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# What factors influence the thermal tolerance of estuarine animals? Interpretation of multiple regression analyses

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**Resumen:** Se realizaron análisis de regresión múltiple para determinar los factores que influyen significativamente en la temperatura letal media durante tres horas (3-h $TL_{50}$), usando cinco especies de crustáceo y 32 de peces capturados en la entrada del canal de una planta térmica estuarina. La temperatura ambiental fue el factor que más frecuentemente afectó, seguida de la salinidad. El método de captura, el tiempo de permanencia de los especímenes en las jaulas antes del experimento y todas las posibles combinaciones de las interacciones de los parámetros estudiados fueron menos significativos.

**Key words:** Heat resistance, acclimation temperature, thermohaline susceptibility, Tropical-subtropical fishes and crustaceans.

The biotic and abiotic factors influencing thermal tolerance on aquatic organisms are photoperiod, seasonal and daily cycles, geographic variations, diet, sex, breeding conditions, age, life cycle stage, salinity, chemicals, body water content and partitioning, oxygen supply, pH, innate and learned behavior, history of thermal exposure, sublethal exposure of limiting factors, and experimental methods (Hutchison 1976, Stauffer 1986, Meador & Kelso 1990, Pepin 1991). Because most heat shock experiments have been done with organisms acclimated in laboratory conditions (Allen & Strawn 1971, Chung & Méndez 1993), their interpretation in the natural environment often has limited senses.

Thus in these experiments, all animals collected in the field have been used directly without laboratory acclimation. Five crustacean and 32 fish species tested were captured from the intake canal of the P.H. Robinson Generating Station, Bacliff, Texas during 16 months, June 1974 throughout September 1975. Various fishing gears such as dip net, lift net, hoop net, traps, seine, trawl, log and revolving screens, hook and line, and cast net were used to collect organisms on daily basis during summer months (June-September) and once a month during non-summer months (October-May). After capture, animals were placed in 45-L ice chest with water from the canal to minimize temperature changes, and were aerated and transported from the intake canal to the laboratory within five minutes. Organisms captured in the afternoon and evening were placed in plastic cages (61-cm in diameter and 63.5-cm deep with 0.325-, 0.65-, or 1.3-cm Doupon Vexar mesh) and in the intake canal for experimentation on the following day. Temperature and salinity measured in the intake canal at the water surface were taken in conjunction with each experiment. They ranged from a low of 8.3°C to a high of 31.6°C and from 2 ppt to 24.8 ppt. All glass, 38-L aquaria were filled with water from the intake canal, aerated to provide

circulation and oxygen, and thermally controlled within 0.1°C. Organisms were exposed for three hours to various predetermined-elevated temperatures (25-41°C). Numbers of the organisms tested in a tank belonging to one species rarely exceeded 10 and, because of availability, was limited to a few for some species (Table 1). Then, median lethal temperature for three-hour (3-h $TL_{50}$), 50% tolerance temperature of the experimental organisms was determined for each species without laboratory acclimation. For details, see Chung and Strawn (1978).

Multiple regression analyses, at 5% significance level, were performed with the 3-h $TL_{50}$ against hydrological parameters, capture methods, days in cages before experiment, and possible interactions between treatments to determine whether these factors significantly affect the 3-h $TL_{50}$. The statistical model used in the analyses was $Y = B_0 + B_1C + B_2T + B_3S + B_4D + B_5TS + B_6TD + B_7SD + e$, where Y = 3-h $TL_{50}$; C = catch method; T = ambient temperature; S = intake and test salinity; D = days in cage before experiment; TS = interaction between intake canal temperature and salinity; TD = interaction between temperature and days in cage before experiment; SD = interaction between salinity and days in cage; and e = random error.

Results of the multiple regression analyses performed on five crustaceans and 32 fishes tested to determine significant factors influencing the 3-h $TL_{50}$ are presented in Table 1.

**Fishing gear:** Each fishing gear should impose different stress on aquatic animals. However, capture method was not a factor significantly affecting the 3-h $TL_{50}$ for all crustaceans and fishes except for three species (Gulf menhaden *Brevoortia patronus*, sea catfish *Arius felis*, and rough silverside *Membras martinica*). This indicates that physiological stresses caused by fishing gears for most species tested do not influence significantly on short term heat shock of three hours. However, handling stress may affect long term thermal tolerance for aquatic organisms.

**Ambient temperature of intake canal:** Intake canal temperature, because it indicates acclimation level, is the most significant factor affecting the 3-h $TL_{50}$ for three crustaceans (grass shrimp *Palaemonetes pugio* and *P. vulgaris* and white shrimp *Penaeus setiferus* and nine fishes (Gulf menhaden, bay anchovy *Anchoa mitchilli*, Atlantic spadefish *Chaetodipterus faber*, crevalle jack *Caranx hippos*, pinfish *Lagodon rhomboides*, sand seatrout *Cynoscion arenarius*, spot *Leiostomus xanthurus*, Atlantic croaker *Micropogon undulatus*, and naked goby *Gobiosoma bosci*). Five of the species that were indifferent to changes in ambient temperatures (ladyfish *Elops saurus*, sailfin fish *Poecilia latipina*, letherjacket *Oligoplites saurus*, mojarra *Eucinostomus* sp., and least puffer *Sphaeroides parvus*) were tested only in the summer (June-September) when intake canal temperatures ranged from approximately 27 to 30°C and were usually around 29°C (Chung & Strawn 1978). Because their thermal acclimation levels would have varied little when they were available for testing, the relationship of ambient temperature to the 3-h $TL_{50}$ should be non-significant. Organisms tested mostly in the summer and a few animals tested in the cool months also showed non-significance because of too few tests at cool temperatures with little varieties. The other 18 species were in this catagory (brown shrimp *Penaeus aztecus*, blue crab *Callinectes sapidus*, threadfin shad *Dorosoma petenenses*, scaled sardin *Harengus pensacolae*, Atlantic toadfish *Opsanus beta*, sheepshead minnow *Cyprinodon variegatus*, Gulf killifish *Fundulus grandis*, mosquitofish *Gambusia affinis*, tidewater silverside *Menidia beryllina*, sheepshead *Archosargus probatocephalus*, silver perch *Bairdiella chrysurus*, spotted seatrout *Cynoscion nebulosus*, red drum *Sciaenops ocellatus*, black drum *Pogonia cromis*, striped *mullet Mugil cephalus*, white mullet *M. curema*, sharptail goby *Gobionellus hastatus*, and southern flounder *Paralichthys lethostigma*). Another possible explanation for non-significance is seasonal differences between acclimation level of the organisms and intake canal temperature. Gain in acclimation level behind ambient temperature when water temperature was rising in the spring. In the fall, as water temperature fell, acclimation lagged behind ambient temperature, and thus, the 3-h $TL_{50}$ was higher than expected based on temperatures at which experimental organisms

TABLE 1

*Results of multiple regression analyses with significance levels for factors affecting 3-h $TL_{50}$, coefficient of determination ($R^2$) and test months*

| Species, N(n) | | Levels of significance | | | | | | | $R^2$ | Months of year |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Gears | Temper-ature | Salin-ity | Days in cage | TS | TD | SD | | |
| **Crustaceans** | | | | | | | | | | |
| 01 | *Palaemonetes pugio*, 75(3681) | 0.44 | 0.00** | 0.14 | - | 0.26 | - | - | 0.83 | 1-12 |
| 02 | *Palaemonetes vulgaris*, 39(1041) | 0.24 | 0.00** | 0.74 | - | 0.84 | - | - | 0.84 | 1-12 |
| 03 | *Penaeus setiferus*, 81(1290) | 0.96 | 0.01* | 0.48 | 0.64 | 0.42 | 0.52 | 0.50 | 0.77 | 6-11 |
| 04 | *Penaeus aztecus*, 92(841) | 0.46 | 0.24 | 0.77 | 0.38 | 0.95 | 0.52 | 0.18 | 0.60 | 5-11 |
| 05 | *Callinectes sapidus*, 64(1036) | 0.07 | 0.08 | 0.77 | - | 0.93 | - | - | 0.59 | 1,5-12 |
| **Fishes** | | | | | | | | | | |
| 01 | *Elops saurus*, 8(21) | - | 0.78 | 0.82 | - | 0.67 | - | - | 0.98 | 8-9 |
| 02 | *Brevoortia patronus*, 64(3016) | 0.04* | 0.00** | 0.00** | 0.31 | 0.84 | 0.61 | 0.61 0.90 | 1-12 | |
| 03 | *Dorosoma petenense*, 9(66) | - | 0.72 | 0.33 | - | 0.55 | - | - | 0.96 | 9-11 |
| 04 | *Harengula pensacolae*, 22(62) | 0.76 | 0.95 | 0.97 | 0.42 | 0.92 | 0.36 | 0.51 | 0.51 | 8-9,11 |
| 05 | *Anchoa mitchilli*, 54(3223) | 0.14 | 0.00** | 0.00** | 0.48 | 0.00** | 0.44 | 0.35 | 0.84 | 1-12 |
| 06 | *Arius felis*, 46(357) | 0.01* | 0.19 | 0.43 | 0.80 | 0.47 | 0.86 | 0.67 | 0.61 | 5-11 |
| 07 | *Opsanus beta*, 40(61) | 0.10 | 0.47 | 0.57 | - | 0.01* | - | - | 0.50 | 2,4-10 |
| 08 | *Cyprinodon variegatus*, 8(12) | - | 0.46 | 0.44 | - | 0.41 | - | - | 0.42 | 6-9,12 |
| 09 | *Fundulus grandis*, 8(11) | - | 0.39 | 0.48 | - | 0.47 | - | - | 0.28 | 6-8,11 |
| 10 | *Gambusia affinis*, 67(2196) | 0.05 | 0.37 | 0.78 | - | 0.72 | - | - | 0.23 | 4-10 |
| 11 | *Poecilia latipinna*, 16(103) | - | 0.78 | 0.74 | - | 0.71 | - | - | 0.17 | 6-8,9 |

Continues

| Species, N(n) | | Levels of significance | | | | | | | $R^2$ | Months of year |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Gears | Temperature | Salinity | Days in cage | TS | TD | SD | | |
| 12 | *Membras martinica*, 49(1227) | 0.02* | 0.76 | 0.94 | 0.51 | 0.96 | 0.81 | 0.57 | 0.33 | 5-12 |
| 13 | *Menidia beryllina*, 6(92) | - | 0.16 | 0.16 | - | 0.11 | - | - | 0.29 | 9-10,12 |
| 14 | *Cheatodipterus faber*, 67(475) | 0.17 | 0.02* | 0.10 | 0.50 | 0.09 | 0.41 | 0.94 | 0.47 | 6-11 |
| 15 | *Caranx hippos*, 14(19) | 0.48 | 0.02* | 0.43 | 0.37 | 0.03* | 0.36 | 1.00 | 0.93 | 6-8 |
| 16 | *Oligoplite saurus*, 19(45) | 0.36 | 0.86 | 0.96 | - | 0.98 | - | - | 0.58 | 6-9 |
| 17 | *Eusinostomus* sp., 14(19) | 0.68 | 0.56 | 0.39 | - | 0.38 | - | - | 0.24 | 7-9 |
| 18 | *Archosargus probatocephalus*, 66(177) | 0.92 | 0.97 | 0.80 | 0.79 | 0.80 | 0.80 | 0.61 | 0.46 | 6-11 |
| 19 | *Lagodon rhomboides*, 87(329) | 0.30 | 0.00** | 0.00** | 0.06 | 0.00** | 0.05 | 0.51 | 0.79 | 2-11 |
| 20 | *Bairdiella chrysura*, 87(647) | 0.61 | 0.13 | 0.22 | 0.20 | 0.13 | 0.13 | 0.12 | 0.40 | 5-11 |
| 21 | *Cynoscion arenarius*, 28(112) | 0.14 | 0.01* | 0.15 | - | 0.17 | - | - | 0.88 | 4,6-11 |
| 22 | *Cynoscion nebulosus*, 24(31) | 0.90 | 0.28 | 0.40 | - | 0.41 | - | - | 0.67 | 6-8,11 |
| 23 | *Leiostomus xanthurus*, 13(31) | - | 0.04* | 0.18 | - | 0.22 | - | - | 0.93 | 3,6,7,9 |
| 24 | *Micropogon undulatus*, 32(102) | -0.00** | 0.01* | 0.00** | 0.01* | 0.00** | 0.17 | 0.83 | 1,3,5- | 12 |
| 25 | *Sciaenops ocellatus*, 9(14) | 0.90 | 0.90 | 0.56 | - | 0.58 | - | - | 0.87 | 5-6,8-9,11 |
| 26 | *Pogonias cromis*, 6(21) | - | 0.13 | 0.13 | - | 0.13 | - | - | 0.84 | 8-9,11 |
| 27 | *Mugil cephalus*, 87(475) | 0.64 | 0.76 | 0.47 | 0.31 | 0.43 | 0.45 | 0.54 | 0.31 | 6-12 |
| 28 | *Mugil curema*, 40(359) | 0.84 | 0.42 | 0.33 | 0.49 | 0.33 | 0.43 | 0.40 | 0.16 | 4-11 |
| 29 | *Gobiosoma bosci*, 54(548) | 0.30 | 0.00** | 0.00** | - | 0.00** | - | - | 0.81 | 1-4,6-12 |
| 30 | *Gobionellus hastatus*, 12(31) | - | 0.11 | 0.14 | - | 0.10 | - | - | 0.12 | 3,7-9 |

Continues

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 31 | *Paralichthys lethostigma*, 15(29) | - | 0.68 | 0.31 | - | 0.32 | - | - | 0.68 | 6-8,11,12 |
| 32 | *Sphoeroides parvus*, 8(20) | - | 0.68 | 0.63 | - | 0.65 | - | - | 0.93 | 6-9 |

Species, N: number of experiments carried out
Species, (n): number of specimens tested
Days in cage: number of days held in a cage prior to experiment
TS: interaction between ambient temperature and salinity
TD: interaction between water temperature and days in cage before experiment
SD: interaction between salinity and days in cage before experiment
*: 5% level of significance
**: 1 % level of significance

were captured (Chung & Strawn 1978). This upholds the general concept in which acclimation rate for aquatic animals needs from several hours to a few days (fast) at an increase in temperature and requires from several days to a few weeks (slow) at a decrease in temperature (Chung 1981, Segnini *et al.* 1993).

**Salinity:** Salinity had a non-significant effect on the 3-h $TL_{50}$ for all crustaceans and all but five fishes (Gulf menhaden, bay anchovy, pinfish, Atlantic croaker and naked goby). The above supports the well known concept that estuarine organisms can survive in a wide range of salinity during temperature shocks for short term periods (Kinne 1971). Salinity is the most important parameter affecting abundance and distribution of the estuarine fishes. Spawning, hatching and early development occur in shallow waters of Galveston Bay. Young fishes penetrate estuaries into upper tributaries receiving freshwater inflow and associated food sources and undergo rapid growth and development (Copeland & Bechtel 1974). Abudance of these fishes drops significantly when salinities exceed below 5 ppt and above 30 ppt (Gallaway & Strawn 1974). Therefore, significance is likely to reflect thermal susceptibility or resistance for these euryhaline species at certain life cycle stages and/or at particular saline conditions.

**Number of days in holding cages before experiment:** Holding organisms in cages in the intake canal for a few days before experiments did not considerably affect on all 3-h $TL_{50}$ except for Atlantic croaker. With this one exception, holding animals in cages apparently did not alter their heat resistance compared with free-living animals. This result increases the importance of the cage-related interactions described below; that is, significant interactions are unlikely to reflect the interaction of the cage damage and other factors considered such as salinity, temperature, and capture method.

**Interaction between water temperature and salinity:** Of the animals tested, only six fishes (bay anchovy, Atlantic toadfish, crevalle jack, pinfish, Atlantic croaker, and naked goby) showed interaction between ambient temperature and salinity. Shrimp (Wisepape 1975) and fishes (Strawn & Dunn 1967) confined in the laboratory acclimated to constant temperature and salinity showed a strong relationship between heat resistance and salinity. The above authors acclimated animals at fixed temperature and salinity, then tested at various constant temperatures and salinities. In this study, capture salinity and test salinity were kept the same because water passing through a power plant does not change in salinity.

**Interaction between temperature and days in cage before experiment:** All crustaceans and fishes except Atlantic croaker showed no interaction between intake canal temperature and days in holding cage before experiment. This indicates no noticeable difference between the temperature acclimation status attained by free-living and caged animals, which in turn suggests no substantial behavioral temperature regulation by organisms living in the intake canal. Lack of such behavioral thermoregulation reflects uniformity of temperature within the intake canal, but not lack of thermoregulatory capability on the part of the organisms.

**Interaction between salinity and days in cage before experiment:** All crustaceans and fishes had non-significant interactions between intake canal salinity and days in holding cage before experiment. Estuarine organisms have a high ability to osmoregulate, and are distributed in a wide range of salinity. The similarity between free-living and caged animals indicates no behavioral salinity regulation within the intake canal. Like temperature, salinity levels were relatively uniform within the intake canal at any particular time (Chung & Strawn 1978).

**Comment:** Overall information indicates that ambient temperature is the major factor affecting the 3-h $TL_{50}$ and that capture salinity is less important. Capture method, holding test animals in cages before expeiment and possible interactions between hydrological parameters and other treatments can be considered to have little effect.

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