PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF *PALYTHOA VARIABILIS* (CNIDARIA, ANTHOZOA) FROM INTERTIDAL ZONE AFTER LOW TIDE EMERSION

RESPOSTAS FISIOLÓGICAS E BIOQUÍMICAS DE *Palythoa variabilis* (CNIDÁRIA, ANTOZOÁRIA) DA ZONA ENTREMARÉS APÓS EMERSÃO DURANTE A MARÉ BAIXA

Luis Felipe Barcena Albertoni[1], Pedro Henrique Curci Brandino[1], Lucas Nascimento Lima[1], Luiza de Castro Mastrangelo Dias[1], Beatriz Rodrigues Guarinão[1], Samuel Coelho Faria[2], Helen Sadauskas-Henrique[1]

[1] Laboratório de Ecofisiologia e Bioquímica de Organismos Aquáticos, Universidade Santa Cecília, Rua Oswaldo Cruz, 11045-907, Santos, Brazil.

[2] Centro de Biologia Marinha, Universidade de São Paulo, Rodovia Manoel Hypólito do Rego, Km 131,50 S/N, São Sebastião - SP, 11600-000

E-mail para contato: albertoniluisfelipe@gmail.com; pedrohenriquecb@hotmail.com

RESUMO – Objetivou-se investigar as respostas bioquímicas no zoantídeo do entre-marés *Palythoa variabilis* diante de exposição aérea seguida de submersão. O regime de marés foi simulado em laboratório a fim de testar que emersão (3 h) seguida de imersão (1 e 3 h) elevará as defesas antioxidantes, a saber a atividade da glutationa S-transferase (GST) e catalase (CAT). Não houve efeito da exposição para CAT, contudo a atividade de GST elevou-se em emersão e em imersão após 1 h, apresentando valores quase 3 vezes maiores em relação ao controle. Os resultados deste trabalho trazem evidências das respostas bioquímicas causadas pela exposição ao ar no coral *Palythoa variabilis* e suas capacidades adaptativas para lidar com este estressor.

Palavras-chave: entremarés; exposição ao ar; Palythoa; DNA; estresse oxidativo.

ABSTRACT – The objective was to investigate the biochemical responses in the intertidal zoanthid *Palythoa variabilis* following aerial exposure followed by immersion. The tidal regime was simulated in the laboratory to test whether emersion (3 hours) followed by immersion (1 and 3 hours) would elevate antioxidant defences, namely the activity of glutathione S-transferase (GST) and catalase (CAT). There was no effect of exposure on CAT; however, GST activity increased during emersion and after 1 hour of immersion, showing values nearly 3 times greater compared to the control. The results of this study provide evidence of the biochemical responses caused by aerial exposure in the coral *Palythoa variabilis* and its adaptive capabilities to cope with this stressor.

Key words: Intertidal; air exposure; Palythoa; DNA; Oxidative stress.

Tipo do trabalho: (x) Iniciação científica ( ) Iniciação tecnológica ( ) Extensão

1 INTRODUCTION

Palythoa variabilis is a widely spread anthozoan commonly found along the western Atlantic coast, from Florida, USA [1], to Santa Catarina, Brazil [2]. It is particularly abundant on the southeastern coast of Brazil. This zoanthid can be found from intertidal areas to the fore-reef bottom, and exhibits great tolerance to a wide range of environmental stressors, hosting symbiotic dinoflagellates (Symbiodiniaceae) inside its tissue [2] [3]. *Palythoa variabilis* is capable of overgrowing coral reefs, causing phase shifts that result in loss of scleractinian coral cover, reducing biodiversity and local ecological functions [3]. During subaerial exposure (SAE), *P. variabilis* produces abundant mucus, which can shelter other marine organisms such as filamentous algae, virus, bacteria, small invertebrates, plankton, and Symbiodiniaceae [4]. *P. variabilis* also present biotechnological potential, including the characterization of lipidic α-amino acids with pro-apoptotic activity [5].

This research aims to understand the physiological responses of *Palythoa Variabilis* from the intertidal zone when exposed to air, through the glutathione S-transferase (GST) and catalase (CAT) activities.

2 MATERIALS AND METHODS

1.2.1. Study area

The study was carried out in São Sebastião, north shore of São Paulo, Brazil, at CEBIMar (Centro de Biologia Marinha da Universidade de São Paulo). The São Sebastião channel (Fig 1) is a marine passage located between São Sebastião island (Ilhabela county) and the mainland (São Sebastião county) in the state of São Paulo, southeastern Brazil (23°49' 40"S – 45° 25' 21"W). The channel has 25 km long, 2 to 7 km wide and a maximum depth of 40 m. The channel currents are driven by the wind, with a NE predominant flow in winter, associated with the arrival of atmospheric fronts. Colonies (N = 6) of *P. variabilis* were collected from the intertidal zone of São Sebastião channel.

2.2.2 *P. Variabilis* collection and fragmentation

The collection was made with a rock pick and hammer. A minimum distance of 5 m between each colony was maintained to minimize genetic similarities. Fragmentation processes were conducted in situ before bringing the corals to the laboratory, and each *P. variabilis* colony

was split into 4 mini-colonies (samples) with a minimum area of 25 cm^2, using surgical instruments and personal protective equipment to avoid any contact with Palytoxin and coral mucus.

3.2.3 Experimental setup

The acclimation took place in a water system composed of 24 glass aquariums with 18 L of recirculating seawater with a 24 h renewal of 25 L/h each for 10 days prior the experiment. Lightning was provided by 12 Maxspect Jump, one for every four aquariums, providing a photosynthetic active radiation (PAR) of 75 µmol quanta/m^2 /s. Water flow was provided by one SarloBetter SB500 (500 L/h) placed inside each aquarium. Salinity (33), pH (8,2) and temperature (24 °C) were monitored daily.

There were four experimental groups with six replicates each (N = 6). Control group (immersion, T0), emersion (T1), 1h re-immersion (T2) and 3 h re-immersion (T3). Samples were immediately stored in -80 ^oC.

4. 2.4 GST and CAT

GST activity was determined as described by Keen [6] using 1-chloro-2,4dinitrobenzene (CDNB) as the substrate. The change in absorbance was recorded at 340 nm in a spectrophotometer (Molecular Devices® SpectraMax M2e), and the enzyme activity were calculated as nmol CDNB conjugate min-1 mg protein-1, using a molar extinction coefficient of 9.6 mM cm^{-1}.

CAT activity was determined as described by Beutler [7]. The rate of enzymatic decomposition of H_2O_2 was measured in a spectrophotometer at 240 nm. Enzyme activity was expressed in µmol H_2O_2 min^{-1} mg $protein^{-1}$.

5.2.5 Data analysis

One-way analysis of variance (ANOVA) was performed. When significance at the 95% level ($p<0.05$) was indicated, Tukey's post-hoc test was applied. The data were presented as mean ± standard deviation of the mean. SigmaStat 3.5 software was used for statistical analyses, and SigmaPlot 11.0 was used for graph generation.

3 RESULTS

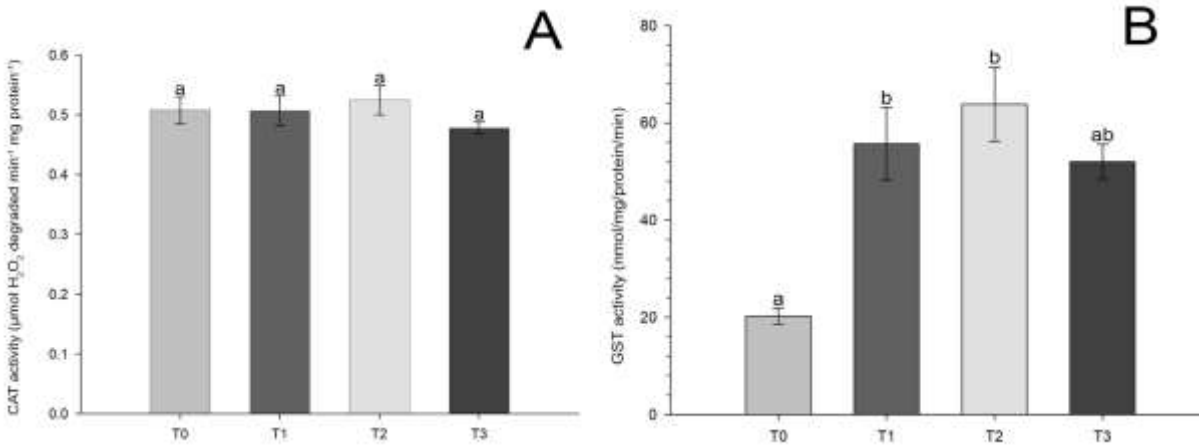

Figure 1: Glutationa S-transferase (A) and Catalase (B) activities in*P. variabilis* after direct exposure from immersion condition to 3 h-emersion (T1) followed by recovery 1 h-recovery (T2) and 3 h-recovery (T3). Values are expressed as mean ± SEM (N = 6). Different letters denote significant differences between compared groups (Tukey's post-hoc test, P < 0,05)

No significant difference was found between Treatment groups for CAT activity. Mean values varied between 0.478 and 0.525 and standard error varied between 0.0102 and 0.0251 (Figure 2A). For GST activity, significant difference was detected between experimental groups. Treatments T1 and T2 presented around three-time higher mean (~60) when compared to T0 (Figure 2B).

4 DISCUSSION

Although the present research had investigated main antioxidant systems, there are other enzymes responsible for neutralising ROS, such as GPX and SOD, which eliminate hydroperoxides and superoxide anions, respectively. Teixeira et al. (2013) observed that for the octocoral *Veretillum cynomorium,* CAT activity increases significantly after 2 h of emersion, differing the present study once there were no significant difference between treatments groups for CAT activity. GST activity increased on T1 and T2 as a response to aerial exposure, possibly avoiding damage.. Giannetto et al. (2017) observed that for mussels *Mytilus galloprovincialis*, GST activity also increases during aerial exposition. A 3 h recovery seems to be a minimum time for *P. variabilis* to return to homeostasis. A similar increase in GST activity following aerial exposure was observed by Teixeira et al. (2013) for the octocoral *Veretillum cynomorium*.

However, the decrease in GST activity occurred after 30 minutes of recovery for *Veretillum cynomorium* (Teixeira et al., 2013), while for *P. variabilis*, GST levels began to decline only after 3 hours of recovery. This suggests that *P. variabilis* may have a prolonged capacity to maintain GST activity compared to *Veretillum cynomorium* during the recovery phase.

5 CONCLUSION

The study concludes that intertidal *P. variabilis* developed adaptations to aerial exposition such as the activation of GST. This study demonstrates the importance of understanding the physiological process that allow these organisms to live and thrive in stressful and unstable environments, such as intertidal zones.

6 REFERENCES

1. Reimer, James Davis, Foord, Colin, Irei, Yuka, Species Diversity of Shallow Water Zoanthids (Cnidaria: Anthozoa: Hexacorallia) in Florida, *Journal of Marine Sciences*, 2012, 856079, 14 pages, 2012. https://doi.org/10.1155/2012/856079

2. Santos, M.A., Kitahara, M.V., Lindner, A. *et al.* Overview of the order Zoantharia (Cnidaria: Anthozoa) in Brazil. *Mar Biodiv* **46**, 547–559 (2016). https://doi.org/10.1007/s12526-015-0396-7

3. Davy SK, Allemand D, Weis VM. 2012. Cell Biology of Cnidarian-Dinoflagellate Symbiosis. Microbiol Mol Biol Rev 76:. https://doi.org/10.1128/mmbr.05014

4. Chimetto, L.A.; Cleenwerck, I.; Alves, N., Jr.; Silva, B.S.; Brocchi, M.; Willems, A.; De Vos, P.; Thompson, F.L. *Vibrio communis* sp. nov., isolated from the marine animals *Mussismilia hispida, Phyllogorgia dilatata, Palythoa caribaeorum, Palythoa variabilis* and *Litopenaeus vannamei*. *Int. J. Syst. Evol. Microbiol.* **2011**, *61*, 362–368.

5. Wilke DV, Jimenez PC, Pessoa C, Moraes MO, Araújo RM, Silva WM, et al. Cytotoxic lipidic α-amino acids from the zoanthid Protopalythoa variabilis from the Northeastern coast of Brazil. J Braz Chem Soc. 2009;20(8):1455-9, http://dx.doi.org/10.1590/S0103-50532009000800010

6. Keen, J. H., Habig, W. H., & Jakoby, W. B. (1976). Mechanism for the several activities of the glutathione S-transferases. *Journal of Biological Chemistry*, *251*(20), 6183–6188. https://doi.org/10.1016/S0021-9258(20)81842-0

7. Beutler, E. Glutathione in Red Blood Cell Metabolism. In *A Manual of Biochemical Methods*; Grunef Strottan: New York, NY, USA, 1975; pp. 112–114.