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Plankton resting stages in recent sediments of Haifa port, Israel (Eastern Mediterranean) - Distribution, viability and potential environmental consequences

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ABSTRACT

Resting stages of plankton were sampled in the surficial sediments in the port of Haifa, Israel, on the eve of a major port enlargement project. We recorded the structure of the assemblages and examined their relationship with different environments within the port. Our findings reveal a remarkably high diversity coupled with low density and the highest number of oligotrich ciliate cyst types recorded from marine sediments. Near the eutrophic and highly polluted zone of the Kishon estuary ciliates were more abundant than elsewhere in the port, whereas dinoflagellates' abundance was reduced, and these trends held true both for full and empty cysts. Some harmful or potentially toxic species, such as *Scrippsiella acuminata*, were widespread in the port. The toxigenic species include *Alexandrium minutum*, *Gymnodinium uncatenatum* and *Lingulodinium polyedrum*. Active cells of the unarmoured, bloom-forming *Akashiwo sanguinea* were identified in the cultures obtained from the incubated sediments.

1. Introduction

The patchy distribution of plankton along horizontal, vertical and temporal gradients (Klais et al., 2016) requires extensive multi-annual sampling efforts to attain adequate knowledge of the plankton assemblages in a particular region (Boero et al., 1996). Many marine phyto- and zoo-planktonic species have complex life cycles, comprising benthic dormant stages (cysts) (Blackburn and Parker, 2005) which may provide information concerning planktonic assemblages that is unobtainable by the intermittent conventional plankton surveys. Cysts may last in sediments much longer than in the water column (Ribeiro et al., 2011), where the occurrence of the planktonic stages is mostly seasonal. Accumulation of cysts, particularly in muddy bottoms in confined environments, characterized by low hydrodynamics and high productivity, constitutes a reservoir of potential biodiversity and ensures the resilience of the species (Boero et al., 1996; Marcus and Boero, 1998). The storage of encysted resting stages in the sediments provides us with multiseasonal, multiannual, occasionally multidecadal integration of the water column assemblages, leading to a better evaluation of plankton diversity. It is not uncommon to identify cysts of species rare in the pelagos and to double the number of species recorded from the plankton (Rubino et al., 2009a). Dinoflagellate cysts are thus useful biological indicators of past and present environmental conditions such as eutrophication (Matsuoka, 1999; Dale, 2009), pollution loads (Sætre et al., 1997; Pospelova et al., 2005; Liu et al., 2012), and blooms of toxic species (Matsuoka et al., 2003). Cyst assemblages may serve as a strategic tool for forecasting toxic blooms (Stock et al., 2007).

Haifa port, enclosing the Kishon estuary, Israel's most polluted river, is impacted by nutrient enrichment and industrial waste water, physically altered by construction and frequent dredging, and prone to establishment of ship-transported alien species (Galil, 2008; Galil and Bogi, 2009; Herut et al. 2011; Herut et al., 2012). We surveyed Haifa port on the eve of a major port enlargement project with the aims of examining the structure of plankton resting stage assemblages, in terms of species richness, diversity and relative abundance, in areas of the port subject to different disturbance regimes and observing the germination patterns of dinoflagellate resting stages in order to estimate their potential to generate blooms.

2. Methods

2.1. Study site

Haifa Port, opened in 1933, is Israel's largest port. It is located on the southern rim of Haifa Bay, the sole natural embayment along the Israeli Mediterranean coast (Fig. 1).

Two rivers, Naaman and Kishon, discharge into the bay, the estuary of the latter debouches into the Kishon port, to the east of the main port. Both the rivers are highly polluted, with the Kishon regarded as the single most polluted river in Israel (Cohen et al., 1993; Herut et al., 1993, 1994), a major source of contaminants to the port's sediments. Mercury

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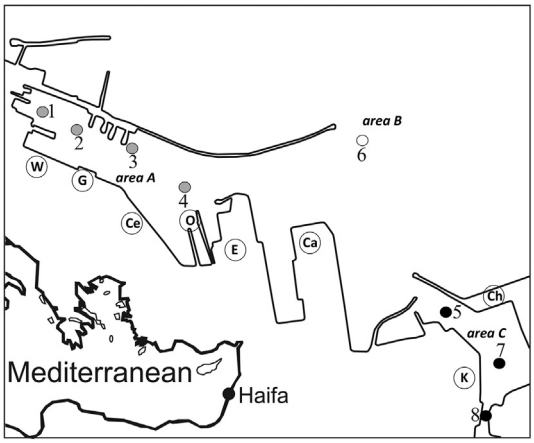

Fig. 1. Location of the sampling sites (stations) and the different zones identified in the port of Haifa, Israel (Eastern Mediterranean) Stations 1, 2, 3, 4 Area A; Station 6 Area B; Stations 5, 7, 8 Area C. The main terminals of the port are indicated W: Western terminal (containers, general and bulk cargo vessels) G: Grain terminal Ce: Central terminal (large passengers and cruise ships) O: Oil terminal E: Eastern terminal (containers) Ca: Carmel terminal (containers) Ch: Chemical terminal (moving and storage of chemicals) K: Kishon terminal (general cargo, bulk cargo vessels).

and other heavy metals (cadium, nickel, copper and chromium) pollute the sediments (Hornung et al., 1989; Herut et al., 2012) as well as tributyltin, PCBs (Polychlororinated biphenyls) and PAHs (Polycyclic aromatic hydrocarbons) (Cohen et al., 1993).

Unlike the extreme oligotrophy of open waters in the Levantine basin, a high load of dissolved nutrients and chl-*a* characterizes the waters of the port and the bay. The organic enrichment, coupled with the physical conditions (microtidal, high radiation, seasonal stratification), resulted in recurrent plankton outbreaks comprising potentially toxic species (Kimor et al., 1996; Kress et al., 1995; Herut and Kress, 1997).

The main port is protected by two breakwaters: the main breakwater, to the north-west, is 2826 m long, while the lee breakwater to the east is 765 m long. The entrance channel between the two breakwaters was 183 m wide and 15 m deep, it has since been deepened to 17.5 m (http://www.haifaport.co.il - last access 6th October 2016). In 2011 the port handled 21,839,000 tons of cargo. The smaller Kishon port, 12 m deep, its entrance channel 80 m wide, includes cargo handling facilities, a chemicals terminal and hinterland container depot. A fishing wharf and a 300 berth recreational marina are located at the mouth of the Kishon river.

The 8 sampled sites (Fig. 1; Table 1) represent different port environments, including frequently dredged areas. "Area A" comprised Stations 1–3, located in the main port, next to the Western terminal (containers, general and bulk cargo vessels), Grain terminal and Central terminal (passenger ships, Ro/Ro), Station 4 was near the Oil terminal. Station 6, located at the entrance of the port next to the main breakwater, represented "Area B". "Area C" comprised Stations 5, 7, 8, located adjacent to the Chemical terminal, the Kishon Quay, and in the channel leading to the fishing wharf and the mouth of the river, respectively.

Table 1
Geographic coordinates, depth and sediment typology of the 8 sampling sites in the port of Haifa.

Station	Area	Lat N	Long E	Depth	Sediment
1	A	32°49.698′	34°59.599′	13.6	Mud - anoxic
2	A	32°49.631′	34°59.760′	10.8	Mud - anoxic
3	A	32°49.555′	35°00.023′	16.4	Mud - oxidized
4	A	32°49.399′	35°00.261′	15.9	Mud - oxidized
5	C	32°48.909′	35°01.521′	12.1	Sand
6	B	32°49.587′	35°01.120′	16.2	Coarse sand
7	C	32°48.701′	35°01.773′	12.3	Mud - oxidized
8	C	32°48.481′	35.01.702′	4.8	Mud - oxidized

2.2. Sediment collection

Sampling took place on August 1st, 2011, aboard the R/V "Etziona", operated by Israel Oceanographic and Limnological Research (IOLR). Surface sediments were collected by means of a Van Veen grab (KahlSico, WA265/SS214, 32 × 35 cm, volume 20 L) with two lids that allowed the collection of undisturbed sediments together with a small amount of water topping the sediment. According to Reidhaar et al. (2016), grab samples are adequate for cyst sampling provided care is taken to avoid disturbance to sediment. Three replicate samples were taken at each site. Only the upper 2 cm of the sediment were collected, representing the layer mostly affected by deposition and resuspension/germination of cysts. In "Area A" (32°49.238 N, 35°1.193E) and "Area C" (32°48.504N, 35°1.709E) surficial sediments (upper 2 cm) for trace metals (i.e. Cd, Cr, Cu, Hg, Ni, Pb, Zn) and total organic carbon (TOC) analysis were sampled on July 14th, 2011, with a stainless steel Van Veen grab (0.08 m^2 area) and transported to the chemical laboratory at the IOLR within hours of sampling, where they were frozen (−20 °C).

Given the high sedimentation rate in the port (2 m yr^{-1} *fide* Cohen et al., 1993) the samples correspond to very recent deposition, including local sediment resuspension during dredging operations.

2.3. Sediment treatment and analysis

Upon collection, the sediment samples were stored in an icebox in the dark, and within three days were transported to the Laboratory of Plankton Ecology at the Institute for Coastal Marine Environment of C.N.R., Taranto, Italy, where they were stored in the dark at 4 °C. An aliquot of sediment (≈2 cm^3) from each sample was treated for the analysis of cysts and another one (≈10 cm^3) was oven-dried for one night at 70 °C to calculate the water content. The wet aliquotes were weighed and screened through a 20 μm mesh (Endecott's LTD steel sieves, ISO3310-1, London, England) using natural filtered (0.45 μm) seawater. The retained fraction was gently ultrasonicated for 1 min and screened again through a sieve battery (200, 75 and 20 μm mesh size), obtaining a fine-grained fraction (20–75 μm) containing protistan cysts and a >75 μm fraction with larger dinoflagellate resting stages (e.g. *Lingulodinium* and *Polykrikos*) and zooplankton resting eggs. Both fractions were suspended in filtered sea-water and stored at 4 °C in the dark until the observation. The material retained onto the 200 μm mesh was discarded. No chemicals were used to dissolve sediment particles in order to preserve calcareous cyst walls.

Qualitative and quantitative analyses were carried out under an inverted microscope (Zeiss Axiovert 200M) equipped with a Nikon Coolpix 990 digital camera, at ×200–320 magnification (×400 for more detailed observation). Both full and whole (i.e. presumably viable) and empty (i.e. germinated) cysts were counted. At least one fifth of the 20–75 μm fraction was analysed. This aliquot was representative of the whole fraction according to a preliminary data analysis based on the relationship between the standard error of the number of *taxa* and the aliquot volume (data not shown) (Bros and Cowell, 1987). The >75 μm fractions were examined in their entirety.

Resting stage morphotypes were identified on the base of published descriptions and the germinated material. Identification was performed to the species level when possible. As a rule, the modern biological names are used. For dinoflagellate cyst morphotypes which active stage is unknown, the paleontological names are used. Quantitative data are reported as cysts × g^{-1} of dry sediment (hereafter cysts g^{-1}).

The sediment samples for trace metals and TOC analysis were lyophilized for 48 h, and sieved through a 250 μm mesh size. TOC was determined by potentiometric titration after digestion with potassium dichromate. Total mercury was measured by mean of a PS Analytical Millennium Merlin mercury analyser with atomic fluorescence detection. Other metals were analysed using flame atomic absorption spectrophotometer Agilent 280 FS AA, and graphite furnace Varian 880 AA and Agilent 240Z AA.

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