

## The evaluation of oxidative stress and quality of spermatozoa in Padanian barbel *Barbus plebejus* and chub *Squalius orientalis*

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### Abstract

The decrease in quality and death of sperm cells are triggered due to the generation of reactive oxygen species (ROS). Hence, the assessment of oxidative damage and antioxidant status are important. For this purpose, activity of antioxidant enzymes [catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD)] and lipid peroxidation [Malondialdehyde (MDA)] were examined in sperm cells of Padanian barbel (*Barbus plebejus*) and chub (*Squalius orientalis*). In addition, duration and percentage of spermatozoa were assessed. Based on our data, GPx ( $43.47 \pm 0.02$  U g<sup>-1</sup> protein) and malondialdehyde ( $2.52 \pm 0.02$  nmol.g<sup>-1</sup> cell) levels in *B. plebejus* were higher compared to spermatozoa of *S. orientalis*. Collectively, alterations in malondialdehyde levels and the antioxidant status could be species-specific differences.

### Introduction

As a benthopelagic fish species, chub (*Squalius orientalis*) is an endemic species and distributed only in Turkey. In the Barbel zone, wild populations of *S. orientalis* are abundant. Chub is a bioindicator species and is used as model a organism (Kupren et al., 2015). *Barbus plebejus* (Teleostei:

Cyprinidae) lives in streams and rivers. It is an indicator species due to feeding on benthic. The interaction of the species with pollutants increases depending on the type of feeding (Minissi et al., 1995). Especially, populations in the barbel zone are influenced by chemical input and loads in water sources (Luigi et al., 2015).

The assessment of oxidative damage and antioxidant status are important because they affect the sperm function. Sperm cells are susceptible as they contain high levels of Polyunsaturated fatty acids (PUFA). The membrane phospholipids of spermatozoa are sensitive to peroxidative damage. In particular, fertilization and embryo development can be affected by the generation of reactive oxygen species (ROS) as oxidative products. Removal of reactive species is accomplished by proactive enzymes [Superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx)] and antioxidants (Chen et al., 2015; Xie et al., 2017). For these reasons, the aim of the current study was to assess sperm motility and oxidative stress status of *B. plebejus* and *S. orientalis*.

### Materials and Methods

For sperm obtainment, *S. orientalis* and *B. plebejus* (2-3 years old) were caught from Solaklı Stream (Trabzon, Turkey) during the reproduction period. Before stripping, the fish were anesthetized with benzocaine (0.5 mg L<sup>-1</sup>). Sperm samples were collected into Eppendorf tubes (2.0 mL) with abdominal massage method. Care was taken not to mix mucus, urine, feces or blood into the samples to prevent contamination. Until analysis, sperm samples were kept on ice packs (4-6 °C).

For antioxidant analysis, sperm samples were centrifuged at 3000xg at 4°C for 15 minutes.

The sperm pellets in the ice bath were diluted with 1.15% KCl at a ratio of 1:10 (w/v) and homogenized. Malondialdehyde (MDA) level, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activity in spermatozoa were analyzed using commercial kits (CAYMAN, Chemical Company, Michigan, USA).

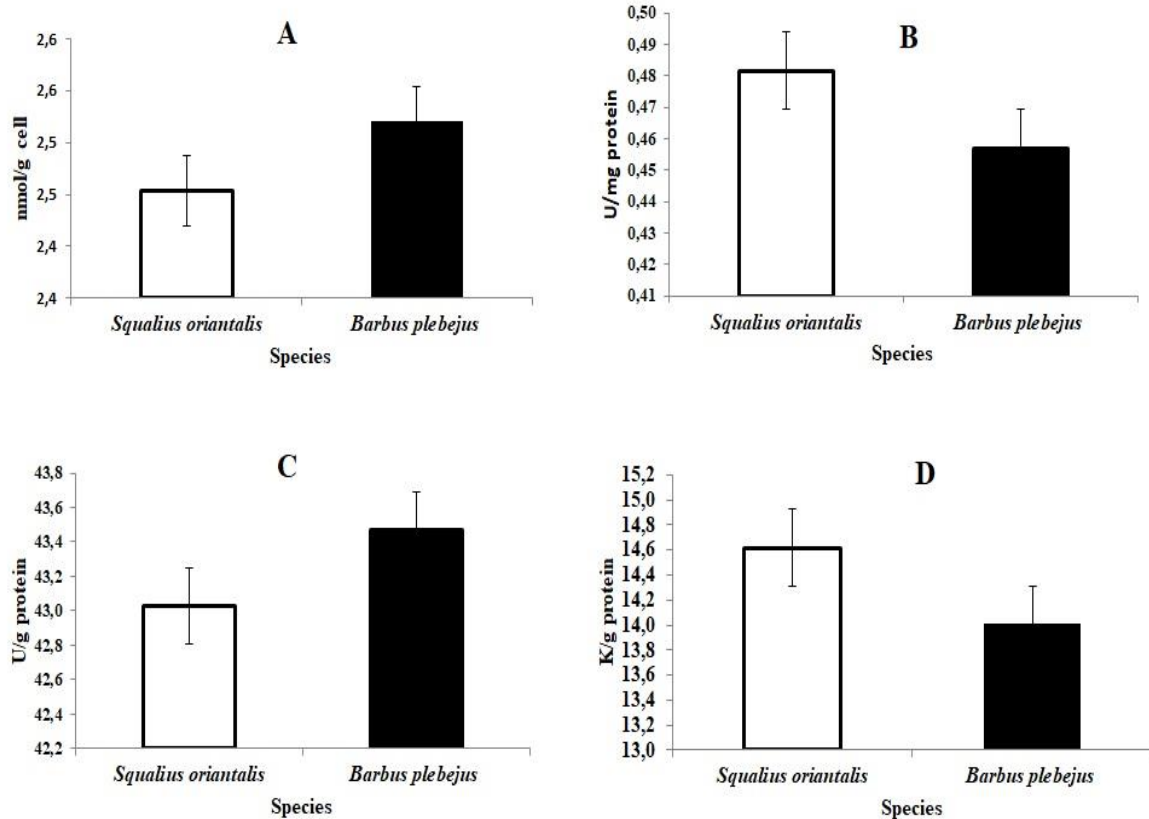
All data are expressed as mean ± standard deviation (SD). An independent sample t-test was applied for differences between species for enzymatic values. Level of significance was accepted as  $P < 0.05$  for all analyses. All statistics were analyzed using the SPSS 14.0 software.

### Results

Sperm quality parameters (mean ± SD) of *S. orientalis* and *B. plebejus* are presented in Table 1. Lipid peroxidation (MDA) and enzymatic (SOD, GPx and CAT) activities are given in Figure 1. GPx ( $43.47 \pm 0.02$  U g<sup>-1</sup> protein) and MDA ( $2.52 \pm 0.02$  nmol g<sup>-1</sup> cell) levels of sperm cells of *B. plebejus* were higher than *S. orientalis*. CAT ( $14.62 \pm 0.88$  k g<sup>-1</sup> protein) and SOD ( $0.48 \pm 0.02$  U mg<sup>-1</sup> protein) activities in *S. orientalis* species were higher than in *B. plebejus* sperm cells.

**Table 1.** Means of sperm quality parameters (mean±SD) in *S. orientalis* and *B. plebejus*.

Parameters	<i>Barbus plebejus</i>	<i>Squalius orientalis</i>
Total length (cm)	11.46±1.52	13.57±1.03
Total weight (g)	14.07±4.66	27.43±6.58
Sperm color	White	White
Sperm volume (ml)	1.08±0.72	0.92±0.44
Motility rate (%)	93.33±2.89	95.00±1.01
Motility duration	78.33±3.17	68.25±2.97



**Figure 1.** A) MDA levels and B) SOD, C) GSH-Px, D) CAT activities of *S. orientalis* and *B. plebejus*.

## Discussion

In sperm cells, defects in membrane integrity, increased cell permeability, enzyme inactivation and decreased fertilization ability may result from lipid peroxidation as a result of excess ROS production (Shiva et al., 2011; Shaliutina-Kolešová et al., 2013). Thus far, studies on the oxidant and antioxidant status in sperm cells and seminal plasma have been conducted in different fish species (*Cyprinus carpio*, *Salvelinus fontinalis*, *Acipenser gueldenstaedtii*, *Acipenser baerii*, *Acipenser ruthenus*, *Salmo coruhensis*, *Oncorhynchus mykiss*) (Shaliutina-Kolešová et al., 2013, 2018; Kocabas et al., 2019). To the authors' knowledge, this is the first study to determine the oxidative stress status of *B. plebejus* and *S. orientalis* spermatozoa.

The present results showed that MDA as an indicator of lipid peroxidation was higher in *B. plebejus* compared to *S. orientalis*

spermatozoa. Sperm cells are protected from oxidative damage through the transition of superoxide anion ( $O_2^-$ ) to hydrogen peroxide ( $H_2O_2$ ) and *S. orientalis* sperm cells have higher SOD activity. The lower levels of MDA in *S. orientalis* sperm cells may be explained by higher levels of SOD activity compared to *B. plebejus*. The increased activity of GPx as an oxidative defense enzyme in *S. orientalis* sperm cells may be related to an inability to prevent cellular stress associated with lipid peroxidation (Kocabas et al., 2018). The variation in sperm cells may be species-specific and the physiology of fish may be affected by different factors (environmental and genetic factors).

## Conclusions

Sperm cells of *S. orientalis* were sensitive to lipid peroxidation compared to *B. plebejus*. Sperm quality and oxidative stress state are related to species differentiation. These data will provide valuable

information for further studies on fish production and broodstock management.

### Ethical approval

The author declares that this study complies with research and publication ethics.

### Data availability statement

The authors declare that data are available from authors upon reasonable request.

### Funding organizations

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