**Estuarine, Coastal and Shelf Science**
October 2016, Volume 180, Pages 190-203
http://dx.doi.org/10.1016/j.ecss.2016.06.029
http://archimer.ifremer.fr/doc/00346/45701/




# First steps of ecological restoration in Mediterranean lagoons: Shifts in phytoplankton communities

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**Abstract :**

Along the French Mediterranean coast, a complex of eight lagoons underwent intensive eutrophication over four decades, mainly related to nutrient over-enrichment from continuous sewage discharges. The lagoon complex displayed a wide trophic gradient from mesotrophy to hypertrophy and primary production was dominated by phytoplankton communities. In 2005, the implementation of an 11 km offshore outfall system diverted the treated sewage effluents leading to a drastic reduction of anthropogenic inputs of nitrogen and phosphorus into the lagoons. Time series data have been examined from 2000 to 2013 for physical, chemical and biological (phytoplankton) variables of the water column during the summer period. Since 2006, total nitrogen and phosphorus concentrations as well as chlorophyll biomass strongly decreased revealing an improvement in lagoon water quality. In summertime, the decline in phytoplankton biomass was accompanied by shifts in community structure and composition that could be explained by adopting a functional approach by considering the common functional traits of the main algal groups. These phytoplankton communities were dominated by functional groups of small-sized and fast-growing algae (diatoms, cryptophytes and green algae). The trajectories of summer phytoplankton communities displayed a complex response to changing nutrient loads over time. While diatoms were the major group in 2006 in all the lagoons, the summer phytoplankton composition in hypertrophic lagoons has shifted towards green algae, which are particularly well adapted to summertime conditions. All lagoons showed increasing proportion and occurrence of peridinin-rich dinophytes over time, probably related to their capacity for mixotrophy. The diversity patterns were marked by a strong variability in eutrophic and hypertrophic lagoons whereas phytoplankton community structure reached the highest diversity and stability in mesotrophic lagoons. We observe that during the re-oligotrophication process in coastal lagoons, phytoplankton shows complex trajectories with similarities with those observed in freshwater lake systems.

**Keywords** : Phytoplankton, Coastal lagoons, Nutrient, Sewage effluents, Re-oligotrophication

## 1. Introduction

Eutrophication has been defined as a suite of adverse symptoms resulting from the nutrient and organic inputs (De Jonge and Elliott, 2011). High biomass decreases light availability, favoring among the primary producers the community that is most competitive for light, *i.e.*, phytoplankton at the expense of macrophytes (Cebrian et al., 2014). This over-production causes a loss of diversity (Schramm, 1999; de Jonge and de Jong, 2002), habitat destruction and mortalities due to anoxia (Smith, 2006; Carlier et al., 2008). These phenomena negatively impact ecosystem health, result in increased vulnerability to disturbances (Heemsbergen et al., 2004; Worm and Lotze, 2006) and loss of ecosystem services (Bullock et al., 2011). In coastal areas, which are characterized by strong demographic growth, eutrophication has become a serious threat since the 1950s (Nixon, 1995). Coastal lagoons are particularly sensitive to eutrophication, since these systems tend to concentrate anthropogenic nutrient inputs (Knopper, 1994; Cloern, 2001; Kennish and Paerl, 2010) due to restricted exchanges with the sea and long water residence time (Boesch, 2002; Kennish and Paerl, 2010; Glibert et al., 2011).

In 2000, The Water Framework Directive (WFD) was established in Europe requiring member states to monitor the ecological and chemical quality state of water bodies and implement ways to achieve good status by 2021 (Sherrard et al., 2006; Cartaxana et al., 2009). Efforts have been made in many parts of the world to combat eutrophication by reducing nutrient inputs from watersheds and initiate ecological restoration. Ecological restoration is well documented in lakes which have been subjected to water quality improvement programs since the 70s (Jeppesen et al., 2005; 2007). Studies on lake restoration have shown that the response trajectories during re-oligotrophication are not simply the inverse of the previous eutrophication processes and are characterized by hysteresis. Accordingly, the recovery of ecosystem functions often lagged behind the reduction of

external nutrient loadings, due to nutrient regeneration from sediments or the persistence of turbid alternative states because of dense blooms of phytoplankton or the presence of a pool of easily resuspendable organic matter (Scheffer et al., 1993; Scheffer and Carpenter, 2003; Sondergaard et al., 2003; Ibelings et al., 2007). Because of these phenomena, re-oligotrophication processes are difficult to understand and predict for degraded systems (Van Donk et al., 2008). Hence, ecological restoration generally takes much longer than water degradation due to eutrophication. At first, it results in modifications of the composition and structure of primary producers communities. Phytoplankton is generally the first autotrophic compartment responding to the change of nutrient availability and other anthropogenic pressures (Livingston, 2000; Paerl et al., 2003). In restored lakes, this response has resulted in considerable changes of phytoplankton biomass, community structure and functional diversity (Ruggiu et al., 1998, Anneville and Pelletier, 2000; Katsiapi et al., 2013).

A functional approach to phytoplankton ecology appears particularly useful to study the adaptive responses of phytoplankton communities to re-oligotrophication. This approach is based on defining the functional traits of species that impact on their performance and survival (Violle et al., 2007) and, thus provides a better understanding of how phytoplankton communities respond to environmental changes. The functional approach has been used to understand how environmental changes or gradients drive phytoplankton community structure (Litchman et al., 2010). Some morphological and physiological traits particularly reflect the phytoplankton adaptations to nutrient availability, such as cell size, maximum growth rate and trophic regime (Litchman and Klausmeier, 2008; Litchman et al., 2010). During re-oligotrophication, the reduction of nutrient inputs could thus favor small cells, which compete more effectively for nutrient uptake and show high growth rates (Chisholm, 1992; Kamenir and Morabito, 2009; Litchman et al., 2010), and mixotrophic species that present some

advantages over strictly autotrophic cells (Anneville and Pelletier, 2000). These functional traits highlight phytoplankton adaptations to the reduction of nutrient availability and represent an interesting tool to evaluate the impact of changing eutrophication status.

Since the implementation of the WFD, coastal waters represent a major issue for management and ecological restoration which has been used to reestablish ecosystems services. So far, little is known about the responses of coastal ecosystems to ecological restoration (Vidal et al., 1999; Duarte et al., 2009; Nixon, 2009). The recent literature describes a diversity of responses to restoration (Boesch, 2002; Elliott et al., 2007; Ho et al., 2008; Duarte et al., 2009), including a decrease of primary production while phytoplankton biomass remained stable (Philippart et al., 2007), reappearance of macrophyte communities (de Jonge and de Jong, 2002), and decrease of biomass and frequency of bloom occurrence (Lie et al., 2011). As shallow lakes, coastal lagoons have been particularly subjected to cultural eutrophication process due to nutrient over-enrichment from watersheds and long residence time (Kennish and Paerl, 2010; Glibert et al., 2011). Ecological restoration of coastal lagoons has started recently and studies of this process are still scarce. Given the high variability and dynamics of these systems, we can expect variable and complex restoration trajectories. During the re-oligotrophication process in a Mediterranean coastal lagoon (Collos et al., 2009), phytoplankton community changes were similar to those observed in some freshwater lakes, *i.e*. characterized by the appearance of dinophytes species and small-sized cyanobacteria (Ruggiu et al., 1998, Kamenir and Morabito, 2009). The results suggest some similar responses of lakes and coastal lagoons to re-oligotrophication process.

In the South of France, to improve the ecological quality of eight eutrophied coastal lagoons close to Montpellier, a drastic and persistent reduction of anthropogenic nutrient inputs has been achieved since December 2005, leading to a dynamic of ecological restoration. In the

framework of a monitoring network, these lagoons and sixteen other lagoons have been monitored from 2000 to 2013 to assess their eutrophication status. These 24 lagoons presented a large eutrophication gradient ranging from oligotrophy to hypertrophy (Souchu et al., 2010), the most eutrophied lagoons showed phytoplankton dominance with high biomasses (>100 µgChla.$L^{-1}$). The phytoplankton size structure was dominated by small eukaryotic algae (3-6 µm) with relatively high growth rates (Bec et al., 2008; 2011).

Prior to the reduction in nutrient loadings, the complex of eight lagoons represented a eutrophication gradient ranging from mesotrophic to hypertrophic, including the most hypertrophic lagoons of the region. This context offered us a unique opportunity to study how the initial eutrophication status of lagoons influences the re-oligotrophication trajectories and to assess the success of ecological restoration in these highly degraded systems. We focused on phytoplankton community shifts to investigate the impact of ecological restoration in coastal lagoons for this range of eutrophication levels. Using data from a 13-year monitoring program, we describe changes of phytoplankton biomass and structure (i.e., size class structure and community composition), by comparing two periods: before and after the nutrient reduction. In addition, after implementation of the nutrient reduction, HPLC pigment analyses were added to the monitoring program. This allowed us to study the dynamics of functional and taxonomic groups as well as phytoplankton diversity, based on pigment biomarkers, during re-oligotrophication trajectories.

## 2. Materials and methods

### *2.1. Studied sites*

The Palavasian lagoon complex is located on the French Mediterranean coast, near Montpellier city (urban population 250 000 inhabitants). Since the 17th century, infilling and human constructions have compartmentalized a large natural lagoon to give rise to the current

142 complex of eight interlinked shallow lagoons covering 38.8 $km^2$ (Fig. 1). A major human
143 intervention was the building of a navigation canal through the natural lagoon oriented NE-
144 SW, named the Rhône-to-Sète canal which divided the complex into two parts (inland and
145 seafront lagoons). As a result, four of the eight lagoons (i.e., the inland lagoons North Ingril,
146 Vic, Arnel, and Méjean lagoons) are bordered by wetland or salt marshes, which can act as a
147 buffer zone and regulate freshwater inputs from the watershed. Four seafront lagoons (South
148 Ingril, Pierre Blanche, Prévost, and Grec lagoons) are located between the Rhône-to-Sète
149 canal and the lido. Among them, South Ingril and Prévost lagoons are connected to the sea
150 through artificial permanent inlets. The inland lagoons are not directly connected to the sea,
151 but receive seawater indirectly by flow through the adjacent seafront lagoons and the Rhône-
152 to-Sète canal, which has many openings. In addition, canalization of the Lez River oriented
153 perpendicular to the Rhône-to-Sète canal has completed the compartmentalization of lagoon
154 complex. The Lez River, the main freshwater source of the Palavasian lagoons, interacts with
155 some lagoons (Arnel, Méjean, Prévost and Grec) through small channels. These multiple
156 canalizations do not account for sufficient water renewal of lagoon waters. The trophic status
157 and main characteristics of the lagoons as well as the sampling stations are listed in Table 1.
158 For Méjean and Prévost lagoons, two zones corresponding to empirically defined
159 hydrodynamic compartments (Souchu et al., 2010), have been considered for sampling
160 stations (see Table 1).
161 Effluents from the wastewater treatment plant of the Montpellier city district were discharged
162 into the Lez River until 2005. Through the many channels described above, the nutrients from
163 these effluents have propagated into the different lagoons, which has resulted in on-going
164 eutrophication from 1965 to 2005. The eutrophication gradient ranged from hypertrophy in
165 lagoons close to the Lez river to mesotrophy in the southwestern lagoons (Souchu et al.,
166 2010). In December 2005, the implementation of a new wastewater treatment plant (Maera),

and a diversion of its effluents through an outfall located 11 km off-shore in the Mediterranean Sea has led to a sudden and drastic reduction of the anthropogenic inputs of nitrogen and phosphorus into the lagoons. This has been estimated as a reduction of 83 % of N and 70 % of P (Meinesz et al., 2013).

*2.2. Data collection*

We exploited the database developed by the Lagoon Monitoring Network (Réseau de Suivi Lagunaire), which comprises data from 2000 to 2013 to assess the eutrophication status of lagoons in the Languedoc-Roussillon region (Souchu et al., 2010). Sampling was carried out during summer periods, when temperature and irradiance are optimal and allow maximal primary production. For the Palavasian lagoon, the ten stations (Table 1) were sampled monthly in June, July, and August from 2000 to 2013. On each date, subsurface water samples were collected with 2 L polypropylene bottles. Temperature and salinity were recorded with a WTW LF 197 field sensor. This database comprised the concentrations of dissolved inorganic nitrogen (DIN = $NO_3$ + $NH_4$ + $NO_2$, µM) and phosphorus (DIP, µM), total nitrogen and phosphorus (TN and TP, µM). It also included water column chlorophyll a concentrations (Chl *a*, µgChla.$L^{-1}$) as a proxy for phytoplankton biomass. Chl a concentration were measured by spectrofluorimetry (Neveux and Lantoine, 1993) with a Perkin-Elmer LS50 B and pico- and nano-phytoplankton abundances ($10^6$ cells.$L^{-1}$) counted with a FACSCalibur flow cytometer (Bec et al., 2011). Based on cytometric analysis, different size classes of phytoplankton were identified: Phycoerythrin-rich picocyanobacteria (≤ 1 µm, PE-CYAN), autotrophic picoeukaryotes (≤3 µm, PEUK), nanoeukaryotes (> 3 µm). The analytical protocols have been described in detail by Souchu et al., (2010) and details on filtration, conservation and analysis of phytoplankton samples have been described by Bec et al.,

(2011). Rainfall data were obtained from Météo France (Fréjorgues station, data publicly available online at *Comparaison climatologique annuelle - Infoclimat*).

*2.3. HPLC determination of pigment diversity*

Since 2006, phytoplankton pigment diversity based on HPLC pigment analysis have complemented the database, to investigate on phytoplankton composition by chemotaxonomic identification of main groups. In the laboratory, exact volumes between 250 to 500 ml of water, depending on phytoplankton biomass, were filtered onto Whatman GF/F filters (47 mm diameter) and stored at -80°C until analysis.

Pigments were extracted with 2.5 mL of 100% methanol in the dark at 4°C for 5 min. Samples were then sonicated 5 times for 10 sec (20 Watts) and spaced by 10 sec in ice to avoid an excessive heating of the extract. After 10 min in the dark at 4°C, extracts were filtered on cellulose acetate filters (0.45 µm pore size) to remove filters and cell debris. An aliquot of 600 µL was diluted with 150 µL of Milli-Q water. A volume of 150 µL of this mix was injected to the HPLC system, a Waters D600 equipment. Chlorophylls and carotenoids were detected by a Waters 2996 photo-diode array detector (optic resolution 1.2 nm), from 400 to 700 nm. Chlorophylls and their derivatives were also detected by a 2475 Multi λ fluorescence detector, from two canals, to optimize the chlorophyll *a* (canal A: 412 nm Excitation – 650 Emission) and the chlorophyll *b* and *c*2 detection (canal B: 440 nm excitation – 650 emission). Pigment extracts were analyzed using the method of Wright et al., (1991) with a flow rate of 1 $ml.min^{-1}$ and a run duration of 29 min. Solvent delivery was programmed following a sequence of three linear gradients as follows (minutes, % solvent A, % solvent B, % solvent C): (0,100,0,0) (2,0,100,0) (18,0,20,80) (21,0,100,0) (24,100,0,0) (29,100,0,0). Solvent A consisted of 80:20 (v/v) methanol: ammonium acetate (0.5 M), solvent B consisted of 90:10 (v/v) acetonitrile : water and solvent C consisted of ethyl acetate.

The HPLC system was calibrated with external standards (DHI Water and Environment, Hørsholm. Denmark). Chromatograms were extracted at 440 nm, and pigments were identified by comparison with a spectral library established from the pigments standards basis and by checking elution order and absorption spectra (Roy et al., 2011), using the software Empower Pro 3. Each peak was checked and the baseline readjusted to minimize errors due to noise. They were then quantified by using peak area, compared to standard calibration curves ($\mu g.L^{-1}$). Pigments dominant in phytoplankton groups were used as markers of these groups. We used fucoxanthin (fuco) as biomarker for fucoxanthin-rich diatoms, peridinin (peri) for dinophytes, and alloxanthin (allo) for cryptophytes (Bustillos-Guzmán et al., 2004). In addition, prasinoxanthin (pras) was used as a biomarker for prasinophytes, while chlorophyll *b* (chl *b*), lutein (lut), violaxanthin (vio), neoxanthin (neo) and zeaxanthin (zea) were considered as characteristic for green algae (Chlorophyta). In the lagoons, the Chlorophyta are mainly represented by chlorophytes and prasinophytes. Zeaxanthin is, however, also present in cyanobacteria (Wright et al., 1991; Sherrard et al., 2006; Eker-Develi et al., 2012). 19'But-fucoxanthin (but-fuco) and 19'Hex-fucoxanthin (hex-fuco) have been used for haptophytes (Wright et al., 1991; Zapata et al., 2000; Paerl et al., 2003).

*2.4. Statistical analysis*

Non-parametric comparisons of Chl *a*, TP, TN concentrations have been performed by Wilcoxon test (R) to compare before (2000-2005) and after (2006-2013) the diversion of the effluents of the waste water treatment. Spearman's rank correlation has been used to describe the links between environmental parameters and biological variables.

The effects of the pre-eutrophication status of the lagoon for its response to the sewage diversion were tested by using two ways ANOVAs (lagoon and time effects for the tested variables: Chl *a* concentration, pico- and nano-eukaryotes abundances). When data did not

satisfy the conditions of applications (normality, homoscedasticity and independence of residuals), they were log-transformed. If they still did not satisfy conditions, we used Permanova (Anderson, 2001). Pairwise comparison with post-hoc Tukey tests helped to separate lagoons into groups. To assess differences of diversity between lagoon and changes in time, diversity index of Shannon (Ds), and evenness using the number of main phytoplankton groups were estimated, from the annual median of summer pigment concentrations. It represented diversity of main phytoplanktonic groups. Two ways ANOVAs were then performed on these indexes to assess if there were differences between lagoons or between years for these two parameters.

To characterize lagoons, spatio-temporal differences in pigment diversity and concentrations were described using non-parametric multivariate analysis of variance (Permanova) and two between-class principal component analyses (between-class PCA), which considers one qualitative variable (here, either the year or the lagoon) (Pélissier et al., 2003). Then, Monte-Carlo tests were used to check significances of differences between groups (Tournois et al., 2013). These PCA were operated on most of the pigments identified, except those rarely identified, with low concentrations, and which did not provide any additional information to this analysis, *e.g*. phaeophorbid *a*, and 19'but-fucoxanthin. All the statistical analyses were operated with the R software (R Core Team, 2013).

## 3. Results

### *3.1 Characterization of the lagoons for the entire monitoring period (2000-2013)*

During summer, both the average temperature and their variations in the lagoons were very similar, *i.e*. around 25 °C with a range between 20 and 31 °C, respectively. A salinity gradient was observed from MW, the less saline lagoon station subject to recurrent freshwater inputs from the Lez River, to IS without freshwater tributaries and connected with the sea by an inlet

(Table 2). The lagoons also presented a trophic gradient, from mesotrophy (IN, IS) to hypertrophy (MW) based on the Chl *a* and total nitrogen and phosphorus concentrations (TN, TP). Based on the entire period (2000-2013), median values of chlorophyll ranged from 3.7 µgChl*a*.$L^{-1}$ at IS to 83.1 µgChl*a*.$L^{-1}$ at MW, and median values of TN and TP ranged from 30.7 and 0.9 µM, respectively at IS to 196 and 12.4 µM, respectively in MW (Table 2). The trophic and salinity gradients appeared inverse in the lagoons as illustrated by significant negative correlations of the three eutrophication indicators (Chl *a*, TN and TP) with salinity ($\rho_{salinity}$= -0.15; $\rho_{TN}$= -0.32; $\rho_{TP}$= -0.31; p-values < 0.05, Spearman correlation, R).

Within the phytoplankton community, the highest values of biomasses and abundances were generally observed before the diversion. Thus, the maximum Chl *a* concentration was 413 µg.$L^{-1}$ observed in June 2004 at MW, in the most hypertrophic lagoon, although a strikingly low value of 0.09 µg.$L^{-1}$ was observed in July 2002 at IN, the inland mesotrophic lagoon (IN). Picoeukaryote abundances ranged from 2.9 x $10^6$ cells.$L^{-1}$ in the inland mesotrophic lagoon (IN) in June 2004 to 2.2 x $10^{13}$ cells.$L^{-1}$ in an inland eutrophic lagoon (AN) in July 2004. Nanoeukaryote abundances ranged from no detected cells in several lagoons (GR, IN, IS, MW) in summer 2001 to 2.4 x $10^9$ cells.$L^{-1}$ at ME in June 2003. Phycoerythrin-rich picocyanobacteria were present in low abundances compared to picoeukaryotes. Their abundances ranged from no detected cells in several lagoons (MW, VC and PB), during the summers of 2001 and 2002, to 1.1 x $10^8$ cells.$L^{-1}$ at MW in June 2004.

*3.2. Changes in eutrophication indicators before and after effluent diversion*

The TN, TP and chlorophyll *a* concentrations showed strong variations during the entire period, which were partly related to the changes before and after effluent diversion. These three variables decreased significantly after the nutrient diversion for all the lagoons (Table 3), showing a decrease of the eutrophication permitted by the implementation of the

diversion. Median values of TN, TP and Chl *a* decreased for the period 2006-2013 (respectively, 47 µM, 1.9 µM and 3.4 µgChla.L$^{-1}$) in comparison to the period 2000-2005 (respectively, 84 µM, 3.8 µM and 23 µgChla.L$^{-1}$). In contrast to the three variables, there was no general trend for the three forms of dissolved inorganic nitrogen ($NO_3$, $NO_2$, $NH_4$) since the concentrations were not systematically different before and after effluent diversion. $NH_4$ represented the main source of DIN before (77%) and after (71%) the effluent diversion. Phosphate concentrations ($PO_4^{3-}$) showed no significant differences between the two periods.

Two-way ANOVA and Permanova (see Methods) showed that the Chl *a* concentrations, the TN and TP concentrations (for Chl *a*, TN and TP concentrations: Two way ANOVA, p-value < 0.05) and the picoeukaryote abundances (Permanova, *p*-values < 0.05) significantly differed among lagoons. Posthoc pairwise Tukey test on Chl *a* concentrations allowed separating the lagoons into three groups (Table S1).

The first group was characterized as hypertrophic and included three lagoons (AR, GR and both stations (ME and MW) of Méjean lagoon). The second group was characterized as eutrophic and comprised three lagoons (PB, VC and both stations (PE and PW) of Prévost lagoon). The third group was characterized as mesotrophic and comprised two lagoons (IN and IS). Within each group, the lagoons showed a similar response to the nutrient reduction (*i.e.* no significant effect of lagoons within groups according to the Anova and posthoc test, p-value > 0.05). Hence, we selected one station in each group to illustrate the impact of trophic status on the lagoon responses to the re-oligotrophication process. For each group, we chose stations in inland lagoons under the direct influence of watershed discharges and more sensitive to potential nutrient loadings than the seafront lagoons. Hence, MW, VC and IN stations were selected to represent the hypertrophic, eutrophic and mesotrophic inland lagoons, respectively.

315 The impact of the trophic status of the selected lagoons on the phytoplankton responses to the
316 re-oligotrophication process was investigated by comparing Chl *a* concentrations (Fig. 2) and
317 picoeukaryote and nanoeukaryote abundances (Fig. 3) before and after the diversion. Chl *a*
318 concentrations were significantly reduced (mean comparison all lagoons, $p < 0.05$) with a
319 decrease of 90.4%, 65.4 % and 79.9 % in the hypertrophic (MW), eutrophic (VC) and
320 mesotrophic (IN) lagoons, respectively (Fig. 2). Picoeukaryote abundances were reduced by
321 60.5% and by 81.8 % in hypertrophic (MW), and mesotrophic (IN) lagoons, respectively (Fig.
322 3). In contrast, in the eutrophic lagoon (VC) for picoeukaryotes the difference before and after
323 the diversion was not significant (mean comparison, p-value > 0.05, see Fig. 3B).
324 Abundances of nanoeukaryotes did not show a global trend for the three lagoons (mean
325 comparison all lagoons, p-value > 0.05). Chl *a* concentrations and picoeukaryote abundances
326 were significantly correlated, showing that the response of the lagoons to the nutrient
327 reduction was particularly well reflected by the picoeukaryotes.
328
329 *3.3. Phytoplankton responses after the reduction of effluent nutrient loads*
330 Since 2006, *i.e.* after nutrient reduction, phytoplankton biomass measured in summer
331 decreased in the three selected lagoons (Fig. 4). The decrease of Chl *a* concentrations was
332 particularly pronounced between 2006 and 2007 and depended on the trophic status of
333 lagoons. Hence, summer Chl *a* concentrations were reduced by 87.7% in MW, 22.4% in VC
334 and 46.2% in IN between 2006 and 2007, reaching mean values of 1.7 $\mu gChla.L^{-1}$ in
335 mesotrophic lagoon (IN) to 9.8 $\mu gChla.L^{-1}$ in hypertrophic lagoon (MW) in the summer of
336 2007 (Fig. 4). Since 2007, the mean Chl *a* concentrations remained below 10 $\mu gChla.L^{-1}$ in
337 the three selected lagoons, except in 2011 in the hypertrophic lagoon (MW) where the mean
338 concentration reached 20.1 $\mu gChla.L^{-1}$.
339

Since 2006, in parallel to phytoplankton biomass, phytoplankton community pigment composition was determined (Table 3). The total summer concentration of accessory pigments in the three selected lagoons showed the same pattern as observed for Chl *a* concentrations, with the highest pigment concentrations measured in hypertrophic lagoon (MW) and the lowest pigment concentrations measured almost in mesotrophic lagoon (IN).

In the hypertrophic lagoon (MW), fucoxanthin was the dominant pigment in 2006, indicating a dominance of the fucoxanthin-rich diatoms (Table 3 and Fig. 5A). Green algae pigments (Chl *b*, lutein, neoxanthin, violaxanthin, prasinoxanthin, and zeaxanthin) showed also high concentrations (more than 5 $\mu g.L^{-1}$) and proportions. We could safely assume that the bulk of zeaxanthin was associated with green algae, since abundances of cyanobacteria were systematically low in the lagoons. In contrast, in the eutrophic lagoon (VC) peridinin showed a high proportion in 2006 (45.4%) indicating a dominance of dinophytes. In the mesotrophic lagoon (IN), proportions of pigments were more equally distributed and fluctuated less than in the other lagoons (Fig. 5) suggesting increased phytoplankton diversity.

The temporal patterns of the mean values of the total accessory pigment concentrations were similar to those of phytoplankton chlorophyll *a* biomass. Concentrations of total accessory pigment strongly decreased between 2006 and 2007, from 80.8% in hypertrophic (MW), 65.8% in eutrophic (VC) and 50.3% in mesotrophic (IN) lagoon but increased in 2011, with 284%, 34.6% and 15.5% in the hypertrophic (MW), eutrophic (VC) and mesotrophic (IN) lagoons, respectively.

Figure 5 shows the time course of the proportions of the accessory pigments in the three selected lagoons. In the hypertrophic lagoon (MW), the fucoxanthin proportion dropped (-99.7%) between summers 2006 and 2007 and this decrease continued until 2011 (Fig. 5A). This reduction of the fucoxanthin concentration coincided with a strong increase of the proportion of green algae pigments (Chl *b*, lutein, neoxanthin, violaxanthin and zeaxanthin)

365 during the same period. The proportion of green algae pigments remained high during all the
366 monitoring and reached almost 80% during four years (up to 85.4% in 2007). The year 2011
367 was marked by strong shift in pigment composition with the lowest fucoxanthin concentration
368 (diatoms) and the highest concentrations of green algae pigments. On the contrary, the year
369 2012 is marked by a decrease of green algae group (4.88% of the pigment proportions). The
370 alloxanthin, marker of cryptophytes, increased after 2006 and its proportion fluctuated
371 between 0.02 % in 2006 and 22.4% in 2013. Peridinin, marker of dinophytes, was below 1%
372 in 2006 and fluctuated between 2007 and 2010. In 2012 when green algae decreased,
373 peridinin concentration strongly increased in association with Chl *c*2 and diadinoxanthin and
374 became the main phytoplanktonic group in 2012 and 2013 (peridinin proportion > 30%).
375 In the eutrophic lagoon (VC), phytoplankton pigment composition was dominated by
376 peridinin, Chl *c*2 and dianodinoxanthin during all the monitoring (Fig. 5B). Peridinin
377 proportions were higher in this eutrophic lagoon than in hypertrophic (MW) and mesotrophic
378 (IN) ones. Peridinin tended to decrease until 2011 (-40.5% in 2008), but highly increased in
379 2012 and 2013 (respectively, 51.2 and 38.5%). In contrast, alloxanthin (cryptophytes)
380 remained stable during the monitoring (up to 10% in proportions). Diatom pigment increased
381 and fluctuated between 2008 and 2011. As for hypertrophic lagoon, the year 2011 was
382 marked by a temporary increase of green algae pigments (up to 40% in proportions).
383 In the mesotrophic lagoon (IN), diatoms and cryptophytes pigments (respectively,
384 fucoxanthin and alloxanthin) were dominant during the 8-year monitoring (Fig. 5C).
385 dinophytes and prasinophytes pigments (respectively peridinin and prasinoxanthin) occurred
386 more since 2010. The proportion of green algae pigments fluctuated, during the entire
387 monitoring period, between 2% in 2008 and 40% in 2011. In the three selected lagoons,
388 19'hex-fucoxanthin and 19'but-fucoxantthin were rarely observed (haptophytes).
389

Based on the chemotaxonomic analyses, the Shannon diversity and Evenness indices (see Methods) have been calculated as proxies for phytoplankton diversity through the 8-year monitoring period (Figure 6A). Globally, the number of main phytoplankton groups significantly changed during the survey. The richness of main taxonomic groups was significantly lower during the period from 2007 to 2009 (4 or 5 groups) than in 2006 and after 2010 (6 groups). The low diversity during these early years reflected that the community was dominated by diatoms, green algae and cryptophytes. Some differences of the richness also appeared depending on the trophic status of lagoons. It was lower in hypertrophic lagoons than in mesotrophic ones, and lower in mesotrophic lagoons than in eutrophic ones. Pooling all years, there was a significant higher diversity (Shannon diversity index Fig. 6A, $D_s$) in the mesotrophic lagoon (IN) compared to the hypertrophic lagoon (MW), but there was no significant difference between mesotrophic (IN) and the eutrophic (VC) lagoons (two-ways Anova and posthoc test, p-value > 0.05). The evenness showed also significant lower values in the hypertrophic lagoon (MW) compared to the mesotrophic (IN) one, because of the green algae dominance in MW (Figure 6B).

In the hypertrophic lagoon (MW), diversity $D_s$ significantly fluctuated during the 8-year monitoring (Fig. 6A). It firstly decreased from 2006 ($D_s$ = 0.49) to 2007 ($D_s$ = 0.20) and then increased between 2007 and 2009 ($D_s$ = 0.70). It decreased again between 2009 and 2011 ($D_s$ close to 0.20) and finally increased to reach a maximal $D_s$ value in 2013 ($D_s$ = 0.74). These changes illustrated shifts in phytoplankton community composition. The low values of $D_s$ in 2007/2008 and in 2010/2011 are related to the predominance of green algae (Fig. 5A) during these four years. Evenness values showed the same patterns in time as that of the Shannon diversity index (Fig. 6B). It strongly decreased when green algae was the dominant phytoplankton group. After 2011, it increased to reach its highest value in 2013.

In contrast, in the eutrophic lagoon (VC), $D_s$ increased from 2006 to 2008 (from 0.36 to 0.71) and stayed relatively stable to reach 0.77 in 2011. The diversity decreased strongly in 2012 ($D_s$ = 0.27, lowest diversity) and stayed lower in 2013 ($D_s$ = 0.50) than between 2007 and 2011. Evenness increased from 2006 to 2009 (0.43 to 0.92) and then fluctuated to finish with an intermediate value (E = 0.61) in 2013.

In the mesotrophic lagoon (IN), diversity and evenness significantly changed over time, decreasing between 2006 and 2008 to reach the lowest values (respectively, 0.47 and 0.63, Figure 6) in 2008. From 2009 to 2010, diversity indices increased and stayed relatively stable to reach the highest values in 2013 (respectively, 0.75 and 0.91, Fig. 6). The lowest diversity and evenness values observed in 2008 were associated to the dominance of two phytoplanktonic groups (diatoms and cryptophytes, Fig. 5C).

*3.4. Lagoons trajectories based on pigment diversity*

In order to study whether the patterns observed for the selected stations are robust for the entire Palavasian lagoon complex, multifactorial analyses were performed using accessory pigment database from the 10 stations of the complex for the 8-year monitoring. It was observed that pigment composition (concentration and diversity) significantly changed among stations (Permanova, F = 5.29, df = 9, p-value = 0.001), and years (Permanova, F = 4.49, df = 7, p-value = 0.001). Moreover, the interaction between the two factors also showed a significant effect (Permanova, F = 1.54, df = 59, p-value = 0.001), indicating that the temporal patterns were different among stations. The principal component analysis (PCA) was used on both lagoon stations (Fig. 7) and years (Fig. 8) to elucidate phytoplankton composition patterns in relation to lagoon trophic status and time. For each of the two PCA, the results were significantly different (Monte-Carlo test, p-value = 0.001), although the ellipses representing each group (either lagoons or by years) overlapped.

The first PCA showed differences between lagoons (Fig. 7). The first axis explained 71% of the total variance, and was mainly driven by pigments markers of the green algae (lut, neo, chl *b*, viola and zea), prasinophytes (prasino), diatoms (fuco, chl *c*2, diadino), associated with total biomass. The second axis explained 16.5% of the total variance and opposed pigments markers of dinophytes (peri) and cryptophytes (allo) in positive against pigment marker of haptophytes (Hfuc) in negative (Fig. 7A). The first axis is correlated with pigments that are characteristic for the phytoplankton groups which are enhanced by eutrophication (i.e., green algae, diatoms) and a marker of eutrophication (Chl *a*). The position of the center of gravity of the ten stations in this projection showed two groups of stations: the three most eutrophic stations (ME, MW, GC) strongly separated from the other ones (Fig. 7B-C). The group of hypertrophic stations presented a very large ellipse suggesting a strong variability of pigment concentrations. Their center of gravity was also located close to Chl *a*, indicating an elevated biomass in these stations compared to the others. More precisely, they are located in two different directions: MW and ME were close to green algae pigments, while GC is closer to pigments from diatoms and cryptophytes indicating a dominance of these three phytoplankton groups with eutrophication. The second group of stations presented lower concentrations of pigments for all the phytoplanktonic groups. Among this group, the centers of gravity of the different stations ranged from positive to negative along the axis 2, reflecting the trophic gradient ranging from the most (AN) to the less eutrophied (IS) station. Their positions along the second axis reflect some differences in the phytoplankton composition along this gradient. Eutrophic lagoons (AN and PB) were close to cryptophytes and dinophytes pigments. On the opposite, mesotrophic lagoons (IN and IS) and Prévost (PE, PW), a lagoon under marine influence, showed a very low phytoplankton biomass and pigment concentrations, and a stronger signal of haptophytes pigment.

The second PCA showed differences between years (Fig. 8). The first axis explained 73% of the total variance, and was mainly driven by the pigment estimating total phytoplankton biomass (Chl *a*), pigment markers of diatoms (fuco) or mostly abundant in diatoms (diadino, Chl *c*2), and pigment marker of prasinophytes (prasino). The second axis explained 17% of the total variance and was mainly driven by pigment marker of cryptophytes (allo) and green algae in positive, opposed to pigment marker of dinophytes (peri) and haptophytes (19'Hex-fuco) (Fig. 8A). The vectors of the pigment markers of cryptophytes and dinophytes were opposed in the first two planes of the PCA (Fig. 8A). The projection of the pigment composition of the ten stations showed that 2006 differed from other years by a stronger concentration of the pigments driving the first axis (Fig. 8B). The phytoplankton biomass was still high in summer 2006, six months after the diversion (Fig. 4), and diatoms were the main group in the ten stations, with a higher fucoxanthin concentration compared to the other pigments that explain the position of the center gravity of this year on the axis 1. The following years are distinct from 2006 along the axis 2, showing a change in phytoplankton biomass and composition (Fig. 8B-C). Their position indicate a strong decrease of the Chl *a* concentrations and shifts in phytoplankton composition with a decrease of diatoms and an increase of green algae principally in 2007 and 2008. From 2007 to 2012, each center of gravity showed a displacement along the axis 2, in the direction of the pigment marker of dinophytes, indicating an increase of the occurrence and the concentration of the peridinin in time. 2011 is characterized by a return to pigment markers of green algae, as described in figure 5. Finally, 2013 also differed from the global trend, with a community composition close from those in 2010, less dominated by dinophytes, more balanced between all the pigments identified.

## 4. Discussion

This study represented a unique opportunity to assess the impact of re-oligotrophication processes in shallow Mediterranean coastal lagoons in conjunction with their contrasting trophic states. The results show that phytoplankton responded quickly, and that the amplitude and qualitative changes depend on the prior eutrophication status of the lagoon.

*4.1. Phytoplankton chlorophyll biomass in heavily eutrophied lagoons*

Before the reduction of the nutrient loadings, the 'Palavasian' lagoons were strongly degraded by regular inputs of nitrogen and phosphorus from treated sewage. The most hypertrophic lagoons Méjean and Grec presented Chl *a* concentrations close to those found in hypertrophic lakes (Jeppesen et al., 1998; Bell and Kalff, 2001). In these lagoons, phytoplankton was the major primary producer, with excessive blooms leading to a complete loss of seagrasses communities and low macroalgal cover probably due to competition for light. Eutrophic symptoms associated to water quality degradation in Palavasian lagoons can be illustrated by the succession of primary producers (Schramm, 1999; Bricker et al., 2008), ranging from a moderate impact, with presence of some macroalgae and seagrasses (IN and IS), to more than the high impact, i.e., dense phytoplankton without macroalgae (ME, MW, GC). The phytoplankton dominance may be related to its strong capacity to compete for dissolved nutrients and light (Cebrian et al., 2014).

After the implementation of the diversion, TN, TP and Chl *a* biomass responded quickly by a strong decrease within two years after the nutrient reduction, whatever the prior trophic status of the lagoons. Although such a decrease has already been observed during re-oligotrophication processes in lacustrine (Ruggiu et al., 1998; Jeppesen et al., 2005; Van Donk et al., 2008), riverine or coastal ecosystems (Kemp et al., 2005; Greening and Janicki, 2006; Xu et al., 2010), it is highlighted that in these coastal lagoons, the response was quick compared to the other systems. Hence, the reduction of chlorophyll *a* in the Tampa bay took 5

514 years (Greening and Janicki, 2006), and the reduction of TN and TP in lakes took about 5 and
515 10-15 years, respectively (Jeppesen et al., 2005). The amplitude of the decrease of Chl *a*
516 biomass was strongly linked to the prior trophic status of the lagoon, and was mainly driven
517 by the decrease of picoeukaryote abundances. Steady-state nutrient conditions observed in
518 these lagoons resulted in massive picoeukaryote blooms, observed particularly in eutrophic
519 and hypertrophic lagoons (Table 2). These picoplanktonic blooms often represented the
520 dominant fraction of Chl *a* biomass throughout summer (Bec et al., 2011) leading to a strong
521 depletion of nutrients in these highly eutrophied systems (Souchu et al., 2010). These
522 phenomena could explain the absence of significant changes for the inorganic nitrogen and
523 phosphorus concentrations after the reduction of nutrient loadings. The rapidity of the
524 responses of coastal lagoons could also be associated to favorable climatic conditions during
525 the five years (2006-2010), characterized by low or no rainfall during summer ("*Comparaison*
526 *climatologique annuelle - Infoclimat*"), reducing non-point source nutrient loads.
527
528 *4.2. Phytoplankton functional taxonomic groups*
529 Small cells (< 10 µm) are the typical dominant phytoplankton in lagoonal algal blooms
530 (Glibert et al., 2010; Bec et al., 2011; Pachés et al., 2014). In the Palavasian complex,
531 phytoplankton community was often dominated by small size classes, most belonging to pico-
532 (2-3 µm) and nanoeukaryotes (3-6 µm) (Bec et al., 2011). Major functional/taxonomic groups
533 were represented by diatoms, green algae, cryptophytes, and dinophytes. Among them,
534 diatoms, green algae and cryptophytes are functional groups composed of fast-growing algae
535 that may have been favored in response to high and fluctuating nutrient loadings (Paerl et al.,
536 2006; Paerl et al., 2010) such as observed in lagoons. However, these functional groups that
537 exhibited contrasting ecological strategies, especially in resource utilization (Litchman et al.,

2007), responded differently to the reduction of effluent nutrient loads in conjunction with lagoon environmental changes (*i.e.* salinity, light, turbulence).

Diatoms were present in all the Palavasian lagoons and were particularly dominant in the most eutrophic lagoons in 2006. In many coastal systems, diatoms dominated the phytoplankton community when silicate concentrations were sufficiently high to sustain their growth (Lie et al., 2011; Burford et al., 2012). In Palavasian lagoons, it has been suggested that silicate concentrations may not be limiting for diatom growth (Souchu et al., 2010). Diatoms, with high maximum nutrient uptake rates and high growth rates, may be favored under high or fluctuating nutrients (Litchman et al., 2007) and are not inhibited by turbulence associated with those nutrient regimes (Margalef, 1978). Moreover, diatoms have a high nitrogen affinity, especially small-sized species (Litchman and Klausmeier, 2008; Litchman et al., 2009) making them really competitive compared to others. Thus, this functional group is well adapted to dominate highly eutrophied waters, particularly in coastal waters under freshwater discharges or sewage influence (Paerl et al., 2010; Lie et al., 2011; Gadea et al., 2013). Furthermore, diatoms with low half-saturation constants for irradiance-dependent growth (Litchman et al., 2007) could be more adapted to the low irradiance associated with high chlorophyll concentration in eutrophic and hypertrophic systems (Sommaruga and Robarts, 1997). In terms of seasonal dynamics, diatoms blooms in temperate coastal systems occur mainly in spring but may last during summer if nutrients are continuously supplied and not entirely consumed (Chisholm, 1992; Agawin et al., 2000; Chang et al., 2003). Throughout the year, the continuous discharge of sewage effluent resulted in high nutrient loadings in lagoons that may sustain diatom growth (Lie et al., 2011; Burford et al., 2012). Then, the reduction of effluent nutrient loadings could affect diatom growth, particularly during the dry summer period, leading to the large decrease of fucoxanthin-rich diatoms. This decrease was particularly observed in the hypertrophic lagoons between 2006 and 2007, suggesting a time

lag between reduction of nutrient loading and changes of phytoplankton community composition. In these lagoons, the phytoplankton composition has shifted towards green algae dominance. Temperature also plays an important role in the phytoplankton seasonal succession. Many green algae (*Chlorella*, *Scenedesmus*, *Cosmarium*) have optimal temperature for growth between 25 and 35°C (Litchman et al., 2010). After 2006, the shift from diatoms to green algae dominance during summer could thus be explained by the shift in nutrient delivery (decreasing external inputs) and by high summer temperatures.

Small *Chlorella*-like algae were the main component of the picoeukaryote community in the eutrophic Palavasian lagoons before the reduction of nutrient loadings (Bec et al., 2011). After 2006, green algae (chlorophytes and prasinophytes) was also a major functional group of phytoplankton community in the 3-6 µm size range and replaced small diatoms especially into hypertrophic lagoons (ME, MW, GC). Because of their high S:V ratio, efficient growth rates and enhanced nutrient uptake rates (Paerl et al., 2003), green algae appear more competitive than other phytoplankton functional groups to use regenerated forms of nutrients in the lagoons (Glibert et al., 2010). Indeed, benthic fluxes are an important source of nutrients (NID and DIP) in coastal lagoons, especially during summer, and may result in high $NH_4$ efflux from the sediment to the water column (Glibert et al., 2010). Even if the effluent diversion has resulted in a strong reduction of the external nutrient supply, internal supply from benthic stocks related to eutrophication could stimulate phytoplankton growth (McGlathery et al., 2007; Burford et al., 2012). Green algae have abilities to use ammonium from regenerated sedimentary stock (Domingues et al., 2011; Donald et al., 2011) and have particularly high affinity for ammonium uptake compared to diatoms and dinophytes (Litchman et al., 2007).

Furthermore, the eutrophication gradient in the Palavasian lagoons is highly linked to freshwaters inputs. Green algae were mainly observed in brackish and hypertrophic lagoons

588 such as Méjean lagoon (Table 2). This phytoplankton functional group is tolerant to low
589 salinity as observed in freshwater and brackish coastal lagoons (Coelho et al., 2007;
590 Cartaxana et al., 2009; Pachés et al., 2014) and is often observed in hypertrophic systems such
591 as lagoons under high freshwater inputs (Torres and delRio, 1995; Bonilla et al., 2005) and
592 lakes (Wasmund and Kell, 1991; Hepperle and Krienitz, 2001). So both low salinities and
593 high trophic status could explain the predominance of chlorophytes in some Palavasian
594 lagoons. Flagellates such as Prasinophytes and cryptophytes, present in the most eutrophic
595 lagoons also seems to be promoted by low salinities (Bonilla et al., 2005).
596 Cryptophytes were present in high proportions in the Palavasian lagoons where they can
597 respond quickly to nutrient loads due to their high growth rate (Paerl et al., 2003). In
598 mesotrophic lagoons, it allowed them to be more competitive than picoeukaryotes that were
599 less abundant. This taxonomic group is well adapted to turbid and low light environments
600 such as coastal and estuarine waters due to photoacclimation (phycobilins and alloxanthin
601 pigments) to low light intensities (Bergmann, 2004; Weng et al., 2009; Fischer et al., 2014).
602 Microscopic observations of the ciliate, *Mesodinium rubrum*, in some Palavasian lagoons
603 suggest that cryptophytes could be also a potential food source for the mixotrophic dinophytes
604 (Paerl et al. 2003; Myung et al., 2011).
605 Dinophytes were scarce or absent in mesotrophic and hypertrophic lagoons in 2006. In many
606 coastal waters, dinophytes occurrence is generally attributed to eutrophication (Anderson et
607 al., 2002; Heisler et al., 2008). However in the coastal lagoons in the South of France,
608 dinophytes have been reported mainly for oligotrophic and mesotrophic marine lagoons
609 (Collos et al., 2009; Bec et al., 2011). Hence, it was suggested that habitat disturbance,
610 species displacement and low turbulence could favor dinophytes occurrences. Due to
611 relatively low growth rates, it is expected that this functional group is better adapted under
612 low-nutrient and low turbulence conditions (Margalef, 1978). After the nutrient reduction, all

lagoons showed increasing proportion and occurrence of peridinin-rich dinophytes with time. In eutrophic lagoons, the phytoplankton composition has shifted from diatoms to dinophytes. This shift has already been observed during the re-oligotrophication of coastal ecosystems caused by a reduction of phosphorus loadings (Yamamoto, 2003; Collos et al., 2009). In these systems, the depletion of inorganic phosphorus may have led to blooms of dinophytes that can utilize dissolved organic phosphorus (Seto Inland Sea) or picocyanobacteria as an additional resource (Thau lagoon). Thus, the ability of dinophytes to grow while the availability of inorganic nutrients decreases could be related to their ability to supplement their photo-autotrophy by mixotrophy (Smayda and Reynolds, 2003; Litchman et al., 2007). Mixotrophy could provide a unique resource niche under steady-state summertime conditions in coastal and estuarine environments (Stickney et al., 2000). In freshwater lakes, mixotrophic flagellate species (dinophytes, chrysophytes, cryptophytes) have appeared or increased during the re-oligotrophication process (Gaedke, 1998; Anneville and Pelletier, 2000; Van Donk et al., 2008). In lake Constance, it has been argued that the mixotrophic properties of *Dinobryon* (chrysophyte) are an advantage in phosphorus-poor waters and its increasing biomass may be explained by the increasing underwater light due to the decreasing biomass of the other phytoplankton groups (Kamjunke et al., 2007). The authors suggested that the increased underwater light availability promoted the autotrophic energy gain (phagotrophic phosphorus gain) of *Dinobryon*. On the other hand, as large phytoplankton biomass blooms decreased, the shift in species composition may have led to greater prevalence of some species such as dinophytes that can fill that niche (Anderson et al., 2002).

*4.3. Phytoplankton trajectories during re-oligotrophication process*

The functional approach was used to understand the phytoplankton community patterns since the re-oligotrophication started in the Palavasian lagoons. The analysis of four major

638 functional phytoplankton groups, through algal strategies and adaptations, revealed that the
639 trajectories of the phytoplankton community displayed a complex response to changing
640 nutrient loads over time. The trajectories of coastal ecosystems during re-oligotrophication
641 may be more complex than expected as other control factors maybe changing at the same time
642 (Duarte et al., 2009).

643 During the 8-year lagoon monitoring, the decrease of chlorophyll biomass is associated to
644 changes of phytoplankton diversity that are strongly linked to the prior trophic status of the
645 lagoons. Diversity, evenness and richness of phytoplanktonic groups were higher in
646 mesotrophic lagoons than in the most eutrophic ones. In ecosystems with high production,
647 diversity is generally reduced by competitive exclusion while it is maximized at
648 "intermediate" disturbance and production level (Huston, 1979; Duarte et al., 2006 and
649 references therein). The diversity changes reflected the modifications of the community
650 structure in response of the reduction of effluent nutrient loads. While Chl *a*, TN and TP
651 concentrations decreased strongly and quickly, the diversity of main phytoplanktonic groups
652 responded to the nutrient reduction over a much longer period. Throughout the monitoring,
653 the diversity patterns were marked by a strong variability in eutrophic and hypertrophic
654 lagoons whereas phytoplankton community structure reached the highest diversity and
655 stability (since 2010) in mesotrophic lagoons. Eutrophic and hypertrophic lagoons are still
656 subjected to environmental fluctuations (i.e. fluctuating nutrient supply) or disturbance related
657 to freshwater inputs from multiple canalization (See Methods) that can explain the
658 fluctuations of phytoplankton diversity. Moreover, as high nutrient availability tends to
659 reduce phytoplankton diversity by favoring fast-growing species (Huston, 1979; Duarte et al.,
660 2006), algal coexistence should be facilitated and thus phytoplankton diversity should
661 increase in time with the decreasing of nutrient availability in lagoons as observed in
662 mesotrophic ones.

Therefore, the responses of lagoon phytoplankton community to re-oligotrophication are not a linear process that remains vulnerable to potential nutrient loads associated to rain events or to interannual climate variations. Indeed, as during the summer of 2011, the re-oligotrophication trend could be temporarily reversed by climatic conditions. The chlorophyll biomass and the proportion of chlorophytes increased in all lagoons. This year presented a record of elevated air temperature (1.5°C warmer than the 1900-2011 average). The spring was exceptionally warm and allowed early phytoplankton growth while summer presented particularly important rainfall and storm events, bringing nutrients to the lagoons (www.meteofrance.fr). The year 2011 represented an exception compared to the 8-year lagoon monitoring period but showed that the phytoplankton trajectories are fragile and can be reversed. In coastal ecosystems, the re-oligotrophication may follow the reduction of sewage nutrient inputs but may be affected by anthropogenic disturbance or by natural phenomena including rainfall events or record flood years (Saeck et al., 2013).

This study showed that changes of phytoplankton community structure and composition are a first step in restoration of water column for the Palavasian lagoons. Even if external nutrient loadings decreased, high internal nutrient loads that have accumulated in the sediments during the eutrophication period may release nutrients in the water column, particularly during summer, delaying the recovery of lagoons in the long term as observed for lakes (Jeppesen et al., 2005; Sondergaard et al., 2007). The increase of the light penetration, permitted by the reduction of the phytoplankton biomass, initiated recently a shift among the primary producers, with the reappearance of the macroalgae particularly in the most eutrophied lagoons (work in progress). Benthic macroalgae can outcompete phytoplankton for nutrients, especially if the major nutrient supply is internal loading form mineralization from sediments (McGlathery et al., 2007). This primary producer reduces the flux of nutrients from the sediment to the water column, which limits the supply of nutrients for phytoplankton growth.

688 By competition for nutrient and light, macroalgae could directly influence the phytoplankton
689 diversity and community structure during the re-oligotrophication process. Inversely to the
690 shift from benthic to pelagic-dominated primary producers occurring during eutrophication
691 (Bricker et al., 2008), Palavasian lagoons could expect a shift from a system pelagic-
692 dominated productivity based on phytoplankton to a more benthic-dominated system based on
693 macroalgae and seagrasses.
694
695 *Acknowledgements*
696 Amandine Leruste was supported by a PhD fellowship from the French Ministry of
697 Education, Higher Education and Scientific Research attributed by the Doctoral School
698 SIBAGHE in Montpellier. This paper uses data from the Lagoon Monitoring Network
699 (Réseau de Suivi Lagunaire) funded by Agence de l'Eau, Région Languedoc-Roussillon and
700 Ifremer. We thank the staff of the Ifremer laboratories in Languedoc-Roussillon for data
701 collection. We thank Annie Pastoureaud and André Vaquer for having initiated the HPLC
702 pigment analysis in the framework of the Lagoon Monitoring Network. This paper is
703 dedicated to the memory of Yves Collos (1949-2015).
704

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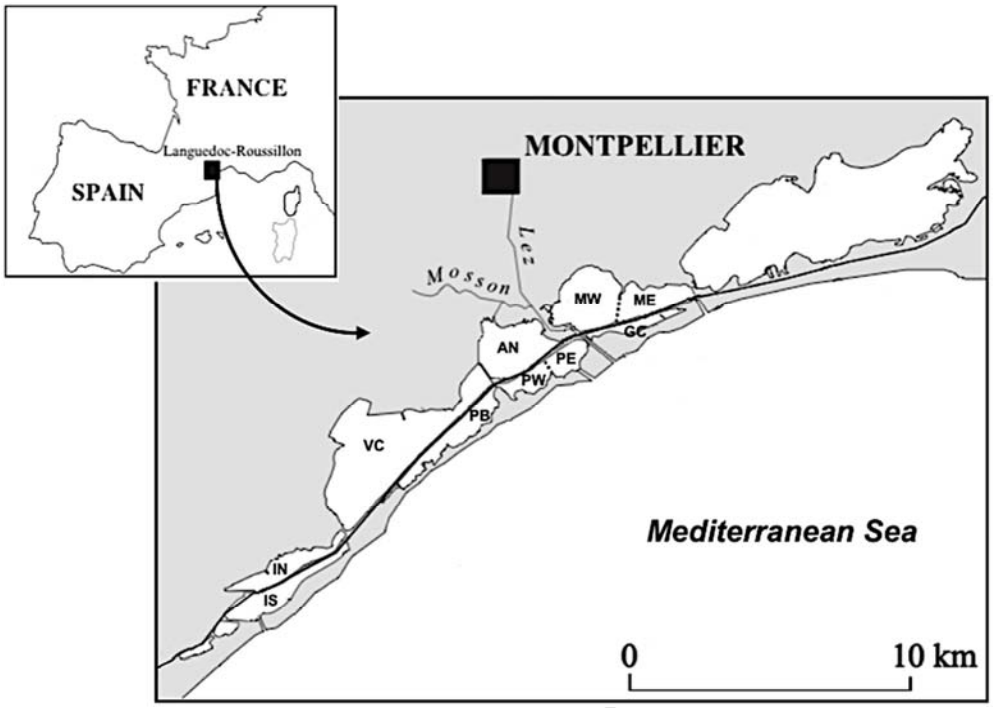
992 **Figures**
993




994

995 Figure 1: Location of the sampling stations, into the eight lagoons of the Palavasian complex,
996 next to the Montpellier city agglomeration. The dark line is represents the Rhône-to-Sète
997 canal.
998

999 Table 1. Characteristics of the 10 stations of the 8 coastal lagoons from the Palavasian
1000 complex. Trophic status, freshwater inputs and seawater exchanges are specified, from
1001 Souchu et al., 2010 and Bec et al., 2011.

| Lagoon | Trophic status | Area ($km^2$) | Volume ($10^6$ $m^3$) | Mean depth (m) | Main freshwater | Connections to the sea | Station | Label of the Station |
|---|---|---|---|---|---|---|---|---|
| Méjean | Hypertrophic | 5.5 | 4.1 | 0.75 | Channel | Indirect | East Méjean | ME |
| | | | | | | | West Méjean | MW |
| Grec | Hypertrophic | 2.7 | 0.7 | 0.30 | Channel | Indirect | Grec | GC |
| Arnel | Hypertrophic | 4.7 | 1.9 | 0.40 | River | Indirect | Arnel | AN |
| Prévost | Eutrophic | 3.8 | 2.9 | 0.75 | Channel | Direct | East Prévost | PE |
| | | | | | | | West Prévost | PW |
| Vic | Eutrophic | 11.5 | 13.8 | 1.2 | Channel | Indirect | Vic | VC |
| Pierre blanche | Eutrophic | 3.7 | 1.5 | 0.4 | Channel | Indirect | Pierre blanche | PB |
| North Ingril | Mesotrophic | 3.2 | 2.2 | 0.6 | Channel | Indirect | North Ingril | IN |
| South Ingril | Mesotrophic | 3.6 | 1.9 | 0.6 | Channel | Direct | South Ingril | IS |

1002

1003 Table 2. Median values and ranges (minimum and maximum values between brackets) of
1004 salinity, temperature, chlorophyll *a*, total nitrogen and total phosphorus concentrations, during
1005 summer periods, from 2000 to 2013.

| Station | Salinity | TN (µM) | TP (µM) | Chl *a* (µgChl *a*.L$^{-1}$) | PE-CYAN ($10^6$ cell/L$^{-1}$) | PEUK ($10^6$ cell/L$^{-1}$) | NANO ($10^6$ cell/L$^{-1}$) |
|---|---|---|---|---|---|---|---|
| MW | 26.8 (13.9 – 38.1) | 196 (42.7 – 527) | 12.4 (2.8 – 30.0) | 83.1 (1.6 – 413) | 0 (0-267) | 1000 (0-9158) | 6.9 (0-16303) |
| ME | 30.0 (19.9 – 36.5) | 108 (37.6 – 296) | 5.6 (1.2 – 17.7) | 42.2 (0.7 – 274) | 1.3 (0-333) | 280 (0.5-4567) | 5.1 (0-4300) |
| GC | 32.0 (22.1 – 39.8) | 149 (36.1 – 432) | 8.0 (1.5 – 32.4) | 73.2 (0.9 – 361) | 3.6 (0-72.9) | 257 (3.7-6526) | 13.1 (0-455) |
| AN | 35.7 (16.9 – 45.9) | 86.3 (34.5 – 298) | 4.6 (1.5 – 27.0) | 47.6 (1.0 – 393) | 0.5 (0-57) | 49.4 (1.3-22000) | 7.6 (0.8-1660) |
| PW | 36.8 (27.1 – 44.0) | 56.0 (25.4 – 137) | 2.4 (0.7 – 6.1) | 12.7 (0.7 – 54.0) | 1.2 (0-18.9) | 10.4 (0.2-1900) | 5.8 (0.5-128) |
| PE | 36.5 (30.7 – 40.5) | 53.0 (15.4 – 188) | 2.3 (0.8 – 8.8) | 15.2 (0.8 – 104) | 2.7 (0-79.4) | 32 (0.4-725) | 10.5 (0.2-627) |
| VC | 38.1 (21.4 – 51.0) | 65.2 (38.2 – 166) | 3.0 (0.8 – 13.0) | 9.9 (1.2 – 50.8) | 0 (0-10.6) | 15.4 (0.4-3200) | 3.5 (0-63.4) |
| PB | 37.3 (23.3 – 48.1) | 69.6 (37.1 – 132) | 3.8 (1.4 – 8.2) | 20.9 (1.3 – 101) | 0.2 (0-32) | 15.8 (1-1975) | 4.3 (0-157) |
| IN | 38.8 (31.9 – 44.1) | 34.5 (16.0 – 80.0) | 1.2 (0.4 – 4.9) | 4.8 (0.1 – 20.8) | 1.7 (0-43) | 16.2 (0.7-1081) | 2.3 (0-18.6) |
| IS | 38.9 (33.8 – 44.5) | 30.7 (9.6 – 75.6) | 0.9 (0.5 – 1.9) | 3.7 (0.2 – 20.5) | 5 (0-237) | 5 (0-237) | 1.7 (0-24.8) |

1006

1007

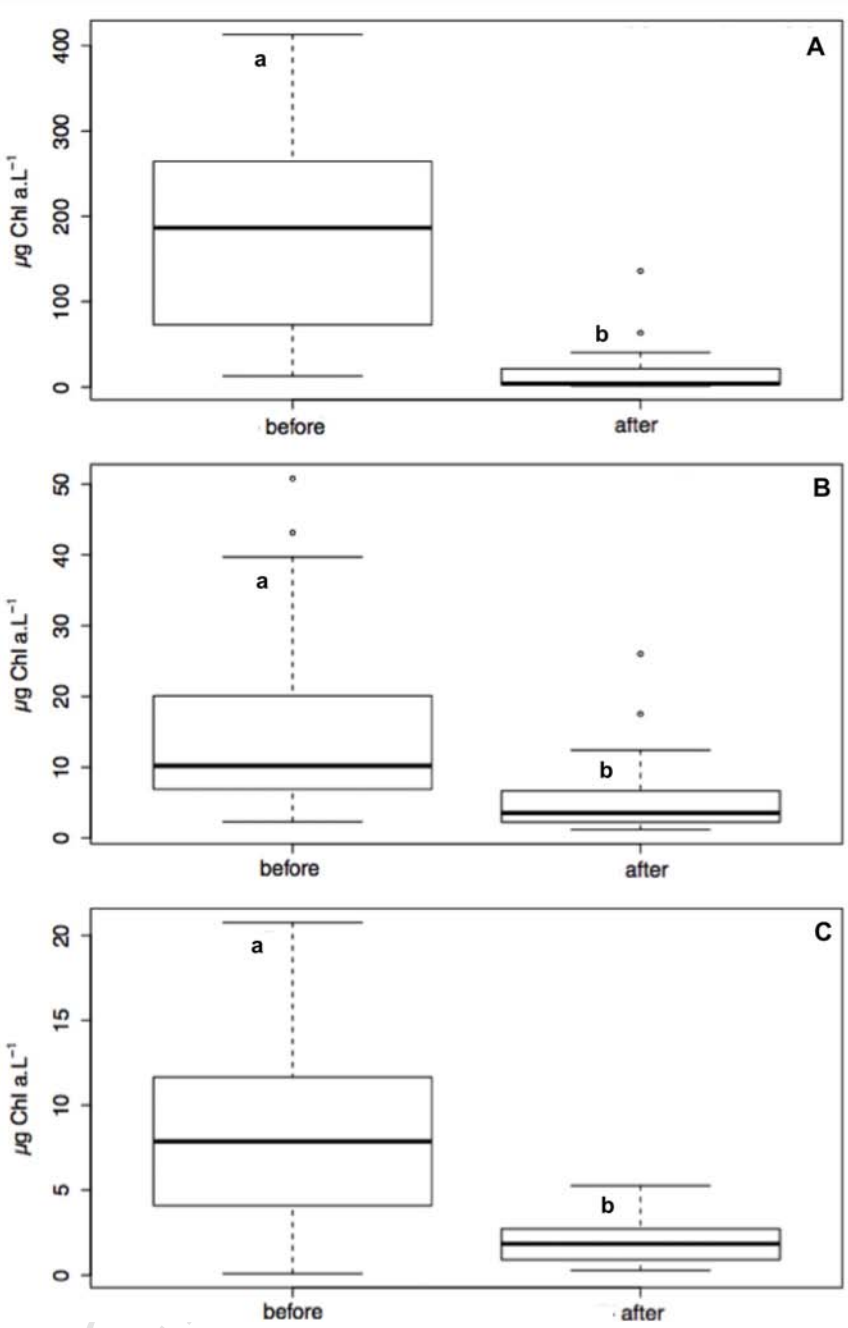



Figure 2. Distributions of Chl *a* concentration in hypertrophic (MW, A), eutrophic (VC, B) and mesotrophic (IN, C) lagoons. Box-and-whisker plots from summer values from 2000 to 2005 (before diversion), and from 2006 to 2013 (after diversion). The whiskers represent the 5th and the 95th percentiles, the outer edges of the boxes represent the 25th and 75th percentiles, and the horizontal line within the boxes represents the median. Significant difference between means before and after is illustrated by different letters (a and b).

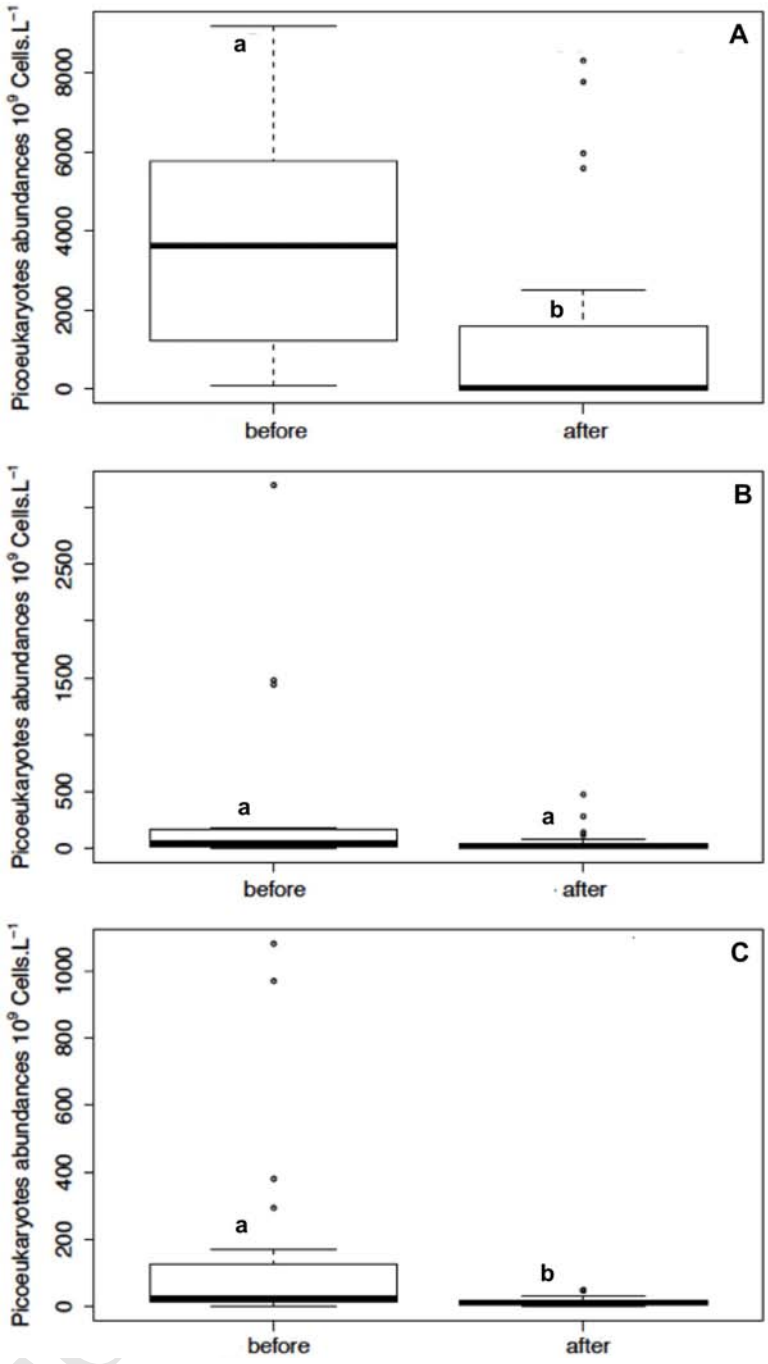
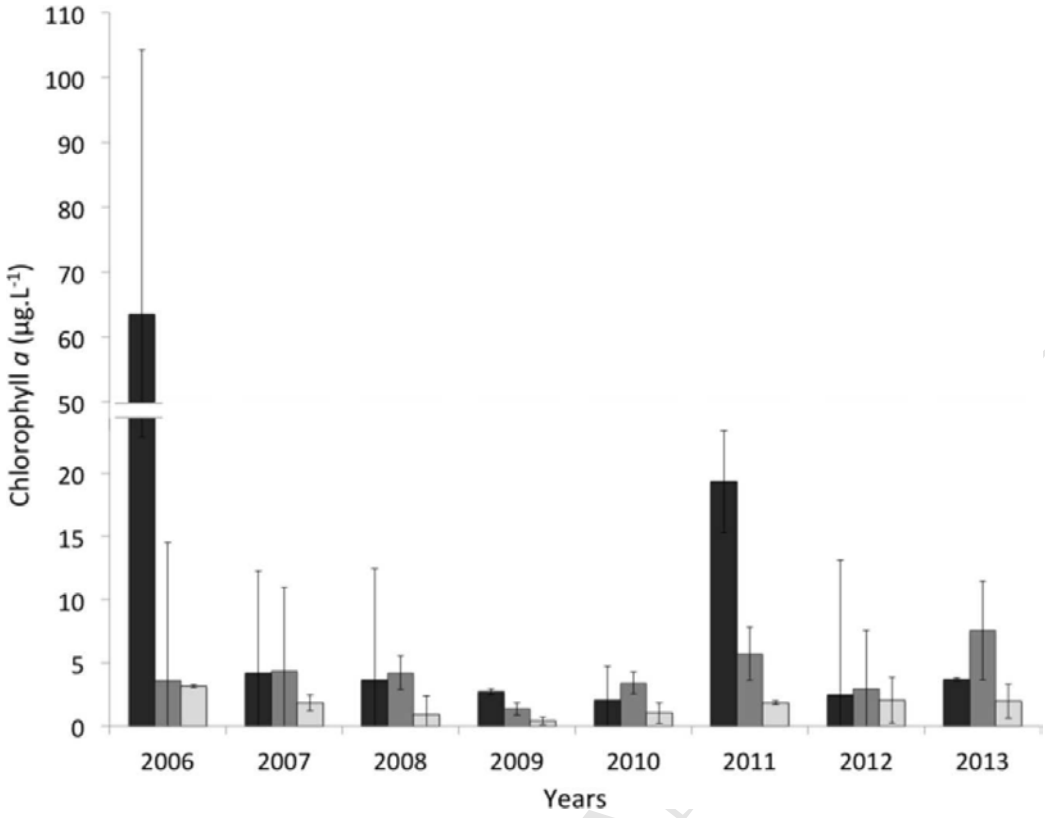



Figure 3. Distributions of picoeukaryote abundances in hypertrophic (MW, A), eutrophic (VC, B) and mesotrophic (IN, C) lagoons. Box-and-whisker plots from summer values from 2000 to 2005 (before diversion), and from 2006 to 2013 (after diversion). The whiskers represent the 5th and the 95th percentiles, the outer edges of the boxes represent the 25th and 75th percentiles, and the horizontal line within the boxes represents the median. Significant difference between mean before and after is illustrated by different letters (a and b).




1025

1026 Figure 4. Summer chlorophyll *a* concentrations in hypertrophic (MW), eutrophic (VC) and
1027 mesotrophic (IN) lagoons from 2006 to 2013. Means of three summer values (June, July,
1028 August). Black, dark gray and light gray indicate MW, VC and IN lagoons, respectively.

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1030 Table 3. Summer concentrations of pigment markers of main phytoplanktonic groups in 2006
1031 in hypertrophic (MW), eutrophic (VC) and mesotrophic (IN) lagoons. Means of three summer
1032 values (June, July, August).

| Pigments and associated taxonomic group | MW | VC | IN |
|---|---|---|---|
| | ($\mu g.L^{-1}$) | | |
| Fucoxanthin (diatoms) | 22.28 | 0.11 | 0.62 |
| Zeaxanthin (cyanobacteria – chlorophytes – prasinophytes) | 4.29 | 0.04 | 0.06 |
| Chlorophyll *b* (green algae) | 1.73 | 0.16 | 0.1 |
| Lutein (chlorophytes – prasinophytes) | 3.06 | 0.07 | 0.06 |
| Neoxanthin (chlorophytes – prasinophytes) | 1.58 | 0.02 | 0.00 |
| Prasinoxanthin (prasinophytes) | 0.68 | 0.08 | 0.06 |
| Alloxanthin (cryptophytes) | 0.01 | 0.16 | 0.16 |
| 19'But-fucoxanthin (haptophytes) | 0.04 | 0.00 | 0.00 |
| 19'Hex-fucoxanthin (haptophytes) | 0.10 | 0.02 | 0.04 |
| Peridinin (dinophytes) | 0.05 | 3.87 | 0.16 |

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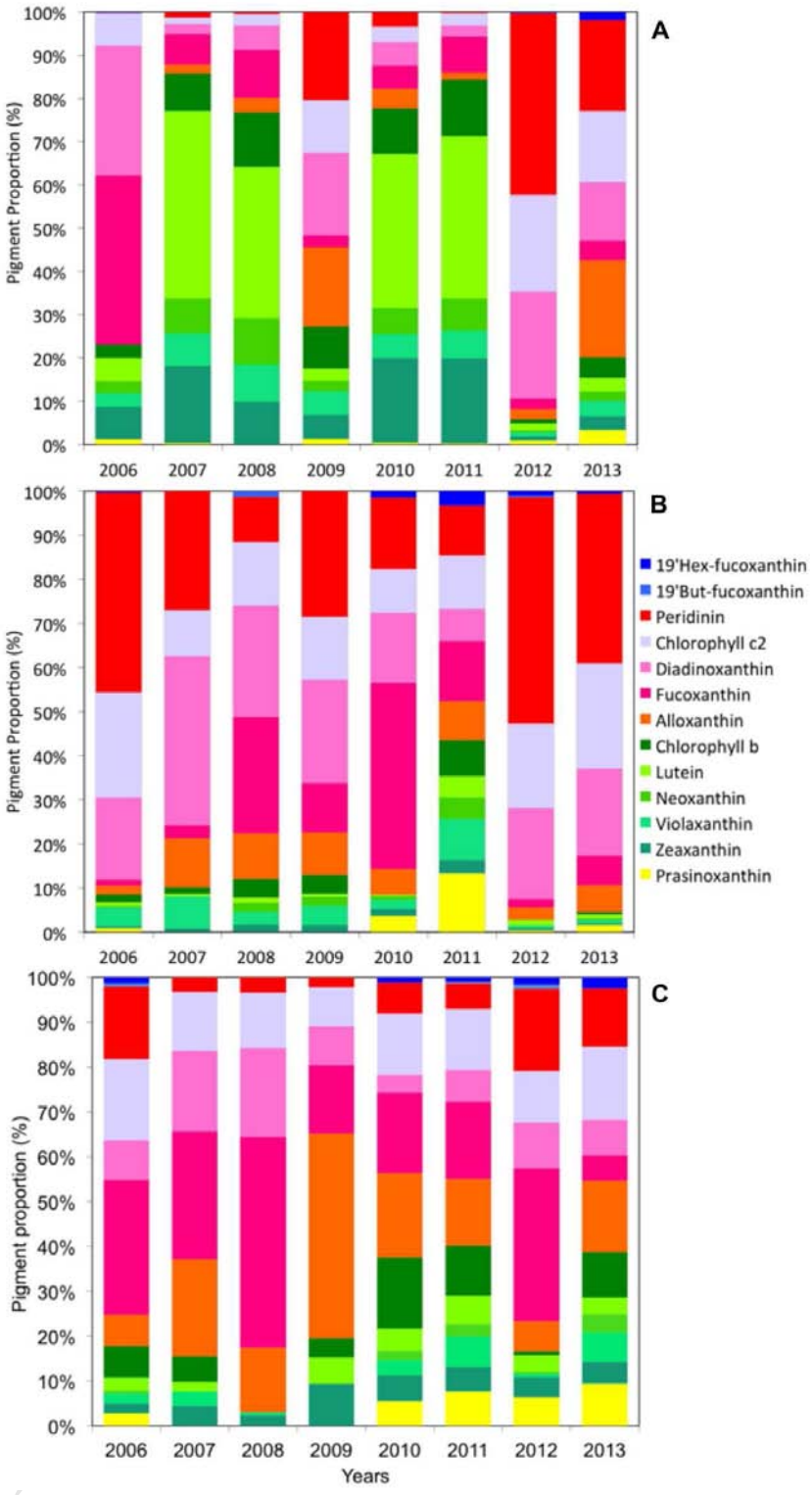


Figure 5. Proportions of accessory pigments in hypertrophic (MW, A), eutrophic (VC, B) and mesotrophic (IN, C) lagoons from 2006 to 2013. Samplings were performed in June, July, August) and the data have been calculated from mean summer values for each year.

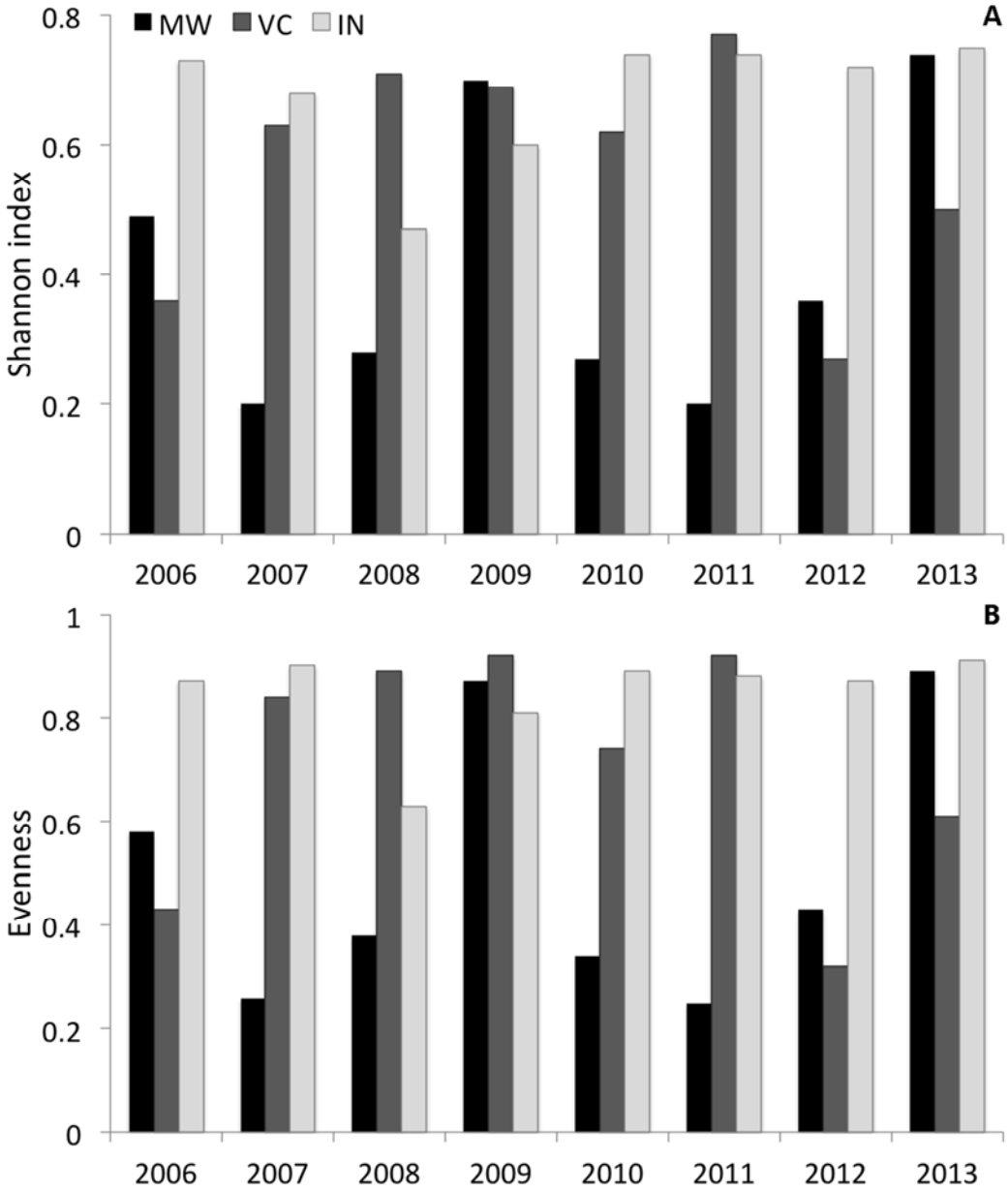


Figure 6. Values of Shannon diversity Index (A) and Evenness (B) of the phytoplankton communities in hypertrophic (MW in black), eutrophic (VC in dark grey) and mesotrophic (IN in light grey) lagoons, based of the phytoplankton pigment composition during summer period (June, July, August) from 2006 to 2013.

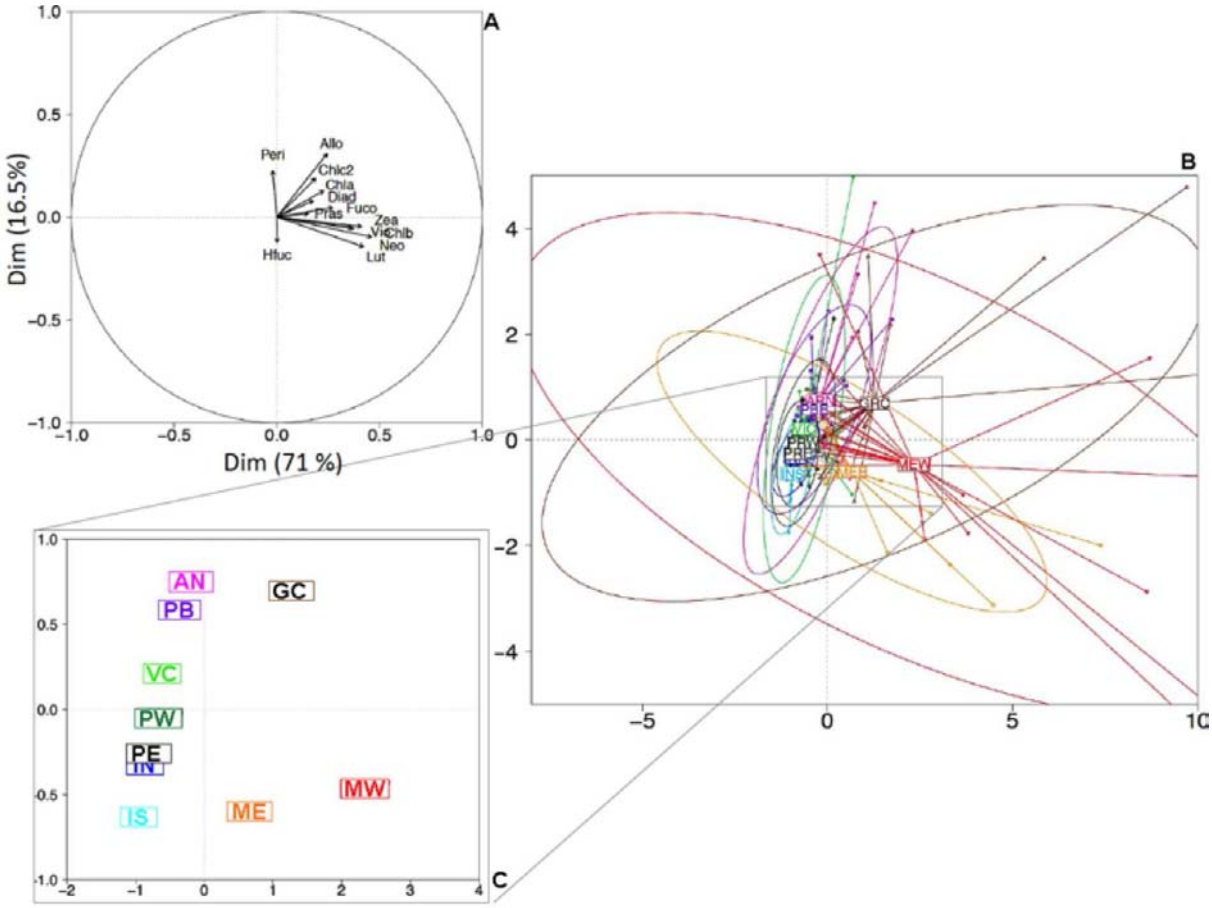


Figure 7. Between-class PCA by stations of the summer monitoring from 2006 to 2013 on the 10 stations in the Palavasian complex. Correlation circle shows a projection of the pigment concentration along the two first axes with different percent of variance between-class. Arrows represent pigments (A) Projection of pigment composition of the ten stations for the 8 years monitoring along the two axes by years, (B) Labels correspond to the center gravity of all the values, and ellipse represent 95% confidence limit of the mean, (C) Position of each center of gravity.

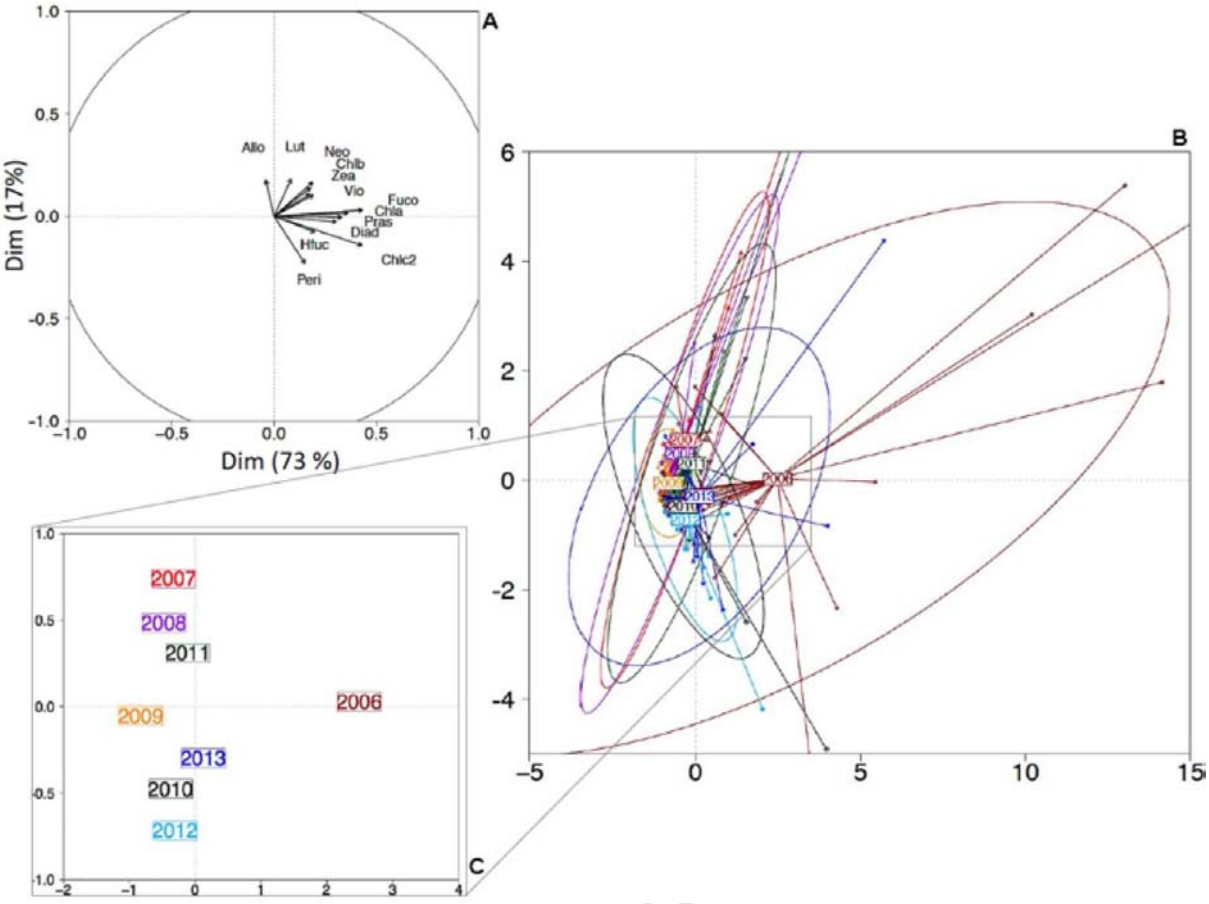


Figure 8. Between-class PCA by years of the summer monitoring from 2006 to 2013 on the 10 stations in the Palavasian complex. Correlation circle shows a projection of the pigment concentration along the two first axes with different percent of variance between-class. Arrows represent pigments (A) Projection of pigment composition of the ten stations for the 8 years monitoring along the two axes by years, (B) Labels correspond to the barycenter of all the values, and ellipse represent 95% confidence limit of the mean, (C) Position of each center of gravity.

**Supplementary Materials**

Table S1. Statistics of mean comparison before and after the effluent diversion, of Chl *a* ($\mu gChla.L^{-1}$), total nitrogen and total phosphorus concentrations ($\mu M$), with hypothesis = concentration before > after (Wilcoxon test, R). Stars precise significance level (* p-value < 0.05, ** p-value < 0.005, *** p-value < 0.0005).

| Lagoons | TN | TP | Chl *a* |
|---|---|---|---|
| MW | 367*** | 359*** | 366*** |
| ME | 363*** | 351*** | 353*** |
| GC | 407*** | 405*** | 418*** |
| AN | 324*** | 318*** | 344*** |
| PW | 271.5*** | 274*** | 286*** |
| PE | 342.5*** | 324*** | 306*** |
| VC | 272* | 295** | 298** |
| PB | 193*** | 183*** | 198*** |
| IN | 312* | 350.5** | 383*** |
| IS | 267.5* | 304* | 376*** |