Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27(2): 87 – 100 (2023)
www.ejabf.journals.ekb.eg

Antioxidant Enzyme Activity and Lipid Peroxidation in Some Marine Species Collected from the Coast of Benghazi, Libya

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ARTICLE INFO

Article History:
Received: Jan. 30, 2023
Accepted: Feb. 27, 2023
Online: March 9, 2023

Keywords:
Antioxidant enzymes,
Lipid peroxidation,
Libya,
Marine species

ABSTRACT

The objective of the present study was to determine the activities of antioxidant enzymes and malondialdehyde levels in various species of bony fishes, cartilaginous fishes and mollusks living at different depths in coastal habitats in the Mediterranean Sea off the coast of Benghazi. Sixteen marine organisms were collected, and the analyses were performed on the muscles and liver. Some species under study are significant for human health and nutrition in addition to their economic importance. The antioxidant enzymes, such as superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx) and terminal markers of lipid peroxidation, thiobarbituric acid reactive substances, expressed as malondialdehyde (MDA) were investigated. The results revealed a link between dietary abundance, trophic behavior, feeding habit and oxidative stress, but the antioxidant response varies depending on the organism.

INTRODUCTION

Oxygen is a critical component of aerobic life in an environment with redox potential; oxygen will invariably produce reactive oxygen species (ROS), such as hydroxyl radical (•OH), superoxide anion ($O2•^-$), hydrogen peroxide (H_2O_2), nitroxide radicals (NO•), and peroxyl radicals (ROO-). Furthermore, ROS is involved in many physiological and biochemical activities of the organism (**Hansel & Diaz, 2021**). A low or moderate level of ROS prevents the host cells against infections and disrupts cell mitosis. In contrast, the high ROS level disrupts the balance between prooxidants and antioxidants, causing oxidative stress. Excess ROS not only reduces the nutritional value of food or feed by oxidizing before consumption but disrupts the physiological function of lipids, proteins, and DNA as well, which results in diseases such as cancer, diabetes, inflammatory diseases, neurodegenerative diseases, aging and immune system damage (**Wang *et al.*, 2021**).

Oxidative stress is an imbalances status in the body's prooxidants and antioxidants. It is usually eliminated by the antioxidant defense system (**Pisoschi *et al.*, 2021**), which includes antioxidant enzymes and low molecular weight antioxidants.

Fishes are at the top of the aquatic food chain, and as vertebrates, they respond strongly to stress (**Koehn *et al.*, 2022**). As a result, they are frequently used as pollutant indicator species in the aquatic environment. A research strategy that is widely recommended today is the assessment of seasonal variations in biomarkers and determining basal levels in model organisms. This effort overcomes the challenges of field studies by incorporating variations in many natural stressors and evaluating the effects of chemical pollution (**Abdel-Hamid *et al.*, 2021**).

The use of fish in environmental monitoring has grown in importance in recent years in studying natural variability and anthropogenic substances, many of which function as prooxidants and accumulate in aquatic environments (**Eide *et al.*, 2021**).

Many studies of antioxidant enzyme activities in aquatic organisms, especially fish, were designed to provide data for comparative studies or to investigate the effects of environmental influences such as diet, seasonal variation and contaminant influence (**Li *et al.*, 2021; Moniruzzaman & Saha, 2021**).

Reactive oxygen species (ROS) are produced due to numerous environmental factors. As poikilothermic organisms, fishes must routinely deal with fluctuations in environmental temperature and metabolic rate, resulting in oscillating ROS levels (**Islam *et al.*, 2022**). The effect of ambient temperature on metabolic activity is directly related to ROS generation and antioxidant status (**Phrompanya *et al.*, 2021**).

Several studies have demonstrated variations in the activity of oxidative stress biomarkers, which have been proposed as biomarkers of pollutant-mediated oxidative stress (**Polverino *et al.*, 2021; Munno *et al.*, 2022**).

The main enzymes that detoxify ROS in all organisms are functionally divided into antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase) and biotransformation phase II components (glutathione-S-transferases- and reduced/oxidized glutathione) (**Tauffenberger & Magistretti, 2021**). The activity of antioxidant enzymes and other biomarkers varies significantly throughout the year, depending on nutrient availability, reproductive status, seasonal growth rate and other factors (**Shaliutina-Kolesova *et al.*, 2018; Khezrian *et al.*, 2020**).

Lipid peroxidation is a free radical-mediated process that generates secondary free radicals, which can directly react with other biomolecules. Lipid peroxidation happens on polyunsaturated fatty acids found in cell membranes and is followed by a radical chain reaction (**Altomare *et al.*, 2021**). For example, the hydroxyl radical is thought to start ROS formation by removing hydrogen atoms, resulting in the formation of lipid radicals that are then converted into conjugated dienes. Furthermore, a peroxyl radical is formed when oxygen is added, which attacks another fatty acid, forming lipid hydroperoxide (LOOH) and a new radical.

Alkanes, malondialdehyde and isoprostanes are meta-stable end-products of lipid peroxidation. Therefore, these compounds have been validated as valuable markers of lipid peroxidation (**Bacou *et al.*, 2021**).

The present study aimed to determine the activity of antioxidant enzymes, such as superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx), and assess the level of lipid peroxidation as malondialdehyde (MDA) in the muscle and liver of various marine species. The relationship between the activity of antioxidant enzymes and MDA content in the muscle and liver of several marine species and their trophic behavior and feeding habits were also investigated.

MATERIALS AND METHODS

Sixteen marine organisms were studied for the activity of antioxidant enzymes and lipid peroxidation off the coast of Benghazi (Table 1). They included twelve bony fish species: *Dentex dentex, Pagellus erythrinus, Sparus aurata, Mullus surmuletus, Oedalechilus labeo, Epinephelus marginatus, Epinephelus caninus, Scorpaena porcus, Dicentrarchus labrax, Euthynnus alliteratus, Saurida undosquamis, Seriola dumerili,* one cartilage fish: *Squalus blainvillei,* and three mollusk species: *Loligo vulgaris, Sepia officinalis* and *Ruditapes decussatus.*

Sample collection and processing

The samples were collected by scuba diving, fishing nets and fishing hooks. The length and weight of the marine organisms studied were measured, and muscle and liver samples were taken from fish and only muscle from mollusks. In the case of fish species, the muscle samples were taken from the middle of the body behind the pectoral fin, below the front dorsal fin and above the upper ventral fin, while in mollusks, it was taken from the outer surface. After weighing the muscle sample, the skin was removed and crushed for analysis by adding distilled water (25 ml) before storage at -20^{o}C until analysis.

Determination of enzyme activities in liver and muscle homogenates

Liver and muscle samples were homogenized in 25ml of phosphate buffer (pH 7.4), and the homogenates were centrifuged for four minutes at 2000*g* to remove debris.

Total superoxide dismutase (SOD) activity was measured based on the enzyme's ability to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium (NBT) dye (**Nishikimi *et al.*, 1972**). Plastic semi-micro-cuvettes contained 1.0ml of working reagent (mix 10 ml of 50 mM phosphate buffer pH = 8.5 and 1ml of NBT 1.0 ml of NADH), 0.1ml of the sample, 0.1ml of phenazine methosulphate,1.0ml of working reagent, 0.1ml of distilled water & 0.1ml of phenazine (control). The reaction was monitored for 5 minutes at 560 nm for the sample and control.

Table 1. Common and scientific names, weights and lengths of the marine species collected

Common name	Scientific name	N	Weight(g) (mean±SD)	Length(cm) (mean±SD)
Common dentex	*Dentex dentex*	3	144 ± 0.62	25.83 ± 0.76
Common Pandora	*Pagellus erythrinus*	3	291.33 ± 72.70	26.66 ± 2.51
Gilthead seabream	*Sparus aurata*	3	351.33 ± 20.42	26.16 ± 0.57
Surmullet	*Mullus surmuletus*	3	169 ± 26.85	23.16 ± 0.50
Boxlip mullet	*Oedalechilus labeo*	3	181.33 ± 15.53	26.83 ± 1.04
Dusky grouper	*Epinephelus marginatus*	3	558 ± 224.13	32.83 ± 2.56
Dogtooth grouper	*Epinephelus caninus*	3	177.33 ± 36.07	24.83 ± 2.02
Black scorpionfish	*Scorpaena porcus*	3	123.33 ± 53.30	18.33 ± 0.76
European seabass	*Dicentrarchus labrax*	3	472.0 ± 42.00	34.00 ± 0.50
Little tunny	*Euthynnus alletteratus*	3	630 ± 181.14	37.66 ± 2.84
Brushtooth lizardfish	*Saurida undosquamis*	3	292.66 ± 43.87	29.66 ± 5.61
Greater amberjack	*Seriola dumerili*	3	375.33 ± 57.14	32.83 ± 2.25
Longnose spurdog	*Squalus blainvillei*	3	305.10 ± 263.18	68.26 ± 27.48
European squid	*Loligo vulgari*	3	66.66 ± 11.01	39 ± 2.64
Common cuttlefish	*Sepia officinalis*	3	124.66 ± 12.85	37.33 ± 2.08
Grooved carpet shell	*Ruditapes decussatus*	3	9.33 ± 1.15	2.66 ± 0.13

Glutathione peroxidase (GPx) activity was measured by observing the rate of NADPH oxidation to $NADP^+$, which is accompanied by a decrease in the absorbance at 340 nm by glutathione reductase and NADPH (**Paglia & Valentina, 1967**). Plastic semi-micro-cuvettes containing 1.0ml of 50 mM phosphate buffer pH 7.0, Triton X-100, 0.1ml of 24 mol NADPH, glutathione reductase, 0.01ml of sample, 0.1ml of hydrogen peroxide, and the assay continued by measuring absorbance at 340nm.

Glutathione reductase (GR) activity was measured at 340nm via measuring the presence of NADPH, which is oxidized to $NADPH^+$. Plastic semi-micro-cuvettes containing 0.050ml of sample and 1.00ml of 100 mmol/ L potassium phosphate buffer pH 7.4 and 1 mmol/L EDTA, 0.10 ml of 50 mmol/L NADPH, and the reaction started. The changes in absorbance was determined per minute over 5 minutes (**DiIlio *et al.* 1983**).

Measurements of thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances were measured in plastic semi-micro-cuvettes containing 0.2ml sample and 1.0ml of 25 mmol/L 2-thiobarbituric acid, and detergent stabilizer for the sample, and 0.2ml of 10 nmol/L malondialdehyde standard and 1.0ml of 25 mmol/L thiobarbituric acid detergent stabilizer for standard (**Satoh, 1978**). The reagents were mixed in a stoppered test tube and heated in a water bath at 95°C for 30 minutes. The mixture was centrifuged at 2000 ×*g* for a few minutes, and the supernatant was measured in a spectrophotometer at 534nm. The absorbance was measured compared to a blank at the same wavelength and expressed as malondialdehyde (MDA) concentration.

Statistical analysis

The results were presented as means ± standard deviation. Before proceeding with the statistical analysis, the data were checked for variance homogeneity. After that, differences in mean values were examined using a one-way or two-way analysis of variance (ANOVA), followed by Tukey's multiple comparison range tests. When $P< 0.05$, differences were reported as statistically significant. The Graph Pad software was used for all statistical analyses.

RESULTS

Antioxidant enzymes activity and malondialdehyde concentration in the muscle

The results of the studied antioxidant enzymes activities, and thiobarbituric reactive substances, expressed as malondialdehyde content in the muscles are shown in Table (2). The highest activity of SOD was detected in the bony fish species *Saurida undosquamis* (250.43±4.02 U/g tissue) and *Scorpaena porcus* (247.09±7.05 U/g tissue), while the lowest activities were in the muscles of *Oedalechilus labeo* (13.57±2.70 U/g tissue) and the mollusk *Loligo vulgari* (21.43±3.10 U/g tissue).

The GR activity differing significantly among the species was studied. The highest activities were recorded in the muscles of the mollusk *Sepia officinalis* (7.89±0.68 U/g tissue) and the bony fish *Dicentrarchus labrax* (7.54±0.07 U/g tissue), while the lowest activities were in the species of *Seriola dumerili* (2.01±0.05 U/g tissue) and the dogfish *Squalus blainvillei* (3.26±0.64 U/g tissue).

Table 2. Antioxidant enzyme activities (U/g) and malondialdehyde content (nmol/g) in the muscle (mean ± SD; n=3)

Marine species	N	SOD	GR	GPx	MDA
Dentex dentex	3	50.33±3.41^{b}	6.60±0.66^{a}	9.33±0.58	451.83±97.6^{b}
Pagellus erythrinus	3	31.67±3.01^{b}	6.50±1.04^{a}	7.46±1.95	613.50±153.2^{a}
Sparus aurata	3	42.19±3.45^{b}	6.56±0.73^{a}	8.35±1.13	352.20±81.9^{b}
Mullus surmuletus	3	211.58±10.12^{a}	6.51±0.83^{a}	9.05±0.35	871.26±5.95^{a}
Oedalechilus labeo	3	13.57±2.70^{c}	4.58±0.77^{b}	8.13±0.75	698.93±103.5^{a}
Epinephelus marginatus	3	41.92±2.22^{b}	5.35±0.28^{a}	9.24±0.21	118.00±0.06^{c}
Epinephelus caninus	3	72.66±9.22^{b}	6.23±0.48^{a}	7.53±0.43	432.53±63.6^{b}
Scorpaena porcus	3	247.09±7.05^{a}	3.40±0.51^{b}	9.40±0.52	524.16±44.11^{a}
Dicentrarchus labrax	3	61.58±14.93^{b}	7.54±0.07^{a}	9.10±0.12	141.53±4.56^{c}
Euthynnus alletteratus	3	67.40±7.39^{b}	6.59±0.77^{a}	7.85±1.06	728.30±29.51^{a}
Saurida undosquamis	3	250.43±4.02^{a}	5.37±0.15^{a}	8.25±1.08	496.53±34.05^{b}
Seriola dumerili	3	132.70±4.28a	2.01±0.05^{b}	7.49±1.40	767.43±133.9^{a}
Squalus blainvillei	3	66.50±6.12^{b}	3.26±0.64^{b}	9.36±0.03	511.10±32.46^{a}
Loligo vulgari	3	21.43±3.10^{c}	6.47±0.90^{a}	7.43±1.62	352.40±45.44^{b}
Sepia officinalis	3	10.78±3.07^{c}	7.89±0.68^{a}	8.60±1.50	175.06±11.43^{c}
Ruditapes decussatus	3	56.94±8.66^{b}	7.46±0.07^{a}	9.42±0.11	651.13±10.42^{a}

Values within a column with different superscript letters are significantly different ($P < 0.05$).

No significant differences ($P< 0.05$) were detected in the GPx enzyme activity in the muscles of the studied species. The highest activities were found in the mollusca *Ruditapes decussatus* (9.42±0.1 U/g tissue) and *Scorpaena porcus* (9.40±0.52 U/g tissue), while the smallest activities were in *Loligo vulgari* (7.43±1.62 U/g tissue) and *Pagellus erythrinus* (7.46±1.95 U/g tissue).

The results of the lipid peroxidation (thiobarbituric acid reactive substances expressed as MDA) analysis of the muscles in different species showed the highest values in *Mullus surmuletus* (871.26±5.95 nmol/g tissue) and *Seriola dumerili* (767.43±133.9 nmol/g tissue), while the lowest values were in *Epinephelus marginatus* (118.0±0.06 nmol/g tissue) and *Dicentrarchus labrax* (141.53±4.56 nmol/g tissue).

Table 3. Antioxidant enzyme activities (U/g) and malondialdehyde content (nmol/g) in the liver (mean ± SD; n=3)

Marine species	**N**	**SOD**	**GR**	**GPx**	**MDA**
Dentex dentex	3	10.53±3.93^{a}	7.64±0.17	8.18±0.10	159.66±49.51^{a}
Pagellus erythrinus	3	69.27±2.05^{b}	7.21±0.05	7.99±0.16	386.40±22.09^{b}
Sparus aurata	3	73.39±3.02^{b}	7.26±0.30	9.85±0.15	561.36±43.99^{b}
Mullus surmuletus	3	117.99±8.20^{b}	7.30±0.18	7.25±1.48	235.50±25.25^{b}
Oedalechilus labeo	3	45.11±4.47^{a}	6.79±0.08	7.72±1.19	158.70±3.26^{a}
Epinephelus marginatus	3	104.03±7.46^{b}	6.62±0.71	8.46±0.47	257.73±9.70^{b}
Epinephelus caninus	3	80.31±21.60^{b}	6.50±0.39	7.55±0.39	108.00±0.07^{a}
Scorpaena porcus	3	140.41±7.03^{b}	6.91±0.07	7.99±0.10	69.10±20.11^{a}
Dicentrarchus labrax	3	62.25±15.39^{a}	7.64±0.05	8.13±0.17	238.00±0.58^{b}
Euthynnus alletteratus	3	101.32±3.08^{b}	6.30±0.32	8.66±1.52	210.00±0.25^{b}
Saurida undosquamis	3	40.06±13.80^{a}	7.58±0.15	9.00±1.00	494.00±1.30^{b}
Seriola dumerili	3	79.33±3.30^{b}	6.07±0.40	8.47±1.25	397.00±30.21^{b}
Squalus blainvillei	3	141.32±8.78^{b}	7.58±0.36	8.27±1.55	290.00±1.56^{b}

Values within a column with different superscript letters are significantly different ($P< 0.05$)

Antioxidant enzymes activity and malondialdehyde concentration in the liver

Table (3) presents the results of the liver's antioxidant enzymes and malondialdehyde contents. The highest activities of SOD were found in the dogfish species *Squalus blainvillei* (141.32±8.78 U/g tissue) and *Scorpaena porcus* (140.41±7.03 U/g tissue). In contrast, *Dentex dentex* (10.53±3.93 U/g tissue) and the bony fish *Saurida undosquamis* (40.06±13.80 U/g tissue) recorded the lowest SOD activity in the liver.

The GR activity in the studied species was not significantly different ($P> 0.05$). The highest GR activity was found in the liver of *Saurida undosquamis* (7.58±0.15 U/g tissue) and the bony fish *Dentex dentex* (7.64±0.17 U/g tissue). In contrast, *Seriola dumerili* (6.07±0.40 U/g tissue) and *Euthynnus alletteratus* (6.30±0.32 U/g tissue) had the lowest GR activity.

There were no significant differences in GPx enzyme activity in the liver between the studied species. However, the bony fish *Sparus aurata* (9.85±0.15 U/g tissue) and *Saurida undosquamis* (9.00±1.00 U/g tissue) showed the highest GPx activity, while *Mullus surmuletus* (7.25±1.48 U/g tissue) and *Epinephelus caninus* (7.55±0.39 U/g tissue) had the lowest activity.

The lipid peroxidation analysis (thiobarbituric reactive acid substances expressed as MDA) of livers from different species revealed significant differences. *Sparus aurata* (561.36±43.99 nmol/g tissue) and *Saurida undosquamis* (494.00±1.30 nmol/g tissue) had the highest values, while *Epinephelus caninus* (108.00±0.07 nmol/g tissue) and *Oedalechilus labeo* (158.70±3.26 nmol/g tissue) had the lowest values.

DISCUSSION

Most countries including Libya rely heavily on marine organisms for protein, fats and vitamins. Therefore, the study's primary goal was to investigate the effects of habits and trophic-environmental behavior on prospective biomarkers in the muscle and liver of several marine species, thereby improving our current understanding of the role of antioxidant enzymes in the oxidative stress for some marine species. Furthermore, abiotic environmental variables such as temperature, oxygen ratios, pH, salinity and pollution, as well as biotic biological components such as feeding behavior and ecological behavior, are the most important indicators of antioxidant enzyme changes in organisms (**Teimouri *et al.*, 2019; Wu *et al.*, 2021**).

Individual fish behavior changes due to changes in the marine environment and internal physiological variables. Learning about the eating habits of various types of fish is one of the first steps in determining their style and nutritional needs. Fish in deep-sea waters, for example, in this study, *Saurida undosquamis*, have different habits and behaviors than those near the coast, and activity in deep-sea waters includes swimming, feeding, reproduction and breathing. Like all living marine organisms, fish require a lot of food, and thus a lack of food may increase the antioxidant defense system's activity and the rate of lipid peroxidation. Food scarcity can be caused by competition between marine organisms or by climatic changes, which increase the stress on energy-generating biochemical mechanisms and the formation of oxygen free radicals in response to the body's nutritional needs (**Hidalgo *et al.*, 2017; Zhang *et al.*, 2022**).

Experiments on animals reveal a difference in antioxidant defense and lipid peroxidation in muscle and liver. For example, *Pagellus erythrinus* showed high MDA concentration, but low SOD, GR and GPx activities, which means improper antioxidant defence in this species. SOD, GR, and GPx activities were higher in *Dentex dentex* muscle as previously described (**Morales *et al.*, 2004; Perez-Jimenez *et al.*, 2017**), but MDA content was not the lowest, which means moderately high oxidative stress because the higher antioxidant enzyme activity was not adequate to inhibit lipid peroxidation.

SOD and GPx activities, as well as MDA level were higher in *Sparus aurata*, while GR activity was higher in *Dentex dentex*. SOD activity and MDA content in the liver of *Dentex dentex* were also low, indicating that antioxidant enzymes other than SOD have importance against ROS-initiated lipid peroxidation. *Pagellus erythrinus* is a carnivorous fish with a high percentage of fats and vitamins in its diet, as well as increased glucokinase (GK) and pyruvate kinase activities (**Cherkas *et al.*, 2020**), which may result in increased non-enzymatic antioxidant levels in the muscle and lower level of antioxidants in the liver. Unlike *S. aurata* relies on herbivory for a portion of its diet, with low or moderate carbohydrate and vitamin levels; therefore, a higher rate of ROS formation would be possible. This could explain the high level of antioxidant enzyme activity against oxidative stress (**Polakof *et al.*, 2008; Moon, 2001**). Some studies suggest that the quality of nutrition and its availability in the environment may cause excess free radical formation in fish (**Wu *et al.*, 2020**).

Human activities impact aquatic ecosystems by changing temperature, oxygen levels, salinity and food availability; seasonal fluctuations affect fish, and the constant rise in temperature and salinity caused by climate change and nutritional behavior may lead to environmental change. For example, the Mediterranean Sea's surface temperature has risen by 0.4^{o}C per decade (**Mengual *et al.*, 2021**). Environmental constraints may also account for differences in enzyme activities among fishes. We should note that *Scorpaena porcus* had higher SOD and GPx activities, and *Dicentrarchus labrax* had higher GR activity than other species, probably due to the activation of different stages of antioxidant defense. Still, *Euthynnus alletteratus* had a higher MDA level, which means improper antioxidant defense or higher oxidative stress. In contrast, *Dicentrarchus labrax* had a lower SOD activity and a MDA content in the muscle, which means that SOD has less importance against ROS-initiated lipid peroxidation. On the contrary, in the liver, SOD activity and MDA content were higher in *Scorpaena porcus*, GR activity was higher in *Dicentrarchus labrax*, and GPx activity was higher in the *Euthynnus alletteratus*, while GR activity and MDA content showed the lowest in *Euthynnus alletteratus* as was found previously (**Sinha *et al.*, 2015**). These results suggest that feeding habits and environment affect oxidative stress and antioxidant defense in different fish species which can be proved by the different feeding types and trophic behavior among the studied species. The most significant higher values of SOD activity in *Scorpaena porcus* and lower values in both *Dicentrarchus labrax* and *Euthynnus alletteratus* could be attributed to climatic changes and their impact on *S. porcus* feeding behavior and living environment, which are increasing the antioxidant defense and the rate of lipid peroxidation (**Nakano *et al.*, 2014; Bal *et al.*, 2021; Nasef, 2021**).

On the Benghazi coast, waste remnants and the odor of urea from sewage can be found, which has become a widely recognized indicator of contamination of human origin. Pollutants include garbage and marine navigation residues, demonstrating humans' negative environmental impact. Seawater pollution contributes to the formation of

ecologically persistent free radicals (**Diarra & Prasad, 2021**). The increased antioxidant enzyme activities and the rate of lipid peroxidation in muscle and liver are probably due to the deposition of waste from plastic and other residues on the bottom. In contrast, *Oedalechilus labeo* lives in open water; therefore, less affected by bottom pollution.

In the Serranidae family, higher SOD, GR, GPx activity and MDA content were found in the *Epinephelus caninus* muscle. In addition, the liver of *Epinephelus marginatus* showed high SOD, GR, and GPx activities and MDA content according to **Francisco *et al.* (2020)**. Although the two species' environments are similar, the high level of antioxidant enzymes and MDA in *Epinephelus marginatus* is due to food diversity. These species consume various foods, ranging from small crustaceans to fish.

The high mineral concentration of human waste from industrial and agricultural operations can cause the high mineral content of the cells. High mineral content can cause oxidative stress and severe cell damage (**Al Naggar *et al.*, 2018**). SOD and GR activities were higher in *Saurida undosquamis*. *Ruditapes decussatus* muscle had higher SOD and GPx activities and MDA content in the liver, as confirmed in previous studies (**Geret *et al.*, 2003; Banni *et al.*, 2009**). The muscle of *Sepia officinalis* had significantly lower SOD, GR, GPx activities and MDA content than *Loligo vulgaris*. *Ruditapes decussatus* recorded high antioxidant enzyme activity and malondialdehyde content due to waste and environmental toxicants, causing increased phytoplankton production. Toxic microalgae blooms, phosphorous, agricultural fertilizer run-off and population sanitation inputs have measurable effects. To assess the effects of environmental factors, the best biomarker was *Ruditapes decussatus* according to the results of this study.

Cartilaginous fish contribute significantly to the functional diversity of marine life. They are a popular species among Benghazi residents, and demand on them is increasing. Fishing boats use nets to catch cartilaginous fish. These fish can be found off the coast of Benghazi at depths of more than 40 meters. To preserve market freshness, some fishing vessels keep them alive in microfibre fishing nets for extended periods, resulting in stress responses from inappropriate fishing and a lack of fishing ethics (**Xie *et al.*, 2021**). Trawls pose a significant threat to marine species and demersal fish, either directly or indirectly, through interactions with fishing nets (**Castro & Van Waerebeek, 2019; Kartal & Sarıışık, 2022**). It should be noted that, in cartilaginous fish muscle, SOD and GPx activities in *Squalus blainvillei* were high. The main reason for the high antioxidant enzyme activity and lipid peroxidation rate is catching and holding the fish in the net. Pressing the fish to stay in the net for a while may activate the antioxidant defense system and increase the rate of lipid peroxidation due to stress.

The findings in the muscles suggest that, the rate of lipid peroxidation in the muscles is higher than in the liver. Two enzymes, GR and GPx, were directly connected in most of the species studied. Furthermore, the increased SOD activity was associated with the increased activity of other antioxidant enzymes.

CONCLUSION

Overall, the current study revealed that the antioxidant enzymes increased in response to oxidative stress, trophic behavior and habitats in different marine organisms. These findings imply that the antioxidant system's capability varies by species and may be related to the ecology of the species. Further research should be conducted on improving the mechanisms of enhancing antioxidants and finding solutions to reduce the high levels of enzymes that may cause long-term damage to marine diversity, resulting in the decline or extinction of the primary food source for much marine fish. This may result in a decrease in the number of these species, or alternatively, their immigration to another location, which also negatively affects marine diversity.

ACKNOWLEDGMENTS

The authors thank the crew of Benghazi Star Fishing vessel and Ahmad Almalah for his help in the fieldwork and in the laboratory. Many thanks to the reviewers and the editor for their constructive comments that helped improve the manuscript.

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