Rev. Biol. Trop., 44(1): 123-126, 1996

Thermoregulatory behavior applied to the culture of *Procambarus clarkii* (Decapoda: Cambaridae)

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(Rec. 5-XI-1993. Rev. 23-V-94. Acc. 20-I-1995)

Abstract: In thermoregulatory behavior of *Procambarus clarkii* studied on organisms acclimated to 20, 23, 26 and 29 °C, body mass did not affect tolerance and resistance. Acclimation temperatures exerted no significant influence on the preferred temperatures nor on the final preferendum (23.4 °C). Nevertheless, acclimation positively affected the low temperature avoidance threshold: the upper incipient lethal temperature by 0.6 °C and the critical thermal maximum (CTM), by 3.2 °C. When culturing *P. clarkii* in Baja California, temperature should be close to the final preferendum, and within the accepted range of 16.9 - 28.5 °C.

Key words: Acclimation, thermoregulation, culture, *Procambarus clarkii,* Baja California, México.

Thermoregulation is influenced by internal body factors; optimum temperatures vary with species age and size and may even change according to time of day (Nichelmann 1983). The "optimum biological temperature" is the environmental temperature which causes no stress to homeothermic organisms. Poikilotherms must adjust their functions to environmental temperature, finding appropriate refuges (Reynolds and Casterlin 1979). Thus, they have developed strategies and adaptive behaviors which may be highly useful in culturing. This paper reports on thermoregulation in *P. clarkii* and how it may be applied to the culturing of the species.

MATERIAL AND METHODS

Crayfish were collected near the dam at La Mision (31°51' N; 116°37' W) in Baja California, México, and kept in 500 l reservoirs at 20 ± 1 °C, pH 7.9, 7.8 mg O_2/l, 670 mg/l hardness ($CaCO_3$), and 137 mg/l alkalinity. The ration provided every three days was 5% of the body weight in food with 35% protein and a 81.4 mg/Kcal protein-energy ratio.

Specimens (150 per acclimation experiment) were kept at 20, 23, 26 and 29 ± 1 °C for 15 to 30 days, with a photoperiod of 12/12 h night/day. Preferred and avoided gravitation temperature experiments of acclimated crayfish were carried out in a horizontal trough (300 cm long, 20 cm in diameter) internally coated with rubber mesh; water depth was 10 cm, with the temperature interval (10-40 °C) surpassing both the minimal and maximal environmental average temperatures registered over the last ten years (Miranda *et al.* 1991). To study the upper incipient lethal temperature (UILT), which relates the mean survival time of the organisms exposed to lethal temperatures, we followed the methodology described by Kilgour *et al.* (1985), where by organisms are abruptly transferred in a series of constant bath temperatures, each differing by 1.5 ±0.2 °C. The oxygen in the bath was 6.6 at the lowest and 5.9 mg/l at the highest temperatures. To study critical thermal maximum (CTM), defined as the "thermal point at which locomotor activity becomes disorganized and the organisms lose their ability to escape from conditions promptly leading to death", bath water was heated at a rate of 1 °C/min (Cowles and Bogert 1944, Paladino *et al.* 1980). Dissolved oxygen in the baths was 8.9-6.3 mg/l.

In all experiments, the organisms were categorized according to Huner (1988) and were divided in to weight categories of 10-20, 20-30 and 30-40 g. Individually-labeled animals were not fed 24 hours prior to each experiment; afterward, they were weighed to 0.1 g and sex was determined.

If preferred temperatures among size groups of diverse acclimation temperatures differed ($P<0.05$), a two-way analysis of the variance was made.

Preferred temperatures were plotted against acclimation temperatures. The point where the preferred temperature curve regulated by visual inspection, intersected the theoretical line slope of 1, was considered the final thermal preferendum (FP).

Upper incipient lethal temperature corresponds to the intercept of the regression line where mortification rate is zero (Kilgour *et al.* 1985). Thermal stress response was potentially related to acclimation temperature. The logarithmic linear regression (Ln) was adjusted by least square and goodness of fit estimated by analysis of residuals (Montgomery and Peck 1982). The significance of the differences observed ($P < 0.05$) was determined by two-way variance analysis.

RESULTS

Mortality during acclimation at 20, 23, 26 and 29 ± 1 °C was less than 1 %; environmental conditions did not prevent organisms from molting. Body mass of acclimated crawfish exerted no influence on preferred temperature ($P > 0.05$), which was close to the final preferendum (23.4 °C) (Fig. 1). It did not affect either the low and high thermal avoidance behavior ($P > 0.05$), nor the upper incipient lethal temperature (UILT) ($P > 0.05$), nor the critical thermal maximum (CTM) ($P > 0.05$). But acclimation temperatures increased ($P < 0.05$) dependent UILT variables (from 34.8 to

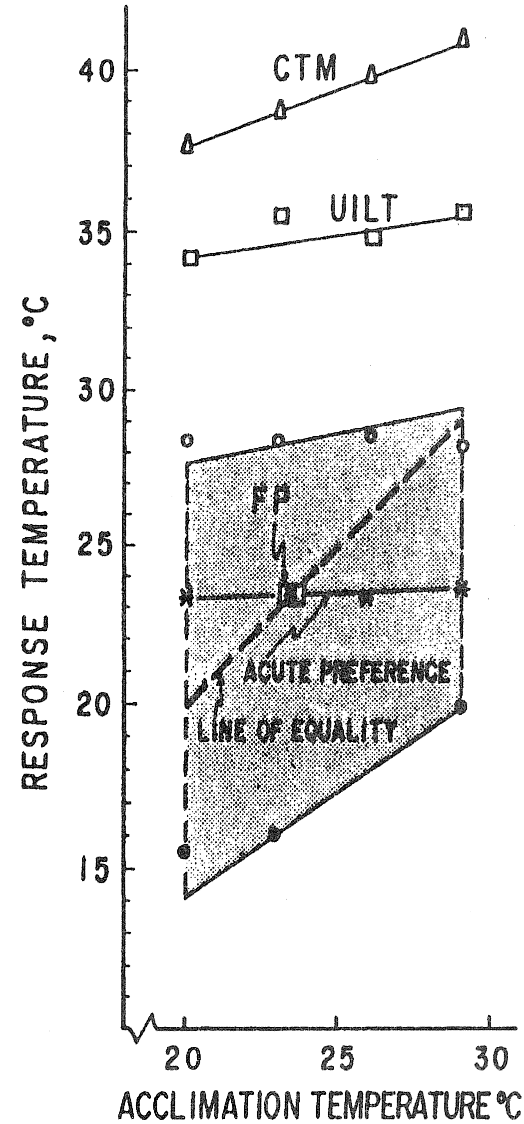

Fig. 1. Shade polygon enclosed the minimal (•) and maximal (•) avoided temperatures; the final preferendum (FP) (23.4 °C) and preferred temperatures (*). Upper incipient lethal temperature (UILT) and critical thermal maximum (CTM). The shaded area comprise 107.1 (°C)^2.

35.4 °C), CTM (from 37.6 to 40.8 °C), and low temperature avoidance threshold significantly (from 15.7 to 19.8 °C) (Fig. 1). For every degree Celcius of acclimation temperature (20-29 °C), preferred temperatures increased 0.04 °C, high and low avoided temperatures increased 0.02

and 0.06 °C, respectively; UILT 0.13 and CTM 0.35 °C. The area of thermal preference for *P. clarkii* of Baja California is 107.1 (°C)^2 (Fig. 1). This area is limited by the high and low avoidance temperatures.

DISCUSSION

A species living in an heterothermic environment must be physiologically tolerant to changes in temperature, adapting its metabolic functions in order to convert food into useful energy for growth or reproduction. Therefore, thermoregulation related findings for *P. clarkii* may be applied to aquaculture if the culture is within the area of thermal preference that includes the final preferendum, within which it must be confined. The area of thermal preference (Giattina and Garton 1982) is a reduced expression of the area within the thermal tolerance polygon proposed by Brett (1956), which for *Pimephales notatus, Perca flavescens* and *Salvenilus fontinalis* covers 338, 239 and 186 (°C)^2 respectively (Giattina and Garton 1982). The thermal area of secure displacement without stress of these organisms is similar to that of *P. clarkii* (107.1 (°C)^2), which reflects its ecological restriction and limited thermal adjustment.

A *P. clarkii* culture restricted to the area of thermal preference can attain improved bodily functions between the preferred (0.04 °C) and the upper avoided temperatures (0.02 °C), but not with the lower avoided temperatures (0.06 °C). The UILT delimits the zone of tolerance of a species where, by definition, only 50% survive extended temperature exposure (Fry 1947). Therefore the UILT (0.13 °C) and the CTM (0.35 °C) exceed the adjustment capacity of *P. clarkii*, since the extended exposure to the UILT can have sublethal effects that, at the CTM, lead to the death of these organisms.

The respective high and low avoidance temperatures for the final preferendum (FP) (23.4 °C) (Espina *et al.* 1993) are 5.0 and 6.8 °C (Fig. 1). Upon relating this interval (= 11.8 °C = 100 %) to the maximal and minimal annual average environmental temperatures (26.2 °C and 7.7 °C) (= 18.5 °C), measures in areas of *P. clarkii* occurrence researched over the last 10 years (Miranda *et al.* 1991), the resulting specific thermal efficience response (STER) covers 63.8 % of the environmental temperature difference.

The cyclic, variable, or regulated culture temperature is very important to the ecological behavior of the species. Since the STER involves the preferred temperatures and the final preferendum, animals are physiologically stable, that is, at their maximum physiological efficiency, free of stress; therefore the population distributes adequately in the area of displacement in reservoirs. Avoided temperature responses by organisms alter movement behavior, and their spatial distribution may cause aggregation problems.

If the environmental high temperature of 40 °C exceeds the STER, this circumstance must be reversal because it exposes culturing organisms to the upper limit of their tolerance, that is, the upper incipient lethal temperature, and, likewise to the critical thermal maximum at which their resistance is limited to short periods. Otherwise, these organisms should be allowed movement to thermal refuges (Giattina and Garton 1982) with lower temperature were thermal efficience increases. Consequently, culturing of *P. clarkii* in Baja California may be practiced in geographic zones where the environmental temperatures are near the final preferendum and do not exceed the STER.

RESUMEN

Se estudió el comportamiento termorregulador de *Procambarus clarkii* con organismos aclimatados a 20, 23, 26 y 29 °C; el peso no influyó la tolerancia y la resistencia de los animales. Las temperaturas de aclimatación no afectaron significativamente las temperaturas preferidas y tampoco el preferendum final (23.4 °C). No obstante, la aclimatación alteró positivamente el umbral térmico inferior de evitación: a temperatura letal incipiente superior en 0.6 °C y la temperatura crítica máxima en 3.2 °C. Para cultivar a *P. clarkii* en Baja California se debe hacer alrededor de la temperatura preferida sin sobrepasar el intervalo de 16.9 a 28.5 °C.

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