Differential Fatty Acids and Stress Biomarkers Responses in Native (Carcinus Aestuarii) and Invasive (Portunus Segnis) Crabs Exposed to Environmental Pollution

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Abstract

The present work aims to determine and compare the tolerance to different stress environmental conditions in two crabs' species the native *Carcinus aestuarii* and the invasive *Portunus segnis*. In this study, *C.aestuarii* appeared more intolerant to environmental stressor as shown by the important changes occurred on the proximate, fatty acids and their essential compounds comparing to *P.segnis*. Our results revealed accumulation of metallic trace elements, macromolecular damages and antioxidant defense systems perturbations mostly in *C.aesuarii* gills and muscles than *P.segnis,* thus highlighting the importance of a multi-markers approach to assess the urban pollution in coastal ecosystems. According to this study, fatty acid profiles and oxidative stress biomarker examination of both crabs indicate that the different environmental stresses significantly influence the tolerance of *C.aestuarii* compared to the invasive *P.segnis* which may provide physiological advantages for the achievement of their invasion in novel ecosystems.

1. Introduction

Tunisian coastal ecosystems are threatened by increasing contamination due to the occurrence of chemical compounds, like polycyclic aromatic hydrocarbons (PAHs) (Barhoumi et al. 2016), pesticides (Mhadhbi et al. 2019) and metallic trace metals (TEs) (Ghribi et al. 2020). These pollutants are released into coastal aquatic ecosystems as a result of human activities such as urbanization, industrialization, and agricultural activities. These xenobiotics affect aquatic organisms causing significant negative health issues and mortality (Bejaoui et al. 2019). Along the Tunisian coast, the Gulf of Gabes represents high value eco-socio-system; it has been recognized through decades as a biodiversity "Hot Spot". This area has been a reservoir of marine organisms, high phytoplankton bloom and a nursery for several fish species (Ben Brahim et al. 2010; Enajjar et al. 2015). However, this shallow coastal highly productive ecosystem have been impacted by tides and submitted to urban/industrial effluents for years (Chifflet et al. 2019). Previous reports demonstrated that the main cause of the disequilibrium of this ecosystem and the degradation of its water quality is the phosphogypsum discharge (Amor et al. 2018). Living marine organisms in the Gulf of Gabes have developed over time physiological adaptations physiological functions that allowed their survival and growth in such highly impacted ecosystem (Hattab et al. 2013). Despite the Gulf of Gabes is exposed to anthropogenic factors altering its natural features, it has been submerged by invasive species that represented an added pressure on its functioning and fisheries. Several exotic invasive species have been recorded in Tunisian coastal ecosystems and specifically in the Gulf of Gabes (Bejaoui et al. 2019).

Biological invasions are a widespread and significant component of human-caused global environmental change where alien species invasion has been favored by new climatic conditions at the expense of native species (Vitousek et al. 1997; Perings et al. 2002). Climate change influences invasive species distribution and behavior by affecting their entry pathways, establishment, spread and colonization of new habitats (Hobbs and Mooney 2005). It has been previously reported that global warming and climate changes affected native communities, altered ecosystem functions and increased the risk of biological invasion. Consequently, invasive species have been considered as the main drivers of global biodiversity loss and ecosystem degradation (Richardson et al. 2010) as they showed a strong tolerance to pollution and environmental stressors (Olden et al. 2006). Observable claims that invasive species are normally more tolerant to unfavorable conditions is still limited (Lenz and al. 2011), and therefore, further investigation is strongly recommended.

Recently, the popular invasion of the ecosystem of the Gulf of Gabes was characterized by the introduction of the blue crab (*Portunus segnis*), which has been introduced to the Mediterranean Sea after the opening of the Suez Canal and quickly became widespread on its coasts (Bejaoui et al. 2017; Annabi et al. 2018; Shaeik et al. 2021; Bejaoui et al. 2021). In Tunisia, its invasive range covers the entire country coasts. It was recorded for the first time in the Gulf of Gabès in 2015, but it has been assumed that its invasion occurred in Tunisian waters for at least two years before (Rabaoui et al. 2015). Like many invasive species, *P. segnis* invasion affected negatively native species, such as *Carcinus aestuarii* (*C. aestuarii*), through direct competition for food and habitat since it displayed faster growth rate and reproductive potential than the native species (Katsanevakis et al. 2014). This native crab (*C. aestuarii*) lives in shallow water and is able to withstand large variations in salinity and temperature, probably due to its tolerance and high adaptability (Yamada and Hauck 2001). *C. aestuarii* inhabited the Mediterranean Sea (estuarine and lagoon ecosystems) and having colonized the Adreatic Sea (Qyli et al. 2020).

In order to examine whether invasive species are more tolerant to stress compared with their native ones, we investigated the physiological responses of two marine crab species (*C. aestuarii* and *P. segnis*) against the environmental changes and trace metal pollution, that have been recently adopted in Tunisian food sector as an important and economically valuable marine products (Mili et al. 2020). Although, several studies investigated the effect of environmental pollution on native crabs, little attention has been paid to assess the tolerance of invasive crabs towards xenobiotics and their possible use as bio-indicators of pollution. Predicting alien invasive species adaptation in response to new dynamic environments, in particularly, environmental pollution presents a further serious challenge.

Up to date, there is no data available on antioxidant defense systems and their response induced by the accumulation of trace elements by comparing invasive and native crab species. For this, this study aimed to perform a comparative analysis of *C. aestuarii* and *P.segnis* to assess whether different realistic stressors can affect their metabolisms where the gills and muscles have different concentrations of trace elements. Therefore, the present study was planned to assess antioxidant networks and biochemical composition by means of established fatty acid profiles, proteins, lipids and carbohydrates contents.

2. Material And Methods

2.1. Sampling area

The Gulf of Gabes is located on the eastern coast of Tunisia (34° 05' 37"; 10° 26' 13"), in the western Mediterranean basin (Figure 1). It covers an area of 90 km^2 which has an average depth of 40 m and a maximum width of 250 km. The Gulf of Gabes is known as the most dynamic ecosystem, devoting around 40% of the national aquatic production (DGPA 2015) and is considered as an important nursery for several species of fish (Enajjar et al. 2015).This ecosystem was recently identified as one of the eleven consensus eco-regions of the Mediterranean and is considered, along with the Venetian Plateau region, to be a

shallow, phytoplankton blooming region known to be oligotrophic, as it is governed by the influx of nutrients generated from Atlantic surface waters from Gibraltar (Krom et al. 2010, Ayata et al. 2017). Despite the hydrodynamics and biogeochemistry of this area, the Gulf of Gabes received important daily industrial and agricultural discharges that affect its quality (Bejaoui et al. 2019).

2.2. Sampling crabs and tissues preparations

A total of 50 sexually mature *P. segnis* and *C. aestuarii* of commercial size (Weight ≈ 180 g and length ≈10 cm) were collected from the Gulf of Gabes at about 50 m depth on spring and winter 2019. Winter season corresponded to the spawning period of both species where gamete release occurred. While, the spring period is marked by the beginning of gametogenesis process in which gametes formation and development in gonads starts (Tureli and Yesilyurt 2017). Samples were carried to the laboratory in aerated box with seawater and acclimated during 6 days. After acclimation, invasive and native crab individuals were sacrificed to remove gills and muscles tissues. Regardless of sex, for the biochemical analysis, organs were immediately homogenized with Tris-HCl buffer (20mM, pH 7.4) in cold, and then centrifuged during 25 min at 10.000 × g. The obtained supernatant extracts were stored at -80°C until proximate and oxidative stress analyses. For trace elements accumulation and fatty acids' analyses, gills and muscles were conserved in liquid nitrogen. Water parameters (Temperature and salinity) were measured several times through the sampling process during spring (T°C = 18.033 ± 0.152; S psu = 38.666 ± 0.155) and winter (T°C = 14.233 ± 0.251; S psu = 37.966 ± 0.152) seasons.

2.3. Metallic trace elements analysis

TEs analysis in muscles and gills tissues was made according to the Carvalho et al (2000) protocol. Each gill and muscle tissues were dried at 150°C until reaching constant weight and measured with precision scale (0.01g dray weight). Dry tissues (1g) were ground to powder using an agate mortar then dried powders were mineralized in Teflon bombs in a closed microwave digestion labstation (Ethos D, Milestone Inc.) using concentrated nitric acid solution (4mL, 69%) and hydrogen peroxide (2mL, %). Digestates were diluted to an appropriate volume of 50 ml prior to being analyzed. TEs concentrations were determined using an inductively coupled plasma mass spectrometry (ICP-MS, Agilent, 7500ceModel). Results were expressed in mg/g of dry weight (DW). Analytical accuracy was checked by analyzing Certified Reference Materials (Charleston, SC, USA) to check the analytical precision. The results obtained for standard reference materials were within the 95% confidence interval and consistent with the certified values for all TEs. The obtained TEs concentrations were compared to maximum permitted limits for human consumption established by Montenegrin national legislation and other international and national guidelines (Table 1).

Table 1
Trace element concentrations (mg/g) in *P. segnis* and *C. aestuarii* muscles and gills collected from the Gulf of Gabes during spring and winter seasons.

			Pb	Cd	Cu	Fe	Ni
Gills	Spring	*P.segnis*	3.541±0.367	0.300±0.037	34.700±2.738	359.458±32.727	1.175±0.214
		C.aestuarii	5.166±0.917***	0.366±0.054**	38.625±1.928**	400.166±24.026*	1.6750.231***
	Winter	*P.segnis*	2.875±0.564#	0.229±0.026#	18.625±1.825###	303.291±11.044##	1.291±0.485
		C.aestuarii	3.208±0.361###	0.291±0.050***##	23.291±2.938**###	349.375±30.813**##	1.250±0.570
Muscles	Spring	*P.segnis*	1.375±0.237	1.333±0.270	16.504±1.562	181.229±44.195	0.445±0.073
		C.aestuarii	1.625±0.209*	1.750±0.262*	22.45±1.932***	208.937±63.484	0.633±0.056***
	Winter	*P.segnis*	1.562±0.233	0.117 0.013###	14.729±1.829	154.854±47.321	0.441±0.028
		C.aestuarii	1.416±0.312	0.131 0.017*###	18.010±1.405**###	148.520±28.274	0.570±0.081**
EU (2006) *mg/g*			0.5	0.5	5	-	-
*FAO (1983)*mg/kg			0.5	0.5	30	-	-
USA (2006)			-	-	1.3	0.3	-
WHO (1993b)			-	-	2	-	0.02

Results are presented as means ± SD (n=6).

Significant differences between *P.segnis* and *C.aestuarii* are detected at 5% as follows: *p<0.05; **p<0.01 and ***p<0.001.

Significant differences between crabs and seasons are detected at 5% as follows: #p<0.05; ##p<0.01 and ###p<0.001.

FAO (1983): Compilation of Legal Limits for Hazardous Substance in Fish and Fishery products (Food and agricultural organization). FAO Fishery circular, No. 464pp,5-1000.

WHO (1993b): Food and drug administration, Guidance document for nickel in shell fish. Washington D.C: DHHS/PHS/FDA/CFSAN/office of seafood; 1993b.

EU (2006): Maximum levels for certain contaminants in foodstuffs, Official Journal of the European Union, L 364/5

USA (2006): United Nations Environment Programme Global Environment Monitoring System/Water Programme (Water Quality for Ecosystem and Human Health)

2.4. Proximate composition analysis

Carbohydrate content was measured in muscles and gills of the invasive and native crabs according to Dubois et al (1956). Total lipids were established according to the method of Frings et al (1972) using phosphor-vanillin buffer. A high-quality United States (U.S) grade of olive oil (Sigma, St. Louis, USA) was used as a standard. The total lipid contents were calculated by reference to a standard curve and expressed as mg/g of wet weight (ww). Protein quantification in the selected organs was estimated through Lowry et al (1951) protocol, using bovine serum albumin (BSA) as a standard and expressed as mg/g of wet weight.

2.5. Fatty acids analysis and nutritional quality indices

The lipid of both gills and muscles crabs was extracted using chloroform/methanol (2v/1v) solution based on Folch et al (1957) method. This solution contained butylated hydroxytoluene (BHT; 0.01%) as an antioxidant. Fatty acid (FA) was trans-methylated according to Cecchi et al (1982) by the addition of sodium methylate ($NaOCH_3$) and sulfuric acid (H_2SO_4). Nonadecanoic acid (C19:0) (Belefonte PA, USA, CRM47885) was added as standard reference to facilitate the identification and calculation of FA. Fatty acid methyl esters (FAMEs) obtained after centrifugation at 3000 tr during 10 min was recuperated and analyzed through a gas chromatography (CPG) which comported a flame ionization detector and a capillary silica column (30 m of length, 250µm of diameter and 0.25 µm of film thickness). The obtained FA profile was integrated using HP chemstation software and identified by comparing the obtained retention times with the standards methyl esters (SUPELCO PUFA-3). The FA composition of gills and muscles were expressed as percent (%).

2.6. Biomarkers analysis

2.6.1. Lipid peroxidation analysis

Malondialdehyde (MDA) level in gills and muscle of *P.segnis* and *C. aestuarii* was estimated basing to Draper and Hadley (1990) at 520 nm, using 1,1,3,3-tetraethoxypropane (TEP) as standard. MDA values were expressed as nmol/mg of protein.

Lipid hydroperoxide (LOOH) level was carried out following Jiang et al (1992) method. The LOOH level was detected at 560 nm and calculated using the ferrous ion oxidation xylenol orange (FOX). Results were expressed as mmol/ mg of protein.

2.6.2. Protein oxidation analysis

Protein carbonyl (PCO) level was determined by a spectrophotometer at 370 nm according to the method of Reznick and Packer (1994). The PCO values were calculated based on the molar extinction coefficient of 2,4-dinitrophénylhydrazine (DNPH; 02.2×104 cm^{-1} M^{-1}) and results were expressed as nmol /mg of protein.

Advanced oxidation protein product (AOPP) level was evaluated following the method of Kayali et al (2006) through a spectrophotometer method at 340 nm. AOPP levels were calculated as nmol/ mg of protein.

2.6.3. Antioxidants status analysis

Metallothioneins (MTs) were determined based the procedure described by Viarengo et al (1997) modified by Petrovic et al (2001). Briefly, supernatants were mixed with cold ethanol and incubate at cold room during 1h. After that, centrifugation was performed to separate the pellets which were washed with 87% ethanol and 1% chloroform. MT level was measured at 412 nm after adding 0.25 M NaCl, 1 mM EDTA and 0.6 mM DTNB to the pellets and the MT concentration was estimated using GSH as a reference standard and expressed as µmol of GSH/ mg of protein.

Reduced glutathione (GSH) was determined according to the method of Ellman (1959). The reaction mixture was prepared with phosphate buffer (0,1M, pH 7.5), salicylic acid (4%) and DTNB (10mM). A yellow product was formed whose absorbance was determined at 412 nm. Reduced glutathione concentration was estimated using a standard curve previously prepared, and expressed as µg/mg of protein.

Glutathion peroxidase (GPx) was measured using GSH as a conjugation substrate based on the method previously described by Flohe and Gunzler (1984). Briefly, 200µL of extract were mixed with 4 mM GSH, 5mMH_2O_2 and 5% TCA, final concentrations. Proteins were precipitated and the supernatant was mixed with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB). Activity was determined spectrophotometrically at 420 nm as µmols GSH consumed min/ mg of protein using an extinction coefficient of 6.22 mM^{-1} cm^{-1}.

Superoxide dismutase (SOD) was determined spectrophotometrically at 580 nm according to Beauchamp and Fridovich (1971), and based on the ability of superoxide dismutase to inhibit the reduction of nitro-blue tetrazolium (NBT) by superoxide anion. One SOD unit was defined as the amount of enzyme required to inhibit 50% of the NBT photo-reduction in comparison with tubes lacking the extracts. Activity was expressed as U/ mg of protein.

Glutathion S-transferase (GST) was determined according to Habig et al (1974). 100µl of gills and muscles supernatants was added to 200µl of reactive solution contained phosphate buffer (pH: 6.5; 100mm), glutathione reduce (GSH; 10mm) and 1-chloro-2,4-dinitrobenzene (CDNB; 60mm). The reaction was measured by calculating the rate of connection between 1-Choloro-2, 4 -dinitrobenzene (CDNB) with reduced L-Glutathion (GSH). This reaction in the range of ultraviolet ray was measured at 340nm. Results were expressed as µmol/ mg of protein.

2.7. Statistical analysis

2.7.1. Anthropogenic indices

Metal pollution index (MPI) was evaluated to compare the total metal content at the different sampling sites and organs (Usero et al. 1997) and was calculated as follows:

Equation (1)

$MPI=(Cf_1 \times Cf_2 \times Cf_3 \times \ldots\ldots Cf_n)^{1/n}$

Where Cf_1, Cf_2, Cf_3 and Cf_n (mg/g) were the average concentration of TEs in each organs and each species.

Bioconcentration factor (BCF) was determined to assess the relationship of TEs levels in invasive and native crabs, and was calculated as follows (Mendoza-Carranza et al. 2016):

Equation (2)

BCF=C native/C invasive

where C_{native} and $C_{invasive}$ (mg/g) stand for the average concentration of TEs in the crabs species.

Individual Mean Bioaccumulation index (IMBI) was calculated according to the equation given by Maes et al (2005) and ranged as follows: 0 < IMBI < 1.

Equation (3)

IMBI=ICi / Cimax /n

where, n is the total member of metals, Ci is the individual metal concentration of the heavy metal I, Cimax is the maximum observed concentration of the heavy metal I.

2.7.2. Data analysis

The resulted data was expressed as mean ± standard deviation (SD. The statistical analysis was performed applying R version 4.0.4 (R Core Team, 2021) (Harrell et al. 2021). Our results were analyzed for normality and homogeneity of variance using Kolmogorov–Smirnov and Levene's tests, respectively. A one-way analysis of variance (ANOVA) was used via Tukey HSD to establish the significant variation between the invasive and native crabs at $p< 0.05$. Principal Component Analysis (PCA) and correlation matrix between tested parameters were run by the statistical software program R version 2.15.2 and applied to assess the association between both crabs' species and measurements parameters in the gills and muscles tissues. The heatmap was generated with the R package heatmaply (Galili et al. 2021).

3. Results

3.1. Metallic trace elements contents in *P. segnis* and *C. aestuarii* muscles and gills

Differences in TEs concentrations between *P. segnis* and *C. aestuarii* were detected and presented in Table 1. In particular, *C. aestuarii* exhibited the highest concentration of TEs in both gills and muscles ($p<0.05$, One-way ANOVA).

Pb, Cd, Cu, Fe and Ni levels were two-fold lesser in *P. segnis* gills than in *C. aestuarii*. Similarly, muscles tissues showed significant lower amounts of TEs (Pb, Cd, Cu and Ni) in *P. segnis* as compared to *C. aestuarii* ($p<0.05$, One-way ANOVA), except Fe which remained constant for both crabs tissues. During the winter, the contents of Cu and Cd in both crabs' tissues decreased significantly when compared to spring (Table 1). Only, gills tissues showed a significant decrease of Pb and Fe during winter in both *P. segnis* and *C. aestuarii* ($p<0.05$, One-way ANOVA). However, Ni contents showed a similar variation in both spring and winter seasons.

3.2. Anthropogenic indices in *P. segnis* and *C. aestuarii* muscles and gills

3.2.1. Metal pollution index (MPI) in P. segnis and C. aestuarii muscles and gills

The results of MPI are presented in Table 2, showing that *C.aestuarii* tissues exhibited the highest values as compared to *P.segnis* ($p<0.05$, One-way ANOVA).Our results demonstrated that MPI values varied significantly between the studied crabs ($p< 0.05$, One-way ANOVA), as evidence by an increase in *C. aestuarii* gills (by 25% and 15% during spring and winter, respectively) and muscles (by 25% during winter) as compared to *P. segnis*.

Table 2
Anthropogenic indices in *P. segnis* and *C. aestuarii* muscles and gills collected from the Gulf of Gabes during spring a

			MPI	IMBI					BCF	
				Pb	Cd	Cu	Fe	Ni	Pb	Cd
Gills	Spring	*P.segnis*	2.925	0.157±0.027	0.160±0.010	0.179±0.016	0.146±0.035	0.174±0.028	1.198±0.172	1.105±
		C.aestuarii	3.632	0.173±0.022*	0.196±0.025**	0.190±0.015*	0.156±0.047	0.184±0.016		
	Winter	*P.segnis*	6.893	0.157±0.018	0.174±0.013	0.173±0.015	0.181±0.016#	0.164±0.030	1.473±0.300#	1.323±
		C.aestuarii	8.668*	0.178±0.028*	0.193±0.051*	0.217±0.009*	0.186±0.011#	0.171±0.023		
Muscles	Spring	*P.segnis*	4.615#	0.166±0.024	0.152±0.030	0.167±0.020	0.154±0.037	0.178±0.012	1.035±0.234	1.493±
		C.aestuarii	5.193#	0.158±0.041	0.164±0.024	0.169±0.014	0.160±0.028	0.173±0.048		
	Winter	*P.segnis*	5.448#	0.164±0.032	0.155±0.05	0.175±0.018	0.168±0.009	0.129±0.048##	1.145±0.209#	1.207±
		C.aestuarii	6.244*#	0.171±0.036	0.179±0.018**	0.169±0.021	0.185±0.015	0.121±0.050###		

Results are presented as means ± SD (n=6).

Significant differences between *P.segnis* and *C.aestuarii* are detected at 5% as follows: *p<0.05; and **p<0.01.

Significant differences between crabs and seasons are detected at 5% as follows: #p<0.05; ##p<0.01 and ###p<0.001.

MPI: metal pollution index; IMBI: bioaccumulation index; BCF: bioconcentration factor.

3.2.2. Bioaccumulation index (IMBI) in P. segnis and C. aestuarii muscles and gills

The distribution of the bioaccumulation index (IMBI) results according to periods and species are given in the Table 2. According to our results data, IMBI was lower in *P.segnis* gills and muscles compared to *C. aestuarii* (p <0.05, One-way ANOVA). Distribution patterns of IMBI in the studied seasons showed significant increases during the spring and winter for Cd, Cu, and Pb in gills of both *P. segnis* and *C. aestuarii* as compared to Fe and Ni which remained stable (p > 0.05, One-way ANOVA). For muscles tissues, only Cd showed a significant enhancement by +43% during the winter season in *C.aestuarii* as compared to *P.segnis*. When compared between seasons, Only IMBI values showed statistical variations as demonstrated by a significant increase of Fe in crabs gills (p<0.05, One-way ANOVA) and a significant decrease of Ni in crabs muscles (p<0.01, One-way ANOVA).

3.2.3. Bioconcentration factor (BCF)in P.segnis and C. aestuarii muscles and gills

The bioconcentration factors (BCF) of TE in *P.segnis* and *C.aestuarii* tissues are presented in Table 2. Based on this table, BCF values greater than 1 was observed for all the tested TEs. Our results showed that BCF, index related to Pb and Cd, increased significantly in gills during winter season by 23% and 20% as compared to the spring. However, no significant change was recorded for BCF index in muscles tissue. Thus, the most significant decreases of the BCF values were observed during winter season for Pb, Cd, Cu and Ni in both gills and muscles (p<0.05, One-way ANOVA).

3.3. Proximate composition in *P. segnis* and *C. aestuarii* muscles and gills

Proximate composition results are presented in Figure 2, showed significant variations between *P. segnis* and *C. aestuarii* gills and muscles organs(p<0.05 One-way ANOVA). There were significant decreases in lipids, proteins and carbohydrates contents in muscles and gills of *C. aestuarii* as compared to *P. segnis* during the two studied period (p<0.05 One-way ANOVA). In turn, during winter, the proximate composition decreased in both *P. segnis* and *C. aestuarii* organs, and reached minimal values as compared to the spring season (Figure 2).

3.4. Fatty acid composition in *P. segnis* and *C. aestuarii* gills and muscles

3.4.1. Fatty acid composition in *P. segnis* and *C. aestuarii* gills

Fatty acid profiles of crabs' gills are summarized in Table 3. Generally, twenty-nine fatty acids were determined and constituted about 94.1–99.3% of the total fatty acids. Large variations in the fatty acid composition of gills tissues were shown among both crabs' species, characterized with a higher proportion of PUFA, SFA than that of MUFAs. These fatty acid profiles were classified into PUFA, which included 44.765–51.951%; SFA, which explained by 31.426–43.204%; followed by MUFA, which composed by 11.066–13.131% of total fatty acids in both *P.segnis* and *C.aestuarii*.

Table 3
Fatty acid composition (%)of *P. segnis* and *C. aestuarii* gills collected from the Gulf of Gabes during spring and winter seasons

	Spring		*Winter*	
	P. segnis	*C. aestuarii*	*P. segnis*	*C. aestuarii*
C14:0	1.506±0.055	1.411±0.012	2.121±0.117###	2.108±0.015###
C15:0	1.183±0.073	1.202±0.225	1.286±0.027###	1.188±0.048
C16:0	14.419±0.773	17.019±0.771**	17.942±1.283##	24.897±0.531**###
C18:0	9.067±0.146	9.718±0.323*	14.232±0.245###	13.991±0.706###
C20.0	3.951±0.361	3.445±0.279	0.549±0.020###	0.712±0.022***###
C22:0	1.298±0.064	1.045±0.070	0.251±0.004###	0.306±0.015**###
∑SFA	31.426±0.906	33.842±0.409**	36.384±1.487###	43.204±0.232*###
C14:1	1.975±0.248	1.222±0.046	1.667±0.084	1.495±0.146#
C15:1	2.312±0.050	2.716±0.593	2.152±0.007###	2.055±0.067
C16:1	4.367±0.488	4.587±1.018	3.919±0.260	3.781±0.544
C18:1	3.378±0.594	3.067±1.127	1.755±0.857#	1.906±0.640
C20:1	0.759±0.121	0.638±0.320	0.348±0.021###	0.259±0.115###
C22:1	0.338±0.044	0.403±0.082	1.222±0.234###	1.862±0.163###
∑MUFA	13.131±1.056	12.635±1.587	11.066±0.849	11.361±1.183
C16:2	3.452±0.385	3.457±0.201	6.710±0.011###	4.448±0.878**
C16:3	3.160±0.234	2.975±0.510	4.038±0.051##	1.136±0.189***##
C16:4	3.019±0.124	3.151±0.095	5.998±0.343###	1.758±0.098***###
C18:2n-6	1.448±0.045	2.350±0.110***	2.056±0.067###	9.870±0.365***###
C18:3n-6	0.414±0.018	0.526±0.028**	0.877±0.081###	3.679±0.109***###
C18:3n-3	3.567±0.438	1.516±0.056***	2.449±0.083##	1.784±0.027***###
C18:4n-3	0.966±0.088	1.045±0.070	2.665±0.100###	1.702±0.144***##
C20:2n-6	0.767±0.051	2.408±0.440**	1.219±0.107##	1.287±0.052##
C20:3n-6	0.454±0.078	1.474±0.217***	0.297±0.028#	2.288±0.081***##
C20:4n-6	2.261±0.238	3.404±0.363**	3.378±0.145##	4.111±0.451*
C20:3n-3	0.454±0.078	0.412±0.038	0.401±0.074	0.271±0.049*##
C20:4n-3	6.771±0.697	1.879±0.083***	0.365±0.038###	0.051±0.005***###
C20:5n-3	2.151±0.196	1.683±0.089**	4.147±0.179###	0.823±0.046***###
C22:2n-6	2.505±0.145	2.579±0.211	1.531±0.508#	3.594±0.528**#
C22:5n-6	1.847±0.054	2.435±0.403*	1.712±0.087#	1.821±0.035#
C22:5n-3	6.521±0.436	5.932±0.128*	3.501±0.290###	1.285±0.048***###
C22 :6n-3	12.186±0.714	10.424±0.392*	10.014±0.355##	4.849±0.774***###
∑PUFA	51.951±0.078	47.656±1.790**	51.366±0.643	44.765±0.899***#
PUFA n-3	32.619±0.432	22.894±0.199***	23.544±0.309###	10.768±0.699***###
PUFA n-6	9.699±0.424	15.177±1.099***	11.074±0.280##	26.653±0.730***###
Results are presented as means ± SD (n=6).				
Significant differences between *P.segnis* and *C.aestuarii* are detected at 5% as follows: *p<0.05; **p<0.01 and ***p<0.001.				
Significant differences between crabs and seasons are detected at 5% as follows: #p<0.05; ##p<0.01 and ###p<0.001.				
∑ : sum ; SFA: saturated fatty acids; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; PUFA n-3: omega 3; PUFA n-6; omega 6.				

In general, dissimilar variations were observed between the two studied crabs' gills. As shown in Table 3, significant increases in SFA levels were observed in gills of *C.aestuarii* as compared to *P. segnis* during the assessed seasons (p<0.05 One-way ANOVA). This increase due mainly to the significant enhancement of palmitic (C16:0), the predominant 010 SFA in all the examined crabs, that contributed approximately to 50% of the total SFA. Additionally, stearic acid (C18:0) was the second abundant SFA, ranging from 9.06–14.23% and showed similar variations in both crabs' gills. Other SFA with odd carbon numbers like myristic acid (C14:0), and pentadecanoic acid (C15:0) were presented in small and similar levels in *P.segnis* and *C.aestuarii* gills. However, arachidic acid (C20:0) and docosenoic acid (C22:0) showed significant increases in *C.aestuarii* gills than in *P. segnis* mainly during the winter season (+29% and +21% respectively).

Similar levels were noticed for MUFA levels in both *P. segnis*and *C. aestuarii* gills between the two studied seasons (p>0.05, one-way ANOVA). This group contained a high proportion of oleic acid (C18:1), palmitoleic acid (C16:1), ranging from 1.75–4.58% followed by myristoleic acid (C14:1) and pentadecenoic acid (C15:1). However, minor levels were noted for gadolic acid (C20:1) and cetoleic acid (C22:1) in both crabs' gills.

The greatest proportion of PUFA was predominated mainly by docosahexaenoic acid (DHA; C22:6n-3, accounted for 4.84–12.18%), eicosapentaenoic acid (EPA; C20:5n-3, accounted for 0.82–4.14%), docosapentaenoic acid(DPA; C22:5*n*-3, ranged between 1.28% and 6.52%), eicosatetraenoic acid (C20:4*n*-3, comprised between 0.05% and 6.77%), and linoleic acid (C18:2n-6, presented between 1.44% and 9.87%*)*.Our results demonstrated that *P.segnis* gills contained significant high levels of *n*-3 PUFA and low levels of total *n*-6 PUFA as compared to *C.aestuarii* in both studied seasons (p<0.001, One-way ANOVA). This lower level of n-3PUFA in *C.aestuarii* gills was proved by the decreases levels of EPA, DHA, C18:3n-3, and C20:4n-3during spring and winter seasons (p< 0.05 One-way ANOVA). Opposite variation was recorded for n-6 PUFA as evidence by statistical enhancement of the arachidonic acid (ARA; C20:4n-6) and it principal precursors (e.g. C18:2n-6 and C20:3n-6) in *C.aestuarii* gills among the two studied seasons when comparing with *P. segnis* (p< 0.05 One-way ANOVA).

Similar trend was documented when compared the two seasons as evidence by an increase of SFA levels, principally C16:0, during winter in both *P. segnis* (+15% and +24%, respectively) and *C. aestuarii* (+27% and +46%, respectively) gills as compared to spring seasons. Concerning MUFA, it level remained stable between crabs gills during the spring as well as winter seasons. Nevertheless, several variation were recorded in the main PUFA composition of gills, showing decreases of n-3 PUFA and DHA and increases of n-6 PUFA and ARA in both *P.segnis* and *C.aestuarii* during winter when compared to spring (p<0.05, One-way ANOVA).

3.4.2. Fatty acid composition in P. segnis and C. aestuarii muscles

In muscles crabs', twenty-nine fatty acids were identified and comprised by 74.09–88.74% of the total fatty acids. These profiles included six SFA (ranged from 20.68–28.84%), six MUFA (ranged from 10.577–21.98%), and seventeen PUFA (ranged from 38.21–46.19%) (Table 4). The levels of SFA and MUFA in both crabs' muscles remained stable during spring, but showed decrease in *C.aestuarii* during the winter by -14% and -13% respectively than spring season. These declines were confirmed by significant depletion of C15:0, C16:0, C15:1, C18:1, C20:1 and C22:1 in *C.aestuarii* muscles (p<0.01, One-way ANOVA).

Table 4
Fatty acid composition (%)of *P. segnis* and *C. aestuarii* muscles collected from the Gulf of Gabes during spring and winter seasons

	Spring		*Winter*	
	P. segnis	*C. aestuarii*	*P. segnis*	*C. aestuarii*
C14:0	2.951±0.583	2.224±0.104	2.526±0.204	2.715±0.036###
C15:0	1.506±0.456	1.295±0.172	1.650±0.090	1.398±0.052**
C16:0	6.061±0.165	8.471±0.359***	11.977±0.589###	9.105±0.415**
C18:0	8.346±0.607	7.238±0.855	9.538±0.733#	8.789±0.418#
C20.0	2.701±0.321	1.251±0.132***	1.810±0.154##	1.787±1.366
C22:0	0.283±0.021	0.208±0.015**	1.339±0.110###	1.502±0.054*###
∑SFA	21.850±0.883	20.689±0.997	28.843±1.766##	25.298±1.620*##
C14:1	0.393±0.004	0.728±0.052***	1.205±0.069###	1.505±0.057**###
C15:1	0.919±0.038	0.945±0.059	1.766±0.081###	1.520±0.143*##
C16:1	1.538±0.137	3.267±0.308***	1.895±0.060##	2.025±0.063*##
C18:1	16.650±1.215	14.897±0.620*	4.660±0.385###	3.533±0.100**###
C20:1	0.944±0.065	1.412±0.050***	1.947±0.059###	1.633±0.114**#
C22:1	0.251±0.044	0.729±0.079***	0.568±0.052###	0.359±0.054**##
∑MUFA	20.697±1.067	21.981±0.656	12.043±0.276###	10.577±0.198***###
C16:2	1.554±0.082	1.998±0.123**	2.321±0.166##	2.600±0.167##
C16:3	0.971±0.076	0.892±0.034	1.100±0.120	1.144±0.185#
C16:4	1.802±0.227	2.637±0.425*	6.127±0.127###	5.916±0.384###
C18:2n-6	11.052±0.428	12.793±0.519**	2.870±0.120###	6.038±0.154***###
C18:3n-6	2.977±0.113	2.811±0.164	1.461±0.106###	1.312±0.039###
C18:3n-3	4.428±0.406	4.873±0.164	1.635±0.167###	1.503±0.040###
C18:4n-3	1.436±0.135	1.420±0.016	1.493±0.027	0.579±0.042***###
C20:2n-6	1.131±0.091	3.413±0.516***	2.788±0.175###	1.900±0.057***##
C20:3n-6	2.234±0.195	2.413±0.046	3.708±0.123###	1.144±0.111***###
C20:4n-6	0.952±0.072	1.663±0.108***	1.284±0.137#	2.210±0.197**##
C20:3n-3	1.684±0.188	1.034±0.182**	0.852±0.055###	0.823±0.033
C20:4n-3	0.084±0.007	0.055±0.012*	0.157±0.011###	0.136±0.008*###
C20:5n-3	4.621±0.365	0.752±0.096***	2.201±0.135###	1.440±0.088***###
C22:2n-6	0.518±0.094	0.671±0.023*	1.535±0.083###	2.186±0.042***###
C22:5n-6	0.667±0.038	2.157±0.152***	4.309±0.295###	3.202±0.068**###
C22:5n-3	2.520±0.491	0.970±0.064**	5.738±0.290###	3.322±0.104***###
C22 :6n-3	7.559±0.370	4.161±0.256***	3.616±0.351###	2.755±0.453*###
∑PUFA	46.196±0.231	44.721±0.372*	43.202±0.728##	38.219±0.801***###
PUFA n-3	22.335±0.267	13.268±0.576***	15.695±0.517###	10.561±0.383***##
PUFA n-6	19.533±0.213	25.924±1.379***	17.957±0.512##	17.995±0.192###
Results are presented as means ± SD (n=6).				
Significant differences between *P.segnis* and *C.aestuarii* are detected at 5% as follows: *p<0.05; **p<0.01 and ***p<0.001.				
Significant differences between crabs and seasons are detected at 5% as follows: #p<0.05; ##p<0.01 and ###p<0.001.				
∑ : sum ; SFA: saturated fatty acids; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; PUFA n-3: omega 3; PUFA n-6; omega 6.				

The obtained data demonstrated that PUFA levels were significantly lower in *C.aestuarii* muscles during spring and winter seasons ($p<0.05$, One-way ANOVA).Similar decreases were revealed for the main PUFA principally n-3 PUFA (-68% and -48%, respectively), DHA (-81% and -31%, respectively) and EPA (-514% and -52%, respectively). Opposite variations were noted for n-6 PUFA, showed highest levels in *C.aestuarii* muscles during spring season (+32%) when

compared to *P.segnis*. However, no change was observed for n-6 PUFA during winter between the two studied crabs. This group was predominantly by ARA and C18:2n-6, showing significant high levels in *C.aestuarii* than *P.segnis* during the two studied periods ($p<0.01$, One-way ANOVA).

Regarding the seasons, our data demonstrated that both native and invasive crabs were characterized by lower amounts of PUFA and MUFA during winter ($p<0.01$, One-way ANOVA). These amounts were mostly related to the decline of C18:1, n-3 PUFA, EPA and DHA in *P.segnis* and *C.aestuarii* muscles during the cold season ($p<0.01$, One-way ANOVA). Conversely, remarkable increases of the SFA levels mainly due to the rise of its main FA such as C16:0 and C18:0 were noticed during winter season for both studied crabs'.

3.4.3. Heatmap analysis of fatty acid composition

The hierarchical clustering based on all determined fatty acids is reported in Figure 3. These fatty acids were presented into a module demonstrating that *C.aestuarii* and *P.segnis* gills clustered together and separated from muscles organs (Figure 3). Our results revealed that gills were the most sensitive organs that show the highest responsive mainly during spring on *C.aestuarii* as compared to *P.segnis* as evidence by high amounts of SFA, n-6 PUFA and their main compounds (ARA, C18:2n-6..).

3.5. Lipid peroxidation indices in *P. segnis* and *C. aestuarii* muscles and gills

Our results showed a significant, increase in MDA levels (22% and 41%, respectively), and LOOH levels (40%, and 39%, respectively) in gills of *C.aestuarii* in comparison with *P.segni s*among the spring and winter seasons (Figure 4). As regards muscles, significant increases were observed during spring for MDA (59%) and LOOH (71%) and during winter only for MDA (72%). However, when comparing the two seasons, both lipid peroxidation indices (MDA and LOOH) were decreased significantly during the winter season ($p<0.05$, One-way ANOVA).

3.6. Protein oxidation indices in *P. segnis* and *C. aestuarii* muscles and gills

Based on the obtained data in Figure 5, there is significantly increased of AOPP and PCO levels in *C.aestuarii* gills during spring (20% and 29% respectively) and winter (70% and 1% respectively) in comparison with *P. segnis*. They also showing significantly increases more than 50%, in *C.aestarii* muscles as compared to *P.segnis* among the spring and winter seasons.

3.7. Antioxidants responses in *P. segnis* and *C. aestuarii* muscles and gills

Antioxidants activities are provided in Table 5. Our results revealed that MTs, GSH, levels and SOD, GPx, GST activities increased in *C. aestuarii* gills in comparison with *P. segnis* ($p<0.01$, One-way ANOVA). Therefore, our results displayed significant increases of MTs (+49%), GSH (+55%), levels and SOD (+67%),GPx (+103%), GST(92%) activities in *C.aestuarii* muscles tissues during winter season as compared to *P. segnis*. Yet, during spring season, the MTs levels showed a significant increase by 81% in *C. aestuarii* muscles when compared to *P. segnis*. However, similar trends were recorded for GSH levels, SOD, GPx and GST activities between *C. aestuarii* and *P. segnis* muscles during spring season. The comparison between seasons showed that winter period was marked by the highest antioxidants activities in both crabs tissues as compared to the spring season ($p<0.05$, One-way ANOVA).

Table 5
Redox status responses in *P. segnis* and *C. aestuarii* muscles and gills collected from the Gulf of Gabes during spring and winter seasons

			MTs (nmol/mg of protein)	GSH (mmol/mg of protein)	SOD (mmol/mg of protein)	GPx (mmol/mg of protein)	GST (mmol/mg of protein)
Gills	Spring	*P.segnis*	0.158±0.031	8.185±0.820	10.925±5.860	50.873±7.750	599.354±50.891
		C.aestuarii	0.211±0.047*	10.149±0.685*	22.008±4.358***	57.411±6.097**	633.886±47.908**
	Winter	*P.segnis*	0.116±0.033	13.938±2.025###	27.622±7.385###	74.134±1.185###	603.375±10.019#
		C.aestuarii	0.178±0.077*	14.795±2.075*###	43.809±5.727***###	98.892±5.322***###	681.170±54.320***#
Muscles	Spring	*P.segnis*	0.044±0.008	4.194±0.452	14.356±2.082	7.647±0.422	104.820±12.308
		C.aestuarii	0.080±0.001**	4.219±0.960	15.419±4.397*	8.049±0.630	201.804±25.105***
	Winter	*P.segnis*	0.053±0.008	4.810±0.845	23.641±6.721###	13.406±4.527###	211.445±37.870###
		C.aestuarii	0.079±0.007**	7.502±0.945**##	39.671±3.239***###	27.303±5.386***###	235.580±28.815***##
Results are presented as means ± SD (n=10).							
Significant difference between crabs' was detected at 5% as follows: *p<0.05; **p<0.01 and ***p<0.001.							
Significant difference between seasons was detected at 5% as follows: #p<0.05; ##p<0.01 and ###p<0.001.							
MTs: metallothioneins; GSH: glutathione; SOD: superoxide dismutase; GPx: glutathion peoxidase; GST: glutathione-S-transferase.							

3.8. Correlation matrix

A Spearman correlation matrix was used to demonstrate the association between the temperature, salinity, proximate, biomarkers parameters, fatty acid groups and trace elements amounts in crabs gills and muscles (Table 6).

Table 6
Correlations between the examined parameters in *P.segnid* and *C.aestuarii* gills and muscles using Sperman test

	Gills							*Muscles*						
	Cd	**Pb**	**Fe**	**Cu**	**Ni**	**T°C**	**Spsu**	**Cd**	**Pb**	**Fe**	**Cu**	**Ni**	**T°C**	**Spsu**
Pr	-0.020	-0.384	-0.354	-0.353	-0.077	0.355	0.225	-0.239	-0.174	-0.247	-0.629 c	-0.567 a	0.354	0.315
Lip	-0.016	-0.414 a	-0.408 a	-0.496 a	0.027	0.427 a	0.400	-0.105	-0.303	-0.137	-0.492 a	-0.423 a	0.361	0.340
Car	-0.182	-0.642 c	-0.654 c	-0.624 b	-0.064	0.462 a	0.543 b	0.059	-0.180	-0.329	-0.651 c	-0.314	0.509 b	0.476 a
MDA	-0.231	0.710 c	0.702 c	0.813 c	0.144	-0.675 b	-0.505 b	0.258	0.018	0.292	0.735 c	0.543 b	-0.433 a	-0.442 a
AOPP	-0.169	0.346	0.649 c	0.689 c	0.272	-0.582 b	-0.317	0.192	0.289	0.200	0.653 c	0.567 b	-0.233	-0.229
PCO	0.181	0.255	0.441 a	0.207	0.044	-0.157	-0.280	0.287	0.185	-0.062	0.493 a	0.388	-0.130	-0.152
LOOH	0.228	0.605 b	0.545 b	0.318	0.253	-0.174	-0.348	-0.096	0.082	0.335	0.748 c	0.301	-0.648 b	-0.648 c
SOD	-0.201	0.785 c	0.784 c	0.813 c	0.018	-0.754 c	-0.589 b	-0.312	-0.146	0.525 b	0.732 c	0.127	-0.753 c	-0.770 c
MT	-0.187	0.361	0.455 a	0.329	0.209	-0.305	-0.403	0.415 a	0.315	0.022	0.612 c	0.788 c	-0.092	-0.116
GPx	-0.310	0.862 c	0.654 c	0.790 c	-0.080	-0.767 c	-0.709 c	-0.275	-0.023	0.465 a	0.769 c	0.172	-0.724 c	-0.753 c
GSH	-0.479a	0.595 b	0.612 b	0.862 c	-0.064	-0.843 c	-0.615 b	-0.161	-0.286	0.548 b	0.684 c	0.195	-0.643 c	-0.652 b
GST	0.104	-0.027	0.175	0.105	-0.043	-0.046	-0.063	0.196	0.100	0.174	0.509 b	0.498 a	-0.453 a	-0.491 a
SFA	-0.452 a	0.653 b	0.735 c	0.960 c	-0.245	-0.946 c	-0.649 b	-0.592 b	-0.050	0.310	0.163	-0.251	-0.808 c	-0.837 c
MUFA	0.270	-0.526 b	-0.286	-0.376	-0.213	0.474 a	0.532 b	0.529 b	0.077	-0.438 a	-0.466 a	0.197	0.970 c	0.975 c
ARA	-0.029	0.633 b	0.744 c	0.785 c	-0.015	-0.651 b	-0.681 b	0.151	0.088	0.269	0.856 c	0.658 b	-0.418 a	-0.446 a
EPA	-0.559 b	-0.415 b	-0.219	0.047	-0.106	-0.189	0.370	-0.346	-0.272	-0.133	-0.584 b	-0.615 b	0.297	0.305
DHA	0.128	-0.845 c	-0.716 c	-0.792 c	0.057	0.725 c	0.736 c	0.034	-0.168	-0.360	-0.684 c	-0.372	0.725 c	0.718 c
PUFA	-0.269	-0.718 c	-0.548 b	-0.409 a	-0.266	0.295	0.578 b	0.230	0.151	-0.442 a	-0.638 c	-0.064	0.765 c	0.767 c
*n*3 PUFA	0.041	-0.811 c	-0.790 c	-0.804 c	-0.057	0.704 c	0.717 c	-0.142	-0.140	-0.294	-0.756 c	-0.535 b	0.542 b	0.546 b
*n*6 PUFA	0.123	0.768 c	0.695 c	0.640 b	-0.039	-0.514 b	-0.735 c	0.656 c	0.334	-0.353	-0.036	0.638 b	0.718 c	0.713 c

Negative correlation is presented with red color as compared to the positive one (blue color).

Values in bold indicate significant correlations at 5%.

Significant level was presented by [a] $p < 0.05$. [b] $p < 0.01$ and [c] $p < 0.001$.

For gills, lipids and carbohydrates contents showed the most negative and significant correlations with Pb ($r \leq -0.414$-; $p<0.05$), Cu ($r \leq -0.496$; $p<0.05$), Fe ($r \leq -0.408$; $p<0.05$), and positive correlations with T° ($r \leq 0.427$; $p<0.05$). However, no significant correlation between protein and trace elements amounts and/or physicochemical parameters was observed (Table 6). Spearman's correlation analysis showed significant positive relations between trace elements amounts (Pb, Cd, Cu, Fe and Ni) and lipid peroxidation (MDA and LOOH), protein oxidation (AOPP and PCO) indices and MTs levels ($r \geq 0.441$; $p<0.05$). Nevertheless, negative correlations were observed between MDA, and AOPP with environmental parameters mostly T°C ($r \geq -0.582$; $p<0.01$). Additionally, GSH

levels showed impotents positive correlations with Pb (r=0.595; p<0.01), Fe (r=0.615; p<0.01), and Cu (r=0.862; p<0.001) and has negative and significant correlation with Cd (r= -0.479; p<0.05). Our results showed that SOD and GPx positively correlated with trace elements accumulation mainly Pb, Fe and Cu (r ≥ 0.654; p<0.001). These antioxidant systems have negative correlations with T°C and Spus (r ≥ -0.615; p<0.01).However, GST activity does not show any correlation with the above parameters. According to Table 6, all trace elements showed significant correlations with fatty acid groups except for Ni. These correlations were negative for EPA, DHA, PUFA and n3PUFA (r ≥ -0.415; p<0.01).and positive for ARA, n6PUFA and SFA (r ≥ 0.633; p<0.01).

For muscles tissues, results showed that Cu and Ni were significantly correlated with carbohydrates, proteins and lipids contents (r ≥ -0.423, p< 0.05) (Table 6). Only carbohydrates have positive correlations with T° (r=0.509; p<0.01) and S psu (r = 0.476; p<0.05). Cu has significant positive correlations with MDA, AOPP, PCO and LOOH levels (r ≥ 0.493; p< 0.05), while MDA and AOPP showed positive correlation with Ni (r ≥ 0.543; p< 0.01). Opposite correlation was found between MDA, LOOH levels and physicochemical parameters as evidence by a negative correlation with T° (r ≥ -0.433; p< 0.05) and S psu (r ≥ -0.442; p < 0.05). According to Table 6, there were also negative correlations between GSH, MT, GPx, SOD, GST and trace elements amounts mainly Cu, Ni, Cd and Fe (r ≥ 0.465; p< 0.05). However, Pb has not any correlations with antioxidants defenses systems. With, all antioxidants biomarkers were correlated negatively with T° and S psu (r ≥ -0.495; p< 0.01) except for MT. Cu, Fe and Ni were the main trace elements that have significant correlations with fatty acid groups (≥ -0.442; p< 0.05). Also, there were positive correlations between abiotic parameters (T°C and Spsu) and DHA, PUFA, n3PUFA and n6PUFA (r ≥ 0.542; p< 0.01).

3.9 Principal compound analysis clusters of all redox status set in P. segnis and C. aestuarii muscles and gills

In Principal component analysis (PCA), the two first principal components explained 82.1% of total variations (Figure 6). Our results revealed that the first axis (modeling 64.8% of total variation) was positively associated with trace elements concentrations (Cd, Pb, Fe, Cu, and Ni), macromolecular damages parameters (MDA, LOOH, PCO and AOPP) as well as antioxidants defenses systems (GSH, MT, GPx, and GST) (Figure 6). This axis showed negative correlations with proteins (p<0.001; r=-0.844) and lipids (p<0.001; r=-0.667) contents. The subsequent axis (modeling 17.3% of the total variation) was positively related with SOD activity and negatively with water parameters (T°C and Spsu). These axes described a physical gradient separating the studied crabs according to organs and seasons (Fig. 6). In general, PCA even allowed us to notice that the gills are the most sensitive organs compared to the muscles because they have an affinity for the accumulation of trace elements. In addition, they have important physiological responses (e.g., levels of MDA, AOPP, MT, etc.) than those observed in muscles. On the other hand, the muscles, especially during the spring season, are associated with the strong environmental variation and the levels of proteins and lipids. Plotting the first two principal components showed that the native *C.aestuarii* crabs are the most sensitive compared to the *P.segnis*.

4. Discussion

One of the mainly universal problems in marine ecosystems is the appearance of invasive species, resulting in a risk to the biodiversity and the ecosystem functions (Mannino et al. 2017; Chan et al. 2019). The entry of invasive species such as *P. segnis* into the gulf of Gabes may possibly affect the regular functioning of *C. aestuarii* as a native species as they may compete for substrate, space and light. Despite the fact that several recent studies have raised concerns about the hazardous effects of TEs accumulation on marine species, no reports are yet available when comparing invasive and native crabs and in particularly from the Tunisian coast. Yet, this study was therefore planned to evaluate and compare the possible toxic effects of TEs bioaccumulation on the both invasive (*P.segnis*) and native (*C.aestuarii*) crabs using a multiple approach (biological, biochemical and cellular markers).

Crabs are intertidal and deposit feeders' species (Iribarne et al. 1997), they have the capacity to bioaccumulate TEs, which are present in both phases (water and sediment) (Karar et al. 2019). The present study demonstrated that *C.aestuarii* is more capable to accumulate TEs and the rate of organs translocation of their measured seems to be greater, compared to *P.segnis*. Our findings clearly showed that *C.aestuarii* has a particular capacity to accumulate Pb, Cd, Cu, Fe and Ni in higher levels in its organs, mainly in the gills. This could be explained by the direct interactions of gills with the surrounding environment (water, sediment…etc) (Chaâbane et al. 2020). Additionally, our results demonstrated that essential elements such as Cu and Fe were detectable during the entire sampling period and were greater than the standard guidelines set by EU (2006), WHO (1993b) and USA (2006). Considering that Cd, Pb and Ni are nonessential toxic TEs, it may be assumed that there is positive bioaccumulation in both crabs' gills since the values were below the standard guideline as recommended by EU (2006) and USA (2006). In line with this, gills commonly tend to bioaccumulate TEs more efficiently than other organs (e.g., muscles, foot and mantle), and this has been well demonstrated in several works carried out on aquatic species, such as the native *Anodonta anatina* and the invasive *Sinanodonta woodiana* (Bielen et al. 2016) and freshwater native *Anodontites trapesialis* and exotic *Limnoperna fortunei* species (Haj et al. 2019). Hence, they partly concluded that gills could reflect greatly the concentration of TEs in surrounding habitat. Our results revealed that all invasive crabs behaved much better in favor of trace element build-up. The higher tolerance of *P. segnis* was evident from the significant decrease of metal pollution and bioaccumulation indices, indicating more efficient resistance mechanism than *C. aestuarii.* A higher tolerance to the accumulation of TEs was translated by the low index of bioaccumulation which can bring physiological advantages for the success of the invasion of *P. segnis* over *C. aestuarii* populations. Bioconcentration of TEs in aquatic species may be influenced by physicochemical extrinsic (e.g., water temperature and salinity) and intrinsic factors (reproductive status …etc) (Pinheiro et al. 2012; Siddiqui and Saher 2021). Thus, significant differences were expected to be observed in crabs among seasons. It has been noted that the accumulation of trace elements in both crabs' organs seems to be more accentuated during winter season which coincides and correlate with the lower values of temperature and salinity (r≥ 0.310;p<0.05). Apparently, this high accumulation could be related to the spawning process which occurred during the cold season as reported by Glamuzina et al (2017).

It has been reported previously that TEs accumulation in aquatic organisms may alter their metabolic functions and the macromolecular structures inducing reactive oxidative stress (ROS) production (Perrat et al. 2013; Hussain et al. 2018; Bejaoui et al. 2019). In this sense, the levels of MDA and LOOH of *C. aestuarii* organs, mostly in the gills, showed significant increases regardless to the high accumulation of TEs than in *P.segnis*. These inductions in the gills of *C. aestuarii* could be due to the structural and functional characteristics of this organ because it is located in direct contact with the external environment and has a small epithelium characterized by poor protection (Compere et al. 1989; Henry et al. 2012).This pattern was previously demonstrated in invasive and native bivalves related to TEs uptake (Rabeh et al. 2019). Additionally, these high responses were more pronounced in gills tissues since TEs were highly

accumulated (r≥ 0.750; p<0.01) and with low environmental conditions (temperature and salinity) (r≥-0.462; p<0.05). Similar to our data, Mansour et al (2020) showed that TEs uptake significantly enhanced the lipid peroxidation products in *Ruditapes decussatus* mostly during the cold season.

The excessive generation of ROS products may in turn maintain the overproduction of hydroxyl radical (.OH) through Fenton reaction, which cause unsaturated fatty acids oxidation, and alter membrane integrity (Ayala et al. 2014; Krumova and Cosa 2016). Our work is the first attempt to compare FA composition of invasive and native crabs' organs that revealing changes in both *P.segnis* and *C.aestuarii* profiles in relation to environmental stressors. These changes were more marked in *C.aestuarii* gills tissues and elucidated by increases of SFA, mainly revealed by the significant enhancement of palmitic (16:0), and stearic (C18:0) acids as compared to *P.segnis*. These results seems to be (i) an evident outcome of the lipid production occurrence (ii) an adaptive response to ensure the high energy demand needed for TEs detoxification in order to promote the membrane stability and (iii) probably ascribed to TEs toxicity. However, *C.aestuarii* muscles showed a decrease of SFA that can reflect the damage causes in lipid metabolism as a shifty reaction beside the induced of ROS production. This supposition is confirmed by the significant negative correlations between the lower SFA levels and TEs accumulation in muscles tissues. Additionally, it is well known that PUFA play a key role in cell development and function (Stillwell and Wassall 2003, Ruxton et al. 2005, Liu et al. 2015). The current results evidently exhibited that PUFA, n-3 PUFA levels mainly DHA and EPA and their respective precursors (ALA, C18:3n-3 and ETA, C20:4n-3) decreased significantly in *C.aestuarii* tissues than *P.segnis.* Such variations were related to TEs accumulation and associated with peroxidation process as consequence of membrane permeability depletion (Filimonova et al. 2016). This supposition is highlighted by the high correlation of PUFA, n-3 PUFA, DHA, and EPA with trace elements uptake (r≥ -0.409; p<0.05). Also, this could be attributed to its important role in mediating immunological and inflammatory responses (Thyrring et al. 2015; Chetoui et al. 2019). Consistent with this, our findings suggest that TEs uptake could be responsible for the decrease of these processes and may provoke simultaneous modifications of gills and muscles structure and function *via* the disruption of their physiological processes. Conversely, n-6 PUFA and ARA, as precursors of eicosanoids cascades; were significantly higher in *C.aestuarii* gills and muscles as compared to *P.segnis* which was highlighted by a positive correlation with TEs uptake. Thus, this enhancement of ARA prove the improvement in the adaptation of *C.aestuarii* to environmental stress mainly the invasion process of *P.segnis*, since it have an important physiological response (Vance and Vance 2002). Our findings were in agreement with previous reports pointed out on *in situ* and *in vivo* works indicating the changes of FA composition of aquatic species concomitant with trace elements uptake or/and exposure (Bejaoui et al. 2019; Fouzai et al. 2020).Yet, this alteration of FA composition during winter season may be attributed to the decrease of temperature and salinity which are known to affect significantly the membrane fluidity (Laurel et al. 2012; Malekar et al. 2018; Guo et al. 2019).

This change in fatty acid composition in organs can affect the function of proteins, mainly those of the membrane known as the vital structural constituents of several bimoleculars (Habeck et al. 2016). In this line, PCO and AOPP have been known as good markers of oxidative stress, reflecting the uncontrolled free radical generation and protein oxidation damage (Soladoye et al. 2015).The present data demonstrated that AOPP and PCO levels increased in *C.aestuarii* than *P.segnis* which might be interpreted as a defense mechanism to the occurrence of protein oxidation stress. As previously described in aquatic organisms, high levels of lipid and protein oxidation are well documented as a signal for macromolecules alteration (Hussain et al. 2018; Bejaoui et al. 2019).These results were further confirmed by a negative correlation found between the contents of proteins, lipids, glycogens and the peroxidation products (MDA, LOOH, AOPP and PCO) (r≥; -0.400; p <0.05) and demonstrated the toxic effect of the TE uptake that targeted the organ functioning and causes cellular stress.

In accordance with this, the generation of peroxidation products in response to TEs accumulation activates antioxidant machinery which includes both antioxidant enzymes and non-enzymatic compounds to neutralize the ROS produced by avoiding potential oxidative damage to cellular components (Ross et al. 2006; Messina et al. 2014). To cope with oxidative stress produced by ROS generation, SOD as the most powerful and the primary detoxification enzyme, removes the superoxide radical through the process of dismutation to oxygen and hydrogen peroxide ($2O_2^{+-} + H^+ \rightarrow H_2O_2 + O_2$) (Ighodaro et al. 2018). Additionally, GPx, a main intracellular enzyme, breakdown the hydrogen peroxides (H_2O_2) to water; and lipid peroxides to their corresponding alcohols principally in the mitochondria (Ng et al. 2007). In our work, SOD and GPx activities in the gills and muscles of *C.aestuarii* were significantly enhanced as compared to *P.segnis* organs. These responses could probably indicate the use of these enzymes during the removal of free radicals excess essentiallyO_2 and H_2O_2 produced following TEs accumulation. On the other hand, GST reached higher activity in *C.aestuarii* than *P.segnis* act in the detoxification of lipid hydroperoxides derived from the TEs uptake in both organs. Our results showed that the increase in GST activities in the gills and muscles promotes greater exclusion of lipid hydroperoxid generated by the radical assault of lipid molecules which is demonstrated by a high positive correlation with lipid peroxidation products (r≥; 0.528; p <0.01). These results align strongly with previous works, where TEs accumulation have been shown to disrupt the redox statut balance of native bivalves *Mytilus galloprovincialis* than the invasive one *Brachidontes pharaonis* (Rabeh et al. 2019). Other findings showed that antioxidants enzymes increased mostly during the winter season that underline the activation of biotransformation and detoxification processes as an effort to minimize the oxidative damage caused by TEs bioaccumulation (Maranho et al. 2015; Uluturhan et al. 2019). Explicitly, GST is a phase II detoxification enzymes that remove the ROS generation products mainly by catalyzing GSH-dependent conjugation and redox reactions (Eroglu et al. 2015). Along with, GSH, is a tripeptide, confers cellular protection by directly reducing free radicals and conjugating endogenous and exogenous electrophiles (Lushchak 2012). This non-enzymatic antioxidant latter was found to increase in *C.aestuarii* gills and muscles as GSH exert its detoxifying function by acting as a direct forager of ROS and electrophilic compounds (Hellou et al. 2012). The enhancement levels of GSH were reported by Campillo et al (2013) and Capo et al (2015) in *R.decussatus* and *P. nobilis* respectively, collected from contaminated sites. Like GSH, metallothionein (MT) as major source of cellular thiols, has a high ability to bind metallic ions (Mao et al. 2012). Additionally, MTs have a key role in the bioaccumulation and detoxification of essential and non-essential TEs and participate in their homeostatic regulation to meet enzymatic and other metabolic demands (Mao et al. 2012). This investigation showed induction of MTs in *C.aestuarii* organs which seems to take part in the detoxification of excessive TEs uptake in both gills and muscles, since MT has the ability to limit the effect of hydroxyl (OH) and superoxide (O_2^-) radicals by removing them (Amiard et al. 2006). This phenomenon has been demonstrated in several aquatic species in the laboratory (Aich et al. 2017) and from contaminated natural habitats (Tremblay et al. 2020). Generally, the notable difference in the antioxidants levels in both crabs' tissues was recorded between seasons. Accordingly, higher redox status responses found in winter in comparison to spring is affected by the implied a relation between the bilogical status and TEs metabolism. Analogous results from previous research on TEs assessment in different aquatic species revealing high antioxidants responses during the cold season (Marques et al. 2018; Uluturhan et al. 2019; Mansour et al. 2020).

Our current work provides evidence to suggest that seasonality could affect the physiology of crabs by altering their metabolic activity and response, causing disruption of biochemical constituents (lipids, proteins, carbohydrates), and leading to the increase of FA degradation. All these modifications seems to be closely related to the paradox of TEs accumulation, environmental conditions and also to the reproductive cycle of crabs as reported previously in several works carried out on aquatic organisms (Karar et al. 2019; Ghribi et al. 2020).

5. Conclusion

According to this study, we can conclude that the accumulation of TEs induces cellular stress, stimulates the biotransformation pathway, and interferes with the antioxidant defenses of the gills and the muscles of *P.segnis* and *C.aestuarii* among seasons. The obtained results highlighted the resistance of *P.segnis* to environmental stressors than *C. aestuarii*. Mainly in winter, the native species are more sensitive, thus reflecting the high toxicity of the absorption of TEs, which reinforces the idea about a greater sensitivity of *C.aestuarii* coupled with the higher redox status responses and the peroxidation processes. Additionally, the evaluation of lipid composition in our present work could be considered as a new tool that helps to disclose the eventual metabolic strategy adopted by native and invasive crabs' species to ensure their resistance against pollution impacts. This knowledge seems essential for the evaluation of the impact of different pollution sources on the ecosystems status to predict the state of the organisms which live there inside the framework of biomonitoring.

Declarations

-**Ethical Approval**: The animals received humane care in accordance with the International Guidelines for Animal Care and with strict adherence to the Ethical Committee of Faculty of Sciences, Sfax, Tunisia, under the ethical approval no. 1204.
-**Consent to Participate:** Not applicable
-**Consent to Publish:** Not applicable
-**Authors Contributions: BS:** Investigation, Formal Analysis and Writing, Original Draft Preparation, Conceptualization, Methodology and Writing, Review and Editing, **CI:** Investigation, Original Draft Preparation, and Writing, Review and Editing, **GF:** Investigation, Original Draft Preparation, and Writing, Review and Editing, **FC:** Formal Analysis and Writing, **TW:** Methodology, **ZS:** Formal and Statistical analyses, **ECM:** Conceptualization, Methodology and Writing, Review and Editing, **SN:** Conceptualization, Methodology and Writing, Review and Editing.

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-**Availability of data and materials:** Not applicable

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Figures

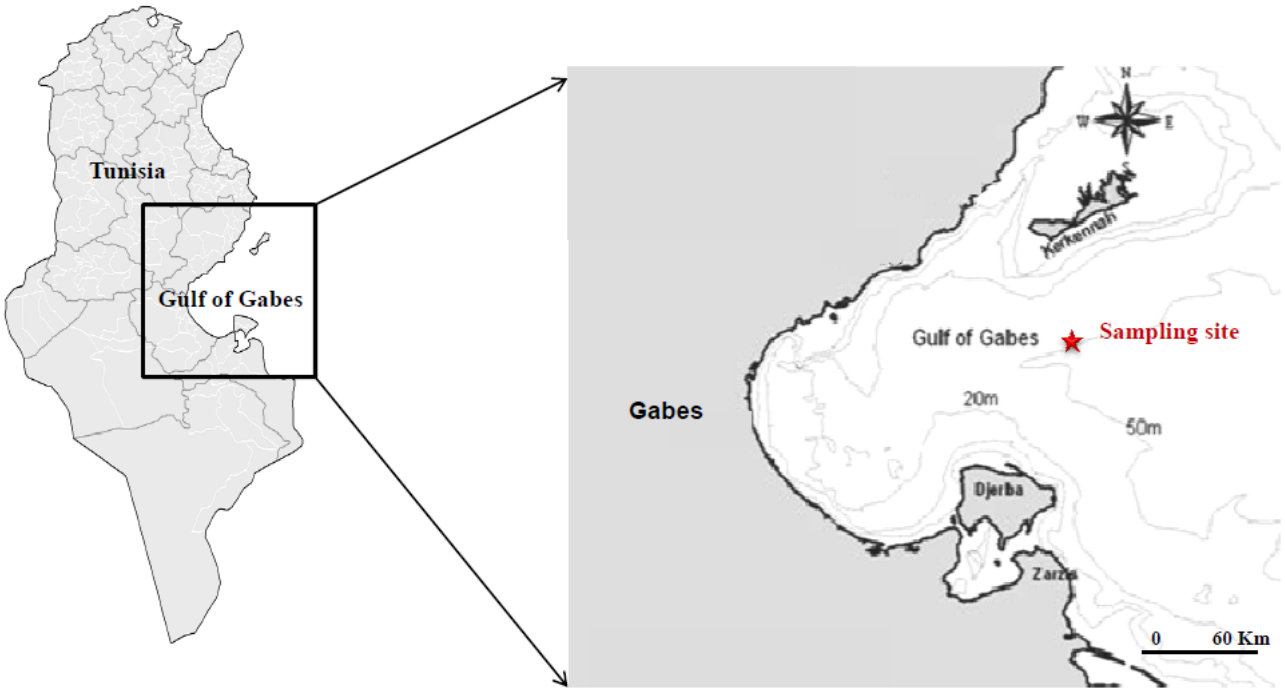

Figure 1

The geographic position of our sampling site in the Gulf of Gabes.

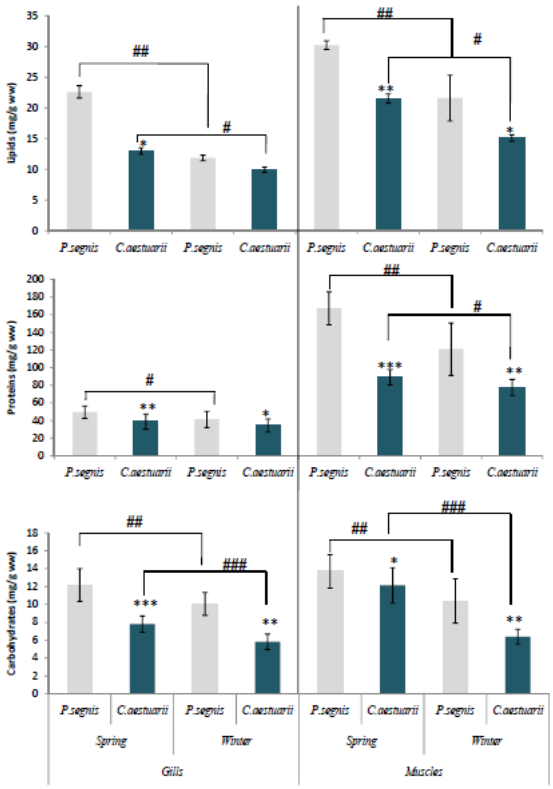

Figure 2

The proximate composition in the muscles and gills of *P.segnis* and *C.aestuarii* collected from Gulf of Gabes during spring and winter seasons.

Results are presented as means ± SD (n=10)

Significant differences between *P.segnis* and *C.aestuarii* are detected at 5% as follows: *p<0.05; **p<0.01 and ***p<0.001.

Significant differences between crabs and seasons are detected at 5% as follows: #p<0.05; ##p<0.01 and ###p<0.001.

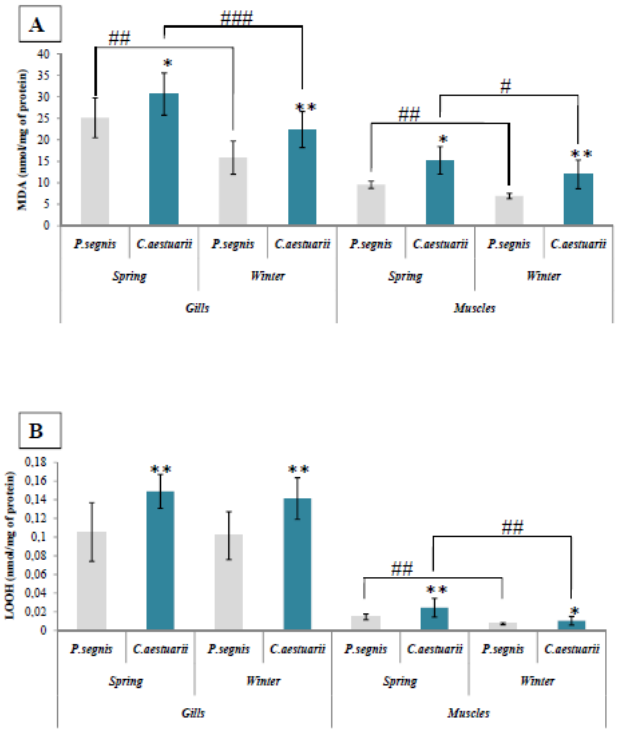

Figure 3

Malondhyaldehide (A) and lipid hydroperoxide (B) levels in the muscles and gills of *P.segnis* and *C.aestuarii* collected from Gulf of Gabes during spring and winter seasons.

Results are presented as means ± SD (n=10)

Significant differences between *P.segnis* and *C.aestuarii* are detected at 5% as follows: *p<0.05and ***p<0.001.

Significant differences between crabs and seasons are detected at 5% as follows: #p<0.05 and ###p<0.001.

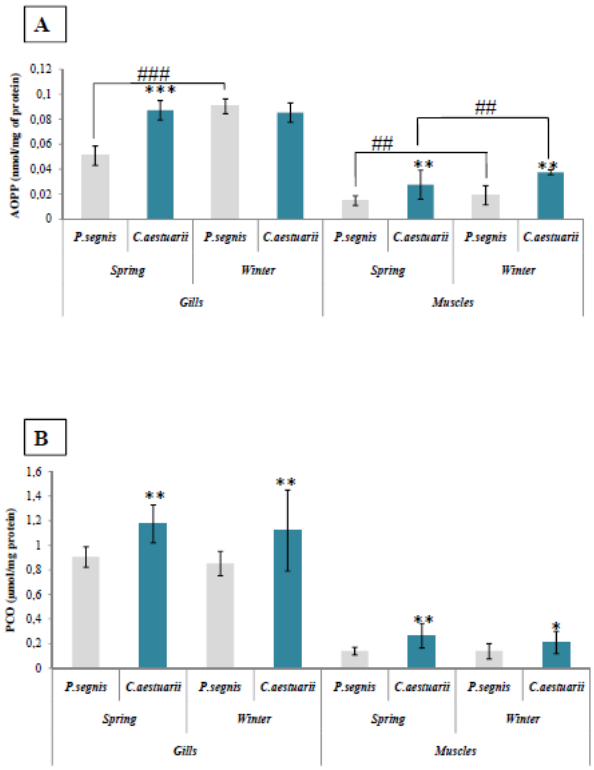

Figure 4

Advanced oxidation of proteins products (A) and proteins carbonyls (B) levels in the muscles and gills of *P.segnis* and *C.aestuarii* collected from Gulf of Gabes during spring and winter seasons.

Results are presented as means ± SD (n=10)

Significant differences between *P.segnis* and *C.aestuarii* are detected at 5% as follows: *p<0.05; **p<0.01 and ***p<0.001.

Significant differences between crabs and seasons are detected at 5% as follows: #p<0.05; ##p<0.01 and ###p<0.001.

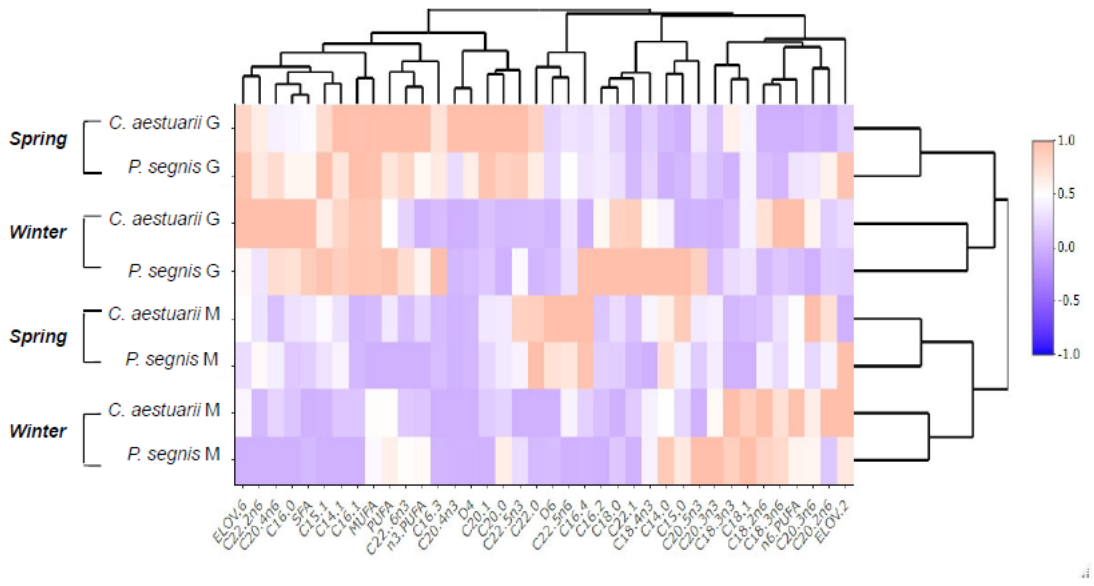

Figure 5

Comparison of fatty acid composition set using Heatmap clustering analysis of in *P.segnis* and *C.aestuarii* gills (G) and muscles (M) collected from Gabes gulf during spring and winter seasons. The red color revealed the peak of FA changes between the studied crabs' organs when compared to blue color.

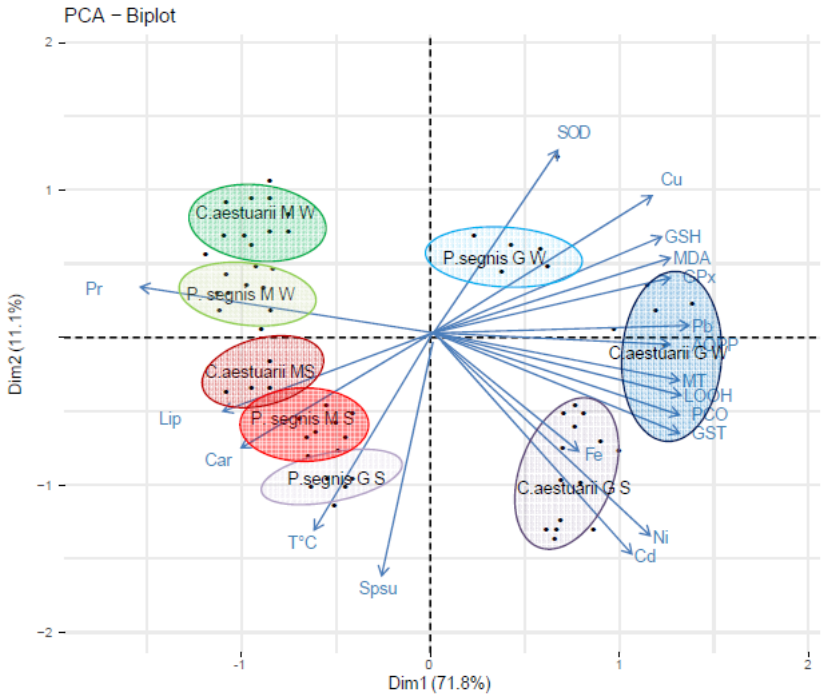

Figure 6

Principal compound analysis score plot of all redox status set determining in *P.segnis* and *C.aestuarii* gills and muscles collected from Gabes gulf during spring and winter seasons. Pr: proteins; Lip: lipids; Car: carbohydrate; T°C: temperature; Spsu: salinity; SOD: superoxide dismutase; GPx: glutathione peroxidase; GST: glutathione-S-trasferase; GSH: glutathione; MT: metallothionein; MDA: malondhyaldehide; LOOH: lipid hydroperoxide; AOPP: advanced oxidation of proteins products; PCO: proteins carbonyls; Cu: cooper; Cd: cadmium; Pb: lead; Fe: iron; Ni: nickel.