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Vertical distribution of division rates in coastal dinoflagellate *Dinophysis* spp. populations: implications for modelling

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ABSTRACT: This study explores vertical heterogeneities of *in situ* division rates (μ) of 3 species of *Dinophysis* and discusses its implications in modelling the population dynamics of these species. Based on a post-mitotic index approach, estimates of μ from vertical net-haul (integrated) samples (μ_{int}) were compared with those from a single depth (oceanographic bottle samples) (μ_z) at the cell maximum during 2 daily cell cycle studies in the Galician Rías Baixas (NW Spain). Additionally, vertical profiles of μ_z were obtained during a field survey in Ría de Vigo. A 2-fold difference was observed between estimates of μ_z from the cell maxima and μ_{int} from integrated samples under stratified conditions. Differences were much larger when the minimum estimates of μ values from different depths (μ_{min}) were compared within single vertical profiles. An exponential growth model was applied to simulate the dynamics of a *D. acuminata* population during a daily cycle in June 1994. Results show that actively dividing cells of a target species may be restricted to narrow layers of the water column. Estimates of μ at different relevant depths during cell cycle studies may be key to determining whether if increased numbers of a target species are due to *in situ* growth or to the balance of imports and exports.

KEY WORDS: *Dinophysis* spp. · Cell cycle · *In situ* division rate · Mitotic index · Cell maximum · Modelling approach

INTRODUCTION

The development of *Dinophysis* spp. populations that produce lipophilic shellfish toxins (LSTs) — okadaic acid (OA), dinophysistoxins (DTXs) and pectenotoxins (PTXs) — represents a major hazard for the Northeast Atlantic shellfish industry (Reguera & Pizarro 2008). Chronic occurrences of *Dinophysis* spp. result in shellfish with LSTs above levels permitted in the EU (Anonymous 1991, 2002) leading to prolonged bans on harvesting in affected areas. To improve prediction of LST outbreaks (including their duration and decline) it is essential to identify the different scenarios — with regard to *in situ* growth, behaviour (vertical migration, aggregation), physically induced accumulation (advection, downwelling, convergence areas) and physical–biological interactions — that may promote numerical increase of *Dinophysis* spp. populations.

The specific division rate, μ, is an important intrinsic parameter in the growth equation that needs to be determined. This parameter estimates the potential for intrinsic division without the interference of losses due to grazing, mortality and physical dispersion (Carpenter & Chang 1988). Even more important for modelling purposes is to estimate μ_{max}, i.e. the maximum potential division rate of the species under optimal conditions when resources are not limiting. In the present study we consider μ_{max} as the maximum division rate estimated for a target species in a given scenario. It is to be expected that μ_{max} of a species, at a given time

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and place, will be observed at its optimum depth in the water column, i.e. the depth where the combination of environmental conditions (resources and physical–biological interactions) are the most suitable for that species' growth and survival.

Dinophysis spp. and other toxin-producing algae (TPA) species that transmit toxins through the food web — even at moderate to low concentrations (10^2 to 10^3 cells l^{-1}) — often constitute a small percentage of the total phytoplankton community. Bulk measurements using chemical indicators such as chlorophyll *a* concentration and primary production are unsuitable for growth studies of these rare TPA species since they reflect the physiology not of the target organisms but of the whole plankton community. Estimates of μ from *in situ* cell cycle studies are time-consuming, but provide realistic information on the division capabilities of the species in question (Reguera et al. 2003).

Estimates of μ in field populations of *Dinophysis* spp. have been obtained either from incubations, on deck or in diffusion chambers (Garcés et al. 1997) or from direct *in situ* high frequency sampling with a mitotic index approach (Table 1). *Dinophysis* spp. often exhibit sharp heterogeneities in their vertical distribution (reviewed in Reguera & Pizarro 2008, Velo-Suárez et al. 2008). To minimize sampling uncertainties derived from low cell concentrations, vertical migration or patchiness (which would result in samples with inadequate numbers of target cells), *in situ* division rate studies of *Dinophysis* spp. have been carried out mainly using vertical net-hauls to obtain integrated samples from the whole water column. In this way, integrated values of μ (μ_{int}) rather than values of μ at specific depths (μ_z) are obtained; however estimates of μ_{max}, i.e. the maximum value of μ in a given scenario, are unobtainable.

Potential growth is often greater than observed net growth, and differences between the two are attributed to cell losses (Garcés & Masó 2001). Net changes in population density result from the balance between gains (growth and physical accumulation) and losses (grazing, mortality and physical dispersion). Knowledge of the variations of these parameters is thus fundamental to understanding the processes leading to bloom formation and maintenance. The contribution of division and mortality rates to *Dinophysis* spp. population dynamics in the Rías Baixas can be analyzed with the use of a simplified biological model. Although these models are generally unable to represent all the variability of an ecosystem, they can provide the necessary framework for exploring different aspects of harmful algae outbreaks (Franks 1997).

In the present study, we compare values of μ estimated from integrated water-column samples (hereafter referred to as 'integrated samples') (μ_{int}) with values based on samples collected at specific depths (μ_z), especially those collected at the depth where the maximum concentration of *Dinophysis* spp., P_{max}, was detected ($\mu_{P_{max}}$). The importance of vertical heterogeneities in division rates of populations and their implications for population dynamic models are discussed.

Table 1. *Dinophysis* spp. *In situ* estimated division rates (mitotic index approach; d^{-1}) of several species of *Dinophysis*. For *D. acuminata*, 1st value: polynomial curve fit; 2nd value: periodic curve fit

Species	Location	Date	μ (d^{-1})	Source
D. acuminata	Long Island, NY, USA	Jul 1997	0.54	Chang & Carpenter (1991)
			0.67	
	Galician Rías, Spain	Jun 1994	0.47	Reguera et al. (2003)
		Oct 1994	0.26	
		Jun 1997	0.09	
		Jun 2005	0.56	S. González-Gil et al. (unpubl.)
	Gullmar Fjord, Sweden	Oct 1995	0.75	Gisselson et al. (1999)
D. acuta	Galician Rías, Spain	Oct 1994	0.65	Reguera et al. (2003)
		Oct 1997	0.33	
		Nov 2005	0.03	Pizarro et al. (2008)
D. caudata	Galician Rías, Spain	Oct 1994	0.24	Reguera et al. (2003)
		Oct 1997	0.25	
D. fortii	Santa Monica Bay, CA, USA	Jun 1975	0.5	Weiler & Chisholm (1976)
D. norvegica	Baltic Sea	Jul–Aug 1998	0.1–0.4	Gisselson et al. (2002)
D. tripos	Galician Rías, Spain	Oct 1994	0.5	Reguera et al. (2003)
D. sacculus	Ebro Delta, Spain	May 1994	0.42	Garcés et al. (1997)
		Jun 1994	0.28	
		Jun 1994	0.38	
		Oct 1994	0.2	

MATERIALS AND METHODS

The present study is based on samples from 2 diel cycle studies conducted on board the RV 'J. M. Navaz' in the Galician Rías in late spring and early autumn 1994 at the time of numerical increases of *Dinophysis* spp. and shellfish harvesting closures. These samples were used to estimate μ on integrated samples (μ_{int}) and at *Dinophysis* spp. cell maxima ($\mu_{P_{max}}$) and to evaluate differences between them. For the late-spring period, an exponential growth model was applied to simulate the population dynamics of *Dinophysis* spp in the Galician Rías. This model was used to determine the influence of both μ_{int} and $\mu_{P_{max}}$ on *Dinophysis* population dynamics.

In addition, differences between μ_z at diverse specific depths, including the depths of cell maxima, were studied with a fine-resolution vertical profile of *Dinophysis* spp.

Diel cycles. ***Dinophysis spp. sampling and cell counts:*** Phytoplankton samples were collected by vertical 20 µm mesh net hauls in the upper 20 to 25 m to obtain integrated samples of the whole water column. To eliminate debris and large zooplankton, samples were passed through a 150 µm mesh and fixed with buffered formaldehyde. Simultaneously, CTD (SBE 25 SEALOGGER) casts were carried out to obtain vertical profiles of temperature, salinity and *in vivo* fluorescence. Oceanographic bottles were used to collect samples at specific depths. Aliquots for phytoplankton counting were immediately fixed with Lugol's iodine acidic solution (Lovegrove 1960).

Daily values of offshore Ekman transport ($m^3\ s^{-1}\ km^{-1}$) were calculated from geostrophic wind data off Cape Finisterre, a representative site for the Rías Baixas, according to Bakun (1973).

The spring cruise (1 to 2 June 1994) was carried out over 22 h at fixed Stn P2 (27 m deep, 42° 21′ N, 8° 47′ W) in Ría de Pontevedra (Fig. 1). Vertical net hauls and inverted 5 l bottle samples were collected at 5, 10, 15 and 20 m every hour from 17:00 to 20:00 h and 00:00 to 14:00 h and every 2 h from 20:00 to 00:00 h. The autumn cruise (27 to 28 October 1994) was carried out during 36 h at fixed Stn V1 (20 m deep, 42° 15′ N, 8° 50′ W) in Ría de Vigo (Fig. 1). Vertical hauls and bottle samples from the cell maxima were collected every 2 h from 13:00 to 17:00 h, hourly from 17:00 to 04:00 h and every half hour from 04:00 to 12:00 h (the time window when cellular division processes take place in *Dinophysis* spp.; Reguera et al. 2003). Depths at which to sample the cell maxima were chosen after reading the CTD profiles and performing quick on-board microscopic observation of concentrated bottle samples from depths where density discontinuities and/or *in vivo* fluorescence were observed. The 5 l bottle samples were concentrated through a collector with a 20 µm end to a final volume of about 50 ml, which was measured to calculate the conversion factor for the cell counts. *Dinophysis* spp. from all Lugol's-fixed samples cells were counted by the Utermöhl (1931) method under a Zeiss (AXIOVERT 135) inverted microscope, after sedimentation from 25 ml columns. Vertical distributions of physical parameters and cell densities were plotted with the Microcal ORIGIN and Golden Software SURFER contour software.

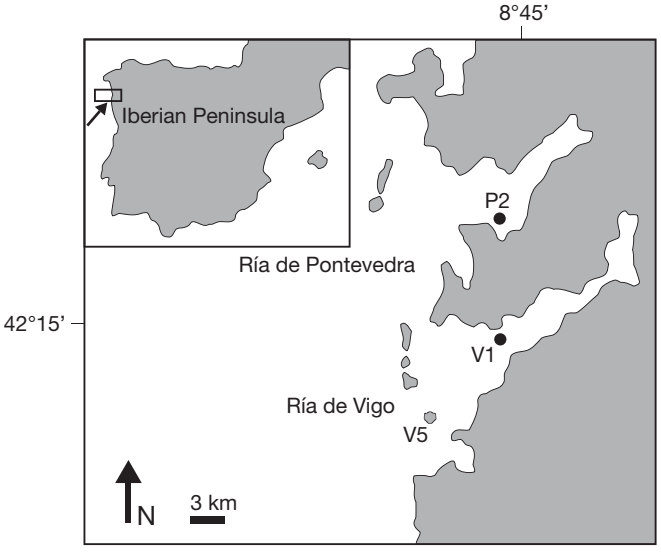

Fig. 1. Rías Vigo and Pontevedra, Iberian Peninsula, showing locations of sampling stations P2, V1 and V5

Estimates of division rates: *In situ* division rates were estimated from the frequency of dividing (paired) and recently divided (incomplete development of the left sulcal list) cells, which were recognized by their distinct morphology as described in Reguera et al. (2003), following the model of Carpenter & Chang (1988):

$$\mu = \frac{1}{n(T_c+T_r)} \sum_{i=1}^{n} (t_s)_i \ln[1 + f_c(t_i) + f_r(t_i)] \quad (1)$$

where μ is the daily average specific division rate, $f_c(t_i)$ is the frequency of cells in the cytokinetic (or paired cells) phase (c) and $f_r(t_i)$ is the half frequency of cells in the recently divided (r) phase in the *i*th sample. T_c and T_r are the duration of the c and r phases, considered as terminal events (*sensu* Carpenter & Chang 1988) in the present study; n is the number of samples taken in a 24 h cycle; and t_s is the sampling interval in hours. In the case of *Dinophysis caudata*, opening pairs (cytokinetic pairs where the dividing cells are not in the same plane) were used instead of paired cells to calculate the frequency of dividing cells. Recently divided cells were counted when incomplete development of the left sulcal list occurred, whether they were single cells or pairs. The duration of the selected terminal events, $T_c + T_r$, was estimated as the interval of time necessary for a cohort of cells to pass from one phase to the next;

in this case, the time interval between time t_0, when the frequency of cells undergoing cytokinesis (f_c) is maximum, and time t_1, when the fraction of recently divided cells f_r is maximum:

$$\frac{1}{2}(T_c + T_r) = (t_0 - t_1) \quad (2)$$

where T_c, T_r, t_1 and t_0 are calculated after fitting a 5th degree Gaussian function to the frequency data.

An average μ (μ_{int}) of the whole water column was estimated from the integrated samples obtained by vertical net hauls, and μ_{Pmax} from the concentrated bottle samples collected at the depth of *Dinophysis* spp. cell maxima (P_{max}).

Modelling approach. An exponential growth model was applied to simulate the population dynamics of *Dinophysis acuminata* on 1 to 2 June 1994.

$$P_t = P_0 \, e^{(\mu + I - E - g)t} \quad (3)$$

where P_t and P_0 are the population densities at times t and 0, respectively; μ is the specific division rate; g is the cell loss rate by grazing; and I and E are the import and export rates, respectively. This equation was applied to both integrated, P_{int}, and cell maximum, P_{max}, counts. Import and export rates include biological vertical migration, physical advection and eddy diffusion. Import and export rates as well as loss rates due to grazing were unknown, and were estimated as a single value (net growth rate, h) calculated every hour before ($\mu = 0$), during ($\mu = \mu_{int}$; μ_{Pmax}) and after division ($\mu = 0$). Values of μ_{int} and μ_{Pmax} were known from the daily cycle results, which showed that a phased-cell division took place from 7:00 to 10:00 h.

Vertical profiles of μ_z. *Dinophysis spp. sampling and cell counts:* Samples were collected on board R/V 'Mytilus' on 21 August 2003 off Ría de Vigo at Stn V5 (100 m deep, 42° 07.43′ N, 8° 58.55′ W) (Fig. 1). Vertical profiles of temperature, salinity and *in vivo* fluorescence were obtained from a CTD (Seabird SBE 911) attached to a rosette sampler (Gral Oceanics 1015) equipped with twelve 10 l oceanographic bottles used to collect water samples from the pycnocline region and from the water layers above and below it. Two kinds of subsamples were taken from each discrete depth (oceanographic bottles) sample: (1) 100 ml was fixed with Lugol's solution, which was used for cell counts; and (2) the rest of the sample was poured into a bucket and gently concentrated by pouring the bucket content through a PVC cylindrical collector with a 20 μm mesh that was kept with its lower half submerged in seawater to avoid cell damage. This concentrate, used to estimate frequency of cells undergoing division, was backwashed and fixed with neutral formaldehyde. *Dinophysis* spp. cells were counted as described above ('Diel cycles').

Estimates of division rates: The maximum frequency approach (McDuff & Chisholm 1982) was used to estimate μ_{min} at each depth (μ_z) in single vertical profiles:

$$\mu_{min} = \ln(1 + f_{max}) \quad (4)$$

where f_{max} is the frequency of dividing cells at each depth. This approach assumes that all cells which divide in a given day can be recognized as undergoing or just having completed mitosis in one sample. The sample should be collected during the appropriate time-window within which peak division is observed. In the case of *Dinophysis acuta* and *D. caudata*, the appropriate time has been confirmed, in several cycle studies, to be 2 to 3 h following sunrise (Reguera et al. 2003, Pizarro et al. 2008).

RESULTS

Hydrographic conditions and phytoplankton distribution in diel cycle experiments

In early June, there was a marked thermohaline stratification and a prominent subsurface chlorophyll maximum (SCM) at 10.5 to 11.5 m (Fig. 2A,B). Downwelling-favourable winds on 31 May (Fig. 3A) promoted a downward movement of the 23.5 σ_t isopycnal and a displacement of the pycnocline and the SCM below 10 m depth at the beginning of the survey (Fig. 3B). Wind direction changed to upwelling-favourable on 1 June and reversed again at the end of the survey (Fig. 3A). In response to the northerly winds, the 23.5 σ_t isopycnal rose to 0–5 m from 00:00 h on 2 June to the end of the cruise (Fig. 3B). The highest concentrations of *Dinophysis acuminata* were found at 10 m at 2:00 h (4379 cells l^{-1}) and at 5 m from 08:00 to 10:00 h (6058 cells l^{-1}) on 2 June (Fig. 3B). The average concentration of *D. acuminata* throughout the cycle was 1280 cells l^{-1} in integrated samples (0 to 20 m).

The October survey took place under typical rainy autumn conditions after the end of the upwelling season. Sampling was carried out during the decline of the autumn maximum of *Dinophysis acuminata* and preceding a very moderate peak of *D. acuta* and the annual maxima of *D. caudata* and *D. tripos*. A homogeneous vertical distribution of temperature (16°C) and salinity (34 to 34.4 psu) was observed below 6 m at Stn V1 (Fig. 2C). Heavy rainfall led to the formation of a strong halocline in the top 4 to 6 m, especially in the first half of the cell cycle sampling (Fig. 2C). There was not a marked SCM (Fig. 2D), temperature in the top 10 m ranged from 15 to 16°C and salinity from 32 to 34.5 psu. In the integrated samples, the average concentration of *D. caudata* (0 to 15 m) was very low (160 cells l^{-1}).

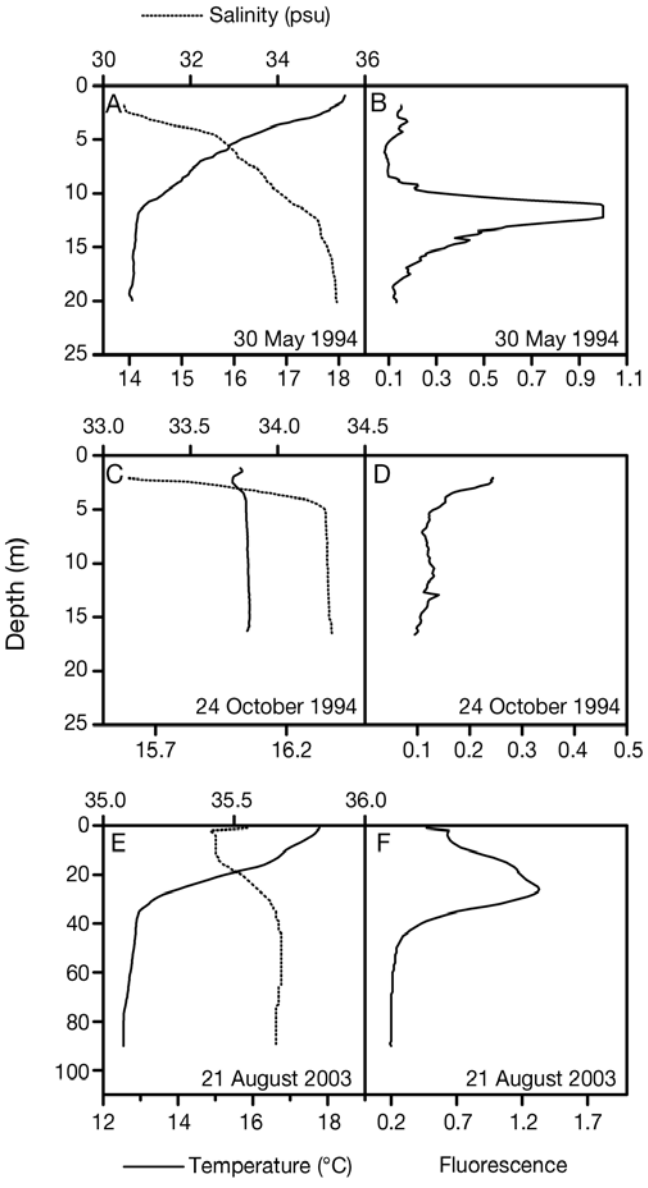

Fig. 2. Vertical profiles of (A,C,E) temperature and salinity, and (B,D,F) *in vivo* fluorescence (arbitrary units). (A,B) Stn P2, 30 May 1994 (prior to the 1–2 June cruise). (C,D) Stn V1, 24 October 1994 (prior to the 27–28 October cruise). (E,F) Stn V5, 21 August 2003

Phase-fraction curves and estimates of μ in integrated and P_{max} samples in diel cycles

June cycle

Fig. 4 shows the distribution of frequencies of dividing (f_c) and recently divided (f_r) cells, as well as the estimated minimum growth rates (μ_{min}) and the mean daily specific μ for *Dinophysis acuminata* during the June cycle, both in integrated samples and at the depth of P_{max}. *D. acuminata* showed a clear-cut phased cell division in both situations. The highest values of f_c (0.11) and f_r (0.17) in integrated samples were lower than at P_{max} (f_c = 0.16, f_r = 0.21). The maximum f_c at P_{max} occurred at 05:00 h (just before sunrise) at 5 m, and at 6:00 h (after sunrise) in the integrated samples. Whilst cytokinesis and sulcal-list regeneration lasted from 05:00 to 10:00 h in integrated samples, these terminal events occurred from 05:00 to 08:00 h at P_{max}. Therefore, cell division was more synchronized at P_{max}.

Estimates of μ and μ_{min}, according to Eqs. (1) & (4), were 0.46 ± 0.05 $d^{-1}$and 0.25 d^{-1}, respectively, in integrated samples, and 0.40 ± 0.04 d^{-1} and 0.21 d^{-1} at P_{max} (Table 2).

October cycle

Fig. 5 shows the distribution of f_c and f_r, as well as μ_{min} and the mean daily specific μ, for *Dinophysis caudata* during the October sampling for both the integrated and the P_{max} (4 to 6 m) samples. The highest values of f_c (0.47) and f_r (0.45) at P_{max} were considerably higher than in integrated samples (f_c = 0.10, f_r = 0.21). While both f_c and f_r were similar and close to 0.50 at P_{max}, maximum values of f_r (up to 0.20) were higher than those of f_c (0.10) in integrated samples. The f_c and f_r maxima were observed at 08:30 and 11:00 h, respectively, at P_{max}, and at 09:00 and 10:30 h in the integrated water column samples. Left sulcal-list regeneration (maximum f_r) at P_{max} was observed from 08:00 to 14:00 h and from 08:00 to 16:00 h in the integrated samples. Thus, f_c lasted longer in the integrated population than at P_{max}. Increasing values of f_c were observed at the end of the cycle (06:00 to 08:00 h) at P_{max}. Both estimates of μ and μ_{min} at P_{max} were double those of the integrated samples.

Modelling results

Results from the application of the exponential growth model to the June 1994 cycle are shown in Fig. 6. Hourly estimates of the net growth rate (h) of the *Dinophysis acuminata* population, both at the cell maximum, P_{max}, and in integrated samples, followed the same pattern. They showed an increase of *D. acuminata* in the dark phase, around midnight (20:00 to 01:00 h), with a peak at 01:00 h that was more pronounced at P_{max} (10 m). From 02:00 to 06:00 h, h fluctuated around zero, and losses were again more pronounced at P_{max}. From 07:00 to 08:00 h, *D. acuminata* concentrations increased at both P_{max} and in the integrated water column, followed by an increase of loss factors, even during division, from 08:00 to 10:00 h. Estimates of h exceeded estimates of growth calcu-

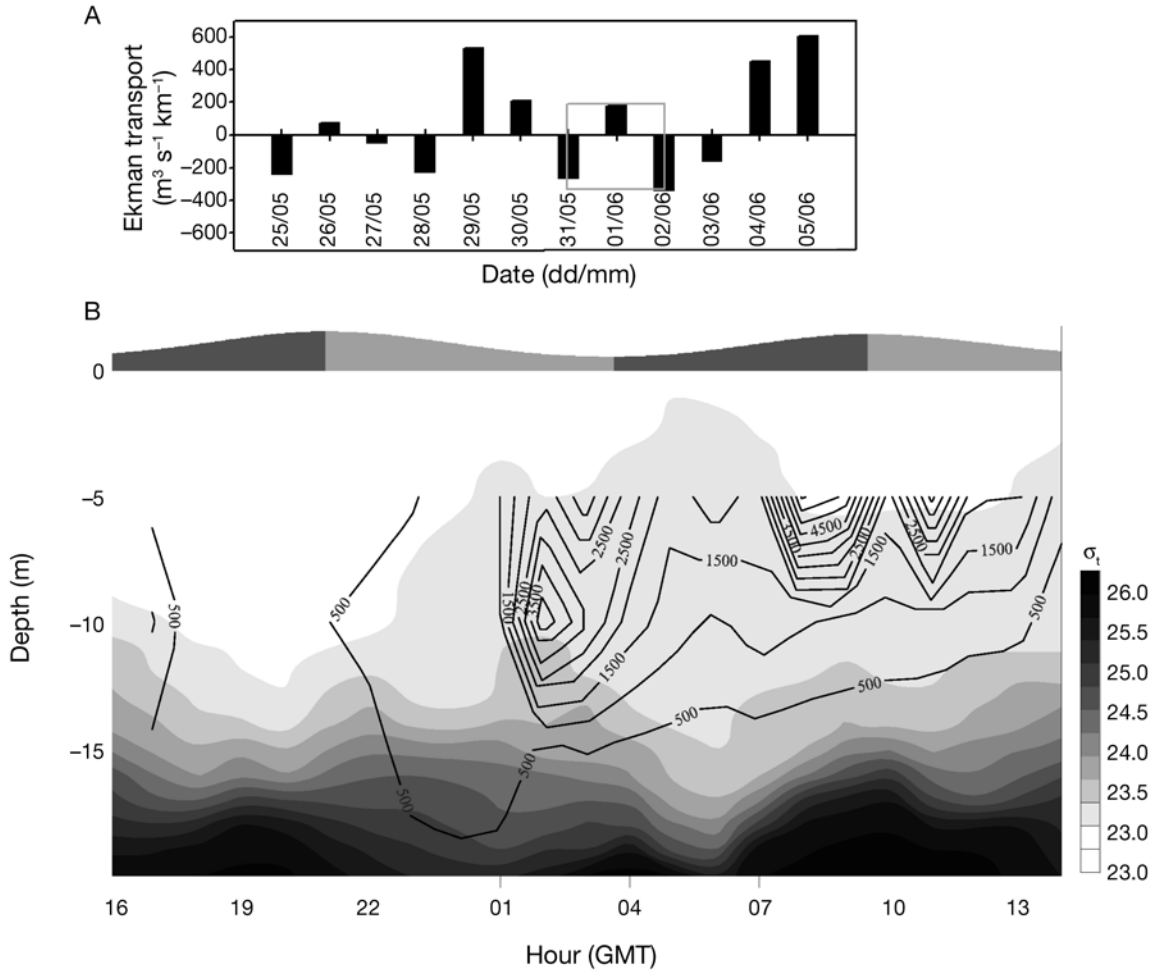

Fig. 3. (A) Ekman transport from 25 May to 3 June 1994 (values from 31 May to 2 June are outlined with a grey box). (B) Vertical distribution of seawater density (σ_t) (gray background shadow) and *Dinophysis acuminata* (cell l^{-1}) (contour lines) obtained from bottle samples at 5, 10, 15 and 20 m at Stn P2 in Ría de Pontevedra from 16:00 (1 June 1994) to 14:00 h (2 June 1994). Tidal state is indicated on the horizontal bar above the panel

lated from division; therefore, the *Dinophysis* spp. population at that moment (07:00 h) not only increased by cellular division but also due to import factors. These gains (07:00 h) and losses (08:00 to 10:00 h) were also higher at P_{max}. Even at 09:00 h, when division occurred, *h* reached a minimum.

Vertical profiles of μ_z values

Hydrographic conditions during the August 2003 sampling on the shelf corresponded to those typical in summer during mild upwelling pulses. Vertical profiles of temperature and salinity (Fig. 2E) showed marked discontinuities at around 20 m and the distribution of *in vivo* fluorescence (Fig. 2F) showed a broad chlorophyll *a* peak around the same depth. *Dinophysis caudata* and *D. acuta* maxima (P_{max}) were located in the 20 m discontinuity layer (Fig. 6). Although both *Dinophysis* spp. were found at this density discontinuity, *D. caudata* was more concentrated in the lower and *D. acuta* in the upper part of the pycnocline.

Table 2. *Dinophysis acuminata* and *D. caudata*. Division rates (d^{-1}) during diel cycles. Data for μ are mean ± SD

Species	Temperature (°C)	Salinity (‰)	$T_c + T_r$ (h)	μ (d^{-1})	μ_{min} (d^{-1})
D. acuminata					
Integrated	17–18	32–33	2.1	0.46 ± 0.05	0.25
Maximum			1.6	0.40 ± 0.04	0.21
D. caudata					
Integrated	15–16	30–35	3.8	0.22 ± 0.02	0.18
Maximum			6.1	0.45 ± 0.05	0.38

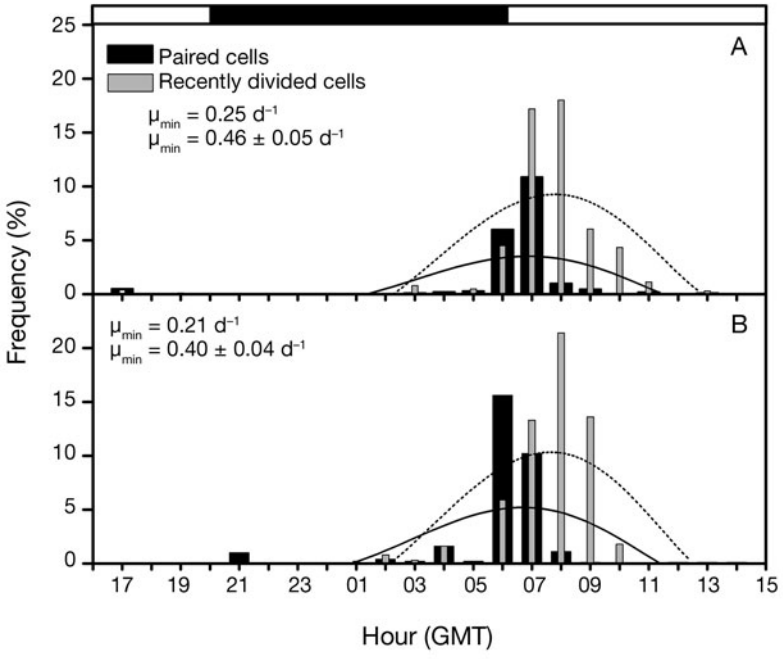

Fig. 4. *Dinophysis acuminata*. Diel distribution of frequencies of paired (dividing) and recently divided cells of *D. acuminata*, fitted to a 5th degree polynomial curve, 1 to 2 June 1994. (A) Integrated samples, (B) at cell maximum. Solid and dashed lines: estimated fitted curve for the frequency of paired cells, and recently divided cells, respectively. Black shading in bar at top: period between sunset and sunrise

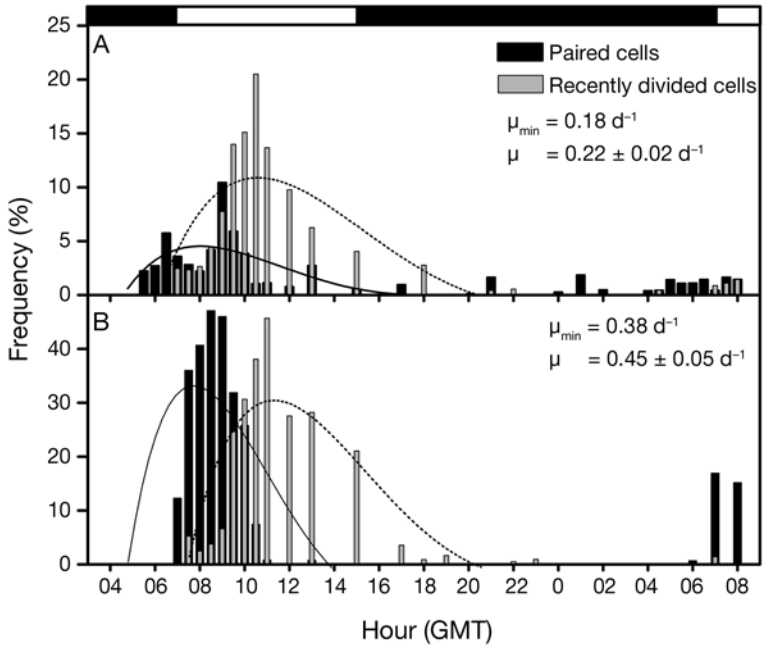

Fig. 5. *Dinophysis caudata*. Diel distribution of frequencies of paired (dividing) and recently divided cells of *D. caudata*, fitted to a 5th degree polynomial curve, 27 to 28 October 1994. (A) Integrated samples, (B) at cell maximum (pycnocline). Solid and dashed lines: estimated fitted curve for the frequency of paired cells, and recently divided cells, respectively. Black shading in bar at top: period between sunset and sunrise

Vertical profiles of μ_{min} at each sampling depth for *Dinophysis acuta* and *D. caudata* are shown in Fig. 7. The highest values of μ_z were found overlapping the P_{max} of both *D. acuta* and *D. caudata*, i.e. their maximum μ_z and μ_{Pmax} values were equal.

DISCUSSION

Species-specific measurements of *in situ* division rates in dinoflagellate populations are scarce (Stolte & Garcés 2006). This is especially true in the case of low biomass populations of TPA species, which often occur embedded in phytoplankton assemblages dominated by diatoms. In these cases, bulk measurement of chlorophyll, primary production and other biochemical markers do not reflect the physiology of the target species. The post-mitotic index method applied in the present study has been shown to provide a simple and reliable way to estimate *in situ* division rates of *Dinophysis* spp., even when these dinoflagellates are present at low to moderate concentrations (10^2 to 10^3 cells l^{-1}) (Reguera et al. 2003).

Dinophysis spp. responsible for diarrhoeic shellfish poisoning (DSP) outbreaks have been labelled slow-growth species (Smayda & Reynolds 2001). Nevertheless, field observations in the Galician Rías show that different species of *Dinophysis* observed during different stages of their population growth and under distinct environmental conditions can exhibit a wide range of values in their intrinsic division rates, between practically zero (Pizarro et al. 2008) and as high as 1 division per day (μ = 0.69 d^{-1}) (see Table 1). Therefore, toxin-producing *Dinophysis* spp. can divide as quickly as many other medium-sized dinoflagellates (e.g. *Alexandrium* spp.). Obviously, these observations depend on the chance of sampling the populations at the right time in their seasonal cycle, and at the right depth where they aggregate. Recently, extremely high division rates (μ = 0.95 d^{-1}) have been obtained in laboratory cultures of *D. acuminata* fed with *Myrionecta rubra* and under continuous illumination (Park et al. 2006). Although growth rates in culture usually include losses due to natural death, these

results suggest that, under optimal conditions of prey availability and light, *D. acuminata* may become a fast-growth species with >1 doubling per day.

Values of μ obtained for *Dinophysis acuminata* during the June cycle, both from integrated samples and from the cell maxima, were of similar magnitude (Table 2). If all possible sources of error (sampling, cell counts, etc.) are considered (Chang & Carpenter 1990), it can be said that *D. acuminata* was dividing at the same rate throughout the whole water column. Therefore, the high densities of *D. acuminata* observed at 5 to 10 m by the end of the diel cycle were not caused by higher division rates in this depth-interval and must have been caused by advection and vertical migration of *Dinophysis* cells swimming upwards during the day.

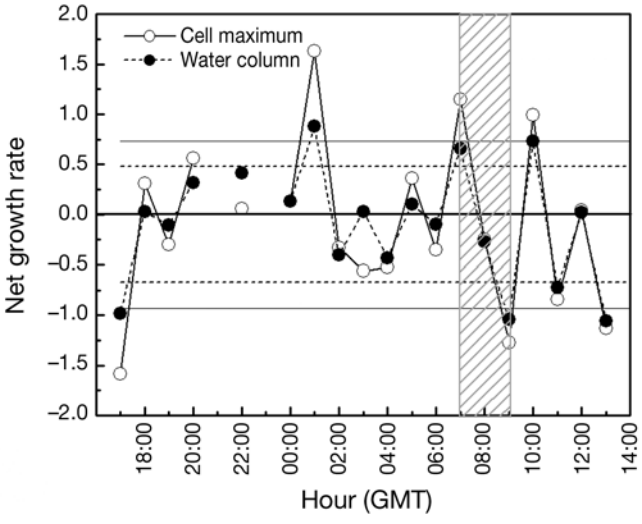

Fig. 6. *Dinophysis acuminata*. Hourly estimates of net growth rates of the *D. acuminata* population from 1 to 2 June 1994 at Ría de Pontevedra. The shaded area indicates when division was considered. Horizontal lines represent the mean ± SD for both model results (net growth-rate estimates) of P_{max} (solid lines) and integrated water samples (dashed lines)

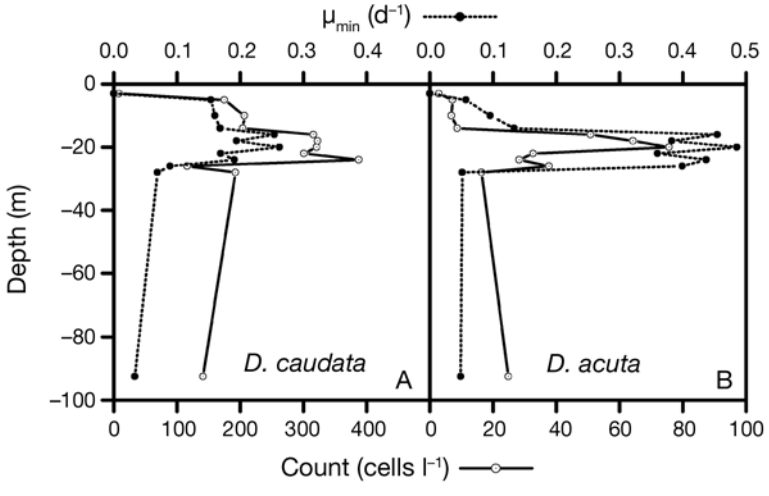

Fig. 7. *Dinophysis caudata* and *D. acuta*. Vertical profiles of μ_z estimates (dashed lines) and cell counts (solid lines) for (A) *D. caudata* and (B) *D. acuta* on 21 August 2003 at Ría de Vigo (Stn V5)

During the October cycle, the estimates of μ_{min} and μ for *Dinophysis caudata* at the cell maxima were double those obtained from integrated samples (Table 2). Therefore, the accumulation of *D. caudata* within the pycnocline on 27 to 28 October may have been a result of actively dividing cells that aggregated around the density discontinuity. The selection and accumulation of harmful dinoflagellates under downwelling conditions and the advection of coastal populations to the Rías have been well documented for the study area (Fraga et al. 1988, Escalera et al. 2006). In the present study, evidence is given to support the view that increased cell numbers of *Dinophysis* spp. in the Rías may result from a combination of advection, usually described as the most important factor in dinoflagellate accumulation during downwelling conditions, and enhanced division rates within the cell maxima. Since μ estimates at P_{max} were double than those from the integrated water column, integrated water column estimates of μ from vertical net hauls in this scenario did not provide a realistic measurement of the population growth. *Dinophysis* spp. often exhibit sharp heterogeneities in their vertical distribution (Velo-Suárez et al. 2008); therefore, estimates of μ from vertical net hauls may conceal much higher division rates occurring in the *Dinophysis* maximum layer.

In the present study, a deterministic model was applied to a population of *Dinophysis acuminata*. Positive values of gain and loss rates from 20:00 to 01:00 h, when division did not occur, showed an increment of import factors for both P_{max} and the integrated water column. Import factors such as advection and vertical migration may have caused the observed increment in *D. acuminata* numbers from 18:00 to 01:00 h. Since horizontal advection by wind or tides would affect rate estimates from both sampling methods, differences between integrated samples and P_{max} μ-estimates could be associated with changes in import or export rates due to swimming behaviour or vertical advection. Significant differences between rates at the P_{max} and those from integrated samples were found in some time intervals, i.e. from 02:00 to 04.00 h, when the *D. acuminata* maximum moved from 10 to 5 m and remained at that depth during the rest of the study (Fig. 3B). The estimated rates and the depth of the P_{max} did not show any further significant differences after 04:00 h (Fig. 3B).

Another peak in net rate occurred during cell division at 07:00 h. Although both μ_{int}

and $\mu_{P_{max}}$ were included in the model, the estimated rates were above 0.5 h^{-1}, which means that advective gains were higher than losses. This moment coincided with high tide so that, in this case, tidal forcing may have added to active division to increase *Dinophysis acuminata* concentrations.

Maximum concentrations of *Dinophysis* spp. have often been related to density gradients, but it is difficult to determine if increased numbers found in these layers are due to *in situ* division rate, physical accumulation or a combination of both (Maestrini 1998). Pizarro et al. (2008) showed that even in the case of a decaying population of *D. acuta*, with a division rate of almost zero (Table 1), *Dinophysis* spp. cells still aggregated in a maximum around the halocline. Based on the results obtained in the present study, it is strongly recommended that division rates should be measured from samples collected at the P_{max} rather than from vertical hauls. During stratified conditions, estimates of μ either from haul samples or from samples collected at discrete depths not associated with P_{max} may give strong underestimates of the potential division rates of the population. Our results also show that the key question — Are the increased numbers of *Dinophysis* spp. at discrete depths due to biology or to physics? — can be answered by obtaining estimates of μ at different and representative discrete depths.

CONCLUSIONS

A comparison of division rates estimated from integrated and depth-specific samples of *Dinophysis* spp. was carried out. The observed differences between μ estimates at the P_{max} and from integrated samples were negligible during the June 1994 cycle, but the *D. acuminata* population was actively dividing (μ = 0.4) in the absence of water column stratification. High cell densities in this case were mainly driven by vertical migration and advection. In contrast, the population of *D. caudata* during the October 1994 cycle provides a good example of numerical increase due to enhanced division in the pycnocline.

Values of μ_z obtained from vertical profiles in 2003 showed the vertical heterogeneity of *Dinophysis* spp. concentration and division. Division rates at P_{max} (20 m) were double in the case of *D. caudata*, and 5 times higher in the case of *D. acuta*, than rates observed in the layers above and below. μ_{int} value of this profile would not have been a realistic measurement of the population growth rate at the time. Vertical heterogeneities in division rate estimates justify the importance of fine-scale sampling to detect layers of actively dividing *Dinophysis* spp. These layers — which may represent hot spots of actively dividing cells, and have a decisive effect on the population dynamics of the target species — can be missed with conventional sampling methods. Although further research is needed, measuring division rates where cells aggregate, i.e. at the cell maxima, provides a more realistic indicator of population growth for *Dinophysis* spp. dynamics and for the application of predictive models.

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