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UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

COMPARATIVE PHYSIOLOGY AND MACROECOLOGY OF THERMAL TOLERANCE IN ECTOTHERMIC VERTEBRATES WITH CORRELATES OF PHYLOGENY AND BIOGEOGRAPHY

A Dissertation

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

By

WILLIAM ISAAC LUTTERSCHMIDT

Norman, Oklahoma

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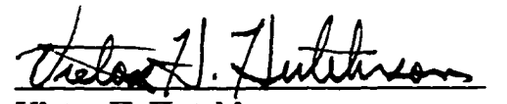
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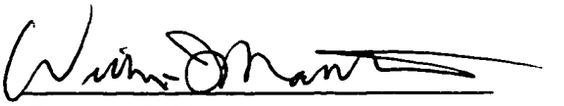
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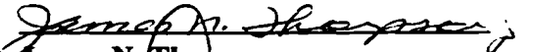
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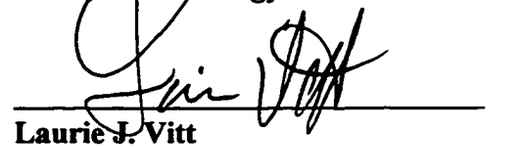
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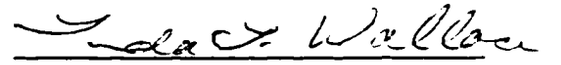
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ABSTRACT

Physiological ecology is an intellectual brew of elements taken from natural history, ecology, and evolution, and operationally tied to the experimental discipline of physiology. ...the bedrock of physiological ecology is a thorough understanding of the natural history of the organisms being studied; not only does such knowledge influence the interpretation of our observations, but it can suggest which species should be studied to determine the rules by which evolution has produced the diversity of life on the planet Earth.
Brian K. McNab 1982
Bulletin of the Ecological Society of America 63(4):346

"Temperature and moisture are the two master limiting factors to the distribution of life on earth" (Krebs 1994). Ecologists have dedicated much study to the understanding of these factors and how they influence species and their biogeography. Organisms have four options in dealing with the climatic condition of their habitats: (1) tolerate the temperature and humidity regardless of possible consequences to fitness, (2) move to more suitable climatic conditions, (3) death, or (4) escape fitness limiting conditions through evolutionary adaptation. It is this forth option which has been one of the primary interests to comparative physiologists and physiological ecologists in explaining (1) how physiological processes function with respect to the environment, (2) how the physiological capacities of an organism enable it to live in conditions not suitable for other species, and (3) how the environment may "fine-tune" or even directionally select for particular physiological processes increasing the relative fitness of future generations.

An organism's distribution and geographic range result from physiological tolerances and its ability to adjust physiological processes to maximize survival, growth, and reproduction under a diversity of environmental factors characteristic to its geographic region. This principle has been described by Liebig's Law of the Minimum

which states that the distribution of species is governed by the environmental factor for which a species has the narrowest tolerance range or least adaptability. As described, physiological ecologists agree that temperature is probably one of the most pervasive factors in determining the distribution of animals. In this work, I address the importance of species specific thermal tolerance in an attempt to explain some macroecological patterns of distribution among fishes. The first two chapters are an evaluation and review of thermal tolerance in studies of comparative physiology. I measured the critical thermal maximum (CTMax) for more than 600 individuals to statistically evaluate the variability associated with the end points loss of righting response (LRR) and onset of spasms (OS) used in determining CTMax. In my review of thermal tolerance, I found 388 of 725 papers to provide data for a historical and taxonomic review with a discussion of various methodologies and uses for the measure of species specific thermal tolerance.

In the third chapter, I investigated the correlates between the comparative physiology of thermal tolerance and macroecological patterns of distribution for several fishes found in Oklahoma. Although temperature and the physiological tolerance for changing thermal regimes are major limiting factors affecting species distribution, studies of macroecology have not adequately addressed the relationship between temperature and an organism's geographic distribution. Studies of macroecology often fail to investigate the underlying physiological factors and tolerances that influence animal distribution. This is one of the first attempts to use a "true" physiological parameter in macroecology.

CHAPTER 1

The Critical Thermal Maximum: Data to Support the Onset of Spasms as the Definitive End Point

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The critical thermal maximum: data to support the onset of spasms as the definitive end point

Abstract: I provide data to support the onset of spasms (OS) as the definitive end-point for determining thermal tolerance with CTMax. I measured CTMax of 610 animals in three vertebrate classes (Actinopterygii, Amphibia, and Reptilia). All animals showed a significantly lower mean loss of righting response (LRR) than OS. A statistical evaluation of the variability associated with the end points LRR and OS also showed that OS is a more precise measure of thermal tolerance.

OS is a more meaningful end-point than LRR because it more closely fits the Cowles and Bogert (1944) original definition of CTMax and occurs at temperatures required for physiological responses such as heat hardening and perhaps the production of some heat shock proteins. The adoption of OS as a standard end-point would allow for valid comparisons of data from different studies and among taxa, an important consideration for comparative phylogenetic analyses. However, I suggest that LRR also should be measured for comparisons with earlier studies.

Introduction

Measures of thermal tolerance have long been used to investigate the thermal capacities of organisms to tolerare their complex holocoenotic environments. The increasing use of data on organismal thermal tolerances indicates the continuing popularity and the

usefulness of such physiological parameters in modern biological investigations. To our knowledge, at least 20 papers have been published within the last year addressing the implications of thermal tolerance to physiological and ecological theory (reviewed in Lutterschmidt and Hutchison *In press*). However, a diversity of experimental methods and terminology are still used by investigators despite pleas for standardizations of test methods (e.g., Hutchison 1976). Standardized methods were again urged (Hutchison 1979; Paladino et al. 1980) with discussion of concerns for making valid comparisons of data from different studies. The problem is further exacerbated by "phylogenetic provincialism" where many authors are unaware of the techniques used and results obtained from diverse taxonomic groups.

Our purpose is to provide general information for the determination of thermal tolerance and data that support the onset of spasms (OS) as the definitive endpoint for determining thermal tolerance with the CTMax method. I provide elsewhere a comprehensive review of thermal tolerance with a discussion of its applications to comparative physiology (see Lutterschmidt and Hutchison *In Press*).

The two major methods for determining thermal tolerance are discussed in Lutterschmidt and Hutchison (*In press*): (1) the static or lethal temperature method, in which time to death at constant test temperatures is measured, and (2) the dynamic method, which involves increasing test temperatures until an appropriate end point is reached (Brett 1944; Fry et al. 1947; Fry 1957). The static method uses statistical techniques from pharmacology (Bliss 1937) to determine the equivalent of an LD_{50} from time mortality curves where the "dosage" is the time animals are exposed to a

constant test temperature until death.

The dynamic method, the critical thermal minima (CTMin) and critical thermal maxima (CTMax), was introduced and defined by Cowles and Bogert (1944) where CTMax was defined as "the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death." This definition was modified by Lowe and Vance (1955) to include statistical parameters. Standardized methods for heating rates and endpoint were added by Hutchison (1961, 1976). Thus, rates of heating from about 0.5 to 1.5 $^{\circ}C\ min^{-1}$ are often used. As temperature increases during a CTMax test an animal usually displays a sequelae of responses: loss of righting response (LRR), sudden onset of muscular spasms (OS), and finally "heat rigor," "coma" or "death." The latter three events are not definitive, difficult to determine (Zweifel 1957), and seldom described fully (Lutterschmidt and Hutchison *In press*).

To assess whether OS is the better CTMax end point, I collected data to (i) compare the LRR and OS within different ectothermic vertebrates and determine which CTMax end point has greater precision and (ii) determine if the same end point demonstrates this greater precision across taxa (i.e., for fish, amphibians and reptiles). To illustrate the importance of standardized methods for the determination of upper temperature tolerance, I discuss the difficulties in using data from studies with widely divergent methods and the importance of comparable thermal tolerance data to ecophysiological theory, comparative physiology, and comparative phylogenetic investigations (Felsenstein 1985). A phylogenetic analysis of these data will be

published in a separate paper.

Materials and Methods

Animal collection and acclimation

I collected 439 fishes representing 24 species and 9 families, 33 salamanders representing 4 species and 2 families, 103 frogs representing 5 species and 3 families, and 35 lizards representing 1 species in Iguanidae. These animals represent 3 vertebrate classes (Actinopterygii, Amphibia, and Reptilia) and 8 orders (Siluriformes, Cypriniformes, Perciformes, Atheriniformes, Cyprinodontiformes, Caudata, Anura, Squamata) (Table 1).

I collected all native fishes by seining streams and reservoirs in central and southcentral Oklahoma. *Tilapia nilotica* were obtained from aquaculture ponds at the University of Oklahoma. Salamanders and frogs were collected from central and southeastern Oklahoma (Cleveland and LaFlore Counties, respectively) and/or obtained from animal suppliers. The lizard *Cophosaurus texanus* was collected from central Kimble County, Texas.

Fishes were acclimatized in Living Stream® aquaria for two weeks at 10 ± 1°C and an LD 12:12 photoperiod; photophase was from 0600 to 1800 h centered on noon CST. I use the term "acclimation" for adjustment to one environmental factor and "acclimatization" for changes to two or more factors, following Folk's (1974) "Rules" for uniform terminology. All measures of thermal tolerance were made between the

second and tenth h of the photophase to reduce possible effects of diel cycles on thermal tolerance (Hutchison 1976). The same acclimation regimes were used for amphibians in an environmental chamber. However, measures of thermal tolerance for amphibians were made between 2000 and 2200 h (second and fourth h) of the scotophase, consistent with the peaks of normal activity. *Cophosaurus texanus* (due to high thermal requirements) were acclimated at 25°C and was also tested during the peak diel activity (i.e., between 1000 and 1600 h or the fourth and tenth h of its photophase). Although different acclimation temperatures influence thermal tolerance (Hutchison and Rowlan 1974), our use of 25°C in acclimation of *Cophosaurus texanus* is valid because I evaluated the variability in end points among individuals of the same species (i.e., pair-wise comparisons) and not the difference in thermal tolerance among species or treatments.

Measures of thermal tolerance

I used the dynamic method, critical thermal maximum (CTMax), for determining thermal tolerance following the methods outlined by Hutchison (1961). I recorded both loss of righting response (LRR) and the onset of opercular spasms in fishes or the onset of muscular spasms in amphibians and reptiles for indices of CTMax with OS being regarded as the definitive end point (Paulson and Hutchison 1987). I recorded T_b for both end points for completeness of data and for statistical evaluation. Body temperature (T_b) for fish LRR was recorded when they lost equilibrium and demonstrated inverted swimming (e.g., Al-Johany and Yousuf 1993; Korhonen and Lagerspetz 1996; Pyron and Beitinger 1993; Rutledge and Beitinger 1989). The OS of

fish were defined by disorganized and high frequency muscular movements, rigidity of the pectoral fins, and, especially, a high frequency quivering of the opercula. All individuals recovered from exposure to LRR and OS. These behaviors seem to fit best the definition of CTMax as "the arithmetic means of the collective thermal points at which locomotor activity becomes disorganized and the animals lose their ability to escape from conditions that will promptly lead to their death" (Hutchison 1961).

I placed each fish in a heating chamber to increase T_b 1°C min^{-1} until LRR and OS were observed. The T_b's of fishes >8.0 cm standard length (SL) and/or 30 g body mass (M_b) were measured by inserting a 36 ga. thermocouple into their urogenital opening (ca. 10 to 15 mm into the lower intestine) prior to testing. T_b's of frogs with a snout-vent-length (SVL) >9.0 cm and/or 75 g were also measured with a thermocouple. T_b's of fishes and frogs of smaller size closely followed chamber-water temperature (Fig. 1), thus the use of thermocouples was unnecessary. All M_b of salamanders and lizards were less than 75 g and no thermocouples were used.

Statistical analyses

Mean LRR and OS were calculated for each species; their associated variances (s^2) and standard errors (SE) are shown also (Table 1). These measures allowed for the evaluation of precision associated with each CTMax end point (i.e., LRR and OS) for each species. I then used paired t-tests to determine significant differences between the means, variances, and standard errors associated with LRR and OS for all fish, and amphibians and reptiles. Paired t-tests also were used to determine if these results of precision associated with LRR and OS were consistent for all taxa combined (Table 2).

An *a priori* natural log transformation for normality was performed on all measures of variance and standard error (Bartlett and Kendall 1946) prior to statistical comparisons of LRR and OS. I determined that all assumptions of normality were met prior to statistical analyses and used SigmaStat 1.0 ® (Jandel Scientific Software Corp., San Rafael, Calif. 1994) for all pairwise comparisons and Statview 4.5® (Abacus Concepts, Berkley, Calif. 1992) for regression analyses.

Animal Care

Animals were collected under authority of permits for scientific collectors issued by the Oklahoma Department of Wildlife Conservation. I followed guidelines for use of live animals in field research (ASIH 1987a, 1987b). Laboratory study protocols were approved by the University of Oklahoma Animal Care and Use Committee (Animal Welfare Assurance Number 73-R-100 on file with the Office for the Protection of Research Risks). The procedures followed were in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Results

Measures and statistical comparisons of thermal tolerance endpoints

All 610 individuals of the 34 species demonstrated LRR at lower temperatures than OS (Fig. 2a, 2b). All paired t-tests showed that mean temperature was significantly lower for LRR than for OS in fishes, amphibians, reptiles and all combined taxa (Table 2). I observed no size-dependent effects on either LRR or OS within species.

Significantly greater variances were also associated with the end-point LRR for fishes, amphibians, reptiles and all combined taxa (Table 2; Figure 2a, 2b). These results showed that OS is a more precise measure of thermal tolerance. An F-test for comparing the homogeneity for within species variances of LRR and OS also showed that these variances differed significantly only when the LRR variance was greater than the OS variance (Table 1).

Regression analyses indicated that critical masses for equal T_b and water/environmental temperature (T_e) were greater than 164.7 and 43.7 g for a frog and fish species, respectively. The relationship between T_e and T_b was highly significant for all four analyses (Fig. 1) with T_e explaining 99.1% of the variance in T_b. This suggests the appropriateness of water as a heating medium for the ease of controlling heating rates for CTMax.

Discussion

Measures and statistical comparisons of thermal tolerance endpoints

There is significantly more variance associated with the end point LRR than OS for all taxa (Table 2). Additionally, tests for the homogeneity of within species variances of LRR and OS show that these variances differed significantly only when the LRR variance was greater than the OS variance (Table 1). Although OS variance was greater than LRR variance for some species (Table 1), the homogeneity for these within species variances of LRR and OS did not differ statistically. Specifically, 31 variances

are compared from paired LRR and OS endpoints for each species. Of these comparisons, 24 show LRR variance to be greater than OS variance; 7 show OS variance to be greater. However, for these seven observations of greater OS variance, there is no significant difference in variance homogeneity between LRR and OS. Of the 24 occasions in which LRR variance is greater, 11 of the 24 comparisons differ significantly ($P < 0.05$). Thus OS is a more valuable and meaningful CTMax end point than LRR because of its greater precision.

A definitive and standard CTMax endpoint: its importance to comparative physiology

Studies in comparative animal physiology involve "the comparing and contrasting of physiological mechanisms, processes, or responses of different species of animals, or of a single species under differing conditions" (Withers 1992). An underlying assumption for any comparative study, however, is that all individuals and species have received identical experimental treatments. Without this assumption, I may attribute differences in physiological capacities for high temperature tolerance to adaptation when aptation was a cause. Inferring evolutionary change in physiological traits and function from invalid comparisons may lead to biased conclusions. I therefore emphasizes the importance for the adoption of a definitive and standard endpoint for measuring CTMax.

I have demonstrated that OS is a more meaningful end point than LRR due to its precision and greater physiological relevance. Although OS is more biologically meaningful, I recommend that both LRR and OS be determined and reported (e.g.,

Berkhouse and Fries 1995) for future comparisons with past studies. I also encourage the use of additional end points, if they are clearly defined, and if the value of OS is determined. Most importantly, when comparing and contrasting thermal tolerance among species from different studies, I recommend that investigators consider possible differences in experimental methods (i.e., acclimation, heating rate, etc.); otherwise ecological and/or evolutionary conclusions from studies with various protocols may be invalid. I advocate OS as the most biologically relevant end point and hope its adoption will facilitate standard definitions and techniques for valid comparisons among future studies.

Conclusion

A plea is once again made for a better appreciation of the factors that influence the thermal tolerance of ectotherms and the methods used for the determination of thermal tolerance under experimental conditions (Hutchison 1976). In nature, animals continually adjust to fluctuations in environmental factors. Because it is not possible to control completely the entire holocoenotic environment and its variations in the laboratory, we must recognize that many environmental factors may influence thermal tolerance and its measurement. Animals' tolerance of a single environmental factor such as temperature is typically greater than their tolerance of combinations of environmental factors studied simultaneously. The modification of thermal tolerance by many environmental factors (e.g., photoperiod, diel and seasonal cycles, respiratory gases, pH, salinity, diet, reproductive status) should be recognized and controlled as far as possible. Thus, tolerance to high temperature eventually must be studied as part of

multiple factorial interactions. The adoption of more standardized methods and terminology will greatly improve communications among investigators and will allow for valid comparisons among their data (Hutchison 1976). This will ultimately facilitate the necessary multiple factor approach and the broader applications of these data to ecophysiological theory (Lutterschmidt and Hutchison In press).

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Table 1. Sample size (n) and the critical thermal maximum (CTMax) end points [loss of righting response (LRR) and the onset of spasms (OS)] are shown with their associated measures of dispersion (variance [s^2] and standard error [SE]) for 35 vertebrate species. The last column shows the results for homogeneity among variances for each end point of CTMax. I used an F-test (Zar 1984) to compare difference between LRR variance and OS variance. Significance is indicated to $P \leq 0.05$ by either "+" if LRR variance > OS variance or "−" if LRR variance < OS variance. A zero indicates nonsignificance. Taxonomic groupings for fish follow Nelson (1994); amphibians and reptiles follow Conant and Collins (1991).

Class	Order	Family	Species	n	LRR(s^2, SE)	OS(s^2, SE)	$P \leq 0.05$
Actinopterygii							
	Siluriformes						
		Ictaluridae					
			Ictalurus punctatus	20	31.32(0.689, 0.186)	33.31(0.294,0.121)	0
	Cypriniformes						
		Catostomidae					
			Ictiobus bubalus	1	29.50	31.30	
		Cyprinidae					
			Campostoma anomalum	18	29.82(0.314, 0.132)	31.75(0.381, 0.145)	0
			Carassius auratus	11	32.05(0.415, 0.194)	35.79(0.087, 0.089)	+
			Cyprinella lutrensis	20	30.70(1.018, 0.226)	34.04(0.800, 0.200)	0
			Hybognathus placitus	26	28.37(1.594, 0.248)	31.75(0.767, 0.172)	0
			Hybopsis amblops	1	30.10	31.70	

	Notemigonus crysoleucas	28	29.13(2.589, 0.304)	33.36(0.540, 0.139)	+
	Notropis atherinoides	8	30.10(0.817, 0.320)	34.10(0.429, 0.231)	0
	Phenacobius mirabilis	2	30.45(0.605, 0.550)	33.35(0.404, 0.450)	0
	Pimephales notatus	29	29.32(0.952, 0.181)	33.70(0.236, 0.090)	+
Atheriniformes					
	Atherinidae				
	Menidia beryllina	20	29.24(3.699, 0.430)	31.59(3.391, 0.412)	0
Cyprinodontiformes					
	Poecilidae				
	Gambusia affinis	20	36.88(0.514, 0.160)	38.47(0.547, 0.165)	0
Perciformes					
	Percidae				
	Etheostoma spectaboli	19	29.80(0.724, 0.195)	31.54(0.412, 0.147)	0
	Moronidae				
	Morone saxatilis	3	29.43(2.813, 0.968)	31.60(0.280, 0.306)	0
	Centrarchidae				
	Lepomis cyanellus	15	31.09(2.099, 0.374)	34.18(4.230, 0.531)	0
	Lepomis gulosus	4	28.98(0.249, 0.250)	32.88(0.049, 0.111)	0
	Lepomis macrochirus	84	30.38(2.748, 0.181)	33.59(1.450, 0.131)	+
	Lepomis megalotis	59	30.87(2.622, 0.211)	34.10(3.949, 0.259)	0
	Lepomis microlophus	3	30.80(4.450, 1.225)	34.10(0.750, 0.500)	0
	Micropterus dolomieui	1	28.30	34.80	
	Micropterus punctulatus	5	30.76(0.173, 0.186)	34.22(0.327, 0.256)	0
	Micropterus salmoides	17	30.69(3.137, 0.430)	33.35(3.599, 0.460)	0
	Cichlidae				
	Tilapia nilotica	25	27.25(7.908, 0.562)	33.56(1.961, 0.280)	+

Amphibia					
	Caudata				
		Salamandridae			
			Notophthalmus viridescens	10	33.84(2.096, 0.458)	37.34(0.112, 0.106)	+
		Plethodontidae			
			Plethodon ouachitae	9	30.64(0.568, 0.251)	33.33(0.338, 0.194)	0
			Plethodon richmondi	4	31.28(0.902, 0.475)	33.48(0.062, 0.125)	+
			Desmognathus ochrophaeus	10	29.23(2.350, 0.485)	32.32(0.593, 0.243)	+
	Anura						
		Ranidae					
			Rana berlandieri	20	37.16(1.682, 0.290)	39.18(0.470, 0.153)	+
			Rana catesbeiana	19	38.88(0.159, 0.091)	40.65(0.188, 0.099)	0
			Rana utricularia	20	36.06(0.280, 0.118)	37.08(0.196, 0.099)	0
		Bufonidae					
			Bufo americanus	24	39.04(0.503, 0.145)	40.86(0.302, 0.112)	0
		Hylidae					
			Hyla chrysoscelis	20	38.02(2.056, 0.321)	40.81(0.367, 0.136)	+
Reptilia							
	Squamata						
		Iguanidae					
			Cophosaurus texanus	35	47.65(1.753, 0.224)	49.42(0.808, 0.152)	+

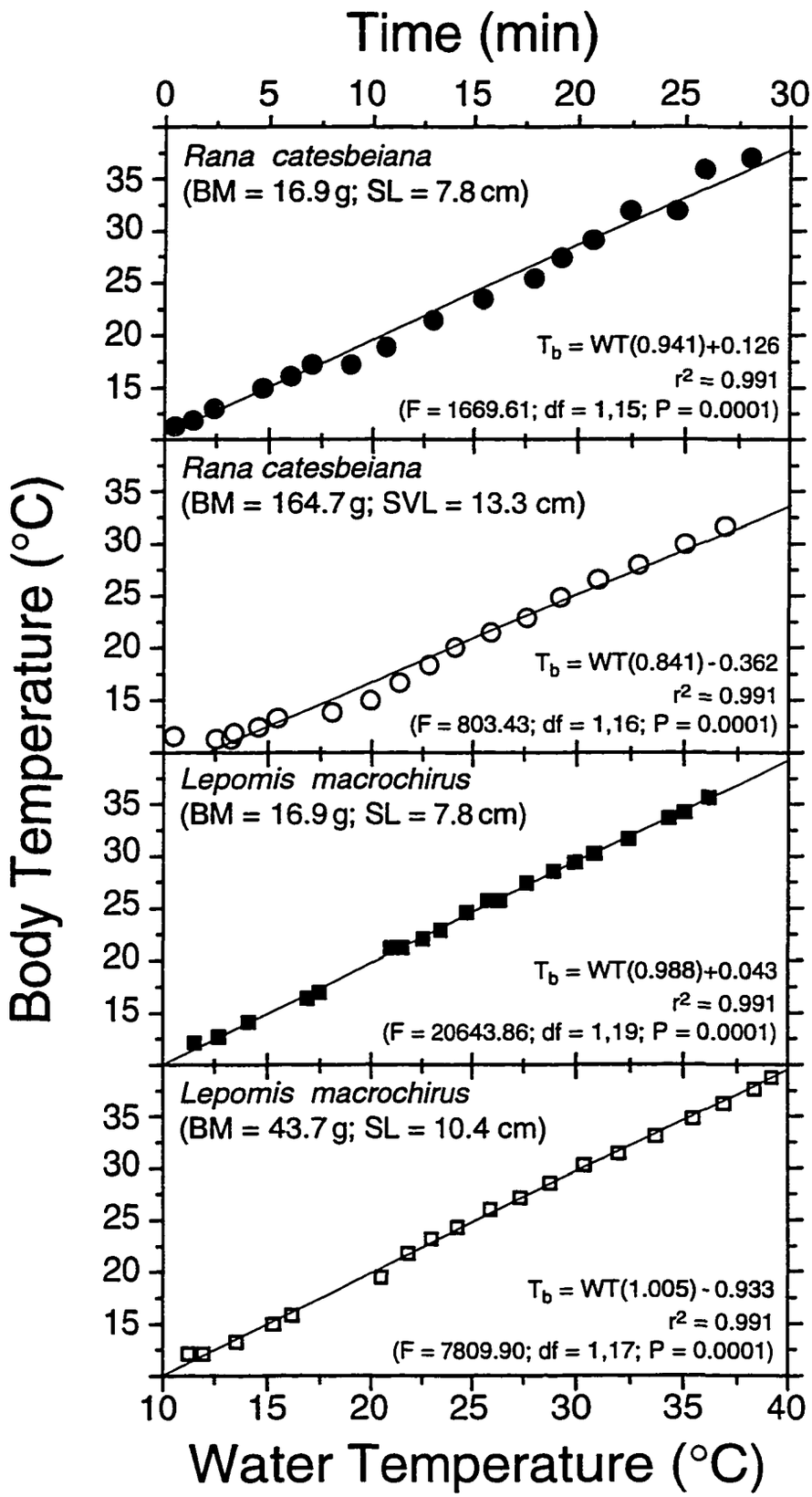
Table 2. Critical thermal maximum (CTMax) end points: results of paired t-tests showing group means ($\bar{x}$), standard deviations (sd), and standard errors (SE). The parameters compared are means and measures of dispersion (i.e., variance and standard error) for loss of righting response (LRR) and the onset of spasms (OS) in fish, amphibians and reptiles, and all taxa combined.

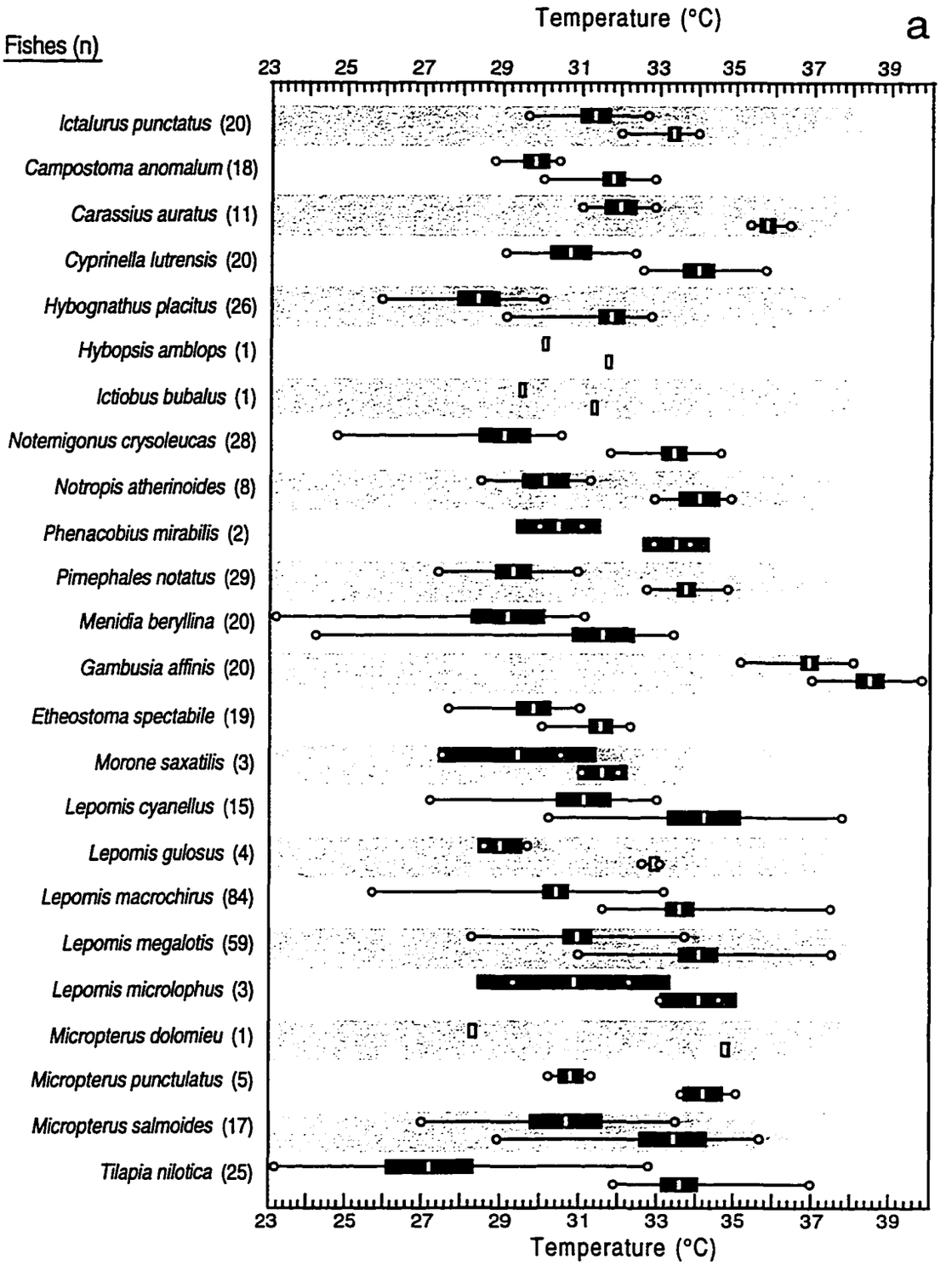
		Values from Paired t-tests		
Comparison	Group	$\bar{x}$	sd	SE
Means of LRR and OS				
Fish	mean LRR	30.22	1.78	0.364
(t = -12.00, df = 23, P < 0.0001)	mean OS	33.42	1.61	0.329
Amphibians and reptiles	mean LRR	36.18	5.374	1.699
(t = -9.65, df = 9, P < 0.0001)	mean OS	38.45	5.041	1.594
All taxa combined	mean LRR	31.97	4.21	0.721
(t = -13.80, df = 33, P < 0.0001)	mean OS	34.90	3.76	0.645
ln(Variances) of LRR and OS				
Fish	ln(LRR s^2)	0.186	1.039	0.227
(t = 3.46, df = 20, P = 0.0025)	ln(OS s^2)	-0.464	1.201	0.262
Amphibians and reptiles	ln(LRR s^2)	-0.109	0.951	0.301
(t = 3.73, df = 9, P = 0.0047)	ln(OS s^2)	-1.305	0779	0.246
All taxa combined	ln(LRR s^2)	0.0907	1.005	0.181
(t = 4.94, df = 30, P < 0.0001)	ln(OS s^2)	-0.7354	1.142	0.205
ln(Standard Errors) of LRR and OS				
Fish	ln(LRR SE)	-1.226	0.596	0.130
(t = 3.47, df = 20, P = 0.0024)	ln(OS SE)	-1.552	0.570	0.124
Amphibians and reptiles	ln(LRR SE)	-1.397	0.596	0.188
(t = 3.75, df = 9, P = 0.0046)	ln(OS SE)	-1.995	0.298	0.094
All taxa combined	ln(LRR SE)	-1.281	0.591	0.106
(t = 4.95, df = 30, P < 0.0001)	ln(OS SE)	-1.695	0.536	0.096

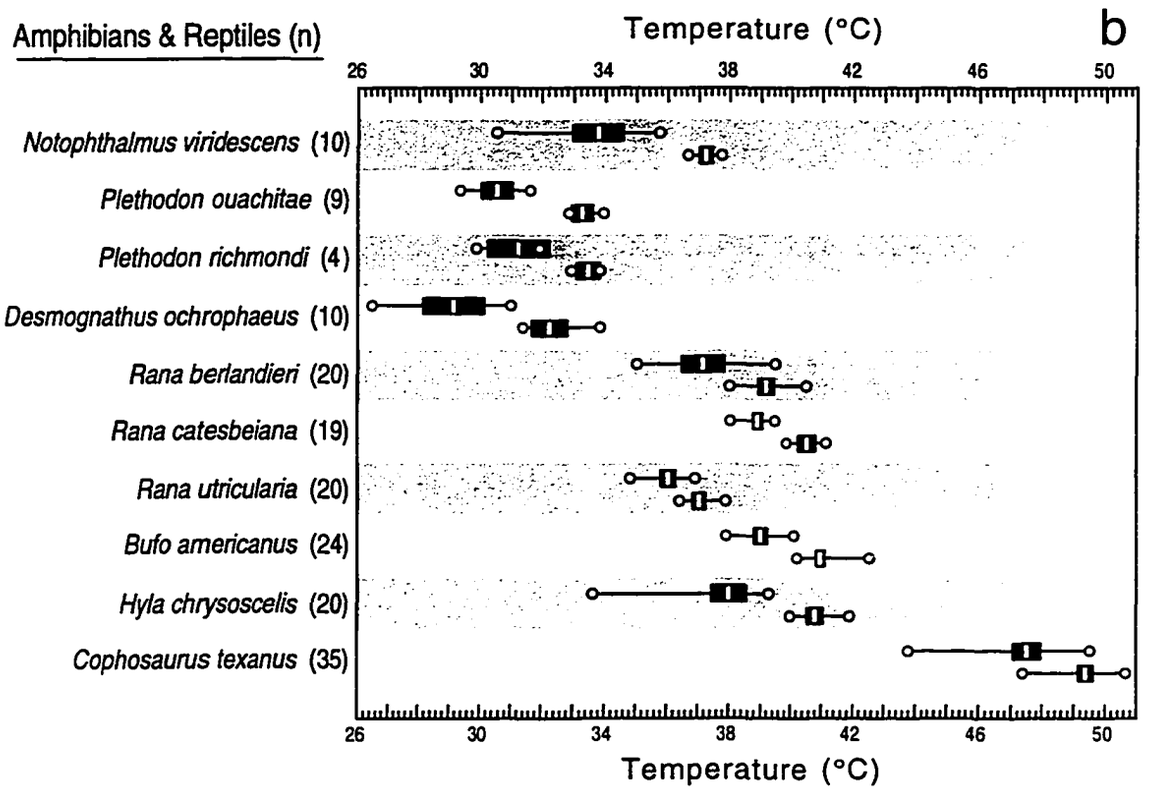
List of Figures

Fig. 1. Relationships between body temperature (T_b) and chamber-water or environmental temperature (T_e) for *Rana catesbeiana* and *Lepomis macrochirus* during heating at a rate of 1°C min^{-1}.

Fig. 2a, 2b. Mean loss of righting response (LRR) and onset of spasms (OS) for fishes (2a) and amphibians and reptiles (2b). Open circles indicate range, black rectangles indicate 2(SE), and white lines at the midpoint of each black rectangle indicate mean CTMax T_b.


Time (min)
0 5 10 15 20 25 30
Rana catesbeiana
(BM = 16.9 g; SL = 7.8 cm)
T_b = WT(0.941)+0.126
r^2 = 0.991
(F = 1669.61; df = 1,15; P = 0.0001)
Rana catesbeiana
(BM = 164.7 g; SVL = 13.3 cm)
T_b = WT(0.841) - 0.362
r^2 = 0.991
(F = 803.43; df = 1,16; P = 0.0001)
Lepomis macrochirus
(BM = 16.9 g; SL = 7.8 cm)
T_b = WT(0.988)+0.043
r^2 = 0.991
(F = 20643.86; df = 1,19; P = 0.0001)
Lepomis macrochirus
(BM = 43.7 g; SL = 10.4 cm)
T_b = WT(1.005) - 0.933
r^2 = 0.991
(F = 7809.90; df = 1,17; P = 0.0001)
Body Temperature (°C)
15 20 25 30 35
10 15 20 25 30 35 40
Water Temperature (°C)


Temperature (°C)
a
Fishes (n)
23 25 27 29 31 33 35 37 39
Ictalurus punctatus (20)
Campostoma anomalum (18)
Carassius auratus (11)
Cyprinella lutrensis (20)
Hybognathus placitus (26)
Hybopsis amblops (1)
Ictiobus bubalus (1)
Notemigonus crysoleucas (28)
Notropis atherinoides (8)
Phenacobius mirabilis (2)
Pimephales notatus (29)
Menidia beryllina (20)
Gambusia affinis (20)
Etheostoma spectabile (19)
Morone saxatilis (3)
Lepomis cyanellus (15)
Lepomis gulosus (4)
Lepomis macrochirus (84)
Lepomis megalotis (59)
Lepomis microlophus (3)
Micropterus dolomieu (1)
Micropterus punctulatus (5)
Micropterus salmoides (17)
Tilapia nilotica (25)
23 25 27 29 31 33 35 37 39
Temperature (°C)


Amphibians & Reptiles (n)
Temperature (°C)
b
26
30
34
38
42
46
50
Notophthalmus viridescens (10)
Plethodon ouachitae (9)
Plethodon richmondi (4)
Desmognathus ochrophaeus (10)
Rana berlandieri (20)
Rana catesbeiana (19)
Rana utricularia (20)
Bufo americanus (24)
Hyla chrysoscelis (20)
Cophosaurus texanus (35)
Temperature (°C)

CHAPTER 2

The Critical Thermal Maximum: History and Critique

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The critical thermal maximum: history and critique

Abstract: I reviewed 725 papers published since Cowles and Bogert (1944) on thermal tolerance to create a data base of studies that used critical thermal maxima (CTMax) or lethal temperature (LT) methods. I found data from 388 of these papers to provide an historical and taxonomic review of various methodologies used in the measurement of thermal tolerance to high temperature.

I conducted this literature review of previous studies to: (i) describe the history of the study of thermal tolerance and show the chronological trends for the use of LT and CTMax methods, (ii) illustrate the diversity of taxa used in thermal tolerance studies, (iii) summarize the diversity of protocols (i.e., end points, heating rates, acclimations, etc.) used for the determination of thermal tolerance, (iv) provide physiological reasons why OS is more meaningful biologically than LRR, and (v) discuss the difficulties in using data from studies with widely divergent methods and the importance of comparable thermal tolerance data to comparative physiology. The adaptation of OS as a standard end point would allow for valid comparisons of data from different studies and among taxa, an important consideration for current investigations of comparative physiology that use the comparative phylogenetic method (Felsenstein 1985).

Introduction

The presence and success of organisms in both time and space are dependent upon a complexity of environmental factors (Odum 1959). Of these factors, temperature is the most pervasive because it directly affects the kinetic energy of reactants (van't Hoff Rule) and thus the biochemical and physiological processes of organisms, including those underlying behavior (Hutchison and Dupré 1992). Temperature and its temperospatial nature mediates the effects of almost all environmental and biological factors explaining a portion of the diversity of physiological and behavioral adaptations among organisms.

In ectotherms, the study of thermal physiology is increasingly complex due to the confounding effects of behavioral plasticity in the regulation of body temperature (T_b). However, in studies of thermal tolerance, particular behaviors (e.g., onset of spasms) are governed by physiological limits to temperature only, eliminating the ambiguity associated with other behavioral measures (e.g., activity temperature). Although the critical thermal maximum (CTMax) may occur at different temperatures among species, the same behavioral response occurs across a diversity of taxa. For these reasons, CTMax is an excellent index and standard for evaluating the thermal requirements and physiology of an organism.

The ease with which temperature is both measured and controlled has resulted in an enormous body of literature dealing with the effects of thermal conditions on organisms, with a large portion of this literature on organismal tolerance to temperature

extremes (Precht et al. 1973; Wieser 1973). Because temperature is so easily measured, protocols (as in CTMax studies) have been highly varied and yet still yield results for analyses. This is unlike more complex protocols (e.g., biochemical assays) in which deviation or inattention to details of a protocol may result in indecipherable or no data.

The two major methods for determining thermal tolerance are (1) the static method which measures the time to death at constant test temperatures, and (2) the dynamic method which involves increasing test temperatures until an end point is reached. The static or lethal temperature (LT) method was developed primarily by F.E.J. Fry and colleagues (Brett 1944; Fry et al. 1942; Fry 1957).

The static method uses statistical techniques from pharmacology (Bliss 1937) to determine the equivalent of an LD_{50} from time mortality curves where the "dosage" is the time animals are exposed to a constant test temperature until death. Median lethal high and low temperatures plotted against acclimation temperature for a species form a polygon that delimits the "zone of resistance" (outside the polygon) from the "zone of tolerance" (inside the polygon). The "ultimate" upper and lower incipient lethal temperatures (UILT and LILT) are also determined by this method and mark the boundary between the zone of tolerance and the zone of resistance. The UILT and LILT are derived from temperatures at which 50% of the population survives an indefinitely long exposure (Fry 1947, 1967). This procedure permits quantification of thermal tolerance by measuring the area of the polygon in square degrees.

The dynamic method, represented by the concepts of critical thermal minimum

(CTMin) and critical thermal maximum (CTMax), was introduced by Cowles and Bogert (1944) with CTMax defined as "the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death." This definition was modified to include statistical variation (Lowe and Vance 1955) and standardized methods to include a constant heating rate that allows deep body temperature to follow ambient test temperatures without a significant time lag (Hutchison 1961, 1976). Slower rates of heating may allow for partial thermal acclimation (Cox 1974; Hutchison 1961, 1976). Thus, rates of heating from about 0.5 to 1.5 °C min^{-1} are often used. As the temperature increases, an animal usually displays a sequence of responses including loss of righting response (LRR), the sudden onset of muscular spasms (OS), and finally "heat rigor," "coma" or "death." The latter three end points are not definitive, difficult to determine (Zweifel 1957), and seldom described fully.

Although the use of these standardized methods has been urged (Hutchison 1979; Paladino et al. 1980) and would allow for comparisons of data from different studies, a wide variety of heating rates and end points remain in use. Even combinations of the static and dynamic methods have been attempted (e.g., Pough and Wilson 1970; Punzo and Rosen 1984; Whitfield and Livezey 1973). The problem is further exacerbated by "phylogenetic provincialism" where many authors are unaware of the techniques used and results obtained from diverse taxonomic groups.

Although the CTMax was first described in reptiles (Cowles and Bogert 1944), various modifications of the method have been used in other ectothermic vertebrates

(e.g., Brattstrom 1963; Matthews 1987) and many invertebrates (e.g., Lagerspetz and Bowler 1993; Lahdes 1995; Korhonen and Lagerspetz 1996; Spidle et al. 1995). The concept has been extended to mammals (Erskine and Hutchison 1982a, 1982b; Wright 1976) including humans (Bynum et al. 1978; Hutchison 1980). The convulsions accompanying heat stroke in humans have been equated to the OS of CTMax in ectothermic vertebrates (Attia and Khogali 1983; Attia et al. 1983).

Lutterschmidt and Hutchison (in press) support OS as the better CTMax end point than LRR by showing that (i) OS within different ectothermic vertebrates had greater precision and (ii) OS shows this greater precision across taxa (i.e., for fish, amphibians and reptiles). To illustrate why OS may be more relevant biologically than LRR and the importance of standardized methods for the determination of upper temperature tolerance, I conducted a literature review of previous studies to: (i) describe the history of the study of thermal tolerance and show the chronological trends in the use of LT and CTMax methods, (ii) illustrate the diversity of taxa used in thermal tolerance studies, (iii) summarize the diversity of protocols (i.e., end points, heating rates, acclimations, etc.) used for the determination of thermal tolerance, (iv) provide physiological reasons why OS is more meaningful biologically than LRR, and (v) discuss the difficulties in using data from studies with widely divergent methods and the importance of comparable thermal tolerance data to ecophysiological and comparative phylogenetic investigations (Felsenstein 1985).

Materials and Methods

Literature Review

I surveyed 1045 papers on thermal tolerance published since Cowles and Bogert (1944). I discarded 320 of the papers which dealt with cold tolerance or cellular/molecular investigations (thus inappropriate for addressing organismal responses). I created a data base of studies where critical thermal maxima (CTMax) or lethal temperature (LT) methods were used with the remaining 725 papers. These were reviewed for the procedures and methods used in determining vertebrate and invertebrate thermal tolerances. I found that 388 of these papers provided original data or review material on measures of organismal thermal tolerance. I used these 388 papers to provide an historical and taxonomic review of various methodologies used in the CTMax literature (Table 1). The total for taxa and methods shown in Table 1 is 604 and not 388 due to papers that contained multiple methods and/or taxa.

Results

Literature review

The best represented taxonomic group in the literature survey was Cypriniformes with 92 accounts. Sixty-four of these thermal tolerance accounts (i.e., 70%) were measured with CTMax. Most of these accounts (53 of 64, 83%) were observations of LRR and not OS. A similar trend was found for the other groups of fishes.

LRR was used more often than OS for reptiles. Only frogs and salamanders

had greater numbers of accounts for OS than LRR. However, studies on anurans also represent the greatest number of accounts (i.e., 14 of 40, 35%) were alternative CTMax end points where used [e.g., increase in evaporative water loss (EWL); (Geise and Linsenmair 1988)].

I also include accounts for several invertebrate classes. Only 22% of all invertebrate accounts used CTMax methods with only 5% of these being observations of OS. The popularity of the LT method for invertebrates may be due to difficulty in observing OS in many invertebrate species. However, investigators have described OS in arachnids (Krakauer 1972), insects (Moulton et al. 1993; O'Neill and O'Neill 1988), and gastropods (Ohsawa and Tsukuda 1955, 1956a, 1956b).

Discussion

History of CTMax

Since Cowles and Bogert (1944) introduced the concept of CTMax, there has been an increasing trend for its use over LT methods (Fig. 1a, 1b). This increase indicates how Cowles and Bogert's classic paper impacted the field of thermal ecology. This trend for CTMax methods may also reflect the ease and the requirement of fewer animals and less time than LT methods. Although Cowles and Bogert's (1944) theoretical concepts and explanations of thermal ecology and tolerance were unparalleled for that time, they did not give detailed explanations and descriptions for an appropriate end point for CTMax. Their measurements of CTMax were taken from tethered reptiles under field

conditions. They allowed animals to struggle toward shade until "effective coordinated movements had ceased." The "coordinated movements" were not described and "loss of righting response" was not mentioned. However, Brattstrom (1968) claimed that "coordinated movements" referred to elimination of righting response. By removing the animal to shade to prevent additional heat absorption, measures of T_b provided indices of CTMax (Cowles and Bogert 1944). Thus, an unstated heating rate, and an unclear definition for the end point in this widely cited work may have led to the diversity of techniques subsequently used in CTMax procedures.

Zweifel (1957) was apparently the first to use and define OS as an end point (defined as "when movements became spasmatic"). Until Lowe and Vance (1955), no defined heating rate and, until Hutchison (1961), no attempts to define clearly the physiological basis for Cowles and Bogert's (1944) observations of "loss of effective coordinated movement" were made. These publications attempted to standardize the experimental protocol for measurement of organismal thermal tolerance with the CTMax method. However, these and later attempts (e.g., Hutchison 1980; Paladino et al. 1980) failed to convince many investigators of the importance and physiological basis of OS in these protocols. The decreasing trend for protocols using the OS end point (Fig. 1c) and an appropriate heating rate may be due to the 11 and 17 year acceptance of diverse methods prior to Lowe and Vance's (1955) and Hutchison's (1961) publications, respectively.

Acclimation and heating rate

The most important influence of all environmental parameters on thermal tolerance is

the thermal history of an organism, especially just before testing. The effects of acclimation on CTMax have been well documented under laboratory conditions (e.g. Hutchison and Rowland 1974). Holland et al. (1974) demonstrated further differences in CTMax for fish acclimated under natural conditions (i.e., acclimatized) to warmer temperatures in a nuclear cooling reservoir. The rates of acclimation to temperature are quite rapid in ectotherms, usually with full acclimation within hours to a few days. The rate is also significantly faster to high than to low temperatures (Hutchison 1976). Less than 24 h is required for full acclimation at temperatures above 20°C but the resistance to very low temperature may be much greater: four days for acclimation from 20 to 4°C in newts (Hutchison 1961) and as much as 20 days for complete acclimation from 26 to 14°C in fishes (Brett 1944, 1946). Exposure to cyclic temperatures with "natural" 24 h periodicities usually results in an acclimation rate faster than exposure to constant temperature equal to the highest value of the cycle (Edney 1964; Heath 1963; Hutchison and Ferrance 1970; Lowe and Heath 1969).

Despite a large body of information on rates of acclimation, some investigators use heating rates that allow acclimation to occur during observations of thermal tolerance. For example, some investigators used a heating rate between 1.0 and 5.0°C h^{-1} (e.g., Baker and Heidinger 1996; Graham 1971; Hecht 1994; Kita et al. 1996; Smale and Rabeni 1995). Others (Guest 1985; Sadler 1979) used rates as slow as 1.0°C day^{-1}. Read and Cumming (1967) with an end point of "death", used a heating rate of 1.0°C per 3.5 days. Such slow rates clearly would allow acclimation to occur during the tests. These examples show that the effects of acclimation on measures of

thermal tolerance have often been ignored and may limit the validity of results to future investigators. The LT method incorporates acclimation as part of the procedure; exposure of seven or more days to constant test temperatures in fishes is common (Fry 1967).

Temperature gain or loss of an organism during heating is dependent on thermal inertia. In addition to physiological influences such as vasomotor changes in circulation (Lillywhite 1987), heating rate is greatly dependent on body mass (M_b) and surface area to volume ratio. Rapid change in environmental temperature (T_e) can cause large lag times between T_e and body temperature (T_b) and may induce possible heat shock effects (Barker et al. 1981; Hutchison and Murphy 1985). Using a heating rate of 1.0°C min^{-1}, I found a highly significant relationship (Lutterschmidt and Hutchison *In press*) between T_b and chamber-water temperature (T_e) for different sized fishes (*Lepomis macrochirus*) and frogs (*Rana catesbeiana*). These results showed that M_b, with no significant lag time between T_e and T_b, could be as great as 165 g with a heating rate of 1.0°C min^{-1} ($R^2 = 0.991$; T_e explained 99.1% of the variation in T_b). I believe that M_b could be considerable greater than 165 g and the R^2 would still be high with the use of an appropriate heating rate (i.e. 1.0°C min^{-1}).

Most researchers recognize these concerns and avoid fast heating rates. However, I found examples of 3.5°C min^{-1} (Lubin and Henschel 1990), 3.8°C min^{-1} (Heatwole et al. 1965) and 10.0°C min^{-1} (Martin and Gentry 1974). The latter rate is probably too fast to avoid heat shock effects and allow T_b to follow T_e without a significant lag time regardless of the organism's small M_b. Some investigators failed to

provide information on heating rate (e.g., Brown 1996; Hirth 1963; Larson 1961; Nietfeldt *et al.* 1980; Punzo and Rosen 1984). Some workers have mixed static and dynamic methods by using a heating rate with the LT method (e.g., Whitfield and Livezey 1973) or constant temperature (static method) with CTMax end points (Sanders and Jacob 1981).

Photoperiod: diel and seasonal cycles

Despite abundant evidence that photoperiod influences thermal tolerance as determined by either the LT (e.g., Hoar 1956; Roberts 1961) or CTMax methods (e.g., Hutchison 1961), many investigators failed to control for day-length. In general, long photophases result in increased heat tolerances, and short photoperiods result in increased cold resistance (Hoar and Robertson 1959). Thus conditions for acclimatization should include control for day length as well as temperature (Hutchison 1976).

Significant diel and seasonal cycles in thermal tolerance occur in most organisms (e.g., Dunlap 1969; Hutchison 1976; Kowalski et al. 1978; Layne et al. 1987). Both types of cycles result from the photoperiod exposures of animals under either laboratory or field conditions (Hutchison 1976). Thus, time of day and season should be considered and reported with all measures of thermal tolerance. Time of day is especially important in the CTMax method where test times are relatively short. In the LT method exposure times to test temperatures are long (up to several days) and will usually mask diel cycles.

CTMax end points

Much of the confusion in selection of an appropriate end point for CTMax derives from the sequelae of symptoms and behaviors observed during heating of an animal to stressful levels (Hutchison 1961; Brattstrom 1968; Paladino et al. 1980). As temperature increases in a test chamber an experimental animal increases activity in an attempt to escape. Bouts of activity are often interspaced with inactive periods. These attempts to escape often continue until a period of inactivity in which LRR occurs, sometimes followed by another period of inactivity, and then the OS. The spasms in ectothermic tetrapods often begin posteriorly with jerky, convulsive movements, spastic opening and closing of the jaw, tremors of the digits (sometimes with an interlocking of digits on hind limbs of salamanders and front limbs of frogs). The spasms may continue, sometimes intermittently, for up to 40 to 50 seconds. The animal is then motionless and a rigidity of the body (rigor) and finally death will follow. Animals usually survive if removed at OS and placed in a cold environment. In fishes the sequelae are similar, but may vary slightly. Becker and Genoway (1979) stated that OS "is much less distinct in fish and cannot be considered a reliable characteristic." I found that a rapid quivering of the opercula, although more difficult to observe than muscular spasms in amphibians and reptiles, serves as a clear and reliable marker for OS. These opercular spasms in fish are usually accompanied by body shuddering, distended gills, and mouth gaping (Lutterschmidt and Hutchison *In press*; Paladino et al. 1980; Schaefer et al. *In press*). With continued heating, death occurs at various intervals after cessation of breathing as marked by a lack of opercular movement. The

quivering of the opercula, however, always occurred in our tests, even when some other sequelae were absent or difficult to detect.

Transections of the central nervous system at various levels and blockage of the myoneural junction in anurans showed that the origin of the stimulus for muscular spasms at OS was anterior to the cerebellum. Likely candidates for the origin of the stimulus include the hypothalamus and motor chain elements such as those in the tectum or tegmentum (Paulson and Hutchison 1987).

Because endotherms defend body temperature changes through a variety of physiological mechanisms, the sequelae during heating to stressful levels are significantly different from those of ectotherms. There is no clear LRR as such in mammals but spasms (convulsions) occur and serve as an excellent end point for CTMax (Erskine and Hutchison 1982a, 1982b; Hutchison and Hart 1984; Wright 1976; Wright et al. 1977). Bynum et al. (1978) suggested that in endotherms, especially humans, the CTMax is defined as "the particular combinations of exposure time at elevated body temperatures that result in either subclinical (CTMs) or clinical (CTMc) injuries." Hutchison (1980) pointed out that: (1) this labeling of the sequelae seen in humans as subsets of CTMax was unnecessary and did not follow the original definition, and (2) the catastrophic clinical symptomatology of heat stroke in humans fits the classic definition of CTMax and OS. As far as I know there have been no CTMax determinations in birds, although LT methods have been used extensively (e.g., Arjona et al. 1990; Bennett et al. 1981; Dawson and Bennett 1981; Johnson and Cowan 1975; Lahiri 1982a, 1982b; Marder and Bernstein 1983; Webb 1987),

especially for poultry (e.g., Ait-Boulahsen et al. 1995; Cogburn et al. 1976; Collier and Schlesinger 1986; Nilsen 1981a, 1981b; Nilsen 1984a, 1984b).

A variety of end points other than LRR and OS have been used as a measure of CTMax. These end points have included a cessation of movement or absence of a response to prodding or similar disturbance (e.g., Buchanan et al. 1988; Dunlap 1968; Hoppe 1978; Kivivuori and Lahdes 1996; Menke and Claussen 1982; Miller and Packard 1974, 1977; Sanborn and Phillips 1996), loss of nictitating membrane response (Krakauer 1970), posterior locomotor paralysis (Lashbrook and Livezey 1970), rigor (Feder and Pough 1975; Pough and Wilson 1970), coma (Gatz 1973; McMahon and Payne 1980), arching of back (Burke and Pough 1976), absence of muscle tone (Pough et al. 1977), cessation of opercular motion in fishes (Chung 1981), failure to move one body length when probed (Appel 1991; Sponsler and Appel 1991), initial and total "disorientation" (Rodrignez et al. 1996), a sharp increase in evaporative water loss (Geise and Linsenmair 1988), knock-down temperature (Jenkins and Hoffmann 1994), inability to gain righting response within 30 sec (Layne et al. 1987), salivation (Hailey and Coulson 1996) and sudden secretion from the paratoid glands (Schmid 1965).

The problem of data interpretation caused by the use of such a diversity of end points has been exacerbated by inappropriate citations. For example, some investigators used end points other then OS, but cited Hutchison (1961) who used spasms (e.g., Brattstrom 1963, 1965; Gatz 1973; Hlohowskyj and Wissing 1985; Lohr et al. 1996; Watenpaugh and Beitinger 1985). Even end points not used in the determination of thermal tolerance have been incorrectly cited as appropriate for

CTMax. Krakauer (1970) "used the loss of a nictitating membrane response (see Hutchison and Dady 1964)." However, the latter authors used this endpoint to measured submergence survival in anurans, not thermal tolerance. Other investigators simply stated that their methods followed Cowles and Bogert (1944), but did not describe an end point (e.g., Curry-Lindahl 1979; Lemos-Espinal and Ballinger 1995) or simply miscited the methods and end points from earlier works (i.e., Cowles and Bogert 1944 and Lowe and Vance 1955) causing confusion (e.g., Brattstom 1968; Graham 1971). Wedemeyer and McLeay (1981) define the CTMax end point as "permanent loss of equilibrium or death" and cite Hutchison (1961) and Paladino and Spotila (1978) as examples; the latter two papers used OS as the end point. Data for CTMax have been given with no description of the methods used (e.g., Bauwens et al. 1995). Becker and Genoway (1979) gave a definition of the CTMax concept with quotation marks and cited Cox (1974) as the source when the material described was originally from Hutchison (1961). Others have used the LT method, but labeled the results as CTMax (e.g., Grande and Andersen 1991). I emphasize that the conclusions from the studies cited in the preceding two paragraphs are mostly valid, ***based upon the end points used.***

The application of the CTMax concept has mostly been to ectothermic vertebrates, but clearly it is applicable to invertebrates. The OS end point works well with most invertebrates where it is marked by spasms of the legs or muscle tremors in arthropods (e.g., Moulton et al. 1993; Perttunen and Lagerspetz 1956; Seymour and Vinegar 1973) and sudden foot extrusion due to spasmodic contractions of the annular

muscles in molluscs (Hamby 1975; Ohsawa and Tsukuda 1955, 1956a). The latter authors, however, did not label their method as "CTMax." Polcyn (1994) used loss of muscle coordination as a measure of heat tolerance in dragonflies; although he does not use the term CTMax, the end point is likely the equivalent of OS.

Death, coma and rigor as end points have seldom been defined. Just when does "death" occur? How is death distinguished from coma and rigor? The physiological point of death is difficult to determine, unless cessation of heart contractions, central nervous system activity, or other criteria are stated as end points and are measured directly. Physiological "death" does not often occur at the time breathing movements stop, especially in aquatic animals with cutaneous and branchial gas exchange structures. For example, I always observed continued heart beats and blood circulation in animals after both LRR and OS, sometimes right up to the point of rigor (i.e., when the body becomes rigid). Unlike the LT method, animals exposed to OS will survive if removed immediately from the heating chamber and placed in a cold temperature. I do not object to the measure of death as an additional end point as suggested by Becker and Genoway (1979); but a clear definition is required and death should not be equated with CTMax, but reported as an additional measurement.

Measures of generalized tissue damage (serum glutamic oxaloacetic and glutamic-pyruvic transaminases [SGOT and SGPT], total proteins and blood urea nitrogen [BUN]) were not altered significantly by exposure to OS in toads (Paulson and Hutchison 1987). Also unaffected were hematocrit, numbers of erythrocytes in circulation, mean cell volumes and hemoglobin concentration. There were significant

increases of blood glucose, lactic acid, creatine phosphokinase (CPK), blood pH, and P_{O_2} and HCO_3 concentration; P_{CO_2} decreased. Frogs exposed to CTMax developed heart lesions (Carlsten et al. 1983). Additional studies on both short- and long-term effects of exposure to LRR and OS are needed.

For many species the tolerance to low temperature may be more important than heat tolerance in governing geographic distribution. Although I have limited our discussion to CTMax, I emphasize that LRR is an appropriate end point for CTMin. LRR fits the Cowles and Bogert's original definition, since the LRR at cold temperatures prevent an animal from moving any significant distance for escape.

At LRR in many animals locomotion is in a fairly straight direction and often persists beyond the points of LRR. For example, fishes can still swim and some reptiles can still move by lateral undulatory motions after LRR and could therefore escape from conditions that would, if continued, lead to their death. Thus LRR does not meet the criteria of the original definition of Cowles and Bogert (1944). Berkhouse and Fries (1995) agree that at LRR in salamanders (*Eurycea nana*), the animals "conceivably could have escaped local high temperatures by random whole body movements" and at the OS "the salamanders effectively were immobile."

Unlike T_b associated with OS, the temperature at LRR is not high enough to produce heat-hardening (Maness and Hutchison 1980). Heat-hardening is a transitory increase in heat tolerance following exposure to lethal high temperatures. Heat-hardening is likely adaptive; it provides a means for acute adjustment to extreme

fluctuations in ambient temperatures (reviewed by Maness and Hutchison 1980). Thus, OS temperatures provide information not available from LRR data.

Frogs, and presumably other vertebrates, can learn to inhibit the righting reflex. Such a response may serve as an ecologically relevant passive-avoidance response to predators, although habituation as an explanation of LRR is not ruled out (Rice and Taylor 1995). Since the inhibition of the righting response easily can be increased by operant-avoidance procedures (reviewed by Suboski 1992) and since species differ in learning abilities with such procedures (Harvey et al. 1981), the LRR as an end point of thermal tolerance may be suspect. No similar mechanisms have been demonstrated or suggested for OS.

Statistical comparisons of the CTMax endpoints LRR and OS

Lutterschmidt and Hutchison (*In press*) demonstrated that there is significantly more variance associated with the end point LRR than OS for a diversity of taxa. Additionally, tests for the homogeneity of paired variances of LRR and OS showed that these variances differed significantly only when the LRR variance was greater than the OS variance. Although OS variance was sometimes greater than LRR variance for some species, the homogeneity for these within species variances of LRR and OS did not differ statistically. These results suggested that OS is a more valuable and meaningful CTMax end point than LRR because of its greater precision.

Ontogenetic differences in LRR occur in the lizard *Cophosaurus texanus* (Lutterschmidt and Durtsche *Submitted*), but no differences in OS were observed among size classes. This further suggests the appropriateness of OS as the definitive

endpoint in determining species specific thermal tolerance.

Heat shock proteins

When cells or whole organisms are exposed to high but sublethal temperatures, as well as to other stressors, synthesis of a set of special proteins is induced. These heat shock proteins (HSP) occur in organisms from archeobacteria to mammals and are thought to protect normal cellular protein during heat (or other) stress and to facilitate cellular recovery after the stress is removed (Craig 1985; Morimoto et al 1990). The HSP response may be a mechanism for survival of what would otherwise be a lethal temperature (Spotila et al. 1989). At least some HSP are in cells under normal (no-heat shock) temperatures and the amount of HSP in ectotherms may be correlated with the mean temperatures of their habitats as well as whole animal thermoresistance (Ulmasov et al. 1992). In adult salamanders synthesis of HSP increased after exposure to OS, but did not always coincide with increased thermal tolerance (Easton et al. 1987). In the frog *Hepidobatrachus laevis* (Carroll 1996) and *Xenopus laevis* (Heikkila et al. 1985) HSP synthesis was correlated with tolerance to heat shock. *Lepidobatrachus* larvae formed two different sets of HSP that were independent of developmental stage or the severity of heat shock. There may be two states of thermal tolerance, a temporary response that does not require synthesis of HSP and a longer term delayed response that does require new HSP synthesis (Boon-Niermeijer et al. 1986). Since the temperatures necessary to induce HSP vary from species to species, as do the molecular weights of the HSP at different temperatures (Lindquist 1986; Lindquist and Craig 1988), the HSP produced at the temperatures of LRR and OS may

vary both quantitatively and qualitatively. The more severe stress of OS may produce different HSP than that of LRR. More studies of the relationships of HSP production at LRR and OS and increased thermal tolerance of the organism are needed (Near et al. 1990).

Predictive models of thermal tolerance

Several investigators have suggested that UILT and CTMax end points may be related in some predictable manner (Becker and Genoway 1979; Cox 1974; Fry 1967). Kilgour et al. (1985) provided a useful model of the static method for determinations of lethal high temperatures in fishes. Applications of the model to data in the literature predicted "UILTs usually within 0.5°C and almost always within 1.0°C of observed values." The model allows predictions of UILT from measurements of shorter term exposures, thus reducing the time usually needed for an accurate measure of LT. Kilgour and McCauley (1986) also constructed a heuristic model in an attempt to reconcile results from the LT and CTMax methods for upper lethal temperatures. Although they concluded that "data from either type of experiment can be used to predict the observations from an experiment of the other type under certain assumptions," they caution that the two methods are both "technically different" and "measure different things." In both the static and dynamic methods thermal tolerance to high temperatures increases with acclimation temperature only to a point; at temperatures above this point thermal tolerance (CTMax or thermal resistance) does not increase. Either a plateau occurs (i.e., often in LT method) or the slope of the line decreases significantly [mostly in CTMax method (Fry 1967; Hutchison 1976)]. Thus,

the lines delimiting resistance polygons determined from static versus dynamic methods are often not parallel. This nonlinearity makes the construction of predictive models difficult. Due to these differences and the limited verification observed, I believe that the model is not yet sufficient for general use, but the results do emphasize the importance of acclimation and heating rates in both the CTMax and LT method. Hopefully new revisions of the model will provide a better tool for valid predictions.

Bennett and Beitinger (1997) discussed the use of thermal tolerance polygons for CTMax and CTMin data, but with LRR as an end point. They show that these polygons are useful in a similar manner to polygons constructed from LT data.

Natural occurrence of CTMax

Over many years critics have suggested that measurements of lethal temperature or CTMax are unrealistic in that such temperatures are seldom experienced by organisms in the field. Such views are exemplified by Rome et al. (1992): "Although compensatory acclimation to CTMax is real, reproducible, and widespread among amphibians, the changes are small in magnitude, and the ecological significance of this response and the benefit to the animal are unproven." According to Feder (1982): "The evident capabilities for behavioral thermoregulation and the paucity of field body temperatures records that are at or near lethal temperatures suggest that amphibians generally do not experience extreme temperature. Rome et al. (1992) also suggest that thermal tolerance is not directly important, but "is correlated with thermal responses that have a more direct bearing on the ecology of amphibians." They then discuss the likelihood that desert amphibians may be an exception. Bradshaw (1992) expresses the

view that desert reptiles may never be exposed to thermal stress in their environment. These critics apparently failed to consider that it is the extremes of environmental conditions, not the means that exert the most selective pressures. Extreme conditions are relatively rare and thus not frequently observed, but over evolutionary time can certainly exert a major influence (Huey and Kingsolver 1989). Indeed, most field biologists recognize that environmental temperatures in many environments can exceed the tolerance limits of ectotherms (e.g., Gunter 1941). These animals have mostly behavioral conditions that approach their thermal tolerance. However, animals trapped in conditions that do not allow escape will die from heat death often before succumbing to other lethal conditions. Examples include desert amphibians (especially tadpoles) in ephemeral pools, fishes trapped in shallow tidal pools or in isolated pools in stream beds, lizards trapped in rocks, aquatic organisms in or near hot springs, etc. (e.g., Bailey 1955; Heatwole and Harrington 1989; Littlewood 1989; Matthews and Hill 1982; O'Neill and O'Neill 1988; Tramer 1977). The concept of "excessive construction" suggests that phenotypic capacities of animals are shaped by rare events that may be critical to an animal's survival, not by routine activities or events; thus, "most aspects of phenotypes will, at any moment of an individual's life, be capable of fulfilling demands much greater than those routinely encountered" (Gans 1979). The assertion by Rome et al. (1992) that tolerance to high temperature is not directly important but is related to other thermal responses more important to an animal's ecology is a caution against a panglossian fallacy (Gould and Lewontin 1979). However, conventional wisdom and direct observations, suggest that animals do

indeed, but rarely, meet lethal thermal conditions in natural environments.

CTMax and its importance to ecophysiological theory

Physiological tolerances are central to an understanding of species distribution and adaptation. Liebig's "Law of the Minimum" states that species distribution is governed by those factors for which a species has the narrowest tolerance range or least adaptability (Bartholomew 1958). The investigation and quantification of thermal tolerance across geographical clines (e.g., Christian et al. 1988; Howard et al. 1983; Lemos-Espinal and Ballinger 1995; Manis and Claussen 1986; Matthews 1986) has allowed for an evaluation for differences in the ability of a species to cope with and adapt to temperature. However, unlike thermal acclimation, temperature adaptation requires genetic change over evolutionary time. Investigators (e.g., Ward and Seely 1996) have examined how harsh thermal environments select for genotypes that enable plasticity in physiological traits. Such investigations are essential to ecophysiological theory for they explain how physiological plasticity for extremes (e.g., CTMax) increases those performance breadths having direct influence upon activity, niche diversification, and geographic distribution that ultimately lead to changes in communities and ecosystems.

Although I do not address thermal tolerance in plants, the amount of recent literature is overwhelming. Many of the studies on plant thermal tolerance address important questions (e.g. Loik and Harte 1996) and should not be overlooked for discussions of ecophysiological theory.

CTMax in comparative physiology and the comparative phylogenetic method

Studies in comparative animal physiology involve "the comparing and contrasting of physiological mechanisms, processes, or responses of different species of animals, or of a single species under differing conditions" (Withers 1992). An underlying assumption for any comparative study, however, is that all individuals and species have received identical experimental treatments. Additionally, current trends in comparative physiology require controls for phylogeny (Felsenstein 1985, 1988), if valid comparisons and contrasts are to be made among taxa. Although these controls for phylogeny have now become common practice in studies of comparative physiology (e.g., Bauwens et al. 1995; Huey and Bennett 1987; Walton 1993; Ward and Seely 1996), the validity of combining data form different studies with various experimental protocols has received little attention. This is an important consideration since investigators of comparative physiology are encouraged to use data from different studies to address more theoretical and evolutionary questions in physiological ecology (e.g., Huey and Bennett 1987; Huey et al. 1991; Snyder and Weathers 1975). Garland et al. (1991) investigated possible evolutionary changes in thermal physiology by using phylogenetic analysis and data from different studies (i.e., Bennett and John-Alder 1986 and Huey and Bennett 1987). This comparative study controlled for both phylogenetic relationships among taxa and valid comparisons of thermal tolerance by using data obtained with identical methodologies.

Correlates between physiology and aspects of behavior or ecology have been frequently explained by adaptations through natural selection (Endler 1986; Feder

1987). The practice of testing correlates of physiological traits among species and then assuming that the results demonstrate adaptation has been criticized for failure to consider the phylogenetic history of the studied organism (Felsenstein 1985, 1988). However, differences in species specific thermal tolerance (due to varying experimental protocols) among species are rarely criticized.

To illustrate the problems with combining data from different studies using varying protocols, I collected species specific thermal tolerance data on *Cyprinella* (*Notropis*) *lutrensis* from seven independent studies (Table 2). The CTMax reported for this species ranged from 31.9 to 39.65°C. I used these seven CTMax values reported for *C. lutrensis* to calculate a grand mean and variance of 35.86 and 6.59°C, respectively. This variance for the combined studies and protocols is more than eight times greater than our reported variance and differs significantly ($F = 8.24$, $df = 6, 19$, $P < 0.001$). Thus, species specific variability for *C. lutrensis* is presumably due to differences in experimental methods (e.g. acclimation temperature [T_a]). If we consider comparisons of independent studies with identical experimental protocols (e.g. Matthews 1986 and 1987; $T_a = 21$°C, or Kowalski et al. 1978, Maness and Hutchison 1980, and Schubauer 1980; $T_a = 15$°C) I find very similar CTMax values among studies. For *C. lutrensis*, Matthews 1986 and 1987 show only a 0.5°C difference in CTMax. More interestingly, Kowalski et al. 1978, Maness and Hutchison 1980, and Schubauer 1980 with identical experimental protocols (Table 2) show only a 0.1°C difference among three different (for taxonomy of that time) *Notropis* species. This suggests that various methods may have greater influence on CTMax than real species

differences.

Our CTMax for *C. lutrensis* is approximately 2°C higher than that reported by the latter three papers. This may be due to seasonal affects of summer thermal regimes prior to measurement of CTMax in early fall; determinations of thermal tolerance in the other studies were made during winter. This observation again emphasizes the importance of similar methods including controls for seasonality.

Conclusion

The tolerances of animals to a single environmental factor such as temperature is typically greater than the tolerance of combinations of environmental factors studied simultaneously. The reproduction of the holocoenotic environment and its variability in the laboratory is not feasible, but the modification of thermal tolerance by many environmental factors (e.g., photoperiod, diel and seasonal cycles, respiratory gases, pH, salinity, diet, reproductive status) should be recognized and controlled as far as possible. Thus, tolerance to high temperature eventually must be studied as part of multiple factorial interactions. The use of standardized methods and terminology will allow for comparisons of data from different investigators and ultimately will facilitate the necessary multiple factor approach (Hutchison 1976).

I have demonstrated that OS is a more meaningful end point than LRR due to its precision and greater physiological relevance. Although OS is more biologically meaningful, I recommend that both LRR and OS be determined and reported (e.g., Berkhouse and Fries 1995) for future comparisons with past studies. I also encourage the use of additional end points, if they are clearly defined, and if the value of OS is

determined. Most importantly, when comparing and contrasting thermal tolerance among species from different studies, I recommend that investigators consider possible differences in experimental methods (i.e., acclimation, heating rate, etc.); otherwise phylogenetic, ecological, and/or evolutionary conclusions from studies with various protocols may be invalid. I advocate OS as the most biologically relevant end point and hope its adoption will facilitate standard definitions and techniques for valid comparisons among future studies.

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Table 1. Taxonomic listing for methods used in determining vertebrate and invertebrate thermal tolerance. (CTMax = critical thermal maximum, OS = onset of spasms, LRR = loss of righting response, LT = lethal temperature)

	CTMax Method\Endpoints						
Taxa	OS	LRR	Other	Unknown	LT	Mixed Methods	TaxaTotals
Mammalia	5	-	-	-	4	-	9
Aves	-	-	-	-	2	1	3
Reptilia							
Serpentes	-	3	-	-	-	1	4
Lacertilia	14	17	4	2	6	2	45
Testudines	5	1	-	-	-	-	6
Crocodylia	-	-	-	-	-	1	1
Amphibia							
Anura	16	9	14	1	11	7	58
Caudata	27	10	3	1	1	3	45
Actinopterygii							
Pleuronectiformes	-	-	-	-	1	-	1
Perciformes	4	33	-	-	11	22	70
Scorpaeniformes	1	1	-	-	1	1	4
Cyprinodontiformes	1	16	3	1	16	3	40
Beloniformes	-	-	2	-	-	-	2
Atheriniformes	-	1	-	-	2	-	3
Salmoniformes	-	12	-	1	19	5	37
Osmeriformes	-	-	-	-	1	-	1
Esociformes	3	-	-	-	1	-	4
Siluriformes	1	4	-	-	2	-	7
Cypriniformes	9	53	1	1	24	4	92
Clupeiformes	-	3	-	-	4	4	11
Anguilliformes	-	-	-	2	1	-	3

Cephalaspidomorphi							
Petromyzontiformes	-	-	-	-	4	-	4
Oligohymenophora	-	-	-	-	3	-	3
Turbellaria	-	-	-	-	1	-	1
Phasmidea	-	-	-	-	1	-	1
Hydrozoa	-	-	-	-	3	-	3
Copepoda	-	-	-	-	3	1	4
Ostracoda	-	-	-	-	1	-	1
Cirripedia	-	-	-	-	2	-	2
Gastropoda	2	1	3	-	12	2	20
Bivalvia	-	-	1	-	12	1	14
Echinoidea	-	-	-	-	1	-	1
Merostomata	-	-	-	-	1	-	1
Branchiopoda	-	-	-	-	3	-	3
Malacostraca	-	5	-	1	39	2	47
Arachnida	2	-	1	-	1	1	5
Insecta	5	1	10	2	25	5	48
Method Totals	95	169	42	12	219	66	604

Table 2. A summary of experimental protocols used in ten different studies to collect CTMax data on the fish *Cyprinella* (*Notropis*) *lutrensis* and three closely related species.

Author(s)	Acclimation Temp.	Photoperiod	Acclimation Length	Heating Rate	End point	CTMax(s^2, n)
Carrier and Beitinger (1988)	20°C	unknown	≥ 1 weeks	0.3°C min^{-1}	LRR	35.03(1.69, 11)
Kowalski et al. (1978)	15°C	LD 12:12	2 to 4 weeks	1.0°C min^{-1}	OS	31.8(0.80, 5)[a]
Lutterschmidt and Hutchison (*In press*)	10°C	LD 12:12	2 weeks	1.0°C min^{-1}	OS	34.04(0.80, 20)
Maness and Hutchison (1980)	15°C	LD 12:12	≥ 1 week	1.0°C min^{-1}	OS	31.9(0.30, 82)
Matthews (1986)	21°C	LD 12:12	14 to 20 days	1.0°C min^{-1}	LRR	35.90(0.17, 15)
Matthews (1987)	21°C	LD 12:12	14 to 20 days	1.0°C min^{-1}	LRR	36.41(1.69, 86)
Mundahl (1990)	unknown	unknown	≤ 3 hours	0.5 to 0.8°C min^{-1}	LRR	36.2(1.00, 6)[b] Rutledge and
Beitinger (1989)	30°C	LD 12:12	≥ 2 weeks	0.3°C min^{-1}	LRR	39.65(0.05, 10)
Smale and Rabeni (1995)[c]	26°C	LD 14:10	63 to 160 days	2.0°C h^{-1}	LRR	38.1(0.18, 11)
Schubauer et al. (1980)	15°C	LD 12:12	3 weeks	1.0°C min^{-1}	OS	31.9(1.00, 8)[d]

[a]CTMax for *Notropis rubellus*

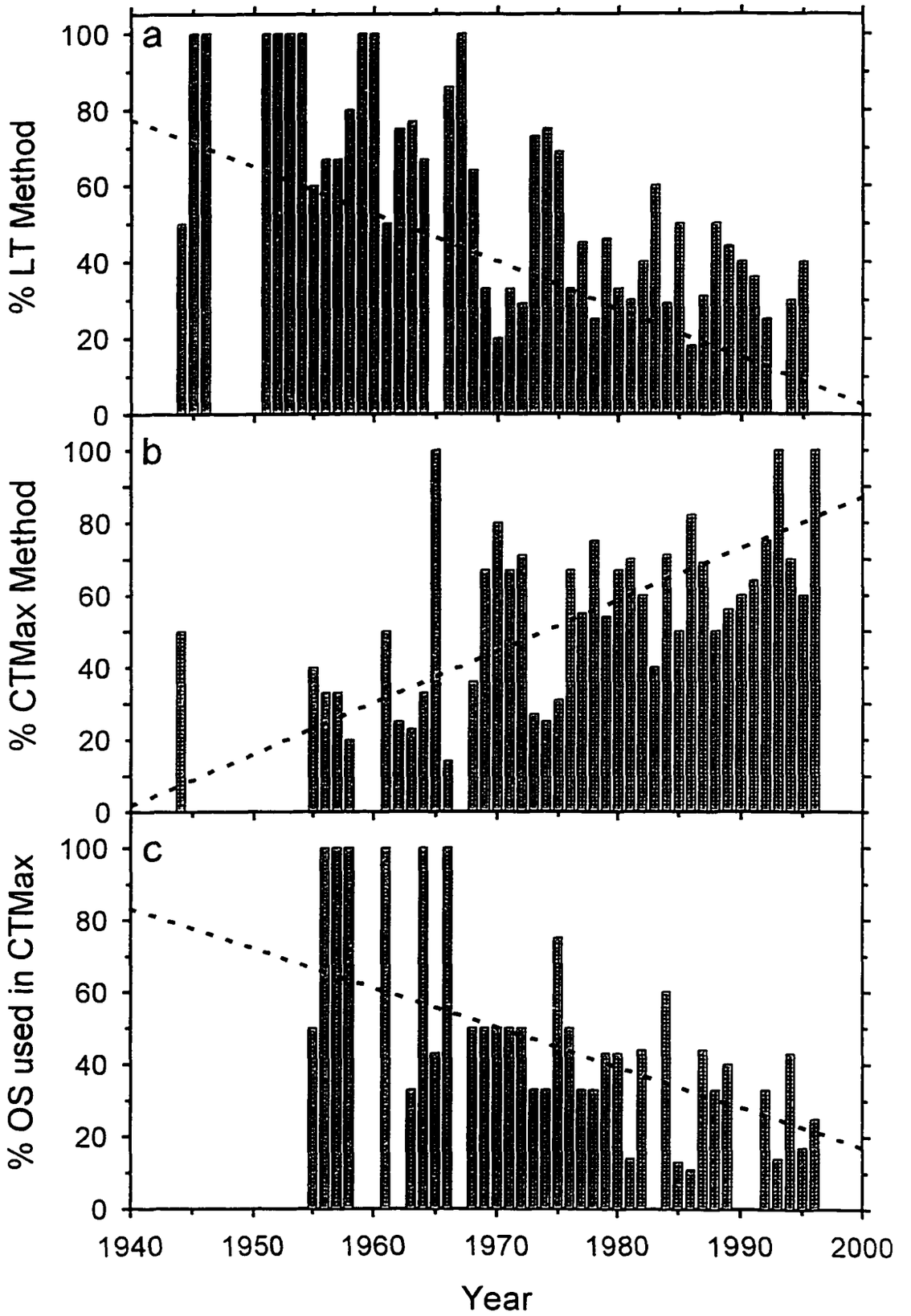
[b]CTMax for *Notropis chrysocephalus*

[c]Authors termed endpoint "critical maximum temperature" and do not use the term critical thermal maximum (CTMax)

[d]CTMax for *Notropis cornutus*

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Fig. 1a - 1c. Chronological trends (shown by dotted lines) for the use of various thermal tolerance methods since Cowles and Bogert's 1944 publication. Figures (a) and (b) illustrate the increased use of critical thermal maximum (CTMax) over lethal temperature (LT) methods. However, (*c*) shows a decrease for the use of the onset of spasms (OS) end point in studies determining thermal tolerance with CTMax methods. Trend lines were generated by SigmaStatat® 1.0 (Jandel Scientific Software Corp., San Rafael, Calif. 1994).


a
b
c
% LT Method
% CTMax Method
% OS used in CTMax
0
20
40
60
80
100
1940
1950
1960
1970
1980
1990
2000
Year

CHAPTER 3

Comparative Physiology of Thermal Tolerance in Fishes: A Macroecological Investigation of Shelford's "Law"

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COMPARATIVE PHYSIOLOGY OF THERMAL TOLERANCE IN FISHES: A MACROECOLOGICAL INVESTIGATION OF SHELFORD'S "LAW"

Abstract.----Distributional patterns of species result in part from their ability to tolerate physiologically the environmental extremes characteristic within their geographical range. Macroecology attempts to reveal relationships between organisms and their environment by investigating statistical patterns of abundance and distribution. In such analyses, species serve as replicates where the relationship between range and body size is used to understand how physical space and ecological resources are partitioned to create unique species' distributions. However, these studies often fail to consider the underlying physiological tolerances that are most likely to be the limiting factors in determining distributions, not the general morphometric measures of body size. I measured high temperature tolerance in 25 species of fishes in an attempt to investigate two subsidiary principles of Shelford's "law" and determine if this physiological parameter is a better predictor of fish distributions than are measures of maximum body size. Although studies of macroecology also emphasize short-term ecological processes and not phylogeny, I accept the alternative perspective in which historical processes may have lasting influences and have therefore accounted for historical effects in our analyses. After phylogenetic controls, I found high temperature tolerance and its species-specific plasticity to predict better the statistical patterns of geographic range size and latitudinal range among the fishes studied. These findings support the idea

that the distribution of species often is governed by the environmental factor for which a species has the narrowest tolerance range or least adaptability.

Correlates between environmental factors and physiological tolerances are central to an understanding of species distribution and adaptation. An organism's geographic distribution largely results from its range of physiological tolerances and its ability to adjust physiological "set-points" to maximize survival, growth, and reproduction. The idea that species have differential survivorship due to differences in their basic requirements and that any one species is only as strong as it weakest link in its ecological chain of requirements was first expressed by Justus Liebig in 1840: "growth of a plant is dependent on the amount of foodstuff which is presented to it in minimum quantity" (*in* Odum 1959). Although Liebig's "law" was coined from his pioneer studies of plant growth and the various limiting nutrients affecting their growth, it has been expanded by many authors (as early as Taylor 1934) to include other factors such as temperature. There are exceptions to this so-called "law", thus referring to this phenomenon as a "law" is inappropriate, in the strictest sense and will therefore be referred to throughout this manuscript as a "rule." Liebig's rule of the minimum is now commonly stated as, "the distribution of species is governed by the environmental factor for which a species has the narrowest tolerance range or least adaptability" (Bartholomew 1958). However, this concept was best expressed by V.E. Shelford's idea that organisms have both an ecological minimum and maximum representing a range of tolerance between an "upper" and "lower" limit (Odum 1959). This concept of the limiting effects of both the maxima as well as the minima was integrated and became know as the "law" of tolerance (Shelford 1913). It was with the use of this law (rule) that the tolerance limits of various plants and animal were

investigated and a newly gained understanding developed for the ecology and distribution of organisms (Odum 1959).

Of the six subsidiary principles of Shelford's rule of tolerance, I was interested in investigating the first two: 1) organisms may have a wide range of tolerances for one factor and a narrow range for another, 2) organisms with wide ranges of tolerance for all factors are likely to be most widely distributed. Most physiological ecologists agree that temperature is probably the most pervasive factor in the holocoenotic environment of an organism limiting distribution (see Hutchison and Dupré 1992). Root (1988) demonstrated that range limits of endotherms may correlate with temperature by showing that the winter distributions of passerine birds in North America correlated with minimum January temperature. The period of reproduction, as stated by the sixth principle of Shelford's rule of tolerance, is usually a critical period when environmental factors are also most likely to be limiting. Pheasants introduced into North America successfully established northern distributions but failed to maintain more southern distributions. Although adult birds could survive, reproduction was limited by the inability of the eggs to tolerate high temperatures (Yeatter 1950). For ectothermic vertebrates, thermal influences are more profound, affecting life histories and population dynamics (e.g., sex determination and ultimately sex ratios in lizards, crocodilians, and turtles [Bull 1987]).

Although, temperature and the physiological tolerance to changing thermal regimes are major limiting factors affecting species distribution (Krebs 1993), studies of macroecology have not investigated Shelford's rule and the possible relationships

between an organism's temperature tolerance and its geographic distribution. The emerging field of macroecology seeks to explain relationships among organisms and their environment by characterizing statistical patterns of abundance, distribution and diversity (Brown 1995). For example, Taylor and Gotelli (1994) and Gaston and Blackburn (1996) investigated the macroecology of the fishes *Cyprinella* and geese (Anseriformes), respectively, using body size to explain geographic range size. However, these studies, as with most macroecological studies, failed to investigate the underlying physiological factors and tolerances that influence animal and plant distribution. Although body size has been used and argued as a physiological and energetically based parameter through allometric equations (Brown 1981, 1995; Brown and Gibson 1983; Brown and Maurer 1987), measures of body size are morphometric parameters only and inferring other interpretations is simply conjecture. Many examples show that animals of similar body size demonstrate diverse energetic demands (e.g. Daniels 1984). Secondly, the use of body size to interpret geographic distribution is, at the least, abstract due to numerous correlated ecological variables including dispersal potential (Glazier 1980), climate and habitat variation (Karr and James 1975), and longitudinal and latitudinal variation in geographical range boundaries (Brown and Maurer 1989; France 1992). Ectotherms and endotherms often exhibit an increase in body size with increasing latitude (Bergmann's rule; Lindsey 1966). High-latitude species also demonstrate greater latitudinal ranges than more tropical, low-latitude species (Rapoport's rule as defined in Stevens 1989; Rapoport 1982; France 1992). Thus, correlates between body size and geographic distribution could result from

underlying latitudinal gradients in each variable (Pagel et al. 1991; Taylor and Gotelli 1994). Many tropical species (e.g., *Bufo marinus* and several species of Iguanidae) of herpetofauna, however, demonstrate latitudinal ranges that do not follow correlates of Rapport's rule (Peters and Donoso-Barros 1970).

Gaston (1990) suggested a bio-physical mechanism by which body size may explain geographic distribution. If small-bodied species are sensitive to climatic disturbance such as varying thermal regimes, they may not be able to maintain large geographic distributions. This idea begins to address the importance of physiological tolerance and acclimatization which may or may not be size dependent, but are definitely species dependent.

I used the physiological parameter of species-specific thermal tolerance *via* the critical thermal maximum (CTMax) to investigate Shelford's law of tolerance in a macroecological study of fish distribution and geographic range size. Because temperature is such a pervasive factor in limiting geographic distribution, measures of physiological plasticity in thermal tolerance are most appropriate for macroecological analyses. Thus, a corollary of Shelford's rule suggests that species with increased thermal tolerance or plasticity in thermal tolerance will have greater geographical ranges. Differences in a species tolerance limits may be directly related to its geographic range and features of habitat utilization (Lillywhite 1987), suggesting that thermal tolerances may be adaptively determined (Stewart 1965; Spellerberg 1972; Lillywhite 1980). Historically, this has been a common theme in comparative physiology, in which correlates between physiological traits and some other organismal

feature were used to explain adaptiveness to particular habitats, environmental regimes, and even geographic distribution. However, adaptation by natural selection cannot explain correlates between physiological traits and behavior/ecology without controls for phylogeny and historical processes (Walton 1993). I used phylogeny as a "null hypothesis" (Kochmer and Handel 1986; Taylor and Gotelli 1994) to account for that portion of interspecific physiological variation that is historical. After adjusting for phylogenetic effects, correlates between thermal tolerance and geographic distribution may reflect underlying ecophysiological processes.

This is the first macroecological investigation to employ a "true" (i.e. not inferred from body size) comparative physiological parameter of species-specific tolerance high temperature to predict statistical patterns of geographic ranges and distribution of fish. I hypothesized that: 1) the physiological performance trait of thermal tolerance and its plasticity will positively correlate with geographic range size and geographic distribution and 2) that high temperature tolerance may predict macroecological patterns better than morphometrics.

METHODS

Fish Collection and Acclimation

I collected 440 fishes representing 25 species and 9 families. All native fish were collected by seining streams and reservoirs in central and south-central Oklahoma. The exotic species, *Tilapia nilotica,* was obtained from aquaculture ponds at the University of Oklahoma. This collection of species from the same geographic region

served as a control for possible differences in thermal tolerance due to geographic variation (Matthews 1986) and differences in climatic regimes. Thus, differences in thermal tolerance were considered to be species-specific differences not confounded by geographic region or variation.

Fishes were collected, acclimatized, and tested between October and February to control for possible seasonal effects upon thermal tolerance (Kowalski et al. 1978; Hutchison 1961; Hoar 1955). Fishes were acclimatized in Living Stream® aquaria for two weeks at 10 ± 1°C and a 12 L:D photoperiod; photophase was from 0600 to 1800 h, centered on noon CST. I used "acclimation" to indicate adjustment to one environmental factor and acclimatization for adjustment to two or more factors, following Folk's "rules" (1974) for uniform terminology.

Measures of Thermal Tolerance

I used the dynamic method, the critical thermal maximum (CTMax), to determine species-specific thermal tolerance of fishes (fig. 1) following the original methods outlined by Hutchison (1961). The onset of opercular spasms (OS) was used as the definitive endpoint for CTMax (Paulson and Hutchison 1987; Lutterschmidt and Hutchison 1997a, 1997b). The OS of fishes is characterized by disorganized and high-frequency muscular movements, rigidity of the pectoral fins, and a high-frequency quivering of the opercula. This behavior seemed to fit best the definition of CTM as "the arithmetic means of the collective thermal points at which locomotor activity becomes disorganized and the animals lose their ability to escape from conditions that

will promptly lead to their death" (Hutchison 1961).

I placed each fish in a heating chamber to increase body temperature (T_b) gradually at 1.0 °C min^{-1} until OS was observed. The T_b of fishes greater than 8.0 cm standard length (SL) or 30 g body mass (M_b) was measured by inserting a thin thermocouple in the urogenital opening (10-15 mm into the lower intestine) of the fish prior to testing. T_b of smaller fish were equal to the chamber's water temperature (Lutterschmidt and Hutchison 1997a). All measures of CTMax were collected between the second and tenth hours of the photophase to reduce possible effects of diel cycles on thermal tolerance (Hutchison 1976).

Statistical and Phylogenetic Analyses

I constructed a phylogeny (fig.2) for 25 fish species from materials presented in Mayden (1992) and Nelson (1994). Because there is no resolved phylogeny for a group that encompasses such taxonomic diversity, this cladogram was constructed from smaller resolved cladograms based upon a variety of characters including behavior, life histories, osteology, and morphology (see material presented in Mayden 1992 for details). In such analyses, the proposed cladogram does not depict a "true" phylogeny but provides a parsimonious hypothesis for the relationship among terminal taxa used in this investigation (Wiley 1981).

Walton (1993) has outlined several analytical methods proposed for incorporating phylogenetic information into comparative studies (e.g., Ridley 1983; Cheverud et al. 1985; Felsenstein 1985; Huey and Bennett 1987; Grafen 1989; Brooks

and McLennan 1991; Harvey and Pagel 1991). Of these analytical methods, I used the independent contrast method (Felsenstein 1985) to reconstruct inferred ancestral trait values which permits the estimation of correlation among quantitative traits. The independent contrast method (i.e., Felsenstein's (1985) method) defines N - 1 independent contrasts (i.e., differences in a quantitative trait) from the phylogenetic relationships among N taxa (Walton, 1993). This method assumes that all traits are normally distributed and that evolutionary change in traits occurs randomly in a manner analogous to Brownian motion (Walton 1993). When independent contrasts are computed, the species' mean phenotypes (continuous traits) are transformed into statistically independent and identically distributed contrasts (Felsenstein 1995). The equation for computing standardized contrasts is:

$$C_i = \frac{(X_a - X_b)}{(V_a + V_b)^{0.5}}$$

where the difference of a trait value (X_a and X_b) between a pair of species (a and b) are divided by the square root of the sum of the branch lengths for the pair of species (V_a and V_b) leading from the most recent common node to the two descendants. These independent contrasts model gradual evolutionary change by assuming that the amount of change is dependent on time, which is relative to branch length (Felsenstein 1985; Martins and Garland 1991). Because actual branch lengths for our phylogeny are unknown, I modeled and considered punctuational change by using relative branch lengths in which all additive branch lengths from the base node to terminal taxa were

equal in length. The punctuated equilibrium model of equal branch lengths (Martins and Garland 1991; Garland et al. 1993) within the phylogeny makes the value of time or length irrelevant, which has the effect of making the amount of change in a trait dependent on the number of bifurcations or species events rather than on time. Despite limited or no data of actual branch lengths of a phylogeny, the use of equal branch lengths is robust (Martins and Garland 1991).

I generated all independent contrasts for measures of thermal tolerance and geographical data with Felsenstein's (1985) independent contrast method within the PDTREE module of the Phenotypic Diversity Analysis Program, 1994 (Garland et al. 1993). I also used an absolute metric of phylogeny with traditional ecophysiological analyses. The absolute metric of phylogeny provided a simple index of "primitiveness" (Gotelli and Pyron 1991), also referred to as an advancement index (Smith 1992) or a phylogenetic distance (Miles and Dunham 1996; Farris 1970), to account for phylogenetic history among taxa. However, this analysis can be used only if taxa within the phylogeny are closely related or can be used on a larger scale phylogeny if few species between terminal taxa are missing from the phylogeny. Because phylogeny of relative relatedness among fishes encompasses such taxonomic diversity and has a large number of species missing between terminal taxa, I used the absolute metric analysis only for the centrarchids. In this case, the number of nodes separating each centrarchid species from the cladogram root (i.e., Centrarchidae) distinguish species characterized by many primitive (pleisiomorphic) character states from species with more derived (apomorphic) character states. Advantages of this absolute metric

included ease of calculation and use in multiregressional analyses to compete with other ecological and physiological predictor variables (Taylor and Gotelli 1994). Norell and Novacek (1992) also found a correspondence between the fossil record and the absolute metric of phylogeny for many vertebrate groups, including teleost fishes. I recognize that this metric does not overcome the nonindependence of species (Felsenstein 1985) and that it ignores extinction of species and phylogenetic change in the absence of speciation (Taylor and Gotelli 1994). However, these other methodologies for analyzing comparative data (e.g. phylogenetic regressions [Felsenstein 1985]) also are not without shortcomings. Many of these methods require large sample size and/or unrealistic assumptions which limit their usefulness (Gotelli and Pyron 1991). Despite these shortcomings, I used the Felsenstein method via the Phenotypic Diversity Analysis Program (Garland et al. 1993; Matins and Garland 1991) to analyze all comparisons and comparison to the absolute metric of phylogeny for Centrarchidae.

Independent contrasts and absolute metrics were analyzed with SigmaStat 1.0® (Jandel Scientific Software Corp. 1994) and Statview 4.5® (Abacus Concepts 1992). I used NTSYS (Applied Biostatistics, Inc. 1994) for matrix generation and analyses of independent contrasts with Mantel tests. All measures of geographic range and thermal tolerance were analyzed for normality and log transformed prior to generation of independent contrasts. An *a priori* natural log transformation for normality was performed on all measures of OS variance (Bartlett and Kendall 1946) prior to generating their independent contrasts. All statistical comparisons were considered significant at $P<0.05$ unless otherwise noted.

Geographic Range Data

Geographic distributions and ranges for all fish species were obtained from comprehensive accounts in Lee et al. (1980). Problems associated with determining geographical ranges are discussed by Taylor and Gotelli (1994); thus I employed Gaston's (1991) definition of geographical ranges as the "extent of occurrence" measured by drawing a smooth border around each species' entire distributional range (Anderson 1984). Each range map was scanned or traced for computer computation of area (km^2) using the following equation:

$$Range = \frac{\left[\left(\frac{Map\ scale \text{ in km}}{Map\ scale \text{ in cm}}\right) \bullet \left(\frac{Distance \text{ between points A and B on map}}{Distance \text{ between points A and B on the Species Range Map}}\right)\right]^2}{\left(Area \text{ of the species range on the Species Range Map in cm}^2\right)^{-1}}$$

I then modified their methods for defining each species' geographical range by using maximum and minimum latitude and latitudinal distribution. I calculated the latitudinal distribution of each species by determining a species' most northern and most southern locality. These localities provided coordinates for degrees of latitude which were then converted to distance (km) from the equator using a model to correct for distance change between each 10° latitude division. Data to construct this model were obtained from List (1984). The distance from the equator of most-southern locality was then subtracted from the most-northern distance from equator to provide a corrected latitudinal range (km) for each species. These two measures of geographic range were regressed against measures of thermal tolerance after controls for

phylogeny. The values used for geographical and latitudinal ranges and maximum body size defined by standard length (SL) for each species are listed in the appendix.

RESULTS

Correlates of body size and geographical range size for fishes used in this investigation were not significant as might be expected from findings of past macroecological studies (fig. 3). These results were consistent both before and after controls for phylogeny. However, the investigation of species-specific thermal tolerance was more meaningful in explaining variation in geographic range size among fishes (fig. 4). Nearly 25% of the variation in geographic range size was explained by species-specific thermal tolerance ($F_{1, 23} = 7.523$; $P = 0.011$). Phenotypic or physiological plasticity in high temperature tolerance (defined by "genetic variation" based on the variability of CTMax among individuals of a species) did not correlate with geographic range size as originally expected. However, correlates for the variability in CTMax were better predictors of geographic range size than body size (table 1).

Because macroecological correlates may be masked by a phylogeny that encompasses such taxonomic diversity, I investigated the same questions using the well defined phylogenies of both the cyprinids (shaded area *a* of fig. 2) and centrarchids (shaded area *b* of fig. 2). For the centrarchids, I found results similar to those for the analyses of all fishes studied. Prior to phylogenetic control, the measure of thermal tolerance (OS) was correlated with only one macroecological variable (i.e., distance

from the equator to its south-central range). After controlling for the effects of phylogeny, I found no correlates between macroecological variables and measures of body size, thermal tolerance, or plasticity in thermal tolerance.

Macroecological variables of cyprinids showed the most significant correlations with measures of body size and physiological performance of CTMax (table 1). However, after controls for phylogeny, measures of species-specific thermal tolerance better explained the variation in geographic range size and latitudinal distribution than did maximum body size (table 2). Mantel tests, used for increased statistical power due to limited sample size of the cyprinid and centrarchid clades, showed the same results for the analyses between macroecological variables and measures of body size and thermal tolerance.

The absolute metric of phylogeny or phylogenetic distance was used only for analyses with the cyprinid and centrarchid clades due to the taxonomic diversity and large number of missing species between terminal taxa of the larger clade of fishes. Primitiveness of species was predicted to correlate negatively with measures of geographic range size (Willis 1922). Thus, larger ranges reflect the long history of a species (i.e., a long-established or primitive species) and recently derived species are confined to small areas near their origin (Brown 1995). Similar logic was used to investigate possible differences in body size and thermal tolerance in which more primitive species with larger ranges may also demonstrate a greater variation in morphology and physiology. Thus I investigated correlates between primitiveness and measures of body size and thermal tolerance. I found no correlations between

phylogenetic distance and measures of geographic range for the centrarchids. An investigation of possible correlates between primitiveness and measures of body size and thermal tolerance also showed no significance. However, analyses of the cyprinid clade showed that primitiveness does negatively correlate with both geographic range size and body size. Primitiveness was not correlated with either thermal tolerance or plasticity in thermal tolerance (table 3).

DISCUSSION

The study of macroecology attempts to reveal relationships between organisms and their environment by investigating statistical patterns of distribution. In such analyses, species serve as replicates to understand how physical space and ecological resources are partitioned by those species to create their unique distributions. Past studies have investigated extensively the associations between geographical range and body size. However, such associations must be viewed cautiously due to the confounding influences of other variables, including phylogeny. Although studies of macroecology emphasize short-term ecological processes and not phylogeny (see Taylor and Gotelli 1994 for discussion), I accept the alternative perspective in which historical processes have lasting influences (Brooks and McLennan 1991) and have therefore accounted for historical effects upon geographic range size, body size, and measures of thermal tolerance in our analyses.

I investigated, *a priori*, geographical range size and latitudinal range both prior to and after controls for phylogeny. For nonphylogenetic and phylogenetic analyses, I

found identical results for the correlates between geographical range size and the measures of body size and CTMax. Results were also consistent for nonphylogenetic and phylogenetic analyses of latitudinal range and the measures of body size and CTMax. These findings of similar results with nonphylogenetic and phylogenetic analyses suggest that correlates between macroecological variables and the measures of body size and thermal tolerance are robust and show the same results regardless of phylogenetic control. This may not be unusual, especially for physiological characters. Walton (1993) showed that the scaling relationships of active and resting oxygen consumption for hylid frogs where statistically similar with nonphylogenetic and phylogenetic analyses. However, I realize that macroecological patterns may be sensitive to phylogeny and speciation, especially for clades that occupy areas with a common climatic history (Taylor and Gotelli 1994) and should therefore be analyzed for possible influences of phylogeny.

Several other macroecological parameters (table 1) may help describe the distribution of a species and were also used prior to phylogenetic controls. However, I found geographic range size and latitudinal range to be the most meaningful measures of distribution and these were used in the phylogenetic analyses. Macroecologists have used geographical range size to investigate possible correlates with body size. I also predicted, *a priori*, that measures of latitudinal range should correlate with species-specific thermal tolerance and a species' ability to tolerate the extremes of its most northern and most southern distribution as might be described by subsidiary principles of Shelford's rule.

For all fishes used in this investigation, I found the measure of high temperature tolerance (i.e., CTMax as determined by OS) to be the best predictor of geographic distribution. This significant correlation was only observed for geographical range and CTMax; no correlation was found for latitudinal range. This sample of species (although not ideal for phylogenetic analyses) was used because all species could be obtained from populations in central and south central Oklahoma. This controlled for possible populational and geographical effects upon high temperature tolerance (e.g., Matthews 1986). Thus I am confident that differences in thermal tolerance are genetically based differences among species and not populational differences or acclimatizational differences from varying environmental regimes in different geographical regions.

Consideration was given to the possibility that macroecological correlates might be masked by my phylogeny of fishes due to this phylogeny encompassing such taxonomic diversity and serving mainly to show relative relationships among terminal taxa. Therefore, I investigated the same questions using the better resolved phylogenies of both the cyprinids (shaded area *a* of fig. 2) and centrarchids (shaded area *b* of fig. 2). Although the same contrast methods were used, the relative relationships of terminal taxa in these groups were separated by very few missing species for the clade. These analyses also indicated that measures of high temperature tolerance were better predictors of geographic distribution than measures of maximum body size. For the centrarchids, I found no significant correlations between macroecological variables and body size or CTMax (table 2). However, correlation

coefficients were greatest for the variance or plasticity in OS (i.e., OS^2) in the analyses of geographical range size. Correlation coefficients for OS and OS^2 were also five times greater than for body size. The lack of statistical significance among macroecological correlates for the centrarchids may be due to very similar high temperature tolerances among the centrarchids used in this investigation. I observed little variation in CTMax among the *Lepomis* and *Micropterus* species (fig. 1). Matthews (in press; 1998) discusses the physicochemical tolerances (which includes high temperature tolerance) of this group. Species within the sunfish clade (*Lepomis*) of the Centrarchidae appear to have, as a group, both relatively high and similar tolerances of temperature and oxygen stress. These findings coincide with the known existence of many *Lepomis* species in relatively harsh environments (Matthews 1987).

Other aspects of centrarchid biology may explain my results. Although there is significant variation in maximum body size among the centrarchids used in this study (i.e., 240 mm SL for *Lepomis machrochirus* to 970 mm for *Macropterus salmoides*), *Lepomis* (which makes up over 60% of the species in the Centrarchid clade, shaded area *b* of fig. 2) are very similar in body size ranging from 250 to 410 mm SL. Secondly, geographical range sizes among the centrarchids may not differ enough to correlate significantly with the little variation in high temperature tolerance and body size. *Lepomis* have very similar range sizes and also demonstrate considerable range overlap (Lee et al. 1980). This significant overlap in range may be most important in explaining the similarity in CTMax among *Lepomis* species (i.e., if one assumes that CTMax results from the acclimatization of a species to its environment and geographic

range). With very similar observations of CTMax, maximum body size, and geographical range size among *Lepomis*, it is not surprising that almost no macroecological correlates were observed. However, CTMax did correlate with the southern locality of range for *Lepomis*; possibly a more meaningful macroecological variable for testing hypotheses of high temperature tolerance. I suggest that *Lepomis* may be inappropriate for future macroecological investigation due to the homogeneity of their ecology.

Several correlates were found for cyprinids. Geographical range size correlated with body size, thermal tolerance, and the plasticity in thermal tolerance. However, thermal tolerance once again explained more of the variation than did body size and correlated strongly with both geographical range size and latitudinal range ($r = 0.806$, $P \leq 0.01$ and $r = 0.716$, $P \leq 0.05$, respectively). Body size seems to be important in correlates of geographical range size, but no correlation was found for latitudinal range and body size. However, thermal tolerance did significantly correlate with latitudinal range (table 2). The number of correlates found between variables of geographical distribution and measures of body size and thermal tolerance for nonphylogenetic (table 1) and phylogenetic (table 2) analyses may be due to the diversity of cyprinid geographical range sizes. The cyprinids also demonstrate a much greater variation in among species-specific high temperature tolerance. Where the centrarchid among-species differences in CTMax was approximately 1°C, the cyprinids demonstrated a range of CTMax from 31.7 to 35.8°C.

Cyprinids are the largest family of fishes with about 2100 species. These fishes

have large diversities of habitats, feeding ecology, and physicochemical tolerances. These diversities are demonstrated by cyprinids occupying a variety of lentic and lotic habitats. Some species are insectivorous while others have behavioral and morphometic modifications for herbivory. Diversities in physicochemical tolerances within cyprinids suggest a recent phylogenetic history of certain species, especially when compared with the centrarchids.

The idea that the primitiveness of a species may correlate with observations of geographic distribution may have been first introduced in 1922 by Willis (Brown 1995). However, this idea has received recent attention in macroecological theory (e.g., Taylor and Gotelli 1994). Willis (1922) found that species with small range sizes outnumbered species with larger ranges. These observations suggested that differences in range size among species may reflect the history of the species. Thus, recently derived species would have small ranges near their origin; long-established species would have larger ranges. Brown (1995) stated that "...study was perhaps the first empirical analysis of the quantitative, statistical distribution of an ecological or biogeographic variable among many species. It was followed by other studies that compiled and interpreted data on the frequency distributions of abundances and body sizes" (Fisher et al. 1943; Preston 1948, 1962a,b; MacArthur 1957; William 1964 and Hutchinson and MacArthur 1959; Van Valen 1973, respectively). Although the analysis and interpretation of those patterns observed by Willis (1922) may be considered quaint today, he is regarded as a pioneer in macroecology (Brown 1995) and such analyses are seen in current literature. Taylor and Gotelli (1994) showed that

primitiveness of the genus *Cyprinella* significantly correlated with geographic range, as I found for the cyprinids. However, unlike the interpretations of Willis (1922), both Taylor and Gotelli (1994) and I found that more primitive species (i.e., species with the smaller number of nodes separating them from the cladogram root) demonstrated larger geographic range. These observations of more primitive species having larger range size also agrees with many groups of fishes (Nelson 1994) and recently derived arthropods (e.g., hymenopterans; Borror et al. 1989).

Macroecologists have overlooked the importance of physiological parameters to macroecological patterns of species distribution. I show that species-specific tolerance to high temperature is a better predictor of macroecological patterns than is the classically used morphometrics of body size. These findings support the subsidiary principles of Shelford's rule that organisms with wide ranges of tolerance are likely to be most widely distributed. Thus, the presence and success of an organism or a group of organisms depends upon a complex of conditions, and any condition which approaches or exceeds the limits of tolerance is that limiting condition or factor. For populations of fishes found in both intermittent streams and more permanent waters of the midwest, both the amount of rainfall and temperature may be important limiting factors to their distribution. Dispersal power for fresh water fishes is limited, and the diversity of species within these habitat represents a historical ability of tolerance which has allowed for their persistence. Matthews (1997) addressed the unanswered question regarding the success of species in harsh environments. Are habitats invaded because of tolerances associated with phylogenetic history, or do species evolve tolerances for

harsh conditions through selection after immigration to those environments? Genetically plastic species within harsh environments may adjust gradually their tolerances or physicochemical selectivity (Matthews 1997). Such adjustment may facilitate dispersal to different habitats and thus ultimately increasing range size temporally. Evidence for adaptation in fishes for thermal tolerance was provided by Zimmerman and Richmond (1981) who found that significant genetic modification took less than 40 years in a fluctuating thermal environment.

A "fixed-tolerance" model may explain extant distributions of some species and is supported by the abrupt and well defined range borders of many fishes (e.g., Winkler 1979 *in* Matthews 1997). Alternatively, populations of species (within limits of zoogeographic chance and biotic pressures) may colonize a diversity of habitats and adapt to local physicochemical conditions (Huey and Slatkin 1976).

These hypotheses of historical versus recent ecological processes may be tested by mapping physiological tolerances or traits onto complete phylogenies (Block and Finnerty 1994). However, difficulties arise when: 1) there are limited phylogenies for the group being studied, 2) the data for physiological traits are unavailable or incomparable (see Lutterschmidt and Hutchison 1997a), and 3) species and data on their physiological traits are difficult to collect.

One may question the importance of temperature and measures of thermal tolerance to the biogeography of fishes. However, fishes within isolated pools of stream beds do experience temperature that lead to their death (Bailey 1955; Matthews and Hill 1982). This is most important when these kills are selective. Such

occurrences, although rare, could actively select for individual within a population with greater genetic plasticity in thermal tolerance. Such scenarios may explain differences in thermal tolerance across geographical clines (e.g., Matthews 1986) and the differences in the ability to cope with and adapt to variation in temperature among species. There is little doubt that temperature exerts some selection pressure on the microevolutionary processes of communities and community structure by selecting against individuals with narrow ranges of thermal tolerance (see Lutterschmidt and Hutchison 1997b for discussion). Investigators (e.g., Ward and Seely 1996) have examined how harsh thermal environments select for genotype that allow for plasticity in physiological traits. Such investigations are essential to ecophysiological theory; they explain how physiological plasticity increases performance breadths that have a direct influence upon activity, niche diversification, and geographic distribution. Similar investigation with physiological parameters may likely prove to be invaluable to macroecologists.

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TABLE 1

CORRELATION COEFFICIENTS OF MACROECOLOGICAL VARIABLES WITH MEASURES OF BODY SIZE (SL), THERMAL TOLERANCE (OS), AND PLASTICITY IN THERMAL TOLERANCE DETERMINED BY SAMPLE VARIANCE (OS^2) FOR NONPHYLOGENETIC ANALYSES

Macroecological Variable	SL	OS	OS^2
FISHES			
Geographical Range	0.179	0.463*	0.205
North-most locality of range	0.032	0.032	0.318
North-central locality of range	0.055	0.045	0.318
South-most locality of range	0.089	0.084	0.257
South-central locality of range	0.110	0.145	0.272
Greatest latitudinal range	0.210	0.187	0.266
Central latitudinal range	0.329	0.336	0.145
CENTRARCHIDS			
Geographical Range	0.407	0.448	0.427
North-most locality of range	0.399	0.055	0.510
North-central locality of range	0.663	0.241	0.508
South-most locality of range	0.520	0.640	0.283
South-central locality of range	0.293	0.711*	0.245
Greatest latitudinal range	0.322	0.522	0.489

Central latitudinal range	0.311	0.358	0.425
CYPRINIDS			
Geographical Range	0.713*	0.810**	0.758*
North-most locality of range	0.597	0.627	0.568
North-central locality of range	0.629	0.713*	0.662
South-most locality of range	0.453	0.480	0.084
South-central locality of range	0.138	0.740*	0.134
Greatest latitudinal range	0.643*	0.685*	0.526
Central latitudinal range	0.500	0.786*	0.404

NOTE.----N = 25, 8, and 10 for fishes, centrarchids, and cyprinids, respectively.

Asterisks indicate probability of correlation coefficients.

*$P \leq 0.05$

**$P \leq 0.01$

TABLE 2

CORRELATION COEFFICIENTS OF MACROECOLOGICAL VARIABLES, GEOGRAPHIC RANGE AND LATITUDINAL RANGE WITH MEASURES OF BODY SIZE (SL), THERMAL TOLERANCE (OS), AND PLASTICITY IN THERMAL TOLERANCE DETERMINED BY SAMPLE VARIANCE (OS^2) FOR PHYLOGENETIC ANALYSES

Macroecological Variable	SL	OS	OS^2
FISHES			
Geographical Range	0.126	0.496**	0.182
	(0.038)	(0.274)*	(0.048)
Greatest latitudinal range	0.292	0.324	0.170
	(0.044)	(0.126)	(0.048)
CENTRARCHIDS			
Geographical Range	0.352	0.344	0.529
	(0.301)	(-0.012)	(0.074)
Greatest latitudinal range	0.110	0.537	0.547
	(0.288)	(0.101)	(0.173)
CYPRINIDS			
Geographical Range	0.726*	0.806**	0.748*
	(0.397)*	(0.682)**	(0.570)*

Greatest latitudinal range	0.630	0.716*	0.555
	(0.385)	(0.572)*	(0.377)

NOTE.----Sample sizes of independent contrasts are N-1. Values in parentheses represent the correlation coefficients from Mantel tests. Asterisks indicate probability.

*P ≤ 0.05

**P ≤ 0.01

TABLE 3

CORRELATION COEFFICIENTS OF PRIMITIVENESS AS DETERMINED BY THE ABSOLUTE METRIC OF PHYLOGENY WITH GEOGRAPHICAL RANGE AND MEASURES OF BODY SIZE (SL), THERMAL TOLERANCE (OS), AND PLASTICITY IN THERMAL TOLERANCE DETERMINED BY SAMPLE VARIANCE (OS^2)

Primitiveness	Range	SL	OS	OS^2
CENTRARCHIDS	0.019	0.398	0.121	0.210
CYPRINIDS	0.722*	0.822**	0.528	0.357

NOTE.----N = 8 and 10 for centrarchids and cyprinids, respectively. Asterisks indicate probability of correlation coefficients.

*$P \leq 0.05$

**$P \leq 0.01$

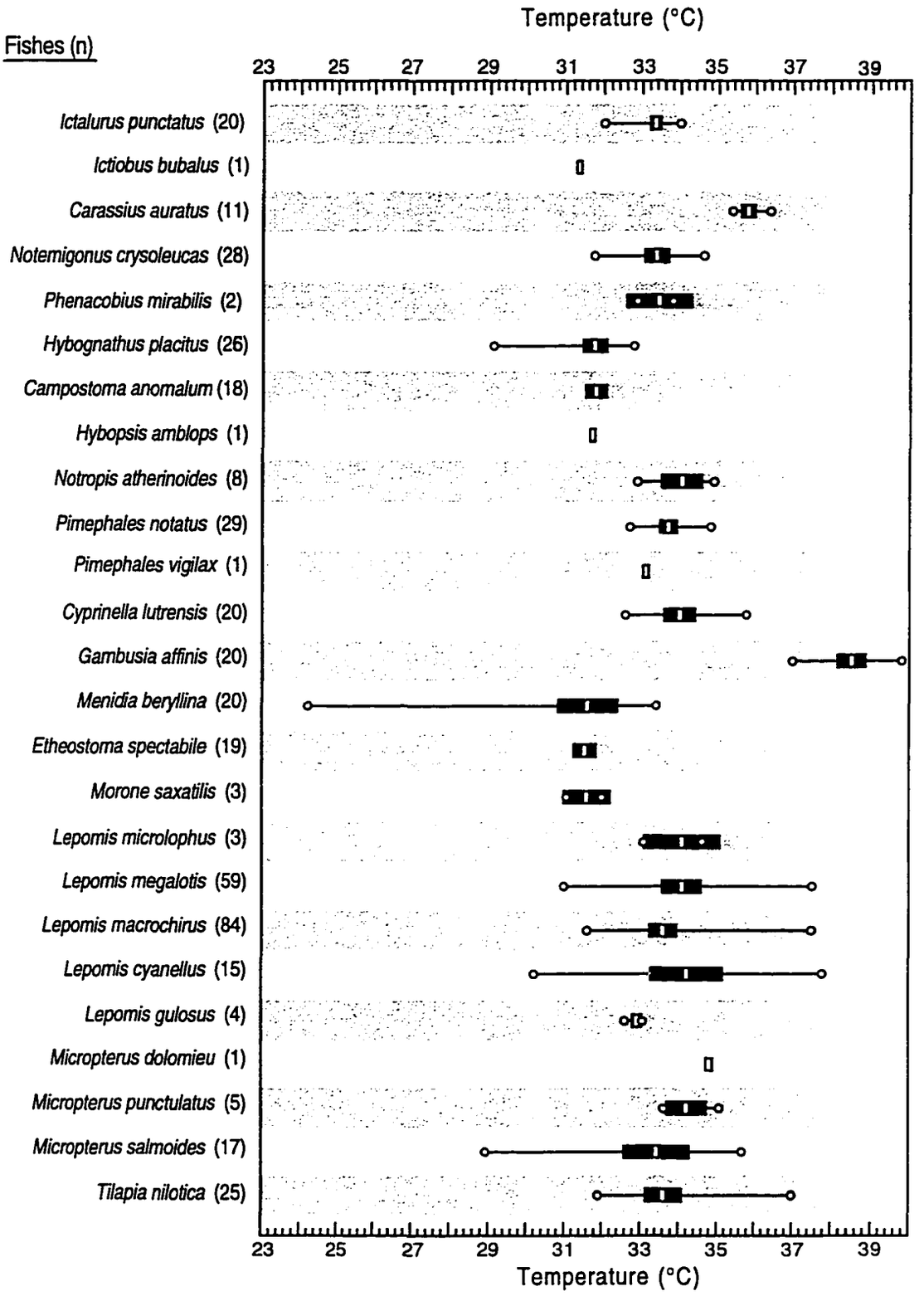
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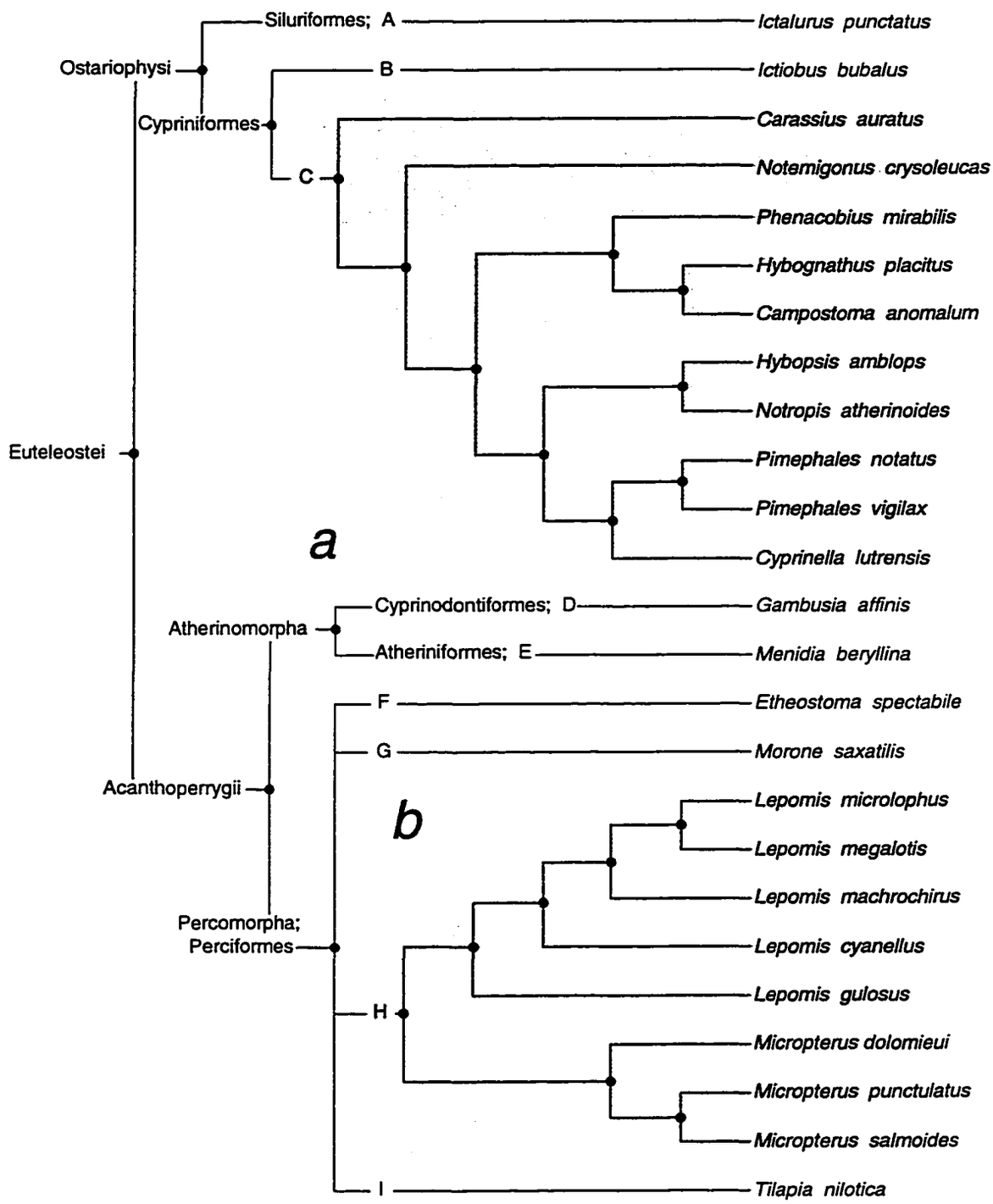
FIG. 1.----Onset of opercular spasms (OS) of fishes modified from Lutterschmidt and Hutchison (1997a). Open circles indicate range, a black rectangle indicates 2(SE), and the white line at the midpoint of the black rectangle indicates the mean CTMax body temperature. Numbers in parentheses next to taxa are sample sizes.

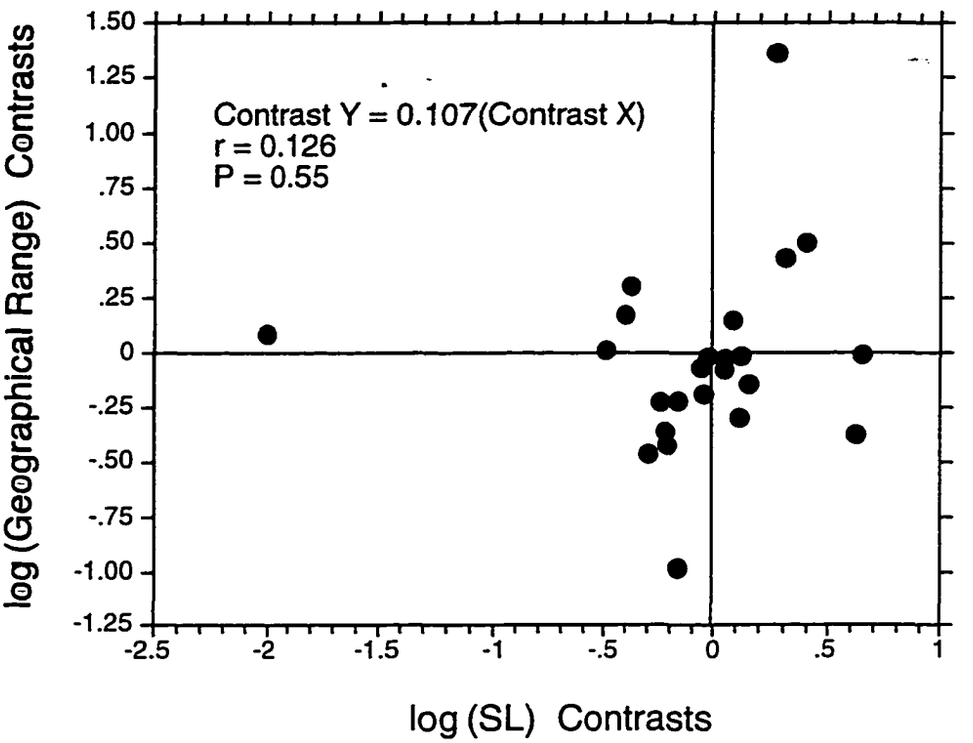
FIG. 2.----Relative phylogenetic relationship among the 25 terminal fish taxa constructed from material presented in Mayden (1992) and Nelson (1994). This proposed cladogram does not depict a true phylogeny but serves rather as a parsimonious or null hypothesis for the relative relationship among the fishes used in this investigation. The shaded areas *a* and *b* represent the cyprinid and centrarchid clades, respectively. Capital letters A through I indicate families to which taxa belong where: A = Ictaluridae, B= Catostomidae, C = Cyprinidae, D = Poecilidae, E = Atherinidae, F= Percidae, G = Moronidae, H = Centrarchidae, and I = Cichlidae.

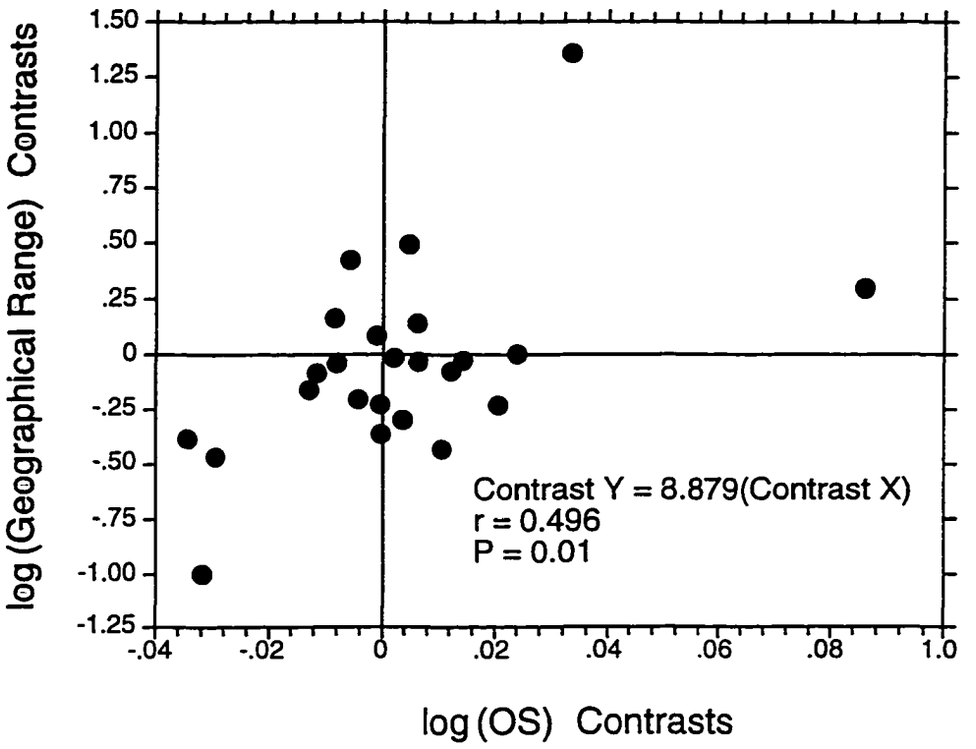
FIG. 3.----Correlation between geographical range size and body size contrasts for fishes with a nonintercept model. Each point represents and independent contrast.

FIG. 4.-----Correlation between geographical range size and species-specific thermal tolerance contrasts for fishes with a nonintercept model for contrast regressions. Each point represents an independent contrast.


Temperature (°C)
Fishes (n)
23 25 27 29 31 33 35 37 39
Ictalurus punctatus (20)
Ictiobus bubalus (1)
Carassius auratus (11)
Notemigonus crysoleucas (28)
Phenacobius mirabilis (2)
Hybognathus placitus (26)
Campostoma anomalum (18)
Hybopsis amblops (1)
Notropis atherinoides (8)
Pimephales notatus (29)
Pimephales vigilax (1)
Cyprinella lutrensis (20)
Gambusia affinis (20)
Menidia beryllina (20)
Etheostoma spectabile (19)
Morone saxatilis (3)
Lepomis microlophus (3)
Lepomis megalotis (59)
Lepomis macrochirus (84)
Lepomis cyanellus (15)
Lepomis gulosus (4)
Micropterus dolomieu (1)
Micropterus punctulatus (5)
Micropterus salmoides (17)
Tilapia nilotica (25)
23 25 27 29 31 33 35 37 39
Temperature (°C)


Siluriformes; A
Ictalurus punctatus
Ostariophysi
B
Ictiobus bubalus
Cypriniformes
Carassius auratus
C
Notemigonus crysoleucas
Phenacobius mirabilis
Hybognathus placitus
Campostoma anomalum
Hybopsis amblops
Notropis atherinoides
Euteleostei
Pimephales notatus
Pimephales vigilax
a
Cyprinella lutrensis
Cyprinodontiformes; D
Gambusia affinis
Atherinomorpha
Atheriniformes; E
Menidia beryllina
F
Etheostoma spectabile
G
Morone saxatilis
Acanthoperrygii
b
Lepomis microlophus
Lepomis megalotis
Percomorpha;
Perciformes
Lepomis machrochirus
Lepomis cyanellus
Lepomis gulosus
H
Micropterus dolomieui
Micropterus punctulatus
Micropterus salmoides
I
Tilapia nilotica


Contrast Y = 0.107(Contrast X)
r = 0.126
P = 0.55
log (Geographical Range) Contrasts
log (SL) Contrasts
1.50
1.25
1.00
.75
.50
.25
0
-.25
-.50
-.75
-1.00
-1.25
-2.5
-2
-1.5
-1
-.5
0
.5
1


log (Geographical Range) Contrasts
1.50
1.25
1.00
.75
.50
.25
0
-.25
-.50
-.75
-1.00
-1.25
-.04
-.02
0
.02
.04
.06
.08
1.0
Contrast Y = 8.879(Contrast X)
r = 0.496
P = 0.01
log (OS) Contrasts

APPENDIX

GEOGRAPHICAL AND LATITUDINAL RANGES AND MEASURES OF MAXIMUM BODY SIZE (STANDARD LENGTH) USED TO GENERATE ALL INDEPENDENT CONSTRASTS

Species	Geographical Range (km^2)	Latitudinal Range (km)	Maximum Body Size (Standard length in mm)
Ictalurus punctatus	4867540	2679.83	1270
Ictiobus bubalus	2650637	2489.78	780
Carassius auratus	58786500	3866.49	300
Notemeigonus crysoleucas	5732954	2944.56	300
Phenacobius mirabilis	1090592	1752.35	120
Hybognathus placitus	1417590	1882.50	130
Campostoma anomalum	2430331	2516.04	220
Hybopsis amblops	604431	912.13	90
Notropis atherinoides	5994585	3593.81	130
Pimephales notatus	2598731	2134.66	110
Pimephales vigilax	1927887	2073.28	89
Cyprinella lutrensis	2476299	2245.02	90
Gambusia affinis	2658790	1622.94	65
Menidia berylina	1363548	1113.82	150
Etheostoma spectabile	1065066	1386.50	20
Morone saxatilis	892589	1923.54	2000
Lepomis microlophus	1400387	1616.66	250

Lepomis megalotis	3301759	2257.96	410
Lepomis macrochirus	2294344	2401.23	240
Lepomis cyanellus	3528864	2567.61	310
Lepomis gulosus	2811967	2085.48	310
Micropterus dolomieui	1696029	1406.10	690
Micropterus punctulatus	1221544	1257.65	610
Micropterus salmoides	3297861	2307.32	970
Tilapia nilotica	4750000	911.39	320

CURRICULUM VITAE
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EDUCATION

Allentown College of St. Francis de Sales, Center Valley, Pennsylvania
B.S., May 1988 (Biology and Secondary Education)

State Teacher's Certification (Biology)
Pennsylvania, May 1988

Southeastern Louisiana University, Hammond, Louisiana
M.S., May 1992 (Biological Sciences)

University of Oklahoma, Norman, Oklahoma
Ph.D. Candidate, May 1995 (Zoology)

RESEARCH INTERESTS

Comparative Physiology
Physiological and evolutionary ecology
Thermal ecology and physiology
Metabolism and energetics
Phenotypic and physiological plasticity
Habitat selection and preferences
Thermal regimes and physical structure of preferred microhabitats of ectotherms
Home range and movement patterns
Telemetry and associated methodologies
Statistics and statistical applications in physiological ecology
Biophysical modeling: operative and standard operative temperatures of ectotherms
Computer modeling and simulation in biology

HONORS, FELLOWSHIPS, AND AWARDS

Deans List, Allentown College, 1987-88.
President, Alpha Sigma Chi (Science Fraternity), Allentown College, 1986-88.
Excellence in Research Award, Allentown College, 1988.
President, Biology Graduate Student Organization, Southeastern Louisiana University, 1990-91.
Student Representative to Graduate Faculty, Southeastern Louisiana University, 1990-91.
Teaching Fellowship, Southeastern Louisiana University, 1991.
Elected Associate Member, Sigma Xi, 1991.

Treasurer, Zoology Association of Graduate Students, University of Oklahoma, 1993.
Graduate Summer Research Assistantship, University of Oklahoma, 1993, 1995
Zoology Department Award for Excellence in Graduate Student Teaching, University of Oklahoma, 1995

GRANTS RECEIVED

Lutterschmidt, W.I. 1989 - 1990. Movement patterns and habitat selection in timber rattlesnakes. Hawk Mountain Sanctuary Research Award. **$1000**

Lutterschmidt, W.I. 1990. Constraints of habitat selection on thermoregulation and preferred body temperature in the timber rattlesnake, *Crotalus horridus*. Sigma Xi, Grants-in-Aid of Research. **$450**

Lutterschmidt, W.I. 1994 and 1997. Field metabolic rates of an endangered rattlesnake in Aruba. President's International Travel Fellowship Grant, Univ. of Oklahoma. **$1000**

Lutterschmidt, W.I. 1995. Herpetofauna of Cucumber Creek, Oklahoma. Nature Conservancy Research and Survey Grant, Oklahoma Chapter. **$2000**

Durtsche, R.D., W.I. Lutterschmidt, M.M. Fuller, K.M. Polivka, J.F. Schaefer. 1996. Osmoregulation and oxygen consumption in aquatic vertebrates. Hach Company (Loveland, Co.), Instrument and Equipment Grant. **$4000**

Lutterschmidt, D.I., W.I. Lutterschmidt, V.H. Hutchison. 1996. Influence of melatonin and chlorpromazine upon the photo- and scotophasic thermal selection of the bullsnake, *Pituophis melanoleucus*. **$500**

Lutterschmidt, W.I. 1997. Thermal tolerance, geographic range, and Liebig's Law: does physiological plasticity matter? Graduate Student Senate Travel Grant **$250**, Graduate College Travel Grant **$350**, and SSAR Travel Grant **$200**.

Total Grant Money Received: **$9750**

PROFESSIONAL EXPERIENCE

TEACHING

William Allen High School, Allentown, PA. Student teaching, Spring 1988. Taught Biology (9th grade honors and gifted classes).

Allentown Central Catholic High School, Allentown, PA. 1988-89. Taught Biology, Chemistry, and Physical Science (Grades 9, 10 and 11).

Southeastern Louisiana University, Department of Biological Sciences, Hammond, LA., Teaching Assistantship, 1989-91. Taught Introductory Zoology.

Oklahoma Museum of Natural History, Norman, OK., 1992-96. Taught summer workshops in Herpetology, Mammalogy, Nature Study, and Stream Ecology.

University of Oklahoma, Department of Zoology, Norman OK. Teaching Assistantship, 1992-Present. Taught Introductory Biology, Ecology, Vertebrate Physiology, and Comparative Vertebrate Anatomy.

PROFESSIONAL AND HONORARY AFFILIATIONS

American Society of Ichthyologists and Herpetologists
Herpetologists' League
Oklahoma Academy of Science

Pennsylvania Academy of Science
Sigma Xi (Associate member)
Society for the Study of Amphibians and Reptiles
Society for Integrative and Comparative Biology:
(Division of Comparative Physiology and Biochemistry)
Southwestern Association of Naturalists

PROFESSIONAL ACTIVITIES

Reviewer for: Herpetological Review
Journal of Herpetology
Journal of the Pennsylvania Academy of Sciences

RELATED COURSES COMPLETED

Undergraduate: Allentown College

Biological Ethics
Biology: Independent Research
Biometry (Statistics)
Botany
Ecology
Education Courses
Environmental Science
Genetics
Molecular Cell Biology and Histology
Natural History of Florida Everglades
Physiology
Student Teaching (Biology)

Graduate: Southeastern Louisiana University

Biostatistics
Coral Reef Ecology
Conservation Biology
Evolutionary Ecology
Herpetology
Plant Ecology
Physiological Ecology
Research Problems
Thesis

Graduate: University of Oklahoma

Molecular Techniques in Field Biology
Evolutionary Ecology
Professional Aspects
Physiological Ecology
Vertebrate Physiology
Vertebrate Natural History
Plant Physiological Ecology

Seminars:
Physiological Ecology
Herpetology
General Biology and Zoology
Ecology (A review of Peters, R.H., 1991. A Critique for Ecology)
Conservation Genetics

PUBLICATIONS

Research Articles

Lutterschmidt, W.I. and H.K. Reinert. 1990. The effect of ingested transmitters upon the temperature preference of the water snake, *Nerodia s. sipedon*. Herpetologica 46(1):39-42.

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Durtsche, R.D., P.J. Gier, M.M. Fuller, W.I. Lutterschmidt, R. Bradley, C.K. Meier, and S.C. Hardy. 1997. Ontogenetic variation in the autecology of the greater earless lizard, *Cophosaurus texanus*. Ecography 20(4):336-346.

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Marvin, G.A. and W.I. Lutterschmidt. Locomotory performance in juvenile and adult box turtles (*Terrapene carolina*): A reanalysis for effects of body size and extrinsic loading using a terrestrial species. Journal of Herpetology (In Press).

Lutterschmidt, D.I., W.I. Lutterschmidt, and V.H. Hutchison. Melatonin and chlorpromazine: thermal selection and metabolic rate in the bullsnake, *Pituophis melanoleucus*. Comparative Biochemistry and Physiology (In Press).

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Lienesch, P.W., W.I. Lutterschmidt, and J.F. Schaefer. Long-term changes in the fish assemblage of a small stream isolated by a reservoir. Copeia (In Review).

Schaefer, J.F., W.I. Lutterschmidt, and L.G. Hill. Physiological performance and stream microhabitat

use by two Centrarchids (*Lepomis megalotis* and *Lepomis macrochirus*). Environmental Biology of Fishes (In Review).

Research Notes: (Techniques, Natural History and Biogeography)

Lutterschmidt, W.I. 1991. An improved laboratory apparatus for examining thermal preferences of reptiles. Herpetological Review 22(3):92-94.

Lutterschmidt, W.I. and J. F. Schaefer. 1996. Mist netting snakes: a technique borrowed from ornithology for sampling semi-aquatic snake populations. Herpetological Review 27(3):131-132.

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Lutterschmidt, W.I., G.A. Marvin, and V.H. Hutchison. 1996. *Rana catesbeiana* (Bullfrog): Record body size. Herpetological Review 27(2):74-75.

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INVITED SEMINARS AND SYMPOSIA

Lutterschmidt, W.I. 1997. Professional aspects of graduate school: the wheres, whens, and whys. Department of Biological Sciences, Southwestern Oklahoma State University, Oklahoma.

Lutterschmidt, W.I. and V.H. Hutchison. 1997. Thermal tolerance, geographic range, and Liebig's Law: does physiological plasticity matter? *In Symposium*. Comparisons and current uses of phylogenetic approaches to ichthyology and herpetology. American Society of Ichthyologists and Herpetologists, University of Washington, Seattle, Washington.

Lutterschmidt, W.I. 1997. Comparative physiology of metabolic plasticity in snakes: a macroecological study. *In Symposium*. Snake ecology for the 21st century. Texas Herpetological Society, University of Texas, Tyler, Texas.

PRESENTATIONS

Lutterschmidt, W.I. and H.K. Reinert. 1989. The effect of ingested transmitters upon the temperature preference of the water snake, *Nerodia s. sipedon*. Pennsylvania Academy of Science, 64th Annual Meeting.

Lutterschmidt, W.I. and H.K. Reinert. 1989. The effect of ingested transmitters upon the temperature preference of the water snake, *Nerodia s. sipedon*. Joint meeting of SSAR, HL, and ASIH. Ann Arbor, MI.

Lutterschmidt, W.I. 1990. The effect of surgically implanted transmitters upon the sprint speed of the checkered garter snake, *Thamnophis marcianus*. Joint meeting of SSAR and HL. New Orleans, LA.

Lutterschmidt, W.I. 1990. The ecology and thermal biology of the timber rattlesnake, *Crotalus horridus*. Lehigh Valley Herpetological Society. Allentown, PA.

Lutterschmidt, W.I. 1991. The use of road cruising for possible determination of habitat selection and activity patterns of snakes in Southern Louisiana. Joint meeting of SSAR and HL. Pennsylvania State University, University Park, PA.

Lutterschmidt, W.I. 1992. The thermoregulatory constraints of the timber rattlesnake, *Crotalus horridus*, within preferred microhabitats. Society for the Study of Reptiles and Amphibians. University of Texas, El Paso, TX.

Lutterschmidt, W.I., G.A. Marvin, and V.H. Hutchison. 1994. Alarm response by a plethodontid salamander (*Desmognathus ochrophaeus*): Conspecific and heterospecific "Schreckstoff". Joint meeting of SSAR and HL. Athens, GA.

Lienesch, P.W., W.I. Lutterschmidt, and J.F. Schaefer. 1996. Long-term changes in the fish assemblage of a small stream isolated by a reservoir. Southwestern Association of Naturalists, 43rd Annual Meeting. McAllen, Texas.

Lutterschmidt, W.I. and V.H. Hutchison. 1996. Measures of thermal tolerance in Oklahoma fishes: A statistical comparison of variability in the endpoints loss of righting response and onset of opercular spasms. Southwestern Association of Naturalists, 43rd Annual Meeting. McAllen, Texas.

Schaefer, J.F., W.I. Lutterschmidt, and L.G. Hill. 1996. Stream microhabitat use by two Centrarchids (*Lepomis megalotis* and *Lepomis macrochirus*). Southwestern Association of Naturalists, 43rd Annual Meeting. McAllen, Texas.

Lutterschmidt, D.I. and W.I. Lutterschmidt. 1996. Efectos de la Melatonia y Clorpromazine enla Seleccion Termica en *Pituophis melanoleucus*. IV Reunion Nacional de Herpetologica. Cuernavaca, Morelos, Mexico.

CURRENT RESEARCH AND MANUSCRIPTS IN PREPARATION

Fontenot, C. and W.I. Lutterschmidt. Observations of temperature preference of *Amphiuma tridactylum* in an aquatic laboratory thermal gradient.

Fontenot, L.W., S.G. Platt, and W.I. Lutterschmidt. A comparison of aquatic snake assemblages from two localities in Southeastern Louisiana: Temporal and spacial changes in community structure.

Lutterschmidt, W.I. and V.H. Hutchison. Nesting behavior and nest temperature variation for a killdeer (*Charadrius vociferus)* in central Oklahoma.

Lutterschmidt, W.I., D.I. Lutterschmidt, C.R. Tracy, and V.H. Hutchison. Time course analysis for the influence of CPZ and melatonin on the thermal selection and temperature preference of *Pituophis melanoleucus*.

Reinert, H.K. and W.I. Lutterschmidt. The thermal preferenda of the timber rattlesnake, *Crotalus horridus*, with a discussion of thermoregulatory constraints within preferred microhabitats.

Lutterschmidt, W.I. Seasonal occurrence of terrestrial activity and movement in the aquatic snake, *Farancia abacura*, in Southeastern Louisiana.

Lutterschmidt, W.I. and C.M. Taylor. Spacial and temporal changes in community structure: A PCA

of seasonal activity and environmental correlates for aquatic and semi-aquatic snake species in a Louisiana wet land.

Lutterschmidt, W.I., R.A. Fiorillo, and J.F. Schaefer. Influences of endoparasite loads (Nematoda) on the physiological performance of two centrarchid fish species, *Lepomus macrochirus* and *Lepomus megalotus*.

Hopla, C. and W.I. Lutterschmidt. Seasonal abundance and over-wintering of the ectoparasites (*Ceratophyllus celsus celsus, Ornithodoros coneanensis, Ixodes baergi, and Oeciacus vicarius*) in cliff swallow nests: An investigation of how site affinity and physiological cold tolerance may influence recolonization.

REFERENCES

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