Oxidation-antioxidation processes and thermal effects on oxidative stress in reptiles

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Abstract

Temperature is the most important abiotic factor and has a direct influence on the physiology of the organism, affecting nearly all other parameters of the living environment of organisms. Ectothermic organisms are highly endangered in the current crisis climate, as they are unable to use metabolic heat to maintain body temperature. Reptiles are ectothermic vertebrates that are also susceptible to temperature fluctuations. Metabolism, muscle and nervous system function and reproduction are closely linked to reptile body temperature. To study the effects of temperature on oxidative stress, it is necessary to describe the generation of reactive oxygen species (ROS) and how organisms can prevent oxidative stress. This article describes the oxidation-antioxidation processes and the oxidative stress caused by thermal effects in reptiles. In the metabolic processes of aerobic organisms, ROS are continuously generated as by-products of oxidation-reduction reactions and are not primarily harmful. Furthermore, ROS are essential for many physiological functions, e.g., for energy production and for processes in the immune system. The potential toxicity of reactive oxygen radicals under physiological conditions is prevented by the antioxidant defence system. Oxidative stress occurs when the balance between oxidation and antioxidation systems is disturbed by excessive amounts of ROS or by the depletion of antioxidants. It is known that the metabolic rate of reptiles correlates with environmental temperature, making them physiologically more sensitive to temperature fluctuations compared to mammals. However, it must be considered that ectothermic organisms have evolved many thermal adaptations through physiological and behavioural measures to mitigate the resulting oxidative stress. However, further research in the fields of ecology, biogeography and evolution is needed to determine the exact effects of temperature on oxidative stress and the resulting changes in life characteristics in wild populations.

Key words: *oxidation-antioxidation processes; thermal effects; oxidative stress; reptiles*

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Introduction

To understand the occurrence of oxidative stress, it is important to emphasize that oxygen is necessary for the life of all aerobic organisms. The oxidation of nutrients as a source of energy is one of the most important prerequisites for the development of higher life forms. Reactive oxygen molecules, known as reactive oxygen species (ROS), are constantly produced during the metabolic processes of aerobic organisms. Free radicals derived from molecular oxygen, which are normally formed as by-products of oxidation-reduction reactions, are not primarily harmful. There are also many important cellular processes in which free radicals have an important physiological function. ROS are generated in appropriate concentrations during normal aerobic metabolism because they are necessary for many physiological functions. For example, they are essential for energy production, they are a necessary component of the process of synthesising thyroid hormones, for carrying out a critical process in the immune system (phagocytosis), for the synthesis of prostaglandin G2 from arachidonic acid, are involved in maintaining vascular tone, and play a critical role in the transmission of signals important for cell communication and function (Ritchie and Frisien, 2022). In addition, leukocytes and macrophages use ROS to destroy foreign bodies such as bacteria and viruses (Čvorišćec and Čepelak, 2009; Andrés et al., 2022, 2023). Thus, it is obvious that oxygen radicals in limited concentrations are an integral part of normal metabolism. The production of ROS depends on the energy consumption of the organism, the availability of oxygen and the efficiency of cell metabolism (Casteilla et al., 2001; Baker et al., 2020). Oxidative stress occurs when the balance between oxidation and antioxidation systems is disturbed by excessive amounts of ROS or the depletion of antioxidants (Scandalios, 2002; Dosek et al., 2007; Zhang et al., 2015).

Temperature is the most important abiotic factor and has a direct influence on the physiology of the organism (Brett, 1971). It affects almost all other parameters of the environment by altering the availability of oxygen in the air and water, it can affect nutrient cycling and influence changes in the acidity of the environment and living organisms (Baker et al., 2020) (Figure 1). Reptiles are ectothermic vertebrates whose internal temperature depends on the environment, making them susceptible to temperature fluctuations (Sheridan and Bickford, 2011: Seebacher et al., 2015; Burraco et al., 2020). Ectotherms are predicted to be highly vulnerable in the current climate crisis as they are unable to utilise metabolic heat to maintain body temperature (Ritchie and Friesen, 2022). Metabolism, muscle work, work of the nervous system and reproduction of reptiles are closely dependent on the temperature of animals (Baker et al., 2020). When an ectothermic organism is outside its thermal optimum zone, in response to stress, energy expenditure increases above the physiological range (Kingsolver et al., 2013; Ritchie and Friesen, 2022). Oxidative stress is the consequence of physiological adaptations with, for example, greater expenditure of energy to the resulting temperature fluctuations outside the thermal optimum (Speakman and Garratt, 2014; Baker et al., 2020; Han et al., 2020). To review the effects of temperature on oxidative stress, it is necessary to describe the generation of ROS and how organisms can prevent oxidative stress. This paper will describe the oxidation-antioxidation processes and the oxidative stress caused as a result of thermal effects in reptiles.

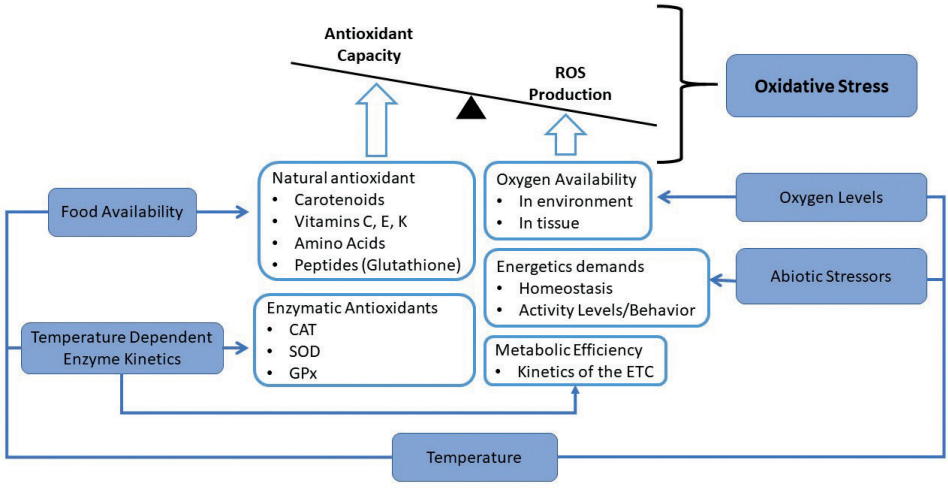

Figure 1. Diagram of the components of the oxidative stress system. Temperature has a major influence on many other important environmental parameters that can challenge the physiology of any organism. Oxidative stress is the confluence of these physiological stressors and organismal responses. CAT – catalase, GPx – glutathione peroxidase, SOD – superoxide dismutase, ROS – reactive oxygen species, ETC – electron transport chain, adapted from Baker et al. (2020).

Free radicals in the organism

Free radicals are particles, ions, atoms, or molecules that have one or more unpaired electrons in their outer shell (Halliwell and Gutteridge, 2007). Free radicals are formed by homolytic cleavage of covalent bonds, whereby each electron remains bound to the neighbouring atom. Unpaired electrons make molecules extremely unstable and reactive. To establish a stable state, they react with the electrons of the nearest molecules. In order to pair their free electron and thus create a stable compound, they enter into reactions with inorganic or organic compounds, such as proteins, lipids, carbohydrates and nucleic acids. For example, they pair with an electron from the outer orbit of another electron, giving or taking them away from other molecules. Free radicals are mainly of organic origin, can react with organic and/or inorganic compounds and have a positive, negative, or neutral charge (Halliwell and Gutteridge, 2007). Once formed, a free radical can initiate a series of chain reactions by reacting with each other or with other less reactive molecules, where the unpaired electrons form chemical bonds releasing energy. The most common reactions involving free radicals are donation of an electron (a reduced radical donates an electron to a non-radical), acceptance of an electron (an oxidised radical accepts an electron from a non-radical), removal of hydrogen, an addition reaction, etc. The half-life of free radicals in biological systems is very short, usually only a few microseconds, making them difficult to quantify (Kehrer, 2000; Horton, 2003; Žura Žaja, 2015).

In the body, *i.e.*, in the cells, there are very low concentrations of free radicals. Healthy cells strive to maintain a balance between the production and elimination

of oxygen radicals. The cells achieve this homeostasis by producing antioxidants, which act as radical scavengers and neutralising the reducing and oxidising molecules produced. If for any reason there is a relative or absolute lack of antioxidants, the cells' ability to remove reactive oxygen molecules decreases and ROS increases. This prevents the adequate recovery of cellular enzymes and directly affects the reduction of the cell's detoxification function, resulting in the flooding of cells with reactive oxygen molecules that can have an unfavourable and even toxic effect, altering cell functions and threatening cell survival or both (Halliwell and Gutteridge, 2007; Ogbuewu et al., 2010; Žura Žaja, 2015). In other words, the disruption of this homeostasis, as a shift in the balance between oxidants and antioxidants in favour of oxidants, is called "oxidative stress", and it represents damage to macromolecules and the development of metabolic disorders (Žura Žaja et al., 2019).

Oxygen radicals can cause several harmful reactions such as lipid peroxidation, damage to DNA, proteins and carbohydrates, or they can oxidise almost any organic molecule by destabilising the nitrogen atoms in DNA, RNA, and amino acids, and in the fatty acid double bonds (Fürst, 1996; Dean et al., 1997; Kehrer, 2000; Jacob and Winyard, 2009). Significant damage to macromolecules alters cell structure and function, disrupting immune system function and repair processes. Finally, free radicals can also have a cytotoxic effect that can lead to cell death (necrosis or apoptosis), chromosomal aberrations, mutations, carcinogenesis, etc. Cell damage caused by free oxygen radicals has been shown to be an etiological factor in the development and pathogenesis of diseases, such as neurodegenerative disorders, inflammation, infertility, viral infections, autoimmune diseases, digestive system disorders, etc. (Ogbuewu et al., 2010).

Reactive oxygen compounds

The electronic configuration of oxygen is responsible for the simultaneous necessity of oxygen for life and the toxicity of oxygen. Oxygen has two unpaired electrons. Therefore, aerobic metabolism is associated with the toxic effect of oxygen due to the oxidation of basic biological molecules, which can alter cell function, threaten cell survival, or both (Halliwell and Gutteridge, 2007; Ogbuewu et al., 2010).

Such an electronic configuration enables the reduction of oxygen. The stepwise reduction of oxygen produces free oxygen

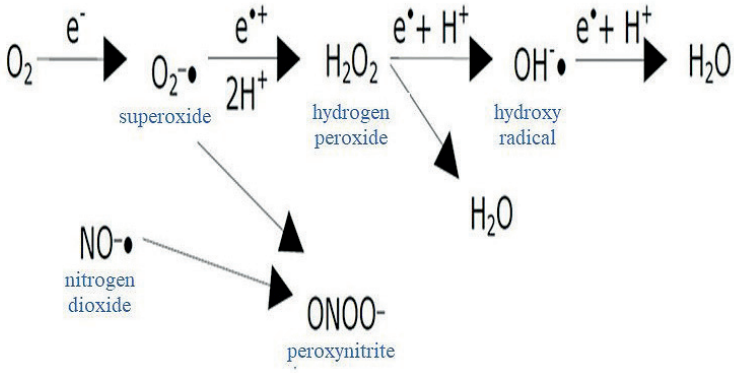

Figure 2. Oxygen reduction and the formation of free radicals (Žura Žaja et al., 2016)

Table 1. Reactive oxygen species (according to Štefan et al., 2007; Žura Žaja et al., 2016)

Free radicals	Molecules that are not free radicals
superoxide anion, $O_2^{\bullet-}$	hydrogen peroxide, H_2O_2
hydroxyl radical, $OH^{\bullet-}$	hypochlorous acid, HClO
lipid peroxyl, $LOO^{\bullet}$	ozone, O_3
lipid alkyl, $LO^{\bullet}$	singlet oxygen, $^1O_2$
hydroperoxyl, $HO_2^{\bullet}$	

radicals in the following order: hydroperoxyl radical (HO_2), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2 oxidising agent), the reduction of which produces a hydroxyl radical (OH^-) (Figure 2).

The term reactive oxygen species includes potentially active, ROS, free radicals and reactive molecules that are not free radicals. ROS are the most abundant radicals in the body, and the most important are the superoxide anion (O_2^{-}) and the hydroxyl radical (OH-). The most important non-free radicals of the biological system are hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hypochlorous acid (HOCl) (Table 1).

Sources of free radicals can be external (such as ionising radiation that causes water cleavage, or smoking due to the high content of radicals in cigarette smoke) or internal, such as enzyme systems of catalysis, activation of neutrophils (example of intentional formation), in the mitochondrial electron transport system (example of accidental formation) (Žura Žaja et al., 2019, 2021, 2023).

The superoxide anion radical is essential for the life of aerobic organisms. The main cause of the formation of superoxide anion radicals is the mitochondrial respiratory chain, in which 13% of oxygen is converted into superoxide radicals under physiological conditions. It acts as a signalling molecule and regulates numerous biological processes such as apoptosis, ageing and senescence (Chiste et al., 2015; Andrés et al., 2023).

Hydrogen peroxide (non-radical) is toxic in high concentrations, and the main danger when it accumulates is the formation of a highly reactive hydroxyl radical in the presence of transition metal ions (such as iron or copper). At the cellular level, the accumulation of hydrogen peroxide can trigger inflammation and even apoptosis. It is very important because it penetrates biological membranes and generates reactive compounds. It modulates and signals the redox metabolism of cells by acting as a messenger and triggering specific oxidations that determine the metabolic response (Andrés et al., 2022).

The hydroxyl radical is the most reactive radical. It reacts with various cellular compounds and can oxidise amino acid residues. It can cleave the structure and chemically alter the bases in DNA, which can lead to mutagenesis and carcinogenesis, and it can attack the lipids of cell membranes, triggering a chain reaction of free radicals known as lipid peroxidation. Due to this process, the membranes become porous, lose their action potential, the function of ion channels changes, enzymes and membrane-bound receptors are inactivated, which leads to the loss of physiological function of the membrane and apoptosis (Andrés et al., 2022).

Superoxide anion and hydrogen peroxide are very selective and therefore less reactive, whereas the hydroxyl radical is very reactive and reacts with all molecules in the cellular environment. Hydroxyl radicals are formed by the combination of

Table 2. Reactive nitrogen species (according to Štefan et al., 2007; Žura Žaja, 2015)

Free radicals	Molecules that are not free radicals
nitric oxide, $NO^{\bullet -}$	nitrosyl, NO^{+}
	nitric acid, HNO_2
	nitrogen trioxide, N_2O_3
nitrogen oxide, $NO_2^{\bullet -}$	peroxynitrite, $ONOO^{-}$
	alkyl peroxy nitrite, ROONO

superoxide anions with hydrogen peroxide. The reaction is catalysed by transition metals in the so-called Haber-Weiss or Fenton reaction (Andrés et al., 2022).

Reactive nitrogen compounds

In addition to ROS, free radicals include reactive nitrogen compounds (RDS), reactive chloride compounds (RCS), reactive bromine compounds (RBS) and reactive sulfur compounds (Halliwell and Gutteridge, 2007).

Nitrogen (N), an essential element of life, is present in the atmosphere as N_2 gas. When the bond of N_2 is broken, N becomes reactive and occurs in various forms. In biological systems, RDS such as nitric oxide (NO^{-}) or nitrogen dioxide (NO_2^{-}) are of importance, while important non-radicals are nitric acid (HNO^{2}) and peroxynitrite ($ONOO^{-}$) (Table 2). RDS is a component of organic molecules such as amino acids, proteins, chlorophyll, haemoglobin, enzymes, nucleic acids (DNA and RNA), humic acids and others. Therefore, the supply of RDS is essential for all life forms but has both beneficial and detrimental effects (Nieder and Benbi, 2021).

The nitric oxide radical does not react immediately with most available biomolecules. It reacts relatively easily with other radicals and usually forms less reactive molecules. Nitric oxide is produced from L-arginine in endothelial, nerve and inflammatory cells by the action of the enzyme nitric oxide synthetase (Moncada and Higgs, 1993; Halliwell and Gutteridge, 2007; Król and Kepinska, 2021). The physiological functions of NO are numerous, including local vasodilatation, regulation of blood pressure, inhibition of platelet aggregation, regulation of cardiac contractility, cell signalling. In the central nervous system, it acts as a neurotransmitter for multiple functions, including memory, while on the periphery it acts via non-adrenergic, non-cholinergic nerves, regulating the functions of various organs by establishing or modulating the immune response (Moncada and Higgs, 1993). In addition to oxygen radicals, macrophages also produce $NO^{\bullet -}$, which they use to destroy pathogenic microorganisms and tumour cells. Nitric oxide is not very toxic, but it easily reacts with hydrogen peroxide or a superoxide anion and produces more toxic compounds that kill bacteria, fungi, helminths, and tumour cells (Stevanović et al., 2011; Król and Kepinska, 2021; Rolf and Benbi, 2022). Nitric oxide in small concentrations has a significant function in reproduction and fertilisation, maintaining sperm motility (Žura Žaja et al., 2019).

Antioxidant defence of the organism

The potential toxicity of reactive oxygen radicals under physiological conditions is prevented by a large number of cytoprotective enzymes and compounds known as antioxidants (Table 3).

An antioxidant is a compound that is present in a low concentration compared to the oxidised substance and significant-

ly delays or prevents the oxidation of that substance.

The mechanisms of action of antioxidants are:

a) Prevention of the formation of ROS
b) Elimination of the generated species by specific enzymes, e.g., superoxide dismutase (SOD), catalase, glutathione peroxidase, etc., and antioxidants in cells and body fluids, such as vitamins C and E, urates, glucose, selenium, ubiquinone, glutathione,
c) Removal of damage and damaged molecules before their accumulation causes further damage (Žura Žaja et al., 2016; Abeyrathne et al., 2022; Hewitt and Degnan, 2023).

Endogenous antioxidants are constantly produced in the body and are divided into enzymatic and non-enzymatic molecules that are cross-linked and interact with each other. They are usually distributed in the cytoplasm and various cell organelles but are also found in the extracellular fluid. Endogenous and exogenous antioxidants neutralise the effect of free radicals and thus influence the development of a more effective immune response (Žura Žaja et al., 2019). It is known that enzymes are mainly localised in the cell, while other antioxidant compounds are localised both within and outside the cells (Abeyrathne et al., 2022; Hewitt and Degnan, 2023).

Cytoprotective enzymes

In multicellular organisms, we find several primary antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), which catalyse complex reaction cascades in which ROS are converted into more stable water and oxygen molecules. For these reactions, they require trace elements (selenium, iron, copper, zinc and magnesium) as co-factors (Young and Woodside, 2001; Rahal et al., 2014; Žura Žaja, 2015). Except for the primary antioxidants, numerous secondary enzymes work in close interaction with antioxidants and provide the necessary cofactors for the basic functions of the enzymatic antioxidants (Hewitt and Degnan, 2023).

Superoxide dismutase is found in almost all eukaryotic cells of plants and animals. It occurs in three forms: mitochondrial, cytosolic, and extracellular. Catalase is involved in the splitting of hydrogen peroxide into water and oxygen.

Glutathione peroxidase is the most important enzyme that breaks down the hydrogen peroxide produced by the action of SOD. It contains selenium and is partially localised in cell membranes. Four forms of this enzyme are known in the body: cellular, gastrointestinal, plasma and phospholipid hydroperoxide glutathione peroxidase, which is found in many tissues (Žura Žaja et al., 2019; Hewitt and Degnan, 2023).

Table 3. Some biologically important antioxidants (according to Čvorišćec and Čepelak, 2009)

Extracellular	Membranous	Cellular
transferrin	alpha-tocopherol	superoxide dismutase
lactoferrin	beta carotene	catalase
haptoglobins	coenzyme Q	glutathione peroxidase
hemopexin		
ceruloplasmin		
extracellular SOD		
urate		

Non-enzymatic antioxidants

Non-enzymatic antioxidants are divided into endogenous (metabolic) and exogenous (dietary intake).

Endogenous antioxidants include lipoic acid, glutathione, L-arginine, uric acid, bilirubin, coenzyme Q10, melatonin, proteins of low molecular weight, which bind heavy metals (metal-chelating proteins), transferrin, etc. (Pham-Huy et al., 2008; Abeyrathne et al., 2022).

Exogenous antioxidants (nutrients) are compounds that cannot be produced in the body and must be taken in with food and/or food supplements, and can be natural or synthetic, for example vitamin E, vitamin C, carotenoids, flavonoids, lycopene, trace elements (Se, Cu, Zn, Mn) (Shinde et al., 2012; Abeyrathne et al., 2022).

Vitamin C or ascorbic acid plays an important role in biological oxidation-reduction processes and cellular respiration, by reducing the α-tocopherol radical, peroxides and other radicals. Vitamin E (lipophilic antioxidant) is an essential component of biological membranes and lipoproteins in the blood. When peroxyl and alkyl radicals are formed during lipid peroxidation, they react primarily with this antioxidant and not with the neighbouring fatty acid. Beta-carotene is a lipid-soluble vitamin A. It acts as an antioxidant, primarily by removing singlet oxygen. Reduced glutathione protects cells from oxidative damage (Žura Žaja et al., 2016).

Oxidative stress

Oxidative stress refers to a shift in the balance of cellular oxidation-reduction reactions in favour of oxidation or excessive formation of radicals, i.e., a loss of balance between the formation of radicals and the ability of the cell to remove them with the help of the antioxidant system. The causes can be mechanical, bacterial, viral, or toxic and result in a loss of the majority of reducing compounds (antioxidants), an increase in oxidising compounds (prooxidants), or an accumulation of molecules that are damaged and altered by the action of free radicals. Oxidative stress can lead to significant disturbances in cell metabolism, including DNA damage, increased intracellular free calcium, damage to membrane ion transporters and proteins, and lipid peroxidation (Scandalios, 2002; Dosek et al., 2007; Zhang et al., 2015; Žura Žaja et al., 2016, 2019, 2023).

Lipid peroxidation

Lipids are functional and structural components of biological membranes, the body's main energy reserves, precursors for vitamins and hormones, involved in intercellular communication and the regulation of gene expression (Catalá, 2009). Lipid peroxidation is the oxidative degradation of lipids triggered by the action of ROS and/or RDS. In this process, free radicals "steal" electrons from lipids (in cell membranes, lipoproteins, and other molecules), resulting in cell damage and loss of integrity, and the process continues with the free radical chain reaction mechanism (Štefan et al., 2007). During lipid peroxidation, the primary products are degraded and secondary products, various aldehydes, are formed (Girotti, 1998; Guéraud et al., 2010). The end products of the lipid peroxidation process are the reactive aldehydes malondialdehyde (MDA) and 4-hydoxynonenal (Žura Žaja et al., 2016, 2019).

The concentration of markers for oxidative status varies considerably in individual tissues (Madeira et al., 2013). Depending on which tissue is damaged, this has an impact on the functions and capabilities of the organs or the entire organism. For example, oxidative damage to the liver can limit the ability to detoxify

the blood and store glycogen (Oettl et al., 2013). Oxidative brain damage can limit cognitive function and affect behaviour and reaction time (Forster et al., 1996), which may include thermoregulatory behaviour. Respiratory surfaces such as lungs and gills accumulate high concentrations of ROS-related products, while brain tissue may show minimal or no changes in response to temperature stress within the same organism (Madeira et al., 2013). Tissue thermal sensitivity is an important factor in measuring the effects of temperature stress. For example, the brain tolerates minimal temperature fluctuations compared to muscle tissue (Kiyatkin, 2010; Ritchie and Frisien, 2022).

Methods of determining oxidative status

The direct determination of free radicals is difficult due to their low concentrations (10-11 mol/L) and their short half-life (milliseconds). It is possible to determine them directly using electron spin resonance (ESR), which is mainly used in scientific research (Čvorišćec and Čepelak, 2009). For the determination of ROS, there are indirect methods for the determination of free oxygen radicals, *i.e.*, for the detection of oxidative stress, based on the following methods:

- Determination of the activity of an enzyme that produces ROS (e.g., myeloperoxidase, NO synthetase...)
- Determination of the concentration of individual antioxidants or the activity of cytoprotective enzymes (SOD, GPx, CAT, vitamins A and C, concentration of extracellular antioxidants)
- Determination of the reaction products of free radicals with various biomolecules (MDA, volatile hydrocarbons, antibodies against oxidized low-density lipoprotein, ...)

Methods with different sensitivity and specificity are mentioned. The methods for determining the total antioxidant status are considered a more appropriate indicator of the active defence mechanism of the organism, and the most commonly used is the determination of the overall antioxidant status (Čvorišćec and Čepelak, 2009). Since markers and tissues generally differ in their biological origin and function, the measured values of oxidative status of different tissues of the same individual may not be correlated. Therefore, the most important question of research interest is the choice of oxidative status indicators, but also the choice of tissue. Pilot studies are useful to test correlations between biomolecular markers and a particular tissue in the same or closely related species (Ritchie and Frisien, 2022).

Thermal effects on oxidative stress in reptiles

Reptiles are ectothermic animals that are not able to significantly increase their body temperature through metabolism, but instead do so by absorbing heat from the environment (Seebacher et al., 2015). There is an ideal temperature for all reptiles, called the thermal optimum (Topt). The thermal optimum is the ideal temperature for physiological processes (performance) and can be a characteristic of an individual, a population, or a species. In contrast to Topt, there are also critical temperatures (CT), high (CTmax) or low (CTmin), which are defined as the point at which an organism switches from aerobic metabolism to anaerobic metabolism (Pörtner, 2002). As temperature rises, the individual's performance increases, and at this point reptiles are able to change their behaviour (exposure or avoidance of heat sources) and thus their body temperature to maintain Topt (Pörtner, 2002). If behav-

ioural changes cannot regulate body temperature, oxidative stress can occur (Ritchie and Frisien, 2022) (Figures 3 and 4).

At or near Topt (within 10-20% of the thermal range of Topt), most cellular functions have likely evolved to operate efficiently to minimise or prevent the inevitable production of ROS from mitochondria and other metabolic sources. An increase in ROS may signal increased gene expression of uncoupling proteins (which reduce proton motive force in mitochondria and thus ROS production), production of endogenous antioxidants that quench ROS, increased activity in repairing molecular damage, or disposal and replacement of dysfunctional mitochondria. All these activities maintain cellular and organismal homeostasis and ultimately fitness. Deviations from Topt can induce hormonal changes by developing and enhancing robust responses and thus resilience to ROS and other potential stressors. However, chronic, and extreme acute deviations from optimal temperature are likely to negatively impact the efficiency of proteins (conformational changes) such as antioxidants and those involved in cellular respiration, signalling pathways and gene expression, thereby increasing ROS

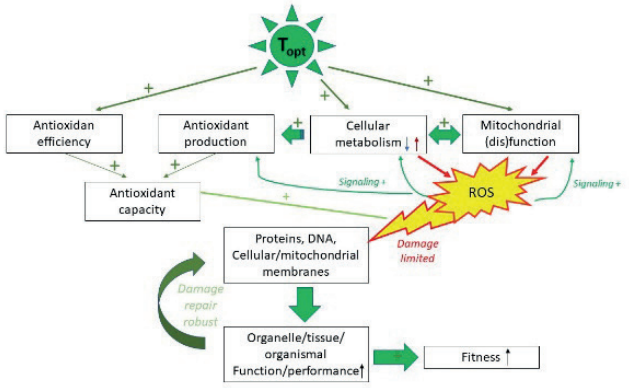

Figure 3.

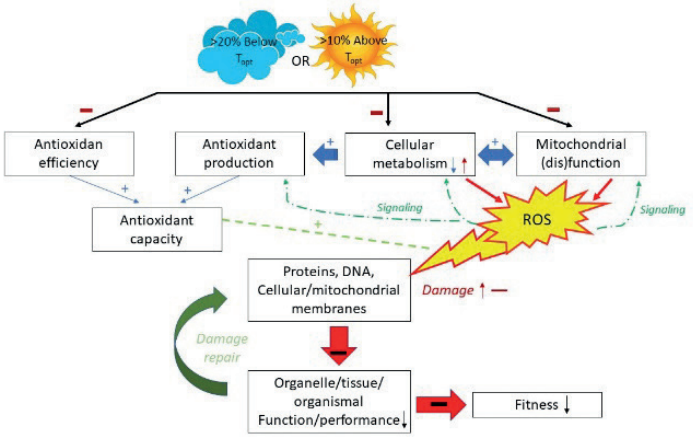

Figure 4.

Figure 3. and **Figure 4.** Possible mechanisms between temperature and oxidative stress in ectothermic vertebrates

production, and decreasing quenching of ROS, leading to oxidative stress damage to macromolecules (proteins, DNA and lipids, e.g., cellular/mitochondrial membranes). The power spectrum of an ectothermic organism influences the extent of change in oxidative status and whether a particular individual (population or species) experiences oxidative stress or eustress, which could have hormonal effects in response to high or low temperatures (according to Ritchie and Frisien, 2022).

Except in extreme situations, it is known that the metabolic rate of reptiles correlates with the ambient temperature, making them physiologically more sensitive to temperature changes compared to mammals (Seebacher et al., 2015). However, it is worth considering that ectothermic organisms have evolved a range of thermal adaptations by selecting genotypes that optimise body functions adapted to the past environment through physiological and behavioural means (Angilletta, 2009). Organisms that are evolutionarily adapted to changing conditions are better able to adapt to temperature changes and mitigate the resulting oxidative stress (Ritchie and Frisien, 2022). In research on acclimatization or adaptation to temperature changes in reptiles, temperature treatments exceeding the optimal temperatures caused significantly less oxidative stress than those in which the temperature was lowered. These results emphasise the importance of phylogeny and adaptation of responses to oxidative stress from environmental conditions during evolution (Zhang et al., 2015; Ritchie and Frisien, 2022). Heat wave stimulation in the corn snake (*Pantherophis guttatus*) was found to have a negative effect on body mass, but it also reduced oxidative damage and probably innate immune function. Furthermore, heatwaves may have complex, modest and even positive physiological effects in some taxa (Stahlschmidt et al., 2017). However, a temperature increases mimicking global warming induced oxidative stress and immunosuppression in a viviparous lizard (*Eremias multiocellata*) (Han et al., 2020).

As mentioned above, oxygen is essential for most life forms, including reptiles, but is also potentially toxic to them due to its conversion to ROS. It is well known that hypoxia (at high altitudes) can increase the production of ROS. Since a limited oxygen supply means less oxygen is available for the terminal uptake of electrons from oxidative phosphorylation, this could lead to an accumulation of reducing equivalents in the mitochondria (Zhang et al., 2015).

The development of many pathological conditions in animals, as well as natural aging, are associated with excessive production of ROS and/or reduced antioxidant capacity. However, many animal species, especially lower vertebrates, can tolerate situations that present a high potential for oxidative stress under natural conditions (anoxia, freezing, immersion, dehydration, sudden and significant temperature changes) (Hermes-Lima and Zenteno-Savin, 2002). Although the animals rely mainly on anaerobic metabolism in such situations, the resumption of normal respiration (upon surfacing, increase in body temperature, etc.) leads to a potentially dangerous situation with excessive ROS production and oxidative stress (Hermes-Lima and Zenteno-Savin, 2002). This condition is comparable to the well-studied oxidative stress during ischemia and reperfusion in mammals (Hermes-Lima et al., 2001).

Tolerance to low temperatures and frost is an important survival strategy for various amphibian and reptile species living in cold climate regions, especially during frosts in winter and spring (Hermes-Lima and Storey, 1993). Hermes-Lima and

Storey (1993) concluded that anoxia and exposure to subfreezing temperatures indeed lead to increased activity of antioxidant enzymes (catalase in muscle and lung, GPX in muscle during freezing and SOD in liver during anoxia). Despite the lack of oxidative metabolism, this phenomenon of increased concentration of antioxidants has been termed "oxidative priming" (Hermes-Lima et al., 2001), meaning that the build-up of antioxidant capacity precedes the actual occurrence of oxidative stress. An increase in the activity of antioxidant enzymes reflects an increase in the rate of synthesis and/or a decrease in their degradation (Hermes-Lima and Zenteno-Savin, 2002). The fact that glutathione (GSH) levels did not change upon freezing in muscle or in frozen and anoxic liver and lung suggests that garter snake cells can adequately maintain antioxidant capacity under conditions of freezing and anoxia (Hermes-Lima and Storey, 1993).

Concluding remarks

Due to global warming, the temperature of the environment is changing, which may alter oxidative stress processes, life history traits and population structure in ectotherms. Additional research in ecology, biogeography and evolution is needed to determine the exact effect of temperature on oxidative stress and the resulting changes in life history traits in wild populations. A better understanding of the effects of temperature on oxidative stress at physiological and behavioural levels, which is critical in ectotherms, can increase knowledge for predicting the effects of climate change on ectotherms and biodiversity. Although some ectothermic organisms have evolved a range of thermal adaptations better than others by selecting genotypes that optimise body functions adapted to past environments through physiological and behavioural means, there is a need to thoroughly investigate the effect of increased and decreased environmental temperatures on oxidative stress in reptiles. Finally, field efforts should focus on characterising behavioural responses (e.g., shifts in refuge use or temporal patterns in daily activity patterns) as a result of a natural heat wave caused by global warming.

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Oksidacijsko-antioksidacijski procesi i toplinski učinci na oksidativni stres u gmazova

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Temperatura je glavni abiotički čimbenik i ima izravan utjecaj na fiziologške procese organizma. Nadalje, temperatura utječe na gotovo sve ostale okolišne čimbenike koji utječu na žive organizme. Predviđa se da će ektotermni organizmi zbog klimatskih promjena biti izloženi velikom riziku zbog njihove ovisnosti o temperaturi okoliša za održavanje tjelesne temperature. Gmazovi su ektotermni

kralješnjaci, koji su kao i ostali ektotermni organizmi osjetljivi na temperaturne promjene. Metabolizam, rad mišića i živčanog sustava te reprodukcija ovisni su o tjelesnoj temperaturi gmazova. Za razumijevanje utjecaja temperature na oksidativni stres, nužno je razjasniti mehanizam nastanka reaktivnih kisikovih spojeva (ROS) te kako spriječiti nastanak oksidativnog stresa. U ovom radu opisat će se oksidacijsko-antioksidacijski procesi i oksidativni stres uzrokovan toplinskim učincima u gmazova. U metaboličkim procesima aerobnih organizama, ROS se kontinuirano stvaraju kao nusproizvodi oksidacijsko-redukcijskih reakcija te primarno nisu štetni. Štoviše, ROS su neophodni za brojne fiziološke funkcije, primjerice za proizvodnju energije i procese imunološkog sustava. Potencijalni štetni učinak reaktivnih kisikovih radikala/spojeva u fiziološkim uvjetima sprječava antioksidativni zaštitni sustav. Oksidativni stres nastaje kada je ravnoteža između oksidacijskog i antioksidativnog sustava poremećena zbog prekomjerne razine ROS-a ili smanjenja antioksidansa. Poznato je da je brzina metabolizma gmazova u korelaciji s temperaturom okoliša, što ih čini fiziološki osjetljivijima na promjene temperature u usporedbi sa sisavcima. No, potrebno je uzeti u obzir da su ektotermni organizmi razvili niz prilagodbi na promjenu temperature, putem fizioloških i bihevioralnih mehanizama te time ublažili nastali oksidativni stres. Međutim, da bi se utvrdio stvarni učinak temperature na oksidativni stres i posljedične promjene životnih obilježja u divljih populacija, potrebna su dodatna istraživanja u područjima ekologije, biogeografije i evolucije.

Ključne riječi: *oksidacijsko-antioksidacijski procesi, toplinski učinci, oksidativni stres, gmazovi*