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Oxidative stress responses of the giant clam (*Tridacna maxima* (Röding, 1798)) to seasonal variations and contaminations on the Red Sea Coast of Egypt

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Abstract: The present study investigated the combined effects of seasonal fluctuations and site-specific contamination along the Egyptian Red Sea Coast using the giant clam *Tridacna maxima* as a biomonitoring tool for oxidative stress. Biomarkers analyzed included malondialdehyde (MDA) as an indicator of lipid peroxidation (LPO), superoxide dismutase (SOD), and glutathione-S-transferase (GST), measured from clam muscle tissues. Specimens were collected from three sites—National Institute of Oceanography and Fisheries (NIOF), Magawish Village, and Al-Hamrawin Harbor—during four seasons (Spring, Summer and Autumn; 2021, and Winter; 2022). Statistical analysis revealed highly significant effects of season, site, and their interaction on all biomarkers (LPO: $p < 0.001$ for season; $p < 0.001$ for site; $p < 0.001$ for interaction; SOD: $p < 0.001$ for season; $p = 0.002$ for site; $p < 0.001$ for interaction; GST: $p < 0.001$ for season; $p < 0.001$ for site; $p < 0.001$ for interaction). Post hoc comparisons confirmed that biomarker responses varied significantly across seasons and among sites, with Al-Hamrawin Harbor showing the highest stress responses, reflecting strong contamination influence, while Magawish and NIOF showed relatively moderate levels. These results demonstrate that *T. maxima* exhibits clear oxidative stress responses to both climatic variability and anthropogenic contamination, underscoring its value as a sensitive bioindicator for environmental monitoring of the Red Sea ecosystem.

Keywords: Tridacna maxima, Biomarkers, Malondialdehyde (MDA), Catalase (CAT), Glutathione-S-transferase (GST).

1. Introduction

The marine ecosystem, which is economically important for the insular population, is threatened by increasing human activities and industrial chemicals **[1]**. Human waste, mass tourism, and chemical pollution have been shown to be the main causes of coral reef degradation **[2]**.

The marine environment could be contaminated by many types of pollutants, which may be organic or mineral. These chemicals exert adverse effects that can be analyzed qualitatively or quantitatively by chemical procedures. However, the complex mixture of these pollutants cannot be assessed **[3]**. Due to the potential synergistic or antagonistic impact of these pollutants on aquatic organisms, chemical procedures cannot be effectively studied. Therefore, the use of appropriate biomarkers to investigate the toxicity of pollutants in aquatic organisms can be considered as an alternative assessment technique of pollutant impact **[4]**. These parameters are studied to assess the adverse effects on the physiological functions of the organisms, and therefore, the effect of environmental variables on population and community can be explained and expected **[4, 5]**.

Antioxidant biomarkers such as malondialdehyde (MDA), as biomarkers of lipid peroxidation (LPO), superoxide dismutase (SOD), and glutathione-S-transferase (GST) can be taken as helpful biomarkers reflecting the toxicity of some pollutants via the generation of oxidative stress **[6]**. Antioxidant enzymes have been shown to vary and can be used as biomarkers of oxidative stress. These biomarkers provide a strong method to assess the impact of many stressors on aquatic animals that evaluate stress in the environment **[7, 8, 9, 10, 11, 12]**.

There is very little information available on the connection between oxidative stress and fitness in aquatic organisms from both clean and contaminated environments.

Malondialdehyde (a biomarker of LPO) is considered a definite product of membrane lipid peroxidation, which is correlated with the presence of oxidative pollutants in the environment through the generation of free radicals by their metabolism **[13]**. Additionally, LPO is an appropriate antioxidant used to assess the efficiency of the antioxidant defense system for protecting against membrane damage caused by pollutants **[14]**.

Superoxide dismutase (SOD) activity is used as a marker involved in the primary defense against oxidative damage. SOD is a common antioxidant found normally in living cells. The basic physiological function of SOD is detoxification and maintaining the redox balance acts as the first line of defense against reactive oxygen species (ROS) and reactive nitrogen

species (RNS). It converts superoxide anion free radicals (O2-) into molecular oxygen (O2) and hydrogen peroxide (H2O2) in the cells [15]. It was found that the activity of SOD is lower in invertebrate compared with vertebrates [16].

Glutathione-S-transferees (GST) are antioxidant enzymes from a family and multi-functional protein involved in the cellular detoxification of pollutants and xenobiotics that play an essential role in protecting against endogenous and exogenous toxic chemicals [17]. GST levels can be changed by a wide range of chemicals due to their role in detoxification processes of xenobiotics. GST activity can be considered a biomarker for several species, such as fish, crustaceans, and mollusks [18].

Tridacna maxima is the most widely distributed giant clam with a large geographic range from the Red Sea and Western Indian Ocean across the Indo-Malay Archipelago to the *Tumato Archipelago* in the central Pacific [18, 19]. It has been traditionally harvested for human consumption and ornamental use. However, the coral reefs they inhabit are in crisis due to the combined stress of human activities, pollutants, and climatic changes. Like bivalve mollusks, *Tridacna maxima* were filter feeders. Consequently, they are regarded as pollutants collector for heavy metals at many locations along the Red Sea Coast of Egypt [20].

T. maxima muscle is thought to be an organ that is exposed to a variety of contaminants and is extremely sensitive to them. Additionally, because humans consume it, it might be considered a significant component of *T. maximum*. So, the present study can be considered an attempt to assess contamination in the marine environment of three sites along the Red Sea Coast of Egypt.

Several studies have been conducted on the Egyptian Red Sea Coasts and have indicated the effects of contaminants on several sites in the Red Sea Coast using them as a biomonitoring tool and oxidative stress as biomarker responses according to the nature of pollutants and human activity that affect the coast [11, 21]. However, To the best of our knowledge, this is the first study to utilize antioxidant biomarkers to evaluate seasonal variations in Tridacna maxima at various sites. Therefore, the present study aims to illustrate the seasonal effects of biochemical responses of bivalves (*T. maxima*) that live in three different sites on the Egyptian Red Sea Coast.

2. Materials and methods

2.1. Study areas

Three locations were chosen along the Red Sea Coast to carry out the present study (**Fig. 1**). These three sites are; the National Institute of Oceanography and Fisheries (NIOF). Magawish Resident village (M) and Al-Hamrawin harbor (H). The first location, the National Institute of Oceanography and Fisheries, is regarded as a healthy environment that is free from pollution. It is situated near the northern edge of the Red Sea, approximately 5 km from the capital of Hurghada, at 27° 17' 1.0.38 "N 33° 46' 15.323". The second site is the Magawish area which lies south of Hurghada City and is situated at 27°6′ 43. 204″N 33° 44′ 38.867"E. This tourist village is distinguished by the presence of different marine activities. The third location, El- Hamrawin, is located at 26° 15:25.362" N 34° 12′ 13.007″ E, about 120 Km South of Hurghada, 60 km south of Safaga, and 20 km north of Al-Quseir. The biggest and oldest phosphate harbors on the Red Sea Coast of Egypt are located in the Al-Hamrawin site.

From each location, 6 specimens of *Tridacna maxima* were collected during the four seasons. The samples of Tridacna maxima were collected seasonally from May 2021 to February 2022.

2.2. Samples' Collection

Samples of *Tridacna maxima* were gathered from the littoral and sublittoral zones at the three investigated sites by snorkeling and scuba diving to a depth of 0.5 to 2.00 m. Six specimens of T. maxima from each position during the four seasons were collected.

The collected T. maxima were similar in size with shell length 15 ± 3 cm and were transferred to the laboratory. Adductor muscles were excised immediately. Tissues were rinsed with ice-cold sterile saline, blotted dry, and weighed. Each muscle was stored at −80 °C until analysis to prevent oxidative degradation.

2.3. Biomarkers Analysis:

The preserved muscle tissues of *T. maxima* were homogenized with an analytical homogenizer in phosphate buffer (pH 7.6) (4 ml for 1 gm of tissues). The homogenate was then centrifuged at 5000 rpm for 30 minutes. Thereafter, the supernatant was used for the determination of oxidative stress biomarkers.

2.4. Oxidative stress biomarkers:

Some relevant oxidative stress biomarkers were determined in the muscle of *T. maxima* including malondialdehyde (MDA) as a lipid peroxidation LPO, assessed using the method of [22, 23]. Superoxide dismutase (SOD) activity was measured following the method of [24] and glutathione S-transferase (GST) was determined according [25]. Measurements were taken at 534 nm for MDA, 480 nm for SOD, and 340 nm for GST using a spectrophotometer (UV 2300). The kits used were obtained from Biomed Diagnostic Company in Giza, Egypt.

2.5. Statistical analysis:

Oxidative stress biomarker results were expressed as mean ± standard deviation (SD). A two-way ANOVA was performed to assess the main and interaction effects of site and season on the measured parameters. Tukey's HSD test was used as a post-hoc comparison to identify significant differences among groups. Statistical analyses were carried out using SPSS software (Version 26) and Microsoft Excel (Office 2016). A significant level of $P \leq 0.05$ was considered.

3. Results

The study found significant variations in oxidative stress indicators in *T. maxima* muscle across three different sites during the four seasons. The statistical analyses demonstrated

that both season and site, as well as their interaction, exerted significant effects on the measured biomarkers (LPO, SOD, GST) in *Tridacna maxima* (**Fig. 2**). Seasonal changes strongly affect LPO, SOD, and GST activities ($p < 0.001$). Also, there is spatial variation among sites; LPO and GST highly significant ($p < 0.001$). SOD also significant but weaker ($p = 0.002$). The interaction between sites and seasons showed highly significant for all variables ($p < 0.001$).

Post-hoc comparisons further revealed distinct seasonal trends. LPO levels were highest in spring, significantly exceeding those in summer, autumn, and winter, while autumn recorded the lowest values. SOD activity followed a similar pattern, peaking in spring but declining sharply in summer, suggesting a seasonal reduction in antioxidant defense capacity during warmer months. In contrast, GST activity was elevated in summer and autumn relative to spring and winter, indicating an increased detoxification response during periods of higher environmental stress. The significant season × site interaction observed for all biomarkers highlights that these seasonal patterns varied among locations, reflecting the combined impact of local environmental conditions and seasonal drivers. Collectively, these results confirm that *T. maxima* exhibit clear, seasonally dependent biomarker responses and can serve as a sensitive biomonitoring tool for assessing site-specific and temporal fluctuations in environmental stress along the Red Sea coast.

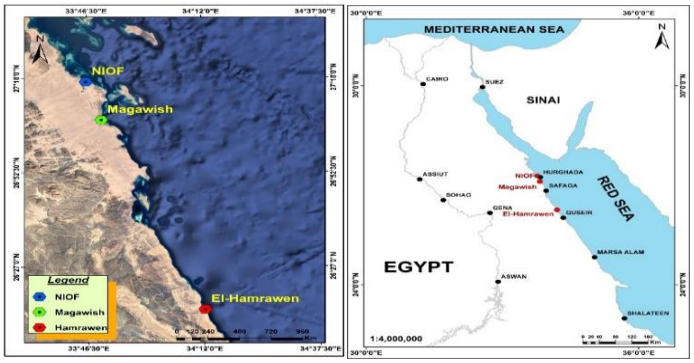

Figure (1): Maps showing the investigated area at three locations along the Red Sea coast, Egypt. Magawish, Al-Hamrawin, and in front of the National Institute of Oceanography and Fisheries (NIOF)

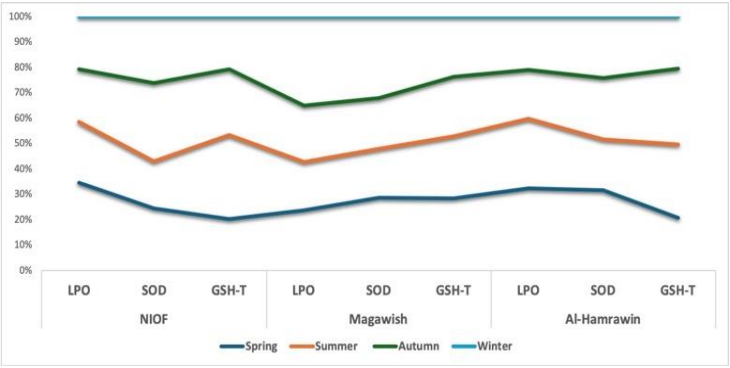

Figure (2): Seasonal variations in the biomarkers (LPO, SOD, and GST) of *Tridacna maxima* across the three study sites: NIOF, Magawish, and Al-Hamrawin.

4. Discussion

Over the past 40 years, human activities along the Egyptian Red Sea coast have escalated, resulting in the introduction of various pollutants like industrial sewage, oil pollution, tourism activities, and solid waste disposal. These contaminants pose a threat to the survival and health of marine bivalves.

Biomarkers are valuable tools for assessing marine coastal pollution and environmental quality [3, 27]. Antioxidant biomarkers in aquatic organisms from polluted, low-polluted, and unpolluted areas show significant differences [5, 16, 28]. Variations in antioxidant biomarkers due to industrial sewage and contaminants from human activities can help distinguish between polluted areas and impacted by human activities [29, 30].

Many studies have used oxidative stress biomarkers as an approach to assess the levels of pollution in different areas. The gonads, gills, mantle, muscles, and digestive glands of bivalves have all been utilized as bioindicators for monitoring marine pollution in these investigations [10, 11, 31, 32].

On the other hand, since the muscles' oxidative stress biomarkers showed a significant difference in differentiation between organisms collected from the most polluted and less polluted locations, it has been demonstrated that these biomarkers can be used as sensitive indicators in the case of severe pollution [32]. Therefore, we selected the muscle of *T. maxima* for monitoring the impact of pollutants in the study sites.The multi-biomarker approach helped determine the pollution levels in the various locations studied, including MDA, SOD, and GST.

The current study demonstrates that the oxidative stress assessments frequently fluctuate throughout each of the four seasons and at the three sites under investigation. The seasonal temperature variations in Egypt can be considered the main reason for varying the antioxidant activities [7]. A further reason might have to do with the different levels of pollutants in the locations where *T. maxima* were collected. The Magawish location is a tourist village that attracts tourists from all over the world and is distinguished by the presence of various human activities, while the National Institute of Oceanography and Fisheries is regarded as an unpolluted site. The Al-Hamrawin site is located near the oldest phosphate harbor, which is a source of chemical contamination [33].

In the current study, the muscle of *T. maxima* was tested for biomarkers since it is an edible bivalve largely harvested and consumed by local populations. Three biomarkers (MDA, SOD, and GST) were determined in the muscle of *T. maxima*. The biomarkers' activities varied greatly among the sites investigated during each of the four seasons.

The product of membrane LPO, MDA, demonstrated a significantly higher elevation in activity across the four seasons at Al-Hamrawin. In contrast, at the Magawish location, it showed a notable elevation during Autumn and Winter and a major decrease during Spring and Summer. It can be suggested that the high MDA value in Al-Hamrawin may be related to the presence of chemicals that induce the production of free radicals, indicating that antioxidative defenses were unable to prevent the deleterious effect of xenobiotics on the lipid membrane of muscle collected from these polluted areas.

Consequently, the high value of MDA is associated with the high concentration of phosphate pollutants, confirming that *T. maxima* was under endogenous and exogenous stress, regardless of the variations in temperature during the four seasons. The lowered MDA activity during spring and Summer; and high activity during Autumn and Winter in Magawish may be correlated with both environmental stress and temperature changes.

The discovery that the MDA activity in Magawish Village was higher in the Winter and Autumn than in the Spring and Summer indicates that the spawning season in the Winter and autumn, which represents endogenous factors as demonstrated by **[12]** in the same species, did not affect this organism. Thus, it can be concluded that the variations in the MDA activity in Al-Hamrawin may be related to both environmental and contaminant stresses, whereas in Magawish, Village they may be related to both environmental and contaminant stresses in spring and summer, and contaminant stress in winter and autumn.

Magawish and Al-Hamrawin showed a significant increase in SOD activity in the *T. maxima* muscle during the Spring and Winter. This confirms that SOD is a fundamental antioxidant present mainly in live cells. The main physiological function of SOD is detoxification and maintaining the redox balance. SOD catalyzes the conversion of superoxide anion free radical into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) in the cells **[15, 34, 35]**. Therefore, the increased value of SOD may be attributed to the contaminants in the two locations due to the inability of SOD to neutralize superoxide ions, preventing the generation of free radicals, as reported by **[36]**. Besides, our outcome has been reported that the increased SOD activity in the biological system is an indication of the toxic effect of xenobiotics **[37]**. In contrast, a marked decrease in SOD activity was observed in Magawish and Al-Hamrawin during the Summer and Autumn seasons. Therefore, the decreased SOD activity during those seasons may result from its neutralization of highly reactive superoxide radicals with prolonged daylight. Therefore, it can be concluded that the increased SOD activity in Magawish and Al-Hamrawin may be the result of the toxic effect of pollutants in Spring, and Winter, but its decreased activity during Summer and Autumn may be related to seasonal variations. In general, there was an external factor (contaminants) that affected SOD activity during Spring and Winter in both locations which cannot render the toxicity of the contaminants on the muscle of the organism studied. Whereas in both locations during Summer and Autumn, there was an intrinsic factor (SOD activity) that rendered the toxicity with prolonged day light and temperature.

Among oxidative stress-related enzymes, glutathione-S-transferases which are a family of enzymes involved in the detoxification of xenobiotics. It has been shown that GST activity was found in the hepatopancreas of *T. maxima* to pollutants in the field **[10]**. However, it is mainly located in the cytosolic fraction in the digestive glands of bivalves **[38]**.

In the current study, GST activity was elevated in the Magawish area during Spring and Winter. This may be related to the increased pollutants to eliminate these pollutants. This suggestion agrees with a study that revealed exposure to different pollutants enhanced GST activity to decrease the pollutants in both field and laboratory studies **[10]**. In this case, GST could neutralize the reactive oxygen species to eliminate the toxic effect of the pollutants on *T. maxima*. On the other hand, GST activity decreased during Summer and Autumn in the same site (Magawish). It is supposed that the level of chemical stress in this location is too high due to tourism activities during these two seasons. The decreased GST activity may be related to another pathway to counteract oxidative stress without the need for animals to enhance GST activity **[39]**. Therefore, we could state that temperature seasonal variation may be a factor. This is contrary to study that indicated GST activity in bivalves increased with the temperature of the water **[7]**.

GST activity was significantly lower in Al-Hamrawin Harbor during the four seasons compared to the reference location (NIOF). This was unexpected as Al-Hamrawin is known to be highly contaminated with phosphate chemicals. Studies have shown that when chemical levels are too high, GST activity can decrease below that of reference locations in bivalves **[40, 41, 42]**. It is possible that oxidative stress was mitigated through alternative pathways, leading to the observed decrease in GST activity despite the need for the organism to enhance it **[39]**. Therefore, it can be concluded that seasonal variations and high levels of phosphate pollutants may impact GST activity in the muscle of *T. maxima*.

5. Conclusion

Our recent study' findings could provide a global overview of pollution levels in various locations along the Egyptian Red Sea Coast. These levels appear to vary both qualitatively and quantitatively due to differences in human activities and types of chemical pollutants present. This study is the first to examine oxidative stress biomarkers (MDA, SOD and GST) in the muscle of giant clam *T. maxima*, across all four seasons. The, results confirm the utility of oxidative stress biomarkers as indicators of environmental pollution and support the idea of periodic biomonitoring of aquatic pollution. Additionally, the fluctuations in oxidative stress activities observed across the four seasons in polluted areas could serve as early warnings of seasonal variations in environmental pollution. Further research is needed to explore the potential of bivalves as a model for detecting the toxic effect of pollutants on aquatic animal and implementing effective conservation measures.

CRediT authorship contribution statement

Conceptualixaton, Mohamed F. El-Sayed [1], Ebtesam A. Yousef[1*], Basma M. Emam[3]; methodology, Mohamed F. El-Sayed [1], Ebtesam A. Yousef[1*], Mohsen Y. Omer[2], Basma M. Emam[3]; software, Basma M. Emam[3]; formal analysis, Basma M. Emam[3]; resources, Mohamed F. El-Sayed [1], Ebtesam A. Yousef[1*], Basma M. Emam[3]; data curation, Basma M. Emam[3]; writing—review and editing, Mohamed F. El-Sayed [1], Ebtesam A. Yousef[1*], Mohsen Y. Omer[2], Basma M. Emam[3]; visualization, Mohamed F. El-Sayed [1], Ebtesam A. Yousef[1*], Mohsen Y. Omer[2], Basma M. Emam[3] ; supervision, Mohamed F. El-Sayed [1],

All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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