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Coupling planktonic and benthic shifts during a bloom of *Alexandrium catenella* in southern Chile: Implications for bloom dynamics and recurrence

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ABSTRACT

Cell abundances and distributions of *Alexandrium catenella* resting cysts in recent sediments were studied along time at two locations in the Chilean Inland Sea exposed to different oceanographic conditions: Low Bay, which is much more open to the ocean than the more interior and protected Ovalada Island. The bloom began in interior areas but maximum cyst concentrations were recorded in locations more open to the ocean, at the end of the Moraleda channel. Our results showed a time lapse of around 3 months from the bloom peak (planktonic population) until the number of resting cysts in the sediments reached a maximum. Three months later, less than 10% of the *A. catenella* cysts remained in the sediments. Maximum cyst numbers in the water column occurred one month after the planktonic peak, when no cells were present. The dinoflagellate assemblage at both study sites was dominated by heterotrophic cysts, except during the *A. catenella* bloom. CCA analyses of species composition and environmental factors indicated that the frequency of *A. catenella* blooms was associated with low temperatures, but not with salinity, chlorophyll *a* concentration, and predator presence (measured as clam biomass). However, resting cyst distribution was only related to cell abundance and location. The occurrence of *A. catenella* cysts was also associated with that of cysts from the toxic species *Protoceratium reticulatum*. By shedding light on the ecological requirements of *A. catenella* blooms, our observations support the relevance of encystment as a mechanism of bloom termination and show a very fast depletion of cysts from the sediments (<3 months), which suggest a small role for resting cyst deposits in the recurrence of *A. catenella* blooms in this area.

1. Introduction

Dinoflagellates are best known as the source of dense and often toxic blooms, with species belonging to the genus *Alexandrium* causing serious episodes of paralytic shellfish poisoning (PSP). In South America, PSP has been recognized as an important public health risk for over a century (Hallegraeff, 1993; Balech, 1995; Lagos, 2003).

The sexual life cycle of *Alexandrium* species includes a dormant benthic stage (resting cyst). A role for benthic cysts has been cited in species dispersion, resistance to unfavorable conditions, population resilience through sexual recombination, and bloom onset and termination (Anderson et al., 1982; Anderson, 1984; Garcés et al., 1999; McGillicuddy et al., 2003). Moreover, given that the toxicity of resting cysts can be higher than that of vegetative cells, resting cyst formation must be taken into account when analyzing the possible effects of a bloom on human health (Dale et al., 1978; Lirdwitayaprasit et al., 1990; Oshima et al., 1992). The roles played by resting cysts in the life cycle and ecology of dinoflagellate species can be very different depending on the species. For example, the life cycles and encystment strategies of *Alexandrium* species are both complex and species-specific

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(for e.g. Figueroa et al., 2006, 2008). Thus, the current approach to the study of dinoflagellate ecology is to reconsider previous general assumptions, by specifically investigating each species.

Exogenous (environmental) factors mainly affect the timing and success of germination whereas endogenous (physiological) factors regulate the germination of resting cysts and determine whether this stage is involved in the short- or long-term survival of the species or serves as a bloom maintenance mechanism (see for e.g. the review by Bravo and Figueroa, 2014, and references therein). Local hydrographic and environmental factors greatly affect the strength and timing of sexual induction, and therefore the success of encystment and germination, while hydrodynamic processes determine the location of cyst deposits (Anderson et al., 2005; Keafer et al., 2005; McGillicuddy et al., 2005; He et al., 2008). Sexual events are known to depend on nutrient levels (e.g. Anderson, 1998; Ellegaard et al., 1998; Figueroa et al., 2005) and the photoperiod (Nuzzo and Montresor, 1999; Sgrosso et al., 2001), but also on the mating system of the species (Kremp, 2013) and the genetic composition of the bloom (Dia et al., 2014). Within the genus *Alexandrium*, the dormancy requirements of *Alexandrium tamarense* cysts differ between deep water and shallow coastal environments (Anderson and Keafer, 1987; Matrai et al., 2005). In *Alexandrium catenella*, dormancy periods are highly variable, ranging from 28 to 97 days (Yoshimatsu, 1984; Hallegraeff et al., 1998). In Mediterranean populations of this species, they are characterized by a gradual rather than a synchronous pattern of germination (Figueroa et al., 2006) that allows for rapid cycling between benthic and planktonic stages (Hallegraeff et al., 1998).

Alexandrium catenella (Dale, 1983) is also an important causative agent of PSP in southern Chile, where it was found for the first time in 1972, in the Magallanes region (Guzmán et al., 1975). Since this report, the intensity and expansion of *A. catenella* outbreaks in Chile have increased (Guzmán et al., 2002; Molinet et al., 2003), with detrimental effects on human health and severely limiting economically important aquaculture activity in the region, which is based on shellfish production (Campodónico et al., 1995). The most recent *A. catenella* bloom, at the end of the austral summer (February–March) of 2009, was one of the most significant with respect to geographic extent and cell density, which in the Aysén region reached up to 1.1×10^6 cells L^{-1} in late March. Sadly, this bloom was directly responsible for the death of two people.

In the present work, to gain further insights into bloom formation by this species and the role of its resting cysts on bloom recurrence, we monitored the abundance and distribution of cells and its resting cysts in both the water column and the surface sediments of two different locations in the Chilean Inland Sea: Low Bay, which is open to the ocean, and Ovalada Island, a more interior and protected area. Our results clarify bloom dynamics and add support to the role of encystment as a mechanism of *Alexandrium catenella* bloom termination. In addition, we discuss on the relevance of resting cysts deposits for the recurrence of blooms in the area, as no long-term cysts beds were found.

2. Material and methods

2.1. Study area

Low Bay (43°49′39″ S–73°57′40″ W) and Ovalada Island (44°04′08″ S–73°42′32″ W) are located in the NW portion of the Chilean Inland Sea (Fig. 1). Both sites are characterized by the presence of large natural beds of clams (*Venus antiqua*), with a total biomass estimate of 1300 and 10,000 tons, respectively (Seguel et al., 2011a). Low Bay (Fig. 1C) is much more open to the ocean than the more interiorly located and protected Ovalada Island (Fig. 1D). In general, this system is characterized by an abrupt bathymetry and a complex coastal morphology, both locations strongly shaped by the oceanic water (Pickard, 1971). The salinity gradient in the upper 50 m ranges between 27 and 33 psu and is mainly influenced by the intense seasonal rainfall, which in this region averages about 3000 mm per year (Pickard, 1971; Silva et al., 1995). Semidiurnal tides have amplitudes ranging from 2 m (neap tides) to 4 m (spring tides) (www.shoa.cl).

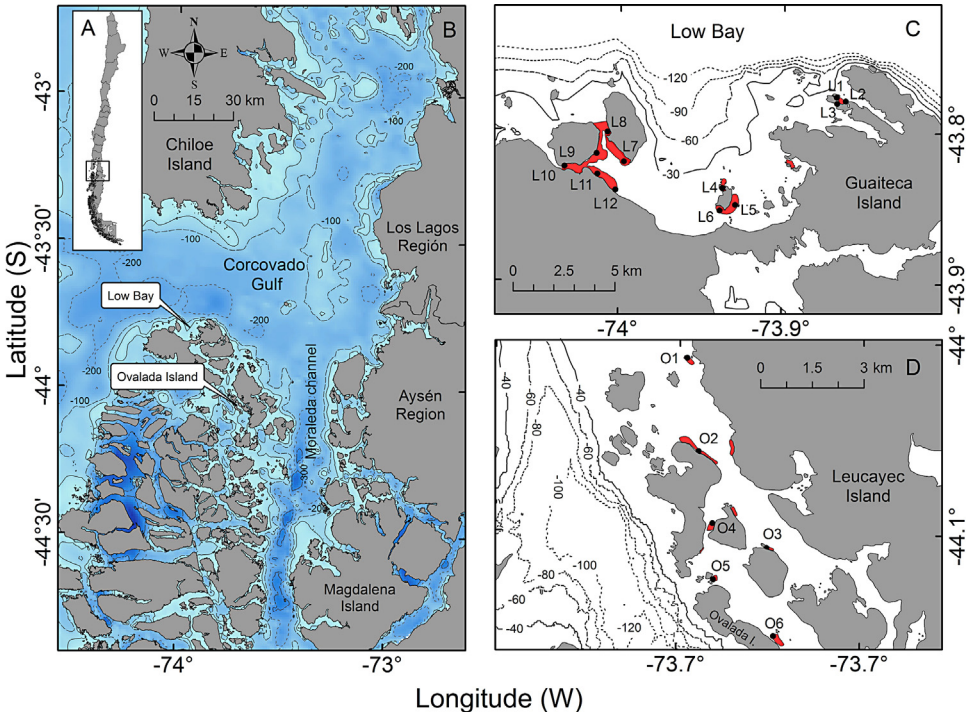

Fig. 1. (A) Study area in southern Chile (*gray rectangle*). (B) The map shows a section of the Chilean Inland Sea, including the location of Low Bay and Ovalada Island. (C) The 12 sampling stations of Low Bay. (D) The six sampling stations at Ovalada Island.

2.2. Field sampling

Between March 2009 and March 2011, surface sediment samples were collected at 12 stations in Low Bay (Fig. 1C) and at six stations of Ovalada Island (Fig. 1D). At each station, two sediment samples were obtained fortnightly from March to August and monthly from September to December. The 414 samples were collected by an autonomous diver using an 8-cm-long corer. The samples were wrapped in aluminum foil and transported to the laboratory. Simultaneously, two water samples for quantitative analyses of potentially harmful microalgae and chlorophyll (chl) *a* measurements were collected close to the sea bottom at each station using a 2.5-L bottle. For quantitative analyses the contents were concentrated through 10-μm Nitex filters (PVC cylindrical collector) and fixed with acidic Lugol's iodine solution for later counting of phytoplankton. The final volume of the concentrate, about 50 mL, was measured to calculate the conversion factor (approximately 50). For Chl *a* and phaeopigments, 200 mL seawater were filtered on Whatman GF/F glass fiber filters in triplicate and immediately frozen (−20 °C) until later analysis by fluorometry, using acetone (90%, v/v) for the pigment extraction (Turner Design TD-700) according to standard procedures (Parsons et al., 1984). Temperature, salinity and depth were recorded at each station using a Star-Oddi DST data loggers.

To evaluate the effects of predation on seed bank cysts, the presence of three species of clams (*Venus antiqua*, *Semele solida*, and *Tawera gayi*) was recorded. Both the density and the biomass of the clams were recorded at each sampling station using six 0.25-m^2 quadrates.

Monthly reports of phytoplankton distribution in the Aysen region (southern Chile) from December 2008 to May 2009 were obtained from the Chilean Monitoring Program at the Instituto de Fomento Pesquero (IFOP) (Guzmán et al., 2010). Plankton samples for quantitative analyses were collected using a dividible hose sampler (Lindahl, 1986) from 0 to 20 m (0–10, 10–20 m), and immediately fixed on board with acidic Lugol's iodine solution (Lovegrove, 1960).

2.3. Cysts and cells counting

The first 3 cm of the cores were transferred to 80-mL flasks and stored at 4 °C until analyzed. The sediments were cleaned and sonicated following the technique described by Matsuoka and Fukuyo (2000). Dinoflagellate cysts were quantified using an Olympus BX40 phase-contrast microscope and a 1-mL Sedgewick-Rafter counting chamber. Three aliquots of each sample were counted. The examined cysts were isolated and photographed using a digital camera Olympus Camedia C-3040-ADU coupled to the microscope (100× magnification).

Quantitative phytoplankton analyses were carried out according to the Uthermöhl method (Uthermöhl, 1958). Samples were sedimented for 12 h in 10-mL columns and then counted at 100× magnification using an inverted light microscope and phase-contrast optics.

2.4. Drifter experiments

Surface drifter experiments were carried out in Ovalada Island and Low Bay, on January 30 and April 5, 2010 respectively, in order to obtain direct measurements of the surface circulation in the area. Progressions of surface drifter trajectories were registered with GPS data-logger every 2 min during a 12-h. Drifter trajectories and surface velocities observed in both areas can be found in the supplementary data (S1–S4).

2.5. Statistical analysis

A two-way hierarchical clustering analysis (HCA) was used to classify the sampling stations with respect to their dinoflagellate cyst assemblages. The HCA was performed on a Euclidean matrix of distances (Legendre and Legendre, 1998) using Ward's linkage clustering method (Ward, 1963). To normalize the distribution and eliminate zero values, the data were first transformed [LN(x + 1)]. To validate the clusters, the silhouette index (Rousseeuw, 1987) was applied:

$$s(i)\frac{b(i)-a(i)}{\max\{a(i),b(i)\}}$$

where $a(i)$ is the average dissimilarity of the i-object to all other objects in the same cluster and $b(i)$ is the minimum average dissimilarity of the i-object to all objects in another cluster (the closest cluster). The silhouette index provides a quantitative and graphical approach to determine the optimal number of clusters. A quantitative measure $s(i)$, known as the "width silhouette," is assigned to each object of the cluster. The width silhouette varies as $-1 \leq s(i) \leq 1$ and indicates the correspondence of one object i with the cluster to which it was assigned. An $s(i)$ value close to 1 indicates that the object i is well classified. An $s(i)$ value near 0 indicates an "intermediate case" in which it is not clear whether the object has been well classified. When $s(i)$ is close to −1, the object has been misclassified (Rousseeuw, 1987).

Canonical correspondence analysis (CCA) was used to evaluate the structure of the dinoflagellate cysts assemblage during the survey and its relationship with environmental variables (temperature, salinity, Chl *a*, pheopigments, and clam biomass). The data were first normalized using a logarithmic transformation [LN(x + 1)] and then organized in a biological matrix that included the abundances of the 28 taxa to which the dinoflagellates cysts had been assigned and one explanatory matrix that included the five environmental variables recorded. A Monte Carlo test was then applied in which 499 permutations were considered in order to determine the significance of both matrices and the canonical axes.

A generalized linear mixed model (GLMM) with logit-link function for negative binomial distribution (McCullagh and Nelder, 1989), was implemented to identify the influence of different environmental factors on *Alexandrium catenella* cysts abundance, using as predictor's variable (fixed terms): *A. catenella* cell densities, sector, chlorophyll *a*, salinity, temperature and clams density. Previously, *A. catenella* cell densities were scaled (mean/standard deviation). Sampling date (months) was used as a random variable (random term). Interaction terms were not used because they can obscure the effects of the individual predictor variables (Gotelli and Ellison, 2004). GLMM is an extension of the generalized linear model (GLM), which provides a more flexible approach for analyzing non-normal data when random effects are present (Bolker et al., 2008). The significance of each explanatory variable was determined using a χ^2 test (Venables and Ripley, 1998).

The analyses were performed using the statistical and programming software R 2.1.12 (R Development Core Team, 2012), packages "ggplot2", "nlme" and "cluster", available through the CRAN repository (www.r-project.org/).

3. Results

3.1. Alexandrium catenella *bloom*

A global view of bloom evolution in the two study sites is provided in Fig. 2, which shows the concentration of *Alexandrium catenella* cells in the water column during the bloom. The bloom started in central areas of the Moraleda channel in December 2008

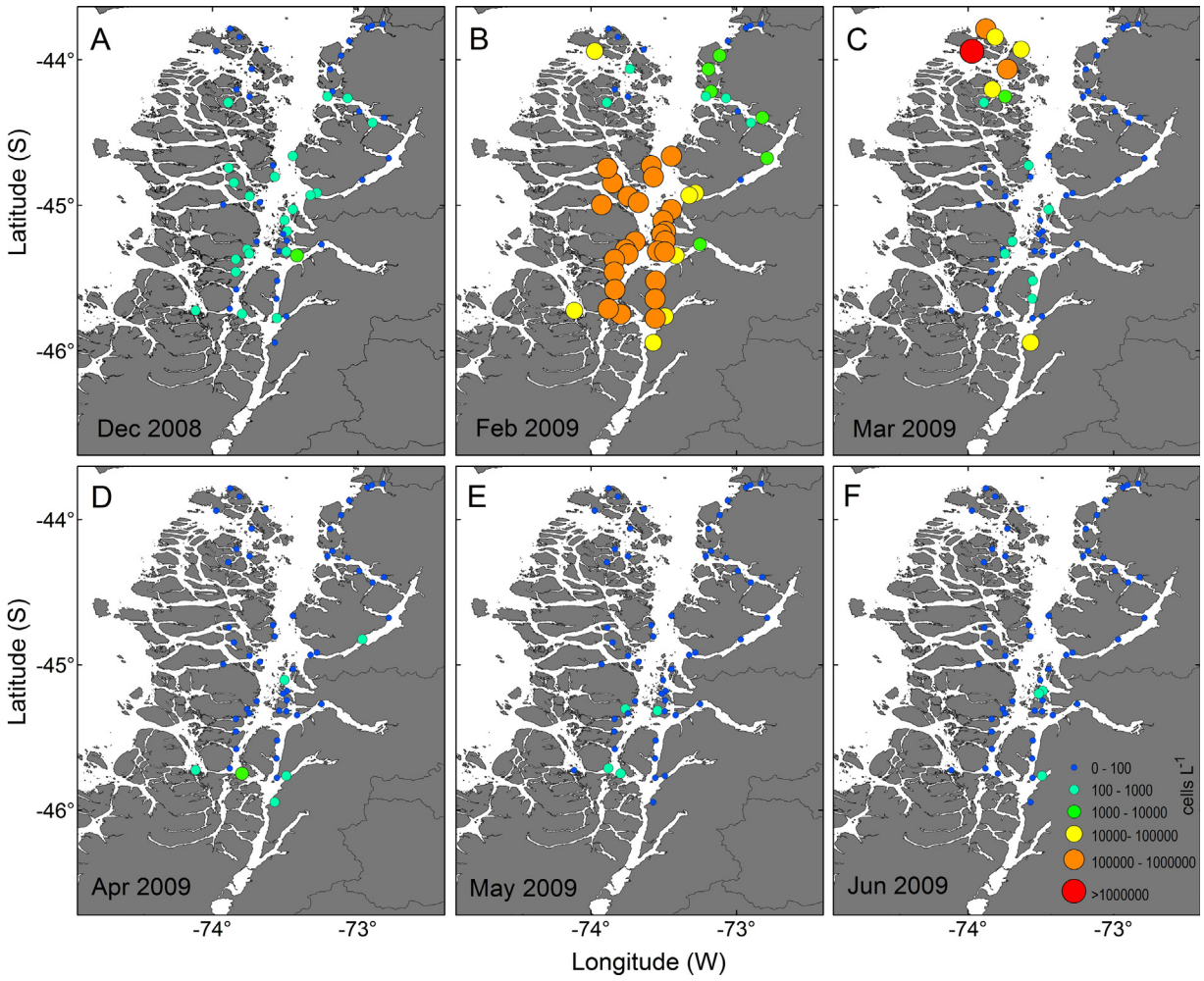

Fig. 2. Spatial and temporal distributions of *Alexandrium catenella* cell density (cells L^{-1}) recorded in a monthly monitoring program carried out in the Aysen region (southern Chile) from December 2009 to May 2010.

and then moved north. The highest cell densities were reached in February but after the bloom spread to the northern, outer areas of the channel the highest densities were recorded in its western portion, in March 2009. The spatial variability was characterized by a pattern of higher abundances toward the southern part of Ovalada Island but with maximum abundances toward the middle of Low Bay. A time course of the average concentrations of planktonic cells, comparing the average concentration of cysts in the water column and in the sediments, is shown in Fig. 3. The highest number of cysts in the water column and in the sediments was reached one month and two months, respectively, after the maximum of planktonic cells (on March 18, 2009). Note that this maximum of *A. catenella* cells (6800 cells L^{-1}) is much lower than the values reported in Fig. 2 (1.1×10^6 cells L^{-1}), which is based on monthly data. Up to 30 cysts L^{-1} were recorded in the water column on April 23, 2009, i.e., one month after cell numbers had reached a maximum by which time they were no longer significant. The maximum concentration of cysts in the sediments (128 cysts mL^{-1}) was recorded on June 5, 2009. The cyst concentration in the water followed a pattern similar to that in the sediments (Fig. 3), with peaks that dissipated after ~40 days.

3.2. Cyst abundance, diversity, and distribution

A list of dinoflagellate cysts from autotrophic and heterotrophic species, including the maximum cyst concentration in each studied location, is shown in Table 1. The diversity of the cyst types corresponded to eight different genera. The maximum cyst concentration was reached at both sites, Low Bay and Ovalada Island, during the bloom of *Alexandrium catenella*, with values of 128 cysts mL^{-1} and 51 cysts mL^{-1}, respectively. A similar maximal distribution was recorded for "round brown cysts," with 76 and 11 cysts mL^{-1}, respectively. The next most relevant cyst maximum was that of *Protoceratium reticulatum* but in contrast to *A. catenella*, a higher number of cysts were detected at Ovalada Island (50 cysts mL^{-1}) than at Low Bay (8 cysts mL^{-1}). The third most abundant species was *Diplopelta parva*, with very similar cyst concentrations at the two sites (20 and 29 cysts mL^{-1} in Low Bay and Ovalada Island, respectively). For the remaining species, fewer than 10 cysts mL^{-1} were recorded, the exception being *Protoperidinium avellanum*. In addition, ours is the first study to document cysts of *Protoperidinium leonis* in the sediments of inshore seas of southern Chile (Fig. 4).

Each dinoflagellate species differed not only in the maximum cyst concentration reached but also in its average abundance throughout the sampling period (Fig. 5). At Ovalada Island, *Alexandrium catenella* cyst concentrations reached a lower maximum value but the subsequent decrease was less dramatic than at Low Bay. The concentration of *Protoceratium reticulatum* cysts peaked twice (maximums around 40 cysts mL^{-1}) at Ovalada Island but was always below 10 cysts mL^{-1} at Low Bay. For the other species, the distribution was quite similar between sites, except in

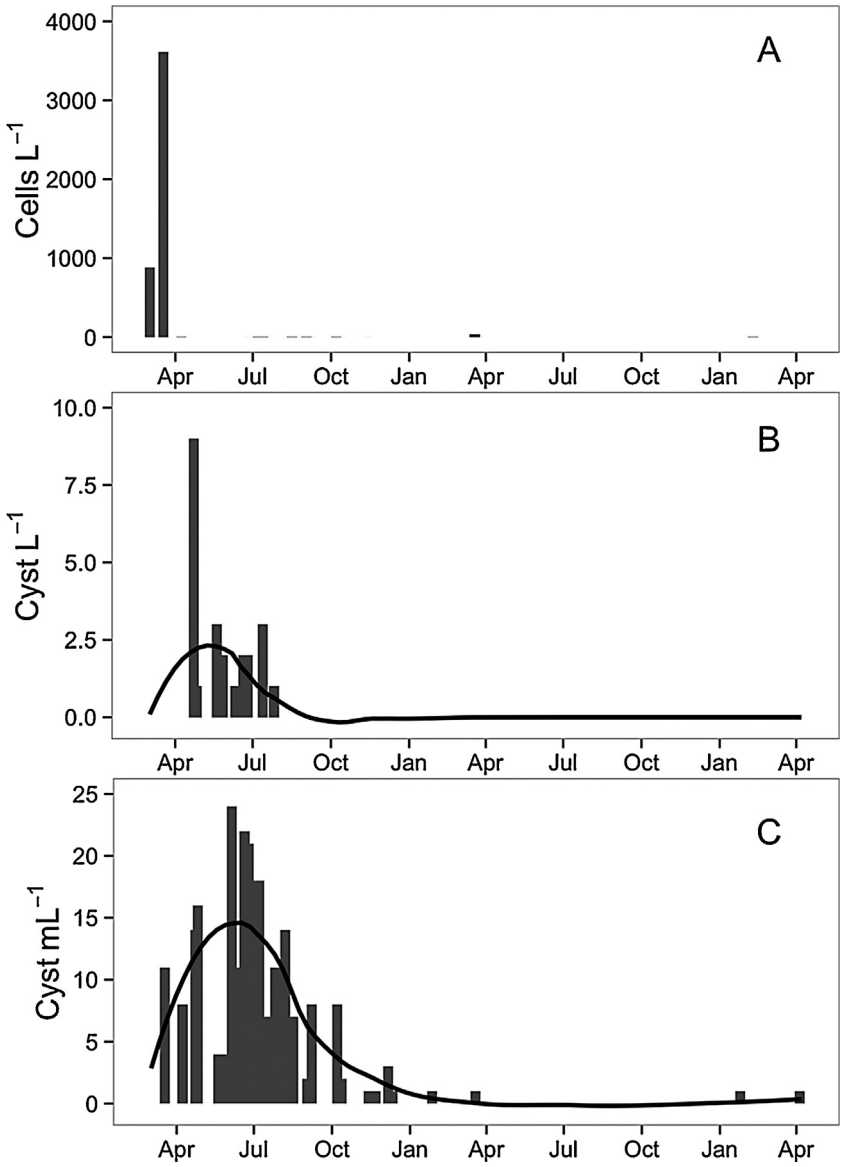

Fig. 3. Mean concentrations of: (a) the cell density of *A. catenella* in the water column, (b) dinoflagellate cyst abundance in the water column, (c) dinoflagellate cyst abundance in sediments of the two study areas, determined from April 2009 to April 2011.

the case of *Scrippsiella* spp., for which maximum numbers were recorded at the end of the sampling period at Low Bay but during early sampling at Ovalada Island. Cysts of *Protoceratium reticulatum*, *Diplopelta parva*, and *Protoperidinium* spp. (“round brown”) were the most frequent and abundant during the sampling period at both sites, although the average values were higher at Ovalada Island. Cysts of the *Gonyaulax spinifera* complex appeared at low abundances and at Low Bay in only one sample, vs. three at Ovalada Island. Unidentified cysts were generally below 2% of the total cyst assemblage.

The relative contribution of autotrophic and heterotrophic cysts to the dinoflagellate cyst pool at each studied site is shown in Figs. 6 and 7. In Low Bay (Fig. 6), the bloom of autotrophic (*Alexandrium catenella*) species occurred between June and August 2009, after which the pool of the dinoflagellate cysts was dominated by heterotrophic species. The total concentration of resting cysts fell below 50 cysts mL^{-1} from September 2009 to March 2010. During April 2010, it climbed to 100 cysts mL^{-1}, with the samples almost exclusively composed of heterotrophic species. Fewer than 10 cysts mL^{-1} were recorded in July 2010, but the concentration increased again in December 2010, reaching peaks in middle areas of Low Bay during January and March 2011. At Ovalada Island (Fig. 7), autotrophic species comprised most of the dinoflagellate assemblage throughout the sampling period (March 2009 to March 2010). Peaks in the concentrations of autotrophic species from July to October at Low Bay were coincident with those at Ovalada Island, even though the bloom at Low Bay was of a longer duration. Another peak in total cyst concentration was determined in January 2010, when autotrophic species accounted for a high percentage of the dinoflagellate assemblage at Low Bay; however, it was not possible to compare this time point to corresponding data from Ovalada Island (missing sample). During March 2010, both the total cyst concentration and the percentage of autotrophic species were higher at Ovalada Island than at Low Bay.

The GLMM analysis showed that *Alexandrium catenella* cell densities and sector contributed significantly ($p < 0.05$) to explain the variability of *A. catenella* cysts. But clams density, temperature, salinity and chlorophyll a not showed significant effects on response variable (Table 4).

Table 1
Diversity and maximum abundance (cysts mL^{-1}) of the 28 types of dinoflagellate cysts recorded from April 2009 to March 2011 in Low Bay and Ovalada Island, Chile. Species code identifying the cyst shown in Fig. 9, nutritional mode (a: autotroph; h: heterotroph), and codes defined in Fig. 4 are indicated.

Dinoflagellates cysts	Code	Nutrition	Maximum (cysts mL^{-1})		Fig. 4 code
			Low Bay	Ovalada Island	
Gonyaucales					
Alexandrium catenella (Wedon *et* Kofoid) Balech	Acat	a	128	51	A
Protoceratium reticulatum (Claparece *et* Lachmann) Bütschli	Pret	a	8	50	B
Gonyaulax spinifera complex (*Nematosphaeropsis*) (Claparece *et* Lachmann)	Nema	a		1	C
Gonyaulax spinifera complex (*Spiniferites mirabilis*) (Claparece *et* Lachmann)	Smir	a		0.3	D
Gonyaulax spinifera complex (*Spiniferites ramosus*) (Claparece *et* Lachmann)	Sram	a	1	1	E
Gymndiniales					
Polykrikos schwartzii Bütschli	Psch	h	1	4	F
Polykriko sp 1.	Psp1	h	1		
Polykriko spp.	Pospp	h	5		
Peridiniales					
Diplopelta parva (Abe) Matsuoka	Dpar	h	20	29	G
Pentapharsodinium dalei Indelicato *et* Loeblich III	Pdal	h	4	1	H
Protoperidinium americanum (Gran *et* Braarud) Balech	Pame	h	5	3	I
Protoperidinium avellanum (Meunier) Balech	Pave	h	15	1	J
Protoperidinium leonis (Pavillard) Balech	Pleo	h	1	1	K, L
Protoperidinium subinerme (Paulsen) Loeblich III	Psub	h	1	1	M
Protoperidinium claudicans (Paulsen) Balech	Pcla	h	1	1	N
Protoperidinium conicoide (Gran) Balech	Pcon	h	3	1	O
Protoperidinium conicum (Gran) Balech	Pconi	h	7	1	P
Protoperidinium denticulatum (Gran & Braarud) Balech	Pden	h	5	2	Q
Protoperidinium excentricum (Paulsen) Balech	Pexc	h	3	1	R
Protoperidinium pentagonum (Gran) Balech	Ppen	h	3	1	S
Protoperidinium sp.	Psp	h	3		
Protoperidinium spp.	Pspp	h	1	6	
Scrippsiella patagonica Akselman *et* Keupp	Spat	a	2		T
Scrippsiella trochoidea (Stein) Loeblich III	Stro	a	1		U
Scrippsiella sp 1.	Ssp1	a		1	V
Scrippsiella spp.	Sspp	a	5	2	
Round brown cyst	Rbro	h	76	11	W
Unidentified cyst	Ucyst		4	2	X

3.3. Species and sites: environmental correlations

Two-way clustering analyses (HCA; Fig. 8) revealed the relationships between the detected species and between those species and the sampling sites. Thus, *Alexandrium catenella* was shown to be strongly associated with *Protoceratium reticulatum*, *Diplopelta parva*, *Protoperidinium avellanum*, and "round brown cysts" but poorly associated with the other species, probably because their occurrence was non-seasonal. Regarding the study sites, there was a very clear the distinction between Low Bay and Ovalada Island. The first partition process, in three nodes, divided the samples in three groups, which accurately segregated the stations at Low Bay from those at Ovalada Island. The former could be further differentiated in two groups, with stations located in more interior areas being more similar to the stations at Ovalada Island.

The biplot of the CCA (Fig. 9) shows the ordination of the species and the environmental variables temperature, salinity, clam biomass, Chl *a*, and pheopigments. CCA1 (canonical coefficient 1) and CCA2 (canonical coefficient 2) carried most of the inertia and explained 86.8% of the variance in the species–environment relationship (Table 2). The environmental variables temperature and Chl *a* explained most of the variance in the environmental relationship (Table 3). The clearest association was between *Spiniferites ramosus* and *Spiniferites mirabilis* and Chl *a*. For

Table 2
Eigenvalues for CCA axes, species–environment correlations, and cumulative percentage (%) variance of species data and the species–environment relationship.

Axes	1	2	3	4	Total inertia
Eigenvalues	0.153	0.020	0.013	0.009	3.4033
Species–environment correlations	0.651	0.381	0.329	0.299	
Cumulative percentage variance					
Of species data	4.5	5.0	5.4	5.7	
Of species–environment relationship	**76.9**	**86.8**	**93.2**	**97.6**	
Sum of all eigenvalues					3.4033
Sum of all canonical eigenvalues					0.199

Table 3
Correlation coefficients between five environmental variables and the first three canonical axes.

Variables	Axis 1	Axis 2	Axis 3
Temperature	**0.8254**	−0.3029	−0.3666
Salinity	0.2505	0.4189	**0.7170**
Chlorophyll *a*	0.3497	**−0.7763**	0.1650
Pheopigments	0.2638	−0.2026	−0.0439
Clam biomass	**−0.5900**	−0.1590	−0.2609

Table 4
Statistical significance of each explanatory variable determined using a χ^2 test (Venables and Ripley, 1998), for variability of *A. catenella* cyst abundance in the sediment, using a generalized linear mixed model (GLMM) with logit-link function for negative binomial distribution. Significant effects ($p < 0.05$) are showed by asterisk.

	Chi square (χ^2)	Degree of freedom	Pr($>\chi^2$)
A. catenella cell densities (scaled)	27.4519	1	1.61E−07***
Sector	17.0716	1	3.60E−05***
Temperature	0.0677	1	0.7947
Chlorophyll *a*	0.1707	1	0.6795
Salinity	0.1421	1	0.7062
Clams density	0.947	1	0.3305

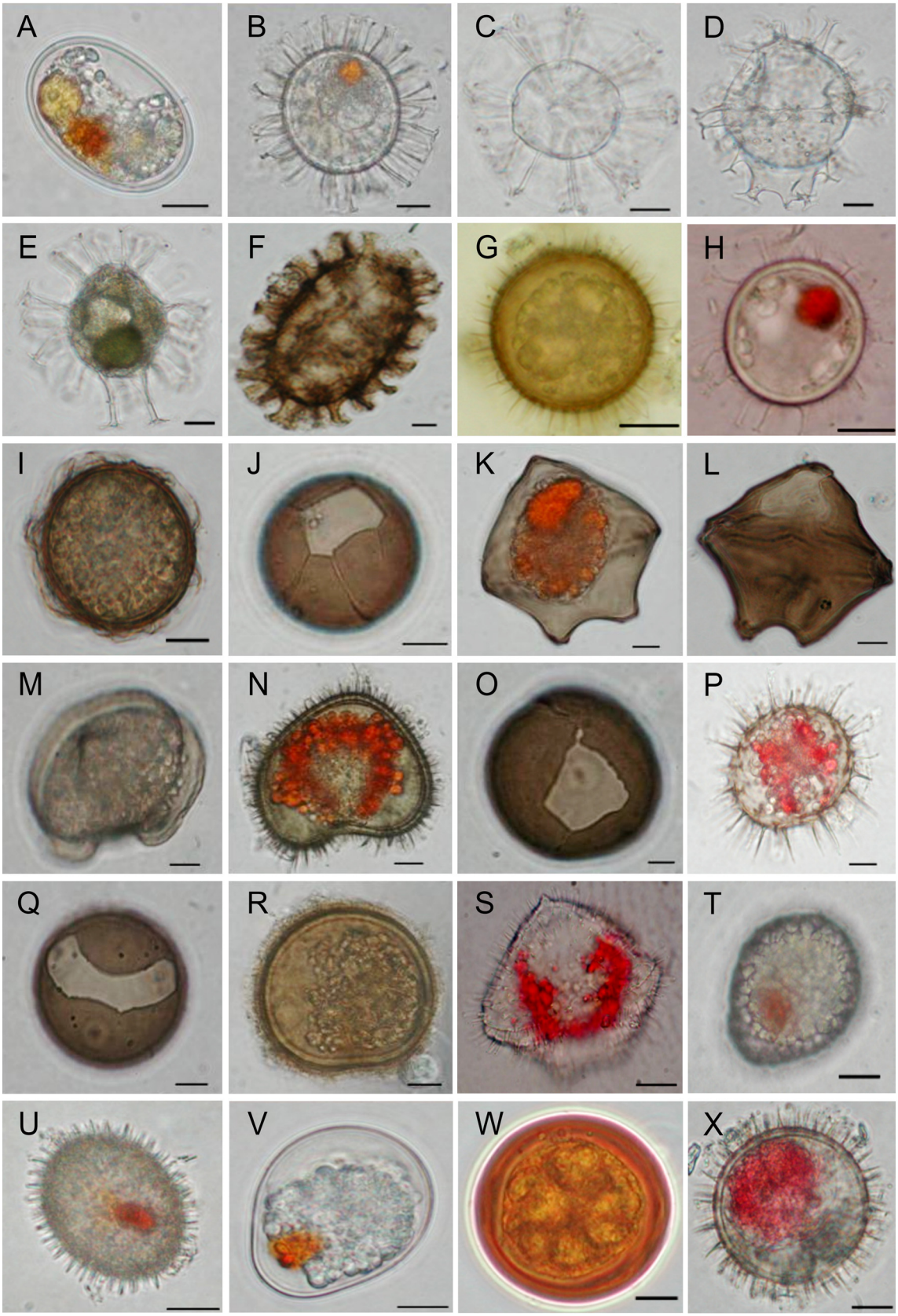

Fig. 4. Dinoflagellate cysts recorded in surface sediments of the study area: (A) *Alexandrium catenella*; (B) *Protoceratium reticulatum*; (C) *Gonyaulax spinifera* complex (Nematosphaeropsis); (D) *Gonyaulax spinifera* complex (*Spiniferites mirabilis*); (E) *Gonyaulax spinifera* complex (Spiniferites ramosus); (F) *Polykrikos schwartzii*; (G) *Diplopelta parva*; (H) *Pentapharsodinium dalei*; (I) *Protoperidinium americanum*; (J) *P. avellanum*; (K) *P. leonis*; (L) *P. leonis* empty cysts; (M) *P. subinerme*; (N) *P. claudicans*; (O) *P. conicoide*; (P) *P. conicum*; (Q) *P. denticulatum*; (R) *P. excentricum*; (S) *P. pentagonum*; (T) *Scrippsiella patagonica*; (U) *S. trochoidea*; (V) *Scrippsiella* sp 1; (W) round brown cyst; (X) unidentified cyst. Calibration bars = 10 μm.

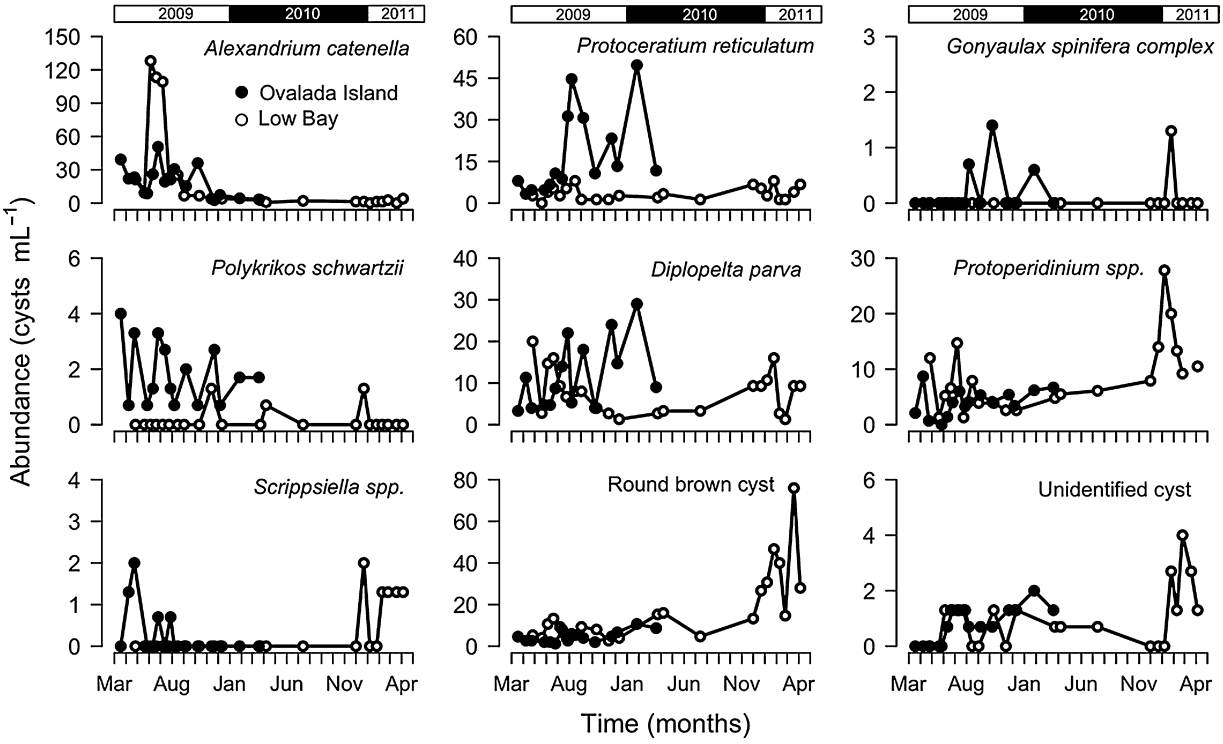

Fig. 5. Maximum abundance of the main dinoflagellate cyst taxa recorded in Low Bay from April 2009 to April 2011 and in Ovalada Island from April 2009 to March 2010. The sampling year is indicated on the horizontal bar above.

Alexandrium catenella, its frequency was weakly associated with a lower water temperature. By contrast, the frequencies of *Protoperidinium subinerme*, *Protoperidinium americanum*, and *Protoperidinium denticulatum* were related to high temperature as well as to salinity and a low clam biomass. *Protoperidinium pentagonum* was also related to a high Chl *a* concentration, whereas the *Gonyaulax spinifera* complex (Nematosphaeropsis) and *Polykrikos* sp. were more sensitive to higher temperatures .

4. Discussion

Most of the studies on dinoflagellate cysts in Southern Chile have focused on the distributions and abundances of two toxic species: *Alexandrium catenella* and *Protoceratium reticulatum* (Lembeye, 2004; Seguel et al., 2005, 2010, 2011b; Seguel and Sfeir, 2010). By choosing two different sites in the Chilean Southern Inland Sea we were able to examine the effects of different environmental and oceanographic conditions on resting cyst abundance, both in the water column and surface sediments, during and a year after a bloom of *A. catenella*. We discuss below our results related to the role of resting cysts in the recurrence of *A. catenella* blooms in this area.

4.1. Composition and distribution of the dinoflagellate cyst pool

The taxonomic composition of the dinoflagellate cyst assemblage investigated in the present work was comparable to that reported for other neritic sites in the South Pacific Ocean (Baldwin, 1987; Bolch and Hallegraeff, 1990; Sonneman and Hill, 1997). However, the diversity of cyst types (eight genera) was higher in our study than in other studies from Southern Chile (Seguel et al., 2005; Alves de Souza et al., 2008; Seguel and Sfeir, 2010). The dinoflagellate assemblage comprised mainly cosmopolitan species such as *Pentapharsodinium dalei*, *Protoperidinium americanum*, *Protoperidinium claudicans*, *Protoperidinium conicum*, and *Protoperidinium avellanum*. These species are characterized by their high tolerance of wide temperature and salinity ranges (Marret and Zonneveld, 2003). Cysts of *Protoperidinium* spp. and the "round brown" cysts were frequent and abundant in the samples and their distribution at the two study sites was similar, which suggests shared ecological requirements and that the "round brown" cysts were in fact members of the *Protoperidinium* genus (Rochon et al., 1999). In addition, to our knowledge, ours is the first reported detection of *Protoperidinium leonis* cysts in sediments of the inshore seas of southern Chile. Gonyaulacales cysts, commonly recorded in other temperate areas (Persson et al., 2000; Joyce, 2004; Orlova et al., 2004), were represented in our study by *Alexandrium catenella*, *Protoceratium reticulatum*, and the *Gonyaulax spinifera* complex. However, cysts of spiniferites occurred at very low abundances and those of *Protoceratium reticulatum* reached important concentrations only in the more interior of the two locations sampled (Ovalada Island). *Protoceratium reticulatum* is a cosmopolitan and yesotoxin-producing species (Satake et al., 1997) and its presence could be related to previously reported detections of these toxins in shellfish from southern Chile (Zhao et al., 1993; Seguel and Sfeir, 2010). According to ours results, this species co-occurred with *A. catenella*. The vegetative cells of spiniferites and *P. reticulatum* are usually found in the inshore seas of southern Chile only at very low abundances (<1 cell L^{-1}) (Lembeye et al., 1997; Uribe et al., 1997; Seguel et al., 2005). However, the abundance of *P. reticulatum* cysts determined in this study was higher than that previously recorded for the fjords and channel system in southern Chile (Seguel et al., 2005). These observations support a heterogeneous distribution of *P. reticulatum* cysts in sediments of the inshore seas of southern Chile.

Cluster analysis of the abundance and diversity of dinoflagellate cyst species segregated the sampling sites into two groups that were likely related to the proportion of autotrophic vs. heterotrophic cysts and the total cyst abundance at each site. The high abundance of heterotrophic dinoflagellate cyst species recorded in the present study is in agreement with previous demonstrations of their predominance in areas with relatively high nutrient

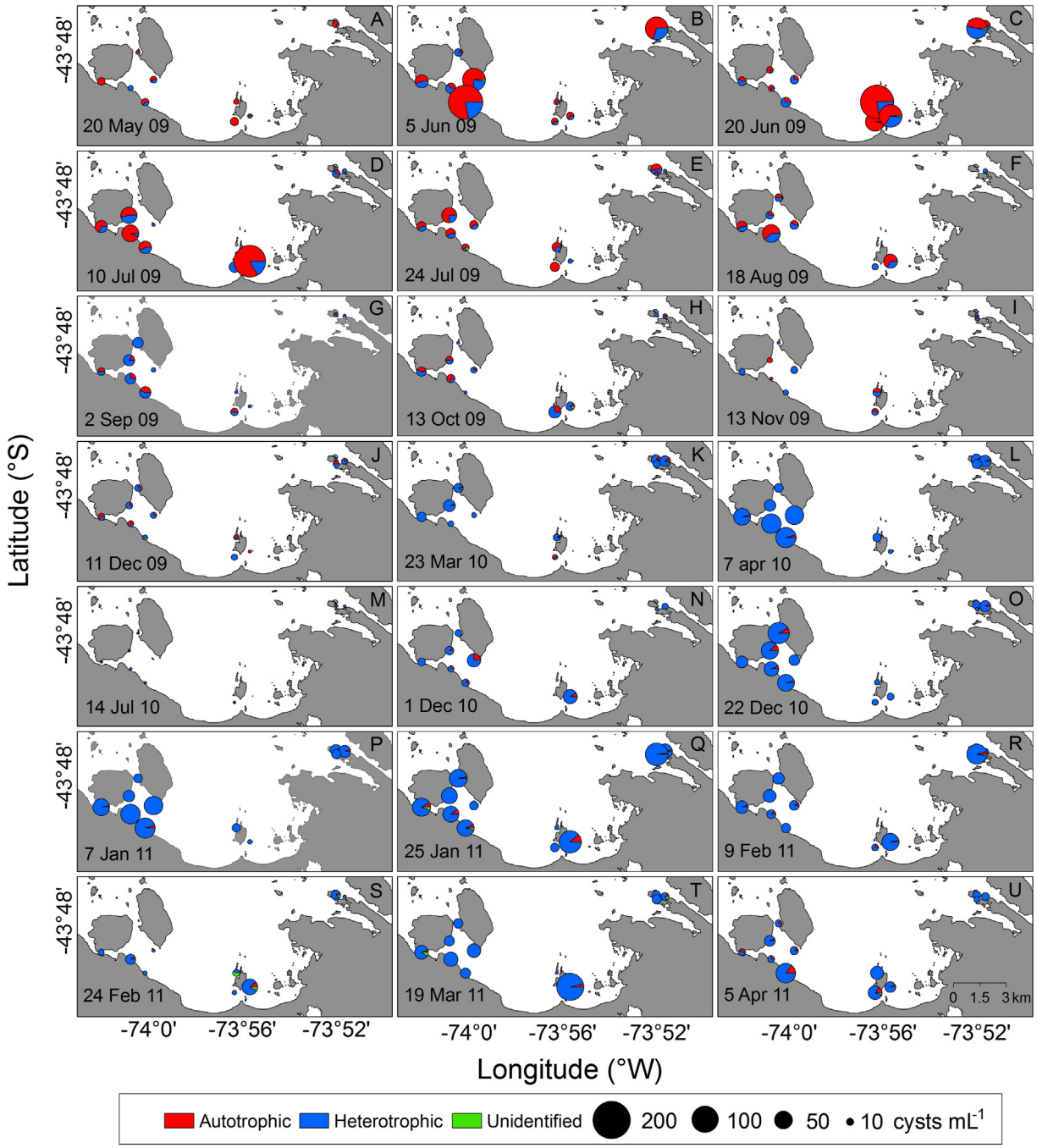

Fig. 6. Spatial and temporal distributions of the total (cyst mL^{-1}) and relative (%) abundances of the dinoflagellate cyst assemblage (heterotrophic, autotrophic, and unidentified) in Low Bay from May 2009 to March 2011.

concentrations (Godhe and McQuoid, 2003; Pospelova et al., 2004; Verleye and Louwye, 2010). As autotrophs and heterotrophs differ in their nutritional requirements (Harland et al., 2003), a determination of the relative contributions of dinoflagellate cyst species belonging to these two trophic groups to the total dinoflagellate assemblage provides important information about the environmental, nutritional, and physico-chemical conditions in the water column at a given site (Dale, 1996; Devillers and de Vernal, 2000; Godhe and McQuoid, 2003; Pospelova et al., 2004). For example, our analyses showed that, throughout the sampling period, heterotrophic cysts (with the exceptions of *Protoceratium reticulatum*, spiniferites, *Polykrikos schawartzii*, and *Diplopelta parva*), were more relevant in the more exterior Low Bay, although this was also where the highest densities of *Alexandrium catenella* cysts were reached during the autotrophic bloom. These results probably reflect both the preferences of the planktonic phase and local circulation patterns, which moved the resting cysts to the north, where they were mainly deposited in the widest part of the Moraleda channel. Our data on surface circulation patterns during a 12 h interval indicate that the sampled area is a very dynamic region, with high current velocities (Supplementary Figures S1–S4). We found the slower current velocities at the more open ocean location of Low Bay (Supplementary Figures S2–S4), where the highest densities of resting cysts were in general found as we mentioned above. This data and the local circulation patterns support the idea that variations in cyst abundance and distribution are not mainly related to bloom proliferation patterns, but indicative of the local oceanographic conditions influencing cyst

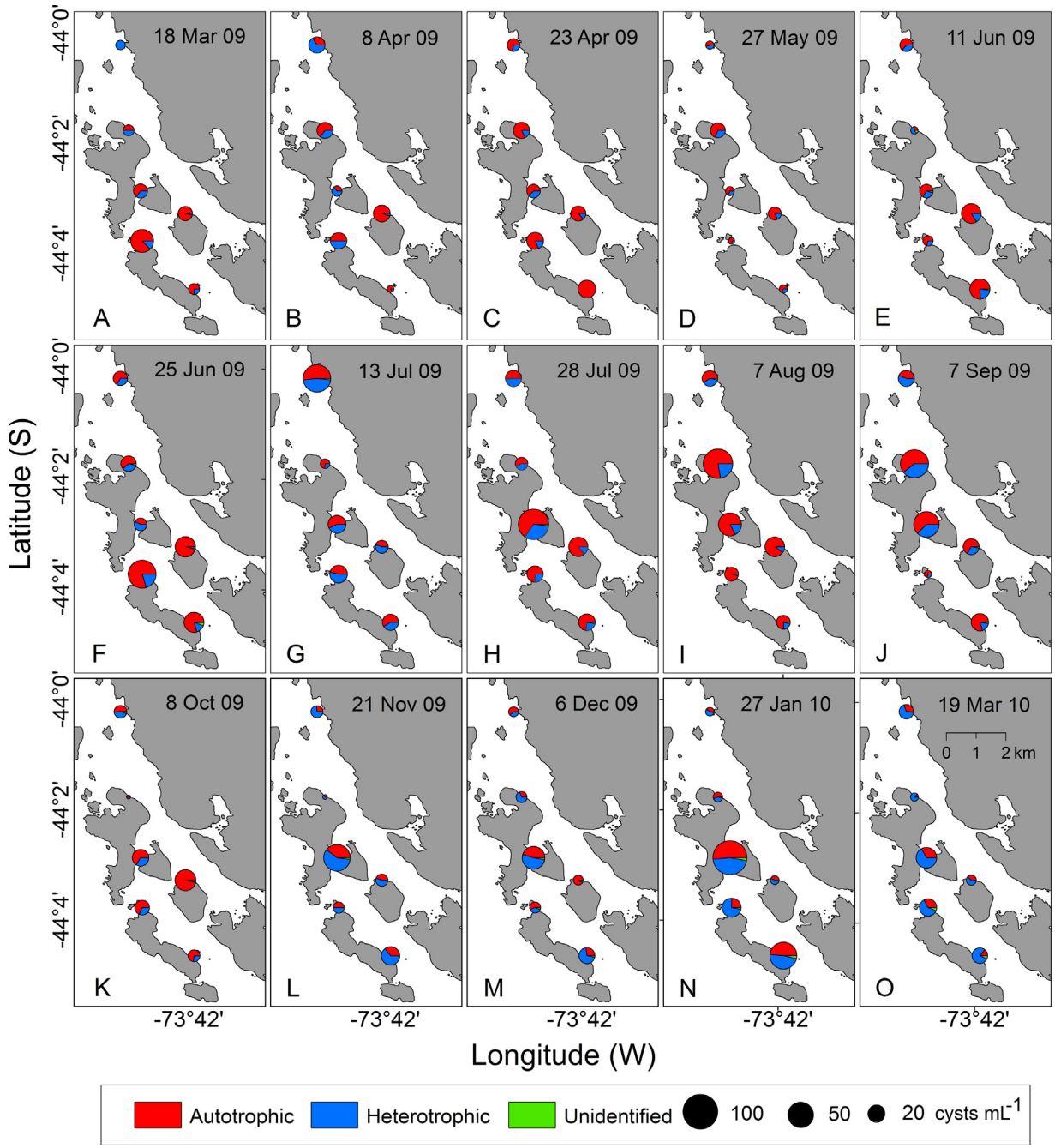

Fig. 7. Spatial and temporal distribution of the total (cyst mL^{-1}) and relative (%) abundances of the dinoflagellate cyst assemblage (heterotrophic, autotrophic and unidentified) at Ovalada Island from April 2009 to March 2010.

sedimentation. The vertical circulation model proposed for the Moraleda channel describes a net flux to the ocean at depths between 0 and 50 m (Silva et al., 1995). Accordingly, even if cyst transport and preservation resulted in the misinterpretation of cyst assemblages in the uppermost core samples of our study, lateral transport can be ruled out in the surface and subsurface waters of the Chilean SE Pacific (Verleye and Louwye, 2010).

4.2. A. catenella *blooms: implications of the encystment/excystment patterns*

For benthic resting cysts, the timing and rate of their formation and germination are major determinants of the termination and recurrence of harmful bloom events (e.g. Anderson and Wall, 1978; Anderson, 1984; Kremp and Heiskanen, 1999; Kremp and Anderson, 2000; Garcés et al., 2004). Going further, they could be important to explain also their magnitude, as the formation of blooms cannot be explained by vegetative growth alone (McQuoid, 2005) and a large part of the winter population of vegetative cells can come from excystment (Ishikawa and Taniguchi, 1996). The short recurrence intervals of *Alexandrium catenella* blooms in Mediterranean harbors support the hypothesis that cyst germination, and not the arrival of off-shore populations, explain the periodicity of blooms (Bravo et al., 2008).

However, the ecological role of resting cysts likely differs depending on the specific ecosystem and the genotypes of the bloom-forming species present within a particular geographic region. In the case of Mediterranean *Alexandrium catenella*,

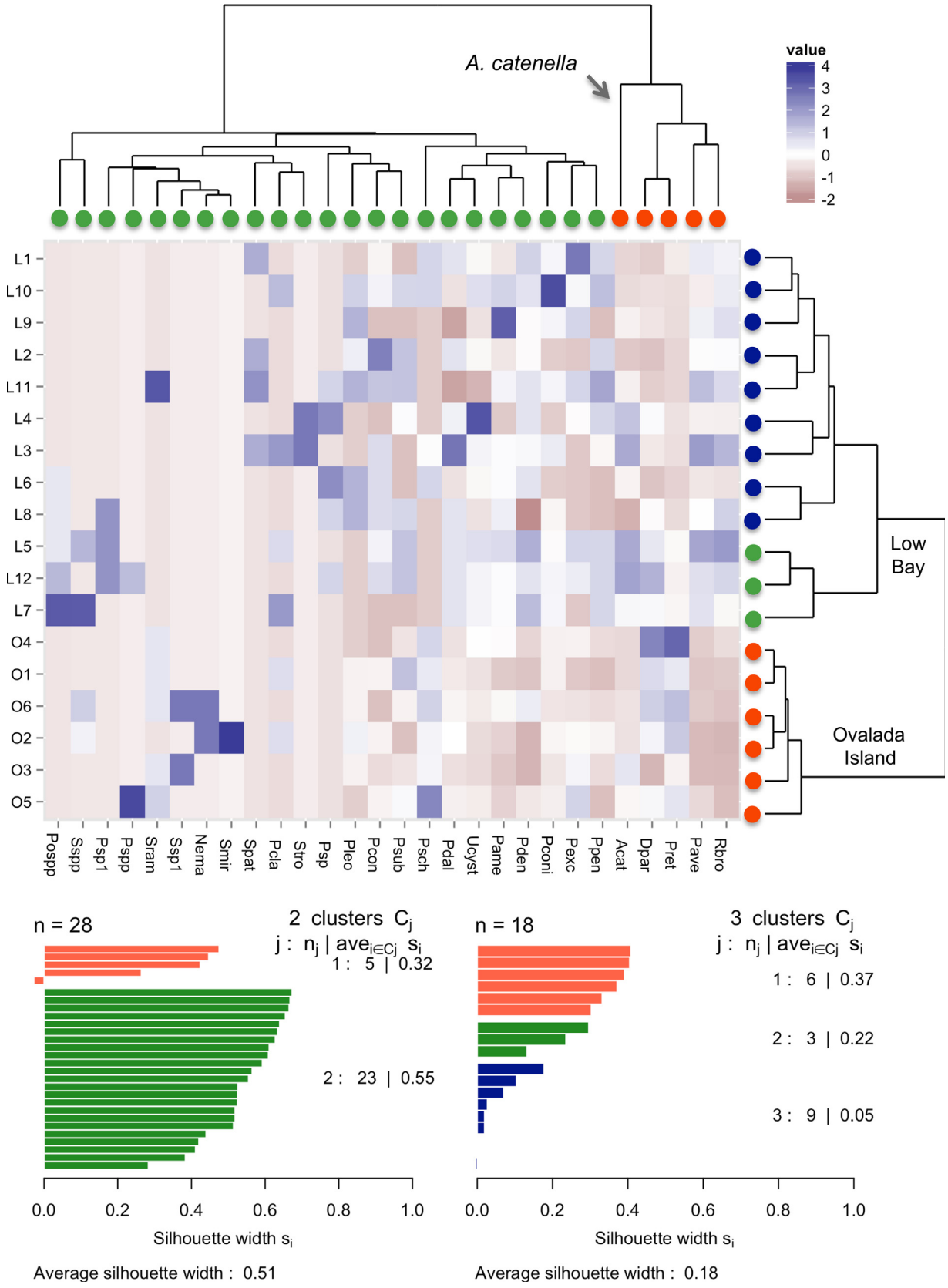

Fig. 8. Upper panel: two-way cluster analysis grouping both the dinoflagellate cyst taxa and the sampling stations. Lower panel: cluster validation using the width silhouette. The silhouette value ranges between −1 and 1, with large positive values indicating distinct clusters with greater intra-cluster than between-cluster similarity.

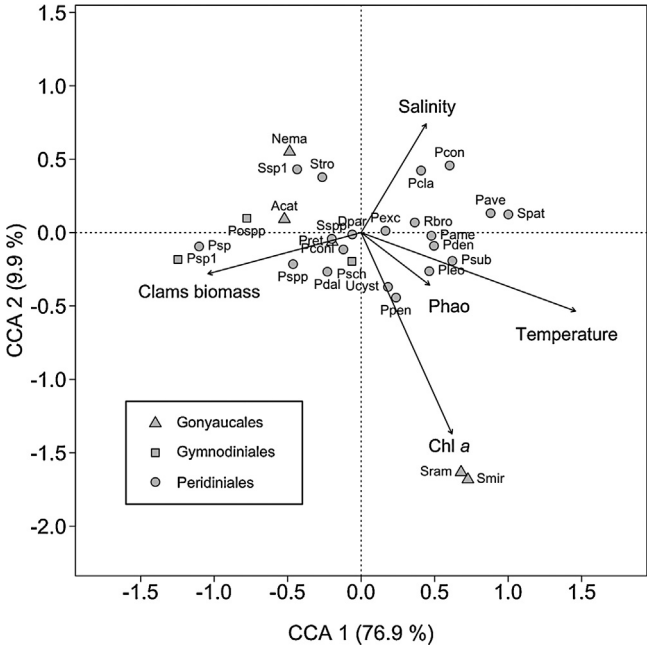

Fig. 9. Diagram of the canonical organization resulting from the canonical correspondence analysis (CCA) for the 28 dinoflagellates cyst types and five environmental variables (temperature, salinity, chlorophyll *a*, pheopigments, and clam biomass). Species codes are indicated in Table 1.

laboratory studies have determined a very short dormancy period that is followed by gradual germination within 5–65 days and strongly influenced by nutritional factors (Figueroa et al., 2006). However, given the high variability in the published data concerning this species, different clades were proposed to have either long or short dormancy requirements, providing, respectively, an effective overwintering strategy or a rapid transition between benthic and planktonic stages (Hallegraeff et al., 1998).

At least part of the described discordances between *Alexandrium catenella* studies can be ascribed to the different species studied. *A. catenella* refers to what are now known to be different species. Indeed, this name is in the process of being restricted to a group of genetically similar strains, as phylogenetic studies of *Alexandrium* have questioned many of the morphologically based classifications of the species within this genus. Several clades (ribotypes) that could not be assigned to *Alexandrium tamarense*, *A. catenella*, or *Alexandrium fundyense* were identified and subsequently assigned to a "*A. tamarense/catenella/fundyense* species complex" (Scholin et al., 1994), later renamed as groups I–V (Lilly et al., 2007) or groups I–IId (Miranda et al., 2012; Wang et al., 2014). Since in some cases there is a clear correlation between clades and geographic origin, *A. catenella* from Chile (group I sensu Lilly et al., 2007, *A. fundyense* according to Wang et al., 2014) is probably the same species as *A. catenella* from Puget Sound (USA), but different from Mediterranean *A. catenella* (group IV sensu Lilly et al., 2007, *A. catenella* according to Wang et al., 2014) and from Australian *A. catenella* (Groups IV/V, *A. catenella*/*Alexandrium australis* according to Lilly et al., 2007 and Wang et al., 2014 respectively).

According to Horner et al. (2011), *Alexandrium catenella* from the Pacific coast of the USA has a mandatory dormancy period of up to 5 months. Unlike Anderson and Keafer (1987), these authors did not find evidence of a synchronous biological clock restricting cyst germination to a specific seasonal time period, as cysts from the study site were able to germinate year round. They instead postulated that a synchronous biological timing mechanism is a strategy of cysts in deep-water beds that are exposed to fairly constant conditions without variation in light and temperature, which is not the case for *A. catenella* cysts in shallow locations as they occur where external environmental cues are able to reach the bottom sediments.

The GLMM analysis showed that *Alexandrium catenella* cell densities and sector contributed significantly to explain the variability of *A. catenella* cysts, suggesting that abundance of resting cysts is a reflection of cyst production. However, values post-bloom show a rapid and total depletion of resting cysts from sediments within a short period of 3 months.

Between bloom periods, resting cysts in sediments decrease due to germination, mortality and predation, and they may be also resuspended to the water column and dispersed to other areas. Therefore, *Alexandrium catenella* cysts in the area either are rapidly resuspended out of the sediments, have been predated or have germinated, therefore having a short dormancy period (<3 months). If this was the case, the dormancy period would be around half as long as that reported for the Puget Sound strains, which presumably belong to the same genetic clade (Horner et al., 2011). Indeed, the resting cyst dynamics reported here at Low Bay and Ovalada Island instead resemble those of *Alexandrium minutum* (Anglès et al., 2012). However, in contrast to *A. minutum*, there was probably no overlap between excystment and encystment processes within the monitored period.

Cyst distribution is influenced by different environmental factors. At small scales, cysts may be spatially distributed as fine-grain-size particles within the basin (Horner et al., 2011) whereas at larger scales cyst distribution is determined by specific physical forcing conditions, which can greatly vary. In this study we accounted for both: on a small scale, the variability between replicate samples was high, consistent with the patchy distribution common to dinoflagellate cyst deposits (Anderson et al., 1982; Horner et al., 2011); at a larger scale, the variability was consistent with local environmental factors and oceanographic circulation patterns within the studied area. Similar to the distribution of the heterotrophic cysts in this study, *Alexandrium catenella* cysts in sediments reached a maximum at Low Bay, in agreement with the vertical circulation model proposed for the Moraleda channel (Silva et al., 1995). The maximum concentration of *A. catenella* cysts in the Low Bay (128 cysts mL^{-1}), as determined in our study, is among the highest reported in Chile, second only to that reported by Seguel and Sfeir (2003) in Tictoc Bay (221.3 cysts mL^{-1}) and about the same as the value reported by Fernández et al. (2005) in Yenecura (127 cysts mL^{-1}). However, these concentrations are much lower than the 8000 cysts mL^{-1} and 4500 cysts mL^{-1} determined by Yamaguchi et al. (2002) in their study of the bays of Tokuyana and Hiroshima (Japan).

The causes underlying the distribution and timing of *Alexandrium catenella* blooms are poorly understood, but specific environmental cues such as temperature are known to be involved (Horner et al., 2011). We found a weak association between *A. catenella* and lower water temperatures, but not with any other of the variables studied, probably reflecting the lack of a seasonal frequency of bloom formation, and therefore of clear environmental correlations This conclusion is supported by the fact that the influence of temperature is not observed in the distribution of *A. catenella* resting, which showed only association to cell abundance and location. However, the seasonal distribution of heterotrophic species allowed finding more clear correlations between environmental variables and abundances. For example, *Protoperidimium* species are known to be seasonal (Jacobson, 1987), detected over a wide range in temperature but many occurring in Spring, which agrees with the association to high temperature and salinity but low predator biomass found in our study for the abundance of some *Protoperidinium* species.

The accumulation of resting cysts in the sediments, so-called resting cyst beds, has been cited to explain the onset and

recurrence of *Alexandrium* blooms (see for e.g. the revision of Anderson et al., 2012). Cysts of some *Alexandrium* species are known to remain viable for up to 100 years (Miyazono et al., 2012), and their presence is a cause for concern even in the absence of cells in the water column. However, *Alexandrium catenella* cysts were rapidly depleted from the surface sediments in the present study, and therefore, we got no evidence pointing to a role of resting cyst beds in the recurrence of blooms in this area. Thus, other mechanisms likely account for the development and recurrence of blooms in this area, for example, the entrance of offshore planktonic populations or cysts. In general, the role of resting cysts in *A. catenella* from southern Chile appears to be more closely related to the dispersion and turnover of genetically recombined genotypes under optimal environmental conditions. Support for this hypothesis will require population genetic studies aimed at establishing the origin of the blooming cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.hal.2014.10.001.

References

Alves de Souza, C., Varela, D., Navarrete, F., Fernández, P., Leal, P., 2008. Distribution, abundance and diversity of modern dinoflagellate cysts assemblages from southern Chile (43–54° S). Bot. Mar. 51, 399–410.

Anderson, D.M., Wall, D., 1978. Potential importance of benthic cysts of *Gonyaulax tamarensis* and *G. excavata* in initiating toxic dinoflagellate blooms. J. Phycol. 14 (2), 224–234.

Anderson, D.M., Aubrey, G., Tyler, M.A., Coats, D.W., 1982. Vertical and horizontal distributions of dinoflagellate cysts in sediments. Limnol. Oceanogr. 27, 757–765.

Anderson, D.M., 1984. Shellfish toxicity and dormant cysts in toxic dinoflagellates blooms. In: Ragelis, E.P. (Ed.), Seafood Toxins. Amer. Chem. Soc. Symposium 505 Series N? 262, Washington DC. pp. 125–138.

Anderson, D.M., Keafer, B.A., 1987. An endogenous annual clock in the toxic marine dinoflagellate *Gonyaulax tamarensis*. Nature 325, 616–617.

Anderson, D.M., 1998. Physiology and bloom dynamics of toxic *Alexandrium* species, with emphasis on life cycle transitions. In: Anderson, D.M., Cembella, A., Hallegraeff, G. (Eds.), Physiological Ecology of Harmful Algal Blooms. Springer-Verlag, Berlin, Heidelberg, pp. 19–48.

Anderson, D.M., Stock, C., Keafer, B., Nelson, A., McGillicuddy, D., Keller, M., Thompson, B., Matrai, P., Martin, J., 2005. *Alexandrium fundyense* cyst dynamics in the Gulf of Maine. Deep Sea Res. II 52, 2522–2542.

Anderson, D.M., Alpermann, T.J., Cembella, A., Collos, Y., Masseret, E., Montresor, M., Truby, E., 2012. The globally distributed genus *Alexandrium*: multifaceted roles in marine ecosystems and impacts on human health. Harmful Algae 14, 10–35.

Anglès, S., Garcés, E., Reñé, A., Sampedro, N., 2012. Life-cycle alternations in *Alexandrium minutum* natural populations from the NW Mediterranean Sea. Harmful Algae 16, 1–11.

Baldwin, P.P., 1987. Dinoflagellate resting cysts from sediments in Marlborough Sounds, New Zealand. N. Z. J. Mar. Freshw. Res. 21, 543–553.

Balech, E., 1995. The Genus *Alexandrium halim* (Dinoflagellata). Sherkin Island Marine Station, Sherkin Island, Co. Cork.

Bolch, C., Hallegraeff, G., 1990. Dinoflagellate cysts in recent marine sediments from Tasmania, Australia. Bot. Mar. 33, 173–192.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H., White, J.S., 2008. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24 (3), 127–135.

Bravo, I., Vila, M., Masó, M., Figueroa, R., Ramilo, I., 2008. *Alexandrium catenella* and *Alexandrium minutum* blooms in the Mediterranean Sea: toward the identification of ecological niches. Harmful Algae 7, 515–522.

Bravo, I., Figueroa, R.I., 2014. Towards an ecological understanding of dinoflagellate cyst functions. Microorganisms 2, 11–32.

Campodónico, I., Lembeye, G., Guzmán, L., 1995. Situación actual de los programas, estudios y actividades relativas a los Florecimientos Algales Nocivos en ChileIn: Second IOC Regional Science Planning Workshop on Harmful Algal Blooms in South America. Workshop Report N° 123, Mar del Plata. .

Dale, B., Yentsch, C.M., Hurst, J.W., 1978. Toxicity in resting cyst of the red tide *Gonyaulax excavata* from deeper water coastal sediments. Science 201, 1223–1225.

Dale, B., 1983. Dinoflagellate resting cysts: benthic plankton. In: Fryxell, G.A. (Ed.), Survival Strategies of the Algae. Cambridge University Press, Cambridge, pp. 69–136.

Dale, B., 1996. Dinoflagellate cyst ecology: modeling and geological applications. In: Jansonius, J., McGregor, D.G. (Eds.), Palynology: Principles and Applications. American Association of Stratigraphic Palynologists Foundation, Utah, pp. 1249–1275.

Devillers, R., de Vernal, A., 2000. Distribution of dinoflagellate cysts in surface sediments of the northern North Atlantic in relation to nutrient content and productivity in surface waters. Mar. Geol. 166 (1–4), 103–124.

Dia, A., Guillou, L., Mauger, S., Bigeard, E., Marie, D., Valero, M., Destombe, C., 2014. Spatiotemporal changes in the genetic diversity of harmful algal blooms caused by the toxic dinoflagellate *Alexandrium minutum*. Mol. Ecol. 23 (3), 549–560.

Ellegaard, M., Kulis, D., Anderson, D., 1998. Cyst of Danish *Gymnodinium nolleri* Ellegaard et Moestrup sp. ined (Dinophyceae): studies on encystment, excystmen and toxicity. J. Plankton Res. 20 (9), 1743–1755.

Fernández, P., Leal, P., Alves, C., Pizarro, G., Varela, D., 2005. Distribución y abundancia de quistes modernos de dinoflagelados en aguas interiores de la zona-austral de Chile (48° 08′ S y 54° 55′ S) con énfasis en *Alexandrium catenella* (Whedon & Kofoid) Balech, XI Congreso Latinoamericano de Ciencias del Mar COLACMAR y XXV Congreso de Ciencias del Mar. 16–20, Mayo, Viña del Mar, Chile, pp. 272.

Figueroa, R.I., Bravo, I., Garcés, E., 2005. Effects of nutritional factors and different parental crosses on the encystment and excystment of *Alexandrium catenella* (Dinophyceae) in culture. Phycologia 44, 658–670.

Figueroa, R.I., Bravo, I., Garcés, E., 2006. Multiples routes of sexuality in *Alexandrium taylori* (Dinophyceae) in culture. J. Phycol. 42, 1028–1039.

Figueroa, R.I., Bravo, I., Garcés, E., 2008. The significance of sexual versus asexual cyst formation in the life cycle of the noxious dinoflagellate *Alexandrium peruvianum*. Harmful Algae 7, 653–663.

Garcés, E., Masó, M., Camp, J., 1999. A recurrent and localized dinoflagellate bloom in a Mediterranean beach. J. Plankton Res. 21 (12), 2373–2391.

Garcés, E., Bravo, I., Vila, M., Figueroa, R., Masó, M., Sampedro, N., 2004. Relationship between vegetative cells and cyst production during *Alexandrium minutum* bloom in Arenys de Mar harbour (NW Mediterranean). J. Plankton Res. 26 (6), 637–645.

Godhe, A., McQuoid, M.R., 2003. Influence of benthic and pelagic environmental factors on the distribution of dinoflagellate cysts in surface sediments along the Swedish west coast. Aquat. Microb. Ecol. 32, 185–201.

Gotelli, N.J., Ellison, A.M., 2004. A Primer of Ecological Statistics. Sinauer Associates, Inc., Sunderland, MA, USA.

Guzmán, L., Campodonico, I., Antunovic, M., 1975. Estudios sobre un florecimiento toxico causado por *Gonyaulax catenella* en Magallanes. IV. Distribución y niveles de veneno paralítico de los mariscos (noviembre de 1972–noviembre de 1973). An. Inst. Patagon. 6 (1/2), 209–223.

Guzmán, L., Pacheco, H., Pizarro, G., Alarcón, C., 2002. *Alexandrium catenella* y veneno paralizante de los mariscos en Chile. In: Sar, E.A., Ferrario, M., Reguera, B. (Eds.), Floraciones Algales Nocivas en el Cono Sur Americano. Instituto Español de Oceanografía, Madrid, pp. 79–120.

Guzmán, L., Vidal, G., Vivanco, X., Arenas, V., Iriarte, L., Mercado, S., Alarcón, C., Pacheco, H., Palma, M., Espinoza, C., Mejías, P., Fernández-Niño, E., Monsalve, J., Pizarro, G., Hinojosa, P., Zamora, C., 2010. Manejo y Monitoreo de las mareas rojas en las regiones de Los Lagos, Aysén y Magallanes. Etapa III 2008–2009, Informe Final. Ministerio de Economía, Fomento y Reconstrucción – Subsecretaría de Pesca, , pp. 205.

Hallegraeff, G., Marshall, J., Valentine, J., Hardiman, S., 1998. Short cyst-dormancy period of an Australian isolate of the toxic dinoflagellate *Alexandrium catenella*. Mar. Freshw. Res. 49, 415–420.

Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase. Phycologia 32, 79–99.

Harland, R., Nordberg, K., Filipsson, H.L., 2003. A high-resolution dinoflagellate cyst record from latest Holocene sediments in Koljö Fjord, Sweden. Rev. Palaeobot. Palynol. 128, 119–141.

He, R., McGillicuddy, D.J., Keafer, B.A., Anderson, D.M., 2008. Historic 2005 toxic bloom of *Alexandrium fundyense* in the western Gulf of Maine: 2. Coupled biophysical numerical modeling. J. Geophys. Res.: Oceans 113,, C07040.

Horner, R.A., Greengrove, C.L., Davies-Vollum, K.S., Gawel, J.E., Postel, J.R., Cox, A.M., 2011. Spatial distribution of benthic cysts of *Alexandrium catenella* in surface sediments of Puget Sound, Washington, USA. Harmful Algae 11, 96–105.

Ishikawa, A., Taniguchi, A., 1996. Contribution of benthic cysts to the population of *Scrippsiella* spp. (Dinophyceae) in Onagawa Bay, northeast Japan. Mar. Ecol. Prog. Ser. 140, 169–178.

Jacobson, D.M., 1987. The Ecology and Feeding Biology of Thecate Heterotrophic Dinoflagellates. Ph.D. Thesis. Woods Hole Oceanographic Institution/Massachusetts Institute of Technology Joint Program 210 pp.

Joyce, L.B., 2004. Dinoflagellate cysts in recent marine sediments from Scapa Flow, Orkney, Scotland. Bot. Mar. 47, 173–183.

Keafer, B.A., Churchill, J.H., McGillicuddy Jr., D.J., Anderson, D.M., 2005. Bloom development and transport of toxic *Alexandrium fundyense* populations within a coastal plume in the Gulf of Maine. Deep Sea Res. II 52, 2674–2697.

Kremp, A., Heiskanen, A.-S., 1999. Sexuality and cyst formation of the spring-bloom dinoflagellate *Scrippsiella hangoei* in the coastal northern Baltic Sea. Mar. Biol. 134, 771–777.

Kremp, A., Anderson, D., 2000. Factors regulating germination of resting cysts of the spring bloom dinoflagellate *Scrippsiella hangoei* from the northern Baltic Sea. J. Plankton Res. 22 (7), 1311–1327.

Kremp, A., 2013. Diversity of dinoflagellate life cycles: facets and implications of complex strategies. In: Lewis, J.M., Marret, F., Bradlay, L. (Eds.), Biological and Geological Perspectives of Dinoflagellates. The Micropalaeontological Society. Special Publications, Geological Society, London, pp. 189–198.

Lagos, N., 2003. Paralytic shellfish poisoning phycotoxins: occurrence in South America. Comments Toxicol. 9, 175–193.

Legendre, P., Legendre, L., 1998. Numerical Ecology, 2nd English ed. Elsevier Science B.V., Amsterdam.

Lembeye, G., Molinet, C., Marcos, N., Sfeir, A., Clement, A., Rojas, X., 1997. Monitoreo de la Marea Roja en las aguas interiores de la X y XI regiones. Informe Final. FIP 95-23b, pp. 62.

Lembeye, G., 2004. Distribución de quistes de *Alexandrium catenella* y otros dinoflagelados en sedimentos de la zona sur-austral de Chile. Cienc. Tecnol. Mar. 27 (2), 21–31.

Lilly, E.L., Halanych, K.L., Anderson, D.M., 2007. Species boundaries and global biogeography of the *Alexandrium tamarense* complex (Dinophyceae). J. Phycol. 43 (6), 1329–1338.

Lindahl, O., 1986. A Dividable Hose for Phytoplankton Sampling. Report of the Working Group on Phytoplankton and Management of Their Effects. International Council for the Exploration of the Sea, C.M.1986/L: 1926, Annex 1983.

Lirdwitayaprasit, T., Nishio, S., Montani, S., Okaichi, T., 1990. The biochemical processes during cyst formation in *Alexandrium catenella*. In: Gráneli, E., Sundstrom, B., Edler, L., Anderson, D.M. (Eds.), Toxic Marine Phytoplankton. Elsevier, New York, pp. 294–299.

Lovegrove, T., 1960. An improved form of sedimentation apparatus for use with an inverted microscope. J. Cons. Int. Explor. Mer. 25 (3), 279–284.

Marret, F., Zonneveld, K.A.F., 2003. Atlas of modern organic-walled dinoflagellate cyst distribution. Rev. Palaeobot. Palynol. 125 (1–2), 1–200.

Matrai, P., Thompson, B., Keller, M., 2005. *Alexandrium* spp. from eastern Gulf of Maine populations: circannual excystment. Deep Sea Res. II 52, 2560–2568.

Matsuoka, K., Fukuyo, Y., 2000. Technical Guide for Modern Dinoflagellate Cyst Study WESTPAC-HAB/WESTPAC/IOC.

McCullagh, P., Nelder, J.A., 1989. In: Generalized Linear Models. Chapman and Hall, London.

McGillicuddy, D., Signell, R., Stock, C., Keafer, B., Keller, M., Hetland, R., Anderson, D., 2003. A mechanism for offshore initiation of harmful algal blooms in the coastal Gulf of Maine. J. Plankton Res. 25 (9), 1131–1138.

McGillicuddy, D., Anderson, D.M., Lynch, D.R., Townsend, D.W., 2005. Mechanisms regulating large-scale seasonal fluctuations in *Alexandrium fundyense* populations in the Gulf of Maine: results from a physical–biological model. Deep Sea Res. II 52 (19–21), 2698–2714.

McQuoid, M.R., 2005. Influence of salinity on seasonal germination of resting stages and composition of microplankton on the Swedish west coast. Mar. Ecol. Prog. Ser. 289, 151–163.

Miranda, L.N., Zhuang, Y., Zhang, H., Lin, S., 2012. Phylogenetic analysis guided by intragenomic SSU rDNA polymorphism refines classification of *Alexandrium tamarense* species complex. Harmful Algae 16, 35–48.

Miyazono, A., Nagai, S., Kudo, I., Tanizawa, K., 2012. Viability of *Alexandrium tamarensis* cysts in the sediments of Funka bay, Hokkaido, Japan: over a hundred year survival times for cysts. Harmful Algae 16, 81–88.

Molinet, C., Lafón, A., Lembeye, G., Moreno, C.A., 2003. Patrones de distribución espacial y temporal de floraciones de *Alexandrium catenella* (Whedon & Kofoid) Balech 1985, en aguas interiores de la Patagonia noroccidental de Chile. Rev. Chil. Hist. Nat. 76, 681–698.

Nuzzo, L., Montresor, M., 1999. Different encystment patterns in two calcareous cyst-producing species of the dinoflagellate genus *Scrippsiella*. J. Plankton Res. 21, 2009–2018.

Orlova, T., Morozova, T., Gribble, K., Kulis, K., Anderson, D., 2004. Dinoflagellate cysts in recent marine sediments from the east coast of Russia. Bot. Mar. 47, 184–201.

Oshima, Y., Bolch, C., Hallegraeff, G., 1992. Toxic composition of resting cyst of *Alexandrium tamarense* (Dinophyceae). Toxicon 30, 1539–1544.

Parsons, T.R., Maita, Y., Lalli, C.M., 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford.

Persson, A., Godhe, A., Karlson, B., 2000. Dinoflagellate cysts in recent sediments from the west coast of Sweden. Bot. Mar. 43, 69–79.

Pickard, G.L., 1971. Some physical oceanographic features of inlets of Chile. J. Fish. Res. Board Can. 28, 1077–1106.

Pospelova, V., Chmura, G., Walker, H., 2004. Environmental factors influencing the spatial distribution of dinoflagellate cyst assemblages in shallow lagoons of southern New England (USA). Rev. Palaeobot. Palynol. 128, 7–34.

R Development Core Team, 2012. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.r-project.org/

Rochon, A., de Vernal, A., Turon, J.L., Matthiessen, J., Head, M.J., 1999. Distribution of Recent Dinoflagellate Cysts in Surface Sediments from the North Atlantic Ocean and Adjacent Seas in Relation to Sea-Surface Parameters. Special Contribution Series. American Association of Stratigraphic Palynologists Foundation, Dallas No. 35.

Rousseuw, P., 1987. Silhouettes: A Graphical Aid to the Interpretation and Validation of Cluster Analysis. J. Comput. Appl. Math. 20, 53–65.

Satake, M., MacKenzie, L., Yasumoto, T., 1997. Identification of *Protoceratium reticulatum* as the biogenetic origin of yessotoxin. Nat. Toxins 5, 164–167.

Scholin, C.A., Herzog, M., Sogin, M.L., Anderson, D.M., 1994. Identification of group and strain-specific genetic markers fro globally distributed *Alexandrium* (Dinophyceae): II. Sequence analysis of a fragment of the LSU rRNA gene. J. Phycol. 30, 999–1011.

Seguel, M., Sfeir, A., 2003. Floraciones algales nocivas en el área sur de la X región y los canales occidentales de la XI región. Resultados crucero CIMAR – Fiordos 8. Informes Preliminares, pp. 137–144.

Seguel, M., Tocornal, M.A., Sfeir, A., 2005. Floraciones algales nocivas en los canales y fiordos del sur de Chile. Cienc. Tecnol. Mar. 28 (2), 5–13.

Seguel, M., Sfeir, A., 2010. Distribución de las toxinas marinas y quistes de dinoflagelados tóxicos en los canales occidentales de la Región de Aysén. Cienc. Tecnol. Mar. 33 (1), 43–55.

Seguel, M., Sfeir, A., Albornoz, V., Gangas, M., Molinet, C., Díaz, P., 2010. Distribucion de los quistes de *Alexandrium catenella* y *Protoceratium reticulatum* (Dinophyceae) en la Region Sur-Austral de Chile. Cienc. Tecnol. Mar. 33 (3), 59–70.

Seguel, M., Molinet, C., Díaz, P., Díaz, M., Varela, D., 2011a. Desarrollo de herramientas de manejo para la planificación de cosechas de bancos naturales de recursos bentónicos contaminados con biotoxinas marinas. Informe Final. Proyecto MR07I1007. Universidad Austral de Chile–Universidad de Los Lagos, , pp. 128.

Seguel, M., Sfeir, A., González, J., Díaz, P., Molinet, C., Labra, G., 2011b. Quistes de dinoflagelados en sedimentos marinos del sur de Chile con énfasis en *Alexandrium catenella* y *Protoceratium reticulatum*. Cienc. Tecnol. Mar. 34 (1-2), 5–17.

Sgrosso, S., Esposito, F., Montresor, M., 2001. Temperature and daylength regulate encystment in calcareous cyst-forming dinoflagellates. Mar. Ecol. Prog. Ser. 211, 77–87.

Silva, N., Sievers, H., Prado, R., 1995. Características oceanográficas y una proposición de circulación, para algunos canales australes de Chile entre 41°20′S y 46°40′S. Rev. Biol. Mar. 30 (2), 207–254.

Sonneman, J.A., Hill, D.R.A., 1997. A taxonomic survey of cyst-producing dinoflagellates from recent sediments of Victorian coastal waters, Australia. Bot. Mar. 40, 149–177.

Uribe, J.C., Guzmán, L., Pizarro, G., Alarcón, C., Iriarte, J.L., 1997. Monitoreo de la Marea Roja en las aguas interiores de la XII Región, Informe Final Proyecto FIP 95-23A. Instituto de Fomento Pesquero, Punta Arena, Chile, pp. 209.

Uthermöhl, H., 1958. Zur Vervollkomnung der quantitativen phytoplankton-Methodik. Mitt. Int. Ver. Limnol. 9, 1–38.

Venables, W.N., Ripley, B.D., 1998. Modern Applied Statistics With S-Plus, 2nd ed. Springer, New York.

Verleye, T.J., Louwye, S., 2010. Recent geographical distribution of organic-walled dinoflagellate cysts in the southeast Pacific (25–53° S) and their relation to the prevailing hydrographical conditions. Palaeogeogr. Palaeoclimatol. Palaeoecol. 298, 319–340.

Wang, L., Zhuang, Y., Zhang, H., Lin, X., Lin, S., 2014. DNA barcoding species in *Alexandrium tamarense* complex using ITS and proposing designation of five species. Harmful Algae 31, 100–113.

Ward, J.H., 1963. Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc. 58 (301), 236–244.

Yamaguchi, M., Itakura, S., Nagasaki, K., Kotani, Y., 2002. Distribution and abundance of resting cysts of the toxic *Alexandrium* spp. (Dinophyceae) in sediments of the western Seto Inland Sea, Japan. Fish. Sci. 68, 1012–1019.

Yoshimatsu, S., 1984. Sexual reproduction of *Protogonyaulax catenella* in culture. II. Mating type. Bull. Plankton Soc. Jpn. 31, 107–111.

Zhao, J., Lembeye, G., Cenci, G., Wall, B., Yasumoto, T., 1993. Determination of okadaic acid and dinophysistoxin-1 in mussels from Chile, Italy and Ireland. In: Smayda, T.J., Shimizu, Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier, Amsterdam, pp. 587–592.