Monitoring of a Potential Harmful Algal Species in the Berre Lagoon by Automated *In Situ* Flow Cytometry

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Abstract

The vicinity of urban activity and industry (petrochemistry) around the Berre lagoon (southeast of France) has induced the degradation of its ecosystem, characterized by a permanent eutrophic state. In particular, a power plant has discharged substantial inputs of enriched freshwater in the lagoon since 1966. Due to these high nutrient inputs and also to regeneration rates, several species of phytoplankton regularly bloom in the lagoon at spring, summer, or autumn. Peaks of phytoplanktonic biomass (>150 µg Chl*a*/dm^3) are generally followed by intense heterotrophic activities leading to O_2 consumption with hypoxic or anoxic episodes. The study of phytoplankton dynamics is thus of primary importance.

Within the framework of an ecological survey, an automated platform of *in situ* instruments was set up in a laboratory located on the "Berre l'Etang" harbor to assess biological and hydrological features of the lagoon at short time scale during October 2011. The phytoplanktonic community was characterized by a Cytosense autonomous flow cytometer (Cytobuoy, Netherlands) operating at high frequency (hourly sampling) in order to detect sudden changes of species compositions and abundances. In parallel, hydrological sensors have measured several physicochemical variables of the water directly pumped from the lagoon (1.5 m depth) to the field laboratory.

In October 2011, among the various phytoplanktonic species optically resolved by the Cytosense, the dinoflagellate *Akashiwo sanguinea* has

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H.-J. Ceccaldi et al. (eds.), *Marine Productivity: Perturbations and Resilience of Socio-ecosystems*,
DOI 10.1007/978-3-319-13878-7_13,

been detected. It is known for causing fish and seabird death when its abundance is high and for having a potential toxicity on human consumed shellfish. On October 6, nutrient concentration combined with a weak hydrodynamic state triggered a large development of this species. Concentration reached up to 450 cells/cm^3, leading to a fast increase in chlorophyll concentration (>20 µg Chl*a*/dm^3). However, these dinoflagellates were quickly affected after a sudden wind (mistral, 330°–360°) event with mean speed exceeding 20 m/s: on October 7, 2011, *A. sanguinea* abundance dropped down below 20 cells/cm^3.

These results, out of reach of more conventional methods with manual sampling, underline the power of *in situ* automated monitoring to follow in near real time the dynamics of phytoplankton in general and also to target some particular species of interest such as harmful algae.

1 Introduction

Phytoplanktonic species play a key role for marine productivity. These primary producers fuel the entire trophic network and modulate biogeochemical cycles (N, C, P, Si, S, Fe). When environmental conditions favor their bloom, some species can be responsible for massive death of organisms, commercial species (Smayda 1997). These events, known as harmful algal blooms (HABs), represent a major risk for the marine fauna, from filter feeders to fishes, but also marine mammals and seabirds, as they can be subject to toxic or nontoxic (anoxia, gill clogging, physiological changes) effects leading to their death (Zigone and Enevoldsen 2000). HABs are caused by numerous species of dinoflagellates, cyanobacteria, diatoms, prymnesiophytes, and raphidophytes which are harmful in many ways. Thus, they require an accurate monitoring to detect them as early as possible.

Phytoplankton analyses are based on their optical properties via microscopy or fluorimetry, both considered as traditional methods used to detect algal proliferations (Anderson et al. 2001). In France, the national program REPHY (REseau de surveillance du PHYtoplancton et des PHY-cotoxines) conducted by IFREMER (Institut Français de Recherche pour l'Exploitation de la MER) is in charge of phytoplankton surveys along the French shore. It aims at enhancing our understanding about factors controlling phytoplanktonic blooms. By means of early warnings, this network also intends for preventing poisoned shellfish consumption by triggering some particular toxic species. Observations are based on microscopic counts which are time consuming and require expertise. The sampling frequency is, typically, from one per month to up to one per week during risky periods (in May/June for most of the sites).

Located in the south of France, the Berre lagoon is one of the largest marine lagoons in Europe and around the Mediterranean Sea. Because it has received a large amount of nutrients over the years, the lagoon used to regularly host algal blooms characterized by the dominance of few species, with extreme biomass (Malkassian 2012). Since 1995, this site benefits from a special attention relying on European (Directive Cadre sur l'Eau (DCE)), regional (Réseau de Suivi Lagunaire (RSL)), and local (Groupement d'Interêt Public pour la Réhabilitation de l'Etang de Berre (GIPREB)) frameworks. In general, lagoons are both highly dynamic and sensitive to human activities because of their intermediate state between land and sea. Besides a long-term monthly monitoring of phytoplankton in the Berre lagoon, it appears that the establishment of the annual budget of primary production is still incomplete. This is likely due to important seasonal changes (with, for instance, sporadic events such as natural flooding or strong wind events) (Gouze et al. 2008).

Even nowadays, little is known about the phytoplankton dynamics at fine scale and about the variables controlling it. In shallow coastal ecosystems, phytoplankton abundances are influenced, at different time scales, by grazing, local inputs (nutrients), and turbulent mixing, as described by Cloern (1996). However, conventional sampling methods are not suitable to assess their changes induced by sudden sporadic events such as wind episodes, freshwater discharges, or spates. There is thus a need for devices able to run automated and high-frequency analysis independently of the weather conditions and of the presence of a person. Such instruments would be very valuable to detect blooms at an early stage and provide a warning about a potential harmful algal bloom. This obviously requires analyses performed at a taxonomic level.

In this context, we report in this study a high-frequency *in situ* monitoring of phytoplankton in the Berre lagoon by means of a Cytosense automated flow cytometer (Cytobuoy b.v., Netherlands). This project has been supported by CNRS EC2CO, FEDER, and the Council of Provence Alpes Côte d'Azur Region. Abundances were measured every hour during October 2011 for species ranging from pico- to small microphytoplankton, some of which identified as harmful such as *Akashiwo sanguinea*.

2 The Berre Lagoon

The Berre lagoon receives freshwater inputs from both three natural rivers and from the artificial channel of a hydroelectric power station (Electricité de France (EDF)) built on the north-eastern shore. The freshwater from the Durance River is thus derived and discharged in the lagoon, bringing with it massive amounts of sediments and organic matter. The lagoon also receives Mediterranean seawater through the Caronte channel. It is subjected to multiple natural (frequent wind events, freshwater discharges, and spates) and anthropic forcing (industries, inhabitations). Due to the proximity of several small cities and from the Marseille metropolis (about 800,000 inhabitants), a pressuring instatement of urban, industrial, and agricultural exploitations has emerged all around the lagoon shore. Since 1966, the main disturbing infrastructure has been the power plant, using and discharging freshwater of the Durance River for electricity supply (by turbines) into the Berre lagoon, with a drastic impact on salinity (by important dilution). All these human activities have generated wastes and inputs that were drained in the lagoon and enriched it, particularly in nitrogen and phosphorus. In 2011, the power plant was responsible for 50 % and 15 % of total nitrogen and phosphorus inputs, respectively. These high inputs combined with the fast regeneration rates of the microbial community (Gouze et al. 2008) have fueled the phytoplanktonic species, and as a result, the water mass has become ultimately eutrophic. For many years, blooms associated with extreme autotrophic biomass (>150 μg Chl*a*/dm^3) and colored waters have been observed until a regulation of the power plant discharges was imposed to EDF in 1994 and 2005 (Malkassian 2012). The salinity gradient between surface and bottom of the lagoon maintains a quasi-permanent stratification of the water column (Nérini et al. 2001) and prevents reoxygenation of the deep layer after the oxygen depletion that follows blooms. Hypoxia or in extreme case anoxia, light attenuation, and toxin production during these blooms have perturbed the entire ecosystem, which remains eutrophic (Fig. 1) and in a "bad" ecological state according to RSL (Réseau de Suivi Lagunaire) evaluation criteria (Mayot et al. 2013).

3 The Experimental Setup

In order to detect sudden changes in composition and abundances of the phytoplanktonic community in the Berre lagoon, the Cytosense (Cytobuoy, Netherlands) flow cytometer was placed in a laboratory nearby the "Berre l'Etang" harbor (Fig. 2).

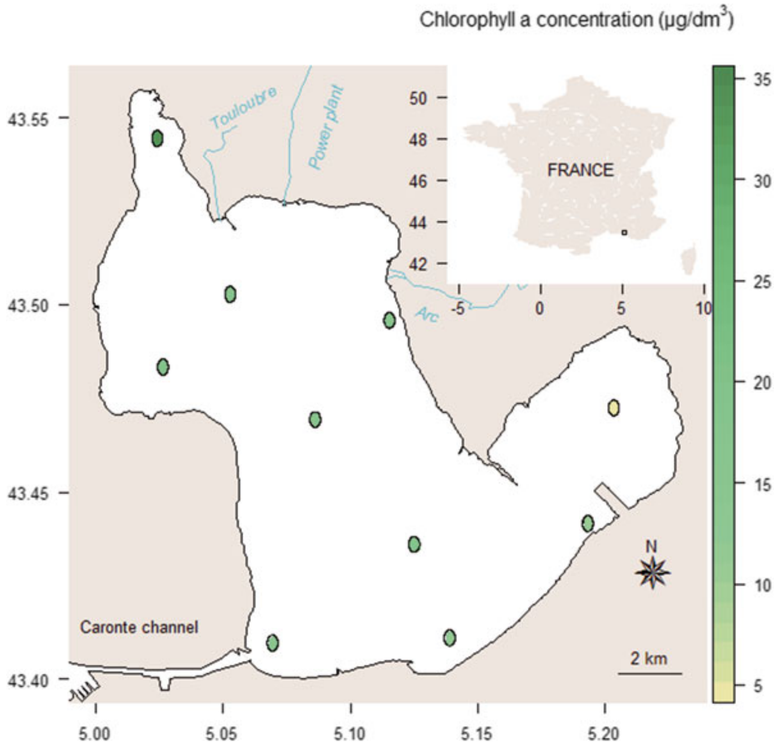


Fig. 1 Surface chlorophyll *a* concentration measured at the 10 hydrological stations in the Berre lagoon for the sampling performed in September 2011

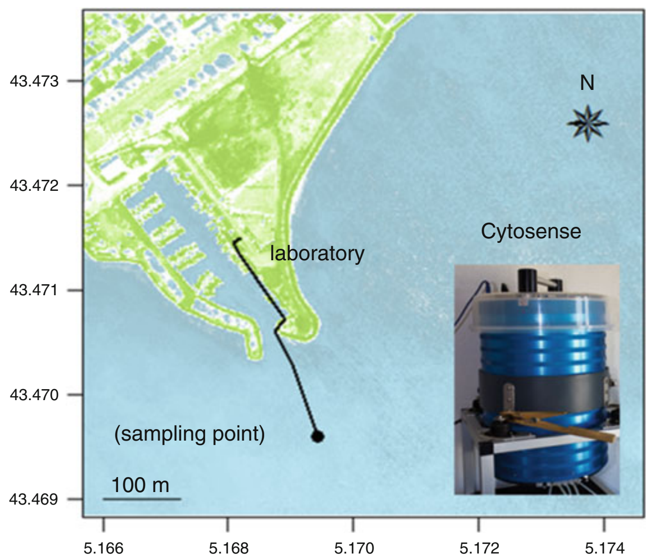


Fig. 2 Location of the sampling point (*black circle*) and the 250 m pipe (*black line*) until the laboratory, on the “Berre l’Etang” harbor. Flow cytometry analyses were automatically run every hour by the Cytosense flow cytometer. When needed, the cytometer could be remotely operated thanks to an Internet connection

This instrument is designed to operate autonomous *in situ* monitoring of a wide number of phytoplanktonic species in the range of size between 1 and 800 µm (Dubelaar et al. 1999). Analyses are made at the single cell level and at high frequency (up to once every 10 min) (Dubelaar and Gerritzen 2000; Peeters et al. 1989). This instrument has been used to reveal the temporal and spatial phytoplankton variability *in situ* (Thyssen et al. 2008). Autotrophic cells are triggered on the basis of their chlorophyll *a* content, which naturally emits red fluorescence. When the cells pass through the light source excitation (a 488 nm laser beam), five optical profiles (two diffusion intensities (forward angle and sideward light scatter) and three fluorescence intensities (red, orange, and yellow fluorescences)) are recorded for each single particle (cell). Cells sharing similar optical properties are therefore grouped together when plotted in 2D projections of the numerous variables collected by the flow cytometer (2 light scatter and three fluorescences, with for each one the area under the curve, the peak and length of the curve, etc.). In addition, an "image-in-flow" device mounted in the Cytosense takes pictures of cells of interest after their passage through the 488 nm laser beam.

In parallel to flow cytometry analyses, hydrological variables were also measured by automated sensors (ISUS for nitrate concentration and Hydrolab probe for temperature, salinity, turbidity, chlorophyll *a* content, pH) in the water pumped from the sampling point (43°28′10.57 N, 5°10′9.91 E, 2.5 m depth) to the field laboratory. Water was carried out to the laboratory through a 250 m hose (50 mm inner diameter) at a flow rate of 30 dm^3/min during 17 min before each analysis (in order to completely flush the entire hose). Each hour, temperature, salinity, turbidity, pH, nitrate, and chlorophyll *a* concentrations were measured by the various sensors installed in an 80 dm^3 tank receiving the pumped water. A dedicated volume of 1 dm^3, set up between the pipe and the 80 dm^3 tank, was used for the flow cytometry analyses performed by the Cytosense. An *in situ* HOBO® Pendant® Temperature/Light Data Logger has been installed next to the pipe inlet in order to measure the incident light intensity at the very sampling depth. A strainer brass was set *in situ* at the end of the hose to prevent biofouling and the passage of larger particles (several millimeters long) which may clog the hose and/or the instruments.

4 Results

4.1 Phytoplankton Clusters Defined by Flow Cytometry

During the October 2011 monitoring, up to 12 separate clusters of photoautotrophic cells (arbitrary labeled C1 to C12) have been resolved thanks to their optical properties recorded by the scanning flow cytometer (Fig. 3). Clusters showed a proportional relation between light scatter (related to cell size) and red fluorescence (related to chlorophyll a pigment content) intensities.

The length of the cells has been determined both from the pictures taken by the image-in-flow and from calibration microspheres (Polysciences) of various sizes (from 1 to 20 μm in diameter). It covered a large range of sizes, from 0.9 ± 0.1 μm (for C12) to 56.4 ± 12.2 μm (for C1) (Table 1). The largest cells pictured by the image-in-flow camera correspond to clusters C1 to C4 and belong to the microphytoplankton class. As revealed by the pictures, they were monospecific and composed of *Akashiwo sanguinea* (Hirasaka) for C1, *Prorocentrum micans* (Ehrenberg) for C2, *Scrippsiella* sp. (Balech) for C3, and *Gymnodinium* sp. (Stein) for C4.

4.2 Dynamics of *Akashiwo sanguinea* and of the Environmental Variables During the Sampling Period

The chlorophyll *a* concentration of the overall phytoplanktonic community strongly varied in the Berre lagoon during the sampling period, with *maxima* correlated to high nitrate concentrations ($n = 215$, correlation coefficient 0.53, $p < 0.001$). Proliferation of microphytoplanktonic cells, including *A. sanguinea*, has led to several peaks of chlorophyll *a* concentration which reached up to 21.81 μg/dm^3 on October 5 at 05:30 pm. Such high biomass have not been detected by the monthly monitoring carried out in the Berre lagoon; actually, in October the lowest concentration has been measured (Fig. 4).

During the sampling period, one major forcing that occurred in the lagoon was a turbulent mixing induced by a strong mistral event (wind from 330° to 360° from October 6 to 10) (Fig. 5).

The mean temperature of the water dropped down by 5.2 °C (from 23.2 ± 0.2 °C to 18.0 ± 0.3 °C between October 6 and 7). Nitrates and micro- and nanophytoplankton concentrations decreased and *Akashiwo sanguinea* abundance dropped

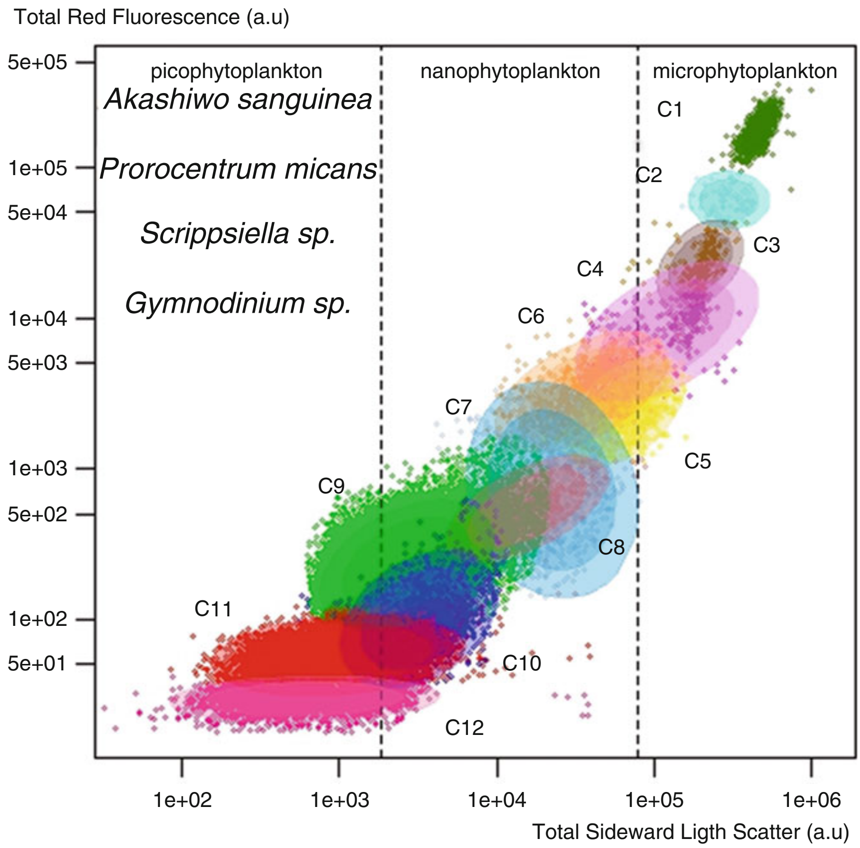
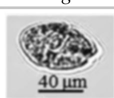
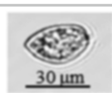
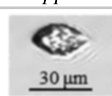
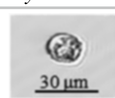


Fig. 3 Cluster description on a red fluorescence (total red fluorescence, arbitrary unit)/light scatter area under the curve (total sideward light scatter, arbitrary unit) cytogram with 0.8 and 0.9 confidence ellipses

Table 1 Mean lengths (µm) of cells composing the various cytometric clusters pictured by the image-in-flow camera

Cluster	C1	C2	C3	C4	C5	C6
	A. sanguinea	*P. micans*	*Scrippsiella* sp.	*Gymnodinium* sp.		
Mean length ± standard deviation						
	56.4 ± 12.2	37.7 ± 10.5	23.3 ± 8.8	20.1 ± 13.5	13.1 ± 4.2	13.2 ± 4.3
	C7	C8	C9	C10	C11	C12
	13.9 ± 5.6	8.6 ± 1.3	3.5 ± 0.5	4.1 ± 1.0	1.1 ± 0.1	0.9 ± 0.1

from 445.5 cells/cm^3 on October 6, 11:00 pm, to 12.2 cells/cm^3 on October 7, 12:00 pm (Fig. 6).

Water turbidity which rose to 15.9 NTU dropped down to 2.02 ± 0.77 NTU as the biggest autotrophic cells decreased in abundance. The maximum light intensity measured *in situ* increased from 9,300 lux to 14,467 lux, while there was no significant difference of the solar irradiation.

5 Discussion

Since the instatement of the EDF power plant on the northern shore of its basin, the Berre lagoon has been subject to recurrent algal blooms in spring, summer, and autumn. In October, these algal proliferations are caused by the persistence of summer-like temperatures combined with the

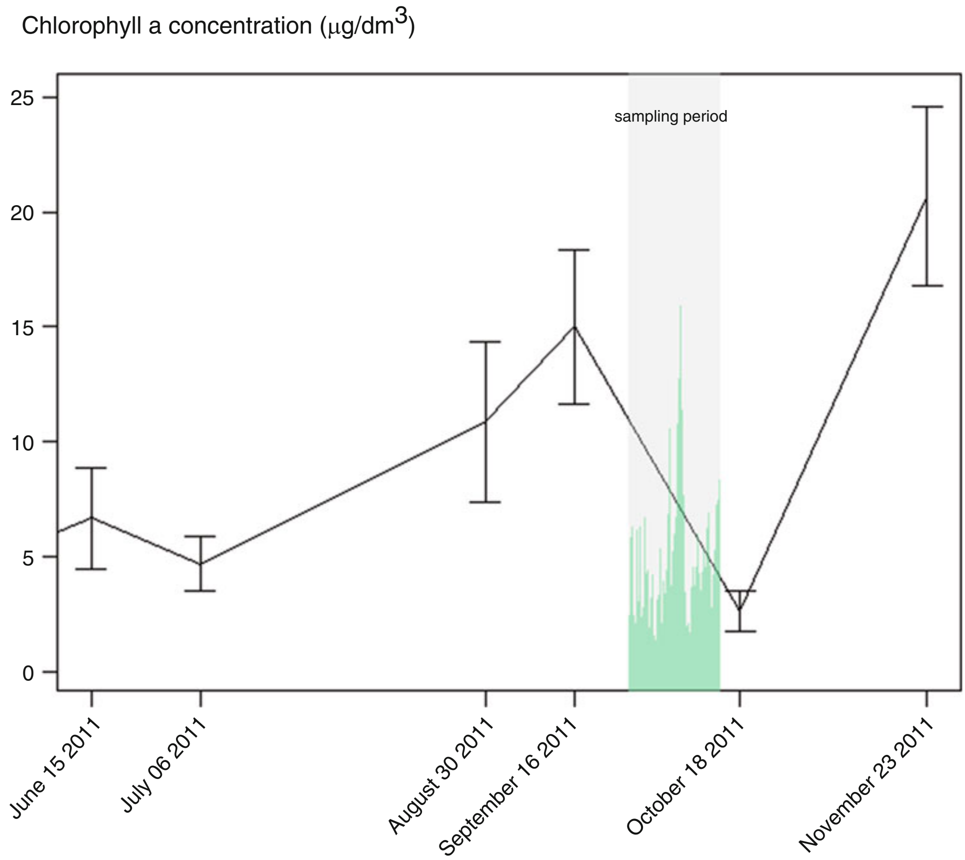


Fig. 4 Chlorophyll *a* concentration measured during the monthly monitoring of the lagoon (*black line*, with the standard deviation, $n = 20$) and during the hourly sampling period from October 5 to 11 (in *green*)

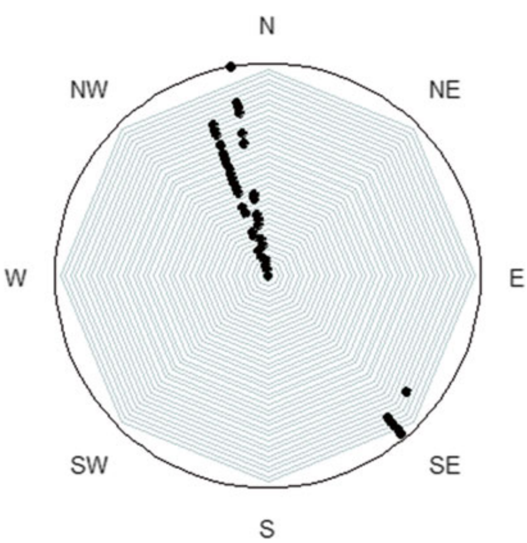


Fig. 5 Distribution of the wind direction with mean speed exceeding 20 m/s during the sampling period

resumption of nutrient supply generated by the power plant activities (Minas 1976). During the sampling period, freshwater discharges reached 150.10^6 m^3 for a volume of the lagoon estimated at 980.10^6 m^3. Mean temperature was 21.4 ± 2.1 °C.

Few dinoflagellate and diatom species like *Prorocentrum minimum* and *Cyclotella* sp. have then always been dominant for both new and regenerated production (Raimbault et al. 2013). Historically, abundances of *P. minimum* could reach 40,000 cells/cm^3 in the lagoon. Before the water policy management, colored waters were observed with concentration of up to 660 cells/cm^3, and fish and mollusk mortality was induced by toxins close to the paralytic shellfish poisoning (PSP) with concentration of 5,184 cells/cm^3 (Belin and Berthome 1988). However, since the first regulation of the power plant discharges

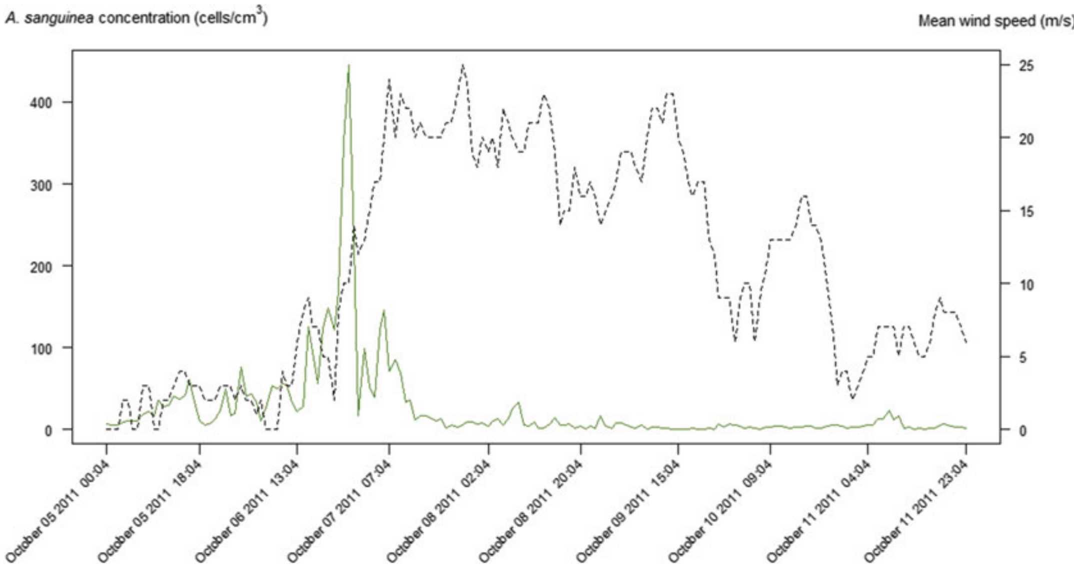


Fig. 6 Dynamics of *A. sanguinea* (*green line*) and mean wind speed (*dashed line*) before, during, and after a strong mistral event

in 1994, the abundance of this species decreased and more eurythermal and euryhaline as well as marine species like *Scrippsiella sp.* were able to develop (Malkassian 2012). *Gymnodinium sp.*, *Akashiwo sanguinea*, *Prorocentrum micans*, and several other species of nanoflagellates are also present in the lagoon (identified by microscopy by the monthly monitoring supervised by the GIPREB). Like *A. sanguinea*, able to grow at temperatures between 10 and 30 °C and salinities between 10 and 40 (Matsubara et al. 2007), these species are well adapted to the important hydrological variations of the lagoon (Nérini et al. 2001).

In the literature, *A. sanguinea* has been referred as a potentially toxic species (Tindall et al. 1984), harmful for fishes, marine birds, mollusks, and human after consumption of contaminated shellfish and corals (Cardwell et al. 1979; Botes et al. 2003; Jessup et al. 2009; Vazquez et al. 2011). However, no biotoxin have been isolated yet and evidences of a direct mortality mode have only been reported recently in case of seabird's stranding with no current toxins involved (Jessup et al. 2009). For Du et al. (2011), a massive red tide dominated by *A. sanguinea* in concentration at only 400 cells/cm^3 was responsible for the mortality of several seabird species. The saponification of their feathers' oil caused by the massive production of surfactant amino acids (MAAs) has led to hypothermia and ultimately to the death of the birds.

In addition to the direct mortality caused by secondary metabolites, harmful algal bloom species can also be indirectly noxious through the exceptional concentration and biomass they reach, especially for benthic species needing oxygen and light. Proliferation of some phytoplanktonic species can be important enough to clog shellfish gills and thus asphyxiate organisms even if the oxygen concentration in the environment is sufficient. The substantial release of phytoplanktonic organic matter derived from the blooms may also favor the oxygen depletion in the water column induced by the respiration of the aerobic heterotrophic prokaryotes.

This compartment is indeed the major responsible for the organic matter remineralization. Manté and Michez (2010) have demonstrated that species richness of the benthic macrofauna of the Berre lagoon is lower than in other Mediterranean lagoons due to frequent anoxia. The thermic or haline stratification of the water column prevents the reoxygenation of the deep layers by gas exchange with the surface, leading to hypoxia or anoxia in extreme cases. The variation of nitrate concentration and salinity during the sampling period with the strong wind (mistral) event clearly demonstrates that the Berre lagoon was in a stratified state in early October. On October 18, vertical profiles of nutrient concentrations showed differences between surface and bottom (GIPREB 2011). The mistral event has contributed to the mixing of the water mass and has favored its reoxygenation.

Due to their capacity to migrate along the water column (i.e., nycthemeral migration), flagellate species including *A. sanguinea* are known to proliferate in worldwide stratified waters (Shipe et al. 2008; Du et al. 2011). Hence, they contribute for a great part to the regenerated production based on nutrient remineralization by the microbial loop in deeper layers. When photosynthesis becomes light limited, flagellates can swim downward the column water to find inorganic regenerated form of nitrogen such as ammonium while nitrates are not consumed.

In October 2011, peaks of phytoplanktonic biomass due to dinoflagellate proliferation were correlated to higher nitrate concentrations. When the chlorophyll *a* content of the lagoon was the highest (21.81 μg/dm^3), *A. sanguinea* measured concentration reached up to 445 cells/cm^3. A recent study conducted on nutrient uptakes and regeneration rate in the water column suggests that pelagic regeneration of ammonium can largely encompass the demand for primary producers' development (Gouze et al. 2008).

As emphasized by Anderson (2009), harmful phytoplanktonic outbreaks are increasing over the years. The first invoked cause is intensification of industrialization and pollution. But global warming is also believed to trigger algal blooms (Moore et al. 2008). Increase of surface temperature is indeed coherent with dinoflagellate proliferation in stratified waters. Moreover, higher exposure to UV due to the depletion of ozone is a factor of control of the biosynthesis of photoprotective MAAs (Litchman et al. 2002).

Climate change could thus enhance the development of harmful dinoflagellate species as well as their deadliness. However, this very study and particularly the effects of the mistral event point out their consequence may not be clear concerning these harmful algal blooms and even display opposite responses. According to the Mermex group (2011), the stratification of water mass could be balanced by more frequent northern wind event like mistral in the Mediterranean Sea.

In this study, the occurrence of a mistral event characterized by a mean speed exceeding 20 m/s was phased with the disappearance of micro- and nanophytoplankton cells. However, wind and storms have been considered as factors of resuspension and dispersal leading to the increase of harmful algal blooms (Anderson 1989; Kremp 2001). Resuspension and dispersal of a high concentrated inoculum of resistant cysts could indeed conduct to massive and sudden proliferation if environmental conditions favor their germination.

6 Conclusion and Perspective

Algal bloom monitoring is a major preoccupation as proliferations of harmful species can induce great economic losses and sanitary issues if they are not anticipated. The Berre lagoon is a brackish water pool known to host regular phytoplanktonic blooms involving harmful and red tides species. As lagoons represent important sources of socio-economic incomes, their management constitutes a major concern for today's society. Since 2000, the European Water Framework Directive policy order regular surveys to state about the eutrophication of these ecosystems, aiming at a coordinate management that could lead to the rehabilitation of hundreds of sites, including the Berre lagoon. This study demonstrates that the deployment of an autonomous flow cytometer in this area constitutes a complementary method to the monthly monitoring of the phytoplanktonic community performed for many years now. During one month, the cytometer has characterized at high frequency the phytoplanktonic assemblage, resolving several clusters ranging from pico- to microphytoplankton. Pictures taken by the instrument helped to identify the largest ones, composed of red tide dinoflagellate species. This strategy combined to hydrological variables records has evidenced a highly dynamic community capable of responding very quickly to sporadic environmental forcing. A fast increase of dinoflagellates' abundance, including the harmful species *Akashiwo sanguinea*, was initiated by the stratification of the water column for which they present physiological advantages compared to other phytoplankton species. The potential outbreak of a harmful algal bloom in the lagoon was however disturbed by the mistral episode with a mean speed exceeding 20 m/s. The possibility of monitoring a great range of phytoplankton, at high frequency and automatically (independently of the weather conditions), with the associated environmental conditions, opens new ways to better understand the circumstances under which harmful algal blooms can arise or disappear. And since they constitute a main effect of eutrophication, autonomous flow cytometry obviously appears appropriate to follow the rehabilitation of the Berre lagoon which is expected after the discharge restrictions imposed to the EDF power plant. The characterization of the resilience of this ecosystem will be eventually determinant for the restoration of certain economical activities, explaining why the interest of such instrumentation does not only stand for fundamental researchers in the field of marine microbiology but also for people, networks, and stakeholders directly or indirectly concerned by eutrophication and the quality of the aquatic environment.

References

Anderson DM (1989) Toxic algal blooms and red tides: a global perspective. In: Red tides: biology, environmental science and toxicology. Elsevier Science Publishing Co., pp 11–16

Anderson DM (2009) Approaches to monitoring, control and management of harmful algal blooms (HABs). Ocean Coast Manag 52:342–347

Anderson DM, Andersen P, Bricelj VM, Cullen JJ, Rensel JE (2001) Monitoring and management strategies for harmful algal blooms in coastal waters, APEC #201-MR-01.1, Asia Pacific economic program, Singapore, and intergovernmental oceanographic commission technical series no. 59, Paris

Belin C, Berthome J-P (1988) Bilan des perturbations phytoplanctoniques observées sur les côtes françaises en 1986. Rapport IFREMER, pp 1–184

Botes L, Smit AJ, Cook PA (2003) The potential threat of algal blooms to the abalone (*Haliotis midae*) mariculture industry situated around the South African coast. Harmful Algae 2:247–259

Cardwell RD, Olsen S, Carr MI, Sanborn EW (1979) Causes of oyster mortality in South Puget Sound. NOAA Technical Memorandum, ERL MESA-39, Environmental Research Laboratories, Marine Ecosystems Analysis Program, Boulder

Cloern JE (1996) Phytoplankton bloom dynamics in coastal ecosystems: a review with some general lessons from sustained investigation of San Francisco Bay, California. Rev Geophys 34:127–168

Du X, Peterson W, McCulloch A, Liu G (2011) An unusual bloom of the dinoflagellate *Akashiwo sanguinea* off the central Oregon, USA, coast in autumn 2009. Harmful Algae 10:784–793

Dubelaar GBJ, Gerritzen PL (2000) CytoBuoy: a step forward towards using flow cytometry in operational oceanography. Sci Mar 64:255–265

Dubelaar GBJ, Gerritzen PL, Beeker AER, Jonker RR, Tangen K (1999) Design and first results of CytoBuoy: a wireless flow cytometer for *in situ* analysis of marine and fresh waters. Cytometry 37:247–254

GIPREB (2011) Bilan du suivi écologique 2011. http://www.etangdeberre.org/upload/file/maxi/rapport-suivi-2011_250.pdf

Gouze E, Raimbault P, Garcia N, Picon P (2008) Nutrient dynamics and primary production in the eutrophic Berre lagoon (Mediterranean, France). Transit Waters Bull 2:17–40

Group M (2011) Marine ecosystems' responses to climatic and anthropogenic forcings in the Mediterranean. Prog Oceanogr 91:97–166

Jessup DA, Miller MA, Ryan J-P et al (2009) Mass stranding of marine birds caused by a surfactant-producing red tide. PLoS ONE 4:e4550

Kremp A (2001) Effects of cyst resuspension on germination and seeding of two bloom- forming dinoflagellates in the Baltic Sea. Mar Ecol Prog Ser 216:57–66

Litchman E, Neale P, Banaszak A (2002) Increased sensitivity to ultraviolet radiation in nitrogen-limited dinoflagellates: photoprotection and repair. Limnol Oceanogr 47:86–94

Malkassian A (2012) Méthodes d'analyse fonctionnelle et multivariée appliquées à l'étude du fonctionnement écologique des assemblages phytoplanctoniques de l'étang de Berre. Thesis. Université de la Méditerranée

Manté C, Michez N (2010) Expertise pour le traitement et l'analyse statistique des données issues du suivi des indicateurs biologiques dans l'étang de Berre. Rapport GIPREB, pp 1–82

Matsubara T, Nagasoe S, Yamasaki Y, Shikata T, Shimasaki Y, Oshima Y, Honjo T (2007) Effects of temperature, salinity and irradiance on the growth of the dinoflagellates *Akashiwo sanguinea*. J Exp Mar Biol Ecol 342:226–230

Mayot N, Gouze E, Malet N (2013) Bilan des apports à l'étang de Berre et état des lieux de l'eutrophisation. Les actes des rencontres LAGUN'R, pp 128–141

Minas M (1976) Production organique primaire dans un milieu saumâtre eutrophe (Etang de Berre). Effets d'une forte dilution (dérivation des eaux de la Durance). Mar Biol 35:13–29

Moore SK, Trainer VL, Mantua NJ, Parker MS, Laws EA, Backer LC, Fleming LE (2008) Impacts of climate variability and future climate change on harmful algal blooms and human health. Environ Health 7:1–12

Nérini D, Manté C, Durbec J-P, Garica F (2001) Une méthode statistique de détermination de séquences caractéristiques dans une série temporelle de plusieurs variables. Application à la physico-chimie des eaux de l'étang de Berre. Earth Planet Sci 332:457–464

Peeters JCH, Dubelaar GBJ, Ringelberg J, Visser JWM (1989) Optical plankton analyser: a flow cytometer for plankton analysis, I: design considerations. Cytometry 10:522–528

Raimbault P, Beker B, Garica N, Fornier M, Gouze E (2013) L'étang de Berre, cycle de la matière, production primaire et communautés phytoplanctoniques. Les actes des rencontres LAGUN'R, pp 143–157

Shipe RF, Leinweber A, Gruber N (2008) Abiotic controls of potentially harmful algal blooms in Santa Monica Bay, California. Cont Shelf Res 28:2584–2593

Smayda TJ (1997) What is a bloom? Limnol Oceanogr 42:1132–1136

Thyssen M, Tarran GA, Zubkov MV, Holland RJ, Gregori G, Burkill PH, Denis M (2008) The emergence of automated high-frequency flow cytometry: revealing temporal and spatial phytoplankton variability. J Plankton Res 30:333–343

Tindall DR, Dickey RW, Carlson RD, Morey-Gaines G (1984) Ciguatoxigenic dinoflagellates from the Caribbean Sea. Seafood Toxin 21:225–240

Vazquez EJ, Lizarraga I, Schmidt CJ, Tapia A, Cortes D, Sandoval F, Tapia A, Guzman JJ (2011) Impact of harmful algal blooms on wild and cultured animals in the Gulf of California. J Environ Biol 32:413–423

Zigone A, Enevoldsen HO (2000) The diversity of harmful algal blooms: a challenge for science and management. Ocean Coast Manag 43:725–748