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# Biogeochemical cycling and phyto- and bacterio-plankton communities in a large and shallow tropical lagoon (Terminos Lagoon, Mexico) under 2009-2010 El Niño Modoki drought conditions

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## Abstract

The 2009-2010 period was marked by an episode of intense drought, known as the El Niño Modoki event. Sampling of Terminos Lagoon (Mexico) was carried out in November 2009 in order to understand the influence of these particular environmental conditions on organic matter fluxes within the lagoon's pelagic ecosystem, and more specifically, on the relations between phyto- and bacterio-plankton communities. The measurements presented here concern biogeochemical parameters (nutrients, dissolved and particulate organic matter, dissolved polycyclic aromatic hydrocarbons [PAHs]), phytoplankton (biomass and photosynthesis) and bacteria (diversity and abundance, including PAH degradation bacteria and ectoenzymatic activities). During the studied period, the water column of Terminos Lagoon functioned globally as a sink, and more precisely as a "nitrogen assimilator", due to the high production of particulate and dissolved organic matter, even though exportation of autochthonous matter to the Gulf of Mexico was weak. We found that "bottom-up" control accounted for a large part of the variability of phytoplankton productivity. Nitrogen and phosphorus stoichiometry mostly accounted for the heterogeneity in phytoplankton and free-living prokaryotes distribution in the lagoon. In the eastern part, we found a clear decoupling between areas enriched in dissolved inorganic nitrogen in the north close to Puerto Real coastal inlet, and areas enriched in phosphate ($PO_4$) in the south close to the Candelaria Estuary. Such a decoupling limited the potential for primary production, resulting in an accumulation of dissolved organic carbon and nitrogen (DOC and DON, respectively) close to the river mouths. In the western part of the lagoon, maximal phytoplankton development resulted from the coupling between Palizada River inputs of nitrate ($NO_3$) and particulate organic phosphorus -PP- (but depleted in $PO_4$) and bacterial activity, transforming PP and dissolved organic phosphorus (DOP) to available $PO_4$. The Chumpan River contributed only marginally to $PO_4$ inputs due to its very low contribution to overall river inputs. The highest dissolved total PAH concentrations were measured in El Carmen Inlet, suggesting anthropogenic pollution of the zone which is probably related to the oil platform exploitation activities in the shallow waters of the south of the Gulf of Mexico. We also found that a complex array of biogeochemical and phytoplanktonic parameters were the driving force behind the geographical distribution of bacterial community structure and activities. Finally, we showed that nutrients brought by the Palizada River supported an abundant bacterial community of PAH-degraders, which are of significance in this important oil production zone.

**Keywords:** biogeochemistry in coastal lagoon, microbial ecology and ecotoxicology, El Niño, lagoon pollution, Gulf of Mexico, Terminos Lagoon

## 1. **Introduction**

Coastal lagoons are complex environments, combining features of shallow inland water bodies wholly or partly sealed off from the adjacent coastal oceans, influenced by tide, river input, precipitation *versus* evaporation balance and surface heat balance. Interactions between freshwater and marine sources generate strong gradients of salinity, light and nutrient availability (Hauenstein and Ramírez, 1986). Biological diversity is generally high in these environments (Milessi et al., 2010). Located in the Southern Gulf of Mexico near Campeche Sound, Terminos Lagoon is one of the largest tropical coastal lagoons worldwide and its recognised environmental importance and protected status are potentially threatened by petroleum-related industrial activities inshore and offshore (García-Ríos et al., 2013). A first tentative budget of salt and nutrients concluded that Terminos Lagoon was slightly autotrophic on a yearly basis (David, 1999), but this assessment was clearly based on scarce environmental data. Chlorophyll-*a* (CHL) concentration and phytoplankton net production have been reported to range from 1 to 17 $\mu g\ L^{-1}$ and from 20 to 300 $gC\ m^{-2}\ a^{-1}$ , respectively (Day et al., 1982), suggesting a potential shift from oligotrophic to eutrophic conditions.

In aquatic ecosystems, bacteria utilize a large fraction (up to 90 %) of primary production, since algal carbon exudates can be the principal source for bacterial production (Cole et al., 1988; Conan et al., 1999). Beside the utilization of a considerable part of the available organic matter, bacterioplankton communities also absorb inorganic nutrients, thus competing with phytoplankton communities (Conan et al., 2007; Hobbie, 1988). The bulk of organic matter is a highly heterogeneous matrix which is primarily composed of complex and refractory substrates (Hoppe et al., 2002), but which also contains labile substrates such as proteins or peptides, oligosaccharides, and fatty acids.. Extracellular enzymes are thus essential to aquatic microorganisms as they allow for the partitioning of complex organic substrates, including high molecular weight compounds which cannot pass through the cell membrane (Arnosti and Steen, 2013). As a function of genetic diversity, the capacity to produce extracellular enzymes is differently distributed in the bacterial community, directly impacting the range of substrates metabolized (Zimmerman et al., 2013). This phenomenon has global-scale implications, since several meta-analyses have clearly evidenced differences in the metabolic capacities of microorganisms from temperate, tropical or high latitude waters (Amado et al., 2013; Arnosti et al., 2011). At a local scale, alteration of the evaporation/precipitation balance due to climate change can be challenging, especially in the case of a coastal lagoon, as it is well known that changes in salinity may alter bacterial diversity and activities (Pedrós-Alió et al., 2000). Local anthropogenic inputs of organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) may also affect bacterial diversity and activities (Aguayo et al., 2014; Jiménez et al., 2011; Rodríguez-Blanco et al., 2010). Indeed, PAHs, which can comprise as much as 25–35 % of total hydrocarbon content in crude oils (Head et al., 2006), are among the most abundant and ubiquitous pollutants in the coastal environment (González-Gaya et al., 2016). These compounds are recognized by the European and US environmental agencies as priority pollutants for the aquatic medium due to their toxicity, persistence and ability to accumulate in the biota (Kennish, 1992). Hence, the presence of PAHs in the marine environment may induce an increase in the indigenous populations of marine bacteria that can break down and utilize these chemicals as a carbon source, the so-called "PAH-degrading bacteria" or "PAH degraders". These bacteria are generally strongly selected in oil-impacted ecosystems, where they may account for 70 to 90% of the total bacterial community (Gutierrez et al., 2014; Head et al., 2006).

Despite their importance, few studies have considered the bacterial communities of tropical inland aquatic ecosystems (Roland et al., 2010) or coastal lagoons (Abreu et al., 1992; Hsieh et al., 2012; MacCord et al., 2013; They et al., 2013) and almost none have dealt with tropical coastal lagoons (Scofield et al., 2015). Among the existing studies, very few have been conducted on bacterial communities and most of the latter have been based on culture-dependent methods (Lizárraga-Partida et al., 1987; Lizárraga-Partida et al., 1986). However, cultivable bacteria represent a very small fraction of total present bacteria (<0.1 %; Ferguson et al., 1984) and culture-independent methods are needed to more accurately assess the diversity and activity of whole bacterial communities in such a vast and understudied system. What is more, Terminos Lagoon is potentially impacted by PAHs, which may come from a diversity of sources including sea-based activities (spills from ships, platforms and pipelines, ballast water discharge, drilling…) but also rivers, surface runoffs and the atmosphere which all carry various urban and industrial wastes (fuel combustion, traffic exhaust emissions...). Nevertheless, to our knowledge, little is known about the PAH content in this ecosystem. Even though Noreña-Barroso et al. (1999) reported on PAH concentrations in the American oyster *Crassostrea virginica* and Rendon-Von Osten et al. (2007), studied PAH concentrations in surface sediments, no data are currently available concerning dissolved PAH concentrations in surface waters of Terminos Lagoon.

Our study aims at evaluating the links between: i) biogeochemical (nutrients, dissolved and particulate organic matter), ii) phytoplanktonic (biomass and photosynthetic activity) and iii) free-living prokaryote (diversity, including PAH-degrading bacteria, and ectoenzymatic activities) parameters in the water column of Terminos Lagoon (Mexico) after a sustained period of minimum river discharge relative to the 2009-2010 El Niño Modoki episode. After having identified the main sources of nutrients in the lagoon (focused on nitrogen and phosphorus), we propose a geographical organization of the ecosystem to explain the distribution of the microbial pelagic communities across the lagoon.

## 2. Materials and methods

### 2.1 Study site and sampling

Terminos Lagoon is a large (1,936 $km^2$, volume 4.65 $km^3$) and shallow (average depth 2.4 m) coastal lagoon located in the Mexican state of Campeche (Fig. 1), 18°20’ to 19°00’ N and 91°10’ to 92°00’ W. Temperature shows low seasonal variation (27 to 33 ºC), but salinity oscillates from brackish to marine waters due to high variability in river runoff (Fichez et al., 2016; Gullian-Klanian et al., 2008). River discharge, precipitation, and groundwater seepage account for 95.44, 4.53 and 0.03 %, respectively. The Chumpan, Candelaria/Mamantel (hereafter Candelaria), and Palizada Estuaries account for 5, 19, and 76 %, respectively of freshwater delivered yearly (~12 $10^9$ $m^3$ $a^{-1}$; i.e. about 2.6 times the lagoon volume) to the lagoon (Fichez et al., 2016). The lagoon is connected to the coastal sea by 2 inlets: El Carmen on the north western side (4 km long) and Puerto Real on the north eastern side (3.3 km in length). About half of the water volume is renewed every 9 days, mostly as a result of tidal exchange. The tide is mainly diurnal, with a mean range of 0.3 m (David and Kjerfve, 1998). Recent results on tidal current modelling (Contreras Ruiz Esparza et al., 2014) reveal both a dynamic inshore current entering the lagoon through Carmen Inlet, flowing through the southern half of the lagoon and exiting through Puerto Real, and a much slower inverse water current flooding the northern central part of the lagoon. This tidally induced hydrodynamic trend generates a counter- clockwise circulation gyre located in the centre of the lagoon, leeward from Carmen Island.

Samples were collected at 0.2 m depth at 35 stations distributed over the whole lagoon (Fig. 1) from the 21st to the 27th of October 2009. In 2009, a yearly cumulated discharge of 4.83 ± 1.71 $10^9$ $m^3$ broke a historical deficit record over the 1992-2011 period for the Palizada River (average yearly cumulative discharge of 7.19 ± 4.22 $10^9$ $m^3$ $s^{-1}$) (Fichez et al., 2016). That exceptional drought period impacted the whole Mesoamerican region during the 2009-2010 El Niño Modoki episode, and resulted in a salinity positive anomaly in Terminos Lagoon that developed most strongly during the post wet season period (Fichez et al., 2016), at the time of our sampling.

A vertical profile of temperature, salinity and fluorescence was carried out at each of the 35 stations with a SeaBird CTD probe (SBE 19) with a precision of 0.01°C for temperature and 0.001 for salinity. Once the profile completed, water was sampled using a 5L Niskin bottle maintained horizontally at 0.2 m below the surface.

### 2.2 Nutrients and Dissolved organic matter

As soon as the Niskin sampler was retrieved on board, a previously acid washed 40 mL Schott® glass vial was rinsed with sampled water, filled, immediately injected with the fluorometric detection reagent for ammonia determination (as described in Holmes et al., 1999), sealed, and stored in the dark for later analysis in the laboratory. Following this, two 30 mL and one 150 mL plastic acid washed vials were then rinsed with sampled water, filled, stored in a specifically dedicated and refrigerated ice cooler, to be later deep-frozen in the laboratory while awaiting analysis of dissolved inorganic and organic nutrients, as follows:

Nitrate ($NO_3$ ± 0.02 µM), nitrite ($NO_2$ ± 0.01 µM), phosphate ($PO_4$ ± 0.01 µM) and silicate ($Si(OH)_4$ ± 0.05 µM) concentrations were measured on a continuous flow Technicon® AutoAnalyzer II (Aminot and Kérouel, 2007), as previously described in Severin et al. (2014). Ammonium ($NH_4$ ± 10 nM) was detected at nanomolar concentrations by fluorometric detection (Holmes et al., 1999) on a Turner Design Trilogy fluorometer.

Samples for dissolved organic matter (DOM) were filtered through 2 precombusted (24h, 450°C) glass fiber filters (Whatman GF/F, 25 mm). 20 mL were collected for dissolved organic carbon (DOC) in precombusted glass tubes, acidified with orthophosphoric acid ($H_3PO_4$), and analyzed by high temperature catalytic oxidation (HTCO) (Cauwet, 1999) on a Shimadzu TOCV analyzer. Typical analytical precision is ± 0.1–0.5 (SD) or 0.2–1 % (CV). 20 mL of samples were collected in Teflon vials for dissolved organic nitrogen (DON) and phosphorus (DOP), and were analyzed by Persulfate wet-oxidation following Pujo-Pay and Raimbault (1994) and Pujo-Pay et al. (1997).

### 2.3 Particulate organic matter, chlorophyll and phaeopigment

A 4 L plastic acid washed container was also used for sub-sampling from the Niskin bottle. This container was rinsed with sampled water, filled, and stored in a dedicated ice cooler while awaiting filtration in the laboratory, as follows: 250 mL of seawater were filtered through a precombusted (24h, 450°C) Whatman GF/F glass filters (25 mm), placed into a Teflon vial and oxidized for particulate organic nitrogen (PON) and phosphorus (PP) measurements (according to Pujo-Pay and Raimbault, 1994). ~1 L was filtered on precombusted (24 h, 450°C) glass fiber filters (Whatman GF/F, 25mm) for particulate organic carbon (POC) and PON measurements. Filters were oven dried at 50°C and stored in washed glass vials and in a desiccator until analysis using a CHN Perkin Elmer 2400following the cruise.

For chlorophyll (CHL), 250 mL samples were filtered using 25 mm diameter Whatman® GF/F filters and immediately stored in liquid nitrogen. CHL and phaeopigment (Phaeo) were later extracted from the filters with 100 % methanol (Marker, 1972), and concentrations were determined by the fluorometric technique (Lorenzen, 1966) on a Turner Design Trilogy fluorometer.

### 2.4 Photosynthetic parameters

Photosynthetic-irradiance parameters ($\alpha$, $P^b_m$ and $I_k$) were measured using the radioactive $^{14}$C-tracer technique (Fitzwater et al., 1982) in a specifically designed homemade incubator. 10x60mL Nunc® culture vials were carefully filled and inoculated with $Na_2H^{14}CO_3$ (final activity of ~0.2 µCi mL$^{-1}$), incubated for 45 min in a 10 light level irradiance gradient (from 0 to 1327 W m$^{-2}$), before being filtered on Whatman GF/F 25 mm filters, rinsed with 10 % HCl, dried at 45°C for 12 h, and placed into scintillation vials. 10 ml of a liquid scintillation cocktail (Ultima Gold uLLT) were added to the set of scintillation vials 6 h before processing in a Beckman Scintillation Counter. The photosynthetic parameters were determined by fitting each obtained curve with the 'hyperbolic tangent model without photoinhibition' proposed by Jassby and Platt (1976).

### 2.5 Measurements of dissolved total PAH concentrations

Dissolved total PAH concentrations were determined by using the EnviroFlu-HC submersible UV fluorometer (TriOS Optical Sensors, Germany), a commercially available instrument dedicated to the *in situ* and real time quantification of PAHs in water. The sensor was calibrated in the laboratory before the cruises following Tedetti et al. (2010) and Sauret et al. (2016). In this work, the mean dissolved total PAH concentrations derived from the sensor are given in ng L$^{-1}$ with a mean coefficient of variation of 10 %.

### 2.6 Abundance of prokaryotes

Free-living prokaryotes were determined by flow cytometry (Mével et al., 2008). 2 mL seawater samples were fixed with 2 % formaldehyde for 1 h at 4°C. A 1 mL sub-sample was incubated with SYBR Green I (Sigma Aldrich, final conc. 0.05% [v/v] of the commercial solution) for 15 min at 20°C in the dark and analysed with a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA) equipped with an air-cooled argon laser (488 nm, 15 mW). Data acquisition and analysis were done with Cell-Quest software (Becton Dickinson). The discrimination of the heterotrophs was undertaken using sybr green to induce a green fluorescence and enable separation of the prokaryotes using the SSC diffraction parameter.

### 2.7 Total and metabolically active bacterial community structure

Nucleic acids were extracted on 0.2 µm-pore-size filters (47 mm, PC, Nucleopore) by filtration of 1 L of pre-filtered (3 µm) water. Co-extraction of DNA and RNA was performed after chemical cell lysis (Ghiglione et al., 1999) with the Qiagen Allprep DNA/RNA extraction kit using the manufacturer's instructions. DNA and cDNA (by M-MLV reverse transcription of 16S rRNA, Promega) were used as a template for PCR amplification of the variable V3 region of the 16S rRNA gene (*Escherichia coli* gene positions 329–533; Brosius et al., 1981). The primer w34 was fluorescently labelled at the 5'-end position with phosphoramidite (TET, Applied Biosystems). CE-SSCP analysis was performed using the 310 Genetic Analyzer and Genescan analysis software (Applied Biosystems), as previously described (Ortega-Retuerta et al., 2012).

### 2.8 Extracellular enzymatic activities

Aminopeptidase, β-glucosidase and lipase were measured using a VICTOR3 spectrofluorometer (Perkin Elmer) after incubations of 2 h at *in situ* temperature with L-leucine-7amido-4-methyl coumarin (LL, 5 µM final), MUF-β-D-glucoside (β-Glc, 0.25 µM final) or MUF-palmitate (Lip, 0.25 µM final). These saturated concentrations and optimized time incubations were determined prior to the extracellular enzymatic activities measurement, as previously described (Van Wambeke et al., 2009).

### 2.9 Quantification of PAH-degrading bacteria by Most-Probable-Number

The quantification of PAH-degrading bacteria was performed by the most-probable-number (MPN) method. A total of 100 µL of each sample was introduced in triplicate into a 48-well microplate with 900 µL of sterile minimum medium, as previously described (Rodríguez-Blanco et al., 2010; Sauret et al., 2016). A mixture of 6 PAHs from 2 to 5 rings (naphthalene, fluorene, phenanthrene, fluoranthrene, pyrene and benzo[a]pyrene) prepared in dichloromethane in equimolar concentration was introduced into each well at a final concentration of 10 µg mL$^{-1}$, as previously described by Sauret et al. (2016). This corresponds to a very high concentration of PAH in nature, i.e. 50 times higher than the values found in the Leghorn harbour (Cincinelli et al., 2001). After 2 weeks of incubation, the change from blue to pink - indicating oxidation of the resazurin contained in the medium - was checked. Based on the flow cytometry quantification of the abundance of bacteria at the beginning of the incubation (see §2.6) and by taking into account the dilution factors where we visually observed color changes after 2 weeks, the traditional MPN table table gave the most probable number of bacteria able to degrade the mixture of six PAHs (Alexander, 1982).

### 2.10 Statistical analysis

Comparative analysis of 16S rDNA- or 16S rRNA-based CE-SSCP fingerprints was carried out with the PRIMER 6 software (PRIMER-E, Ltd., UK) using Bray-Curtis similarities. We used the similarity profile test SIMPROF (PRIMER 6) to check that a specific sub-cluster can be recreated by randomly permuting the entry ribotypes and samples, when using hierarchical agglomerative clustering. The significant branch (SIMPROF, $p<0.05$) was used as a prerequisite for defining bacterial clusters, and clusters were reported on non-metric multidimensional scaling (MDS) representation.

Canonical correspondence analysis (CCA) was used to investigate the variations in the CE-SSCP profiles under the constraint of our set of environmental variables, using CANOCCO software (version 5.0), as previously described in Berjeb et al. (2011). Significant variables (i.e. variables that significantly explained changes in 16S rDNA- and 16S rRNA-based fingerprints) in our data set were chosen using a forward-selection procedure. Explanatory variables were added until further addition of variables failed to contribute significantly ($p< 0.05$) to a substantial improvement in the model's explanatory power. Environmental parameters were previously transformed according to their pairwise distributions, and Spearman's rank pairwise correlations between the transformed environmental variables were used to determine their significance with Statel v2.7.

## 3. Results

### 3.1 Distribution of physical parameters

At the studied period, Terminos Lagoon was characterized by a north west-south east positive temperature gradient from >30 to about 27°C (Fig. 2A). Salinity was maximal at Puerto Real Inlet (37.50) and along the southern limits of El Carmen Island, intermediate at Candelaria and Chumpan River mouths, and minimal (21.57) close to the Palizada River (Fig. 2B).

### 3.2 Distribution of biogeochemical parameters

Nitrate and ammonium concentrations (Fig. 2C and 2D) were maximum close to the Palizada embouchure (16.6 and 0.3 µM, respectively) and to the Puerto Real Inlet (2.5 µM in $NO_3$ and the highest $NH_4$ concentration of 1 µM). In the rest of the lagoon, $NO_3$ concentrations were quite low and homogeneous (close to the detection limit of 0.01 µM). $NH_4$ concentrations were more variable with minimum values on the northern side of the lagoon, and concentration in the range 0.1 to 0.3 µM on the southern inshore side.

The distribution pattern for $PO_4$ (Fig. 2E) differed significantly from N-nutrients. Minimum concentrations (<0.05 µM) were measured in the western part of the lagoon under the influence of the Palizada

River, indicating very low $PO_4$ inputs from that river as opposed to nitrogen-nutrients. $PO_4$ concentrations were also low (<0.10 µM) in the centre of the lagoon. The highest $PO_4$ concentration was measured in front of the Chumpan River (0.17 µM), whereas significant inputs in the eastern part came from Candelaria River (0.13 µM) and Puerto Real Inlet (0.12 µM).

The distributions of dissolved organic carbon (DOC; Fig. 2F), nitrogen (DON; Fig. 2G) and phosphorus (DOP; Fig. 2H) concentrations followed a pattern comparable to that of $PO_4$. The higher concentrations of DOC, DON and DOP were measured in the south eastern part of the lagoon. However, the maximal DOC and DON concentrations (> 400 and 20 µM, respectively) were measured in front of Candelaria River, whereas the maximal DOP concentrations were observed in front of the Chumpan River (> 1 µM). Lowest concentrations <200, 5 and 0.1 µM of DOC, DON and DOP were measured in front of the Palizada River mouth and DOC even spread along the northern shore of Carmen Island. Significant Spearman's rank correlations ($n=35$, $p<0.05$) were found between DON and DOC ($\rho=0.64$), DOP ($\rho=0.64$) and temperature ($\rho=-0.32$).

The 3 rivers were clearly the main sources of particulate organic nitrogen (PON) and phosphorus (PP) in the lagoon (Fig. 3). PON reached a maximum concentration of 9.3 µM in front of the Chumpan Estuary and progressively decreased while spreading to the north (Fig. 3A). Concerning PP, the Palizada River was the main source with concentrations close to 0.9 µM, progressively decreasing to 0.6 µM while spreading along the southern shore toward the Chumpan Estuary and 0.5 µM in the north-eastern drift toward Puerto Real passage (Fig. 3B). Significant Spearman's rank correlations ($n=35$, $p<0.05$) were found between PP and PON ( $\rho=0.73$), $NO_3$ ($\rho=0.57$) and salinity ($\rho=-0.56$).

### 3.3 Photosynthetic pigment and activity

Chlorophyll (CHL) and phaeopigment (Phaeo) followed a convergent distribution pattern (Fig. 3C and 3D) with maximum concentrations close to or in the vicinity of the Palizada mouth (>6 $\mu gCHL\ L^{-1}$ and ~2 $\mu gPhaeo\ L^{-1}$). A range of 1-6 $\mu gCHL\ L^{-1}$ and 1-2 $\mu gPhaeo\ L^{-1}$ was encountered in the western part of the lagoon. Concentrations <1 $\mu gCHL\ L^{-1}$ and 1-2 $\mu gPhaeo\ L^{-1}$ were mostly confined to the eastern part. On a global view, Phaeo accounted for 28 ± 8 % of CHL on average, thus attesting to rather active phytoplankton communities. Significant Spearman's rank correlations ($n=35$, $p<0.05$) were found between CHL and Phaeo ($\rho=0.82$) or PP ($\rho=0.74$).

The maximum rate of carbon production per unit of chlorophyll at light saturation ($P^b_m$, Fig. 3E) was minimal (<0.5 $mgC\ mgCHL^{-1}\ h^{-1}$) in the Palizada plume in association with the maximum Phaeo:CHL ratio measured (>44 %). Maximum $P^b_m$ values in excess of 8.0 mgC $mgCHL^{-1}$ $h^{-1}$ were measured close the Chumpan Estuary in an area of low Phaeo:CHL ratio (<25 %).

### 3.4 Bacterial abundance and extracellular enzymatic activities

Free-living prokaryote abundance ranged from 1.0 to 4.8 $10^6$ cell $mL^{-1}$ (mean=2.8 $10^6$ cell $mL^{-1}$, SD=0.9 $10^6$ cell $mL^{-1}$, n=35), with maximum values observed in the Puerto Real passage and close to the river mouths (Candelaria and Chumpan Rivers), with the exception of the Palizada River which showed the highest river-lagoon gradient from maximum to minimal values cited above (Fig. 3F).

Cell specific aminopeptidase (Leu-MCA), and phosphatase (MUF-P) activities reached maximum values close to the mouths of the Palizada and Chumpan Rivers (33, and 131.9 fmol $L^{-1}$ $h^{-1}$ $cell^{-1}$, respectively (Fig. 4A, and 4B). Cell specific lipase activity (MUF-Lip) was maximum (10.9 fmol $L^{-1}$ $h^{-1}$ $cell^{-1}$; Fig. 4C) from the Chumpan River mouth northward towards El Carmen Island, crossing the lagoon approximately in its middle following the isotherms (Fig. 2A). Much lower activities were found over most of the lagoon for all the activities (mean values in fmol $L^{-1}$ $h^{-1}$ $cell^{-1}$ are 12.6 ± 8.4 for Leu-MCA, 12.1 ± 24.2 for MUF-P and 2.4 ± 2.6 for MUF-Lip). Significant Spearman's rank correlations (n=35, p<0.01) were found between aminopeptidase activities and DOC (ρ=-0.27), PON (ρ=0.33) and to a greater extent, between phosphatase activities and $PO_4$ (ρ=-0.46), PP (ρ=0.60), $NO_3$ (ρ=0.69), CHL (ρ=0.53).

### 3.5 Dissolved PAH concentrations and estimated abundance of bacterial PAH-degraders

Dissolved total PAH concentrations (Fig. 5A) were higher close to the El Carmen Inlet (332 ng $L^{-1}$) and relatively lower close to Palizada River (187 ng $L^{-1}$) and to the Chumpan River mouth (166 ng $L^{-1}$). They were generally lower in the rest of the lagoon (<130 ng $L^{-1}$). Quantification by MPN counts showed high enrichment of PAH-degraders close to Palizada River (estimated at 4.6 $10^4$ cells $mL^{-1}$, equivalent to 4.4 % of free-living prokaryotes) (Fig. 5B). Lower values were found close to the Chumpan River mouth (estimated at 4.7 $10^3$ cells $mL^{-1}$, equivalent to 0.2 % of free-living prokaryotes), and commonly represented less than 0.1 % of the free-living prokaryote abundance in the rest of the lagoon. Quantification by MPN counts showed significant, if low, Spearman's rank correlation with dissolved total PAH concentrations (ρ=0.37, p<0.05, n=35). MPN count correlations (p<0.05, n=35) were stronger with PP (ρ=0.65) and CHL (ρ=0.53).

### 3.6 Spatial distribution of total and metabolically active bacteria by CE-SSCP fingerprints.

Bacterial community structure defined as a function of 16S rDNA-based fingerprints from each sample singled out 3 individual stations (Palizada River, El Carmen Inlet and Candelaria River) and aggregated 5 groups of stations (Fig. 6A). Three of these groups included a large number of samples: cluster I grouped 9 stations located in the north-eastern part of the lagoon close to Puerto Real Inlet; cluster II grouped 9 stations positioned in the middle of the lagoon north from Chumpan River to Carmen Island; cluster III grouped 8 stations situated to the south west of Carmen Island. Two other groups with fewer stations identified intermediated communities found between El Carmen Inlet and the Palizada River in the western part of the lagoon (cluster V; stations 2, 4, 6) and in the middle of the lagoon, close to the Candelaria River (cluster IV; stations 22, 24, 27).

Metabolically active bacterial communities as a function of 16S rRNA-based fingerprints singled out 2 stations (Palizada River and El Carmen Inlet) and aggregated 5 groups of stations which are slightly different from the DNA-based clusters (Fig. 6B). Three of these groups included a large number of samples: cluster I formed the largest cluster with 15 stations located in the eastern part of the lagoon; cluster II grouped 9 stations in the middle of the lagoon north of Chumpan River to Carmen Island; cluster III grouped 5 stations in the north western part of the lagoon, close to El Carmen Inlet. Two other groups with fewer stations showed intermediate communities found close to the Palizada River mouth (cluster IV; stations 6 and 8) and further east (cluster V; stations 9 and 12).

### 3.7 Environmental drivers of the total and active prokaryote community structures

To analyse the main environmental factors controlling the spatial distribution of total (Fig. 7A) and active (Fig. 7B) prokaryote communities, we performed a canonical correspondence analysis (CCA). In both DNA- and RNA- based analysis, the cumulative percentage of variance of the species-environment relationship indicated that the first and second canonical axes explained 48 % and 24 % of the total variance, respectively for DNA and 45 % and 31 % for RNA. The remaining axes accounted for less than 14 % of the total variance each, and thus were not considered as significant enough.

In the DNA-based CCA, the first canonical axis was positively correlated with $NO_3^-$ and CHL and negatively correlated with concentration of DOC, DOP, DON and oxygen. In the RNA-based CCA, the first canonical axis was positively correlated with $NO_3^-$ and PAHs and negatively correlated with the concentration of POC, PON, oxygen, salinity, $PO_4$ and CHL. The concomitant effect of those parameters explained 27 % and 40 % (ratio between the sum of all canonical eigenvalues and the sum of all eigenvalues) of the changes in bacterial community structure found in the DNA- and RNA-based fractions, respectively (Figure 7).

## 4. Discussion

### 4.1 Biogeochemical characteristics of Terminos Lagoon under low river discharge conditions

With a contribution of about 76 % to river inputs in the lagoon (Fichez et al., 2016; Jensen et al., 1989), Palizada River delivers most of the new nitrogen input as nitrate and ammonium. High concentrations in nitrogen were also measured in the Puerto Real Inlet, suggesting a second nitrogen source from coastal seawater. These two sources have clearly different impacts on primary producer development and activity as shown by the Phaeo:CHL ratio (<20 % in the vicinity of the Palizada River, but >30 % close to the Puerto Real Inlet) and $P^b_m$ values (low in the Palizada area and higher close to the inlet). So, despite greater chlorophyll degradation (indicated by high Phaeo concentrations), phytoplanktonic cells were more productive under the influence of waters from the Gulf of Mexico when compared to those under the river's influence. Specifically, there were similar nutrients, DOM and POM concentrations for the two zones and we measured a similar potential primary production per unit volume (27.5 and 30.2 mgC $m^{-3}$ $h^{-1}$ for Palizada River and Puerto Real Inlet, respectively). However, the chlorophyll stock was about 2-fold lower in the area of the inlet (6.3 and 3.9 mgCHL $m^{-3}$ for Palizada River and Puerto Real Inlet, respectively). This is in apparent contradiction with what has traditionally been reported on the influence of river inputs in coastal areas, which generally largely enhance primary productivity (see for example the Rhone River in the Mediterranean Sea; Pujo-Pay et al., 2006). Decreasing turbidity along the estuarine to inlet transect is a first factor explaining the seaward offset of phytoplankton productivity. But higher grazing activity by herbivores in the coastal waters or in the vicinity of the inlet could be an explanation to further justify the conjunction of higher Phaeo concentrations together with active phytoplankton physiology (this requires further study).

Moreover, Day *et al.* (1982) demonstrated that small additions of filtered mangrove water had a stimulatory effect on pelagic primary production in Terminos Lagoon. This observation was later confirmed by Rivera-Monroy *et al.* (1998), who also evidenced a large temporal variability in stimulating effect, and a rapid inhibition due to variable humic substance concentrations. The relative decrease of productivity close to the Palizada plume could be due to humic matter, as we also found relatively high concentrations in dissolved PAHs (see hereafter §4.4). Finally, it is clear that bottom-up control of the system (by nutrients and/or humic substances) drove the differential responses of phytoplankton productivity in the eastern and western part of the lagoon, probably in conjunction with grazing activity (top-down control).

At the time of our study, Palizada River and Puerto Real Inlet were major sources of nitrogen to the lagoon. Sediments are generally considered to be a significant internal source of nutrients in shallow coastal ecosystems, but they may also be a net sink of dissolved nitrogen, at least during certain times of the year (Sundbäck et al., 2000; Tyler et al., 2003). Rivera-Monroy et al. (1995a) measured nitrogen fluxes between Estero Pargo (an unpolluted tidal creek), and a fringe mangrove forest in Terminos Lagoon. They reported that mangrove sediments were a sink of $NO_3$ and $NH_4$ throughout the year. Denitrification, the dissimilatory reduction of $NO_3$ to produce $N_2O$ and $N_2$, was considered as the main process contributing to $NO_3$ loss. However, direct measurements of denitrification rates in the fringe and basin mangroves of Terminos Lagoon indicated a low sink of $NO_3$ (Rivera-Monroy et al., 1995b), contrary to what has been evidenced in other mangrove forests (i.e. Twilley, 2013). This was later confirmed by Rivera-Monroy *et al.* (Rivera-Monroy et al., 2007), who hypothesised that most of the inorganic nitrogen was retained in the sediments and not lost via denitrification. They also measured a decoupling between sources of nitrogen and phosphorus, and because P is a limiting nutrient, they assumed that the dominant source was from tidal inputs as opposed to remineralization from organic matter in the sediment. During our study, Origel Moreno (2015) found that benthic carbon mineralization consumed a large proportion (between 67 and 86 %) of the pelagic carbon production. These values are at the upper end of the range calculated for sub-tropical lagoons (Grenz et al., 2010; Machado and Knoppers, 1988) and indicate high biological activity in the sediments. Additionally, Origel Moreno (2015) estimated that 50 to 95 % of nitrogen was mineralized in the sediment through various N-consuming processes and also that nitrogen was more efficiently mineralized than phosphorus.

Our large scale study considering the whole lagoon brings some information about the potential origin of phosphorus in the water column. It is clear from our measurements that phosphate distribution in the lagoon is disconnected from nitrogen. This impacts the stoichiometry of particulate organic matter (N:P ratio) through the whole lagoon, as shown by the surprising and relative low values of the PON:PP ratio (<13) at all stations (indicating a particulate nitrogen deficit), with the exception of those located in the southwest part of the lagoon where a canonical Redfield ratio of 16 was measured (Fig. 8). To sustain their growth requirement, primary producers have the ability to decouple their consumption of phosphorus and nitrogen in respect to a variable metabolic plasticity (Conan et al., 2007). In comparison to the two previously discussed main sources of $NO_3$ and $NH_4$ (Palizada River and Puerto Real Inlet) located in the west and north part of the lagoon, we identified two distinct main sources of $PO_4$ in Terminos Lagoon during the sampled period: (i) river inputs from the Candelaria and Chumpan in the south part, even though their contribution to the overall river discharge is low, and (ii) mineralization of organic phosphorus (PP and DOP) by prokaryote activity (coherent with ectoenzymatic activities; see hereafter §4.2). Note that the major source of PP in the lagoon was the Palizada River, whereas accumulation of DOP was measured between the Palizada and Chumpan Rivers in the south west of the lagoon. In this area, distribution of dissolved oxygen was minimal compared to the rest of the lagoon, which is coherent with high rates of organic matter mineralization in the water column. Finally during our study, the dominant source of $PO_4$ was not tidal flooding as hypothesized by Rivera-Monroy *et al.* (2007), but rather the mineralization of organic matter by free-living prokaryotes in the water column. If this conclusion appears valid in the context of weak river discharges, further studies will be necessary to test its potential extension to other environmental conditions (rainy periods, river flooding, tidal amplitude...).

### 4.2 Relationship between biogeochemical conditions and prokaryotic activities

Our analysis of biogeochemical trends in Terminos Lagoon has been combined with the study of the spatial distribution of prokaryotic extracellular activity. Bacterial aminopeptidase and lipase extracellular activities play a key function in the transformation of biopolymer into small monomers, since a large part of organic matter is in the form of large size molecules, whereas small molecules only (<600 Da) are directly assimilable by bacteria (Weiss et al., 1991). The expression of aminopeptidase activity indicates the absence of direct bacterial assimilation of dissolved organic matter and their ability to actively release enzymes outside the cells (Van Wambeke et al., 2009). Moderate but significant negative correlations were found between aminopeptidase activity per cell and DOC concentration in Terminos Lagoon ($\rho=0.27$, $n=35$, $p<0.01$). Higher DOC concentrations associated with lower aminopeptidase activity suggest a higher amount of labile organic matter for bacteria. The high aminopeptidase activity in the Palizada River plume confirmed the presence of recalcitrant organic matter from terrestrial origin, as opposed to minimum activities in Puerto Real marine waters or in the Candelaria mouth, where DOC concentrations were maximal. Lipase activities showed different trends, with higher activities found in the middle of the lagoon north of the Chumpan River to Carmen Island. We previously published results indicating that ambient quantity and quality of hydrolysable acyl-lipids clearly couple with the measurement of their *in situ* hydrolysis rates (Bourguet et al., 2009). The differences between spatial distributions of ectoenzymatic aminopeptidase and lipase activities suggest that organic matter from different composition resided in the central zone of Terminos Lagoon, a result in strong agreement with a recent study on hydrodynamics that identified a large circulation cell in the same central area (Contreras Ruiz et al., 2014). Unfortunately, the contribution of the protein or lipid pool to total organic matter was not measured at the time of the study, which may have strengthened our hypothesis concerning the role of the composition of organic matter in the spatial distribution of extracellular enzyme activities. This lack of information may explain the very low or absent correlation found between extracellular activities and measured biogeochemical parameters.

Phosphatase activity is well known to be controlled by the availability of soluble reactive phosphorus (Van Wambeke et al., 2009). This activity was essentially observed in the vicinity of the Palizada River, which is the main source of PP in the lagoon, but not in Puerto Real Inlet, which are the two $PO_4$-depleted zones which indirectly influence the stoichiometry of particulate organic matter, as discussed above (Fig. 8). Thus, a zone with clear phosphatase activity but which is P-depleted means very low P-availability for phytoplanktonic growth. This observation is consistent with the low phytoplankton productivity observed, indicating weak C-fixation rates in the Palizada mouth, which strengthens our bottom-up control hypothesis. Extracellular phosphatase activity was significantly ($p<0.05$, $n=35$) negatively correlated with $PO_4$ ($\rho=-0.46$) and positively correlated with PP ($\rho=0.60$). Our data therefore converge with the model previously proposed by Robadue *et al.* (2004) which predicted a different behaviour between the eastern and western sides of the lagoon in terms of both water budget and ecosystem functioning, a distinction which is mostly driven by the respective influences of the Palizada River discharge in the west and the Puerto Real marine water inputs in the north east.

### 4.3 Prokaryotic community structure and ectoenzyme activities

Molecular fingerprinting (such as CE-SSCP) and next-generation sequencing technologies generally yield converging results (Ghiglione et al., 2005; Ghiglione and Murray, 2012; Ortega-Retuerta et al., 2012; Sauret et al., 2015), evidencing clear shifts in bacterial community structure as a function of changes in biogeochemical characteristics (Ghiglione et al., 2005). Numerous factors can regulate microorganism population dynamics, often simultaneously, and the literature contains evidence (Berdjeb et al., 2011; Fuhrman et al., 2013; Ghiglione et al., 2008) underlining the importance of relevant statistical analysis to investigate the relative importance of environmental factors in predicting the bacterial community dynamics. It is generally recognized that the expression of ectoenzyme activities could result from species selection and population dynamics (Martinez et al., 1996), and the zonation of prokaryotic community structure in the eastern, middle and western parts of the lagoon agree with such a paradigm. The community composition in the eastern part could be divided into two sub-clusters corresponding to the respective influences of the Palizada River mouth and El Carmen Inlet. Both DNA- and RNA-based fingerprinting show that the Palizada River and El Carmen Inlet hosted distinct prokaryotic communities, as previously observed in transition zones such as rivers (Ortega-Retuerta et al., 2012) or lagoon mouths (Rappé et al., 2000). The relation between community composition and ectoenzyme activities was particularly evident when the lipase and aminopeptidase rates are considered. Lipase activity was magnified in the middle of the lagoon with a south to north increasing gradient from Chumpan River to Carmen Island that coincided with specific communities (cluster II in both DNA- and RNA-based fingerprinting). Other communities were found in the western part under the influence of the Palizada River, where higher aminopeptidase activity was measured.

The combination of DNA and RNA strengthens our observations, as DNA-based analysis alone would have failed to distinguish between active, dormant, senescent or dead cells, and would thus prevent assessment of the level of activity of each detected bacterial population (Rodríguez-Blanco et al., 2010). Even though the abundance of bacteria in the sea is high, only a small fraction is considered to be metabolically active (Del Giorgio and Bouvier, 2002). Bacterial growth rate has been shown to correlate with cellular rRNA content (Kemp et al., 1993); therefore, information on cellular activity may be obtained by tracking reverse-transcribed 16S rRNA (Lami et al., 2009). In the present study, we focused on the free-living prokaryotes and disregarded the particle-attached fraction by pre-filtering the water by 3 µm, which allowed to eliminate the problem of DNA eukaryotic chloroplasts which may have biased our results in the context of gradients of productive zones. The combination of DNA and RNA results in Terminos Lagoon showed similar trends, with total and active communities presenting a strong zonation between the eastern, middle and western parts of the lagoon, to which could be added smaller transition zones located around major sources of coastal (El Carmen Inlet) and river inputs (Palizada and Candelaria). Here, the combination of DNA and RNA showed similar tendencies within the total and active communities presenting eastern, middle and western distribution across the lagoon. These results indicate that most of the free-living bacterial communities detected by molecular fingerprinting (DNA-based) were active (RNA-based) within the lagoon, with the exception of the local transition zones between the lagoon waters and the coastal (El Carmen Inlet) or rivers (Palizada and Candelaria).

### 4.4 Biogeochemical parameters and PAHs drive the prokaryotic community structure

Through the use of direct gradient multivariate ordination analyses, we demonstrate that a complex array of biogeochemical parameters was the driving force behind prokaryotic community structure shifts in Terminos Lagoon. Physico-chemical parameters such as nitrate, oxygen, dissolved organic matter (DOC, DON, DOP) and chlorophyll *a* acted in synergy to explain bacterial assemblage changes in rDNA level. Some differences were observed to explain the geographical patterns of the metabolically active bacterial communities (rRNA level). Indeed, salinity, particulate organic matter (PON, PP) and phosphate were needed in addition to nitrate, oxygen and CHL parameters already outlined on rDNA level. The variance explained by the environmental variables selected by the statistical model represented 27 % and 40 % of the variability at the

DNA and RNA level, respectively. Further studies are therefore needed to elucidate the unexplained variance of the model, due to other parameters not taken into account in our study, such as ecological relationships between bacterial communities themselves, or top-down control by predation and viral lysis (Ghiglione et al., 2016).

The concentration of dissolved total PAHs was also a significant explanatory variable of the metabolically active bacterial community structure. PAHs are considered the most toxic component of crude oil to marine life and are ubiquitous pollutants in the coastal environment (Kennish, 1992). Our study was performed just before the 2010 Deepwater Horizon (DWH) blowout in the Gulf of Mexico, but several offshore oil platforms exist in the shallow waters of Campeche Bank in the southern part of the Gulf of Mexico, for example, in the Campeche field (Cheek-1) which is only 60 km north of Terminos Lagoon (Warr et al., 2013). The coast of Campeche itself was also impacted by the 1979 *Ixtoc I* platform oil spill, roughly 100 km northwest of Terminos Lagoon (Warr et al., 2013). PAHs concentrations in Terminos Lagoon indicated an input into the lagoon from El Carmen Inlet (maximal concentration of 332 ng $L^{-1}$) that mostly impacted the eastern part, with concentration <130 ng $L^{-1}$ in the rest of the lagoon. We observed a high enrichment of PAH-degraders in the south eastern part of the lagoon, with low but significant correlation with PAH concentrations ($\rho=0.37$, $p<0.05$, n=35). This enrichment was particularly high (estimated at 4.6 $10^4$ cell $mL^{-1}$, equivalent to 4.4 % of the free-living prokaryotes abundance) in the Palizada River mouth. Nitrogen fertilization from allochthonous inputs from the Palizada River may be crucial for PAHs degradation potential in Terminos Lagoon. Indeed, it is well accepted that bacterial degradation of hydrocarbon (carbon source for bacteria) is dependent on nutrients to re-equilibrate the C:N:P ratio (Sauret et al., 2015; Sauret et al., 2016). Some halotolerant bacteria such as *Marinobacter hydrocarbonoclasticus* sp. 17 (Grimaud et al., 2012) may have the capability to degrade PAHs and survive in rivers, lagoons and seawater.. Further studies using PAH-stable isotopes coupled with pyrosequencing (Dombrowski et al., 2016; Sauret et al., 2016) are necessary to identify the dynamic of these functional communities in Terminos Lagoon. Using similar approaches, previous reports have shown that the pollutant content and PAHs, in particular, were responsible for the dynamic of bacterial community structure in the sediment of Bizerte Lagoon, Tunisia (Ben Said et al., 2010). Such a massive impact of pollutants was not observed here, possibly because of the difference in the degree of pollution between the two areas (moderately contaminated in Terminos Lagoon *versus* highly contaminated in Bizerte). Metabolically active bacterial community structure in Terminos Lagoon was significantly impacted by PAH-pollution, even though it did not exceed the effect of other environmental parameters and their specificity at each geographical location.

## 5. Conclusions

This study provides a new original set of biogeochemical characteristics for one of the largest shallow tropical coastal lagoons. Due to the 2009-2010 El Niño Modoki episode, climatic conditions in Terminos Lagoon region were exceptionally dry at the time of our sampling, hence potentially indicative of future environmental conditions resulting from the predicted trends in climate change in the Centro American region. We evidenced a clear distinction in ecosystem functioning between the eastern and western parts of the lagoon. Most of the oceanic water entering through the inlets spread toward the south-east where dissolved organic matter accumulated. This area did not support significant phytoplankton development. In the west, we hypothesized a balance shift between top-down and bottom-up control to explain the different responses in terms of phytoplankton productivity. The decoupling between nitrogen inputs brought by oceanic waters and the Palizada River, and phosphate inputs from the Chumpan River did not allow for phytoplankton C-fixation. Most of the phytoplankton biomass was aggregated around the Palizada River mouth (which carried most of the freshwater into the lagoon), in a P-depleted area (low phosphate concentration and high bacterial phosphatase activity). Bacterial ectoenzyme activities were mainly observed in the middle of the lagoon, along a south to north cross section stretching from the Chumpan River up to Carmen Island. Maximum mineralization activities were found in this area, which coincided with high extracellular lipase and aminopeptidase activities and low DOC and $O_2$ concentrations. The lagoon produced significant quantities of particulate and dissolved organic matter thanks to i) nutrient inputs from the rivers, ii) to uncoupling between nitrogen and phosphate, and iii) to prokaryotic activities, but in the end, most of this autochthonous matter was internally processed or stored and only a little was exported to the Gulf of Mexico coastal waters. During our study therefore, the water column of Terminos Lagoon functioned globally as a kind of "nitrogen assimilator". Highest PAH concentrations were measured in El Carmen Inlet, suggesting anthropogenic pollution in this zone which is probably related to the oil platform exploitation activities in the shallow waters of the south of the Gulf of Mexico and, more locally, to the efflux from El Carmen harbour which serves as a logistical support to the oil extraction industry. We also evidenced the importance of nitrogen fertilization from the Palizada River, which seems to support an abundant prokaryotic community of PAH-degraders.

Another significant outcome of our study has been (i) to link the spatial distribution of ectoenzymatic activities with changes in prokaryotic community structure and (ii) to show that a combination of a complex set of physical and biogeochemical parameters was necessary to explain the changes in prokaryotic community structure. This study also emphasizes the use of direct multivariate statistical analysis to keep the influence of pollutants in perspective, without denying the role of other physico-chemical variables to explain the dynamic of prokaryotic community structure in polluted areas.

Our study provides an extensive dataset efficiently mixing biogeochemical status with information on phytoplankton and prokaryotic structure and dynamics. This has never before been measured in Terminos Lagoon and the outcome offers a strong base of information and reflexion for future studies on this essential coastal system and the potential environmental conditions which might prevail as a consequence of future climate change. Further studies are needed to compare our dataset with high river input regime conditions and to asses both how this might affect the observed uncoupling between nitrogen and phosphate, as well as the dominant source of phosphorus and its consequences on primary production and prokaryotic activities. Finally, the role of top-down control should also be investigated in order to better understand the variability of the observed responses.

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**Figure legends**

**Figure 1**: Study site location and distribution of the 35 sampled stations in the lagoon.

**Figure 2**: Mapped distribution of the physico-chemical parameters measured in Terminos Lagoon in October 2009 for **A.** temperature (°C); **B.** salinity; **C.** nitrate concentrations ($NO_3$ in µM); **D.** ammonium concentrations ($NH_4$ in µM); **E.** phosphate concentrations ($PO_4$ in µM); **F.** dissolved organic carbon concentrations (DOC in µM); **G.** dissolved organic nitrogen concentrations (DON in µM); and **H.** dissolved organic phosphorus concentrations (DOP in µM).

**Figure 3**: as Figure 2 for **A.** particulate organic nitrogen concentrations (PON in µM); **B.** particulate organic phosphorus concentrations (PP in µM); **C.** total chlorophyll concentrations (CHL in mg.m$^{-3}$); **D.** phaeopigments (Phaeo in mg.m$^{-3}$); **E.** maximum photosynthetic rate normalized to chlorophyll ($P^b_m$ in mgC.mgCHL$^{-1}$.h$^{-1}$); **F.** free-living prokaryotes abundance ($10^6$ cell.mL$^{-1}$)

**Figure 4**: as Figure 2 for **A.** aminopeptidase activities (fmol.L$^{-1}$.h$^{-1}$.cell$^{-1}$); **B.** phosphatase activities (fmol.L$^{-1}$.h$^{-1}$.cell$^{-1}$); and **C.** lipase activities (fmol.L$^{-1}$.h$^{-1}$.cell$^{-1}$)

**Figure 5**: as Figure 2 for **A.** total dissolved PAHs (ng.L$^{-1}$); and **B**. the most-probable-number (MPN in count)

**Figure 6:** Multidimensional scaling (MDS) plot of the total (**A**) and metabolically active (**B**) prokaryotic community structures as determined from CE-SSCP profiles based on the Bray–Curtis similarity index. Clusters were determined according to the SIMPROF similarity profile test ($p<0.05$).

**Figure 7:** Canonical correspondence analysis of total (A) and active (B) bacterioplankton community structure from the 35 samples using physico-chemical parameters. Arrows point in the direction of increasing values of each variable. The length of the arrows indicates the degree of correlation with the represented axes. The position of samples relative to arrows is interpreted by projecting the points on the arrow and indicates the extent to which a sample prokaryotic community composition is influenced by the environmental parameter represented by that arrow. The variance explained by the environmental variables selected by the model represent 27 % and 40 % of the variability at the DNA and RNA level, respectively.

**Figure 8**: as Figure 2 for NOP:PP ratio

Figure 1

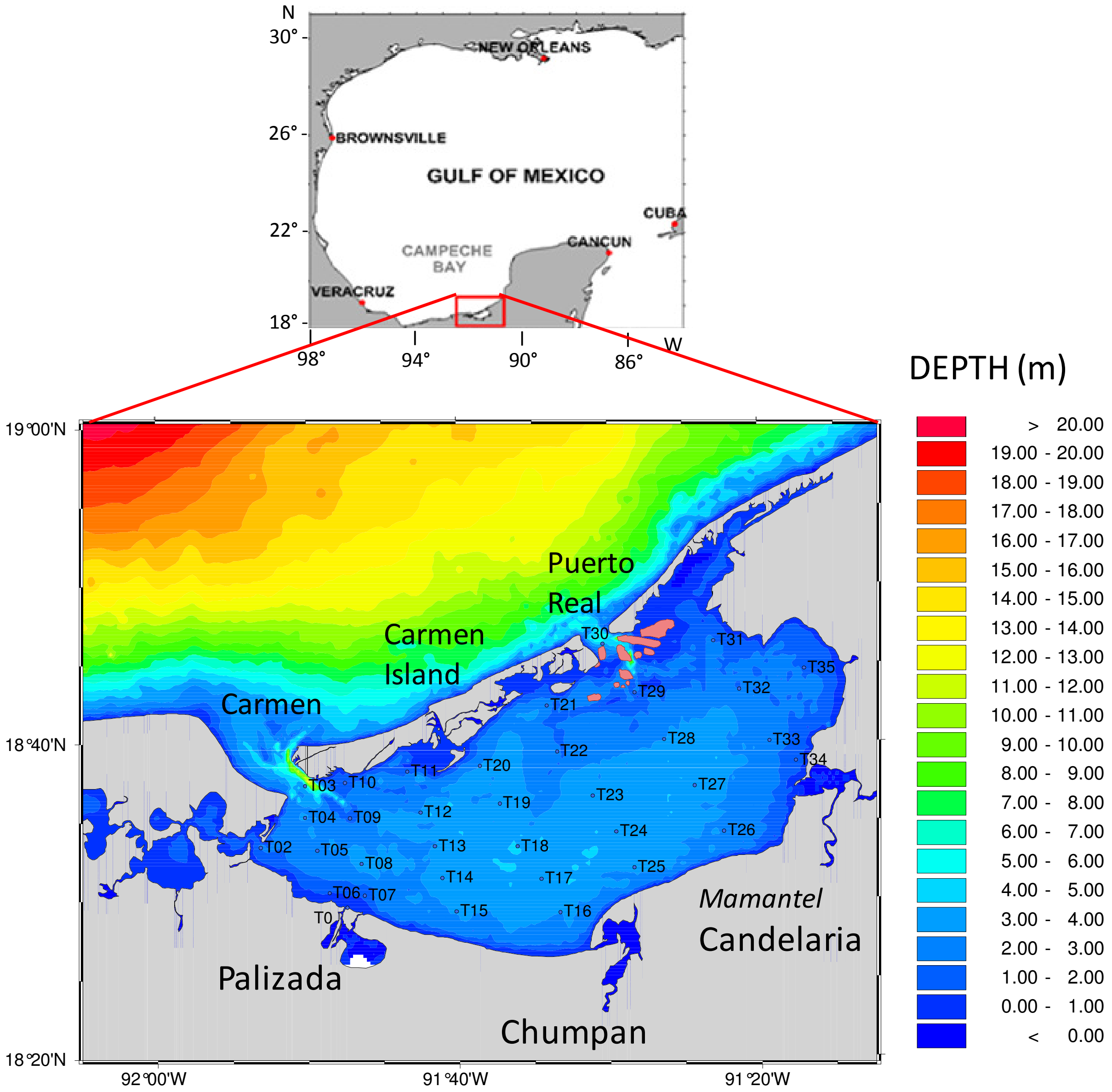

N
30°
26°
22°
18°
98°
94°
90°
86°
W
NEW ORLEANS
BROWNSVILLE
GULF OF MEXICO
CUBA
CANCUN
CAMPECHE BAY
VERACRUZ
19°00'N
18°40'N
18°20'N
92°00'W
91°40'W
91°20'W
Puerto Real
Carmen Island
Carmen
Palizada
Chumpan
Mamantel
Candelaria
T0
T02
T03
T04
T05
T06
T07
T08
T09
T10
T11
T12
T13
T14
T15
T16
T17
T18
T19
T20
T21
T22
T23
T24
T25
T26
T27
T28
T29
T30
T31
T32
T33
T34
T35
DEPTH (m)
> 20.00
19.00 - 20.00
18.00 - 19.00
17.00 - 18.00
16.00 - 17.00
15.00 - 16.00
14.00 - 15.00
13.00 - 14.00
12.00 - 13.00
11.00 - 12.00
10.00 - 11.00
9.00 - 10.00
8.00 - 9.00
7.00 - 8.00
6.00 - 7.00
5.00 - 6.00
4.00 - 5.00
3.00 - 4.00
2.00 - 3.00
1.00 - 2.00
0.00 - 1.00
< 0.00

Figure 2

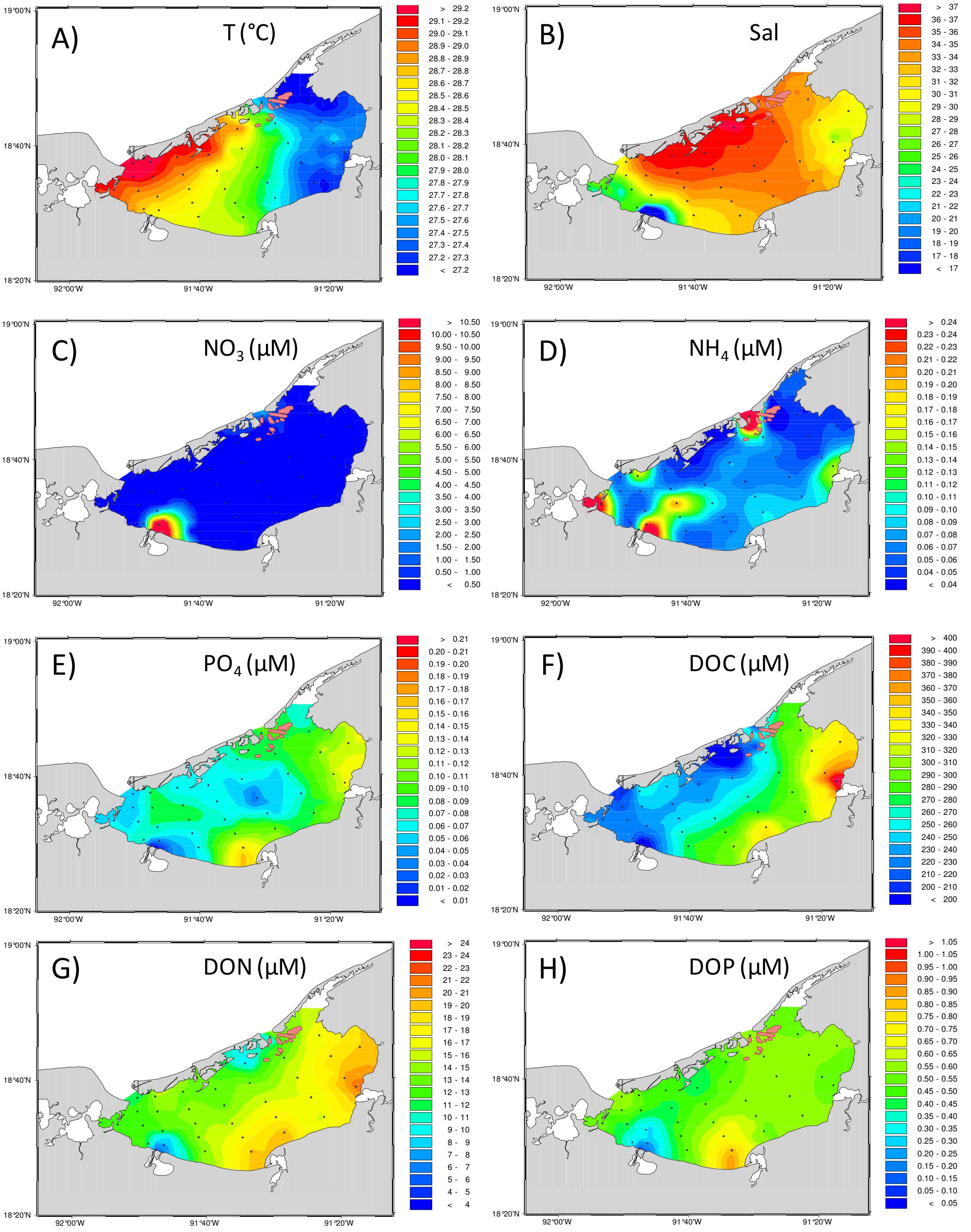
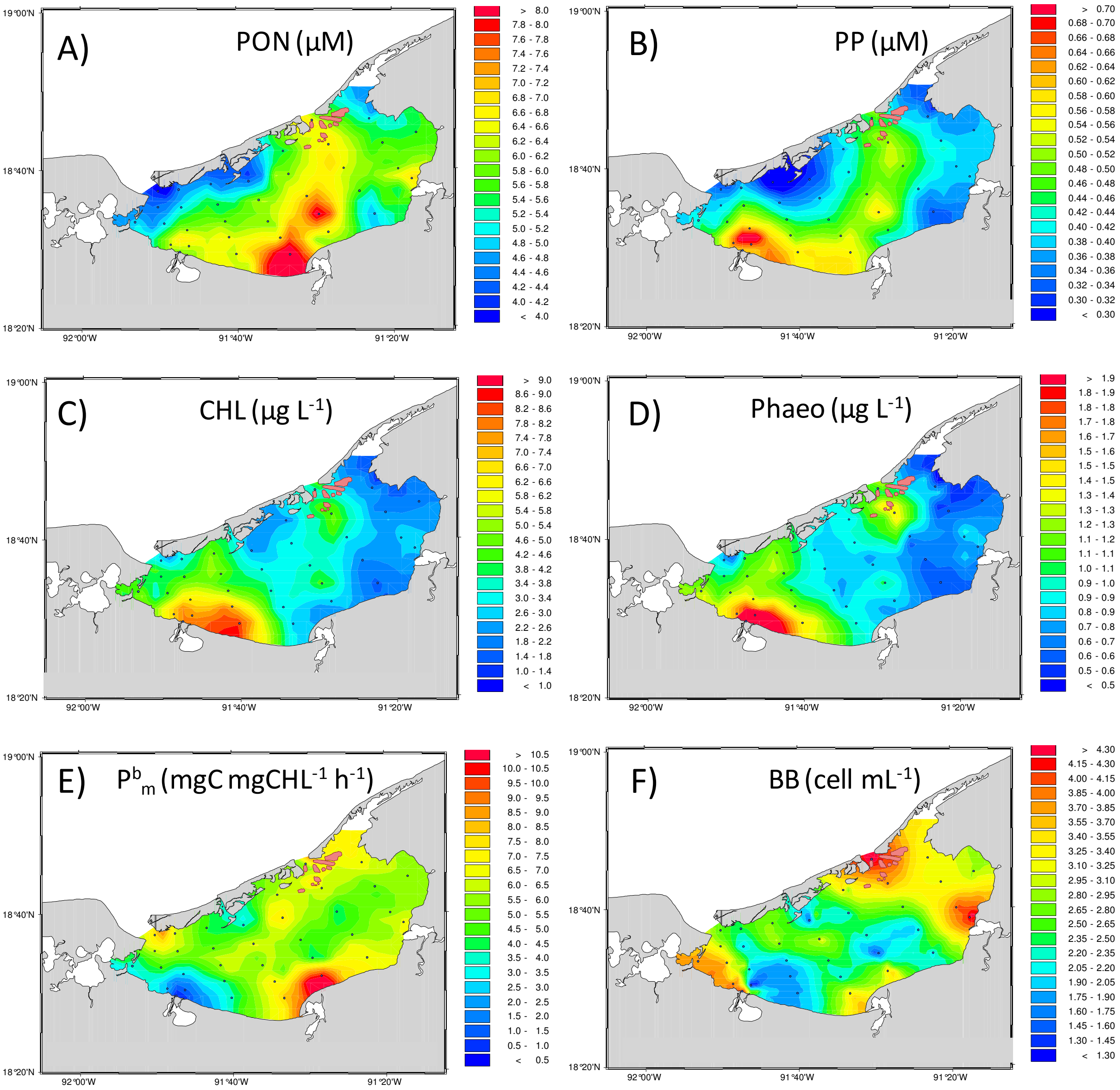
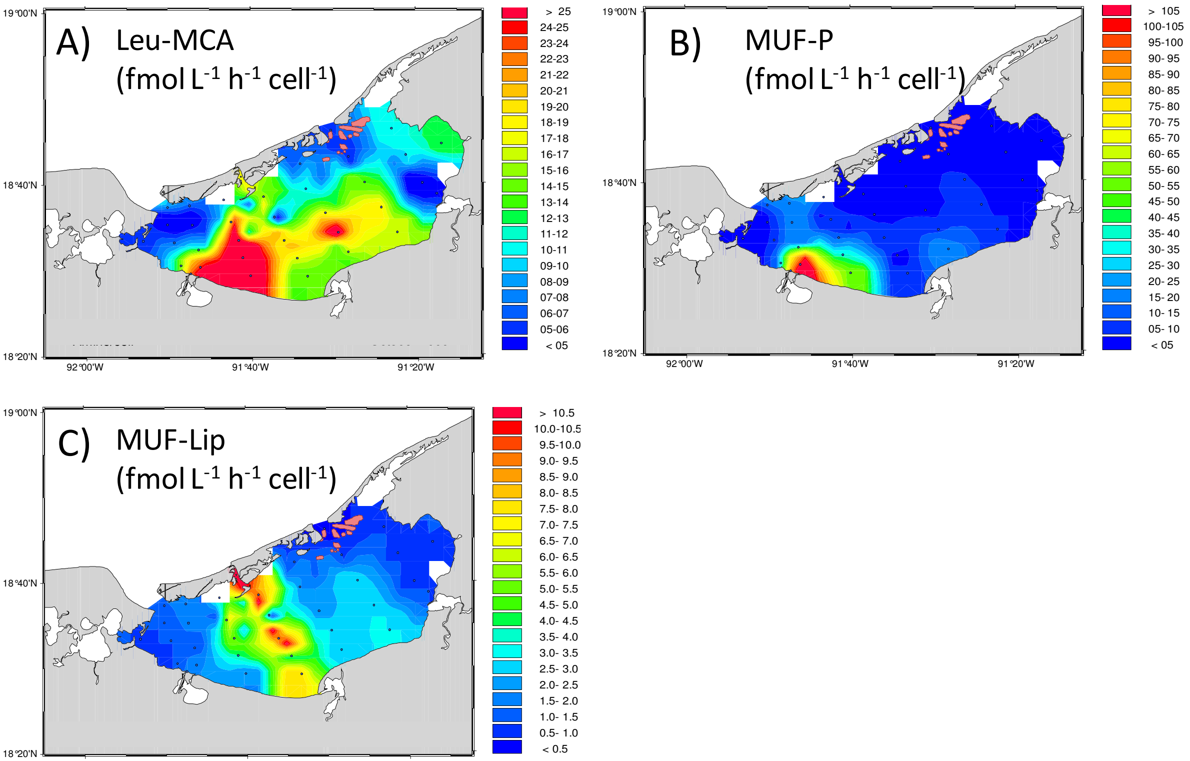

A)
T (°C)
> 29.2
29.1 - 29.2
29.0 - 29.1
28.9 - 29.0
28.8 - 28.9
28.7 - 28.8
28.6 - 28.7
28.5 - 28.6
28.4 - 28.5
28.3 - 28.4
28.2 - 28.3
28.1 - 28.2
28.0 - 28.1
27.9 - 28.0
27.8 - 27.9
27.7 - 27.8
27.6 - 27.7
27.5 - 27.6
27.4 - 27.5
27.3 - 27.4
27.2 - 27.3
< 27.2
B)
Sal
> 37
36 - 37
35 - 36
34 - 35
33 - 34
32 - 33
31 - 32
30 - 31
29 - 30
28 - 29
27 - 28
26 - 27
25 - 26
24 - 25
23 - 24
22 - 23
21 - 22
20 - 21
19 - 20
18 - 19
17 - 18
< 17
C)
NO$_3$ (µM)
> 10.50
10.00 - 10.50
9.50 - 10.00
9.00 - 9.50
8.50 - 9.00
8.00 - 8.50
7.50 - 8.00
7.00 - 7.50
6.50 - 7.00
6.00 - 6.50
5.50 - 6.00
5.00 - 5.50
4.50 - 5.00
4.00 - 4.50
3.50 - 4.00
3.00 - 3.50
2.50 - 3.00
2.00 - 2.50
1.50 - 2.00
1.00 - 1.50
0.50 - 1.00
< 0.50
D)
NH$_4$ (µM)
> 0.24
0.23 - 0.24
0.22 - 0.23
0.21 - 0.22
0.20 - 0.21
0.19 - 0.20
0.18 - 0.19
0.17 - 0.18
0.16 - 0.17
0.15 - 0.16
0.14 - 0.15
0.13 - 0.14
0.12 - 0.13
0.11 - 0.12
0.10 - 0.11
0.09 - 0.10
0.08 - 0.09
0.07 - 0.08
0.06 - 0.07
0.05 - 0.06
0.04 - 0.05
< 0.04
E)
PO$_4$ (µM)
> 0.21
0.20 - 0.21
0.19 - 0.20
0.18 - 0.19
0.17 - 0.18
0.16 - 0.17
0.15 - 0.16
0.14 - 0.15
0.13 - 0.14
0.12 - 0.13
0.11 - 0.12
0.10 - 0.11
0.09 - 0.10
0.08 - 0.09
0.07 - 0.08
0.06 - 0.07
0.05 - 0.06
0.04 - 0.05
0.03 - 0.04
0.02 - 0.03
0.01 - 0.02
< 0.01
F)
DOC (µM)
> 400
390 - 400
380 - 390
370 - 380
360 - 370
350 - 360
340 - 350
330 - 340
320 - 330
310 - 320
300 - 310
290 - 300
280 - 290
270 - 280
260 - 270
250 - 260
240 - 250
230 - 240
220 - 230
210 - 220
200 - 210
< 200
G)
DON (µM)
> 24
23 - 24
22 - 23
21 - 22
20 - 21
19 - 20
18 - 19
17 - 18
16 - 17
15 - 16
14 - 15
13 - 14
12 - 13
11 - 12
10 - 11
9 - 10
8 - 9
7 - 8
6 - 7
5 - 6
4 - 5
< 4
H)
DOP (µM)
> 1.05
1.00 - 1.05
0.95 - 1.00
0.90 - 0.95
0.85 - 0.90
0.80 - 0.85
0.75 - 0.80
0.70 - 0.75
0.65 - 0.70
0.60 - 0.65
0.55 - 0.60
0.50 - 0.55
0.45 - 0.50
0.40 - 0.45
0.35 - 0.40
0.30 - 0.35
0.25 - 0.30
0.20 - 0.25
0.15 - 0.20
0.10 - 0.15
0.05 - 0.10
< 0.05
19°00'N
18°40'N
18°20'N
92°00'W
91°40'W
91°20'W

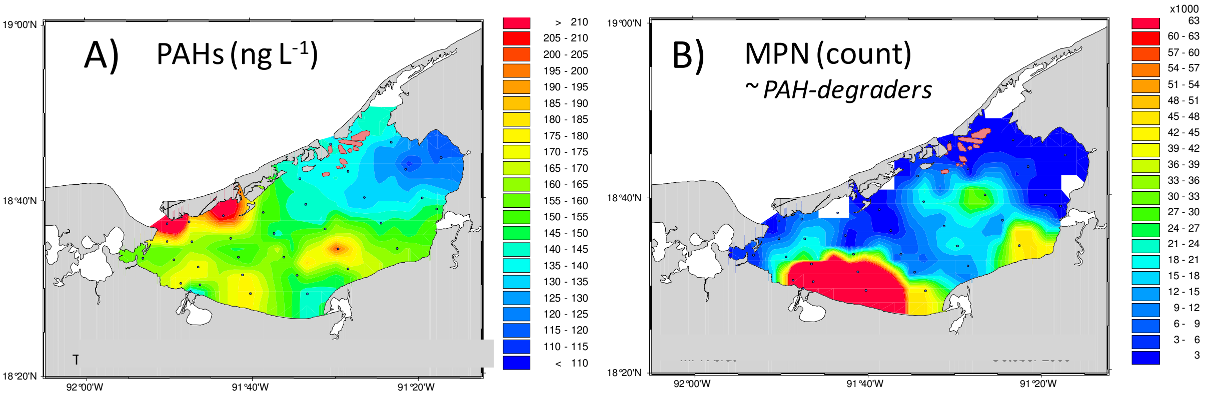
Figure 3


A)
PON (µM)
> 8.0
7.8 - 8.0
7.6 - 7.8
7.4 - 7.6
7.2 - 7.4
7.0 - 7.2
6.8 - 7.0
6.6 - 6.8
6.4 - 6.6
6.2 - 6.4
6.0 - 6.2
5.8 - 6.0
5.6 - 5.8
5.4 - 5.6
5.2 - 5.4
5.0 - 5.2
4.8 - 5.0
4.6 - 4.8
4.4 - 4.6
4.2 - 4.4
4.0 - 4.2
< 4.0
B)
PP (µM)
> 0.70
0.68 - 0.70
0.66 - 0.68
0.64 - 0.66
0.62 - 0.64
0.60 - 0.62
0.58 - 0.60
0.56 - 0.58
0.54 - 0.56
0.52 - 0.54
0.50 - 0.52
0.48 - 0.50
0.46 - 0.48
0.44 - 0.46
0.42 - 0.44
0.40 - 0.42
0.38 - 0.40
0.36 - 0.38
0.34 - 0.36
0.32 - 0.34
0.30 - 0.32
< 0.30
C)
CHL (µg L-1)
> 9.0
8.6 - 9.0
8.2 - 8.6
7.8 - 8.2
7.4 - 7.8
7.0 - 7.4
6.6 - 7.0
6.2 - 6.6
5.8 - 6.2
5.4 - 5.8
5.0 - 5.4
4.6 - 5.0
4.2 - 4.6
3.8 - 4.2
3.4 - 3.8
3.0 - 3.4
2.6 - 3.0
2.2 - 2.6
1.8 - 2.2
1.4 - 1.8
1.0 - 1.4
< 1.0
D)
Phaeo (µg L-1)
> 1.9
1.8 - 1.9
1.8 - 1.8
1.7 - 1.8
1.6 - 1.7
1.5 - 1.6
1.5 - 1.5
1.4 - 1.5
1.3 - 1.4
1.3 - 1.3
1.2 - 1.3
1.1 - 1.2
1.1 - 1.1
1.0 - 1.1
0.9 - 1.0
0.9 - 0.9
0.8 - 0.9
0.7 - 0.8
0.6 - 0.7
0.6 - 0.6
0.5 - 0.6
< 0.5
E)
Pbm (mgC mgCHL-1 h-1)
> 10.5
10.0 - 10.5
9.5 - 10.0
9.0 - 9.5
8.5 - 9.0
8.0 - 8.5
7.5 - 8.0
7.0 - 7.5
6.5 - 7.0
6.0 - 6.5
5.5 - 6.0
5.0 - 5.5
4.5 - 5.0
4.0 - 4.5
3.5 - 4.0
3.0 - 3.5
2.5 - 3.0
2.0 - 2.5
1.5 - 2.0
1.0 - 1.5
0.5 - 1.0
< 0.5
F)
BB (cell mL-1)
> 4.30
4.15 - 4.30
4.00 - 4.15
3.85 - 4.00
3.70 - 3.85
3.55 - 3.70
3.40 - 3.55
3.25 - 3.40
3.10 - 3.25
2.95 - 3.10
2.80 - 2.95
2.65 - 2.80
2.50 - 2.65
2.35 - 2.50
2.20 - 2.35
2.05 - 2.20
1.90 - 2.05
1.75 - 1.90
1.60 - 1.75
1.45 - 1.60
1.30 - 1.45
< 1.30
19°00'N
18°40'N
18°20'N
92°00'W
91°40'W
91°20'W

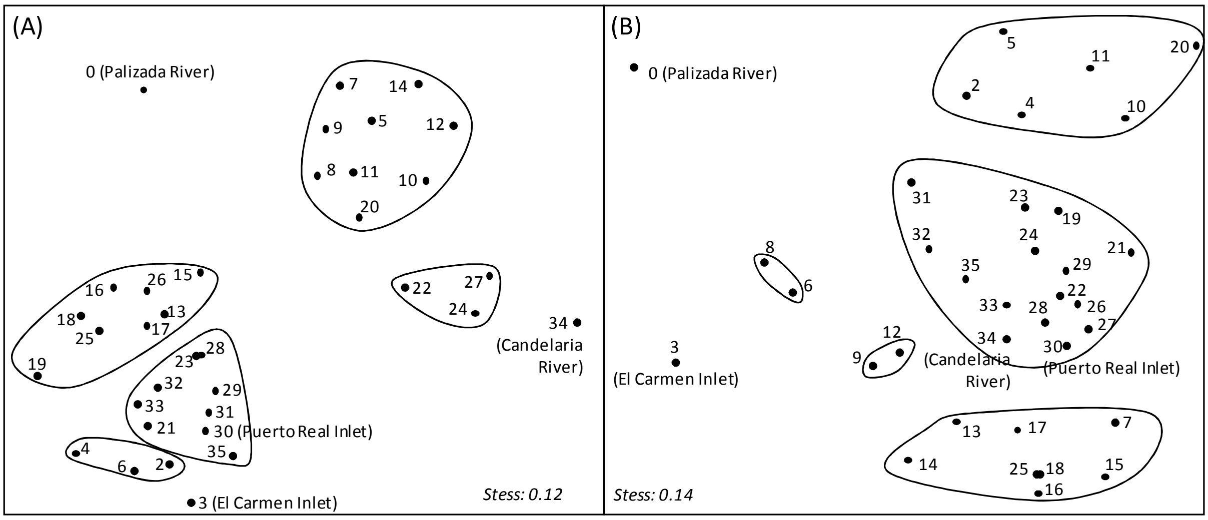
## Figure 4



## Figure 5



# Figure 6



# Figure 7

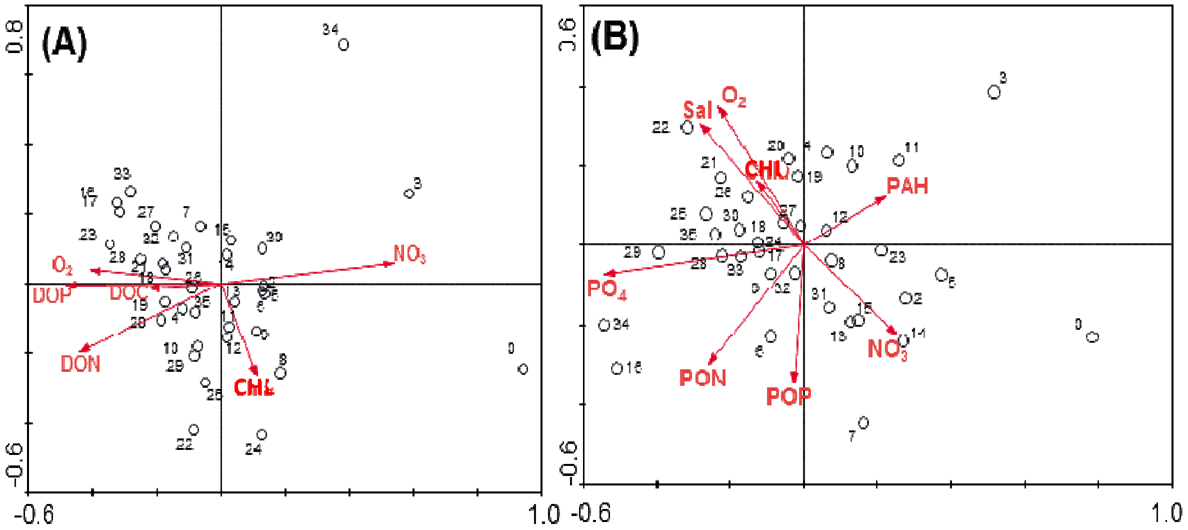
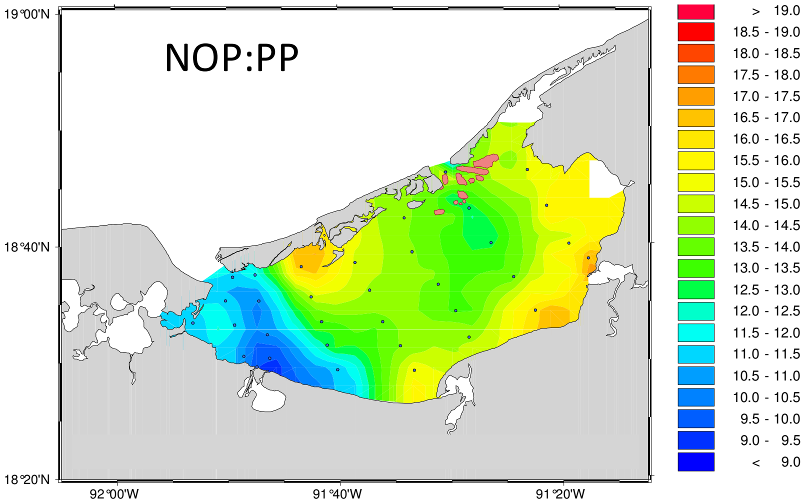


Figure 8


NOP:PP
19°00'N
18°40'N
18°20'N
92°00'W
91°40'W
91°20'W
> 19.0
18.5 - 19.0
18.0 - 18.5
17.5 - 18.0
17.0 - 17.5
16.5 - 17.0
16.0 - 16.5
15.5 - 16.0
15.0 - 15.5
14.5 - 15.0
14.0 - 14.5
13.5 - 14.0
13.0 - 13.5
12.5 - 13.0
12.0 - 12.5
11.5 - 12.0
11.0 - 11.5
10.5 - 11.0
10.0 - 10.5
9.5 - 10.0
9.0 - 9.5
< 9.0