

Antioxidant capacity and reactive oxygen metabolites in lizard (*Podarcis siculus*) skeletal muscle tissues – preliminary results



A. Shek Vugrovečki, J. Miljković*, I. Žura Žaja, L. Pađen, D. Šikić, T. Gojak, M. Glogoški, D. Lisičić, S. Ana Blažević and J. Aladrović

Abstract

There are few studies on oxidative stress in reptiles and there is a lack of basic knowledge on oxidative stress in the tissues of the widely distributed Italian wall lizard (*Podarcis siculus*). The aim of the present preliminary study was to test and compare the biological antioxidant potential (BAP), total superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and derivatives of reactive oxygen metabolites (d-ROM) of muscle tissue between two populations of *P. siculus*. Two free-living groups of eight adult males were included in each group (N=16). One group was caught on the island of Pag (at Novalja), and the second from the mainland (at Knin) in Croatia. Animals were kept in the same, optimal conditions for two months. After muscle tissue homogenisation and centrifugation in the supernatant, the activities of GSH-Px, SOD and concentrations of BAP and d-ROM were determined using commercial biochemical kits on a biochemical

analyser. The results of this study showed that although there were no statistically significant differences in the values of oxidants and antioxidant parameters determined in muscle tissue supernatants between these two populations, values of all determined parameters were higher in the Pag group especially BAP values that approached statistical significance. No differences were found in d-ROM/BAP and GSH-Px/SOD ratios, but all parameters showed significant positive correlations, with the strongest positive correlation between GSH-Px and BAP, and SOD and BAP. These findings can serve as a base for future studies with a larger sample size, different tissue, different locations, between sexes or between species at the same location.

Key words: biological antioxidant potential; total superoxide dismutase; glutathione peroxidase; reactive oxygen metabolites; muscle; *P. siculus*

Ana SHEK VUGROVEČKI, DVM, PhD, Associate Professor, Josip MILJKOVIĆ*, (Corresponding author, e-mail: josip.miljkovic@vef.unizg.hr), DVM, Assistant, Ivona ŽURA ŽAJA, DVM, PhD, Associate Professor, Lana PAĐEN, DVM, PhD, Assistant Professor, Jasna ALADROVIĆ, DVM, PhD, Full Professor, Faculty of Veterinary Medicine, University of Zagreb, Croatia; Dunja ŠIKIĆ, mag. biol. exp., Research Assistant, Tomislav GOJAK, mag. biol. exp., Research Assistant, Marko GLOGOŠKI, PhD, mag. biol. exp., Research Assistant, Duje LISIČIĆ, mag. biol. exp., PhD, Associate Professor, Sofia Ana BLAŽEVIĆ, mag. biol. exp., PhD, Assistant Professor, Division of Animal Physiology, Faculty of Science, University of Zagreb, Croatia

Introduction

Over the last century, human activities have caused a variety of changes that affect wildlife habitats, either directly (urbanisation and deforestation) or indirectly (habitat pollution and climate change). Animals living in a modified environment can either adapt, be forced out a habitat, or become extinct (Beaulieu and Costantini, 2014).

One highly adaptable species is the Italian wall lizard, *Podarcis siculus* (Rafinesque-Schmaltz, 1810). It is an opportunistic lizard capable of adapting to a range of habitats, often using man-made objects or ornamental plants as shelter (Corti and Cascio, 2002; Rocha, 2022). In the process of adaptation to different environments, individuals are faced with different stressful stimuli and develop defence mechanisms against oxidative stress. Therefore, this adaptable species can serve as a good research model.

The term reactive oxygen species (ROS) includes free oxygen radicals, e.g., superoxide anion radicals, hydroxyl radicals, hydroperoxyl radicals, singlet oxygen (Valdivia et al., 2007). In low/moderate concentrations, they are required for physiological activities such as intracellular cell signalling and homeostasis, cell death, immune defence against pathogens, and the induction of mitogenic reactions (Dröge, 2002). A balance between reduction and oxidation is essential for physiological cellular functions. Free radicals are produced in the organism as a byproduct of cellular aerobic metabolism, but can also be induced by external sources such as UV light, ionising radiation, nutrition, stress, etc. (Madkour, 2019). Therefore, aerobic organisms have an antioxidant system that is responsible for preventing, neutralising, or repairing ROS-induced damage (Madkour, 2019). The antioxidant systems

consisting of enzymes and non-enzymatic antioxidants play a crucial role in regulating concentrations of these free radicals (Devasagayam et al., 2004). The enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) are among the more important and better-studied antioxidants (Halliwell, 1999; Aguirre and Lutz, 2004; Valdivia et al., 2007; Gil-del Valle, 2011; Zhang et al., 2015; Richie and Friesen, 2022). When there is an imbalance between ROS production and the capacity of antioxidant systems to detoxify reactive intermediates, a condition known as oxidative stress (OS) can occur. Free radicals produced in excess oxidise DNA, proteins, and lipids, and are involved in the pathogenesis of various diseases (Halliwell, 1999; Aguirre and Lutz, 2004; Valdivia et al., 2007; Gil-del Valle, 2011).

The superoxide radical formed during the respiratory chain in the mitochondria, SOD converts to hydrogen peroxide. The removal of hydrogen peroxide is catalysed by GSH-Px. SOD and GSH-Px were selected as representatives of the vital enzymatic antioxidants in skeletal muscle due to high number of mitochondria (Dong and Tsai, 2023).

Derivatives of reactive oxygen metabolites (d-ROM) is a collective term that includes both oxygen-centred free radicals and non-radical oxygen derivatives, while the biological antioxidant potential (BAP) provides an estimate of mainly non-enzymatic molecules such as uric acid, bilirubin, and vitamins E and C in the sample (Iorio, 2010) and shows the systemic antioxidant properties (Kusano et al., 2016). We chose the derivatives of reactive oxygen metabolites (d-ROM) because they are now considered the 'gold standard' for measuring overall systemic oxidative status in humans and animals. The oxidative balance of lizard muscle tissue was measured in homogeneous samples using

the d-ROM assay, which measures the concentration of hydroperoxide, a reactive oxygen metabolite resulting from the attack of ROS on organic substrates (and therefore reflecting oxidative damage). To the extent of our knowledge, d-ROM has not been determined in reptiles, but it is commonly used (Giordano et al., 2015) in avian and mammalian studies and provides valuable data about oxidative damage. Overall, data on BAP concentrations in muscle tissue samples are sparse, especially in wild animals or reptiles.

The ratio of d-ROM and BAP, known as the oxidative stress index (OSi), has been evaluated as a new biomarker of redox status in rams (Oikonomidi et al., 2017) and dairy cows (Benzie and Strain, 1996). The OSi was proposed by Abuelo et al. (2012) as an indicator of an animal's risk of developing oxidative stress. Those authors concluded that it is important to evaluate not only the concentrations of oxidants and concentrations/activity of antioxidants separately, but also to analyse their relationship through a ratio or proportion, since an imbalance between oxidants and antioxidants defines the concept of oxidative stress (Castillo et al., 2005).

According to different authors, measuring the level of oxidative stress is extremely important for determining the fitness of animals and populations, as high levels of oxidative stress can affect the survival and reproduction of animals (Costantini, 2008; Monaghan et al., 2009; Beaulieu et al., 2013).

Lizards are a commonly used model for studies investigating whole organism performance and the physiology of muscle contraction (Anderson and Roberts, 2020). Optimal muscle performance is critical for prey capture, escape from predators, and many other behaviours (Higham et al., 2011). As in other tissues, oxidative

metabolism generates free radicals that cause peroxidation, enzyme inhibition and DNA damage in muscle cells. Therefore, the functionality of the antioxidant system against free radicals is important to protect muscle cells and tissues (Masuda et al., 2002). In mammals, the muscle cells consume a large amount of oxygen, which is significantly increased during physical exertion. The leg muscles have an increased ability to generate force very quickly (Anderson and Roberts, 2020). This O₂ consumption is associated with the continuous generation of ROS (Powers et al., 2011) and moderate amounts of free radicals are required for various adaptive responses. Low levels of oxidants in muscles at rest ensure normal force production, and oxidants during exercise improve the body's ability to resist damage (Lian et al., 2022). Muscle cells contain many different antioxidant enzymes and two of these are GSH-Px and SOD. The metabolic profile of reptiles can be generalised as one of limited aerobic capacity compared to birds and mammals. However, the activities of antioxidant enzymes vary in the highly heterogeneous skeletal muscle, depending on the metabolic properties of the muscle fibres. Slow-twitch oxidative fibres, for example, process more antioxidant enzyme activities than fast ones (Ji et al., 1992).

The inclusion of oxidative stress indicators as part of a biomarker system could serve as a reference in areas where disease and pollution are known to be a threat to reptile populations or could be used as a baseline prior to determining environmental effects of various causes. There are few studies on oxidative stress in reptiles (Hermes-Lima and Storey, 1993; Willmore and Storey, 1997) and there is a lack of basic knowledge on oxidative stress in the tissues of the widely distributed Italian wall lizard. Costantini et al. (2010) stated that quantifying free radical production,

oxidative damage, and antioxidant capacity can provide new insights into understanding interspecific variation in lifestyle.

Accordingly, the aim of this study was for the first time to obtain baseline data on the antioxidants and oxidative status in muscle tissue of a widespread and adaptable lizard species, *P. siculus*.

Materials and methods

Ethics and welfare approval statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. We obtained a permit for lizard sampling from the Croatian Ministry of Environmental and Nature Protection Energy (Class: AP/L612-07 I 18-481 65; No: 517-05-1-1-20-6), permit of the Ethics Committee of the Faculty of Science, University of Zagreb, Croatia (Class: 643-02/21-01/1, No. 251-58-10617-21-1) and permit of the Ethics Committee of the Faculty of Veterinary Medicine, University of Zagreb, Croatia (Class: 640-01/23-02/05, No. 251-61-01/139-23-34).

Field sampling and housing conditions

Field sampling was conducted in September 2020 at two locations - Novalja on the island of Pag, and the surroundings of the city of Knin, Croatia. Individuals were sampled in the morning hours after coming out of their shelters to warm up. A total of 16 adult males of *P. siculus* (Rafinesque-Schmaltz, 1810) were captured by noosing at both locations, eight individuals at Knin (44°02'N 16°12'E/44.04°N 16.20°E/4.04) and eight individuals around Novalja on the island of Pag (44°33'22"N 14°53'08"E/44.55611°N14.88556°E/44.55611). Lizards were captured and individually transported in cloth bags to the Department of Animal Physiology, Faculty of Science, University of Zagreb during the same day. The survival rate was 100%. At the department, lizards were held individually in transparent plastic boxes filled with peat as a substrate, with a plastic tube closed at one end that served as a hiding place and a stone to mimic the natural environment. A heating bulb was placed above each box, which served as a

Table 1. Nutritional profile of insects *Gryllus assimilis*

Chemical composition (in dry matter) of <i>Gryllus assimilis</i> (provided by commercial producer)	
Total proteins	65.0%
Methionine	0.7%
Lysine	2.9%
Total fat	15.5%
Palmitic acid	25.0%
Oleic acid	25.0%
Linoleic acid	40.0%
Total ash (total minerals)	5.2%
Calcium	1.2%
Phosphorus	1.3%
Raw fibre	8.0%

Table 2. Vitamin supplement composition

Chemical composition of vitamin supplement provided by commercial producer	
Crude Protein (min)	14.0%
Crude Fat (min)	1.2%
Crude Fiber (max)	8.0%
Moisture (max)	12.0%
Ash (max)	9.0%
Calcium (min)	4.4%
Calcium (max)	4.6%
Salt (max)	0.00275%
Potassium (min)	0.0033%
Sulfur (min)	0.011%
Magnesium	2 ppm
Copper	2.5 ppm
Zinc	6.5 ppm
Iodine	0.75 ppm
Manganese	6.5 ppm
Choline	81.44 mg/lb
Menadione	0.907 mg/lb
Biotin	0.004 mg/lb
Inositol	4.989 mg/lb
Beta Carotene	4.082 mg/lb
Vitamin D ₃	9,979 IU/lb
Vitamin E	45.3 IU/lb

heat source during midday (from 11 am to 1 pm). UV lamps needed by lizards to maintain normal physiological function were installed, and were kept on from 8 am to 6 pm. Temperature and humidity were kept between 20 and 25°C and 40 and 60%. Lizards were fed crickets (*Gryllus sp.*) dusted with vitamins and calcium as a dietary supplement, water was given *ad libitum*. The chemical composition of crickets and vitamin supplement is shown in Tables 1 and 2. Lizards were kept in

these conditions until they were sacrificed in December 2020.

Tissue samples

Muscle tissue of the left hind leg (expected muscles as shown in Pereira et al. (2015): ambiens, fibularis longus, flexor tibialis internus, gastrocnemius, ileofibularis, puboischiotibialis) was removed from animals immediately after sacrificing by decapitation and severing of the spinal

cord in accordance with the British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1, weighted and stored at -20°C until analysis. After thawing, the muscle tissue was homogenised in 0.14 mol/L KCl using an Ultra-Turrax T25 Basic Homogenizer (IKA, Germany). The ratio of tissue mass to buffer volume was 1:10 and the tissue was homogenised for 60 seconds (3 x 20 seconds with cooling intervals of 10 seconds) at 13500 rpm. The

homogenates were centrifuged for 30 minutes at 10000 x g and at a temperature of 4°C in a Sigma 3 K 15 centrifuge (Sigma, Germany).

Analysis of derivatives of reactive oxygen metabolites (d-ROM)

The concentration of reactive oxygen metabolites in tissues was determined by d-ROM ready-made kit (Diacron International, Italy) on an Architect c4000

Table 3. Descriptive statistics of biological antioxidant potential (BAP), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), reactive oxygen metabolites (d-ROM) and the Oxidative Stress index (Osi) in lizard skeletal muscle from Knin and Pag, Croatia

Variable	Location							
	Knin				Pag			
	N	Mean	Std. Dev.	Std. Error	N	Mean	Std. Dev.	Std. Error
BAP (mmol/g tissue)	8	110	58	20	8	283	240	85
GSH-Px (mU/g tissue)	8	5.5	3.7	1.3	8	12.1	11.1	3.9
d-ROM (U Carr/g tissue)	8	934	854	302	7	1257	1006	380
SOD (mU/g tissue)	8	501	314	111	8	1000	805	284
Osi (U Carr/mmol)	8	0.008	0.005	0.002	7	0.006	0.004	0.002

N – number of valid samples; Mean – arithmetic mean; Std. Dev – standard deviation; Std. Error – standard error. The difference is considered significant if $P < 0.05$.

Table 4. T-test for the unpaired data: biological antioxidant potential (BAP), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), reactive oxygen metabolites (d-ROM) and a Oxidative Stress index (Osi) in lizard skeletal muscle from Knin and Pag, Croatia

Variable	T-tests;					
	Grouping: location Group 1: Knin		Group 2: Pag		P	t-value
	Mean Knin	Std. Dev. Knin	Mean Pag	Std. Dev. Pag		
BAP (mmol/g tissue)	110	58	283	240	0.07	-1.98
GSH-Px (mU/g tissue)	5.5	3.7	12.1	11.1	0.14	-1.58
d-ROM (U/g tissue)	934	854	1257	1006	0.51	-0.67
SOD (U Carr/g tissue)	501	314	1000	805	0.12	-1.64
Osi (U Carr/mmol)	0.008	0.005	0.006	0.004	0.59	0.56

Mean – arithmetic mean; Std. Dev – standard deviation. The difference is considered significant if $P < 0.05$

biochemical analyzer (Abbott, USA). The result of the d-ROM test was expressed in Carratelli units (U CARR) per gram of tissue. One U CARR represents 0.08 mg/dL hydrogen peroxide.

Analysis of biological antioxidant potential (BAP)

The antioxidant capacity of skeletal muscle tissue of lizards was determined using the BAP test. The antioxidant potential in tissues was measured with an Architect c4000 biochemical analyser (Abbott, USA) using a commercial kit (Diacron International, Italy). The BAP concentration is given in mmol per gram of tissue.

The oxidative stress index was calculated from concentrations of d-ROM and BAP and expressed as UCarr/mmol.

Analyses of antioxidative enzyme activity

The enzyme activity of glutathione peroxidase and superoxide dismutase in tissues were determined using a Randox (Ireland) kit on an Architect c4000 biochemical analyzer (Abbott, USA). GSH-Px and SOD activity are expressed in units per gram of tissue.

Statistical methods

The results were statistically analysed using the statistical program STATISTICA version 12 (StatSoft, Tulsa, USA). The d-ROM, BAP, Osi, GSH-Px and SOD values from the skeletal tissue of lizards from Pag and Knin were expressed as mean \pm standard deviation. The normality of the distribution was checked using the Kolmogorov-Smirnov test. The t-test for unpaired data was used to test for differences between lizards from two sites. The relationship between the observed indicators was examined by Pearson's correlation. Differences were considered statistically significant at $P < 0.05$.

Results

Descriptive statistics are presented in Table 3. A two-sample t-test was performed to compare the concentrations of d-ROM and BAP, and the activities of GSH-Px and SOD in skeletal muscle tissue of lizards from Knin (group 1) and Pag (group 2). Results of t-test for unpaired data are given in Table 2. Table 4 shows that there was no significant difference in the analysis, although samples from the Pag location showed higher enzyme activity and BAP concentration, but also higher d-ROM.

A Person's correlation coefficient was presented in Table 5. There was a strong or very strong positive correlation between all the analytes. BAP and GSH-Px, and BAP and SOD showed a very strong positive correlation, whereas SOD and GSH-Px showed a strong positive correlation, and d-ROM and BAP, GSH-Px and SOD displayed a positive correlation (Table 5). These correlations suggest that as one variable increases, the others tend to increase as well, particularly between BAP, GSH-Px, and SOD which are strongly and positive correlated with one another.

Discussion

This study gives the first insight into the oxidative/antioxidative properties of muscle tissue in male *P. siculus*. Our data showed that the values of the analysed markers of oxidative stability in the muscle tissue of *P. siculus* from two different locations kept under optimal conditions for three months did not show significant statistical differences, though strong and very strong positive correlation were found between the analysed parameters.

Most of the previous research on oxidative stress in reptiles examined the effects of exogenous conditions such as thermal effects (Hermes-Lima and Ken-

Table 5. Pearson's correlation matrix between biological antioxidant potential (BAP), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), reactive oxygen metabolites (d-ROM) in lizard skeletal muscle

Variable	Correlation matrix				
	BAP (mmol/L)	GSH-Px (mU/g tissue)	d-ROM (U Carr/g tissue)	SOD (mU/g tissue)	
BAP (mmol/L)	Pearson's r	-	-	-	-
	p-value	-	-	-	-
GSH-Px (mU/g)	Pearson's r	0.97***	-	-	-
	p-value	<0.001	-	-	-
d-ROM (UCarr/g)	Pearson's r	0.85*	0.79*	-	-
	p-value	0.015	0.033	-	-
SOD (mU/g)	Pearson's r	0.99***	0.95**	0.83*	-
	p-value	<0.001	=0.001	=0.021	-

Marked correlations are significant * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

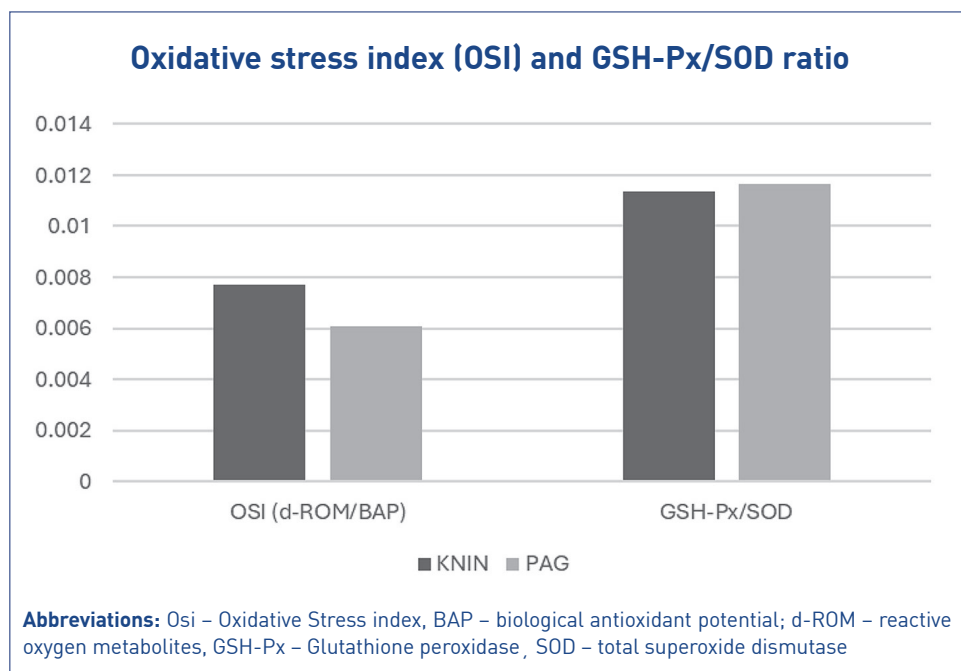


Figure 1. Oxidative stress index (ratio between d-ROM and BAP) and the ratio between GSH-Px and SOD

neth, 1998; Voituron et al., 2006), hypoxia (Valdivia et al., 2007) and environmental pollution (Gavrić et al., 2017; Simbula et al., 2021). It is known that stress responses when an ectothermic organism experiences an ambient temperature outside its optimal thermal range cause various physiological costs (Kingsolver et al., 2013) and one of these is oxidative stress (Speakman et al., 2015; Baker et al., 2020). As Valdivia et al. (2007) stated, the measurement of oxidative stress is an effective tool for assessing the health of a reptile. Constantini et al. (2010) emphasised that differences in oxidative stress between sexes or populations of the same species and changes in an individual's oxidative status across the stages of its life cycle, as reported for domesticated zebra finches (Alonso-Alvarez et al., 2006), are just some of the issues that need to be studied more intensively. However, it is difficult to compare the results of different studies since the research to date involves different species, different methods of analyte determination, and different expressions of indicator values. The activities of GSH-Px in the muscle tissue of lizards in this study are similar to the results on snakes in full activity after hibernation (Gavrić et al., 2017). The results of Hermes-Lima and Storey (1993) on the muscle tissue of the garter snake *T. s. parietalis* and by Zhang et al. (2015) on the muscle tissue of the higher altitude lizard *Phrynocephalus vlangalii* showed similar values of GSH-Px and SOD activities.

Earlier studies showed that most reptiles studied rely heavily on anaerobic energy production for muscle activity (Gleeson et al., 1980). However, more recent studies (Bonine et al., 2001, 2005) found that the composition of muscle fibre types can vary greatly between lizard species. In our study, we determined the GSH-Px and SOD activities in the muscle tissue of the hind legs of *P. siculus* for the first time.

In this study, the activity of GSH-Px and SOD determined in lizards caught on the island of Pag was twice that compared to the Knin group, with a significance level of $P=0.12$ and $P=0.14$, respectively. Such results are probably due to the small sample size included in the study and the high variability of the data. GSH-Px and SOD are related enzymes, under aerobic conditions SOD catalyses the dismutation of superoxide radical into hydrogen peroxide, which removes GSH-Px. The mentioned connection was also proven in this study by the high correlation of these enzymes ($r=0.95$; $P<0.001$, Table 5) and the same GSH-Px/SOD ratio in both groups of animals (Pag 0.012, Knin 0.011).

In our study, the Pag group had higher levels of d-ROM ($M = 1257 \pm 1006$ U Carr/g tissue) than the Knin group ($M = 934 \pm 854$ U Carr/g tissue), although this was not statistically significant. As d-ROM has never been determined in lizard tissue to the author's knowledge, it is difficult to discuss, though the Pag group had a somewhat higher production of d-ROM. The concentration of free radicals in muscles depends on the level of activity, according to Gavrić et al. (2017) who found higher values in snakes after hibernation.

Similar values for d-ROM as reported here were published by Criscuolo et al. (2010), who found that d-ROM levels were higher in the haemolymph of short-lived male tarantulas (which also had higher superoxide production and lower antioxidant defences) than in the haemolymph of their long-lived females (which also had lower superoxide production and higher antioxidant defences).

The Knin group had lower BAP concentrations ($M = 110 \pm 58$ $\mu\text{mol/g}$ tissue) than the Pag group ($M = 283 \pm 240$ $\mu\text{mol/g}$ tissue). This increase in non-enzymatic but also in GSH-Px and SOD activity could be induced by higher d-ROM values in the

Pag group. Using a similar method, Evelson et al. (2001) determined a similar concentration of non-enzymatic antioxidants in the heart of rats.

Many researchers conclude that it is important to evaluate not only the concentrations of oxidants and antioxidants separately, but to also analyse their relationship to each other, because it is the imbalance between oxidants and antioxidants that defines the concept of OS (Castillo et al., 2005; Abuelo et al., 2013) and could provide information about the adaptation and status of the antioxidant enzyme system. Abuelo et al. (2013) explained that an increase in the ratio could mean a higher risk of oxidative stress. In this study, OSi was extremely low in both groups, which could be interpreted as very effective protection against antioxidants in lizard skeletal muscle or decrease of oxidative processes due to optimal husbandry (Knin OSi=0.008±0.005 UCarr/mmol; Pag OSi=0.006±0.004 UCarr/mmol). In addition, some authors state that the values of the ratios between the antioxidant enzymes themselves should be considered more often than their individual specific activities to better assess how much and whether an organism can be protected from oxidative stress damage (Castillo et al., 2005; Abuelo et al., 2013).

Since the studied lizards were kept under the same conditions for a longer period, all previously reported causes of changes in oxidant/antioxidant levels could be excluded in this study. It was also hypothesised that exposure to different habitat factors over a prolonged period leads to certain adaptive traits that make native species more resistant to environmental extremes (Constantini, 2008).

In their review, Ritchie and Friesen (2022) pointed out the importance of geological, biogeographical, and evolutionary studies of oxidative stress and wild reptile

populations in particular, and that the study of responses to oxidative stress at physiological and behavioural levels is essential to make valuable predictions that could expand knowledge about the effects of climate change on ectotherms and biodiversity. In addition, Beaulieu and Costantini (2014) stated that another attractive aspect of oxidative stress markers for conservationists is their universality and that the results of different studies can be applied to bacteria, humans, or any species with aerobic metabolism and even to anaerobic organisms exposed to oxygen.

Conclusion

In this study, we determined antioxidant capacity and reactive oxygen metabolites in skeletal muscle tissues in the lizard *Podarcis siculus* of the family Lacertidae. From these preliminary results, we can conclude that in *P. siculus* from different locations, intrapopulation differences had no significance influence on the oxidative status of animals, and this is a good basis for further research into the influence of other effects in the field. The results of this preliminary study can serve as a basis for future studies with a larger sample size, at different sites, between sexes or between different species at the same site.

Finally, markers of oxidation status could be used to assess the impact of human activities on natural animal populations, but also to make predictions about individual survival or reproductive prospects, which could have significance for conservation.

Acknowledgements

This study is a part of a project funded by the support from the Croatian national science foundation HRZZ-UIP-2019-04-8469 – Dopaminska regulacija kompetitivnog ponašanja

u kohabituirajućim populacijama guštera *Podarcis siculus* i *Podarcis melisellensis* (BOLDer) (project leader Blažević, Sofia Ana, HRZZ - 2019-04). The funding source was not involved in any stage of the research and publication of the results.

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Antioksidativni kapacitet i reaktivni metaboliti kisika u tkivima skeletnih mišića guštera (*Podarcis siculus*) – preliminarni rezultati

Dr. sc. Ana SHEK VUGROVEČKI, dr. med. vet., izvanredna profesorica, Josip MILJKOVIĆ, dr. med. vet., asistent, dr. sc. Ivona ŽURA ŽAJA, dr. med. vet., izvanredna profesorica, dr. sc. Lana PAĐEN, dr. med. vet., docentica, Veterinarski fakultet Sveučilište u Zagrebu, Hrvatska; Dunja ŠIKIĆ, mag. biol. exp., znanstvena novakinja, Tomislav GOJAK, mag. biol. exp., znanstveni novak, dr. sc. Marko GLOGOŠKI, mag. biol. exp., znanstveni novak, dr. sc. Duje LISIČIĆ, mag. biol. exp., izvanredni profesor, dr. sc. Sofia Ana BLAŽEVIĆ, mag. biol. exp., docentica, Zavod za fiziologiju životinja, Prirodoslovno-matematički fakultet, Sveučilište u Zagrebu, Hrvatska

Ravnoteža između redukcije i oksidacije ključna je za pravilne fiziološke stanične funkcije, a antioksidativni obrambeni sustavi koji se sastoje od enzimatskih i neenzimatskih antioksidansa imaju ključnu ulogu u regulaciji tih slobodnih radikala. Cilj je ovoga rada bio uspoređujući dvije populacije *Podarcis siculus* po prvi put ispitati i usporediti antioksidativni status - biološki antioksidativni potencijal (BAP), ukupnu superoksid dismutazu SOD i glutacion peroksidazu (GSH-Px) te reaktivne metabolite kisika (ROM), iz mišićnog tkiva. U ovoj smo studiji usporedili oksidativnu stabilnost i reaktivne metabolite kisika između dvije slobodnoživuće skupine od ukupno 8 odraslih mužjaka guštera. Jedna skupina je uhvaćena na otoku Pagu (grad Novalja), a druga oko grada Knina, Hrvatska. Životinje su tijekom dva mjeseca držane u istim, optimalnim uvje-

tima. U pripremljenom tkivnom nadtalogu gotovim kitovima na biokemijskom analizatoru određene su: aktivnosti glutacion peroksidaze, superoksid dismutaze, koncentracija neenzimskih antioksidativnih molekula i koncentracija reaktivnih metabolita kisika, izračunati su i omjeri između odabranih analita. Rezultati ovog istraživanja pokazali su da ne postoje statistički značajne razlike u vrijednostima pokazatelja oksidativno/antioksidativnog statusa između ove dvije populacije guštera *P. siculus*. Nije utvrđena ni razlika u omjeru između pokazatelja, a ni značajna korelacija među njima. Iako nisu statistički značajni, ovi nalazi mogu poslužiti kao osnova za buduće studije s većim uzorkom, različitim lokacijama, spolom ili između različitih vrsta na istoj lokaciji.

Ključne riječi: oksidacijski/antioksidacijski status, gušter, mišić, *P. siculus*