Effects of salinity on *ZOANTHUS SOCIATUS* (Cnidaria: Anthozoa): Is low salinity a limiting factor?

Efeitos osmóticos em *Zoanthus sociatus* (Cnidaria:Anthozoa): A baixa salinidade é um fator limitante?

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ABSTRACT
Abiotic factors, such as variations on salinity, exert influence on the animal distribution in the intertidal zone, including zoanthids. This study evaluated the osmotic, morphological and ethological effects of salinity variations on tropical zoanthid *Zoanthus sociatus*. In order to analyze the hypothesis of osmotic conformation, the zoanthid was submitted to salinity stress. To estimate the osmotic capabilities of the species studied, specimens collected in beach rocks were taken alive to the laboratory and maintained in water collected from the site. The osmoregulatory ability of *Z. sociatus* was determined by measuring the hemolymph osmolality under various salinity conditions and comparing it to the medium osmolality. Zoanthid *Z. sociatus* is able to present osmotic conformation in hemolymph salinity in a wide range of external salinity values. The bleaching frequency was high in low salinities and the mortality rate was high after two days of experiment. This experiment shows for the first time the importance of osmotic conformation in a tropical zoanthid and discusses the role of low salinity as a limiting factor for survival and distribution of these important animals in tropical coastal reefs.
Key-words: Salinity, zoanthids, intertidal zone, bleaching.

RESUMO
Fatores abióticos, como variações na salinidade, exercem influência na distribuição dos animais na zona intermaré, incluindo os zoantídeos. Este estudo avaliou os efeitos osmóticos, morfológicos e etológicos das variações de salinidade no zoantídeo tropical *Zoanthus sociatus*. Para analisar a hipótese de osmoconformação, o zoantídeo foi submetido ao estresse salino. Para estimar as capacidades osmóticas da espécie estudada, espécimes foram coletados em recifes de arenito e levados vivos para o laboratório na água em que foram coletados. A capacidade osmorregulatória de *Z. sociatus* foi determinada mensurando a osmolalidade da hemolinfa em várias condições de salinidade e comparando com a osmolalidade do meio. O zoantídeo *Z. sociatus* é capaz de osmoconformação da salinidade da hemolinfa dentro de uma grande variação de valores de salinidade externa. A frequência de branqueamento foi alta em baixas salinidades e a taxa de mortalidade é alta após 2 dias de experimento. Este experimento demonstra pela primeira vez a importância da osmoconformação no zoantídeo tropical e discute o papel da baixa salinidade como um fator limitante para a sobrevivência e distribuição desses animais importantes nos recifes costeiros tropicais.
Palavras-chaves: Salinidade, zoantideos, zona intermaré, branqueamento.

I. Introduction

The study of osmoregulation has led to a multitude of analyses at various levels of biological organization (i.e. organisms, cells and molecules), especially in coastal marine invertebrates, which, for being often exposed to salinity fluctuations in their natural habitat, constitute good model systems for studying the effects of osmolarity changes. For example, osmotic regulation was studied in poriferans, mollusks, crustaceans and cnidarians (RAND et al*.,* 2000).

Under the extreme conditions existing in intertidal ecosystems, slight differences in osmoregulatory capabilities among species may have large influence on the intertidal community structure. Salinity is often an important environmental factor that affects the horizontal distribution of marine invertebrates living in coastal areas (JAHN et al. 2006).

The nature of substratum is extremely important to these animals and is one of the major controllers of their distribution; however, abiotic factors such as variations on salinity also influence the distribution scales of the intertidal zone (COSTA JR. et al., 2002). Thus, a relationship is observed between the degree of tolerance to physicochemical factors of the environment and the distribution of most intertidal species. The community structure and the distribution of the organisms on rocky substrata are dependent on abiotic factors and inter and intraspecific relationships that act as controllers of the local equilibrium (TKACHENKO; ZHIRMUNSKY, 2002).

Osmoregulatory mechanisms are employed to counteract osmotic stresses and to maintain differences between the extracellular medium and the external environment, providing an adequate osmotic environment to the cells. Changes in the extracellular concentrations determine the gain or loss of cell water, which is reflected as a change in weight. Early studies considered that the volume regulatory mechanisms involved just fluxes of inorganic ions. The participation of organic solutes was only later demonstrated (AMADO et al., 2006).

Cnidaria are of high ecological significance in reef environments, since they develop associations with an array of other invertebrates and, besides that, consolidated substratum environments provide improved stability and protection to a great diversity of species. Zoanthidea corresponds to an abundant group in tropical regions, particularly on beach rocks. Cnidaria that belong to this taxon are widely spread and usually abundant in sea communities of tropical environments and, despite their relevance, the knowledge about them is still scarce (COLLINS, 2002; BOSCOLO; SILVEIRA, 2005).

So far, no information has been published on the salinity tolerance and optimal salinity levels for the zoanthid *Zoanthus sociatus*, although their natural distribution appears to be dependent on salinity (RABELO et al. 2007). This study investigated the osmoregulatory capability of tropical zoanthid *Zoanthus sociatus* (Cnidaria, Zoanthidea), which is very common in Tropical South Atlantic and Caribbean coast. Thus, the questions considered in this study were: Does the salinity variation of the medium affect the osmolarity of body fluids of this zoanthid? Is the zoanthid considered an osmoregulatory animal? Do morphological and/or ethological modifications occur with salinity variations?

II. Material and Methods

Animals

The colonies of *Zoanthus sociatus* Ellis, 1767 display a greenish color, due to the presence of photosynthesizing microalgae. Colonies of *Z. sociatus* may form dense mats on intertidal reef zones, also abundant in the subtidal zone. Polyps are expanded when submerged and are highly resistant to environmental disturbances, showing a well developed regenerative capability (BABCOCK; RYLAND, 1990; RABELO et al. 2007).

In order to estimate the osmoregulatory capabilities of the species studied, specimens collected in beach rocks were taken alive to the laboratory in water from which they were collected. Specimens of *Zoanthus sociatus* were collected from Paracuru beach in the State of Ceará, Northeastern Brazil. Paracuru beach is in the west region of the State of Ceará, 90 km from the capital, Fortaleza (03°23'53.0" S, 39°00'38.8" W), comprehending an extensive belt of beach rocks with highly conspicuous tide pools. These formations are constituted of beach rocks that occur mostly in the intertidal zones. Due to the geographical location, Ceará shore is characterized by environmental stability, given the steady temperature and salinity, with no considerable seasonal changes, like in many tropical reefs of the world. For these experiments, individuals were collected between April and June 2008.

Experiments

In the laboratory, the animals were kept in an aerated aquarium at the salinity of the water from which they had been collected (35) and at ± 26°C laboratory controlled temperature (the annual average temperature at the reef, under natural light and dark conditions), with daily provision of natural food. The day before hemolymph extraction, the specimens were not fed. The control group was treated with the same salinity of the natural environment (35) and without food during the experiment. The water was not changed during the experiment to enable a fast implementation (48 h).

The osmoregulatory ability of aquatic animals can be determined by measuring the hemolymph osmolality under various conditions of salinity and comparing the values to the medium osmolality (ROMANO; ZENG, 2006). After a 24-hour acclimation period, the animals (10 in each treatment) were transferred from the original salinity to salinities of 0, 10, 20, 30, 40 and 50. Seawater, diluted with distilled water, was used in experiments at different salinities. The osmotic concentration of hemolymph was determined after the exposure. A single hemolymph sample was extracted from the gastrovascular cavity of each zoanthid, by puncturing with a 50µl syringe. Hemolymph salinity and water osmolalities were measured with a refractometer (ATAGON – 1 PPM 0-50%).

Statistical analysis

Data are presented as mean ± standard deviation. Data normalization was performed with the Kolmogorov-Smirnov test. For the salinity range tested in the osmoregulation experiments (0-50), the relationship between hemolymph and water osmolalities was examined using the linear regression analysis. The relationship between the two variables may be a functional dependence (Zar, 1984), that is, the dependent variable is the hemolymph salinity and the independent variable is water salinity of the medium.

The dependence of Y (hemolymph salinity) on X (water salinity) found in the sample (i.e., b # 0) does not necessarily mean that the population presents any dependence (β # 0). The preceding Ho (β=0) may be tested using the analysis of variance (ANOVA). The statistical analysis was performed using GraphPad Instat Statistical Package Version 3.01 and GraphPad Prism 4.0.

III. Results

Figures 1 and 2 show data of corporal salinity of zoanthid *Z. sociatus* before the experiments. For cnidarian *Z. sociatus*, data were obtained from the results after the Gauss curve, according to the normality test (KS=0.2856, P=0.0537). Mean values for the species did not differ significantly from the values for the medium. The data analysis supports the hypothesis that *Z. sociatus* may be isosmotic in relation to the medium, whose mean salinity is 38.

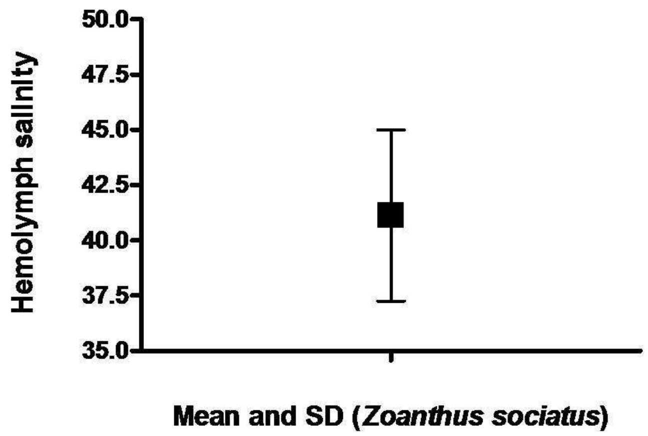

Figure 1 - Mean and standard deviation of hemolymph salinity in *Zoanthus sociatus* before the experiment.

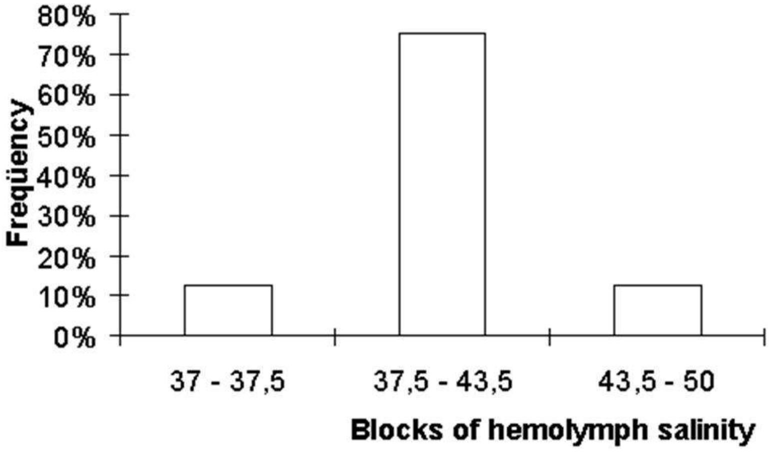

Figure 2 - Histogram of hemolymph salinity in *Zoanthus sociatus* before the experiment.

Z. sociatus did not show morphological or ethological modifications regarding the medium salinity. For *Z. sociatus*, the salinity variation (0 – 50) did not show any influence on mouth opening; instead, it maintained the mouth closed. Neither swelling nor wilting was observed in zoanthids submitted to different salinities.

The study observed that, for *Z. sociatus*, the probable osmotic stress caused a bleaching reaction. Bleaching was observed in lower salinities (Figure 3), but not in high salinities (40 and 50) and in the control group.

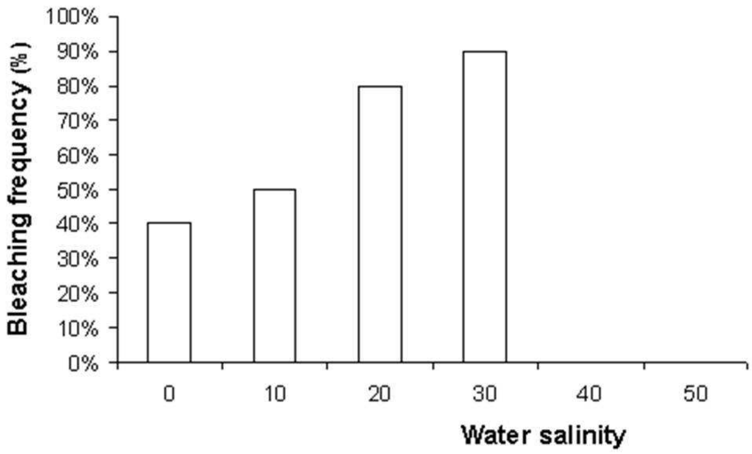

Figure 3 - Bleaching frequency of *Zoanthus sociatus* in experimental water salinity treatments (n=20 in each treatment).

The osmotic behavior in relation to the salinity variations (Figure 4) suggests that *Z. sociatus* is an osmoconformer in relation to the medium, i.e., it acts according to the environmental variation, keeping a similar osmolarity, which explains the absence of swelling and wilting. The regression analysis (Figure 5) was significantly different from zero (F test, P=0.0003) and highlighted the strong statistical correlation (r^2= 0.98) between the dependent (corporal salinity) and independent (medium salinity) variables.

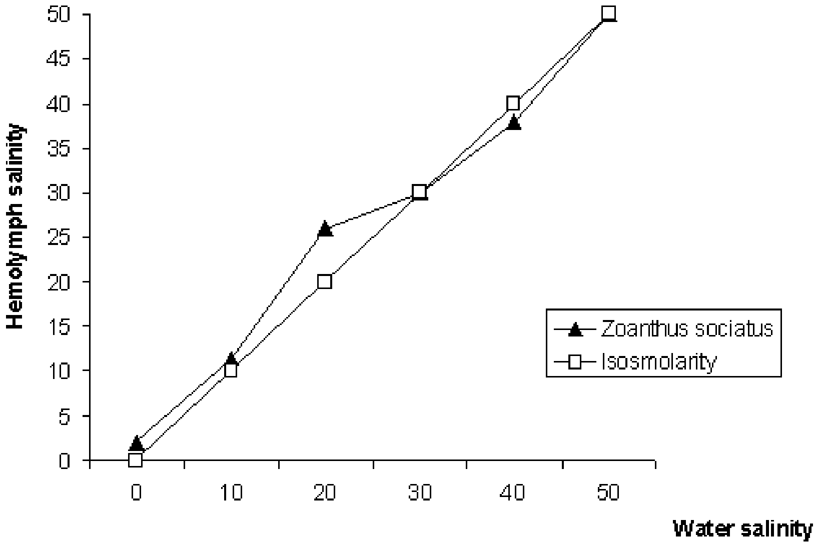

Figure 4 - Osmotic conformation of body fluids in *Zoanthus sociatus.*

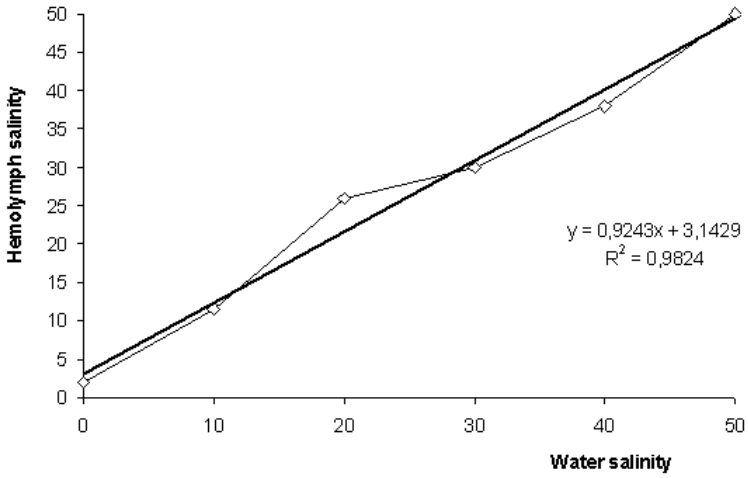

Figure 5 - Linear regression of hemolymph salinity of *Zoanthus sociatus* and water salinity.

After six hours of experiment with different salinities, no mortality was observed in the studied species. However, after 48 hours of experiment, the death rate of 100% was obtained in all treatments, except in control, which had no mortality.

IV. Discussion

Cnidarians maintain a condition of isoosmolarity in relation to the medium. The survival in low salinities depends on the ability to survive the body fluid dilution. It is generally assumed that the distribution and survival of cnidarians are affected by a number of physical factors, such as temperature and salinity. However, surprisingly little knowledge is available about survival limits in the different stages of the life cycle of cnidarians. Estenohaline characteristics can lead to fast death within a few days under conditions of osmotic stress (ARAI, 1997).

Environmental factors, such as drought, salinity and temperature extremes, have limited the survival of species like zoanthids. Organisms that live in habitats where these factors are a major issue have developed a few adaptations to survive in these environments. They accumulate organic solutes, such as polyhydric alcohols, free amino acids and quaternary ammonium and/or tertiary sulphonium compounds, in response to osmotic stress. The accumulation of these solutes in response to osmotic stress is a metabolic adaptation found in stress-tolerant invertebrates and vertebrates, suggesting convergent evolution for this trait (TKACHENKO; ZHIRMUNSKY, 2002; JAHN et al., 2006; RABELO et al. 2007).

Reimer (1971b), in a study conducted on *Zoanthus solanderi*, demonstrates that salinity variations promote bleaching. Bleaching consists in a rupture of the symbiotic relationship between the zoanthids and the photosizing dinoflagellate, which provokes death or egress of the zoanthid tissues of the microalgae. Leão et al. (2008) addresses the bleaching events that occur in reefs around the world and verify that, during this phenomenon, corals and zoanthids become more susceptible to diseases, infections and parasites; thus, the reef environment as a whole becomes fragile and vulnerable. One of the main reasons for that is the synergy between low salinity and high temperatures in reef environments (BEGER et al., 2003; ALUTOIN et al., 2005; KIKUCHI et al., 2010).

The survival of zoanthids in low salinities is directly related to the capability of diluting their body fluids, as suggested by ARAI (1997) for cnidarians. It shows that, in invertebrates, just as in vertebrates and microorganisms, to overcome a hypoosmotic shock that makes the water input lead to osmolysis, the cell will release organic osmolytes, especially free amino acids and their derivatives through its membrane (BROWN, 1976). MUTHIGA and SZMANT (1987) remark that osmoconformatory and osmoregulatory strategies are common in zoanthids.

Reimer (1971a) demonstrates that mouth opening in *Zoanthus* is related to peptides. The mouth closure might be related to the absence of food, as it happens with anemones (HICKMAN et al., 2004). Mouth opening in *Z. sociatus*, however, may also show relations to food availability, as suggested by Reimer (1971a). Muthiga and Szmant (1987) remark that zoanthids may close their mouths to reduce osmotic stress. Because *Z. sociatus* is an osmoconformer, ethological modifications, like mouth opening, do not have a great influence in osmotic regulation. Thus, the mouth closure shown by *Z. sociatus* during this experiment is probably due to the lack of food.

Muthiga and Szmant (1987) report that mortality usually varies with the experiment duration, with higher rates occurring within two to five days. Most organisms have, at least, a limited capacity to respond to an osmotic or ionic challenge by rapidly changing existing transport mechanisms. Acclimation responses increase the overall capacity of an organism to perform a physiological function. The acclimation response is similar or identical to phenotypic plasticity; its presence or absence will often determine the ability of an animal to live in certain habitats and, thus, determine the ecological limits of the species distribution (MCCORMICK; BRADSHAW, 2006).

Low salinities may possibly act as a limiting factor in the distribution and survival of *Z. sociatus*, mainly in coastal areas that are subject to freshwater exposure, such as estuaries. Estuarine environments are probably the most stressful aquatic biotopes, where abiotic variables, such as salinity, can abruptly change across both spatial and temporal scales. Hence, since organisms need to maintain specific osmotic gradients between their body fluids and the environment to remain in good physiological conditions, their ability to tolerate osmotic stress can be considered as a mandatory requirement for their establishment and maintenance in estuaries. Euryhalinity is, consequently, a necessary characteristic for estuarine inhabitants (PÉQUEUX, 1995). Although labeled as euryhaline, *Zoanthus* cannot tolerate long exposures to salinity variations (mainly in low salinity), which may explain its absence in estuarine zones (RABELO et al. 2007; BARRADAS et al. 2010)

V. References

ALUTOIN, S. et al. Effects of multiple stressors copper and reduced salinity on the metabolism of the hermatipic coral *Porites lutea*. *Marine Environmental Research,* Amsterdam, v.52, n. 3, p. 289-299, 2005.

AMADO, E. M.; FREIRE, C. A.; SOUZA, M. M. Osmoregulation and tissue water regulation in the freshwater red crab *Dilocarcinus pagei* (Crustacea, Decapoda), and the effect of waterborne inorganic lead. *Aquatic toxicology,* Amsterdam, v.79, p. 1-8, 2006.

ARAI, M. N. *A Functional Biology of Scyphozoa.* New York: Chapman and Hall, 1997.

BABCOCK, R. C.; RYLAND, J. S. Larval development of a tropical zoanthid (*Protopalythoa* sp.). *Invertebrate Reproduction Development,* Newcastle, v.17, n .3, p. 229-239, 1990.

BARRADAS, J. I. et al. Spatial distribution of benthic macroorganisms on reef flats at Porto de Galinhas Beach (northeastern Brazil), with special focus on corals and calcified hydroids. *Biotemas (UFSC)*, Florianópolis, v. 23, p.1-11, 2010.

BEGER, M.; JONES, G. P.; MUNDAY, P. L. Conservation of coral reef biodiversity: a comparison of reserve selection procedures for corals and fishes. *Biological Conservation,* Amsterdam, v.111, p. 53-62, 2003.

BOSCOLO, H. K.; SILVEIRA, F. L. Reproductive biology of *Palythoa caribaeorum* and *Protopalythoa variabilis* (Cnidaria, Anthozoa, Zoanthidea) from the southeastern coast of Brazil. *Brazilian Journal of Biology,* São Carlos, v.65, n.1, p.29-41, 2005.

BROWN, A. D. Microbial water stress. *Bacteriological reviews*, Bethesda, v.40, p. 803-846, 1976.

COLLINS, A. G. Phylogeny of medusozoa an the evolution of cnidarian life cycles. *Journal of Evolutionary Biology,* Boston, v.15, p.418-432, 2002.

COSTA JR., O. S.et al.. Spatial and seasonal distribution of seaweeds on coral reefs from Southern Bahia, Brazil. *Botanica Marina,* Scotland, v.45, p. 346-355, 2002.

HICKMAN, C.P.; ROBERTS, L.S.; LARSON, A. Princípios Integrados de Zoologia. 11. ed. Rio de Janeiro: Guanabara Koogan, 2004.

JAHN, M.P. Osmotic effect of choline and glycine betaine on the gills and hepatopancreas of the *Chasmagnathus granulata* crab submitted to hyperosmotic stress. *Journal of Experimental Marine Biology and Ecology,* Amsterdam, v.334, p.1-9, 2006.

KIKUCHI, R. K. P.; LEÃO, Z. M. A. N. ; OLIVEIRA, M. D. M. Conservation status and spatial patterns of AGRRA vitality indices in Southwestern Atlantic Reefs. *Revista de Biología Tropical*, San José, v. 58, p. 1-31, 2010.

LEÃO, Z. M. A. N.; KIKUCHI, R. K. P.; OLIVEIRA, M. D. M. Branqueamento de corais nos recifes da Bahia e sua relação com eventos de anomalias térmicas nas águas superficiais do oceano. *Biota Neotropica (Edição em Português. Online)*, São Paulo, v. 8, p. 1-10, 2008.

MCCORMICK, S. D.; BRADSHAW, D. Hormonal control of salt and water balance in vertebrates. *General and Comparative Endocrinology,* Amsterdam, v.147, p. 3-8, 2006.

MUTHIGA, N. A.; SZMANT, A. M. The effects of salinity stress on the rate of aerobic respiration and photosynthesis in the hermatipic coral *Siderastrea siderea*. *Biological Bulletin,* Woods Hole, v. 173, p.539-551, 1987.

PÉQUEUX, A. Osmotic regulation in crustaceans. *Journal of Crustacean Biology,* Virginia, v. 15, p. 1-60, 1995.

RABELO, E. F. et al. Distribuição de Zoantídeos (Cnidaria:Zoanthidea) em Recifes de Arenito: Influência da Competição com Macroalgas. In: CONGRESSO LATINO AMERICANO DE CIENCIAS DO MAR – COLACMAR, XII.,2007,Florianópolis. Livro de resumos., 2007, v. 2, p. 213-214.

RAND, R.P.; PARSEGIAN, V.A.; RAU, D.C. Intracellular osmotic action**.** *Cellular and Molecular Life Sciences,* Basel, v.57, p. 1018-1032, 2000.

REIMER, A. A. Chemical control of feeding behavior and role of glicina in the nutrition of *Zoanthus* (Coelenterata: Zoanthidea). *Comparative Biochemistry and Physiology, Part A: Physiology,* Amsterdam, v.39, n.4, p. 743-759, 1971a.

________. Observations on the relationships between several species of tropical zoanthids (Zoanthidea, Coelenterata) and their zooxanthellae. *Journal of Experimental Marine Biology and Ecology,* Amsterdam,v.7,n.2, p. 207-214, 1971b.

ROMANO, N.; ZENG, C. The effects of salinity on the survival, growth and haemolymph osmolality of early juvenile blue swimmer crabs, *Portunus pelagicus*. *Aquaculture*, Amsterdam,v. 260, p. 151-162, 2006.

SEBENS, K. P. Intertidal distribution of Zoanthids on the Caribbean Coast of Panama: Effects of predation and desiccation. *Bulletin of Marine Science,* Miami,v.32, n.1, p.316-335, 1989.

TKACHENKO, K. S.; ZHIRMUNSKY, A. V. Distribution of sessile invertebrates in a rocky sublittoral community off Rimsky-Korsakov Islands (Sea of Japan). *Marine Ecology*, Boston, v.23, n. 3, p. 253-267, 2002.

ZAR, J.H. *Biostatistical Analysis*, United States: Prentice Hall, 1984.