

Effects of Temperature and Salinity on the Survival Rates of *Coxicerberus ramosae* (Albuquerque, 1978), an Interstitial Isopod of a Sandy Beach on the Coast of Brazil

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ABSTRACT

The tolerance to the combined effects of temperature and salinity was investigated in the interstitial isopod Coxicerberus ramosae (Albuquerque, 1978), a species of intertidal zone of sandy beaches in Rio de Janeiro, Brazil. The animals were collected on Praia Vermelha Beach. The experiments lasted 24 h and nine salinities and seven temperatures were used for a total of 63 combinations. Thirty animals were tested in each combination. The species showed high survival in most of the combinations. The temperature of 35 °C was lethal and at 5 °C, the animals tolerated only a narrow range of salinities. The statistical analyses showed that the effects of temperature and salinity were significant on the survival, which confirmed the euryhalinity and eurythermy of this species.

Key words: Interstitial, isopod, resistance, temperature, salinity. *Coxicerberus*

INTRODUCTION

Temperature and salinity are considered to be the most important physiological factors influencing marine organisms (Kinne, 1971). The sandy coast between the sea and the continental domain is very unstable with respect to several environmental variables, especially these factors (Coineau, 1985). The distribution of organisms in the interstitial habitat can be better understood through the concomitant study of the effects of specific factors in the laboratory and field. Tolerance and resistance tests can be used to determine the survival capacity of a species in relation to certain factors (Krauss and Found, 1975).

The combined effects of temperature and salinity on the survival of marine animals have been demonstrated in many marine organisms, mainly crustaceans (Todd and Dehnel, 1960; Segal and Burbancki, 1963; Haefner, 1969; Jansen, 1970; Ong and Costlow, 1970; Jones, 1972; Biggs and McDermott, 1973; Sandifer, 1973; Christiansen and Costlow, 1975; Rosenberg and Costlow, 1976; Vlasblom et al., 1977; Young and Hazlett, 1978; Rothlisberg, 1979; Dawirs, 1979; Johnson, 1980; Azis and Greenwood, 1981; Gaudy et al., 1982; Yagi and Ceccaldi, 1983; Roddy et al., 1984; Preston, 1985; Vinuesa et al., 1985; Harms, 1986; Moreira et al., 1982, 1986; Blazskowski and Moreira, 1986; Cadman and Weinstein, 1988;

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Avis, 1988; Menet et al., 1991; Young, 1991; Qiu and Qian, 1997, 1998; Lemaire et al., 2002; Paula et al., 2003; Thiagarajan et al., 2003; Aktas et al., 2004.) The importance of these effects and the need to consider these factors jointly were emphasized by Kinne (1963, 1964 and 1970). Kinne (1970) noted that salinity could modify the effects of temperature and alter the temperature range of many biological processes. In turn, temperature can also modify the effects of salinity. Several experimental studies on interstitial species have been carried out (Gray, 1966 a and b; 1968; Jansson, 1962, 1966, 1967, 1968; Krauss and Found, 1975; Vernberg and Coull, 1975, 1981; Wieser and Schiemer, 1977; Milliou, 1977, 1996; Milliou and Moraitou-Apostolopoulou, 1991). However, similar studies have never been done on interstitial isopods.

Many species of microcerberid isopods live exclusively in the interstitial habitat and most of them inhabit marine and continental waters. The more primitive species probably originated during ocean regressions, dating from the Lower Cretaceous. Many microcrustaceans that presently inhabit continental underground waters had marine ancestors, which initially colonized the interstitial waters of sandy coasts (Boutin and Coineau, 1991).

The genus *Coxicerberus* includes only marine species, whereas the majority of continental species are members of other microcerberidean genera which have more primitive characters (Wägele, Voelz and McArthur, 1995). In Brazil, five species of *Coxicerberus* have been described. *Coxicerberus ramosae* is very abundant on sandy beaches in Rio de Janeiro (Albuquerque, 1978). The aim of this work was to test the resistance of *C. ramosae* to the combined effects of temperature and salinity.

MATERIAL AND METHODS

Praia Vermelha Beach, located in Guanabara Bay in the state of Rio de Janeiro, Brazil, at 22° 57'S and 043° 9'W, is part of the Atlantic tropical area. The beach is exposed and its sand is very homogeneous and coarse. The flow of continental underground water is considerable, causing a salinity gradient in the interstitial water. In this beach, the temperature of the interstitial water

varies from 20 to 30°C, and the salinity from 27 to 36 (Albuquerque and Genofre, 1999).

Isopods were collected in the resurgence zone (Salvat, 1964) by the Karaman-Chappuis method (Coineau, 1971). The animals were taken to the laboratory in pots transported in insulated containers. The tolerance of the species under laboratory conditions was studied through two separate tests. In each test, three replicates with 10 animals each were placed in Petri dishes containing filtered sea water at salinity 35 and 22°C in a BOD chamber in darkness. A total of 60 animals were tested, 30 per test. During the experiments, the animals were not fed. The dishes were initially examined after six hours, and every 24 h thereafter. Animals which did not react to the touch of a needle were considered dead, and the experiments ended when the last individual was dead.

To verify the combined effects of temperature and salinity on the species resistance, the experiments included seven temperatures (5, 10, 15, 20, 25, 30 and 35°C) and nine salinities (5, 10, 20, 30, 35, 40, 50, 60, 70) for a total of 63 combinations. The animals were transferred directly to the experimental conditions according to Dorgelo (1976). Thirty individuals were placed in Petri dishes (10 replicates per group) and they were submitted to temperature and salinity combinations. The experiments finished after 24 h. The animals were not fed during this period. A total of 1890 individuals were tested.

Low-salinity water was obtained by diluting sea water with distilled water. High-salinity water was made by freezing sea water. Salinity was measured with an American Optical/TD salinometer/refractometer. The survival rate was calculated at the end of each experiment.

Two-way analysis of variance (ANOVA) with a 95% confidence interval ($P < 0.01$) was used. All data were tested for normality (Kolmogorov-Smirnov test), and homoscedasticity (Cochran test) to assess the variation in survival of the different temperature and salinity combinations. The Tukey test was used to verify the difference among the treatments (Zar, 1999). The correlation between the survival percentages and the temperature and salinity was estimated by multiple regression analysis. The statistical tests were done using Statistica for Windows v.6.0.

RESULTS

Under the laboratory conditions, 50% of the individuals survived almost 30 days in the first experiment and 24 days in the second (Fig. 1). A

survival rate of 100% for *C. ramosae* was shown in the first 24 h on the two experiments, which indicated excellent tolerance under the laboratory conditions.

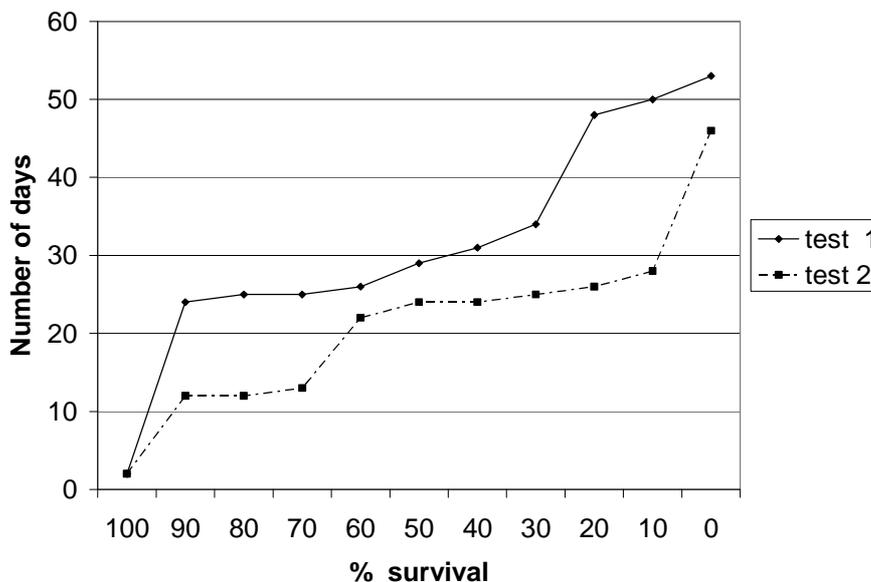


Figure 1 - Tolerance of *Coxicerberus ramosae* to laboratory conditions.

The results of the experiments using 63 temperature and salinity combinations showed high tolerance of this species to most of the combinations tested (Fig. 2 and 3). Survival rate was high for all the temperatures, except at 35°C. This temperature was lethal and almost all the individuals died in the salinities tested. At 10, 15 and 30°C, the survival was more than 50% in the salinities from 10 to 60. At 20°C, the animals only died in the salinity of 5, and at 25°C, they died only at salinity of 70 (Fig. 2).

The survival of *C. ramosae* was high at almost all the salinities and more than 50% survival was observed, except at 5 and 70. The percentage

survival of species was very high at salinity from 30 to 40 in a wide range of temperatures, and the mortality after 24 h was practically null. All the individuals secreted a mucous layer at low temperatures (5 and 10°C) at salinity 5. At 50 and 60 of salinity, the species presented more than 50% of survival from 5 to 30°C. In salinity 70, 50% of survival was observed only at 20°C. After seven hours of experiments, when the animals were submitted to extreme salinities (5 and 70), they became incapable of moving around and only moved their appendages when stimulated by a needle. At 5 of salinity, the species only showed higher resistance at 25°C (Fig. 3).

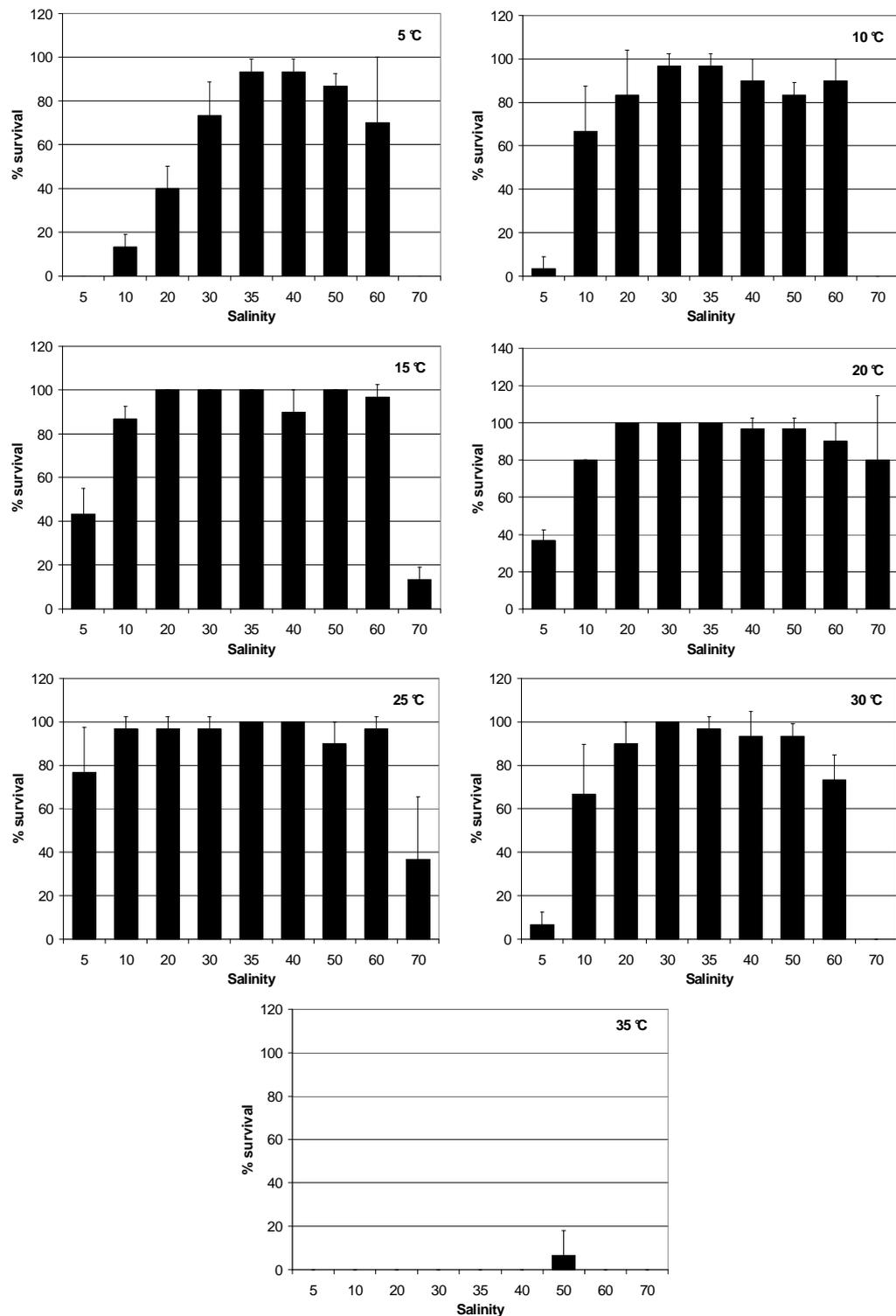


Figure 2 - Mean survival of *Coxicerberus ramosae* at different temperatures.

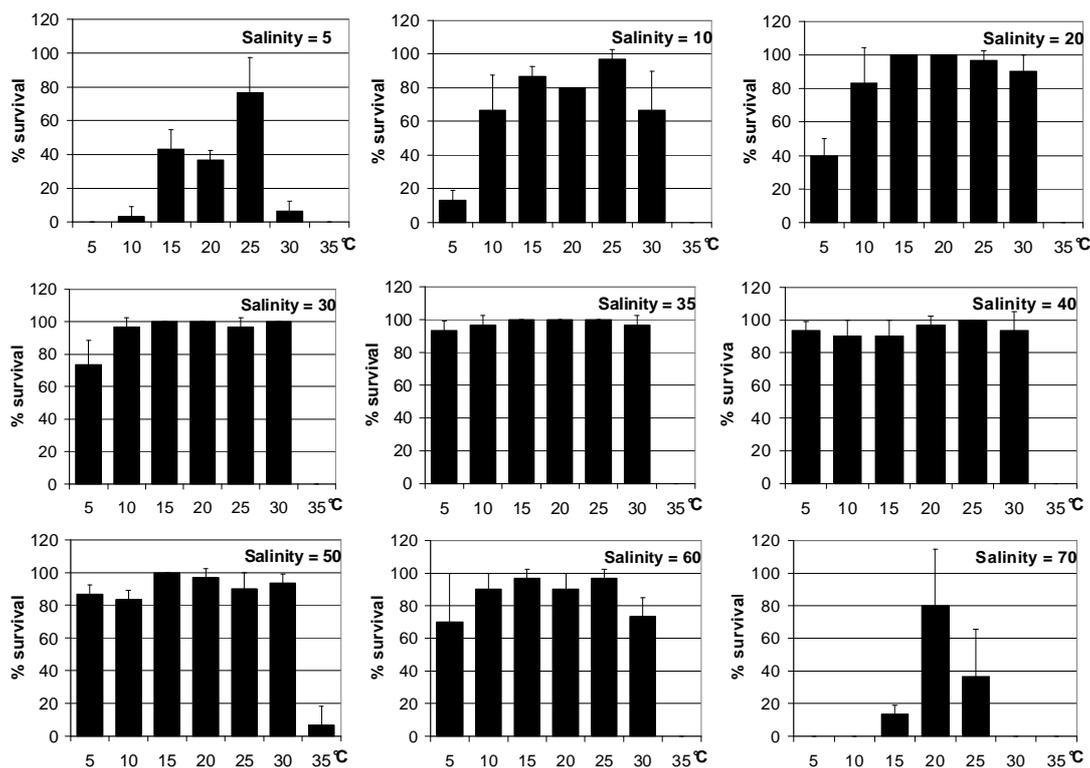


Figure 3 - Mean survival of *Coxicerberus ramosae* at different salinities.

The analysis of variance of the survival data showed that temperature, salinity and interaction effects were highly significant (Table 1). The Tukey test showed that extreme treatments of

temperature (5° and 35°C) and salinity (5, 10 and 70) were responsible for the higher differences (Table 2).

Table 1 - ANOVA comparing the effects of temperature and salinity on *Coxicerberus ramosae* survival. T= temperature; S=salinity; TxS= combined effect of temperature and salinity; SS=sum of squares; MS= mean squares.

	SS	d.l.	MS	F	p
Intercept	748000.5	1	748000.5	6015.834	<0.001
T	145818.0	6	24303.0	195.458	<0.001
S	105689.9	8	13211.2	106.252	<0.001
TxS	45124.9	48	940.1	7.561	<0.001
Error	15666.7	126	124.3		

Table 2 - Tukey test applied to the different temperature and salinity treatments (95% confidence interval).S=Salinity; T=Temperature.

S	post- test	T°C	Post-test
5	≠ 10;20;30;35;40;50;60	5	≠ 10;15;20;25;30;35
10	≠ 5;20;30;35;40;50;60;70	10	≠ 5;20;25;35
20	≠ 5;10;35;70	15	≠ 5;20;30;35
30	≠ 5;10;70	20	≠ 5;10;15;30;35
35	≠ 5;10;20;70	25	≠ 5;10;15;30;35
40	≠ 5;10;70	30	≠ 5;20;25;35
50	≠ 5;10;70	35	≠ 5;10;15;20;25;30
60	≠ 5;10;70		
70	≠ 10;20;30;35;40;50;60		

The different combinations of temperature and salinity showed significant but slight positive correlation ($R^2=0.06192137$) with the survival percentage (Table 3). In the partial results of

multiple regressions, only temperature was statistically significant ($P=0.001$), showing a negative correlation with the percentage survival.

Table 3 - Multiple regression analysis of the combined effects of salinity and temperature on *Coxicerberus ramosae* (95% confidence interval). T= Temperature; S= Salinity

	Beta	Std. Err.	B	Std. Err.	t(186)	p-level
Intercept			81.09441	8.133581	9.97032	0.000000
T	-0.247307	0.071017	-1.00529	0.288681	-3.48236	0.000619
S	0.027576	0.071017	0.05404	0.139174	0.38830	0.698239

$R^2 = 0.06192137$

F (2.186) = 6.1388 $p < 0.00262$

DISCUSSION

The results indicated a high resistance of *C. ramosae* between salinities 10 to 60 and temperatures 10 to 30 °C, respectively. This indicated a high degree of euryhalinity and eurythermy for this species, which is common among coastal interstitial animals. Similar results were obtained by Jansen (1970), who observed that the isopod *Sphaeroma hookeri* was well-adapted to survive in a wide range of salinities, but that its survival was reduced at high and low temperatures. The same results were found with the isopod *Jaera albifrons* (Sjöberg, 1967). *Sphaeroma hookeri*, a common species in estuaries, easily tolerated low salinities (Jansen, 1970). For *C. ramosae*, the survival rate was high in very high salinities, probably because this interstitial isopod lives in the intertidal zone.

C. ramosae tolerated high temperature (30°C) better than low (5°C) and also tolerated better salinity variation in high temperatures. This behavior is common among tropical species (Vernberg and Vernberg, 1972; Moreira et al., 1982).

The production of mucus seems to be a response to stressful conditions. Interstitial protozoan secrete mucus, and remain immobile when they are submitted to stressful salinities (Jansson, 1968). All the individuals of *C. ramosae* secreted a mucous layer at the salinity 5 in low temperatures (5 and 10°C). This occurred by the abrupt transfer of the individuals to stressful salinities in temperatures very different from that in which they lived in nature. This mechanism reduced the tegument permeability of animal, therefore reducing its water absorption (Kinne, 1964).

The decrease of the organism's activity in stressful salinities was observed by Jansson (1966) in the Mystacocarida *Derocheilocaris remanei* when submitted to salinity of 60. *C. ramosae* showed same behavior at salinity of 70.

Certain species better tolerate low salinities in low temperatures and high salinities in high temperatures. Other species better tolerate the low/high combination (Kinne, 1971). In the present study, low and high salinities were better tolerated at high temperatures. Tropical animals seem usually to survive better at low salinities in combination with high temperatures (Vernberg and Vernberg, 1970).

Krauss and Found (1975) observed that *Derocheilocaris remanei* (Mystacocarida) showed the lowest survival at high temperature x low salinity combination. On the contrary, *C. ramosae* showed the lowest survival in the combination of low temperature x low salinity. Present results indicated a tolerance limit between the salinities of 60 and 70, because at 70 after 3 h, 80% of animals were dead, whereas at 60 at the same temperature, all of them were alive after 24 h.

The statistical analysis indicated that the factor which determined the largest mortality was temperature, probably because the animals were exposed to extreme temperatures (5 and 35°C). Hummon (1975) found similar results with interstitial Gastrotricha, where temperature was the main factor for the mortality observed. It is known that the range of ecological temperature of an animal is more restricted than the experimental ones. The best survival conditions for *C. ramosae* were characterized by the combination 15 and 20°C from 20 to 35 of salinity.

Results showed that the species is well adapted to wide variations of salinity and temperature. *C.*

ramosae is, therefore, an euryhaline and eurythermic species, and temperature and salinity were not probably responsible for its spatial and temporal distribution on the Praia Vermelha Beach.

These results seemed to confirm the hypothesis of the marine origin of continental of Microcerberids through the interstitial habitat (Coineau and Boutin, 1992), because these physiological characteristics facilitated the entry of these species into the continental underground waters during several geological periods.

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RESUMO

A resistência aos efeitos combinados de temperatura e salinidade foi investigada no isópode intersticial *Coxicerberus ramosae* (Albuquerque, 1978) encontrado comumente na zona intertidal de praias arenosas do Rio de Janeiro, Brasil. Os exemplares foram coletados na Praia Vermelha. Os experimentos tiveram a duração de 24 horas e nove salinidades e sete temperaturas foram utilizadas, perfazendo um total de 63 combinações. Em cada combinação de T e S foram testados 30 animais. A espécie mostrou um grande percentual de sobrevivência na maior parte das combinações. A temperatura de 35^oC foi letal e na temperatura de 5^oC a espécie resistiu a uma faixa estreita de salinidades. As análises estatísticas mostraram que tanto os efeitos da temperatura como da salinidade e da interação entre estes fatores foram significativos na sobrevivência da espécie. O alto percentual de sobrevivência da espécie nas diferentes combinações de temperatura e salinidade, confirma, portanto, a eurialinidade e a euritermia da espécie, características comuns das espécies intersticiais litorais.

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