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Are mussels and oysters capable of reducing the abundances of *Picochlorum* sp., responsible for a massive green algae bloom in Thau lagoon, France?

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Abstract :

After an exceptional hydroclimatic year, a “massive green algae bloom”, dominated by the autotrophic picoeukaryote Picochlorum sp, was observed in 2018–2019 in the Thau lagoon. Oyster farmers informed decision-makers and scientists about an alarming halt to growth, loss of flesh weight and abnormal mortality in commercial size oysters, whereas mussel farmers observed no adverse effects. Two hypotheses were tested. First, Mediterranean mussels are capable of filtering Picochlorum spp. whereas Pacific and flat oysters are not. Second, picophytoplankton filtration rates vary with the size of the oyster, spats having greater ability than juveniles and commercial size oysters. A series of experiments was conducted in February 2019 using a series of beakers containing (i) three different types of solutions (1 L): water from the Thau lagoon containing Picochlorum at a rate of 45.2 million cells.L−1 (P) or diluted to 20.7 million cells. L−1 (D) and a solution of Isochrysis galbana containing 9.4 million cells. L−1; (ii) in the absence or presence of five types of bivalves: Pacific oysters of three different sizes (spat, juvenile and commercial size), flat oysters, and Mediterranean mussels of commercial size. Four separate water samples were taken during a 40-min incubation period to measure fluctuations in picophytoplankton and nanophytoplankton abundances using flow cytometry. Retention efficiency (%) was compared according to species and size. In contrast to Pacific and flat oysters, mussels with an average weight of 21.4 g, were able to drastically reduce the abundances of Picochlorum and depleted nearly one million Picochlorum cells per minute at 11.4 °C. These results suggest that the 1228 t of mussels that died in 2018 before the Picochlorum bloom could have helped limit the Picochlorum bloom if they had survived the heat and anoxia.

Highlights

► Unlike oysters, mussel can drastically reduce the abundance of 1–3-µm picophytoplankton. ► Oysters of commercial size cannot retain <2-µm picoeukaryotes and cyanobacteria. ► The ability of Pacific oyster spat to retain 2–3-µm picoeukaryotes was significant.

Keywords : Crassostrea gigas, Ostrea edulis, Mytilus galloprovincialis, Filtration, Cytometry, Phytoplankto, n Picoeukaryote, Picocyanobacteria

1. Introduction

Coastal environments are currently threatened by global change, including the effects of excessive anthropogenic inputs and climate change. Several intense microalgae blooms (Galimany et al. 2020), anoxia (Fallensen et al. 2000, Diaz and Rosenberg 2011) and massive mortality events (Diaz and Rosenberg 1995, Lomstein et al. 2006, Thronson and Quigg 2008) have been recorded worldwide that had significant impacts on biodiversity, fisheries and aquaculture.

This was the case of the Thau lagoon in 2018-2019. Thau lagoon, located on the Mediterranean Sea in the south of France, was a productive environment, where 7,856 t of Pacific oysters (*Crassostrea gigas*), 2,843 t of Mediterranean mussels (*Mytilus galloprovincialis*) were produced in 2017 (Le Gal 2019), along with flat oysters (*Ostrea edulis*) to a lesser extent. While no problem of oxygen had been observed since 2006 (Derolez et al. 2020a), in summer 2018, an anoxic event had a severe impact on the functioning of the ecosystem. This event destroyed the entire stock of mussels (1,218 t) and more than 30% of oyster production (2,703 t), causing losses of nearly 5.9 million euros (Prefect of Hérault 2018). This phenomenon also led to the mortality of many fish species and benthic invertebrates (Richard & Fiandrino 2018). The anoxic event was followed in the autumn of 2018 by a massive bloom, dominated by picophytoplankton of the genus *Picochlorum* sp. (< 3 µm autotrophic picoeukaryote: Lagarde et al. 2021). Since the colour of the water was surprisingly green, we characterized this phenomenon as “massive green algae bloom”. An exceptional biomass of 26.8 $\mu g.L^{-1}$ was observed in December 2019 in the Marseillan farming area of the lagoon (Fig. 1A, REPHY, 2021). Such exceptional phytoplankton biomasses have not been observed since the creation of Ifremer's phytoplankton observation and monitoring network in Thau lagoon in 1998 (REPHY 2021, Derolez et al. 2020b). The phytoplankton in Thau lagoon were reported to be composed of pico, nano- and micro-phytoplankton (Vaquer et al. 1996), and the picophytoplankton of

phycoerythrin-rich picocyanobacteria and autotrophic picoeukaryotes (Bec et al. 2011). Typically, the highest abundances of picoeukaryotes are found in summer (Vaquer et al. 1996), and their contribution to Chl*a* biomass can be high in the Thau lagoon in this period (Bec et al. 2005), with the smallest autotrophic picoeukaryote of the genus, *Ostreococcus tauri* as the main component (Vaquer et al. 1996, Bec et al. 2005). Nevertheless, the contribution of picoeukaryotes had never previously reached more than 75% in terms of biomass and almost 100% in terms of abundances as it did during the massive green algae bloom (Lagarde et al. 2021), with record abundances of one to 1,4 billion cells per liter observed in January 2019 (Lagarde et al. 2021). Picophytoplankton in the Thau lagoon are usually well controlled by protists (Bec et al. 2005), and a bloom of this size has never previously been observed. As observed by the Ifremer oyster observatory (Ecoscopa), the bloom arrested growth and caused loss weight of flesh in Pacific oysters in the autumn of 2018 and the winter of 2019 (Fig. 1B, C, Fleury et al. 2020). During the same period, oyster mortalities were declared to policy makers. Analyses performed by the Ifremer mollusc pathology network (REPAMO) showed that the mortalities were not linked to a referenced pathogen. A similar massive bloom caused by this kind of picophytoplankton *(*formerly *Nannochloris*) was reported in the Salses-Leucate lagoon in the 1980s (Boutière et al. 1982), and led to the decline of oysters and mussels in the farms (Boutière et al. 1982). Scientists linked this to a malnutrition phenomenon, which was assumed to caused by the excessive number of particles in the water (Boutière et al. 1982).

Given the unprecedented nature of the phenomenon and its impact on the shellfish farming profession, the State authorities responsible for the shellfish farming sector asked regional scientists to examine the links between *Picochlorum* and farmed shellfish, in an experimental laboratory study.

Farmed shellfish are filter-feeding bivalves, i.e., they have the ability to filter water and the particles it contains through their gills. The anatomy of bivalves varies with the species (Gosling

et al. 2015). Mussels and Pacific and flat oysters have a mantle, gills and labial palps, equipped with cilia, to circulate water and direct nutrient particles into their mouth (Winter 1978, Gosling 2015) where the particles are ingested and transferred to their digestive system or released as pseudo-faecal matter (Gosling 2015) through the exhalation siphon in the case of mussels or the mantle in the case of oysters. As Pouvreau et al. (1999) wrote: food uptake by suspension feeders depends on 1) retention efficiency (RE) which is the fraction of particles of a given size retained during one single passage across the gill, and 2) pumping rate (PR) defined as the volume of water processed by the bivalve per unit time. Riisgard et al. (2001) noted that different terms are used in the literature for expressing the rate of water processing of bivalves and this sometimes causes confusion. Riisgard et al. (2001) state that the definition of filtration rate is the same as pumping rate but generally differs from clearance rate, which is defined as volume of water completely cleared of suspended particles per unit of time. Nevertheless, the filtration rate can be equivalent to the clearance rate if the suspended particles are 100% efficiently retained by the bivalve gills (Riisgard et al. 2001, Gosling et al. 2005). Riisgard et al. (2001) presented a review of methods to analyse these parameters to determine filtration capacities of bivalves.

Thus, the literature shows that the filtration capacities (pumping, retention and so clearance rates) differ in the three studied species. At equal dry weight (1 g) and at 15 °C, clearance rate of Mediterranean mussel was estimated to 4.08 litres of water in one hour, vs. an equivalent volume of 3.7 litres per hour for the Pacific oyster and the flat oyster (Supplementary material, see references and Fig1 for estimation). Their filtration rate increases with the weight of the organism following an allometric law (Winter 1978, Gosling 2015). Thus, when these rates are related to weight, smaller organisms have higher filtration activity than larger ones (Winter 1978). These relationships are also positively influenced by temperature (Gosling 2015, Koojman et al. 2010). According to relationships reported in the literature (Supplementary

material-Table 1), the clearance rate of a 1-g dry-weight bivalve is a function of the temperature gradient and species, with a mean clearance rate ranging from 1.5 to 9.5 $L.DWg^{-1}.h^{-1}$ for *Crassostrea gigas*, vs. 1.7 to 7.2 $L.DWg^{-1}.h^{-1}$ for *Ostrea edulis* and 1.8 to 9.4$L.DWg^{-1}.h^{-1}$ for *Mytilus galloprovincialis* at 6°C and 25°C respectively (Supplementary material-Figure 1). In addition to their filtration rates, particle retention efficiency is known to vary with the size of the particle and the species of bivalve (Riisgård 1988). Commercial size oysters are known to totally retain particles larger than 4-5 µm (Barillé et al. 1993, Dupuy et al. 2000a). They thus ingest prokaryotes adsorbed to suspended matter, nano- (3-20 µm) and micro- (20-200 µm) plankton, associated with the functional groups: protists, phytoplankton and zooplankton (Barillé et al. 1993, Dupuy et al. 1999, 2000a, Troost et al. 2009). While commercial size oysters were previously reported to be unable to efficiently retain picoparticles (0-3 µm) (Dupuy et al. 2000a), a recent *in situ* study showed that spat of Pacific oysters were able to reduce autotrophic picoeukaryote abundances (Richard et al. 2019). The capacity to retain small particles, such as picoplankton, thus varies according to the size of the oyster and to the anatomy of its gill system, which is known to change during development (Cannuel & Beninger 2006). However, a recent study in the Thau Lagoon (Correia-Martins et al. 2022) suggested that picophytoplankton are poorly retained by the newly developed gills of *Crassostrea gigas* post-larvae, which could be critical for larval settlement and metamorphosis in overabundances. The majority of studies of the retention capacity of mussels (Strohmeier et al. 2012a, Cranford et al. 2016, Sonier et al. 2016) have concerned blue mussels (*Mytilus edulis*). A series of experiments (Cranford et al. 2016) showed that mussels had a maximum particle retention rate > 8-11 µm and that this rate gradually decreased, with a retention rate of 50-60% for particles of 4 µm and of 30-40% for particles of 2 µm. Sonier et al. (2016) reported a 20% retention rate for particle sizes ranging from 0.2 to 2 µm, while Strohmeier et al. (2012) reported retention rates ranging between 14-64% and 12-86% for particles of 1 µm and 4 µm, respectively. Other species of

mussels, including *Mytella Charruana*, were also shown to able to reduce the abundances of bacteria by filtration (Galimany et al. 2017).

These different reports raise questions about the capacity of the different bivalve species reared in the Thau lagoon to filter *Picochlorum sp.*. During the massive green algae bloom, several shellfish farmers observed that the presence of mussels in the storage basins allowed the water to become clearer over time, which was not the case in the presence of commercial size oysters. Other farmers reported having observed the phenomenon with oyster spats. In the present study, two hypotheses were tested. First, Mediterranean mussels are capable of filtering *Picochlorum* that Pacific and Flat oysters cannot. Second, picophytoplankton filtration varies with the size of the bivalve, and oyster spat has a higher filtration capacity than juvenile or commercial size oysters. A series of incubations was carried out with different species of bivalves (*Crassostrea gigas, Mytilus galloprovincialis, Ostrea edulis*) and with Pacific oysters of different sizes (spat, juvenile, commercial size). As the overall efficiency of particle retention can also vary depending on the concentration of particles (Barillé et al. 1993) and the nature of the seston (Shumway et al. 1985, Barillé et al. 1993, Dupuy et al. 2000a), the experiments were conducted using phytoplankton solutions containing varying concentrations of pico- and nano-phytoplankton. To evaluate retention efficiency, three types of solutions were used: water from the Thau lagoon diluted to different extents, and a culture solution of one nanophytoplankton species, *Isochrysis galbana*, which is frequently used as feed for bivalves to evaluate retention efficiency (Wilson 1983, Riisgård 1988).

2. Materials and methods

The experiment was carried out between the 8th and 13th of February, 2019 at the Ifremer research station in the city of Sète, in an unheated room in order to be as close as possible to the water temperature of the Thau lagoon (9 °C) at that time of year.

2.1. Biological material

2.1.1. Phytoplankton solution

Samples were taken on the 11th of February 2019 at the Bouzigues station in the Thau lagoon to measure the concentration of *Picochlorum* using flow cytometry in order to prepare the dilution strategy. The concentration of *Picochlorum* was estimated at 60 x 10^6 cells.L^{-1}.

Three phytoplankton solutions were prepared from this sample: the first, called solution P, corresponded to raw water containing *Picochlorum*. To prepare the two other solutions, the lagoon water (P) was filtered on 0.7 µm GF/F filters to obtain water (F) free of pico- and nano-phytoplankton, but with the same salinity (36) and chemical composition as that of P. The second solution (D) was prepared with 8.33 L of P (theoretically 60 x 10^6 cells.L^{-1}) diluted in 11.67 L of filtered seawater F in order to approximate the concentrations usually observed in the Thau lagoon during the same period (25 x 10^6 cells.L^{-1} : Bec et al. 2005). The third solution was prepared with filtered seawater (F) inoculated with a strain of *Isochrysis galbana* (I) usually used to feed bivalves in hatcheries. This strain (concentration: 10^{10} cells.L^{-1}) was supplied by GreenSea (Mèze, Hérault/Occitanie, France). If 50 mL of *Isocrhysis galbana* are used to inoculate 19.95 L of filtered seawater, the theoretical concentration of *Isochrysis galbana* should be around 25 x 10^6 cells. L^{-1} i.e. as close as possible to the concentrations of picophytoplankton in solution D. The effect of bivalve filtration has not been tested on *Isochrysis galbana* at higher concentration since it was impossible to carry out 72 incubations on the same day with the staff (3 scientists) and equipment (54 beakers) available for the study. The P, D and I solutions were homogenised using magnet bars and acclimatised in refrigerated chambers at 9 °C, i.e. the temperature of the Thau lagoon during sampling.

2.1.2 Bivalves

Three species of bivalves were used in the experiment: the Pacific oyster (C: *Crassostrea gigas)*, the flat oyster (O: *Ostrea edulis*) and the Mediterranean mussel (M: *Mytilus galloprovincialis*). The flat oysters were bred and supplied by *Le cercle des huîtres* (Jean-Marc Deslous Paoli), a shellfish farm corporation in the Thau lagoon. The mussels, originating from Vigo in Spain, were supplied by Gaec Le Rocher (Emmanuel Fournier). Three sizes of Pacific oyster were used: oyster spat (s) that passed through a 6-mm sieve (Cs), juvenile (j) oysters of the appropriate size to be stuck on ropes and pass through a 20 mm sieve (Cj) and individuals of the appropriate size to be marketed, and corresponding to calibre 3, i.e. weighing between 66 and 85 g (C). C were reared in the Thau lagoon in the Bouzigues shellfish farm zone by Deslous Paoli. The Cs and Cj were supplied by the France Naissain hatchery. Prior to the experiments, on the 8th of February 2019, 30 individuals of each type of bivalve were measured using a calliper and weighed on a precision balance at the Ifremer Sète station. The average size and total wet weight (shell + flesh) of the Cs individuals were 12.4 mm & 0.25 g; for Cj: 38.7 mm & 3.8 g; for C: 82.5 mm & 71.6 g; for O: 72.1 mm & 47.4 g, and for M: 69.2 mm & 21.4g (Supplementary material-Figure 2).

2.2. Aim and experimental set-up

The experiment was based on a simple incubation method in closed systems, which is typically used to assess the efficiency of particle retention by bivalve filtration (Riisgård 1988, Shumway et al. 1985, Dupuy et al. 1999, Riisgård et al. 2001). This method has the advantage of being inexpensive, reproducible and can be used to simultaneously test the influence of several factors on changes in phytoplankton cell abundances over time in the different systems. Incubation series were carried out in 2 L borosilicate beakers in the presence or absence of five types of bivalves (M: Mussel, O: Flat oyster, Cs, Cj, C: spat, juveniles or commercial size Pacific

oysters). The beakers were filled with three types of solution (I, D, P), at a temperature close to that of the lagoon (11.4 °C). The aim of the incubation series was to evaluate variations in picophytoplankton and nanophytoplankton abundances over time in order to calculate the percentage of abatement in the beakers due to filtration by the bivalves. As the filtration activity of bivalves varies according to the weight of the organism and the temperature, before the experiments started, it was necessary to 1) calculate the theoretical clearance rate for each bivalve tested according to its weight and the water temperature of the lagoon, 2) calculate the time required for the bivalve to filter the total volume contained in the beaker, 3) adjust the abundance of bivalves in a beaker for each treatment so that the time required to filter the total volume of the beaker was equivalent for each treatment, and finally 4) determine an incubation time and sampling times that were adequate for this experiment and equivalent across treatments The approach and results of these four steps are described in the supplementary material. This preliminary analysis showed that abundance of bivalves did need to be adjust to 1 for mussels, flat oysters and commercial size-Pacific oysters vs. 4 for juveniles and 13 for spat of Pacific oysters. Incubation time did need to be set at 1 hour and measurement time steps at 0, 10, 20, 40 minutes (Supplementary material).

2.3 Experimental design and sampling

The experimental design of this study consisted of the three types of phytoplankton solution described above, (P stands for "raw water with *Picochlorum*", D for "dilute" raw water, I for "*Isochrysis*") and six different beaker contents (Figure 2). The beakers either contained bivalves, or not (W: water with no bivalve). Control in the absence of bivalve was preferred to control with an empty shell to compare phytoplankton dynamics observed in ecosystems exploited or not by shellfish culture. The bivalves were previously held in cups containing 13 spats of Pacific oyster (Cs), four juveniles of Pacific oyster (Cj), one Pacific oyster (PO), one

flat oyster (O), or one mussel (M) of commercial size (Figure 2). The cups were prepared prior to the experiments on the 11th of February 2019. Bivalves were acclimated in aquaria containing lagoon water for 24 h prior to the incubations.

On the 12th of February 2019, the phytoplankton solutions, which were previously kept in climate chambers at 9 °C, were homogenised using magnetic stirrers and then transferred to six beakers per solution at a rate of 1 L per beaker. The 18 beakers were randomly distributed in batches of six on three tables in the incubation room, which was kept quiet and dark. The contents of each beaker were randomly allocated after the cups containing the different bivalves had been arranged. Samples (1 mL) of the water were taken after homogenisation by three operators using a pipette. Each operator took care of six randomly assigned beakers. Sampling was timed and 30 seconds were left between two beakers. The first sample (T0) was taken immediately after the bivalves had been gently spooned into the bottom of the beakers. Subsequent samples were taken after 10, 20 and 40 minutes of incubation (Figure 2) for analysis of phytoplankton abundances using flow cytometry.

This experiment was replicated three times on the 12th of February 2019 using new beakers, solutions and bivalves each time. The experiments were first performed with replicate 1, then 2 and then 3; hence, a total of 54 beakers were used. The 54 beakers were not fitted with aeration systems since incubation duration was judged as too short (1h) for bivalve respiration to significantly reduce oxygen levels in 1L-solution at 11.4°C. Measurements done at the end of the second series of experiments showed that mean oxygen concentration was 97,5 ± 2,7 % in the absence vs. 94,7 ± 2.3 % in the presence of shellfish whatever the studied solution (P, D, I). Thus, no impact was presumed on bivalve filtration. A total of 216 samples were taken and fixed with 50 µL of 4% formalin before being stored at -80 °C for subsequent analysis of pico- and nano-phytoplankton concentrations.

2.4 Abundances of pico- and nano-phytoplankton

Pico- and nano-phytoplankton abundances were determined using a FACSCalibur cytometer according to the protocol detailed by Bec et al., (2011). This method, which is suitable for counting small (i.e. < 20 µm) bacterial and phytoplankton cells, enables counting and characterisation of particles in suspension based on different fluorescence criteria (natural or artificial), size and granulometry. Driven by a pressurised liquid flow, the cells pass one by one at high speed in front of a laser beam (488 nm). Chlorophyll *a* and phycoerythrin related fluorescence signals (FL3 and FL2, respectively), as well as diffraction signals of the light emitted at different angles (forward and side scatter) were measured for each cell. The measurement of Forward Scatter (FSC) allows for the discrimination of the cells by size. The Side Scatter measurement (SSC) provides information about the internal complexity (i.e. granularity) of the cells. Samples were analysed with a mixture of fluorescent beads of various nominal sizes (Polysciences, 1, 3, 6 and 10 µm in size). The size classes of natural populations have been estimated from the average of FSC values of populations relative to FSC of fluorescent beads (Bec et al 2011). Two analyses were performed of each sample to be sure to include all organisms between 1 and 20 µm (i.e. pico- (< 3 µm) and nanophytoplankton (3-20 µm) in size). Thus, based on their fluorescence and size, one population of picocyanobacteria (CYANO, < 1 µm), two populations of autotrophic picoeukaryotes (PEUK1, 2-3 µm; PEUK2, 1-2 µm) and one population of nanophytoplankton (NANO > 3 µm) were identified (Appendix 1A). *Picochlorum* was associated with the size characteristics of the PEUK1 population. The *Isochrysis* culture was between 6 µm and 10 µm in size (Appendix 1B). Cell concentrations are expressed as 10^6 cells.L^{-1}.

2.5. Flux measurements or percentage of abatement

Using the pico- and nano-phytoplankton abundance data, fluxes (Δ) were calculated from the difference between the abundances measured after 40 minutes and those measured at the initial time (0). Negative values corresponded to consumption and/or sedimentation, while positive values corresponded to production. A percentage of abatement, also called relative retention efficiency (Dupuy et al. 1999), was determined by subtracting the concentrations measured at 40 minutes from those measured at the initial time (0) and linking them as a percentage to the initial phytoplankton abundance. The calculations were performed at the scale of each beaker, giving a total of 54 values.

In this experimental context, we define abatement as a decrease in the concentrations of phytoplankton cell in the water. The term "abatement" was preferred to the term "retention" of particles since our observations allowed us to identify a decrease in the concentrations of cells in the water in the presence of bivalves but not to confirm their retention by the gill filter, their potential ingestion and assimilation by the bivalves studied. Indeed, it is possible that, after having passed through the gill system, these particles are rejected by the bivalves in the form of pseudo-faecal matter instead of being ingested and assimilated.

2.6. Data illustration and statistical analysis

Data analysis (illustrations and statistical tests) were performed with JMP version 12 and PRIMER. Two series of PERMANOVAs, based on Euclidean distances, were performed to test 1) the effect of the type of bivalve, the time, and their interaction on the cell abundances of each group of phytoplankton analysed (PEUK1, PEUK2, CYANO, NANO) for each of the phytoplankton solutions tested (P, D, I); 2) the influence of the type of solution, bivalve and their interactions on the flux values in %. *A posteriori* tests with PRIMER were used to compare the means according to the different treatment levels. The results of the post-hoc tests are indicated in the figures by lower case letters.

3. Results

3.1. Initial abundances of pico- and nano-phytoplankton

At initial time (0) and in the absence of bivalves, the contribution to total abundances of each of the four phytoplankton groups (CYANO, PEUK2, PEUK1, NANO) was similar in the P and D solutions (Figure 3). Total abundances were represented by 96.5% by autotrophic picoeukaryotes, mostly by *Picochlorum* (70.8%), characterised by the PEUK1 population of 2-3 µm in size (Figure 3). The relative contributions of CYANO and NANO were 1.7 and 1.8%, respectively. The total abundances of pico- and nano-phytoplanktonic cells were 57.3 and 25.3 x 10^6 cells.L^{-1} for P and D, respectively. Solution I consisted of 100% NANO, corresponding to *Isochrysis galbana* at a concentration of 9.4 ± 0.6 x 10^6 cells.L^{-1}. This concentration was lower than predicted (25 x 10^6 cells.L^{-1}), probably because of a wrong estimation of the concentration of the strain supplied by GreenSea, or because of a wrong homogenisation of the strain before subsampling of 50 mL and dilution in the filtered water.

3.2. Changes in pico- and nano-phytoplankton abundances over time as a function of the presence of the five types of bivalve and of the solutions tested

Permanova analyses (Table 1) showed that autotrophic picoeukaryote abundances (PEUK1 and PEUK2) varied significantly with “Bivalve x Time” interaction in both P and D solutions.

In both solutions, PEUK1 and PEUK2 abundances varied significantly over time, with a significant linear decrease in the presence of mussels (Figure 4AB), with higher R^2 and lower p values in P than in P (PEUK1: $p < 0.0001$, R^2=0,93 in P vs. p =0,0135, R^2 = 0,47 in D; PEUK2: p=0.0017, R^2=0.64 in P vs. p=0.0088, R^2=0.51 in D. PEUK1 abundances decreased by almost 87% after 40 minutes in solution P (0: 45.25 vs. 40: 5.94 x 10^6 cells.L^{-1}, Figure 4A), and by

50% in solution D (0: 20.74 vs. 40: 9.74 x 10^6 cells.L^{-1}, Figure 4A). PEUK2 abundances decreased by a factor of 2 in solution P (40: 17.7 vs. 0: 8.26 x 10^6 cells.L^{-1}, Figure 5B) and by a factor of 1.5 in solution D (0: 8.24 to 40: 5.25 x 10^6 cells.L^{-1}, Figure 4B).

Picocyanobacteria (CYANO) abundances also varied significantly according to "Bivalve x Time" interactions in solution P (Table 1), but not in solution D (Table 1) while significant linear relation was observed between CYANO and time in both solutions with higher R^2 and lower p values in P than in D (CYANO: $p < 0.001$, R^2=0.82 in P vs. $p = 0.0292$, R^2=0.39 in D. In solution P, CYANO abundances (Figure 4C) decreased by 50% after 40 minutes in the presence of mussels and more particularly during the last 20 minutes (20: 0.97 vs. 40: 0.54 x 10^6 cells.L^{-1}, Figure 4C).

Nanophytoplankton (NANO) abundances varied significantly according to "Bivalve x Time" interaction in solutions P and I. In solution D, abundances varied only according to Time and Bivalve with no effect of "Bivalve x Time" interaction (Table 1; Figure 4D). *A posteriori* tests showed that in the presence of mussel, there was a significant decrease in NANO abundances over time, i.e. by a factor of 2.2 in solution P (0 : 1.55 vs. 40 : 0.7 cells.L^{-1}, Figure 4D), by a factor of 2 in solution D (0 : 0.50 vs. 40 : 0.25 cells.L-1 , Figure 4D) and by a factor of 5.9 in solution I (0 : 10.04 vs. 40 : 1.7 x 10^6 cells.L^{-1} , Figure 4D).

In the presence of mussels (M), the decrease in the abundances of PEUK1, PEUK2, CYANO and NANO was significant during time incubation. This was not the case for the abundances observed in the presence of other bivalves (Figure 4).

3.2.Percentage decrease in cell abundances according to the presence of the five types of bivalve and the three types of solution tested

Permanova analysis showed that the percentage change in phytoplankton abundances observed after 40 minutes varied according to the Bivalve, not to the Solution and their interaction for the four categories of organisms PEUK1, PEUK2, CYANO, NANO (Table 2).

The results were synthetized in Figure 5. While positive fluxes were attributed to production in the case of PEUK1, CYANO and NANO in the absence of bivalves (W: Figure 5A, C, D), negative fluxes, illustrating a decrease in abundances, were observed in the presence of certain bivalves with more or less variability depending on the organism studied. For small Pacific oysters (Cs), a significant decrease in the abundances of PEUK1 and NANO was observed with higher percentages for nanophytoplankton than picoeukaryotes (NANO: -41.9%, PEUK1: -10%). In the presence of Cj and C, there was no significant decrease in the abundances of PEUK1 and PEUK2 (Figure 5AB, whereas a respective 22.6% and 12.3% abatement in NANO abundances was observed (Figure 5D). With flat oysters (O), significant abatements of 6.6% PEUK1 and 6.4% NANO were observed (Figure 5AD). The percentage abatement of PEUK1, PEUK2 and NANO was significantly higher in the presence of mussels than in the presence of other bivalves (Figure 5ABD). The mean percentages of abatement were 69.8% for PEUK1; 44.3% for PEUK2; 70% for NANO whatever the P, D and I solutions (Figure 5ABD). While an abatement of CYANO abundances was observed in the presence of mussels (M: 42, 3%), no abatement was observed in the presence of Pacific oysters (Cs, Cj, C) or flat oysters (O) (Figure 5C).

4. Discussion

As a result of the crisis caused by the massive green algae bloom in 2018-2019 in the Thau lagoon, the aim of this study was to test the capacity of different species (Pacific oysters: *Crassostrea gigas*, Flat oysters: *Ostrea edulis*), Mediterranean mussel: *Mytilus galloprovincialis*) and different sizes (spat, juvenile, commercial size) of Pacific oysters to

reduce abundances of *Picochlorum*, using samples of water collected from the Thau lagoon in February 2019.

While cell abundances of picoeukaryotes in the lagoon reached 1.2 billion per litre in January 2019 (Lagarde et al. 2021), their abundances were around 55 million per litre at the time of the experiments. The picoeukaryote bloom was thus already decaying at the time of the experiment, with abundances more than 20 times lower than at their peak. These abundances nevertheless remained abnormally high compared to the abundances usually observed in February in the lagoon (25×10^6 cells.L^{-1} : Bec et al. 2005). Autotrophic picoeukaryotes are known to contribute significantly, in February, up to 40%, to phytoplankton biomass in the Thau lagoon (Bec et al. 2005). The remaining 60% is generally made up of nanophytoplankton (3-20 µm) and microphytoplankton (> 20 µm). At the time of this study, 96.5% of the phytoplankton communities were autotrophic picoeukaryotes, which is exceptional for the Thau lagoon. Two populations of autotrophic picoeukaryotes were observed. The first (PEUK1), which comprised the majority (72.5%), corresponded to cells of size 2-3 µm, mainly *Picochlorum* as observed in Lagarde et al. (2021). The second population of smaller size (1-2 µm) could correspond to several species of autotrophic picoeukaryotes including *Bathycoccus* and *Micromonas* (Lagarde et al. 2021). Associated with these two picoeukaryote populations, picocyanobacteria and nanophytoplankton were also quantified by flow cytometry but each contributed less than 2% to total abundances. The abundances of picocyanobacteria and nanophytoplankton were of the order of one million cells per litre, which is very low compared to the abundances observed in the lagoon (Derolez et al. 2020a), in particular picocyanobacteria abundances, which can reach 80 times higher values in summer (Bec et al. 2011, Lagarde et al. 2018) and 300 times higher values were observed during the anoxic event in Thau lagoon (Derolez et al. 2020a).

Two types of solutions, at different dilution rates, were prepared for this experiment using lagoon water. In both cases, this study demonstrated that at 11.4 °C, only the Mediterranean mussel was able to drastically reduce the abundances of picophytoplankton, in particular the dominant population (PEUK1) 2-3 µm in size, considered as *Picochlorum*. These results confirmed the observations of shellfish farmers. Indeed, before our study, some of them reported that, the colour of their tank water, originally green, became transparent over time in presence of mussels, in contrast to commercial size oysters. Only mussels seemed to be able to clear this green water These observations were in agreement with those made on *Mytilus edulis* with picophytoplankton (Strohmeier et al. 2012b, Cranford et al. 2016, Sonier et al. 2016, 2021) and also with the findings of Galimany et al. (2020), with other mussel species (*Ischadium recurvum, Mytella charruana, Perna viridis*) and *Picochlorum* sp.

With an equivalent theoretical filtration capacity, the percentage abatement of picoplanktonic particles was higher in the presence of mussels than in the presence of flat oysters or of Pacific oysters, regardless of their size. This result is probably related to the smallest porosity of the mussel gill filter. Thus in 40 minutes, mussels with an average weight of 21.4 g were able to filter out up to 87% of the abundances of *Picochlorum* contained in a litre of water at a temperature of 11.4 °C when the concentration of *Picochlorum* was 45.2 million cells per litre. In the diluted solution, the percentage abatement was lower (53%). This result is probably due to the fact that in addition to the nature and size of the particles, bivalve filtration activity also depends on the concentration of phytoplankton (Wilson 1983, Barillé et al. 1993). This aspect is discussed below.

The capacity of mussels to reduce picoeukaryote concentrations was higher for the PEUK1 population including *Picochlorum* (69.8%, 2-3 µm) than for the PEUK2 population (44.3%, 1-2 µm) or for picocyanobacteria (42.3%, < 1 µm), which were smaller in size. Retention rates of these fine particles were close to those observed for blue mussels (1 µm: 14-64%, Strohmeier

et al. 2012). The abatement rate of nanophytoplankton was equivalent (70%) to that of *Picochlorum*. This result is linked to the position of the laterofrontal cilia. The gill filter of mussels is thought to be composed of 50% of 2-3 µm mesh and 50% of 5-6 µm mesh (Dral 1967). In this case, the PEUK2 and CYAN populations would be less well retained by the cilia than *Picochlorum* and *Isochrysis*, which are both bigger than or the same size as the mesh of the gill filter. By contrast, the capacity of oysters to retain picocyanobacteria at 1 x 10^6 cells.L^{-1} was zero at 11.4 °C. The picocyanobacteria were probably too small to be trapped by the oyster gills in addition to which, their concentration was too low. Indeed, other species of oysters, such as *Crassostrea virginica* have been shown to be able to reduce the abundances of bacteria, but in that study, the abundances were 300 times higher (3 x 10^5 cells.ml-1, *i.e*. 300 x 10^6 cells.L^{-1}: Galimany et al. 2017) than those in our study. At these high abundances, bacteria probably form aggregates, which are more easily retained by the bivalve gills.

The results of the analysis of variations in picoeukaryote concentrations in the presence of oysters over time did not clearly reveal the potential of oysters to retain picophytoplankton, thereby confirming the observations of Dupuy et al. (2000) for Pacific oyster of commercial size. Nevertheless, calculation of the abatement rate revealed a certain abatement of *Picochlorum* by flat oysters (O: 6.6%) and by small Pacific oysters (Cs: 15.9%). The hypothesis that oyster spat are able to retain *Picophytoplankton* more efficiently than juvenile and commercial size oysters was thus confirmed. Nevertheless, the reduction in *Picochlorum* in presence of flat oysters or of Pacific oyster spat was respectively 4 and 10 times lower than in presence of mussels. In the same way, eastern oysters (*Crassostrea virginica)* were shown to be able to reduce abundances of *Picochlorum* by filtration but with a lower clearance rate than that achieved by green (*Perna viridis)* or charru mussels (*Mytella charruana,* Galimany et al. 2020). However, it is important to note that in the latter study, the *Picochlorum* were 5-6 µm in

size (Galimany et al. 2020), meaning they were more efficiently retained by the gills of the bivalves than the smaller *Picochlorum* (2-3 µm), present in the Thau Lagoon.

Since our experiments were performed with theoretically equivalent filtration capacities, we expected that the rate of removal for nanophytoplankton would not differ with the type of bivalve since, *a priori*, all the bivalves studied are able to retain nanophytoplankton (Dupuy et al. 2000b, Strohmeier et al. 2012b, Cranford et al. 2016). But it was not the case. The abatement rates for nanophytoplankton ranged from 6.4% to 42.9% depending on the species, with higher rates for spat (Cs) and juvenile oysters (Cj), followed by commercial size Pacific oysters (C) and finally flat oysters (O). Although these rates were higher for nanophytoplankton than for picophytoplankton, they were still relatively low. This result is probably due to the low concentrations of nanophytoplankton in the P & D solutions (0.97 and 0.5 x 10^6 cells.L^{-1}) and to the moderately high concentrations in the culture solution (9.4 x 10^6 cells.L^{-1}).

Riisgård et al. (2003) demonstrated regulation of the opening valve and filtration activity of bivalves in response to algal concentration levels. When algal cells were offered to unfed bivalves (*Mytilus edulis*, *Cardium edule*, *Mya arenaria*), the animals soon opened their siphons/valves simultaneously with a pronounced increase of the filtration rate. By contrast, when open and actively filtering bivalves experienced decreasing algal concentrations below a certain level, this lead within a few hours to a reduced opening state and cessation of filtration activity (Riisgård et al. 2003). Higgins (1980) did the same observation with oysters (*Crassostrea virginica*). For flat oysters (*Ostrea edulis*),Wilson (1983) demonstrated that although the retention rate of *Isochrysis galbana* cells is 100% at low concentrations (≈ 2 x 10^6 cells.L^{-1}), the pumping rate is minimal. So lower quantities are retained at these low concentrations than those observed at 24 x 10^6 cells.L^{-1} when the pumping rate is optimal and when the retention rates are still very high (Wilson 1983). Above the 24 x 10^6 cells.L^{-1}

threshold, the pumping rate and retention rate dropped to reach minimum values at 500 million cells per litre.

While Wilson's (1983) experiment was carried out at 20 °C, i.e. close to the optimum for the flat oyster and the Pacific oyster (Supplementary material-Table 1), the present study was carried out at 11.4 °C, closer to the temperatures measured in the Thau lagoon during the study period (9 °C) and to the reference temperature for mussels (15 °C: Supplementary material-Table 1). This finding could also explain the lower rates of nanophytoplankton cell removal by oysters compared to mussels.

Finally, this result could also be due to a difference in filtration behaviour. Oysters, especially flat oysters, may be more sensitive to the stress caused by handling and bring placed in a beaker than mussels. Indeed, mussels were seen to start filtering the water in the first few seconds, which was not necessarily the case for flat oysters and commercial size Pacific oysters. To avoid potential stress caused by handling, these experiments could be repeated in a continuous flow system (Barillé et al. 1993, Galimany et al. 2011, Strohmeier et al. 2012b, Cranford et al. 2016) to allow the animals to acclimate to the measurement chamber. Nevertheless, this experiment already provides a first overview of the potential of different species and sizes of bivalves reared in Thau to reduce the concentrations of *Picochlorum* observed in the lagoon during the bloom decay phase.

5. Conclusions and Perspectives

This study demonstrated that, unlike Pacific oysters and flat oysters, mussels with an average weight of 21.4 g were capable of drastically reducing the abundances of *Picochlorum* in a one-litre solution, thereby extracting almost one million *Picochlorum* cells per minute at 11.4 °C. These results suggest that the 1,228 tonnes of mussels that died in 2018 before the *Picochlorum* bloom could have helped to limit the *Picochlorum* bloom if they had survived the heat and

anoxia. Thus, it may be important to keep stocks of mussels in the Thau lagoon. However, it may be even more important to harvest the mussels in the lagoon before the summer heat, to avoid the massive mortality observed in recent years due to global warming.

It is also important to note that these experiments show that mussels are able to extract a defined quantity of *Picochlorum* at a given time. It is now necessary to work on the fate of these cells, i.e. whether they are ingested and assimilated by the mussels and not rejected in the form of pseudo-faeces. *A priori*, some producers in the lagoon obtained good growth of their mussels at the time of the *Picochlorum* efflorescence. Sonier et al. (2016) reported that picophytoplankton significantly contributes to mussel growth in intensive culture environments where the percentage of picophytoplankton in phytoplankton biomass is high. If the observations of Wilson (1983) are applicable to mussels and *Picochlorum* cells, it would be interesting to identify the threshold at which the retention rate decreases. Above this threshold, there would be a risk that the mussel's gills become clogged and that the mussel would coat the *Picochlorum* cells with mucus before expelling them in the form of pseudo-faeces. Further analysis could identify the proportion of assimilated to rejected cells using an isotopic approach like that used by Galimany et al. (2020) and Sonier et al. (2021). Such a study could use a *Picochlorum* strain at several concentrations, up to 2 billion cells, as observed in hypereutrophic lagoons. Finally, it would be interesting to reproduce this study along a temperature gradient to evaluate interactions between seasonal variability of bivalves and *Picochlorum*. Further studies would be next needed to understand the interactions between mussel farming and *Picochlorum*. At the ecosystem scale, a study could be conducted using a modelling approach, as did Gray et al. (2021) in Florida, to estimate the top-down role of bivalve in regulating microalgae bloom, taking account of hydrodynamics, time residence, population orientation, filtration capacities of bivalve and regeneration of this microalgae. The results of such studies will pave the way

for better management of massive green algae blooms in shellfish exploited ecosystems around the world.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

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Table 1 Permanova results (p-values) testing the effect of bivalve presence, time and their interactions on abundances of the 4 organism groups (PEUK1, PEUK2, CYANO, NANO) for each solution (P, D, I). The p values < 0.05 shown in bold indicate either an effect of the Time x Bivalve interaction, or an effect of Time whatever the bivalve type, or of the Bivalve whatever the Time.

	P				D				I
	PEUK1	PEUK2	CYANO	NANO	PEUK1	PEUK2	CYANO	NANO	NANO
Bivalve	**0.001**	**0.012**	**0.009**	**0.001**	**0.009**	**0.001**	0.406	**0.001**	**0.001**
Time	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.185**	0.935	**0.007**	**0.001**
Time x Bivalve	**0.001**	**0.001**	**0.001**	**0.001**	**0.04**	**0.011**	0.11	0.632	**0.002**

Table 2 Permanova results (p-values) testing the effect of Solution, Bivalve presence and their interactions on the percentage change in the four groups of organisms (PEUK1, PEUK2, CYANO, NANO). The p values < 0.05 shown in bold indicate an effect of the Bivalve presence whatever the type of Solution.

	PEUK1 %	PEUK2 %	CYANO %	NANO %
Solution	0.352	0.433	0.138	0.34
Bivalve	**0.001**	**0.001**	**0.001**	**0.001**
Solution x Bivalve	0.386	0.141	0.911	0.345

Figure captions

Figure 1: A) Chl*a* biomass (µg.L^{-1}), Mean (± Standard Error: SE) of B) Total individual weight (g) and C) Contribution of flesh to total weight (%) of Pacific oysters (*Crassostrea gigas*) reared on ropes at the Marseillan farming site in the Thau lagoon, and observed from 2016 to the beginning of 2019 as part of the Ifremer REPHY (A) and Ecoscopa (B, C) networks. Green bars include the anoxic event, succeeded by the massive green algae bloom.

Figure 2: Experimental design composed of 3 solutions (Thau lagoon water P, diluted Thau lagoon water D, filtered Thau lagoon water containing *Isochrysis* I), with 6 different contents (absence of bivalves: i.e. Water W vs. presence of bivalves: 3 sizes of Pacific oysters (*Crassostrea gigas*: C): spat (Cs), juvenile (Cj) and commercial size (C), Flat oyster (*Ostrea edulis*: O) and Mediterranean mussel *Mytilus galloprovincialis*: M), 3 replicates, 4 sampling times (0, 10, 20, 40 minutes) for a total of 54 beakers and 216 water samples.

Figure 3: Mean (± SE) A) contribution to total abundance (%) and B) abundances (10^6 cells.L^{-1}) of the 4 groups of phytoplanktonic organisms: picocyanobacteria (CYANO), picoeukaryotes 1 (PEUK1), picoeukaryotes 2 (PEUK2) and nanophytoplankton, observed in the P, D and I solutions, and measured at T0 in three beakers in the absence of bivalves (W).

Figure 4: Mean (± SE) abundances (x 10^6 cells.L^{-1}) of A) autotrophic picoeukaryotes PEUK1, B) PEUK2, C) picocyanobacteria CYANO, D) nanophytoplankton NANO, measured in the absence (W) or presence of bivalves, i.e. 3 sizes of *Crassostrea gigas*: spat (Cs), juvenile (Cj) and commercial size (C), Flat oyster (*Ostrea edulis*: O) and Mediterranean mussel *Mytilus galloprovincialis*: M), according to 3 solutions (Thau lagoon water P, diluted Thau lagoon water D, filtered Thau lagoon water containing *Isochrysis* I: right axis). Results of the post tests are indicated by lower case letters. Means associated with different letters varied significantly over time in the same bivalve. The absence of a letter indicates no difference between means over

time in the same bivalve. The abundances of CYANO and NANO did not vary significantly with “Bivalve x Time” interactions. Thus no post-hoc test was performed within the interaction.

Figure 5: Mean (± SE) percentage change (Δ: between 40 vs. 0 time; ± SE) in abundances of A) PEUK1, B) PEUK2, C) CYANO, D) NANO according to the absence (W) or presence of bivalves, i.e. 3 sizes of *Crassostrea gigas*: spat (Cs), juvenile (Cj) and commercial size (C), Flat oyster (*Ostrea edulis*: O) and Mediterranean mussel *Mytilus galloprovincialis*: M), calculated from data observed in the three solutions, P, D and I. The letters indicate a significant difference according to the type of bivalve for each of the 4 phytoplankton groups.

Figures

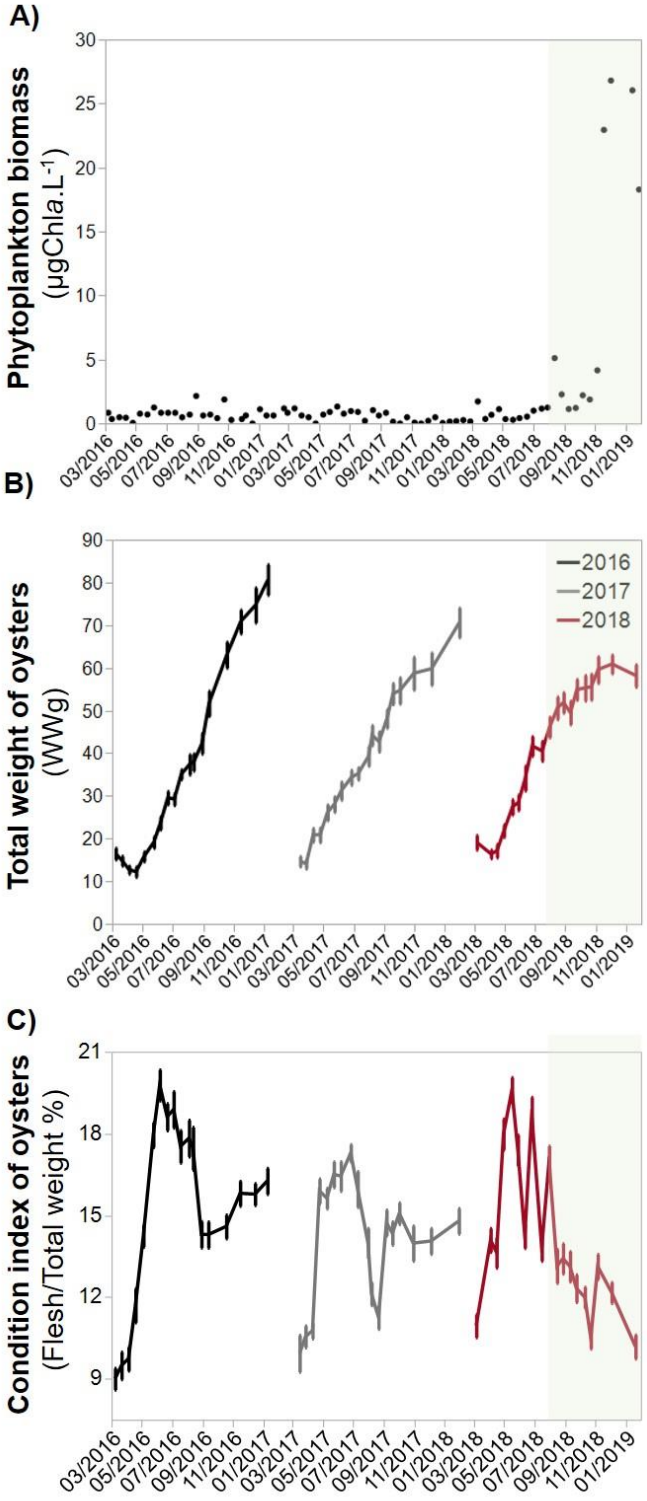


Figure 1. Richard et al.

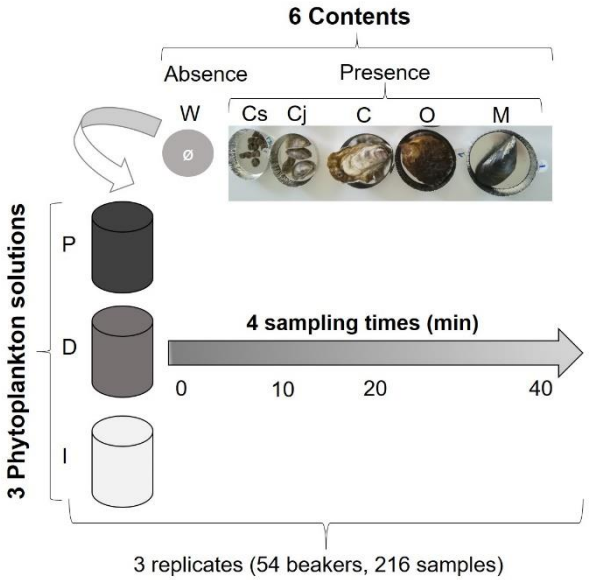


Figure 2. Richard et al.

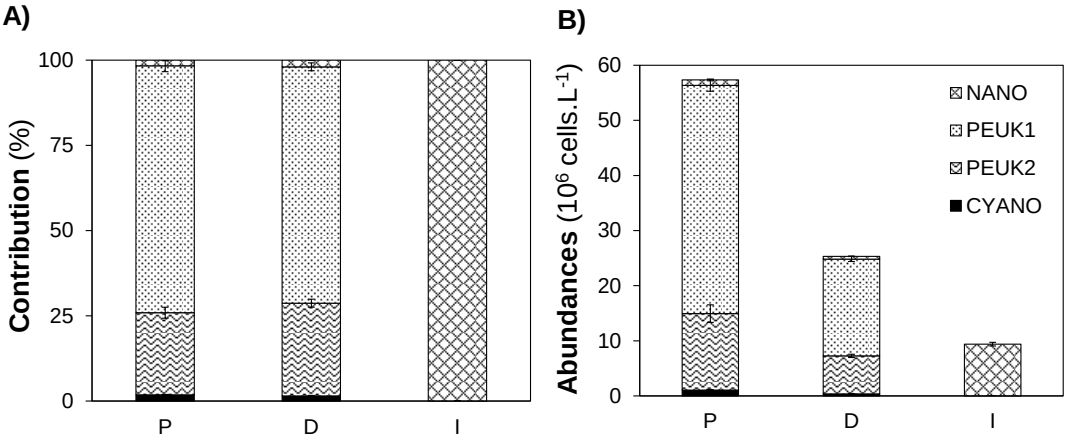


Figure 3. Richard et al.

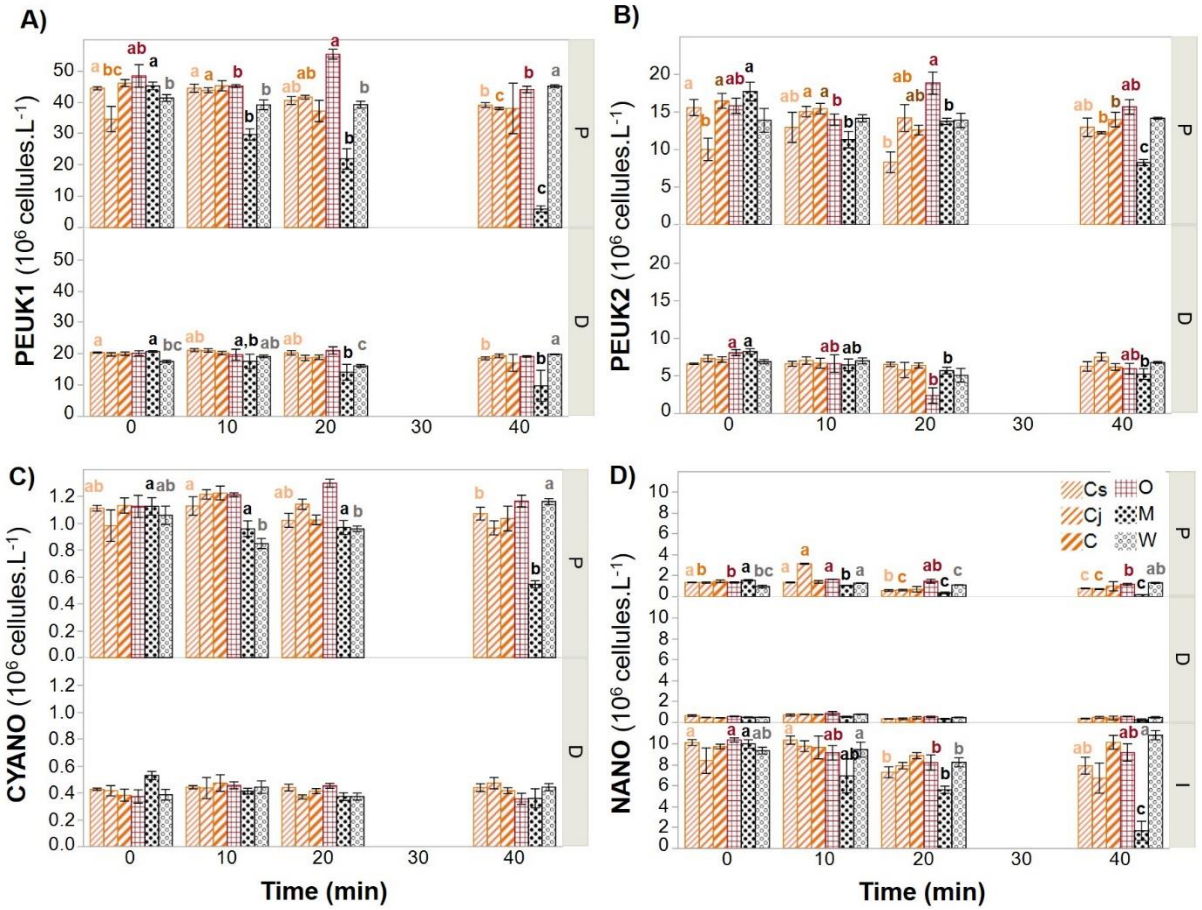


Figure 4. Richard et al.

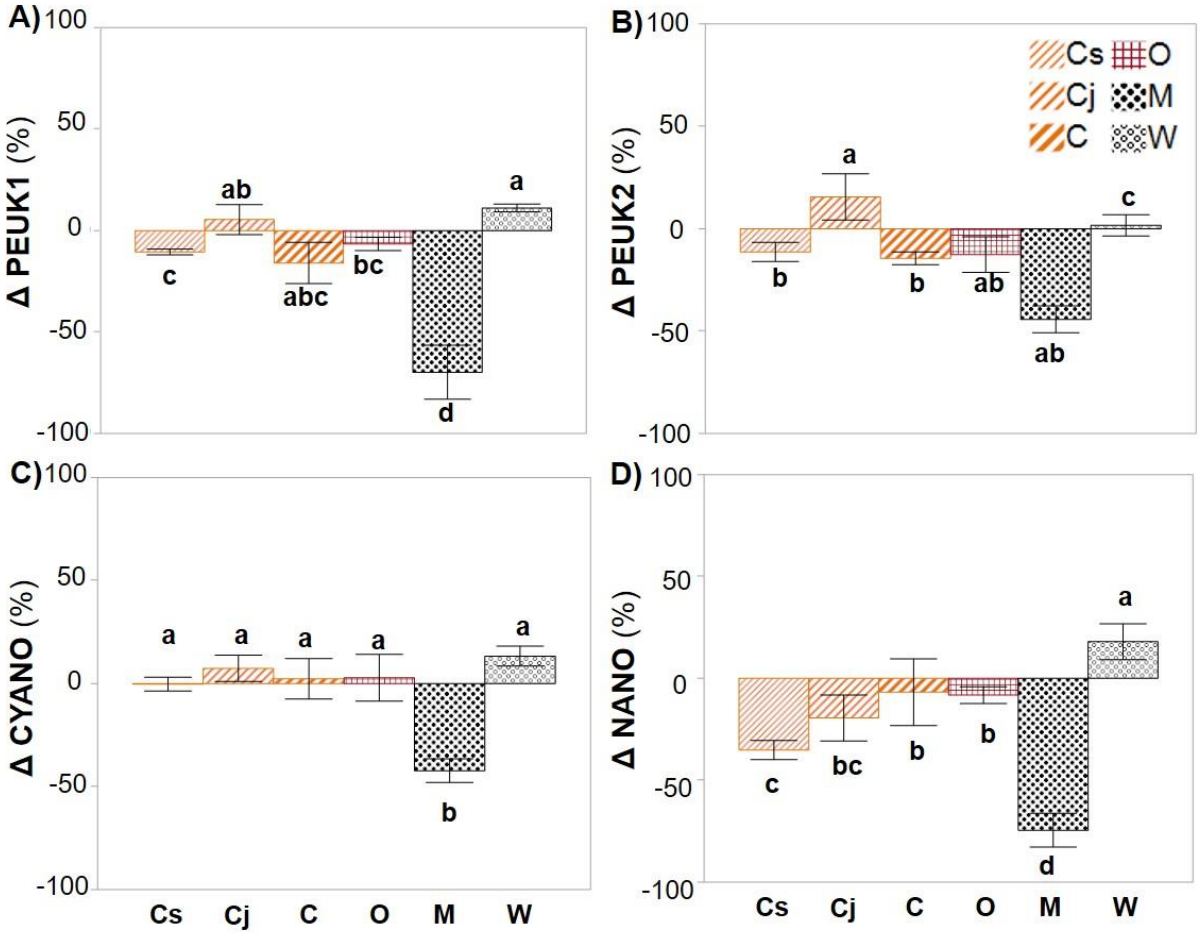


Figure 5. Richard et al.

Supplementary materials: Four preliminary steps to determine abundances of bivalves, incubation duration and sampling time

1. Determination of theoretical filtration rates for each type of bivalve as a function of their weight and the temperature of the lagoon (11.4 °C)

Using values from the literature, Arrhenius' law (Table 1a), allometric relationships (Table 1b) and biometric relationships (Table 1c), the filtration rate for each of the bivalves was determined at 11.4 °C as a function of their individual total weight (Figure 1). The filtration rate of the five bivalves selected for the experiment theoretically varied from 0.28 $L.h^{-1}$ for Cs to 3.82 $L.h^{-1}$ for C at 11.4 °C (Figure 2). Flat oysters (O) and Mediterranean mussels (M) theoretically had an equivalent individual filtration rate of approximately 2.6 L.h-1 (Table 1, Figure 2).

2 Determination of the time required for the bivalves to filter the entire volume in the beaker

The time required for the bivalves to filter the entire volume contained in the beakers, (1 L in this experiment) was previously determined according to the individual biomass of the bivalves, the temperature and the volume of solution (Table 1) using the following formula

$t = (V/Ye) \times 60$

where t is time in minutes, V is the volume of solution in the beaker in litres, Ye ($L.h^{-1}$) is the theoretical filtration rate of the bivalve for an individual of weight (We) according to its weight at 11.4 °C.

3 Adjustment of the abundance of bivalves to obtain an equivalent filtered volume and incubation time for each treatment

In order to ensure the incubation time was equivalent between treatments, the abundance of small (Cs) and intermediate (Cj) Pacific oysters were adjusted so that the volume filtered by these organisms was equivalent to that filtered by a larger organism (C: Table 2). The number (N) of Cs and Cj individuals (N) was determined using the following formula:

N (Cs or Cj) = Ye C/Ye (Cs or Cj)

where Ye is the experimental metabolic rate for an individual of weight (We)

The theoretical volume (expressed in L) filtered per hour by all individuals in the beaker was determined as follows: V= N x Ye

The abundance of Cs was therefore set at 13 individuals and that of Cj at 4 individuals, while the abundance of large Pacific oysters (C), flat oysters (O) or Mediterranean mussels (M) was 1 (Table 2).

According to these theoretical calculations, after adjusting for abundance, the time needed for the bivalves to filter 1 L of solution from the beaker was of the same order of magnitude (16.5 to 22.9 minutes) regardless of the species (Table 2), which was not the case with no adjustment for abundance, as the filtration time of a Cs was 13.7 times less than that of a large C oyster while their biomass was 286 times lower. If we had based our calculations on equivalent biomass, we would have set the abundance of Cs at 286 individuals. The 286 individuals would then theoretically have taken less than a minute to filter a litre of water, which would have been significantly less than the time required for C, O and M bivalves and far too fast to be analysed correctly.

4 Determination of incubation and sampling times

The incubation time and water sampling times were determined according to the time required for the bivalves to theoretically filter the entire volume of solution contained in the beaker by

regularly including sampling times upstream and downstream of this period, with an initial time (0 min), immediately after the bivalve was gently placed in the bottom of the beaker using a spoon. As the time required to filter one litre was estimated to be between 16.5 and 22.9 minutes depending on the type of bivalve after adjusting for abundance (Table 2), we considered that it would be interesting to sample water after 10 minutes and 20 minutes of contact with the bivalve, i.e. before the entire volume of the solution was theoretically filtered, and after 40 minutes, i.e. when the volume had been filtered at least once.

Tables of supplementary material

Table 1 Summary of standard clearance rates, allometric coefficients, Arrhenius temperatures and reference temperatures for the three species studied and associated references

	Standard Clearance rate: Ys = K1 = a ($L.h^{-1}.DWg^{-1}$)	Allometric coefficient b	Temperature (°C)	References	Arrhenius Temperature TA (Kelvin)	Reference Temperature T (Kelvin= T°C + 273.15)	References
Crassostrea gigas	4.82	0.44	19	*Bougrier et al. 1995*	5800	292.15	*Bernard et al. 2011*
Mytilus galloprovincialis	4.08	1.06	15	*Van Erkom Schurinkand Griffiths 1992*	7146	288.15	*DEB TOOL**
Ostrea edulis	6.55	0.46	20-22	*Pouvreau et al. 1999*	8129	294.15	*DEB TOOL**

* http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/

[a] Arrhenius equation: $K(T) = K1 \exp (TA/T1-TA/T)$ (Koojman et al. 2010) where K(T) is the metabolic rate measured at T, (K1) is the optimal metabolic rate measured at optimal temperature (T1), and TA is the Arrhenius temperature.

[b] Allometric equation: $Ye = a\ We^{b}$ (Golsing et al. 2015) & $Ys = (Ws /We)^{b} \times Ye$ (Bayne et al. 1987) where Ye is the experimental metabolic rate per individual of weight We, and Ys is the standard metabolic rate for an individual of standard flesh weight (Ws, 1gDW), a is the standard metabolic rate and b is the allometric coefficient.

[c] biometric relation: Pacific oyster: Flesh weight = 2.8% Total weight (ecoscopa network: 94-2017), mussel: flesh weight = 4.3% total weight (SBTag2017)

Table 2 Theoretical clearance rate per individual at 11.4 °C, the Time each individual took to filter 1 L of solution; Theoretical cleared volume per hour after adjusting for abundance; Time needed at 11.4 °C to filter the volume of solution contained in the beaker after adjusting for abundance in the different treatments, *i.e.* types of bivalve (Pacific oysters (*Crassostrea gigas*) of spat size (Cs), juvenile size (Cj) and commercial size (C), flat oysters (*Ostrea edulis*: O) and mussels (*Mytilus galloprovincialis*: M)).

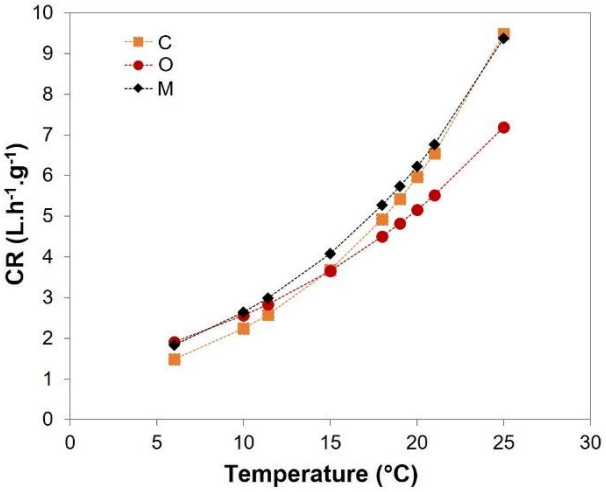
Treatment	Theorical clearance rate (Ye) ($L^{-1}.h^{-1}$) per individual at 11.4°C	Time to clear 1L of solution (min) for a given individual	Number of individuals per beaker (N per treatment)	Theoretical cleared volume per hour for each treatment	Time to clear 1L of solution (min) for each treatment
Cs	0.28	215.3	13	3.64	16.5
Cj	0.83	72.3	4	3.32	18.0
C	3.82	15.7	1	3.82	15.7
O	2.62	22.9	1	2.62	22.9
M	2.66	22.5	1	2.66	22.5

Figure captions of supplementary material

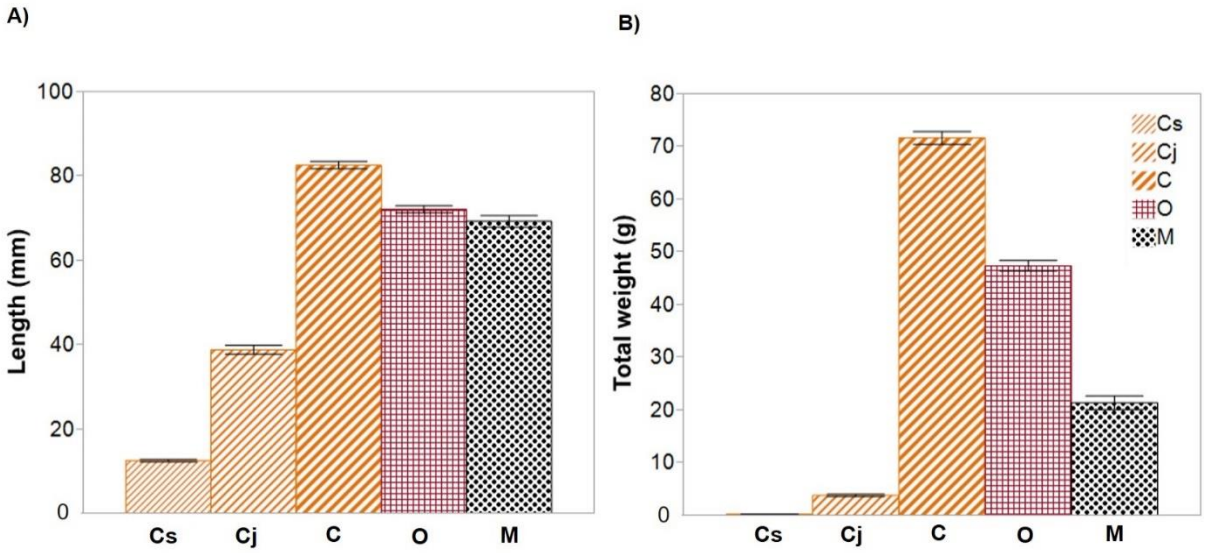
Figure 1: Individual clearance rate (CR in L per hour) by Pacific oyster (*Crassostrea gigas*: C), Flat oyster (*Ostrea edulis*: O) and Mediterranean mussel (*Mytilus galloprovincialis*: M) of 1 g dry flesh weight, calculated according to a gradient of water temperature in Celsius. A bivalve of 1 g dry flesh weight corresponds to a 36-g Pacific oyster, a 47-g flat oyster and a 23-g mussel.

Figure 2: Mean (± Standard Error: SE) of length (A) and total weight (B) measured in 30 individuals of each type of bivalve: Pacific oyster (*Crassostrea gigas*) spat (Cs), juvenile (Cj) and commercial size (C), flat oyster (*Ostrea edulis*: O) and mussel (*Mytilus galloprovincialis*: M).

Figure 3: Individual Clearance rate (CR in L per hour) calculated at 11.4 °C as a function of total weight and of the species. The squares frame the weight and filtration rates of the 5 types of bivalves studied: Pacific oyster (*Crassostrea gigas*) spat (Cs), juvenile (Cj) and commercial size (C), flat oyster (*Ostrea edulis*: O) and mussel (*Mytilus galloprovincialis*: M).



Supplementary material-Figure 1. Richard et al.



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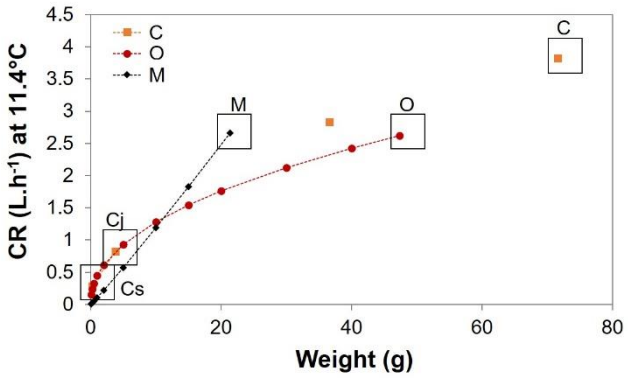
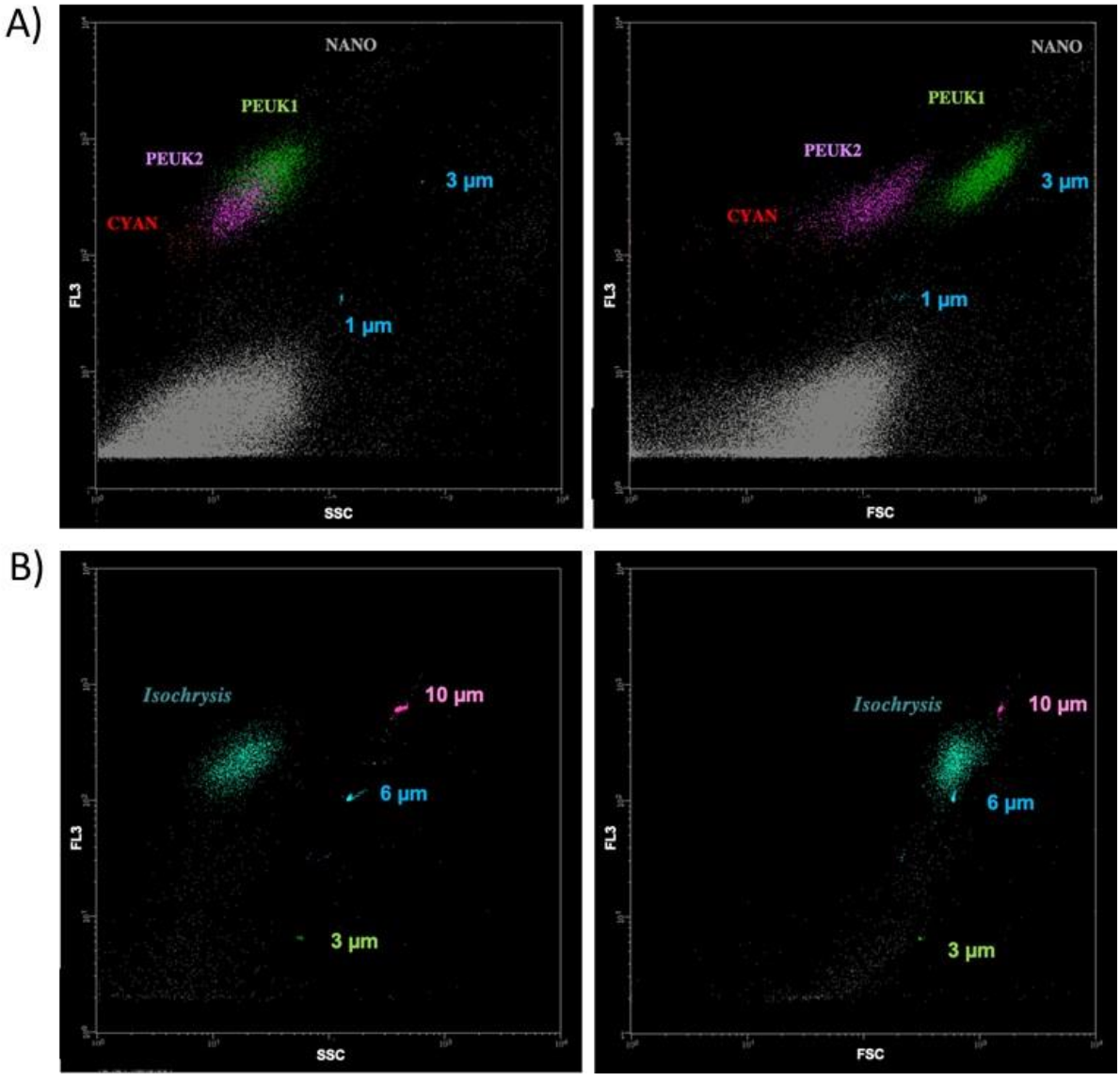


Supplementary material-Figure 3. Richard et al.

Appendix

Figure 1: Dotplots of cytometric analysis of phytoplankton observed in A) Thau lagoon (P) and B) *Isochrysis* (I) samples. Population of cyanobacteria (CYAN), picophytoplankton (PEUK1, PEUK2) and nanophytoplankton (NANO) were distinguished according to red fluorescence (FL3) and Side or Forward Scatter (SSC, FSC).



In the Thau lagoon sample (Figure 1A), PEUK2 population was less than 1-2 µm in size. The PEUK1 population, considered as *Picochlorum*, was closer to 2-3 µm. The picocyanobacteria (CYAN) were discriminated based on lower FL3 and FSC than picoeukaryote populations, and

the detection of FL2 fluorescence (not shown). Their size was under 1 µm. The NANO population was larger than the 3 µm beads and their abundances appear to be very low (Figure1A). No PEUK1, PEUK2 and CYANO was observed in *Isochrysis* solution (Figure 1B). The *Isochrysis* population was between 6 µm and 10 µm in size (B)

Highlights:

Unlike oysters, mussel can drastically reduce the abundance of 1-3-µm picophytoplankton.

Oysters of commercial size cannot retain < 2-µm picoeukaryotes and cyanobacteria.

The ability of Pacific oyster spat to retain 2-3-µm picoeukaryotes was significant.