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Changes in the annual harmful algal blooms of *Alexandrium minutum*: effects of environmental conditions and drainage basin inputs in the Rance estuary (Brittany, France)

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Abstract – Time series of physico-chemical data and concentrations (cell L^{-1}) of the toxic dinoflagellate *Alexandrium minutum* collected in the Rance macrotidal estuary (Brittany, France) were analyzed to understand the physico-chemical processes of the estuary and their relation to changes in bloom development from 1996 to 2009. The construction of the tidal power plant in the north and the presence of a lock in the south have greatly altered hydrodynamics, blocking the zone of maximum turbidity upstream, in the narrowest part of the estuary. *Alexandrium minutum* occurs in the middle part of the estuary. Most physical and chemical parameters of the Rance estuary are similar to those observed elsewhere in Brittany with water temperatures between 15–18 °C, slightly lowered salinities (31.8–33.1 PSU), low river flow rates upstream and significant solar radiation (8 h day^{-1}). A notable exception is phosphate input from the drainage basin which seems to limit bloom development: in recent years, bloom decline can be significantly correlated with the decrease in phosphate input. On the other hand, the chemical processes occurring in the freshwater-saltwater interface do not seem to have an influence on these occurrences. The other hypotheses for bloom declines are discussed, including the prevalence of parasitism, but remain to be verified in further studies.

Keywords: Harmful algae / dinoflagellate / nutrients / eutrophication / drainage basin / land runoff

1 Introduction

For several decades, harmful algal blooms (so-called HABs) have spread around the world, becoming a problem for human health and the exploitation of marine resources. Many of these toxic events can have a significant economic impact on the coastal communities whose livelihood depends on marine resources (Hoagland et al. 2002).

Following cases of food poisoning caused by consumption of shellfish in Southern Brittany, France, Ifremer (*Institut Français pour l'Exploitation de la Mer* – French Research Institute for Exploitation of the Sea) decided to set up and operate a phytoplankton monitoring network called REPHY (*Réseau de surveillance du Phytoplancton et des phycotoxines* – Phytoplankton and phycotoxin monitoring network) in 1983. REPHY became operational in 1984 and today covers the entire French coast, including the coastline of Corsica. Its main objective is to monitor all shellfish farming, fishing and harvesting areas by detecting the presence of microalgae which are potentially toxic for marine animals and/or consumers of shellfish.

A summary of 20 years of production zone monitoring (Belin 2004) showed that each year in France numerous shellfish farming and harvesting zones closed for several weeks due to the presence of biotoxins in the shellfish. Over the last 20 years, 55% of nationwide closures of shellfish production zones have affected Brittany and more specifically the Finistère area and were caused by the presence of toxins (Amnesic shellfish poisoning, ASP, Diarrhetic shellfish poisoning, DSP and Paralytic shellfish poisoning, PSP). In particular, the southern Breton coasts have been extensively affected by HABs, where the species *Dinophysis acuminata* has been responsible for 85% of the toxic events over the last 20 years.

In the northern part of Brittany, at the entrance to the English Channel, the marine currents are, on average, four times stronger than in the southern part of this region (Cambon 2008). This hydrodynamic structure appears to be unfavorable for toxic algal blooms which are rarer there than in the southern part. The most recent event occurred in 2003 and it was caused by the ichthyotoxic species *Karenia mikimotoi* (Vanhoutte-Brunier et al. 2008). However, in the numerous small estuaries which characterize this northern coastline, blooms of *Alexandrium minutum* develop on a regular

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basis (Aber Benoit and Aber Wrac'h inlets, Penzé river, bay of Morlaix, Jaudy and Rance estuaries).

All of these estuarine sites are oyster farming areas for the Pacific oyster (*Crassostrea gigas*) and many inter-zone transfers of shellfish are made during the rearing cycle. Each year, cysts of *Alexandrium minutum* can be transported during these inter-zone transfers between the sites (Wyatt and Jenkinson 1997; Vila et al. 2001).

These hypotheses are backed up by taxonomic studies which have shown that cells of *Alexandrium minutum* in the Rance estuary are characterized in morphological terms by the absence of a ventral pore on the 1' plate, like the other strains isolated in estuaries further west. Phylogenetic studies confirm that they belong to the global clade (strains from Europe and Australia) (Lilly et al. 2005) while differing slightly from the strains of Southern Brittany, which are generally characterized by the presence of this ventral pore (Nézan, pers. com.).

Alexandrium minutum was noticed for the first time in 1985 in Southern Brittany and then observed in the Aber Wrac'h sea inlet in Northern Brittany in 1988 (Nézan et al. 1989; Le Doux et al. 1990; Morin et al. 2000). From 1990, it was regularly observed in the Bay of Morlaix and the Penzé estuary; here, blooms of several million cells L^{-1} were seen each year. A few years later, *Alexandrium minutum* was observed in all the estuaries of Northern Brittany, but without reaching such a large development (a maximum of approximately 1 million cells L^{-1} was reached in the Rance estuary in 1996).

More recently, a new event has occurred in the bay of Brest where *Alexandrium minutum* has bloomed with up to 41 million cells L^{-1} (Chapelle et al. 2015).

Although numerous studies have focused on the development of *Alexandrium minutum* in these areas, no study until now has reviewed what is known about blooms of this species in the Rance estuary, a macrotidal estuary that has been modified by the construction of a tidal power plant at its mouth. Our study aims to describe the spatiotemporal development of blooms of this toxic dinoflagellate in the Rance estuary, with regard to the functioning of the drainage basin, from 1996 to 2009, especially in relation to supplied nutrients. Our objective is also to verify whether *A. minutum* bloom development in the Rance estuary presents similar temporal and environmental patterns compared to other estuaries in Brittany (Spring-Summer, water temperature >15 °C, lowered salinities, lower tidal range, rich-nutrients, and hydrodynamic stability).

2 Material and methods

2.1 Rance estuary

The Rance estuary is 16 km long and 2.5 km wide at its broadest point and is a natural environment that has been significantly altered by two large man-made structures. Downstream is the first tidal power plant in the world, which was built between 1963 and 1966. It is 750 m long, including a lock, a 24-unit power plant and 1 movable 6-gates dam, and upstream is Le Châtelier lock which limits the southward progression of seawater. There are three large centers of urban development to the north and south, i.e. Saint-Malo (50 000 inhabitants) at the mouth, Dinard (pop. 15 000), and Dinan (pop. 12 000). The lateral drainage basins (175 km^2) are less urbanized and have numerous villages located along the estuary (Fig. 1a).

The 80 km-long Rance river has a low flow rate (average 7 $m^3\ s^{-1}$) with low water levels of 0.5 $m^3\ s^{-1}$ and ten-year floods of 60 $m^3\ s^{-1}$. A 909 km^2 drainage basin, traditionally used for farming activities, drains into the Rance river. On this drainage basin, with the exception of an industrial laundry in Caulnes, agrifood is the main industry.

During spring tides at the equinox, the tidal range at Saint-Malo is 13.5 m. The tidal power plant's presence and operation reduce this tidal range by 40% in the retention basin (22 km^2) where the tidal range varies from 7 to 8 m at spring tide and 2.5 m at neap tide (Fig. 1b). Upstream from the dam, the lowest level of the water never drops below 4 m (Navy rating). This modification in tidal ranges has led to a decrease in the surfaces that uncover during tides and represent no more than 50% of the retention basin's surface area. Before construction of the plant, the intertidal domain covered 70% of the surface area (Retière 1989) (Figs. 1a–1c).

Another specificity of this estuary, due to the tidal power plant's operation, is the duration of slack waters that alter the functioning of the estuary and promotes development of blooms when turbulence is low (Bolli et al. 2007). On the side of the open ocean, the slack water periods last about fifteen minutes, whereas in the basin, they are artificially extended for several hours, during which time the currents are weak or non-existent. Where this estuary profile has not been modified in a significant way, since the opening of the power station, upstream of Port Saint-Hubert, the sedimentation is in the order of 0.5 m to 2.0 m year per year (Fig. 1c).

With 9 000 $m^3\ s^{-1}$ at neap tides and 18 000 $m^3\ s^{-1}$ at spring tides, the oscillating volumes are very high at the tidal power plant in comparison to the volumes of freshwater input by the drainage basin at Le Châtelier lock.

2.2 Source of data used to study the estuarine physico-chemical parameters

In the framework of the network to monitor water quality in estuaries in Brittany, the governmental authority DDTM (*Direction Départementale Territoires et Mer*) in Saint-Malo ensures the policing of water quality by implementing a network of sampling sites on the estuary and its tributaries. Samples were collected about six times per year at six stations, especially during spring and summer (April, June, August), periods of *Alexandrium minutum* blooms, going from upstream of Châtelier lock to upstream of the power plant (Le Minihic), two hours before low water and below the surface (1 m to 1.50 m depth) (Fig. 1a). Data for oxygen, pH, nitrate nitrogen (soluble reactive nitrogen, SRN), phosphates (soluble reactive phosphorus, SRP), silicate and suspended particulate matter were extracted from the DDTM database.

2.3 Source of data for *Alexandrium minutum*

Data on *Alexandrium minutum* concentrations in water and temperature/salinity were collected in the framework of the national phytoplankton monitoring network in France (REPHY).

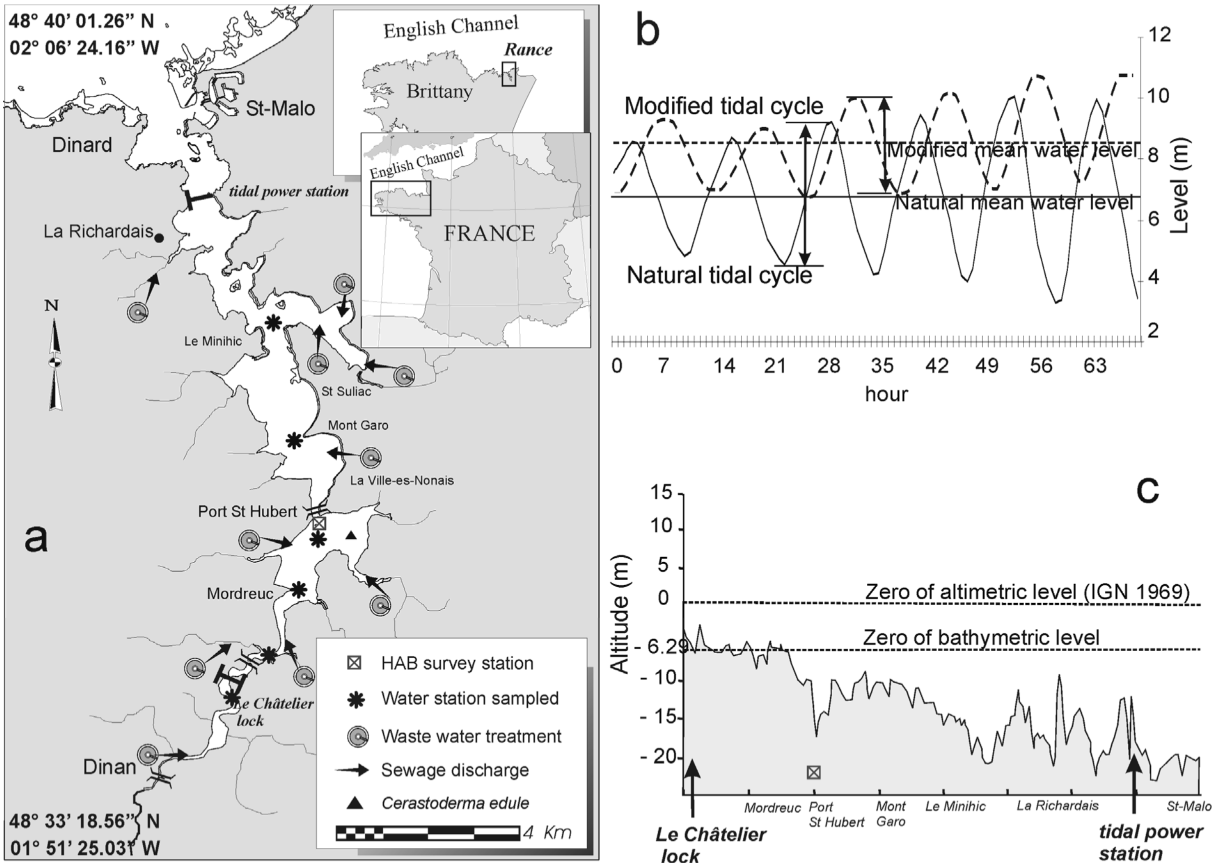

Fig. 1. Rance estuary, place names, location of water quality monitoring stations and inputs (a) ; example of tidal cycles in the maritime part of the Rance (dotted line) and at sea (solid line), from February 9, 2010 midnight to February 11, 2010 11:00 PM, based on EDF and SHOM data, showing mean water level differences, amplitude differences and the duration of slack waters (b); topography of the floor redrawn from Kasouk, Deffontaines, 2009 (c).

For the Rance estuary, the sampling station is located at Port Saint-Hubert (Figs. 1a and 1c). Only taxa that are toxic (or potentially toxic) for human health or for marine fauna were targeted and counted. Samples were collected all year round, twice a month, except during the "risk period" between late May and August, when sampling was done on a weekly basis. Water samples were taken using a HYDRO-BIOS Standard Water Sampler, below the surface (1 m to 1.50 m depth), one hour before high water, and then fixed with acidic Lugol (2.5 ml L^{-1}) and stored at low temperature in the dark. For counting, after homogenizing the sample, a 10 ml aliquot was left to settle for six hours in an Utermöhl chamber (1958), and analyzed using an inverted phase contrast microscope (Olympus IMT2 or Nikon TE2000-U). The number of toxic cells was expressed per liter of seawater (cells L^{-1}). Once checked and validated, the data were stored in the national IFREMER QUADRIGE[2] database. The dataset used in this study comes from the counts performed between 1996 and 2009.

PSP (Paralytic Shellfish Poisoning) measurements were made on cockles (*Cerastoderma edule*) sampled near Port Saint-Hubert (500 m away from the left bank) (Fig. 1a), using the bioassay method (Anon 2005).

In addition to the monitoring network, in the year 2000, 13 sites were sampled between Le Châtelier lock and the tidal power plant (Figs. 1a and 9) during May and June, at low water and high water, in order to better understand the spatial distribution of *Alexandrium minutum*. The parameters measured in the water were the *Alexandrium* cell count, temperature (Testo 915-1), salinity (WTW LF 323), and turbidity (HACH 2100).

2.4 Environmental data

The meteorological data used (rainfall, solar radiation) were supplied by the METEO-France station at Dinard airport. The flow rate data for the Rance river come from the Saint-Jouan-de-l'Isle measuring station, managed by the government authority DREAL (*Direction Régionale de l'Environnement, de l'Aménagement et du Logement*). Estimation of the flow rates at Le Châtelier lock was computed using the following formula supplied by the DREAL: $Q_{\text{downstream}} = Q_{\text{upstream}} X(1 + k(S_{\text{downstream}} - S_{\text{upstream}})/S_{\text{upstream}})$, where $k = 0.8$ (non-schistose soil with losses due to infiltration), $S_{\text{downstream}} = 938\ \text{km}^2$ and $S_{\text{upstream}} = 143\ \text{km}^2$.

The tidal coefficients were provided by the SHOM (French Navy hydrographic and oceanographic service). In France, sea charts are published by the SHOM. They use as references: for altitude, the zero of the general levelling of France, frame of reference of IGN: for the depth, the hydrographic level zero (or zero of the sea charts), which is selected in the vicinity of the water level of astronomical low tide. The tidal coefficient is calculated for a high tide. It is the quotient of the semi-diurnal tidal range by the mean value of the tidal range for the equinox spring tides, in Brest (6.1 m). It is a number without unit ranging between 20 and 120.

The Winter NAO (North Atlantic Oscillation) Index data were provided by the Climate Analysis Section, NCAR, Boulder, USA, (Hurrell 1995).

2.5 Data analysis

Descriptive analyses of the parameters at Port Saint-Hubert from 1996 to 2009 were carried out and a dilution line along the salinity gradient for each parameter was made. The data were processed using Addinsoft – XLSTAT software to generate box plots. Box plots also provided a graphic summary of the dispersion (mean, median and quartiles $Q1$, $Q3$) of these factors as a function of salinity, all seasons and all years. The limits were calculated as:

- lower limit, low $L = X(i)$ such that $\{X(i) - [Q1 - 1.5(Q3 - Q1)]\}$ is minimal and $X(i) = Q1 - 1.5(Q3 - Q1)$.
- Upper limit, up $L = X(i)$ such that $\{X(i) - [Q3 + 1.5(Q3 - Q1)]\}$ is minimal and $X(i) = Q3 + 1.5(Q3 - Q1)$.

In order to understand the development of *Alexandrium minutum* over the 14 years of monitoring with respect to environmental, hydrological or meteorological parameters, two multivariate analyses were performed. The data were processed using Addinsoft – XLSTAT software to compute descriptive statistics, to test the regressions and perform multivariate analyses such as multiple correspondence analysis (MCA) and principle component analysis (PCA) (Chatfield and Collins 1980).

An initial analysis was done using measurements taken in the water, with the same frequency, at the Port Saint-Hubert monitoring site (temperature and salinity), flow rate data (river and tidal coefficient for the sea) and daily sunlight measurements. Since, in these data, no simple linear correlation between the number of cells in water and environmental variables was found, the quantitative variables (*Alexandrium minutum* concentrations and environmental parameters) were transformed into qualitative variables, and the resulting contingency table thus obtained (Table 1) was analyzed using an MCA (Benzécri 1992; Chatfield and Collins 1980). The class limits for environmental variables were established from the quartiles of each parameter. The number of occurrences corresponds to the total number of samples collected between 1996 and 2009 for which both features were observed. The value of transforming quantitative variables into qualitative ones (which leads a priori to a loss of information) is that MCA enables non-linear relationships between qualitative characters to be taken into account.

To supplement this descriptive analysis of the links between environmental factors (in salt water) and the concentrations of *Alexandrium minutum* (performed over the entire observation period), a PCA was used in the second step to describe the inter-annual variability of *Alexandrium minutum* concentrations in relation to inputs from the Rance river, upstream of Le Châtelier lock (Table 2).

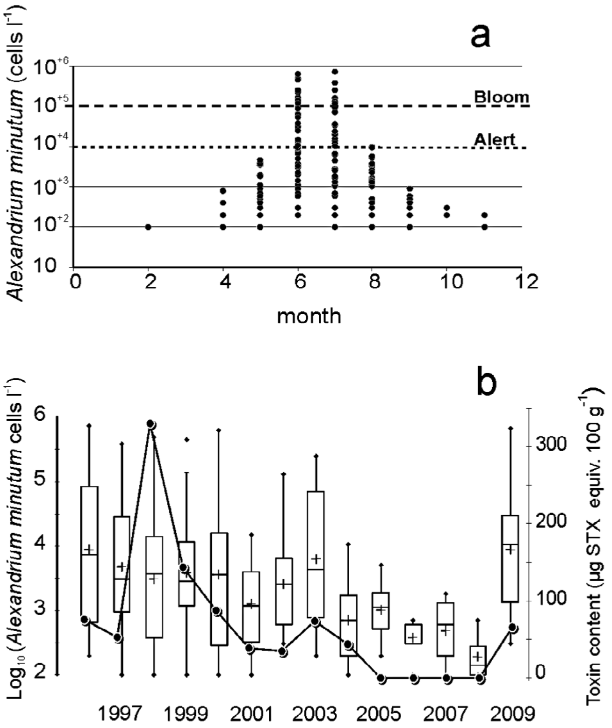

Fig. 2. Monthly distribution of concentrations of Alexandrium minutum established from 14 years of monitoring (each point represent a count) (a) and yearly evolution of concentrations of A. minutum (box plot) compared with maximum toxicity in Cerastoderma edule flesh (solid line and black circle) at Port Saint-Hubert (expressed as μg STX equiv. 100 g^{-1}) from 1996 to 2009 (b).

A linear regression (Addinsoft – XLSTAT software) was used to model the quantitative dependent variable Y (*Alexandrium minutum*) through a linear combination of quantitative explanatory variable, X (fluxes PO_4). The determinist model is written for observations as follows: $Y_i = a_1 X_{ki} + e_i$, where Y_i is the value observed for the dependent variable for observation i, X_{ki} is the value taken by variable k for observation i, and e_i is the error of the model. The model is found by using the least squares method (the sum of squared errors e_i^2 is minimized).

3 Results

3.1 Temporal changes of *Alexandrium minutum* and environmental parameters at Port Saint-Hubert: time series 1996–2009

The distribution of *Alexandrium minutum* observed in the water at Port Saint-Hubert, from 1996 to 2009, showed that this species was mainly observed from April to September and more rarely in February, October and November (Fig. 2a). The alert threshold established at 10 000 cells L^{-1} by the national phytoplankton monitoring network and bloom levels (100 000 cells L^{-1}) occurred in June and July, and more rarely in August. During the 14 years of monitoring, the observed blooms never exceeded one million cells L^{-1}.

Table 1. Contingency table comparing *A. minutum* classes and environmental parameter classes at Port Saint-Hubert between 1996 and 2009. The class limits for environmental variables are established from the quartiles of each parameter: Temp1, Temperature (class1) $\leqslant$10.1 °C; Temp2, Temperature (class2) range 10.1 and 15.4 °C; . . . Sal2, Salinity (class2) range 31.8 and 33.1; . . . Tide2, tidal coefficient (class2) range 55 and 72; . . . Flow2, Flow of the Rance river (class2) range 0.2 and 0.6 $m^3\ s^{-1}$; . . . Sun2, Daily sunlight (class2) range 1 and 4 $h\,day^{-1}$; 5DaySun1, Sum of 5 consecutive Daily sunlight $\leqslant$14 h 5 $days^{-1}$; . . . NoAlex, absence of *A. minutum* in the water sample; LowAlex, number of *A. minutum* range 10^2 and 10^3 cells L^{-1}; Alert, number of A. minutum range 10^3 and 10^4 cells L^{-1} corresponding to the alert level of the monitoring network REPHY; Bloom, number of A. minutum $\geqslant 10^4$ cells L^{-1} corresponding to the bloom level of the monitoring network REPHY. The number of occurrences corresponds to the total number of samples collected between 1996 and 2009 for which both features were observed. The Chi-square test of independence between the lines and columns gives a calculated *p*-value (<0.0001) lower than the level of significance alpha =0.05. The null hypothesis H0 can be rejected; there is a link between the lines and columns of the table.

	NoAlex	LowAlex [100–1000[	Alert [1000–10 000[	Bloom $\geqslant$10 000
Temp1 $\leqslant$10.1 °C	107	4	0	0
Temp2]10.1–15.4 °C]	83	20	6	1
Temp3]15.4–18.6 °C]	41	20	20	32
Temp4 > 18.6 °C	38	22	34	14
Sal1 $\leqslant$31.8	90	11	8	3
Sal2]31.8–33.1]	63	17	14	19
Sal3]33.1–33.9]	62	18	16	15
Sal4 >33.9	52	20	22	10
Tide1 $\leqslant$55	64	24	14	11
Tide2]55–72]	64	14	19	14
Tide3]72–85]	65	13	13	16
Tide4 >85	76	15	14	6
Flow1 $\leqslant$0.2 $m^3\ s^{-1}$	57	22	23	9
Flow2]0.2–0.6 $m^3\ s^{-1}$]	39	25	20	26
Flow3]0.6–1.3 $m^3\ s^{-1}$]	73	12	16	10
Flow4 >1.3 $m^3\ s^{-1}$	100	7	1	2
Sun1 $\leqslant$1 $h\,day^{-1}$	89	8	6	8
Sun2]1–4 $h\,day^{-1}$]	71	16	11	12
Sun3]4–8 $h\,day^{-1}$]	59	22	21	8
Sun4 >8 $h\,day^{-1}$	50	20	22	19
5DSun1 $\leqslant$14 h 5 $days^{-1}$	100	7	2	2
5DSun2]14–23 h 5 $days^{-1}$	77	16	8	9
5DSun3]23–35 h 5 $days^{-1}$]	51	22	20	17
5DSun4 >35 h 5 $days^{-1}$	41	21	30	19

Table 2. Annual means of variables used for Principal Components Analysis. *P*, phosphates concentration in the river upstream of Le Châtelier lock ($\mu mol\ L^{-1}$); meanflow, annual mean flow of the river ($m^3\ s^{-1}$); rain, annual mean rainfall at Dinard (mm); Flux *P*, annual mean phosphates concentration *X* flow of the river ($10^{+3}\ \mu mol\ s^{-1}$); LogAlex, annual mean of Log_{10} (A. minutum per liter) observed at Port Saint-Hubert; N, annual mean nitrogen concentration in the river upstream of Châtelier lock ($\mu mol\ L^{-1}$); Flux *N*, annual mean nitrogen concentration *X* flow of the river ($10^{+3}\ \mu mol\ s^{-1}$); NAO Index Data provided by the Climate Analysis Section, NCAR, Boulder, USA, Hurrell (1995); Sun, annual mean daily sunlight at Dinard (h).

Year	*P*	Meanflow	Rain	Flux *P*	LogAlex	*N*	Flux *N*	NAO	Sun
1996	6.7	4.5	105.8	30.8	3.95	270.53	1232.12	−3.78	4.6
1997	10.6	2.6	102.6	27.4	3.68	148.18	382.86	−0.17	5.2
1998	3.2	7.4	136.4	23.9	3.48	384.99	2830.87	0.72	4.3
1999	3.2	9.7	140.6	30.4	3.58	379.28	3671.06	1.70	4.4
2000	1.8	10.7	146.3	19.7	3.56	452.42	4825.08	2.80	4
2001	2.2	12.2	141.6	27.2	3.10	398.86	4878.38	−1.90	4.7
2002	2.4	5.8	135.0	14.2	3.41	285.75	1672.92	0.76	4.6
2003	5.0	5.7	96.7	28.4	3.80	262.23	1491.27	0.20	5.4
2004	1.1	7.1	146.1	8.0	2.85	358.89	2549.66	−0.07	4.4
2005	2.9	4.0	121.6	11.4	3.01	258.29	1022.76	0.13	4.6
2006	1.2	5.7	55.0	6.6	2.58	331.70	1889.59	−1.09	4.6
2007	1.2	6.6	66.8	7.6	2.69	413.27	2711.22	2.80	4.5
2008	1.1	8.4	70.9	9.1	2.29	369.77	3114.58	2.10	4.7
2009	1.1	6.5	63.7	7.0	3.95	270.80	1766.47	−0.40	5.5

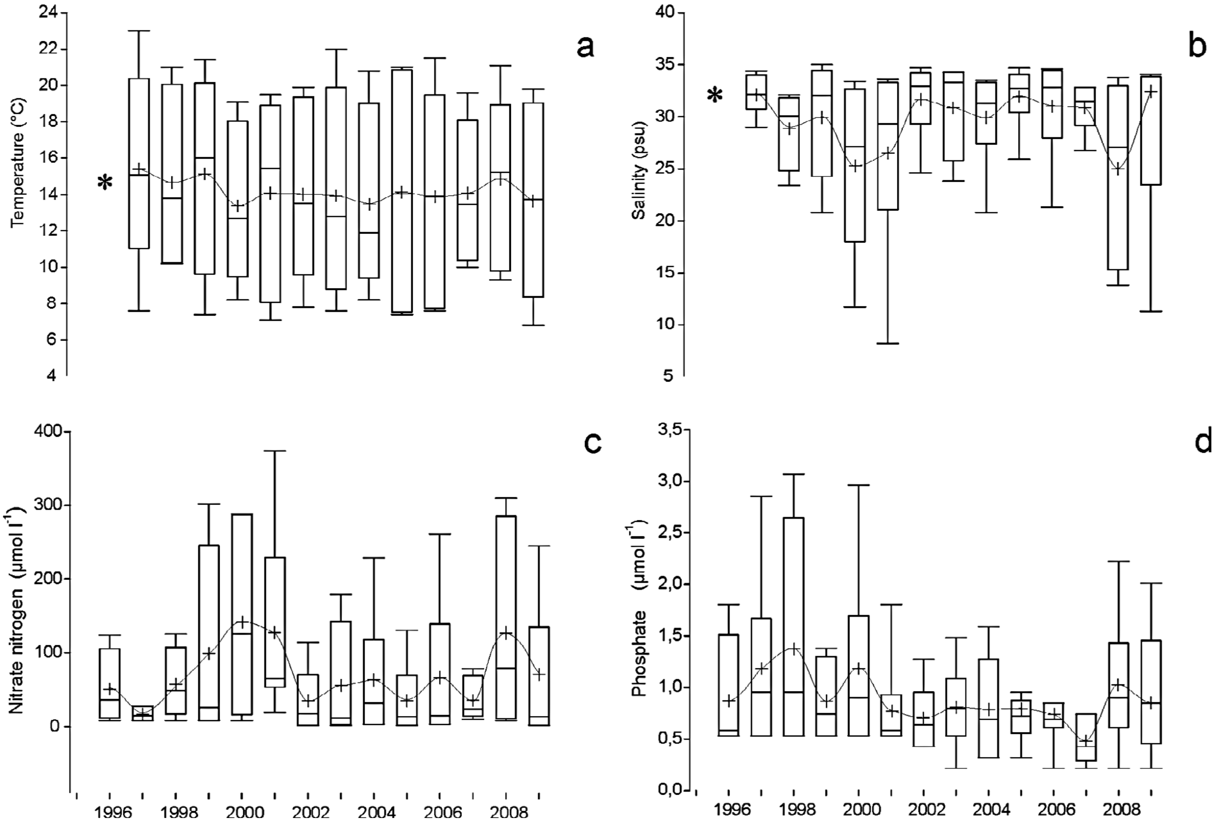

Fig. 3. Distribution of temperatures (°C) (a), salinity (PSU) (b), nitrate nitrogen (μmol L^{-1}) (c) and phosphate (μmol L^{-1}) (d) at Port Saint-Hubert from 1996 to 2009 (all years and all seasons). The average is displayed as a cross +, and the median as a black line. A Solid line connects the means in all figures (∗ *not enough data*).

From 1996 to 2000, maximum cell concentrations varied between 730 000 and 630 000 cells L^{-1} (Fig. 2b). There was a significant decrease in 2001 and from 2003 until 2008, a decrease in concentration was noted every year. However, in 2009, a larger development of 670 000 cells L^{-1} was measured again. The year 2009 appear to be atypical in this time series.

From 1997 to 2009 at Port Saint-Hubert, the average maximum temperature was measured in 1997 (15.4 °C with a delta = 15.3 °C) and the minimum in 2004 (13.3 °C) (Fig. 3a). The salinity shows strong interannual variations (on average 25–32 PSU) due to years with more precipitation (METEO-France Data) (Fig. 3b). Nitrate nitrogen concentrations appear to follow the same trend with a peak in 2000–2001 (143 μmol L^{-1}) and then decreased (~20 μmol L^{-1}) with higher concentrations in 2008 and 2009 (Fig. 3c). The average phosphate concentrations vary from 1.4 μmol L^{-1} in 1998 to 0.5 μmol L^{-1} in 2007, and are noticeably in constant decline since 2000 (Fig. 3d).

Although there is no relationship between the number of cells observed in the water (Fig. 2b) and temperature, salinity or the amount of nitrogen at Port Saint-Hubert ($R^2 \sim 0.2$), only a tenuous relationship with the phosphate concentration may be observed ($R^2 \sim 0.3$) (Fig. 3d). These two parameters, *Alexandrium minutum* cell concentrations and phosphate, seemed to follow the same decreasing trend.

Given that, in our data, there was no simple linear correlation between the number of cells in water and environmental variables, quantitative variables (*Alexandrium minutum* cell concentrations and environmental parameters) were transformed into qualitative variables to perform an MCA. This analysis (Fig. 4, Table 1) showed that the development of *Alexandrium minutum* blooms was mainly linked to temperature (Temp3 range of 15.5 °C to 18.5 °C) (Fig. 4, upper left quadrant) and low river flow rates (Flow2 range of 0.22 to 0.58 m^3 s^{-1}), and to a lesser extent to salinities (Sal2) from 31.8–33.1 PSU, tidal coefficients (Tide3) between 72 and 85 and once the bloom began (Alert) to lower coefficients (Tide1, Tide2) of 55 to 72. Daily solar radiation was significant, greater than 497 min day^{-1} (Sun4).

However, (Fig. 4, right part), *Alexandrium* was absent under winter environmental conditions characterized by temperatures <10 °C (Temp1), high fresh water discharges (Flow4), little sunlight (Sun1) and low salinities (Sal1).

Considering these hydro-climatic conditions, the decrease in blooms of *Alexandrium minutum* beginning in 2000–2003 cannot be explained. Thus a PCA was performed on the annual means of parameters measured in fresh water, upstream of Le Châtelier lock, the main source of nutrients (Fig. 5, Table 2).

These parameters characterize the main river inputs to the estuary (annual mean discharge, nitrate nitrogen and phosphate concentrations (μmol L^{-1}) and nitrate nitrogen and phosphates fluxes). The annual mean meteorological conditions (rainfall, annual mean daily solar radiation and the annual NAO index calculated over December, January, February and March) were also taken into consideration. Because the year

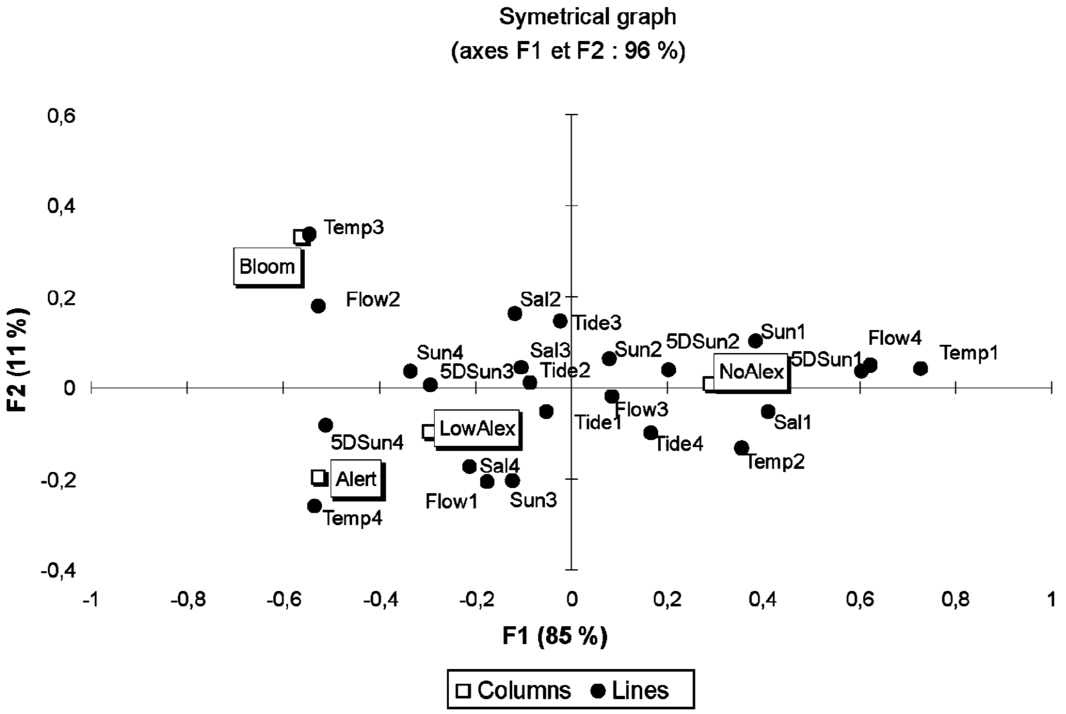

Fig. 4. Multiple Correspondence Analysis on table comparing *A. minutum* classes (white square) and environmental parameter classes (black dots) at Port Saint-Hubert between 1996 and 2009: Temp1, Temperature (class1) ⩽10.1 °C; Temp2, Temperature (class2) range 10.1 and 15.4 °C; . . . Sal2, Salinity (class2) range 31.8 and 33.1; . . . Tide2, tidal coefficient (class2) range 55 and 72; . . . Flow2, Flow of the Rance river (class2) range 0.2 and 0.6 $m^3 s^{-1}$; . . . Sun2, Daily sunlight (class2) range 1 and 4 $h\,day^{-1}$; 5DaySun1, Sum of 5 consecutive Daily sunlight ⩽14 h 5 $days^{-1}$; . . . NoAlex, absence of *A. minutum* in the water sample; LowAlex, number of *A. minutum* range 10^2 and 10^3 cells L^{-1}; Alert, number of A. minutum range 10^3 and 10^4 cells L^{-1} corresponding to the alert level of the monitoring network REPHY; Bloom, number of A. minutum ⩾10^4 cells L^{-1}

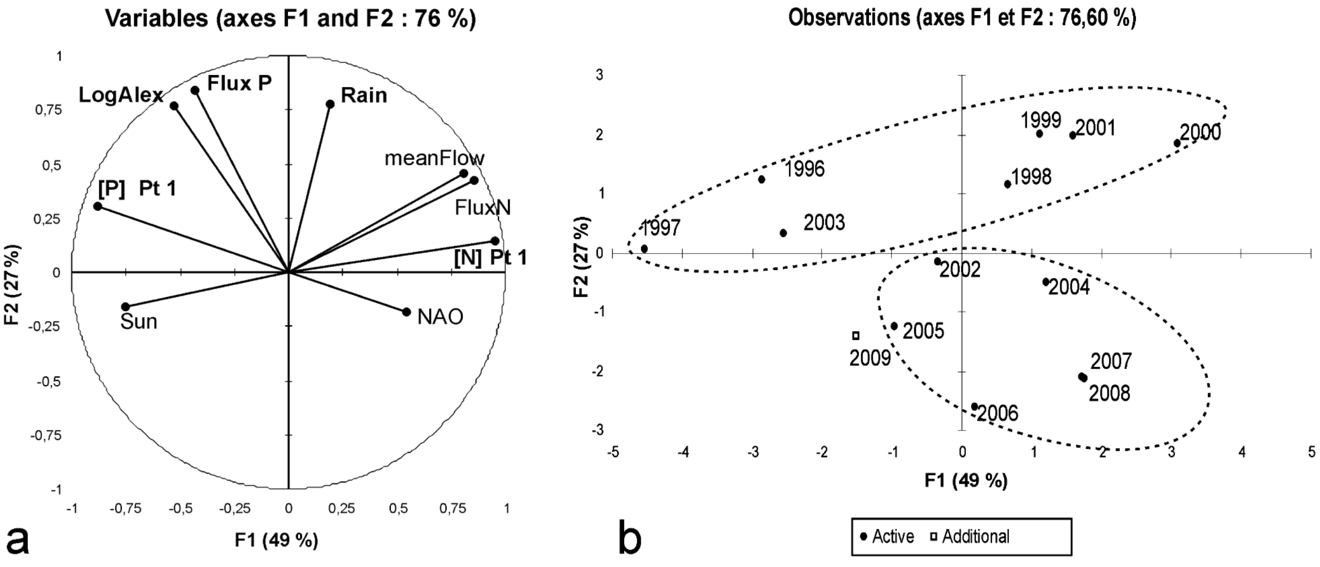

Fig. 5. Correlation circle for variables (a) and projection of years on the first 2 factorial axes (b): [P] Pt1, phosphates concentration in the river upstream of Châtelier lock (μmol L^{-1}); meanFlow, annual mean flow of the river ($m^3 s^{-1}$); Rain, annual mean rainfall at Dinard (mm); Flux P, annual mean phosphates concentration × flow of the river (10^{+3} μmol s^{-1}); LogAlex, annual mean of Log_{10} (A. minutum per liter) observed at Port Saint-Hubert; [N] Pt1, annual mean nitrogen concentration in the river upstream of Le Châtelier lock (μmol L^{-1}); FluxN, annual mean nitrogen concentration × flow of the river (10^{+3} μmol s^{-1}); NAO, Index Data provided by the Climate Analysis Section, NCAR, Boulder, USA, Hurrell (1995); Sun, annual mean daily sunlight at Dinard (h).

2009 seems atypical with respect to the previous ones, it was considered an outlier in PCA and was not part of the computations.

Over 76% of the total inertia was taken up in the factorial plane 1–2. Axis 1 was defined by phosphate concentrations in fresh water, in contrast to nitrate nitrogen concentrations (Fig. 5). Although nitrate nitrogen concentrations were closely linked to the mean river flow rates (0.84), the same does not hold true for phosphate concentrations. However, *Alexandrium minutum* concentrations appear to be strongly correlated to phosphate concentrations and above all to annual phosphate fluxes (0.85). Rainfall, phosphates flux and *Alexandrium minutum* concentrations characterize axis 2.

Projection of the years on the factorial plane thus shows two sets whose distribution on the vertical plane strongly correlated to the annual quantity of *Alexandrium minutum*, phosphate fluxes and rainfall (Figs. 5a and 5b). These two sets extend over the horizontal plane following the average annual discharge rates, the phosphate concentrations which flow into the estuary and the nitrate nitrogen concentrations and fluxes. In the background is the atypical year 2009, characterized by an *Alexandrium minutum* bloom, with a low phosphate input, a decreasing nitrate nitrogen input and lower mean annual rainfall than in the previous years (Fig. 5).

The trend in phosphate fluxes into the estuary, as well as the maximum concentrations of *Alexandrium minutum* observed at Port Saint-Hubert since 1996 are shown in Figure 6. With the exception of the year 2009, a significant relationship can be established between the decrease in *Alexandrium minutum* and the reduction of phosphate fluxes. 73% of the variability of maximum concentrations of *Alexandrium minutum* is explained by the phosphate fluxes. Given the fact that the probability corresponding to the *F* value (Fisher test) is lower than 0.0001, it would be taking a lower than 0.01% risk in assuming that the null hypothesis (no effect of the two explanatory variables) is wrong.

Lastly, the maximum concentrations of saxitoxins measured in the flesh of cockles (*Cerastoderma edule*) near Port Saint-Hubert, between 1996 and 2009, showed no direct relationship with the maximum number of *Alexandrium* cells (Fig. 2b). In spite of higher concentrations of cells measured in 1996, 2000 and 2009, the highest level of saxitoxins was measured in 1998, which corresponded to a concentration of 493 000 cells L^{-1} in the water column.

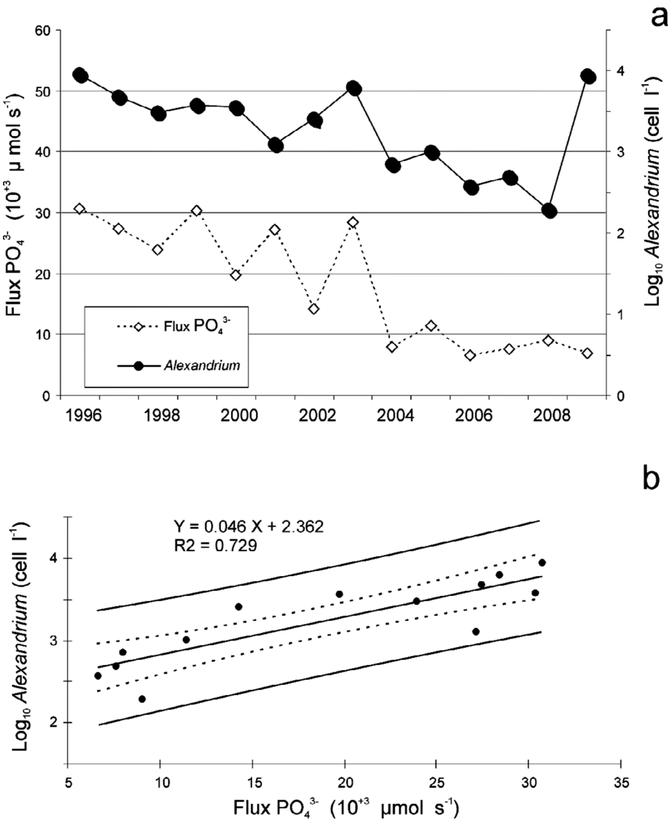

Fig. 6. Trend of annual mean concentrations of Alexandrium minutum at Port Saint-Hubert (solid line, black point) and annual mean phosphate fluxes at Le Châtelier lock (dashed line, gray diamond) from 1996 to 2009 (a) and regression between annual mean concentrations of Alexandrium minutum and annual mean phosphate fluxes (b).

3.2 Variations of physico-chemical parameters along the estuary and spatial distribution of *Alexandrium minutum* in 2000

For salinity, three zones could be identified according to their respective heterogeneity. The first zone, between Le Châtelier lock and Mordreuc, was characterized by the strongest dispersion. This is the area where fresh and sea water mix (brackish water) with salinities ranging from ~5 to ~20 PSU. The distribution of suspended particulate matter (SPM) along the salinity gradient displays higher values for the salinity range [1–5 PSU] in the zone located near Le Châtelier lock (Fig. 7a). On average, the values range from 25 mg L^{-1} in the Rance river to 40 mg L^{-1} where fresh waters mix with more saline waters.

The second zone is an intermediate zone between Mordreuc and Port Saint-Hubert whose salinity varied from ~20 to 30 PSU, area where the peak of *Alexandrium minutum* was observed. Following a decrease in the salinities comprised between 5 and 10 PSU, the average level of SPM slightly rises once again at 25 to 30 PSU.

To the north of Port Saint-Hubert is the third zone, an area of marine waters where salinities ranged from 30 to 35 PSU, the less dispersion.

Likewise, the upstream estuary showed two larger primary production zones, one located in the vicinity of the lock and the other located before the widening of the estuary, where salinities range from 15 to 25 PSU. It was also in the intermediate zone between the two production peaks that the pH dropped after reaching its maximum level during the mixing of fresh water and saline water (Figs. 7b and 7c). Dissolved oxygen varies according to the distribution of chlorophylls, with two slight depletions (Fig. 7d).

There is a sharp dilution gradient for nitrate nitrogen between fresh and marine waters. On average, concentrations range from ~420 μmol L^{-1} to ~24 μmol L^{-1} (Fig. 8a).

Contrary to nitrate nitrogen, concentrations of phosphates noticeably rise (~6 μmol L^{-1}) in the same zone downstream

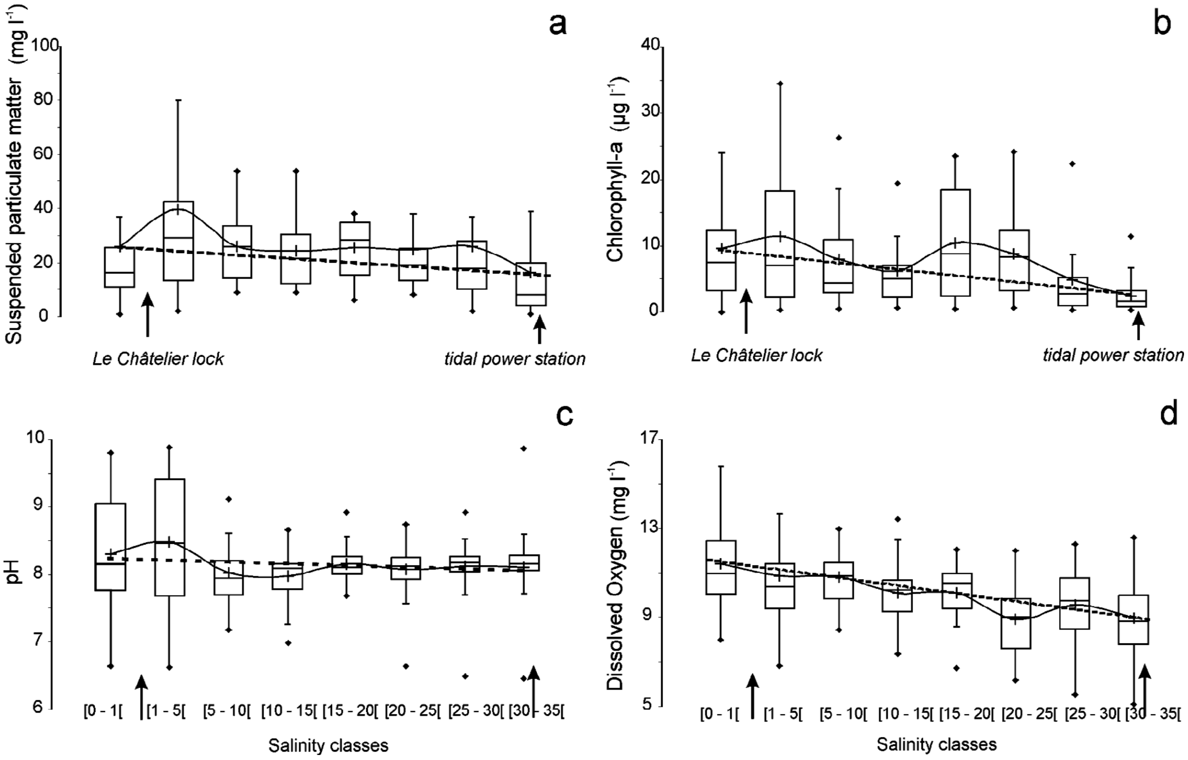

Fig. 7. Distribution of suspended particulate matter (mg L^{-1}) (a), chlorophyll (μg L^{-1}) (b), pH (c), dissolved oxygen (mg L^{-1}) (d), in water as a function of salinity from 1994 to 2009 (all years and all seasons). Arrows indicate the locations of Le Châtelier lock and tidal power station. Dashed, theoretical dilution line if conservativity between upstream and downstream; solid line connects the means, dilution average recorded for each parameter.

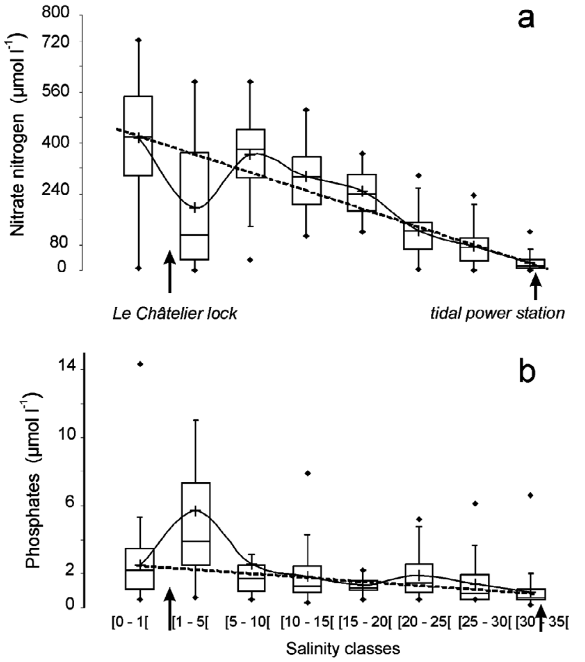

Fig. 8. Distribution of concentrations of nitrate nitrogen (μmol L^{-1}) (a) and phosphates (μmol L^{-1}) (b) along the salinity gradient from 1994 to 2009 (all years and all seasons). Arrows indicate the locations of Le Châtelier lock and tidal power station. Dashed, theoretical dilution line if conservativity between upstream and downstream; solid line connects the means, dilution average recorded for each parameter.

from the lock (Fig. 8b). A smaller input (~2 μmol L^{-1}) of phosphates also exists in the Mordreuc zone (salinity of 20–25 PSU), previously described as a sector slightly depleted in oxygen.

In 2000, at Port Saint-Hubert, the maximum *Alexandrium minutum* concentration was observed in June (624 000 cell L^{-1}), with a low tidal coefficient (46), a tidal range of 3.09 m between high water and low water, high water slack of three hours, low water slack lasting 50 min., water temperature and salinity of 19 °C and 31.9 PSU and a fresh water discharge rate of 4.47 m^3 s^{-1} at Le Châtelier lock.

Five days before the maximum concentration, the peak of *Alexandrium minutum* (146 500) was observed upstream in the water column at high water, 5 km from the lock, with a 19 °C water temperature, 33.2 PSU salinity, 2.4 NTU turbidity, in the area where the estuary becomes wider and shallower (Mordreuc) (Figs. 1c, 9a and 9c). Since the tidal power plant was built, this zone has been characterized by shallow bathymetry and significant silting (2 m in some places) (Kasouk and Deffontaines 2009). The presence of *Alexandrium minutum* was confirmed over the entire estuary, from the least saline zones near Le Châtelier lock, to the marine waters not far from the tidal power plant, as well as in neighboring coves.

At low tide (Figs. 9b and 9d), the cells maxima (85 500) were observed between Mont Garo (9 km) and Port Saint-Hubert (6.8 km), with an 18.3 °C water temperature, 32.5 PSU of salinity and 2.4 NTU of turbidity.

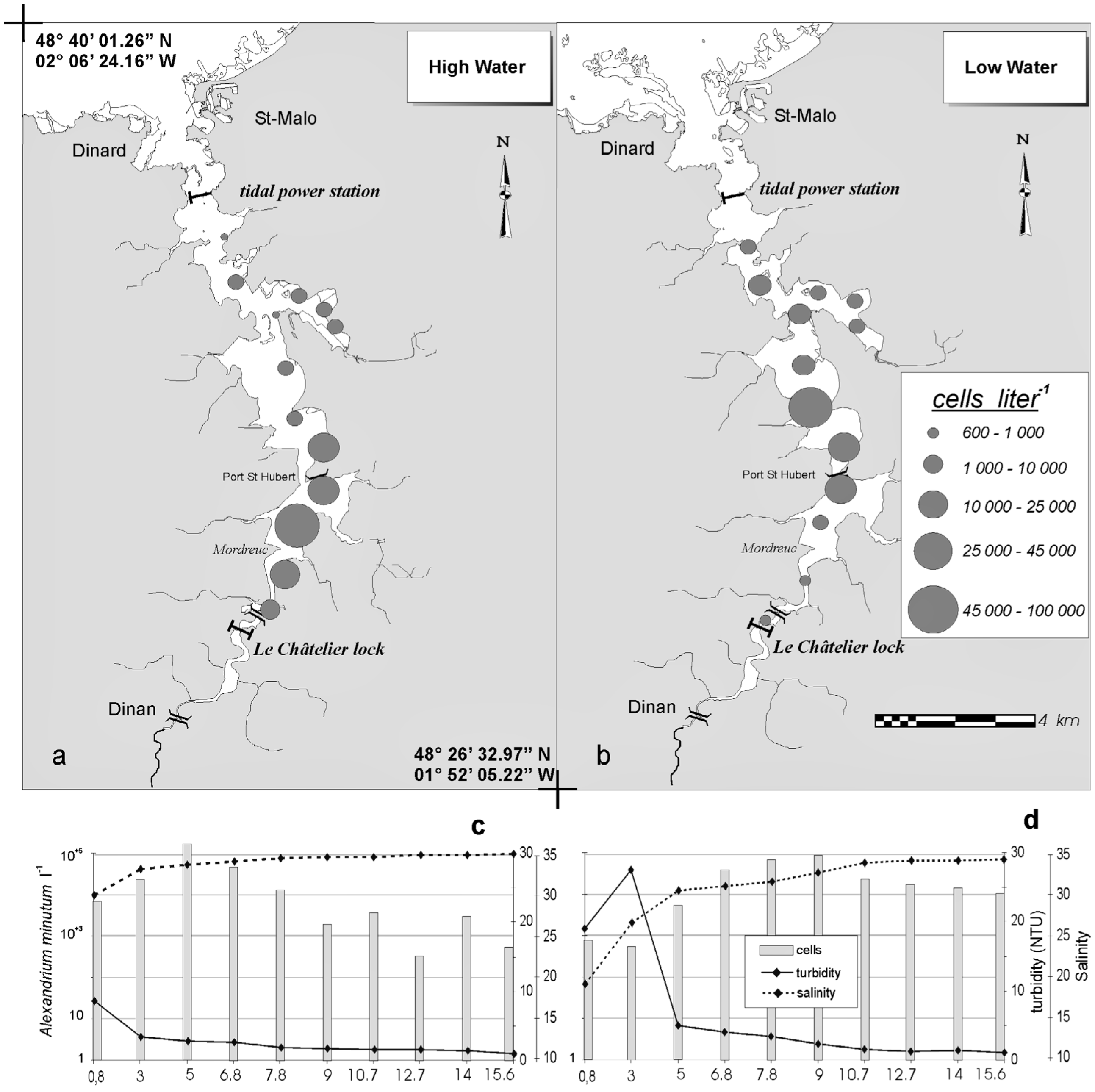

Fig. 9. Spatial distribution of Alexandrium minutum in the Rance estuary and variations of salinity (dash line), turbidity (solid line) and cells of Alexandrium minutum (histogram) along the kilometric gradient, from the lock, at high water (a,c) (June 21, 2000) and low water (b,d) (June 27, 2000).

4 Discussion

In the estuarine environment, all environmental factors are highly influenced by salinity and this is particularly true as far as nutrient concentrations are concerned (Liss 1976).

The Rance estuary is indeed a water body which is heavily modified, as defined by the WFD (Water Framework Directive), by the presence of a tidal power plant downstream and Châtelier lock upstream. Before the tidal power plant construction, the zone of brackish waters extended as far as Port Saint-Hubert (Retière 1989). The power plant has significantly altered hydrodynamic conditions.

However, although the hydrological and hydrodynamic conditions and the benthic communities are disrupted by the new functioning of the water body (Desroy and Retière 2004), similarities can be found with estuaries which are not subjected to the same constraints. This means that the Rance estuary functions similar to other estuaries with maximum turbidity in the upstream section, varying according to river flow rates and tides (Allen et al. 1980).

Contrary to nitrate nitrogen, the decrease in phosphate concentrations after 1998 has not been correlated to discharge rates. Thus nitrate nitrogen would mainly originate from farming, due to land runoff, whereas phosphates would come from anthropogenic and industrial discharges.

Although the hydrodynamic parameters have been modified in the Rance estuary in comparison with other estuarine sites in Northern Brittany, the conditions of *Alexandrium minutum* development are similar to those described by Morin et al. (2000) in the Penzé estuary or in the bay of Brest (Chapelle et al. 2015): an optimal temperature between 15 and 18 °C, slightly freshened waters (31.8–33.1 PSU), lower river flow rates upstream, blooms beginning during weak tides and significant solar radiation. These parameters are not very different from what has been noted for blooms of *Alexandrium minutum* in Southern Europe, except for a much cooler temperature (10.2 °C) at the start of the bloom (Delgado et al. 1990).

This suggests that *Alexandrium minutum* requires stable and fairly smooth hydrodynamic conditions (high residence time, low turbulence), significant sunlight and sufficient nutrient input, particularly in nitrate nitrogen and phosphates (Bolli et al. 2007; Smayda 2008). In the Rance river, these conditions are met in the middle part of the estuary between Mordreuc and Port Saint-Hubert, where the water is shallow (Fig. 1c) with a tidal slack water period artificially extended up to several hours (Fig. 1b), slightly freshened, less turbid than in the upstream zone and supplied by a drainage basin with large farming activity and urban development. However, seeing the significant masses of water put into play by the tidal power plant (9000 to 18 000 $m^3\ s^{-1}$), the dilution and dispersion of cells are high along the estuary. This can explain why the blooms observed in this estuary have never exceeded one million cells L^{-1} during the 14 years of monitoring.

Contrary to another species, *Alexandrium catenella,* which has appeared and bloomed in the Thau lagoon since 1995 following a sharp decline in phosphate inputs in the drainage basin (Jauzien et al. 2010), *Alexandrium minutum* in the Rance has shown a decrease similar to that of the phosphate inputs since 1996. As described for the Penzé estuary (Maguer et al. 2004; Andrieux-Loyer et al. 2008; Chapelle et al. 2010), in the Rance, there is always a surplus of nitrate nitrogen with N/P ratios which rose on average from 66 in 1996 to 427 in 2001. Following a slight drop in 2002, the ratio stabilized at around 400 until 2008.

Information obtained about the watershed, indicates that between 1995 and 2004, there were many upgrades of the collective sewage systems to bring them into compliance in regards to phosphates, which reduced the daily input to the natural environment by tenfold on average. Although *A. minutum*, like other species in the *Alexandrium* genus, has been described as being a "storage specialist" of phosphates, using its stocks for cell growth in periods of depletion (Labry et al. 2008; Yamamoto and Tarutani 1999), a prolonged decrease here of this nutrient progressively limits the extent and appearance of the blooms, as our results show to occur over 14 years. Since the phosphate inputs to the drainage basin were both low and stable since 2006, the year 2009 with a slightly drop in N/P ratio (264 on average) and a bloom with 670 000 cells L^{-1} remains an outlier in this time series.

This outlier could be explained by the hypothesis of diffusive phosphate flux coming from the sediment a short time before the bloom. Indeed, in the Penzé estuary, these inputs can make up over half of the inputs from the river (Andrieux-Loyer et al. 2008). But, in this atypical year 2009, it is also possible that the storm, with winds of 19.5 $m\,s^{-1}$ (METEO-France Data), which occurred a week beforehand, could have resuspended the sediment and remobilized bioavailable phosphates in the water column.

As regards the toxicity measured in shellfish (Fig. 2), except in 1998 and 1999, there is a tenuous linear relationship between the concentration of toxins measured in *Cerastoderma edule* near Port Saint-Hubert and the maximum number of *Alexandrium minutum* cells counted at the same point ($Y = 0.0001X$, $R^2 = 0.5$). The toxicity decreased, year by year, with the number of cells observed, and thus with the decrease of phosphates. And yet, this fact appears to be in contradiction with the various studies by authors who worked on the limiting effect of phosphates and the production of toxins by *Alexandrium minutum* in the laboratory (Maestrini et al. 2000; Grzebyk et al. 2003; Frangopulos et al. 2004; Lim et al. 2010). However, although there is a direct relationship between toxicity in shellfish and the population of this microalgae, on the cellular level the relationship between toxicity and nutrients is more complex (Smayda 2008). Several antagonistic phenomena come into play. On the one hand, the toxicity of *Alexandrium minutum* increases but the intensity of the bloom declines and, on the other, the diversity and number of other microalgae can increase. Consequently, the toxicity will be "diluted" in the bolus ingested by the shellfish and will be perceived with less intensity.

Even though physico-chemical parameters enable us to understand the increase or decrease of harmful algae blooms, the biological parameters should not be neglected, e.g. Smayda (2008) who showed the effect of zooplankton grazing on HABs. The influence of parasitism is the other hypothesis that could explain this outlier. Actually, as demonstrated by Montagnes et al. (2008), unlike parasites, grazing alone can hardly contain the development of an *Alexandrium* bloom. Henceforth, in the Penzé estuary located some 150 km to the west, the rapid and virulent proliferation of parasites regulates the population of this dinoflagellate (Chambouvet et al. 2008; Chambouvet and Guillou 2009). After an initial phase of introduction, settling and development, in the absence of a suitable predator, *Alexandrium minutum* has become sensitive to *Amoebophrya* sp. type parasites and no longer proliferates as intensely.

Presently, for the Rance estuary we do not have more information about grazing or proliferation of parasites. These factors remain to be verified in a further study.

The distribution of cysts mapped in Brittany by Erard-Le Denn et al. (1993) shows the potential presence of cysts of *Alexandrium minutum* on some 30 sites. And yet only a few of these sites show recurrence of blooms. Therefore, it would be interesting to understand why geographically similar estuaries that are contaminated by cysts do not systematically favor the development of an HAB.

Likewise, in the English Channel and above all in the Bay of Biscay, *Alexandrium minutum* has been regularly observed for several years (in coastal waters and further offshore). The description of environmental factors favoring the development of this species provides elements which are vital for in-depth understanding of how ecosystems function and of HAB determinism.

Nevertheless, although there is a scientific consensus for linking HAB and eutrophication (Heisler et al. 2008), we must bear in mind that the cause is not single, but multiple: destabilization of the pelagic food web due to overfishing bringing about a modification in grazing (Granéli 2005), parasitism of some microalgae (Chambouvet et al. 2008), or simply a conjunction of favorable or unfavorable natural phenomena, ranging from degradation of the organic matter which supplies nutrients to meteorological and hydrodynamic parameters.

5 Conclusion

The analysis of a time series of 14 years on the Rance estuary demonstrated the prevalent role of phosphate inputs (concentration × flow rate) from the drainage basin on the development of blooms of *Alexandrium minutum.* In spite of dephosphatation, denitrification and other processes that occur where fresh and seawater meet, the inputs measured upstream of the lock most significantly explain the decrease of this microalga in the middle of the estuary from 1996 to 2008. However, although phosphate limits growth of this species, it is not the limiting parameter, since this nutrient is stored in the sediment. When the species is present and hydrological and meteorological conditions are favorable, both chronic and occasional inputs of phosphates promote its development (Heisler et al. 2008). This example also shows that improvements made on the watershed in order to reduce a source of pollution (either agricultural or urban) can quickly have perceivable effects on the development of an HAB (Anderson et al. 2002). In this case, given the potential stocks of nitrate nitrogen, inputs of this nutrient must be managed in order to obtain a lasting effect.

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