



A comparative and evolutionary approach to oxidative stress in fish: A review

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1 **A comparative and evolutionary approach to oxidative stress in fish: a review**

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46 **Running title:** Oxidative stress in fish

Abstract

Oxidative stress results from an imbalance between the production of reactive oxygen species and the antioxidants defenses, in favour of the former. In recent years, the association between oxidative processes, environmental change and life histories has received much attention. However, most studies have focused on avian and mammalian taxonomic groups, with less attention given to fish, despite their ecological and socio-economic relevance. Here we present a review of the extrinsic and intrinsic factors that influence oxidative processes in fish, using a comparative and evolutionary approach. We demonstrate that oxidative stress plays a key role in shaping fish's responses to environmental change as well as life history strategies. We focus on representative examples to compare and contrast how levels of oxidative stress respond to changes in temperature, salinity and oxygen availability. Furthermore, we describe how emerging threats (i.e., pollution) affect oxidative stress parameters in fish. Oxidative stress indicators are increasingly being used as biomarkers to understand the mechanisms of various human-induced stressors, but also to understand the physiological consequences of how animals are distributed in space and time and influenced by different life stages. Despite the expansion of the field of ecological oxidative stress, we are only beginning to understand the complex ways in which oxidative stress may interact with both extrinsic and intrinsic factors in fish. We conclude with a research agenda for oxidative research on fish and note that there is need for further research particularly in the area of life-history strategies and ecological implications of oxidative status, as this type of research has the potential to help us understand patterns and dynamics relevant to fish conservation.

Keywords: antioxidants, evolution, fish, oxidative ecology, oxidative stress, reactive oxygen species

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1. Introduction

Oxygen in the Earth's atmosphere became present approximately 2.4 billion years ago and was highly reduced (below 5%) until approximately 600 million years before present. Prior to this time, the Earth was dominated by anaerobic organisms (Kasting and Siefert 2002). Archean cyanobacteria evolved the ability to photosynthesize (Nisbet and Sleep 2001), after which the abundance of carbon dioxide and water as reductants, along with the availability of sunlight, greatly increased the production of oxygen, leading to the evolution of other photosynthetic organisms (Falkowski et al. 2004). Over time, atmospheric oxygen increased greatly, modifying both aquatic and terrestrial habitats. These changes brought along forceful selective pressures on the remaining anaerobic organisms, resulting in a world populated by aerobic species.

The presence of atmospheric oxygen comes with its own set of challenges, the most important one being the formation of oxygen-derived free radicals – an atom or molecule with an unpaired electron – or of non-radical reactive oxygen species (ROS) (e.g., hydrogen peroxide; hypochlorous acid) (Halliwell and Gutteridge 2015). The discovery of ROS in biological systems occurred approximately 60 years ago, and was immediately linked to diseases and aging (Harman 1956). The study of these ROS and oxidative stress (i.e., generation of biomolecular oxidative damage due to an imbalance between ROS and protective antioxidant mechanisms) has since become a “hot topic” in medicine, molecular biology, physiology and, more recently, ecology. While there is extensive literature on the underlying mechanisms of oxidative stress (Costantini 2008, 2014; Dowling and Simons 2009; Metcalfe and Alonso-Alvarez 2010; Blount et al. 2015), along with many reviews on specific aspects of oxidative stress in aquatic environments (marine environments (Lesser 2006), or environmentally-induced oxidative stress in aquatic animals (Lushchak 2011)), we lack a unifying review that accounts for all aspects

surrounding oxidative stress in fish. While other reviews have mainly focused on birds and mammals (e.g., Costantini 2008; Metcalfe and Alonso-Alvarez 2010), a similar review is lacking for fish.

Fish generate many ecosystems services (reviewed in Holmlund and Hammer 1999; Lynch et al. 2016) and are remarkably diverse (Helfman et al. 2009). They live in waters that vary from entirely fresh to hypersaline, from the shallows to the abyss, from supersaturated (with oxygen) to hypoxic, from polar seas to tropical oxbow lakes. They also vary dramatically in size, morphology and life history. Their important role in aquatic ecosystems (and for humans) coupled with this immense diversity makes them relevant models for further exploring oxidative stress in ecology. Moreover, because of the direct and indirect effects of human activities on fish (see Gray 1997; Dudgeon et al. 2006), there is need and desire to characterize the ways in which humans are potentially influencing their biology, including influences that affect oxidative stress in aquatic organisms. Here we present a comprehensive summary of oxidative stress in fish. This review will focus on the extrinsic and intrinsic factors associated with oxidative stress and antioxidants in fish and provide representative examples of the main findings associated with these factors, using a comparative and evolutionary approach. We then summarize the current gaps in knowledge in this field and provide suggestions for future research. Given that the assays for ROS are not taxon-specific, we avoid in-depth discussion of the technical aspects of *in vivo* and *in vitro* ROS assays and analytical methods because they are covered elsewhere (see e.g., “Free Radicals in Biology and Medicine” by Halliwell and Gutteridge 2015).

1.1 Reactive oxygen species, antioxidants and oxidative stress in fishes

Atmospheric oxygen has two unpaired electrons in its ground state, making oxygen paramagnetic, a characteristic that prevents it from easily interacting with organic molecules unless it is activated (Asada and Takahashi 1987; Cadenas 1989; Fridovich 1998; Halliwell and Gutteridge 2015). The partial reduction of oxygen leads to the production of reactive intermediates such as superoxide radical ($O_2^{\bullet-}$), singlet oxygen (1O_2), hydrogen peroxide (H_2O_2), and the hydroxyl radical (HO^{\bullet}) (Asada and Takahashi 1987; Cadenas 1989; Fridovich 1998; Halliwell and Gutteridge 2015), referred to as ROS. All photosynthetic and aerobic organisms produce ROS, through their normal metabolism, from the mitochondrial electron transport chain at (1) complex I (NADH dehydrogenase) and (2) between ubiquinone and complex III (Brookes 2005). The superoxide radical is typically the first ROS formed, eventually leading to the formation of hydrogen peroxide and finally hydroxyl radicals which are, chemically, the most damaging of the ROS (Asada and Takahashi 1987; Cadenas 1989; Fridovich 1998; Halliwell and Gutteridge 2015), but uncertain how it affects organismal biology and fitness. While not a radical species, H_2O_2 can pass easily through membranes and is longer lived than most oxygen-derived radical species. In the presence of metal ions with changeable valence, H_2O_2 can be converted to the highly reactive hydroxyl radical which is capable of extracting electrons and protons from macromolecules (nucleic acids, proteins, lipids and carbohydrates) that it comes in contact with, thereby generating more unpaired electrons and radical species.

The production of ROS is positively associated with the concentration of oxygen within an organism (Jamieson et al. 1986). When the increase in ROS production exceeds an organism's ability to quench these reactive species with the use of antioxidants, oxidative stress occurs, damaging cellular components (Asada and Takahashi 1987; Cadenas 1989; Fridovich 1998; Halliwell and Gutteridge 2015). However, ROS can also serve as signaling molecules, with

higher sensitivity at low concentrations, at which their signaling effects are more pronounced (Dröge, 2002; Valko et al. 2007). To protect themselves against the potentially highly damaging ROS, organisms have evolved a system to either prevent or repair the effects of oxidative stress. Prevention comes in the form of antioxidants, which can either be enzymatic (e.g., superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX)) or non-enzymatic molecules (e.g., ascorbic acid (vitamin C), glutathione, α -tocopherol (vitamin E), carotenoids and other small molecules (Cadenas 1997; Valko et al. 2007) in nature. Table 1 presents a summary of the commonly measured oxidative markers and antioxidants in fish biology.

Aquatic animals, like all other organisms, must have a balance between the production of ROS and antioxidant defenses (Winston 1991; Winston and Di Giulio 1991; Kelly et al. 1998; Valavanidis et al. 2006), and are of particular interest as they experience a multitude of stressors such as temperature fluctuations, osmotic stress (in the case of anadromous species), alterations in oxygen availability, pollution, and other anthropogenic impacts, which can directly affect free radical chemistry (Lushchak 2011).

2. Extrinsic factors affecting oxidative stress

While ROS are produced intrinsically through the mitochondrial electron transport chain or the activity of immune cells, environmental stressors can also result in the production of reactive species (reviewed by Lushchak 2011). Aquatic environments include freshwater and marine systems, each bringing their own sets of constraints and challenges. In this section, we review the extrinsic factors associated with oxidative stress, and provide examples of the ways in which these factors may influence fish's levels of ROS.

2.1 Temperature

It is predicted that global climate change will increase water temperature by 2°C by the end of the 21st century (IPCC 2013), with the potential to severely impact physiological mechanisms across a wide range of organisms. It is believed that organisms living in thermally stable environments (e.g., Arctic and Antarctic regions) have a reduced ability to cope with the effects of increasing temperature (Huey and Hertz 1984), but how this temperature increase will affect cellular mechanisms is still poorly understood, especially those related to redox chemistry (Almroth et al. 2015). According to known thermodynamics principles, an increase in temperature is associated with an increased metabolic rate (Q10 Effect), leading to an increase in oxygen consumption, an increased flux at the level of the electron transport chain, and a greater production in ROS (Halliwell and Gutteridge 2015). Consequently, one would expect a higher production of ROS when water temperatures are higher. This is especially true for stenothermal fish species of the Arctic and Antarctic regions, which have very narrow thermal windows (Levins 1968). Life at the Poles has led to the evolution of a wide range of adaptations in fish, such as the increased presence of polyunsaturated fatty acids (PUFAs; Tomanek 2010) and increased mitochondrial density (Bottino et al. 1967; Fangue et al. 2009), in order to maintain energy production and metabolic rates at lower temperatures (Cheng and Detrich 2007). Antarctic icefish (*Notothenioidei* sp., Nototheniidae) have also lost their red blood cells and hemoglobin over evolutionary time as a result in living in oxygen-rich Antarctic waters. While these mechanisms are adaptive at cold temperatures, increasing water temperatures may render them maladaptive, especially in the context of oxidative stress (e.g., increased production of ROS in the mitochondria and increased likelihood of oxidation for PUFAs; Halliwell and Gutteridge 2015; Reznick and Packer 1994).

A study on the bald notothen (*Pagothenia borchgrevinki*, Nototheniidae), a species endemic to Antarctica (Clarke and Johnston 1996), exposed fish to an increase in temperature and examined the effects of such increase on antioxidant defenses, protein and lipid damage and transcriptional regulation of genes involved in redox chemistry (Almroth et al. 2015). The study showed that acute (12 hour) exposure to increasing temperature led to increased antioxidant defenses, but that these levels were similar to baseline levels when fish were exposed to chronic temperature elevation (3 weeks). These findings were also accompanied by greater levels of oxidative damage suggesting that, although stenothermal fish can modulate their antioxidant capacity, they can only do so in a transient manner, the latter which remains insufficient in quenching the increased ROS production accompanying the increase in metabolic rate with increased temperature (Almroth et al. 2015). It has been suggested that the accumulation of damage products from oxidative stress can lead to a decrease in fitness and proper physiological function (Sohal 2002); a situation which is likely to occur with the predicted ocean warming temperature across the globe. Hofmann et al. (2000) found that in one Polar fish species (emerald rockcod *Trematomus bernacchii*, Nototheniidae) the ability to induce heat shock proteins (which are for example important to help detoxification from oxidized proteins) of all size classes following either thermal or chemical stress was absent, possibly lost during evolution in these cold and thermally stable environments. *In vivo* metabolic labelling experiments that involved injection of ³⁵S-labelled methionine and cysteine into whole fish previously subjected to a heat stress of 10°C yielded no evidence for synthesis of any size class of heat shock protein (Hofmann et al. 2000). *In vivo* labelling experiments with isolated hepatocytes similarly showed significant amounts of protein synthesis, but no indication of enhanced expression of any class of heat shock proteins. Induction of chemical stress through exposure to the heavy metal cadmium

also failed to induce synthesis of heat shock proteins. However, Western analysis revealed that both the inducible and constitutively expressed forms of Hsp 70 chaperones are present in this species (Hofmann et al. 2000).

Mitochondria of Antarctic icefish also have higher densities of phospholipids per mg of mitochondrial proteins compared with related species from temperate regions (O'Brien and Mueller 2010). The high density of lipid-rich mitochondria in oxidative muscles of icefishes enhances oxygen delivery in the absence of haemoglobin and myoglobin (O'Brien 2011). However, these phospholipids are rich in PUFAs, which place these fish at increased risk of oxidative damage both in normal and in increased water temperatures. Additionally, it was found that several tissues in icefishes have lower levels of antioxidants compared with related temperate species (Witas et al. 1984; Cassini et al. 1993; Abele et al. 2012). Heart mitochondria of icefishes were shown to be more tightly coupled electron transfer than those of red-blooded fishes at 2 or 10°C, which increased the production of reactive species in icefishes when the electron transport chain was disrupted (Mueller et al. 2011). The activity of superoxide dismutase and the non-enzymatic antioxidant capacity per mg of mitochondrial proteins did not differ between icefishes and red-blooded species, but the non-enzymatic antioxidant capacity normalized to mitochondrial phospholipid content was significantly lower in icefishes than in red-blooded fishes. It was also found that the membrane susceptibility to peroxidation was only detectable in icefishes at 1°C and not in red-blooded species (Mueller et al. 2011). These constitutive differences contribute to make icefishes more vulnerable to oxidative stress than red-blooded temperate fishes when exposed to a thermal challenge. However, as with heat shock proteins, icefishes also appear to have lost the ability of upregulating antioxidant enzymes in response to oxidative stress. Mueller et al. (2012) found that levels of oxidized proteins and

lipids increased in the heart ventricle of some icefishes but not in red-blooded species in response to warming. Despite an increase in oxidative damage in hearts of icefishes, there was no activation of the antioxidant response: neither transcript levels nor activity of antioxidants increased in any tissue of any species in response to exposure to the critical thermal maximum (thermal limit above which the ambient temperature becomes lethal).

Recent studies on Antarctic Notothenioidei fish showed, however, that their antioxidants might have the potential to buffer oxidative damage induced by increased temperatures. Enzor and Place (2015) have examined the potential synergistic effects that increased water temperature and partial pressure of CO₂ have on the level of protein damage in Emerald rockcod, bald notothen, and dusky rockcod (*Trematomus newnesi*, Nototheniidae), and combined these measurements with changes in antioxidant enzymes (SOD and CAT). Their findings indicated that the activity of both SOD and CAT displayed only small changes across treatments and tissues. Short-term acclimation to decreased seawater pH and increased temperature resulted in significant increases in oxidative damage. Surprisingly, despite no significant change in antioxidant capacity, cellular damage returned to near basal levels, and in *T. bernacchii*, significantly decreased, after long-term acclimation. Although, these data suggest that notothenioid fish may have an antioxidant capacity necessary to offset predicted future ocean conditions, it remains unclear if this capacity comes with physiological trade-offs that would impact on fitness traits. While the above is generally true, studies have also shown that the opposite can also occur. For example, Malek et al. (2004) found that a decrease in ambient water temperature appeared to induce oxidative stress in the skeletal muscle of adult zebrafish by upregulating genes related to oxygen and ROS metabolism as well as the response to oxidative stress. Similarly, compared to gilthead sea breams (*Sparus aurata*) acclimated to 20°C, fish

maintained for 10 days at 8°C had higher levels of liver oxidative damage (thiobarbituric acid reactive substances; TBARS) and nitric oxide, as well as downregulated antioxidant genes (Ibarz et al. 2010).

There exists a wide range of studies surveying the effects of temperature on oxidative stress markers and antioxidants. These studies show that, although polar fish species might be more sensitive to increased water temperatures, fish species from other regions are not out of risk. Bagnyukova et al. (2007) investigated the effects of a rapid transfer from cold to warm temperatures on oxidative damage as well as antioxidant defenses in goldfish (*Carassius auratus*, Cyprinidae), and found that increases and/or decreases in oxidative markers were highly dependent on the tissues. Furthermore, it was concluded that short-term exposure to warm temperature disturbed multiple oxidative stress indicators, but only marginally impacted antioxidant capacity. Heise et al. (2006a) showed that at critical and severe temperature elevations (considered critical and severe for the species), the North Sea eelpout (*Zoarces viviparus* L., Zoarcidae) had increased oxidative damage markers (TBARS) and reduced enzymatic activity of SOD. Another study on the North Sea eelpout demonstrated that cold exposure increased oxidative stress parameters only during the recovery phase at control temperature, while antioxidant capacity remained stable throughout (Heise et al. 2006b). Lushchak and Bagnyukova (2006) showed that goldfish exposed to high temperature (35°C) had elevated SOD levels in the brain, liver and kidney. This effect was reversed when fish were allowed to recover at 21°C. The same study also found that SOD activity was highly correlated to levels of lipid peroxidation products, which suggests that these products might play a role in the upregulation of antioxidants. A study on the coastal fish, the Rock goby (*Gobius paganellus*, Gobiidae) further demonstrated that, while some tissues (muscle, liver) may suffer increased

oxidative damage when fish being exposed to increasing water temperatures, other tissues (gills) may be unresponsive because their basal antioxidant defences are already very high (Vinagre et al. 2014). These results might indicate that basal antioxidant protection is kept high in those tissues that may have a stronger impact on health and, ultimately, fitness. Altogether, these findings also suggest that the effects of temperature on oxidative stress mechanisms may be tissue- and species-dependent and that species may have evolved different mechanisms to deal with the oxidative challenges induced by thermal stress. Finally, we should not forget that conspecific populations may also differ in the way they regulate their redox state, depending on the selective pressures under which they evolved. A nice example is provided by Nikinmaa et al. (2013), which analysed transcription and redox enzyme activities at a steady state and in response to an acute temperature change in three populations of three-spined sticklebacks (*Gasterosteus aculeatus*, Gasterosteidae). They found that there was divergence in markers linked to antioxidant response, indicating that processes regulating the redox balance may be important targets of selection underlying adaptive divergence in this fish species.

2.2 Oxygen availability

The introduction of molecular oxygen into Earth's atmosphere billions of years ago has allowed animals to produce energy more efficiently (Falkowski et al. 2004). With this new ability also came a dependence on oxygen and sensitivity to changes in oxygen availability for most organisms, though some species are exceptions. In fact, ectothermic animals have developed ways to cope and survive extreme oxygen conditions (Welker et al. 2013). In the 1990s, scientists started to observe an increase in antioxidant levels under hypoxic conditions, a phenomenon later coined "preparation for oxidative stress" by Hermes-Lima et al. (1998). More

specifically, anoxia- and hypoxia-tolerant fish appear to have an anticipatory response during low-oxygen conditions by increasing their antioxidant capacity under low oxygen conditions (hypoxia, anoxia) to enhance their ability to quench ROS production upon return to normal oxygen concentrations (Lushchak and Bagnyukova 2006).

In the oceans, oxygen is not distributed evenly (Sewell and Fage 1948). Deep sea regions are often characterized as poorly oxygenated areas, and may provide refuges against oxidative stress for deep sea fish (Janssens et al. 2000). Furthermore, increased depth is associated with a decrease in metabolic rate (Childress 1995), resulting in lower ROS production in body tissues. Consequently, one could expect that deep sea fish face less oxidative stress, offering opportunities to study the mechanisms that evolved to regulate oxidative balance under low oxygen conditions. Janssens et al. (2000) found that reduced metabolic needs in deep sea fish were linked to lower antioxidant activities of SOD and GPX, but that CAT activity was neither affected by species depth nor metabolic rate. These findings suggest that SOD and GPX are primarily used against metabolically induced ROS, while CAT likely serves another purpose (Janssens et al. 2000). In fish that do not live in the deep sea, hypoxic conditions also appear to induce increased antioxidant activity, most notably that of SOD and catalase (in goldfish liver (Lushchak et al. 2001), and in common carp (*Cyprinus carpio*, Cyprinidae) liver (Lushchak et al. 2005b)).

In general, the production of ROS is correlated to the amount of oxygen within an organism (Jamieson et al. 1986). Logically, an increase in oxygen levels increases the chance of electrons to escape the complexes within the electron transport chain, and consequently form ROS. Fish have evolved ways to either avoid environments rich in oxygen or to intrinsically reduce their efficiency for extracting environmental oxygen (Lushchak 2011). Goldfish (*C.*

auratus) exposed to hyperoxic conditions experienced greater levels of oxidative stress (Lushchak et al. 2005a). Similar observations were made in Atlantic salmon (*Salmo salar*, Salmonidae; Olsvik et al. 2005) and Senegal sole (*Solea senegalensis*, Soleidae; Salas-Leiton et al. 2009). Hypoxic conditions can also induce increased activities of CAT and GPX in goldfish liver and brain, respectively (Lushchak et al. 2001). Similar observations were found in the common carp (*Cyprinus carpio*, Cyprinidae) liver (Lushchak et al. 2005b). Oehlers et al. (2007) also showed that glutathione S-transferase (GST) levels increased under low oxygen availability in medaka (*Oryzias latipes*, Adrianichthyidae). Hypoxia was found to increase oxidative stress levels in the rotan (*Perccottus glenii*, Odontobutidae) by increasing protein carbonyls in the brain, liver and skeletal muscle relative to control fish (Lushchak and Bagnyukova 2007). Additionally, lipid peroxidation products increased in all tissues, while thiols appeared to decrease with hypoxia. SOD increased significantly in the liver of hypoxic fish, while the activity of other enzymes appears to have decreased during hypoxia.

While the majority of studies demonstrate that hyperoxic conditions lead to increases in oxidative stress levels, some studies also show the opposite trend to be true. It is possible that oxidative stress, in the context of oxygen availability, is somewhat species-dependent. For example, fish inhabiting estuarine environments are exposed daily to episodes of anoxia and reoxygenation because they become exposed to air during low tides and their tissues are reoxygenated when the water level increases during high tides. Cycles of dissolved oxygen in estuaries can range from anoxia (complete lack of oxygen), hypoxia (oxygen concentrations lower than normoxia) to various levels of supersaturation (200–300% higher than normoxia) over short time periods (Ross et al. 2001). Estuarine fish, as well as estuarine invertebrates, have evolved several metabolic adaptations to tolerate short-term anoxia, such as the use of

fermentable fuels to produce energy and allow depression of metabolic rate (Storey and Storey 1990; Brooks and Storey 1997; Ross et al. 2001).

2.3 Salinity

In marine environments, the absorption of solar radiation as well as the presence of hydrothermal vents cause the greatest production of ROS (Mopper and Kieber 2000; Tapley et al. 1999). Marine fish species tend to experience additional environmental changes in comparison to freshwater fish due to the highly variable environment. Many fish species will undergo large salinity changes due to their life history, for example as they migrate from freshwater rivers into marine waters (e.g., salmonids), or as they migrate on a global scale from waters with high to low salinity and vice versa (e.g., tuna, billfish). Salinity changes impose physiological and behavioural responses such as osmoregulatory demands. There is evidence that the stress associated by changes in salinity causes an increase in the production of ROS (Liu et al. 2007).

When olive flounder (*Paralichthys olivaceus*, Paralichthyidae) were exposed to seawater for a 48h period, GPX and GST activity levels increased, demonstrating their role in quenching the increased production of ROS (Choi et al. 2008). Enzyme activities for CAT, GPX and SOD were measured in sturgeons (*Acipenser naccarii*, Acipenseridae) gradually acclimated to seawater. After 20 days spent in sea water, muscle water content, plasma osmolality and cellular constants has returned to normal, indicating that osmoregulatory processes were at work. However, cortisol levels, antioxidant activity and lipid peroxidation showed abnormal values, demonstrating that osmoregulation had caused significant physiological effects, consequently increasing oxidative stress (Martinez-Alvarez et al. 2002). Taken together, these findings suggest

that changes in salinity induce oxidative stress in fish. Fish that undergo freshwater to marine transitions, and vice versa, may have evolved ways to cope with the increased ROS production imposed by this transition such as an anticipatory antioxidant build up in the months prior to migration (Birnie-Gauvin et al. *in review*). Furthermore, we may expect that pelagic fish are less resistant to oxidative stress given that they do not undergo these periodic changes in salinity, however this kind of comparative research is lacking in the field. Given the obvious association between environmental factors and free radicals, fisheries science may benefit from greater inclusion of oxidative ecology in an effort to better understand how fish will cope with changing environmental conditions.

3. Emerging threats: pollution and land use changes

Aquatic environments are increasingly receiving pollutants in the form of agricultural and industrial chemicals which, being taken up by fish, may initiate free radical processes (reviewed in Lushchak 2016). Often these contaminants form radical species themselves, either through chemical or metabolic conversions.

3.1 Metals and industrial pollutants

Mercury represents a serious contaminant, especially in tropical environments where gold mining results in the spreading of mercury (Malm 1998). Monteiro et al. (2010) found that mercury exposure leads to elevated levels of oxidative stress in matrinxã (*Brycon amazonicus*, Characidae). Additionally, their findings suggest that oxidative processes may be the main pathway by which contaminants induce toxicity in fish. Catfish (*Channa punctatus* Bloch, Channidae) exposed to paper mill effluent, both in the short-term and the long-term, showed a

time-dependent increase in glutathione levels, GPX activity and GST activity in the liver (Ahmad et al. 2000). The same study also found that gills and kidney were less resistant to oxidative damage due to a lower antioxidant capacity. Oxidative stress was studied in the red blood cells of Nile tilapia (*Oreochromis niloticus*, Cichlidae) in fish from a fish farm and from a polluted area of a reservoir (Bainy et al. 1996). Erythrocytes were found to be more susceptible to oxidative damage in fish from the polluted sites due to an increased oxygen uptake. The same fish showed an increase in SOD activity, and a decrease in both CAT activity and glutathione content; all of which are indicative of oxidative stress. Similar results were found in African catfish (*Clarias gariepinus*, Channidae) obtained from the Ogun River of Nigeria, heavily polluted by heavy metals from local industries (Farombi et al. 2007), where SOD activity increased in liver, kidney and heart, but decreased in gills, and a decrease in CAT activity was consistently observed. Glutathione concentrations also increased in liver, kidney and heart, but decreased in gills. A similar study revealed that Indian freshwater fish (*Wallago attu*, Siluridae) from the highly polluted Panipat site had significantly higher levels of oxidative stress markers than fish from the less polluted Agra site (Pandey et al. 2003); a finding which was also observed in the European eel, in a tissue-specific manner (Ahmad et al. 2006), and the pearl cichlid (*Geophagus brasiliensis*, Cichlidae; Wilhelm Filho et al. 2001). Data obtained from such comparative studies demonstrate that antioxidant systems are a sensitive biochemical indicator of pollution exposure, and can be used to measure susceptibility to toxins (Almar et al. 1998).

Silver nanoparticles are becoming a growing concern in aquatic environments. To test their consequences on fish, Choi et al. (2010) exposed adult zebrafish (*Danio rerio*, Cyprinidae) to silver nanoparticles, and showed increased levels of malondialdehyde (a by-product of lipid peroxidation and measured by the TBARS assay) and total glutathione, as well as a decrease in

both CAT and GPX activities. In addition, an induction of DNA damage was observed. These findings suggest that silver nanoparticle toxicity is associated with oxidative stress in zebrafish under laboratory conditions. Copper (Cu) is another element that tends to accumulate in fish tissues, generally at higher doses than in the environment (Chevreuil et al. 1995). Cu is an excellent Fenton reagent, with a greater ability than iron to convert H₂O₂ to the hydroxyl radical. In the eel, a single 24h exposure to Cu (2.5µM) caused a significant increase in GST concentration and a significant decrease in reduced glutathione content in gills (Ahmad et al. 2005). In kidney tissues, Cu exposure led to decreases in CAT activity, reduced glutathione and GPX activity, as well as an increase in GST activity. These results provide evidence that exposure to heavy metals in fish can unsettle the redox balance and alter antioxidant defences.

3.2 Pesticides and agricultural pollutants

Pesticides have been shown to cause adverse effects in fish as they find their way into water systems (reviewed in Slaninova et al. 2009). Organochlorine pesticides were banned in many countries in the 1970s (still used in some tropical countries however) but their residues remain in the environment to this day. A prime example of such pesticide is dichlorodiphenyltrichloroethane (DDT) which causes an accumulation of lipid peroxides and protein carbonyls in fish (Grune 2000; Dalle-Donne et al. 2003). Fenthion, an organophosphate, causes large elevations of oxidized glutathione (GSSG; an indicator of oxidative stress) in the brain of Nile tilapia at sublethal concentrations (Piner et al. 2007). A wide range of pesticides were found to have similar impacts in fish (reviewed in Slaninova et al. 2009). Aquatic environments are affected by other anthropogenic contaminants like xenobiotics, which can exert effects on the redox chemistry of fish (Rajkumar and Milton 2011). Ekambaram et al. (2014)

investigated such effects in the brain tissue of flathead grey mullet (*Mugil cephalus*, Mugilidae) and found that fish exposed to xenobiotics had significant increases in lipid peroxides and protein carbonyls, along with decreases in reduced glutathione and total antioxidant capacity. This suggested that water pollutants can increase oxidative damage and reduce antioxidant systems in fish. Another noxious pollutant of concern in aquatic ecosystems is fly ash leachate, which can have severe physiological effects on fish (Ghio et al. 2002). Spotted snakehead (*Channa punctate*, Channidae) exposed to fly ash leachate had higher lipid peroxidation and higher antioxidant enzyme activities than control fish. These effects were found to be most prevalent in gill tissues, suggesting that gills are the most vulnerable to pollutants (Ali et al. 2004).

3.3 Land use changes

Recent studies have shown that landscape modifications, like urbanization and agriculture, can have serious negative repercussions on adjacent aquatic environments (Allan 2004). More specifically, these landscape alterations can impact nutrient inputs, dissolved oxygen concentration and temperature regimes (Meador and Goldstein 2003) which, in turn, can impact community structure and biodiversity (Allan 2004). Physiological metrics have been used to evaluate the extent of anthropogenic impacts on individuals. For example, blood-based indicators (i.e., glucocorticoids, oxidative stress indicators) are highly sensitive to stressors associated with human activities and environmental change (Barton 2002; Cooke and Suski 2008).

To date, very few studies have considered changes in land use as drivers for free radical processes. However, Blevins et al. (2013) found that creek chub (*Semotilus atromaculatus*,

Cyprinidae) from agricultural and forested streams displayed no significant differences in glutathione concentration after high temperature exposure and acclimation to low temperature. A similar study by Blevins et al. (2014) found that creek chub from two stream types did not differ in plasma glutathione concentrations. Fish that swim in urban river systems are often exposed to high levels of contaminants, combined with the increased flow rates associated with channelized rivers (Winter et al. 2004); two factors which have the potential cause oxidative stress (Aniagu et al. 2006). While no studies that we know of have investigated this particular association, fish that were initially exercised to exhaustion had much lower performances when they were previously exposed to a polluted site (McKenzie et al. 2006).

Brinkmann et al. (2010) investigated the effects of sediment re-suspension on oxidative markers in rainbow trout by simulating a 5-day flood event; a phenomenon becoming more and more common in today's changing climate. While this approach demonstrated that sediment re-mobilization leads to the uptake of sediment-bound pollutants, there were no significant alterations in oxidative enzymatic activities. It was suggested that a 5-day event may not be sufficient to allow for the detection of changes at the protein and enzyme level. Alternatively, it is possible that antioxidants levels were sufficiently high to buffer against the oxidative stress levels in the rainbow trout utilized in this study.

The impact of contaminants on the oxidative balance might also depend on the interaction with other stressors. For example, under normoxia, exposure of silver catfish (*Rhamdia quelen*, Channidae) to manganese increased lipid peroxidation in brain and kidney, increased glutathione in brain and decreased CAT activity in both brain and kidney (Dolci et al. 2013). Conversely, moderate hypoxia was able to prevent manganese-induced lipid peroxidation

in brain and to reduce it in kidney; glutathione was increased in brain, while activity of CAT was reduced in kidney and brain tissues (Dolci et al. 2013).

These findings suggest that in general, pollution of aquatic environments is reflected physiologically in fish by perturbing normal free radical processes, leading to increases in oxidative damage and disturbance of antioxidant defences. It would therefore be advisable to consider oxidative ecology in fisheries science and monitoring, given the extent of pollution in many waterbodies and the fish species that live within them.

4. Life histories and other intrinsic factors associated with oxidative stress

Life history theory is based on the assumption that increased allocation of resources into one function results in the diversion of resources from other functions (Stearns 1992). A classic example is reproductive trade-offs where increased breeding efforts result in lower future fecundity or survival (Stearns 1992). Other life history traits can be viewed using the same principle. An example of this is migration; investing more energy reserves into migration diverts such reserves from other functions such as growth, reproduction and survival. While there is extensive literature linking oxidative stress to life histories in birds (Costantini 2008) and mammals (Martin and Grotewiel 2006), there is a lack of literature regarding fish species. Recently, the role of ROS has received considerable attention as one of the key players in determining life-history strategies among various animal taxa.

4.1 Reproductive strategy

When resources are allocated to reproduction, an organism can no longer use these resources (e.g., energy, time, nutrients, etc.) for self-maintenance. The costs associated with

reproduction have been widely studied and documented (Reznick 1992), though the underlying causes of these costs have only recently been investigated (Zera and Harshman 2001). Reproduction is a highly demanding activity which elevates metabolic rate for an extended period of time, and likely induces an increase production in ROS (Alonso-Alvarez et al. 2004). However, fish (and other organisms) have evolved different breeding styles, each accompanied by their own benefits and constraints. Semelparity is defined by a single breeding event, after which an individual will die. In this case, fish may not invest resources into generating strong antioxidant defenses at all because all resources have been allocated to a single reproductive event. One might expect antioxidant capacity to be quite low during spawning of semelparous fish species. In pink salmon (*Oncorhynchus gorbuscha*, Salmonidae), oxidative damage and antioxidant capacity changed on a tissue-specific basis during spawning migration (Wilson et al. 2014). More specifically, both DNA damage and antioxidant capacity were lower at the spawning grounds when compared to similar parameters from fish at the river entrance. These results demonstrate that oxidative stress, as well as antioxidant defense and repair, are tissue-specific during spawning migration, and support the prediction that semelparity is associated with little resource allocation toward antioxidant defenses. Alternatively, fish may be attempting to lower oxidative damage by consuming more antioxidants in order to produce eggs of higher quality.

As a life history strategy, fish may reduce investments in their own protection in favor of egg antioxidant protection which is essential for hatching success and survival chances (Fontagné et al. 2006). Taylor et al. (2015) measured oxidative stress indicators and antioxidants in the plasma, heart, brain and liver of adult female sockeye salmon (*Oncorhynchus nerka*, Salmonidae) and developing offspring from three distinct populations from the Fraser river. The

study revealed that oxidative stress and antioxidant status in the offspring were not affected by maternal oxidative stress. Rather, it appears that offspring produce their antioxidants (mainly glutathione) endogenously in the later stages of development, probably due to the fact that semelparous mothers invest the better part of their resources into spawning migration rather than antioxidant protection during the migration (Taylor et al. 2015). Other studies have demonstrated that newly hatched fish and embryos rely heavily on maternal antioxidants, and subsequently generate endogenous antioxidants with time (Fontagné et al. 2006; Hung et al. 1981; Koshio et al. 1994).

In contrast, iteroparity is defined by multiple breeding events throughout life. Iteroparous species are expected to be less sensitive to the resource-based trade-off associated with reproduction (Stearns 1992). The first evidence of an association between reproduction and antioxidant defenses in iteroparous species was shown in zebra finches (Alonso-Alvarez et al. 2004). The study demonstrated that reproduction decreases antioxidant defences, which may suggest that oxidative stress is a cost of reproduction. One could assume a similar pattern in iteroparous fish, but evidence for this is lacking. Despite its scarcity, studies linking oxidative stress to life histories in iteroparous fish do exist. In smallmouth bass (*Micropterus dolomieu*, Centrarchidae), a study revealed that paternal care was negatively correlated with oxidative stress resistance, but oxidative stress markers (lipid peroxides, protein carbonyl groups and 8-hydroxy-2-deoxyguanosine (8-OHdG)) did not increase as a result of parental care (Wilson et al. 2012). The cumulative effect of oxidative stress across the reproductive lifespan of an organism has been suggested to be the cause of the age-associated decline in performance observed in iteroparous species (Kirkwood and Austad 2000).

Individual strategies of investing in mate choice may also result in different oxidative stress states. For example, Pike et al. (2007) showed that male sticklebacks fed on a low-carotenoid diet allocated more carotenoids to their nuptial colouration in an attempt to maintain sexual attractiveness. Not only were they not chosen by females in mate choice trials, but also their capital investment in sexual colouration was paid in terms of higher oxidative damage and lower survival (Pike et al. 2007). Furthermore, Dijkstra et al. (2011) revealed that red morphs and blue morphs, *Pundamilia nyererei* and *Pundamilia pundamilia* (Cichlidae), respectively, showed differences in oxidative stress levels during reproduction. Red males had significantly higher oxidative stress levels than blue males during reproduction, but only when aggression and color were expressed to a maximum, suggesting that red morphs trade off increased aggressiveness with physiological costs. These differences were attributed to carotenoid allocation in the skin of red males (Dijkstra et al. 2011).

4.2 Diet and food deprivation

In recent years, the term dietary oxidative stress has been coined to describe an imbalance between pro-oxidants and antioxidants that results from an insufficient supply of nutrients (Sies et al. 2005). Additionally, it has been suggested that the extent to which oxidative damage is imposed on organisms is directly related to the ability to produce antioxidants and their effectiveness in oxidative stress defenses (Diguiseppi and Fridovich 1984). Many antioxidants contributing to a fish's antioxidant capacity originate from its diet, especially fat-soluble antioxidants as they cannot be synthesized *de novo* by animals (Goodwin 1984). Consequently, we may expect fish with different diets to cope with oxidative stress in different ways.

Selenium is an essential micronutrient used in many physiological functions, such as growth, development and antioxidant defences (Rayman 2000). It is a component of GPXs and thioredoxin reductases among many others, giving it a crucial role for cellular protection against oxidative stress (Rayman 2000). Fontagné-Dicharry et al. (2015) demonstrated that plant-based diets should be supplemented with selenium in rainbow trout fry (*Oncorhynchus mykiss*, Salmonidae). Fish supplemented with selenium yeast had a significantly higher reduced:oxidized glutathione ratio compared to fish that were fed with sodium selenite or non-supplemental diets. Furthermore, Rider et al. (2009) found that supra-supplementation of selenium yeast did not affect oxidative stress prior to a exposure to a stressor in the form of daily handling and confinement. However, a trend toward higher GPX post-stress in selenium supplemented rainbow trout was observed. These findings suggest that physical stressors can increase the rate of selenium utilisation and, consequently, commercial fish diets should be supplemented with selenium (Rider et al. 2009). Similar findings were also observed in the crucian carp (*Carassius auratus gibelio*, Cyprinidae; Zhou et al. 2009).

Radi et al. (1985) found that SOD activity was generally higher in herbivorous fish (grass carp (*Ctenopharyngodon idella*, Cyprinidae) and silver carp (*Hypophthalmichthys molitrix*, Cyprinidae)) than in omnivorous fish (barbel (*Barbus barbus*, Cyprinidae), crucian carp and common carp (*C. carpio*, Cyprinidae)), suggesting that plant-based diets may have micronutrients which are absent or in lower levels in other food sources. Alternatively, it is possible that these plants have higher levels of pro-oxidants which may induce mild oxidative stress in fish, and cause the marked upregulation in antioxidant enzyme activities. In the Senegalese sole, the activity of CAT and SOD were found to be significantly higher in the liver of fish fed high lipid diets, while low-lipid diets appeared to minimize susceptibility to oxidative

stress, suggesting that energy sources can affect the oxidative status of these fish (Rueda-Jasso et al. 2004). Polyunsaturated fatty acids, though an essential energy source, come with the added cost of lipid peroxidation (Sargent et al. 1999), and may provide an explanation for the higher activities of enzymatic antioxidants. Taken together, these findings suggest that omnivorous and herbivorous fish may cope with oxidative stress differently by using different components of their diet to generate antioxidants.

Food deprivation can also have a wide range of physiological impacts (e.g., accelerated ageing, toxicity to chemicals), most of which can be attributed to the production of ROS (Robinson et al. 1997). For example, in the gilt-head bream (*Sparus aurata*, Sparidae), it was found that both complete and partial food deprivation for a 3-week period significantly increased malondialdehyde (a measure of lipid peroxidation by the TBARS assay) and GSSG levels (Pascual et al. 2003). The same study also showed that these levels returned to normal levels when fish were returned to control conditions (2% w/w food/fish). Similar results were obtained in a study on common dentex (*Dentex dentex*, Sparidae) where fish deprived of food for 5 weeks showed significant increases in lipid peroxidation, SOD, CAT and GPX and a significant decrease in GR activity (Morales et al. 2004). These findings show that changes in food availability or food quality during an organism's lifetime may impact its ability to produce antioxidants, defend against oxidative stress and have impacts on its fitness.

4.3 Physical activity

It was postulated in 1988 by Alessio and Goldfarb that exercise may cause oxidative stress due to an increase in oxygen metabolism at the level of the mitochondria. This association has been experimentally shown in mammalian studies (Bejma and Ji 1999), but has only been

studied in fish twice that we know of. After exhaustive exercise, adult chub (*Leuciscus cephalus*, Cyprinidae) had reduced antioxidant capacity (SOD and GSH concentration) compared to non-exercised control fish (Aniagu et al. 2006). Exercised fish also showed significantly higher DNA damage, but demonstrated no significant changes in SOD activity or GSH concentration relative to control fish. The authors reported that the degree of oxidative stress in exercised fish may not have been sufficient to elicit a defensive response or that the antioxidant defense mechanisms may have been evoked at a different timescale than the one used for the exhaustive swim and the tissue sampling. It is also possible that the antioxidant defenses were sufficient to handle the small amounts of oxidative stress which occurs during exercise, but not exhaustive swim.

In another study, Amérand et al. (2010) investigated the effects of exposure to 10.1 MPa (equivalent to 1,000 m in depth) hydrostatic pressure on silver European eel (*Anguilla Anguilla*, Anguillidae) oxidative stress parameters. The pressure exposure, which is associated with eel spawning migration, resulted in an inverse correlation between ROS production and metabolic rate, suggesting that oxygen consumption can be elevated without the harmful oxidative damage, usually sustained during increased metabolic rate associated with continuous swimming (Amérand et al. 2010).

While the link between oxidative stress and physical activity may appear to be obvious, evidence suggests that the association may be more complex than first thought in fish. The few studies on the topic have observed variable results in response to different degrees of exercise, limiting the generality of their conclusions. Physical effort in fish is often associated with important aspects of fitness and survival such as reproduction or predator avoidance. Given our current lack of understanding of the links between physical activity and oxidative stress parameters, further studies are needed.

4.4 Aging and senescence

The idea that aging and oxidative stress are linked has been around for decades (Harman 1956). As a result, a shift in the delicate balance between the production of ROS and antioxidant defences result in a deterioration of physiological functions, and is in fact thought to be the major cause of senescence (Murphy et al. 2011). However, very few studies investigated the effects of age on antioxidant protection in fish. The free-radical theory of ageing emerged more than 50 years ago, postulating that the production of ROS increases with age, and that antioxidant defenses decline (Harman 1956). While this link has been established in humans, very little information is available for fish and the correlation between antioxidant defenses and age remains unclear (Martinez-Alvarez et al. 2005).

Wdzieczak et al. (1982) found that the younger fish of various species (roach [*Rutilus rutilus* L., Cyprinidae], carp, burbot [*Lota lota* L., Lotidae], the bream [*Abramis brama* L., Cyprinidae], the perch [*Perca fluviatilis*, Percidae], silver carp [*Hypophthalmichthys molitrix*, Cyprinidae], sea bass [*Dicentrarchus labrax*, Moronidae], eel, and scorpion fish [*Scorpaena porcus*, Scorpaenidae]) had higher antioxidant capacity than older fish. Otto and Moon (1996) compared the enzymatic activity of antioxidants in rainbow trout and black bullhead (*Ameiurus melas*, Ictaluridae) from two age classes; 1+ and 3+. They found that the activities of SOD and GR declined with age in liver and extrahepatic tissues, while age-associated changes were not observed for GPX and CAT. In the freshwater murrel (*Channa punctatus*, Channidae), brain and liver GPX activity decreased throughout maturation but not during senescence where enzyme activities appeared to increase in the liver and remain stable in the brain (Nayak et al. 1999). Similarly, Sanz et al. (2001) found that the activities of GPX and CAT increased with age in the

plasma and erythrocytes of sturgeon (*A. naccarii*, Acipenseridae). The broad spectrum of observations described here suggests that long-lived species, such as the sturgeon, have evolved ways to increase their antioxidant capacity with age, while other more short-lived species appear to have a relatively constant decline in antioxidant defenses throughout life. These differences could play a significant role in expanding the lifespan of iconic long-lived fish species. The aforementioned studies are cross-sectional. We highlight the importance of doing longitudinal studies here, in order to better understand how life-history trajectories are linked to oxidative status regulation.

5. Summary and research needs

Fish are amongst some of the most diverse (Helfman et al. 2009) yet imperiled groups of organisms on Earth (Richter et al. 1997). Consequently, it is particularly relevant to study fish to understand how they will cope with future environmental change, and to understand the underlying physiological mechanisms associated with life history strategies. Oxidative stress, as well as antioxidant defenses, can be modulated in complex ways, and may play a key role in the coping mechanisms that fish will use to adapt to environmental variability. Based on our review, it became apparent that life histories of fish are likely to be significantly influenced by oxidative stress and antioxidant capacity through interactions with growth, reproduction, and body maintenance, and hence future fitness and survival (Costantini 2008; Metcalfe and Alonso-Alvarez 2010, Figure 1).

The last decade has seen explosive development in the field of ecological oxidative stress (see Speakman et al. 2015). However, birds (Wiersma et al. 2004; Costantini 2008) and mammals (Bergeron et al. 2011; Ble-Castillo et al. 2005) continue to be the best-studied

taxonomic groups. Fish typically have more variable reproductive strategies and environments in which they can live in comparison to other species (e.g., warm vs cold; well oxygenated vs poorly oxygenated). Given these circumstances, fish may have evolved ways to cope with oxidative stress that differ from mechanisms observed in other taxa. Furthermore, fish may offset the costs of oxidative processes due to their indeterminate growth (see Charnov and Berrigan 1991) compared to species with determinate growth patterns. In addition, most studies investigating life histories have been correlative, with contradicting results, and few have been carried out in the wild (Costantini 2014; Speakman et al. 2015). The need for more field studies is pressing, especially within ectotherms (including fish), which would provide a more complete understanding of the implications of oxidative stress in the wild. There is also a need for a wider range of diversity of model species which may yield unexpected insights in the field, and help partition contrasting results in the current literature. There is a need for additional research with non-lethal endpoints, especially in the context of life-histories; following changes in oxidative status of a fish over time could be highly informative. In the last two decades, only 117 research articles have been published specifically on the oxidative ecology of fish (Figure 2), suggesting that this area of research is largely unexplored.

Furthermore, no studies that we know of have used a comparative approach to investigate differences in oxidative coping mechanisms among fish with different life cycles, predatory tactics, feeding strategies, and that live in different habitats. Evidence also suggests that oxidative parameters can be used to monitor population health, though the majority of studies investigating that link have focused on birds (e.g., Beaulieu et al. 2013). Given that antioxidant defenses are typically associated with fertility and survival while oxidative stress negatively affects reproduction and growth, the use of fish as models to study oxidative parameters to

examine population health would be valuable. Experimental approaches are also highly valuable in providing fundamental information on oxidative stress mechanisms (i.e, using transgenic fish for targeted gene mutation, e.g., Carvan III et al. 2011). Further research is needed to validate the development of techniques to experimentally manipulate oxidative status and antioxidant capacity (e.g., buthionine sulfoximine and N-acetylcysteine manipulation) in fish (e.g., reviewed in Koch and Hill 2016). For the aforementioned reasons, research on oxidative stress in fish is essential to fully understand redox chemistry in an ecological context. The field will benefit from studies on the oxidative ecology of fish by providing us with a better understanding of the underlying mechanisms that likely play a role in determining important population dynamics significant to fisheries science as a whole.

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Table 1. Oxidative stress measures commonly used in fish biology. We note that at present there is no “preferred” method(s) to measure oxidative stress in fish. Study objectives and design, technical capacity and financial resources, tissue type, sampling method, among other issues, will all influence the relative applicability of a given biomarker and method. There are certainly some approaches that seem to be more commonly used in research on fish (e.g., GSH, DNA damage, ORAC) but that does not necessarily mean that they are the best. Here we provide a brief summary of some of the more commonly used oxidative stress measures used in fish noting that this is a rapidly evolving research realm.

OS Biomarker	Method of detection	Applications
Protein carbonyls	Protein carbonyl formation (Levine et al. 1990; Stadman and Berlett 1997)	Levels of protein damage, fragmentation; insight into overall oxidative stress levels
DNA damage	8-hydroxy-2'-deoxyguanosine assay (Kasai 1997)	Levels of DNA damage; insight into overall oxidative stress levels
Lipid peroxidation	Thiobarbituric acid reactive substances test (Draper et al. 1993)	Levels of lipid damage; insight into overall oxidative stress levels
Catalase (CAT)	CAT enzymatic activity assay (Sinha et al. 1972)	Insight into antioxidant defenses; higher activities may be associated with higher levels of H ₂ O ₂
Superoxide dismutase (SOD)	SOD activity assay (Beauchamp and Fridovich 1971; Oyanagui 1984)	Insight into antioxidant defenses; higher activities may be associated with higher levels of O ₂ • ⁻
Glutathione peroxidase (GPX)	GPX activity assay (Paglia and Valentine 1967; Flohé and Günzler 1984)	Insight into antioxidant defenses; higher activities may be associated with higher levels of ROS
Glutathione reductase (GR)	GR activity assay (Carlberg and Mannervik 1975; Wheeler et al. 1990)	Insight into antioxidant defenses; GR reduces GSSG back to GSH; higher activities of GR may be associated with higher levels of GSSG
Glutathione (GSH); total glutathione (TGS); glutathione disulfide (GSSG)	Glutathione assay (Akerboom and Sies 1981; Smith et al. 1988)	Provides insight into oxidative damage (GSH to GSSG ratio or vice versa) and antioxidant defenses (GSH)
Vitamin C (ascorbic acid)	Ascorbic acid assay (Roe and Kuether 1943; Deutsch and Weeks 1965)	Insight into antioxidant defenses (provides an electron to quench ROS)

Vitamin E (α -tocopherol)	Vitamin E assay (Prieto et al. 1999)	Insight into antioxidant defenses (peroxyl radical scavenger)
Low molecular weight antioxidants	Oxygen radical absorbance capacity (ORAC) assay (Cao et al. 1993)	Insight into total low molecular weight antioxidant defenses.

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Figure Captions

Figure 1. Interaction of intrinsic and extrinsic factors on oxidative stress in fish. The interplay between environmental factors and biological (life-history) factors in the context of oxidative biology. The level of oxidative stress experienced by a fish likely plays an important role in shaping its life history strategies, which in turn affects the type of environment the fish lives in.

Figure 2. Publications in oxidative ecology of fish. Number of publications per year obtained from a Web of Science search within the years of 1900 to 2015, using the following Boolean search: ((fish*)AND(oxidative stress))OR((fish*)AND(antioxidant*)). The search was further refined for the fields of Ecology and Evolutionary Biology. The results were then processed to identify relevant studies using keywords within the article titles and abstract.