Pseudo-nitzschia multistriata
Alexandrium minutum
Gymnodinium catenatum
Gyrodinium sanus
Planomeiocyte
Gamete
Vegetative
Zygote
Alexandrium catenella
Cyst
Alexandrium minutum
Alexandrium minutum
Protoperidinium
Gymnodinium instriatum
Gymnodinium catenatum
Alexandrium minutum

Bloom dynamics and life cycle strategies of two toxic dinoflagellates in a coastal upwelling system (NW Iberian Peninsula)

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ABSTRACT

A study of *Gymnodinium catenatum* and *Alexandrium minutum* blooms on the Galician coast was conducted from 2005 to 2007 in order to increase knowledge of the mechanisms governing recurrent blooms of these species. Considerable differences in their bloom dynamics were observed. *G. catenatum* blooms occurred in autumn and winter, following the pattern previously reported in the literature: they began off-shore and were advected to the Galician rias when a relaxation of the coastal upwelling occurred. On the other hand, *A. minutum* blooms developed inside embayments in spring and summer during the upwelling season and were associated with water stability and stratification. Both the vegetative population and the cyst distribution of *A. minutum* were related to less saline water from freshwater river outputs, which support a saline-gradient relationship postulated herein for this species. Dinoflagellates may produce both long-term double-walled cysts (resting) and short-term pellicle cysts. Resting cyst deposition and distribution in sediments showed that seeding occurred during the blooms of both species. However, the relationship between the cyst distribution in the sediments in Baiona Bay and the intensity and occurrence of *G. catenatum* blooms, suggests that the latter are not directly related to resting cyst germination. Moreover, the results presented in the present study point to other difference between the two species, such as the detection of pellicle cysts only for *A. minutum*. Finally, we discuss how the life cycle strategies of these two species may help to explain the different mechanisms of bloom formation reported herein.

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1. Introduction

Harmful algal blooms (HABs) are recurrent events in Galician waters produced by species that may cause paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP) and amnesic shellfish poisoning (ASP). There are two PSP-producing species in the area: *Gymnodinium catenatum* Graham 1943 and *Alexandrium minutum* Halim 1960 (Fraga et al., 1984). Blooms of these toxic dinoflagellates affect aquaculture resources to such an extent that a monitoring programme of potentially harmful phytoplankton species was established more than three decades ago. However, research on their bloom dynamics has been unequal and has focused more on *G. catenatum*, because its blooms affect a larger area. On the other hand, local studies of the bloom dynamics of *A. minutum*, previously cited as *Gonyaulax tamarense* (Blanco et al., 1985), have been very scarce and almost limited to phytoplankton monitoring information.

Galicia is located in the NW of the Iberian Peninsula, and is the northern limit of the NW African eastern boundary upwelling system. Along the western Iberian margin, north winds in spring and summer cause a displacement of surface waters off-shore due to Ekman transport and the upwelling of relatively cold nutrient-rich subsurface waters near the coast. On the other hand, southwestern winds in autumn and winter drive warm and nutrient-depleted surface waters toward the coast, where they may cause downwelling (Fraga, 1981; Tilstone et al., 1994). An upwelling index (Bakun, 1973) centered on 43°N11°W and based on surface pressure maps has been found to be a good indicator of this coastal upwelling, i.e. when the index is positive the subsurface waters upwell, causing an increase in the salinity and a decrease in surface temperature (Álvarez-Salgado et al., 1993).

Where the coast is fairly straight, as happens on the Saharan or Portuguese coasts, the upwelling system works as is typically defined in the classical literature. In the Galician rias, which comprises bays oriented more or less perpendicular to the coast, the upwelling becomes more complex. The interaction of coastal upwelling with upwelling-favorable local winds and the estuarine circulation makes the upwelling very intense in the inner parts of the rias, where it causes a high primary production in surface waters (e.g. Fraga, 1981; Varela, 1992; Figueiras et al., 2002). Moreover, river runoff creates a surface layer of much lesser density than the bottom waters of the rias, which flows into the

ocean. Negative upwelling index values have been associated with retention and sinking of surface water near the mouth of the rias (Castro et al., 1997).

Blooms of the toxic chain-forming dinoflagellate *G. catenatum* have been associated with upwelling relaxation when winds reverse (Fraga et al., 1988) or with downwelling (Tilstone et al., 1994). During the upwelling season, the phytoplankton has been described to be dominated by diatoms in the rias and dinoflagellates towards the shelf associated with the upwelling front (Tilstone et al., 1994). When the upwelling relaxes, warm dinoflagellate-populated off-shore waters are advected to the rias and their accumulation in the downwelling front is promoted. The strong swimming capabilities of chain-forming dinoflagellates such as *A. affine* and *G. catenatum* have been proposed as an ecological advantage in downwelling circumstances (Fraga et al., 1989). Indeed, toxic dinoflagellates in Galician waters have been described to appear in late summer or early autumn coinciding with the end of the upwelling season. The dynamics of the blooms of *G. catenatum* has been reported by several authors to follow a pattern dominated by physical parameters (Fraga et al., 1988; Sordo et al., 2001; Crespo and Figueiras, 2007).

On the other hand, *A. minutum* blooms have been reported in the region inside the Ria of Ares (Blanco et al., 1985) in summer; they were not associated with upwelling relaxation but rather with northern winds and stratified waters. All over the world, the blooms of this species have been related to water stability, salinity stratification and local nutrient-rich freshwater inputs, particularly in estuaries, harbors and lagoons (Blanco et al., 1985; Giacobbe et al., 1996; Maguer et al., 2004; Vila et al., 2005; Bravo et al., 2008).

Among all the features that dinoflagellate species may use in their life strategy, resting stage formation and germination, as well as the factors regulating them, have been related to the seasonality of their populations (Walker, 1984). Therefore, cyst-beds have been proposed for promoting the re-establishment of the vegetative population and enabling species to extend their distribution (Anderson and Wall, 1978; Steidinger and Haddad, 1981). *G. catenatum* and *A. minutum* are both known to form a resting cyst during their sexual cycle (Anderson et al., 1988; Bolch et al., 1991). However, major differences can be observed in their life cycles: (1) homothallism and heterothallism are observed in *G. catenatum* (Figueroa et al., 2006) but only heterothallism in *A. minutum* (Figueroa et al., 2007); (2) planozygote division may be undergone in both species but division capacity of fusing gametes after syngamia was only observed in *G. catenatum*, which may indicate a more flexible life cycle for the latter species (Figueroa et al., 2006); (3) the presence of chains of cyst-like stages observed during a bloom of *G. catenatum* also indicates that the sexual life cycle of this species may be more complex (Figueroa et al., 2008); and (4) the *A. minutum* resting cyst has a dormancy period of 30–45 days (Figueroa et al., 2007), compared with 6–10 days for *G. catenatum* (Bravo and Anderson, 1994; Figueroa et al., 2006).

In this paper we describe the different environmental niches associated with the bloom-pattern of *G. catenatum* and *A. minutum* in Galician waters, and discuss how the characteristics of the life strategy of each species match the different ecological niches in which they develop.

2. Methods

2.1. Description of Baiona Bay

Baiona Bay is located south of the Ria of Vigo (Fig. 1). Its hydrography is highly influenced by the coastal morphology and
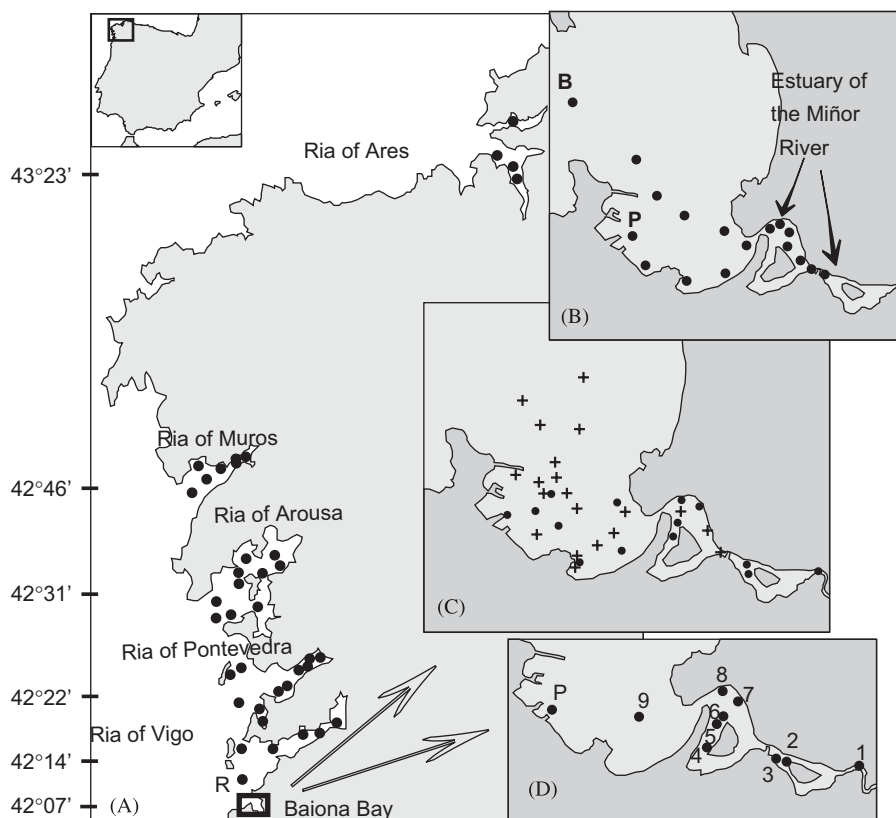
Fig. 1. Maps of the study area showing sampling stations in the four Rías Baixas, the Ria of Ares, Baiona Bay and the estuary of the Miñor River; (A and B) stations where phytoplankton, temperature and salinity measurements were performed; (C) sediment sampling carried out in 2005 (crosses) and 2007 (circles); and (D) situation of the traps for the spatial study of cyst fluxes.

by the freshwater input from the Miñor River (Fig. 1B). The harbor of Baiona is situated south of the bay and has strongly contributed to making this part an artificial sedimentary basin. The estuary of the Miñor River extends 1–2 km up the river (Fig. 1B) and is greatly influenced by tides: in most parts of it, the water depth fluctuates from 2 m at high tide to zero at low tide, when only the river water remains. Both the Ria of Ares and Baiona Bay, in which blooms of *A. minutum* are common, are oriented with their mouth open to the north, so while north winds cause general upwelling in the area, in these two bays they may cause a local downwelling.

2.2. Upwelling index, hydrographic data and phytoplankton sampling

The upwelling index was calculated using the average daily module and north component of the geostrophic winds in a $2° \times 2°$ cell centered at 43°N11°W. Average daily geostrophic winds were estimated from atmospheric surface pressure maps, provided at 6 h intervals by the Agencia Española de Meteorología (AEMET) (Lavin et al., 1991). The phytoplankton samples and physical data from the whole Galician coast from 2005 to 2007 were collected in the framework of the Phytoplankton Monitoring Programme carried out throughout each year by the Technological Institute for Marine Environment Control of the Xunta de Galicia (INTECMAR). Sampling was performed weekly during the 3 years at 25 stations (Fig. 1A and station B in Fig. 1B). Temperature and salinity profiles were obtained using a CTD Seabird 25. Integrated water samples were taken from each station at depths of 0–5, 5–10 and 10–15 m, using a pipe sampler (Lindahl, 1986). *A. minutum* and *G. catenatum* were quantified from water subsamples (25 mL) from each layer fixed with Lugol's iodine solution according to the Utermöhl method. Moreover, in the framework of this research project (SEED, http://www.icm.csic.es/bio/projects/seed/), other phytoplankton surveys were performed in order to study *A. minutum* blooms and carry out specific life cycle studies: (1) samplings were performed weekly from May to August 2006 at 7–9 stations located in Baiona Bay and on 2 August at 7 stations more in the estuary of the Miñor River (Fig. 1B); and (2) samples were collected weekly from one station

situated to the south of Baiona Bay (named as P in Fig. 1B) from August 2006 to December 2007. Phytoplankton samples were collected at 0.5 and 2 m depth by a manual suction pump and samples were fixed with formaldehyde at a final concentration of 1%. Temperature and salinity profiles were obtained by a handheld probe system (YSI Model 30M). *A. minutum* was identified by thecal plate tabulation (Balech, 1995) after a few drops of the fluorescent dye Calcofluor White M2R (Fritz and Triemer, 1985) had been added to the sedimenting chamber in order to stain the dinoflagellate plates. The chambers were examined and *A. minutum* were counted under $400 \times$ magnification in an inverted epifluorescence microscope with UV excitation light and a UV filter set.

A one-way ANOVA test and a Kolmogorov–Smirnov Z-test were applied to temperature and salinity, respectively, from stations R in the Ria of Vigo and P in Baiona Bay (Fig. 1A, B) in order to determine the differences between the means (for temperature) and differences in the shapes of the distributions (for salinity). Moreover, one-way ANOVA tests were applied to cell sizes of *A. minutum* observed on 26 July and 2 August 2006.

2.3. Cyst study in sediment traps

Two experiments were performed in sediment traps:

(1) A temporal study was carried out by means of one sediment trap placed at station P in the south of Baiona Bay (Fig. 1B) from 1 August 2006 to 30 December 2007. The trap consisted of two cylindrical plastic collectors (height 30 cm, diameter 5.5 cm, aspect ratio 5.45), which were placed in a metal disk tied to a surface buoy by a rope in such a way that the trap was always moored at a depth of 1 m below the surface. The buoy was anchored to a floating dock in Baiona marina. Settled material from the trap was collected every week, coinciding with the phytoplankton sampling at the same station. Samples from the trap were sieved to retain the 10–100 μm size fractions and collected in filtered seawater in a final volume of 50 mL, which was kept at 4 °C in the dark and observed using light microscopy in vivo within 48 h. For
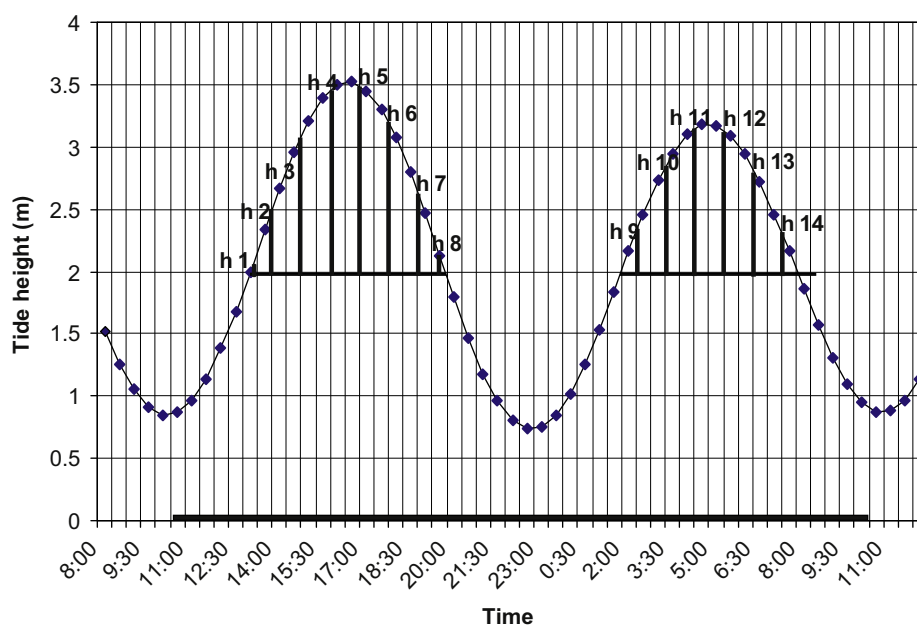

Fig. 2. Tide cycle in the Ria of Vigo, showing tide heights during the trap experiment performed on 27 and 28 June in Baiona Bay and the estuary of the Miñor River; h_n represents the water column above the trap (station 4) for each hour; the bar in the x-axis represents the time the experiment lasted.

quantification of cysts, 5 mL were diluted 3 times with filtered seawater and one subsample of 1 mL was observed for cyst quantification in a phytoplankton counting chamber. Resting cysts of *A. minutum* were identified by the thick wall and particular yellow accumulation body (Bolch et al., 1991), whereas pellicle cysts lacked a distinct thick wall and had a less condensed cytoplasm (Bravo et al., 2010).

(2) Other trap experiments were performed from 27 to 28 July and from 1 to 2 August 2006 at two stations in Baiona Bay and eight stations in the estuary of the Miñor River in order to study the spatial distribution of cyst deposition (Fig. 1D). The two traps in the bay were the same as that described above for the previous experiment. The eight traps placed in the estuary were adapted to be driven into the sediment by means of a long steel nail. The sediment collectors of these traps were smaller (height 14 cm, diameter 4 cm, aspect ratio 3.5) in order to adapt to shallow waters, and they were placed 5 cm above the sediment. The experiments lasted 24 h. Because of the tide cycle in the region (Fig. 2), the traps from the estuary were submerged in the water during different periods of time depending on the tide height at each station (Table 1). In order to compare all cyst fluxes, for each station the heights of water above the trap each hour were added up and subsequently the cyst numbers were standardized to 1 m (as an example the heights for the trap placed at station 4 are indicated in Fig. 2).

2.4. Cyst study in sediment

In order to study cyst distribution in the sediment of Baiona Bay, three sediment samplings were performed at 14–19 stations (Fig. 1C) in May 2005, December 2005 and March 2007. Samples were collected by a scuba diver placing plastic cylindrical corers (20 cm long × 3 cm base diameter) into the sediment in such a way that the vertical sediment profile resulted in a depth range 0 to 8–10 cm. In order to prevent the loss of the sediment surface cysts, the water over the sediment was also sampled and brought to the laboratory. Samples were stored in darkness and at 4 °C temperature until they were processed within 1 month from sampling. The profiles were cut every cm to quantify the cyst concentrations of the first four centimeters. The sediment was processed by sieving and density gradient following the methodology described by Bravo et al. (2006). Resting cysts of *A. minutum* and *G. catenatum* were counted from subsamples (3 mL) in an Utermöhl sedimentation chamber using a Zeiss Axiovert 135 inverted microscope at 400× magnification. The cysts were identified by means of the following features: brown color, microreticulated wall and size for *G. catenatum* (Anderson et al., 1988; Bravo and Ramilo, 1999), and thick and transparent color of the wall and yellow accumulation body for the resting cyst of *A. minutum* (Bolch et al., 1991).

3. Results
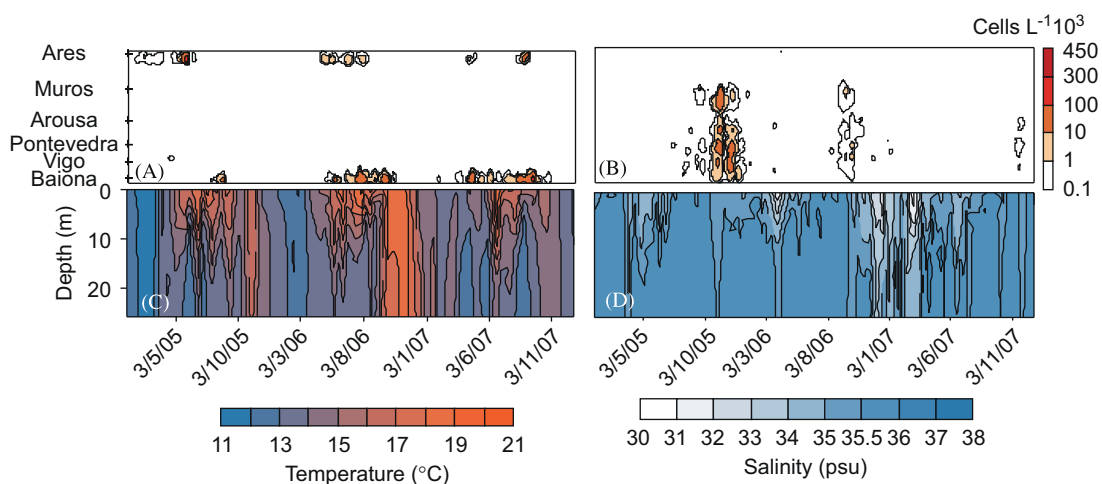
3.1. Development of the blooms and relationship with hydrodynamic conditions

The populations of *A. minutum* and *G. catenatum* on the Galician coast displayed a clear seasonality but followed very different patterns in time, geographical distribution and association with hydrodynamic features (Fig. 3).

A. minutum blooms occurred recurrently in spring and summer at two inshore estuarine locations, Baiona Bay and the Ria of Ares (Figs. 1A, 3A), which are highly freshwater-influenced regions. Cell densities higher than 10^3 cells L^{-1} were associated with stratified waters, which are characteristic in Galician rias in spring and summer (Fig. 3C, D). At least two blooms were commonly detected in both environments, one in spring and one in summer. Details of these blooms were studied in Baiona Bay from May 2006 to December 2007.

Three features characterize the *A. minutum* blooms and the associated hydrodynamic conditions:

(1) They occur in spring and summer. *A. minutum* was a common species in the phytoplankton community in Baiona Bay from early spring to late summer, but had an irregular distribution.

Table 1
Tide heights (m) of stations showed in Fig. 1D.

| Station	Height
1	2.5
2	2.7
3	2.2
4	1.8
5	2.8
6	1.9
7	2.0
8	1.4

Fig. 3. Cell concentration distribution of (A) *Alexandrium minutum* and (B) *Gymnodinium catenatum* on the Galician coast, and (C) temperature and (D) salinity at station B from January 2005 to December 2007.

Most cell-density peaks of its temporal distribution were no more than 1 week long (Fig. 4B). Although very few cells were usually detected in winter, a maximum of 4×10^2 cells L^{-1} was observed on 2 February 2007. Temperatures and salinities at surface in the station P (Baiona Bay) are shown in Fig. 4C. The

peak of cell densities reached 1×10^6 cell L^{-1} on 26 July 2006 and was associated with temperatures higher than 20 °C, the highest in the studied period. In spring and summer, positive upwelling indices (UI) (Fig. 4A) are associated with higher salinities and lower temperatures in the rias due to the
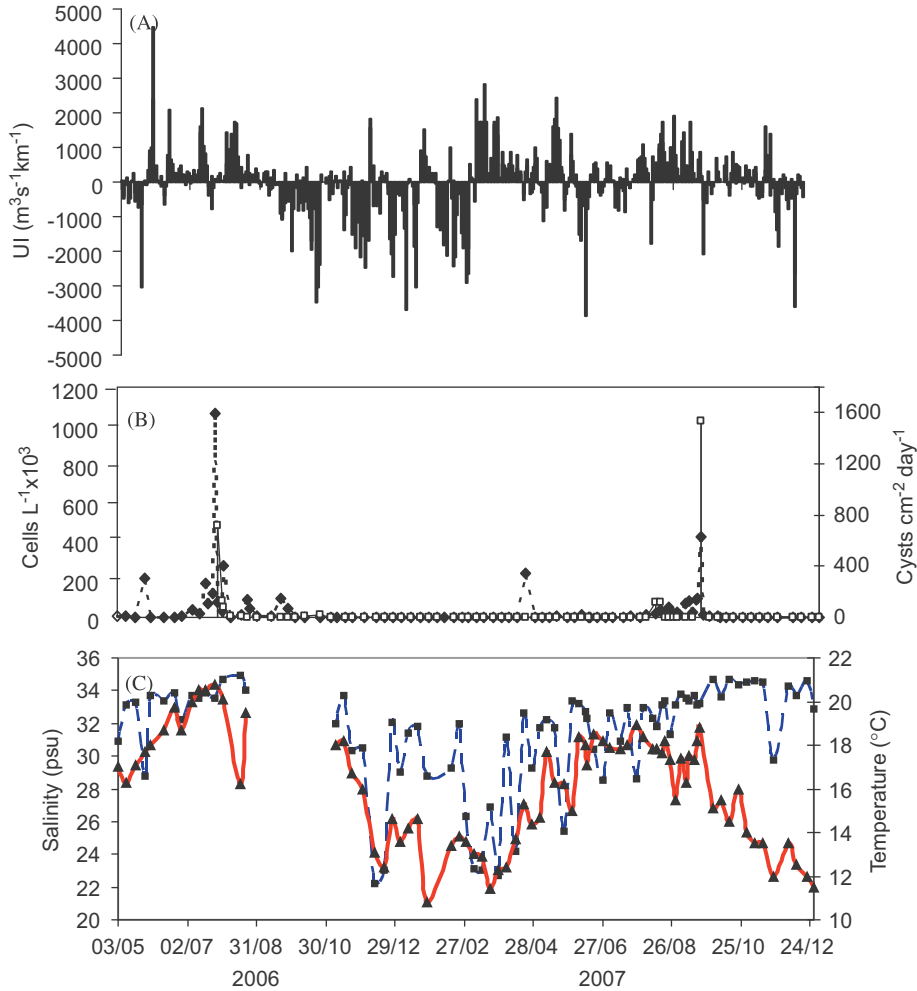
Fig. 4. (A) Upwelling index (UI), (B) vegetative cells (diamonds) and resting cysts (squares) of *Alexandrium minutum*, and (C) surface temperature (triangles) and salinity (squares) at station P, inside Baiona Bay, from May 2006 to December 2007. Positive and negative values of UI indicate favorable conditions for upwelling and downwelling, respectively.
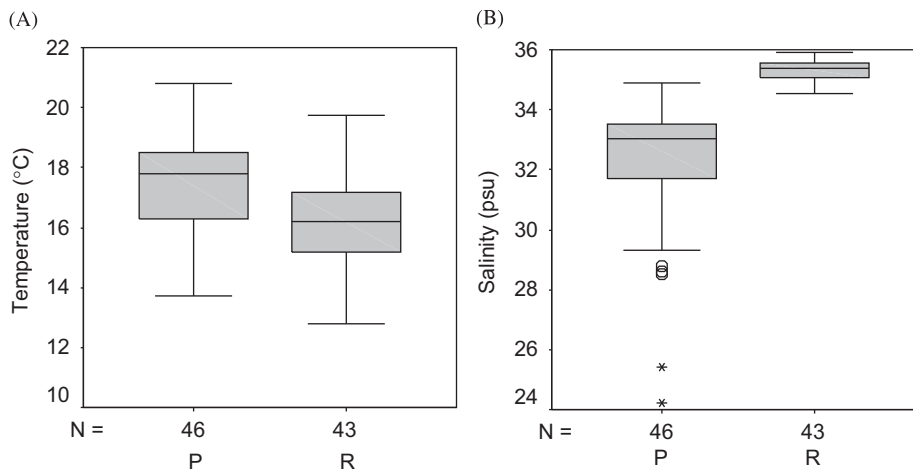

Fig. 5. Means and interquartile ranges for (A) temperature and (B) salinity inside Baiona Bay (station P) and the Ria of Vigo (station R) in spring and summer 2006 and 2007.
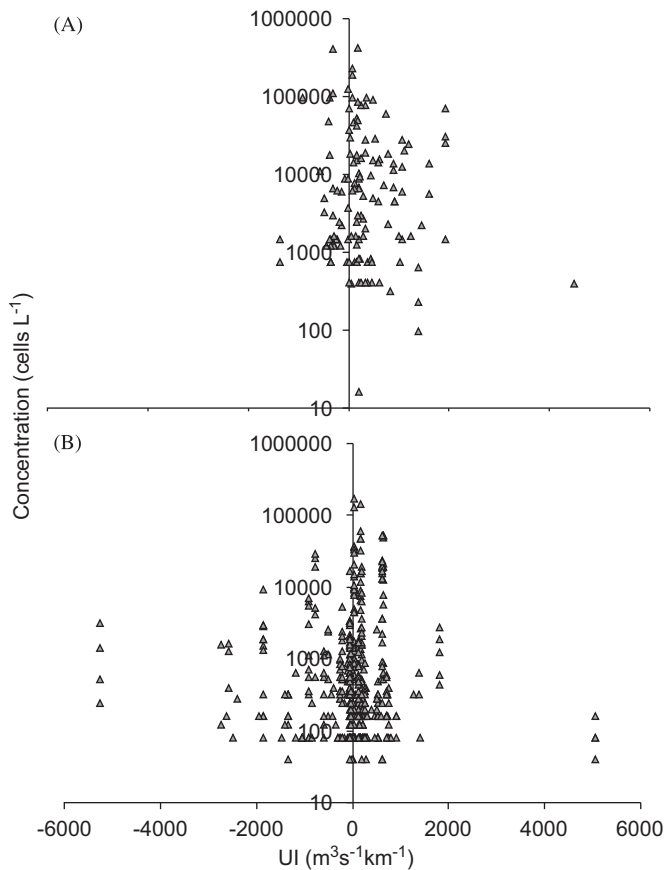
Fig. 6. Scatterplots of the cell concentrations of (A) *Alexandrium minutum* and (B) *Gymnodinium catenatum* in Galician waters from January 2005 to December 2007 against upwelling indexes. Only concentrations above zero are shown.

entrance of cold and saline water through the bottom. However, in the inner part of the rias and inside embayments such as Baiona Bay, this upwelled water is sun-heated and lowers the salinity due to river runoff. Consequently, there were significant differences in spring and summer in the temperature and salinity averages ($p < 0.01$) between the values of these variables at a station located inside Baiona Bay (station P), where *A. minutum* blooms developed, and a station in the mouth of the Ria of Vigo (station R) (Fig. 5).

(2) The *A. minutum* population was associated mostly with positive UI (Fig. 6A). The intensity of the bloom varied inversely with intensity of upwelling, 70% of cell densities over 10^4 cell L^{-1} occurring at UI lower than 5×10^2 m^3 s^{-1} km^{-1}. The most intensive bloom lasted from mid-July to early August 2006, coinciding with upwelling indexes below 250 m^3 s^{-1} km^{-1} for more than 1 week and reaching up to 1×10^6 cell L^{-1} on 26 July 2006 (Fig. 4).

(3) The spatial distribution of cells was associated with a salinity gradient induced by the river discharges. On 26 July 2006, during the top of the bloom, at low tide when all discharged water from the estuary is distributed in the bay, the highest concentrations of cells were observed in the south associated with less saline water (Fig. 7A, B), whereas no cells were detected at the outermost station (station B in Fig. 1B). On 2 August, when the bloom declined, at high tide when seawater floods into the inner part of the estuary, the highest concentration of cells (up to 3×10^5 cell L^{-1}) was observed inside the estuary, associated with salinities of 32–34 psu (Fig. 7C, D). On that date, 1.8×10^4 cells L^{-1} were detected at the B station (Fig. 1B).

G. catenatum blooms were also recurrent in the region, though following a very different pattern to that of *A. minutum* blooms (Fig. 3A, B). They occurred in autumn and winter and appeared in
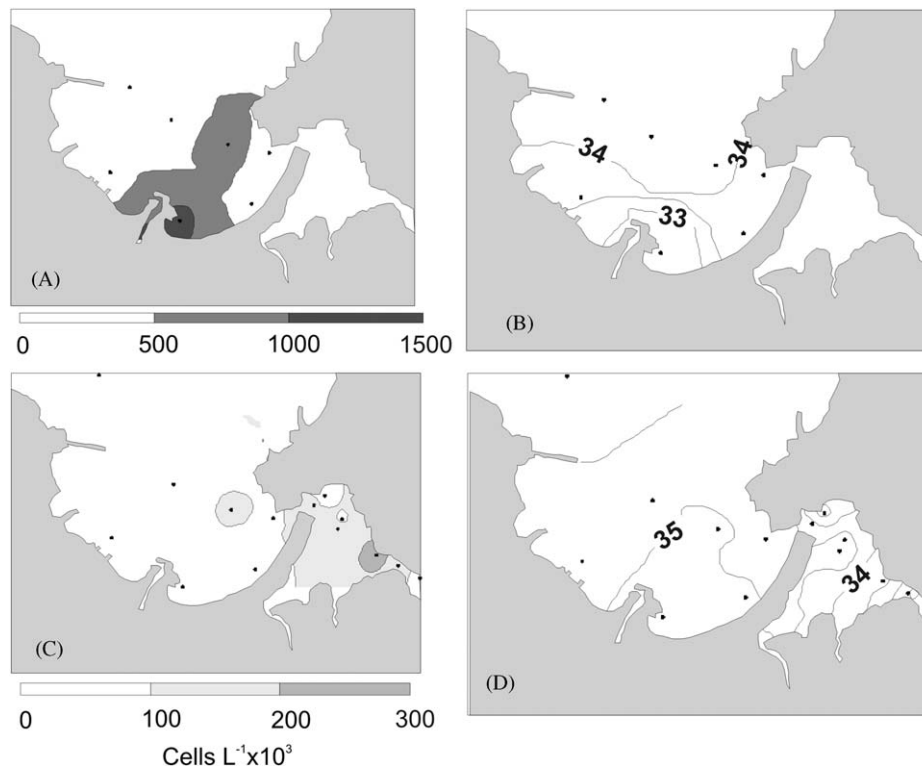
Fig. 7. Spatial distribution of *Alexandrium minutum* cell concentrations and salinity during (A and B) low tide on 26 July 2006 inside Baiona Bay and during (C and D) high tide on 2 August 2006 inside Baiona Bay and in the estuary of the Miñor River.
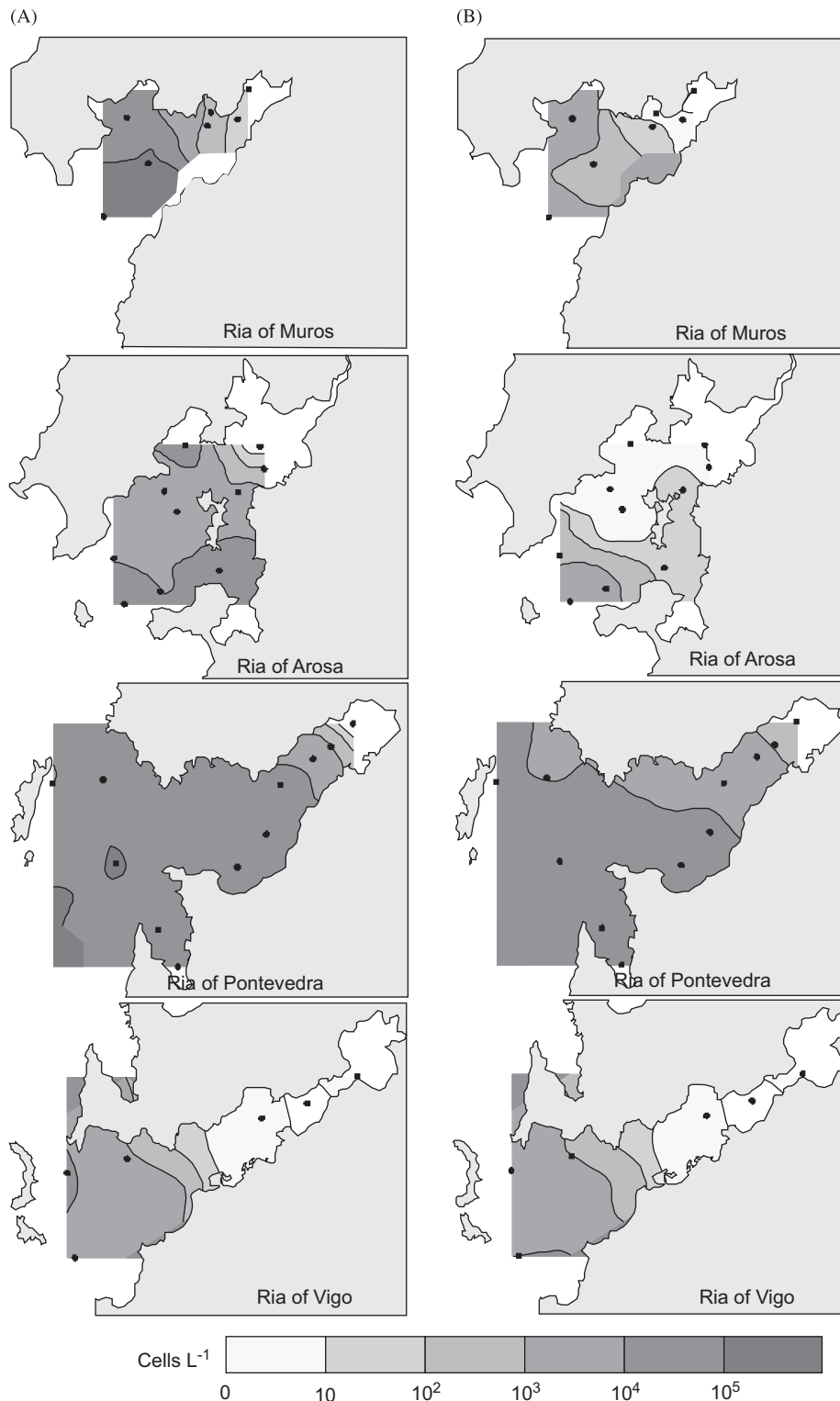
(A)   (B)

Fig. 8. Spatial distribution of cell concentrations of *Gymnodinium catenatum* in the Rías Baixas (A) on 8 November and (B) on 5 December 2005.

2005, 2006 and 2007 but in very different concentrations. The most intensive bloom occurred in autumn 2005, when cell concentrations reached up to 6.3×10^5 cell L^{-1}. In 2006 and 2007, the maximum concentrations were 2×10^4 and 6×10^2 cell L^{-1}, respectively. The blooms developed in autumn and affected all four Rías Baixas (Fig. 3B). In 2005, when the most intensive bloom occurred, a few cells were first seen in early July at the outermost station of Ria of Arousa and in late July, August and

September in all the Rías Baixas. In autumn, the bloom developed in two peaks, reaching maximum concentrations of 6.3×10^5 cells L^{-1} in the northeast Ria of Muros on November 8 (Fig. 8A). A second peak of cell concentrations on December 5 reached up to 9.2×10^4 cells L^{-1} in the Ria of Pontevedra (Fig. 8B). The spatial distribution of cells followed the same pattern in both summer and autumn. The highest concentrations were always detected in the mouth of the rias, only 7% of the cells being at the innermost

stations. Although the bloom declined in December, concentrations of cells lower than 10^3 cells L^{-1} were occasionally detected until spring and summer 2006. In that year, the autumn bloom reached a maximum of only 2×10^4 cells L^{-1} (Fig. 3B). The spatial distribution was different from that of the previous year because 53% of the population of the autumn bloom was detected at the innermost stations and 35% in the mouth of the rias. The increase of cells in autumn was associated with autumn downwelling when the water column inside the rias is mixed, showing temperatures of 14–17 °C in 2005 and 16–19 °C in 2006, in contrast to the stratified water column described in the upwelling season (Fig. 3C, D). The relationship between *G. catenatum* concentrations and the UI is shown in Fig. 6B. *G. catenatum* was mostly associated with negative or bellow $250 \, m^3 \, s^{-1} \, km^{-1}$ UI, although part of the distribution also included higher values corresponding to the phase of the 2005 bloom that occurred in November and December, when alternating upwelling and downwelling events supported the bloom for a longer time. This feature and sexual stages during the 2005 bloom are described by Figueroa et al. (2008).

3.2. Life cycle events

A. minutum sexual stages such as the planozygote or the gamete are hardly distinguishable from vegetative cells by light microscopy. Only mature planozygotes have been reported to be bigger and darker than vegetative cells and gametes (Figueroa et al., 2007). During the summer bloom of 2006, dark biflagellate planozygotes were observed in live samples on 26 July and 2 August, coinciding with the maximum and the decline of cell concentrations. We performed two studies on those dates in order to check sexuality occurrence in the field: (1) classification by cell size, and (2) trap experiments to distinguish different types of cysts and perform cyst flux measurements.

Cell sizes showed significant differences ($p < 0.01$) between 26 July (22.4 ± 2.3 µm long, 20.0 ± 2.4 µm wide) and 2 August 2006 (26.7 ± 3.2 µm long, 22.6 ± 2.3 µm wide) (mean ± sd, $n = 346$). The presence of mature planozygotes on 2 August is quite apparent in the size class of 19–36 µm length and 16–31 µm width against 16–27 µm length and 13–26 µm width on 26 July (Fig. 9).

During the temporal study of cyst sedimentation carried out by means of a trap experiment in Baiona Bay during 2006 and 2007, two types of cysts of *A. minutum* were detected in the traps: (1) double-walled resting cysts which matched the original description of Bolch et al. (1991) and originated from sexual reproduction and (2) pellicle cysts whose origin is unknown, and whose morphology is described by Bravo et al. (2010). Up to 7×10^2 and 1.5×10^3 resting cysts $cm^{-2} \, day^{-1}$ were detected in the bay in late July 2006 and middle September 2007, respectively, coinciding with the largest annual peaks of vegetative cells (Fig. 4). They were also observed in very low concentrations (1.2×10^2 resting cysts $cm^{-2} \, day^{-1}$) in mid-August 2007. The trap experiment performed to study spatial distribution of cyst fluxes in the area during the peak of vegetative cells that occurred in late July 2006 showed a spatial heterogeneity in resting cyst deposition. Major differences in resting cyst deposition were detected among traps from different stations. Cyst fluxes of $7.2 \times 10^2 \pm 1.7 \times 10^2$ resting cysts $cm^{-2} \, day^{-1}$ were observed in the bay and from $2.3 \times 10^2 \pm 2 \times 10^2$ to $12.3 \times 10^3 \pm 3.3 \times 10^3$ resting cysts $cm^{-2} \, day^{-1}$ in the estuary (Table 2). The same pattern occurred during the decline of the bloom in early August 2006, when cyst deposition ranged from $1.2 \times 10^2 \pm 10^2$ to $14.2 \times 10^3 \pm 10^3$ resting cysts $cm^{-2} \, day^{-1}$ (Table 2).

G. catenatum sexual stages were studied during the 2005 bloom. The following life cycle stages were detected in water
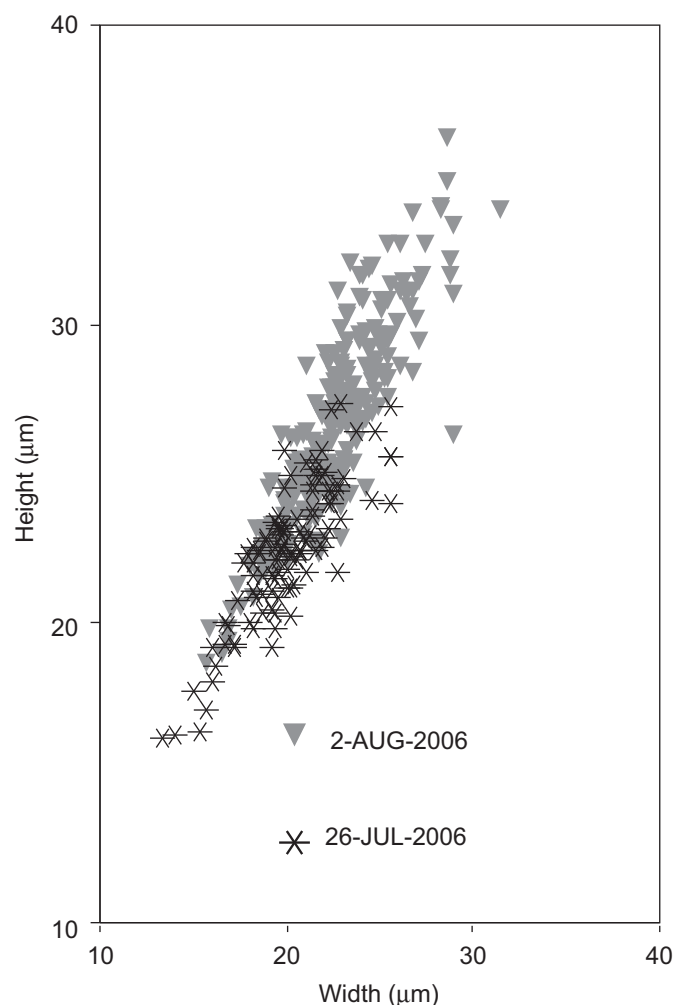

Fig. 9. Cell size distribution of *Alexandrium minutum* in Baiona Bay during the 2006 bloom.

Table 2
Means and standard deviations ($n = 2$) of resting cyst fluxes (cysts $cm^{-2} \, day^{-1}$) of *Alexandrium minutum* in Baiona Bay and in the estuary of the Miñor River.

Trap station[a]	27–28 July 2007	1–2 August 2007
1 (inner estuary)	1400 ± 140	750 ± 203
2 (inner estuary)	6250 ± 590	3000 ± 810
3 (inner estuary)	4560 ± 1220	2470 ± 1260
4 (middle estuary)	1170 ± 290	925 ± 620
5 (middle estuary)	12290 ± 3330	14250 ± 1060
6 (middle estuary)	1670 ± 320	820 ± 280
7 (middle estuary)	970 ± 980	340 ± 100
8 (middle estuary)	230 ± 200	160 ± 80
9 (bay)		120 ± 100
P (bay)	720 ± 170	

[a] See the position of stations in Fig. 1D.

samples: vegetative cells, fusing gametes, planozygotes and resting cysts. The morphology of these life cycle stages and features of sexual process during this bloom are described by Figueroa et al. (2008). The occurrence of all sexual stages showed a clear temporal distribution. Planozygotes were detected from 1 November to 29 December; on 13 December the planozygote: vegetative cell ratio showed a significant increase (Fig. 10A).
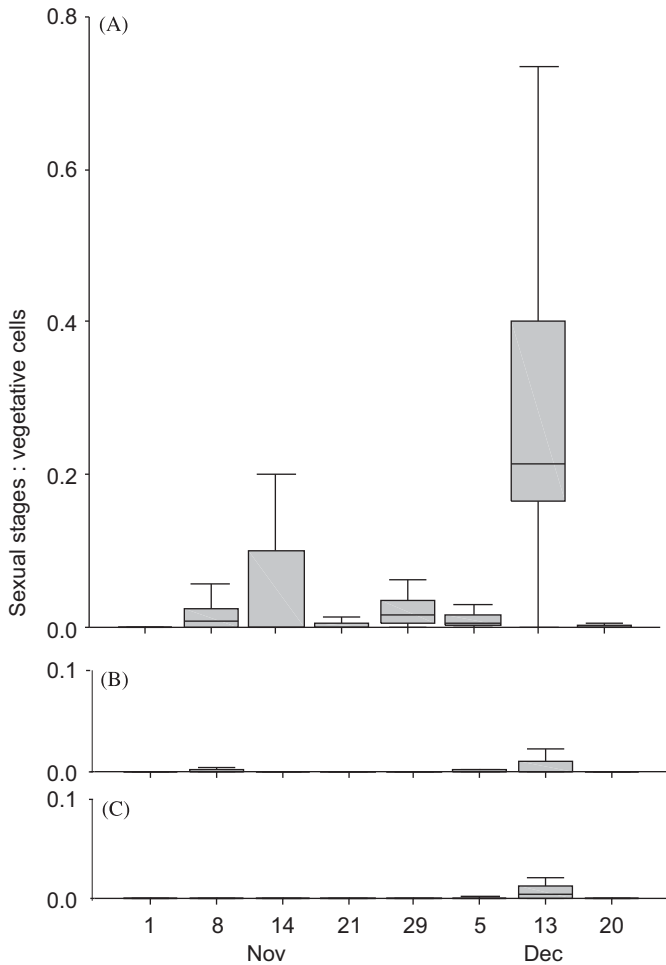
Fig. 10. Ratios of concentrations of (A) planozygotes to vegetative cells, (B) cysts to vegetative cells and (C) fusing gametes to vegetative cells of *Gymnodinium catenatum* in the Galician rias during the 2005 bloom. Means and interquartile ranges are shown.

Up to 1.9×10^4 planozygotes L^{-1} were detected on that date in the outer part of the Ria of Pontevedra, coinciding with the maximum number of vegetative cells. Cysts and fusing gamete concentrations were lower in number than planozygotes although they showed the same temporal pattern (Fig. 10B, C). On the other hand, the sexual stage:vegetative cell ratio did not show differences in either depth or spatial distribution. The study of cell sizes of the whole population by dates showed a considerable cell size increase on December 13 and 20 (Fig. 11).

3.3. Cyst distribution in the sediment

All the cysts from the sediments taken into account in the present study matched the original descriptions of the resting cysts of the two studied species (Bolch et al., 1991; Anderson et al., 1988). The cyst distribution studies revealed considerable differences between species. The concentration of the cysts of *G. catenatum* and *A. minutum* in the sediment of Baiona Bay was highly related to the previous occurrence of the blooms. No cysts of *G. catenatum* were observed in May 2005, but the concentration was high (up to 1.9×10^3 cysts cm^{-3} of wet sediment) in December 2005 after the occurrence of the bloom of this species in the autumn. The maximum was in the first cm of sediment depth while the average in the first four centimeters of sediment
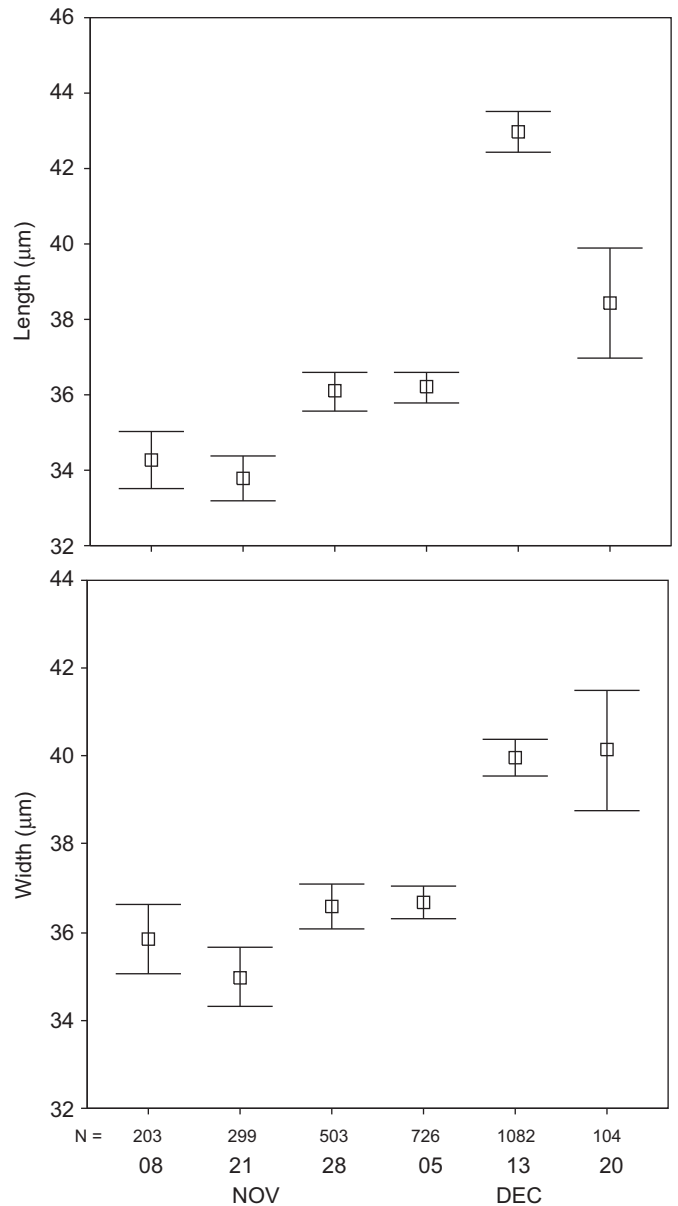
Fig. 11. Cell sizes of whole populations of *Gymnodinium catenatum* in the Galician rias during the 2005 bloom. Means and confidence interval (95% level) are shown.

(Fig. 12A) showed far lower values. In March 2007, resting cyst concentrations of this species underwent a considerable decrease (up to 80 cysts cm^{-3} of wet sediment) (Fig. 12B) and the cysts were homogeneously distributed in the 4 cm of sediment depth. *A. minutum* resting cysts showed a different pattern of distribution in both time and space. In December 2005, when the concentration of *G. catenatum* cysts was high, that of *A. minutum* cysts was very low (maximum of 20 cysts cm^{-3} of wet sediment) (Fig. 12C). However, higher concentrations (up to 4×10^2 cysts cm^{-3} of wet sediment) were detected in March 2007. The average of the cysts of *A. minutum* in the first 4 cm of sediment in December 2005 and March 2007 are depicted in Fig. 12C and D, respectively. In both surveys, *A. minutum* cysts were distributed quite homogeneously in the vertical profile of sediment. The comparison of the cyst distribution pattern of the two species showed interesting differences. The concentration of *G. catenatum* cysts in the southwestern part of the area was related to the entrance of more saline water from the external part
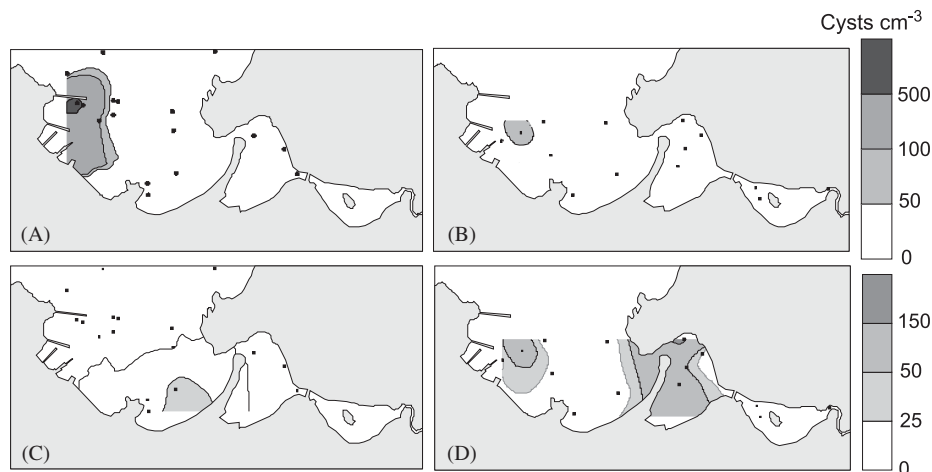
Fig. 12. Distribution of cysts (average of the first 4 cm of sediment, expressed as cysts cm^{-3} of wet sediment) of *Gymnodinium catenatum* and *Alexandrium minutum* on (A and C) December 2005 and (B and D) March 2007.

of the bay, whereas the concentration of *A. minutum* cysts in the southeastern part was related to the less saline water from the river (compare Fig. 12A and B with C and D).

4. Discussion

G. catenatum has been reported among other dinoflagellates – e.g. the also toxigenic *A. catenella* – to be a phytoplankton species that has evolved through seasonal succession in upwelling systems (Kudela et al., 2005). The higher swimming capabilities of these chain-forming dinoflagellates have been reported to give them competitiveness to succeed in these ecosystems (Fraga et al., 1989). However, upwelling habitats also exhibit a great variety of spatial and temporal heterogeneities that lead to many ecological niche types in which typical non-upwelling species may develop. In this paper we present data that corroborate *G. catenatum* as a negative upwelling-associated species, and we compare it with *A. minutum*, which is reported to be a coastal embayment species, in the same area.

The differentiation of the environmental features of each species shows interesting results. In Galician waters, the blooms of *A. minutum* and *G. catenatum* showed a very different spatial and seasonal pattern. The former bloomed in spring and summer associated with moderately positive upwelling indices, whereas the latter bloomed in autumn associated with negative upwelling indices that reflect coastal downwelling. The blooms of *G. catenatum* studied in this paper followed the pattern already reported in the literature, occurring in autumn coinciding with the relaxation of the upwelling that drives the cells inshore of the rias where they accumulate and grow (Fraga et al., 1988; Crespo et al., 2006). Those of *A. minutum*, on the other hand, developed inside coastal embayments in spring and summer, associated with stratification and the dominance of positive upwelling indices. The fact that the intensity of the bloom varied inversely with the intensity of the upwelling indices suggests that, although the blooms occur with northerly winds, the intensity may be critical for maintaining the population inside the rias. The inverse relationship between wind intensity and dinoflagellate bloom formation and duration has been reported previously (Yamamoto et al., 2002; Basterretxea et al., 2005). The blooms of *A. minutum* were associated with an embayment or a small ria, and with less saline water from the river situated in the south of the bay. These features clearly show that the dynamics of *A. minutum* in Galician rias seems to be more related to red tide-forming dinoflagellates

in a semi-enclosed environment than to the pattern explained above for *G. catenatum*. Discolored red tides of *Lingulodinium polyedrum*, several species of *Gonyaulax* and *Myrionecta rubra* (=*Mesodinium rubrum*), were already reported by Margalef (1956) in Galician rias, associated with long periods of great stability and lack of circulation between the rias and the sea. In these conditions, diatoms are lost by sedimentation being replaced by more-competitive dinoflagellates. This matches well with the characteristics of the blooms of *A. minutum* in other regions. Blooms of *A. minutum* occur all over the world associated with salinity stratification and local nutrient-rich freshwater inputs (Blanco et al., 1985; Cannon, 1990; Chang et al., 1996; Erard-Le Denn, 1997). Moreover, very intensive blooms of this species occurring in the Mediterranean seem to be restricted to nutrient-enriched semi-enclosed coastal sites, particularly harbors, estuaries and lagoons (Giacobbe et al., 1996; Vila et al., 2005; Bravo et al., 2008).

Margalef (1978) pointed out that the different life histories of phytoplankton are viewed as a result of selection under different environmental pressures. As such, the two different mechanisms of bloom formation of *G. catenatum* and *A. minutum* should be supported by different life strategies in the Galician rias, as has already been described in the literature (Bravo and Anderson 1994; Figueroa et al., 2006, 2007). There are two main differences between the life cycles of these species: the sexual cycle flexibility and the capability of forming different types of benthic stages. The higher capacity of *G. catenatum* to interchange between haploid and diploid forms has been evidenced by life cycle studies in both culture and nature by Figueroa et al. (2006, 2008), who describe the division capacity of cultured and wild *G. catenatum* planozygotes, division and encystment of fusing gamete pairs after syngamia, and an even shorter dormancy period than previously reported (Bravo and Anderson, 1994) for the sexual resting cysts (6 days). These characteristics may support the conclusion that sexuality is rather a frequent and flexible route for this species. Also, new sexual stages were recently described in a natural bloom of this species (Figueroa et al., 2008). Although the origin of these stages, described as chains of both reticulated and non-reticulated cysts, is unknown, the morphology of the chained cysts suggests a sexual origin, indicating that important features of this dinoflagellate sexuality are still unknown. Although planozygotes of *A. minutum* can also divide, division percentages in culture are lower (Figueroa et al., 2007) and the versatile cycle of *G. catenatum* was not described for *A. minutum* (Figueroa et al., 2007).

A. minutum can form a pellicle cyst in addition to resting double-walled cysts (Laabir et al., 2007; Garcés et al., 2004; Bolli et al., 2007; Bravo et al., 2010). However, although these thin-walled cysts of *G. catenatum* have also been described in cultures (Blackburn et al., 2001), their viability was not tested and they have not been reported elsewhere. The pellicle cysts of *A. minutum* underwent similar oscillations to those of vegetative cells, which could be due to changes in wind intensity and direction (Bravo et al., 2010). In such a dynamic environment, those pellicle cysts can probably play an important role in maintaining the population in a particular protected area—due to the strong northerly winds in the area the cysts could help to avoid dispersion and keep the population inside Baiona Bay. The sedimentation of pellicle short-dormancy cysts of *A. taylori* at night has been described as a mechanism for avoiding the dispersion caused by wind, which minimizes advective losses of cells and promotes the blooms of this species reported on Mediterranean beaches (Basterretxea et al., 2005; Garcés et al., 2002).

The Iberian upwelling system should be understood as a variety of ecological niches based on different geographical configurations in which each species is adapted. Hence, there will be a variety of adaptive features for all the species common to upwelling systems. The variety of sub-habitats that can be found in upwelling systems will match with a variety of species with different adaptive strategies. The two species referred to in the present paper are only one example. Another toxic species reported in upwelling systems is *Lingulodinium polyedrum*, which shows a dynamic similar to that of *A. minutum* in Galician waters, appearing in summer and related to cyst beds (Blanco, 1990). However, the life cycles of the two species differ clearly in the type of heterothallism and the sexual pellicle differences on cyst formation (Figueroa and Bravo, 2005; Figueroa et al., 2007).

It appears, in principle, that life cycles are more species-specific than environments. *A. catenella* has been reported as a phytoplankton species from the South African upwelling system. The life cycle for this species shows considerable differences from that of *G. catenatum*, the typical species from the Iberian upwelling system. For example, it shows a simple heterothallic behavior, a longer dormancy period and no reported planozygote division (Figueroa et al., 2005). However, all these characteristics need to be confirmed because a higher number of strains may be needed to find a complex heterothallic behavior, the dormancy period varies greatly between *A. catenella* isolates and even within the same clonal cross, and the planozygote study was performed with only old planozygotes, which are known to lose the capacity of division (Figueroa et al., 2006, 2008). Additionally, it is necessary to carry out the life cycle and species definition of *A. tamarense* complex species before trying to reach any conclusion on their adaptive strategies.

Further information is needed on dinoflagellate biology and ecology in order to link the complexity of adaptive strategies and life cycles with the ecological mosaics of the multiple sub-habitats that can exist in upwelling systems. However, for an oceanic species, as seems to be the case of *G. catenatum*, closing the sexual cycle in the water column might be more favorable than going through the benthos by means of a resting cyst. In this scenario, the strategies of both *G. catenatum* and *A. minutum*, the latter based on either fast (pellicle, short-lived cysts) or slow (resting, long-lived cysts) benthic transitions, seem to fit a logical pattern.

The phytoplankton seasonality in a particular area has been related to the seeding strategies that provide the inoculum for subsequent blooms. This long-term survival approach shown by many dinoflagellate populations is species-specific, and develops through different life cycle strategies. In the case of *G. catenatum* blooms in Galician waters, both vegetative cells (Fraga et al., 1988) and resting cysts (Figueiras and Pazos, 1991) have been proposed

as being used as a seeding population. Our data on cyst distribution in Baiona Bay and the Ria of Vigo from Figueroa et al. (2008) proves that high cyst seeding occurred in the area. These authors also showed that a high proportion of the cyst germination occurred only three months after the bloom, while there was no bloom in the water. Furthermore, the data from the present paper show that cyst concentrations and blooms in Baiona Bay are not directly related since the 2005 bloom occurred without there being cysts in the sediment, and the seeding from that bloom was not followed by a corresponding large bloom either in the following months or in autumn. These observations corroborate the hypothesis of Figueroa et al. (2008) that the bloom in 2005 was caused by an inoculum derived from off-shore vegetative populations. However, it cannot be ruled out that the resting cysts left in the sediment made some contribution to inoculating cells into the water column in 2006 and 2007. This could explain why in the summer and autumn of 2006 and 2007 the proportion of vegetative cells was higher at the internal stations, whereas in 2005 it was not.

The transitions from planktonic to benthic stages in *A. minutum* have been proven to be very important in its dynamics on the Mediterranean coast (Garcés et al., 2004). The data presented here indicate that seeding of this species also occurred during blooms at Atlantic sites. The recurrence of blooms in Baiona Bay is probably due to cyst germination since, unlike blooms of *G. catenatum*, they show no evidence of arrival of off-shore population. Moreover, the data on cyst sedimentation in the traps and cyst distribution in the sediments showed that resting cyst accumulation was related to freshwater input from the river, which strengthens the ecological trend of populations of this species. It is noteworthy that in the contiguous Ria of Pontevedra there is a bay with the same orientation as Baiona Bay and the Ria of Ares, but no blooms of *A. minutum* have ever been reported there. This difference might be explained by the absence of freshwater inputs in that bay, which makes it different from the others. In contrast, *G. catenatum* cyst distribution in the sediment of Baiona Bay was associated with more saline water from off-shore, which further supports the differences in the bloom strategies of these two species. The explanation for *A. minutum* is confirmed by the results obtained in cultures. An inverse relationship between *A. minutum* resting cysts and salinity was detected during an experiment based on a three-factorial design by Figueroa et al. (submitted). These authors showed that encystment was regulated by temperature and salinity. Salinity was the primary independent variable and had a negative effect on both cyst per cell production and cyst yield (cysts L^{-1}).

5. Conclusions

The present study was carried out in natural populations and focused on elucidating the life strategies of two phytoplankton species based on the differentiation of life cycle stages in field samples. The data presented herein indicate that vegetative population and cyst data can be used to discriminate within the same region between different blooming strategies shown by two dinoflagellate species, which are related to the seasonal pattern defined by upwelling and downwelling episodes. The distinct blooming strategies of *G. catenatum* and *A. minutum* corresponded well with their different life cycle histories. Resting cyst deposition occurred for both species during the period covered by the present study. However, the data presented herein suggest that cysts play a very different role in each species. Specifically, a direct relationship between bloom intensity and cyst distribution was not observed for the population of *G. catenatum*. This was consistent with the high interchange between haploid and diploid

forms characteristic of this species, suggesting that sexual reproduction in *G. catenatum* is quite frequent and that its seasonal blooms are not related to cyst germination. By contrast, the presence of both vegetative populations and resting cysts of *A. minutum* was strongly related to less saline inshore habitats. Moreover, the data support the importance of resting cysts in the recurrence of *A. minutum* blooms and that the occurrence of these blooms does not depend on the delivery of off-shore populations.

Acknowledgments

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