Population dynamics of *Dinophysis acuminata* in the Ría de Pontevedra (NW Spain): Physical and biological coupling in a coastal upwelling system

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**Abstract**

Fine resolution measurements of phytoplankton and physical parameters were made from 31 May to 14 June 2005 in the coastal upwelling system of the Ría de Pontevedra (NW Spain). A sequence of upwelling-relaxation-upwelling-downwelling events was recorded with a moored Acoustic Doppler Current Profiler (ADCP). During the upwelling pulses, *Dinophysis acuminata* populations were found aggregated in near-surface patches (up to 8 x10^3 cells L^{-1}) located in the warmer (15-18°C) surface (0-6 m) waters. In contrast, on 13 June 2005, when downwelling conditions occurred, *D. acuminata* reached its highest concentration (9 x 10^3 cells L^{-1}) and spread in a near surface layer throughout the whole Ría. In this study, results from the examination of tides, currents, temperature, salinity, satellite images and wind records, together with the fine-scale vertical distribution of plankton species highlight the potential role of physical processes in promoting and transporting *D. acuminata* populations in the Ría de Pontevedra. Understanding both local fine-scale circulation patterns, regional physical processes and physical-biological coupling at different scales will improve our knowledge of the spatial and temporal occurrence of *D. acuminata* blooms in coastal upwelling systems. 

**Keywords:** *Dinophysis acuminata*, physical-biological interactions, harmful algae blooms, coastal upwelling system

1. Introduction

Diarrhetic Shellfish Poisoning (DSP) is a gastrointestinal disease resulting from ingestion of shellfish contaminated with lipophilic shellfish toxins (LSTs). Chronic occurrences of toxin-producing *Dinophysis* spp. cause the accumulation of DSP toxins in shellfish above regulatory levels. These harmful events, which even with low biomass can contaminate seafood, constitute the main threat for the Northeast Atlantic shellfish industry (Reguera and Pizarro, 2008). In the Galician Rías Baixas (NW Spain), *Dinophysis acuminata* populations are extremely persistent and can be found under a wide range of salinity (30-35.5 psu) and temperature (12-22°C). Abundance and seasonal patterns of *D. acuminata* populations show an alternation of peaks and troughs from March to October, through the whole upwelling season, with a significant increase during autumn downwelling events (Reguera et al., 1993; Escalera et al., 2006).

Different mechanisms have been suggested for the control of the development of *D. acuminata* blooms. Turbulence and current may act to dissipate or concentrate *D. acuminata* cells while the population numbers may change due to intrinsic features (division, mortality) and physical-biological interactions. In most studies of *Dinophysis* spp., its maximum cell concentrations have been often related to marked temperature- and salinity-driven density gradients in the water column (Maestrini, 1998;
Horizontal confinement within mesoscale eddies and offshore transportation have also been pointed as key mechanisms involved in bloom development (Lunven et al., 2005; Xie et al., 2007; Escalera et al., 2010).

Park et al. (2006) first reported on the maintenance of *D. acuminata* cultures by providing the photosynthetic *Mesodinium rubrum*. This breakthrough has contributed to a increase our knowledge of the physiology and biology of *Dinophysis*. Culture results suggested that *D. acuminata* is an obligate mixotroph that requires light, nutrients and live ciliate prey for long-term survival (Kim et al., 2008). Understanding the strategies by which *D. acuminata* maintains itself in the plankton community such long periods is a goal that is possible to reach now with the combination of laboratory and field experiments.

However, right now, our understanding of the biological, physical and chemical processes that regulate *D. acuminata* blooms in the field is still limited. Difficulties in understanding *D. acuminata* distributions in highly dynamics regions such as the Rías Baixas arise from a mismatch between the scales at which the biological and physical measurements are routinely made in field surveys and the scales of the mesoscale structures that influence plankton communities and processes. Thus, one of the keys to understand local changes observed in *D. acuminata* dynamics in the Rías lies in explaining how physical and biological processes interact to promote the bloom development, maintenance and decline.

During May- June 2005, a 2 week multidisciplinary cruise was carried out in Ría de Pontevedra with advanced instrumentation that allowed high resolution vertical sampling of physical and biological properties of the water column during an upwelling-downwelling cycle. This paper reports on the observed physical-biological interactions that may be responsible for the distribution and abundance of *D. acuminata* in the study area.

### 2. Material & Methods

#### 2.1. The study area

The Ría de Pontevedra was selected as the best location for studies related to *Dinophysis* spp. based on historical monitoring records of cell abundance and shellfish harvesting closures (Blanco et al., 1998). Ría de Pontevedra is a flooded tectonic valley located on the NW coast of the Iberian Peninsula (Fig 1). Hydrodynamics of the Ría are mainly driven by the wind regime (Prego et al., 2001). Northerly winds promote upwelling of cold, salty and nutrient-rich Eastern North Atlantic Central Water (ENACW) from spring to early autumn (March to October). Upwelling forces a two-layer density induced positive circulation, characterized by the outflow of surface waters and the compensating inflow of upwelled water at the bottom (Wooster et al., 1976) (Fig 2A). In autumn and winter (October to March) southerly winds are predominant in the area and the circulation reverses. Shelf water enters the Ría at the surface and there is a compensating outflow at the bottom (Prego et al., 2001) (Fig 2B). Changes in wind forcing lead to rapid changes (<24 h; Sousa 1995) in the oceanographic conditions of the Ría, which in turn force significant changes in plankton distribution and ecology (Tilstone et al., 2000).

#### 2.2. Sampling overview

Studies were carried out on board R/V *Mytilus* from 31 May to 14 June 2005. Sample locations are shown in Fig 3. The IFREMER high-resolution particle size/CTD/video analyzer (IPSAP) was used to obtain a fine scale vertical distribution of physical and biological parameters (depth, temperature, salinity, PAR and in vivo fluorescence) and of suspended particles in the water column. This profiler is able to collect samples from specific depths where structures of interest, chlorophyll maximum, pycnoclines and maximum particle load, are detected by means of a peristaltic pump on deck, connected in one end to the profiler with a 45-m hose, and with a water flow from a hose in the other end. Samples from each depth can be either collected directly from the profiler (unconcentrated natural samples) or the flowing water can be filtered through a set of superimposed framed meshes (150-70-20 µm) to obtain size-fractionated concentrations. The meshes are placed on top of a container that keeps the plankton fractions suspended in free-flowing seawater, and allows their recovery with the least possible damage to the cells.

Vertical net-hauls (20-µm mesh) and size-fractioned (20-70 µm) concentrates from specific depths (profiler) were collected to obtain a quick quasi real-time qualitative information on live phytoplankton. These samples were immediately examined on board under a Zeiss AX-IOVERT 135 microscope (100X, 400X) with epifluorescence and helped to make choices on the stations and depths where sampling efforts should be focused.

Plankton samples were collected according to the objectives pursued: i) Unconcentrated natural samples fixed with acidic Lugol’s solution for quantification of the phytoplankton community and of the ciliate *M. rubrum*, and ii) 500 mL to 1 L seawater samples concentrated through...
20-µm filters to a final volume of 50 mL, and fixed with Lugol’s for quantification of Dinophysis spp.

2.3. Meteorological and oceanographic observations

Sea surface temperature (SST) images from the Advanced Very High Resolution Radiometry (AVHRR) satellite sensor were obtained from NERC (Plymouth). Chlorophyll-a concentration was estimated by NERC from MODIS satellite sensor data using the OC3 algorithm.

Hourly mean values of wind speed and direction were obtained from the Seawatch buoy off Cabo Silleiro, at 42° 7.8’N, 9° 23.4’W (www.puertos.es; Fig 1), a representative site for studying the oceanographic conditions on the western Galician shelf (Torres et al., 2003).

Current velocity profiles were measured continuously at station 2 (Fig 1) with a bottom mounted Acoustic Doppler Current Profiler (ADCP) (RD Instruments, 614.4 kHz) moored from 30 May-13 June 2005. Reported current velocities are based on 2 min averages (bin size: 1 m) from raw ADCP data processed using WinADCP software (RD Instruments). ADCP distance to the surface was stimulated by post-processing the echo amplitude profile of pings.

2.4. Phytoplankton counts

Phytoplankton and M. rubrum counts were carried out under an inverted microscope (Nikon ECLIPSE 2000) following the Utermöhl (1931) method. The volume of the sedimentation columns (10-25 mL) was chosen depending on the plankton biomass (columns of 10 mL when chlorophyll a values were higher than 15 µg L⁻¹) to prevent the overlay of phytoplankton cells at the bottom of the chamber. Phytoplankton abundance was determined to species level when possible. Two transects were counted at 400X magnification to include the smaller and more abundant species and the whole bottom of the chamber at 100X to enumerate larger species. To count Dinophysis spp., 3-10 mL aliquots of the concentrated samples (1 L to 50 mL; factor: 1:20) were placed in sedimentation chambers, and the whole surface of the chamber scanned at a magnification of 100X, so that the detection limit was 5 cell L⁻¹.

3. Results

3.1. Oceanographic conditions during the survey

Winds measured at Silleiro buoy during the study period are plotted (Fig 4A) as well as tides (Fig 4B) and ADCP subtidal currents at 5 m from the bottom (Fig 4C) and 38 m from the bottom (Fig 4D), which serve as an estimation of near-bottom and near-surface currents. The figure shows that the effect of wind forcing in currents is modulated by tides. Northerly upwelling winds before the cruise induced an Ekman pattern of outflowing surface currents and inflowing in bottom layers. The coincidence of that upwelling pulse with neap tides result in a domination of the wind induced Ekman forcing, and no reversal of current direction with tide was found. This contrasts with the upwelling pulse around 06 June, when bottom (near-surface) currents were outflowing (inflowing) during ebb (flood) in spite of Ekman forcing.

The upwelling pulse at the beginning of the cruise did not result in appearance of upwelled waters on the surface (Fig 5). On 2 and 3 June, currents show that the relaxation of upwelling induced a surface inflowing, bottom inflowing pattern. Surface waters previously exported to the shelf came back into the Ría. Contrastingly, the longer upwelling pulse from 03-08 June is seen in satellite imagery to induce the appearance of cold and chlorophyll-a rich surface water (Fig 6) on the shelf exported from the Ría. Reversal of wind from 09 June induces a surface inflowing bottom outflowing at the mooring location and entrance of shelf waters (Fig 5).

3.2. Distribution of D. acuminata

Fig 7 show the horizontal distribution of D. acuminata and M. rubrum cell maxima at each station sampled in the Ría de Pontevedra during the survey. From 31 May to 10 June, patches of high D. acuminata cell abundance (5x10³ cell L⁻¹) were found moving around the Ría and almost never associated with the 27 °C isopycnal (10 m), where diatoms were dominant. Dinophysis acuminata was almost always aggregated in the warmer (15-18°C) surface (0-6 m) waters, with the species maximum associated with the diurnal thermocline (Fig 8, Fig 9, and Fig 10). One isolated patch of 10³ cell L⁻¹ was found below the 27 °C isopycnal at 21 m depth in cold upwelled waters on 31 May (sta 3; Fig 10). In this case, D. acuminata occurred within the ENACW nutrient-rich waters below the diatom TL at the pycnocline.

High density (5x10³ cell L⁻¹; sta 4 and 2) patches of M. rubrum, were also found within the same depth range, especially on 06-07 June (Fig 7). On 13 June, when downwelling conditions caused piling of warmer shelf waters in the Rías and disruption of stratification, D. acuminata reached its highest concentration (9x10³ cell L⁻¹) and spread throughout the whole Ría (Fig 7). This species formed a continuous near-surface TL located in the warmer waters associated with the diurnal
cruise. Thus, *D. acuminata* was still most abundant in the surface layer and not associated with the diatoms at the 27°σt isopycnal (Fig 8, Fig 9, and Fig 10).

### 4. Discussion

Previous observations (Blanco et al., 1998; Tilstone et al., 2000) supported the view that in the Galician Rías Baixas strong upwelling pulses disperse dinoflagellate populations offshore. However, this study shows that substantial numbers (> 10^3 cell L^-1) of *D. acuminata* persisted in Ría de Pontevedra throughout an upwelling-downwelling cycle. This species, which in 2005 persisted in Ría de Pontevedra throughout an upwelling event that substantial numbers (*ff*) in the surface layer and not associated with the diatoms at thermocline. Thus, *D. acuminata* Rías Baixas strong upwelling pulses disperse dinoflagellates in near surface patches that were located in the top 6 m and associated with the diurnal thermocline. During relaxation, and in association with downwelling pulses, *Dinophys*is appeared in high numbers (> 2x10^3 cell L^-1) and spread throughout the Ría.

The occurrence of high numbers of *Dinophys*is spp. in the Galician Rías has been associated with two different scenarios: *in situ* growth favored by periods of stratification between moderate upwelling pulses, and downwelling events that promote accumulation of large dinoflagellates, including *Dinophys*is spp., particularly at the end of the upwelling season (Reguera et al., 1995).

In our cruise, estimates of the intrinsic division rate (µ) of *D. acuminata* by the mitotic index approach (Reguera et al., 2003) during upwelling (03 June) and relaxation (09 June) showed moderate values of µ (µ_min = 0.16 d^-1), whereas those during downwelling conditions (13-14 June) were much higher (µ_min = 0.25 d^-1, µ_max = 0.56 d^-1) (González-Gil et al., 2010). Therefore, downwelling pulses, at least those of the magnitude measured during this cruise, did not seem to inhibit *D. acuminata* division. The observed numerical increase would have resulted from both *in situ* division and physically-driven accumulation, which selects for motile dinoflagellates under downwelling conditions (Fraga et al., 1988). Aggregation around a density gradient, with or without significant *in situ* division, may produce the frequent observations of *Dinophys*is maxima in stratified waters. A recent 24-h study of an autumn bloom of *D. acuta* in Ría de Pontevedra (Pizarro et al., 2008) showed that even in the case of a decaying population, with a division rate of almost zero, *Dinophys*is cells aggregated in a maximum around a shallow halocline.

The presence of *D. acuminata* at 21 m in the northern mouth of the Ría on 31 May (sta 3) deserves special attention. Given that high cell abundances of *D. acuminata* were always recorded at the surface after this date, this deeper-water *D. acuminata* population, transported into the Rías by inflowing water may have acted as an inoculum, and migrated from the upwelled waters into warmer subsurface waters to rebuild the pre-existing population. A similar mechanism could explain the presence of the only *D. acuminata* cell maximum observed within the Chl a maximum at station 2 during the second upwelling pulse on 07 June. In a 10-yr time series study in the same Ría, Pazos et al. (2005) established a relation between late initiation of *D. acuminata* populations and the lack of spring upwelling events. A similar mechanism was described by Townsend et al. (2001) for the transport of *Alexandrium* spp. cells by the Eastern Maine Coastal Current from offshore waters into the Gulf of Maine. Thus, upwelling pulses can represent a pathway through which recurrent *D. acuminata* populations, that wax and wane over the upwelling season (March-October) in the Galician Rías Baixas, are entrained from the adjacent shelf.

A seasonal trend in the vertical distribution of the population, from early growth starting in deeper water, to late stages aggregating near the surface, has been described for *D. acuminata* in the Rías (Escalera et al., 2006). Variations in the vertical distribution of the cell maxima for mixotrophic *Dinophys*is spp. could be attributed to different feeding behavior during different phases of the population. It was surprising that during this study, *D. acuminata* cell maxima were located in the top 5 m, where inorganic nutrients were depleted, high light intensities could produce photosynthesis inhibition, and where wind-forced surface currents were strongest. It was observed that there was a change in cell appearance, progression from small non-vacuolated cells to highly vacuolated, large cells (González-Gil et al., 2010), possibly related to feeding on patches of *M. rubrum* that were also found in the surface waters on 06-07 June. It may therefore be heterotrophic feeding, and the distribution of its specific prey (*M. rubrum*), that caused *D. acuminata* to be located in the upper layers at this phase of the population growth.

The presentation of the results although brief suggest that the interplay of physical forcing in this upwelling influenced shelf shapes a very variable environment. The presence of the Galician Rías, embayments where complex adjustment to wind and tidal forcing occurs enhances variability and increases the range of niches for *D. acuminata* to exploit.
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Figure 1: (A) Map of NW Iberian Peninsula and the location of the SeaWatch Buoy off Cabo Silleiro; (B) Map of the Ría de Pontevedra showing the location of the sta 2-ADCP mooring site
Figure 2: Simplified diagrams of the vertical circulation of the Galician Rías during: (A) upwelling and (B) downwelling
Figure 3: Spatial and temporal distribution of IPSAP profiler sampling sites.
Figure 4: Winds measured at Silleiro buoy (A) tides (B) and ADCP subtidal currents at 5 m from the bottom (C); and 38 m from the bottom (D) from 28 May to June 2005.
Figure 5: AVHRR-SST nighttime images for the study period. At the location of Silleiro buoy, blue arrow represent surface winds and black arrow near-surface (3m) subtidal currents. At the ADCP mooring location, currents at 5 m (black arrows) and 38 m (red arrows) from the bottom are plotted.
Figure 6: MODIS chlorophyll-a images for the study period. At the location of Silleiro buoy, blue arrow represent surface winds and black arrow near-surface (3m) subtidal currents. At the ADCP mooring location, currents at 5 m (black arrows) and 38 m (red arrows) from the bottom are plotted.
Figure 7: Horizontal distribution of *D. acuminata* and *M. rubrum* (cell maxima for each station) in the Ría de Pontevedra during the survey. (No *M. rubrum* data available on 10 and 13 June).
Figure 8: Vertical distribution and abundance of *D. acuminata* from 31 August to 13 June at station 1 (see location in Fig 3). Colour and contour lines: (A) temperature, (B) salinity and (C) Chl *a* concentration.
Figure 9: Vertical distribution and abundance of *D. acuminata* from 31 August to 13 June at station 2 (see location in Fig 3). Colour and contour lines: (A) temperature, (B) salinity and (C) Chl *a* concentration.)
Figure 10: Vertical distribution and abundance of *D. acuminata* from 31 August to 13 June at station 3 (see location in Fig 3). Colour and contour lines: (A) temperature, (B) salinity and (C) Chl *a* concentration.)