Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v35i1.10970

Osmoregulation in tropical zoanthid *Protopalythoa variabilis* (Cnidaria: Anthozoa)

Marcelo de Oliveira Soares[1*] and Leonardo Peres de Souza[2]

*[1]Instituto de Ciências do Mar, Laboratório de Plâncton e Análise Ambiental, Universidade Federal do Ceará, Av. da Abolição, 3207, 60165-081, Meireles, Fortaleza, Ceará, Brazil. [2]Centro de Ciências, Departamento de Biologia, Campus do Pici, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil. *Author for correspondence. E-mail: bio_marcelo@yahoo.com.br*

ABSTRACT. This study evaluated the physiological, morphological and ethological effects of salinity variations on the tropical zoanthid *Protopalythoa variabilis*. This zoanthid was submitted to different salinity levels to probe the hypothesis of osmoregulation. Specimens collected in beach rocks from Northeastern Brazil were taken alive to the laboratory in their original water. The osmoregulatory ability of *P. variabilis* can be determined by measuring the hemolymph osmolality under various salinity conditions and comparing with the osmolality of the medium. The zoanthid *P. variabilis* is a “weak regulator”, as it only osmoregulates within a narrow range of external salinity values, and its hemolymph osmolality drops, approximately in parallel with the isosmotic line, when the medium salinity falls below a certain limit. Ethological and morphological modifications under different salinities are discussed. This experiment shows for the first time the importance of osmotic regulation in the tropical zoanthid *P. variabilis.*

Keywords: anthozoan, salinity, physiology, reefs.

Osmorregulação no zoantídeo tropical *Protopalythoa variabilis* (Cnidaria: Anthozoa)

RESUMO. Este estudo avaliou os efeitos fisiológicos, morfológicos e etológicos no zoantídeo tropical *Protopalythoa variabilis* em diferentes níveis de salinidade. Para testar a hipótese de osmorregulação, o zoantídeo foi submetido a diferentes variações de salinidade. Os indivíduos foram coletados em recifes de arenito no Nordeste brasileiro e foram levados vivos para o laboratório na água em que foram recolhidos. A capacidade osmorregulatória de *P. variabilis* foi determinada pela medição da osmolaridade da hemolinfa em diferentes condições de salinidade e comparadas com a osmolaridade do meio. O zoantídeo *P. variabilis* é um “regulador fraco” visto que osmorregula dentro de uma estreita faixa de valores de salinidade externa e quando a salinidade do meio cai abaixo de um certo limite, ocorre redução da osmolaridade da hemolinfa, aproximadamente em paralelo com a linha isosmótica. As mudanças etológicas e morfológicas em diferentes salinidades são discutidas. Este experimento demonstra pela primeira vez a importância da regulação osmótica no zoantídeo tropical *P. variabilis*.

Palavras-chave: antozoário, salinidade, fisiologia, recifes.

Introduction

There are several life forms in the intertidal zone: barnacles, crabs, corals, zoanthids, echinoderms and algae; all of them properly adapted to the conditions of such environment, like the periodic exposure to sunlight and the lack of water during low tides and intense hydrodynamism (COSTA JR. et al., 2002).

Abiotic factors, as the variation in wave energy, type of substratum, light intensity, salinity and temperature, influence the intertidal zone, regarding the distribution and life cycles. Environmental factors, such as drought, salinity and temperature extremes, are limiting factors for species survival. Organisms that live in habitats where these factors are a major issue have developed some adaptations to survive in these environments (JAHN et al., 2006).

Most organisms have 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 often determines the capacity of an animal to live in certain habitats and, thus, determines the ecological limits to species distributions (MCCORMICK; BRADSHAW, 2006).

According to Péqueux (1995), there are two broad categories of euryhaline invertebrates in respect to osmotic regulation: the "strong regulators", which can maintain their hemolymph osmolality almost constant in a wide range of external salinity values, and the "weak regulators", which only osmoregulate within a narrow range of external salinity values and whose hemolymph osmolality drops, more or less in parallel with the isosmotic line, when the medium salinity falls below a certain limit (PRUSCH, 1983).

The Order Zoanthidea members are colonial anthozoarians, usually connected through a stolon on their lower parts. Their numerous tentacles are arranged in one or two rows. They are common in coral reefs and beach rocks along the tropical coasts (BOSCOLO; SILVEIRA, 2005).

Presently, there is no published information about the salinity tolerance and optimal salinity levels for the zoanthid *P. variabilis*. This study investigated the osmoregulatory capability of tropical zoanthid *P. variabilis* (Cnidaria, Zoanthidea), which is very common in the Brazilian and Caribbean coasts. Thus, the questions considered in this study were: Does the salinity variation of the medium affect the osmolarity of body fluids in this zoanthid? Is the zoanthid considered an osmoregulator animal? Do morphological and/or ethological changes occur with the salinity variation?

Material and methods

Animals

The species *P. variabilis* is represented by brown colonial polyps of coriaceous texture, not much concentrated, reaching max. 1.5 cm high, connected on basal portion through a tube-shaped filamentous stolon. They are usually associated with *Zoanthus* colonies, which display a green color due to their photosynthesizing microalgae (BARRADAS et al., 2010). *P. variabilis* may appear isolated or forming aggregations on sand or consolidated substrata, where it competes for space against other zoanthids. The long column allows this species to live buried in sand with the oral disk on the surface (BOSCOLO; SILVEIRA, 2005). The species is very common in the Southeast and Northeast coast of Brazil.

Experiments

Specimens of *P. variabils* (n = 120) were collected in the Paracurú beach, State of Ceará, Northeastern Brazil. Paracurú beach is situated in the west region of the State of Ceará, 90 km from the capital, Fortaleza (03°23'53,0"E, 39°00'38,8"W), encompassing an extensive belt of beach rocks with highly conspicuous tide pools. These formations are constituted of beach rocks that occur majorly in intertidal zones. Specimens were collected randomly with spatulas in the sandstone reefs. Subsequently, animals were taken alive to the laboratory in plastic bags with their original water. For these experiments, individuals were collected between April and June 2005. In the laboratory, animals were kept in aerated aquaria at the salinity of their original water (38‰) and at ± 20ºC (annual average temperature in the reef, under natural light and dark conditions), and daily supplied with *Artemia* sp. The experimental group and control was not fed the day before and during the experiment.

The osmoregulatory ability of aquatic animals can be determined by measuring the hemolymph osmolality under various salinity conditions and comparing with the medium osmolality (ROMANO; ZENG, 2006). After a 24-hour acclimation period, animals (20 animals in each treatment) were transferred from the original salinity (38‰ to salinities of 0, 10, 20, 30, 40 and 50). Seawater, diluted with distilled water, was used to obtain low salinities, and NaCl P.A was added in the sea water to obtain a high concentration. The ethological changes were accompanied (like oral disc closure, wilting, and swelling) in the experiment. The osmotic concentration of the hemolymph was determined before the experiments and after a 6-hour exposure. A single hemolymph sample was extracted from the gastrovascular cavity of each zoanthid by puncturing with a 50 μL syringe. Hemolymph salinity and water osmolalities were measured with a refractometer (ATAGON - 1E PPM 0-50%).

Statistical analysis

Data are presented as mean ± standard deviation. The normalization of data was performed with the Kolmogorov-Smirnov test. The groups were compared using one-way analysis of variance (ANOVA) within treatment groups, always with an α value of 0.05. Tukey's multiple comparison test was applied after ANOVA to compare the analyzed groups (ZAR, 1984). The statistical analyses were performed using GraphPad Instat Statistical Package Version 3.01 and GraphPad Prism 4.0.

Results

Figure 1 show the data of corporal salinity of the zoanthid *P. variabilis* before the experiments. Corporal salinity of *P. variabilis* was 38.3 ± 1.41, approved through Kolmogorov-Smirnov normality test (KS = 0.2162, $p > 0.10$). Mean values of species were not significantly different from the values of the medium. The data analysis supports the hypothesis that zoanthid *P. variabilis* is isosmotic in relation to the medium, whose mean salinity is 38‰.

Results regarding morphological variations related to the salinity changes (Figure 1) evidenced the wilting (probably water loss to the medium) and swelling (probably water gain from the medium) in *P. variabilis.* The highest values of wilting occurred under salinity ranging from 35 to 50; these results were due to the high salt concentration in the medium, which implies water flow out of the body. On the other hand, the highest values of swelling were observed under salinity ranging from 0 to 30; these results were due to the low salt concentration in the medium, which indicates water flow into the animal's body. Such effects were observed in *P. variabilis*, due to the gradient difference.

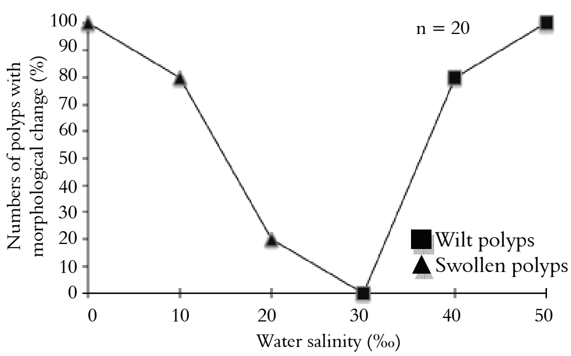

Figure 1. Frequency of *P. variabilis* polyps with morphological changes under different salinity conditions (n = 20 at each treatment).

The variation of salinity in the different treatments was accompanied by ethological changes (like oral disc closure, to lessen water and salt exchanges with the hostile medium) (Figure 2). It shows that the behavior of *P. variabilis* consists in closing the oral disc under salinities other than those in the control treatment or those closer to their natural environment. This behavior is probably an attempt to reduce the osmotic stress generated by the osmotic gradient.

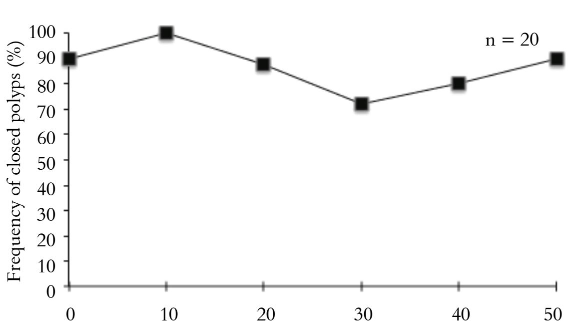

Figure 2. Ethological aspects: Frequency of closed polyps of *P. variabilis* under different salinity conditions (n = 20 at each treatment).

The mean values registered for the hemolymph from *P. variabilis* in the experimental groups differed significantly (ANOVA test, $p < 0.0001$), evidencing that this species is not a "strong regulator". *P. variabilis* presents an osmoregulatory behavior; it is a hyperregulator under salinities ranging from 0 to 20, and a hyporegulator under salinities ranging from 30 to 50. For *P. variabilis*, no linear relationship of isosmolarity was observed among the variables suggesting that it is an osmoregulator (Figure 3, Table 1).

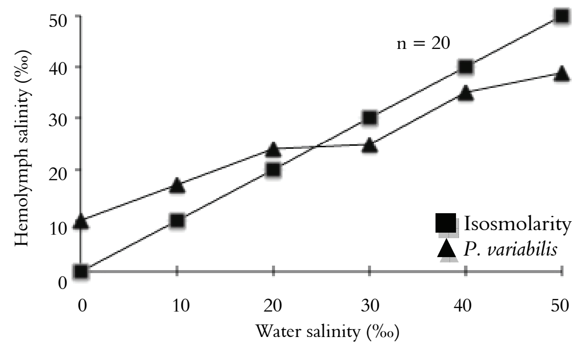

Figure 3. Osmotic regulation in the zoanthid *P. variabilis*.

Table 1. Tukey's multiple comparison test between the water salinity treatments (NS = Non significant; S = Significant).

Tukey's multiple comparison test	P value	Significance
0 vs. 10	$p > 0.05$	NS
0 vs. 20	$p < 0.001$	S
0 vs. 30	$p < 0.01$	S
0 vs. 40	$p < 0.001$	S
0 vs. 50	$p < 0.001$	S
10 vs. 20	$p > 0.05$	NS
10 vs. 30	$p > 0.05$	NS
10 vs. 40	$p < 0.001$	S
10 vs. 50	$p < 0.001$	S
20 vs. 30	$p > 0.05$	NS
20 vs. 40	$p < 0.01$	S
20 vs. 50	$p < 0.001$	S
30 vs. 40	$p < 0.05$	S
30 vs. 50	$p < 0.01$	S
40 vs. 50	$p > 0.05$	NS

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

Discussion

According to Rand et al. (2000), the study on osmoregulation gave rise to a multitude of analyses at various levels of biological organization (i.e. organisms, cells and molecules), especially with coastal marine invertebrates, which, being often exposed to salinity variations in their natural habitat, are good models for the study about the effects of osmolarity changes. For example, the osmotic regulation was studied in poriferans, mollusks, crustaceans and cnidarians.

Salinity is often an important environmental factor that influences the horizontal distribution of marine invertebrates living in coastal areas (BARRADAS et al., 2010). The survival of zoanthids under low salinities is directly related to the ability of diluting the body fluids in cnidarians. These animals keep a condition of isosmolarity in relation to the medium (GATES; MAYFIELD, 2007), like the tropical zoanthid *P. variabilis*.

The study showed that in invertebrates, just as vertebrates and micro-organisms, to overcome a hypoosmotic shock that causes water input that may lead to osmolysis, the cells will release organic osmolytes, especially free amino acids and their derivatives, through its membrane (JAHN et al., 2006), which explains the morphological modifications in zoanthid *P. variabilis*.

Reimer (1971) showed that in zoanthids, the opening of oral disc is related to peptides. Oral disc closure may, in turn, be related to the lack of food, as observed with anemones. However, oral disc opening in *P. variabilis* may also show a relationship to food availability, as suggested by Reimer (1971). Muthiga and Szmant (1987) observed that zoanthids may close their oral disc to reduce the osmotic stress. In this study, the ethological aspect, like oral disc closure, is probably related to an attempt to reduce osmotic stress under low or high salinities, due to the lack of food in the experiments.

Gates and Mayfield (2007) verified that osmoconformatory and osmoregulatory strategies are rather common in zoanthids. Muthiga and Szmant (1987) addressed this subject observing that osmoregulatory mechanisms are little known in corals. There are two broad categories of euryhaline invertebrates in respect to hyperosmotic regulation: the “strong regulators”, which can maintain their hemolymph osmolality almost constant in a wide range of external salinity values, and the “weak regulators”, which only osmoregulate within a narrow range of external salinity values and whose hemolymph osmolality drops, more or less in parallel with the isosmotic line, when the medium salinity falls below a certain limit (PÉQUEUX, 1995). The zoanthid considered in this study is a “weak regulator”.

Muthiga and Szmant (1987) argued that mortality usually varies with the experiment duration, with higher rates occurring within two to five days. Thus, it is evident that, despite being euryhalines, these organisms do not survive an exposure to long-term variations in salinity, which explains their absence in estuarine regions in tropical countries

The assessment on osmotic stress has been the aim of studies with several organisms, such as bacteria, plants, invertebrates and mammals. In researches with invertebrates, information found is mainly about crustaceans and mollusks (GILLES; GOFFINET, 1991; RUMSEY, 1973) while little is known about cnidarians. This experiment shows for the first time the importance of osmotic regulation in the tropical zoanthid *P. variabilis.* However, other further studies should be developed, such as the evaluation of organic molecules involved in osmoregulation processes, to increase the knowledge about the osmotic effects of salinity on tropical zoanthids.

Conclusion

The studied species is one of the most important species in reef ecosystems in Northeast Brazil. The comprehension of mechanisms of adaptation in function of salinity change, can provide important information about the resilience of communities facing environmental changes. Future research may focus on how biochemical and genetic mechanisms control the morphological and ethological alterations described in the present study.

Acknowledgements

We are grateful to Dr. Ana de Fátima Fontenele Urano Carvalho. This study was supported by a grant of Funcap and CNPq.

References

BARRADAS, J. I.; AMARAL, F. D.; HERNÁNDEZ, M. I. M.; FLORES-MONTES, M. J.; STEINER, A. Q. Spatial distribution of benthic macroorganisms on reef flats at Porto de Galinhas Beach (northeastern Brazil), with special analysis in zoanthids. **Biotemas**, v. 23, n. 2, p. 61-67, 2010.

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**, v. 65, n. 1, p. 29-41, 2005.

COSTA JR., O. S.; ATTRILL, M. J.; PEDRINI, J. A.; DE-PAULA, J. C. Spatial and seasonal distribution of seaweeds on coral reefs from Southern Bahia, Brazil. **Botanica Marina**, v. 45, n. 1, p. 346-355, 2002.

GATES, R. D.; MAYFIELD, A. B. Osmoregulation in anthozoan-dinoflagellate symbiosis. **Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology**, v. 147, n. 1, p. 1-10, 2007.

GILLES, R.; GOFFINET, G. Effects of osmotic shock on the ultrastructure of cell nuclei in euryhaline and stenohaline crustaceans. **Tissue and Cell**, v. 23, n. 6, p. 909-915, 1991.

JAHN, M. P.; CAVAGNI, G. M.; KAISER, D.; KUCHARSKI, L. C. 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**, v. 334, n. 1, p. 1-9, 2006.

MCCORMICK, S. D.; BRADSHAW, D. Hormonal control of salt and water balance in vertebrates. **General and Comparative Endocrinology**, v. 147, n. 1, 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**, v. 173, n. 3, p. 539-551, 1987.

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

PRUSCH, R. D. Evolution of invertebrate homeostasis: Osmotic and ionic regulation. **Comparative Biochemistry and Physiology**, v. 76, n. 4, p. 753-761, 1983.

RAND, R. P.; PARSEGIAN, V. A.; RAU, D. C. Intracellular osmotic action. **Cellular and Molecular Life Sciences**, v. 57, n. 1, 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**, v. 39, n. 4, p. 743-759, 1971.

ROMANO, N.; ZENG, C. The effects of salinity on the survival, growth and haemolymph osmolality of early juvenile blue swimmer crabs, *Portunus pelagicus*. **Aquaculture**, v. 260, n. 1, p. 151-162, 2006.

RUMSEY, T. J. Some aspects of osmotic and ionic regulation in *Littorina littorea* (L.) (Gastropoda, Prosobranchia). **Comparative Biochemistry and Physiology Part A: Physiology**, v. 45, n. 2, p. 327-344, 1973.

ZAR, J. H. **Bioestatistical analysis**. 2nd ed. New York: Prentice-Hall, 1984.

Received on August 23, 2010.
Accepted on February 15, 2011.