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Do differences in the activities of carbohydrate metabolism enzymes between Lake Whitefish ecotypes match predictions from transcriptomic studies?☆

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ABSTRACT

Transcriptomic studies are facilitating the search for the molecular bases of adaptation in natural populations, but the impact of these differences in mRNA content on animal physiology are often unknown. One way to determine if molecular changes have the potential to influence animal physiology and performance is to test for correlated changes at higher levels of biological organization, including enzyme activity. Here, we measure the activities of carbohydrate metabolism enzymes to test if previously documented genetic and transcriptomic variation between 'dwarf' and 'normal' Lake Whitefish ecotypes are associated with corresponding changes in enzyme activity (measured as maximal rate, V_{max}) in liver and skeletal muscle. We use laboratory-reared fish from the same populations as prior transcriptomic studies and find that white muscle mRNA content is a good predictor of glycolytic and glycogen metabolism enzyme activity, and dwarf whitefish have evolved higher activities than normal whitefish. However, the differences in hepatic mRNA content found between ecotypes in prior studies are not associated with comparable changes in enzyme activity. For example, dwarf whitefish have lower enzyme activities, but higher transcript abundances for two glycolytic enzymes compared to normal whitefish. Overall, we find that transcriptomic studies successfully highlight evolutionary variation in enzyme activities, but not always in the direction predicted, indicating that a variety of tissue-specific regulatory mechanisms contributed to the evolution of energy metabolism in Lake Whitefish.

1. Introduction

A major goal in comparative physiology is to understand the mechanisms linking genotype to phenotype (Mykles et al., 2010). In particular, evolutionary physiologists are interested in determining how genetic and molecular variation contributes to differences in physiology, performance and fitness within and among populations and species (Barrett and Hoekstra, 2011; Savolainen et al., 2013). A common approach to detecting intra-specific molecular variation has been to measure transcriptomic divergence and then identify candidate genes, pathways and biochemical networks associated with ecologically-important phenotypic divergence (Pavey et al., 2010; Alvarez et al., 2015; Pardo-Diaz et al., 2015). However, evolutionary variation in mRNA content is often buffered at the level of protein abundance and differences in protein abundance can arise via translational or post-translational mechanisms (e.g. Khan et al., 2013; Battle et al., 2015). Therefore, it is important to test if changes in mRNA abundance are associated with comparable changes in protein content, enzyme activity, and cellular function among populations and species when studying the molecular bases for phenotypic evolution (Dalziel et al., 2009; Diz et al., 2012; Evans, 2015). Agreement between changes in gene expression and enzyme activities supports the hypothesis that transcriptomic variation has the potential to influence physiology and performance, but is rarely tested among populations of wild, non-model organisms (but see Pierron et al., 2009; Rees et al., 2011; Nikinmaa et al., 2013; Rokyta et al., 2015).

The Lake Whitefish (*Coregonus clupeaformis*) is one species for which genomic and transcriptomic studies have detected extensive molecular variation associated with local adaptation and ecological speciation (Bernatchez et al., 2010). The Lake Whitefish is a freshwater, salmonid

Abbreviations: ALDO, aldolase; GPI, phosphoglucose isomerase; PFK, phosphofructokinase; TPI, triosephosphate isomerase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PK, pyruvate kinase; LDH, lactate dehydrogenase; CK, creatine phosphokinase; PYG, glycogen phosphorylase; GYS, glycogen synthase; GPD, glycerol 3 phosphate dehydrogenase

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Table 1
Summary of genomic, transcriptomic, biochemical, and whole-organismal studies comparing central energy metabolism and phenotypes associated with energy expenditure and production in dwarf and normal Lake Whitefish ecotypes. For information on the specific genes within each biochemical pathway listed below see Tables 2, S1 and S2.

Reference	Tissue	Populations (rearing conditions)	Traits or loci measured	Divergence in energy metabolism between dwarf and normal?
Rogers et al. (2002)	–	Témiscouata dwarf & Alymer normal pure crosses and F1 hybrids (lab-reared)	Swimming activity	*Yes* — dwarf fish swim more actively than normal fish
Rogers and Bernatchez (2005)	–	Témiscouata dwarf & Alymer normal pure crosses and backcrosses (lab-reared)	Growth	*Yes* — normal fish grow more quickly than dwarf fish and sexually mature at a later age
Rogers and Bernatchez (2007)	–	Témiscouata dwarf & Alymer normal backcrosses (lab-reared)	Swimming activity, growth, QTL mapping, genome scan	*Yes* — selection on loci (anonymous) associated with higher swimming activity and decreased growth rate in dwarf fish
Derome et al. (2006)	White muscle	Cliff Lake & Indian Pond (wild)	mRNA content (3.7 K microarray)	*Yes* — variation among ecotypes in mRNA content for glycolytic genes, oxidative phosphorylation genes, creatine kinase and glycerol 3 phosphate dehydrogenase. Direction of expression change varied among lakes and genes.
Derome et al. (2008)	White muscle	Témiscouata dwarf & Alymer normal pure crosses and backcrosses (lab-reared)	mRNA content (16 K microarray)	*Yes* — lower mRNA content of oxidative phosphorylation genes and higher mRNA content of creatine kinase in dwarf than normal fish, and differential expression of glycolytic genes that varied in direction among lakes and genes.
Whiteley et al. (2008)	Brain	Témiscouata dwarf & Alymer normal pure crosses and backcrosses (lab-reared)	mRNA content (16 K microarray)	*Yes* — lower mRNA content of oxidative phosphorylation genes in dwarf than normal fish.
St-Cyr et al. (2008)	Liver	Cliff Lake & Indian Pond (wild); Témiscouata dwarf & Alymer normal pure crosses (lab-reared)	mRNA content (16 K microarray)	*Yes* — dwarf fish had higher mRNA content for glycolytic genes and lower mRNA content for glycerol 3 phosphate dehydrogenase compared to normal fish. Differential expression of oxidative phosphorylation and citric acid cycle genes that varied in direction among lakes and genes.
Nolte et al. (2009)	Whole embryos, juveniles	Témiscouata dwarf & Alymer normal pure crosses (lab-reared)	mRNA content (16 K microarray)	*No* — in embryos. *Yes* — in juveniles. Dwarf fish had higher mRNA content for glycolytic and glycogenolytic genes, creatine phosphokinase and glycerol 3-phosphate dehydrogenase than normal fish. Differential expression of oxidative phosphorylation genes that varied in direction among genes.
Renaut et al. (2009)	Whole embryos	Témiscouata dwarf & Alymer normal pure crosses, F1 crosses and backcrosses (lab-reared)	mRNA content (16 K microarray)	*Yes* (embryos) — dwarf fish had lower mRNA content of an oxidative phosphorylation gene. *Yes* (juveniles) — dwarf juveniles had higher mRNA content of glycolytic genes, glycogenolytic genes and glycerol 3-phosphate dehydrogenase, and lower mRNA expression of oxidative phosphorylation genes than normal fish. Creatine kinase genes were differentially expressed but the direction of change varied among lakes and isoforms. One creatine kinase isoform (CK-B) showed transgressive segregation.
Jeukens et al. (2009)	White muscle, Liver	Cliff Lake & Indian Pond (wild); Témiscouata dwarf & Alymer normal pure crosses (lab-reared)	mRNA content of candidate genes (RT-PCR)	*Yes* — wild and lab-reared dwarf fish had a higher mRNA content for a glycolytic gene in muscle (LDH A).
Jeukens et al. (2010)	Liver	Cliff Lake (wild)	mRNA content (RNA-seq)	*Yes* — wild dwarf fish had higher mRNA content for glycerol 3 phosphate dehydrogenase and most glycolytic, glycogenolytic, and glycogenic genes than normal fish. Differential expression of gluconeogenic, citric acid cycle and oxidative phosphorylation genes that varied in direction among lakes and genes.
Renaut et al. (2010)	–	Cliff Lake (wild), Témiscouata dwarf & Alymer normal backcrosses (lab-reared)	Genetic divergence (SNP genotyping)	*Yes* — differences in allele frequencies of creatine kinase isoforms and glycolytic, citric acid cycle and oxidative phosphorylation genes.
Renaut and Bernatchez (2011)	Whole embryos	Témiscouata dwarf & Alymer normal pure crosses & backcrosses, normal and malformed (lab-reared)	mRNA content (16 k microarray)	*No* — differences in energetic gene expression detected.
Renaut et al. (2011)	–	Cliff, Indian, Webster, East & Témiscouata Lake (wild); Témiscouata dwarf & Alymer normal pure crosses (lab-reared)	Genetic divergence (SNP genotyping)	*Yes* — differences in allele frequencies of glycolytic, gluconeogenic and oxidative phosphorylation genes. One glycolytic gene (TPI) associated with condition factor.
Jeukens and Bernatchez (2012)	–	Cliff Lake & Indian Pond (wild)	Genetic divergence of candidate gene (Sanger sequencing)	*Yes* — differences among ecotypes in allele frequencies of a citric acid cycle gene (cytoplasmic MDH) and signatures of selection in promoter.
Evans and Bernatchez (2012)	White muscle	Cliff & Webster lakes (wild)	mRNA content (RT-PCR)	*Yes* — dwarf fish generally had a higher mRNA content of oxidative phosphorylation genes.
Evans et al. (2012)	Blood, kidney, brain, gill	Cliff, Indian, East & Témiscouata lakes (wild)	Red blood cell morphology, hemoglobin isoform and mRNA content	*Yes* — wild dwarf fish had more cathodic hemoglobins, red blood cells with larger nuclei, and higher hemoglobin mRNA content in the brain.
Evans et al. (2013)	Ventricle, gill, brain	Cliff, Indian, East & Témiscouata lakes (wild)	Tissue masses and gill surface area	*Yes* — wild dwarf fish have smaller gills and slightly larger ventricles. Brain size

(continued on next page)

Table 1 (*continued*)

Reference	Tissue	Populations (rearing conditions)	Traits or loci measured	Divergence in energy metabolism between dwarf and normal?
				varied among lakes.
Hébert et al. (2013)	–	Cliff Lake (wild)	Genetic divergence (Sequencing)	*Yes* — differences in allele frequencies of creatine kinase and glycolytic, gluconeogenic and oxidative phosphorylation genes.
Filteau et al. (2013)	White muscle, brain	Témiscouata dwarf & Alymer normal pure crosses and backcrosses (lab-reared)	Gene co-expression (mRNA content - 16 K microarray)	*Unknown* – muscle – data on pure parental crosses not available. *No* — brain
Dion-Côté et al. (2014)	Whole embryos	Témiscouata dwarf & Alymer normal pure crosses and backcrosses (lab-reared)	mRNA content (RNA-seq)	*Yes* — dwarf embryos had higher mRNA content of creatine kinase and glycolytic, citric acid cycle, and oxidative phosphorylation genes. Many glycolytic, citric acid cycle, oxidative phosphorylation and fatty acid metabolism genes are under-expressed in malformed backcross embryos, indicating these loci are involved in hybrid dysfunction.
Dalziel et al. (2015)	White muscle, red muscle, heart	Témiscouata dwarf & Alymer normal pure crosses (lab-reared, same fish as used in this study)	Enzyme activities	*Yes* — dwarf fish had a higher percentage of red muscle, larger ventricle, higher white muscle mitochondrial content and higher activities of enzymes involved in the citric acid cycle and oxidative phosphorylation in red and white muscles than normal fish.
Laporte et al. (2016)	Liver, gill, brain	Témiscouata dwarf & Alymer normal pure crosses (lab-reared, same fish as used in this study)	Enzyme activities	*Yes* — dwarf whitefish had smaller brains, larger livers, and higher activities of oxidative phosphorylation enzymes in the liver than normal fish. Gill size was similar among ecotypes.
Dalziel et al. (2017)	Heart, liver, white muscle	Cliff, Indian, East & Témiscouata lakes (wild)	Tissue masses and enzyme activities	*Yes* — all dwarf whitefish populations have a higher percentage of red muscle, three have a larger liver, and two have larger ventricles than sympatric normal populations. Differences among lakes in the direction of variation in hepatic and white muscle glycolytic, glycogenolytic, glycogenic, oxidative phosphorylation, and citric acid cycle enzyme activities among ecotypes.

fish found in lakes and rivers across North America (Scott and Crossmann, 1998). Much of the species' current distribution was covered by ice during the Pleistocene glaciation, separating populations into different refugia for up to ~60,000 years (Bernatchez and Dodson, 1990, 1991; Pigeon et al., 1997; Jacobsen et al., 2012; Rougeux et al., in press). When the glaciers retreated (~12–20,000 years ago), several lineages came into secondary contact and have since undergone continued genetic and phenotypic divergence, leading to the evolution of the limnetic, 'dwarf' ecotype from an ancestral epi-benthic, 'normal' ecotype (Bernatchez et al., 2010). The dwarf ecotype has evolved repeatedly, and independently, across the species range (Vuorinen et al., 1993; Bernatchez et al., 1996; Pigeon et al., 1997), suggesting a strong role of natural selection in evolutionary divergence.

In lakes within the St. John River Basin (Maine, USA and Québec, Canada), the dwarf form has evolved from populations of the Acadian glacial lineage and extant normal populations are derived from the Atlantic lineage (Pigeon et al., 1997; Lu et al., 2001). The dwarf ecotype is hypothesized to have evolved in response to competition for limited resources and ecological opportunity (Landry et al., 2007; Landry and Bernatchez, 2010), and is a more active swimmer with a more streamlined body, lower condition factor, higher active metabolic rate, slower growth rate, earlier maturity, and more limnetic-like feeding morphology than the ancestral-like, normal ecotype (Bernatchez et al., 1999; Trudel et al., 2001; Rogers et al., 2002; Rogers and Bernatchez, 2005; Laporte et al., 2015; Laporte et al., 2016). Quantitative trait loci associated with phenotypic divergence among ecotypes display evidence of selection and further argue that these dwarf ecotypes have evolved via natural selection (Rogers et al., 2002; Rogers and Bernatchez, 2005, 2007; Bernatchez et al., 2010; Gagnaire et al., 2013a; Laporte et al., 2015).

In the past decade, a combination of transcriptomic (Derome et al., 2006, 2008; St-Cyr et al., 2008; Whiteley et al., 2008; Jeukens et al., 2009, 2010; Nolte et al., 2009; Renaut et al., 2009; Evans and Bernatchez, 2012; Filteau et al., 2013; Dion-Côté et al., 2014) and genomic (Renaut et al., 2010, 2011; Hébert et al., 2013; Gagnaire et al., 2013b; Laporte et al., 2015) studies have documented the molecular variation associated with adaptive divergence between whitefish ecotypes. While hundreds of candidate genes have been detected, one functional category consistently varies between ecotypes from multiple different lakes in the St. John River Basin: central energy metabolism (Tables 1, S1, S2; Bernatchez et al., 2010). This suggests that, as in many other species, differences in energy metabolism are a key contributor to local adaptation (e.g. Eanes, 2011; Zera, 2011; Marden, 2013; Cheviron et al., 2014).

In particular, dwarf whitefish tend to have a higher white muscle mRNA content of genes encoding glycolytic and glycogen metabolism enzymes and lower mRNA content of oxidative phosphorylation genes than normal whitefish when reared under common laboratory conditions (Derome et al., 2008; Jeukens et al., 2009; Nolte et al., 2009; Table 2). In liver, lab-reared dwarf whitefish also have higher glycolytic mRNA content than normal whitefish, but oxidative phosphorylation genes show little divergence (St-Cyr et al., 2008). Recently, Dalziel et al. (2015) found that the activities of oxidative phosphorylation enzymes in white muscle are higher in lab-reared dwarf whitefish than normal whitefish, opposite to findings for mRNA content in the same lab-reared populations (Derome et al., 2008; Nolte et al., 2009; Fig. 1). Furthermore, Laporte et al. (2016) found that the activities of hepatic oxidative phosphorylation enzymes are higher in lab-reared dwarf than normal whitefish, despite little difference in mRNA content (Fig. 1). This disparity between mRNA content and enzyme activity suggests that translational or post-translational regulation may underlie the evolution of energy metabolism in Lake Whitefish (Suarez and Moyes, 2012).

The primary goal of this study was to test if the activities of enzymes participating in carbohydrate metabolism in the liver and white muscle varied among ecotypes as predicted by differences in mRNA abundance

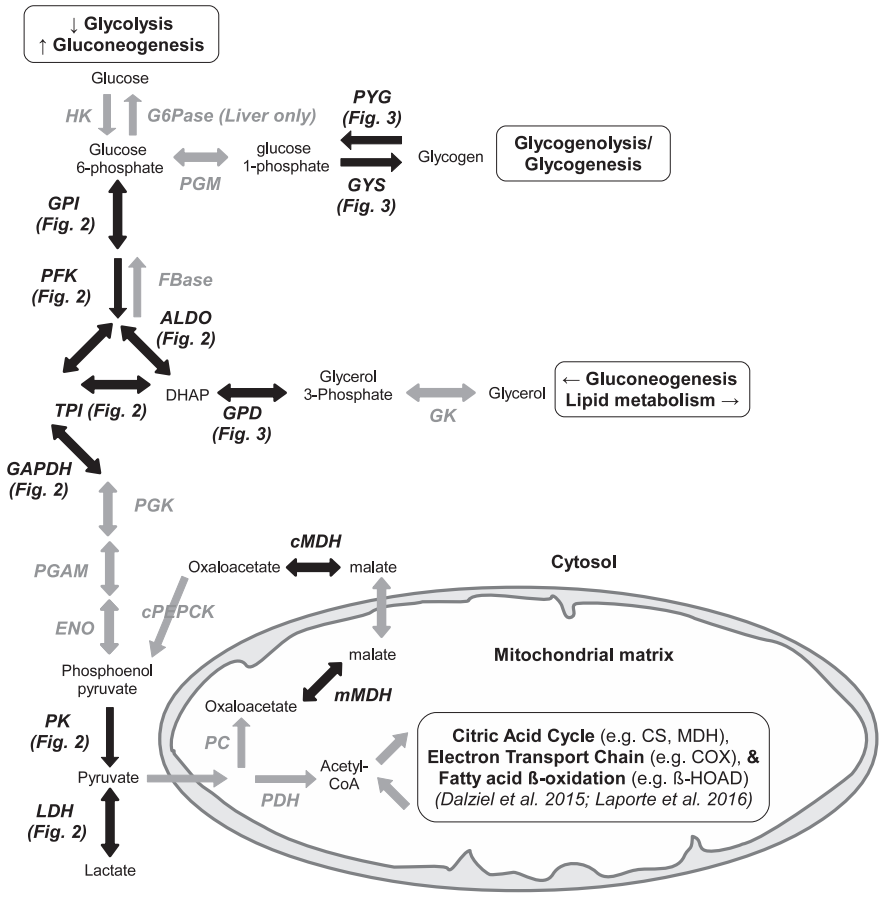

Fig. 1. Summary of biochemical pathways measured in this paper and associated studies (Dalziel et al., 2015; Laporte et al., 2016). Enzyme names are in bold and italicized and those measured in the lab-reared crosses used in this study in black text (current paper, Dalziel et al., 2015, or Laporte et al., 2016) and unmeasured enzymes in grey. Substrates are in plain text, and pathway names are boxed. Many intermediates are omitted and only the cytosolic isoform of phosphoenolpyruvate carboxykinase (PEPCK), is displayed for clarity. Abbreviations are as follows: hexokinase (HK), phosphoglucose isomerase (GPI), phosphofructokinase (PFK), aldolase (ALDO), triosephosphate isomerase (TPI), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), phosphoglycerokinase (PGK), phosphoglycerate mutase (PGAM), enolase (ENO), pyruvate kinase (PK), lactate dehydrogenase (LDH), glucose 6-phosphatase (G6Pase), phosphoglucomutase (PGM), glycogen phosphorylase (PYG), glycogen synthase (GYS), fructose 1,6-bisphosphatase (FBPase), glycerol 3 phosphate dehydrogenase (GPD), glycerokinase (GK), malate dehydrogenase (MDH), pyruvate carboxylase (PC), pyruvate dehydrogenase complex (PDH), beta hydroxyacyl CoA dehydrogenase (β-HOAD), cytochrome *c* oxidase (COX), citrate synthase (CS). Candidate enzymes were chosen based upon prior transcriptomic and genetic studies of Lake Whitefish dwarf and normal ecotypes (reviewed in Table 1, S1 and S2).

detected in prior studies (Tables 1, 2, S1, S2; Derome et al., 2008; St-Cyr et al., 2008; Jeukens et al., 2009; Nolte et al., 2009). mRNA content was measured in the same populations, but not the same individuals, in which we measure enzyme activities. Therefore, this study focuses upon population-level variation among ecotypes, not individual variation. We also note that differences among ecotypes in gene expression and enzyme activity vary among populations of wild Lake Whitefish ecotypes found at different points on the speciation continuum (Evans and Bernatchez, 2012; Dalziel et al., 2017, Tables 1, S1 and S2); in this paper we focus on a set of laboratory-reared dwarf and normal populations on which the majority of transcriptomic, genomic and quantitative genetic studies have been conducted (reviewed by Bernatchez et al., 2010; Bernatchez, 2016). Secondly, by rearing fish in a common laboratory environment and varying their activity levels (Dalziel et al., 2015; Laporte et al., 2016), we were able to test if differences in enzyme activities are likely to be genetically based and characterize the extent of phenotypic plasticity in glycolytic (i.e., GPI, PFK, ALDO, TPI, GAPDH, PK, LDH), glycogenolytic (i.e., PYG) and glycogenic (i.e., GYS) enzyme activities. Finally, we measured enzyme activities in red skeletal muscle, for which we have no prior gene expression data, to better understand how different tissue-types contribute to divergence among ecotypes (Trudel et al., 2001; Rogers et al., 2002; Rogers and Bernatchez, 2005).

2. Materials and methods

2.1. Experimental families & swim-training methods

We studied Témiscouata Lake dwarf and Alymer Lake normal whitefish bred and reared in the lab by Dalziel et al. (2015) and Laporte et al. (2016). These fish are from the same populations used in prior transcriptomic studies (Derome et al., 2006; St-Cyr et al., 2008; Jeukens et al., 2009; Nolte et al., 2009), and reared under similar conditions in the same facility at Université Laval, but are not the same individuals. To make crosses, parents were caught in Témiscouata Lake (dwarf whitefish, Acadian lineage, 47°36′N, 68°45′W) and Aylmer Lake (normal whitefish, Atlantic lineage, 45°50′N, 71°26′W) and brought back to the Laboratoire de Recherche en Sciences Aquatiques (LARSA, Université Laval, Québec, Canada) for breeding. Gametes from multiple females (seven to nine) and males (seven to fourteen) were mixed to ensure the genetic background of these crosses was representative of the populations. Crosses were made following the methods of Rogers et al. (2002) and Nolte et al. (2009) and reared in a flow through system under identical temperatures and lighting schedules, and fed *Artemia* nauplii enriched with Selco and algae. To minimize genetically based differences in body size among ecotypes (Rogers et al., 2002), and the effects of allometric variation on enzyme activities (Moyes and LeMoine, 2005), the normal cross was kept at a slightly higher density than dwarf whitefish (but all tanks were < 0.8 kg/m^3) for the first 16 months of life, at which point fish were sampled. At the end of the experiment 16-month old dwarf fish weighed 4.89 g ± 0.16 g and were 8.57 cm ± 0.10 cm long, while 16 month old normal fish weighed 5.06 g ± 0.21 g, and were 8.06 ± 0.12 cm long. There were no significant differences in mass among groups (Dalziel et al., 2015), but dwarf whitefish were more slender than normal whitefish as expected from genetically based differences in shape (Laporte et al., 2015).

We also tested the effect of swim-training on enzyme activities. We tested this variable because dwarf and normal whitefish show genetically based differences in the propensity for swimming, such that limnetic dwarf whitefish are more active swimmers than benthic normal whitefish (Rogers et al., 2002). Furthermore, carbohydrate metabolism enzymes often show phenotypic plasticity with exercise training in fish (e.g. Martin-Perez et al., 2012). The swim-training experiment is more

fully described in Dalziel et al. (2015) and Laporte et al. (2016) and began when fish were ~16 months of age. We used 129 fish (64 dwarf and 65 normal) and swam 'trained' fish for four to seven months, for 6 h per day, at speeds of ~1.2–1.4 body lengths per second (BL/s). This speed is approximately 33% of this species' critical swimming speed based upon data from Bernatchez and Dodson (1985) and was chosen to mimic the limnetic foraging of wild dwarf whitefish. Fish were randomly assigned to 'control' or 'swim-training' tanks (dwarf and normal whitefish combined) and all sampling and measurements were conducted 'blind', as the two ecotypes cannot be easily distinguished by morphology and were identified via genotyping after the experiment was finished (described by Dalziel et al., 2015).

2.2. Sample collection

Full information on sample collection is given by Dalziel et al. (2015). Briefly, after euthanizing fish we collected tissues for enzyme assays by cutting a 3 mm thick muscle 'steak' ~3 mm posterior to the cloaca with a razor blade. We then dissected the liver and removed the gallbladder. All samples were immediately frozen in liquid nitrogen prior to storage at − 80 °C. Red and white muscle fibres were separated just prior to enzyme assays from frozen steaks (see next section). All protocols were approved by Université Laval's animal care committee (Protocol 82178).

2.3. Activities of metabolic enzymes in skeletal muscles and liver

2.3.1. Sample preparation

We measured enzyme activities in red and white muscle samples isolated from frozen steaks and whole livers. See Supplementary Tables S3–S5 for sample sizes for each tissue, which ranged from 6 to 16 individuals per experimental treatment (ecotype and swim-training treatment) per tissue. Glycolytic enzymes and creatine phosphokinase were measured in tissues homogenized in 20 volumes (white muscle and liver) or 60 volumes (red muscle) of chilled buffer following Moyes et al. (1997) and Martinez et al. (2006). This included the enzymes: phosphoglucose isomerase (GPI, EC 5.3.1.9), phosphofructokinase (PFK, EC 2.7.1.11), aldolase (ALDO, EC 4.1.2.13), triosephosphate isomerase (TPI, EC 5.3.1.1), glyceraldehyde 3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12), pyruvate kinase (PK, EC 2.7.1.40), lactate dehydrogenase (LDH; EC 1.1.1.27), and creatine phosphokinase (CPK, EC 2.7.3.2). Glycolytic enzymes and CPK from white muscle were measured from − 80 °C frozen homogenates from Dalziel et al. (2015) and liver glycolytic enzymes were measured in new homogenates frozen prior to assays to match freeze/thaw conditions.

Enzymes participating in glycogenesis, glycogenolysis and glycerol metabolism were measured in liver and white muscle samples only (not red muscle). We measured the activities of active and total glycogen phosphorylase (PYG; 2.4.1.1), active and total glycogen synthase (GYS; EC 2.4.1.11), and glycerol 3 phosphate dehydrogenase (GPD; EC 1.1.1.8) following the methods of Milligan (2003) and Driedzic et al. (2006) with some modifications. Specifically, samples were homogenized in 4 mL Wheaton glass homogenizers kept at on ice with 10 volumes of buffer (50 $mmol^{-1}$ imidazole, 5 mM EDTA, 5 mM EGTA, 5 mM DTT, 1 mM Roche Pefabloc SC, 1 × Roche PhosSTOP, pH 7.5), with DTT, phosphatase inhibitors, and protease inhibitors added just prior to use.

2.3.2. Enzyme assays

All assays were optimized to ensure that substrates, cofactors, and linking enzymes were not limiting and were conducted at 26 °C with a 96 well plate spectrophotometer (Spectramax 190, Molecular Devices, Sunnyvale, CA, USA). All samples were assayed in triplicate, and background reaction rates were subtracted. Tissue protein content was measured in quadruplicate on all samples with Bradford Reagent and a protein standard curve composed of bovine serum albumen (Sigma-Aldrich).

Final reaction concentrations for GPI, PFK, PK, LDH, and CPK are described by Dalziel et al. (2015). Final reaction concentrations for ALDO were 10 mM KCl, 0.2 mM NADH, 5 U/mL GPD, 14.5 U/mL TPI, and 0.75 mM fructose 1,6-bisphosphate in 100 mM Hepes, pH 7.4. The TPI reaction was conducted in 10 mM KCl, 0.2 mM NADH, 10 U/mL GPD, 2.9 mmol glyceraldehyde 3-phosphate in 100 mM Hepes, pH 7.4, and GAPDH with 10 mM KCl, 2 mM $MgCl_2$, 3.1 mM ATP, 0.2 mM NADH, 8 U/mL PGK, and 2.8 mM phosphoglycerate in 100 mM Hepes, pH 7.4. PYG was measured in the presence of 15 mM $MgSO_4$, 0.5 mM DTT, 0.5 mM NADP, 0.25 mM EDTA, 1 U/mL glucose-6-phosphate dehydrogenase, 1.5 U/mL phosphoglucomutase, 0.01 mM glucose 1,6-bisphosphate, 2 mg/mL glycogen, in 50 mM potassium phosphate, pH 7.3. GYS was measured with 70 mM KCl, 4 mM $MgCl_2$, 0.5 mM PEP, 0.2 mM NADH, 5 U/mL LDH, 5 U/mL PK, 2 mg/mL glycogen in 50 mM Tris, pH 7.8. Cytoplasmic GPD was measured with 0.15 mM NADH, and 2 mM DHAP in 50 mM imidazole, pH 7.2.

2.4. Statistical tests and comparisons to studies of mRNA content

All statistical analyses were conducted with R v3.1.1 (R Development Core Team, 2014). In cases where there was a significant effect of size on enzyme activities we used the residuals from the best-fit least-squared linear regression against mass in subsequent analyses. To test the effects of ecotype (fixed effect, dwarf or normal) and treatment (fixed effect, swim-trained or control) we ran a mixed effects linear model using the nlme package in R with tank nested as a random effect (two-way nested ANOVAs; Pinheiro et al., 2015). All plots were created with ggplot2 (Wickham, 2009). We qualitatively compare our enzyme activity data to previously collected mRNA content measured in white muscle and liver of adult fish (> three years old) and whole-juveniles (four months old) from the same populations (Alymer normal and Témiscouata dwarf) reared in the same facility at Université Laval (Table 2: Derome et al., 2008; St-Cyr et al., 2008; Jeukens et al., 2009; Nolte et al., 2009). All comparisons of enzymes and mRNA are at the population level and not among individual fish. Transcriptomic data from lab-reared embryos, juveniles and adults and other populations of adult whitefish collected from the wild are summarized in the supplemental material (Tables S1, S2; Derome et al., 2006, 2008; St-Cyr et al., 2008; Jeukens et al., 2009, 2010; Nolte et al., 2009; Renaut et al., 2009, 2010; Whiteley et al., 2008; Evans and Bernatchez, 2012; Jeukens and Bernatchez, 2012; Filteau et al., 2013; Gagnaire et al., 2013a, 2013b; Hébert et al., 2013; Dion-Côté et al., 2014; Dalziel et al., 2017).

3. Results

3.1. Activities of metabolic enzymes

3.1.1. White and red muscle

All seven glycolytic enzymes had higher activities in dwarf white muscle than normal white muscle when expressed per mg muscle protein (Fig. 2) and per g tissue (Table S3). A similar trend was found in red muscle, but the difference between ecotypes was much smaller and only phosphoglucose isomerase had a significantly higher activity in dwarf than normal whitefish after differences in total muscle protein content were taken into account (Fig. 2; Table S4; protein content measured by Dalziel et al., 2015). The white muscle of dwarf whitefish also contained higher total activities of glycogen phosphorylase, glycogen synthase, and glycerol 3-phosphate dehydrogenase, than normal whitefish (Fig. 3 and Table S3). The latter 3 enzymes were not measured in red muscle. We found no effect of training on any muscle enzymes after accounting for differences in tissue protein content (Figs. 2, 3, Tables S3, S4).

3.1.2. Liver

In liver, five of the seven measured glycolytic enzymes differed

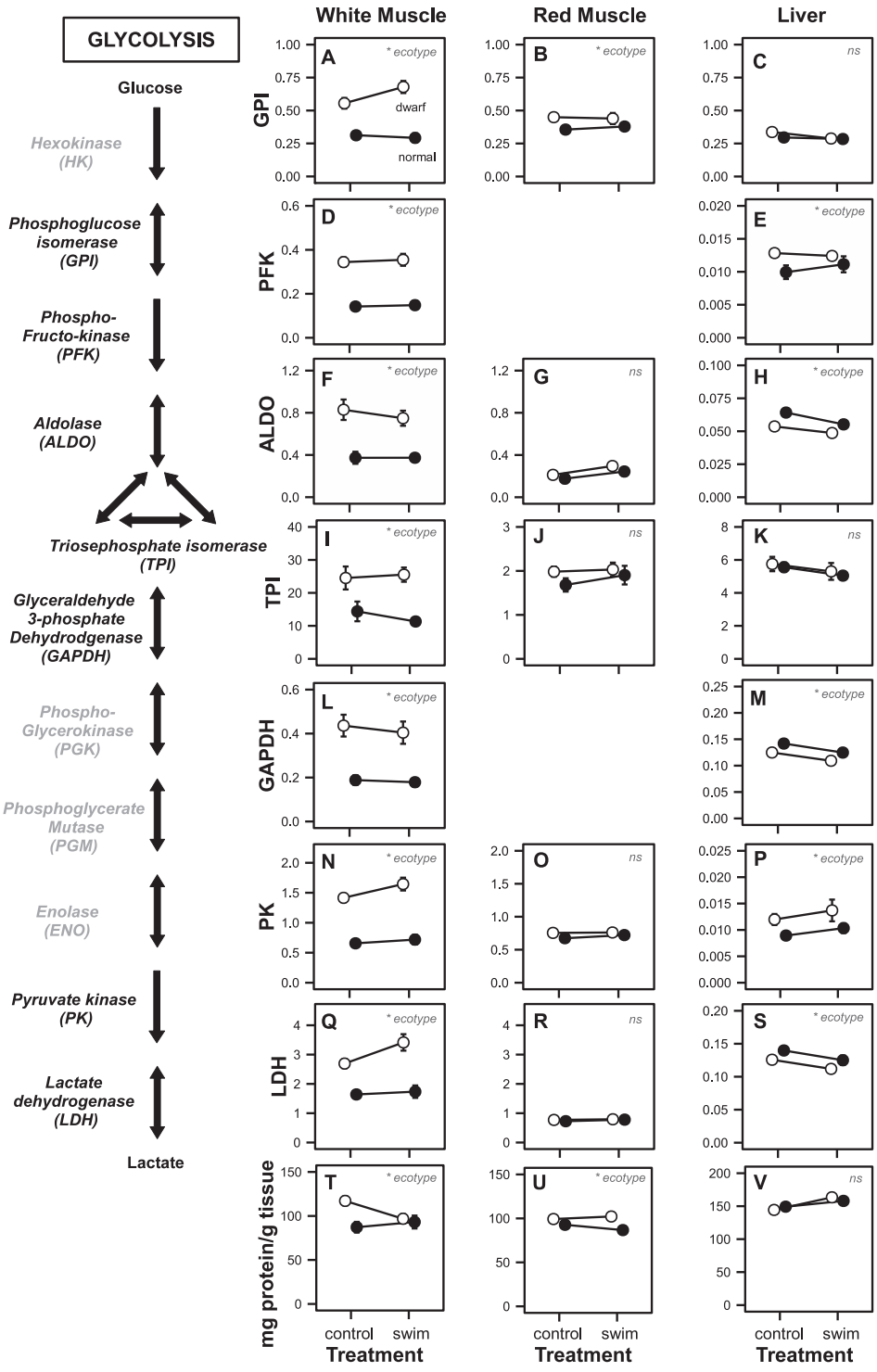

Fig. 2. Activities of glycolytic enzymes in the skeletal muscles and liver of dwarf (white circles) and normal (black circles) whitefish reared in the laboratory under control conditions (control) or with swim-training (swim). Activities are expressed as μmol·min^{-1}·mg of total muscle protein^{-1} and presented as the grand means ± SEM of tank means for each ecotype and treatment (n = 4 tanks in each treatment). Statistical analyses included all individuals in a mixed-effects, nested, two-way ANOVA model. No significant treatment or interaction effects were detected. Significant effects of ecotype are noted with an asterisk and non-significant effects of ecotype are noted with an 'ns'. Samples sizes range from 6 to 15 individuals per group per tissue and full information on sample sizes, statistical results and data expressed per g tissue^{-1} are presented in Supplementary Tables S3–S5.

significantly between ecotypes. However, the direction of variation was enzyme-specific; some of these enzymes were more active in dwarf whitefish livers while others had higher activities in normal whitefish livers (Fig. 2). With respect to hepatic glycogen metabolism, dwarf whitefish had slightly, but not significantly, higher activities of total glycogen phosphorylase (p = 0.09), while glycogen synthase did not differ between ecotypes (Fig. 3). There were no differences in hepatic glycerol 3 phosphate dehydrogenase activity among ecotypes (Fig. 3). We found no effect of training on any hepatic enzymes after accounting for differences in tissue protein content (Figs. 2, 3, Table S5).

3.2. Variation in enzyme activities and mRNA content between ecotypes

We compared differences in enzyme activities per gram tissue between ecotypes to previously measured differences in mRNA content per total RNA in liver and white muscle of lab-reared whitefish (Derome et al., 2008; St-Cyr et al., 2008; Jeukens et al., 2009; Tables 1 and 2). We note that enzyme activities and mRNA content were measured in different individuals from the same populations (Alymer normal and Témiscouata dwarf), so all comparisons are at the population level. We also compared our white muscle enzyme activities to differences in

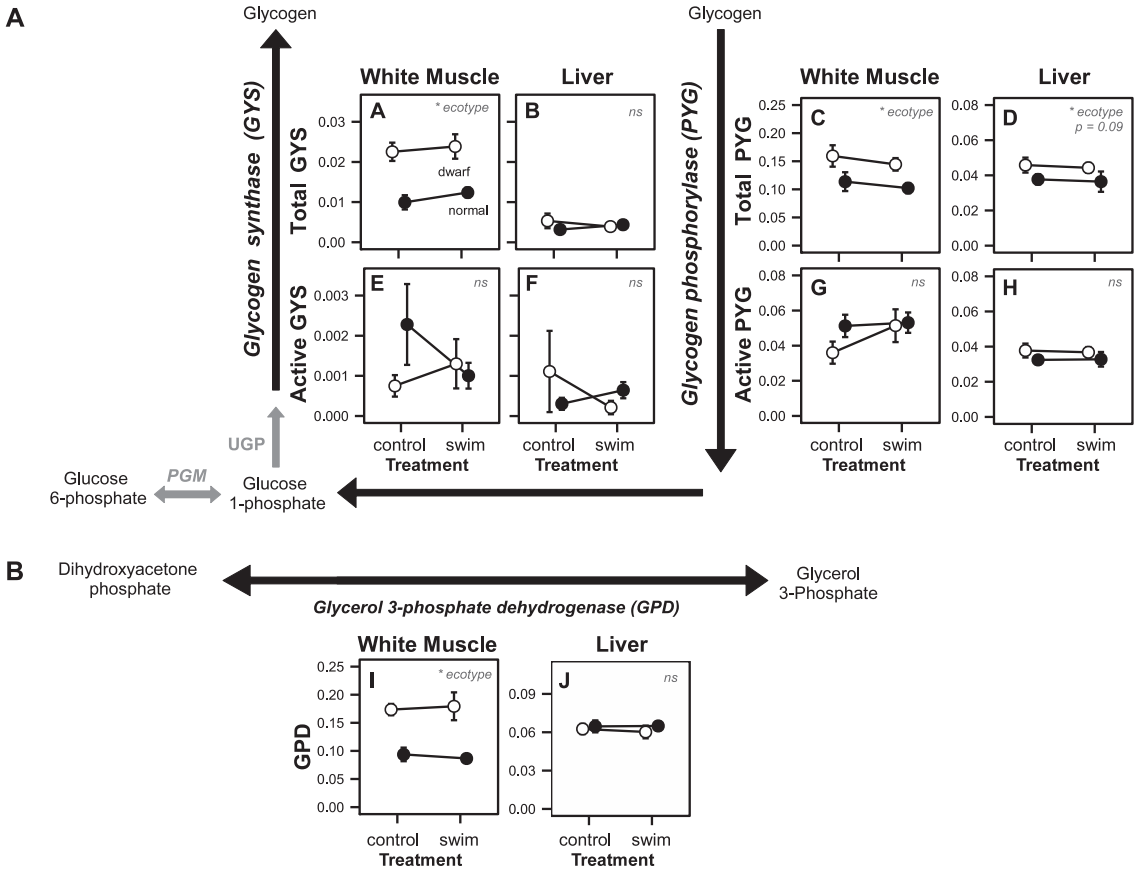

Fig. 3. Enzyme activities of (A) glycogen phosphorylase (PYG), glycogen synthase (GYS), and (B) glycerol 3-phosphate dehydrogenase (GPD) in white muscle and liver of dwarf (white circles) and normal (black circles) whitefish reared in the laboratory under control conditions (control) or with swim-training (swim). Enzymes not measured in this study are noted in grey and many pathway components are excluded for clarity. Additional abbreviations: phosphoglucomutase (PGM), UDP–glucose pyrophosphorylase (UGP), glycerol kinase (GK), no significant differences (ns). Results are presented as in Fig. 2, with full statistical results, sample sizes (9–16 individuals per group per tissue) and data expressed per g tissue^{-1} in Supplementary Tables S3–S5.

muscle-specific isoform expression of lab-reared, whole-juvenile dwarf and normal whitefish (Nolte et al., 2009). Measures of mRNA content and enzyme activities from other, wild-caught sympatric pairs of ecotypes are summarized in Tables S1 and S2 to assess convergence among independently evolved pairs.

3.2.1. White muscle

We found that some differences in mRNA content among ecotypes in adult white muscle (Derome et al., 2006; Jeukens et al., 2009), predicted enzymatic differences among ecotypes. Namely, white muscle pyruvate kinase and lactate dehydrogenase mRNA content and enzyme activities were both higher in dwarf than normal whitefish (Table 2). Alternatively, there were no differences in mRNA content (Derome et al., 2008), but significant differences in enzyme activities among ecotypes for phosphoglucose isomerase, phosphofructokinase, triosephosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, and glycogen phosphorylase. No mRNA content data for the muscle isoforms of aldolase and glycogen synthase or the mitochondrial glycerol 3 phosphate dehydrogenase isoform was available as probes for these genes were not on the microarray used by Derome et al. (2008) (von Schalburg et al., 2005).

We also compared differences in whole-juvenile whitefish mRNA content of muscle-specific isoforms (Nolte et al., 2009) to white muscle enzyme activities among ecotypes, as > 50% of tissue is normally white muscle in fish (Johnston et al., 2011). The whole juvenile fish studied by Nolte et al. (2009) were at a more similar life stage to our experimental fish and their mRNA content was found to be a good predictor of ecotype differences in enzyme activities in our white muscle samples. In particular, juvenile dwarf whitefish had higher mRNA content and enzyme activities for all measured glycolytic and glycogenolytic enzymes, including the muscle specific isoforms for phosphofructokinase, aldolase, pyruvate kinase, and glycogen phosphorylase (Table 2). The only enzyme for which mRNA content did not predict the direction of change between dwarf and normal whitefish was the glycerol 3 phosphate dehydrogenase cytoplasmic isoform, which showed no variation in mRNA content but higher enzymatic activities in dwarf whitefish. Differences in enzyme activity (1.4–2.4 × higher in dwarf when expressed per mg protein) were generally greater than differences in mRNA content (1.2–1.6 × higher in dwarf when expressed per g total RNA) among ecotypes (Table 2).

3.2.2. Liver

In contrast to white muscle, divergence among ecotypes in hepatic glycolytic and glycogenolytic mRNA content did not effectively predict variation in carbohydrate metabolism enzyme activities (Table 2). St-Cyr et al. (2008) found a higher mRNA content for aldolase (both muscle and liver isoforms) and glyceraldehyde 3-phosphate dehydrogenase, but we found enzyme activities to be higher in normal whitefish (Fig. 2, Table 2). Furthermore, St-Cyr et al. (2008) found no differences between ecotypes in the mRNA content of phosphofructokinase, pyruvate kinase, lactate dehydrogenase and glycogen phosphorylase, while we found higher enzyme activities in dwarf whitefish for phosphofructokinase, pyruvate kinase and glycogen phosphorylase and lower activities of lactate dehydrogenase (Figs. 2, 3, Table 2). Finally, we detected no difference in glycerol 3 phosphate dehydrogenase, while St-Cyr et al. (2008) found a lower mRNA content

Table 2
mRNA content and enzyme activities of carbohydrate metabolism genes in adult tissues and whole-juvenile lab-reared whitefish (Témiscouata dwarf and Alymer normal ecotypes). mRNA abundances were measured with the 3.7 K salmonid microarray (Rise et al., 2004; Derome et al., 2006), 16 K salmonid microarray (von Schalburg et al., 2005; Derome et al., 2008; St-Cyr et al., 2008) or RT-PCR (Jeukens et al., 2009) and normalised to total RNA. Data are presented as dwarf/normal values; values > 1 are higher in dwarf fish (in bold), while values < 1 are higher in normal fish (italicized). Relevant tissue-specific isoforms are in black, while other isoforms are in grey. An '?' indicates values were not measured and 'ns' indicates that there was no significant difference in activity/abundance. Data from multiple probes for the same gene are presented as averages, except when trends varied among probes. Gene names follow conventions for humans. Data for gene expression and genetic divergence in wild populations are summarized in Tables S1 and S2.

Pathway	Enzyme	Enzyme activity/ mg protein (Dwarf/Normal)	Enzyme activity/ g tissue (Dwarf/Normal)	Gene	mRNA content/ total mRNA (Dwarf/Normal); Adult tissue [1-3]	mRNA content/ total mRNA (Dwarf/Normal); Whole juveniles [4]
WHITE MUSCLE						
Glycolysis	Phosphoglucose isomerase (GPI)	**1.8**	**2.4**	GPI	ns [1]	**1.2**
	Phosphofructokinase (PFK)	**2.4**	**3.3**	PFK-B/L (liver)	ns [1]	ns
				PFK-M (muscle)	ns [1]	**1.2**
	Aldolase (ALDO)	**2.2**	**3.0**	ALDO-A (muscle)	? [1]	**1.6**
				ALDO-B (liver)	*0.7* [1]	ns
				ALDO-C (brain)	ns [1]	**1.2**
	Triose phosphate isomerase (TPI)	**1.8**	**2.4**	TPI	ns [1]	**1.5**
	Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	**2.2**	**3.0**	GAPDH	ns [1]	**1.5**
				GAPDH-testis specific	ns [1]	ns
	Pyruvate kinase (PK)	**2.2**	**2.9**	PK-M (muscle)	**1.1** [1]	**1.4**
				PK-L,C (liver & erythrocyte)	ns [1]	ns
	Lactate dehydrogenase (LDH)	**1.6**	**2.2**	LDH-A (muscle & liver)	? [1], **1.6** [3]	?
				LDH-B (heart)	ns [1]	**1.2**
				LDH-C (testes)	?	?
Glycogenolysis	Glycogen phosphorylase (PYG) - total	**1.4**	**1.9**	PYG-M (muscle)	ns [1]	**1.3**
				PYG-B (brain)	ns [1]	**1.2**
				PYG-L (liver)	?	?
Glyconeogenesis	Glycogen Synthase (GYS) – total	**2.2**	**2.8**	GYS-1 (muscle)	?	?
				GYS-2 (liver)	?	?
Linking carbohydrate and lipid metabolism	Glycerol 3-phosphate dehydrogenase, cytoplasmic (GPD)	**1.8**	**2.6**	GPD1(cytoplasmic)	ns [1]	ns
				GPD2 (mitochondrial)	?	?
LIVER						
Glycolysis	Phosphoglucose isomerase (GPI)	ns	ns	GPI	ns [2]	
	Phosphofructokinase (PFK)	**1.3**	**1.2**	PFK-B/L (liver)	ns [2]	
				PFK-M (muscle)	ns [2]	
	Aldolase (ALDO)	*0.8*	*0.8*	ALDO-A (muscle)	**1.3** [2]	
				ALDO-B (liver, CB502483)	**1.3** [2]	
				ALDO-B (liver, CA062426)	ns [2]	
				ALDO-C (brain)	ns [2]	
	Triose phosphate isomerase (TPI)	ns	ns	TPI	ns [2]	
	Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	*0.9*	*0.9*	GAPDH	**1.3-1.7** [2]	
				GAPDH-testis specific	ns [2]	
	Pyruvate kinase (PK)	**1.4**	**1.3**	PK-M (muscle)	ns [2]	
				PK-L,C (liver & erythrocyte)	ns [2]	
	Lactate dehydrogenase (LDH)	*0.9*	*0.9*	LDH-A (muscle & liver)	?	
				LDH-B (heart)	ns [2]	
				LDH-C (testes)	?	
Glycogenolysis	Glycogen phosphorylase (PYG or PYG) - total	ns	**1.2**	PYG-M (muscle)	ns [2]	
				PYG-B (brain)	ns [2]	
				PYG-L (liver)	?	
Glyconeogenesis	Glycogen Synthase (GYS or GYS) - total	ns	ns	GYS-1 (muscle)	?	
				GYS-2 (liver)	?	
Linking carbohydrate and lipid metabolism	Glycerol 3-phosphate dehydrogenase, cytoplasmic (GPD)	ns	ns	GPD1 (cytoplasmic)	*0.7* [2]	
				GPD2 (mitochondrial)	?	

References: (1) Derome et al. (2008), (2) St-Cyr et al. (2008), (3) Jeukens et al. (2009), (4) Nolte et al. (2009).

in dwarf whitefish (Fig. 3, Table 2). The two genes for which St-Cyr et al.'s (2008) hepatic mRNA content and our enzyme activities agreed were phosphoglucose isomerase and triosephosphate isomerase, for which no differences in activity or mRNA content were detected (Table 2).

4. Discussion

Transcriptomic studies are commonly used to survey the molecular variation underlying phenotypic divergence in natural populations (Alvarez et al., 2015). However, not all transcriptomic variation influences phenotypes at higher levels of biological organization, so it is important to test if differences in mRNA content are associated with corresponding changes in protein content or enzyme activity (reviewed by Diz et al., 2012). The best way to test for an effect of transcriptomic divergence is to directly manipulate mRNA abundance in a controlled genetic background (e.g. RNAi, morpholinos; reviewed by Dalziel et al., 2009; Pardo-Diaz et al., 2015). However, this may not be possible when specific loci are not known, adaptation is polygenic, or organisms are not amenable to genetic manipulations, as is the case with Lake Whitefish (see Bernatchez, 2016). Therefore, we tested if previously detected transcriptomic variation (Derome et al., 2008; St-Cyr et al., 2008; Jeukens et al., 2009; Nolte et al., 2009) between lab-reared dwarf and normal whitefish leads to corresponding changes in enzyme activity between ecotypes from the same populations. We found: 1) dwarf whitefish have higher white muscle activities of all measured enzymes of glycolytic and glycogen metabolism than normal whitefish and that some of these vary as predicted by prior transcriptomic studies in

whitefish from a variety of age classes from the same populations, 2) differences in carbohydrate metabolism enzyme activities among ecotypes in red muscle follow similar trends, but are much lower than in white skeletal muscle, 3) hepatic activities of glycolytic and glycogen metabolism enzymes vary in an enzyme-specific manner between ecotypes and enzyme activities do not vary as predicted from transcriptomic surveys in adult fish from the same populations, and 4) differences in hepatic and muscle enzyme activities in these populations of dwarf and normal whitefish are genetically based (present in lab-bred and reared fish), and do not vary with moderate swim-training.

4.1. Do mRNA content and enzyme activity vary among ecotypes in a similar fashion?

Prior transcriptomic studies of lab-reared adult whitefish (> 3 years old, ~23–29 cm fork length) found that glycolytic mRNA content was higher in white muscle and liver of dwarf than normal whitefish (Derome et al., 2008; St-Cyr et al., 2008; Jeukens et al., 2009). Similarly, in whole lab-reared juveniles (4 months old, ~0.8 g, fork length not measured, body composition similar to adult fish) glycolytic and glycogenolytic mRNA content was higher in dwarf compared to normal whitefish (Nolte et al., 2009). Because these transcriptomic studies predate our work, and samples are no longer available, we could not measure enzyme activities in tissues from the same individuals; hence our goal was to test for evolutionary divergence among ecotypes at the population-level in our 16 month old juvenile fish (~5–9 cm fork length, ~5 g Dalziel et al., 2015; Laporte et al., 2016). It is possible that allometric variation due to differences in the age and size of the individuals used in prior transcriptomic and our enzymatic studies has occurred as glycolytic enzyme activities and mRNA content often show positive scaling with body size in fishes (Moyes and LeMoine, 2005). However, unless scaling varies between ecotypes, dwarf and normal whitefish should be subject to similar levels of scaling. With respect to measures in whole-juvenile whitefish (Nolte et al., 2009), we accounted for differences in tissue content (pure muscle vs. whole-juveniles) by only comparing muscle-specific isoform gene expression.

Finally, we note that we measured maximal enzyme velocity (V_{max}), which is the product of enzyme concentration ([E]) and turnover number (catalytic events per active site per unit time, k_{cat}). Thus, if the coding regions of carbohydrate metabolism genes have evolved differences in k_{cat}, the link from protein content to enzyme activity may differ among ecotypes. Furthermore, evolutionary variation in translational or post-translational regulation may also change the relationship from mRNA content to enzyme activity among ecotypes, leading to mismatch in the ratios dwarf/normal mRNA content and enzyme activity.

4.1.1. White muscle

We found that two of seven genes (PK and LDH) had both a higher mRNA content (Derome et al., 2008; Jeukens et al., 2009) and higher white muscle enzyme activity in dwarf versus normal whitefish, suggesting that their activities are transcriptionally regulated. The five other glycolytic genes showed no difference in mRNA content among ecotypes in adult white muscle (Derome et al., 2008), but higher enzyme activities in dwarf than normal whitefish, arguing for a role of post-transcriptional regulation. However, if allometric scaling varies between dwarf and normal ecotypes or divergence in expression only occurs at specific life-stages the comparisons of adult mRNA content to enzyme activity in juveniles may not be appropriate. Indeed, divergence in muscle-isoform specific, whole-juvenile, carbohydrate metabolism gene expression (Nolte et al., 2009) is greater than gene expression divergence among ecotypes in adult muscle (Derome et al., 2008). We found strong concordance between the dwarf/normal ratios of mRNA content from four month old whole-juvenile fish (Nolte et al., 2009) and of enzyme activities in white muscle of our 16 month old fish for all possible comparisons of glycolytic genes and glycogen phosphorylase; in all cases, dwarf whitefish had higher mRNA content and enzyme activities.

For all genes showing corresponding divergence in enzyme activities and mRNA content among ecotypes in either adult muscle or whole-juvenile samples the divergence in enzyme activities was larger than that in mRNA content. This could indicate that further translational or post-translational mechanisms amplify any transcriptomic variation. However, enzyme activities and mRNA content are expressed relative to different denominators in these studies (total protein and total RNA, respectively), so variation in the amount of total RNA per mg protein needs to be taken into account to determine which regulatory steps contribute to differences in enzyme activities (e.g. Dalziel et al., 2005; Martin-Perez et al., 2012).

4.1.2. Liver

In contrast to white muscle, liver gene expression in adult whitefish (St-Cyr et al., 2008) did not predict enzyme activity variation among ecotypes for any genes, suggesting that transcriptomic variation does not contribute to divergence in hepatic enzyme activities. It is possible that this discordance is due to individual variation in mRNA content and enzyme activities, but since genetically-based divergence in hepatic enzyme activities between ecotypes were larger than variation within ecotypes this does not seem likely. Unfortunately, we used all hepatic tissues for enzyme assays so cannot directly test this hypothesis by measuring mRNA content in the same individuals. Another possibility is that divergence among ecotypes varies at different ages or sizes, as was found for gene expression in white muscle (Derome et al., 2008 vs. Nolte et al., 2009).

It is also possible that variation in the coding regions of these genes may influence enzyme activities (V_{max}, a combination of enzyme concentration and specific activity) such that differences in specific activity, and not enzyme concentration, underlie differences in activity between ecotypes. Previous genomic studies have found evidence for genetic divergence in metabolic genes, but these SNPs were primarily detected in non-coding regions, arguing that regulatory variation is more important for the evolution of enzymes in energy metabolism among whitefish ecotypes (Renaut et al., 2010, 2011; Hébert et al., 2013). A further examination of the full coding regions of glycolytic/glycogen metabolism genes is required to fully evaluate this hypothesis, but the concordance between mRNA content and activity in isoforms and genes that are also expressed in white muscle (e.g. GPI, TPI, G-APDH, LDH-A) argues that this is not likely the major mechanism leading to transcriptomic and enzymatic discordance. Finally, it is possible that translational or post-translational mechanisms buffer variation in mRNA content among ecotypes (e.g. GPD), and lead to previously undetected divergence (e.g. PFK, PK, LDH, PYG) or reverse the effects of mRNA variation (e.g. ALDO, GAPDH).

Transcriptional regulation is clearly an important mechanism underlying the evolution of phenotypic traits (Wray, 2007), but recent proteomic studies have found that regulatory processes at other levels of biological variation are also critical (Diz et al., 2012; Albert et al., 2014; Bauernfeind et al., 2015). For example, much variation in global protein content is explained by factors other than mRNA content (Vogel and Marcotte, 2012). While about 50–85% of genes show a positive correlation between mRNA and protein levels, normally < 30% of variation is explained, suggesting translational/post-translational mechanisms also contribute to evolutionary variation in protein content (e.g. Ghazalpour et al., 2011; Skelly et al., 2013; Battle et al., 2015). These correlations among mRNA and protein levels vary widely among tissues, cellular components and functional categories of genes (e.g. Ghazalpour et al., 2011; Skelly et al., 2013; Wu et al., 2013; Wang et al., 2015; Rokyta et al., 2015). Therefore, our finding that differential enzyme activity has likely evolved via transcriptional mechanisms in white muscle, and translational/post-translational mechanisms in liver is plausible. Furthermore, while glycolytic genes are well-known to be transcriptionally regulated, there is also substantial translational and post-translational control (reviewed by Pilkis and Granner, 1992;

Desvergne et al., 2006; Dang et al., 2008; Tripodi et al., 2015). Indeed, evidence for post-transcriptional control of glycolytic protein content during local adaptation has been found for heart ENO and GAPDH by Rees et al. (2011) in *Fundulus heteroclitus* populations.

4.2. Do enzymes within a biochemical pathway co-vary among ecotypes?

Pathway flux can evolve as a result of catalytic or regulatory differences in single enzymes (e.g. Olson-Manning et al., 2013, 2015) or a few enzymes that have majority flux control (e.g. Eanes, 2011; Lavington et al., 2014). Flux may also evolve as the result of variation in 'master' regulatory factors controlling the expression or activity of all pathway genes (e.g. hypoxia inducible factor 1 co-regulates glycolytic gene expression during hypoxia; Semenza, 2011). By measuring the activities of seven of the eleven enzymes in glycolysis, we were able to determine if changes in enzyme activity among dwarf and normal whitefish occurred in only a few (indicating changes in enzyme specific coding regions or regulatory evolution), or in many glycolytic enzymes (co-variation, suggesting the evolution of a trans-regulatory factor influencing the whole pathway).

In the white muscle, all seven glycolytic enzymes had ~2 fold higher activities in dwarf than normal whitefish. Dwarf whitefish also had higher total activities of glycogen metabolism enzymes (GYS, PYG) and an enzyme coordinating carbohydrate and fat metabolism (GPD). The evolutionary up-regulation of all measured enzymes, including those known to operate near capacity during exercise, such as PYG (Eanes et al., 2006), indicates that dwarf whitefish have evolved a higher potential for carbohydrate metabolism. Nolte et al.'s (2009) finding that juvenile dwarf whitefish also have a higher mRNA content of many of these genes compared to normal whitefish suggests that variation in transcriptional 'master regulator(s)' may underlie evolutionary divergence in carbohydrate metabolism in white muscle. Indeed, expression quantitative trait locus studies (eQTL) in Lake Whitefish white muscle have mapped expression 'hotspots' for genes involved in glycolysis (GAPDH, PK, TPI) and carbohydrate metabolism (GPD) (Derome et al., 2008). The identity of these eQTL loci remains to be determined.

In liver, there was divergence in many glycolytic enzymes between the two ecotypes, but these enzymes did not show a coordinated pattern of divergence as in white muscle. In particular, normal whitefish had higher activities of three glycolytic enzymes (ALDO, GAPDH, and LDH), dwarf whitefish had higher activities of two enzymes (PFK and PK), and two enzymes did not vary among ecotypes (TPI and GPI). These findings, in combination with a lack of correspondence between mRNA content and enzyme activities in liver, suggest that enzyme-specific coding regions or translational/post-translational mechanisms may have led to evolutionary divergence. These differences in regulation among enzymes within a pathway make it difficult to predict the cumulative effects, if any, on glycolytic pathway flux. We do note that some of these enzymes, including ALDO, GAPDH, and LDH, are reversible enzymes that catalyze both glycolytic and 'reverse' gluconeogenic reactions. ALDO, GAPDH, and LDH are more highly expressed in normal whitefish, while PFK and PK, which act only in glycolysis, are more highly expressed in dwarf whitefish. Furthermore, PFK is a classic 'non-equilibrium' enzyme regulated via allosteric modulators and thought to exert strong control over glycolysis (reviewed by Hochachka and Somero, 2002). Based upon these observations we hypothesize that dwarf whitefish have a higher per gram capacity for hepatic glycolysis, but that normal whitefish may have a higher per gram hepatic capacity for gluconeogenesis.

Current data regarding the expression of gluconeogenic-specific genes in these populations are equivocal: St-Cyr et al. (2008) found no differences in the hepatic mRNA content of fructose 1,6-bisphosphatase (FBPase) or cytoplasmic phosphoenolpyruvate carboxykinase (cPEPCK), and the microarray used in that study lacked probes for the mitochondrial PEPCK (mPEPCK), glucose 6-phosphatase (G6Pase), and pyruvate carboxylase (PC). Jeukens et al.'s (2010) RNA-seq study of the livers of wild whitefish from Cliff Lake found that FBPase-2 was higher in normal whitefish livers, but mPEPCK and G6Pase were higher in dwarf whitefish livers. Gluconeogenic enzymes display transcriptional, post-transcriptional, translational and post-translational regulation, so overall hepatic gluconeogenic capacity should be compared among ecotypes in future experiments to test the hypothesis that normal whitefish have a higher hepatic capacity for gluconeogenesis (Enes et al., 2009; Jitrapakdee, 2012). Furthermore, Laporte et al. (2016) found that dwarf whitefish have a relatively larger liver than normal whitefish, which should accentuate increases in hepatic glycolytic capacity in dwarf whitefish, but might cancel out potential tissue-specific increases in gluconeogenic capacity in normal whitefish when whole-liver metabolic capacity is measured.

4.3. Which tissues show energetic divergence among dwarf and normal ecotypes?

Studies of whole-animal behaviour and metabolism have found that dwarf whitefish are more active swimmers with a higher active metabolic rate that grow more slowly and mature earlier than normal whitefish (Trudel et al., 2001; Rogers et al., 2002; Rogers and Bernatchez, 2005). To help determine how individual tissues contribute to variation in whole-animal metabolism we compared liver, white muscle and red muscle. We found that dwarf whitefish have higher activities of carbohydrate metabolism enzymes in white muscle, that differences in the activities of glycolytic enzymes also evolved in liver, but that there was little divergence in red muscle. These data agree with our previous work finding substantial divergence in the activities of aerobic energy metabolism enzymes in white muscle and liver, but less variation in red muscle (Dalziel et al., 2015; Laporte et al., 2016). Instead it seems that divergence in the oxidative red muscle occurs via changes in the relative proportions of this fibre-type relative to white muscle, as dwarf whitefish have a higher percentage of red muscle (Dalziel et al., 2015, 2017).

Previous studies on other metabolically active tissues, such as brain and heart, also indicate that divergence in size and/or energy metabolism has occurred. In particular, normal whitefish more highly express genes involved in energy metabolism in the brain and have a larger normalised brain size (Whiteley et al., 2008; Laporte et al., 2016, but see Evans et al., 2013), while dwarf whitefish have larger normalised ventricles and show little divergence in energetic enzyme activities per mg heart protein (Evans et al., 2013; Dalziel et al., 2015, 2017). Together, these physiological studies show that changes in whole-animal metabolism between dwarf and normal ecotypes are associated with variation in the size of the brain, heart, liver and red muscle as well as changes in the activities of aerobic energy metabolism, glycolysis, glycogenolysis, and glycogenesis per gram tissue in white muscle, liver and brain.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cbpb.2017.08.001.

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