Using gene expression to identify the most suitable environmental conditions for juvenile deepwater redfish (*Sebastes mentella*) growth and metabolism in the Estuary and the Gulf of St. Lawrence

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Abstract

Deepwater redfish *Sebastes mentella* will be among the most important resource-sustaining commercial bottom-fish fisheries in the years to come in the Estuary and Gulf of St. Lawrence (EGSL). In 2011, 2012, and 2013, three strong cohorts were recruited to the stock; their abundance in 2018 was 80 times higher than that of the 1993–2012 period. The main goal of this work was to deepen our knowledge of their growth regulation and metabolism in order to identify molecular indicators and determine how they are influenced by natural environmental conditions. Fish weight and water temperature explained 11% of the variation in relative mRNA levels of specific gene targets in liver and muscle among seven sites where deepwater redfish were captured in the EGSL. The relative expression of liver insulin-like growth factor-1 (*igf-1*) and white muscle A-chain lactate dehydrogenase (*ldh-a*) correlate positively with weight, whereas heavy chain muscle myosin (*myo*), heart citrate synthase (*cs*), and white muscle pyruvate kinase (*pk*) correlate negatively. The relative expression of heart cytochrome c oxidase subunit 1 (*cox-1*) and white muscle insulin-like growth factor-1 receptor isoform a (*igf-1ra*) correlate negatively with temperature. Deepwater redfish from the estuary were smaller than those caught at other sites. Since the growth potential of deepwater redfish was strongly correlated with temperature (being enhanced by higher temperatures), this study suggests an ecological advantage for this species in a climate-warming context.

Keywords: Redfish, Growth, Metabolism, Transcriptomics, Temperature.

Introduction

The redfish fishery in the Estuary and Gulf of St. Lawrence (EGSL) has historically targeted two species, the Acadian redfish (*Sebastes fasciatus*) and the deepwater redfish (*Sebastes mentella)*. The two species look very similar but can be distinguished genetically and to a lesser extent morphologically (DFO, 2018a). In 2011, 2012, and 2013, three strong cohorts recruited to the stock. Genetic analyses have indicated that these cohorts were dominated by deepwater redfish, and all the deepwater redfish caught for this study belong to a unique ecotype or genetic group known as "*S. mentella* gulf," which is the only one present in the EGSL (DFO, 2018b; Benestan *et al.*, 2021). Research survey data show that the abundance of deepwater redfish juveniles in 2018 was 80 times higher than their average abundance for the 1993–2012 period (DFO, 2018a). Indeed, the Department of Fisheries and Ocean Canada (DFO) data suggests that redfish (both Acadian and deepwater redfish) should become the most important resource sustaining commercial bottom-fish fisheries in the years to come in the EGSL (DFO, 2018a). Most of the recent information for deepwater redfish in the EGSL comes from summer trawl surveys, and there is little information for other seasons on diet, distribution, and movements (Senay *et al.*, 2021). Deepwater redfish is a benthic fish remaining on or near the sea bottom during the daytime and rising higher in the water column at night, presumably to feed (Scott and Scott, 1988). The group of prey contributing the most to deepwater redfish diet is zooplankton (32%), followed by shrimp (29%) and other invertebrates (17%) (DFO, 2018a; Senay *et al.*, 2021).

Understanding deepwater redfish stock dynamics requires knowledge on the physiology of the species, including growth and metabolism. However, studying the physiology of deepwater

redfish presents specific challenges because of the low survival rate of fish caught in a deep environment (Saborido-Rey *et al.*, 2004). Maintaining deepwater redfish in a rearing environment that mimics their natural environmental conditions is almost impossible because of the depth and pressure encountered in nature. The transcriptomics study of genes involved in growth and metabolic regulation may offer new investigative tools that can overcome sampling and rearing challenges and allow us to evaluate the physiological response in their original environment.

In fishes, growth is controlled at the endocrine level, especially by the growth hormone (GH) / insulin-like growth factor-1 (IGF-1) axis (Björnsson, 1997; Wood *et al.*, 2005; Duan *et al.*, 2010; Vélez *et al.*, 2017). This axis is modulated by both biotic and abiotic conditions, such as the nutritional or thermal environment (Beckman, 2011; Reindl and Sheridan, 2012; Breves *et al.*, 2016). GH is synthesized by the pituitary gland and regulates many functions, including somatic growth, energy metabolism, reproduction, digestion, osmoregulation, and immune response (Reinecke *et al.*, 2005; Kawaguchi *et al.*, 2013). It also stimulates the production of IGF-1 in the liver (Meier *et al.*, 2009; Volkoff *et al.*, 2010; Bergan-roller and Sheridan, 2018; Vélez *et al.*, 2018). Its main effects on growth are thought to be via regulation of IGF-1 release (Beckman *et al.*, 2004; Reinecke *et al.*, 2005; Picha *et al.*, 2006; Beckman, 2011; Kawaguchi *et al.*, 2013). It has been shown that measures of IGF-1 (plasma concentration and liver mRNA levels) are also strongly correlated with specific growth rate in several teleost species and could be used as a proxy for growth (reviewed by Beckman, 2011). Indeed, plasma IGF-1 has a significant positive correlation with liver *igf-1* mRNA and specific growth rate in species closely related to deepwater redfish, such as olive rockfish *Sebastes serranoides* (Hack *et al.*, 2018) and copper rockfish *Sebastes caurinus* (Hack *et al.*, 2019) as well as in other fish species such as rainbow trout *Oncorhynchus mykiss* (Gabillard *et al.*, 2003) and chinook salmon *O. tshawytscha* (Pierce *et*

al., 2005). Plasma IGF-1 also has a significant positive correlation with muscle *igf-1* mRNA in the hybrid striped bass *Morone chrysops* × *Morone saxatilis* (Picha *et al.*, 2008). In cabezon *Scorpaenichthys marmoratus*, another species closely related to deepwater redfish, Strobel *et al.* (2020) found not only a significant positive correlation between plasma IGF-1 and specific growth rate, but also evidence that fasting affected *igf-1* mRNA in liver but not in white muscle, indicating a negative correlation between muscle *igf1-r* mRNA and growth rate/food intake. In muscle tissue, IGF-1 has a role in the regulation of metabolism, facilitating the uptake of substrates that contribute to muscle growth and may promote the expression of other important genes involved in the myogenesis process (fiber regulation, activation of satellite cells, proliferation, differentiation, and maturation) (e.g., Duan *et al.*, 2010; Vélez *et al.*, 2016). Specifically, the myosin (*myo*) gene is involved in the two final stages of the myogenesis process, i.e., differentiation and maturation (Vélez *et al.*, 2017).

Another important aspect to consider when studying growth is metabolism, since somatic growth is the result of the energetic balance between assimilated and consumed energy (Saborido-Rey and Kjesbu, 2005). In fishes, the activities of muscle pyruvate kinase (PK) and lactate dehydrogenase (LDH), indicators of anaerobic glycolysis, have been demonstrated to be correlated with growth (Pelletier *et al.*, 1993a). In heart and red muscle, the activities of citrate synthase (CS) and cytochrome c oxidase (COX) have been shown to be good indicators of the Krebs cycle and mitochondrial activity (aerobic metabolism) (*Salvelinus alpinus*: Le François *et al.*, 2005; *Anarhichas minor*: Desrosiers *et al.*, 2008; *Paralabrax nebulifer*: Yang and Somero, 1996; different Antarctic fish species: Torres and Somero, 1988). LDH and PK activities have been shown to increase in spotted wolffish *Anarhichas minor* during development (Desrosiers *et al.*, 2008) and were positively correlated with growth rate in Atlantic cod *Gadus morhua*

(Pelletier *et al.*, 1993b) and body size in walking catfish *Clarias betranchus* (Tripathi and Verma, 2004). Davies and Moyes (2007) showed that the mass-specific activity of CS scaled negatively with body size, the glycolytic enzyme PK showed positive scaling, and the ratio of mass-specific PK to CS enzyme activity increased with body size, whereas the ratio of *pk* to *cs* mRNA transcripts was unaffected in largemouth bass *Micropterus salmoides* and smallmouth bass *Micropterus dolomieu*, suggesting that the enzyme relationships were not due simply to transcriptional regulation of both genes.

The main goal of this work was to deepen our knowledge of growth regulation (liver *igf-1*, white muscle *igf-1ra* and *myo*) and metabolism (heart *cox-1* and *cs*, white muscle *pk* and *ldh-a*) in deepwater redfish. We hypothesized that the expression of genes coding for hormones regulating growth (in liver and muscle) and for enzymes regulating metabolism (in liver, muscle, and heart) could be indicators of growth characteristics at different sampling sites and thus be indicative of habitat suitability for juveniles. We aimed to explore how characteristics of the capture sites could influence gene expression. Deepwater redfish were captured in various zones of the EGSL that differ in terms of dissolved oxygen, temperature, salinity, depth, and deepwater redfish biomass. This approach allowed us to obtain insight on which habitats would be the most suitable for deepwater redfish growth and condition factor. This information is valuable in a management context.

Methods

Redfish sampling

Redfish were captured alive during the August 2018 scientific campaign carried out at seven sites in the EGSL (AG: Anticosti–Gaspé, ES: Estuary, GA: Gyre d'Anticosti, LC: Laurentian Channel, EC: Esquiman Channel, AC: Anticosti Channel, CSt: Cabot Strait; Figure 1) by DFO aboard the CCGS *Teleost* with a Campelen 1800 trawl. Data from 670 redfish individuals (random subsamples taken from the entire catch) were considered for this study. The tows were planned to last 15 minutes at a speed of ~3 knots. Bottom measurements of dissolved oxygen, temperature, and salinity along with depth data and standardized redfish biomass (weight of all individuals in a catch standardized for tow duration) were collected at sampling stations (AG, LC, EC, and AC: one sampling station; GA and CSt: two sampling stations; ES: three sampling stations). Once fish were caught, they were put into baskets to weigh the entire catch by station (hereafter called biomass). From these baskets, we randomly selected fish from among those that met the following three requirements: (1) the fish was alive; (2) fish length was between 20 and 23 cm; and (3) the fish had not regurgitated its stomach. We took tissue samples from the first 20 and the others were measured for the DFO survey. We did not have control over the number of stations per site: logistical decisions were based on the multiple research objectives of the survey. Salinity, temperature, and depth were measured using an SBE 19plus V2 SeaCAT CTD (Sea-Bird Scientific, Bellevue, WA, USA), and dissolved oxygen was measured using an Optode 4831 (Aanderaa, Bergen Norway). Data are reported in Table 1.

Fork lengths were measured on all fish (Table 2). At each site, 20 deepwater redfish with lengths between 20 and 23 cm were sacrificed by severing the spinal cord and were immediately dissected on ice for tissue samplings. This size standardization ensured that immature individuals belonging to the same cohort (2011–2013; Brassard *et al.*, 2017) were used for the gene response

evaluation (Table 3) and that the gene response was only related to growth and not gonad maturation. This decision was made based on information that was available when the project was planned. In the meantime, a study based on gonad histology that is currently in progress has indicated that most fish larger than 20 cm are undergoing maturation or are mature (C. Senay, unpublished results). However, we examined the gonads of all fish sampled for gene expression and found no visual evidence of developed gonads. Tissue samples from heart, liver, and a piece of epaxial muscle from just beneath the dorsal fin were immediately stored in RNA*later* (Invitrogen, Waltham, MA USA) at –20°C pending further analysis of relative gene expression (liver: *igf-1*; white muscle: *igf-1ra*, *myo*, *pk*, *ldh-a*; heart: *cox-1*, *cs*; Table 4). We were careful not to include the gallbladder to avoid RNAses. A piece of the pectoral fin was also removed from all individuals and stored in 100% ethanol for DNA-based species identification.

Fish condition

The lengths and weights of all captured deepwater redfish were measured (n = 670). The Fulton condition factor (K) was estimated for each sampled fish using the following formula:

$$K = 100(W/L^3)$$

where W is the wet body weight in grams and L is the fork length in centimeters. For condition factor calculation, we assumed isometric growth since similarly sized individuals were used. Larger values (>1) signified that a fish was heavy (and potentially had greater energy and fat reserves) for a given size (Fulton, 1904; Nash *et al.*, 2006).

Genetic identification of individuals

186 *DNA extraction* – For each fish sampled for gene expression (n = 140), DNA was extracted from
187 20 mg of fin tissue (wet mass) using the DNeasy blood and tissue kit (Qiagen, Inc., Mississauga,
188 ON, Canada). The final concentration was adjusted to 50 ng ml^{-1} for genetic analysis.
189
190 *Genetic analysis* – Genetic analysis was conducted according to Valentin et al. (2014), with slight
191 modifications, using the Qiagen multiplex PCR kit (Qiagen). Four of the original 13 markers
192 allowing species identification (Seb9, Seb25, Seb31, and Seb33) were used to discriminate
193 species. Electrophoresis was conducted on an ABI 3130 genetic analyzer (Applied Biosystems,
194 Waltham, MA, USA) using 0.1 µl of the GeneScan™ 1200 LIZ™ dye size standard (Thermo
195 Fisher Scientific, Waltham, MA, USA) for each sample. The GeneMapper® Software v5.2
196 (Thermo Fisher Scientific) was used to perform data analysis/genotyping. The R pipeline
197 EasyAssign (version 0.1.0; https://github.com/GenomicsMLI-DFO/SebAssign) was used to
198 assign samples to species. Genetic analysis indicated that 6% of the sampled fish were *S.*
199 *fasciatus* (captured at AG and GA), and species was undetermined in 23% of the fish. Of the 140
200 fish sampled, 95 individuals were identified as « *S. mentella* gulf » (the threshold used for the
201 assignment was 95%); from these, we randomly selected 10 fish per sampling site—a total of
202 70—that were used for gene expression analyses (Table 3).
203
204 ***Gene expression***
205 *Extraction of total RNA* – RNA was extracted from 30 mg of liver, heart, and epaxial muscle (wet
206 mass) from 10 deepwater redfish per site (total of 70), using the RNeasy Plus Universal Mini Kit
207 (liver; Qiagen, Inc.) or RNeasy Fibrous Tissue Kit (heart and muscle; Qiagen, Inc.) and diluted to
208 a final concentration of 200 ng μL^{-1} RNA. RNA purity, quality, concentration, and absorbance

ratio 260/280 were determined by SYBR Safe DNA Gel Stain 2% agarose gel electrophoresis (ChemiDoc XRS+ system, Biorad, CA, USA) and spectrophotometry (NanoVue Plus, GE Healthcare, Pittsburgh, PA, USA).

Reverse transcription – Reverse transcription of messenger RNA (mRNA) into complementary DNA (cDNA) was performed in duplicate using the Quantitect reverse transcription kit (Qiagen, Inc., Mississauga, ON, Canada). cDNA was then diluted to a final concentration of 200 ng μL^{-1}, separated into aliquots, and kept frozen at –20°C until further analysis. Integrity was verified and cDNA concentrations were measured using a NanoVue Plus spectrophotometer. The efficiency of reverse transcription was verified by quantitative polymerase chain reaction (qPCR) using serial dilutions of a representative pool of cDNA samples collected from different sampling sites and compared to the ideal slope of –3.3.

PCR amplification, sequencing, and assembly of partial cDNAs – Since there were no available sequences for target and reference genes in deepwater redfish, primers were designed using sequences from closely related or other marine fish species (Suppl. Table 1). PCRs were performed in 25 µL reaction volumes containing 12.5 µL of AmpliTaq Gold 360 (Applied Biosystems), 0.5 µL of 360 GC enhancer (Applied biosystems), 2.5 µL of cDNA, 1.25 µL each of forward and reverse primers (20 mM), and 7 µL of nuclease-free H_2O. Reactions were amplified under a thermal profile at 95°C for 10 min, 40 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min and 20 s, followed by 7 min at 72°C. PCR products were then tested by gel electrophoresis on 2% agarose gels. The amplified PCR products were purified using the QIA quick PCR purification kit (Qiagen) and forward and reverse sequenced using the BigDye

232 Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) with the ABI PRISM 3130 Genetic
233 Analyzer (Applied Biosystems). For each gene, the sequence obtained was compared with the
234 sequence used for primer design using the BLAST® software (Altschul *et al.*, 1990). Sequence
235 lengths and percentages of similarity to the reference sequences are presented in Supp. Table 1.
236
237 *Design of primers and probes – S. mentella* TaqMan primers and probes were designed using the
238 Primer Express software version 3.0 (Applied Biosystems) for each reference and target gene
239 (Table 4).
240
241 *Real-time PCR analysis and gene quantification* – Gene expression was quantified by qPCR
242 performed in triplicate on deepwater redfish samples using a QuantStudio 3 Real Time PCR
243 System (Applied Biosystems). Each reaction consisted of 2 µL of diluted cDNA, 5 µL of
244 TaqMan Fast Advanced Mix (Applied Biosystems), 0.5 µL of Custom TaqMan Gene Expression
245 Assays (Applied Biosystems), and 2.5 µL of sterile water, for a total volume of 10 µL. The
246 thermal cycling of qPCR was done in two steps: (1) 2 min at 50°C for optimal AmpErase uracil-
247 N-glycosylase activity followed by 20 s at 95°C to activate DNA polymerase, and (2) 45
248 denaturation cycles for 1 s at 95°C and annealing / extension for 20 s at 60°C. Cycle thresholds
249 (C_T) were obtained with the QuantStudio Design Analysis software (ThermoFisher Connect).
250
251 The relative quantification of gene expression was calculated according to the 2-$\Delta\Delta C_T$ method of
252 Livak and Schmittgen (2001), with C_T being a threshold cycle:
253
254 $$2^{-\Delta\Delta C_T} = 2^{-(\Delta C_{Te} - \Delta C_{Tc})}$$

where C_{Te} = C_T of the target gene - C_T of the reference genes for sample x, and C_{Tc} = C_T of the target gene - C_T of the reference genes for the calibrator (see below). In this study, the calibrator was deepwater redfish sampled from Cabot Strait (the deepest, saltiest, and most highly oxygenated site). The stability of reference gene expressions was verified with Expression Suite version 1.0, where the score was calculated according to Vandesompele *et al.* (2002). The score is a measure of the stability of genes in the qPCR analysis, which can vary according to the tissue: the lower the score, the more stable the expression. The reference genes used were 18S, β-actin, and EF1α, and the best combination of scores was kept for each tissue. For accurate averaging of the reference genes, we used the geometric mean instead of the arithmetic mean because the former better controls for possible outlier values and abundance differences between the different genes (Vandesompele et al., 2002). The efficiency of the qPCR was verified for each gene, and percent efficiency values are reported in Table 4.

Statistical analyses – Length, weight, Fulton condition factor, and qPCR data were compared using one-way ANOVA ($\alpha < 0.05$), with site as the explanatory variable. To assess ANOVA assumptions, data normality was verified using the Kolmogorov-Smirnov test and homoscedasticity was tested using the Levene test. If significant differences occurred, post-hoc HSD Tukey tests were used when homoscedasticity was verified and multiple-range Games-Howell tests were applied in cases of heteroscedasticity.

An exploratory principal component analysis was run to verify whether fish from each site differed according to gene expression data, but no clear pattern discriminating the sites emerged (see Suppl. Fig. 1). Relationships were examined between the response variables *(igf-1, igf-1ra,*

279 *myo*, *cox-1*, *cs*, *pk*, *ldh-a*) and the explanatory variables describing site-scale characteristics
280 (dissolved oxygen, temperature, depth, and deepwater redfish biomass at the entire capture site)
281 and individual-scale characteristics (length, weight, and Fulton condition factor of analyzed
282 individuals) using the 70 deepwater redfish on which qPCR analyses had been done. To do so, a
283 global canonical redundancy analysis (RDA: *rda* function in the R vegan package) was run with
284 all explanatory variables, and model probability and adjusted coefficients of determination
285 (adjusted R^2) were calculated. The adjusted R^2 was quantified (*RsquareAdj* function in the R
286 vegan package), and it accounts for the number of observations and number of degrees of
287 freedom in the fitted model (Peres-Neto *et al.*, 2006; Legendre *et al.*, 2011).
288
289 Variation partitioning was conducted to determine the relative contribution of site-scale
290 characteristics (dissolved oxygen, temperature, salinity, depth, and total biomass of captured
291 deepwater redfish at the sampling site) and individual-scale characteristics (length, weight, and
292 Fulton condition factor of the analyzed individuals) to explain gene expressions (*varpart* function
293 in the R vegan package). This method requires multiple partial RDAs to quantify the variance
294 explained exclusively and jointly by groups of variables.
295
296 A selection of variables contributing to the explained variation was achieved by using both
297 forward and backward selection as well as a stopping criterion (*ordiR2step* function in the R
298 vegan package; Blanchet *et al.*, 2008). This criterion limits overfitting by preventing selected
299 variables included in the reduced model from explaining more variation than the full model
300 developed with all explanatory variables. A triplot was produced with a type 1 scaling to
301 illustrate distances among objects (i.e., individuals) and relationships with selected environmental
302 variables based on the reduced model using only significant explanatory variables. In such a

representation, the distance between objects are Euclidean distances (objects closer to each other have similar variable values), while the angles between the vectors of response variables are meaningless. The angles between vectors of the response variables and explanatory variables reflect linear correlation.

Results

Individual fish characteristics

The lengths, weights, and Fulton condition factors of 670 deepwater redfish were significantly different among the sites (length: $F= 65.62$, $p < 0.0001$; weight: $F= 42.80$, $p < 0.0001$; Fulton condition factor: $F= 46.67$; $p < 0.0001$) (Table 2). Deepwater redfish captured in LC, EC, and CSt were the longest, while those captured in ES were the shortest (in average 42% shorter; Table 2). Fish from LC, EC, and CSt were also significantly heavier than those from ES (Table 2). Deepwater redfish from CSt had the highest Fulton condition factor (Table 2) while those captured at ES had the lowest (24% lower than CSt). Condition factors were intermediate in deepwater redfish captured at other sites. Weight, length, and condition factor followed same tendencies in the 10 fish per site that were sampled for genomic analysis (Table 3).

Genes involved in growth regulation. The relative expression of *igf-1* in liver was the lowest at ES, where it was 68% lower than at CSt or AG ($F = 3.97$, $p = 0.0019$; Figure 2A) and intermediate to all other sites. The relative expression of its receptor, *igf-1ra,* in white muscle was 476% higher in deepwater redfish captured at ES compared to those captured at AG, GA, and LC, with intermediate values at the other sites ($F = 4.60$, $p < 0.0001$; Figure 2B). The relative

expression of white muscle *myo* was the lowest in individuals captured at CSt ($F = 2.73$, $p = 0.0210$; Figure 2C) while it was significantly higher—by 189%—at AC, but no significant correlation was detected at ES or LC because interindividual variations were too high. *Myo* expression was intermediate at other sites.

Genes involved in metabolism. In white muscle, a 424% higher expression of *pk* was observed in individuals captured at ES ($F = 9.10$, $p < 0.0001$; Figure 3A) compared to those from CSt, GA, and LC, with intermediate values at other sites. Deepwater redfish captured at AG had a significantly higher expression of *ldh-a* (298%) compared to those in fish captured at all other sites except for CSt ($F = 3.84$, $p = 0.0025$; Figure 3B). The relative expression of *cox-1* in the heart was significantly higher in juveniles captured at ES (212%) compared to those captured at CSt, AG, LC, and AC, with intermediate values found in fish captured at the other sites ($F = 2.97$, $p = 0.019$; Figure 3C). Fish captured in EC had higher expressions of *cs* (204%) compared to fish captured in GA and LC ($F = 3.50$, $p = 0.0046$; Figure 3D).

Relationships between genomic indicators and site-scale and individual-scale characteristics

Correlations among explanatory variables were investigated. Salinity was excluded from the analysis because it was highly correlated with depth (Pearson's $r = 0.92$). The RDA model including all variables (individual-scale and site-scale) was significant ($p = 0.001$) and explained 18% of the variation in individual genomic indicators (adj $R^2 = 18\%$). Variation partitioning revealed that site-scale characteristics alone explained 12% of the variation ($p = 0.002$) and individual-scale characteristics alone explained 6% ($p = 0.011$); no shared fraction was explained by both types of variables together (Figure 4A). Variable selection indicated that weight and

temperature had the strongest effect on genomic indicators: their importance was similar (weight, $F = 5.28$, $p = 0.002$; temperature, $F = 5.224$, $p = 0.006$), and the reduced model explained 11% of the variation ($p = 0.001$).

An RDA triplot illustrating the reduced model showed that deepwater redfish captured in ES were found in cold water and had the lowest weight (Figure 4B). The relative expressions of *igf-1* in liver and *ldh-a* in white muscle were positively correlated to weight, whereas *cs* in heart as well as *myo* and *pk* in white muscle were negatively correlated to weight. The relative expressions of heart *cox-1* and liver *igf-1ra* were not strongly correlated with weight, but they were negatively correlated with temperature. Other genomic indicators were not strongly affected by temperature.

Discussion

In this study, we hypothesized that the expression of genes involved in growth and metabolism could provide information about growth characteristics and habitat suitability for deepwater redfish juveniles. Since weight and temperature explained most variations in the genomic indicators, this analysis highlights the relationships of genes correlated with these variables. The importance of temperature could be the key to understanding growth differences of deepwater redfish in the EGSL.

Transcriptomic indicators and fish weight

The lowest and the highest relative expression of liver *igf-1* were associated with the sites where the smallest and the heaviest deepwater redfish were captured, respectively. This positive correlation between weight and liver *igf-1* has been previously described in different fish (e.g.,

Beckman, 2011), including rockfish *Sebastes serranoides* reared under laboratory conditions, and indicates that differences in expression may be attributed to the quantity of ingested food (Hack *et al.*, 2018). We also expected that high liver *igf-1* expression would be correlated with a high expression of white muscle *igf1-ra*, since IGF-1 binds to receptors on the surface of muscle cells to exert its action on muscle growth. However, the reverse situation was observed at ES and CSt, with low expressions of *igf-1* occurring with high expressions of *igf-1ra*. Similar results were reported for *Sebastes* by Hack *et al.* (2019), suggesting a negative relationship between muscle *ig1r* expression and fasting-associated decline in muscle *igf-1* expression that could indicate reduced muscle growth. Nevertheless, it is interesting to note that the opposite situation was observed in deepwater redfish captured at AG: the highest *igf-1* expression occurred with the lowest *igf-1ra* expression, even though the weights of individuals captured at this site were intermediate to those of fish captured at ES and CSt.

Deepwater redfish captured at ES also exhibited the highest relative expression of white muscle *pk*. High expression of muscle *pk* has been associated with starvation in rainbow trout *Oncorhynchus mykiss* and may indicate the breakdown of muscle proteins to compensate for the lack of food intake (Johansen and Overturf, 2006). Results from a parallel study currently in progress (S. Brown-Villemin – Pers. Comm.) and made on the same fish as those used in our study showed that 70% of the stomachs from ES were empty while 90% of those from CSt were full. This possible starvation could explain why the fish caught in ES were smaller and had the highest *pk* expression. Furthermore, analysis of stomach content revealed that in both sites deepwater redfish were feeding on zooplankton but the occurrence of each zooplankton taxon identified differed.

The expression of *ldh-a* in white muscle was positively correlated with weight, but our data did not show any spatial pattern across the EGSL. A similar positive correlation was reported in *Sebastes goodei*, but negative correlations have also been found in *Sebastolobus alascanus* (Vetter and Lynn, 1997) and *Synaphobranchus kaupii* (Bailey *et al.*, 2005). It has been also reported that LDH activity might be sensitive to environmental oxygen levels (Vetter and Lynn, 1997) and very sensitive to changes in nutritional condition during food limitation (Yang and Somero, 1996). According to this evidence, we could expect that deepwater redfish from CSt (highest dissolved oxygen concentration) and ES (lowest dissolved oxygen concentration) would show significantly different *ldh* expressions, but this was not the case. Indeed, there was no correlation between dissolved oxygen content and relative gene expressions in the EGSL. Unfortunately, we have no data from stomach contents to examine potential nutritional differences, that could explain our results across the EGSL.

The relative expression of white muscle *myo* was negatively correlated with weight in our study and differed only between fish captured at CSt and AC. These fish had significantly different weights, but no differences in terms of either length or condition factor. Contradictory results have been reported in gilthead sea bream *Sparus aurata* and rainbow trout, with negative correlations between weight and *myo* being reported by Azizi *et al.* (2016) and Overturf and Hardy (2001) and no correlation being reported by Alami-Durante *et al.* (2010) and Vélez *et al.* (2018). Therefore, the difference in *myo* gene expression observed between CSt and AC could be related to other factors, such as diet. However, an experiment with juvenile rainbow trout indicated that dietary differences were not responsible for the expression of different myogenic factors, including *myo* (Alami-Durante *et al.*, 2010).

In general, CS activity typically decreases in larger individuals, and a negative correlation with weight has been reported in black bass, sunfish (*Lepomis gibbosus*, *Lepomis macrochirus*; Davies and Moyes, 2007), barred sand bass (*Paralabrax nebulifer*; Yang and Somero, 1996), and *Sebastolobus altivelis* (Vetter and Lynn, 1997). Indeed, aerobic metabolic activity is thought to scale negatively with size due to economies in the cost of oxygen transport with increasing body size (Vetter and Lynn, 1997). However, it should be reminded that the gene expression does not necessarily represent direct changes in protein levels due to the action of post-transcriptional mechanisms, that may lead to a disparity between mRNA abundance and enzyme activity (Craig *et al.*, 2007; Vagner and Santigosa, 2011; Velki *et al.*, 2017).

One may argue that the fish size standardization we did during sampling may have masked weight and length differences at different sites. However, we were able to find strong evidence that there are significant differences in gene expression between ES and the other sites. These results would suggest that even the slower-growing individuals at CSt would have a better growth potential than the faster-growing individuals at ES. Such differences in growth potential between sites could result from biotic and abiotic differences between sites that could lead to (1) a disparity in the regulatory pathway at molecular levels , as found in this study; (2) higher metabolic costs that left less energy available for growth; or (3) differences in the nutritional quality of prey.

Transcriptomic indicators and temperature conditions

In ectotherms, growth strongly depends on environmental conditions, especially temperature (Fry, 1971). In deepwater redfish, the relative expression of heart *cox* was negatively correlated with temperature. This was clearly illustrated in our study when comparing the different capture

sites: fish caught in ES (the coldest zone) showed a higher expression of heart *cox* compared to the warmest sites (CSt, AG, LC, AC). Cold acclimation in wild fish typically leads to an increase in COX activity (Bremer and Moyes, 2011) due to an increase in mitochondrial gene expression. This may be a compensatory mechanism to overcome the negative thermodynamic effects of cold on processes relying on enzymatic reactions (e.g., Nathanailides, 1996). Indeed, changes in water temperature can have pronounced effects on physiological processes such as muscle and cardiovascular function as well as metabolism and growth (e.g., Little *et al.*, 2020). In Atlantic salmon S*almo salar*, COX activity increased rapidly with lower temperature (Nathanailides, 1996) In contrast, no relationship was observed between COX activity and temperature in Atlantic cod (Pelletier *et al.*, 1995). Contrary to our findings, *ldh* expression in black rockfish *Sebastes schlegelii* increased with warmer temperatures (Song *et al.*, 2019). Facing cold temperature, there are two metabolic strategies available for ectotherms: (1) to increase metabolic rate to compensate for temperature-mediated decreases in the metabolic rate, or (2) to decrease metabolic rate to reduce energy consumption (Song *et al.*, 2019). Deepwater redfish from ES, where water is the coldest, seem to use a combination of both: anaerobic metabolism (*ldh-a*) is down-regulated and aerobic metabolism (*cox-1*) is up-regulated.

The relative expression of white muscle *igf-1ra* was negatively correlated with temperature. It is surprising that this gene did not show any strong correlation with weight and yet did with temperature. The mechanisms mediating the effects of temperature on growth—and more specifically, on the regulation of the GH–IGF1 axis—are not well understood.

The EGSL can be separated into three distinct depth layers characterized by temperature conditions: the surface layer, the cold intermediate layer, and the deepwater layer. The deepwater layer, where deepwater redfish were captured and where temperatures range around 5–6°C (> 250 m) throughout the year (Galbraith *et al.*, 2019), is mostly isolated from exchanges with the surface and is very stable across depth (150–500m). The temperature difference observed during the survey between the coldest (ES) and the warmest (CSt) sites was 0.7°C. However, according to Bourdages *et al.* (2019), the mean temperature in August 2018 (time of the sampling) at 300 m was 5.6°C in ES and 6.8°C in CSt (a difference of 1.2°C). Considering the stability of the deepwater layer, this difference could impact deepwater redfish physiology. This has been found to be the case in other fish species: Ghinter *et al.* (2021) found that a difference of 2°C can exceed the optimal temperature range for growth in Greenland halibut *Reinhardtius hippoglossoides* in the bottom waters of the EGSL. This suggests that even small increases in temperature associated with global change are likely to have strong effects on sebaste physiology and thereby on populations and fisheries (e.g., Little *et al.*, 2020; Ghinter *et al.*, 2021).

Gene expression and other physicochemical variables

Other variables (salinity, depth, and redfish biomass) do not appear to correlate with gene expression in deepwater redfish in the EGSL, but deepwater redfish captured in ES differed from those from the other sites. ES is also characterized by low oxygen content, but low oxygen conditions were also found at GA and AC. However, general fish condition (length, weight, and condition factor) seemed to be better at GA and AC compared to ES. Indeed, our analysis did not confirm any correlation with oxygen levels.

Considering the data from our study, energy costs seem to be higher in ES than in other sites, as suggested by the up-regulation of *cs* and *cox-1*, but we are not able to precisely identify the factors explaining this. Because energy may be diverted from growth when gonad maturation begins, it is important to compare individuals at the same developmental stage. Differences among sampling sites could be related to differences in maturation stage that were not visually observable during sampling. Even though we do not have data to support this statement, it seems unlikely that gonad maturation would have been more advanced in the coldest areas (with the smallest fish). Future studies targeting fish of different sizes from each site would be useful to address this question.

Conclusion

Deepwater redfish from the same cohorts captured at different sites present different patterns of gene expression that are related to their weight and to the temperature conditions of the sites. We found that (1) the relative expressions of liver *igf-1* and white muscle *ldh-a* were positively correlated with weight; (2) white muscle *pk* and *myo* as well as heart *cs* were negatively correlated with weight; and (3) white muscle *igf-1ra* and heart *cox* were negatively correlated with temperature. We thus suggest that liver *igf-1*, white muscle *igf-1ra* and *pk*, and heart *cox-1* could be used as growth indicators for surveys in the field. Since weight of deepwater redfish is positively correlated with temperature (even enhanced at higher temperatures), this study suggests an ecological advantage for this species in a climate-warming context. For this reason, monitoring the gene expression response related to increased water temperatures will certainly improve our understanding of this species' population dynamics and help deepen further to better understand physiological mechanisms underlying the observed differences. More broadly, transcriptomics could be an important tool for investigating growth in a deep-sea fish whose

514 physiology is difficult to study. However, the inclusion of more ecological variables such as prey
515 abundance could help gain a better understanding of fish physiology in future studies.
516
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528
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530 M.A.M. contributed to data generation, data analysis, and manuscript preparation; M.V
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532 analysis, and manuscript preparation; and C.A. contributed with ideas, data generation, data
533 analysis, and manuscript preparation.
534
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Figure Captions

Figure 1. Deepwater redfish sampling sites in the Estuary and Gulf of St Lawrence. Circle sizes indicate oxygen saturation (µmol/kg) and colours indicate depth. AG: Anticosti–Gaspé, ES: Estuary, GA: Gyre d'Anticosti, LC: Laurentian Channel, EC: Esquiman Channel, AC: Anticosti Channel, CSt: Cabot Strait.

Figure 2. Relative changes in gene expression (mean ± SE) of three target genes related to growth in deepwater redfish captured at the sampling stations (AG: Anticosti–Gaspé, ES: Estuary, GA: Gyre d'Anticosti, LC: Laurentian Channel, EC: Esquiman Channel, AC: Anticosti Channel, CSt: Cabot Strait). (A) liver insulin-like growth factor 1 (*igf-1*), (B) white muscle insulin-like growth factor 1 receptor (*igf-1r*), (C) muscle myosin (*myo*). The dashed horizontal lines indicate the normalized values for the calibrator group (CSt: the deepest, saltiest, and most highly oxygenated site). Different letters indicate significant differences among sites (Tukey

HSD multiple comparison tests when data were homoscedastic [*igf-1*] and Games-Howell tests when data were heteroscedastic [*igf-1r, myo*]; $\alpha = 0.05$).

Figure 3. Relative changes in gene expression (mean ± SE) of four target genes related to metabolism in deepwater redfish captured from the sampling stations (AG: Anticosti–Gaspé, ES: Estuary, GA: Gyre d'Anticosti, LC: Laurentian Channel, EC: Esquiman Channel, AC: Anticosti Channel, CSt: Cabot Strait). (A) white muscle pyruvate kinase (*pk*), (B) white muscle lactate dehydrogenase (*ldh*), (C) heart cytochrome c oxidase (*cox*), (D) heart citrate synthase (*cs*). The dashed horizontal line indicates the normalized values for the calibrator group (CSt: the deepest, saltiest, and most highly oxygenated site). Different letters indicate significant differences among sites (Tukey HSD multiple comparison tests when data were homoscedastic [*ldh, cs*] and Games-Howell tests when data were heteroscedastic [*pk, cox*]; $\alpha = 0.05$).

Figure 4. (A): Site-scale and individual-scale analyses. Variation partitioning, relationships between gene expression (Y) and site variables (X1: dissolved oxygen, temperature, depth, and redfish biomass) and individual variables (X2: length, weight, Fulton index). (B): Redundance analysis. *Scaling 1*- distance triplot (object focused). Selected explanatory variables are in black, indicators are in red, and fish from different sites are indicated by different symbols.

Suppl. Figure 1. Principal component analysis (PCA) of the variables of each site (left) and gene expression database (right; *igf-1, igf-1r, myo, cox, cs, pk, ldh*) associated with capture sites for deepwater redfish. Different colours represent different capture sites.