Monitoring the Health State of Some Species from the Egyptian Mediterranean Coast North of Alexandria Caught by Trawling Net

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ABSTRACT

Man's adverse impacts on the aquatic environment are constantly present. The purpose of this study was to determine the health state of five fish species caught by trawl net, including *Argyrosomus regius*, *Solea vulgaris*, *Sparus aurata*, *Nemipterus randalli*, and *Eutrigla gurnardus* that showed a rise in the Egyptian catch ratio and became recognized as among commercial species in the recent ten years by trawl net from the Mediterranean coast of Egypt, northern Abu Qir Bay, during the winter of 2023. The concentrations of heavy metals (Cr, Pb, As, and Cd) were investigated in muscle tissues by using ICP-OES inductively coupled plasma atomic emission spectroscopy. The results showed that the accumulation of the examined metals was below the standard maximum permissible limit. MPI decreased in the order *S. vulgaris*> *S. aurata* > *N. randalli* > *E. gurnardus* > *A. regius*. The enzyme activities of Na^+/K^+ and Ca^{2+}ATPase showed significant differences ($P< 0.05$) between the examined species. The highest activity appeared in liver tissue, followed by brain and kidney tissues, then gill, with muscle showing the least activity. This was done to investigate the status of ion regulation and energy state. Oxidant variables and antioxidants in muscle tissues revealed a significant elevated difference ($P< 0.05$) in lipid peroxidation content (LPO), GPX, SOD, and CAT between *S. vulgaris* and *S. aurata* comparable to the other species and a significant reduction in total thiol (T-SH) and protein thiol (P-SH). This study demonstrated the impact of the health state of some species of trawl net catch in the presence of environmental multi-stressors to illustrate and increase the precision of the monitoring ecological consequences of environmental changes.

INTRODUCTION

Overall organisms in natural ecosystems face a variety of simultaneous environmental stressors which alter with location and time. Deciphering the intricate relationships between environmental stressors (natural or artificial) and evaluating their causal effects on organisms is necessary to fully appreciate the effects of environmental variation on individual health as well as the long-term effects on population health and dynamics (**Doney *et al*., 2012**). Environmental changes can negatively impact fish life history features and fitness, resulting in long-lasting changes to basal metabolic rate. Anthropogenic or environmental disruptions can lead to long-term alterations in physiological functions. Most physiological biomarkers offer early warning indicators of

declining environmental conditions, relapses, and recovery dynamics, which may be advantageous to overfished populations **(Killen *et al*., 2013; Killen, 2014)**. Physiological states and environmental influences likely have stronger correlations than common morphometric markers such as growth rate or body condition indices. Numerous physiological conditions considered as early warning systems, encourage proactive fisheries management and provide management with information on the mechanisms underlying observed population trends and finally support conservation efforts aimed at preserving the marine ecosystem and biodiversity, which are essential to human health as well as economic and social activities associated with the marine environment **(Brosset *et al*., 2021)**.

The study of complex ecosystems through ecology can often be completed in a few steps by assessing the impact of a small number of carefully selected variables on the health and fitness of individual organisms **(Doney *et al*., 2012; Schull *et al*., 2023)**. However, because interactions among numerous ecological stresses (both internal and external to the organism) may induce interactive repercussions, the overall impact of diverse environmental pressures on individual organisms may either surpass or fall short of their projected cumulative effects. This may differ from the limited impact of the single-stressor-based models **(Crain *et al*., 2008; Côté *et al*., 2016)**. Therefore, multifactorial studies are essential to assess the combined effects of different environmental pressures on particular species **(Rosa & Seibel, 2008; Brosset *et al*., 2021)**. Integrative modeling approaches consider the potential direct and indirect causal pathways by which environmental variables may impact the health and fitness of organisms. Estuaries and coastal areas are examples of dynamic ecosystems where these strategies could be very helpful. These regions of great biological productivity are located where the open sea and land meet. In addition to receiving significant inputs of organic matter and nutrients from human and natural sources, such as agricultural and urban runoff, they also act as repositories for pollutants and other dangerous materials **(Losso & Ghirardini, 2010)**. The significance of physiological processes for an individual's fitness and health performance cannot be overstated **(Williams *et al*., 2008; Bryndum-Buchholz *et al*., 2019)**. Therefore, there is an urgent need for multifactorial studies that assess the cumulative effects of numerous environmental stresses on various organisms **(Brosset *et al*., 2021; Schull *et al*., 2023)**.

The meagre *(Argyrosomus regius,* Asso, 1801) are found in both inshore and offshore shelf areas, typically between 15 and 100 meters deep **(Griffiths & Heemstra, 1995)**. It is marine, brackish, benthopelagic oceanodromous **(Riede, 2004)**, and its depth ranges from 15 - 300m. They feed on fishes and swimming crustaceans (**Schneider, 1990; Kır *et al*., 2017**). The gilthead seabream (*Sparus aurata*, Linnaeus 1758) is considered a carnivore fish. It consumes a wide range of food organisms such as shellfish, mussels, and oysters favoring gastropods **(Pita *et al*., 2002)**. It is marine,

brackish, and demersal, and its depth ranges from 1- 150m (**Muus & Nielsen, 1999**). *Solea vulgaris* (Quesnel, 1806) are fish species classified as flatfishes in marine shallow water (**Nelson, 1976**). *Solea vulgaris* eats a wide variety of prey, including copepods, polychaetes, algae, seagrasses, mollusks, and amphipods (**El-mor & Ahamed, 2008**). The Randall's threadfin bream (*Nemipterus randalli*) is one of the lessepsian species that migrated from the Red Sea to the Mediterranean Sea via the Suez Canal. In the Northeastern Mediterranean Sea, it affected the food web dynamics in a coastal ecosystem and boosted the commercial value of the fisheries (**Akgun & Akoglu, 2023**). It is non-migratory, marine, and demersal, with a depth range of 20 to 450 meters (**Goldshmidt *et al*., 1996**). The grey gurnard *(Eutrigla gurnardus*, Linnaeus, 1758) is a marine, brackish, demersal fish found at depths between 10 and 340 meters (**Mytilineou *et al*., 2005**). They primarily eat prawns and shore crabs as well as fish, including gobies, flatfish, juvenile herring, and sand eels (**Viler, 1983; Frimodt, 1995; Vinogradov *et al*., 2014**).

The purpose of this study was to assess the health of *Argyrosomus regius, Sparus aurata, Solea vulgaris, Nemipterus randalli,* and *Eutrigla gurnards* in a multi stressor environment

MATERIALS AND METHODS

Location of study

The examined samples were collected in January 2023 during the winter season. By the bottom trawler caught five species: *Argyrosomus regius, Solea vulgaris*, *Sparus aurata*, *Nemipterus randalli,* and *Eutrigla gurnardus*; these five species showed a rise in the Egyptian catch ratio and became recognized as among commercial species in the recent ten years. The sampling locations were limited in the range of 10- 100 meters depths in Alexandria, Egypt's Northern Abu Qir Bay (Fig. 1). The El-Tabia pumping station exposes Abu-Qir Bay, which is situated in the eastern region of Alexandria and is regarded as a significant fishing location, to industrial and agricultural wastes (**El Nemr *et al*., 2012**).
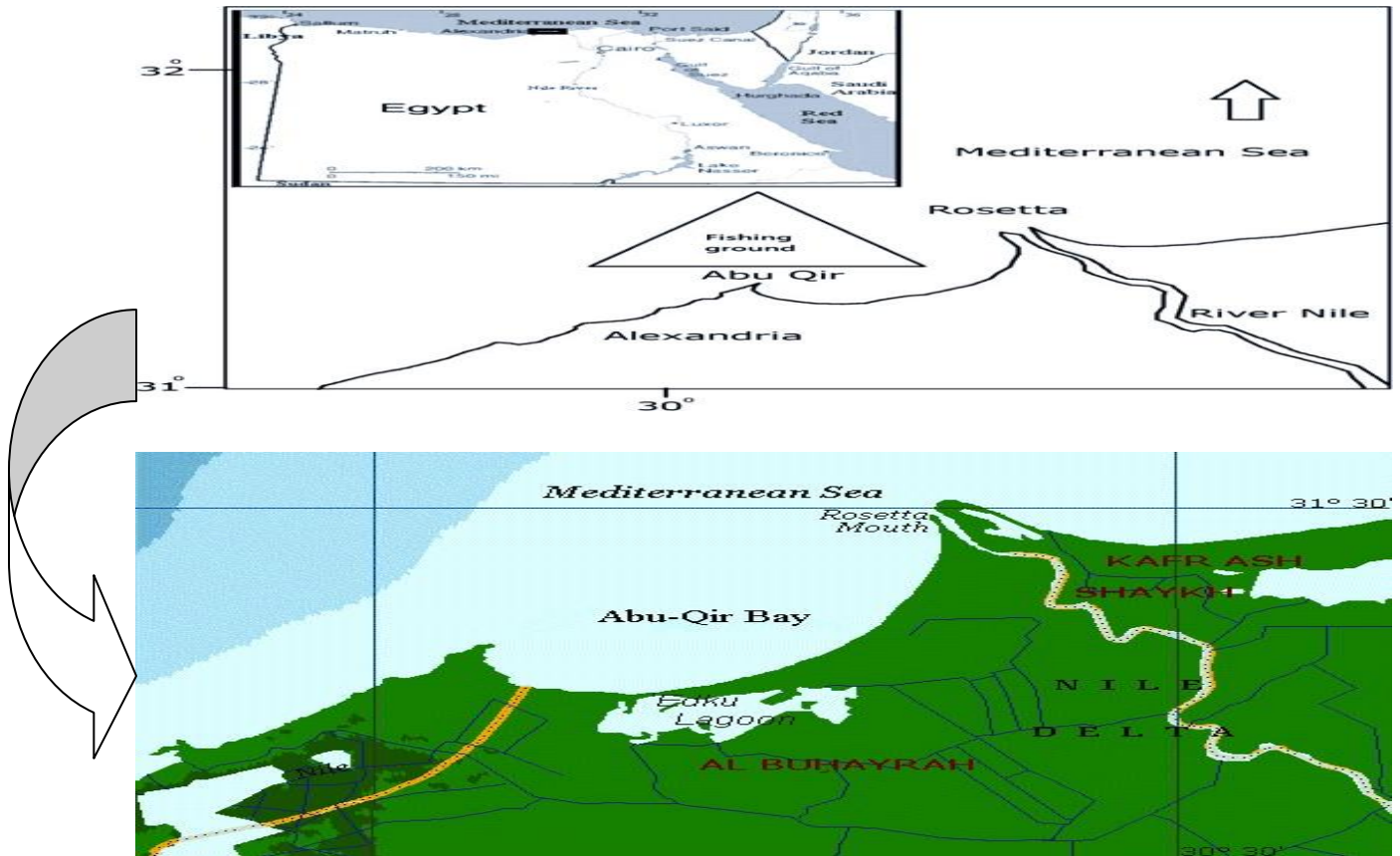
Fig. 1. Map of Northern Abu Qir Bay, Alexandria, Mediterranean Sea, Egypt, where fishing samples were collected using trawling nets (El Nemr *et al*., 2012; El Haweet, 2013)

Samples collection

Five fish species—*Argyrosomus regius* (length range: 38- 55cm), *Solea vulgaris* (length range: 12- 26 cm), *Sparus aurata* (length range: 16- 25cm), *Nemipterus randalli* (length range: 17-23 cm), and *Eutrigla gurnardus* (length range: 15- 22cm)—were randomly collected during the winter of 2023 using an appropriate fishing gear with the assistance of local fishermen. Samples were transported in ice-cold boxes to the laboratory and frozen at -20°C for analysis.

Argyrosomus regius   *Nemipterus randalli*

Eutrigla gurnards   *Sparus aurata*

Solea vulgaris

Ethical clearance

The author declares that the sampling of this species under investigation was done following the guidelines of the international conventions for the use of animals in scientific research.

Samples preparation

The skin was quickly and carefully peeled off after the frozen samples had defrosted and been cleaned in deionized water. The brain, liver, kidney, gills, and white dorsal muscles were quickly and thoroughly isolated. The tissues were then weighed and cleaned in an isotonic NaCl saline solution. For an antioxidant examination, the tissue homogenate (10% w/ v) was prepared using an electrical homogenizer in a ratio of 1–9 times ice-cold phosphate buffer (0.1M), pH 7.4.

Calculating the metal pollution index (MPI) and heavy metal concentrations

The content of heavy metals in the fish under examination's white dorsal muscles was calculated. 0.5g of the muscle tissue samples were dissolved at $85^{o}C$ in a 5ml concentrated HNO3 solution until the process was completed. The filter paper was used to filter samples and diluted up to 25 milliliters of distilled water (**Mohamed *et al*., 2017**). The metals were examined using an Agilent 5100 VDV ICP-ES instrument. The inductively coupled plasma was used in ICP-OES's inductively coupled plasma atomic emission spectroscopy.

The total quantity of metal content in various fish species was compared using MPI using the equation provided by (**Usero *et al*., 1997**):

$$MPI= (M_1 X M_2 X M_3 X \ldots\ldots X Mn)\ 1/n$$

Where, Mn is the metal concentration (µg/ g wet tissues), and n is the number of metals studied.

Ca^{2+}ATPase and Na^+/K^+ Activities

Tissues from the gill, white dorsal muscle, kidney, liver, and brain were quickly and meticulously separated and cleaned in normal saline. Samples were weighed and homogenized by a homogenizer set at 35,000rpm in an 11:10 w:v 65mM L−1 imidazole buffer (pH 7.4). To quantify the amount of inorganic phosphate (Pi) released as the activity of ATPases in the crude homogenate during the hydrolysis of the substrate ATP at 25ºC, as reported by a method of **Üner et al. (2005)**. Additionally, the incubation medium was prepared according to the method of **Ames and Dubin (1956)**.

Lipid peroxidation quantification

The terminal product produced in the breakdown of polyunsaturated fatty acid-mediated by free radical Malondialdehyde (MDA) was quantified as thiobarbituric acid reactive substances (TBARS) according to the method of **Buege and Aust (1978)**.

Assessment of the antioxidant activity of muscle tissue

The investigated samples were analyzed to ascertain the level of Superoxide Dismutase (SOD) activity in the muscle tissue supernatant **(Paoletti & Mocali, 1990)**. The catalase (CAT) concentration in the muscle supernatant was measured using the **Aebi (1984)** method. Glutathione peroxidase (GPx) activity was determined using the **Paglia and Valentine (1967)** method. The levels of total thiol (T-SH) and protein thiol (P-SH) in muscle tissue were assessed according to **Sedlak and Lindsay (1968)**.

Statistical analysis

After one-way ANOVA was performed for data analysis, GraphPad Prism version 5.0 was used for multiple Tukey test comparisons, and *P*-values <0.05 were considered statistically significant. Each reading reflects the mean values ± SD.

RESULTS

Concentrations of heavy metals (µg/g wet weight) in muscle tissue

In Table (1) the accumulation of Cd revealed non-significant differences (*P* >0.05) among the examined species but revealed significant differences (*P*< 0.05) in the accumulation of Pb, As, and Cr between *Solea vulgaris , Sparus aurata,* and other examined species. The means of accumulation of Cd ranges from 0.005- 0.007µg/ g wet weight tissues, with all values below the international standards, and they decreased in

the order *Eutrigla gurnardus> Solea vulgaris > Sparus aurata > Argyrosomus regius>Nemipterus randalli.* The accumulation of Pb in muscle ranged from 0.050-0.098, with all values below the international standards, and they decreased in the order *Solea vulgaris > Sparus aurata > Eutrigla gurnardus > Argyrosomus regius > Nemipterus randalli.* The means accumulation of As in muscle tissues ranges from 0.30 - 0.63µg/ g wet weight tissues, with all values below theinternational standards, and they decreased in the order *Solea vulgaris> Sparus aurata > Eutrigla gurnardus > Argyrosomus regius > Nemipterus randalli.* The accumulation of Cr in muscles ranges from 0.17- 0.36, with all values below the international standards, and they decreased in the order *Solea vulgaris > Sparus aurata > Nemipterus randalli > Eutrigla gurnardus> Argyrosomus regius.* MPI decreased in the order *Solea vulgaris>Sparus aurata > Nemipterus randalli > Eutrigla gurnardus > Argyrosomus regius.*

Table 1. The accumulation of heavy metal concentrations (µg/g wet weight) in fish species' muscle tissue with the metal pollution index and the global maximum permissible limits (MPLs)

Species	Cd	Pb	AS	Cr	MPI
Argyrosomus regius	0.006±0.0001	0.053±0.002	0.31±0.001	0.17±0.003	0.064
Solea vulgaris	0.0069±0.0002	0.098*±0.003	0.63*±0.004	0.36*±0.002	0.111
Sparus aurata	0.0064±0.0001	0.078*±0.002	0.55*±0.001	0.33*±0.004	0.098
Nemipterus randalli	0.005±0.0001	0.050±0.001	0.30±0.003	0.31±0.003	0.069
Eutrigla gurnardus	0.007±0.0001	0.064±0.005	0.32±0.002	0.21±0.001	0.074
FAO (1983)	0.05	0.5	0.12-1.0	1.0	
EC (2006)	0.05	0.3	-	-	
FAO/WHO (1989)	0.1	0.5	-	-	
FAO(2003)	0.05	0.2	-	-	
USFDA (1993)				12-13	

Values represent the mean ± SD., (*) significant differences.

Na$^+$ /K$^+$, Ca^{2+}ATPase activities

The enzyme activities of Na$^+$ / K$^+$ showed significant differences ($P< 0.05$) between the examined species (Fig. 2). The highest activity appeared in liver tissue, followed by brain and kidney tissues, then gill, with muscle showing the least activity. The enzyme activity of Ca^{2+}ATPase showed significant differences ($P< 0.05$) between the examined species (Fig. 3). The highest activity appeared in liver tissue, followed by brain and kidney tissues, then gill, with muscle showing the least activity.
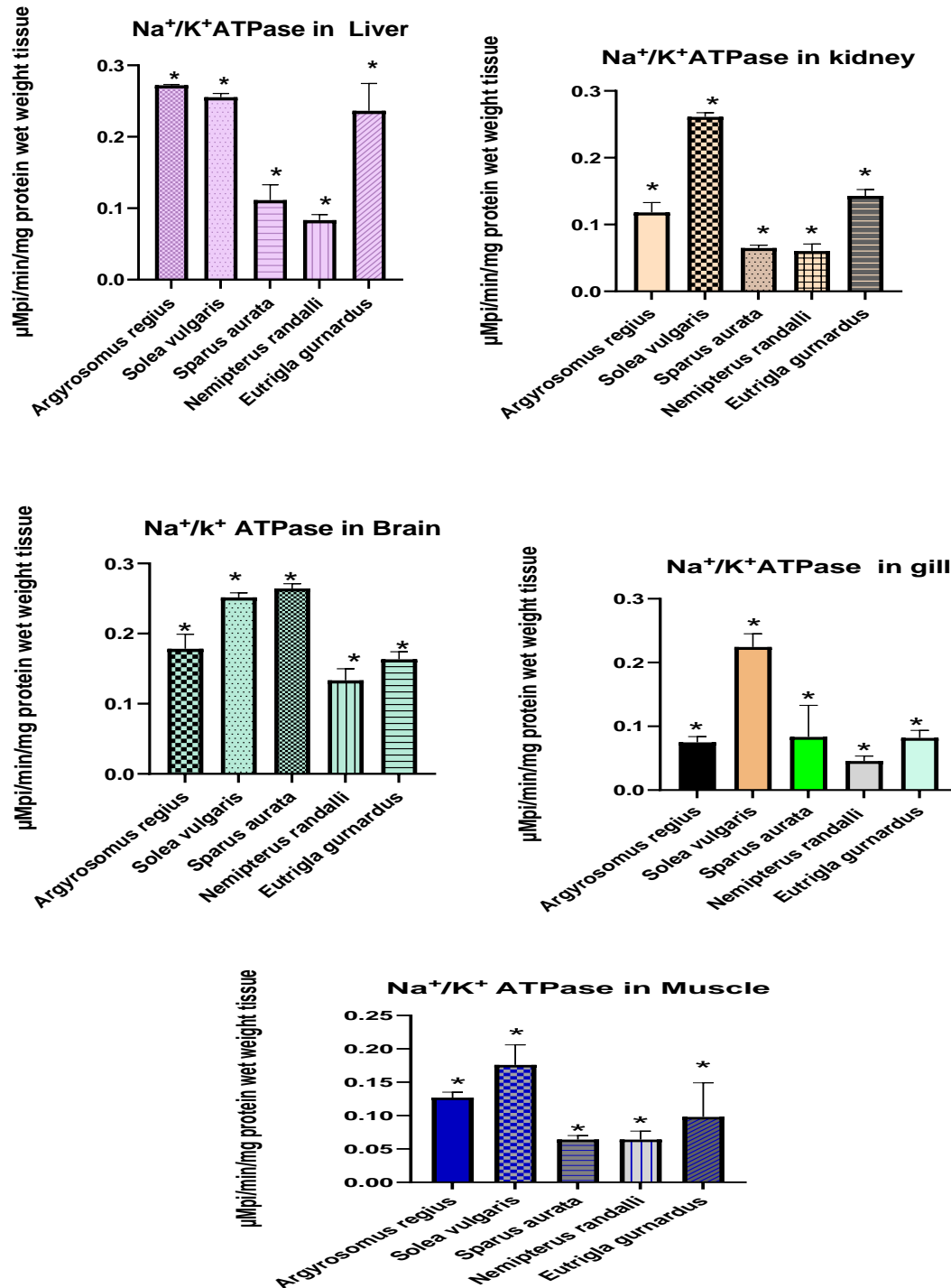
Fig. 2. The activity of Na$^+$/K$^+$ ATPase in white dorsal muscle, kidney, brain, gill, and liver tissues for five species: *Argyrosomus regius* , *Solea vulgaris*, *Sparus aurata*, *Nemipterus randalli*, and *Eutrigla gurnardus*. The bottom trawl net samples. The sampling locations were limited to 10- 100m depths in Alexandria, Egypt's Northern Abu Qir Bay. Each reading represents the Mean ± SD, n=6 fish. Significant at *P*< 0.05 between 5 species
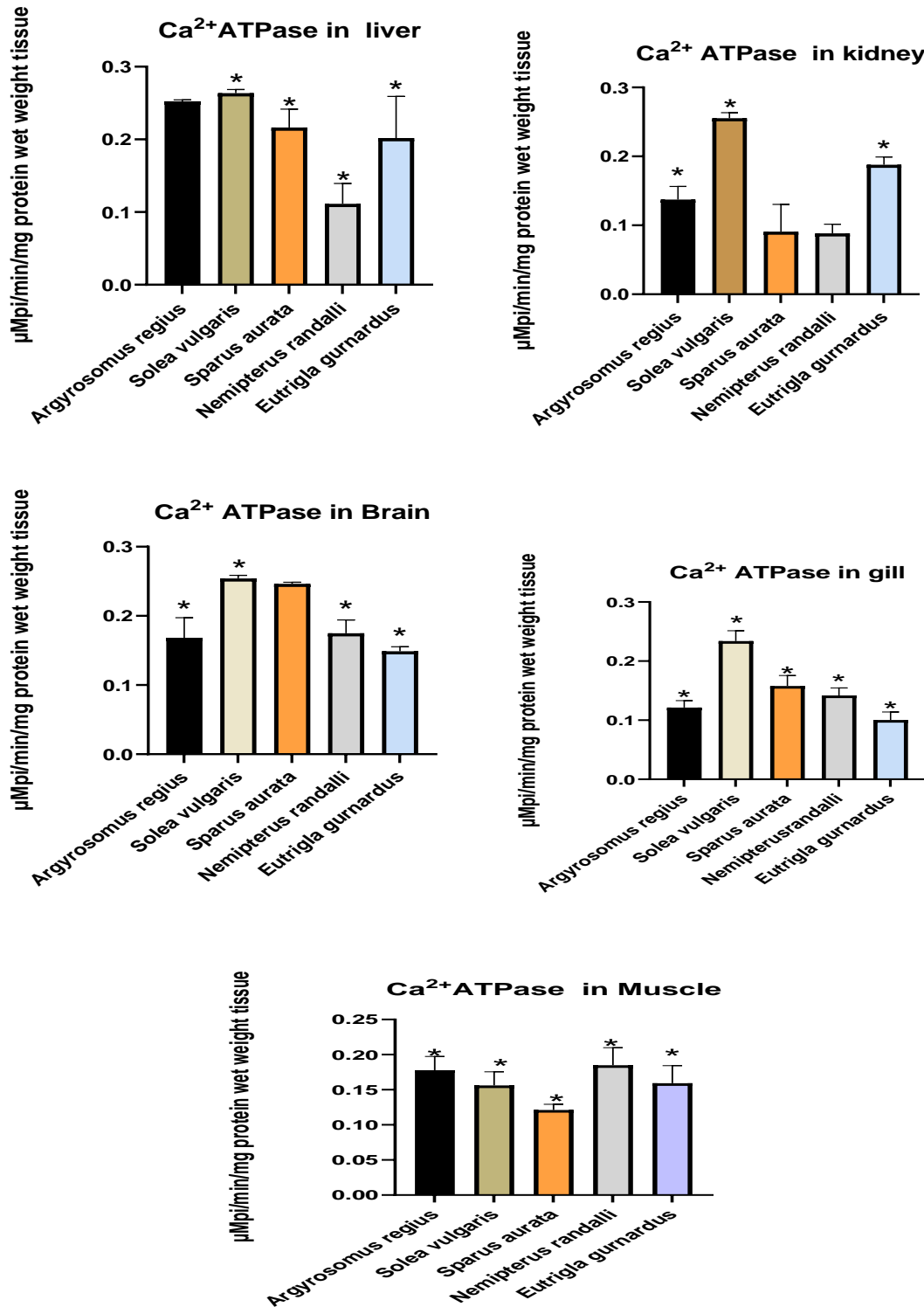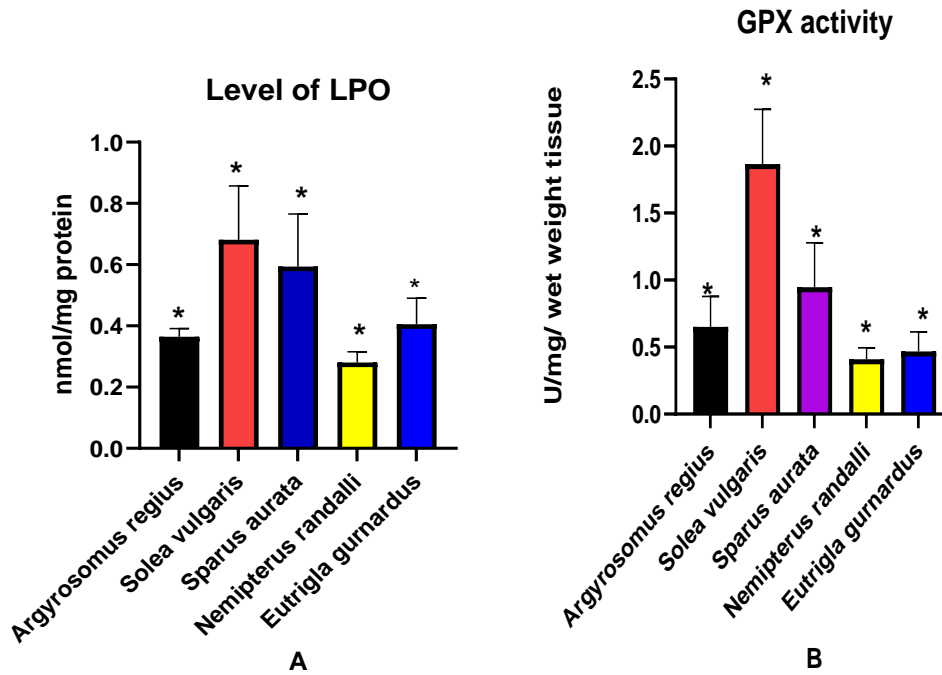
Fig. 3. The activity of Ca^{2+}ATPase in liver, brain, kidney, gill, and dorsal muscle tissues for five species: *Argyrosomus regius*, *Solea vulgaris*, *Sparus aurata*, *Nemipterus randalli*, and *Eutrigla gurnardus*. The bottom trawl net samples. The sampling locations were limited to 10- 100m depths in Alexandria, Egypt's Northern Abu Qir Bay. Each reading represents the Mean ± SD of n=6 fish. Significant at *P*< 0.05 between 5 species
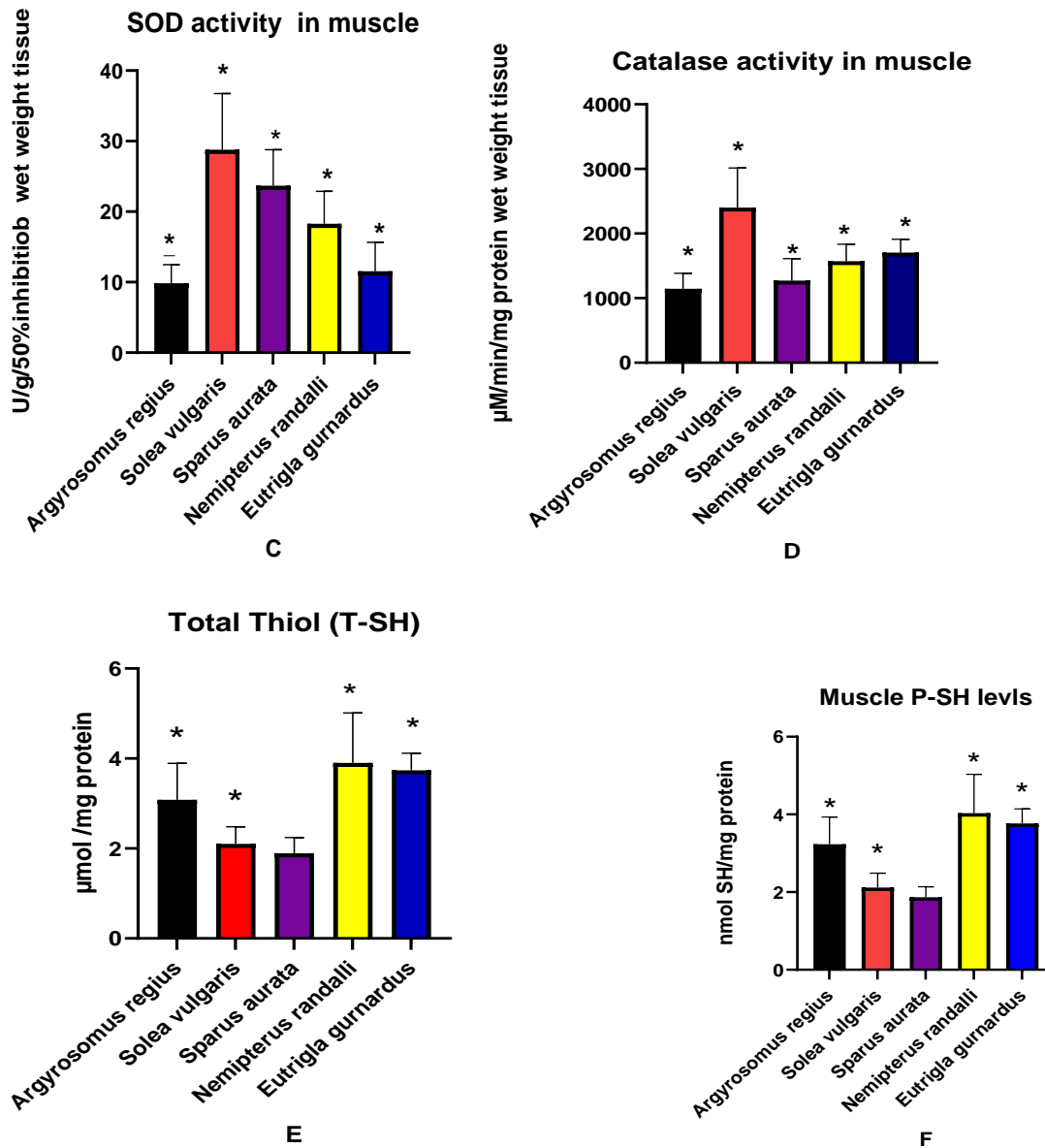
Oxidant variables and antioxidant activities

Muscle level of LPO showed a significant difference ($P< 0.05$) between the examined species (Fig. 4). The highest level of LPO appeared in *Solea vulgaris,* followed *by Sparus aurata.* The response activities of antioxidant enzymes GPX, SOD, and CAT showed significant differences ($P< 0.05$) between the examined species The highest activities of GPX, SOD, and CAT appeared in *Solea vulgaris,* followed by *Sparus aurata*. Muscle levels of non-enzymatic antioxidant content of T-SH, and P-SH showed significant differences ($P< 0.05$) between the examined species. The least levels of T-SH and P-SH appeared in *Solea vulgaris,* followed by *Sparus aurata*.

Fig. 4. Oxidant variables and antioxidant status in muscle tissues for five species: *Argyrosomus regius*, *Solea vulgaris*, *Sparus aurata*, *Nemipterus randalli*, and *Eutrigla gurnardus*. (A) Level of LPO, (B) GPX activity, (C) SOD activity (D) CAT activity, level of T-SH, and level of muscle P-SH. The bottom trawl net samples. The sampling locations were limited to 10- 100m depths in Alexandria, Egypt's Northern Abu Qir Bay. Each reading represents the Mean ± SD, n=6 fish. Significant differences appear between 5 species at *P*< 0.05

DISCUSSION

There were differences in the concentrations of the studied metals between the examined species. MPI decreased in the order *Solea vulgaris> Sparus aurata > Eutrigla gurnardus > Nemipterus randalli > Argyrosomus regius*. The highest accumulation appeared in *Solea vulgaris* followed by *Sparus aurata* probably due to the difference in feeding habitats among the examined species. These results are similar to the study of **Salem and Ayadi (2016)**, in which *Solea vulgaris* revealed the highest accumulation of metal in comparison to *Diplodus annularis* and *Liza aurata* from the southwestern Mediterranean. Aquatic animals are a part of the food chain and can become contaminated by heavy metals **(Perugini *et al*., 2014)**. The toxicity of heavy metals and the possibility of their accumulation in the biota are significant issues for both human health and environmental protection. According to **Nabavi *et al*. (2013)**, the species differences in heavy metal concentrations may have been caused by differences in size, classification, biological zones, and trophic levels.

The contents of Cd

Cadmium (Cd) is regarded as a very toxic non-essential heavy metal that is not required for the biological functions carried out by living things. Accordingly, even at low concentrations, Cd may be harmful to living beings (**Tsui *et al*., 2004)**. Cd levels in the present study ranged from 0.005- 0.007µg/ g wet weight tissue, generally below all international standards and below the ranges in the literature, 0.03- 0.52µg/ g for muscle from the Egyptian Mediterranean Sea **(EL-Moselhy, 1996)**, 0.02- 0.24µg/ g for muscles of fish from the Black Sea coasts **(Topcuoğlu *et al*., 2002)**, 0.3- 0.12µg/ g for muscles from the Tuzla Lagoon, Mediterranean Sea region **(Dural *et al*., 2007)**, 0.09- 0.27µg/ g for muscle tissues for fish from the Egyptian Mediterranean Sea µg/ g wet weight **(Hussein & Khaled, 2014)** and below **(Karim *et al*., 2016)**, 0.71- 2.48×10^{-3} mg/ kg of dry matter caught on the Mediterranean coast from the North East of Morocco.

• The contents of Pb

Fish are susceptible to the non-essential metal lead (Pb). Fish that are exposed to high levels of Pb experience negative effects on their development, metabolism, growth rates, and survival. They also produce more mucus. Pb may have detrimental health effects such as neurotoxicity and nephrotoxicity **(Ylmaz *et al*., 2010)**. This is because Pb and Ca ions have similar deposition and mobilization from bones, as previously discovered by **Moore and Ramamoorthy (1984)**. In our study, Pb levels ranged from 0.050- 0.098µg/ g for muscle tissues below all international standards and below Pb levels in the literature, have been reported in the range of 0.22- 0.85µg/ g for fish from the middle Black Sea **(Tuzen, 2003)**, 0.33- 0.93µg/ g for fish from the Black and Aegean eas for muscles **(Uluozlu *et al*., 2007)**, and from 98.9 to 60.65×10^{3} mg/ kg of dry matter for fish from the Mediterranean coast in the northeast of Morocco **(Karim *et al.,* 2016)**.

• The contents of As

One metalloid element that is commonly found in aquatic environments is arsenic (As). It is a significant and widespread environmental toxin that has an impact on the health of all living things due to both natural and man-made processes (**Rossman, 2003**). Fish growth rate, immune system function, and reproduction are all impacted by prolonged exposure to high As concentrations in marine habitats (**Datta *et al*., 2009**). Since organic forms are known to be less dangerous than inorganic forms (**Sanz *et al*., 2005**). The mean content of As levels from this investigation ranged from 0.31- 0.63μg/ g wet weight which is below the international standard, and below As levels in the literature ranging from 1.62 to 5.01mg kg^{-1} wet weight (**Storelli *et al*., 2005**), below the range of **Ansel (2021)** with the range of 1.2561– 3.8562mg kg^{-1}/wet weight from the western Algerian stock and in *Thunnus thynnus* from the Mediterranean Sea.

• The contents of Cr

Chromium (Cr) is a necessary trace metal. The form of Cr that is physiologically useful is essential for the metabolism of glucose. According to estimates, the average human needs approximately 1μg per day (**FAO, 1983; Abdallah, 2013**). In the present study, Cr content in muscles ranged from 0.17 to 0.36μg/ g, which is in the same range as reported by **Ansel (2021)** with values ranging from 0.1254 to 0.4002mg/ kg wet weight from the western Algerian stock. These values are below the ranges reported by **Masoud *et al*. (2007)**, who found Cr levels ranging from 0.23 to 1.26μg/ g in fish muscle from Alexandria coastal waters, Egypt. They are also below the values reported by **Hussein and Khaled (2014)**, with a range of 0.74 to 0.86μg/ g wet weight in muscle tissues of fish from the Egyptian Mediterranean Sea.

In the present study, the enzyme activities of Na^+ / K^+ and Ca^{2+}ATPase increased significantly etween the examined species. The highest activity appeared in liver tissue followed by brain, and kidney tissues followed by gill, and muscle showed the least activity. In general, the highest activities of Na^+ / K^+ and Ca^{2+}ATPase appeared in *Solea vulgaris* comparable to other examined species probably due to their high metal content which causes oxidative stress so Na^+ / K^+ and Ca^{2+}ATPase increased in response to stress this result agreed with the previous study of **Wang *et al*. (2012)**, after exposure to five different types of environmental stresses, the sensitivity of the gene expression of V-H ATPase and Na^+/K^+-ATPase of the white shrimp *Litopenaeus vannamei* was examined by quantitative real-time PCR (bacteria, pH, Cd, salinity and low temperature). The findings show that the two genes are responsive to salinity more than other stresses and are implicated in all types of stress responses. Shrimp's anti-stress system in response to environmental stress may involve ATPases.

The study of **Verbost *et al*. (1988)** demonstrated that there was direct competition between Ca^{2+} and Cd^{2+} and that Cd^{2+} had a high affinity for Ca2+ binding sites on the ATPase. According to **Mcgeer *et al*. (2012)**, exposure to Cd can also result in the

disruption of Na ions, which can inhibit Na^+/K^{+-}ATPase activity (**Atli & Canli, 2007**). This is in line with the findings of **Wang *et al*. (2017)**, who determined that hypoxia caused ATPase activity in *Larimichthys crocea* exposed to temperature and salinity variations to first decrease and then increase. These findings had a significant impact on the physiological and biochemical aspects of *L. crocea* of two sizes.

ATPase enzymes are necessary for intracellular and cellular functions. Cell membranes are actively crossed by them to transfer cations (**Das & Mukherjee, 2003; Temiz *et al.*, 2018**). Ion and acid-base regulation is another major physiological energy drop since membrane ion pumps such as $Na^{+/}K^+$ATPase and Ca^{2+}ATPase demand ATP and other transport systems depend on the ion gradients these pumps create (**Larsen *et al*., 2014**). It provides the energy required to transport metabolites and maintains the ionic balance (**Temiz *et al*., 2018; Waugh, 2019**). Na^+/K^+ATPase, a major ATP consumer, accounts for 10– 20% of cellular metabolism (**Pan *et al*., 2015**). Calcium ion adenosine triphosphatase is a vital protein involved in the transport of calcium ions across the cell membrane, necessary to maintain intracellular calcium ion homeostasis. This ubiquitous protein is membrane lipid-dependent (**Temiz *et al*., 2018; Waugh, 2019**), and its physiological phase and chemical composition are largely determined by the lipid bilayer around it. An increase in Na^+/K^+ATPase activity in response to abiotic stressors that alter intracellular ion concentrations and/or the ion gradients between the intracellular and exterior environments is what causes elevated ATP consumption. Osmoregulators show a more pronounced modification of Na^+/K^+ATPase activity in response to environmental stresses such as osmotic stress (**Huang *et al*., 2010**) as compared to osmoconformers, osmoregulators showed a more robust reactivity (**Whiteley *et al*., 2018; Ivanina *et al*., 2020**). Abiotic stresses such as high temperatures (**Nattie, 1990**), hypoxia (**Wang *et al*., 2017**), or ocean acidification (**Melzner *et al*., 2020**), can disturb acid-base homeostasis. The ion transport systems need more energy as a result of these stressors. According to various studies (**Wood *et al*., 2008, 2010; Stumpp *et al*., 2012; Frieder *et al*., 2017; Pan *et al*., 2015; Clark, 2020**), compensatory increases in ion and acid-base transport activities account for the raised energy costs of biomineralization in marine calcifies under scenarios of ocean acidification. Low pH and high CO_2 have also been shown to improve the Na^+/K^+ATPase energy allocation in mantle tissues and echinoderm larvae (**Frieder *et al*., 2017**).

In the present study, the level of LPO increased in *Solea vulgaris* and *Sparus aurata* comparable to other examined species. The activity of main antioxidant enzymes SOD, CAT, and GPX increased significantly in *Solea vulgaris* comparable to other species. Increased level of LPO was found to be correlated with increased Na^+/K^+ATPase activity in different tissues (brain, kidney, liver, gill, and muscle tissues. This suggests the existence of a compensatory mechanism in which high activity of this enzyme would be required to regulate the loss of ions caused by the increase in LPO processes. This result

agree with those of **Rangasam *et al*. (2022)**, who elucidated that the evaluation of Na^+/K^+ ATPase activity as a biomarker of the toxicity of microplastic pollutants revealed that CAT activity decreased and increased the level of LPO and gill Na^+/K^+ ATPase activity. The zebrafish exposed to polyethylene microplastics to environmentally relevant concentrations of 5 and 50µg/ L altered their oxidative and antioxidant responses. Although oxidizing chemicals such as hydrogen peroxide are well-known to produce and mediate oxidative stress, LPO is increasingly being acknowledged as a significant mediator of mortality and illness. Since lipids are the main component of cellular membranes, they are crucial for maintaining the structural integrity of cells. Proteins and nucleic acids can be covalently modified by an excessive lipid oxidation, altering the physicochemical properties of biological membranes **(Gaschler *et al*., 2017)**. GPx enzymes function as co-substrates in the conversion of LPO to the appropriate alcohols. Lipid peroxides in cells are regulated by GPx enzymes, which are crucial. Lipid peroxides accumulate when this enzyme is dormant and frequently result in cell death **(Friedmann *et al*., 2014)**. The results of **Kovacik (2017)** study indicated that metals increased the activity of several enzymes, including metallothionein (MT), glutathione S-transferase (GST), glutathione reductase (GR), glutathione (GSH), and glutathione S-transferase (GST-Px). An imbalance between antioxidant and oxidant states is referred to as oxidative stress. This imbalance can have a reversible effect on the redox status of cell compartments **(Viña *et al*., 2018)**. However, when certain repair mechanisms such as antioxidants (enzymatic and non-enzymatic) are unable to keep up with the rapid rate of oxidation, oxidative damage harmful and irreversible bimolecular damage caused by free radicals on lipids and proteins of cell constituents may occur **(Winterbourne, 2015)**. In controlling both the production and elimination of ROS, aquatic organisms have sophisticated, multilayer antioxidant systems that function to either completely eradicate or significantly lessen ROS molecules and their detrimental consequences **(Lushchak, 2016)**. The antioxidant enzymes CAT and SOD are the first line of defense against reactive oxygen species (ROS). They are activated to detoxify and counteract the harmful effects of ROS by converting H_2O_2 to water (H_2O) and oxygen (O_2) by the CAT enzyme and the superoxide anion (O_2) to hydrogen peroxide (H_2O_2) by the SOD enzyme **(Halliwell, 2017)**. The impairment and reduction of antioxidant status in living organisms cause the suppression of development, reproduction, and survival to decrease future fitness, and form characteristics of life history impacts **(Sopinka *et al*., 2016; Birnie-Gauvin *et al*., 2017)**. The current study found that the levels of non-enzymatic antioxidants, specifically T-SH and P-SH, decreased in the muscles of *Solea vulgaris* and *Sparus aurata*. This reduction in the thiol content is likely related to increased production of reactive oxygen species (ROS) since thiols oxidize ROS irreversibly when they interact with them **(Requejo *et al*., 2010)**. These findings corroborate the findings of earlier research **(Baldissera *et al*., 2018; Freitas Souza *et al*., 2019)**, which found that the infected tilapias had lower PSH levels in their liver and kidney. According to **Freitas**

Souza *et al*. (2019), thiols (SH groups) are essential for the proper operation of cells and tissues. Conversely, a deficiency or reduction in thiol levels, or their oxidation, results in oxidative stress and the emergence of diseases. Protein thiols and non protein thiols are any low-molecular-weight thiol compounds that have a sulfhydryl group (-SH) in their structure **(Nassar *et al.*, 2014)**. These compounds are found in the majority of plants, bacteria and all human tissues **(Ferrat *et al*., 2003)** in addition to and fish **(Freitas Souza *et al*., 2019)**. These are considered to be antioxidants that work through a variety of mechanisms, such as (i) being components of the thiol/disulfide redox buffer as a whole, (ii) functioning as metal chelators, (iii) sating radicals, (iv) acting as substrates for specific redox reactions, and (v) acting as specific reductants.

CONCLUSION

This study demonstrates the impact of the health state of some species of trawl net catch in the presence of environmental multi-stressors to illustrate and increase the precision of the monitoring ecological consequences of environmental changes.

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