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Fibrocapsa japonica (Raphidophyceae) occurrence and ecological features within the phytoplankton assemblage of a cyclonic eddy, offshore the Eastern Alboran Sea

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Abstract

The Raphidophycean *Fibrocapsa japonica* Toriumi & Takano was detected for the first time offshore in the Eastern Alboran Sea (Western Mediterranean) in October 2006. Its distribution appeared very localised and atypical, as it was abundant in the open waters of a cyclonic eddy. Microscope counts of the natural phytoplankton assemblages revealed that *F. japonica* was dominant in the microplankton, together with Dinophyceae, within and below the cyclonic nutrient-rich dome (25 - 30 m). Bacillariophyceae were the primary microplanktonic fraction in only a few samples on the edges of the eddy. Moderately high abundances of *F. japonica* (maximum of 60 cells ml^{-1}), with preliminary cysts and many cells aggregated in mucous nets, indicated a senescent phase. Despite the Atlantic origin of the cyclonic water masses, the molecular identification and the water temperature of 15 °C, which could have favoured pre-cyst formation, would suggest a Mediterranean source for *F. japonica* cells. Finally, we hypothesise that *F. japonica*, which is generally a coastal species, could have a coastal origin. In fact, *F. japonica* was mainly detected at a depth of 40-60 m at the coastal sites and at 25-30 m at the cyclonic dome. *F. japonica* generally being a coastal species, it can be hypothesised that it was entrapped by the cyclonic eddy, which typically detaches from the coastal waters, and thus carried offshore to the cyclonic waters.

Keywords: *Fibrocapsa japonica*; Harmful Algal Bloom (HAB); Western Mediterranean Sea; cyclonic eddy; 5.8S –ITS rDNA.

Introduction

Fibrocapsa japonica Toriumi & Takano (Raphidophyceae) is a potentially ichthyotoxic marine microalga first reported in Japan (Okaichi, 1972), where it was associated with mass fish mortality events (Toriumi & Takano, 1973; Okaichi, 1989). The exact mechanism of icthyotoxicity in Raphidophyceae is unknown, but it has been linked to several different processes: an abundant production of mucous that clogs fish gills (Fu, 2003), production of reactive oxygen species (ROS) that asphyxiate the fish (Oda *et al*., 1997) and the production of haemolytic compounds (Fu *et al*., 2004) and/or brevetoxins (Khan *et al*., 1996). The presence of brevetoxins in *F. japonica* was recently debated (Guidi-Rontani *et al*., 2010; Pezzolesi *et al*., 2010; Band-Schmidt *et al*., 2012), and thus the overall toxicity of this species is likely due to a combination of the above-mentioned factors acting together (Pezzolesi *et al*., 2010; de Boer *et al*., 2012).

Experiments conducted on *F. japonica* have shown that it has low nutrient uptake efficiency, and its growth should therefore be favoured in high-nutrient conditions, which are frequently encountered in the stratified shallow coastal and brackish waters (Riegman *et al*., 1996; de Boer *et al*., 2004; Handy *et al*., 2005; Cucchiari *et al*., 2008) where Raphidophyceae are typically detected.

F. japonica is known to bloom in temperate and tropical coastal waters worldwide, including the Pacific and Atlantic American coasts (Loeblich & Fine, 1977; Smayda & Villareal, 1989; Tomas, 1998; Del Carmen Cortés *et al*., 2003; Verity, 2010), Korean and Chinese waters (Lee *et al*., 2001), the Arabian Sea (Härnström *et al*., 2009), southern Australia and New Zealand (Rhodes *et al*., 1993). *F. japonica* has been reported in coastal European waters as well: in the French Atlantic (Billard, 1992), in the North Sea (Vrieling *et al*., 1995) and Baltic Sea waters (Vershinin & Orlova, 2008), as well as in the Mediterranean Sea, along both the Tyrrhenian Sea and the Adriatic Sea coasts (Cucchiari *et al*., 2008, and references therein). Kooistra *et al*. (2001) detected a high degree of polymorphisms among the nuclear ribosomal DNA ITS regions from different *F. japonica* strains, likely due to hybridisation mechanisms, and suggested that the exchange of discharged ship ballast waters and/or aquaculture may have induced the expansion of *F. japonica*'s disjointed distribution range.

Although some Raphidophyceae were identified in samples collected along the Malaga coast by Mercado *et al*. (2005) and in the brackish waters of the coastal Nador lagoon (Morocco) by Daoudi *et al*. (2009), *F. japonica* had previously never been detected in the open oceanic waters of the Western Mediterranean Sea.

The Western Mediterranean Sea, and the Alboran Sea and the Algerian Basin in particular, are strongly hydrodynamic basins. As shown in Figure 1 (top), Atlantic water enters the investigated area at Gibraltar and proceeds eastward as Modified Atlantic Water (MAW, S < 36.5 and T > 20 °C, Millot, 1999). It continues as a jet along the Spanish coast following an anticyclonic circulation, gradually mixing with the resident waters and producing subsequent mesoscale structures (Rodriguez *et al*., 1998; Morán & Estrada, 2001). It then turns southward as the Almeria-Oran jet, lying on top of the Levantine Intermediate Water (LIW, S > 37.5 and T < 15°C, Millot, 1999). Lacombe & Richez (1982) defined the Atlantic Mediterranean Interface (AMI) as the sub-surface mixing interface between MAW and LIW (S = 37.0-37.5 and T = 15-20 °C). Finally, MAW continues eastward along the Algerian coast as the unstable 'Algerian Current', which may produce further mesoscale structures (Millot, 1999; Send *et al*., 1999). These hydrodynamic features impart a high degree of environmental variability both in light and nutrient availability, and enhanced productivity is typically detected in these locations (Fiala *et al*., 1994; Morán *et al*., 2001; Leblanc *et al*., 2004). Moreover, these mesoscale structures, which are generally large (tens of kilometres) and long-lived (from weeks to months, Millot, 1999), catch and mix coastal and offshore waters and transport them for long distances in their eastward drifting (Send *et al*., 1999).

Cyclonic eddies are generally more productive than anticyclonic ones (Fiala *et al*., 1994; Rodriguez *et al*., 1998) and many authors have documented the abundance of Bacillariophyceae at upwelling waters in the Alboran Sea, in particular large and/or chain-forming genera like *Chaetoceros*, *Guinardia*, *Leptocylindrus*, *Nitzschia*, *Proboscia*, *Pseudonizschia*, *Rhizosolenia*, *Thalassionema* and *Thalassiosira* (Gould & Wiesenburg, 1990; Fiala *et al*., 1994; Videau *et al*., 1994; Rodriguez *et al*., 1998; Arin *et al*., 2002; Mercado *et al*., 2005) are reported. Conversely, anticyclonic eddies and low-nutrient MAW are generally dominated by nano- and picoplankton (Claustre *et al*., 1994; Fiala *et al*., 1994; Videau *et al*., 1994; Rodriguez *et al*., 1998; Jacquet *et al*., 2002; Massi *et al*., 2006; Nuccio *et al*., 2007).

We report the presence of *F. japonica* offshore in the Eastern Alboran Sea for the first time in the autumn of 2006. This species was collected offshore in the waters of a cyclonic eddy and not in coastal shallow waters, which are the usual conditions for Raphidophyceae. *F. japonica* atypical distribution is described here together with the main environmental conditions, i.e., water column structure and nutrient concentrations. Furthermore, the abundance and composition of the phytoplankton assemblage are analysed and compared with the available data on phytoplankton for that area. The purpose of this work is to inquire and assess the eco-physiological features of *F. japonica* which may explain its detection in high abundance in the unusual conditions of the upwelling cyclonic waters.

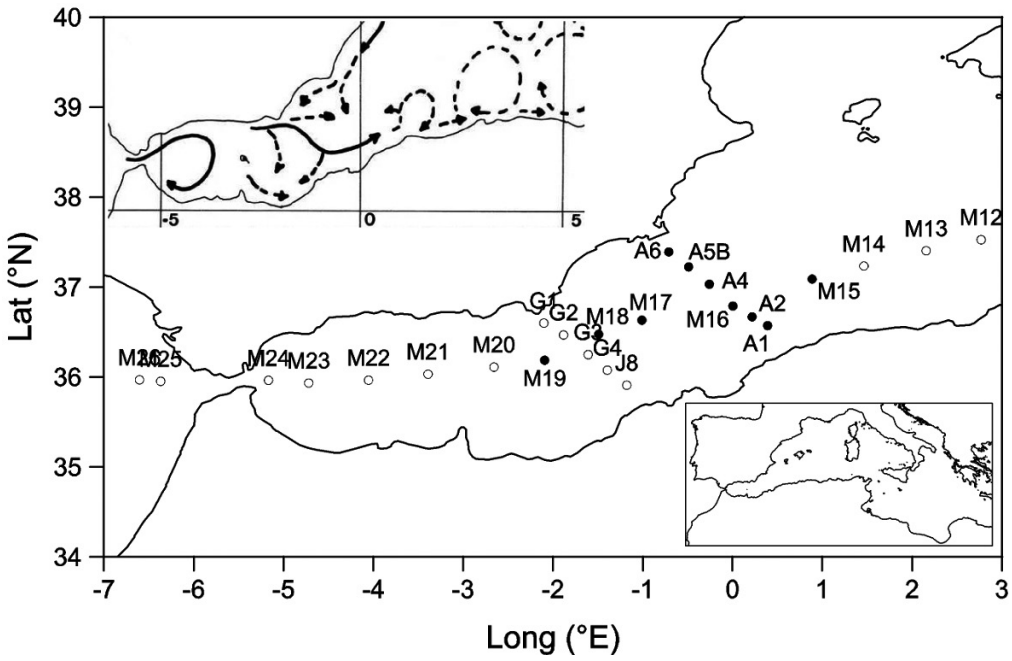

Fig. 1: Map showing the sampling locations in the Western Mediterranean Sea. Full symbols indicate the stations extensively analysed in the present study: M15-M19 and A1-A6. The window on top shows typical patterns of circulation of Modified Atlantic Water (MAW) in the Western Mediterranean Sea: continuous lines indicate steady paths and dashed lines outline the mesoscale currents throughout the year (modified from Millot, 1999).

Materials and Methods

Sampling strategy and in situ measurements

Samples were collected during the MEDBIO06-MEDGOOS13 oceanographic cruise (R/V Urania, CNR) in the Western Mediterranean Sea. The sampling took place along three crossing transects: a long NE-SW transect (the M-transect) and two short NW-SE transects (the A-transect and G-transect). This paper focuses extensively on data collected between -2° and 1° long. E and 36° and 37° lat. N (October 13th-16th 2006) along the M-transect (E-W: stations sts. M15, M16, M17, M18 and M19) and the A-transect (NW-SE: sts. A1, A2, M16, A4, A5B and A6, Fig. 1). At each station, temperature, conductivity and fluorescence emission along the water column were measured by a SBE-911 plus CTD system (Seabird) equipped with a fluorometer (SeaTech). Water samples for nutrient and biological analysis were collected in 12-L Niskin bottles that were mounted on a rosette sampler (General Oceanic, 24x). After examining the fluorometric contours, water samples were collected at discrete depths between the surface and 100-150 m.

Pigment and nutrient analysis

Water samples (4 l) were filtered onto GF/F filters (47 mm, Whatman) and stored at -20 °C until their analysis by high performance chromatography (HPLC). Total chlorophyll *a* (chlorophyll *a* + divinyl chlorophyll *a* if present) was detected by HPLC (Class VP HPLC system, Shimadzu) according to Vidussi *et al*. (1996) and Barlow *et al*. (1997).

Samples of 50-100 ml were collected in polyethylene flasks and immediately fixed with mercury chloride at a final concentration of 20 mg ml^{-1} $HgCl_2$ in the sample (Kirkwood, 1992). Inorganic nitrogen (i.e., nitrates and nitrites), phosphate and silicate concentrations were determined by an AutoAnalyzer3 (Bran-Luebbe) equipped with the AACE software (AutoAnalyzer Control and Evolution) according to standard methods (Saggiomo *et al*., 2010). Only the nitrogen (i.e., nitrates and nitrites) data are presented here.

Microscopic analysis

Samples of 250-500 ml were collected in dark polyethylene flasks and immediately fixed with neutralised formalin at a final concentration of 1%. Samples were analysed within a few months of sampling by counting cells after sedimentation of 50-100 ml on an inverted optical phase-contrast microscope (objective 40 x, ZEISS IM, Zingone *et al*., 2010). A random cell counting strategy was chosen and at least 700 optical fields, corresponding to minimum ¼ of the sedimented volume, were observed on the chamber bottom. The taxa examined fell into the following groups: Bacillariophyceae, Dinophyceae, Raphidophyceae, Prymnesiophyceae coccolithophores and a heterogeneous group denoted "other plankton" that generally included nanoplanktonic flagellates belonging to Cryptophyceae, Chrysophyceae, Haptophyta excluding coccolithophores, Prasinophyceae, < 10 mm flagellates and incertae sedis cells not identified at the species level. The primary sources used for taxonomic identification were those reported in Zingone *et al*. (2010).

Microscopic analyses were performed on the samples collected from the stations from M15 to M25 (Fig.1), with a total of 104 observed samples.

Statistical analysis

Temperature, salinity, density, nutrients, chlorophyll *a* and total phytoplanktonic and *F. japonica* abundance data were subjected to Principal Component Analysis (PCA, Hotelling, 1933). Data were standardised in cases by the ratio between each data and the square root of the sum of squares. Statistica 7.0 software (StatSoft) was used for this analysis.

Genomic DNA extraction and PCR amplification detection assay

Formalin-fixed samples were collected by centrifugation at 3500 rpm for 15 min at room temperature, and the supernatant was gently discarded; filtered seawater was added, and the samples were centrifuged again at 3500 rpm for 15 min at room temperature. This step was repeated twice. The pellets were then stored at -80 °C pending molecular analyses. Pellets were extracted for their total genomic DNA using the DNeasy Plant Kit (Qiagen Valencia, CA, USA) according to the manufacturer's instructions. Purified genomic DNA was quantified on agarose gel using the serially diluted DNA Marker Lambda (MBI Fermentas, Germany) and a gel-doc apparatus (Bio-Rad, Hercules, CA, USA).

Species-specific *F. japonica* primers were derived from Penna *et al*. (2007), and nested PCR amplification was carried out as described in Penna *et al*. (2010). Eukaryotic-specific primers targeting the 5.8S-ITS regions (Adachi *et al*., 1994) were used in the first PCR. The PCR-amplified products from the PCR assay using the universal eukaryotic primers and not directly visualised on agarose gel were used as the template in the nested PCR amplification with species-specific primers for *F. japonica* using 1 µl of the first PCR-amplified product. PCR amplifications were performed in an applied Biosystem DNA Thermo Cycler 2720 (Foster City, CA, USA).

Results

Hydrology and nutrient concentrations

A cyclonic eddy was detected between 2°W and 1°E and 36° and 37°N by means of the hydrological and hydrodynamic measurements conducted along the eastern Alboran Sea (Ribotti & Borghini, 2006). The eddy was 100 km wide and 400-500 m deep. Its edges were situated between the coastal sts. A6 (Spanish coast) and A1 (Algerian coast), and its dome was evident in the strong subsurface alo-thermocline (around 25 m deep) between sts. M16-A2-A4-M17 (Fig. 2). Particularly, in the area under examination, the

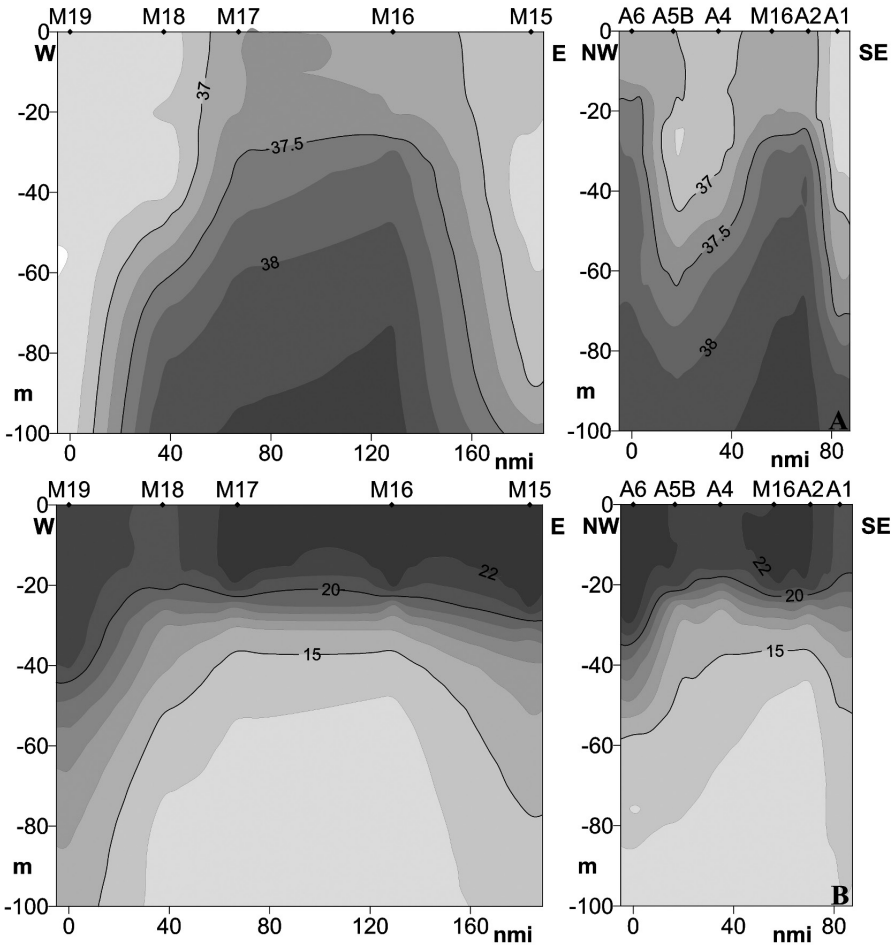

Fig. 2: Seawater A) salinity and B) temperature along the 2 transects. Bold lines denote the A) 37.0 and 37.5 isohaline and the B) 15 °C and 20 °C isotherms, respectively.

38.3 isohaline reached low depths at sts. M16 and A2 at 90 and 80 m, respectively (Fig. 2A), thus showing the presence of LIW (S > 37.5) at shallow depths at these two stations. Lighter MAW (S = 36.5-36.9) occupied the water column down to 100 m at st. M19 (Fig. 2A). The subsurface AMI was around 25-30 m deep at sts. M16, M17 and A2, respectively, while LIW upwelled and mixed with downwelling MAW at sts. M15 and M19, which were located on the edges of the eddy (Fig. 2A and B). With respect to temperature, the AMI layer was between 15 and 20 °C and a steep thermocline separated the colder LIW (< 15 °C) from the warmer surface MAW (> 20 °C, Fig. 2B).

In addition to these hydrological features, the analysis of the SeaWiFS map of surface chlorophyll *a* distribution in the Western Mediterranean Sea as obtained from the NASA Ocean Color website (http://reason.gsfc.nasa.gov/Giovanni) for 8-day averages between the 8th and 15th October 2006 (Fig. 3), clearly shows the presence of the cyclonic eddy between 2°W and 1°E.

Nutrient distribution seemed to be related to the hydrodynamics of the eddy too: high nitrogen concentrations were detected in the upwelling LIW between sts. M16-M17 (9 μM), at the AMI (sts. M16-M17-A2, Fig. 4), and also at surface in the dome of the eddy, where higher values than the adjacent nutrient-depleted surface waters were found (< 0.5 μM, Fig. 4). Phosphate and silicate followed a similar trend, with concentrations in the eddy always > 0.05 μM and > 2 μM respectively (Fani, 2008).

Phytoplankton biomass, densities and composition

The highest chlorophyll *a* concentrations were found at the top of the eddy (25-30 m) and deeper along its edges (40-45 m, Fig. 5A), with the maximum value of 4.7 mg m^{-3} at st. M17 25 m. Values > 1 mg m^{-3} were found in surface waters and at the AMI along the A-transect, while concentrations did not reach 0.2 mg m^{-3} in the adjacent surface waters (Fig. 5A).

Phytoplankton was primarily located within the eddy (Fig. 5A), and high cell abundances were detected in the surface and AMI waters at sts. M16-M17-A2 (up to 200 x 10^3 cells l^{-1} at st. M17 25 m, Fig. 5A) or along the edges of the eddy (sts. M15-M18 and sts. A1-A4-A5B, Fig. 5A). Samples at these stations contained high abundances of *Fibrocapsa japonica*, which co-dominated the assemblages with Dinophyceae.

F. japonica distribution was localised in the AMI and in the surface waters of the eddy (sts. M16, M17, A2),

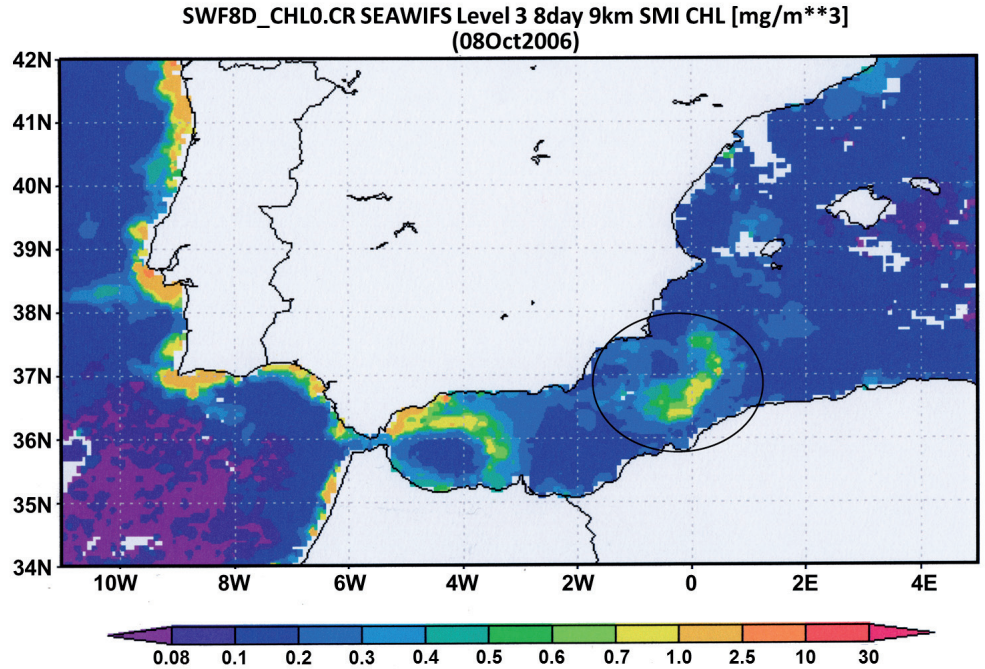

Fig. 3: SeaWiFS map of surface chlorophyll *a* distribution in the Western Mediterranean Sea at the time of sampling: the circle encloses the cyclonic eddy. Data are integrated on 8 days (8th-15th October 2006), web source: http://reason.gsfc.nasa.gov/Giovanni.

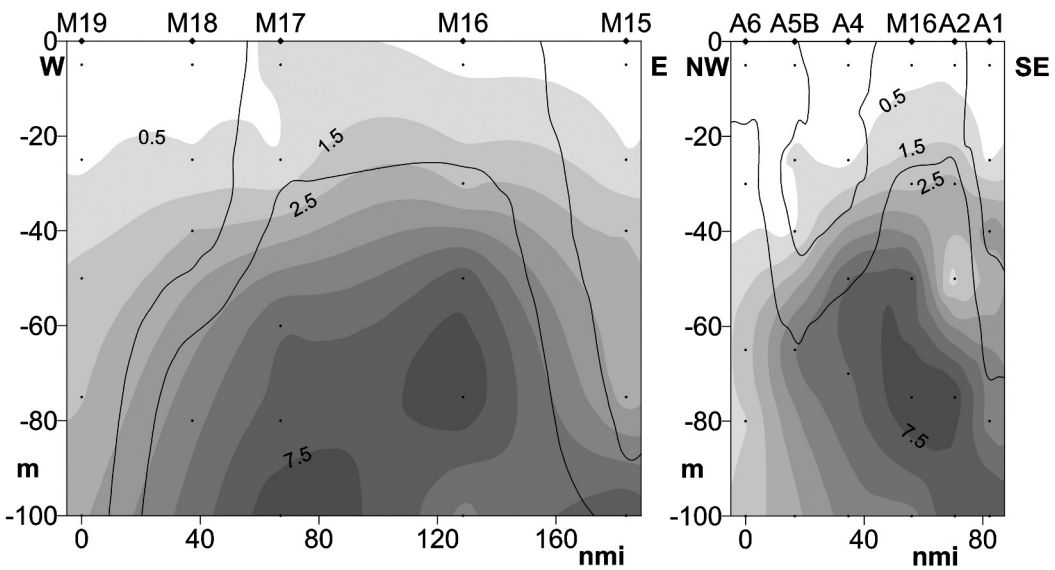

Fig. 4: Seawater inorganic nitrogen concentration (nitrates + nitrites, µM) overlapped to the 37.0 and 37.5-isohaline (bold lines).

and in deeper water of its coastal edges (sts. A1 and A6, Fig. 5B), with the highest abundance at st. M17 25 m, with 60 x 10^3 cells l^{-1}, representing 29% of the assemblage. Conversely, *F. japonica* was absent to the west, from sts. M18 to M25 and from G1 to J8 (Fani, 2008).

Cells appeared round to ovate, 15-30 x 12-20 µm. Due to the fixation, many *F. japonica* individuals appeared "raspberry"-shaped under the microscope with discharged mucous threads (Fig. 6A and B). Nevertheless, the majority of the cells still exhibited the typical rod-shaped posterior mucocysts (arrow in Fig. 6A and B), which made identification easier (Hara & Chihara, 1985), and they were often aggregated in a mucous net.

PCR amplifications were performed on the same samples to identify and confirm the presence of *F. japonica*. These samples contained mixed phytoplankton communities including the target species. Only after nested PCR amplifications performed on water samples using species-specific primers was *F. japonica* positively identified. PCR products of the expected size (180 bp) for the target species were detected in the samples containing *F. japonica* cells, confirming the microscopic identification.

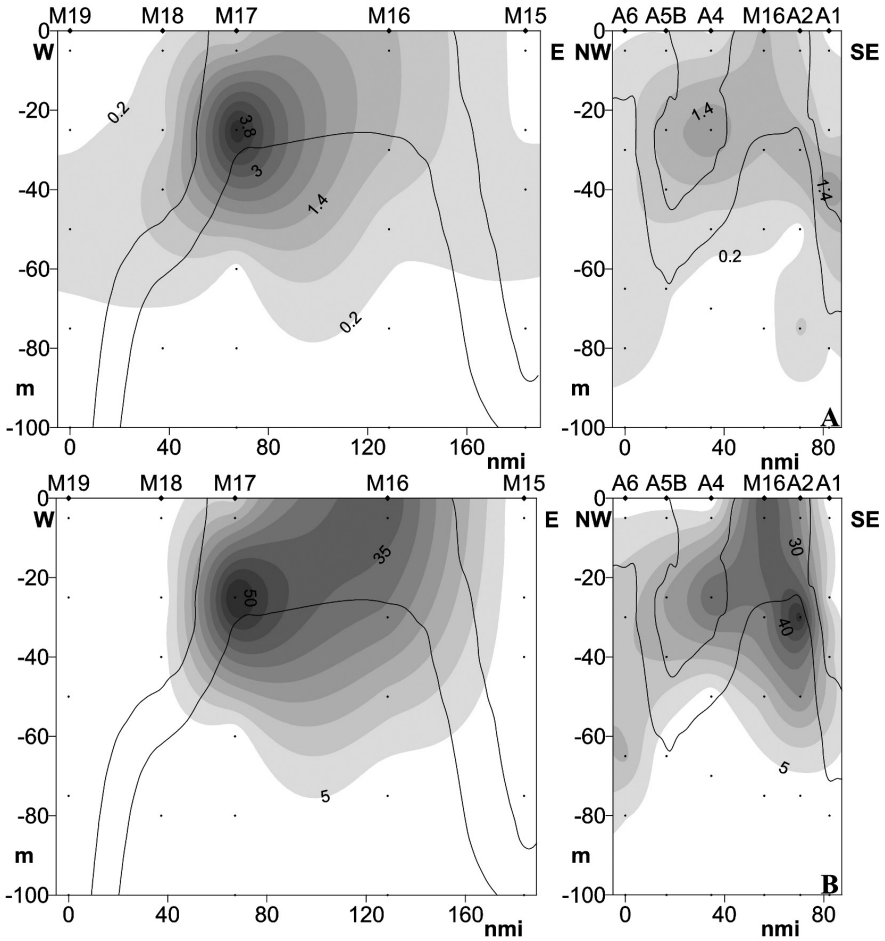

Fig. 5: A) Total chlorophyll *a* concentration (chlorophyll *a* + divinyl-chlorophyll *a*, mg m^{-3}) and B) *Fibrocapsa japonica* cell number map (x 10^3 cells l^{-1}) overlapped to the isohalines (bold lines) as in Fig. 4.

A few surface (0-25 m, st. M17) and deep (50 m, sts. A2-A4) samples were rich in spherical dark cells as well. These cells were smaller than vegetative *F. japonica* cells and contained dark round bodies, which were likely precysts of this species (de Boer, Groningen, personal communication, Fig. 6C).

Together with *F. Japonica,* Dinophyceae and the "other plankton" fraction were the main component of the phytoplankton assemblages in the eddy (Fig. 7).

High Dinophyceae abundances were recorded (Fig. 7A), up to 90 x 10^3 cells l^{-1} at sts. M17 25 m and A4 25 m. Dinophyceae were generally distributed between 0 and 50 m within the eddy, but they were primarily abundant at the AMI. Among thecates, the most abundant genus was *Heterocapsa* (*H. minima* Pomroy, *H. niei* (Loeblich) Morrill & Loeblich III, *H. rotundata* (Lohmann) G. Hansen, and *H. pygmaea* A. R. Loebl.), with 65 x 10^3 cells l^{-1} and 54 x 10^3 cells l^{-1} in the two above mentioned samples, respectively. Among the athecates, the most common species observed was the heterotrophic *Gyrodinium fusus* (Meunier) Akselman (= *Gyrodinium fusiforme* Kofoid et Swezy) at mean values of 2 x 10^3 cells l^{-1}. Moreover, a few harmful species of dinoflagellates were observed as well: *Gymnodinium catenatum* Graham, *Karenia* sp., *Lingulodinium polyedrum* (Stein) Dodge, *Prorocentrum* spp., *Gonyaulax hyalina* Ostenfeld and Schmidt and *Gonyaulax polygramma* Stein.

A relatively high contribution of nanoflagellates (other plankton) to the phytoplankton assemblage of the eddy was observed, mainly in surface waters at sts. A2, M16, M17 (up to 50 x 10^3 cells l^{-1}, Fig. 7B). The bulk of this group was represented by tetra- and biflagellates ≤ 10 µm, likely belonging to Prasinophyceae, Haptophyta excluding coccolithophores, and discoloured protists as *Leucocryptos marina* (Braarud) Butcher.

Bacillariophyceae were abundant only in a few subsurface samples, at the coastal sites (st. A1, 282 x10^3 cells l^{-1}) and at the edges of the eddy (st. M18, 88 x10^3 cells l^{-1}, Fig. 8A). These samples were dominated by a nanoplanktonic centric diatom (diameter: 3-12 µm), whose cells were frequently aggregated in colonies and interconnected by dark threads, identified as *Thalassiosira partheneia* Schrader. Microplanktonic diatoms belonging to the genera *Cylindrotheca*, *Detonula*, *Pseudo-nitzschia* were detected on the NW side of the eddy at the AMI (st. M16 30 m, st. A5B 40 and 65 m, st. A6 65 m), though their concentrations were always lower than 20 x 10^3 cells l^{-1} (Fig. 8A).

Similarly, high concentrations of Prymnesiophyceae

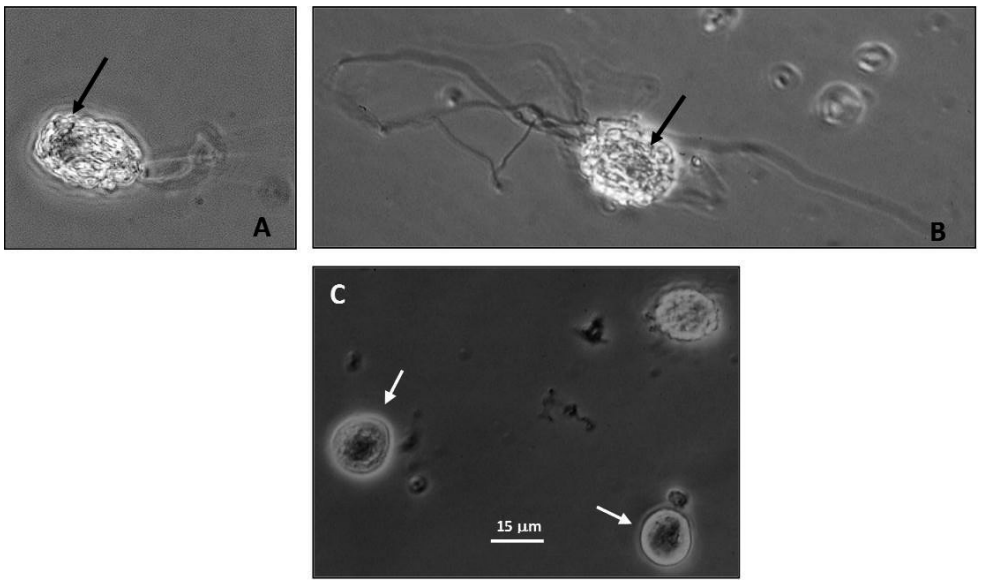

Fig. 6: Pictures of *F. japonica* with A) and B) discharged mucous threads and the rod-shaped posterior mucocysts (indicated by the arrows) clearly visible, and C) a vegetative raspberry-like cell (top right) and two pre-cysts (indicated by the arrows). Cell diameter was generally 15-30 µm.

(Fig. 8B) were found along the edges of the eddy and at the coastal stations at depths around 40-60 m. The highest values of 93 x 10^3 cells l^{-1} at st. M15 represented around 80% of the phytoplankton assemblage, followed by the other maxima at st. A5B (80 x 10^3 cells l^{-1}) and st. A1 (63 x 10^3 cells l^{-1}, Fig. 8B). *Emiliania huxleyi* (Lohmann)

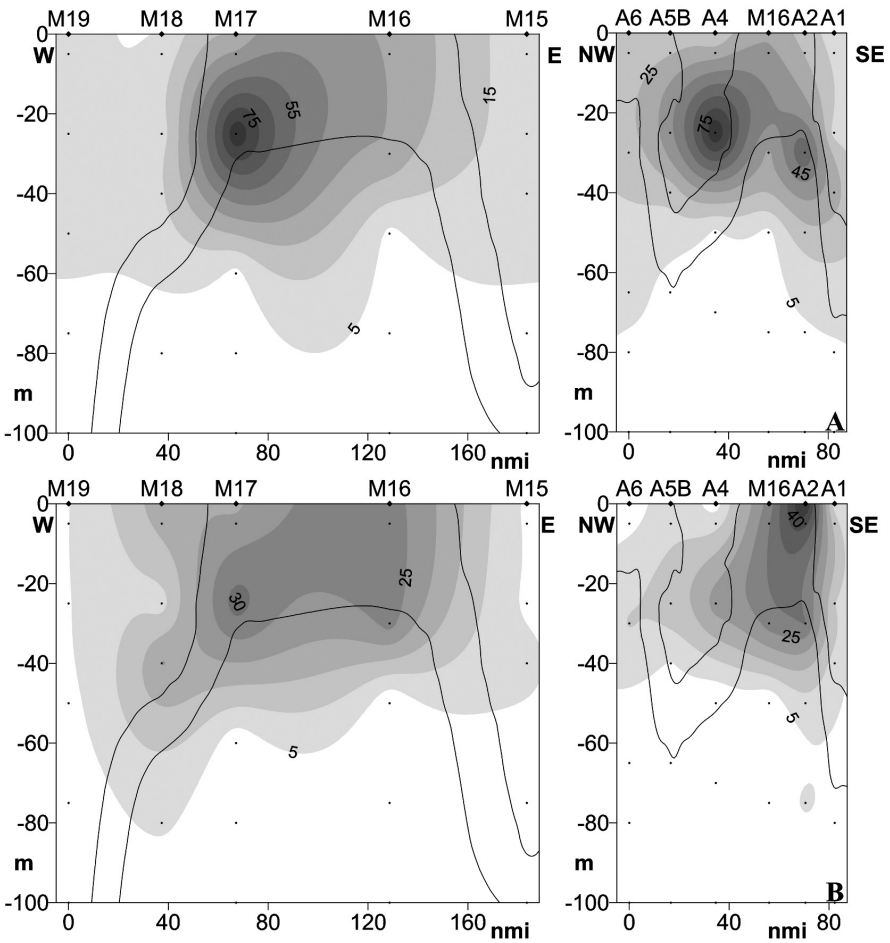

Fig. 7: A) Dinophyceae, and B) "other plankton" (Cryptophyceae, Chrysophyceae, Haptophyta excluding coccolithophores, Prasinophyceae, < 10 mm flagellates and incertae sedis cells) cell number map (x 10^3 cells l^{-1}) overlapped to isohalines (bold lines) as in Fig. 4.

Hay et Mohler (up to 55 x 10^3 cells l^{-1} at st. A5B 40 m) and *Gephyrocapsa oceanica* Kamptner (up to 40 x 10^3 cells l^{-1} at st. M15 40 m) were generally co-dominant species in a variable relative ratio. *Calciosolenia murrayi* Gran, and *Algirosphaera robusta* (Lohmann) Norris were recorded in large numbers too, with maxima of 7 x 10^3 cells l^{-1} (st. M19 50 m) and 17 x 10^3 cells l^{-1} (st. M17 25 m) respectively.

Principal Component Analysis (PCA)

The PCA correlation matrix indicates that the first principal component PC1 (eigenvalue = 4.47) is responsible for 46.65% of the total variance, while the second component PC2 (eigenvalue = 2.39) corresponds to 26.51% of the total variance. PC1 deals with the hydrodynamics of the water column, showing that temperature varies inversely with nutrients, density and salinity along the water column. As expected, density correlates well with salinity (r = 0.844), nitrogen (r = 0.782) and silicate (r = 0.780), while temperature has a negative or very low correlation when related to any other variable. Moreover, nitrogen correlates well with both silicate (r = 0.801) and phosphate (r = 0.541). PC2 is inversely linked to phytoplankton biomass and abundance, and thus concerns with biological activities. Finally, chlorophyll *a* concentrations correlate well with both cell number (r = 0.735) and *F. japonica* abundance (r = 0.799).

The projection of the variables onto the factor-plane indicates that salinity, density and nutrients are positively correlated to PC1, but temperature is negatively linked to it (Fig. 9A). Furthermore, salinity, density and nutrients are slightly negatively or not at all (nitrogen) correlated to PC2, while temperature correlates positively with this component. Finally, chlorophyll *a* and cell number correlate negatively with both PC1 and PC2.

Along the PC1-axis, deep samples (> 50 m) are on the right side, samples from the AMI are near the origin, and MAW samples are on the left side (Fig. 9B). Along the PC2-axis, low abundance samples from LIW are found on top of the axis (i.e., they are positively correlated with both PC1 and PC2), while rich samples are found downward along it, with the highest abundance, st. M17 (25 m), at its bottom.

Discussion

These results report the first detection of the potentially harmful Raphidophycean *F. japonica* in the offshore surface

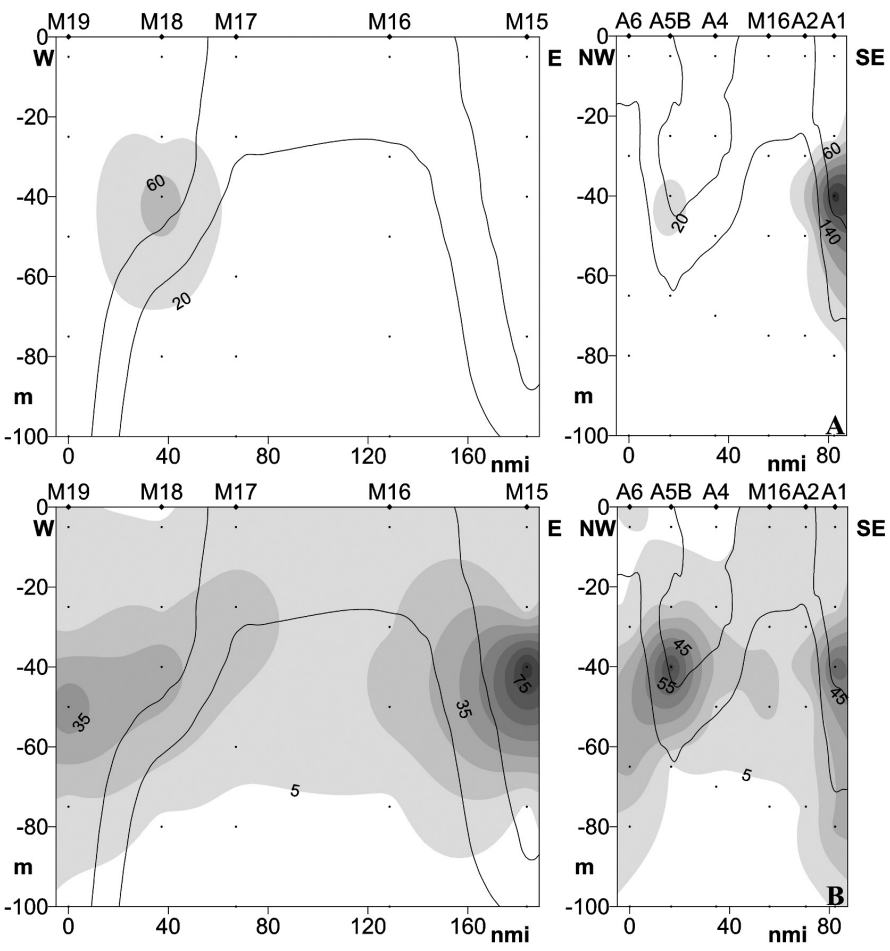

Fig. 8: A) Bacillariophyceae, and B) Prymnesiophyceae cell number map (x 10^3 cells l^{-1}) overlapped to isohalines (bold lines) as in Fig. 4.

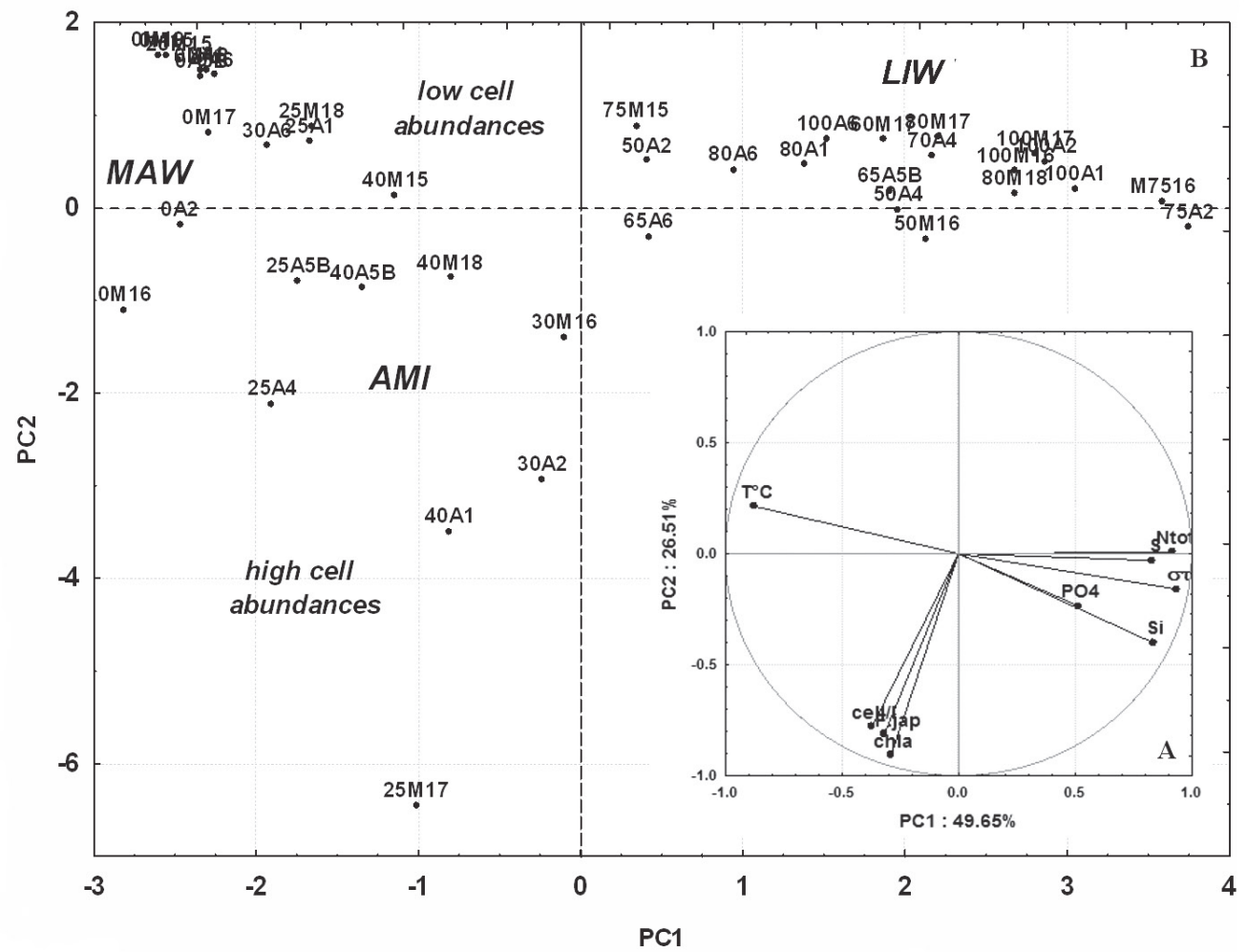

Fig. 9: A) A projection of the variables onto the factor plane, where the x-axis is PC1 and the y-axis is PC2, and B) a projection of the cases onto the factor plane, where the x-axis is PC1 and the y-axis is PC2. MAW is Modified Atlantic Water; AMI is the Atlantic Mediterranean Interface; LIW is Levantine Intermediate Water.

and subsurface waters of a cyclonic eddy in the eastern Alboran Sea. Although our survey did not follow the evolution of the bloom in the first half of October, we sampled in the upwelling area of nutrient-rich LIW that controlled the fertilisation of the euphotic zone both within and below the AMI, so that the highest chlorophyll *a* and phytoplankton abundances were found at the AMI, 25-30 m deep. This data confirms that the phytoplankton distribution was strongly coupled to nutrient input and agrees with the findings by Morán *et al*. (2001), who described a cyclonic eddy, in the same season (October) and approximately located in the same area, with similar nutrient concentrations and chlorophyll *a* maxima (> 1.4 mg m^{-3}) in its central and outer waters. So the hydrodynamic conditions were the major constraint on the distribution of phytoplankton biomass and assemblages, making the primary contribution to the samples variance, as evidenced by the PC analysis along the PC1 axis.

The surface and sub-surface biomass maxima within the eddy were dominated by *Fibrocapsa japonica* and Dinophyceae. *F. japonica* was detected by both microscopy and molecular PCR methods, disclosing its presence offshore in an oceanic eddy rather than in a coastal or brackish system, preferential habitat for Raphidophyceans. Microscopic analysis of the samples from this campaign did not reveal the presence of *F. japonica* eastward or westward of the eddy, confirming its confinement to the cyclonic waters only.

Cell abundances of *F. japonica* may reach as high as 10^7 cells l^{-1} in brackish ponds in a typical bloom event (Liu *et al*., 2008). The maximum cell abundance observed in this study (60 x 10^3 cells l^{-1}) was far less than these bloom concentrations. Nevertheless *F. japonica*'s abundances were of the same order of magnitude as the average microplanktonic densities which are detected at upwellings in the Western Mediterranean (Fiala *et al.*, 1994; Gómez *et al*., 2000; Mercado *et al*., 2005). On the other hand, *F. japonica* cells often appeared aggregated in mucous nets, and mucous production is often related to stress conditions (i.e., nitrogen limitation, Decho, 1990; de Boer *et al*., 2005). It is possible that the quickly variable conditions experienced by *F. japonica* could have limited its maximum abundance, so that in offshore waters this species didn't find the optimal conditions for blooming (due to the vertical mixing with cold waters, the unstable features of the water masses within the eddy,

and so on), and it is possible that it cannot ever reach high typical bloom concentrations offshore, i.e. this finding within the eddy could have been just an occasional one. Thus, it could be assumed that those assemblages had consumed nutrients (mainly nitrogen) upwelled in the eddy and were in the declining phase of a bloom during the sampling.

Furthermore, the abundance of the athecate *Gyrodinium fusus* might have been due to its grazing on *F. japonica*, thus contributing to the bloom decline. This has been documented by Nakamura *et al*. (1992) for the mixotrophy of *Gyrodinium dominans* grazing on *Chattonella antiqua*, and by Tillmann & Reckermann (2002) for *Oblea rotunda* and *Oxyrrhis marina* grazing on *F. japonica*.

F. japonica is considered as a eurithermal species. Different geographic strains show similar optimum growth temperature ranges, though these generally lie between 20 and 26 °C (Khan *et al*., 1996; de Boer *et al*., 2005; Cucchiari *et al*., 2008). It can survive at low temperatures by forming cysts (Yoshimatsu, 1987; de Boer *et al*., 2005; Cucchiari *et al*., 2010). Yoshimatsu (1987) reported that cyst germination was linked to low temperature, with high rates of germination after cyst storage at 12 °C, while Cucchiari *et al*. (2010) suggested that a temperature of 15 °C could be low enough to complete cyst formation for Adriatic *F. japonica* strains when kept in the dark.

A few observed samples were rich in *F. japonica* pre-cysts, similar to the preliminary cysts described by Cucchiari *et al*. (2010). The pre-cysts formation could confirm the end of the bloom in adverse conditions, induced by the low nutrient conditions in surface waters and by the LIW temperature of 14.5 °C in the deep samples, as proposed for the Adriatic *F. japonica* strains (Cucchiari *et al*., 2010). Cysts of *F. japonica* have already been detected in northwestern Adriatic sediment samples by molecular methods, confirming the presence of *F. japonica* resting stages in the area where this species blooms. In this study, molecular PCR was used to reveal the presence of this harmful species in a peculiar ecosystem. For future investigations, it will be useful to characterise the genotype of *F. japonica* isolates in this area to compare with other isolates from distinct Mediterranean areas.

In our study, the highest *F. japonica* abundances were recorded under conditions of temperature ranging from 15.8 °C (st. A2, 30 m) to 22.4 °C (st. M16, 0 m), and salinity values from 36.6 (st. M17 25 m) to 37.7 (st. A2, 30 m). Both the induction of preliminary cysts at T = 14.5 °C and the presence of *F. japonica* at field salinity values > 37 could suggest a Mediterranean origin. The salinity ranges enabling cell growth vary between strains, and the Adriatic strain has a higher optimum salinity range (S = 30-38, Cucchiari *et al*., 2008) compared to those in the Wadden and North Seas (S = 25-35, Khan *et al*., 1996; de Boer *et al*., 2004).

The distributions of the other phytoplanktonic classes were patchy and appeared to be confined to preferential depths. Nanoflagellates primarily occurred in MAW, confirming previous findings of higher abundances of the smallest fractions in nutrient-depleted surface waters (Claustre *et al*., 1994; Rodriguez *et al*., 1998; Morán *et al*., 2001). *Emiliania huxleyi* and *Gephyrocapsa oceanica* were abundant in the AMI along the edges of the eddy, which agrees with their typical distribution in fronts and isopycnal mixing layers in the Western Mediterranean Sea (Estrada *et al*., 1999; Mercado *et al*., 2005; Massi *et al*., 2006).

The contribution of microplanktonic Bacillariophyceae is large in cyclonic eddies, especially when compared to anticyclonic ones, where nano- and picoplankton generally dominate (Fiala *et al*., 1994; Rodriguez *et al*., 1998; Massi *et al*., 2006; Nuccio *et al*., 2007). In the sampling area, the colony-forming diatom *Thalassiosira partheneia* was actually found, although only in a few samples, and it was dominant particularly at 40 m in coastal sts. A1 and M18. Blooms of *Thalassiosira partheneia* Schrader have frequently been reported in adjacent deep waters (Gould & Wiesenburg, 1990) along the Almeria-Oran Front and in the deep chlorophyll maximum in a single station between the MAW jet and an anticyclonic eddy (Fiala *et al*., 1994). Nevertheless, as assessed and discussed so far, *F. japonica* and Dinophyceae were dominant elsewhere in the eddy.

The taxonomic composition of the phytoplankton assemblages from cell counts confirmed the diagnostic patterns of pigment distribution, particularly fucoxanthin (the diagnostic pigment for Raphidophyceae and Bacillariophyceae) concentration was 1.19 mg m^{-3} at st. M17 (25 m) and 0.51 mg m^{-3} at st. A1 (40 m), corresponding to the highest cell abundances of *F. japonica* and *T.* cf. *partheneia*, respectively (Fani, 2008).

The synthetic picture arising from the PCA along the PC2 axis, probably tied to biological activity, shows lower cell abundances in the MAW than in the AMI, where higher cell abundances could be related to a previous consumption of nutrients by phytoplankton. Thus, we could differentiate between the AMI fast-growing assemblages (with rapid nutrient uptake) and the nutrient-recycling ones in the MAW. When focusing on *F. japonica*, the PCA reiterated that *F. japonica* cells were in a diminishing phase, as already discussed by means of other features (low cell abundances, organisms often aggregated in mucous nets as a result of stress conditions, presence of pre-cysts), and that *F. japonica* growth did occur in cyclonic waters, in contrast to the usual knowledge of the preferential conditions for its growth.

About the local source of *F. japonica*, we could assume a coastal origin, considering the high hydrodynamic character of mesoscale structures in the Alboran Sea, typically catching and detaching waters from the coasts. *F. japonica* cells might have been caught from a coastal assemblage along the Spanish coast, entrapped within the Almeria-Oran jet and finally carried offshore within the cyclonic waters. Nevertheless, *F. japonica* was collected in deeper (40-60 m) samples at the coastal sts. A6 and A1, leading us to suppose that cysts from Spanish coasts and/or Algerian sediments could have been resuspended in the cyclonic waters, where they found conditions (i.e., nutrients and light) which al-

lowed the germination and growth of the vegetative stages.

To conclude, this investigation on the natural phytoplankton assemblages of the Eastern Alboran Sea highlighted *F. japonica* tolerance for a wide range of temperature and salinity conditions, likely triggering its excystment and encystment depending on the sharp time and space variations of the environment. Among the microplanktonic algae, this Raphidophycean species revealed itself as a good competitor, taking advantage of the high nutrients present in cyclonic waters.

For this reason, further investigation should evaluate harmful flagellates' behaviour in highly variable conditions by means of experimental simulation and modelling. Since eddies may transport water and their phytoplankton assemblages at great distances, they could act as a 'pelagic seed bank' from which seed stocks are dispersed, as suggested by Smayda (2002) for dinoflagellate vegetative cells at frontal zones, and behave as a floating ecosystem (the 'oases in the Mediterranean desert' by Kerr, 1986 and Claustre *et al*., 1994).

Hence, mesoscale structures should be monitored not only for their key role in primary production enhancement, but also because they could work as a major microalgal dispersal mechanism, expanding the distribution range of harmful species if favourable conditions are encountered. Thus, the role of the Alboran Sea as a corridor for potentially toxic species from the Atlantic eastward (Gómez, 2003) should be confirmed.

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