Temperature and metal exposure affect membrane fatty acid composition and transcription of desaturases and elongases in fathead minnow muscle and brain

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Abbreviations

ARA	Arachidonic acid
Cd	Cadmium
CI	Condition index
DHA	Docosahexanoic acid
EPA	Eicosapentanoic acid
FA	Fatty acid
FADS	Fatty acid desaturases
HSI	Hepatosomatic index
HVA	Homeoviscous adaptation
LC-PUFA	Long chain polyunsaturated fatty acid
MUFA	Monounsaturated fatty acid
Ni	Nickel
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid

Abstract

In this study, we tested the hypothesis that metal exposure affected the normal thermal response of cell membrane FA composition and of elongase and desaturase gene transcription levels. To this end, muscle and brain membrane FA composition and FA desaturase (*fads2*, *degs2* and *scd2*) and elongase (*elovl2*, *elovl5* and *elovl6*) gene transcription levels were analysed in fathead minnows (*Pimephales promelas*) acclimated for eight weeks to 15, 25 or 30°C exposed or not to cadmium (Cd, 6 µg/l) or nickel (Ni, 450 6 µg/l). The response of membrane FA composition to temperature variations or metal exposure differed between muscle and brain. In muscle, an increase of temperature induced a decrease of polyunsaturated FA (PUFA) and an increase of saturated FA (SFA) in agreement with the current paradigm. Although a similar response was observed in brain between 15 and 25˚C, at 30˚C, brain membrane unsaturation was higher than predicted. In both tissues, metal exposure affected the normal thermal response of membrane FA composition. The transcription of desaturases and elongases was higher in the brain and varied with acclimation temperature and metal exposure but these variations did not generally reflect changes in membrane FA composition. The mismatch between gene transcription and membrane composition highlights that several levels of control other than gene transcription are involved in adjusting membrane FA composition, including post-transcriptional regulation of elongases and desaturases and *de novo* phospholipid biosynthesis. Our study also reveals that metal exposure affects the mechanisms involved in adjusting cell membrane FA composition in ectotherms.

Key words: cell membranes; temperature; metals; fatty acids; desaturases; elongases

1. Introduction

Cell membranes are highly sensitive to temperature, affecting their physical properties and consequently the functioning of embedded proteins (Hochachka and Somero, 2002). Under cold temperatures, the overall packing order of membrane phospholipids increases, causing a decrease of membrane fluidity. At the opposite, an increase of temperature induces phospholipid disorder and enhances fluidity. To counteract temperature effects, poikilotherms remodel membrane phospholipid fatty acid (PLFA) composition, a process known as homeoviscous adaptation (Hazel and Williams, 1990; Henderson et al., 1995; Sinensky, 1974; Wodtke and Cossins, 1991).

Long chain polyunsaturated fatty acids (LC-PUFA) such as arachidonic acid (ARA, 20:4n-6), eicosapentanoic acid (EPA, 20:5n-3) and decosahexanoic acid (DHA, 22:6n-3) are essential for cell functioning. As major constituents of membrane phospholipids, they control membrane fluidity and influence protein activity and membrane function (Hashimoto et al., 2006; Horrocks and Farooqui, 2004; Stillwell and Wassall, 2003). In addition, DHA and ARA have an important role in neural function and development (Innis et al., 1999; Salem et al., 2001). Two groups of enzymes, desaturases and elongases, are responsible for the regulation of membrane PLFA composition following changes in temperature (Hazel and Livermore, 1990; Trueman et al., 2000b). The biosynthesis of LC-PUFA from 18:2n-6 and 18:3n-3 involves desaturases and elongases. Desaturases incorporate double bonds at a specific position of the acyl chain (Guillou et al., 2010) and can be divided into two families: steroyl-CoA desaturases (SCD) and fatty acid desaturases (FADS) (Marquardt et al., 2000). Elongases catalyze the elongation process by inserting 2 carbons at a time (Jakobsson et al., 2006). Recently, a variety of desaturases and elongases involved in the PUFA biosynthetic pathway have been cloned and numerous desaturase families have been identified in marine and freshwater species (Tocher et al., 2006; Zheng et al., 2004). The Δ6 FADS2 desaturase appears to be more common compared to the Δ5 FADS2 desaturase. Desaturases vary among species. In Atlantic salmon, a unifunctional Δ5 FADS2 desaturase has been reported, while in zebrafish (*Danio rerio*), rabbitfish (*Siganidae Siganus*) and pike silverside (*Chirostoma*

estor) three bifunctional Δ6/Δ5 FADS2 desaturases have been identified (Fonseca-Madrigal et al., 2014; Hastings et al., 2004). Regarding elongases, the first that has been reported, ELOVL5, was characterised in zebrafish (*Danio rerio*) (Agaba et al., 2004) and subsequently in several other species (Agaba et al., 2005; Monroig et al., 2013). As for ELOVL2, to date it has been reported in many species, such as Atlantic salmon, rainbow trout and zebrafish (Gregory and James, 2014; Monroig et al., 2009; Monroig et al., 2013). The extent to which fish can convert 18:2n6 and 18:3n3 to LC-PUFA varies among species and depends on their assemblages of desaturase and elongase enzymes. Palmitic acid (16:0) and stearic acid (18:0) are converted to 16:1n7 and 18:1n9 by SCD, that performs a desaturation at the Δ9 position of these fatty acids (Guillou et al., 2010). Since they do not possess Δ12 or Δ15 desaturases to desaturate 18:1n9 to 18:2n6 (LOA) and then to 18:3n3 (ALA), fish need to acquire these essential fatty acids through food. Then, LOA and ALA are converted to LC-PUFA through a series of enzymatic reactions (Fig. 1). DHA can be synthesized by two pathways. In the first one, often referred to as the "Sprecher shunt pathway", EPA undergoes two elongations to obtain 24:5n-3 followed by a Δ6 desaturation and a chain shortening (Sprecher, 2000). The second one is more direct and it involves Δ4 desaturation of 22:5n-3 (Li et al., 2010). It was long considered that vertebrates produced DHA from EPA only via the Sprecher shunt pathway and did not possess a Δ4 desaturation step, but the existence of an alternative pathway for DHA production from EPA via direct Δ4-desaturation has been recently demonstrated (Li et al., 2010). Once produced, PUFA are incorporated into membrane phospholipids by specific acyltransferases. It appears that freshwater fish have the enzymatic capacity to perform LC-PUFA biosynthesis (Agaba et al., 2005; Hastings et al., 2004; Morais et al., 2009) while marine fish exhibit low activity of desaturases and elongases such as Δ5 FADS2 desaturase and ELOVL2 elongase (Morais et al., 2012; Tocher et al., 2006). This difference may be explained by the higher abundance of LC-PUFA in marine compared to freshwater food webs. In marine ecosystems, the higher availability of LC-PUFA may have induced the loss of biosynthetic capacities for LC-PUFA

153 in fish, while in contrast their lower availability in freshwater food webs may be the
154 responsible for the persistence of desaturases and elongases (Leaver et al., 2008).
155 The effects of temperature on metal uptake in aquatic organisms have been
156 abundantly studied and consistently reported to increase with increasing temperature
157 (Cherkasov et al., 2007; Grasset et al., 2016; Mubiana and Blust, 2007; Nichols and
158 Playle, 2004). Several studies have also investigated the effects of variations in
159 acclimation temperature on cell membrane PUFA composition and consistently
160 reported that cold acclimation yields to an increase in cell membrane polyunsaturation
161 (Grim et al., 2010; Hazel, 1995; Hazel and Williams, 1990; Kraffe et al., 2007). Metal
162 exposure may also alter membrane structure by stimulating lipid peroxidation, a
163 complex sequence of reactions leading to the oxidation of polyunsaturated fatty acids
164 (Ramanathan et al., 1994; Viarengo et al., 1990). Since susceptibility to lipid
165 peroxidation increases with membrane unsaturation, cold acclimated fish may be more
166 vulnerable to lipid peroxidation.
167 To our knowledge, our previous study on yellow perch (Fadhlaoui and Couture,
168 2016) was the first to investigate the combined effects of temperature and metal
169 exposure (Cd and Ni) on membrane fatty acid composition. We selected Cd and Ni since
170 these metals are found in elevated concentrations in many Canadian areas subjected to
171 metal mining and smelting (Couture et al., 2008; Pyle and Couture, 2011). In the present
172 study, we examined the response of cell membrane PLFA to the same stressors in
173 another species of freshwater fish which, in contrast to yellow perch, has a clear
174 preference for warmer water (Hasnain et al., 2010), hence providing an interspecific
175 comparison. The transcription level of genes encoding for desaturases and elongases
176 has been studied in freshwater species in response to temperature acclimation but
177 never to our knowledge in fish exposed to a combination of temperature and metal
178 stresses, a question that is particularly relevant since we have shown in yellow perch
179 that metal exposure modifies the response of cell membrane PLFA to temperature
180 acclimation (Fadhlaoui and Couture, 2016). The objectives of our study are *i)* to provide
181 an interspecific comparison of temperature and metal induced modifications in muscle

and brain cell membrane PLFA; *ii)* to investigate the response of gene transcription levels of desaturases (*fads2*, *degs2* and *scd2*, encoding respectively for Δ5/6 desaturase, Δ4 desaturase and stearoyl-CoA desaturase (Δ9 desaturase)) as well as elongases (*elovl2*, *elovl5* and *elovl6*) encoding respectively ELOVL2, ELOVL5 and ELOVL6 to metal exposure and variations in temperature; and *iii*) to investigate the relationships between the cell membrane PLFA composition of different tissues and desaturase and elongase transcription levels.

2. Materials and methods

2. 1 Fish and experimental design

Adult fathead minnows (*Pimephales promelas*) were obtained from Aquatic Research Organisms (ARO, Hampton (NH), USA). In a temperature-controlled room at the INRS-ETE, fish were acclimated for two weeks to laboratory conditions at 25°C with a 16 h light and 8 h dark photoperiod. During this period, fish were fed daily with frozen brine shrimp (*Artemia salina*). After this acclimatization period, 22 fish were placed in each of nine 45L aquaria to start experimental conditions. Temperature was reduced (in 3 aquaria) or raised (in 3 aquaria) at a rate of 2°C per day to reach a low (15°C) and a high temperature (30°C) while the last three aquaria were maintained at 25˚C. For each temperature, one aquarium was used as a control (uncontaminated), a second aquarium was contaminated by Cd and the last with Ni. Physico-chemical parameters (pH, temperature, nitrites and nitrates) were controlled daily. Aquarium water was renewed twice a week with reconstituted water ([Ca^{2+}] 70 µM, [Cl^{-}] 129 µM, [K^{+}] 12 µM, [Mg^{2+}] 13 µM, [Na^{+}] 179 µM, [$SO4^{2-}$] 63 µM). When desired temperatures were reached, metal exposure was begun by the addition of Ni or Cd stock solutions to the water of one aquarium at each temperature. Values in Cd aquaria were 5.7 ± 0.35 µg/L (n=59, corresponding to the number of water samples collected during the exposure period in Cd-contaminated aquaria) and 456 ± 14 µg/L (n=59) in Ni aquaria. These concentrations were chosen to reflect aqueous concentrations commonly found in Canadian aquatic environments influenced by mining activities (Campbell et al., 2003). Metal

concentrations were analysed with ICP-MS after every water change to correct and maintain the desired concentration. At the end of the exposure period (8 weeks), fish were sacrificed by a blow to the head. Biometric measures (length and weight) were taken, then fish were dissected. Six fish were randomly selected for fatty acid analysis in muscle and brain as well as muscle gene transcription. As fathead minnow brain was very small, a second group of six fish was selected to perform gene transcription analysis. For fatty acid analysis, muscle and brain samples were stored at −80°C. For quantitative real time PCR (RT-qPCR) measurements, samples were stored in tubes filled with RNAlater. All procedures on fish were approved by our institutional animal care committee.

2. 2 Lipid extraction and phospholipid fatty acid analysis

Total lipids were extracted from 100 mg muscle and whole brains according to Folch et al. (1957). The detailed procedure for lipid extraction and membrane fatty acids composition was described in Fadhlaoui and Couture (2016). Total lipids were separated into polar and neutral lipids. The fraction containing polar lipid was then esterified to obtain fatty acid methyl esters (FAME). The resultant FAMEs were analyzed by gas chromatography- flame ionization detector (GC-FID) and the relative FAME content was determined by comparing chromatograms with reference standards (mixtures of 37 fatty acids, NHI-F, fatty acid methyl ester mix, PUFA NO.2, animal source and fatty acid methyl esters kit (Sigma-Aldrich, Canada)).

2. 3 Gene transcription level analyses

In order to obtain the sequences coding for *fads2*, *degs2*, *scd2*, *elovl2*, *elovl5* and *elovl6* in *P. promelas*, we first searched these sequences in the well-described transcriptome of *Danio rerio*. Then, these sequences were blasted against the genome of *P. Promelas* (WGS JNCD01) using Blast algorithm and BioEdit software. The obtained sequences were then blasted against the NR database. For each gene, specific primers

were determined from the most conserved regions (Blast results are given in Appendix 1) using the Primer3plus software.

Total RNA was extracted from muscle and brain using Absolutely RNA RT-PCR Miniprep Kit (Agilent) according to the manufacturer's instructions. Tissues were weighed (20 mg muscle and the whole brain) and homogenized in a RNA lysis buffer using a tissue homogenizer (MP Fastprep) for 40s. For each sample, RNA quality was evaluated by electrophoresis on a 1% agarose gel and concentrations as well as purity were determined by spectrophotometry (Nanodrop 8000). Then, first-stand cDNA was synthesised from 400 ng of total brain or muscle RNA using GoScript Reverse Transcription System (Promega) according to manufacturer's instructions. Following the reverse transcriptase reaction, cDNA was diluted 6-fold for the muscle and 10-fold for the brain. Real-time PCR reactions were then performed in an MX3000P (Stratagene; 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s and 72°C for 30 s). Each 20 µL reaction contained 12.5 µL of GoTaq qPCR master mix (Promega), 5 µL template and the specific primer pairs at a final concentration of 250 nM each. The reaction specificity was determined for each reaction by gel electrophoresis and from the dissociation curve of the PCR product. This was obtained by following the SyberGreen fluorescence level during a gradual heating of the PCR products from 60 to 95 °C. Amplification efficiencies for all primer sets were calculated; all values proved to be sufficient to allow direct comparison of amplification plots according to the ΔΔCt method (Livak and Schmittgen, 2001). Relative quantification of gene expression was achieved by concurrent amplification of the *β-actin* endogenous control. Hence, during our experiment, total RNAs were quantified and 400 ng was used to be reverse-transcribed. During the subsequent qPCR amplifications, the output cycle corresponding to the *β-actin* was examined. No significant difference was observed on the output cycle of *β-actin* among fish groups ($p > 0.05$), demonstrating the relevance of *β-actin* as reference gene.

2. 4 Muscle and brain metal analyses

Metal quantification was performed on the same samples used for fatty acid analysis and gene transcription in the muscle, while in the brain metal analysis was only performed on the samples used for gene expression. Samples were lyophilised in acid-washed (HNO_3) Eppendorf tubes. Certified reference material (TORT-2 from National Research Council of Canada) as well as blanks were also analyzed to assess analytical accuracy and recovery rates (104.3±5 % and 92.5±2.7 for Cd and Ni, respectively; n=3). After lyophilisation, samples were weighed and digested in trace metal grade nitric acid (100 µl/mg dry sample) over 2 days at room temperature, followed by 24h in trace metal grade hydrogen peroxide (40 µl/mg dry sample) and finally diluted in ultrapure water. The concentrations of Cd and Ni were determined using inductively coupled plasma mass spectrometry (ICP-MS, Thermo Elemental, Model X-7).

2. 4 Calculation of indices and statistical analysis

The indices were calculated according to following formulae:

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

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

The Unsaturation Index (UI) is the sum of the % unsaturated fatty acids multiplied by their number of double bonds (= ΣMUFA + ΣUFAx2 + Σ3UFAx3 + Σ4UFAx4 + Σ5UFAx5 + Σ6UFAx6). Data were expressed as mean ± SEM (n = 6).

Comparisons among mean values from different stressor combinations were performed using one-way analysis of variance (ANOVA), after checking assumptions of normality (Kolmogorov-Smirnov) and homoscedasticity of the error terms (Levene). When the assumptions were not met as deduced from ad-hoc tests, a Wilcoxon / Mann-Whitney or Kruskal-Wallis test was applied. If significant effects were detected, the Tukey-Kramer HSD was used to determine whether means between pairs of samples were significantly different from one another. Subsequently, Spearman correlations were carried out to

294 investigate correlations among the various parameters monitored. The interactions of
295 temperature and Cd on membrane fatty acid composition as well as desaturase and
296 elongase transcription levels were determined by two-way ANOVA. Statistical analyses
297 were made with JMP 11.0.0 (SAS Institute Inc.).

298 ## 3. Results

299 ### 3. 1 Fish condition
300
301 No mortality was observed in Cd-exposed fish. However, Ni addition induced 100%
302 mortality in the warmer aquaria (25°C and 30°C). Therefore, in this paper, we discuss
303 the interaction of temperature and metals only for Cd and the comparison of Ni vs. Cd
304 effects was performed only for cold-acclimated fish (15˚C).
305 The condition index (CI) was higher in fish acclimated to 15˚C compared to fish
306 acclimated at the warmer temperature (Table 1). While Cd exposure had no influence
307 on fish condition at the warmer temperatures, at 15˚C the condition of Cd-exposed fish
308 was higher than for controls. The HSI was also negatively impacted by increasing
309 temperature, with higher values in control fish at 15˚C compared to 25˚C and 30˚C, but
310 it was not affected either by Cd or Ni (Table 1). Both HSI and CI were positively
311 correlated in all fish pooled (Spearman coefficient= 0.71, $p<0.001$, n=42).
312

313 ### 3. 2 Tissue metal concentrations
314
315 The concentration of Cd in muscle showed a significant increase in warm-
316 acclimated, Cd-exposed fish, and was higher at 30°C compared to 25°C. At 15˚C,
317 exposure to Cd did not lead to a significant accumulation of the metal in muscle (Table
318 1). However, in brain, Cd concentration increased only at 25°C and no significant
319 accumulation was measured at 15°C compared to controls (Table 1). In both muscle and
320 brain, a significant accumulation of Ni was observed in fish exposed to that metal at
321 15°C (Table 1). Surprisingly, Cd exposure also led to an increase of muscle Ni
322 concentrations at all temperatures tested compared to controls, although the increase

was not significant for fish acclimated to 30˚C compared to controls at the same temperature.

3. 3 Membrane fatty acid composition in muscle and brain

Muscle membranes of cold-acclimated fish were richer in PUFA (specifically n-3 PUFA) and poorer in SFA than in warm-acclimated fish at 30°C (Appendix 2 and Table 2). However, the difference between fish at 15°C and 25°C was not significant and membrane PLFA were similar. The ratio of unsaturated to saturated fatty acids varied with temperature and was higher in cold-acclimated fish. The percentage of DHA (22:6n-3) and EPA (20:5n-3) decreased at 30°C but was unchanged at 25°C compared to 15˚C (Appendix 2). Exposure to either Ni or Cd at 15˚C or to Cd at 25˚C did not lead to noteworthy effects on membrane PLFA composition (Appendix 2). However, at the highest temperature, SFA decreased and PUFA increased significantly in Cd-exposed fish, mainly due to a sharp increase in 22:6n-3 (Appendix 2). In this condition, membrane composition was similar to that of fish acclimated at the colder temperatures (Appendix 2 and Table 2). The two-way ANOVA showed that SFA, PUFA, U/S ratio n-3 PUFA and DHA were significantly affected by the interaction of temperature and Cd exposure (Table 3).

In brain tissue, membrane composition was not strongly different in fish acclimated to 15°C compared to 30°C (Appendix 3 and Table 4), except for MUFA that increased at 30°C. Between 15°C and 25°C the DBI, PUFA, DHA and EPA decreased significantly (Appendix 3 and Table 4). Brain membrane FA proved to be rather rich in MUFA. In addition, they displayed high levels of n-9 UFA (between 24 and 34% of total fatty acids), mostly due to an elevated percentage of 18:1n9, compared to muscle membranes, in which n-9 UFA did not exceed 19% of the total fatty acids. The two-way ANOVA showed that in the brain, all FA groups were affected by temperature, but none were affected by Cd alone. Nonetheless, a significant interaction of Cd and temperature was observed on SFA, MUFA (and specifically n-9 UFA) and the U/S ratio (Table 3).

3. 4 Desaturase and elongase transcription levels

The transcription level of genes of both desaturases and elongases was different between muscle and brain tissues. In the muscle, desaturase and elongase transcription levels were different among exposure conditions (Figs. 2), but did not clearly respond to changes in acclimation temperature or metal exposure. Specifically, *fads2* was maintained at the same level under 15°C and 25°C in the presence or absence of Cd or Ni. However, under Cd exposure at 30°C, *fads 2* transcription level decreased significantly (Fig. 2A). The transcription level of *degs2* increased notably at 15°C under Ni exposure (Fig. 2B), while *scd2* presented the same transcription level independently of temperature changes but it decreased significantly at 30°C in Cd-exposed fish (Fig. 2C). The transcription level of *elovl2* was not affected at all by temperature or metal exposure while that of *elovl5* increased slightly at 30°C compared to 15˚C in control fish and was strongly stimulated in metal-exposed fish acclimated at the colder temperature (Fig. 2E) and *elovl6* decreased significantly under Cd exposure in warm-acclimated fish (Fig. 6E). The two-way ANOVA detected a significant interaction of Cd and temperature on *fads2*, *scd2*, *elovl5* and *elovl6* in the muscle (Table 3).

Brain desaturase transcription levels were consistently higher at low temperature but their response to metal exposure varied. Specifically, *fads2* transcription level was nearly twice higher at 15°C compared to 25°C and 30°C and stimulated by Cd, but at 25˚C only (Fig. 3A). The same trend of thermal response was observed for *degs2*, with a higher transcription level at 15°C than at warmer conditions. However, in contrast to *fads2*, it was down-regulated by Cd at 15˚C (fig. 3B). Finally, the *scd2* transcription level was not significantly affected by metal exposure although Ni exposure at 15˚C and Cd exposure at all tested acclimation temperatures tended to decrease it (Fig. 3C). The transcription of two of the three elongase genes examined, *elovl2* and *elovl5*, decreased with increasing acclimation temperature. At 15°C, under Cd exposure, *elovl*2 decreased while it increased in Ni-exposed fish and reached the highest values of all experimental conditions (Fig. 3D). Metal exposure did not affect *elovl5* transcription level (Fig. 3E). The transcription level of *elovl6* was not affected by either by temperature or metal

exposure (Fig. 3F). The two-way ANOVA detected a significant interaction of Cd and temperature on *fads2* and *elovs2* (Table 3).

4. Discussion

4. 1 Temperature effects on Cd accumulation

The greater Cd accumulation in the muscle of warm acclimated fish may be explained by the elevated metabolic rate at high temperature. Among factors that affect metal uptake, ventilation rate and ionoregulatory processes have been reported to increase with temperature (Massabuau and Tran, 2003; Wang et al., 2005). In the brain, Cd accumulation did not follow the same trend as observed in muscle and increased only at 25°C. Metals can be transported by the blood and cross the blood-brain barrier to enter into the brain (Rouleau et al., 2003). In cold-exposed fish, blood flow can be reduced, to slow down the cooling of brain in order to maintain neuronal activities (van den Burg et al., 2005). It is likely that the lower brain Cd concentrations at 30˚C compared to 25˚C also results from a reduction of brain irrigation, however this mechanism remains to be investigated.

4. 2 Effects of temperature and metals on morphometric indices

An increase in temperature from 15˚C to 25˚C and 30˚C negatively affected both the CI and the HSI of our fathead minnows, consistent with a previous study on the same species (Lapointe et al., 2011) and also supported by Grasset et al. (2014) for yellow perch. The absence of negative effects of Cd accumulation on either of the two condition indicators also agree with our previous study, Fadhlaoui and Couture (2016) as well as that of Grasset et al. (2016) in yellow perch. In fathead minnows, combined exposure to heat stress and aqueous or dietary Cu exposure did not either affect fish condition more than heat stress alone (Lapointe et al., 2011). Hence, in both species, the effects of heat stress on indicators of energy storage are far more important than metal exposure at environmentally relevant concentrations. Nickel did not affect either

410 the condition indicators examined at 15˚C. However, in contrast to Cd, we cannot rule
411 out that exposure to Ni, combined to heat stress, led to a critical reduction of fish
412 condition and yielded the massive mortality observed at 25 and 30˚C. Hasnain et al.
413 (2010) have reported an upper lethal temperature of 31.3˚C for fathead minnows
414 (upper incipient lethal temperature, or UILT; temperature at which 50% mortality occurs
415 in a population). Our study suggests that Ni, but not Cd, reduces the UILT in fathead
416 minnows. Regarding the higher values of HSI that we observed at the lower
417 temperature, others have suggested that an increase of liver size may be an adaptive
418 response to compensate the decrease in enzyme activities (Kent et al., 1988; Seddon,
419 1997; Weber and Bosworth, 2005). Given the positive correlation between HSI and CI,
420 our study suggests that 15°C is an optimal temperature for fathead minnow growth.
421 In our previous study (Fadhlaoui and Couture, 2016), we performed a similar
422 experiment using yellow perch, a fish considered as eurythermal and tolerant to a wide
423 range of contaminants including Cd and Ni (Couture et al., 2015; Eastwood and Couture,
424 2002; Rajotte and Couture, 2002). Perch were exposed to the same aqueous
425 concentrations of Cd and Ni as in this study for seven weeks (compared to eight weeks
426 in this study for fathead minnows) at either 9˚C or 28˚C. Yet, in spite of the upper
427 temperature exceeding the UILT of 25.5˚C reported by Hasnain et al. (2010) for yellow
428 perch, metal exposure did not induce mortality. Our studies combined confirm
429 experimentally that yellow perch is more tolerant than fathead minnows to Ni at
430 elevated temperatures, but their experimental design does not allow to conclude on
431 their respective tolerance to aqueous Cd or heat stress, alone or in combination.

432 **4. 3 Effects of temperature and metals on membrane fatty acids**
433

434 Although exposure to both 25°C and 30°C negatively impacted the condition of
435 fathead minnows, the fatty acid composition of their muscle only differed at the highest
436 temperature tested, with substantial decreases in LC-PUFA and an enrichment in SFA in
437 muscle PLFA of fish acclimated at 30˚C compared to 15˚C. This thermal response of
438 fathead minnow muscle membrane PLFA, largely driven by changes in the proportion of

439 DHA, was highly similar to our observations for yellow perch (Fadhlaoui and Couture
440 2016) and consistent with the theory of HVA.
441 Even though in our experiment, exposure to Ni, but not Cd, at 15˚C led to a
442 substantial increase in muscle concentrations of the metal, neither metal affected
443 muscle membrane PLFA composition, except for a global decrease of n-6 UFA in metal-
444 exposed fish. All n-6 PUFA except 18:2n6 were decreased in Ni-exposed fish, including
445 20:2n-6, 20:3n6, 20:4n-6 and 22:4n6, but the trends of decrease of the same individual
446 FA in Cd-exposed fish were non-significant. Interestingly, in yellow perch, exposure to
447 both metals at the colder temperature tested (9˚C) also induced a sharp decrease in n-6
448 UFA (Fadhlaoui and Couture, 2016), suggesting a common mechanism of action in the
449 two species. Our observation that 18:2n6 was higher in Ni-exposed fish suggests an
450 inhibition of the n-6 elongase and desaturase pathways. Compared to yellow perch in
451 which SFA, MUFA and PUFA were modified by metal exposure, the overall response of
452 fathead minnow muscle PLFA to metal exposure at low temperature remained modest.
453 The mechanisms involved in metal-induced modifications of cell membrane PLFA may
454 be more sensitive in yellow perch than in fathead minnows at colder temperatures.
455 However, since here, the coldest temperature was 15˚C, we cannot exclude that the
456 response of fathead minnows to metal exposure at 9˚C may have been similar to the
457 response of yellow perch.
458 Exposing fathead minnows to Cd strongly modified the response of muscle cell
459 membrane PLFA composition to increasing temperatures, as highlighted by significant
460 interactions between the two stressors on SFA and PUFA in general, and n-3 PUFA in
461 particular. Much like we reported earlier for yellow perch, the heat-induced decrease in
462 PUFA observed in control fish at 30°C was counteracted by Cd. However, in fathead
463 minnows the Cd-induced increase in membrane polyunsaturation was subtler than for
464 perch. While in perch exposure to Cd at 28˚C resulted in an extent of muscle membrane
465 PLFA polyunsaturation comparable to fish acclimated 19˚C lower, exposing fathead
466 minnows acclimated to 30˚C to Cd made their muscle membrane PLFA comparable to
467 control fish acclimated at 15 and 25˚C. Given the mortality that resulted from combining

468 elevated temperatures and aqueous Ni exposure in fathead minnows in our study, we
469 do not know whether Ni interfered more strongly than Cd with the temperature-
470 induced adjustments of muscle membrane PLFA, although we can hypothesize this to be
471 the case since in perch, the interference of Ni was even stronger than for Cd (Fadhlaoui
472 and Couture 2016). To our knowledge, there is no report in the literature other than our
473 own studies about metal effects on cell membrane composition and its thermal
474 response in fish or any other organism.

475 The decrease in SFA in the muscle of fish acclimated at 30˚C when exposed to Cd
476 was mainly due to the total inhibition of the sharp increase in 16:0 observed in control
477 fish at that temperature. While the % composition of 16:0 remained between 12 and
478 15% in all other temperature and metal exposure scenarios examined, it reached 30% in
479 control fish acclimated at 30˚C, suggesting a normal thermal acclimation response for
480 the species. Fatty acid synthase (FAS) is a rate-limiting enzyme in the *de novo*
481 biosynthesis of fatty acids that catalyzes the reaction leading to palmitic acid (16:0)
482 synthesis (Smith et al., 2003). Previous studies on Cd-exposed crabs have reported an
483 impairment of lipid metabolism involving a decrease of FAS activity (Liu et al., 2016;
484 Yang et al., 2013). We therefore hypothesize that the observed inhibition of SFA
485 synthesis in the muscle of Cd-exposed fathead minnows acclimated to 30˚C may be
486 related to a Cd-induced inhibition of FAS, accentuated by the rise of temperature which
487 stimulated the accumulation of this metal.

488 Fathead minnow brain cell membrane PLFA generally differed from muscle, with
489 higher proportions of MUFA, especially oleic acid (18:1n-9) in the former, and they
490 responded more subtly to changes in acclimation temperature and metal exposure. In
491 contrast to muscle for which changes in membrane PLFA were maximal at the extremes
492 of the acclimation temperature spectrum, in the brain, the decrease of PUFA in
493 response to elevated temperature was only observed between 15°C and 25°C. The
494 reversal of the trend when further increasing temperature to 30˚C suggests an
495 impairment of the normal response to thermal acclimation. Interestingly, Buda et al.
496 (1994) reported that the brain cell membrane PLFA from *Cyprinus carpio* acclimated to

497 temperatures 20˚C apart (23°C-25°C vs. 5°C), did not change their composition, but they
498 did not examine an intermediate temperature as we did in our study. (Farkas et al.,
499 2000) demonstrated that the composition of cold-water fish brain was characterized by
500 an abundance of DHA (mainly 18:1/22:6 phosphatidylethanolamine), but that its level
501 decreased with an increase of temperature, as we also observed between 15 and 25˚C.
502 Also in agreement with our study for fathead minnows, cold adaptation in various
503 teleost species has been reported to be accompanied by higher proportions of PUFA in
504 brain membranes, while MUFA remained constant (Logue et al., 2000). Several studies
505 have shown that cell membrane functions depending on viscosity are disrupted at high
506 temperatures (Cossins and Prosser, 1978; Friedlander et al., 1976). We hypothesize that
507 for fathead minnows, 30°C represented a critical temperature forcing fish to make
508 adjustments to their brain cell membrane PLFA in order to maintain membrane-
509 associated neuronal functions that are influenced by membrane fluidity. Indeed, in their
510 experiment in which Buda et al. (1994) acclimated *Cyprinus carpio* to two extreme
511 temperatures 20˚C apart, they also hypothesized that the absence of modifications in
512 the proportions of highly polyunsaturated fatty acids such as DHA in their brains
513 involved other mechanisms aiming at maintaining neuronal properties.
514 Like our observations for muscle, Cd exposure did not affect brain cell membrane
515 PLFA at 15˚C. The significant decrease in n-3 PUFA in the brain of Ni-exposed fish points
516 to a substantial difference in the modes of action of this metal between the two tissues
517 examined. In muscle, Ni did not affect n-3 FA except for a sharp increase in 20:4n-3, but
518 induced a reduction of n-6 FA. Considering the increase of the minor FA 18:4n-3
519 accompanied by a decrease of 22:6n-3, Ni exposure appears to induce an alteration of
520 n-3 PUFA biosynthesis in the brain of cold-acclimated fish. Since neither 20:5n-3 nor
521 22:5n-3 were affected by Ni exposure, our data suggests that the activity of $\Delta 4$
522 desaturases was affected by the presence of Ni.
523 In spite of the substantial accumulation of Cd in the brain of fish acclimated to 25°C,
524 cell membrane PLFA general characteristics like the DBI and PUFA percentages were not
525 affected by Cd. Yet, there was a substantial decrease in 16:0 and in the shorter chain n-9

17:1n-9, while longer chained n-9 18:1n-9, 20:1n9 and 24:1n9 increased, resulting in an overall increase of n-9 UFA. We do not know whether Cd specifically induced decreases in 16:0 or increases in n-9 FA in brain, although we hypothesized for muscle (above) that Cd may inhibit FAS leading to a decrease of 16:0. Regardless, our results suggest that although Cd induced changes in brain cell membrane composition through some yet unknown mechanism, decreases in some FAs were compensated by increases in others, presumably in order to maintain membrane properties and function. Interestingly, at the opposite of what we observed in muscle, even in the absence of a significant Cd accumulation in the brain of fish acclimated at 30°C, the substantial decrease in SFA observed in control fish was reversed in Cd-exposed fish, due to increases in several SFA, yielding U/S ratios comparable to those of fish acclimated to lower temperatures, in the presence or absence of metals. Intriguingly, the effect of Cd observed at 25˚C on SFA was the opposite of what was observed in fish acclimated 5˚C warmer. Hence, even though fathead minnows appear capable of maintaining the overall PLFA composition of their brain cell membranes much more efficiently than for their muscle, temperature, Cd and their interaction interfere substantially with SFA and n-9 MUFA. Given the lack of Cd accumulation in the brain of fish acclimated to 30˚C compared to 25°C, we cannot suggest that the observed changes are due to direct interactions of Cd with membrane PLFA. We can therefore hypothesize that Cd-induced changes in brain membrane PLFA are at least in part due to the interference of this metal with lipogenic processes in other tissues, involved in the production of fatty acids destined for incorporation in brain phospholipids.

4. 4 Relationships between membrane fatty acid and the transcription of desaturases and elongases

In fathead minnow muscle, although temperature globally affected the transcription levels of *degs2* and *scd2* genes, a decrease of acclimation temperature (comparing 25˚C or 30˚C with 15˚C) did not affect desaturase and elongase transcription levels except for *elovl5* that was slightly lower in fish acclimated at 15˚C compared to 30˚C. Hence, the

556 substantial temperature-induced modifications of muscle membrane PLFA composition,
557 including an increase in PUFA in cold-acclimated fish, were not reflected by the
558 transcription levels of genes involved in controlling its composition. Previous studies
559 have reported an induction of desaturase transcription and activity in fish during cold
560 acclimation to restore membrane fluidity (Hsieh and Kuo, 2005; Tiku et al., 1996;
561 Wodtke and Cossins, 1991). Given the absence of response at the transcriptional level in
562 our study, the increase in PUFA in the muscle of cold-exposed fish may be related to the
563 activation of latent enzymes, as reported by others (Tiku et al., 1996; Trueman et al.,
564 2000a). In these studies fish acclimation was brief compared to our study. Therefore, it
565 is possible that in our study desaturases genes initially responded to temperature
566 decrease before returning to basal levels at the end of exposure. Yet, future studies
567 should investigate the time course of the transcriptomic response during thermal
568 acclimation.

569 Exposure to Ni at 15˚C affected the transcription level of genes, but in an opposite
570 direction compared to changes in membrane composition, which, as discussed in
571 Section 4.3, suggested an inhibition of n-6 biosynthetic pathway. In our study, this
572 pathway included the genes *fads2*, *degs2*, *elovl2* and *elovl5*, also shared by the n-3
573 biosynthetic pathway. In Ni-exposed fish, *degs2* transcription level was the highest, but
574 the corresponding product of this $\Delta 4$ desaturase in the n-3 pathway, DHA, was not
575 modified compared to control fish. Likewise, *elovl5* was induced under Ni exposure, yet,
576 n-6 PUFA, mainly 20:4n-6 and 22:4n-6, decreased, while 22:5n-3 was unaffected by Ni
577 exposure. Clearly, the upregulation of these genes in Ni-exposed fish did not lead to an
578 enhancement of the products of their pathways, but suggests that Ni inhibited the
579 desaturase and elongase enzymes for which they encode. These increases in gene
580 transcription levels would suggest a compensatory mechanism.

581 In contrast to our observations in fish acclimated at 15˚C, for which Cd exposure did
582 not affect gene transcription level except for an enhancement of *elovl5*, in Cd-exposed
583 fish acclimated at 30˚C, *fads2*, *degs2*, *scd2* and *elvol6* decreased significantly compared
584 to control fish, in contradiction with the sharp increase in PUFA and DBI in Cd-exposed

585 fish. Since elongases and desaturases are responsible of modifications in cell membrane
586 PUFA concentrations and DBI, if the transcription of their genes was downregulated in
587 Cd-exposed fish, this suggests that their activity was upregulated, through some
588 unknown mechanism. Nevertheless, we have to consider that the regulation of
589 membrane lipid composition also involves adjustments of FA incorporation into
590 membrane PL, membrane turnover and PL formation by acylation re-acylation cycles
591 (Hazel, 1984). During the membrane turnover process, FA may be incorporated into
592 membrane PL by direct acylation of lysophosphate (Van Den Bosch, 1980). Therefore, in
593 Cd-exposed fish at warm temperatures, the increase in PUFA may also involve a
594 stimulation of these re-acylation processes.

595 In the brain of our fathead minnows, the transcription levels of desaturases and
596 elongases appeared much greater than in muscle. Aliyu-Paiko et al. (2013), evaluating
597 the transcription of these enzymes in different tissues of *Channa striata*, have also
598 shown that their levels were higher in the brain and liver compared to muscle. Others
599 have also reported that desaturases (Tocher et al., 2006; Zheng et al., 2004) and
600 elongases (Carmona-Antonanzas et al., 2011; Xue et al., 2014) were highly expressed in
601 brain tissue. Higher transcription of desaturases and elongases in the brain highlight
602 their important role in neuronal tissue, particularly for DHA and EPA. As mentioned
603 earlier (section 4.3), brain membrane PL contained a greater amount of MUFA
604 compared to muscle, dominated mainly by oleic acid (18:1n-9). This fatty acid is
605 synthesised through a desaturation of stearic acid (18:0) by the Δ9 desaturase encoded
606 by *scd2*, which in our study was higher in brain compared to muscle. This molecular
607 species is important in brain to control the biophysical properties of membranes under
608 temperature variations. The presence of 18:1n-9 in the sn-1 position of phospholipids
609 increases their surface area compared to the combination 18:0/22:6n-3 (Zabelinskii et
610 al., 1995) and decreases consequently the electrostatic interaction between head group
611 regions (Michaelson et al., 1974). The rate of synaptic fusion rate depends on PLFA
612 composition. A high ratio of 18:1n-9/22:6n-3 improves fusion and assists in maintaining
613 signal transduction at low temperature. Like for 18:1n-9, DHA-containing PL in the brain

are important for signal transduction and information processing (Farkas et al., 2000). Moreover, in our study, a long-chain fatty acid, 24:1n-9, was more abundant in brain membrane compared to muscle as reported by others (Bell and Tocher, 1989; Tocher and Harvie, 1988). This fatty acid is an elongation product of oleic acid (18:1n-9), which is itself a desaturation product of 18:0 (Thomassen et al., 1985) and is essential for different neuronal functions.

In the brain of our fish, the transcription of desaturases (*fads2* and *degs2*) and elongases (*elovl5*) showed a substantial decrease in 25°C-acclimated fish compared to cold acclimated-fish. This decrease was consistent with the modifications of membrane composition as described earlier (section 4.3). Previous studies examining temperature effects on transcription levels of these genes have reported that stearoyl-CoA desaturase plays an important role in the metabolism of membrane fatty acids aiming at regulating membrane fluidity following temperature fluctuations (Tiku et al., 1996; Trueman et al., 2000). Furthermore, the transcription level of *scd2* desaturase (Hsieh and Kuo, 2005; Tiku et al., 1996; Trueman et al., 2000; Xu et al., 2015) and elongase (Mellery et al., 2016; Norambuena et al., 2015; Ren et al., 2013) were reported to be up-regulated under cold temperature in different species, in agreement with our study.

In our fathead minnows exposed to 30°C, the transcription of brain desaturases (*fads2, degs2* and s*cd2*) and elongases (*elovl6* and *elovl5*) decreased, according to normal response to temperature increase. Surprisingly membrane structure did not correspond to the observed variations in transcription and their fatty acid composition was almost similar to that of cold exposed-fish, especially in relation to SFA, PUFA and BDI, suggesting that post-transcriptional and *de novo* phospholipid biosynthetic mechanisms acted to prevent a decrease in brain membrane unsaturation following acclimation to this extreme temperature. The high level of PUFA observed in these fish in spite of the decrease of desaturase and elongase transcription levels likely reflects their critical importance in the function of neural tissue, discussed above.

Metal exposure modified the transcription levels of desaturases and elongases in the brain of our fish. In cold exposed fish, *degs2* and *elovl2* decreased under Cd exposure

643 without leading to modifications in membrane FA composition. In Ni-exposed fish,
644 *elovl2* transcription was induced, while in contrast PUFA, particularly n-3 PUFA,
645 decreased significantly, suggesting that the upregulation of elongases aimed at
646 counteracting Ni-induced effects on brain membrane PUFA. It has been demonstrated
647 that *elovl2* and *elovl5* are regulated by sterol regulatory element binding protein
648 (SREBP) transcription factors (Qin et al., 2009). Studies on Atlantic salmon have shown
649 that the expression of SREBP was affected by modifications of PUFA content (Minghetti
650 et al., 2011; Morais et al., 2009; Zheng et al., 2005). Thus, we suggest that the increase
651 of *elovl2* under Ni exposure was regulated by a positive feedback loop in response to a
652 decrease of PUFA, in an attempt to increase n-3 PUFA, especially DHA that was
653 significantly reduced in Ni-exposed fish.
654 Exposure to Cd did not affect the transcription levels of elongases or desaturases in
655 fish acclimated to the warmer temperatures, except for *fads2* which was induced by Cd
656 at 25°C. Brain membrane composition however did not reflect the changes in *fads2*
657 transcription level since the main products of the Δ5/Δ6 desaturases (DHA, EPA and
658 ARA) were unchanged. In partial support of our study, Cd has been reported to induce
659 Δ6 desaturase activity in rat (Kudo and Waku, 1996), although Δ9 desaturase (*scd2*)
660 activity decreased. Their study examined enzyme activity and not gene transcription
661 level, so a direct comparison with our study is risky. To our knowledge, only one study
662 examined Cd effects on fish brain desaturase transcription levels. The authors reported
663 that Cd induced the transcription of Δ9 desaturase (Balla and Hermesz, 2009), contrary
664 to our study in which brain *scd2* transcription levels were not affected by metal
665 exposure.
666 In conclusion, our study supports that temperature acclimation affected muscle and
667 brain PLFA differently. In muscle, temperature-induced changes in membrane
668 composition agreed with the HVA theory in contrast to the brain, which was more
669 unsaturated than predicted at the highest acclimation temperature. Desaturase and
670 elongase transcription was higher in the brain, in agreement with the high level of
671 MUFA and PUFA in these membranes and the important role that LC-PUFA play in

672 neuronal functions. Metal exposure modified the normal response to temperature,
673 inducing major modifications of membrane PLFA through some yet unknown
674 mechanism. Temperature and metal induced modifications in desaturase and elongase
675 transcription levels did not systematically correspond to the observed changes in
676 membrane PFLA, suggesting that post-transcriptional regulation of elongases and
677 desaturases as well as other membrane biosynthetic processes may be involved.

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679

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Table 1: Condition index, hepatosomatic index, cadmium and nickel concentration in muscle and brain (µg. g^{-1} dry weight) of fathead minnows after eight weeks of exposure to each experimental condition. Data are expressed as mean ± SEM (n=6)

		15°C	15°C-Cd	15°C-Ni	25°C	25°C-Cd	30°C	30°C-Cd
Condition index		1.10±0.01 ^{b}	1.26±0.04 ^{a}	1.19±0.02 ^{ab}	0.75±0.02 ^{c}	0.70±0.06 ^{c}	0.69±0.02 ^{c}	0.78±0.01 ^{c}
Hepatosomatic index		1.50±0.08 ^{ab}	1.22±0.16 ^{abc}	1.32±0.09 ^{ab}	0.67±0.06 ^{d}	0.82±0.02 ^{cd}	0.96±0.03 ^{bcd}	1.07±0.06 ^{bc}
Ni	**Muscle**	0.11±0.01 ^{c}	0.43±0.05 ^{b}	1.59±0.16 ^{a}	0.15±0.02 ^{c}	0.57±0.07 ^{b}	0.38±0.09 ^{bc}	0.58±0.08 ^{b}
	Brain	0.27±0.03 ^{b}	0.28±0.07 ^{b}	3.61±0.34 ^{a}	0.20±0.10 ^{b}	0.38±0.10 ^{b}	0.41±0.10 ^{b}	0.34±0.10 ^{b}
Cd	**Muscle**	0.05±0.01 ^{c}	0.17±0.01 ^{c}	0.04±0.01 ^{c}	0.02±0.00 ^{c}	1.03±0.12 ^{b}	0.05±0.01 ^{c}	1.32±0.07 ^{a}
	Brain	0.22±0.10 ^{b}	0.31±0.05 ^{b}	0.04±0.01 ^{c}	0.13±0.06 ^{c}	1.04±0.14 ^{a}	0.03±0.00 ^{c}	0.28±0.09 ^{b}

Table 2: Fatty acid groups and families (% of total fatty acids) in muscle membranes of fathead minnows under different exposure conditions. Data are expressed as mean ± SEM (n=6) and are calculated for each individual of the same condition. Different superscript letters within a row indicate significant differences between conditions ($p<0.05$).

	15°C	15°C-Cd	15°C-Ni	25°C	25°C-Cd	30°C	30°C-Cd
SFA	18.5±0.3^{cd}	12.7±0.7^{d}	18.0±0.4^{cd}	21.5±0.4^{b}	22.3±0.7^{bc}	34.0±1.4^{a}	22.8±0.9^{b}
MUFA	16.1±0.9^{b}	14.5±3.5^{b}	22.9±0.9^{ab}	16.4±0.9^{b}	16.8±1.5^{b}	29.1±2.0^{a}	22.2±0.9^{ab}
PUFA	66.0±0.9^{ab}	69.1±3.5^{a}	58.5±1.0^{ab}	63.1±1.0^{ab}	63.0±2.0^{ab}	38.5±1.9^{c}	53.6±2.2^{b}
n-9 UFA	10.2±0.7^{c}	10.3±1.2^{c}	11.7±0.7^{bc}	12.5±0.7^{bc}	12.3±0.9^{bc}	19.4±1.7^{a}	14.4±0.4^{b}
n-3 UFA	49.7±1.0^{ab}	54.3±2.7^{a}	44.2±1.3^{ab}	47.8±1.3^{ab}	47.3±2.4^{ab}	22.9±1.0^{c}	41.5±2.1^{b}
n-6 UFA	16.3±0.2^{a}	14.8±0.4^{b}	13.1±0.7^{c}	15.1±0.7^{abc}	15.6±1.1^{b}	15.3±2.2^{abc}	12.1±0.9^{c}

Table 3: Effects of temperature, Cd and their interaction as determined by two-way ANOVA analysis of FA groups, FA families and transcription level of elongases and desaturases in fathead minnow muscle and brain. Values in bold represent a significant effect of temperature, Cd or their interaction ($p<0.05$).

	Muscle			Brain		
	Temperature	**Cd**	**Temperature x Cd**	**Temperature**	**Cd**	**Temperature x Cd**
SFA	**<0.0001**	**0.0130**	**0.0030**	**0.0406**	0.5338	**0.0014**
MUFA	**<0.0001**	**0.0444**	0.1185	**0.0408**	0.8243	**0.0037**
PUFA	**<0.0001**	**0.0001**	**0.0002**	**0.0096**	0.8257	0.5908
U/S Ratio	**<0.0001**	**0.0026**	**0,0383**	**0.0182**	0.2099	**0.0002**
n-9 UFA	**0.0042**	0.5152	0.7228	**0.0113**	0.0849	**0.0178**
n-3 UFA	**<0.0001**	**<0.0001**	**<0.0001**	**0.0085**	0.3383	0.2465
n-6 UFA	0.2153	0.1476	0.2882	**0.0139**	0.3109	0.2531
fads2	0.1336	0.1426	0.3862	**<0.0001**	**0.0044**	**0.0318**
degs2	**0.0223**	0.4013	**0.0113**	0.7014	**0.0318**	0.0728
scd2	**0.0045**	0.5442	**0.0034**	**<0.0001**	0.1173	0.2304
elovl2	0.7704	0.7289	0.1294	**0.0006**	0.2424	**0.0005**
elovl6	0.3873	**0.0384**	**0.0017**	0.5876	0.1176	0.9821
elovl5	0.5766	0.4746	**0.0080**	**0.0138**	0.4254	0.1174

Table 4: Fatty acid groups and families (% of total fatty acids) in brain membranes of fathead minnows under different exposure conditions. Data are expressed as mean ± SEM (n=6) and are calculated for each individual of the same condition. Different superscript letters within a row indicate significant differences between conditions ($p<0.05$).

	15°C	15°C-Cd	15°C-Ni	25°C	25°C-Cd	30°C	30°C-Cd
SFA	25.8±2.6^{ab}	27.8±1.6^{ab}	31.5±1.5^{a}	33.03±2.9^{a}	25.1±2.3^{ab}	18.8±0.8^{b}	28.0±1.9^{a}
MUFA	28.6±0.6^{b}	28.9±1.5^{b}	30.4±1.4^{ab}	29.5±2.5^{ab}	37.7±2.5^{a}	37.4±1.1^{a}	30.0±3.2^{ab}
PUFA	45.6±2.1^{a}	43.2±2.7^{a}	38.1±1.9^{b}	34.3±0.7^{b}	37.1±4.2^{ab}	43.8±0.9^{a}	41.9±4.9^{ab}
n-9 UFA	24.0±0.5^{b}	24.7±1.2^{b}	26.7±1.4^{ab}	25.4±2.1^{b}	34.3±2.4^{a}	29.9±2.1^{ab}	28.2±3.1^{ab}
n-3 UFA	40.5±1.9^{a}	37.8±2.1^{ab}	32.9±1.9^{b}	29.3±0.7^{c}	32.0±4.3^{abc}	36.5±1.9^{b}	30.3±1.9^{bc}
n-6 UFA	5.3±0.4^{ab}	4.7±0.6^{b}	5.2±0.1^{ab}	4.7±0.5^{b}	5.0±0.3^{ab}	7.0±0.8^{ab}	11.3±3.6^{a}

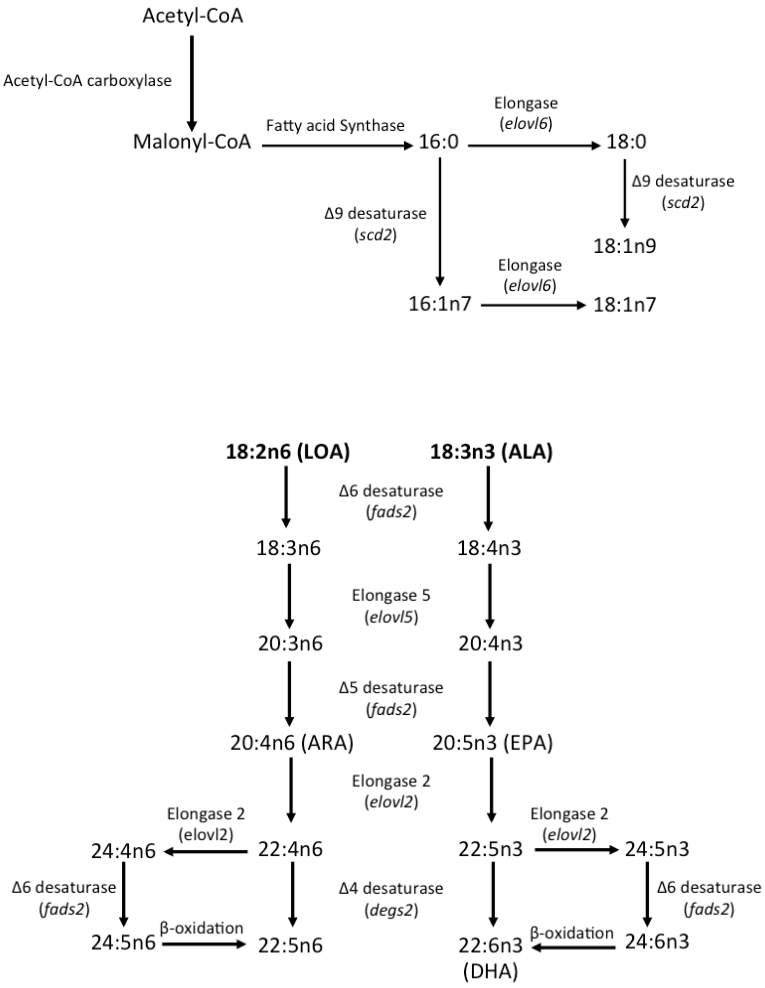


Figure 1: The biosynthesis pathway of monounsaturated and long-chain polyunsaturated fatty acids in fish

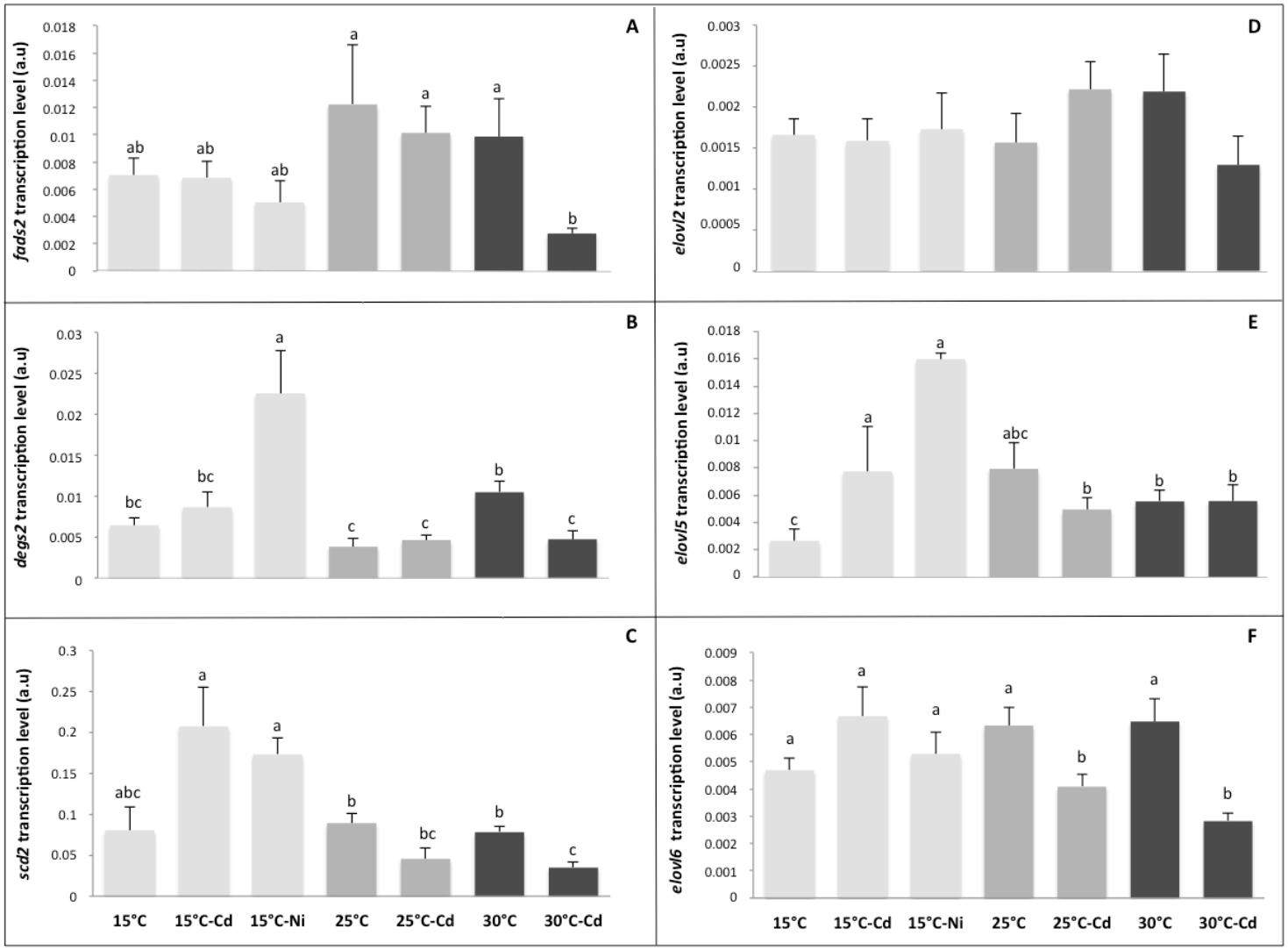


Figure 2: Transcription level of desaturase genes *fads2* (A), *degs2* (B), *scd2* (C) and elongase genes *elovl2* (D), *elovl5* (E), *elovl6* (F) in fathead minnow muscle after eight weeks of exposure to each experimental condition. Data are expressed as mean SEM (n=6) and significant differences between means are indicated by different letters ($p<0.05$)

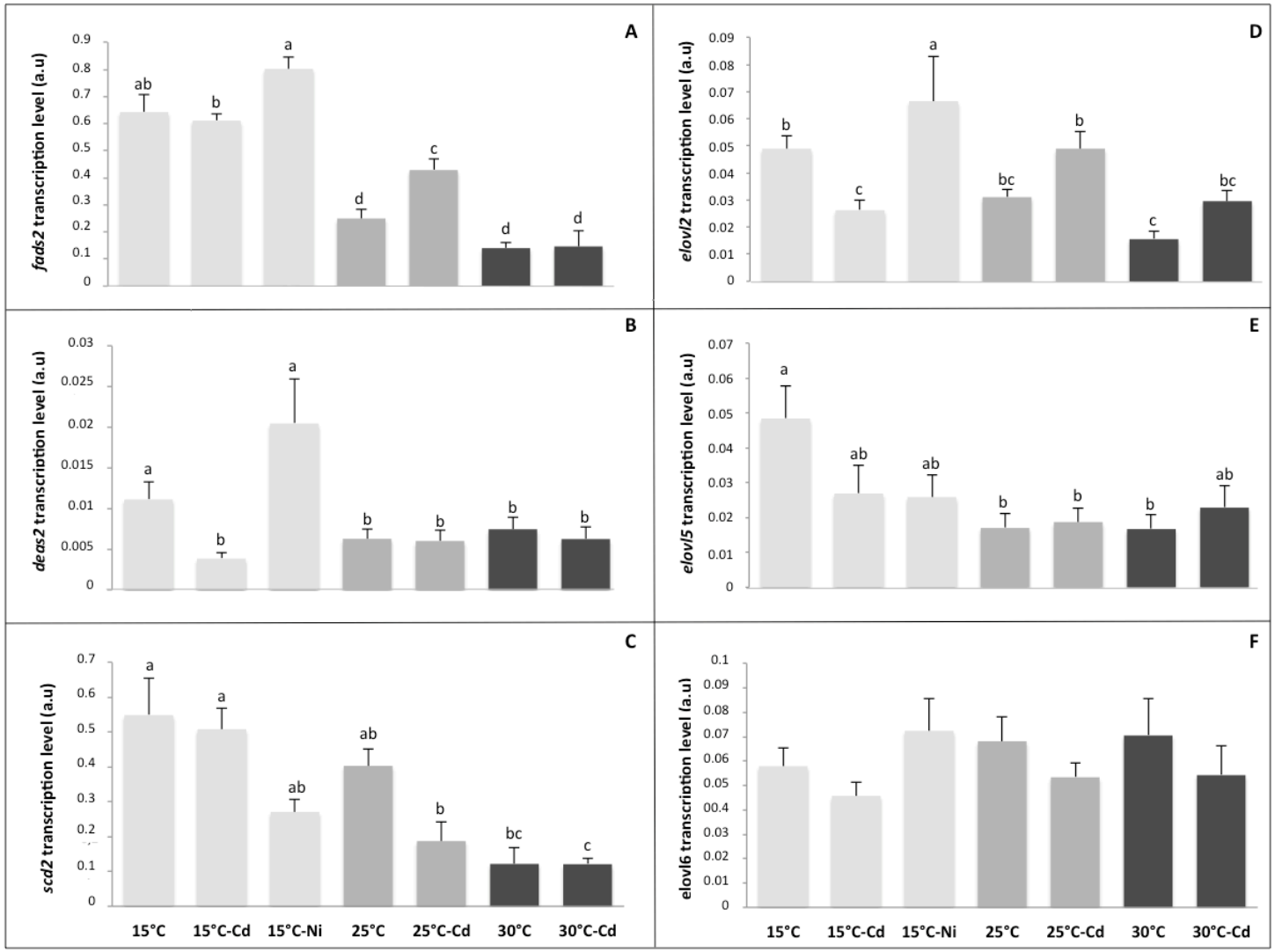
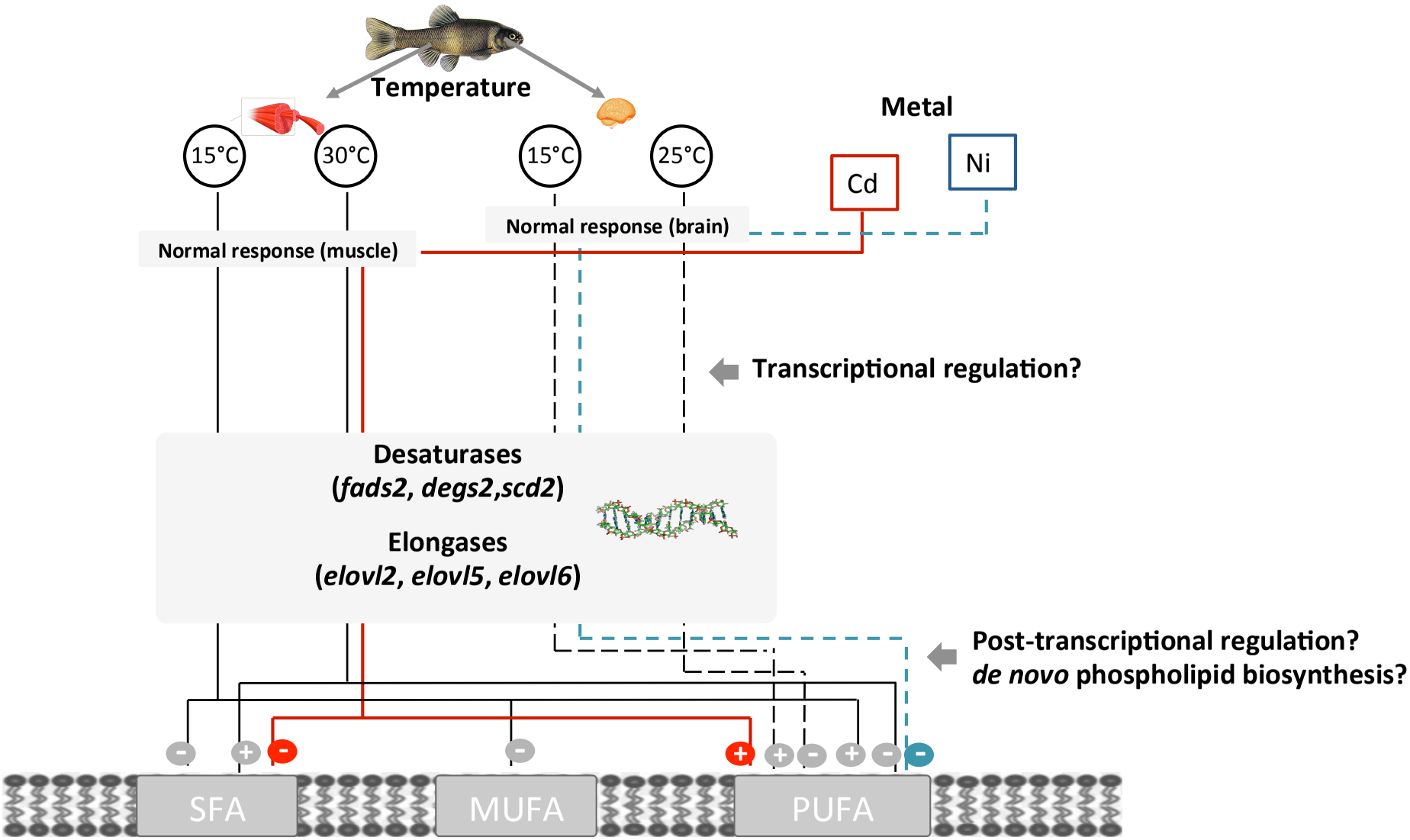


Figure 3: Transcription level of desaturase genes *fads2* (A), *degs2* (B), *scd2* (C) and elongase genes *elovl2* (D), *elovl5* (E), *elovl6* (F) in fathead minnow brain after eight weeks of exposure to each experimental condition. Data are expressed as mean SEM (n=6) and significant differences between means are indicated by different letters ($p<0.05$)

Highlights

- Fathead minnows were exposed to cadmium or nickel at 15, 25 or 30°C
- Cell membrane fatty acid composition varied with acclimation temperature
- Muscle and brain fatty acid composition responded differently to temperature
- Metal exposure affected the thermal response of membrane composition in both tissues
- Desaturase and elongase transcription levels did not reflect membrane modifications


Temperature
15°C
30°C
15°C
25°C
Metal
Cd
Ni
Normal response (muscle)
Normal response (brain)
Transcriptional regulation?
Desaturases
(fads2, degs2,scd2)
Elongases
(elovl2, elovl5, elovl6)
Post-transcriptional regulation?
de novo phospholipid biosynthesis?
SFA
MUFA
PUFA