



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

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Published in:
Fish and Fisheries

Link to article, DOI:
[10.1111/faf.12215](https://doi.org/10.1111/faf.12215)

Publication date:
2017

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Birnie-Gauvin, K., Costantini, D., Cooke, S. J., & Willmore, W. G. (2017). A comparative and evolutionary approach to oxidative stress in fish: A review. *Fish and Fisheries*, 18(5), 928-942.
<https://doi.org/10.1111/faf.12215>

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1 **A comparative and evolutionary approach to oxidative stress in fish: a review**
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8 *Accepted in Fish and Fisheries*
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46 **Running title:** Oxidative stress in fish

47 **Abstract**

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

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

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93 **1. Introduction**

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

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

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

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

136

137 **1.1 Reactive oxygen species, antioxidants and oxidative stress in fishes**

138 Atmospheric oxygen has two unpaired electrons in its ground state, making oxygen
139 paramagnetic, a characteristic that prevents it from easily interacting with organic molecules
140 unless it is activated (Asada and Takahashi 1987; Cadenas 1989; Fridovich 1998; Halliwell and
141 Gutteridge 2015). The partial reduction of oxygen leads to the production of reactive
142 intermediates such as superoxide radical ($O_2^{\bullet-}$), singlet oxygen (1O_2), hydrogen peroxide (H_2O_2),
143 and the hydroxyl radical (HO^{\bullet}) (Asada and Takahashi 1987; Cadenas 1989; Fridovich 1998;
144 Halliwell and Gutteridge 2015), referred to as ROS. All photosynthetic and aerobic organisms
145 produce ROS, through their normal metabolism, from the mitochondrial electron transport chain
146 at (1) complex I (NADH dehydrogenase) and (2) between ubiquinone and complex III (Brookes
147 2005). The superoxide radical is typically the first ROS formed, eventually leading to the
148 formation of hydrogen peroxide and finally hydroxyl radicals which are, chemically, the most
149 damaging of the ROS (Asada and Takahashi 1987; Cadenas 1989; Fridovich 1998; Halliwell and
150 Gutteridge 2015), but uncertain how it affects organismal biology and fitness. While not a radical
151 species, H_2O_2 can pass easily through membranes and is longer lived than most oxygen-derived
152 radical species. In the presence of metal ions with changeable valence, H_2O_2 can be converted to
153 the highly reactive hydroxyl radical which is capable of extracting electrons and protons from
154 macromolecules (nucleic acids, proteins, lipids and carbohydrates) that it comes in contact with,
155 thereby generating more unpaired electrons and radical species.

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

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

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

175

176 **2. Extrinsic factors affecting oxidative stress**

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

183

184 2.1 Temperature

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

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

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

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

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

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

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

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

299 oxidative damage when fish being exposed to increasing water temperatures, other tissues (gills)
300 may be unresponsive because their basal antioxidant defences are already very high (Vinagre et
301 al. 2014). These results might indicate that basal antioxidant protection is kept high in those
302 tissues that may have a stronger impact on health and, ultimately, fitness. Altogether, these
303 findings also suggest that the effects of temperature on oxidative stress mechanisms may be
304 tissue- and species-dependent and that species may have evolved different mechanisms to deal
305 with the oxidative challenges induced by thermal stress. Finally, we should not forget that
306 conspecific populations may also differ in the way they regulate their redox state, depending on
307 the selective pressures under which they evolved. A nice example is provided by Nikinmaa et al.
308 (2013), which analysed transcription and redox enzyme activities at a steady state and in
309 response to an acute temperature change in three populations of three-spined sticklebacks
310 (*Gasterosteus aculeatus*, Gasterosteidae). They found that there was divergence in markers
311 linked to antioxidant response, indicating that processes regulating the redox balance may be
312 important targets of selection underlying adaptive divergence in this fish species.

313

314 **2.2 Oxygen availability**

315 The introduction of molecular oxygen into Earth's atmosphere billions of years ago has
316 allowed animals to produce energy more efficiently (Falkowski et al. 2004). With this new
317 ability also came a dependence on oxygen and sensitivity to changes in oxygen availability for
318 most organisms, though some species are exceptions. In fact, ectothermic animals have
319 developed ways to cope and survive extreme oxygen conditions (Welker et al. 2013). In the
320 1990s, scientists started to observe an increase in antioxidant levels under hypoxic conditions, a
321 phenomenon later coined "preparation for oxidative stress" by Hermes-Lima et al. (1998). More

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

326 In the oceans, oxygen is not distributed evenly (Sewell and Fage 1948). Deep sea regions
327 are often characterized as poorly oxygenated areas, and may provide refuges against oxidative
328 stress for deep sea fish (Janssens et al. 2000). Furthermore, increased depth is associated with a
329 decrease in metabolic rate (Childress 1995), resulting in lower ROS production in body tissues.
330 Consequently, one could expect that deep sea fish face less oxidative stress, offering
331 opportunities to study the mechanisms that evolved to regulate oxidative balance under low
332 oxygen conditions. Janssens et al. (2000) found that reduced metabolic needs in deep sea fish
333 were linked to lower antioxidant activities of SOD and GPX, but that CAT activity was neither
334 affected by species depth nor metabolic rate. These findings suggest that SOD and GPX are
335 primarily used against metabolically induced ROS, while CAT likely serves another purpose
336 (Janssens et al. 2000). In fish that do not live in the deep sea, hypoxic conditions also appear to
337 induce increased antioxidant activity, most notably that of SOD and catalase (in goldfish liver
338 (Lushchak et al. 2001), and in common carp (*Cyprinus carpio*, Cyprinidae) liver (Lushchak et al.
339 2005b)).

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

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

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

368 fermentable fuels to produce energy and allow depression of metabolic rate (Storey and Storey
369 1990; Brooks and Storey 1997; Ross et al. 2001).

370

371 **2.3 Salinity**

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

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

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

400

401 **3. Emerging threats: pollution and land use changes**

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

406

407 **3.1 Metals and industrial pollutants**

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

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

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

437 both CAT and GPX activities. In addition, an induction of DNA damage was observed. These
438 findings suggest that silver nanoparticle toxicity is associated with oxidative stress in zebrafish
439 under laboratory conditions. Copper (Cu) is another element that tends to accumulate in fish
440 tissues, generally at higher doses than in the environment (Chevreuil et al. 1995). Cu is an
441 excellent Fenton reagent, with a greater ability than iron to convert H₂O₂ to the hydroxyl radical.
442 In the eel, a single 24h exposure to Cu (2.5µM) caused a significant increase in GST
443 concentration and a significant decrease in reduced glutathione content in gills (Ahmad et al.
444 2005). In kidney tissues, Cu exposure led to decreases in CAT activity, reduced glutathione and
445 GPX activity, as well as an increase in GST activity. These results provide evidence that
446 exposure to heavy metals in fish can unsettle the redox balance and alter antioxidant defences.

447

448 **3.2 Pesticides and agricultural pollutants**

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

460 investigated such effects in the brain tissue of flathead grey mullet (*Mugil cephalus*, Mugilidae)
461 and found that fish exposed to xenobiotics had significant increases in lipid peroxides and
462 protein carbonyls, along with decreases in reduced glutathione and total antioxidant capacity.
463 This suggested that water pollutants can increase oxidative damage and reduce antioxidant
464 systems in fish. Another noxious pollutant of concern in aquatic ecosystems is fly ash leachate,
465 which can have severe physiological effects on fish (Ghio et al. 2002). Spotted snakehead
466 (*Channa punctate*, Channidae) exposed to fly ash leachate had higher lipid peroxidation and
467 higher antioxidant enzyme activities than control fish. These effects were found to be most
468 prevalent in gill tissues, suggesting that gills are the most vulnerable to pollutants (Ali et al.
469 2004).

470

471 **3.3 Land use changes**

472 Recent studies have shown that landscape modifications, like urbanization and
473 agriculture, can have serious negative repercussions on adjacent aquatic environments (Allan
474 2004). More specifically, these landscape alterations can impact nutrient inputs, dissolved
475 oxygen concentration and temperature regimes (Meador and Goldstein 2003) which, in turn, can
476 impact community structure and biodiversity (Allan 2004). Physiological metrics have been used
477 to evaluate the extent of anthropogenic impacts on individuals. For example, blood-based
478 indicators (i.e., glucocorticoids, oxidative stress indicators) are highly sensitive to stressors
479 associated with human activities and environmental change (Barton 2002; Cooke and Suski
480 2008).

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

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

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

500 The impact of contaminants on the oxidative balance might also depend on the
501 interaction with other stressors. For example, under normoxia, exposure of silver catfish
502 (*Rhamdia quelen*, Channidae) to manganese increased lipid peroxidation in brain and kidney,
503 increased glutathione in brain and decreased CAT activity in both brain and kidney (Dolci et al.
504 2013). Conversely, moderate hypoxia was able to prevent manganese-induced lipid peroxidation

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

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

512

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

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

524

525 **4.1 Reproductive strategy**

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

528 reproduction have been widely studied and documented (Reznick 1992), though the underlying
529 causes of these costs have only recently been investigated (Zera and Harshman 2001).
530 Reproduction is a highly demanding activity which elevates metabolic rate for an extended
531 period of time, and likely induces an increase production in ROS (Alonso-Alvarez et al. 2004).
532 However, fish (and other organisms) have evolved different breeding styles, each accompanied
533 by their own benefits and constraints. Semelparity is defined by a single breeding event, after
534 which an individual will die. In this case, fish may not invest resources into generating strong
535 antioxidant defenses at all because all resources have been allocated to a single reproductive
536 event. One might expect antioxidant capacity to be quite low during spawning of semelparous
537 fish species. In pink salmon (*Oncorhynchus gorbuscha*, Salmonidae), oxidative damage and
538 antioxidant capacity changed on a tissue-specific basis during spawning migration (Wilson et al.
539 2014). More specifically, both DNA damage and antioxidant capacity were lower at the
540 spawning grounds when compared to similar parameters from fish at the river entrance. These
541 results demonstrate that oxidative stress, as well as antioxidant defense and repair, are tissue-
542 specific during spawning migration, and support the prediction that semelparity is associated
543 with little resource allocation toward antioxidant defenses. Alternatively, fish may be attempting
544 to lower oxidative damage by consuming more antioxidants in order to produce eggs of higher
545 quality.

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

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

559 In contrast, iteroparity is defined by multiple breeding events throughout life. Iteroparous
560 species are expected to be less sensitive to the resource-based trade-off associated with
561 reproduction (Stearns 1992). The first evidence of an association between reproduction and
562 antioxidant defenses in iteroparous species was shown in zebra finches (Alonso-Alvarez et al.
563 2004). The study demonstrated that reproduction decreases antioxidant defences, which may
564 suggest that oxidative stress is a cost of reproduction. One could assume a similar pattern in
565 iteroparous fish, but evidence for this is lacking. Despite its scarcity, studies linking oxidative
566 stress to life histories in iteroparous fish do exist. In smallmouth bass (*Micropterus dolomieu*,
567 Centrarchidae), a study revealed that paternal care was negatively correlated with oxidative
568 stress resistance, but oxidative stress markers (lipid peroxides, protein carbonyl groups and 8-
569 hydroxy-2-deoxyguanosine (8-OHdG)) did not increase as a result of parental care (Wilson et al.
570 2012). The cumulative effect of oxidative stress across the reproductive lifespan of an organism
571 has been suggested to be the cause of the age-associated decline in performance observed in
572 iteroparous species (Kirkwood and Austad 2000).

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

585

586 **4.2 Diet and food deprivation**

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

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

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

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

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

636

637 **4.3 Physical activity**

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

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

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

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

664

665 4.4 Aging and senescence

666 The idea that aging and oxidative stress are linked has been around for decades (Harman
667 1956). As a result, a shift in the delicate balance between the production of ROS and antioxidant
668 defences result in a deterioration of physiological functions, and is in fact thought to be the major
669 cause of senescence (Murphy et al. 2011). However, very few studies investigated the effects of
670 age on antioxidant protection in fish. The free-radical theory of ageing emerged more than 50
671 years ago, postulating that the production of ROS increases with age, and that antioxidant
672 defenses decline (Harman 1956). While this link has been established in humans, very little
673 information is available for fish and the correlation between antioxidant defenses and age
674 remains unclear (Martinez-Alvarez et al. 2005).

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

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

695

696 **5. Summary and research needs**

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

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

710 taxonomic groups. Fish typically have more variable reproductive strategies and environments in
711 which they can live in comparison to other species (e.g., warm vs cold; well oxygenated vs
712 poorly oxygenated). Given these circumstances, fish may have evolved ways to cope with
713 oxidative stress that differ from mechanisms observed in other taxa. Furthermore, fish may offset
714 the costs of oxidative processes due to their indeterminate growth (see Charnov and Berrigan
715 1991) compared to species with determinate growth patterns. In addition, most studies
716 investigating life histories have been correlative, with contradicting results, and few have been
717 carried out in the wild (Costantini 2014; Speakman et al. 2015). The need for more field studies
718 is pressing, especially within ectotherms (including fish), which would provide a more complete
719 understanding of the implications of oxidative stress in the wild. There is also a need for a wider
720 range of diversity of model species which may yield unexpected insights in the field, and help
721 partition contrasting results in the current literature. There is a need for additional research with
722 non-lethal endpoints, especially in the context of life-histories; following changes in oxidative
723 status of a fish over time could be highly informative. In the last two decades, only 117 research
724 articles have been published specifically on the oxidative ecology of fish (Figure 2), suggesting
725 that this area of research is largely unexplored.

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

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

743 **Acknowledgments**

744 S.J.C. is supported by the Natural Sciences and Engineering Research Council (NSERC) of
745 Canada (E.W.R. Steacie Fellowship, the Discovery Grant Program and the Strategic Grant
746 Program) and the Canada Research Chairs Program. W.G.W. is supported by NSERC of Canada
747 (Discovery Grant Program and CREATE Programs). D.C. is supported by the Research
748 Foundation of Flanders (FWO).

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1349 **Table 1. Oxidative stress measures commonly used in fish biology.** We note that at present
 1350 there is no “preferred” method(s) to measure oxidative stress in fish. Study objectives and
 1351 design, technical capacity and financial resources, tissue type, sampling method, among other
 1352 issues, will all influence the relative applicability of a given biomarker and method. There are
 1353 certainly some approaches that seem to be more commonly used in research on fish (e.g., GSH,
 1354 DNA damage, ORAC) but that does not necessarily mean that they are the best. Here we provide
 1355 a brief summary of some of the more commonly used oxidative stress measures used in fish
 1356 noting that this is a rapidly evolving research realm.
 1357

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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1359 **Figure Captions**

1360

1361 **Figure 1. Interaction of intrinsic and extrinsic factors on oxidative stress in fish.** The
1362 interplay between environmental factors and biological (life-history) factors in the context of
1363 oxidative biology. The level of oxidative stress experienced by a fish likely plays an important
1364 role in shaping its life history strategies, which in turn affects the type of environment the fish
1365 lives in.

1366

1367 **Figure 2. Publications in oxidative ecology of fish.** Number of publications per year obtained
1368 from a Web of Science search within the years of 1900 to 2015, using the following Boolean
1369 search: ((fish*)AND(oxidative stress))OR((fish*)AND(antioxidant*)). The search was further
1370 refined for the fields of Ecology and Evolutionary Biology. The results were then processed to
1371 identify relevant studies using keywords within the article titles and abstract.

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