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Oligotrophication and emergence of picocyanobacteria and a toxic dinoflagellate in Thau lagoon, southern France

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Abstract:

Time series data have been examined in Thau lagoon (Southern France) from 1972 to 2006 for water temperature, salinity, nutrients and from 1987 to 2006 for phytoplankton. A first main trend identified is an increase in mean annual water temperature (1.5 °C over 33 years or 0.045 °C/year) that was not evenly distributed among seasons. The highest rate of increase was in the spring (+ 3.0 °C over 33 years), followed by summer (+ 2.0 °C) and fall (+ 1.7 °C). In winter, no significant increase over the 33 year period could be found. A second clear trend is a large decrease in soluble reactive phosphorus (SRP) concentration over the same 33 year period (summer values decreased from 10 µM to 1 µM, while winter values decreased from 3 µM to undetectable at present). Nitrate concentrations depended mainly on rainfall events and watershed runoff. Ammonium data were too fragmentary to be useful. N/P ratios expressed the traditional way of DIN/SRP cannot be used for phytoplankton that are not strict autotrophs. The recent and almost simultaneous appearance of both picocyanobacteria (mostly *Synechococcus*) and the toxic dinoflagellate *Alexandrium catenella* in Thau seem to be related to reduced nutrient loading and the increase in water temperature. *A. catenella* blooms occur either in the spring or the fall when water temperature is near 20 °C and remains so for several weeks with winds speeds below 2–3 m s^{-1}. Picocyanobacterial growth is stimulated by increased summer temperatures, and lowered SRP levels provide picocyanobacteria an ecological advantage over other phytoplankton classes, in particular diatoms such as *Skeletonema costatum* whose cell densities have decreased over the last 8 years in summer and fall, but not in winter. An hypothesis is presented according to which *A. catenella* is not stimulated by increased temperatures, but is able to use picocyanobacteria for growth, and this provides this organism an additional resource over other strictly autotrophic phytoplankton. On a more general level, our data do not support the hypothesis that increased nutrient loading leads to harmful blooms of dinoflagellates. Instead, a combination of habitat disturbance and species displacement seems to lead to such blooms.

Keywords : Thau lagoon ; long term variability ; oligotrophication ; picocyanobacteria ; *Alexandrium catenella* ; *Skeletonema costatum*

Regional index term : Mediterranean

1. Introduction

The recent increase in harmful algal blooms (HAB) in coastal areas is generally attributed to eutrophication. However, the cause-effect relationship is not always well established (Taylor et al., 1994; Smayda, 1997; Sellner et al., 2003). The main problem in relating HAB to environmental factors is that we lack a long term perpective on the occurrence of such blooms (Maso and Garcés, 2006). Time series data are important to understand processes and scales of phytoplankton variability and have revealed impacts of both increasing (Cloern, 2001; Cadée and Hegeman, 2002; Smith, 2006) and decreasing (Ruggiu et al., 1998; Anderson et al., 2002; Philippart et al., 2007) nutrient loads and concentrations on phytoplankton dynamics, sometimes with unexpected results. For example, in the Seto Inland Sea, reductions in nutrient load led to a decrease in phytoplankton biomass, but also to an increase in toxic phytoplankton species (Anderson et al., 2002 ; Imai et al., 2006).

The Thau lagoon has been recently invaded by a toxic dinoflagellate, *Alexandrium catenella* (Lilly et al., 2002) that is affecting economically important aquaculture activities such as oyster farming. Recent studies have focused on the nitrogenous nutrition of this dinoflagellate (Collos et al., 2004 ; 2006) and its ability to retrieve the limiting resources from this environment. Based on their results of growth kinetics under inorganic nitrogen (N) or phosphorus (P) limitation, Matsuda et al. (1999) suggested that *A. catenella* could not become dominant in waters subjected to inorganic N or P limitation. It is therefore a paradox that *A.*

catenella blooms occur in Thau Lagoon where dissolved inorganic N or P are limiting most of the time (Collos et al., 1997; Souchu et al., 1998 ; 2001).

Here we place this recent phenomenon in a wider context using a long term data base on physical, chemical and biological factors that have evolved over the last 30 years.

2. Material and methods

2.1. Study site

The Thau lagoon is a shallow marine lagoon located on the French Mediterranean coast (43°24'N-3°36'E) covering 75 km^2 (Fig. 1). It has a mean depth of 4 m, with a maximum depth of 10 m. The lagoon is connected to the sea by 3 narrow channels. Three oyster farming zones are located along the northwestern shore. The lagoon represents 10% of French oyster production and is the main oyster production center on the Mediterranean. Since 1998, it has experienced recurrent blooms of *Alexandrium catenella* that periodically threaten economic activities.

2.2 Physical variables

The Ifremer observation network provided records of surface water temperature and salinity (monthly means). Over the study period, the number of stations ranged from 2 to 11. Sampling frequency varied from 1 to 8 samples per month. Rainfall values (monthly means) on the watershed were obtained from the National Meteorological Board local station of Sète (Fig. 1). North Atlantic Oscillation (NAO) index values were obtained from http://www.cgd.ucar.edu/cas/jhurrell/indices.data.html#naostatseas.

Western Mediterranean Oscillation (WeMO) index values were obtained from http://www.ub.es/gc/English/wemo.htm. The latter index corresponds to the surface atmospheric pressure difference between Padua (Italy) and San Fernando (Spain). The Thau lagoon being located about half-way between those two points, local weather patterns are likely to be influenced by such pressure differences.

2.3. Nutrients

Samples for ammonium determination were immediately fixed and measured at the laboratory using the method of Koroleff (1976). Nitrate was measured according to Wood et al. (1967), nitrite according to Bendschneider and Robinson (1952), soluble reactive phosphorus (SRP) according to Murphy and Riley (1962), and silicate according to Mullin and Riley (1955). Potential nutrient limitation was assessed according to the criteria of Justic et al. (1995) :

N limitation: DIN < 1µM ; DIN/SRP < 10 ; Si/DIN > 1

P limitation: SRP < 0.1 µM ; DIN/SRP > 22 ; Si/SRP > 22

Si limitation: Si < 2 µM ; Si/SRP < 10 ; Si/DIN < 1

2.4. Biological variables

Chlorophyll a was estimated from 90% acetone extracts and fluorimetry (Holm-Hansen et al. 1965) or spectrofluorometry (Neveux and Lantoine, 1993).

Picophytoplankton abundances were estimated by flow cytometry. From 1990 to 1996, samples were analyzed with a Bruker Spectrospin ACR-1400-SP fitted with a mercury arc lamp and a 365 nm band-pass filter (Vaquer et al. 1996). From 1996 on, a Becton Dickinson FACSCalibur flow cytometer, fitted with a 488 nm laser was used. Samples (1000 µl) were fixed by 2% (final concentration) formaldehyde (Troussellier et al., 1995) and stored in liquid nitrogen. Eukaryotic phytoplankton cells were discriminated on the basis of light diffraction (FSC Forward Scatter, related to cell size) and red fluorescence emissions (chlorophyll *a*, wavelength > 650 nm). Picocyanobacteria were identified by their orange fluorescence emissions (phycoerythrin, 542-585 nm). All samples were analyzed with a mixture of fluorescent beads of 0.96 and 1.8 µm diameter ("Fluoresbrite" YG beads, Polysciences, Inc., Warrington, PA) in order to normalize all parameters and to discriminate pico- and nanophytoplankton. Duplicate subsamples acquisitions were run for 6 min and were performed at a medium rate (25-30 µl min^{-1}). Data were logged using CellQuest software, and analyzed with "AttractorsTM" software (Becton Dickinson, Inc., USA).

The identification of *Synechococcus* was done by electron microscopy.

Nano-microphytoplankton. The REPHY Ifremer network started monitoring the Thau lagoon for phytoplankton cells greater than 10 µm equivalent cell diameter and toxic species in 1987 by optical microscopy. Sampling was carried out at least twice a month at station B. Approximately 900 samples were analyzed for this time series.

3. Results

3.1 Physical variables

Individual water temperature values ranged from 4 to 29 °C. Mean annual values ranged from 13.8 (1980) to 16.7 °C (2003). Over the 33 year period, the increase was significant and about 1.5°C, or 0.045°C/year. But this increase was not evenly distributed among seasons (Fig. 2). On a seasonal basis, the highest rate of increase was in the spring (+ 3.0°C over 33 years), followed by summer (+ 2.0°C) and fall (+1.7°C). In winter, no significant increase over the 33 year period could be found.

Concerning mean monthly values, a significant negative correlation between temperature anomalies and the WeMO index could be found ($r^2 = 0.051$, n=321, $p< 0.01$). The correlation was strongest for the month of May ($r^2 = 0.370$, n=23, $p<0.01$). No overall relationship could be found with the NAO index, but in July, temperature anomalies were positively and significantly correlated with NAO values.

Individual salinity values ranged from 28.5 to 40.0. Mean annual values ranged from 32.6 to 38.0, with no apparent trend over the period, but those values were positively correlated with the NAO winter index in a highly significant way ($r^2= 0.378$, n=31 , $p< 0.01$).

Mean annual rainfall values ranged from 279 mm/year in 1998 to 1310 mm/year in 1990, with no apparent trend over the period. Monthly values ranged from zero in January 1983 to 342 mm/month in October 1979. No relationship could be found with the NAO index. But the rainiest months (> 310 mm/month) were associated with negative WeMO index values.

3.2 Nutrients

Data on ammonium concentrations were fragmentary. From several published data sets (Casellas et al., 1990; Collos et al., 1997, 2005a; Souchu et al., 2001), a seasonal trend could be identified with high (above 5 µM, and up to 10 µM) values in the fall (September to November) and low (less that 2 µM) during the rest of the year. Nitrate ranged between undetectable (< 0.05 µM) and 22 µM, with no long term temporal trend. The nitrate concentrations were closely related to rainfall events (Fig. 3). The Thau ecosystem functions most of the year as a continuous culture with a low background of phytoplankton biomass as Chl a (0.1 – 2 µg/l) growing on regenerated production. Following rainfall events, a nutrient pulse arrives from the watershed in the lagoon and triggers a phytoplankton bloom using nitrate as a N source such as during the November 1993 *Thalassiosira* bloom (Collos et al. 1997). Silicate ranged between 0.4 and 43 µM, with no temporal trend. Soluble reactive phosphorus (SRP) ranged between undetectable and 10 µM over the 30 year period. There was a steady decline in concentrations with time (Fig. 4) due to efforts to reduce eutrophication. Superimposed on this general trend were seasonal trends with SRP peaks in summer due to release from sediment enhanced by temperature (Mazouni et al. 1996). But overall, a large decrease in SRP concentrations occurred over the 30 year period. Summer values decreased from 10 µM to 1 µM while winter values decreased from 3 µM to undetectable (< 0.03 µM) at present.

Individual values of NO3/SRP over 30 years ranged between 0.05 and 27.7 without any apparent trend. There was a clear seasonal trend in DIN/SRP ratios whose range matched long term trends in NO3/SRP ratios. For example, in 1993, low DIN/SRP values (0.4-0.5) were recorded in summer. DIN ranged from 0.4 to 0.5 μM and SRP from 1.0 to 1.1 μM, indicating N limitation according to the criteria of Justic et al. (1995). In winter, high DIN/SRP values (8 to 29) were observed. DIN ranged from 1.7 to 4.6 μM and SRP was around 0.2 μM, indicating no limitation by either N or P according to the same criteria.

3.3 Phytoplankton

Cyanobacteria

Between November 1991 and February 1994 a monthly sampling program recorded mean values between 19 and 192 x 10^3 cells/l (mean from 5 stations in Thau lagoon), but much higher values (around 5000 x 10^3 cells/l) at one reference station in the Mediterranean Sea (Vaquer et al. 1996). By 1998-1999, maximum cell densities reached 400-700 x 10^3 cells/l (mean from 4 stations in Thau lagoon). Cell densities remained around those values until the summer of 2003 when individual values up to 11 x 10^8 cells/l and mean values up to 400 x 10^6 cells/l were observed (Fig. 4). Most of these were of the *Synechococcus* genus, as identified by electron microscopy.

Diatoms

The main planktonic genera present in Thau lagoon were *Asterionellopsis, Chaetoceros, Ditylum, Pseudo-nitzschia, Rhizosolenia, Skeletonema costatum, Thalassionema* and *Thalassiosira*. Mean annual cell densities of *S. costatum* reached a peak of about 2,000 cells/ml in 1996, corresponding to a record low NAO winter index (-3.78). Cell densities then steadily declined over the next 10 years to reach 7 cells/ml in 2005. However, the decline was not evenly distributed and was more pronounced in spring, summer and fall than in winter (Table 1). The other genera did not show such trends.

Alexandrium catenella

First reports of this species were made in 1995 (20,000 cells/l), following 8 years of monitoring without observation of this species. The first toxic event was reported in 1998 (Lilly et al. 2002). Thereafter, maximum cell densities increased with time until 2004, up to 15×10^6 cells/l (Fig. 4), with more pronounced blooms in the fall than in the spring.

4. Discussion

The long term increase in mean annual water temperature is similar to that found in other coastal environments at similar latitudes (Ohtaki et al., 1992 ; Soletchnik et al., 1998; Goffart et al., 2002). For example, in the Seto Inland Sea, the annual increases in air and water temperature were 0.07 and 0.06 °C/year respectively (Ohtaki et al., 1992) over the period 1970-1989. However, it is not so much mean annual values rather than values recorded over smaller time scales such as the seasonal ones (Fig. 2), that appear to be important for phytoplankton, as will become evident below.

Mean annual salinity was correlated with the NAO winter index in a highly significant way. However, neither mean annual or seasonal water temperature, nor rainfall were correlated with NAO. There was no correlation between rainfall and salinity, indicating that it is probably a balance between evaporation, rainfall and exchanges with the Mediterranean Sea that controls salinity in Thau lagoon, or that rainfall data are not representative of the situation in the watershed. Possibly, the low inertia of this shallow environment (mean depth = 4 m) regarding water temperature makes it less sensitive to farfield (climate) influences and more dependent on local weather conditions. The correlation between temperature anomalies and the WeMO index values, similar to that found in the Gulf of Valencia (Martin-Vide and Lopez-Bustins, 2006), supports this possibility.

Ammonium data were too fragmentary to be used on the long term. From results of a seasonal study with monthly sampling, ammonium could be related to the phaeophytin a/chlorophyll a ratio (Collos et al., 2005a) and the relationship was interpreted as a general decomposition of phytoplankton communities during fall. The lack of continuous ammonium data also prevented us from using the DIN/SRP ratio, specially as ammonium could represent 100% of DIN at times. In as much as *A. catenella* is known to use urea and possibly other organic N compounds as N sources (Carlsson et al., 1998; Dyhrman and Anderson, 2003; Collos et al., 2004; Jeong et al. 2005), as well as organic P (Matsuda et al., 1999), the use of the N/P ratio under the traditional form of DIN/SRP does not seem applicable to this species or other phytoplankton species that are not strict autotrophs. Irmish (1991) has shown that adding urea to the DIN could change the N/P ratios in the Baltic Sea by a factor of 3 to 7.

Probably the most useful data set is the one shown in Fig. 4, where low to undetectable SRP concentrations coincided with the appearance of picocyanobacteria (around 1994) and *A. catenella* (around 1995). The decrease in SRP was due mainly to the implementation of sewage treatment plants in the 1970s (La Jeunesse and Elliott, 2004). Phosphorus stored in shellfish represented about 20% of total inputs by human activities, and the contribution of shellfish to limiting eutrophication is probably maximal due to current legal limits on the area allocated to shellfish farming (La Jeunesse and Elliott, 2004). The simultaneous oligotrophication and appearance of *A. catenella* blooms in Thau lagoon bear a striking similarity to events in the Seto Inland Sea where, citing Anderson et al. (1992) : « as the waters became less eutrophic and large biomass blooms decreased, there was a shift in species composition, leading to a greater prevalence of some that are responsible for shellfish poisonings in humans, such as *Alexandrium tamarense* and *A. catenella* ».

Among a series of marine Mediterranean lagoons that can be ordered along a eutrophication gradient characterized by chlorophyll a and total phosphorus (Fig. 5), Thau appears to be one of the less eutrophied ones, yet harbors a toxic dinoflagellate. Biogeochemical aspects of this eutrophication gradient are being treated elsewhere (Souchu et al., in preparation). A similar situation occurs in another local lagoon, Leucate, that is even less eutrophied than Thau, but in which the toxic dinoflagellate *Dinophysis acuminata* blooms recurrently (Le Bec et al., 1997). Thus it seems, at least from those two examples, that HABs are not related to eutrophication of the Mediterranean coastal zone.

This led us to examine climate change as a possible driver of the recent development of HABs in Thau lagoon. Concerning *A. catenella*, the occurrence of several blooms between 2000 and 2003 led us to look for a relationship between environmental variables and cell densities. The relationship with surface temperature is shown in Fig. 6. It can be seen from these data that, for a bloom to occur, there has to be a period during which surface temperature remains near 20°C (± 1°C) and wind speed below 2-3 $m.s^{-1}$. When the water temperature increases rapidly in the spring, as in 2001, no bloom develops. The same phenomenon occurs in the fall if the water temperature decreases too rapidly, as in 2002. Therefore, the general long term increase in water temperature in Thau does not seem to have a direct effect on *A. catenella,* since it is a physical window, identified so far by water temperatures near 20°C, that provides conditions for bloom development. This variable may also act as a proxy for some other variable such as water column stability or turbulence. Regarding the sensitivity of *Alexandrium catenella* to turbulence, results differ among investigators (Sullivan et al., 2003; Collos et al., 2004; Bolli et al., 2007) probably reflecting differences in experimental conditions. Our data indicate that wind events could act either directly on growth rate or dissipate the bloom by advection.

Concerning picocyanobacteria, there seems to be an effect of temperature on their development. As shown in Fig. 7, while relatively low summer cell densities (below 100 cells/µl) were recorded before 2003, they increased about 10 times during the summer of 2003 when temperatures reached 28°C in August - the highest values recorded for the last 33 years. Then in 2004, summer temperatures went back to below 25°C and picocyanobacterial cell densities also decreased to previous levels. In 2006, the increasing trend was reproduced. Hence, there appears to be a clear threshold effect of temperature on picocyanobacterial abundance in Thau lagoon. These results are consistent with previous findings that showed picocyanobacterial gross growth rates in Thau Lagoon are related to water temperature in a significant way (Bec et al., 2005).

Another factor possibly contributing to picocyanobacterial development is the lowered SRP concentration in recent years. Table 2 summarizes a literature review of half-saturation constants (Ks) for SRP uptake by several classes of phytoplankton. Such comparisons are fraught with difficulties : apart from differences in experimental conditions, one problem is the possibility of multiphasic uptake of SRP that is common in unicellular algae (Jeanjean et al., 1970; Rivkin and Swift, 1982; Jansson, 1993). As mentioned by Rivkin and Swift (1982), “Direct curve fitting might not discriminate between the various phases and would therefore overestimate both the Km and Vmax”. In order to minimize those risks, and retain the phase of uptake of ecological significance, we have selected those studies where the SRP addition was 20 µM at most. A second selection was done by rejecting studies using P sufficient cells. When raw data were available, and visual inspection revealed multiphasic kinetics, Ks were recalculated as in Collos et al. (2005b). When several Ks values were obtained for one species (at several dilution rates for example), only the average value was retained in order to weigh each species equally. The data set obtained is not exhaustive, but is probably representative of the published studies on that topic. Freshwater and marine data were pooled. For the Chlorophyceae, freshwater species represented 95% of the data. For the Cyanophyceae, there were 8 marine studies and 12 freshwater ones, without significant differences in Ks. However, Ks of freshwater *Synechococcus* (mean of 0.17 µM for n=6) were much lower than those of marine *Synechococcus* (mean of 0.94 µM for n=4). This difference comes mainly from the high Ks value for *Synechococcus* WB7803 (Donald et al. 1997) which may be due to methodological biases since more recent studies indicate Ks as low as 0.13 µM for the same strain (Scanlan, 2003). Recent evidence from natural populations of *Synechococcus* from the Mediterranean (Moutin et al., 2002; Tanaka et al., 2003) indicate Ks values for SRP uptake lie between 0.02 and 0.05 µM.

For some members of the phytoplankton, the number of cases documented in Table 2 is clearly not sufficient to draw meaningful conclusions. But for at least the first four classes, it is clear that cyanobacteria, and more particularly picocyanobacteria such as *Synechococcus*, appear to be well equipped to scavenge low levels of SRP from the environment.

The development of picocyanobacteria upon or following decreases in SRP has also been reported from other aquatic environments. For example, in Lago Maggiore (Italy), autotrophic picoplankton, mainly *Synechococcus*, developed following oligotrophication of the lake (Ruggiu et al., 1998; Callieri and Piscia, 2002). In Saidenbach Reservoir (Germany), the decrease in SRP induced a development of cyanobacteria in summer at the expense of diatoms (Horn, 2003). In both cases, the maximum SRP levels during the eutrophication phase were much lower than those experienced in Thau (1 and 0.5 µM respectively in Lago Maggiore and Saidenbach Reservoir), but the levels corresponding to cyanobacteria development were similar to those in Thau lagoon (0.25 and 0.07 µM, respectively).

The decrease in mean annual cell numbers of *S. costatum* (Fig. 4) in Thau lagoon over the last ten years is particularly striking as it ranges over almost 3 orders of magnitude (from

2,600 down to 7 cells/ml) and it is concurrent with the increase in picocyanobacteria and *A. catenella*. Those opposing trends suggest species interactions between *S. costatum* and either picocyanobacteria or *A. catenella*. For example, in 1993, *S. costatum* was dominant in June and N uptake measurements showed that it was well adapted to the nutrient regime (Collos et al. 2003). However, the cell density of this species progressively decreased from 1996 on (Fig. 4), but the decrease was not evenly spread among seasons. As shown in Table 1, during the picocyanobacteria "explosive" phase (1999 on), the decrease in *S. costatum* was most pronounced in spring (significant decrease, $p<0.05$), summer (significant decrease, $p<0.05$) or fall, but not in winter. In as much as picocyanobacteria develop mostly in summer, it can be suggested that they displaced *S. costatum* during that season. In spring and fall, which are seasons during which *A. catenella* blooms, this probably also contributed to the displacement of *S. costatum* by taking up dissolved inorganic nutrients that were common ressources to both species. The decrease in SRP therefore seems to have "closed" a niche (defined as a resource, sensu Smayda, 2002, and here defined as SRP concentrations) to diatoms such as *S. costatum*. Picocyanobacteria were able to outcompete and displace this previously dominant species by their ability to exploit low SRP levels (Table 2). This situation is somewhat similar to that in Saidenbach Reservoir (Horn, 2003) where the diatom *Fragilaria crotonensis* was displaced by cyanobacteria when SRP levels fell below 0.1 µM.

Thus, the combination of two main controlling factors, temperature and SRP, that are evolving in opposite directions could help explain the appearance and development of picocyanobacteria such as *Synechococcus* in Thau lagoon.

Finally, there may be a trophic link that could also contribute to the quasi simultaneous appearance of both picocyanobacteria and *A. catenella* in this environment. It was recently shown that 17 species of dinoflagellates (including *A. catenella*) were able to graze upon the picocyanobacterium *Synechococcus* (Jeong et al., 2005). Comparisons of N-based growth and

N uptake rates by *A. catenella* (Collos et al., 2007) indicate that this species grew mainly on ammonium and urea as N sources, but an unknown N source was periodically important and had to be taken into account to support the observed growth rates. Particulate N in the form of picocyanobacteria could therefore provide this supplement in limiting nutrient to *A. catenella* and give it an ecological advantage over strictly autotrophic phytoplankton. Independently of the direction of change in trophic status generally invoked, such a scenario is compatible with the habitat disturbance hypothesis (Smayda, 2002) leading to HAB occurrences.

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References

Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries 25, 704-726.

Bec, B., Husseini-Ratrema, J., Collos, Y., Souchu, P., Vaquer, A., 2005. Phytoplankton seasonal dynamics in a Mediterranean coastal lagoon: emphasis on the picoeukaryote community. J. Plankton Res. 27, 881-894

Bendschneider, K., Robinson, R.J., 1952. A new spectrophotometric method for the determination of nitrite in seawater. J. Mar. Res. 11, 87-96.

Bolli, L., Llaveria, G., Garcés, E., Guadayol, O., van Lenning, K., Peters, F., Berdalet, E., 2007. Modulation of ecdysal cyst and toxin dynamics of two Alexandrium (Dinophyceae) species under small-scale turbulence. Biogeosciences Discuss. 4, 893-908.

Cadée, G.C., Hegeman, J., 2002. Phytoplankton in the Marsdiep at the end of the 20th century: 30 years monitoring biomass, primary production, and *Phaeocystis* blooms. J. Sea Res. 48, 97-110.

Callieri, C, Piscia, R., 2002. Photosynthetic efficiency and seasonality of autotrophic picoplankton in Lago Maggiore after its recovery. Freshwater Biol. 47, 941-956.

Casellas, C., Pena, G., Picot, B., Illes, S., Bontoux, J., 1990. Nutrients in Thau lagoon: grouping of contiguous geographical zones. Water Res. 24, 1479-1489.

Cembella, A.D., Antia, N.J., Harrison, P.J., 1984. The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae: a multidisciplinary perspective: part 1. Crit. Rev. Microbiol. 10, 317-391.

Cloern, J.E., 2001. Our evolving conceptual model of the coastal eutrophication problem. Mar. Ecol. Progr. Ser. 210, 223-253.

Collos, Y., Vaquer, A., Bibent, B., Slawyk, G., Garcia, N., Souchu, P., 1997. Variability in nitrate uptake kinetics of phytoplankton communities in a Mediterranean coastal lagoon. Estuar. Coastal Shelf Sci. 44, 369-375.

Collos, Y., Vaquer, A., Bibent, B., Souchu, P., Slawyk, G., Garcia, N., 2003. Responses of coastal phytoplankton to ammonium and nitrate pulses: seasonal variations of uptake and regeneration of nitrogenous nutrients. Aquatic Ecology 37, 227-236.

Collos, Y., Gagne, C., Laabir, M., Vaquer, A., Cecchi, P., Souchu, P., 2004. Nitrogenous nutrition of *Alexandrium catenella* (Dinophyceae) in cultures and in Thau lagoon, southern France. J. Phycol. 40, 96-103.

Collos, Y., Husseini-Ratrema, J., Bec, B., Vaquer, A., Lam Hoai, T., Rougier, C., Pons, V., Souchu, P., 2005a. Pheopigment dynamics, zooplankton grazing rates and the autumnal ammonium peak in a Mediterranean lagoon. Hydrobiol. 550, 83-93

Collos, Y., Vaquer, A., Souchu, P., 2005b. Acclimation of nitrate uptake by phytoplankton to high substrate levels. J. Phycol. 41, 466-478.

Collos, Y., Lespilette, M., Vaquer, A., Laabir, M., Pastoureaud, A., 2006. Uptake and accumulation of ammonium by *Alexandrium catenella* during nutrient pulses. Afr. J. Mar. Sci. 28, 313-318.

Collos, Y., Vaquer, A., Laabir, M., Abadie, E., Laugier, T., Pastoureaud, A., Souchu, P., 2007. Contribution of several nitrogen sources to growth of *Alexandrium catenella* during blooms in Thau lagoon, Southern France. Harmful Algae 6, 781-789.

Donald, K.M., Scanlan, D.J., Mann, N.H., Joint, I., 1997. Comparative phosphorus nutrition of the marine cyanobacterium Synechococcus WH7803 and the marine diatom *Thalassiosira weissflogii*. J. Plankton Res. 19, 1793-1813.

Doremus, C, 1982. Geochemical control of dinitrogen fixation in the open ocean. Biol. Oceanogr. 1, 429-436.

Duarte, C.M., 1995. Submerged aquatic vegetation in relation to different nutrient regimes. Ophelia 41, 87-112.

Fu, F.-X., Zhang, Y., Bell, P.R.F., Hutchins, D.A., 2005. Phosphate and growth kinetics of *Trichodesmium* (Cyanobacteria) isolates from the Northe Atrlantic Ocean and the Great Barrier Reef, Australia. J. Phycol. 41, 62-73.

Fu, F.-X., Zhang, Y., Feng, Y., Hutchins, D.A., 2006. Phosphate and ATP uptake and growth kinetics in axenic cultures of the cyanobacterium *Synechococcus* CCMP 1334. Eur. J. Phycol. 41, 15-28.

Goffart, A., Hecq, J.H., Legendre, L., 2002. Changes in the development of the winter-spring phytoplankton bloom in the Bay of Calvi (NW Mediterranean) over the last two decades: a response to changing climate ? Mar. Ecol. Prog. Ser. 236, 45-60.

Holm-Hansen, O., Lorenzen, C.J., Holmes, R.W., Strickland, J.D.H., 1965. Fluorometric determination of chlorophyll. J. Cons. Perm. Int. Explor. Mer 30, 3-15.

Horn, H., 2003. The relative importance of climate and nutrients in controlling phytoplankton growth in Saidenbach Reservoir. Hydrobiol. 504, 159-166.

Imai, I., Yamaguchi, M., Hori, Y., 2006. Eutrophication and occurrences of harmful algal blooms in the Seto Inland Sea, Japan. Plankton Benthos Research 1, 71-84.

Irmisch, A., 1991. Investigations on the urea uptake by phytoplankton in the Baltic sea. Acta Hydrochim. Hydrobiol. 19, 39-44.

Isvanovics, V., Shafik, H.M., Présing, M., Juhos, S., 2000. Growth and phosphate uptake kinetics of the cyanobacterium, *Cylindrospermopsis raciborskii* (Cyanophyceae) in throughflow cultures. Freshwater Biol. 43, 257-275.

Jansson, M., 1993. Uptake, exchange, and excretion of orthophosphate in phosphate-starved *Scenedesmus quadricauda* and *Pseudomonas* K7. Limnol. Oceanogr. 38, 1162-78.

Jeanjean, R., Blasco, F., Gaudin, C., 1970. Etude des mécanismes d'absorption de l'ion phosphate par les Chlorelles. C. R. Acad. Sci. (Paris) 270, 2946-2949.

Jeong, H.J., Park, J.Y., Nho, J.H., Park, M.O., Ha, J.H., Seong, K.A., Jeng, C., Seong, C.N., Lee, K.Y., Yih, W.H., 2005. Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. Aquatic Microb. Ecol. 41, 131-143.

Justic, D., Rabalais, N.N., Turner, R.E., Dortch, Q., 1995. Changes in nutrient structure of river-dominated coastal waters – stoichiometric nutrient balance and its consequences. Estuar. Coast. Shelf Sci. 40, 339-356.

Koroleff, F. 1976. Determination of nutrients. In: Grasshoff, K. (Ed.), Methods of Seawater

Analysis, Verlag Chemie, Weinheim, pp. 117-182.

Kromkamp, J., Van den Heuvel, A., Mur, L.R., 1989. Phosphorus uptake and photosynthesis by phosphate-limited cultures of the cyanobacterium *Microcystis aeruginosa*. Br. Phycol. J. 24, 347-355.

La Jeunesse, I., Elliott, M., 2004. Anthropogenic regulation of the phosphorus balance in the Thau catchment-coastal lagoon system (Mediterranean Sea, France) over 24 years. Mar. Poll. Bull. 48, 679-687.

Le Bec, C., Belin, C., Gaertner, J.C., Beliaeff, Raffin, B., Ibanez, F., 1997. Séries temporelles du réseau de surveillance du phytoplancton (REPHY). Etude de deux zones de la côte Ouest Méditerranée. Oceanol. Acta 20, 101-108.

Lehman, J. T., Botkin, D.B., Likens, G.E., 1975. The assumptions and rationales of a computer model of phytoplankton population dynamics. Limnol. Oceanogr. 20, 343-364.

Lilly, E.L., Kulis, D.M., Gentien, P., Anderson, D.M., 2002. Paralytic shellfish poisoning toxins in France linked to a human-introduced strain of *Alexandrium catenella* from the western Pacific: evidence from DNA and toxin analysis. J. Plankton Res. 24, 443-452.

Martin-Vide, J., Lopez-Bustins, J.A., 2006. The Western Mediterranean Oscillation and rainfall in the Iberian Peninsula. Int. J. Climat. 26, 1455-1475.

Maso, M., Garcés, E., 2006. Harmful microalgae blooms (HAB); problematic and conditions that induce them. Mar. Poll. Bull. 53, 620-630.

Matsuda, A., Nishijima, T., Fukami, K., 1999. Effects of nitrogenous and phosphorus nutrients on the growth of toxic dinoflagellate *Alexandrium catenella*. Nippon Suisan Gakkaishi 65, 847-855.

Mazouni, N., Gaertner, J.C., Deslous-Paoli, J.M., Landrein, S., Geringer d'Oedenberg, M., 1996. Nutrient and oxygen exchanges at the water-sediment in a shellfish farming lagoon (Thau, France). J. Exp. Mar. Biol. Ecol. 205, 91-113.

Moutin, T., Thingstad, T.F., Van Wambeke, F., Marie, D., Slawyk, G., Raimbault, P., Claustre, H., 2002. Does competition for nanomolar phosphate supply explain the predominance of the cyanobacterium *Synechococcus* ? Limnol. Oceanogr. 47: 1562-1567.

Moutin, T., Van Den Broeck, N., Beker, B., Dupouy, C., Rimmelin, P., Le Bouteiller, A., 2005. Phosphate availability controls *Trichodesmium* spp. biomass in the SW Pacific Ocean. Mar. Ecol. Prog. Ser. 297, 15-21.

Mullin, J.B., Riley, J.P., 1955. The colorimetric determination of silicate with special reference to sea and natural waters. Anal. Chim. Acta 12, 162-176.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31-36.

Nalewajko, C., Lean, D.R.S., 1980. Phosphorus. In: Morris, I. (Ed.), The physiological ecology of phytoplankton. University of California Press, Berkeley, CA, pp. 235-258.

Neveux, J., Lantoine, F., 1993. Spectrofluorometric assay of chlorophylls and phaeopigments using the least squares approximation technique. Deep-Sea Res. 40, 1747-1765.

Ohtaki, E., Yamashita, E., Fujiwara, F., 1992. Atmospheric carbon-dioxide variations at coastal site, Sibukawa, in Seto Inland Sea, Japan. 2. Observation from 1980 to 1989. Theor. Appl. Climatol. 45, 177-181.

Philippart, C.J.M., Beukema, J.J., Cadée, G.C., Dekker, R., Goedhart, P.W., Van Iperen, J.M., Leopold, M.F., Herman, P.M.J., 2007. Impacts of nutrient reduction on coastal communities. Ecosystems 10, 95-118.

Rivkin, R. B., Swift, E., 1982. Phosphate uptake by the oceanic dinoflagellate *Pyrocystis noctiluca*. J. Phycol. 18, 113-120.

Ruggiu, D., Morabito, G., Panzani, P., Pugnetti, A., 1998. Trends and relations among basic phytoplankton characteristics in the course of the long-term oligotrophication of Lake Maggiore (Italy). In: Alvarez-Cobelas, M., Reynolds, C.S., Sanchez-Castillo, P., Kristiansen,

J. (Eds.), Phytoplankton and trophic gradients. Hydrobiol. 369/370, 243-257.
Scanlan, D.J., 2003. Physiological diversity and niche adaptation in marine *Synechococcus*. Adv. Microb. Ecol. 47, 1-64.
Schramm, W., 1999. Factors influencing seaweed responses to eutrophication: some results from EU-project EUMAC. J. Appl. Phycol. 11, 69-78.
Sellner, K.G., Doucette, G.J., Kirkpatrick, G.J., 2003. Harmful algal blooms: causes, impacts and detection. J. Ind. Microbiol. Biotechnol. 30, 383-406.
Smayda, T.J., 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol. Oceanogr. 42, 1137-1153.
Smayda, T.J., 2002. Adaptive ecology, growth strategies and the global bloom expansion of dinoflagellates. J. Oceanography 58, 281-294.
Smith, V.H., 2006. Responses of estuarine and coastal marine phytoplankton to nitrogen and phosphorus enrichment. Limnol Oceanogr 51, 377-384.
Souchu, P., Gasc, A., Collos, Y., Vaquer, A., Tournier, H., Bibent, B., Deslous-Paoli, J.M., 1998. Biogeochemical aspects of bottom anoxia in a Mediterranean lagoon (Thau, France). Mar. Ecol. Prog. Ser. 164, 135-146.
Souchu, P., Vaquer, A., Collos, Y., Landrein, S., Deslous-Paoli, J.M., Bibent, B., 2001. Influence of shellfish farming activities on the biogeochemical composition of the water column in Thau lagoon. Mar. Ecol. Prog. Ser. 218, 141-152.
Sullivan, J.M., Swift, E., Donaghay, P.L., Rines, J.E.B., 2003. Small-scale turbulence affects the division rate and morphology of two red-tide dinoflagellates. Harmful Algae 2, 183-199.
Tanaka, T., Rassoulzadegan, F., Thingstad, T.F. 2003. Measurements of phosphate affinity constants and phosphorus release rates from the microbial food web in Villefranche Bay, northwestern Mediterranean. Limnol. Oceanogr. 48, 1150-1160.

Taylor, F.J.R., Haigh, R., Sutherland, T.F., 1994. Phytoplankton ecology of Sechelt Inlet, a fjord system on the British Columbia coast. II. Potentially harmful species. Mar. Ecol. Progr. Ser. 103, 151-164.

Troussellier, M., Courties, C., Zettelmaier S., 1995. Flow cytometric analysis of coastal lagoon bacterioplankton and picophytoplankton: fixation and storage effects. Estuar. Coast. Shelf Sci. 40, 621-633.

Vadstein, O.A., Olsen, Y., 1989. Chemical composition and phosphate uptake kinetics of limnetic bacterial communities cultured in chemostats under phosphorus limitation. Limnol. Oceanogr. 34, 1989, 939-946.

Vaquer, A., Troussellier, M., Courties, C., Bibent, B., 1996. Standing stock and dynamics of picophytoplankton in the Thau lagoon (northwest Mediterranean coast). Limnol. Oceanogr. 41, 1821-1828.

Wood, E.D., Armstrong, F.A.J., Richard, F.A., 1967. Determination of nitrate in sea water by cadmium copper-reduction to nitrite. J. Mar. Biol. Ass. U.K. 47, 23-31.

Yamamoto, T., Tarutani, K., 1996. Growth and phosphate uptake kinetics of *Alexandrium tamarense* from Mikaya Bay, Japan. In: Yasumoto, T., Oshima, Y., Fukuyo, Y. (Eds.), Harmful and toxic algal blooms. IOC/UNESCO, Paris, pp. 293-296.

Yamamoto, T., Tarutani, K., 1999. Growth and phosphate uptake kinetics of the toxic dinoflagellate *Alexandrium tamarense* from Hiroshima Bay, Japan. Phycol. Res. 47, 27-32.

Table 1. Seasonal trends in mean cell densities (10^6 cells/l) at different seasons for *Skeletonema costatum* in Thau lagoon. Surface samples from station B. Numbers in parentheses denote number of observations. Maximal values in bold.

Year	WINTER		SPRING		SUMMER		FALL	
1999	0.12	(9)	**3.2**	(8)	0.22	(6)	**0.07**	(7)
2000	1.01	(9)	1.2	(7)	**0.49**	(10)	0.00	(6)
2001	0.27	(6)	0.5	(7)	0.13	(6)	0.03	(7)
2002	0.26	(6)	0.16	(7)	0.27	(7)	0.07	(7)
2003	0.01	(7)	0.003	(6)	0.003	(6)	0.02	(7)
2004	0.04	(6)	0.08	(8)	0.013	(7)	0.002	(6)
2005	0.003	(7)	0.0002	(6)	0.014	(7)	0.014	(6)
2006	**2.28**	(7)	0.001	(7)	0.012	(6)	0.0006	(7)

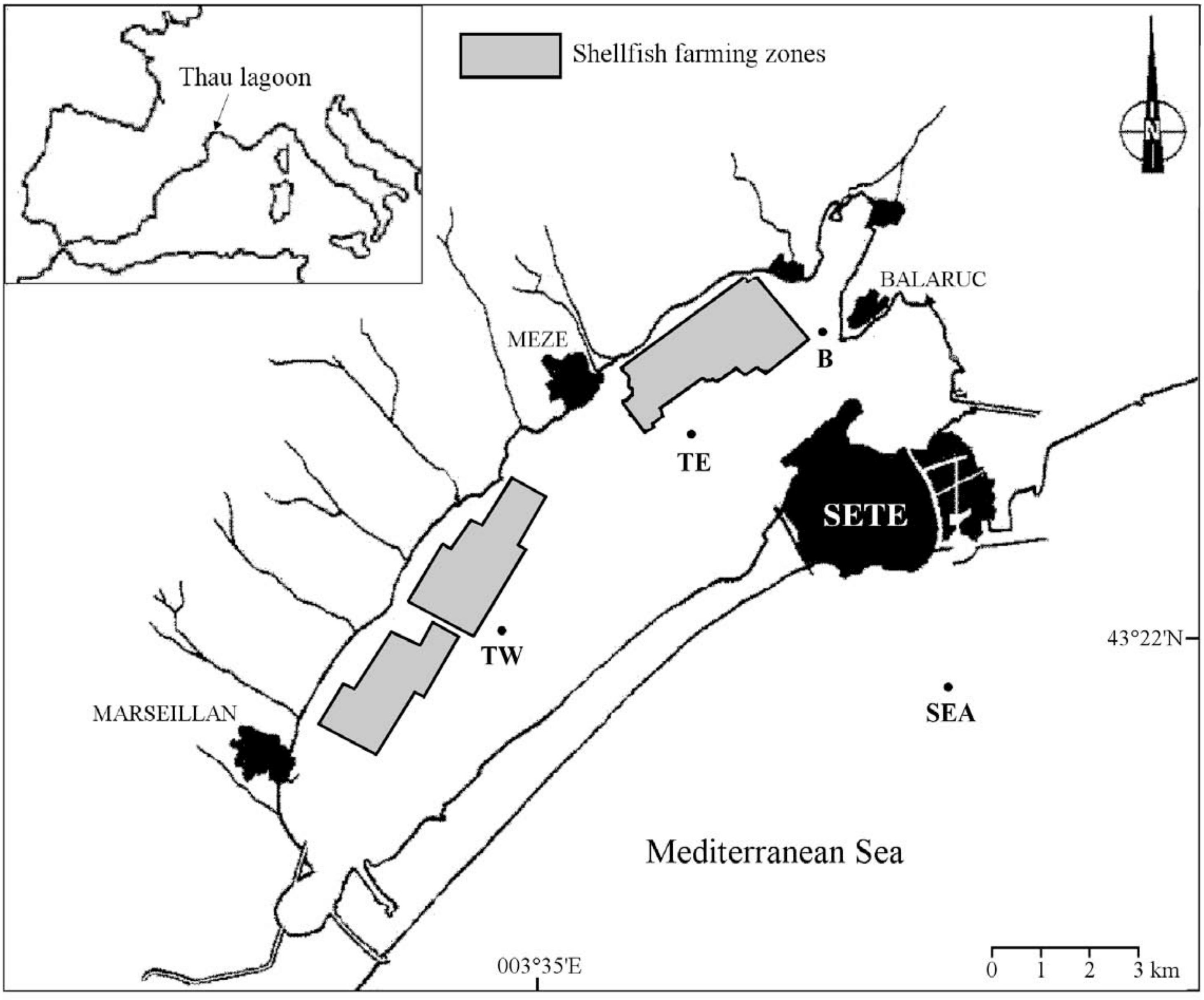
Table 2. Half-saturation constants for soluble reactive phosphorus (K_{SRP}) during uptake by unicellular algae. Data from compilations by Lehman et al. (1975), Nalewajko and Lean (1980), Doremus (1982), Cembella et al. (1984), Vadstein & Olsen (1989), Donald et al. (1997), Smayda (1997b) and Fu et al. (2006), with additional data from Fu et al. (2005), Kromkamp et al. (1989), Isvanovics et al. (2000), Moutin et al. (2002, 2005), Yamamoto & Tarutani (1996, 1999). Data selected for maximum SRP additions of 20 µM. « All » refers to both nutrient depleted and nutrient replete conditions.

Class	conditions	n	K_{SRP}
Chlorophyceae	All	18	2.5
	P deficient	13	2.6
Cyanophyceae	All	29	0.7
	P deficient	20	0.7
Diatomophyceae	All	24	1.3
	P deficient	19	1.3
Dinophyceae	All	13	1.8
	P deficient	8	2.4
Euglenophyceae	All	5	4.6
Prymnesiophyceae	All	3	1.3
Raphidophyceae	All	3	1.7

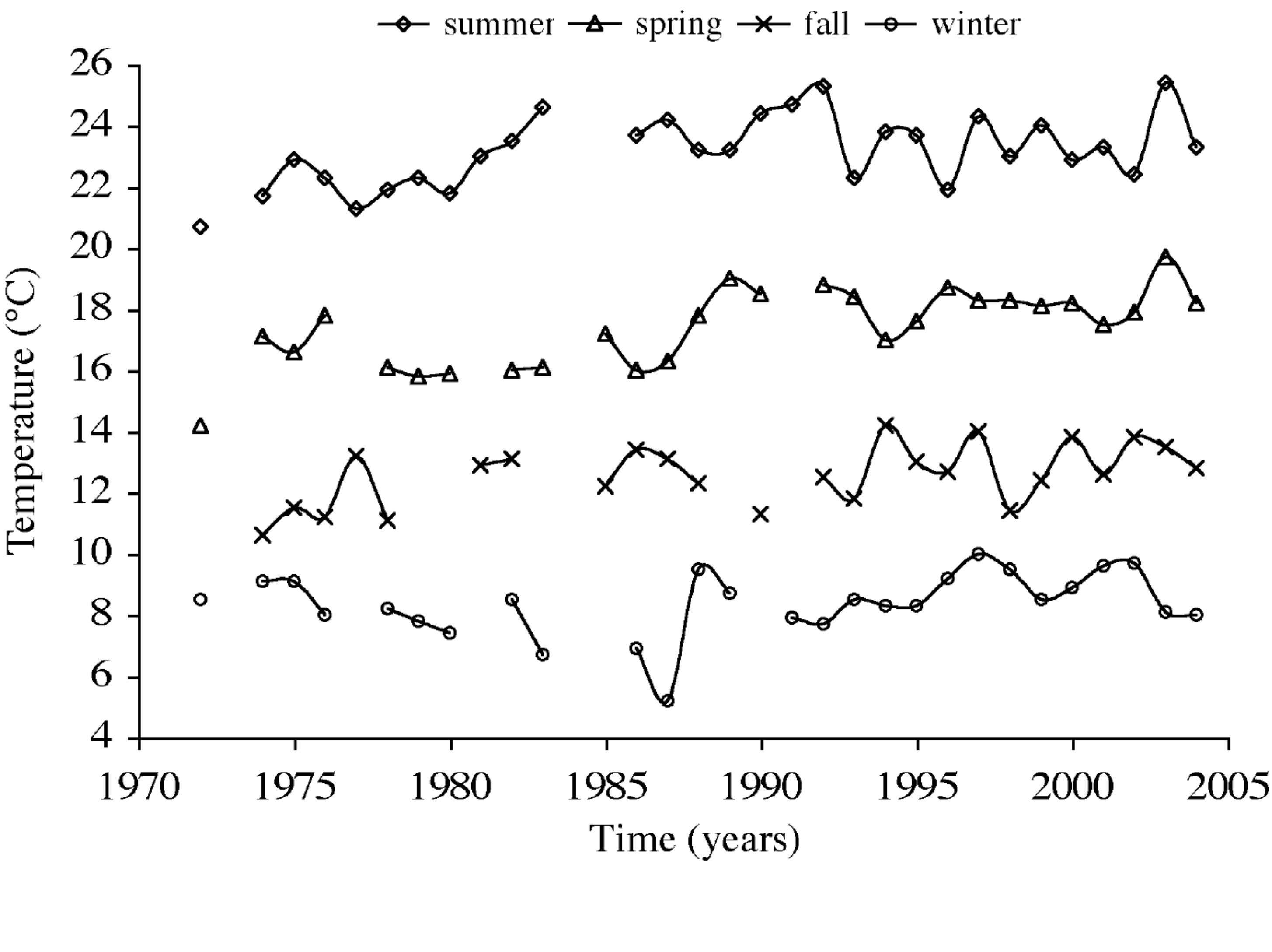
Figure captions

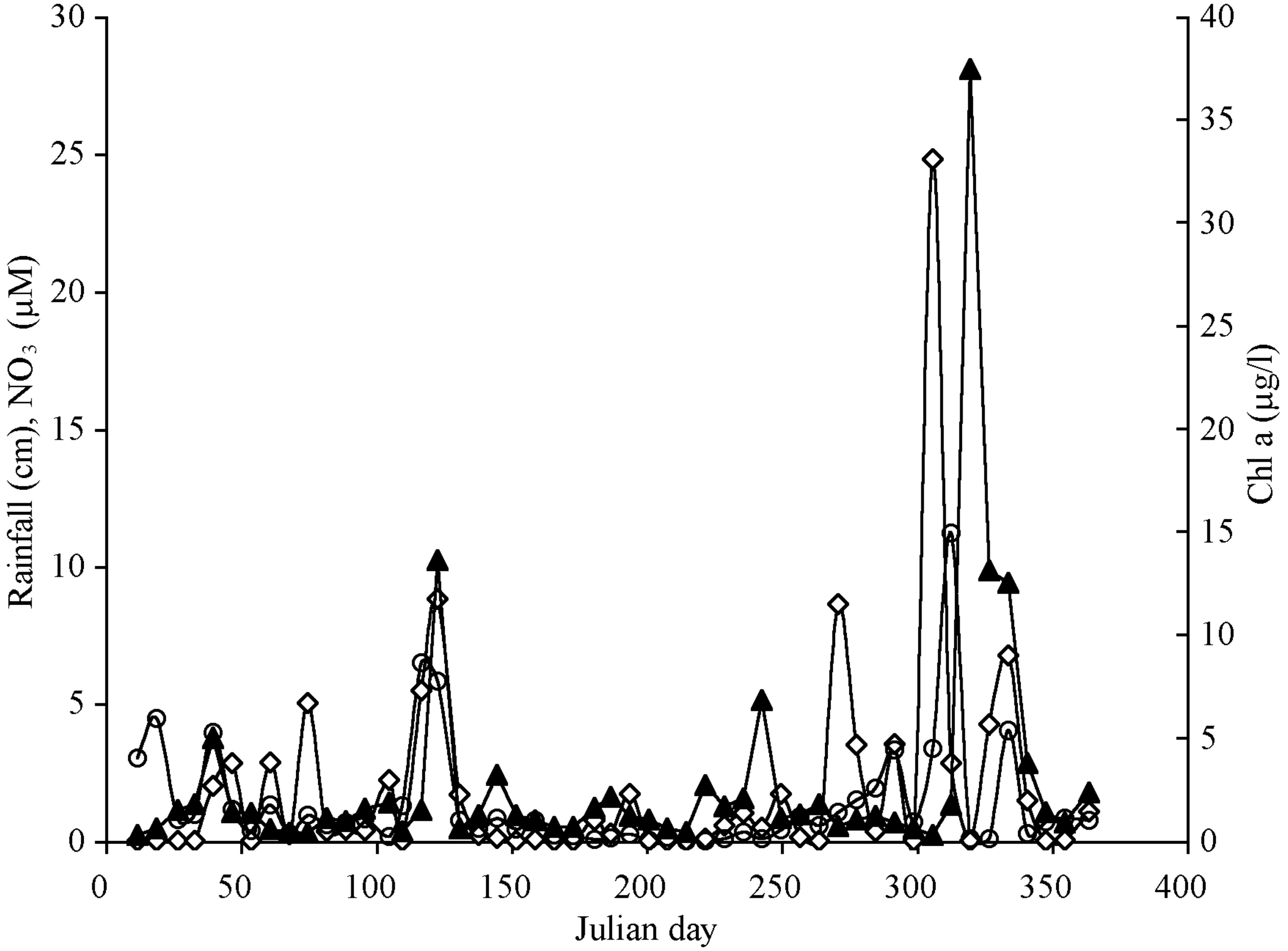
1. Study site and station locations. Urban areas in black, shellfish farming areas in grey.
2. Mean seasonal surface water temperature (°C) in Thau lagoon as a function of time. Average values from between 2 and 11 stations depending on year.
3. Rainfall events, nitrate and chlorophyll a concentrations in Thau lagoon in 1993. Open diamonds: rainfall (cm); open circles: nitrate (µM); black triangles: chlorophyll a (µg/l)
4. Soluble reactive phosphorus (µM; black squares), *Synechococcus* (*Syne*, 10^8 cells/l; open circles), *Alexandrium catenella* (*A. cat*, 10^6 cells/l; open triangles) and *Skeletonema costatum* (*S. cost*, 10^5 cells/l; diamonds, no data before 1987) cell densities.
5. Chlorophyll a concentration (Chl-a) vs. total phosphorus concentration (TP) in 23 French Mediterranean lagoon waters. Values correspond to medians of pooled summer values (June, July and August) from 1998 to 2003 (From Souchu et al., in prep) for the Ifremer network "Réseau de Suivi Lagunaire" (http://www.rsl.cepralmar.com). Field observations allowed lagoons to be grouped according to the phanerogam-macroalgae and/or phytoplankton succession (Duarte, 1995; Schramm, 1999). The least eutrophicated lagoons correspond to transparent waters and a dominance of climax species such as phanerogams (stars). The next group still includes climax species but also proliferating macroalgae (squares). The next higher eutrophication level leads to the disappearance of climax species but proliferating macroalgae can still develop (diamonds). The final stage corresponds to the quasi-exclusive dominance of phytoplankton (triangles). Symbols within circles represent lagoons in which dinoflagellate HAB events have been recorded.

6. Water temperature and *Alexandrium catenella* blooms occurring in spring (SPR) and in fall (FAL) from 2000 to 2003. Bloom events are shown as thick black horizontal lines. A: Spring events (Julian days 91 to 181, i.e. April to June); B: Fall events (Julian days 244 to 334, i.e. September to November).
7. Mean *Synechococcus* cell densities (10^6 cells/l; bars) and mean water temperature (°C; lines with circle symbol) in summer (2001-2006) at stations TE (black symbol) and TW (open symbol).

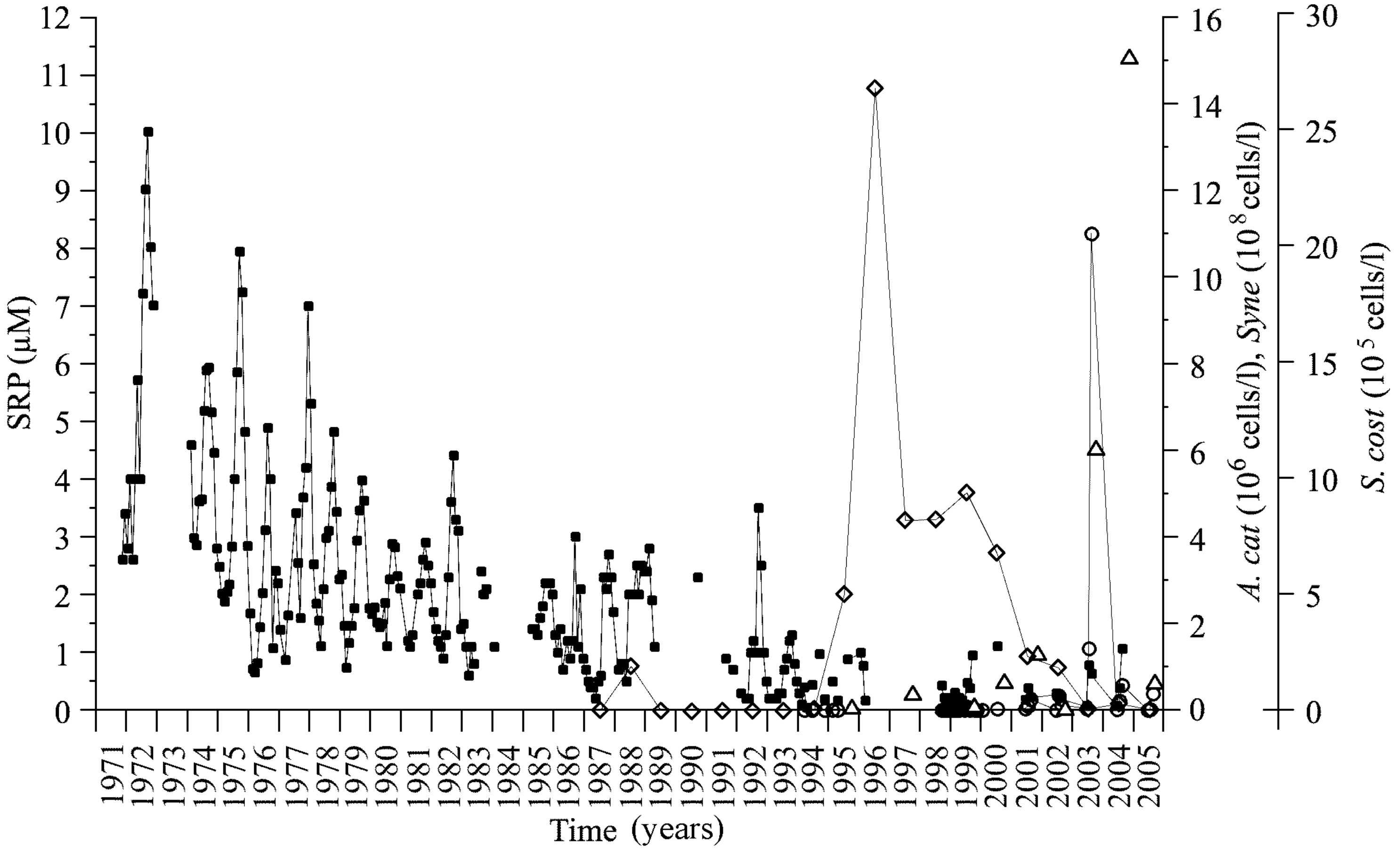


Shellfish farming zones
Thau lagoon
MEZE
BALARUC
B
TE
SETE
TW
MARSEILLAN
SEA
43°22'N
Mediterranean Sea
003°35'E
0
1
2
3 km


summer
spring
fall
winter
Temperature (°C)
26
24
22
20
18
16
14
12
10
8
6
4
1970
1975
1980
1985
1990
1995
2000
2005
Time (years)



30
25
20
15
10
5
0
40
35
30
25
20
15
10
5
0
0
50
100
150
200
250
300
350
400
Julian day
Rainfall (cm), NO3 (μM)
Chl a (μg/l)


SRP (µM)
0
1
2
3
4
5
6
7
8
9
10
11
12
A. cat (10^6 cells/l), Syne (10^8 cells/l)
0
2
4
6
8
10
12
14
16
S. cost (10^5 cells/l)
0
5
10
15
20
25
30
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
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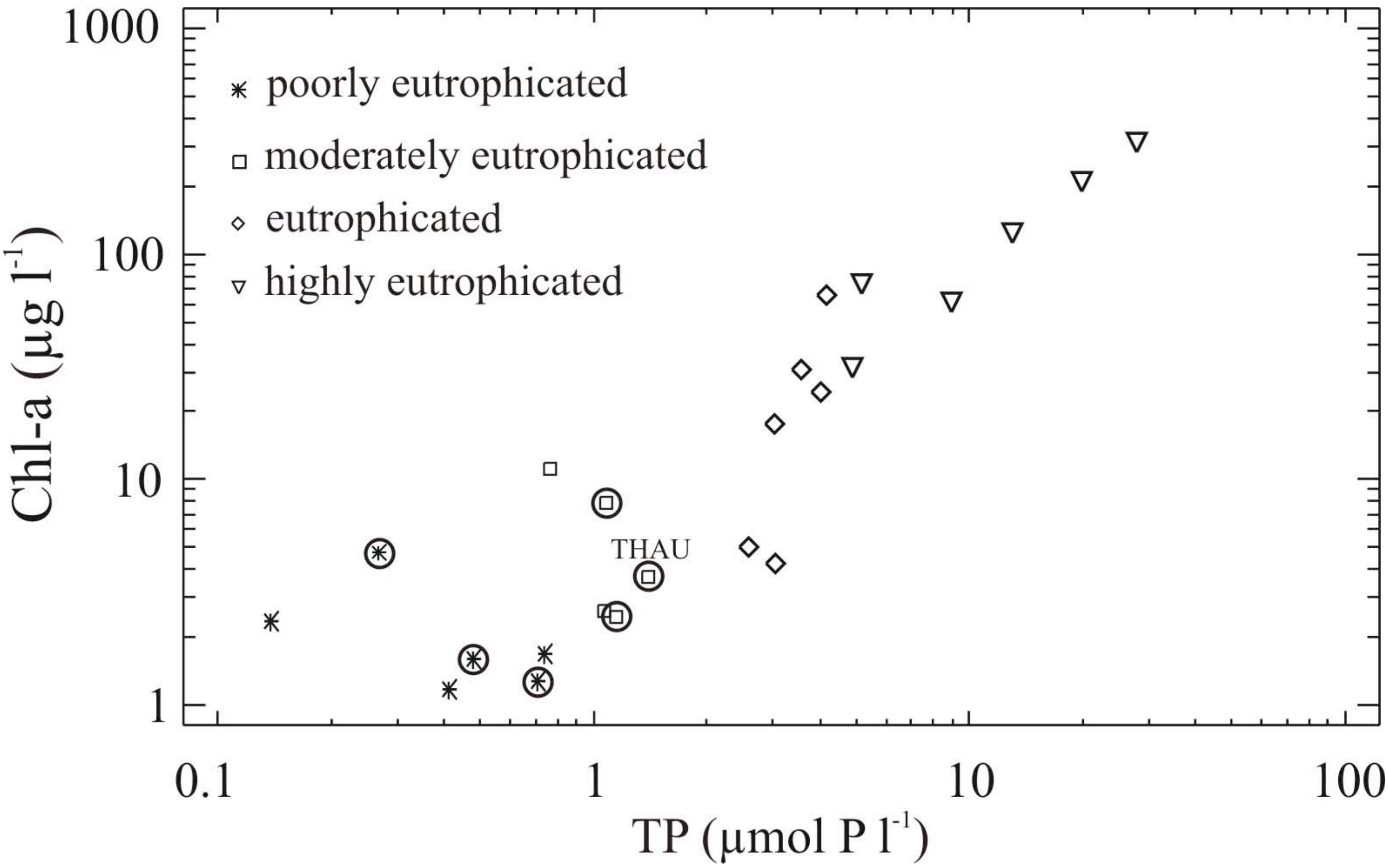
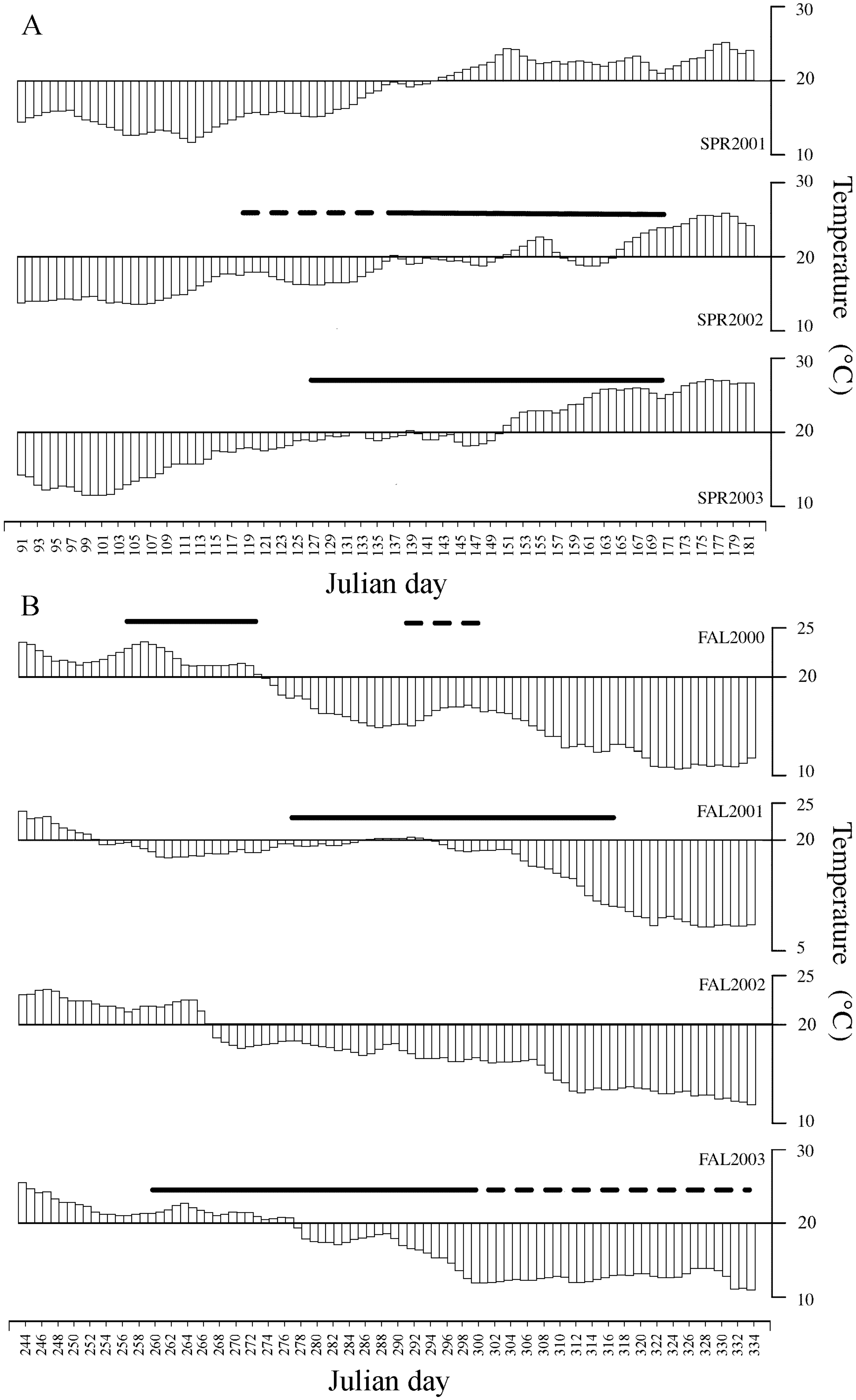
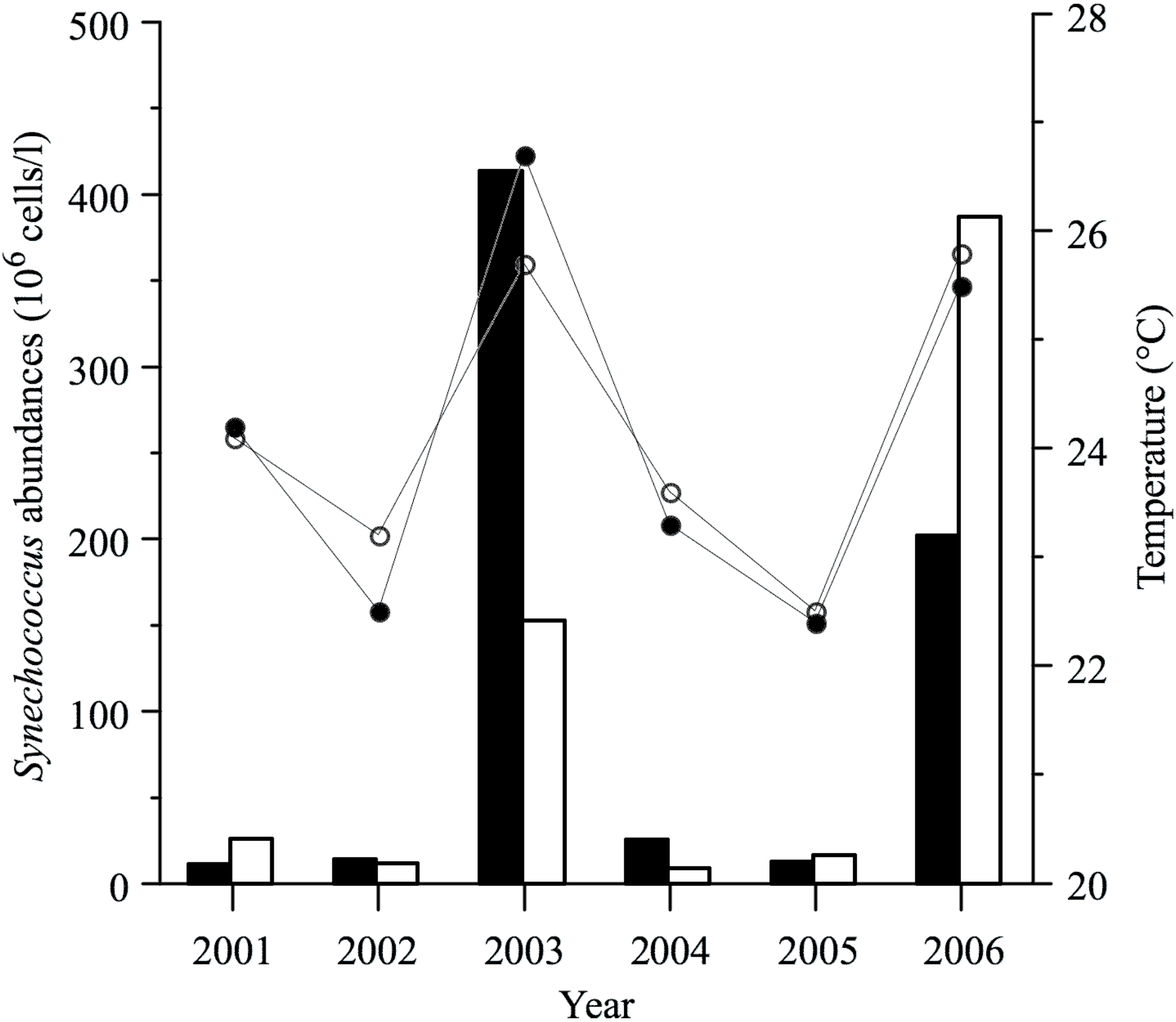
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
Time (years)



poorly eutrophicated
moderately eutrophicated
eutrophicated
highly eutrophicated
THAU
Chl-a (μg l-1)
TP (μmol P l-1)
1000
100
10
1
0.1
1
10
100



A
SPR2001
SPR2002
SPR2003
Temperature (°C)
Julian day
B
FAL2000
FAL2001
FAL2002
FAL2003
Temperature (°C)
Julian day



500
400
300
200
100
0
Synechococcus abundances (10^6 cells/l)
28
26
24
22
20
Temperature (°C)
2001
2002
2003
2004
2005
2006
Year