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Response of Phytoplankton Assemblages From Naturally Acidic Coastal Ecosystems to Elevated $p\text{CO}_2$

Natalia Osma^{1,2*}, Laura Latorre-Melín¹, Bárbara Jacob³, Paulina Y. Contreras⁴, Peter von Dassow^{1,5,6} and Cristian A. Vargas^{1,2,4}

¹ Millennium Institute of Oceanography (IMO), University of Concepción, Concepción, Chile, ² Department of Aquatic Systems, Faculty of Environmental Science, University of Concepción, Concepción, Chile, ³ Patagonian Ecosystems Research Center (CIEP), Coyhaique, Chile, ⁴ Center for the Study of Multiple-Drivers on Marine Socio-Ecological Systems (MUSELS), University of Concepción, Concepción, Chile, ⁵ Department of Ecology, Pontifical Catholic University of Chile, Santiago, Chile, ⁶ UMI 3614 Evolutionary Biology and Ecology of Algae, CNRS, Sorbonne University, Pontificia Universidad Católica de Chile, Universidad Austral de Chile, Station Biologique, Roscoff, France

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*Correspondence:

Natalia Osma
natalia.osma@imo-chile.cl

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The interplay of coastal oceanographic processes usually results in partial pressures of CO_2 ($p\text{CO}_2$) higher than expected from the equilibrium with the atmosphere and even higher than those expected by the end of the century. Although this is a well-known situation, the natural variability of seawater chemistry at the locations from which tested organisms or communities originate is seldom considered in ocean acidification experiments. In this work, we aimed to evaluate the role of the carbonate chemistry dynamics in shaping the response of coastal phytoplankton communities to increased $p\text{CO}_2$ levels. The study was conducted at two coastal ecosystems off Chile, the Valdivia River estuary and the coastal upwelling ecosystem in the Arauco Gulf. We characterized the seasonal variability (winter/summer) of the hydrographic conditions, the carbonate system parameters, and the phytoplankton community structure at both sites. The results showed that carbonate chemistry dynamics in the estuary were mainly related to seasonal changes in freshwater discharges, with acidic and corrosive conditions dominating in winter. In the Arauco Gulf, these conditions were observed in summer, mainly associated with the upwelling of cold and high $p\text{CO}_2$ ($>1,000 \mu\text{atm}$) waters. Diatoms dominated the phytoplankton communities at both sites, yet the one in Valdivia was more diverse. Only certain phytoplankton groups in this latter ecosystem showed a significant correlations with the carbonate system parameters. When the impact of elevated $p\text{CO}_2$ levels was investigated by $p\text{CO}_2$ manipulation experiments, we did not observe any significant effect on the biomass of either of the two communities. Changes in the phytoplankton species composition and abundance during the incubations were related to other factors, such as competition and growth phases. Our findings highlight the importance of the natural variability of coastal ecosystems and the potential for local adaptation in determining responses of coastal phytoplankton communities to increased $p\text{CO}_2$ levels.

Keywords: climate change, environmental variability, carbonate chemistry, community structure, coastal upwelling, estuary

1. INTRODUCTION

Human activities over the last two centuries have triggered changes in the global climate system at a pace unprecedented over the past 300 Myr (Caldeira and Wickett, 2003). On a global scale, the air-sea surface gas exchange has promoted an oceanic uptake of about one third of the recent anthropogenic CO_2 emissions (Sabine et al., 2004; Gruber et al., 2019), leading to an alteration of the seawater carbonate chemistry and a reduction in seawater pH of 0.1 units (Hoegh-Guldberg and Bruno, 2010). This process is well-known as ocean acidification (OA, Orr et al., 2005) and is expected to further decrease average oceanic pH between 0.14 and 0.35 units by the end of the century (Caldeira and Wickett, 2003), which will likely have substantial consequences for marine ecosystems (Bopp et al., 2013).

Models predicting future changes in the carbonate chemistry associated to OA can be fairly accurately applied in the open ocean, where the environmental heterogeneity is rather low (Hofmann et al., 2011). Nevertheless, anticipating the synergic effects of OA in coastal ecosystems becomes more complex, owing to the high fluctuations in the physical-chemical properties in short temporal and spatial scales that occurs in these regions (Booth et al., 2012; Waldbusser and Salisbury, 2014). The partial pressure of CO_2 ($p\text{CO}_2$) in the coastal domain exhibits a higher natural variability related both to biological processes, such as respiration and photosynthesis (Shamberger et al., 2011; Buapet et al., 2013; Saderne et al., 2013), as well as to oceanographic processes including the upwelling of deep waters and the discharge of fresh water from rivers (e.g., Cao et al., 2011; Vargas et al., 2016). The dynamic interplay of these coastal processes may result in $p\text{CO}_2$ levels in surface waters higher than expected from the equilibrium with the atmosphere (Hofmann et al., 2011; Wallace et al., 2014). One factor significantly contributing to $p\text{CO}_2$ variability in coastal areas is the discharge of riverine waters (Cao et al., 2011; Vargas et al., 2016). These waters are typically more acidic and corrosive than oceanic waters as they present low pH values and a reduced buffering capacity linked to their low alkalinity (Raymond and Cole, 2003; Vargas et al., 2018). In addition, the remineralization of the organic matter transported by rivers by heterotrophic organisms results in high DIC and $p\text{CO}_2$ levels. In this regard, estuarine environments constitute one naturally acidified coastal ecosystem, where the $p\text{CO}_2$ values can reach several thousands microatmospheres (Borges et al., 2006; Wallace et al., 2014), representing a potential source of CO_2 to the atmosphere. The other example is observed in upwelling-influenced coastal zones, where the upwelling-favorable winds promote the transport to the surface of naturally acidic subsurface waters characterized by low pH (<8) and high $p\text{CO}_2$ (>600 μatm) levels, resulting in a decrease of the seawater pH in the nearshore area (Feely et al., 2008). However, at the same time, these nutrient-rich upwelled waters stimulate primary production, which lower the concentration of dissolved inorganic carbon (DIC), ultimately reducing the levels of $p\text{CO}_2$. Owing to such local acidification processes in the coastal zone, marine organisms inhabiting this areas are constantly exposed to low pH/high $p\text{CO}_2$ conditions and, therefore, may be operating at the limit of their physiological tolerances. Further acidification of

the water through OA may lead to a synergistic effect (Gruber, 2011) and bring their physiology beyond these thresholds. Conversely, it may also occur that local adaptation to these highly variable environments confers on these organisms a higher resilience capacity to face sudden changes and increases in the $p\text{CO}_2$ conditions (e.g., Duarte et al., 2013; Vargas et al., 2017). All these potential effects and consequences are still a matter of investigation.

The impact of high $p\text{CO}_2$ levels on the phytoplankton community has been previously characterized in several distinct ecosystems, e.g., the Northern Sea (Burkhardt et al., 2001), equatorial Pacific (Tortell et al., 2002) or Southern ocean (Tortell et al., 2008; Trimborn et al., 2013), among many others. However, these studies have shown contrasting responses of the phytoplankton community structure to increased CO_2 levels. For instance, at high $p\text{CO}_2$ conditions, Tortell et al. (2002) observed a decrease in the abundance of diatoms and an increase of the non-siliceous groups whereas Tortell et al. (2008) reported an increase in primary production and abundance of the chain-forming diatoms *Chaetoceros* spp. Hare et al. (2007), in turn, showed that diatoms were replaced by nanoflagellates under these same conditions. Therefore, there is not a clear consensus about which groups will be benefited in a high $p\text{CO}_2$ environment. In this light, knowing the carbonate system dynamics of the ecosystems where the phytoplankton communities inhabit may be crucial to correctly interpret the outcome of these investigations (Hofmann et al., 2011; Vargas et al., 2017). Unfortunately, this information is usually lacking in the investigation of the impact of OA.

In the present work, we provide insights into the natural seasonal variability of the carbonate system and the phytoplankton community structure and physiology at two contrasting coastal areas off central Chile: an estuarine ecosystem (Valdivia River estuary), and a coastal upwelling ecosystem influenced by freshwater discharges (Arauco Gulf). In addition, the response of these phytoplankton communities to elevated $p\text{CO}_2$ levels is studied using CO_2 manipulation experiments. The objective of this study was 2-fold: (1) to evaluate the relationship between the seasonal variability of the seawater chemistry and the phytoplankton community at two contrasting coastal ecosystems, and (2) to study the response of these communities to elevated $p\text{CO}_2$ levels. Our hypothesis was that phytoplankton communities inhabiting highly variable, naturally acidic coastal ecosystems, such as estuaries, river-influenced areas and/or coastal upwellings, are adapted to high $p\text{CO}_2$ conditions.

2. METHODS

2.1. Description of Study Sites

In this study we investigated two coastal ecosystems off Central Chile which experience naturally high and variable $p\text{CO}_2$ conditions: The river-influenced coastal upwelling area off Concepción, in the northern part of the Arauco Gulf (36° 42'–58' S, 73° 12'–17' W), and the Valdivia River estuarine system (39° 46'–53' S, 73° 25' W) (Figure 1). The Arauco Gulf is an equatorward facing embayment characterized by intense seasonal upwelling events between September and March (austral Spring-Summer) caused by an increase in the southwesterly wind stress

(Sobarzo et al., 2007; Letelier et al., 2009). During these events, surface waters over the continental shelf edge are typically supersaturated in CO_2 , resulting in strong across-shore $p\text{CO}_2$ gradients (Torres et al., 2011). In its northern part, the Arauco Gulf is influenced by freshwater discharges from the Biobío River, whose plume typically moves southward within the bay. Average flow rates of this river range between 200 and 3,000 $\text{m}^3 \text{s}^{-1}$ (Valle-Levinson et al., 2003), with maximum values observed during the rainy season in winter (June–August) and minimum values during summer (January–March). Likewise, Biobío River runoff constitutes a significant source of nutrients (silicate, nitrate and phosphate), trace metals and dissolved organic and inorganic carbon (DOC and DIC) for the adjacent coastal ocean (Pérez et al., 2015; Vargas et al., 2016). The Valdivia River estuary, in turn, constitutes one of the most important estuarine systems from central-southern Chile in terms of mean area and freshwater discharges. Hydrographic conditions reveals that it is a partially mixed type estuary, widely affected by tidal cycles (Pino et al., 1994). It presents a seasonal rainfall regime, with an annual average freshwater discharge of 592 $\text{m}^3 \text{s}^{-1}$ and maximum and minimum flow rates in austral winter (July) and summer (March), respectively (Garcés-Vargas et al., 2013).

In this study, we conducted four sampling campaigns between 2014 and 2016, both during austral winter and summer; two in the Valdivia River estuary (August 2014 and January 2015) and two in the Arauco Gulf (September 2015 and January 2016). Each sampling was accomplished in 1 day and consisted in an along-shore transect of five stations (Figure 1), covering areas influenced by fresh-water discharges and areas without this influence.

2.2. Sampling and Hydrographic Measurements

The hydrological information on daily river flows were obtained from the Dirección General de Aguas (www.dga.cl) of the Chilean Ministry of Public Work. Seasonal variability in the freshwater discharges of the Biobío and Valdivia Rivers during the sampling years is shown in Figure 1. During each campaign, the water column was characterized through vertical profiles of temperature, salinity and oxygen recorded using a SeaBird SBE-19 plus conductivity-temperature-depth (CTD) equipped with a Westar fluorometer. Water samples for chlorophyll *a* (Chl *a*; 200–300 mL), nutrients [NO_3^- , NO_2^- , PO_4^{3-} , and $\text{Si}(\text{OH})_4$; 25 mL], DIC and the $\delta^{13}\text{C}$ isotopic signature of DIC (40 mL), as well as for phytoplankton abundance (250 mL) were collected with 5 L Niskin bottles at 2, 10, 25, and 50 m (only at GA3 and GA5) in the Arauco Gulf, and at 1, 3, and 5 m (plus two additional depths at 8 and 10 m only at VE4 and VE5) in the Valdivia River estuary. In addition, samples for Total Alkalinity (TA) in the Arauco Gulf and for pH in the Valdivia estuary were obtained at the same sampling depths, which were used to estimate both the $p\text{CO}_2$ and the saturation state of aragonite (Ω_{arag}) (see below). Chl *a* samples were directly filtered through GF/F (total Chl *a* concentration), and stored at -20°C until analysis in the laboratory by fluorometry (Turner Design TD-700) according to Parsons et al. (1984). Water samples for nutrients analysis were

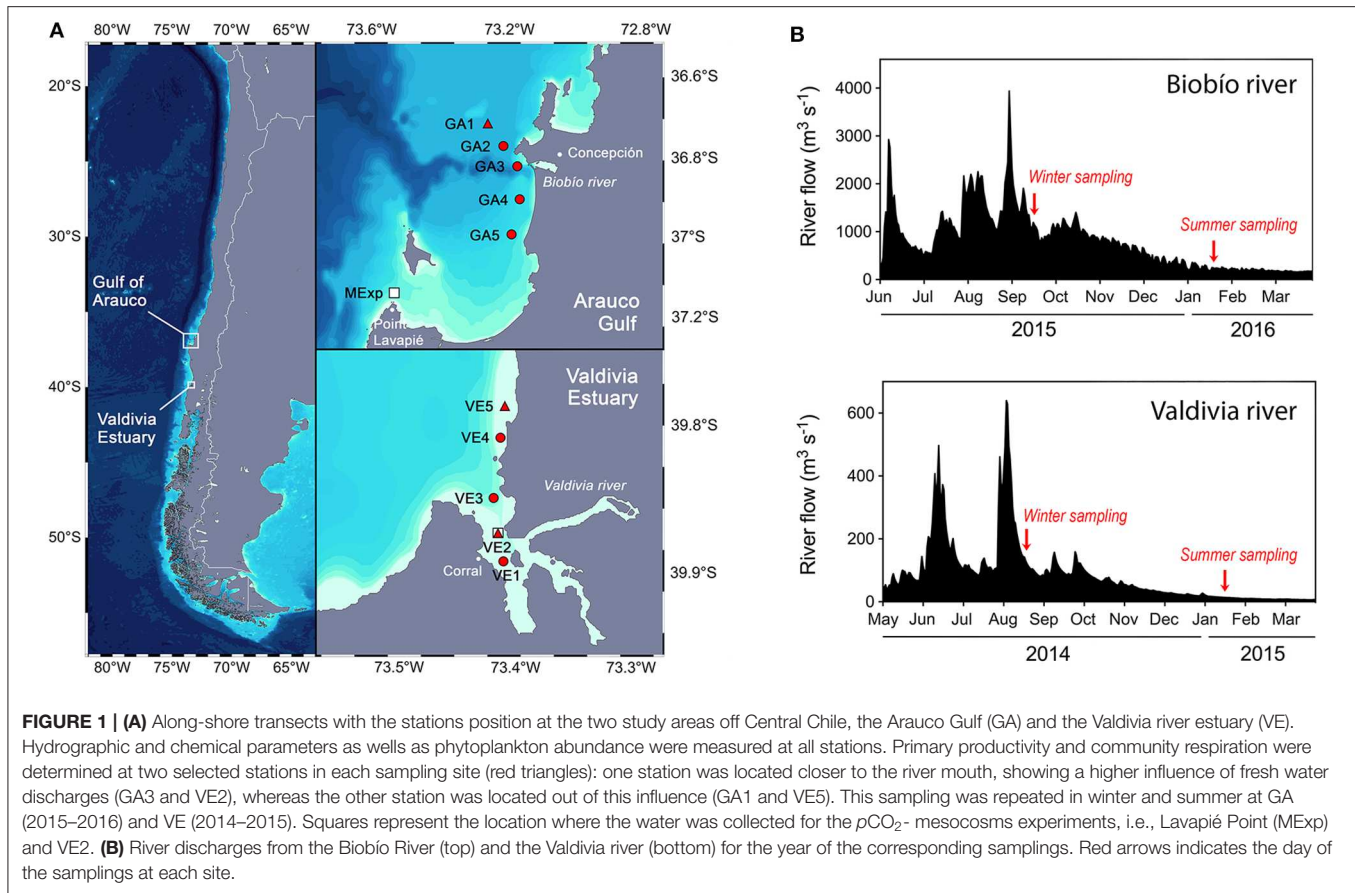
pre-filtered (GF/F), frozen (-20°C) and subsequently analyzed following (Strickland and Parsons, 1968).

Gross primary productivity (GPP) and community respiration (CR) was estimated at two stations in each study area (Figure 1), both during winter and summer campaigns. One station was selected at the inner part of the Valdivia River estuary (VE2) and another one offshore in Corral Bay (VE5), at 20 km from the coast. Similarly, in the Arauco Gulf, one station was located inside the Biobío river plume (GA3) at 4 km from the coast, and the other one northward outside the influence of this river plume (GA1), at ~ 40 km from the coast. *In situ* incubations to determine GPP and CR were conducted at two depth levels (1 and 3 m) in the Valdivia River estuary and at three depth levels within the euphotic zone (2, 10, and 15 m) in the Arauco Gulf. Discrete-depth measurements of GPP and CR were integrated to 5 and 20 m depths (in Valdivia estuary and the Arauco Gulf, respectively) using the trapezoidal approximation.

Finally, two additional water samplings were carried out to perform the carbonate system manipulation experiments with phytoplankton assemblages from both coastal upwelling and estuarine ecosystems. In the Valdivia River estuary, about 70 L of surface water was collected using 10 L-Niskin bottles at the inner part of the estuary (VE2) in August 2014. Here, the CO_2 manipulation experiments were conducted at the Coastal Station of Calfuco (Universidad Austral de Chile, $39^\circ 78' \text{S}$, $73^\circ 39' \text{W}$). In the Arauco Gulf, the sampling took place in November 2016 at the upwelling center off Lavapié Point ($37^\circ 08' \text{S}$, $73^\circ 34' \text{W}$). A similar water volume (~ 70 L) was collected using a suction pump in clean plastic containers and was immediately transported to the mesocosm facilities at the Marine Biological Station of Dichato (Universidad de Concepción, $36^\circ 33' \text{S}$, $72^\circ 39' \text{W}$). Major physical-chemical parameters, such as temperature, salinity, nutrient concentration and CO_2 system parameters (i.e., DIC and pH), as well as Chl *a* concentration and phytoplankton abundance were likewise determined during these field collections in order to have the *in situ* conditions of the experimental water.

2.3. Analysis and Estimation of the Carbonate System Parameters

All the parameters were analyzed according to Riebesell et al. (2010). Seawater pH was measured potentiometrically in a 25 ml-thermostatted cell at $25 \pm 0.1^\circ\text{C}$ using a Metrohm 713 pH meter (input resistance $> 1,013 \text{ Ohm}$, 0.1 mV sensitivity, and nominal resolution 0.001 pH units) with a glass combined double junction Ag/AgCl electrode (Metrohm model 6.0219.100), calibrated with 8.089 Tris buffer (25°C) according to DOE (1994). pH values are given on the total hydrogen scale (pH_T). For DIC and its $\delta^{13}\text{C}$ isotopic measurements, a subsample of 40 ml was collected with a sterile syringe, filtered through a GF/F filter (previously pre-combusted at 450°C for 4–5 h) directly into 40 ml glass 200 Series I-CHEM[®] vials and poisoned with a saturated solution of HgCl_2 to stop biological activity. The septa of the vials were substituted by butyl rubber septa to prevent diffusion of CO_2 (DOE, 1994). Samples were run on an OI Analytical total inorganic carbon-total organic carbon (TIC-TOC) Analyzer



(Aurora Model 1030), first run to determine the parts per million carbon organic/inorganic concentration and then for the $\delta^{13}\text{C}$ isotopic signature. The TIC-TOC analyzer was interfaced with a Finnigan Mat DeltaPlus isotope ratio mass spectrometer for analysis by continuous flow. Data were normalized using internal standards. The analytical precision was 2% for the quantitative DIC measurements and $\pm 0.2\text{‰}$ for the isotopes. All the analysis were conducted in the G. G. Hatch Stable Isotope Laboratory at the University of Ottawa, Canada. Samples for total alkalinity (TA) were collected in 250 ml- glass bottles, fixed with a saturated solution of HgCl_2 , and subsequently measured in the laboratory by potentiometric titration with HCl following the closed-cell method described in DOE (1994).

pH and DIC values (Valdivia estuary), and TA and DIC values (Arauco Gulf) were used in the software CO2SYS (Pierrot et al., 2006) to calculate the rest of the carbonate system parameters (i.e., $p\text{CO}_2$, TA and pH_T in Valdivia estuary and Arauco Gulf, respectively) and the Ω_{arag} . Temperature, salinity, and nutrient [PO_4^{3-} and $\text{Si}(\text{OH})_4$] data were likewise included in these calculations. The carbonic acid dissociation constants (K_1 and K_2) were taken from Mehrbach et al. (1973) as refit by Dickson and Millero (1987) for marine waters (salinity > 30 psu) and those by Millero (2010) for estuarine waters (salinity between 1 and 30 psu). The KHSO_4 equilibrium constant determined by Dickson (1990) was applied for all calculations.

2.4. Phytoplankton Community Structure, Gross Primary Production (GPP), and Community Respiration (CR)

Phytoplankton samples for microscopy were fixed with 2% acidic Lugol solution and analyzed following the Utermöhl technique (Utermöhl et al., 1958), with classification until the lowest possible taxonomical level, using an Olympus IX51 microscope. GPP and CR were estimated from changes in the dissolved oxygen (DO) concentrations after incubating BOD bottles at *in situ* light conditions and in the dark (Strickland, 1960). At all samplings, the incubation time was <12 h and 3–4 replicates of each light condition (i.e., *in situ* and dark) were run. DO was determined before and after the incubation using the OxyMini[®] optode system (WPInst.). Net community production (NCP) was calculated as the difference in the DO concentration between light (GPP) and dark (CR) BOD bottles after the incubation.

2.5. Carbonate System Manipulation Experiments

Once in the laboratory, the seawater collected at each sampling site for the experiments was pre-filtered through a 200 μm mesh to eliminate large zooplankton. After acclimation to the corresponding $p\text{CO}_2$ treatment for 12 h (Valdivia estuary) or 22 h (Arauco Gulf), seawater was transferred to polycarbonate

20L- bottles to start the experiments. Semi-automatic mesocosm systems for seawater carbonate chemistry manipulation of similar characteristics were employed at the two locations. The different $p\text{CO}_2$ treatments were attained by continuously bubbling the seawater with either ambient (low $p\text{CO}_2$ treatment) or CO_2 -enriched air (high $p\text{CO}_2$ treatment). Air/ CO_2 mixtures were produced using a bulk technique, based on mixing filtered, dry air with ultra pure CO_2 at a known flow rate using Mass Flow Controllers (MFC; Aalborg Model GFC). In the Valdivia River estuary the low $p\text{CO}_2$ level was set at $\sim 380 \mu\text{m}$ (control level, IPCC 2014), whereas in the Arauco Gulf the low $p\text{CO}_2$ level was set at $700 \mu\text{m}$ corresponding to the *in situ* value during field sampling (i.e., “control” treatment). For the estuarine community, the high $p\text{CO}_2$ treatment was $\sim 900 \mu\text{m}$ whereas, for the coastal upwelling area, we considered an experimental treatment of $1600 \mu\text{m}$ $p\text{CO}_2$. The upwelled water was already CO_2 -saturated ($700 \mu\text{m}$ $p\text{CO}_2$), and therefore we assumed a higher ΔCO_2 of around $900 \mu\text{atm}$. In both cases, these high $p\text{CO}_2$ levels can naturally occur in these ecosystems (Vargas et al., 2017). Four replicates per treatment were run during the experiments, that lasted for 5 days and were conducted under temperature controlled conditions and 12/12 days/night light regime. The water inside the incubation bottles was gently mixed using rotor-powered blades, in order to prevent sedimentation of the phytoplankton cells. Subsamples for nutrients, carbonate system parameters, chlorophyll *a* and phytoplankton abundance were collected every day (except nutrients in the Arauco Gulf, which were sampled *in situ*, and on days 2 and 4 during the experiments) and analyzed as stated above.

2.6. Statistical Analyses

Pairwise comparisons were conducted applying the Student *t*-test and were considered significant at $p < 0.05$. The phytoplankton community was characterized using the Shannon-Wiener Diversity index (H'), Margalef's species richness (d), and Pielou's evenness index (J_0) at each station and sampling time, using logarithms to the base 2 in the calculation of H' . It should be noted that these indices are not true species-level diversity indexes as some of the organisms could only be identified to the genus level (e.g., *Chaetoceros* spp. and *Thalassiosira* spp.). The correlation between environmental and biological variables was addressed by means of the Spearman correlation coefficient, which were considered significant at $p < 0.05$. Furthermore, a Generalized Linear Model (GLM) was applied to significantly correlated variables to evaluate the dependence between the biological variables and the environmental factors at each study site.

3. RESULTS

3.1. Hydrography

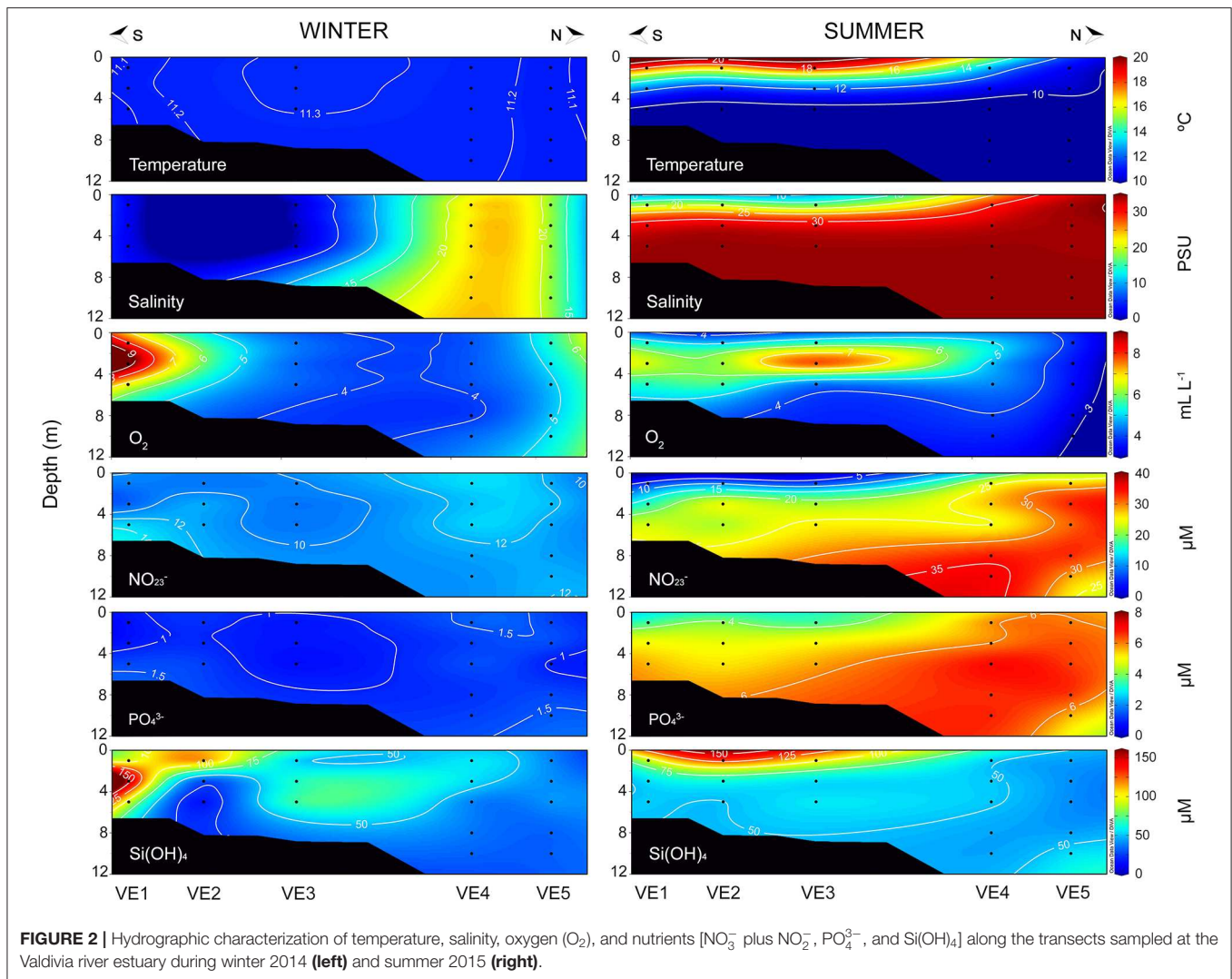
Clear seasonal differences in the hydrographic conditions were observed at the two sampling sites. The Biobío and Valdivia rivers discharges showed a typical annual pattern during the sampling years, with maximum values in winter and minima in summer (Figure 1B). Accordingly, fresh water influence was higher in winter at both areas, although in Valdivia, almost the entire

water column at the inner estuarine stations was dominated by riverine waters (VE1-VE3, Figure 2) whereas in the Arauco Gulf this influence was limited to the surface waters in the southernmost stations (GA3-GA5, Figure 3). The water column along the transects was well-mixed and characterized by low temperatures ($11\text{--}12^\circ\text{C}$) and oxygenated waters ($> 3 \text{ ml L}^{-1}$) during this season. In summer, in contrast, the warming of the surface waters caused the stratification of the water column, with temperatures decreasing 9°C in the upper 5 m in the Valdivia estuary (Figure 2) and 4°C in the upper 20 m in the Arauco Gulf (Figure 3). In the Valdivia estuary, the reduced river flow during this season limited the influence of freshwater to the first 4 m at the inner part of the estuary (VE1-VE3), whereas the influence of more oceanic waters characterized by higher nutrient levels (both $\text{NO}_3^- + \text{NO}_2^-$ and PO_4^{3-}) increased. In the Arauco Gulf, the low oxygen values ($< 1 \text{ ml L}^{-1}$) together with the high PO_4^{3-} concentrations below 20 m showed the influence of an upwelling of oxygen-poor nutrients-rich subsurface waters in summer, especially at stations GA1, GA2, and GA5 (Figure 3). However, $\text{NO}_3^- + \text{NO}_2^-$ concentration did not follow the same pattern as the concentrations in summer were lower than in winter, which may be attributed to an enhanced nitrogen uptake by phytoplankton during the spring-summer blooms. Si(OH)_4 concentrations, in turn, were significantly higher in Valdivia than in the Arauco Gulf (*t*-test, $p < 0.05$) but were associated with freshwater inputs at both sites, with maximum values at the Biobío river mouth (GA3) in the Arauco Gulf and at the inner estuarine stations (VE1-VE3) in Valdivia.

3.2. Carbonate System Dynamics at Two Coastal Ecosystems

As with the hydrographic conditions, carbonate system parameters showed marked seasonal differences. The dynamics of the carbonate system in the Valdivia estuary were driven by the interplay between predominant freshwater discharges in winter and the influence of more oceanic waters in summer (Figure 4). In winter, the water column at the inner part of the estuary (VE1-VE3) was characterized by low DIC concentrations ($< 1,250 \mu\text{mol kg}^{-1}$), low TA ($< 1,700 \mu\text{mol kg}^{-1}$), low $p\text{CO}_2$ levels ($< 300 \mu\text{atm}$), and corrosive conditions ($\Omega_{\text{arag}} < 1$), due to low alkalinity/salinity conditions associated to high freshwater inputs from the Valdivia river (Figure 1B). Depleted $\delta^{13}\text{C}_{\text{DIC}}$ values indicated the contribution of riverine DIC to the total pool of DIC in this estuarine portion. The pH_T values showed intermediate values during the winter campaign, ranging between 7.60 and 7.99 units. In summer, however, the pH_T ranged from as low as 7.48 at the stations outside of the river plume (i.e., VE4 and VE5) to as high as 8.03 at the surface waters of VE1 and VE3 stations. The influence of oceanic waters extended from the coastal stations to the inner portion of the estuary during this season, being characterized by low pH_T , and high DIC concentrations ($> 1,700 \mu\text{mol kg}^{-1}$), TA values ($> 2,000 \mu\text{mol kg}^{-1}$), and $p\text{CO}_2$ levels ($> 700 \mu\text{atm}$) (Figure 4).

In the Arauco Gulf, the highest pH_T values were recorded at the surface waters in winter, ranging between 8.19 and 8.56 units (Figure 5), while the lowest DIC concentrations (1535

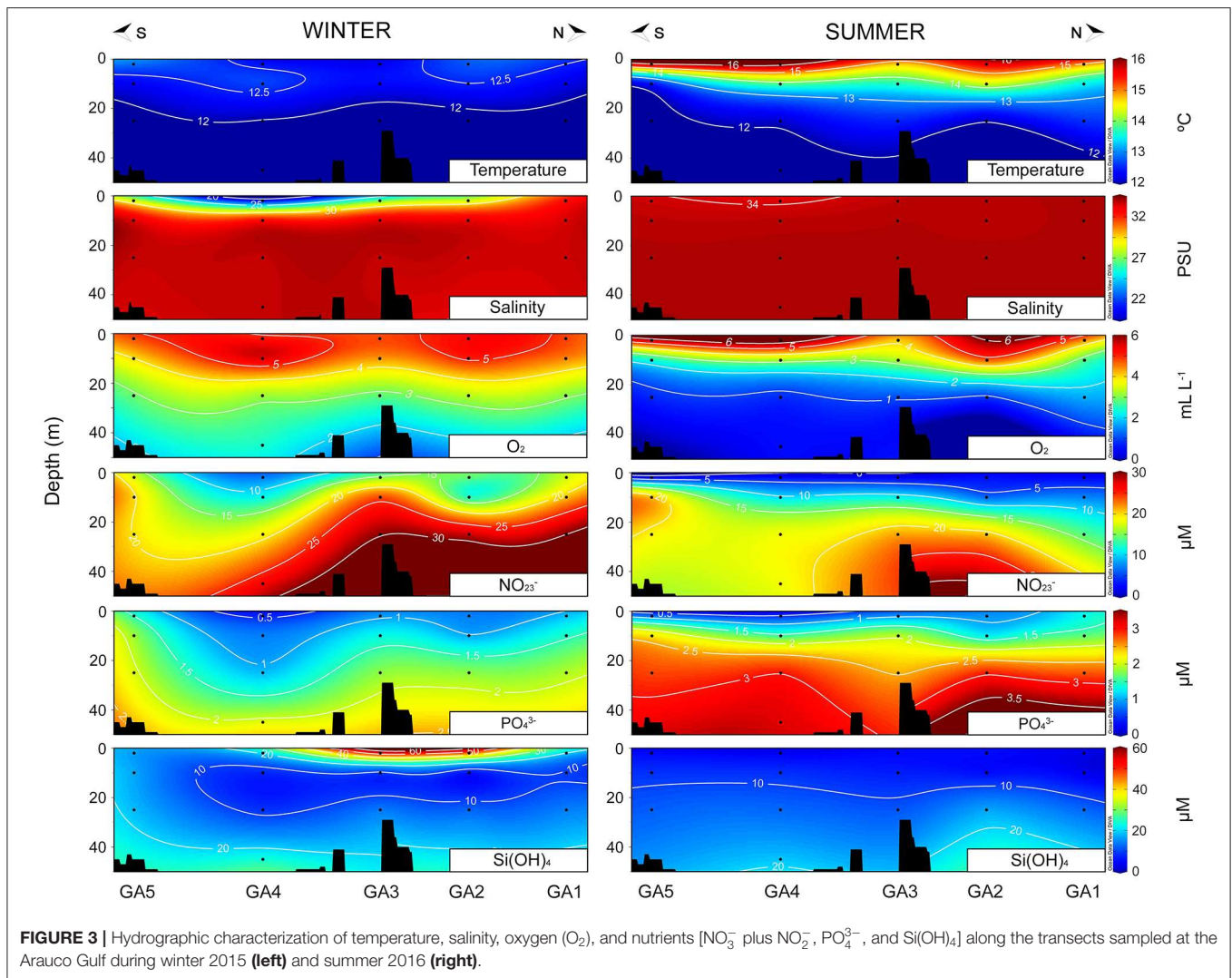


$\mu\text{mol kg}^{-1}$) and TA levels ($1,731 \mu\text{mol kg}^{-1}$) for this area were determined at the river mouth station GA3, associated with higher freshwater inputs from the Biobío river during this season (Figure 1B). However, the riverine DIC contribution to the DIC pool in the Arauco Gulf was not evident from the $\delta^{13}\text{C}_{\text{DIC}}$ values. The $p\text{CO}_2$ levels remained relatively low ($<300 \mu\text{atm}$) throughout most of the transect in winter and Ω_{arag} values revealed supersaturation conditions with respect to aragonite. In summer, in contrast, all the carbonate system parameters (except the TA, which showed a relatively constant value of $2,298 \pm 8 \mu\text{mol kg}^{-1}$ along the transect) reflected the upwelling of acidic and corrosive subsurface waters mainly at stations GA1, GA2, and GA5 (Figure 5). These subsurface waters were also characterized by low pH values (<7.9 units), high DIC concentration ($>2,100 \mu\text{mol kg}^{-1}$), high $p\text{CO}_2$ levels ($>700 \mu\text{atm}$), and Ω_{arag} undersaturation conditions (<1), as compared to other stations (i.e., GA3 and GA4) and season (i.e., winter). $\delta^{13}\text{C}_{\text{DIC}}$ values also evidenced the influence of fractionated DIC pool ($<1\%$) associated to low oxygen and corrosive subsurface waters.

In general terms, the more acidic waters (low pH/ high $p\text{CO}_2$) were observed in summer at both the Arauco Gulf and the Valdivia estuary, caused by the influence of subsurface upwelled waters and coastal oceanic waters, respectively. However, in contrast to the Arauco Gulf, the more corrosive conditions $\Omega_{\text{arag}} (<1)$ in the water column at the Valdivia estuary were recorded in winter, coinciding with the larger freshwater inputs, which are characterized by a reduced buffering capacity associated to the low TA values (Figure 5, Figure S1).

3.3. Temporal and Spatial Variations of the Phytoplankton Community

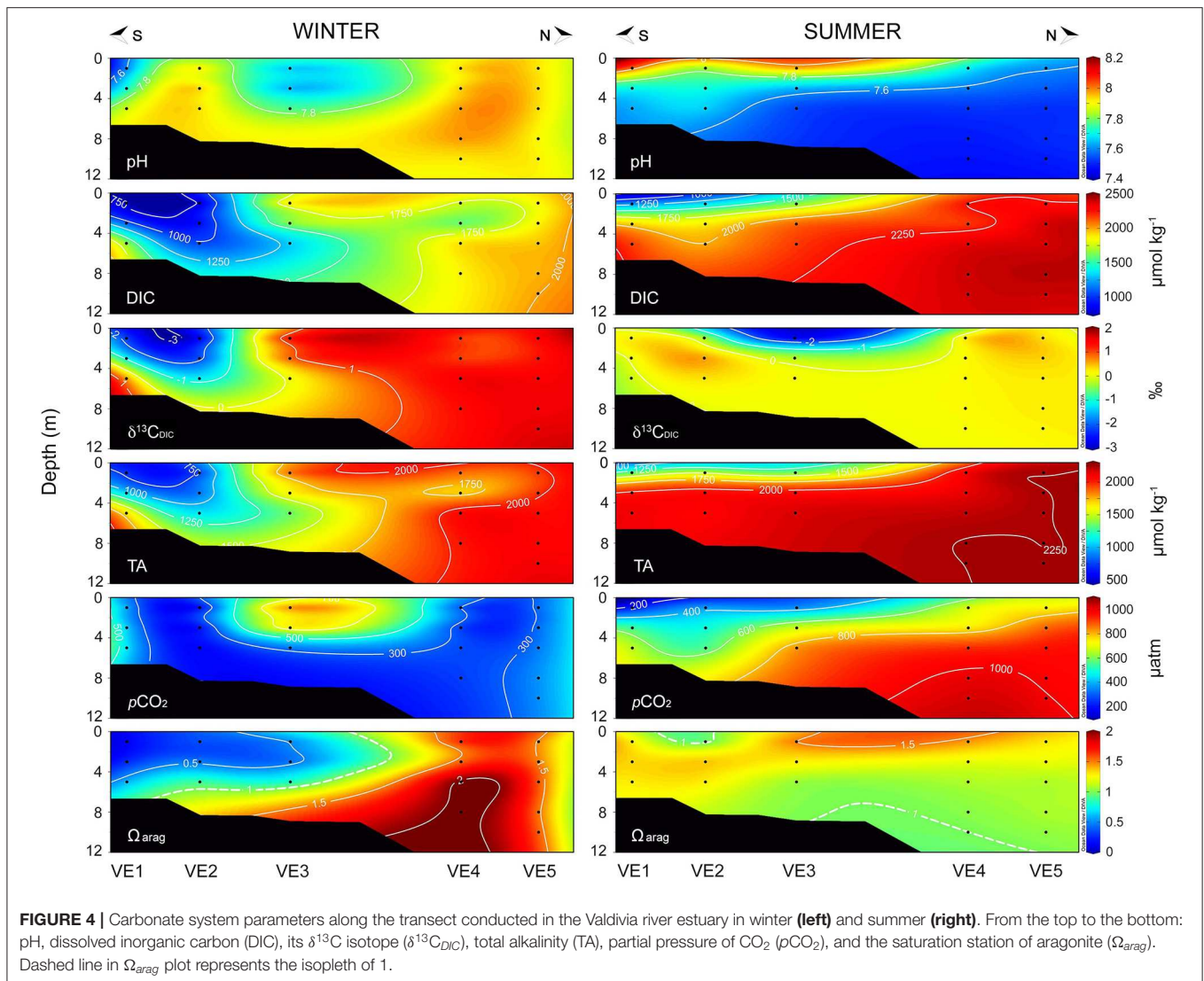
Seasonal differences in the Chl *a* concentration and the abundance of the dominating phytoplankton groups were larger than spatial variations along the transects at both samplings sites. Vertical profiles in the Valdivia estuary showed low Chl *a* concentrations ($<2.5 \mu\text{g L}^{-1}$) in winter at all stations and depths (Figure 6), with integrated values (over the first 5 m) ranging between 5.3 and 8.4 mg m^{-2} (Table 1). In summer, this



concentration increased at all stations but mainly at the inner estuarine section (VE1–VE3), where it achieved maximum values between 7 and 18 $\mu\text{g L}^{-1}$ at subsurface waters and integrated values in the water column of 44.6–65.5 mg m^{-2} (Table 1). Similarly, the cell abundance of the different phytoplankton groups was about one order of magnitude larger in summer (Figure 6), even though the community was more diverse and the species richness was higher in winter (Table 1). Diatoms were the most abundant group throughout the year, contributing between 50 and 94% to the total abundance (Table S1), although there was a seasonal change in the dominant species. In winter, *Aulacoseira granulata* and *Thalassionema nitzschioides* accounted for most of the phytoplankton abundance, whereas in summer *Chaetoceros* spp. and *Skeletonema costatum* dominated the water column. A distinct distribution of less abundant groups was also observed along the transect. For instance, chlorophytes were only collected at the inner estuarine stations (Figure 6), where they were relatively abundant in winter (14–29%, Table S1). Other groups, such as dinoflagellates were present throughout the entire

water column, at all stations and samplings, but their relative contribution to the total community changed likely associated to the influence of more oceanic waters as it was higher both at VE4 and VE5 stations and in summer all along the transect (between 10 and 36%, Table S1).

In the Arauco Gulf the seasonal patterns of Chl *a* concentration and the cell abundance were similar as in Valdivia, being higher in summer than in winter (Figure 7). However, the latter was one order of magnitude higher in the Arauco Gulf during the same season, with maximum values reaching more than 3×10^6 cells L^{-1} at surface waters at GA2 (Figure 7) and integrated values (over the first 20 m) of 88.5×10^9 cells m^{-2} (Table 1). According to the diversity and species richness indexes (Table 1), the plankton community in this coastal ecosystem was less diverse and showed lower species richness in summer, but it was strongly dominated by diatoms throughout the year (relative abundance between 91 and 99%, Table S2). As in Valdivia, there was a seasonal change in the species composition at each station. *Chaetoceros* spp. was the most abundant group



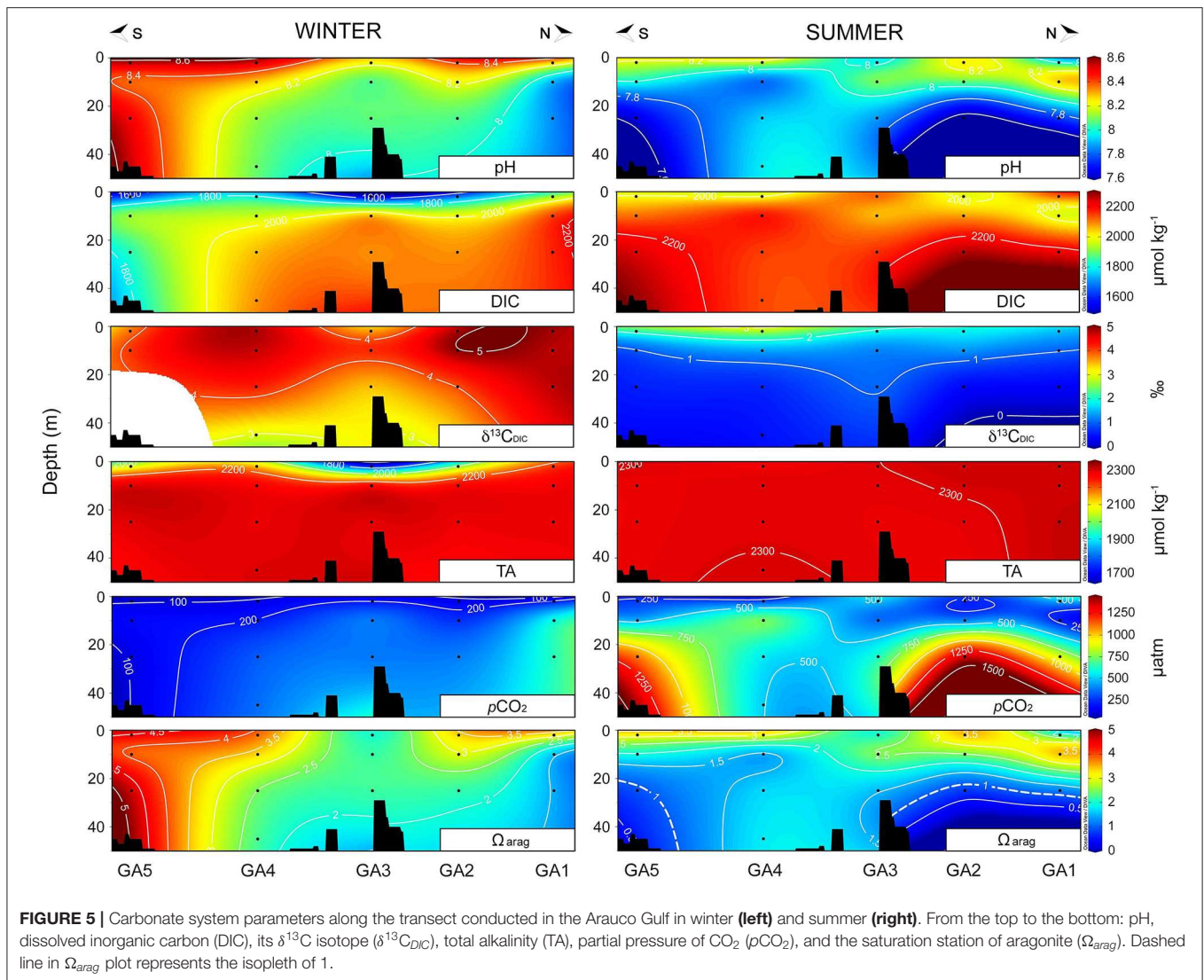
at stations GA1 and GA2 in winter (80–86%), whereas the same genus dominated the phytoplankton community at stations GA4 and GA5 in summer (88–82%, **Table S2**). The northernmost stations (GA1–GA3) were dominated by the chain-forming diatom *Leptocylindrus danicus* in summer. It is noteworthy that GA3, the station located at the Biobío river mouth (**Figure 1**), presented the highest diversity and species richness values in winter, not showing a clear dominance of any species or genera (**Table S2**). The contribution of dinoflagellates was low in this area both in winter and summer, ranging from 0.2 to 8.9%, with maximum integrated cell abundances of 35.6×10^7 cells m^{-2} at GA3.

As expected, the largest gross primary production rates (GPP) were recorded during summer campaigns at both study sites (**Table 2**), with values being one order of magnitude higher ($>4,000$ mg C $\text{m}^{-2} \text{d}^{-1}$) in the Arauco Gulf than in the Valdivia River estuary. Seasonal differences in the community respiration (CR) were observed between sites as well. In Valdivia, the highest CR levels were determined at

the inner estuarine stations in winter. In the Arauco Gulf, in contrast, the highest values were measured in summer at the more oceanic station (GA1), coinciding with the highest GPP rates supported by the upwelling of nutrient-rich subsurface waters (**Figure 3**). The GPP/CR ratio showed a similar trend at the two coastal ecosystems (**Table 2**), with significantly lower values recorded during winter campaigns (t -test, $p < 0.05$).

3.4. Relationship Between Environmental and Biological Variables

Several significant correlations were obtained between the structure and physiological rates of phytoplankton communities and the abiotic factors, such as the hydrographic properties and the carbonate system parameters. **Tables 3, 4** includes these correlations as well as the results of the GLM model applied to elucidate the role of each environmental factor in predicting the biological variables in Valdivia and the Arauco Gulf, respectively. We observed distinct relationship



of the environmental and biological factors between the two sites.

In the Arauco Gulf, the variability of the Chl *a* concentration, the abundance of the different taxonomic groups and the physiological rates were largely explained (44–71%) by variations in temperature and nutrients concentration (Table 4). None of the carbonate system parameters was significantly correlated to the considered biological variables in this ecosystem. In contrast, in the Valdivia estuary, the carbonate chemistry in the water column showed a significant role in modulating the abundance of certain taxonomic groups. For instance, change in the Ω_{arag} values explained 14% of the variance in the Chl *a* concentration and 23–40% of the seasonal variability observed in the abundance of euglenophytes, ciliates and silicoflagellates (Table 3). The abundance of diatoms and dinoflagellates, in turn, was correlated to the salinity and nutrients concentration, even though the multivariate linear approach considering these factors was only able to explain significantly the variance in dinoflagellates (31%).

3.5. Response of Coastal Phytoplankton Communities to Short-Term Exposure to Elevated $p\text{CO}_2$ Levels

Seawater parameters during field collection (i.e., *in situ*) and for the experimental set up are given in Figures 8, 9. It is noteworthy that the pH/ $p\text{CO}_2$ levels established for the two experimental treatments (low/high) were maintained constant over the 5-days experiments, as reflected by the continuous monitoring of pH and $p\text{CO}_2$ values. Natural assemblages of planktonic communities from estuarine and coastal upwelling ecosystems showed distinct responses during the experimental period. In the Valdivia River estuary, the *in situ* values of pH and $p\text{CO}_2$ were of 7.93 and 162 μatm (VE2 in Figure 5, winter sampling), respectively. For the experiment, however, we decided to set the low $p\text{CO}_2$ /high pH treatment (hereinafter “low treatment”) at 380 μatm , a typical and recommended value for control treatments in CO_2 manipulation experiments (IPCC,

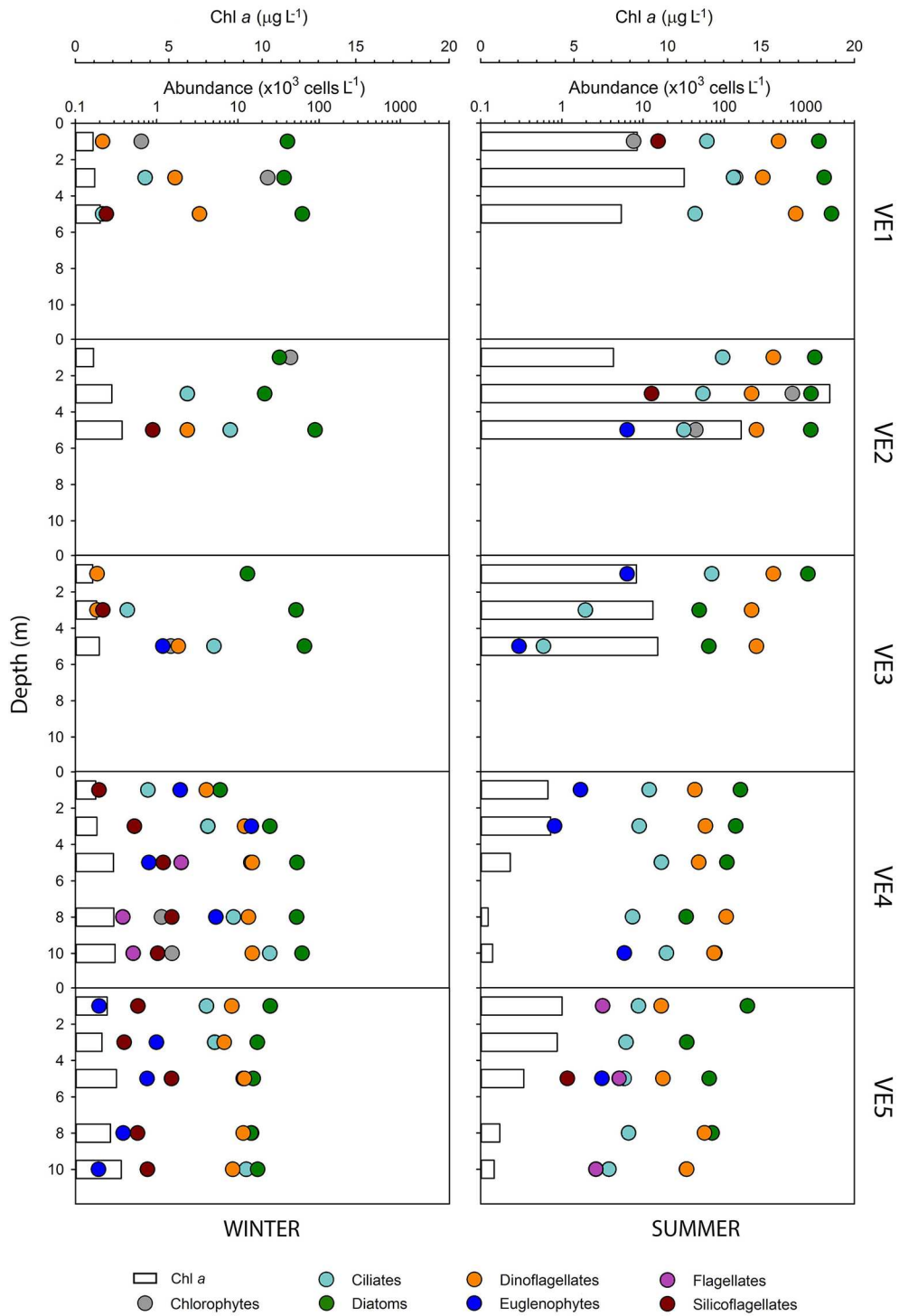


FIGURE 6 | Cell abundance of the phytoplankton groups (*dots*) dominating the community in the Valdivia river estuary during winter (**left**) and summer (**right**). Note that the abundance is given in log-scale. The bars represents the chlorophyll a concentration at each station and during each seasonal sampling. The legend at the bottom applies to all the panels.

TABLE 1 | Phytoplankton community biodiversity and species richness through the Shannon-Wiener diversity index (H'), Pielou's evenness index (J_0), and Margalef's species richness (d) at both study areas.

Stations	Int. Chl <i>a</i> (mg m^{-2})	Int. abundance ($\times 10^6$ cells m^{-2})	H'	J_0	d
VALDIVIA ESTUARY					
VE1	5.30–46.08	275.40–11462.68	2.75–2.14	0.74–0.69	3.19–1.29
VE2	8.36–65.51	304.59–9944.58	1.94–2.30	0.60–0.70	1.90–1.61
VE3	5.40–44.61	207.86–4110.04	2.29–2.04	0.68–0.62	2.29–1.70
VE4	6.50–16.29	226.32–1018.41	2.66–1.81	0.81–0.61	2.11–1.30
VE5	8.39–19.25	180.05–623.50	2.28–1.61	0.79–0.57	1.40–1.20
ARAUCO GULF					
GA1	54.10–131.81	552.57–77002.21	0.83–0.24	0.40–0.10	0.53–0.50
GA2	57.81–197.65	2595.14–88513.84	0.57–0.70	0.25–0.30	0.61–0.49
GA3	32.93–151.63	1139.98–22736.50	2.09–0.56	0.79–0.27	0.93–0.41
GA4	74.77–118.82	992.66–2801.09	0.82–0.49	0.46–0.30	0.36–0.27
GA5	35.81–54.04	1295.29–14165.36	1.14–0.37	0.59–0.17	0.43–0.49

The integrated chlorophyll *a* (Chl *a*) concentration and phytoplankton abundances are also given, being the integrated depth of 5 m in the Valdivia estuary and of 20 m in the Arauco Gulf. The left and right values in the range corresponds to the winter and summer values, respectively.

2014) and the high $p\text{CO}_2$ /low pH treatment (hereinafter “high treatment”) at 900 μatm . Both the Chl *a* concentration and the abundance of phytoplankton groups in general showed an exponential increase during this experiment (Figure 8). Thus, Chl *a* values in the low treatment, for instance, increased from as low as 1 $\mu\text{g L}^{-1}$ at initial conditions to as high as 15 $\mu\text{g L}^{-1}$ in 5 days. No significant differences were detected between the low and high treatments at the end of the incubation (t -test, $p > 0.05$). The fast increase in the phytoplankton growth rates was reflected in the nutrient ratios (Figure 8). While the Si/N ratio decreased steadily (from ca. 11 to 9), the N/P ratio increased toward the end of the experiments in both treatments (from ca. 9 to 25). In this regard, there was no significant differences in the N uptake rates between the low and high treatments. The phytoplankton community in these estuarine waters was mainly dominated by diatoms (65% of the total abundance), in agreement to that observed under natural conditions (Figure 6). Most of the plankton groups increased their abundance during the incubation at both treatments, except silicoflagellates, which were only present at the beginning of the experiment. Euglenophytes and dinoflagellates showed a moderate increase in both treatments, whereas diatoms and flagellates exhibited the largest increase and were the most abundant groups at the end. When the effect of the elevated $p\text{CO}_2$ level was tested after the 5-days incubation, we only observed significant differences (t -test, $p < 0.01$) in the abundance of diatoms and flagellates, being higher in the low treatment. A detailed examination of the species composition of diatoms revealed that the contribution of less abundant species (<1% of the total abundance, grouped as “Other diatoms,” Figure 8), which corresponded mostly to freshwater diatoms, as well as of *Chaetoceros* spp. decreased toward the end of the experiment. Conversely, taxa, such as *Thalassiosira* spp. and *Skeletonema costatum* increased, mainly at the high $p\text{CO}_2$ treatment.

In the Arauco Gulf, the *in situ* pH and $p\text{CO}_2$ levels were 7.65 and 706 μatm , respectively (Figure 8). These values were considered for the low treatment as a measurement of control conditions in upwelling waters, whereas the high treatment was set at 1,600 μatm of $p\text{CO}_2$, considering a $\Delta p\text{CO}_2$ of 900 μatm with respect to the control conditions. Nutrient ratios N/P and Si/N remained constant at ca. 11 and 0.73, respectively, indicating no significant uptake of N by phytoplankton and no nutrient limitation during the experiment. Likewise, no significant effect of $p\text{CO}_2$ levels was observed in the concentration of Chl *a*, although it decreased from about 2 $\mu\text{g L}^{-1}$ at *in situ* and initial values to 0.6 $\mu\text{g L}^{-1}$ at the end of the incubation. Similarly, the abundance of diatoms decreased with time at both the high and low treatments, although this decrease was two times higher at high $p\text{CO}_2$ conditions than at low $p\text{CO}_2$ conditions (13.5 $\times 10^3$ cells $\text{L}^{-1} \text{d}^{-1}$ vs. 5.9 $\times 10^3$ cells $\text{L}^{-1} \text{d}^{-1}$). This decrease in the cell abundance coincided with an increase in the aggregates formation, as revealed by visual observations of the incubation bottles. The abundance of dinoflagellates, in contrast, did not show any influence of elevated $p\text{CO}_2$ levels nor a decrease with time. In this experiment, the diatom community was dominated by *Thalassiosira* spp. As in the estuarine ecosystem, the less abundant diatom species decreased toward the end of the experiment irrespective of the treatment.

4. DISCUSSION

4.1. The Natural Environmental Variability of Hydrography and Carbonate System for Estuarine and Coastal-Upwelling Phytoplankton Communities

A frequent research gap in OA studies is the lack of information about the natural variability of the seawater chemistry at the locations where the organisms are collected. However, it is increasingly more evident that this knowledge is critical to correctly interpret the outcome of these investigations (e.g., Boyd et al., 2016; Vargas et al., 2017), particularly in studies conducted at highly variable and/or naturally acidic coastal ecosystems. In the present work, we have described the physical-chemical properties of the water column (in terms of temperature, salinity, dissolved oxygen, nutrients concentration and carbonate system parameters) at each study area both during winter and summer, as we expected to record different carbonate chemistry conditions associated to the distinct oceanographic processes dominating the water column during these two seasons.

The seasonal variability in the hydrographic conditions and the phytoplankton community in the Valdivia river estuary and the adjacent coastal area were mostly associated to changes in the extent of the river plume along the transect, which was primarily linked to seasonal changes in the freshwater discharges, as previously described for this area (Vargas et al., 2003; Giesecke et al., 2017). In winter, the low $\text{NO}_3^- + \text{NO}_2^-$ and PO_4^{3-} concentrations typical for this system (Pérez et al., 2015) together with the limited solar irradiance characteristic at this latitude (Iriarte et al., 2007; González et al., 2010) limited the phytoplankton growth and favored a net heterotrophic system,

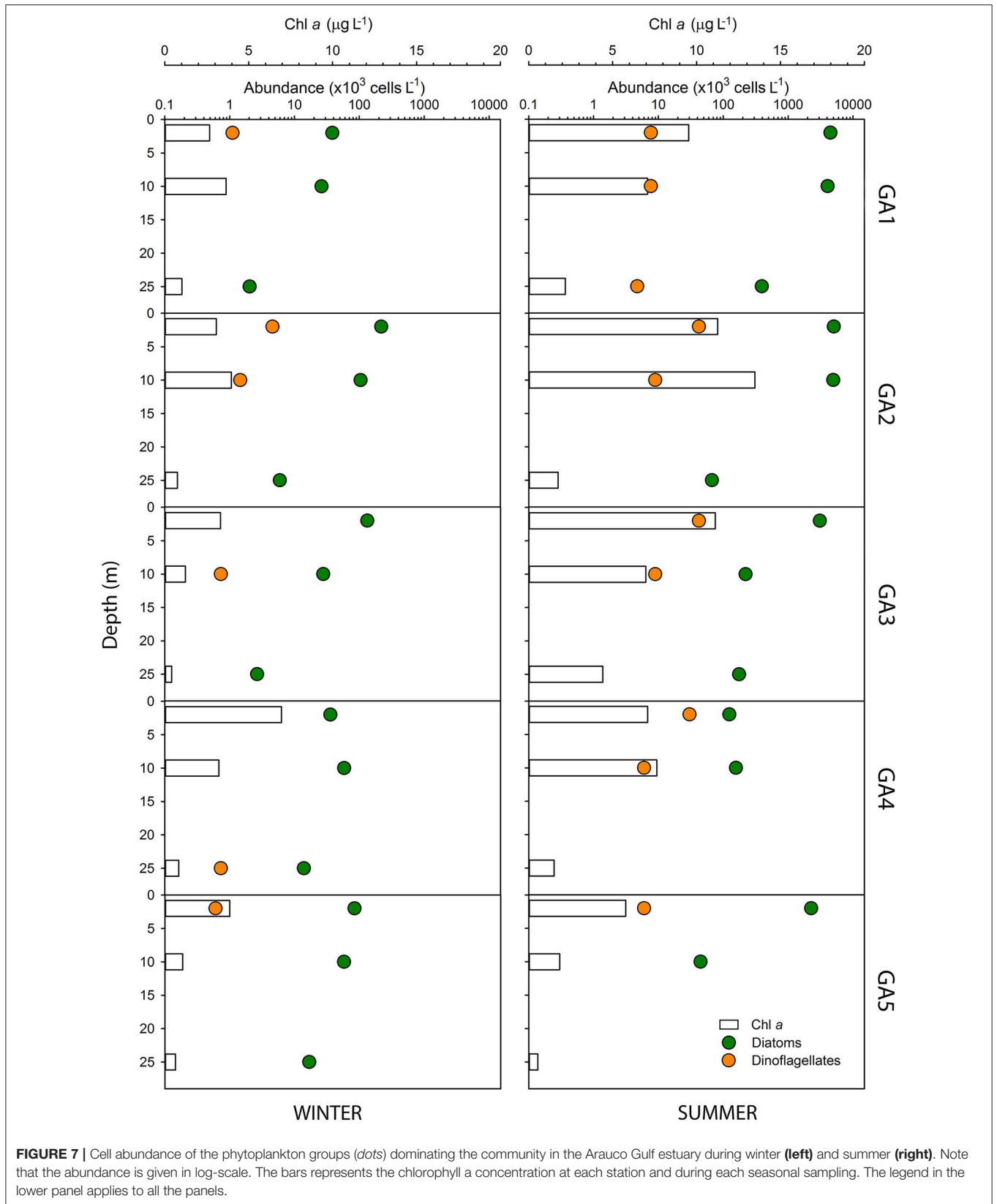


TABLE 2 | Integrated gross primary productivity (GPP), community respiration (CR), net community production (NCP), and the GPP/CR ratio at two station of the Valdivia estuary and the Arauco Gulf during winter and summer.

Stations	Season	GPP	CR	NCP	GPP/CR
VALDIVIA ESTUARY					
VE2	Winter	11.89 ± 2.32	118.15 ± 23.43	-106.26 ± 17.34	0.10 ± 0.06
	Summer	469.37 ± 52.15	62.62 ± 9.98	406.75 ± 43.56	7.50 ± 2.31
VE5	Winte	19.62 ± 3.32	6.66 ± 1.84	12.97 ± 2.43	2.95 ± 0.47
	Summer	295.84 ± 25.22	16.89 ± 1.89	278.95 ± 32.21	17.52 ± 2.99
ARAUCO GULF					
GA1	Winter	231.22 ± 34.68	236.97 ± 30.81	-5.75 ± 0.75	0.98 ± 0.13
	Summer	4699.09 ± 704.86	1222.82 ± 158.97	3476.27 ± 521.44	3.84 ± 0.50
GA3	Winter	140.34 ± 28.07	154.41 ± 23.16	-14.07 ± 1.83	0.91 ± 0.14
	Summer	2311.12 ± 346.67	718.31 ± 143.66	1592.81 ± 318.56	3.22 ± 0.42

VE2 and GA3 were located close to the Valdivia and the Biobío river mouths (see **Figure 1**), respectively, being influenced by freshwater discharges, mainly in winter. VE5 and GA1 were located out of the river plume influence. The integrated depths are 5 m in the Valdivia estuary and 20 m in the Arauco Gulf. The units for GPP, CR and NCP are $\text{mg C m}^{-2} \text{d}^{-1}$, while the GPP/CR is unitless.

where the CR exceeded GPP (**Table 2**). This high CR was likely associated to the consumption of the organic matter transported by the river. Furthermore, as the relative abundance of the microheterotrophic components of the plankton community was not significantly higher during this time (**Table S1**), the enhanced respiration could be attributed to an increase in the pico- and nanoplankton community associated to freshwater discharges (Iriarte et al., 2018; Cuevas et al., 2019). In summer, the reduction of the river runoff led to a strong stratification in this tidal estuary, with considerable implications for the distribution of Chl *a* in the water column. The surface waters of lower salinity showed low phytoplankton biomass, increasing within the pycnocline and below (**Figure 6**), which is a typical pattern observed in other estuaries and fjord areas (Alldredge et al., 2002; Jouenne et al., 2007). Phytoplankton composition along the transect shifted from a freshwater-water species dominated community during winter toward a marine species dominated community during summer, in agreement to that reported by Giesecke et al. (2017).

The hydrographic features in the Arauco Gulf follow a seasonal pattern, with higher upwelling events during austral spring/summer (Valle-Levinson et al., 2003; Sobarzo et al., 2007) and with a considerable influence of freshwater discharges from the Biobío River in winter (Vargas et al., 2016). Furthermore, water column stratification in this region results from the influence of both temperature and salinity (Sobarzo et al., 2007). Accordingly, our results showed that thermal stratification dominated the water column in summer (associated with an increase in the solar irradiance) whereas salinity stratification was stronger in winter, particularly at those stations influenced by the river plume (GA3–GA5, **Figure 3**). The transport of nutrient-rich subsurface waters to the euphotic zone in summer promoted the phytoplankton growth, reaching Chl *a* values ($>10 \mu\text{g L}^{-1}$) common for this coastal region (Iriarte and Bernal, 1990; Vargas et al., 2007). Likewise, the nutrient concentrations measured during our samplings are within the range of previously reported values (Vargas et al., 2013; Pérez et al., 2015). The relatively low $\text{NO}_3^- + \text{NO}_2^-$ values (as compared to the values in winter), together with the high phytoplankton biomass, suggest that our

summer sampling took place after an intense upwelling episode. The composition of the phytoplankton taxa found in this study in the Arauco Gulf was in good agreement with that already described for this ecosystem (Anabalón et al., 2007; González et al., 2007) as well as for other upwelling areas (Kudela et al., 2005). On the other hand, the high GPP values found in this ($1.4\text{--}4.7 \text{ g C m}^{-2} \text{d}^{-1}$) and previous studies (e.g., Daneri et al., 2000; Vargas et al., 2007) highlights the importance of the Humboldt current system off central Chile as one of the most productive systems in the world.

Carbonate chemistry dynamics at both sampling sites were likewise associated to the major local oceanographic forcing's dominating the water column. In Valdivia, the more corrosive conditions were observed at the inner estuarine section in winter, associated with the larger river freshwater discharges (**Figure 4**). This low salinity water was characterized by low DIC concentration and TA (**Figure S1**), which limited the buffering capacity of the system and lead to $\Omega_{\text{arag}} < 1$. Similar undersaturation conditions have been reported for other estuarine and river plume environments (e.g., Cao et al., 2011; Wallace et al., 2014). In summer, the subsurface intrusion of more oceanic waters, characterized by high $p\text{CO}_2$ and low pH values, was responsible of the undersaturation conditions at the offshore stations (**Figure 4**). The high pH and low $p\text{CO}_2$ levels observed at the surface waters of the inner estuarine portion was most likely caused by the high photosynthetic rates observed during this season (**Table 2**) (Hinga, 2002). In the Arauco Gulf, the more corrosive conditions were registered in summer and were associated to the upwelling episode that apparently happened before our sampling. The subsurface waters were characterized by low pH (<7.6) and high $p\text{CO}_2$ ($>1,000 \mu\text{atm}$) levels, which led to undersaturation conditions with respect to Ω_{arag} . This is a common feature observed in this (Vargas et al., 2016) and other upwelling ecosystems (e.g., Feely et al., 2008; Hofmann et al., 2011). In winter, the larger influence of freshwater discharges caused the low DIC and TA values determined at the river mouth station (GA3) (Vargas et al., 2016). Overall, the values of the carbonate system parameters determined in this study for both

TABLE 3 | Relationship between biological variables (Y) and environmental factors (X_i) at the Valdivia river estuary.

Variables (Y)	Factors (X_i)	Spearman ^a R ²	GLM		
			σ	p-value	R ²
Chl a	Ω_{arag}	0.14 [†]	1.87	>0.05	0.14 ^{n.s.}
Diatoms	Salinity	0.12 [†]	-2.28×10^3	>0.05	0.01 ^{n.s.}
	PO_4^{-3}	0.21 ^{**}	74.17×10^3	>0.05	
Dinoflagellates	Salinity	0.36 ^{***}	2.03×10^3	>0.05	0.31 ^{**}
	NO_{23}^-	0.17 [†]	-19.75×10^3	<0.01	
	PO_4^{-3}	0.47 ^{***}	80.04×10^3	>0.01	
Chlorophytes	Temperature	0.12 [†]	5.03×10^3	>0.05	0.03 ^{n.s.}
	pH	0.11 [†]	-282.49×10^3	>0.05	
	$p\text{CO}_2$	0.13 [†]	-0.31×10^3	>0.05	
	NO_{23}^-	0.17 [†]	5.63×10^3	>0.05	
	Si(OH)_4	0.12 [†]	0.39×10^3	>0.05	
Euglenophytes	Ω_{arag}	0.40 ^{***}	2.20×10^3	<0.05	0.08 [†]
Ciliates	PO_4^{-3}	0.14 [†]	2.66×10^3	>0.05	0.04 ^{n.s.}
	Ω_{arag}	0.23 ^{**}	9.86×10^3	>0.05	
Flagellates	Si(OH)_4	0.12 [†]	-6.35	>0.05	0.01 ^{n.s.}
Silicoflagellates	pH	0.31 ^{***}	2.52×10^3	>0.05	0.02 ^{n.s.}
	$p\text{CO}_2$	0.24 ^{**}	-0.38	>0.05	
	Si(OH)_4	0.13 [†]	18.57	>0.05	
	Ω_{arag}	0.25 ^{**}	-16.78	>0.05	
	GPP	Si(OH)_4	0.62 [†]	0.67	>0.05
Diversity	Salinity	0.67 [†]	0.01	>0.05	0.69 [†]
	NO_{23}^-	0.45 [†]	-0.02	>0.05	
	PO_4^{-3}	0.45 [†]	-0.13	>0.05	
	Sp. richness	Salinity	0.67 [†]	-0.04	>0.05
	NO_{23}^-	0.63 ^{**}	0.01	>0.05	
	PO_4^{-3}	0.63 ^{**}	-0.07	>0.05	

Only variables with significant correlations are presented and were considered to conduct the generalized linear model (GLM). The regression coefficient of predicting factors (σ), their significance in the model (p-value) as well as the adjusted-R² for the model are given. ^aThe significance of the R² values is defined as follows: *p < 0.05, **p < 0.01, ***p < 0.001, n.s. not significant (p > 0.05).

sites are comparable to those included in recent seasonal analysis of the carbonate chemistry conducted in the same areas by Pérez et al. (2015) and Vargas et al. (2016, 2017). In summary, both sites represent ecosystems which naturally experience very large ranges in carbonate system parameters, despite the important differences in the hydrographical causes of this variability and the particular combinations of carbonate system and other chemical parameters that result.

Previous studies have thus reported the dynamics of the hydrography, carbonate chemistry and phytoplankton community structure separately at the two study sites. However, to our knowledge, this is the first time that the relationship between abiotic factors including the carbonate system parameters and the phytoplankton is investigated along the Chilean coast. We observed significant negative correlations between carbonate system parameters and cell abundances only in certain phytoplankton taxa at the estuarine community (Table 3), e.g., silicoflagellates in which changes in the pH and

TABLE 4 | Relationship between biological variables (Y) and environmental factors (X_i) at the Arauco Gulf.

Variables (Y)	Factors (X_i)	Spearman ^a R ²	GLM		
			σ	p-value	R ²
Chl a	Temperature	0.84 ^{***}	0.82	>0.05	0.71 ^{***}
	NO_{23}^-	0.68 ^{***}	-0.23	<0.01	
	PO_4^{-3}	0.33 ^{***}	-0.32	>0.05	
Diatoms	Si(OH)_4	0.39 ^{***}	-0.03	>0.05	
	Temperature	0.78 ^{***}	485.50×10^3	>0.05	0.44 ^{***}
	NO_{23}^-	0.60 ^{***}	-66.91×10^3	>0.05	
	PO_4^{-3}	0.22 ^{**}	215.53×10^3	>0.05	
Dinoflagellates	Si(OH)_4	0.30 ^{**}	-15.60×10^3	>0.05	
	Temperature	0.63 ^{***}	5.38×10^3	<0.01	0.53 ^{***}
	NO_{23}^-	0.61 ^{***}	-105.80	>0.05	
	PO_4^{-3}	0.18 [†]	612.94	>0.05	
GPP	Si(OH)_4	0.39 ^{***}	-5.43	>0.05	
	Temperature	0.58 ^{**}	271.98	<0.05	0.65 ^{**}
	NO_{23}^-	0.71 ^{**}	12.48	>0.05	
DCR	Temperature	0.50 [†]	26.26	>0.05	0.66 [†]
	Salinity	0.40 [†]	7.38	>0.05	
	NO_{23}^-	0.68 ^{**}	-0.49	>0.05	
	Si(OH)_4	0.35 [†]	1.10	>0.05	
Diversity	Temperature	0.47 [†]	1.01	>0.05	0.65 ^{n.s.}
	Salinity	0.47 [†]	-0.35	>0.05	
	NO_{23}^-	0.56 [†]	0.12	>0.05	
	Si(OH)_4	0.69 ^{**}	0.04	>0.05	

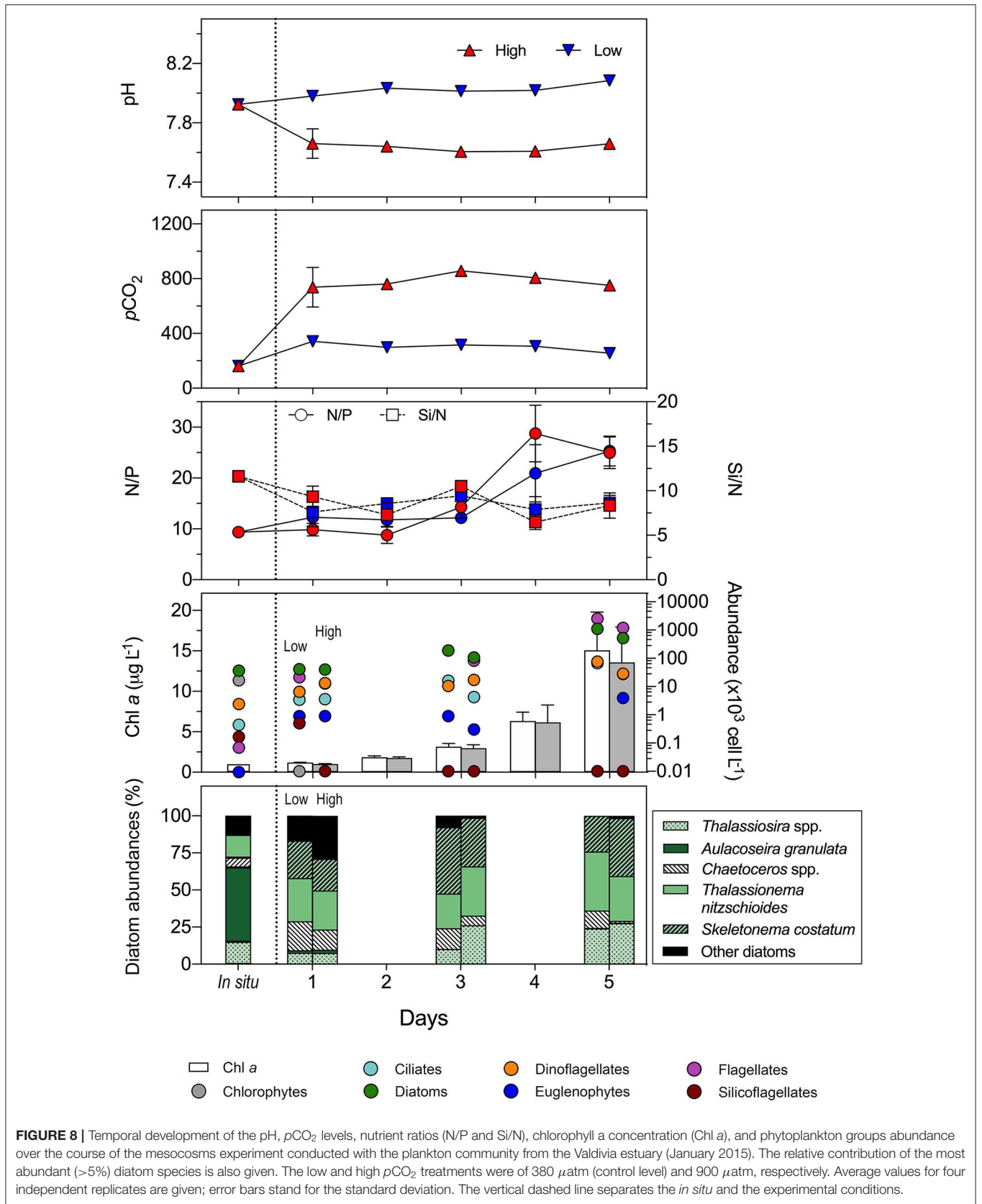
Only variables with significant correlations are presented and were considered to conduct the generalized linear model (GLM). The regression coefficient of predicting factors (σ), their significance in the model (p-value) as well as the adjusted-R² for the model are given. ^aThe significance of the R² values is defined as follows: *p < 0.05, **p < 0.01, ***p < 0.001, n.s. not significant (p > 0.05).

Ω_{arag} values explained about one fourth of the changes in their cell abundance. Other phytoplankton groups, such as diatoms and dinoflagellates, were mainly correlated to nutrient concentrations and to other abiotic factors, such as salinity, in the estuarine ecosystem, and temperature in the coastal upwelling ecosystem.

4.2. Influence of Elevated $p\text{CO}_2$ on Contrasting Phytoplankton Assemblages

A principal aim of the present study was to investigate how phytoplankton communities that experience high carbonate system variability and naturally acidified conditions respond to increases in the $p\text{CO}_2$ levels. This study and the previous studies cited show that the annual range of $p\text{CO}_2$ variation is higher in the Arauco Gulf (~ 200 – $1,600 \mu\text{atm}$) than in the Valdivia River estuary (~ 150 – $1,000 \mu\text{atm}$). These differences were taken into account in the design of the tested experimental conditions, adjusting the tested CO_2 conditions to be more comparable relative to what the communities naturally experience.

Prior to evaluating the effect of increased $p\text{CO}_2$, an overall look at the response of the communities investigated here showed that they exhibited opposite behavior during the incubation, despite that both were dominated by diatoms and presented similar initial values of Chl a and total cell abundance. Regardless



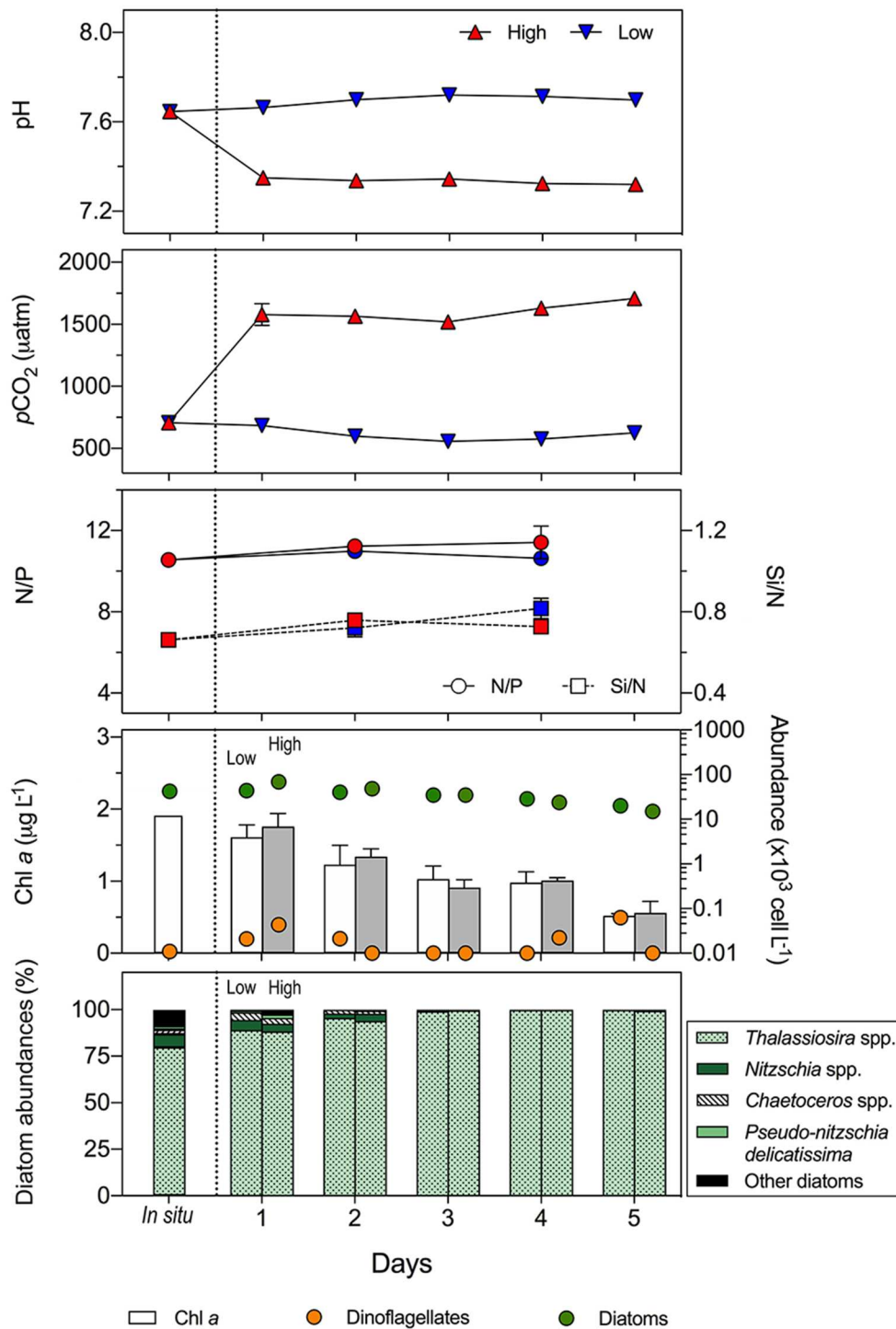


FIGURE 9 | Temporal development of the pH, pCO₂ levels, nutrient ratios (N/P and Si/N), chlorophyll a concentration (Chl a), and phytoplankton groups abundance over the course of the mesocosms experiment conducted with the plankton community from the Arauco Gulf (November 2016). The relative contribution of the most abundant (>1%) diatom species is also given. The low and high pCO₂ treatments were of 700 μatm (*in situ* pCO₂ level during the sampling) and 1,600 μatm, respectively. Average values for four independent replicates are given; error bars stand for the standard deviation. The vertical dashed line separates the *in situ* and the experimental conditions.

of the $p\text{CO}_2$ treatment, the community from the Valdivia River estuary showed an exponential increase of both the Chl *a* concentration and the abundance of most of the plankton groups (Figure 8), while the phytoplankton community from the Arauco Gulf presented a decreasing trend of the same biological variables toward the end of the incubation (Figure 9). Since the two experiments were conducted under nutrient-sufficient conditions, we argue that the reason for this contrasting response is that the two communities were at different growth phases when they were collected from the field, which is a common situation when conducting experiments with naturally-phytoplankton assemblages. In general, the phytoplankton from the Valdivia river estuary was more diverse and was actively growing as reflected by the increase in the N/P ratio (Figure 9), which evidenced an enhanced N uptake to support the high growth rates. The phytoplankton from the Arauco Gulf, however, was in a stationary growth phase, according to the constant N/P and Si/N ratios measured during the experiment, and were largely dominated (>75%) by one single diatom genera, *Thalassiosira* spp. These results suggest that this latter community was currently in a post-bloom phase. During this phase, diatoms increase the production of transparent exopolymeric particles (TEP) which enhances cells stickiness and contributes to the subsequent formation of aggregates (Alldredge et al., 1995; Kioerboe et al., 1996; Thornton, 2002) as observed during our experiments. This aggregates formation is likely the cause of the observed cell abundance decrease in both $p\text{CO}_2$ treatments during the experiment with this coastal-upwelling community (Figure 9). In any case, considering that the purpose of this work was to evaluate and compare the response of two distinct coastal phytoplankton communities to rising $p\text{CO}_2$, this premise was fulfilled, as the results showed that they differed not only in their taxonomic composition but also in their growth phase.

Owing to the key role of phytoplankton in aquatic food webs as major primary producers, the impact of high $p\text{CO}_2$ conditions on these organisms has been the focus of intense research over the last two decades. These investigations, however, have yielded contrasting results. For instance, some studies with natural phytoplankton assemblages have reported a stimulating effect of elevated $p\text{CO}_2$ on growth rates and primary productivity (Kim et al., 2006; Riebesell et al., 2007; Tortell et al., 2008). Hare et al. (2007), in turn, reported constant primary production under elevated CO_2 in the Bering Sea but a decrease in the diatom contribution to the phytoplankton community. On the other hand, an inhibitory effect has been observed in the formation of the calcareous plankton skeletons, like that of coccolithophorids (Riebesell et al., 2000; Delille et al., 2005), and more recently, other studies have likewise reported negative effects of increased CO_2 in non-calcifying organisms (Feng et al., 2010; Yoshimura et al., 2010; Hama et al., 2011). In the present work, we observed no detectable impact of increased CO_2 on Chl *a* concentration in none of the two phytoplankton communities studied (Figures 8, 9), which aligns well with results from previous micro- and mesocosm OA experiments conducted with diatom-dominated phytoplankton assemblages under the same nutrient sufficient conditions (e.g., Engel et al., 2014; Bach et al., 2017).

Although there was no significant impact on total phytoplankton biomass, there were some possible effects on particular phytoplankton functional groups or even specific taxa within functional groups, particularly in the estuarine community. For example, the total numerical abundances of flagellates, diatoms, and dinoflagellates, was about 25–50% lower under the high $p\text{CO}_2$ treatment. This also suggests that the Chl *a* per cell must have on average been higher under this treatment, to account for the lack of an effect on total Chl *a* concentrations, although we have no direct data at the cellular level for this. Increases in Chl *a* per cell in response to high $p\text{CO}_2$ have been previously reported in multiple dinoflagellates (e.g., Eberlein et al., 2016; Hennon et al., 2017). However, Chl *a* per cell in diatoms has been reported to increase modestly (Crawford et al., 2011) to stay the same (e.g., Hennon et al., 2017) or decrease (Jacob et al., 2017) under high $p\text{CO}_2$. Within the diatoms, the genera *Skeletonema* and *Thalassiosira* appeared to benefit more than others. Although *in situ* the community was dominated by freshwater diatoms, such as *A. granulata* (which represented 50% of the numerical abundance), after 5 days these were completely replaced by marine groups. The freshwater diatoms thus probably represent a component that is not growing locally but is advected in, along with other particulate and dissolved organic carbon contributed from the river. *Chaetoceros* spp. also showed modest decrease toward the end.

The mechanisms underlying differences in the response of distinct phytoplankton to elevated $p\text{CO}_2$ can be complex to unravel. On the one hand, a key factor in determining the success of specific functional groups, like diatoms, is their carbon concentrating mechanisms (CCM, Burkhardt et al., 2001; Cassar et al., 2004). Free CO_2 in the ocean is at limiting levels for RuBisCO, the primary carboxylating enzyme used in photosynthesis (Beardall and Raven, 2004). To overcome this constraint, many phytoplankton species have developed an intracellular mechanisms that converts bicarbonate ions (HCO_3^-) to CO_2 at the active site of this enzyme, thereby supporting higher carbon fixation rates than what would be possible if photosynthesis only relied on diffusive CO_2 uptake (Giordano et al., 2005). The proportion at which both processes operate (i.e., direct CO_2 uptake vs. HCO_3^- uptake) varies largely among species (e.g., Reinfelder, 2011; Matsuda and Kroth, 2014), as they differ in the efficiency and regulation of their CCMs (Burkhardt et al., 2001; Rost et al., 2003; Trimborn et al., 2008). Increases in the seawater CO_2 may directly benefit phytoplankton species primarily relying on diffusive CO_2 uptake or those with inefficient CCMs. Species that rely on a resource-intensive CCM, in turn, could also potentially benefit from a downregulation of the CCM in the future, which will allow them to optimize the energy and resources allocation (Rost et al., 2008; Eggert et al., 2014). Nevertheless, this CCM downregulation at increased $p\text{CO}_2$ has been observed to occur primarily under nutrient-limitation conditions (Taucher et al., 2015), and depends on the capacity of each species to switch between carbon acquisition mechanisms. On the other hand, low pH can potentially affect key metabolic rates, such as the ones involved in the maintenance of intracellular homeostasis, which could ultimately counteract the positive effect

of CO_2 fertilization (Wu and Gao, 2010; Bach and Taucher, 2019).

The response of the phytoplankton community from the coastal-upwelling ecosystem in the Arauco Gulf showed no significant effect of short-term exposure to increased $p\text{CO}_2$ in the abundance of either of the two groups that integrated the community, i.e., diatoms and dinoflagellates (Figure 9). This finding supports our initial hypothesis that exposure to high CO_2 conditions will not challenge phytoplankton communities inhabiting highly variable and acidic habitats. In agreement with our results, a recent review by Bach and Taucher (2019) showed that diatom communities in oceanic environments responded more frequently to rising $p\text{CO}_2$, with negative effects, than in coastal, estuarine, or benthic environments. It is currently beyond doubt that environmental variability can play a key role in promoting local adaptation of organisms so that they tolerate a larger range of pH and $p\text{CO}_2$ levels, which are usually characteristics of coastal ecosystems in both temporal and spatial scales (Duarte et al., 2013; Vargas et al., 2017). One study that reflects the importance of local adaptation was the meta-analysis conducted by Vargas et al. (2017) along the Chilean coast. These authors showed that organisms inhabiting areas with high mean $p\text{CO}_2$ and high $p\text{CO}_2$ variability exhibited lower negative effects on physiological traits to elevated $p\text{CO}_2$ conditions, suggesting that local adaptation can play a key role in setting sensitivity of species to changes in $p\text{CO}_2$. In agreement to that reported by these authors for other marine organisms, such as copepods, gastropods or mussels, our results suggest that phytoplankton communities inhabiting the coastal upwelling ecosystem in the Gulf of Arauco are locally adapted to the naturally occurring high $p\text{CO}_2$ levels (Figure 5; Vargas et al., 2017), so that short-term exposure to elevated $p\text{CO}_2$ levels does not challenge them.

5. CONCLUSIONS

Our results have shown that changes in the carbonate chemistry in the coastal water may have distinct implications for the phytoplankton communities inhabiting an estuarine and a coastal upwelling systems. Increases of $p\text{CO}_2$ can modify the structure of the estuarine phytoplankton community by decreasing the cell abundance of specific phytoplankton groups, which could have potential bottom-up effects for higher trophic levels. Contrarily, the phytoplankton community from the coastal upwelling ecosystem appears to be locally adapted to

changes in the $p\text{CO}_2$ levels. Given the high CO_2 values naturally occurring in this coastal ecosystem, other environmental drivers, such as eutrophication and/or warming may have a larger impact on this phytoplankton assemblage in the future. Overall, these findings highlight the importance of knowing the habitat-specific natural variability of seawater chemistry to better understand the potential impact of elevated $p\text{CO}_2$ conditions at the individual, community and ecosystem levels.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

CV and BJ contributed the concept and design of the study. LL-M, BJ, and PC collected the samples during field samplings, conducted the experiments, and analyzed the samples. NO, PC, and CV organized the database and analyzed the datasets. NO drafted the manuscript with contributions from CV and PD. All authors contributed to the manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2020.00323/full#supplementary-material>

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

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