Journal of Experimental Marine Biology and Ecology
April 2008, Volume 358, Issue 2, Pages 124-135
http://dx.doi.org/10.1016/j.jembe.2008.01.025

Competition for phosphorus between two dinoflagellates: A toxic Alexandrium minutum and a non-toxic Heterocapsa triquetra

C. Labry[a], *, E. Erard–Le Denn[a], A. Chapelle[a], J. Fauchot[a], A. Youenou[a], M.P. Crassous[a], J. Le Grand[a] and B. Lorgeoux[a]

[a] Ifremer Brest, Dyneco/Pelagos, BP 70, 29280 Plouzané, France

*: Corresponding author : C. Labry, email address : Claire.Labry@ifremer.fr

Abstract:

The understanding of the dominance of one species with respect to others is a pertinent challenge in HAB growth dynamics studies and the nutrient supply mode is one of the factors potentially involved. The competition for phosphorus (P) between a toxic species, Alexandrium minutum, and a non-toxic species, Heterocapsa triquetra, was studied (1) along a gradient of P depletion, (2) testing different P depletion degrees before a single PO4 supply and (3) experimenting different PO4 supply frequencies. In conditions of PO4 depletion, H. triquetra stopped growing after two days both in monospecific and mixed batch cultures whereas A. minutum grew progressively from day 2 until the end of the experiment. This time-lag growth of A. minutum is associated to its ability to store P intracellularly and then mobilize it for cell division when P depletion becomes severe. Heterocapsa triquetra outcompeted A. minutum when it was submitted to less than three days of P depletion before the pulse. In contrast, A. minutum outcompeted H. triquetra after more than three days of depletion. This transition was related to the capacity for A. minutum to increase its cell PO4 uptake rate in a higher proportion to face potential PO4 supply. As a result of this physiological acclimatation to P starvation, A. minutum consumed the whole PO4 pulse supplied after 3 to 10 days of P depletion. This resulted in a reduction of H. triquetra growth. These two acclimatations were confirmed in a P limited semi-continuous culture experiment testing several PO4 supply frequencies (1, 2, 4, 6 day intervals). These experiments revealed that A. minutum is a "storage specialist" species for P, which uptakes PO4 pulses for luxury consumption, survives depletion periods and, then, utilizes P for cell growth. In contrast, H. triquetra is more a "velocity adapted" species, which utilizes PO4 just after supply to increase their cell division rate.

Keywords: *Alexandrium minutum*; *Heterocapsa triquetra*; Dinoflagellates; Phosphorus storage; Pulse; Ecophysiology; Batch culture; Semi-continuous culture

1. Introduction

In the competition between phytoplanktonic species, different factors and intrinsic properties can interplay : allelopathic mechanisms (Arzul and Gentien, 2005), nutrient acquisition modalities (Gaedeke and Sommer, 1986; Ghosh et al., 1999; Sommer, 1984), light gradient (Huisman et al., 1999), selective predation from zooplankton (Guisande et al., 2002), parasitism (Erard Le Denn et al., 2000; Park et al., 2004), life cycle properties… For one species, the success of proliferation results from a particular conjunction between several factors, becoming favourable at one time and one place (Anderson et al., 2002; Smayda and Reynolds, 2001).
Intrinsic properties of harmful species are particularly studied because of their capacity to proliferate in coastal waters and their economical impacts on human activities. For these species, the challenge is to identify and evaluate the most important factors affecting their growth dynamics. *Alexandrium minutum* and *Heterocapsa triquetra* are known to reach very high concentrations (> 10^6 cells l^{-1}) to the detriment of other coexistent species (Erard-Le Denn, 1991; Kononen et al., 2003; Labib and Halim, 1995; Lindholm and Nummelin, 1999; Litaker et al., 2002a; Vila et al., 2005). Proliferations of *A. minutum* are of particular concern because of its ability to produce paralytic shellfish poisoning (PSP) toxins. This species is, therefore, frequently responsible for harmful algal blooms in coastal waters (Usup et al., 2002; Vila et al., 2005; Yoshida et al., 2000). Conversely, *H. triquetra* is non-toxic but it significantly contributes to the total phytoplankton biomass and may be harmful, due to its ability to form dense blooms (Lindholm and Nummelin, 1999; Litaker et al., 2002a). *Heterocapsa triquetra* was reported to be associated with *Alexandrium* species on several occasions, such as with *A. minutum* in the estuary of Penzé, northern Brittany, France (Morin et al., 2000), or with *A. tamarense* in the eastern coast of USA (Anderson and Stolzenbach, 1985) and Hong Kong waters (Lu and Hodgkiss, 2004). *Heterocapsa triquetra* is therefore a potential competitor of *Alexandrium* species in natural assemblages. Investigations on the competition between these species both in monospecific and mixed cultures are crucial to better understand their specific ecological niches in terms of growth potential. Such experiments would also provide information on physiological parameters, such as growth and nutrient uptake processes, useful for the calibration of ecophysiological (cell quota based) models for these species.

Among the different factors, which may affect the competition between *H. triquetra* and *A. minutum*, previous studies evaluated their allelochemical potential. Some reported that *A. minutum*, as other *Alexandrium* species, exhibit allelopathic properties (Fistarol et al., 2004; Tillmann and John, 2002). In contrast, *H. triquetra* is not known to have allelopathic or hemolytic activities, nor to inhibit competitors by direct cell contact, as it was reported for the morphologically analogous species *Heterocapsa circularisquama* (Oda et al., 2001; Uchida et al., 1999). The preliminary studies of Erard-Le Denn et al. (2003) on competition between *H. triquetra* and *A. minutum* (strain AM89BM) did not detect any allelopathic effect between the two species during their exponential growth phase (culture filtrate method). However, an effect was observed when the culture filtrate of each species in stationary phase was used as new medium for the other target species. Arzul et al. (1999) reported similar results working on the same strain of *A. minutum* against *C. gracile* and *K. mikimotoï*. Thus, when considering the capacity of *A. minutum* and *H. triquetra* to grow together exponentially, allelopathy does not seem to be an important factor in competition between the two algal species.

Among the other factors possibly involved, light is probably not a key factor. Dinoflagellates are able to migrate in the water column to fulfil their light energy demand and avoid high surface irradiance during the day (Fauchot et al., 2005; Passow, 1991). Phototactic movements of *H. triquetra* was observed in the field (Anderson and Stolzenbach, 1985; Litaker et al., 2002b) and in culture (Braarud and Pappas, 1951). Natural *A. minutum* populations were found to have vertical migratory behaviour closely correlated to the light-dark cycle (Labib and Halim, 1995). In contrast, the nutrient supply mode is known to affect the species composition of phytoplankton assemblages (Sommer, 1984), including those with dinoflagellates (Yamamoto and Hatta, 2004). Numerous studies have been conducted to test this hypothesis, of effect of nutrient supply on multispecific cultures or natural assemblages in freshwater environments (Gaedeke and Sommer, 1986; Ghosh et al., 1999; Sommer, 1984). There are few surveys on multispecific cultures of marine species, particularly harmful ones. Riegman et al. (1996) reported that *H. triquetra,* in mixed cultures containing dinoflagellates and Prymnesiophyceae, was a good competitor in the case of excess nutrients whereas it was sensitive to PO_4 limitation. *Alexandrium tamarense* was a poor competitor under both nutrient limited and repleted conditions. Davidson et al. (1999) demonstrated that, in N and Si limited conditions, the lowest cell density and

cell volume were observed for *A. minutum* in competition with a diatom and a flagellate. Furthermore, Erard-Le Denn et al. (2003) showed that *H. triquetra* always outgrew *A. minutum* in the case of excess nutrients, regardless of salinity and light conditions. The one and only experimented condition under which *A. minutum* outcompeted *H. triquetra* involved a severe 10 day PO_4 limitation period followed by a PO_4 pulse.

The aim of the present study was to explore more deeply the importance of PO_4 supply mode in presence of two species in competition, *A. minutum* and *H. triquetra* in cultures. Different phosphorus (P) depletion conditions, followed by a single PO_4 supply, as well as different PO_4 supply frequencies, were tested. The first conditions were experimented in batch cultures to gradually reach a gradient of P depletion. Conversely, PO_4 supply frequencies were tested in semi-continuous cultures with the same dilution rate for all cultures. Under these conditions, nutrient supplies other than PO_4 were always renewed (every day) and were never depleted such as expected in batch. The observed responses in batch and semi-continuous cultures were evaluated in terms of growth rate, phosphate uptake and phosphorus storage ability.

2. Material and Methods

2.1. Strain and culture conditions

Alexandrium minutum (AM89BM) and *Heterocapsa triquetra* (HT99PZ) were isolated in northern Brittany (France) from the Morlaix estuary in 1989 and the Penze estuary in 1999. Both strains were routinely grown in sterilized (autoclaved 20 min at 120°C) prefiltered natural seawater enriched with f/2 medium (Guillard and Ryther, 1962). For the present study, cultures were grown at 18°C, at a salinity of 27, with an overhead illumination of 200 µE m^{-2} s^{-1} in a 14:10 light/dark (L:D) cycle. Each sampling was carried out 5 to 6 hours after the beginning of the light phase.

2.2. Batch culture experiments

A first experiment on monospecific and mixed cultures was conducted (experiment A, exp. A). This experiment was then partly replicated for monospecific cultures (experiment B, exp. B) to measure additional parameters (Fig. 1). For both experiments, cells were preconditioned twice in a medium containing the equivalent of 25 % of the concentrations present in f/2 medium for phosphate (9 µM) and nitrate (220 µM) and concentrations present in f/2 medium for the other nutrients. For experiment A, *A. minutum* and *H. triquetra* cells were inoculated either alone (monospecific culture, single batch) or together (mixed culture, triplicate batch) in 4.5 L batch cultures containing no phosphate and 200 µM nitrate. The initial cell concentration of each species was 500 cells ml^{-1}. These cultures are named hereafter "PO_4 depleted" cultures (Fig. 1). Cell and PO_4 concentrations were monitored 1, 2, 3, 5, 7 and 10 days after inoculation. For each sampling date, a subsample (400 ml) was also transferred to a 1 L flask and was submitted to a 4 µM PO_4 supply (KH_2PO_4). These latter cultures are named "PO_4 pulsed" cultures (Fig. 1). Cell and PO_4 concentrations were monitored once a day for 8 days. For experiment B, additional parameters, such as intracellular C, N, P contents and cell volume, were measured on monospecific cultures (triplicate batch), at 1, 2, 3, 4, 5, 7 and 10 days after inoculation. These cultures are the "PO_4 depleted" cultures of exp. B (Fig. 1).

The cell division rate was used as the reference for the specific growth rate in the rest of the manuscript i.e. measured as increase in cell densities. This allows to consider the possibility of a time delay between phosphate uptake and subsequent increase in cell concentration, as it is the case for phytoplankton growth in a varying nutrient regime (Collos, 1986; Davidson et al., 1993). The cell specific growth rate (µ) was calculated by plotting logarithm of cells versus time and determining the slope according to the least squares criterion. A minimum of three sampling points was included in the calculation. The initial cell PO_4 uptake rate in the "PO_4 pulsed" cultures was calculated from the difference in PO_4 concentrations between day 0 and day 1 divided by mean cell concentration.

2.3. Semi-continuous culture experiments

Cells were preconditioned in 4L monospecific batch cultures containing concentrations for PO_4 equivalent to one fourth of f/2 medium concentrations for 10 days (concentrations equivalent to f/2 medium for other nutrients). They were then submitted to a semi-continuous mode with the same medium for 10 days, at a dilution rate of 0.15 d^{-1} (renewal once a day, 5 hours after the beginning of the light phase). Cells were then inoculated in 2 L cultures containing no phosphate and f/2 conditions for all other nutrients. The initial (day 0) cell concentration was 4500 cells ml^{-1} for each species. Four frequencies (every 1, 2, 4 or 6 days) of PO_4 supply were then tested and for each frequency, 1 monospecific culture of each species as well as 2 mixed cultures (replicates) were prepared. From day 1, the cultures were daily renewed at 0.15 d^{-1} with f/2 medium except for PO_4 concentrations. The latter were adjusted proportionally according to the period of supply: 9, 18, 36 and 54 µM PO_4 for 1, 2, 4 or 6 day intervals. The corresponding concentrations in the cultures just after supply considering the dilution rate, were 1.35, 2.7, 5.4 and 8.1 µM PO_4. Under these conditions, the dilution events were the same for all cultures and PO_4 quantities received by these cultures were well-balanced. The only difference between the cultures was the frequency of PO_4 supply.

For each dilution event, the cultures were gently stirred to ensure the homogeneous distribution of cells before automatic medium removal with a peristatic pump. Then, the new medium was added manually. Gentle circular manual movement was preferred to magnetic stirring, which could alter the physiological state of *A. minutum* cells through the induction of cell Carbon stress (works of Probert, 1999, on the same strain). A dilution rate of 0.15 d^{-1} was chosen, since it was a good compromise between P limited conditions and sufficient renewal of the medium. Cell and PO_4 concentrations were measured in the withdrawn volume and monitored daily as well as the intracellular P content in monospecific cultures. Cell specific growth rates were calculated from day to day using the exponential growth equation and taking into account the dilution rate of the cultures.

2.4. Analyses

For *A. minutum* and *H. triquetra* enumerations, samples were fixed with a few drops of Lugol's iodine and cells were counted with an inversed optical microscope (Utermöhl method). A mean cell diameter was determined for monospecific cultures on fixed cells using an image analyser coupled to an optical microscope (user software developed by Ifremer). The method consists in measuring several lengths of a cell at various angles from the horizontal axis. A mean length value is calculated, providing an equivalent spherical diameter (ESD) compatible with the calculation of the cell volume as a sphere. Such cell ESD determination was considered appropriate since the shape of *A. minutum* was considered sub-spherical (Balech, 1989), spherical (Probert, 1999), or ellipsoidal (Hillebrand et al., 1999) and that of *H. triquetra* as two symmetrical cones (Hillebrand et al., 1999). 100 cells were analysed for each replicate culture.
Samples for PO_4 determination were very carefully filtered on glass fiber filters (Whatman GF/F) with a syringe filtration system. PO_4 were analysed on an autoanalyser AACE Bran and Lubbe (Tréguer and Le Corre, 1975). Samples for particulate C, N, and P were filtered on precombusted (12 h at 400°C) 25 mm Whatman GF/D filters and filters were deep frozen (- 20 °C). Particulate C and N were analysed on a CN VarioEL III Elementar analyser after carbonate removal (Aminot and Kerouel, 2004). Particulate phosphorus was determined using high temperature method (Solorzano and Sharp, 1980). C, N and P cell quotas (Q_C, Q_N, Q_P) were obtained by dividing particulate C, N, P by the corresponding cell number taking into account filtered volumes.

3. Results

3.1. Batch culture experiments

The "PO_4 depleted" cultures (exp. A and B)
Cell concentrations and growth rate evolution
The temporal variations in cell concentrations in the monospecific "PO_4 depleted" cultures were similar for exp. A and exp. B (Fig. 2a): Heterocapsa triquetra cell concentrations increased until day 2, and then stabilized or decreased, whereas A. minutum cells kept on growing. We observed similar growth curves in the mixed cultures (Fig. 2b), where A. minutum reached higher cell concentrations than H. triquetra after day 3. Less than 20 % standard deviation was obtained when triplicate cultures were

conducted (mixed cultures of exp. A and monospecific cultures of exp. B), indicating a relatively low variability of responses. In addition, temporal evolutions of cell concentrations in mixed and monospecific cultures of exp. A and B were similar. However, higher cell concentrations and no lag phase were observed during exp. A compared to exp. B. These differences might be explained by a higher inoculum for exp. A than for exp. B (630 against 383 ± 42 cells ml^{-1} for A. minutum and 570 against 493 ± 100 cells ml^{-1} for H. triquetra). Furthermore, the natural seawater used for cultures (Atlantic seawater) was collected at two different times. Even if the addition of inorganic nutrients was identical, the composition and concentration of dissolved organic matter of these natural seawaters were probably different. This might have also contributed to the difference in responses between the two experiments.

Therefore, our results show that there was no significant difference between the response of each species in the mixed cultures and their response in isolation (exp. A). Each species adjusted similarly to depleted conditions, regardless of the presence of other species.

Quotas Q_P, Q_C, Q_N and cell volume evolution (exp. B)

In the "PO_4 depleted" cultures of exp. B, the temporal evolution of A. minutum Q_P showed a slight increase during the lag phase followed by a strong decrease between days 2 and 3 and a slower decrease later on (Fig. 3a). In contrast, H. triquetra Q_P did not exhibit important variations. The minimum values were reached on day 10 for both species and were similar (6.5 ± 0.6 pgP $cell^{-1}$ for A. minutum and 6.1 ± 0.4 pgP $cell^{-1}$ for H. triquetra). However the Q_P range was larger in A. minutum cells (6.5 to 23 pgP $cell^{-1}$) than in H. triquetra cells (6.1 to 9.2 pgP $cell^{-1}$). For both species, Q_C and Q_N increased and then stabilized (Fig. 3b, c). Alexandrium minutum Q_C was on average 1.5 times higher than H. triquetra Q_C throughout the depletion period. In these monospecific cultures, the higher concentrations of A. minutum cells compared to H. triquetra cells after day 4 (Fig. 2a) is, therefore, also accompanied by a higher A. minutum C biomass.

The initial (day 0) values of Q_C, Q_N and Q_P for H. triquetra cells were approximately half those of A. minutum cells. Thus their initial C:N, C:P, and N:P ratios were not significantly different (Fig. 3d, e, f). The decrease in Q_P until the end of the experiment resulted in an increase of C:P and N:P ratios for both species, the highest values being reached by A. minutum. In A. minutum cells, the C:N ratio decreased during the lag phase and then increased during the growth phase. In contrast, H. triquetra C:N ratio increased during the growth phase and decreased during the saturation phase.

Flat size distributions resulted in large standard deviations in cell volumes (Fig. 4a). However, our results show that cell volumes tended to increase after day 4 for *A. minutum* and from day 1 to day 5 for *H. triquetra* (Fig. 4a). It corresponded to a slight decrease in normalized Q_C (i.e. Q_C per unit of cell volume) for *A. minutum*, whereas for *H. triquetra*, normalized Q_C remained stable after day 2 (Fig. 4b).

The "PO_4 pulsed" cultures (exp. A)

Cell concentrations and growth rate evolution

A pronounced shift from a H. triquetra dominance was observed in the "PO_4 pulsed" cultures (Fig. 5), after 1 to 2 days of P depletion to an A. minutum dominance after 3 days of P depletion. Our results reveal three different growth patterns, depending on the duration of the depletion period (Fig. 5, Fig. 6a, b, Table 1). First, after 1 to 2 days of P depletion and pulse, H. triquetra outgrew A. minutum. Second, after 3 to 5 days of depletion and pulse, the growth rates of the two species were not significantly different. However, the exponential growth phase was longer and, thus, the stationary phase mean cell concentrations were higher in A. minutum than in H. triquetra. Third, after 7 to 10 days of depletion and pulse, A. minutum outgrew H. triquetra.

Conversely, in the monospecific "PO_4 pulsed" cultures, H. triquetra always exhibited higher growth rates and higher cell concentrations in stationary phase compared to A. minutum, regardless of the duration of the P depletion period (Fig. 6c, d, Table 1). For all monospecific cultures, the growth rate decreased during the first three days of depletion and, then, increased until day 7. These results show that the cells adjusted favourably to depleted conditions from day 3 to the end of the experiment (Fig. 6c, Table 1).

PO_4 concentrations and initial cell PO_4 uptake rate evolution

In monospecific cultures, H. triquetra totally consumed the 4 µM PO_4 pulse in more than 1 day (between 2 to 4 days) regardless of the duration of the previous depletion period (data not shown). In contrast, in mixed or monospecific cultures, A. minutum took several days to deplete this PO_4 pulse after only 1 or 2 days of depletion. After 3 days of depletion, the pulse was consumed in less than 1

day (data not shown). After 1 day of P depletion, H. triquetra exhibited the highest cellular PO_4 uptake rate (Fig. 7). However, from 2 to 5 days of depletion, A. minutum cellular PO_4 uptake rate increased and exceeded H. triquetra uptake rate. Moreover, after three days, A. minutum PO_4 uptake rate was underestimated (Fig. 7, dotted line) since the sampling time interval (24 h) was too long to estimate an actual uptake rate.

3.2. Semi-continuous culture experiments

Cell concentrations and growth rate evolution

In the monospecific cultures, H. triquetra reached higher cell concentrations than A. minutum in all conditions whereas, in the mixed cultures, A. minutum always outgrew H. triquetra (Fig. 8). This outcome occurred earlier when the PO_4 supply interval was shorter (1 and 2 days against 4 and 6 days; Fig. 8). In the monospecific cultures for 1 and 2 day intervals, cell concentrations increased exponentially as in batch culture mode after a lag period. Then, both species concentrations reached a steady state starting at day 10. Their respective mean growth rates (0.21 d^{-1} and 0.19 d^{-1} for A. minutum ; 0.16 d^{-1} and 0.16 d^{-1} for H. triquetra) were close to the theoretical growth rate at steady state (0.16 d^{-1}) which was calculated from the dilution rate (D) and the theoretical formula [-ln(1-D)]. For the 4 and 6 day intervals, cell density in the monospecific cultures increased and oscillated after the second pulse. Heterocapsa triquetra cells increased immediately 24 h after each pulse, while A. minutum cells increased more progressively or showed a 1 to 2 day delay before increasing (Fig. 8). No cell density fluctuation was detected in the mixed cultures. Alexandrium minutum cells kept increasing whereas H. triquetra cells progressively decreased. For all cultures, PO_4 became undetectable after day 4 (data not shown). Thus, after day 4, the PO_4 pulses were not visible anymore in the ambient PO_4 concentrations 24 hours after supply.

Q_P evolution

For all monospecific cultures (Fig. 9), Q_P initially increased during the lag period, then decreased during the exponential phase. The evolution of Q_P confirmed that the same steady state was reached for 1 and 2 day periods. A relatively stable Q_P was observed after day 10 with a mean value of 6.7 $\pm$ 0.9 pgP $cell^{-1}$ for A. minutum (n=16) and 4.1 $\pm$ 0.4 pgP $cell^{-1}$ (n=18) for H. triquetra. For 4 and 6 day periods, A. minutum and H. triquetra Q_P increased the day after the pulse, with a greater mean increase factor, for A. minutum (2.1) than for H. triquetra (1.5). Q_P reached a maximum value of 21.5 pgP $cell^{-1}$ for A. minutum and 18.3 pgP $cell^{-1}$ for H. triquetra.

4. Discussion

This study shows that *A. minutum* dominates *H. triquetra* in three situations, (1) in conditions of moderate to severe P limitation, (2) when a PO_4 pulse succeeds to moderate or severe P limitation and (3) when PO_4 pulses are regularly (1 to 6 day intervals) added to a P limited medium. However, before definitively drawing conclusion on the effect of the PO_4 supply mode on the competition between the two species in the present experiments, the absence of other processes, which could have affected the response of both species, will be discussed. Then, the physiological processes, which can explain the dominance of *A. minutum* in conditions involving P limitation will be analysed. Finally the ecological advantages of these physiological abilities for *A. minutum* will be explored.

No effect of allelopathy, phagotrophy or planozygote formation

The present strain of *A. minutum* is known to have allelopathic properties against some species, such as *Chaetoceros gracile* and *Karenia mikimotoï* and not against other, such as *Scrippsiella trochoïdea* (Arzul et al. 1999). In the present PO_4 depleted conditions, the absence of allelopathy is supported by the similar growth response observed for each species in isolation and in mixed cultures. Therefore, the lack of allelopathic effect between *A. minutum* and *H. triquetra* observed by Erard-Le Denn et al. (2003) during the exponential growth phase in condition of excess nutrients is also supported in severe PO_4 depleted conditions. We suppose allelopathic processes did not affect the competition in the intermediate P limited conditions of the semi-continuous experiments but we cannot totally exclude it. Previous studies also reported that *H. triquetra* was capable of phagotrophic uptake of some

flagellates (Legrand et al., 1998) and dinoflagellates (Litaker et al., 2002a). However, the fact that there was no effect of the co-occurrence of the two species in the present PO_4 depleted cultures supports the absence of phagotrophy in these cultures, even though inorganic P was lacking. In addition, cell engulfment was never observed in the mixed batch and semi-continuous cultures, neither in field samples of the Penzé estuary (north Brittany, France) where both species coexist (Morin et al., 2000). Thus, our observations suggest that allelopathy and phagotrophy did not affect the competition between the present *A. minutum* and *H. triquetra* strains.

A cell volume enlargement was observed in the monospecific PO_4 depleted cultures of *H. triquetra* (up to 62 %) and *A. minutum* (up to 68 %). For *A. minutum*, a cell with a diameter above 28 µm may be a planozygote (work of Probert, 1999 on the same strain). In addition the induction of sexuality leading to the formation of planozygotes was found to occur in P limited batch cultures via the reduction of cellular N (Probert, 1999). The occurrence of planozygotes in the present cultures would change the calculation of the initial cell PO_4 uptake rate, as a planozygote is not equivalent to a single vegetative cell in term of nutrient uptake capacity. However, *A. minutum* cells above 28 µm in diameter were detected only on day 10 and represented 12 % of the total population (data not shown). There is, therefore, no significant effect of a potential induction of sexuality in the present cultures. The difference with the results of Probert (1999) could be linked to his use of a multitube (25 ml volume) approach, which was more favourable to planozygote production than our sampling from one large culture submitted to daily stirring (Anderson and Lindquist, 1985). However, even if P limitation might have affected N uptake during the growth phase compared with C fixation (increase in particulate C:N ratio), cellular N were always higher (> 150 pgN $cell^{-1}$ after day 1) than the threshold level (around 100 pgN $cell^{-1}$) thought to be necessary for gametogenesis. This confirms that the increase in *A. minutum* cell volume was not related to the formation of planozygotes. This cell enlargement was partly correlated to cell Carbon quota ($R^2 = 0.531$, $p < 0.0001$ for *A. minutum* and $R^2 = 0.761$, $p < 0.0001$ for *H. triquetra*). As cell volume normalized Q_C slightly decreased, a greater degree of vacuolation could also, to a lesser extent, explain this cell enlargement for *A. minutum* (Flynn et al., 1996).

Consequently, our results show that the PO_4 supply mode affected the competition between *A. minutum* and *H. triquetra* in the present experiments, with negligible effects of allelopathy, phagotrophy or gametogenesis.

Physiological abilities of *A. minutum* in conditions involving P limitation

The present mixed batch experiments show that *A. minutum* competed with *H. triquetra* most successfully after three days of P depletion followed either by a single PO_4 pulse or by more severe P depletion. This shift was clearly the consequence of favourable physiological acclimatations of *A. minutum* to P intracellular stress. In the monospecific cultures, this stress appeared as a sharp drop in its P cell content (Q_P).

A first acclimatation was an increase in the cell PO_4 uptake rate as highlighted by the evolution of the initial uptake rate of the PO_4 pulse with P depletion. Although both species responded to this stress by a large increase, the increase in cell PO_4 uptake rate was higher in *A. minutum* than in *H. triquetra*. When 4 µM PO_4 were added to mixed cultures, *A. minutum* monopolized the supplied PO_4 for its growth, while *H. triquetra* growth rate declined. These results show that *A. minutum* has the capacity to drastically increase its cell PO_4 uptake rate to face potential PO_4 supply. This ability represents a physiological advantage compared to *H. triquetra,* when moderate to severe P limited conditions are followed by a PO_4 enrichment. This result was confirmed when different PO_4 supply frequencies were tested in semi-continuous mode. In the monospecific cultures, four days after the beginning of the experiment, all the PO_4 pulses were consumed in less than 24 h and the P cell quotas were always higher for *A. minutum*, suggesting a higher cell PO_4 uptake rate. In the mixed cultures, *A. minutum* always outgrew *H. triquetra*. This dominance occurred earlier when the PO_4 supply interval was shorter. In these latter conditions, PO_4 concentrations after supply were lower and *A. minutum* cells, with higher PO_4 uptake rates, may have monopolized this PO_4, thus reducing *H. triquetra* growth. When the PO_4 supply interval was longer, particularly for 6 day interval, PO_4 concentrations were higher after supply. The two species may have shared this PO_4 for their growth. They balanced their growth rate with the dilution rate until finally *A. minutum* prevailed against *H. triquetra*.

The ability of *A. minutum* to store P for a delayed or progressive growth represents another favourable physiological acclimatation of *A. minutum*. In PO_4 depleted conditions, the progressive growth of *A.*

minutum during 10 days of depletion corresponded to the use of stored P (strong decrease of Q_P). In contrast, *H. triquetra* stopped growing after two days, which corresponded to lower Q_P at the beginning of the experiment. In the monospecific semi-continuous cultures, *A. minutum* cells greatly increased their P cell quota 24 hours after the 4 and 6 day PO_4 pulses with a subsequent delayed or progressive growth thereafter. *Alexandrium minutum* is, therefore, capable to store P, which can be then mobilized for growth. Conversely, *H. triquetra* cells immediately used their uptaken PO_4 to increase their cell growth rate.
The dominance of *A. minutum* in conditions involving P limitation can, therefore, be explained by higher PO_4 uptake capacity than that of *H. triquetra* and the ability to store P and use it later for growth.

Ecological advantages of P storage ability and high PO_4 uptake capacity

P storage ability is commonly evaluated from the "luxury coefficient" defined by Droop (1974). It corresponded to the ratio between the maximum P cell quota (Q_{Pmax}) determined in non-limited P situations and the minimum P cell quota (Q_{Pmin}), or subsistence quota, determined in P limited conditions. From the present experiments, the ranges of Q_P for *A. minutum* and *H. triquetra* are respectively 6.5 to 23 pgP $cell^{-1}$ and 4.1 to 18.3 pgP $cell^{-1}$. The minimum value found here for *A. minutum* Qp is lower than the one found by Probert (1999) (17 pgP $cell^{-1}$) but close to the one measured by Bechemin et al. (1999) on the same strain (5.9 pgP $cell^{-1}$). The maximum Q_P value is half of the maximum value measured by Probert (1999) in P repleted conditions (48 pgP $cell^{-1}$). To our knowledge, for *H. triquetra*, no Q_P data exist in the literature. However, previous investigations on *H. triquetra* provided a maximum Q_P of 23.3 pgP $cell^{-1}$ in P repleted conditions (unpublished data). Finally, considering values from the present study and values from the literature on the same *A. minutum* strain, the ranges of Qp for *A. minutum* and *H. triquetra* are respectively 5.9 to 48 pgP $cell^{-1}$ and 4.1 to 23.3 pgP $cell^{-1}$. The corresponding Q_{Pmax} / Q_{Pmin} ratios are respectively 8.1 and 5.7. There is no large difference between these ratios and P storage ability of *A. minutum* is lower than the storage ability observed for *A. tamarense* (36, cited in Yamamoto and Tarutani, 1999). Furthermore, freshwater species known to be competitive in P limited environments generally exhibit ratios > 30 (Ducobu et al., 1998; Spijkerman and Coesel, 1998). However, this comparison between Q_{Pmax} and Q_{Pmin} is not ecologically relevant, indeed Q_{Pmax} and Q_{Pmin} values originated from different growth situations (limited cultures or not, batch or semi-continuous) and can not indicate storage capacity. In addition, P storage capacity evaluated by the range of Q_P does not systematically reflect the actual cell production based on this nutrient stored (Spijkerman and Coesel, 1998). In contrast, the present study demonstrates, for the first time, the capacity of *A. minutum* to efficiently mobilize stored P for delayed growth. The ability of *H. triquetra* to use immediately uptaken PO_4 for cell division is also clearly highlighted by our experiments.

Among the patterns of species behaviours in the competition for nutrients - the term "behaviour" is preferred to "strategy" - *H. triquetra* is a "velocity-adapted" species for P (Sommer, 1984), a "growth" response species (Collos, 1986), which utilizes PO_4 just after supply to increase its cell division rate. *Heterocapsa triquetra* growth is, therefore, favoured in conditions of excess nutrients as previously observed (Erard-Le Denn et al., 2003; Riegmann et al. 1996). *Alexandrium minutum* appears more like a "storage specialist", which uptakes PO_4 pulses for luxury consumption (storage), survives depletion periods and, then, utilizes PO_4 for cell growth. According to Collos (1986), such behaviour is an ecological advantage when the nutrient pulsing frequency is lower than the cell division rate. Conversely, high frequency is favourable to species with a strong coupling between growth rate and nutrient uptake such as *H. triquetra*. As a consequence, in the present semi-continuous mixed cultures, *H. triquetra* should have been favoured by 1 and 2 day intervals and *A. minutum* favoured by 4 and 6 day intervals. However, surprisingly, *A. minutum* always dominated in all interval cases. In this particular case of competition, the dominance of *A. minutum* is also explained by its higher capacity of cell PO_4 uptake, as explained in the previous section. Thus both physiological abilities, storage and uptake capacity, must be taken into account to understand the outcome of the competition.
Alexandrium species were reported to have low PO_4 affinity for growth (Frangopulos et al., 2004; Matsuda et al., 1999) and uptake (Yamamoto and Tarutani, 1999), in contrast with high PO_4 affinity species, which are generally not dinoflagellates (Smayda, 1997). *Alexandrium* species should not be favoured in P limited environments. However their storage capacity balances this disadvantage, such as demonstrated here for *A. minutum* and previously suggested for *A. tamarense* (Yamamoto and Tarutani, 1999). It would allow these species to take advantage of migration from poor to rich PO_4

waters or to benefit from isolated PO_4 inputs. This is a substantial advantage to face interspecific competition.

5. Conclusion

The present study provides new information about competitive behaviours of *A. minutum* and *H. triquetra* under different PO_4 supply modes. In addition, the present investigations also offer new parameter values of the growth, phosphate uptake and storage processes of *A. minutum* and *H. triquetra,* which can be useful for ecophysiological models. This study is the first to present physiological parameters for *H. triquetra*, such as the range of Q_C, Q_N, Q_P, C:P, C:N and N:P values, which had never been measured before. Two favourable physiological acclimatations were highlighted from the present experiments for *A. minutum* compared with *H. triquetra* : (1) the ability to store P intracellularly and then mobilize it for cell division when P depletion becomes severe and (2) the capacity to increase its cell PO_4 uptake rate in higher proportion, to face potential PO_4 supply. *Alexandrium minutum* appears to be a “storage specialist” and *H. triquetra* a “velocity adapted” species. *Heteroscapsa triquetra* is favoured in the case of excess nutrients, whereas *A. minutum* is favoured, when P depleted conditions alternate with P supplies. These physiological abilities of *A. minutum* are additional advantages to face competition and may partly explain their capacity to dominate the phytoplankton community *in situ* and to form dense harmful blooms.

Acknowledgements

This research was supported by the French "Programme National Environnement Côtier" in the Thematic Research Action 3 on Harmful Algal Blooms and by the European Interreg projects, ICREW (Improving Coastal and Recreational Waters) and FINAL (Forecasting Initiation of blooms of toxic Algae). We wish to thank P. Bodenes for illustrations and reviewers for their constructive comments.

References

Aminot, A., Kerouel, R., 2004. Mesures des concentrations en carbone, azote et phosphore organiques particulaires. In: Ifremer (Ed.), Hydrologie des écosystèmes marins. Paramètres et analyses, pp. 194-214.

Anderson, D.M., Stolzenbach, K.D., 1985. Selective retention of two dinoflagellates in a well-mixed estuarine embayment: the importance of diel vertical migration and surface avoidance. Mar. Ecol. Prog. Ser. 25, 39-50.

Anderson, D.M., Lindquist, N.L., 1985. Time course measurements of phosphorus depletion and cyst formation in the dinoflagellate *Gonyaulax tamarensis* Lebour. J. Exp. Mar. Biol. Ecol. 86, 1-13.

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

Arzul, G., Gentien, P., 2005. Allelopathic interactions among marine microalgae. In: Rao, D.V.S. (Ed.), Algal cultures, analogues of blooms and applications. Science Publishers, Enfield and Plymouth, pp. 131-161.

Arzul, G., Seguel, M., Guzman, L., Erard-Le Denn, E., 1999. Comparison of allelopathic properties in three toxic *Alexandrium* species. J. Exp. Mar. Biol. Ecol. 232, 285-295.

Balech, E., 1989. Redescription of *Alexandrium minutum* Halim (Dinophyceae) type species of the genus *Alexandrium*. Phycologia 28, 206-211.

Bechemin, C., Grzebyk, D., Hachame, F., Hummert, C., Maestrini, S.Y., 1999. Effect of different nitrogen/phosphorus nutrient ratios on the toxin content in *Alexandrium minutum*. Aquat. Microb. Ecol. 20, 157-165.

Braarud, T., Pappas, I., 1951. Experimental studies on the dinoflagellate *Peridinium triquetrum* (Ehrb.) Lebour. Avh. Nor. Vidensk.-Akad. Oslo 1. Mat.-Naturvidensk. Kl. 2, 1-23.

Collos, Y., 1986. Time-lag algal growth dynamics: biological constraints on primary production in aquatic environments. Mar. Ecol. Prog. Ser. 33, 193-206.

Davidson, K., Cunningham, A., Flynn, K.J., 1993. Modelling temporal decoupling between biomass and numbers during the transient nitrogen-limited growth of a marine phytoflagellate. J. Plankton Res. 15, 351-359.

Davidson, K., Wood, G., John, E.H., Flynn, K.J., 1999. An investigation of non-steady-state algal growth. I. An experimental model ecosystem. J. Plankton Res. 21, 811-837.

Droop, M.R., 1974. The nutrient status of algal cells in continuous culture. J. Mar. Biol. Ass. U.K. 54, 825-855.

Ducobu, H., Huisman, J., Jonker, R.R., Mur, L.R., 1998. Competition between a prochlorophyte and a cyanobacterium under various phosphorus regimes: Comparison with the Droop model. Journal-of-Phycology 34, 467-476.

Erard-Le Denn, E., 1991. Recent occurrence of red tide dinoflagellate *Alexandrium minutum* Halim from the north western coasts of France. In: Park, J., Kim, H. (Eds.), Recent Approaches on Red Tides. Proceedings of "1990 Korean-French Seminar on Red tide, 9-10 Nov 1990", pp. 85-98.

Erard-Le Denn, E., Crassous, M.P., Youenou, A., Martin-Jezequel, V., Harang, B., V., M., 2003. Etude de la competition entre especes lors du developpement d'une efflorescence toxique à *Alexandrium minutum*, Rapport Ifremer PNEC-ART3, pp. 25.

Erard Le Denn, E., Chretiennot Dinet, M.J., Probert, I., 2000. First report of parasitism on the toxic dinoflagellate *Alexandrium minutum* Halim. Estuar. Coast. Shelf Sci. 50, 109-113.

Fauchot, J., Levasseur, M., Roy, S., 2005. Daytime and nighttime vertical migrations of *Alexandrium tamarense* in the St. Lawrence estuary (Canada). Mar. Ecol. Prog. Ser. 296, 241-250.

Fistarol, G.O., Legrand, C., Selander, E., Hummert, C., Stolte, W., Granéli, E., 2004. Allelopathy in *Alexandrium* spp.: effect on a natural plankton community and on algal monocultures. Aquat. Microb. Ecol. 35, 45-56.

Flynn, K., Jones, K.J., Flynn, K.J., 1996. Comparisons among species of *Alexandrium* (Dinophyceae) grown in nitrogen- or phosphorus-limiting batch culture. Mar. Biol. 126, 9-18.

Frangopulos, M., Guisande, C., deBlas, E., Maneiro, I., 2004. Toxin production and competitive abilities under phosphorus limitation of *Alexandrium* species. Harmful algae 3, 131-139.

Gaedeke, A., Sommer, U., 1986. The influence of the frequency of periodic disturbances on the maintenance of phytoplankton diversity. Oecologia 71, 25-28.

Ghosh, M., Prasad, K.V.R., Mehta, S.K., Gaur, J.P., 1999. Response of a natural phytoplankton assemblage to pulsed supply of phosphorus in semicontinuous cultures. Ann. Limnol-Int. J. Limnol. 35, 23-29.

Guillard, R.R.L., Ryther, J.H., 1962. Studies on marine plankton diatoms.I *Cyclotella nana* Husted and *Detonela confervacea* (Cleve) Gran. Can. J. Microbiol. 8, 229-239.
Guisande, C., Frangopulos, M., Maneiro, I., Vergara, A.R., Riveiro, I., 2002. Ecological advantages of toxin production by the dinoflagellate *Alexandrium minutum* under phosphorus limitation. Mar. Ecol. Prog. Ser. 225, 169-176.
Hillebrand, H., Dürselen, C.D., Kirschtel, D., Pollingher, U., Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol. 35, 403-424.
Huisman, J., Van Oostveen, P., Weissing, F.J., 1999. Species dynamics in phytoplankton blooms: incomplete mixing and competition for light. Am. Nat. 154, 46-68.
Kononen, K., Huttunen, M., Hallfors, S., Gentien, P., Lunven, M., Huttula, T., Laanemets, J., Lilover, M., Pavelson, J., Stips, A., 2003. Development of a deep chlorophyll maximum of *Heterocapsa triquetra* Ehrenb. at the entrance to the Gulf of Finland. Limnol. Oceanogr. 48, 594-607.
Labib, W., Halim, Y., 1995. Diel vertical migration and toxicity of *Alexandrium minutum* Halim red tide, in Alexandria, Egypt. Mar. Life 5, 11-17.
Legrand, C., Graneli, E., Carlsson, P., 1998. Induced phagotrophy in the photosynthetic dinoflagellate *Heterocapsa triquetra*. Aquat. Microb. Ecol. 15, 65-75.
Lindholm, T., Nummelin, C., 1999. Red tide of the dinoflagellate *Heterocapsa triquetra* (Dinophyta) in a ferry-mixed coastal inlet. Hydrobiologia 393, 245-251.
Litaker, R.W., Tester, P.A., Duke, C.S., Kenney, B.E., Pinckney, J.L., Ramus, J., 2002a. Seasonal niche strategy of the bloom-forming dinoflagellate *Heterocapsa triquetra*. Mar. Ecol. Prog. Ser. 232, 45-62.
Litaker, R.W., Warner, V.E., Rhyne, C., Duke, C.S., Kenney, B.E., Ramus, J., Tester, P.A., 2002b. Effect of diel and interplay variations in light on the cell division pattern and in situ growth rates of the bloom-forming dinoflagellate *Heterocapsa triquetra*. Mar. Ecol. Prog. Ser. 232, 63-74.
Lu, S., Hodgkiss, I.J., 2004. Harmful algal bloom causative collected from Hong Kong waters. Hydrobiologia 512, 231-238.
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.
Morin, P., Erard-Le Denn, E., Maguer, J.F., Madec, C., Videau, C., Le Grand, J., Mace, E., 2000. Etude des causes de proliférations de microalgues toxiques en mer : cas d' *Alexandrium*. Université Bretagne Occidentale, Ifremer Brest, 135 pp.
Oda, T., Sato, Y., Kim, D., Muramatsu, T., Matsuyama, Y., Honjo, T., 2001. Hemolytic activity of *Heterocapsa circularisquama* (Dinophyceae) and its possible involvement in shellfish toxicity. J. Phycol. 37, 509-516.
Park, M.G., Yih, W., Coats, D.W., 2004. Parasites and phytoplankton, with special emphasis on dinoflagellate infections. J. Euk. Microbiol. 51, 145-155.
Passow, U., 1991. Vertical migrations of *Gonyaulax catenata* and *Mesodinium rubrum*. Mar. Biol. 110, 455-463.
Probert, I., 1999. Sexual reproduction and ecophysiology of the marine dinoflagellate *Alexandrium minutum* Halim. PhD thesis, University of Westminster, Ifremer Brest, London, pp. 99.
Riegman, R., De Boer, M., De Senerpont Domis, L., 1996. Growth of harmful marine algae in multispecies cultures. J. Plankton Res. 18, 1851-1866.
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., Reynolds, C.S., 2001. Community assembly in marine phytoplankton: application of recent models to harmful dinoflagellate blooms. J. Plankton Res. 23, 447-461.
Solorzano, L., Sharp, J.H., 1980. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnol. Oceanogr. 25, 754-758.
Sommer, U., 1984. The paradox of the plankton: fluctuations of phosphorus availability maintain diversity of phytoplankton in flow-through cultures. Limnol. Oceanogr. 29, 633-636.
Spijkerman, E., Coesel, P.F.M., 1998. Different response mechanisms of two planktonic desmid species (chlorophyceae) to a single saturating addition of phosphate. J. Phycol. 34, 438-445.
Tillmann, U., John, U., 2002. Toxic effects of *Alexandrium* spp. on heterotrophic dinoflagellates: an allelochemical defence mechanism independent of PSP-toxin content. Mar. Ecol. Prog. Ser. 230, 47-58.
Tréguer, P., Le Corre, P., 1975. Manuel d'analyse des sels nutritifs dans l'eau de mer. Université de Bretagne Occidentale, Brest, 110 pp.
Uchida, T., Toda, S., Matsuyama, Y., Yamaguchi, M., Kotani, Y., Honjo, T., 1999. Interactions between the red tide dinoflagellates *Heterocapsa circularisquama* and *Gymnodinium mikimotoi* in laboratory culture. J. Exp. Mar. Biol. Ecol. 241, 285-299.

Usup, G., Pin, L.C., A., A., Teen, L.P., 2002. *Alexandrium* (Dinophyceae) species in Malaysian waters. Harmful Algae 1, 265-275.
Vila, M., Giacobbe, M.G., Masó, M., Gangemi, E., Penna, A., Sampedro, N., Azzaro, F., Camp, J., Galluzzi, L., 2005. A comparative study on recurrent blooms of *Alexandrium minutum* in two Mediterranean coastal areas. Harmful Algae 4, 673-695.
Yamamoto, T., Tarutani, K., 1999. Growth and phosphate uptake kinetics of the toxic dinoflagellate *Alexandrium tamarense* from Hiroshima Bay in the Seto Inland Sea, Japan. Phycological Res. 47, 27-32.
Yamamoto, T., Hatta, G., 2004. Pulsed nutrient supply as a factor inducing phytoplankton diversity. Ecol. Model. 171, 247-270.
Yoshida, M., Ogata, T., Thuoc, C.V., Matsuoka, K., Fukuyo, Y., Hoi, N.C., Kodama, M., 2000. The first finding of toxic dinoflagellate *Alexandrium minutum* in Vietnam. Fisheries Science 66, 177-179.

Figures

Fig. 1 : Diagram of batch culture experiments (A and B) including monospecific cultures of *Alexandrium minutum* (*Am*) and *Heterocapsa triquetra* (*Ht*) and mixed cultures of both (*Am* + *Ht*)

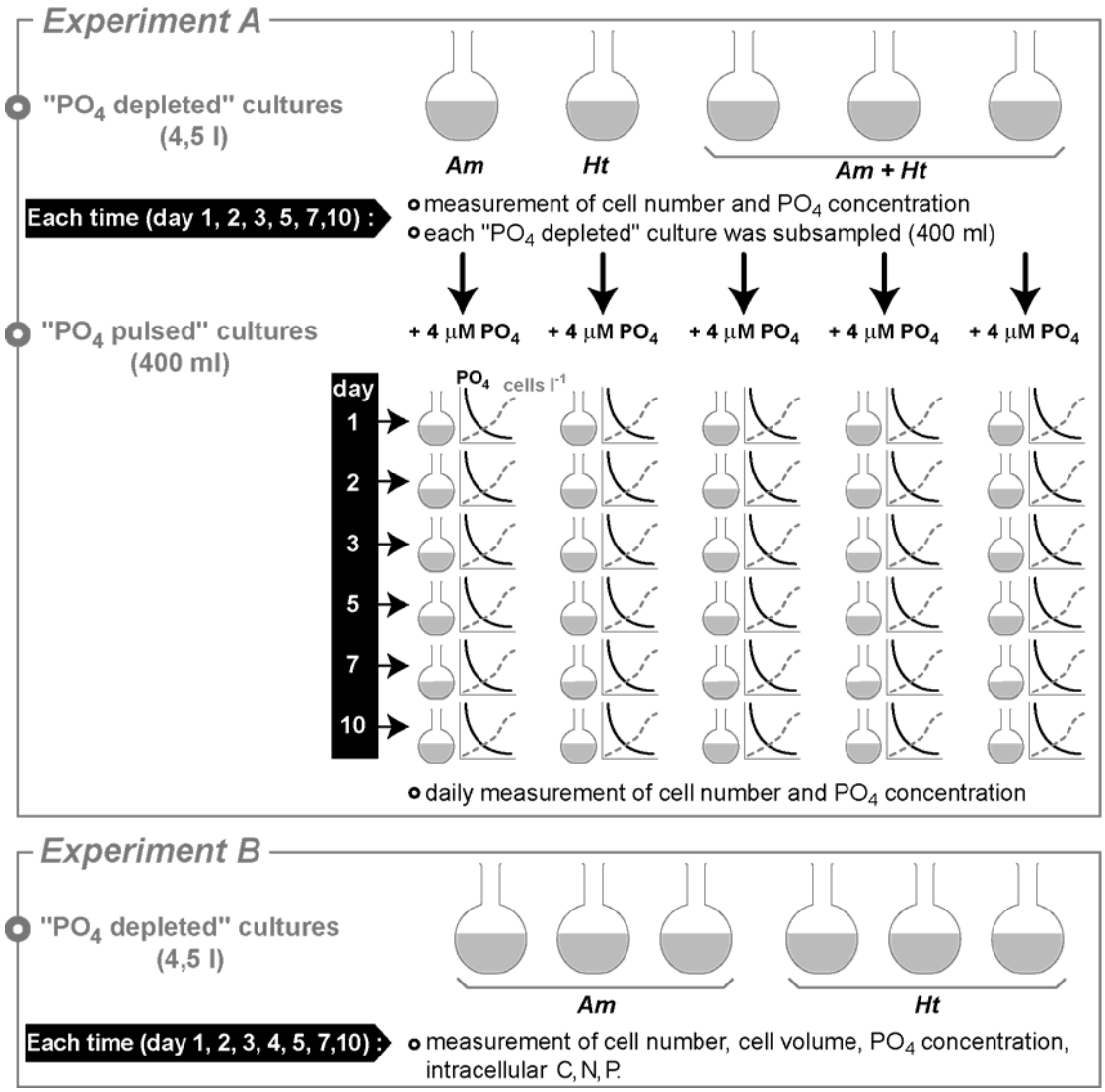


Fig. 2 : Evolution of *A. minutum* (Am) and *H. triquetra* (Ht) cell concentrations in the "PO_4 depleted" monospecific cultures (a, exp. A and B) and mixed cultures (b, exp. A). Symbols linked with full line correspond to exp. A and with dashed line to exp. B. Error bars illustrate the standard deviation corresponding to culture triplicates

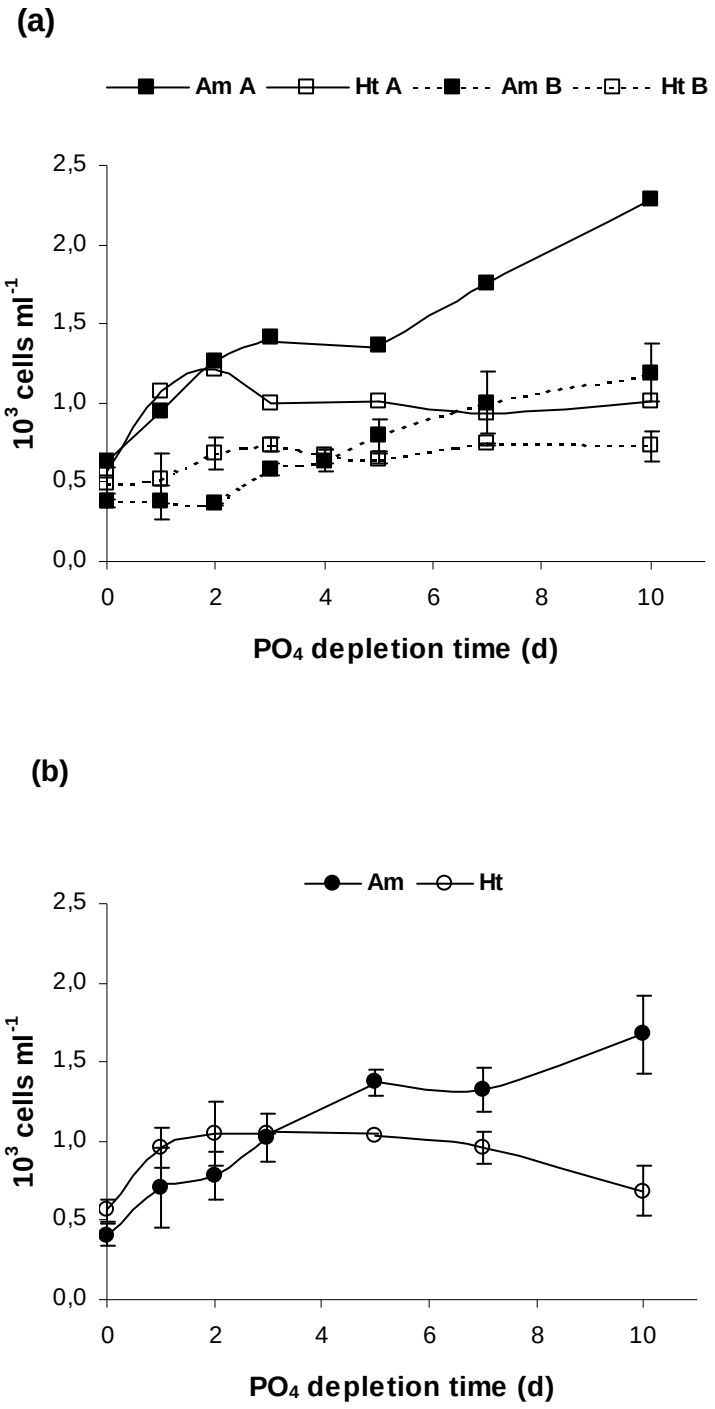


Fig. 3 : Evolution of Q_P, Q_C, Q_N (a, b, c), C:P, N:P and C:N (d, e, f) atomic ratios in the "PO_4 depleted" monospecific cultures (exp. B) of *A. minutum* (*Am*) and *H. triquetra* (*Ht*) versus the PO_4 depletion time. Error bars illustrate the standard deviation corresponding to culture triplicates.

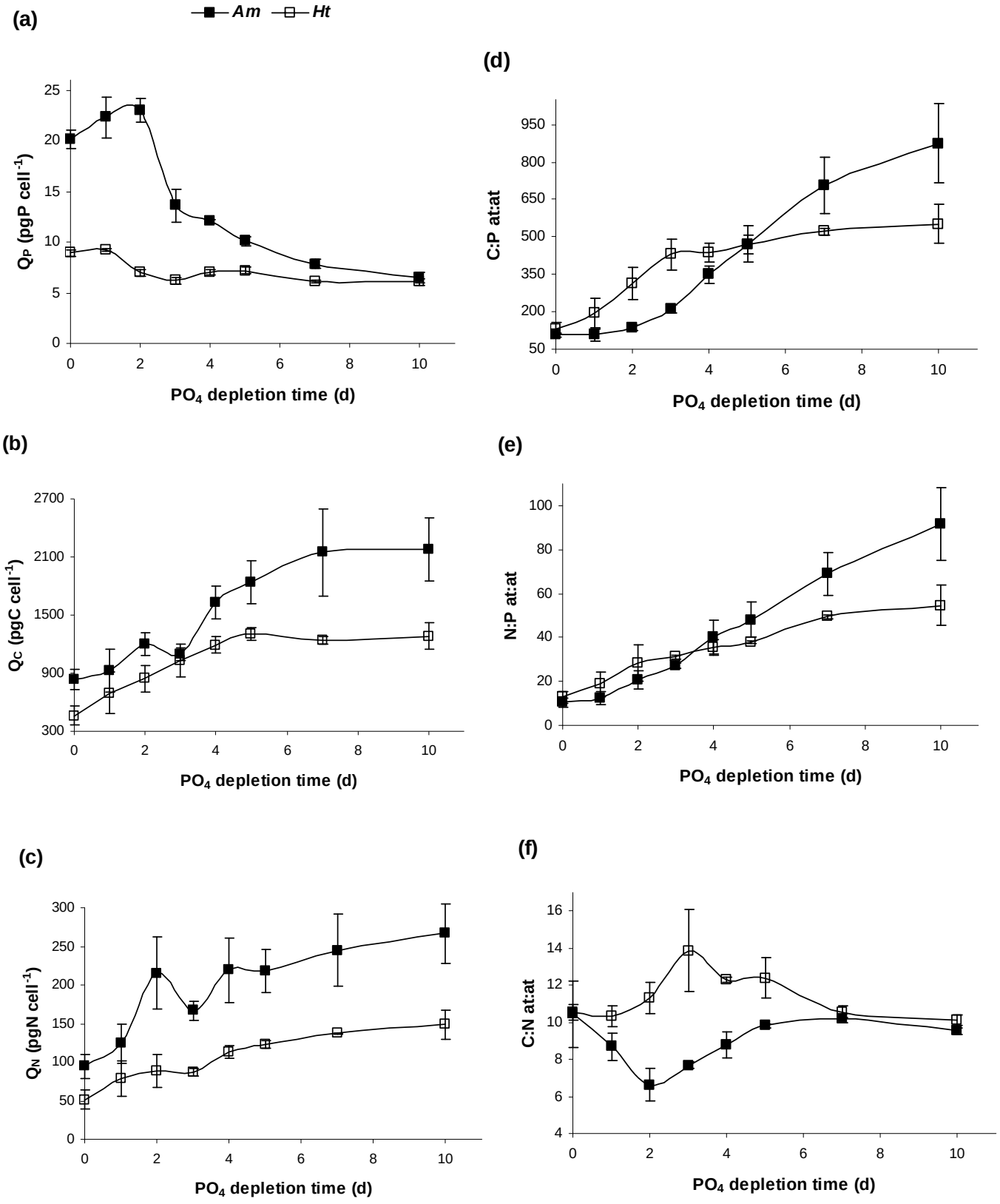


Fig. 4 : Evolution of the cell volume (a) and normalized Q_C (i.e. Q_C per unit of cell volume) (b) in the "PO_4 depleted" monospecific cultures (exp. B) of *A. minutum* (*Am*) and *H. triquetra* (*Ht*) versus the PO_4 depletion time. Error bars illustrate the standard deviation corresponding to 300 analysed cells.

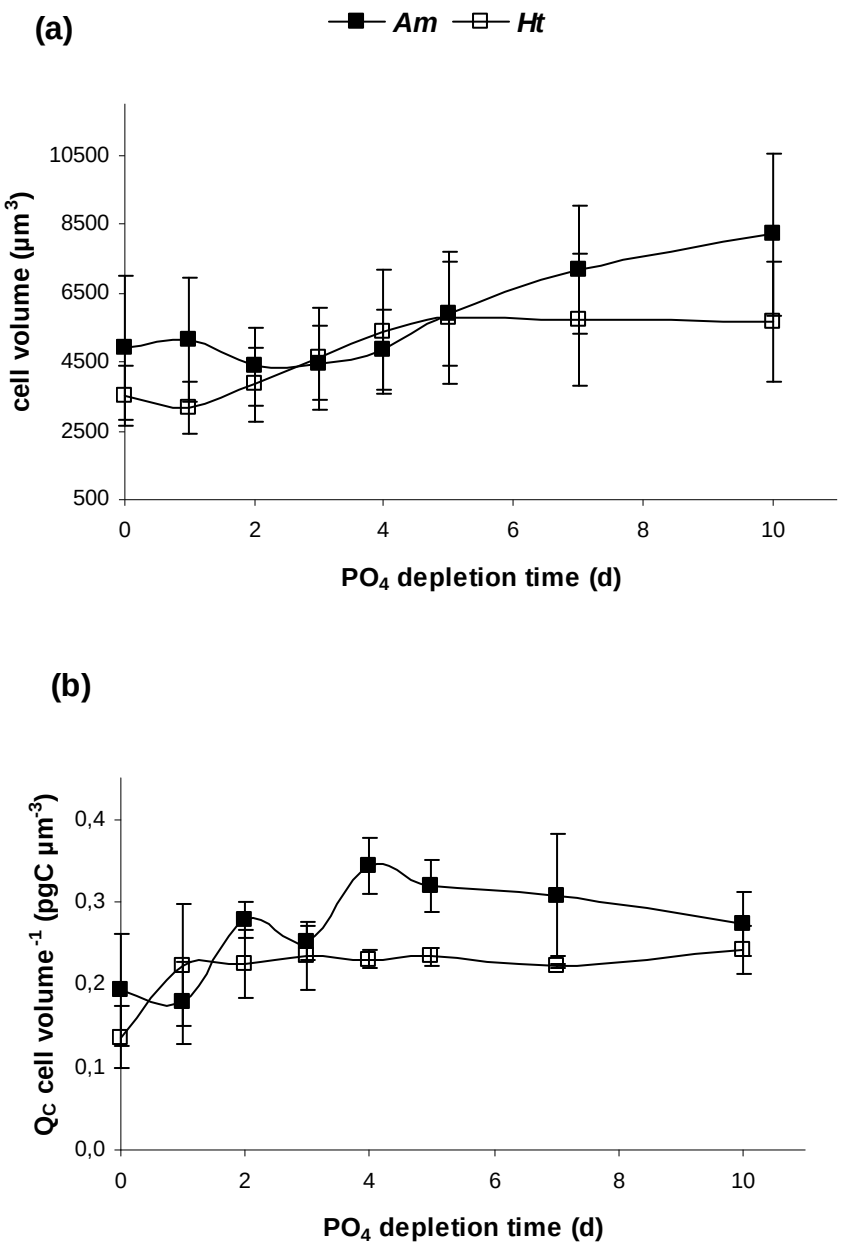


Fig. 5 : Evolution of *A. minutum* (*Am*) and *H. triquetra* (*Ht*) cell concentrations in the "PO_4 pulsed" mixed cultures after 1, 2, 3, 5, 7 or 10 day exposure to a PO_4 depleted medium (exp. A). Error bars illustrate the standard deviation corresponding to culture triplicates

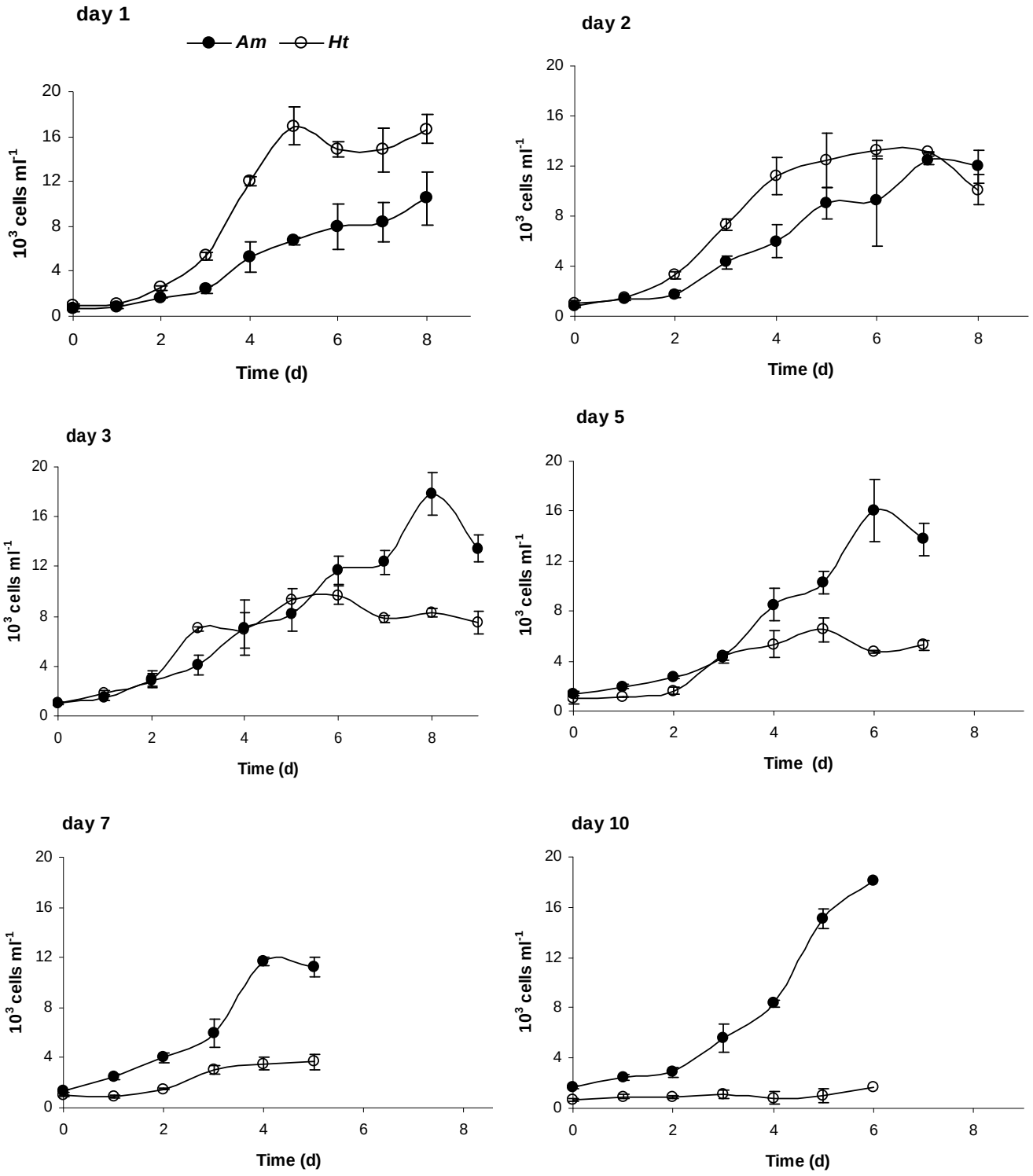


Fig. 6 : Evolution of specific growth rate μ (a, c) and stationary phase cell concentrations (b, d) of *A. minutum* (*Am*) and *H. triquetra* (*Ht*) in the "PO_4 pulsed" mixed (a, b) and monospecific (c, d) cultures (exp. A) versus the previous PO_4 depletion time. Error bars correspond to 95 % confidence interval.

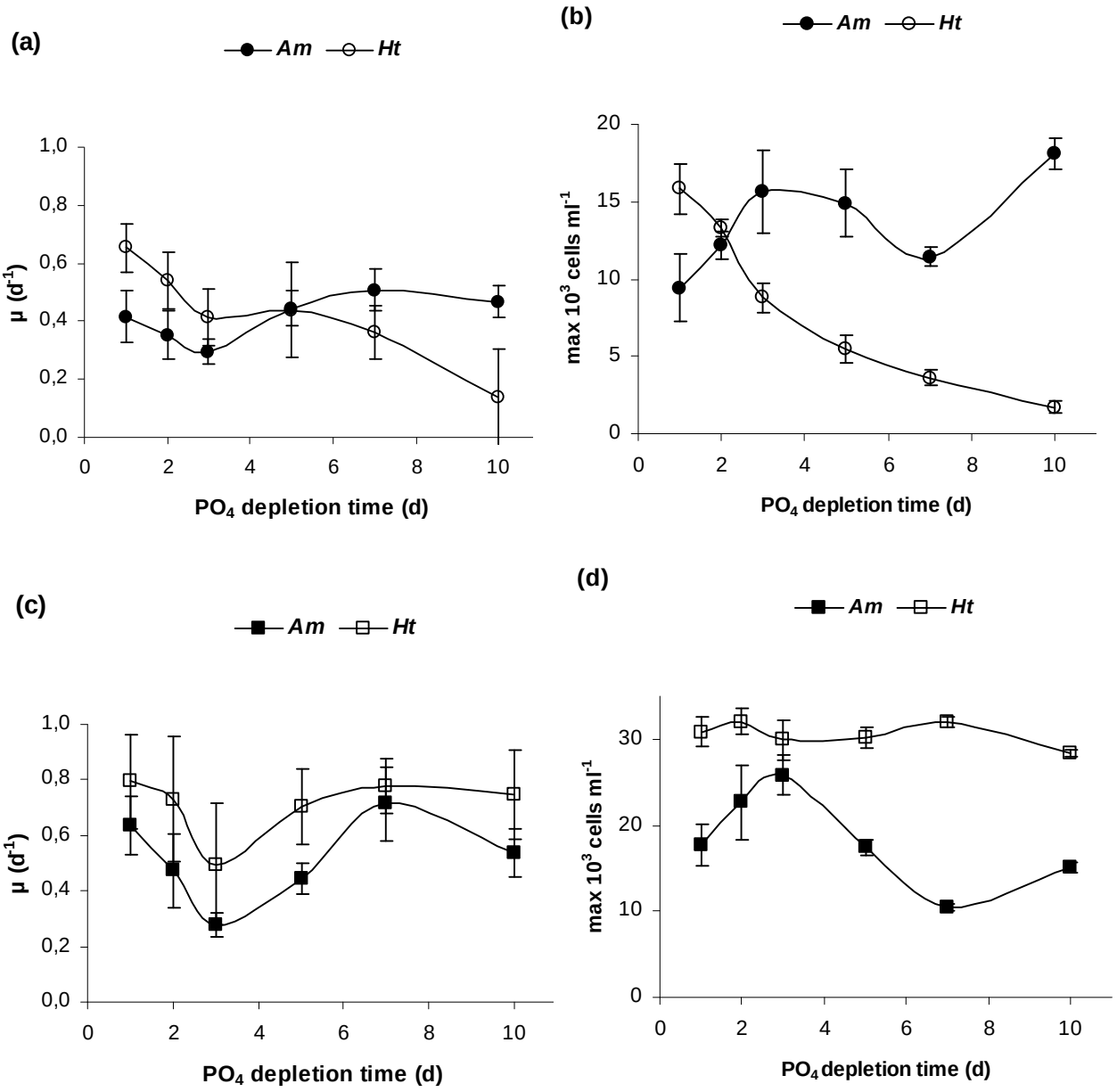


Fig. 7 : Evolution of the initial cell PO_4 uptake rate in the "PO_4 pulsed" monospecific cultures of *A. minutum* (*Am*) and *H. triquetra* (*Ht*) versus the previous PO_4 depletion time (exp. A). After three days, *A. minutum* PO_4 uptake rate was underestimated (dotted line) as the sampling time interval (24 h) was too long to estimate an actual uptake rate.

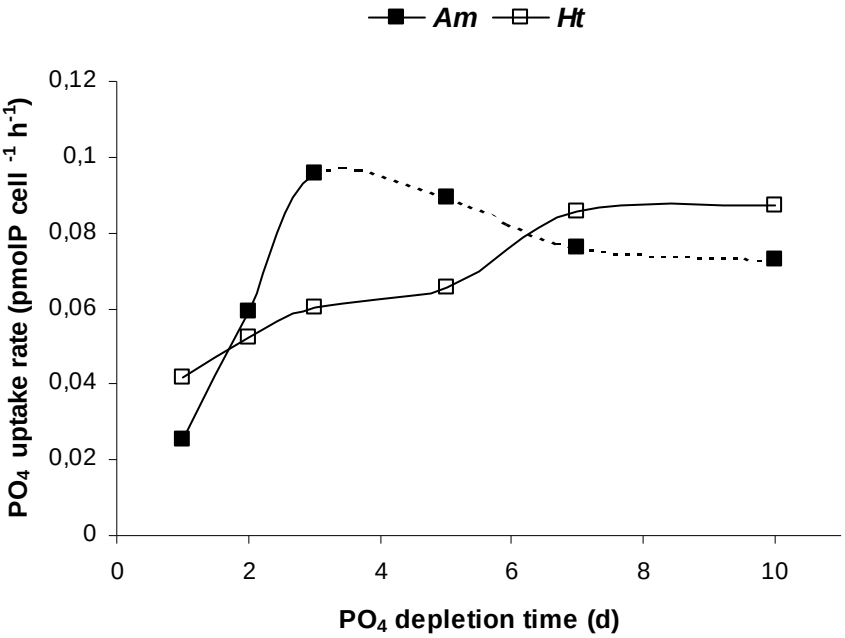


Fig. 8 : Evolution of *A. minutum* (*Am*) and *H. triquetra* (*Ht*) cell concentrations in the semi-continuous monospecific (square) and mixed (circle, +) cultures, to which PO_4 was added every 1, 2, 4 or 6 days. Weak *A. minutum* cell concentrations at 13 days over 1 and 2 day intervals were probably artefacts because of incomplete homogeneisation. Arrows indicate the day of PO_4 supply. One-day intervals are not illustrated. Error bars illustrate the standard deviation corresponding to culture replicates

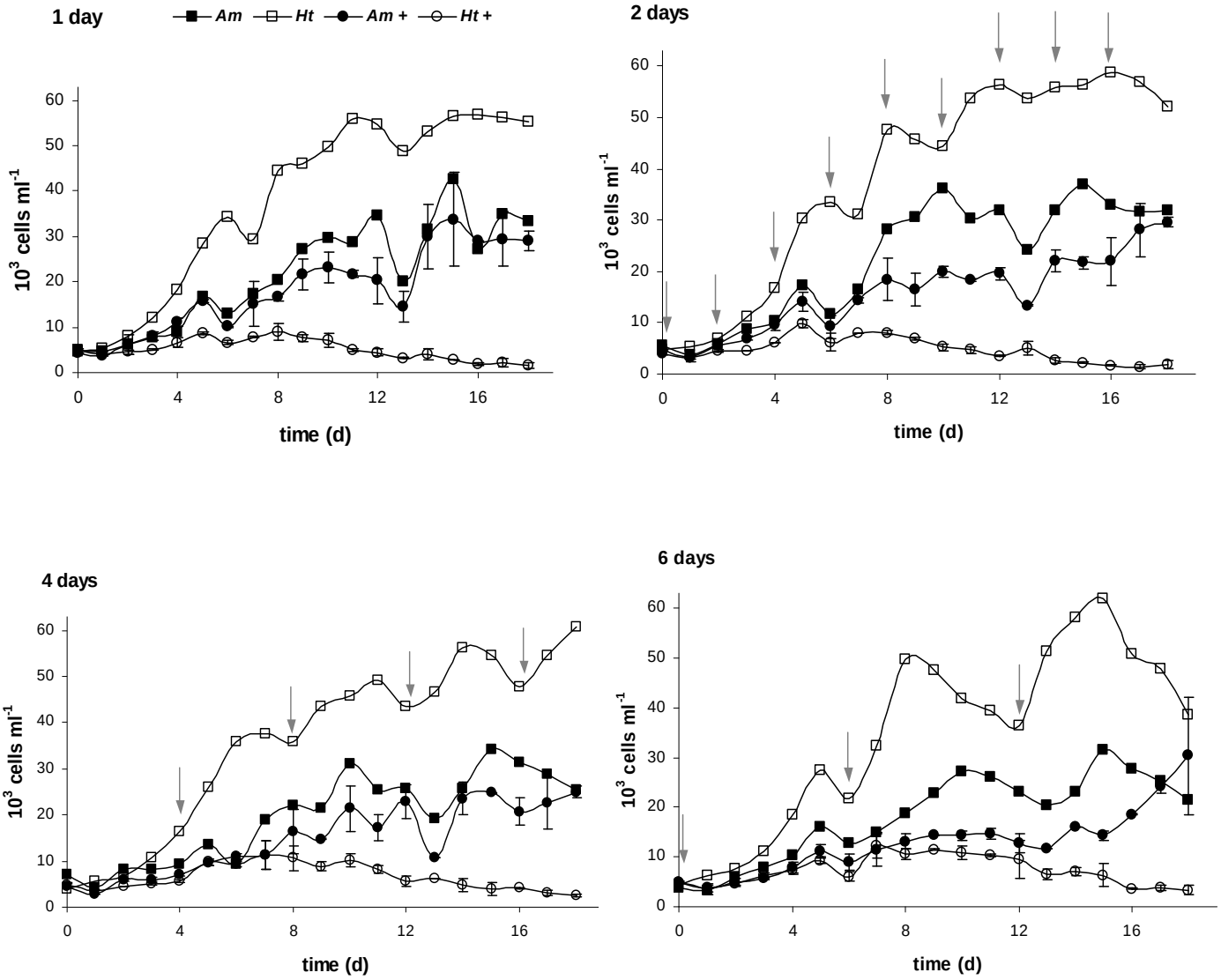
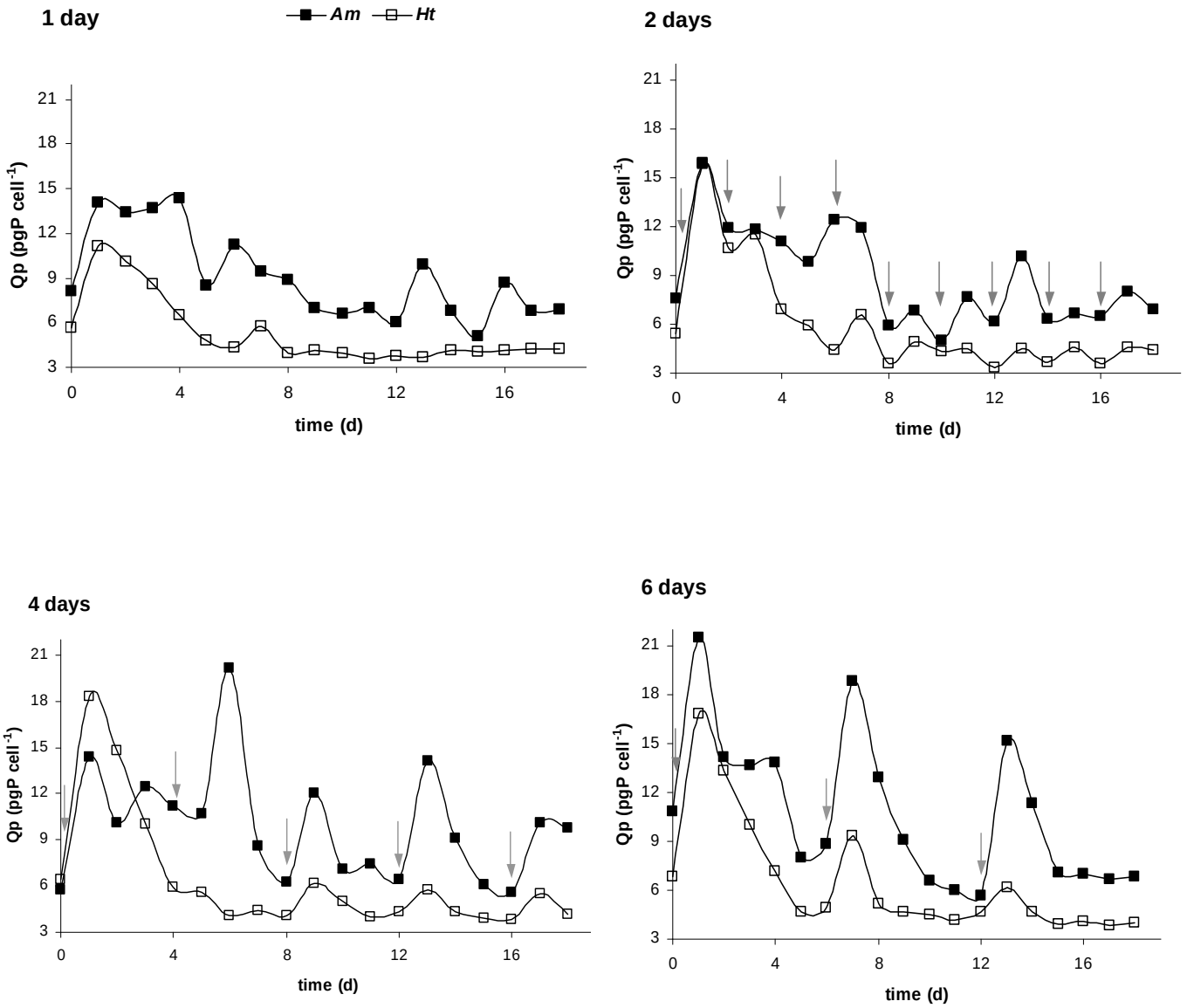


Fig. 9 : Evolution of Q_P in the monospecific semi-continuous cultures of *A. minutum* (*Am*) and *H. triquetra* (*Ht*) to which PO_4 was added every 1, 2, 4 or 6 days. High *A. minutum* Q_P at 13 days over 1 and 2 day intervals were probably overestimated since incomplete homogeneisation may have generated abnormally low cell concentrations. Arrows indicate the day of PO_4 supply. One-day intervals are not illustrated.



Table

Table 1 : Mean specific growth rate ± 95 % confidence interval of *A. minutum* and *H. triquetra* in the "PO_4 pulsed" mixed and monospecific cultures versus the previous PO_4 depletion time (exp. A).

PO_4 depletion time (d)	***A. minutum* growth rate (d^{-1})**		***H. triquetra* growth rate (d^{-1})**	
	monosp.	mixed	monosp.	mixed
1	0.63 ± 0.11	0.42 ± 0.09	0.80 ± 0.17	0.65 ± 0.08
2	0.47 ± 0.13	0.35 ± 0.08	0.73 ± 0.22	0.54 ± 0.10
3	0.28 ± 0.04	0.30 ± 0.04	0.50 ± 0.22	0.41 ± 0.10
5	0.44 ± 0.05	0.45 ± 0.06	0.70 ± 0.14	0.44 ± 0.16
7	0.71 ± 0.13	0.51 ± 0.07	0.78 ± 0.10	0.36 ± 0.09
10	0.54 ± 0.09	0.47 ± 0.06	0.75 ± 0.16	0.14 ± 0.17