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Influence of toxic phytoplanktons and heavy metals in the antioxidant response of a common herbivorous fish species (*Sarpa salpa*)

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ABSTRACT

Within the framework of a biosurveillance of the marine species on the Tunisian coasts, we studied the Sarpa salpa (L), a fish that is consumed by the people living in this region. There is a seasonal occurrence of hallucinogen effects observed when this fish is consumed by humans. The objective of this work was to correlate the antioxidant response of fish to environmental toxic phytoplanktons and metal exposure. The salema that lives around the Island of Kerkennah is primarily an herbivorous fish during all seasons. We observed an increased expression of catalase (CAT) and glutathione peroxidase (GPx) in certain organs compared to the control fish, D.annularis. There was a "season-depending" cumulative effect, appearing in the organs starting with the liver, followed by the brain, and finally the muscle. Moreover, it increases according to the size of the animal and thus to the amount of food it consumes. The hallucinogen effect that the consumption of S.salpa has on humans is parallel to the seasonal variation in the epiphytes that are co-ingested by these fish with their food. Furthermore, heavy metals accumulation in organs of these fish showed a significant variation between the two species ($P<0.05$). A significant correlation ($P<0.01$) was also observed between the total toxic dinoflagellates and the antioxidant response: CAT and GPx in liver, brain, and muscle for all seasons and all sizes together. Our work indicates that, toxic phytoplanktons and heavy metals accumulation are responsible for the increase of antioxidant activities in the organs of S. salpa.

Key words: *S.salpa*, Toxic dinoflagellate, Antioxidant activities, Heavy metals.
Abbreviations: *S.salpa, Sarpa salpa; D.annularis, Diplodus annularis; VI, vacuity index; P.oceanica, Posidonia oceanica; GPx, glutathione peroxidase; CAT, catalase.*

INTRODUCTION

Sarpa salpa, also known as salema porgy, is an herbivorous sea fish that preferentially feeds on *Posidonia oceanica* throughout the year [1-2] and is used for human consumption in the Mediterranean region. Due to its low cost, these fish are predominantly on the menu of the lower income class. An important observation is the presence of ciguateric species that live as epiphytes on the leaves of *P. oceanica* [3]; they are co-ingested by the *S.salpa* as part of their diet. *D.annularis* presented an interest rule ecological, economic and aquacultural [4-5].

The Gulf of Gabes is a threatened biotope mainly due to the pressure of anthropogenic expansion and dumping of large quantities of phosphogypsum and other chemical products which severely impacted benthic habitats [6]. It has been shown that epiphytes of seagrass are sensitive to environmental changes [7]. For example, various studies reported increases in epiphyte biomass parallel with nutrient enrichment [8], eutrophication and water quality. This has led in the Gulf of Gabes to a substantial proliferation of microalgae and particularly of toxic dinoflagellates [9].

Such proliferation of undesirable microalgae has been shown to result in increasing problems in both coastal and estuarine environments [10-11].

Heavy metals can be classified as potentially toxic (arsenic, cadmium, lead, mercury, nickel, etc.), probably essential (vanadium, cobalt) and essential (copper, zinc, iron, manganese, selenium) [12]. Toxic elements can be very harmful even at low concentration when ingested over a long time period. The essential metals can also produce toxic effects when the metal intake is excessively elevated [13]. Water pollution leads to fish contaminated with toxic metals, from many sources, e.g. industrial and domestic waste water, natural runoff and contributory rivers [14-15]. Fish, generally accumulate contaminants from aquatic environments, have been largely used in food safety studies. Heavy metals discharged into the marine environment can damage both marine species diversity and ecosystems, due to their toxicity and accumulative behavior [16-17]. In the sea, pollutants are potentially accumulated in marine organisms and sediments, and subsequently transferred to man through the food chain [18]. For this reason, determination of chemical quality of aquatic organisms, particularly the contents of heavy metals in fish is extremely important for human health [19].

It is known for a wide range of environmental pollutants such as heavy metals and ciguatoxin (neurotoxins) that they can induce oxidative stress in aquatic animals including fish. The generation of reactive oxygen species (ROS) is commonly associated with cellular injures due to alterations in DNA, proteins and membranes [20]. Lipid peroxidation estimation has been found to have a high predictive value as a biomarker of this effect [21]. Also, antioxidant enzymes have been proposed as biomarkers of contaminant-mediated oxidative stress in a variety of marine organisms and their induction reflects a specific response to pollutants or toxins [22]. Because of, on one hand the possible effects on the fish and secondly in relation to consumption of the fish by humans, it is relevant to study the oxidative stress in fish.

The objective of this work was to correlate the antioxidant response of fish to environmental toxic phytoplankton and heavy metals exposure.

MATERIALS AND METHODS

Feeding behaviour and food composition of *S.salpa*

Fish collection

The study was carried out in the Island of Kerkennah (Gulf of Gabes, South East Tunisia). This archipelago is characterized by extensive *P. oceanica* seagrass meadows. Specimens of *S. salpa* were collected between January 2006 and January 2007; 59 specimens in winter, 57 in spring, 57 in summer and 55 in autumn. Total length (TL) of the fish was measured to the nearest 0.1 cm and weigh to the nearest 0.1 g. Their sizes ranged from 12.8 to 28 cm and the fish were divided into three classes; adults (3–7 years: large size) TL > 20 cm; subadults (2 years: medium size) 17 cm < TL < 20 cm; and young (1 year: small size) TL < 17 cm. Immediately after capture, fish were dissected, the guts were removed and preserved in a 4% formalin solution. Also the liver, brain and muscle (including dark muscle) were removed, rinsed with ice-cold saline and stored at −80 °C until further analysis. In the laboratory, prey identification was carried out to the lowest possible taxonomic level. Species abundance and wet weight were recorded to the nearest ±0.001 g after removal of surface water by blotting paper.

As control we collected between January 2006 and January 2007 the annular seabream *D. annularis* (Linnaeus, 1758) (belonging to the same biotope and the same family). Fifty specimens of *D. annularis* were collected in winter; 48 in spring; 51 in summer and 52 in autumn and were processed as described above.

Additionally, a second batch of fish adult *S. salpa* and *D. annularis* were collected for heavy metal assay in the autumn season from September to November (2006) in the same region. Specimens were brought to the laboratory on ice and total length and weight of the samples were measured. Immediately after arrival at the lab the fishes were eviscerated, the organs dissected and rapidly frozen at (−80 °C) and stored until use.

Analysis of the stomach contents

For each specimen collected whose stomach contained seagrass leaves and epiphytes, we determined the composition and food source. Therefore the stomach contents were washed in a Petri dish and studied under a microscope. Food items were sorted into large taxonomic groups and when possible identified to species level according to Fischer et al. [23]. The diet of the *S.salpa* was characterized using the vacuity index (VI) (1). Analysis of the vacuity index in time will inform us about the dietary behavior of the fish and allow us to formulate seasonal or monthly rhythms.

$$VI = \frac{\text{Number of empty stomachs}}{\text{Number of investigated stomachs}} \times 100 \quad (1)$$

The second parameter is the relative mass of item *i* (2) where item *i* can be a group, a family, a genus or a species.

$$\text{Relative mass (i) (\%)} = \frac{\text{Mass of the item (i)}}{\text{Total mass of stomachs contents}} \times 100 \quad (2)$$

Taxonomic identification and quantification of phytoplankton species in the stomach contents

The content of each stomach was put in a beaker, 500 ml of sea water was added and the mixture was shaken, filtered (75 µm pore size) and the flow-through preserved with 3% formol. Phytoplankton classification and counting was performed using an inverted microscope following the method proposed by Uthermöhl [24] after fixation with a Lugol's iodine solution [25].

The concentration of phytoplankton (non-toxic diatoms) and of toxic epiphytic dinoflagellates associated with ciguatera fish poisoning (e.g., *Prorocentrum sp*., *Ostreopsis sp*., *Coolia sp.*, and *Amphidinum sp.*) was recorded.

Seasonal variation in cellular stress, heavy metals and toxicity in tissue of different organs

Biochemical assays in fish organs

The frozen liver, brain and muscle cell samples were homogenized (Ultra Turrax T25, Germany) 1/2, w/v) in an ice cold buffer (TBS: 50 mM Tris, 150 mM NaCl, pH 7.4) and centrifuged (5000 g, 30 min, 4 °C); supernatants were frozen at (−80°C).

Protein quantification

In tissues of fish protein contents were measured according to the method of Lowry et al. [26] using bovine albumin serum as standard.

Determination of antioxidant enzyme activities in tissues of fish

* Catalase (CAT) activity was assayed by the method of Aebi [27]. Enzymatic reaction was initiated by adding an aliquot of 20 µl of the homogenized tissue and the substrate (H2O2) to a concentration of 0.5 M in a medium containing 100 mM phosphate buffer (pH 7.4). Changes in absorbance were recorded at 240 nm. CAT activity was calculated in terms of µmole H_2O_2 consumed/min/mg of protein.

* Glutathione peroxidase (GPx) the assessment of GPx activity was determined using a commercial kit (catalog No. RS 505; Randox, Ltd.,). GPx catalyzes the oxidation of GSH by cumene hydroperoxide. In the presence of GSH reductase and NADPH, the oxidized GSH is immediately converted to the reduced form with a concomitant oxidation of $NADPH^{-}NADP^{+}$. The decrease in absorbance at 340 nm was measured [28]. The enzyme activity was expressed as nmoles of GSH oxidized/min/mg protein.

Metal concentrations in fish organs

For this aspect, 15 *S.salpa* and 14 *D.annularis* were collected, their biometric data recorded (Table 1) and tissues used to determine the heavy metal content as described in materials and methods. Each organ sample (liver, viscera except liver and muscle including dark muscle) was treated according to the method described by Hamza-Chaffai et al. [6]. Lead (Pb), copper (Cu), and nickel (Ni) were analyzed on an atomic absorption spectrophotometer (HITACHI Z 8200) using the Zeeman Effect (Amiard et al. 1987). This methodology has been validated through international intercalibration exercises [29].

Statistical analysis

Data are presented as average ± standard deviation. The calculations were performed on groups of five animals each and the differences were examined by a two-way analysis of variance (fixed factors: size and season), followed by the Fisher test (Stat View) and the significance was accepted at $^{*}P<0.05$. Also, correlation coefficients (R) were calculated for all sizes and all seasons together using the Pearson correlation.

RESULTS

Different aspects describing the feeding of *S.salpa*

Nature of the stomach contents of the S. salpa

Over the one year period over which this study was conducted (January 2006 to January 2007), we found that the diet of large- and medium-sized *S.salpa* was composed of the following varieties of marine flora: marine Phanerogams (seagrass): *P.oceanica*; red algae: *Petiola stupulaceae*; *Grateloupia sp.*; *Ceramium sp.*; *Sphacelaria pluma;* Brown Algae: *Dictyota dichotoma*; *Cytoseira sp* and the Cyanophyta: *Lyngbia sp.*. In spite of the abundance of the *Caulerpa prolifera* in the biotope, this species did not appear in the stomach contents.

Vacuity index (VI)

To study the dietary behaviour of *S.salpa*, we examined 228 stomachs, 104 of which contained macrophytes species. The *S.salpa* were captured at night, when the fish are generally most active because they are feeding. The individuals caught were hauled onboard the following morning, therefore some may have stayed in the net for several hours and their capture may have occurred before the ingestion of prey or after digestion. As a result, many specimens had an empty stomach at the moment they were collected.

The seasonal variation of the mean vacuity index for the medium and large size classes showed a decrease in spring (31.57%) and summer (40%) relative to winter (78.9%) or autumn (66.10%), indicating that *S. salpa* fed significantly more in the first two periods compared to winter. The mean annular VI for *S.salpa* was 54.38%.

Diet of the Sarpa salpa

P.oceanica is the year around the major constituent of the diet of large- and medium-sized *S.salpa* (yearly average >50%). However we observed significant variation between the seasons. A striking difference was found in winter; the large *S.salpa* generally fed less on *P.oceanica* leaves which comprised (7.34%) of their diet with a large contribution of red algae (87.18%) and brown algae (5.77%) (fig.1). Unlike the large, the medium sized fish almost exclusively fed on *P.oceanica* leaves in winter 97.34 % (fig.1).

During spring, the fish tend to eat more as demonstrated by a lower vacuity index (table 1). During spring both classes of fish nourish on a diverse diet of seagrass, brown algae, red algae and for the medium-sized fish also on Cyanophyta *Lyngbia* sp. The high contribution of *Cyanophyta Lyngbia sp*. in their diet (up to 40%) is certainly not because of preference but rather because of the abundance of these algae in the environment (fig.1).

Summer is the season preceding the reproductive period for large-sized *S.salpa*. Adult fish increased their grazing of *P.oceanica* leaves, reaching (79.5%) during this season, whereas the medium incorporated also a significant percentage of *Lyngbia sp.* (42.18%) into their diet (fig.1).

Both medium and large size classes attained maximum grazing *P.oceanica* leaves (64.31%; 67.20% respectively) during autumn (fig. 1). We noticed that its food spectrum in this period was much diversified with a multitude of algae. So was the brown alga *Cytoseira sp.* only in this season identified with a percentage of 4.24%.

Evaluation of the phytoplankton composition

The toxic phytoplankton species observed in the stomach contents of *S.salpa* were the dinoflagellates *Prorocentrum rathymum*, *Prorocentrum lima*, *Prorocentrum concavum*, *Ostreopsis siamensis*, *Coolia monotis* and *Amphidinum carterae*. Seasonal variation of the toxic phytoplankton was compared to the total number of phytoplankton in the *P.oceanica* meadow. We found that the proportions of the toxic species followed the same pattern as found for the total phytoplankton population (Fig.2).

Seasonal variation of antioxidant activities for S.salpa

Antioxidant enzyme activities in several organs and compartments of the *S.salpa* specimens were studied. The results obtained were compared with those found for the control fish, the annular seabream *D.annularis* (from the same biotope). The organs were selected on the basis of functional criteria, which made them preferential targets, i.e., xenobiotic metabolism (liver) and the known neurotoxic effect of toxic dinoflagellates ingested by *S.salpa* (brain) and muscle being the preferred part of the fish used for human consumption.

We noted several differences:

During winter, no significant variation (P>0.05) was observed for the antioxidant activities catalase (CAT) and gluthation peroxidise (GPx) in large and medium size classes of *S. salpa* as well as in *D. annularis* (Fig .3-4).

In spring, we noticed a significant increase (P<0.05) for the antioxidant enzyme activities studied: catalase (CAT) and Glutathion peroxidase (GPx) which affected only the liver of *S.salpa* of large and medium sizes (Fig.3-4).

In summer, we observed a significant increase (P<0.05) for the activity of catalase (CAT) in liver of the *S.salpa* for both sizes large and medium (fig.3-4). In addition, during this season, we observed a significant variation (P<0.05) for both large and medium size classes in the liver for the activity glutathione peroxidase (GPx). In the same way, we observed a significant variation (P<0.05) for the two enzyme activities catalase (CAT) and glutathione peroxidase (GPx) in the brain only for the large size class.

In autumn, we observed a significant variation (P<0.05) for the enzyme activity of catalase in the liver at the large and medium size classes. Moreover, we noticed that there also exists a significant variation (P<0.05) for the antioxidant enzyme activity in the brain and muscle for the large size class of *S.salpa* (fig.3-4). The activity of glutathione peroxidase (GPx) enzyme is expressed in the three organs for the large size class of *S.salpa* (P<0.05) (fig. 3-4). However, looking at the graphs it seems like there is the same antioxidant response in *D.annularis* (despite the lower activity level, most probably due to interspecies metabolism rate).

Concentration of heavy metals in different organs

The mean concentrations of lead, copper and nickel in the liver, muscle (including dark muscle) and viscera (viscera except liver) of *S.salpa* and *D.annularis* are shown in Table 3. We observed a significant increased concentration of all three heavy metals in each of the three tissues; viscera, liver and muscle of *S.salpa* investigated compared to the control fish *D.annularis*.

Inter-seasonal Correlation between the total toxic dinoflagellates and the antioxidant activities in different organs of *S.salpa*

The antioxidant activities in *S.salpa* shows a cumulative effect over the consecutive seasons, beginning in spring with a maximum effect during autumn, and affecting an increasing number of organs first the liver, then the brain, and finally the muscle. This effect further increase with the size of the animal which might be related to the amount of *P.oceanica* leaves consumed that are enriched with toxic epiphytic phytoplankton in a similar seasonal pattern.

To justify and consolidate our precedent observations, we started a series of tests of correlation between the total toxic dinoflagellates in the stomach contents of *S. salpa* and the profiles of antioxidant activities in fish organs. Pearson correlation analysis is listed in table 3. There were a significant positive correlation between the total toxic dinoflagellates and the level of antioxidant activities measured in the liver, the brain and the muscle for all seasons and all sizes together.

DISCUSSION

Feeding behaviour of *S.salpa*

Over the year, we see a fluctuating pattern in the vacuity index for *S.salpa*. This can probably be explained by the feeding behaviour of *S.salpa*. Grazing in June-September is done in massive schools that actively feed on *P.oceanica* to accumulate reserve for the winter period when they eat less, and to prepare adult fish for reproduction. *S. salpa* has a single period of maximum spawning: from mid-September to mid-October followed by a period of intensive settlement at the end of November. In March, when adults migrate to deep waters, juveniles live and feed in shallow, rocky bottoms [1]. This result in mean annular VI for *S.salpa* that is rather weak (54.38%) in contrast to that found for *D.annularis* (91.48%) [30]. Our findings are consistent with this behaviour, before egg-laying the *S. salpa* nourishes and stores lipids for the sake of their genital product maturation.

The grazing behaviour for the medium and large size classes of *S. salpa* is in essence only different in winter. The medium sized in winter almost exclusively feed on seagrass whereas the large sized feed on red algae. In spring, both classes have a mixed diet of with near equal contribution of seagrass, cyanophyta and brown algae (and in case of large sized also red algae). In summer and autumn the contribution of seagrass increases. These results are comparable to the results found by Alcoverro et al. [31]. Adult leaves, which showed the greatest colonization by epiphytes, were preferred by herbivores throughout the year at all depths [32-33]. The variation of the toxic phytoplankton in stomach contents of *S. salpa* follows a seasonal trend with a peak in summer.

Seasonal variation in antioxidant activities in the *S.salpa* as a result of changes in their diet

In winter, no significant variation in antioxidant enzyme activities was observed in *S.salpa* at the level of the various organs studied as compared to the control fish, *D. annularis*. It would seem that this absence of significant variation is due to the absence of toxic epiphytic dinoflagellates on the leaves of *P. oceanica* consumed by *S. salpa*. In spring, there was a significant increase in catalase and glutathione peroxidase activities in the liver of the *S.salpa*. The increase in the percentage of *P. oceanica* as the food source of large- and medium-sized fish could explain the

difference found between the antioxidant enzyme responses at the hepatic level in the different size ranges. Moreover, we noticed an increase in antioxidant enzyme activities that is certainly due to the presence of toxic dinoflagellates in the stomach contents of *S.salpa* with a percentage equal to 1.14%. In summer, *P. oceanica* leaves showed greatest colonization by epiphytes owing to the fact that the surrounding water was calmer. *S.salpa* had maximum periods of grazing on *P. oceanica* leaves during summer and autumn. This has an effect on the resulting antioxidant activities. We also noticed a significant variation of catalase and glutathione peroxidase activities in the liver of both large- and medium-sized fish. This proves that the main catalase is highly expressed during the period of contamination by toxic phytoplankton because of the elimination of ROS, supporting the results obtained by [34-35]. There was also a significant variation of catalase and glutathione peroxidase activites in the brain of the large-sized *S.salpa*. In autumn, the percentage of ciguateric species compared to the other seasons was most important (5.26%). During this season, fish from both size classes consumed *P.oceanica* leaves as the preferential food source (>50%), which is in agreement with the results found by Peirano et al. [1]. Differences in grazing might be explained by different behavior of the grazers. Massive schools of *S.salpa* actively feed on *P.oceanica* leaves in summer to accumulate reserves for reproduction during autumn. This behavior is reflected in the rate of antioxidant activities, and both size classes showed significant variation in liver catalase activity. The activities of glutathione peroxidase were expressed in the three organs of the large-sized *S.salpa*. These results enabled us to conclude that there was a peak in toxicity in autumn, and that toxicity increased enormously in the brain in autumn and even accumulated in the muscle of the large-sized fish. The metabolism of toxic compounds frequently results in the formation of ROS, which significantly contribute to their toxicity [36].

Accumulation of heavy metals: additional source of antioxidant activities

S. salpa and *D .annularis* have very different eating habits which takes place in different trophic chains. *D. annularis* is omnivorous fish that feeds on worms, mollusks, Crustaceans algae, *P.oceanica* leaves and as secondary prey annelids [37]. *S. salpa* is a herbivorous sea fish that nourishes preferentially on the seagrass *P. oceanica* through the year [32-2]. Several studies suggest employing seagrasses as bio-indicators of coastal waters metal contamination [38]. *P. oceanica* may have a greater bioaccumulation capacity for all the metals considered except Hg and may reflect both contaminations in the water column and in sediment [38]. This could explain the quantities of heavy metals bioconcentrated significantly in organs of *S.salpa* as compared to the control fish, *D. annularis*. The accumulation of heavy metals in organs of *S.salpa* might contribute to the increase of antioxidant activities in our study.

We compared our average values of copper in fish organs (1.86- 9.54 $\mu g.g^{-1}$) with the Canadian food standards (Cu: 100 $\mu g.g^{-1}$), Hungarian standards (Cu: 60 $\mu g.g^{-1}$) and the range of international standards (Cu: 10-100 $\mu g.g^{-1}$) this showed that our values are lower than the guideline. The lead values in fish organs were found to be in range of 1.40 -1.70 $\mu g.g^{-1}$. These values were lower than those reported in the range of international standards for Pb in fish is 0.5-10 $\mu g.g^{-1}$. Nickel values in fish organs were found to be in range of 1.01-2.25 $\mu g.g^{-1}$. Nickel contents in the literature have been reported in the range of 0.11–12.88 µg/g dry weight in fish species from Iskenderun Bay, Northern East Mediterranean Sea, Turkey [40], 0.02–3.97 µg/g in seafoods from Marmara, Aegean and Mediterranean sea in Turkey [41].

Inter-seasonal correlation between the total toxic dinoflagellates and the antioxidant response for the *S.salpa*

The induction of the increase in antioxidant activities response was a logical answer to the exposure to toxic substances generated by toxic dinoflagellates and heavy metals accumulation. *S. salpa* and *D. annularis* has very different eating habits, take place in different trophic chain. This explains the quantities of heavy metals bioconcentrated significantly in organs of *S. salpa* as compared to the control fish, *D. annularis*. The accumulation of heavy metals in organs of *S. salpa* might be implicated in the increase of antioxidant response in our study. So, the accumulation of heavy metals in organs of *S. salpa* may intervene, but phytoplankton is most influential because of the toxic dinoflagellates consumed by *S. salpa*. The control fish, *D. annularis*, does not consume toxic dinoflagellates and does not show an antioxidant response like *S. salpa*. It appears that antioxidant activities are generally increased in the presence of toxic phytoplankton in the stomach contents of *S. salpa*.

Table.1. Biometric data (average ± SE) of fish from the coastal waters of the Island of Kerkennah (Gulf of Gabes; South East Tunisia) during autumn 2006.

Location	Species	N	Length (cm)	Weight (g)
Island of Kerkennah	*S.salpa*	15	21.9±0.66	113.86±1.50
	D.annularis	14	20.14±0.44	105.93±1.50

Table.2. Mean values (µg/g.dry weight) with standard deviations of copper, nickel and lead in various body organs of two fish species *S.salpa* and *D.annularis* collected from the Island of Kerkennah (Gulf of Gabes; South East Tunisia) during autumn 2006.

Fish Species	Heavy metals	Liver	Muscle	Viscera
Diplodus annularis				
	Cu	2.46±0.28	1.11±0.01	2.00±0.04
	Ni	0	0	0.62±0.06
	Pb	1.12±0.09	0	0.38±0.19
Sarpa salpa				
	Cu	9.54±0.50*	1.86±0.11*	7.48±0.78*
	Ni	1.01±0.11*	1.22±0.04*	2.25±0.15*
	Pb	1.70±0.18*	0	1.40±0.18*

Values are mean ± SE; n=8;
**P≤0.05 significant from control;*

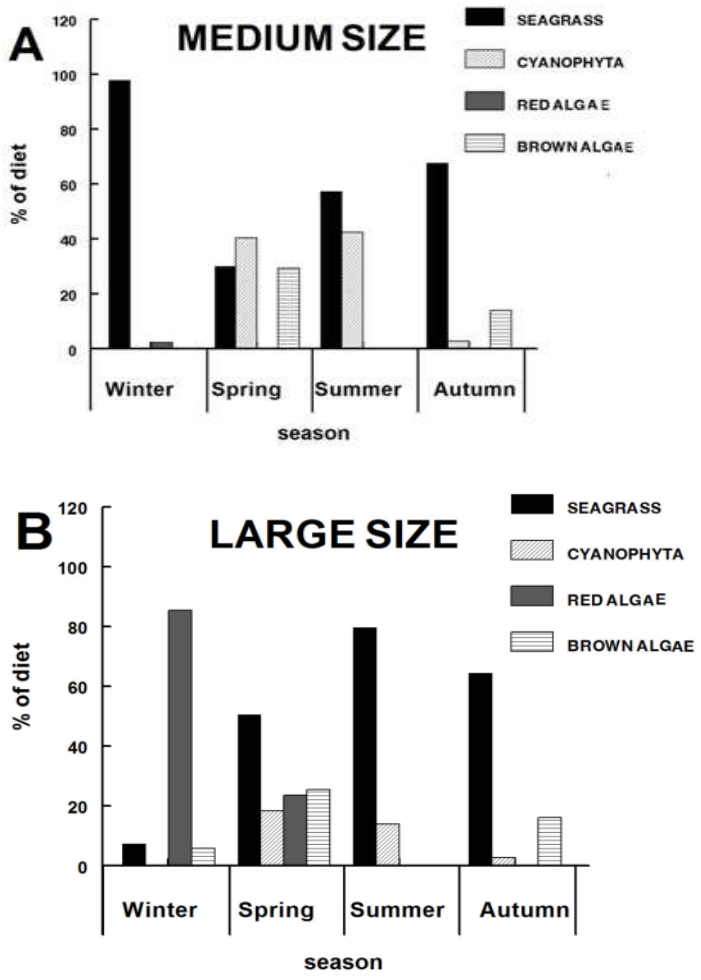

Fig. 1 Grazing behaviour of *Sarpa salpa*. Diet composition of *Sarpa salpa* was determined in the stomach content for two size classes; subadults (TL<20 cm) (A) and adults (TL>20) (B) in terms of percentage of seasonal grazing (%).

Table.3. Correlation matrix (Pearson test) between the total toxic dinoflagellates in stomach contents of *S.salpa* and the antioxidant activities of catalase (CAT) and gluthathione peroxidase (GPx) for all seasons and all sizes together. (P<0.01numbers of parameters 3 and number of analyzed samples 48).**

	Total toxic dinoflagellate	CAT activity in brain	CAT activity in muscle	CAT activity in liver	GPx activity in brain	GPx activity in muscle	GPx activity in liver
Total toxic dinoflagellate	**1**						
CAT activity in brain	0.643**	**1**					
CAT activity in muscle	0.503**	0.689**	**1**				
CAT activity in liver	0.506**	0.481**	0.428**	**1**			
GPx activity in brain	0.513**	0.752**	0.727**	0.353**	**1**		
GPx activity in muscle	0.455**	0.677**	0.708**	0.446**	0.818**	**1**	
GPx activity in liver	0.564**	0.820**	0.667**	0.519**	0.739**	0.742**	**1**

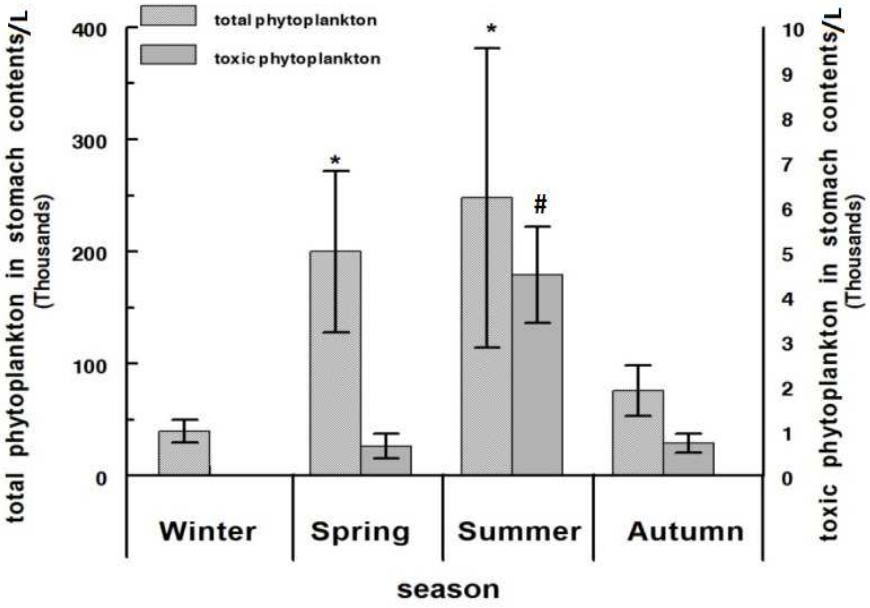

Fig.2. Seasonal variation of the toxic phytoplankton. The total number of phytoplankton counted in (1 L) of water from stomach contents of *Sarpa salpa* was analyzed.

**P<0.05: For total phytoplankton compared to autumn or winter;*

@P<0.05: For toxic phytoplankton compared to autumn, winter or spring.

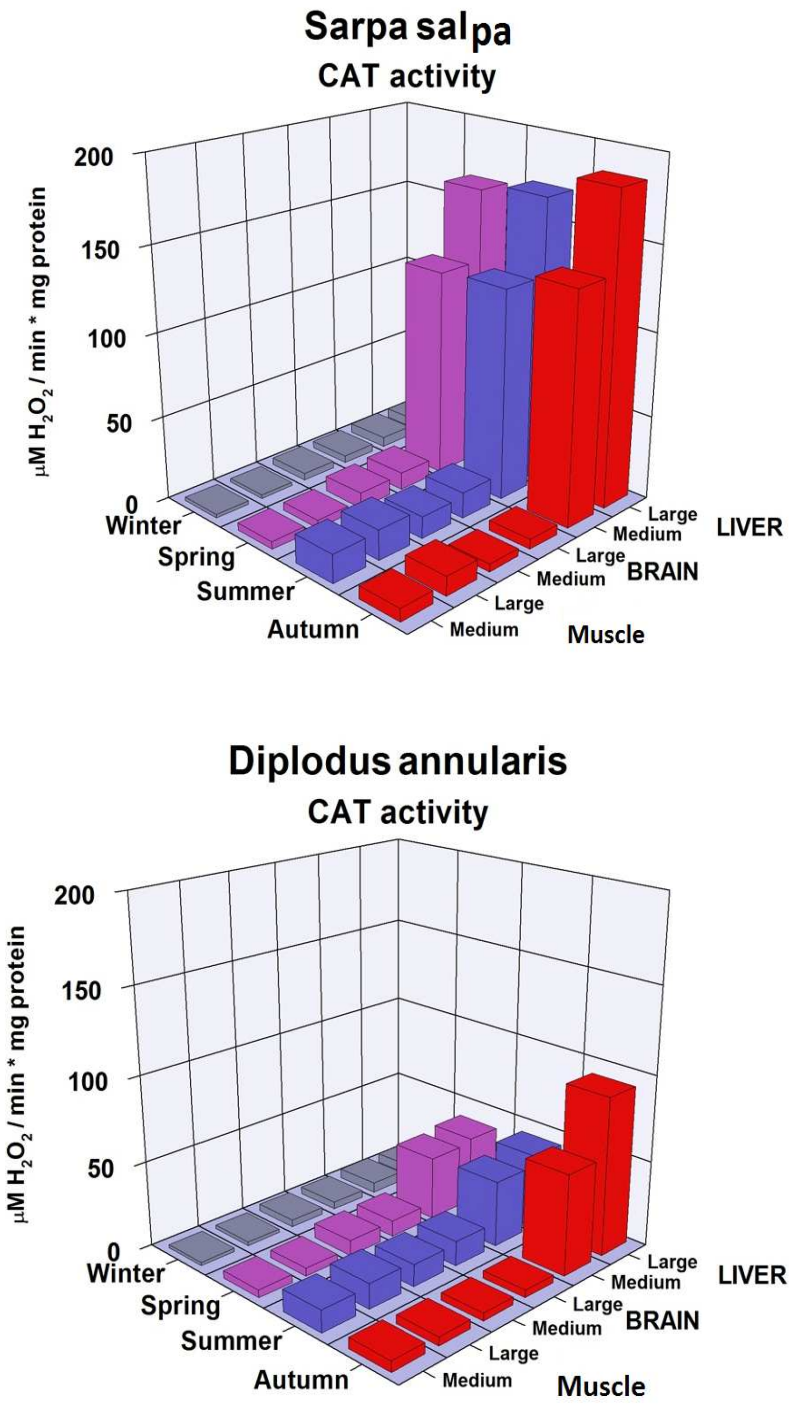

Figure 3. Catalase activity [µM H2O2 min^{-1} * mg $protein^{-1}$] in the liver, brain, and muscle of (a) *Sarpa salpa* compared to (b) the control fish *Diplodus annularis* the large and medium size classes.

The values represent the mean. The standard deviation was smaller than 10% of the mean in all cases and was therefore not included for clarity.

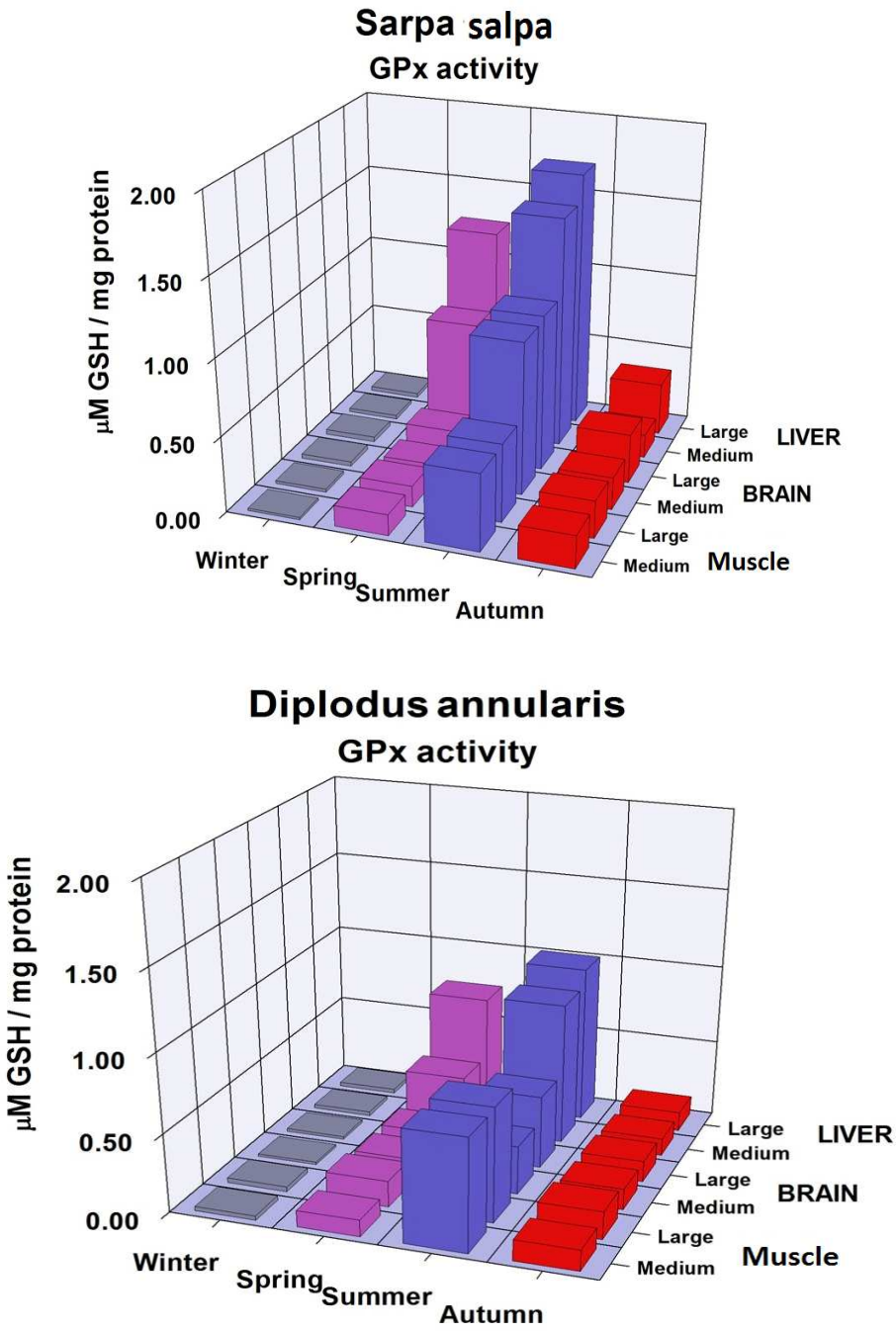

Figure 4. Glutathione peroxidase activity [µMGSH min^{-1} * mg $protein^{-1}$] in the liver, brain, and muscle during winter, spring, summer, and autumn in *Sarpa salpa* (a) compared to the control fish *Diplodus annularis* (b) for the large and medium size classes

The values represent the mean. The standard deviation was smaller than 10% of the average in all cases and was therefore not included for clarity.

CONCLUSION

We noticed a significant correlation between the total toxic dinoflagellates in the stomach contents and the antioxidant activities at the side of liver, the brain and the muscle for all seasons and all sizes together. In addition we found that the toxicity in fish organs correlates with the rate of dietary intake containing *P.oceanica* leaves which are rich in toxic epiphytic phytoplankton of a seasonal nature. Our work strongly indicates that, toxic dinoflagellates and heavy metals accumulation are responsible for the increase of antioxidant activities in fish organs of *S.salpa*.

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