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OXIDATIVE STRESS PHYSIOLOGY IN ANIMALS: REACTIVE OXYGEN SPECIES AS A CENTRAL MEDIATOR OF THE RESPONSE TO ENVIRONMENTAL STRESSORS

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ABSTRACT
Aerobic life is, in a sense, a bargain. Oxygen fuels the energy metabolism that animals depend upon, yet the very chemistry that extracts energy from nutrients also leaks partially reduced oxygen species back into the cell. These reactive oxygen species (ROS), together with reactive nitrogen species, sit at the heart of a continuous physiological balancing act: at low and tightly controlled levels they serve as second messengers and help shape cellular signalling, whereas when their generation outpaces the antioxidant machinery they begin to damage lipids, proteins and nucleic acids. This imbalance, termed oxidative stress, has become over the last three decades one of the more useful organising concepts in comparative and environmental physiology. Here we argue that oxidative stress is best understood not as a disease state but as a central mediator that translates a broad range of environmental challenges-thermal extremes, fluctuating oxygen, shifting salinity, food limitation and chemical pollution-into integrated physiological, life-history and fitness outcomes. We summarise the principal sources of ROS in animal tissues, the enzymatic and non-enzymatic defences that hold them in check, and the redox-sensitive signalling networks, the Keap1–Nrf2 axis in particular, that couple perturbation to adaptive gene expression. We then examine how individual stressors modulate oxidative balance, why the resulting responses are so often context-dependent, and how phenomena such as hormesis, preparation for oxidative stress and cross-tolerance complicate any simple dose–response logic. We close by highlighting persistent methodological problems and several directions that, in our view, deserve closer attention.

Keywords: *Reactive oxygen species; oxidative stress; antioxidant defence; environmental stressors; redox signalling; comparative physiology; hormesis.*

1. INTRODUCTION

Few biochemical ideas have travelled as far across biology as oxidative stress. What began as a fairly narrow observation in mammalian biochemistry-that aerobic metabolism inevitably generates partially reduced derivatives of molecular oxygen-has since grown into a framework used by ecologists, toxicologists, aquaculturists and gerontologists alike [1,2]. The reason is not hard to find. Almost every activity an animal undertakes, from swimming and foraging to mounting an immune response or simply surviving a cold night, carries a metabolic cost, and metabolism is the chief domestic source of ROS [3]. Whenever the rate at which these species are produced exceeds the cell's capacity to remove them or to repair their damage, the redox balance tips and oxidative stress ensues [1,4].

For a long while oxidative stress was framed mainly in the language of pathology and ageing. Although that emphasis made sense, looking back, it was also a little restrictive. Animals within thewild are not laboratory patients; they contend with temperature swings, hypoxic waters, salinity gradients, periodic food shortages and a rising burden of anthropogenic pollutants, and each of these can disturb the production–elimination balance of ROS [5]. A

increasing body of research currently views oxidative stress as a physiological hub, where information from the environment is translated into modifications in cellular function, the distribution of resources among conflicting demands, and ultimately in survival and reproduction.[6]. That shift in perspective is what we try to capture in this review.

The aim is therefore twofold. First, we revisit the underlying physiology: where ROS come from, how they are neutralised, and how redox state is sensed and signalled. Second, and more centrally, we ask how this machinery responds to the environmental stressors animals actually meet, and why those responses prove so frequently inconsistent between studies, tissues and taxa.

2. Reactive oxygen species: chemistry, sources and targets

The term ROS is a convenient umbrella for a chemically diverse set of molecules, only some of which are true radicals. The primary species is the superoxide anion (O2•−), formed by a one-electron reduction of molecular oxygen. Superoxide is not, in itself, especially reactive, but it is the gateway to more troublesome derivatives: its dismutation yields hydrogen peroxide (H2O2), and in the presence of transition metals such as iron or copper, H2O2 can be converted through Fenton chemistry into the hydroxyl radical (•OH), which reacts more or less indiscriminately with whatever biomolecule it happens to meet [7,8]. Alongside these, reactive nitrogen species-peroxynitrite above all-broaden the picture, so that oxidative stress in practice usually means a mixture of oxidative and nitrosative pressure [4,9].

Table 01. Major reactive oxygen and nitrogen species, their principal cellular sources and their primary biological targets.

Reactive species	Symbol	Principal cellular source(s)	Main reactivity / targets
Superoxide anion	$O_2^{\bullet-}$	Mitochondrial ETC (Complexes I, III); NADPH oxidases; xanthine oxidase	Moderately reactive; oxidises [4Fe–4S] clusters; precursor of H_2O_2 and $ONOO^-$
Hydrogen peroxide	H_2O_2	Dismutation of $O_2^{\bullet-}$ (SOD); peroxisomal oxidases; NOX4	Diffusible signalling molecule at low levels; oxidises protein thiols; Fenton substrate
Hydroxyl radical	•OH	Fenton / Haber–Weiss reaction (Fe^{2+}, $Cu^+ + H_2O_2$)	Extremely reactive and short-lived; attacks lipids, proteins and DNA indiscriminately
Singlet oxygen	$^1O_2$	Photosensitisation; some peroxidase reactions	Oxidises membrane lipids, proteins and guanine bases
Peroxyl / alkoxyl radicals	ROO• / RO•	Propagation of lipid peroxidation chains	Sustain and amplify membrane lipid oxidation
Nitric oxide	•NO	Nitric oxide synthases (NOS)	Signalling (vasodilation, neurotransmission); precursor of $ONOO^-$
Peroxynitrite	$ONOO^-$	Reaction of $O_2^{\bullet-}$ with •NO	Nitrates tyrosine residues; damages lipids, proteins and DNA

Where do these species come from? The mitochondrial electron transport chain is the classic answer, and it remains the dominant endogenous source in most tissues. Electrons that escape, mainly at Complexes I and III, reduce oxygen prematurely, and the leak rate is sensitive to membrane potential, oxygen tension and the supply of substrate [3,10]. Mitochondria are not the only contributors, however. NADPH oxidases generate superoxide deliberately, for signalling and for host defence; peroxisomes release H2O2 during the β-oxidation of fatty acids; and enzymes such as xanthine oxidase become important under particular conditions, ischaemia and reperfusion being the obvious example [8,9]. The cellular sources, in short, are multiple and at least partly regulated, which already hints that ROS production is not merely an accident of breathing.

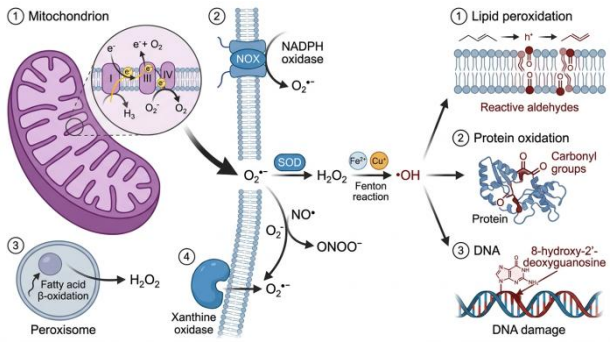

Figure 01: The main cellular sources of reactive oxygen species in animal tissues and their principal

macromolecular targets-lipids, proteins and DNA. The mitochondrial electron transport chain is the dominant endogenous source (heavy arrow); the superoxide → hydrogen peroxide → hydroxyl-radical cascade and the formation of peroxynitrite are shown at the centre.

The targets are just as varied. Polyunsaturated fatty acids in membranes are oxidised in self-propagating chains of lipid peroxidation, generating reactive aldehydes that go on to inflict further damage; proteins accumulate carbonyl groups and lose function; and DNA suffers a range of base modifications, of which 8-hydroxy-2′-deoxyguanosine is the most familiar marker [4,8]. The downstream consequences span everything from subtle shifts in enzyme activity to mutagenesis and cell death, depending on the intensity of the insult and on how long it lasts.

3. The antioxidant defence network

Animals are far from defenceless against this onslaught. A layered system of antioxidants keeps steady-state ROS concentrations low, and it is the relationship between this system and the rate of ROS production-rather than production on its own-that determines whether oxidative stress actually occurs [11,12]. The enzymatic arm is usually described first. The superoxide dismutases (SOD), present in cytosolic, mitochondrial and extracellular forms, accelerate the conversion of superoxide to hydrogen peroxide. Catalase and the glutathione peroxidases (GPx) then dispose of that peroxide, the former chiefly in peroxisomes and at high peroxide loads, the latter relying on reduced glutathione across a much wider concentration range [11]. Behind these sit the thioredoxin and peroxiredoxin systems, which have turned out to be central both to peroxide removal and to redox signalling, together with the glutathione- and thioredoxin-reductases that regenerate the reduced cofactors at the expense of NADPH.

Table 02: The principal enzymatic and non-enzymatic antioxidants of animals and their main functions.

Antioxidant	Type / location	Main function or reaction
Enzymatic antioxidants		
Superoxide dismutase (SOD)	Cytosol (Cu/Zn), mitochondria (Mn), extracellular	$2\ O_2^{\bullet-} + 2\ H^+ \rightarrow H_2O_2 + O_2$
Catalase (CAT)	Peroxisomes	$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$
Glutathione peroxidase (GPx)	Cytosol, mitochondria (Se-dependent)	$H_2O_2 + 2$ GSH → $2\ H_2O$ + GSSG; reduces lipid hydroperoxides
Glutathione reductase (GR)	Cytosol	GSSG + NADPH + H^+ → 2 GSH (regenerates reduced glutathione)
Peroxiredoxins (Prx)	Cytosol, mitochondria	Thiol-based reduction of H_2O_2 and peroxynitrite
Thioredoxin / thioredoxin reductase (Trx / TrxR)	Cytosol, mitochondria	Maintain protein-thiol redox state; recycle peroxiredoxins
Glutathione-S-transferase (GST)	Cytosol	Phase-II conjugation and detoxification of electrophiles
Non-enzymatic antioxidants		
Glutathione (GSH)	Cytosol (most abundant thiol)	Direct scavenger and enzyme cofactor; GSH/GSSG redox buffer
Ascorbic acid (vitamin C)	Aqueous phase	Radical scavenger; regenerates α-tocopherol
α-Tocopherol (vitamin E)	Membranes (lipid phase)	Chain-breaking antioxidant; halts lipid peroxidation
Carotenoids	Membranes (lipid phase)	Quench singlet oxygen; scavenge peroxyl radicals
Uric acid	Plasma / cytosol	Scavenges peroxynitrite, singlet oxygen and hydroxyl radical
Polyphenols / flavonoids	Dietary	Radical scavenging and transition-metal chelation

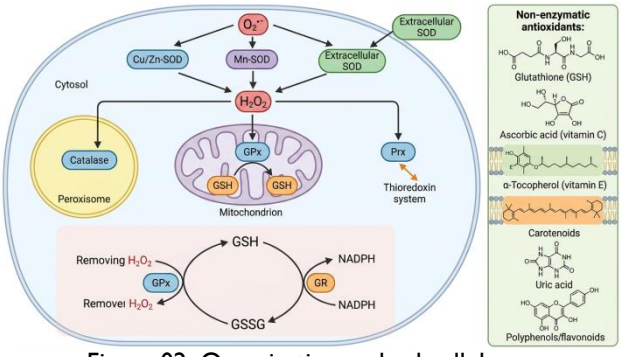

Figure 02: Organisation and subcellular compartmentalisation of the antioxidant defence network. Superoxide is converted by SOD isoforms to hydrogen peroxide, which is removed by catalase, glutathione peroxidase and the peroxiredoxins; the glutathione redox cycle (GPx / GR) and the principal non-enzymatic antioxidants are also shown.

The non-enzymatic component is chemically more heterogeneous. Glutathione, the most abundant low-molecular-weight thiol, is the workhorse here, and the

ratio of its reduced to oxidised forms (GSH/GSSG) is frequently taken as an index of cellular redox state [9]. To this are added a range of dietary and endogenous small molecules: ascorbate (vitamin C), the tocopherols and tocotrienols (vitamin E) that defend membranes, carotenoids, uric acid, and in many animals a variety of polyphenols acquired from food [12]. One point that is sometimes underappreciated is that the antioxidant network is compartmentalised and partly redundant; saturating or removing a single component does not necessarily produce stress, because others can compensate, at least within limits.

It is worth adding that more antioxidant is not invariably better. Several of these molecules turn pro-oxidant under certain conditions, and maintaining a high antioxidant capacity is itself metabolically and nutritionally costly-a consideration that becomes important once trade-offs enter the discussion [12].

4. Redox balance, eustress and redox signalling

A purely damage-centred view of ROS is now widely regarded as too narrow. Low and controlled fluxes of hydrogen peroxide, in particular, behave as genuine signalling molecules, reversibly oxidising cysteine residues on target proteins and thereby altering their activity. It has proved useful to distinguish oxidative eustress, the mild, physiological end of the spectrum that supports redox regulation, from oxidative distress, the supraphysiological end at which signalling is disrupted and damage accumulates [1,13]. The same molecule, then, may be beneficial or harmful depending only on its concentration and its location, which is part of why this field has been so prone to apparent contradictions.

Cells sense and respond to shifts in redox state through several pathways, but the best characterised in animals is the Keap1–Nrf2–ARE axis. Under basal conditions the transcription factor Nrf2 is held in the cytoplasm by Keap1 and continuously marked for degradation. Oxidation or covalent modification of reactive cysteines on Keap1 releases Nrf2, which then translocates to the nucleus and switches on a battery of antioxidant and detoxification genes, among them those encoding glutathione-synthesising enzymes, peroxiredoxins and phase-II conjugating enzymes [14]. In effect this is an adaptive feedback loop: a rise in oxidant load triggers a compensating rise in defensive capacity. Other redox-sensitive regulators, including various MAP-kinase cascades and the hypoxia-inducible factors, interact with this system, so the response to any given perturbation is seldom the work of a single pathway [13].

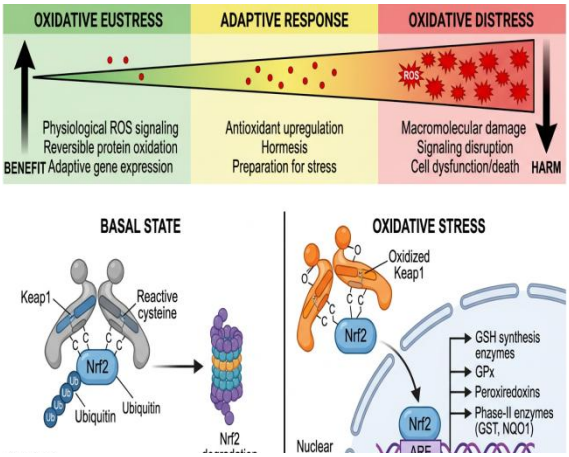

Figure 03: The redox continuum from oxidative eustress (low, regulatory ROS) through an adaptive response to oxidative distress (macromolecular damage), and the Keap1–Nrf2–ARE switch. Under basal conditions Nrf2 is degraded; oxidation of Keap1 cysteines releases Nrf2 to the nucleus, where it drives transcription of antioxidant and phase-II genes.

Instead of being a passive indicator of metabolic errors, redox status is seen as a controlled cellular variable that is monitored and altered similarly to pH or intracellular calcium [1].

5. Oxidative stress as a central mediator

If redox state is a regulated variable that tracks oxidant load, and if virtually every environmental challenge an animal faces alters that load, then oxidative stress occupies a natural position as an integrator — a common currency through which different stressors come to exert overlapping effects [5,6]. This is the central idea we want to develop. Rather than treating temperature, hypoxia, salinity and pollution as separate problems, each with its own self-contained physiology, it is often more illuminating to ask how each of them shifts the production–elimination balance of ROS, and how the organism then reallocates resources in response.

The strongest version of this argument comes from life-history theory. It was proposed that oxidative stress mediates the classic trade-offs between growth, reproduction and self-maintenance, on the grounds that the activities enhancing current performance tend to raise ROS production, while protecting the soma against the resulting damage is itself costly [6]. Reactive oxygen species have even been described as universal constraints on life-history evolution, a phrase that nicely captures the ubiquity of the problem even if it perhaps overstates its uniformity [15]. Empirically the support has been mixed, and there has been a good deal of debate over whether reproduction reliably increases oxidative damage; but the conceptual appeal of a single mechanistic hub linking metabolism, environment and fitness is hard to deny, and it has

reshaped the way many physiologists now design their experiments [16].

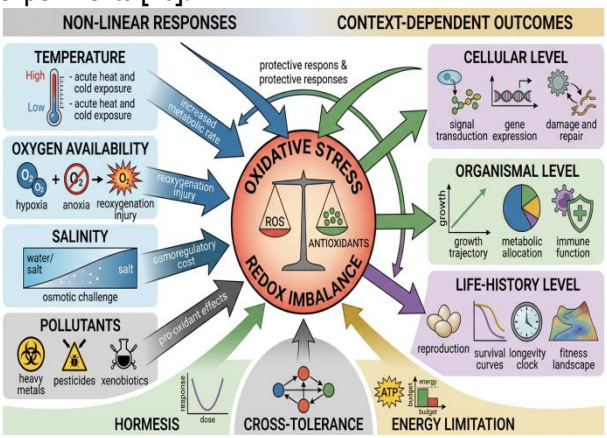

Figure 04: Oxidative stress as a central node that integrates diverse environmental stressors (temperature, oxygen fluctuation, salinity and pollutants) and translates them into physiological, life-history and fitness outcomes. Responses are typically non-linear and are further shaped by hormesis, cross-tolerance and the energy available for antioxidant defence.

6. ENVIRONMENTAL STRESSORS AND THE OXIDATIVE RESPONSE

After establishing that framework, we address the pressures themselves. The following examples are not all-inclusive, but they demonstrate the unifying function of oxidative balance as well as the unpleasant variability that any truthful account must deal with.

6.1 Temperature

Particularly in ectotherms, whose body temperature follows that of their surroundings, temperature is arguably the most thoroughly researched environmental factor influencing oxidative balance. The intuitive expectation is very straightforward: oxidative damage should increase with temperature since warming increases metabolic rate, which in turn enhances mitochondrial ROS generation. The real world has shown out to be far messier. Elevated temperature does not consistently increase oxidative damage in vertebrate ectotherms, according to a meta-analysis of experimental temperature manipulations; acute and acclimation treatments behave quite differently, and the results heavily depend on phylogeny and the thermal regime to which the animals are adapted [17]. While organisms from fluctuating settings frequently survive without any discernible harm, those from stable habitats typically exhibit obvious oxidative responses to heat stimulus [17]. The naive metabolic prediction is blunted by a number of factors, including the effects of temperature on membrane fluidity and mitochondrial uncoupling, as well as the overexpression of antioxidant enzymes whose catalytic rates are temperature-dependent [5]. It's also important to remember that coldexposure and rapid rewarming, rather than heat as such, can be potent generators of ROS in their own right.

6.2 Oxygen availability: hypoxia, anoxia and reoxygenation

The paradox of fluctuating oxygen is instructive. One might think that low oxygen would completely reduce oxidative pressure by restricting the substrate for ROS generation. The damaging phase, though, usually arrives not during hypoxia but on reoxygenation, when a tissue primed by low oxygen is abruptly re-exposed to it, in a sequence reminiscent of ischaemia–reperfusion injury in mammals [18]. What is remarkable is that many animals which routinely endure anoxia or severe hypoxia-certain fish, frogs and turtles, diving mammals, estivating snails, freeze-tolerant insects-do not merely tolerate this but appear to pre-empt it. The phenomenon, termed preparation for oxidative stress, involves the upregulation of antioxidant defences during the low-oxygen phase itself, ahead of the oxidative burst that accompanies recovery [18,19]. The first clear hint came from a striking increase in catalase activity in the muscle of garter snakes during freezing exposure [20], and the same pattern has since been documented across a remarkable range of animal phyla [19]. It is a neat illustration of an anticipatory, as opposed to a merely reactive, physiological strategy [21].

6.3 Salinity and osmotic challenge

Changes in environmental salinity oblige aquatic animals to perform osmotic work, and that work has an oxidative dimension. Osmoregulatory tissues such as the gills are metabolically active, and shifts in salinity have repeatedly been linked to altered ROS production and to changes in antioxidant enzyme activity [5]. The bioenergetic cost of maintaining ion gradients, and the close coupling between osmoregulation, mitochondrial activity and oxidative status, has been singled out as an area still in need of systematic study [22]. Once again the responses are far from uniform: whether a particular salinity change provokes oxidative damage depends on the species' osmoregulatory strategy and on its prior acclimation, much as it does with temperature.

6.4 Pollutants and xenobiotics

Human-borne pollutants make up a large and still-growing category of oxidative stressors. Iron, copper, cadmium, and chromium are only a few of the transition metals that either directly or indirectly encourage the production of reactive oxygen species (ROS) through redox cycling and Fenton-type reactions or by depleting antioxidants like glutathione [4,9]. ROS are often produced during the enzymatic biotransformation of organic xenobiotics, such as many industrial chemicals and pesticides [5]. Because of this, oxidative damage indicators and antioxidant enzymes are now often utilised biomarkers in aquatic ecotoxicology to identify exposure before overt toxicity manifests [5]. Although the overall use of

oxidative endpoints as indicators of pollution is now well established, the interpretation is not always clear because organisms might build compensatory antioxidant responses that conceal the underlying harm.

Table 04 (Previous studies): Representative studies and syntheses on environmentally induced oxidative stress in animals.

Organism / group	Stressor	Main oxidative response(s) reported	Ref.
Aquatic animals (broad synthesis)	Temperature, oxygen, salinity, pollutants	Each factor can shift the ROS production–elimination balance; antioxidant enzymes and damage markers serve as biomarkers	[5]
Vertebrate ectotherms (meta-analysis, 42 species)	Elevated temperature / CO_2 acidification	Warming did not uniformly raise oxidative damage; antioxidant enzymes up-regulated mainly at higher temperatures; outcome depends on phylogeny and acclimation	[17]
Garter snake (Thamnophis sirtalis)	Freezing / anoxia	Marked rise in muscle catalase; antioxidant defences up-regulated ahead of reoxygenation ("preparation for oxidative stress")	[20]
Hypoxia / anoxia-tolerant vertebrates and invertebrates	Hypoxia–reoxygenation	CAT, SOD, GPx and GSH increased during low O_2; oxidative damage occurs chiefly on reoxygenation	[18, 19]
Estivating land snails	Metabolic depression / estivation	Antioxidant capacity raised during dormancy as anticipatory protection	[21]
Coastal marine invertebrates	Salinity / osmotic change	Osmotic work in gills coupled to mitochondrial activity; altered ROS production and antioxidant enzyme activity	[22]
Fish (comparative / evolutionary synthesis)	Multiple environmental stressors	Oxidative status and antioxidant configuration vary with species and environmental history	[26]

Responses are summarised qualitatively from the cited works; specific quantitative values should be taken from the primary sources.

7. MEASURING OXIDATIVE STRESS: BIOMARKERS AND THEIR PITFALLS

Quantifying oxidative stress sounds more straightforward than it is. No single measurement captures the whole phenomenon, so researchers rely on a panel of markers, each reporting on a different facet of the redox system. Lipid peroxidation is commonly estimated through malondialdehyde or the broader thiobarbituric-acid-reactive substances (TBARS) assay, and through more specific products such as the isoprostanes; protein oxidation through carbonyl content; and oxidative DNA damage through 8-hydroxy-2′-deoxyguanosine [8]. On the defence side, the activities of SOD, catalase and GPx, total antioxidant capacity, and the GSH/GSSG ratio are all in routine use [11].

Table 03: Commonly used biomarkers of oxidative stress in animals and their interpretive value.

Biomarker	What it reports	Interpretation / notes
Markers of oxidative damage		
Malondialdehyde (MDA) / TBARS	Lipid peroxidation	Widely used and inexpensive, but non-specific and assay-sensitive
4-Hydroxynonenal (4-HNE)	Lipid peroxidation (reactive aldehyde)	Forms protein adducts; more specific than TBARS
F_2-isoprostanes	Lipid peroxidation	Specific and stable; a reliable index of lipid oxidation
Protein carbonyls	Protein oxidation	General, stable marker of oxidative protein damage
8-OHdG / 8-oxo-dG	Oxidative DNA damage	Mutagenic lesion; measurable in tissue and urine

Antioxidant and redox status		
SOD activity	Antioxidant enzyme	Ambiguous: a rise may mean protection or early stress
Catalase / GPx activity	Antioxidant enzymes	Best interpreted together with SOD and damage markers
GSH/GSSG ratio	Cellular redox state	A fall indicates an oxidative shift
Total antioxidant capacity (FRAP, ORAC, TEAC)	Global antioxidant capacity	Integrative but non-specific; complements single markers

Every one of these has its limitations, and the inconsistency between them is a constant source of annoyance. Particularly unclear are the activities of antioxidant enzymes, where a reduction may indicate either active downregulation or depletion, while an increase may indicate an effective defensive response or, equally, the early stage of harm [16]. Numerous widely used tests are non-specific and susceptible to sample handling, with TBARS being the archetypal offender. Partly for these reasons the field has gradually shifted towards using several complementary markers together, and towards cautious, comparative interpretation rather than reliance on any single number [16]. It has long been argued that the uncritical use of these methods has muddied the literature, and that careful methodology matters here as much as anywhere in biochemistry [23].

8. HORMESIS, ADAPTIVE RESPONSES AND CROSS-TOLERANCE

A theme running through everything above is that the relationship between stressor intensity and oxidative outcome is rarely linear. Mild oxidative challenges often improve subsequent stress resistance rather than harming the organism, a biphasic dose–response widely known as hormesis [24]. One specific example is the preparation-for-oxidative-stress response to low oxygen; another is the enhancement of antioxidant capacity with modest temperature change or moderate exercise.Theadaptive logic lies in the fact that a sublethal dose of oxidant acts as a signal, through pathways such as Keap1–Nrf2, that primes the defensive system before a larger insult arrives [14,24].

Another intriguing ecological outcome of this anticipatory logic is cross-tolerance. Exposure to one stressor can provide some resistance to another since so many stressors converge on the same oxidative machinery and signalling pathways. An animal may be more resilient to a chemical or osmotic assault if it is accustomed to mild hypoxia or a variable temperature regime [25]. The fundamental idea of the energy-limited tolerance to stress paradigm is that the ability to mount these responses is limited by energy and resources; under prolonged or compounded stress, the bioenergetic budget that finances antioxidant defence may simply run out [25]. In other words, adaptation is real but limited, and when multiple stressors push simultaneously, the same plasticity that shields animals from moderate variation may fail [26].

9. ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS

The implications of considering oxidative stress as a primary mediator extend far beyond the individual cell. Oxidative balance should have an impact on growth rates, reproductive investment, signal integrity, and lifespan if controlling ROS influences how animals distribute their resources [6]. Because the same pigments cannot be used for both display and antioxidant protection at the same time, carotenoid-based ornaments have been interpreted in behavioural and evolutionary ecology, for instance, as honest signals of an individual's ability to cope with oxidative stress [6]. The basic logic-that an animal's physiological state limits what it can afford to advertise-retains substantial explanatory power even though the data is truly disputed and some of the initial enthusiasm has since subsided.

Oxidative physiology is increasingly brought up in conversations about environmental change at the population and species level. Oxidative components are present in thermal tolerance, hypoxia tolerance, and pollution sensitivity, and there is true variety in the configuration of oxidative systems among taxa [16]. This diversity is itself shaped by selection and the environmental history of lineages, according to comparative and evolutionary methods, especially in fish and other ectotherms [26]. Knowing it is important for both academic purposes and for predicting which creatures will be most at risk as climates warm and habitats deteriorate.

10. OUTSTANDING QUESTIONS AND FUTURE DIRECTIONS

Several gaps stand out. The first is methodological: until biomarkers are standardised and validated across taxa, comparing oxidative stress between studies will remain hazardous, and meta-analyses will continue to be hampered by sheer heterogeneity [16,17]. Second, most experimental work still examines single stressors in isolation, whereas wild animals routinely face them in combination, and the interactions between stressors acting on a shared oxidative system are still poorly mapped [25]. Third, we understand the signalling side considerably better than the whole-organism side; linking molecular events at the level of Keap1–Nrf2 to consequences for survival and reproduction is difficult, and relatively few studies manage it convincingly [6].

Finally, the balance between the signalling and the damaging roles of ROS-the eustress–distress boundary-is hard to pin down experimentally, and yet it is precisely where much of the interesting biology resides [1,13]. Progress on any of these fronts would sharpen what is already among the more integrative tools available to environmental and comparative physiology.

11. CONCLUSION

Oxidative stress has matured from a concept rooted in disease and ageing into a broad organising principle for understanding how animals meet environmental challenge. The argument we have tried to make is that ROS, far from being mere metabolic waste, sit at a crossroads where the chemistry of aerobic life, the regulation of gene expression and the ecology of stress all intersect. Although the immediate causes of thermal extremes, oxygen fluctuations, salinity changes, and chemical pollution are different, they all share an oxidative currency, and the organism's response-whether it is damaged, signals adaptively, or avoids the insult entirely-is controlled by the same defensive and regulatory mechanisms. Non-linearity, hormesis and cross-tolerance, and the persistent challenge of accurately detecting redox state complicate the picture; these issues are not minor details but rather essential components of the system. Oxidative stress provides one of the more unifying perspectives in animal physiology when handled carefully, paying attention to method and context. As animal ecosystems continue to change, this perspective is expected to become even more pertinent.

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