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Dinoflagellate cyst production in the north-western Adriatic Sea

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Abstract

A sediment trap study was conducted in the Gulf of Venice, north-western Adriatic Sea, from April to December 2005 to assess relationships between planktonic dinoflagellates and cyst production. Every month, CTD profiles and discrete samplings for phytoplankton, nutrients and particulate matter were conducted. Cyst fluxes spanned from 90 to 127,600 cysts m⁻² d⁻¹ and major peaks were due to a small cyst attributed to cf. *Biecheleria* and to calcareous cysts of *Scrippsiella trochoidea*. A good correspondence between cyst fluxes in sediment traps and the presence of the corresponding vegetative cells in the water column was detected for *Lingulodinium polyedrum*, and species of the genera *Gonyaulax* and *Protoperidinium*. A PCR method applied to surface sediment samples allowed the identification of a number of potentially harmful dinoflagellate cysts (*Alexandrium minutum*, *A. taylorii*, *Lingulodinium polyedrum* and *Protoceratium reticulatum*).

Keywords: Dinoflagellates, cysts, sediment traps, NW Adriatic Sea, *Scrippsiella*, *Biecheleria*.

Introduction

Many dinoflagellates produce cysts during their life cycle (Head, 1996) and these resting stages often play an important role in population dynamics (e.g., Marcus & Boero, 1998; Dale, 2001; Anderson *et al.*, 2014). The switch from actively growing cells to resting cysts can in fact contribute to a bloom termination (e.g., Kremp & Heiskanen, 1999), while the germination of cysts can represent the inoculum for future blooms in the water column (e.g., Anderson *et al.*, 2005; Wang *et al.*, 2007; Ishikawa *et al.*, 2014). The formation of resting cysts might represent an adaptive trait for protists living in highly variable coastal environments. These organisms can grow in the water column during favorable environmental windows and then sink onto surface sediments, where they constitute cyst banks that 'anchor' planktonic species to a specific environment (Anderson & Wall, 1978; Wyatt & Jenkinson, 1997; McGillicuddy *et al.*, 2003; Mizushima, 2004; Anderson *et al.*, 2005). Cyst banks include populations originating over several years and thus represent a source of phenotypic and genotypic diversity (Alpermann *et al.*, 2009). Cysts are surrounded by a thick and decay-resistant cell wall and contain high amounts of reserve materials; these features allow them to remain viable in the bottom sediments for years (Ribeiro *et al.*, 2011). These resting stages can survive in ballast waters or in shellfish stocks traded over long distances, and can thus represent a way of dispersal through

space and time. This is particularly relevant in the case of harmful species, where efforts have been made to elucidate the role of resting stages in the apparent spreading of harmful algal blooms (e.g., Joyce *et al.*, 2005; Bolch & De Salas, 2007).

Factors controlling cyst production and the physiological mechanisms involved have not been clearly defined, and laboratory experiments at times produced results that do not match with field observations. Research carried out with cultured strains suggested that encystment is related to the onset of stress conditions (Pfiester & Anderson, 1987), in particular nutrient limitation (e.g., Anderson & Lindquist, 1985; Figueroa *et al.*, 2005). However, field and laboratory studies could not always find a link between cyst production and nutrient limitation (Anderson *et al.*, 1983; Zonneveld *et al.*, 2009). Other factors, such as day length (Sgrosso *et al.*, 2001), temperature shifts (Kremp *et al.*, 2009), a combination of these factors (Pospelova *et al.*, 2010) or, in heterotrophic species, food limitation (Nagai *et al.*, 2002; Saito *et al.*, 2007) might trigger or modulate the formation of resting cysts.

Many studies reported qualitative and quantitative information on dinoflagellate cysts in marine sediments and extensive distribution maps, covering broad geographic ranges, are now available (e.g., Zonneveld *et al.*, 2013). However, much fewer are the studies that estimated cyst fluxes along the water column in coastal areas (e.g., Fujii & Matsuoka, 2006; Pospelova *et al.*, 2010;

Price & Pospelova, 2011; Shin *et al.*, 2012; Bringue *et al.*, 2013; Fertouna-Bellakhal *et al.*, 2014) and even fewer are those that related cyst production with the seasonal pattern of the planktonic dinoflagellate assemblage (e.g., Montresor *et al.*, 1998; Godhe *et al.*, 2001; Balkis *et al.*, 2016). Cyst production generally shows a marked seasonality, both in terms of total fluxes and species assemblages, with production peaks often related to the increase of water temperature (Shin *et al.*, 2012).

The northern Adriatic Sea is a highly productive area, where phytoplankton communities are typically dominated in terms of abundance by diatoms and phytoflagellates throughout the year, while dinoflagellates contribute, on average, only a limited percentage of the population (Bernardi Aubry *et al.*, 2012). However, during spring and summer, when dinoflagellates show peaks of abundances, they can account for approximately half of the total phytoplankton biomass (Bernardi Aubry *et al.*, 2006). A number of studies focused on cyst abundances in surface sediments in the northern Adriatic Sea (e.g. Nichetto *et al.*, 1995; Sangiorgi *et al.*, 2005; Penna *et al.*, 2010), among which a survey carried out in surface sediments has shown that cyst abundances were maximal in areas corresponding to the Po River plume (Rubino *et al.*, 2000; Zonneveld *et al.*, 2009). In this area, the abundance of dinoflagellate cysts has been also quantified along a sediment core dating back 150 yrs and shifts

between non-eutrophic and eutrophic periods have been detected based on the composition of the cyst assemblage (Sangiorgi & Donders, 2004).

The aims of this study are: (i) to estimate dinoflagellate cyst fluxes at an offshore station of the Gulf of Venice (NW Adriatic Sea), (ii) to assess the relationship between the abundances of planktonic dinoflagellates in the water column and the production of resting cysts, and (iii) to assess the abundance of dinoflagellate cysts in surface sediments focusing on harmful species.

Methods

Study area and sampling

The sampling site (station C10, 45.25° N 012.76° E) was located in the Gulf of Venice (NW Adriatic Sea) (Fig. 1), at 36 km from the coast, with a bottom depth of 30 m. The area is characterized by the input of important outflows of continental freshwater (Po and Adige rivers) into its western side. This hydrological feature is the main driving force for the circulation pattern of water masses in the Adriatic Sea. An open cyclonic circulation characterizes the basin during winter, when the unstable physical structure of the water column induces the formation of coastal fronts and freshwater flows southward along the western coastline. The beginning of thermal stratification in the spring is usually coupled with an increase of freshwater

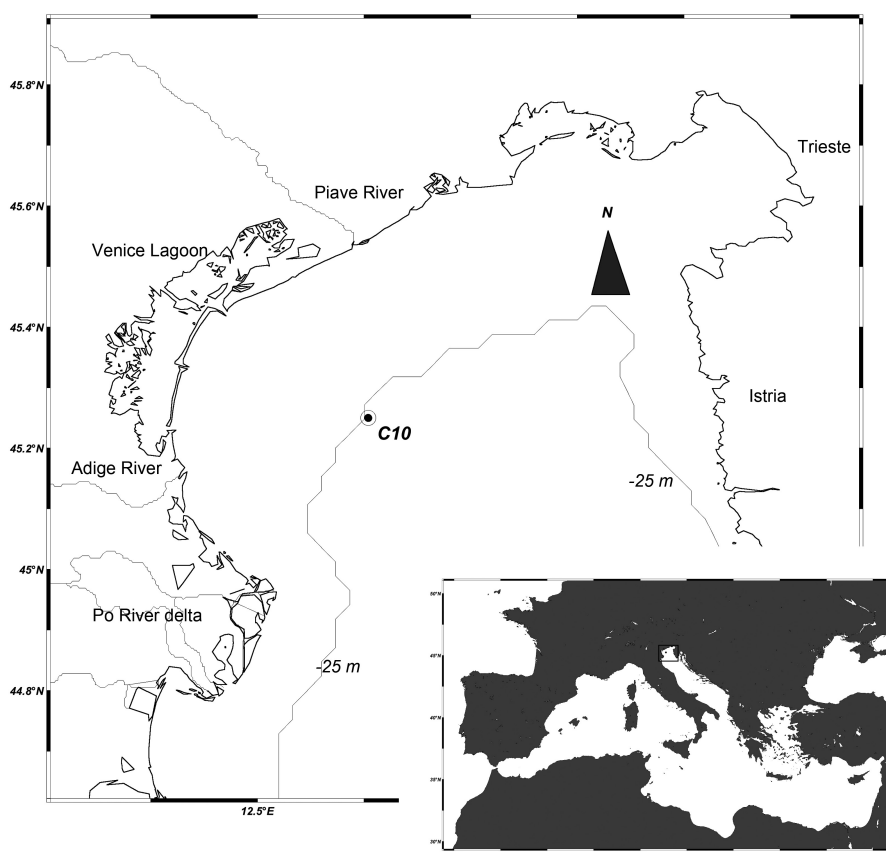


Fig. 1: Map of the Gulf of Venice (insert of the Mediterranean Sea) with the location of the sampling station C10.

discharge, and the low salinity surface layer flows eastwards, producing a wide mesoscale structure that extends southwards till the Po River mouth (Socal *et al.*, 2008). Usually, in the presence of vertical stability and strong winds from NE (Bora wind), a branch of the river plume can flow eastward, reaching the Istria peninsula, and determines the formation of a double gyre with semi-enclosed circulation and opposite direction patterns (Ursella *et al.*, 2006). Cyclonic and anticyclonic structures that develop during the summer in the northern and southern parts of the basin, respectively, are conducive to cross-basin transport of the west side coastal Po River-affected waters.

Sampling was carried out from 22 April to 25 December 2005. A sediment trap was deployed for the entire study period, whereas CTD profiles (Idronaut model 316, Milano, Italy) were carried out on a monthly basis. The mooring system was equipped with current meter and a sediment trap (cylindrical-conic Technicap model 4/3, La Turbie; France) placed 4 m from the bottom. The trap had a collection area of 0.05 m² and was connected to a 12-sample carousel that sealed one collector every ten days. To prevent cell degradation, the collecting bottles were filled with a preservative consisting of 4% buffered formalin solution in 250 ml of seawater filtered through Millipore 0.2 mm filters. After collection, samples were stored in the dark at 4°C until laboratory analyses of total organic carbon, total mass flux (TMF) and cyst abundance.

Surface sediment samples were collected in October 2005 and January 2006 by SCUBA divers using three 50 ml syringes with cut-off bases. Sediment samples were extruded from the syringe and the top 2 cm layer (i.e., 10 cm³ of wet sediment) was cut with a razor, and stored in the dark at 4 °C. These three samples were used to estimate: (i) sediment granulometry, (ii) organic carbon content and the dry weight, and (iii) the abundance of dinoflagellate cysts, respectively for each sampling.

Water samples for phytoplankton, dissolved nutrients, total suspended matter (TSM) and organic carbon (OC) analysis were collected at monthly intervals with Niskin bottles at three different depths (surface, intermediate and near bottom). Samples for TSM, OC and nutrient analyses were immediately filtered on GF/F Whatman pre-weighted glass-fiber filters (25 mm diameter, nominal porosity 0.7 µm) and frozen at -20 °C until analysis. Samples for phytoplankton analysis were immediately fixed with hexamethylenetetramine neutralized formaldehyde to a final concentration of 0.8%. Vertical net samples were collected with a 20 µm mesh phytoplankton net and fixed with neutralized formaldehyde to a final concentration of 1.6%.

Nutrient and total suspended matter analysis

Samples for nutrient analysis (NO₂, NO₃, NH₄ and PO₄) were analyzed using a Syssta Autoanalyzer (Anagni, Italy), according to Grasshoff *et al.* (1999). TSM dry weight was determined gravimetrically after drying at 60 °C.

OC content was determined by a Perkin-Elmer 2400 CHN elemental analyser (Waltham, MA, USA), after removing inorganic carbon by vapor phase acidification following the method of Hedges & Stern (1984).

Treatment of sediment trap samples

Sediment trap samples were processed to determine the Total Mass Flux (TMF), the organic carbon (OC) and the cyst abundance and composition. Sediment trap samples were treated following Heussner *et al.* (1990) and swimmers (i.e., zooplankton and occasionally small fish that are thought to actively enter sediment traps) were manually removed. To determine the total mass flux (TMF) and organic carbon, three subsamples (with variable volumes from 1/20 to 1/280 of the sample volume depending on concentration) for each parameter were filtered through Whatman GF/F filters, pre-washed and pre-combusted overnight at 500 °C. To estimate the splitting accuracy, variation coefficients for the three replicate samples were calculated from weight determinations: these ranged from 0.27 to 11.44%, with an average of 2.02%. Filters for TMF determination were dried at 60 °C and then weighed (Strickland & Parsons, 1972). TMF was expressed as g m⁻² d⁻¹. The OC was determined as illustrated in the ‘*Nutrient and total suspended matter analysis*’ paragraph and expressed as percent of TMF.

For the quantification of cysts in sediment traps, a subsample of variable volume (2.5 to 20 ml, depending on the quantity of settled material) was collected after a careful homogenization, sonicated for 2 min with a Branson 2510 sonicator (Branson Ultrasonics, CT, USA), prefiltered onto a 100 µm mesh stainless sieve and then sieved on a 10 µm stainless sieve (Retsch, Haan, Germany). The material collected on the 10 µm sieve was transferred into a plastic tube, adjusted to 7.5 ml with filtered sea water (FSW) and stored at 4 °C until the quantitative analyses were carried out (within 1 month after sample collection). Before the analyses, the slurry was homogenized, and two replicates (from 200 to 1000 µl) were transferred to test tubes and diluted with FSW to a final volume of 10 ml for the microscopy analysis.

Treatment of surface sediment samples

The first sediment sample was used to determine granulometry, i.e. the sand fraction less than 63 µm. The sample was wet sieved, using A.S.T.M. sieves and processed following Wentworth (1922).

The second sample was used to measure the dry weight (by drying the filter at 105 °C for one week) then analyzed the OC content following the method of Hedges & Stern (1984) (as illustrated in the “*Nutrient and total suspended matter analysis*” paragraph). The OC was expressed as percent of dry weight.

The third sample (10 cm³) was used to assess cyst abundance and composition by microscopic and molecular methods. The sample was sonicated and sieved

as described above for trap samples. A 14 ml subsample was taken, diluted in 40 ml of FSW and divided into four tubes (10 ml each). Each tube was treated following the Bolch (1997) method: after separation of the organic fraction from the sediment using a differential gradient of sodium polytungstate (SPT), the pellet was suspended by adding 5 ml FSW and fixed with Lugol. Three tubes were used for microscope analysis and one for molecular PCR analysis.

Resuspension assessment

To estimate the fraction attributed to the autochthonous settling of organic matter of planktonic origin (primary flux) and that attributed to the bottom sediment resuspension (secondary flux) in the trap samples, a simplified label approach was used based on two end-member's mixing as proposed by Gasith (1975) and applied in the northern Adriatic Sea by Matteucci & Frasconi (1997) and Giani *et al.* (2001). As indicators of the primary flux, the value of 12.3% of organic carbon (OC) in the total suspended matter was used. This value represents the highest percentage of organic carbon on TSM measured in the water column during the study period and was measured in May 2005, during a phytoplankton bloom. As indicators of the resuspension, the value of OC percentage in the sediment was chosen. This value was 0.34% (measured at October 2005), in consideration that the seasonal variability of this parameter was extremely low (De Lazzari *et al.*, 2006).

The relative contribution of primary flux to the gross sedimentation in each trap-sample was calculated as follows:

$$F_p = F_t (C_i - C_r) / (C_p - C_r)$$

where F_p is the sedimentation rate of autochthonous fraction ($\text{g} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$), F_t is the gross sedimentation (TMF as $\text{g} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$), C_i is the OC% in the i -th sample, C_r is OC% in the resuspension end member and C_p is OC% in the primary flux end member. The relative contribution of resuspension fraction (F_r) was then calculated as $F_t - F_p$. Then contribution of cysts derived from sediment resuspension was calculated for each trap sample by multiplying the factor F_r by the cyst abundances measured in sediment samples at the same station.

Microscope analysis

Trap and sediment samples for cyst analysis and water samples for phytoplankton analysis were homogenized and a subsample (0.5-10 ml, 1 ml and 25-50 ml, respectively) was settled in an Utermöhl chamber and analyzed with an inverted microscope (Axiovert 35, Zeiss, Oberkochen, Germany) equipped with phase contrast, following the Utermöhl method (Edler & Elbrächter, 2010).

Cysts were counted on the entire sedimentation chamber at magnifications of 200 and 400. Phytoplankton counts were performed on 1-4 transects at 400x magnification to enumerate a minimum of 200 cells. The entire chamber was then analyzed at 200x to obtain a more accurate esti-

mate of large-sized dinoflagellates. Phytoplankton abundances were expressed as cells l^{-1} and, to allow comparison with cyst fluxes, as cells m^{-2} , after trapezoid integration of cell concentration data at the three sampling depths.

The cysts of *cf. Biecheleria* were prepared for SEM analysis following the method illustrated in Siano & Montresor (2005). Samples were placed on a Nucleopore (Nucleopore, Pleasanton, CA) polycarbonate filter, dehydrated in an ethanol series (10, 30, 50, 70, 80, 90, 95 and 100%), and treated in a Critical Point Dryer (Polaron CPD 7501, Newhaven, GB). The filter was mounted on a stub, sputter coated with gold-palladium for observation under a Philips XL20 (Philips, Eindhoven, The Netherlands).

Molecular PCR analysis

Lugol-fixed sediment samples obtained as reported above were centrifuged at 4,000 rpm for 20 min at room temperature, the supernatant was gently discarded and 20 ml of filtered seawater was added, and the samples were again centrifuged at 4,000 rpm for 20 min at room temperature (Penna *et al.*, 2010). The cyst pellet was resuspended in 1 ml of sterile MilliQ water and centrifuged at 12,000 rpm for 5 min at room temperature; the supernatant was discarded and the pellet was frozen at -80°C until analyses. The procedure of processing cyst pellets, and the genomic DNA extraction and purification were carried out according to Penna *et al.* (2010). Purified genomic DNA was quantified on agarose gel using serially diluted Lambda DNA Marker (MBI Fermentas, Germany) and a gel-doc apparatus (Bio-Rad, Hercules, CA, USA).

Primers for the genus *Alexandrium* and species-specific primers for *A. minutum*, *A. taylorii*, *A. mediterraneum*, *Lingulodinium polyedrum* and *Protoceratium reticulatum* designed in the 5.8S rDNA-ITS regions were derived from Penna *et al.* (2007), while primers specific for *A. tamutum* were designed in this study. The primers were designed in the ITS-5.8S rDNA regions based on all *Alexandrium* species and other related dinoflagellate consensus ITS-5.8S rRNA gene sequences available from GenBank by using OLIGO ver. 6.65. The species-specific primers for amplification of 157 bp of *A. tamutum* were Ftam (5'-TCATGCTCGTTCTTTGCTTAC-3') and Rtam (5'-GAAGGTGCAAATTACGTTCA-3'). The primers were synthesized by Eurofins MWG operon (Ebersberg, Germany).

Amplification from sediment samples for the detection of different taxa was performed in an Applied Biosystems DNA Thermo Cycler 2720 (Foster City, CA, USA). PCR amplifications were carried out directly using genus- or species-specific primers or by following two steps as follows: an initial PCR using eukaryotic primers targeting the ITS-5.8S gene; then, a second (i.e. nested) PCR reaction, using genus and species-specific primers on amplified products of ribosomal genes as described in Penna *et al.* (2010).

Results

Environmental parameters

Temperature was one of the main factors regulating water stratification and varied from 7.5 to 25.0 °C in the period February to December 2005 (Fig. 2a). Decreases of salinity in the surface layers were observed at May 25th, and October 20th representing clear freshwater pulses influencing the water column stability (Fig. 2b).

The dissolved inorganic nitrogen (DIN, i.e. the sum of dissolved nitrates, nitrites and ammonia) ranged from 0.11 to 89.75 $\mu\text{mol l}^{-1}$ (average in the sampling period $5.8 \pm 17.17 \mu\text{mol l}^{-1}$, Fig. 2b). Maximum values of DIN were observed on October 20th 2005 (Fig. 2b), while minima were observed in summer when the nitric component of DIN was almost completely depleted. Phosphates never reached particularly high concentrations, ranging between: 0.02 and 0.75 $\mu\text{mol l}^{-1}$ (avg $0.13 \pm 0.14 \mu\text{mol l}^{-1}$), with maximum values at surface on 20 October 2005 (data not shown).

Cyst flux

A total of 14 dinoflagellate cyst taxa has been identified in sediment trap samples, eleven of which are illustrated in Fig. 3. Other taxa not illustrated are the cysts of *Scrippsiella trochoidea*, a complex of at least six cryp-

tic species (D'Onofrio *et al.*, 1999; Zinssmeister *et al.*, 2011), Protoperidinium and of *Diplopsalis*. The cysts attributed to the *Scrippsiella trochoidea* complex were oval, 20-45 μm long and 20-40 μm wide, and surrounded by calcareous spinous processes of variable lengths. Small spherical organic-walled cysts (approximately 15 μm in diameter) that in light microscopy appeared surrounded by a thin mucus layer collecting detritus on their surfaces were often recorded in the sediment trap samples (Fig. 3a). When observed with SEM, their wall surfaces showed faint reticulation and numerous short (2 μm) conical processes (Fig. 3b). These cysts were identified as a cyst of cf. *Biecheleria* (Moestrup *et al.*, 2009). Another dinoflagellate frequently recorded in autumn was the cyst of *Lingulodinium polyedrum*, with a spherical central cyst body (diameter 40-50 μm) covered by numerous hollow and slender conical processes, variable in size, with pointed tips ornamented by minute microgranules (Fig. 3c). Cysts attributed to *Alexandrium minutum* or to *A. tamutum* - the cysts of the two species are morphologically identical (Montresor *et al.*, 2004) - were roughly hemispherical, pale in color, covered by a thin layer of mucilage and with a prominent orange-red accumulation body (Fig. 3d). Other cyst taxa were also detected, such as *Spiniferites* cysts, which is the micropaleontologic name

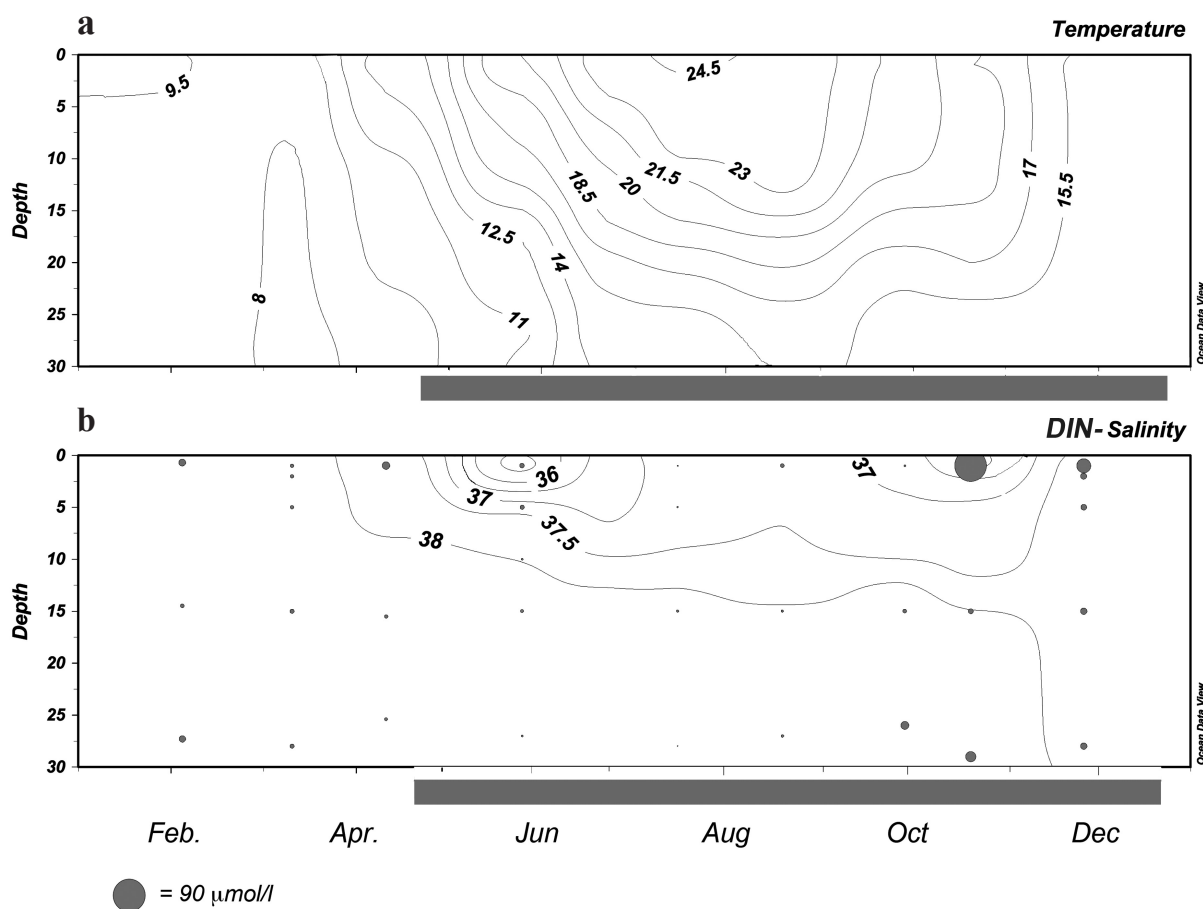


Fig. 2: Vertical profiles of temperature (a), salinity and total dissolved nitrogen (DIN) (b). Circle areas are proportional to DIN concentration (reference scale on the left corner of the x axis). Gray bars below the x axis mark the period of trap deployment.

of *Gonyaulax* (Fig. 3e). These *Spiniferites* cysts species presented bifurcate and trifurcate processes, the cyst wall was usually colorless and the shape of the main body varied from spherical to ellipsoidal with a diameter of between 30 and 50 μm . Among *Protoperidinium* cysts, cysts of *P. compressum* (Fig. 3f), *P. conicum* (Fig. 3g), *P. subinerme* (Fig. 3h) and *P. claudicans* (Fig. 3i) were recorded. Round cysts with an organic brown, smooth wall, identified here as 'round brown cysts' and attributable to the micropaleontological genus *Brigantedinium* (cysts of *Protoperidinium*) or to cysts of *Diplopsalis* were also relatively abundant in our samples. In one single sample, cysts of *Protoceratium reticulatum* were recorded (Fig. 3j). These cysts were round (diameter ca. 50 μm) and ornamented by numerous thin tubular processes of variable morphology, slender to stout,

with capitate tips. Finally, two rare (in terms of frequency and abundance) calcareous cyst taxa were recorded in trap samples; they have been identified as *Pentapharsodinium tyrrhenicum* (Fig. 3k) and *Posoniella tricarinelloides* (Fig. 3l). The first has a round to slightly oval body covered by a calcareous layer of variable thickness. Diameter of the cyst body (calcareous layer excluded) is 25-32 μm , and the calcareous layer is 2-15 μm thick. The *Posoniella tricarinelloides* cyst was spherical and surrounded by a thick layer of calcareous crystals randomly arranged, which formed two parallel transversal ridges. The diameter of the cyst body was around 25 μm .

Cyst fluxes ranged in number from a minimum of 90 ± 10 (September, first week) to maximum of $127,600 \pm 5,414$ cysts $\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ (August, second week) (Fig. 4a).

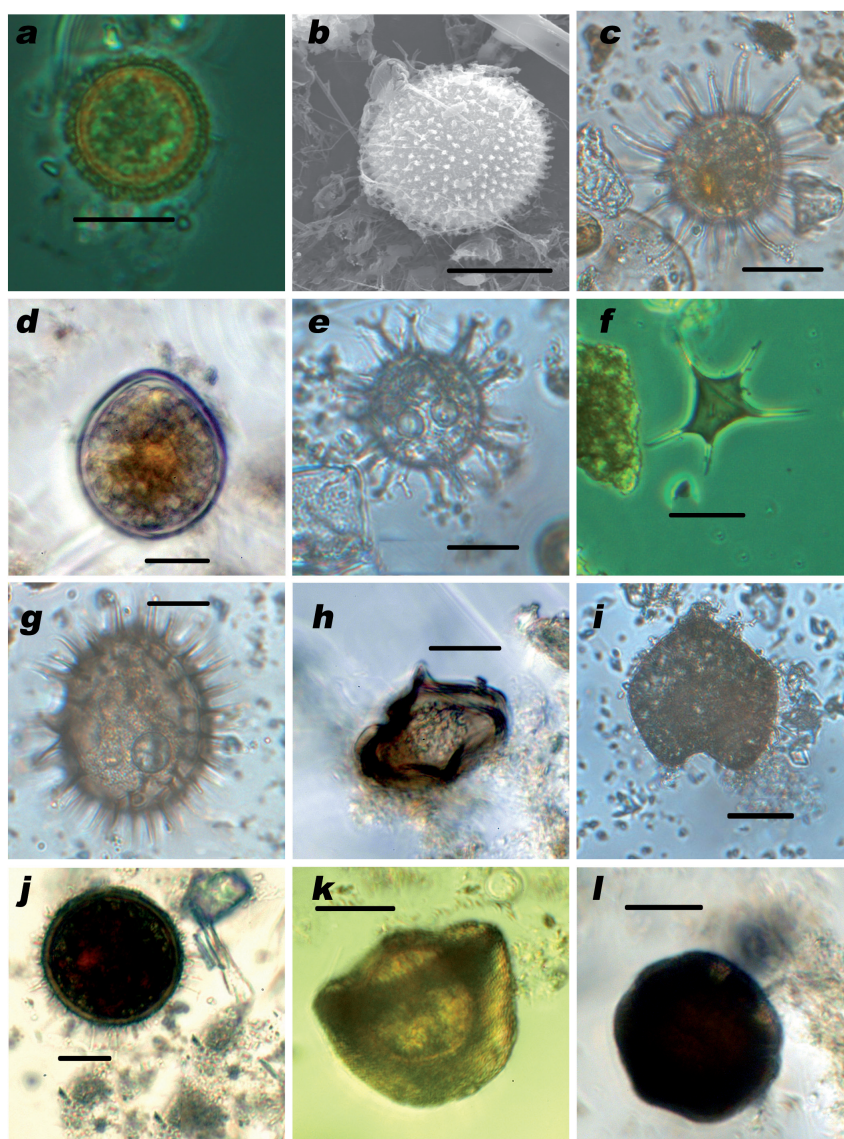


Fig. 3: Light microscope (LM) and Scanning electron microscope (SEM) images of selected cyst species identified in the study. a-b) cyst of cf. *Biecheleria*, LM (a) and SEM (b); c) cyst of *Lingulodinium polyedrum*; d) cyst of *Alexandrium minutum*/ *A. tamutum*; e) *Spiniferites* sp.; f) cyst of *Protoperidinium compressum*; g) cyst of *Protoperidinium conicum*; h) cyst of *Protoperidinium subinerme*; i) cyst of *Protoperidinium claudicans*; j) cyst of *Protoceratium reticulatum*; k) *Pentapharsodinium tyrrhenicum*; l) *Posoniella tricarinelloides*. Scale bars: 10 μm (a-b); 30 μm (c, f, i) 20 μm d, e, g-h,j-l).

At the beginning of the sampling period (May), the cyst flux ranged from $10,400 \pm 221$ to $22,500 \pm 636$ cysts \cdot m⁻² \cdot d⁻¹ (Fig. 4a). The spring cyst assemblage mainly consisted of cf. *Biecheleria* cysts (50-60%), unidentified round brown cysts and cysts of *S. trochoidea* (Fig. 4b). Later on, from the middle of June to the middle of July, rather low cyst fluxes (from $1,300 \pm 721$ to $2,400 \pm 305$ cysts \cdot m⁻² \cdot d⁻¹) were detected and the species composition was similar to those of May, with the small cf. *Biecheleria* cysts accounting for the highest percentage. At the beginning of August, a sharp increase in fluxes of *S. trochoidea* cysts occurred and they reached the highest abundances ($99,600 \pm 814$ cysts \cdot m⁻² \cdot d⁻¹) on 14 August, when they dominated (72 \pm 9%) the cyst assemblage (Fig. 4b). Ten days later, a sudden decrease of two orders of magnitude in cyst fluxes was observed and these low levels were maintained until the middle of September, when the composition of the cyst assemblage changed. The late summer-autumn period (September-December) was characterized by the dominance of *Lingulodinium polyedrum* and *Alexandrium minutum*/*A. tamutum* cysts, with the former prevailing in late September-October and the latter in November-December. In autumn, cyst fluxes were rather constant and ranged between $5,000 \pm 487$ and $14,100 \pm 1,296$ cysts \cdot m⁻² \cdot d⁻¹. Other cysts were found with lower fluxes, such as those of gen-

era *Protoperidinium*, *Diplopsalis*, *Gonyaulax* (as different *Spiniferites* species) and *Protoceratium reticulatum*. A few cysts of *Posoniella tricarinelloides* were detected in the August samples. Empty cysts, mainly belonging to *Lingulodinium polyedrum*, represented a minor fraction (2-4 %) that was even lower in correspondence with maximum fluxes.

Temporal trends of dinoflagellate taxa in the water column and cysts in the sediment traps

The abundances of dinoflagellates in the water column ranged from 560 to 105,000 cells l⁻¹ ($7 \cdot 10^6$ to $1.1 \cdot 10^9$ cells m⁻²). The most abundant taxa were unidentified Gymnodiniales, *Protoperidinium* spp., *Prorocentrum* spp. and the *Scrippsiella trochoidea* complex. For some taxa, a correspondence between the presence of vegetative cells in the water column and the flux of the relative cysts in the sediment traps was detected (Tab.1; Fig. 5). A good match was observed between the abundance of species of the genus *Gonyaulax* in the water column and that of *Spiniferites* spp. in trap samples (Fig. 5a) and for *Lingulodinium polyedrum* and its cysts (Fig. 5b). On the contrary, no such relation was observed for the spiny calcareous *Scrippsiella trochoidea* cysts and its corresponding vegetative stages, which were one of the most rep-

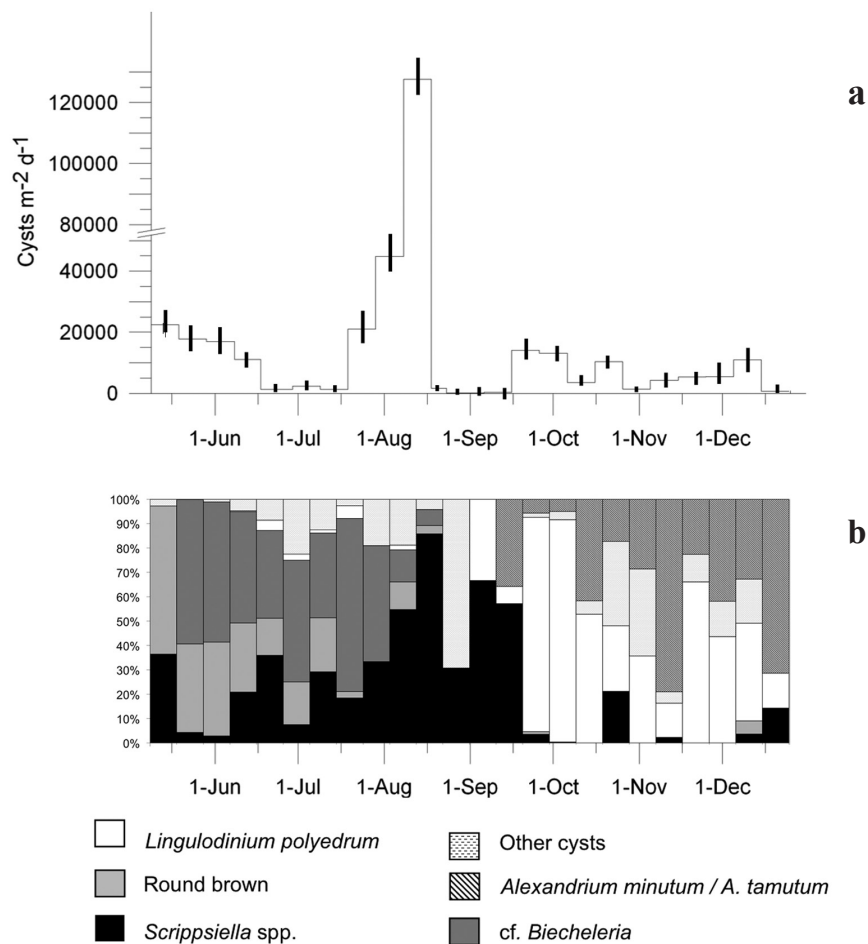


Fig. 4: Dinoflagellate cyst fluxes (cysts \cdot m⁻² \cdot d⁻¹) at station C10 and relative abundances of the main dinoflagellate cyst taxa in sediment traps.

representative taxa in the phytoplankton (Fig. 5c). Peaks of *cf. Biecheleria* cysts were measured in correspondence of relatively high abundances of small naked dinoflagellates until the end of August, but the abundance of vegetative cells was rather high also during the following months, when this cyst morphotype was not detected in the sediment traps (Fig. 5d). Semi-quantitative data of dinoflagel-

late species detected in the vertical net samples further supported the matching vegetative cells and cysts in trap samples for *Protoperidinium* spp. in spring (Fig. 5e) and *Alexandrium minutum/A. tamutum* (Fig. 5f). The latter cysts were constantly recorded, albeit with low abundances, in sediment traps during autumn, and vegetative cells were present in water and net samples in the same period.

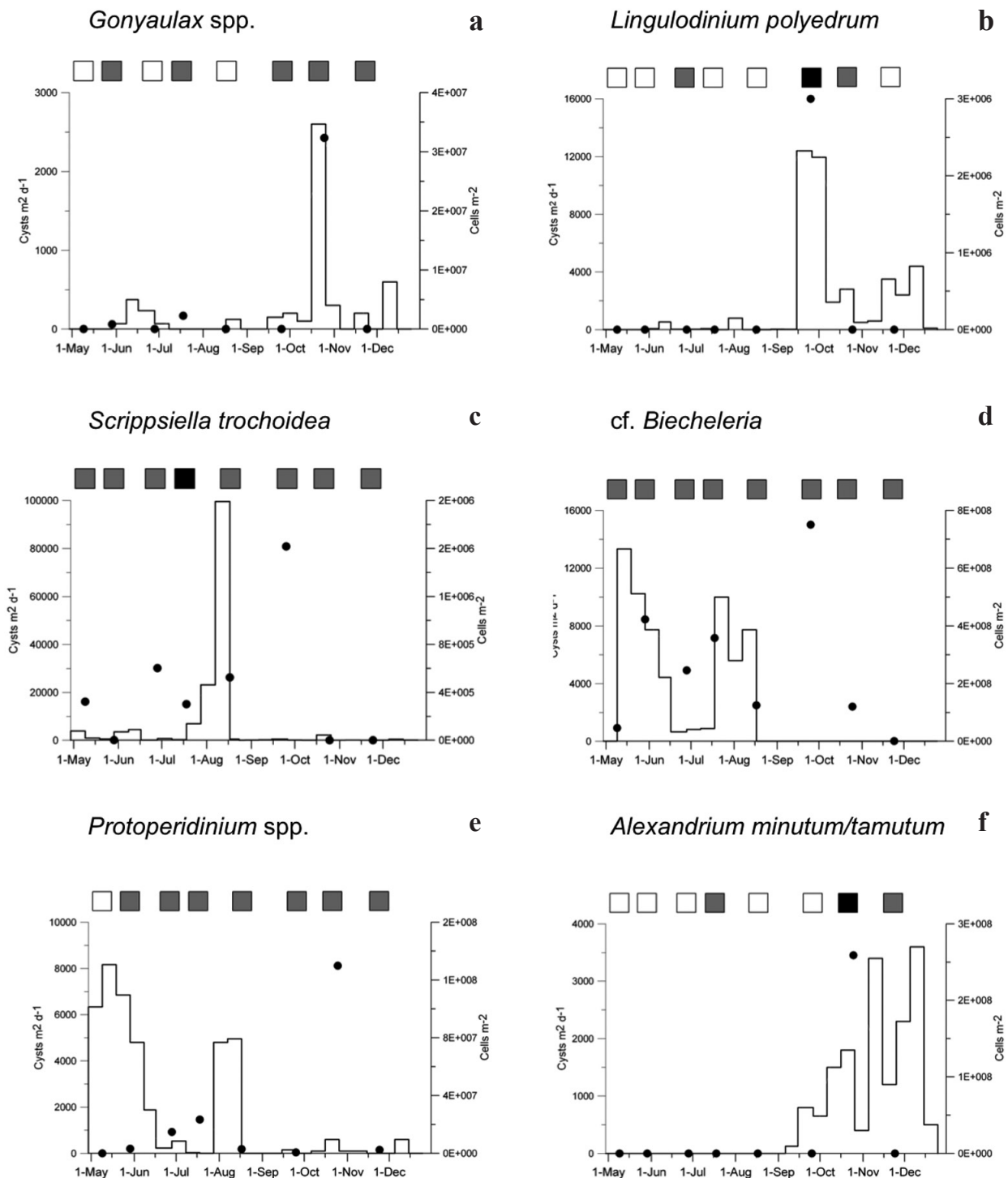


Fig. 5: Cysts fluxes (cysts·m⁻²·d⁻¹; bars) and cell abundances integrated over the water column (cells m⁻², black circles) for selected dinoflagellate species. Squares on the top of the figure represent the presence of the species in vertical net samples (white = absent; gray = present; black = abundant). a) *Spiniferites* cysts and *Gonyaulax* spp.; b) *Lingulodinium polyedrum*; c) *Scrippsiella trochoidea*; d) *cf. Biecheleria* cysts and small gymnodinioids; e) *Protoperidinium* spp.; f) *Alexandrium minutum/tamutum*.

Cyst abundance and composition in surface sediments

The sediment was classified as “silty sand” and contained 73% sand. The cyst abundance was lower in the October 2005 sample (299 ± 18 cysts·g⁻¹·dw) as compared to January 2006 (466 ± 95 cysts·g⁻¹·dw). In October 2005, *Scrippsiella trochoidea* cysts dominated ($28 \pm 15\%$), followed by *Lingulodinium polyedrum* ($21 \pm 2\%$), round brown cysts ($18 \pm 10\%$), and by *Protoperidinium oblongum*, *Spiniferites* (cysts of *Gonyaulax* spp.), *Alexandrium minutum-tamutum* and *Pentapharsodinium dalei*. In January 2006, *Scrippsiella trochoidea* represented $36 \pm 8\%$, round brown $22 \pm 6\%$, and *L. polyedrum* $14 \pm 3\%$.

The PCR assay detected the presence of various *Alexandrium* species (*A. taylorii*, *A. minutum* and *A. tamutum*), *Lingulodinium polyedrum* and *Protoperidinium reticulatum*. In particular, species-specific PCR amplification products were obtained based on the corresponding size fragments of 297, 212, 157, 383, 382 bp, respectively.

Fluxes of organic and inorganic material and estimates of resuspension

A high variability of the total mass flux (TMF) was observed, with values ranging from 0.39 to 105.17 g m⁻² d⁻¹ (mean value 21.22 ± 33.53) (Fig. 6), with the highest values in October and December 2005. The organic carbon averaged $5.78 \pm 1.18\%$, and the higher organic fraction was observed in spring-summer seasons (up to 14%). The peaks in TMF generally corresponded to the minimum in organic fraction (Fig. 6).

Resuspension was high in autumn-winter (more than 80%), while it was approximately 40-50% in spring-summer with a marked decrease (13%) in August 2005. The

abundances of cysts in sediments were used to estimate the amount of resuspended cysts in the sediment traps. In general, the pattern of total cyst fluxes was opposite to that of resuspension and the contribution of cysts derived from resuspension processes never exceeded 0.3%, while it increased in July in correspondence of cyst flux minima (even reaching 100%).

Discussion

Dinoflagellate cyst fluxes

Dinoflagellate cyst fluxes recorded at the sampling station in the north-western Adriatic Sea ranged between 90 ± 10 and $127,600 \pm 5,414$ cysts m⁻² day⁻¹. The sampling interval of the sediment traps was rather narrow, enabling assessment of the temporal variability of cyst fluxes with good resolution. The highest cyst fluxes were recorded during the warmest period; in this case, the water column was stratified and DIN concentrations were low.

A comparison of our data with dinoflagellate cyst fluxes in other temperate coastal areas is hampered by the different methods used to estimate cyst fluxes. Some studies in fact used palynological methods based on the use of heavy acids, which destroy calcareous resting cysts that are often abundant in coastal areas. This is the case in two of the extensive studies that presented data on dinoflagellate cyst production in the Strait of Georgia and in Saanich Inlet (Canada) (Pospelova *et al.*, 2010; Price & Pospelova, 2011). Cyst fluxes were extremely high in Saanich Inlet with values comprising between $\sim 149,000$ and $\sim 2,400,000$ cysts m⁻² day⁻¹ with an assemblage dominated by cysts of heterotrophic species in spring and summer and *Spiniferites* cysts during the winter months (Price & Pospelova, 2011).

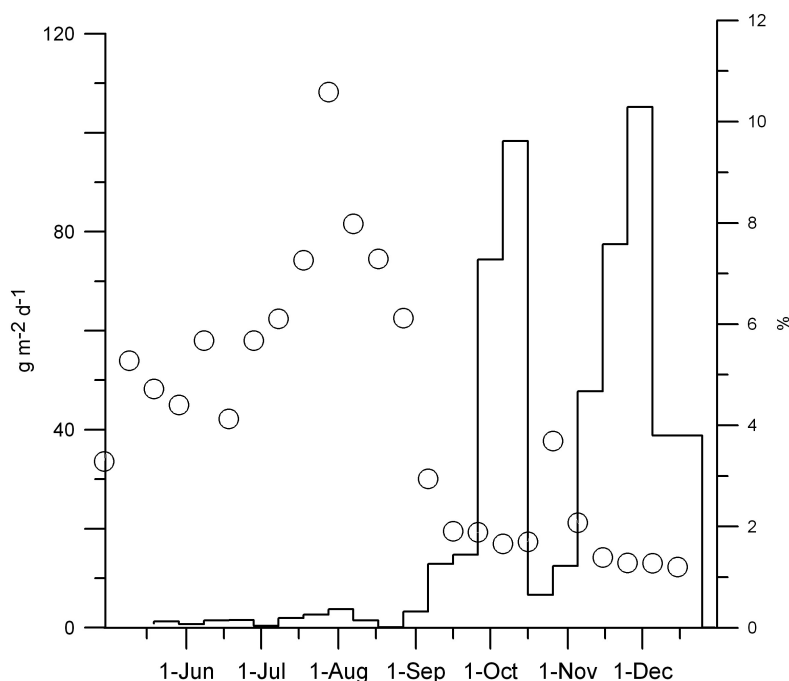


Fig. 6: Total mass flux (g·m⁻²·d⁻¹; bars) and organic carbon flux expressed as percentages of the total mass flux (circles).

Table 1. List of dinoflagellate taxa recorded as vegetative cells in net samples and as cysts in sediment traps; species marked in bold are known to produce cysts. Gray blocking highlight the co-occurrence of both vegetative cells in the water column and cysts in sediment traps.

	28-Apr-05		25-May-05		21-Jun-05		14-Jul-05		18-Aug-05		27-Sep-05		20-Oct-05		25-Nov-05	
	WC	TRAP	WC	TRAP	WC	TRAP	WC	TRAP	WC	TRAP	WC	TRAP	WC	TRAP	WC	TRAP
Alexandrium cf. minutum Halm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alexandrium cf. tamutum M.Montresor, A.Beran & U.John	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alexandrium cf. taylorii Balech	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Amphidinium</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dinophysis caudata</i> Saville-Kent	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dinophysis fortii</i> Pavillard	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dinophysis norvegica</i> Claparède & Lachmann	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dinophysis ovata</i> Claparède & Lachmann	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dinophysis sacculus</i> Stein	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dinophysis tripos</i> Gourret	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Diplopsalis lenticula</i> Bergh	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Goniodoma</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Gonyaulax fragilis</i> (Schütt) Kofoid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gonyaulax polygramma Stein	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gonyaulax spinifera Kofoid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gonyaulax spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gymnodinium spp.	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Gyrodinium</i> spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Histonella</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Heterocapsa rotundata</i> (Lohmann) G. Hansen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Kofoidinium velleioideis</i> Pavillard	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lingulodinium polyedrum (F.Stein) J.D.Dodge	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Noctiluca scintillans</i> (Macartney) Kofoid & Swezy	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Oxytoxum</i> spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Oxytoxum variabile</i> Schiller	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Phalacroma rotundatum</i> (Claparède & Lachmann) Kofoid & Michener	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Podolampas palmipes</i> Stein	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Prorocentrum cordatum</i> (Ostenfeld) J.D.Dodge	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Prorocentrum dactylus</i> (Stein) Dodge	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Prorocentrum dentatum</i> Stein	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Prorocentrum micans</i> Ehrenberg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Protoceratium reticulatum (Claparède & Lachmann) Bütschli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Protoperidinium bipes</i> (Paulsen) Balech	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Protoperidinium cf. crassipes (Kofoid) Balech	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Protoperidinium cf. brevipes (Paulsen) Balech	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Protoperidinium cf. steinii (Jørgensen) Balech	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Protoperidinium claudicans</i> (Paulsen) Balech	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Protoperidinium conicum</i> (Gran) Balech	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Protoperidinium depressum (Bailey) Balech	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Protoperidinium diabolus</i> (Cleve) Balech	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Protoperidinium granii</i> (Ostenfeld) Balech	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Protoperidinium ovum</i> (Schiller) Balech	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Protoperidinium divergens (Ehrenberg) Balech	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Protoperidinium steinii (Jørgensen) Balech	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Protoperidinium spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pyrocystis lunula</i> (Schütt) Schütt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Scrippsiella sp.	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tripos candelebrus</i> (Ehrenberg) F. Gomez	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tripos declinatus</i> (G. Karsten) F. Gomez	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tripos extensus</i> (Gourret) F. Gomez	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tripos furca</i> (Ehrenberg) F. Gomez	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tripos fusus</i> (Ehrenberg) F. Gomez	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tripos gibberus</i> (Gourret) F. Gomez	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tripos hexacanthus</i> (Gourret) F. Gomez	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tripos horridus</i> (Cleve) F. Gomez	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tripos macroceros</i> (Ehrenberg) F. Gomez	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tripos massiliensis</i> (Gourret) F. Gomez	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tripos pentagonus</i> (Gourret) F. Gomez	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tripos trichoceros</i> (Ehrenberg) F. Gomez	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tryblionella compressa</i> (J.W.Bailey) Poulin	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Lower values (from 600 to 336,200 cysts m⁻² day⁻¹) were recorded in the Strait of Georgia, where again cysts of heterotrophic species dominated the assemblage, with the exception of very high production rates of *Alexandrium* cysts recorded one year in the spring (Pospelova *et al.*, 2010).

Better comparisons could be performed with studies that examined the whole cyst assemblage collected in the sediment traps without palynological treatments. An annual study carried out in a bay along the southern coast of Korea (Shin *et al.*, 2012) detected fluxes comprised between 0.10×10⁵ and 35.97×10⁵ cysts m⁻² day⁻¹, with remarkable peaks during the summer months, when the assemblage was noticeably dominated by heterotrophic *Polykrikos* cysts. Cysts of *Scrippsiella trochoidea* were abundant in summer-autumn and *Gymnodinium catenatum* cysts were present during summer. The cyst yield of the sediment traps measured in the present study was on average 1.45±0.54 10⁴ cysts m⁻² d⁻¹ and was one order of magnitude lower than that reported in a study in the Gulf of Naples, Mediterranean Sea (Montresor *et al.*, 1998). This might be due to the fact that in the northern Adriatic Sea diatoms and phytoflagellates dominate throughout the year (Totti *et al.*, 2005; Bernardi Aubry *et al.*, 2006), while in the Gulf of Naples dinoflagellate abundances are generally higher (Montresor *et al.*, 1998; Ribera d'Alcalà *et al.*, 2004). Some commonalities may be detected between the timing of cyst fluxes in the Gulf of Naples and in the Gulf of Venice. In both areas, the cyst assemblage was often dominated by calcareous cysts (mostly *Scrippsiella trochoidea* and *S. crystallina*-like), which showed peaks in late August-October but also in the early spring, a period that was not sampled in the present study. Small-sized cysts that produced gymnodinioid cells upon germination peaked in late spring-summer (Montresor *et al.*, 1998); they included also spiny cysts similar to those identified as cf. *Biecheleria* in the present study. Gonyulacales cysts (*Spiniferites*, *Lingulodinium polyedrum*, *Alexandrium* and *Protoceratium reticulatum*) were detected in autumn in the Gulf of Venice, while these taxa characterized the summer period in the Gulf of Naples.

One of the concerns when we approached this study was the influence of resuspension processes on cyst flux measurements, considering that this process can be critical in shallow water environments characterized by strong hydrodynamics, such as the northern Adriatic basin (Giani *et al.*, 2001). The estimated resuspension varied from 13 up to 87%. However, considering (i) the low abundances of cysts in bottom sediments, and (ii) the fact that resuspension was higher when cyst flux was at minimum levels, it was estimated that the contribution of resuspended cysts in the trap samples never exceeded 1%. The relatively low impact of resuspension on cyst fluxes might be further supported by the fact that very few empty cysts were recorded in the sediment traps.

Dinoflagellate cysts fluxes and cells in the water column

In a few studies (Montresor *et al.*, 1998; Godhe *et al.*, 2001; Wang *et al.*, 2007), attempts were made to relate the timing and abundance of cyst fluxes with the corresponding concentration of motile cells in the water column. This is not straightforward, due to differences in sampling intervals, problems in the identification of motile taxa at the species level, advection processes that affect the water column, etc., but it can provide some valuable information. High fluxes of calcareous cysts, dominated by spiny cysts of the *Scrippsiella trochoidea* complex, are the hallmark of all studies carried out in coastal temperate areas and, in some cases, a relatively good match has been reported between these cysts and *S. trochoidea*-like cells in the water column (this study, Montresor *et al.*, 1998, Wang *et al.*, 2007). In the present study, high production rates of *S. trochoidea* cysts were recorded in August, and may have been stimulated by the relatively low nutrient concentration as described in Rengefors *et al.* (1996) and/or may have coincided with the end of the bloom period of this species. However, the high abundance of *S. trochoidea* recorded in the water column at the beginning of October was not followed by high cyst production rates.

A relatively good match was also observed between the presence of *Gonyaulax* spp. in water and net samples and the peak of *Spiniferites* cysts at the end of October, and between the records of *L. polyedrum* in the water column and the production of cysts in the subsequent weeks. *Spiniferites* belongs to the group with affinities to high temperature waters as described in Zonneveld *et al.* (2009) so our detection in autumn could be related to this feature. It is interesting to point out that cysts of *Lingulodinium polyedrum* were not detected in the traps deployed in the Gulf of Naples, while they were repeatedly recorded in the Gulf of Venice from October to December. This may be explained by the preference of this species for lower salinity and relatively high nutrient concentration (Lewis & Hallet, 1997; Zonneveld *et al.*, 2009, 2013; Balkis *et al.*, 2016). *Lingulodinium polyedrum* cysts are indeed used as a proxy for eutrophic conditions (Dale, 2009) and the quantification of its abundance in a sediment core collected close to our sampling area (southern the Po River delta) allowed the estimation of multidecadal trends of eutrophication in the area (Sangiorgi & Donders, 2004).

The match between vegetative cells of the genus *Protoperidinium* and its cysts and small gymnodinioids in the water column and cf. *Biecheleria* cysts in the sediment traps was not very good. This is most probably because the two taxa, *Protoperidinium* and small gymnodinioids, include many different species, some producing cysts and some others not, thus impairing a more precise comparison.

Cysts in sediments and detection of potentially harmful species

Cyst concentration in the surface sediments collected at the two stations off the Venice Lagoon were in the lower range of abundance, when compared with data gathered at other coastal stations in the Mediterranean Sea (Rubino *et al.*, 2000; Giannakourou *et al.*, 2005; Satta *et al.*, 2010, 2013). The highest cyst abundances have been generally recorded in secluded areas, such as harbors or bays, where the limited hydrodynamics favors the accumulation of sinking cysts within the sediments (e.g., Satta *et al.*, 2010, 2013). The station sampled in the present study was located in a relatively shallow area along the coast, where surface sediments might be mixed by heavy storms and/or trawling activity (Cavaleri & Stefanon, 1980; Pranovi *et al.*, 2000). The total estimated fluxes of dinoflagellate cysts was $3.46 \cdot 10^6$ cysts \cdot m⁻² over 6.5 months (May-December 2005). The total cyst concentration in the surface sediments was of $7.64 \pm 1.67 \cdot 10^6$ cysts \cdot m⁻² at the same site, considering that 2 g of dry weight of sediment roughly corresponded to 1 cm³ of wet sediment. Cyst concentration in the upper centimeters of sediments was thus in the same order of magnitude of the annual cyst fluxes. When comparing annual cyst fluxes with cyst concentration in the sediments, we have however to consider (i) that active bioturbation in oxygenated sediments can transport resting stages in deeper layers (Anderson *et al.*, 1982; Godhe & Mcquoid, 2003), (ii) that some cysts can have a fast turnover time and germinate (Nuzzo & Montresor, 1999), and (iii) that trawling activities might mix sediments and affect vertical cyst distribution in sediments (Giannakourou *et al.*, 2005).

The composition of cyst assemblages in the sediments was dominated by calcareous cysts of *Scrippsiella trochoidea*. This cyst morphotype is produced by a genetically diversified group of autotrophic peridinioid dinoflagellates that share gross morphology and plate pattern of *S. trochoidea*, but might include different cryptic species (Montresor *et al.*, 2003; Gottschling *et al.*, 2012). Cysts of *Scrippsiella trochoidea* are reported as dominant in almost all surveys of coastal areas over a broad latitudinal range (Giannakourou *et al.*, 2005; Wang *et al.*, 2007; Pitcher & Joyce, 2009; Shin *et al.*, 2012). This could be explained considering that *Scrippsiella trochoidea*-like dinoflagellates are rather abundant in the plankton and that they have relatively high encystment rates (e.g., Sgroso *et al.*, 2001, Wang *et al.*, 2007). The cyst assemblage recovered in the surface sediments more or less matched that recorded in the sediment traps, with the notable exception of cf. *Biecheleria* cysts, which showed notable fluxes in the sediment traps but were not detected in the sediments. This might be due to different reasons, such as rapid degradation of these small-sized cysts in the sediments, active and selective predation by detritivorous benthic organisms (Persson & Rosenberg, 2003), or their

lower sinking rates in sodium polytungstate gradient that was used to separate cysts from the mineral component.

Molecular PCR techniques with species-specific probes applied to identify potentially harmful dinoflagellates in the sediment samples permit the identification of *Alexandrium taylorii*, *A. tamutum*, *A. minutum*, *Lingulodinium polyedrum*, and *Protoceratium reticulatum* resting stages in the sediments. Cysts of *L. polyedrum* were regularly detected also by the examination of samples under light microscopy, while cysts of *A. tamutum* and *A. minutum* were detected but could not be identified to species level due to their identical morphologies. While *A. tamutum* is not known to produce toxins (Montresor *et al.*, 2004), *A. minutum* produces PSP-toxins and this species has been also recorded in the northern Adriatic Sea, highlighting the importance of correct identification of the two species (Pistocchi *et al.*, 2012). Cysts of *Protoceratium reticulatum* were not detected in the sediment samples by the microscopy method, but were sporadically recorded in the sediment traps. Their concentrations in the sediments might have been very low and beyond the detection limits of the microscope method. The cysts of *Alexandrium taylorii* were instead never recorded during this study by microscopy, in the sediments, nor in the traps. A possible explanation is that the cyst of this species is a featureless round-oval cyst (Figueroa *et al.*, 2006) that might have been misidentified as *A. minutum* or *A. tamutum*. The highly specific and sensitive PCR-based assay thus proved to be very effective in the detection of resting stages of potentially toxic species (Penna *et al.*, 2010). Blooms of the above-mentioned harmful species have not been recorded in the Gulf of Venice, where the most abundant harmful dinoflagellates belong to the genus *Dinophysis* (Bernardi Aubry *et al.* 2000). Nevertheless, toxic events attributed to *Alexandrium* spp. have been reported repeatedly in the northern Adriatic Sea, close to the Po River delta (Poletti *et al.*, 1998, Pistocchi *et al.*, 2012), and our study shows that resting stages are present also in the Gulf of Venice.

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