Identification of unique microbiomes associated with harmful algal blooms caused by *Alexandrium fundyense* and *Dinophysis acuminata*

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Abstract: Biotic interactions dominate plankton communities, yet the microbial consortia associated with harmful algal blooms (HABs) have not been well-described. Here, high-throughput amplicon sequencing of ribosomal genes was used to quantify the dynamics of bacterial (16S) and phytoplankton assemblages (18S) associated with blooms and cultures of two harmful algae, *Alexandrium fundyense* and *Dinophysis acuminata*. Experiments were performed to assess changes in natural bacterial and phytoplankton communities in response to the filtrate from cultures of these two harmful algae. Analysis of prokaryotic sequences from ecosystems, experiments, and cultures revealed statistically unique bacterial associations with each HAB. The dinoflagellate, *Alexandrium*, was strongly associated with multiple genera of Flavobacteria including *Owenweeksia* spp., *Maribacter* spp., and individuals within the NS5 marine group. While Flavobacteria also dominated *Dinophysis*-associated communities, the relative abundance of Alteromonadales bacteria strongly co-varied with *Dinophysis* abundances during blooms and *Ulvibacter* spp. (Flavobacteriales) and *Arenicella* spp. (Gammaproteobacteria) were associated with cells in culture. Eukaryotic sequencing facilitated the discovery of the endosymbiotic, parasitic dinoflagellate, *Amoebophrya* spp., that had not been regionally described but represented up to 17% of sequences during *Alexandrium* blooms. The presence of *Alexandrium* in field samples and in experiments significantly altered the relative abundances of bacterial and phytoplankton by both suppressing and promoting different taxa, while this effect was weaker in *Dinophysis*. Experiments specifically revealed a negative feedback loop during blooms whereby *Alexandrium* filtrate promoted the abundance of the parasite, *Amoebophrya* spp. Collectively, this study demonstrates that HABs formed by *Alexandrium* and *Dinophysis* harbor unique prokaryotic and eukaryotic microbiomes that are likely to, in turn, influence the dynamics of these HABs.

Keywords: *Alexandrium*, bacteria, *Dinophysis*, HAB, microbiome, microbial, sequencing

Introduction

The spatial and temporal expansion of harmful algal blooms (HABs) is a globally recognized phenomenon (Hallegraeff, 1993; Glibert et al., 2005). HABs associated with human health syndromes, for example, paralytic shellfish poisoning (PSP) and diarrhetic shellfish poisoning (DSP), are a growing human health and economic concern in many coastal regions (Anderson et al., 2008; Anderson et al., 2012). These dinoflagellate-related HABs can be associated with substantial economic losses due to the closure of shellfish beds containing toxic shellfish (Hoagland et al., 2002; Koukaras and Nikolaidis, 2004; Jin and Hoagland, 2008; Jin et al., 2008). Beyond economic effects, there is evidence that *Alexandrium* can cause significant alterations in estuarine plankton communities which may ultimately alter food webs, fisheries, and biogeochemical cycling (Weissbach et al., 2010; Hattenrath-Lehmann and Gobler, 2011; Weissbach et al., 2011; Weissbach et al., 2012; Hattenrath-Lehmann et al., 2015b). In New York, the PSP- and DSP-producing dinoflagellates, *Alexandrium* and *Dinophysis*, respectively, occur in succession (Hattenrath-Lehmann et al., 2013) and provide a unique opportunity to study potential shifts in plankton community composition over the course of successive toxin-producing blooms, which currently remains unknown.

Interactions among plankton assemblages play a central role in food web productivity, elemental cycling, and ecosystem function (Daly and Smith, 1993; Falkowski et al., 2008; Sunagawa et al., 2015; D'Alelio et al., 2016). Among phytoplankton, harmful algae utilize an array of ecological strategies to form blooms including the production of allelopathic chemicals and nutritional strategies such as mixotrophy that allow them to outcompete co-occurring phytoplankton (Smayda, 1997; Smayda, 2002; Glibert and Legrand, 2006). Further, bacteria are known to influence and be influenced by phytoplankton (Lima-Mendez et al., 2015; Ramanan et

al., 2016). Bacteria can affect harmful algal bloom formation, maintenance and termination by lysing harmful algae (Su et al., 2011; Inaba et al., 2013; Li et al., 2015b), inducing (Adachi et al., 1999; Adachi et al., 2003; Mayali et al., 2007) or preventing cyst formation (Adachi et al., 2002), altering physiology (Jauzein et al., 2015), and through symbiotic relationships (Croft et al., 2005; Kazamia et al., 2012). There is also evidence that bacteria degrade HAB toxins (Manage et al., 2009; Shetty et al., 2010) and influence toxin production (Albinsson et al., 2014; Lelong et al., 2014) in a variety of HAB species and some studies have documented bacterial modification of gene expression in HABs (Moustafa et al., 2010; Kazamia et al., 2012).

During HABs, the ability of a singular algal species to gain dominance is dependent upon physical, chemical and complex biological interactions within the planktonic community. To gain insight regarding the factors facilitating HABs, an assessment of the whole planktonic community is highly desirable as it provides information regarding competitive interactions among the HABs and plankton species as well as identifies indicator species that may precede or succeed the bloom species. High-throughput sequencing is an ideal tool for such an assessment as it can better identify rare, less abundant species and picoplankton compared to traditional light microscopy (Xiao et al., 2014). To date, only a few studies have utilized high-throughput sequencing to provide a detailed assessment of bacterial communities associated with HABs (Sison-Mangus et al., 2014; Li et al., 2015a; Yang et al., 2015), none of which has targeted the PSP- or DSP- producing dinoflagellates, *Alexandrium* and *Dinophysis*, respectively. In addition, none of these studies has used experimental approaches to understand the factors driving changes in planktonic communities associated with HABs, and the bacterial assemblages associated with the DSP-producing dinoflagellate, *Dinophysis,* have yet to be described.

Here, high-throughput amplicon sequencing was used to assess bacterial (16S) and phytoplankton assemblages (18S) associated with the harmful algae, *Alexandrium fundyense* and *Dinophysis acuminata*, during successive toxic blooms in Northport Bay, NY, USA. To gain insight regarding factors driving succession, experiments were performed assessing plankton community responses to filtrate from cultures of these two harmful algae. Finally, size fractionation was used to describe and compare free-living and potential epiphytic or intracellular bacterial assemblages in both blooms as well as cultures of *Alexandrium* and *Dinophysis*. Collectively, this study reveals a novel data set describing the unique microbiomes associated with each of these HABs, as well as the mechanism by which *Alexandrium* shapes its microbiome.

Materials and Methods

Study site sampling

Field samples were collected on a weekly basis from April through July during 2011 from a site in Northport Harbor, New York (40.8916°N, 73.3572°W; site 2, Hattenrath et al., 2010), which is a shallow (2 - 4 m), well mixed, eutrophic system within the southeastern portion of the Northport-Huntington Bay complex, located on the southern shore of Long Island Sound. During 2011, the *Alexandrium* bloom led to the closure of >7,000 acres of shellfish beds in Northport and Huntington Bays due to the presence of saxitoxin-contaminated shellfish. Further, while no closures were implemented, DSP toxins were found in shellfish throughout Northport Bay due to the presence of the okadaic acid-producing *Dinophysis* bloom (Hattenrath-Lehmann et al., 2013). Whole water samples were preserved in Lugol's iodine. Aliquots were settled in counting chambers and plankton were identified and enumerated using an inverted light microscope (Hasle, 1978). Cells larger than 10 µm were identified to at least genus level and grouped as autotrophic

105 nanoflagellates, dinoflagellates, and diatoms. Densities of *Dinophysis* were enumerated using a 1
106 mL Sedgewick-Rafter slide under a compound microscope using concentrated water samples
107 preserved in Lugol's iodine as described in Hattenrath-Lehmann et al. (2013). Densities of
108 *Alexandrium fundyense* were enumerated using a molecular probe developed by Anderson et al.
109 (2005) and described in Hattenrath et al. (2010). Briefly, aliquots of phytoplankton concentrates
110 (formalin and then methanol preserved) were hybridized with an oligonucleotide probe specific
111 for the NA1 North American ribotype *Alexandrium fundyense/catenella/tamarense* (this particular
112 ribotype of the *Alexandrium tamarense* species complex has recently been revised to *A. fundyense*
113 (John et al., 2014)) with Cy3 dye conjugated to the 5' terminus (5'-/5Cy3/AGT GCA ACA CTC
114 CCA CCA-3'). Cells were enumerated using a Nikon epifluorescence microscope with a Cy3™
115 filter set (Anderson et al., 2005).

116 For molecular analysis, whole seawater from Northport Harbor was filtered onto a 0.2μm
117 polycarbonate filter and immediately frozen at -80°C. Additionally, in the field a 2L concentrated
118 sample was created by filtering successively through a 200μm and 20μm mesh filter, backwashing
119 onto a 20μm polycarbonate filter and immediately flash frozen in liquid nitrogen and stored at -
120 80°C. Select sample dates were used to compare phytoplankton and bacterial assemblages in the
121 0.2 and 20 µm size fractions at the peaks of both the *Alexandrium* (9 May) and *Dinophysis* (27
122 June) blooms to compare free-living and particle-associated bacterial assemblages.

123 *Cultures and culturing conditions*

124 Locally isolated cultures, *Alexandrium fundyense* (NPB8; Northport Bay, NY) and
125 *Dinophysis acuminata* (Meetinghouse Creek, NY) were used for this study (Hattenrath-Lehmann
126 and Gobler, 2011; Hattenrath-Lehmann and Gobler, 2015). Algal cultures were grown in sterile
127 *f*/2 medium (Guillard and Ryther, 1962) with a salinity of 25 PSU, made with autoclaved and 0.2

128 µm-filtered aged coastal Atlantic Ocean water (40.79698N, 72.46068W), at 18°C in an incubator
129 with a 12:12 h light:dark cycle, illuminated by a bank of fluorescent lights that provided a light
130 intensity of ~100 µmol quanta m^{-2} s^{-1} to cultures. Given the previous documentation of negative
131 effects of antibiotics on *Dinophysis* cultures (Hattenrath-Lehmann and Gobler, 2015), antibiotics
132 (stock solution, Thermo Scientific HyClone Penicillin (10,000U/mL) Streptomycin
133 (10,000µg/mL) in 0.85% NaCl) were only added to *Alexandrium* cultures at a final concentration
134 of 1% by volume to discourage microbial contamination. The mixotrophic, *Dinophysis acuminata*
135 culture was maintained by using a three-step culturing process (Park et al., 2006) as described in
136 Hattenrath-Lehmann and Gobler (2015). For experiments (see *Filtrate experiments*), *Dinophysis*
137 cultures were fed *M. rubrum* at a ~1:1 ratio four times during a two-week period (every three to
138 four days) prior to the start of the experiment to ensure that cells were healthy and in exponential
139 growth phase. To compare bacterial assemblages in cultures to those found in a natural
140 phytoplankton community, aliquots of cultures were filtered onto 0.2µm (whole bacterial
141 assemblage) and 20µm polycarbonate (to capture potentially epiphytic and cell-associated
142 bacteria) filters and immediately frozen at -80°C. The bacteria captured on the 0.2 µm but not 20
143 µm filter were considered free-living bacterial assemblages, whereas those on the 20 µm filter only
144 were considered attached, epiphytic, or intracellular.

145 *Filtrate Experiments*

146 Experiments were performed to assess the natural plankton community's response to the
147 addition of environmentally relevant densities of *Alexandrium* and *Dinophysis*. While the
148 allelopathic effects of *Alexandrium* on natural phytoplankton (Fistarol et al., 2004; Tillmann et al.,
149 2009; Hattenrath-Lehmann and Gobler, 2011) and bacterial communities (Weissbach et al., 2010)
150 have been examined, the allelochemical potential of *Dinophysis* is currently unknown. Filtrate

(0.2µm) was used for experiments (AlexADD and DinoADD= *Alexandrium* and *Dinophysis* addition experiments, respectively) to eliminate the introduction of bacteria into experimental treatments (Hattenrath-Lehmann and Gobler, 2011). During 2014, filtrate experiments were conducted with triplicate, 330ml bottles half-filled with unamended water from Northport Bay, NY, and half-filled with two types of filtrate (0.2µm) as follows: a control was established whereby half of the bottle was filled with 0.2µm filtered seawater, and for the treatment, half of the bottle was 0.2µm filtered *Alexandrium* (1,320 cells mL^{-1} prior to filtration; 14 May) or *Dinophysis* (600 cells mL^{-1} prior to filtration; 18 June) culture. Both Northport Bay water and cell-free *Alexandrium* and *Dinophysis* medium was obtained by gentle filtration (<5psi) through a sterile 0.2 µm Millipore Steritop filter. To ensure that the effects seen by the addition of *Alexandrium* or *Dinophysis* filtrate were due to allelochemicals and not nutrients, saturating concentrations of N (88µM), P (3.6µM), and Si (88µM) as well as vitamins and trace metals (both at *f*/2 concentrations) were added to all bottles. All experimental bottles were incubated at ambient light and temperature for 48 h in Shinnecock Bay at the Stony Brook Southampton Marine Science Center (Gobler et al., 2004). At the end of the incubation, contents of each experimental bottle was filtered onto 0.2 µm polycarbonate filters that was then preserved as above for molecular analysis, and sequenced as individual biological replicates in triplicate.

DNA extraction, Illumina Sequencing and Analysis

To extract nucleic acids, 1 mL of cetyltrimethyl ammonium bromide (CTAB) buffer with fresh beta-mercaptonethanol was added to the 0.2µm and 20µm polycarbonate filters, vortexed, heated to 50°C for 20 minutes, and frozen at -80°C until processing. Genomic DNA extraction was performed using the CTAB method (Dempster et al., 1999). Following extraction, double-stranded DNA was quantified on a Qubit® fluorometer using a dsDNA BR Assay kit. Samples

were normalized to an equal quantity of DNA for sequencing and were sent to Molecular Research Labs (Shallowater, Texas, USA) for amplicon sequencing. The 16S rRNA gene V4 variable region (~300bp) was amplified using bacterial primers A519F: 5´CAG CMG CCG CGG TAA and 802R: 5´TAC NVG GGT ATC TAA TCC (Klindworth et al., 2013). The V7/V8 region of the 18S rRNA gene (~450bp) was amplified using primers 1183F: 5´AAT TTG ACT CAA CAC GGG and 1631aR: 5´TAC AAA GGG CAG GGA CG (Hadziavdic et al., 2014). For each sample, an identifying barcode was placed on the forward primer and a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) was performed. The following PCR conditions were used: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, and a final elongation step at 72°C for 5 minutes. Successful amplification was determined by visualizing PCR products using a 2% agarose gel. Samples (27 for 18S and 31 for 16s, Table S1 and S2, respectively) were pooled together for each respective primer region in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were then purified using calibrated Ampure XP beads and subsequently used to prepare a DNA library by following Illumina TruSeq DNA library. Paired-end (2x300) sequencing was performed on an Illumina MiSeq following the manufacturer's guidelines. Sequence data was processed using the Quantitative Insights Into Microbial Ecology v1.9.1 pipeline (QIIME, http://qiime.org; Caporaso et al., 2010b). Raw sequences were depleted of barcodes, paired-end reads joined, depleted of primers, demultiplexed, and quality filtered using the default parameters in QIIME. The resulting quality filtered sequences were then clustered into operational taxonomic units (OTUs) at 97% similarity with UCLUST (Edgar, 2010) using the open reference clustering protocol and SILVA release v119 (http://www.arb-silva.de/) as the reference set. The representative sequence set was aligned using PyNAST

(Caporaso et al., 2010a) and taxonomically classified using UCLUST (Edgar, 2010). For 18S specifically, all non-algal OTUs were not considered in order to focus specifically on algal assemblages. Since species specificity with the QIIME pipeline was typically not possible, representative sequences for the most abundant OTUs were extracted and species specificity (percent identity) was determined using BLAST. In some cases, algal species were not identified (i.e. *Thalassiosira* spp.) due to multiple species having 100% identity with sequences. Similarly, for 16S, since our focus was on prokaryotes, all chloroplast and mitochondria (Table S2) related sequences were removed from OTU tables and not further considered in analyses.

For the V7/V8 region of the 18S rDNA, the 27 samples generated 2,847,120 paired end reads with an amplicon size of ~450bp. After quality filtering and joining reads a total of 2,035,933 reads clustered at 97% identity into 24,004 OTUs. Overall, algae represented 35 to 98% of total reads (prior to removal of non-algal OTUs) with an average of 86% for all 27 samples (Table S1). For the 519F/802R region of 16S rDNA, the 31 samples generated 4,458,146 paired end reads with an amplicon size of ~300bp. After quality filtering and joining reads a total of 3,676,342 reads clustered at 97% identity into 47,760 OTUs (Table S2). The 921,186 and 45,876 reads assigned as chloroplasts and mitochondria, respectively, were removed from the dataset and not considered in analyses (Table S2).

Statistical Analysis

Multivariate statistical analyses were conducted using PAST v3.11 (Hammer et al., 2001). Specifically, a similarity percentage (SIMPER) using Bray-Curtis metrics was conducted to determine the taxa responsible for differences in relative abundances between groups and principal component analyses (PCA) were also conducted to determine the groups of variables that behaved in a similar manner. Non-metric multidimensional scaling (NMDS) using a Bray-

Curtis metric was conducted in RStudio (vegan package, metaMDS) to assess the similarity of bacterial community composition among all samples. Student t-tests were used to compare arcsine square root transformed data of relative abundance data sets using Sigma Plot 11.0. If data failed normality, non-parametric, Mann-Whitney U-tests were used for such comparisons.

Results

Phytoplankton and bacterial assemblages of Northport Bay

During spring 2011, *Alexandrium fundyense* was present in Northport Harbor from late March through late May, with peak densities occurring on 9 May (25,300 cells L^{-1}) and a smaller secondary peak (6,600 cells L^{-1}) on 16 May (Fig. 1A). A *Dinophysis acuminata* bloom succeeded the *Alexandrium bloom* with maximal densities occurring on 27 June (1.3 million cells L^{-1}) with cells present from late April through September (Fig. 1A). Mean (±SD) temperature and surface salinities were 15.8±1.4˚C and 23.9±0.4 during the peak of the *Alexandrium* bloom (3 – 16 May), and 22.8±1.4˚C and 23.4±0.3 during the peak of the *Dinophysis* bloom (21 June -6 July). Mean (±SD) nitrate, ammonium, phosphate and silicate concentrations were 14.4±6.3µM, 2.2±2.1µM, 0.9±0.4µM and 26.6±8.2µM during the peak of the *Alexandrium* bloom, and 7.4±7.3µM, 0.7±0.6µM, 0.8±0.7µM and 37.1±18.5µM during the peak of the *Dinophysis* bloom. Water column temperature was the only physio-chemical parameter that differed between the blooms being significantly higher during the peak of the *Dinophysis* bloom (t-test; $p<0.001$).

Over the course of the time series, phytoplankton community sequence diversity changed dramatically (Fig. 1B, C). In late April, Bacillariophyceae (diatoms) represented 64% of the algal, V7/V8 sequences (Fig. 1B) being comprised mostly of *Skeletonema* spp. (29%) and *Rhizosolenia setigera* (22%; Fig. 1C). During the peak of the *Alexandrium* bloom (3 – 16 May; Fig. 1A), the community shifted to one dominated by the class Dinophyceae (dinoflagellates; 21-

78%) and Litostomatea (*Mesodinium rubrum* was the only member present within this class; 2-69%; Fig. 1B). During the *Alexandrium* bloom, the dinoflagellate community was a mixed assemblage dominated *by Heterocapsa rotundata* (1-9%), *Heterocapsa triquetra* (1-21%), *Amoebophrya* spp. (1-17%) and *Alexandrium* (1 - 3%; Fig. 1C). During the demise of the *Alexandrium* (1 June) bloom, the algal community briefly shifted back to a diatom-dominated community (58-75%) comprised mostly of *Thalassiosira* spp. (28-46%) and *Skeletonema* spp. (13-20%) before reverting back to a dinoflagellate-dominated (30-90%) community during the peak of the *Dinophysis* bloom (21 June- 6 July; Fig. 1) and beyond. In contrast to *Alexandrium*, during the peak of the *Dinophysis* bloom, *Dinophysis* represented the majority of algal sequences (24-70%; Fig. 1). At the phyla level, a similarity percentages analysis (SIMPER) identified Litostomatea (38%), Dinophyceae (30%) and Bacillariophyceae (27%) as the primary drivers (cumulative=95%; average dissimilarity=53.66) of differences between the peak *Alexandrium* and peak *Dinophysis* communities (Fig. 1). At the genus-species level, *Dinophysis acuminata* (29%), *Mesodinium rubrum* (26%), *Skeletonema* spp. (14%) and *Amoebophrya* spp. (6%) accounted for 75% of the differences seen between the two bloom communities (SIMPER, average dissimilarity=77.78; Fig. 1).

Over the course of both blooms the phyla Bacteroidetes (24-37%) and Proteobacteria (41-60%) were the dominant contributors to the bacterial community (Fig. 2B). Within the phyla Bacteroidetes, the order Flavobacteriales comprised 20-32% of 519F/802R sequences (Fig. 2C) and within the phyla Proteobacteria, Rhodobacterales (9-15%), Rickettsiales (1-8%) and SAR11 clade (2-11%) of the Class Alphaproteobacteria, and Alteromonadales (3-8%) and Oceanospirillales (4-10%) of the Class Gammaproteobacteria dominated (Fig. 2C). At the phyla level, a similarity percentages (SIMPER) analysis identified ‘Unassigned’ (43%), Proteobacteria

266 (31%) and Bacteroidetes (17%) as the primary drivers (cumulative=91%; average
267 dissimilarity=21.57) of differences between the peak *Alexandrium* and peak *Dinophysis* bacterial
268 communities (Fig. 2). During the peak of the *Alexandrium* bloom, the relative abundances of
269 Proteobacteria and Bacteroidetes were significantly (t-test, $p<0.01$) greater than during the
270 *Dinophysis* bloom, while the relative abundances of 'Unassigned' sequences were significantly
271 (t-test, $p<0.001$) lower (Fig. 2B). At the order level, 'Unassigned' (41%), Flavobacteriales
272 (18%) and SAR11 clade (13%) account for 72% of the differences seen between the two bloom
273 communities (SIMPER, average dissimilarity=22.36; Fig. 2C). The relative abundances of
274 Flavobacteriales and the SAR11 clade were significantly (t-test, $p<0.01$) greater during the
275 *Alexandrium* bloom compared to during the *Dinophysis* bloom (Fig. 2C). At the genus level, the
276 NS5 marine group (11%) and an uncultured bacterium (Rhodobacteracea; 11%) were the
277 dominant sequences during the peak of the *Alexandrium* bloom while the peak of the *Dinophysis*
278 bloom was dominated by 'Unassigned' sequences (21%; Table 1).

279 Phytoplankton and bacterial assemblages differed across size fractions (0.2µm vs. 20µm)
280 during the peak of the *Alexandrium* (9 May) and *Dinophysis* (27 June) blooms (Fig. 3). Among
281 phytoplankton, while Litostomatea (=*Mesodinium*; 69%) and Dinophyceae (21%) dominated the
282 0.2µm size fraction, Dinophyceae (82%) dominated the 20µm fraction at the peak of the
283 *Alexandrium* bloom (9 May; Fig. 3A). At the genus/species level, *Mesodinium rubrum* (69%)
284 and *Amoebophrya* spp. (14%) dominated the whole community while *Alexandrium fundyense*
285 and *Amoebophrya* spp. comprised 23 and 41% of the 20µm size fraction, respectively (Fig. 3B).
286 The relative abundances of Proteobacteria and Bacteroidetes shifted from 59 to 76%, and 34 to
287 14% in the 0.2 and 20 µm size fractions, respectively (Fig. 3C). At the order-level, the major
288 drivers of differences (SIMPER) between the size fractions were the Flavobacteriales,

289 Rhodobacterales and 'other' bacteria which shifted from 32 to 11%, 15 to 6% and 19 to 58%, in
290 the 0.2 and 20 µm size fractions, respectively (Fig. 3D). At the genus level, the NS5 marine
291 group (Flavobacteriales) dominated 0.2µm size fraction while the *Perlucidibaca*
292 (Gammaproteobacteria, Moraxellaceae) dominated the 20µm size fraction (Table 1). The peak
293 of the *Dinophysis* bloom (27 June) was dominated by Dinophyceae (>90%), specifically
294 *Dinophysis acuminata* (>70%), in both the 0.2 and 20 µm size fractions (Fig. 3A, B). The
295 relative abundances of Proteobacteria and Bacteroidetes shifted from 44 to 53%, and 28 to 17%
296 in the 0.2 and 20 µm size fractions, respectively (Fig. 3C). At the order-level, the major drivers
297 of differences (SIMPER) between the size fractions were the Rickettsiales, Flavobacteriales, and
298 Rhodobacterales which shifted from 3 to 13%, 22 to 13% and 15 to 7%, in the 0.2 and 20 µm
299 size fractions, respectively (Fig. 3D).

300 ***Filtrate experiments using natural communities***

301 The addition of *Alexandrium* filtrate significantly altered both bacterial and
302 phytoplankton assemblages of Northport Bay (Fig. 4). Specifically, for bacterial sequences, the
303 addition of *Alexandrium* filtrate yielded a community in which the relative abundances of
304 Bacteroidetes were significantly (t-test, $p<0.001$) greater, while Proteobacteria, Cyanobacteria,
305 'other' bacteria and 'Unassigned' relative abundances were significantly (t-test, $p<0.01$) lower
306 compared to the control (Fig. 4). At the order level, the addition of *Alexandrium* filtrate caused
307 the relative abundances of Flavobacteriales to significantly (t-test, $p<0.001$) increase, while
308 Rhodobacterales, Rickettsiales, the SAR11 clade, Alteromonadales, Oceanospirillales, 'other'
309 bacteria, and 'Unassigned' relative abundances all significantly decreased (t-test, $p<0.05$)
310 compared to controls (Fig. 4). The addition of *Alexandrium* filtrate significantly (t-test, $p<0.05$)
311 decreased an 'uncultured bacterium' from the Rhodobacterales while significantly (t-test,

312 $p<0.05$) increasing the relative abundances of *Owenweeksia* spp. and the NS5 marine group.
313 The *Alexandrium* filtrate treatment was dominated by sequences of the NS5 marine group and
314 *Owenweeksia* spp, both from the Flavobacteriales, while 'Unassigned' and an 'uncultured
315 bacterium' from the Rhodobacterales dominated the control (Table 1). In regards to algal
316 sequences, the addition of *Alexandrium* filtrate significantly (t-test, $p<0.001$) increased relative
317 abundances of Dinophyceae and Dictyochophyceae but significantly (t-test, $p<0.05$) decreased
318 relative abundances of Litostomatea (*Mesodinium*) and 'other' phytoplankton (Fig. 4). The
319 addition of *Alexandrium* filtrate led to a significant increase in the relative abundances of
320 *Amoebophrya* spp., Dictyochophyceae, 'other Dinophyceae' and *Gyrodinium* spp. (t-test,
321 $p<0.05$), while *Mesodinium rubrum* and 'other algae' relative abundances significantly decreased
322 (t-test, $p<0.05$; Fig. 4). The addition of *Dinophysis* filtrate caused no significant changes in the
323 relative abundances among bacterial or phytoplankton sequences at any level of classification.
324 ***Bacterial assemblages associated with cultures***
325 The relative abundances of bacterial sequences differed between size fractions in both
326 *Alexandrium* and *Dinophysis* cultures (Fig. 5). In *Alexandrium* cultures, Bacteroidetes and
327 Proteobacteria were the most abundant, with relative abundances of Bacteroidetes being 85% in
328 the 0.2µm size fraction and 65% in the 20µm size fraction and Proteobacteria being 13 and 32%
329 in these two fractions, respectively (Fig. 5A). At the order-level, Flavobacteriales dominated
330 both size fractions, with relative abundances of 82 and 63% in the 0.2 and 20µm size fractions,
331 respectively (Fig. 5B). The dominant bacterial genus in *Alexandrium* cultures was *Maribacter*
332 spp. (Order Flavobacteriales) with relative abundances of 65% and 50% in the 0.2 and 20µm size
333 fractions (Table 1). In *Dinophysis* cultures, Proteobacteria and Bacteroidetes were also the most
334 abundant sequences present, with relative abundances of 76 and 59%, and 11 and 35% in the 0.2

335 and 20μm size fractions, respectively (Fig. 5A). At the order-level, Rhodobacterales and
336 Flavobacteriales were the dominant sequences in *Dinophysis* cultures, with relative abundances
337 of 39 and 17%, and 7 and 31% in the 0.2 and 20μm size fractions, respectively (Fig. 5B). Genus
338 specific information demonstrates that *Arenicella* spp. (Gammaproteobacteria; 22%) and
339 *Ulvibacter* spp. (Flavobacteriales; 23%) dominated the 0.2 and 20 μm size fractions of
340 *Dinophysis* cultures, respectively (Table 1).

341 **Discussion**

342 Interactions among plankton shape the succession of communities and broadly influence
343 food web dynamics. Bacteria play a central role in marine food webs and engage in complex
344 interactions with phytoplankton (Falkowski et al., 2008; de Vargas et al., 2015; Lima-Mendez et
345 al., 2015; Sunagawa et al., 2015; Ramanan et al., 2016). An important first step in describing
346 phytoplankton-bacterial interactions and associations is identifying the covariance of members
347 within these assemblages. Traditional microscopy, however, often underestimates phytoplankton
348 species diversity (Xiao et al., 2014) and dated methodologies such as terminal restriction
349 fragment length polymorphisms (TRFLP) and denaturing gradient gel electrophoresis (DGGE)
350 fail to completely and/or accurately describe bacterial community composition (Zhang et al.,
351 2007; Koch et al., 2014; Samarajeewa et al., 2015). In contrast, the use of high-throughput
352 amplicon sequencing during this study allowed for a precise assessment of bacterial (16S) and
353 phytoplankton assemblages (18S) associated with blooms of two harmful algal species,
354 *Alexandrium fundyense* and *Dinophysis acuminata*. Prokaryotic sequences in field,
355 experimental, and culture samples revealed unique bacterial consortia associated with
356 *Alexandrium* and *Dinophysis*. Further, the use of 18S sequencing revealed less abundant as well
357 as difficult to identify species, such as the parasitic dinoflagellate, *Amoebophrya* spp.

Collectively, these findings describe the precise microbiome associated with these HABs and the extent to which they are shaped by these HABs.

The amplicon sequencing of the V7/V8 region of 18S rDNA during this study provided a series of novel insights that microscopy could not have facilitated. For example, sequencing revealed the presence of picoplankton (i.e. *Picomonas* spp.), algae that do not preserve well for microscopy (i.e. *Heterosigma akashiwo*; Chang et al., 1990), and the dominance of the parasitic dinoflagellate, *Amoebophrya* spp. The dinoflagellate, *Amoebophrya* is ~5µm in its free-living infectious dinospore form, intracellular in its trophont form, and short-lived in its free-swimming vermiform stage (Park et al., 2013; Velo-Suárez et al., 2013). Globally, *Amoebophrya* spp. is known to cause the demise of *Alexandrium fundyense* blooms (Taylor, 1968; Velo-Suárez et al., 2013; Brosnahan et al., 2015), but has never been observed in association with *Alexandrium* blooms in the mid-Atlantic region of the US, despite decades of *Alexandrium* studies in this region (Schrey et al., 1984; Colin and Dam, 2002; Hattenrath et al., 2010; Hattenrath-Lehmann and Gobler, 2011; Hattenrath-Lehmann et al., 2015b; Zhuang et al., 2015). In the present study, however, *Amoebophrya* was found to be universally present and, at times, highly abundant in field samples, accounting for up to 17% of 18S sequences. It was not, however, observed microscopically. Considering the higher relative abundance (1-17%) of *Amoebophrya* during the peak of the *Alexandrium* bloom and its greater dominance (41%; Fig. 3) in the >20µm size fraction relative to the 0.2µm fraction, it is likely that *Alexandrium* was its target host (Velo-Suárez et al., 2013). Given the presence of *Heterocapsa triquetra* during the peak and demise of the *Alexandrium* bloom and the presence of *Amoebophrya* after the demise of the *Alexandrium* bloom, and the inability of dinospores to survive >10 days in the water column (Coats and Park, 2002), *H. triquetra* may have been a secondary host. Alternatively, there may have been

381 different strains of *Amoebophrya* infecting each of these dinoflagellates given host-specificity for
382 this parasite is known to be at the species or strain level (Coats et al., 1996; Coats and Park,
383 2002; Park et al., 2013). The discovery of *Amoebophrya* spp. in this region is ecologically
384 relevant given the its role in the occurrence, duration, and termination of these toxic HABs
385 (Velo-Suárez et al., 2013; Brosnahan et al., 2015; Ralston et al., 2015) and was facilitated
386 specifically by the high-throughput sequencing approached used here.

387 Many species of the *Alexandrium* are known to be allelopathic (Tillmann et al., 2009).
388 During this study, consistent with prior studies in this system (Hattenrath-Lehmann and Gobler,
389 2011; Hattenrath-Lehmann et al., 2015b), a clear allelopathic signal was observed in the field as
390 during the peak of the *Alexandrium* bloom diatoms levels significantly decreased compared to
391 before and after the *Alexandrium* bloom. In addition, *Alexandrium* filtrate, presumably enriched
392 in allelochemicals (Fistarol et al., 2004; Tillmann et al., 2009; Hattenrath-Lehmann and Gobler,
393 2011), significantly decreased the relative abundance of 'other algae' and *Mesodinium rubrum*
394 but significantly enhanced the relative abundances of *Amoebophrya* spp. and *Gyrodinium* spp.
395 Consistent with this, prior studies of the same *Alexandrium* species using microscopy
396 demonstrated it was capable of enhancing dinoflagellate densities while allelopathically
397 depressing diatom and nanoflagellate abundances (Hattenrath-Lehmann and Gobler, 2011) as
398 well as *Mesodinium* densities (Fistarol et al., 2004). The higher relative abundance of
399 *Amoebophrya* spp. in response to *Alexandrium* allelochemicals has not been previously reported,
400 but may represent a host-parasite cellular signaling mechanism (Yoshino et al., 2001; Hemphill
401 et al., 2006; Ressurreição et al., 2016). In an ecosystem setting, this would create a negative
402 feedback loop during *Alexandrium* blooms whereby increasing *Alexandrium* densities and
403 allelochemicals may ultimately promote infections by *Amoebophrya* spp. and lead to bloom

404 demise. This hypothesis is consistent with observations in the Northeast US (i.e. New England)
405 where *Amoebophrya* spp. plays a prominent role in the demise of *Alexandrium* blooms (Velo-
406 Suárez et al., 2013; Brosnahan et al., 2015; Ralston et al., 2015). While the effects of
407 *Alexandrium* on planktonic organisms vary, it is clear that *Alexandrium* can shape phytoplankton
408 communities via inhibiting or promoting the growth of other algae.

409 While the sequencing of 18S rDNA facilitated a series of new discoveries during this
410 study, some differences between sequencing analyses and microscopy were detected (Fig. S1).
411 In some cases, sequencing was not species-specific (e.g. *Thalassiosira* spp., *Prorocentrum* spp.)
412 while in other cases, for certain taxa (Euglenoids), genes were not amplified during sequencing
413 but species were quantifiable via microscopy (Fig. S1). These issues are likely, in part, due to
414 informatic shortcomings as the current eukaryotic SILVA database does not contain sequences
415 for some genera or species (i.e. *Prorocentrum gracile*, *Eutreptiella* spp.) that were identified via
416 light microscopy in our samples. These issues could also be related to the primers used in this
417 study as even 'universal' primers do not amplify all taxa (Hadziavdic et al., 2014) with
418 additional primer sets and regions of interest (e.g. ITS, *cox*1, *rbcL* regions) needed to attain
419 species-specificity within particular genera (e.g. Raho et al., 2008; Hamsher et al., 2013;
420 Fernandes et al., 2014; Wallace and Gobler, 2015). Further, it is possible that differences
421 between sequencing and microscopy could arise from species specific differences in copies of
422 rDNA (Zhu et al., 2005; Godhe et al., 2008). Of specific concern is the high copy numbers of
423 rDNA in eukaryotes with larger genomes (Prokopowich et al., 2003) such as that commonly seen
424 in dinoflagellates (Zhu et al., 2005). We attempted to circumvent this potential issue, however,
425 by using relative abundances in effort to normalize for these differences. Further, we note that,
426 beyond the close correspondence between OTUs and microscopically quantified dinoflagellates

427 of interest the ability to observe the *Alexandrium* allelopathic signal (as mentioned above) in the
428 dynamics of field populations gives us confidence in our approach indicating that high or
429 variable copy numbers of dinoflagellates did not obfuscate environmentally important trends
430 such as allelopathy. The increased diversity and, in some cases, lack of species specificity with
431 sequencing compared to microscopy was also demonstrated by Xiao et al. (2014), who therefore
432 recommended using dual amplicon sequencing and microscopy approaches for maximal
433 detection and identification.

434 In a manner consistent with prior studies of HABs including *Cochlodinium polykrikoides*,
435 *Alexandrium* spp., *Pseudonitzschia* spp., and *Akashiwo sanguinea* (Wichels et al., 2004; Jasti et
436 al., 2005; Kaczmarska et al., 2005; Garces et al., 2007; Hasegawa et al., 2007; Sison-Mangus et
437 al., 2014; Park et al., 2015; Yang et al., 2015), bacterial populations associated with *Alexandrium*
438 and *Dinophysis* cultures, field samples, and experiments were dominated by Proteobacteria
439 (alpha- and gammaproteobacteria) and Bacteroidetes (Flavobacteria). A principal component
440 analysis (PCA) comparing *Alexandrium* and *Dinophysis* abundances with the relative
441 abundances of bacterial (16S) sequences revealed that both HABs were associated with specific
442 bacterial groups. At the order-level, 73% of the data set was explained by three principal
443 components. The first principal component (PC 1) explained 40% of the variance and was
444 comprised of *Alexandrium* densities, Flavobacteriales, the SAR11 clade (alphaproteobacteria)
445 and Oceanospirillales (gammaproteobacteria; Table 2). PC2 explained an additional 19% of the
446 variance with *Dinophysis* abundances and Alteromonadales (gammaproteobacteria) co-varying
447 together and being inversely correlated to Rickettsiales (alphaproteobacteria; Table 2). Finally,
448 in PC3 'other bacteria' and Rhodobacterales (alphaproteobacteria) co-varied together and were

449 inversely correlated to 'unassigned bacteria' and explained an additional 14% of the variance
450 (Table 2).

451 While both *Alexandrium* and *Dinophysis* cultures and field populations were dominated
452 by Proteobacteria (alpha- and gammaproteobacteria) and Bacteroidetes (Flavobacteria), these
453 HABs had distinct microbiomes. Consistent with PC1 of the PCA, the relative abundances of
454 Flavobacteriales and the SAR11 clade were significantly greater during the *Alexandrium* bloom
455 compared to during the *Dinophysis* bloom, and in cultures of *Alexandrium*, Flavobacteriales (Fig.
456 5) represented the majority of sequences (>63%) of both size fractions indicating that they were
457 primarily epiphytic and/or intracellular associated. At the genus-level, the NS5 marine group
458 (Flavobacteriales), an uncultured bacterium (Rhodobacteracea), and *Owenweeksia* spp. were
459 commonly the dominant genera among *Alexandrium*-associated field samples with *Perlucidbaca*
460 spp. (order Pseudomonadales) and *Limnobacter* spp. (order Burkholderiales) found at the peak of
461 the *Alexandrium* bloom in the >20micron size fraction. Further, *Alexandrium* cultures were
462 dominated by the Flavobacteria, *Maribacter* spp., which represented 65% and 50% of the whole
463 and >20 µm size fractions, respectively, the latter suggesting a direct cellular association (Table
464 1). In contrast, *Dinophysis* had a greater association with Proteobacteria and 'unassigned
465 bacteria' (Table 1). For example, 'unassigned bacteria' and an uncultured bacterium
466 (Rhodobacteracea) dominated *Dinophysis* related field samples. In addition, *Marivita* spp. (order
467 Rhodobacterales), *Arenicella* spp. (Gammaproteobacteria), and *Ulvibacter* spp. (order
468 Flavobacteriales) were found in cultures of *Dinophysis* (Table 1), with the latter two genera
469 found in the >20 µm size fraction and thus directly associated with cells. Given that our cultures
470 of *Dinophysis* are incapable of survival when exposed to even modest levels of antibiotics
471 (Hattenrath-Lehmann and Gobler, 2015), it is plausible that these bacteria may have an

472 important, nutritional, symbiotic relationship with this alga (Croft et al., 2005). Further, while
473 the microbiome of *Dinophysis* prey in culture may alter the bacterial communities, given the
474 high similarity of the bacterial communities in the two size fractions in culture (Fig. 6) it would
475 seem the prey do not appreciably contribute to *Dinophysis* microbiomes. Collectively, high-
476 throughput sequencing revealed the unique microbiomes of these HABs.

477 The positive association between *Alexandrium* and the Flavobacteriales identified in PC1
478 was also evident during incubation experiments. *Alexandrium* filtrate significantly suppressed
479 some Proteobacteria (e.g. Rhodobacterales, Rickettsiales, the SAR11 clade, Alteromonadales,
480 Oceanospirillales) but strongly promoted the relative abundance of *Owenweeksia* spp. and the
481 NS5 marine group, both Flavobacteriales (Table 1, Fig. 4). Among the Flavobacteriales, the
482 impact of *Alexandrium* filtrate on *Owenweeksia* spp. was the most remarkable as filtrate (22%)
483 enhanced relative abundances 11-fold compared to the control (2%) over the 48 h experiment.
484 Similarly, through TRFLP analysis, Weissbach et al. (2010) demonstrated that continuous
485 additions of the two strains of *Alexandrium* resulted in bacterial communities that differed from
486 untreated communities. Moreover, the enhancements of Flavobacteria with the addition of
487 *Alexandrium* filtrate is consistent with observations during *Alexandrium* blooms and in cultures
488 as Flavobacteria made up a significant portion of these communities. In addition, some of these
489 effects could have also been due to bacterial stimulation via the introduction of organic matter
490 either leaked from *Alexandrium* cells and thus in the filtrate itself or from allelopathically lysed
491 phytoplankton due to the presence of *Alexandrium* during blooms. Both such processes occur
492 during *Alexandrium* blooms and thus are likely to contribute to the close correspondence
493 between bacterial communities established during experiments and blooms.

494 The consistency of bacterial clade-HAB associations among the field samples,
495 experiments, and in culture evidences the ability of these dinoflagellates to shape surrounding
496 bacterial communities. Non-metric multidimensional scaling (NMDS) analyses revealed that
497 bacterial communities associated with *Dinophysis* and *Alexandrium* during blooms, experiments,
498 and in culture were distinct and statistically different from each other at both the order- and
499 genus-level (Fig 6). The single exception to this was bacteria from the *Dinophysis* experiment
500 (DINOADD) where the control and filtrate treatment grouped closely to each other and were not
501 significantly different (Fig. 6), supporting the hypothesis that *Dinophysis* has weaker control of
502 bacterial communities compared to *Alexandrium*. Regardless, bacterial communities do seem to
503 play a significant role in *Dinophysis* survival as the addition of antibiotics was found to be lethal
504 to *Dinophysis* cultures (Hattenrath-Lehmann and Gobler, 2015). At the genus-level, field-based
505 samples (including experiments) were more closely related to one another than both cultures,
506 with the *Dinophysis* culture being more similar to the field samples than the *Alexandrium*
507 culture, a finding likely related to the presence of antibiotics in *Alexandrium,* but not in
508 *Dinophysis,* cultures (Fig. 6). Further, we note that the low levels of antibiotics added upon
509 initial inoculation of the stock *Alexandrium* culture (see methods) likely degraded during the two
510 weeks prior to collecting samples for sequencing bacterial amplicons in cultures and creating
511 filtrate for experiments (Kummerer, 2009). Bacteria associated with *Dinophysis* and
512 *Alexandrium* blooms and experiments were also clearly and statistically separated from each
513 other, supporting the concept that each of these HABs is associated with their own core
514 microbiome (Sunagawa et al., 2015). Finally, NMDS revealed the manner in which
515 *Alexandrium* filtrate transformed bacterial communities as at the order- and genus-level the

516 experimental addition of *Alexandrium* filtrate resulted in a bacterial community statistically
517 distinct from the control treatment (Fig 6).

518 While sequencing of field, experimental, and culture samples revealed unique bacterial
519 associations with the HABs formed by *Alexandrium* and *Dinophysis*, precise algal-bacterial
520 interactions are uncertain but can be hypothesized from the literature. For example, bacteria
521 from the Alteromonas group have been shown to inhibit cyst formation in *Alexandrium* (Adachi
522 et al., 1999; Adachi et al., 2002; Adachi et al., 2003) and displayed decreasing relative
523 abundance over the course of the bloom which may have allowed cyst formation at the end of
524 this event (Anglés et al., 2012). Flavobacteria, the most abundant group found in a majority of
525 the field, experimental, and culture samples, especially those associated with *Alexandrium*, are
526 known to facilitate macromolecule conversion (Buchan et al., 2014). Teeling et al. (2016), for
527 example, found an increase in the gene frequency and diversity of carbohydrate-active enzyme
528 families associated with increased relative abundances of Flavobacteria during the spring bloom
529 in the North Sea. More specifically, they found that the NS5 marine group, which was a
530 dominant group in bloom samples (Table 1), was rich in glycoside hydrolase family fucosidases
531 (Teeling et al., 2016). Hence, these bacteria may play a role in nutrient and organic matter
532 cycling during *Alexandrium* and *Dinophysis* blooms which are strongly promoted by organic
533 compounds and regenerated forms of nutrients (Hattenrath et al., 2010; Hattenrath-Lehmann et
534 al., 2015a).

535 The use of high throughput sequencing during this study provided a more detailed
536 assessment of bacterial communities associated with HABs than previously described. In some
537 cases, findings here were consistent with prior studies using older technologies. For example,
538 Adachi et al. (2003) found that Pseudomonads co-dominated *Alexandrium* blooms in Japan,

539 while during this study *Pseudomonas* spp. was associated with the >20µm size fraction of
540 *Alexandrium* cultures and thus likely attached or intracellular (Table 1). In contrast to some prior
541 studies using older technologies (plate isolation, DGGE, FISH) concluded that bacteria from the
542 Rosebacter clade were a dominant bacteria associated with *Alexandrium* (Adachi et al., 2003;
543 Wichels et al., 2004; Jasti et al., 2005; Garces et al., 2007). During this study, Roseobacter
544 (order Rhodobacterales), while present in all samples, was not dominant within *Alexandrium*
545 cultures or field samples (<1% of OTUs). This difference is partly expected as older
546 technologies were incapable of describing whole microbial communities. The present study,
547 therefore, represents a novel data set as high-throughput sequencing has not been used to
548 describe microbial communities associated with *Alexandrium* or *Dinophysis*, in an ecosystem
549 setting or in culture.

550 High-throughput sequencing studies continue to expand our understanding of planktonic
551 communities (de Vargas et al., 2015; Lima-Mendez et al., 2015; Sunagawa et al., 2015; Yang et
552 al., 2015). Ocean sequencing studies such as the Tara Oceans study have demonstrated that
553 global eukaryotic diversity has yet to be fully described (de Vargas et al., 2015) but that biotic
554 interactions are better predictors of prokaryotic and eukaryotic community structure than abiotic
555 factors (Lima-Mendez et al., 2015). Bacteria are known to influence and be influenced by
556 phytoplankton (Bratbak and Thingstad, 1985; Lima-Mendez et al., 2015; Ramanan et al., 2016),
557 and anthropogenic nutrient loading and climate change are facilitating a global expansion of
558 HABs (Heisler et al., 2008; Hallegraeff, 2010; Anderson et al., 2012, Gobler et al. submitted).
559 Going forward, high-throughput sequencing will be an important tool for assessing the manner
560 and extent to which anthropogenic processes influence interactions among HABs and bacterial
561 communities.

In summary, this study defined distinct microbiomes associated with HABs formed by the toxic dinoflagellates *Alexandrium* and *Dinophysis*. While *Alexandrium* was found to directly and strongly shape bacterial and algal communities, microbial communities associated with *Dinophysis* were more likely a consequence of prevailing biogeochemical conditions and/or other biotic interactions. While some members of the microbial consortia associated with these HABs may aid in promoting these events (e.g. nutrient and organic matter regeneration by Flavobacteria), others may promote bloom demise (e.g. *Amoebophrya* spp). Regardless, the identification of the precise microbiome associated with each of these HABs opens a series of new lines of research to better understand the interactions among microbes during these events.

Acknowledgements

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578 **Tables**

579 **Table 1.** Dominant genus in size fractionated field, experimental and culture samples based on
580 relative abundances of 16S sequences. AlexADD= *Alexandrium* filtrate addition experiment.

Sample	Size fraction (µm)	Dominant lineages	Relative abundance (%)
***Alexandrium* peak**			
(3-May to 16-May)	0.2	NS5 marine group (Flavobacteriales)	11
		Rhodobacteracea_uncultured bacterium (Rhodobacterales)	11
		Owenweeksia (Flavobacteriales)	7
***Dinophysis* peak**			
(21-June to 6-July)	0.2	Unassigned	21
		Rhodobacteracea_uncultured bacterium (Rhodobacterales)	7
		Owenweeksia (Flavobacteriales)	5
		NS5 marine group (Flavobacteriales)	5
AlexADD experiment			
Control	0.2	Unassigned	12
		Rhodobacteracea_uncultured bacterium (Rhodobacterales)	9
		NS5 marine group (Flavobacteriales)	9
+*Alexandrium* filtrate	0.2	*Owenweeksia* (Flavobacteriales)	23
		NS5 marine group (Flavobacteriales)	11
		Rhodobacteracea_uncultured bacterium (Rhodobacterales)	5
Size fractionated field samples			
9-May	0.2	NS5 marine group (Flavobacteriales)	12
		Rhodobacteracea_uncultured bacterium (Rhodobacterales)	11
	20	*Perlucidibaca* (Pseudomonadales)	17
		Limnobacter (Burkholderiales)	7
27-Jun	0.2	Unassigned	19
		Rhodobacteracea_uncultured bacterium (Rhodobacterales)	8
		Owenweeksia (Flavobacteriales)	6
	20	Unassigned	23
Culture			
Alexandrium	0.2	*Maribacter* spp. (Flavobacteriales)	65
		Owenweeksia (Flavobacteriales)	4
	>20	*Maribacter* spp. (Flavobacteriales)	50
		Pseudomonas spp. (Pseudomonadales)	7
Dinophysis	0.2	*Arenicella* spp. (Gammaproteobacteria)	22
		Rhodobacteracea_uncultured bacterium (Rhodobacterales)	11
		Marivita spp. (Rhodobacterales)	9
	>20	*Ulvibacter* (Flavobacteriales)	23
		Arenicella spp. (Gammaproteobacteria)	19

581

582 **Table 2.** Factors loadings for the three principal components of correlations between
583 *Alexandrium fundyense* and *Dinophysis acuminata* abundances and the relative abundances of
584 16S sequences at the Order level. Values in bold type indicate the groupings of the 3 PCs that
585 described 73% of the variance in the data.

Parameter	PC 1	PC 2	PC 3
Alexandrium	**0.40717**	0.21792	-0.1701
Dinophysis	-0.2599	**0.41213**	-0.0677
Flavobacteriales	**0.38334**	-0.0078	-0.1262
Rhodobacterales	0.24455	0.33997	**0.46422**
Rickettsiales	0.28505	**-0.448**	-0.1479
SAR11 clade	**0.36586**	-0.1897	-0.1144
Alteromonadales	0.13214	**0.6085**	-0.074
Oceanospirillales	**0.34149**	0.1532	0.12701
Unassigned bacteria	-0.4099	0.08095	**-0.4408**
Other bacteria	-0.2066	-0.1733	**0.69586**
% of variance explained	40	19	14

586

587

588

589 **Figure Legends:**

590 **Figure 1.** A) 2011 Log *Alexandrium fundyense* and *Dinophysis acuminata* densities (cells L^{-1}).
591 Red and blue panel represent the peaks of the *Alexandrium* and *Dinophysis* blooms respectively.
592 B) & C) are the relative abundances of algal sequences over the course of the 2011 *Alexandrium*
593 and *Dinophysis* blooms at Class- and genus/species-level, respectively. The red and blue boxes
594 represent the dates included in the peaks of the *Alexandrium* and *Dinophysis* blooms,
595 respectively.
596
597 **Figure 2.** A) & B) are the relative abundances of bacterial sequences over the course of the 2011
598 *Alexandrium fundyense* and *Dinophysis acuminata* blooms at Phylum- and Order-level,
599 respectively. The red and blue boxes represent the dates included in the peaks of the
600 *Alexandrium* and *Dinophysis* blooms, respectively.
601
602 **Figure 3.** Relative abundances of size fractioned (0.2µm=whole bacterial assemblage and
603 20µm= potentially endosymbiotic or epiphytic bacteria) algae and bacterial communities during
604 the peak of the *Alexandrium fundyense* (May 9th) and *Dinophysis acuminata* (June 27th) bloom.
605 A) & B) are relative abundances of algal assemblages at Class- and genus/species-level,
606 respectively. C) & D) are relative abundances of bacterial assemblages at Phylum- and order-
607 level, respectively.
608
609 **Figure 4.** Relative abundances of bacterial (16s) and algal (18s) sequences for control and
610 *Alexandrium fundyense* culture filtrate additions during an experiment conducted using the
611 natural phytoplankton community of Northport Bay.
612
613 **Figure 5.** Relative abundances of size fractioned (0.2µm=whole bacterial assemblage and
614 20µm= potentially endosymbiotic or epiphytic bacteria) bacterial communities associated with
615 *Alexandrium fundyense* and *Dinophysis acuminata* cultures. A) & B) are relative abundances of
616 bacterial assemblages at Phylum- and Order-level, respectively. Alex= *Alexandrium* and DA=
617 *Dinophysis*.
618
619 **Figure 6**. Relative abundances of bacterial community composition data analyzed via non-metric
620 multidimensional scaling using a Bray-Curtis metric. 95% confidence interval was drawn using
621 the standard deviation of the points from the centroid of the cluster using a chi-squared
622 distribution. Top: Order-level; Bottom: Genus-level. AlexADD and DinoADD= *Alexandrium*
623 and *Dinophysis* addition experiments, respectively.

624
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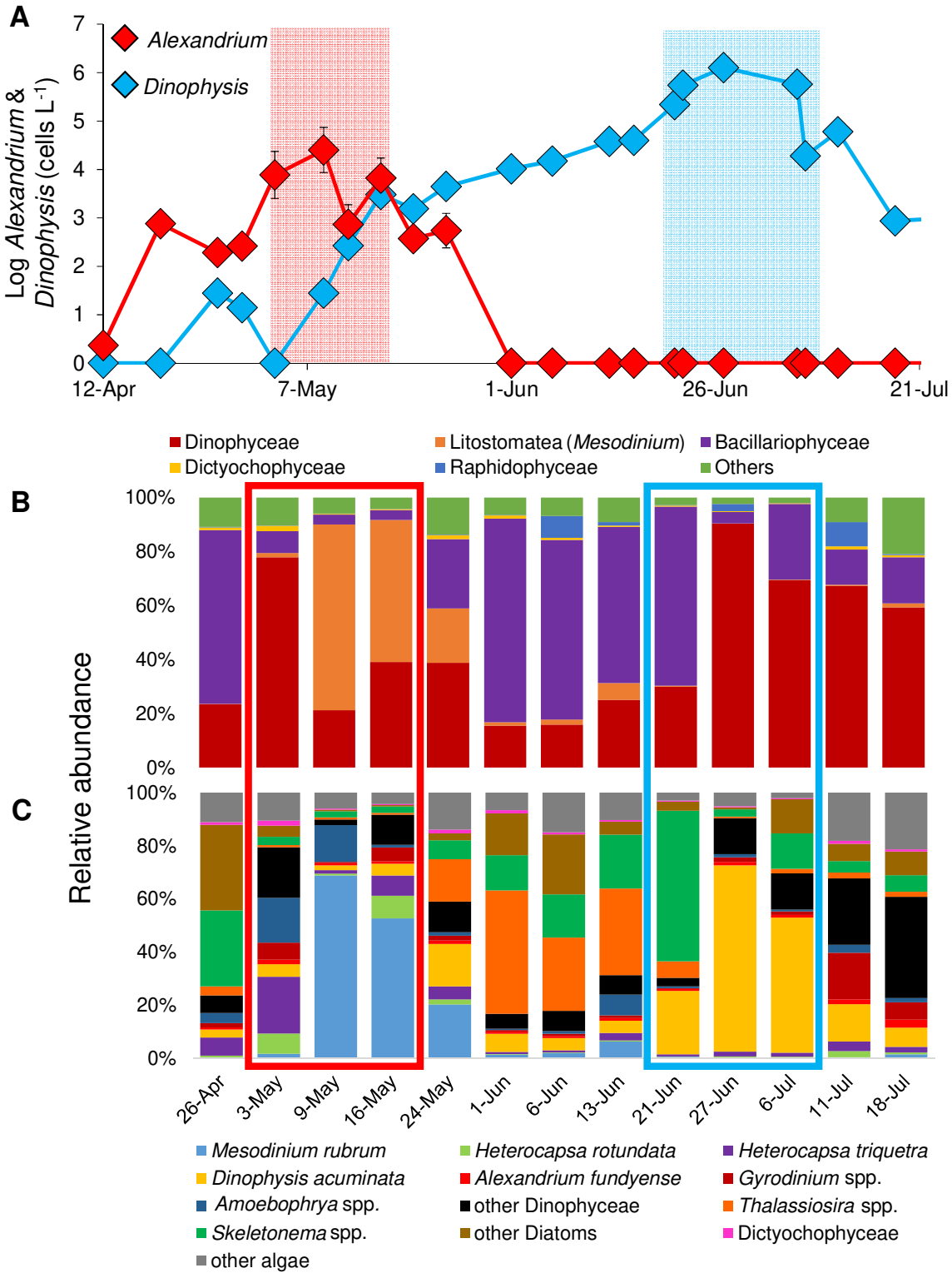
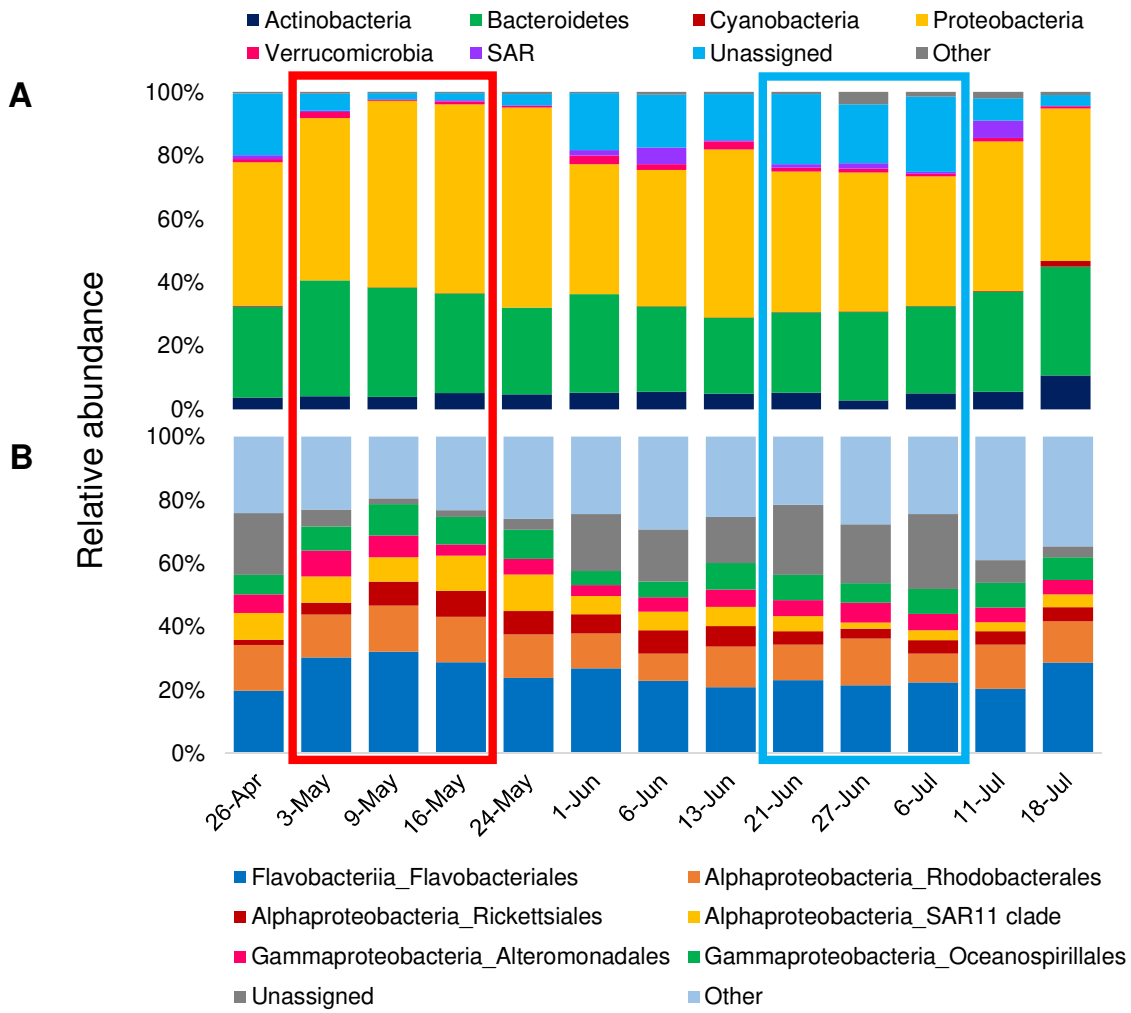
627

628

Figure 1.


A
Alexandrium
Dinophysis
Log Alexandrium & Dinophysis (cells L-1)
0
1
2
3
4
5
6
7
12-Apr
7-May
1-Jun
26-Jun
21-Jul
B
C
Dinophyceae
Litostomatea (Mesodinium)
Bacillariophyceae
Dictyochophyceae
Raphidophyceae
Others
Relative abundance
100%
80%
60%
40%
20%
0%
26-Apr
3-May
9-May
16-May
24-May
1-Jun
6-Jun
13-Jun
21-Jun
27-Jun
6-Jul
11-Jul
18-Jul
Mesodinium rubrum
Heterocapsa rotundata
Heterocapsa triquetra
Dinophysis acuminata
Alexandrium fundyense
Gyrodinium spp.
Amoebophrya spp.
other Dinophyceae
Thalassiosira spp.
Skeletonema spp.
other Diatoms
Dictyochophyceae
other algae

632 **Figure 2.**

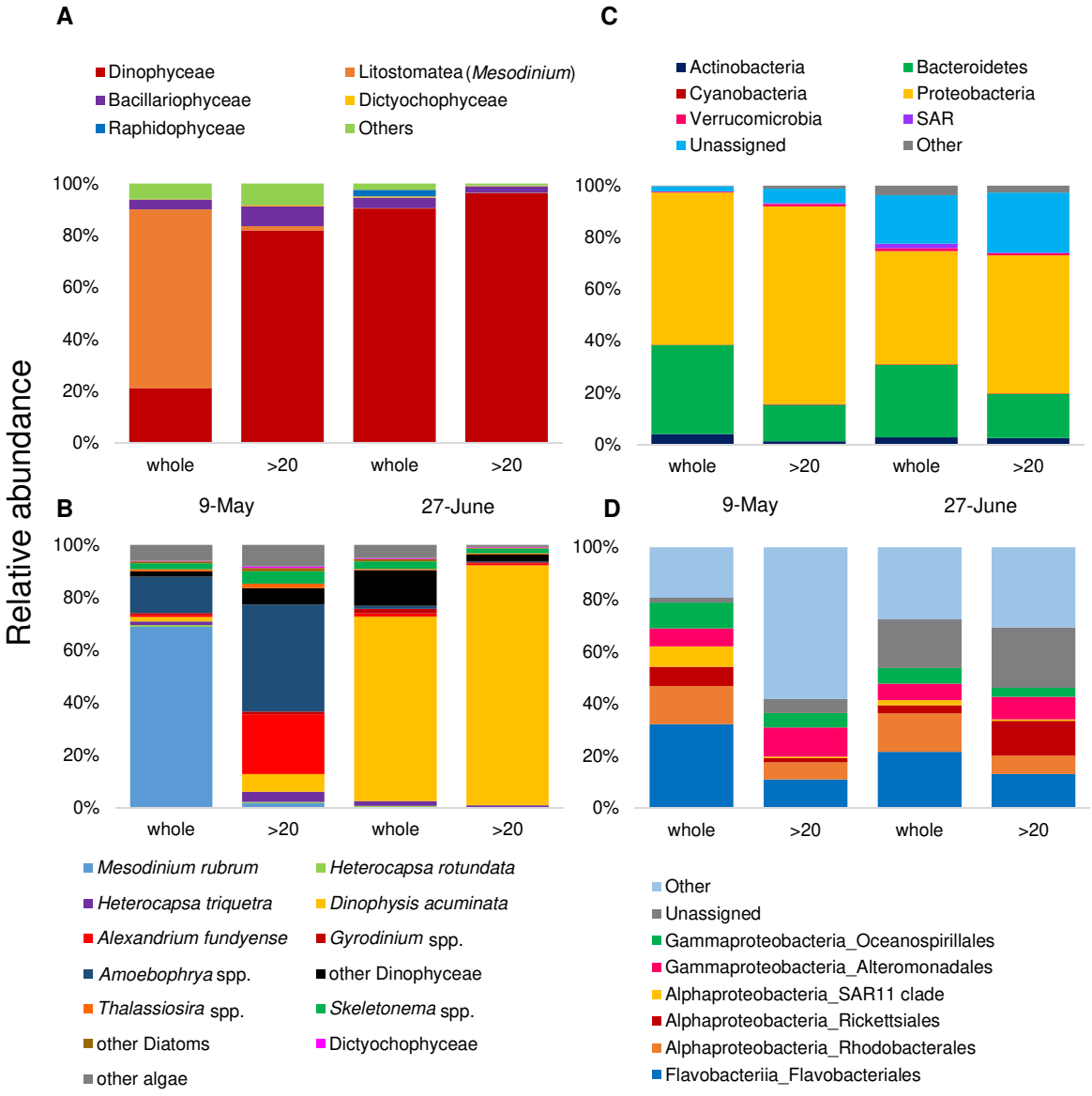


633

634

635 **Figure 3.**

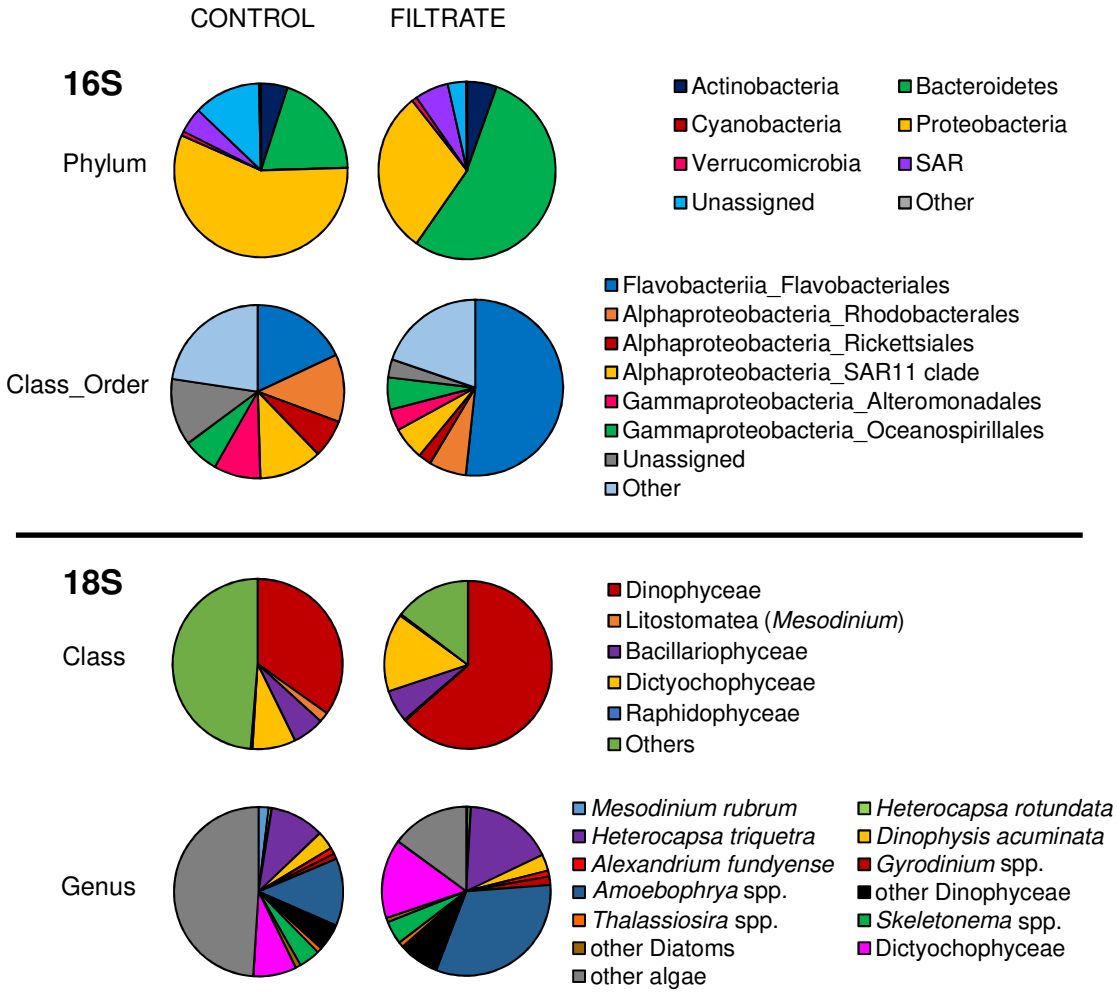
636



637

638 **Figure 4.**

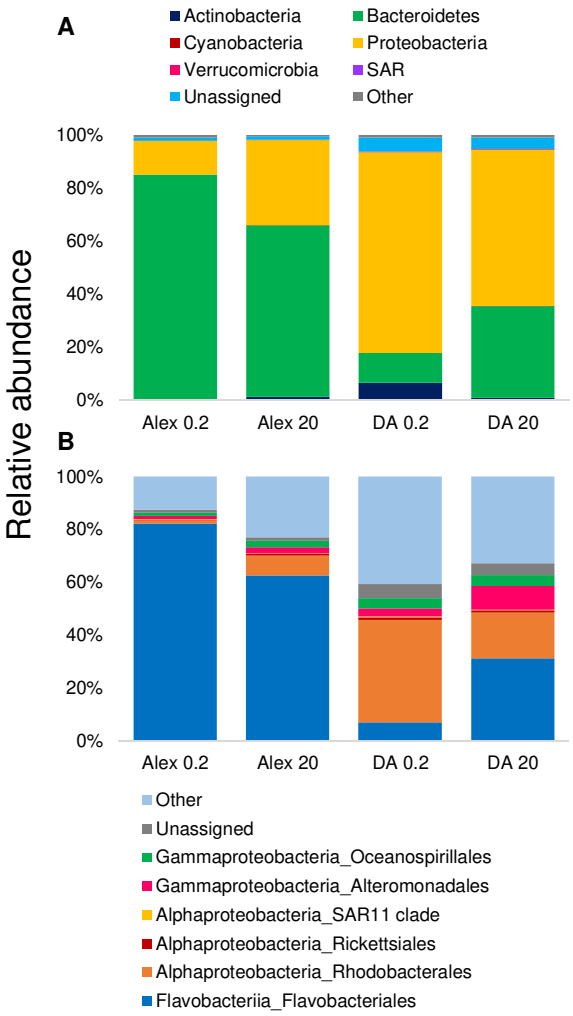
639



640

641 **Figure 5.**

642

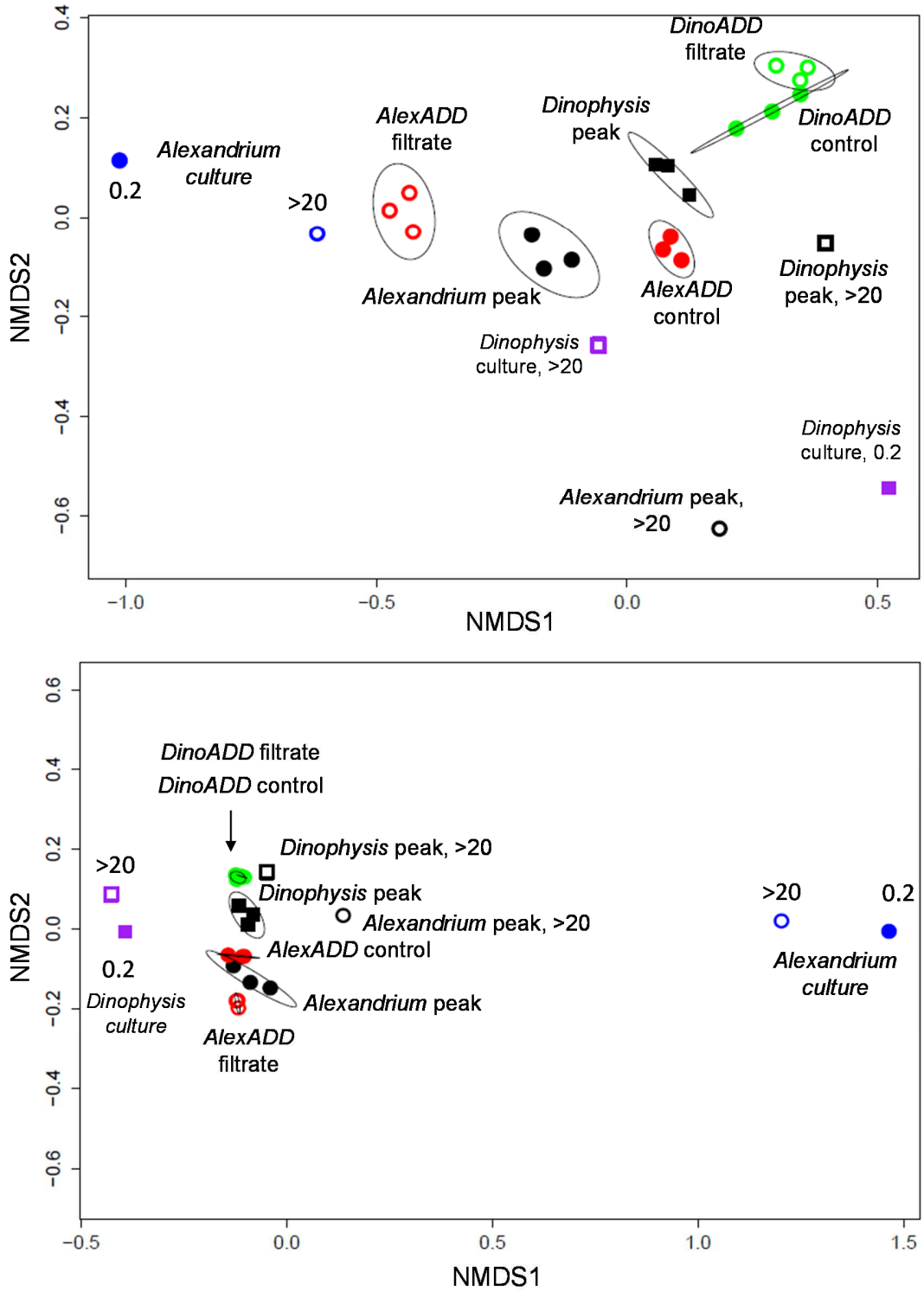


643

644

645 **Figure 6.**

646



647

Supplementary Tables:

Table S1. QIIME outputs for the sequencing of the V7/V8 region of the 18S rRNA gene, including split libraries output (demultiplexed reads), total UCLUST assigned reads and the total number of algal assigned reads for time series, and experimental (AlexADD and DinoADD) samples and size fractions. % algal reads= (total number of algal assigned reads divided by the total number of UCLUST assigned reads) x 100. AlexADD and DinoADD= *Alexandrium* and *Dinophysis* addition experiments, respectively.

Table S2. QIIME outputs for the sequencing of the V4 variable region of the 16S rRNA gene, including split libraries output (demultiplexed reads), total UCLUST assigned reads and the total number of chloroplast and mitrochondria assigned reads for time series, culture and experimental (AlexADD and DinoADD) samples and size fractions. AlexADD and DinoADD= *Alexandrium* and *Dinophysis* addition experiments, respectively.

661 **Table S1.**

size fraction	sample	split libraries output	UCLUST assigned total reads	UCLUST assigned algal reads	% algal reads
	Unassigned	452,066	422,175	368,882	87
	Time series				
0.2	26-Apr	31,929	29,348	26,846	91
0.2	3-May	28,258	25,853	22,474	87
0.2	9-May	61,523	59,143	42,010	71
0.2	16-May	48,520	46,110	29,703	64
0.2	24-May	80,920	75,313	51,810	69
0.2	1-Jun	89,280	83,505	75,665	91
0.2	6-Jun	68,263	63,774	60,089	94
0.2	13-Jun	30,803	28,569	25,816	90
0.2	21-Jun	49,241	47,141	45,233	96
0.2	27-Jun	54,582	52,036	49,461	95
0.2	6-Jul	48,736	46,407	44,808	97
0.2	11-Jul	27,608	25,112	20,421	81
0.2	18-Jul	47,917	45,505	15,960	35
>20	9-May	40,229	37,271	13,130	35
>20	27-Jun	165,270	160,618	157,252	98
	AlexADD				
0.2	Control 1	30,067	28,038	19,026	68
0.2	Control 2	34,058	31,639	21,540	68
0.2	Control 3	34,181	31,658	22,807	72
0.2	+ Alex 1	40,162	38,019	15,376	40
0.2	+ Alex 2	50,539	47,958	31,614	66
0.2	+ Alex 3	92,503	86,572	77,747	90
	DinoADD				
0.2	Control 1	62,340	60,223	54,201	90
0.2	Control 2	61,152	59,263	57,016	96
0.2	Control 3	68,612	66,813	65,018	97
0.2	+ Dino 1	142,996	138,346	133,817	97
0.2	+ Dino 2	79,649	77,177	75,334	98
0.2	+ Dino 3	125,951	122,347	119,425	98
	Total	2,147,355	2,035,933	1,742,481	86

662

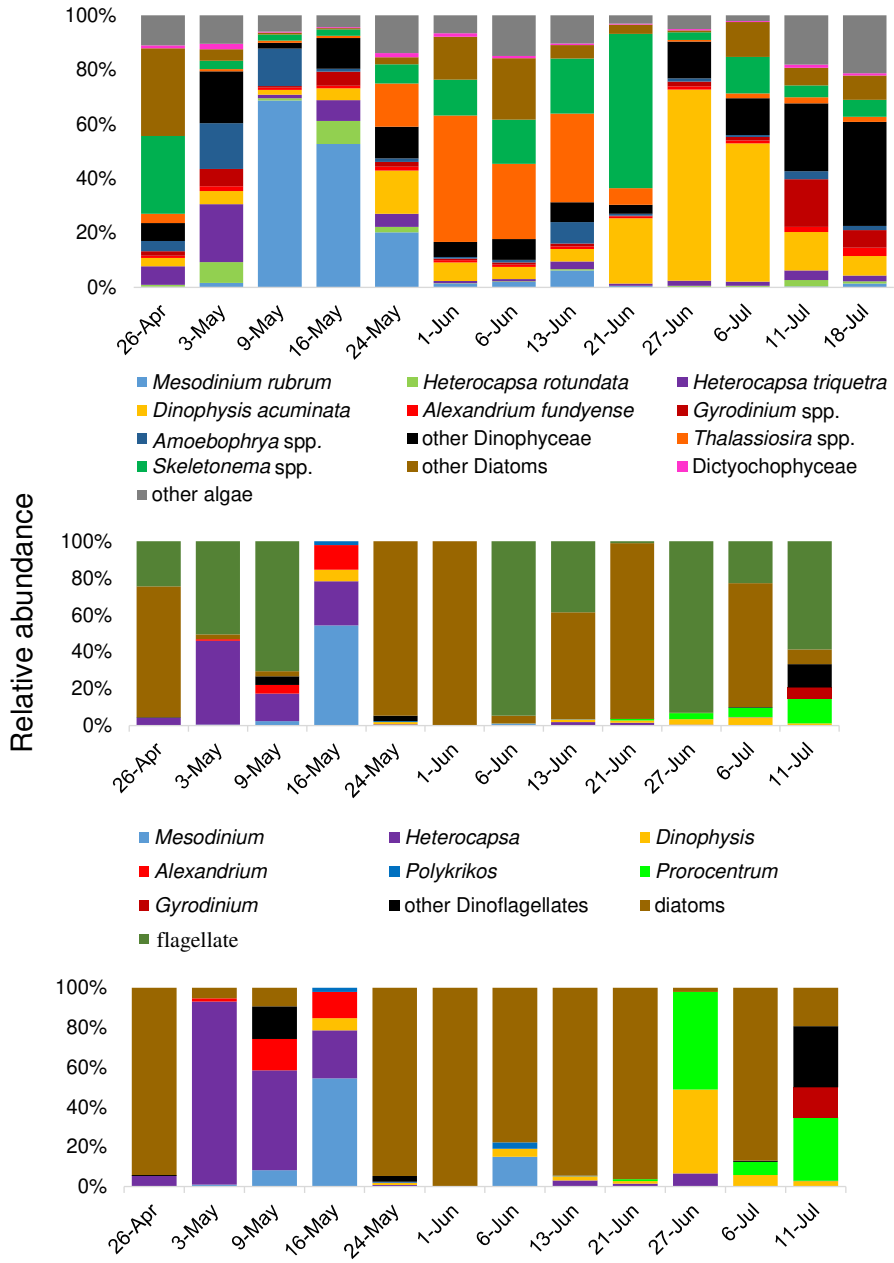
663 **Table S2.**

size fraction	sample	split libraries output	UCLUST assigned total reads	# of reads assigned as chloroplasts	# of reads assigned as mitochondria
	Unassigned	1,082,447	944,829	225,807	13,221
	Time series				
0.2	26-Apr	132,919	121,718	24,179	520
0.2	3-May	65,801	60,886	11,582	1,189
0.2	9-May	59,293	57,110	6,722	287
0.2	16-May	127,767	121,621	17,105	287
0.2	24-May	159,942	151,458	24,739	389
0.2	1-Jun	111,623	107,084	25,012	403
0.2	6-Jun	129,811	124,963	36,802	598
0.2	13-Jun	126,142	119,359	33,780	390
0.2	21-Jun	136,191	129,218	41,072	624
0.2	27-Jun	112,113	97,688	62,953	2,022
0.2	6-Jul	142,151	130,826	47,116	265
0.2	11-Jul	125,039	112,586	28,535	311
0.2	18-Jul	51,908	47,364	8,239	120
>20	9-May	119,419	84,142	20,636	1,022
>20	27-Jun	31,256	26,519	14,093	2,917
	Cultures				
0.2	*Alexandrium*	24,501	17,791	276	18
>20	*Alexandrium*	35,735	17,671	610	28
0.2	*Dinophysis*	47,245	45,579	10,714	91
>20	*Dinophysis*	42,093	40,907	21,171	50
	AlexADD				
0.2	Control 1	102,215	86,926	22,539	2,680
0.2	Control 2	132,743	111,867	28,990	3,136
0.2	Control 3	112,746	96,890	22,847	2,719
0.2	+ Alex 1	142,295	124,812	26,068	4,274
0.2	+ Alex 2	116,393	102,937	23,517	3,456
0.2	+ Alex 3	142,580	126,575	24,613	4,162
	Dinoadd				
0.2	Control 1	56,338	54,590	12,460	70
0.2	Control 2	122,331	118,238	22,241	179
0.2	Control 3	55,046	53,238	13,883	77
0.2	+ Dino 1	58,050	56,188	14,160	73
0.2	+ Dino 2	67,803	65,657	17,208	124
0.2	+ Dino 3	122,383	119,105	31,517	174
	Total	4,094,319	3,676,342	921,186	45,876

664

Supplementary Figures:

Fig. S1. QIIME species specific analysis (Top). Lugol's preserved samples counted via light microscopy. This includes flagellates that were mostly dominated by Euglenoids (Middle). Lugol's preserved samples counted via light microscopy with flagellate removed (Bottom). Lower legend is used for both 'Middle' and 'Bottom' figures.



673 **References**

674 Adachi, M., Kanno, T., Matsubara, T., Nishijima, T., Itakura, S., Yamaguchi, M., 1999.
675 Promotion of cyst formation in the toxic dinoflagellate *Alexandrium* (Dinophyceae) by
676 natural bacterial assemblages from Hiroshima Bay, Japan. Marine Ecology Progress
677 Series 191, 175-185.
678 Adachi, M., Kanno, T., Okamoto, R., Itakura, S., Yamaguchi, M., Nishijima, T., 2003.
679 Population structure of *Alexandrium* (Dinophyceae) cyst formation-promoting bacteria in
680 Hiroshima Bay, Japan. Appl. Environ. Microbiol. 69(11), 6560-6568.
681 Adachi, M., Matsubara, T., Okamoto, R., Nishijima, T., Itakura, S., Yamaguchi, M., 2002.
682 Inhibition of cyst formation in the toxic dinoflagellate *Alexandrium* (Dinophyceae) by
683 bacteria from Hiroshima Bay, Japan. Aquat. Microb. Ecol. 26(3), 223-233.
684 Albinsson, M.E., Negri, A.P., Blackburn, S.I., Bolch, C.J.S., 2014. Bacterial community affects
685 toxin production by *Gymnodinium catenatum*. PLoS One 9(8), e104623.
686 Anderson, D.M., Burkholder, J.M., Cochlan, W.P., Glibert, P.M., Gobler, C.J., Heil, C.A.,
687 Kudela, R.M., Parsons, M.L., Rensel, J., Townsend, D.W., 2008. Harmful algal blooms
688 and eutrophication: Examining linkages from selected coastal regions of the United
689 States. Harmful Algae 8(1), 39-53.
690 Anderson, D.M., Cembella, A.D., Hallegraeff, G.M., 2012. Progress in understanding harmful
691 algal blooms: paradigm shifts and new technologies for research, monitoring, and
692 management. Ann Rev Mar Sci 4, 143-176.
693 Anderson, D.M., Kulis, D.M., Keafer, B.A., Gribble, K.E., Marin, R., Scholin, C.A., 2005.
694 Identification and enumeration of *Alexandrium* spp. from the Gulf of Maine using
695 molecular probes. Deep Sea Res. I 52(19-21), 2467-2490.
696 Anglés, S., Garces, E., Hattenrath-Lehmann, T.K., Gobler, C.J., 2012. In situ life-cycle stages of
697 *Alexandrium fundyense* during bloom development in Northport Harbor (New York,
698 USA). Harmful Algae 16, 20-26.
699 Bratbak, G., Thingstad, T.F., 1985. Phytoplankton-bacteria interactions: an apparent paradox?
700 Analysis of a model system with both competition and commensalism. Mar Ecol Prog
701 Ser 25(1), 23-30.
702 Brosnahan, M.L., Velo-Suárez, L., Ralston, D.K., Fox, S.E., Sehein, T.R., Shalapyonok, A.,
703 Sosik, H.M., Olson, R.J., Anderson, D.M., 2015. Rapid growth and concerted sexual
704 transitions by a bloom of the harmful dinoflagellate *Alexandrium fundyense*
705 (Dinophyceae). Limnol. Oceanogr. 60(6), 2059-2078.
706 Buchan, A., LeCleir, G.R., Gulvik, C.A., Gonzalez, J.M., 2014. Master recyclers: features and
707 functions of bacteria associated with phytoplankton blooms. Nat Rev Micro 12(10), 686-
708 698.
709 Caporaso, J.G., Bittinger, K., Bushman, F.D., DeSantis, T.Z., Andersen, G.L., Knight, R., 2010a.
710 PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics
711 26(2), 266-267.
712 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K.,
713 Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights,
714 D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M.,
715 Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T.,
716 Zaneveld, J., Knight, R., 2010b. QIIME allows analysis of high-throughput community
717 sequencing data. Nature methods 7(5), 335-336.

718 Chang, F.H., Anderson, C., Boustead, N.C., 1990. First record of a *Heterosigma*
719 (Raphidophyceae) bloom with associated mortality of cage-reared salmon in Big Glory
720 Bay, New Zealand. New Zealand Journal of Marine and Freshwater Research 24(4), 461-
721 469.
722 Coats, D.W., Adam, E.J., Gallegos, C.L., Hedrick, S., 1996. Parasitism of photosynthetic
723 dinoflagellates in a shallow subestuary of Chesapeake Bay, USA. Aquat. Microb. Ecol.
724 11(1), 1-9.
725 Coats, D.W., Park, M.G., 2002. Parasitism of photosynthetic dinoflagellates by three strains of
726 *Amoebophrya* (Dinophyta): Parasite survival, infectivity, generation time, and host
727 specificity. J. Phycol. 38(3), 520-528.
728 Colin, S.P., Dam, H.G., 2002. Latitudinal differentiation in the effects of the toxic dinoflagellate
729 *Alexandrium* spp. on the feeding and reproduction of populations of the copepod *Acartia*
730 *hudsonica*. Harmful Algae 1(1), 113-125.
731 Croft, M.T., Lawrence, A.D., Raux-Deery, E., Warren, M.J., Smith, A.G., 2005. Algae acquire
732 vitamin B_{12} through a symbiotic relationship with bacteria. Nature 438(7064), 90-93.
733 D'Alelio, D., Libralato, S., Wyatt, T., Ribera d'Alcalà, M., 2016. Ecological-network models
734 link diversity, structure and function in the plankton food-web. Scientific Reports 6,
735 21806.
736 Daly, K.L., Smith, W.O., 1993. Physical-biological interactions influencing marine plankton
737 production. Annual Review of Ecology and Systematics 24, 555-585.
738 de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., Lara, E., Berney, C., Le
739 Bescot, N., Probert, I., Carmichael, M., Poulain, J., Romac, S., Colin, S., Aury, J.-M.,
740 Bittner, L., Chaffron, S., Dunthorn, M., Engelen, S., Flegontova, O., Guidi, L., Horák, A.,
741 Jaillon, O., Lima-Mendez, G., Lukeš, J., Malviya, S., Morard, R., Mulot, M., Scalco, E.,
742 Siano, R., Vincent, F., Zingone, A., Dimier, C., Picheral, M., Searson, S., Kandels-Lewis,
743 S., Acinas, S.G., Bork, P., Bowler, C., Gorsky, G., Grimsley, N., Hingamp, P., Iudicone,
744 D., Not, F., Ogata, H., Pesant, S., Raes, J., Sieracki, M.E., Speich, S., Stemmann, L.,
745 Sunagawa, S., Weissenbach, J., Wincker, P., Karsenti, E., 2015. Eukaryotic plankton
746 diversity in the sunlit ocean. Science 348(6237).
747 Dempster, E.L., Pryor, K.V., Francis, D., Young, J.E., Rogers, H.J., 1999. Rapid DNA extraction
748 from ferns for PCR-based analyses. Biotechniques 27, 66-68.
749 Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics
750 26(19), 2460-2461.
751 Falkowski, P.G., Fenchel, T., Delong, E.F., 2008. The microbial engines that drive Earth's
752 biogeochemical cycles. Science 320(5879), 1034-1039.
753 Fernandes, L.F., Hubbard, K.A., Richlen, M.L., Smith, J., Bates, S.S., Ehrman, J., Léger, C.,
754 Mafra Jr, L.L., Kulis, D., Quilliam, M., Libera, K., McCauley, L., Anderson, D.M., 2014.
755 Diversity and toxicity of the diatom *Pseudo-nitzschia* Peragallo in the Gulf of Maine,
756 Northwestern Atlantic Ocean. Deep Sea Research Part II: Topical Studies in
757 Oceanography 103, 139-162.
758 Fistarol, G.O., Legrand, C., Selander, E., Hummert, C., Stolte, W., Graneli, E., 2004. Allelopathy
759 in *Alexandrium* spp.: effect on a natural plankton community and on algal monocultures.
760 Aquat. Microb. Ecol. 35(1), 45-56.
761 Garces, E., Vila, M., Rene, A., Alonso-Saez, L., Angles, S., Luglie, A., Maso, M., Gasol, J.M.,
762 2007. Natural bacterioplankton assemblage composition during blooms of *Alexandrium*

763 spp. (Dinophyceae) in NW Mediterranean coastal waters. Aquat. Microb. Ecol. 46(1), 55-
764 70.
765 Glibert, P.M., Anderson, D.M., Gentien, P., Granéli, E., Sellner, K.G., 2005. The global,
766 complex phenomena of harmful algal blooms. Oceanography 18(2), 136-147.
767 Glibert, P.M., Legrand, C., 2006. The diverse nutrient strategies of harmful algae: focus on
768 osmotrophy, In: Graneli, E., Turner, J.T. (Eds.), Ecology of Harmful Algae, Ecological
769 Studies. Springer-Verlag, Berlin Heidelberg, Germany, pp. 163-175.
770 Gobler, C.J., Deonarine, S., Leigh-Bell, J., Gastrich, M.D., Anderson, O.R., Wilhelm, S.W.,
771 2004. Ecology of phytoplankton communities dominated by *Aureococcus*
772 *anophagefferens*: the role of viruses, nutrients, and microzooplankton grazing. Harmful
773 Algae 3(4), 471-483.
774 Godhe, A., Asplund, M.E., Härnström, K., Saravanan, V., Tyagi, A., Karunasagar, I., 2008.
775 Quantification of diatom and dinoflagellate biomasses in coastal marine seawater samples
776 by real-time PCR. Appl. Environ. Microbiol. 74(23), 7174-7182.
777 Guillard, R.R., Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana*
778 Hustedt, and *Detonula confervacea* Cleve. Can. J. Microbiol. 8(2), 229-239.
779 Hadziavdic, K., Lekang, K., Lanzen, A., Jonassen, I., Thompson, E.M., Troedsson, C., 2014.
780 Characterization of the 18S rRNA gene for designing universal eukaryote specific
781 primers. PLoS One 9(2), e87624.
782 Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase.
783 Phycologia 32(2), 79-99.
784 Hallegraeff, G.M., 2010. Ocean climate change, phytoplankton community responses, and
785 harmful algal blooms: A formidable predictive challenge. J. Phycol. 46(2), 220-235.
786 Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological statistics software
787 package for education and data analysis. Palaeontologia Electronica 4(1), 9pp.
788 Hamsher, S.E., LeGresley, M.M., Martin, J.L., Saunders, G.W., 2013. A comparison of
789 morphological and molecular-based surveys to estimate the species richness of
790 *Chaetoceros* and *Thalassiosira* (Bacillariophyta), in the Bay of Fundy. PLoS One 8(10),
791 e73521.
792 Hasegawa, Y., Martin, J.L., Giewat, M.W., Rooney-Varga, J.N., 2007. Microbial community
793 diversity in the phycosphere of natural populations of the toxic alga, *Alexandrium*
794 *fundyense*. Environ. Microbiol. 9(12), 3108-3121.
795 Hasle, G.R., 1978. The inverted microscope method. Monogr. Oceanogr. Meth. 6, 88-96.
796 Hattenrath-Lehmann, T., Gobler, C.J., 2015. The contribution of inorganic and organic nutrients
797 to the growth of a North American isolate of the mixotrophic dinoflagellate, *Dinophysis*
798 *acuminata*. Limnol. Oceanogr. 60(5), 1588-1603.
799 Hattenrath-Lehmann, T.K., Gobler, C.J., 2011. Allelopathic inhibition of competing
800 phytoplankton by North American strains of the toxic dinoflagellate, *Alexandrium*
801 *fundyense*: Evidence from field experiments, laboratory experiments, and bloom events.
802 Harmful Algae 11, 106-116.
803 Hattenrath-Lehmann, T.K., Marcoval, M.A., Berry, D.L., Fire, S., Wang, Z., Morton, S.L.,
804 Gobler, C.J., 2013. The emergence of *Dinophysis acuminata* blooms and DSP toxins in
805 shellfish in New York waters. Harmful Algae 26(0), 33-44.
806 Hattenrath-Lehmann, T.K., Marcoval, M.A., Mittlesdorf, H., Goleski, J.A., Wang, Z., Haynes,
807 B., Morton, S.L., Gobler, C.J., 2015a. Nitrogenous nutrients promote the growth and

toxicity of *Dinophysis acuminata* during estuarine bloom events. PLoS One 10(4), e0124148.

Hattenrath-Lehmann, T.K., Smith, J.L., Wallace, R.B., Merlo, L.R., Koch, F., Mittelsdorf, H., Goleski, J.A., Anderson, D.M., Gobler, C.J., 2015b. The effects of elevated CO_2 on the growth and toxicity of field populations and cultures of the saxitoxin-producing dinoflagellate, *Alexandrium fundyense*. Limnol. Oceanogr. 60(1), 198-214.

Hattenrath, T.K., Anderson, D.M., Gobler, C.J., 2010. The influence of anthropogenic nitrogen loading and meteorological conditions on the dynamics and toxicity of *Alexandrium fundyense* blooms in a New York (USA) estuary. Harmful Algae 9(4), 402-412.

Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W.C., Dortch, Q., Gobler, C.J., Heil, C.A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H.G., Sellner, K., Stockwell, D.A., Stoecker, D.K., Suddleson, M., 2008. Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae 8(1), 3-13.

Hemphill, A., Vonlaufen, N., Naguleswaran, A., 2006. Cellular and immunological basis of the host-parasite relationship during infection with *Neospora caninum*. Parasitology 133(Pt 3), 261-278.

Hoagland, P., Anderson, D., Kaoru, Y., White, A., 2002. The economic effects of harmful algal blooms in the United States: estimates, assessment issues, and information needs. Estuaries 25(4), 819-837.

Inaba, N., Watanabe, T., Sakami, T., Nishi, H., Tahara, Y., Imai, I., 2013. Temporal and spatial distribution of algicidal and growth-inhibiting bacteria in the coastal sea of southwest Japan. J. Plankton Res.

Jasti, S., Sieracki, M.E., Poulton, N.J., Giewat, M.W., Rooney-Varga, J.N., 2005. Phylogenetic diversity and specificity of bacteria closely associated with A*lexandrium* spp. and other phytoplankton. Appl. Environ. Microbiol. 71(7), 3483-3494.

Jauzein, C., Evans, A.N., Erdner, D.L., 2015. The impact of associated bacteria on morphology and physiology of the dinoflagellate *Alexandrium tamarense*. Harmful Algae 50, 65-75.

Jin, D., Hoagland, P., 2008. The value of harmful algal bloom predictions to the nearshore commercial shellfish fishery in the Gulf of Maine. Harmful Algae 7(6), 772-781.

Jin, D., Thunberg, E., Hoagland, P., 2008. Economic impact of the 2005 red tide event on commercial shellfish fisheries in New England. Ocean & Coastal Management 51(5), 420-429.

John, U., Litaker, R.W., Montresor, M., Murray, S., Brosnahan, M.L., Anderson, D.M., 2014. Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonomy: The introduction of five species with emphasis on molecular-based (rDNA) classification. Protist 165(6), 779-804.

Kaczmarska, I., Ehrman, J.M., Bates, S.S., Green, D.H., Léger, C., Harris, J., 2005. Diversity and distribution of epibiotic bacteria on *Pseudo-nitzschia multiseries* (Bacillariophyceae) in culture, and comparison with those on diatoms in native seawater. Harmful Algae 4(4), 725-741.

Kazamia, E., Czesnick, H., Thi, T.V.N., Croft, M.T., Sherwood, E., Sasso, S., Hodson, S.J., Warren, M.J., Smith, A.G., 2012. Mutualistic interactions between vitamin B_{12}-dependent algae and heterotrophic bacteria exhibit regulation. Environ. Microbiol. 14(6), 1466-1476.

852 Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013.
853 Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-
854 generation sequencing-based diversity studies. Nucleic Acids Research 41(1), e1-e1.
855 Koch, F., Burson, A., Tang, Y.Z., Collier, J.L., Fisher, N.S., Sañudo-Wilhelmy, S., Gobler, C.J.,
856 2014. Alteration of plankton communities and biogeochemical cycles by harmful
857 *Cochlodinium polykrikoides* (Dinophyceae) blooms. Harmful Algae 33(0), 41-54.
858 Koukaras, K., Nikolaidis, G., 2004. *Dinophysis* blooms in Greek coastal waters (Thermaikos
859 Gulf, NW Aegean Sea). J. Plankton Res. 26(4), 445-457.
860 Kummerer, K., 2009. Antibiotics in the aquatic environment--a review--part I. Chemosphere
861 75(4), 417-434.
862 Lelong, A., Hégaret, H., Soudant, P., 2014. Link between domoic acid production and cell
863 physiology after exchange of bacterial communities between toxic *Pseudo-nitzschia*
864 *multiseries* and non-toxic *Pseudo-nitzschia delicatissima*. Marine Drugs 12(6), 3587-
865 3607.
866 Li, J., Zhang, J., Liu, L., Fan, Y., Li, L., Yang, Y., Lu, Z., Zhang, X., 2015a. Annual periodicity
867 in planktonic bacterial and archaeal community composition of eutrophic Lake Taihu.
868 Scientific Reports 5, 15488.
869 Li, Y., Zhu, H., Lei, X., Zhang, H., Cai, G., Chen, Z., Fu, L., Xu, H., Zheng, T., 2015b. The
870 death mechanism of the harmful algal bloom species *Alexandrium tamarense* induced by
871 algicidal bacterium *Deinococcus* sp. Y35. Frontiers in Microbiology 6, 992.
872 Lima-Mendez, G., Faust, K., Henry, N., Decelle, J., Colin, S., Carcillo, F., Chaffron, S., Ignacio-
873 Espinosa, J.C., Roux, S., Vincent, F., Bittner, L., Darzi, Y., Wang, J., Audic, S., Berline,
874 L., Bontempi, G., Cabello, A.M., Coppola, L., Cornejo-Castillo, F.M., d'Ovidio, F., De
875 Meester, L., Ferrera, I., Garet-Delmas, M.-J., Guidi, L., Lara, E., Pesant, S., Royo-
876 Llonch, M., Salazar, G., Sánchez, P., Sebastian, M., Souffreau, C., Dimier, C., Picheral,
877 M., Searson, S., Kandels-Lewis, S., Gorsky, G., Not, F., Ogata, H., Speich, S.,
878 Stemmann, L., Weissenbach, J., Wincker, P., Acinas, S.G., Sunagawa, S., Bork, P.,
879 Sullivan, M.B., Karsenti, E., Bowler, C., de Vargas, C., Raes, J., 2015. Determinants of
880 community structure in the global plankton interactome. Science 348(6237).
881 Manage, P.M., Edwards, C., Singh, B.K., Lawton, L.A., 2009. Isolation and identification of
882 novel microcystin-degrading bacteria. Appl. Environ. Microbiol. 75(21), 6924-6928.
883 Mayali, X., Franks, P.J.S., Azam, F., 2007. Bacterial induction of temporary cyst formation by
884 the dinoflagellate *Lingulodinium polyedrum*. Aquat. Microb. Ecol. 50, 51-62.
885 Moustafa, A., Evans, A.N., Kulis, D.M., Hackett, J.D., Erdner, D.L., Anderson, D.M.,
886 Bhattacharya, D., 2010. Transcriptome profiling of a toxic dinoflagellate reveals a gene-
887 rich protist and a potential impact on gene expression due to bacterial presence. PLoS
888 One 5(3), e9688.
889 Park, B.S., Kim, J.-H., Kim, J.H., Gobler, C.J., Baek, S.H., Han, M.-S., 2015. Dynamics of
890 bacterial community structure during blooms of *Cochlodinium polykrikoides*
891 (Gymnodiniales, Dinophyceae) in Koren coastal waters. Harmful Algae In press.
892 Park, M.G., Kim, S., Kim, H.S., Myung, G., Kang, Y.G., Yih, W., 2006. First successful culture
893 of the marine dinoflagellate *Dinophysis acuminata*. Aquat. Microb. Ecol. 45(2), 101-106.
894 Park, M.G., Kim, S., Shin, E.-Y., Yih, W., Coats, D.W., 2013. Parasitism of harmful
895 dinoflagellates in Korean coastal waters. Harmful Algae 30, Supplement 1, S62-S74.
896 Prokopowich, C.D., Gregory, T.R., Crease, T.J., 2003. The correlation between rDNA copy
897 number and genome size in eukaryotes. Genome 46(1), 48-50.

898 Raho, N., Pizarro, G., Escalera, L., Reguera, B., Marin, I., 2008. Morphology, toxin composition
899 and molecular analysis of *Dinophysis ovum* Schutt, a dinoflagellate of the "*Dinophysis*
900 *acuminata* complex". Harmful Algae 7(6), 839-848.
901 Ralston, D.K., Brosnahan, M.L., Fox, S.E., Lee, K.D., Anderson, D.M., 2015. Temperature and
902 residence time controls on an estuarine harmful algal bloom: Modeling hydrodynamics
903 and *Alexandrium fundyense* in Nauset estuary. Estuaries Coasts 38(6), 2240-2258.
904 Ramanan, R., Kim, B.-H., Cho, D.-H., Oh, H.-M., Kim, H.-S., 2016. Algae–bacteria interactions:
905 Evolution, ecology and emerging applications. Biotechnology Advances 34(1), 14-29.
906 Ressurreição, M., Elbeyioglu, F., Kirk, R.S., Rollinson, D., Emery, A.M., Page, N.M., Walker,
907 A.J., 2016. Molecular characterization of host-parasite cell signalling in *Schistosoma*
908 *mansoni* during early development. Scientific Reports 6, 35614.
909 Samarajeewa, A.D., Hammad, A., Masson, L., Khan, I.U.H., Scroggins, R., Beaudette, L.A.,
910 2015. Comparative assessment of next-generation sequencing, denaturing gradient gel
911 electrophoresis, clonal restriction fragment length polymorphism and cloning-sequencing
912 as methods for characterizing commercial microbial consortia. Journal of Microbiological
913 Methods 108, 103-111.
914 Schrey, S.E., Carpenter, E.J., Anderson, D.M., 1984. The abundance and distribution of the toxic
915 dinoflagellate, *Gonyaulax tamarensis*, in Long Island estuaries. Estuaries 7(4), 472-477.
916 Shetty, K.G., Huntzicker, J.V., Rein, K.S., Jayachandran, K., 2010. Biodegradation of polyether
917 algal toxins–Isolation of potential marine bacteria. Journal of environmental science and
918 health. Part A, Toxic/hazardous substances & environmental engineering 45(14), 1850-
919 1857.
920 Sison-Mangus, M.P., Jiang, S., Tran, K.N., Kudela, R.M., 2014. Host-specific adaptation
921 governs the interaction of the marine diatom, *Pseudo-nitzschia* and their microbiota. The
922 ISME Journal 8(1), 63-76.
923 Smayda, T.J., 1997. Harmful algal blooms: Their ecophysiology and general relevance to
924 phytoplankton blooms in the sea. Limnol. Oceanogr. 42(5), 1137-1153.
925 Smayda, T.J., 2002. Adaptive ecology, growth strategies and the global bloom expansion of
926 dinoflagellates. J. Oceanogr. 58(2), 281-294.
927 Su, J., Yang, X., Zhou, Y., Zheng, T., 2011. Marine bacteria antagonistic to the harmful algal
928 bloom species *Alexandrium tamarense* (Dinophyceae). Biological Control 56(2), 132-
929 138.
930 Sunagawa, S., Coelho, L.P., Chaffron, S., Kultima, J.R., Labadie, K., Salazar, G., Djahanschiri,
931 B., Zeller, G., Mende, D.R., Alberti, A., Cornejo-Castillo, F.M., Costea, P.I., Cruaud, C.,
932 d'Ovidio, F., Engelen, S., Ferrera, I., Gasol, J.M., Guidi, L., Hildebrand, F., Kokoszka, F.,
933 Lepoivre, C., Lima-Mendez, G., Poulain, J., Poulos, B.T., Royo-Llonch, M., Sarmento,
934 H., Vieira-Silva, S., Dimier, C., Picheral, M., Searson, S., Kandels-Lewis, S., Bowler, C.,
935 de Vargas, C., Gorsky, G., Grimsley, N., Hingamp, P., Iudicone, D., Jaillon, O., Not, F.,
936 Ogata, H., Pesant, S., Speich, S., Stemmann, L., Sullivan, M.B., Weissenbach, J.,
937 Wincker, P., Karsenti, E., Raes, J., Acinas, S.G., Bork, P., 2015. Structure and function of
938 the global ocean microbiome. Science 348(6237).
939 Taylor, F.J.R., 1968. Parasitism of the toxin-producing dinoflagellate *Gonyaulax catenella* by the
940 endoparasitic dinoflagellate *Amoebophrya ceratii*. Journal of the Fisheries Research
941 Board of Canada 25(10), 2241-2245.
942 Teeling, H., Fuchs, B.M., Bennke, C.M., Krüger, K., Chafee, M., Kappelmann, L., Reintjes, G.,
943 Waldmann, J., Quast, C., Glöckner, F.O., Lucas, J., Wichels, A., Gerdts, G., Wiltshire,

944 K.H., Amann, R.I., 2016. Recurring patterns in bacterioplankton dynamics during coastal
945 spring algae blooms. eLife 5, e11888.
946 Tillmann, U., Alpermann, T.L., da Purificacao, R.C., Krock, B., Cembella, A., 2009. Intra-
947 population clonal variability in allelochemical potency of the toxigenic dinoflagellate
948 *Alexandrium tamarense*. Harmful Algae 8(5), 759-769.
949 Velo-Suárez, L., Brosnahan, M.L., Anderson, D.M., McGillicuddy, D.J., 2013. A quantitative
950 assessment of the role of the parasite *Amoebophrya* in the termination of *Alexandrium*
951 *fundyense* blooms within a small coastal embayment. PLoS One 8(12), e81150.
952 Wallace, R.B., Gobler, C.J., 2015. Factors controlling blooms of microalgae and macroalgae
953 (*Ulva rigida*) in a eutrophic, urban estuary: Jamaica Bay, NY, USA. Estuaries Coasts
954 38(2), 519-533.
955 Weissbach, A., Béchemin, C., Genauzeau, S., Rudström, M., Legrand, C., 2012. Impact of
956 *Alexandrium tamarense* allelochemicals on DOM dynamics in an estuarine microbial
957 community. Harmful Algae 13, 58-64.
958 Weissbach, A., Rudström, M., Olofsson, M., Béchemin, C., Icely, J., Newton, A., Tillmann, U.,
959 Legrand, C., 2011. Phytoplankton allelochemical interactions change microbial food web
960 dynamics. Limnol. Oceanogr. 56(3), 899-909.
961 Weissbach, A., Tillmann, U., Legrand, C., 2010. Allelopathic potential of the dinoflagellate
962 *Alexandrium tamarense* on marine microbial communities. Harmful Algae 10(1), 9-18.
963 Wichels, A., Hummert, C., Elbrächter, M., Luckas, B., Schütt, C., Gerdts, G., 2004. Bacterial
964 diversity in toxic *Alexandrium tamarense* blooms off the Orkney Isles and the Firth of
965 Forth. Helgoland Marine Research 58(2), 93-103.
966 Xiao, X., Sogge, H., Lagesen, K., Tooming-Klunderud, A., Jakobsen, K.S., Rohrlack, T., 2014.
967 Use of high throughput sequencing and light microscopy show contrasting results in a
968 study of phytoplankton occurrence in a freshwater environment. PLoS One 9(8),
969 e106510.
970 Yang, C., Li, Y., Zhou, B., Zhou, Y., Zheng, W., Tian, Y., Van Nostrand, J.D., Wu, L., He, Z.,
971 Zhou, J., Zheng, T., 2015. Illumina sequencing-based analysis of free-living bacterial
972 community dynamics during an *Akashiwo sanguine* bloom in Xiamen sea, China.
973 Scientific Reports 5, 8476.
974 Yoshino, T.P., Boyle, J.P., Humphries, J.E., 2001. Receptor-ligand interactions and cellular
975 signalling at the host-parasite interface. Parasitology 123 Suppl, S143-157.
976 Zhang, R., Liu, B., Lau, S.C.K., Ki, J.-S., Qian, P.-Y., 2007. Particle-attached and free-living
977 bacterial communities in a contrasting marine environment: Victoria Harbor, Hong Kong.
978 FEMS Microbiol. Ecol. 61(3), 496-508.
979 Zhu, F., Massana, R., Not, F., Marie, D., Vaulot, D., 2005. Mapping of picoeucaryotes in marine
980 ecosystems with quantitative PCR of the 18S rRNA gene. FEMS Microbiol. Ecol. 52(1),
981 79-92.
982 Zhuang, Y., Zhang, H., Hannick, L., Lin, S., 2015. Metatranscriptome profiling reveals versatile
983 N-nutrient utilization, CO_2 limitation, oxidative stress, and active toxin production in an
984 *Alexandrium fundyense* bloom. Harmful Algae 42, 60-70.

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