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ARTICLE

Temperature, oxygen, and diet modulate gene transcription and metabolic capacities in yellow perch

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Abstract: To unambiguously interpret the impacts of environmental contamination on fish condition, it is important to understand the confounding effects of natural environmental factors. In this study, we measured the effects of temperature (11, 20, and 28 °C), oxygen level, and dietary restriction on yellow perch (*Perca flavescens*) using biometric endpoints and enzymatic and transcriptomic endpoints in liver. Fulton's condition factor (FCF) and pyloric cæca index were significantly correlated (Spearman's coefficient = 0.18; p value = 0.0002) and decreased under heat stress. Both hypoxia and dietary restriction also negatively affected FCF. These changes, indicating modifications in growth and energy accumulation, were also detected at the enzymatic level. Glucose-6-phosphate dehydrogenase activity was only affected by temperature, whereas nucleoside diphosphate kinase activity decreased under hypoxic and restricted diet conditions. Temperature stress was also observed at the transcription level for genes associated with energy metabolism, oxidative stress response, and apoptosis. This study will contribute to a better understanding of the influences of natural stressors on these biomarkers of condition and metabolic capacities, which are commonly used in ecotoxicological studies.

Résumé : Pour interpréter de manière non ambiguë les impacts de la contamination du milieu sur la condition des poissons, il importe de comprendre les effets de confusion des facteurs environnementaux naturels. Nous avons mesuré les effets de la température (11, 20 et 28 °C), de la concentration d'oxygène et de la restriction alimentaire sur la perchaude (*Perca flavescens*) en utilisant des aboutissements biométriques et des aboutissements enzymatiques et transcriptomiques dans le foie. Le facteur de condition de Fulton (FCF) et l'indice du caecum pylorique étaient significativement corrélés (coefficient de Spearman = 0,18; valeur de p = 0,0002) et diminuaient dans des conditions de stress thermique. L'hypoxie et la restriction alimentaire avaient également un effet négatif sur le FCF. Ces changements, qui reflètent des modifications de la croissance et de l'accumulation d'énergie, ont également été notés au niveau enzymatique. Seule la température avait une incidence sur l'activité de la glucose-6-phosphate déshydrogénase, alors que l'activité de la nucléoside diphosphate kinase diminuait dans des conditions d'hypoxie et de restriction alimentaire. Un stress thermique a également été observé au niveau de la transcription pour les gènes associés au métabolisme énergétique, au stress oxydatif et à l'apoptose. L'étude contribuera à une meilleure compréhension des influences de facteurs de stress naturels sur ces biomarqueurs de la condition et des capacités métaboliques couramment utilisés dans les études écotoxicologiques. [Traduit par la Rédaction]

Introduction

Climate change may have deleterious impacts on the fertility, growth, and biodiversity of aquatic, terrestrial, and aerial animals (Cochrane et al. 2009), and freshwater ecosystems are particularly sensitive to these changes (Bates et al. 2008). An increase of temperature in aquatic ecosystems is known to affect physiological processes in fish (Rosenzweig et al. 2007; Cochrane et al. 2009), and it often leads to lower aqueous oxygen concentrations as the solubility of oxygen is inversely related to water temperature. Because variations in temperature and oxygen concentrations affect all components of freshwater ecosystems, food availability can also be predicted to be altered (Bates et al. 2008; Noyes et al. 2009).

As ectothermic organisms, fish possess mechanisms to compensate for temperature variations and their effects on the properties and function of cell components. Changes in the rate of biochemical reactions and in cell membrane fluidity have been observed in response to temperature variations (Guderley 2004). Rises of temperature can increase growth rates and food conversion efficiency (Cochrane et al. 2009). Temperature changes have also been shown to disturb lipid peroxidation and antioxidant defense (Bagnyukova et al. 2007), to induce changes in the activities of the different heat-shock proteins (Buckley et al. 2006), and to lead to changes in energy metabolism, lipid, and carbohydrate levels (Vergauwen et al. 2010).

Hypoxia can cause direct mortality but more commonly results in sublethal effects, such as reduced growth or activity (Arend et al. 2011). Thus, within the range of physiological tolerance, in response to hypoxic conditions, increases of lactate dehydrogenase (LDH) and superoxide dismutase (SOD) activities were observed in spot croaker (*Leiostomus xanthurus*) gills (Cooper et al. 2002). Numerous modifications were also reported in gene transcription levels as a response to hypoxia in zebrafish (*Danio rerio*; Ton et al. 2003). Modifications in the transcription level of genes involved in ATP production and gluconeogenesis have also been reported in the longjaw mudsucker (*Gillichthys mirabilis*) under hypoxia (Gracey

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et al. 2001). Food restriction can also negatively affect fish condition (Weber and Bosworth 2005), induce oxidative stress (Pascual et al. 2003; Morales et al. 2004; Bayir et al. 2011), or lead to changes in the transcription of genes encoding for key metabolic enzymes, such as glucose-6-phosphate dehydrogenase (G6PDH) and nucleoside diphosphate kinase 22 (NDPK) (Masuda et al. 2009). Thermal stress is also well known to induce responses at the transcriptomic level, including genes involved in protection against apoptosis and lipid metabolism (Smith et al. 2013).

In addition to these natural stressors, contaminants may also affect aquatic organisms. A better understanding of contaminant effects requires that stress responses due to environmental variables be distinguished from those induced by contaminants. Because of its high tolerance to temperature fluctuations and contaminants and its ubiquitous presence in Canadian lakes, yellow perch (*Perca flavescens*) is an ideal species to study the effects of environmental stressors on freshwater fish. In particular, yellow perch has been extensively studied in the context of metal contamination in Canadian mining and smelting regions (Kraemer et al. 2005; Couture and Pyle 2008; Rasmussen et al. 2008). Therefore, the objective of this study was to investigate the effects of changes in temperature, oxygen, and food consumption on biometric, enzymatic, and transcriptomic indicators that were shown in earlier studies to respond to metal contamination in yellow perch. Fulton's condition factor (FCF) and pyloric cæca index are biometric indicators commonly reported to be affected by metal contamination (Couture and Rajender Kumar 2003; Iles and Rasmussen 2005; Gauthier et al. 2009). Similarly, the activities of G6PDH (involved in antioxidant defense), NDPK (indicator of biosynthesis), and LDH (indicator of anaerobic capacities) have also been reported to be affected by metal contamination (Levesque et al. 2002; Couture and Pyle 2008; Lapointe and Couture 2010). Finally, we measured the transcription levels of five genes encoding for proteins involved in energy production, response to oxidative stress, and apoptosis (*glucose-6-phosphate dehydrogenase* (*g6pdh*), *cytochrome c oxidase* (*cco*), *superoxide dismutase* (*sod*), *diablo-like protein* (*diablo*), and *heat shock protein 90kDa beta member 1* (*hsp90b1*)). These genes were selected on the basis of a previous microarray study in our research group that made use of the same experimental fish as this study (Bougas et al. 2013).

Materials and methods

Fish

Young of the year yellow perch were purchased from Kinmount Fish Farm (Kinmount, ON, Canada) and transported to the Laboratoire de Recherche en Sciences Aquatiques (LARSA) at Université Laval (Québec, QC), where they were transferred to a 1 m^3 circular tank. They were maintained for acclimatization for 1 month and fed daily with frozen brine shrimp (*Artemia salina*) with a ration corresponding to 3% of their biomass. During the two first weeks of acclimatization, the temperature was progressively raised from 17 to 20 °C (intermediate temperature). At the end of the acclimatization period, 25 fish were placed in each 40 L glass aquarium, at a density of 160 g biomass per aquarium. A temperature of 20 °C with constant water flow was maintained for an additional acclimatization period of three weeks, after which experimental conditions were initiated.

At the end of the experiment, fish were sacrificed by a blow to the head, then measured, weighed, and immediately dissected. Pyloric cæca, liver, kidney, and muscle were collected and immediately frozen in liquid nitrogen for further analysis. Biometric measures were performed for all the individuals.

Exposure conditions

Each condition, described below, was replicated in three separate aquaria, yielding a total of 75 fish per condition. Fish were divided among various projects, and a number were set aside for method optimizations. For this project, to examine physiological and transcriptomic endpoints, 18 fish were randomly sampled from each experimental condition: 10 yellow perch were used to measure gene expression using qRT–PCR, and 8 were used to measure enzyme activities. After acclimatization, yellow perch were exposed to different conditions of temperature, hypoxia, or dietary restriction for 45 days. The temperature was gradually increased or reduced at a rate of 2 °C per day to reach the experimental temperatures. Fish were exposed to low (11 °C), intermediate (20 °C), or high (28 °C) temperature in three aquaria for each temperature, without changes in other conditions (100% O_2 and food ration at 3% of biomass per day). The intermediate temperature condition was chosen as a control for hypoxia and restricted diet conditions. Hypoxia (75% O_2 saturation, achieved by limiting the air exchange and water aeration) was maintained in three aquaria, with other conditions as in the control (20 °C and food ration at 3% of biomass per day). In the last three aquaria, fish were maintained under a restricted diet (0.75% of biomass per day, 20 °C, and 100% O_2 saturation). This restricted diet level was chosen based on a previous study (Gauthier et al. 2008) where this ration led to a significant reduction of perch growth.

Sample analysis

Biometric measures

Fish condition was estimated using two biometric measurements. FCF was calculated with the following equation: FCF = (weight (g)/length $(cm)^3$) × 1000 (Nash et al. 2006). The pyloric cæca index was calculated as described by Gauthier et al. (2009), using the following equation: $W_{corr} = (W_m/W_f)^b W_c$ where W_{corr} is the corrected mass of the pyloric cæca, W_m is the mean of the fish mass for the dataset, W_f is the fish mass, b is the slope of the logarithmic relationship between fish mass and cæcum mass (0.3109 for this dataset), and W_c is the uncorrected mass of the pyloric cæca. The pyloric cæca index is a good indicator of recent feeding history of fish and of food availability in their environment (Gauthier et al. 2008).

Enzyme analyses

Enzyme activities were measured using a UV/Vis spectrophotometer (Varian Cary 100; Varian Inc., Palo Alto, Calif., USA) on a microplate at room temperature (20 °C). Reactions were recorded over a period of 5 min, and a linear portion of at least 3 min was used to calculate reaction rates.

All chemicals were bought from Sigma-Aldrich (Canada), except for thymidine diphosphate (TDP), which was purchased from Plenum Scientific Research Inc. Livers were homogenized in ice-cold buffer (pH 7.5, 20 mmol·L^{-1} HEPES, 1 mmol·L^{-1} EDTA, 0.1% Triton X-100) for three bursts of 20 s using an Ultra Turrax T25 tissue homogenizer.

Reaction conditions for the enzymes selected in this study were as follows:

Nucleoside-diphosphate kinase (NDPK; EC 2.7.4.6): Imidazole buffer (imidazole 50 mmol·L^{-1}, $MgCl_2$ 20 mmol·L^{-1}, KCl 70 mmol·L^{-1}), pH 7.5, β-NADH 0.24 mmol·L^{-1}, adenosine triphosphate (ATP) 2 mmol·L^{-1}, phospho(enol) pyruvate (PEP) 1.1 mmol·L^{-1}, LDH and PK in excess, TDP 70 mmol·L^{-1} (omitted in controls).

Glucose 6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49): Imidazole buffer (imidazole 50 mmol·L^{-1}, $MgCl_2$ 25 mmol·L^{-1}, KCl 70 mmol·L^{-1}), pH 7.5, β-nicotinamide adenine dinucleotide phosphate (NADP) 0.3 mmol·L^{-1}, glucose-6-phosphate 200 mmol·L^{-1} (omitted in controls).

Lactate dehydrogenase (LDH; EC 1.1.1.27): Phosphate buffer 100 mmol·L^{-1}, pH 7.0, β-nicotinamide adenine dinucleotide (β-NADH) 0.16 mmol·L^{-1}, and pyruvate 0.5 mmol·L^{-1}.

RNA extractions and qRT–PCR analysis

Total RNA was extracted from liver of 40 fish corresponding to 10 individuals per condition (temperature, oxygen, restricted

Table 1. Description of the selected genes for qRT–PCR and the primers used for each.

Gene name	Gene symbol	Biological pathway	Forward primer	Reverse primer
glucose-6-phosphate dehydrogenase	*g6pdh*	Glucose metabolism	CCAAATGCTCTGCCTGGTTGCCA	TCCCCACATATGACCCAGCACCA
diablo-like protein	*diablo*	Regulation of programmed cell death	ATGGCAGCCGAGGCAGCAAAC	TCCGAGCTTCCCCCACCTGC
heat shock protein 90kDa beta member 1	*hsp90b1*	Negative regulation of programmed cell death	GCGTCAGATCTGGCTGTGGTTCTG	CGGAGCATGCGCTCTATCCTCTGT
cytochrome c oxydase	*cco*	Mitochondrial respiratory chain	AAAACCCCCTGCCATCTCCCAA	TGCCAGCGGCAAGAACAGGT
superoxide dismutase	*sod*	Antioxidant defense	TGAGCAGGAGGAGGGTTCATCCCC	CCTGCACTGATGCACCCGTTTGT
β-actin	*β-actin*	Reference gene	GCCTCTCTGTCCACCTTCCA	GGGCCGGACTCATCGTACT

diet). Liver RNA extractions were performed using the PureLink RNA mini kit from Ambion following the manufacturer's protocol, and a DNase treatment (10 U·μL^{-1}) was made on the entire RNA sample. RNAs were reverse-transcripted into cDNA with a high-capacity cDNA reverse transcription kit (Applied Biosystems, Life Technologies, Carlsbad, Calif., USA).

The five genes selected for this study were chosen from a parallel microarray study of our research group that made use of the same experimental fish as this study (Bougas et al. 2013). Specific primers were determined using PrimerBlast (NCBI software) (Table 1). qRT–PCR analyses were performed in triplicate using Fast SYBR Green Master Mix (Applied Biosystems) in the 7500 Fast Real-Time PCR System (Applied Biosystems) following the protocol developed by Pierron et al. (2009). The transcript abundance for each gene was calculated as relative quantification normalized (RQ) according to the expression of the β-actin gene as a reference gene.

Statistical analysis

Statistical analyses to measure the differences between the five groups of fish for biometric, enzymatic, and transcript abundances were performed using R software (version 2.13.0). Comparisons among fish groups were performed using analysis of variance (ANOVA, p value < 0.05) after verifying the homoscedasticity and the normality of the data. ANOVAs were followed by Tukey–Kramer post-hoc tests (p value < 0.05) or Wilcoxon Mann–Whitney post-hoc tests (p value < 0.05).

To assess correlations among FCF and transcript abundances, enzymatic activities, and the pyloric cæca index, Spearman coefficients (α = 0.05) were calculated to test for correlation using JMP software (version 7.0; SAS Institute Inc., Cary, N.C., USA) on the ln-transformed data.

Results

Biometric response to environmental stressors

Temperature, hypoxia, and food restriction significantly affected FCF. Fish exposed to the coldest temperature (11 °C) exhibited the highest FCF, whereas FCF values in fish exposed to high temperature (28 °C) or dietary restriction were significantly lower than in fish from any other conditions. Finally, fish exposed to hypoxia had a significantly lower FCF compared with fish from the intermediate and low temperature groups (Fig. 1).

FCF was positively correlated to the other biometric index, the pyloric cæca index (Table 2). FCF was positively correlated with the expression of *g6pdh* and with the activity of G6PDH and NDPK.

The pyloric cæca index was highest in fish exposed to the low temperature and lowest in fish exposed to the high temperature. Hypoxia and food restriction yielded intermediate values (Fig. 2).

Response of enzyme activities to environmental stressors

The activities of the three enzymes examined varied significantly among experimental conditions. Liver NDPK activity (Fig. 3*a*) was lowest in fish exposed to hypoxia or dietary restriction and about three-fold lower than in controls. In contrast, exposure to low or high temperature did not lead to significant changes in liver NDPK activity.

Liver G6PDH activity was only affected by temperature changes (Fig. 3*b*); at both low and high temperatures, the increase in activity was statistically the same compared with the intermediate temperature. Liver G6PDH and NDPK activities were strongly positively correlated (Spearman's coefficient = 0.78; p value < 0.0001; n = 40).

Liver LDH activity was affected by temperature with lower values in cold compared with intermediate and high temperatures (Fig. 3*c*). Food restriction also led to an important decrease in liver

Fig. 1. Fulton's condition factor for each exposure condition (mean ± SE; n = 150 for the medium temperature and n = 75 for other conditions). Bars with different letters differ significantly (p < 0.05; Tukey post-hoc test).

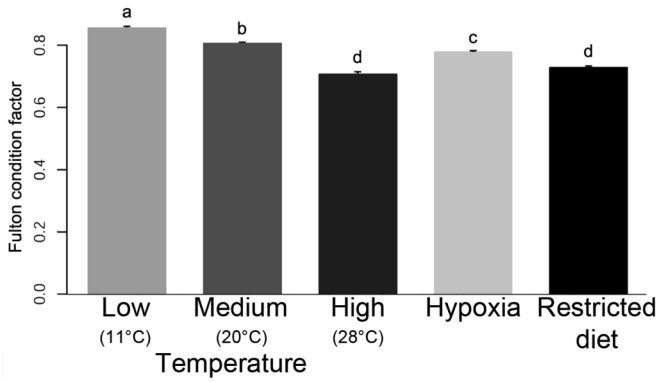


Table 2. Spearman correlations with p values between Fulton's condition factor (FCF) and the other variables examined.

Analysis	n	Spearman correlation	p
Pyloric cæca index	450	0.18*	0.0002
diablo–RQ	50	−0.11	0.42
g6pdh–RQ	50	0.34*	0.009
hsp90b1–RQ	50	0.12	0.39
cco–RQ	50	0.22	0.11
sod–RQ	50	0.01	0.93
NDPK (UI·g wet mass^{-1})	40	0.32*	0.04
G6PDH (UI·g wet mass^{-1})	40	0.43*	0.005
LDH (UI·g wet mass^{-1})	40	−0.02	0.88

Note: RQ corresponds to the relative quantification normalized to a reference gene, the *β-actin* gene. *, significant at p < 0.05.

Fig. 2. Pyloric cæca index for each exposure condition (mean ± SE; p value < 0.05; n = 150 for the medium temperature and n = 75 for other conditions). Bars with different letters differ significantly ($p < 0.05$; Tukey post-hoc test).

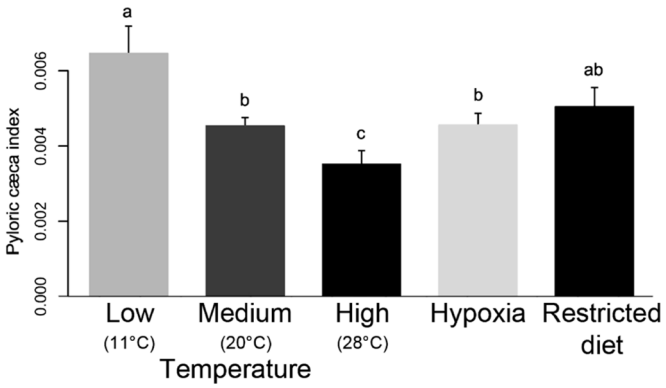
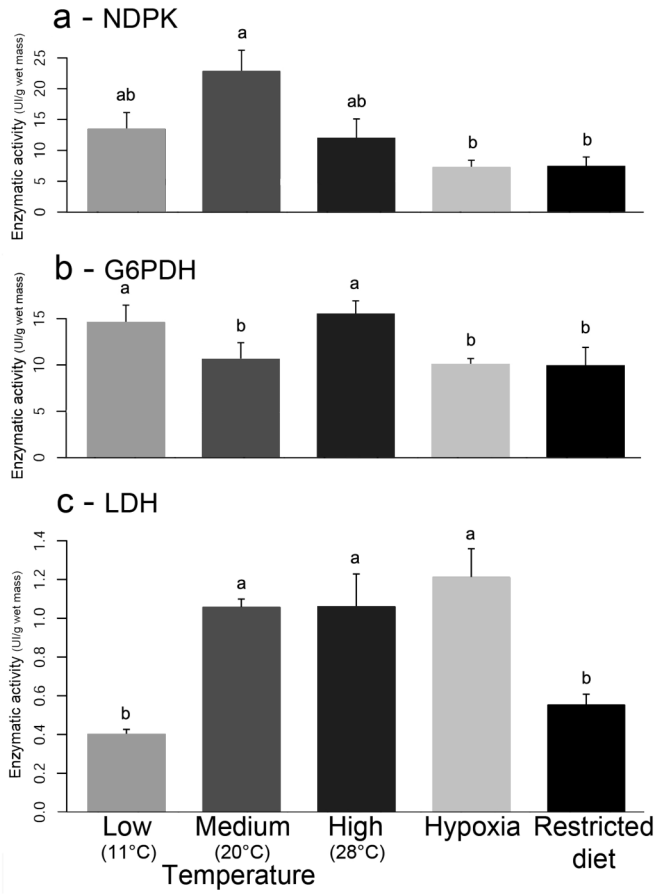


Fig. 3. Liver enzyme activities of (*a*) NDPK, (*b*) G6PDH, and (*c*) LDH for each exposure condition (mean ± SE; n = 8 in each condition). Bars with different letters differ significantly ($p < 0.05$).

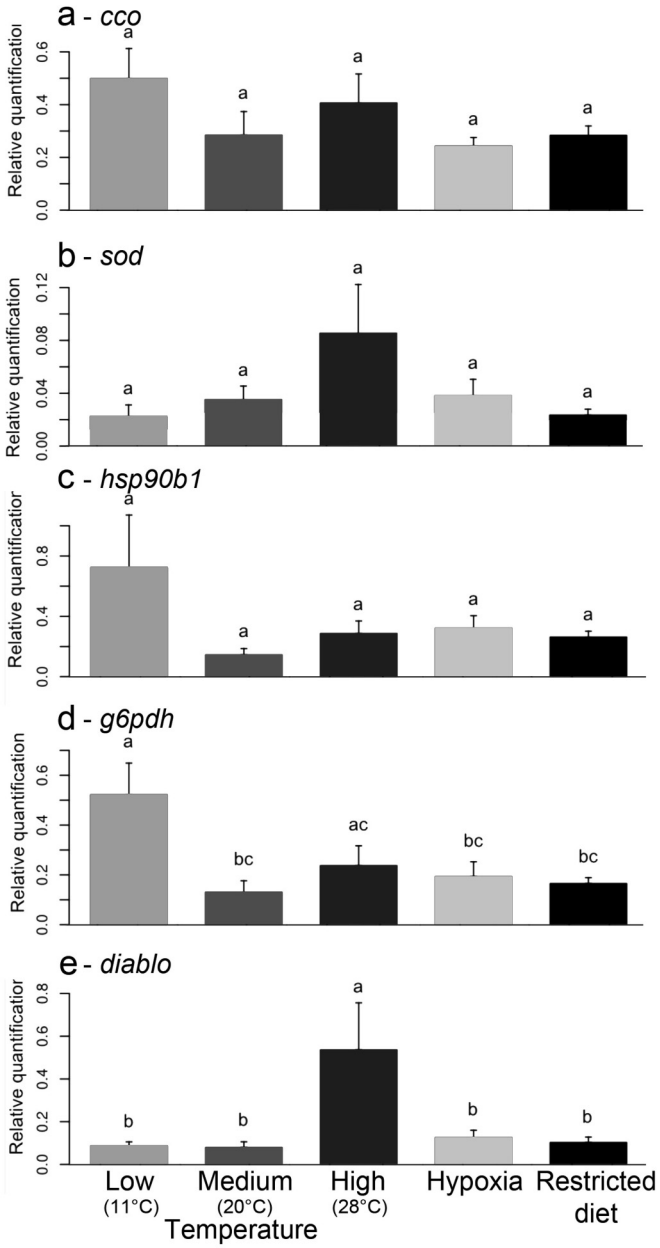


LDH activity. Surprisingly, there was no effect of hypoxia on liver LDH activity.

Gene transcription levels among different experimental conditions

Only temperature affected the transcription levels of the genes examined (Fig. 4). There was no evidence that hypoxia or food restriction affected gene transcription. A high variability in gene transcription levels combined with low sample size may have prevented several trends from being statistically significant,

Fig. 4. Liver transcription levels of (*a*) *cco*, (*b*) *sod*, (*c*) *hsp90b1*, (*d*) *g6pdh*, and (*e*) *diablo* genes as measured by qRT–PCR for each exposure condition (mean ± SE; n = 10 in each condition). Bars with different letters differ significantly ($p < 0.05$).



namely for *cco*, *sod*, and *hsp90b1*. The *g6pdh* gene showed a five-fold increase in transcription levels in fish from the low temperature (11 °C) compared with the control (20 °C) (Fig. 4*d*). The transcription level of the apoptotic gene (*diablo*) showed a statistically significant increase at 28 °C compared with the control and the other conditions, with a transcription level almost 30 times higher than for the other temperature conditions (Fig. 4*e*).

Discussion

Temperature effects

Changes in temperature are known to modify the development and growth of yellow perch (Staggs and Otis 1996). Our results illustrate these modifications, as indicated by FCF, which decreased significantly with an increase of the thermal regime, suggesting that among the three temperatures tested, 11 °C was optimal for

growth and energy accumulation. This is supported by the other morphometric biomarker studied, the pyloric cæca index, which also decreased with increasing temperatures. The pyloric cæca index has been reported to reflect food availability in yellow perch (Gauthier et al. 2008), but in our experiment this is unlikely given that fish from all temperature groups were fed the same ration. An alternative explanation is that an increase in metabolic rate with temperature may lead to higher dietary requirements, leading to an effective under-feeding in fish exposed to the warmer temperatures. This hypothesis is supported by the high correlation we observed between FCF and pyloric cæca index, as also reported by Gauthier et al. (2008).

Temperature-induced changes in fish condition were to some extent reflected by enzyme activities. In particular, the increased activity of liver G6PDH at low temperature corresponded to high values of FCF and pyloric cæca index. This enzyme is involved in the pentose phosphate pathway, which provides reducing energy for the cells, for cell growth and proliferation, and fatty acid synthesis. At low temperature, the increase of G6PDH activity, therefore, reflects the accumulation of energy stores. In agreement with G6PDH activity, the same thermal response was observed for the transcription levels of the *g6pdh* gene (Fig. 4*d*), suggesting a steady state between RNA and protein levels of glucose 6-phosphate dehydrogenase in yellow perch liver.

Exposing perch to a high temperature not only led to a decrease in condition, but it also induced a thermal stress sufficient to trigger an apoptotic response indicative of oxidative stress, as indicated by the sharp increase of the transcription level of *diablo* gene transcription level. Indeed, as shown in studies on apoptosis and cancer cells, diablo proteins act as activators of apoptotic cell death (Portt et al. 2011; Wong 2011). Our results are also supported by a parallel study in which a microarray revealed that the transcription levels of genes involved in apoptosis, such as the *jun oncogene, diablo-like protein*, and *transcriptional regulator myc* genes, were upregulated in yellow perch maintained at 28 °C (Bougas et al. 2013). Studies in other fish species have also linked heat stress to increased apoptosis (Yabu et al. 2001; Buckley et al. 2006; Ito et al. 2008). Interestingly, it has been shown that in cell lines, an increase in G6PDH activity led to improved defenses against oxidative stress-induced cell death, especially because this enzyme also plays a role as an antioxidant in response to oxidative stress by generating NADPH (Tian et al. 1999; Fico et al. 2004). Because exceeding the optimal temperature leads to modifications of mitochondrial properties, which in turn increase oxidative stress (Pörtner 2002), the increased liver G6PDH activity and gene transcription level in heat-stressed perch observed in our study may also be interpreted as an antioxidant cellular response aimed at limiting apoptosis.

Food restriction and hypoxia

In designing this study, we chose the intermediate temperature (20 °C) to expose fish to hypoxia and dietary restriction, as this temperature is typical of that encountered in yellow perch habitat at the end of spring. Therefore, in the discussion below, the intermediate temperature group will serve as a reference to compare the metabolic and genomic responses of fish to hypoxia and dietary restriction.

Food restriction and hypoxia induced similar responses for most of the endpoints examined. Hypoxia induced a slight but significant decrease of FCF, in agreement with the literature. Chabot and Dutil (1999) showed that the growth rate of Atlantic cod (*Gadus morhua*) was directly proportional to the degree of water oxygenation. However, in contrast to its reaction to heat stress, the pyloric cæca index did not respond to hypoxia, suggesting that under hypoxic stress, energy derived from ingested food was diverted towards combatting stress instead of growth. In support of this hypothesis, liver NDPK activity was strongly affected by hypoxia, with a four-fold decrease compared with control (20 °C) conditions. Furthermore, because neither G6PDH activity nor gene transcription level were affected by hypoxia, this strongly supports the view that energy demand remained high in hypoxic fish. However, this energy was likely diverted towards fighting the metabolic consequences of oxygen reduction and oxidative stress, as it has been shown in many other studies (Cooper et al. 2002; Zhang et al. 2009; Sussarellu et al. 2010), leading to a reduction in biosynthesis and consequently in fish condition. Nevertheless, the lack of response of liver LDH activity, an indicator of anaerobic capacities, in hypoxic fish was unexpected. Indeed, there are reports for other fish species that hypoxia induced increases in LDH activity in other tissues such as the gill and brain (Cooper et al. 2002; Mandic et al. 2012). However, our results agree with the study of Mandic et al. (2012) who did not observe increased liver or muscle anaerobic capacities in sculpins exposed to hypoxia.

Fish response to food restriction was remarkably similar to the response to hypoxia, with the exception of liver LDH activity, which was significantly lower in food-restricted fish. The decrease in FCF in food-restricted fish demonstrates that the condition was severe enough to induce a mobilization of reserves to sustain metabolism, a well-established phenomenon in the literature (Gauthier et al. 2008). Although the relative pyloric cæca mass did not respond to food intake reduction, this is likely due to the slow downward response of this parameter to reductions in feeding regime in yellow perch, in contrast to its faster positive response under conditions of increasing food availability. Indeed, in an earlier experiment, we demonstrated that in yellow perch fed the same maximal and minimal rations as in this study, a decrease of feeding rate during six weeks did not lead to a significant decrease of this index, whereas it increased significantly after six weeks of feeding at the maximal rate (Gauthier et al. 2008).

Changes in LDH activity with food ration, as reported here, are well documented. For yellow perch, Gauthier et al. (2008) reported that muscle LDH activity in yellow perch was strongly and positively related to feeding rate. The same phenomenon has been documented for Atlantic cod (Pelletier et al. 1993; Dutil et al. 1998). In these earlier studies, muscle LDH activity was positively correlated with condition factor, a phenomenon that was interpreted by Houlihan et al. (1988) as an indication that tissue cytosolic proteins like LDH act as protein stores that can be rapidly mobilized in situations of fasting. In contrast, Couture et al. (1998) reported a decrease of liver LDH activity with increasing condition in Atlantic cod, but an actual increase when LDH activity was calculated on the basis of lipid-free liver mass. This is because Atlantic cod accumulate large quantities of lipids in their livers during periods of intense feeding, unlike yellow perch, which accumulate visceral lipids instead. Hence, our study supports evidence that feeding increases liver LDH activity in fish.

Finally, it is important to note that because yellow perch exposed to hypoxia and food restriction were maintained at 20 °C and that this temperature was not optimal for growth, although typical of temperatures to which these fish are subjected during the growth season, it is possible that the biometric, physiological, and transcriptomic responses to hypoxia and food restriction measured in this study could have been more pronounced if fish had been maintained at a colder temperature. Future studies should examine the combined effects of these stressors to better understand how they may interact in nature to affect indicators of growth.

In conclusion, wild fish are exposed to multiple stressors, which affect to various extents biomarkers of growth and condition. This study has demonstrated that the condition factor is affected by heat stress, hypoxia, and dietary restriction, whereas the pyloric cæca index was only affected by temperature, at least in the five-week time course of the experiment, which would correspond to short-term variations in food availability in the wild. The transcription level of the *diablo* gene was only, but very strongly, affected by temperature, so this biomarker should be prioritized in

multi-stress studies of wild yellow perch to identify potential heat stress. In addition, as demonstrated here the temperature stress can affect several physiological and genomic biomarkers commonly used in ecotoxicological studies. For instance, our study revealed that G6PDH activity is influenced by environmental temperature. Therefore, as for the condition factor or pyloric cæca index, its use in field studies should be coupled with measurement of the *diablo* gene to ensure that differences among study groups are not due to heat stress. In contrast, because NDPK activity was affected by hypoxia and food restriction but not by temperature, interpretation of differences in its activity in ecotoxicological studies can ignore variations in water temperature but should be prudent about potential differences in oxygen levels and food availability among the populations compared.

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