EVALUATION OF LONG-TERM EFFECTS OF POLLUTION ON SPARUS AURATA AND DICENTRARCHUS LABRAX IN MEDITERRANEAN AND RED SEAS

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ABSTRACT

To predict the possible effects of climate change on marine organisms and water quality, this study evaluated the long-term effects of pollution on Sparus aurata & Dicentrarchus labrax in Alexandria on the Mediterranean Sea and Suez on the Red Sea concerning their seasonal variation. The physicochemical factors pertaining to seasonal variation in mean temperatures at the Mediterranean and the Red Seas were documented. They ranged from 17.26 –25.73°C with a mean value of 22.01±0.31 in Alexandria and from 17.35 – 29.98 °C with a mean value of 24.27 ±0.54 °C in Suez. The collected water samples had mean concentrations of dissolved oxygen (DO) ranging from 3.94-8.28 (6.77±0.42 mg/L) in Alexandria to 4.65–9.38 (7.08±0.53 mg/L) in Suez. The oxidizable organic matter (OOM) mean varied between 5.40- 10.4 with a mean value of 8.10±0.76 mg/L in Alexandria and 5.74–11.06 with a mean value of 8.77±0.84 mg/L in Suez. Mean salinity differed between 37.36 - 39.62 with a mean value of 38.42 ± 0.23 in Alexandria and 40.28 -42.92 with a mean value of 41.63 ± 0.28 at Suez. The mean pH values varied from 7.96 – 8.57 with a mean value 8.30 ± 0.04 in Alexandria while ranging from 7.61 - 8.34 with a mean value 8.07±0.11 in Suez and thus indicating that the water was slightly alkaline. Temperature had a major impact on the liver tissue's catalase (CAT) and Glutathione S transferase (GST) activities. All values of CAT & GST activities in Suez were higher than in Alexandria. CAT activity in the Mediterranean Sea during summer showed lower values than in the Red Sea, which were $3.76 \pm 0.08 \& 4.18 \pm 0.04 \text{ U/mg}$ protein respectively. On the contrary, the activity of GST in Seabass Dicentrarchus labrax with gilt-head bream Sparus aurata were 120.18 ±1.37 & 123.75 ±1.28 U/mg protein respectively at the Red Sea. In comparison, the activity was lower in the Mediterranean Sea in the summer than in the Red Sea, which was 116.55 ± 0.75 & 119.18 ± 0.68 U/mg protein respectively.

Keywords: Pollution, Sparus aurata, Dicentrarchus labrax, Mediterranean Sea, Red Sea

INTRODUCTION

Concern about the concentrations of agricultural and industrial pollutants in the water and the possible consequences of this contamination on both human health and the welfare of animal populations has grown over the past ten years. We may anticipate finding the earliest signs of environmental catastrophe in places like these since the seas and lakes serve as the final resting place for contaminants from industrial effluents or agricultural applications via rivers and other water channels (Uddin and Jeong, 2021).

The aquatic environment, precisely the water quality, is thought to be the primary determinant of the health and sickness of both farmed and wild fish. One of the main reasons providing a substantial threat to the existence of aquatic creatures is the pollution of the marine environment by inorganic and organic pollutants (Ahmed *et al.*, 2016).

Aquatic ecosystems have frequently been employed in bioassays to ensure water quality. The creation of biological monitoring methods based on the ability to detect water contamination quickly in the presence of low levels of natural toxicants and other substances. Since fish can metabolize, concentrate, and store waterborne toxins, they provide good research animals for examining the consequences of various contaminants in water samples. Fish can be used as a screening tool for substances that may be teratogenic and carcinogenic to humans since they frequently react to toxicants similarly to higher vertebrates. The major use of a fish-based model system is to map the distribution and impact of pollution in the aquatic environment (UN, 2016; Jin *et al.*, 2020a, b).

Pollutants work by altering the biological or structural properties of biomolecules. Early diagnostic methods for measuring biological effects and evaluating environmental quality are biomarkers for water pollution. They are described as a variation in biological reaction at the organismal level. The most common abiotic factor influencing an organism's biology is its temperature (Beitinger and Lutterschmidt, 2011). According to Pörtner and Farrell (2008), the organism's sensitivity to heat is thus essentially exploited for changes in marine ecosystems brought about by climate change. According to the hypothesis known as oxygen- and capacity-limited thermal tolerance (OCLTT), temperature in highly oxygenated

waters seriously restricts the aerobic range of aquatic ectotherms because of the limited capacity of the circulatory and ventilatory systems to match oxygen demand (Pörtner, 2004, 2010; Pörtner and Farrell, 2008).

As oxygen levels are limited during warming and cooling, organisms gradually use their anaerobic metabolism, antioxidant defenses, and heat-shock response as examples of protective mechanisms to keep the sub-organism level within their thermal tolerance limits or thermal windows (Pörtner, 2004). The Critical Thermal Maximum (CTmax) and Minimum (CTmin) are useful relative proxies for the temperatures at which fish are unable to escape conditions that would ultimately result in thermal death when assessing the thermal tolerance limits or thermal windows of fish (Becker and Genoway, 1979; Beitinger *et al.*, 2000; Beitinger and Lutterschmidt, 2011).

The significance of figuring out physiological limits to various environmental forces has been emphasized in order to develop productivity estimates (Deutsch *et al.*, 2015; Marras *et al.*, 2015). It is crucial to comprehend how ectotherm organisms react to seasonal temperature extremes and pinpoint the mechanisms at play in the responses to understand the consequences of global warming on the thermal tolerance of a particular ectotherm organism.

By achieving the main objective of this study, environmental pollution could be monitored through the evaluation of the physicochemical properties of water pollutants and studying the direct effects on aquatic biota through the assessment of antioxidant enzymes so the aquatic fish would be conserved. It is essential to comprehend how various stresses limit the physiology and distribution of species in order to forecast the possible effects of climate change on marine life. Due to the economic significance of seabass (*Dicentrarchus labrax*) and gilt-head bream (*Sparus aurata*) in both the Mediterranean and Red Seas, we assessed the effects of seasonal variations in seawater temperature on organismal and sub-organismal traits related to the thermal performance of fish samples.

MATERIALS AND METHODS

Sample collection

Water samples and two different types of fish samples (Gilt-head bream *Dicentrarchus labrax* and the sea bass *Sparus aurata*) were collected from various polluted sites from Alexandria and Suez at their seasonal variation (Autumn, Summer, Winter, and spring).

Sample processing

At a midstream location and during the low tide period, water samples were collected four times a year at a depth of roughly 10 to 20 cm. About 1000 milliliters of water made up each sample. To separate the suspended materials, a portion of each sample was taken and passed through the Whatman 41 filter paper. To protect the water samples from fungus or other pathogens, an alkaline potassium iodide solution was added after sampling. After being delivered to the laboratory, the bottles were properly labeled and kept in the refrigerator pending additional examination (Mahrous *et al.*, 2020). The fish were randomly collected from the Mediterranean and Red Seas in four seasons (Autumn, Summer, Winter, and spring) and divided into four groups with four fish within every group. Before the experiment began, every fish was prepared overnight. The groups served as standard control and the pollutants were detected in fish samples by antioxidant enzymes assay (Hoseinifar *et al.*, 2020).

Evaluation of water sample physicochemical characteristics

The physicochemical water quality analysis was conducted using five key parameters: the concentration of dissolved oxygen (DO), the temperature, oxidizable organic matter (OOM), Salinity, and pH. In compliance with the Standard Procedures for the Examination of Water and Wastewater (2012), measurements were taken on water samples. A mercury thermometer was used to measure the water samples' temperature on-site (APHA, 2005). The concentrations of dissolved oxygen were determined using APHA's standard methodology (2005), via DO meter thermo scientific Model ORION Star A213. Every analysis was done in triplicate, and the average result was used.

The permanganate oxidation method (FAO, 1975) was used to determine the OOM, which is computed using the equation that follows.

OOM mgO₂ /L = (V blank-V sample) x 8 x 1000 x N_{Na2S2O3}

V of sample

The JENWAY 4520 Conductivity meter was used to measure the salinity. Using a portable pH meter (JENWAY 3510 digital pH meters) and taking the required safety precautions during the sampling and standardization procedures, the pH values of water samples were estimated to be approximately 0.1 units in situ.

Biochemical analysis (antioxidant enzymes assay)

Using up 0.5 g/L of Tricaine methanesulfonate (MS222) to induce deep anesthesia, the fish were put to sleep and the livers of four of the individuals were removed, measured $(\pm 10-5 \text{ g})$, and stored at -80 °C for biochemical analysis. The liver tissues were used to quantify every measurement. The liver is a major site for lipid peroxidation despite having a high metabolic rate (Pörtner *et al.*, 2005).

According to Habig *et al.* (1974), a tiny amount of liver from each fish was removed and infused with 0.16 mg of heparin in phosphate buffer saline solution (pH 7.4) to eliminate any blood cells. The samples remain stable for a minimum of one month if the supernatants from centrifugation are kept at -80 °C until the enzyme activities are ascertained. Antioxidant enzymes namely CAT and GST levels following the manufacturer's instructions, were measured twice using the available kits. Antioxidant biomarker kits were obtained from Biodiagnostics Co., Cairo, Egypt. All supplementary chemicals used in this study were of high purity grade obtained from commercial sources.

Catalase (CAT) activity:

The highly sensitive, straightforward, and direct Catalase Activity Assay Kit (Colorimetric method) can measure the amount of catalase present in a variety of biological samples, including all cell and tissue lysates. The catalase in the sample combines with oxygen and hydrogen peroxide (H2O2) in the catalase activity assay protocol. A product that can be measured colorimetrically at OD 510 nm is produced when the unconverted H2O2

reacts with a probe (Aebi, 1984). As a result, the signal obtained is inversely proportional to the catalase activity found in the sample. As little as 1 μ U of catalase activity can be detected by the kit. Catalase has two distinct roles: (1) it catalyzes the conversion of H2O2 into H2O and O2; and (2) it oxidizes H donors, including phenols, methanol, ethanol, and formic acid, by consuming catalyses' kinetics deviates from the expected trend. Hence, it is impossible to measure enzyme activity at substrate saturation or to calculate the Ks. The enzymatic breakdown of H₂O₂ is a first-order reaction, as opposed to reactions that occur at substrate saturation, and its rate is always proportional to the amount of peroxide present. Hence, the experiment must be performed with relatively low quantities of H₂O₂ to prevent a quick reduction in the initial rate of the reaction (about 0.01 M).

Glutathione S transferase (GST):

By using 1-chloro-2,4-dinitrobenzene as a substrate and incubating reduced glutathione at 25 °C and monitoring the absorbance increase at 340 nm, the amount of GST activity was ascertained. A single GST unit was described as the quantity of enzyme needed to catalyze one micromole of 2,4 dinitrophenyl-S-glutathione every minute (Habig *et al.*, 1974).

Statistical Analysis

The means \pm SD are used to represent all of the results from the water physiochemical parameters and enzyme activity assay. One-way analysis of variance (ANOVA) was utilized to compare and determine whether there were any significant differences between the groups. For a two-tailed test, for statistical significance, a probability of p < 0.05 was used.

RESULTS AND DISCUSSION

Water sample physicochemical characteristics.

The physicochemical parameters, which include temperature, pH, oxidizable organic matter (OOM), dissolved oxygen (DO) concentration, salinity, and pH, were measured for samples taken during various seasons from the Mediterranean and Red Seas. The results are displayed in both Table 1 and Figure 1. Seasonal mean temperatures concerning their

836

variation for the four seasons at the Mediterranean & Red seas ranged from 17.26 - 25.73°C with a mean value of 22.01±0.31& 17.35 – 29.98 °C respectively. The results of this study were in line with The World Health Organization's standards (WHO, 2017a), the Department of Public Health Engineering Ministry (DPHE, 2019) and the United States Environmental Protection Agency (USEPA, 2012). They all agreed that these values were appropriate for aquatic life and household activities. The biotic community and aquatic populations may not be limited in their ability to survive by changes in water temperature (Whitehead et al., 2009; Islam et al., 2019). A marginal variation in the average seasonal temperature existed between Alexandria and Suez. The water temperature at Suez (24.27 ± 0.54 °C) was two degrees higher than in Alexandria. Due to aquatic life's wide tolerance range for temperature, the temperature of the water might not matter as much. water temperature can significantly impact DO in contaminated water (Machender et al., 2013; Ahmed et al., 2016). Pollution significantly impacts dissolved oxygen levels because when they go below 2 mg/l, marine life experiences severe physiological stress that could result in mortality. This is because both municipal waste loads and industrial waste promote chemical breakdown and oxidation (Whitehead et al., 2018). The water becomes more loaded with organic compounds and pathogens. Consequently, the amount of nutrients is reduced and the anoxic conditions worsen together with the rise of other negative consequences. Water discharged from industrial areas need an increased oxygen concentration to facilitate chemical oxidation and breakdown (Whitehead et al., 2018; Islam et al., 2018; Uddin and Jeong 2021).

Because of this, the amount of DO in water is regarded as a reliable predictor of its quality (Hussein *et al.*, 2013). The mean DO concentrations of the collected water samples varied from 3.94–8.28 with a mean value of 6.77±0.42 mg/L in Alexandria and 4.65–9.38 with a mean value of 7.08±0.53 mg/L in Suez (Table 1 & Fig 1). The present study demonstrated DO concentration at four seasons. Aquatic life's survival is enhanced by such a value (Uddin and Jeong, 2021). The following guidelines for DO concentration are approved: 6 mg/L for drinking water, 4-5 mg/L for amusement, 4-6 mg/L for fish and other

tamed animals, and 5 mg/L for commercial use (WHO, 2017a, 2017b; DPHE, 2019). Hence, the water was almost suitable for those purposes, and according to these seasonal and point-based observations, it can be stated that all these values were within the permissible limits (Hasan *et al.*, 2019). Oxidizable organic matter (OOM) mean varied between 5.40-10.4 with a mean value of 8.10 ± 0.76 mg/L & 5.74-11.06 with a mean value of 8.77 ± 0.84 mg/L for the seasons at the Mediterranean & Red seas.

The salinity mean varied between 37.36-39.62 with a mean value of 38.42 ± 0.23 at Alexandria and 40.28-42.92 with a mean value of 41.63 ± 0.28 at Suez. The mean pH values varied from 7.96-8.57 with a mean value 8.30 ± 0.04 in Alexandria while ranging from 7.61-8.34 with a mean value 8.07 ± 0.11 in Suez and thus indicating that the water was slightly alkaline (Table 1 & Fig 1). Ahmed et al. (2015) and Afrin et al. (2016) observed that the pH values of the water ranged from 6.98 -8.0 respectively, which were consistent with the values found in this study.

The seasonal mean pH value, taking into account seasonal variation, was found in Table 1 to be within the acceptable range for a variety of uses, including irrigation, household, and recreational activities. Surface water systems typically have a pH between 6.5 and 8.5, which is also the ideal range for irrigation and fish culture. According to the standards of the Department of Environment in Bangladesh (DoE, 1997), the ideal pH range for irrigation and fish culture is between 6.5 and 8.0, while surface water systems require a pH between 6.5 and 8.5. Fish and other aquatic invertebrates are more prevalent in freshwater systems when the water is alkaline, as noted by Parker *et al.* (1992). In the present study, the pH values remained rather constant in different investigated seasons, where the pH range of 7 to 8 was maintained. As a result, pH has no effect on the distribution of aquatic populations (Table 1 & Fig. 1).

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Mohammed, Omneya et al.

Table 1: The seasonal values of physiochemical parameters in water samples collected from

| COLLECTI | PARAMETE | | | ANNUA | | | |
|------------|---|--|---|--|--|--|---|
| ON SITES | R | | Spring | Summer | Autumn | Winter | L MEAN |
| | Water (°C) | Range | 21.94- | 23.76- | 21.77- | 17.26- | 17.26-25.73 |
| | | Mean±S | 23.81 | 25.73 | 22.69 | 18.33 | 22.01±0.31 |
| | | D | 22.95±0.39 | 25.16±0.48 | 22.46±0.24 | 17.49 ± 0.12 | |
| Alexandria | | | b | a | b | с | |
| | DO (mg/l) | Range | 6.02–7.37 | 3.94-7.22 | 5.16-6.95 | 7.31-8.28 | 3.94-8.28 |
| | | Mean±S | 6.81±0.31a | 6.12±0.74 ^b | 6.41 ± 0.42^{ab} | 7.76 ± 0.20^{a} | 6.77±0.42 |
| | | D | b | | | | |
| | OOM (mg/l) | Range | 6.11-10.4 | 6.13-9.52 | 7.62 -9.72 | 5.40 - 8.53 | 5.40-10.4 |
| | | Mean±S | 8.76±0.95 ^a | 8.04±0.75 ^{ab} | 8.32±0.53 ^{ab} | $7.29\pm0.81^{\circ}$ | 8.10±0.76 |
| | ~ | D | | | | | |
| | Salinity | Range | 38.29- | 37.83- | 37.36- | 38.05- | 37.36–39.62 |
| | | Mean±S | 38.72 | 39.62 | 38.69 | 38.74 | 38.42±0.23 |
| | | D | 38.51±0.09 | 38.58±0.38 | 38.16±0.29 | 38.45±0.15 | |
| | рН | Range | 8.03-8.21 | 8.39-8.57 | 8.45-8.53 | 7.96-8.31 | 7.96-8.57 |
| | | Mean±S | 8.11±0.04 | 8.47±0.04 | 8.49±0.04 | 8.15 ± 0.04 | 8.30±0.04 |
| | | D | | | | | |
| | | | | ã | | | |
| | Parameter | | | Sea | son | | nnual mean |
| | Parameter | | Spring | Sea Summer | son Autumn | Winter | nnual mean |
| | Parameter Water (°C) | Range | Spring 23.61- | Sea Summer 29.28- | son Autumn 22.79- | Winter 17.35- | nnual mean 17.35–29.98 |
| | Parameter Water (°C) | Range Mean±S | Spring 23.61- 25.82 | Sea Summer 29.28- 29.98 | Son Autumn 22.79- 25.09 | Winter 17.35- 21.94 | nnual mean 17.35–29.98 24.27±0.54 |
| | Parameter Water (°C) | Range Mean±S D | Spring 23.61- 25.82 24.87 ± | Sea Summer 29.28- 29.98 29.61 ± | Son Autumn 22.79- 25.09 23.65 0.501 | Winter 17.35- 21.94 18.97 ± | nnual mean 17.35–29.98 24.27±0.54 |
| Suez | Parameter Water (°C) | Range Mean±S D | Spring 23.61- 25.82 24.87 ± 0.48 ^b | Sea Summer 29.28- 29.98 29.61 ± 0.14 ^a 5.24 - 5.54 | son Autumn 22.79- 25.09 23.65 ±0.50b | Winter 17.35- 21.94 18.97 ± 1.03 ^c | nnual mean 17.35–29.98 24.27±0.54 |
| Suez | Parameter Water (°C) DO (mg/l) | Range Mean±S D Range | Spring 23.61- 25.82 24.87 ± 0.48 ^b 5.52-7.81 | Sea Summer 29.28- 29.98 29.61 ± 0.14 ^a 5.34-7.54 5.48 ± | son Autumn 22.79- 25.09 23.65 ±0.50b 4.65-7.83 | Winter 17.35- 21.94 18.97 ± 1.03° 7.03-9.38 | Innual mean 17.35–29.98 24.27±0.54 4.65–9.38 |
| Suez | Parameter Water (°C) DO (mg/l) | Range Mean±S D Range Mean±S | $\begin{array}{r} \textbf{Spring} \\ 23.61-\\ 25.82\\ 24.87 \pm \\ 0.48^{b} \\ 5.52-7.81\\ 7.01 \pm \\ 0.51^{ab} \end{array}$ | Sea Summer 29.28- 29.98 29.61 ± 0.14 ^a 5.34-7.54 6.48 ± 0.46 ^b | | Winter 17.35- 21.94 18.97 ± 1.03 ^c 7.03-9.38 8.53 ± 0.51 ^a | nnual mean 17.35–29.98 24.27±0.54 4.65–9.38 7.08±0.53 |
| Suez | Parameter Water (°C) DO (mg/l) | Range Mean±S D Range Mean±S D | $\begin{array}{r} \textbf{Spring} \\ 23.61-\\ 25.82\\ 24.87 \pm \\ 0.48^{b} \\ 5.52-7.81\\ 7.01 \pm \\ 0.51^{ab} \\ \hline \end{array}$ | Sea Summer 29.28- 29.98 29.61 ± 0.14 ^a 5.34-7.54 6.48 ± 0.46 ^b 6.40 ± 0.46 ^b | | Winter 17.35 - 21.94 $18.97 \pm$ 1.03° $7.03-9.38$ $8.53 \pm$ 0.51° | nnual mean 17.35–29.98 24.27±0.54 4.65–9.38 7.08±0.53 |
| Suez | Parameter Water (°C) DO (mg/l) OOM (mg /l) | Range Mean±S D Range Mean±S D Range | $\begin{array}{r} \textbf{Spring} \\ \hline 23.61- \\ 25.82 \\ 24.87 \pm \\ 0.48^{b} \\ \hline 5.52-7.81 \\ 7.01 \pm \\ 0.51^{ab} \\ \hline 5.74-10.69 \\ \hline 5.74-10.69 \\ \hline \end{array}$ | $\begin{array}{r} \textbf{Sea} \\ \hline \textbf{Summer} \\ 29.28-\\ 29.98 \\ 29.61 \pm \\ 0.14^a \\ 5.34-7.54 \\ 6.48 \pm \\ 0.46^b \\ \hline \textbf{6.92-10.75} \\ 6.92-10.75 \\ \hline \textbf{a} \\ \textbf{b} \\ \hline \textbf{c} \\ \textbf{c} $ | $\begin{array}{r} \textbf{son} \\ \hline \textbf{Autumn} \\ 22.79- \\ 25.09 \\ 23.65 \\ \pm 0.50b \\ 4.65-7.83 \\ 6.50 \pm \\ 0.67^{b} \\ 8.27-11.06 \\ 8.27-11.06 \\ \end{array}$ | Winter 17.35 - 21.94 $18.97 \pm$ 1.03° $7.03-9.38$ $8.53 \pm$ 0.51^{a} $6.19-9.33$ $7.05 + 33$ | nnual mean 17.35–29.98 24.27±0.54 4.65–9.38 7.08±0.53 5.74–11.06 2.77±0.94 |
| Suez | Parameter Water (°C) DO (mg/l) OOM (mg/l) | Range Mean±S D Range Mean±S D Range Mean±S | $\begin{array}{r} \textbf{Spring} \\ \hline 23.61- \\ 25.82 \\ 24.87 \pm \\ 0.48^{b} \\ \hline 5.52-7.81 \\ 7.01 \pm \\ 0.51^{ab} \\ \hline 5.74-10.69 \\ 8.65 \pm \\ 1.24^{ab} \\ \end{array}$ | Sea Summer 29.28- 29.98 29.61 \pm 0.14 ^a 5.34-7.54 6.48 \pm 0.46 ^b 6.92-10.75 8.73 \pm 0.84 ^a | $\begin{array}{r} \textbf{son} \\ \hline \textbf{Autumn} \\ 22.79- \\ 25.09 \\ 23.65 \\ \pm 0.50b \\ 4.65-7.83 \\ 6.50 \pm \\ 0.67^{b} \\ 8.27-11.06 \\ 9.74 \pm \\ 0.50^{a} \\ \end{array}$ | Winter 17.35 - 21.94 $18.97 \pm$ 1.03° $7.03-9.38$ $8.53 \pm$ 0.51^{a} $6.19-9.33$ $7.95 \pm$ 0.66^{b} | nnual mean 17.35–29.98 24.27±0.54 4.65–9.38 7.08±0.53 5.74–11.06 8.77±0.84 |
| Suez | Parameter Water (°C) DO (mg/l) OOM (mg/l) | Range Mean±S D Range Mean±S D Range Mean±S D | $\begin{array}{r} \textbf{Spring} \\ 23.61-\\ 25.82\\ 24.87 \pm \\ 0.48^{b} \\ 5.52-7.81\\ 7.01 \pm \\ 0.51^{ab} \\ 5.74-10.69\\ 8.65 \pm \\ 1.24^{ab} \\ 40.76 \\ \end{array}$ | $\begin{array}{r} {\color{black} \textbf{Sea}} \\ \hline \textbf{Summer} \\ 29.28- \\ 29.98 \\ 29.61 \pm \\ 0.14^a \\ \hline 5.34-7.54 \\ 6.48 \pm \\ 0.46^b \\ \hline 6.92-10.75 \\ 8.73 \pm \\ 0.84a^b \\ \hline 41.82 \\ \end{array}$ | $\begin{array}{r} \textbf{son} \\ \hline \textbf{Autumn} \\ 22.79- \\ 25.09 \\ 23.65 \\ \pm 0.50b \\ 4.65-7.83 \\ 6.50 \pm \\ 0.67^{b} \\ 8.27-11.06 \\ 9.74 \pm \\ 0.59^{a} \\ 40.07 \\ \end{array}$ | Winter 17.35 - 21.94 $18.97 \pm$ 1.03° $7.03-9.38$ $8.53 \pm$ 0.51^{a} $6.19-9.33$ $7.95 \pm$ 0.69^{b} | nnual mean 17.35–29.98 24.27±0.54 4.65–9.38 7.08±0.53 5.74–11.06 8.77±0.84 40.28, 42.62 |
| Suez | Parameter Water (°C) DO (mg/l) OOM (mg /l) Salinity | Range Mean±S D Range Mean±S D Range Range Mean±S | $\begin{array}{r} \textbf{Spring} \\ 23.61-\\ 25.82\\ 24.87 \pm \\ 0.48^{b} \\ 5.52-7.81\\ 7.01 \pm \\ 0.51^{ab} \\ 5.74-10.69\\ 8.65 \pm \\ 1.24^{ab} \\ 40.76- \\ 42.11 \\ \end{array}$ | $\begin{array}{r} {\color{black} \textbf{Sea}} \\ \hline \textbf{Summer} \\ 29.28- \\ 29.98 \\ 29.61 \pm \\ 0.14^a \\ 5.34-7.54 \\ 6.48 \pm \\ 0.46^b \\ 6.92-10.75 \\ 8.73 \pm \\ 0.84a^b \\ 41.82- \\ 42.92 \\ 42.92 \\ \end{array}$ | $\begin{array}{r} \textbf{son} \\ \hline \textbf{Autumn} \\ 22.79- \\ 25.09 \\ 23.65 \\ \pm 0.50b \\ 4.65-7.83 \\ 6.50 \pm \\ 0.67^{b} \\ 8.27-11.06 \\ 9.74 \pm \\ 0.59^{a} \\ 40.97- \\ 42.95 \\ \end{array}$ | Winter 17.35 - 21.94 $18.97 \pm$ 1.03^{c} $7.03-9.38$ $8.53 \pm$ 0.51^{a} $6.19-9.33$ $7.95 \pm$ 0.69^{b} 40.28 - | nnual mean 17.35–29.98 24.27±0.54 4.65–9.38 7.08±0.53 5.74–11.06 8.77±0.84 40.28–42.92 41.62±0.28 |
| Suez | Parameter Water (°C) DO (mg/l) OOM (mg /l) Salinity | Range Mean±S D Range Mean±S D Range Mean±S D Range Mean±S | $\begin{array}{r} \textbf{Spring} \\ 23.61-\\ 25.82\\ 24.87 \pm \\ 0.48^{b} \\ 5.52-7.81\\ 7.01 \pm \\ 0.51^{ab} \\ 5.74-10.69\\ 8.65 \pm \\ 1.24^{ab} \\ 40.76- \\ 42.11\\ 41.40 + \end{array}$ | $\begin{array}{r} \textbf{Sea} \\ \hline \textbf{Summer} \\ 29.28-\\ 29.98 \\ 29.61 \pm \\ 0.14^a \\ 5.34-7.54 \\ 6.48 \pm \\ 0.46^b \\ 6.92-10.75 \\ 8.73 \pm \\ 0.84a^b \\ 41.82-\\ 42.92 \\ 42.94 \\ 41.4 \\ \end{array}$ | $\begin{array}{r} \textbf{son} \\ \hline \textbf{Autumn} \\ 22.79- \\ 25.09 \\ 23.65 \\ \pm 0.50b \\ 4.65-7.83 \\ 6.50 \pm \\ 0.67^{b} \\ 8.27-11.06 \\ 9.74 \pm \\ 0.59^{a} \\ 40.97- \\ 42.05 \\ 41.64 \\ \end{array}$ | Winter 17.35 - 21.94 $18.97 \pm$ 1.03^{c} $7.03-9.38$ $8.53 \pm$ 0.51^{a} $6.19-9.33$ $7.95 \pm$ 0.69^{b} 40.28 - 41.72 40.06 | nnual mean 17.35–29.98 24.27±0.54 4.65–9.38 7.08±0.53 5.74–11.06 8.77±0.84 40.28–42.92 41.63±0.28 |
| Suez | Parameter Water (°C) DO (mg/l) OOM (mg /l) Salinity | Range Mean±S D Range Mean±S D Range Mean±S D Range Mean±S D | $\begin{array}{r} \textbf{Spring} \\ 23.61-\\ 25.82\\ 24.87 \pm \\ 0.48^{b} \\ 5.52-7.81\\ 7.01 \pm \\ 0.51^{ab} \\ 5.74-10.69\\ 8.65 \pm \\ 1.24^{ab} \\ 40.76- \\ 42.11\\ 41.49 \pm \\ 0.28^{b} \\ \end{array}$ | $\begin{array}{r} \textbf{Sea} \\ \hline \textbf{Summer} \\ 29.28-\\ 29.98 \\ 29.61 \pm \\ 0.14^a \\ 5.34-7.54 \\ 6.48 \pm \\ 0.46^b \\ 6.92-10.75 \\ 8.73 \pm \\ 0.84a^b \\ 41.82-\\ 42.92 \\ 42.44 \pm \\ 0.24^a \end{array}$ | $\begin{array}{r} \textbf{son} \\ \hline \textbf{Autumn} \\ 22.79-\\ 25.09 \\ 23.65 \\ \pm 0.50b \\ 4.65-7.83 \\ 6.50 \pm \\ 0.67^{b} \\ 8.27-11.06 \\ 9.74 \pm \\ 0.59^{a} \\ 40.97- \\ 42.05 \\ 41.64 \pm \\ 0.25^{ab} \\ \end{array}$ | $\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$ | nnual mean 17.35–29.98 24.27±0.54 4.65–9.38 7.08±0.53 5.74–11.06 8.77±0.84 40.28–42.92 41.63±0.28 |
| Suez | Parameter Water (°C) DO (mg/l) OOM (mg /l) Salinity | Range Mean±S D Range Mean±S D Range Mean±S D Range Mean±S D | $\begin{array}{r} \textbf{Spring} \\ 23.61-\\ 25.82\\ 24.87 \pm \\ 0.48^{b} \\ 5.52-7.81\\ 7.01 \pm \\ 0.51^{ab} \\ 5.74-10.69\\ 8.65 \pm \\ 1.24^{ab} \\ 40.76- \\ 42.11\\ 41.49 \pm \\ 0.28^{b} \\ 7.74.821 \end{array}$ | $\begin{array}{r} \textbf{Sea} \\ \hline \textbf{Summer} \\ 29.28-\\ 29.98 \\ 29.61 \pm \\ 0.14^a \\ 5.34-7.54 \\ 6.48 \pm \\ 0.46^b \\ 6.92-10.75 \\ 8.73 \pm \\ 0.84a^b \\ 41.82-\\ 42.92 \\ 42.44 \pm \\ 0.24^a \\ \hline 7.06 \times 24 \end{array}$ | $\begin{array}{r} \textbf{son} \\ \hline \textbf{Autumn} \\ 22.79- \\ 25.09 \\ 23.65 \\ \pm 0.50b \\ 4.65-7.83 \\ 6.50 \pm \\ 0.67^{b} \\ 8.27-11.06 \\ 9.74 \pm \\ 0.59^{a} \\ 40.97- \\ 42.05 \\ 41.64 \pm \\ 0.25^{ab} \\ 7.61 \times 22 \end{array}$ | Winter 17.35 - 21.94 $18.97 \pm$ 1.03^c $7.03-9.38$ $8.53 \pm$ 0.51^a $6.19-9.33$ $7.95 \pm$ 0.69^b 40.28 - 41.72 $40.96 \pm$ 0.33^b | nnual mean 17.35–29.98 24.27±0.54 4.65–9.38 7.08±0.53 5.74–11.06 8.77±0.84 40.28–42.92 41.63±0.28 7.61_8.34 |
| Suez | Parameter Water (°C) DO (mg/l) OOM (mg /l) Salinity pH | Range Mean±S D Range Mean±S D Range Mean±S D Range Mean±S D | $\begin{array}{r} \textbf{Spring} \\ 23.61-\\ 25.82\\ 24.87 \pm \\ 0.48^{b} \\ 5.52-7.81\\ 7.01 \pm \\ 0.51^{ab} \\ 5.74-10.69\\ 8.65 \pm \\ 1.24^{ab} \\ 40.76-\\ 42.11\\ 41.49 \pm \\ 0.28^{b} \\ 7.74-8.31\\ 8.07 \pm 0.14 \\ \end{array}$ | $\begin{array}{r} \textbf{Sea} \\ \hline \textbf{Summer} \\ 29.28-\\ 29.98 \\ 29.98 \\ 29.61 \pm \\ 0.14^a \\ \hline 5.34-7.54 \\ 6.48 \pm \\ 0.46^b \\ \hline 6.92-10.75 \\ 8.73 \pm \\ 0.84a^b \\ \hline 41.82-\\ 42.92 \\ 42.44 \pm \\ 0.24^a \\ \hline 7.96-8.34 \\ 8.12 \pm 0.08 \\ \hline \end{array}$ | $\begin{array}{r} \textbf{son} \\ \hline \textbf{Autumn} \\ 22.79- \\ 25.09 \\ 23.65 \\ \pm 0.50b \\ 4.65-7.83 \\ 6.50 \pm \\ 0.67^{b} \\ 8.27-11.06 \\ 9.74 \pm \\ 0.59^{a} \\ 40.97- \\ 42.05 \\ 41.64 \pm \\ 0.25^{ab} \\ \hline 7.61-8.22 \\ 7.92 \pm 0.12 \\ \end{array}$ | $\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$ | nnual mean 17.35–29.98 24.27±0.54 4.65–9.38 7.08±0.53 5.74–11.06 8.77±0.84 40.28–42.92 41.63±0.28 7.61–8.34 8.07±0.11 |

the Mediterranean & Red Seas





Figure 1: Seasonal distributions in the Mediterranean and Red seas of a) temperature (°C);
b) dissolved oxygen (DO) (mg/L); c) oxidizable organic matter (OOM) (mg/L); d)
Salinity; e) pH

Catalase (CAT) activity & Glutathione S transferase (GST)

Temperature had a significant impact on the liver tissue's CAT and GST activities. All values of CAT & GST activity in Suez were higher than in Alexandria, (Table 2 & Fig. 2). It increased with summer temperatures, resulting in higher CAT activity of Red Sea Gilt-head bream *Sparus aurata* and Seabass *Dicentrarchus labrax* where it reached 4.03 \pm 0.04 and 4.37 \pm 0.04 U/mg protein respectively. In Alexandria, CAT activity during summer showed lower values than in Suez, being 3.76 \pm 0.08 & 4.18 \pm 0.04 U/mg protein respectively.

In the Red Sea, the activity of GST in Gilt-head bream *Sparus aurata* and Seabass *Dicentrarchus labrax* were 120.18 \pm 1.37 & 123.75 \pm 1.28 U/mg protein respectively. However, GST activity was lower in the Mediterranean *Sparus aurata* and Seabass *Dicentrarchus labrax* (116.55 \pm 0.75 & 119.18 \pm 0.68 U/mg protein respectively) during summer than in the Red Sea. These results revealed that the Seabass *Dicentrarchus labrax* was more affected than the Gilt-head bream *Sparus aurata*. This might be due to the physiological characterization of the fish sample.

As we know the result is related to the fish species and very useful was barcoded the fish analyzed (Di Finizio *et al.*, 2007). As seen in the present analysis, we can summarize that enzyme activity correlates with pollution, so the Red Sea is more polluted than the

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Journal of Environmental Sciences (JES) Faculty of Graduate Studies and Environmental Research, Ain Shams University

Mohammed, Omneya et al.

Mediterranean. The most critical biochemical biomarkers used recently for pollution assessment in fish are some enzymes related to the detoxification of toxic tools and their metabolites. The key pathogenic factors are an imbalance between oxidation and antioxidant systems and excessive reactive oxygen species (ROS) generation (Ahmadifar *et al.*, 2021). It has been explained that excess ROS, which damages tissue proteins, causes a large number of lipid peroxides and additional oxidative damage (Nakano and Wiegertjes, 2020; Saheli *et al.*, 2021). CAT & GST enzymes are implicated in the endogenous enzymatic antioxidant mechanisms that conserve the redox homeostasis and modulate the antioxidant system (Livingstone, 2001; Abdel-Tawwab and Wafeek, 2017; Hoseinifar *et al.*, 2021). Enzymes from the family of CAT & GST are considered very important for protection against any damage resulting from potentially reactive compounds combined with endogenous molecules, so they are finally removed by the body (D'Errico et al., 2018). The enzyme activity is known to be a vital biomarker of exposure to different environmental pollutants either terrestrial or aquatic (Fasulo *et al.*, 2015; Guerriero *et al.*, 2017 a,b).

Table 2: Seasonal values of the activity of CAT & GST (U/mg protein) in the liver tissues

 of Gilt-head bream Sparus aurata and Seabass Dicentrarchus labrax collected

 from Alexandria and Suez.

| | | GILT-HEA | AD BREAM | SEABASS | | | | | | | |
|------------|---|-------------------------|--|--------------------------------|--------------------------------------|--|--|--|--|--|--|
| Samples | | Sparus aurata | | Dicentrarchus labrax | | | | | | | |
| Alexandria | | | | | | | | | | | |
| Season | | CAT | GST | CAT | GST | | | | | | |
| Spring | 4 | $2.77\pm0.04^{\rm c}$ | $104.13 \pm 0.46^{\circ}$ | 2.92 ± 0.06^{c} | 106.14 ± 0.66^c | | | | | | |
| Summer | 4 | 3.76 ± 0.08^{a} | $116.55\pm0.75^{\text{a}}$ | 4.18 ± 0.04^{a} | 119.18 ± 0.68^a | | | | | | |
| Autumn | 4 | 3.40 ± 0.06^{ab} | ${\begin{array}{c}{111.58} \pm \\ {0.95}^{ab}\end{array}}$ | 3.59 ± 0.06^{b} | 113.35 ± 0.91^{b} | | | | | | |
| Winter | 4 | 2.91 ± 0.05^{bc} | $109.05\pm0.68^{\text{b}}$ | 3.21 ± 0.03^{bc} | 110.40 ± 1.08^{b} | | | | | | |
| Suez | | | | | | | | | | | |
| | | CAT | GST | CAT | GST | | | | | | |
| Spring | 4 | $2.89\pm0.05^{\rm c}$ | $108.85 \pm 0.65^{\circ}$ | $3.08\pm0.02^{\rm c}$ | $111.45 \pm 1.29^{\circ}$ | | | | | | |
| Summer | 4 | 4.03 ± 0.04^{a} | $120.18\pm1.37^{\text{a}}$ | 4.37 ± 0.04^{a} | 123.75 ± 1.28^a | | | | | | |
| Autumn | 4 | 3.61 ± 0.04^{b} | 115.25 ± 1.54^{b} | 3.84 ± 0.07^{b} | 118.93 ± 0.62^{b} | | | | | | |
| Winter | 4 | $3.15 \pm 0.05^{\circ}$ | $11\overline{3.83 \pm 0.96^{b}}$ | $3.\overline{35 \pm 0.04}^{c}$ | $11\overline{4.08 \pm 1.50^{\circ}}$ | | | | | | |

*The data are expressed as mean \pm SEM. ^{a,b,c:} Mean values within tissue with unlike superscript letters were significantly different (*P*<0.05).

The present study is, to our knowledge, the first report of the combined effects of seasonal seawater temperature on functional traits linked with the thermal performance of a notothenioid fish. By examining both organismal and sub-organismal responses in Gilt-head bream *Sparus aurata* & Seabass *Dicentrarchus labrax*, we found a reduction in the thermal tolerance range under temperatures with a decrease in the Mediterranean Sea than in the Red Sea revealed through an elevated Do level. An oxidative stress condition was also detected in the liver tissues. Such responses may have significant consequences under the present trajectories of climate change.

Changes in the antioxidant enzyme were response for the different temperatures on the liver tissue, wherefore the effects of potentially higher ROS generated under changing environmental conditions (Lushchak, 2014). Overall, these results indicate the generation of

an oxidative stress condition in the Seabass *Dicentrarchus Labrax* under warmer temperatures. These findings suggest that this species does not maintain a constant high antioxidant defense level, as the Gilt-head bream *Sparus aurata* compensates for the predicted temperature (Enzor and Place, 2014).



Figure 2: The activity (U/mg protein) of CAT & GST in the liver tissues of Gilt-head bream and Seabass collected from the Mediterranean & Red Sea during the four seasons.

* The data are expressed as mean \pm SD. ^{a,b,c:} Mean values within tissue with unlike superscript letters were significantly different (*P*<0.05).

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تقييم التأثير طويل المدى للتلويم على الدنيس والقاروس في البدر المتوسط والبدر الأحمر

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مستخلص البحجم

للتنبؤ بالتأثيرات المحتملة لتغير المناخ على الكائنات البحرية ونوعية المياه، قامت هذه الدراسة بتقييم التأثيرات طويلة المدى للتلوث على سمك الدنيس Sparus aurata وسمك القاروص Dicentrarchus labrax في الإسكندرية على البحر الأبيض المتوسط والسويس على البحر الأحمر مع الاخذ بعين الاعتبار التغير الموسمي. تم توثيق العوامل الفيزيائية والكيميائية المتعلقة بالتغير الموسمي في متوسط درجات الحرارة في البحر الأبيض المتوسط والبحر الأحمر. وتراوحت درجة الحرارة من 17.26 إلى 25.73 درجة مئوية بمتوسط 22.01 ± 0.31 درجة مئوية في الإسكندرية ومن 17.35 إلى 29.98 درجة مئوية بمتوسط 24.27 ± 0.54 درجة مئوية في السويس. كان لعينات المياه المجمعة متوسط تركيزات للأكسجين المذاب (DO) تتراوح بين 3.94–8.28 (6.77±0.42 مجم/لتر) في الإسكندرية إلى 4.65–9.38 (7.08±0.53 مجم/لتر) في السويس. تراوح متوسط المواد العضوية القابلة للأكسدة (OOM) بين 10.4–5.40 بمتوسط قيمة 8.10±0.76 ملجم/لتر في الإسكندرية و5.74–11.06 بمتوسط قيمة 8.77±0.84 ملجم/لتر في السويس. وتراوح متوسط الملوحة بين 37.36 - 39.62 بمتوسط 38.42±0.23 في الإسكندرية، و 40.28 – 42.92 بمتوسط 41.63±0.28 في السويس. تراوح متوسط قيم الأس الهيدروجيني من 7.96 إلى 8.57 بمتوسط 8.30 ± 0.01 في الإسكندرية بينما تراوح من 7.61 إلى 8.34 بمتوسط 8.07 في السويس مما يشير إلى أن المياه كانت قلوية قليلاً. كان لدرجة الحرارة تأثير كبير على أنشطة انزيم الكاتليز (CAT) وانزيم الجلوتاثيون إس ترانسفيريز (GST) في أنسجة الكبد. وكانت جميع قيم أنشطة CAT وGST في السويس أعلى منها في الإسكندرية. أظهر نشاط CAT في البحر الأبيض المتوسط خلال فصل الصيف قيمًا أقل مما كانت عليه في البحر الأحمر، والتي بلغت 3.76 ± 0.08 و 4.18 ± 0.04 وحدة / ملغ بروتين على التوالي. وعلى العكس من ذلك، بلغ نشاط GST في سمك القاروص Dicentrarchus labrax مع الدنيس GST في سمك القاروص 120.18 ±1.37 و 123.75 ±1.28 وحدة/ملجم بروتين على التوالي في البحر الأحمر. وبالمقارنة، كان النشاط أقل في البحر الأبيض المتوسط في الصيف منه في البحر الأحمر، والذي كان 116.55 ±0.75 و 119.18 ±0.68 وحدة/ ملجم بروتين على التوالي.

الكلمات المفتاحية: التلوث، الدنيس، القاروس، البحر المتوسط، البحر الأحمر

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848