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# Impact of temporal change of salinity to biodefense mechanism of *Haliotis gigantea*

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## Introduction

The increasing of extreme precipitation events that cause the cumulative levels of hyposaline stress in coastal marine system were investigated in this present study. Extreme heavy rains and fresswater runoff that linked with climate change [1,2] caused a disruption the important thresholds for survival and metapopulation dynamic; contributed to the shifting distribution range in blue mussel congeners, *Mytilus trossulus* and *Mytilus galloprovincialis* [3]; and lowering the lysozyme activity on manila clam, *Ruditapes philippinarum* [4].

In this report, the physiological responses to hyposaline stress of Giant abalone, *Haliotis gigantea* were examined. Giant abalone is an intertidal species, that is commercially important products in Japan, China, and Korea [5]. Intertidal species such as Giant abalone has been proven to be excellent study systems for evaluating climate change [6]. Temporary salinity changes were exposed on Giant abalone to evaluate the impact on the biodefense mechanism in term of survival, lysozyme activity and phenoloxidase activity.

## Materials and methods

Giant abalone, *Haliotis gigantea* (shell length 25.54 ± 1.51 mm; shell width 17.40 ± 1.09; weight 2.12 ± 0.30 g) were kept at indoor laboratory using recirculating tanks (23 x 18 x 17 cm), with artificial sea water (LIVESea® Salt, DELPHIS, Japan), aerated and acclimated for 7 days at 20°C and 32 psu salinity. Dry kelp were given 6.5 mg individual$^{-1}$ day$^{-1}$ as feeds. Sea water was changed as needed, by measuring water quality using handheld colorimeter for water quality measurement of aquaculture (HACH DR890, HACH Co., Ltd., USA).

Two experimental treatment were employed, 20 psu and 26 psu, and one control treatment 34 psu, with 10 abalones/treatments and triplicates. Animals were exposed for 3 h target salinity (20 and 26 psu) by taking out appropriate amount of seawater in the tank and change it with freshwater added by degrees. After 3 h hyposaline exposed, concentrated seawater solution were added to maintain the salinity into the initial state (32 psu). The threatment were continously carried out after 24h recovery period. Shell size and survival were recorded for the duration of 30 days.

Hemolymph were collected by homogenizing the carcass using tissue homogenizer. The equal volume of phosphate buffer (0.1 M, pH 7) were added into the homogenized tissue, then stirred firmly. The mixture were centrifuged at 4000 rpm for 30 min. Supernatant were used to analize lysozyme activity [7] and phenoloxidase activity [8].

## Results and discussions

No significant different ($p > 0.05$) on survival rate (Fig. 1) of abalane kept on 32 psu (87%), 26 psu (77%), and 20 psu (80%). Figure 2 shows the increasing of Δ absorbance following the reactions of hemolymph with the substrate that represent the lysozyme activity from hemolymph of Giant abalone. Temporary hyposaline stress resulted in a significant ($p < 0.05$) reduction in phenoloxidase activity compared with control animals (Fig. 3). During the esperimental period, water quality for rearing the Giant abalone were under the threshold (Table 1).

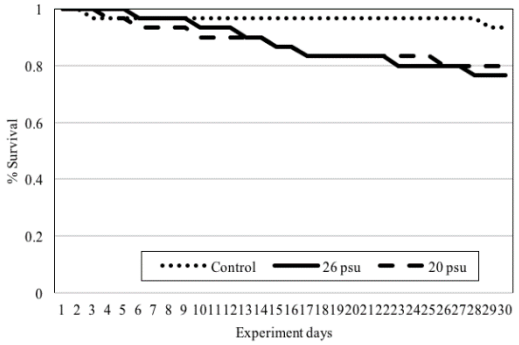


**Fig. 1.** Survival of *Haliotis gigantea*, expressed as percentage of abalones surviving at a temporary hyposaline stress (control, 20 and 26 psu for 3 hours). One-way ANOVA: no significant difference ($p > 0.05$).

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External stress factors such as temperature and salinity are known to be able to affect the immune parameters of marine gastropods species such as Giant abalone [9]. As reported in the previous study [4], lysozyme activity was affected by the temporary change on salinity. Moreover, the present study support the indication that the sudden thyposaline stress affect the biodefence parameter, in term of lowering the phenoloxidase activity of Giant abalone.

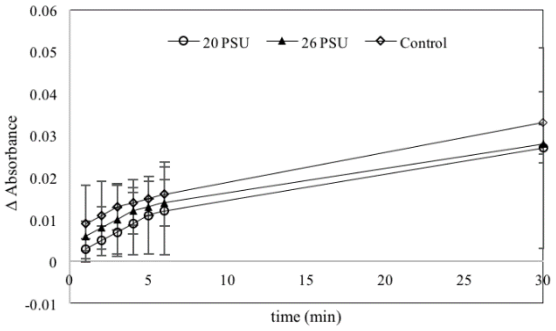


**Fig. 2.** Lysozyme activity in haemolymph of abalone *Haliotis gigantea* exposed to different temporary salinity for 30 days. Values are mean of Δ absorbance of bacterial suspension, *Micrococcus lysodeikticus* at 450 nm following the addition of samples from three individuals per tanks per treatments.

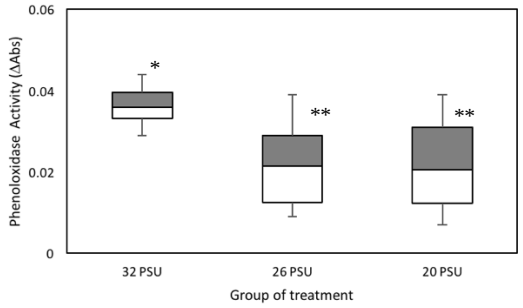


**Fig. 3.** Phenoloxidase activity in haemolymph of abalone *Haliotis gigantea* exposed to different temporary salinity for 30 days. Values are mean of Δ Absorbance of L-DOPA at 490 nm following the addition the samples from three individuals per tanks per treatments. Box plot with different stars are significant different ($p < 0.05$).

**Table 1.** Mean of water quality parameter on the experiment of 30-day exposure a temporary salinity on Giant abalone, *Haliotis Gigantea*

| Parameter | Control | 26 psu | 20 psu |
|---|---|---|---|
| $NO_3^-$ (mg/L) | 2.15 ± 0.07 | 0.90 ± 0.12 | 2.00 ± 0.02 |
| $NO_2^-$ (mg/L) | 0.11 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.01 |
| $NH_3$ (mg/L) | 0.06 ± 0.01 | 0.03 ± 0.01 | 0.04 ± 0.01 |
| $PO_4^{3-}$ (mg/L) | 0.28 ± 0.02 | 0.36 ± 0.05 | 0.17 ± 001 |
| pH | 8.22 ± 0.16 | 8.11 ± 0.14 | 8.01 ± 0.10 |

## Conclusions

The present study was designed to evaluate the effect of temporary change in salinity on survival, lysozyme activity and phenoloxidase activity of Giant abalone, *Haliotis gigantea*. This study has shown that 3 hours hyposaline stress (20 psu and 26 psu) for 30 days did not affect the survival of Giant abalone. However, Manila clams exposed temporary hyposaline stress of 20 and 26 psu had a lower lysozyme activity, and lower phenoloxidase activity Overall, this study strengthens the idea that under the temporary hyposaline stress, Giant abalone might be susceptible by threat of disease due to low lysozyme activity and phenoloxidase activity.

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