Deep-Sea Research II 101 (2014) 249–254

Contents lists available at ScienceDirect

Deep-Sea Research II

journal homepage: www.elsevier.com/locate/dsr2

Phased cell division, specific division rates and other biological observations of *Dinophysis* populations in sub-surface layers off the south coast of Ireland

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ARTICLE INFO

Available online 29 May 2013

Keywords:
Dinophysis
Dinophysis acuminate
Dinophysis acuta
Specific growth rate
Thin layers

ABSTRACT

The proportions of viable cells of *Dinophysis* spp. that were paired (dividing) and recently divided during a cell cycle were measured on populations of *D. acuta* and *D. acuminata* observed off the south coast of Ireland in July 2007 and July 2009. Both species exhibited phased cell division in 2009 with maximum frequency of division (f_{max}) 2 h after sunrise. Different patterns of division (timing of f_{max}) were shown by *D. acuta* in 2007, when the population aggregated in a thin layer was transported by a coastal jet flow. High resolution (decimetre-scale) profiles within the thin layer showed large differences in the vertical distribution of biological properties (feeding status, mortality). Values of the specific growth rate μ were compared to estimates derived in similar fashion from observations on *Dinophysis* populations elsewhere. Different patterns exhibited by the same species in different regions may be attributed to adaptations to latitudinal differences (length of photoperiod). The question of whether phased cell division always occurs in *Dinophysis* populations, and the incorporation of the potential specific division rate into models of *Dinophysis* growth are discussed. Comprehensive field data sets demonstrate the impact of the results on the coherence of *Dinophysis* populations during their transport along the Irish coast in jet-like flows towards sites of intensive shellfish culture.

1. Introduction

Estimates of the specific (or per capita) growth rate (μ, d^{-1}) of potentially harmful algal species in the marine environment are vital for their insertion into the biological component of physical-biological coupled models with which harmful algal blooms (HABs) can be predicted (GEOHAB, 2011). This parameter estimates the potential for intrinsic division without the interference of losses due to grazing, mortality and physical dispersion (Carpenter and Chang, 1988). Further, in situ estimates of μ are essential to determine whether fast increases in a population are due to active growth, physical accumulation or a combination of physical-biological interactions. Net growth rates (μ minus losses due to cell lysis) for several species that can be cultured relatively easily in the laboratory, such as those from the genus *Alexandrium* and *Pseudo-nitzschia*, have now been estimated as functions of temperature, salinity, irradiance or nutrients (Stolte and Garcés, 2006). Similar rates for *Dinophysis* are scarce due to the difficulty of maintaining the organism in mixotrophic cultures (Park et al., 2006), and information on in situ specific growth rates is very limited, in particular due to their typically low cell density in water samples. A valuable approach for deriving specific growth rates for this genus lies in estimates based on the mitotic index approach (McDuff and Chisholm, 1982; Chang and Carpenter, 1991). This is because populations divide in phase and it is possible to recognise morphologically different stages in the (vegetative) cell cycle of *Dinophysis* that are 'terminal events', i.e. periods between any point in the cell cycle and the end of mitosis (Mitchison, 1971) that are unambiguous and reliable (Reguera et al., 2003).

Use of the cell-cycle stage markers referred to above has shown that in western (Long Island Sound, Chang and Carpenter, 1991) and eastern (Galician Rías, Reguera et al., 2003) Atlantic coastal waters, Mediterranean Sea (Ebro River Delta, NE Spain, Garcés et al., 1997) and Gulf of Mexico (Campbell et al., 2010) *Dinophysis* divides in phase (i.e. all dividing cells do so within a limited time window) and there are clear peaks in the frequencies of dividing nuclei and cells and of recently divided cells which are observed after the onset of light. In contrast, no phased division was

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http://dx.doi.org/10.1016/j.dsr2.2013.05.031

observed during a cell cycle study with *Dinophysis acuminata* in the Gullmar Fjord, Skagerrak, SE Sweden (Gisselson et al., 1999).

This paper reports estimates of specific growth rates (μ) from in situ cell cycle studies on *Dinophysis* populations carried out off the southwest coast of Ireland, and compares the cell cycle patterns with those observed elsewhere. Special attention is paid to the high resolution vertical distribution of biological parameters within a thin layer of *D. acuta* occurring in Irish shelf waters in July 2007.

2. Material and methods

2.1. Field sampling

Water samples for measurements on *Dinophysis* populations were obtained in July of both 2007 and 2009 at sampling stations located in the Celtic Sea off the southwest coast of Ireland (Fig. 1). In July 2007, the research vessel was tracking a subsurface thin layer, within a patch of 10 km × 7 km, as part of a major cruise (HABIT 2007), but was anchored at a fixed station for the 24 h cell cycle study (Farrell et al., 2012); in July 2009, samples during a mixed bloom of *D. acuta* and *D. acuminata* were taken at a fixed station.

Two kinds of water samples were collected for microplankton analyses and for cell cycle studies respectively. Water samples for microplankton quantitative analyses (raw samples) were obtained using either 10-L Niskin bottles mounted on a CTD (SBE 9–11) rosette or pumped peristaltically to the surface from specific depths from the intake end of a hose mounted on a CTD Ifremer Particle Size Analyser Profiler (IPSAP; Lunven et al., 2012). Samples were preserved in Lugol's iodine and stored in 50-mL cell culture bottles. Water samples for cell cycle studies (concentrated samples) in July 2007 were obtained by filtering 2 L of water collected within the detected thin layer of *Dinophysis* through a 20-μm filter that was resuspended in 30 mL of filtered seawater with Lugol's iodine. This procedure was followed to make sure a sufficient number of *Dinophysis* cells were collected. Samples for the cell cycle studies in 2009 were collected by using a 20-μm mesh net with an opening diameter of 40 cm; this net was lowered and raised slowly (0.5 m s^{-1}) to a depth of 50 m to obtain an integrated water column sample. In addition, during the 2007 sampling, the IFREMER "Fine-Scale Sampler" (FSS) (Lunven et al., 2005) was deployed to study the fine-scale vertical distribution of *D. acuta* cells and of their specific growth rate (μ_{min}) within the 5-m thin layer. This FSS system consists of a ladder-like structure on which 15 2-L oceanographic bottles are mounted horizontally, 20 cm apart between the central axes of two consecutive bottles. Deployment of the FSS was at a time (08:00UTC) when all cells either undergoing cell division or recently divided could be expected to be recognised. The FSS was gently lowered to the target depth, chosen from the IPSAP profile readings, and allowed to drift horizontally for a few metres so that undisturbed water was sampled. Its design, featuring a large fin and smooth minimal flow inside the bottles, produced minimal disturbance of the water layers. The 15 bottles were closed simultaneously by means of an electromagnetic trigger. On retrieval of the FSS, the water from each bottle was filtered through a 20-μm filter material collected on the filter was resuspended in 30 mL of filtered seawater with Lugol's iodine.

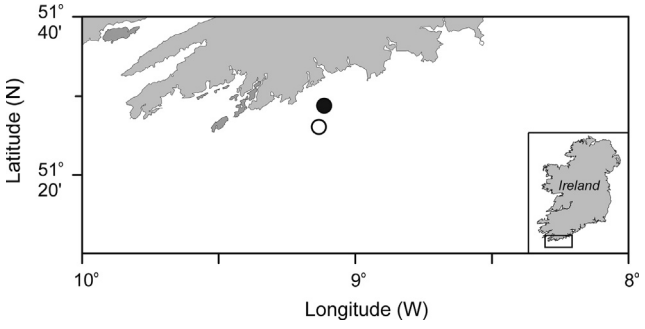

Fig. 1. Map of southwest Ireland showing position of diel stations for the *Dinophysis* growth observations made in July 2007 (filled circle) and July 2009 (open circle).

2.2. Dinophysis analyses and frequency of vacuolation and mortality

Samples for quantitative analyses of *Dinophysis* cells were examined using an inverted microscope (Olympus CKX41) at × 100 and × 200 magnification. Cell counts were enumerated using the settlement bottle technique (McDermott and Raine, 2010) by scanning the entire base of a filled 50 mL cell culture bottle (detection limit: 40 cells L^{-1}). Cell counts of the FSS samples were carried out under an inverted microscope (Nikon ECLIPSE TE2000-S) according to the Utermöhl (1931) method. The densities of *Dinophysis* spp. were estimated after the enumeration of specimens from the whole sedimentation chamber at a magnification of 100 × (detection limit: 5 cells L^{-1}).

The frequency of cells containing digestive vacuoles (vacuolated cells) was used as a proxy for recent phagotropic feeding. Either empty thecae (with only one half of the left sulcal list, counted as 1 specimen every 2 thecae) or empty cells of *D. acuta* were interpreted as 'dead cells'. A minimum of 200 individuals were scanned and the whole sedimentation chamber was searched in samples with a lower abundance of *D. acuta*. The frequency of vacuolated cells was estimated by dividing the number of vacuolated individuals between the total number of "alive" (i.e. with noticeable cellular content in the Lugol's fixed sample), vacuolated and non-vacuolated cells. Mortality frequency was estimated by dividing the number of dead cells between the total number of alive and dead specimens.

2.3. Estimates of specific growth rates

Cell cycle studies to obtain estimates of *Dinophysis* specific growth rates lasted for at least 24 h and samples were taken at 2–3 h intervals. In situ specific growth rates (μ) were estimated from the frequency of dividing (paired) and recently (incomplete development of the left sulcal list) divided cells of *D. acuminata* and *D. acuta*, which were recognised by their distinct morphology as described in Reguera et al. (2003). A minimum of 200 individuals of each species were examined and the specific growth rate estimated following the model of Carpenter and 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 (d^{-1}) is the daily average specific growth 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 ith sample. T_c and T_r are the duration of the c and r phases, considered as terminal events 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.

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