.75	34.7	0.22	0.93	43.0
6	1.21	0.66	28.9	0.29	0.92	38.0
			$\bar{x} = 29.6$			$\bar{x} = 37.8$

mass ratio could be a most important factor in supplying oxygen to the body tissues at high ambient temperatures. In *P. neomexicanus*, the oxygen pulse decreased at higher temperatures indicating that increased cardiac output was not accompanied by a comparable increase in oxygen supply to the tissues. This was probably due to limitations in respiratory surface area for oxygen diffusion resulting in lower blood oxygen tensions despite increased cardiac output.

The cause of the dip in both the M-T curves and heart rate curves between 15° and 20° C in *A. hardii* and *P. neomexicanus* is not apparent from the data obtained in this study. Temperatures around 15° C may represent an environmental optimum for these species where fluctuations in temperature around this optimum have a reduced effect on metabolic processes. This could result in greater metabolic efficiency at temperatures near this optimum.

The difference in desiccation rate between *A. hardii* and *P. neomexicanus* may

have been due to behavioral differences. Both species exhibited coiling behavior soon after being placed in the desiccation chamber. *A. hardii* tended to remain in this position longer and was able to move into an area below the incurrent port on occasion due to its small size. In this area of the desiccation chamber there would have been less turbulence and consequently less drying effect of the air. Thus it is possible that *A. hardii* may not have been subjected to the same drying conditions as *P. neomexicanus*.

A comparison of the vital limits of desiccation in *P. neomexicanus* with other species in the genus indicates that it has a higher tolerance to desiccation than the eastern plethodons and is more like the western species with respect to this parameter. Highton (1962) placed *P. neomexicanus* with the eastern small *Plethodon* group but indicates that it has differentiated greatly from them all. The high tolerance to desiccation in *P. neomexicanus* is additional evidence that it has been separated from the eastern group for some time and adapted to the more xeric conditions of the Jemez Mountains. *P. neomexicanus* is similar to the western *Plethodon* in its desiccation tolerance which is an additional difference from eastern *Plethodon* to those listed by Highton (1962).

Although there are no data available on oxygen consumption over a wide range of temperatures for representatives of either the eastern or western *Plethodon*, data on oxygen consumption of *P. cinereus* at temperatures between 15 and 20° C from Evans (1939) give a Q_{10} of approximately 1.00 and the rate of oxygen consumption for *P. cinereus* was less than that obtained for *P. neomexicanus* in this study. This suggests that *P. cinereus* may have an M-T curve similar to *P. neomexicanus* but this needs to be substantiated. The high resistance to desiccation and requirement of fairly low ambient temperatures to obtain sufficient oxygen to meet its metabolic requirements in *P. neomexicanus*, indicate that the dispersal of this form from the east across Oklahoma to the Jemez area in New Mexico could have occurred during the Pleistocene when the climate in that area was considerably cooler and wetter than at present as suggested by Blair (1958). Additional data on metabolic responses to temperature and moisture for western and eastern species of *Plethodon* could be extremely valuable in

interpreting phylogenetic affinities and distribution patterns in the genus.

The vital limit of desiccation in *A. hardii* was lower than that of western *Aneides*. However, comparable data for the eastern *Aneides aeneus* are not available. Also there are no comparable data on metabolic-temperature responses in other species of *Aneides*.

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