



Potential effects of climate change on the growth response of the toxic dinoflagellate *Karenia selliformis* from Patagonian waters of Chile

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ABSTRACT

Northern Patagonia (41–44°S) is affected by climatic, hydrological and oceanographic anomalies, which in synergy with processes such as global warming and acidification of the coastal oceans may affect the frequency and intensity of harmful algal blooms (HABs). Greater frequency of HABs has been reported in the southeastern Pacific Ocean, including blooms of the toxic dinoflagellate *Karenia selliformis*, causing massive mortality of marine fauna in the oceanic and coastal areas of Patagonia. The objective of this study was to determine the effects of temperature and pH interaction on the growth of *K. selliformis* (strain CREAN_KS02), since these factors have wide seasonal fluctuations in the Patagonian fjord ecosystem. The CREAN_KS02 strain isolated from the Aysén Region (43°S) was used in a factorial experiment with five pH levels (7.0, 7.4, 7.7, 8.1 and 9.0) and two temperatures (12 and 17 °C) during a period of 18–21 days. Results indicated a significant effect of temperature and pH interaction on growth rate (range 0.22 ± 0.00 to 0.08 ± 0.01 d⁻¹) and maximum density (range $13,710 \pm 2,616$ to $2,385 \pm 809$ cells mL⁻¹) of *K. selliformis*. The highest density and growth of *K. selliformis* was found at 12 °C with a reduced pH (7.0–7.7). The results suggest that the current environmental conditions of coastal Patagonia, waters of low temperature and relatively low pH, may be favorable for the development of blooms of this species during autumn. We suggest that there is natural plasticity of *K. selliformis* in a wide pH range (7.0–8.1) but in a narrow low temperature range (10.6–12.9 °C), values that are typically recorded in the oceanic region of northern Patagonia. In contrast, in an extreme climate change scenario (ocean warming and coastal acidification) in northern Patagonia, a negative effect on the growth of *K. selliformis* may be expected due to amplification of the acidification effects caused by the thermal stress of high temperature water.

1. Introduction

Karenia selliformis is a toxic dinoflagellate of the family Kareniaceae, which has generated important monospecific blooms worldwide. These blooms produce active gymnodimines (GYM-A, GYM-B and GYM-C) with neurotoxic characteristics, which act as neuromuscular blockers by altering the nicotine receptors of acetylcholine (Assunção et al., 2017). It has been suggested that most species of Kareniaceae produce

ichthyotoxic polyunsaturated fatty acids (PUFA), whose toxicity may increase due to peroxidation of lipids in the presence of reactive oxygen species (Seki et al., 1995; Miles et al., 2000; Marrouchi et al., 2010; Mooney et al., 2010; Mardones et al., 2017; Assunção et al., 2017). Blooms of *Karenia* spp. have caused massive mortality of fish, and GYMs have been detected in shellfish, including green shell mussels, blue mussels, scallops, cockles, clams and abalones (Kharrat et al., 2008; Medhioub et al., 2009; Marrouchi et al., 2010; Naila et al., 2012; Feki-

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Sahnoun et al., 2020).

Blooms of *K. selliformis* occur widely around the world, in heterogeneous oceanographic ranges of temperature, salinity and nutrients: New Zealand (Stirling, 2001; Haywood et al., 2004), Kuwait (Heil et al., 2001), Túnez (Munday et al., 2004; Feki-Sahnoun et al., 2020); Gulf of Mexico (Haywood et al., 2007; Steidinger et al., 2008; Brand et al., 2012); France (Bire et al., 2002) and Australia (Takahashi et al., 2007). Previous studies carried out on *K. selliformis* reported optimal environmental conditions for growth (concentrations higher than 10^4 – 10^5 cells L^{-1}) in a wide range of temperature (12–28 °C) and salinity (38–42) in the Gulf of Gabès during 1997–2007 (Feki-Sahnoun et al., 2017). In the coastal system of Kuwait Bay, Arabian Sea, a bloom of *K. selliformis* with an abundance of 10^4 cells L^{-1} was associated with a 20-fold increase in inorganic nitrogen (up to 60 μM) and with an increase in orthophosphate concentration (1.5 μM), suggesting that coastal eutrophication contributed significantly to the development of blooms of this species (Heil et al., 2001; Al-Yamani et al., 2015).

Karenia selliformis shows high physiological plasticity against heterogeneous environmental factors. Blooms of this species associated with temperatures of 13.5 and 15 °C (Clement et al., 2001; Uribe and Ruiz, 2001; Guillou et al., 2002; Mardones et al., 2020) have been recorded in coastal waters of the southeastern Pacific Ocean (Inner Sea of Chiloé, 42°S and the Magellan Strait, 53°S). A high growth rate of *K. selliformis* ($0.41 \pm 0.03 d^{-1}$) was recorded in experimental conditions with a strain isolated from the Archipelago of Guaitecas, Southern Chile (43°S) at salinity-temperature of 30/18 °C, with a maximum abundance ($11,800 \pm 904$ cells mL^{-1}) at 30/15 °C (Mardones et al., 2020). It was reported that *K. selliformis* (strain GM94GAB), isolated from north of Sfax, Gulf of Gabès, Tunisia, did not show significant differences in growth rate for salinity treatments (36 to 44), but rates varied depending on the temperature (15, 17, 20 °C) (Medhioub et al., 2009). The wide spectrum of responses of different strains to environmental factors combined under experimental conditions (e.g. scenarios of ocean warming and acidification) indicates the need to determine the interactive effects of key factors on the growth of *K. selliformis* from Patagonian waters.

The first bloom in Chilean waters related to the genus *Karenia* (Strain AF318247; Guillou et al., 2002) occurred in the Chiloé Archipelago (42°S) in March–April 1999, with densities up to 8×10^6 cells L^{-1} (Clement et al., 2001; Guillou et al., 2002; Mardones et al., 2020). In April 1999, two unidentified “morphotypes” of *Gymnodinium* similar to *G. mikimotoi* produced a bloom of up to 4.3×10^4 cells L^{-1} in surface waters of the Magellan Strait (52°S; Clement et al., 2001; Uribe and Ruiz, 2001). The latest event of wide spatial distribution in the Chilean coast (from 37 – 45°S) related to *K. selliformis* occurred during the austral summer of 2018 (Mardones et al., 2020). *Karenia selliformis* events usually produce mortality of marine species, including echinoderms (sea urchins and sea stars), mollusks (snails, limpets, and octopuses), and fish (salmon) (Clement et al., 2001; Uribe and Ruiz, 2001; Mardones et al., 2020).

Chemical changes in seawater associated with ocean acidification include an increase in dissolved inorganic carbon concentration (Roleda and Hurd, 2012), which in excess (mainly HCO_3^- and CO_2) may favor photosynthetic processes and phytoplankton growth (Raven, 1997; Gattuso and Hansson, 2011). This depends on the capacity to invest energy used by the Carbon Concentration Mechanism (CCM), the composition of the phytoplankton assemblage and the photosynthetic efficiency of autotrophs growing in low- CO_2 environment levels (Singh et al., 2014). For example, some noxious dinoflagellates have little or no capability to use bicarbonate, and their photosynthesis depends on active absorption of CO_2 (Bercel and Kranz, 2019; Li et al., 2021).

A future increase of CO_2 in coastal systems combined with other environmental factors driven by climate change (high temperature and high solar radiation) and local anthropogenic contamination (coastal eutrophication) would produce environmental and biogeographic mosaics favorable for the development of toxic microalga species such as

K. selliformis (Gao et al., 2019). It has been suggested that in future scenarios of global change (i.e. high pCO_2 and high temperatures), blooms of noxious algae may be more frequent in coastal areas worldwide (Hallegraeff, 2010; Lian et al., 2022; Takagi et al., 2022; Borges et al., 2022; Zhang, 2022), including northern Patagonia (Van Leeuwen et al., 2021; Trainer et al., 2020). The evidence shows strong seasonal and daily variation in the carbonate system in Patagonian coastal surface waters (Vergara-Jara et al., 2019; Vellojin et al., 2022). Some particular and still unknown interactions among pH, pCO_2 and temperature could potentially be keys to trigger blooms of *K. selliformis* that generate negative effects on artisanal fishing, intensive aquaculture and marine biodiversity in coastal areas of southern Chile due to its high toxicity.

Different future drivers (climate change, coastal acidification, eutrophication) are expected to produce a series of positive or negative effects on the growth of HAB species. There is currently little information about the metabolic responses and growth of *K. selliformis* in relation to the dynamics of the variables that compose the carbonate system (pH and pCO_2). Therefore, this study focused on evaluating experimentally the response of the growth rate of the *K. selliformis* to a combination of temperature and pH, which are highly variable seasonally and over years in the Patagonian marine system.

2. Materials and methods

2.1. Study area

The coastal area of the Guaitecas Archipelago is located in the northern part of the channels of western Patagonia, adjacent to the southern extreme of the Archipelago of Chiloé (Corcovado Gulf, 43°50'S). The climate of the region is modulated by the seasonal variability of the west winds that produce abundant precipitation in winter, > 3000 mm $year^{-1}$ (Pickard, 1971; Sauter, 2020). The effect of river runoff is less significant in this area of north Patagonia, observed salinity varies from 27 to 33 Sp (practical salinity, no unit: PSS-78, is reported as Sp; UNESCO, 1981) and temperature from 9 to 14 °C (Silva et al. 1998; Molinet et al. 2008). However, anomalies in temperatures of $+ 2$ °C are observed in the coastal area of Patagonia (Garreaud, 2018; Pujol et al., 2022), because of large-scale atmospheric circulation modes generated by El Niño Southern Oscillation (ENSO) and the Southern Annular Mode (SAM).

The main inorganic nutrients vary seasonally on the surface; nitrate ranges from 6.2 to 32.7 μM , orthophosphate from 0.01 to 2.0 μM , and silicic acid from 0.09 to 33.3 μM (Buchan and Quinones, 2016). The most important water masses in this coastal system are the Estuarine Water (EW) on the surface (0–30 m), the Modified Subantarctic Water (MSAAW) at intermediate depth (30–100 m), and the Subantarctic Water (SAAW) below 100 m (Silva and Guzmán, 2006; Silva and Palma, 2008; Silva and Vargas, 2014).

2.2. Experimental setting

The *K. selliformis* CREAN_KS02 strain was isolated from the Guaitecas Archipelago (43°57'S, 73°45'W; Fig. 1) in March 2018 from a multi-specific bloom of the family Kareniaceae that extended from the coast of the Bio-Bio Region (~37°S) to the Aysén Region (~46°S) in summer/early-autumn (January–March) of 2018 (Mardones et al., 2020). This strain was cultured in L1 medium (Guillard and Ryther, 1962) and salinity of 33 under a light–dark regimen of 12:12 h at 100 μmol photon $m^{-2} s^{-1}$ using white-cold fluorescent light. The salinity *in vitro* was the same as that recorded *in situ* in the sampling (33 Sp). We carried out a factorial (5x2) experiment to analyze the combined effect of 5 pH levels (7.0, 7.4, 7.7, 8.1, and 9.0) and two temperatures (12 and 17 °C) on the growth of *K. selliformis*. Experimental treatments (2 temperatures \times 5 pH) were performed in triplicate using sterile flasks, each with 45 mL of L1 culture medium with 5 mL of headspace (total number of flasks =

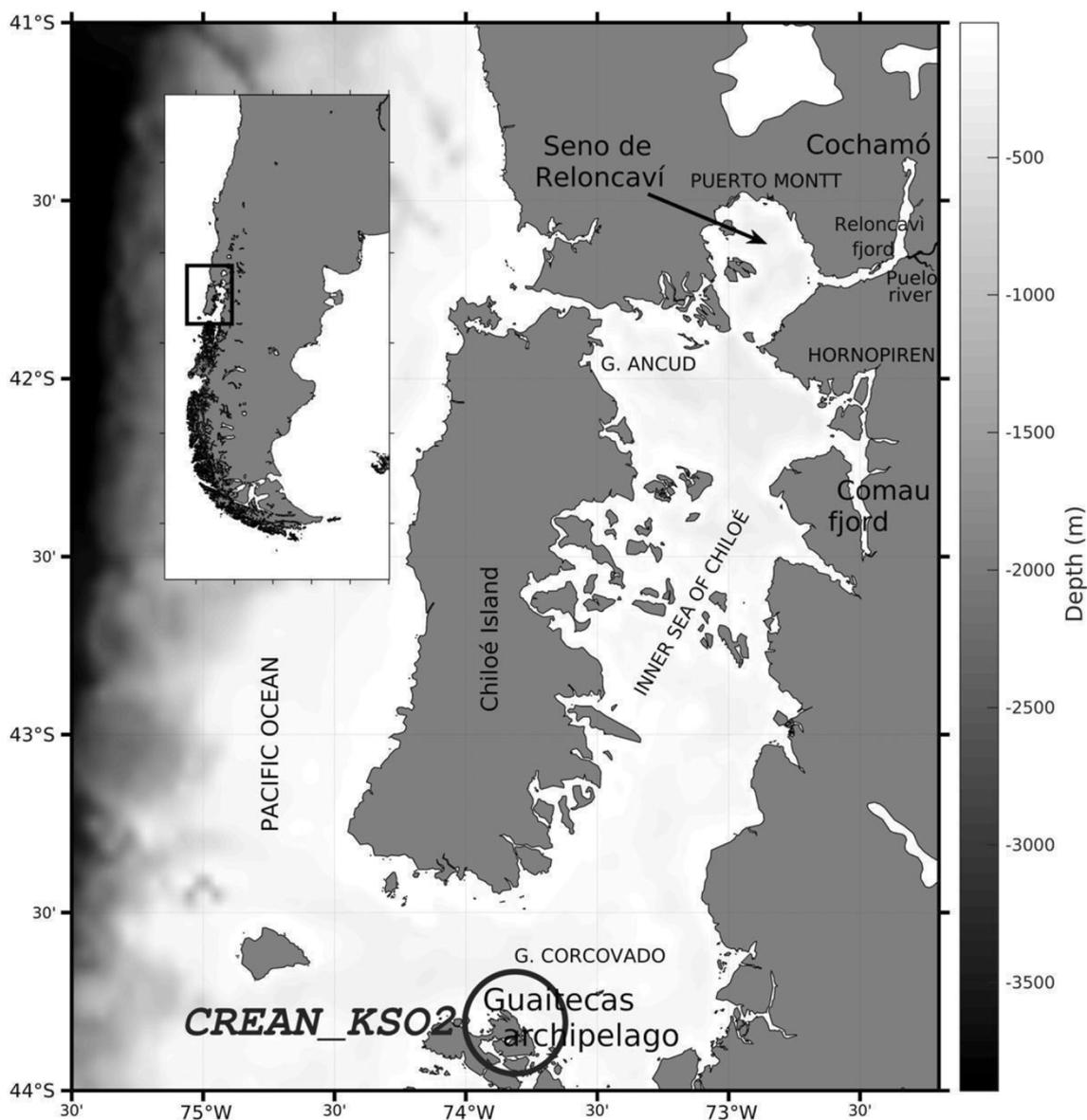


Fig. 1. Study area where the CREAN_KSO2 strain of *K. selliformis* was isolated during a bloom in the coastal area of the Guaitucas Archipiélago (43°S), Aysén Region in March 2018.

120, this experimental design minimizes changes in the carbonate chemistry due to sample manipulation). The pH_T was modified (the initial pH was 7.94 ± 0.03) using the acid/base method that simulates the conditions of chemical changes in the carbonate of seawater (Riebesell et al., 2010; Leal et al., 2017; Wells et al., 2021). Equal volumes of 0.4 M HCl and 0.4 M $NaHCO_3$ were prepared to obtain the three treatments of lower pH (pH_T 7.01, 7.41, and 7.71), while equal volumes of 0.4 M NaOH and 0.4 M $NaHCO_3$ were added for the two treatments with high pH (pH_T 8.10 and 9.00) (Riebesell et al., 2010). The acid/base method was used, considering that the organisms of this group (dinoflagellates) have better growth in stratified conditions, so it is always sought to perform this type of culture without bubbling. CO_2 bubbling can also cause cells to be damaged by the flow of bubbles and can cause alterations in the experiment. The initial experimental concentration was standardized with an inoculation volume of 800 cells mL^{-1} from a culture in the exponential phase. The experiment was carried out for 18–21 days, sampling every 3–6 days to complete a total of 4 (excluding T_0) to estimate cell density (cells mL^{-1}) and growth rate (divisions day^{-1}).

A temperature of 12 °C was chosen as an optimal temperature for the

growth of *Karenia selliforme*, based on results of previous experiments (Mardones et al., 2020) and information on the environmental variability of the temperature in the area where the bloom of this species took place (Silva and Palma, 2006; Pérez-Santos et al., 2021; Saldías et al., 2021). Five °C was added to the early autumn average of 12 °C, to have a temperature similar to a future scenario of temperature change in northern Patagonia. The basification/acidification treatments were (i) pH 9.0, representing the microalga post-bloom or preindustrial condition in the open ocean, (ii) pH 8.1, the mean of the open ocean (atmosphere–ocean equilibrium), (iii) pH 7.7, the worst projected scenario of acidification (RCP8.5; IPCC, 2013), and (iv) a lower value (pH < 7.7), recorded for the ocean surface in coastal areas influenced by freshwater runoff (Vergara-Jara et al., 2019). The pH/pCO_2 values selected in this study exceed the extreme values predicted for future scenarios associated with acidification of the open ocean (Cai et al., 2011; Melzner et al., 2013; Wallace et al., 2014; Raven et al., 2020).

Our goal in this experimental design was to simulate sudden changes in temperature and pH that often occur within the fjords due to the tidal regime that leads to freshwater input/output. Also, considering that the main purpose of our study is to assess the scenarios of how HABs behave

in the interphase between more stable offshore coastal waters and complex and highly dynamic fjord waters, our experimental design assessed the effect of sudden changes in abiotic variables on the physiological response of *K. selliformis*. Thus, established dinoflagellate cultures were immediately split into various treatments.

2.3. Growth rate

Cell density was estimated for each sample by quantification of buffered Lugol-fixed cells using a Sedgewick-Rafter chamber under an inverted light microscope (Motic® AE31 Elite Research Grade Inverted Microscope). Three replicates were used to estimate the mean and the growth rate μ (day^{-1}), as:

$$\mu = \frac{\ln\left(\frac{c_2}{c_1}\right)}{(t_2 - t_1)} \quad (1)$$

Where c_1 and c_2 are the cell densities (cell mL^{-1}) at the beginning (t_1) and end (t_2) of the incubation period, respectively.

2.4. Measures of pH and parameters of carbonate chemistry

Seawater pH during the experiment (days 0, 6, 11, 14 and 18) was measured as total scale pH (pH_T) in water samples from the experimental units, using an Orion ROSS ultra pH/ATC, 8302BNUMD pH electrode connected to a Thermo Scientific Orion 3 Star pH portable meter. The calibration curve of the electrode was determined using pH 7 and 10 buffers with equilibrated temperature (color-coded, trackable by NIST). A Tris buffer standardized with a seawater buffer was used to measure pH in the total scale. At the beginning of the experiment (t_0), water samples were fixed with mercuric chloride and stored in the dark at low temperature ($< 7^\circ\text{C}$) in 50 mL gas-tight containers for analysis of total alkalinity (A_T). A_T was measured using the closed cell titration method (Dickson et al., 2007). The variables A_T , pH_T , salinity and temperature ($^\circ\text{C}$) were used to estimate the chemical carbonate parameters (pCO_2 , CO_2 , DIC, HCO_3^- and CO_3^{2-} ; Table 1) of seawater with the SWCO₂ program (Hunter, 2007).

2.5. Statistical analysis

To determine the effect of the interaction of pH and temperature on the growth of the *K. selliformis* strain, a factorial analysis of variance (ANOVA; Zar, 2010) based on a regression model of the response variables (maximal density and growth rate) was performed. Both variables were transformed to log10 to assess the assumptions of normality, linearity and homoscedasticity, using the Shapiro-Wilk, linearity and Bartlett tests, respectively. The residuals model was plotted (Residuals vs Fitted, Normal Q-Q, Scale-Location and Residuals vs Leverage). Based on the results of the analyses, we conclude that our data meet the

assumptions of normality and homoscedasticity, indicating that they are adequate to perform an ANOVA and a post hoc parametric test. Post-hoc multiple comparisons were carried out with Tukey HSD tests to determine significant differences between pairs of treatment effects (Zar, 2010). A null hypothesis of no difference in the response variables was used in all analyses, with a significance level of $p < 0.05$. Analyses were performed using the R software v.3.4.4–2018.

3. Results and discussion

In the current scenario of global climate change (warming, acidification, deoxygenation), alterations have been reported in the physical and chemical processes that modulate ocean dynamics, causing important (positive or negative) effects on the metabolism, abundance distribution, and structure of phytoplankton communities (Raven and Beardall, 2021). For species that develop HABs, and specifically for *K. selliformis* due to its wide distribution in coastal and oceanic waters, it is key to recognize the ranges of values of the main factors which trigger its growth, especially in scenarios of future changes the ocean will undergo (Feki et al., 2008; Mardones et al., 2020). The results of this study and other previous studies of *K. selliformis*, as well as, other species that are classified as HABs, show their response to possible conditions imposed by future scenarios and eliminate the widespread concept of increasing HABs in future scenarios.

During summer/early-autumn (January–March), 2018 an intense bloom of the Kareniaceae family was recorded in off the southern Chilean Coast (Mardones et al., 2020). During the spring bloom, the highest values of total chlorophyll-a (Chl-a; as proxy of phytoplankton biomass) were $> 5 \text{ mg m}^{-3}$ in January, mainly in the coastal area of the Bio-Bio Region ($\sim 37^\circ\text{S}$). However, a wide spatial extension of relatively high mean Chl-a values ($\sim 1\text{--}3 \text{ mg m}^{-3}$) with respect to the values observed in February and March were observed in the southern area ($\sim 42\text{--}46^\circ\text{S}$) from the inner to the open coastal waters (Fig. 2A).

The bloom was decreasing in the north in February, but the southern coastal area and the interior channels of the Aysén Region ($43\text{--}46^\circ\text{S}$) mostly maintained the same Chl-a pattern ($\sim 1\text{--}3 \text{ mg m}^{-3}$; Fig. 2 b). Lower Chl-a values were found in March, but localized areas with mean Chl-a values of $\sim 1\text{--}2 \text{ mg m}^{-3}$ were found in the coastal waters from 36°S to 42°S and in the inner waters of Chiloé (Fig. 2 c). Low temperatures ($\sim 12\text{--}15^\circ\text{C}$) were recorded along the exposed coast from the Bio-Bio to Aysén regions during January to March, with the lowest temperature values ($\sim 12\text{--}13^\circ\text{C}$) in the Guaitecas Archipelago ($\sim 43^\circ\text{S}$) and the inner sea of Chiloé (Fig. 2 d-f). Previous studies in the Guaitecas Archipelago reported water temperature of 12 to 15°C from summer to autumn (February to April), with high primary productivity and phytoplankton biomass ($5\text{--}10 \text{ mg Chl-a m}^{-3}$; Buchan and Quiñones, 2016).

The coastal region of northern Patagonia ($41\text{--}44^\circ\text{S}$) has undergone high local interannual variability of climatic, hydrological, and

Table 1

Chemical parameters of carbonate calculated using total alkalinity (A_T), pH_T , practical salinity (34 Sp) of each pH_T treatment: 7.00, 7.40, 7.7, 8.1 and 9.0 at 12 and 17°C for the initial sampling time. Mean \pm SD ($n = 3$).

T $^\circ\text{C}$	Parameter	Nominal seawater pH_T treatment									
		7.0		7.4		7.7		8.1		9.00	
12	pH_T	6.94	\pm 0.01	7.34	\pm 0.03	7.63	\pm 0.01	7.99	\pm 0.01	8.89	\pm 0.01
	A_T ($\mu\text{mol Kg}^{-1}$)	2059.25	\pm 8.04	2018.86	\pm 14.64	2037.39	\pm 28.84	2038.68	\pm 16.99	2045.82	\pm 4.03
	DIC ($\mu\text{mol Kg}^{-1}$)	2221.05	\pm 6.24	2034.68	\pm 19.09	1971.93	\pm 31.78	1842.10	\pm 20.68	1268.57	\pm 3.71
	HCO_3^- ($\mu\text{mol Kg}^{-1}$)	2021.97	\pm 7.33	1928.93	\pm 16.90	1870.24	\pm 30.89	1689.98	\pm 21.87	766.09	\pm 5.76
	CO_2 ($\mu\text{mol Kg}^{-1}$)	184.20	\pm 3.78	70.11	\pm 4.46	35.35	\pm 1.51	13.85	\pm 0.55	0.78	\pm 0.02
	pCO_2 (μatm)	5361.35	\pm 110.03	2040.59	\pm 129.76	1028.93	\pm 44.02	403.08	\pm 15.88	22.82	\pm 0.47
17	pH_T	7.01	\pm 0.01	7.39	\pm 0.02	7.70	\pm 0.01	8.08	\pm 0.01	8.99	\pm 0.01
	A_T ($\mu\text{mol Kg}^{-1}$)	2059.25	\pm 8.04	1998.86	\pm 20.10	1975.73	\pm 19.02	2016.35	\pm 44.50	2045.82	\pm 4.03
	DIC ($\mu\text{mol Kg}^{-1}$)	2193.04	\pm 3.95	2000.28	\pm 23.53	1890.72	\pm 17.86	1779.57	\pm 46.23	1201.38	\pm 9.18
	HCO_3^- ($\mu\text{mol Kg}^{-1}$)	2016.41	\pm 6.81	1899.42	\pm 21.77	1787.69	\pm 16.77	1607.01	\pm 45.17	659.35	\pm 11.82
	CO_2 ($\mu\text{mol Kg}^{-1}$)	159.55	\pm 3.69	61.53	\pm 2.74	28.90	\pm 0.69	10.70	\pm 0.57	0.54	\pm 0.02
	pCO_2 (μatm)	4643.89	\pm 107.39	1790.87	\pm 79.81	841.18	\pm 19.95	311.36	\pm 16.62	15.67	\pm 0.64

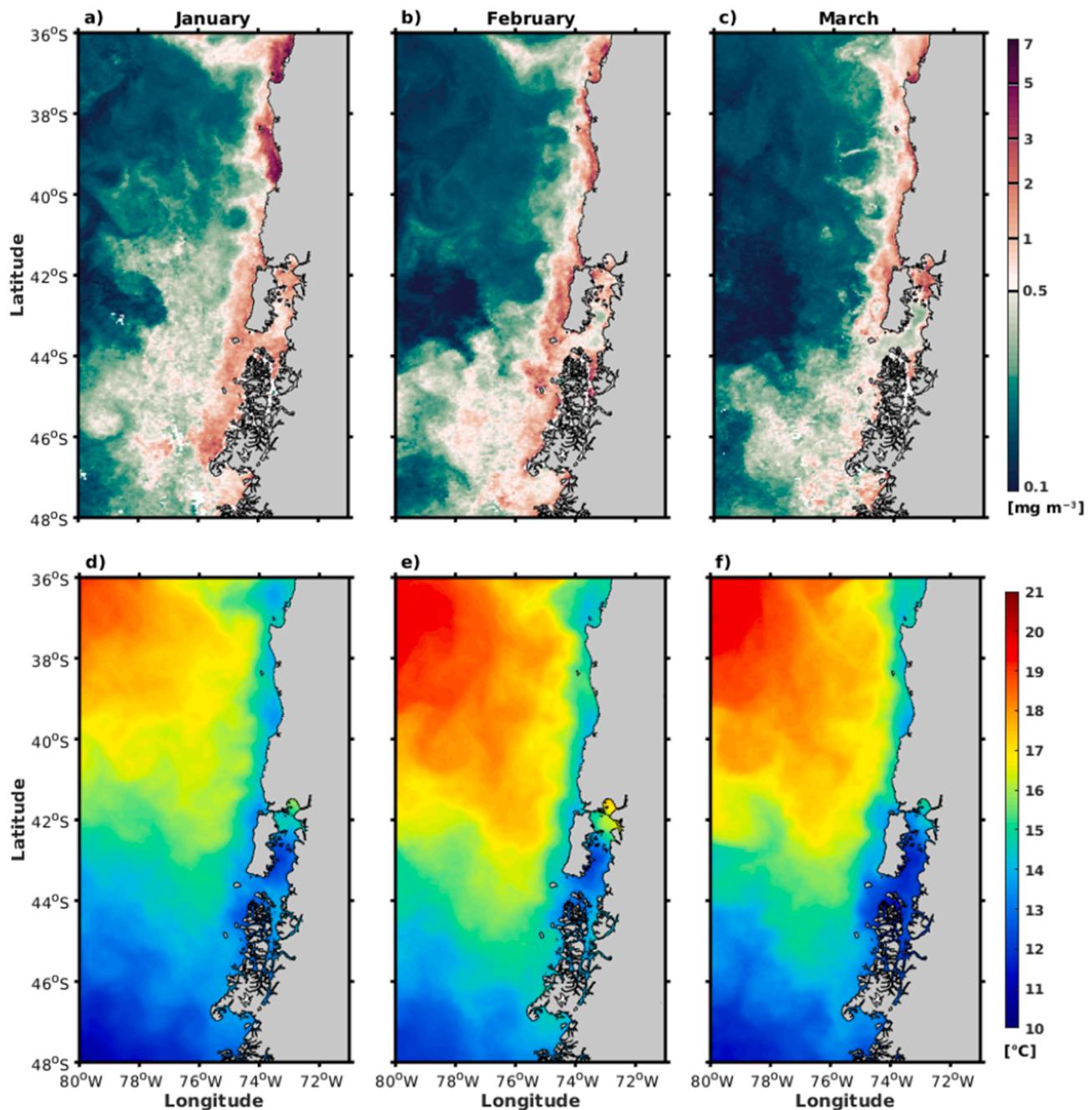


Fig. 2. Monthly mean of total chlorophyll-a (Chl-a; a - c) and sea surface temperature (SST; d - f) in January, February and March 2018 in the coastal area of Chile from the Bio-Bio (37°S) to Aysén (47°S) Regions. The Chl-a data were obtained from the Copernicus-GlobColour product of the Copernicus Marine and Environment Monitoring Service (CMEMS; <https://resources.marine.copernicus.eu/products>; `oceancolour_glo_chl_l3_nrt_observations`) with 0.3 km of spatial resolution and the SST data were obtained from the Multi-scale Ultra-high Resolution Sea Surface Temperature (MUR-SST; https://podaac.jpl.nasa.gov/Multi-scale_Ultra-high_Resolution_MUR-SST) product with a spatial resolution of 1 km.

oceanographic processes, in synergy with the global processes of climate change (Lara et al., 2016; León-Muñoz et al., 2018; Aguayo et al., 2019). In the last few years there has been a decrease in precipitation in the fjord system of northern Patagonia, which has led to a notorious decrease in the input of freshwater to the fjord system (Aguayo et al., 2019). This tendency to decrease of river freshwater discharge influences the adjacent coastal ocean, shallowing the haline stratification and increasing the mixing and advection deep waters rich in nutrients, low pH and high $p\text{CO}_2$, which are associated with a greater potential frequency of HABs in northern Patagonia in the near future (Iriarte, 2018; León-Muñoz et al., 2018; Van Leeuwen et al., 2021).

3.1. Maximum *K. Selliformis* cell density

Maximum cell density in the exponential phase of the *K. selliformis* strain in the 10 treatments indicated a temperature \times pH interaction (Fig. 3 A; ANOVA test, $p < 0.05$, $F = 12.8$). The mean maximum cell density of *K. selliformis* was obtained with pH 7.0, 7.4, 7.7 and 8.1 at 12 °C (Fig. 3 A; Test Tukey, $p < 0.05$). The maximum cell density at 12 °C varied from $13,710 \pm 2,616$ to $11,153 \pm 898$ cells mL^{-1} , compared the treatments at 17 °C ($8,686 \pm 1,965$ to $2,385 \pm 809$ cells mL^{-1} ; Table 2). All pH treatments at 12 °C (except pH 9.0) were in the exponential phase at the end of the trial period (Fig. 4 A). In contrast, the pH treatments at

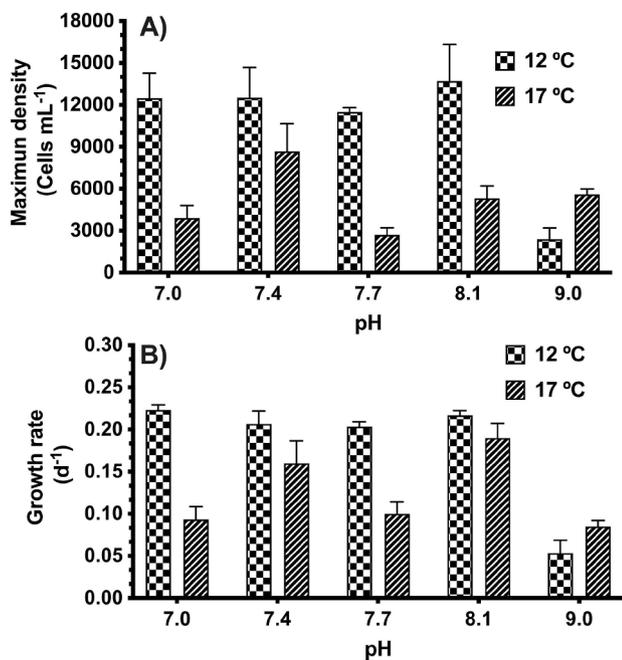


Fig. 3. Combined effect of temperature (°C) and pH on maximum cell density (A) and growth rate (B). Bars indicate the means and vertical lines above the bars show the standard deviation (±SD; n = 3).

Table 2

Culture growth parameters of *K. selliformis* (CREAN_KSO2), maximum cell density (AB_{Max}) and specific mean growth rate (μ) under combinations of temperature (°C) and pH (mean ± SD).

Treatments (Temperature * pH)	AB _{Max} (cells mL ⁻¹)	μ (d ⁻¹)
1 (12 °C + 7.0)	12,486 ± 1,784	0.22 ± 0.01
2 (12 °C + 7.4)	12,520 ± 2,151	0.21 ± 0.01
3 (12 °C + 7.7)	11,153 ± 898	0.21 ± 0.01
4 (12 °C + 8.1)	13,710 ± 2,616	0.22 ± 0.00
5 (12 °C + 9.0)	2,386 ± 809	0.06 ± 0.02
6 (17 °C + 7.0)	3,913 ± 891	0.09 ± 0.01
7 (17 °C + 7.4)	8,686 ± 1,965	0.16 ± 0.02
8 (17 °C + 7.7)	2,690 ± 520	0.10 ± 0.01
9 (17 °C + 8.1)	5,313 ± 883	0.19 ± 0.02
10 (17 °C + 9.0)	5,590 ± 398	0.08 ± 0.01

17 °C were in a phase of cell decrease on day 14 except for pH 7.0, which was still in the exponential phase at the end of the experiment (Fig. 4 B). A previous study with the CREAN_KSO2 strain performed by Mardones et al. (2020), where a temperature of 15 °C was used as a scenario for warmer summers and 18 °C as an extreme climate event, found higher abundance at 15 °C (11,800 ± 904 cells mL⁻¹; Mardones et al., 2020). Our study obtained a similar density at 12 °C (13,710 ± 2,616 cells mL⁻¹), considered as the current average temperature within the fjords, and 17 °C, a future scenario but also the temperature that the water surface commonly reaches during summer. There were positive effects on maximum cell density for pH 7.0 to 8.1. Our study suggests that a scenario of seasonal (autumn–winter) coastal acidification and relatively cold waters (current conditions) in northern Patagonia are favorable for the growth of *K. selliformis*.

Positive effects on maximum density have also been recorded under acidified conditions for other HAB species, for example, positive effects on the growth of *Amphidinium carterae* were observed under experimental conditions of 1000 μatm and pH 7.81 (Li et al., 2020), as well as *Karenia mikimotoi*, with maximum cell density under experimental conditions of 1075 μatm CO₂ and pH 7.80 (Wang et al., 2019). Lian et al., (2022) found that high levels of CO₂ (800 and 1200 μatm) significantly promoted the growth of *Alexandrium minutum* compared to

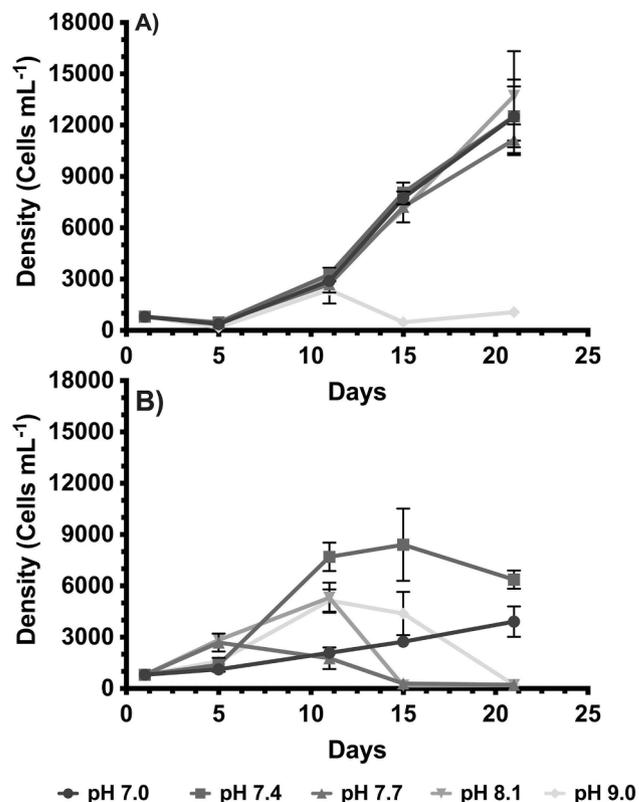


Fig. 4. Mean values of cell density (±SD; n = 3) of *K. selliformis* (CREAN_KSO2) observed in the factorial experiment based on different pH levels (7.0, 7.4, 7.7, 8.1, 9.0) combined with two temperatures, 12 (A) and 17 °C (B). Symbols represent the mean and the bars the standard deviation of the cell counts in triplicate.

the group exposed to current CO₂ levels (420 μatm). These authors observed that at 800 μatm CO₂ the exponential growth phase extended longer and higher cell abundance was reached (6 × 10⁴ cells mL⁻¹). A density of 2.02 ± 0.19 × 10⁷ cells L⁻¹ was obtained when *Alexandrium tamarense* was exposed to 1000 μatm CO₂ (pH 7.75, experimental), which was significantly higher than cultures exposed to 395 μatm CO₂ (pH 8.23, control) (Pang et al., 2017). The increment of cell density under low pH could be a response associated with the availability of CO₂ in the environment, because dinoflagellate cells cease to use metabolic energy for the Carbon Concentration Mechanism (CCM) and instead it is used for growth (Fu et al., 2008; Ou et al., 2017). This cellular process has been explained as a down-regulation of CCM under high CO₂ (2000 μatm) for *Karenia mikimotoi*, related to increase of growth rate (Hu et al., 2017).

3.2. *K. Selliformis* growth rate

There was a significant effect of temperature and pH in the growth rate of the *K. selliformis* strain (Fig. 3 B; ANOVA: $p < 0.05$, $F = 12.8$). The pH treatments 7.0, 7.4, 7.7 and 8.1 at 12 °C had a higher mean growth rate (0.22 ± 0.00 day⁻¹, Table 2) than the pH treatments at 17 °C (Fig. 3 B; Tukey test: $p < 0.05$). Growth rate values at 12 and 17 °C were lower than the optimal growth reported in other studies of this species (0.34 ± 0.14 day⁻¹ and 0.41 ± 0.03 day⁻¹) at 20 °C (strain GM94GAB, isolated in 1994 from the north of Sfaz, Gabès Gulf) and 18 °C (CREAN_KSO2 strain, isolated from the GUAITECAS Archipelago) (Medhioub et al., 2009; Mardones et al., 2020). In contrast, a study with *Karenia mikimotoi* showed that high temperatures (18–20 °C) and low pH (7.5) were favorable for cell division, with a high growth rate of 0.37 day⁻¹ at 20 °C with pCO₂ of ~ 2000 μatm (Hu et al., 2017). Interestingly, results with

the Chilean *K. selliformis* strain indicated optimal growth rates at relatively low temperature (12 °C), low pH (7.0 and 7.7) and high pCO₂ (~5000 and ~ 1000 µatm) (Mardones et al., 2020). These results show that different strains and species respond differently to experimental conditions, depending upon their tolerance capacity, adaptation, plasticity in the original habitat, and culture conditions (Table 3). The wide spectrum of responses observed under different experimental scenarios may be due to inherent differences in physiology related to the habitats where these strains lived before the experimental conditions, the range of time when the physiological response was measured and generation times (days to months; Tortell et al., 2008).

3.3. Future scenarios for *K. Selliformis* in Patagonia

Because of the recent blooms of noxious microalgae in northern Patagonia between 2016 and 2021, influenced by climate and hydrological/oceanographic drivers, it is important to study the autecology of HAB species, especially to establish ranges of key factors values under regional environmental conditions. pH values vary from 7.0 to 9.0 in coastal systems like fjords and estuaries during the year, where the water column is influenced by freshwater from glacier melting, rivers and/or deep waters with high rates of re-mineralization and respiration (Macedo et al., 2001; Hansen, 2002; Hinga, 2002; Vergara-Jara et al., 2019; Raven et al., 2020; Vellojin et al., 2022).

The experimental results of this study indicated a strain-specific positive response of the Chilean *K. selliformis* strain with high density and growth rate under a wide range of pH (7.0–8.1) at a temperature of

12 °C (Fig. 5). These physical–chemical characteristics are observed in Patagonian coastal waters (41–47°S), where from summer to the beginning of autumn the mean temperature is ~ 12 °C and the pH varies from 7.7 to 8.1 (Alarcón et al., 2015; Vergara-Jara et al., 2019; Narváez et al., 2019; Vellojin et al., 2022). The negative effects on the growth of *K. selliformis* may be associated with thermal stress generated by temperatures of 17 °C, which may exceed the maximum temperature (thermal threshold) at which this strain can develop bloom events (Fig. 5). An important effect of temperature has been previously shown by Mardones et al. (2020); water temperature usually ranges from 9 to 14 °C in the area where the *Karenia* bloom occurred (Silva et al. 1998; Molinet et al. 2008). A temperature of 17 °C was not recorded during the bloom of *K. selliformis* (see Fig. 2). In this study we showed that this temperature is an additive stressor for the *K. selliformis* strain in southern Chile, inhibiting its growth. A clear example that strains isolated from different oceanographic conditions can differ highly in physiological response, is the fact that Chilean *K. selliformis* strains are the only ones that do not produce gymnodimines among all strains reported in the literature (Mardones et al., 2020).

A bloom of *K. selliformis* was reported at temperatures lower than 14 °C in coastal waters of Tunisia (Feki-Sahnoun et al., 2020). A bloom of *K. selliformis* was reported in Hokkaido, Japan with high cell density (>1,000 cells mL⁻¹) in a temperature range of 11.0–17.3 °C. Also, high cell densities (10,560 cells mL⁻¹ and 9,600 cells mL⁻¹) were found in Toyokoro and Hiroo, Japan at 11.9 and 11.3 °C, respectively (Iwataki et al., 2022), similar to the temperature during the 2018 bloom in Chilean Patagonia. This suggests that strains of *K. selliformis* may have a

Table 3

Species of phytoplankton used in trials to evaluate the effects of temperature, pH and pCO₂ on cell density and specific growth rate (μ). Positive or negative responses to conditions of acidification and high temperatures are indicated (+or -).

Phytoplankton species	Maximum density (cell mL ⁻¹)	Temp (°C)	pH (+or -)	pCO ₂ (µatm)	Response (+or -)	Source
<i>Karenia selliformis</i>	13,710	12	8.10	403	+	This trial
<i>Karenia selliformis</i>	11,800	15	-	-	-	Mardones et al.,2020
<i>Karenia mikimotoi</i>	35,000	20	7.80	1075	+	Wang et al., 2019
<i>Amphidinium carterae</i>	35,000	23	7.81	1000	+	Li et al., 2020
<i>Alexandrium minutum</i>	60,000	20	-	800	+	Lian et al., 2022
<i>Alexandrium tamarense</i>	2.02 × 10 ⁷	20	7.75	1000	+	Pang et al., 2017
	μ (d ⁻¹)					
<i>Karenia selliformis</i>	0.22	12	8.10	403	+	This trial
<i>Karenia selliformis</i>	0.41	18	-	-	+	Mardones et al.,2020
<i>Karenia selliformis</i>	0.34	20	-	-	+	Medhioub et al., 2009
<i>Karenia mikimotoi</i>	0.37	-	7.50–7.52	2000	+	Hu et al., 2017
<i>Karenia mikimotoi</i>	0.65	20	7.80	1000	+	Li et al., 2021
<i>Karenia brevis</i>	0.43	25	-	1000	+	Errera et al., 2014
<i>Karenia brevis</i>	0.24	26	7.89	780	+	Bercel and Kranz, 2019
<i>Alexandrium catenella</i>	0.18	20	8.10	400	-	Seto et al., 2019
<i>Alexandrium catenella</i>	0.20	20	7.80	1000	+	Seto et al., 2019
<i>Alexandrium catenella</i>	0.28	-	8.01	369	-	Mardones et al., 2017
<i>Alexandrium catenella</i>	0.34	-	8.12	313	-	Mardones et al., 2017
<i>Alexandrium tamarense</i>	0.31	20	7.75	1000	+	Pang et al., 2017
<i>Alexandrium minutum</i>	1.25	25	~7.73	1000	+	Thangaraj et al., 2022
<i>Alexandrium affine</i>	0.60	26	~7.73	1000	+	Lee et al., 2021
<i>Alexandrium pacificum</i>	0.58	24	~7.73	1000	+	Lee et al., 2021
<i>Scrippsiella</i> sp.	0.34	20	7.80	1000	+	Seto et al., 2019
<i>Amphidinium carterae</i>	0.45	20	8.10	400	-	Seto et al., 2019
<i>Amphidinium carterae</i>	0.41	20	7.80	1000	+	Seto et al., 2019
<i>Amphidinium carterae</i>	~0.7	20	7.60	~1000	+	Bausch et al., 2019
<i>Akashiwo sanguinea</i>	~0.40	20	~7.74	1000	+	Ou et al., 2017
<i>Akashiwo sanguinea</i>	~0.40	28	~7.84	1000	+	Ou et al., 2017
<i>Akashiwo sanguinea</i>	0.38	25	-	-	+	Chen et al., 2015
<i>Gambierdiscus polyneisensis</i>	0.20	-	8.40–8.20	-	-	Longo et al., 2020
<i>Karlodinium veneficum</i>	~0.28	-	7.94	747	-	Fu et al., 2010
<i>Karlodinium veneficum</i>	0.47	20	8.03	445	-	Müller et al., 2019
<i>Ceratium fusus</i>	0.32	-	8.73	-	-	Söderberg and Hansen, 2007
<i>Ceratium furca</i>	0.26	-	9.08	-	-	Söderberg and Hansen, 2007
<i>Ceratium tripos</i>	0.14	-	8.92	-	-	Söderberg and Hansen, 2007
<i>Protoceratium reticulatum</i>	~0.23	-	-	1000 – 5000	+	Pierangelini et al., 2017
<i>Proocentrum minimum</i>	~0.7	-	-	750	+	Fu et al., 2008

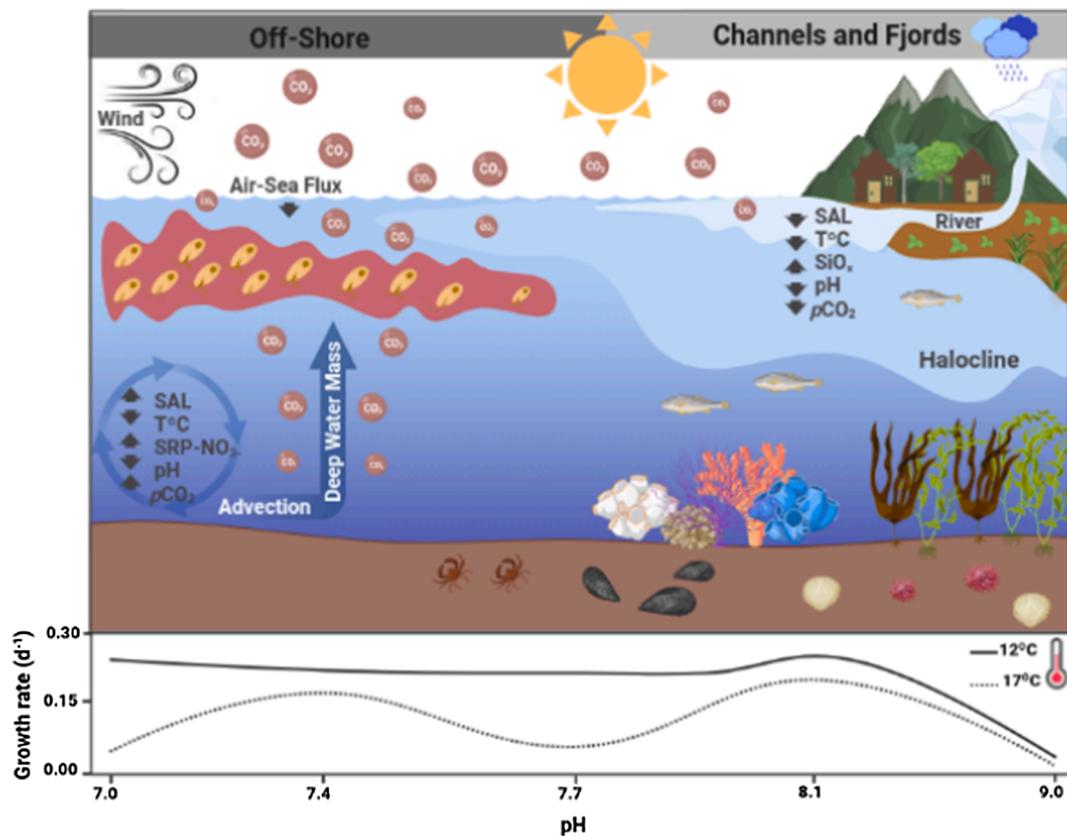


Fig. 5. Schematic representation of possible ocean warming consequences in synergy with CO₂ and pH variability on *K. selliformis* growth response in the Patagonian marine system. Current scenario generated by the combination of temperature (12 °C) with variable pH conditions that are within the natural and seasonal variability of Patagonian fjords. This scenario includes relatively cold water and haline stratification in summer and autumn. This scenario is favorable for *K. selliformis*, whose growth may be high in conditions of high CO₂ and nutrients from advection of deep waters. Energy costs decrease when populations grow at the surface of a water column with CO₂ available for photosynthesis, due to the Carbon Concentration Mechanism (CCM). In the climate change scenario (17 °C), *K. selliformis* undergoes the conditions of a warming of the coastal ocean (+5 °C) in synergy with variable pH values (< 8.0), producing a negative response in growth. This may be explained by the temperature of 17 °C, which may surpass the optimal environmental window of this species, generating thermal stress, which has been related to amplification of the effects of acidification. We postulate that the combination of these conditions causes several kinds of cell damage, such as: oxidative stress, decrease in the photorespiration rate, reduction in the thermal dissipation capacity, changes in the efficient CCM, which finally result in less growth of this species. In both scenarios (Fig. 5) of the combination of temperatures of 12 and 17 °C with pH 9.0 and low CO₂, a decrease in the growth of *K. selliformis* is expected due to the low availability of CO₂ needed for photosynthesis.

positive physiological response at temperatures below 15 °C, therefore adapted to a Subantarctic environment. This emphasizes the importance of knowing the specific optimal physiological temperature of each strain under a scenario of changing ocean (Taucher et al., 2015).

Mardones et al., (2020) showed that *K. selliformis* is poorly adapted to low salinity, nearshore conditions where no *K. selliformis* bloom has been observed yet. However, this species is observed in offshore coastal waters (Fig. 5), where high salinity, low temperature, high nutrient concentrations, and low pH and CO₂ (Galán et al., 2021) could stimulate its growth. These oceanographic conditions are often driven by the upwelling deep water favored by the weather patterns of winds in late spring and early summer (Narváez et al., 2019). Finally, more frequent cold events in Patagonia in the last decade (Mora-Soto et al., 2022) could be viewed as providing a beneficial habitat of cold oceanic waters and could have important consequences on coastal phytoplankton structure.

Historical and projected changes in the world's eastern boundary upwelling systems show a trend toward upwelling intensification in poleward regions. This condition might lead to: 1) mitigation of warming in nearshore regions; 2) stratification of the water column, and 3) a shallow mixed layer. These conditions can reduce genetic variation in populations and favor species with tolerance to low temperatures and low pH (Gaitán-Espitia et al., 2017; Bograd et al., 2023).

In a scenario of warming and acidification of the oceans in northern Patagonia, the appearance of algal blooms of the species *K. selliformis*

(CREAN_KS02) will be less frequent according to our results, considering the low growth rate at 17 °C with low pH. These are contradictory results in comparison with other species of harmful microalgae that have shown favorable growth in these scenarios (i.e. *Alexandrium catenella* (Mardones et al., 2017; Seto et al., 2019), *Amphidinium carterae* (Seto et al., 2019; Bausch et al., 2019; Li et al., 2020), *Akashiwo sanguinea* (Chen et al., 2015; Ou et al., 2017), *Karenia brevis* (Errera et al., 2014; Berceel and Kranz, 2019; Table 3).

Effects of increased acidification caused by thermal stress in a species of symbiotic coral dinoflagellate (*Symbiodinium* sp.) included changes in the uptake and assimilation of carbon concentration and alteration of the photoprotective mechanisms, annulling adaptation to acidification (Anthony et al., 2008). It has also been shown that decreases in pH may affect cellular processes depending on proton pumps, such as the absorption of nutrients essential for growth, and generate oxidative stress (Beardall and Raven, 2004; Giordano et al., 2005; Suffrian et al., 2011; Wells et al., 2015; Bautista-Chamizo et al., 2019). Although this study showed a positive response of the growth of *K. selliformis* in a range of lower pH which occurs naturally in the Patagonia coastal system, future studies should consider the effects of the interaction of pH and inorganic nutrients (nitrate, ammonium, orthophosphate), since HABs may be associated with events of advection of deep waters (Fig. 5) or continuous input of loads of nitrogen and phosphorus from aquaculture activity (Soto and Norambuena, 2004; Iriarte et al., 2005, 2007, Olsen et al.,

2014, 2017).

4. Conclusions

This study showed the interactive effects of temperature and pH in the maximum cell density and growth rate of the Chilean *K. selliformis* strain obtained from the northern section of Patagonia. The main result indicates the maximum density and growth rate for the strain of *K. selliformis* were obtained under the combined effect of the temperature of 12 °C (typical for Patagonian waters) with a lower pH range (7.0–7.7). The environmental conditions observed in the coastal system of Patagonia, low temperature (currently 12 °C, this low temperature is typical of autumn and spring in the Patagonian system, not in the period (summer) where *K. selliformis* blooms are more recurrent) and acidification (current and future scenarios) would be favorable for the development of blooms of *K. selliformis*, given by its natural plasticity in the pH range of 7.57 to 8.24 and temperature values from 10 to 13 °C recorded seasonally in Patagonia. In contrast, the temperature of 17 °C combined with low pH (7.0–7.7), which simulate the conditions of future warming of coastal waters, produced a negative effect on the maximum cell density and growth rate of *K. selliformis*. Based on the marked seasonal variability in pH and temperature along the coast of Chile and the *K. selliformis* strain behavior observed in this experiment, we hypothesize an increase in the occurrence of *K. selliformis* during summer and autumn months in the coastal zone from 37 to 47°S.

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

The authors do not have permission to share data.

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