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Regional variation of gene regulation associated with storage lipid metabolism in American glass eels (*Anguilla rostrata*)

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ABSTRACT

Variation in gene regulation may be involved in the differences observed for life history traits within species. American eel (*Anguilla rostrata*) is well known to harbor distinct ecotypes within a single panmictic population. We examined the expression of genes involved in the regulation of appetite as well as lipid and glycogen among glass eels migrating to different locations on the Canadian east coast and captured at two different periods of upstream migration. Gene expression levels of three reference and five candidate genes were analyzed by real-time PCR with Taqman probes in recently captured wild glass eels. All gene transcripts were detected in glass eels. Of the five candidate genes, bile salt activated and triacylglycerol lipases were respectively 7.65 and 3.25 times more expressed in glass eels from the St. Lawrence estuary than in those from Nova Scotia, and there was no effect related to the two-week difference in capture date. These two genes explained 82.41% of the dissimilarity between the two rivers. In contrast, glycogen phosphorylase, ghrelin, and leptin receptor genes showed no significant differences in gene transcription. These results confirmed at the molecular level an observation that was recently made at the phenotypic level that glass eels from the St. Lawrence estuary have a greater capacity to use lipid reserves to sustain their metabolic needs. These observations add to the body of evidence supporting the hypothesis that regional phenotypic variation observed in American eel is determined early in life and that part of this variation is likely under genetic control.

1. Introduction

American eel, *Anguilla rostrata*, is a facultative catadromous fish (i.e., it performs a non-obligatory trophic migration to fresh water). It is widely distributed througout eastern North America and is associated with a unique reproductive site in the Sargasso Sea (Tsukamoto et al., 1998). Consequently, the species is composed of a single, genetically homogeneous panmictic population (Côté et al., 2013). The leptocephalus larvae migrate toward the continental slope where they metamorphose into glass eels (Tesch, 2003). Since the 1980s, a 99% decrease in glass eel recruitment has been observed in the St. Lawrence River, which used to support an important fishery in Canada (Cairns et al., 2014; Castonguay et al., 1994a, 1994b; MacGregor et al., 2008). This species was recently declared "threatened" in Canada (COSEWIC, 2012).

In contrast, recruitment has remained relatively stable along the Atlantic coast of Canada, and this regional discrepancy in recruitment despite panmixia has puzzled managers for many years. In addition to regional recruitment variation, pronounced regional phenotypic variation has been documented in American eel. In particular, eels colonizing the St. Lawrence River and Lake Ontario are characterized as being much older and with a larger size at sexual maturity but with a slower growth rate compared to eels from the coastal areas, as well as being 100% females (Côté et al., 2015; Jessop, 2010). While it has been traditionally believed that such phenotypic variation is entirely controlled by the environment (i.e., reflecting pure phenotypic plasticity), there is growing evidence that regional functional genetic variation may also play a role.

Differences in growth and profiles of gene transcription have been documented between eels from the St. Lawrence River and from the Canadian Atlantic coast when reared in common environments for three years (Côté et al., 2009, 2014, 2015). In addition, latitudinal variations in RNA/DNA ratios have been observed, with both lower body condition and higher RNA/DNA ratios in high and low latitudes (Laflamme et al., 2012; 47–49°N [Eastern Canada] vs. 30–32°N [Florida–South Carolina]). Moreover, evidence for spatially varying selection resulting in regional variations in allele frequencies have been documented for genes involved in lipid and carbohydrate metabolism (Gagnaire et al., 2012). More recently, Pavey et al. (2015) provided strong evidence that the phenotypic differences observed between two ecotypes (fresh water from the St. Lawrence River vs. the brackish/salt water from the east coast of Canada) have a polygenic basis for genes that are mainly

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involved in vascular and morphological development, calcium ion regulation, growth, transcription factors, and olfactory receptors. In brief, the notion that regional phenotypic variation observed in American eel is only due to phenotypic plasticity is no longer tenable, and genetic variation resulting from spatially varying selection must therefore also be taken into consideration for a full understanding of the complexity of eel life history.

Glass eels rely on the energy stored during the larval stage to achieve their migration toward feeding habitats (Boëtius and Boëtius, 1989), which could be freshwater, estuarine, or marine (Arai, 2012; Cairns et al., 2004; Jessop et al., 2002; Lamson et al., 2006; Pavey et al., 2015). In a recent study on the energetic status of American glass eels captured along the east coast of Canada (Nova Scotia and the St. Lawrence estuary), Gaillard et al. (2015) showed that there was no relationship between energy status and salinity preference, but there were differences in energy storage strategies between capture sites. Glass eels captured in the St. Lawrence estuary had a higher level of glycogen and lower reserves of triacylglycerol than those captured on the Atlantic coast. There is also some evidence that glass eels resume feeding once they are totally pigmented, but some individuals may need to feed to complete their upstream migration (e.g., Harrison et al., 2014). Indeed, non-pigmented glass eels with full stomachs were observed in Grande Rivière Blanche (GRB), which is a tributary to the St. Lawrence estuary (C. Côté, pers. comm.), and non-pigmented glass eels captured in both GRB and Nova Scotia were feeding in the lab (Côté et al., 2009). How the relative expression for genes involved in lipid and carbohydrate metabolism as well as appetite regulation varies in glass eels from different sites needs to be clarified.

In this context, we tested 1) whether spatial or temporal differences in the expression of genes involved in lipid and glycogen metabolism differed between glass eels migrating to Nova Scotia on the Atlantic coast or in the St. Lawrence estuary; and 2) whether genes regulating appetite would be more expressed in glass eels captured in the St. Lawrence estuary, supporting the hypothesis of early feeding. To achieve these goals, we used real-time PCR to measure the transcription level of five candidate genes: triacylglycerol lipase (TAGL), bile salt activated lipase (BAL), glycogen phosphorylase (GPase), ghrelin (GHRL), and leptin receptor (LEP-R).

2. Materials and methods

2.1. Sampling

Glass eels were captured by a commercial elver fishery in the Mersey River (MR; 44°02′ N, 64°42′ W), Nova Scotia (Atlantic coast), on 26–28 March and 20–21 April 2012 and by members of our research team in Grande-Rivière-Blanche (GRB; 48°47′ N, 67°41′ W), Québec (St. Lawrence River), on 2–6 and 18–21 June 2012 (Boivin et al., 2015). These periods represent the early arrival of glass eels in this area (Côté et al., 2009). Glass eel captures began two hours before the nighttime high tide and lasted for three hours. Samplers waded into river mouths and captured eels using dip-nets and headlamps. Glass eels were transferred by car to the wet-lab facility at Maurice-Lamontagne Institute (IML; Fisheries and Oceans Canada) in buckets containing water from the estuary. The introduction and transfer of glass eels from Nova Scotia to Québec were done under conditions specified in the license obtained from Fisheries and Oceans Canada. A total of 40 individuals were sampled for the present study: 10 glass eels per river site and date of capture (MR, March; MR, April; GRB, early June; GRB, late June). Glass eels were anesthetized in 0.68 mM MS222 (ethyl 3-aminobenzoate methanesulfonate; Sigma-Aldrich). Total body length and wet mass were measured, and pigmentation stage was determined according to Haro and Krueger (1988). Pigmentation results are presented in Gaillard et al. (2015). Glass eels were individually stored at −20 °C in RNALater until analyses.

2.2. Candidate and reference genes

Five candidate genes were studied: triacylglycerol lipase (TAGL), which catalyzes the breakdown of triacylglycerol in several tissues (Murray et al., 2003; Tocher and Sargent, 1984); bile salt activated lipase (BAL), the most important digestive lipase in teleosts (Iijima et al., 1998; Murray et al., 2003; Patton et al., 1975, 1977); glycogen phosphorylase (GPase), an enzyme that catalyzes the rate-limiting step in glycogenolysis (Brown and Cori, 1961); and ghrelin (GHRL) and leptin receptor (LEP-R), which are hormonal factors generally considered to regulate appetite (Lin et al., 2000; Unniappan and Peter, 2005). The use of only one reference gene in qPCR analysis is not recommended (Bustin et al., 2009), and reference gene expression can vary from one tissue to another (Olsvik et al., 2005). Vandesompele et al. (2002) recommended the use of three reference genes for the reliable normalization of a pool of normal tissues in order to avoid relatively large errors caused by the use of one reference gene. Based on previous eel studies (Weltzien et al., 2005), the reference genes chosen for the present work were acidic ribosomal protein (ARP) and cytochrome B (CytB); we also used elongation factor 1 (EF1), which was reported to be very stable in Atlantic salmon (Olsvik et al., 2005).

2.3. Whole body grinding

Glass eels were individually homogenized dry with liquid nitrogen using a Precellys Dual homogenizer coupled with a cooling system (Precellys, Bertin Technologies) in CKMix 50 R containing beads for hard tissue grinding. Samples were ground using three cycles of 26 s at 5800 rpm, with a 30 s time lapse separating each cycle. The resulting powder was held at −80 °C until RNA extraction.

2.4. Total RNA extraction

Total RNA was extracted from 10 mg dry mass of homogenate powder using an RNeasy® Fibrous Tissue Kit (Quiagen Inc., ON, Canada) and was diluted to obtain a final concentration of 200 ng μl^{-1}. Total RNA purity, quality, and concentration were determined using electrophoresis on 2% agarose gel stained with ethidium bromide (0.05 mg ml^{-1}) (Alpha Imager® HP System, Alpha-InnoTech, Alpha Imager 3400 software, ProteinSimple) and the 260 nm/280 nm absorbance ratio using a NanoVue Plus spectrophotometer (GE Healthcare, QC, Canada) before reverse transcription.

2.5. Reverse transcription

Reverse transcription was done using a Quantitect® Reverse Transcription Kit (Qiagen Inc., ON, Canada). The cDNA samples obtained were diluted to a final concentration of 20 ng μl^{-1}, separated into aliquots, and kept frozen at −20 °C until further analysis. cDNA integrity and concentrations were verified with a NanoVue Plus spectrophotometer. Reverse transcription efficiency was verified using serial dilutions of a pool of four RNA samples from different origins and dates of capture to ascertain slopes of −3.3. qPCR analyses were performed in triplicate (Biorad MyiQ I cycler, Bio-Rad Laboratories, Inc., ON, Canada) using IQ™ SYBR® Green Supermix (Bio-Rad Laboratories Inc., ON, Canada) and an iCycler iQ™ Real-Time PCR on one reference gene (EF1). Linear regression of the serial dilution curve was done with MIciyiQ Software version 1.0 (Bio-Rad, USA), giving an efficiency of 94.5% ($y = -3.4603\times + 10.341$; correlation coefficient: 0.997).

2.6. Specific sequences and design of Taqman primers and probes

For GHRL and reference genes, primers were designed based on available mRNA sequences from genus *Anguilla* found in the National Center for Biotechnology Information (NCBI) bank using Primer-Blast