ICES Journal of Marine Science

ICES Journal of Marine Science (2022), 79(2), 382–393. https://doi.org/10.1093/icesjms/fsab269

Original Article

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

Maria Angelica Martínez-Silva [1,*], Marie Vagner[2], Caroline Senay[3], and Céline Audet [1]

[1]*Institut des sciences de la mer de Rimouski, Université du Québec à Rimouski, 310 allée des Ursulines, Rimouski, QC, Canada*
[2]*Laboratoire des Sciences de l'Environnement Marin, UMR 6539 (CNRS/Univ Brest/IRD/Ifremer), Plouzané, France*
[3]*Institut Maurice-Lamontagne, Pêches et Océans Canada, Mont-Joli, QC, Canada*

[*]*Corresponding author: tel: (+1) 418 724-1650; e-mail: mariaangelica.martinezsilva@uqar.ca.*

Martínez-Silva, M.A., Vagner, M., Senay, C. and Audet, C. Using gene expression to identify the most suitable environmental conditions for growth and metabolism of juvenile deepwater redfish (*Sebastes mentella*) in the Estuary and the Gulf of St. Lawrence. – ICES Journal of Marine Science, 79: 382–393.

Received 1 June 2021; revised 10 December 2021; accepted 13 December 2021; advance access publication 11 January 2022.

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 *igf-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: growth, metabolism, redfish, temperature, transcriptomics.

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, Fisheries and Oceans Canada (DFO) data suggest 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 the 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. Transcriptomic studies of the genes involved in growth and metabolic regulation may offer new investigative tools that can overcome sampling and rearing challenges and allow us to evaluate physiological responses 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 *Oncorhynchus 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 cosnsider 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; and 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, and 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 min 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; and 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

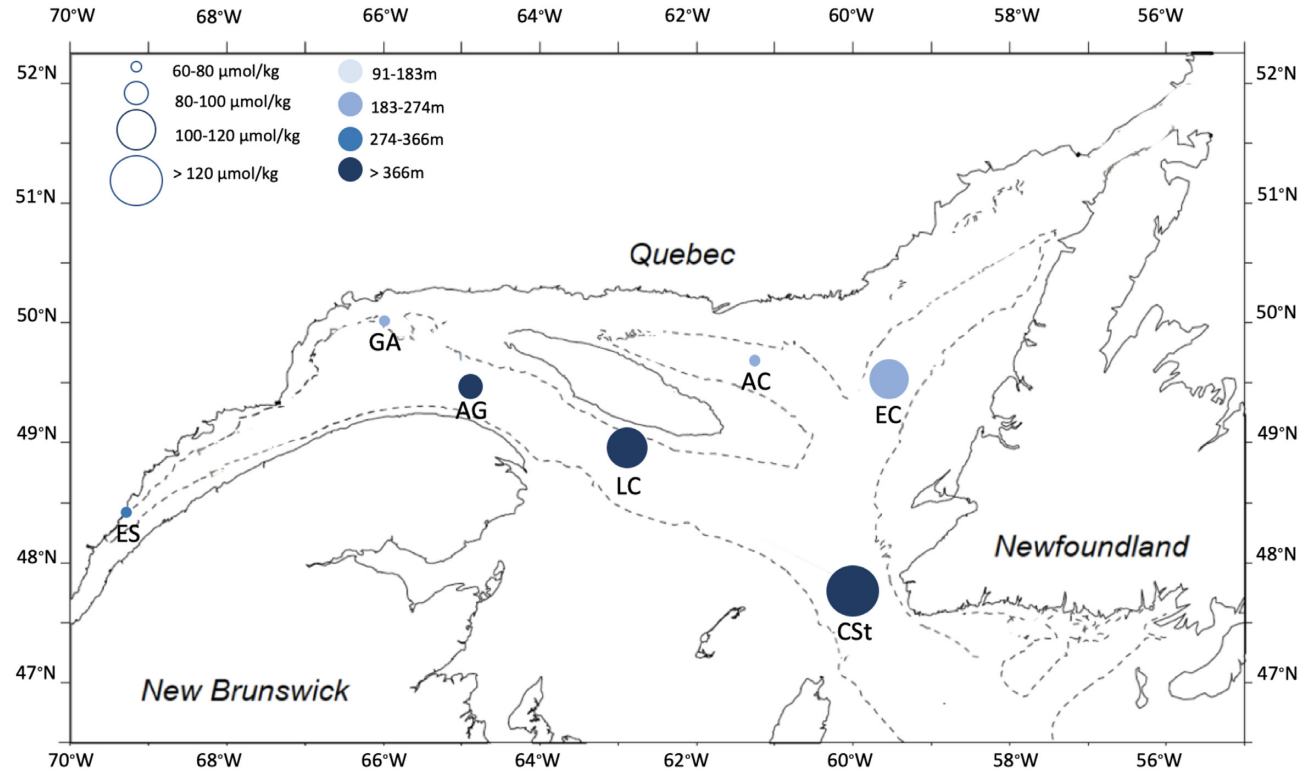

Figure 1. Deepwater redfish sampling sites in the Estuary and Gulf of St Lawrence. Circle sizes indicate oxygen saturation (µmol kg^{-1}) and colours indicate depth. AG: Anticosti–Gaspé, ES: Estuary, GA: Gyre d'Anticosti, LC: Laurentian Channel, EC: Esquiman Channel, AC: Anticosti Channel, and CSt: Cabot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

Table 1. Position, biomass (entire catch per site), number of fish measured, and bottom physicochemical parameters at each sampling site in the EGSL. (AG, LC, EC, and AC: one sampling station; GA and CSt: two sampling stations; and ES: three sampling stations).

Site	Long (°W)	Lat (°N)	Biomass (kg)	Measured fish	Oxygen (μmol kg^{-1})	Temp (°C)	Salinity	Depth (m)
Anticosti–Gaspé (AG)	64.669	49.357	272.46	51	88.61	6.27	34.76	384
Estuary (ES)	69.330	48.360	151.68	171	60.85 ± 3.48	5.67 ± 0.15	34.45 ± 0.06	306 ± 29.37
Gyre Anticosti (GA)	66.046	49.768	2310.34	61	75.16 ± 10.56	5.73 ± 0.52	34.47 ± 0.29	255 ± 86.97
Laurentian Channel (LC)	62.944	48.840	738.64	77	120.00	6.28	34.89	375
Esquiman Channel (EC)	59.419	49.421	3984.93	81	118.31	6.37	34.46	240
Anticosti Channel (AC)	61.356	49.514	2187.14	86	71.31	6.03	34.43	245
Cabot Strait (CSt)	60.042	47.786	357.68	145	158.51 ± 2.18	5.89 ± 0.03	34.95 ± 0.01	501 ± 8.49

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 fish 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), 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 (Senay, unpublished data). 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) at −20°C pending further analysis of relative gene expression (liver: *igf-1*; white muscle: *igf-1ra*, *myo*, *pk*, and *ldh-a*; and 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.

Table 2. Number of deepwater redfish measured per site (total 670) with their mean (± *SD*) weight, length, and Fulton condition factor. For each column, different letters indicate significant differences among sites (ANOVA with Games–Howell post-hoc tests because data were heteroscedastic; $\alpha = 0.05$).

Site	Measured fish	Length (cm)	Weight (g)	Fulton
Anticosti–Gaspé (AG)	51	22.48 ± 5.62 (b)	147.8 ± 62.87 (c)	1.294 ± 01.7 (b)
Estuary (ES)	171	15.59 ± 4.83 (d)	55.6 ± 41.87 (d)	1.071 ± 0.19 (c)
Gyre d'Anticosti (GA)	61	19.34 ± 6.53 (c)	134.2 ± 116.13 (c)	1.228 ± 0.25 (b)
Laurentian Channel (LC)	77	27.07 ± 8.08 (a)	365.6 ± 349.75 (ab)	1.271 ± 0.16 (b)
Esquiman Channel (EC)	81	25.48 ± 8.00 (a)	255.8 ± 229.02 (b)	1.278 ± 0.18 (b)
Anticosti Channel (AC)	86	19.2 ± 6.22 (c)	137.4 ± 124.65 (c)	1.217 ± 0.22 (b)
Cabot Strait (CSt)	145	28.09 ± 7.06 (a)	371.7 ± 294.11 (a)	1.412 ± 0.13 (a)

Table 3. Mean ± (*SD*) weight, length, and Fulton condition factor for the ten redfish individuals per site used for genetic analysis. For each column, different letters indicate significant differences among sites (ANOVA; Tukey HSD multiple comparison tests when data were homoscedastic (weight) and Games–Howell tests when data were heteroscedastic (length, fulton; $\alpha = 0.05$).

Site	Length (cm)	Weight (g)	Fulton
Anticosti–Gaspé (AG)	22.45 ± 1.2 (ab)	146.3 ± 22.3 (b)	1.290 ± 0.12 (bc)
Estuary (ES)	20.88 ± 1.1 (b)	115.5 ± 10.4 (c)	1.279 ± 0.17 (bc)
Gyre d'Anticosti (GA)	22.39 ± 0.6 (a)	150.6 ± 12.2 (ab)	1.342 ± 0.10 (bc)
Laurentian Channel (LC)	22.13 ± 0.9 (ab)	130.8 ± 16.8 (bc)	1.203 ± 0.09 (c)
Esquiman Channel (EC)	22.05 ± 0.5 (ab)	137.5 ± 16 (bc)	1.280 ± 0.11 (bc)
Anticosti Channel (AC)	22.1 ± 0.8 (ab)	146.5 ± 20.6 (b)	1.350 ± 0.09 (b)
Cabot Strait (CSt)	22.51 ± 0.6 (a)	174.1 ± 22.5 (a)	1.521 ± 0.13 (a)

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:

$$K = 100\left(W/L^3\right),$$

where W is the wet body weight in g and L is the fork length in cm. 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

DNA extraction

For each fish sampled for gene expression ($n = 140$), DNA was extracted from 20 mg of fin tissue (wet mass) using the DNeasy blood and tissue kit (Qiagen, Inc., Mississauga, ON, Canada). The final concentration was adjusted to 50 ng ml^{-1} for genetic analysis.

Genetic analysis

Genetic analysis was conducted according to Valentin *et al.* (2014), with slight modifications, using the Qiagen multiplex PCR kit (Qiagen, Inc.). A total of four of the original 13 markers allowing species identification (Seb9, Seb25, Seb31, and Seb33) were used to discriminate species. Electrophoresis was conducted on an ABI 3130 genetic analyzer (Applied Biosystems, Waltham, MA) using 0.1 μl of the GeneScan™ 1200 LIZ™ dye size standard (Thermo Fisher Scientific, Waltham, MA) for each sample. The GeneMapper® Software v5.2 (Thermo Fisher Scientific) was used to perform data analysis/genotyping. The The R Package EasyAssign (https://github.com/biodray/EasyAssign"\t "_blank") was used to assign samples to species. Genetic analysis indicated that 6% of the sampled fish were *S. fasciatus* (captured at AG and GA), and species was undetermined in 23% of the fish. Of the 140 fish sampled, 95 individuals were identified as "*S. mentella* gulf" (the threshold used for the assignment was 95%); from these, we randomly selected ten fish per sampling site (for a total of 70) □that were used for gene expression analyses (Table 3).

Gene expression

Extraction of total RNA

RNA was extracted from 30 mg of liver, heart, and epaxial muscle (wet mass) from ten deepwater redfish per site (total of 70), using the RNeasy Plus Universal Mini Kit (liver; Qiagen, Inc.) or RNeasy Fibrous Tissue Kit (heart and muscle; Qiagen, Inc.) and diluted to 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 (Chemi-Doc XRS + system, Biorad, CA) and spectrophotometry (NanoVue Plus, GE Healthcare, Pittsburgh, PA).

Reverse transcription

Reverse transcription of messenger RNA (mRNA) into complementary DNA (cDNA) was performed in duplicate using the Quantitect reverse transcription kit (Qiagen, Inc.). 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

Table 4. Information on partial sequences and probes obtained for *S. mentella*. For each studied gene, we present the PCR amplicon size (number of base pairs; bp), the designed qPCR primers and probes, and the % efficiency (this is presented as a slope; the ideal is −3.3 and the accepted range is −3.58 to −3.10). Abbreviations are as follows: *igf-1*: insulin like growth factor 1 (liver); *igf-1ra*: IGF-1 receptor (white muscle); *myo*: myosin heavy chain (white muscle); *pk*: pyruvate kinase (white muscle); *ldh-a*: lactate dehydrogenase (white muscle); *cox-1*: cytochrome oxidase (heart); *cs*: citrate synthase (heart); *ef-1a*: elongation factor 1 alpha; *18s*: 18s ribosomal; and *b-actin*: beta actin.

Gene	Amplicon size (bp)	% Efficiency (slope)	qPCR primers and probes (5′-3′)
igf-1	177	97.63 (−3.38)	F—GCTGAGGACGCACAGCAGTA R—CAAGAGTGCGATGTGCTGTATCT P—TGAGAGGGTGTGGCTAC
igf-1ra	113	100.08 (−3.32)	F—GCCTGTACACCTGAGGACATCA R—CCACCGAGTCCAGGTAGCA P—ATCCATGCGGATTGAG
myo	755	94.92 (−3.45)	F—TGGAGCTTACCTTGGCCAAA R—TCAGGTTCTTCACCTTGTTCTCAGT P—TGGAGAAGGAGAAACAT
pk	185	94.54 (−3.46)	F—CCTGCAGTTCGGTGTGGAT R—GCGGATGAAGGAGGCAAA P—ACGGAGTCGACATGGT
ldh-a	121	107.71 (−3.15)	F—GGGAGCAAGCCATACTTTTAGC R—GCAAGGTCAGGAATTGAATTTGA P—TCTGGCAAAATCCT
cox-1	237	93.44 (−3.49)	F—TGAAAAGATTGTCAGGTCGACTGA R—CGCCCCTGGCTGGTAAT P—CTCCTGCGTGGGCTA
cs	229	99.68 (−3.33)	F—TCAGCGCCGCCATCA R—GGAGTATGCCCGTGCAAAA P—CTCTGAATAGCGAGAGCG
ef-1a	813	100.38 (−3.32)	F—CGGCCGACGGGTACAGT R—GCCCCTGCAGGACGTCTAC P—CCAATACCGCCAATTT
18s	842	99.00 (−3.35)	F—CTTTCGCTTTCGTCCGTCTT R—GTATTGTGCCGCTAGAGGTGAA P—CGCCGGTCCAAGAA
b-actin	507	101.99 (−3.28)	F—ACCATCGGCAATGAGAGGTT R—CGAGGAAGGATGGCTGGAA P—TTGCCCAGAGGCC

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 (Supplementary Table S1). 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, Inc.) and forward and reverse sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) with the ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). For each gene, the sequence obtained was compared with the sequence used for primer design using the BLAST® software (Altschul *et al.*, 1990). Sequence lengths and percentages of similarity to the reference sequences are presented in Supplementary Table S1.

Design of primers and probes

Deepwater redfish TaqMan primers and probes were designed using the Primer Express software version 3.0 (Applied Biosystems) for each reference and target gene (Table 4).

Real-time PCR analysis and gene quantification

Gene expression was quantified by qPCR performed in triplicate on deepwater redfish samples using a QuantStudio 3 Real Time PCR System (Applied Biosystems). Each reaction consisted of 2 μl of diluted cDNA, 5 μl of TaqMan Fast Advanced Mix (Applied Biosystems), 0.5 μl of Custom TaqMan Gene Expression Assays (Applied Biosystems), and 2.5 μl of sterile water, for a total volume of 10 μl. The thermal cycling of qPCR was done in two steps: (1) 2 min at

50°C for optimal AmpErase uracil-N-glycosylase activity followed by 20 s at 95°C to activate DNA polymerase, and (2) 45 denaturation cycles for 1 s at 95°C and annealing/extension for 20 s at 60°C. Cycle thresholds (C_T) were obtained with the QuantStudio Design Analysis software (ThermoFisher Connect).

The relative quantification of gene expression was calculated according to the 2-$\Delta\Delta C_T$ method of Livak and Schmittgen (2001), with C_T being a threshold cycle:

$$2^{\Delta\Delta C_T} = 2^{-(\Delta C_{Te} - \Delta C_{Tc})},$$

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 % 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 (Supplementary Figure S1). Relationships were examined between the response variables *(igf-1, igf-1ra, myo, cox-1, cs, pk,* and *ldh-a*) and the explanatory variables describing site-scale characteristics (dissolved oxygen, temperature, depth, and deepwater redfish biomass at the entire capture site) and individual-scale characteristics (length, weight, and Fulton condition factor of analysed individuals) using the 70 deepwater redfish on which qPCR analyses had been done. To do so, a global canonical redundancy analysis (RDA: *rda* function in the R vegan package) was run with all explanatory variables, and model probability and adjusted coefficients of determination (adjusted R^2) were calculated. The adjusted R^2 was quantified (*RsquareAdj* function in the R vegan package), and it accounts for the number of observations and number of degrees of freedom in the fitted model (Peres-Neto *et al.*, 2006; Legendre *et al.*, 2011).

Variation partitioning was conducted to determine the relative contribution of site-scale characteristics (dissolved oxygen, temperature, salinity, depth, and total biomass of captured deepwater redfish at the sampling site) and individual-scale characteristics (length, weight, and Fulton condition factor of the analysed individuals) to explain gene expressions (*varpart* function in the R vegan package). This method requires multiple partial RDAs to quantify the variance explained exclusively and jointly by groups of variables.

A selection of variables contributing to the explained variation was achieved by using both forward and backward selection as well as a stopping criterion (*ordiR2step* function in the R vegan package; Blanchet *et al.*, 2008). This criterion limits overfitting by preventing selected variables included in the reduced model from explaining more variation than the full model developed with all explanatory variables. A triplot was produced with a type 1 scaling to illustrate distances among objects (i.e. individuals) and relationships with selected environmental 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: $F = 42.80$, $p < 0.0001$; and 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 (on average of 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 for the ten 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 ($F = 3.97$ and $p = 0.0019$; 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 ($F = 4.60$ and $p < 0.0001$; Figure 2b). The relative expression of white muscle *myo* was lowest in individuals captured at CSt ($F = 2.73$ and $p = 0.0210$; 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 ($F = 9.10$ and $p < 0.0001$; 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 ($F = 3.84$ and $p = 0.0025$; Figure 3b). The relative expression of *cox-1* in the heart

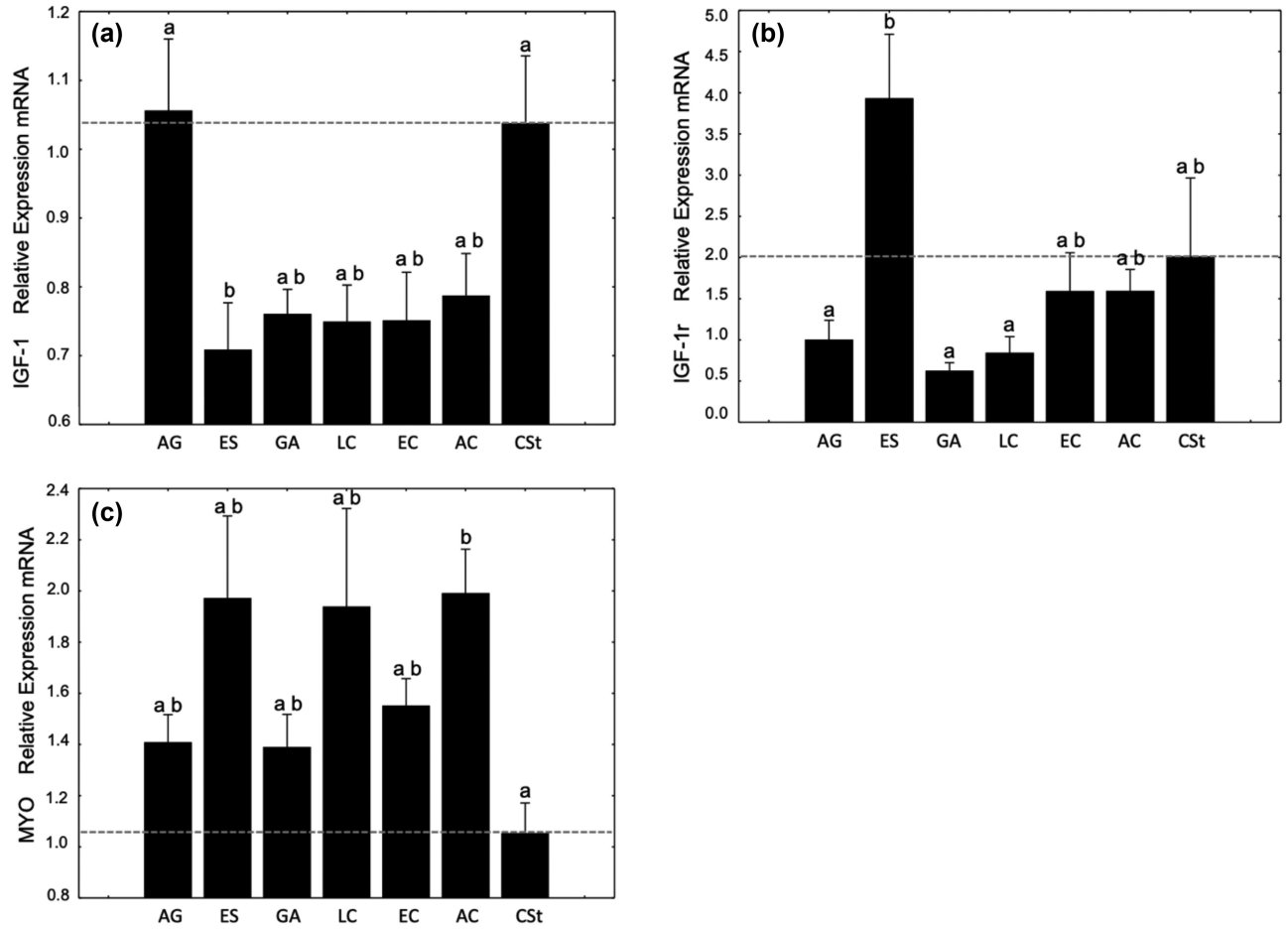

Figure 2. Relative changes in gene expression (mean ± SE) of three target genes related to growth in deepwater redfish captured at the sampling stations (AG: Anticosti–Gaspé, ES: Estuary, GA: Gyre d'Anticosti, LC: Laurentian Channel, EC: Esquiman Channel, AC: Anticosti Channel, and CSt: Cabot Strait). (a) liver insulin-like growth factor 1 (*igf-1*), (b) white muscle insulin-like growth factor 1 receptor (*igf-1r*), and (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*]; $\alpha = 0.05$).

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 ($F = 2.97$ and $p = 0.019$; Figure 3c). Fish captured at EC had higher expressions of *cs* (204%) compared to fish captured in GA and LC ($F = 3.50$ and $p = 0.0046$; 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: $F = 5.28$, $p = 0.002$ and 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 investigation, we hypothesized that studying the gene expression of growth and metabolism regulators could provide information on growth characteristics and indicate 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.

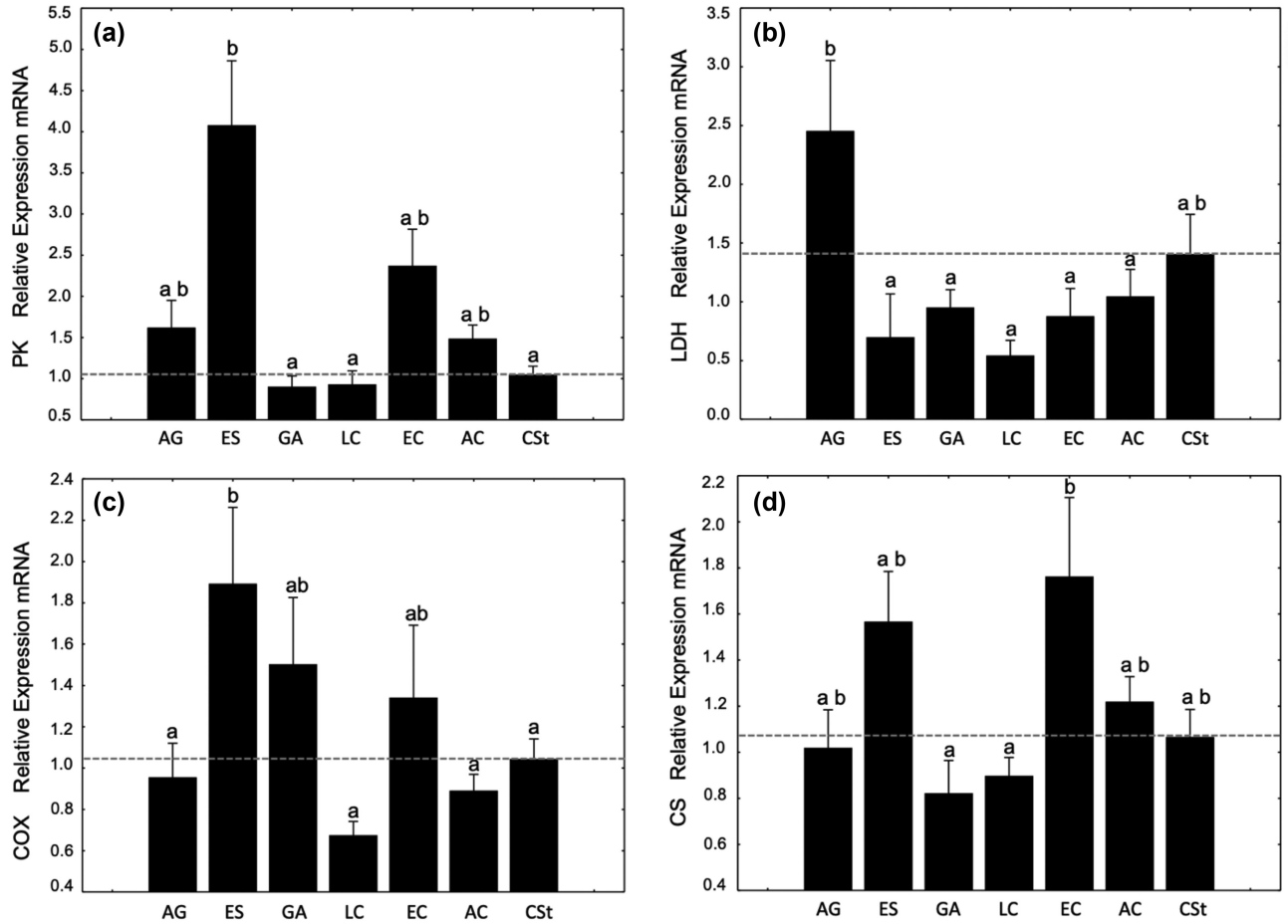

Figure 3. Relative changes in gene expression (mean ± SE) of four target genes related to metabolism in deepwater redfish captured from the sampling stations (AG: Anticosti–Gaspé, ES: Estuary, GA: Gyre d'Anticosti, LC: Laurentian Channel, EC: Esquiman Channel, AC: Anticosti Channel, and CSt: Cabot Strait). (a) white muscle pyruvate kinase (*pk*), (b) white muscle lactate dehydrogenase (*ldh*), (c) heart cytochrome c oxidase (*cox-1*), and (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-1*]; $\alpha = 0.05$).

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 expressions of liver *igf-1* were associated with the sites where the smallest and 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 *S. 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 muscle *pk* expression has been associated with starvation in rainbow trout *O. 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 could explain why the fish caught in ES were smaller and had the highest *pk* expression due to possible starvation. Furthermore, analysis of stomach content revealed that deepwater redfish at both sites were feeding on zooplankton, but zooplankton taxon composition 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*

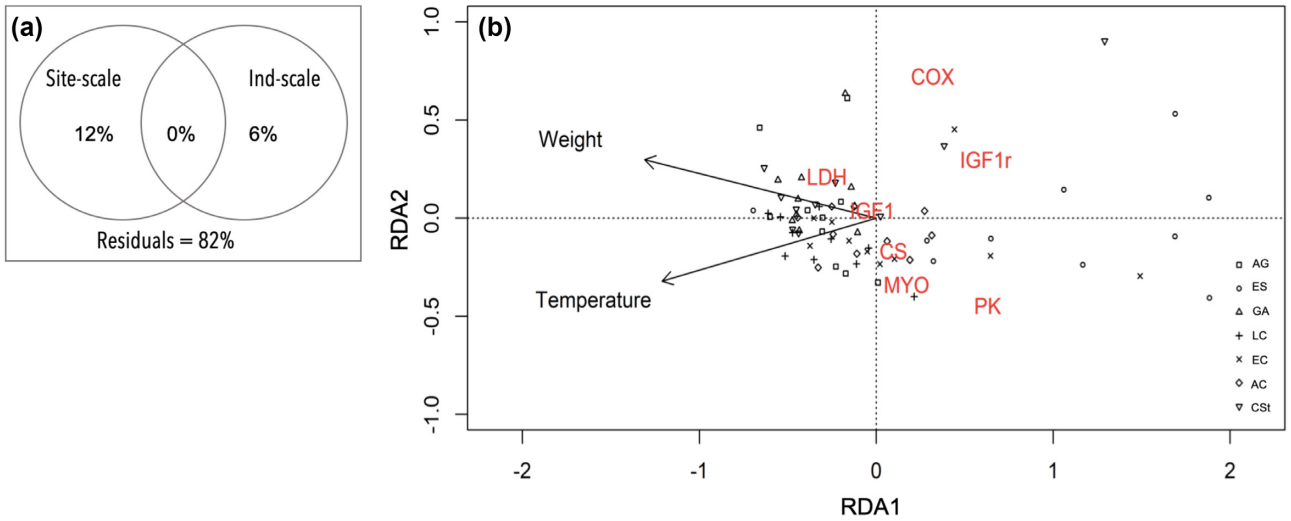

Figure 4. (a): Site-scale and individual-scale analyses. Variation partitioning, relationships between gene expression (Y) and site variables (X1: dissolved oxygen, temperature, depth, and redfish biomass) and individual variables (X2: length, weight, and Fulton index). (b): Redundance analysis. *Scaling 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.

goodei, but negative correlations were 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) and ES (lowest dissolved oxygen) 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 to examine potential nutritional differences, which 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 *O. mykiss*, 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 largemouth bass *M. salmoides* and smallmouth bass *M. dolomieu*, sunfish *Lepomis gibbosus* and *Lepomis macrochirus* (Davies and Moyes, 2007), barred sand bass *Paralabrax nebulifer* (Yang and Somero, 1996), and longspine thornyhead *Sebastolobus altivelis* (Vetter and Lynn, 1997). 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 remembered 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 were 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 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-1* 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-1* compared to the warmest sites (CSt, AG, LC, and AC). Cold acclimation in wild fish typically leads to an increase in COX activity due to an increase in mitochondrial gene expression (Bremer and Moyes, 2011). 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 *Salmo salar,* COX activity increased rapidly with lower temperature (Nathanailides, 1996) In contrast, no relationship was observed between COX activity and temperature in Atlantic cod *G. morhua* (Pelletier *et al.*,

1995). Contrary to our findings (no correlation between *ldh-a* expression and temperature), *ldh* expression in black rockfish *Sebastes schlegelii* increased with warmer temperatures and showed differences among tissues: expressions were higher in liver and brain and lower in muscle (Song *et al.*, 2019). There are two metabolic strategies available to ectotherms: (1) increased metabolism to compensate for temperature-mediated decreases in the metabolic rate or (2) suppressed metabolic rate to reduce energy consumption (Song *et al.*, 2019). Deepwater redfish from ES seem to use a combination of both: anaerobic metabolism (*ldh-a*) is down regulated and aerobic metabolism (*cox-1*) is up regulated in cold water.

The relative expression of white muscle *Igf-1ra* was negatively correlated with temperature. It is surprising that this gene does not show any strong correlation with weight and yet does 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. Nevertheless, increases in temperature associated with global climate change are likely to have strong effects on fish physiology and thereby on populations and fisheries (e.g. Little *et al.*, 2020; Ghinter *et al.*, 2021).

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 (150–500 m). 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 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 deep-water 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.

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, and 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, 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 found to correlate positively 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-1* 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 field surveys. Since the 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 us identify the physiological mechanisms underlying observed differences. More broadly, transcriptomics could be an important tool for investigating growth in a deep-sea fish whose physiology is difficult to study. The inclusion of more ecological variables such as prey abundance could improve our understanding of fish physiology in future studies.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Data availability statement

Data available on request. The data underlying this article be shared on reasonable request to the corresponding author.

Contributions

M.A.M.S. contributed to data generation, data analysis, and manuscript preparation; M.V. contributed to data analysis and manuscript preparation; C.S. contributed to data generation, data analysis, and manuscript preparation; and C.A. contributed with ideas, data generation, data analysis, and manuscript preparation.

Funding information

This project was part of the partnership program "Return of groundfish in the Estuary and Northern Gulf of St. Lawrence," co-funded by Fisheries and Oceans Canada (DFO) and Ressources Aquatiques Québec (RAQ)—Regroupement stratégique des Fonds de recherche du Québec.

Acknowledgements

We are grateful to all who provided technical and scientific assistance in the laboratory and field. Special thanks to Renée Gagné and Sarah Brown-Vuillemin (Institut des sciences de la mer de Rimouski) and Hugues Benoit, Jean-François Lussier, Denis Bernier, Pierre-Marc Scallon-Chouinard, Marie-Julie Roux, and Jérome Gagnon (Fisheries and Oceans Canada), who contributed to deepwater redfish collection; Geneviève Parent and Éric Parent (Fisheries and Oceans Canada), who helped with sequencing; and Hugo Bourdages (Fisheries and Oceans Canada) who planned the

scientific campaign. This research was supported by the Fonds des partenariats 2017–18 *Retour des poissons de fond dans l'estuaire et le nord du golfe du Saint-Laurent* of Fisheries and Oceans Canada and by the *Ressources Aquatiques Québec* research network (*Regroupement stratégique des Fonds de recherche du Québec*).

References

Alami-Durante, H., Médale, F., Cluzeaud, M., and Kaushik, S. J. 2010. Skeletal muscle growth dynamics and expression of related genes in white and red muscles of rainbow trout fed diets with graded levels of a mixture of plant protein sources as substitutes for fishmeal. Aquaculture, 303: 50–58.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. Journal of Molecular Biology, 215: 403–410.

Azizi, S., Nematollahi, M. A., Mojazi Amiri, B., Vélez, E. J., Salmerón, C., Chan, S. J., and Navarro, I. 2016. IGF-I and IGF-II effects on local IGF system and signaling pathways in gilthead sea bream (*Sparus aurata*) cultured myocytes. General and Comparative Endocrinology, 232: 7–16.

Bailey, D. M., Genard, B., Collins, M. A., Rees, J. F., Unsworth, S. K., Battle, E. J. V, and Bagley, P. M. 2005. High swimming and metabolic activity in the deep sea eel *Synaphobranchus kaupii* revealed by integrated *in situ* and *in vitro* measurements. Physiological and Biochemical Zoology, 78: 335–346.

Beckman, B. R., Fairgrieve, W., Cooper, K. A., Mahnken, C. V. W., and Beamish, R. J. 2004. Evaluation of endocrine indices of growth in individual postsmolt coho salmon. Transactions of the American Fisheries Society, 133: 1057–1067.

Beckman, B. R. 2011. Perspectives on concordant and discordant relations between insulin-like growth factor 1 (IGF1) and growth in fishes. General and Comparative Endocrinology, 170: 233–252.

Benestan, L., Rougemont, Q., Senay, C., Normandeau, E., Parent, E., Rideout, R., and Bernatchez, L. 2021. Population genomics and history of speciation reveal fishery management gaps in two related redfish species (*Sebastes mentella* and *Sebastes fasciatus*). Evolutionary Applications, 14: 588.

Bergan-Roller, H. E., and Sheridan, M. A. 2018. The growth hormone signaling system : insights into coordinating the anabolic and catabolic actions of growth hormone. General and Comparative Endocrinology, 258: 119–133.

Björnsson, B. T. 1997. The biology of salmon growth hormone : from daylight to dominance. Fish Physiology and Biochemistry, 17: 9–24.

Blanchet, F. G., Legendre, P., and Borcard, D. 2008. Modelling directional spatial processes in ecological data. Ecological Modelling, 215: 325–336.

Bourdages, H., Brassard, C., Desgagnes, M., Galbraith, P., Gauthier, J., Legare, B., and Nozores, C. 2019. Preliminary results from the groundfish and shrimp multidisciplinary survey in August 2018 in the Estuary and Northern Gulf of St. Lawrence. DFO Canadian Science Advisory Secretariat Research Document 2018/036. Fisheries and Oceans Canada. iv + 87p.

Brassard, C., Bourdages, H., Duplisea, D., Gauthier, J., and Valentin, A. 2017. L'état des stocks de sébaste (*Sebastes fasciatus* et s. *mentella*) de l'unité 1 (golfe du saint-laurent) en 2015L'état des stocks de sébaste (*Sebastes fasciatus* et s. *mentella*) de l'unité 1 (golfe du saint-laurent) en 2015. DFO Canadian Science Advisory Secretary Research Document 2017/023. Pêches et Océans Canada, Ottawa. ix + 52p.

Bremer, K., and Moyes, C. D. 2011. Origins of variation in muscle cytochrome c oxidase activity within and between fish species. Journal of Experimental Biology, 214: 1888–1895.

Breves, J. P., Phipps-Costin, S. K., Fujimoto, C. K., Einarsdottir, I. E., Regish, A. M., Thrandur, B., and Mccormick, S. D. 2016. Hepatic insulin-like growth-factor binding protein (igfbp) responses to food restriction in atlantic salmon smolts. General and Comparative Endocrinology, 233: 79–87.

Craig, P. M., Wood, C. M., and McClelland, G. B. 2007. Oxidative stress response and gene expression with acute copper exposure in zebrafish (*Danio rerio*). American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 293: R1882–R1892.

Davies, R., and Moyes, C. D. 2007. Allometric scaling in centrarchid fish: origins of intra- and inter-specific variation in oxidative and glycolytic enzyme levels in muscle. Journal of Experimental Biology, 210: 3798–3804.

Desrosiers, V., Le François, N. R., Tveiten, H., Andreassen, I., and Blier, P. U. 2008. Ontogenesis of catabolic and energy metabolism capacities during the embryonic development of spotted wolffish (*Anarhichas minor*). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 150: 200–206.

DFO. 2018a. Units 1 + 2 redfish management strategy. Canadian Science Advisory Secretariat Science Advisory Report 2018/033. Fisheries and Oceans Canada.

DFO. 2018b. Assessment of redfish stocks (*Sebastes mentella* and *S. fasciatus*) in units 1 and 2 in 2017. Canadian Science Advisory Secretariat Science Advisory Report 2018/032. Fisheries and Oceans Canada.

Duan, C., Ren, H., and Gao, S. 2010. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: roles in skeletal muscle growth and differentiation. General and Comparative Endocrinology, 167: 344–351.

Fry, F. E. 1971. The effect of environmental factors on the physiology of fish. Fish Physiology, 6: 1–98.

Fulton, T. W. 1904. The rate of growth of fishes. 22nd Annual Report of the Fishery Board of Scotland, 3: 141–241.

Gabillard, J. C., Weil, C., Rescan, P. Y., Navarro, I., Gutiérrez, J., and Le Bail, P. Y. 2003. Effects of environmental temperature on IGF1, IGF2, and IGF type i receptor expression in rainbow trout (*Oncorhynchus mykiss*). General and Comparative Endocrinology, 133: 233–242.

Galbraith, P. S., Chassé, J., Caverhill, C., Nicot, P., Gilbert, D., Lefaivre, D., and Lafleur, C. 2019. Conditions océanographiques physiques dans le golfe du Saint-Laurent en 2018. Canadian Science Advisory Secretariat Science Advisory Document 2019/046. Pêches et Océans Canada. iv + 83p.

Ghinter, L., Lambert, Y., and Audet, C. 2021. Juvenile greenland halibut (*Reinhardtius hippoglossoides*) growth in the context of rising temperature in the Estuary and Gulf of St. Lawrence. Fisheries Research, 233: 1–10.

Hack, N. L., Strobel, J. S., Journey, M. L., Beckman, B. R., and Lema, S. C. 2018. Response of the insulin-like growth factor-1 (Igf1) system to nutritional status and growth rate variation in olive rock fish (*Sebastes serranoides*). Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 224: 42–52.

Hack, N. L., Cordova, K. L., Glaser, F. L., Journey, M. L., Resner, E. J., Hardy, K. M., and Beckman, B. R. 2019. Interactions of long-term food ration variation and short-term fasting on insulin-like growth factor-1 (IGF-1) pathways in copper rockfish (*Sebastes caurinus*). General and Comparative Endocrinology, 280: 168–184.

Johansen, K. A., and Overturf, K. 2006. Alterations in expression of genes associated with muscle metabolism and growth during nutritional restriction and refeeding in rainbow trout. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 144: 119–127.

Kawaguchi, K., Kaneko, N., Fukuda, M., Nakano, Y., Kimura, S., Hara, A., and Shimizu, M. 2013. Responses of insulin-like growth factor (IGF)-I and two IGF-binding protein-1 subtypes to fasting and refeeding, and their relationships with individual growth rates in yearling masu salmon (*Oncorhynchus masou*). Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 165: 191–198.

Le François, N. R., Lamarre, S. G., and Blier, P. U. 2005. Is white muscle anaerobic glycolysis capacity indicative of competitive ability in Arctic charr? Journal of Fish Biology, 66: 1167–1176.

Legendre, P., Oksanen, J., and Tter Braak, C. J. F. 2011. Testing the significance of canonical axes in redundancy analysis. Methods in Ecology and Evolution, 2: 269–277.

Little, A. G., Loughland, I., and Seebacher, F. 2020. What do warming waters mean for fish physiology and fisheries? Journal of Fish Biology, 97: 328–340.

Livak, K. J., and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods, 25: 402–408.

Meier, K. M., Figueiredo, M. A., Kamimura, M. T., Laurino, J., Maggioni, R., Pinto, L. S., and Dellagostin, O. A. 2009. Increased growth hormone (GH), growth hormone receptor (GHR), and insulin-like growth factor i (IGF-I) gene transcription after hyperosmotic stress in the brazilian flounder *Paralichthys orbignyanus*. Fish Physiology and Biochemistry, 35: 501–509.

Nash, R. D. M., Valencia, A. H., Geffen, A. J., and Meek, A. 2006. The origin of fulton's condition factor — setting the record straight. Fisheries, 31: 236–238.

Nathanailides, C. 1996. Metabolic specialization of muscle during development in cold-water and warmwater fish species exposed to different thermal conditions. Canadian Journal of Fisheries and Aquatic Sciences, 53: 2147–2155..

Overturf, K., and Hardy, R. W. 2001. Myosin expression levels in trout muscle: a new method for monitoring specific growth rates for rainbow trout *Oncorhynchus mykiss* (Walbaum) on varied planes of nutrition. Aquaculture Research, 32: 315–322.

Pelletier, D., Guderley, H., and Dutil, J.-D. 1993a. Does the aerobic capacity of fish muscle change with growth rates?. Fish Physiology and Biochemistry, 12: 83–93.

Pelletier, D., Guderley, H., and Dutil, J.-D. 1993b. Effects of growth rate, temperature, season, and body size on glycolytic enzyme activities in the white muscle of atlantic cod (*Gadus morhua*). Journal of Experimental Zoology, 265: 477–487.

Pelletier, D., Blier, P. U., Dutil, J.-D., and Guderley, H. 1995. How should enzyme activities be used in fish growth studies?. Journal of Experimental Biology, 198: 1493–1497.

Peres-Neto, P. R., Legendre, P., Dray, S., and Borcard, D. 2006. Variation partitioning of species data matrices: estimation and comparison of fractions. Ecology, 87: 2614–2625.

Picha, M. E., Silverstein, J. T., and Borski, R. J. 2006. Discordant regulation of hepatic IGF-I mRNA and circulating IGF-I during compensatory growth in a teleost, the hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). General and Comparative Endocrinology, 147: 196–205.

Picha, M. E., Turano, M. J., Tipsmark, C. K., and Borski, R. J. 2008. Regulation of endocrine and paracrine sources of igfs and Gh receptor during compensatory growth in hybrid striped bass (*Morone chrysops* x *Morone saxatilis*). Journal of Endocrinology, 199: 81–94.

Pierce, A. L., Shimizu, M., Beckman, B. R., Baker, D. M., and Dickho, W. W. V. 2005. Time course of the GH /IGF axis response to fasting and increased ration in chinook salmon (*Oncorhynchus tshawytscha*). General and Comparative Endocrinology, 140: 192–202.

Reindl, K. M., and Sheridan, M. A. 2012. Peripheral regulation of the growth hormone-insulin-like growth factor system in fish and other vertebrates. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 163: 231–245.

Reinecke, M., Björnsson, B. T., Dickhoff, W. W., McCormick, S. D., Navarro, I., Power, D. M., and Gutiérrez, J. 2005. Growth hormone and insulin-like growth factors in fish: where we are and where to go. General and Comparative Endocrinology, 142: 20–24.

Saborido-Rey, F., Garabana, D., and Cerviño, S. 2004. Age and growth of redfish (*Sebastes marinus, S . mentella,* and *S . fasciatus*) on the flemish cap (Northwest Atlantic). ICES Journal of Marine Science, 61: 231–242.

Saborido-Rey, F., and Kjesbu, O. S. 2005. Growth and Maturation Dynamics. Spanish National Research Council. 26pp.

Scott, W. B., and Scott, M. G. 1988. Atlantic fishes of Canada. Canadian Bulletin of Fisheries and Aquaculture Sciences, 219: 731.

Senay, C., Ouellette-Plante, J., Bourdages, H., Bermingham, T., Parent, G., Chabot, D., and Duplisea, D. 2021. Unit 1 redfish (*Sebastes mentella* and *S. fasciatus*) stock status in 2019 and updated information on population structure, biology, ecology, and current fishery closures. DFO Canadian Science Advisory Secretariat Research Document 2021/015. Canadian Science Advisory Secretariat. xi + 119p.

Song, M., Zhao, J., Wen, H. S., Li, Y., Li, J. F., Li, L. M., and Tao, Y. X. 2019. The impact of acute thermal stress on the metabolome of the black rockfish (*Sebastes schlegelii*). Plos ONE, 14: 1–23.

Strobel, J. S., Hack, N. L., Label, K. T., Cordova, K. L., Bersin, T. V., Journey, M. L., and Beckman, B. R. 2020. Effects of food deprivation on plasma insulin-like growth factor-1 (Igf1) and igf binding protein (Igfbp) gene transcription in juvenile cabezon (*Scorpaenichthys marmoratus*). General and Comparative Endocrinology, 286: 113319.

Torres, J. J., and Somero, G. N. 1988. Metabolism, enzymic activities and cold adaptation in antarctic mesopelagic fishes. Marine Biology, 98: 169–180.

Tripathi, G., and Verma, P. 2004. Scaling effects on metabolism of a teleost. Journal of Experimental Zoology, 301A: 718–726.

Vagner, M., and Santigosa, E. 2011. Characterization and modulation of gene expression and enzymatic activity of delta-6 desaturase in teleosts: a review. Aquaculture, 315: 131–143.

Valentin, A. E., Penin, X., Chanut, J. P., Power, D., and Sévigny, J. M. 2014. Combining microsatellites and geometric morphometrics for the study of redfish (*Sebastes* spp.) population structure in the Northwest Atlantic. Fisheries Research, 154: 102–119.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., and Speleman, F. 2002. Accurate normalization of real-time RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology, 3: 1–13.

Vélez, E. J., Lutfi, E., Azizi, S., Montserrat, N., Riera-Codina, M., Capilla, E., and Navarro, I. 2016. Contribution of in vitro myocytes studies to understanding fish muscle physiology. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 199: 67–73.

Vélez, E. J., Lutfi, E., Azizi, S., Perelló, M., Salmerón, C., Riera-Codina, M., and Ibarz, A.. 2017. Understanding fish muscle growth regulation to optimize aquaculture production. Aquaculture, 467: 28–40.

Vélez, E. J., Perelló, M., Azizi, S., Moya, A., Lutfi, E., Pérez-Sánchez, J., and Calduch-Giner, J. A. 2018. Recombinant bovine growth hormone (rBGH) enhances somatic growth by regulating the GH-IGF axis in fingerlings of gilthead sea bream (*Sparus aurata*). General and Comparative Endocrinology, 257: 192–202.

Velki, M., Meyer-Alert, H., Seiler, T. B., and Hollert, H. 2017. Enzymatic activity and gene expression changes in zebrafish embryos and larvae exposed to pesticides diazinon and diuron. Aquatic Toxicology, 193: 187–200.

Vetter, R. D., and Lynn, E. A. 1997. Bathymetric demography, enzyme activity patterns, and bioenergetics of deep-living scorpaenid fishes (genera *Sebastes* and *Sebastolobus*): paradigms revisited. Marine Ecology Progress Series, 155: 173–188.

Volkoff, H., Hoskins, L. J., and Tuziak, S. M. 2010. Influence of intrinsic signals and environmental cues on the endocrine control of feeding in fish: potential application in aquaculture. General and Comparative Endocrinology, 167: 352–359.

Wood, A. W., Duan, C., and Bern, H. A. 2005. Insulin-like growth factor signaling in fish. International Review of Cytology, 243: 215–285.

Yang, T. H., and Somero, G. N. 1996. Activity of lactate dehydrogenase but not its concentration of messenger RNA increases with body size in barred sand bass, *Paralabrax nebulifer* (Teleostei). The Biological Bulletin, 191: 155–158.

Handling Editor: Lorenz Hauser