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Rami Bouzenda
Laboratory of Applied Animal
Biology, Faculty of Sciences,
Department of Biology
University Badji Mokhtar of
Annaba, Annaba, Algeria

Noureddine Soltani
Laboratory of Applied Animal
Biology, Faculty of Sciences,
Department of Biology
University Badji Mokhtar of
Annaba, Annaba, Algeria

Mohamed El Hadi Khebbeh
Laboratory of Cellular
Toxicology, Faculty of Sciences,
Department of Biology
University Badji Mokhtar of
Annaba, Annaba, Algeria

Correspondence
Mohamed El Hadi Khebbeh
Laboratory of Cellular
Toxicology, Faculty of Sciences,
Department of Biology
University Badji Mokhtar of
Annaba, Annaba, Algeria.

Assessment of pollution in the Gulf of Annaba (Algeria) by monthly measurements of two biomarkers in a fish species *Liza aurata*

Rami Bouzenda, Noureddine Soltani and Mohamed El Hadi Khebbeh

Abstract

The present study aimed to assess water quality of the Gulf of Annaba by monthly measurements of two biomarkers of pollution during one year (December 2011 to November 2012) in the fish *Liza aurata* (Teleostei, Mugilidae) sampled in two different sites, Sidi Salem a polluted site and Hnaya as control site. This fish species was chosen because of its large consumption in our country and its sensitivity against several pollutants. Activities of liver glutathione S-transferase (GST) and brain acetylcholinesterase (AChE), respectively biomarkers of oxidative stress and neurotoxicity, were determined. Environmental parameters (temperature, dissolved oxygen and salinity) were also monitored. Results demonstrated that the physico-chemical data put in evidence of the temporal fluctuations without station effects. *L. aurata* showed differential biomarker responses according to abiotic factors and of the anthropogenic pressure of pollutants. An increase in GST activity was observed in fish from the polluted site Sidi Salem compared to the control site Hnaya suggesting induction of detoxification system. Moreover, a significant inhibition in AChE activity was recorded in fishes from the polluted site, which could reflect contamination by neurotoxic pollutants such as heavy metals. Overall, obtained data indicate that *L. aurata* constitutes a useful tool as sentinel organism for biomonitoring of aquatic pollution.

Keywords: Gulf of Annaba, biomonitoring, fish, *Liza aurata*, biomarkers, GST, AChE

1. Introduction

The pollution of aquatic ecosystems by several pollutants is an important environmental problem [1, 2]. During the last decade, various studies have shown that hydrocarbons [3], metals [4, 5], organophosphorus compounds (OPs), pesticides and herbicides [6, 7, 8] considerably contaminate different compartments of industrialized coastal regions. The absorption of some pollutants takes place in Humans mostly via the intake of contaminated food. Mollusks are present in our diet, they are great bio accumulators even if they originate from sites in which the levels of such contaminants are considered low [9] and could be considered 'potentially' dangerous for consumers [10].

Pollution of aquatic environment can be estimated in water, sediment and also in marine organisms [11]. According to [12] a bioindicator is an organism or a set of organisms that allows, by reference to biochemical, cytological, physiological, ecological or ethological variables, in a practical and safe way, to characterize the status of an ecosystem or an Eco complex and to highlight as early as possible their changes, natural or caused. Fishes form a large part of the human diet, so it is not surprising that most studies have been conducted on edible fish [13, 14, 15]. Mugilidae or mules are widely used as bioindicator species of pollution [16, 17]. These species inhabit the mouths and marginal-littoral zones, are known by their bio concentration of pollutants [18, 19, 20]. However, the choice of bioindicator species and biochemical biomarkers based on their sensitivity to chemical stress also requires taking into account natural, annual, seasonal and circadian fluctuations; Of those related to the aquatic environment: temperature, salinity, pressure, dissolved oxygen; Of those inherent in the stage of development; Of those intrinsic to individuals such as age, maturity, sex, nutritional status, and even those associated with adaptive processes such as parasitism [21, 22]. The environmental risk assessment involves the use of biomarkers designed to highlight an early stage of pollution [23, 24]. Many biochemical and cellular biomarkers have been studied in aquatic organisms, and particularly in fish and bivalve mollusks [25, 26]. Glutathione S-transferase (GST) are a multiple-enzyme family involved in phase II detoxification processes [26] and are used as biomarkers of several

groups of pollutants including metals and organophosphorus [27, 28]. Acetylcholinesterases (AChE) play an important role in the functioning of the neuromuscular system by preventing continuous muscular contraction [29]. AChE activity has been proposed as a biomarker of exposure to several chemicals such as organophosphorus compounds [30], and also by other contaminants such as metals, synthetic detergents, some components of fuel oils and algal toxins [31, 32].

This study aims to evaluate the water quality of the Gulf of Annaba by monthly measuring activities of two enzyme biomarkers of pollution in a fish *Liza aurata* Risso, 1810 (Teleostei, Mugilidae) and some physico-chemical parameters

of the marine environment (temperature, salinity and dissolved oxygen), in two different sites, Sidi Salem (polluted site) located in the Gulf of Annaba near coastal and industrial activities and Hnaya (control site) located out of the gulf considered to be far from contamination and less polluted.

2. Materials and methods

2.1 Study area

Fishes *L. aurata* were collected from December 2011 to November 2012 at Gulf of Annaba located in the eastern Algeria near the Tunisian-Algerian border in the region of Annaba (Fig.1).



Fig 1: Location of sampling sites in region of Annaba (Northeast Algeria). (Site 1: Hnaya as control site; Site 2: Sidi Salem as polluted site).

The fishes were collected in two sites: (Fig. 1), the site 1 (Hnaya 36°N54'24.0, 08°E 7'35) and the site 2 (Sidi Salem 36° 50' N, 7° 47' E). Sidi Salem site was known to be more polluted than Hnaya site because of its location near industrial and coastal activity areas, which contains several pollutants, originate from urbanism factory rejects.

2.2 Determination of physico-chemical parameters of water

Physico-chemical parameters were monthly measured from December 2011 to November 2012. Temperature, dissolved oxygen and salinity of water of the two sites were measured in duplicate *in situ* using a multi-parameter WTW type 2F30104211 (Multi 340 I/Set).

2.3 Collection and preparation of samples

L. aurata of similar size (24.63 ± 0.46 cm) and weight (255.77 ± 6.17 g) were monthly fished ($n=6$ for each month and site) between December 2011 and November 2012 from each site and were immediately transferred to our laboratory and dissected. Liver and brain were used as biological material for the quantification of GST and AChE activities respectively. Liver is kept in homogenization buffer (20 ml of Phosphate buffer 0.1M pH=6, 1.71g sucrose) using ultrasound. The homogenate is then centrifuged at 14000 rpm for 30 minutes. The recovered supernatant was used for the determination of liver GST activity and protein content. Brain is homogenized for a few seconds in 1ml of detergent solution [38. 03 mg EGTA (éthylène glycol-bis, β -aminoéthyl éther N N N 'N' tetra-acétique), 1 ml triton X 100%, 5.845 g NaCl, 80 ml 10 mM Tris buffer] and centrifuged at 5000 rpm for 5 minutes. The recovered supernatant was used for determination of brain AChE activity and protein concentration.

2.4 Glutathione S-transferase activity

GST activity was quantified according to the colorimetric method [33] and expressed as μM per min per mg of proteins, of providing a substrate for enzyme (usually 1-chloro-2,4 dinitrobenzene CDNB which reacts readily with many form of GST) and glutathione. The catalyzed reaction of conjugation of this two products leads to the formation of a new molecule that absorbs light at 340 nm. Activity was expressed as $\mu\text{mol}/\text{min}/\text{mg}$ proteins.

2.5 Acetylcholinesterase activity

Determination of AChE activity was performed using a method described by [34] with the use of acetylthiocholine (ASCh) as substrate. The activity rate was measured as change in absorbance/min at 412 nm (extinction coefficient $1.36 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$). Activity was expressed as nmol/min/mg protein.

2.6 Protein quantification

The proteins were quantified according to the method of [35], using bleu brilliant of Coomassie (G250, Merck) as reagent and bovine serum albumin (BSA, Sigma) as standard protein. The absorbance was read at a wavelength of 595 nm.

2.7 Statistical tests

Data are expressed as mean \pm standard deviation (SD). All statistical calculations were performed with the MINITAB Software (Version 16, Penn State College, PA, USA). Data from physico-chemical parameters water and enzyme activities (GST, AChE) were tested using two-way analysis of variance (ANOVA). Differences between sites were determined by Tukey's test. A significant difference was assumed when $p < 0.05$.

3. Results

3.1 Physico-chemical characteristics of water from the two sites

The different values (temperature, salinity and dissolved O₂) monthly measured in water were presented in table 1. Obtained results showed seasonal variations in the two sampling sites along a year (table 2). The lowest temperature was recorded in February and the highest in August with a maximum values of 28.5 °C at site 1 (Hnaya) and 28.8 °C at site 2 (Sidi Salem).

Salinity showed the highest values in summer and autumn period, with a maximum value of 37 psu at site 1 (Hnaya) and 39.9 psu at site 2 (Sidi Salem) during the month of October. However, salinity reached their minimum value 26.6 Psu in March at site 1(Hnaya) and 28.1 Psu at site 2.

Values recorded for the dissolved oxygen in the two sites showed significant differences, the highest results was registered in January at both sites with 15.2 and 11.3 mg/L respectively at site 1 and 2 while the lowest was recorded in August (4.1 and 2.8 mg/L) in sites 1 and 2, respectively.

Table 1: Monthly variation of temperature (°C), salinity (psu) and dissolved oxygen (mg/L) during one year in the two studied sites.

Months Parameters		Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
Temperature (°C)	Site 1	12.5	12.2	12.1	13.8	16	20.4	23.7	26	28.5	25	23.9	18
	Site 2	13.9	13.2	12.5	14.8	18.2	22.6	24.1	27.1	28.8	26.3	23.4	18.7
Salinity (psu)	Site 1	31.7	28.2	26.8	26.6	28.1	31.4	34.8	36.3	36.6	36.7	37	36.9
	Site 2	31.2	31.2	31.2	28.1	31	34.5	37.9	38.7	39.4	39.7	39.9	38.8
Dissolved oxygen (mg/L)	Site 1	15.2	15.1	13.3	13.7	12.1	8.4	6.9	4.2	4.1	4.8	5.8	12.4
	Site 2	11.3	9.9	8.3	8.3	7.1	6.7	5.6	3.5	2.8	2.8	4.2	7.3

Table 2: Two-way ANOVA (site, month) on physico-chemical water parameters from the two sites.

Physical parameters	P (Site)	P (Month)	P (Site vs Month)
Temperature (°C)	0.000 ***	0.000 ***	0.000 ***
Salinity (psu)	0.212	0.306	0.471
Dissolved oxygen (mg/L)	0.000 ***	0.000 ***	0.000 ***

(***significant at p<0.001).

3.2 Glutathione S-Transferase Activity

Monitoring of the variation in the content of GST ($m \pm s$) ($\mu\text{M}/\text{min}/\text{mg}$ protein) in *Liza aurata* liver from the two sites revealed the existence of monthly significant fluctuations (Fig. 2). GST content increased and the maximum values were recorded in August for the site 1 (Hnaya) and 2 (Sidi Salem), (48.60 and 92.54 $\mu\text{M}/\text{min}/\text{mg}$ protein) respectively. The lowest values were recorded in January for both sites (35.54 at site 1 and 52.45 $\mu\text{M}/\text{min}/\text{mg}$ protein at site 2). In addition, GST activity in liver of *L. aurata* from site 2 was significantly higher than those of individuals from site 1 during all months of the year. Significant effects ($P < 0.001$) of site ($F = 207.97$; $df = 1, 120$) and months ($F = 1740.60$; $df = 11, 120$) were revealed by two-way ANOVA test (Table 3).

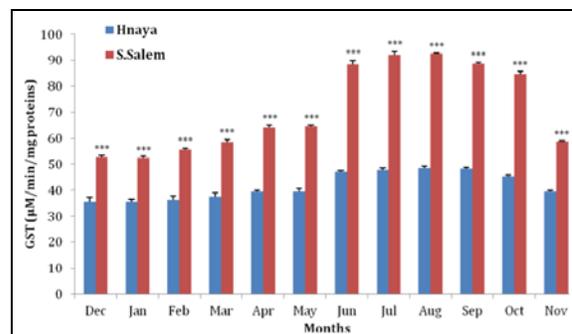


Fig 2: Monthly variation of glutathione-S-transferase (GST) activity ($\mu\text{M}/\text{min}/\text{mg}$ protein) in liver of *L. aurata* collected from two studied sites (December 2011 to November 2012). ($m \pm SD$; $n = 6$; ***: Significantly different at $p < 0.001$).

Table 3: Two-way ANOVA (site, month) on GST activity data in *L. aurata* liver.

Sources of variation	DF	SS	MS	Fobs	P
Site	1	31119.28	31119.28	207.97	0.000***
Month	11	15712.21	1428.28	1740.60	0.000***
Interaction Site/Month	11	4258.37	387.12	471.74	0.000***
Residual error	120	98.48	0.82	-	-
Total	143	51188.33	-	-	-

(DF: Degrees of freedom; SS: Sum of squares; MS: Mean squares). (***: Significantly different at $p < 0.001$).

3.3 Acetylcholinesterase activity

AChE activity was evaluated on *Liza aurata* brain fished in the two sites (Fig. 3). Obtained results showed significant fluctuations of this activity along a year. AChE activities from site 02 (Sidi Salem) were lower than those recorded in site 01 (Hnaya) suggesting an inhibition of AChE activities in site 02 (Sidi Salem). The lowest values were observed in August at

the two sites of study, with values of 29.96 ± 2.385 nmol/min/mg protein for site 01 (Hnaya), and 13.72 ± 0.953 nmol/min/mg protein for site 02 (Sidi Salem). The maximum values were observed in January. Results were confirmed by the two-way ANOVA since a significant ($P < 0.001$) effect of both site ($F = 2544.10$; $df = 1, 120$) and month ($F = 284.51$; $df = 11, 120$) was noted (Table 4).

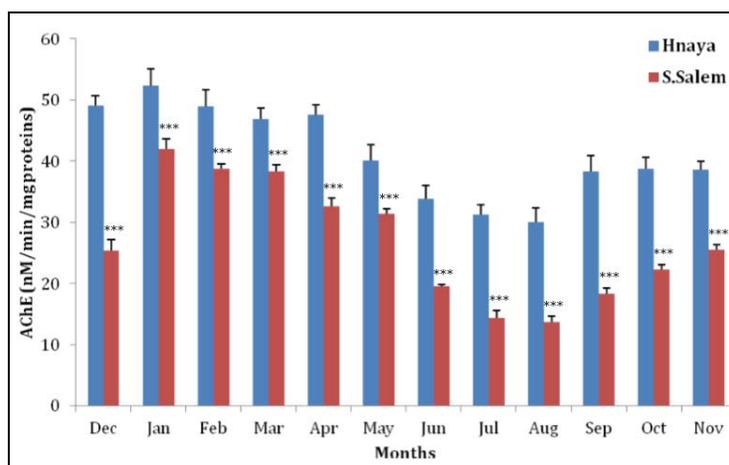


Fig 3: Monthly variation of Acetylcholine esterase activity (AChE) (nM/min/mg protein) in brain of *L. aurata* collected from two studied sites (December 2011 to November 2012). (m \pm SD ; n= 6 ; ***: Significantly different at p< 0.001).

Table 4: Two-way ANOVA (site, month) on AChE activity data in *L. aurata* brain.

Sources of variation	DF	SS	MS	Fobs	P
Site	1	7513.70	7513.70	2544.10	0.000***
Month	11	9243.03	840.28	284.51	0.000***
Interaction Site/Month	11	703.68	63.97	21.66	0.000***
Residual error	120	354.41	2.95	-	-
Total	143	17814.81	-	-	-

(DF: Degrees of freedom; SS: Sum of squares; MS: Mean squares; ***: Significantly different at p< 0.001).

4. Discussion

Among the several natural abiotic factors, oxygen level, temperature and salinity are the most likely to modulate oxidative stress [36], as well as immunological parameters in aquatic animals [37, 38]. The physico-chemical parameters of water (temperature, salinity and dissolved oxygen) monthly registered at the two sites (Hnaya and Sidi Salem) during one year (December 2011 to November 2012) showed seasonal fluctuations; temperature recorded revealed the existence of two major thermal periods in the two sites, a cold period that extends from October to August with maximum values in August at both sites (28.5°C for Hnaya and 28.8°C for Sidi Salem). Significant differences were observed between each month and an interaction sites/months was also observed. Increase in temperature in the S. Salem site compared to Hnaya may be due to domestic and industrial releases. Fluctuations of this abiotic parameter were related to the local climatic conditions and more particularly to air temperature [39, 40, 41]. The highest salinity values were observed in October at both sites with 37°C for Hnaya and 39.9°C for Sidi Salem due to combined action of high temperatures which cause strong evaporation and decreased precipitation; While low water evaporation rates and heavy winter precipitation showed low salinity [42], which explains the values recorded during January to April in both sites.

Dissolved oxygen is an essential component of most aquatic organisms. Fluctuations in dissolved oxygen content in water are related to seasonal variations in temperature, sea levels and salinity. In this work, a significant drop in oxygen levels was observed from June to October and an increase from November to March in both sites. These variations were probably due to the presence of plants, oxidizable matter and aerobic germs [43]. A reduction in O₂ concentration also disrupts reproduction and locomotion [44, 45]. Moreover, higher temperature lead to an increase in oxygen consumption and consequently to an enhancement of ROS (Reactive Oxygen Species) generation [46].

Analysis of AChE activity, as a biomarker in different tissues provides a method of diagnosing poisoning and detecting contamination by anti-cholinesterase pesticides [47, 31] and also by other contaminants such as metals, synthetic detergents, some components of fuel oils and algal toxins [48, 49, 50]. Inhibition of AChE, which is responsible for the degradation of acetylcholine, will result in excessive stimulation of cholinergic nerves, resulting in tremors and convulsions [51].

GST is an important phase II enzyme that catalyzes the conjugation of reduced glutathione (GSH) to cellular components damaged by ROS attack, leading to their detoxication [52]. The GST activity, as a biomarker of defense, participates also in anti-oxidative defenses [53] and can be triggered by some pollutants [54, 55]. It was also reported that activity of some GST iso-enzymes could be induced by substrates such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenols (PCBs) [56].

In this current study, evaluation of biomarkers (AChE, GST) revealed seasonal variations as reported [57]. Inhibition of AChE activity was observed in the warm months (June to September) at the two sites. The same observations were noted in *Mytilus galloprovincialis* [58], *Donax trunculus* [59] and *Cerastoderma glaucum* [60] with increasing temperature. Enzymatic activity could be modulated by natural factors such as seawater temperature, biotoxins or cyanobacteria and salinity [61, 62]. The maximum values of GST in *L. aurata* liver were recorded in the warmer months (June to October) at the two sites; this was related to environmental factors such as temperature. Indeed, the temperature could influence the activity of enzyme systems by altering all biological and physiological functions of animals [63]. Other factors could influence GST activity such as the reproductive cycle [64], age [65], salinity and dissolved oxygen [66]. Environment contamination probably played a role, especially in site 2 (Sidi Salem) compared to site 1 (Hnaya). We found the lowest levels of AChE activity and the higher values of GST in site 02 (Sidi Salem) compared with values measured in site 01

(Hnaya). This suggested that potential pollutants like pesticides might be highly at Sidi Salem site, which is located near industrial area that produced phosphoric fertilizers and pesticides, such organophosphates. This site in the Gulf of Annaba is a recipient of a large amount of contaminants from urban, agricultural, harbor and industrial activities^[67, 68, 69].

In contrast, site 1 (Hnaya) was relatively less polluted. The high levels of AChE activity and less levels of GST as compared with Sidi Salem site confirmed this. It was considered as a reference Site. Our results were consistent with studies on *Donax trunculus* from the coast of Annaba (Algeria)^[60, 70, 71] who reported an inhibition in AChE and an induction in GST activities in the site of Sidi salem polluted by heavy metals compared to a less polluted site. Similarly, an inhibition of AChE was observed in clams *Ruditapes philippinarum* exposed to neurotoxic pollutants present in the water of agricultural land drainage^[72] and in *Donax trunculus* from a multi-contamination site by the intensive agricultural and industrial activities in comparaisson with the reference site in the Gulf of Tunis^[73].

5. Conclusion

It can be concluded that activities of GST and AChE measured in a fish species *Liza aurata* were influenced by environmental factors (temperature, dissolved oxygen and salinity) this was confirmed by the seasonal variations of these enzymes during the study period. In addition, site 2 (Sidi Salem) was more polluted compared to site 1 (Hnaya). The fish species tested was useful as species to assess environmental effects of pollution in this study.

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