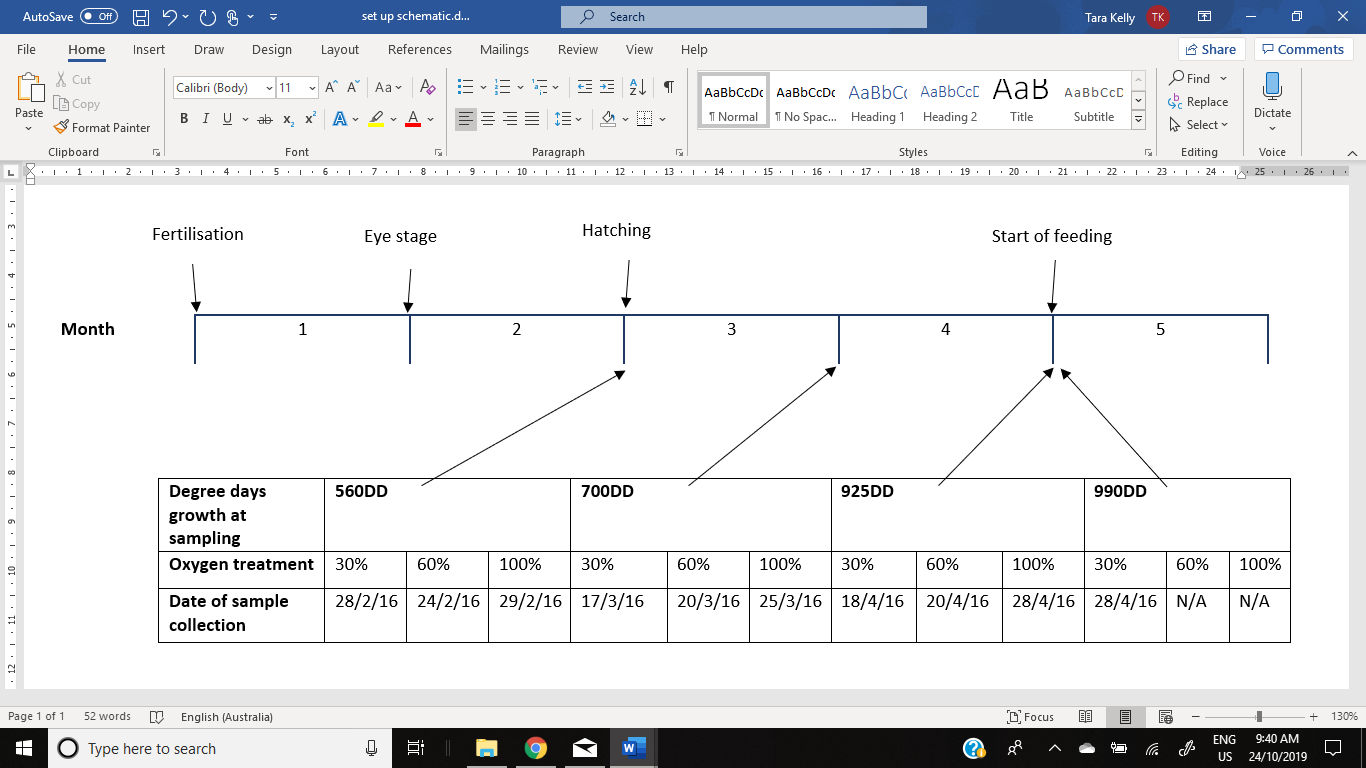
**Low oxygen stress during early development influences regulation of hypoxia-response genes in farmed Atlantic salmon (*Salmo salar*)**

**Tara Kelly**\***,co, Hanne Johnsen†,**‡,**co, Erik Burgerhout†,co, Helge Tveiten†,**§**, Tina Thesslund†, Øivind Andersen** **†,**\*\***, Nicholas Robinson†,**\*

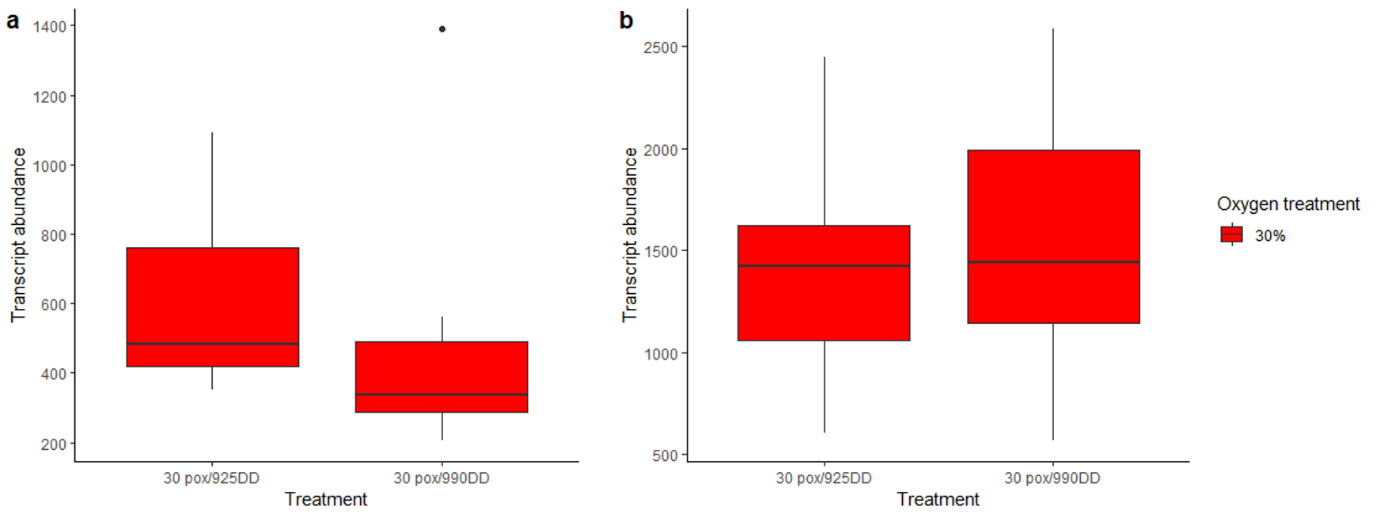
**Supplementary Materials**

**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 |