Quantification of the role of acclimation temperature in temperature tolerance of fishes

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Synopsis

The relative effect of acclimation temperature on temperature tolerance was estimated from a geometrical partitioning of the temperature tolerance polygon of a fish species into three distinct zones relative to four key tolerance temperatures. This approach yields a middle tolerance zone which is independent of acclimation temperature bounded by upper and lower acclimation dependent zones. Acclimation dependent and independent temperature tolerance zones can be quantified by either areal or linear methods. Both methods were applied to quantify the effect of acclimation temperature in 21 species of temperate fishes for which temperature tolerance polygons were available. Temperature tolerance polygon areas of these 21 species ranged from 468 to 1380°C^2 and are linearly related ($r^2 = 0.93$, $p < 0.001$) to ultimate incipient upper lethal temperatures. Although areal and linear partitioning methods yielded similar acclimation independent and dependent tolerances, estimates from the areal method incorporates additional information concerning the shape of the temperature tolerance polygon, in particular lower and upper lethal temperature plateaus. Mean combined acclimation dependent and independent tolerance areas of these 21 species were not different, indicating that acclimation effectively doubles the temperature tolerance polygon. Mean lower acclimation dependent area was nearly three times greater than mean upper acclimation dependent area, suggesting that acclimation plays a larger role in tolerance of low rather than high temperatures. Among these 21 species, temperature tolerance of brook charr and sheepshead minnow were the least and most affected by acclimation temperature, respectively.

Introduction

Among the myriad of biological, physical and chemical factors which influence the temperature tolerances of fishes (see Hutchison 1976), acclimation temperature is considered to be among the most critical. Studies as long ago as Davenport & Castle (1896), Loeb & Wasteneys (1912), Hathaway (1927) and Sumner & Doudoroff (1938) indicated that the lethal thermal limits are strongly effected by the temperatures that fish experience prior to tests. Reporting either a single temperature tolerance value or even a range of temperature tolerances for a species is meaningless without indicating the pretest temperature acclimation state.

In the laboratory, temperature tolerances of fishes are usually measured via either temperature dynamic (i.e., critical thermal methodology, CTM) or static (i.e., incipient lethal temperature, ILT) methodologies. Both of these methods quantify temperature tolerance in a random sample of fish; however, they do not yield similar tolerance values because of differences in experimental techniques and endpoints. These differences were first discussed by Fry et al. (1946), Fry (1967), Hutchison (1976) and numerous

authors since, including Bennett & Beitinger (1997). The static technique was developed by Fry et al. (1942) from a pharmacological dose–response method of Bliss (1937). In this approach (also termed the Fry or plunge method) a temperature lethal to exactly 50% of a random sample of fish is estimated by instantaneous transfer (plunging) of groups of fish from various constant acclimation temperatures into a series of static test temperatures near the expected lower and upper temperature limits of a species. Mortality is recorded over time and estimates of the lower and upper temperatures tolerated by 50% of the sample for various exposure times, e.g., 12, 24, 48 or 96 h, are constructed from a relationship between percentage mortality and test temperatures. Since thermal resistance usually is not linear with respect to time, data are often transformed into probits and plotted against the logarithm of time (Fry et al. 1942, 1946). The static method does not confuse time with temperature or allow partial acclimation of fishes during trials, and because death is the endpoint, measures physiological thermal tolerance (Fry 1947). Results are reported as incipient lower and incipient upper lethal temperatures, i.e., ILLTs and IULTs, for fishes acclimated to specific temperatures and can be summarized graphically as a temperature tolerance polygon (Fry et al. 1942). The area of the polygon expresses the temperature tolerance in units of $°C^2$ and hence, reflects the degree of eurythermicity of a species. As such, the temperature tolerance polygon is equivalent to Hutchinson's (1965) fundamental thermal niche of a species. In the first application of the static method (Fry et al. 1942) ILLTs and IULTs were measured in goldfish, *Carassius auratus*, acclimated to a series of temperatures between 1 to 38°C. Goldfish had ultimate lower and upper lethal temperatures of 0 and 41°C and a thermal tolerance polygon area of 1220°C^2.

In general, as acclimation temperature increases, both upper and lower tolerance limits of a species increase. The former represents a gain in heat tolerance and the latter is a loss of 'cold' tolerance. Four different rates of acclimation can be measured relative to temperature tolerance (Brett 1946): rates of heat gain and heat loss, and rates of gain and loss of 'cold' tolerance. Often these rates are reported as time required to fully adjust temperature tolerance limits, and have been the subject of many studies (e.g., Loeb & Wasteneys 1912, Hathaway 1927, Sumner & Doudoroff 1938, Doudoroff 1942, Brett 1946, Davies 1973, Chung 1981, Bennett et al. 1998). Estimates of the amount of time needed by various fishes to reacclimate, i.e., adaptively change their lethal thermal limits, in these studies range from 1 to 20 days and are dependent upon the species, and the direction and magnitude of temperature change.

Although virtually all studies of temperature tolerance of fishes include information concerning acclimation temperature(s), to our knowledge no one has attempted to quantify the effect of acclimation temperature on the temperature tolerance of a species. In this paper we develop, employ and compare two separate but related methods to quantify the effect of acclimation temperature on the temperature tolerance of a variety of fish species.

Methods

Our approach is based on a geometric partitioning of a species temperature tolerance polygon into three separate zones relative to four key tolerance temperatures: the ultimate ILLT and ultimate IULT, the highest ILLT and lowest IULT. For demonstration of our partitioning approach, a temperature tolerance polygon was generated from the mean tolerance data of the 21 species of fishes incorporated in this paper and given in Figure 1. The upper line represents the IULT boundary which extends from 23.0 to 31.1°C and the lower line is the ILLT boundary which ranges from 0.0 to 10.8°C. The ultimate IULT and ultimate ILLT equal the absolute highest and lowest temperature, respectively, tolerated by a species. They represent the genetic, i.e., fundamental, limits of temperature tolerance of a species and vary interspecifically. The ultimate IULT is usually estimated as the intersection of the IULT boundary with an isothermal line drawn through the temperature tolerance polygon. At this point, IULT equals acclimation temperature. The ultimate ILLT of many freshwater species of fish may be less than 0°C, however, usually it is reported as 0°C owing to difficulties in achieving sub-0°C temperatures in freshwater and interpreting the importance of sub-0°C temperatures in the life of freshwater fishes. The lowest IULT equals the IULT of fish acclimated to 0°C, and is obtained by extrapolation from a regression of IULT on acclimation temperature. The highest ILLT is the ILLT for fish acclimated to the species ultimate IULT. Graphically it appears as the intersection of the ILLT boundary with a perpendicular line 'dropped' from the ultimate IULT.
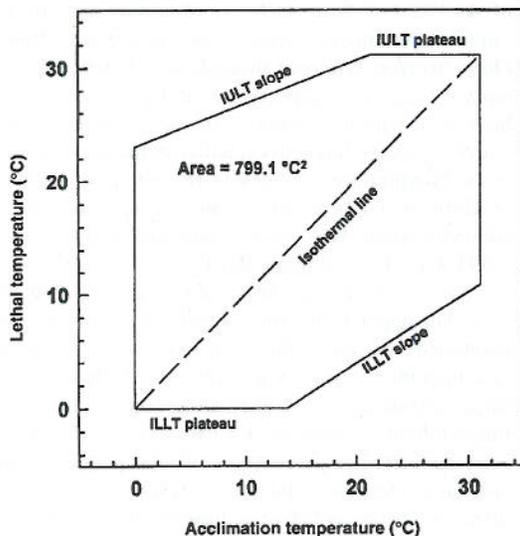
Figure 1. Labeled temperature tolerance polygon derived from the mean data of 21 species of fishes. ILLT and IULT equal incipient lower and upper lethal temperatures, respectively. The lower line represents the ILLT boundary and the upper line represent the IULT boundary. Plateaus occur in both lower and upper boundaries. Slopes relating change in ILTs over the nonplateau portions of the upper and lower lethal temperature boundaries equal 0.386°C and 0.634°C per 1.0°C change in acclimation temperature, respectively.

Partitioning a species temperature tolerance polygon via these four key tolerance temperatures yields an upper acclimation dependent zone which is a function of the arithmetic difference between the ultimate and lowest IULTs; it represents heat gain via acclimation. Similarly a lower acclimation dependent zone is generated which is a function of the ultimate ILLT (usually 0°C) and the highest ILLT; it represents gain of 'cold' tolerance via acclimation. These two zones quantitatively represent the effect of acclimation temperature on upper and lower temperature tolerances, respectively. This leaves a middle zone, mathematically defined as the arithmetic difference between a species lowest IULT and highest ILLT which is independent of acclimation temperature. We termed this the intrinsic temperature tolerance zone. These zones of acclimation dependent and independent temperature tolerance can be quantified by either areal or linear methods. In the former, the area of each zone is measured in units of °C², and in the latter the linear distance in °C is measured. Following quantification either by integration of areas or calculation of
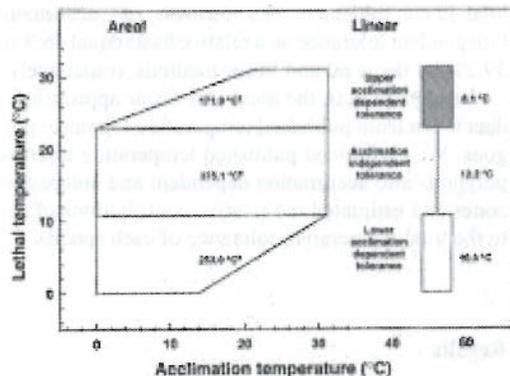

Figure 2. Application of the areal and linear methods to partition temperature tolerance into acclimation independent, lower and upper acclimation dependent tolerances for data presented in Figure 1. The four key partitioning temperatures are ultimate IULT (31.1°C), ultimate ILLT (0.0°C), highest ILLT (10.8°C) and lowest IULT (23.0°C) which separate the polygon (a) and linear tolerance (b) into three zones relative to the effect of acclimation temperature.

linear distances, the relative contributions of the lower and upper acclimation dependent zones (separately and combined) and the acclimation independent zone to the total temperature tolerance of species can be estimated and compared.

Again for demonstration purposes both areal and linear methods are illustrated in Figure 2 which was derived from the mean tolerance data of 21 species of fishes examined in this study and originally plotted in Figure 1. This 'homogenized' polygon has an area of 799.1°C² and linear tolerance extending from 0.0 to 31.1°C. The four key partitioning temperatures (ultimate IULT = 31.1°C, lowest IULT = 23.0°C, highest ILLT = 10.8°C and ultimate ILLT = 0.0°C) partition the polygon (panel A) and linear tolerance (panel B) into three distinct zones of tolerance relative to acclimation temperature. Quantifying these tolerances zones via the areal approach yields an acclimation independent area of 375.1°C², upper acclimation dependent area of 171.0°C² and lower acclimation dependent area of 253.0°C². The combined acclimation dependent tolerance is 424.0°C² which equals 53.1% of the total polygon area. The linear method yields an acclimation independent tolerance of 12.2°C (23.0 − 10.8°C), upper acclimation dependent tolerance of 8.1°C (31.1 − 23.0°C) and lower acclimation dependent tolerance of 10.8°C (10.8 − 0.0°C). Here the combined acclimation dependent tolerance equals 18.9°C or 60.8% of

total linear tolerance. The intrinsic, i.e., acclimation independent tolerance on a relative basis equal 46.9 and 39.2% by the areal and linear methods, respectively.

We applied both the areal and linear approaches to data taken from published temperature tolerance polygons. We partitioned published temperature tolerance polygons into acclimation dependent and independent zones and estimated the relative contributions of each to the total temperature tolerance of each species.

Results

Temperature tolerance polygons determined by the ILT method were found for 21 species including 8 cyprinids, 6 salmonids and single species from 7 other fish families published between 1942 (goldfish, Fry et al.) and 1997 (sheepshead minnow, *Cyprinodon variegatus*, Bennett & Beitinger 1997). Nearly all of these are temperate species from North America (Table 1). A majority of the polygons were taken from Hart (1947, 1952) and Brett (1952). Polygon areas for these 21 species range from 468 to 1380°C^2 and linear temperature tolerances extend from a minimum of 23.7°C (0.0 to 23.7°C) to a maximum

of 43.1°C (-1.5 to 41.6°C). Minimum and maximum temperature tolerances occurred in chum salmon, *Oncorhynchus keta*, and sheepshead minnow, *Cyprinodon variegatus*, respectively. Polygon shapes are hexagonal with the exception of 5 of the 6 salmonids which have curvilinear lower lethal temperature boundaries. Polygon area is linearly related (p < 0.0001) to ultimate IULT (Figure 3) and is expressed in the following regression model: polygon area (°C^2) = $-593.9 + 44.85$ (ultimate IULT, °C), $r^2 = 0.93$.

Prior to discussing the results, we compared the lower and upper acclimation dependent (separately and combined) and the acclimation independent temperature tolerance estimates generated by both areal and linear approaches. Tolerance areas (see Table 2) and linear tolerance distances for each species (Figure 4) were converted to percentages of total polygon areas and linear temperature tolerances, respectively, to make direct comparisons between these methods. Estimates from both methods were highly significantly correlated (r ranged from 0.967 to 0.992, all p < 0.0001, see Table 3, row 4); however, mean percentages estimated by the two methods were highly significantly different (Table 3, row 3). The linear approach generated larger lower, upper and combined acclimation tolerance zones. Not only were all linear acclimation

Table 1. Temperature tolerance polygons (°C^2) and linear temperature tolerances (°C) for 21 species of fishes. Entries are ordered by increasing polygon area and sources are given.

Species	Polygon, °C^2	Linear tolerance, °C	Reference
Oncorhynchus keta	468	0.0–23.7	Brett (1952)
Oncorhynchus nerka	505	0.0–24.9	Brett (1952)
Oncorhynchus kisutch	528	0.0–24.8	Brett (1952)
Oncorhynchus tshawytscha	529	0.0–24.9	Brett (1952)
Coregonus artedii	541	0.0–26.0	Edsall & Colby (1970)
Salvelinus fontinalis	625	0.0–25.3	Fry et al. (1946)
Menida menida	699	1.4–32.6	Brett (1970)
Perca flavescens	742	0.0–29.7	Hart (1947)
Notropis atherinoides	747	0.0–30.7	Hart (1947)
Catostomus commersonni	770	0.0–29.3	Hart (1947)
Rhinichthys atratulus	790	0.0–29.3	Hart (1952)
Girella nigricans	800	0.0–32.5	Doudoroff (1942)
Notropis cornutus	803	0.0–31.0	Hart (1947)
Semotilus atromaculatus	808	0.0–30.3	Hart (1952)
Pimephales notatus	884	0.0–33.3	Hart (1952)
Pimephales promelas	903	0.0–33.2	Hart (1947)
Notemigonus crysoleucas	940	0.0–34.7	Hart (1952)
Lepomis macrochirus	954	0.0–36.0	Reynolds & Casterlin (1979)
Ictalurus nebulosus	1162	0.0–37.4	Brett (1944)
Carassius auratus	1220	0.0–41.0	Fry et al. (1942)
Cyprinodon variegatus	1380	-1.5–41.6	Bennett & Beitinger (1997)
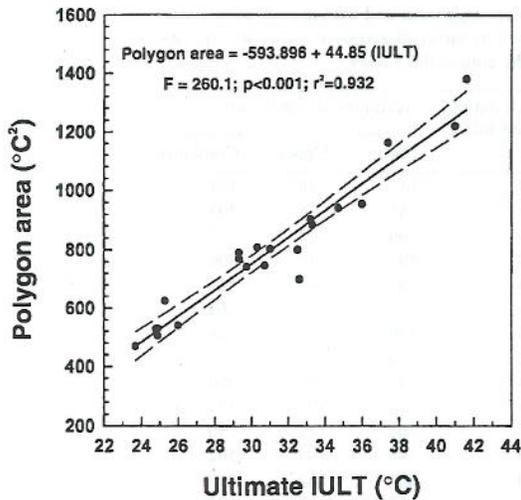
Figure 3. Relationship between temperature tolerance polygon area (°C²) and ultimate IULT (°C) for 21 species of fishes. Actual data points, line of best fit, 95% confidence limits on the line of best fit, linear regression model, probability of the model from ANOVA and coefficient of determination (r^2) are given.

dependent means greater, on an individual species basis, lower and upper acclimation dependent estimates were greater via the linear method in 20/21 and 21/21 species, respectively.

The major difference between the two partitioning approaches is that the areal method takes into account the shapes of the lower and upper lethal temperature boundaries. Both the upper and lower temperature tolerance boundaries of all species contain plateaus (see Figure 1, Table 4). Once a plateau is reached there is no increase in IULT or no decrease in ILLT with further changes in acclimation temperature. Mean lower and upper plateaus of the 21 species equaled 14.6 and 9.6°C, respectively, and the mean lower plateau is highly significantly longer than the upper plateau (paired t test, p = 0.008). Extreme lower and upper temperature tolerance plateaus are seen in brook charr, *Salvelinus fontinalis* (Figure 5) and white sucker, *Catostomus commersoni*, respectively. Brook charr have a lower lethal plateau of 23°C which indicates that charr acclimated to temperatures between 0 and 23°C have ILLTs of 0°C. In the white sucker, the ultimate IULT is achieved at an acclimation temperature of 15.2°C and remains constant to 29.3°C, yielding an upper lethal temperature plateau of 14.1°C. The longer the plateau,

the more closely the shape of a temperature tolerance polygon shifts from a hexagon to a square.

We expressed the plateaus given in Table 4 relative to the total temperature tolerance range of each species to determine if the magnitude of the temperature tolerance plateaus differentially influenced estimates of acclimation dependent temperature tolerance by the two methods. For example, the lower and upper plateaus of the bluegill, *Lepomis macrochirus*, equaled 10.2 and 8.8°C, respectively. Dividing each by the total temperature tolerance range of bluegill, 36.0°C, yields a relative lower temperature plateau of 0.283, a relative upper temperature plateau of 0.244 and a relative combined plateau $((10.2 + 8.8)/(36.0 \times 2))$ of 0.264. Possible relationships between relative plateaus and differences between acclimation dependent tolerance estimates made by the two methods were investigated by correlation analyses. All correlations were highly significant: relative lower plateau was inversely correlated to differences between lower acclimation dependent estimates (r = −0.71, p = 0.0003), relative upper plateau was inversely correlated to differences between upper acclimation estimates (r = −0.72, p = 0.0002), and the relative combined lower and upper plateaus was inversely correlated to differences in the total acclimation dependent estimates (r = −0.85, p < 0.0001). These inverse correlations indicate that as either lower or upper (or both) lethal temperature plateaus become shorter, acclimation dependent tolerance estimates of the two partitioning methods diverge. Two species with exactly the same ultimate IULT and lowest IULT, but differences in the length of their upper temperature tolerance plateaus will yield the same linear upper acclimation dependent zone but will vary in their upper acclimation dependent areas. Average relative combined plateau equaled 0.384 and ranged between 0.204 and 0.609. This nearly threefold difference between minimum and maximum values suggests fairly large interspecific variation. For these reasons, we concluded that the areal method yields a better estimate of the effect of acclimation temperature on temperature tolerance, and in the remainder of this paper we have limited our discussion to results of the areal partitioning method.

Table 2 lists temperature tolerance polygons partitioned into acclimation independent and acclimation dependent (lower, upper and combined) areas for the 21 species. Combined acclimation dependent temperature tolerance ranged from 69 (*Salvelinus fontinalis*)

Table 2. Lower, upper and combined acclimation dependent and acclimation independent tolerances (rounded to the nearest °C²) partitioned by the areal approach for 21 fish species. Summarizing descriptive statistics are given in the bottom three rows.

Species	Polygon	Acclimation independent	Acclimation dependent		
			Lower	Upper	Combined
Oncorhynchus keta	468	317	113	38	151
Oncorhynchus nerka	505	302	137	66	203
Oncorhynchus kisutch	528	335	149	44	193
Oncorhynchus tshawytscha	529	223	187	119	306
Coregonus artedii	541	195	202	144	346
Salvelinus fontinalis	625	556	32	37	69
Menida menida	699	265	199	229	428
Perca flavescens	742	332	174	236	410
Notropis atherinoides	747	293	249	205	454
Catostomus commersonni	770	448	215	107	322
Rhinichthys atratulus	790	500	168	122	290
Girella nigricans	800	298	309	193	502
Notropis cornutus	803	393	285	125	410
Semotilus atromaculatus	808	431	208	169	377
Pimephales notatus	884	366	311	207	518
Pimephales promelas	903	352	362	189	551
Notemigonus crysoleucas	940	428	350	162	512
Lepomis macrochirus	954	352	296	295	591
Ictalurus nebulosus	1162	701	228	233	461
Carassius auratus	1220	399	497	324	821
Cyprinodon variegatus	1380	392	642	346	988
Mean	799	375	253	171	424
± one standard deviation	241	114	134	89	212
Arithmetic range	468–1380	195–701	32–642	37–346	69–988

to 988°C² (*Cyprinodon variegatus*). Expressed relative to total temperature tolerance polygons, these combined acclimation dependent temperature tolerances equal 11.0 and 71.6%, respectively. Figure 5 presents partitioned temperature tolerance polygons for these two species.

Acclimation independent and combined acclimation dependent areas were neither correlated ($r = 0.003$, $p = 0.987$) nor were their means (375.1 and 424.0°C²) significantly different (paired t test, $p = 0.36$). Overall the combined acclimation dependent areas comprised $51.1 \pm 14.5\%$ (mean ± one standard deviation) of the total temperature tolerance area of these species (Table 3); therefore, on the average, acclimation temperature effectively doubles the area of the temperature tolerance polygons of these 21 species.

Lower ($r = 0.84$), upper ($r = 0.82$) and combined ($r = 0.88$) acclimation dependent tolerance areas are highly significantly correlated to polygon area (all $p < 0.0001$). Not surprisingly lower and upper acclimation dependent areas are highly significantly

correlated ($r = 0.79$); however, the mean lower acclimation dependent area, 253.0°C² is highly significantly larger than the mean upper acclimation dependent area, 89.2°C² (paired t test, $p < 0.0001$) indicating that changes in acclimation temperature have a greater effect on tolerance of low rather than high temperatures.

Additional evidence to support our claim that acclimation temperature has a greater influence on tolerance of low rather than high temperatures derives from considerations of slopes relating acclimation temperature to ILTs. Slopes, i.e., $\Delta ILT/\Delta$ acclimation temperature, were calculated over the nonplateau portions of both ILLT and IULT boundaries of each species (Table 4). All slopes relating IULT to acclimation temperature are linear and varied from 0.131 (brook charr) to 0.634 (yellow perch, *Perca flavescens*). These extreme slopes indicate that a change of 1.0°C in IULT results from changes of 4.5 and 1.6°C, respectively, in acclimation temperature. The mean (± standard deviation) IULT slope is 0.386 ± 0.145 for all 21 species suggesting
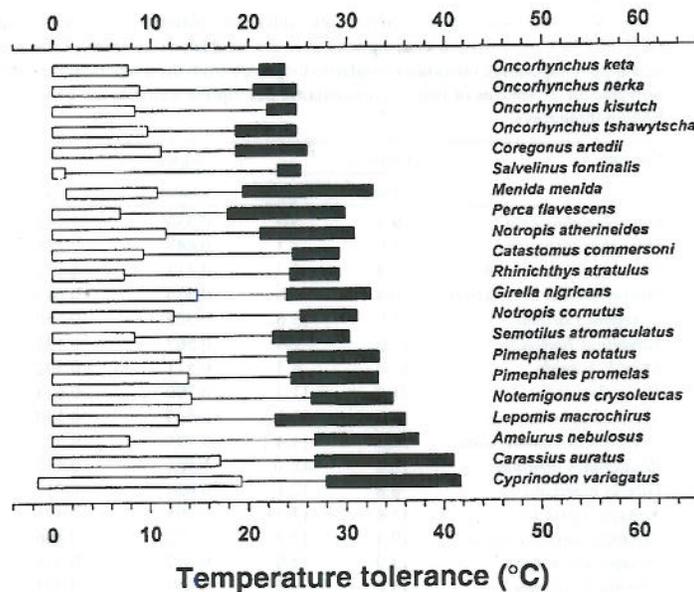
Figure 4. Temperature tolerance of 21 species of fishes partitioned by the linear approach into lower (open rectangles) and upper acclimation dependent (closed rectangles) and acclimation independent (—) zones.

Table 3. Comparison of areal and linear approaches to partition relative (%) temperature tolerances into acclimation dependent and independent contributions to total temperature tolerance in 21 species of fishes. Descriptive statistics are mean ± one standard deviation. Statistics listed in the bottom two rows (t values and correlation coefficients, r) are derived from paired t tests and Pearson product-moment correlations, respectively. Probabilities from all paired t tests and correlation analyses were highly statistically significant (p < 0.0001).

	Acclimation independent	Acclimation dependent		
		Lower	Upper	Combined
Areal method	48.8 ± 14.6	30.6 ± 9.2	20.5 ± 7.9	51.1 ± 14.6
Linear method	40.5 ± 15.5	34.3 ± 10.0	25.2 ± 9.3	59.5 ± 15.5
t values	18.7	6.5	9.3	18.7
r	0.992	0.967	0.979	0.992

that on the average, a 2.6°C change in acclimation temperature changes IULT by 1°C.

Slopes relating ILLT to acclimation temperature (Table 4) ranged from 0.412 (coho salmon, *Oncorhynchus kisutch*) to 0.953 (fathead minnow, *Pimephales promelas*). For these extremes, changes of 2.4 and 1.05°C in acclimation temperature result in a 1.0°C change in lower lethal temperatures. Mean (± standard deviation) ILLT slope equaled 0.661 ±

0.140, indicating that on the average a 1.6°C change in acclimation temperature produced a 1.0°C change in the ILLT. The response of the ILLT boundary to acclimation temperature is not always linear. In 5 of the 6 salmonids ILLTs and acclimation temperature are curvilinear. The mean slope relating ILLT to acclimation temperature is highly significantly greater than the mean slope for the IULT boundary (paired t test, t = 6.3, p ≪ 0.0001). This latter finding supports our

Table 4. Lower and upper temperature tolerance plateaus (°C) and slopes (Δ°C ILT/Δ°C acclimation temperature) over the nonplateau portions of the lower and upper temperature tolerance boundaries estimated from the temperature tolerance polygons of 21 species of fishes. Summarizing descriptive statistics are given in the bottom three rows.

Species	Plateaus		Slopes	
	Lower	Upper	Lower	Upper
Oncorhynchus keta	9.7	5.4	0.550[1]	0.142
Oncorhynchus nerka	5.0	7.3	0.447[1]	0.223
Oncorhynchus kisutch	4.4	5.7	0.412[1]	0.152
Oncorhynchus tshawytscha	6.4	13.6	0.524[1]	0.549
Coregonus artedii	5.0	13.6	0.528[1]	0.590
Salvelinus fontinalis	23.0	7.8	0.567	0.131
Menida menida	12.4	4.3	0.511	0.502
Perca flavescens	21.0	11.2	0.790	0.634
Notropis atherinoides	12.7	13.1	0.645	0.540
Catostomus commersonni	16.5	14.4	0.725	0.323
Rhinichthys atratulus	16.4	18.1	0.566	0.455
Girella nigricans	8.8	12.1	0.624	0.417
Notropis cornutus	15.4	13.2	0.795	0.321
Semotilus atromaculatus	19.4	13.2	0.772	0.456
Pimephales notatus	13.9	11.0	0.677	0.418
Pimephales promelas	18.6	9.5	0.953	0.371
Notemigonus crysoleucas	13.8	3.4	0.682	0.265
Lepomis macrochirus	10.2	8.8	0.501	0.490
Ictalurus nebulosus	22.7	5.1	0.531	0.325
Carassius auratus	17.3	4.9	0.722	0.393
Cyprinodon variegatus	17.2	10.1	0.788	0.410
Mean	13.8	9.8	0.633	0.386
± one standard deviation	5.77	4.04	0.138	0.145
Arithmetic range	4.4–23.0	3.4–18.1	0.412–0.953	0.131–0.634

[1] Although these ILLT boundaries were curvilinear, average linear slopes were computed.

claim that acclimation temperature has a greater effect on lower than upper temperature tolerance.

Discussion

Temperature tolerance data portrayed as a polygon conveys important information concerning the physiology and ecology of a species, and the shape of a polygon offers a clue to the role acclimation temperature plays in the temperature tolerance of a species. In this study we have described two approaches to quantify the effect of acclimation temperature on temperature tolerance by partitioning temperature tolerance into acclimation independent and dependent areas. We have considered 18 measurement and 22 derived variables concerning various aspects of a species temperature tolerance polygon including the four key tolerance temperatures (ultimate ILLT, ultimate IULT, highest ILLT and lowest IULT), plateaus in the upper and lower tolerance boundaries, slopes relating acclimation temperature and ILT over the nonplateau portions of the tolerance boundaries and estimates of acclimation independent (intrinsic), lower, upper and combined acclimation dependent tolerance (quantified by two separate methods) in 21 species of fishes which provide nearly an infinite number of statistical comparisons. Nevertheless, we have restricted our discussion to several major findings, and realize that our findings may be limited by our data base, e.g., tropical fishes may have different temperature tolerance and acclimation temperature relationships than those found here.

Comparisons of the areal and linear approaches to quantify the effect of acclimation temperature on temperature tolerance demonstrated that the areal method produces better estimates since it includes information
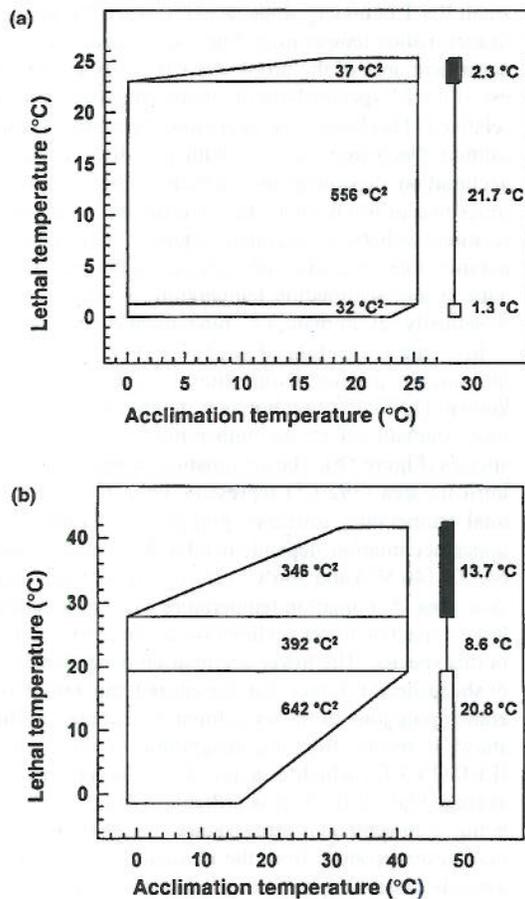
(a)

(b)

Figure 5. Areal and linear partitioned temperature tolerance polygons for the species exhibiting the least and greatest effect of acclimation temperature on temperature tolerance: a – temperature tolerance of the brook charr, *Salvelinus fontinalis*, is only mildly affected by acclimation temperature; b – acclimation temperature greatly expands the temperature tolerance polygon of sheepshead minnow, *Cyprinodon variegatus*.

concerning the shapes of the upper and lower lethal temperature boundaries, in particular the effect of tolerance plateaus on tolerance areas. Just as the thermal tolerance of an organism can be far better expressed as an area than as two points, one the upper and one lower lethal temperature (Fry et al. 1942), acclimation temperature dependent and intrinsic tolerance estimates are better measured as areas than linear distances.

Polygon areas in the 21 species of fishes range from 468 to 1380°C² for chum salmon and sheepshead

minnow, respectively. Not only is polygon area of the latter approximately three times larger than the former, it is the largest reported temperature tolerance area for any species of fishes indicating that the sheepshead minnow is the most eurythermal fish species known. The six species of salmonids have the six smallest polygon areas among the 21 species included in this study. The mean polygon of these salmonids was $533 \pm 52°C^2$ (mean ± standard deviation) which is considerably smaller than the overall mean polygon area of 799.1°C². Although polygon area was significantly correlated (p < 0.054) to 13 different individual variables, the ultimate IULT is the best single predictor of polygon area (Figure 3). Fully 93.2% of variation in polygon areas is accounted for by linear changes in ultimate IULT. Inclusion of additional independent variables would improve our predictions of polygon area by a mere 6.8%.

The general shape of the temperature tolerance polygons of all species with the exception of five of the six salmonids was hexagonal. Non-linearity in the five salmonids occurred in the ILLT boundary. Fry (1971) suggests that nonlinear changes in lethal temperature boundaries probably reflect different causes of death. Considerable variation was seen in the length of lower and upper temperature tolerance plateaus of the 21 species. Lower plateaus ranged from 4.4 to 23.0°C and upper plateaus from 3.4 to 18.1°C. Expressed relative to total temperature tolerance, plateaus extend from 17.7 to 90.9% and 9.8 to 61.8% of lower and upper ILT boundaries, respectively. The mean lower plateau was significantly longer, 4.0°C, than the mean upper plateau (paired t test, p = 0.008). Also polygon area was significantly correlated to lower (r = 0.56, p = 0.008) but not upper (p = 0.54) plateaus. Along a plateau, ILTs remain constant in the face of changes in acclimation temperature. Longer plateaus translate to larger acclimation dependent areas.

The amount of a temperature tolerance polygon partitioned into upper acclimation dependent tolerance is determined by the arithmetic difference between a species ultimate IULT and lowest IULT and the length of the IULT plateau. Similarly the lower acclimation dependent tolerance area is a function of the difference between a species ultimate ILLT and highest ILLT and the length of the ILLT plateau. Since all six of these variables exhibit considerable variation, it is not surprising that estimates of lower, upper and combined acclimation dependent temperature tolerances among

the 21 species vary by 2.0, 0.9 and 1.4 orders of magnitude, respectively.

Mean combined acclimation dependent and acclimation independent areas in the 21 species were not significantly different, and on a relative basis each accounted for approximately 50% of total polygon area. Relative combined acclimation dependent tolerance was mildly correlated with polygon area (r = 0.455, p = 0.038) indicating that the effect of acclimation temperature increases with increases in polygon area. Noteworthy exceptions appear upon closer inspection of these data. Separating the 21 species into three groups of seven species each relative to combined acclimation dependent tolerance yields some interesting results. These three groups have relative combined acclimation dependent tolerance ranging from 11.0 to 41.8, 46.7 to 60.8 and 61.0 to 71.6%, respectively. Although the two species (sheepshead minnow and goldfish) with the largest temperature tolerance polygons have the largest relative combined acclimation dependent tolerance, the relative combined acclimation dependent tolerance of *Ictalurus nebulosus* with the third largest polygon ranks seventeenth. The relative combined acclimation dependent tolerance of four of the six salmonids are in the lowest group, but cisco, *Coregonus artedii* has the third highest relative acclimation dependent tolerance. Finally, the relative combined acclimation dependent tolerances of five of the eight cyprinids fall into the middle group, however, *Rhinichthys atratulus* is in the lowest and *Pimephales notatus* and goldfish are in the highest groups, respectively. The large interspecific variation in acclimation dependent and independent tolerances do not appear to be completely explained on a phylogenetic basis, hence they should be investigated on species by species basis relative to the temperatures in their natural habitats.

In terms of acclimation independent and dependent tolerances the brook charr and sheepshead minnow represent the extremes. Of the total temperature tolerance polygon ($625°C^2$) of the brook charr, $556°C^2$ or 89% is unaffected by acclimation temperature. The shape of this species polygon approximates a square (Figure 5, panel a). This large intrinsic temperature tolerance results from a combination of factors including an extremely long lower lethal plateau (23.0°C) and a highest ILLT of only 1.3°C which produces a lower acclimation dependent area of only $32°C^2$. In addition, the small upper acclimation dependent area of $37°C^2$ is a result of a 2.3°C difference between the ultimate IULT and lowest IULT (25.3 and 23.0°C) which yields a

small IULT boundary slope, 0.131°C per 1.0°C change in acclimation temperature. The combined acclimation dependent area of the brook charr is easily the smallest of the 21 species both on an absolute ($69°C^2$) and relative (11%) basis. The next closest species is chum salmon, *Oncorhynchus keta*, with a relative combined acclimation dependent area which is more than 2.9 times that of brook charr. Brook charr has an unusual relationship between acclimation temperature and temperature tolerance since its realized temperature tolerance at any acclimation temperature is similar to its genetically set ultimate, i.e., fundamental, limits.

In contrast, sheepshead minnow not only possesses the largest piscine temperature tolerance polygon known ($1380°C^2$), acclimation temperature has its most dramatic effects on temperature tolerance of this species (Figure 5b). The acclimation independent, i.e., intrinsic area ($392°C^2$) represents only 28.4% of the total temperature tolerance polygon. The lower and upper acclimation dependent tolerance encompasses $642°C^2$ (46.5%) and $346°C^2$ (25.1%) of the total tolerance area. Acclimation temperature has a particularly large impact on lower acclimation dependent tolerance in this species. The lower acclimation dependent area of sheepshead is larger than the entire temperature tolerance polygons of all six salmonids included in this study. It results from the magnitude of the highest ILLT (19.3°C) which is a full 8.5°C larger than the average highest ILLT. It is difficult to think of 19.3°C being a lower lethal temperature. Decreasing acclimation temperature from the ultimate IULT (41.5°C) towards lower temperatures produces a dramatic gain of 'cold' tolerance in sheepshead minnow at the rate of 0.79°C per 1°C change in acclimation temperature. Temperature acclimation plays a major role in extending the temperature tolerance of the sheepshead minnow.

Lower and upper acclimation dependent tolerance areas were highly correlated suggesting that if acclimation temperature effects tolerance of low temperatures, it also will effect tolerance of high temperatures. Results clearly demonstrate that acclimation temperature effects tolerance of low temperature more greatly than tolerance of high temperatures in a majority of these 21 species. Not only were lower acclimation dependent tolerance areas nearly three times larger than upper acclimation dependent tolerance area, the ILLT boundary varies more markedly in response to changes in acclimation temperature. On the average a 1°C change in IULT requires a 2.6°C change in acclimation

temperature, whereas the same change in ILLT requires only a 1.6°C change in acclimation temperature.

The nearly three-fold difference between lower and upper acclimation dependent areas indicates that the intrinsic tolerance area is not positioned equidistantly between the ultimate ILLT and IULT. On the average, the upper boundary of the intrinsic area is nearly 3°C closer to the ultimate IULT than the lower boundary is to the ultimate ILLT. The closer proximity of the intrinsic tolerance area to higher temperatures could compensate for the smaller slopes relating acclimation temperature and upper temperature tolerance.

Temperature tolerance acclimation is a compensatory, reversible change in tolerance limits of a species in response to changes in environmental temperature. Tolerance acclimation, similar to capacity acclimation which specifically refers to various biochemical/physiological rate functions, is adaptive, i.e., serves to maintain or re-establish internal homeostasis permitting survival in the face of temperature dynamic environments. For example, fishes typically exhibit seasonal changes in temperature tolerance endpoints which essentially bracket seasonal water temperatures, providing a temperature tolerance safety margin. The internal processes responsible for tolerance acclimation are not known but obviously involve changes in biochemistry, particularly enzyme variants.

All 21 species of fishes included in this study exhibited varying abilities to extend their lower and upper thermal tolerance limits by acclimation. The small acclimation dependent tolerance of brook charr suggests that it possess little ability for temperature tolerance acclimation. Its realized tolerance is similar to the genetically set or fundamental tolerance regardless of temperature acclimation state. Over its entire biokinetic range of 0.0–25.3°C, ILLTs and IULTs increase by only 1.3 and 2.3°C (Figure 5a). In contrast, dramatic shifts in temperature tolerance limits suggest that the sheepshead minnow possesses a large tolerance acclimation ability. Over its biokinetic range (−1.5 − 41.6°C), ILLTs and IULTs change by 20.8 and 13.7°C (Figure 5b), respectively. These changes in upper and lower temperature tolerance limits from intrinsic levels (19.3–27.9°C) indicate the pre-eminent role of tolerance acclimation in this species. On the average, acclimation effectively doubles the size of the temperature tolerance polygon, however, large interspecific variation indicates that each species should be investigated on an individual basis relative to its natural thermal environment.

References cited

Bennett, W.A. & T.L. Beitinger. 1997. Temperature tolerance of the sheepshead minnow, *Cyprinodon variegatus*. Copeia 1997: 77–87.

Bennett, W.A., T.L. Beitinger & R.W. McCauley. 1998. Rates of gain and loss of heat tolerance in channel catfish. Trans. Amer. Fish. Soc. 127: 1051–1058.

Bliss, C.I. 1937. The calculation of the time–mortality curve. Ann. Appl. Biol. 24: 815–852.

Brett, J.R. 1944. Some lethal temperature relations of Algonquin Park fishes. Univ. Toronto Studies Biol. Series No. 52, Pub. Ont. Fish. Res. Lab. 63: 1–49.

Brett, J.R. 1946. Rate of gain of heat-tolerance in goldfish (*Carassius auratus*). Univ. Toronto Studies Biol. Series No. 53, Pub. Ont. Fish. Res. Lab. 64: 7–28.

Brett, J.R. 1952. Temperature tolerance in young Pacific salmon, genus *Oncorhynchus*. J. Fish. Res. Board Can. 9: 265–323.

Brett, J.R. 1970. Environmental factors, part I. Temperature. pp. 513–560. *In:* O. Kinne (ed.) Marine Ecology, Wiley, London.

Chung, K.S. 1981. Rate of acclimation of the tropical saltmarsh fish *Cyprinodon dearborni* to temperature changes. Hydrobiologia 78: 177–181.

Davenport, C.B. & W.E. Castle. 1896. Studies in morphogenesis, III. On the acclimatization of organisms to high temperatures. Arch. Entwick. 2: 227–249.

Davies, W.D. 1973. Rates of temperature acclimation for hatchery reared striped bass fry and fingerlings. Prog. Fish-Cult. 35: 214–217.

Doudoroff, P. 1942. The resistance and acclimatization of fishes to temperature changes. I. Experiments with *Girella nigricans* (Ayres). Biol. Bull. 83: 219–244.

Edsall, T.A. & P.J. Colby. 1970. Temperature tolerance of young-of-the-year cisco, *Coregonus artedii*. Trans. Amer. Fish. Soc. 99: 526–531.

Fry, F.E.J. 1947. Effects of the environment on animal activity. Univ. Toronto Studies Biol. Series No. 55, Pub. Ont. Fish. Res. Lab. 68: 1–62.

Fry, F.E.J. 1967. Responses of vertebrate poikilotherms to temperature. pp. 375–409. *In:* E.H. Rose (ed.) Thermobiology, Academic Press, New York.

Fry, F.E.J. 1971. The effect of environmental factors on the physiology of fish. pp. 1–98. *In:* W.S. Hoar & D.J. Randall (ed.) Fish Physiology, Volume 4, Environmental Relations and Behavior, Academic Press, New York.

Fry, F.E.J., J.R. Brett & G.H. Clawson. 1942. Lethal limits of temperature for young goldfish. Rev. Can. Biol. 1: 50–56.

Fry, F.E.J., J.R. Brett & K.F. Walker. 1946. Lethal temperature relations for a sample of young speckled trout (*Salvelinus*

fontinalis). Univ. Toronto Studies Biol. Series No. 55, Pub. Ont. Fish. Res. Lab. 66: 9–35.

Hart, J.S. 1947. Lethal temperature relations of certain fish of the Toronto region. Trans. Royal Soc. Can. 41: 57–71.

Hart, J.S. 1952. Geographic variations of some physiological and morphological characters in certain freshwater fish. Univ. Toronto Studies Biol. Series No. 60, Pub. Ont. Fish. Res. Lab. 72: 1–79.

Hathaway, E.S. 1927. Quantitative study of the changes produced by acclimatization in the tolerance of high temperatures by fishes and amphibians. Bull. U.S. Bur. Fish. 43: 169–192.

Hutchinson, G.E. 1965. The niche: an abstractly inhabited hypervolume. pp. 26–78. *In:* The Ecological Theatre and the Evolutionary Play, Yale University Press, New Haven.

Hutchison, V.H. 1976. Factors influencing thermal tolerance of individual organisms. pp. 10–26. *In:* G.W. Esch & R.W. McFarlane (ed.) Thermal Ecology II, U.S. Nat. Tech. Inf. Serv., Springfield.

Loeb, J. & H. Wasteneys. 1912. On the adaptation of fish (*Fundulus*) to high temperatures. J. Exp. Zool. 12: 543–557.

Reynolds, W.W. & M.E. Casterlin. 1979. Behavioral thermoregulation and the 'final temperature preferendum' paradigm. Amer. Zool. 19: 211–224.

Sumner, F.B. & P. Doudoroff. 1938. Some experiments upon temperature acclimatization and respiratory metabolism in fishes. Biol. Bull. 74: 403–429.