***Background***

Arcartia tonsa is a marine copepod species with a global distribution and an important members of marine food chains. As oceans warm, these marine copepods serve as indicators of overall ecosystem health and plasticity. The health of A.tonsa has implications for the economic viability of many fisheries, making it important to study how environmental changes impact the species. One thousand A.tonsa individuals were collected from a coastal ecosystem in Groton, CT and raised for three generations prior to exposure to treatments. Copepods were experimentally evolved for 20 generations in ambient environments (line - AA), or high temperature high CO2 environments (line - HH) with 4 replicates each. Copepods were then reciprocally transplanted to AA or HH conditions for 3 generations, resulting in 4 distinct treatments (AAHH, AAAA, HHHH,HHAA) with 4 replicates. After one (F1) and three (F3) generations, 20 individuals were collected from each replicate for all treatments and used to extract RNA. RNA was sequenced using 150 bp paired end reads on the Illumina NovaSeq 6000 platform for RNAseq. Sequencing was conducted by Novogene who also handled the library preparation. The goal of the experiment was to understand how a) both evolved and transplant environment impact gene expression and b) time in transplant environment impacts gene expression. I hypothesize that copepods’ evolved environment (“line”) will have a greater impact on gene expression, but that this will degrade with time in the transplant environment (“environment”).

***Bioinformatics Pipline***

Sequence data was received as a .fastq file of reads and quality scores. Files were viewed in FastQC then trimmed using trimmomatic which paired and removed adapters and filtered sequences for minimum quality and length. Data was visualized with FastQC to confirm improvement, after which the transcriptome was assembled using Trinity. Reads were mapped to the transcriptome then transcripts were quantified using Salmon to generate a count file with counts for each transcript in all samples.

The count file and metadata were imported into R and counts per transcript were rounded then analyzed using the DESeq2 package. A DESeq object was created with the model: design = ~line + environment + line:environment to capture the impact of line, environment, and their interaction on gene expression. Genes with less than 10 reads per sample were filtered out to omit those with low expression. The DESeq model was ran using the DESeq function and was used to plot a principal component analysis. Two additional models were created to test for the impact of each model term on gene expression using the likelihood ratio test (LRT). Specifically, the models were design = ~ environment + line and design = ~ line + environment. These models were then used to determine which genes were impacted by line, environment, and their interaction. Genes IDs were extracted with the results function, ordered by adjusted pvalue and filtered to contain only significant genes. Overlap in significant genes was determined and plotted as a Venn diagram of genes unique and shared among model terms.

***Results***

The average number of reads per gene and sample were 11931 and 18166143 respectively for F1 and 11221 and 17727744 respectively for F3. When comparing PCAs, F3 shows more clustering by environment whereas the F1 shows more clustering by line (fig. 1). Further, in the F3 generation there is a strong impact of the environment in which copepods were evolved (line) on differential expressed genes (DEGs) (fig. 2a), with 1465 unique DEGs. This is nearly a this is nearly a 10-fold increase from the F1 generation (fig. 2b). There is also a nearly 2-fold increase from F1 to F3 in DEGs due environment (fig. 2a,b). Further, we see a strong decline in genes attributed to the interaction of line and environment from F1 to F3 (fig.2a,b) Importantly, 49, 54, and 105 DEGs are consistently attributed to the copepods’ line, environment, or their interaction from F1 and F3, however the polarity and magnitude of these DEGs vary between F1 and F3. The significant increases in gene expression both as a result of line and environment Chart, scatter chart

Description automatically generatedindicate an ability to adapt to new environments, but also a legacy effect of evolved environments.

**b.**

**a.**

Figure 1. Principe component analysis (PCA) for generations F1 and F3. Over 50% of variance is explained by PC1 + PC2

Diagram, venn diagram

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**Venn Diagram of DEGs attributed to each model term**

Figure 2. Venn diagram showing DEGs attributed to line, environment, or their interaction in the a) F1 and b) F3 generations

*A.tonsa* copepods appear capable of adapting to sudden warming events, as indicated by changes in DEGs attributed to environment from F1 to F3. Additionally, there is some legacy effect of line as indicate by the large number of line attributed DEGs in the F3 generation. The impact of the line on gene expression indicated that 20 generations of experimental evolution does significantly alter gene expression even after transplanting. Further as the model showed significant differences in gene expression between both line and environment, we know that HH environments do alter the physiology of copepods. Initally we see strong indication of interaction in the F1 generation, however interaction associated DEGs are less prevalent in F3. All these results combined could indicate adaptive capacity in the expression of some genes, while others are less flexible. It is apparent that time in a given environment has an impact on gene expression; in the future it would be useful to understand the expression levels in the generations leading up to transplant as well as in more generations post-transplant (e.g. up to F10). It would also be useful to understand how environment is impacting phenotypic traits of copepods like body size (per Rice et.al. 2014), to connect gene expression with the physical nature of the organism.