

Response to Reviewer 1 Comments

Comments and Suggestions for Authors : The authors describe a characterization study of differentially transcribed genes associated with muscle growth in an economically important fish species. The rationale for the study was presented and the experimental design for the analyses of the production traits are reasonable. The authors selected fish from the fastest growing and fish from the slowest fish for their differential transcriptomic study. The experiments were well described with the minor exceptions presented below. The data led the authors to conclude that fish that ate more grew faster. This is not an unexpected observation. While it is important to continue to investigate the transcriptional and translational mechanisms of fish growth, the use of these data was not discussed by the authors. For instance, were they looking for biomarkers for broodstock selection or did the study help inform nutritional strategies? Because the mechanisms of muscle growth, especially in indeterminate growers is of great importance to the elucidation of the potential for resolving issues like sarcopenia, cachexia, space flight, muscular dystrophy, and spinal cord injury these types of studies are still important. Therefore, a couple of issues should be addressed by the authors if they choose to revise the manuscript.

Response: Thank you for your comments and suggestions on our manuscript. We appreciate your positive feedback on the rationale and experimental design of our study, as well as your acknowledgement of the importance of investigating the transcriptional and translational mechanisms of fish growth. We agree that further discussion on the potential applications of our data, such as biomarkers for broodstock selection or nutritional strategies, would be valuable and will consider including it in our revisions. We also appreciate your reminder of the broader implications of studying muscle growth mechanisms beyond economic considerations, and will strive to address these issues in our revised manuscript. Thanks again for your thoughtful feedback.

Point 1: The use of a single reference gene is always problematic since not all reference genes are stable under different conditions, like differential nutritional planes. The authors should discuss the use of a single reference gene based on the study of Jacob, et al. 2012 (attached).

Response 1:

Thank you for providing the article. I have read it and learned a lot from it. The article discusses the importance of selecting suitable reference genes for data normalization in Reverse Transcription - quantitative Polymerase Chain Reaction (RT-qPCR). The authors aimed to review the literature since the implementation of the MIQE guidelines, compare various algorithms for expression stability, and identify reliable reference genes for human cancer cell lines. They reviewed 37 publications since 2009, with two-thirds of patient samples and one-third cell lines. They identified HSPCB, RRN18S, and RPS13 as the most stable reference genes in their selection of 25 cancer cell lines. The authors recommend establishing a set of at least three suitable reference genes selected by at least three stability algorithms in advance of experiments. However, that article presented results in human cancer cells, and as for the study in fish, most of the papers only used a single reference gene (β -actin)

as reference gene. Here are some articles from recent years that support this:

1. Huang, J., *et al.* Heterozygous depletion of pik3r1 improves growth and feed conversion efficiency in Gibel carp (*Carassius gibelio*). *Aquaculture* 2021;545.
2. Li, R., *et al.* Characterization and expression profiles of muscle transcriptome in Schizothoracine fish, *Schizothorax prenanti*. *Gene* 2019;685:156-163.
3. Sun, Y., *et al.* Transcriptome analysis reveals the molecular mechanisms underlying growth superiority in a novel grouper hybrid (*Epinephelus fuscogutatus* ♀ × *E. lanceolatus* ♂). *BMC Genet* 2016;17:24.
4. Yang, Y., *et al.* Transcriptional profiling of skeletal muscle reveals starvation response and compensatory growth in *Spinibarbus hollandi*. *BMC Genomics* 2019;20:938.
5. Lu, X., *et al.* Transcriptome analysis of grass carp (*Ctenopharyngodon idella*) between fast-and slow-growing fish. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, 2020, 35: 100688.

I appreciate your feedback and will consider the possibility of using multiple reference genes in future research. Thank you again for your valuable input.

Point 2: There are English grammar issues that need to be addressed. These are not serious, as the manuscript is well written except for these minor issues.

Response 2: Thank you for your feedback on our manuscript. We appreciate your positive assessment of the overall quality of our writing. We apologize for any English grammar issues that may have arisen and will address them in our revisions. We take all feedback seriously and strive to improve the clarity and accuracy of our writing. Thank you again for your helpful comments.

Point 3: Were the melting curves and efficiency of the primers evaluated. Since it appears that all primer/template reactions were conducted under the same conditions it is important that all of the primers behaved the same under those same conditions. This is important for providing a basis for confidence in the interpretation of the data from the experiments. For a discussion of this issue a publication by Bustin, et al. 2009 is provided. It is suggested that the authors response reflects the suggestions of MIQE presented in the Bustin publication.

Response 3: Thank you for your comments and suggestions on our manuscript. The melting curves and efficiency of the primers were evaluated in our study. All primer/template reactions were conducted under the same conditions to ensure consistency. We followed the MIQE guidelines for qPCR experiments and have provided a detailed description of our experimental procedures in the Methods section of the manuscript. We agree with the importance of evaluating primer efficiency and melting curves, as discussed in the Bustin et al. 2009 publication, and have taken steps to ensure the reliability and reproducibility of our results.

Table 1 The specific primers for the RT-PCR

Numer	Gene	Primers	Sequences (5' - 3')	Tm/°C
A	<i>myh1</i>	RT-MYH1_F	TCAAGCAGAAGCAGCGTGAGGAG	66.8
		RT-MYH1_R	CGGATAGCACAGAGCCTTCAGCAT	66.8

B	<i>myh4</i>	RT-MYH4_F	TGATGCCAAGAGTGCCTGCTACG	67.2
		RT-MYH4_R	CTGTCCTCTCCTCCTGAGTGTCCA	64.4
C	<i>gtr12</i>	RT-GTR12_F	ACCTGCTCATCTCCATGACCTTCC	65.5
		RT-GTR12_R	TATCTCCTCCAGCGTGCACCT	65.8
D	<i>tril6</i>	RT-TRI16_F	CGCACAAGCACACTCATCACAAGA	65.8
		RT-TRI16_R	TCATATGGTGGCGGCTTCACTTCT	66.9
E	<i>crem</i>	RT-CREM_F	ATAACGCCAGCTCCAGCCTTCC	66.6
		RT-CREM_R	CGGGCAGCTTCCCTGTTCTTCA	68.3
F	<i>ttc9a</i>	RT-TTC9A_F	TGGAGAATGCAGAGCTGGAGTGTT	65.1
		RT-TTC9A_R	CGGATCGGTACAGAGCCTTGAAGT	65.9
G	<i>jun</i>	RT-JUN_F	CTCTCCTGATGTGGGCTTGCTGAA	67.5
		RT-JUN_R	CTCCTGCTCGTCGGTGACATTCTT	66.7
H	<i>tri65</i>	RT-TRI65_F	CCAGCAGAGAATCCAGGACAGAGA	64.6
		RT-TRI65_R	TCCATCAGACGGATCAGGTCAGTG	65.8
I	<i>fmo5</i>	RT-FMO5_F	CGGTGGTTGGAGGAGGAAGTTCAG	68.0
		RT-FMO5_R	AATGCTCGCTCTGTCTGGCTCTG	65.8
J	<i>b3gt2</i>	RT-B3GT2_F	GGCGGGTGTCTTACTCCAGTTGT	64.3
		RT-B3GT2_R	AGGCGTTGTGCTTGTTGCTCTG	65.1
K	<i>tnni2</i>	RT-TNNI2_F	CGACAAGGTGGACGAGGAGAGGTA	66.5
		RT-TNNI2_R	AGCATGGAGTCAGCGGACATACG	66.1
L	<i>pprc1</i>	RT-PPRC1_F	ACCGCTGCTACTGTGTCTGCTC	62.0
		RT-PPRC1_R	GGTGAGGGTTTGGTGGACTTGCTA	66.4
M	<i>ghr</i>	RT-GLIS1_F	GCAGCAAGGATGTCTGTACGGAGA	65.5
		RT-GLIS1_R	AGAGTGAGAGGCGGACGGAGTT	64.1
N	<i>ptgis</i>	RT-PTGIS_F	TCTGGCAGCAGTGAAGAAGGAGTT	64.5
		RT-PTGIS_R	GCAGCGGTGAGTCTGAGTGTCT	61.7
O	<i>sdhb</i>	RT-SDHB_F	GCTGTCTGTCTGTTCTGTTGTGG	63.5
		RT-SDHB_R	CCGAACCATCACCATCATGCCAGA	70.6
P	<i>stc2</i>	RT-STC2_F	GAAGTGTGGAGACGAGGTCAGGTT	63.5
		RT-STC2_R	GTGGCTGTGGATTGCTGTGGAAG	66.4
Q	<i>nr4a1</i>	RT-NR4A1_F1	CCTTGGTGGGAAATGGCTATGTGG	68.5
		RT-NR4A1_R1	GCTACTGCGTTGTGGCTGAATGAA	66.4
R	<i>nr4a1_1</i>	RT-NR4A1_1_F2	AACGCCTCCTGTCAGCACTACG	64.2
		RT-NR4A1_1_R2	CGATTCCGCCTCCTCTTGTCCA	68.1
S	<i>pde4d</i>	RT-PDE4D_F	CTCCATCTCAGCCGACAAGTTCCA	67.4
		RT-PDE4D_R	TACTGCCACTGTAGCCCTCCTCTT	63.6
T	<i>s41a1</i>	RT-S41A1_F	CGGAGATCGTGGTGGCATTGGA	69.5
		RT-S41A1_R	TCATCACTCTCGTTGCTCTGGCTT	65.2
U	<i>pdp1</i>	RT-PDP1_F	CGAGCCAGAGATCACGTACCACAA	66.2
		RT-PDP1_R	CCACGATACGAACCACCTCCTGTC	66.6
V	<i>tutla</i>	RT-TUTLA_F	TGGCTCATCCTCAGCAGCAACAT	66.6
		RT-TUTLA_R	ACATTGACAGACTCGCTCGGTTCA	65.8
W	<i>klf13</i>	RT-KLF13_F	TGGCAAATCATCCCACCTCAAAGC	69.1

		RT-KLF13_R	CGGAGCGGGCGAACTTCTTACT	66.8
X	<i>htr1a</i>	RT-HTR1A_F	CGGCTGGCATCTCCTTCGCTAT	67.5
		RT-HTR1A_R	CGCTGGAGTGAGAGTCATCATCCT	64.5
		RT-MXRA5_F	GGCTCCAAGGTAGAGGCACCATAC	65.3
Y	<i>mxra5</i>	RT-MXRA5_R	GCAGGACAGCAAGGTCTCCATCA	66.1
		RT-actb-F	GATCTGGCATCACACCTTCTAC	56.2
Z	<i>actb</i>	RT-actb-R	CACCGGAGTCCATGACAATAC	57.5

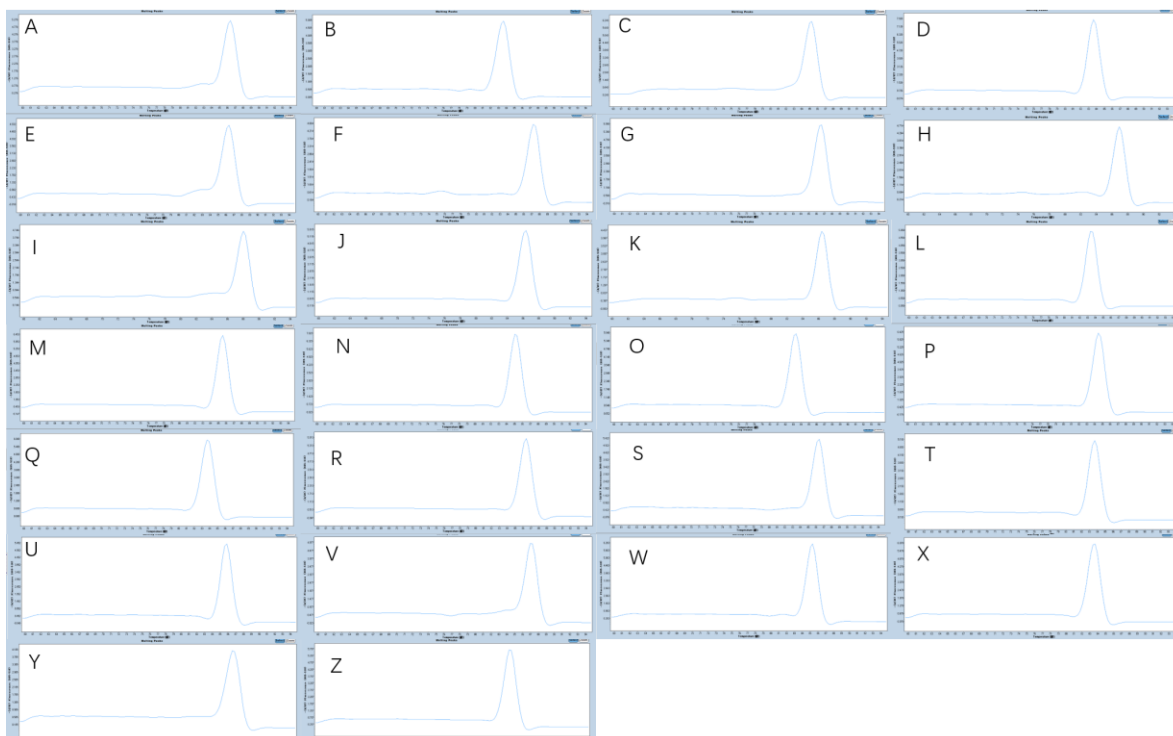


Fig 1 the melting curves of the primers

Point 4: line 151. The authors state that they used 10ul of DNA. The amount of sample which would be conveyed by volume (concentration) of the sample is more informative and allows replication of the experiments in independent laboratories.

Response 4: Thank you very much for this question. Firstly, we appreciate the opportunity to clarify this point. Secondly, we apologize for the serious error in our description here. Our original intention was to express that we used a system containing 100 ng cDNA in 10 μ l, but due to negligence in writing, this part of the content was written as 10 μ l cDNA in the manuscript. We have corrected this section and added more details. We are very sorry for such a low-level mistake and will be more careful in the future to prevent such errors from happening again.

XThe revised content: Each reaction system had a total volume of 10 μ L, containing 100 ng of cDNA, 0.5 μ L of each primer (10 μ M), 5 μ L of Power SYBR Green PCR MasterMix, and the remaining volume was supplemented with double-distilled water to reach 10 μ L.