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Research Article

Role of *Oxyrrhis marina* as a key predator in regulating phytoplankton dynamics during red tide events of 2018-2019 in the San Jorge Bay, Antofagasta, Chile

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ABSTRACT. *Oxyrrhis marina* has been reported as an active predator in marine ecosystems, leading us to hypothesize that its grazing activity may impact the dynamics of species during red tide (RT) events in the San Jorge Bay (SJB). We conducted a study on developing one of the largest RT events observed in the SJB between June 2018 and May 2019. We isolated and identified the abundant microorganisms present during the bloom using microscopy techniques. Additionally, we evaluated the grazing activity of *O. marina* as a predator on phytoplankton species previously isolated from the SJB. Controlled laboratory experiments were conducted with *O. marina* and six different prey species at a concentration of 1×10^5 cells mL^{-1} (1:1) under controlled photoperiod, irradiance, and temperature conditions. The results revealed that the most abundant species during the RT event were *Prorocentrum triestinum*, *P. micans*, and *O. marina*. Regarding grazing preference, after 72 h, *O. marina* exhibited a significantly higher consumption rate of *P. triestinum* (19 cells mL^{-1}) compared to the less consumed *P. micans* (8,627 cells mL^{-1}). The observed strong preference of *O. marina* for *P. triestinum* suggests its potential role as a key controller of phytoplankton upwelling during RT periods. This study suggests that the diversity of microorganisms during red tide periods in SJB, Antofagasta, would be regulated by physicochemical and biological factors.

Keywords: *Oxyrrhis marina*; microalgae, dinoflagellate; diatoms; red tide; grazing; northern Chile

INTRODUCTION

Heterotrophic flagellate protists play an important ecological role in marine planktonic microbial communities, channeling a large proportion of primary carbon production to larger organisms (Bachy et al. 2022, Meira et al. 2022, Villanueva et al. 2022). These microorganisms are the dominant component of the plankton in terms of abundance and biomass (Bachy et al. 2022), demonstrating an enormous morphological diversity (Wisecaver & Hackett 2011). The dinoflagellate *Oxyrrhis marina* is widely distributed in coastal environments. Some research has shown that these dinoflagellates dominate grazing activities in aquatic microbial food webs (Sherr & Sherr 2002, 2007, Jung et al. 2021) and are primarily responsible for the decline of phytoplankton in marine ecosystems (Tillman & Reckermann 2002, Jeong et al. 2003, Davidson et al. 2005, Martel 2006, Abbate 2022), which is consumed daily between 60-70% (Calbet & Landry 2004). *O. marina* has shown remarkable versatility in its prey preferences (Martel 2009, Roberts et al. 2011, Meunier et al. 2012), being able to feed on bacteria (<1 µm), small microalgae (2-4 µm) (Hansen et al. 1996, Jeong et al. 2008, Rodríguez-Martínez et al. 2022), flagellates (4-17 µm) (Jeong et al. 2001, 2003, 2007a,b, Martin-Cereceda et al. 2008, Benico et al. 2019) and can feed on protist species as large as itself, such as *Cricosphaera elongata* (20-30 µm) (Droop 1966, Dodge & Crawford 1971). In this regard, studies have been carried out on the grazing of *O. marina*. For example, Deore et al. (2022) identified 24 exometabo-

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lites, suggesting that they may be associated with signs or indicators of disturbance due to the grazing of *Dunaliella tertiolecta* (prey) mediated by *O. marina* (predator). Poulson-Ellestad et al. (2016) reported a similar trend in which grazing of *O. marina* on *Emiliania huxleyi* increased with the accumulation of nine metabolites. On the other hand, dinoflagellates produce toxins such as pectenotoxins that are found as a minor fraction associated with okadaic acid or its derivatives, so their effect alone as producers of diarrheal symptoms in humans is unknown. The yessotoxin is a low-toxicity toxin. Therefore, its real inclusion among the biotoxins that cause diarrheal intoxication syndrome is discussed (Burgess & Shaw 2001, James et al. 2002, Krys & Fremy 2002). However, these toxins could be a factor that influences the increase or decrease of some microorganisms that are affected or favored by their presence.

San Jorge Bay (SJB), located in Antofagasta, Chile, has a semi-closed and open orientation towards the south with a depth that reaches 160-190 m and is exposed to winds (south and southwest) that are the main forcing of its circulation (Escribano & Hidalgo 2001, Escribano et al. 2002). On the other hand, its internal waters are characterized by presenting temperature records between 2 to 3°C higher than the waters in the external sector of the bay (Piñones et al. 2007). Its waters are retained through a cyclonic gyre (Escribano & Hidalgo 2001, Piñones et al. 2007), favoring an increase in temperature due to the high solar radiation characteristic of these latitudes (Stewart 2003) and a greater daily fluctuation of this parameter (Kaplan et al. 2003). On the other hand, the dynamics of the Humboldt Current System would influence the circulation pattern of the bay on primary productivity, including persistent upwelling fronts and sporadic "El Niño" events (Escribano & Hidalgo 2001). This phenomenon significantly influences the dominance and proliferation of specific microorganisms throughout the year (Stewart 2003). The particularities of the SJB generate special interest in studying the microorganisms associated with it, mainly in periods of red tides (RT).

Considering this background, this study assessed the abundance of microorganisms and the predominant genera found during the 2018-2019 RT event in SJB, located in northern Chile's Antofagasta region. Additionally, we aimed to investigate the grazing activity of *O. marina* on the microalgae species present in the bay through laboratory experiments. The primary objective was to elucidate whether the grazing behavior of *O. marina* is linked to the abundance of microorganisms responsible for RT occurrences.

MATERIALS AND METHODS

Sampling

The samples were collected monthly (12 months) between the years 2018-2019 in three different intertidal points from the SJB at the coordinates: St1 (23°42'09.9"S-70°25'29.3"W), St2 (23°42'06.4"S-70°25"28.2"W) and St3 (23°42'00.8"S-70°25'26.4"W) (Fig. 1). The samples were taken in triplicate in previously sterilized 250 mL Schott glass bottles, then the samples were taken to the laboratory of the Center for Bioinnovation (CBIA, by its Spanish acronym) of the University of Antofagasta for analysis.

Relative abundance

The cells were counted through a Utermöhl chamber (435025; W. Reichmann), with a 100 mL column for 24 h, using 0.01% formalin to decant the ciliated organisms. The microorganisms were observed using an inverted Olympus IX71 microscope with 4x/10x magnification.

Isolation and maintenance of microorganisms

Microorganisms were isolated by capillary pipetting (Hoshaw & Rosowski 1973) with an Olympus IX71 inverted microscope. Isolated cells were cultured in 12-well microplates (CLS3595; Merck) with 3 mL of modified F/2 (designated MESO-MR1). The composition per liter of culture medium was: $NaNO_3$ 900 µM; $NaH_2PO_4 \times 2H_2O$ 40 µM; $NaHCO_3$ µM; $ZnSO_4 \times 7H_2O$ 245 nM; $CoSO_4 \times 7H_2O$ 5.69 nM; $MnSO_4 \times 4H_2O$ 2.42 µM; $Na_2MoO_4 \times 2H_2O$ 6.1 nM; Na_2SeO_3 1 nM; $NiCl_2 \times 6H_2O$ 6.3 nM; $Na_2EDTA \times 2H_2O$ 8.29 µM; $FeCl_3 \times 6H_2O$ 6.56 µM; $C_{63}H_{88}CoN_{14}O_{14}P$ 1.47 nM; $C_{12}H_{17}N_4OS$ 297 nM; $C_{10}H_{16}N_2O_3S$ 4.09 nM; HBO_3 1.86 M $NH_2C(CH_2OH)_3$ 250 mM; $Na_2SiO_3 \times 9H_2O$ 120 µM.

The plates were kept at 18°C with a photoperiod of 12:12 h light:dark and irradiance between 30-60 µmoL m^{-2} s^{-1}. After 15 days, the initial cultures were transferred to 250 and 500 mL cell culture dishes and maintained with medium renewal fortnightly. In addition, to be used as prey in the experimental tests, strains of green microalgae and diatoms obtained from the collection of laboratory strains were used, which were *Tetraselmis marina*, *Tetraselmis* sp., *Phaeodactylum tricornutum*, and *Navicula* sp.

Registration of images of prey and predator

The ZOE BIO-RAD fluorescent cell imager recorded the images of the experimental tests, and the samples were fixed with 0.01% formalin. Prey and predator

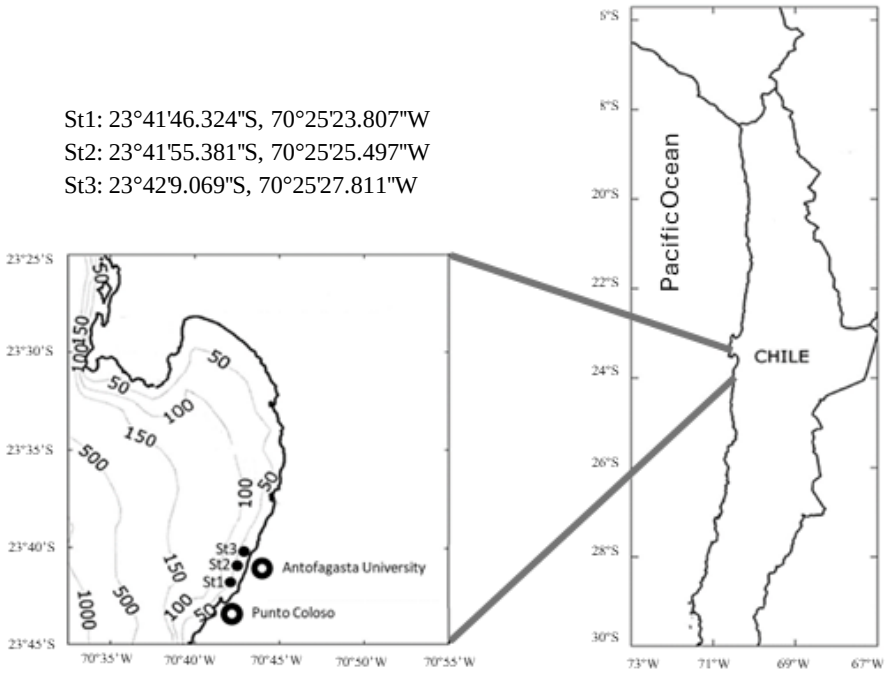

Figure 1. Map of San Jorge Bay showing the locations of the three sampling stations (●): St1, St2, and St3 during a sampling year.

were observed under a fluorescent microscope by confocal laser scanning (Leica TCS SP8, Germany) using a 40x 1.4 NA Zeiss Plan Apochromat objective lens LAS X software (Leica Application Suite for Advanced Fluorescence).

Grazing activity assay

The dinoflagellate *O. marina* was used as a predator, whose cultures were centrifuged at 1,500 rpm for 10 min and acclimatized in autoclaved filtered seawater at 0.2 µm for five days in 75 mL flasks. The predator did not feed for two weeks before the experience.

T. marina, *Tetraselmis* sp., *P. tricornutum*, *Navicula* sp., *P. triestinum*, and *P. micans* prey were used in the exponential phase, concentrated by centrifugation, and resuspended in filtered seawater. Flasks with 250 mL of culture medium were used to evaluate grazing activity. Each prey was placed separately plus the predator *O. marina* in triplicate at a concentration of 1×10^5 cells mL^{-1} (1:1), with photoperiod 12:12 h light:dark, irradiance between 30-60 µmol m^{-2} s^{-1}, and the flasks were incubated at 18°C.

Cell concentration was assessed at 72 h using a flow cytometer (FCM, (Jazz BD FACS standard cell sorter/BD Biosciences, San Jose, CA) equipped with an argon ion excitation laser (488 nm), detectors forward (FS) and lateral (SS) light scattering and four fluorescence detectors corresponding to four different wavelength intervals: 530 ± 40, 585 ± 29, 692 ± 40 and >750 nm. Detector 692/40 was used (excited at 488) to detect predator/prey cell populations in the dot plots based on their forward scatter (FSC) (relative size).

Statistical analyzes

Data are expressed as mean ± standard deviation and compared by one-way ANOVA analysis of variance. The Student's *t*-test was used to analyze the single comparison. Statistical significance was established at $P \leq 0.05$ using GraphPad Prism version 5.01 software.

RESULTS

Relative abundance

The sampled dinoflagellate community exhibited a remarkable morphological diversity, displaying a significant degree of heterogeneity across various genera, including *Prorocentrum*, *Ceratium*, *Dinophysis*, *Oxyphysis*, *Oxyrrhis*, and *Scripsiella* (Fig. 2). These genera demonstrated varying degrees of abundance throughout the different months surveyed. Notably, the genus *Prorocentrum* dominated the sampled period, displaying the highest abundance in 8 out of the 12 months analyzed. The specific percentage abundances recorded were as follows: August (36%), September

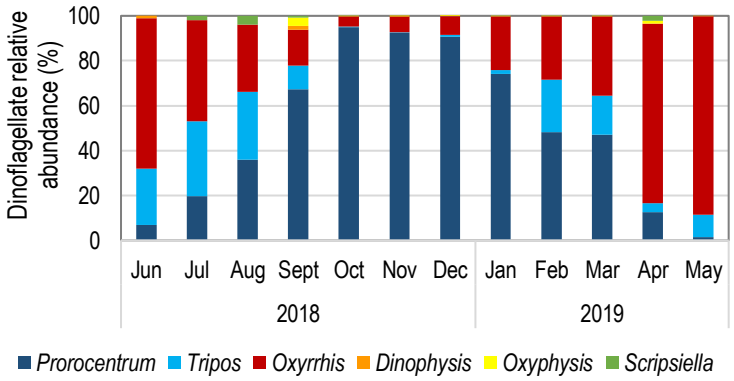

Figure 2. Relative abundance of dinoflagellates was assessed monthly during a sampling year. The genus *Prorocentrum* was the most abundant during the sampled period, followed by the genus *Oxyrrhis*, the genus *Tripos*, and, to a lesser extent, the genus *Dinophysis*, *Oxyphysis*, and *Scripsiella*.

(67%), October (95%), November (92%), December (91%) in the year 2018, and January (74%), February (48%), and March (47%) in the year 2019. Among the species identified via bright field optical microscopy, *P. triestinum* and *P. micans* were the most frequently observed, with the latter species exhibiting the highest abundance (Fig. 4). The genus *Oxyrrhis* was the second group that presented more abundance in 4 of the 12 months sampled. The highest percentages were in June at 67%, July at 45% in 2018, April at 80%, and May at 88% in 2019 (Fig. 2). The species identified in greater abundance was *O. marina*.

Dinoflagellates of the genus *Tripos* were the third most abundant group. However, they were not dominant in any of the months sampled. The months that presented the highest abundance percentage were June, 25%, and July, 33% of 2018. The species identified by microscopy were *Tripos balechi*, *T. furca*, *T. tripos* and *T. fusus*. Finally, the dinoflagellates observed in lower abundance and only in a few months were from the genus *Dinophysis*, *Oxiphysis*, and *Scripsiella* (Fig. 2).

Oxyrrhis initially exhibited a low concentration during the fifth month of the phytoplankton bloom onset (October 2018). However, starting from the sixth month (November 2018), *Oxyrrhis* concentration showed a notable increase, coinciding with a simultaneous decline in the abundance of the genus *Prorocentrum* (Fig. 2). To investigate the dynamics between these two dominant genera, a correlation coefficient analysis was conducted, revealing a strong inversely proportional linear correlation between *Oxyrrhis* and *Prorocentrum* (r = -0.997), which indicates that as the concentration of *Oxyrrhis* increased, the abundance of *Prorocentrum* decreased throughout the event (Fig. 3).

Isolated microorganisms

Three different species of dinoflagellates recognized for their morphological characteristics and identified as *O. marina*, *P. triestinum*, and *P. micans* were isolated. The isolated strains were kept in flasks with modified F/2 (MESO-MR1) and incubated in a room heated at 18°C with a photoperiod of 12:12 h light:dark and irradiance between 30-60 µmol m^{-2} s^{-1}.

Grazing activity

Prey and predator images were recorded to obtain the length and width of the cells used in the grazing test. Images were taken using Leica TCS SP8 confocal microscopy, 40x objective, and LAS X software. Scale bar = 25 µm. In addition, the autofluorescent activity (natural intracellular pigments) of the microorganisms under study was observed: green for rhodopsin in *O. marina*, red for chlorophyll in microalgae, diatoms, and dinoflagellates (Fig. 5).

In the cytometric analysis evaluated, the ingestion or preference of the predator *O. marina* in the consumption of food of different phytoplankton prey classified as Chlorophyta (*Tetraselmis* sp. and *T. marina*), diatoms (*Navicula* sp. and *P. tricornutum*) and dinoflagellates (*P. micans* and *P. triestinum*). As controls, the separate recording of each prey and predator at 0 h (beginning of the experiment) was considered. The emitted autofluorescence was used to visualize them in the dot plot without applying fluorescent probes. The prey shows greater fluorescence through the 692/40 (488) detector, and the

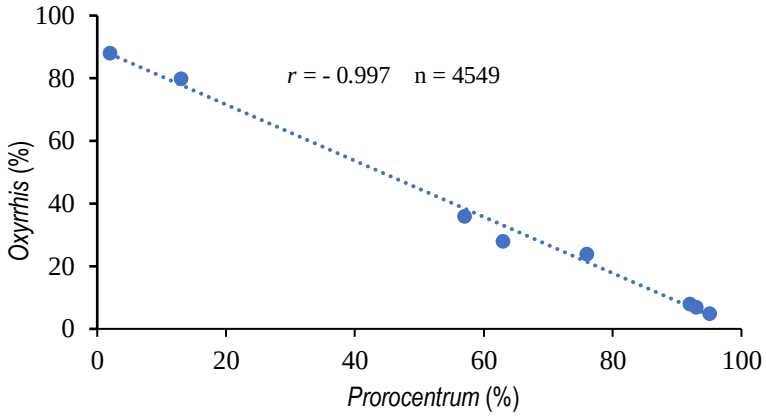

Figure 3. Correlation coefficient analysis showed that when the proportion of *O. marina* increased, an inversely proportional trend was observed, decreasing the concentration of dinoflagellates of genus *Prorocentrum* during the phytoplanktonic bloom between October 2018 and May 2019. Correlation coefficient (r) and total number of microorganisms (*Prorocentrum* + *Oxyrrhis*) used in the analysis (n).

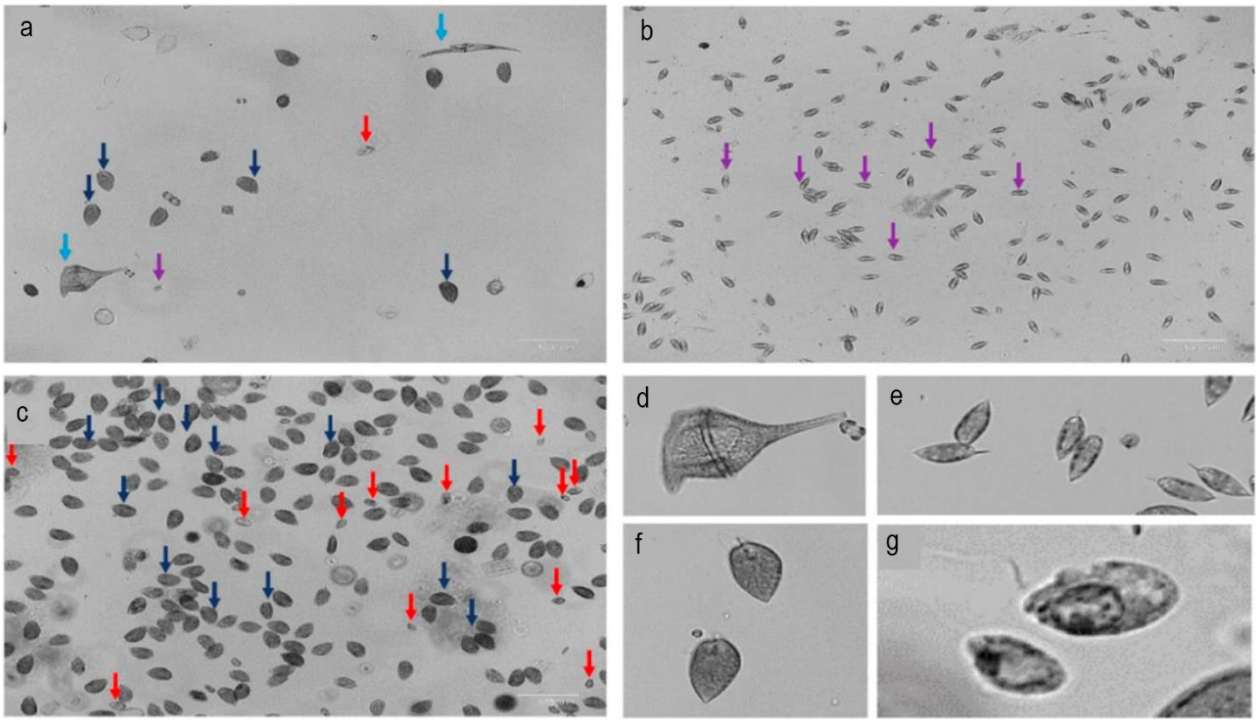

Figure 4. Dinoflagellates observed during a) October, b) November, c) December, related to relative abundance, d) *Tripos* sp. (light blue arrow in photo a), e) *P. triestinum* (purple arrow in photo b), f) *P. micans* (blue arrow in photo a), and g) *O. marina* (red arrow in photo c). Cells were recorded within a bright field using the ZOE Fluorescent Cell Imager.

predator *O. marina* shows greater volume and cell size through the forward scattering of light (FSC) (Fig. 6).

The cloud of events obtained in the controls of each prey and predator was compared with the treatments (predator + prey). The results at 72 h showed that the predator *O. marina* could reduce the abundance of prey in the treatments in different proportions (Fig. 6 blue events), reflecting a higher or lower consumption depending on the prey species.

At the beginning of the experience, the predator had two weeks of fasting. After 72 h, different displacements of predator events were recorded, identifying four different populations (based on the position of the events in the diagram), which we defined as PRF

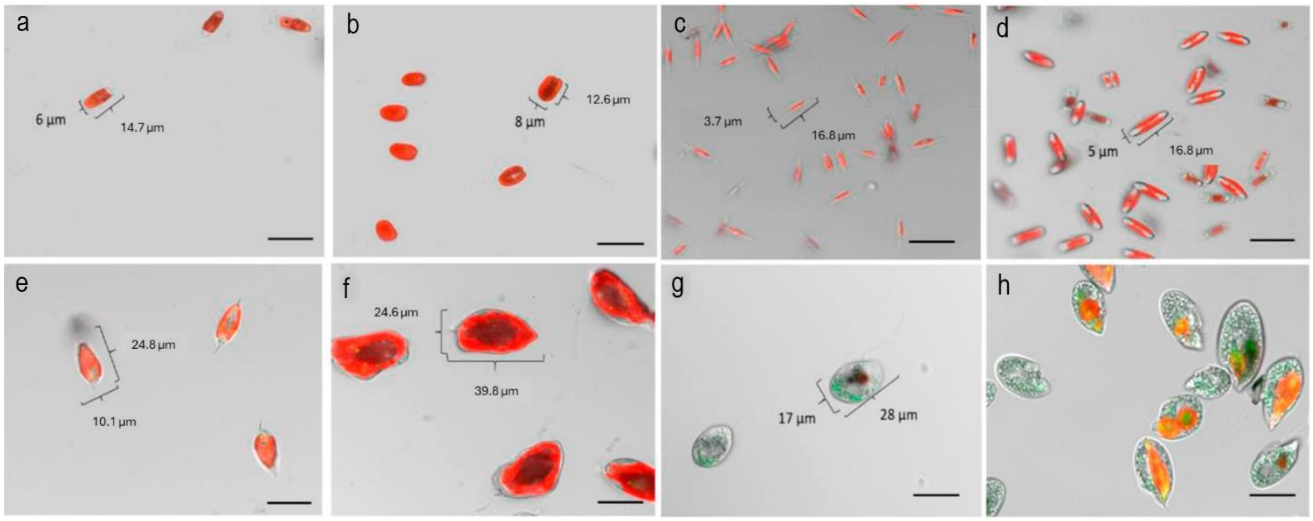

Figure 5. Light microscopy images of vegetative cells, including the length and width of the cells used in the predation assay. a) *Tetraselmis marina*, b) *Tetraselmis* sp., c) *Navicula* sp., d) *P. tricornutum*, e) *P. triestinum*, f) *P. micans*, g) *Oxyrrhis marina* and h) grazing activity of *O. marina* (green cell) on *P. triestinum* (orange cells). Images were taken by Leica TCS SP8 confocal microscopy, using a 40x 1.4 NA Zeiss Plan Apochromat objective lens, LAS X software. Scale bar = 25 µm.

(predator ready to feed, green color), PF (predator feeding, purple color), JFP (just fed predator, red color) and P (prey, blue color) . The treatments RPF, PF, and JFP groups are related to the predator *O. marina*. When comparing their position or location with that in the event diagram of the controls, we infer that the different observed intensities of the predator in the diagram are related to the predator *O. marina*. When comparing their position or location with the event diagram of the controls, we infer that the different intensities observed in the diagram are related to the feeding stage of the predator *O. marina* (Fig. 6a).

Of all the prey species evaluated, after 72 h, a greater preference for consumption was observed first for *P. triestinum* (Fig. 6a) and secondly for *Navicula* sp. (Fig. 6b), showing in both that after this time, the prey is once again in the PRF state, that is, ready to receive more food. The third most preferred prey was *T. marina* (Fig. 6c) and, in fourth place, *Tetraselmis* sp. (Fig. 6d). In both, it was observed that the predator was found at the time of registration in the PRF, PF, and JFP phases. The fifth prey, *P. tricornutum*, showed that the predator was in the JFP phase, that is, recently fed (Fig. 6e; red color). However, the events of the prey P show that these did not decrease, indicating no greater prey consumption (Fig. 6e; blue color). The sixth prey, *P. micans* (Fig. 6f), revealed a higher intensity of events in the PRF/PF phases and a lower intensity in the JFP phase. As in the previous prey, *P. micans* reflected a high intensity of prey events (Fig. 6f; blue color), which would indicate less preference.

To better understand the cells consumed by the predator *O. marina* in the cytometric analysis at 72 h of treatment, the cells that remained in the samples after consumption and converted into cells mL^{-1} were analyzed. The data obtained in cells mL^{-1} of the prey obtained after 72 h of treatment are displayed in Table 1.

DISCUSSION

This study comprehensively assessed the morphological diversity of dinoflagellates present in the analyzed samples. The predominant species identified were *P. triestinum* and *P. micans*, followed by *O. marina*. The species *Tripos balechi*, *T. furca*, *T. tripos*, and *T. fusus* were also observed, albeit in lower abundance. Lastly, the genera *Dinophysis*, *Oxiphysis*, and *Scripsiella* were identified, but with relatively limited representation. Species identification was performed qualitatively through microscopy, comparing the obtained images and characteristics with the information available in Tomas (1997). Avalos et al. (2019), in their study conducted in the same period and location, reported the initial expansion of this phenomenon from the coast to the open sea, spanning a distance of 30 km and extending over 600 km along the coastline from the north to the south of Antofagasta in 2018. This bloom further expanded in 2019, reaching a

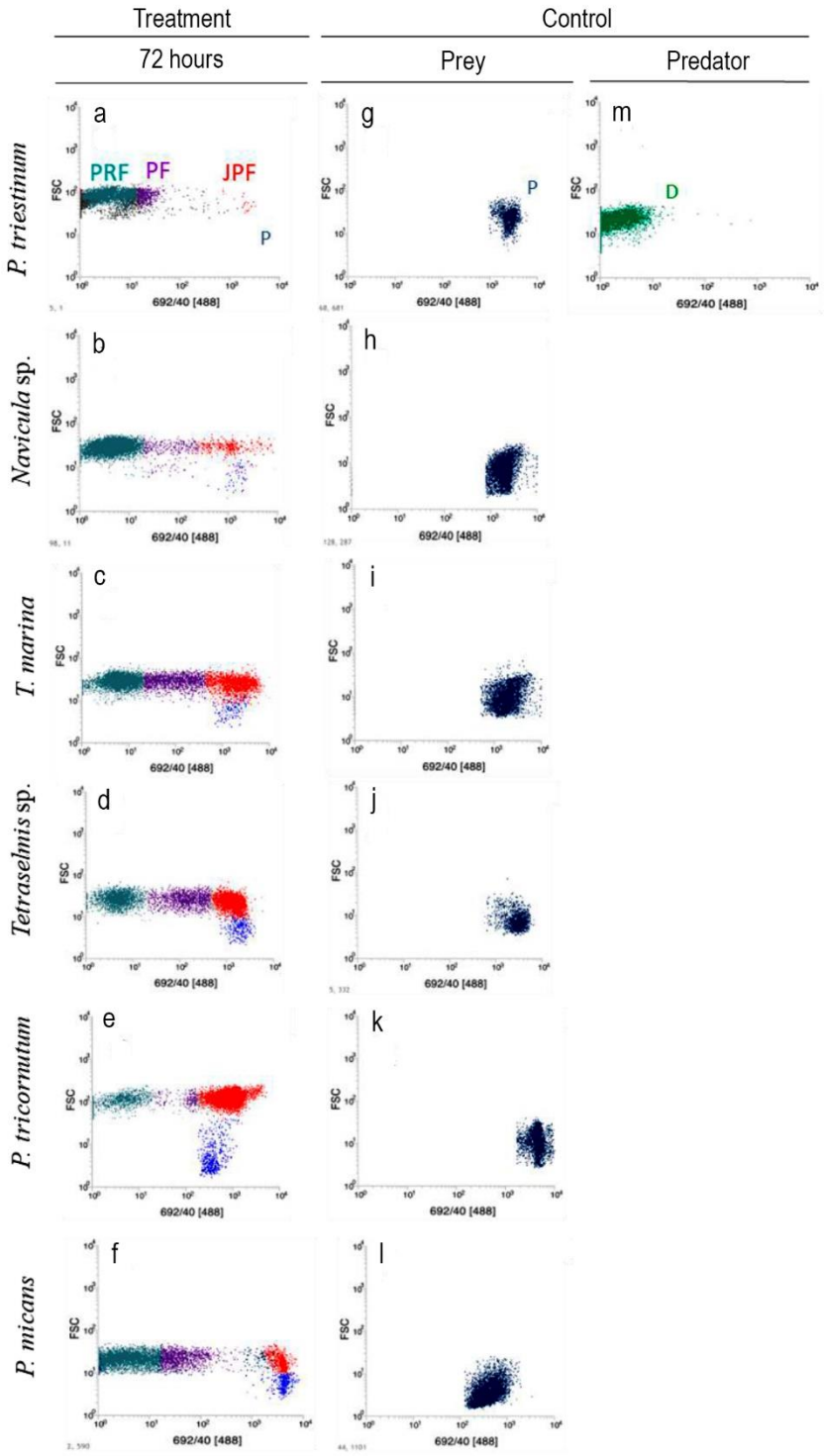

Figure 6. Grazing activity of *Oxyrrhis marina* on different prey during 72 h. PRF: predator ready to feed (green color); PF: predator ready to feed (green color); JFP: just fed predator (red color) and blue color for prey cells. a) *Tetraselmis marina*, b) *Tetraselmis* sp., c) *Phaeodactylum tricornutum*, d) *Navicula* sp., e) *Prorocentrum triestinum*, f) *Procentrum micans*, g-l) preys, m) control *O. marina*. The forward scatter (FSC) signal can be employed for the discrimination of cells based on size.

Table 1. Prey preferences recorded after 72 h of treatment ± standard deviation. The number of cells counted indicates what remained in the sample after consumption of *O. marina*, the lower the value shows greater preference.

Species	Cells mL^{-1}
Prorocentrum triestinum	19 ± 2
Navicula sp.	25 ± 27
Tetraselmis marina	257 ± 75
Tetraselmis sp.	319 ± 61
Phaeodactylum tricornutum	1167 ± 370
Prorocentrum micans	8627 ± 77

length of 1,500 km and extending to the coast of Peru near the city of Arequipa. Chlorophyll-*a* data obtained from NASA OceanColor Web (https://oceancolor.gsfc.nasa.gov/) by these authors indicated values above 10 mg m^{-3} until the beginning of March 2019, classifying it as one of the largest and longest RT events in the history of Chile.

Mackey et al. (2012) discussed the role of Monterey Bay in California, USA, as an incubator for characteristic RT microorganisms, such as dinoflagellates belonging to the genera *Prorocentrum*, *Ceratium*, *Dinophysis*, *Alexandrium*, and *Scripsiella*. Similarly, in both Monterey Bay and SJB, an abundance of dinoflagellate genera was observed, highlighting the ecological similarities and potential incubatory characteristics shared between these bays.

Using relative abundance analysis, we identified a temporal alternation in the presence of microorganisms, specifically *Prorocentrum* and *Oxyrrhis*, which were most abundant between June 2018 and May 2019. *Prorocentrum* exhibited the highest abundance compared to *Oxyrrhis* from September 2018 to January 2019. It could be attributed to several factors, including environmental conditions such as higher water temperatures during this period and nutrient upwellings in the Bay that favored the development of *Prorocentrum*. Additionally, increased toxins associated with these species may have controlled the growth of other phytoplankton microorganisms. Furthermore, optimal conditions influenced the predation dynamics of *O. marina* against *Prorocentrum*.

On the other hand, correlation coefficient analysis conducted between the *Oxyrrhis* and *Prorocentrum* genera revealed a strong correlation (R = -0.997) between these variables. These findings suggest that during RT events, the abundance of *Oxyrrhis* and *Prorocentrum* genera may be mutually controlled through grazing in conjunction with other physicochemical variables, which indicates that the presence and relative abundance of either *Oxyrrhis* or *Prorocentrum* during the RT event are influenced by these factors, ultimately determining their population dynamics throughout the event's duration. Various microalgae species produce lipophilic toxins accumulated by bivalve filter feeders, posing a danger to food safety and human health. Shellfish exploitation is determined by the toxic potential of local strains and the biotransformations of toxins by the exploited bivalve species (Díaz et al. 2022). In this regard, Avalos et al. (2019) carried out toxin analyses at five points of the SJB during 2018-2019; in all the samples, dominated *Prorocentrum* sp., and the least observed genera were *Ceratium*, *Peridinium*, *Dinophysis*, *Protoperidinium*, *Gymnodinium*, and others. All the samples contained average values of PTX1 (pectenotoxin^{-1}, 0.001 pg cell^{-1}), PTX2 (pectenotoxin^{-2}, 0.006 pg cell^{-1}), and YTX (yesotoxin, 0.002 pg cell^{-1}). Studies carried out in four different sites of the SJB Antofagasta during the years 2019-2020 by De los Rios-Escalante et al. (2022) found a high abundance of *Dinophysis* in February 2019. A second group, which combines four sites sampled in February 2019, presented a high abundance of *Gymnodinium* and *Ceratium* and a high abundance of *Prorocentrum* in 2020. The results of these authors show us that the variation in the abundance of the microorganisms present in the bay could depend on the physical-chemical conditions the microorganism faces. During the summer of 2007, Álvarez et al. (2011) identified the dinoflagellate *P. reticulatum* (Dinophyceae) in an RT event that occurred in Bahía Mejillones (23º10'S, 70º45'W), located north of Antofagasta. Yessotoxin was the only toxin present, with concentrations ranging from 0.2 to 0.4 pg cell^{-1}. Another study by Díaz et al. (2022) in Chile, focusing on lipophilic toxins, revealed the presence of species such as *Dinophysis acuminata* and *P. reticulatum* in coastal waters of all regions of Chile. These species were found to be frequent and very abundant producers of toxins. However, unlike most European strains, these Chilean strains were rich in pectenotoxins and lacked okadaic acid (OA), which has implications for human health. Additionally, *Dinophysis acuta*, suspected to be the primary cause of diarrheal shellfish poisoning outbreaks, was found predominantly in the southern regions of Chile. *P. triestinum* and *P. micans* were identified in greater abundance at our work site. We reviewed the scientific literature of these species regarding abundance studies and toxicity records in Chile. The information on this topic is very scarce, and most of the information is found in studies carried out in other countries on these species. Therefore, this work would be an important contribution to the knowledge of the abundance and record of the presence of the species identified in the

largest RT event recorded on the coast of Chile. Although *P. micans* was considered a non-toxic species in 1994, the worst marine mortality was recorded off the South African coast with a 30 km stretch of the St. Helena Bay coastline (Matthews & Pitcher 1996) caused by the entrapment of *P. micans*, marine organisms died from suffocation and hydrogen sulfide poisoning (generated by anaerobic bacteria). Similarly, *P. triestinum* generated high mortality in the same bay in 2017 due to significant anoxia (Ndhlovu et al. 2017). The dinoflagellate *O. marina* was our study site's third most abundant species. This species of approximate size between 15-40 µm is widely distributed in coastal environments and has been widely reported as a predator of planktonic protists (Roberts et al. 2011). In our work, we observed an approximate size between 15-30 µm of this species. Martin-Cereceda et al. (2008) observed the predation capacity of *O. marina* on organisms such as *G. amphinema* (5.6 µm), *Chilomonas paramecium* (20 µm), and *Methanophrys* sp. (40 µm), their results indicate that the size of the prey moderated the range of ingestion. Hansen et al. (1996) concluded that *O. marina* selectively grazes larger prey, such as *Rhodomonas* sp. and *Tetraselmis suecica* (>7 µm), relative to smaller species such as *Emiliania huxleyi*, *Isochrysis galbana*, *Micromona pusilla*, and *Nannochloris* sp. (1 to 5 µm). These results indicate the relevance of the size or biovolume of the prey in the predatory activity of *O. marina*.

Flow cytometry has now facilitated the rapid analysis of phytoplankton compared to epifluorescence microscopy, considering that phytoplankton varies in size (0.8 to 200 µm) and pigmentation (variations in chlorophyll-*a*, *b*, *c*; phycoerythrin and phycocyanin), whose properties have favored its discrimination by cytometry (Dubelaar & Jonker 2000). In this work, we study predation through cytometry using *O. marina*, which has been used as a model predator to investigate the selective feeding of different species of prey (Flynn et al. 1996, Hansen et al. 1996, Davidson et al. 2005). Our findings revealed that *O. marina* significantly reduced the concentration of five of the six prey species examined. The order of preference, from highest to lowest, was *P. triestinum*, *Navicula* sp., *T. marina*, *Tetraselmis* sp., *P. tricornutum*, and finally, the least-preyed upon *P. micans*. Our results demonstrated that *O. marina* exhibited a stronger preference for *P. triestinum*. In line with our findings, You et al. (2020) reported, for the first time, that *O. marina* preys on *P. triestinum*, supporting our observations. Therefore, our results confirm their research. Also, our results indicate a preference for *P. triestinum* as it is one of the most consumed species out of the seven evaluated. *P. triestinum* has a cell wall comprising cellulose. The thecal plates are delicate with a smooth surface, with peripheral trichocyst pores and thickened edges present (Bursa 1959, Dodge 1965, 1975, 1982, Toriumi 1980, Hasle et al. 1996). This characteristic may be one of the reasons for the greater preference in the consumption of *P. triestinum* due to its thecal plates concerning the cell wall of green microalgae and the diatom silicate frustule. Roberts et al. (2011) discuss that *O. marina* consumes various prey and has different feeding preferences, but much remains to be learned about the feeding of this planktonic herbivorous protist. *O. marina* has been shown to consume bacteria (<1 µm) to cells (>20 µm), although optimal prey size is smaller than itself (Hansen et al. 1996, Davidson et al. 2011). Finally, besides the nutrition obtained through phagotrophy, *O. marina* can survive by absorbing dissolved organic molecules in the laboratory. This mechanism can be used in saprobic environments (Lowe et al. 2011).

Several studies have investigated the feeding behavior of *O. marina* and its ability to discriminate among prey based on size, biochemical composition, and charge (Hammer et al. 1999, 2001, Wootton et al. 2007). Conducted experiments using artificial pearls and found that *O. marina* had a higher ingestion rate for 4 µm pearls compared to 1 µm pearls. Additionally, it was observed that *O. marina* could discriminate artificial particles with high surface charge, such as carboxylate and silicate beads.

Wootton et al. (2007) discussed the ability of *O. marina* to distinguish between beads with different biochemical surface compositions. They found that *O. marina* significantly preferred mannose-BSA-coated beads over N-acetylgalactosamine-BSA-coated beads. Despite ingesting artificial particles, *O. marina* prefers live prey (Hammer et al. 1999, Wootton et al. 2007). While artificial prey can provide insights into feeding behavior, there are still unidentified cues provided by live prey that induce capture.

Comparing studies on prey selectivity is challenging due to differences in experimental design, initial predator-prey inoculum, measured parameters, and experimental duration. Roberts et al. (2011) stated that determining the prey parameters that drive selective feeding in interspecies prey selectivity studies is difficult. Factors such as prey size, motility, cell surface properties, and the release of dissolved chemical signals (including chemo-attractants, chemo-repellents, and toxins) vary among prey species. Controlling and manipulating these variables in experiments is complex, and currently, it is challenging to interpret how prey

parameters influence the grazing preferences of heterotrophic protists in general.

There is consistent evidence that heterotrophic protists (ciliates and heterotrophic dinoflagellates), rather than copepods, are the main consumers of phytoplankton (Calbet & Landry 2004), including harmful algal blooms (HAB) species in marine systems (Calbet et al. 2003, Kim & Jeong 2004). HAB drivers are complex, often site-specific, and not exclusively linked to nutrient enrichment (Davidson et al. 2014, Michalak 2016). Other factors, such as antagonists, can control the formation of HAB (Almeda et al. 2018). Our results building on this research provide a valuable contribution to comprehending better the factors driving the dynamics of microorganism abundance during RT events along the shores of SJB in Antofagasta, northern Chile. Moreover, our study sheds light on the associated toxins produced by these microorganisms. The knowledge gained from our research will enable further understanding and comparisons of microorganism dynamics in the SJB with those of other coastal regions worldwide.

CONCLUSIONS

The study allowed to identify five genera of potentially toxic dinoflagellates (*Prorocentrum*, *Ceratium*, *Dinophysis*, *Oxyphysis* and *Scrippsiella*) in SJB, Antofagasta, during the 2018-2019 RT event. *Prorocentrum* was the most abundant (8 of the 12 months sampled) with the most frequent species being *P. triestinum* and *P. micans*. It was followed by *Oxyrrhis* (4 of the 12 months sampled) with the species *O. marina*. The analyses revealed a temporal alternation between *Prorocentrum* and *Oxyrrhis* during the sampling period. Furthermore, the results suggest that *Oxyrrhis* and *Prorocentrum* may regulate their density through grazing during red tide events. On the other hand, environmental conditions probably promoted the grazing dynamics of *O. marina* on phytoplankton, with a particular preference for *Prorocentrum*. After 72 h, the feeding behavior of the predator *O. marina* revealed its ingestion and preference, from greatest to least, for *P. triestinum*, *Navicula* sp., *T. marina*, *Tetraselmis* sp., *P. tricornutum* and *P. micans*. The preference for *P. triestinum* suggests *O. marina* as a controller of phytoplankton during the red tide phenomenon.

Credit author contribution

H. Cameron: writing-original draft, conceptualization, validation, methodology; Y. Leyton: writing draft, supervision, review and editing; C. Riquelme: funding acquisition, project administration. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no potential conflict of interest in this manuscript.

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