COMPARATIVE TRANSCRIPTOMICS ACROSS ATLANTIC SALMON *Salmo salar* SMOLTS, EUROPEAN SEABASS *Dicentrarchus labrax* AND RAINBOW TROUT *Oncorhynchus mykiss* UNDERGOING ACUTE STRESS INDUCED BY TRANSPORT

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Context

Unravelling new knowledge on molecular markers signalling acute stress in farmed fish is essential for better monitoring, improve animal welfare and health, and reduce mortality. This study aimed to evaluate physiologic and metabolic effects of acute stress induced by transport in three fish species: rainbow trout (Oncorhynchus mykiss), Atlantic salmon (Salmo salar), and European seabass (Dicentrarchus labrax).

Experimental setup

Trials were conducted at CIIMAR, Riasearch and University of Cádiz facilities, respectively.
After acclimatisation, fish were divided in one group of fish that was immediately sampled to serve as a non-stressed control. Additionally, two groups of fish were transferred each to three oxygen saturated containers and transported by a transport van for 2 (trout), 6 (salmon) or 4 (seabass) hours. Upon arrival, half were immediately sampled while the remaining fish were put in the original system and sampled at 24h post-arrival (n=18 per sampling time, except seabass n=12).

RNASeq

Total RNA was extracted from skin tissue and pooled (n=3-4 samples per treatment) for RNAseq on an Illumina sequencer at a commercial provider. Raw sequence reads were trimmed and filtered using fastp [1], to remove adapter sequences and low-quality reads. Transcripts were quantified using the pseudoalignment method implemented in kallisto [2]. Differentially expressed genes (DEGs) between the different treatments and the control samples at time 0 (i.e., before transport) were determined using DESEQ2 [3]. DEGs were further analysed using g:profiler [4] and ShinyGO [5].

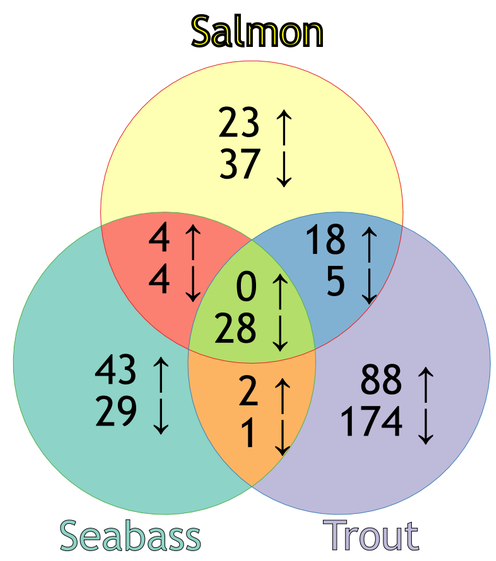

Figure 1 - Venn diagram indicating overlap among enriched GO terms related to up (↑) and down (↓) regulated genes in the three species: rainbow trout in blue, Atlantic salmon in yellow and European seabass in green. The two salmonids (trout and salmon) share more GO terms reflecting their shared evolutionary history, although most are exclusive to each species.

Results

All species showed hundreds of DEGs after being submitted to transport stress (Table 1 and Figure 2) with seabass showing the lowest number of DEGs and salmon with the highest. Preliminary analysis showed different gene pathways regulated during acute stress (Figure 1 and 3), with the two salmonids sharing more genes. In addition, the European seabass seems to be nearly recovered by 24h, while both salmonid species still showed many DEGs at 24h highlighting interspecific variability in stress recovery.

Table 1 - Number of DEGs in rainbow trout, Atlantic salmon and European seabass after transport, and after a 24h recory period. Second column refers to up regulated genes, and third colun to down-regulatede genes. A) Only genes with an adjusted p-value lower than 0.05 and absolute fold change greater than 2 were considered. B) Only genes with an adjusted p-value lower than 10^{-6} and absolute fold change greater than 2 were considered.

A

Species	Up (stressed / recovery)	Down
Rainbow trout	773 / 451	736 / 935
Atlantic salmon	978 / 1397	864 / 3465
European seabass	351 / 613	246 / 124

B

Species	Up (stressed / recovery)	Down
Rainbow trout	288 / 54	71 / 154
Atlantic salmon	462 / 654	209 / 1607
European seabass	175 / 0	115 / 0

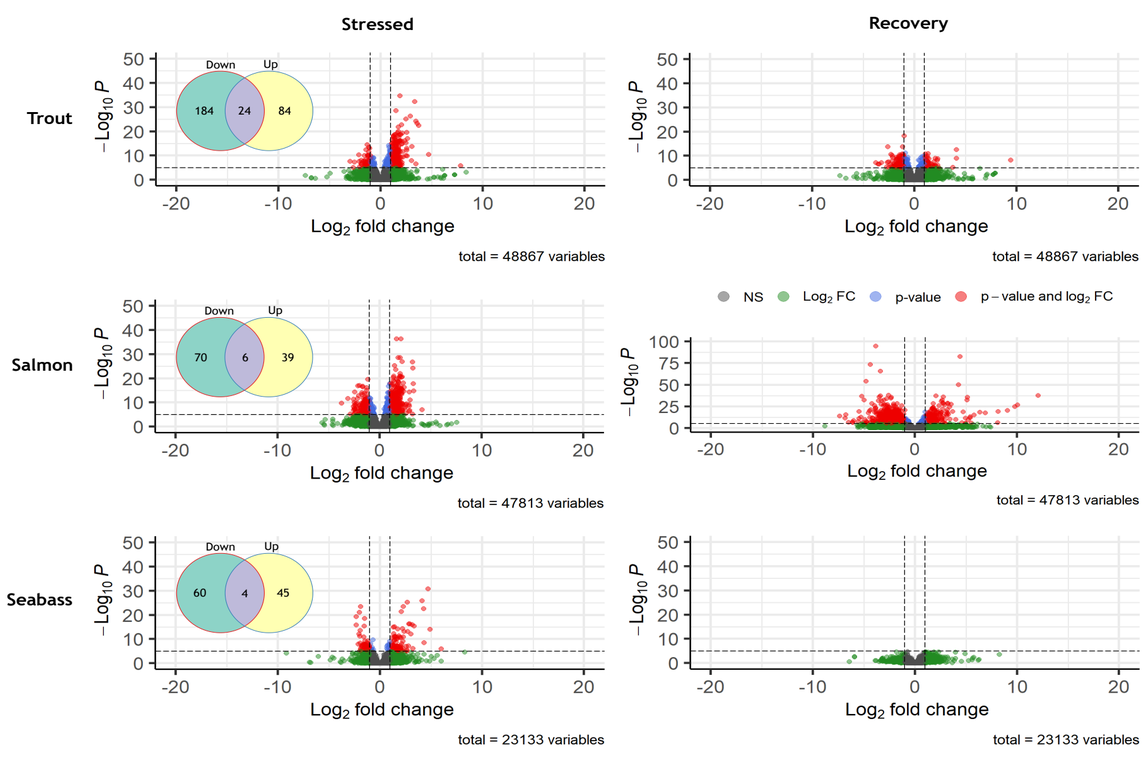

Figure 2 - Volcano plots of differentially expressed genes for stressed (left) and recovered (right) fish: rainbow trout (top), Atlantic salmon (middle), and European seabass (bottom). Highlighted are genes with a p value < 10^{-6} and absoulute value of fold change > 1. Venn diagrams represent number of enriched GO terms down and up-regulated and their overlap.

Pathway enrichment

GO term enrichment analysis revealed common or related terms both up and down regulated after a period of stress, namely genes related to circadian rythm and response to stimulus are highly represented in upregulated DEGs in all species, while terms related to nucleic acids regulation and transcription, and biosynthetic processes are enriched in down regulated DEGs. Species specific DEGs include cellular components in trout such as cornified envelope (up) and mitochondrial membrane (down); in salmon, transporter activity (up) and metabolic processes (down); and in seabass, negative regulation of processes (up) and development (down).

Figure 3 -Gene Ontology (GO) terms enrichment analysis for rainbow trout (top), Atlantic salmon (middle), and European seabass (bottom). Left charts represent terms linked to upregulated genes, while right charts indicate terms linked to downregulated genes. Highlighted terms represent related GO terms (see legend).

Conclusion

Our results highlight interspecific variability in reaction to stress, but also the similarity in some of the molecular pathways affected by stress, particularly in evolutionary more closely related species, such as salmon and trout. Further analysis with microRNAs and proteomics will give us a broad molecular signature for transport related stress with a potential for monitoring health in farmed fish.

References

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