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MANESS, JOSEPH DALE
HEAT HARDENING IN VERTEBRATE ECTOTHERMS.

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HEAT HARDENING IN VERTEBRATE ECTOTHERMS

APPROVED BY

Victor H. Bennett

Howard Haines

Charles Carpenter

Frank J. Fontaine

DISSERTATION COMMITTEE

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HEAT HARDENING IN VERTEBRATE ECTOTHERMS

ABSTRACT

1. Heat hardening is a transitory increase in heat tolerance following a sub-lethal exposure to lethal high temperatures.
2. Within 1-2 hours of an initial exposure to the critical thermal maximum (CTM), the CTM's of two species of amphibians and two species of fish had increased significantly above the initial level and then decreased to the initial level of tolerance within 24 hours.
3. Experiments with exposures to sub-CTM temperatures and multiple exposures to the CTM indicated that hardening requires exposure to the CTM and may be the maximum CTM attainable by the animal.
4. Diel and seasonal variation had significant effects on hardening ability.
5. Field evidence suggests that heat hardening is adaptive in that it provides an acute means of adjustment to extreme fluctuations in diurnal temperatures.

INTRODUCTION

The profound influence of temperature on ectotherms has stimulated a multitude of studies on the mechanisms of adjustment to changing thermal environments (for reviews see Rose, 1967; Whittow, 1970; Prosser, 1973; Precht, 1973). Thermal tolerance and adjustment of thermal tolerance are of particular interest because an animal's survival under a given set of thermal conditions determines its presence or absence in a particular region or habitat (Brett, 1956; Hutchison, 1961; Fry, 1967; Brattstrom, 1970; Fry, 1971; Hutchison, 1976). Prior to this study, investigations of adjustment of thermal tolerance dealt primarily with acclimation or acclimatization. Acclimation refers to adjustment to change in a single environmental factor and acclimatization refers to adjustment to several factors (Folk, 1974; Hutchison, 1976). Both these processes require from several days to weeks for completion. Recently, a mechanism of acute adjustment of tolerance, requiring only a few hours, has been proposed; temperature hardening.

Temperature hardening is well known in plants where exposure to extreme temperatures causes plants to become

more "hardy" or resistant to temperature stress. Alexandrov (1964) extended this concept to animal cells, reporting that the temperature of cessation of protoplasmic streaming (death) could be raised in cells briefly exposed to lethal temperatures. Precht (1973, p419) expanded the concept to whole animals and defined temperature hardening as a "quick, usually transitory, adaptation to high and low temperatures." Hardening is distinct from acclimation in that hardening is a rapid, short term response to brief exposures to temperatures in the lethal range. Acclimation is a slower, longer lasting response to temperatures within normal ranges (Hutchison and Maness, 1979). We will examine only hardening responses to high temperature (heat hardening), because levels of cold tolerance are difficult to determine in ectotherms.

Although several field observations (Lowe and Heath, 1969; Pough and Wilson, 1970; Otto, 1974) suggest the presence of heat hardening, hardening in animals has been demonstrated in only two laboratory studies. Hutchison (1961) heated Notopthalmus viridescens to their critical thermal maximum (CTM), allowed them to recover at their acclimation temperature for increasing intervals, and reheated them to a second CTM. The second CTM was significantly higher than the initial CTM at the shorter recovery intervals, decreasing to the initial level

following the longer intervals. Although Hutchison did not refer to this phenomenon as heat hardening, it fits the criteria for hardening which were later defined by Alexandrov (1964) and Precht (1973). Basedow (1969), working in Precht's lab, applied Hutchison's method to the newt, Triturus vulgaris, and achieved similar results. He reported, however, that these data and results he obtained with two fish, Idus idus and Anquilla vulgaris, were insufficient to distinguish hardening from acclimation.

If hardening exists, it will be more rapid in its onset and decay than acclimation, a determination which can be readily made with the CTM method. Both Alexandrov (1964) and Precht (1973) state that such an increase in tolerance may be simply rapid acclimation due to the extreme temperatures employed. If this is the case, heating the animals to just below the CTM should also cause a significant increase in tolerance. No significant increase in tolerance will mean that hardening is distinct from acclimation in that hardening requires exposure to lethal temperatures. If one exposure to the CTM increases tolerance, a second exposure could cause a further increase in tolerance and a third exposure might increase tolerance still more. However, if tolerance levels do not increase following multiple exposures, the hardening CTM may be the maximum CTM attainable by the

animal. Because the CTM is the criterion used for heat hardening, hardening may be influenced by some of the same factors as the CTM, i.e. diel and seasonal variation. This study attempts to answer these questions for two species of amphibians and two species of fish.

MATERIALS AND METHODS

Animals

The Rana berlandieri (Rio Grande leopard frogs collected in Sinaloa, Mexico) were obtained from Nasco (Fort Atkinson, Wisconsin) and the Notophthalmus viridescens (red-spotted newts) were obtained from Zetts Fish Hatcheries (Pennsylvania). We collected the Notropis lutrensis (red shiner) from Walnut Creek (McClain County, Oklahoma) and the Pimephales promelas (fat head minnow) from a pond at the University of Oklahoma Fisheries Lab (Noble, Oklahoma) which had been stocked with fish from the Zetts Fish Hatcheries the previous year. We acclimatized the amphibians in Sherer environmental chambers (Sherer-Gillette Co., Marshall, Michigan) at 15°C and a LD 12:12 photoperiod. Twice a week, we fed mealworms and newborn mice to the frogs and earthworms and commercial Xenopus Food (Nasco) to the newts. We acclimatized the fish in "Living Stream" Frigid Units (Frigid Units Inc., Toledo, Ohio) under three sets of conditions, LD 12:12 and 15°C and natural photoperiod and 15°C and 20°C. We fed the fish ground Xenopus Food every other day. All animals were acclimatized at least

one week and fasted at least one feeding period prior to testing.

Critical Thermal Maximum

We determined the CTM's by the method described by Hutchison (1961), heating the animals in water at a rate of 1°C/min. Frog temperatures were taken cloacally; the fish and newt body temperatures were considered to be the same as the water adjacent to the animal. The CTM was measured at the onset of spasms, characterized by spasmodic gaping of the mouth and twitching of the limbs in amphibians or by spasmodic opercular movements, mouth gaping, and twitching of the pectoral fins in fish. Immediately following the CTM, the animals were returned to their acclimatization conditions for recovery.

Heat Hardening

We used Hutchison's (1961) "Repeated-CTM" method to determine the presence of heat hardening, using recovery intervals ranging from 30 minutes to 72 hours. A minimum of 10 animals were used for each recovery interval and all initial CTM's were combined for the data analysis. Heat hardening was judged to have occurred if a significant ($P \leq 0.05$) increase in CTM above the initial level was recorded.

Effect of Sub-CTM Temperatures

We heated the animals to 1-1.5°C below their

previously determined initial CTM and allowed them to recover at their acclimatization conditions for periods of 1-24 hours. Following recovery, we heated the animals to their CTM, allowed them to recover for the same interval, and reheated them to a second CTM. A minimum of 10 animals were used at the beginning of each determination. If the initial CTM of the preheated animals was significantly greater ($P \leq 0.05$) than the previously determined initial CTM, exposure to high sub-CTM temperatures could cause heat hardening or heat hardening and rapid acclimation may be the same phenomenon. We also examined the effect of preheating on hardening ability.

Effect of Multiple Exposures to CTM

We heated the animals to their CTM five times in succession, allowing them to recover between each heating at their acclimatization conditions for periods of 1-24 hours. We determined that multiple exposures to CTM had an effect if the CTM continued to increase significantly ($P \leq 0.05$) with each repetition.

Diel and Seasonal Variation

At four hour intervals, we heated animals to their CTM, allowed them to recover for one hour, and reheated them to their CTM. We considered diel variation to be a factor if the CTM for one or more periods was significantly

($P \leq 0.05$) higher or lower than the other periods. We determined that hardening ability was affected by diel variation if the differences between the initial CTM and the repeat CTM were significant for some periods and non-significant for others.

At approximately three month intervals, we heated Notropis lutrensis, collected October 1976, to their CTM, allowed them to recover for one hour, and reheated them to their CTM. These fish were from a single population, collected on the same date, and maintained throughout the experiment at 15°C and under a natural photoperiod. No fish was tested more than once. Seasonal variation in CTM was considered a factor if the CTM of any season was different ($P \leq 0.05$) from the other seasons. There was seasonal variation in hardening ability if significant hardening occurred in one or more seasons but not in the others.

Data Analysis

Data were analyzed with a one-way analysis of variance (ANOVA) and Duncan's multiple range test (Sokal and Rohlf, 1969) at a $P \leq 0.05$ level of significance. We excluded from the data analysis all animals which failed to recover from the CTM because by definition, they had exceeded the CTM.

RESULTS

In all four species tested, the second CTM was significantly higher than the initial CTM within 1-2 hours of the first exposure and then had returned to the initial level within 24 hours (Figs. 1-2).

Preheating to sub-CTM temperatures had mixed effects. Preheating had no effect on the initial CTM of R. berlandieri and only untreated animals showed any hardening ability (Fig. 3). In N. viridescens, the preheated initial CTM was significantly greater than that of the untreated group after the one hour recovery interval, but was non-significant at all other intervals. There was no significant hardening in preheated animals (Fig. 3). The results with fish paralleled those in amphibians. Preheating had no effect on N. lutrensis and again there was no significant hardening in preheated animals. Initial CTM's of preheated P. promelas were significantly higher than those of unheated animals at recovery intervals of 60, 90, and 120 minutes and were non-significant at the longer intervals. Hardening ability in preheated animals was non-significant at all recovery intervals.

In all species, multiple repetitions of the CTM show no significant increase in CTM beyond that following the first recovery interval. However, there was a slight increase in mortality with further repetitions, and a general, though non-significant decrease in tolerance in some of the experiments (Fig. 4).

There was no significant diel variation in initial or repeat CTM in all four species (for example see Fig. 5). There were, however, periods in which hardening ability was significant and those when it was not. In all four species, significant hardening ability occurred from early to late afternoon, although N. viridescens had additional periods of significant hardening at 2000 and 2400 CST.

Notropis lutrensis had significant seasonal variation in CTM and hardening ability. The CTM in May was significantly higher than October and February. The fish showed significant hardening ability only in October and May (Fig. 6).

DISCUSSION

Our results demonstrate that heat hardening exists in the animals tested. The increase in tolerance 1-2 hours following the initial exposure to CTM is faster than the published rates of acclimation of the CTM, which range from 24-96 hours (Hutchison, 1961; Brattstrom and Lawrence, 1962; Brattstrom, 1968; Hutchison and Ferrance, 1970). Basedow (1969) used a "shock transfer" method which involved a rapid transfer of the animal from a moderate adaptation (acclimation) temperature to an extreme adaptation temperature. He observed significant increases in tolerance requiring 8-12 hours in the fish Idus idus and Anguilla vulgaris. This rate, however, is still slower than the rates of increase due to hardening. More important than the differences in the rates of increase in tolerance, are the rates of return to initial levels of tolerance. All animals had returned to the initial levels of tolerance within 24 hours or less. The published rates of acclimation to low temperatures or the rates of reacclimation to low temperatures after acclimation to high temperatures are slower than the rates of acclimation to high temperatures. These rates of

acclimation or reacclimation range from 48-96 hours (Hutchison, 1961; Brattstrom and Lawrence, 1962; Brattstrom, 1968; Dunlap, 1968; Hutchison and Ferrance, 1970). Thus the rates of onset and decay of hardening are strikingly different from the rates of acclimation.

The results of the preheating experiments are puzzling. The rate of acclimation is dependent on the temperature of acclimation (Brattstrom and Lawrence, 1962; Brattstrom, 1968; Hutchison and Ferrance, 1970); the higher the temperature of acclimation, the more rapid the rate of acclimation. The significant effects of preheating on N. viridescens and P. promelas therefore occurred at much shorter intervals than would be expected if the acclimation rate is dependent on acclimation temperature. Although preheating temperatures were below the CTM (onset of spasms), they are still above lethal temperatures which require greater exposure time for effect. In some cases the animals had lost their righting response, which is sometimes used as a CTM endpoint (i.e. Brattstrom, 1963). Perhaps these animals which showed significant preheating effects were exposed to temperatures high enough to induce hardening. Hutchison (1961) found preheating had no effect on the CTM and Basedow (1969) found that preheating increased heat resistance of newts less than exposure to the resistance temperature (CTM). Alexandrov (1964) found

no increase in heat resistance of cells until they reached lethal temperature. These findings, combined with our data, strongly suggest that heat hardening in animals requires exposure to CTM temperatures. However, further studies are needed to determine if this increase in tolerance is due to hardening, rapid acclimation, or possibly a response to heat damage (Precht, 1973).

Multiple exposures to the CTM do not continue to increase tolerance after the first exposure. The CTM is the highest temperature tolerated by an animal, even though the animal is exposed only briefly. Thus, it is reasonable to expect that any adjustment resulting from this exposure would be the maximum adjustment of tolerance attainable, the "ultimate CTM." Indeed, the adjustment of heat resistance due to acclimation proceeds only to some upper limit or plateau (Fry, 1958, 1967; Hutchison, 1976) and our results are consistent with that idea.

Diel variation in CTM has been demonstrated by a number of workers (Kosh and Hutchison, 1968; Johnson, 1971, 1972a, 1972b, 1976; Spellerberg and Hoffman, 1972). Therefore, the lack of significant diel variation in CTM in our study does not preclude its presence. However, the diel variation in hardening ability is certainly suggestive of an adaptive mechanism, because significant periods of hardening ability, early to late afternoon,

correspond to periods of peak environmental temperature.

Seasonal variation also seems to be a factor in both CTM and hardening ability, with the greatest magnitude of adjustment corresponding to the warmest season. Seasonal variation of CTM or temperature resistance has been demonstrated by several workers (Hoar, 1955; Hoar and Robertson, 1959; Hutchison, 1961; Lucas and Reynolds, 1967; Feder and Pough, 1975; Kowalski et al., 1978). The difficulty of measuring seasonal variation in a single population, under identical acclimation and test conditions, make results in this and other studies hard to evaluate.

Although laboratory evidence tends to support the existence of heat hardening in animals, its adaptive value must be demonstrated in the field. The laboratory results indicate that hardening would be adaptive for animals in environments with extreme fluctuations in diurnal temperatures. There are some field studies which support this idea.

The best field evidence for heat hardening is found in fish, particularly desert fish. Lowe and Heath (1969) observed desert pupfish, Cyprinodon macularius, active in the field at temperatures of 40-41°C while adjacent waters at temperatures as low as 30°C were available. These activity temperatures are near laboratory CTM temperatures (41-43°C) and above the

highest temperature (36.5°C) at which the fish can survive long enough to become acclimated in the laboratory. They suggested that this increased tolerance might be due to the cyclical nature of the thermal environment and that the fish might spend brief amounts of time at the highest available temperatures and then return to cooler water for periods of recovery and repair. This observation is consistent with findings that acclimation to cyclical temperature regimes increases the rate and scope of thermal acclimation (Heath, 1963; Hutchison and Ferrance, 1970; Feldmeth et al., 1974). Otto (1974) observed western mosquitofish, Gambusia affinis, in water at 42°C, approximately 4°C above their laboratory upper incipient lethal (38.3°C) (Otto, 1973). He tested fish acclimated to various constant and cycling temperature regimes. He varied the time at the peak temperature in the cyclic regimes and used lethal, supralethal, and sublethal temperatures as peak temperatures. Only the cyclical regimes with sublethal exposures to lethal or supralethal peak temperatures produced tolerances comparable to those observed in the field.

Fish appear to be utilizing heat hardening to increase their thermal tolerance behaviorally in the field. It is reasonable that fish from non-desert habitats would also show this adaptation because although aquatic environmental temperatures may not change as

rapidly as those in the terrestrial environment, the aquatic environment does not provide the variety of microhabitat "escape routes" as temperatures approach lethal limits. For this reason, it may be more difficult to demonstrate heat hardening in the field for terrestrial vertebrates. There is, however, one possible example of heat hardening in terrestrial vertebrates.

Pough and Wilson (1970) found that juvenile spotted salamanders, Ambystoma maculatum, sheltered under rocks near a pond, increased their CTM from a low of 38.6°C in early morning to a high of 39.7°C in the afternoon on sunny days. They termed this phenomenon "natural daily acclimation" because laboratory tests under constant conditions showed no daily cycle in CTM. While they never found living salamanders under rocks at temperatures greater than 32°C, laboratory tests showed the salamanders did not move from under rocks until the under-rock temperatures reached 36°C. However, once the salamanders left the rocks, they moved onto sand at substrate temperatures of 38°C. This temperature is very close to the morning CTM and could cause an increase in tolerance via hardening. If these laboratory tests reflect what occurs in nature, the salamanders very well could be behaviorally utilizing heat hardening to increase their tolerance if all under-rock temperatures should exceed 32°C (Hutchison and Maness, 1979). In a similar study,

Pough (1974) found eastern red efts, Notopthalmus v. viridescens, occupied the warmest regions under bark even though cooler regions were readily available. Therefore, terrestrial ectotherms, as well as aquatic ectotherms, may be capable of increasing their thermal tolerance via heat hardening.

Our results combined with these published field observations strongly suggest an adaptive role for heat hardening. Species from habitats with extreme fluctuations in diurnal temperatures may utilized heat hardening to survive lethal environmental temperatures, but several questions remain. The adaptive value of heat hardening in desert habitats seems obvious, but hardening may also be adaptive in more temperate habitats such as the intermittent rivers and streams of the Great Plains. While the usefulness of hardening in aquatic habitats seems clear, evidence from terrestrial habitats is lacking, as is evidence for the presence and adaptive value of hardening in other vertebrate ectotherms and endotherms. In addition to the studies with other vertebrates, the biochemical and physiological basis of acclimation of thermal tolerance needs to be elucidated. Perhaps only then can we distinguish between the processes of hardening and acclimation and determine if hardening is truly adaptive or simply a cellular response to damage caused by high temperatures.

LITERATURE CITED

- Alexandrov, V. Ya. (1964) Cytophysiological and cytoecological investigations of resistance of plant cells towards the action of high and low temperature. Q. Rev. Biol. 39, 35-77.
- Basedow, T. (1969) Über die auswirkung von temperatur-schocks auf die temperaturresistenz poikilothermer wassertiere. Eine untersuchung zum problem der thermischen schockanpassung bei tierern. Int. Rev. ges. Hydrobiol. 54, 765-789.
- Brattstrom, B. H. (1963) A preliminary review of the thermal requirements of amphibians. Ecology 44, 238-255.
- Brattstrom, B. H. (1968) Thermal acclimation in anuran amphibians as a function of latitude and altitude. Comp. Biochem. Physiol. 24, 93-111.
- Brattstrom, B. H. (1970) Amphibia. In Comparative Physiology of Thermoregulation Vol. I. Invertebrates and Nonmammalian Vertebrates (Edited by G. C. Whittow), pp. 135-166. Academic Press, New York.

- Brattstrom, B. H. and P. Lawrence. (1962) The rate of thermal acclimation in anuran amphibians. Physiol. Zool. 35, 148-156.
- Brett, J. R. (1956) Some principles in the thermal requirements of fishes. Q. Rev. Biol. 31, 75-87.
- Dunlap, D. G. (1968) Critical thermal maximum as a function of temperature of acclimation in two species of hyliid frogs. Physiol. Zool. 41, 432-439.
- Dunlap, D. G. (1969) Evidence for a daily rhythm of heat resistance in the cricket frog, Acris crepitans. Copeia 1969, 852-854.
- Feder, M. E. and F. H. Pough. (1975) Temperature selection by the red-backed salamander, Plethodon c. cinereus (Green) (Caudata: Plethodontidae). Comp. Biochem. Physiol. 50A, 91-98.
- Feldmeth, C. R., E. A. Stone, and J. H. Brown. (1974) An increased scope for thermal tolerance upon acclimating pupfish (Cyprinodon) to cycling temperatures. J. comp. Physiol. 89, 39-44.
- Folk, G. E., Jr. (1974) Textbook of Environmental Physiology. Lea and Febiger, Philadelphia.
- Fry, F. E. J. (1958) The experimental study of behavior in fish. Proc. Indo-Pac. Fish. Counc. 3, 37-42.

- Fry, F. E. J. . (1967) Responses of vertebrate poikilotherms to temperature. In Thermobiology (Edited by A. H. Rose), pp. 375-409. Academic Press, New York.
- Fry, F. E. J. (1971) The effect of environmental factors on the physiology of fish. In Fish Physiology VI. Environmental Relations and Behavior (Edited by W. S. Hoar and D. J. Randall), pp. 79-88. Academic Press, New York.
- Heath, W. G. (1963) Thermoperiodism in the sea-run cutthroat trout. Science 142, 486-488.
- Hoar, W. S. (1955) Seasonal variation in the resistance of goldfish to temperature. Trans. Roy. Soc. Can. 49, 25-34.
- Hoar, W. S. and G. B. Robertson. (1959) Temperature resistance of goldfish maintained under controlled photoperiods. Can. J. Zool. 37, 419-428.
- Hutchison, V. H. (1961) Critical thermal maxima in salamanders. Physiol. Zool. 34, 92-125.
- Hutchison, V. H. (1976) Factors influencing thermal tolerances of individual organisms. In Thermal Ecology II (Edited by G. W. Esch and R. W. McFarlane), pp. 10-26. U.S. Natl. Tech. Inform. Serv., Springfield, Virginia.
- Hutchison, V. H. and M. R. Ferrance. (1970) Thermal tolerances of Rana pipiens acclimated to daily temperature cycles. Herpetologica 26, 1-8.

- Hutchison, V. H. and J. D. Maness. (1979) The role of behavior in temperature acclimation and tolerance in ectotherms. Amer. Zool. (In Press).
- Johnson, C. R. (1971) Daily variation in the thermal tolerance of Litoria caerulea (Anura: Hylidae). Comp. Biochem. Physiol. 40A, 1109-1111.
- Johnson, C. R. (1972a) Diel variation in the thermal tolerance of Litoria gracilentia (Anura: Hylidae). Comp. Biochem. Physiol. 41A, 727-730.
- Johnson, C. R. (1972b) Thermal relations and daily variation in the thermal tolerance in Bufo marinus. J. Herpetol. 6, 35-38.
- Johnson, C. R. (1976) Diel variation in the thermal tolerance of Gambusia affinis affinis (Pisces: Peociliidae). Comp. Biochem. Physiol. 55A, 337-340.
- Kosh, R. J. and V. H. Hutchison. (1968) Daily rhythmicity of temperature tolerance in eastern painted turtles. Copeia 1968, 244-246.
- Kowalski, K. T. , J. P. Schubauer, C. I. Scott, and J. A. Spotila. (1978) Interspecific and seasonal differences in the temperature tolerance of stream fish. J. Thermal Biol. 3, 105-108.
- Lowe, C. H. and W. G. Heath. (1969) Behavioral and physiological responses to temperature in the desert pupfish Cyprinodon macularius. Physiol. Zool. 42, 53-59.

- Lucas, J. J. and W. W. Reynolds. (1967) Temperature selection by amphibian larvae. Physiol. Zool. 40, 159-171.
- Mahoney, J. J. and V. H. Hutchison. (1969) Photoperiod acclimation and 24-hour variations in the critical thermal maxima of a tropical and a temperate frog. Oecologia 2, 143-161.
- Otto, R. G. (1973) Temperature tolerance of the western mosquitofish. J. Fish. Biol. 5, 575-585.
- Otto, R. G. (1974) The effects of acclimation to cyclic thermal regimes on heat tolerance of the western mosquitofish. Trans. Amer. Fish. Soc. 103, 331-335.
- Pough, F. H. (1974) Natural daily temperature acclimation of eastern red efts, Notopthalmus v. viridescens (Rafinesque) (Amphibia: Caudata). Comp. Biochem. Physiol. 47A, 71-78.
- Pough, F. H. and R. E. Wilson. (1970) Natural daily temperature stress, dehydration, and acclimation in juvenile Ambystoma maculatum (Shaw) (Amphibia: Caudata). Physiol. Zool. 43, 194-205.
- Precht, H. (1973) Limiting temperatures of life functions. In Temperature and Life (By H. Precht, J. Christophersen, H. Hensel, and W. Larcher), pp. 400-440. Springer-Verlag, New York.
- Prosser, C. L. (1973) Comparative Animal Physiology 3rd ed. W. B. Saunders, Philadelphia. 966p.

- Rose, A. H. (1967) Thermobiology. Academic Press,
New York. 653 p.
- Sokal, R. R. and F. J. Rohlf. (1969) Biometry. The
Principles and Practices of Statistics in
Biological Research. W. H. Freeman, San Francisco.
776 p.
- Spellerberg, I. F. and K. Hoffman. (1972) Circadian
rhythm in lizard critical minimum temperature.
Die Naturwissenschaften 11, 517-518.
- Whittow, G. C. (1970) Comparative Physiology of
Thermoregulation Vol. I. Invertebrates and
Nonmammalian Vertebrates. Academic Press, New
York. 333 p.

FIGURE LEGENDS

Fig. 1. -- Heat hardening in amphibians. Top - Rana berlandieri. Bottom - Notophthalmus viridescens. Time elapsed between the initial CTM at time 0 and the second exposure is shown on the abscissa. An asterisk (*) indicates a significant difference between the initial CTM and the indicated second exposure; horizontal line, the mean; vertical line, the range; rectangles, two standard errors of the mean; and the numbers at the top, the sample size.

Fig. 2. -- Heat hardening in fish. Top - Notropis lutrensis. Bottom - Pimephales promelas. The manner of presentation is the same as Fig. 1.

Fig. 3. -- Effect of preheating to sub-CTM temperatures on the CTM. Top - Rana berlandieri. Bottom - Notophthalmus viridescens. The time intervals between the control initial and repeat CTM's and the preheated initial and repeat CTM's are on the abscissa. The remainder of the presentation is the same as Fig. 1.

Fig. 4. -- Effect of multiple exposures on the CTM. Top -

Rana berlandieri. Bottom - Notopthalmus viridescens.

Time intervals between the initial CTM at time 0 and successive exposures are on the abscissa. The remainder of the presentation is the same as Fig. 1.

Fig. 5. -- Diel variation of CTM and hardening ability - Notopthalmus viridescens. The time of day (CST) is on the abscissa. The dark bar on the abscissa represents scotophase; asterisks(*), significant hardening ability; solid line, initial CTM; and the dashed line, repeat CTM. The remainder of the presentation is the same as Fig. 1.

Fig. 6. -- Seasonal variation in CTM and hardening ability - Notropis lutrensis. The dates of the initial and repeat CTM determinations are on the abscissa. The asterisks (*) represent significant hardening ability; plain rectangles, initial CTM; and the shaded rectangles, repeat CTM. The remainder of the presentation is the same as Fig. 1.











