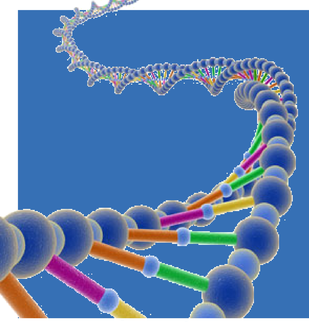

Combined effects of temperature and metal contamination on membrane fatty acid composition, desaturases and elongases in fathead minnow (*Pimephales promelas*)

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Introduction

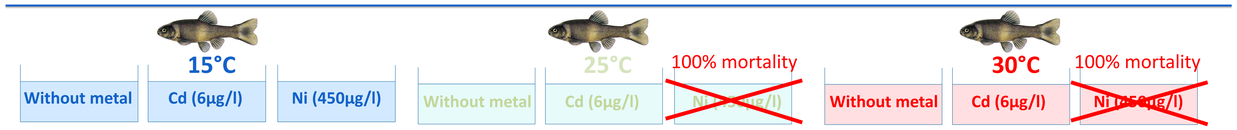
Long-chain polyunsaturated fatty acids (LC-PUFA), are essential for multiple physiological processes, including the maintenance of cell membrane structural integrity [1,2,3,4]. Biosynthesis of these fatty acids involves sequential desaturation and elongation of PUFA precursors. Two groups of enzymes are implicated in this process: desaturases (FADS) which incorporate double bonds into fatty acyl chains and elongases (ELOVL) which catalyze the condensation step in the elongation process [5]. In ectotherms, temperature influences the extent of unsaturation of biological membranes, cold-acclimated animals expressing a higher percentage of membrane phospholipid polyunsaturation compared to warm-acclimated conspecifics [6]. This process is known as homeoviscous adaptation (HVA) and it ensures membrane function and integrity for a range of acclimation temperatures, likely through modulations of desaturase and elongase gene transcription and activity. Some metals, such as Cd and Ni, can induce the production of reactive oxygen species (ROS), which may in turn lead to lipid peroxidation, PUFA being particularly vulnerable to ROS.

Study objectives

The aim of this study is to understand the combined effects of temperature and metal contamination (Cd and Ni) in fathead minnow (*Pimephales promelas*) muscle and brain on

(i) the fatty acid composition of membrane phospholipids;
(ii) the transcription level of desaturase and elongase genes;
(iii) Differences in desaturase and elongase transcription between the two tissues

Materials and methods



Lipid extraction and FAME analysis

Chloroform/methanol extraction

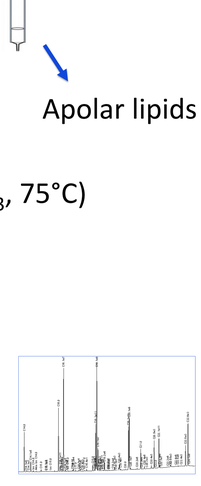
Polar lipids Apolar lipids

Esterification (BF_3, 75°C)

FAME

Purification

GC-FID



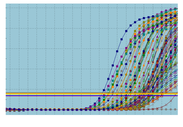
Lipid extraction and FAME analysis

RNA extraction and purification

Reverse transcription

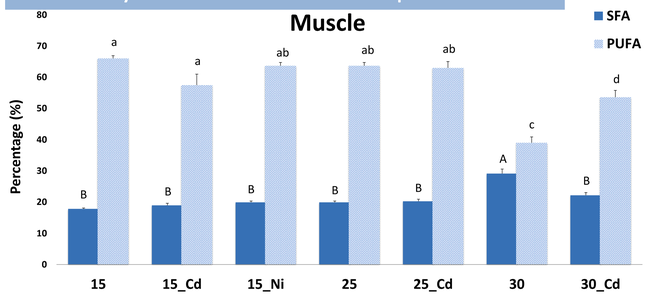
cDNA

Real-time quantitative PCR

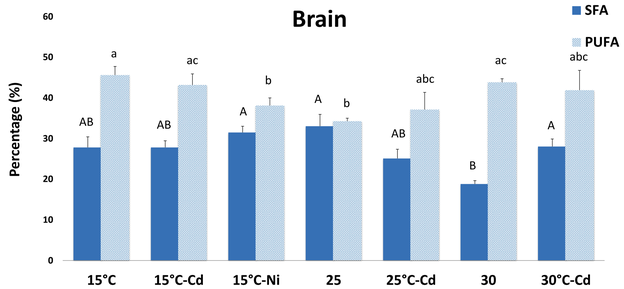


Results and discussion

Fatty acid membrane composition

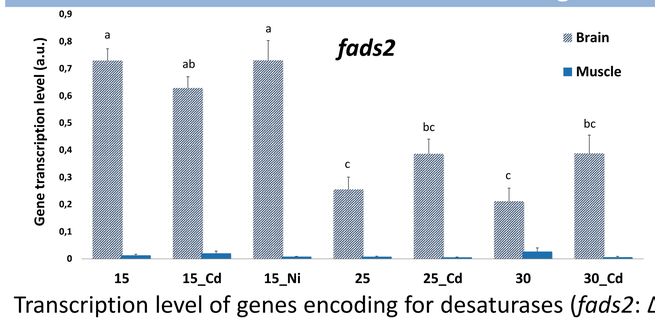


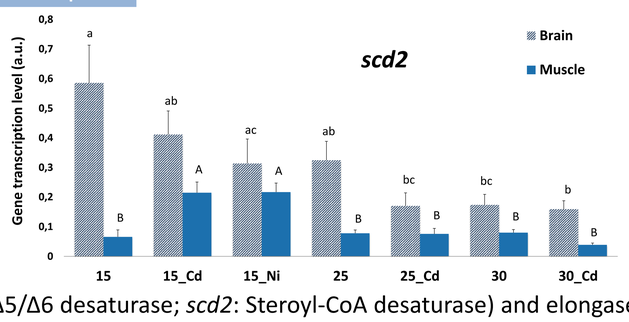
During warm acclimation (30°C) PUFA percentage decreased and SFA increased compared to 15°C and 25°C.
Agrees with the HVA theory.
Cd exposure interfered with the normal warm acclimation response of cell membrane composition at 30°C



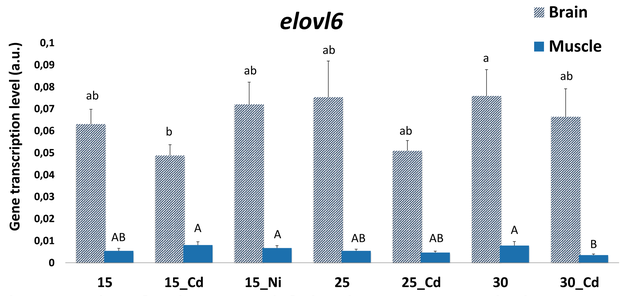
FA composition of brain phospholipids varied little with temperature acclimation compared to muscle.
Except for a decrease at 15°C under Ni exposure, PUFA percentage remained largely unaffected by metals.

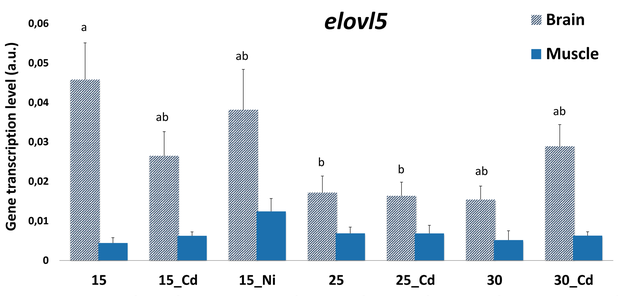
Tissue distribution of desaturase and elongase trasnscripts





Transcription level of genes encoding for desaturases (*fads2*: Δ5/Δ6 desaturase; *scd2*: Steroyl-CoA desaturase) and elongases (*elovl6* and *elovl5* encoding respectively for elongase6 and elongase5) varied between tissues: brain tissue showed a higher level compared to muscle.
Desaturase and elongase genes were induced at low temperatures. Temperature and metal combinations had different effects on the transcription level of desaturase and elongase genes, which did not always correspond to the modifications in fatty acid composition.





In the muscle of Cd-exposed fish, desaturase and elongase transcription levels remained mostly unchanged in spite of a decrease in PUFA percentages. Inversely for Ni at 15°C, despite the increase of gene transcription levels (*elovl6*, *scd2*), PUFA concentrations remained unchanged, suggesting a regulation at the post-transcriptional level.
In the brain, desaturase and elongase genes were mostly induced at low temperature and their level of transcription was lower at 25°C and 30°C. In contrast, PUFA percentage in warm-acclimated fish did not differ from the percentage in cold-acclimated fish. Upregulation of gene expression at colder temperatures likely compensates for cold-induced reductions in enzyme activity, allowing maintenance of PUFA concentrations. This regulation is important as the functions of neural tissues depend highly on membrane structure and processes.

Conclusions

- Combined heat stress (30˚C) and Ni exposure exceeded the metabolic tolerance of fathead minnows, leading to 100% mortality.
- Temperature-induced adjustments in cell membrane phospholipid composition in muscle agree with HVA.
- In contrast to muscle, brain phospholipid composition is largely maintained regardless of temperature, likely due to requirements for neural function.
- Metal exposure affected the normal response of membrane composition to temperature acclimation in muscle, but not in brain.
- Temperature and metal combinations had different effects on desaturase and elongase gene transcription levels.

References

[1] Csoboz, B., G. E. Balogh, E. Kusz, I. Gombos, M. Peter, T. Crul, B. Gungor, L. Haracska, G. Bogdanovics, Z. Torok, I. Horvath and L. Vigh (2013). "Membrane fluidity matters: hyperthermia from the aspects of lipids and membranes." Int J Hyperthermia **29**(5): 491-499.
[2] Hall, J. M., C. C. Parrish and R. J. Thompson (2002). "Eicosapentaenoic acid regulates scallop (Placopecten magellanicus) membrane fluidity in response to cold." Biological Bulletin **202**(3): 201-203.
[3] Hazel, J. R. (1995). "Thermal Adaptation in Biological-Membranes - Is Homeoviscous Adaptation the Explanation." Annual Review of Physiology **57**: 19-42.
[4] Hazel, J. R. and E. E. Williams (1990). "The Role of Alterations in Membrane Lipid-Composition in Enabling Physiological Adaptation of Organisms to Their Physical-Environment." Progress in Lipid Research **29**(3): 167-227.
[5] Jakobsson, A., R. Westerberg and A. Jacobsson (2006). "Fatty acid elongases in mammals: Their regulation and roles in metabolism." Progress in Lipid Research **45**(3): 237-249.
[6] Williams, E. E. and J. R. Hazel (1994). "Thermal Adaptation in Fish Membranes - Temporal Resolution of Adaptive-Mechanisms." Temperature Adaptation of Biological Membranes: 91-106.

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