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Comparing diatom and *Alexandrium catenella/tamarense* blooms in Thau lagoon: Importance of dissolved organic nitrogen in seasonally N-limited systems

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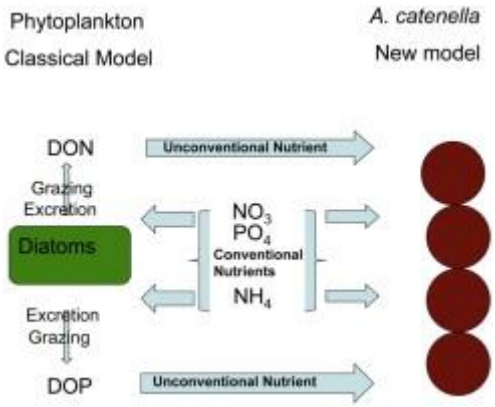
Abstract:

Diatom blooms in Thau lagoon are always related to rain events leading to inputs of inorganic nutrients such as phosphate, ammonium and nitrate through the watershed with time lags of about 1 week. In contrast, blooms of *Alexandrium catenella/tamarense* can occur following periods of 3 weeks without precipitation and no significant input of conventional nutrients such as nitrate and phosphate. Field results also indicate a significant drop (from 22–25 to 15–16 μM over 3 days) in dissolved organic nitrogen (DON) at the bloom peak, as well as a significant inverse relationship between *A. catenella/tamarense* cell density and DON concentrations that is not apparent for diatom blooms. Such dinoflagellate blooms are also associated with elevated (6–9 μM) ammonium concentrations, a curious feature also observed by other investigators, possibly the results of ammonium excretion by this organism during urea or other organic nitrogen assimilation.

The potential use of DON by this organism represents short cuts in the nitrogen cycle between plants and nutrients and requires a new model for phytoplankton growth that is different from the classical diatom bloom model. In contrast to such diatom blooms that are due to conventional (nitrate, phosphate) nutrient pulses, *Alexandrium catenella/tamarense* blooms on the monthly time scale are due to organic nutrient enrichment, a feature that allows net growth rates of about 1.3 d^{-1}, a value higher than that generally attributed to such organisms.

Geographical abstract:



Highlights

► Diatom and dinoflagellates blooms are compared at the same site. ► *Alexandrium catenella* blooms can be fueled by dissolved organic nitrogen. ► Ammonium peak is a signal of dissolved organic nitrogen utilization. ► Dinoflagellates net growth rate can exceed that of diatoms under N limited conditions. ► *Alexandrium catenella* net growth rate in situ can reach 1.3 day^{-1}.

Abbreviations

- DIN, dissolved inorganic nitrogen;
- DON, dissolved organic nitrogen;
- HABs, harmful algal blooms;
- PON, particulate organic nitrogen;
- SRP, soluble reactive phosphorus

Keywords: *Alexandrium catenella/tamarense* ; Non classical blooms ; Organic nitrogen ; Ammonium ; Growth rate

Introduction

Historically, marine phytoplankton ecologists have focused on the annual, high biomass, diatom-dominated spring bloom in the North Atlantic (Parsons et al. 1988) and many models are based on such a scheme (Fasham et al. 1990; Neumann 2000; Lavoie et al. 2009). The recent development and/or ensuing monitoring of harmful algal blooms (HABs), and more particularly those due to dinoflagellates, have called this diatom model into question (Heisler et al. 2008; Wyatt, 2010). As mentioned by Heisler et al. (2008), we need to "move away from simplistic inorganic nutrient-dose-yield models ". Quoting Wyatt (2010): "the long-standing agricultural hypothesis … should be given less prominence", …, "we need to explore different kinds of models and hypotheses to improve our understanding of phytoplankton dynamics". Smayda (2002) has outlined the contrasting bloom behavior of diatoms and dinoflagellates in descriptive terms, and Smayda (2008) has reviewed the linkage (or lack of it) between HABs and nutrient oversupply. Recent attempts to model mixotrophic organisms (Flynn and Mitra 2009) has proven challenging (Glibert et al. 2010) because of the complexity of their physiology, so that models dealing with mixotrophic dinoflagellates such as *Alexandrium tamarense* do not explicitly include the use of dissolved organic nutrients (Yamamoto et al. 2002; Yamamoto & Seike, 2003). Overall, there is a lack of quantitative data on such rate processes (Glibert et al. 2010).

In Thau lagoon (Southern France), the recent appearance (1995) and recurrent development (up to 250 µg Chla L^{-1}) of HABs (*Alexandrium catenella/tamarense*) have taken place in a long-term context of oligotrophication (Souchu et al. 1998; Collos et al., 2009) that tends to support the lack of relationship with "conventional" nutrient supply. In the same waters, episodic rain events bring DIN and SRP from the watershed leading to diatom blooms (up to 40 µg Chla L^{-1}). Thus this site is well fitted to carry out a comparative study of diatom and dinoflagellate blooms.

Our objectives are first to identify general trends over the last 20 years (1990-2011) in Thau lagoon and relationships between environmental variables such as water temperature and salinity and the major phytoplankton groups such as diatoms and dinoflagellates. We then focus on more detailed physiological mechanisms underlying bloom development, more specifically dynamics of nutrient such as nitrogen (N) that is generally limiting phytoplankton growth in this environment (Collos et al. 1997; Souchu et al. 1998, 2001). Both diatom and *A. catenella/tamarense* blooms are described and compared using ancillary environmental data.

Material and methods

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 three narrow channels. Three oyster farming zones are located along the northwestern shore. The lagoon represent 10% of French oyster production and is the main oyster production center on the Mediterranean. Samples for diatom blooms were taken from station ZA (Figure 1) at 1 m depth. *A. catenella /tamarense* blooms were monitored in Angle Creek (station A5), where such blooms always originate and develop (Collos et al. 2004, 2007, 2009; Genovesi et al. 2011). Samples for ammonium (NH_4^+) determination were fixed onboard and measured using the method of Koroleff (1976) with a precision of 5% and detection limit of 0.05 µM. Filtration and storage of samples were performed within 1 h of sampling. The contents of the sampling bottles were pre-filtered through a 200 µm screen to remove large zooplankton, stored in acid pre-cleaned polycarbonate bottles and taken to the laboratory for filtration in an all-glass filtering system with a vacuum under 10 cm Hg. This system allowed the measurement of dissolved and particulate nitrogen forms on the same sample. Filtration was made through a pre-combusted (450°C for 6 h) Whatman glass-fibre filter (GF/F) which was lyophilized and

saved for later analysis of particulate organic carbon (PC) and nitrogen (PN) with a Perkin-Elmer CHN 2400 analyser. Filtrates were frozen immediately at -20°C in pre-combusted Pyrex flasks, and stored for analysis within 2 months (Macdonald and McLaughin, 1982). They were then gently thawed at ambient temperature before analyses for soluble reactive phosphorus (SRP), urea, nitrate (NO_3^-) and nitrite (NO_2^-). For the determination of reactive silicate (Si), filtrates were stored at 4°C in polycarbonate bottles until their analysis within a few weeks. The simultaneous determination of dissolved organic nitrogen (DON) and phosphorus (DOP) was performed using the photo-oxidation method (Armstrong and Tibbits, 1968). Before 2003, dissolved organic nitrogen, NO_2^- and NH_4 were transformed into NO_3^- by a 2000 W medium pressure mercury vapour lamp (Heraeus Model EQ 2023 ref. 56070152). The calibration with 20 different compounds of nitrogen and phosphorus required an irradiation time of 24 h for optimal oxidation that gave results comparable with high temperature catalytic oxidation methods (HTCO) within 10% (Gasc, 1993). Beginning in 2003, concentrations of total nitrogen in water (TN) were measured on pre-filtered water by chemical oxidation with potassium persulfate in hot alkaline medium in Teflon vials adapted from Raimbault and Slawyk (1991). Briefly, 5 ml of reagent mix were added to 40 mL of sample and autoclaved in Teflon vials at 120°C (1 bar) for 1 h. The various nitrogen forms (except N_2) were oxidized to nitrate that was then measured according to Tréguer and Le Corre (1975). Analysis of SRP, NO_3^-, NO_2^- and Si were made in triplicate by a Technicon AutoAnalyser II (Tréguer and Le Corre, 1975) with a precision of 1%. Urea was measured according to Goeyens et al. (1998).

Concentrations of DON were calculated by subtracting the concentration of NH_4^+ and NO_3^-+ NO_2^- from the TDN concentration. The standard deviation (SD) of the DON concentration was calculated by propagation of uncertainty with the following equation:

$$SD = (S^2_{TDN} + S^2_{NH4} + S^2_{NO3/NO2})^{1/2}$$

where S^2 is the variance of the three measurements (Bevington and Robinson, 2002).

For chlorophyll *a* (Chl *a*) determination, 750 mL of water were filtered through a GF/F filter which was stored in petrislides® and immediately frozen at 20°C. Analyses were performed within a few weeks using the fluorometric method for pre-2000 samples (Yentsch and Menzel, 1963). After 2000, Chl a, b, and c and pheopigments a, b, and c were measured by spectrofluorometry (Neveux and Lantoine, 1993). Phytoplankton cell counts were done according to Utermöhl (1958) with 10 ml sedimentation chambers. Detection limit was 100 cells L^{-1}.

Net growth rates are calculated from regression equation between natural log of Chl *a* (diatoms) or cell numbers (*A. catenella*) and time (Guillard, 1973). Significance tests were carried out on the slope of the regression line.

In order to quantify the spatial heterogeneity of *A. catenella* blooms, we carried out an extensive survey using 25 stations over a grid of about 1x2 km in Angle Creek on 25 September 2003 (Fig. 1). Table 1 shows the coefficient of variations (CVs) of mean values for each variable. In addition, the same grid was covered at 12 other dates between 19 September and 6 November 2003 for *A. catenella* cell densities only. For those data, the CVs of mean cell densities ranged between 80 and 188%. In spite of this patchiness, cell densities of *A. catenella* at one station (A5) were significantly related to the mean of the other 24 stations (r^2=0.444, n=24, $p<0.001$).

For time series (1990-2011), we used the Ifremer database for temperature, salinity, total diatoms and total dinoflagellates. Correlation matrices are built up using mean, maximum and third quartile values (after log transformation for diatom and dinoflagellate cell counts). Here we use salinity as a proxy for freshwater (and inorganic nutrient) input into the lagoon from the watershed.

One way ANOVAs are carried out and Durbin-Watson tests are used to check for serial autocorrelation of residues. We also introduced time lags between variables that were suggested by more detailed analyses of blooms (see below).

Results

Both diatoms and dinoflagellates present bimodal temporal distribution of mean monthly cell densities over a yearly cycle, with maxima in March and June for diatoms, and March and September for dinoflagellates.

Concerning the March blooms, time series data indicated a significant inverse relationship between mean diatom cell densities in March and mean salinities in January-February (Fig. 2). Durbin-Watson tests indicated that there was no serial autocorrelation between residues. There were also weaker significant inverse relationships using maximum salinity values and third quartile diatom cell densities, but in it was not the case for dinoflagellate cell densities. In the fall, the dinoflagellate bloom was positively related to water temperature (Fig. 3)

We illustrate both kinds of blooms by several examples as follows.

Diatom blooms

Figure 4 illustrates one example of a *Skeletonema* spp. bloom at the end of February 1996, reaching 50 millions cells per liter (other species less than 10,000 cells L^{-1} and dinoflagellates less than 2,500 cells L^{-1}). Record rainfall in December 1995-January 1996 (data not shown) led to elevated nitrate (30 μM) and ammonium (15 μM) levels that fed the bloom and decreased as chlorophyll a biomass increased up to a peak of about 35 μg L^{-1}. Ammonium reached undetectable levels in the early phase of the bloom, and nitrate was not totally exhausted as it remained around 3-5 μM at the end of the bloom. SRP was limiting phytoplankton growth as it reached nearly undetectable (0.01 μM) levels at bloom peak.

DIN/SRP ratios ranged from 16 to 682 (at bloom peak). DIN (nitrate+nitrite+ammonium) was always above 4 μM. Silicate was always above 1 μM (data from Souchu et al. 2001). DON did not change significantly (linear regression of DON vs time: r^2=0.095, n=18) throughout the bloom (10-17 μM). PN increased from a background of about 3 μM up to 20 μM at bloom peak.

Another diatom bloom example is taken from a November 1993 *Thalassiosira* bloom following a major rain event in early November that led to a pulse of nitrate (up to 11 μM) and ammonium (up to 4 μM) and a chl a peak of about 37 μg L^{-1} a week later (Fig. 5). Both ammonium and nitrate were exhausted by that time. SRP was 0.07 μM and DIN was 0.3 μM at bloom peak. DIN/SRP ratios ranged from 2.5 to 28.5 with a value of 4 at bloom peak. Silicate was always above 2.6 μM. DON ranged between 17 and 29 μM over the 72 days of monitoring with no obvious trend. (linear regression of DON vs time: r^2=0.308, n=11). PN increased from a background of about 1.5 μM to a maximum of 9 μM at bloom peak.

Dinoflagellate blooms

Generally, the above relationship between salinity and diatom cell densities (Fig. 2) could not be reproduced for dinoflagellates. Instead, a significant positive relationship was found between water temperature and dinoflagellate cell densities in September (Fig. 3). In order to investigate further the factors behind *A. catenella/tamarense* blooms that are taking place mainly in the fall, we used a DIN supply estimated from rainfall vs. *Alexandrium* cell nitrogen relationship. Rainfall was converted to DIN supply through the watershed using equations obtained from relationships between DIN and rainfall over the previous week (Fig. 6). *A. catenella/tamarense* cells were converted to PN using values (5 to 25 pmol N $cell^{-1}$) from Collos et al. (2004) determined in cultures. Plotting N demand estimated from minimum

and maximum equivalent PN vs DIN supply (Fig. 7) led to categorize the blooms in two groups: in 1998, 1999, 2000, 2001 and 2003, DIN supply from the watershed following rain events could not account for the blooms, while it could in later years (2004 and 2005) for which data were available. No bloom took place in 2002.

A first example of *A. catenella/tamarense* blooms is shown in Fig. 8 where a period of 80 days is covered (1 September to 20 November in 2000 and 2001). In 2000, a major bloom took place in mid-September with a peak cell density of 625,000 cells L^{-1} on 18 September, representing 35 μg chl a L^{-1} and 24 μM PN (Collos et al. 2007). While no nutrient data were available for this period, the most striking feature is the lack of rainfall over the 3 weeks preceding this bloom. As concentrations of DIN are significantly ($p<0.05$) related to rainfall cumulated over the previous week (Fig. 6) at this site, this was the earliest indication that such dinoflagellate blooms did not follow the usual patterns shown in Fig. 4 and 5 and the classical diatom model. In addition, the major rain event in mid-October 2000 did not lead to a dinoflagellate bloom, but to a small diatom bloom of Chaetoceros and Skeletonema. In 2001, there was no rain during the week before the *A. catenella/tamarense* bloom (1.27×10^6 cells L^{-1} on 5 November) that was preceded by a mixed diatom bloom (485,000 *Pseudo-nitzschia* cells L^{-1} and 275,000 *Chaetoceros* cells L^{-1}).

In the category of blooms not explained by DIN supply through the watershed from rainfall, another bloom was sampled in much more detail (Fig. 9) and at two stations (A00 and A5) in Angle Creek during the 2003 fall period (15 September to 16 October). The *A. catenella/tamarense* cell density reached a peak of 770,000 cells L^{-1} at station A5 and 136,000 cells L^{-1} at station A00 on October 2. A rain event (65 mm) on September 22 led to a small pulse of ammonium (2-3 μM) and nitrate (4-7 μM). DIN/SRP ratios ranged between 2.0 and 9.9, the latter value being due to the ammonium maximum observed at bloom peak. Otherwise DIN/SRP ratios were always below 6.6 during the bloom period, so that N was the

limiting nutrient. But the most striking features concerned significant (unpaired t test, $p=0.0009$) decreases in DON between September 29 (25.5 and 22.4 µM at stations A00 and A5 respectively) and October 2 (16.2 and 15.5 µM at station A00 and A5 respectively), and ammonium peaks of 6 µM (station A00) and 9.5 µM (station A5) coinciding with peak cell densities (Fig. 9), compared to background values of 0.5 – 1 µM. DON minima (15-16 µM) also coincided with cell maxima at both stations. Urea ranged between 0.5 and 2.8 µgatN L^{-1} without apparent trends with time.

Finally, *A. catenella/tamarense* cell densities were positively and significantly related ($p<0.001$) to ammonium concentrations and negatively ($p<0.05$) to DON (Fig. 10).

Net growth of *A. catenella/tamarense* during this event can be estimated around 1.3 d^{-1} ($p<0.05$) from pooled data at stations A00 and A5 between September 18 and 22. In spite of the very patchy distribution of this species (typical coefficients of variations of mean cell densities range from 97 to 169%), this high value is confirmed by pooled data from 25 stations within Angle Creek (net growth rate = 1.36 d^{-1}, $p<0.001$).

Discussion

The significant inverse relationship between mean diatom cell densities and mean salinities (Fig. 2) and the lack of such relationship for dinoflagellates clearly suggest a dichotomy in the environmental factors influencing diatom and dinoflagellate blooms. Here we use salinity as a proxy for inorganic nutrient supply from freshwater input through the watershed.

Concerning diatom blooms, the examples given here (Fig. 4 and 5) are consistent with traditional patterns of phytoplankton blooms in temperate waters. They can be explained by simple N budgets where decreases in DIN match increases in PON. For example, in 1996, the increase in PON (about 17 µM) could be easily accounted for by the DIN decrease (about 40

250 μM). According to the criteria of Justic et al. (1995), the *Skeletonema* bloom (Fig. 4) took
251 place under conditions of P limitation, and that of *Thalassiosira* (Fig. 5) under conditions of
252 both N and P limitation.
253 Concerning dinoflagellate blooms, the relationship between water temperature and cell
254 densities is probably not a direct one, as water temperature is likely to represent water
255 stability or turbulence in our situation (Laanaia et al. 2013). Dinoflagellate species other than
256 *A. catenella/tamarense* in Thau include *Dinophysis acuminata* and *D. sacculus*, *Gyrodinium*
257 *spirale*, *Heterocapsa triquetra*, *Peridinium quinquecorne* and *Protoperidinium bipes*. Most of
258 them are heterotrophs so that *A. catenella/tamarense* can probably be considered as
259 representative of those other dinoflagellates that bloom in Thau lagoon. Although there is
260 some evidence that *A. catenella/tamarense* blooms can be triggered by urea pulses involving
261 lag times of one week (Collos et al., 2007), here we document blooms that occur following
262 long (up to 3 weeks) periods without rain events. The eastern part of the lagoon where those
263 blooms always originate is a collapsed karstic system that does not receive significant
264 amounts of groundwater during dry periods. Generally, karstic systems in our area feed
265 lagoons that are oligotrophic (Souchu et al. 2010).
266 In contrast to other sites such as the Seto Inland Sea where blooms of *A. tamarense*
267 occur under conditions of P limitation (Yamamoto et al. 2002; Yamamoto & Seike, 2003),
268 blooms of *A. catenella/tamarense* in Thau lagoon always occur under conditions of N
269 limitation, assessed either from dilution bioassays (Collos et al. 2004, 2007) or DIN/SRP
270 ratios. For example, during the fall 2003 bloom (Fig. 9), the DIN/SRP ratio was always below
271 6.5, except at the bloom peak, when ammonium also reached a maximum value.
272 While there was no apparent decrease in DON during the diatom blooms (Fig. 4 and
273 5), this was the case during the *A. catenella/tamarense* bloom of fall 2003 (Fig. 9). DON
274 minima coincided with peaks in cell densities at both stations. Although there is a lot of

variability involved (Fig. 9), the inverse relationship between DON and *A. catenella/tamarense* cell densities tends to support the hypothesis that this organism uses DON directly, as already shown in cultures of the same species (Carlsson et al. 1998; Collos et al. 2004; Loureiro et al. 2009).

In the Seto Inland Sea, Yamamoto et al. (2004) reported that "a decrease of DOP and DON seemed to coincide with *A. tamarense* blooms, suggesting utilization of DOM by *A. tamarense*". The same authors reported a DON peak a week following a *S. costatum* bloom in spring 1997 in northern Hiroshima Bay (Japan) and an *A. tamarense* bloom 3 weeks later.

Our data are also strikingly similar to those from Long Island (USA) coastal waters (Laroche et al. 1997) where, in a N limited system, DON decreased from about 35 μM to 15 μM during a bloom of *Aureococcus anophagefferens*.

Concerning the major ammonium peak during the bloom, such features have been reported by other investigators. Taylor et al. (1994) noted that "…the (*A. catenella*) bloom was associated with elevated ammonium concentrations" in a British Columbia fjord in September 1989. Yamamoto et al. (2010) reported a positive relationship between *A. tamarense* cell densities and ammonium (up to 350 μM) in Osaka Bay in April 2007. Hattenrath et al. (2010) observed NH_4 levels up to 3 μM at *A. fundyense* bloom peak in spring 2007 compared to background values of 0.5-1 μM before and after the bloom in a New York estuary. However, this pattern was not reproduced a year later. In our case, the high (6-9 μM) ammonium values do not seem to be due to an artefact such as internal ammonium release induced by cell rupture during filtration (Collos 2002) because the DON decreased rather than increased with increasing cell densities (Fig. 9). Grazing estimates from dilution experiments carried out on the same samples (Collos et al. 2007) indicate that grazing was nil on October 1 and therefore could not have contributed to the ammonium peak on October 2. These ammonium pulses could be due to overflow metabolism during assimilation of urea or other

300 organic compounds, as already reported for this (Jauzein et al., 2008) or other (Uchida, 1976)
301 dinoflagellate species.
302 Historically, the direct use of DON by phytoplankton has been very difficult to prove
303 in presence of bacteria that can convert DON to ammonium. In the case of *A. catenella*, the
304 strongest case for direct DON use probably comes from the work of Loureiro et al. (2009)
305 where it was shown, from uptake kinetics considerations, that the low ammonium
306 concentrations could not support the observed growth rates and DON had to be used directly.
307 In the present case, the highly significant positive relationship between *A.*
308 *catenella/tamarense* cell densities and ammonium (Fig. 9) argues against direct ammonium
309 use. Rather, ammonium is produced by *A. catenella/tamarense* during DON assimilation.
310 This is consistent with culture work for this (Jauzein et al. 2008) and other species (Price and
311 Harrison 1988 and references therein; Munoz-Blanco et al. 1990) but under natural
312 conditions, to our knowledge, *Alexandrium* blooms are the only ones to present this
313 peculiarity (Taylor et al., 1994; Hattenrath et al., 2010; Yamamoto et al. 2010). Incidentally,
314 although *A. catenella/tamarense* appears to be associated with high ammonium
315 concentrations, it should not be considered as an "indicator" species because the ammonium
316 is a result and not the cause of its presence.
317 Estimates of DON uptake from our field data range from 4 to 16 μmol N 10^{-6} cells d^{-1}, and are
318 consistent with estimates obtained in cultures of *A. catenella* (Carlsson et al. 1998; Loureiro
319 et al. 2009). Taking an average value of 10 pmol N $cell^{-1}$ (Collos et al. 2004), this leads to N
320 based growth rates of between 0.4 and 1.6 d^{-1}. Those values are consistent with net growth
321 rates (1.3-1.4 d^{-1}) based on changes in cell densities during the bloom. Those values are the
322 highest ever reported for this species (Stolte and Garcés, 2006; Anderson et al., 2012), but are
323 not unreasonably high. Smayda (1996) obtained similar growth rate values by incubating a
324 dinoflagellate culture under natural irradiance. This shows that laboratory culture conditions

and specially low irradiances (say 50-100 µmol photons.m^{-2}.s^{-1}) lead to low growth rates that are not representative of field situations. Interferences in our estimates could come from advection and vertical migration. Concerning the vertical migration, it happens on a daily time scale, but our estimates range over 4 days of measurements, so this factor is averaged out. Concerning advection, this bloom took place during a calm period (wind events disperse the blooms, Laanaia et al. 2013), so that there was not much water mass movement. There was no downward flow of water as no major river flows in the creek, and the blooms shown in Fig. 8 and 9 occurred during dry periods.

Finally, we emphasize that our field estimate of growth is based on data from 25 stations within the creek, so that this intensive sampling gives a representative picture of the situation and averages out any heterogeneity in *Alexandrium* biomass levels.

The Alexandrium growth rates are also higher than net growth rates for diatom blooms described in Fig. 4 and 5 (0.70 and 0.35 d^{-1} respectively). Thus, this dinoflagellate can occasionally grow faster than diatoms and this feature partly explains its periodic dominance. We suggest that this higher growth rate is due to its ability to use DOM as already shown in cultures (Carlsson et al. 1998; Legrand and Carlsson, 1998; Loureiro et al. 2009). This provides *A. catenella/tamarense* with an ecological advantage relative to other phytoplankton groups that cannot use DOM. Those include diatoms that are generally considered to have higher growth rates than dinoflagellates (Banse, 1982, Stolte and Garcés, 2006). Still, there are a few observations, carried out under natural irradiance (Smayda 1996; Garcés et al. 1998), showing that *Alexandrium* may exhibit relatively high growth rates (1.0-1.3 d^{-1}).

As mentioned by Glibert et al. (2010): “quantitative data about the role of mixotrophy in nutrition, growth, and blooms are lacking ». Here we provide such data for *A. catenella/tamarense*, illustrating some of the “ecophysiological diversity” (Smayda, 2002) of such organisms. In order to improve models of dinoflagellate growth that still cannot describe

such blooms correctly, we suggest taking into account dissolved organic matter as a nutrient source, as already suggested a long time ago (Antia et al. 1980, 1991) and using higher values of maximal growth rates that are probably underestimated in current models of dinoflagellate growth.

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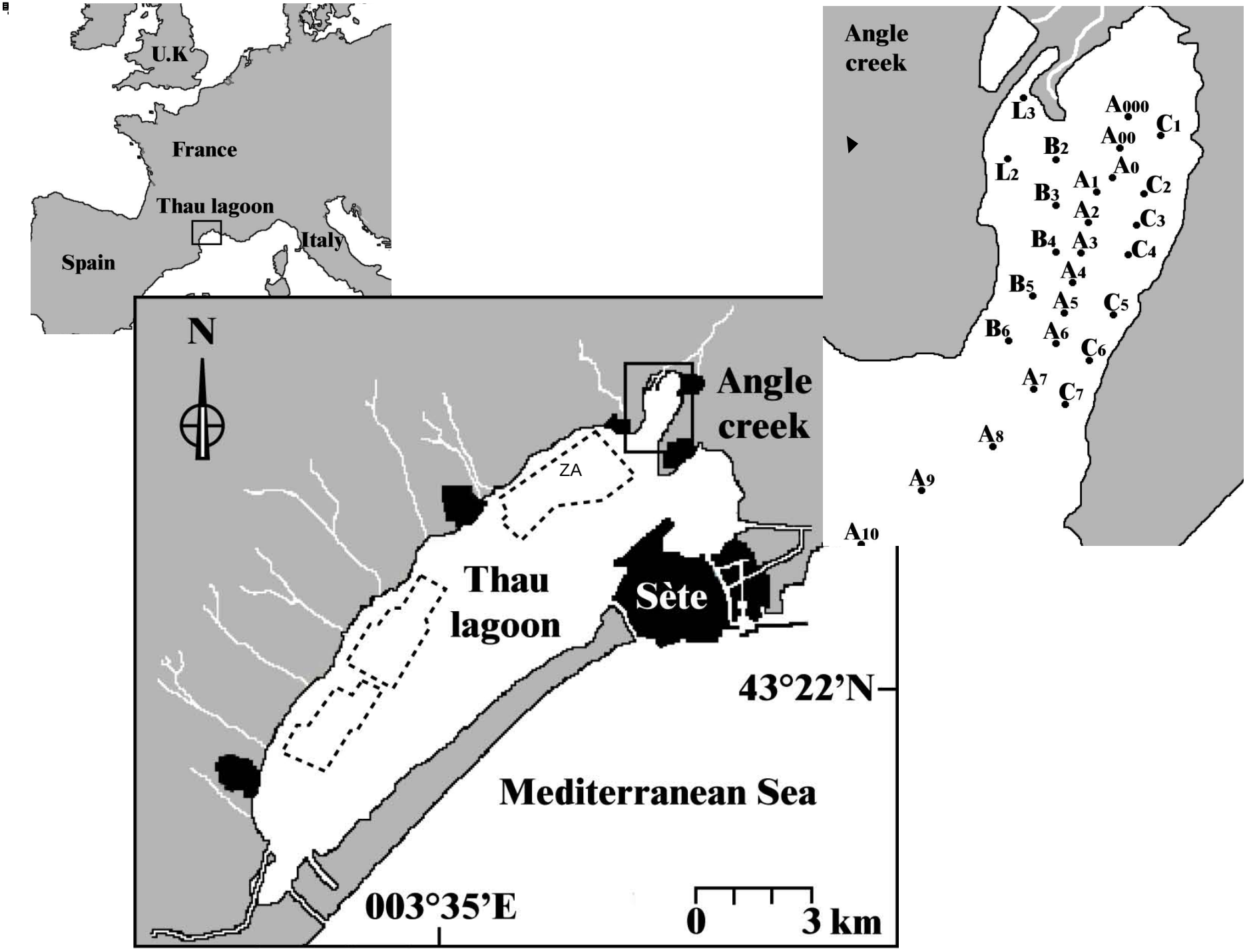
Figure legends

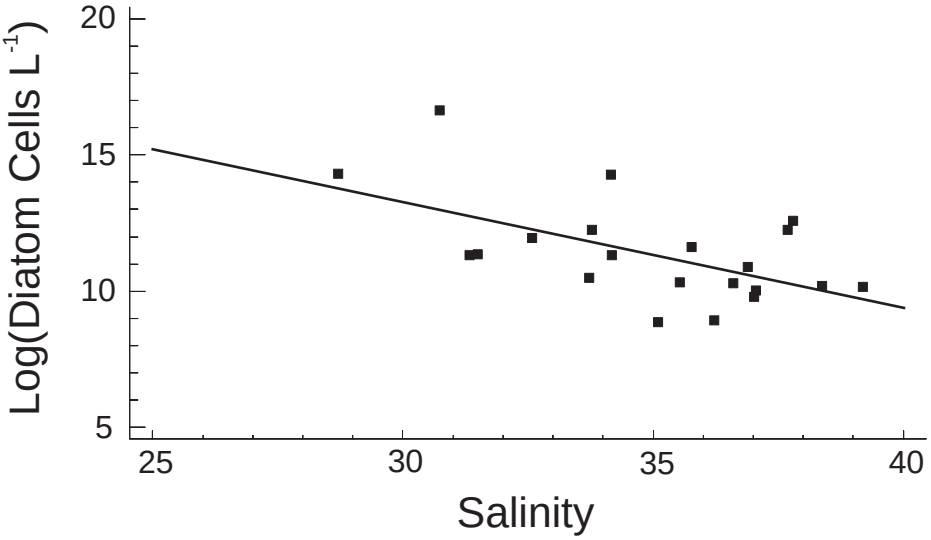
1. Study site with station locations. Urban areas in black. Oyster farming areas within stipples.
2. Mean diatom cell densities (cells per liter) in February-March as a function of mean salinities in January-February (1990-2011). r = -0.572; p = 0.007
3. Mean dinoflagellate cell densities (cells per liter) in September as a function of mean temperatures in September (1990-2011). r = 0.592; p = 0.005
4. Winter diatom (*Skeletonema* spp.) bloom in Thau lagoon (February 1996). A: Rainfall and salinity; B: ammonium, nitrate and soluble reactive phosphorus (SRP); C: Chlorophyll a (Chl a), dissolved organic nitrogen (DON), particulate nitrogen (PN).
5. Fall diatom (*Thalassiosira* spp.) bloom in Thau lagoon (November 1993). A: Rainfall and salinity; B: ammonium, nitrate and soluble reactive phosphorus (SRP); C: Chlorophyll a (Chl a), dissolved organic nitrogen (DON), particulate nitrogen (PN).
6. Dissolved inorganic nitrogen concentrations in Angle Creek (station A5) as a function of rainfall (log transformed) cumulated over the previous week. Curve is regression equation: $y = \exp(0.18 + 0.37x)$; $r^2 = 0.515$; n = 15; $p<0.005$
7. N demand estimated from *A. catenella* cell quotas (diamonds: minimum values, squares: maximum values) and cell counts at the height of the blooms (62 to 99 % of total cell counts) as a function of DIN supply estimated from rain integrated over the previous week (1998-2005). Line is regression equation of slope 1.

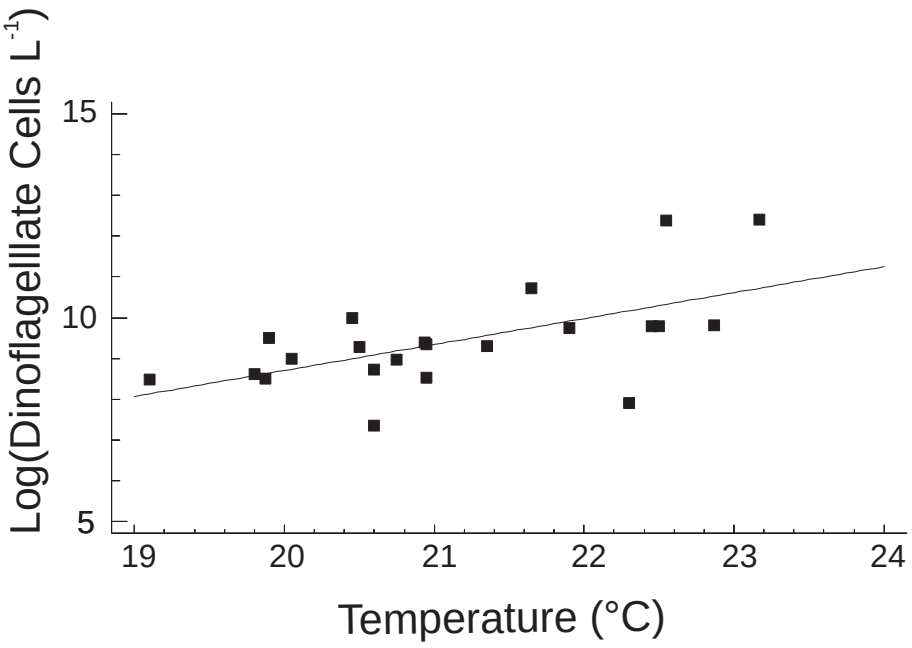
8. Fall 2000 and 2001 *Alexandrium catenella/tamarense* bloom in Thau lagoon. A: 2000; B: 2001. Cumulative rainfall over the previous week, salinity and *A. catenella/tamarense* cell numbers.
9. Fall 2003 *Alexandrium catenella/tamarense* blooms at station A5 in Thau lagoon. A: rainfall, salinity and *A. catenella/tamarense* cell densities. B: ammonium, nitrate, and dissolved organic nitrogen (DON).
10. *Alexandrium catenella/tamarense* cell densities as a function of ammonium (closed squares) and DON (open squares) during the fall 2003 bloom. Pooled data from two stations (A00 and A5).

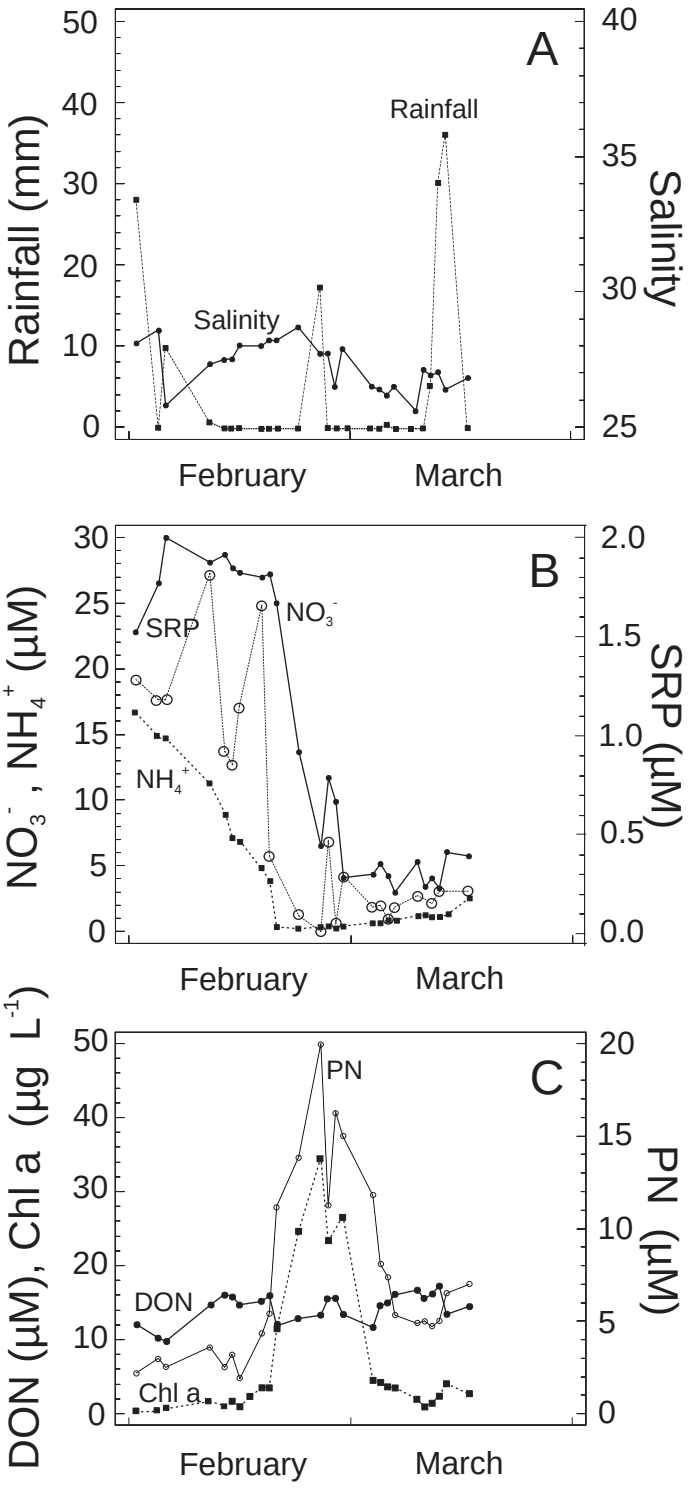
    For ammonium: $Log(Acat) = 0.31NH4+9.4$; $r^2 = 0.263$; $n = 18$; $p<0.05$

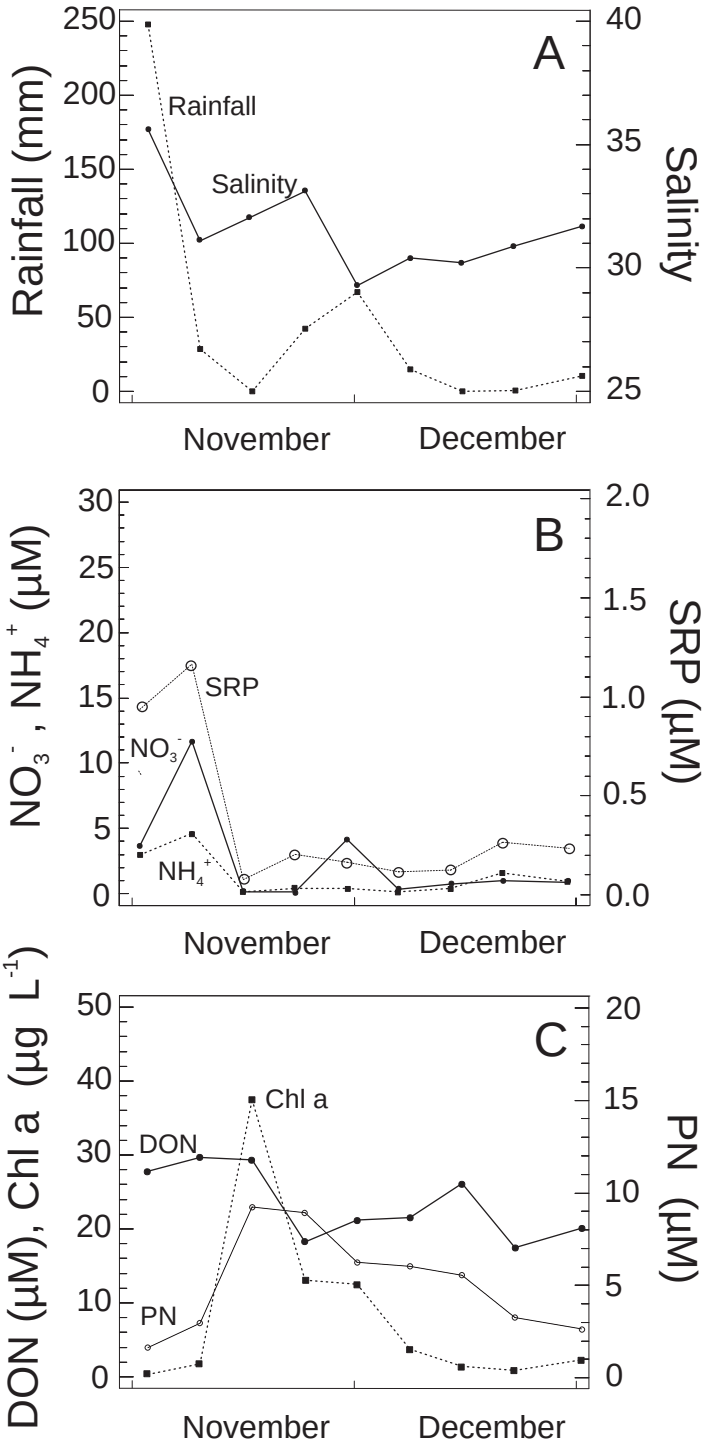
    For DON: $Log(Acat) = -0.19DON+13.9$; $r^2 = 0.388$; $n =15$; $p<0.02$


U.K
France
Thau lagoon
Italy
Spain
N
Angle creek
ZA
Thau lagoon
Sète
43°22'N
Mediterranean Sea
003°35'E
0
3 km
Angle creek
L3
A000
C1
A00
B2
L2
A0
A1
C2
B3
A2
C3
B4
A3
C4
A4
B5
A5
C5
B6
A6
C6
A7
C7
A8
A9
A10


Log(Diatom Cells L-1)
20
15
10
5
25
30
35
40
Salinity


Log(Dinoflagellate Cells L-1)
15
10
5
19
20
21
22
23
24
Temperature (°C)


A
Rainfall
Salinity
Rainfall (mm)
Salinity
February
March
B
SRP
NO_3^-
NH_4^+
NO_3^- , NH_4^+ (µM)
SRP (µM)
C
PN
DON
Chl a
DON (µM), Chl a (µg L^{-1})
PN (µM)


A
Rainfall
Salinity
Rainfall (mm)
Salinity
250
200
150
100
50
0
40
35
30
25
November
December
B
SRP
NO_3^-
NH_4^+
NO_3^- , NH_4^+ (µM)
SRP (µM)
30
25
20
15
10
5
0
2.0
1.5
1.0
0.5
0.0
November
December
C
Chl a
DON
PN
DON (µM), Chl a (µg L^{-1})
PN (µM)
50
40
30
20
10
0
20
15
10
5
0
November
December

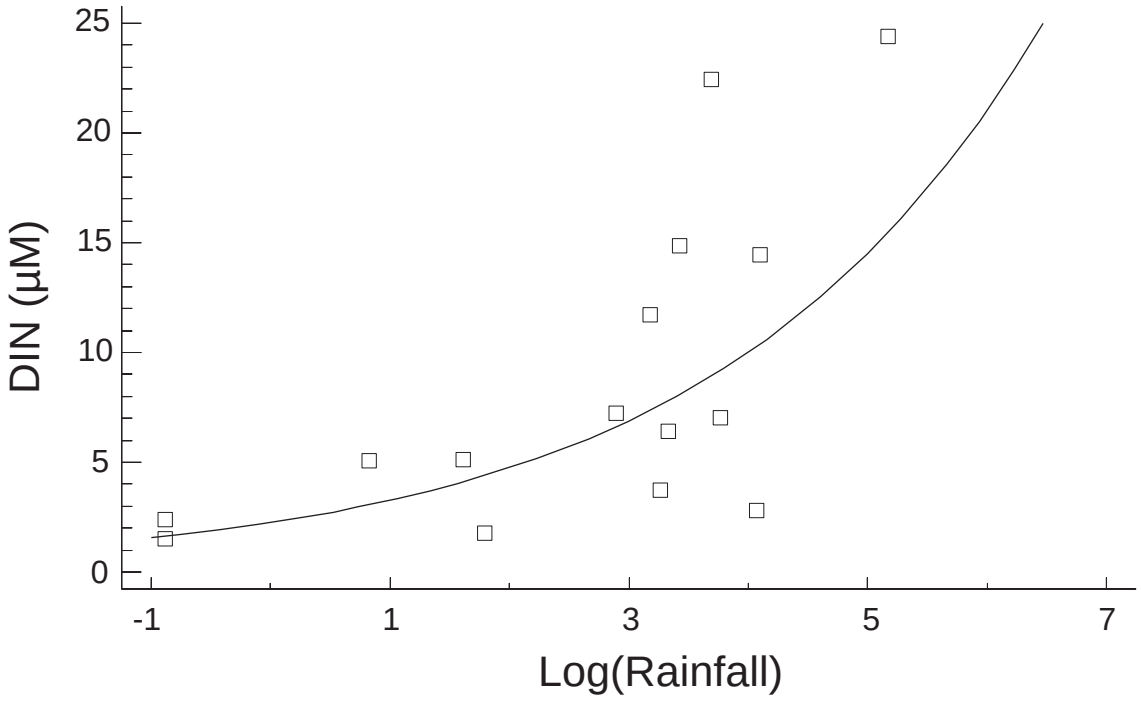
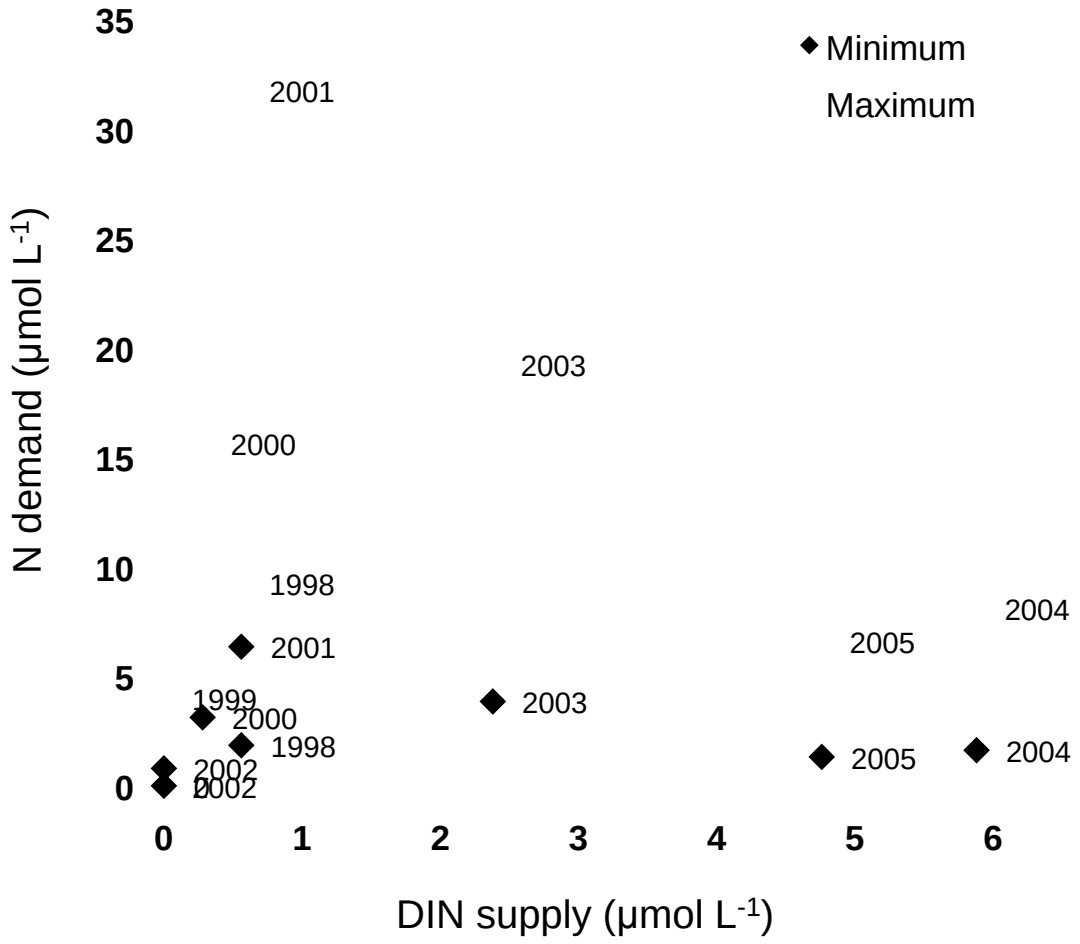
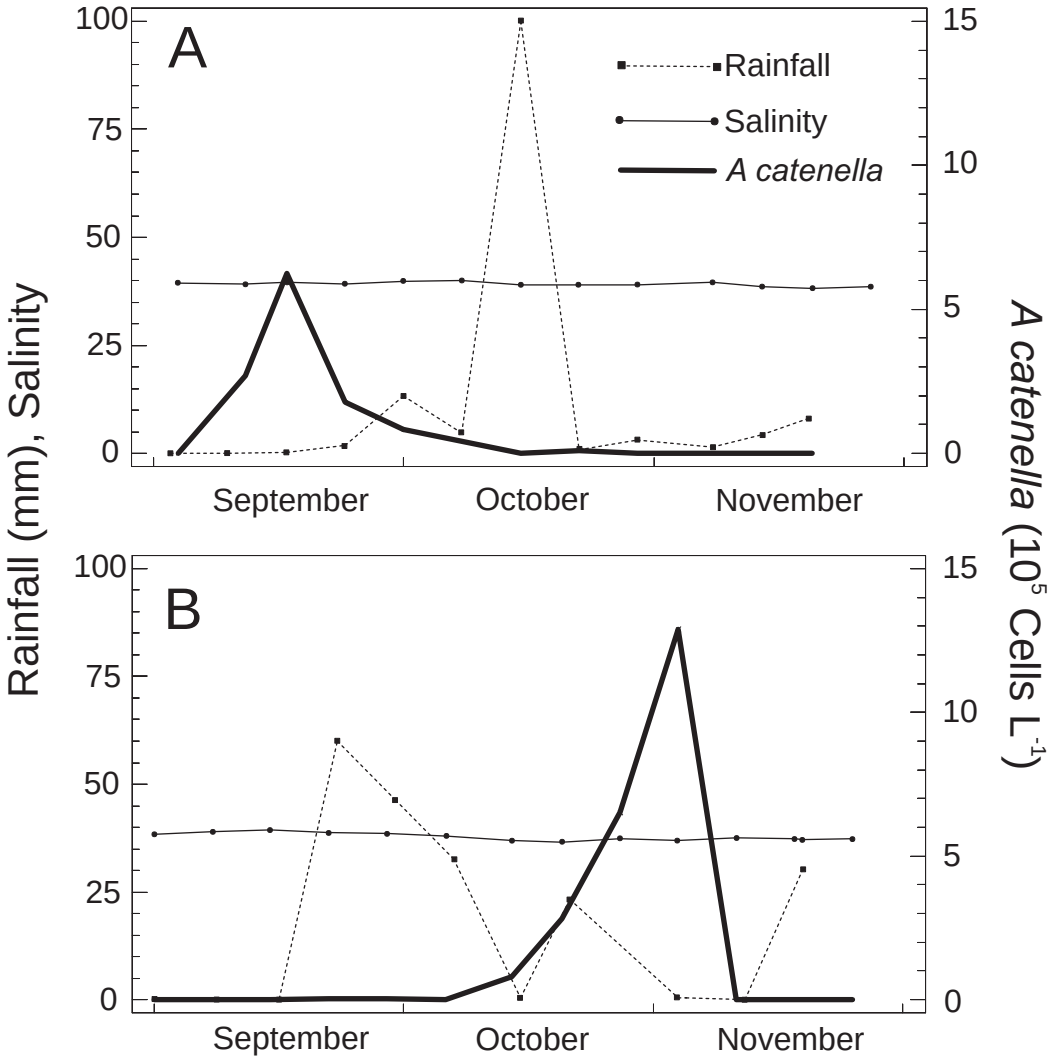
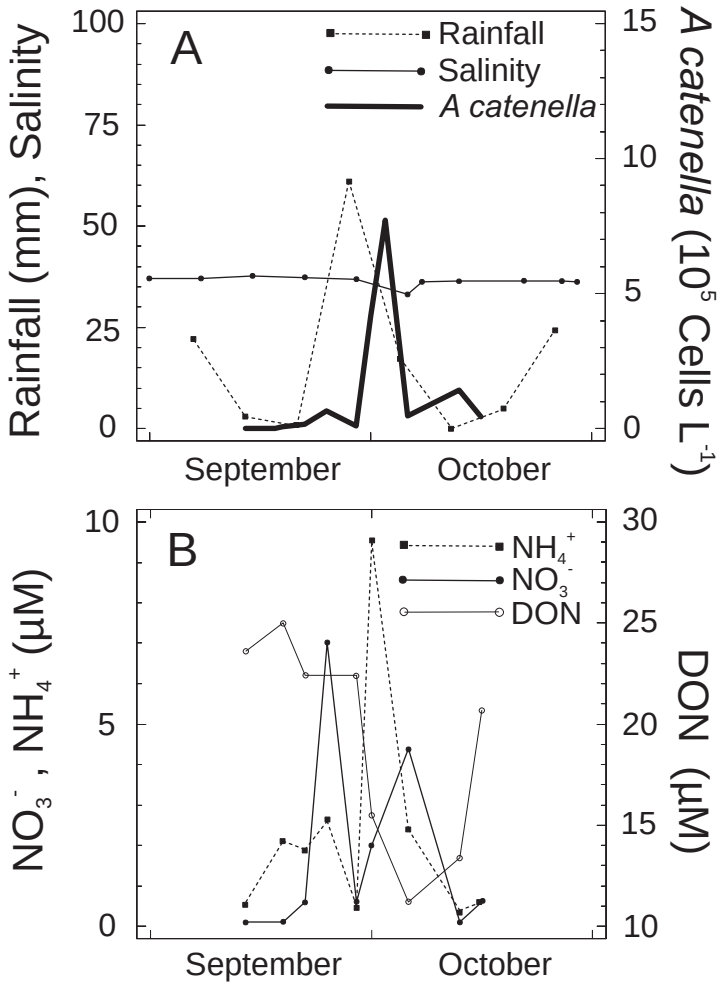

25
20
15
10
5
0
DIN (µM)
-1
1
3
5
7
Log(Rainfall)

Figure 7


35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
N demand (µmol L-1)
DIN supply (µmol L-1)
Minimum
Maximum
2001
2003
2000
1998
2001
2004
2005
1999
2000
2003
1998
2005
2004
2002
2002


A
B
Rainfall
Salinity
A catenella
Rainfall (mm), Salinity
A catenella (10^5 Cells L^-1)
100
75
50
25
0
15
10
5
0
September
October
November


A
Rainfall
Salinity
A catenella
Rainfall (mm), Salinity
A catenella (10^5 Cells L^-1)
100
75
50
25
0
15
10
5
0
September
October
B
NH4+
NO3-
DON
NO3- , NH4+ (µM)
DON (µM)
10
5
0
30
25
20
15
10
September
October

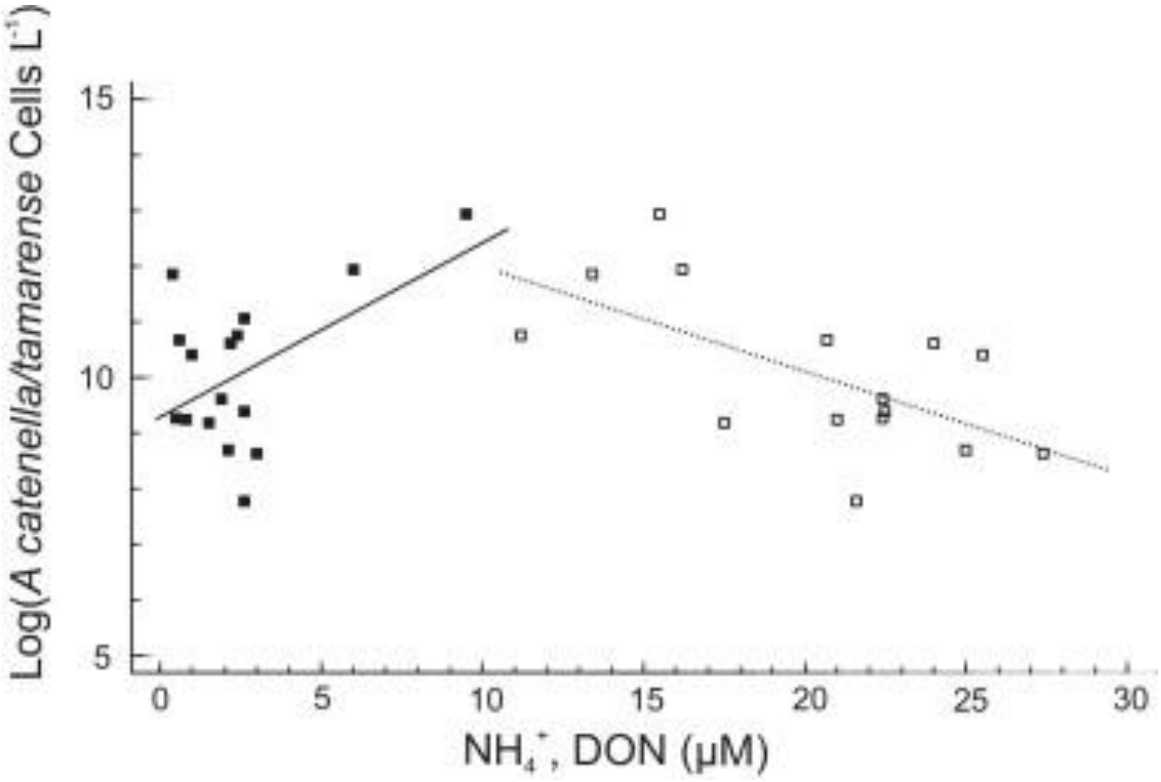

Log(A catenella/tamarense Cells L-1)
15
10
5
0
5
10
15
20
25
30
NH4+, DON (µM)

Table 1. Spatial heterogeneity of physical, chemical and biological variables in Angle Creek. Data from 25 stations on 25 September 2003

Variable	Coefficient of variation (%)
Salinity	5
Nitrate	65
Nitrite	16
Ammonium	49
DON	14
SRP	18
Silicate	11
A. catenella	122