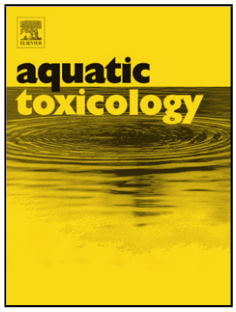

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Combined effects of temperature and metal exposure on the fatty acid composition of cell membranes, antioxidant enzyme activities and lipid peroxidation in yellow perch (*Perca flavescens*)

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Highlights

- The fatty acid composition of yellow perch muscle at 9°C was enhanced in monounsaturated and polyunsaturated fatty acids compared to fish maintained at 28°C
- The thermal adjustment of muscle phospholipid fatty acid profiles is likely due to modifications of desaturase and elongase activities
- Exposure to Ni and Cd modified muscle phospholipid fatty acid composition in a temperature-dependent manner
- The higher fatty polyinsaturation in cold-acclimated fish did not increase their vulnerability to peroxidation.
- Lower concentrations of malondialdehyde were measured in warm-acclimated, Ni-exposed fish, suggesting an overcompensation of antioxidant mechanisms that could explain their lower condition.

Abstract

The aim of this study was to investigate the combined effects of temperature and metal contamination (cadmium and nickel) on phospholipid fatty acid composition, antioxidant enzyme activities and lipid peroxidation in fish. Yellow perch were acclimated to two different temperatures (9°C and 28°C) and exposed either to Cd or Ni (respectively 4 µg/L and 600 µg/L) for seven weeks. Superoxide dismutase, catalase, glutathione-S-transferase, glutathione peroxidase activities and glutathione concentration were measured as indicators of antioxidant capacities, while malondialdehyde concentration was used as an indicator of lipid peroxidation. Poikilotherms including fish counteract the effects of temperature on phospholipid fatty acid ordering by remodelling their composition to maintain optimal fluidity. Accordingly, in our

study, the fatty acid composition of yellow perch muscle at 9°C was enhanced in monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) compared to fish maintained at 28°C, in agreement with the theory of homeoviscous adaptation. Using ratios of various fatty acids as surrogates for desaturase and elongase activities, our data suggests that modification of the activity of these enzymes is responsible for the thermal acclimation of phospholipid fatty acid profiles. However, this response was altered under Ni and Cd exposure: PUFA decreased (specifically n-6 PUFA) while the proportion of saturated fatty acids increased at 9°C, whereas at 28°C, PUFA increased to proportions exceeding those observed at 9°C. Lipid peroxidation could be observed under all experimental conditions. Both enzymatic and non-enzymatic antioxidant defense systems acted cooperatively to cope with oxidative stress leading to lipid peroxidation, which was not affected by temperature acclimation as indicated by malondialdehyde concentration, in spite of a higher polyinsaturation in cold-acclimated fish which would be predicted to increase their vulnerability to peroxidation. However, in warm-acclimated, Ni-exposed fish, in which the highest proportion of PUFA was observed, lower concentrations of malondialdehyde were measured, suggesting an overcompensation of antioxidant mechanisms in these fish which could represent a substantial metabolic cost and explain their lower condition.

Abbreviations

ARA Arachidonic acid (C20:4 n-6)

CAT Catalase

Cd Cadmium

CI Condition index

D5D Δ5-desaturase

D6D Δ6-desaturase

DHA Docosahexaenoic acid (C22:6 n-3)

ELOVL2 Elongase 2

64 **EPA** Eicosapentaenoic acid (C20:5 n-3)
65 **GPx** Glutathione peroxidase
66 **GSH** Reduced glutathione
67 **GST** Glutathione-S-transferase
68 **HSI** Hepatosomatic index
69 **LPO** Lipid peroxidation
70 **MDA** Malondialdehyde
71 **MUFA** Monounsaturated fatty acids
72 **n-3 PUFA** Omega-3 series polyunsaturated fatty acids
73 **n-6 PUFA** Omega-6 series polyunsaturated fatty acids
74 **n-9 PUFA** Omega-9 polyunsaturated fatty acids
75 **Ni** Nickel
76 **PI** Peroxidation index
77 **PUFA** Polyunsaturated fatty acids
78 **SCD-16** stearoyl-CoA-desaturase
79 **SCD-18** stearoyl-CoA-desaturase
80 **SFA** Saturated fatty acids
81 **SOD** Superoxide dismutase

82

83 Keywords: Fish; Cell membranes; Phospholipid fatty acids; Temperature; Cadmium; Nickel;
84 Antioxidant enzymes; Lipid peroxidation

1. Introduction

Biological membranes are semipermeable barriers surrounding cells and organelles. They are composed of a lipid bilayer and a variety of proteins. Membranes are highly sensitive to temperature fluctuations. This sensitivity is due to the effects of temperature on membrane lipids and consequently on the proteins embedded in the membranes (Hochachka and Somero, 2002). The internal temperature of fish and other poikilothermic organisms largely reflects ambient environmental temperature. These organisms counteract the effects of fluctuations in environmental temperature on the properties and function of their cell membranes by remodelling membrane lipids, a process known as homeoviscous adaptation (Hazel, 1995) involving changes in phospholipid head groups, acyl-chain composition and cholesterol content (Hazel and Williams, 1990). Shifts in phospholipid classes surrounding proteins modulate their activity (Frick et al., 2010; Robinson, 1993). This homeoviscous adaptation ensures the maintenance of membrane functions (Hazel, 1995; Kraffe et al., 2007; Pernet et al., 2007). Desaturases and elongases are the key enzymes involved in fatty acid synthesis and remodelling pathways. It was demonstrated that cold-challenged ectothermic fish display an upregulation of these enzymes to restore the fluidity of cold-rigidified membranes (Tiku et al., 1996; Tocher et al., 2004; Trueman et al., 2000).

Aerobic organisms depend on oxygen for energy production through oxidative phosphorylation. Reactive oxygen species (ROS) are constantly generated during normal cell metabolism. These species include superoxide anion (O_2–), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$) and others (Halliwell and Gutteridge, 1999). Organisms cope with increasing ROS production by up-regulating their antioxidant defense system through non-enzymatic (glutathione, ascorbic and uric acid, tocopherols, etc.) and enzymatic components (Livingstone, 2003). General cellular antioxidant enzymes are superoxide dismutase (SOD, converts $O_2^{\cdot -}$ to H_2O_2), catalase (CAT, reduces H_2O_2 to H_2O), glutathione peroxidase (GPx, detoxifies H_2O_2 or organic hydroperoxides produced, for example, by lipid peroxidation (Halliwell and Gutteridge, 1999)) and glutathione S-transferase (GST, catalyzes the conjugation of glutathione (GSH) with various electrophilic substances). If antioxidant systems fail to eliminate excessive ROS production, significant damage can occur including DNA damage, protein degradation, enzyme inactivation and lipid peroxidation (Halliwell and Gutteridge, 1999). Indeed, ROS that possess sufficient energy to

remove a hydrogen atom within lipid chains from methylene groups (-CH_2-), can initiate lipid peroxidation (Girotti, 1985). During initiation, a lipid radical (L·) produced after the abstraction of a hydrogen atom reacts with dioxygen to generate a lipid peroxyl radical (LOO·). This peroxyl radical then reacts with another lipid to generate another radical (L·) and a lipid hydroperoxide (LOOH). It has been demonstrated that more polyunsaturated fatty acids, and hence membranes with a higher degree of unsaturation, are more prone to LPO (Lin and Huang, 2007). The intensity of LPO is assessed by measuring the concentrations of primary products, lipid peroxides or end products of LPO such as MDA and other aldehydes (Halliwell and Gutteridge, 1999). Common products of LPO released within the non-polar interior of biological membranes affect membrane stability by disruption of the non covalent bonds (e.g., *van der Waals* interactions). Furthermore, LOOH damages membrane integrity by affecting lipid-lipid and lipid-protein interactions (Kuhn and Borchert, 2002). In addition to damaging membrane physical properties, LPO can be deleterious by affecting membrane protein functions. For example, the activity of Na^+/k^+-ATPase was reduced by approximately 90% after LPO in brain synaptosomes (Chakraborty et al., 2003).

Thermal stress induces oxidative stress in ectotherms (Bagnyukova et al., 2003; Bocchetti et al., 2008; Verlecar et al., 2007). An increase of temperature stimulates metabolic processes, enhances oxygen consumption and consequently may increase ROS production (Lushchak, 2011).

Toxic trace metals induce uncontrolled reactive species production and oxidative stress. Cadmium (Cd) and nickel (Ni) are widespread pollutants in aquatics systems. Previous investigations have demonstrated that Cd does not generate ROS directly. Instead, Cd-induced oxidative stress results from the displacement of endogenous Fe leading to ROS generation (Schlenk and Benson, 2003; Valko et al., 2006). In turn, this affects GSH and thiol concentrations and antioxidant enzyme activities and can lead to lipid peroxidation (Sevcikova et al., 2011; Valko et al., 2005; Wang and Wang, 2009). Nickel is also highly toxic to living organisms. It can mediate directly or indirectly the oxidation of macromolecules (DNA, lipids and proteins) (Palermo et al., 2015). It can induce oxidative stress through ROS formation via Haber-Weiss/Fenton reactions (Torreilles and Guerin, 1990), depletion of intracellular free radical scavengers such as GSH (Krezel et al., 2003) or inhibition of the activity of antioxidant enzymes

(Attig et al., 2014; Kubrak et al., 2012).

In a natural environment, organisms are typically exposed to multiple stressors, including natural factors, such as changes in temperature, oxygen concentrations or food availability, and anthropogenic stressors, such as contaminants. Several studies have investigated the effects of these stressors separately on different fish species and an increasing number of studies have examined their combined effects (Cai and Curtis, 1990; Cailleaud et al., 2007; Grasset et al., 2016 ; Kefaloyianni et al., 2005; Tocher et al., 2004). Yet, to our knowledge, this is the first study to examine the responses of cell membrane phospholipid fatty acid composition, lipid peroxidation and oxidative stress to variations in acclimation temperature and metal exposure. We investigated this question in the muscle of yellow perch (*Perca flavescens*), a freshwater fish species commonly found in areas affected by metal contamination. To this end, fish were acclimated to a cold or a warm temperature under clean conditions or combined with environmentally-relevant aqueous concentrations of Cd or Ni. Membrane phospholipid fatty acid composition was measured, along with indicators of oxidative stress (MDA) and oxidizability (PI) of membrane phospholipids and cellular antioxidant capacities (SOD, CAT, GST, GPx, GSH).

2. Materials and methods

2.1 Experimental design: thermal acclimation and metal exposure

Yellow perch (*Perca flavescens*) were obtained from Trevor Thomas, Abbey Road Fish Farm, (Wainfleet, ON) and transported to the Laboratoire de Recherche en Sciences Aquatiques (LARSA) at Université Laval (Québec, QC) for thermal acclimation. Fish were maintained in a $1m^3$ circular tank for one month to be acclimated to laboratory conditions at a temperature of 20°C. During this period, fish were fed with Hikari® frozen brine shrimp (*Artemia salina*) with a daily ration of 3% of their biomass. Following acclimation, 25 fish were placed in each of six 40 L aquaria and experimental conditions were started. Although yellow perch tolerate a wide range of temperatures, literature reports that they have a preference for warmer temperatures around 20-22˚C (Huh et al., 1976; Rasmussen and Brinkmann, 2015; Tidwell et al., 1999) However, a recent study from our laboratory (Grasset et al., 2014) as well as preliminary studies

indicated that the growth of yellow perch is optimal at colder temperatures, around 9-11˚C. In the same studies, we also observed that, although inducing heat stress, 28˚C was easily tolerated by yellow perch. Therefore, in our study we selected 9 and 28˚C as experimental temperatures since these presented a wide range with a comparable separation from the acclimation temperature. After acclimation at 20˚C, temperature was reduced (in 3 aquaria) or raised (in 3 aquaria) at a rate of 2°C per day to reach a low (9°C) and a high (28°C) temperature. When the desired temperatures were reached, metal exposure was initiated following the procedures described in Grasset et al. (2016). For Cd, measured water concentrations were 3.98 ± 0.23 µg/L (35.4 ± 2.0 nM) (mean ± SE, n=56; 2 temperatures, 2 aquaria per temperature, two samplings per week). Values in Ni exposure aquaria were 605.9 ± 13.3 µg Ni/L (10.3 ± 0.2 µM) (n=56). Concentrations of both metals in control aquaria were consistently below 0.01 µg/L. These metal concentrations were selected as realistic in contaminated environments (Couture et al., 2008; Gauthier et al., 2006). After 7 weeks of exposure, fish were sacrificed by a blow to the head. Biometric measures (length and weight) were taken, then fish were dissected and muscle samples were stored at −80°C for fatty acid, enzyme and lipid peroxidation analyses. Eight fish per condition (2 temperatures for control, Cd and Ni-exposed fish) were randomly selected for this study. The remainder of the fish were dedicated to another study (Grasset et al., 2016). All procedures were approved by our institutional animal care committee and followed the guidelines of the Canadian Council on Animal Care.

2.2 Sample preparation for enzyme and GSH assays

Muscle samples (40-50 mg) were thawed on ice, diluted 10-fold with ice-cold buffer (pH 7.5; 20 mM HEPES; 1 mM EDTA; 0.1% Triton X-100) and homogenized for two bursts of 20 s using an Ultra Turrax T25 homogenizer (Janke and Kunkel, IKA-labortechnik, Staufen, Germany). During homogenization, samples and homogenates were kept on ice. Once homogenized, three 20 µL aliquots were kept in separate tubes and stored at -80°C for SOD, CAT and GPx assays whereas GST and GSH were analyzed the same day. Assays were performed using a UV/Vis Cary 50 spectrophotometer equipped with a microplate reader (Varian Inc., Palo Alto, CA).

205 **2.3 Enzyme assays**
206
207 All enzyme activities were measured using a UV-Vis spectrophotometer (Varian Cary 100;
208 Varian Inc., Palo Alto, CA) with 96-well microplates at room temperature (20°C). Reactions were
209 recorded over a period of five minutes and linear sections of at least three minutes were
210 selected to calculate reaction rates. The activity of GST (EC 2.5.1.18) was measured according to
211 Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The reaction
212 mixture contained phosphate buffer (50mM, pH 6.5), CDNB (1mM), GSH (6mM, omitted in
213 controls), in a final volume of 200 µL (including 10 µL of sample). The formation of S -2,4-dinitro
214 phenyl glutathione conjugate was monitored for 5 min by following its absorbance at 340 nm.
215 GST activity was expressed in IU (µmol of substrate converted to product per min) per g of
216 muscle (wet weight) using an extinction coefficient of 9.6 mM cm^{-1}.

217 The activities of SOD, CAT and GPx were determined using assay kits (Nos. 706002 and
218 707002 and 703102 for SOD, CAT and GPx, respectively) purchased from Cayman Chemical
219 Company Inc. (Ann Arbor, Michigan USA) in supernatants obtained by centrifugation of
220 homogenates at 5000 g for 5 min, 10 000 g for 15 min or 10 000 g for 15 min at 4°C for SOD, CAT
221 and GPx respectively. Activities were expressed as UI mg $protein^{-1}$. SOD activity (EC 1.15.1.1)
222 was determined by the detection of superoxide radicals generated by xanthine oxidase and
223 hypoxanthine (one unit of SOD represents the amount of enzyme required for 50% dismutation
224 of superoxide radical). CAT (EC 1.11.1.6) activity was determined using a colorimetric assay by
225 measuring formaldehyde formed with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole as
226 chromogen. GPx activity (EC 1.11.1.9) was determined by the measure of $NADP^+$ absorbance
227 decrease at 340 nm, which is directly proportional to GPx activity.

228 **2.4 Reduced glutathione (GSH) assay**
229
230 GSH was determined according to Ellman (1959) by measuring the absorbance of 5,5-dithiobis-
231 2-nitrobenzoic acid (DTNB)-GSH conjugates at 412 nm and expressed as nmol GSH per mg of
232 protein. The concentration was determined in the initial homogenates used for enzyme assays.
233 Samples were treated with trichloroacetic acid (TCA, 10% w/v) to remove molecules and
234 enzymes that can hinder GSH analysis, then centrifuged at 8872 g for 10 min. Fifty µL of

supernatant was mixed with 230 µL of Tris–HCl buffer (0.8 M Tris/HCl, 0.02 M EDTA, pH 8.9) and 40 µL of 0.01 M DTNB (5,5'-dithiobis-(2-nitrobenzoic acid), Ellman's reagent). The reaction mixture was incubated for 5 min at room temperature. The concentration of GSH was calculated using a GSH standard curve.

2.5 Protein determination

Protein concentration was measured on muscle homogenates according to the dye-binding procedure of Bradford (1976) using bovine serum albumin as standard. Absorbance was measured at 580 nm and protein concentration was expressed as mg protein per g wet weight.

2.6 Lipid peroxidation

The peroxidative damage to lipids that occurs with free radical generation and results in the production of malondialdehyde (MDA) was assessed. Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) according to the instructions of an assay kit (10009055) purchased from Cayman Chemical Company Inc. (Ann Arbor, Michigan USA). Muscle samples (25 mg) were homogenised in 250 µL of specific buffer (50 mM Tris-HCl, pH 7.6, containing 150 mM sodium chloride, 1% Tergitol (NP-40), 0.5% sodium deoxycholate, and 0.1% SDS). Homogenates were centrifuged at 1 600 g for 10 min at 4°C. The MDA in the sample reacted with thiobarbituric acid (TBA) under high temperature (100°C) to generate the MDA-TBA adduct. MDA was measured by colorimetry at 530 nm. The concentration was expressed as nmol/mg protein.

2.7 Lipid extraction and phospholipid fatty acid analysis

Total lipids were extracted from 100 mg muscle samples with 21 volumes of chloroform/methanol (2:1, v/v) containing 0.01% (w/v) butylated hydroxytoluene (BHT) as antioxidant (Folch et al., 1957) After homogenisation, 5 mL of NaCl (0,73%) were added to precipitate proteins and the mixture was centrifuged 15 min at 1000 g. Two phases separated by a protein layer were obtained: an upper chloroform phase that contains lipids and a lower aqueous phase. The chloroform layer was removed and the solvent evaporated in a TurboVap® under a nitrogen flow.

Polar and neutral lipid fractions were separated by passing samples through a 500 mg, 3 mL

265 silica column (Sigma-Aldrich, CA). Neutral lipids were eluted by rinsing with 10 mL chloroform,
266 then polar lipids were eluted with 10 mL of methanol.
267 Fatty acids are difficult to analyze because they are highly polar and tend to form hydrogen
268 bonds. Reducing their polarity facilitates their analysis by gas chromatography and facilitates
269 separation by number and position of unsaturations. The esterification reaction of fatty acids to
270 methyl esters involves the condensation of the carboxyl group of an acid and the hydroxyl group
271 of an alcohol in the presence of a catalyst. Fatty acids from polar fractions were esterified with 2
272 mL of BF_3 (Boron trifluoride-methanol solution 14% in methanol) for 1h at 75°C. After cooling,
273 fatty acid methyl esters (FAME) were extracted with 3 mL of water (to remove the catalyst- BF_3)
274 followed by 3 mL of petroleum ether. To remove impurities and BHT, samples were purified on
275 a silica column by rinsing with 5 mL petroleum ether and then eluting with 10 mL petroleum
276 ether containing 5% diethyl ether.
277 Finally, the organic solvent was evaporated under nitrogen, FAME were dissolved in hexane,
278 transferred to 300 µL screw-capped vials and stored at -20°C to be analysed by gas
279 chromatography with a flame ionization detector (GC-FID, Agilent Technologies, 7890D GC
280 system) equipped with a fused silica capillary column (Agilent Technologies: 30 m, 0,250 mm
281 I.D, 0,25 µm). The injection was made at a constant pressure. The oven was programmed to
282 begin at an initial temperature of 140°C increasing to 170°C at a rate of 6.5°C / min, then to
283 200°C at a rate of 2.75°C / min for 14 min and finally the temperature rose to 230°C at a rate of
284 3°C / min for 12 min. FAMEs were identified by comparing chromatograms with standard
285 reference mixtures of 37 fatty acids, NHI-F, fatty acid methyl ester mix, PUFA NO.2, animal
286 source and fatty acid methyl esters kit (Sigma-Aldrich, Canada).

287 **2.8 Kidney metal analysis**
288
289 Metal analysis was performed by ICP-MS on the kidneys of all fish exposed to metal and
290 temperature combinations for this and another parallel study (Grasset et al., 2016) where
291 analytical procedures are detailed. Kidney was selected for metal analysis because other
292 analyses were prioritized for liver and muscle of these small fish in the larger project. Since in
293 contaminated yellow perch, kidney, liver and muscle concentrations are correlated with each
294 other (Couture et al., 2008; Pyle et al., 2005), kidney metal concentrations reflect accumulation

in other tissues, although most metals, including Cd and Ni, typically accumulate much more in fish kidney and liver than in muscle (Pannetier et al., Submitted; Pyle et al., 2005).

2.9 Calculation of indices and statistical analysis

The indices were calculated according to the following formulas:

Condition index **(CI)** =[Weight (g) / (Fish length (mm))^3]*100.

Hepatosomatic index **(HSI)** = (Liver weight (g) / Fish weight (g)) × 100

Peroxidation index **(PI)** = 0.025*(%monoenoics)+1*(%dienoics)+2*(%trienoics)+4*(% tetraenoics)+ 6*(% pentaenoics)+ 8*(% hexaenoics), where monoenoics, dienoics, trienoics, tetraenoics, pentaenoics and hexaenoics are fatty acids containing 1, 2, 3, 4, 5 and 6 double bonds, respectively (Almaida-Pagan et al., 2012).

Desaturase activities were estimated as the product/precursor ratios of individual fatty acids according to the following formulas: Δ9-desaturase = stearoyl-CoA-desaturase = [16:1 (n-7)/16:0] and [18:1 (n-9)/18:0], Δ5-desaturase = 20:5n3/20:4n3 and Δ6-desaturase = 20:4n3/18:3n3**.** The indices of elongase activity were calculated using (22:2n6/20:2n6) and (22:5n3/20:5n3) ratios.

Data were expressed as mean ± SE (n = 8) and statistical analyzes were made with JMP 11.0.0 (SAS Institute Inc.). Comparisons among mean values from different stressor combinations were performed using one-way analysis of variance (ANOVA). If the test showed significant differences and the data were normally distributed, a Tukey-Kramer HSD test was performed to determine the significance level (p-value). When data were nonparametric, a Wilcoxon / Mann-Whitney or Kruskal-Wallis test was applied. Subsequently, Spearman correlation was carried out to investigate correlations among the various parameters monitored. The interactions of temperature and metals on the main fatty acid classes were determined by two-way ANOVA. For this analysis, a variable termed “metal” was created that combined Cd and Ni exposed fish.

A principal component analysis (PCA) was performed to highlight relationships among tested parameters. Resulting biplots represented projections on extracted principal components and two-dimensional loadings of tested parameters as arrows. The parameter significance is represented by arrow length and direction relative to plotted components.

3. Results

3.1 Fish condition and metal contamination

Fish exposed to Ni at 9˚C accumulated this metal significantly in their kidney compared to controls and Ni accumulation was enhanced 4-fold at 28˚C compared to 9˚C (Table 1). In control fish, kidney Ni concentrations were low and not affected by temperature. Exposure to Cd also led to a significant kidney accumulation of this metal at both temperatures. Like for Ni, fish exposed to the higher temperature accumulated higher concentrations of Cd in their kidney. However, in contrast to Ni, kidney Cd concentrations were also higher in control fish at 28˚C compared to 9˚C. Indeed, kidney Cd concentrations in control fish at 28˚C were comparable to values measured in fish exposed to Cd at 9˚C.

In control fish, an increase of temperature did not affect the CI but strongly and negatively affected the HSI yielding a 3-fold decrease of relative liver size (Table 1). Neither Cd nor Ni affected the HSI. Although the CI of fish at 9˚C was not affected by metals, at 28˚C fish exposed to Ni expressed lower values of the CI. The HSI was positively correlated to the CI in fish from all conditions combined (Spearman correlation = 0.6025; $p<0.0001$; $n=48$).

3.2 Fatty acid composition of membrane phospholipids

Several differences were found in the fatty acid composition of muscle membrane phospholipids from fish exposed at 9°C and 28°C in the presence or absence of Cd or Ni (Table 2). The principal fatty acids of total membrane phospholipids from yellow perch muscle were 22:6n3>16:0>20:5n3>18:1n9≈18:2n6≈20:4n6>18:1n7≈18:0. The relative abundance of major (>1%) and minor (<1%) fatty acids was affected by both temperature and metals (Table 2). The slight increase of SFA at the warmer temperature was not significant (Table 3) and none of the individual SFA varied significantly with temperature (Table 2). In contrast, MUFA, dominated by 18:1n9, decreased at high temperature (Table 3). At 9°C under Cd and Ni exposure, SFA and MUFA increased compared to controls at 9°C although the Ni-induced increase in MUFA was not significant, while at 28°C neither SFA nor MUFA varied appreciably following metal exposure,

353 leading to a significant metal-temperature interaction (Table 3). The PI was lowest in control fish
354 acclimated to 28˚C, but highest in Ni-exposed fish at that same elevated temperature.
355 Moreover, total PUFA and n-3 PUFA, of which DHA (22:6n3) is the most important, were
356 strongly positively correlated to the PI (Table 4).

357 In all conditions, PUFA were more abundant than SFA and MUFA and were dominated by
358 20:5n3 and 22:6n3. This FA category varied in the opposite direction compared to SFA and was
359 affected by temperature and metals (Table 3). There were significantly higher proportions of
360 several individual n-3 and n-9 FA at 9˚C compared to 28˚C (Table 2). However, the two-way
361 ANOVA only identified a significant effect of temperature on n-9 PUFA, since metals strongly
362 affected n-3 PUFA composition but differently at each temperature, leading to a significant
363 metal-temperature interaction but masking the temperature effect (Table 3). Indeed, n-3 PUFA
364 (the family that dominated PUFA) was 1.5-fold lower at 28°C compared to 9°C when only
365 control fish were considered and the difference was significant (one-way ANOVA, data not
366 shown). In contrast, n-6 PUFA exhibited higher proportions at 28˚C and decreased in metal-
367 exposed fish at both temperatures, although only significantly at 9˚C. There was, however, no
368 significant temperature-metal interaction on n-6 PUFA (Table 3).

369 ### 3.3 Estimated activity of fatty acid desaturases and elongases

370 Estimated muscle D9D activity decreased significantly with an increase of temperature but it
371 was not affected by metal exposure (Table 5). Estimated D6D and D5D activities did not differ
372 between 9°C and 28°C. However, the presence of Ni and Cd had an effect on D6D: at 9°C, Cd
373 and Ni exposure induced an increase of this index compared to 9°C controls. The same was
374 observed for Ni exposure at 28°C, with a 3-fold increase compared to control fish at the same
375 temperature. The activity of the ELOVL2 elongase was significantly influenced by temperature,
376 expressing much lower values at 28˚C compared to 9˚C. Metals also affected ELOVL2 activity,
377 but in opposite directions at 9°C and 28°C, both Cd and Ni leading to strong decreases compared
378 to same-temperature controls at 9˚C, but Cd yielding a significant increase at 28˚C.

3.4 Antioxidant enzyme activities

Muscle CAT and SOD activities showed different responses to temperature compared to metal exposure. Catalase activity was much more affected by temperature than by metals. Its activity drastically decreased at 28°C compared to 9°C ($p<0.0001$; Figure 1). Slight but non-significant increases were observed under Cd and Ni exposure at both temperatures. In contrast to CAT, SOD activity was not affected by temperature ($p>0.5$; Figure 2). At 9°C, both Cd and Ni exposure decreased SOD activity. At 28°C, Ni exposure also decreased SOD activity, but Cd exposure increased it, yielding the highest muscle SOD activity of all conditions tested. Like SOD, muscle GPx activity was not affected by temperature (Figure 3). Interestingly, it reacted to metal exposure in opposite directions compared to SOD. At 9°C, under Cd and Ni exposure, GPx activity increased by roughly 25% relative to 9°C controls. At 28°C, it increased significantly under Ni exposure ($p=0.001$), to reach the highest values among experimental conditions. Like SOD and GPx, temperature did not affect GST activity (Figure 4). Although the enzyme was insensitive to metals at 28°C, at the colder temperature, its activity increased two-to three fold following exposure to either Cd or Ni.

3.5 Glutathione

Glutathione levels were strongly affected by temperature and were much higher at 28°C than at 9°C (Figure 5). The non-significant trend of decrease that can be observed in Ni-exposed fish at 9°C became strongly significant at 28°C. On the other hand, Cd did not affect at all GSH levels at either temperature.

3.6 Lipid peroxidation

The concentration of lipid peroxides, expressed in terms of MDA concentrations, was not significantly affected by temperature (Figure 6). Exposure to Cd did not affect lipid peroxidation at either temperature, but Ni exposure led to strong decreases of MDA concentrations at both temperatures ($p<0.01$ at 9°C and $p<0.001$ at 28°C). Indeed, in the whole dataset, muscle MDA concentration was strongly and negatively correlated to kidney Ni concentration (Table 6), MDA concentration was weakly, but negatively correlated with the PI.

3.7 Relationships among indicators of oxidative stress and antioxidant capacities

The activity of SOD displayed strong negative correlations with CAT, GST and GPx activities, but a positive correlation with GSH concentrations (Table 6). Catalase and GST activities were weakly positively correlated with each other, and the two enzymes negatively correlated with GSH concentrations. Likewise, glutathione peroxidase activity, which showed a positive correlation with GST activity, correlated negatively with GSH concentrations. There was no relationship between MDA concentration and any indicator of antioxidant capacities. The CI increased significantly with muscle CAT activity and MDA and GST concentrations, but was negatively correlated with GSH concentrations.

To facilitate interpretation of the relationships between antioxidant enzymes and lipid peroxidation and to define patterns of biomarker response to temperature and metal stress combinations, we performed a Principal Component Analysis (PCA; Figure 7). The analysis led to two relevant components that accounted for nearly 81% of the overall variability. The first principal component (PC 1) explained 55.9% of the total variance and was characterised firstly by the high loading of CAT and GST, correlated positively, and on the other hand by SOD and GSH, which correlated negatively. This first axis appeared to be associated to temperature specific responses, since fish from the two exposure temperatures were clearly separated along that axis, with fish exposed to 9˚C to the right, those exposed to 28˚C to the left, and very little overlap. This analysis highlights that high values of CAT, GST and GPx activities were related to low temperature, while elevated values of SOD activity and GSH concentrations were related to the high temperature condition. The second component explained 33.2% of total variance and MDA concentration showed the highest loading (0.97) on that axis. Principal component 2 (PC2) allowed a fair degree of separation of fish according to metal exposure conditions, with control fish associated to the top (positive) values of PC2, while Ni-exposed fish tended to gather at the opposite (negative) side, with intermediate values for Cd-exposed fish. Thus, the PCA supports that lower values of lipid peroxidation, as indicated by MDA concentrations, were found in Ni-exposed fish compared to Cd-exposed or control fish.

4. Discussion

4.1 Effects of temperature on membrane composition

Cell membranes of poikilotherms subjected to variations in temperature restructure their phospholipids to maintain cellular integrity. In our study, the proportion of UFA, including PUFA and MUFA, was higher in muscle phospholipids of fish acclimated to the colder temperature, in agreement with the general theory of homeoviscous adaptation (Hazel, 1972; Hazel et al., 1991). The major decrease of DHA in the muscle of warm-acclimated fish supports the role of this major fatty acid in the thermal response of cell membrane phospholipid composition. Previous studies have reported this observation and demonstrated that phospholipids containing 22:6n3 are important in controlling membrane fluidity at low temperatures (Logue et al., 2000; Tiku et al., 1996). More recently, Snyder et al. (2012) observed in freshwater alewives (*Alosa pseudoharengus*) a remodelling of polar lipids at cold temperatures. Unsaturated fatty acids (mainly DHA) greatly increased compared to SFA.

The remodelling of cell membrane fatty acid composition in response to ambient temperature variations involves desaturase and elongase enzymes, but direct measurement of their activities is difficult. In our study, we used product-to-precursor ratios as surrogate measures of desaturase and elongase indices (Cormier et al., 2014) to examine the contribution of these enzymes in temperature-induced membrane remodelling. The higher activity of D9D at 9°C compared to 28°C corresponded to higher proportions of MUFA and PUFA. This enzyme is one of key enzymes involved in the unsaturation of SFA, inserting double bonds into fatty acids at the C9-C10 position. Unsaturating fatty acids at this position confers a disordering effect within membranes which enhances fluidity (Barton and Gunstone, 1975). The ELOVL-2 elongase catalyzes the elongation of fatty acid chains leading to the formation of long chain fatty acids such as DHA. Its higher activity in our cold-acclimated fish likely also played a role in the increased proportions of UFA.

4.2 Effects of metals on membrane composition and interaction with temperature

Exposure to Cd and Ni modified the normal response of cell membrane phospholipid composition to temperature acclimation in the muscle of our yellow perch. Most importantly, the increase in PUFA in cold-acclimated fish compared to warm-acclimated fish was largely counteracted by exposure to Cd and Ni. In contrast, in metal-exposed warm-acclimated fish, muscle phospholipid PUFA content increased to levels comparable to those observed in cold-acclimated control fish. These modifications observed in metal-exposed fish generated two alternative hypotheses that our experimental design does not allow to tease apart conclusively: Either metals modify desaturase and elongase activities which results in changes in FA composition, or the metal modifies the properties of phospholipid bilayers and the response reflects a compensatory adjustment of its composition in order to preserve membrane function. Evidence from our data lends support to the latter hypothesis. For instance, the decrease of the estimated ELOVL2 elongase activity 9°C in Cd-exposed fish compared to controls at the same temperature corresponds to a decrease in PUFA content, which is dominated by DHA. Since this fatty acid is synthesized through two successive elongations (Tocher et al., 2003), our results could suggest that the decrease in PUFA following Cd exposure was the result of elongase inhibition in cold-acclimated fish. However, kidney Cd accumulation was higher in fish acclimated to 28˚C compared to fish at 9˚C. Hence, an enhancement of the direct inhibition of elongases and desaturases by Cd would be expected in warm-acclimated fish. Yet, in the latter fish, exposure to Cd yielded higher PUFA and DHA percentages compared to control fish at the same temperature and estimated elongase activity increased to levels similar to values calculated in cold-acclimated fish. Jones et al. (1987) reported a decrease of PUFA content on marine diatom (*Asterionella glacialis*) under Cd exposure, which they explained by an inhibition of the Δ6-desaturase caused by the formation of Cd complexes with thiol-containing enzymes involved in lipid biosynthesis. Our study does not support their hypothesis. Similarly, in our Ni-exposed fish, estimated Δ5-desaturase and ELOVL2 elongase activities decreased at 9°C whereas Δ6-desaturase increased at 28°C, which argues against a direct effect of metals on elongases and desaturases.

4.3 Influence of temperature and metals on lipid peroxidation and indicators of antioxidant capacities

In the present study, the correlations established between SOD, CAT and GPx activities support that these enzymes act jointly to prevent ROS-induced oxidative damage. The dismutation of superoxide anions by SOD generates hydrogen peroxide, which is converted to water and oxygen by CAT and GPx. The enzyme SOD represents the primary defense against excess ROS production (Halliwell and Gutteridge, 2007). In our study, Cd and Ni exposure modified muscle SOD activity, but temperature by itself did not, although, as discussed above, metal accumulation was stimulated at the higher temperature. Since metal-induced variations in muscle SOD activity differed between cold and warm-acclimated fish, this suggests an interaction between temperature and metal exposure on antioxidant defense mechanisms. Interestingly, in cold-acclimated fish, exposure to either Cd or Ni led to decreases in SOD activity. However, although the accumulation of Cd in warm-acclimated fish induced SOD activity and led to a positive correlation between kidney Cd concentration and muscle SOD activity, the reverse was observed for Ni. Hence, the response of SOD activity to Cd exposure was temperature-dependent, while the response to Ni exposure was not. In a parallel study under the same conditions (Grasset et al., 2016), warm-acclimated yellow perch exposed to Ni expressed a sharp increase of SOD activity in their liver, but Cd did not affect the hepatic activity of this enzyme. Hence, as also demonstrated earlier for wild perch (Pierron et al., 2009), the response of antioxidant defense mechanisms to metal contamination is tissue and metal-specific.

The strong positive correlation between muscle SOD activity and glutathione concentrations that we report here reveals an active upregulation of these components of the antioxidant system through complex differential responses to increases in temperature and metal exposure. The increase of GSH concentrations in perch acclimated to 28°C agrees with a study by Bagnyukovaa et al. (2007) on various tissues of goldfish (*Carassius auratus)* following an increase of temperature from 3°C to 23°C. Furthermore, our observation of a depletion of GSH concentrations in Ni-exposed fish is supported by earlier studies which attributed this phenomenon to either Ni-mediated ROS formation leading to GSH depletion, or to a direct

519 interaction of Ni with GSH leading the formation of Ni-GSH complexes (Misra et al., 1990;
520 Salnikow et al., 1994).

521 Like for SOD, in our study muscle GPx activity was not affected by temperature, but increased
522 following exposure to Cd and Ni. Others have demonstrated that GPx activity is stimulated by
523 metal exposure in toadfish (*Halobatrachus didactylus*) (Soares et al., 2008) and mussels (*Mytilus*
524 *galloprovincialis*) (Tsangaris et al., 2007). Furthermore, GPx activity has been considered to be
525 complementary to CAT activity to ensure optimal protection against oxidative stress by
526 scavenging H_2O_2 deriving from superoxide anion dismutation (Dabas et al., 2012; Halliwell and
527 Gutteridge, 1999). In all conditions combined, like for GPx, the activity of CAT in the muscle of
528 our perch was inversely correlated to SOD activity. Hence, contrary to our observations for SOD,
529 temperature strongly negatively affected muscle CAT activity, but the enzyme seemed little
530 affected by metals. The negative correlation between SOD and CAT illustrates the
531 complementary nature of these two components of the antioxidant system. Indeed, the
532 superoxide anion ($O2^{\cdot-}$) generated by SOD activity has been reported to have an inhibitory effect
533 on CAT activity (Kono and Fridovich, 1982).

534 The response of muscle GST activity to temperature and metals contrasted with the other
535 biomarkers of the antioxidant system measured. Although GST activity was not sensitive to
536 temperature, it responded to metal exposure by expressing sharp increases in activity, but only
537 in cold-acclimated fish. This enzyme has been shown by others to increase with thermal stress
538 and metal exposure in aquatic organisms. For example, Madeira et al. (2013) reported that GST
539 activity in the muscle of several estuarine fish species (*Diplodus vulgaris*, *Diplodus sargus*,
540 *Dicentrarchus labrax* and *Liza ramada*) increased with thermal stress. As for any other stressor,
541 the extent of response to heat stress clearly varies as a function of the intensity and duration of
542 the stressor. The lack of thermal response of GST activity in our study likely reflects interspecific
543 differences in thermal tolerance and exposure protocols. For metal exposure, Pretto et al.
544 (2011) reported an increase of GST activity in the liver of silver catfish (*Rhamdia quelen*)
545 exposed to different concentration of Cd for 7 and 14 days and Kubrak et al. (2012) presented
546 similar conclusions in goldfish *Carassius auratus* (spleen) exposed to three concentrations of Ni.

4.4 Influence of membrane composition on lipid peroxidation and indicators of antioxidant capacities

In the discussion below, we hypothesize that metal accumulation in whole kidney tissue is correlated to metal concentrations in the vicinity of the muscle phospholipid bilayers, which is likely given the correlations in metal concentrations among tissues reported in the literature for contaminated fish (see Section 2.8), and the co-variation of total tissue metal concentrations and concentrations measured in membrane-containing subcellular components (Couture et al., 2015; Rosabal et al., 2015).Temperature greatly affected kidney metal accumulation, with higher accumulation of both Cd and Ni at the elevated temperature suggesting a higher risk of toxicity. However, since the phospholipids in the cell membranes of warm-acclimated fish were less polyunsaturated, and hence less vulnerable to metal-induced lipid peroxidation, these two unrelated consequences of temperature acclimation could act in opposite directions, decreasing potential differences in metal-induced lipid peroxidation between the two acclimation temperatures. Yet, metal exposure affected the normal response of cell membrane phospholipid composition to temperature and the PI, an index reflecting the vulnerability of fatty acids to oxidation, indicated that the greater risk of membrane lipid peroxidation was found in warm-acclimated, Ni-exposed fish.

The concentration of MDA, the only direct measurement of lipid peroxidation in our study, was not affected by temperature, in agreement with the PI that did not differ between control fish acclimated to either temperature. However, exposure to both metals, but in particular to Ni, had a stronger influence on both MDA and PI in warm-acclimated compared to cold-acclimated fish, but surprisingly the two parameters varied in opposite directions. Hence, in Ni-exposed warm-acclimated fish, expressing a very high proportion of oxidation-prone PUFA as indicated by elevated PI values, lipid peroxidation was lowest. Indeed, PUFA with a high degree of unsaturation such as ARA (20:4n6), EPA (20:5n3) and DHA (22:6n3) exhibit a greater oxidizability compared to other membrane fatty acids (Cosgrove et al., 1987). For example, the oxidizability of DHA (six double bonds) is about 7.5 fold more important than that of 18:2n6 (two double bonds) (Holman, 1957). Here, warm-acclimated fish exposed to Ni accumulated the metal to high concentrations and this negatively affected their condition. The composition of their muscle membranes was modified by Ni exposure in such a way as to increase their vulnerability

to oxidative stress, but the opposite response was observed. Our study therefore suggests that under these conditions, the depletion of glutathione by ROS or through complexation with Ni combined with an increase of GPx activity overcompensated the risk of LPO. The metabolic cost of this overstimulation of the antioxidant response may be partly responsible for the lower condition of these fish.

5. Conclusion

Data from this study provide novel information about combined temperature and metal effects on fatty acid membrane composition, antioxidant defense system and lipid peroxidation. The higher PUFA content in the muscle cell membranes of cold-acclimated yellow perch allowing maintenance of membrane fluidity and function is consistent with the theory of homeoviscous adaptation. Our study also supports that cold acclimation of membrane composition results from modifications in the activity of key elongases and desaturases. However, under metal exposure, the normal response of cell membrane composition to thermal acclimation was reversed, either through a direct effect on elongase and desaturase activity or as a consequence of metal-induced modifications of membrane properties. Under most conditions studied, the antioxidant system did not prevent accumulation of MDA, suggesting that LPO is a normal occurrence in perch muscle. However, exposure to Ni and particularly at the warmer temperature appears to have triggered a massive response of the antioxidant system, leading to a substantial decrease of LPO in spite of the higher polyinsaturation of membrane lipids in these fish. Future studies should examine the implications of these observations for cell energetics and membrane function.

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Wang, M.H., Wang, G.Z., 2009. Biochemical response of the copepod *Tigriopus japonicus* Mori experimentally exposed to cadmium. Arch Environ Contam Toxicol 57, 707-717.**Figure 1:** Catalase (CAT) activity in yellow perch muscle among exposure conditions. Data expressed as mean ± SE (n=8). Means with different superscript letters differ significantly ($P \leq 0.05$).

Figure 2: Superoxide dismutase (SOD) activity in yellow perch muscle among exposure conditions. Data expressed as mean ± SE (n=8). Means with different superscript letters differ significantly ($P \leq 0.05$).

Figure 3: Glutathione peroxidase (GPx) activity in yellow perch muscle among exposure conditions. Data expressed as mean ± SE (n=8). Means with different superscript letters differ significantly ($P \leq 0.05$).

Figure 4: Glutathione-S-transferase (GST) activity in yellow perch muscle among exposure conditions. Data expressed as mean ± SE (n=8).
Means with different superscript letters differ significantly ($P \leq 0.05$).

Figure 5: Glutathione concentrations (mmol mg^{-1} of protein) in yellow perch muscle among exposure conditions. Data expressed as mean ± SE (n=8). Means with different superscript letters differ significantly ($P \leq 0.05$).

Figure 6: Lipid peroxidation level expressed as MDA concentration in yellow perch muscle among exposure conditions. Data expressed as mean ± SE (n=8). Means with different superscript letters differ significantly ($P \leq 0.05$).

Figure 7: Principal component analysis (PCA) of antioxidant defense enzymes activities and lipid peroxidation in yellow perch muscle among the different conditions. Each point represents an individual fish. ● 9˚C; ▲9˚C+Cd; ■ 9˚C+Ni; ○ 28˚C; △ 28˚C+Cd; □ 28˚C+Ni.

Figure 1

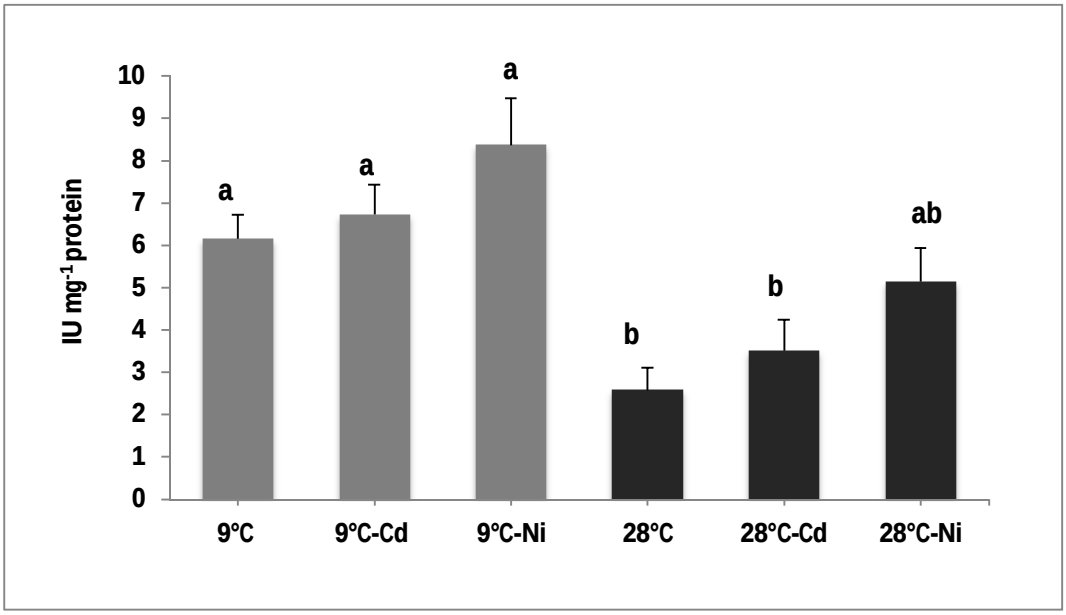

Figure 2

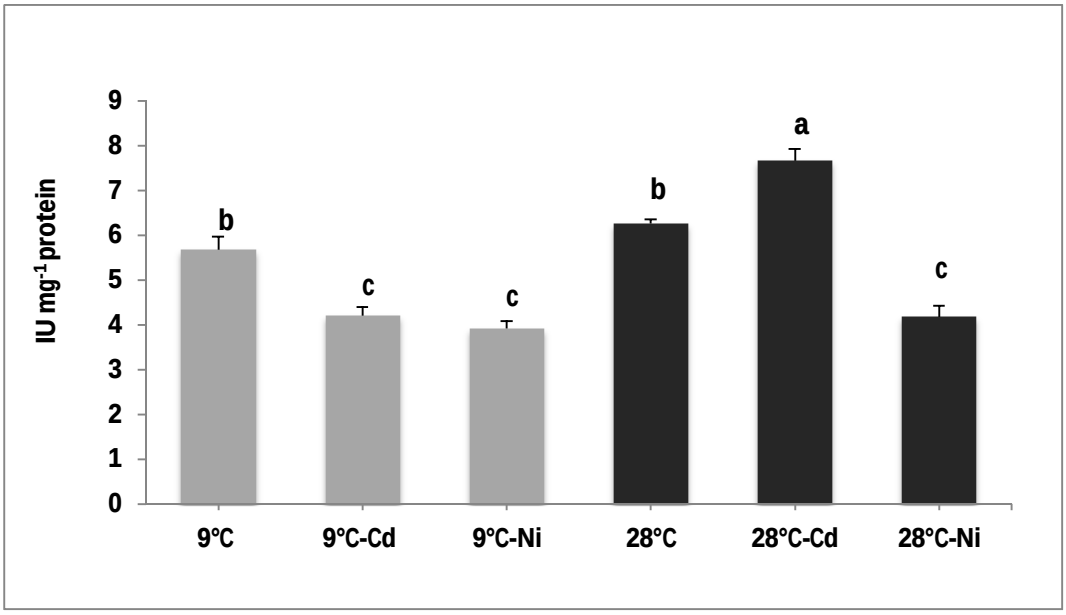

Figure 3

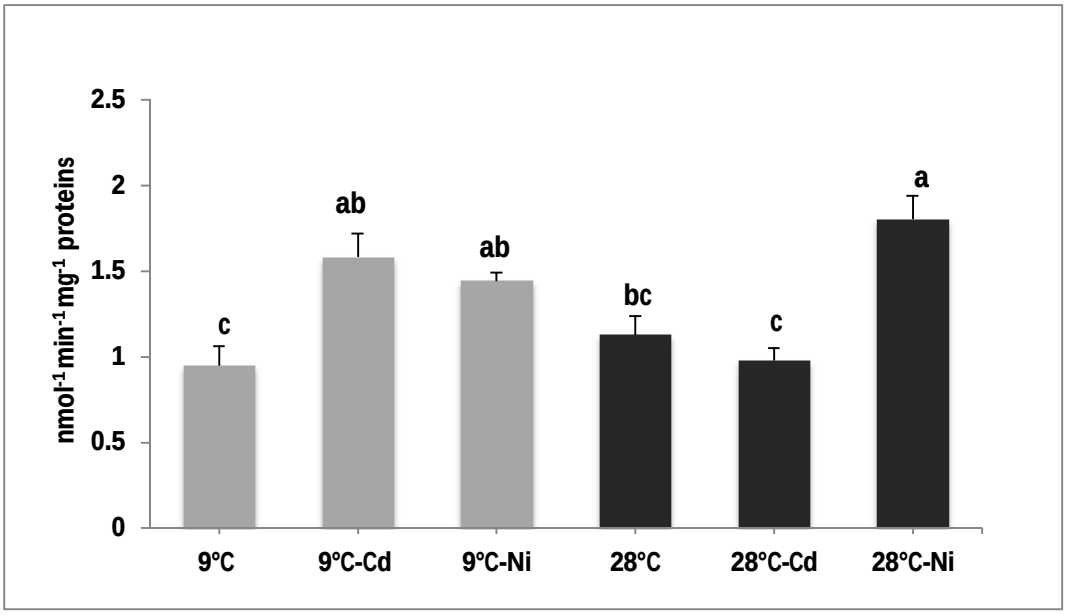

Figure 4

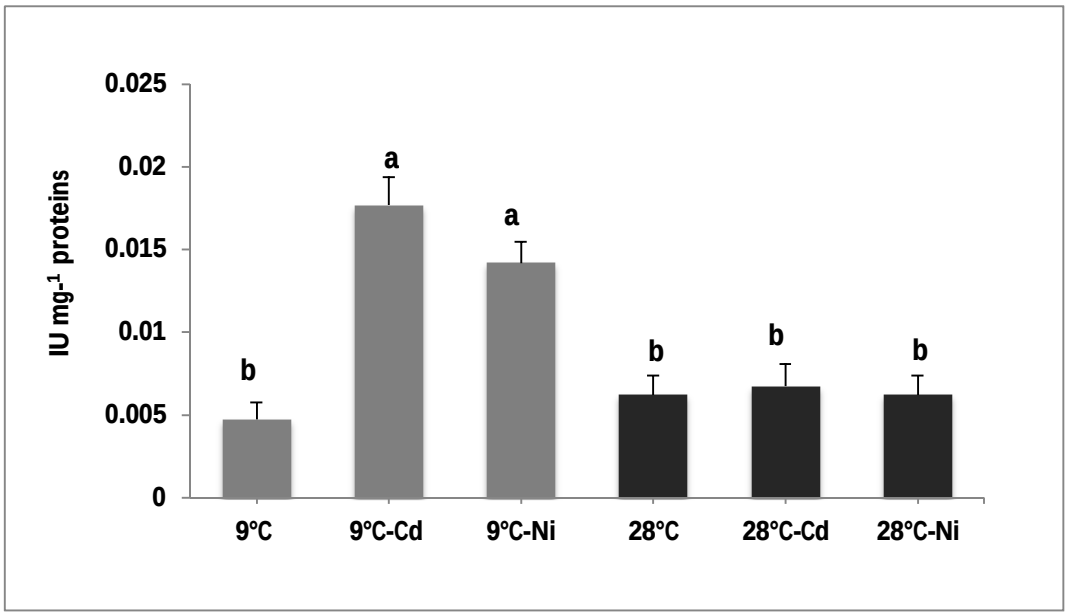

Figure 5

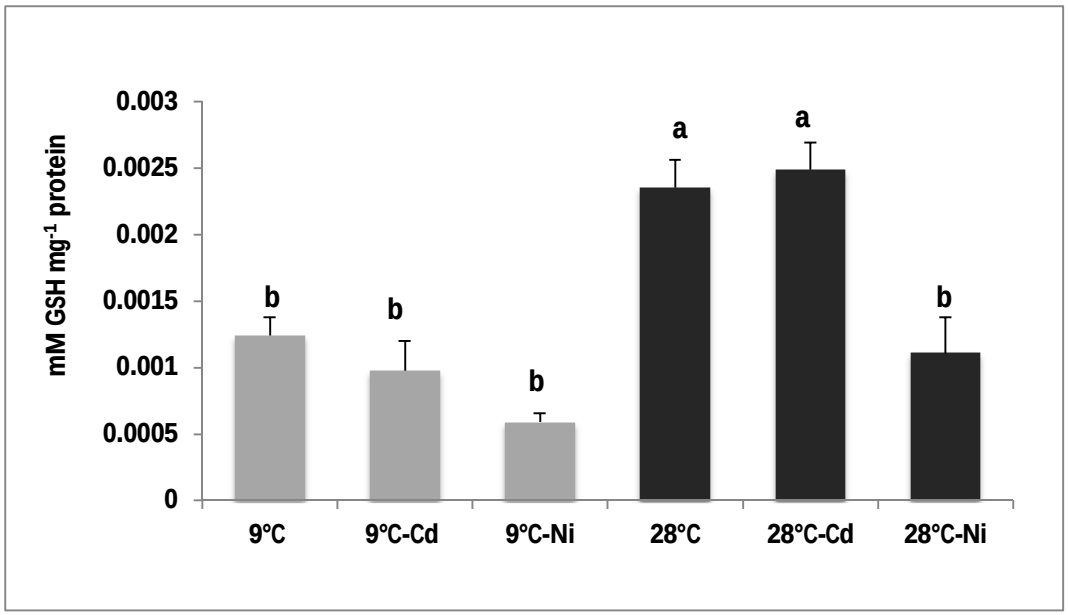

Figure 6

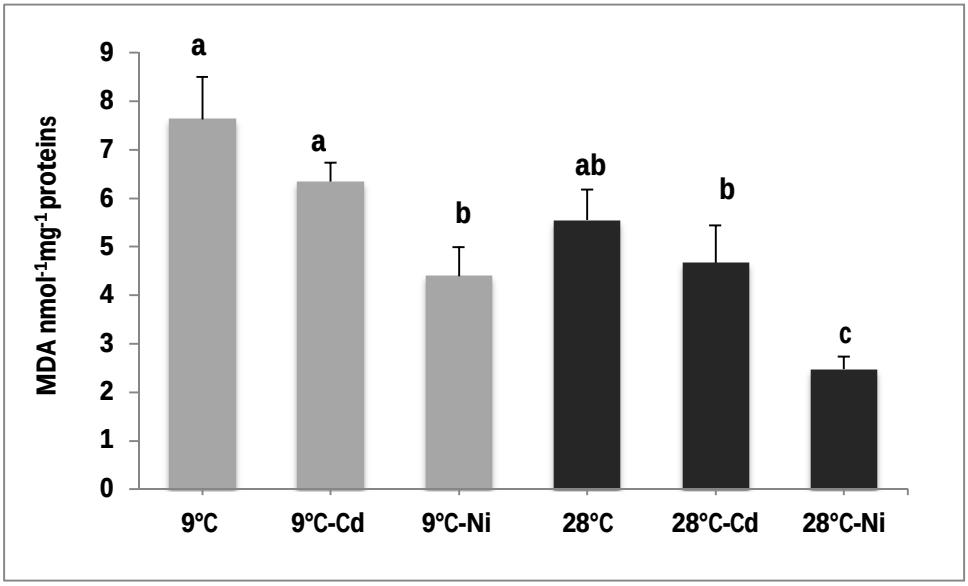

Figure 7

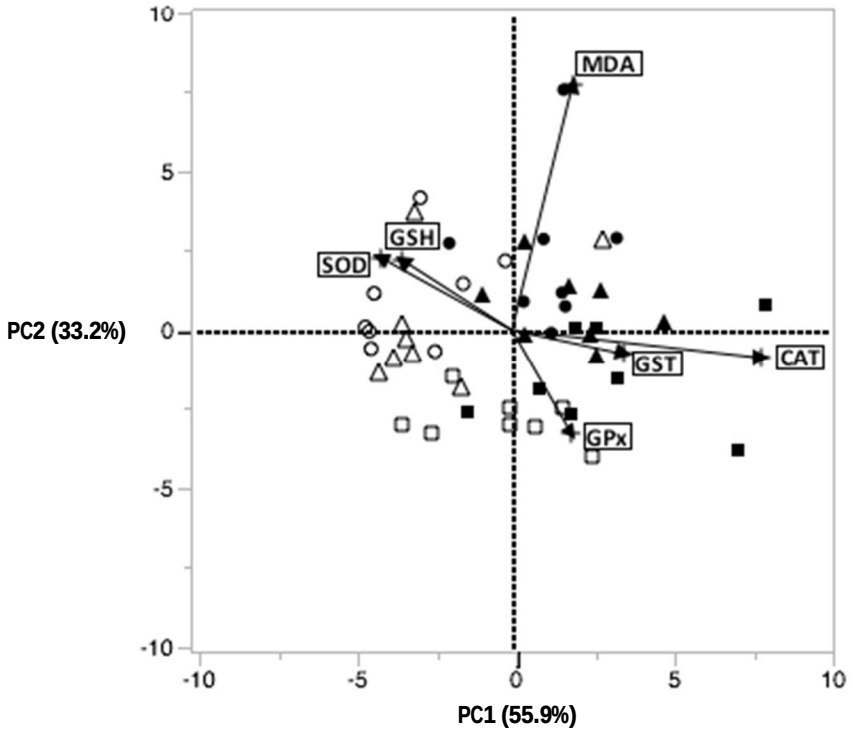

Table 1: Mean of Ni and Cd concentration (μg g^{-1} dry weight) in yellow perch kidney, hepatosomatic index and condition index (mean ± SE; n=8). Means with different superscript letters differ significantly as determined by one-way ANOVA ($p \leq 0.05$).

	9°C	9°C+Cd	9°C+Ni	28°C	28°C+Cd	28°C+Ni
Kidney Ni	0.43 ± 0.03^{c}	0.28 ± 0.07^{c}	6.11 ± 0.47^{b}	0.67 ± 0.05^{c}	0.31 ± 0.01^{c}	23.52 ± 1.33^{a}
Kidney Cd	0.47 ± 0.08^{c}	1.97 ± 0.12^{b}	0.31 ± 0.02^{c}	2.37 ± 0.11^{b}	5.31 ± 0.74^{a}	1.46 ± 0.05^{bc}
HSI	2.30 ± 0.14^{a}	2.45 ± 0.31^{a}	2.27 ± 0.10^{a}	0.72 ± 0.04^{b}	0.63 ± 0.03^{b}	0.76 ± 0.05^{b}
CI	1.04 ± 0.02^{ab}	1.02 ± 0.01^{a}	1.02 ± 0.01^{a}	0.91 ± 0.02^{bc}	0.86 ± 0.02^{bc}	0.82 ± 0.02^{d}

Table 2: Fatty acid composition (percentage of total fatty acids) of total phospholipids in yellow perch muscle from each exposure condition (mean ± SE; n=8). Means with different superscript letters differ significantly as determined by one-way ANOVA ($p \leq 0.05$).

	9°C	9°C+Cd	9°C+Ni	28°C	28°C+Cd	28°C+Ni
C14:0	0.9±0.2^{bc}	1.7±0.16^{a}	1.6±0.3^{ab}	1.6±0.7^{ac}	0.4±0.4^{c}	0.7±0.2^{c}
C15:0	0.04±0.04^{ab}	0.2±0.08^{a}	0.15±0.08^{ab}	0.08±0.05^{ab}	1.02±0.67^{ab}	0.00±0.00^{b}
C16:0	13±0.6^{b}	15.9±0.9^{a}	15.8±0.3^{a}	12.7±1.2^{b}	13 ±0.7^{b}	14.2±0.9^{b}
C17:0	0.09±0.06^{ab}	0.3±0.07^{a}	0.3±0.08^{a}	0.1±0.07^{ab}	0.0±0.0^{b}	0.08±0.06^{ab}
C18:0	2.7±0.3^{b}	2.9±0.3^{ab}	3.7±0.1^{ab}	3.6±0.4^{ab}	4.3±0.4^{a}	4.2±0.4^{a}
C22:0	0.8±0.3^{ab}	0.1±0.1^{ab}	0.0±0.0^{b}	1.6±0.3^{a}	1.3±0.6^{ab}	1.2±0.5^{ab}
C24:0	0.2±0.2^{ab}	0.0±0.0^{b}	0.0±0.0^{b}	0.9±0.3^{a}	0.0±0.00^{b}	0.08±0.08^{b}
C14:1n5	0.0±0.0^{b}	7.7±3.2^{a}	1.8±0.71^{b}	0.2±0.1^{b}	0.0±0.0^{b}	0.0±0.0^{b}
C16:1n7	1.8±0.1	1.5±0.1	1.5±0.07	1.4±0.2	1.8±0.2	1.2±0.2
C17:1n9	1.2±0.1^{b}	1.1±0.1^{a}	1.3±0.2^{b}	2.01±0.2^{ab}	2.9±0.5^{b}	1.8±0.5^{ab}
C18:1n7	5.9±1.2^{a}	1.8±0.7^{ab}	1.9±1.2^{ab}	4.9±0.9^{b}	3.1±1.01^{ab}	4.3±0.9^{ab}
C18:1n9	7.8±1.2	7.3±1.03^{abc}	8.2±0.4^{a}	5.7±0.9^{c}	6.05±1.01^{bc}	5.7±0.9^{c}
C20:1n9	0.6±0.1^{a}	0.5±0.1^{ab}	0.7±0.1^{a}	0.1±0.07^{c}	0.0±0.0^{c}	0.2±0.1^{bc}
C22:1n9	0.0±0.0	0.0±0.0	0.03±0.03	0.0±0.0	0.0±0.0	0.04±0.04
C18:2n6	5.8±0.3^{a}	4.9±0.4^{ab}	6.03±0.4^{a}	5.6±0.9^{ab}	3.6±0.4^{ab}	3.5±0.2^{b}
C20:2n6	0.1±0.08	0.0±0.0	0.0±0.0	0.08±0.08	0.0±0.0	0.2±0.1
C18:3n6	0.0±0.0	0.0±0.0	0.09±0.01	0.09±0.01	0.0±0.0	0.0±0.0
C18:3n3	1.9±0.4^{a}	1.4±0.3^{ab}	2.1±0.3^{a}	1.01±0.5^{ab}	0.2±0.2^{b}	0.5±0.2^{b}
C18:4n3	0.3±0.1^{a}	0.04±0.04^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
C20:4n6	3.9±0.3^{b}	3.5±0.3^{b}	4.3±0.3^{ab}	6. 9±1.6^{a}	4.4±0.7^{ab}	6±0.3^{ab}
C20:4n3	0.7±0.2^{ab}	1.2±0.1^{a}	1.3±0.07^{a}	0.3±0.1^{b}	0.3±0.2^{b}	0.5±0.2^{b}
C22:4n6	7.7±2.5^{a}	0.4±0.2^{b}	0.9±0.09^{b}	10±4.5^{a}	12±4.4^{abc}	4.8±1.6^{a}
C20:5n3	9.7±0.7^{ab}	9.2±0.8^{ab}	9.5±0.5^{ab}	7.6±1.08^{b}	8.8±0.6^{ab}	10.8±0.4^{a}
C22:5n3	2.9±0.3^{a}	2.8±0.2^{ab}	2.9±0.2^{ab}	1.7±0.3^{bc}	1.07±0.4^{c}	2.5±0.3^{ab}
C22:6n3	28.7±2.8^{ab}	24.9±1.5^{bc}	25.2±2^{bc}	20.3±2.6^{c}	26.1±2.4^{abc}	34.9±1.4^{a}
PI	330.48±13.07^{bc}	292.57±5.56^{bc}	329.44±8.19^{bc}	289.9±12.19^{c}	324.41±13.43^{b}	399.05±6.71^{a}

1062 **Table 3**: Fatty acid groups of membrane phospholipids in yellow perch muscle from each exposure condition (mean ± SE; n=8). Means
1063 with different superscript lowercase letters (9°C) or uppercase letters (28°C) differed significantly as determined by one-way ANOVA ($p \leq$
1064 0.05). The significance of combined effects of temperature and metal exposure as determined by two-way ANOVA is also reported, with
1065 F values in bold representing significant effects of temperature, metal exposure (Cd and Ni combined) or their interaction.

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	9°C			28°C			Two-way ANOVA		
	Control	**Cd**	**Ni**	**Control**	**Cd**	**Ni**	**Temperature**	**Metal**	**Interaction**
SFA	17.7±0.5 ^{b}	21.2±1.02 ^{a}	21.7±0.3 ^{a}	20.6±1.6 ^{A}	19.7±0.8 ^{A}	20.5±0.7 ^{A}	0.9387	0.138	0.0504
MUFA	12.9±1.2 ^{b}	20.4±2.3 ^{a}	14.6±0.7 ^{ab}	10.7±0.4 ^{A}	11.3±1.0 ^{A}	12.2±2.1 ^{A}	**0.0008**	**0.0418**	**0.0498**
PUFA	62±1.7 ^{a}	47.6±3.03 ^{b}	52.4±2.5 ^{b}	48.2±4.8 ^{B}	56.7±1.4 ^{AB}	63.8±1.6 ^{A}	0.3567	0.1409	**0.0001**
n-3 PUFA	44.4±3.6 ^{a}	40.7±2.6 ^{a}	41.2±2.3 ^{a}	28 ±2.1 ^{B}	36.7±3.1 ^{B}	49.6±1.9 ^{A}	0.1422	**0.0241**	**0.0023**
n-6 PUFA	17.5±1.3 ^{a}	8.8±0.7 ^{b}	9.3±0.8 ^{b}	23.3±3.5 ^{A}	19.8±3.3 ^{A}	14.5±1.3 ^{A}	**0.0006**	**0.0031**	0.4361
n-9 PUFA	8.5±0.5 ^{a}	7.8±0.6 ^{a}	8.7±0.3 ^{a}	5.9±0.6 ^{A}	6.1±0.4 ^{A}	5.9±0.2 ^{A}	**<.0001**	0.7283	0.5534

Table 4: Spearman correlations among fatty acid groups and related indices in yellow perch muscle from the various exposure conditions combined (n=48). Numbers in bold indicate significant correlations. Asterisks denote the significance level of the correlations: *0.05; **0.01; ***0.001.

	SFA	MUFA	PUFA	PI	n-3 PUFA	n-6 PUFA	n-9 PUFA
MUFA	0.0368	1					
PUFA	0.0526	-0.2253	1				
PI	0.0422	-0.2104	**0.5819*** **	1			
n-3 PUFA	0.0105	0.0582	**0.6135*** **	**0.3149*** **	1		
n-6 PUFA	-0.3579	**-0.3433** **	**-0.0737***	-0.0539	-0.5667	1	
n-9 PUFA	0.2531	**0.239*** **	0.309	0.0757	**0.3214***	-0.358	1
Ni	0.0877	**-0.1506***	0.365	0.3487	**0.4077***	-0.1459	-0.1836
Cd	0.0404	-0.0961	**-0.0669***	-0.0553	-0.2596	0.2957	**-0.3466*** **

Table 5: Estimated fatty acid desaturase and elongase activities in yellow perch muscle from each exposure condition (mean ± SE; n=8; n.c. = not calculated).

	9°C	9°C+Cd	9°C+Ni	28°C	28°C+Cd	28°C+Ni
D9D	3.02±0.2^{a}	2.7±0.34^{a}	2.2±0.3^{a}	1.6±0.5^{b}	1.4±0.1^{b}	1.4±0.1^{b}
D5D	10.1±0.7^{a}	8.4±1.6^{a}	7.1±0.4^{b}	9.5±2.6^{ab}	9.1±0.4^{a}	14.2±2.7^{a}
D6D	0.4±0.1^{b}	0.8±0.1^{a}	0.7±0.07^{a}	0.3±0.2^{b}	n.c	0.9±0.3^{a}
ELOVL2	1.3±0.3^{a}	0.09±0.04^{b}	0.2±0.02^{b}	0.5±0.1^{b}	1.6±0.7^{a}	0.9±0.3^{ab}
ELOVL2'	0.3±0.02^{ab}	0.3±0.02^{a}	0.3±0.01^{abc}	0.2±0.03^{abc}	0.1±0.04^{bc}	0.2±0.02^{c}

D9D: Δ9-desaturase (stearoyl-CoA-desaturase, 18:1n9/18:0); **D5D:** Δ5-desaturase (20:5n3/20:4n3)**; D6D:** Δ6-desaturase (20:4n3/18:3n3)**; ELOVL2:** Elongase 2 (22:4n6/20:4n6)**; ELOVL2':** Elongase 2' (22:5n3/20:5n3)

Table 6: Spearman correlations among biometric (CI), biochemical (SOD, CAT, GST, GSH and MDA) variables and kidney Cd and Ni concentrations in yellow perch muscle from the various exposure conditions combined (n=48). Numbers in bold indicate significant correlations. Asterisks denote the significance level of the correlations: *0.05; **0.01; ***0.001.

	SOD	**CAT**	**GPx**	**GST**	**GSH**	**MDA**	**PI**	**CI**
CAT	**-0.4363****	1						
GPx	**-0.5281*****	0.2268	1					
GST	**-0.4737***	**0.4121***	**0.2043****	1				
GSH	**0.6989*****	**-0.3997****	**-0.265***	**-0.3697****	1			
MDA	0.1459	-0.0484	-0.2522	-0.0277	0.1132	1		
PI	0.1427	-0.1275	0.1471	-0.3362	0.0504	**-0.2557***	1	
CI	-0.1914	**0.4615*****	-0.2117	**0.5337*****	**-0.3853*****	**0.2904****	-0.2251	1
Cd	**0.6136*****	**-0.4275*****	-0.3068	-0.1411	**0.5661*****	-0.0818	-0.0553	**-0.2317***
Ni	**-0.4439****	0.0912	**0.5699****	-0.1443	**-0.2565***	**-0.5349*****	0.3487	**-0.4663*****