

Ocean acidification effects on metabolic gene expression in juvenile rockfish (*Sebastes* spp.)



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Introduction

Adverse effects of ocean acidification (OA) add to the growing list of anthropogenic disturbances already affecting fish stocks. Compared with invertebrates, fish were previously thought to be relatively tolerant to OA due to their higher capacity for acid-base regulation. However, recent work has shown negative impacts on fish physiology, e.g. lack of discrimination between olfactory cues in the clownfish (Munday et al., 2009).

Rockfish (*Sebastes* spp.) are an ecologically and economically important fisheries species, and are a valuable marine resource to the state of California (Lea et al., 1999). **We recently tested the effects of chronic low pH exposure (7.2, 7.5, 7.8, and control 8.0) on swimming physiology of juvenile copper (*Sebastes caurinus*) and blue (*S. mystinus*) rockfish and found that blue rockfish appear to be more tolerant to OA.** Copper rockfish had a significantly lower critical swimming speed and aerobic scope compared with controls, whereas blue rockfish exhibited no change. It is possible that juvenile blue rockfish are pre-adapted or acclimated to OA because of exposure to upwelling conditions during development.

Changes in gene expression may elucidate the underlying molecular mechanisms that led to reductions in swimming performance and aerobic scope in copper rockfish (e.g. a shift from aerobic to anaerobic metabolism). **Using next generation RNA sequencing technology (RNA-Seq), we are examining changes in gene expression in muscle tissue of copper and blue rockfish exposed to OA conditions.**

Here, we present the first step in this process: the construction and characterization of our *de novo* transcriptome assembly for rockfish, a requirement for RNA-Seq analyses with non-model species that have no reference genome.

Methods

Sample preparation:

- White muscle tissue was dissected from 31 juvenile copper rockfish exposed to four pH conditions for 4-5 months (7.2, 7.5, 7.8, and 8.0 pH units).
- Total RNA was extracted using a Qiagen RNeasy kit (Qiagen, Valencia, CA).
- cDNA libraries were constructed using the NEBNext Ultra Directional RNA Library Prep kit for Illumina.
- cDNA libraries were sequenced on an Illumina HiSeq machine (Illumina, Inc.) - 1 paired end and 1 single end lane with 7-8 individuals multiplexed per lane.

Transcriptome assembly & annotation:

- A copper rockfish *de novo* transcriptome assembly was constructed using Trinity software (Haas et al., 2013) using 15 individuals.
- Sequences were annotated against an NCBI non-redundant database for teleost fish and SwissProt to identify putative genes and categorize them using gene ontology or functional categories.

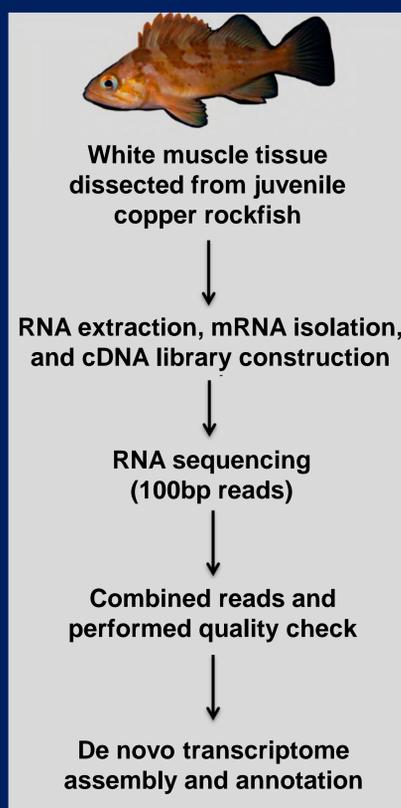


Figure 1. Sample prep and *de novo* assembly workflow.

Literature Cited

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Results

De novo Assembly Characterization:

- There are a total of 90,804 isoforms and 56,992 genes in our assembly (Table 1).
- Many genes have more than one isoform, with an average of 1.59 isoforms per gene (Fig. 2).
- An N50 value of 1,852 (Table 1) indicates that our assembly is well constructed and of good quality.
- N50 value is a standard statistic of transcript assembly used to measure the quality of the assembly and is a weighted mean of contig size.
- GC content is 48.24% (Table 1).

Table 1. Copper rockfish *de novo* transcriptome assembly and longest isoform per gene statistics.

Sequencing and assembly statistics	
Total number of isoforms	90,804
Total number of genes	56,992
Total number of bases	121,945,631
Minimum sequence length	201
Maximum sequence length	59,752
Median contig length	454
Average contig length	953.43
N10	5,064
N20	3,748
N30	2,964
N40	2,373
N50	1,852
Percent GC	48.24

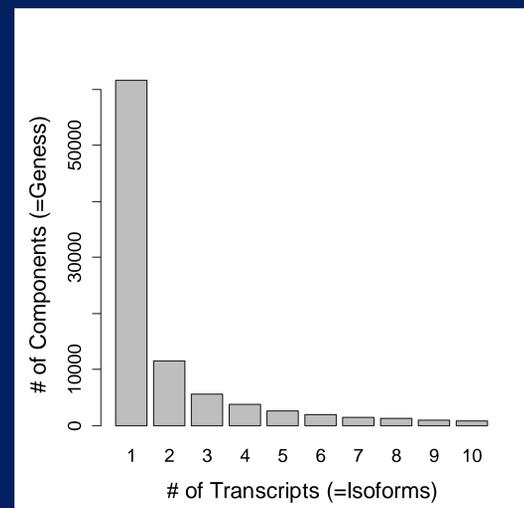


Figure 2. Histogram of number of isoforms per gene in the copper rockfish *de novo* transcriptome assembly.

Annotation Summary:

- 30,059 of the 90,804 contigs were successfully annotated, with a yield of 33.1% of contigs having one or more hits against the SwissProt BLAST database.
- We are currently BLASTing against 8 previously sequenced teleost fish.

Discussion and Future Work

Our *de novo* assembly and annotation produced a transcriptome assembly for copper rockfish containing 56,992 genes (Table 1), 33.1% of which possessed significant homology to a known protein product in the high quality manually annotated SwissProt database. Most genes had only one expressed isoform, but many had >10, matching previous work in vertebrates (e.g. Gonzalez-Porta et al., 2013).

Fish genomes contain ~26,000 protein-coding genes (zebrafish; Collins et al., 2012), yet our assembly is 1.5x larger than this. This may be an artifact of the assembly process (e.g. multiple short contigs may represent different portions of the same gene). Although our assembly size appears to be larger than expected, it is in line with a recent *de novo* assembly for black-faced blennies (Schunter et al., 2014).

Future Work:

- Sequences from juvenile copper rockfish exposed to ambient (8.0 pH) and OA treatments (7.8, 7.5, and 7.2 pH) will be mapped to this assembly and counted to determine which metabolic genes are differentially expressed among treatments.
- Our *de novo* transcriptome assembly can be used by other researchers interested in studying the effects of other environmental stressors (e.g. increased temperature, hypoxia) on rockfish gene expression.

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