



Fasting ameliorates oxidative stress: A review of physiological strategies across life history events in wild vertebrates

David C. Ensminger¹, Andrea Salvador-Pascual¹, B. Gabriela Arango¹, Kaitlin N. Allen¹, José Pablo Vázquez-Medina^{*}

Department of Integrative Biology, University of California, Berkeley, USA

ARTICLE INFO

Keywords:

Antioxidants
Lipid peroxidation
Reactive oxygen species
Redox balance
Starvation

ABSTRACT

Fasting is a component of many species' life history due to environmental factors or behavioral patterns that limit access to food. Despite metabolic and physiological challenges associated with these life history stages, fasting-adapted wild vertebrates exhibit few if any signs of oxidative stress, suggesting that fasting promotes redox homeostasis. Here we review mammalian, avian, reptilian, amphibian, and piscine examples of animals undergoing fasting during prolonged metabolic suppression (e.g. hibernation and estivation) or energetically demanding processes (e.g. migration and breeding) to better understand the mechanisms underlying fasting tolerance in wild vertebrates. These studies largely show beneficial effects of fasting on redox balance via limited oxidative damage. Though some species exhibit signs of oxidative stress due to energetically or metabolically extreme processes, fasting wild vertebrates largely buffer themselves from the negative consequences of oxidative damage through specific strategies such as elevating antioxidants, selectively maintaining redox balance in critical tissues, or modifying behavioral patterns. We conclude with suggestions for future research to better elucidate the protective effects of fasting on oxidative stress as well as disentangle the impacts from other life history stages. Further research in these areas will facilitate our understanding of the mechanisms wild vertebrates use to mitigate the negative impacts associated with metabolically-extreme life history stages as well as potential translation into therapeutic interventions in non-fasting-adapted species including humans.

1. Introduction

1.1. Oxidants, antioxidants, and oxidative stress

Free radicals are short-lived chemical species containing one or more unpaired electrons (Slater, 1984; Cheeseman and Slater, 1993). Oxygen-derived free radicals and their related non-radical reactive species (e.g. H_2O_2) are collectively known as reactive oxygen species (ROS). Mitochondria generate ROS as byproducts of aerobic metabolism during the reduction of molecular oxygen (O_2) in the electron transport chain (Babcock, 1999; Semenza, 2007). The sequential one-electron reduction of O_2 produces superoxide radical ($O_2^{\bullet-}$; Sies, 1997) which undergoes

dismutation to yield hydrogen peroxide (H_2O_2 ; Loschen et al., 1974), the highly reactive hydroxyl radical ($HO^{\bullet}$; Florence, 1984), and water. The reaction of transition metals with $O_2^{\bullet-}$ and H_2O_2 (e.g. Fenton reaction) also yields $HO^{\bullet}$ (Butler and Halliwell, 1982), which generates alkyl or peroxy radicals when acting on fatty acids (Nelson et al., 1994; Sies, 1997). $O_2^{\bullet-}$ can also react with nitric oxide ($NO^{\bullet}$) to generate peroxynitrite ($ONOO^-$), a strong oxidant classified as a reactive nitrogen species (Blough and Zafiriou, 1985; Koppenol et al., 1992; Radi, 2018). In addition to the electron transport chain, several enzymes generate ROS as their main catalytic products. Major oxidant-generating enzymes include xanthine oxidase (XO; McCord et al., 1985), NADPH oxidases (Babior, 1999), the membrane-bound microsomal monooxygenase

Abbreviations: ROS, reactive oxygen species; XO, xanthine oxidase; SOD, superoxide dismutases; GPx, glutathione peroxidases; Prdx, peroxiredoxins; GSH, glutathione; GR, glutathione-disulfide reductase; GSSG, glutathione disulfide; Nrf2, nuclear factor erythroid 2-related factor 2; IF, intermittent fasting; CR, caloric restriction; 4-HNE, 4-hydroxy-2-nonenal; MDA, malondialdehyde; HO-1, heme oxygenase; RF, Ramadan-related fasting; BAT, brown adipose tissue; GRP75, glucose-regulated protein 75; HSP70, heat shock protein 70; HIF-1, hypoxia-inducible factor 1; RAS, renin-angiotensin system; GST, glutathione S-transferase; TBARS, thiobarbituric acid reactive substances.

^{*} Corresponding author at: Department of Integrative Biology, 1005 Valley Life Science Building, #3140, Berkeley, CA 94720-3140, USA.

E-mail address: jpv-m@berkeley.edu (J.P. Vázquez-Medina).

¹ Equal contribution.

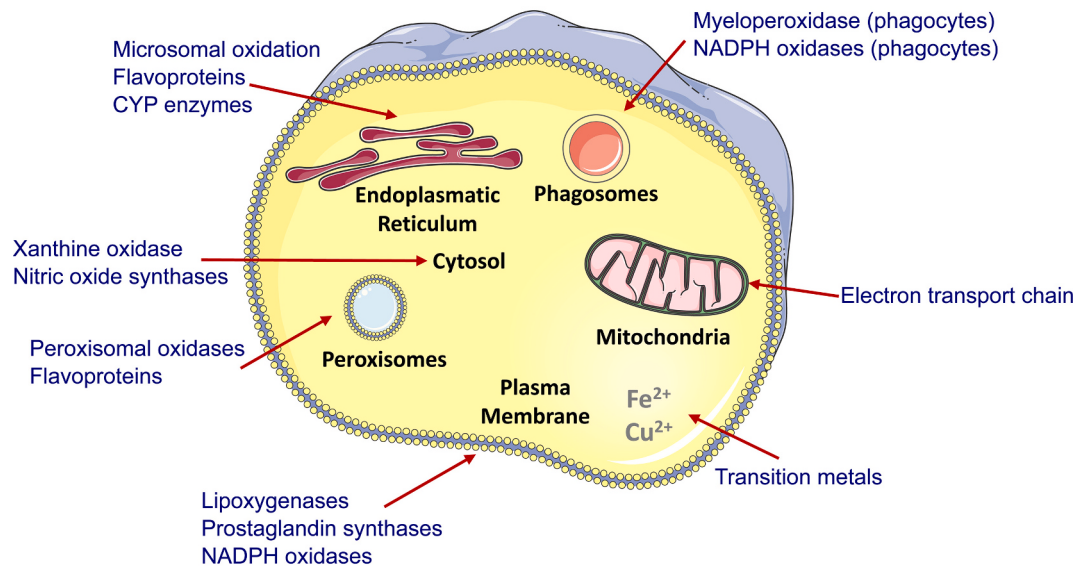
<https://doi.org/10.1016/j.cbpa.2021.110929>

Received 14 December 2020; Received in revised form 17 February 2021; Accepted 21 February 2021

Available online 26 February 2021

1095-6433/© 2021 Elsevier Inc. All rights reserved.

A) Oxidant sources



B) Antioxidant defenses

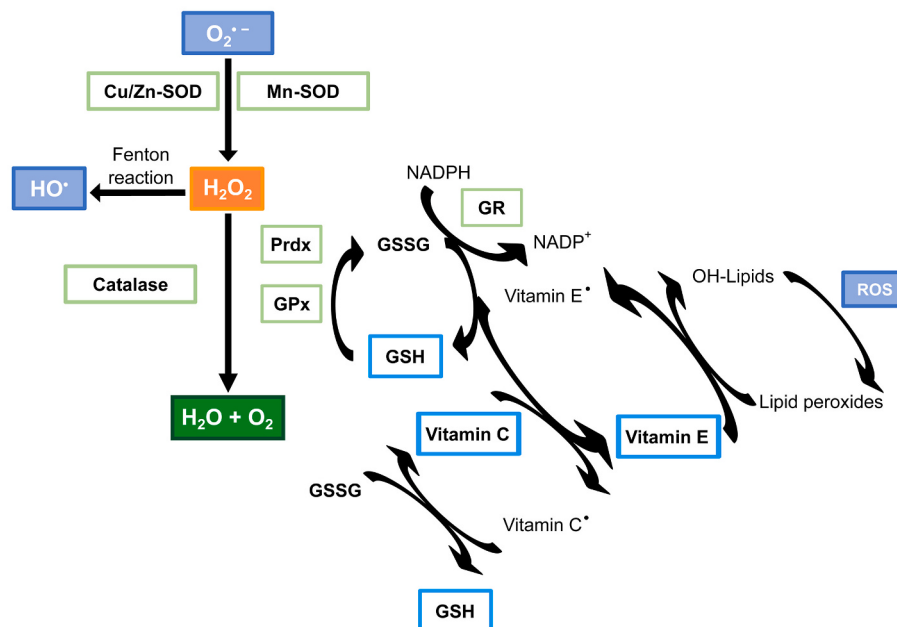


Fig. 1. Endogenous sources of oxidant generation (A) and antioxidant defenses (B).

system (cytochromes P450; Zangar et al., 2004), nitric oxide synthases (NOS; Miller et al., 1997), and membrane-associated enzymes such as lipoxygenases and cyclooxygenase (Cho et al., 2011). The main endogenous sources of oxidant generation are summarized in Fig. 1A.

Increased oxidant generation or decreased antioxidant levels can cause oxidative stress, a landmark of multiple pathological conditions (Sies, 1985; Gospodaryov and Lushchak, 2012), aging (Sies, 1985; Viña et al., 2013), and frailty (Inglés et al., 2014; Viña et al., 2018). Oxidative stress induces oxidative damage to proteins, lipids, carbohydrates, and nucleic acids, subsequently impairing the physiological functions of these molecules (Bokov et al., 2004). Lipids are highly susceptible to oxidative damage via lipid peroxidation (Cheeseman and Slater, 1993; Rikans and Hornbrook, 1997), which can be reversed by enzymes that either reduce phospholipid hydroperoxides or hydrolyze and re-acylate phospholipid fatty acyl bonds (Ursini et al., 1982; Sevanian et al., 1983;

Fisher et al., 2018). Proteins also scavenge a high proportion of ROS (Davies et al., 1999); however, in contrast to lipids, protein oxidation is usually irreversible (Dean et al., 1993; Shacter, 2000) and dysfunctional oxidized proteins must be degraded by the proteasome (Grune et al., 1997). Oxidative stress disrupts physiological redox signaling (Jones, 2006), which is typically maintained by low levels of oxidants (oxidative eustress; Sies, 2019) including H_2O_2 , a well-known second messenger for numerous physiological functions across phyla (Suzuki et al., 1997; Veal and Day, 2011).

Antioxidants maintain ROS levels within a physiological range by acting at three tiers of protection: prevention, interception, and repair (Sies, 1993). Low concentrations of antioxidants delay or inhibit the oxidation of cellular substrates (Halliwell, 1990; Halliwell and Gutteridge, 1995). Antioxidant enzymes detoxify oxidants by converting these compounds into less harmful molecules: superoxide dismutases (SOD)

Box 1

Reactive species, antioxidants, oxidative damage products and redox sensitive transcription factors mentioned in this review.

Reactive species	Antioxidants		Oxidative damage		Redox-sensitive transcription factors
	Enzymatic	Non-enzymatic	Lipid peroxidation products	Protein oxidation products	
Superoxide radical ($O_2^{\bullet-}$)	Superoxide dismutases (SOD)	Glutathione (GSH)	Malondialdehyde (MDA)	Nitrotyrosine	Nuclear factor erythroid 2-related factor 2 (Nrf2)
Hydrogen peroxide (H_2O_2)	Catalase	Vitamins (C and E)	F2-isoprostanes	Protein carbonyls	Hypoxia-inducible factor 1 (HIF-1)
Hydroxyl radical ($HO^{\bullet}$)	Glutathione peroxidases (GPx)	Uric acid	4-hydroxy-2-nonenal (4-HNE)		Nuclear factor Kappa B (NF- κ B)
Lipid radicals (e.g., peroxy: $ROO^{\bullet}$)	Peroxioredoxins (Prdx)		Lipid hydroperoxides		
Nitric oxide ($\bullet NO$)	Glutathione- disulfide reductase (GR)				
Peroxynitrite ($ONOO^-$)					

convert $O_2^{\bullet-}$ into O_2 and H_2O_2 (McCord and Fridovich, 1969), while glutathione peroxidases (GPx; Cohen and Hochstein, 1963), catalase (Mueller et al., 1997), and peroxiredoxins (Prdx; Kang et al., 1998) decompose H_2O_2 into water and O_2 (Halliwell, 1974), preventing the formation of $HO^{\bullet}$. The particular role of each of those enzymes in H_2O_2 detoxification depends on subcellular localization and intracellular H_2O_2 levels (Chance et al., 1979).

Excessive oxidant production can saturate antioxidant enzymes. Non-enzymatic antioxidants prevent existing ROS from participating in chain reactions by deactivating them into non-reactive products or by transferring the radical function to compartments less vulnerable to oxidative damage (Sies, 1997). Glutathione (GSH) is the main non-enzymatic antioxidant in mammalian cells (Dickinson and Forman, 2002). GSH can scavenge ROS directly and also serves as a cofactor for several antioxidant enzymes including glutaredoxins, GPx, and some Prdx (Fernandez-Marcos and Nóbrega-Pereira, 2016). GSH is synthesized *de novo* from glutamine, cysteine and glycine in a two-step enzymatic reaction catalyzed by glutamate-cysteine ligase and GSH synthetase. The cellular pool of GSH is maintained by glutathione-disulfide reductase (GR), which recycles GSH from glutathione

disulfide (GSSG; Harlan et al., 1984). GSH also contributes to maintaining the reduced state of non-enzymatic antioxidants vitamin C (May et al., 1996) and vitamin E (Scholz et al., 1989). Fig. 1B illustrates the main endogenous antioxidant defenses.

The nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates the expression of GR and glutamate-cysteine ligase, maintaining GSH homeostasis. Nrf2 is activated in response to oxidative stress (Harvey et al., 2009; Nguyen et al., 2009; Ma, 2013). Upon oxidation of key cysteine residues in Nrf2's cytosolic inhibitor Kelch-like ECH-associated protein 1, Nrf2 translocates into the nucleus where it binds to antioxidant-response elements in the promoter region of several antioxidants and other cellular defense genes (Nguyen et al., 2009; Ma, 2013). Therefore, Nrf2 mediates an adaptive response to physiological challenges that induce oxidative eustress (Galiè et al., 2019; Sies, 2020). Upregulation of endogenous antioxidants via Nrf2 activation is a key mechanism used by several species adapted to cope with environmental and metabolic changes that increase ROS generation (Giraudo-Billoud et al., 2019). Box 1 lists the major reactive species, antioxidants, oxidative damage products and redox-sensitive transcription factors reviewed in this manuscript.

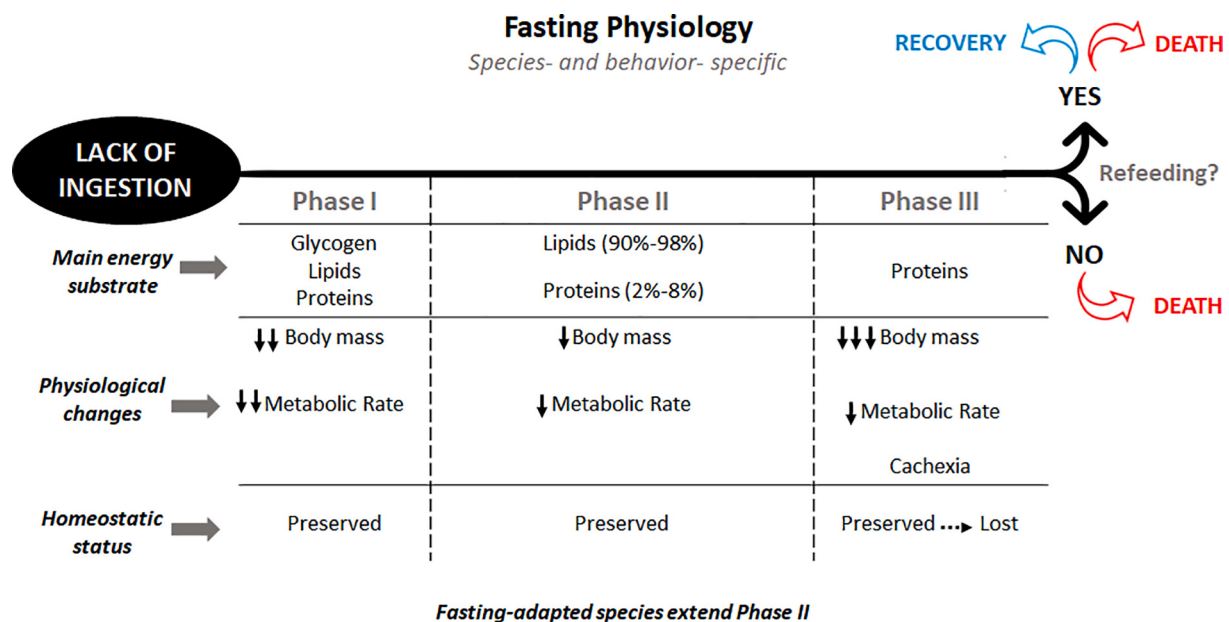


Fig. 2. Metabolic responses to fasting.

The timeline from fasting to starvation follows a continuum that is species- and behavior-specific. The predominant metabolized substrate changes for each of the 3 phases of fasting. Body mass decreases at a different pace during each phase, and metabolic rate slows progressively. Fasting-adapted species typically extend Phase II to spare protein catabolism. Phase III involves the high risk of losing homeostasis with consequent organ failure and death unless the animal refeeds to recover.

1.2. Fasting ameliorates oxidative stress: results from humans and biomedical models

Fasting and starvation are characterized by the lack of ingestion and the use of endogenous reserves as energy sources (McCue, 2010). Although these terms are often used interchangeably, fasting and starvation differ in the state of metabolic homeostasis. Fasting animals adapt to maintain homeostasis and preserve organ function. In contrast, starvation leads to the loss of homeostatic control, compromised organ function and eventually death (Castellini and Rea, 1992; McCue, 2012). Fasting begins with Phase I, a relatively short period characterized by a daily loss of body mass and a decline in metabolic rate. During Phase I, substrates derived from the last ingested meal are no longer available and body reserves of glycogen, lipids, and protein become the sources of energy to sustain cellular function (Secor and Carey, 2016). During Phase II, the use of amino acids decreases while lipid oxidation and the resultant production of ketone bodies increase (Cahill, 2006). These adjustments in substrate utilization allow maintenance of tissue function and structure (Nordøy and Blix, 1991; Castellini and Rea, 1992; Secor and Carey, 2016). A transition into Phase III occurs if feeding does not resume before the depletion of lipid stores. Phase III is characterized by increased protein catabolism (Castellini and Rea, 1992; Lindgård et al., 1992; Secor and Carey, 2016). During Phase III, loss of body mass accelerates, exacerbated by lean tissue degradation (cachexia). Phase III induces death from starvation if the animal cannot recover with refeeding (Viscarra and Ortiz, 2013; Secor and Carey, 2016). Thus, the traditional view considers that the progression of fasting/starvation follows three discrete, sequential phases defined by specific physiological changes such as loss of body mass, or by the main fuel substrate used during each phase. However, there is controversy surrounding the separation into phases, and how the continuity in the physiological responses fits in the traditional model (McCue, 2010). In clinical models, the substrate used to generate ATP is a well-known indicator of the progression from fasting to starvation: reliance on glucose oxidation at the early stage, the transition to lipid oxidation, and, finally, to lean tissue degradation and protein catabolism (Viscarra and Ortiz, 2013). Fasting-adapted species, however, exhibit extraordinary control over metabolic fuel utilization. Many fasting-adapted vertebrates (e.g. seals, penguins) consume lipid-heavy diets and likely do not meet the bulk of their energetic needs via glucose oxidation. In addition, fasting-adapted animals typically extend Phase II to spare their protein reserves, thereby prolonging vital organ function (Boismenu et al., 1992; Castellini and Rea, 1992; McCue, 2012). Fig. 2 summarizes the current understanding of fasting physiology and how fasting-adapted species differ from the traditional laboratory models.

Interventional studies with biomedical models show that fasting optimizes energy metabolism, boosts cellular protection, and reduces inflammation and oxidative stress (Longo and Mattson, 2014). Fasting also prevents the development of aging-related diseases, and is thus considered a behavioral anti-aging intervention (de Cabo et al., 2014). However, identification of the specific fasting regimens that improve health and protect against disease remains challenging. Intermittent fasting (IF) is the most commonly studied fasting regime in rodents and humans (Mattson et al., 2017; de Cabo and Mattson, 2019). During IF, the eating pattern alternates between periods with little or no energy intake (e.g., 16–48 h) and periods of regular food ingestion (Mattson et al., 2017). Of note, IF is becoming a popular alternative to caloric restriction (CR), a prolonged reduction in caloric intake without malnutrition. While CR benefits health and aging, IF appears to improve health outcomes to a greater extent (Anton et al., 2018; de Cabo and Mattson, 2019).

A reduction in oxidative stress caused by both IF and CR is one of the main positive effects of these interventions (Sohal and Weindruch, 1996; Longo and Mattson, 2014). Studies in rodents and macaques provide valuable knowledge about how IF and CR affect oxidative stress. In mice, daily 30% CR for 6 months decreases the levels of the lipid peroxidation

product 4-hydroxy-2-nonenal (4-HNE) and protein carbonyls (a marker of protein oxidation), while lowering the GSH:GSSG ratio (a marker of oxidative stress), via sirtuin 3-mediated SOD2 activation (Qiu et al., 2010). CR also attenuates oxidative stress in male rats on a high fat diet. Moreover, a 40% reduction in caloric intake over 10 weeks decreases the lipid peroxidation product malondialdehyde (MDA) and reverses the increased expression of hepatic Nrf2 and heme oxygenase-1 (HO-1). Similarly, CR normalizes SOD2 levels in adipose tissue of rats on a high fat diet (Park et al., 2012). The first study to localize and quantify oxidative damage in aging mammalian skeletal muscle included a CR intervention in rhesus macaques. Here, Zanai and colleagues found that CR attenuates age-dependent increases in 4-HNE-modified proteins in skeletal muscle after 10 years of CR (Zainal et al., 2000). Moreover, CR in old rhesus macaques prevented an increase in lipid peroxidation (plasma isoprostanes levels, 8-isoPGF) and improved the animals' metabolic profile (Mattison et al., 2012). Overall, the interventions in non-human biomedical models show a remarkable effect of both IF and CR on mitigating lipid peroxidation.

Most findings from human studies recapitulate the results from rodent and rhesus macaque work. In overweight asthmatic adults, alternate day CR for 8 weeks decreases circulating 8-isoPGF, nitrotyrosine (a marker of protein nitration), protein carbonyls, and 4-HNE. CR for 6 months also reduces DNA damage (Johnson et al., 2007). A similar but longer CR intervention lowered urinary isoprostanes while short-term fasting in women reduced urinary 8-isoPGF and MDA levels (Lee et al., 2006). Similarly, the first human trial of early time restricted feeding (a 6-hour feeding period with dinner before 3 p.m.) showed decreased 8-isoPGF levels in overweight men with diabetes (Sutton et al., 2018). Interestingly, a modified 3 week IF intervention of a combined 25% reduction in caloric intake with feasting days (consuming 175% of caloric intake) did not promote significant changes in oxidative stress markers (Wegman et al., 2015), but overall, studies in humans, macaques and rodents show that fasting ameliorates oxidative stress.

During the holy month of Ramadan, Muslims abstain from food consumption between dawn and sunset. Thus, Ramadan-related fasting (RF) presents an opportunity to study the effects of intermittent fasting on oxidative stress in humans (Alharbi et al., 2017); however, variations in diet and lifestyle accompanying RF have led to contradictory results. A recent meta-analysis examined changes in MDA in healthy subjects before and after Ramadan, concluding that RF causes a minor reduction in MDA (Faris et al., 2019). Other studies, however, show that RF decreases erythrocyte MDA and total carotenoids in plasma, but has no effect on serum MDA, plasma protein carbonyls, or other antioxidants (Ibrahim et al., 2008). Similarly, a study conducted in healthy volunteers shows that urinary 8-isoPGF levels remain unchanged during RF but increase one month later (Faris et al., 2012). In contrast, diabetic patients show improved lipid profiles and glycemic control after RF. Moreover, both diabetic patients and nondiabetic subjects show a reduction in serum MDA and increased blood GSH during the last week of RF. Furthermore, MDA levels are lower and GSH is higher after six weeks of RF compared to pre-fasting conditions (Al-Shafei, 2014). RF also increases the expression of Nrf2 and SOD2 in nondiabetic obese subjects. Therefore, RF may reduce the risk of developing obesity-related disorders, such as diabetes, in which oxidative stress constitutes a significant risk factor (Meigs et al., 2007; Madkour et al., 2019). Overall, despite contradictory results related to variations in dietary composition and lifestyle, these studies suggest that upregulation of the endogenous antioxidant defense system during RF boosts protection against oxidative stress. Together, studies in humans and biomedical models demonstrate that various fasting regimes attenuate oxidative stress, though these species are not naturally adapted to fast and the regimes studied typically limit food deprivation periods to 48 h.

In contrast to the biomedical studies described above, several vertebrates undergo prolonged periods of food deprivation as part of their life histories. In nature, fasting is an adaptive response to overcome lack

of food availability and facilitate natural biological cycles. Even in the presence of food, some species allocate their time and energy to other activities related to different life history traits instead of eating (e.g. hibernation, estivation, migration, molting, reproduction, maturation, and predator avoidance; Castellini and Rea, 1992; McCue, 2010; Secor and Carey, 2016). Here, we review the physiological strategies wild vertebrates use to prevent or attenuate oxidative stress during naturally fasting conditions.

2. Fasting as a life history component in the natural world

2.1. Mammals

A wide range of mammals utilize fasting within the context of varied life histories. Bears, ground squirrels, and bats fast while hibernating during seasons of food scarcity. Seals, in comparison, fast during several energetically expensive life history stages including breeding and post-natal development. Each of these taxa faces unique metabolic challenges with respect to managing oxidative stress while fasting.

Winter conditions typically constrain food availability, requiring animals to adopt varied metabolic and behavioral strategies to avoid starvation. Two such strategies involve altering the means by which animals obtain food during this period: some species stockpile food while others migrate to regions with greater food availability. Hibernation stands in contrast to both of these strategies; hibernating animals undertake extended fasts during which they minimize energetic requirements and rely on existing body nutrient stores rather than attempt to mitigate limited food availability. Decreased metabolic and physical activity during hibernation raises the risk of muscle atrophy and dys-regulated redox signaling (Pellegrino et al., 2011), which may negatively impact fitness upon emergence from hibernation. Despite these potential risks, bears and squirrels remain protected against excessive muscle loss and tissue injury during hibernating fasts (Lohuis et al., 2007; Wei et al., 2018; Chazarin et al., 2019).

Ground squirrels are well known for their extreme hibernations, which generally occur as bouts of torpor and arousal. During torpor squirrels allow body temperature to cool substantially, sometimes to near freezing (Hut et al., 2002). Metabolic activity is reduced up to 95% and perfusion of many tissues limited for days at a time. Periodic rewarming events (interbout arousals) driven by non-shivering thermogenesis in brown adipose tissue (BAT) increase metabolic rate and reperfuse ischemic tissues (Boyer and Barnes, 1999). During non-shivering thermogenesis metabolic substrates are consumed but ATP is not produced; rather, the cycle yields heat (Chaffee et al., 1975). The use of this strategy by a fasting animal appears paradoxical, particularly as comparable reperfusion events impact redox balance in many non-hibernating species and characterize pathological human conditions including myocardial infarction and ischemic stroke (Granger and Kvietys, 2015). Antioxidants are thus essential in preserving redox balance during hibernating fasts in squirrels, though expression profiles appear to be species- and tissue-specific, and complete avoidance of oxidative damage is not observed in all tissues.

Metabolic activity in BAT drives rewarming during interbout arousals in squirrels and this tissue thus may be at risk for oxidative stress during hibernating fasts. Nrf2 expression increases in BAT from 13-lined ground squirrels (*Spermophilus tridecemlineatus*) during all torpor stages and late arousal periods (Morin et al., 2008). Nrf2 regulates the expression of SOD and GPx (Ma, 2013); accordingly, SOD expression and activity are higher in BAT from hibernating than active European (*Citellus citellus*) and arctic ground squirrels (*Spermophilus parryi*) (Buzadzić et al., 1990; Yan et al., 2006), and no increases in lipid peroxidation (conjugated dienes) or nuclear factor kappa B, which drives ischemic inflammation, are observed in BAT from torpid 13-lined ground squirrels (Carey et al., 2000). Interestingly, expression of glucose-regulated protein 75 (GRP75), the mitochondrial form of the stress-responsive chaperone heat shock protein 70 (HSP70), is low in

BAT from torpid 13-lined ground squirrels (Carey et al., 1999), though animals were not sampled during the non-shivering thermogenesis-dependent arousal stage in this study. These data suggest that BAT remains protected against oxidative stress throughout hibernation despite potential oxidant generation by mitochondria during non-shivering thermogenesis.

Protection of the squirrel brain and heart against oxidative stress during hibernation likely depends on hypoxia-inducible factor 1 (HIF-1) and Nrf2 activation. In arctic ground squirrels, arterial P_{O_2} remains high during torpor but declines rapidly during arousal as whole animal oxygen consumption rates increase (Ma et al., 2005). Accordingly, brain HIF-1 α protein levels increase in late arousal, concomitant with declining arterial P_{O_2} (Ma et al., 2005) and there is no evidence for increased oxidative damage in the brain of hibernating versus euthermic animals (Orr et al., 2009). Nrf2 levels are highest in the brain during late torpor and early arousal in both Daurian (*Spermophilus dauricus*) and 13-lined ground squirrels (Morin et al., 2008; Wei et al., 2018). In Daurian ground squirrels, brain MDA and H_2O_2 levels also increase during late torpor (immediately prior to arousal) but decline during the arousal period, potentially due to increased Nrf2-dependent expression of the antioxidant enzymes SOD1 and GPx1 during interbout arousal (Wei et al., 2018). Heart levels of Nrf2, H_2O_2 , and MDA are similar to those observed in brain, with Nrf2 activation during late torpor and early arousal mitigating increases in MDA and H_2O_2 via increased expression of SOD1 and HO-1 (Morin et al., 2008; Wei et al., 2018). Therefore, squirrels appear to mitigate oxidative damage to the brain and heart during hibernation via upregulation of the hypoxia- and redox-sensitive transcription factors HIF-1 and Nrf2 and their target genes.

Fasting animals rely heavily on lipid stores to fuel metabolism, but several organs such as the brain remain obligate consumers of glucose. Gluconeogenesis in the liver produces most of the glucose required by glucose-dependent tissues during fasting, but this process demands the catabolism of other substrates. Torpor substantially decreases substrate oxidation and mitochondrial respiration rates in the liver of 13-lined ground squirrels, though free radical leak is higher in this tissue in torpor than during interbout arousals (Brown et al., 2012). Concomitant increases in Nrf2 and GRP75 expression in the liver during torpor and early arousal may limit leak-related oxidative damage in this tissue, though this has not been measured specifically in 13-lined ground squirrels (Carey et al., 1999; Morin et al., 2008). However, hibernating arctic ground squirrels display decreased available GSH without increasing MDA or protein carbonyls (Orr et al., 2009), and liver MDA levels decrease rapidly after torpor in Daurian ground squirrels, suggesting a high capacity for recovery after hibernation (Wei et al., 2018).

Ground squirrels appear to prioritize protection of BAT, brain, heart, and liver against oxidative stress during hibernating fasts. However, universal avoidance of oxidative damage in all tissues is energetically expensive as it requires constitutive synthesis of antioxidants and therefore may be infeasible in a fasting animal. Interestingly, lipid peroxidation levels (conjugated dienes), nuclear factor kappa B activation, and GSH oxidation increase in the gut of hibernating 13-lined ground squirrels, indicating that the ischemic, fasting gut does experience oxidative stress during hibernation, despite increased expression of GRP75 (Carey et al., 1999, 2000, 2003). Together, the above studies suggest that squirrels utilize tissue-specific strategies for managing oxidative stress during hibernating fasts.

Contrary to squirrels, hibernating bears maintain relatively high body temperatures, decoupling metabolic suppression from core temperature (Hissa et al., 1994; Tøien et al., 2011). Studies of fasting in hibernating bears have focused on skeletal muscle, blood, and liver due to technical constraints in obtaining tissues such as heart and brain. Hibernating brown bears (*Ursus arctos*) upregulate expression of cytosolic but not mitochondrial antioxidants in skeletal muscle, and down-regulate expression of mitochondrial complexes I, II and III (Chazarin et al., 2019). Nrf2-dependent antioxidant levels increase and MDA levels decrease in skeletal muscle from hibernating brown bears, suggesting

that suppressed mitochondrial activity limits oxidative damage during hibernation, and that Nrf2-dependent Prdx6 expression may prevent or repair lipid peroxidation resulting from oxidant generation (Li et al., 2015; Fisher et al., 2018; Chazarin et al., 2019). Skeletal muscle from hibernating American black bears (*Ursus americanus*) upregulates expression of genes involved in protein synthesis (Fedorov et al., 2009); this may minimize muscle atrophy and loss of strength despite inactivity during hibernation (Lohuis et al., 2007; Hershey et al., 2008). Interestingly, both the ubiquitin-proteasome and autophagy pathways are activated in skeletal muscle from Japanese black bears (*Ursus thibetanus japonicus*) one week post-hibernation, though simultaneous inhibition of myostatin appears to protect against predicted rates of atrophy in these animals (Miyazaki et al., 2019). Ecological and seasonal contexts also play a major role in bears' resistance to muscle atrophy. In polar bears (*Ursus maritimus*), winter hibernating muscle protein loss is predicted to be approximately twice as high as for black and brown bears, and these losses are further compounded by summer food shortages in polar climates (Whiteman et al., 2017).

In circulation, black bears display increased MDA levels in red blood cell membranes and plasma during hibernation (Chauhan et al., 2002), but markers of systemic inflammation (C-reactive protein, pentraxin-related protein) do not increase in hibernating brown bears (Stenvinkel et al., 2013). As red blood cells turn over more frequently than other somatic cell types, bears may tolerate limited oxidative damage to these cells while protecting other tissues. As in muscle, liver from hibernating American black bears upregulates expression of genes involved in protein synthesis, including several elongation factors. However, GPX3 and SOD2 expression were lower in the liver of hibernating versus summer active bears (Fedorov et al., 2009). Liver from hibernating Japanese black bears upregulates expression of gluconeogenesis-related genes while down-regulating expression of genes involved in amino acid catabolism. Increased hepatic expression of uncoupling protein 2 during hibernating fasts may also protect against oxidant generation in the presence of accumulated fatty acids (Shimozuru et al., 2012). Though the systems-level metabolic strategies for tolerating extended fasting differ between bears and squirrels, it appears that both employ tissue-specific strategies to mitigate oxidative damage.

Hibernating fasts in bats have been understudied but are potentially interesting from the perspective of redox balance. Active bats rely on flight, which is an energetically expensive form of locomotion that carries the potential for oxidative damage. In hibernating bat species, however, flight during active periods may stimulate a conditioning response to oxidant generation (Gómez-Cabrera et al., 2008; Gomes et al., 2012). Indeed, no changes in brain MDA were observed for two bat species (*Myotis ricketti* and *Rhinolophus ferrumequinum*) across torpor, arousal, or active states and reactive oxygen and nitrogen species generation remained relatively consistent. Brains from hibernating bat species have less MDA than non-hibernating species (*Rhinolophus leschenaultia* and *Cynopterus sphinx*), regardless of hibernation stage, suggesting that the brains of hibernating bats are protected against oxidative damage, though the predominant antioxidants vary with species (Yin et al., 2016).

In contrast to the prolonged metabolic suppression and inactivity observed in hibernating mammals, many marine mammals fast simultaneously with energy-demanding processes, including reproduction, migration, and molting (Vázquez-Medina et al., 2011a, 2011b, 2012). Among these, the interaction between oxidative stress and fasting has been most intensively studied in elephant seals (*Mirounga spp.*), which breed, lactate, molt, and mature during prolonged terrestrial fasts. Energetic output for male and female adult northern elephant seals (*Mirounga angustirostris*) fasting during the breeding season is 3 to 6 times their predicted standard metabolic rate (Crocker et al., 2001, 2012). High energy outputs in the absence of nutrient intake carry the risk of increased oxidant generation and oxidative damage, which may further exacerbate energetic demands as oxidized lipids and proteins must be

enzymatically repaired or removed. Plasma XO activity increases across the breeding season in male elephant seals, as does SOD and GPx activities. However, males show increased oxidative damage to lipids and DNA in circulation across the breeding season, suggesting that the observed increase in antioxidant activity does not completely prevent oxidative damage in these animals as the breeding season progresses (Sharick et al., 2015). Female elephant seals do not engage in physical fighting during the breeding season but must produce energy-rich milk while fasting. Plasma XO activity increases across the breeding fast in females. Catalase and GPx activities also increase, but SOD activity and vitamin C levels do not change across lactation. There is no evidence for oxidative damage to DNA or lipids associated with the lactating fast in females, though circulating levels of nitrotyrosine are higher in females later in the lactation period (Sharick et al., 2015). In addition to breeding, adult elephant seals undertake an annual terrestrial molt of approximately one month, during which the entire pelage is replaced. As described earlier, fasting and physical inactivity typically drive skeletal muscle atrophy. In elephant seals, however, muscle mass and respiratory capacity are maintained during the (physically inactive) molting fast, perhaps due to the maintenance of antioxidant gene expression, which does not decline (Worthy et al., 1992; Wright et al., 2020).

Weaned elephant seal pups fast on land for 2-3 months prior to departing on their first marine foraging trip. During this terrestrial fast pups mobilize blubber stores to fuel an increase in mass-specific blood and muscle oxygen stores, which support extended breath hold diving (Champagne et al., 2012). The renin-angiotensin system (RAS) is activated during this fast (Ortiz et al., 2000), and is known to drive oxidative damage in other species via the activation of NADPH oxidases. Despite RAS activation, and increased XO and NADPH oxidase expression and activity during the postweaning fast, elephant seal pups do not show increased oxidative damage in circulation or skeletal muscle (Vázquez-Medina et al., 2010, 2013; Soñanez-Organis et al., 2012). Protection in these animals is potentially driven by HIF-1 α stabilization, Nrf2 activation and glutathione biosynthesis, all of which increase across the fast (Vázquez-Medina et al., 2011c, 2013; Soñanez-Organis et al., 2013). Accordingly, expression and activity of the Nrf2 dependent antioxidant enzymes Prdx6, glutathione S-transferase (GST), GPx, and GR are elevated in muscle late in the fast, as are catalase expression and activity and expression of both the mitochondrial and cytosolic isoforms of SOD (Vázquez-Medina et al., 2010, 2011c). SOD, catalase, and GPx activities also increase in red blood cells across the postweaning fast (Vázquez-Medina et al., 2010). Serum vitamin C levels in these animals are maintained across the postweaning fast but are generally low, perhaps due to the increased expression and activity of enzymatic antioxidants as diving capacity develops (Boaz et al., 2012). Oxidative damage in blubber has not been comprehensively assessed in fasting elephant seal pups, but a recent transcriptomics study shows an increase in blubber GPx3 expression across the fast, while expression of peroxidase-like protein and GPx7 - both of which have lost peroxidase activity - decreases during fasting in these animals (Martinez et al., 2018). Together, the redox changes and limited oxidative stress observed during prolonged fasting in elephant seals mimic those of short duration protective fasts in humans, but elephant seals can sustain these fasts for months without incurring substantial oxidative damage.

2.2. Birds

Birds experience many challenging physiological conditions that can cause oxidative stress such as seasonal migration and breeding (Costantini et al., 2007; Colominas-Ciuró et al., 2017); these behaviors often coincide with fasting. For example, many species migrate from low to high food quality areas annually (Berthold, 2001). During these trips, migrating birds are fasting and exhibit elevated metabolic rates due to constant exercise (McWilliams et al., 2004). While lack of food during such an energetically demanding time requires these animals to rely on energy from fat and protein stores (Berthold, 2001), these fasts may be

protective by stimulating the antioxidant system (Jenni-Eiermann et al., 2014; Schull et al., 2016) or decreasing tissue-specific metabolic demands (Dewasmes et al., 1980). Similar to migration, some sea birds undergo intermittent fasting during the breeding season (Costantini and Bonadonna, 2010; Colominas-Ciuró et al., 2017; Pap et al., 2018) suggesting that the protective benefits of fasting may extend across multiple life history stages. By comparing adaptive strategies among these different life history stages, we can better understand the role of fasting in redox homeostasis in birds.

While traveling facilitates resource acquisition in migratory birds, such as European robins (*Erithacus rubecula*) and garden warblers (*Sylvia borin*), these species experience intense endurance exercise and high metabolic rates that can cause oxidative damage (Berthold, 2001; Cooper-Mullin and McWilliams, 2016). However, through fasting and other physiological adjustments, migratory birds largely avoid excessive oxidative damage. Whereas birds typically have higher basal metabolic rates than terrestrial mammals, birds' decreased proportion of electron flow across the mitochondrial membrane limits mitochondrial $O_2^{\cdot-}$ production compared to mammals (Ku and Sohal, 1993; Perez-Campo et al., 1998). Limited mitochondrial ROS generation combined with an effective enzymatic antioxidant system, elevated serum total antioxidant capacity, and optimized fuel utilization may limit oxidative damage in birds (Gutiérrez et al., 2019). Additionally, a decreased metabolic rate due to fasting may further prevent oxidative damage (Dewasmes et al., 1980). Fasting migratory birds, such as the ruff (*Philomachus pugnax*), primarily utilize lipid and protein as fuel sources, which likely generate less mitochondrial ROS than carbohydrates (Weber, 2009; Kuzmiak et al., 2012). Storing and metabolizing lipids can lead to elevated lipid peroxidation (Skríp et al., 2015), but the lipid composition of avian cells makes them less susceptible to lipid peroxidation than mammalian cells (Hulbert et al., 2007). Additionally, uric acid, a byproduct of protein catabolism, is both the main nitrogenous waste in birds (Wright, 1995) and a primary non-enzymatic antioxidant (Halliwell and Gutteridge, 1995). Taken together, these studies suggest that birds remain largely protected from oxidative stress due to the composition of their cell membranes, the type of fuel they utilize, and increased levels of non-enzymatic antioxidants while fasting.

Despite decreased ROS generation, high uric acid levels, and the potential benefits of fasting, some birds exhibit oxidative stress due to biotic and abiotic factors associated with migration (Costantini et al., 2007; Jenni-Eiermann et al., 2014; Bodey et al., 2019). European robins captured during migration show elevated protein carbonyls and GPx activity in red blood cells compared to resting birds, with both parameters negatively correlated with protein, but not fat stores (Jenni-Eiermann et al., 2014). In contrast, there are positive correlations between plasma hydroperoxides and total antioxidant capacity, and both fat, and protein stores in garden warblers and barn swallows (*Hirundo rustica*) while migrating (Costantini et al., 2007). In addition to body condition, abiotic factors such as environmental temperature are associated with elevations in lipid peroxidation (MDA) in light bellied brent geese (*Branta bernicla*; Bodey et al., 2019). Strikingly, body condition has no impact on lipid peroxidation in this species, suggesting that the relationship between redox homeostasis and body condition may be species-specific or at least tissue-specific (Wei et al., 2018). Additionally, migratory birds in general show higher MDA levels and non-enzymatic antioxidant capacity than non-migratory birds, suggesting a fundamental difference in redox balance due to migration and fasting (Eikenaar et al., 2017). While the confluence of migration and fasting can still lead to oxidative stress, behavioral strategies that decrease oxidant generation or increase antioxidant pools may prevent oxidative damage.

Birds utilize specific strategies that promote redox homeostasis to prepare for migration-induced oxidative stress. In birds partaking in long migrations, circulating antioxidant capacity increases while lipid peroxidation (thiobarbituric acid reactive substances; TBARS) decreases across the pre-migratory season (Gutiérrez et al., 2019). This physiological preparation may be increased by the consumption of food high in

dietary antioxidants, such as fruits and polychaetes (Alan et al., 2013; Martínez-Curci et al., 2015). Additionally, pre-migratory fat stores are positively associated with non-enzymatic antioxidant capacity and lipid peroxidation (Skríp et al., 2015); increased lipid peroxidation may represent a cost of increasing fat supply and utilization (Pérez-Rodríguez et al., 2015; Skrip et al., 2015). Stopovers can achieve similar elevations in antioxidant levels as pre-migratory behaviors (Skríp et al., 2015; Eikenaar et al., 2020). During these stopovers, garden warblers reduce circulating oxidative damage (hydroperoxides; Skrip et al., 2015) independent of the amount of food consumed or body fuel stores gained (Eikenaar et al., 2020). Interestingly, though oxidative damage decreases across the stopover, non-enzymatic antioxidant capacity does not vary, suggesting an alternative driver in the modulation of oxidative damage (Eikenaar et al., 2020) such as upregulation of antioxidant enzymes. Though migration is a confounding factor when assessing the relationship between fasting and oxidative stress in birds, we can tease apart patterns to elucidate the role of fasting on oxidative stress by comparing migration with other challenging physiological conditions that coincide with fasting.

Breeding, similar to migration, carries physiological costs for many avian species (Costantini and Bonadonna, 2010) as reproduction is a major driver of oxidative stress in birds (Wiersma et al., 2004; Pap et al., 2018). Increased breeding effort elevates plasma MDA, and higher hatching success is positively correlated with lipid peroxidation (as measured by TBARS) in breeding females (Alonso-Alvarez et al., 2010; Pap et al., 2018). However, while increased breeding effort did not lead to changes in survival rate between years, oxidative damage was negatively correlated and GSH levels were positively correlated with survival rates of barn swallows (Pap et al., 2018). Interestingly, during breeding both old and young birds have higher levels of oxidative damage than middle aged birds, suggesting that oxidative stress may be associated with both senescence and selective mortality (Nussey et al., 2008; Alonso-Alvarez et al., 2010). Combined, these data suggest that oxidative damage is negatively correlated with survival, but it is not clear whether breeding in particular or the related fasting drives these changes in redox homeostasis (Schull et al., 2016).

As various avian species frequently cope with fasting periods during the breeding season, these studies provide both an ecological and mechanistic lens to examine oxidative stress. In addition to the species described above, recent studies have begun to explore how fasting impacts oxidative stress in breeding penguins (Schull et al., 2016; Colominas-Ciuró et al., 2017). Initial data suggest that while short-term fasting in king penguins (*Aptenodytes patagonicus*) increases SOD activity, prolonged fasting increases hydroperoxides (Schull et al., 2016). Moreover, refeeding leads to decreased hydroperoxides, but there is a positive correlation between the rate of mass gain and hydroperoxide levels after the foraging trip in advance fasting individuals only (Schull et al., 2016). These results suggest that while fasting-derived oxidative damage is largely avoided in king penguins, there may be potential long-term effects on oxidative damage from foraging due to increased foraging effort or fasting duration. Fasting-associated increases in antioxidants may protect penguins from oxidant generation during breeding, as neither incubation nor chick rearing while fasting increases hydroperoxide levels (Colominas-Ciuró et al., 2017), however, antioxidant levels (total plasma antioxidant capacity and total non-protein thiols) decrease in incubating adult Magellanic penguins (*Spheniscus magellanicus*). These data suggest that incubating eggs may be more demanding than chick rearing due to potential antioxidant depletion (Colominas-Ciuró et al., 2017). Therefore, fasting may help limit oxidative stress in penguins, however, the underlying mechanism of this effect and its applicability to other species are still unknown.

Examining the impacts of fasting on mitochondrial function and metabolic hormones may help elucidate the mechanisms by which fasting mitigates oxidative stress. Early work shows that fasting in king penguins decreases mitochondrial oxygen consumption and uncoupling protein activity (Rey et al., 2008). While decreased mitochondrial

respiration could limit oxidant generation, a decrease in uncoupling proteins would undermine this effect (Harper et al., 2004). A recent study proposed that animals living in extreme conditions may have evolved physiological mechanisms to protect against environmental or oxidative stress (Stier et al., 2019). In king penguins, elevated basal levels of glucocorticoids are positively correlated with increased GSH and decreased mitochondrial efficiency (Stier et al., 2019). There are likely other mechanisms modulating redox homeostasis besides glucocorticoids, as basal glucocorticoids increase during advanced fasting in adults (Cherel et al., 1988) but not in chicks (Le Ninan et al., 1988). However, the increase in glucocorticoids due to fasting is present in other species and offers a promising avenue to understand how fasting regulates redox balance (Angelier et al., 2015). House sparrows (*Passer domesticus*) show similar results to king penguins as glucocorticoids decrease MDA in these animals (Vágási et al., 2018), suggesting that the protective nature of glucocorticoids and fasting may be broader than in *Spheniscidae* alone. Blue petrels (*Halobaena caerulea*) that fast for long periods have higher antioxidant capacity and lower oxidative damage than shorter fasting Antarctic Prions (*Pachyptila desolata*; Costantini and Bonadonna, 2010), which further supports the idea of a protective role for fasting in redox homeostasis. Taken together, data from birds corroborates mammalian studies that show a protective effect of fasting on oxidative stress. Across endothermic species and life history stages, fasting is associated with elevated antioxidants and decreased oxidative damage. Due to a temporal overlap between fasting and physiologically expensive activities such as reproduction and migration, the mechanisms underlying such effects remain largely elusive, however, studies in birds highlight the potential roles of fuel utilization, mitochondrial function, and glucocorticoids in sustaining redox balance in naturally fasting animals.

2.3. Reptiles and amphibians

Fasting-associated metabolic depression allows ectothermic vertebrates to optimize the use of endogenous energy reserves in challenging environmental conditions. Some amphibians and reptiles can reduce their metabolic rate by 60–90% and fast for over a year (Secor and Carey, 2016). Fluctuations in tissue oxygen availability during arousal, however, likely increase ROS generation. Thus, preparation for oxidative stress (Hermes-Lima et al., 2015) or rapid increases in antioxidant defenses during arousal are important strategies for preventing or mitigating oxidative damage.

Similar to mammals, many reptiles and amphibians cope with oxidative stress using physiological and metabolic adjustments associated with fasting. Tegu lizards (*Salvator merianae*) decrease their metabolic rate by 80% upon entering dormancy during subtropical hibernation. This transition decreases intestinal GSH, GPx, and GST, but lipid hydroperoxides also decrease, suggesting that winter dormancy in Tegu lizards does not induce oxidative stress despite antioxidant depletion (Moreira et al., 2018). In contrast, the European common lizard (*Lacerta vivipara*) survives freezing during hibernation by upregulating SOD and GPx (Voituron et al., 2006). Transcriptomic and proteomic analyses in hibernating Central bearded dragons (*Pogona vitticeps*) show upregulation of genes and proteins involved in antioxidant defense and mitochondrial upkeep in the brain, muscle and heart (Capraro et al., 2019). Moreover, freezing in wood frogs (*Rana sylvatica*) increases ferritin heavy chain and SOD2 protein abundance (Gupta et al., 2020), while freezing in Garter snakes (*Thamnophis sirtalis parietalis*) increases muscle GSH, catalase and GPx, along with lung catalase, suggesting that freezing activates systems involved in peroxide removal in preparation for increased ROS generation during thawing (Hermes-Lima and Storey, 1993). Likewise, grass snakes (*Natrix natrix*) increase GST activity after hibernation. This increase likely mitigates oxidative damage associated with reoxygenation, possibly via reduction of lipid hydroperoxides (Yang et al., 2001), despite decreases in GR and catalase in this species (Gavric et al., 2015). Paradoxically, high metabolic rates

may also help reptiles avoid oxidative damage during arousal. Grass snakes acclimated to warm temperatures (32°C) have 7-fold higher metabolic rates compared to cold-acclimated snakes (18°C). Hydroperoxide levels are lower in warm- than in cold-acclimated snakes, suggesting that high metabolic rates help limit oxidative stress (Bury et al., 2018). While the mechanism underlying such reductions in lipid hydroperoxides remains unknown, the glutathione system plays an integral role in this process and overall maintenance of redox homeostasis across many species of hibernators.

In contrast to the protection against oxidative stress described above, some reptiles and amphibians incur selective oxidative damage associated with hibernating fasts. The soft-shelled turtle (*Pelodiscus sinensis*) experiences lipid peroxidation (as measured by TBARS) in the spleen, in which vitamin C levels are nearly exhausted during hibernation (Chen et al., 2019), while the heart, brain and liver remain protected (Baker et al., 2007). These animals activate Nrf2 and upregulate SOD, catalase, and GPx3 expression upon arousal (Zhang et al., 2017). Vitamin C levels, which decrease during hibernation in most soft-shelled turtle tissues, recover to pre-hibernating or even higher levels and are associated with decreased splenic MDA during arousal (Chen et al., 2019). Similarly, increased vitamin C levels during arousal from hibernation provide neuronal cytoprotection to pond slider turtles (*Trachemys scripta*) (Rice et al., 2002). In contrast, Asian toads (*Duttaphrynus melanostictus*) display increased lipid peroxidation (as measured by TBARS), protein oxidation and a shift toward GSSG in the liver and brain during hibernation despite high vitamin C levels (Sahoo and Patnaik, 2020), and hibernation suppresses antioxidant defenses inducing oxidative stress in *Nanorana parkeri* frogs (Niu et al., 2018). Overall, these results are consistent with findings in hibernating mammals, which show species-, tissue-, and time-specific patterns of antioxidant protection. Moreover, these results also suggest that some species including Asian toads and *N. parkeri* frogs likely mitigate oxidative damage by upregulating antioxidant defenses upon arousal.

Dry conditions, high temperatures, and low food availability pose a threat to ectotherms; some frog species estivate to mitigate the detrimental effects of these conditions. In green-striped burrowing frogs (*Cyclorana alboguttata*) estivation decreases metabolism by 70%, muscle and heart mitochondrial H₂O₂ generation by 88–94% (Reilly et al., 2014), and causes global changes in gene expression (Reilly et al., 2013). Muscle genes differentially expressed during this period participate in cytoskeletal remodeling, energy metabolism, redox homeostasis, the cellular stress response and apoptotic signaling (Reilly et al., 2013). Moreover, estivation maintains or increases SOD activity and expression, HSP70 abundance, and total antioxidant capacity; accordingly, lipid peroxidation (MDA) does not increase during estivation (Hudson et al., 2006; Young et al., 2013a, 2013b). Similarly, the Brazilian Caatinga's frog (*Proceratophrys cristiceps*) prepares for arousal-induced ROS generation by increasing GSH, catalase and GPx during estivation (Moreira et al., 2020). Thus, transcriptional and biochemical changes during estivation help frogs conserve cellular functions and avoid oxidative stress and muscle atrophy during arousal.

Hibernation and estivation represent energy-saving strategies which coincide with fasting. In contrast, some reptiles also fast during energetically expensive capital breeding seasons. In the asp viper (*Vipera aspis*) reproduction increases lipid peroxidation despite upregulation of antioxidant defenses in pregnant snakes (Stier et al., 2017), suggesting that oxidative stress is a cost of reproduction. In loggerhead sea turtles (*Caretta caretta*) plasma SOD decreases as the nesting season progresses (Perrault and Stacy, 2018). Although oxidative damage markers have not been measured concurrently, these results suggest that oxidative damage may be a cost of reproduction in sea turtles. Thus, while most fasting amphibians and reptiles avoid oxidative damage derived from hibernation and estivation, oxidative stress is likely a trade-off for reproduction in fasting capital breeders.

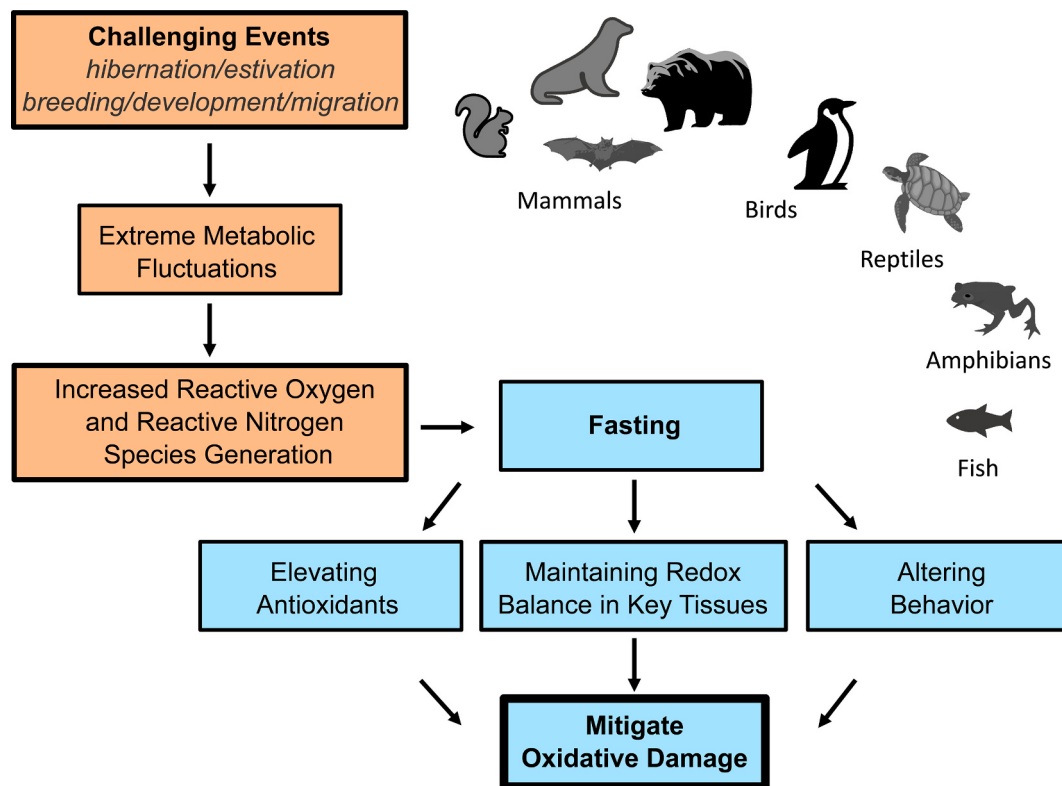


Fig. 3. Responses that mitigate oxidative stress in fasting-adapted species.

Hibernation, estivation, breeding, maturation, or migration alter metabolic rates in a variety of animals. These fluctuations often elevate rates of reactive oxygen and reactive nitrogen species generation, increasing the potential for oxidative damage. Fasting-adapted organisms such as seals, bears, squirrels, penguins, and turtles elevate antioxidant levels, prioritize redox balance in key tissues, and alter behavior to mitigate oxidative damage.

2.4. Fish

Temperate salmonids fast during migration, though their fasting strategies vary widely. Adult salmonids incur reproductive costs due to long migrations (Healey, 2001). In contrast, parr optimize migration using bimodal feeding; they either fast during the autumn-winter months and defer migration for a year, or feed and migrate sooner (Metcalf and Thorpe, 1992). Additionally, when exposed to cold temperatures, trouts (Salmonidae) stop feeding or defer food assimilation (Simpkins and Hubert, 2000). While some studies have measured the effects of fasting on oxidative stress during natural conditions, most reports evaluate changes in redox homeostasis during experimental conditions in salmonids and other teleost species.

Fasting promotes cold resistance in zebrafish by modulating lipid catabolism and autophagy, and by increasing SOD, GPx, and HSPA5 expression (Danio rerio; Lu et al., 2019). Similarly, fasting upregulates catalase, GPx, GR, and mitochondrial and cytosolic SOD activities in estivating African slender lungfish (*Protopterus dolloi*; Page et al., 2010). Moreover, intermittent fasting increases liver activity of SOD and lysozyme in crucian carp (*Carassius auratus*; Li et al., 2019). Short-term fasting (2 days) simulates Nrf2 and upregulates SOD1, SOD2, catalase GPx, and GST in skeletal muscle of juvenile Chinese perch (*Siniperca chuatsi*; Wu et al., 2020), while fasting for seven days increases GST, GR, GPx and catalase activities in catfish (*Hoplosternum littorale*) gills (Rossi et al., 2015), and hepatic SOD, GPx, GST, and catalase expression in rockbream (*Oplegnathus fasciatus*; Nam et al., 2005). Overall, these data show that fasting upregulates enzymatic antioxidants across multiple fish species.

In contrast, experimental long-term (21–28 days) food deprivation increases lipid peroxidation (as measured by TBARS) in catfish (Rossi et al., 2015), and decreases hepatic GST and GPx expression in rainbow trout (*Oncorhynchus mykiss*; Salem et al., 2007) and zebrafish (Drew

et al., 2008). Moreover, food deprivation for two weeks reduces hepatic metabolic capacity while increasing mitochondrial H_2O_2 generation in brown trout (*Salmo trutta*; Salin et al., 2018). These results show that while short-term fasting prevents oxidative stress by modulating endogenous antioxidants in fish, prolonged food deprivation promotes ROS generation and oxidative damage (Furne and Sanz, 2018).

Detrimental effects of long migrations in salmon include reduced offspring output and oxidative stress (Shpak and Proulx, 2007). Hepatic lipid peroxidation increases through the return journey in the kidney of Chinook salmon, *Oncorhynchus tshawytscha*. Similarly, lipid hydroperoxides increase with migration in juvenile Chinook salmon while vitamin E levels decrease (Welker and Congleton, 2005). Moreover, hepatic and ovary thiamine (vitamin B1) concentrations decrease along the spawning run in Atlantic salmon (*Salmo salar*). These changes are associated with increased hepatic MDA and decreased glucose-6-phosphate dehydrogenase activity (Vuorinen et al., 2020), which is critical for maintaining intracellular GSH levels (Salvemini et al., 1999). Moreover, the antioxidant response is tissue-specific in Pacific salmon (*Oncorhynchus* spp.) in which DNA damage and total antioxidant capacity increase at the end of migration in plasma and brain, but not in liver, red, or white muscle (Wilson et al., 2014). Overall, these studies suggest that long migrations promote oxidative stress in salmon by decreasing antioxidant defenses.

Behavioral strategies during reproductive migrations ameliorate oxidative stress in glass eels (*Anguilla anguilla*). Eel hatchlings can become temporary estuarine residents, which have lower oxidative stress compared to their oceanic counterparts (Bolliet et al., 2017). Similarly, salmon undergo a pre-migration fasting period triggered by fluctuations in water chemistry (e.g., salinity, pH), which stimulates oxidative phosphorylation and glycolytic potential, likely decreasing ROS generation (Miller et al., 2009). Moreover, migrating salmonids (*Salmo trutta*) have higher GSH levels and total antioxidant capacity than

Table 1

Protective redox mechanisms altered by fasting across different life history events and taxa.

	Taxonomic group	Life history event	Redox strategy	Citation
Mammals	Squirrels	Hibernation	↑ NRF2, HIF-1α, SOD1, GPx1, HO-1, GRP75 ↓ body temp, metabolic rate	Carey et al. (1999, 2000, 2003); Ma et al. (2005) Morin et al. (2008); Ma (2013); Wei et al. (2018)
	Bears	Hibernation	↑ NRF2, Prdx6, uncoupling protein 2 ↓ mitochondrial electron transfer chain complexes I, II, and III	Chauhan et al. (2002); Fedorov et al. (2009); Shimozuru et al. (2012); Stenvinkel et al. (2013); Li et al. (2015); Chazarin et al. (2019)
	Bats	Hibernation	↑ antioxidants, conditioning response	Gómez-Cabrera et al. (2008); Gomes et al. (2012); Yin et al. (2016)
	Elephant seals	Breeding, maturation	↑ Nrf2, XO, SOD, GPx, catalase, HIF-1α, GSH, Prdx6, GST, GR	Vázquez-Medina et al. (2010, 2011c, 2013); Soñanez-Organis et al. (2012, 2013); Sharick et al. (2015)
Birds	Migratory birds	Migration	↑ Lipid and protein utilization, GPx, glucocorticoids, consumption of foods high in antioxidants, antioxidant upregulation ↓ mitochondrial O ₂ ⁻ generation, metabolic rate, carbohydrate utilization	Ku and Sohal (1993); Perez-Campo et al. (1998); Hulbert et al. (2007); Weber (2009); Kuzmiak et al. (2012); Jenni-Eiermann et al. (2014); Eikenaar et al. (2017, 2020); Vágási et al. (2018)
	Penguins	Breeding	↑ SOD, GSH, glucocorticoids ↓ mitochondrial oxygen consumption	Rey et al. (2008); Schull et al. (2016); Colominas-Ciuró et al. (2017); Stier et al. (2019)
Reptiles	Lizards, frogs, snakes, turtles	Dormancy, hibernation, arousal	↑ Nrf2, SOD, GPx, GSH, catalase, GST, vitamin C, mitochondrial upkeep	Hermes-Lima and Storey (1993); Yang et al. (2001); Rice et al. (2002); Voituron

Table 1 (continued)

	Taxonomic group	Life history event	Redox strategy	Citation
Amphibians	Frogs	Estivation, arousal	↑ SOD, HSP70, GSH, catalase, GPx	et al. (2006); Gavric et al. (2015); Zhang et al. (2017); Bury et al. (2018); Moreira et al. (2018); Capraro et al. (2019); Chen et al. (2019)
				Hudson et al. (2006); Young et al. (2013a, 2013b); Moreira et al. (2020)
Fish	Fish	Cold exposure, migration, reproduction	↑ Nrf2, SOD, GPx, HSPa5, catalase, GPx, GR, lysozyme, GST, glycolytic potential, GSH, total antioxidant capacity, HSP70 Choose to migrate or not	Page et al. (2010); Nam et al. (2005); Miller et al. (2009); Iq and Shu-Chien (2011); Rossi et al. (2015); Birnie-Gauvin et al. (2017); Bolliet et al. (2017); Lu et al. (2019); Li et al. (2019)

residents, and future migration date is negatively correlated with antioxidant capacity, suggesting that antioxidant potential is associated with migratory capacity in salmonids (Birnie-Gauvin et al., 2017). Overall, these results show that behavioral strategies and environmentally-dependent antioxidant responses mitigate migration-induced oxidative stress in glass eels and migrating salmon.

Fasting associated with mouthbrooding (Faber-Hammond et al., 2019), increases circulating hydroperoxides while decreasing gill catalase expression in East African cichlids (*Astatotilapia burtoni*; Keller et al., 2017; Sawecki et al., 2019). In contrast, mouthbrooding increases abundance of HSP70 and natural killer cell enhancing factor, which has peroxidase activity, in oral mucus of tilapia fish (*Oreochromis spp.*; Iq and Shu-Chien, 2011). In smallmouth bass (*Micropterus dolomieu*), parental care decreases feeding opportunities (Hanson et al., 2009), but does not increase lipid hydroperoxides, protein or DNA oxidation (Wilson et al., 2012). Thus, these studies show that while energetically expensive reproductive behaviors that limit food consumption alter redox homeostasis in fish, they do not always result in oxidative damage. This further highlights the importance of measuring antioxidants as well as oxidative damage markers when examining oxidative stress.

3. Conclusions and future directions

Prolonged food deprivation in wild vertebrates accompanies a range of extreme or energetically expensive life history events. Notably, fasting-adapted animals avoid starvation during prolonged food deprivation by using lipids as a fuel source, effectively extending Phase II, but increasing the risk of lipid peroxidation. Oxidative stress is also a potential consequence of oxygen fluctuations resulting from prolonged metabolic suppression and inactivity (hibernation, estivation) or

energetically-demanding processes (migration, reproduction), which may jeopardize fitness. Naturally fasting-tolerant vertebrates, however, are well-equipped to handle oxidative stress associated with these life history events and share some similarities (Fig. 3), though some of the molecular strategies for coping with these conditions appear to be species-, tissue-, and life history stage-specific (Table 1). Despite the broad range of strategies employed, the redox-sensitive transcription factor Nrf2 plays a major role in the maintenance of redox homeostasis during fasting across taxa. Additionally, selective maintenance of redox balance in specific tissues such as the brain and heart suggests that complete prevention of oxidative damage is often prohibitively expensive and that animals can maintain fitness by protecting key tissues while allowing selective injury in other repairable systems such as lipid stores.

Disentanglement of the metabolic and molecular components of fasting from concurrent life states (e.g. migration, reproduction) remains challenging, but expanded sampling regimes, inter-species comparisons, and a combination of field and laboratory studies when possible, will bolster our current understanding of how fasting-tolerant species cope with metabolic challenges while preventing oxidative damage. Moreover, the study of these physiological responses in naturally fasting-adapted species can provide a better understanding of the protective effects of fasting in non-adapted species including humans, with subsequent implications for the prevention of metabolic and age-associated pathologies and possibly lifespan extension.

In addition to the myriad strategies employed by different taxa for coping with prolonged fasting, generalization is further complicated by the range of assays employed in the studies reviewed in this manuscript. TBARS, a popular colorimetric assay of lipid peroxidation, is not specific for lipid peroxidation products (MDA) unless these are detected by mass spectroscopy. F2-isoprostane levels are a more accurate measure of lipid peroxidation, though true specificity is again only achieved using mass spectroscopy (Forman et al., 2015). The determination of protein carbonylation is the most extensively used marker for protein oxidation, but not all the methods are equal in robustness, accuracy, and reliability (Suzuki et al., 2010; Rogowska-Wrzesinska et al., 2014). Though fasting regimes differ between natural events in wild animals and interventions in humans and laboratory models, the similarities in the redox response underscore potentially similar adaptive mechanisms behind the beneficial effects of fasting on ameliorating oxidative stress in humans.

Declaration of Competing Interest

None.

Acknowledgements

DCE is supported by the National Science Foundation Postdoctoral Fellowship in Biology Program (Grant # DBI 1907155). BGA is supported by the UC Berkeley Chancellor's Fellowship. KNA is supported by the National Science Foundation Graduate Research Fellowship Program (Grant # DGE 1752814) and a UC Berkeley Fellowship. JPV-M is supported by UC Berkeley startup funds.

References

- Alan, R.R., McWilliams, S.R., McGraw, K.J., 2013. The importance of antioxidants for avian fruit selection during autumn migration. *Wilson J. Ornithol.* 125, 513–525.
- Alharbi, T.J., Wong, J., Markovic, T., Yue, D., Wu, T., Brooks, B., Hetherington, J., Seimon, R., Gibson, A.A., Toth, K., Silveira, S., Sainsbury, A., Little, T.J., 2017. Brief report. Ramadan as a model of intermittent fasting: effects on body composition, metabolic parameters, gut hormones and appetite in adults with and without type 2 diabetes mellitus. *Obes. Med.* 6, 15–17.
- Alonso-Alvarez, C., Pérez-Rodríguez, L., García, J.T., Viñuela, J., Mateo, R., 2010. Age and breeding effort as sources of individual variability in oxidative stress markers in a bird species. *Physiol. Biochem. Zool.* 83, 110–118.
- Al-Shafei, A.I., 2014. Ramadan fasting ameliorates oxidative stress and improves glycemic control and lipid profile in diabetic patients. *Eur. J. Nutr.* 53, 1475–1481.

- Angelier, F., Wingfield, J.C., Parenteau, C., Pellé, M., Chastel, O., 2015. Does short-term fasting lead to stressed-out parents? A study of incubation commitment and the hormonal stress responses and recoveries in snow petrels. *Horm. Behav.* 67, 28–37.
- Anton, S.D., Moehl, K., Donahoo, W.T., Marosi, K., Lee, S.A., Mainous, A.G., Leeuwenburgh, C., Mattson, M.P., 2018. Flipping the metabolic switch: understanding and applying the health benefits of fasting. *Obesity* 26, 254–268.
- Babcock, G.T., 1999. How oxygen is activated and reduced in respiration. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12971–12973.
- Babior, B.M., 1999. NADPH oxidase: an update. *Blood, J. Am. Soc. Hematol.* 93, 1464–1476.
- Baker, P.J., Costanzo, J.P., Lee, R.E., 2007. Oxidative stress and antioxidant capacity of a terrestrially hibernating hatchling turtle. *J. Comp. Physiol. B* 177, 875–883.
- Berthold, P., 2001. *Bird Migration: A General Survey*, 2nd ed. Oxford University Press, New York.
- Birnie-Gauvin, K., Peiman, K.S., Larsen, M., Baktoft, H., Aarestrup, K., Willmore, W., Cooke, S.J., 2017. Oxidative stress and partial migration in a salmonid fish. *Can. J. Zool.* 95, 829–835.
- Boaz, S.M., Champagne, C.D., Fowler, M.A., Houser, D.H., Crocker, D.E., 2012. Water-soluble vitamin homeostasis in fasting northern elephant seals (*Mirounga angustirostris*) measured by metabolomics analysis and standard methods. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 161, 114–121.
- Bodey, T.W., Cleasby, I.R., Blount, J.D., Vigfusdottir, F., Mackie, K., Bearhop, S., 2019. Measures of oxidative state are primarily driven by extrinsic factors in a long-distance migrant. *Biol. Lett.* 15, 20180750.
- Boismenu, C., Gauthier, G., Larochelle, J., 1992. Physiology of prolonged fasting in greater snow geese (*Chen caerulescens atlantica*). *Auk* 109, 511–521.
- Bokov, A., Chaudhuri, A., Richardson, A., 2004. The role of oxidative damage and stress in aging. *Mech. Ageing Dev.* 125, 811–826.
- Bolliet, V., Claveau, J., Jarry, M., Gonzalez, P., Baudrimont, M., Monperrus, M., 2017. Migratory behavior, metabolism, oxidative stress and mercury concentrations in marine and estuarine European glass eels (*Anguilla anguilla*). *Physiol. Behav.* 169, 33–40.
- Boyer, B., Barnes, B., 1999. Molecular and metabolic aspects of mammalian hibernation. *Bioscience* 49, 713–724.
- Brown, J.C.L., Chung, D.J., Belgrave, K.R., Staples, J.F., 2012. Mitochondrial metabolic suppression and reactive oxygen species production in liver and skeletal muscle of hibernating thirteen-lined ground squirrels. *Am. J. Phys. Regul. Integr. Comp. Phys.* 302, R15–R28.
- Bury, S., Cichoń, M., Bauchinger, U., Sadowska, E.T., 2018. High oxidative stress despite low energy metabolism and vice versa: Insights through temperature acclimation in an ectotherm. *J. Therm. Biol.* 78, 36–41.
- Butler, J., Halliwell, B., 1982. Reaction of iron-EDTA chelates with the superoxide radical. *Arch. Biochem. Biophys.* 218, 174–178.
- Buzadzić, B., Spasić, M., Saicić, Z.S., Radojčić, R., Petrović, V.M., Halliwell, B., 1990. Antioxidant defenses in the ground squirrel *Citellus citellus*. 2. The effect of hibernation. *Free Radic. Biol. Med.* 9, 407–413.
- de Cabo, R., Mattson, M.P., 2019. Effects of intermittent fasting on health, aging, and disease. *N. Engl. J. Med.* 381, 2541–2551.
- de Cabo, R., Carmona-Gutierrez, D., Bernier, M., Hall, M.N., Madeo, F., 2014. The search for antiaging interventions: from elixirs to fasting regimens. *Cell* 157, 1515–1526.
- Cahill, G.F., 2006. Fuel metabolism in starvation. *Annu. Rev. Nutr.* 26, 1–22.
- Capraro, A., O'Meally, D., Waters, S.A., Patel, H.R., Georges, A., Waters, P.D., 2019. Waking the sleeping dragon: gene expression profiling reveals adaptive strategies of the hibernating reptile *Pogona vitticeps*. *BMC Genomics* 20, 460.
- Carey, H.V., Sills, N.S., Gorham, D.A., 1999. Stress proteins in mammalian hibernation. *Am. Zool.* 39, 825–835.
- Carey, H.V., Frank, C.L., Seifert, J.P., 2000. Hibernation induces oxidative stress and activation of NF-κB in ground squirrel intestine. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 170, 551–559.
- Carey, H.V., Rhoads, C.A., Aw, T.Y., 2003. Hibernation induces glutathione redox imbalance in ground squirrel intestine. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 173, 269–276.
- Castellini, M.A., Rea, L.D., 1992. The biochemistry of natural fasting at its limits. *Experientia* 48, 575–582.
- Chaffee, R.R.J., Allen, J.R., Arine, R.M., Fineg, J., Rochelle, R.H., Rosander, J., 1975. Studies on thermogenesis in brown adipose tissue in temperature-acclimated *Macaca mulatta*. *Comp. Biochem. Physiol. A Physiol.* 50, 303–306.
- Champagne, C.D., Crocker, D.E., Fowler, M.A., Houser, D.S., 2012. Fasting physiology of the pinnipeds: the challenges of fasting while maintaining high energy expenditure and nutrient delivery for lactation. In: McCue, M.D. (Ed.), *Comparative Physiology of Fasting, Starvation, and Food Limitation*. Springer, Berlin, Heidelberg, pp. 309–336.
- Chance, B., Sies, H., Boveris, A., 1979. Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* 59, 527–605.
- Chauhan, V.P.S., Tsiouris, J.A., Chauhan, A., Sheikh, A.M., Brown, W.T., Vaughan, M., 2002. Increased oxidative stress and decreased activities of Ca²⁺/Mg²⁺-ATPase and Na⁺/K⁺-ATPase in the red blood cells of the hibernating black bear. *Life Sci.* 71, 153–161.
- Chazarin, B., Ziemianin, A., Evans, A.L., Meugnier, E., Loizon, E., Chery, I., Arnemo, J. M., Swenson, J.E., Gauquelin-Koch, G., Simon, C., Blanc, S., Lefai, E., Bertile, F., 2019. Limited oxidative stress favors resistance to skeletal muscle atrophy in hibernating brown bears (*Ursus arctos*). *Antioxidants (Basel, Switzerland)*, 8.
- Cheeseman, K.H., Slater, T.F., 1993. An introduction to free radical biochemistry. *Br. Med. Bull.* 49, 481–493.

- Chen, B., Niu, C., Yuan, L., Zhang, W., 2019. Physiological responses in vitamin C system during hibernation in juvenile Chinese soft-shelled turtle *Pelodiscus sinensis*. *J. Oceanol. Limnol.* 37, 767–776.
- Cherel, Y., Robin, J.P., Walch, O., Karmann, H., Netchitailo, P., Le Maho, Y., 1988. Fasting in king penguin. I. Hormonal and metabolic changes during breeding. *Am. J. Physiol. Integr. Comp. Physiol.* 254, R170–R177.
- Cho, K.-J., Seo, J.-M., Kim, J.-H., 2011. Bioactive lipoygenase metabolites stimulation of NADPH oxidases and reactive oxygen species. *Mol. Cell* 32, 1–5.
- Cohen, G., Hochstein, P., 1963. Glutathione peroxidase: the primary agent for the elimination of hydrogen peroxide in erythrocytes. *Biochemistry* 2, 1420–1428.
- Colominas-Ciuró, R., Bertellotti, M., Carabajal, E., D'Amico, V.L., Barbosa, A., 2017. Incubation increases oxidative imbalance compared to chick rearing in a seabird, the Magellanic penguin (*Spheniscus magellanicus*). *Mar. Biol.* 164, 99.
- Cooper-Mullin, C., McWilliams, S.R., 2016. The role of the antioxidant system during intense endurance exercise: lessons from migrating birds. *J. Exp. Biol.* 219, 3684–3695.
- Costantini, D., Bonadonna, F., 2010. Patterns of variation of serum oxidative stress markers in two seabird species. *Polar Res.* 29, 30–35.
- Costantini, D., Cardinale, M., Carere, C., 2007. Oxidative damage and anti-oxidant capacity in two migratory bird species at a stop-over site. *Comp. Biochem. Physiol. Toxicol. Pharmacol.* 144, 363–371.
- Crocker, D.E., Williams, J.D., Costa, D.P., Le Boeuf, B.J., 2001. Maternal traits and reproductive effort in northern elephant seals. *Ecology* 82, 3541–3555.
- Crocker, D.E., Houser, D.S., Webb, P.M., 2012. Impact of body reserves on energy expenditure, water flux, and mating success in breeding male northern elephant seals. *Physiol. Biochem. Zool.* 85, 11–20.
- Davies, M.J., Fu, S., Wang, H., Dean, R.T., 1999. Stable markers of oxidant damage to proteins and their application in the study of human disease. *Free Radic. Biol. Med.* 27, 1151–1163.
- Dean, R.T., Gieseg, S., Davies, M.J., 1993. Reactive species and their accumulation on radical-damaged proteins. *Trends Biochem. Sci.* 18, 437–441.
- Dewasmes, G., Le Maho, Y., Cornet, A., Groscolas, R., 1980. Resting metabolic rate and cost of locomotion in long-term fasting emperor penguins. *J. Appl. Physiol.* 49, 888–896.
- Dickinson, D.A., Forman, H.J., 2002. Glutathione in defense and signaling: lessons from a small thiol. *Ann. N. Y. Acad. Sci.* 973, 488–504.
- Drew, R.E., Rodnick, K.J., Settles, M., Wacyk, J., Churchill, E., Powell, M.S., Hardy, R.W., Murdoch, G.K., Hill, R.A., Robison, B.D., 2008. Effect of starvation on transcriptomes of brain and liver in adult female zebrafish (*Danio rerio*). *Physiol. Genomics* 35, 283–295.
- Eikehaar, C., Källstig, E., Andersson, M.N., Herrera-Dueñas, A., Isaksson, C., 2017. Oxidative challenges of avian migration: a comparative field study on a partial migrant. *Physiol. Biochem. Zool.* 90, 223–229.
- Eikehaar, C., Winslott, E., Hessler, S., Isaksson, C., 2020. Oxidative damage to lipids is rapidly reduced during migratory stopovers. *Funct. Ecol.* 34, 1215–1222.
- Faber-Hammond, J.J., Coyle, K.P., Bacheller, S.K., Roberts, C.G., Mellies, J.L., Roberts, R. B., Renn, S.C.P., 2019. The intestinal environment as an evolutionary adaptation to mouthbrooding in the Astotilapia burtoni cichlid. *FEMS Microbiol. Ecol.* 95.
- Faris, M.A.-I.E., Hussein, R.N., Al-Kurd, R.A., Al-Farajeh, M.A., Bustanji, Y.K., Mohammad, M.K., 2012. Impact of Ramadan intermittent fasting on oxidative stress measured by urinary 15-f(2t)-isoprostane. *J. Nutr. Metab.* 2012, 802924.
- Faris, M.A.-I.E., Jahrami, H.A., Obaideen, A.A., Madkour, M.I., 2019. Impact of diurnal intermittent fasting during Ramadan on inflammatory and oxidative stress markers in healthy people: Systematic review and meta-analysis. *J. Nutr. Intermed. Metab.* 15, 18–26.
- Fedorov, V.B., Goropashnaya, A.V., Tøien, Ø., Stewart, N.C., Gracey, A.Y., Chang, C., Qin, S., Pertea, G., Quackenbush, J., Showe, L.C., Showe, M.K., Boyer, B.B., Barnes, B.M., 2009. Elevated expression of protein biosynthesis genes in liver and muscle of hibernating black bears (*Ursus americanus*). *Physiol. Genomics* 37, 108–118.
- Fernandez-Marcos, P.J., Nóbrega-Pereira, S., 2016. NADPH: new oxygen for the ROS theory of aging. *Oncotarget* 7, 50814–50815.
- Fisher, A.B., Vasquez-Medina, J.P., Dodia, C., Sorokina, E.M., Tao, J.-Q., Feinstein, S.I., 2018. Peroxiredoxin 6 phospholipid hydroperoxidase activity in the repair of peroxidized cell membranes. *Redox Biol.* 14, 41–46.
- Florence, T.M., 1984. The production of hydroxyl radical from hydrogen peroxide. *J. Inorg. Biochem.* 22, 221–230.
- Forman, H.J., Augusto, O., Brigelius-Flohe, R., Dennery, P.A., Kalyanaram, B., Ischiropoulos, H., Mann, G.E., Radi, R., Roberts, L.J., Vina, J., Davies, K.J.A., 2015. Even free radicals should follow some rules: a guide to free radical research terminology and methodology. *Free Radic. Biol. Med.* 78, 233–235.
- Furne, M., Sanz, A., 2018. Starvation in fish – sturgeon and rainbow trout as examples. In: Preedy, V., Patel, V.B. (Eds.), *Handbook of Famine, Starvation, and Nutrient Deprivation: From Biology to Policy*. Springer International Publishing, Cham, pp. 1–16.
- Galiè, M., Covi, V., Tabaracci, G., Malatesta, M., 2019. The role of nrf2 in the antioxidant cellular response to medical ozone exposure. *Int. J. Mol. Sci.* 20.
- Gavric, J., Prokic, M., Despotovic, S., Gavrilovic, B., Radovanovic, T., Borkovic-Mitic, S., Pavlovic, S., Sačić, Z., 2015. Biomarkers of oxidative stress and acetylcholinesterase activity in the blood of grass snake (*Natrix natrix* L.) during prehibernation and posthibernation periods. *Braz. Arch. Biol. Technol.* 58, 443–453.
- Giraud-Billoud, M., Rivera-Ingraham, G.A., Moreira, D.C., Burmester, T., Castro-Vazquez, A., Carvajalino-Fernández, J.M., Dafre, A., Niu, C., Tremblay, N., Paital, B., Rosa, R., Storey, J.M., Vega, I.A., Zhang, W., Yepiz-Plascencia, G., Zenteno-Savin, T., Storey, K.B., Hermes-Lima, M., 2019. Twenty years of the “Preparation for Oxidative Stress” (POS) theory: ecophysiological advantages and molecular strategies. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 234, 36–49.
- Gomes, E.C., Silva, A.N., de Oliveira, M.R., 2012. Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. *Oxidative Med. Cell. Longev.* 2012, 756132.
- Gómez-Cabrera, M.-C., Domenech, E., Viña, J., 2008. Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. *Free Radic. Biol. Med.* 44, 126–131.
- Gospodaryov, D., Lushchak, V., 2012. Oxidative stress: cause and consequence of diseases. In: Lushchak, V., Gospodaryov, D. (Eds.), *Oxidative Stress and Diseases*. IntechOpen.
- Granger, D.N., Kvietys, P.R., 2015. Reperfusion injury and reactive oxygen species: the evolution of a concept. *Redox Biol.* 6, 524–551.
- Grune, T., Reinheckel, T., Davies, K.J., 1997. Degradation of oxidized proteins in mammalian cells. *FASEB J.* 11, 526–534.
- Gupta, A., Brooks, C., Storey, K.B., 2020. Regulation of NF-κB, FHC and SOD2 in response to oxidative stress in the freeze tolerant wood frog, *Rana sylvatica*. *Cryobiology* 97, 28–36.
- Gutiérrez, J.S., Sabat, P., Castañeda, L.E., Contreras, C., Navarrete, L., Peña-Villalobos, I., Navedo, J.G., 2019. Oxidative status and metabolic profile in a long-lived bird preparing for extreme endurance migration. *Sci. Rep.* 9, 17616.
- Halliwell, B., 1974. Superoxide dismutase, catalase and glutathione peroxidase: solutions to the problems of living with oxygen. *New Phytol.* 73, 1075–1086.
- Halliwell, B., 1990. How to characterize a biological antioxidant. *Free Radic. Res. Commun.* 9, 1–32.
- Halliwell, B., Gutteridge, J.M.C., 1995. The definition and measurement of antioxidants in biological systems. *Free Radic. Biol. Med.* 18, 125–126.
- Hanson, K.C., Abizaid, A., Cooke, S.J., 2009. Causes and consequences of voluntary anorexia during the parental care period of wild male smallmouth bass (*Micropterus dolomieu*). *Horm. Behav.* 56, 503–509.
- Harlan, J.M., Levine, J.D., Callahan, K.S., Schwartz, B.R., Harker, L.A., 1984. Glutathione redox cycle protects cultured endothelial cells against lysis by extracellularly generated hydrogen peroxide. *J. Clin. Invest.* 73, 706–713.
- Harper, M.-E., Bevilacqua, L., Hagopian, K., Weindrich, R., Ramsey, J.J., 2004. Ageing, oxidative stress, and mitochondrial uncoupling. *Acta Physiol. Scand.* 182, 321–331.
- Harvey, C.J., Thimmlappa, R.K., Singh, A., Blake, D.J., Ling, G., Wakabayashi, N., Fujii, J., Myers, A., Biswal, S., 2009. Nrf2-regulated glutathione recycling independent of biosynthesis is critical for cell survival during oxidative stress. *Free Radic. Biol. Med.* 46, 443–453.
- Healey, M.C., 2001. Patterns of gametic investment by female stream-and ocean-type chinook salmon. *J. Fish Biol.* 58, 1545–1556.
- Hermes-Lima, M., Storey, K.B., 1993. Antioxidant defenses in the tolerance of freezing and anoxia by garter snakes. *Am. J. Phys.* 265, R646–R652.
- Hermes-Lima, M., Moreira, D.C., Rivera-Ingraham, G.A., Giraud-Billoud, M., Genaro-Mattos, T.C., Campos, É.G., 2015. Preparation for oxidative stress under hypoxia and metabolic depression: revisiting the proposal two decades later. *Free Radic. Biol. Med.* 89, 1122–1143.
- Hershey, J.D., Robbins, C.T., Nelson, O.L., Lin, D.C., 2008. Minimal seasonal alterations in the skeletal muscle of captive brown bears. *Physiol. Biochem. Zool.* 81, 138–147.
- Hissa, R., Siekkinen, J., Hohtola, E., Saarela, S., Hakala, A., Pudas, J., 1994. Seasonal patterns in the physiology of the European brown bear (*Ursus arctos arctos*) in Finland. *Comp. Biochem. Physiol. A Physiol.* 109, 781–791.
- Hudson, N.J., Lehnert, S.A., Ingham, A.B., Symonds, B., Franklin, C.E., Harper, G.S., 2006. Lessons from an estivating frog: sparing muscle protein despite starvation and disuse. *Am. J. Phys. Regul. Integr. Comp. Phys.* 290, R836–R843.
- Hulbert, A.J., Pamplona, R., Buffenstein, R., Buttemer, W.A., 2007. Life and death: metabolic rate, membrane composition, and life span of animals. *Physiol. Rev.* 87, 1175–1213.
- Ibrahim, W.H., Habib, H.M., Jarrar, A.H., Al Baz, S.A., 2008. Effect of Ramadan fasting on markers of oxidative stress and serum biochemical markers of cellular damage in healthy subjects. *Ann. Nutr. Metab.* 53, 175–181.
- Inglés, M., Gambini, J., Carnicero, J.A., García-García, F.J., Rodríguez-Mañas, L., Olaso-González, G., Dromant, M., Borrás, C., Viña, J., 2014. Oxidative stress is related to frailty, not to age or sex, in a geriatric population: lipid and protein oxidation as biomarkers of frailty. *J. Am. Geriatr. Soc.* 62, 1324–1328.
- Iq, K.C., Shu-Chien, A.C., 2011. Proteomics of buccal cavity mucus in female tilapia fish (*Oreochromis spp.*): a comparison between parental and non-parental fish. *PLoS One* 6, e18555.
- Jenni-Eiermann, S., Jenni, L., Smith, S., Costantini, D., 2014. Oxidative stress in endurance flight: an unconsidered factor in bird migration. *PLoS One* 9, e97650.
- Johnson, J.B., Summer, W., Cutler, R.G., Martin, B., Hyun, D.-H., Dixit, V.D., Pearson, M., Nassar, M., Tellejohann, R., Maudsley, S., Carlson, O., John, S., Laub, D. R., Mattson, M.P., 2007. Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. *Free Radic. Biol. Med.* 42, 665–674.
- Jones, D.P., 2006. Redefining oxidative stress. *Antioxid. Redox Signal.* 8, 1865–1879.
- Kang, S.W., Chae, H.Z., Seo, M.S., Kim, K., Baines, I.C., Rhee, S.G., 1998. Mammalian peroxiredoxin isoforms can reduce hydrogen peroxide generated in response to growth factors and tumor necrosis factor-α. *J. Biol. Chem.* 273, 6297–6302.
- Keller, I.S., Salzburger, W., Roth, O., 2017. Parental investment matters for maternal and offspring immune defense in the mouthbrooding cichlid *Astatotilapia burtoni*. *BMC Evol. Biol.* 17, 264.
- Koppenol, W.H., Moreno, J.J., Pryor, W.A., Ischiropoulos, H., Beckman, J.S., 1992. Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. *Chem. Res. Toxicol.* 5, 834–842.

- Ku, H.H., Sohal, R.S., 1993. Comparison of mitochondrial pro-oxidant generation and anti-oxidant defenses between rat and pigeon: possible basis of variation in longevity and metabolic potential. *Mech. Ageing Dev.* 72, 67–76.
- Kuzmiak, S., Glancy, B., Sweazea, K.L., Willis, W.T., 2012. Mitochondrial function in sparrow pectoralis muscle. *J. Exp. Biol.* 215, 2039–2050.
- Le Ninan, F., Cherel, Y., Sardet, C., Le Maho, Y., 1988. Plasma hormone levels in relation to lipid and protein metabolism during prolonged fasting in king penguin chicks. *Gen. Comp. Endocrinol.* 71, 331–337.
- Lee, K.-H., Bartsch, H., Nair, J., Yoo, D.-H., Hong, Y.-C., Cho, S.-H., Kang, D., 2006. Effect of short-term fasting on urinary excretion of primary lipid peroxidation products and on markers of oxidative DNA damage in healthy women. *Carcinogenesis* 27, 1398–1403.
- Li, H., Benipal, B., Zhou, S., Dodia, C., Chatterjee, S., Tao, J.-Q., Sorokina, E.M., Raabe, T., Feinstein, S.I., Fisher, A.B., 2015. Critical role of peroxiredoxin 6 in the repair of peroxidized cell membranes following oxidative stress. *Free Radic. Biol. Med.* 87, 356–365.
- Li, T., Qi, M., Gatesoupe, F.-J., Tian, D., Jin, W., Li, J., Lin, Q., Wu, S., Li, H., 2019. Adaptation to fasting in Crucian Carp (*Carassius auratus*): gut microbiota and its correlative relationship with immune function. *Microb. Ecol.* 78, 6–19.
- Lindgård, K., Stokkan, K.A., Le Maho, Y., Groscolas, R., 1992. Protein utilization during starvation in fat and lean Svalbard ptarmigan (*Lagopus mutus hyperboreus*). *J. Comp. Physiol. B.* 162, 607–613.
- Lohuis, T.D., Harlow, H.J., Beck, T.D.I., Iaizzo, P.A., 2007. Hibernating bears conserve muscle strength and maintain fatigue resistance. *Physiol. Biochem. Zool.* 80, 257–269.
- Longo, V.D., Mattson, M.P., 2014. Fasting: molecular mechanisms and clinical applications. *Cell Metab.* 19, 181–192.
- Loschen, G., Azzi, A., Richter, C., Flohé, L., 1974. Superoxide radicals as precursors of mitochondrial hydrogen peroxide. *FEBS Lett.* 42, 68–72.
- Lu, D.-L., Ma, Q., Wang, J., Li, L.-Y., Han, S.-L., Limbu, S.M., Li, D.-L., Chen, L.-Q., Zhang, M.-L., Du, Z.-Y., 2019. Fasting enhances cold resistance in fish through stimulating lipid catabolism and autophagy. *J. Physiol.* 597, 1585–1603.
- Ma, Q., 2013. Role of nrf2 in oxidative stress and toxicity. *Annu. Rev. Pharmacol. Toxicol.* 53, 401–426.
- Ma, Y.L., Zhu, X., Rivera, P.M., Tjøen, Ø., Barnes, B.M., LaManna, J.C., Smith, M.A., Drew, K.L., 2005. Absence of cellular stress in brain after hypoxia induced by arousal from hibernation in Arctic ground squirrels. *Am. J. Phys. Regul. Integr. Comp. Phys.* 289, R1297–R1306.
- Madkour, M.I., El-Serafi, T., Jahrami, H.A., Sherif, N.M., Hassan, R.E., Awadallah, S., Faris, M.A.-I.E., 2019. Ramadan diurnal intermittent fasting modulates SOD2, TFAM, Nrf2, and sirtuins (SIRT1, SIRT3) gene expressions in subjects with overweight and obesity. *Diabetes Res. Clin. Pract.* 155, 107801.
- Martinez, B., Khudiyakov, J., Rutherford, K., Crocker, D.E., Gemmell, N., Ortiz, R.M., 2018. Adipose transcriptome analysis provides novel insights into molecular regulation of prolonged fasting in northern elephant seal pups. *Physiol. Genomics* 50, 495–503.
- Martínez-Curci, N.S., Azpiroz, A.B., Isacch, J.P., Elías, R., 2015. Dietary relationships among Nearctic and Neotropical migratory shorebirds in a key coastal wetland of South America. *Emu (Aust. Ornithol.)* 115, 326–334.
- Mattison, J.A., Roth, G.S., Beasley, T.M., Tilmont, E.M., Handy, A.M., Herbert, R.L., Longo, D.L., Allison, D.B., Young, J.E., Bryant, M., Barnard, D., Ward, W.F., Qi, W., Ingram, D.K., de Cabo, R., 2012. Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. *Nature* 489, 318–321.
- Mattson, M.P., Longo, V.D., Harvie, M., 2017. Impact of intermittent fasting on health and disease processes. *Ageing Res. Rev.* 39, 46–58.
- May, J.M., Qu, Z.C., Whitesell, R.R., Cobb, C.E., 1996. Ascorbate recycling in human erythrocytes: role of GSH in reducing dehydroascorbate. *Free Radic. Biol. Med.* 20, 543–551.
- McCord, J.M., Fridovich, I., 1969. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* 244, 6049–6055.
- McCord, J.M., Roy, R.S., Schaffer, S.W., 1985. Free radicals and myocardial ischemia. The role of xanthine oxidase. In: Peter, P., Poole-Wilson, P.A. (Eds.), *Advances in Myocardiology*. Springer US, Boston, MA, pp. 183–189.
- McCue, M.D., 2010. Starvation physiology: reviewing the different strategies animals use to survive a common challenge. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 156, 1–18.
- McCue, M.D., 2012. An introduction to fasting, starvation, and food limitation. In: McCue, M.D. (Ed.), *Comparative Physiology of Fasting, Starvation, and Food Limitation*. Springer, Berlin, Heidelberg, pp. 1–5.
- McWilliams, S.R., Guglielmo, C., Pierce, B., Klaassen, M., 2004. Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. *J. Avian Biol.* 35, 377–393.
- Meigs, J.B., Larson, M.G., Fox, C.S., Keaney, J.F., Vasan, R.S., Benjamin, E.J., 2007. Association of oxidative stress, insulin resistance, and diabetes risk phenotypes: the Framingham offspring study. *Diabetes Care* 30, 2529–2535.
- Metcalfe, N.B., Thorpe, J.E., 1992. Anorexia and defended energy levels in overwintering juvenile salmon. *J. Anim. Ecol.* 61, 175.
- Miller, K.M., Schulze, A.D., Ginther, N., Li, S., Patterson, D.A., Farrell, A.P., Hinch, S.G., 2009. Salmon spawning migration: metabolic shifts and environmental triggers. *Comp. Biochem. Physiol. Part D. Genomics Proteomics* 4, 75–89.
- Miller, R.T., Martásek, P., Roman, L.J., Nishimura, J.S., Masters, B.S., 1997. Involvement of the reductase domain of neuronal nitric oxide synthase in superoxide anion production. *Biochemistry* 36, 15277–15284.
- Miyazaki, M., Shimozuru, M., Tsubota, T., 2019. Skeletal muscles of hibernating black bears show minimal atrophy and phenotype shifting despite prolonged physical inactivity and starvation. *PLoS One* 14, e0215489.
- Moreira, D.C., Welker, A.F., Campos, É.G., de Souza, S.C.R., Hermes-Lima, M., 2018. Subtropical hibernation in juvenile tegu lizards (*Salvator merianae*): insights from intestine redox dynamics. *Sci. Rep.* 8, 9368.
- Moreira, D.C., Carvajalino-Fernández, J.M., Silva, W.P., Kuzniewski, F., Navas, C.A., de Carvalho, J.E., Hermes-Lima, M., 2020. Preparation for oxidative stress in *Proceratophrys cristiceps* (Anura, Odontophrynidae) naturally estivating in the Brazilian Caatinga. *Sci. Total Environ.* 723, 137957.
- Morin, P., Ni, Z., McMullen, D.C., Storey, K.B., 2008. Expression of Nrf2 and its downstream gene targets in hibernating 13-lined ground squirrels, *Spermophilus tridecemlineatus*. *Mol. Cell. Biochem.* 312, 121–129.
- Mueller, S., Riedel, H.D., Stremmel, W., 1997. Determination of catalase activity at physiological hydrogen peroxide concentrations. *Anal. Biochem.* 245, 55–60.
- Nam, Y.K., Choi, Y.S., Choi, B.N., Kim, K.H., Kim, S.K., Kim, D.S., 2005. Alteration of antioxidant enzymes at the mRNA level during short-term starvation of rockbream *Oplegnathus fasciatus*. *Fish. Sci.* 71, 1385–1387.
- Nelson, M.J., Cowling, R.A., Seitz, S.P., 1994. Structural characterization of alkyl and peroxy radicals in solutions of purple lipoygenase. *Biochemistry* 33, 4966–4973.
- Nguyen, T., Nioi, P., Pickett, C.B., 2009. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *J. Biol. Chem.* 284, 13291–13295.
- Niu, Y., Cao, W., Zhao, Yaofeng, Zhai, H., Zhao, Yao, Tang, X., Chen, Q., 2018. The levels of oxidative stress and antioxidant capacity in hibernating *Nanorana parkeri*. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 219–220, 19–27.
- Nordoy, E.S., Blix, A.S., 1991. Glucose and ketone body turnover in fasting grey seal pups. *Acta Physiol. Scand.* 141, 565–571.
- Nussey, D.H., Coulson, T., Festa-Bianchet, M., Gaillard, J.-M., 2008. Measuring senescence in wild animal populations: towards a longitudinal approach. *Funct. Ecol.* 22, 393–406.
- Orr, A.L., Lohse, L.A., Drew, K.L., Hermes-Lima, M., 2009. Physiological oxidative stress after arousal from hibernation in Arctic ground squirrel. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 153, 213–221.
- Ortiz, R.M., Wade, C.E., Ortiz, C.L., 2000. Prolonged fasting increases the response of the renin-angiotensin-aldosterone system, but not vasopressin levels, in postweaned northern elephant seal pups. *Gen. Comp. Endocrinol.* 119, 217–223.
- Page, M.M., Salway, K.D., Ip, Y.K., Chew, S.F., Warren, S.A., Ballantyne, J.S., Stuart, J.A., 2010. Upregulation of intracellular antioxidant enzymes in brain and heart during estivation in the African lungfish *Protopterus dolloi*. *J. Comp. Physiol. B.* 180, 361–369.
- Pap, P.L., Vincze, O., Fülöp, A., Székely-Béres, O., Patrás, L., Péntzes, J., Vágási, C.I., 2018. Oxidative physiology of reproduction in a passerine bird: a field experiment. *Behav. Ecol. Sociobiol.* 72, 18.
- Park, S., Park, N.-Y., Valacchi, G., Lim, Y., 2012. Calorie restriction with a high-fat diet effectively attenuated inflammatory response and oxidative stress-related markers in obese tissues of the high diet fed rats. *Mediat. Inflamm.* 2012, 984643.
- Pellegrino, M.A., Desaphy, J.-F., Brocca, L., Pierno, S., Camerino, D.C., Bottinelli, R., 2011. Redox homeostasis, oxidative stress and disuse muscle atrophy. *J. Physiol.* 589, 2147–2160.
- Pérez-Campo, R., López-Torres, M., Cadenas, S., Rojas, C., Barja, G., 1998. The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach. *J. Comp. Physiol. B.* 168, 149–158.
- Pérez-Rodríguez, L., Romero-Haro, A.A., Sternalski, A., Muriel, J., Mougeot, F., Gil, D., Alonso-Alvarez, C., 2015. Measuring oxidative stress: the confounding effect of lipid concentration in measures of lipid peroxidation. *Physiol. Biochem. Zool.* 88, 345–351.
- Perrault, J.R., Stacy, N.I., 2018. Note on the unique physiologic state of loggerhead sea turtles (*Caretta caretta*) during nesting season as evidenced by a suite of health variables. *Mar. Biol.* 165, 71.
- Qiu, X., Brown, K., Hirschey, M.D., Verdin, E., Chen, D., 2010. Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. *Cell Metab.* 12, 662–667.
- Radi, R., 2018. Oxygen radicals, nitric oxide, and peroxynitrite: redox pathways in molecular medicine. *Proc. Natl. Acad. Sci. U. S. A.* 115, 5839–5848.
- Reilly, B.D., Schlupali, D.I., Cramp, R.L., Ebert, P.R., Franklin, C.E., 2013. Frogs and estivation: transcriptional insights into metabolism and cell survival in a natural model of extended muscle disuse. *Physiol. Genomics* 45, 377–388.
- Reilly, B.D., Hickey, A.J.R., Cramp, R.L., Franklin, C.E., 2014. Decreased hydrogen peroxide production and mitochondrial respiration in skeletal muscle but not cardiac muscle of the green-striped burrowing frog, a natural model of muscle disuse. *J. Exp. Biol.* 217, 1087–1093.
- Rey, B., Halsey, L.G., Dolmazon, V., Rouanet, J.-L., Roussel, D., Handrich, Y., Butler, P.J., Duchamp, C., 2008. Long-term fasting decreases mitochondrial avian UCP-mediated oxygen consumption in hypometabolic king penguins. *Am. J. Phys. Regul. Integr. Comp. Phys.* 295, R92–R100.
- Rice, M.E., Forman, R.E., Chen, B.T., Avshalumov, M. V., Cragg, S.J., Drew, K.L., 2002. Brain antioxidant regulation in mammals and anoxia-tolerant reptiles: balanced for neuroprotection and neuromodulation. *Comp. Biochem. Physiol. Toxicol. Pharmacol.* 133, 515–525.
- Rikans, L.E., Hornbrook, K.R., 1997. Lipid peroxidation, antioxidant protection and aging. *Biochim. Biophys. Acta Mol. basis Dis.* 1362, 116–127.
- Rogowska-Wrzęsinska, A., Wojdyla, K., Nedić, O., Baron, C.P., Griffiths, H.R., 2014. Analysis of protein carbonylation — pitfalls and promise in commonly used methods. *Free Radic. Res.* 48, 1145–1162.
- Rossi, A., Cazenave, J., Bacchetta, C., Campana, M., Parma, M.J., 2015. Physiological and metabolic adjustments of *Hoplosternum littorale* (Teleostei, Callichthyidae) during starvation. *Ecol. Indic.* 56, 161–170.
- Sahoo, D., Patnaik, P., 2020. Oxidative stress markers and antioxidant defense in hibernating common Asian toads, *Duttaphrynus melanostictus*. *Arch. Biol. Sci.* 72, 23–30.

- Salem, M., Silverstein, J., Rexroad, C.E., Yao, J., 2007. Effect of starvation on global gene expression and proteolysis in rainbow trout (*Oncorhynchus mykiss*). *BMC Genomics* 8, 328.
- Salin, K., Villasevil, E.M., Anderson, G.J., Auer, S.K., Selman, C., Hartley, R.C., Mullen, W., Chinopoulos, C., Metcalfe, N.B., 2018. Decreased mitochondrial metabolic requirements in fasting animals carry an oxidative cost. *Funct. Ecol.* 32, 2149–2157.
- Salvemini, F., Franzé, A., Iervolino, A., Filosa, S., Salzano, S., Ursini, M.V., 1999. Enhanced glutathione levels and oxidoreistance mediated by increased glucose-6-phosphate dehydrogenase expression. *J. Biol. Chem.* 274, 2750–2757.
- Sawicki, J., Miros, E., Border, S.E., Dijkstra, P.D., 2019. Reproduction and maternal care increase oxidative stress in a mouthbrooding cichlid fish. *Behav. Ecol.* 30, 1662–1671.
- Scholz, R.W., Graham, K.S., Gumprecht, E., Reddy, C.C., 1989. Mechanism of interaction of vitamin E and glutathione in the protection against membrane lipid peroxidation. *Ann. N. Y. Acad. Sci.* 570, 514–517.
- Schull, Q., Viblanc, V.A., Stier, A., Saadaoui, H., Lefol, E., Criscuolo, F., Bize, P., Robin, J.-P., 2016. The oxidative debt of fasting: evidence for short- to medium-term costs of advanced fasting in adult king penguins. *J. Exp. Biol.* 219, 3284–3293.
- Secor, S.M., Carey, H.V., 2016. Integrative physiology of fasting. *Compr. Physiol.* 6, 773–825.
- Semenza, G.L., 2007. Life with oxygen. *Science* 318, 62–64 (80).
- Sevanian, A., Muakkassah-Kelly, S.F., Montestruque, S., 1983. The influence of phospholipase A2 and glutathione peroxidase on the elimination of membrane lipid peroxides. *Arch. Biochem. Biophys.* 223, 441–452.
- Shacter, E., 2000. Quantification and significance of protein oxidation in biological samples. *Drug Metab. Rev.* 32, 307–326.
- Sharick, J.T., Vázquez-Medina, J.P., Ortiz, R.M., Crocker, D.E., 2015. Oxidative stress is a potential cost of breeding in male and female northern elephant seals. *Funct. Ecol.* 29, 367–376.
- Shimozuru, M., Kamine, A., Tsubota, T., 2012. Changes in expression of hepatic genes involved in energy metabolism during hibernation in captive, adult, female Japanese black bears (*Ursus thibetanus japonicus*). *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 163, 254–261.
- Shpak, M., Proulx, S.R., 2007. The role of life cycle and migration in selection for variance in offspring number. *Bull. Math. Biol.* 69, 837–860.
- Sies, H., 1985. Oxidative stress: introductory remarks. *Oxidative Stress* 501, 1–8.
- Sies, H., 1993. Strategies of antioxidant defense. *Eur. J. Biochem.* 215, 213–219.
- Sies, H., 1997. Oxidative stress: oxidants and antioxidants. *Exp. Physiol.* 82, 291–295.
- Sies, H., 2019. Chapter 13 - oxidative stress: eustress and distress in redox homeostasis. In: Fink, G. (Ed.), *Fink Biochemistry, and Pathology*. Academic Press, pp. 153–163.
- Sies, H., 2020. Oxidative eustress and oxidative distress: introductory remarks. In: *Oxidative Stress*. Academic Press, pp. 3–12.
- Simpkins, D.G., Hubert, W.A., 2000. Drifting invertebrates, stomach contents, and body conditions of juvenile rainbow trout from fall through winter in a Wyoming tailwater. *Trans. Am. Fish. Soc.* 129, 1187–1195.
- Skip, M.M., Bauchinger, U., Goymann, W., Fusani, L., Cardinale, M., Alan, R.R., McWilliams, S.R., 2015. Migrating songbirds on stopover prepare for, and recover from, oxidative challenges posed by long-distance flight. *Ecol. Evol.* 5, 3198–3209.
- Slater, T.F., 1984. Free-radical mechanisms in tissue injury. *Biochem. J.* 222, 1–15.
- Sohal, R.S., Weindruch, R., 1996. Oxidative stress, caloric restriction, and aging. *Science* 273, 59–63 (80).
- Soñanez-Organis, J.G., Vázquez-Medina, J.P., Zenteno-Savín, T., Aguilar, A., Crocker, D.E., Ortiz, R.M., 2012. Prolonged fasting increases purine recycling in post-weaned northern elephant seals. *J. Exp. Biol.* 215, 1448–1455.
- Soñanez-Organis, J.G., Vázquez-Medina, J.P., Crocker, D.E., Ortiz, R.M., 2013. Prolonged fasting activates hypoxia inducible factors-1 α , -2 α and -3 α in a tissue-specific manner in northern elephant seal pups. *Gene* 526, 155–163.
- Stenvinkel, P., Fröbert, O., Anderstam, B., Palm, F., Eriksson, M., Bragfors-Helin, A.-C., Qureshi, A.R., Larsson, T., Friebe, A., Zedrosser, A., Josefsson, J., Svensson, M., Sahdo, B., Bankir, L., Johnson, R.J., 2013. Metabolic changes in summer active and anuric hibernating free-ranging brown bears (*Ursus arctos*). *PLoS One* 8, e72934.
- Stier, A., Dupoué, A., Picard, D., Angelier, F., Brischoux, F., Lourdais, O., 2017. Oxidative stress in a capital breeder (*Vipera aspis*) facing pregnancy and water constraints. *J. Exp. Biol.* 220, 1792–1796.
- Stier, A., Schull, Q., Bize, P., Lefol, E., Haussmann, M., Roussel, D., Robin, J.-P., Viblanc, V.A., 2019. Oxidative stress and mitochondrial responses to stress exposure suggest that king penguins are naturally equipped to resist stress. *Sci. Rep.* 9, 8545.
- Sutton, E.F., Beyl, R., Early, K.S., Cefalu, W.T., Ravussin, E., Peterson, C.M., 2018. Early time-restricted feeding improves insulin sensitivity, blood pressure, and oxidative stress even without weight loss in men with prediabetes. *Cell Metab.* 27, 1212–1221. e3.
- Suzuki, Y.J., Forman, H.J., Sevanian, A., 1997. Oxidants as stimulators of signal transduction. *Free Radic. Biol. Med.* 22, 269–285.
- Suzuki, Y.J., Carini, M., Butterfield, D.A., 2010. Protein carbonylation. *Antioxid. Redox Signal.* 12, 323–325.
- Tøien, Ø., Blake, J., Edgar, D.M., Grahm, D.A., Heller, H.C., Barnes, B.M., 2011. Hibernation in black bears: independence of metabolic suppression from body temperature. *Science* 331, 906–909 (80).
- Ursini, F., Maiorino, M., Valente, M., Ferri, L., Gregolin, C., 1982. Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides. *Biochim. Biophys. Acta* 710, 197–211.
- Vágási, C.I., Pátraş, L., Pap, P.L., Vincze, O., Mureşan, C., Németh, J., Lendvai, Á.Z., 2018. Experimental increase in baseline corticosterone level reduces oxidative damage and enhances innate immune response. *PLoS One* 13, e0192701.
- Vázquez-Medina, J.P., Crocker, D.E., Forman, H.J., Ortiz, R.M., 2010. Prolonged fasting does not increase oxidative damage or inflammation in postweaned northern elephant seal pups. *J. Exp. Biol.* 213, 2524–2530.
- Vázquez-Medina, J.P., Olgun-Monroy, N.O., Maldonado, P.D., Santamara, A., Knigsberg, M., Elsner, R., Hammill, M.O., Burns, J.M., Zenteno-Savín, T., 2011a. Maturation increases superoxide radical production without increasing oxidative damage in the skeletal muscle of hooded seals (*Cystophoracristata*). *Can. J. Zool.* 89, 206–212.
- Vázquez-Medina, J.P., Soñanez-Organis, J.G., Burns, J.M., Zenteno-Savín, T., Ortiz, R.M., 2011b. Antioxidant capacity develops with maturation in the deep-diving hooded seal. *J. Exp. Biol.* 214, 2903–2910.
- Vázquez-Medina, J.P., Zenteno-Savín, T., Forman, H.J., Crocker, D.E., Ortiz, R.M., 2011c. Prolonged fasting increases glutathione biosynthesis in postweaned northern elephant seals. *J. Exp. Biol.* 214, 1294–1299.
- Vázquez-Medina, J.P., Zenteno-Savín, T., Ortiz, R.M., 2012. Coping with physiological oxidative stress: a review of antioxidant strategies in seals. *J. Comp. Physiol. B* 182, 741–750.
- Vázquez-Medina, J.P., Soñanez-Organis, J.G., Rodríguez, R., Viscarra, J.A., Nishiyama, A., Crocker, D.E., Ortiz, R.M., 2013. Prolonged fasting activates Nrf2 in post-weaned elephant seals. *J. Exp. Biol.* 216, 2870–2878.
- Veal, E., Day, A., 2011. Hydrogen peroxide as a signaling molecule. *Antioxid. Redox Signal.* 15, 147–151.
- Viña, J., Borrás, C., Abdelaziz, K.M., García-Vallés, R., Gómez-Cabrera, M.C., 2013. The free radical theory of aging revisited: the cell signaling disruption theory of aging. *Antioxid. Redox Signal.* 19, 779–787.
- Viña, J., Borrás, C., Gómez-Cabrera, M.C., 2018. A free radical theory of frailty. *Free Radic. Biol. Med.* 124, 358–363.
- Viscarra, J.A., Ortiz, R.M., 2013. Cellular mechanisms regulating fuel metabolism in mammals: role of adipose tissue and lipids during prolonged food deprivation. *Metabolism* 62, 889–897.
- Voituron, Y., Servais, S., Romestaing, C., Douki, T., Barré, H., 2006. Oxidative DNA damage and antioxidant defenses in the European common lizard (*Lacerta vivipara*) in supercooled and frozen states. *Cryobiology* 52, 74–82.
- Vuorinen, P.J., Rokka, M., Rittanen, T., Käkälä, R., Nikonen, S., Pakarinen, T., Keinänen, M., 2020. Changes in thiamine concentrations, fatty acid composition, and some other lipid-related biochemical indices in Baltic Sea Atlantic salmon (*Salmo salar*) during the spawning run and pre-spawning fasting. *Helgol. Mar. Res.* 74, 10.
- Weber, J.-M., 2009. The physiology of long-distance migration: extending the limits of endurance metabolism. *J. Exp. Biol.* 212, 593–597.
- Wegman, M.P., Guo, M.H., Bennion, D.M., Shankar, M.N., Chrzanowski, S.M., Goldberg, L.A., Xu, J., Williams, T.A., Lu, X., Hsu, S.I., Anton, S.D., Leeuwenburgh, C., Brantly, M.L., 2015. Practicality of intermittent fasting in humans and its effect on oxidative stress and genes related to aging and metabolism. *Rejuvenation Res.* 18, 162–172.
- Wei, Y., Zhang, J., Xu, S., Peng, X., Yan, X., Li, X., Wang, H., Chang, H., Gao, Y., 2018. Controllable oxidative stress and tissue specificity in major tissues during the torpor-arousal cycle in hibernating Daurian ground squirrels. *Open Biol.* 8.
- Welker, T.L., Congleton, J.L., 2005. Oxidative stress in migrating spring chinook salmon smolts of hatchery origin: changes in vitamin E and lipid peroxidation. *Trans. Am. Fish. Soc.* 134, 1499–1508.
- Whiteman, J.P., Harlow, H.J., Durner, G.M., Regehr, E.V., Rourke, B.C., Robles, M., Amstrup, S.C., Ben-David, M., 2017. Polar bears experience skeletal muscle atrophy in response to food deprivation and reduced activity in winter and summer. *Conserv. Physiol.* 5, cox049.
- Wiersma, P., Selman, C., Speakman, J.R., Verhulst, S., 2004. Birds sacrifice oxidative protection for reproduction. *Proc. Biol. Sci.* 271 (Suppl), S360–S363.
- Wilson, S.M., Gravel, M.-A., Mackie, T.A., Willmore, W.G., Cooke, S.J., 2012. Oxidative stress associated with paternal care in smallmouth bass (*Micropterus dolomieu*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 162, 212–218.
- Wilson, S.M., Taylor, J.J., Mackie, T.A., Patterson, D.A., Cooke, S.J., Willmore, W.G., 2014. Oxidative stress in Pacific salmon (*Oncorhynchus* spp.) during spawning migration. *Physiol. Biochem. Zool.* 87, 346–352.
- Worthy, G.A.J., Morris, P.A., Costa, D.P., Le Boeuf, B.J., 1992. Moulting energetics of the northern elephant seal (*Mirovanga angustirostris*). *J. Zool.* 227, 257–265.
- Wright, P.A., 1995. Nitrogen excretion: three end products, many physiological roles. *J. Exp. Biol.* 198, 273–281.
- Wright, T.J., Davis, R.W., Holser, R.R., Hüeckstädt, L.A., Danesi, C.P., Porter, C., Widen, S.G., Williams, T.M., Costa, D.P., Sheffield-Moore, M., 2020. Changes in northern elephant seal skeletal muscle following thirty days of fasting and reduced activity. *Front. Physiol.* 11, 564555.
- Wu, P., Wang, A., Cheng, J., Chen, L., Pan, Y., Li, H., Zhang, Q., Zhang, Jiaqi, Chu, W., Zhang, Jianshe, 2020. Effects of starvation on antioxidant-related signaling molecules, oxidative stress, and autophagy in juvenile Chinese Perch skeletal muscle. *Mar. Biotechnol.* 22, 81–93.
- Yan, J., Burman, A., Nichols, C., Alila, L., Showe, L.C., Showe, M.K., Boyer, B.B., Barnes, B.M., Marr, T.G., 2006. Detection of differential gene expression in brown adipose tissue of hibernating arctic ground squirrels with mouse microarrays. *Physiol. Genomics* 25, 346–353.
- Yang, Y., Cheng, J.Z., Singhal, S.S., Saini, M., Pandya, U., Awasthi, S., Awasthi, Y.C., 2001. Role of glutathione S-transferases in protection against lipid peroxidation. Overexpression of hGSTA2-2 in K562 cells protects against hydrogen peroxide-induced apoptosis and inhibits JNK and caspase 3 activation. *J. Biol. Chem.* 276, 19220–19230.
- Yin, Q., Ge, H., Liao, C.-C., Liu, D., Zhang, S., Pan, Y.-H., 2016. Antioxidant defenses in the brains of bats during hibernation. *PLoS One* 11, e0152135.

- Young, K.M., Cramp, R.L., Franklin, C.E., 2013a. Each to their own: skeletal muscles of different function use different biochemical strategies during aestivation at high temperature. *J. Exp. Biol.* 216, 1012–1024.
- Young, K.M., Cramp, R.L., Franklin, C.E., 2013b. Hot and steady: elevated temperatures do not enhance muscle disuse atrophy during prolonged aestivation in the ectotherm *Cyclorana alboguttata*. *J. Morphol.* 274, 165–174.
- Blough, N. V, Zafiriou, O.C., 1985. Reaction of superoxide with nitric oxide to form peroxonitrite in alkaline aqueous solution. *Inorg. Chem.* 24, 3502–3504.
- Zainal, T.A., Oberley, T.D., Allison, D.B., Szweda, L.I., Weindruch, R., 2000. Caloric restriction of rhesus monkeys lowers oxidative damage in skeletal muscle. *FASEB J.* 14, 1825–1836.
- Zangar, R.C., Davydov, D.R., Verma, S., 2004. Mechanisms that regulate production of reactive oxygen species by cytochrome P450. *Toxicol. Appl. Pharmacol.* 199, 316–331.
- Zhang, W.-Y., Niu, C.-J., Chen, B.-J., Yuan, L., 2017. Antioxidant responses in hibernating Chinese soft-shelled turtle *Pelodiscus sinensis* hatchlings. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 204, 9–16.