Following diatom response to ocean acidification in mesocosm experiments with metatranscriptome sequencing indicates contrasting CO_2 responses in *Skeletonema* and *Thalassiosira* diatoms

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Background

- Fossil fuel combustion has raised atmospheric pCO_2 from ~277 ppm in 1750 to the present level of ~400 ppm[1, 2].
- Increased levels of dissolved CO_2 gas in seawater perturbs carbonate chemistry in the ocean, which results in a decreased buffering capacity, or ocean acidification (OA)[3].
- The ocean sequesters approximately 1/3 of anthropogenic CO_2 and has been the only net sink for CO_2 in the past 200 years[4, 5].
- Diatoms are responsible for ~40% of oceanic primary production based upon photosynthetic fixation of CO_2, which is aided by a carbon-concentrating mechanism (CCM), which uses bicarbonate transporters (BCTs) to take up HCO_3- and carbonic anhydrases (CAs) to interconvert HCO_3- and CO_2[6, 7, 8].
- There are several distinct classes of BCTs and CAs found in diatoms, including the substrate specific SLC4 and non specific SLC26 BCTs, as well as the α, β, δ, γ, ζ, and θ CAs[9, 10, 11, 12].
- The ability to regulate and conserve energetic costs associated with the CCM may influence diatom community composition in a higher CO_2 future
- This study used mesocosm CO_2 manipulation incubations to examine the diatom community response to changes in pCO_2.

Methods

- Seawater from Vineyard Sound, MA was collected in March of 2014, pre-filtered to remove large grazers (200 µm), and amended with nutrients.
- pCO_2 levels were manipulated to approximate pre-industrial conditions (< 215 ppm pCO_2), present day pCO_2 in the open ocean (330-390 ppm pCO_2), and future pCO_2 projections (>780 ppm pCO_2)[1, 2].
- Chlorophyll, nitrate and nitrite, phosphate, silicic acid, and pCO_2 levels (alkalinity, dissolved inorganic carbon, and pH) were measured and recorded throughout the experiment.
- After 20 days of incubation, biomass was collected on 3 µm pore-size polyester filter by gentle filtration, flash frozen in liquid nitrogen, and stored at -80 °C until DNA and RNA extraction.
- For amplicon sequencing from the extracted DNA, diatom-specific primers to amplify the V4 region of the 18S rDNA gene were modified to include Illumina MiSeq sequencing adapters[13, 14].
- Between 100,000-500,000 2x250 bp reads were sequenced per amplicon library and adapter sequences were trimmed from raw reads using CutAdapt[15]
- DADA2[16] was used to de-noise reads, merge read pairs, remove chimeras, and trim on length (less than 390 bp or greater than 410 bp[13]) and taxonomy was assigned using the PR2 DB[17].
- Metatranscriptomes were sequenced from RNA, with equal amounts of total RNA pooled from like-replicates pre library preparation (polyA mRNA enrichment).
- Approximately 30 million 2x100 bp reads were sequenced per metatranscriptome library.
- Reads were trimmed (Trimmomatic)[18] and mapped (BWA-MEM[19] and SAMtools[20] or DIAMOND[21]) to the MMETSP database[22], which was clustered at 98% ID per species in CD-HIT-EST[23, 24] .
- Differential gene expression was inferred separately for each species, using the raw counts in Analysis of Sequence Counts[25].

Metatranscriptome reads and amplicon ASVs dominated by *Skeletonema* and *Thalassiosira*

Figure 1

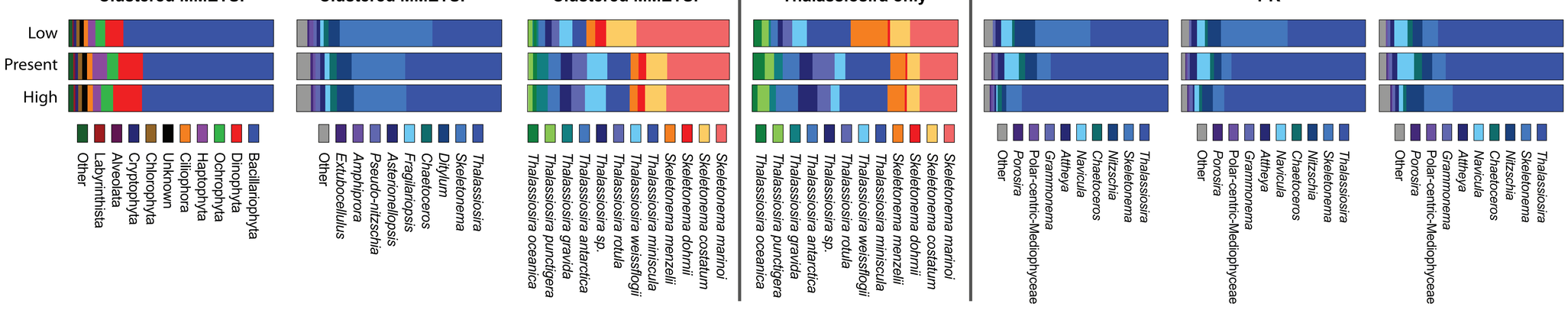
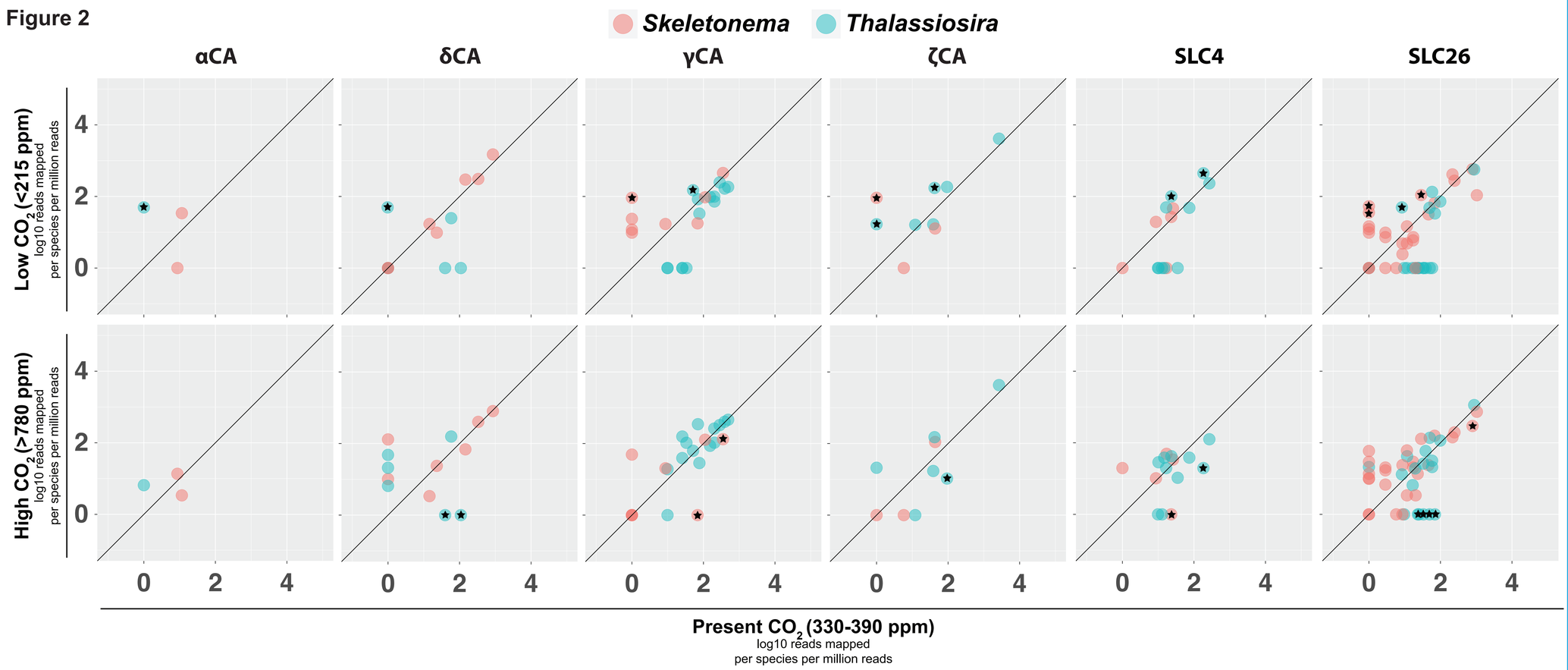


Figure 1: A series of bar graphs showing the taxonomic proportion of metatranscriptpome reads (MMETSP DB) and ASVs (PR2 DB). Metatranscriptome reads were mapped to proteins in the MMETSP DB using DIAMOND (--top 0 –more-sensitive –evalue .00001) and the top hits were binned and summed on the phyla level, which indicated that most of the reads were mapping to the Bacillariophyta (Figure 1A), mainly *Skeletonema* and *Thalassiosira* spp. (Figure 1B and 1C).
The presence of *Skeletonema* and *Thalassiosira* was confirmed by a stringent mapping step (BWA MEM -T 60 –aM –k 10; samtools view -q 60 -b) against *Skeletonema* and *Thalassiosira* nucleotide sequences from the clustered MMETSP DB (1D).
Diatom specific V4 amplicon sequencing, ASV assignment and binning by genus confirms that *Skeletonema* and *Thalassiosira* were present in these incubations (1E).
This is consistent with previous studies that found both of these diatoms in Massachusetts Bay during the month of March[26].

Skeletonema regulates the **γ**CAs, **ζ**CAs, and **SLC26** BCTs — ***Thalassiosira*** regulate **α**CAs, **δ**CAs, **ζ**CAs, **SLC4** BCTs, and **SLC26** BCTs

Figure 2



★ Indicates >= 0.95 posterior probability of a 2-fold change in high or low CO_2 relative to the present-day approximation.

Figure 2: A series of 1:1 lines showing the expression of CAs and BCTs from *Thalassiosira* and *Skeletonema*. Each point on the plot represents a transcript. Per-transcript expression values shown are normalized based on the total number of reads mapped over all transcripts in that species and then log10 transformed. Differential gene expression was inferred separately for each species, using the raw counts in Analysis of Sequence Counts[25]. Genes with at least 0.95 posterior probability of a 2-fold change were considered differentially expressed and are indicated by the black stars if they are up in low CO_2 or down in high CO_2, as would be expected for genes important for CCM function.

Conclusions

- The disparity in the CO_2-sensitivity of the δCAs might be explained by the observation that *Thalassiosira* and *Skeletonema* have distinct sub-types of δCAs[11].

- CAs use different metal cofactors: γ CAs can substitute zinc cofactors with iron, while the δ can substitute zinc with cobalt[27, 28].

- The expression of the BCTs suggest that these two diatoms use different mehanisms of inorganic carbon uptake: *Skeletonema* uses diffusive CO_2 uptake and/or non-specific SLC26-mediated HCO_3- transport, whereas *Thalassiosira* uses substrate specific SLC4 HCO_3- transport[9, 10].

- These diatoms' co-existence may be partially explained by their reliance on CAs that use different metal cofactors or their use of different inorganic carbon pools (CO_2 or HCO_3-).

- If *Skeletonema* is relying on diffusive CO_2 uptake rather than active HCO_3- transport, *Thalassiosira* diatoms' ability to CO_2-sensitively regulate the SLC4 transporters might afford it an energetic benefit and competitive advantage over *Skeletonema* in high CO_2.

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