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## **Stress responses in fishes: from molecular to evolutionary processes**

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1 **Abstract**

2 In the context of global changes, fish are increasingly exposed to multiple stressors that have  
3 cascading effects from molecules to the whole individual, thereby affecting wild fish populations  
4 through selective processes. In this review, we synthesize recent advances in molecular biology and  
5 evolutionary biology to outline some potentially important effects of stressors on fish across  
6 biological levels. Given the burgeoning literature, we highlight four promising avenues of research.  
7 First, (1) the exposure to multiple stressors can lead to unexpected synergistic or antagonistic  
8 effects, which should be better taken into account to improve our predictions of the effects of actual  
9 and future human activities on aquatic organisms. Second, (2) we argue that such interactive effects  
10 might be due to switches in energy metabolism leading to threshold effects. Under multiple stress  
11 exposure, fish could switch from a “compensation” strategy, i.e. a reallocation of energy to defenses  
12 and repair to a “conservation” strategy, i.e. blocking of stress responses leading to strong  
13 deleterious effects and high mortality. Third, (3) this could have cascading effects on fish survival  
14 and population persistence but multiscale studies are still rare. We propose emerging tools merging  
15 different levels of biological organization to better predict population resilience under multiple  
16 stressors. Fourth (4), there are strong variations in sensitivity among populations, which might arise  
17 from transgenerational effects of stressors through plastic, genetic, and epigenetic mechanisms. This  
18 can lead to local adaptation or maladaptation, with strong impacts on the evolutionary trajectories of  
19 wild fish populations. With this review, we hope to encourage future research to bridge the gap  
20 between molecular ecology, ecotoxicology and evolutionary biology to better understand the  
21 evolution of responses of fishes to current and future multiple stressors in the context of global  
22 changes.

23 **Keywords:** *multiple stressors, metabolic strategy, adaptation, evolutionary ecotoxicology,*  
24 *transgenerational effects*

25

## 26 **Introduction**

27

28 Due to global changes, wild fish populations are exposed to multiple environmental stressors, such  
29 as global warming, pollution or emerging pathogens, which have attracted a number of exciting  
30 research questions in the last decades (Woodward et al. 2010; Crozier and Hutchings 2014; Schulte  
31 2014; Matthaei and Lange 2016; Lange et al. 2018). For instance, what are the consequences of  
32 multiple environmental stressors on fish at the molecular, physiological and whole organism levels?  
33 How are stress responses integrated across levels of biological organization, especially under  
34 multiple stressors? Why are some populations able to cope with environmental stressors while some  
35 others dwindle? What are the long-term consequences of stressors across generations and the  
36 evolutionary mechanisms involved?

37 To address these questions, a large body of research has been dedicated to the molecular and  
38 physiological pathways involved in the stress response, with a rise in the literature focusing on fish  
39 in the last decades (Barton 2002; Killen et al. 2013; Schulte 2014; Schreck et al. 2016; Balasch and  
40 Tort 2019). In parallel, other studies have focused on the evolutionary mechanisms explaining  
41 phenotypic and genetic divergence across fish populations exposed to different levels of  
42 perturbations (Crozier and Hutchings 2014; Reid et al. 2016; Whitehead et al. 2017), leading to the  
43 emerging field of evolutionary ecotoxicology (Bickham 2011; Coutellec and Barata 2011; Oziolor  
44 et al. 2016, 2017a; Brady et al. 2017a). However, much remains to be done to bridge the gap  
45 between studies examining the impacts of multiple stressors on cells and organisms and studies  
46 examining the evolutionary causes of variations in genotypes and sensitivity across populations.  
47 This is partly because different fields of biology (e.g. molecular ecology, ecotoxicology,  
48 ecophysiology, evolutionary biology) have developed different approaches. In this review, we

49 attempt to build on recent advances in molecular and evolutionary ecology to give a synthetic view  
50 of the evolution of stress response in fish across levels of biological organization.

51

## 52 *Definitions and objectives*

53 The stress response *per se* (i.e. the physiological response triggered by stressors) is a complex series  
54 of biochemical, physiological and behavioral adjustments to environmental variations, that can  
55 enable the maintenance of internal homeostasis and survival under some conditions (Bonga 1997;  
56 Chrousos 1998; Barton 2002). Stress responses can also have negative long-term consequences  
57 depending on the level/duration/number of stressors (Schulte 2014; Balasch and Tort 2019). Some  
58 stress responses are mediated by changes in stress hormones levels (catecholamines and  
59 glucocorticoids, especially cortisol), while some responses are directly mediated by the stressor  
60 itself, for instance temperature-inducible molecular changes in Heat Shock Proteins (HSP) or stress-  
61 induced epigenetic changes (Iwama et al. 1998; Feil and Fraga 2012; Schulte 2014; Rey et al. 2016;  
62 Schreck et al. 2016). We will here adopt a broad definition of stress responses and consider  
63 responses that are initiated by stress hormones or directly by the stressor itself, following the  
64 definition given in Schulte et al. (2014). We chose this broad definition of stress responses for two  
65 reasons. First, some mechanisms underpinning some stress-induced behavioral, reproductive or  
66 morphological changes are still unknown. Second, there is now recent evidence that direct effects of  
67 stressors play a larger role than previously thought, for instance through epigenetic changes (e.g.  
68 Rey et al. 2016).

69 Interestingly, empirical studies bring contrasted results depending on the species,  
70 population, and stressor(s), making it difficult to predict the responses of aquatic organisms in their  
71 natural environment (Lavergne et al. 2015; Pédrón et al. 2017b, 2017a; Attard et al. 2018). In this  
72 review, we synthesize recent advances in molecular biology (especially omics approaches) and

73 evolutionary ecotoxicology. We focus on abiotic thermal (changes in temperature) and chemical  
74 stressors (contaminants and more generally, altered water quality), because they are of increasing  
75 concern in wild and farmed fish populations due to global changes (Schinegger et al. 2012, 2018).  
76 We also include biotic stressors such as predators and parasites because they are also affected by  
77 global changes (e.g. invasive predators and emerging diseases) (Rahel and Olden 2008; Altizer et  
78 al. 2013; Cable et al. 2017) and because they can interact with abiotic stressors (Thilakaratne et al.  
79 2007; Blonar et al. 2009; Marcogliese and Pietrock 2011; Schmitz et al. 2016). However, the  
80 combined effects of multiple biotic and abiotic stressors on fish across levels of biological  
81 organization are still poorly known (Jackson et al. 2015; Schinegger et al. 2016a). This highlights  
82 the need of multiscale studies from molecules to populations to better understand their effects on  
83 aquatic organisms.

84         Based on the existing literature, we propose four promising avenues of future research. We  
85 first (1) propose that exposure to multiple stressors results in complex interactive effects (synergism  
86 or antagonism) across biological scales, which need to be taken into account in future studies.  
87 Second, (2) we suggest that such effects might result from switches from a “metabolic  
88 compensation strategy” to a “metabolic conservation strategy”, when a certain threshold or  
89 combination of stressor(s) is reached, resulting in contrasted deleterious effects at higher levels of  
90 biological organization. Third, (3) we expose how this might differentially affect individual fitness  
91 and population persistence, potentially explaining why some populations resist to perturbations  
92 while others dwindle. Fourth, (4) we then review evidence of demonstrated interpopulation  
93 variability in fish stress responses and expose how trans-generational effects of stressors could have  
94 strong consequences for the evolutionary trajectories of wild populations, leading in some cases to  
95 extinction or to local adaptation. We believe that developing such promising avenues of future  
96 research will significantly enhance our understanding of fish response to current and future stressors  
97 in the context of global changes (Table 1).

99       (1) *Interactive effects of multiple stressors*

100 Most existing studies focus on the effect of one specific stress factor in isolation. Due to multiple  
101 anthropogenic activities and global changes, aquatic organisms are however often exposed to  
102 several stressors at once (Matthaei and Lange 2016; Schinegger et al. 2016b, 2018). Evaluating  
103 these multistress effects remains however a challenge: complex interactions between stressors can  
104 be expected, because different kinds of stressors trigger different physiological pathways that can  
105 interfere (Killen et al. 2013; Segner et al. 2014). Experimental studies using specific combinations  
106 of stress factors have for instance unveiled complex interactive effects of thermal, toxic or biotic  
107 stressors (Marcogliese et al. 2005; Holmstrup et al. 2010; McBryan et al. 2013; Gandar et al. 2017b,  
108 2017a; Pédrón et al. 2017a), resulting in different types of interactions between stressors (Folt et al.  
109 1999; Christensen et al. 2006; Côté et al. 2016; Liess et al. 2016).

110       *Synergism* occurs when individuals exposed to a stressor have a lower resistance or  
111 tolerance to another stressor (Liess et al. 2016; Lange et al. 2018). For instance, an increase in  
112 temperature can amplify the deleterious effects of contaminants, because increased temperature can  
113 increase metabolic rates and assimilation of contaminants in fish (Noyes et al. 2009). In addition,  
114 thermal stress can disrupt the ability of fish to set up an adequate stress response, thereby impairing  
115 their ability to limit the deleterious effects of contaminants (Kennedy and Ross 2012; Gandar et al.  
116 2015, 2017b). Inversely, the metabolic costs of pollutant exposure may disturb the molecular and  
117 physiological processes involved in thermal acclimation (Gordon 2005; López-Olmeda and  
118 Sánchez-Vázquez 2011). In these cases, synergism between stressors can be expected. As a  
119 consequence, more severe deleterious effects at the cell and individual levels can be observed under  
120 multiple stressors compared to single stressors with potential important negative consequences for  
121 fish survival and population persistence in the wild. For instance, pesticides have a higher negative

122 effect on goldfish *Carassius auratus* when they are combined with a thermal stress (Gandar et al.  
123 2015, 2017b, 2017a; Jacquin et al. 2019).

124 On the opposite, *antagonism* occurs when exposure to one stressor limits or decreases the  
125 effects of another stressor (Folt et al. 1999; Jackson et al. 2015), especially when physiological  
126 adaptations to one stressor are beneficial against the exposure to additional stressors (e.g. co-  
127 tolerance) (Vinebrooke et al. 2004). For instance, some families of Atlantic salmon *Salmo salar* that  
128 are tolerant to high-temperature are also more tolerant to hypoxia, because of increased heart  
129 ventricle size and myoglobin levels (e.g. Anttila et al. 2013). Fish are also interacting with natural  
130 stressors and a number of other species (predators, parasites) in their environment, which may also  
131 change the outcomes of stressor exposure (Holmstrup et al. 2010). For instance, a growing number  
132 of studies showed that fish exposed to contaminants are more susceptible to predation due to  
133 neurotoxic effects and disruption of social interactions by pollution (e.g. Lurling and Scheffer 2007;  
134 Tudorache et al. 2008). In addition, fish exposed to contaminants can be more susceptible to  
135 parasitism. For instance, Nile tilapia *Oreochromis niloticus* exposed to contaminated sediments have  
136 more gill monogean parasites due to immunosuppressive effects of toxicants (Sanchez-Ramirez et  
137 al. 2007). Conversely, parasitism can increase the sensitivity of hosts to pollutants in three-spined  
138 sticklebacks *Gasterosteus aculeatus* (Pascoe and Cram 1977). Thermal stress can also have strong  
139 effects on host-parasite interactions. For instance, the occurrence and negative effects of the  
140 emerging Proliferative Kidney Disease (PKD) on brown trout *Salmo trutta* are increased by water  
141 warming, with dramatic consequences for European salmonid populations (Okamura et al. 2011;  
142 Bruneaux et al. 2017).

143 Experimental studies manipulating the composition of communities, and field studies  
144 evaluating these multiple stress effects with realistic biotic interactions should now be conducted to  
145 refine our ability to predict the effects of future stressors on aquatic wildlife (Hahn 2011).

146



147 (2) *Metabolic strategies in response to multiple stressors*

148 Recent studies help unraveling the underpinning mechanisms explaining these complex multiple  
149 stressor effects. During the last decade, a growing body of research used “omics” tools, providing  
150 new insights into physiological pathways involved in fish stress responses (Gonzalez and Pierron  
151 2015; Oziolor et al. 2017b). These “omics” methods have been applied at different levels of  
152 biological organization (i.e. genomics, transcriptomics, proteomics, metabolomics) to evaluate the  
153 response of fish to temperature (e.g. Kullgren et al. 2013; Bermejo-Nogales et al. 2014a; Yang et al.  
154 2016), altered water quality and pollution (e.g. Li et al. 2016; Oziolor et al. 2017b; Ortiz-Villanueva  
155 et al. 2018), pathogens (e.g. Lü et al. 2014), and recently to multiple stressors (Gandar et al. 2017b;  
156 Pédrón et al. 2017a). Although response patterns can vary according to the species and the nature,  
157 intensity or duration of stressors, most studies highlight the central role of defense systems and  
158 metabolism (Kassahn et al. 2009). More specifically, stress responses generally involve changes in  
159 defense systems (e.g. chaperone proteins HSP, antioxidant systems, immunity), and more  
160 importantly, energy metabolism (e.g. Evrard et al. 2013; Bermejo-Nogales et al. 2014a; Gandar et  
161 al. 2015, 2017b; Naour et al. 2017). For instance, a transcriptomic approach showed that most  
162 transcript changes of delta smelt *Hypomesus transpacificus* exposed to salinity stress are related to  
163 metabolic processes (Komoroske et al. 2016). Naour et al. (2017) also showed that stressed red  
164 cusk-eel *Genypterus chilensis* have altered expression levels of energy metabolism pathways,  
165 especially gluconeogenesis, triacylglyceride catabolism, cholesterol and fatty acid biosynthesis.

166 More generally, under single stressor exposure, energy and oxygen consumption is generally  
167 increased to face the cost of stress response, as shown in bluegill sunfish *Lepomis macrochirus*  
168 exposed to wastewater effluents (Du et al. 2018). However, responses to multiple stressors are often  
169 very different compared to single stressors. For instance, goldfish exposed to multiple thermal and  
170 toxic stressors show on the contrary a decrease of energy consumption, a decrease of amino acid  
171 metabolism, energy production and defense systems (Gandar et al. 2017a). Metabolism and the

172 regulation of energy balance are thus central in the understanding of (multi)stress responses  
173 (Kassahn et al. 2009). Below we propose a theoretical framework based on metabolic strategies  
174 proposed by Sokolova et al. (2012; 2013) to better predict the impacts of single vs. multiple  
175 stressors across levels of biological organization.

176         The tolerance to environmental stressors depends largely on the energy available, the  
177 efficiency of acquisition and conversion of food, but also on the ability to mobilize energy resources  
178 to compensate for the metabolic cost of defenses (Sokolova et al. 2012; Sokolova 2013). The  
179 constraint of maintaining the aerobic metabolic capacity determines the stress tolerance of  
180 organisms and therefore their chances of survival (Kassahn et al. 2009). In addition, the  
181 bioenergetic costs of maintaining homeostasis and defense systems while repairing damages  
182 increase with the intensity and number of stressors, and this can lead to different metabolic  
183 strategies and result in strong threshold effects (Sokolova et al. 2012). In other words, the effects of  
184 stressors are very different depending on the duration, dose and combinations of stressors because  
185 alternative metabolic strategies can be displayed. Below, we describe the two main types of  
186 metabolic responses and the associated physiological effects that can be expected under single vs.  
187 multiple stressors (Fig 1).

188         Specifically, when the dose, duration and/or number of stressors is low, fish might display a  
189 “*metabolic compensation*” strategy (sensu Sokolova 2013). Fish implement a complete stress  
190 response from molecules to the whole individual. In a nutshell, cortisol and catecholamine levels  
191 are increasing, leading to the reallocation of energetical resources to defence mechanisms and  
192 maintenance (e.g. detoxification, immunity, damage repairs), which enables maintaining  
193 homeostasis and survival. For instance, stressed gilthead sea bream *Sparus aurata* show increased  
194 mitochondrial metabolism (Bermejo-Nogales et al. 2014) and goldfish exposed to single stressor  
195 (thermal or toxic stressor alone) show increased energy consumption and oxidative defenses  
196 (Gandar et al. 2017a). Because the energy demand for maintenance increases, the energy available

197 for growth, reproduction, activity and storage of reserves decreases (Sokolova 2013) (Fig 1). This  
198 can lead to a temporary reduction in body mass, growth and/or reproduction. For instance, fathead  
199 minnows *Pimephales promelas* exposed to temporary anthropogenic pollution in urban streams  
200 show reduced egg production (Crago et al. 2011). However, negative long-term consequences of  
201 single stressors for fitness might be reduced, if defence and repair mechanisms have been efficiently  
202 mobilized, thereby reducing long-term irreversible cellular damage and mortality in the long term  
203 (Fig 1). Accordingly, goldfish exposed to single thermal or toxic stressor alone show limited  
204 irreversible cellular damage in their gills and liver, which may ensure long-term survival (Jacquin et  
205 al. 2019). This is a “*metabolic compensation*” strategy where the energy is allocated to stress  
206 resistance, allowing the organism to efficiently face stressors with limited long-term damage. In this  
207 case, strong short-term changes in metabolism, immune and anti-oxydant defenses might be  
208 observed at the molecular and physiological level, but with low damage at the cellular level. At the  
209 individual level, this could translate into low effects on mortality and fish fitness can be temporarily  
210 maintained (Fig 1). Long-term studies in wild populations are however needed to formally test these  
211 fitness effects.

212 In contrast, when the dose, duration and/or number of stressors is high, fish could display a  
213 “*metabolic conservation*” strategy (sensu Sokolova 2013) (Fig 1). In such conditions, the energy  
214 demand for maintenance might be too high (i.e. beyond the aerobic metabolic capacity of the fish),  
215 or there might be a disruption of aerobic metabolism, leading to a decrease in the energy available  
216 below the energy demand (Sokolova et al. 2012). In both cases, aerobic metabolism cannot cover  
217 the energy demand. Metabolism can then switch to partial anaerobic to cover the costs of  
218 maintenance. Organisms can then enter into a state of “*metabolic shutdown*”, i.e. a suppression of  
219 all metabolic activity above basal metabolism, a decreased catabolism of carbohydrates and amino  
220 acids. This phenomenon, called a “*conservation strategy*” (Sokolova et al. 2012, 2013), has been  
221 observed under multiple stress exposure. For instance, gilthead seabream exposed to multiple

222 stressors (water level changes and chasing) show a blocked stress response (no cortisol increase)  
223 and a switch from aerobic to anaerobic metabolism (Bermejo-Nogales et al. 2014). Because stress  
224 responses are blocked and defense systems are not triggered, no effects on routine behaviors and  
225 growth and reproduction are expected (Fig 1). However, strong deleterious effects on cellular  
226 structure (high levels of cellular lesions and DNA damage) can be expected. For instance, goldfish  
227 conjointly exposed to high temperature and pesticides show reduced metabolism associated with  
228 increased cellular lesions of the gills and liver (Gandar et al. 2017a, 2017b; Jacquin et al. 2019).  
229 This can then translate into high mortality and low fitness, with potential detrimental effects for  
230 population persistence (de Montaudouin et al. 2010). However, empirical studies testing the long-  
231 term effects of these “*compensation*” vs “*conservation*” metabolic strategies on fish fitness and  
232 populations are now needed to formally test these hypotheses.

233         Taken together, the existing studies show that the stress tolerance and long-term effects of  
234 stressors largely depends on the energy balance and metabolic capacity of the fish, especially in a  
235 multistress context. However, the specific stimuli triggering a switch from a “*compensation*” to a  
236 “*conservation*” metabolic strategy remain to be determined. Identifying the thresholds and  
237 combinations of stressors leading to each metabolic strategy is a major challenge for future  
238 research. To tackle this question, we need more empirical studies in realistic ecological conditions  
239 to better understand when and how the physiological endpoints of individuals are reached, and to  
240 help refining environmental risks posed by multiple anthropogenic stressors.

241

### 242 (3) *Cascading effects of stressors from molecules to populations*

243 As explained above, exposure to single vs multiple stressors thus have strong consequences across  
244 levels of biological organization, depending on the metabolic strategy adopted, with potentially  
245 strong consequences for population persistence. However, most existing studies focused on the

246 molecular and cellular levels, and less work is devoted to the population level. Here we highlight  
247 some potential effects of damage induced by single vs multiple stressors on individual fitness and  
248 population persistence (Fig 2).

249 As seen above, exposure to moderate and/or single stressors is expected to lead to a  
250 “*compensation*” metabolic strategy (Sokolova 2013), resulting in limited oxidative and cellular  
251 damage (Fig 1). This is because Reactive Oxygen Species (ROS) induced by moderate/single  
252 stressors (Malek et al. 2004; Slaninova et al. 2009; Lushchak 2011; Cui et al. 2014), might be  
253 rapidly neutralized via the anti-oxidant and repair machinery that is fully triggered under a  
254 compensation metabolic strategy (Fig 1). This is expected to result in limited irreversible damage to  
255 DNA, cells and organ functions and low effects on individual survival and fitness (e.g. Gandar et al.  
256 2015, Jacquin et al. 2019). If individuals can maintain their long-term fitness under single or  
257 moderate stress conditions, one can expect a low risk of population extinction on the long term (e.g.  
258 de Mautaudoin et al. 2010).

259 In contrast, under multiple stressors, individuals often display a “*conservation*” metabolic  
260 strategy which leads to a metabolic shutdown blocking the anti-oxidant and repair machinery (Fig  
261 1). Excessive oxidative stress under multiple stress exposure can lead to irreversible DNA and cell  
262 damage (Valavanidis et al. 2006; Raphael et al. 2014; Santos et al. 2017), with potential impacts for  
263 organ function, fish survival and fitness (Costa et al. 2011; Xing et al. 2012; Birnie-Gauvin et al.  
264 2017). Amplified negative effects of multiple stressors are thus expected on fish survival (Wang et  
265 al. 2017). This might have severe consequences for population persistence for at least two reasons.

266 First, alterations of cell structure and cycle (through activations of the tumor protein p53 for  
267 instance) can lead to enhanced cancer risk (Hagenaars et al. 2013; Topal et al. 2014), as shown in  
268 zebrafish *Danio rerio* exposed to multiple pesticides (Weber et al. 2013) or in goldfish exposed to  
269 increased temperature and pesticides levels (Gandar et al. 2017b). At the population level, this

270 could lead to increased cancer prevalence and high extinction risk in fish populations exposed to  
271 multiple environmental stressors (Baumann 1984, 1998; Black and Baumann 1991), although more  
272 empirical studies are necessary to unravel the complex interactions between individual and  
273 environmental determinants of cancer occurrence and extinction in wild populations (Hochberg and  
274 Noble 2017; Vittecoq et al. 2018).

275         Second, excessive oxidative stress observed under multiple stressors can affect DNA  
276 structure and integrity (Cachot et al. 2006; Polard et al. 2011; Santos et al. 2017). In some cases,  
277 this can lead for instance to accelerated attrition of telomeres that are non-coding, repetitive  
278 sequences located at the ends of chromosomes, protecting them during replication. Individuals that  
279 have stress-shortened telomeres have accelerated senescence, and diminished longevity (Bize et al.  
280 2009; Herborn et al. 2014; Bernadotte et al. 2016; Reichert and Stier 2017). Interestingly,  
281 environmental stressors such as heat stress and pollutants have been shown to increase telomere  
282 attrition in various vertebrates including fish (Boonekamp et al. 2014; Blévin et al. 2016; Debes et  
283 al. 2016; Simide et al. 2016; McLennan et al. 2017; Angelier et al. 2018), and this has been  
284 associated with enhanced extinction risk at the population level (Dupoué et al. 2017). Integrative  
285 markers such as telomeres could thus be highly relevant because they integrate stressors effects  
286 from molecules to populations and help anticipating the extinction risks of wild fish populations  
287 exposed to multiple stressors.

288         Ultimately, predicting the emerging properties of aquatic systems exposed to multiple  
289 stressors would need more integrative approaches to better anticipate extinction risk at the  
290 population level. Biological systems respond to multiple perturbations as multiple-level  
291 interactions, within networks of cellular traits, connected physiological processes and interspecies  
292 interactions (Geist 2011; Segner et al. 2014; Balasch and Tort 2019). Theoretical frameworks  
293 examining the dynamics of cellular to organismal and population extinction risk have been  
294 proposed (Connon et al. 2012). For instance, synthetic biology (Kitano 2002) or the Adverse

295 Outcome Pathways (AOP) assessment aim to establish associations between different levels of  
296 biological organization and ultimately on population extinction risk (Ankley et al. 2010). This  
297 methodology has been applied to high-density transcription data in fathead minnows, to relate  
298 chemical exposure to disruption of the hypothalamus-pituitary-gonadalendocrine pathway leading  
299 to reprotoxic effects and decreased reproduction in fish populations (Perkins et al. 2011). However,  
300 empirical data on population dynamics and extinction risk are still lacking to fully implement such  
301 methodologies under (multi)stress exposure and to better integrate the evolutionary responses of  
302 stressed wild populations.

303

#### 304 (4) Population variability and evolutionary effects of stressors

305 Interestingly, a growing number of studies highlights a high variability in sensitivity to stressors  
306 among wild fish populations exposed to contrasted environmental conditions. For instance, different  
307 populations of European flounder *Platichthys flesus* present different sensitivities to temperature,  
308 hypoxia and contamination (Lavergne et al. 2015; Pédrón et al. 2017b). Wild guppies *Poecilia*  
309 *reticulata* evolving under contrasted PAHs pollution regimes in Trinidadian rivers have different  
310 morphology and behaviors (Rolshausen et al. 2015; Jacquin et al. 2017). Atlantic killifish *Fundulus*  
311 *heteroclitus* living in industrially polluted sites show enhanced pollution tolerance, indicating rapid  
312 adaptation to extreme pollution (Reid et al. 2016; Whitehead et al. 2017). Such examples have led  
313 to the rise of the emerging field of evolutionary toxicology, which is a promising route towards a  
314 better understanding of long-term population responses to environmental stressors (Bickham 2011;  
315 Coutellec and Barata 2011; Brady et al. 2017b; Hamilton et al. 2017; Oziolor et al. 2017b), while  
316 raising some exciting challenges for future research. For instance, the observed population  
317 divergence can sometimes result in adaptation (enhanced fitness) or to maladaptation (decreased  
318 fitness) in stressful environments, and the conditions in which adaptive divergence occurs are not  
319 clear yet (Loria et al. in press.; Ghalambor et al. 2007; Merilä and Hendry 2014). In addition, the

320 evolutionary mechanisms leading to divergence among populations are still debated, because  
321 genetic, plastic and epigenetic processes often interact to shape their evolutionary trajectories  
322 (Merilä and Hendry 2014; Merilä 2015; Beaman et al. 2016) (Fig 2). We discuss some of these  
323 mechanisms below.

324 Under some conditions, genetic local adaptation can occur. Due to selective and neutral  
325 processes, some specific resistant genotypes can be fixed in natural populations exposed to  
326 stressors, leading to local adaptation, i.e. enhanced fitness in stressful environments. However,  
327 empirical evidence of such genetic-based local adaptation in fish is still scarce. One of the best  
328 documented example is the killifish (e.g. Oziolor et al. 2014; Reid et al. 2016). *Fundulus*  
329 *heteroclitus* living in urban estuaries show strong genetic variations in toxicity mediating genes  
330 (such as the aryl hydrocarbon receptors AHR) which confer them a high tolerance to normally  
331 lethal levels of pollution (Reid et al. 2016, Whitehead et al. 2017). Interestingly, different  
332 populations having evolved in different sites show distinct molecular variations in genes connected  
333 to signaling and detoxification pathways. This suggests that different genetic combinations might  
334 have been selected for to confer the same adaptive tolerance in different killifish populations (Reid  
335 et al. 2016). High standing genetic diversity is thus crucial to determine the evolutionary potential  
336 of fish populations exposed to stressors.

337 One major challenge is however to predict the evolutionary responses to multiple stressors.  
338 For instance some genetic adaptations might confer advantages against additional stressors, such as  
339 in some *Daphnia magna* populations co-adapted to warming and increased toxicity (Zhang et al.  
340 2018). In other cases, adaptation to one stressor might impede adaptation to another stressor, such  
341 as in European flounder populations exposed to contamination that are less able to respond to  
342 thermal stress (Lavergne et al. 2015). Modelling approaches might help determining the effects of  
343 multiple environmental stressors on selective processes and population extinction risk. For instance,



344 an individual-based demo-genetic model predicted the combined effects of river flow and ocean  
345 warming on salmon population resilience and genetic evolution (Piou and Prévost 2013).

346 In addition, plasticity and transgenerational plasticity (parental effects) are major drivers of  
347 rapid phenotypic divergence among populations under stress exposure, because they can enable  
348 individuals with a given genotype to rapidly adjust their phenotypes to environmental conditions  
349 (Hendry et al. 2008; Merilä and Hendry 2014; Beaman et al. 2016). For instance, thermal plasticity  
350 enable fish to rapidly adjust their metabolism to environmental temperature (Narum et al. 2013).  
351 Transgenerational plasticity through parental effects is also an important evolutionary mechanism,  
352 because it can directly translate the effects of parental stress environment on offspring phenotype  
353 (Mousseau 1998). However, parental effects can have negative or positive effects on offspring  
354 fitness.

355 On one hand, negative parental effects are expected for instance if there is a mismatch  
356 between parental and offspring environmental conditions, which is the case in stochastic  
357 environments (Bateson et al. 2014). In addition, intrinsic costs of plasticity and stress response can  
358 also reduce offspring fitness (Ghalambor et al. 2007; Räsänen and Kruuk 2007). For instance,  
359 descendants of cortisol-treated females have a lower tolerance to thermal stress in Atlantic salmon  
360 (Eriksen et al. 2006, 2007), and stressed parents of Atlantic cod *Gadus morhua* sire offspring with  
361 malformations and decreased survival (Morgan et al. 1999). Toxic stressors can also trigger the  
362 induction of genes coding for oxidative and cellular stress across generations in zebrafish (Armant  
363 et al. 2017). In addition, DNA alterations resulting from oxidative stress in parental gonadic cells  
364 decrease the quality of gametes and offspring (Devaux et al. 2015; Herráez et al. 2017). In these  
365 cases, negative maladaptative effects of transgenerational stress are expected on offspring fitness.

366 On the other hand, some parental effects can be beneficial for offspring fitness, if they  
367 preadapt the young to the same conditions encountered by parents (Mousseau 1998). For instance,

368 parents of three-spined sticklebacks *Gasterosteus aculeatus* exposed to parasites can improve  
369 offspring response to the same parasites later on (Kaufmann et al. 2014). Sheepshead minnows  
370 *Cyprinodon variegatus* adaptively program their offspring growth to the temperature they will  
371 experience, which improves their growth and survival (Salinas and Munch 2012). However, there is  
372 often a trade-off between positive and negative effects generated by trans-generational stress  
373 effects. For instance, positive trans-generational effects of UV radiation on offspring survival are  
374 associated with increased costs on molecular damage and infection rate in guppies (Ghanizadeh  
375 Kazerouni et al. 2017). These examples suggest that transgenerational effects of stressors are more  
376 important than previously thought, but that their associated costs can cause adaptive or maladaptive  
377 plasticity depending on conditions and underpinning mechanisms.

378         Recent studies have unveiled some mechanisms underpinning transgenerational effects of  
379 stressors and have highlighted the crucial role of epigenetics in adaptive processes (Beldade et al.  
380 2011; Rey et al. 2016; Danchin et al. 2019). Epigenetic changes in DNA in response to stressors  
381 (such as changes in methylation and expression levels) are more frequent than previously thought,  
382 although their functional effects are not always clearly characterized (Nikinmaa and Rytönen  
383 2011). Stress exposure can cause strong epigenetic changes in some genes of parents that are  
384 transmitted to their eggs and embryos (Feil and Fraga 2012; Mirbahai and Chipman 2014; Pierron  
385 et al. 2014; Vandegehuchte and Janssen 2014; Cavalieri and Spinelli 2017). For instance, stress  
386 induce epigenetic changes in genes regulating defense and metabolism pathways, cell formation and  
387 development, bones and muscles formation, as well as neurologic development (e.g. Mirbahai and  
388 Chipman 2014; Vandegehuchte and Janssen 2014). Interestingly, these epigenetic changes are  
389 transmitted to offspring, and thus impact the evolutionary trajectory of populations. For instance,  
390 offspring of stressed sticklebacks *Gasterosteus aculeatus* have divergent brain transcriptomes and  
391 different levels of expression of DNA methylases, which is likely affecting their behavior and  
392 fitness across generations (Metzger and Schulte 2016, 2017). These studies suggest that

393 transgenerational stress effects and stress tolerance depend partly on epigenetic effects, and that  
394 adaptation (or maladaptation) to environmental stressors might well be faster than previously  
395 thought (Fig 2). Under global changes, the pace of environmental changes likely exceeds the rate of  
396 genetic evolutionary changes, so that such rapid plastic and epigenetic responses to environmental  
397 stressors might be crucial and result in local adaptation depending on the rate of environmental  
398 changes (Hendry et al. 2008; Crozier and Hutchings 2014; Rey et al. 2016; Danchin et al. 2019).  
399 However, more studies are needed to characterize the resulting effects on population adaptation and  
400 persistence.

401         In addition, determining which evolutionary mechanisms are responsible for population  
402 divergence is still a challenge, because a combination of all selective processes is likely to occur.  
403 For instance, adaptive plasticity depends on genetic variations, and there is often a selection for  
404 different levels of plasticity, which complicates the evolutionary trajectories of fish evolving in  
405 stressful environments (Kawecki and Ebert 2004; Ghalambor et al. 2007; Bateson et al. 2014;  
406 Beaman et al. 2016). Consequences for population persistence will also strongly depend on standing  
407 intraspecific variations in existing populations and gene flow, and on spatio-temporal scale of  
408 environmental perturbations (Fitzpatrick and Reid n.d.; Ghalambor et al. 2007; Merilä and Hendry  
409 2014; Hendry 2016; Hamilton et al. 2017; Hendry et al. 2017; Whitehead et al. 2017). One of the  
410 challenges for future studies will be to take into account these spatio-temporal scales of variations,  
411 and to integrate the effects of multiple stressors to better predict the effects of human activities on  
412 the evolutionary trajectories of wild fish populations.

413

## 414 **Conclusion**

415 To conclude, existing studies highlight the need to use multiple stress approaches across biological  
416 scales to better understand the effects of human-driven perturbations on fish populations. Recent

417 literature highlight the central role of metabolism in the stress response of fish from molecules to  
418 the whole individual. Depending on the duration, level and combination of stressors, fish can set up  
419 different metabolic strategies and energy allocation patterns, which could explain the high  
420 variability in deleterious effects on fish fitness and population persistence. Importantly, some of  
421 these effects are transmitted across generations with potential impacts on the evolutionary  
422 trajectories of wild populations. With this review, we hope to encourage future studies to bridge the  
423 gap between molecular and evolutionary approaches to better predict the impacts of present and  
424 future stressors on aquatic wildlife under global changes.

425

426

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435

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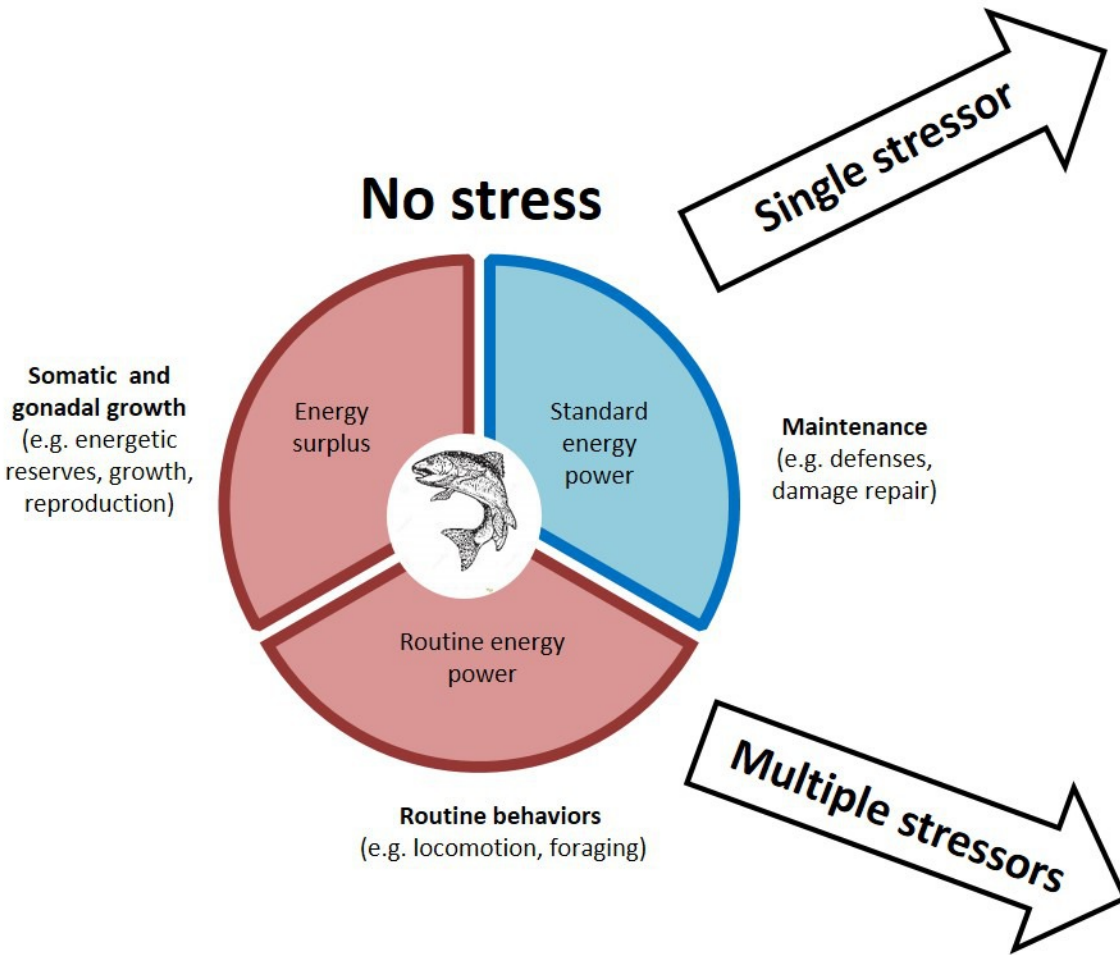
806 **Fig 1.** Schematic representation of theoretical allocation of energy following the concept of  
807 metabolic capacity of Fry (1947) and metabolic strategies of Sokolova (2013). Under normal  
808 conditions (no stress, on the left), the total energy available (represented by the circle) is first  
809 allocated to maintenance and damage repair (standard energy power), then to routine behaviors  
810 (locomotion, foraging). The energy surplus is invested in somatic and/or gonadal growth (growth  
811 and reproduction). Under exposure to a single stressor, a “compensation strategy” response is  
812 observed: the energy allocated to maintenance increases, at the expense of routine behaviors, and  
813 growth. This stress response usually leads to limited irreversible damage to cells and tissues, and  
814 low fitness effects. Under multiple stressors exposure, the energy available might be insufficient to  
815 cover the costs of maintenance, leading to a “conservation strategy”: the stress response is blocked  
816 and no reallocation of energy is observed. In this case, high level of irreversible cellular damage is  
817 expected, with increased mortality and deleterious effects on fitness (adapted from Sokolova et al.  
818 2013, Gandar et al. 2015).

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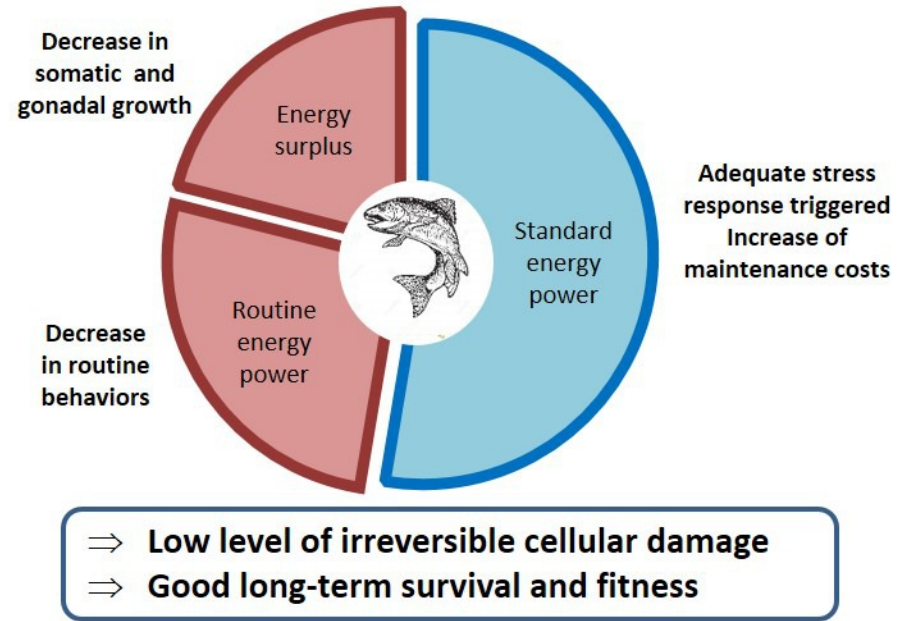
820 **Fig 2:** Fish stress responses across levels of biological organisation, from molecules to  
821 populations.

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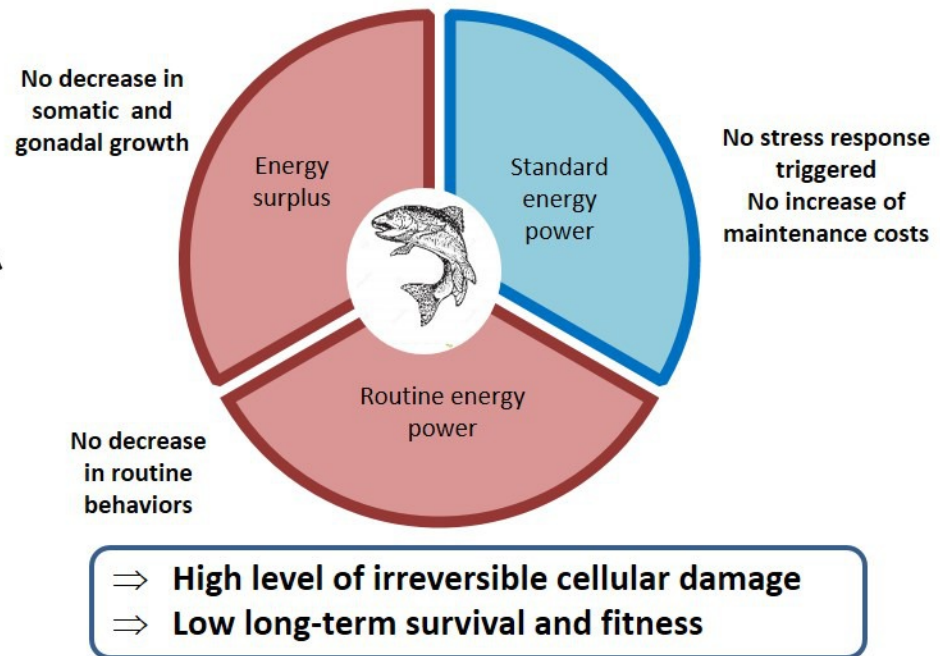




## « Compensation strategy »



## « Conservation strategy »



# Multiple environmental stressors



## Molecular responses

DNA damage, telomere erosion  
DNA methylation & expression

## Cellular responses

Changes in cell metabolism & life cycle  
Oxidative damage  
Immunity

## Individual responses

Maintenance, behavior  
Growth  Reproduction  
(Trade-offs)  
**Fitness**



## Transgenerational effects

Genetic, plastic and epigenetic effects



## Population responses

Variations in sensitivity



## Evolutionary response

Adaptation or maladaptation

Biological level of organisation

**Table 1.** *Summary of promising questions and future prospects*

**Question 1:** *What are the effects of multiple abiotic and biotic stressors across biological levels? Are synergic or antagonistic effects more frequent than previously thought?*

**Current limitations and future prospects:** Multiple stressors can have unexpected synergistic or antagonistic interaction effects on fish depending on the level of biological organization considered. Empirical studies in semi-controlled conditions from molecules to populations will help unravelling such interaction effects.

**Question 2:** *What can explain these interactions effects? Are different metabolic strategies set up under single vs. multiple stressors?*

**Current limitations and future prospects:** The metabolic strategy framework (compensation vs conservation strategy, *sensu* Sokolowa 2013) is a powerful framework for a better understanding of the high variability of stress responses observed in wild populations. Fish could switch from a “compensation” to a “conservation” metabolic strategy when exposed to multiple stressors, with contrasted effects across biological levels (Fig 1). More empirical studies are needed to test this promising theoretical framework.

**Question 3:** *What are the consequences on individual fitness and population persistence? Are there emerging tools to better link different levels of biological organization, from molecules to populations?*

**Current limitations and future prospects:** Most studies have focused on molecular pathways involved in the stress response while other studies compared individual fitness and population dynamics across environments. Integrated biomarkers and theories linking molecular and population effects could be powerful tools to bridge the gap between small- and large-scale effects of environmental stressors (Fig 2).

**Question 4:** *Why do different populations differ in their sensitivity to stressors? Can local adaptation occur and which evolutionary processes are involved (genetic, plastic, epigenetic effects)?*

**Current limitations and future prospects:** Molecular studies have enhanced our understanding of genetic and epigenetic processes underpinning adaptive processes, but they have been rarely applied to wild populations exposed to stressors. Future studies tracking molecular, genetic and epigenetic changes among stressed and unstressed fish populations while taking into account their evolutionary history will give insight into the evolutionary potential of wild fish populations exposed to multiple global changes.

**Graphical abstract**

