DISTINGUISHING BETWEEN ENVIRONMENTAL AND METAL STRESS IN WILD YELLOW PERCH (*Perca flavescens*) USING GENOMIC, PHYSIOLOGICAL AND BIOMETRIC INDICATORS

Julie GRASSET[1,2], Bérénice BOUGAS[1], Eric NORMANDEAU[2], Peter G.C.CAMPBELL[1], Louis BERNATCHEZ[2] and Patrice COUTURE[1]

[1] INRS-ETE, 490 de la Couronne, G1K 9A9, Québec QC-Canada / [2] IBIS, Université Laval, Pavillon Charles-Eugène Marchand, 1030 avenue de la Médecine, G1V 0A6, Québec QC-Canada

julie.grasset@ete.inrs.ca

Introduction

Freshwater ecosystems are highly vulnerable to climate change[1]. In particular, temperature variations in aquatic environments affect biological processes in fish[2]. For yellow perch (*Perca flavescens*), effects of temperature have been reported at different levels of organization, from gene expression[3] to the condition factor[4].

In ecotoxicological studies, it is important to be able to distinguish between stress responses due to environmental variables from those induced by contaminants. Furthermore, in the context of a changing climate, we need a better understanding of the combined effects of contaminants and natural stressors.

The objective of this study is to investigate the effects of variations of environmental variables such as temperature, hypoxia and food restriction on the physiology and gene transcription level of yellow perch. The long term objective of this study is to discriminate the specific signature of metal contamination stress in wild fish.

Materials and methods

Lab exposure

- 3 aquariums/condition
- 25 fish/aquaria
- Young-of-the-year fish
- Fish from a fish farm
- 6 weeks of exposure

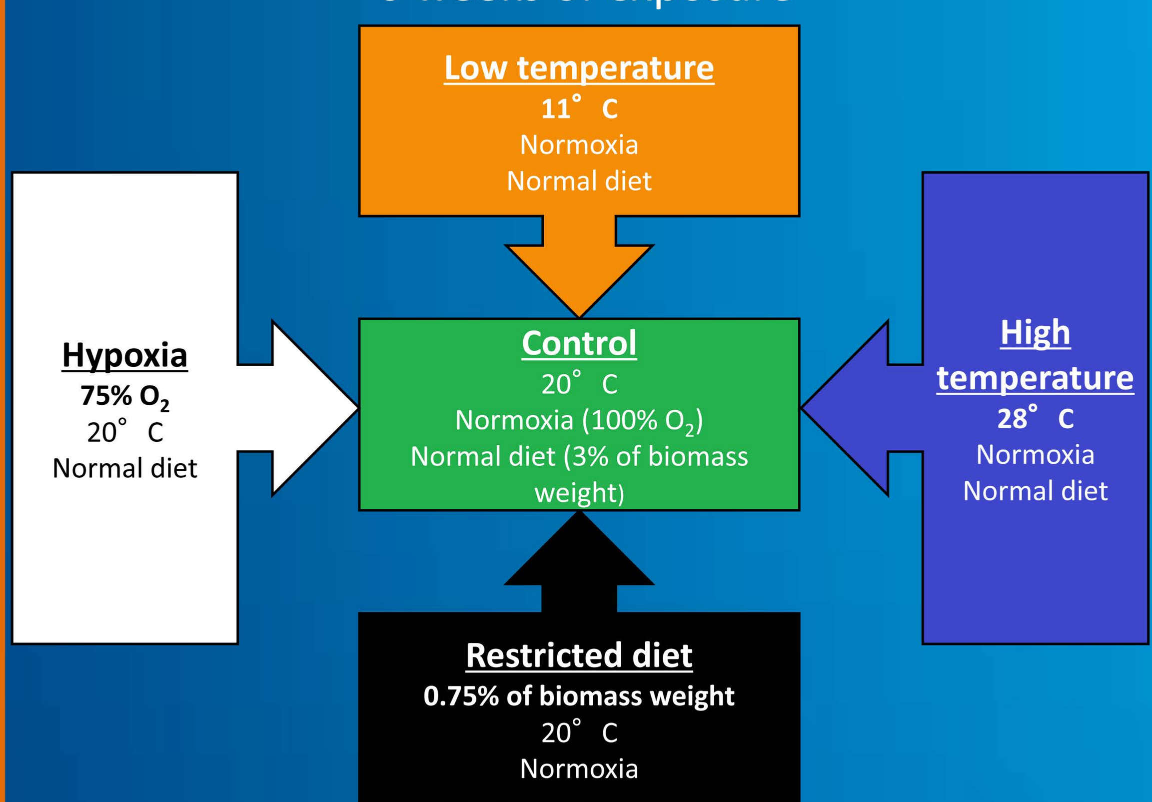

Figure 1: Description of the exposure conditions

Analyses

- Fulton index (condition factor) $FCF=\left(\frac{M}{L^a}\right)\times C$
- Gene expression
  - in the liver (8 samples per condition)
  - 1000 genes microarray
  - 8 genes on qRT-PCR including:
    - Diablo-like protein (*diablo*): apoptosis induction
    - Glucose-6-phosphate dehydrogenase (*g6pdh*): Cell growth and proliferation, synthesis of fatty acids, production of nucleic acids

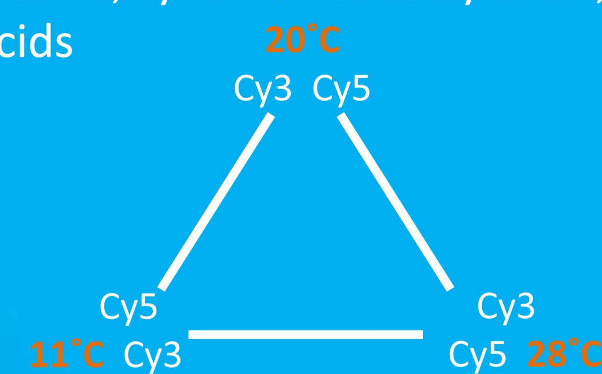

Figure 2: Example of a loop experimental design with dye-swap using the microarray (8 biological replicates by conditions)

- Enzymatic activities
  - in the liver (8 samples per condition)
  - 8 enzymes including:
    - Nucleoside diphosphate kinase (NDPK): biosynthetic capacities of cells
    - Glucose-6-phosphate dehydrogenase (G6PDH): Cell growth and proliferation, synthesis of fatty acids, production of nucleic acids

Results

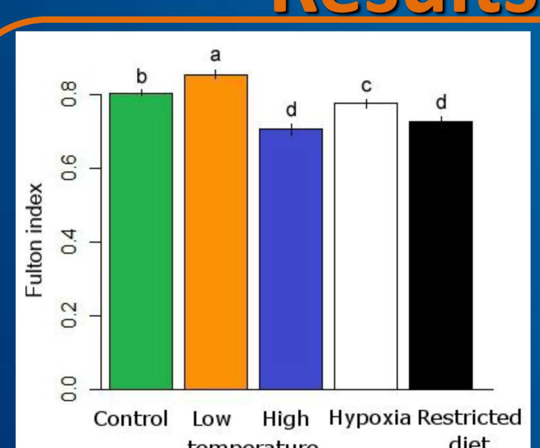

Figure 3 : Fulton index of yellow perch for each variable tested (Pairwise t-test, p-value < 0.05)

- **Biometric indicator (FCF)**

The Fulton Condition Factor significantly decreased with an increase in temperature, as well as under hypoxia and food restriction.

⇒ Indicates that heat, hypoxia and food restriction within the range that can be encountered in the field by this species are stressful conditions reducing energy accumulation in yellow perch.

Results

- **Gene expression**

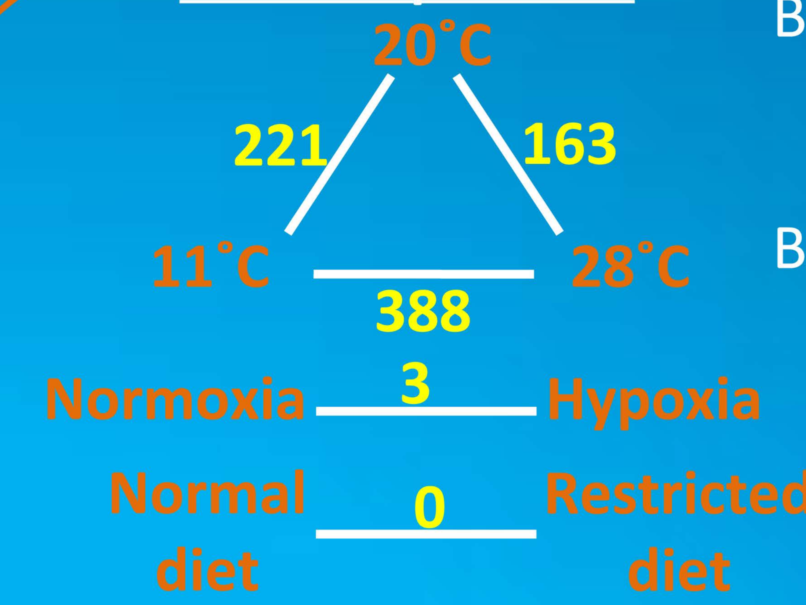

Figure 4: Number of genes showing a significant difference of expression among the different conditions using the microarray (ANOVA, FDR=0.05)

Biological functions represented by overexpressed genes:

- Regulation of cell death and apoptosis
- Biosynthetic processes.

Biological functions represented by underexpressed genes:

- Lipid metabolism
- Ion transport
- Vitamin metabolism
- Sexual differentiation

- **Enzymatic activities** (µmol/min/g)

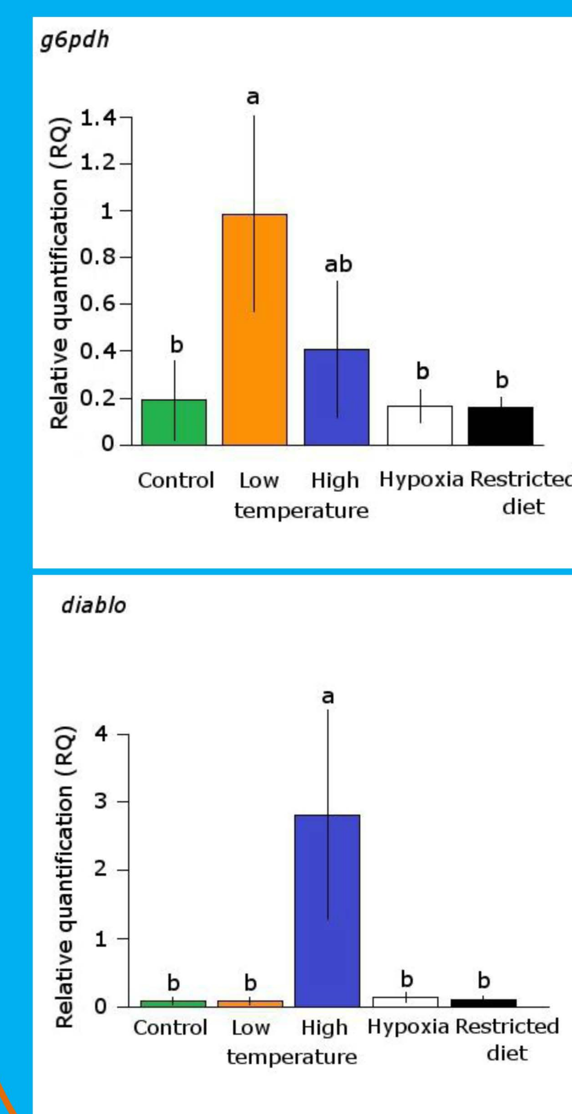

Figure 5: Level of gene expression (*g6pdh* and *diablo*) between the different conditions (Tuckey test, p-value < 0.05)

- Increase of transcription and enzymatic activity of the glucose-6-phosphate dehydrogenase in cold water

⇒ Suggests an increase of growth and proliferation of cells, and of the synthesis of nucleic and fatty acids when the temperature is low. This can be correlated to the increase of the FCF.

- Induction of apoptosis in hot water, regulation in other conditions

⇒ Suggests that heat stress induced the ultimate response to stress

- Decrease of NDPK activity in stressful condition

⇒ Suggests that the stressors examined divert energy away from growth processes

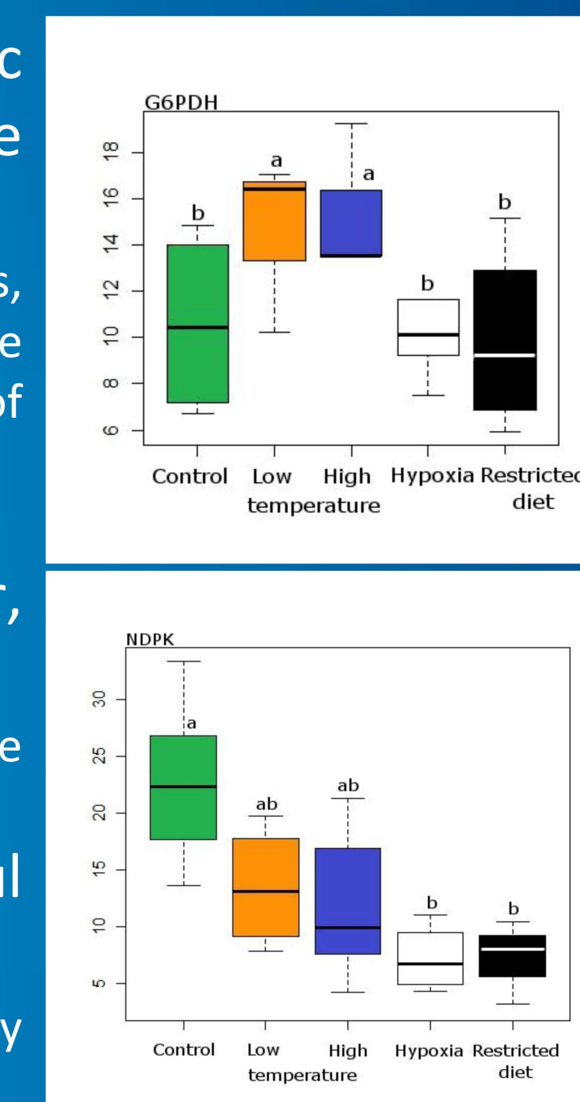

Figure 6 : Enzymatic activities (G6PDH and NDPK) among the different conditions (Pairwise t-test, p-value < 0.05)

Conclusion

The stress induced by an increase in temperature, a decrease of oxygen levels or in food availability, affects the physiological condition of yellow perch. Hypoxia and food restriction lead to simultaneous decreases in the condition factor and in liver activities of NDPK and G6PDH, clearly indicating a reduced capacity for energy accumulation and growth. In contrast, heat stress was the only stressor that induced apoptosis. Heat stress also had a strong negative impact on the condition factor and liver NDPK activity, but liver G6PDH activity remained elevated, suggesting that lipid metabolism was not affected.

Wild fish are naturally exposed to important variations in temperature, oxygen levels and food availability. For wild yellow perch, studies have also reported that metal contamination induces apoptosis, affects the condition factor[5], the expression of several genes and the activities of enzymes including NDPK[6] and G6PDH[7]. Our study highlights the importance of characterizing and considering the effects of natural stressors on biomarkers of metal stress in wild fish.

References

[1] Bates *et al.* 2008, IPCC Secretariat
[2] Cochrane *et al.* 2009, FAO
[3] Glémet *et al.* 2007, Can. J. Fish. Aquat. Sci.
[4] Kitchell *et al.* 1977, J. Fish. Res. Bd. Can.
5 Gauthier *et al.* 2009, Ecotoxicol. Environ. Saf.
6 Couture et Kumar. 2003, Aq.Tox.
[7] Levesque *et al.* 2002, Aq. Tox.

INRS Université d'avant-garde · UNIVERSITÉ LAVAL · IBIS · VALE · CRSNG NSERC · Canada Research Chairs · Chaires de recherche du Canada