INFLUENCE OF DESICCATION TOLERANCE ON THE ECOLOGY OF *Balanus amphitrite* DARWIN, 1854 (CRUSTACEA: CIRRIPEDIA).

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ABSTRACT

Desiccation tolerance was studied in the intertidal barnacle *Balanus amphitrite* Darwin, 1854, in order to characterize its response to air exposure in relation to vertical distribution by means of laboratory and field experiments. After 48 hours of exposure to 0% relative humidity only 20% of the animals died, the remaining died between this time and 120 hours. While at 75% relative humidity, survival was 60% after 120 hours. The fact that mortality at both humidity conditions was greatly increased after 48 hours of exposure, could be related to the tolerance limit for *B. amphitrite* under these experimental conditions.

However, in field experiments adults of this species can withstand air exposure for very long periods in a shore level at least 0.5 m above the vertical limit of distribution, thus suggesting that desiccation is not the only factor determining such distribution limit.

Keywords: Desiccation tolerance, intertidal, adaptation, barnacle, Cirripedia

INTRODUCTION

Intertidal animals must face two major kind of chalenges derived from the periodic exposure to water and air: the maintenance of metabolic activity of the tissues in two different respiratory conditions and desiccation due to evaporation from permeable surfaces, especially respiratory ones (Burnett, 1988).

Among invertebrates two possible strategies can be recognized in order to solve the compromise between maintaining some degree of metabolic activity and avoid excessive loss of water. Mobile intertidal animals like some brachyuran crabs maintain an important rate of aerobic metabolism during emersion, restoring lost water by different strategies (Felgenhauer & Abele, 1983; Wolcott, 1976). For sessile organisms, as some bivalve mollusks, the reported strategy for avoiding desiccation stress is to isolate themselves by closing the valves of the shell during exposition; this isolation from the source of oxygen implies the switching to anaerobic metabolism and the concomitant accumulation of toxic end products (Burnett,1988). However, most intertidal bivalves are able to maintain some degree of aerobic metabolism by periodical openning of the shell, that allows air ventilation; this behavior is known as shell gaping (Lent, 1968; Truchot, 1990).

In spite of their sessile habits, barnacles have been cited as displaying a respiratory strategy similar to that of active intertidal animals. Barnes & Barnes (1957) have suggested that these animals continue interacting with the aerial environment by expelling the water contained within the mantle and adjusting the operculum plates to allow air access to the tissues.

Since barnacles are sessile animals, the duration of aerial exposure depends entirely upon their vertical position and tide amplitude. The evaporative water loss during such periods is also influenced by temperature and relative humidity. Vertical zonation, occupation of crevices in upper levels and the apparent poorness of barnacle species on tropical coasts are indirect evidence of the effect of desiccation on the ecology of this group (Foster, 1971, 1987; Connell, 1974). Desiccation tolerance together with respiratory strategy during emersion are two important physiological factors to be taken into account in studies of vertical distribution of these species.

Balanus amphitrite Darwin, 1854 is a cosmopolitan intertidal barnacle widely distributed in temperate and subtropical coasts, being a conspicuous member of the intertidal community of Quequén, Buenos Aires Province, Argentina. López Gappa et al. (1993) reported that desiccation is possibly the major stressing factor in the mentioned community.

The aim of this work is to study the desiccation tolerance of adult *B. amphitrite* and its importance on vertical distribution of this species, by means of field and laboratory experiments.

MATERIALS AND METHODS

Laboratory experiment:

Animals were collected from Punta Carballido, 5 km from Quequén, Buenos Aires Province, Argentina (38°34'S, 58°38'W). Animals were maintained in the laboratory in a glass aquarium with artificial sea water (24 ‰) prepared with H.W. Marinemix salts added to dechlorinated tap water. Water temperature was maintained at 20± 1°C with a photoperiod of 12 L : 12 D. The environmental parameters were representative of the site and season of sampling (López Gappa et al., 1990).

Seventy individuals previously blotted dry with absorbent paper, were weighed at a precision of 0.1mg (W_0) and randomly sorted to each one of 14 desiccators. Desiccators consisted of 125 ml glass recipients, containing a desiccating substance at the bottom and a plastic mesh screen separating the animals from the substance. Two relative humidity conditions were tested, 0% R.H. (silicagel at the bottom) and 75% R.H. (saturated NaCl solution).

All the animals of one desiccator (n = 5) were weighed at 1, 3, 6, 12, 24, 48, or 120 hours from the beginning of the experiment. The survival of each animal was tested by reimersing it in the acclimation aquarium and searching for cirral activity during 30 minutes. After testing survival, animals were dried in an oven at 70°C until constant weight (dry weight). Data corresponding to 120 hours and 0 % R.H. were eliminated from the analysis because all individuals died in this treatment.

Total water content was defined as the difference between initial weight (W_0) and dry weight (W_d). Water loss at each time interval was calculated as the difference between W_0 and the weight of the same animal after being removed from the desiccator (W_t); the ratio between this value and total water content was regarded as proportional water loss (Jones & Simmons, 1982).

Mean proportional water loss obtained from five individuals at each different humidity condition and time of exposure was compared by means of two way ANOVA, followed by GT2 multiple comparisons (Sokal & Rholf, 1981). Regression lines were fitted for each relative humidity versus time, the obtained slopes were regarded as the instantaneous water loss rates and compared through t-test for equality of slopes.

Field experiment:

The experiment consisted on the transference of animals from their original position at 0.8 m above mean lower low water spring tides (MLLWST) to 1.65 m above MLLWST. Animals settled at the original level are covered by water about 20% of the time throughout the year, and at the experimental level animals are submerged only by spring tides and extraordinary tides caused by south east wind storms. Twenty individuals of *Balanus amphitrite* were removed from their original location and transferred to a higher level, original density and distribution were maintained. After testing survival as described for laboratory assay, each animal was attached with epoxy cement to the head of a screw, previously fixed to the rock. Twenty five animals were removed and handled as the experimental ones, but re-attached to their original location, these individuals were considered as control ones.

Survival was recorded during two years by the end of every season. Survival time was compared between control and experimental group by means of Mann Whitney U test (Sokal Rohlf, 1981).

RESULTS

Laboratory experiment

Proportional water loss was significantly different among times of exposure (p) and also between relative humidity conditions ($p< 0.05$). For both humidity conditions, water loss at 48 hours was significantly different ($p< 0.001$) from any other time interval (Table I).

Regression analysis was significant for both humidity conditions ($p< 0.01$), being rejected the hypothesis for equality between slopes ($p< 0.001$). The calculated slopes can be considered as the instantaneous rate of water loss for each humidity. Regresion lines, equations and R^2 values are shown in Figure 1.

Table I: Percentage water loss standard deviation and percentage survival for *Balanus amphitrite* exposed to air at two relative humidity conditions. Vertical lines indicate not significant differences between periods of exposure as result of multiple comparisons of percentage water loss by GT2 method.

	0% Relative Humidity		75% Relative Humidity	
Time of Exposure	Percentage water loss	Percentage Survival	Percentage water loss	Percentage survival
1 hour	10.9 ± 2.9	100	2.7 ± 1.1	100
3 hours	14.2 ± 6.1	100	6.6 ± 3.6	100
6 hours	16.5 ± 2.6	100	6.1 ± 1.3	100
12 hours	18.7 ± 3.5	100	10.0 ± 2.1	100
24 hours	28.6 ± 6.6	100	11.3 ± 1.4	100
48 hours	38.3 ± 7.8	80	24.6 ± 6.1	100
120 hours	______	0	46.3 ± 13.1	60

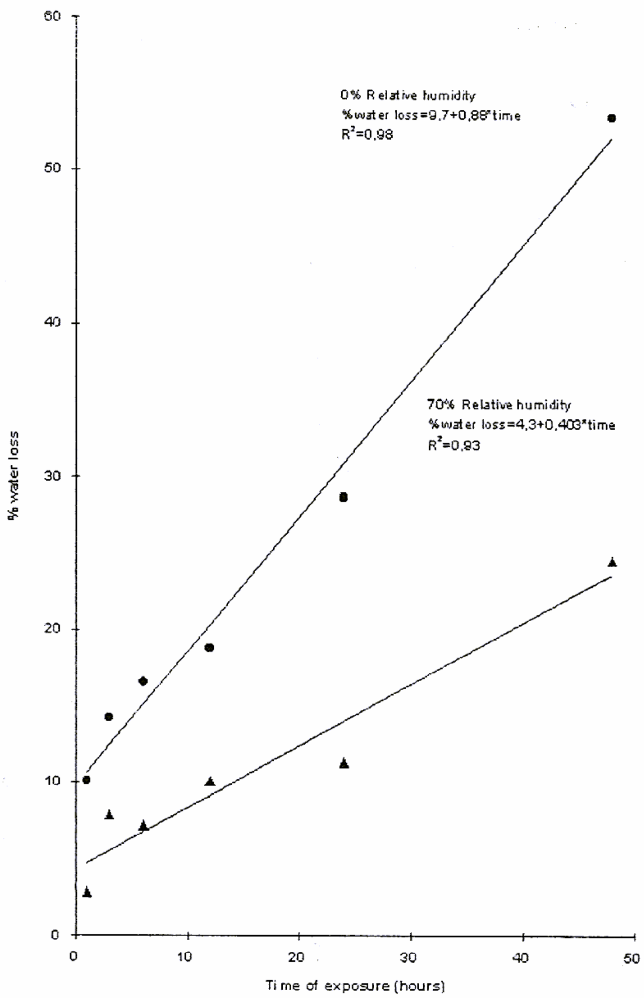

Figure 1: Linear regression of percentage water loss during air exposure of *Balanus amphitrite* at two different humidity conditions.

Field experiment:

Table II shows percentage mortality at different times for both control and transferred barnacles. Survival times for control and experimental groups were not significantly different. (Mann Whitney statistic U = 253.5; P = 0.467).

Table II. Percentage mortality for *Balanus amphitrite* transferred from 0.8 m (control) above mean lower low water spring tides (MLLWST) to 1.65 m (experimental group) above MLLWST.

	% Mortality	
Time (days)	Control	Experimental Group
98	28	25
169	36	45
297	76	70
383	80	90
465	88	90
524	92	90
636	100	95
749		100

DISCUSSION

Desiccation has been reported as a very important factor determining the upper limit of distribution in barnacles (Connell, 1974; Foster, 1971, 1987). The success of all species in colonizing intertidal habitats is directly related to their tolerance to desiccation and/or its capacity to avoid water loss.

In the rocky shore of Quequén, predation and competition for space are not important factors influencing the distribution of *B. amphitrite* because population density is very low and typical predators (e.g. carnivorous gastropods as *Thais* and *Nucella*) are absent (personal observation). On the other hand, the stressing effect of desiccation is often enhanced by the occurrence of very low tides during the high temperature hours at later spring (López Gappa et al., 1993).

Both time of exposure and relative humidity have significant effects on water loss of *B. amphitrite*. An initial, abrupt loss of water was observed after the first hour in 0% RH exposed animals and after the third hour in 75% RH ones. This result along with the observation that in 75% RH barnacles were wet after the first hour in spite of being blotted dry at the beginning of the experiment, indicates that these animals expel the water contained within the mantle cavity when air-exposed. Such respiratory strategy agrees with that reported by Barnes & Barnes (1957), and probably allows this species to maintain a moderate degree of aerobic metabolism during emersion, thus avoiding the accumulation of toxic end products of anaerobic metabolism. Moreover, the fact that *B. amphitrite* can tolerate a water loss above 40%, considered a high desiccation tolerance among crustaceans (Jones & Greenwood, 1982), also indicates the capability of this species to maintain ventilation in air.

Truchot (1990) pointed out that the degree at which conditions of emersion affect desiccation rate of a given species is a factor determining its reliance on air breathing. However, *B. amphitrite* shows an encreased water loss rate at the most desiccating medium tested, thus suggesting that this species relies in air ventilation even when its desiccation rate is significantly affected by the exposure conditions. This species does not seem to posses a regulatory mechanism for reducing the rate of O_2 uptake and consequently desiccation in the acute conditions of this laboratory experiment.

Field experiments suggest that adults of this species can withstand air exposure for very long periods in a shore level at least 0.5 m above the natural limit for vertical distribution. Thus desiccation of adult animals appears not to be the only factor determining the upper limit of the distribution of *B. amphitrite* in the study area.

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