



Transcriptomic Responses of Four Pelagophytes to Nutrient (N, P) and Light Stress

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Pelagophytes are abundant picophytoplankton within open ocean ecosystems and the causative algae of harmful brown tide blooms in estuaries. The physiological capabilities facilitating the ecological success of pelagophytes in these diverse ecosystems remains poorly understood. Here, we investigated the transcriptional response of two coastal pelagophytes (*Aureococcus anophagefferens* and *Aureoumbra lagunensis*) and two open ocean pelagophytes (*Pelagococcus subviridis* and *Pelagomonas calceolata*) to conditions commonly found within the marine ecosystems where they thrive: low concentrations of nitrogen (N), phosphorus (P), or light. OrthoMCL was used to generate a total of 62,653 orthologous groups (OGs) with only a small fraction of these OGs (2,776 or 4.4%) being shared among all four pelagophytes. Of the commonly shared OGs, 8% were significantly differentially abundant under low N, P, or light with the majority associated with energy and lipid metabolism. Distinct responses among pelagophytes included increased abundance of transcripts encoding phosphate transporters (*Aureococcus*) and transcripts encoding a pyrophosphatase (*Aureococcus* and *Pelagomonas*) under low P, the expression of a suite of organic nitrogen-degrading enzymes under low N (*Aureoumbra* and *Pelagomonas*), increased abundance of transcripts encoding flavodoxins relative to ferredoxins (*Pelagomonas*) and transcripts encoding lysophospholipase (*Pelagococcus*) under low light, and both increases and decreases in abundance of transcripts encoding selenoproteins in all pelagophytes except *Pelagococcus*. Collectively, this study provides new information on the expressed gene complement of these poorly characterized taxa and demonstrates that these pelagophytes possess a combination of shared and unique physiological features that likely facilitate their adaptation to distinct environmental conditions.

Keywords: comparative transcriptomes, pelagophytes, nutrient limitation, light limitation, *Aureococcus anophagefferens*, *Aureoumbra lagunensis*, *Pelagococcus subviridis*, *Pelagomonas calceolata*

INTRODUCTION

Pelagophytes are important members of ocean plankton communities as they are numerically abundant and ubiquitously distributed across marine ecosystems (Simon et al., 1994; Andersen et al., 1996; John et al., 2007). Of the known pelagophytes, four genera (*Aureococcus*, *Aureoumbra*, *Pelagomonas*, and *Pelagococcus*) have received increasing attention due to their ability to form

harmful algal blooms (*Aureococcus* and *Aureoumbra*; Gobler and Sunda, 2012) and their prevalence in open ocean ecosystems (Pelagomonas and Pelagococcus; Lewin et al., 1977; Dupont et al., 2014). Flow cytometric and electron microscopic analyses were initially used to demonstrate that the pelagophyte *Pelagomonas* sp. is one of the most abundant picoeukaryotes in the North Atlantic and Pacific subtropical oceans (Simon et al., 1994). More recent studies utilizing sorting flow cytometry and sequencing have revealed that *Pelagomonas calceolata* is ubiquitously distributed across world oceans (Cuvelier et al., 2010; Worden et al., 2012) and can be numerically dominant within the deep chlorophyll maximum (DCM) (Dimier et al., 2009; Li et al., 2013) where it is thought to be responsible for the majority of nitrate assimilation (Dupont et al., 2014). Less is known about the distribution of *Pelagococcus subviridis*, but it appears to be widely distributed in the ocean (Lewin et al., 1977) and carries a unique pigment profile of fucoxanthin and a 19'-butyloxyfucoxanthin with diadinoxanthin and diatoxanthin (Vesk and Jeffrey, 1987). In coastal ecosystems, the bloom-forming pelagophytes *Aureococcus anophagefferens* and *Aureoumbra lagunensis* are notorious for their ability to form brown tides that damage shellfisheries and seagrass meadows and disrupt energy flow to upper trophic levels (Gobler and Sunda, 2012).

While the prevailing environmental conditions in coastal and open ocean ecosystems can differ radically, some characteristics of turbid estuaries may mimic those of the subsurface chlorophyll maximum (e.g., low light, low-to-moderate nutrient levels, and abundance of organic matter, Cullen, 1982, 2015; Gobler et al., 2011). Brown tide blooms often occur during periods of low dissolved inorganic nitrogen (DIN) and phosphorus (DIP) (Gobler et al., 2004, 2013a). Both *Aureococcus* and *Aureoumbra* can assimilate dissolved organic nitrogen (DON) and phosphorus (DOP) for growth (Gobler et al., 2004; Muhlstein and Villareal, 2007), and the levels of DON and DOP can significantly decrease during the course of a brown tide, suggesting active degradation of compounds in these nutrient pools (Gobler et al., 2004, 2013a). Further, the ability of *Aureococcus* and *Aureoumbra* to utilize DON and DOP has been highlighted in previous genomic, transcriptomic, and proteomic studies (Berg et al., 2008; Gobler et al., 2011; Wurch et al., 2011b, 2013; Sun et al., 2012; Frischkorn et al., 2014). Lastly, *Pelagomonas*, *Aureococcus*, and *Aureoumbra* are all capable of maintaining maximal growth rates under low light conditions such as those found at the DCM or under high biomass bloom conditions (Dimier et al., 2009; Gobler et al., 2011, 2013a). While *Aureococcus* and *Aureoumbra* share a similar ecological niche in estuaries (Gobler and Sunda, 2012) and *Pelagococcus* and *Pelagomonas* reside in open ocean ecosystems (Lewin et al., 1977; Dupont et al., 2014), it appears *Aureococcus* is phylogenetically more similar to *Pelagomonas* than to *Aureoumbra*, based upon plastid genome comparisons (Deyoe et al., 1995; Worden et al., 2012), suggesting potential other shared genetic traits.

During the past two decades, genome sequencing has provided significant insight into the physiological potential of multiple species of phytoplankton (Palenik et al., 2003; Gobler et al., 2011; Lin et al., 2015). With continued development of

sequencing techniques and bioinformatic approaches, assessment of messenger RNA transcript abundances, also known as transcriptomes, have been used to bridge the information gap between genomic potential and phytoplankton response to ecosystem conditions (Keeling et al., 2014; Mock et al., 2016). Transcriptomics has revealed how this genomic potential in phytoplankton responds to a variety of nutrient regimes (Dyhrman et al., 2012; Bender et al., 2014; Frischkorn et al., 2014) and phytoplankton group-specific metabolic responses to varying environments (Alexander et al., 2015; Harke et al., 2015).

While *Aureococcus* is one of the few eukaryotic algae with a sequenced genome (CCMP1984, Gobler et al., 2011), there is relatively little known about gene content of other pelagophytes or how members of this group respond to changes in their environment. These changes are important as they shape not only the physiology of the organisms in isolation but also how they interact with competitors (Wurch et al., 2019) and predators (Gann et al., 2020a,b). Here, we compared the transcriptomic responses of two coastal pelagophytes, *Aureococcus anophagefferens* and *Aureoumbra lagunensis*, and two open ocean pelagophytes, *Pelagococcus subviridis* and *Pelagomonas calceolata*, to conditions commonly found within the ecosystems where they thrive: low levels of N, P, or light.

MATERIALS AND METHODS

Culture Maintenance

Two coastal pelagophytes, *Aureococcus anophagefferens* (CCMP1850 isolated from Great South Bay, NY, United States, 40.651°N, -73.152°W, in 1998) and *Aureoumbra lagunensis* (CCMP1510 isolated from Laguna Madre, TX, United States, 27.471°N, -97.320°W, in 1992), and two oceanic pelagophytes, *Pelagococcus subviridis* (CCMP1429 isolated from the subarctic Pacific, 49.917° N, -145.117°, isolation date unknown) and *Pelagomonas calceolata* (CCMP1214 isolated from the subtropical Pacific, 30.833°N, -136.833°, in 1973), were used for experiments. Cultures were grown in modified GSe medium (Tang and Gobler, 2009) made with boiled and then filter-sterilized (0.22 μm) North Atlantic Ocean seawater (40.290°N, -71.989°W) with a final salinity of 32.5 and amended with 60 μM NH₄Cl and 5 μM K₂HPO₄. All cultures were maintained with a final concentration of 1% antibiotic solution (primary stock was a mixture of 10,000 I.U. penicillin and 10,000 μg mL⁻¹ streptomycin; Mediatech Inc., United States) to minimize bacterial contamination. Cultures were maintained with a 14:10 h light:dark cycle, illuminated by a bank of fluorescent lights (T-12 medium bipin) providing 100 μmol photons m⁻² s⁻¹ for *Aureococcus* and *Aueoumbra* and 50 μmol photons m⁻² s⁻¹ for *Pelagomonas* and *Pelagococcus*. *Aureococcus*, *Aureoumbra*, and *Pelagomonas* were grown at 21 ± 1° C whereas *Pelagococcus*, isolated from the subarctic Pacific, was grown at 14 ± 1°C.

Experimental Design

To investigate the transcriptomic responses of these pelagophytes to conditions commonly present in their native ecosystems, cultures were incubated under starting conditions of either low

P ($1 \mu\text{M PO}_4^-$), low N ($30 \mu\text{M NH}_4^+$), or low light ($20 \mu\text{E m}^{-2} \text{ s}^{-1}$) to elicit stress responses in comparison with replete control cultures ($5 \mu\text{M PO}_4^-$, $60 \mu\text{M NH}_4^+$, $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Low P and low N cultures were incubated at the same light intensity ($100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for *Aureococcus* and *Aueoumbra* and $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for *Pelagomonas* and *Pelagococcus*) as control cultures, while low light cultures were incubated with the same nutrient concentration as control cultures ($60 \mu\text{M NH}_4^+$, $5 \mu\text{M PO}_4^-$). Triplicate 450 mL cultures were inoculated with $\sim 3.5 \times 10^5$ cells mL^{-1} harvested from exponentially growing maintenance cultures and monitored daily at 2 pm each day to avoid diel variation. *In vivo* fluorescence was measured on Turner Designs TD-700 fluorometer (EM filter of $> 665 \text{ nm}$ and EX filter of $340\text{--}500 \text{ nm}$; Sunnyvale, CA, United States). Maximum quantum efficiency of photosystem II (F_v/F_m) was estimated from *in vivo* (F_v) and DCMU (3,4-dichlorophenyl-1,1-dimethylurea)-enhanced *in vivo* fluorescence (F_m) of each replicate culture on the same fluorometer (EM filter of $410\text{--}600 \text{ nm}$ and EX filter of $300\text{--}400 \text{ nm}$; Parkhill et al., 2001). All readings were blank corrected using sterile culture media. The addition of DCMU blocks the electron transfer between photosystem I and photosystem II, maximizing the fluorescence (Parkhill et al., 2001). F_v/F_m has been utilized as an indicator of nutrient status with values of ~ 0.6 under nutrient replete conditions and decreasing to ~ 0.3 under nutrient deplete conditions (Parkhill et al., 2001; Harke and Gobler, 2013). Bulk alkaline phosphatase activity (APA) was measured daily on the TD-700 fluorometer (EM filter of $410\text{--}600 \text{ nm}$ and EX filter of $300\text{--}400 \text{ nm}$) using 4-methylumbelliferyl phosphate at $250 \mu\text{M}$ concentration (Hoppe, 1983). Lugol's iodine-preserved cells (Yoon, 2016) were quantified with a $50 \mu\text{m}$ aperture using a Beckman Multisizer 3 Coulter Counter (Fullerton, CA, United States). Growth rates were calculated for 3 days before harvest.

RNA Extraction and cDNA Sequencing

Physiological parameters were used to determine a harvesting time for each treatment. F_v/F_m and APA were utilized as indicators of nutrient stress (N or P, respectively), in low N and low P cultures while growth rates were utilized as an indicator of physiological status under low light. For each species, control cultures were harvested during exponential growth phase while low N treatments were harvested when F_v/F_m was reduced relative to the replete control. Low P treatments were harvested when APA was elevated relative to the replete control and low light treatments were harvested when cultures established a stable but slower growth rate relative to the replete control. In some cases, the differences in APA or F_v/F_m between treatments and the control were present but not significant at the $p < 0.05$ level. Harvest timing differed by treatment and species, occurring at the last timepoint plotted in **Figure 1**. At the time of harvest, cell pellets of each culture were collected by centrifuging the entire volume of each biological replicate ($n = 3$) in an IEC CL31R Multispeed centrifuge (Thermo Electron Corporation, Milford, MA, United States) for 8 min at $1300 \times g$ at culture growth temperatures stated above. After supernatant removal, the pellets

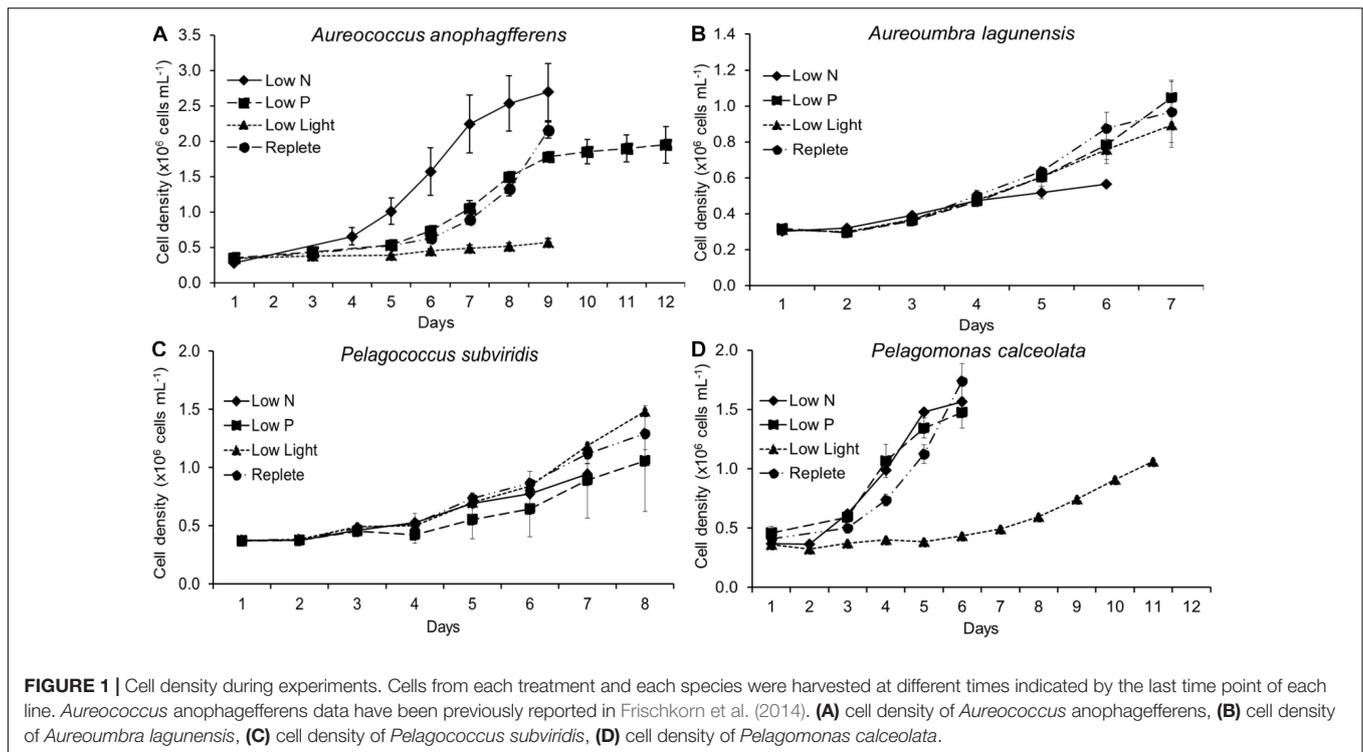
were transferred to 2 mL microcentrifuge tubes and centrifuged again under the same conditions and any remaining supernatant was removed. Concentrated cells were immediately frozen in liquid nitrogen and stored at -80°C . Total processing time from experimental conditions to flash freezing was $< 20 \text{ min}$.

Total RNA of each biological replicate ($n = 3$ per treatment) was extracted with an UltraCleanTM Plant RNA Isolation kit (Mo Bio Laboratories Inc., CA, United States) according to manufacturer's instructions. As sequencing of biological replicates was not available through the Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP; Keeling et al., 2014), replicates were then pooled to average across biological variability in transcriptional response between replicate flasks. Residual DNA was digested using an Ambion Turbo DNA-freeTM kit according to manufacturer's instructions and NanoDropTM 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, United States) was used to confirm no DNA contamination of RNA. RNA quantity was assessed with an Invitrogen Qubit Q32855 (Invitrogen, Carlsbad, CA, United States) and RNA quality was assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, United States). As a part of the MMETSP, library prep and sequencing was performed at the National Center for Genome Resources (NCGR) using an Illumina TruSeq RNA Sample Preparation Kit to generate libraries from $\sim 2 \mu\text{g}$ of RNA. Sequencing of 50 base pair paired-end reads from each library was performed on an Illumina HiSeq 2000, generating 2 Gbp of sequence per library. Sequence data are available on the iMicrobe repository¹ with the identification numbers MMETSP0914 – MMETSP0917 for *Aureococcus anophagefferens* (0914 = replete control, 0915 = low N, 0916 = low P, 0917 = low light), MMETSP0890 – MMETSP0893 for *Aureoumbra lagunensis* (0890 = replete control, 0891 = low N, 0892 = low P, 0893 = low light), MMETSP0882 – MMETSP0885 for *Pelagococcus subviridis* (0882 = replete control, 0883 = low N, 0884 = low P, 0885 = low light), and MMETSP0886 – MMETSP0889 for *Pelagomonas calceolata* (0886 = replete control, 0887 = low N, 0888 = low P, 0889 = low light).

De novo Assembly, Annotations, OrthoMCL Orthologous Grouping, and Expression Analysis

As part of the MMETSP, a combined assembly of all treatments fusing NCGR's pipeline (Batch Parallel Assembly version 2.0) was performed for each species. Low quality reads were trimmed using a String Graph Assembler with swinging average at Q15 (SGA; Simpson and Durbin, 2012). After reads shorter than 25 base pairs were discarded, preprocessed sequences were assembled with ABySS using 20 unique k-mer sizes between $k = 26$ and $k = 50$ (Simpson and Durbin, 2012). ABySS was run requiring a minimum k-mer coverage of 5, popping at > 0.9 branch identity, with the scaffolding flag disabled to avoid over reduction of divergent regions. The assembled reads (= unities) from all k-mer assemblies were combined and grouped at 98%

¹<http://data.imicrobe.us>



sequence identity using CD-Hit (Li and Godzik, 2006). Then, CAP3 was used to identify minimum 100 bp overlaps between the resultant contigs and assembled them into larger sequences (Huang and Madan, 1999), and the resulting contigs were paired-end scaffolded using ABySS (Simpson et al., 2009). GapCloser, a part of the SOAP *de novo* package, was used to close gaps created during scaffolding. Redundant sequences were removed using CD-Hit and contigs less than 150 bp were filtered out (Li and Godzik, 2006). RSEM (Li and Dewey, 2011) was used to generate read counts with default parameters.

Initial annotations were carried out by NCGR using ESTScan (Iseli et al., 1999) to predict coding sequences with a *Bacillariophyta* scoring matrix. Peptide predictions over 30 amino acids in length were annotated. Matches against the UniProtKB/Swiss-Prot database were generated using blastp (Altschul et al., 1990). Coding sequences were also characterized using HMMER3 (Zhang and Wood, 2003) against Pfam (Bateman et al., 2004), TIGRFAM (Haft et al., 2001), and SUPERFAMILY (Gough et al., 2001) databases. In addition, contigs from each species were mapped to the KEGG database (Kanehisa et al., 2006) using the KEGG Automatic Annotation Server² on complete or draft genome mode.

Predicted peptide sequences were clustered into orthologous groups (OGs) using OrthoMCL (Li et al., 2003) by running all versus all Blastp with default settings: a *P*-value cutoff of $1e^{-5}$ and an inflation value (-I) of 1.5, which provides the best granularity (i.e., cluster tightness; Li et al., 2003). OrthoMCL was

performed *via* virtual workbench in the Discovery Environment of the iPlant Collaborative³ (Stein, 2008; Oliver et al., 2013). Total OGs generated from OrthoMCL analysis included OGs with single-copy contigs. The differentially abundant OGs were divided into three groups: OGs that were shared among all four pelagophytes (here called “core OGs”), OGs that were found in two or three species (here called “shared OGs”), and OGs that were found in a single species (here called “unique OGs”). To characterize biochemical pathways, KEGG annotations from above were assessed for contigs within OGs. In addition, as the majority of OGs that are involved in N and P metabolism do not have KEGG annotations (Marchetti et al., 2012; Alexander et al., 2015), the initial MMETSP annotations from NCGR pipeline were also used. OGs associated with P metabolism were assigned to the KEGG energy metabolism module, which also includes N metabolism.

Sequence reads were aligned to contigs using Burrows-Wheeler Alignment tool (BWA; Li and Durbin, 2009). Read counts were summed at the OG level by each treatment and each species. An Analysis of Sequence Counts (ASC; Wu et al., 2010) was then performed on OGs from each species separately by comparing each treatment to the replete control. ASC estimates the prior distribution by modeling biological variability using the data itself. This empirical Bayesian method has been used in other marine phytoplankton studies without sequenced replicates (Dyhrman et al., 2012; Konotchick et al., 2013; Kopf et al., 2015; Harke et al., 2017). A statistically significant increase or decrease

²<http://genome.jp/tools/kaas/>

³<https://de.iplantcollaborative.org/de/>

in abundance relative to replete was determined using a fold-change greater than or equal to 2 and a posterior probability greater than 0.95 (Wu et al., 2010). Significant differences were represented as \log_2 transcripts per million (TPM). The differentially abundant OGs were divided into three groups: OGs that were shared among all four pelagophytes (here called “core OGs”), OGs that were found in two or three species (here called “shared OGs”), and OGs that were found in a single species (here called “unique OGs”). The core and shared OGs were used to assess the common responses among pelagophytes while the unique OGs were used to assess the response of each species to the experimental conditions. Core, shared, and unique differentially abundant OGs were displayed in Ratio-Average (RA) plots of taxa-specific differential gene expression in response to treatments using R.

Transcriptome Completeness Analysis Using BUSCO

For quantitative assessment of transcriptome completeness, Benchmarking Universal Single-Copy Orthologs⁴ (BUSCO v2.0) was performed using a eukaryotic lineage to classify matches. The covered matches are classified with BUSCO notations of complete, fragmented, and missing genes. Complete genes are genes when gene lengths are within two standard deviations of BUSCO groups mean length, fragmented genes are when genes are only partially recovered, and missing genes are when genes are not recovered (Simão et al., 2015).

Statistics

Differences in growth rates, F_v/F_m , and APA among cultures were assessed *via* One-Way Analysis of Variance (ANOVA) and *post hoc* Tukey tests using Sigma Plot 11.0 (Systat Software Inc., San Jose, CA, United States). Bray-Curtis dissimilarity analysis was performed using the ‘vegan’ version 2.5 package in RStudio⁵ on core and shared OGs with significantly different expression patterns among four pelagophytes to quantify the compositional dissimilarity across all treatments. For simplicity, significantly differentially expressed OGs will be referred to as differentially abundant henceforth.

RESULTS

Culture Growth in Different Treatments

The timing of cell harvest was set to assure replete or resource-stressed conditions were present for each culture (Figure 1). At the time of harvest, growth rates of nutrient-replete cultures (= control) were significantly higher than those of all three experimental conditions for all species ($p < 0.05$; One-Way ANOVA; Tukey test) except for *Aureoumbra*, for which growth rates in low P and low light were similar to the control and *Pelagococcus*, for which growth rates in low P were similar to control (Table 1). Photosynthetic efficiencies (F_v/F_m) were lower than that of the control in all species for low N treatments

but only significantly different in *Aureococcus* and *Pelagomonas* ($p < 0.05$; One-Way ANOVA; Tukey test; Table 1). APA in low P cultures was significantly higher than control cultures on the day of harvest for all species except for *Aureococcus* in which APA was below $0.001 \text{ nmol mL}^{-1} \text{ h}^{-1}$ in the replete condition ($p < 0.05$; One-Way ANOVA; Tukey test; Table 1).

OGs Comparison

A total of 62,653 OGs were generated from the 78,780 peptides predicted from contigs, comprising 8,482 OGs for *Aureoumbra*, 15,578 OGs for *Pelagomonas*, 17,033 OGs for *Pelagococcus*, and 21,560 OGs for *Aureococcus* (Figures 2A,B). OGs were divided into three groups: core OGs found in all species, shared OGs found in at least two species, and unique OGs found in only one species (Figures 2A,C). The core set of 2,776 OGs accounted for a relatively small proportion of the total OGs in each species, ranging from 13% in *Aureococcus* to 33% in *Aureoumbra*, while the proportions of shared and unique OGs were much higher (Figure 2C). Of the total OGs, 26,508 OGs were composed of single copy contigs and 36,145 OGs were composed of multiple copy contigs (Supplementary Figure S1). Among the significantly differentially abundant OGs (4,879), 60% were shared OGs while core and unique OGs were responsible for 14% and 25% of the DE OGs, respectively (Supplementary Table S1). Of the shared OGs, 65% were not assigned a KEGG pathway. Considering all of the core and unique OGs, 77% and 54% were assigned a function within central metabolism (Supplementary Table S1). For *Aureococcus*, *Aureoumbra*, and *Pelagomonas*, > 60% of BUSCOs were complete, while 7~11% were fragmented and < 30% was missing (Supplementary Figure S2). In contrast, *Pelagococcus* had the least completed transcriptome with 39% of BUSCOs complete and 40% of BUSCOs missing (Supplementary Figure S2). For core OGs, 32~48% were completed while more than 50% of core OGs were missing among four pelagophytes (Supplementary Figure S2). Full OrthoMCL and ASC results are reported in Supplementary Tables S2, S3.

Significant Changes in Relative Abundance of OGs Under Resource Stress

Raw read counts summed at the OG level were analyzed using ASC to identify differentially abundant OGs within each species and Bray-Curtis dissimilarity of OGs was used to assess compositional dissimilarity among four pelagophytes across treatments. Overall, pelagophytes responded to environmental stress differently. Response to low light was most different from responses to low N and low P among pelagophytes except for *Aureococcus*, which showed the most divergent response to low P and similar responses to low N and low light (Figure 3). Principal component analyses (PCA) of these responses (Supplementary Figure S3) highlighted that each pelagophyte had a species-specific signal that was greater than the specific responses to individual stressors.

Of the total OGs generated, 4,879 OGs were differentially abundant among treatments with 936 OGs significantly increased

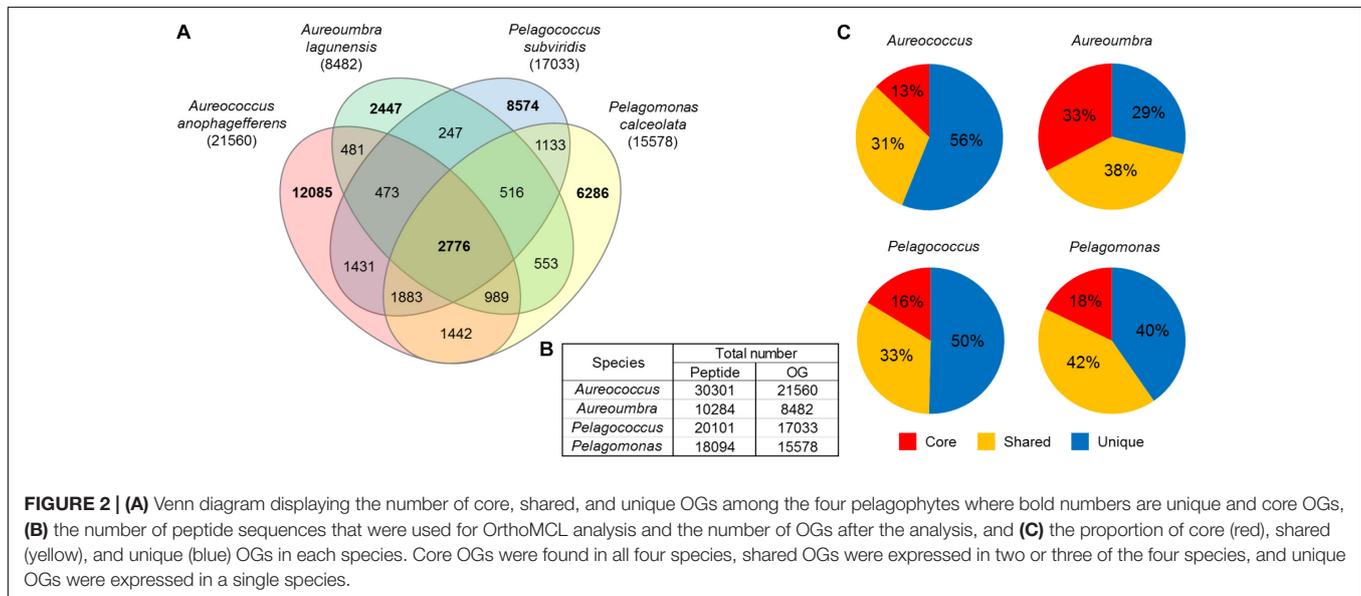
⁴<http://busco.ezlab.org/>

⁵<http://www.rstudio.com/>

TABLE 1 | Alkaline phosphatase activity, photosynthetic efficiency (F_v/F_m) and growth rate for 3 days at the exponential stage.

Species	Alkaline phosphatase activity ($\text{nmol mL}^{-1} \text{h}^{-1}$)		F_v/F_m				Growth rate (d^{-1})			
	Replete	Low P	Replete	Low N	Low P	Low Light	Replete	Low N	Low P	Low Light
<i>Aureococcus anophagefferens</i>	–	–	0.58 ± 0.02	$0.47 \pm 0.00^{**}$	0.51 ± 0.01	$0.52 \pm 0.01^*$	0.44 ± 0.01	$0.09 \pm 0.02^{**}$	$0.04 \pm 0.02^{**}$	$0.07 \pm 0.02^{**}$
<i>Aureobrya lagunensis</i>	0.07 ± 0.03	$0.20 \pm 0.05^{**}$	0.63 ± 0.02	0.57 ± 0.04	0.67 ± 0.04	0.61 ± 0.01	0.24 ± 0.03	$0.09 \pm 0.02^*$	0.27 ± 0.02	0.19 ± 0.05
<i>Pelagococcus subviridis</i>	0.08 ± 0.04	$0.15 \pm 0.03^{**}$	0.57 ± 0.04	0.52 ± 0.02	0.58 ± 0.03	0.59 ± 0.01	0.18 ± 0.03	$0.01 \pm 0.01^{**}$	0.13 ± 0.03	$0.03 \pm 0.02^{**}$
<i>Pelagomonas calceolata</i>	0.02 ± 0.00	$0.07 \pm 0.01^{**}$	0.54 ± 0.03	$0.48 \pm 0.02^*$	$0.35 \pm 0.02^{**}$	$0.57 \pm 0.01^*$	0.43 ± 0.01	$0.23 \pm 0.001^{**}$	$0.16 \pm 0.11^*$	$0.18 \pm 0.04^{**}$

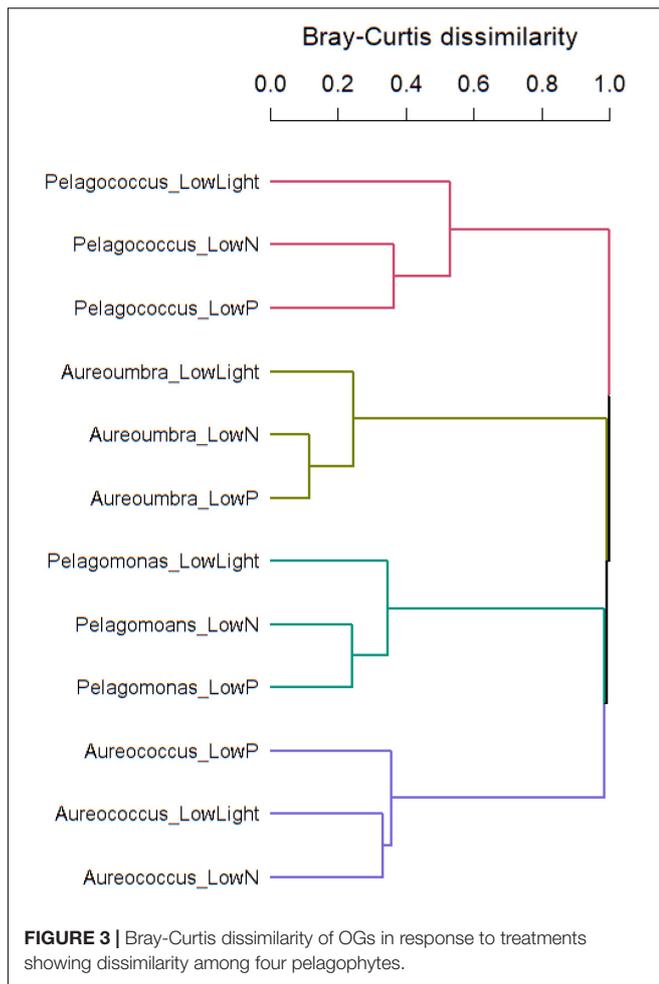
Asterisks indicate significant changes relative to the replete control ($*p < 0.05$ and $**p < 0.001$; One-Way ANOVA). APA in *Aureococcus* was not measured. *Aureococcus* data have been reported in Frischkorn et al. (2014).



and 3,943 OGs significantly decreased relative to the control (Figure 4). Among the total OGs that had increased relative transcript abundance compared to the replete condition, a disproportionate number (483 or 52%) came from *Pelagomonas* while 1,867 (or 47%) of the total OGs with significantly decreased relative transcript abundance the majority were from *Aureococcus* (Figure 4). The treatment eliciting the largest number of OGs with differentially abundant transcripts relative to replete varied among species; in low N for *Aureobrya* (882 OGs), low P for *Aureococcus* (983 OGs), and low light for *Pelagomonas* (838 OGs) and *Pelagococcus* (101 OGs; Figure 4). There was no significant response to low P for *Aureobrya* or *Pelagococcus* (Figure 4). The largest number of OGs with significantly increased relative abundance under low N occurred in *Aureobrya* with 155 OGs (18%) and *Pelagomonas* with 132 OGs (46%) under low P, and 213 OGs in low light (Figure 4) while the largest number of OGs with a significant decrease in relative abundance varied among species; low N for *Aureobrya* (727 OGs, 82%), low P for *Aureococcus* (910 OGs, 93%), and low light for *Pelagomonas* (625 OGs, 75%; Figure 4).

For all four pelagophytes, there was a significant correlation between the relative decrease in growth rate compared to the control in each treatment and the percentage of OGs that had significantly decreased abundance in that treatment (Supplementary Figure S4).

While most of the KEGG-annotated and differentially abundant OGs were from core and shared OGs (3,655 OGs), the fraction of the differentially abundant total core/shared KEGG-annotated OGs differed by species; 83% for *Aureococcus* (1,755 OGs) and *Pelagococcus* (137 OGs), 77% for *Pelagomonas* (1,295 OGs), and 50% for *Aureobrya* (468 OGs; Figure 5A). The fraction of unique KEGG-annotated OGs among the KEGG-annotated and differentially abundant OGs also differed by species; 50% for *Aureobrya* (460 OGs), 23% for *Pelagomonas* (381 OGs), and 17% for *Aureococcus* (355 OGs) and *Pelagococcus* (28 OGs; Figure 5A). The number of KEGG-annotated differentially abundant OGs per treatment mostly ranged from 59 to 187 in *Aureococcus*, *Aureobrya*, and *Pelagomonas*, with an overall smaller response observed in low-light *Aureobrya* and both low-light and low N *Pelagococcus* (13 – 16; Figure 5B).



Like patterns observed when considering all OGs, the treatment eliciting the largest number of KEGG-annotated differentially abundant OGs was low N for *Aureoumbra* (163), low P for *Aureococcus* (102), and low light for the open ocean pelagophytes, *Pelagomonas* (187) and *Pelagococcus* (16) (**Figure 5B**). Across species, a larger proportion of the differentially abundant OGs were attributed to energy metabolism, genetic information processing, carbohydrate and lipid metabolism, and transport systems (**Figure 5B**). The remaining KEGG-annotated OGs were dominated by six KEGG modules; genetic information processing (18%), energy metabolism (18%), carbohydrate and lipid metabolism (17%), nucleotide and amino acid metabolism (16%), transport system (15%) and cellular processes (14%) (**Figure 5B**).

Differentially Abundant OGs Related to Major Pathways

Differentially abundant OGs related to N metabolism included OGs encoding amino acid metabolism and N-relevant transporters as well as OGs associated with cleavage of organic N compounds (**Figure 6A**). Under N stress, OGs encoding arginase, cathepsin, urease, aspartate aminotransferase,

aminotransferase, and an aminohexanoate cleavage enzyme all significantly increased in abundance in *Pelagomonas* (**Figure 6A**). Other pelagophytes had far fewer differentially abundant OGs associated with N stress, although an OG encoding urease increased in *Aureoumbra* and a second OG encoding aminotransferase had decreased relative abundance in *Pelagomonas*. Under low P, OGs related to inorganic pyrophosphatase were significantly increased in abundance in *Aureococcus* and *Pelagomonas*. *Aureococcus* also had OGs encoding phosphate transporters that were significantly increased in abundance as well as an alkaline phosphatase OG that was significantly decreased in abundance (**Figure 6B**).

Low light was the treatment which elicited the largest number of differentially abundant OGs in *Pelagomonas*. Under low light, *Pelagomonas* had a flavodoxin OG with significantly increased relative abundance, five ferredoxin OGs with significantly lower relative abundance, and one ferredoxin OG with significantly higher relative abundance (**Figure 6C**). In contrast, *Aureococcus* had three ferredoxin OGs with higher relative abundance under low light (**Figure 6C**). The relative abundance of OGs encoding two lysophospholipases with one increased and another decreased under low light conditions for *Pelagococcus* as well as one decreased for *Pelagomonas* (**Figure 6C**). Lastly, an OG encoding a photosystem II protein had increased in abundance *Aureoumbra* in response to light stress (**Figure 6C**).

All treatments elicited a transcriptomic response in genes encoding selenocysteine-containing proteins, except for *Pelagococcus* (**Figure 7**). Methyltransferase OGs were a primary component of the differentially abundant selenocysteine-related OGs with a total number of 26 OGs that both decreased (18 OGs) and increased (8 OGs) in relative abundance across all treatments (**Figure 7**). OGs encoding thioredoxins also largely decreased in all treatments for all pelagophytes except *Aureococcus* and *Pelagomonas* (**Figure 7**). Lastly, OGs encoding glutaredoxin were observed to significantly increase and decrease in relative abundance for *Pelagomonas* and *Aureoumbra* (**Figure 7**).

Various types of OGs encoding transporters were differentially abundant in all four pelagophytes with the largest responses in *Aureococcus* (48 OGs) and *Pelagomonas* (43 OGs), with the majority exhibiting significantly decreased relative abundance (44 OGs for *Aureococcus* and 26 OGs for *Pelagomonas*) across treatments (**Figure 8**). OGs encoding phosphate transporters were found to be responsive in *Aureococcus* at low P with significant increased relative abundance (**Figures 6, 8**). *Aureococcus* also had more OGs encoding ion and metal transporters with significantly increased relative abundance than other pelagophytes (**Figure 8**). A complete list of annotations for OGs and contigs can be found in **Supplementary Table S4** and a list of differentially abundant OGs and their relevant information can be found in **Supplementary Table S1**.

DISCUSSION

In this study, transcriptional profiles were compared for four pelagophytes –*Aureococcus*, *Aureoumbra*, *Pelagococcus*, and *Pelagomonas* under low nutrient (N, P) and low light conditions

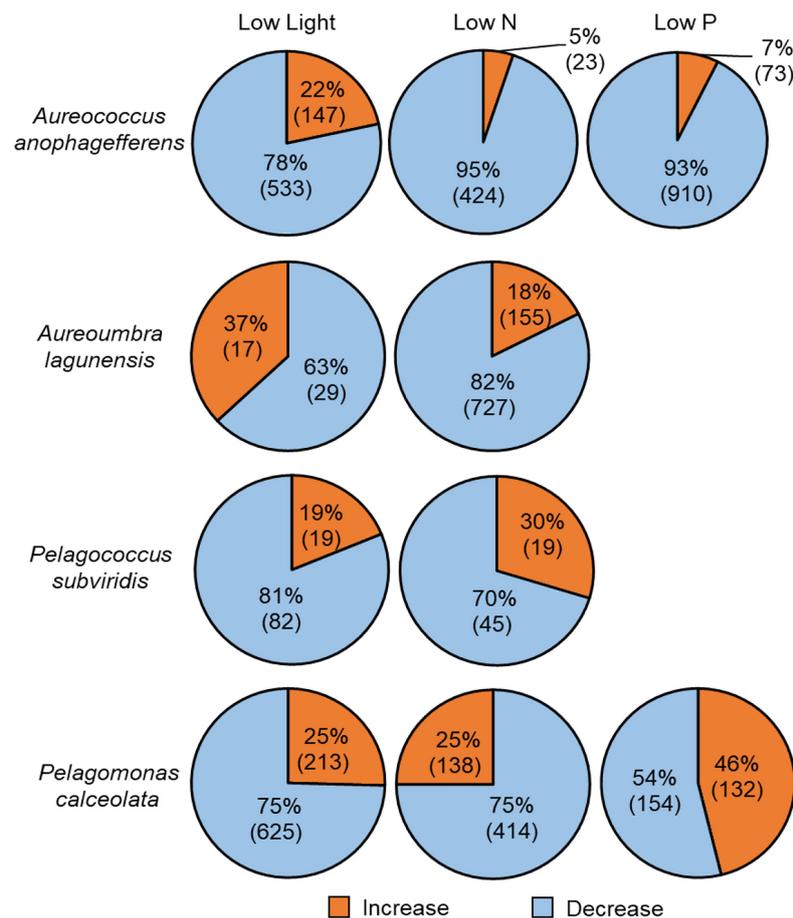


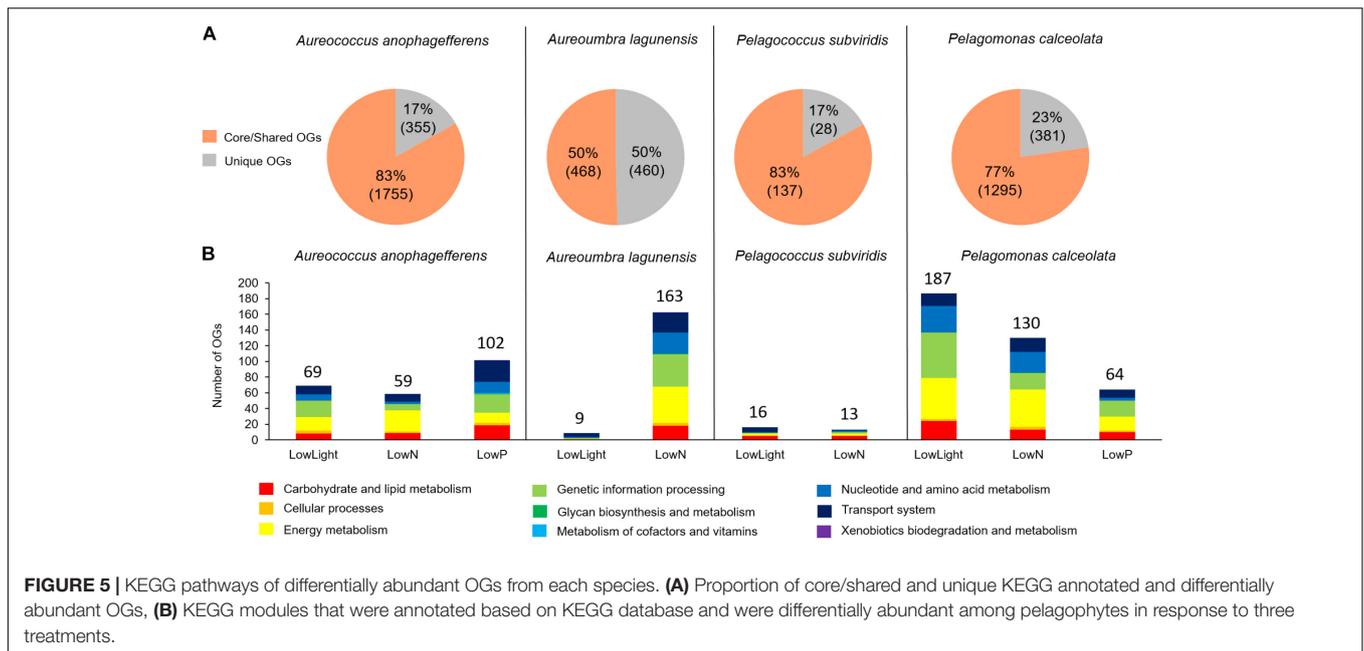
FIGURE 4 | Proportion of all differentially abundant OGs relative to control in each species across treatments and number in parenthesis indicates the number of differentially abundant OGs. Blue denotes significant decreases in relative abundance and orange denotes significant increases in relative abundance. No differentially abundant OGs were detected in the low P treatment for *Aureoumbra lagunensis* or *Pelagococcus subviridis*.

that are commonly found in the ecosystems where they thrive and compete with other phytoplankton. The comparative analysis of transcriptomes provided insight regarding functional traits that may be essential for pelagophytes in such conditions. Changing abundances of transcripts representing light-related, N-related, P-related, selenocysteine-related, and transport were a common response among pelagophytes under similar conditions, indicating these gene sets may be associated with pathways that permit pelagophytes to persist and, in some cases, dominate marine ecosystems.

Core and Shared Significantly Abundant OGs in Pelagophytes

Orthologous proteins have been used to assess functional traits shared among multiple phytoplankton strains (Alexander et al., 2020) or species (Bender et al., 2014; Di Dato et al., 2015; Simmons et al., 2016; Harke et al., 2017). In this study, two-thirds of KEGG-assigned, differentially abundant OGs were core (present in all species) or shared (present in two or three species) among all pelagophytes suggesting

similar pathway-level responses to nutrient and light stress (Figure 5A). Shared responses were also observed across these conditions for *Aureococcus* strains (Frischkorn et al., 2014). Of the four species considered, *Aureococcus* and *Pelagomonas* had the largest number of KEGG-assigned differentially abundant OGs, perhaps reflecting a broader response by these organisms to resource stress and their close phylogenetic relationship (Deyoe et al., 1995; Worden et al., 2012). Additionally, the intensity of some responses is also reflective of the intensity of resource stress within experimental treatments. While cell harvests were timed to occur when growth rates were slowed and physiological measurements were indicative of a resource stress response, the relative degree of stress differed among species examined and may have influenced the number and intensity of OG responses in each species. Lastly, in some cases, responses could be jointly attributed to minor variation in growth stage. While control cultures were always in exponential growth, some nutrient treatments were nearing or entering stationary phase (e.g., *Pelagococcus*), and slowed growth was associated with fewer differentially abundant OGs (Supplementary Figure S4).



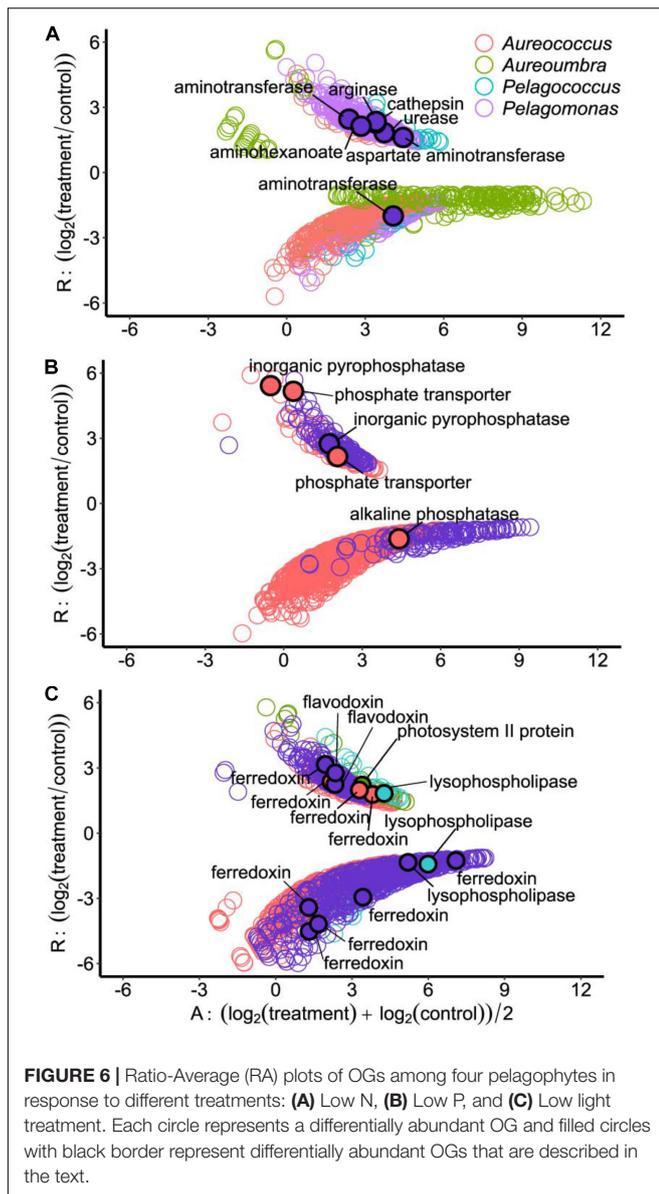
Of note, the total number of OGs (21,560) for *Aureococcus* in this study was nearly twice the number of genes (11,520) reported for its genome (Gobler et al., 2011). The transcriptomes presented here are from a different strain (CCMP1850) than that of the sequenced genome (CCMP1984) which may account for some differences; however, prior efforts mapping transcriptome contigs to the *Aureococcus* genome revealed that some of these contigs map to the same gene, suggesting presence of isoforms, mRNA fragments, or assembly errors (Li and Jiang, 2012; Frischkorn et al., 2014). Further, *Aureococcus* was recently shown to be diploid which could influence transcriptome assembly (Huff et al., 2016). Finally, recent sequencing efforts using Nanopore sequencing technology for multiple strains of *Aureococcus*, including the strain studies here suggest *Aureococcus* has ~25,000 predicted genes (John Archibald, Dalhousie University; pers. comm.), a number highly similar to the number of predicted OGs by the current study (21,560).

Differentially Abundant OGs Associated With Low N

In coastal ecosystems, the pelagophytes *Aureococcus* and *Aureoumbra* are able to proliferate when DIN levels are low by utilizing DON (Gobler et al., 2004, 2013a). Measurement of ^{15}N uptake rates per cell using sorting flow cytometry revealed that *Aureococcus* assimilates urea faster than co-occurring picoplankton (Kang et al., 2017). Further, the whole genome study of *Aureococcus* identified more genes associated with organic N metabolism compared to other phytoplankton (Gobler et al., 2011) and a proteomic study of *Aureoumbra* highlighted the potential utilization of regenerated, extracellular, organic nutrients (Sun et al., 2012). Responses to low N in the current study for these two species were less clear despite strong overall transcriptional and physiological responses to N stress. For example, only one *Aureoumbra* urease ortholog was significantly decreased in relative abundance under low N (**Supplementary**

Table S4) while no response was observed in *Aureococcus* among shared OGs. In prior studies with *Aureococcus*, this species displayed both increased and decreased transcription of urea transporters under N stress (Berg et al., 2008; Wurch et al., 2011b; Dong et al., 2014). This is either due to differing limiting conditions between studies, variations in growth stage relative to resource stress, or the conservative nature of ASC in identifying significant response as has been documented in other studies (Frischkorn et al., 2014; Harke et al., 2017).

With regards to the open ocean species, under low N conditions while *Pelagococcus* was lack of the significantly abundant transcripts associated with low N, *Pelagomonas* displayed a significantly increased abundance of transcripts for genes related to the cleavage of organic N compounds, suggesting that increased expression of these enzymes may be a survival strategy of *Pelagomonas* under low N conditions. *Pelagomonas* is commonly found in oligotrophic open oceans and is highly abundant at DCM (Simon et al., 1994; Andersen et al., 1996), especially at the deeper depth within the DCM (Latasa et al., 2017). In the DCM of the eastern North Pacific subtropical ocean, *Pelagomonas* is estimated to be responsible for most of the nitrate assimilation, expressing genes for nitrate transporters, carbamoyl-phosphate synthase, and a ferredoxin-dependent glutamate synthase in support of this process (Dupont et al., 2014). However, in low nitrate surface waters (Johnson et al., 2010), the transcriptional profile of *Pelagomonas* shifts to predominantly stress proteins and chaperones, suggesting nutrient stress (Dupont et al., 2014). The ability of *Pelagomonas* to catabolize DON compounds under DIN-limited conditions, as evidenced here by increased abundance of cathepsin, urease, and arginase transcripts (involved in the breakdown of proteins and the urea cycle), could allow this species to compete with co-existing picoplankton such as *Prochlorococcus*, which is known to assimilate DON at a high rate in open ocean ecosystems (Zubkov et al., 2003) where bacterially mediated nutrient



regeneration exceeds nitrate production (Azam et al., 1994). We hypothesize that *Pelagomonas* may vertically partition its physiological strategies with cells in low nitrate surface waters using DON and those within the DCM using nitrate. The ability to exploit organic N sources, such as that observed here in *Pelagomonas*, is consistent with modeling studies where enhanced nutrient recycling, in part, supports picoplankton growth in a warmer ocean (Flombaum et al., 2020).

Differentially Abundant OGs Associated With Low P

While phytoplankton in the open ocean are usually considered N-limited, phosphate is present at only nanomolar concentrations in surface oligotrophic oceans (Martiny et al., 2019), forcing many microbes to rely on DOP for growth

(Dyhrman et al., 2007). In contrast to the open ocean, DIP and DOP are relatively copious in shallow coastal waters due to fluxes from sediments (Boynton and Kemp, 1985; Sundby et al., 1992), rapid regeneration in the water column (Benitez-Nelson, 2000) and/or input from riverine discharge (Hellweger et al., 2016). However, previous studies have shown that in some cases brown tides can be P-limited and may significantly reduce DOP levels as the bloom peaks in biomass (Gobler et al., 2004; Wurch et al., 2019). Laboratory growth experiments (Muhlstein and Villareal, 2007) as well as genomic and transcriptomic analyses (Gobler et al., 2011; Wurch et al., 2011b; Frischkorn et al., 2014) have suggested that *Aureococcus* possesses the ability to utilize P from a variety of organic compounds. As such, both coastal and open ocean pelagophytes could experience P stress *in situ*. *Aureococcus* has a robust response to P-stress with the induction of genes and proteins related to phosphate transport, the metabolism or DOP and arsenate detoxification in both culture and field populations (Wurch et al., 2011b, 2019; Frischkorn et al., 2014). Further, genes involved P uptake and metabolism such as pyrophosphatase and phosphate transporters had increased relative abundance in *Pelagomonas* populations in the DCM relative to surface waters of the eastern North Pacific subtropical ocean (Dupont et al., 2014). Consistent with this literature, *Aureococcus* and *Pelagomonas* transcriptomes were characterized by altered transcript abundance of phosphate transporter, inorganic pyrophosphatases and or arsenate detoxification OGs. One *Aureococcus* phosphate transporter OG showed decreased relative abundance in low P while two others increased (**Supplementary Table S4**). The affinities of these differentially abundant phosphate transporters in *Aureococcus* may be varied, as differences in the expression of phosphate transporters have been described previously in the proteomic response of *Aureococcus* to P deficiency (Wurch et al., 2011a) and in studies of other marine phytoplankton (Chung et al., 2013; Monier et al., 2012) and higher plants (Mitsukawa et al., 1997). Additionally, the *Aureococcus* phosphate transporter OGs contained a single contig in each OG, suggesting that multiple OGs encoding phosphate transporters in *Aureococcus* more likely represent different types of transporters than copies of the same phosphate transporter, consistent with genomic observations (CCMP1984, Gobler et al., 2011).

Pyrophosphatases hydrolyze inorganic pyrophosphate to produce two molecules of phosphate (Chiou and Lin, 2011). In higher plants, high expression of inorganic pyrophosphatase genes is observed under P-deficient conditions (Hernández-Domínguez et al., 2012). Both coastal and open ocean pelagophytes (i.e., *Aureococcus* and *Pelagomonas*) displayed increased abundance of OGs encoding inorganic pyrophosphatase under low P (and for *Pelagomonas* also under low N; **Supplementary Table S4**), suggesting the use of intracellularly recycled phosphate originating from pyrophosphate may be a common molecular response that allows pelagophytes to survive extended periods of low P. Finally, *Pelagomonas* displayed an increased abundance of an arsenite methyltransferase OG under low P (**Supplementary Table S4**). Since inorganic phosphate transporters cannot discriminate between phosphate and arsenate, phytoplankton have been shown to accumulate high levels of arsenate when

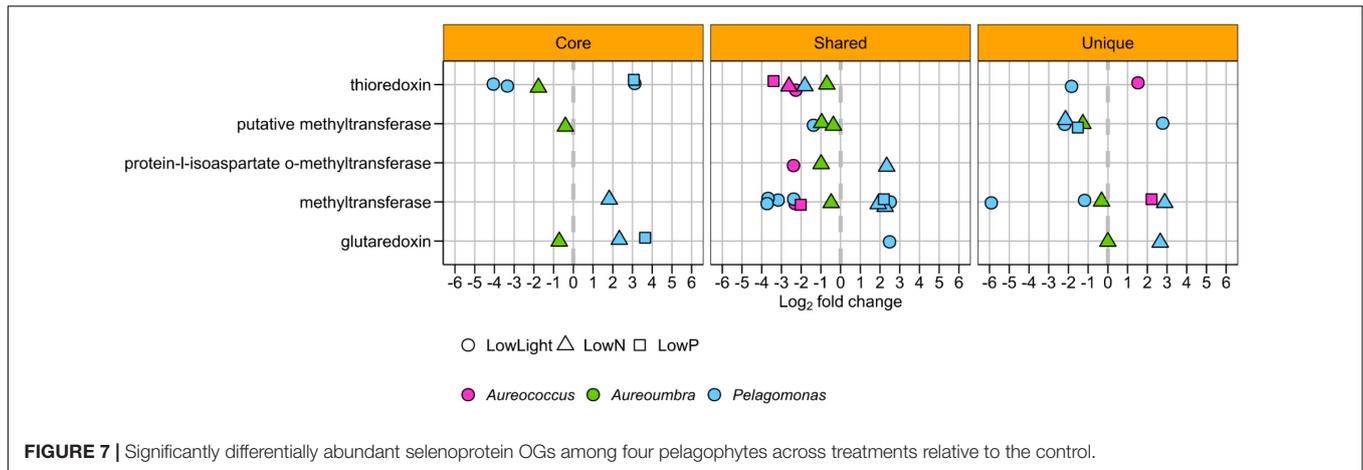


FIGURE 7 | Significantly differentially abundant selenoprotein OGs among four pelagophytes across treatments relative to the control.

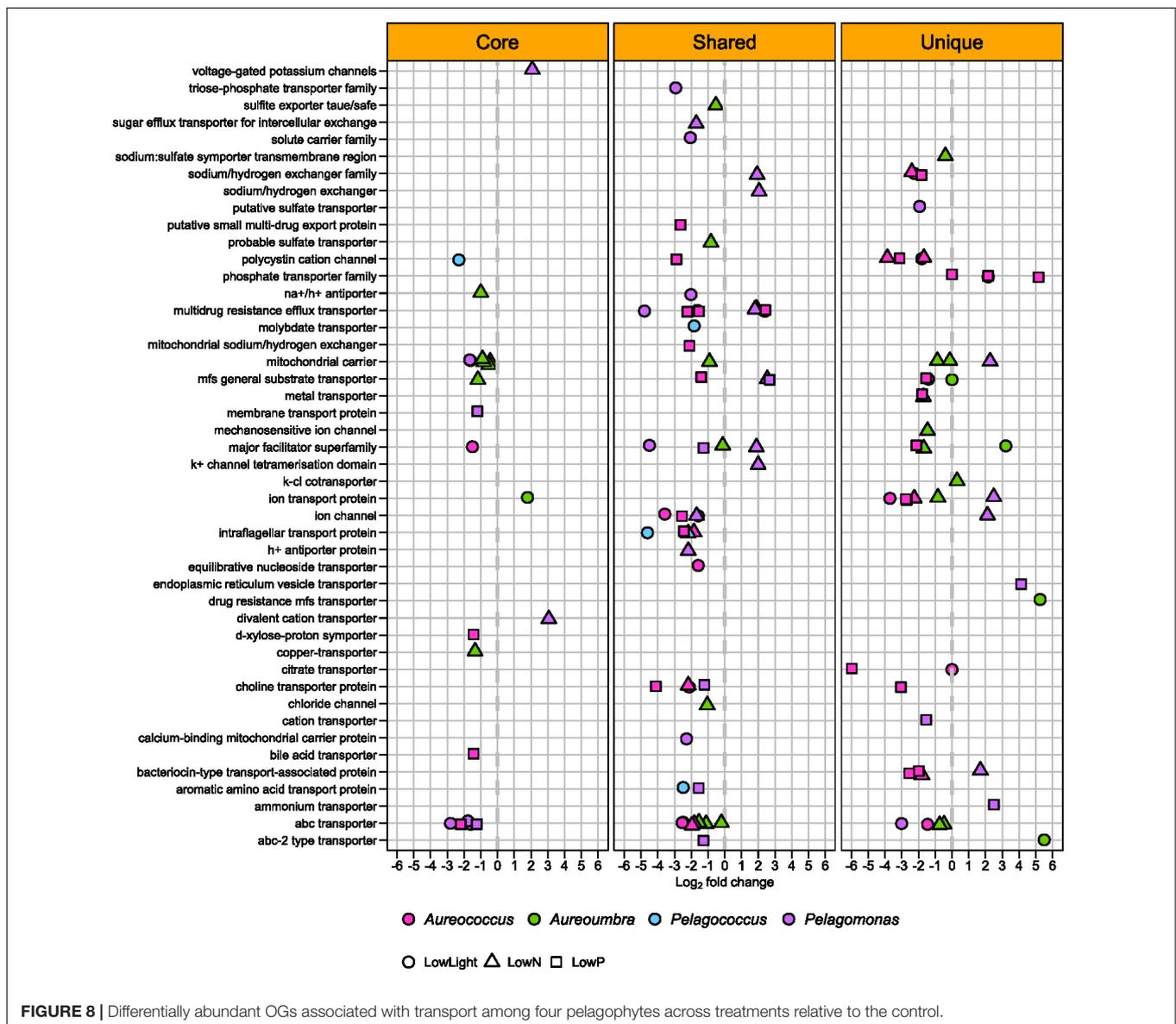


FIGURE 8 | Differentially abundant OGs associated with transport among four pelagophytes across treatments relative to the control.

P is limited (Budd and Craig, 1981; Hellweger et al., 2003; Silver and Phung, 2005). Phytoplankton often reduce arsenate to arsenite intracellularly and use arsenite methyltransferase to make dimethylarsinite, which has lower cellular toxicity than arsenate or arsenite (Hellweger et al., 2003). The P modulation of transcripts for an arsenite translocating ATPase was previously reported in both *Aureococcus* culture and field populations (Wurch et al., 2019), consistent with the P modulation of arsenate detoxification in pelagophytes.

In contrast to *Aureococcus* and *Pelagomonas*, transcriptional responses to low P in *Aureoumbra* and *Pelagococcus* were muted. The P-stress in *Aureoumbra* and *Pelagococcus* might not have been sufficient enough to induce significant differences expression of all genes involved in P stress compared to replete cultures. *Aureoumbra* has an extremely high N:P ratio (> 100) and forms blooms within high salinity, low P lagoon, suggesting that *Aureoumbra* has a high tolerance to P-limitation (Liu et al., 2001). Unlike other pelagophytes, physiological characteristics of *Pelagococcus* have been rarely investigated but given the responses here, it is assumed that *Pelagococcus* is also has a high tolerance to P-limitation.

Differentially Abundant OGs Associated With Photosynthesis and Lipid Metabolism

While all experimental conditions altered the expression of OGs related to lipid metabolism (e.g., lysophospholipase) and photosynthesis (e.g., flavodoxin and ferredoxin) in the four pelagophytes (**Supplementary Table S4**), only the low light condition had significantly increased expression of these OGs: lysophospholipase in *Pelagococcus*, ferredoxin in *Aureococcus*, and ferredoxin and flavodoxin in *Pelagomonas*. Lysophospholipases are located in cell membranes and cleave lysophospholipids to liberate fatty acids that are then metabolized or used in membrane restructuring (Pride et al., 2013). For most of these pelagophytes, the OGs decreased in abundance when growth rate was limited, but *Pelagococcus* showed an increase in expression of one lysophospholipase OG under low light suggesting this pelagophyte may more actively rearrange and recycle cell membranes compared to other pelagophytes in response to low light conditions. While the biogeographic distribution of *Pelagococcus* is poorly described, like *Pelagomonas*, this alga may also specialize at the DCM (Dupont et al., 2014) where acclimation to low light may be an important survival strategy.

Ferredoxin delivers electrons generated *via* photosynthesis to metabolic processes (Hase et al., 2006) and can be replaced with the isofunctional flavodoxin which, unlike ferredoxin, does not require iron (Fe) as a co-factor (Sancho, 2006). Low light induced an increase in the number of transcripts for OGs encoding ferredoxin in *Aureococcus* whereas *Pelagomonas* displayed a significant increase in abundance for flavodoxin OGs and significant decreases in abundance for 5 of 6 ferredoxin OGs. *Pelagomonas* also showed increased abundance of flavodoxin in low N and low P, along with changes in ferredoxin transcript levels in low N (**Supplementary Table S4**). Iron (Fe) is typically more limited in the open ocean (albeit

particularly in surface waters) than coastal waters (Boyd and Ellwood, 2010). Increased abundance of enzymes associated with photosynthesis during low light adaptation, including ferredoxin, creates a large Fe demand (Hase et al., 2006). As *Aureococcus* commonly occurs in coastal waters and therefore is exposed to high levels of Fe (Gobler et al., 2002), it may never be Fe-limited, and thus likely relies exclusively on ferredoxin for photosynthesis. The decline in both flavodoxin and ferredoxin OGs in low N for *Aureoumbra* suggests a different response in this organism, perhaps a general decline in photosynthesis as indicated by a decline in photosystem II protein-coding gene transcripts in low N (**Supplementary Table S4**). In contrast, *Pelagomonas*, whose pelagic habitats are low in Fe, may increase the abundance of flavodoxin under low light to assist its dominance in the DCM while coping with the potential co-stress of Fe and light.

Differentially Abundant OGs Associated With the Selenoproteome

Selenium is an essential trace element, being incorporated into selenocysteine (Sec) proteins during translation, including the oxidoreductases formate dehydrogenase H, glutathione peroxidase, methionine sulfoxide reductase, and thioredoxin (Kim et al., 2015). Selenocystein-related proteins are often involved in regulating cellular redox homeostasis (Stadtman, 1996; Lobanov et al., 2009), playing this role in all three domains of life (Hawkes and Alkan, 2010). *Aureococcus* possesses the largest and the most diverse selenoproteome identified to date (Gobler et al., 2013b) and many selenocysteine-related OGs were differentially abundant by all of the pelagophytes except for *Pelagococcus* during this study (**Figure 7**). While all pelagophytes, except for *Pelagococcus*, had thioredoxin OGs which decreased in relative abundance under resource-stressed conditions, *Aureococcus* and *Pelagomonas* also had thioredoxin OGs which increased in relative abundance under low light and low P and decreased in low N (**Figure 7**), potentially indicating these proteins were important for combating cellular stress under these conditions (Stadtman, 1996; Lobanov et al., 2009). While the regulation and role of the selenoproteome in these pelagophytes requires additional study to fully resolve, here we highlight transcripts that were responsive to light and nutrients, thus highlighting targets for further work.

Differentially Abundant OGs Associated With Ion Transport

While OGs coding for various types of transporters were significantly increased or decreased in relative abundance in all four pelagophytes under all three stresses, the largest response was found in *Aureococcus* (48 OGs out of 106 OGs), which displayed decreased relative abundance in transcripts for many transporters (42 OGs out of 106 OGs) in all treatments. *Aureococcus* is known to possess more genes coding for molybdenum, copper, and nickel-containing enzymes and other metal and ion transporters compared to competing algae, a trait permitting *Aureococcus* to access abundant metals in coastal environments to form high biomass blooms (Gobler et al., 2011). The lowered relative transcript abundance of all of these

transporters in *Aureococcus* in all treatments is likely related to their slowed growth and thus lowered requirement for metals and ions as well as the need to allocate energy to other, more vital cellular processes. In contrast, the open ocean alga, *Pelagomonas*, had a more balanced change in transporters with increased relative abundance in transcripts for 17 OGs and decreased relative abundance in transcripts for 26 OGs, perhaps reflecting a switch from low to high affinity transporters that permit persistence in a low resource environment (Levy et al., 2011).

CONCLUSION

In summary, nutrient and light stress triggered significant changes in abundance patterns for OGs encoding genes associated with energy and lipid metabolism among four pelagophytes. Specific OGs that were differentially abundant under low N, low P, and low light included genes associated with cleavage of organic N compounds, intracellular P recycling, lipid hydrolysis, electron transport, and redox homeostasis. Collectively, this study reveals core, shared and unique aspects of both the coastal pelagophyte and the open ocean pelagophyte transcriptomes and how they are uniquely modified in response to stress. Moving forward, these transcriptomes will serve as a critical reference database for further evaluating how pelagophytes thrive in such a diversity of environments.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

CG, MH, and DB contributed to conception and design of the study. MH performed the experiments. YK and MH organized the database and performed the statistical analysis. YK, MH, and DB developed and refined methods. CG and YK wrote the first draft of the manuscript. All authors contributed to manuscript preparation, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.636699/full#supplementary-material>

Supplementary Figure 1 | The number of OGs that have single contigs or multiple contigs present within the total OGs.

Supplementary Figure 2 | BUSCO analysis comparison of four pelagophyte transcriptomes for quantitative assessment of assemblies. **(A)** Completeness of four pelagophytes transcriptomes (Total). **(B)** Completeness of core OGs. Complete indicates contigs when contig lengths are within standard deviations of BUSCO groups mean length, fragmented indicates contigs when contigs are only partially recovered, and missing indicates contigs when contigs are not recovered.

Supplementary Figure 3 | Principal component analysis (PCA) of OGs. AaLl, *Aureococcus anophagefferens* in low light; AaLn, *Aureococcus anophagefferens* in low N; AaLp, *Aureococcus anophagefferens* in low P; ALLl, *Aureoumbra lagunensis* in low light; ALLn, *Aureoumbra lagunensis* in low N; ALLp, *Aureoumbra lagunensis* in low P; PsLl, *Pelagococcus subviridis* in low light; PsLn, *Pelagococcus subviridis* in low N; PsLp, *Pelagococcus subviridis* in low P; PcLl, *Pelagomonas calceolata* in low light; PcLn, *Pelagomonas calceolata* in low N; PcLp, *Pelagomonas calceolata* in low P.

Supplementary Figure 4 | Regression plot depicting the significant relationship ($p < 0.01$) between the relative difference in growth rate for each treatment relative to the control and the percentage of OG with decreased abundance in each treatment for *Aureococcus anophagefferens* (Aa), *Aureoumbra lagunensis* (Al), *Pelagococcus subviridis* (Ps), and *Pelagomonas calceolata* (Pc).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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