**Low oxygen stress during early development influences regulation of hypoxia-response genes in farmed Atlantic salmon (*Salmo salar*)**

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**Supplementary Materials**

**A**

**B**



**Figure S1.** Outline of experimental set up, from egg fertilisation through to start-feeding at approximately 4 months showing the incubation temperatures for each treatment throughout the experiment (A) and dates/stages of development sampled (B).



**Figure S2.** RNA-seq count data for *egln3* (a) and *hbae* (b) in Atlantic salmonreared in 30% O2 until 925DD and 990DD.

**Table S1.** Outline of primers used for qPCR

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Direction** | **Sequence (5' -> 3')**  | **Accession** | **Chromosome** | **Efficiency**  |
| *egln1\_L* | Forward | CGAGCAACAGAATGACACAGC | XM\_014131242.1 | 12 | 2.06 |
|  | Reverse  | GCAGGCGGATTTGAACGT |  |  |  |
| *egln2* | Forward | CTGGGCTGCCGTTCTATAATG | NM\_001165346.1 | 9 | 1.80 |
|  | Reverse  | ACTGTGGCTGCCCTCAATG |  |  |  |
| *egln3* | Forward | GGACCGGATATGTGAAACATGTG | XM\_014194431.1 | 1 | 1.96 |
|  | Reverse  | GCCTTCCAGTTCTTGTTCAGGTA |  |  |  |
| *hbae*  | Forward | CGAGCTGCACGCCTTCA | NM\_001140924.1 | 6 | 1.99 |
|  | Reverse  | GGATGTTGTGGTTGATGATCTTGA |  |  |  |

Efficiency = (10^(-1/slope)

**Table S2.** Pyrosequencing assays. Outline of assays used for DNA methylation analysis.

|  |  |  |  |
| --- | --- | --- | --- |
| **Assay** | **Direction** | **Sequence (5' -> 3')** | **Position CpG site** |
| 1 | Forward PCR Primer | TGGTGTGAGTTTAGATTTGTTATAGAA | 4, 5 |
|  | Reverse PCR Primer | CAATAATTCAACCTTTCTCACTCT |  |
|  | Sequencing Primer | ATAGAATAGAATAGAATTTTTGTGA |  |
| 2 | Forward PCR Primer | TGTAGAGTGAGAAAGGTTGAATTAT | 6, 7, 8 |
|  | Reverse PCR Primer | AAAACCAAACAATCAAATCCAAAACTAACC |  |
|  | Sequencing Primer | AGAAAGGTTGAATTATTGAAA |  |

**Table S3.** Spearman’s correlation between relative expression and methylation at promoter CpG sites in *egln3* (n=36).

|  |  |  |
| --- | --- | --- |
| **CpG site** | **p-value** | **Correlation**  |
| **6** | 0.002757 | -0.4974898 |
| **8** | 0.002189 | -0.4941665 |
| **6+8** | 0.000382 | -0.5600938 |
| **All sites (4-8)** | 0.08201 | -0.2937858 |

**Table S4.** Top twenty enriched gene ontology terms associated with differentially expressed genes comparing: A) 30% O2 990DD with 100% O2 925DD, B) 30% O2 990DD with 60% O2 925DD, C) 60% O2 925DD with 100% O2 925DD, and D) 30% O2 990DD with 30% O2 925DD.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **A** |  |  |  |  |
| **GO.ID** | **Term** | **Annotated** | **Significant** | **Expected** | **Fisher.classic** |
| GO:0003735 | structural constituent of ribosome | 231 | 177 | 53.91 | < 1e-30 |
| GO:0005198 | structural molecule activity | 564 | 246 | 131.62 | 2.30E-27 |
| GO:0016675 | oxidoreductase activity, acting on a hem... | 38 | 33 | 8.87 | 1.80E-16 |
| GO:0016676 | oxidoreductase activity, acting on a hem... | 38 | 33 | 8.87 | 1.80E-16 |
| GO:0015002 | heme-copper terminal oxidase activity | 38 | 33 | 8.87 | 1.80E-16 |
| GO:0004129 | cytochrome-c oxidase activity | 38 | 33 | 8.87 | 1.80E-16 |
| GO:0015078 | proton transmembrane transporter activit... | 138 | 73 | 32.21 | 4.50E-14 |
| GO:0009055 | electron transfer activity | 98 | 55 | 22.87 | 2.60E-12 |
| GO:0016491 | oxidoreductase activity | 911 | 276 | 212.6 | 4.10E-07 |
| GO:0016651 | oxidoreductase activity, acting on NAD(P... | 56 | 28 | 13.07 | 1.20E-05 |
| GO:0051920 | peroxiredoxin activity | 10 | 8 | 2.33 | 0.00025 |
| GO:0050136 | NADH dehydrogenase (quinone) activity | 17 | 11 | 3.97 | 0.00033 |
| GO:0003954 | NADH dehydrogenase activity | 17 | 11 | 3.97 | 0.00033 |
| GO:0016655 | oxidoreductase activity, acting on NAD(P... | 17 | 11 | 3.97 | 0.00033 |
| GO:0008137 | NADH dehydrogenase (ubiquinone) activity | 17 | 11 | 3.97 | 0.00033 |
| GO:0016706 | oxidoreductase activity, acting on paire... | 23 | 13 | 5.37 | 0.00061 |
| GO:0003899 | DNA-directed 5'-3' RNA polymerase activi... | 43 | 20 | 10.04 | 0.00071 |
| GO:0034062 | 5'-3' RNA polymerase activity | 43 | 20 | 10.04 | 0.00071 |
| GO:0097747 | RNA polymerase activity | 43 | 20 | 10.04 | 0.00071 |
| GO:0003723 | RNA binding | 635 | 181 | 148.19 | 0.00122 |
|  |  |  |  |  |  |
| **B** |  |  |  |  |
| **GO.ID** | **Term** | **Annotated** | **Significant** | **Expected** | **Fisher.classic** |
| GO:0005506 | iron ion binding | 60 | 26 | 6.15 | 2.10E-11 |
| GO:0048037 | cofactor binding | 123 | 39 | 12.61 | 2.10E-11 |
| GO:0140104 | molecular carrier activity | 22 | 14 | 2.25 | 1.60E-09 |
| GO:0005344 | oxygen carrier activity | 21 | 13 | 2.15 | 1.10E-08 |
| GO:0019825 | oxygen binding | 21 | 13 | 2.15 | 1.10E-08 |
| GO:0020037 | heme binding | 44 | 19 | 4.51 | 1.30E-08 |
| GO:0046906 | tetrapyrrole binding | 45 | 19 | 4.61 | 2.00E-08 |
| GO:0019842 | vitamin binding | 30 | 13 | 3.07 | 2.60E-06 |
| GO:0016829 | lyase activity | 41 | 14 | 4.2 | 2.90E-05 |
| GO:0005215 | transporter activity | 272 | 49 | 27.88 | 3.20E-05 |
| GO:0050662 | coenzyme binding | 59 | 17 | 6.05 | 5.00E-05 |
| GO:0031406 | carboxylic acid binding | 26 | 10 | 2.66 | 0.00013 |
| GO:0043177 | organic acid binding | 26 | 10 | 2.66 | 0.00013 |
| GO:0031418 | L-ascorbic acid binding | 10 | 6 | 1.02 | 0.00016 |
| GO:0022857 | transmembrane transporter activity | 216 | 39 | 22.14 | 0.00021 |
| GO:0016705 | oxidoreductase activity, acting on paire... | 38 | 12 | 3.89 | 0.00025 |
| GO:0048029 | monosaccharide binding | 11 | 6 | 1.13 | 0.00033 |
| GO:0016830 | carbon-carbon lyase activity | 15 | 7 | 1.54 | 0.00035 |
| GO:0016491 | oxidoreductase activity | 225 | 39 | 23.06 | 0.00051 |
| GO:0016675 | oxidoreductase activity, acting on a hem... | 14 | 6 | 1.43 | 0.00163 |
|  |  |  |  |  |  |
| **C** |  |  |  |  |
| **GO.ID** | **Term** | **Annotated** | **Significant** | **Expected** | **Fisher.classic** |
| GO:0003735 | structural constituent of ribosome | 217 | 71 | 39.4 | 1.50E-07 |
| GO:0016675 | oxidoreductase activity, acting on a hem... | 39 | 18 | 7.08 | 5.50E-05 |
| GO:0016676 | oxidoreductase activity, acting on a hem... | 39 | 18 | 7.08 | 5.50E-05 |
| GO:0015002 | heme-copper terminal oxidase activity | 39 | 18 | 7.08 | 5.50E-05 |
| GO:0004129 | cytochrome-c oxidase activity | 39 | 18 | 7.08 | 5.50E-05 |
| GO:0009055 | electron transfer activity | 91 | 30 | 16.52 | 0.00048 |
| GO:0140110 | transcription regulator activity | 743 | 170 | 134.9 | 0.00049 |
| GO:0003700 | DNA-binding transcription factor activit... | 695 | 158 | 126.19 | 0.00103 |
| GO:0005198 | structural molecule activity | 508 | 118 | 92.23 | 0.00197 |
| GO:0015370 | solute:sodium symporter activity | 58 | 20 | 10.53 | 0.0022 |
| GO:0016772 | transferase activity, transferring phosp... | 1277 | 270 | 231.86 | 0.0024 |
| GO:0015081 | sodium ion transmembrane transporter act... | 121 | 35 | 21.97 | 0.00243 |
| GO:0016779 | nucleotidyltransferase activity | 105 | 31 | 19.06 | 0.00296 |
| GO:0015291 | secondary active transmembrane transport... | 149 | 41 | 27.05 | 0.00304 |
| GO:0016859 | cis-trans isomerase activity | 41 | 15 | 7.44 | 0.00401 |
| GO:0005328 | neurotransmitter:sodium symporter activi... | 41 | 15 | 7.44 | 0.00401 |
| GO:0003755 | peptidyl-prolyl cis-trans isomerase acti... | 41 | 15 | 7.44 | 0.00401 |
| GO:0005326 | neurotransmitter transporter activity | 43 | 15 | 7.81 | 0.00668 |
| GO:0015293 | symporter activity | 76 | 23 | 13.8 | 0.00697 |
| GO:0015294 | solute:cation symporter activity | 76 | 23 | 13.8 | 0.00697 |
|  |  |  |  |  |  |
| **D** |  |  |  |  |
| **GO.ID** | **Term** | **Annotated** | **Significant** | **Expected** | **Fisher.classic** |
| GO:0003824 | catalytic activity | 7117 | 1947 | 1752.42 | 8.20E-13 |
| GO:0016853 | isomerase activity | 124 | 61 | 30.53 | 2.60E-09 |
| GO:0140097 | catalytic activity, acting on DNA | 101 | 52 | 24.87 | 5.20E-09 |
| GO:0048037 | cofactor binding | 527 | 186 | 129.76 | 1.70E-08 |
| GO:0016491 | oxidoreductase activity | 915 | 288 | 225.3 | 8.30E-07 |
| GO:0050662 | coenzyme binding | 293 | 108 | 72.15 | 1.70E-06 |
| GO:0008135 | translation factor activity, RNA binding | 142 | 59 | 34.96 | 6.40E-06 |
| GO:0019842 | vitamin binding | 125 | 51 | 30.78 | 4.70E-05 |
| GO:0003678 | DNA helicase activity | 33 | 19 | 8.13 | 5.40E-05 |
| GO:0016635 | oxidoreductase activity, acting on the C... | 7 | 7 | 1.72 | 5.50E-05 |
| GO:0003743 | translation initiation factor activity | 101 | 43 | 24.87 | 5.50E-05 |
| GO:0051540 | metal cluster binding | 68 | 31 | 16.74 | 0.00013 |
| GO:0051536 | iron-sulfur cluster binding | 68 | 31 | 16.74 | 0.00013 |
| GO:0008233 | peptidase activity | 805 | 243 | 198.22 | 0.00014 |
| GO:0016787 | hydrolase activity | 2941 | 801 | 724.16 | 0.00018 |
| GO:0070011 | peptidase activity, acting on L-amino ac... | 770 | 232 | 189.6 | 0.00022 |
| GO:0003917 | DNA topoisomerase type I activity | 6 | 6 | 1.48 | 0.00022 |
| GO:0008177 | succinate dehydrogenase (ubiquinone) act... | 6 | 6 | 1.48 | 0.00022 |
| GO:0046923 | ER retention sequence binding | 6 | 6 | 1.48 | 0.00022 |
| GO:0016645 | oxidoreductase activity, acting on the C... | 23 | 14 | 5.66 | 0.00024 |