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**Abstract**

Aquatic systems are subjected to various sources of stress due to global changes, such as increasing temperature and pollution. A major challenge for the next decade will be to evaluate the combined effects of these multiple stressors on organisms and ecosystems. For organisms submitted to chemical, biological or physical stressors, the capacity to set up an efficient adaptive response is a fundamental prerequisite for their long-term survival and performance. In this study, goldfish (*Carassius auratus*) were subjected to individual and combined pesticide mixtures and increased temperatures to evaluate their adaptive response in multistress conditions from the molecular to the individual level. Fish were exposed for 16 days to a mixture of pesticides at environmental relevant concentrations (S-metolachlor, isoproturon, linuron, atrazine-desethyl, aclonifen, pendimethalin and tebuconazole) and at two temperatures (22 °C and 32 °C). Three major physiological traits of the stress response were measured: the hormonal response (i.e. plasma cortisol), the metabolic balance from molecular to individuals' levels (metabolomics, cellular energy allocation, energy reserves and global condition indexes), and the cellular defense system induction (SOD, CAT and GST). Results show that (1) environmentally relevant concentrations of pesticides lead to significant responses in fish at all biological levels; (2) the metabolic response depends on the nature of stress (thermal vs. chemical); and (3) fish may be unable to set up an efficient adaptive response when chemical and thermal stresses were combined, with adverse outcomes at the individuals' level.

**Keywords:** Pesticides mixtures, Climate Change, Temperature, Metabolomics, Nuclear Magnetic Resonance NMR, Oxidative stress, Goldfish

## 1. Introduction

Global changes are increasingly exposing aquatic systems to multiple stressors, but predicting their effects on aquatic organisms at different biological levels remains a challenge. The stress response is a set of adaptive physiological responses of organisms submitted to a stimulus (a stressor) perceived as dangerous, that helps maintaining homeostasis (Selye, 1950; Barton, 2002). For this purpose, the activation of the sympathetic nervous system and Hypothalamic-Pituitary-Adrenal (HPA) axis initiates a cascade of physiological changes triggered by catecholamines (i.e. adrenaline) and corticosteroid (i.e. cortisol) stress hormones. When stress persists, organisms enter in a phase of resistance characterized by the induction of cellular defense systems (Selye, 1946; 1950). The bioenergetic cost of the defense is supported by metabolic trade-offs between maintenance, activity, growth, reproduction and energy storage (Calow, 1991; Handy et al., 1999; Marchand et al., 2004). When stress is chronic or exceeds the defense and compensation capacities, adverse effects on survival and fitness of organisms may occur (Selye, 1950; Barton, 2002).

France is the leading European country in terms of agricultural area (Alim'agri, 2012) and the fourth largest consumer of pesticides in the world (Bonnefoy, 2012). The 15 most frequently detected molecules in surface waters are herbicides and fungicides, essentially used to limit weeds production and control pathogen invasion in field crops and viticulture (Butault et al., 2010). While the adverse effects of insecticides on non-target species are subject to a broad consensus within the scientific community, the toxicity of herbicides at environmentally relevant concentrations for aquatic vertebrates remains largely controversial (Solomon et al., 2008, 2013; Van Der Kraak et al., 2014). Previous studies focused almost exclusively on the effects of a single molecule. Studies on the effects of complex and realistic mixtures of pesticides on aquatic vertebrates are extremely rare, which can lead to a dramatic underestimation of the risks for wildlife (Hayes et al., 2006). At the same time, aquatic systems are submitted to environmental changes, such as increasing temperature. Interactions between contaminants and water temperature can be particularly deleterious for ectothermic species as the temperature directly affects many biochemical and physiological processes (López-Olmeda, and Sánchez-Vázquez, 2011; Manciocco et al., 2014). Higher temperature leads to increased energy demand. By affecting the metabolic level of individuals, the temperature can change the ability of aquatic organisms to effectively respond to chemical contamination, and *vice versa* (Lemly, 1996; Kennedy and Ross, 2012; Sokolova, 2013). The majority of studies thus shows a synergistic effect of temperature on the toxicity of pollutants, especially in aquatic species (Schiedek et al., 2007; Noyes et al., 2009; Holmstrup et al., 2010; Laetz et al., 2014). Inversely, the exposure to pollutants may affect the temperature tolerance in fish species (Manciocco et al., 2014). However, there is a lack of knowledge about combined effects between temperature and pesticide mixtures on aquatic vertebrates. In previous studies (Gandar et al., 2015, 2017), we exposed goldfish (*Carassius auratus*) to higher temperature and a mixture of herbicides and fungicides at realistic concentrations for 96 hours. Results showed significant cross effects between temperature and pesticides on behavioral responses, energy reserves and hepatic proteome profile, suggesting that combined thermal and

68 chemical stressors impaired the stress response of the goldfish to individual stressors (Gandar et al., 2015,  
69 2017). However, the effects of a longer exposure on fish global performance across biological levels remain to  
70 be determined.

71 Therefore, the aim of this study was to assess the adaptive response to stress of goldfish submitted to  
72 single and combined chemical and thermal stresses, integrating responses from the molecular to the whole  
73 organism level. To that end, goldfish were exposed during 16 days to a mixture of six herbicides (S-  
74 Metolachlor, Isoproturon, Linuron, Aclonifen, Atrazine-desethyl and Pendimethalin) and one fungicide  
75 (Tebuconazol) to a total concentration of  $42 \mu\text{g.L}^{-1}$  at two temperatures ( $22 \text{ }^\circ\text{C}$  and  $32 \text{ }^\circ\text{C}$ ). The stress response  
76 was investigated at 6 h, 96 h and 16 d of exposure, including plasma cortisol concentration, hepatic induction  
77 of antioxidant enzymatic defenses (superoxide dismutase SOD, catalase CAT) and enzymatic system of  
78 detoxification Glutathion-S-Transferase (GST), hepatic metabolome response by  $^1\text{H-NMR}$  metabolomic  
79 analysis, energy reserves and cellular energy allocation in liver and white muscle, somatic indexes (muscle and  
80 hepatic indexes) and global condition of fish (Fulton's condition factor).

## 81 2. Materials and methods

### 82 2.1. Pesticide mixture

83 The objective of the study was to characterize fish responses to environmentally relevant pesticide  
84 mixtures at different temperatures. The mixture was developed on the basis of the pesticide contamination of  
85 the Save River (France), assessed from March 2008 to November 2009 (Pollard et al., 2011) as described by  
86 Gandar et al. 2017. According to the analysis conducted by Pollard et al. (2011) and Taghavi et al. (2011), we  
87 selected six herbicides and one fungicide using three criteria: the frequency of detection, the concentration  
88 and the representation of the different families of molecules detected. Selected molecules are S-metolachlor,  
89 Isoproturon, Linuron, Tebuconazol, Aclonifen, Atrazine-desethyl and Pendimethalin (Supplementary Table 1).

90 Pesticide concentrations in water were measured after 16 d of exposure. Water samples of 1 mL were spiked  
91 with internal standards solution (Simazine D10, Atrazine-desethyl D7, Isoproturon D6, Linuron D6, Metolachlor  
92 D6, Pendimethalin D5, Tebuconazole D6) and directly analyzed on an Agilent liquid chromatographic system  
93 coupled with a triple quadrupole mass spectrometer (Agilent LC 1290 Infinity / 6460 Triple quadrupole)  
94 equipped with an electrospray ionization source used in positive mode (ESI+) (adapted from Gamain et al.,  
95 2016). Pesticides were quantified using a quantification transition. Analytical method was validated in terms of  
96 calibration linearity, specificity, and limits of quantifications ( $0.005$  to  $0.5 \mu\text{g.L}^{-1}$ ). Recoveries of samples of  
97 fortified mineral water were evaluated for water analysis (from 89 to 116%). Control calibrating standards  
98 were also injected every 15 samples and analytical blanks were performed. All solvents for chemicals analysis  
99 were at least of analytical grade. The measured concentration of pesticides showing a slight decrease  
100 compared to expected values (total concentration of  $38.7 \mu\text{g.L}^{-1}$ , Supplementary Table 1). Pesticide standards  
101 were obtained from Sigma-Aldrich (St. Louis, MO, USA): S-metolachlor (CAS-No: 87392-12-9,

102 PESTANAL<sup>®</sup>, 98,4% pure), Isoproturon (CAS-No: 34123-59-6, PESTANAL<sup>®</sup>, 99% pure), Linuron (CAS-No: 330-55-  
103 2, PESTANAL<sup>®</sup>, 99,7% pure), Atrazine-desethyl (CAS-No: 6190-65-4, PESTANAL<sup>®</sup>, 99,5% pure), Aclonifen (CAS-  
104 No: 74070-46-5, PESTANAL<sup>®</sup>, 99,8% pure), Pendimethalin (CAS-No: 40487-42-1, PROWL<sup>®</sup>, 98,8% pure),  
105 Tebuconazol (CAS-No:107534-96-3, PESTANAL<sup>®</sup>, 99,3% pure). Acetone (CAS: 67-64-1, Fisher Chemical, HPLC  
106 solvent) was purchased from Fisher Scientific (Illkirch, France). Scintillation liquid (Flo-Scint II<sup>™</sup>) was purchased  
107 from Perkin Elmer (Waltham, Massachusetts, USA).

## 108 2.2. Animal care

109 Fish were purchased from the fish farming Carpio (Consac, France) in the size range 10-12 cm. They  
110 were first acclimatized for two weeks in opaque tanks under controlled conditions (18 °C with a 12:12 h light  
111 regime). Water was aerated and dechlorinated prior to fish introduction. Half of the water was renewed every  
112 day and fish were fed daily with commercial pellets. No mortality occurred during the acclimation period.  
113 Experimental procedures were conducted under French animal handler's certificate n<sup>o</sup>.31-103, giving  
114 authorization to experiment on living vertebrates.

## 115 2.3. Experimental design

116 Fish were exposed to a pesticide mixture and/or temperature increased to obtain four conditions: the  
117 CONTROL group (fish at 22 °C non-exposed to pesticides), the TEMP group (fish at 32 °C non-exposed to  
118 pesticides), the PEST group (fish exposed to pesticides at 22 °C) and the PEST\*TEMP groups (fish exposed to  
119 pesticides at 32 °C) as previously described (Gandar et al., 2017). Goldfish were placed in 60 L opaque aquaria  
120 in a temperature controlled room (22 °C, 12 fish per aquarium and 2 aquaria per condition\*exposure time).  
121 Prior to pesticide exposure, the water of the TEMP and PEST\*TEMP groups was gradually heated (1 °C/day) to  
122 reach 32 °C. Fish were then exposed for 6 h, 96 h or 16 d to the mixture of pesticides. Water was renewed by  
123 half every day. Temperature, pH, oxygenation and conductivity were assessed 1 h after the water renewal  
124 (Supplementary Table 2). Ammonium, nitrites and nitrates concentrations were measured by colorimetry at  
125 each time of exposure (Supplementary Table 2). Fish were fed daily and no mortality occurred during  
126 exposures.

127 Twelve fish per condition were randomly sampled at 6 h, 96 h and 16 d. Fish were euthanized with an  
128 overdose of benzocaine. Blood samples were collected from the caudal vein by puncturing using a heparinized  
129 syringe and centrifuged. Plasma samples were kept at -80 °C. Fish were weighted (to the nearest 10 mg) and  
130 measured (fork length to the nearest mm). Livers and white muscles were collected, weighted (to the nearest  
131 0.1 mg), frozen in liquid nitrogen and kept at -80 °C.

## 132 2.4. Plasma cortisol concentration

133 Plasma cortisol concentration was assessed by radioimmunoassay (RIA) as previously described  
134 (Désautés et al., 1997). The plasma cortisol concentration was expressed in ng.mL<sup>-1</sup>.

## 2.5. Metabolomic analysis

## 2.5.1. Metabolite extraction

Metabolites were extracted from 50 mg tissue samples with a methanol/chloroform/water (2.0:2.0:1.8) solvent mixture using a Twostep protocol adapted from Lin et al. (2007) and Wu et al. (2008) with slight modifications. Briefly, livers of fish were ground and weighted. A 50 mg sample was homogenized using a Fastprep® homogenizer with methanol (4 mL.g<sup>-1</sup>) and Milli-Q water (0.85 mL.g<sup>-1</sup>) and vortexed for 5 s. Samples were then mixed with chloroform (4 mL.g<sup>-1</sup>) and Milli-Q water (2 mL.g<sup>-1</sup>) and vortexed during 5 s, incubated 15 min at 4 °C and then centrifuged at 2800 g for 15 min. The entire upper layer was collected and kept at -20 °C.

2.5.2. 1D-<sup>1</sup>H NMR spectroscopy and spectral preprocessing

Samples were evaporated using a SpeedVac and resuspended in a phosphate buffer (pH 7.4) prepared in a deuterated water (D<sub>2</sub>O) and containing 1 mM of sodium 3-trimethylsilyl-2,2,3,3-d<sub>4</sub>-propionate (TMSP, internal chemical shift standard). Samples were centrifuged and 600 µl of supernatant were transferred in 5 mm NMR tubes. NMR Spectra were acquired at 600 MHz with a Bruker Avance spectrometer (Bruker, Karlsruhe, Germany) with a SampleJet autosampler using a NOESY 1D sequence to remove the water signal. For 1D spectra, 256 scans were collected into 64,000 data points with a relaxation delay of 2 s. The free induction decays (FID) were Fourier transformed and spectra were phased, baseline corrected and calibrated (TMSP, δ 0 ppm) using TOPSPIN v2.1 software (Bruker, Karlsruhe, Germany). A variable size bucketing was used and 102 buckets were selected according to resonance signals and multiplicity between 9.4 and 0.5 ppm, excluding residual water signal. The signal intensity in each bucket was integrated using AMIX software (version 3.9.13, Bruker, Karlsruhe, Germany). Data were normalized with respect to the total spectrum intensity using mean-centering and Pareto scaling to allow comparison between samples.

## 2.5.3. Statistical analysis

Supervised Partial Least Square-Discriminant Analysis (PLS-DA) was used to assess the discrimination between groups. OSC filter was applied prior to the analysis to remove the analytical and biological variations that were not related to group discrimination (Beckwith-Hall et al., 2002). Q<sup>2</sup> (predictability of the model, threshold of 0.4) and R<sup>2</sup> (total explained variations) values were used to confirm the validity of the models. Permutation tests (200 iterations) were then applied to assess PLS-DA model robustness. The Variable Importance in Projection (VIP, weight of variables for each component; value > 1.0 - arbitrary threshold) was used to determine discriminant buckets. Comparisons between conditions were performed using Kruskal-Wallis tests and post-hoc tests (significance threshold = 0.05).

SIMCA P and R softwares were used respectively for PLS-DA analysis and Kruskal-Wallis test.

## 167 2.5.4. Peak assignments

168 The discriminant metabolites were identified using the literature, home-made and freeware databases  
 169 such as the Biological Magnetic Resonance Data Bank ([www.bmrb.wisc.edu/](http://www.bmrb.wisc.edu/)) (Ulrich et al., 2008) and the  
 170 Human Metabolome Database ([www.hmdb.ca/](http://www.hmdb.ca/)) (Wishart et al., 2007). To confirm the chemical structure of  
 171 metabolites of interest, 2D  $^1\text{H}$ - $^1\text{H}$  COSY (Correlation Spectroscopy) and 2D  $^1\text{H}$ - $^{13}\text{C}$  HSQC (Heteronuclear Single  
 172 Quantum Coherence Spectroscopy) NMR experiments were performed on selected samples. The spectral  
 173 assignment was based on matching 1D and 2D data to reference spectra.

## 174 2.6. Energy reserves and cellular energy allocation

175 Energetic reserves at the molecular and cellular level are essential to sustain the stress responses of  
 176 fish. Total carbohydrate, lipid and protein contents and oxygen consumption rate in the electron transport  
 177 system (ETS) were measured in liver and white muscle of fish with protocols adapted from De Coen and  
 178 Janssen (1997). Carbohydrate concentration ( $\mu\text{g}\cdot\text{mg}^{-1}$  of tissue) was quantified using a microplate  
 179 spectrophotometer at 492 nm against glucose in TCA. Protein concentration ( $\mu\text{g}\cdot\text{mg}^{-1}$  of tissue) was quantified  
 180 by adding Bradford reagent and reading absorbance at 595 nm against bovine serum albumin in 0,2 N NaOH  
 181 (Bradford, 1976). Total lipid concentration ( $\mu\text{g}\cdot\text{mg}^{-1}$  of tissue) was measured by reading absorbance at 525 nm  
 182 against tripalmitin in chloroform. The ETS activity ( $\text{mJ}\cdot\text{mg}^{-1}\cdot\text{tissue}\cdot\text{h}^{-1}$ ), was measured by absorbance reading at  
 183 490 nm when iodinitrotetrazolium (INT) was added and during 3 min. The molar extinction coefficient of  
 184  $15,900\text{ M}^{-1}\text{cm}^{-1}$  was used.

185 Carbohydrate, protein and lipid concentrations were transformed in their energetic values ( $\text{mJ}\cdot\text{mg}^{-1}$  of  
 186 tissue) using their enthalpy of combustion (17, 24 and  $39.5\text{ kJ}\cdot\text{g}^{-1}$  respectively). Available energy (AE), energy  
 187 consumption (EC) and cellular energy allocation (CEA) in liver and white muscle were then calculated according  
 188 to Verslycke et al. (2004):

$$189 \quad \text{AE} = \text{carbohydrates} + \text{proteins} + \text{lipids} \quad (\text{mJ}\cdot\text{mg}^{-1}\text{ tissue}) \quad (1)$$

$$190 \quad \text{EC} = \text{ETS activity} \quad (\text{mJ}\cdot\text{mg}\text{ tissue}^{-1}\cdot\text{h}^{-1}) \quad (2)$$

$$191 \quad \text{CEA} = \frac{\text{AE}}{\text{EC}} \quad (\text{h}^{-1}) \quad (3)$$

## 193 2.7. Antioxidant and detoxification enzymes

194 The antioxidant system is a central component of the defense system against chemical and thermal  
 195 stressors. The activities of the SOD, CAT and GST were measured in the liver of fish by spectrophotometry.  
 196 Protocols were adapted from Feirrer et al. (2015). Briefly, tissue (50 mg) was homogenized at 4 °C using a  
 197 FastPrep® in K-phosphate buffer (1 mL, 0.1 M pH 7.8; 100 mM K-phosphate, 20% (v/v) glycerol and 0.2 mM  
 198 fluoride phenylmethylsulphonyl). Homogenates were centrifuged at 10,000 g for 15 min at 4 °C. Supernatants

(post-mitochondrial fraction, PMS) were distributed in 4 volumes of 100 mL and stored at -80 °C for two weeks.

Protein concentrations were determined with the Bradford method. CAT activity was determined at 240 nm by measuring the breakdown of H<sub>2</sub>O<sub>2</sub> (Babo and Vasseur, 1992). SOD activity was measured at 340 nm for 5 minutes against standard SOD (Paoletti et al., 1986). GST activity was assessed at 340 nm for 5 min by measuring the conjugation between 1-chloro- 2,4-dinitrobenzene and reduced glutathione (Habig et al., 1974). The enzyme activity was expressed in units per mg of proteins (U.mg<sup>-1</sup>).

## 2.8. Somatic and condition indexes

At the individual level, energetic reserves and condition indexes are an essential component of the physiological state of the fish. Hepatosomatic index (HSI) and white muscle index (MI) reflect the metabolic status of the liver (Chellappa et al., 1995; Zheng et al., 2013) and white muscles (Moon and Johnston, 1980) respectively. They were calculated as follow:

$$HSI = \frac{\text{liver weight (g)}}{\text{total weight(g) - liver weight (g)}} \times 100 \quad (4)$$

$$MI = \frac{\text{muscle weight (g)}}{\text{total weight(g)}} \times 100 \quad (5)$$

The Fulton's condition factor (FCF) is an index of the "well-being" of fish and reflects their global metabolic status (Smolders et al., 2005). It was calculated from weight and length of fish as follow:

$$FCF = \frac{\text{weight (g)}}{\text{length (cm)}^3} \quad (6)$$

## 2.9. Statistical analyses

Except for the metabolomics data, differences between groups were analyzed with ANOVA software and LSD post-hoc tests after validation of the assumption of homogenous variances with Levene test. Canonical analyses were used to integrate data from plasma cortisol concentration, AE and CE in liver and muscle, somatic indexes (HSI and MI) and condition factor (FCF) of fish. The distance between groups was calculated for each time of exposure with the Mahalanobis distance, and the obtained p-value was used to determine significant separation with a threshold of 0.05.

## 3. Results

### 3.1. Endocrine, metabolic and physiological responses

The direction and significance of the endocrine, metabolic and physiological responses of fish to individual and combined chemical and thermal stresses are presented in Table 4. The mean values for all parameters are

229 listed in the Supplementary Table 3. Compared to the CONTROL group, PEST exposure significantly increased  
230 plasma cortisol at T6 h, hepatic carbohydrates at T6 h and T16 d, hepatic proteins at T6 h, liver EC at T6 h,  
231 lipids and AE in muscle at T96 h and HSI at T6 h and T16 d. A decrease was found in liver lipids. At T6 h, the  
232 TEMP group showed increases in cortisol, hepatic protein concentration and EC in liver and white muscle. A  
233 decrease was observed in muscle AE and CEA. No effects were found at T96 h and T16 d (Table 4, Table S3).

234 The PEST\*TEMP group showed an increase of plasma cortisol at T6 h compared to CONTROL, but the  
235 concentration was similar to that observed in both individual stress groups (Supplementary Table 3). There  
236 was no other effect compared to CONTROL at T6h. On the opposite, PEST\*TEMP significantly increased muscle  
237 lipids, AE and CEA, HSI and MI at T16d. A significant decrease in the FCF was observed at T16d. PEST\*TEMP  
238 also tended to decrease the plasma cortisol concentration compared to CONTROL group at T16d ( $p=0.053$ , LSD  
239 post-hoc test).

240 Plasma cortisol concentration, liver and muscle AE and CE, somatic indexes (HSI and MI) and condition factor  
241 (FCF) of fish were integrated in canonical analyses associated to Mahalanobis distance computation to  
242 measure the separation between groups. Canonical representations for each exposure time and Mahalanobis  
243 distances between CONTROL and stressed groups were shown in the Fig. 1. Results showed a stress-specific  
244 and time-dependent response of fish with an opposite trend between individual and combined stress effects.  
245 The canonical analysis and Mahalanobis distances showed a significant separation between the CONTROL  
246 group and both the TEMP and PEST groups at T6 h (Fig. 1A), but not at T96 h and T16 d (Fig. 1B and C  
247 respectively). On the opposite, the distance between the PEST\*TEMP and the CONTROL groups was non-  
248 significant after 6 h but increased over time to become significant after 16 d.

### 249 3.2. $^1\text{H-NMR}$ metabolomic analysis

250 Representative 600 MHz  $^1\text{H}$  NMR spectra of control and exposed goldfish obtained from aqueous  
251 extracts of liver tissues at time-point 16 days were shown in Supplementary Fig. 1. Primary metabolites have  
252 been annotated in the spectra. OSC-PLS-DA score plots obtained for each exposure duration are shown in Fig.  
253 2.

254 At T6 h, a valid and robust model ( $A=3$  latent component,  $R^2=52.7\%$ ,  $Q^2=0.37$ ) has been built for the  
255 integrated spectra: the score plot of the PLS-DA (Fig. 2A) showed a clear separation between the group of  
256 fishes at 22 °C and the group of fishes at 32 °C, regardless pesticides exposure.

257 36 buckets had a VIP value  $> 1.0$  and was statistically different by the Kruskal–Wallis test. These  
258 differences corresponded to 12 metabolites, according to 2D NMR spectra annotation. High temperature  
259 (TEMP) increased glutamine, reduced glutathione, phosphorylcholine, taurine, and decreased UDP and UTP  
260 compared to CONTROL group. The PEST\*TEMP and the TEMP groups were not clearly separated on the OSC-  
261 PLS-DA score plot (Fig. 2A). However, PEST\*TEMP exposure significantly decreased proline, UDP and UTP levels

262 compared to control fish, and PEST exposure significantly increased glutamine, reduced glutathione, succinate,  
263 choline, AMP, NAD<sup>+</sup> and decreased maltose level compared to control fish (Table 1 and Fig. 2D).

264 At T96 h, the score plot of the PLS-DA showed a clear separation between the 22 °C group and the 32  
265 °C group along the first latent component, and between the exposed fish and the non-exposed fish for the 32  
266 °C group (Fig. 2B). This analysis generated a PLS-DA model with three latent components, with R<sup>2</sup><sub>Y</sub> = 54.3%  
267 and Q<sup>2</sup> = 0.34. Thirty buckets were identified as discriminant in the metabolic profiles, corresponding to 8  
268 metabolites. Despite close metabolic fingerprints for these two groups, PEST exposure significantly decreased  
269 AMP compared to CONTROL. In the TEMP group, significant increase was observed for maltose, while proline,  
270 malonate, AMP, NAD<sup>+</sup>, UDP and UTP decreased. PEST\*TEMP exposure significantly decreased proline,  
271 succinate, AMP, NAD<sup>+</sup>, UDP and UTP (Table 2 and Fig. 2E).

272 At T16 d, same patterns were observed in the score plot (Fig. 2C) but difference between exposed and  
273 non-exposed fishes at 32 °C was more pronounced. 15 metabolites were found significant between at least  
274 two groups. As compared to CONTROL, decreases were found in isoleucine, leucine, valine, alanine, proline,  
275 lysine, glutamine, creatine, taurine, lactate, AMP, NAD<sup>+</sup> and increases were found in maltose for the  
276 PEST\*TEMP group. TEMP condition increased significantly reduced glutathione and phosphorylcholine, and  
277 decreased proline compared to CONTROL. No discriminant metabolites were found for PEST exposure (Table 3  
278 and Fig. 2F).

### 279 3.3. Defense system induction

280 SOD, CAT and GST activities were measured in the liver of fish (Fig. 3). An increase of CAT activity was  
281 observed in the PEST group at T6 h. CAT and SOD activity decreased in the PEST\*TEMP group at T96 h and  
282 then increased at T16 d. GST activity tended to increase in the PEST group at T6 h compared to the CONTROL  
283 and was significantly higher than in the PEST\*TEMP group. No effects were observed at T96 h and T16 d. The  
284 TEMP group showed no response of these enzyme activities.

## 286 4. Discussion

287 The aim of this study was to assess the adaptive response of goldfish submitted to individual and  
288 combined chemical and thermal stress. We first characterized the primary endocrine stress response (plasma  
289 cortisol), then secondary responses (hepatic induction of enzymatic defense systems and metabolic responses  
290 in liver and white muscles) and tertiary responses (global condition of fish). We found stress-specific and time-  
291 dependent responses of goldfish at all biological levels observed. Multivariate analyses showed two  
292 contrasting patterns of response (1) Fish exposed to single chemical or thermal stressors had a significant  
293 stress response at T6 h followed by a recovery at T96 h and T16 d from the molecular to the individual levels  
294 (significant stress response and low deleterious effects across biological levels). In contrast, (2) fish exposed to

295 combined chemical and thermal stressors had no stress response at T6 h, followed by important deleterious  
296 changes at T16 d from the molecular to the individual levels (absence of stress response and high deleterious  
297 effects across biological levels).

298 4.1 Responses to individual stress (PEST and TEMP groups): an adapted and coordinated early  
299 response.

300 4.1.1 Cortisol response to single stressors

301 In fish exposed to single stressors, we found an increase of plasma cortisol concentration at T6h in all  
302 stressed groups compared to the CONTROL groups. Several studies have shown an increase in plasma cortisol  
303 in fish exposed to herbicides (Waring and Moore, 2004; Soso et al., 2007), fungicides (Hashim and Zaki, 2005;  
304 Tierney et al., 2006) or temperature increase (Lyytikäinen et al., 2002; Fernandino et al., 2013). Our results  
305 show that both pesticide mixture and temperature increase triggers an endocrine stress response, showing  
306 that fish have initiated a general stress response. It was associated to the induction of a number of antioxidant  
307 defense systems, depending on the nature of the stress. For instance we observed an increase in CAT and GST  
308 activities, glutathione, and glutamine - on which glutathione synthesis depends - and taurine.

309 4.1.2 Antioxydant response to single stressors

310 CAT and SOD catalyze the reduction of ROS, participating to their detoxification (Regoli et al., 2011;  
311 Regoli and Giuliani, 2014). In another hand, GST intervenes in the detoxification of both ROS and lipophilic  
312 chemicals, such as pesticides, by catalyzing their conjugation with glutathione. Induction of CAT, SOD and/or  
313 GST are consistent with results of previous studies in fish exposed to herbicides (Jin et al., 2010; Guilherme et  
314 al., 2012; Stara et al., 2012; Xing et al., 2012; Blahová et al., 2013; Nwani et al., 2013; Sinhorin et al., 2014) and  
315 high temperature (Gorbi et al., 2005; Madeira et al., 2013; Yin et al., 2013). We observed a transient increase  
316 activity of CAT and GST in the PEST group at T6 h, suggesting an increase in ROS production under the pesticide  
317 exposure, although SOD activity remained at the control level. GST activity increased also suggesting an  
318 induction of the hepatic detoxification process. In contrast, no responses of CAT, SOD and GST activity were  
319 observed in the TEMP group, despite the fact that temperature increase is a well-known pro-oxidant factor in  
320 ectothermic species (Cui et al., 2014).

321 Glutathione is a well-known ROS scavenger and was used as a biomarker of oxidative stress in many  
322 studies (Regoli et al., 2011). Glutathione synthesis depends on the cellular concentration of glutamate and  
323 glutamine (DeBerardinis and Cheng, 2010). NMR analysis showed an increase of these molecules at T6 h in  
324 both the PEST and TEMP groups. Considering their extensive variety of critical roles in cell function and  
325 antioxidant defenses, the increase of glutamine in the liver of fish can be considered as an important response  
326 to both chemical and thermal stressors.

327 Thermal stressor also increased hepatic taurine levels at T6 h and decreased malonate at T96 h.  
328 Taurine is a sulfur-amino acid which counteracts oxidative stress, although specific mechanisms are poorly

understood (Kumar et al., 2009; Rosemberg et al., 2010). On the opposite, malonate induces mitochondrial potential collapse and ROS production, leading to cell death (Fernandez-Gomez et al., 2005). Consequently, increased taurine level and decreased malonate may act in the same way to prevent ROS induced damages in thermal exposed fish.

An increase in phospholipid precursor concentrations (choline and derivative) in both groups may also be a response to lipid peroxidation caused by oxidative stress and requiring the synthesis of novel phospholipids (Li et al., 2014; Xu et al., 2015).

#### 4.1.3 Metabolic response to single stressors

At the same time, an increase in energy consumption is observed in the liver - increased liver energy consumption (EC) at T6 h in both the PEST and TEMP groups - and, for the TEMP group, in the muscle. The increase in basal metabolism is classic in fish subjected to stress and corresponds to the bioenergetic cost of the induction of systems of protection, detoxification and repair of damage. Increased ETS activity was shown in ectotherm species in response to chemical exposure and temperature increase (Gagné et al., 2006, 2007; Sappal et al., 2015; Schmidlin et al., 2015), which is consistent with our results. ETS activity reflects the production of ATP by mitochondria which depends directly on the cellular energy demand (Gagné et al., 2007; Smith et al., 2012). The induction of defense systems, including detoxification, protection and reparation processes, is a costly process which increases the cellular energy demand and requires metabolic compensations (Sherwood et al., 2000; Trudel et al., 2010; Sokolova et al., 2012). Proteins are the most costly energy molecules to synthesize (Jorgensen, 1988; Smith et al., 2001). Increased hepatic protein concentrations were observed at T6 h in both the PEST and TEMP exposed fish. These increases may reflect the induction of defense systems, including antioxidant enzymes or heat shock proteins (Viant et al., 2003). Increased bioenergetic cost due to defense protein induction was shown in fish exposed to heat shock (Viant et al., 2003) or pollutants (Smith et al., 2001). We can also note that protein concentrations increased at T6 h but not at T96 h or T16 d, which is consistent with the induction of antioxidant defenses and the increased energy consumed. In PEST exposed fish, the co-occurring increases observed at T6 h in liver EC, AMP and succinate, an intermediary product of the TCA cycle, showed a metabolic compensation to the increased energy requirement. In a consistent way, hepatic glucose and lipids, which are important substrates for the energy production pathways, showed important decreases at T6 h. However, we found an increase in total carbohydrate concentration in liver at T6 h and T16 d. Glycogen is the common form of storage of carbohydrates in cells and increased glycogen deposition is a classic metabolic response to cortisol (Mommsen et al., 1999). Maltose was also significantly decreased in livers after 6 h of pesticides exposure. Maltose as a disaccharide could yield two monosaccharides of glucose upon hydrolysis. Maltose degradation could serve as an energy supplement to replenish pesticides induced energy demand (Li et al., 2017). In the TEMP group, energy consumption increased at T6 h both in liver and white muscle, associated with significant decreases in muscular AE and CEA. In the tolerance range, a higher temperature affects biochemical kinetics and enzyme

364 activity leading to basal and active metabolism increased (Cech et al., 1985; López-Olmeda and Sánchez-  
365 Vázquez, 2011; Manciooco et al., 2014). The increased energy consumption observed in liver and muscle at T6  
366 h, and the decreased available energy in muscle are consistent with systemic metabolic acceleration under  
367 higher temperature. The drop observed at T6 h in the muscle CEA reflected a decreased metabolic status.  
368 Inversely, the hepatic CEA remained constant despite the increased energy consumption. A similar dichotomy  
369 between muscular and hepatic metabolic responses to heat shock was observed in *Oncorhynchus mykiss*  
370 (Viant et al., 2003). Under stress, lipid and protein reserves in white muscles are released into the blood  
371 system, carried to the liver and used as substrates for gluconeogenesis and protein synthesis (Mommsen et al.,  
372 1999). These results show that higher temperature induce an increased energy demand and metabolic  
373 compensation through the whole organism. The vast majority of metabolic responses to higher temperature  
374 disappeared at T96 h and T16 d, suggesting that thermal exposed fish reached an adapted physiological status.  
375 So, with increased temperatures, the increase in metabolism is also directly related to the effect of  
376 temperature on the enzymatic activity and kinetics of biochemical reactions. This energy cost is supported by  
377 an increase in ATP production by ETS as well as glycolysis, Krebs cycle and gluconeogenesis from liver and / or  
378 muscle proteins or lipids.

#### 379 4.1.4 Physiological response to single stressors

380 Finally, the lack of effect on the general condition of the fish and the disappearance of most of these  
381 responses at the end of the experiment suggest that the fish have an effective response to achieve a more  
382 appropriate physiological state. However, some effects persist at T16 d in the liver of the PEST group,  
383 especially for the HSI that increase at T6 h and T16 d. HSI increase was shown in fish exposed to pesticides  
384 (Arnold et al., 1995; Biagianti-Risbourg and Bastide, 1995; Bacchetta et al., 2014). HSI is correlated to the  
385 metabolic status of the liver, and may reflect increased glycogen storage (Chellappa et al., 1995; Zheng et al.,  
386 2013). But a swollen HSI may also be due to sequestration of lipophilic pollutants in lipid droplets (Biagianti-  
387 Risbourg and Bastide, 1995), increased detoxification capacities (Arnold et al., 1995; Bacchetta et al., 2014) or  
388 degenerative changes in the liver tissue (Arnold et al., 1995; Guardiola et al., 2014). Histopathological analyzes  
389 could determine whether this effect is indicative of histological damage caused by pesticides, increased liver  
390 detoxification capacities or pesticide trapping in lipid vacuoles. We can therefore conclude that pesticide  
391 exposure induced an increased energy demand in the liver of the goldfish, probably due to defense system  
392 induction, and metabolic compensations. While other responses shut down, the increased HSI, MI and hepatic  
393 carbohydrate concentration observed at T16 d indicated persisting metabolic and/or histological perturbations  
394 in goldfish exposed to the pesticide mixture. However, compared to fish exposed to combined stressors, fish  
395 exposed to single stressors had lower deleterious effects of treatments at the individual level.

396 To summarize, fish exposed to single stressors displayed a significant cortisol response with  
397 coordinated effects on the metabolism and energetic reserves and low deleterious effects at different time  
398 scales. These results are consistent with a metabolic compensation strategy, i.e. the set-up of a coordinated

399 stress responses and metabolic responses with a depletion of energetic reserves to maintain homeostasis and  
400 limit the deleterious effects of single stressors (Sokolova et al., 2013). However, it is not clear yet whether fish  
401 can implement such coordinated stress response when exposed to a combination of stressors that may have  
402 synergistic or antagonistic effects.

403 4.2 Responses to combined stressors (PEST \* TEMP group): an inhibited early response and significant  
404 effects from the molecular to the individual scale at T16 d.

#### 405 4.2.1 Cortisol response to combined stressors

406 Cortisol level in fish subjected to both an increase in temperature, and pesticides mixture increased at  
407 T6 h. Surprisingly, combined stressors did not lead to an additional increase in cortisol secretion. This result  
408 shows that fish under thermal stress does not respond to pesticide exposure by an increase in cortisol  
409 secretion. First, a possible explanation is that pesticide mixture toxicity decreases with the increase in  
410 temperature. However, the vast majority of studies on aquatic vertebrates showed an opposite effect (Gluth  
411 and Hanke, 1984; Ferrando et al., 1987; Osterauer and Köhler, 2008; Rohr and Palmer, 2013). Second, the  
412 exposure to a first stressor may affect the normal function of the HPI axis and prevent the induction of a stress  
413 response to a subsequent stressor (Romero, 2004; Auperin and Geslin, 2008). In this last case, the  
414 hyporesponsive HPI axis to a consecutive stressor could shut down the fish ability to perform an appropriate  
415 stress response (Romero, 2004, 2010).

416 It is also interesting to note that cortisol concentration tended to decrease in the PEST\*TEMP group  
417 compared to controls after 16 days of pesticide exposure ( $p=0.053$ , LSD post-hoc test). A lower level of cortisol  
418 is generally observed in organisms submitted to an extreme stress (Romero, 2004, 2010). This decrease could  
419 be due to pesticide induced endocrine perturbation of the HPI axis function. Studies have shown that fish  
420 exposed to chronic chemical stressors such as pesticides display a decreased corticosteroid response to a  
421 second (Cericato et al., 2008, 2009; Bisson and Hontela, 2002; Nascimento et al., 2012; Koakoski et al., 2014).  
422 This inhibition could be linked to a toxic effect of pesticides, including tebuconazole, on interrenal cells or a  
423 regulation of the activity of the HPI axis at the level of the hypothalamus or pituitary (Koakoski et al., 2014;  
424 Zhang et al. 2015). Endocrine disruption of the synthesis of steroid hormones, i.e. cortisol and/or sex  
425 hormones can have important consequences on the fitness and survival of fish, affecting physiological traits  
426 and behaviors associated with stress adaptation and reproduction (Ankley et al., 2002).

#### 427 4.2.2 Antioxydant responses to combined stressors

428 Surprisingly, all defense systems and metabolic responses observed in response to individual stresses  
429 were inhibited in fish exposed to multiple stressors at early time. The PEST\*TEMP exposed fish showed no  
430 response of CAT, GST, glutathione, glutamine, taurine, choline and phosphorylcholine at T6 h. Moreover, CAT  
431 and SOD activities were reduced compared to control fish at T96 h. Similar patterns of reciprocal inactivation  
432 of defense systems were shown in *Pimephales promelas* exposed to copper and heat shock (Lapointe et al.,

433 2011). Moreover, it is well established that ROS (e.g. superoxide) oxidizes non enzymatic antioxidant defenses  
434 and inactivates several enzymes including CAT (see a review in Valavanidis et al., 2006). Inhibition of the stress  
435 defenses may indicate an overproduction of ROS under combined chemical and thermal stresses which  
436 exceeded the antioxidant capacity of the cells. Inhibition of defense system reflects an antagonistic effect of  
437 thermal and chemical stressors on the overall stress response. Interestingly, CAT and SOD activity increased  
438 significantly at T16 d while other responses were still shut down. This delayed response emphasizes the  
439 adverse effect of chemical and thermal induced oxidative stress on cellular components, with increased risk of  
440 cell and tissue injuries.

#### 441 4.2.3 Metabolic responses to combined stressors

442 At the molecular level, a decrease in the concentration of a large number of metabolites indicates a  
443 significant disturbance of amino acid metabolism, energy production and defense against oxidative stress. In  
444 the PEST\*TEMP group, the metabolic responses to individual stressors measured at T6 h disappeared,  
445 including the responses observed at the molecular, cellular and tissue levels. In particular, the ETS activity in  
446 both liver and muscle decreased compared to the PEST and TEMP groups. The decreased ETS activity under  
447 combined stressors could result from (1) perturbations in the normal function of the ETS or (2) a decreased  
448 mitochondrial densities in cells (Lannig et al., 2006; Sokolova and Lannig, 2008; Lavergne et al., 2015). In  
449 ectothermic species, mitochondrial plasticity plays a critical role in the metabolic response to environmental  
450 stressors (Strobel et al., 2013). The incapacity of fish to maintain an elevated energy production under multiple  
451 stress exposures could explain the absence of induction of the defense system observed at T6 h.  
452 Consequently, it could potentiate oxidative damages on cellular components, including proteins. The creatine  
453 phosphocreatine system plays a critical role in cellular energy metabolism (Sun et al., 2012; Wang et al., 2017).  
454 Thus, the decreased creatine, found under combined exposure stressors at T16 d strengthens the perturbation  
455 of energy metabolism which is consistent with the above conclusion.

456 PEST\*TEMP exposed fish showed significant decreases in amino-acids levels as soon as T6 h, with  
457 reduced levels of proline and lysine. At T16 d, reduced concentrations were found for isoleucine, leucine,  
458 valine, alanine, proline, lysine, glutamate, glutamine, creatine and taurine, indicating an important  
459 perturbation of amino-acid metabolism and/or oxidative damages on proteins.

#### 460 4.2.4 Physiological responses to combined stressors

461 At the tissue level, the increase in HSI and the deposition of lipids in the muscles also indicate  
462 disturbances in energy metabolism, sequestration of pesticides in lipid vacuoles and / or tissue damage.  
463 Finally, a significant decrease in the overall condition of the fish (FCF) is visible at T16 d. When fish are  
464 subjected to both combined stressors, the general stress response is inhibited, at least initially, with significant  
465 individual-level effects on overall fish health. Given that the stress response is fundamental in enabling  
466 organisms to acclimate to changes in their environment, its inhibition under multiple stressors seems to have

important consequences on fitness and fish performance affecting physiological traits and behavioral responses related to stress adaptation and reproduction (Pankhurst and Van Der Kraak, 2011).

## 5. Conclusion

Taken together, these results indicate that (1) single thermal and chemical stressors lead to a general stress response and a metabolic compensation strategy, i.e. the set-up of a coordinated stress response and depletion of energetic reserves limiting adverse effects at higher biological levels. The lack of effect on the general condition of the fish (i.e. tertiary response) suggests that the stress response set up is adapted and effective. However, (2) the patterns of metabolic response and defensive systems strongly depended on the nature of the stress (thermal vs. chemical). Finally, (3) fish were unable to establish an effective stress response when chemical and thermal stressors were combined, with deleterious effects at the individual level. Exposure to combined chemical and thermal stressors disrupts the stress response and results in a decrease in the overall condition of the fish, indicating a synergistic effect of temperature and pesticide mixture on goldfish. These results also outline the central role of metabolism in these stress responses. The decrease in cellular energy metabolism when the two stresses are combined is consistent with the shift from a compensation strategy to a metabolic conservation strategy (Sokolova et al., 2013). In other words, exposure to a combination of stressors might exceed the metabolic capacity of the fish, leading to an early inhibition of the stress response (lower level of cortisol, antioxidant defenses, and lower depletion of energy reserves) with potential high deleterious effects at the individual level.

To our knowledge this study is the first to evaluate the combined effects on the fish stress response of temperature warming and water contamination by pesticide mixtures with an integrated molecule- individual scale. The combined analysis of omic approaches, here metabolomics, and more conventional biochemical approaches seems to be a powerful way to evaluate the response of organisms undergoing multiple stresses. Future studies should now consider different combinations of stressors and evaluate their effects on individuals across biological levels, from the molecules to the whole individual, while taking into account the inter-individual variability of sensitivity. Because global changes are exposing organisms to a combination of stressors at the same time, this will help understanding and predicting the responses of natural populations to actual and future stresses.

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## 502 References

503 Alim'agri, 2012. Agriculture, Agri-food and Forest data. French Ministry of Agriculture, Agro-food and  
504 Forest report. [WWW Document]. URL <http://agriculture.gouv.fr/ministere/alimagri-chiffres-cles-2012>

505 Ankley, G.T., Kahl, M.D., Jensen, K.M., Hornung, M.W., Korte, J.J., Makynen, E.A., Leino, R.L., 2002.  
506 Evaluation of the Aromatase Inhibitor Fadrozole in a Short-Term Reproduction Assay with the Fathead Minnow  
507 (*Pimephales promelas*). *Toxicol. Sci.* 67, 121–130. doi:10.1093/toxsci/67.1.121

508 Arnold, H., Pluta, H.-J., Braunbeck, T., 1995. Simultaneous exposure of fish to endosulfan and  
509 disulfoton *in vivo*: ultrastructural, stereological and biochemical reactions in hepatocytes of male rainbow  
510 trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* 33, 17–43. doi:10.1016/0166-445X(95)00006-P

511 Auperin, B., Geslin, M., 2008. Plasma cortisol response to stress in juvenile rainbow trout is influenced  
512 by their life history during early development and by egg cortisol content. *Gen. Comp. Endocrinol.* 158, 234–  
513 239. doi:10.1016/j.ygcen.2008.07.002

514 Babo, S., Vasseur, P., 1992. *In vitro* effects of Thiram on liver antioxidant enzyme activities of rainbow  
515 trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* 22, 61–68. doi:http://dx.doi.org/10.1016/0166-445X(92)90036-M

516 Bacchetta, C., Rossi, A., Ale, A., Campana, M., Parma, M.J., Cazenave, J., 2014. Combined toxicological  
517 effects of pesticides: A fish multi-biomarker approach. *Ecol. Indic.* 36, 532–538.  
518 doi:10.1016/j.ecolind.2013.09.016

519 Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in  
520 circulating corticosteroids. *Integr. Comp. Biol.* 42, 517–525. doi:10.1093/icb/42.3.517

521 Baudiffier, D., 2012. Modes de perturbation de la stéroïdogénèse testiculaire et de la spermatogénèse  
522 chez le poisson zèbre (*Danio rerio*) par des fongicides azolés. Université Rennes 1.

523 Beckwith-Hall, B.M., Brindle, J.T., Barton, R.H., Coen, M., Holmes, E., Nicholson, J.K., Antti, H., 2002.  
524 Application of orthogonal signal correction to minimise the effects of physical and biological variation in high  
525 resolution <sup>1</sup>H NMR spectra of biofluids. *Analyst* 127, 1283–1288. doi:10.1039/b205128c

526 Biagiante-Risbourg, S., Bastide, J., 1995. Hepatic perturbations induced by a herbicide (atrazine) in  
527 juvenile grey mullet *Liza ramada* (Mugilidae, Teleostei): an ultrastructural study. *Aquat. Toxicol.* 31, 217–229.  
528 doi:10.1016/0166-445x(94)00065-x

529 Bisson, M., Hontela, A., 2002. Cytotoxic and endocrine-disrupting potential of atrazine, diazinon,  
530 endosulfan, and mancozeb in adrenocortical steroidogenic cells of rainbow trout exposed *in vitro*. *Toxicol.*  
531 *Appl. Pharmacol.* 180, 110–117. doi:10.1006/taap.2002.9377

532 Blahová, J., Plhalová, L., Hostovský, M., Divišová, L., Dobšíková, R., Mikulíková, I., Štěpánová, S.,  
533 Svobodová, Z., 2013. Oxidative stress responses in zebrafish *Danio rerio* after subchronic exposure to atrazine.  
534 Food Chem. Toxicol. 61, 82–85. doi:10.1016/j.fct.2013.02.041

535 Bonnefoy, N., 2012. Information Report on Behalf of the Joint Mission of Information on Pesticides and  
536 Their Impact on Health and the Environment. Report of the French Senate (in French). Paris, France.

537 Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of  
538 protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.  
539 doi:http://dx.doi.org/10.1016/0003-2697(76)90527-3

540 Butault, J.-P., Dedryver, C.-A., Gary, C., Guichard, L., Jacquet, F., Meynard, J.-M., Nicot, P., Pitra, M.,  
541 Reau, R., Sauphanor, B., Savini, I., Volay, T., 2010. Ecophyto R&D: What Ways to Reduce the Use of Pesticides?  
542 Summary of the study report (in French). France.

543 Calow, P., 1991. Physiological costs of combating chemical toxicants: Ecological implications. Comp.  
544 Biochem. Phys. C. 100, 3–6. doi:10.1016/0742-8413(91)90110-F

545 Cericato, L., Neto, J.G.M., Fagundes, M., Kreutz, L.C., Quevedo, R.M., Finco, J., da Rosa, J.G.S., Koakoski,  
546 G., Centenaro, L., Pottker, E., Anziliero, D., Barcellos, L.J.G., 2008. Cortisol response to acute stress in jundiá  
547 *Rhamdia quelen* acutely exposed to sub-lethal concentrations of agrichemicals. Comp. Biochem. Phys. C. 148,  
548 281–286. doi:10.1016/j.cbpc.2008.06.008

549 Cericato, L., Neto, J.G.M., Kreutz, L.C., Quevedo, R.M., da Rosa, J.G.S., Koakoski, G., Centenaro, L.,  
550 Pottker, E., Marqueze, A., Barcellos, L.J.G., 2009. Responsiveness of the interrenal tissue of Jundiá (*Rhamdia*  
551 *quelen*) to an *in vivo* ACTH test following acute exposure to sublethal concentrations of agrichemicals. Comp.  
552 Biochem. Phys. C. 149, 363–7. doi:10.1016/j.cbpc.2008.09.002

553 Chellappa, S., Huntingford, F.A., Strang, R.H.C., Thomson, R.Y., 1995. Condition factor and  
554 hepatosomatic index as estimates of energy status in male three-spined stickleback. J. Fish Biol. 47, 775–787.  
555 doi:10.1111/j.1095-8649.1995.tb06002.x

556 Cui, Y., Liu, B., Xie, J., Xu, P., Habte-Tsion, H.M., Zhang, Y., 2014. Effect of heat stress and recovery on  
557 viability, oxidative damage, and heat shock protein expression in hepatic cells of grass carp  
558 (*Ctenopharyngodon idellus*). Fish Physiol. Biochem. 40, 721–729. doi:10.1007/s10695-013-9879-2

559 DeBerardinis, R.J., Cheng, T., 2010. Q's next: the diverse functions of glutamine in metabolism, cell  
560 biology and cancer. Oncogene 29, 313–24. doi:10.1038/onc.2009.358

561 De Coen, W. M. and Janssen, C. R. 1997. The use of biomarkers in *Daphnia magna* toxicity testing. IV.  
562 Cellular Energy Allocation: A new methodology to assess the energy budget of toxicant-stressed *Daphnia*  
563 populations. J. Aquat. Ecosyst. Stress Recovery.6, 43–55.

- 564 Désautés, C., Bidanel, J.P., Mormède P. 1997. Genetic Study of Behavioral and Pituitary-Adrenocortical  
565 Reactivity in Response to an Environmental Challenge in Pigs. *Physiol. Behav.* 62,337-345.
- 566 Fernandez-Gomez, F.J., Galindo, M.F., Gómez-Lázaro, M., Yuste, V.J., Comella, J.X., Aguirre, N., Jordán,  
567 J., 2005. Malonate induces cell death via mitochondrial potential collapse and delayed swelling through an  
568 ROS-dependent pathway. *Brit. J. Pharmacol.* 144, 528–37. doi:10.1038/sj.bjp.0706069
- 569 Fernandino, J.I., Hattori, R.S., Moreno Acosta, O.D., Strüssmann, C.A., Somoza, G.M., 2013.  
570 Environmental stress-induced testis differentiation: Androgen as a by-product of cortisol inactivation. *Gen.  
571 Comp. Endocr.* 192, 36–44. doi:10.1016/j.ygcen.2013.05.024
- 572 Ferrando, M.D., Andreu-Moliner, E., Almar, M.M., Cebrian, C., Nuñez, a., 1987. Acute toxicity of  
573 organochlorinated pesticides to the European eel, *Anguilla anguilla*: The dependency on exposure time and  
574 temperature. *B. Environ. Contam. Tox.* 39, 365–369. doi:10.1007/BF01688297
- 575 Ferreira, N.G.C., Morgado, R., Santos, M.J.G., Soares, A.M.V.M., Loureiro, S., 2015. Biomarkers and  
576 energy reserves in the isopod *Porcellionides pruinosus*: The effects of long-term exposure to dimethoate. *Sci.  
577 Total Environ.* 502, 91–102. doi:10.1016/j.scitotenv.2014.08.062
- 578 Gagné, F., Blaise, C., André, C., Salazar, M., 2006. Effects of pharmaceutical products and municipal  
579 wastewaters on temperature-dependent mitochondrial electron transport activity in *Elliptio complanata*  
580 mussels. *Comp. Biochem. Physiol. C.* 143, 388–393. doi:10.1016/j.cbpc.2006.04.013
- 581 Gagné, F., Blaise, C., André, C., Pellerin, J., 2007. Implication of site quality on mitochondrial electron  
582 transport activity and its interaction with temperature in feral *Mya arenaria* clams from the Saguenay Fjord.  
583 *Environ. Res.* 103, 238–246. doi:10.1016/j.envres.2006.05.006
- 584 Gandar, A., Jean, S., Canal, J., Marty-Gasset, N., Gilbert, F., Laffaille, P., 2015. Multistress effects on  
585 behavior and physiology of *Carassius auratus*. *Environ. Sci. Pollut. R.* 3, 3323–3331. doi:10.1007/s12192-010-  
586 0223-9
- 587 Gandar, A., Laffaille, P., Marty-Gasset, N., Viala, D., Molette, C., Jean, S. 2017. Proteome response of  
588 fish under multiple stress exposure: Effects of pesticide mixtures and temperature increase. *Aquat Toxicol.*  
589 184, 61-77. doi: 10.1016/j.aquatox.2017.01.004
- 590 Gamain, P., Gonzalez, P., Cachot, J., Pardon, P., Tapie, N., Gourves, P.Y., Budzinski, H., Morin, B., 2016.  
591 Combined effects of pollutants and salinity on embryo-larval development of the Pacific oyster, *Crassostrea*  
592 *gigas*. *Mar. Environ. Res.*, 113, 31-38. doi: 10.1016/j.marenvres.2015.11.002
- 593 Gluth, G., Hanke, W., 1984. A comparison of physiological changes in Carp, *Cyprinus carpio*, induced by  
594 several pollutants at sublethal concentration - II. The dependency on the temperature. *Comp. Biochem.*  
595 *Physiol. C.* 79, 39–45. doi:10.1016/0742-8413(84)90160-9

596 Gorbi, S., Baldini, C., Regoli, F., 2005. Seasonal variability of metallothioneins, cytochrome P450, bile  
597 metabolites and oxyradical metabolism in the European eel *Anguilla anguilla* L. (Anguillidae) and striped  
598 mullet *Mugil cephalus* L. (Mugilidae). Arch. Environ. Con. Tox. 49, 62–70. doi:10.1007/s00244-004-0150-9

599 Guardiola, F.A., González-Párraga, P., Meseguer, J., Cuesta, A., Esteban, M.A., 2014. Modulatory effects  
600 of deltamethrin-exposure on the immune status, metabolism and oxidative stress in gilthead seabream  
601 (*Sparus aurata* L.). Fish. Shellfish. Immun. 36, 120–9. doi:10.1016/j.fsi.2013.10.020

602 Guilherme, S., Gaivão, I., Santos, M.A., Pacheco, M., 2012. DNA damage in fish (*Anguilla anguilla*)  
603 exposed to a glyphosate-based herbicide – Elucidation of organ-specificity and the role of oxidative stress.  
604 Mutat. Res-Gen Tox. En. 743, 1–9. doi:10.1016/j.mrgentox.2011.10.017

605 Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-Transferases. The first enzymatic step in  
606 mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.

607 Handy, R.D., Sims, D.W., Giles, A., Campbell, H. A., Musonda, M.M., 1999. Metabolic trade-off between  
608 locomotion and detoxification for maintenance of blood chemistry and growth parameters by rainbow trout  
609 (*Oncorhynchus mykiss*) during chronic dietary exposure to copper. Aquat. Toxicol. 47, 23–41.  
610 doi:10.1016/S0166-445X(99)00004-1

611 Hashim, E.F., Zaki, M.S., 2005. Assessment of the hazardous effect of fungicide Dithane on *Clarias*  
612 *lazera* (catfish) including haematological, biochemical, and immunological parameters. Arab Univ. J. Agric. Sci.  
613 13, 1005–1018.

614 Hayes, T.B., Case, P., Chui, S., Chung, D., Haeffele, C., Haston, K., Lee, M., Mai, V.P., Marjuoa, Y., Parker,  
615 J., Tsui, M., 2006. Pesticide mixtures, endocrine disruption, and amphibian declines: Are we underestimating  
616 the impact? Environ. Health Persp. 114, 40–50. doi:10.1289/ehp.8051

617 Holmstrup, M., Bindesbøl, A.M., Oostingh, G.J., Duschl, A., Scheil, V., Köhler, H.R., Loureiro, S., Soares,  
618 A.M.V.M., Ferreira, A.L.G., Kienle, C., Gerhardt, A., Laskowski, R., Kramarz, P.E., Bayley, M., Svendsen, C.,  
619 Spurgeon, D.J., 2010. Interactions between effects of environmental chemicals and natural stressors: A review.  
620 Sci. Total Environ. 408, 3746–3762. doi:10.1016/j.scitotenv.2009.10.067

621 Hontela, A., 1997. Endocrine and physiological responses of fish to xenobiotics: Role of  
622 glucocorticosteroid hormones. Rev. Toxicol. 1, 1–46.

623 Jin, Y., Zhang, X., Shu, L., Chen, L., Sun, L., Qian, H., Liu, W., Fu, Z., 2010. Oxidative stress response and  
624 gene expression with atrazine exposure in adult female zebrafish (*Danio rerio*). Chemosphere 78, 846–852.  
625 doi:10.1016/j.chemosphere.2009.11.044

626 Jorgensen, C.B., 1988. Metabolic costs of growth and maintenance in the toad, *Bufo bufo*. J. Exp. Biol.  
627 138, 319–331.

- 628 Kennedy, C.J., Ross, P.S., 2012. Stress syndromes: Heightened bioenergetic costs associated with  
629 contaminant exposure at warm temperatures in teleosts. *Integr. Environ. Asses.* 8, 202–204.  
630 doi:10.1002/ieam.1261
- 631 Koakoski, G., Quevedo, R.M., Ferreira, D., Oliveira, T.A., da Rosa, J.G.S., de Abreu, M.S., Gusso, D.,  
632 Marqueze, A., Kreutz, L.C., Giacomini, A.C.V., Fagundes, M., Barcellos, L.J.G., 2014. Agrichemicals chronically  
633 inhibit the cortisol response to stress in fish. *Chemosphere* 112, 85–91.  
634 doi:http://dx.doi.org/10.1016/j.chemosphere.2014.02.083
- 635 Kumar, P., Prasad, Y., Patra, A.K., Ranjan, R., Swarup, D., Patra, R.C., Pal, S., 2009. Ascorbic acid, garlic  
636 extract and taurine alleviate cadmium-induced oxidative stress in freshwater catfish (*Clarias batrachus*). *Sci.*  
637 *Total Environ.* 407, 5024–5030. doi:10.1016/j.scitotenv.2009.05.030
- 638 Laetz, C.A., Baldwin, D.H., Hebert, V.R., Stark, J.D., Scholz, N.L., 2014. Elevated temperatures increase  
639 the toxicity of pesticide mixtures to juvenile coho salmon. *Aquat Toxicol.* 146, 38–44.
- 640 Lannig, G., Cherkasov, A.S., Sokolova, I.M., 2006. Temperature-dependent effects of cadmium on  
641 mitochondrial and whole-organism bioenergetics of oysters (*Crassostrea virginica*). *Mar. Environ. Res.* 62, 79–  
642 82. doi:10.1016/j.marenvres.2006.04.010
- 643 Lapointe, D., Pierron, F., Couture, P., 2011. Individual and combined effects of heat stress and aqueous  
644 or dietary copper exposure in fathead minnows (*Pimephales promelas*). *Aquat. Toxicol.* 104, 80–85.  
645 doi:10.1016/j.aquatox.2011.02.022
- 646 Lavergne, E., Pedron, N., Calves, I., Claireaux, G., Mazurais, D., Zambonino-Infante, J., Le Bayon, N.,  
647 Cahu, C., Laroche, J., 2015. Does the chronic chemical contamination of a European flounder population  
648 decrease its thermal tolerance? *Mar. Pollut. Bull.* 95, 658–64. doi:10.1016/j.marpolbul.2015.01.006
- 649 Lemly, A.D., 1996. Winter stress syndrome: an important consideration for hazard assessment of  
650 aquatic pollutants. *Ecotox. Environ. Safe.* 34, 223–227. doi:10.1006/eesa.1996.0067
- 651 Li, M., Wang, J., Lu, Z., Wei, D., Yang, M., Kong, L., 2014. NMR-based metabolomics approach to study  
652 the toxicity of lambda-cyhalothrin to goldfish (*Carassius auratus*). *Aquat. Toxicol.* 146, 82–92.  
653 doi:10.1016/j.aquatox.2013.10.024
- 654 Li, M., Ruan L.Y., Zhou J.W., Fu Y.H., Jiang L., Zhao H., Wang J.S., 2017. Metabolic profiling of goldfish  
655 (*Carassius auratus*) after long-term glyphosate-based herbicide exposure. *Aquat. Toxicol.* 188, 159–169.  
656 http://dx.doi.org/10.1016/j.aquatox.2017.05.004
- 657 Lin, C.Y., Wu, H., Tjeerdema, R.S., Viant, M.R., 2007. Evaluation of metabolite extraction strategies  
658 from tissue samples using NMR metabolomics. *Metabolomics* 3, 55–67. doi:10.1007/s11306-006-0043-1

659 López-Olmeda, J.F., Sánchez-Vázquez, F.J., 2011. Thermal biology of zebrafish (*Danio rerio*). J. Therm.  
660 Biol. 36, 91–104. doi:10.1016/j.jtherbio.2010.12.005

661 Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101,  
662 13–30. doi:10.1016/j.aquatox.2010.10.006

663 Lytikäinen, T., Pylkkö, P., Ritola, O., Lindström-Seppä, P., 2002. The effect of acute stress and  
664 temperature on plasma cortisol and ion concentrations and growth of Lake Inari Arctic charr, *Salvelinus*  
665 *alpinus*. Environ. Biol. Fish. 64, 195–202. doi:10.1023/A:1016017822700

666 Madeira, D., Narciso, L., Cabral, H., 2013. Influence of temperature in thermal and oxidative stress  
667 responses in estuarine fish. Physiol. Part A 166, 237–243. doi:http://dx.doi.org/10.1016/j.cbpa.2013.06.008

668 Maltby, L., Brock, T.C.M., van den Brink, P.J., 2009. Fungicide Risk Assessment for Aquatic Ecosystems:  
669 Importance of Interspecific Variation, Toxic Mode of Action, and Exposure Regime. Environ. Sci. Technol. 43,  
670 7556–7563. doi:10.1021/es901461c

671 Manciooco, A., Calamandrei, G., Alleva, E., 2014. Global warming and environmental contaminants in  
672 aquatic organisms: The need of the etho-toxicology approach. Chemosphere 100, 1–7.  
673 doi:10.1016/j.chemosphere.2013.12.072

674 Marchand, J., Quiniou, L., Riso, R., Thebaut, M.-T., Laroche, J., 2004. Physiological cost of tolerance to  
675 toxicants in the European flounder *Platichthys flesus*, along the French Atlantic Coast. Aquat. Toxicol. 70, 327–  
676 343. doi:http://dx.doi.org/10.1016/j.aquatox.2004.10.001

677 McCarty, R., 2010. Fight-or-Flight Response, in: Encyclopedia of Stress. Elsevier Inc., pp. 62–64.

678 Mommsen, T., Vijayan, M., Moon, T., 1999. Cortisol in teleosts: dynamics, mechanisms of action, and  
679 metabolic regulation. Rev. Fish Biol. Fish. 9, 211–268. doi:10.1023/a:1008924418720

680 Moon, T.W., Johnston, I. A., 1980. Starvation and the activities of glycolytic and gluconeogenic  
681 enzymes in skeletal muscles and liver of the plaice, *Pleuronectes platessa*. J. Comp. Physiol. B 136, 31–38.  
682 doi:10.1007/BF00688619

683 Nascimento, C.R.B., Souza, M.M., Martinez, C.B.R., 2012. Copper and the herbicide atrazine impair the  
684 stress response of the freshwater fish *Prochilodus lineatus*. Comp. Biochem. Physiol. C. 155, 456–61.  
685 doi:10.1016/j.cbpc.2011.12.002

686 Norris, D.O., 1997. The Mammalian Adrenal Glands and Comparative Aspects of Vertebrate Adrenals:  
687 Cortical and Chromaffin Cells, in: Norris, D.O. (Ed.), Vertebrate Endocrinology. Academic Press, New York, pp.  
688 299–355.

- 689 Noyes, P.D., McElwee, M.K., Miller, H.D., Clark, B.W., Van Tiem, L. A., Walcott, K.C., Erwin, K.N., Levin,  
690 E.D., 2009. The toxicology of climate change: Environmental contaminants in a warming world. *Environ. Int.*  
691 35, 971–986. doi:10.1016/j.envint.2009.02.006
- 692 Nwani, C.D., Nagpure, N.S., Kumar, R., Kushwaha, B., Lakra, W.S., 2013. DNA damage and oxidative  
693 stress modulatory effects of glyphosate-based herbicide in freshwater fish, *Channa punctatus*. *Environ.*  
694 *Toxicol. Phar.* 36, 539–547. doi:http://dx.doi.org/10.1016/j.etap.2013.06.001
- 695 Osterauer, R., Köhler, H.R., 2008. Temperature-dependent effects of the pesticides thiacloprid and  
696 diazinon on the embryonic development of zebrafish (*Danio rerio*). *Aquat. Toxicol.* 86, 485–494.  
697 doi:10.1016/j.aquatox.2007.12.013
- 698 Pankhurst, N.W., Van Der Kraak, G., 2011. Effect of stress on reproduction and growth of fish, in:  
699 Iwama, G.K., Pickering, A.D., Sumpter, J.P., Schreck, C.B. (Eds.), *Fish Stress and Health in Aquaculture*.  
700 Cambridge University Press, Cambridge, UK, pp. 73–93.
- 701 Paoletti, F., Aldinucci, D., Mocali, A., Caparrini, A., 1986. A sensitive spectrophotometric method for  
702 the determination of superoxide dismutase activity in tissue extracts. *Anal. Biochem.* 154, 536–541.  
703 doi:http://dx.doi.org/10.1016/0003-2697(86)90026-6
- 704 Polard, T., Jean, S., Gauthier, L., Laplanche, C., Merlina, G., Sánchez-Pérez, J.M., Pinelli, E., 2011.  
705 Mutagenic impact on fish of runoff events in agricultural areas in south-west France. *Aquat. Toxicol.* 101, 126–  
706 134. doi:10.1016/j.aquatox.2010.09.014
- 707 Regoli, F., Giuliani, M.E., 2014. Oxidative pathways of chemical toxicity and oxidative stress biomarkers  
708 in marine organisms. *Mar. Environ. Res.* 93, 106–117. doi:http://dx.doi.org/10.1016/j.marenvres.2013.07.006
- 709 Regoli, F., Giuliani, M.E., Benedetti, M., Arukwe, A., 2011. Molecular and biochemical biomarkers in  
710 environmental monitoring: A comparison of biotransformation and antioxidant defense systems in multiple  
711 tissues. *Aquat. Toxicol.* 105, 56–66. doi:10.1016/j.aquatox.2011.06.014
- 712 Rohr, J.R., Palmer, B.D., 2013. Climate change, multiple stressors, and the decline of ectotherms.  
713 *Conserv. Biol.* 27, 741–751. doi:10.1111/cobi.12086
- 714 Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. *Trends Ecol.*  
715 *Evol.* 19, 249–255. doi:http://dx.doi.org/10.1016/j.tree.2004.03.008
- 716 Romero, L.M., 2010. Fight or Flight Responses, *Encyclopedia of Animal Behavior*. Elsevier.  
717 doi:10.1016/B978-0-08-045337-8.00261-8
- 718 Rosemberg, D.B., da Rocha, R.F., Rico, E.P., Zanotto-Filho, A., Dias, R.D., Bogo, M.R., Bonan, C.D.,  
719 Moreira, J.C.F., Klamt, F., Souza, D.O., 2010. Taurine prevents enhancement of acetylcholinesterase activity

720 induced by acute ethanol exposure and decreases the level of markers of oxidative stress in zebrafish brain.

721 Neuroscience 171, 683–92. doi:10.1016/j.neuroscience.2010.09.030

722 Sappal, R., MacDougald, M., Fast, M., Stevens, D., Kibenge, F., Siah, A., Kamunde, C., 2015. Alterations  
723 in mitochondrial electron transport system activity in response to warm acclimation, hypoxia-reoxygenation  
724 and copper in rainbow trout, *Oncorhynchus mykiss*. Aquat. Toxicol. 165, 51–63.  
725 doi:http://dx.doi.org/10.1016/j.aquatox.2015.05.014

726 Schiedek, D., Sundelin, B., Readman, J.W., Macdonald, R.W., 2007. Interactions between climate  
727 change and contaminants. Mar. Pollut. Bull. 54, 1845–1856. doi:10.1016/j.marpolbul.2007.09.020

728 Schmidlin, L., von Fumetti, S., Nagel, P., 2015. Copper sulphate reduces the metabolic activity of  
729 *Gammarus fossarum* in laboratory and field experiments. Aquat. Toxicol. 161, 138–145.  
730 doi:http://dx.doi.org/10.1016/j.aquatox.2015.02.005

731 Selye, H., 1950. Stress and the General Adaptation Syndrome. Bmj. Brit. Med. J. 1, 1383–1392.  
732 doi:10.1136/bmj.1.4667.1383

733 Selye, H., 1946. The General Adaptation Syndrome and the disease of adaptation. J. Clin. Endocr.  
734 Metab. 6, 117–230.

735 Sherwood, G.D., Rasmussen, J.B., Rowan, D.J., Brodeur, J., Hontela, A., 2000. Bioenergetic costs of  
736 heavy metal exposure in yellow perch (*Perca flavescens*): in situ estimates with a radiotracer (<sup>137</sup>Cs)  
737 technique. Can. J. Fish. Aquat. Sci. 57, 441–450. doi:10.1139/cjfas-57-2-441

738 Sinhörin, V.D.G., Sinhörin, A.P., Teixeira, J.M. dos S., Miléski, K.M.L., Hansen, P.C., Moreira, P.S.A.,  
739 Kawashita, N.H., Baviera, A.M., Loro, V.L., 2014. Effects of the acute exposition to glyphosate-based herbicide  
740 on oxidative stress parameters and antioxidant responses in a hybrid Amazon fish surubim (*Pseudoplatystoma*  
741 *sp.*). Ecotox. Environ. Safe. 106, 181–7. doi:10.1016/j.ecoenv.2014.04.040

742 Smith, R.A.J., Hartley, R.C., Cochemé, H.M., Murphy, M.P., 2012. Mitochondrial Pharmacology. Trends  
743 Pharmacol. Sci. 33, 341–52. doi:10.1016/j.tips.2012.03.010

744 Smith, R.W., Blaney, S.C., Dowling, K., Sturm, A., Jönsson, M., Houlihan, D.F., 2001. Protein synthesis  
745 costs could account for the tissue-specific effects of sub-lethal copper on protein synthesis in rainbow trout  
746 (*Oncorhynchus mykiss*). Aquat. Toxicol. 53, 265–277. doi:10.1016/S0166-445X(01)00171-0

747 Smolders, R., Coen, W. De, Blust, R., 2005. Integrative measures of toxicant exposure in zebra fish  
748 (*Danio rerio*) at different levels of biological organization, in: Ostrander, G.K. (Ed.), Techniques in Aquatic  
749 Toxicology - Volume 2. pp. 5–7.

750 Sokolova, I.M., Lannig, G., 2008. Interactive effects of metal pollution and temperature on metabolism  
751 in aquatic ectotherms: implications of global climate change. Clim. Res. 37, 181–201. doi:10.3354/cr00764

- 752 Sokolova, I.M., Frederich, M., Bagwe, R., Lannig, G., Sukhotin, A. a., 2012. Energy homeostasis as an  
753 integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Mar. Environ.*  
754 *Res.* 79, 1–15. doi:10.1016/j.marenvres.2012.04.003
- 755 Sokolova, I.M., 2013. Energy-Limited Tolerance to Stress as a Conceptual Framework to Integrate the  
756 Effects of Multiple Stressors. *Integr. Comp. Biol.* 53, 597-608. doi:10.1093/icb/ict028
- 757 Solomon, K.R., Carr, J.A., Preez, L.H. Du, Giesy, J.P., Kendall, R.J., Smith, E.E., Kraak, G.J. Van Der, 2008.  
758 Effects of Atrazine on Fish, Amphibians, and Aquatic Reptiles: A Critical Review. *Crit. Rev. Toxicol.* 38, 721-772.
- 759 Solomon, K.R., Dalhoff, K., Volz, D., Van Der Kraak, G., 2013. Effect of herbicides on fish, in: Tierney,  
760 K.B., Farrell, A., Brauner, C.J. (Eds.), *Organic Chemical Toxicology of Fishes. Fish Physiology: Volume 33.* pp.  
761 369–409.
- 762 Soso, A.B., Barcellos, L.J.G., Ranzani-Paiva, M.J., Kreutz, L.C., Quevedo, R.M., Anziliero, D., Lima, M.,  
763 Silva, L.B. da, Ritter, F., Bedin, A.C., Finco, J.A., 2007. Chronic exposure to sub-lethal concentration of a  
764 glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundiá (*Rhamdia*  
765 *quelen*). *Environ. Toxicol. Phar.* 23, 308–313. doi:10.1016/j.etap.2006.11.008
- 766 Stara, A., Machova, J., Velisek, J., 2012. Effect of chronic exposure to simazine on oxidative stress and  
767 antioxidant response in common carp (*Cyprinus carpio L.*). *Environ. Toxicol. Phar.* 33, 334–43.  
768 doi:10.1016/j.etap.2011.12.019
- 769 Strobel, A., Leo, E., Pörtner, H.O., Mark, F.C., 2013. Elevated temperature and pco2 shift metabolic  
770 pathways in differentially oxidative tissues of *Notothenia rossii*. *Comp. Biochem. Phys. B.* 166, 48–57.  
771 doi:10.1016/j.cbpb.2013.06.006
- 772 Taghavi, L., Merlina, G., Probst, J.-L., 2011. The role of storm flows in concentration of pesticides  
773 associated with particulate and dissolved fractions as a threat to aquatic ecosystems - Case study: the  
774 agricultural watershed of Save river (Southwest of France). *Knowl. Manag. Aquat. Ecosyst.* 400, 06.  
775 doi:10.1051/kmae/2011002
- 776 Tierney, K.B., Taylor, A.L., Ross, P.S., Kennedy, C.J., 2006. The alarm reaction of coho salmon parr is  
777 impaired by the carbamate fungicide IPBC. *Aquat. Toxicol.* 79, 149–57. doi:10.1016/j.aquatox.2006.06.003
- 778 Trudel, M., Goto, D., Wallace, W.G., 2010. Bioenergetic responses of a benthic forage fish (*Fundulus*  
779 *heteroclitus*) to habitat degradation and altered prey community in polluted salt marshes. *Can. J. Fish. Aquat.*  
780 *Sci.* 67, 1566–1584. doi:10.1139/F10-082
- 781 Ulrich, E.L., Akutsu, H., Doreleijers, J.F., Harano, Y., Ioannidis, Y.E., Lin, J., Livny, M., Mading, S., Maziuk,  
782 D., Miller, Z., Nakatani, E., Schulte, C.F., Tolmie, D.E., Kent Wenger, R., Yao, H., Markley, J.L., 2008.  
783 *BioMagResBank. Nucleic Acids Res.* 36, D402–D408. doi:10.1093/nar/gkm957

- 784 Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullou, M., Press, A.I.N., Press, A.I.N., 2006.  
785 Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants.  
786 Environ. Chem. 64, 178–189. doi:10.1016/j.ecoenv.2005.03.013
- 787 Van Der Kraak, G.J., Hosmer, A.J., Hanson, M.L., Kloas, W., Solomon, K.R., 2014. Effects of Atrazine in  
788 Fish, Amphibians, and Reptiles: An Analysis Based on Quantitative Weight of Evidence, Crit. Rev. Toxicol. 44, 1-  
789 66. doi:10.3109/10408444.2014.967836
- 790 Viant, M.R., Werner, I., Rosenblum, E.S., Gantner, A.S., Tjeerdema, R.S., Johnson, M.L., 2003.  
791 Correlation between heat-shock protein induction and reduced metabolic condition in juvenile steelhead trout  
792 (*Oncorhynchus mykiss*) chronically exposed to elevated temperature. Fish Physiol. Biochem. 29, 159–171.  
793 doi:10.1023/B:FISH.0000035938.92027.81
- 794 Verslycke, T., Roast, S. D., Widdows, J., Jones, M. B. and Janssen, C. R. 2004. Cellular energy allocation  
795 and scope for growth in the estuarine mysid *Neomysis integer* (Crustacea: Mysidacea) following chlorpyrifos  
796 exposure: a method comparison. J. Exp. Mar. Bio. Ecol. 306, 1–16.
- 797 Wang Y., Zhu W., Wang D., Teng M., Yan J., Miao J., Zhou Z., 2017. <sup>1</sup>H NMR-based metabolomics  
798 analysis of adult zebrafish (*Danio rerio*) after exposure to diniconazole as well as its bioaccumulation behavior.  
799 Chemosphere. 168, 1571-1577. <http://dx.doi.org/10.1016/j.chemosphere.2016.11.157>
- 800 Waring, C.P., Moore, A., 2004. The effect of atrazine on Atlantic salmon (*Salmo salar*) smolts in fresh  
801 water and after sea water transfer. Aquat. Toxicol. 66, 93–104. doi:10.1016/j.aquatox.2003.09.001
- 802 Wishart, D.S., Tzur, D., Knox, C., Eisner, R., Guo, A.C., Young, N., Cheng, D., Jewell, K., Arndt, D.,  
803 Sawhney, S., Fung, C., Nikolai, L., Lewis, M., Coutouly, M.-A., Forsythe, I., Tang, P., Shrivastava, S., Jeroncic, K.,  
804 Stothard, P., Amegbey, G., Block, D., Hau, D.D., Wagner, J., Miniaci, J., Clements, M., Gebremedhin, M., Guo,  
805 N., Zhang, Y., Duggan, G.E., Macinnis, G.D., Weljie, A.M., Dowlatabadi, R., Bamforth, F., Clive, D., Greiner, R., Li,  
806 L., Marrie, T., Sykes, B.D., Vogel, H.J., Querengesser, L., 2007. HMDB: the Human Metabolome Database.  
807 Nucleic Acids Res. 35, D521–526. doi:10.1093/nar/gkl923
- 808 Wu, H., Southam, A.D., Hines, A., Viant, M.R., 2008. High-throughput tissue extraction protocol for  
809 NMR- and MS-based metabolomics. Anal. Biochem. 372, 204–212. doi:10.1016/j.ab.2007.10.002
- 810 Xing, H., Li, S., Wang, Z., Gao, X., Xu, S., Wang, X., 2012. Oxidative stress response and  
811 histopathological changes due to atrazine and chlorpyrifos exposure in common carp. Pestic. Biochem. Phys.  
812 103, 74–80. doi:10.1016/j.pestbp.2012.03.007
- 813 Xu, H.-D., Wang, J.-S., Li, M.-H., Liu, Y., Chen, T., Jia, A.-Q., 2015. <sup>1</sup>H NMR based metabolomics  
814 approach to study the toxic effects of herbicide butachlor on goldfish (*Carassius auratus*). Aquat. Toxicol. 159,  
815 69–80. doi:10.1016/j.aquatox.2014.11.020

816 Yin, F., Sun, P., Peng, S., Tang, B., Zhang, D., Wang, C., Mu, C., Shi, Z., 2013. The respiration, excretion  
817 and biochemical response of the juvenile common chinese cuttlefish, *Sepiella maindroni* at different  
818 temperatures. *Aquaculture* 402-403, 127–132. doi:10.1016/j.aquaculture.2013.03.018

819 Zhang X., Zhong Y., Tian H., Wang W., Ru S., 2015. Impairment of the cortisol stress response mediated  
820 by the hypothalamus–pituitary–interrenal (HPI) axis in zebrafish (*Danio rerio*) exposed to monocrotophos  
821 pesticide. *Comp. Biochem. Phys. C.* 176-177, 10-16. <http://dx.doi.org/10.1016/j.cbpc.2015.07.003>  
822 Lu, Z., Liu, C.-X., Chen, Q.-L., Tan, X.-Y., Zhu, Q., Gong, Y., 2013. Differential effects of acute and chronic zinc  
823 (Zn) exposure on hepatic lipid deposition and metabolism in yellow catfish *Pelteobagrus fulvidraco*. *Aquat.*  
824 *Toxicol.* 132-133, 173–181. doi:<http://dx.doi.org/10.1016/j.aquatox.2013.02.002>

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Fig. captions :

Fig. 1. PLS-DA scores (top) and loadings plot (bottom) plots of integrated  $^1\text{H-NMR}$  spectra of liver extracts for each time point independently.

For scores plots, each dot represents an observation (fish), projected onto first (horizontal axis) and second/third (vertical axis) PLS-DA variables. Temperature is shown in different colors: 22 °C (CONTROL) in black, 32 °C in blue; exposition to pesticides is shown in different symbols: box for non-exposed fish, four-point star for exposed fish. The black ellipse determines the 95% confidence interval, which is drawn using Hotelling's T2 statistic. For loading plot, the horizontal axis corresponds to chemical shifts (ppm) and the vertical axis the loading value. The color bar corresponds to the value of the loading in the discrimination model. The positive part of the loadings plot represents metabolites higher for group(s) in the left section of the scores plot whereas the negative part of the loadings plot represents metabolites that are higher for group(s) in the right section of the score plot. Corresponding loading plots color coded according to the correlation coefficients from blue to red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

A. T6h liver extracts ( $A=3$ ,  $R^2=52.7\%$ ,  $Q^2=0.368$ ). B. T96h liver extracts ( $A=3$ ,  $R^2=54.3\%$ ,  $Q^2=0.343$ ). C. T16d liver extracts ( $A=2$ ,  $R^2=55.4\%$ ,  $Q^2=0.507$ ). D. T6h liver extracts. E. T96h liver extracts. T16d liver extracts.

Fig. 2. Canonical representations of endocrine, physiological and metabolic responses of goldfish to individual and combined chemical and thermal stresses.

Fish were exposed for 6 h, 96 h or 16 d to a mixture of pesticides (PEST), temperature increased (TEMP) or a combination of pesticide mixture and temperature increased (PEST\*TEMP). Integration of endocrine, metabolic and physiological responses was realized using canonical analysis and Mahalanobis distance calculs. Blue diamonds: position of variables on the first canonical plan. Red squares: mean positions of groups. Blue boxes: Mahalanobis distances between exposed groups and control group. Asterisks: significant Mahalanobis distances with \*: p-value<0.05, \*\*: p-value<0.01. Abbreviations: AE: available energy; EC: energy consumed; HSI: hepaosomatic index; MI: muscle index; FCF: Fulton's condition factor.

Fig. 3. Defense system induction in goldfish exposed to individual and combined chemical and thermal stresses.

The activity of CAT, SOD and GST were measured in the liver of goldfish exposed to pesticide mixture (PEST), temperature increased (TEMPS) or a combination of both stresses (PEST\*TEMP). Boxplots at 6 h, 96 h and 16 d are presented. N=12.

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**Table 1. Fold-change of discriminant metabolites identified by  $^1\text{H}$  NMR metabolomics approach in aqueous liver extracts of *Carassius auratus* exposed to pesticide mixture (PEST), temperature increased (TEMP) or both (PEST\*TEMP) compared to CONTROL group at T6 h.**

	Metabolites	Chemical shift <sup>a</sup> $\delta^1\text{H}$ (ppm)	FC <sup>b</sup> PEST	P <sup>c</sup>	FC TEMP	P	FC PEST*TEMP	P
Amino-acids	Glutamine	2.14 (m), 2.45(m), 3.78(t)	1.43	**	1.32	**	1.32	
	Proline	2.00(m), 2.04(m), 2.35(m), 3.34(m), 3.41(m), 4.14(dd)	1.15		0.72		0.65	*
Organic acids	Glutathione (reduced)	2.17(q), 2.55(m), 2.98(ddd), 3.80(m), 4.57(m)	1.29	**	1.27	**	1.14	
	Succinate	2.40(s)	1.29	**	1.00		0.97	
	Taurine	3.27(t), 3.43(t)	1.15		1.18	**	1.09	
Sugars	Maltose	3.29(dd), 3.43(t), 3.56-4.00, 4.66(d), 5.24(d), 5.42(d)	0.91	**	0.99		0.98	
Phosphagens	Choline	3.20(s), 3.53(m), 4.07(m)	1.49	***	1.15		1.08	
	Phosphoryl-choline	3.22(s), 3.60(m), 4.17(m)	1.31		1.78	*	1.54	
Nucleotides	AMP	4.03(m), 4.38(m), 4.52(dd), 6.15(d), 8.24(s), 8.56(s)	1.40	***	1.14		1.00	
	NAD <sup>+</sup>	4.24(m), 4.35-4.44, 4.47-4.56, 6.03(d), 6.10(d), 8.14(s), 8.20(dd), 8.41(s), 8.84(d), 9.15(d)	1.40	***	1.20		1.08	
	UDP/UTP	4.22-4.30, 4.38-4.47, 5.98(m), 7.99(d)	1.28		0.79	***	0.74	*

<sup>a</sup>Multiplicity : s, singlet; d, doublet; t, triplet; q, quartets; m, multiplet. <sup>b</sup>FC, Fold Change = exposed samples/control samples. <sup>c</sup>P values were calculated using a nonparametric Kruskal-Wallis test. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

**Table 2. Fold-change of discriminant metabolites identified by  $^1\text{H}$  NMR metabolomics approach in aqueous liver extracts of *Carassius auratus* exposed to pesticide mixture (PEST), temperature increased (TEMP) or both (PEST\*TEMP) compared to CONTROL group at T96 h.**

	Metabolites	Chemical shift <sup>a</sup> $\delta^1\text{H}$ (ppm)	FC <sup>b</sup> PEST	P <sup>c</sup>	FC TEMP	P	FC PEST*TEMP	P
Amino-acids	Proline	2.00(m), 2.04(m), 2.35(m), 3.34(m), 3.41(m), 4.14(dd)	1.11		0.69	***	0.85	***
Organic acids	Malonate	3.14(s)	0.87		0.75	*	0.77	
	Succinate	2.40(s)	1.02		0.92		0.74	**
Sugars	Maltose	3.29(dd), 3.43(t), 3.56-4.00, 4.66(d), 5.24(d), 5.42(d)	1.03		1.24	***	1.04	
Nucleotides	AMP	4.03(m), 4.38(m), 4.52(dd), 6.15(d), 8.24(s), 8.56(s)	0.85	***	0.83	***	0.68	***
	NAD <sup>+</sup>	4.24(m), 4.35-4.44, 4.47-4.56, 6.03(d), 6.10(d), 8.14(s), 8.20(dd), 8.41(s), 8.84(d), 9.15(d)	0.95		0.82	***	0.73	***
	UDP/UTP	4.22-4.30, 4.38-4.47, 5.98(m), 7.99(d)	0.94		0.72	***	0.57	***
Non identified		1.09(d)	1.00		0.68	***	1.50	***
		3.09(m)	0.97		0.72	**	0.91	**

<sup>a</sup>Multiplicity : s, singlet; d, doublet; t, triplet; q, quartets; m, multiplet. <sup>b</sup>FC, Fold Change = exposed samples/control samples. <sup>c</sup>P values were calculated using a nonparametric Kruskal-Wallis test. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

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**Table 3. Fold-change of discriminant metabolites identified by  $^1\text{H}$  NMR metabolomics approach in aqueous liver extracts of *Carassius auratus* exposed to pesticide mixture (PEST), temperature increased (TEMP) or both (PEST\*TEMP) compared to CONTROL group at T16 d.**

	Metabolites	Chemical shift <sup>a</sup> $\delta^1\text{H}$ (ppm)	FC <sup>b</sup> PEST	P <sup>c</sup>	FC TEMP	P	FC PEST*TEMP	P
Amino-acids	Alanine	1.48 (d), 3.77 (q)	1.01		1.05		0.82	**
	Glutamine	2.14 (m), 2.45(m), 3.78(t)	0.97		1.27		0.71	***
	Isoleucine	0.94 (t), 1.01 (d), 1.26 (m), 1.46 (m), 1.98(m), 3.68(d)	1.00		0.81		0.57	***
	Leucine	0.96(d), 0.97(d), 1.70(m), 3.73 (t)	0.96		0.84		0.55	**
	Lysine	1.44(m), 1.50(m), 1.73(m), 1.90(m), 3.02(t), 3.77(t)	1.04		1.04		0.68	**
	Proline	2.00(m), 2.04(m), 2.35(m), 3.34(m), 3.41(m), 4.14(dd)	0.93		0.83	**	0.64	**
	Valine	0.99(d), 1.04(d), 2.28(m), 3.62(d)	0.98		0.92		0.62	***
Organic acids	Creatine	3.04(s), 3.93(s)	0.94		0.76		0.55	***
	Glutathione (reduced)	2.17(q), 2.55(m), 2.98(ddd), 3.80(m), 4.57(m)	0.94		1.65	***	0.99	
	Lactate	1.33(d), 4.10(q)	1.05		0.99		0.83	*
	Taurine	3.27(t), 3.43(t)	1.02		0.96		0.86	***
Sugars	Maltose	3.29(dd), 3.43(t), 3.56-4.00, 4.66(d), 5.24(d), 5.42(d)	0.88		0.99		1.08	***
Phosphagens	Phosphoryl-choline	3.22(s), 3.60(m), 4.17(m)	1.04		1.68	*	1.18	
Nucleotides	AMP	4.03(m), 4.38(m), 4.52(dd), 6.15(d), 8.24(s), 8.56(s)	1.03		1.08		0.83	**
	NAD <sup>+</sup>	4.24(m), 4.35-4.44, 4.47-4.56, 6.03(d), 6.10(d), 8.14(s), 8.20(dd), 8.41(s), 8.84(d), 9.15(d)	1.06		1.12		0.69	***
Non identified		3.09(m)	1.12		0.97		0.67	**

<sup>a</sup>Multiplicity : s, singlet; d, doublet; t, triplet; q, quartets; m, multiplet. <sup>b</sup>FC, Fold Change = exposed samples/control samples. <sup>c</sup>P values were calculated using a nonparametric Kruskal-Wallis test. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

**Table 4. Endocrine, metabolic and physiological responses in goldfish exposed to individual and combined chemical and thermal stresses.**

Variables	T6h			T96h			T16d		
	PEST	TEMP	PEST*TEMP	PEST	TEMP	PEST*TEMP	PEST	TEMP	PEST*TEMP
<b>Cortisol</b>	↑*	↑*	↑*	-	-	-	-	-	↓°
<b>Liver</b>									
carbohydrates	↑*	-	↑°	-	-	-	↑*	-	-
proteins	↑*	↑**	-	-	-	-	-	-	-
lipids	↓*	-	-	-	-	-	-	-	-
AE	-	-	-	-	-	-	-	-	-
EC	↑*	↑**	-	-	-	-	-	-	-
CEA	↓°	-	-	-	-	-	-	-	-
<b>White Muscle</b>									
carbohydrates	-	-	-	-	-	-	-	-	-
proteins	-	-	-	-	-	-	-	-	-
lipids	-	↓°	-	↑*	-	-	-	-	↑*
AE	-	↓*	-	↑*	-	-	-	-	↑*
EC	-	↑*	-	-	-	-	-	-	-
EA	-	↓***	-	-	-	-	-	-	↑*
<b>HSI</b>	↑**	-	-	-	-	-	↑*	-	↑**
<b>MI</b>	-	-	-	-	-	-	↑*	-	↑**
<b>FCF</b>	-	-	-	-	-	-	-	-	↓**

Fish were exposed for 6 hours, 96 hours or 16 days to a mixture of pesticides (PEST), temperature increased (TEMP) or a combination of both (PEST\*TEMP). At each time, ANOVA analyses followed by LSD post-hoc tests were performed. Red arrows indicate increased values compared to CONTROL group while green arrows correspond to decreased values. Asterisks: significance of the differences compared to CONTROL group with: °: p<0.1, \*: p<0.05, \*\*: p<0.01, \*\*\*: p<0.001. Abbreviations: AE: available energy; EC: energy consumed; CEA: cellular energy allocation; HSI: hepatosomatic index; MI: muscle index; FCF: Fulton's condition factor.

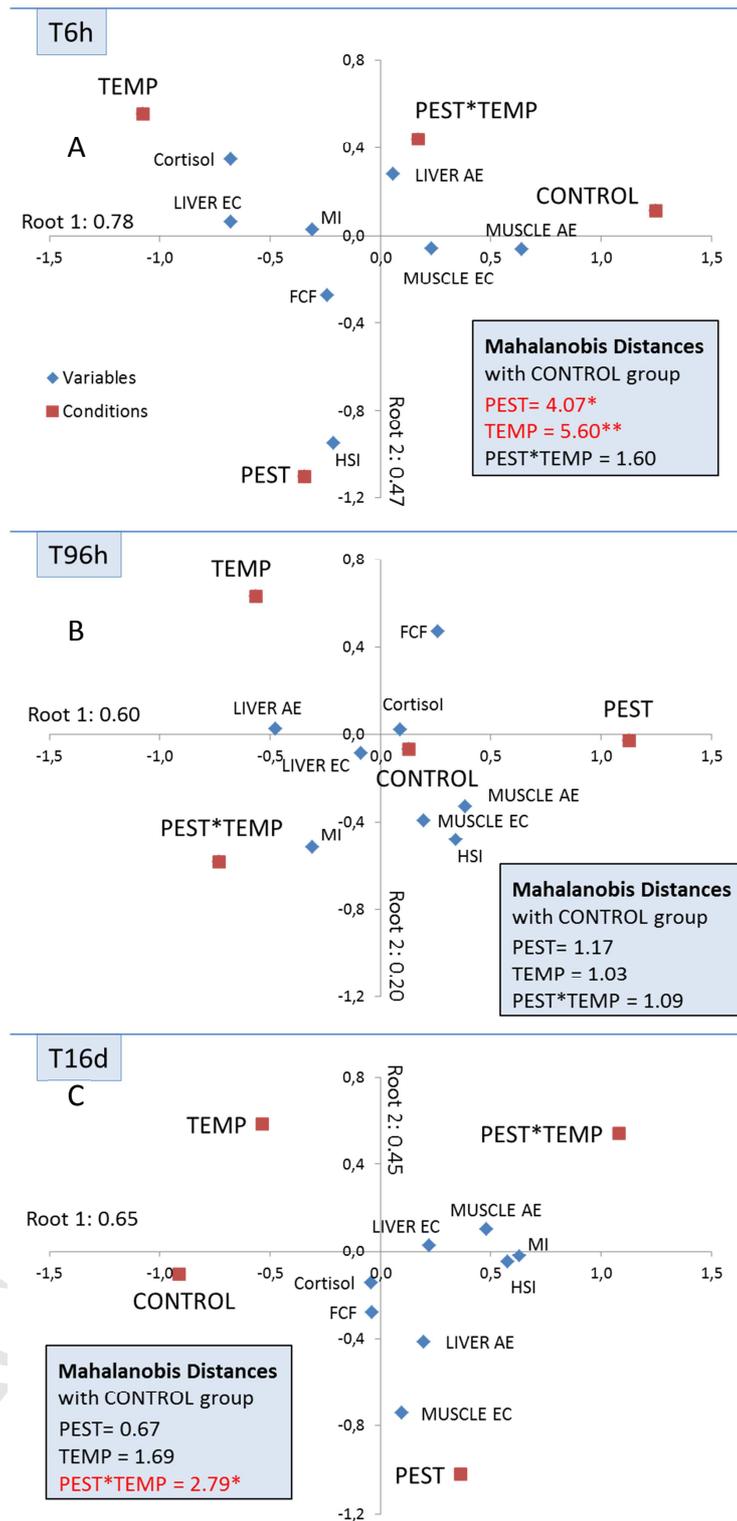


Fig. 1. Canonical representations of endocrine, physiological and metabolic responses of goldfish to individual and combined chemical and thermal stresses.

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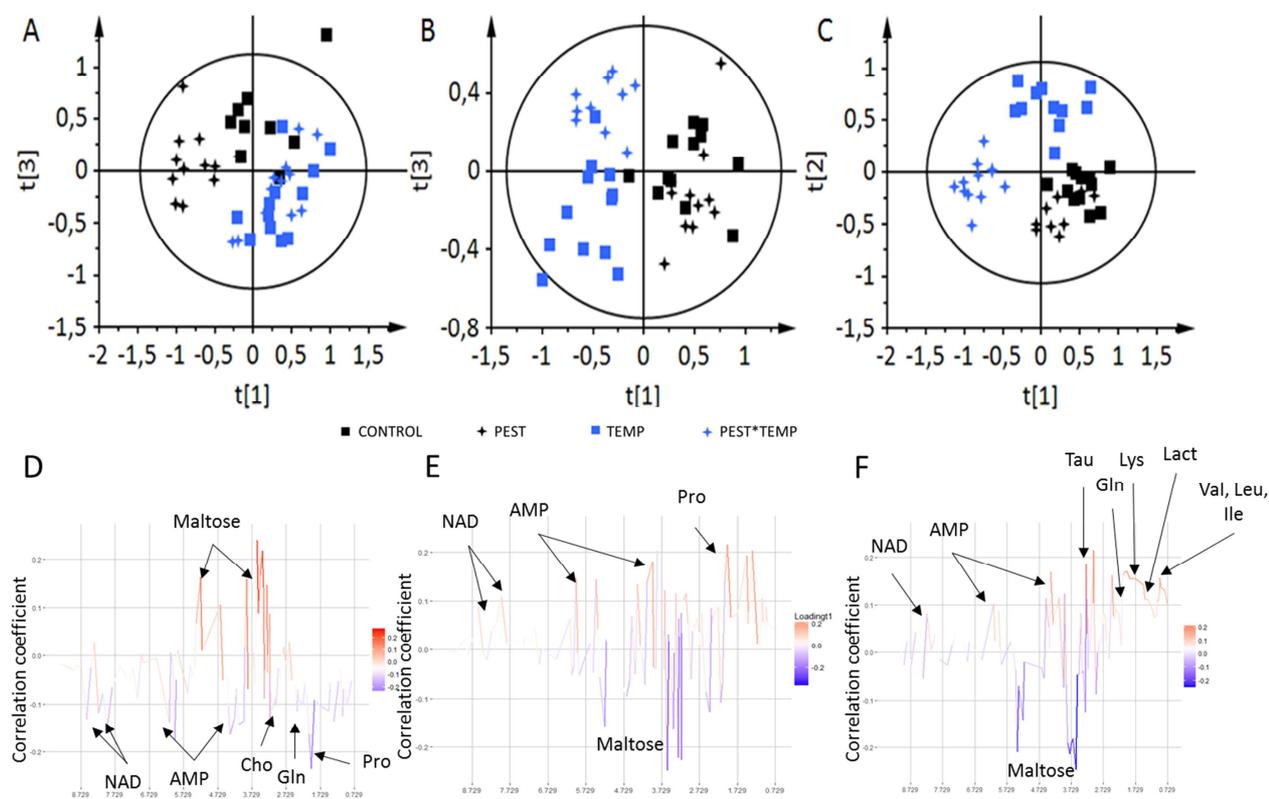


Fig. 2. PLS-DA scores (top) and loadings plot (bottom) plots of integrated  $^1\text{H-NMR}$  spectra of liver extracts for each time point independently.

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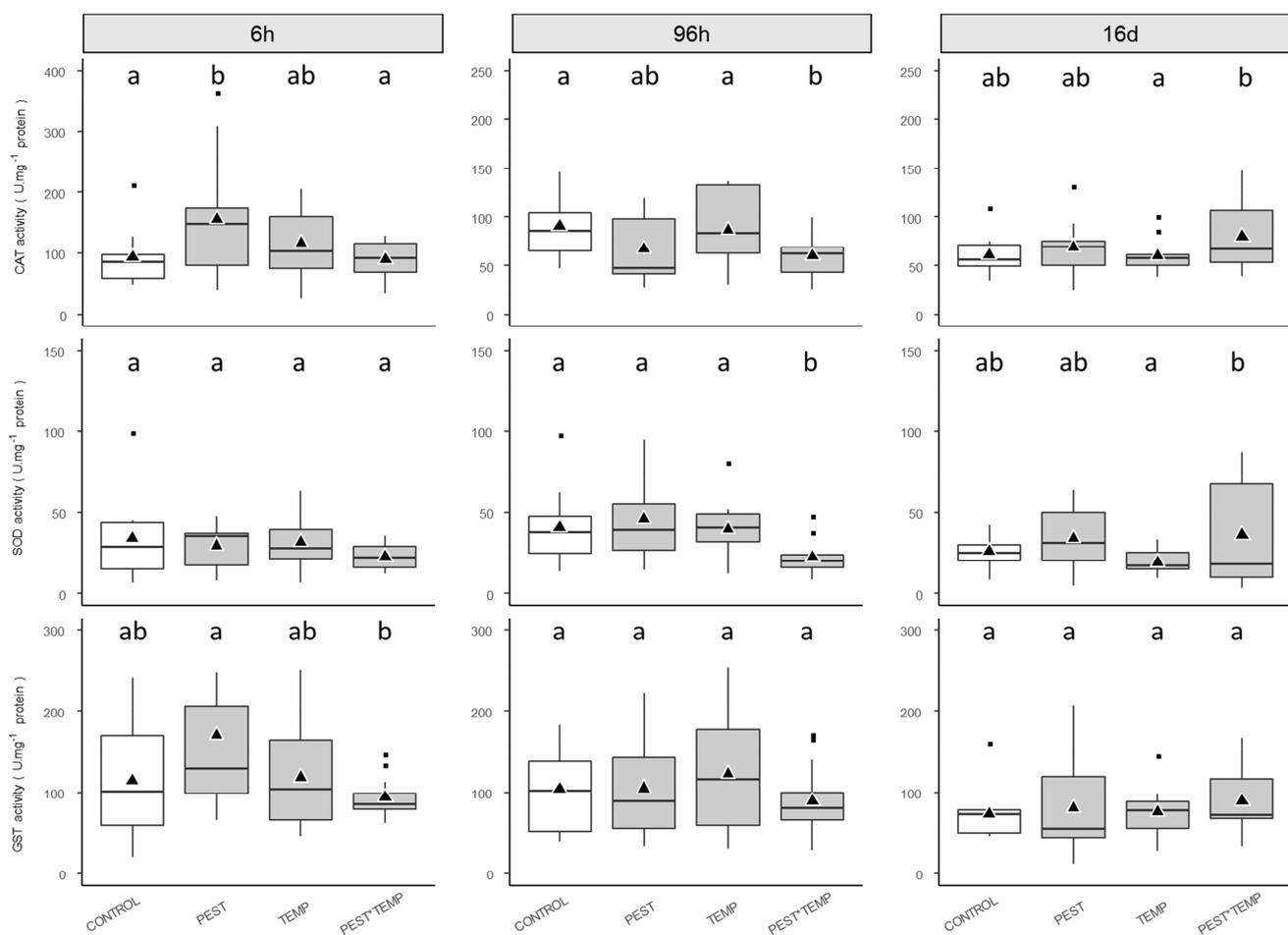


Fig. 3. Defense system induction in goldfish exposed to individual and combined chemical and thermal stresses.

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## Highlights

- Goldfish were exposed to a pesticide mixture and elevated temperatures for 16 days
- Metabolomics and biochemical approaches were conducted
- Adapted and coordinated early responses to individual stressors were observed
- Multistress reduced earlier endocrine response, antioxidant defense and metabolic compensation

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