DIEL OBSERVATIONS ON POPULATIONS OF *DINOPHYSIS* AND *ALEXANDRIUM* SPP. (*DINOPHYCEAE*) FROM THE NW ADRIATIC SEA

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Quanti-qualitative changes in *Dinophysis* and *Alexandrium* populations from the Gulf of Venice – NW Adriatic Sea – were studied on a short time scale (44 h cycles). Under hydrographic conditions of marked thermo-haline stratification (July 1995), *D. sacculus* populations assembled in the surface waters (12 24 γ_t isopycnals). Division processes took place mostly in the early hours of the morning and at 14:00 h, 4 to 10 % of the population consisted of morphotypes of reduced size (possibly gametes resulting from successive cell divisions). Similar percentages were also observed in October for *D. caudata*, with a maximum division between 06:00 h and 10:00 h. During this month, coexisting populations of *Alexandrium* cf. *fundyense* and *A.* cf. *tamarense* displayed rhythmic changes in cell density that slightly differed from those exhibited in July by *A. pseudogonyaulax*, suggesting a circadian control of the cell cycle rather than a response to hydrographic changes.

Key words: Phytoplankton, toxic dinoflagellates, *Alexandrium, Dinophysis,* vertical migration, diel cycles, Gulf of Venice, Adriatic Sea

Introduction

Dinoflagellates belonging to the genera *Dinophysis* and *Alexandrium* include well-known producers of diarrhoetic and paralytic shellfish poisoning (DSP and PSP, respectively). Previous reports from the northern Adriatic Sea have studied

the seasonal occurrence of various *Dinophysis* and *Alexandrium* species, as well as their toxicity (e. g. HONSELL et al. 1992, 1995, DELLA LOGGIA et al. 1993, BASTIANINI et al. 1994). Short time scale cycles of dinoflagellates from the above genera have been given less attention to date, although diel rhythmicities may characterize important processes like e.g. cell division and vertical migration. Phased cell division and in situ observations on potential growth rates of *Dinophysis* species have been reported from Atlantic and NW Mediterranean waters (e.g. REGUERA et al. 1996, GARCES et al. 1997). Likewise, rhythmic division has also been shown under laboratory conditions in other dinoflagellates, indicating that it occurs only at certain times of day allowed by the circadian clock (e.g. SWEENEY 1984).

Here we examine the short-time scale changes in cell density, distribution, and life cycle stages of *Dinophysis* and *Alexandrium* species from the Gulf of Venice, as well as their possible relationships with exogenous forcing factors. Morphological information on the thecate vegetative stage of *Alexandrium pseudogonyaulax* is provided as a complement to published descriptions (BALECH 1995, MONTRESOR et al. 1993, mostly dealing with the resting stage).

Materials and methods

Dinophysis and *Alexandrium* assemblages were collected from the NW Adriatic Sea (Gulf of Venice) at a shallow station close to the mouth of the River Adige (45° 09.00 N, 12° 23.15 E, maximum depth: 20 m). Samplings were carried out in July and October 1995 at 4 h intervals over a 44 h period using Niskin bottles (sampling depths: 0.5, 5, 10, 15, and 18 m) and 10–20 µm mesh nets for vertical hauls over the entire water column. CTD profiles were recorded at 2 h intervals with an 801 Ocean Seven Idronaut probe. Samples were fixed with neutralized formaldehyde or glutaraldehyde, and morpho-cytological analyses were made by light and epifluorescence microscopy (blue excitation, 487909 Zeiss filter set). Specimens of *Alexandrium pseudogonyaulax* were post-fixed in osmium tetroxide (1 % in diluted seawater), dehydrated through Nuclepore filters using an increasing series of ethanol, and critical point dried. Filters were mounted on stubs, gold-coated, and analyzed with a Philips XL20 scanning electron microscope at 5 kV.

Cell densities were determined by light microscope counts over the whole area of Utermöhl settling chambers (100 mL). Percentages of terminal division stages (TDS), i.e. cells undergoing cytokinesis to cells still attached in pair, were estimated for *Dinophysis sacculus* and *D. caudata* and in situ potential growth rates (μ_{min}) were obtained applying the method of VAULOT (1992). In situ μ_{min} values were compared with those obtained in the laboratory keeping zooplankter-free assemblages – dominated by *D. sacculus* or *D. caudata* – under controlled conditions. Field conditions were simulated using appropriate temperatures and light : dark cycles (*D. sacculus*: 22 °C and 16 : 8 h LD; *D. caudata*: 20 °C and 12 : 12 h LD). Estimates of growth rates in the laboratory also involved the diel analysis at 1–2 h intervals of binucleate stages, after staining with acetocarmine (SHEEHAN and HRAPCHAK 1980).

Results and discussion

Dinophysis spp.

Dinophysis assemblages were dominated in July by *D. sacculus*, whereas other species were present at lower density, according to the picture previously given for northern Adriatic areas (BASTIANINI et al. 1994). Vegetative cells of *D. sacculus* and possible stages of gametogenesis (Fig. 1) appeared under hydrographic conditions of strengthened water column stability (average $\Delta\gamma_t \cdot m^{-1} = 1.9$ units at the top 5 m and 0.2 units in the underlying waters). In July, water warming (surface T = 24 °C) and enhanced surface dilution (minimum salinity = 11 psu) led to thermohaline stratification, with a marked pycnocline in the upper layer (Fig. 2a). Here, on two consecutive days, division processes increased near sunrise with a maximal frequency of terminal division stages at 06 : 00 h (Fig. 2b). The growth rate of *D. sacculus* observed in situ was 0.10–0.12 div day^{-1} (μ_{min}), close to the values obtained in the laboratory, using both μ_{min} and net μ via cell counts (0.04–0.17 div day^{-1}). μ_{min} values were comparable with those reported from the NW Mediterranean (GARCÉS 1998) and with those described for related species, but were lower than μ's estimated by other means (see Table 1 and MAESTRINI 1998 for a summary). Although the μ_{min} approach may underestimate the true μ, we found a good correspondence with the net growth rate from the laboratory.

Tab. 1. Growth rates (divisions day^{-1}) of *Dinophysis* spp. as estimated by the Vaulot's method (μ_{min}), cell cycle analysis (μ), and carbon uptake (^{14}C).

Species	μ_{min} [1]	μ [2]	μ (^{14}C)	Reference
D. sacculus	0.10–0.12[3]			This study
D. sacculus	0.10–0.14	0.17–0.42		GARCÉS 1998
D. sacculus	0.24[4]			GARCÉS et al.1997
D. acuminata	0.12–0.22			REGUERA et al. 1996
D. acuminata		0.67		CHANG and CARPENTER 1991
D. acuminata			0.52–0.73	GRANELI et al. 1995
D. acuminata			0.59 (max.)	GRANELI et al. 1997
D. acuta	0.11			REGUERA et al. 1996
D. acuta			0.36–0.45	GRANELI et al. 1995
D. acuta			0.41 (max.)	GRANELI et al. 1997
D. norvegica			0.63 (max.)	GRANELI et al. 1997
D. caudata	0.30–0.42[5]			This study
D. caudata	0.19			REGUERA et al. 1996
D. fortii	0.50			WEILER and CHISHOLM 1976
D. tripos	0.21			REGUERA et al. 1996

[1] Duration of cell cycle phases not included (see also ANTIA et al. 1990);
[2] Duration of cell cycle phases included;
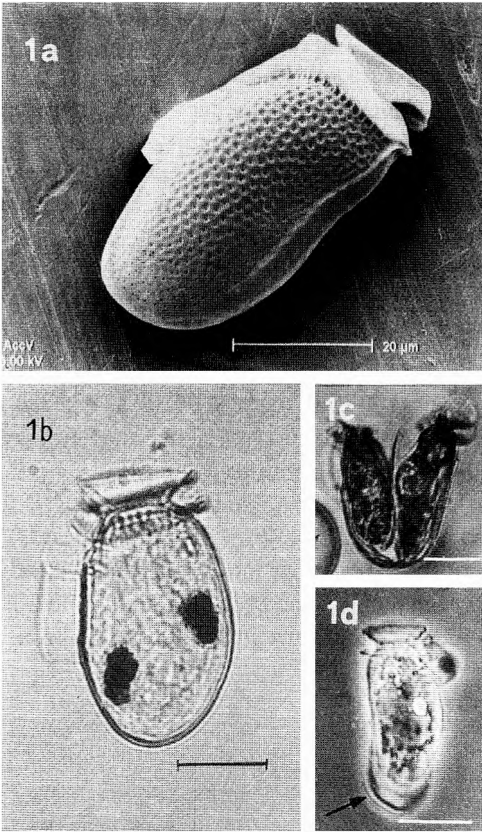[3] Lab: 0.04–0.17; [4] Average: 0.14; [5] Lab: 0.35–0.45.

Fig. 1. Life stages of *Dinophysis sacculus* (SEM and LM). NW Adriatic and laboratory specimens.
(a) Vegetative cell.
(b) Binucleate stage.
(c) Terminal division phase with two small cells inside the parental theca.
(d) Gamete-like dimorphic stage (inherited thecal half arrowed). Scale bars = 20 μm.

The density of *D. sacculus* in the Gulf of Venice was maximal at 14:00 h (0 m) but never exceeded 400 cells L^{-1}, collapsing in the subsequent dark hours. However, *D. sacculus* concentrations up to 4200 cells L^{-1} have been reported during the summer period in the gulf waters (BASTIANINI et al. 1994). At 14:00 h, 4 to 10 % of the population showed morpho-cytological features typical of gametes (Fig. 1d), including small size (length = 38–46 μm) and weak autofluorescence (see also GIACOBBE and GANGEMI 1997). No migratory behaviour was displayed throughout the water column by this population, which was nearly always confined to the top layer (0–5 m, Fig. 2c) in correspondence with chlorophyll maxima due to the growth of diatoms (see also SOCAL and BIANCHI 1989). Neither were downward displacements observed during the nocturnal drop in cell density at the surface (Fig. 2c). This validates mesocosm experiments with related species (*D. acuminata*), showing either the absence of diel migrations or vertical migrations limited to a few meters (0–6 m, see MAESTRINI 1998 for a review).

D. sacculus populations were almost completely replaced in October by *D. caudata* at various life stages (Fig. 3). During this month, a thermal destratification of the water column took place, with surface to bottom temperature differences limited to 2–3 °C (versus 8 °C in July). However, a marked pycnocline still persisted in the upper layer (0–2 m, average $\Delta\gamma_t$ = 6.8 units) (Fig. 4a).
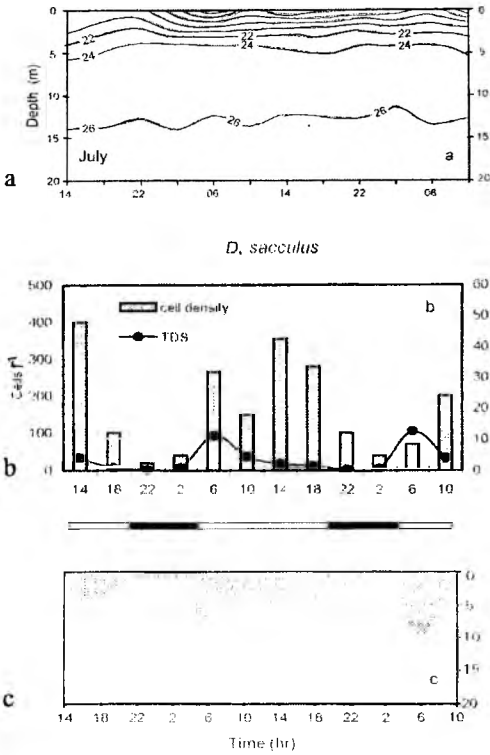
Fig. 2. (a) Isopycnal trends in the Gulf of Venice (4-6 July 1995). Contours at 2 γ_t intervals.

(b) *Dinophysis sacculus*. Coincident timing of cell density and terminal division stages (TDS, %) at 0.5 m.

(c) Time changes in vertical distribution of *D. sacculus* (light area: cells absent; dark area: cells present; max 400 cells L⁻¹). Bars indicate light and dark periods.
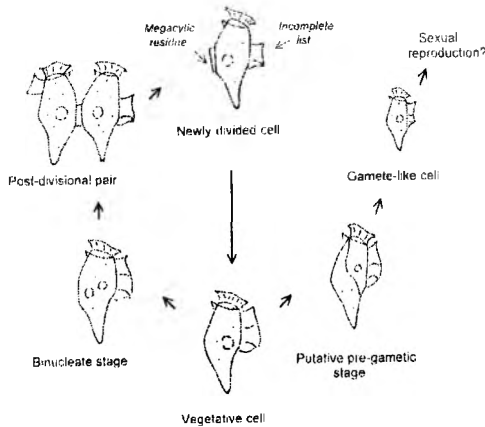

Fig. 3. Schematic diagram with reproductive stages of *Dinophysis caudata*. Asexual cycle on the left; possible sexual pathway on the right.

TDS frequency and density of *D. caudata* showed a 24 h periodicity, increasing between 06:00 h and 10:00 h (Fig. 4b). On two occasions, percentages of division stages were maximal at 10:00 h (52 % and 35 %). The potential growth
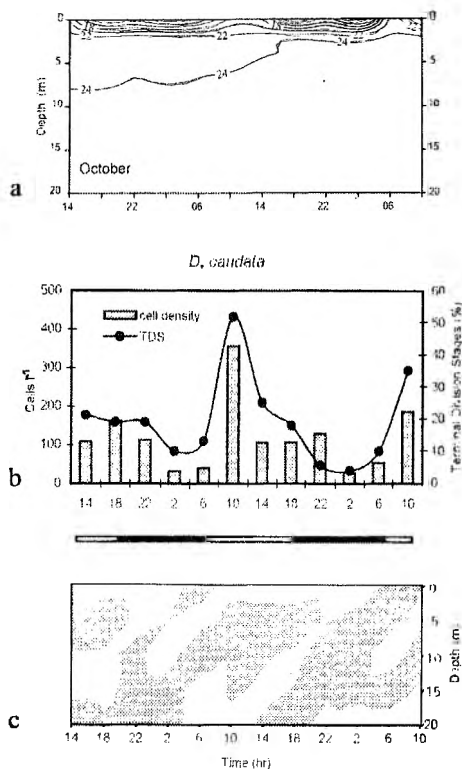
Fig. 4. (a) Isopycnal trends in autumn 1995 (October 3 5). Contours at 2 γ_t intervals.

(b) *D. caudata*. Coincident timing of cell density (values integrated over the water column) and terminal division stages (TDS, %).

(c) Space-time route of *D. caudata* populations (light area: cells absent; dark area: cells present; max 400 cells L^{-1}). Bars indicate light and dark periods.

rate of *D. caudata* determined in situ was 0.30–0.42 div day^{-1} versus 0.35–0.45 div day^{-1} from the laboratory (μ_{min} and net μ). In both cases, μ_{min} values were higher than in *D. sacculus* (Table 1). Unlike this species, *D. caudata* cells were spread over the entire water column (Fig. 4c) mostly assembling at 5 m. Gamete--like, small morphotypes (Fig. 3) constituted 4 % of the total (cell length × width: 70 × 34 μm; usual size: 90 × 44 μm). Upward displacements of the population were evident between 2:00 h and 6:00 h, leading to the appearance of cells at the surface just before sunrise (Fig. 4c). This suggests a possible endogenous rhythm of migration rather than a positive phototactic response or a passive vertical transport, in the absence of any hydrographic changes.

Alexandrium spp.

Alexandrium populations were represented in July by *A. pseudogonyaulax* (subgenus *Gessnerium*) and in October by *A.* cf. *tamarense* and *A.* cf. *fundyense* (subgenus *Alexandrium*). Assemblages of *A. pseudogonyaulax* were localized at the depth of 15 m only, just below the 26 γ_t isopycnal. This species, whose morphological characteristics are illustrated in Figure 5, resembles *A. taylori* (see GIACOBBE and YANG 1999) and *A. satoanum* in gross outline (BALECH 1995), although it can be distinguished by a peculiar, conspicuous ventral pore in its first apical plate (1'). The cell density of *A. pseudogonyaulax*, which produces a hepatotoxic metabolite rather than PSP-toxins (goniodomin A: TERAO et al.
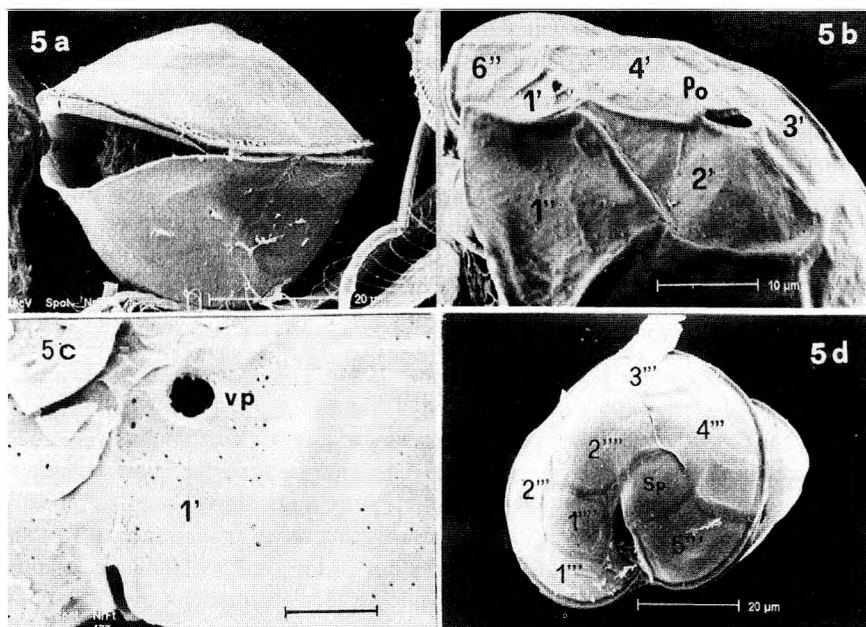
Fig. 5. *Alexandrium pseudogonyaulax* from natural populations. SEM.
(a) Specimen in early ecdysis.
(b) Epithecal view showing the complete disconnection of 1' and Po plates.
(c) 1' plate with a wide ventral pore (vp) near the right anterior margin. Scale bar = 2 μm.
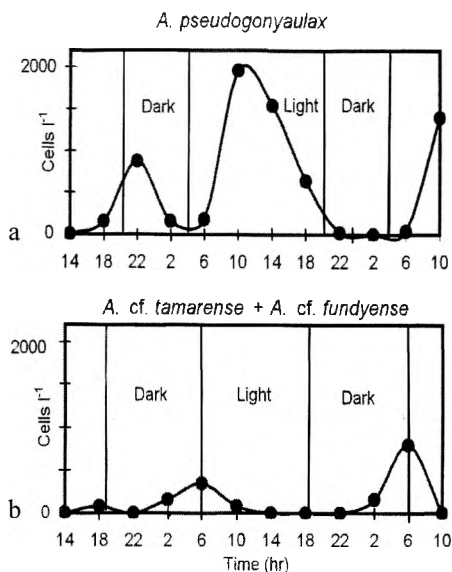(d) Hypothecal pattern with posterior sulcal plate (Sp) sloped to the right.

Fig. 6. Diel changes in densities of (a) *Alexandrium pseudogonyaulax* (July, 15 m) and (b) *A.* cf. *tamarense* plus *A.* cf. *fundyense* (October, 5 m).

1990), increased during the early light period up to a mid-morning peak (max.: 2 000 cells L^{-1} at 10:00 h); however, on one occasion there was also a slight increase at 22:00 h (Fig. 6a).

In contrast, populations of *A.* cf. *tamarense* and *A.* cf. *fundyense*, also present in other northern Adriatic areas (e.g. HONSELL et al. 1992), were often concentrated at 5 m – just below a more diluted water layer – with a negligible presence at the other points of the water column. This did not allow the evidencing of any migratory behaviour over the depths examined. In October, the total density of *A.* cf. *tamarense* plus *A.* cf. *fundyense* started to increase halfway through the dark period and peaked at sunrise (800 cells L^{-1}, Fig. 6b).

Conclusions

In July 1995, populations of *D. sacculus* from the Gulf of Venice were concentrated in the surface waters and displayed no migratory behaviour throughout the water column, although vertical migrations might have been limited to microlayers within the pycnocline. This hypothesis is consistent with other authors' observations of a migratory rhythm in cultured assemblages of this species (DELGADO et al. 1996), with cyclic surface fluctuations over the photoperiod closely comparable to our field profiles. Alternatively, the periodic nocturnal drop in density at the surface could be explained by coactive events, such as an increased grazing pressure, or by dynamic physical processes of horizontal dispersal. However, the sharp short-term oscillations in surface salinity, linked to the tidal regime, and the low potential growth rates observed support the last assumption, suggesting a diel transport of this population by flood and ebb currents, with corresponding cell dispersal or accumulation. Despite the interferences in the population growth due to tidal influence, the in situ division rhythm of *D. sacculus* displayed a 24 h periodicity, being apparently set in motion by the onset of the light period.

A circadian rhythm in cell division or migration is also likely in *D. caudata* in the light of the observation at regular intervals (24 hr) of enhanced division processes or vertical displacements of the population, in the absence of substantial, coinciding changes in physical conditions of the water column.

Although the interpretation of field data may be confused by horizontal and vertical transport, migration, or by parasite-effects due to tidal influence (SOURNIA 1974), the timing of *Alexandrium* densities too showed a clear rhythmic pattern, with a slight phase shift in the different species possibly due to different division times. Circadian periodicities can however be proven exclusively under constant laboratory conditions (SWEENEY 1984). Thus, whether or not such rhythms are controlled by endogenous or exogenous factors (e.g. photoperiod, light intensity, nutrient supply) remains to be established through laboratory experiments.

Aknowledgements

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