River-specific gene expression patterns associated with habitat selection for key hormones-coding genes in American glass eels (*Anguilla rostrata*)

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Abstract

The glass eel stage in American Eel (*Anguilla rostrata)* marks the onset of the catadromous migration into estuarine or freshwater habitats, and the endocrine mechanisms underlying this habitat selection are still not well understood. Using a candidate genes approach, the aim of this study was to test for different patterns of gene expression related to 1) salinity preferences and/or 2) capture site to predict physiological differences between migratory behaviors. We performed analyses revealing the expression of genes coding for key hormonal factors or their receptors on American glass eels collected at the mouths of three rivers on the east coast of Canada (Grande-Rivière-Blanche in the St. Lawrence estuary, Rivière-Saint-Jean on

the Gaspé Peninsula, and Mersey River in Nova Scotia) that displayed different salinity preferences (brackish/salt/fresh water) in laboratory conditions. Transcripts from genes coding for PRL, TSH, type 2-iodothyronine deiodinase (DIO-2), thyroid receptors ($THR\alpha_a$, $THR\alpha_b$), GH, IGF-1, and their respective receptors GH-R_1 and IGF-1R were all detected in glass eels. No differences in the expression patterns were detected pertaining to salinity preference, but strong differences were found among rivers. Rivière-Saint-Jean glass eels, which were the longest and the least pigmented among the three rivers, were characterized by the highest expressions of PRL, DIO-2, and $THR\alpha_b$. Those from Grande-Rivière-Blanche showed an increase in IGF-1R. Glass eels captured in these two rivers exhibited the highest expression of GH and GH-R_1. Overall, these results confirm gene × environment interactions at the gene expression level when glass eels settle into their continental habitat. As such, our results also support the concept of the presence of different ecotypes in the Atlantic Canadian Coast and in the Estuary and Gulf of St-Lawrence.

Introduction

The glass eel stage in American Eel *Anguilla rostrata* marks the end of the oceanic migration of the leptocephalus larvae and the onset of catadromous migration into estuarine or freshwater habitats (Cairns et al. 2004; Jessop et al. 2002; Pavey et al. 2015; Tesch 2003). The range of distribution of *A. rostrata* along the coast of North America is large and covers tropical, temperate and subarctic areas. Facultative catadromy (non-obligatory trophic migration to fresh water) has only recently been documented in eels and Tsukamoto et al. (1998) were the first to describe a “sea eel” ecophenotype being an ecological sub-unit adapted to a particular habitat under environmental influences (Turesson 1922). In European glass eel (*Anguilla anguilla*), facultative catadromy has been suggested to be under endocrine control through phenotypic plasticity mechanisms, and different ecophenotypes (freshwater *vs.* brackish/saltwater) were associated with differences in osmoregulatory ability (Edeline et al. 2005b). The freshwater type exhibited a salinity preference for freshwater and colonized river habitats. Glass eels exhibiting freshwater preference were also characterized by high locomotor activity, and poor growth performance traits that were described as promoting the colonization of fresh waters (Edeline et al. 2005b). This ”freshwater type” also had high thyroid gland activity (higher plasma levels of thyroxine compared to triiodothyronine) and high thyroid hormone levels relative to glass eels of

the ”saltwater type” (Edeline et al. 2004). Edeline et al. (2005a) also showed that immersion in thyroxine enhanced locomotor activity while immersion in thiourea modified rheotaxis, supporting an active role of thyroid hormones during glass eel migrations. In contrast to those with a freshwater preference, the brackish/saltwater type of European glass eels exhibited brackish/saltwater preference, low locomotor activity, high growth performance (Edeline et al. 2005b), and low thyroid activity with low thyroid hormone levels, especially thyroxine (Edeline et al. 2004). This ecophenotype colonized marine and estuarine habitats (Edeline et al. 2009). Weak thyroid activity was hypothesized to affect sensitivity to olfactory cues, intestine development, and rheotaxis (Edeline 2005). Based on results obtained in the European Eel, Edeline et al. (2009) proposed a strategy related to condition to be underlying the expression of different ecophenotypes. Thus, it is predicted from these previous studies that high energetic status, high thyroid activity, but a low level of growth hormone secretion should characterize the freshwater ecotype, while low energetic status, low thyroid activity, but a high level of growth hormone secretion should characterize the brackish/saltwater ecotype (Edeline et al. 2005a, 2005b; Edeline 2007).

A recent population genomics study on American Eel showed that American yellow and silver eels colonizing fresh water and brackish/salt water in Eastern Canada can be genetically distinguished and reclassified with high accuracy, supporting the occurrence of a genetic basis for the different ecotypes in this species (Pavey et al. 2015). In contrast to an ecophenotype, an ecotype is an ecological subunit that is morphologically, physiologically, and genetically adapted to a habitat and, if transplanted into different habitat, its differences would be retained as they are fixed genetically (Turesson, 1922). Moreover, rearing of American glass eels in different salinity conditions also resulted in different growth performance according to their geographic origin, confirming this notion in young stages (Côté et al. 2009). In addition, transcriptomic differences were observed for polygenic traits and genes involved in many physiological functions related to both salinity rearing conditions and glass eel origin (Côté et al. 2014). However, using American glass eels captured at different locations on the Canadian east coast, Boivin et al. (2015) showed that although most did not make a choice between fresh and salt water, they usually preferred fresh water no matter what their geographic origin.

The aim of this study was to test whether differences in hormones and/or hormone receptor genes were associated with differences in the settlement habitat of American glass eels.

We studied gene expression tools, an approach that was successfully used previously both in American glass eel (Gaillard et al. 2015; 2016) and embryo and larval European Eel (Politis et al. 2017) and studied American glass eels colonizing rivers previously associated with different ecotypes (Mersey River on the Atlantic coast: brackish/saltwater ecotype; Rivière-Saint-Jean and Grande-Rivière-Blanche located in the Gulf and St. Lawrence Estuary: freshwater ecotype; Pavey et al. 2015), and according to their salinity preference (Boivin et al. 2015). Since the St. Lawrence Estuary glass eels were associated with the freshwater ecotype (Pavey et al. 2015), we hypothesized that expression of prolactin, hyper-osmoregulatory hormone for freshwater adaptation and coding genes for the thyroidal axis would be higher compared to eels from the Atlantic coast (brackish/saltwater ecotype). We also hypothesized that higher expressions of the somatotropic axis would be present in those from the southern location (smaller and heavier glass eels) compared to the northern ones. Considering the model for European Eel (Edeline et al. 2009), we predicted that American glass eels with freshwater preference would have a higher expression of thyroid function and lower expression of coding genes for the somatotropic axis compared to those exhibiting a brackish/saltwater preference. Finally, using glass eels exhibiting different salinity preferences, we tested whether the endocrine model underlying the expression of salinity preference suggested by Edeline et al. (2005, 2009) for European glass eel could apply to American Eel using gene expression analysis tools.

<A> Materials and Methods

Sampling. —

Glass eels were captured during new and full moon at their earliest arrival in the estuaries of three east coast Canadian rivers at different upstream distances relative to the Sargasso Sea (Figure 1): Mersey River, Nova Scotia, 26–28 March, 20–21 April 2012 (n = 3209); Rivière-Saint-Jean, Québec, 16–21 May, 28 May – 3 June 2012 (n = 636); and Grande-Rivière-Blanche, Québec, 2–6 June, 18–21 June 2012 (n = 1657). At Mersey River, glass eels were captured with fish nets by a commercial elver fishery; at Grande-Rivière-Blanche, we captured glass eels with fish nets; and at Rivière-Saint-Jean, glass eels were captured in the river estuary using two trap nets operated by the Ministère Forêt Faune et Parcs (see Boivin et al. 2015). Sampling was identical for glass eels captured in each of the three rivers.

River-specific gene expression patterns in recruits of American glass eels

On the day after capture, glass eels were transferred to the Maurice-Lamontagne Institute according to the procedures of the Canadian Council on Animal Care to assess salinity preference (Boivin et al. 2015). They were placed in a thermostatic chamber and kept unfed (see Boivin et al. 2015 for detailed information about the experimental system). After 48h of acclimatization, behavioural experiments took place. The experimental setup was used to assess freshwater preference (% of eels that chose fresh water, FW), saltwater preference (% of eels that chose salt water, SW) and brackish water preference (% of eels that remain in brackish water, BW). Three glass tanks (31.5 × 27 × 61 cm) provided triplicate measurements for each experiment. Acclimation salinity and salinity into the experimental tanks were 18‰. Two funnels, connected to filtering flasks, were inserted into each tank. Fresh and sea (salinity 33‰) water were gravity-delivered into the neck of the flasks at a rate of 180 mL/min, offering a binary choice between flows of FW and SW. An overflow drain allowed any excess water to be evacuated throughout the experiments. Charcoal-filtered dechlorinated tap water was used as FW while BW and SW were prepared by adding either FW or synthetic salts (Instant Ocean) to sand-filtered St. Lawrence Estuary water (salinity 20–25‰). The tests were done in darkness to minimize the stress associated with the manipulations and because glass eels are mainly active at night in natural habitats. See Boivin et al. 2015 for more detailed information about evaluation of the experimental bias of the apparatus. For all experiments, an average of 71 ± 34 individuals, selected to ensure a sufficient number of replicates, were placed at the beginning of each experiment in the BW-filled waiting chamber for an acclimation period of 30 min after which the water flows were activated for a 30 min experimental period. Both the acclimation and experimental periods were conducted in darkness in order to minimize the stress associated with the manipulations and because glass eels are mainly active at night in natural habitats. A preference experiment was run for each glass eel arrival (two per river, see Fig. 1) and each experiment was performed in triplicate tanks (see Boivin et al. 2015). At the end of the experiments, all glass eels that chose FW from the triplicate experimental tanks were pooled, as were those that chose SW and those that remained in BW. From these, 10 “FW”, 10 “SW”, and 10 “BW” glass eels, for a total of 20 per salinity per river, were individually anaesthetized in 0.68 mM MS-222 (ethyl 3-aminobenzoate methanesulfonate; Sigma-Aldrich). For this study, we used 10 individuals classified as FW, SW, or BW for each river, for a total of 90 glass eels (Figure 1). Total body length (from the tip of the snout to the tip of the caudal fin; ± 1mm) and wet mass (± 1 mg) were measured. Pigmentation stage was

155 determined according to Haro and Krueger (1988). Glass eels were rinsed with brackish water,
156 gently blotted dry, transferred to 1.5 ml tubes filled two-thirds full with RNAlater® (Sigma-
157 Aldrich®, ON, Canada), and kept frozen overnight at 4°C before being stored at −20°C until
158 molecular analyses. In total, 90 individuals, 30 from each river, were sampled for further analyses
159 (Figure 1)
160
161 *Candidate and reference genes.* —
162 Nine candidate genes were studied: the subunit thyroid stimulating hormone β (TSH-β), Type
163 2-iodothyronine deiodinase (DIO-2), thyroid hormone receptors α_a and α_b (THRα_a and THRα_b),
164 prolactin (PRL), growth hormone (GH) and its receptor 1 (GH-R_1), and insulin-like growth
165 factor 1 (IGF-1) and its receptor (IGF-1R). TSH-β subunit is a proxy of TSH that stimulates the
166 thyroid gland to produce thyroxine (Han et al. 2004; e.g., MacKenzie et al. 2009); DIO-2 is the
167 major isoform that converts the pro-hormone thyroxine into bioactive triiodothyronine (e.g.,
168 Gomes et al. 2014); and THRα_a and THRα_b mediate the biological activity of thyroid hormones
169 binding with triiodothyronine (e.g., Gomes et al. 2014). PRL is well known for its central role in
170 freshwater osmoregulatory processes (ionic and osmotic balance) (Manzon 2002; Sakamoto and
171 McCormick 2006). GH stimulates growth through IGF-1 activation, has a direct effect on
172 growing tissues, and regulates lipid mobilization (e.g., Dai et al. 2015). GH-R_1 binds specifically
173 with GH to initiate the actions of GH (Ozaki et al. 2006a, 2006b). In fishes, IGF-1 mediates the
174 action of GH, which promotes somatic growth during cellular differentiation and mitogenesis
175 processes in muscle tissues, during development of the nervous system, and in bones, and it has a
176 critical role in neural induction by binding with IGF-1R, which triggers its actions (Dai et al.
177 2015; Escobar et al. 2011; Perrot et al. 1999). GH and IGF-1 are also associated with saltwater
178 acclimation (e.g., Sakamoto and McCormick 2006).
179 The qPCR analysis must be normalized using internal standards, the reference genes, for
180 which transcription is assumed to be constant. The use of only one reference gene in qPCR
181 analyses is not recommended (Bustin et al. 2009), and the expression of reference genes can vary
182 from one tissue to another (Olsvik et al. 2005). Vandesompele et al. (2002) recommended the use
183 of three reference genes for the reliable normalization of a pool of normal tissues in order to
184 avoid relatively large errors caused by the use of one reference gene. Based on previous eel

studies (Weltzien et al. 2005; Gaillard et al. 2016), the reference genes chosen for the present work were acidic ribosomal protein (ARP), cytochrome B (CytB), and elongation factor 1 (EF1).

Whole-body grinding. —

Glass eels were individually dry-homogenized 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, and cycles were separated by 30 s. The resulting powder was held at −80°C until RNA extraction.

Total RNA extraction. —

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

Reverse transcription. —

Reverse transcription was done in duplicate using the Quantitect® Reverse Transcription Kit (Qiagen Inc., ON, Canada). The cDNA samples obtained were diluted to a final concentration of 20 ng/µl, separated into aliquots, and kept frozen at −20°C until further analysis. cDNA integrity and concentrations were verified using a NanoVue Plus spectrophotometer. Reverse transcriptase efficiency was verified using serial dilutions of a pool of four RNA samples from different origins and dates of capture and compared with the ideal slope of −3.3. qPCR analyses were performed in triplicate (Bio-Rad MyiQ iCycler, 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) and one candidate gene (PRL). Linear regression of the serial dilution curves were done with MyiQ Software v 1.0 (Bio-Rad, USA), giving an efficiency of 94.5% for the reference gene ($y = -3.4603x + 10.341$; $r = 0.997$) and 97.4% for the candidate gene ($y = -3.3187x + 22.533$; $r = 0.935$).

Specific sequences and design of Taqman primers and probes. —

Except for the IGF-1R sequence which was obtained from the draft annotated American Eel genome (Pavey et al. 2016), the mRNA sequences for the reference and target genes were not available for American Eel in the GeneBank databases. Therefore, oligonucleotide primers were designed using Primer-Blast for each reference and candidate gene of interest based on available mRNA sequences from genus *Anguilla* found in the National Center for Biotechnology Information (NCBI) bank (http://www.ncbi.nlm.nih.gov/tools/primer-blast/; website accessed 23-11-2015). Primers were ordered from Integrated DNA Technologies™ (Coralville, IA, USA) and diluted to 20 μM before use. GenBank numbers for sequences were found on the NCBI website, and forward and reverse primer sequences are reported in Table 1. A pool of randomly chosen cDNA samples (rivers and salinity preferences) was used with primers for amplifications (all in duplicate) by polymerase chain reaction (PCR) with iCycleriQ™ Real-Time PCR (Bio-Rad, USA) using the Ampli Taq Gold® 360 Master Mix Kit (Applied Biosystems, CA, USA). The quality and integrity of each PCR product or amplicon were verified by electrophoresis on 2% agarose gels with ethidium bromide (0.05 mg/ml) containing a PCR marker (Sigma-Aldrich®, ON, Canada). Single fragments were obtained for all reference and candidate genes except for THRα_a, for which two fragments were obtained. Both THRα_a amplicons were isolated with the Ezna® Gel Extraction Kit (Omega Bio-Teck, GA, USA) before purification and were thereafter treated separately. Amplicons were purified on columns using the QIAquick PCR Purification Kit (Qiagen Inc., ON, Canada), and purified amplicons were sequenced in forward and reverse directions with associated primers and the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA). Unincorporated dye terminators from sequencing reactions were removed using the Ultra-Step® Dye Terminator Removal Kit (Omega Bio-Teck, GA, USA). Isolated fragments containing dye-labeled dideoxynucleotide triphosphates (ddNTPs) were dried for 20 min using a Speed Vac (Savant AS 160 Automatic) and suspended in fomamide; fragments were analyzed using a 3 130 Genetic Analyzer (Applied Biosystems-Hitachi) and POP-7™ polymer (Life Technologies™, ON, Canada). Sequence assembly and alignment verification were done with Sequencher 5.2.4 software (Genes Codes Inc.).

Alignments between the sequence obtained and the sequence used for primer design were performed for each gene. Supplemental Table 1 reports specific sequences obtained for each gene. Except for IGF-1R, the percentages of identity between sequences obtained from glass eels and sequences from GenBank are also presented in Supplemental Table 1. For THRα_a, the longest sequence that showed the highest homology score was retained for the study. TaqMan probes were designed using Primer Express 3.0 software (Applied Biosystems®) and were obtained from Life Technologies™ (Mairway, ON, Canada) (Table 1).

Real-time PCR assays and quantification. —

qPCR were performed in triplicate on glass eel samples with the ABIPRISM® 7900 HT Sequence Detection System (Applied Biosystems®). The 10 µl of volume for each reaction was made up of 2 µl cDNA (10^{-2} ng/µl), 5 µl TaqMan Advanced Mix (Life Technologies™, ON, Canada), 2.5 µl sterile water, and 0.5 µl TaqMan probe specific to a gene (Life Technologies™, ON, Canada). Thermal cycling of qPCR consisted of two steps: 1) 2 min at 50°C for optimal AmpErase® uracil-N-glycosylase activity followed by 10 min at 95°C to activate the AmpliTaq Gold® DNA Polymerase, and 2) 45 cycles of denaturing at 95°C for 30 s and annealing/extend at 60°C for 1 min. Cycle thresholds (C_T) were obtained using Expression Suite 1.0 software (Applied Biosystems, Foster City, CA).

Relative quantification of gene expression was calculated according to the 2^{-DDC_T} method of Livak and Schmittgen (2001):

$$2^{-DDC_T} = 2^{-(DC_{Te} - DC_{Tc})}$$

where $C_{Te} = C_{T\ candidate\ gene} - C_{T\ reference\ genes}$ for sample *x* and

$C_{Tc} = C_{T\ candidate\ gene} - C_{T\ reference\ genes}$ for the calibrator (see below).

In this study, the calibrator was the group of glass eels sampled at MR (the most southern river) that exhibited BW preference (absence of choice for either FW or SW). The calibrator always represents the 1.0 fold expression level, and other individuals and groups are expressed in these units. Non-detectable expressions (threshold cycles greater than 38) were given the same C_T value of 40 instead of eliminating fish that showed no expression, thus avoiding an overestimation of the global expression level. For this reason, normalization for prolactin and TSH-β were above 1.0.

qPCR validation. —

qPCR efficiency was verified for each TaqMan probe (slopes close to -3.3, all $R^2 > 0.98$; Table 1). A serial dilution of 10^{-1} to 10^{-5} or, when the signal was low, 4^{-1} to 4^{-5} (IGF-1, THRα_a) or 2^{-1} to 2^{-5} (PRL, TSH-β), was performed on a new pool of eight randomly selected cDNA samples within samples of different origin and salinity preference. qPCR analyses for each TaqMan probe were performed on the pool with the same protocol described above, except that the 2 µl cDNA (10^{-2} ng/µl) was replaced by 2 µl of the pooled cDNA. Suitability, stability, and validation of quantitative qPCR reference genes were verified with Expression Suite 1.0 software, where the score was calculated according to Vandesompele et al. (2002). The score is a gene stability measure in qPCR analyses that may vary with tissues: the lower the score, the more stable the expression. The gene-stability measures for the three reference genes were 0.786, 0.836, and 0.714 for ARP, CytB, and EF1, respectively (calculated using ExpressionSuite software). The three reference genes exhibited very little variation among subsamples (Supplementary Figure 1).

Le Cren condition index and statistical analyses. —

The Le Cren condition index (Kn) was calculated as described in Gaillard et al. (2015). Linear regressions of log10-transformed length and wet mass were made using data from all individuals that expressed a salinity preference. The constants were determined from the regression line obtained ($y = -5.4551 + 2.6137\ x$; $r^2 = 0.50$; $n = 1143$). The residual distribution of Kn was verified by fitting a Henry line ($F_{1, 1140} = 1786.65$; $P < 0.0001$; $r^2 = 0.61$). As demonstrated by Gaillard et al. (2015), Kn is both a condition index and an indicator of triacylglycerol and glycogen content. Because Gaillard et al. (2015) already showed that Kn, wet mass, length, and pigmentation stage did not differ according to salinity preference, only the presence of river effect was tested for these variables.

The relative quantification of gene expression ($2^{-\Delta\Delta C_T}$) for the nine candidate genes was analyzed using two-way permutational multivariate analysis of variance (PERMANOVA; $\alpha < 0.05$, 9999 permutations, type III sums of square) with the PERMANOVA+ add-on (v 1.02) in PRIMER (v 6.1.1.12). The two fixed factors were river (level = 3) and salinity (level = 3). Missing data (29 data out of 810) were replaced by the mean of the subsample in a salinity

preference – river group (García et al. 2015). Distance-based tests for homogeneity of multiple dispersions (PERMDISP) were verified for the two factors to determine if data needed transformation, and we found that no transformations were required (river: F = 1.7571, *P (perm)* = 0.2421; salinity: F = 1.1642, *P (perm)* = 0.3832). Thus a Bray-Curtis similarity matrix was constructed for relative gene expression data. Even though this type of statistical approach has been first developed for ecological studies, it is now used in gene expression (e.g. Ferrier et al. 2013) or metagenomic studies (e.g. Tamki et al. 2001). When PERMANOVA tests detected a factor effect, pair-wise comparisons were done. Finally, to explore dissimilarities between groups, we performed multidimensional scaling plot analysis (MDS). Similarity percentage analysis (SIMPER) was also run to identify the relative contribution of each gene and biological trait to the differences observed within one factor. Because SIMPER results were similar to those obtained with the MDS (results not shown), only the MDS results are presented. One-way ANOVAs ($\alpha = 0.05$) were run to test for specific differences in gene expression for each candidate gene or biological traits using STATISTICA v 10.0 software (http://www.statsoft.com; website accessed 21-11-2015) when PERMANOVA indicated significant factor effects. ANOVAs were followed by Tukey multiple comparison tests ($P < 0.05$). Normality and homoscedasticity of data were verified with the Kolmogorov–Smirnov and Levene tests, respectively. Pigmentation index data were analyzed with the non-parametric Kruskal-Wallis test.

<A> Results

<B> Gene expression and salinity preference

At the glass eel stage, no salinity preference was associated with the expression of any candidate gene since no factor or interaction effects were detected (salinity preference: df = 2, pseudo-F = 0.92891, *P (perm)* > 0.05; salinity preference × river: df = 4, pseudo-F = 1.2209, *P (perm)* > 0.05). However, patterns of gene expression differed among the three rivers (river: df = 2, pseudo-F: 4.1295, *P (perm)* < 0.0005). Differences were most apparent between RSJ and MR glass eels (pairwise tests: Grande-Rivière-Blanche & Mersey River: t = 1.5866, *P (perm)* < 0.05; Grande-Rivière-Blanche & Rivière-Saint-Jean: t = 2.0927, *P (perm)* < 0.005; MR & RSJ: t = 2.3426, *P (perm)* < 0.001), which was also confirmed by the MDS analysis (Figure 2). Differences in the expression of PRL, DIO-2, and GH on the MDS horizontal axis and the

338 expression of TSH-β and IGF-1R explained most of the differences among rivers. The
339 expression of TSH-β and IGF-1R were strongly positively correlated, and a positive correlation
340 was also observed between DIO-2 and PRL (Figure 2A, C). MDS results suggested that high
341 expression of DIO-2 and PRL characterized the Rivière-Saint-Jean glass eels, while those from
342 Grande-Rivière-Blanche were characterized by stronger expression of IGF-1R, TSH-β, and GH
343 (Figure 2A, C).
344 PRL expression was 10.3 times higher in Rivière-Saint-Jean glass eels than in the
345 calibrator (Mersey River-brackish water preference), while PRL expression in Mersey River and
346 Grande-Rivière-Blanche were similar and close to the expression level observed in the calibrator
347 group (Figure 3A). DIO-2 expression was 2.7 higher in Rivière-Saint-Jean glass eels compared
348 to the calibrator group, and this expression level was significantly higher than those observed in
349 the two other rivers (although DIO-2 was 1.7 higher in Grande-Rivière-Blanche than in Mersey
350 River; Figure 3B). GH expression was 2 and 2.4 higher in the glass eels from Rivière-Saint-Jean
351 and Grande-Rivière-Blanche compared to the calibrator group and significantly higher than the
352 value observed in Mersey River glass eels (Figure 3C). IGF1-R expression was 1.3 times higher
353 in Grande-Rivière-Blanche glass eels compared to the calibrator group, and this level was
354 significantly higher than observed in the other two rivers (Figure 3D). Contrary to our prediction,
355 the expression of TSH-β did not differ among rivers (Figure 3E). THRα_b expression in RSJ was
356 slightly more elevated (1.64) than in the calibrator group and significantly higher than levels
357 measured in Mersey River and Grande-Rivière-Blanche glass eels (Figure 3F). Finally, the
358 expression of GH-R_1 was significantly higher (1.3) in Rivière-Saint-Jean compared to Mersey
359 River, with intermediate expression levels in GRB (Figure 3G). Again, expression in Mersey
360 River was very close to that of the calibrator. The expressions of THRα_a (Figure 3H) and IGF-1
361 (Figure 3I) were similar among rivers.
362
363 <B> Phenotypic traits
364 The longest glass eels were captured in Grande-Rivière-Blanche and Rivière-Saint-Jean
365 (Figure 4A). The Rivière-Saint-Jean and Mersey River glass eels were 1.2 heavier than those
366 captured in GRB and had a higher condition index (Figure 4B, C). Glass eels captured in
367 Rivière-Saint-Jean were generally non-pigmented (pigmentation index close to 1), while
368 pigmentation at the lateral line was present in both Mersey River and Grande-Rivière-Blanche

(median of pigmentation index from 2 to 4) (Figure 4D). MDS analysis indicated that phenotypic differences among rivers were largely associated with variations in length and pigmentation status.

<A> Discussion

The first goal of this study was to investigate whether the endocrine model underlying salinity preference suggested by Edeline et al. (2009) for European glass eel could apply to American Eel. A second goal was to test whether there were different hormones gene expression patterns associated with the different habitats colonized by glass eels. To achieve these goals, comparative analyses of gene expression were performed according to salinity preference and site of capture of glass eels. While we found limited support for different expression patterns according to salinity preference, pronounced differences in gene expression were observed among rivers or origin. However, the observed patterns did not generally correspond to our working hypotheses and predictions. Thus, we found no difference in the expression of genes coding for the thyroxine/triiodothyronine axis or for the GH/GH-R/IGF-1/IGF1-R axis according to salinity preference. These results coupled with those demonstrating the absence of differences related to energy storage status according to salinity preference and the presence of strong differences between origin of glass eels at gene expression and cellular level (Gaillard et al. 2015, 2016) confirm that the "hypothetical endocrine mechanism for the control of glass eel migratory plasticity" suggested for European glass eel (Edeline et al. 2009) does not apply to American glass eel.

<B> Differential pattern of gene expression among sites of capture

The presence of different American Eel ecotypes in the Maritimes and in the St. Lawrence River is supported by strong evidence based on growth and sex determination phenotypic attributes (Côté et al. 2015) and a genome-wide association study (Pavey et al. 2015). Based on genome-wide genotypic differences Pavey et al. (2015) associated the St. Lawrence River ecotype to a freshwater ecotype, and the Atlantic Canada ecotype to a brackish/saltwater ecotype. Thus, both intra- and inter-ecotype differences were found, the most pronounced being between Mersey River and Rivière-Saint-Jean, both harbouring a different ecotype. Rivière-Saint-Jean glass eels were characterized by the highest expression of prolactin, DIO-2, and

THRα_b, a hormonal gene expression pattern expected for a freshwater ecotype. They were also the least pigmented, indicating that they were the least developed. DIO-2 expression was also higher in Grande-Rivière-Blanche compared to Mersey River glass eels. Considering that both Rivière-Saint-Jean and Grande-Rivière-Blanche contain the freshwater ecotype, their higher DIO-2 expression compared to the calibrator group could be associated with freshwater colonization. Even though there was no specific difference in the expression of TSH-β, MDS analysis discriminated Grande-Rivière-Blanche glass eels through their expression of this hypothalamic factor, which stimulates thyroxine production. The results of the MDS analysis showed a clear correlation between the PRL and DIO-2 expression patterns, thus a link between activation of transcripts from the thyroidal axis and the freshwater ecotype cannot be dismissed.

Glass eels seem to be prepared for the osmotic challenge posed by the saltwater–freshwater transition, given that they have twice as many prolactin cells compared to leptocephali (in *A. japonica*; Arakawa et al. 1992) and they develop a multi-layered oesophageal mucosa (in *A. anguilla*; Ciccotti et al. 1993). Considering the important role of prolactin in freshwater adaptation (Sudo et al. 2013), it is not surprising to observe a higher expression level in RSJ than in MR glass eels. However, we expected a similarly high level of prolactin in Grande-Rivière-Blanche glass eels, especially since Côté et al. (2014) observed differences of gene expression for two unique transcripts associated with prolactin function between Grande-Rivière-Blanche glass eels and those from Nova Scotia (Mersey River) prior to their river entrance, but this was not the case for PRL here. However, these results showed that activation of transcripts of prolactin was high in unpigmented freshwater glass eels that just achieved metamorphosis from leptocephali into glass eel. This finding is in agreement with a previous study done by Arakawa et al. (1992).

Complex patterns for coding genes of the somatotropic axis were observed in glass eels from the three rivers. Higher levels of GH and GH-R_1 expression were observed in the freshwater ecotype, and these glass eels were longer than those captured in Mersey River. Recently, Politis et al. (2017) studying the larval stage of European eel, showed that GH expression was higher at higher temperature. Here, temperature may not play such a role as temperatures in northern areas (Grande-Rivière-Blanche and Rivière-Saint-Jean) are expected to be colder than in the Nova Scotia littoral zone. Expression of the IGF-1 receptor was also higher in Grande-Rivière-Blanche glass eels. Although not statistically different, IGF-1 expression also

tended to be more elevated in these two rivers. Previous studies on American Eel found different effects of origin on growth of glass and yellow eels (Boivin et al. 2015; Côté et al. 2009, 2015). For example, glass eels from the brackish/saltwater ecotype had a higher wet mass and grew faster regardless in controlled conditions of the salinity of rearing water than did those from the freshwater ecotype (Boivin et al. 2015; Côté et al. 2009, 2015). Thus, a stimulated hormone-encoding gene for the somatotropic axis would have been expected in Mersey River glass eels. On the contrary, all indicators remained low compared to glass eels from the two other rivers caught at their arrival in the river system. Perhaps stimulation of transcripts of the somatotropic axis occurs later after the entrance into the rivers and/or with the resumption of food in the brackish/saltwater ecotype. Indeed, Pavey et al. (2015) showed enrichment in allelic frequencies of growth factor receptor binding at the yellow eel stage.

Many factors could be involved in the regulation of growth, and determining the exact role of the observed response must await more detailed studies on tissue-specific functions even though tissue puncture is delicate at this stage. Indeed, fasting should affect the somatotropic axis in a dissimilar fashion between ecotypes and these differences could also reflect differences in osmoregulatory and energy storage strategies. In fact, GH could regulate lipid mobilization by increasing lipid depletion from adipose tissues in fasting fish (e.g., Dai et al. 2015). Recently, Gaillard et al. (2016) detected a 25-fold higher expression of lipolysis enzymes in Grande-Rivière-Blanche glass eels than in Mersey River ones and demonstrated that glass eels from the freshwater ecotype have a better ability to mobilize efficiently lipid storage at recruitment. A possible activation of transcripts of GH could be related to lipid depletion. However, the presence of different patterns in GH and GH-R expression certainly supports the presence of transcriptomic differences in the freshwater and saltwater eel ecotypes despite the fact that they belong to a single panmictic population (Côté et al. 2013).

<B> No effect of salinity preference on gene expression

The absence of a salinity preference effect on gene expressions may seem counterintuitive considering the abundant literature on the environmental influence on salinity preference of *A. Anguilla* glass eels (Creutzberg 1961; Tosi et al. 1988); *Fundulus grandis* juveniles (Miller et al. 1983); *Oncorhynchus keta*, *O. gorbuscha*, *O. tshawytscha*, *O. nerka*, and *O. kisutch* pre-smolts (McInerney 1964; Otto and McInerney 1970); and *Leuresthes sardine*

462 postlarvae (Reynolds and Thomson 1974). Considering that American Eel is very euryhaline,
463 salinity preferences could be seen as an index of migratory capacity similar to the smoltification
464 transformation (Otto and McInerney 1970) or to genetic cognitive capacities as suggested in
465 European glass eels (Podgorniak et al. 2015). Overall, the presence of very different endocrine
466 gene expression patterns observed in glass eels captured on the Canadian east coast add to the
467 growing volume of evidence that both phenotypic plasticity as well as spatially varying selection
468 processes are present in this species. Divergent lipolysis capacity in American glass eel has also
469 been demonstrated for individuals captured at the same time and sites of capture (Gaillard et al.
470 2016). Gene × environment and origin × salinity effects can influence growth patterns and the
471 expressions of genes representing many functional groups in American glass eels arriving on the
472 Canadian east coast (Côté et al. 2009, 2014). Latitudinal variations in RNA/DNA ratios have
473 been detected throughout the entire distribution range (Laflamme et al. 2012), and evidence of
474 differing patterns of selection along environmental gradients (spatially varying selection) was
475 inferred to explain shifts in allele frequencies involved in metabolism (e.g. lipid and sugar
476 metabolism, development of respiratory function, development of heart muscle) within the time
477 frame of a single generation despite the panmictic reproduction mode in American Eel (Gagnaire
478 et al. 2012; Pavey et al. 2015).
479
480 <B> Gene expression of the thyroid axis and eel development
481 Since the development of glass eels captured in Rivière-Saint-Jean seemed to be less
482 advanced than in the other two rivers, the higher expressions of thyroid activity indicators could
483 be related to developmental processes. The thyroid axis has been shown to be involved in fish
484 metamorphosis (Power et al. 2001; Sudo et al. 2014). Specifically, iodothyronine deiodinases
485 have been shown to control developmental phases in teleost fishes (e.g., Jarque and Piña 2014).
486 Kawakami et al. (2013) showed higher expressions of thyroid hormone α_a and α_b receptors during
487 the larval stage of the Japanese eel with a decrease at the onset of metamorphosis followed by a
488 peak at later stages of metamorphosis. In the Japanese conger eel, *Conger myriaster*, the
489 expression of these two thyroid hormone receptors peaked at metamorphosis and the expression
490 of the isoform α_a was higher than that of α_b. Moreover, triiodothyronine in Japanese Eel was
491 primarily triggered during metamorphosis while thyroxine peaked at the end of the
492 metamorphosis and at the glass eel stage (Yamano et al. 2007).

493 In American Eel, hormonal concentration at the yellow eel stage indicated that those that
494 were colonizing rivers had the highest thyroxine concentrations (Castonguay et al. 1990).
495 Considering that both Edeline et al. (2004) and Castonguay et al. (1990) showed only slight
496 differences in triiodothyronine concentrations between freshwater and brackish/marine glass and
497 yellow eels, this may argue for a role related to metamorphosis to explain high expression levels
498 of genes related to the thyroid axis in Rivière-Saint-Jean**.** However, the role of development
499 processes involved in the hormone-encoding genes for thyroid axis remains unclear considering
500 the variations in response of gene expression profiles for this axis among our rivers along with
501 variations in the stage of pigmentation. Moreover, the number of individuals did not allow
502 including the time of capture for consideration in the present study. This is an important question
503 for future studies.
504
505 <*B*> Gene expression profiles of the thyrotropic axis
506 We analyzed different indicators of the thyrotropic axis, and we expected similar
507 differences in gene expressions along the whole axis (from thyroid stimulating hormone to
508 receptors for thyroid hormones), which was not the case. The different portions of this axis are
509 regulated by different mechanisms (for a review see Orozco and Valverde-R 2005), and it could
510 be relevant trying to find at which level of regulation differences between ecotypes occur.
511 Indeed, Sudo et al. (2014) found increased thyroid hormone levels without an increase in the
512 expression of TSH-β during Japanese eel metamorphosis (Sudo et al. 2014). Body concentration
513 measurements of circulating thyroid hormones would have provided suitable information.
514 Unfortunately our attempts to perform such measures were unsuccessful in the sense that levels
515 were always below the detection threshold of the RIA kit used even when we worked with pools
516 of four individuals that were shorter, younger and had less plasma compared to European glass
517 eels at their entrance into the rivers.
518
519 **Conclusions**
520 This study highlights that endocrine mechanisms underlying recruitment in glass eels
521 depend on their ecotype and vary along geographic sites, since ecotypes are adapted to live under
522 varying environmental conditions. Our results show that the larger size of the freshwater ecotype
523 could be explained by their GH/GH-R_1 and IGF-1R gene expression and that high GH/GH-R_1

and high type 2-deiodinase mRNA levels could be important traits allowing this ecotype to reach remote and colder Canadian estuaries. The regulatory mechanisms of the thyroidal and somatotropic axes that could explain the different geographic hormonal gene expression patterns as well as how these differences are programmed during the glass eel stage remain to be elucidated. The demonstration of differential molecular phenotypes between ecotypes and rivers at recruitment stage supports the view that the origin of glass eels potentially used in stocking practices should be taken into account in management decisions. Namely, the choice of the geographic site to be used in resettlement programs should take into account ecotypic variation (e.g. freshwater vs. salt-brackish ecotypes, sensu Pavey et al. 2015) in order to respect ecological and genetic integrity of supplemented contingents.

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References

Arakawa, E., T. Kaneko, K. Tsukamoto and T. Hirano. 1992. Immunocytochemical detection of prolactin and growth hormone cells in the pituitary during early development of the Japanese eel, *Anguilla japonica* (Endocrinology). Zoological Science 9:1061-1066.

River-specific gene expression patterns in recruits of American glass eels

555 Boivin, B., M. Castonguay, C. Audet, S. A. Pavey, M. Dionne and L. Bernatchez. 2015. How
556 does salinity influence habitat selection and growth in juvenile American eels *Anguilla* rostrata?
557 Journal of Fish Biology 86:765-784.
558
559 Bustin, S. A., V. Benes, J. A. Garson, J. Hellemans, J. Huggett, M. Kubista, R. Mueller, T.
560 Nolan, M. W. Pfaffl, G. L. Shipley, J. Vandesompele and C. T. Wittwer. 2009. The MIQE
561 guidelines: minimum information for publication of quantitative real-time PCR experiments.
562 Clinical Chemistry 55:611-622.
563
564 Cairns, D. K., J. C. Shiao, Y. Iizuka, W. N. Tzeng and C. D. MacPherson. 2004. Movement
565 patterns of American eels in an impounded watercourse, as indicated by otolith microchemistry.
566 North American Journal of Fisheries Management 24:452-458.
567
568 Castonguay, M., J.-D. Dutil, C. Audet and R. Miller. 1990. Locomotor activity and concentration
569 of thyroid hormones in migratory and sedentary juvenile American eels. Transactions of the
570 American Fisheries Society 119:946-956.
571
572 Ciccotti, B. E., E. Macchi, A. Rossi, E. Cataldi and S. Cataudella. 1993. Glass eel (*Anguilla*
573 *anguilla*) acclimation to freshwater and seawater: morphological changes of the digestive tract.
574 Journal of Applied Ichthyology 9:74-81.
575
576 Côté, C. L., M. Castonguay, M. Kalujnaia, G. Cramb and L. Bernatchez. 2014. In absence of
577 local adaptation, plasticity and spatially varying selection rule: a view from genomic reaction
578 norms in a panmictic species (*Anguilla rostrata*). BMC Genomics 15:403.
579
580 Côté, C. L., M. Castonguay, G. Verreault and L. Bernatchez. 2009. Differential effects of origin
581 and salinity rearing conditions on growth of glass eels of the American eel *Anguilla rostrata*:
582 implications for stocking programmes. Journal of Fish Biology 74:1934-1948.
583

584 Côté, C. L., P. A. Gagnaire, V. Bourret, G. Verreault, M. Castonguay, and L. Bernatchez. 2013.
585 Population genetics of the American eel (*Anguilla rostrata*): FST = 0 and North Atlantic
586 Oscillation effects on demographic fluctuations of a panmictic species. Molecular Ecology 22:
587 1763-1776.
588
589 Côté, C. L., S. A. Pavey, J. A. Stacey, T. C. Pratt, M. Castonguay, C. Audet and L. Bernatchez.
590 2015. Growth, female size, and sex ratio variability in American eel of different origins in both
591 controlled conditions and the wild: Implications for stocking programs. Transactions of the
592 American Fisheries Society 144 :246-257.
593
594 Creutzberg, F. 1961. On the orientation of migrating elvers (*Anguilla vulgaris* Turt.) in a tidal
595 area. Netherlands Journal of Sea Research 1:257-338.
596
597 Dai, X., W. Zhang, Z. Zhuo, J. He and Z. Yin. 2015. Neuroendocrine regulation of somatic
598 growth in fishes. Science China Life Sciences 58 :137-147.
599
600 Edeline E., 2005. Facteurs du contrôle de la dispersion continentale chez l'anguille. PhD thesis,
601 Paul Sabatier Toulouse III University.
602
603 Edeline, E., A. Bardonnet, V. Bolliet, S. Dufour and P. Elie. 2005a. Endocrine control of
604 *Anguilla anguilla* glass eel dispersal: effect of thyroid hormones on locomotor activity and
605 rheotactic behavior. Hormones and Behaviour 48:53-63.
606
607 Edeline, E., S. Dufour, C. Briand, D. Fatin and P. Elie. 2004. Thyroid status is related to
608 migratory behavior in *Anguilla anguilla* glass eels. Marine Ecology Progress Series 282:261-
609 270.
610
611 Edeline, E., S. Dufour and P. Elie. 2005b. Role of glass eel salinity preference in the control of
612 habitat selection and growth plasticity in *Anguilla anguilla*. Marine Ecology Progress Series
613 304:191-199.
614

River-specific gene expression patterns in recruits of American glass eels

615 Edeline, E., S. Dufour and P. Elie. 2009. Proximate and ultimate control of eel continental
616 dispersal spawning migration of the European eel. Pages 433-461*in* G. van den Thillart, S.
617 Dufour and J. C. Rankin, editors. Spawning Migration of the European Eel. Fish & Fisheries
618 Series, vol. 30. Springer, Dorrecht, Netherlands.
619
620 Escobar, S., E., N. Fuentes, E. Poblete, J. A. Valdés, D. Safian, A. E. Reyes, M. Álvarez and A.
621 Molina 2011. Molecular cloning of IGF-1 and IGF-1 receptor and their expression pattern in the
622 Chilean flounder (*Paralichthys adspersus*). Comparative Biochemistry and Physiology- Part B:
623 Biochemistry & Molecular Biology 159:140-147.
624
625 Ferrier Maurice, C., H.J. Haiser and P.J. Turnbaugh. 2013. Xenobiotics shape the physiology and
626 gene expression of the active human gut microbiome. Cell 152, 39–50.
627
628 Gagnaire, P.-A., E. Normandeau, C. Côté, M. Møller Hansen and L. Bernatchez. 2012. The
629 genetic consequences of spatially varying selection in the panmictic American Eel (*Anguilla*
630 *rostrata*). Genetics 190:725-736.
631
632 Gaillard, M., L. Bernatchez, R. Tremblay and C. Audet. 2015. Regional variation in energy
633 storage strategies in American glass eels from Eastern Canada. Comparative Biochemistry and
634 Physiology - Part A: Molecular & Integrative Physiology 188:87-95.
635
636 Gaillard, M., S. A. Pavey, C. L. Côté, L. Bernatchez and C. Audet. 2016. Regional variation of
637 gene regulation associated with energy metabolism in American glass eels (*Anguilla rostrata*).
638 Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology
639 196:30-37.
640
641 García, S., J. Luengo and F. Herrera 2015. Data preprocessing in data mining. Springer
642 International Publishing, Switzerland.
643

644 Gomes, A. S., R. N. Alves, I. Rønnestad and D. M. Power. 2014. Orchestrating change: the
645 thyroid hormones and GI-tract development in flatfish metamorphosis. General and Comparative
646 Endocrinology 220:2-12.
647
648 Han, Y., I.-C. Liao, W.-N. Tzeng and J. Y.-L. Yu. 2004. Cloning of the cDNA for thyroid
649 stimulating hormone beta subunit and changes in activity of the pituitary-thyroid axis during
650 silvering of the Japanese eel, Anguilla japonica. Journal of Molecular Endocrinology 32:179-
651 194.
652
653 Haro, A. J. and W. H. Krueger 1988. Pigmentation, size, and migration of elvers (*Anguilla*
654 *rostrata* (Lesueur)) in a coastal Rhode Island stream. Canadian Journal of Zoology 66:2528-
655 2533.
656
657 Jarque, S. and B. Piña 2014. Deiodinases and thyroid metabolism disruption in teleost fish.
658 Environmental Research 135:361-375.
659
660 Jessop, B., J.-C. Shiao, Y. Iizuka and Tzeng W.-N. 2002. Migratory behaviour and habitat use by
661 American eels *Anguilla rostrata* as revealed by otolith microchemistry. Marine Ecology Progress
662 Series 233:217-229.
663
664 Kawakami, Y., K. Nomura, H. Ohta and H. Tanaka. 2013. Characterization of thyroid hormone
665 receptors during early development of the Japanese eel (*Anguilla japonica*). General and
666 Comparative Endocrinology 194 :300-310.
667
668 Laflamme, S., C. Côté, P.-A. Gagnaire, M. Castonguay, L. Bernatchez. 2012. RNA/DNA ratios
669 in American glass eels (*Anguilla rostrata*): evidence for latitudinal variation in physiological
670 status and constraints to oceanic migration? Ecology and Evolution 2:875-884.
671
672 Livak, K. J. and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-
673 time quantitative PCR and the 2−ΔΔCT method. Methods 25:402-408.
674

675 MacKenzie, D. S., R. A. Jones and T. C. Miller. 2009. Thyrotropin in teleost fish. General and
676 Comparative Endocrinology 161:83-89.
677
678 Manzon, L. A. 2002. The role of prolactin in fish osmoregulation: a review. General and
679 Comparative Endocrinology 125:291-310.
680
681 McInerney, J. E. 1964. Salinity preference: an orientation mechanism in salmon migration.
682 Journal of the Fisheries Research Board of Canada 21:995-1018.
683
684 Miller, C. A., A. J. Fivizzani and A. H. Meier. 1983. Water temperature influences salinity
685 selection in the Gulf killifish, *Fundulus grandis*. Canadian Journal of Zoology 61:1265-1269.
686
687 Olsvik, P. A., K. K. Lie, A.-E. O. Jordal, T. O. Nilsen and I. Hordvik. 2005. Evaluation of
688 potential reference genes in real-time RT-PCR studies of Atlantic salmon. BMC Molecular
689 Biology 6:21-30.
690
691 Orozco, A. and C. Valverde-R. 2005. Thyroid hormone deiodination in fish. Thyroid 15:799-
692 813.
693
694 Otto, R. G. and J. E. McInerney. 1970. Development of salinity preference in pre-smolt coho
695 salmon, *Oncorhynchus kisutch*. Journal of Fisheries Research Board of Canada 27:793-800.
696
697 Ozaki, Y., H. Fukada, Y. Kazeto, S. Adachi, A. Hara and K. Yamauchi. 2006a. Molecular
698 cloning and characterization of growth hormone receptor and its homologue in the Japanese eel
699 (*Anguilla japonica*). Comparative Biochemistry and Physiology - Part B: Biochemistry &
700 Molecular Biology 143:422-431.
701
702 Ozaki, Y., H. Fukada, H. Tanaka, H. Kagawa, H. Ohta, S. Adachi, A. Hara and K. Yamauchi.
703 2006b. Expression of growth hormone family and growth hormone receptor during early
704 development in the Japanese eel (*Anguilla japonica*). Comparative Biochemistry and Physiology
705 - Part B: Biochemistry & Molecular Biology 145:27-34.

Pavey, S. A., J. Gaudin, E. Normandeau, M. Dionne, M. Castonguay, C. Audet and L. Bernatchez. 2015. RAD sequencing highlights polygenic discrimination of habitat ecotypes in the panmictic American eel. Current Biology 12:1666-1671.

Pavey, S. A., M. Laporte, E. Normandeau, J. Gaudin, L. Létourneau, S. Boisvert, J. Corbeil, C. Audet and L. Bernatchez 2016. Draft genome of the American eel (*Anguilla rostrata*). Molecular Ecology Resources 17:806-811.

Perrot, V., E. B. Moiseeva, Y. Gozes, S. J. Chan, P. Ingleton and B. Funkenstein. 1999. Ontogeny of the insulin-like growth factor system (IGF-I, IGF-II, and IGF-1R) in gilthead seabream (*Sparus aurata*): expression and cellular localization. General and Comparative Endocrinology 116:445-460

Podgorniak, T., A. Angelini, S. Blanchet, E. de Oliveira, F. Pierron and F. Daverat. 2015. Climbing experience in glass eels: A cognitive task or a matter of physical capacities? Physiology and Behavior 151:448-455.

Politis, S. N., D. Mazurais, A. Servili, J.-L. Zambonino-Infante, J. J. Miest, S. R. Sørensen, J. Tomkiewicz and I. A. E. Butts. 2017. Temperature effects on gene expression and morphological development of European eel, *Anguilla anguilla* larvae. PLoS ONE 12: e0182726.

Power, D., L. Llewellyn, M. Faustino, M. A. Nowell, B. T. Björnsson, I. Einarsdottir, A. V. Canario, G. E. Sweeney. 2001. Thyroid hormones in growth and development of fish. Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology 130:447-459.

Reynolds, W. W. and D. A. Thomson. 1974. Ontogenetic change in the response of the Gulf of California grunion, *Leuresthes sardina* (Jenkins & Evermann), to a salinity gradient. Journal of Experimental Marine Biology and Ecology 14:211-216.

736 Sakamoto, T. and S. D. McCormick. 2006. Prolactin and growth hormone in fish
737 osmoregulation. General and Comparative Endocrinology 147:24-30.
738
739 Sasai, S., F. Katoh, T. Kaneko and K. Tsukamoto. 2007. Ontogenic change of gill chloride cells
740 in leptocephalus and glass eel stages of the Japanese eel, *Anguilla japonica*. Marine Biology
741 150:487-496.
742
743 Sudo, R., A. Okamura, M. Kuroki and K. Tsukamoto. 2014. Changes in the role of the thyroid
744 axis during metamorphosis of the Japanese eel, *Anguilla japonica*. Journal of Experimental
745 Zoology - Part A: Ecological and Integrative Physiology 321:357-364.
746
747 Sudo, R., H. Suetake, Y. Suzuki, J. Aoyama and K. Tsukamoto 2013. Profiles of mRNA
748 expression for prolactin, growth hormone, and somatolactin in Japanese eels, *Anguilla japonica*:
749 the effect of salinity, silvering and seasonal change. Comparative Biochemistry and Physiology -
750 Part A: Molecular & Integrative Physiology 164:10-16.
751
752 Tamaki, H., C.L. Wright, X. Li, O. Lin, C. Hwang, S. Wang, J. Thimmapuram, Y. Kamagata and
753 W.-T. Liu. 2011. Analysis of 16S rRNA amplicon sequencing options on the Roche/454 next-
754 generation titanium sequencing platform. PLoS ONE 6(9): e25263.
755
756 Tesch, F.-W. 2003. The Eel, 5th Edition. Wiley-Blackwell, Oxford.
757
758 Tosi, L., L. Sala, C. Sola, A. Spampanato and P. Tongiorgi. 1988. Experimental analysis of the
759 thermal and salinity preferences of glass-eels, *Anguilla anguilla* (L.), before and during the
760 upstream migration. Journal of Fish Biology 33:721-733.
761
762 Tsukamoto, K., L. Nakal and W.-V. Tesch. 1998. Do all freshwater eels migrate? Nature 396:
763 635-636.
764
765 Turesson, G. 1922. The genotypical response of the plant species to the habitat. Hereditas 3:211-
766 350.

Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe, F. Speleman. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology 3:research0034.

Weltzien, F.-A., C. Pasqualini, P. Vernier, S. Dufour. 2005. A quantitative real-time RT-PCR assay for European eel tyrosine hydroxylase. General and Comparative Endocrinology 142:134-142.

Wilson, J., A. Leitão, A. Gonçalves, C. Ferreira, P. Reis-Santos, A.-V. Fonseca, J. da Silva, J. Antunes, C. Pereira-Wilson and J. Coimbra 2007. Modulation of branchial ion transport protein expression by salinity in glass eels (*Anguilla anguilla* L.). Marine Biology 151:1633-1645.

Yamano, K., K. Nomura and H. Tanaka. 2007. Development of thyroid gland and changes in thyroid hormone levels in leptocephali of Japanese Eel (*Anguilla japonica*). Aquaculture 270:499-504.

River-specific gene expression patterns in recruits of American glass eels

Captions

Figure 1 Map showing sampling sites. 1: Mersey River, Nova Scotia (MR), brackish/saltwater ecotype; 2: Rivière-Saint-Jean, Gulf of St. Lawrence, Québec (RSJ), freshwater ecotype; 3: Grande-Rivière-Blanche, St. Lawrence River, Québec (GRB), freshwater ecotype. The dates of capture and a schema representing the experimental design (10 glass eels per river and per salinity preference; Boivin et al. 2014) are also shown.

Figure 2 Multidimensional scaling (MDS) of Bray-Curtis similarities from gene expressions database and vector plots associated. Only vector plots that contributed the most to dissimilarities between rivers, are shown ($\rho > 0.6$ on one MDS axis). Vector plots characterize the grouping on the MDS plot, i.e., direction of the vectors is dictated by elevated gene expressions (a) and elevated biological trait measurements (b). Each river was averaged in the MDS by the factor river × salinity, to maximize readability on the 2-D ordination (c). GRB: Grande-Rivière-Blanche; MR: Mersey River: RSJ: Rivière-Saint-Jean. Large dashed circles indicate freshwater ecotype and light dashed circle indicates salt/brackish ecotype. FW: fresh water; SW/BW: salt/brackish water.

Spearman correlations of the vector plots were : (a) $\rho = -0.84$ for type 2-iodothyronine deiodinase (DIO-2), $\rho = -0.93$ for prolactin (PRL), $\rho = -0.71$ for growth hormone (GH), $\rho = -0.40$ for thyroid stimulating hormone (TSH-β), and $\rho = -0.30$ for insulin-like growth factor 1 receptor (IGF-1R) on the horizontal axis of MDS 1, and $\rho = -0.37$ for DIO-2, $\rho = -0.09$ for PRL, $\rho = 0.55$ for GH, $\rho = -0.62$ for TSH-β, and $\rho = 0.82$ for IGF-1R on the vertical axis of MDS 2; (b) $\rho = -0.64$ for length, $\rho = 0.75$ for pigmentation stage (Pig.) on MDS 1 axis, and $\rho = -0.74$ for length, $\rho = -0.11$ for Pig. on MDS 2 axis.

Figure 3 Relative changes in gene expression of nine candidate genes in glass eels captured in the Mersey River (MR), Rivière-St-Jean (RSJ), and Grande-Rivière-Blanche (GRB). The calibrator values (indicated by horizontal lines) were determined from MR glass eels with a preferrence for brackish water (mean ± SE): A) Prolactine (PRL), B) type 2-iodothyronine deiodinase (DIO-2), C) growth hormone (GH), D) insulin-like growth factor 1 receptor (IGF-1R), E) thyroid stimulating hormone (TSH-β), F) thyroid hormone receptor α_b (THRα_b), G) growth hormone receptor 1 (GH-R$_1$), H) thyroid hormone receptor α_a (THRα_a), and I) insulin-

like growth factor 1 (IGF-1). The dashed horizontal lines indicate the normalized values for the calibrator group. Different letters indicate significant differences among rivers (one-way ANOVA, $\alpha = 0.05$).

Figure 4 Biological trait measurements: (A) length, (B) wet mass, (C) LeCren condition index, Kn, and (D) pigmentation stage. MR: Mersey River, RSJ: Rivière-St-Jean, GRB: Grande-Rivière-Blanche. Mean ± SE are presented for (A), (B), and (C). A box plot of the median, 25 and 75% percentiles, minimum and maximum values are shown for pigmentation stage (D). The asterisk (non parametric analysis) and different letters (parametric statistical analysis) indicate significant differences among rivers.

River-specific gene expression patterns in recruits of American glass eels

827 **Table 1** List of each GenBank accession numbers, species' sequences forward (F) and reverse
828 (R) primers, Taqman primers F, R and Taqman probes (P), of the reference and candidate genes.
829 qPCR efficiencies (equation of linear regression and its coefficient of determination, R^2) are also
830 presented.

River-specific gene expression patterns in recruits of American glass eels

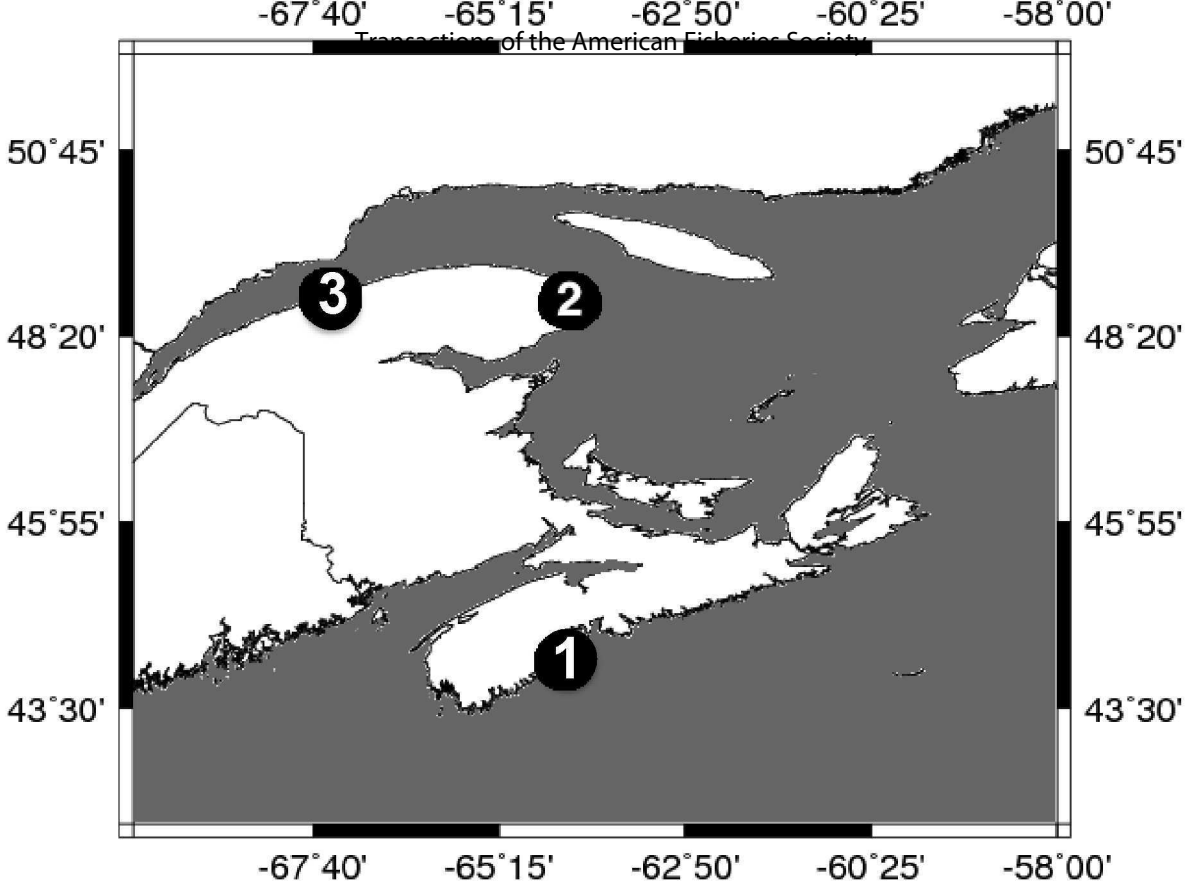
Coding Genes	GenBank access. No.	Forward and reverse primers used for sequencing	Taqman forward, reverse, and probes used for qPCR	qPCR efficiency
Acidic ribosomal protein: ARP	*A. anguilla* (AY763793)	F:GCCACGTGGAAGTCCAACTA R:CAGGAGTTTCTCCAGAGCGG	F:TCTCCCTGCGTGCAAAGG P:TGGTGCTGATGGGC R:CTTGCGCATCATGGTGTTCT	y = −3.1958 x + 34.388 R^2 = 0.99926
Cytochrome B: CytB	*A. anguilla* (AF006714)	F:CATCTGCCGAGACGTCAACT R:ATCTGCCCTCATGGAAGCAC	F:TCATCTGCCGAGACGTCAAC P:ATGGATGATTAATTCGC R:GAGGCCCCATTTGCATGTAG	y = −3.1254 x + 32.523 R^2 = 0.99922
Elongation factor 1, EF1	*A. japonica* (AB593812)	F:CCTGAAGCCTGGTATGGTGG R:TACGTTGCCACGACGGATTT	F:TTGCCCCTGCCAACGT P:ACCACTGAGGTCAAGTC R:GGGACTCATGGTGCATTTCC	y = −3.3354 x + 33.755 R^2 = 0.99974
Prolactin, PRL	*A.anguilla* (X69149)	F:CTTCCCTCCAAACCCCTCAC R:CCTTGGAGGAGGAGCCAATC	F:GGCGCCATCTACAGCAAAAC P:AGGGAACTGCAGGACC R:CCCAGAGCTCAGGCTGTTG	y = −3.2675 x + 29.078 R^2 = 0.98724
Thyroid stimulating hormone β, TSH-β	*A. japonica* (AY158008.1)	F:GCCCTCCAAGATGAGAGTGG R:GCAGACTGGAGGCTCTTACC	F:GCAGGACAGGTTCTCTCCATCT P:CCTGTGGACTACACGCT R:TCACACTCTGGTTTCTCCACGTA	y = −3.2842 x + 28.009 R^2 = 0.98643
Thyroid hormone receptors α_a, THRα_a	*A. japonica* (AB678206.1)	F:TCGTTTTGTTGCAGGGGTTG R:CTTTCATTGGCAAGCTCCCG	F:CGATGCAGAAAGTGGTGATACC P:TGAGACCGACCGCTC R:CAAATCTCCACTGCTCTTGTCTCA	y = −3.6152 x + 24.467 R^2 = 0.99738

River-specific gene expression patterns in recruits of American glass eels

Thyroid hormone receptors $\alpha_{b,}$ THRα_b	*A. japonica* (AB678207.1)	F:CTCACGTGCGTGGAGAAGAT R:GTCTGCTTCACACCTCCTGG	F:GCTGGCGTTCGAACACTACA P:AACTACCGCAAGCACA R:TGGGCCAGAAGTGAGGAATG	y = −3.1052 x + 40.300 R^2 = 0.99847
Type 2-iodothyronine deiodinase, DIO-2	*A. japonica* (AB199797.1)	F:AAGCTGGGCTTCAGTAGCAG R:CCTCTGAGCAGCCAGAACTC	F:CCTGCTGGACTTTGCGTCAT P:CGACCGTCCTTTAGTG R:GTGGCCGAGCCAAAGTTG	y = −3.3820 x + 40.970 R^2 = 0.99624
Growth hormone, GH	*A. anguilla* (AY616666.1)	F:CCCAGGTTAAGGGGCTGATG R:CAAGCCCAATCCCTCACACA	F:ATCTCCTCAGCCCTGATCCA P:TCATGGGTGTATCCTC R:AAGCATCGCTCAGGGTCTTC	y = −3.1047 x +25.839 R^2 = 0.99967
Growth hormone receptor 1, GH-R_1	*A. japonica* (AB180476.1)	F:CATCTTCACCCCTTGCCTGT R:TACTGGGTCCAGACGGATGT	F:GGGTCTTCTTCGCCACTAGCT P:CTGCCCAGCGACTG R:TGACCGAGTAGTCCGGACACT	y = −3.3030 x + 38.281 R^2 = 0.99985
Insulin like growth factor 1, IGF-1	*A. japonica* (AB353115.1)	F:GCTGCAGTTTGTGTGTGGAG R:TCTGATGCACCTCCTTGCAG	F:CAGGCTATGGATCCAGCTCAA P:ACGGTCACACAATCG R:GCAGCACTCGTCGACTATGC	y = −3.3309 x + 25.418 R^2 = 0.99202
Insulin like growth factor 1 receptor, IGF-1R	*A. rostrata*	*Pavey, unpublised data*	F:CGAGCGCATCGAGTTCCT P:AACGAGGCGTCCGTC R:GGTGGCAGTTGAACTCCTTCA	y = −3.2673 x + 40.298 R^2 = 0.99755

831

832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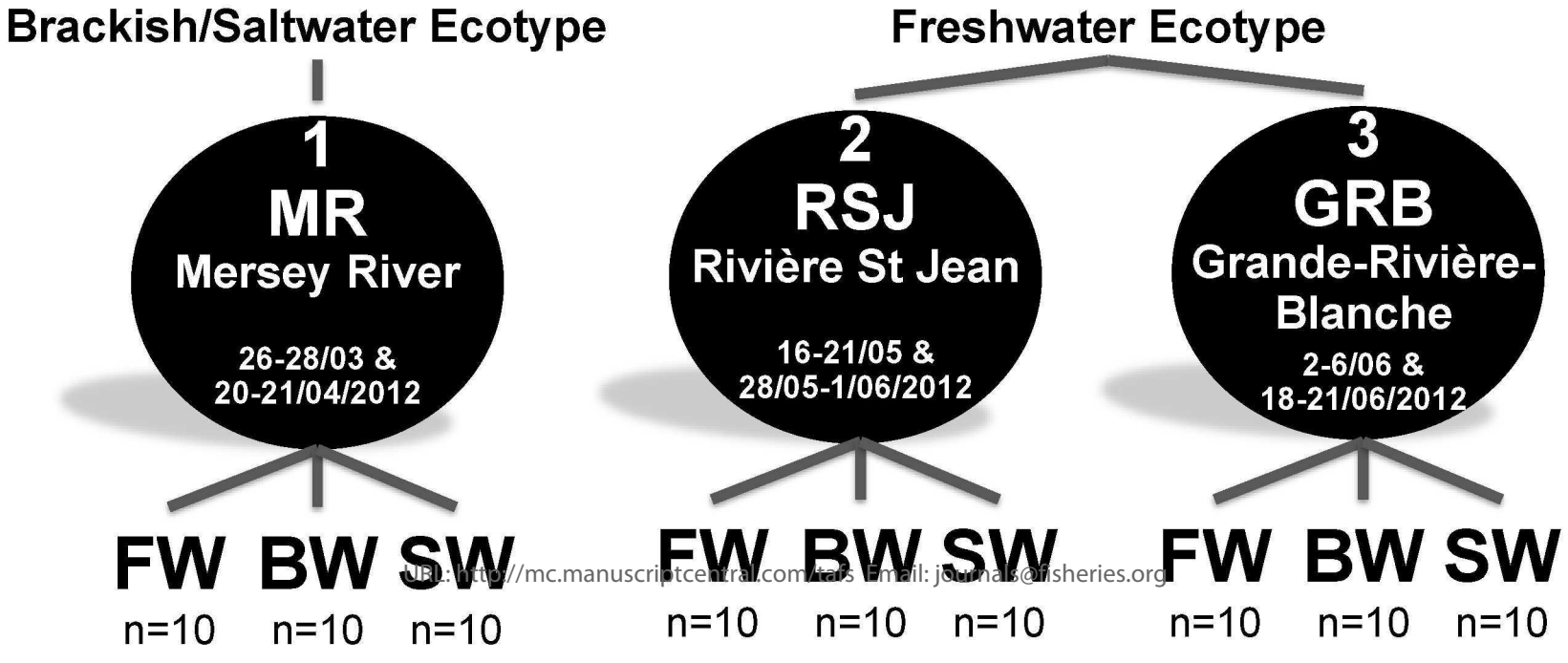
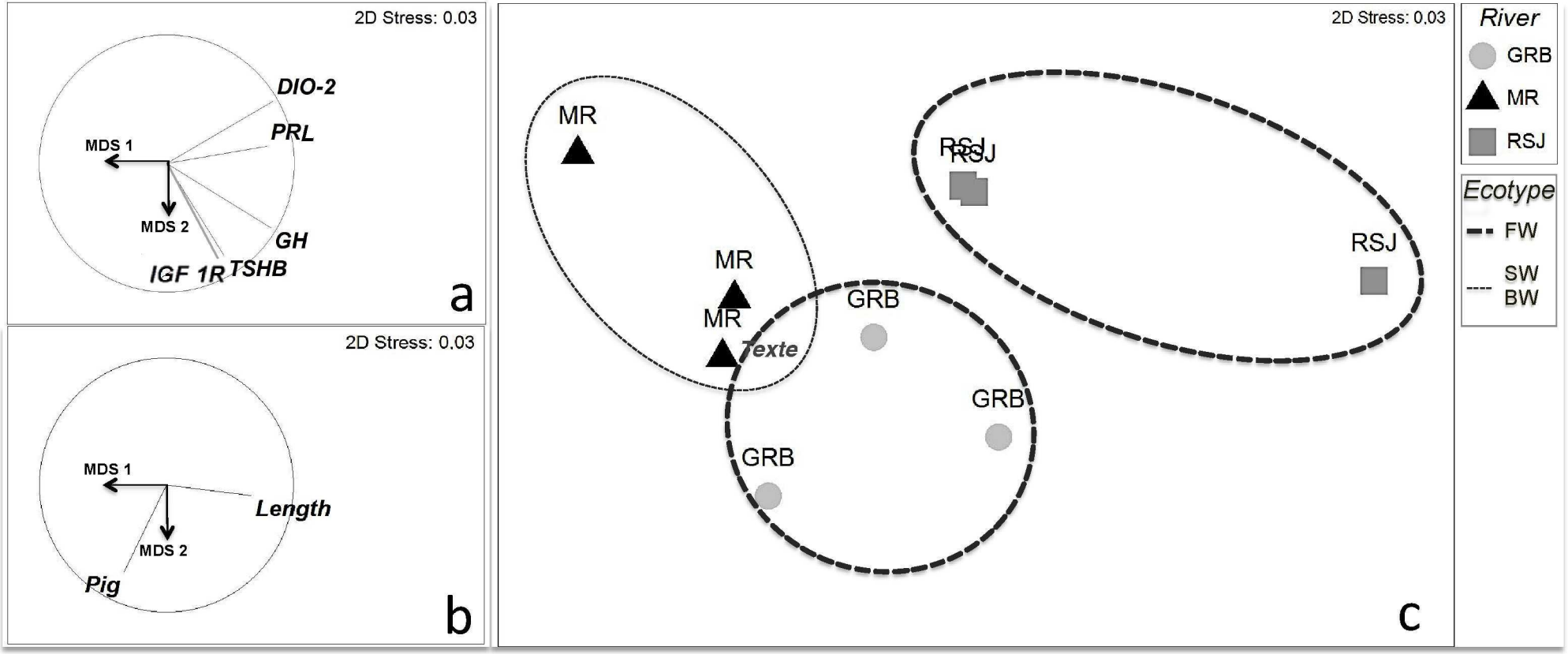
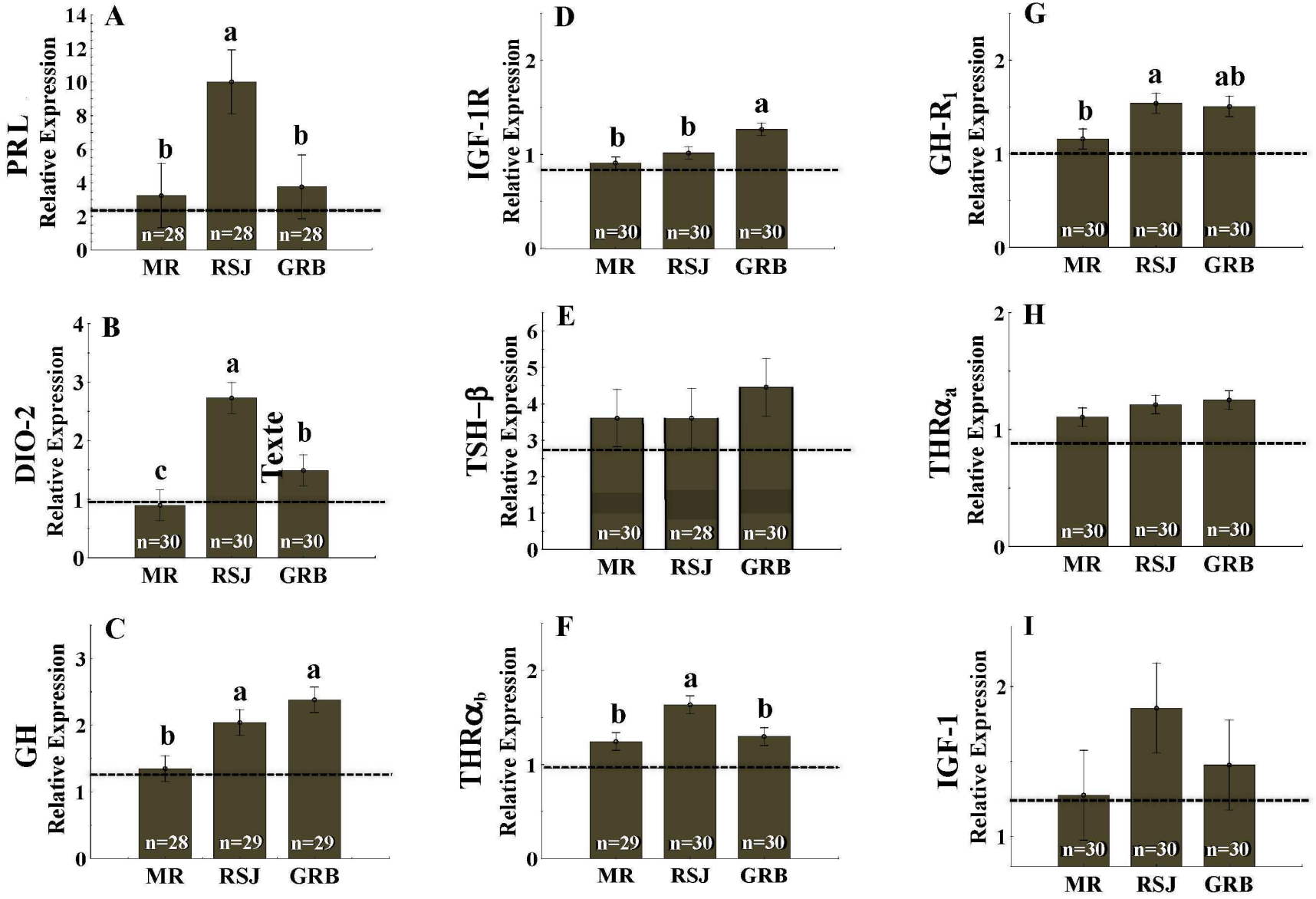
48°20'
45°55'
43°30'
3
2
1


Brackish/Saltwater Ecotype
Freshwater Ecotype
1
MR
Mersey River
26-28/03 &
20-21/04/2012
2
RSJ
Rivière St Jean
16-21/05 &
28/05-1/06/2012
3
GRB
Grande-Rivière-
Blanche
2-6/06 &
18-21/06/2012
FW BW SW
n=10 n=10 n=10
FW BW SW
n=10 n=10 n=10
FW BW SW
n=10 n=10 n=10


2D Stress: 0.03
DIO-2
PRL
MDS 1
MDS 2
GH
IGF 1R
TSHB
a
2D Stress: 0.03
MDS 1
Length
MDS 2
Pig
b
2D Stress: 0.03
MR
RSJ
RSJ
RSJ
MR
MR
Texte
GRB
GRB
GRB
c
River
GRB
MR
RSJ
Ecotype
FW
SW
BW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PRL
Relative Expression
b
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b
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MR
RSJ
GRB
D
IGF-1R
Relative Expression
b
b
a
n=30
n=30
n=30
MR
RSJ
GRB
G
GH-R1
Relative Expression
b
a
ab
n=30
n=30
n=30
MR
RSJ
GRB
B
DIO-2
Relative Expression
c
a
b
n=30
n=30
n=30
MR
RSJ
GRB
E
TSH–β
Relative Expression
n=30
n=28
n=30
MR
RSJ
GRB
H
THRαa
Relative Expression
n=30
n=30
n=30
MR
RSJ
GRB
C
GH
Relative Expression
b
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MR
RSJ
GRB
F
THRαb
Relative Expression
b
a
b
n=29
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MR
RSJ
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Relative Expression
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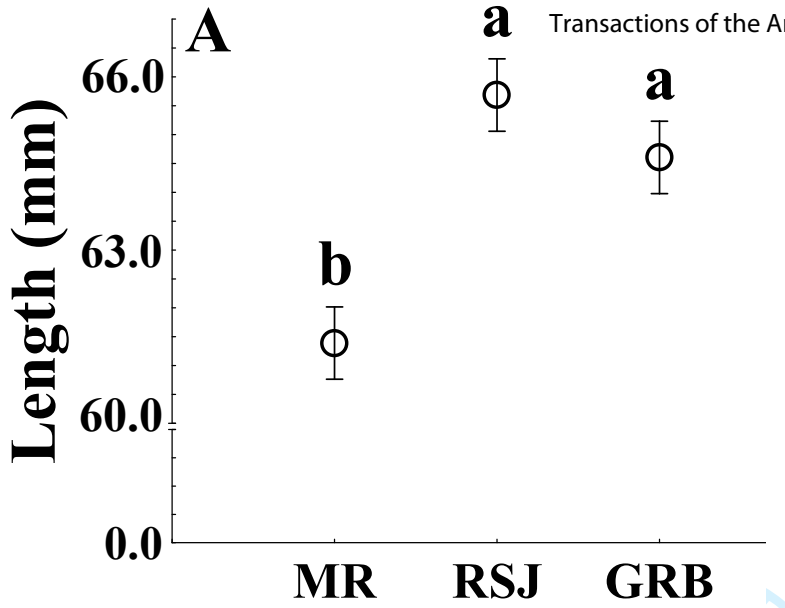
RSJ
GRB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Length (mm)
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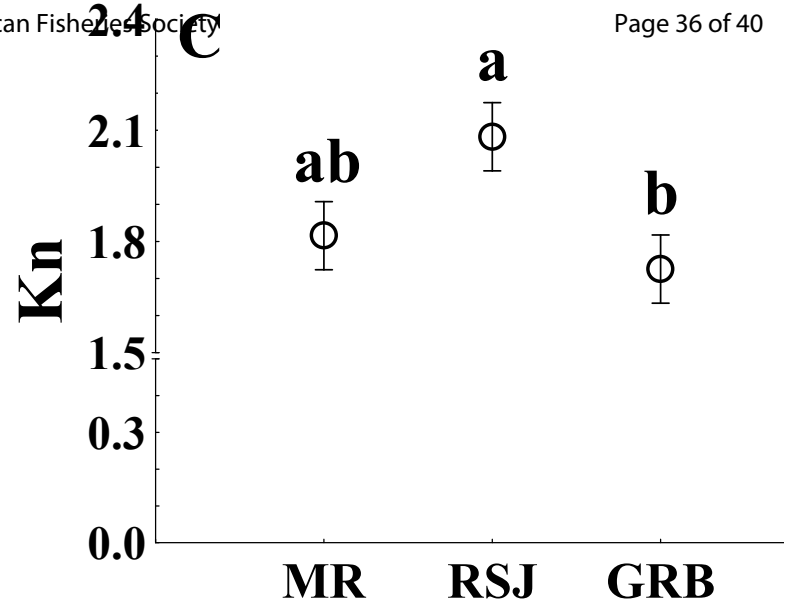
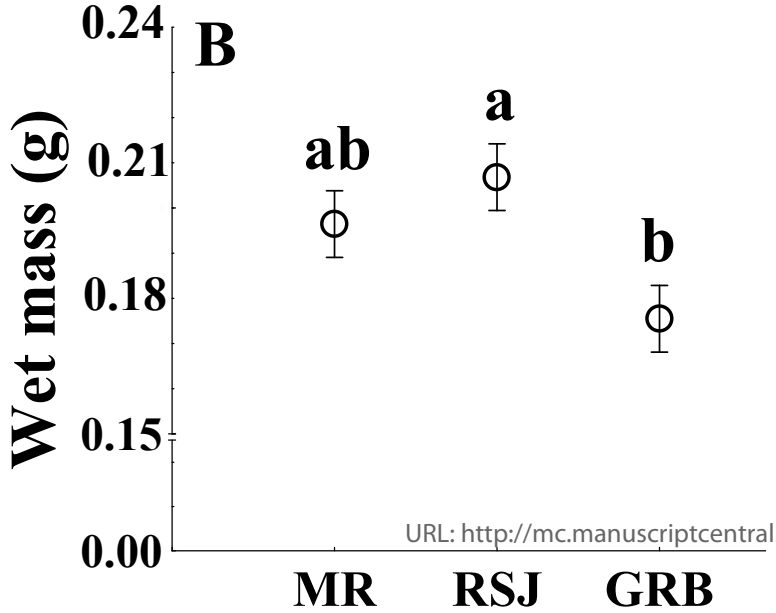
60.0
0.0
a
a
b
MR
RSJ
GRB


C
Kn
2.4
2.1
1.8
1.5
0.3
0.0
a
ab
b
MR
RSJ
GRB


B
Wet mass (g)
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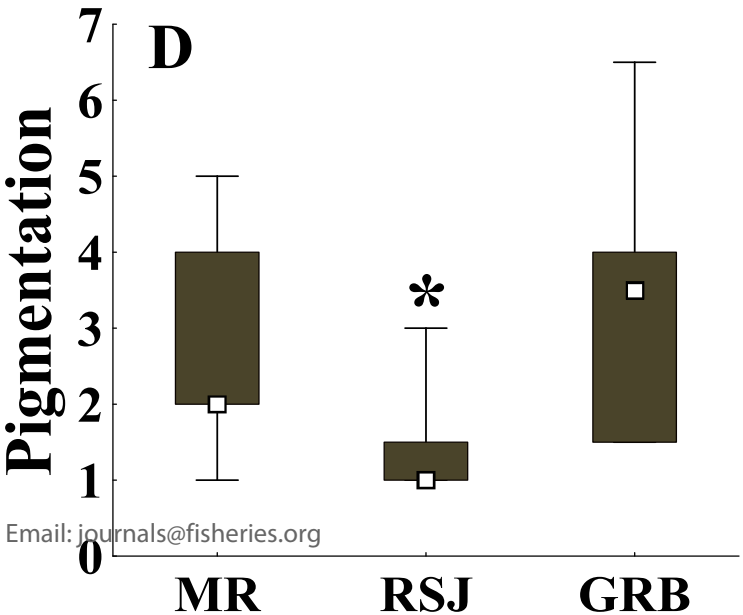
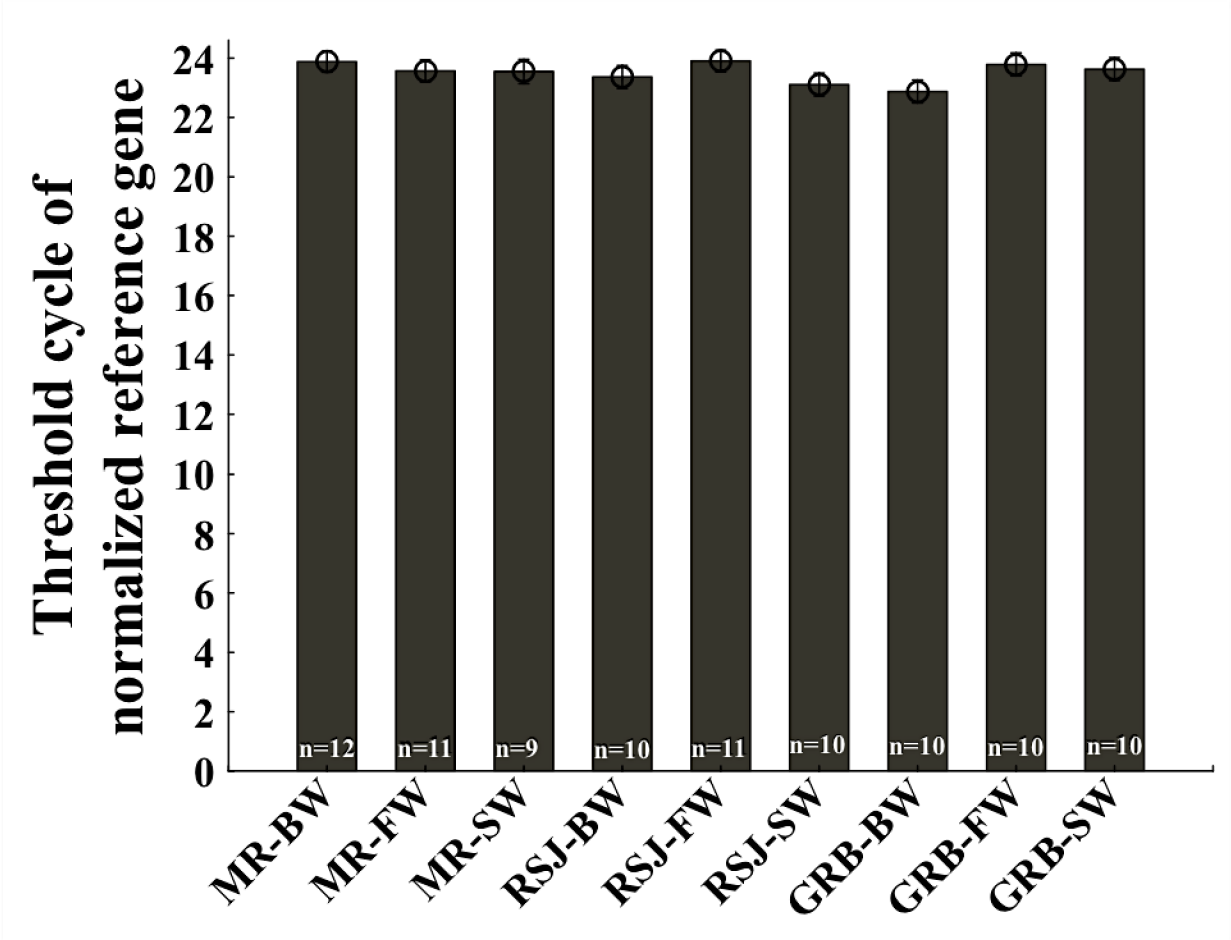
0.18
0.15
0.00
ab
a
b
MR
RSJ
GRB


D
Pigmentation
7
6
5
4
3
2
1
0
*
MR
RSJ
GRB


Threshold cycle of normalized reference gene
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22
20
18
16
14
12
10
8
6
4
2
0
n=12
n=11
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n=11
n=10
n=10
n=10
n=10
MR-BW
MR-FW
MR-SW
RSJ-BW
RSJ-FW
RSJ-SW
GRB-BW
GRB-FW
GRB-SW

River-specific gene expression patterns in recruits of American glass eels

Supplement material 1

Specific sequences (expressed sequence tag [EST] or coding DNA sequence [CDS] for IGF-1R) listed for each gene with their numbers of base pairs. Except for IGF-1R, the percentage of similarity and the score obtained between these sequences and the GenBank species with the best alignment results are also provided. Alignment results were extracted using the Basic Local Alignment Search tool, Blast® (http://blast.ncbi.nlm.nih.gov; website accessed 12-06-2014). Reference genes: ARP: acidic ribosomal protein, CytB: cytochrome B, and EF1: elongation factor 1; candidate genes: PROL: prolactine, TSH-β: thyroid stimulating hormone β, THRα_a and THRα_b: thyroid hormone receptors α_a and α_b, DIO-2: Type 2-iodothyronine deiodinase, GH: growth hormone, GH-R_1: growth hormone receptor 1, IGF-1: insulin-like growth factor 1, and IGF-1R: insulin-like growth factor 1 receptor. Underlined sections indicate positions of forward and reverse Taqman primers and double underlined sections indicate positions of Taqman probes.

River-specific gene expression patterns in recruits of American glass eels

Gene	Specific expressed sequence tag of *Anguilla rostrata* (number of base pairs)	Results of Alignment
ARP	TGCCACGTGGAAGTCCAACTATTTTATGAAAATCATCCAACTCTTGGATGAGTACCCCAAGTGCTTC ATTGTGGGGGCCGACAATGTGGGCTCCAAGCAGATGCAGACCATCCGCCTCTCCCTGCGTGCAAAGG CTGTGGTGCTGATGGGCAAGAACACCATGATGCGCAAGGCCATCCGTGGCCATCTGGAGAACAACC CCGCTCTGGAGAAACTCCTGAAATCCCTATTTTCCAAAGCCCGAAGGCTTTGCATATGTAATTTGCCA TAAAACACCTTTTTGCCAAGAAGGTAAATTCCTC (302bp)	AY63793.1 *(A. anguilla)* 99%, 399
CytB	TTCATCTGCCGAGACGTCAACTATGGATGATTAATTCGCAACCTACATGCAAATGGGGCCTCATTCTT CTTTATCTGCCTATACCTTCACATTGCCCGAGGACTTTACTACGGCTCATATCTTTACAAAGAAACAT GAAACATTGGAGTCGTATTATTCCTATTAGTAATAATAACAGCATTCGTKGGGTATGTGCTTCCATGA GGGCAGATAAAGAA (218bp)	KJ546053 *(A. anguilla)* 99%, 375
EF1	TTCCTGAAGCCTGGTATGGTGGTGACCTTTGCCCCTGCCAACGTGACCACTGAGGTCAAGTCTGTGG AAATGCACCATGAGTCCCTGCCTGAGGCTCTTCCCGGTGACAATGTTGGCTTCAACGTCAAGAACGT CTCTGTCAAGGAAATCCGTCGTGGCAACGTAAA (167bp)	HM367094 *(A. japonica)* 100%, 303
PROL	TTCTTCCCTCCAAACCCCTCACGACAAGGACCAGGCTTTGAGAGTGCCGGAATCAGAGCTGCTGTCC CTCGCCCGCGCGCTCCTGCTGTCCTGGAACGATCCCCTGCTCCTGCTCGCCTCCGAGGCGCCCACGCT GTCCCATCCGCAGAACGGCGCCATCTACAGCAAAACAAGGGAACTGCAGGACCAGTCCAACAGCCT GAGCTCTGGGCTGGACAGGCTGATTCACAAGATTGGCTCCCTCCTTCCAAAGAAAA (257bp)	AY616666 *(A. anguilla)* 99%, 359
TSH-β	TGCCCTCCAAGATGAGAGTGGTCCTGTTGGCCAGCGGCGTCCTCTGCCTGTTAGCAGGACAGGTTCT CTCCATCTGCAGTCCTGTGGACTACACGCTGTACGTGGAGAAACCAGAGTGTGACTTCTGCGTGGCC ATCAACACCACCATCTGCATGGGCTTCTGCTATTCCCTGGTAAGAGCCTCCAGTCTGCAA (194bp)	AY158008.1 *(A. japonica)* 99%, 348
THRα$_a$	TTTTCGTTTTGTTGCAGGGGTTGAAATATCGCTTGCCGTGTAGTGTATTGNACTAGCTAGCTAGCCAG CTAGCGAAATATTAAAGCAAGCGCTGGTCACTGAGCCTGCTTGGACTTCTTAATTTCTCTCTCAAAAG AGACATATTTAAAACTACAAAGACGTAAGCATACGATGCAGAAAGTGGTGATACCCTGAGACCGAC CGCTCACCCCCCTGAGACAAGAGCAGTGGAGATTTGCCGGGAGCTTGCCAATGAAAGA (260bp)	AB678206.1 *(A. japonica)* 99%, 459

River-specific gene expression patterns in recruits of American glass eels

THRα_b	TTTTCACGTGCGTGGAGAAGATCGAGAAGTGCCAGGAGACATAMCTGCTGGCGTTCGAACACTACA TCAACTACCGCAAGCACAACATTCCTCACTTCTGGCCCAAGCTGCTGATGAAGGTGACAGACCTGCG CATGATCGGGGCGTGCCACGCCAGCCGGTTCCTGCACATGAAGGTGGAGTGCCCCACCGAACTCTTC CCCCCGCTCTTCCTGGAGGTCTTCGAAGACCAGGAGGTGTGAAGCAGACAA (251bp)	AB678207.1 *(A. japonica)* 98%, 435
DIO-2	TTTAGCTGGGCTTCAGTAGCAGGACAGGCGATGAGTGTAACCTGCTGGACTTTGCGTCATCCGACCG TCCTTTAGTGGTCAACTTTGGCTCGGCCACCTGACCCCCCTTCATCAGCCACCTGCCTGCCTTCCGGC AGCTGGTGGAAGAGTTCTCGGATGTGGCCGACTTTCTCCTGGTCTACATTGACGAGGCTCACCCCTCC GACATCTGGGCGGCACCAGCAGTGGAGGCGCATTCCTTCAAAGTACAGAAGCACCGCAGCCTTGAG GAGCGAGTTCTGGCTGCTCAGAGGAA (295bp)	AB199797.1 *(A. japonica)* 99%, 514
GH	AGCTCATAGTTTATCGCCCTCTATCTGTCAGGACGGGTACTTGCTGCGCATCTCCTCAGCCCTGATCC AGTCATGGGTGTATCCTCTGAAGACCCTGAGCGATGCTTTCTCAAACAGCCTGATGTTTGGGACCTCT GATGGGATCTTTGATAAGCTGGAGGACCTGAACAAGGGCATCAATGAATTAATGAAGGTAAAATGG GGTAA (207bp)	AY616666.1 *(A. anguilla)* 99%, 359
GH-R$_1$	TCATCTTCACCCCTTGCCTGTTGCTAGGTGCCGGAGCCTCGCCGGAAGGAGGCGCGGAGACGCCGTC CGAAGCCCCCCCCGCAGGGCCCCCACTTCACCGGCTGCTTGTCTCGGGAGCAGGAGACGTTCCGTTGC TGGTGGAGTGCCGGAAGCTTCCGGAACCTGACGGAACCCGGGGCCCTGAGGGTCTTCTTCGCCACTA GCTCCCTGCCCAGCGACTGGAAGGAGTGTCCGGACTACTCGGTCACCGTGCCGAACGAGTGCTATTT CAACAAGAGCTACACATCCGGCTGGACCCAATAA (302bp)	AB180476.1 *(A. japonica)* 97%, 501
IGF-1	AACAGAGGTTTTATTTCAGTACCACAGGCTATGGATCCAGCTCAAGACGGTCACACAATCGTGGCAT AGTCGACGAGTGCTGCTTCCAGAGCTGTGAGCTACGGCGACTGGAGATGTACTGCGCACCAGTGAA ACCTGGCAAGGCTGCCCGGTCCGTCAGGGCACAGCGCCACACGGACATNCCAAAAACCCAGAAGAA ACAAATGTCCGGTAATANTCWCCCATSCTGCAAGGAGGTGCATCAGAA (247bp)	AB353115.1 *(A. japonica)* 96%, 416
IGF-1R	GACGGAAGGGCGGTGTACGTGCCGGACGAGTGGGAGGTCCCGCGGGAGAAGATCACTCTGTCTCGG GAGCTGGGCCAGGGCTCGTTCGGCATGGTGTACGAGGGCATCGCCAAGGGCGTGGTCAAGGACGAG CCCGAGACGCGCGTGGCCATCAAGACGGTCAACGAGTCGGCCAGCATGCGCGAGCGCATCGAGTTC CTCAACGAGGCGTCCGTCATGAAGGAGTTCAACTGCCACCACGTGGTGCGTCTGCTGGGCGTGGTTT CGCAGGGTCAGCCCACTCTGGTCATAATGGAGCTGATGACCCGTGGAGACCTCAAGAGTCACCTGCG GTCTCTGCGGTCCCAGGAG (351bp)	Pavey et al. Unpublished data.

River-specific gene expression patterns in recruits of American glass eels