

Monitoring bacterial composition and assemblage in the Gulf of Corcovado, southern Chile: Bacteria associated with harmful algae

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ABSTRACT

Harmful Algal Blooms (HABs) have caused damage to the marine environment in Isla San Pedro in the Gulf of Corcovado, Chile. While rising water temperature and artificial eutrophication are the most discussed topics as a cause, marine bacteria is a recent attractive parameter as an algal bloom driver. This study monitored algal and bacterial compositions in the water of Isla San Pedro for one year using microscopy and 16S rRNA metabarcoding analysis, along with physicochemical parameters. The collected data were analyzed with various statistical tools to understand how the particle-associated bacteria (PA) and the free-living (FL) bacteria were possibly involved in algal blooms. Both FL and PA fractions maintained a stable bacterial composition: the FL fraction was dominated by Proteobacteria (α -Proteobacteria and γ -Proteobacteria), and Cyanobacteria dominated the PA fraction. The two fractions contained equivalent bacterial taxonomic richness (c.a. 8,000 Operational Taxonomic Units) and shared more than 50% of OTU; however, roughly 20% was exclusive to each fraction. The four most abundant algal genera in the Isla San Pedro water were *Thalassiosira*, *Skeletonema*, *Chaetoceros*, and *Pseudo-nitzschia*. Statistical analysis identified that the bacterial species *Polycycloporans algicola* was correlated with *Pseudo-nitzschia* spp., and our monitoring data recorded a sudden increase of particle-associated *Polycycloporans algicola* shortly after the increase of *Pseudo-nitzschia*, suggesting that *P. algicola* may have regression effect on *Pseudo-nitzschia* spp. The study also investigated the physicochemical parameter effect on algal-bacterial interactions. Oxygen concentration and chlorophyll-*a* showed a strong correlation with both FL and PA bacteria despite their assemblage differences, suggesting that the two groups had different mechanisms for interacting with algal species.

1. Introduction

Harmful algal blooms (HABs), which are phenomena resulting from exponential phytoplankton overgrowth, have been observed globally on almost every coast at increased frequency and intensity in recent years (Hallegraeff et al., 2021). Some microalgae can produce toxins, which accumulate in bivalves and cause illness in higher trophic levels of organisms that digest the contaminated shellfish. Even phytoplankton that do not secrete toxins can change and damage the ecosystem when high

biomass accumulates. Therefore, HABs negatively affect the environment, the health of living organisms, and even the economy (Anderson 1994; Glibert et al., 2005; Anderson et al., 2012). Although research into HABs has been progressing over time, their precise mechanism has not been fully elucidated. Many studies have reported that physical and geographical factors in the ocean control HABs (Hallegraeff 1993; Anderson 1994; Glibert et al., 2005; Anderson et al., 2012; Hallegraeff et al., 2021), but bacteria have also attracted attention in recent years as a HAB-driving factor (Bertrand et al., 2015; Amin et al., 2015; Ramanan

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et al., 2016; Seymour et al., 2017).

Interactions between phytoplankton and bacteria in the ocean play an essential role in carbon and nutrient cycling and stabilizing aquatic food webs. Most primary production becomes dissolved organic matter (DOM), which is used by heterotrophic bacteria, making it a major carbon flow pathway in the marine environment (Azam et al., 1983; Ducklow and Carlson, 1992; Azam and Malfatti, 2007; Thornton, 2014; Liu et al., 2020). Therefore, bacterial variability in the marine environment can alter carbon flux and, in turn, the phytoplankton community (Azam, 1998; Vargas et al., 2007; Bertrand et al., 2015; Seymour et al., 2017; Valdés-Castro et al., 2022). Given the critical role of bacteria in oceanic biogeochemical cycles, it is important to understand the dynamics of bacterial communities and their relationship with phytoplankton communities. Therefore, the focus of this study was to gather information on marine bacteria composition and diversity over time and examine how they may respond to HAB dynamics.

The study site was on the coast of Isla San Pedro (Los Lagos, Chile), located at the entrance of the Gulf of Corcovado and the southeastern end of Chiloé Island. This area is famous for different ecosystem services; salmon and mussel aquaculture has been the most important activity in recent decades (Bjørndal and Aarland 1999, and the area is also known for its frequent HAB outbreaks (Quiñones et al., 2019). For instance, shellfish aquaculture was affected by a bloom of *Pseudo-nitzschia australis* in 2000. The amnesic shellfish poisoning (ASP) toxin secreted by this HAB species contaminated the blue mussel *Mytilus chilensis* at a toxin concentration above the regulatory limit, leading to the coastal closure and mussel harvesting restriction for over one month (Díaz et al., 2019). Similarly, salmon industries in this region have been repeatedly damaged by paralytic shellfish poisoning (PSP) toxins from blooms of *Alexandrium catenella*, resulting in long-term coastal closures (Clément et al., 2002; Molinet et al., 2003; Fuentes-Grünwald et al., 2008; Crawford et al., 2021). Blue mussels and salmon businesses generate the highest annual revenues in Chile, and most production depends on this region (Molinet et al., 2010; Bjørndal and Aarland, 1999; Díaz et al., 2019; Quiñones et al., 2019). Consequently, the coast closure of this area due to HABs brings severe environmental and economic harm to local people and to the country.

Several studies on the Gulf of Corcovado to identify algal species and survey quantities using microscopy are ongoing for early HAB warning purposes (Yarimizu et al., 2020). The Instituto de Fomento Pesquero (IFOP) has been collecting ecological data on the Gulf of Corcovado since 2006. In addition, considerable efforts have been made to investigate responsible HAB species in this region (Mardones et al., 2010 and 2020; Varela et al., 2012; Paredes et al., 2019; Crawford et al., 2021). However, from the perspective of searching for a HAB driver, few data are available on bacteria in the Gulf of Corcovado. Furthermore, there is no time course survey on the bacterial community and composition in the area, although this may help elucidate how bacterial diversity changes could respond to HAB dynamics. The most relevant information available for this area is research carried out by some groups reporting on microbial composition at a few time points in the channels of Chilean Patagonia, further south of the Gulf of Corcovado (Gutiérrez et al., 2018; Valdés-Castro et al., 2022). Therefore, the present study monitored the seawater of Isla San Pedro in the Gulf of Corcovado biweekly for one year using 16S rRNA gene sequence analysis. This study further compared the taxonomic richness of particle-associated (PA) and free-living (FL) bacterial assemblages of the site using various statistical tools. Concurrently, phytoplankton species and physicochemical oceanographic data were collected to seek environmental parameters involved in the algae–bacteria association. Finally, a hypothetical microbiome interaction was derived from network analyses. These findings provide an insight into the understanding of algae–bacteria dynamics in the water of Isla San Pedro in the Gulf of Corcovado.

2. Materials and methods

The information on materials and reagents is listed in Table SI.

2.1. Sampling

The study period was from March 2019 to March 2020 (fall: March–May; winter: Jun–Aug; spring: Sep–Nov; summer: Dec–Feb). The sampling station was 1 km off the coast of Isla San Pedro, located at the entrance of the Gulf of Corcovado (−43.313; −73.662) (Figure S1 and Figure S2). The sampling methods generally followed the protocol of Yarimizu et al. (2020, 2021). Water was collected at 10-m depth biweekly with a deployed conductivity–temperature–depth (CTD) sampler from a survey ship. Chlorophyll-*a* (chl-*a*), water temperature, salinity, and DO were measured using a CTD sampler. The following work was performed on the vessel during the sampling: 5 L of the water sample was transferred into a triple-washed plastic container. From the collected water, 1 L was immediately filtered through a 1- μm tandem filter membrane to collect PA bacteria, followed by filtration through a 0.22 μm membrane to collect FL bacteria. These filtered membranes were stored in a freezer to be used for DNA extraction and sequencing. For nutrient analysis, 100 mL of the water sample was immediately filtered through a 0.22- μm pore syringe filter, and the filtrate was stored in a freezer. For microscopic phytoplankton identification, 200 mL of the water sample was concentrated $\times 20$ using a filter set, and the concentrated samples (10 mL) were fixed with Lugol to a final concentration of 1%. The Lugol-fixed samples were stored in a refrigerator at 4°C.

2.2. Laboratory analysis

The analytical methods are described in Yarimizu et al. (2020, 2021). Briefly, nutrient analysis was performed for NO₂, NO₃, PO₄, and Si(OH)₄ using an AQ400 discrete analyzer per United States Environmental Protection Agency (USEPA) procedure 40 C.F.R. part 136. Phytoplankton species were identified and quantified from 1 mL of Lugol-fixed sample using microscopy. The oceanographic data, including chl-*a*, water temperature, salinity, and dissolved oxygen (DO), were transferred from the CTD sampler. DNA was extracted from the filtered membranes using the Chelex-buffer method, followed by high-throughput amplicon sequencing using Illumina MiSeq with the primer sets in Table SII and index primers listed in Yarimizu et al. (2021). The meteorology data (precipitation and atmospheric temperature) were obtained from the open-access website; Dirección General de Aeronáutica Civil - Dirección Meteorológica de Chile Climáticos (<http://www.meteochile.gob.cl/PortalDMC-web/index.xhtml>), with the station code 430001 (Quellón AD, −43.13417°, −73.63194°).

2.3. Statistical analysis

The sequences were retrieved in fastq files and processed with DADA2 package v. 1.14.1 (Callahan et al., 2016) of R 3.6.0 (R Team, 2007) through trimming, quality filtering, dereplication, counting unique sequences, sample inference, merging into contigs, and removing chimeric sequences in default mode, but `-truncLen=290,240` was used to match the quality of sequence reads (Table SIII) (Yarimizu et al., 2021). Taxonomic assignment was based on small subunit (SSU) rRNA gene sequence in SILVA release 138 (Yilmaz et al., 2014). SILVA is the name from Latin *silva*, or forest (<http://www.arb-silva.de>) of a web resource database of aligned rRNA gene sequences and supplementary online services. Singletons, mitochondria, and chloroplasts were removed, and all samples were rarified to even sequencing depth based on the lowest sequencing depth. Sequences were clustered to operational taxonomic units (OTUs) at a 99% similarity level and merged into the same genus if multiple species records coincided within the same genus.

The R package ‘microeco’ (v. 0.6.1) was used for the following analyses (Liu et al., 2021). Chao-1 and Shannon were used as two indices of alpha diversity. Significant differences in the OTU count and alpha diversity indices were determined using paired t-tests. Beta diversity was measured using the Bray–Curtis dissimilarity index and was further evaluated by visualizing non-metric multidimensional scaling (nMDS). Linear discriminant analysis effect size (LEfSe) (Segata et al., 2011) was performed to compare the relative abundance of different bacterial genera between PA and FL groups. Taxa were considered significant based on a linear discriminant analysis (LDA) score greater than 4.0 and

a p-value less than 0.01. A distance-based redundancy (dbRDA) analysis was used to evaluate the variation in beta diversity between FL and PA bacterial communities based on physicochemical parameters. Spearman’s rank correlation test was carried out to examine the correlation between physicochemical parameters and the four most abundant algal species. Networks were constructed using an average of the distance matrices created from Sparse Correlations for Compositional data (SparCC) (Friedman and Alm, 2012), Spearman’s correlation and Sparse Inverse Covariance estimation for Ecological Association and Statistical Inference (SpiecEasi v.1.0.2) (Kurtz et al., 2015), where the networks

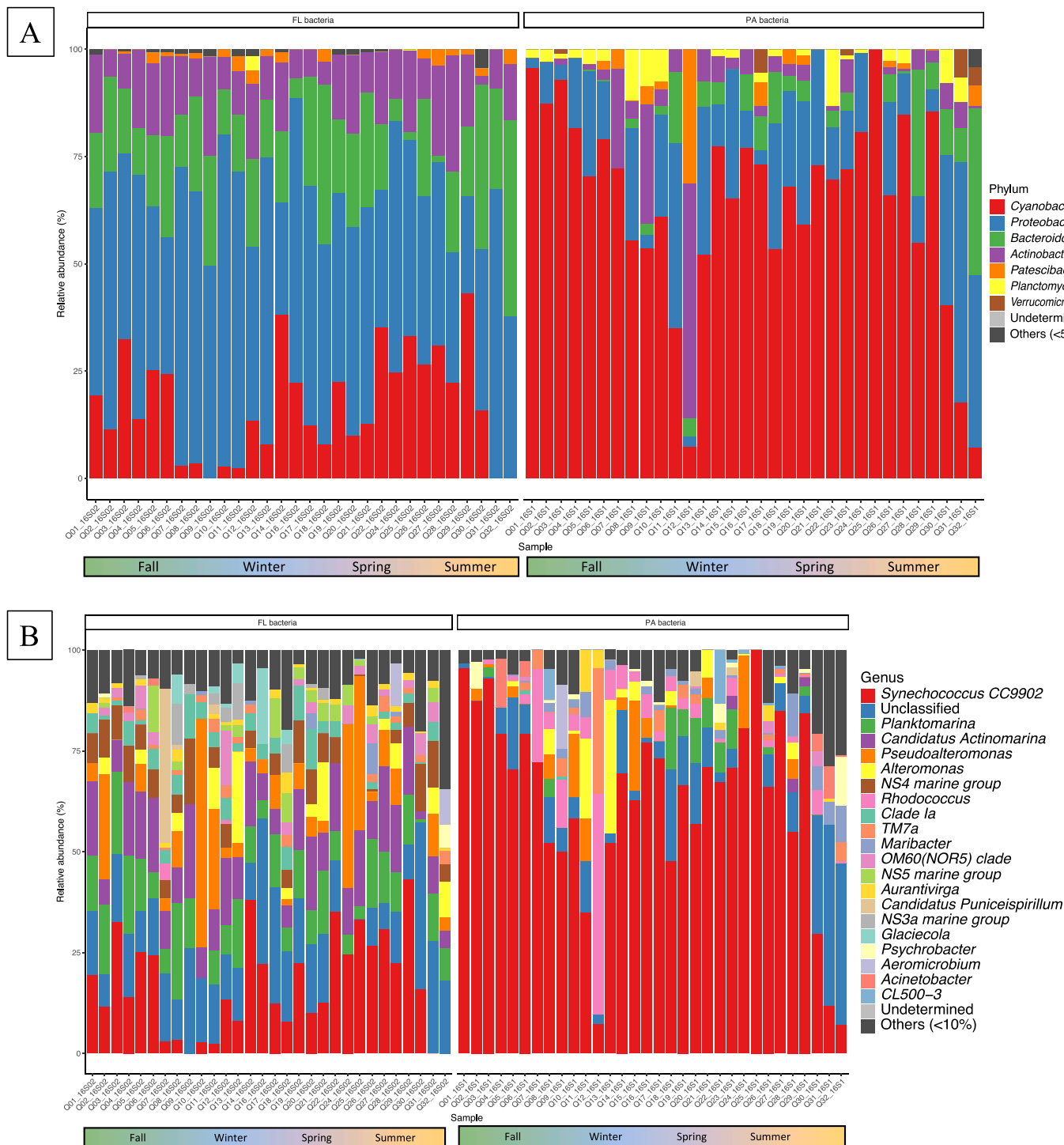


Fig. 1. Relative abundance of bacteria in the Isla San Pedro water in the Gulf of Corcovado Analysis was performed by 16S rRNA gene amplicon sequence for samples collected at 10-m depth from March 2019 to March 2020 to obtain a bacterial composition at A) phylum and B) genus levels.

were constructed using the 'Meinshausen and Bühlmann (mb)' method to find bacterial genera possibly associated with the algal species and physicochemical factors.

2.4. Phylogenetic tree

The 46 sequences classified as *Polycycloporans* were searched by blastn in the 16S/ITS database of Genbank (v. 20.220.611). All sequences with an identity greater than 90% and 100% coverage were obtained (a total of 15 sequences). After alignment with the sequences by the MAFFT online service (Kato et al., 2019), a phylogenetic tree was constructed using the neighbor-joining method with bootstrap value of 1000 by RAxML (v. 8.2.12) (Stamatakis, 2014), and visualized using FigTree (v. 1.4.4).

3. Results

3.1. Bacterial composition

The bacterial composition in the 10-m depth water of Isla San Pedro in the Gulf of Corcovado was monitored from March 2019 to March 2020 using 16S rRNA gene amplicon sequence analysis. The phylum composition of FL bacteria was stable throughout the year and dominated by Proteobacteria, accounting for more than 50%, followed by Bacteroidetes, Actinobacteria, and Cyanobacteria (Fig. 1). The phylum composition of PA bacteria varied, depending on the sampling date, although a pattern was seen with the most dominant group being Cyanobacteria, followed by Actinobacteria and Proteobacteria (Fig. 1).

The class composition of FL bacteria was also stable throughout the year: α -Proteobacteria and γ -Proteobacteria were the most dominant groups, followed by Bacteroidia and Acidimicrobia (Figure S3). The dominant class of PA bacteria was Oxyphotobacteria, followed by Actinobacteria and γ -Proteobacteria, and less frequently by α -Proteobacteria. The class composition of PA bacteria had higher variability during spring (Figure S3).

FL bacteria were classified within orders: Synechococcales, Rhodobacteriales, Flavobacteriales, Enterobacteriales, Pseudomonadales, SAR-11, and Actinomarinales (Figure S4). These order groups were almost evenly distributed throughout the year. The prevailing order group of PA bacteria was Synechococcales throughout the year (Figure S4).

The genera of FL bacteria included *Synechococcus*, *Planktomarina*, *Candidatus*, *Pseudoalteromonas*, and *Alteromonas* (Fig. 1). The genus *Synechococcus* CC9902 strongly dominated PA bacterial composition (Fig. 1). Among the PA bacterial community, *Synechococcus* was significantly high in summer than in winter ($p < 0.01$) (Figure S5A). In FL bacterial community, no seasonally unique bacterial genus was identified by the statistical analysis ($p > 0.01$) (Figure S5B).

3.2. Diversity of FL and PA bacteria

Taxonomic richness between the FL and PA fractions was compared by OTU. There were 8355 OTUs obtained from the FL fraction during the study period and 8843 OTUs from the PA fraction. The two fractions shared 3959 OTUs, accounting for 56.4% of total OTUs, while 23.2% of OTUs were exclusive to the FL fraction and 20.4% to the PA fraction (Figure S6). The taxa that determined phylogenetic community differences across the FL and PA groups were evaluated by LEfSe (Figure S6). The identified representative FL bacterial species were the genus of Clade Ia, *Candidatus actinomarina*, NS4 marine group, *Planktomarina*, NS5 marine group, and *Pseudoalteromonas*. The identified representative PA bacterial genus were *Halioxenophilus*, *Marinobacter*, *Psychrobacter*, *Rhodococcus*, and *Synechococcus* CC9902. The diversity of the FL and PA bacteria were computed by the Shannon diversity index and Chao-1 index, and an apparent inequality showed between these groups ($p < 0.01$) (Figure S7A). The difference in microbial communities between the FL and PA bacteria was assessed by Bray–Curtis dissimilarity and

showed a clear distinction in diversity (Figure S7B). The community dissimilarity was also supported by a dendrogram representing a different hierarchical clustering (Figure S8). The diversity of FL and PA bacteria were also different among the seasons (Figure S7C, E). The communities of PA bacteria were also different among the seasons (Figure S7F), while those of FL bacteria were not different (Figure S7D).

3.3. Temporal variation in dominant phytoplankton species

Phytoplankton species were identified and quantified by an inverted microscopy (Olympus CKX 42) at intervals. *Chaetoceros* spp. dominated phytoplankton composition throughout the study period. *Thalassiosira* spp. were fewer in number but also notably detected throughout the year (Fig. 1A). *Skeletonema* spp. occasionally showed a high relative abundance, especially during the austral fall and winter of the study period. *Pseudo-nitzschia* spp. dominated the composition at a few time points during the austral summer of the study period (Fig. 2A). In terms of absolute value, increased total cell count was observed during the austral summer, with the highest peak of over 4000 cells ml⁻¹ on 5 Mar 2020 (Fig. 2B). The most abundant species on 5 Mar 2020 was *Chaetoceros*, accounting for approximately 2500 cells ml⁻¹, followed by *Thalassiosira* and *Skeletonema* spp. A high abundance of *Pseudo-nitzschia* spp. was detected on three consecutive sampling dates during the austral summer, with a peak of 1261 cells ml⁻¹ on 24 Jan 2020.

3.4. Temporal variation in meteorological and physicochemical data

The dataset is provided in Table SIV. The summary is provided in Fig. 3. The summary of the max, min, and mean of each parameter is provided in Table SV.

3.4.1. Meteorology

The precipitation and atmospheric temperature were obtained at Quellón city, the closest meteorological station (−43.13417°, −73.63194°) from the sampling point. The average temperature of the city was 9.9°C, with a maximum of 13.9°C in January 2020 and a minimum of 6.3°C in August 2019. The average monthly cumulative precipitation of the city was 109 mm, with a maximum of 222 mm in July 2019 and a minimum of 55 mm in March 2020. The city is typically drier during the summer, from January to March.

3.4.2. Chl-a

The average chl-a at the sampling point was 2.9 $\mu\text{g l}^{-1}$. There were two chl-a spikes, Peak 1 (24 Jan 2020) and Peak 2 (28 Feb 2020), with 16.6 $\mu\text{g l}^{-1}$ and 17.9 $\mu\text{g l}^{-1}$, respectively. This was consistent with the microscopy data, showing the two peaks of increased absolute cell counts. The dominant phytoplankton species of Peak 1 was *Pseudo-nitzschia*, accounting for 1261 cells ml⁻¹ out of 1366 cells ml⁻¹. Peak 2 was dominated equally by *Chaetoceros* and *Thalassiosira*, accounting for 1299 cells ml⁻¹ and 1291 cells ml⁻¹ out of 3336 cells ml⁻¹, respectively.

3.4.3. Water temperature

There was no dramatic change in water temperature at the sampling point during the study. The average water temperature of the sampling point was 11.0°C, a maximum of 12.2°C on 4 Jan 2020 and a minimum of 9.9°C on 17 Oct 2019.

3.4.4. Salinity

There was no dramatic change in salinity at the sampling point during the study. Salinity remained between 32.7 PSU (Practical Salinity Unit) and 33.8 PSU, with an average of 33.3 PSU.

3.4.5. DO

The average DO of the sampling point was 7.1 mg l⁻¹, with a maximum of 9.0 mg l⁻¹ on 21 Mar 2019, and a minimum of 4.6 mg l⁻¹ on 12 Mar 2020.

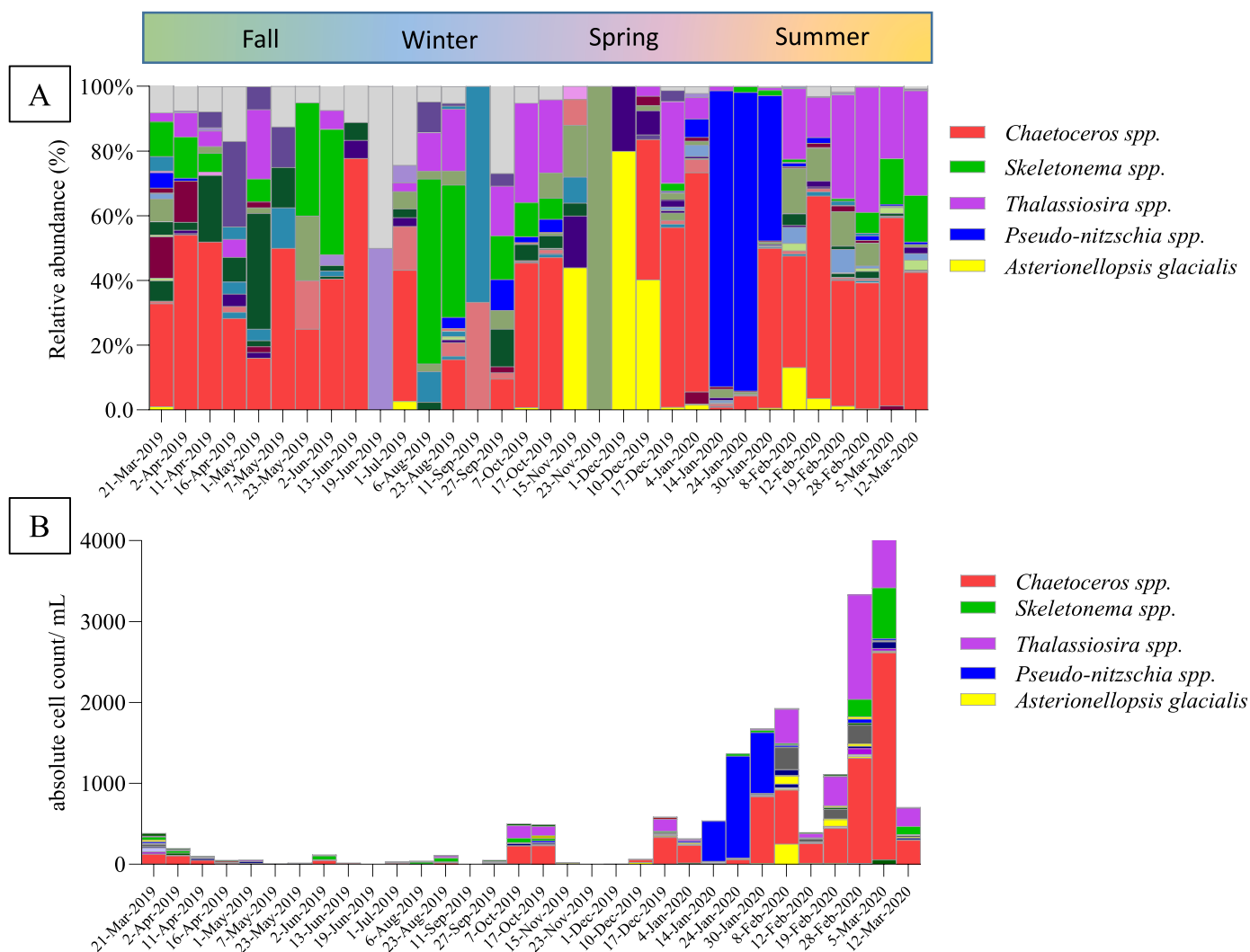


Fig. 2. Phytoplankton species in Isla San Pedro waters in the Gulf of Corcovado: Phytoplankton species and cell counts were recorded at every timepoint by microscopy from March 2019 to March 2020; A) relative abundance, B) absolute phytoplankton cells mL⁻¹.

3.4.6. Nitrogen

The nitrogen content showed variability during the study period. The average concentration of total nitrogen N_T (sum of NO_3 and NO_2) at the sampling point was 10.63 μM , with a maximum of 23.28 μM on 2 Jun 2019. NO_3 values were almost always 10-fold higher than NO_2 values, and therefore N_T dominantly represented NO_3 . The mean NO_3 was 10.04 μM , while the mean NO_2 was 0.59 μM .

3.4.7. Phosphate

PO_4 content was very low and stable throughout the study period. The average PO_4 at the sampling point was 1.27 μM , with a maximum of 1.92 μM on 12 Feb 2019.

3.4.8. Silicate

The average $Si(OH)_4$ at the sampling point was 4.21 μM , with a maximum of 35.74 μM on 2 Apr 2019. Silicate exceeded 15 μM at three intervals (2 Apr, 1 May, and 6 Aug in 2019) during austral fall and winter.

3.5. Algae–bacteria interaction

During the study period, the four most abundant algal species in the sampling point were *Pseudo-nitzschia* spp., *Chaetoceros* spp., *Skeletonema* spp., and *Thalassiosira* spp. (Fig. 2). Bacterial species possibly associated

with these algal species were searched for using network analysis, and the genus *Polycyclovorans* was identified as being correlated with *Pseudo-nitzschia* spp. (Fig. 4). Therefore, the relative abundance of *Polycyclovorans* obtained from 16S rRNA sequence analysis and *Pseudo-nitzschia* spp. obtained by microscopy was plotted in the time course (Fig. 5), which showed that the PA *Polycyclovorans* was selectively high on 30 Jan, shortly after the peak in *Pseudo-nitzschia* on 24 Jan in 2020. Network analysis further showed that *Polycyclovorans* was directly connected to *Pseudo-nitzschia* and indirectly related to chl-*a* through *Pseudo-nitzschia*, and bacterial species of *Fluvivirga* and *Alteromonas* were directly and indirectly connected to chl-*a*, respectively (Fig. 4). DO and water transparency were connected to a larger bacterial network regardless of connection to phytoplankton species (Fig. 4). To assign the species, the 46 OTU sequences of *Polycyclovorans* obtained were examined by BLASTn against the 16S/ITS database in Genbank. All 46 OTUs were most similar to *P. algicola*, with 98% identity and 100% coverage (Figure S9).

3.6. Physicochemical influence on bacterial communities

The physicochemical influence on bacterial communities was evaluated by dbRDA, in which both FL and PA bacterial groups were similarly correlated with the physicochemical parameters (Fig. 6) despite these bacterial groups differing in composition and diversity (Figure S6).

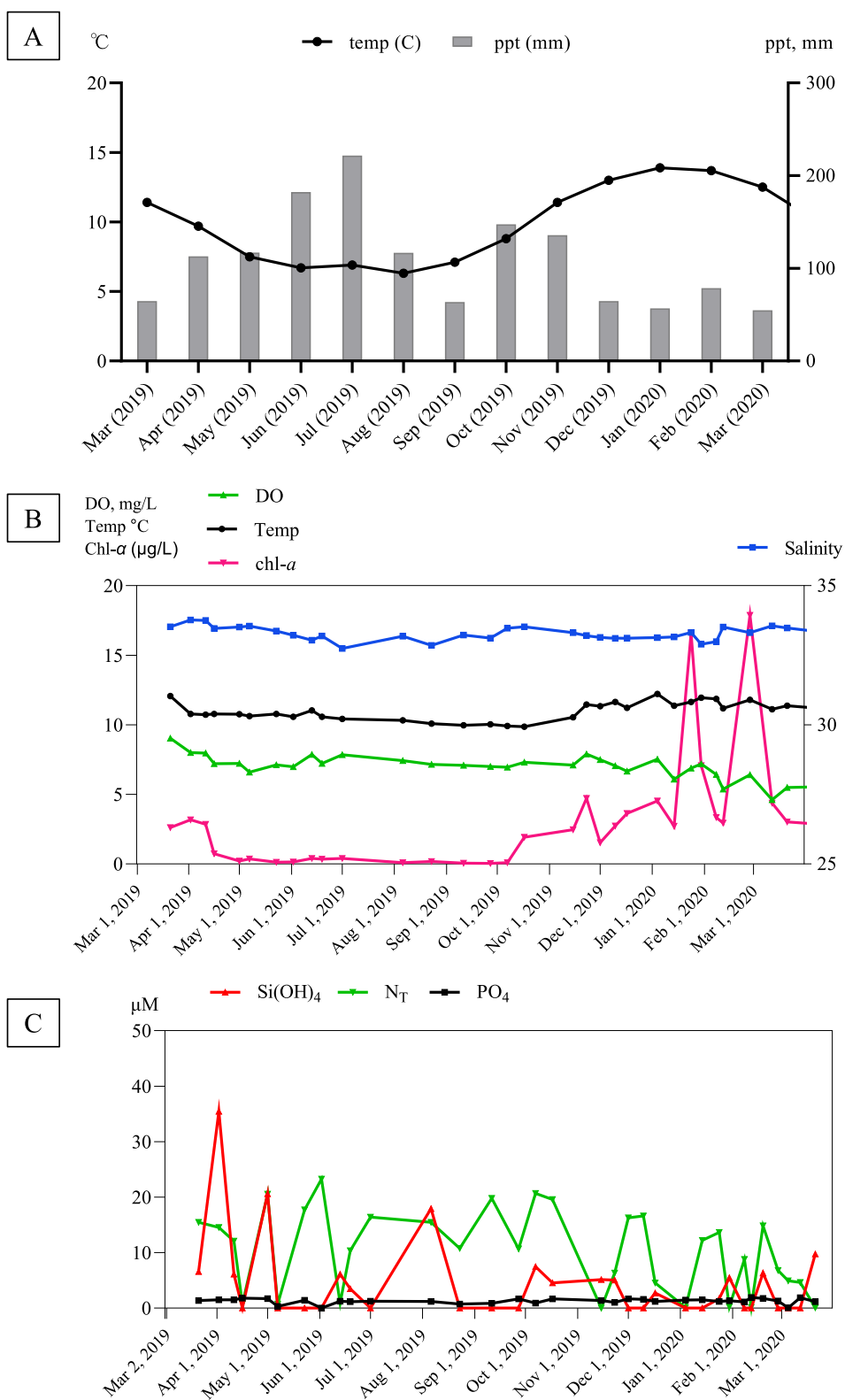


Fig. 3. Temporal variation in meteorology and physicochemical properties from March 2019 to March 2020: (A) Precipitation and atmospheric temperature at Quellón city, (B) water temperature, salinity, DO, and chl- α of Isla San Pedro waters in the Gulf of Corcovado, and (C) total nitrogen, PO $_4$, and Si(OH) $_4$ of Isla San Pedro waters in the Gulf of Corcovado.

Specifically, the first and second axes of the redundancy model collectively accounted for 39.2% of the total variation ($R^2 = 0.194$), but neither axes could separate the FL and PA bacterial communities (Fig. 6). Among the physicochemical parameters, chl- α showed the

strongest correlation with both FL and PA bacteria (Fig. 6). Pearson's correlation coefficient supported the alpha diversity of the PA bacterial community negatively and strongly correlated with chl- α and water temperature, while that of the FL bacteria positively and strongly

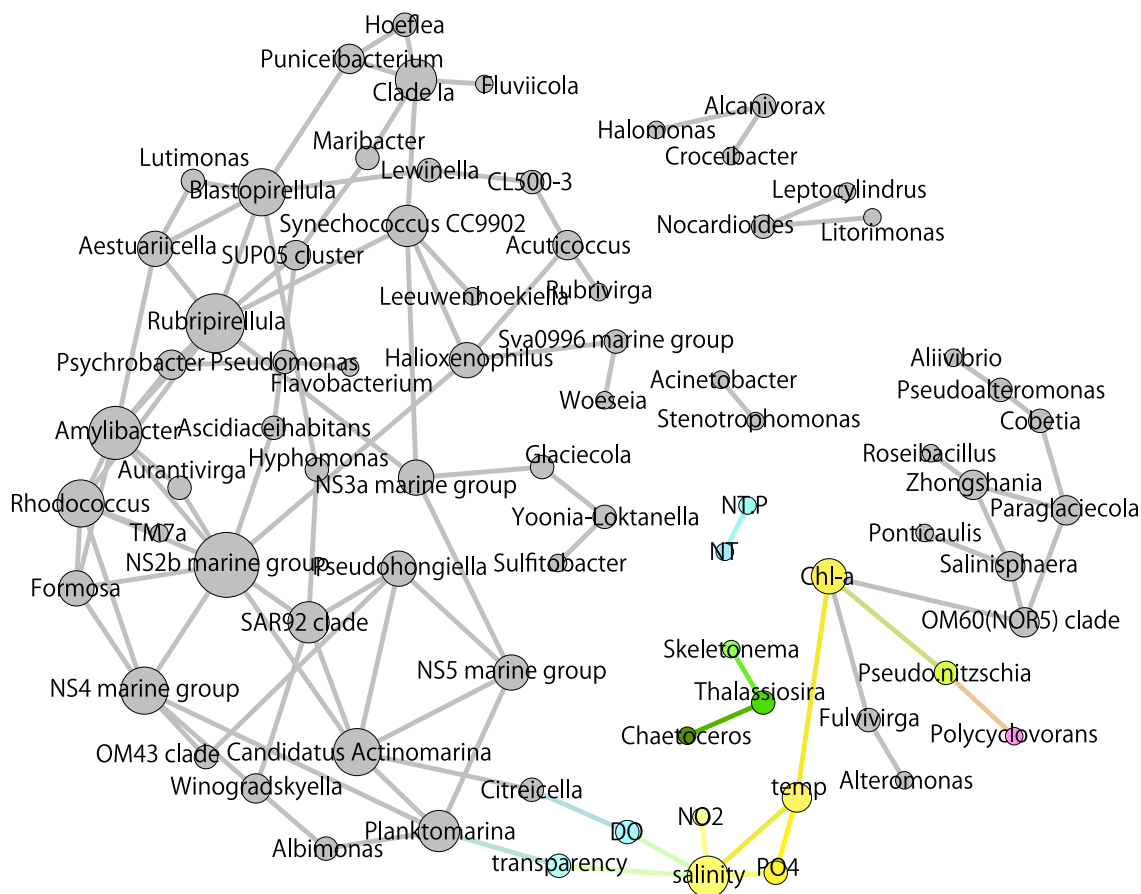


Fig. 4. Taxon network in the Isla San Pedro waters in the Gulf of Corcovado: Network analysis was performed with SpiecEasi to find bacterial species possibly associated with algal species and their connection to physicochemical factors. The figure shows the microbiome network linking bacteria, algae, and physicochemical factors. Light blue and yellow: environmental data; light green and green: algae; pink: algae-associated bacteria.

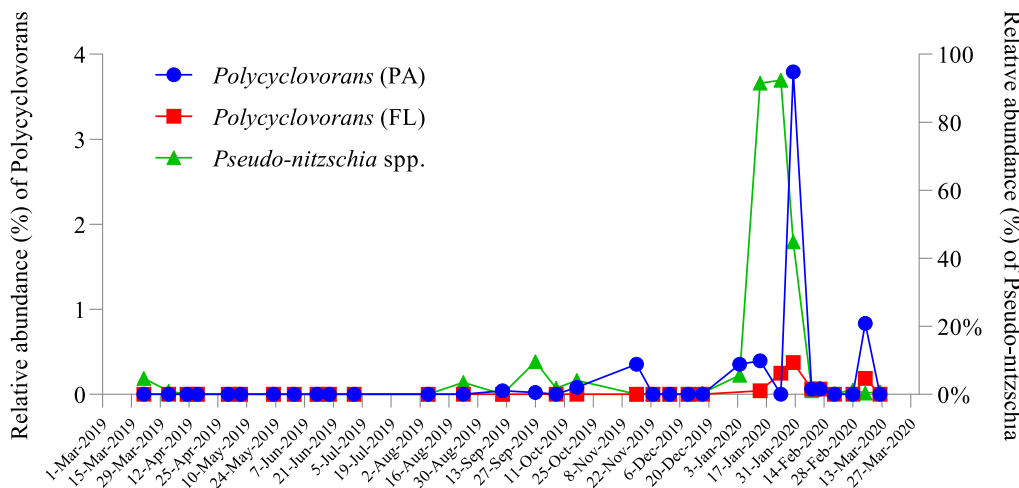


Fig. 5. Relative abundance of *Pseudo-nitzschia* and *Polycyclovorans* in the Isla San Pedro water in the Gulf of Corcovado: The relative abundance of free-living (FL) *Polycyclovorans* and PA *Polycyclovorans* was obtained from 16S rRNA metabarcoding analysis, and that of *Pseudo-nitzschia* spp. was obtained by microscopy. The peak of particle-associated (PA) *Polycyclovorans* was detected on 30 Jan, after the peak of *Pseudo-nitzschia* on 24 Jan 2020.

correlated with DO (Fig. 7).

3.7. Physicochemical influence on algal species

Spearman’s rank correlation coefficient (r) was used to assess how strongly each physicochemical parameter was related to the four most

abundant algal species in Isla San Pedro waters in the Gulf of Corcovado. *Pseudo-nitzschia* spp. and *Chaetoceros* spp. showed a moderate positive correlation with chl-a, while *Skeletonema* spp. and *Thalassiosira* spp. showed a weak positive correlation with chl-a (Fig. 8). In addition, the water temperature was positively correlated with *Pseudo-nitzschia* spp. and *Chaetoceros* spp. (Fig. 8). In contrast, *Chaetoceros* spp. was

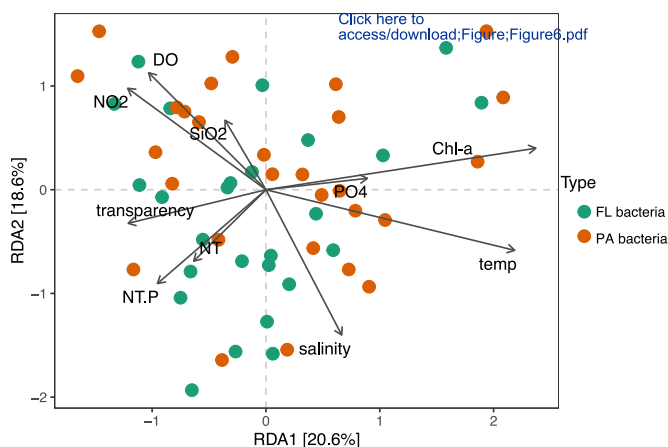


Fig. 6. Redundancy model of bacterial correlation with physicochemical parameters visualized by dbrDA ordination plot: The two dissimilarity matrices - (FL) and particle-associated (PA) bacteria - were evaluated for correlation with physicochemical parameters and visualized for beta diversity variation between the two bacterial communities.

negatively correlated with DO and transparency, while *Thalassiosira* spp. was negatively correlated with DO. None of these algal species correlated with the chemical parameters throughout the study period (Fig. 6).

4. Discussion

The present study investigated bacterial composition and diversity in the Isla San Pedro water in the Gulf of Corcovado from the perspective of algal bloom factors. The bacterial composition of the FL group was stable throughout the 1-year study period. Proteobacteria, specifically the classes of α -Proteobacteria and γ -Proteobacteria, dominated the FL fraction, and this observation was supported by many previous studies on marine bacteria taxonomic diversity and composition (Crespo et al., 2013; Miller et al., 2020; Valdés-Castro et al., 2022). The FL fraction in this study also contained Bacteriodota, Actinobacteriota, and Cyanobacteria, which have also been previously reported as dominant bacteria associated with phytoplankton in the marine environment, including in the southern Chilean ocean and estuaries (Crespo et al., 2013; Gutiérrez et al., 2018; Miller et al., 2020; Valdés-Castro et al., 2022).

The bacterial composition of the PA group fluctuated more than the

FL group in the Isla San Pedro water. Nevertheless, Cyanobacteria, specifically the *Synechococcus* genus, clearly dominated the PA fraction. This may be particularly true for shallow water, as represented by this sampling site, where microbial mats are complex and co-occurring populations have a high degree of biogeochemical interactivity (Mackey et al., 2017). Azam (2004) reported that *Synechococcus* is one of the most abundant oceanic primary producers. Crespo et al. (2013) investigated the bacterial assemblages in water at depths of 0–2000 m in the northwestern Mediterranean Sea and reported that Cyanobacteria represented >15% of the total bacterial abundance in the coastal sample of the PA fraction. In addition, Miller et al. (2020) studied six global basins and concluded that shallow-water communities were enriched with Cyanobacteria.

The number of taxa identified in the FL and PA fractions of the Isla San Pedro water was equivalent (approximately 8000 OTU each). Which fraction contains a greater variety of bacterial species probably depends on how easily and importantly the bacteria interact with co-occurring populations in the geographic location and water depth (Mackey et al., 2017). For instance, the PA fraction reportedly contained richer taxa than the FL fraction in the Mediterranean and Laptev Sea (Acinas et al., 1999; Ghiglione et al., 2007; Crespo et al., 2013; Kellogg and Deming 2009). Greater taxonomic richness in the PA fraction than in the FL fraction has also been reported in Arroyo Burro coastal lagoon samples (Lamontagne and Holden, 2003). In contrast, a research group reported that the FL community of the ultra-oligotrophic eastern Mediterranean Sea was taxonomically richer than the PA community (Rosenberg et al., 2021).

Despite their equal taxonomic richness, the bacterial assemblages of the FL and PA fractions differed in the Isla San Pedro water. A clear difference in bacterial assemblages between the two fractions has been observed in prior studies (Acinas et al., 1999; Ghiglione et al., 2007; Crespo et al., 2013; Kellogg and Deming, 2009; Lamontagne and Holden, 2003; Rosenberg et al., 2021). In this study, while roughly 50% of the total taxa were common to both fractions, each fraction contained the species likely to represent uniqueness, such as *Candidatus actinomarina*, *Planktomarina* spp., and *Pseudoalteromonas* spp. for the FL group and *Synechococcus* spp., *Halioxenophilus* spp., *Maribacter* spp., *Psychrobacter* spp., and *Rhodococcus* spp. for the PA group. Increasing evidence suggests that algae–bacteria interaction is specific, and PA bacteria require particular adaptations to attach to the pairing algae, perhaps moving to an appropriate distance so that they can effectively use the carbon resources and other available nutrients (Azam and Malfatti,

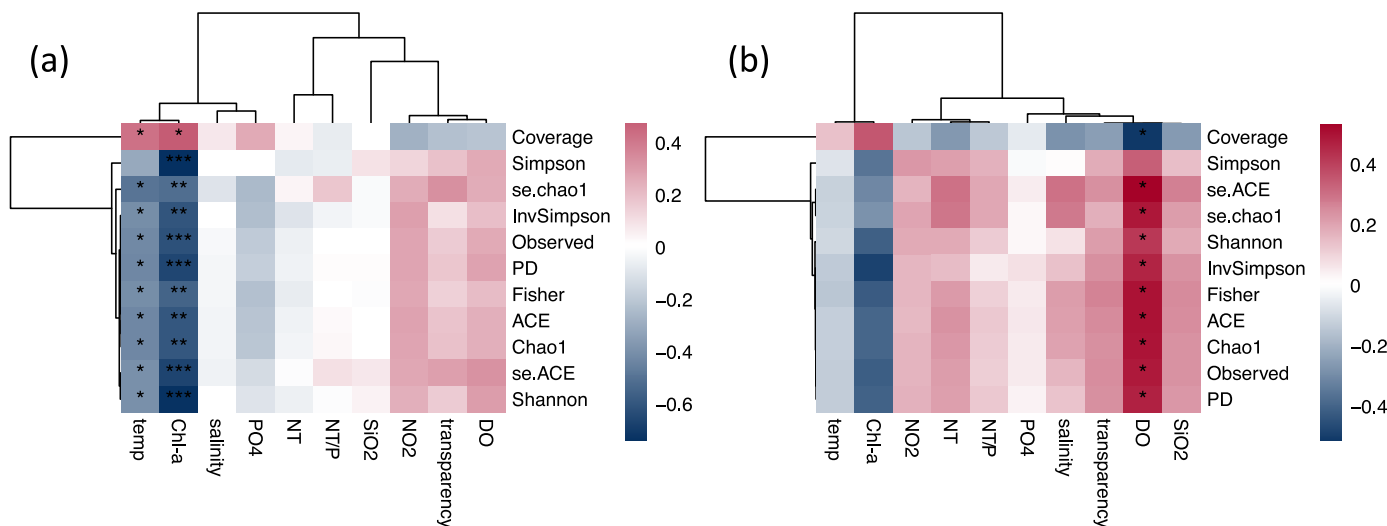


Fig. 7. Pearson's correlation coefficient of bacterial alpha diversity and physicochemical parameters: The correlation between environmental parameters and alpha diversity index of a) particle-associated (PA) bacteria and b) free-living (FL) bacteria is shown here. The correlation coefficients are significant at $p < 0.01$ *, 0.001 **, and 0.0001 ***.

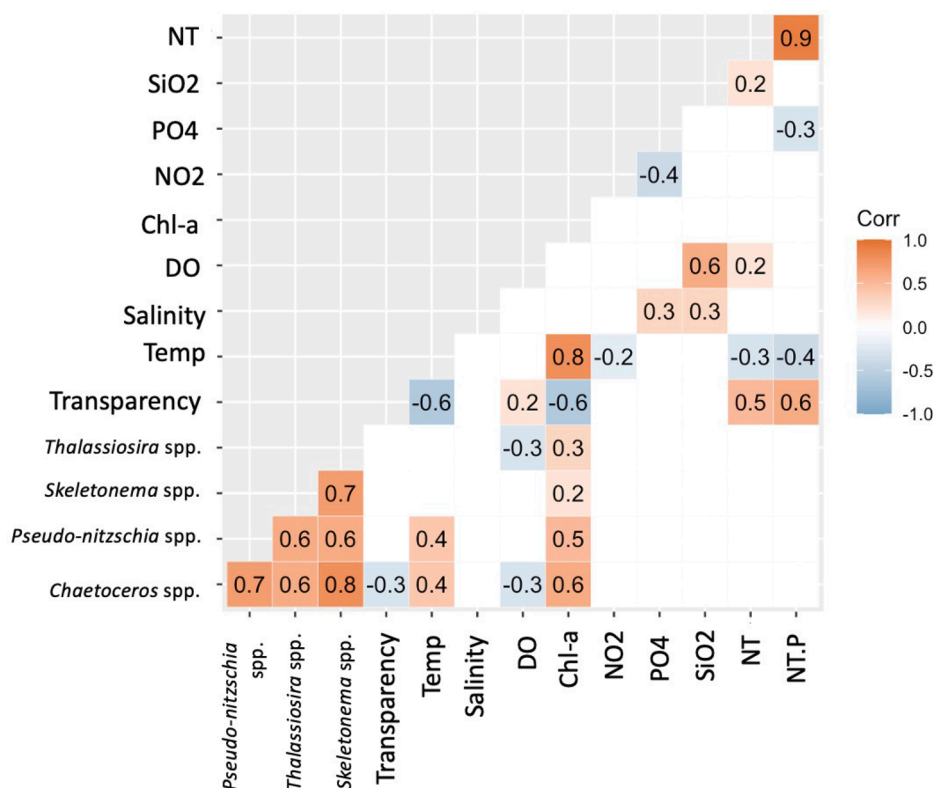


Fig. 8. Spearman's rank correlation coefficient: Correlation was evaluated between physicochemical parameters and the four most abundant algal species in the Isla San Pedro waters in the Gulf of Corcovado collected from March 2019 to March 2020. The value in each point represents a correlation coefficient significant at $p < 0.05$. Insignificant values are not shown.

2007; Bertrand et al., 2015; Amin et al., 2015; Ramanan et al., 2016; Seymour et al., 2017; Mayali 2018). Therefore, it is reasonable that the PA assemblage differed from the FL assemblage.

Both FL and PA bacterial communities in the Isla San Pedro water were strongly correlated to chl-*a* despite their assemblage differences, suggesting that the two groups had different mechanisms for interacting with algal species. The PA bacterial relationship with algal species can be explained by their mutualism and antagonism. For instance, the PA bacteria may be high-nutrient-loving bacteria, and they avoid nutrient-depleted situations by attaching to the pairing algal species (Lyons et al., 2010; Lyons and Dobbs, 2012; Crepo et al., 2013; Yung et al., 2016). PA bacteria can also act as predators: they swim up to the algal cell surface, use their hydrolases, and kill the metabolically coupled algal species (Mayali and Azam, 2004). Freshly lysed algae comprise organic matter and support the robust growth of bacteria (Bidle and Azam, 1999; Azam and Malifatti, 2007). In many cases, the relationship between clustering bacteria and phytoplankton is extraordinarily complex and variable, and the biochemistry of the algae–bacteria interaction needs to be characterized (Azam and Malifatti, 2007).

The most abundant algal genera in the Isla San Pedro water were *Thalassiosira*, *Skeletonema*, *Chaetoceros*, and *Pseudo-nitzschia*. Roughly half of *Pseudo-nitzschia* spp. are known to produce domoic acid, a neurotoxin responsible for a neurological disorder in humans, and thus a bloom of this species can be detrimental to this area, whose aquaculture is world-famous. Therefore, finding a bloom driver of this species can help aquaculture managers put appropriate countermeasures in place. From a perspective of bacteria as a factor involved in HABs, this study identified *Polycyclovorans algicola* as a potential pairing bacterium of *Pseudo-nitzschia* spp. from the network analysis. The monitoring data clearly showed their correlation: PA *Polycyclovorans algicola* selectively increased shortly after a peak in *Pseudo-nitzschia* bloom, suggesting that *Polycyclovorans algicola* may have regression effect on *Pseudo-nitzschia* spp. To the best of our knowledge, the interaction of *Polycyclovorans*

algicola and a *Pseudo-nitzschia* bloom has never before been reported from temporal environment monitoring. Thompson et al. (2018) reported a culture study showing the detection of *Polycyclovorans algicola* from microalgal cells isolated from coastal water and enriched with crude oil using Catalyzed Reporter Deposition Fluorescence in situ Hybridization (CARD-FISH). Nevertheless, little information is available on specific bacterial species involved in *Pseudo-nitzschia* in general, despite efforts made in algae–bacteria studies. For instance, Kaczmarek et al. (2005) reported that eight bacterial strains isolated from *Pseudo-nitzschia multiseries* cultures belonged to Alphaproteobacteria, Gammaproteobacteria, and Bacteroidetes clades. Similarly, Guannel et al. (2011) presented that Gammaproteobacteria, Roseobacter clade, and Flavobacteria coexist with *Pseudo-nitzschia* multiseries. In addition to *Polycyclovorans algicola*, network analysis showed that *Fluvivirga* and *Alteromonas* were related to chl-*a* values, suggesting that monitoring these two bacteria may lead to warnings of algal growth. Notably, microbiome network construction tools still face challenges such as sampling resolution, selection of sequencing data and pipeline, data preprocessing, confounding factors, and network interpretation, which can easily alter outcomes (Armitage and Jones, 2019; Faust, 2021). Nonetheless, microbiome network inference algorithms are improving every year, and network analysis is becoming a popular tool for examining microbial interactions in a diverse and complex community. Performing subsequent monitoring using a validation study protocol will ensure the reliability of the network analysis results.

Other factors are, of course, intricately involved in HAB mechanisms, which is the main reason why HAB prediction of HABs has been extremely challenging. For the Isla San Pedro water in the Gulf of Corcovado, all four of the most abundant algal species showed a positive correlation with chl-*a* measurements. *Pseudo-nitzschia* spp. and *Chaetoceros* spp. positively correlated with water temperature, while *Chaetoceros* spp. and *Thalassiosira* spp. were negatively correlated with DO. Most of these observations—namely physical influences on algal

growth—are consistent with prior studies. Water temperature is particularly a well-studied factor known to influence algal blooms (Kavak and Karadogan, 2012; Aguilera et al., 2013; Kumar et al., 2016; Gobler et al., 2017; León-Muñoz et al., 2018). In addition, geographical factors are encompassed in HABs. For instance, Patagonian fjords experience freshwater runoff to the marine environment during the austral spring–summer due to ice melt, and an influence on the ecosystem—including bacterial and algal biomass, community structure, and dynamics—is expected (González et al., 2013; Gutiérrez et al., 2018; Valdés-Castro et al., 2022). Surprisingly, this study did not find seasonal physicochemical factors that could explain the variation in beta diversity between the FL and PA bacterial communities. One explanation for this is that there was no dramatic fluctuation in physical and chemical parameters during the study period. Another explanation is that more than 50% of bacterial taxa are shared between the FL and PA bacterial communities in the study site, and these shared bacteria may be able to adapt and control the communities, regardless of the physicochemical factors.

5. Conclusions

This study presented differences and similarities between FL and PA bacterial taxa in the Isla San Pedro water and discussed how their assemblages changed over time. It also provided an insight into the algae–bacteria interaction with physicochemical factors. Our investigation identified an association of PA *Polycyclovorans algicola* with *Pseudo-nitzschia* spp. in the study site. Although further studies are necessary, *Polycyclovorans algicola* may be used as a possible *Pseudo-nitzschia* bloom termination indicator in the future. Ultimately, this work contributes to the baseline knowledge of how the bacterial community composition and its functional diversity will respond to algal dynamics in Isla San Pedro water.

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Data archiving

The sequencing results obtained in this study were registered into NCBI under accession number PRJNA854153.

CRediT authorship contribution statement

So Fujiyoshi: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Visualization, Data curation, Writing – original draft, Project administration. **Kyoko Yarimizu:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Visualization, Data curation, Writing – original draft, Project administration. **Gonzalo Fuenzalida:** Conceptualization, Validation, Resources, Writing – review & editing. **Marco Campos:** Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing. **Joaquín-Ignacio Rilling:** Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing. **Jacqueline J. Acuña:** Writing – review & editing, Project administration. **Pedro Calabrano Miranda:** Methodology, Validation, Validation. **Emma-Karin Cascales:** Methodology, Validation, Validation. **Ishara Perera:** Methodology, Software, Formal analysis, Validation, Visualization, Data curation. **Oscar Espinoza-González:** Resources, Project administration. **Leonardo Guzmán:** Resources, Project administration. **Milko A. Jorquera:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **Fumito Maruyama:** Conceptualization, Investigation, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

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