

Ecophysiological behavior of *Caquetaia kraussii* (Pisces: Cichlidae) exposed to different temperatures and salinities

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Received 24-IX-1999. Corrected 19-VII-2000. Accepted 19-IX-2000.

Abstract: Tropical river sardine, *Caquetaia kraussii*, captured from La Aguá lagoon (Sucre State, Venezuela) were acclimatized for four weeks at 22, 24, 30 and 32°C and at 0, 5, 10, 15 and 17 ‰ salinity. To evaluate effects of thermal response to acclimatization level, the fish were transferred suddenly from lower temperatures (22 and 24°C) to higher ones (32 and 30°C) respectively. Then thermal resistance time was measured at the lethal temperature of 40.9°C for 30 days. We considered that acclimatization process completed when resistance time was stabilized at the new temperature regime. For the saline effect, the concentrations of sodium and potassium were measured in the tissues at each treatment: gills, white muscle, gut and heart. The results showed that thermal tolerance increased rapidly in 3 h with a 6°C rise in temperature (from 24 to 30°C) and in 24 h with a 10°C rise (22 to 32°C). With decreasing temperatures, the acclimatization level reached its lowest in 11 days with a 6°C decrease (from 30 to 24°C) and in 14 days with a 10°C decrease (32 to 22°C). *Caquetaia kraussii* regulates as much sodium as potassium in gills and white muscle tissues at all salinity levels tested; however, gut and heart tissues showed significantly different regulations among salinities examined.

Keywords: Thermal acclimatization, salinity, electrolytic concentration, *Caquetaia kraussii*.

Temperature and salinity are the abiotic factors that have the strongest influence on organisms because they involve functional and structural alterations through changes in the osmotic concentration, promoting modification of behavior of a species inside an ecosystem. Also, salinity may establish differences in physiological effects in the embryonic development depending on other environmental factors, particularly temperature (Bennett & Judd 1992, Battaglani & Talbot 1993, Chung & Strawn 1994).

Knowledge of these two factors is very useful in studying tropical organisms in coastal lagoons, temporary shallow water lagoons, lakes and estuaries, since they are exposed to

temperature fluctuations during the day and night and to salinity changes due to evaporation during dry periods or dilution during rainy seasons (Segnini *et al.* 1993, Segnini de Bravo & Chung 1997).

Fish living in a freshwater environment have higher rates of glomerular filtration and renal excretion of water and salts than those living in a seawater environment, because there is a hypoosmotic relation to their environment. Therefore, they have less electrolytic concentration than the seawater variety, absorbing Na⁺, K⁺ and Cl⁻ ions, mainly in the digestive tract and desalinizing the water at gill levels. In euryhaline fish, the glomerular filtration rate

and urine flow changes in the direction of ion concentrations, which means that it passes from a lower saline ion concentration to a higher one and vice versa. Although the nature of this mechanism is not known, it may be assumed that these changes are promoted in renal functions; furthermore they may participate in circulatory and hormonal functions (Canahan *et al.* 1986, Salman & Eddy 1988).

In spite of the physiological importance and ecological consequences of temperature and salinity on the stenohaline and euryhaline organisms, the available information is scarce. The aim of this work is to study the influence of acclimatization temperature and salinity in river sardine *Caquetaia*, which is a great food source for its excellent flavour, few bony axial skeleton, low fat and high protein contents.

MATERIALS AND METHODS

The organisms were captured in "La Aguá" lagoon, near Chiguana, Estado Sucre, Venezuela (10°30'N and 63°41'W) and transferred to the laboratory in insulated containers to avoid abrupt changes of temperature.

Later on, groups of 200 fish of 13.0 ± 1.5 mm in standard length (L) were acclimatized to temperatures of 22, 24, 30 and 32°C, respectively (the temperature range in La Aguá lagoon during the year). For their acclimatization, aquaria with a 300 l capacity, a thermal controller, a heater and a contact thermometer to regulate water temperature with a variation of $\pm 0.01^\circ\text{C}$, were used. Acclimatization rates of two temperature changes were studied: a difference of 6°C (24 to 30°C) and of 10°C (22 to 32°C). Before the experiment and after transfer up to 30 days, 10 fish, taken at random with replica, were exposed to the lethal temperature of 40.9°C to measure their thermal resistance time. We considered that acclimatization level had been completed when the survival time of the organisms progressively stabilized at a higher or lower temperature.

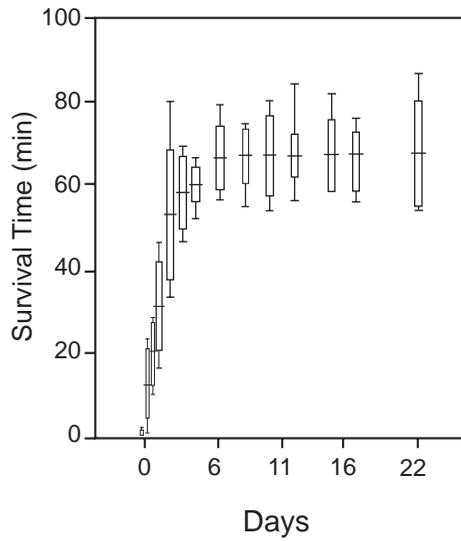
To study the incipient lethal salinity, three groups of 20 fish (10.0 ± 3.3 mm L), acclima-

tized at 23°C with their respective replica and control were placed in freshwater aquaria. Then salinity was increased daily by 2‰ up to 15‰. After this, the increment was 1‰ per day, because it is known that in Colombia this species can be found in 14‰ salinity water (Hurtado 1975).

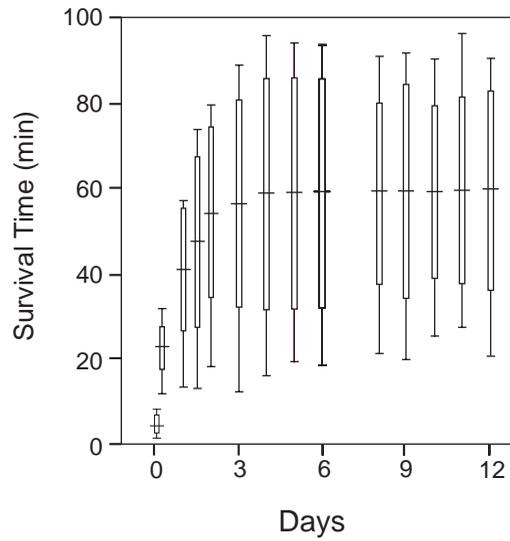
To obtain the Na^+ and K^+ ion concentrations, groups of 10 fish and their replica (80 ± 10 mm L) were acclimatized to five salinities (0, 5, 10, 15 and 17‰) at a constant temperature of 23°C. Each experimental salinity was reached in the way described previously. After a 4 week saline acclimatization, the fish were sacrificed by beheading; heart, muscular, gill and gastric tissue samples were taken, using a scalpel, taking care of always cutting in the same place and approximately the same amount (complete heart, complete gut, 4 thin sheets of gills and segments of white muscle). They were weighed immediately after the dissection, placed in plastic bags and then frozen at -17°C until the ion concentration was determined. The tissues were placed separately (muscle, heart, gill and gut) in a Kjeldahl flask. Nitric acid (3ml, 50%) was added and organic matter destroyed by being boiled for approximately 5-8 min. After this, a 5 ml liquid sample with the intratissular ions was obtained and placed in assay tubes to determinate ionic concentrations later on. The determination of ionic concentration of sodium and potassium was carried out in a direct reading by flame photometer. The samples were diluted in a 1: 10 ratio. Before carrying out photometric readings, the apparatus was calibrated using NaCl and KCl solutions of known concentrations.

The results obtained during the experimentation were subjected to a statistical treatment of a confidence level of 95%, using a variance analysis and an *a posteriori* test using Duncan's simultaneous comparison method in order to determine significant differences between a) the average values of thermal survival and exposure time (days) for each acclimatization exchange rate used and b) the average values of concentrations of each ion and salinities measured in the tissues tested.

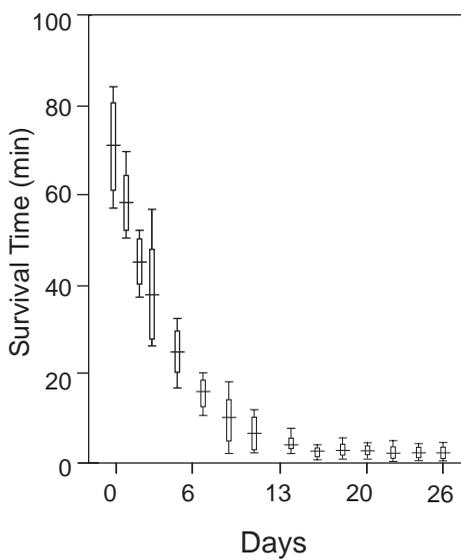
a) 22°C to 32°C



b) 24°C to 30°C



c) 32°C to 22°C



d) 30°C to 24°C

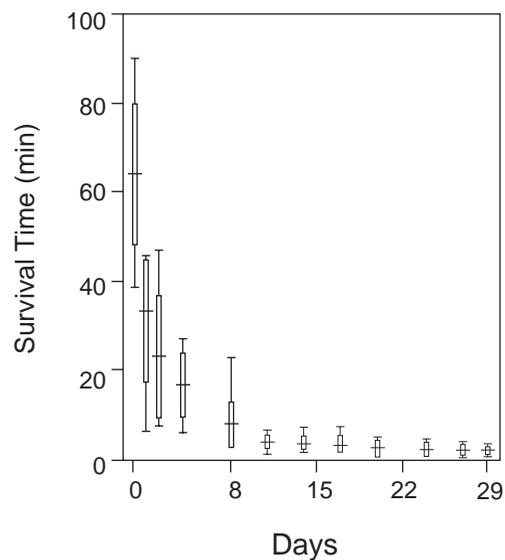


Fig. 1. Thermal resistance of *Caquetaia Krausii* acclimatized during 4 weeks at: a) 22°C, changed to 32°C. b) 24°C, changed to 30°C. c) 32°C, changed to 22°C and d) 30°C, changed to 24°C and exposed to the lethal temperature of 40.9°C.

TABLE 1

Variance analysis of survival time of *Caquetaia kraussii* acclimatized during four weeks to:
 a) 22°C, changed to 32 °C. b) 32°C, changed to 22 °C. c) 24°C, changed to 30 °C. d) 30 °C, changed to 24 °C
 and exposed to lethal temperature of 40.9 °C for each change in the rates of acclimatization

Source	D.F.	S.C.	M.C.	F _s
A: 22 to 32 °C				
Between groups	13	43858.5800	3373.7370	33.6311 ***
Intragroups	70	7022.1100	100.3159	
Total	83	50880.6900		
B: 32 to 22 °C				
Between groups	14	44321.7300	3165.8380	107.8279 ***
Intragroups	75	2202.0080	29.3601	
Total	89	46523.7400		
C: 24 to 30°C				
Between groups	13	37213.9700	2862.6130	5.5264 ***
Intragroups	126	65266.9400	517.9916	
Total	139	102480.9000		
D: 30 to 24°C				
Between groups	11	38531.9400	3562.9030	53.9614 ***
Intragroups	108	7010.8240	64.9150	
Total	119	45542.7600		

TABLE 2

Sodium and potassium ion average values ($\mu\text{Eq/g}$) obtained for *Caquetaia kraussii* tissues acclimatized at 23°C during four weeks at different salinities

Tissue	Ion	SALINITY (‰)						
		0	5	10	15	17	18	20
Muscle	Na ⁺	33.95	35.91	37.01	37.15	36.57	91.44	109.51
	K ⁺	110.59	108.68	102.57	100.00	91.03	64.10	60.05
Gills	Na ⁺	115.27	120.31	120.17	128.86	112.19	188.12	201.79
	K ⁺	68.21	46.62	49.64	54.05	46.66	38.64	10.32
Gut	Na ⁺	97.64	139.14	347.46	303.87	340.30	350.43	392.40
	K ⁺	83.49	112.54	162.94	118.16	100.59	60.94	70.72
Heart	Na ⁺	117.67	244.44	197.58	241.38	243.20	270.15	305.33
	K ⁺	106.87	106.87	164.76	103.82	110.56	70.53	47.82

RESULTS

The results showed a straight and positive relationship between acclimatization temperature and thermal tolerance of *Caquetaia kraussii* (Fig. 1). The variance analysis applied to thermal resistance time during reacclimatization periods at the lethal temperature of 40.9°C showed a highly significant effect (Anova, $P < 0.01$), on the increasing temperatures from 22 to 32°C (df: 13/70; F: 33.63) and from 24 to 30°C (df: 13/126, F: 5.53). Similar results were obtained for the decreasing conditions from 32 to 22°C (df: 14/75; F: 107.83) and from 30 to 24°C (df: 11/108; F: 53.96) for each thermal exchange rate (Table 1). An analysis of Fig. 1 showed that thermal resistance times measured before increasing temperatures from 24 to 30°C and from 22 to 32°C were significantly lower than those measured after temperature increased. On the other hand, for temperatures decreasing from 30 to 24°C and 32 to 22°C, thermal resistance times during 8-9 days after transfer were significantly higher than the remainder.

In Table 2, average values of ionic concentration of sodium and potassium are shown (in $\mu\text{Eq/g}$) for *Caquetaia kraussii*, acclimatized at 23°C and subjected to different environmental salinities during 4 weeks. The variance analysis show that a significant difference does not exist (Anova, $P > 0.05$) for the values related to sodium and potassium ions in muscular and gill tissues, but a significant difference did exist for gut ($F_s = 52.247$ for Na^+ and 15.770 for K^+) and heart tissues ($F_s = 7.771$ for Na^+ and 6.71 for K^+).

DISCUSSION

The acclimatization involves a series of physiological changes within genetically regulated limits of each species (Segnini *et al.* 1993). The natural cycle of temperature produces a thermal resistance higher than constant acclimatization temperature in the laboratory (Ciurcina & Chung 1983). Because of that the

cyclic thermal period allows to the species to maintain a tolerance maximal with a recovery time to thermal stress. In this research it was found that *Caquetaia kraussii* have a wide adaptation capacity to an abrupt temperature increase (Fig. 1a, b): 3 h after being transferred, their thermal resistance time begin to increase and they reach full acclimatization at a new temperature regime from 48 to 72 h, depending on whether the increment is of 6°C or 10°C. These results imply that rapid acclimatization behavior of *Caquetaia kraussii* is due to adaptation mechanisms to temperature increases in their natural environment (La Aguá lagoon).

In the case of acclimatization to lowering temperatures, a fast decrease of thermal resistance is observed in those organisms exposed to a 6°C decrease (Fig. 1c,d). When the acclimatization temperature difference is 10°C, thermal resistance of this species diminishes slowly. This thermal decrease occurs abruptly at first (at 24 h), followed by two smaller decreases at a close interval (48 and 72 h), continuing with another remarkable descent (120 h) and later on, three small decreases (144, 216 and 288 h) until reaching full acclimatization at 336 h (14 days), where the variances are so small that they are not significant. On the other hand, in the 6°C difference decrease, the thermal resistance time occurs in three stages: an abrupt loss in the first 24 h, then a marked descent from 2 to 4 days to conclude in the stability phase from that time onwards. Possibly, *Caquetaia kraussii* have a lesser capacity to diminish their acclimatization temperature.

The results obtained from 6°C difference for *Caquetaia kraussii* acclimatization temperature were similar to those reported by Chung (1985) for another species belonging to the same family, *Oreochromis mossambicus*: they required two days for temperature increase, but acclimatization rate for temperature decrease was not similar. While *Oreochromis mossambicus* needed 14 days, *Caquetaia kraussii* only required 11 days. Segnini *et al.* (1993) reported one day to allow *Mugil curema* to reach their full acclimatization in a temperature increase from 24 to 30°C and from 28

to 34°C and 6 to 8 days in a temperature decrease at the same rates. The mechanism of different acclimatization types to temperature increase and decrease can be correlated with the fish's metabolism, since temperature acts as a controlling factor of the state of molecular activation of the metabolic chain components (Wootton 1990), increasing in this way, the rate at which the chemical transformations take place. Therefore, decrease in water temperature diminishes the fish's metabolism. On the other hand, a temperature increase speeds up its metabolism and, consequently, it is possible to assume that the acclimatization rate can be diminished at lower temperatures. The organisms that live in lagoons and shallow waters can be acclimatized to temperature changes during the day and they do not lose their acclimatization level during the night. These organisms have the advantage of tolerating higher temperatures, and they displace the thermal tolerance zone at high temperature levels (Bettinger & Fitzpatrick 1979, Chung & Strawn 1994).

On the other hand, it is necessary to note that *Caquetaia kraussii* acclimatized at 5, 10, 15 and 17‰ showed a 100% survival rate during the experimentation (40 days). When the salinity was changed at 18‰, the survival rate diminished to 97%, and at salinity 19‰, the survival rate was 95%. When the salinity was 20‰, the survival rate observed was 50% at 24 h and a 100% death toll at 36 h. The opercular movement stayed in a range between 74 and 76 bit/min, until salinity 15‰ and increased to 83-90 bit/min at higher salinities (19-20‰). The previous results show that the lethal incipient salinity for this species is 17‰. This is the limit at which they can be acclimatized, because beyond this concentration, the fish ingest little food. When the salinity was increased to 19‰, the fish stopped eating completely. When the salinity reached 20‰ the fish remained most of the time at the bottom of the aquarium and they were aggressive in defending their territory. If a fish lost a scale through the aggression of another fish, it ascended immediately to the surface of the water and it was almost immobile until it died. They presented a

dark color, characteristic of a stress condition, as well as a slimy consistency. Their weight diminished 50%.

As we can observe, Na⁺ and K⁺ ions can regulate muscular and gill tissues, but they did not do so with gut and heart tissues. *Caquetaia kraussii* regulate the sodium and potassium ions separately but do not do both at the same time, mainly at 10‰ salinity. This seems to be the salinity limit in which this species begins to stimulate all its osmoregulatory system or stress strategy to compensate the salinity. Also, we can observe that *Caquetaia kraussii* abruptly modify their preferential temperature at this salinity. Segnini & Chung (1989) reported these changes (26.7 at 0‰, 25.6 at 5‰, 31.3 at 10‰ and 27.8°C at 15‰) according to environmental salinity. These species modify their final preferendum (28.3 at 30.0°C) when living in a hyperosmotic environment. These results confirm previous findings presented by Finstad *et al.* (1989) who pointed out that the ionic content in fish depends on temperature when the salinity is altered.

The processes of physiological adaptation in the gills to salinity changes are gradual and involve synthesis and destruction of molecular components of the epithelial transport system while monovalent ions (Na⁺ and K⁺) are excreted by the gills (Towle 1981, Langdon & Thorpe 1984).

At 5 and 10, 15 and 17‰ salinities, the physiological mechanisms of regulation to excrete the excess absorbed by the gills are stimulated, indicating that gills are highly permeable to sodium ions in both directions, and that saline balance is achieved by the adjustment of gain or loss of salts. All these mechanisms are very similar to the circulatory and hormonal system. They indicate that to maintain the sodium balance in teleost in a seawater environment, external potassium is necessary. It is thought that in seawater, the potassium ion is exchanged for the sodium ion which is eliminated through the blood into the seawater, and that this effect might be responsible for the maintenance of the sodium ion gradient concentration through the gills. The

exchange suggests that activated adenosintri-fosfatase by sodium and potassium (ATPase carrier) may have a function in the ionic exchange of the gills (Meador & Kelso 1990)

C. kraussii in salinities higher than 17‰ diminished the potassium levels in all the tissues studied, suffering a hypotacemia that brings the organism to death. These results confirm those reported by Robertson (1984), who studied the content of sodium and potassium in *Anguilla anguilla* muscle and found that sodium ion increased in the animals adapted to freshwater, even though the potassium ion diminished. For *Caquetaia kraussii*, potassium ion lowering was so significant that muscular paralysis occurred. The loss of potassium in the cell interior produces hyperpolarization of nervous and muscular tissues that prevent their transmission from the electric potentials to the nervous signals through these tissues.

From the results, it is possible to conclude that *Caquetaia kraussii* is an oligohaline fish that regulates the sodium and potassium ions at gills and muscle until the salinity reached (17‰), which is considered to be the incipient lethal level.

ACKNOWLEDGEMENTS

This work was supported by Research Council, Universidad de Oriente (CI-5-019-00642/94-97). We want to thank to Gordon Hart from University of Oriente for reviewing the manuscript.

RESUMEN

La sardina tropical de río, *Caquetaia kraussii*, capturada en la laguna La Aguá (Estado Sucre, Venezuela) fue aclimatada durante cuatro semanas a la temperatura de 22, 24, 30 y 32°C y a 0, 5, 10, 15 y 17 ‰ de salinidad. Para evaluar los efectos de respuestas térmicas a los niveles de aclimatación, los peces fueron transferidos abruptamente desde las temperaturas bajas (22 y 24°C) hasta las altas (32 y 30°C) respectivamente. Se midió entonces la resistencia térmica a la temperatura letal de 40.9°C durante 30 días. Se consideró que los peces habían alcanzado completamente su aclimatación cuando se estabilizaba al nuevo régimen de

temperatura. Para el efecto salino, las concentraciones de sodio y potasio fueron determinadas en los tejidos: branquial, muscular, gástrico y cardíaco. Los resultados mostraron que la tolerancia térmica aumentaba rápidamente en 3 h cuando la diferencia de temperatura era de 6°C (24 a 30°C) y en 24 h cuando la diferencia era de 10°C (22 a 32°C). En los descensos de temperaturas, el nivel de aclimatación se alcanzó a los 11 días cuando el intercambio se hizo desde 30 a 24°C y en 14 días con un descenso de 10°C (32 a 22°C). Los resultados indican que *Caquetaia kraussii* regula tanto sodio como potasio a nivel muscular y branquial a todos los niveles de salinidad estudiados; sin embargo a nivel gástrico y cardíaco se observaron regulaciones significativamente diferentes entre las salinidades examinadas.

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