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Vanucci, S.; GUIDI, FLAVIO; PISTOCCHI, ROSSELLA; Long, R. A.: Phylogenetic structure of bacterial assemblages co-occurring with *Ostreopsis cf. ovata* bloom. HARMFUL ALGAE 55, p. 269-271. ISSN 1568-9883.

DOI: 10.1016/j.hal.2016.04.003

The final published version is available online at: <http://dx.doi.org/10.1016/j.hal.2016.04.003>

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Phylogenetic structure of bacterial assemblages co-occurring with *Ostreopsis cf. ovata* bloom

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Abstract

Extensive blooms of the toxic epiphytic/benthic dinoflagellate *Ostreopsis cf. ovata* are being reported with increasing frequency and spatial distribution in temperate coastal regions including the Mediterranean. These blooms are of human and environmental health concern due to the production of isobaric palytoxin and a wide range of ovatoxins by *Ostreopsis cf. ovata*. Bacteria-microalga interactions are important regulators in algal bloom dynamics and potentially toxin dynamics. This study investigated the bacterial assemblages co-occurring with *O. cf. ovata* (OA) and from ambient seawaters (SW) during the early and peak phases of bloom development in NW Adriatic Sea. Fractions of the bacterial assemblages co-occurring with *O. cf. ovata* (OA) and more closely associated to the mucilage layer (LA) embedding *O. cf. ovata* cells were also reported.

In total, 14 bacterial phyla were detected by targeted 454 pyrosequencing of the 16S rRNA gene. The dominant bacterial phyla in the OA assemblages were *Proteobacteria* and *Bacteroidetes*; while at the class level *Alphaproteobacteria* were the most abundant (83 and 66% relative abundance early and peak bloom phases) followed by *Flavobacteria* (7 and 19% early and peak phases). *Actinobacteria* and *Cyanobacteria* were of minor importance (<5% of the relative bacterial abundance each). *Gammaproteobacteria* showed a notable presence in OA assemblage only at the early phase of the bloom (genus *Haliea* 13%). The *Alphaproteobacteria* were predominantly composed by the genera *Ruegeria*, *Jannaschia* and *Erythrobacter* which represented about half of the total phylotypes' contribution of OA at both early and peak phases of the *O. cf. ovata* bloom, suggesting interactions between this consortium and the microalga. Moreover, the highest contribution of *Ruegeria* (30% of the total phylotypes) was observed at the early phase of the bloom in LA assemblage.

Microbial assemblages associated with the ambient seawaters while being also dominated by *Alphaproteobacteria* and *Flavobacteria* were partially distinct from those associated with *O. cf. ovata* due to the presence of genera almost not retrieved in the latter assemblages.

Keywords: *Ostreopsis*; Toxic dinoflagellate; HAB associated bacteria; Pyrosequencing; Bacterial diversity; *Ruegeria*; *Haliea*; *Jannaschia*

1 Introduction

Extensive blooms of toxic epiphytic/benthic dinoflagellate *Ostreopsis cf. ovata* are currently reported with increasing frequency and area distribution in the Mediterranean Sea (Viala et al., 2001; Aigizaki and Nikoaidis, 2006; Mangiajoco et al., 2011; Funari et al., 2015). The dinoflagellate grows epiphytically forming brownish mucilage mats on macroalgae but also on other biotic and abiotic substrata in shallow and sheltered waters (Viala et al., 2001; Aigizaki and Nikoaidis, 2006; Totti et al., 2010; Accoroni et al., 2011). Moreover, *O. cf. ovata* is often found in plankton samples due to resuspension from the benthic substrata (Viala et al., 2001; Aigizaki and Nikoaidis, 2006; Seina and Orvola, 2010; Totti et al., 2010). Mediterranean *O. cf. ovata* produces palytoxin-like compounds, namely isobaric palytoxin and a wide range of ovatoxins (OVTX a to k; Garcia-Artares et al., 2014; Brissard et al., 2015; Tartaglione et al., 2016) under both field and culture conditions (e.g., Accoroni et al., 2011; Ciminiero et al., 2011; Ciminiello et al., 2012a,b; Pezzolesi et al., 2012, 2014; Scafo et al., 2012; Pezzolesi et al., 2012; Pezzolesi et al., 2014; Vanucci et al., 2012a). The blooms can have a severe impact on human health causing intoxications through marine aerosol inhalation and contact (Gaiti et al., 2005; Kermarec et al., 2008; Tichadou et al., 2010; Funari et al., 2015). They also strongly affect invertebrate benthic communities causing

massive mortalities (Accoroni et al. 2011; Faimali et al. 2012; Gorbi et al. 2012, 2013; Care et al. 2015). Moreover, bioaccumulation of palytoxin-like compounds has been reported for bivalve mollusks and herbivorous echinoderms (e.g. Aigizaki et al. 2011; Amzi et al. 2012; Furan et al. 2013; Brissard et al. 2014). Driven by the negative impacts of *O. cf. ovata* Mediterranean outbreaks, research efforts have been aimed at identifying the environmental conditions and factors that trigger or regulate the microalgal bloom dynamics (Aigizaki and Nikoaidis 2006; Totti et al. 2010; Grané et al. 2011; Pistocchi et al. 2011; Mangiaajo et al. 2011; Pistocchi et al. 2011; Accoroni et al. 2015a,b). One of the major factors affecting *Ostreopsis* blooms is hydrodynamic regime, as consistently higher abundances are found under low hydrodynamism and in sheltered sites compared with exposed ones (Chang et al. 2000; Shears and Ross 2009; Mabrouk et al. 2011; Seina et al. 2014).

In parallel to the physicochemical factors, a greater appreciation about the significance of the bidirectional interactions between microalgae and bacteria in terms of regulating harmful algal blooms (HABs) has developed (Kodama et al. 2006; Loureiro et al. 2011). Culture-based studies on harmful planktonic dinoflagellates have shed light on several effects of bacteria on algal cellular physiology, which ultimately influence cell growth dynamics, production, degradation and/or modification of algal toxins (e.g. Hod et al. 2001; Long et al. 2003; Su et al. 2005; Azanza et al. 2006; Donovan et al. 2009; Green et al. 2010; Wang et al. 2010; Uncited references; Boich and Subramanian 2011; Bolch et al. 2011; Santos and Azanza 2012). Patterns of association between cultured harmful dinoflagellates and specific bacterial groups such as *Alpha* and *Gammaproteobacteria*, *Cytophaga*, *Flavobacteria*, *Bacteroides* and some subgroups, primarily the *Roseobacter* clade and the *Alteromonas*, have been observed (Amaro et al. 2005; Jasti et al. 2005; reviewed by Garcés et al. 2007; Green et al. 2010 and references therein; Onda et al. 2015). Moreover, several members of the *Roseobacter* clade (e.g. phyotypes of *Phaeobacter* and *Ruegeria* genera) associated with marine algae and/or isolated from non-toxic and toxic dinoflagellate cultures are known as some of the most effective colonizers of surfaces in the coastal environments (e.g. Sighom and Buchan 2009 and references therein; Eifantz et al. 2013). Furthermore, *Roseobacters* have the capability to produce potent antibacterial compounds, mainly affecting non-*Roseobacter* phyotypes, giving selective advantage to this clade (Long and Azam 2001; Brinkhoff et al. 2004; Bruhn et al. 2007). In addition, it has been recently demonstrated that the interaction between dinoflagellates and these bacteria can be mutually antagonistic or switch between both (Wagner Döber et al. 2010; Wang et al. 2014, 2015), depending on algal physiological status as aging algae will induce the production of algicidal compounds by bacteria (e.g. *Phaeobacter gallaeciensis*, Seyedsayamdost et al. 2011, 2014; *Ruegeria* sp. TM1040, Ricca et al. 2012) which could have an important role in the declining stages of algal blooms (Ricca et al. 2012).

Field impact of these associations or how elements of natural bacterial assemblages interact with the HAB population, however, is still poorly known (Mayali and Azam 2004; Mayali et al. 2008), outlining the need for *in-situ* studies assessing phylogenetic diversity and its temporal changes of the natural co-occurring bacterial populations during HABs. The limited studies on harmful planktonic dinoflagellates' microbiota generally converge on a broad feature for the dominance of the two bacterial groups, the *Rhodobacterales* (*Alphaproteobacteria*) and *Cytophaga*, *Flavobacteria*, *Bacteroides* (*Bacteroidetes*) during blooms (Garcés et al. 2007; Jones et al. 2010; Park et al. 2015).

The ecological role of bacterial assemblages associated with epiphytic/benthic toxic dinoflagellates has received considerably less attention than for their planktonic counterparts. Previous studies relied upon bacterial plate isolation from cultured dinoflagellates (e.g. *Ostreopsis lenticularis* and *Gambierdiscus toxicus*, Tosteson et al. 1989; *Prorocentrum lima*, Lafay et al. 1995; Prokic et al. 1998; *G. toxicus*, Sakami et al. 1999). More recently, Pérez Guzmán et al. (2008) showed that about half of total bacteria associated with *O. lenticularis* cultures was made up by a single species belonging to *Cytophaga*, *Flavobacteria*, *Bacteroides*, complex. In contrast, Ruh et al. (2009) found *Alphaproteobacteria* to be the largest group in monocultures of *Coolia monotis* and *Ostreopsis ovata*. A laboratory study provided evidence that bacteria interfere indirectly with *Ostreopsis cf. ovata* growth, toxin production, and likely on toxin degradation (Vanucci et al. 2012b). Currently, there is no phylogenetic data on the natural bacterial assemblages associated with *O. cf. ovata* blooms, and more generally with blooms of benthic dinoflagellates.

The present study focuses on the phylogenetic characterization of the bacterial assemblages co-occurring over the early and the peak phases of a benthic *O. cf. ovata* bloom at Passetto station. The Passetto station (Conero Riviera) has been classified as a hot spot area for *Ostreopsis cf. ovata* blooms by the Italian Agency for the Protection and Environmental Research (SPRA 2012). In this region, *O. cf. ovata* summer blooms occur regularly, and cell abundances are among the highest recorded along Mediterranean coasts (Mangiaajo et al. 2011; Accoroni et al. 2012, 2015a). This site is a semi-enclosed bay sheltered by a natural reef and characterized by a mostly rocky bottom and shallow depth (up to 2 m). The shore is subjected to a moderate anthropogenic impact during the summer season (Marini et al. 2002), being a popular area for summer holidays, when it is also subjected to tramping by swimmers. This site is also characterized by the presence of small caves derived from human boring of the natural cliffs, with some wastewater discharge facilities. A clear and important role of the hydrodynamic conditions on *Ostreopsis* blooms' trend has been recognized in this area (Totti et al. 2010; Accoroni et al. 2011, 2012). Recently, Accoroni et al. (2015a) have proposed a conceptual model for annual *Ostreopsis cf. ovata* blooms in the Northern Adriatic Sea based on the synergic effects of hydrodynamics, temperature, and the N:P ratio of water column nutrients, pointing out that calm conditions appeared to be a prerequisite for blooms development. Indeed, low hydrodynamism would favor *O. cf. ovata* proliferation by facilitating macroalgal colonization, juxtaposing of the microalgal cells and forming mucilage mats (Vial et al. 2001; Totti et al. 2010) which are known to be hotspots for microorganisms interactions (e.g. Coe et al. 2014a,b; Carreira et al. 2015).

The aims of the present study were: (i) assessing the phylogenetic composition of bacterial assemblages co-occurring with *O. cf. ovata* at the early and the peak phases of the bloom in order to highlight most prominent bacterial associations; (ii) comparing bacterial assemblages associated with *O. cf. ovata* with those from ambient seawaters for evaluating possible contribution of autochthonous bacteria to the former ones. The bacterial assemblages were assessed by high-throughput parallel tag sequencing (454 pyrosequencing).

2 Materials and methods

2.1 Study area and samples collection

Bacterial assemblages associated with *Ostreopsis cf. ovata* aggregates (OA) (i.e. *O. cf. ovata* colonizing macrophytes) and those associated with the ambient seawaters (SW) were collected at the early and at the peak phases (19 September and 2 October 2012 respectively) of an *O. cf. ovata* bloom along the coast of North western Adriatic Sea (Passetto, Italy, 43°36'38"~~N~~~~and 13°32'20"~~~~N~~ and 13°32'20" E).

Surface temperature (approximately 0.5 m depth) and salinity were recorded ~~in situ~~ by a YS Pro Plus probe. Samples from six to eight dinoflagellate colonized macrophytes for microorganisms (i.e. *O. streopsis* *cf. ovata* and bacteria) cell counting and bacterial assemblages structure analysis were base cut using a sterile blade and immediately collected in 700 mL aseptic reclosable polyethylene bags with minimal seawater. Ambient seawater samples for microbial assemblages were collected in acid washed autoclaved 1 L polypropylene bottles. Additional water samples for nutrient analysis were collected in polyethylene bottles close to the sampled macrophytes. All samples were stored on ice and in the dark for transport to the laboratory.

Nutrient samples were prepared by filtering through Whatman GF/F filters (nominal pore size 0.7 µm) and stored at -20 °C until analysis. Nitrate, nitrite, ammonium, and phosphate concentrations were determined according to the methods described by Strickland and Parsons (1972) using an Autoanalyzer QuAatro Axflow.

2.2 ~~O. cf. ovata~~*O. cf. ovata* and bacterial enumeration

For determination of *O. streopsis* *cf. ovata* epiphytic abundances, dinoflagellate colonized macrophyte samples were treated following the procedure described in Totti et al. (2010) and fixed with 1% Lugol solution (Thronsdén 1978). Seawater subsamples for *O. cf. ovata* planktonic abundances were also fixed with 1% Lugol solution. After fixation, both epiphytic and planktonic *O. cf. ovata* cell counts were performed following Utermöhl method (Hasle 1978) using a Zeiss Axioplan inverted microscope at 320~~x~~ magnification under bright field and phase contrast illumination.

Bacterial abundances were assessed for OA and SW subsamples, and for OA subsamples pre-filtered onto sterilized 11 µm pore size filters (Millipore). All subsamples were fixed with 0.2 µm prefiltered formaldehyde (2%) and bacterial enumerations were carried out following the method described by Shibata et al. (2006). Briefly, aliquots were concentrated onto 0.2 µm pore size Anodisc filters (Whatman, 25 mm diameter), stained with 100 µL of 8X SYBR Gold (Life Technologies), mounted onto microscopic slides, and stored at -20 °C. Enumeration was performed using epifluorescence microscopy (Nikon Eclipse 80i, magnification 1000~~x~~) under blue light excitation, counting at least twenty fields per sample and a minimum of 300 cells. Abundances of bacteria more closely associated with the mucilage layer embedding *O. streopsis* *cf. ovata* cells, here defined as the bacterial fraction retained onto 11 µm pore size filters (i.e. mucilage layer bacterial assemblage, LA), were estimated by subtracting bacterial counts obtained for 11 µm prefiltered OA subsamples to counts obtained for OA subsamples.

2.3 DNA samples processing and extraction

For harvesting bacterial assemblages associated with *O. streopsis* *cf. ovata* (i.e. OA), dinoflagellate colonized macrophyte samples were shaken in the storage water (3 min) to allow the detachment of *O. cf. ovata* cells, then up to 100 mL of the suspension was collected under low vacuum onto 0.2 µm pore size Supor 200 PES filters (Pall Corporation/Pall Life Sciences). In order to assess the bacterial phylotypes more closely associated with the mucilage layer embedding *O. cf. ovata* cells (LA), aliquots of the same suspension were collected onto sterilized 11 µm pore size filters (Millipore). Hence, LA assemblages represent fractions of the entire bacterial assemblages (OA) collected onto 0.2 µm pore size filters. Samples for DNA analysis of seawater bacterial assemblages (SW) were collected as previously described for storage water. All filters were stored at -80 °C in sterile 2 mL microcentrifuge tubes until analysis. Filters were shredded under sterile conditions, and DNA from cells on the filters was extracted using the UltraClean Soil DNA isolation kit (MoBio Laboratories) according to the manufacturer's instructions. DNA concentrations and purity were determined with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE).

2.4.3.1 16S rDNA pyrosequencing

For pyrosequencing analysis, extracted DNA samples from three replicates were pooled together and diluted to 10 ng µL⁻¹. Initial amplification of the hypervariable V1-V2 region of the bacterial 16S rDNA was performed on total DNA from samples. Master mixes for these reactions were prepared with Qiagen HotStar Hi-Fidelity Polymerase Kit (Qiagen, Valencia, CA), forward primer composed of the Roche Titanium Fusion Primer A (5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG-3'), a 10 bp Multiplex Identifier (MD) sequence (Roche, Indianapolis, IN) unique to each of the samples, and the universal bacteria primer 8F (5'-AGAGTTTGATCCTGGCTCAG-3'). The reverse primer was composed of the Roche Titanium Primer B (5'-CCTATCCCTGTGTGCTTGCGCAGTCTCAG-3'), the identical 10 bp MD sequence as the forward primer, and the reverse bacteria primer 338R (5'-GGTGGGTGGGTAGGAGT-GCTGCCTCCCGTAGGAGT-3'). Amplification in triplicate of each sample was performed under the following conditions: an initial denaturing step at 94 °C for 5 min, followed by 35 cycles of denaturing at 94 °C for 45 s, annealing at 50 °C for 30 s, and extension at 72 °C for 90 s, then a final extension at 72 °C for 10 min, and a final hold at 4 °C. Samples with pooled replicates were gel-purified individually using the Qiaquick Gel Extraction Kit (Qiagen, Valencia, CA) and combined at equimolar ratios. The 16S rDNA amplicons from the pooled samples were sequenced on a Roche 454 Genome Sequencer FLX Titanium instrument using the GS FLX Titanium XLR70 sequencing reagents and protocols (Microbiome Core Facility, Chapel Hill, NC). Initial data analysis and base pair calling were performed by Research Computing at UNC (Chapel Hill, NC).

2.5.4 Sequence processing and diversity analysis

The 16S rRNA gene amplicons data were processed through the RDP pyrosequencing pipeline (<http://pyro.cme.msu.edu>). Pre processing included screening and removing of short reads (<250 bp) and low quality reads. After sorting based on sequence tags and trimming of primer and tag sequences, derived high quality reads were checked for artificial chimeric formations using the Uchime algorithm. Community taxonomy information was obtained using the RDP classifier tool (Wang et al. 2007) and those sequences either related to chloroplasts or not belonging to the Domain Bacteria were discarded from further analysis. Then samples were standardized to the size of the smallest library (10,349 reads) by randomly subsampling the datasets. Sequences were aligned with the nfernal aligner and operational taxonomic units (OTUs) were clustered at a 97% similarity level by the furthest neighbor algorithm. The representative sequence for each cluster was assigned according to the minimum sum of the square of distances between sequences within a cluster (Cole et al. 2014a,b). Taxa abundances were normalized at phylum, class and genus level based on the average 16S rRNA copy number values reported by rrnDB database (Stoddard et al. 2014). When the 16S copy number for a specific taxon was not available in the database, the average value for the upper taxonomical level was considered. Alpha diversity was analyzed through rarefaction curves and diversity estimators (Chao 1, Shannon index and evenness) were calculated.

2.65 Statistical analysis

All statistical analysis except for Metastats analysis were performed with the PAST software package for Windows (Hammer et al. 2001). Differences in *Ostreopsis* cf. *ovata* cell numbers, bacterial abundances and diversity estimators within and between sampling times were assessed through Student's *t* test. Beta diversity was addressed by non-metric multidimensional scaling (NMDS) performed using a Bray-Curtis similarity matrix of OTUs abundance data. Community level differences between groups were tested by analysis of similarities (ANOSIM) and Similarity Percentage analysis (SIMPER) was utilized to identify the OTUs most contributing to the dissimilarity between bloom phases and assemblages. Finally, Metastats (White et al. 2009) was used for detection of differences in contribution of bacterial OTUs among samples. Statistical significance was set at $p < 0.05$ for all the analysis.

3 Results

3.1 Environmental conditions and cell abundances

Sampling was carried out at an early and at the peak phases of the *Ostreopsis* cf. *ovata* bloom (1st and 2nd sampling times, respectively). Surface seawater temperature and salinity were 22.2 °C and 34.7 and 21.5 °C and 36.8 at early and peak, respectively. Dissolved inorganic nitrogen (DIN, i.e. NH_4^+ plus NO_2^- plus NO_3^-) was 9.40 μM at the early and 0.55 μM at the peak phase of the bloom. While phosphate concentration (PO_4^{3-}) was 0.31 μM at both sampling times, leading to a N:P ratio of 30.2 and 1.8 (1st and 2nd sampling, respectively).

Abundances of *Ostreopsis* cf. *ovata* cells colonizing macrophytes (OA) were two fold higher at the peak phase than at the early phase of the bloom (mean value: 2.06×10^6 vs. 9.49×10^5 cells g^{-1} fw, $p < 0.05$, Student's *t* test). Cell densities of *O. cf. ovata* in ambient seawater samples (SW) were on average 1.98 and 3.10×10^4 cells L^{-1} at the early and peak phase, respectively ($p > 0.05$, Student's *t* test).

Bacterial abundances were approximately two fold higher in both OA and LA assemblages at the peak compared to the early phase of the bloom (OA mean values: 6.62×10^6 cells mL^{-1} vs. 3.06×10^6 cells mL^{-1} ; LA mean values: 1.49×10^6 cells mL^{-1} vs. 0.68×10^6 cells mL^{-1} , $p < 0.01$, all Student's *t* test), whereas abundances in SW samples did not change significantly between the two sampling periods (mean value: 7.94×10^5 cells mL^{-1} vs. 6.34×10^5 cells mL^{-1} , $p > 0.05$, Student's *t* test). Overall, bacterial abundances were almost one order of magnitude higher in OA than in SW samples ($p < 0.01$, Student's *t* test).

3.2 Diversity of microbial assemblages

A total of 73,641 high quality reads spanning the 16S rDNA V1-V2 hypervariable region were used in the final analysis (average length: 287 bp). This yielded 4765 different OTUs at 97% similarity from the whole dataset. Rarefaction analysis based on OTUs indicated that sampling did not achieve complete coverage except for SW sample at the early phase (SW, Fig. 1). Curves reached higher number of OTUs per reads at the peak than at the early bloom phase for all the assemblages. The number of bacterial OTUs and estimated species richness (Chao 1), together with Shannon diversity (H') and evenness (J') and evenness (J), reached the highest values in OA assemblage at the peak phase of the bloom (OA), whereas the lowest values were found for bacteria more closely associated with the mucilage layer embedding *Ostreopsis* cf. *ovata* cells at the early phase (i.e. LA), except for Chao1 (Table 1). OTUs number and Shannon index were significantly higher at the peak than at the early phase ($p < 0.05$, all Student's *t* test), whereas the other indexes did not show significant differences between phases and assemblages ($p > 0.05$, all Student's *t* test). The majority of OTUs, including the most abundant ones, were shared among assemblages, while unique OTUs (31.6% of the total OTUs in OA and 30.9% in SW assemblages) were mainly represented by singletons (~60%).

Fig. 1 Diversity of bacterial assemblages associated with *O. cf. ovata* (OA) mucilage layer bacterial assemblages (LA) and seawater bacterial assemblages (SW) at the early and peak phase of the bloom (and respectively). Rarefaction curves were computed on bacterial OTUs at a dissimilarity level of 3%.

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Table 1 Bacteria diversity parameters during *O. cf. ovata* bloom. Summary of total high quality sequences after normalization to the smallest library (Reads), number of bacterial operational taxonomic units detected at 97% identity (OTUs), estimated species richness (Chao 1), Shannon diversity index (H') and evenness (J') obtained from pyrosequencing of bacterial assemblages associated with *O. cf. ovata* (OA) mucilage layer bacterial assemblages (LA) and seawater bacterial assemblages (SW) at the early and peak phase of the bloom (and respectively).

Sample	Reads	OTUs	Chao 1	H'	J'
OA	10,349	1621	3229	5.28	0.715
OA	10,349	2214	4078	6.36	0.826
LA	10,349	1322	2582	4.63	0.644
LA	10,349	2177	3886	6.31	0.821
SW	10,349	1394	1798	5.79	0.800
SW	10,349	1921	3337	6.18	0.817

As expected, higher numbers of OTUs were shared between OA and LA compared to SW assemblages, and higher percentages of shared OTUs were observed at the peak than at the early phase of the bloom (data not shown). These findings were supported by the NMDS plot displaying Bray-Curtis similarities between samples (Fig. 2), which revealed that OA temporal samples were more similar than SW temporal samples (54 and 39% similarity, respectively), while the assemblages were more similar at the peak than at the early bloom phase (60 and 46% similarity). Nevertheless, ANOSIM test did not underline significant differences between assemblages or bloom phases ($p > 0.05$ for both comparisons). SIMPER analysis showed that seven OTUs explained 20% of the dissimilarity either between assemblages or between bloom phases: four OTUs were related to *Rhodobacteraceae* and three to the genera *Erythrobacter* (Alphaproteobacteria), *Haliea* (Gammaproteobacteria) and *Propionibacterium* (Actinobacteria), respectively (Table 2, Table S1).

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Table 3 Supplementary Table S1 related to this article can be found in the online version at doi 10.1016/j.hal.2016.04.003

The number of specific phylotypes (i.e. OTUs detected in only one kind of assemblage at both bloom phases) were 295 for OA and 82 for SW assemblage and all the phylotypes of them were rare (i.e. <1% of the total reads in a sample) accounting for 6.2 and 1.7% of the total OTUs respectively. While more than half of the OA specific OTUs belonged to the *Rhodobacteraceae* family, SW specific OTUs were more uniformly distributed between taxa (data not shown).

3.3 Microbial assemblages composition and comparisons

Overall 14 different bacterial phyla 20 classes and 165 genera were recovered ~~supplementary Table S2–S3 and Supplementary Tables S2–S4~~ respectively) All microbial assemblages either related to *C. streopsis* cf *ovata* (i.e. both OA and LA assemblages) or with the ambient seawaters (SW) were dominated by *Proteobacteria* (50.9–85.7%) followed by *Bacteroidetes* (12.7–36.0%) and *Actinobacteria* (0.6–6.3%) *Cyanobacteria* was the fourth most represented phylum yet never exceeding 2.5% in relative abundance (Table S2) Phyla *Fusobacteria*, *Firmicutes* and *Planctomycetes* were present at abundances just above 1% only in SW while *Parcubacteria* reached values ~1.5% in both SW and *O. cf. ovata*-related assemblages (OA and LA) at the bloom peak phase The remaining phyla *Acidobacteria*, *Chloroflexi*, *Chlorobi*, *Spirochaetes*, *TM7* and *Deinococcus*, *Thermus* were rare representatives (i.e. <1% Table S2)

~~ISSJ~~Appendix ASupplementary dataSupplementary data associated with Supplementary Tables S2–S4 related to this article can be found in the online version at doi:10.1016/j.hal.2016.04.003

Assemblages related to *Ostreopsis* cf *ovata* (OA and LA) showed predominance of *Alphaproteobacteria* at both phases of the bloom (66.0 to 84.4% OA and LA respectively Fig 3) Genera *Ruegeria* and *Jannaschia* (*Roseobacter* clade) along with *Erythrobacter* were the most abundant ones with the highest values for *Ruegeria* and *Erythrobacter* at the early phase (30.2% in LA and 23.8% in OA respectively) and for *Jannaschia* at the peak phase (21.1% in OA Fig 4) primarily due to contribution of the three OTUs #6, #164 and #516 respectively (Table 2) Moreover the OTU #40 assigned to *Rhodobacteraceae* had also a consistent contribution in OA and LA assemblages particularly at the early bloom phase (Table 2) Other *Rhodobacteraceae* genera such as *Litoreibacter*, *Loktanelle*, *Loktanelle*, *Paracoccus* showed contributions around 3% (Fig 4) Overall contribution of OTUs assigned to *Alphaproteobacteria* were as significantly higher in *O. cf. ovata*-related assemblages (OA and LA) compared to SW assemblages ($p < 0.05$ Metastats analysis) In addition OTUs significantly more abundant in OA and LA than in SW assemblages were all affiliated to the *Rhodobacteraceae* family including one *Ruegeria* (OTU #1637) and several *Jannaschia*-related OTUs (i.e. OTUs #2431, #2072, #6042, #4675) Within the *O. cf. ovata*-related assemblages OTU #2431 was significantly more present in OA than LA samples ($p < 0.05$ Metastats analysis) Class *Gammaproteobacteria* was mainly represented by OTUs belonging to the genus *Haliea* at the early phase (>10% primarily OTU #35 Table 2) and then shifting to *Granulosicoccus* at the peak phase (~3%) The remaining three *Proteobacteria* classes (*Delta-Epsilon-Epsilon* and *Betaproteobacteria* Table S3) were rare Other notable groups showed an inverse trend with respect to *Alphaproteobacteria* (Fig 3) Members of *Flavobacteria* (7.2 and 22.2% OA and LA) followed by *Sphingobacteria* showed the highest contributions at the peak phase as well as *Ilumatobacter* (6% *Actinobacteria*) (Table 3).

Fig. 3 Percent distribution of the dominant classes (≥1% in at least one of the samples) in bacterial assemblages associated with *O. cf. ovata* (OA) mucilage layer bacterial assemblages (A) and in seawater bacterial assemblages (SW) at the early and peak phase of the bloom (and respectively) as revealed from pyrosequencing data normalized for number of ribosomal operons per genome. Others represent the classes with less than 1% of relative abundance individually. Abbreviations: Alpha: *Alphaproteobacteria*; Gamma: *Gammaproteobacteria*; Delta: *Deltaproteobacteria*; Epsilon: *Epsilonproteobacteria*; Flavo: *Flavobacteria*; Spingo: *Sphingobacteria*; Actino: *Actinobacteria*; Cyano: *Cyanobacteria*; Fusob: *Fusobacteria*.

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Fig. 4 Relative contribution of the major bacterial genera ($\geq 1\%$ in at least one of the samples) in bacterial assemblages associated with *O. cf. ovata* (OA) mucilage layer bacterial assemblages (A) and seawater bacterial assemblages (SW) at the early phase (A) compared to the peak phase (B) of the bloom as revealed from pyrosequencing data normalized for number of ribosomal operons per genome. Vertical lines separate groups of different phyla or classes. Abbreviations: α Alphaproteobacteria, γ Gammaproteobacteria, δ Deltaproteobacteria, ϵ Epsilonproteobacteria, Flavo Flavobacteria, Sp Sphingobacteria, Act Actinobacteria, Cy Cyanobacteria, Fu Fusobacteria, Par Parvulimicrobia, S Incertae Sedis.

Table 3 Major bacteria OTUs leading to dissimilarity—Similarity percentage analysis (SIMPER)—showing contribution of the seven OTUs explaining 20% of the dissimilarity between assemblages and bloom phases. The number of sequences per OTU for each of the samples are also reported. (Table 3 does not exist in the manuscript. Please remove completely Table 3 and the relative legend from the article.)

at text: **Table 3**

OTU (Table 3 does not exist in the manuscript. Please remove completely Table 3 and the relative legend from the article.)	Closest relative RD classifier	No. of sequences per OTU						Contribution to dissimilarity (%)	
		OA	OA	LA	LA	SW	SW	Assemblages	Bloom phases
6	<i>Ruegeria</i>	1519	462	2268	644	579	647	7.09	4.97
40	<i>Rhodobacteraceae</i>	812	412	1184	583	437	330	3.51	2.64
35	<i>Haliea</i>	568	9	638	10	23	48	2.83	2.86
33	<i>Rhodobacteraceae</i>	13	20	13	32	692	49	2.22	3.37
7	<i>Propionibacterium</i>	0	15	1	14	418	14	1.32	2.27
516	<i>Jannaschia</i>	219	278	91	153	28	61	1.27	1.56
164	<i>Erythrobacter</i>	355	247	333	193	88	167	1.22	1.63

Ambient seawater bacterial assemblages were also dominated by *Alphaproteobacteria* (48–60%) although with a significant lower contribution with respect to *O. cf. ovata*-related assemblages (OA and LA). This class showed the highest contribution at

the peak phase of the bloom primarily with *Erythroba* *Jannaschia* and *Ruegeria* (Fig 4) While OTU #40 (*Rhodobacteraceae*) overlapped with the *O cf ovata* related assemblages (OA and LA) OTU #33 (also a *Rhodobacteraceae*) had a large contribution only in SW (Table 2) Differently from *O cf ovata*-related assemblages *Haliea* accounted only for 1.3% at both samplings whereas *Granulosicoccus* was about 3% at the peak phase as in the former ones interestingly the contribution of members affiliated to *Vibrio* wereas significantly higher in SW than in OA and LA assemblages (e.g. OTU #76 $p < 0.05$ Metastats analysis) where the genus was essentially absent Similarly members belonging to *Haliscobenobacter* (2% early phase) the most abundant representative of *Sphingobacteria* had a significantly higher contribution in SW with respect to *O cf ovata*-related assemblages (OA and LA) (OTU #335 $p < 0.05$ all Metastats analysis) Whereas *Flavobacteria*-affiliated (24 and 18% SW and SW) did not differ significantly between assemblages ($p > 0.05$ all Metastats analysis) Notably *Propionibacterium* (*Actinobacteria*) was found at high relative abundance only in SW (12.7% primarily OTU #7 Table 2) whereas its contribution was negligible in OA and LA assemblages A similar pattern was also observed for *Cetobacterium* (*Fusobacteria* Fig 4)

When comparing the two phases of the bloom several *Flavobacteria* OTUs (e.g. OTUs #265 #678 #733 #1340) were significantly more abundant at the peak phase ($p < 0.05$ Metastats analysis) Contribution of OTU #208 related to *Granulosicoccus* (*Gammaproteobacteria*) as well as OTUs #150 and #366 affiliated with genus *Arcobacter* (*Epsilonproteobacteria*) were also differentially higher at the peak than at the early phase ($p < 0.05$ Metastats analysis) Lastly *Ilumatobacter* (*Actinobacteria*) GpIIa (*Cyanobacteria*) and *Parcubacteria Incertae Sedis* showed the highest relative abundances at the peak phase for all assemblages and contributions of a large number of OTUs assigned to these genera were significantly higher at the peak than at the early phase of the bloom ($p < 0.05$ Metastats analysis) Fig 5 summarizes bacterial assemblages' main shifts in relative abundance at genus level between early and peak phase of the bloom

Fig. 5 Major changes in relative abundance at genus level and class level (inset) between peak phase (SW) and early phase (OA) of the bloom in bacterial assemblages associated with *O cf ovata* (OA) and seawater bacterial assemblages (SW). Differences were calculated by subtracting percent ages recorded at the peak from values at the early phase of the bloom. The positive values denote higher contribution at the peak as opposed to the early phase.

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4 Discussion

4.1 General

Abundances of *Ostreopsis cf ovata* cells recorded during this study in both *O cf ovata* (OA) and ambient seawater (SW) assemblages were in the range of values reported previously for the same area and more generally for Mediterranean Sea (Totti et al 2010 Mangialajo et al 2011 and references therein Accoroni et al 2015a Carnicer et al 2015). Bloom development occurred under stable weather conditions, low hydrodynamism, and accompanied by a drop of nitrogen concentration at the peak phase. The limited sampling (two timepoints) does not allow to infer on relationship dynamics between inorganic nutrients and *O cf ovata* bloom. The physicochemical conditions, the onset bloom N:P ratio, and nutrient concentration changes observed in this study

however do fulfill the recent conceptual model proposed for *O. cf. ovata* blooms in this region (Accoroni et al 2015a). The model postulates that calm conditions are a prerequisite for blooms and only when this state is established temperature and suitable N:P ratio will have a decisive effect (Accoroni et al 2015a). Bacterial cell densities reported for the ambient seawaters were in the range of abundances found during non-toxic or harmful microalgal blooms (e.g. Lamy et al 2009; Jones et al 2010; Mayali et al 2011; Park et al 2015).

Diversity indexes of bacterial assemblages associated with *O. cf. ovata* (OA) were comparable with pyrosequencing derived values reported for a broad range of bacterial benthic eukaryote associations (Webster et al 2010; Lee et al 2011; Carlos et al 2013; He et al 2014) and for shallow water sediments (Wang et al 2013; Liu et al 2015; ~~Piccini et al 2015~~; Piccini and García-Alonso 2015), the latter being considered among the most diverse environments (Lozupone and Knight 2007). While the rarefaction curves indicated that species richness was not fully sampled, they do suggest a higher bacterial richness at the peak than at the early phase of the bloom in accordance with Chao1 and Shannon estimators.

The comparison between *C. streopsis* *cf. ovata* (OA) and ambient seawater (SW) assemblages did not indicate a clear distinction in terms of alpha and beta diversity, differently from other studies that compared benthic eukaryote associated bacteria with the surrounding seawater bacteria (Webster et al 2010; Lee et al 2011; Carlos et al 2013; He et al 2014). In fact, in this study, assemblages differed mostly in terms of the OTU relative contribution rather than in the presence or absence of specific phylotypes, as the latter were detected in very low abundances. Cells of *O. cf. ovata* and its mucilage layer adhere only loosely to the substrata and can be easily resuspended in the water column, particularly as blooms progress and mats become heavier (Totti et al 2010; Mangialajo et al 2011). This phenomenon may explain the higher Bray-Curtis similarities found among samples at the peak than at the early bloom phase and a portion of the phylogenetic overlap between OA and SW assemblages, as revealed by the NMDS plot.

4.2 Bacterial assemblages associated with ~~*O. cf. ovata*~~ *O. cf. ovata*

In this study, *Alphaproteobacteria* was the dominant class of the bacterial assemblages associated with *C. streopsis* *cf. ovata* (OA) during both bloom phases (83 and 66% relative abundance, early and peak), followed by *Flavobacteria* that showed the highest contribution at the peak phase (up to 19%). The same trend was also observed for the bacterial fraction more closely associated to the mucilage layer embedding the *O. cf. ovata* cells (LA). These main bacterial composition features and trends are consistent with previous ones reported for non-toxic phytoplankton blooms (Fandino et al 2001, 2005; Brussaard et al 2005; Alderkamp et al 2006; Lamy et al 2009) and also for the few available planktonic HABs (Garcés et al 2007; Hasegawa et al 2007; Jones et al 2010; Park et al 2015), with the exception for Yang et al (2012, 2015).

The highest contributions found in this study for several *Flavobacteria*-affiliated OTUs at the peak than at the early bloom phase are in accordance with the main metabolic traits ascribable to the members of this class, being recognized as specialists for degradation of particulate organic matter and high molecular weight compounds (e.g., cellulose, chitin and proteins; Kirchman 2002; Rink et al 2007; ~~García-Añares et al 2014~~; Gómez Pereira et al 2010; ~~Gómez Pereira et al 2010~~; Fernández Gómez et al 2013; Buchan et al 2014). As such, these OTUs probably respond as secondary colonizers (Elifantz et al 2013) to the wider pool of refractory organic matter such as phyto-detritus as the bloom proceeded. Members of *Actinobacteria*, the third most abundant phylum, generally recognized as organotrophic bacteria able to decompose recalcitrant and poorly accessible substrates at later stages of microbial succession (Holt et al 1994; Zakharova et al 2013; Bagatini et al 2014), were present predominately at the peak phase with *Ilumatobacter* as the main representative. This genus has been recently found abundant during degradation processes of freshwater diatoms (Zakharova et al 2013) and at the stationary growth phase of freshwater cultured phytoplankton (Bagatini et al 2014) and other algal cultures (Green et al 2015). In this context, this genus might be directly related to the degradation of *C. streopsis* *cf. ovata* senescent cells but also probably responding to the epiphytic diatoms that colonize the macrophytes throughout the year, co-occurring in minor proportion with *O. cf. ovata* and presumably undergoing to nutrient stress during the bloom (Totti et al 2010; Carnicer et al 2015). At the early phase of the bloom, *Gammaproteobacteria* (~6%) associated with *O. cf. ovata* assemblages (OA) were mainly represented (13%) by the recently designated genus *Haliae* (Urios et al 2008), primarily with OTU #35. Members and sequences of *Haliae* have been found in surface waters of the North-western Mediterranean Sea (Urios et al 2008, 2009; Lucena et al 2010). In addition, representatives have been reported in the presence of methane source in oxic water layers (Mau et al 2013) and in association with an *A. kashiwo* sanguine bloom event (Yang et al 2015), suggesting that phylotypes affiliated to this genus may be involved in the dynamics of methane that is produced through not yet identified microorganisms under summer phytoplankton bloom by using dimethylsulfoniopropionate (DMSP) (Damm et al 2008, 2010; Dickschat et al 2015). Overall, the limited contribution of *Gammaproteobacteria* reported here is partially consistent with that one found for assemblages co-occurring with *Alexandrium* spp. blooms in which specific groups such as *Alteromonadaceae* could not be retrieved in significant proportions (Garcés et al 2007). By contrast, Yang et al (2012, 2015) found bacterial assemblages dominated by *Gammaproteobacteria* and *Alphaproteobacteria* during *A. sanguinea* blooms, with an increase of the former at the decline phase (Yang et al 2012), suggesting an algicidal activity for this class, as also proposed for *Cochlodinium polykrikoides* (Park et al 2015), that conversely it could not be inferred by data reported here. This investigation, however, was limited to the early and peak phases of the bloom and did not sample its decline phase. The remaining phyla associated with the *O. cf. ovata* assemblages (OA) were rare (<1%). Representatives from *Acidobacteria*, *Chloroflexi*, *Chlorobi*, *Fusobacteria*, *Firmicutes*, *Spirochaetes*, *TM7* and *Deinococcus* *Thermus* were observed. While their relative abundances were low, it cannot be precluded their importance in the nutrient dynamics and interactions within the assemblages.

Focusing on *Alphaproteobacteria*, a *Rhodobacteraceae* consortium composed by *Ruegeria*, *Jannaschia* and the OTU #40 (closely related to *Roseovarius*, Table S1) together with *Erythrobacter* appears to be associated with the *O. cf. ovata* assemblages (OA), representing more than half of the total phylotypes' contribution at both phases of the bloom, with the highest values at the early phase for OTU #40, *Ruegeria* and *Erythrobacter* and at the peak phase of the bloom for *Jannaschia*. Additionally, more than half of the *O. cf. ovata* specific OTUs were affiliated to the *Rhodobacteraceae*, remarking the crucial role of this family in the bacterial-algal interactions (e.g. Buchan et al 2014). Genus *Erythrobacter* is a very relevant component of the marine planktonic communities, becoming in some cases one of the most dominant groups in eutrophic coastal environments (Shiba and Simidu 1982; Frette et al 2004), adapted to grow on refractory carbon (Frette et al 2004). Phylotypes belonging to *Erythrobacter* have been found associated with cultured microalgae (e.g. *Skeletonema costatum*; Jasti et al 2005; *Coolia monotis*; Ruh et al 2009), macroalgae (Burke et al 2011), colonial ascidians (Martínez García et al 2007), phytoplankton blooms (Borsodi et al 2013; Yang et al 2015).

and also endosymbiont (e.g. in *Pyrodinium bahamense* var. *compressum* Azanza et al. 2006). Moreover, as in this case, *Erythrobacter* has been found dominant together with *Jannaschia* in limestone biofilm (Berdoulay and Salvado 2009), suggesting a potential relationship between these two aerobic anoxygenic phototrophic (AAnP) genera.

The *Rhodobacteraceae* are among the most abundant and ecologically relevant coastal marine bacteria; their ecological niches range from free-living plankton to symbiont to biofilm pioneers (reviewed by Geng and Belas 2010; Elifantz et al. 2013; Hahnke et al. 2013; Elifantz et al. 2013). These bacteria, in fact, show high metabolic versatility, including aerobic anoxygenic photosynthesis, the degradation of the algal osmolyte dimethylsulfoniopropionate (DMSP), and the synthesis of bioactive metabolites such as tropodithietic acid (TDA), which has potent antibacterial properties (Geng and Belas 2010; Hahnke et al. 2013 and references therein). In this study, *Rhodobacteraceae* may have also taken advantage of the high DIN levels (mainly nitrate) detected at the early phase of the bloom, as they are able to use nitrate as a terminal electron acceptor to sustain an active energy metabolism also in the absence of oxygen (Wagner Döbler and Biebl 2006; Wagner Döbler et al. 2010; Riedel et al. 2015). Diel anoxia may be expected in *Ostreopsis* cf. *ovata* mats, as regularly recorded in photosynthetic biofilms at night as a consequence of intense respiration, as often observed in microbial mats (Steunou et al. 2008). Follow-up studies of diel and longitudinal gene expression in response to physicochemical fluctuations is necessary to further elucidate these microbial consortial interactions. The genus *Ruegeria* phylotypes have been retrieved from surface ocean waters of most climatic zones in both coastal zones and open oceans (e.g. Gram et al. 2010; Lai et al. 2010), in tidal flat sediments (Oh et al. 2011), associated with marine invertebrates (e.g. Menezes et al. 2010; Lee et al. 2012; Kim et al. 2014), but also being reported in association with cultured toxic dinoflagellates (e.g. *Pfiesteria piscicida* Alavi et al. 2001; *Alexandrium catenella* Amaro et al. 2005; *Pyrodinium bahamense* var. *compressum* Onda et al. 2015). Representatives of *Ruegeria* synthesize TDA prominently under static conditions (Bruhn et al. 2007; Porsby et al. 2008; Geng and Belas 2010, 2011; D'Alvise et al. 2014) and mainly inhibiting non-*Roseobacter* phylotypes (e.g. members of *Vibrio* among *Gammaproteobacteria*, Bruhn et al. 2005, 2007; *Flavobacteriia*, and *Actinobacteriia*, Brinkhoff et al. 2004; Rabe et al. 2014), thus enhancing *Roseobacters* symbiosis by limiting competition from other species and then promoting algal growth (Miller and Belas 2006; Geng and Belas 2010; Seyedsayamdost et al. 2011 and references therein). In this study, the high contribution of *Ruegeria* in LA (30% of the total phylotypes) would warrant further investigation to assess bacterial biofilm formation during *O. cf. ovata* bloom development. High relative abundances of *Jannaschia* related clones have been reported in the Baltic Sea (Salka et al. 2008), in tidal flat sediments (Yoon et al. 2010; Fang et al. 2014; Piccini and García Alonso 2015; Piccini et al. 2015), in association with algal cultures (Green et al. 2015) but also as endosymbionts (Apprill et al. 2009). Previous studies have shown that members of genera *Ruegeria* and *Jannaschia* are attracted by DMSP (e.g. *Ruegeria* sp. TM1040, Miller and Belas 2004, 2006; Geng and Belas 2010; *Jannaschia* sp., Apprill et al. 2009) and catabolize it (Moran et al. 2007; Todd 2012; Todd et al. 2012 and references therein; Reisch et al. 2013; Dickschat et al. 2015 and references therein), as many *Roseobacters* (González et al. 2000; Newton et al. 2010; Wagner Döbler et al. 2010; Riedel et al. 2015). Dimethylsulfoniopropionate is produced by several toxic dinoflagellates (reviewed by Caruana and Malin 2014), including *O. cf. ovata* (Vanucci et al. in press).

At the peak of the bloom, *Jannaschia* overcame *Ruegeria*, while this study does not allow to infer on conditions and/or bacterial-algal interactions which would have favored the growth of one genus over the other and their individual effects on *Ostreopsis* cf. *ovata* bloom dynamics. It is well known that *Roseobacters* are involved in algal bloom decline processes and may exert significant control over phytoplankton biomass (González et al. 2000; Mayali et al. 2008; Teeling et al. 2012; Buchan et al. 2014). Particle-associated phylotypes of this clade have been also noted as being highly antagonistic to other bacteria (Long and Azam 2001). Recently, it has been demonstrated that *Roseobacters* dinoflagellates relationship shifts from a mutualistic to a pathogenic phase in response to ageing cells (Wagner Döbler et al. 2010; Wang et al. 2014, 2015) by producing algicidal compounds induced by breakdown products released in the case of aging algae (e.g. *Phaeobacter gallaeciensis*, *P. inhibens*, Seyedsayamdost et al. 2011, 2014; *R. pomeroyi*, Riclea et al. 2012). The presence of aging and potentially nutrient-stressed *O. cf. ovata* cells would increase with bloom progression, likely inducing a shift from a mutualistic to an antagonistic interaction. Further studies focusing on the potential transition from mutualistic to antagonistic interactions between *O. cf. ovata* and the associated *Roseobacters* are needed.

4.3 Bacterial assemblages in ambient seawaters

Ambient seawater bacterial assemblages (SW) were partially distinct from those associated with *Ostreopsis* cf. *ovata* (OA), primarily containing taxa almost not retrieved in the latter and also by showing a significant lower contribution of OTUs assigned to *Alphaproteobacteria* compared to *O. cf. ovata* assemblages. Moreover, SW assemblages, while sharing the major genera of this class with OA assemblages, were also characterized by a large contribution of OTU #33 (closely related to *Salinibacter*, Table S1) at early bloom, not observed in the same high proportion in OA assemblages. Seawater assemblages contained members of OTUs belonging to *Vibrio*, *Propionibacterium*, *Halicomonas*, and *Cetobacterium*, which were absent or in low abundance in the OA assemblages. These genera are commonly connected with host organisms or sewage pollution (Vorobjeva 1999; Finegold et al. 2003; Mulder and Deinema 2006; Ceccarelli and Colwell 2014). This finding was not surprising as the sampling area is moderately affected by anthropogenic impact during summer season (Accoroni et al. 2011). Dynamics between benthic hosts' associated and the surrounding seawater bacteria can be tightly coupled, as the latter can serve as a major seeding source for epibiotic consortium, as well as the former may diffuse into the surrounding planktonic assemblages (He et al. 2014; Singh and Reddy 2014; He et al. 2014; Cleary et al. 2015). Moreover, some bacterial taxa possess many surface colonization traits (e.g. *Rhodobacteraceae*) and can be cosmopolitans living in both habitats. Whereas other taxa may be specialists of water column and lack the capacity to live in certain interfaces (e.g. He et al. 2014) or being selectively excluded in the presence of competitive bacteria associated to the benthic substrata (e.g. Singh and Reddy 2014), as it could be the case for *Vibrio* and *Propionibacterium*. Thus, it appears that the retrieved allochthonous bacteria (i.e. bacteria anthropogenic impact related) harbored in the ambient seawaters marginally affect bacterial assemblages associated with *O. cf. ovata* during bloom development, whereas dislodgment and resuspension from *O. cf. ovata* mats could be possibly responsible for some bacterial diffusion into seawater assemblages, as also suggested by similarities pattern between samples in the NMDS plot.

5 Conclusions

Dinoflagellate-bacteria relationships are known to range from mutualistic to antagonistic interactions; however, we are just starting to appreciate the more ephemeral and subtle aspects (Kodama et al. 2006; Geng and Belas 2010;

Wagner Döber et al 2010; Wang et al 2014 2015) In this study it has been found a strong association of a core bacteria genera with *C. streopsis* cf. *ovata* during the two investigated boom phases. Under the correct physicochemical conditions noted earlier a positive interaction between the consortium of *Alphaproteobacteria* and *O. cf. ovata* favoring the dinoflagellate proliferation and the boom development phase it is hypothesized. Low hydrodynamism is definitely considered a prerequisite for *Ostreopsis* boom development (Aizaki and Nikoaidis 2006; Mangiaajo et al 2008; Totti et al 2010; Pistocchi et al 2011; Accoroni et al 2015a). Stable weather conditions could also favor *Ruegeria* colonization and biofilm formation sustaining this mutualistic/beneficial phase although further investigations are needed to assess this hypothesis. Moreover a focus on the role of both *Ruegeria* and *Jannaschia* in the boom termination processes warrants future research considering the bivalent interaction role played by *Roseobacters* in relation to a galactate (Seyedsayamdost et al 2011 2014). The era of high throughput sequencing will allow further detailed investigations on bacteria co-occurring with epiphytic/benthic harmful galactate blooms at the consortia and cellular levels and their interactions

~~and Todd et al., 2012.~~**Acknowledgements**

This research was supported by Italian PR N2009 project by ~~M-UR and the M-UR and the F-AMU~~ College of Science and Technology. We are grateful to Dr. Mauro Marini (SMAR CNR Ancona) for providing nutrient data and to Natasha Butz (Microbiome Core Facility UNC Chapel Hill, North Carolina, USA) for technical assistance in sequencing. ~~Appendix A Supplementary data~~The following are the supplementary data to this article: [SS]

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Multimedia Component 1

Table S1 Closes matches from the NCBI GenBank database based on sequence similarity of the seven OTUs explaining 20% of the dissimilarity between assemblages and bloom phases as revealed by SIMPER analysis.

Multimedia Component 2

Table S2 Relative abundance (in %) of all the phyla detected by pyrosequencing of bacterial assemblages associated with *O. ovata* (OA), mucilage layer bacterial assemblages (LA), and seawater bacterial assemblages (SW) at the early and peak phase of the bloom (I and II, respectively). Rare phyla accounted for less than 1% in percentage.

Multimedia Component 3

Table S3 Relative abundance (in %) of all the classes detected by pyrosequencing analysis. Rare classes accounted for less than 1% in percentage.

Multimedia Component 4

Table S4 Relative abundance (in %) of all the genera detected by pyrosequencing analysis. Rare genera accounted for less than 1% in percentage.

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