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Antioxidant enzymes and lipid peroxidation in sturgeon *Acipenser naccarii* and trout *Oncorhynchus mykiss*. A comparative study

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Abstract

Enzymatic antioxidant activities (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase) and lipid peroxidation were determined in different tissues (gills, heart, digestive tract, liver, white muscle, skin, red blood cells, swimbladder) of trout (*Oncorhynchus mykiss*) and sturgeon (*Acipenser naccarii*). Total tissue antioxidant activity was, except for catalase, generally higher in trout than sturgeon, with the reverse tendency displayed by the liver. In both species, lipid peroxidation in the digestive tract showed higher values compared to the other tissues. Sturgeon, compared to trout, had higher energy content stored as fat in liver and muscle and thus appeared to have an effective safeguard against oxidation, as shown by low lipid peroxidation.
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1. Introduction

Like all aerobic organisms, fish are also susceptible to the attack of reactive oxygen species and, as a consequence, have an antioxidant defence system, as demonstrated by works dating primarily to the 1970s. Several circumstances promote the antioxidant defence response in fish. Factors intrinsic to the fish itself, such as age, phylogenetic position, and feeding behaviour, as well as environmental factors such as the type of diet supplied, daily or seasonal changes in temperature, dissolved oxygen, toxins present in the water, pathologies, or parasites, can either fortify or weaken antioxidant defences (Felton, 1995; Martínez-Álvarez et al., 2005). Most of the research on oxidative stress in fish focuses on toxicological aspects, such as the effects of different xenobiotics on antioxidant–enzyme activities, induction of biotransformation processes as well as on the intensity of lipid peroxidation and other biomarkers of oxidative damage (Di Giulio et al., 1989; Winston and Di Giulio, 1991). Despite these parameters having been used as biomarkers for contaminants, a review of the related literature disclosed no clear trend, since the fish response depends on several variables such as the species, tissue, the antioxidant parameter itself, time of exposure, and contaminant concentration, besides the other physiological and environmental changes mentioned above.

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Studies on oxidative stress in fish have opened a number of research lines on fish physiology in recent years (Rudneva, 1997, 1999; George et al., 2000a,b; Ross et al., 2001; Martínez-Álvarez et al., 2002; Morales et al., 2004). Further studies would provide more precise information concerning the response of antioxidant defences under different circumstances as well as on the regulatory mechanisms of this response, which will no doubt benefit aspects related to fish farming and production.

A number of enzymes are known to have major antioxidant activity, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR). An imbalance between the oxidizing processes and the antioxidant defence systems of an organism causes oxidative stress, leading to tissue damage, which, in the case of lipids, can be quantified by the formation of peroxides (Halliwell and Gutteridge, 2000).

Rainbow trout (*Oncorhynchus mykiss*) is one of the first fish to be farmed and among the first in which its physiology has become quite well known, as is the case of many teleost fish. Currently, some European countries, such as Italy and Spain, are beginning to farm the sturgeon *Acipenser naccarii*. This catadromous species, also called the Adriatic sturgeon, migrates seasonally from the rivers Po, Ticino and Adigge to the Adriatic Sea (Clementi et al., 1999). Recent studies (Garrido-Ramos et al., 1997; Hernando et al., 1999; De la Herrán et al., 2004) support the contention that the historical distribution of this species includes certain Spanish rivers. This opens the possibility of investigating different aspects from this primitive fish group dating to the Triassic in order to clarify its oxidative metabolism and antioxidant defences and thereby benefit fish farming (Martínez-Álvarez et al., 2002).

In the present work, a comparative study is made concerning the activities of the antioxidant enzymes SOD, CAT, GPX, GR as well as lipid-peroxidation levels in gills, heart, digestive tract, liver, white muscle, skin, red blood cells, and swimbladder of the sturgeon *A. naccarii* and the trout *O. mykiss*, maintained under routine fish-farming conditions.

2. Materials and methods

2.1. Fish

The experimental animals, the sturgeon (*A. naccarii*; age 1+; 656.0±15 g, $n=10$) and the rainbow trout (*O. mykiss*; age 1+; 289.5±5 g, $n=10$) were obtained from the fish farm Sierra Nevada S.L. (Riofrío, Granada, Spain). The maintenance and feeding conditions were those of the fish farm. Both fish species were fed the same artificial diet (Le Gouesant, France), with an approximate chemical composition of 48% of protein, 14% lipid, 11.4% ash, and 15% carbohydrates. The water temperature was maintained at 14±1 °C.

2.2. Sampling

The animals were quickly captured and killed after being anaesthetized by immersion, until sedate, in a clove-essence solution. Blood samples were taken from the caudal vessels using heparinised syringes in less than 1 min and transferred to heparinised tubes held on ice until centrifugation. Immediately after blood collection, heart, liver, complete digestive tract (from oesophagus to the anus), swimbladder, and a portion of gills, white muscle and skin (both from anterior dorsal region) were removed in situ, immediately frozen in liquid nitrogen and kept at −80 °C until analysed.

Table 1
Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR) activity in different tissues of trout (T) and sturgeon (S)

	Gills		Heart		Digestive tract		Liver	
	T	S	T	S	T	S	T	T
SOD (U/mg protein)	46.79±7.55	28.20±4.98	655.81±41.81	612.06±45.01	707.94±34.38	240.33*±17.95	834.69±28.15	967.63*±48.69
CAT (U/mg protein)	29.98±2.71	39.43±5.19	5.76±1.02	4.10±0.34	31.64±9.57	114.65*±8.36	116.74±11.36	159.09*±10.33
GPX (mU/mg protein)	159.19±15.14	137.83±14.96	112.10±9.05	104.36±7.87	667.29±47.14	333.80*±40.99	66.79±8.30	105.27*±8.58
GR (mU/mg protein)	28.30±3.94	7.47*±0.53	9.78±1.42	4.62*±0.65	330.80±55.93	174.25±21.36	27.21±3.43	46.21±9.45

Data are means±S.E.M. ($n=10$).
* Significant differences between trout (T) and sturgeon (S) for the same tissue ($p<0.05$).

2.3. Treatment of samples

Blood was centrifuged at 650×*g* for 10 min to separate red blood cells (RBC) from plasma, and the haemolysate supernatant from RBC was obtained according to Marcon and Filho (1999). This briefly consisted of carefully washing RBC with a 9 ‰ saline solution at an approximate ratio of 1:3 (v/v); the mix was centrifuged at 650×*g* for 5 min and supernatant removed. This was repeated twice. Then RBC were added to a buffer solution (20 mM Tris–HCl pH 7.8, 10% glycerol (v/v) and 0.1% triton X-100 (v/v)) at a ratio of 1:9 (v/v) and frozen at −20 °C for 12 h to break up the cells. After this time, cells were centrifuged at 5000×*g* for 10 min at 4 °C. Supernatant containing RBC content was frozen at −80 °C until required for analysis.

Tissue samples were homogenized in ice-cold buffer (100 mM Tris–HCl, 0.1 mM EDTA and 0.1% triton X-100 (v/v), pH 7.8) at a ratio of 1:9 (w/v). Homogenates were centrifuged at 30,000×*g* for 30 min in a Centrikon H-401 centrifuge. After centrifugation, the supernatant was collected and frozen at −80 °C until analysed.

2.4. Analytical methods

All enzymatic assays were carried out at 25±0.5 °C using a PowerWave$_x$ microplate scanning spectrophotometer (Bio-Tek Instruments, USA) in duplicate in 96-well microplates (UVStar®, Greiner Bio-One, Germany). The enzymatic reactions were started by the addition of the tissue extract, except for SOD where xanthine oxidase was used. The specific assay conditions were as follows.

Catalase (CAT; EC 1.11.1.6) activity was determined by measuring the decrease of H_2O_2 concentration at 240 nm according to Aebi (1984). The reaction mixture contained 50 mm potassium phosphate buffer (pH 7.0) and 10.6 mM H_2O_2 freshly prepared.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured spectrophotochemically by the ferricytochrome *c* method using xanthine/xanthine oxidase as the source of superoxide radicals. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.1 mM xanthine, 0.013 mM cytochrome *c* and 0.024 IU ml^{-1} xanthine oxidase. One activity unit was defined as the amount of enzyme necessary to produce a 50% inhibition of the ferricytochrome *c* reduction rate measured at 550 nm (McCord and Fridovich, 1969).

Glutathione peroxidase (GPX; EC 1.11.1.9) activity was measured following the method of Flohé and Günzler (1984). Blood samples were treated with KCN solution (Drabking reagent; Sigma Chemical Co.) to avoid haemoglobin interference. A freshly prepared glutathione reductase solution (2.4 U ml^{-1} in 0.1 M potassium phosphate buffer, pH 7.0) was added to a 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM EDTA, 1 mM sodium azide, 0.15 mM NADPH and 0.15 mM H_2O_2. After the addition of 1 mM GSH (reduced glutathione), the NADPH-consumption rate was monitored at 340 nm.

Glutathione reductase (GR; EC 1.6.4.2) activity was assayed as described by Calberg and Mannervik (1975), with some modifications, by measuring the oxidation of NADPH at 340 nm. The reaction mixture consisted of 0.1 M sodium phosphate buffer (pH 7.5), 1 mM EDTA, 0.63 mM NADPH and 0.15 mM GSSG.

Except for SOD, for which the arbitrary units have already been mentioned, for other enzymatic activities, one unit of activity is defined as the amount of enzyme required to transform 1 μmol of substrate/min under the above assay conditions. Enzyme activities were standardised to the total tissue-protein concentration in gill (4.09±0.62–5.12±0.47 mg/ml), heart (5.84±0.42–5.95±0.34 mg/ml), digestive tract (1.24±0.09–1.64±0.13 mg/ml), liver (12.57±0.96–10.55±0.6 mg/ml), white muscle (6.87±0.29–4.80±0.25 mg/ml), skin

White muscle		Skin		RBC		Swimbladder	
T	S	T	S	T	S	T	S
29.57±2.00	26.84±2.81	23.05±1.71	9.45*±2.12	12.92±1.40	2.20*±0.14	495.75±79.42	262.47*±26.72
46.21±6.87	4.27*±0.59	0.38±0.05	0.97*±0.09	64.17±3.88	79.43*±4.30	5.52±0.67	4.97±0.92
100.18±4.14	155.22*±10.12	126.29±6.78	216.06*±17.33	100.27±6.00	63.24*±6.73	215.08±37.09	282.38±39.18
817.94±64.81	698.76±85.09	2.56±0.22	1.72±0.37	1.17±0.10	2.55*±0.36	66.76±8.17	2.85*±0.57

(5.78±0.36–4.80±0.35 mg/ml), RBC (78.65±1.66–76.91±6.55 mg/ml) and swimbladder (1.78±0.22–3.06±0.39 mg/ml) of trout and sturgeon, respectively, determined by the Biuret–Folin method (Ohnishi and Barr, 1978) using bovine serum albumin as a standard.

Lipid-peroxidation levels were determined by quantifying the concentration of thiobarbituric-acid-reacting substances (TBARS), expressed as nmol/g tissue, according to Buege and Aust (1978).

All biochemicals, including substrates, coenzymes and purified enzymes, were obtained from Roche (Mannheim, Germany) or Sigma Chemical Co. (USA). All other chemicals came from Merck (Darmstadt, Germany) and were of the reagent grade.

2.5. Statistical analysis

The results are expressed as means±S.E.M. The differences between species in a tissue for each enzyme activity were tested for significance using the Student's *t*-test ($p<0.05$). For the analysis of the dependence between two variables, the Pearson correlation coefficient was estimated and data were adjusted by linear regression. For all the statistical analyses the software SPSS 11.5 for Windows was used.

3. Results

The highest SOD values in both species were found in the liver. The heart, digestive tract, and swimbladder followed the hepatic values, while in RBC the activity was the lowest, these values being slightly lower than in skin, muscle and gills, where the values were quite similar (Table 1). Also, except in the liver, where the values for this enzyme proved to be significantly higher in sturgeon than in trout, the SOD values of the tissues were either similar (white and cardiac muscle and gills), or significantly higher in trout than in sturgeon (Table 1).

The CAT activity, as in the case of SOD, was higher in the liver of both species. In the sturgeon, liver CAT activity was followed by that of the digestive tract, RBC, gills, heart, swimbladder and skin. In the trout, CAT values of the liver were followed by those of erythrocytes, white muscle, digestive tract, and gills, white heart, swimbladder, and skin. The CAT activity in white muscle of trout was significantly higher than in the sturgeon, while in gills, heart, and swimbladder the activity was very similar in both species, and in the rest of the tissues (liver, digestive tract, skin, and RBC) it was significantly higher in the sturgeon than in trout.

In both species the highest GPX activities were found in the digestive tract, with intermediate values in the white muscle, skin, gills, and swimbladder, while the lowest values were found in the liver, heart, and RBC. The values for heart, gills, and swimbladder were similar in the trout and sturgeon, whereas in the liver, muscle, and skin, higher values were found in the sturgeon. The trout showed higher activities only in the digestive tract and RBC.

The GR values found in the different tissues of both species followed a similar pattern, where the highest activity was found in white muscle, followed by the digestive tract, and skin, with RBC values showing the lowest values. Slightly higher values were found in the heart, swimbladder, and gills. Except in the liver and RBC, the trout showed higher activities than in the sturgeon. A noteworthy difference was found in white muscle of both species, where the activity of this enzyme was far higher than in the other tissues.

In terms of lipid peroxidation (Fig. 4), the values in the digestive tract were 10- to 20-fold higher compared to the other tissues. In liver, lipid peroxidation in sturgeon was significantly higher than in trout. On the contrary, the lipid peroxidation of white muscle and skin in trout was higher than in the sturgeon. In trout the levels found in gills and skin, although lower than in the digestive tract, were higher than in the other tissues, where the values were lower and very similar in both species.

4. Discussion

SOD is the first enzyme to respond against oxygen radicals (McCord and Fridovich, 1969) and is the one that offers the greatest response to oxidative stress (Winston and Di Giulio, 1991). Any situation that increases oxygen consumption by the mitochondria proportionally increases the generation of O_2^- (Camougrand and Rigoulet, 2001). Experimental tests in animals demonstrate a correlation between SOD and tolerance to oxygen toxicity (Pérez-Campo et al., 1993).

Table 1 shows that regardless of the species, SOD activity is higher in the liver. This is also reflected by Fig. 1A and B, which shows, for each enzyme, the relative percentage between tissues in each species, giving the value of 100 to the tissue that presents the highest activity. It is known that vertebrate liver exhibits high metabolism and oxygen consumption, and it probably best represents the status of antioxidant defences in organisms, and therefore, it is frequently referred to in the literature (Chance et al., 1979; Davies, 1991; Wilhelm-Filho et al., 1993). Thus, our results found in

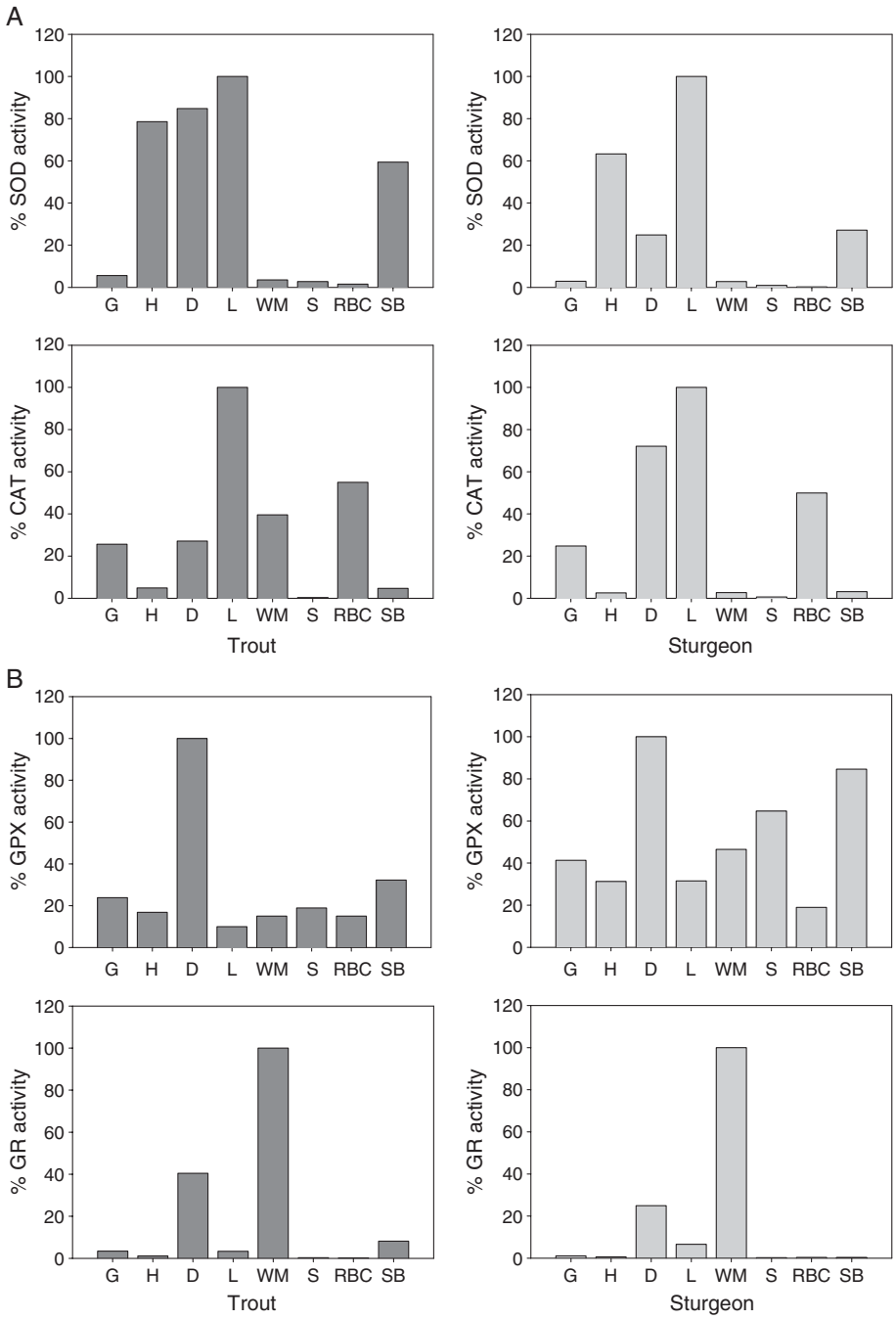

Fig. 1. (A) SOD (superoxide dismutase) and CAT (catalase) activity in different tissues of trout and sturgeon (G, gills; H, heart; D, digestive tract; L, liver; WM, white muscle; S, skin; RBC, red blood cells; SB, swimbladder). For each plot, the mean value with the highest specific activity was considered as 100%. (B) GPX (glutathione peroxidase) and GR (glutathione reductase) activity in different tissues of trout and sturgeon (G, gills; H, heart; D, digestive tract; L, liver; WM, white muscle; S, skin; RBC, red blood cells; SB, swimbladder). For each plot, the mean value with higher specific activity was considered as 100%.

trout with the example of teleosts and in the sturgeon as an acipenserid (condrostean) agree with the literature in reference to the other fish, both elasmobranchs as well as teleosts (Wdzieczak et al., 1982; Cassini and Albergoni, 1993; Wilhelm-Filho and Boveris, 1993; Wilhelm-Filho et al., 1993; Avci et al., 2005).

Also, in all tissues, except in liver, SOD activity in trout was greater than in sturgeon (Table 1). This is also

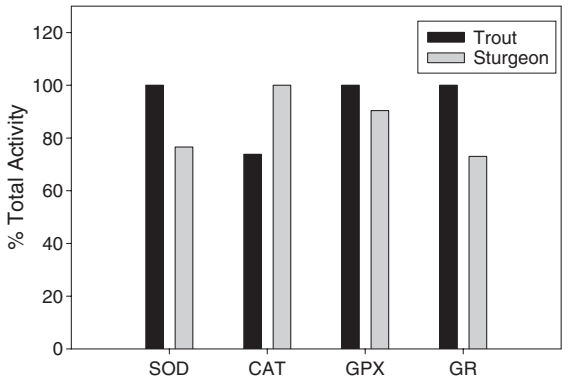

Fig. 2. Total tissues activity in trout and sturgeon. Values for each enzyme are expressed as a percentage in which the value obtained in the species with maximum total activity is 100%.

reflected in the percentage of activity in the totality of the tissues (Fig. 2) and in the liver (Fig. 3), where for each enzyme the value of 100 corresponds to the species that showed the highest value of enzymatic activity. The sturgeon is a epibenthonic fish and less active than the trout. Kieffer et al. (2001) found that the resting consumption rates for Atlantic sturgeon (*Acipenser oxyrhynchus*) and shortnose sturgeon (*Acipenser brecirostrum*) were 10–20% lower than those of rainbow trout. Also, it has been found that SOD activity in the RBC of horse mackerel (*Trachurus mediterraneus*) and pickerel (*Spicara smaris*) as well as pelagic fast-swimming fish species were significantly higher than in the RBC of bottom-pelagic and bottom fish species (Rudneva, 1997). The lower oxygen consumption by sturgeon may explain the lower activity of the antioxidant enzymes. Another possible explanation, according to some authors, is that antioxidant enzymes appear to correlate with phylogenetic position, where more ancestral species exhibit less activity (Rabie et al., 1972; Smith, 1976; Tappel et al., 1982). Also, Rudneva (1997) reported that the high levels of urea, glutathione, and vitamin K found in *Squalus acanthias* (elasmobranch) might compensate for the limited enzymatic antioxidant system, and this researcher assumed that the low-molecular-weight antioxidants appeared earlier in the evolutionary process than did the enzymes that play a key role in the defence against oxidative stress in ancient animals. It is known that the sturgeon, like elasmobranchs, can increase its urea content in the blood by osmoregulatory mechanisms (Martínez-Álvarez et al., 2002) and can also synthesise vitamin C (Moreau et al., 1999).

The circumstances discussed above might explain why SOD activity in trout is greater than in sturgeon in different tissues but not in the liver. This may be because, among other factors, sturgeon liver synthesises a great amount of lipids, as indicated by the activities of some enzymes involved, such as glucose-6-phosphate dehydrogenase and malic enzyme, which present 8- to 9-fold higher values than in trout liver (Morales et al., unpublished results).

Furthermore, in sturgeon, lipid storage in this tissue can reach 80% of dry matter (Sanz et al., 1997). In turn, these lipids are rich in unsaturated and highly unsaturated fatty acids (García-Gallego et al., 1999), which exhibit a very strong tendency towards oxidation (Kok et al., 1994; Fang et al., 2003). It is logical, as commented above, that greater ROS-generating activity in a tissue should prompt a higher level of antioxidants (López-Torres et al., 1993).

CAT activity, found mainly in peroxisomes, is associated with elevated concentrations of H_2O_2. We detected higher activity of this enzyme (Table 1, Fig. 1A) in the liver and RBC in both species. These results agree with many other works (Matkovics et al., 1977; Wdzieczak et al., 1982; Gabryelak et al., 1983; Wilhelm-Filho et al., 1993). Also, we found high activities in the digestive tract, the lowest values corresponding to white muscle, especially in sturgeon, followed in ascending order by heart, skin, and swimbladder, while gills registered intermediate values. It should be highlighted that, with respect to the overall group of tissues, CAT activity in the sturgeon was greater than in the trout (Table 1, Fig. 2). In the liver of both species a positive relationship was found between SOD and CAT, as reported for other fish (Wilhelm-Filho et al., 1993; Marcon, 1996). In the rest of the tissues, this relationship was not found, and thus in RBC, regardless of the species, low values for SOD activity were associated with high CAT activity. It is possible that the function of SOD, necessary for the formation of H_2O_2

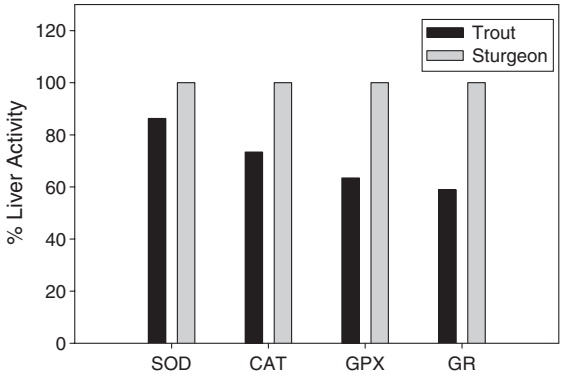

Fig. 3. Total liver activity in trout and sturgeon. Values for each enzyme are expressed as a percentage in which the value obtained in the species with maximum activity is 100%.

on which CAT acts, might also involve the intervention of other enzymes, such as glycolate oxidase or urate oxidase (Nagai et al., 1999). On the other hand, based on the example of the digestive tract of the trout, high values of SOD activity may be associated with a low CAT activity, which in a certain way could be offset by a high GPX and GR activity, as found in this tissue.

GPX catalyses the reduction of H_2O_2 derived from oxidative metabolism as well as peroxides from oxidation of lipids and is considered the most effective enzyme against lipid peroxidation (Winston and Di Giulio, 1991). Its activity is considered complementary to CAT activity, being especially suited for hydroperoxide detoxification at low substrate concentrations (Pérez-Campo et al., 1993; Halliwell and Gutteridge, 2000). The digestive tract showed the highest levels in both species, with intermediate values in the white muscle, skin, gills and swimbladder, and the lowest values appeared in the heart and RBC (Table 1, Fig. 1B). In both species tissues such as gills, digestive tract, RBC and swimbladder, we found that when CAT augmented GPX declined and vice versa. CAT activity in gills was lower in trout than in sturgeon, while GPX activity showed a tendency to higher values, although not significantly. The same can be stated for the digestive tract and RBC, where the differences were statistically significant (Table 1). On the contrary, in white muscle and swimbladder, CAT activities showed higher values in trout compared to sturgeon, while GPX activity was lower. It is known that there is a certain enzyme compartment in the cell, with catalase being associated with peroxisomes and the GPX with cytoplasm and other organelles. Assays on mammal RBC showed that the activity of these enzymes would be regulated by H_2O_2 levels, with CAT and GPX activity being associated with high and low levels of H_2O_2, respectively (Davies, 2000; Halliwell and Gutteridge, 2000). Although CAT activity in the sturgeon was higher than in the trout, GPX activity was lower. Also, in the digestive tract of the trout, there were GPX levels that were double those found in sturgeon. In the trout, as opposed to the sturgeon, the fat reserve is perivisceral. This fact is probably related to the higher GPX activity in the digestive tract of the trout, protecting the digestive tube against the peroxidation of this lipid reserve.

The role of GR is fundamental for GPX activity, maintaining the cytosolic concentration of reduced glutathione (Tian et al., 1999; Leopold and Loscalzo, 2000) and needs NADPH to carry out its function. For both species, GR activity was greatest in white muscle and digestive tract, while skin and RBC showed the lowest values, but the overall values were higher in trout than in sturgeon. Regarding the high GR activity in the white muscle of both species, as there was no positive relationship with GPX, this high activity could be partly explained by the lower availability of reduced glutathione reserves in this tissue and in both species, with respect to the rest of the tissues. The low GPX activity found in trout and sturgeon white muscle agrees with studies in red muscle of mammals (Halliwell and Gutteridge, 2000). Also, Nagai et al. (1999) found no activity in white muscle of 3 fish species. However, the digestive tract, another tissue with marked GR activity, also registered high GPX activity. Future studies to determine the availability of glutathione in the different tissues in trout and sturgeon will help to elucidate these discrepancies.

Considering the results for each tissue in both species, we should emphasize that liver showed the highest SOD and CAT antioxidant activity, both enzymes appearing to have an important role in combating the sequential generation of superoxide radical ($O_2.^-$) and hydrogen peroxide (H_2O_2) from the intense metabolic activity characteristic of this tissue. In addition, greater antioxidant activity would be necessary to avoid the oxidation of the fat accumulated in the liver of the sturgeon.

The heart, another tissue with intense metabolic activity, appears to defend itself from superoxide anions, fundamentally by strong SOD activity, and, in H_2O_2 reduction, GPX, CAT, and GR participate but none predominantly, as occurs in the liver or RBC, where CAT is strongly induced.

In the digestive tissue, we also found high enzymatic antioxidant activities and, in this case, not only the SOD, CAT, and GR but also GPX values were higher than in other tissues, mainly in trout. Despite the high

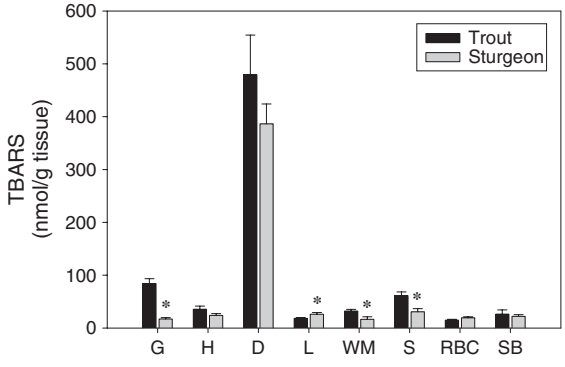

Fig. 4. TBARS levels on different tissue in trout and sturgeon (G, gills; H, heart; D, digestive tract; L, liver; WM, white muscle; S, skin; RBC, red blood cells; SB, swimbladder). Data are means±S.E.M. ($n=10$) *Significant differences between trout and sturgeon for the same tissue ($p<0.05$).

antioxidant activity, we found lipid-peroxidation levels 10- to 20-fold higher than in other tissues, especially in trout (Fig. 4). Although a positive correlation between the levels of TBARS and GPX ($p<0.05$) was found, it appears to be insufficient in the digestive tissue, which, as commented above, is surrounded by perivisceral fat in trout.

The swimbladder is also a tissue that stands out from the rest in terms of its enzymatic antioxidant activity, with a relatively high GPX, registering the lowest peroxidation values. These results would explain the need for antioxidant protection of a tissue that besides its hydrodynamic functions is a reservoir of oxygen and, consequently, is subjected to high oxygen tensions. This is especially true in trout, which is a physoclistous fish, as opposed to the physostomous sturgeon, which fills its swimbladder by swallowing air. This circumstance could explain why the enzymatic antioxidant activity in the swimbladder of the trout was greater than in the sturgeon (Table 1). This antioxidant protection has also been shown by other authors, and in this sense in toadfish (*Opsanus tau*) the SOD activity of swimbladder was found to be higher than that of any other body tissue examined (Halliwell and Gutteridge, 2000).

In white muscle, the antioxidant enzymes found were not very high; however, the GR activity was notably higher than in the other tissues. In any case, the enzymatic activities proved partly responsible for the minimum levels of lipid peroxidation (Fig. 4). Sturgeon white muscle registered markedly lower lipid-peroxidation values despite that the percentage in fat exceeded that of trout, reaching 36% of the dry matter (Sanz et al., 1997) with fat rich in n3 PUFAs and HUFAs, as in hepatic tissue (García-Gallego et al., 1999).

In RBC, free radicals are continuously generated in relation to their function of oxygen transport (Saltman, 1989; Nagababu and Rifkind, 2000), and in nucleated RBC in fish, related to the presence of mitochondria (Falcioni et al., 1987; Tiano et al., 2000). The results show low levels of antioxidant activity in most of the enzymes, except in CAT, in comparison to other tissues, apparently indicating that this activity would be sufficient to cover the antioxidant needs in RBC, as reflected by the low levels found for lipid peroxidation in this tissue (Fig. 4). On the other hand, the relatively high CAT activity could indicate a more important role of this enzyme in relation to GPX in the reduction of H_2O_2.

Gills and skin are tissues that overall can be considered to have less enzymatic antioxidant activity of all the tissues determined (Table 1, Fig. 1A and B). The lipid peroxidation in gills and skin was almost double that of the other tissues, except for the digestive tract, especially in trout (Fig. 4). The gills appear to be susceptible to oxidation, partly because of their defensive phagocytic activity (Parihar and Dubey, 1995) and partly for presenting fewer antioxidant resources in comparison with other tissues, such as liver (Fatima et al., 2000).

Further studies, particularly comparative ones which take into consideration different physiological situations, will shed more light on the functional complexity of the sturgeon and trout antioxidant defences with the aim of optimizing farming conditions of both species.

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