Influence of agriculture and aquaculture activities on the response of autotrophic picoplankton in Laguna Macapule, Gulf of California (Mexico)

Aída Martínez-López[1*], Cristian Hakspiel-Segura[1], Diana Cecilia Escobedo-Urías[2] and Bárbara González-Acosta[1]

Abstract

Introduction: The lagoon is a component of coastal zones, whose populations of autotrophic picoplankton (APP) remain largely unstudied. These lagoons display high-nutrient productivity and additionally may also be subjected to anthropogenic activities. This study selected Laguna Macapule, located on the eastern shore in the mid-region of the Gulf of California, due to the fact that a drainage network servicing the surrounding agricultural region (>230,000 hectares under cultivation) directs irrigation runoff, shrimp farm effluents, and urban wastewater containing large quantities of nutrients to be discharged into this lagoon. We propose to identify the APP's response to various types of environmental and anthropogenic influence in this highly impacted coastal lagoon.

Methods: Two sites (separated by 2.7 km) were monitored from December 2007 to December 2008. One, located at the entrance to Laguna Macapule (oceanic influence) and the other a discharge canal (eutrophic conditions) inside the lagoon at El Tortugón.

Results: APP was the numerically dominant phytoplankton fraction (15×10^6 to 620×10^6 cells L^{-1}) with coccoidal cyanobacteria as the dominant fraction throughout the year. Peak levels were reached in spring-early autumn and they were the second largest contributor to biomass. Abundance of APP cells corresponds to the lagoon's eutrophic status. Maximum numbers and a higher average of APP were recorded at the El Tortugón channel during the warm season (months with SST higher than 24 °C). The general positive relationship of the APP's annual cycle at both sites as well as a negative relationship with heterotrophic nanoflagellates (HNF) abundance, supports the idea that natural forcing, in particular sea surface temperature (SST) is the predominant influences on APP's seasonal variability.

Conclusions: Distinguishable significant differences in APP abundances and nutrients were recognizable between the two sites. The interplay of these variables contributed to lower densities of APP in winter and high densities in spring-early autumn. N:P = ~4 suggests that spring-early autumn abundance of the APP autotrophic component was sustained by urea from shrimp farm discharge water. Thus, a total nutrient-based approach is likely the most suitable tool for establishing nitrogen limitation of biological production in Laguna Macapule and similarly impacted ecosystems around the world.

Keywords: Aquaculture, Autotrophic picoplankton, Eutrophic conditions, Heterotrophic ciliates, Heterotrophic nanoflagellates, Urea

* Correspondence: amartin@ipn.mx
[1]Centro Interdisciplinario de Ciencias Marinas-IPN (CICIMAR), Av. IPN s/n, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23096, Mexico
Full list of author information is available at the end of the article

Martínez-López et al. Ecological Processes (2017) 6:6

Page 2 of 11

Introduction

Autotrophic picoplankton (APP) is recognized as a ubiquitous and abundant component of phytoplanktonic communities (Marshall 2002) represented primarily by prokaryotic coccoidal cyanobacteria (Flombaum et al. 2013) and prochlorophytes (Chisholm et al. 1988). There are also small eukaryotes with a potentially large representation (Johnson and Sieburth 1982; Jardillier et al. 2010). In the 1970s, high densities of APP were discovered in low-nutrient, temperate oceanic areas (Johnson and Sieburth 1979; Waterbury et al. 1979). Since then, most studies have focused on oligotrophic systems, which corroborated the contribution of APP to pelagic food webs and their role in carbon and mineral cycling (Barber and Hiscock 2006; Flombaum et al. 2013). Thus, the paradigm that APP was important primarily in oceanic waters was established. Subsequently, abundant evidence has shown that APP is a major component of the phytoplankton community in coastal and continental shelf areas (e.g., Calvo-Díaz and Morán 2006; Linacre-Rojas et al. 2010; Gunbua et al. 2012). Within coastal zones, there remains a specific subclass region where populations of APP, to a great extent, are unstudied. These are lagoons that display high-nutrient productivity, as well as those threatened by anthropogenic activities. Studies of ecosystems affected by eutrophication and imbalanced N:P ratios provide evidence of large abundances of APP ($>100 \times 10^6$ cells L^{-1}) (Badylak and Phlips 2004; Murrell and Caffrey 2005; Gaulke et al. 2010). In nitrogen-limited environments, APP displays a distinct competitive advantage (Raven 1998); however, the APP responses to seasonal changes in coastal systems remain unclear, mainly because there is limited documentation of the annual cycle.

In the Gulf of California, with numerous lagoons, the subtropical climate favors seasonal nutrient enrichment because rainfall is prevalent mainly in the summer, which leads to rapid nutrients runoff. Many lagoons receive nutrient inputs from fertilization of shrimp farms and crop fields, as well as the load derived from urban settlements that use streams as waste disposal channels. These sources of nutrient pollutants affect phytoplankton overall abundance and seasonal variability. Laguna Macapule is one of over 15 coastal water bodies along coast of Sinaloa. It was selected for this study because it is impacted by several nutrient sources of disparate origins. It receives large quantities of nutrients from irrigation runoff that are collected in a drainage network servicing the surrounding agricultural region (>230,000 ha under cultivation), shrimp farm effluents, and urban wastewater. These nutrient sources should favor microphytoplankton growth; instead, some studies indicate that smaller, coccoid cyanobacteria and flagellates are the dominant groups under conditions typified by high concentrations of ammonium and low inorganic N:P nutrient ratios of less than 16:1 (Poot-Delgado 2006). This suggested that APP could make an important contribution to the base of the trophic web structure. At present, there are no published annual data on the abundance of APP in Mexican coastal systems in the Gulf of California.

This study is the first compilation and will serve as a baseline for future surveys that document abundance and seasonal variability of APP in the Laguna Macapule, with the aim of understanding the small-size fraction of the planktonic food web in coastal systems affected by anthropogenic pressures.

Methods

Study site

Laguna Macapule is a small lagoon (38 km²) in northwestern Sinaloa (25° 18′ N, 108° 42′ W; Fig. 1) with an average depth of 2.3 m, a maximum depth of 8 m, and a water residence time of approximately 2.2 days (Magaña-Álvarez 2004). Physical and chemical conditions in this part of the Gulf of California are mainly influenced by wind forcing, evaporation in warm months, and occasional heavy rainfall (Álvarez-Borrego et al. 1978; Castro et al. 2000). Persistent northwesterly winds lasting from a few hours to a week or longer in the winter–spring seasons contribute to lower water temperature and force the initiation of offshore coastal upwelling (Martínez-López et al. 2008). This entrains nutrient-rich water into the area, which is eventually dispersed into coastal lagoons through tidal action (Escobedo-Urías et al. 2007). During summer and autumn, there is advection of oligotrophic tropical water into the area forced by prevalent southeastern winds (Bernal et al. 2001). The rainy season, from June through October, typically contributes more than 90% of the annual rainfall (Magaña-Álvarez 2004).

Inside the lagoon, two sampling points were selected because of their contrasting characteristics. One site is located at the inlet of the lagoon, which exhibits open ocean water conditions year round. The other (El Tortugón) is located at the back of the lagoon in front of a discharge mouth, one of several drainage channels that enter the lagoon after passing through mangroves. This natural tidal channel receives discharge from a small settlement, two shrimp farms, and agricultural runoff from the backland. This site is strongly influenced by fresh water runoff during the rainy season, which carries agricultural effluents. Numerous others shrimp farms are located along the length of the inner shore of the lagoon.

Sampling procedures and analyses

Monthly samples were taken from December 2007 through December 2008 at the two sampling stations (2.7 km far away) at high tide. The tides in this lagoon
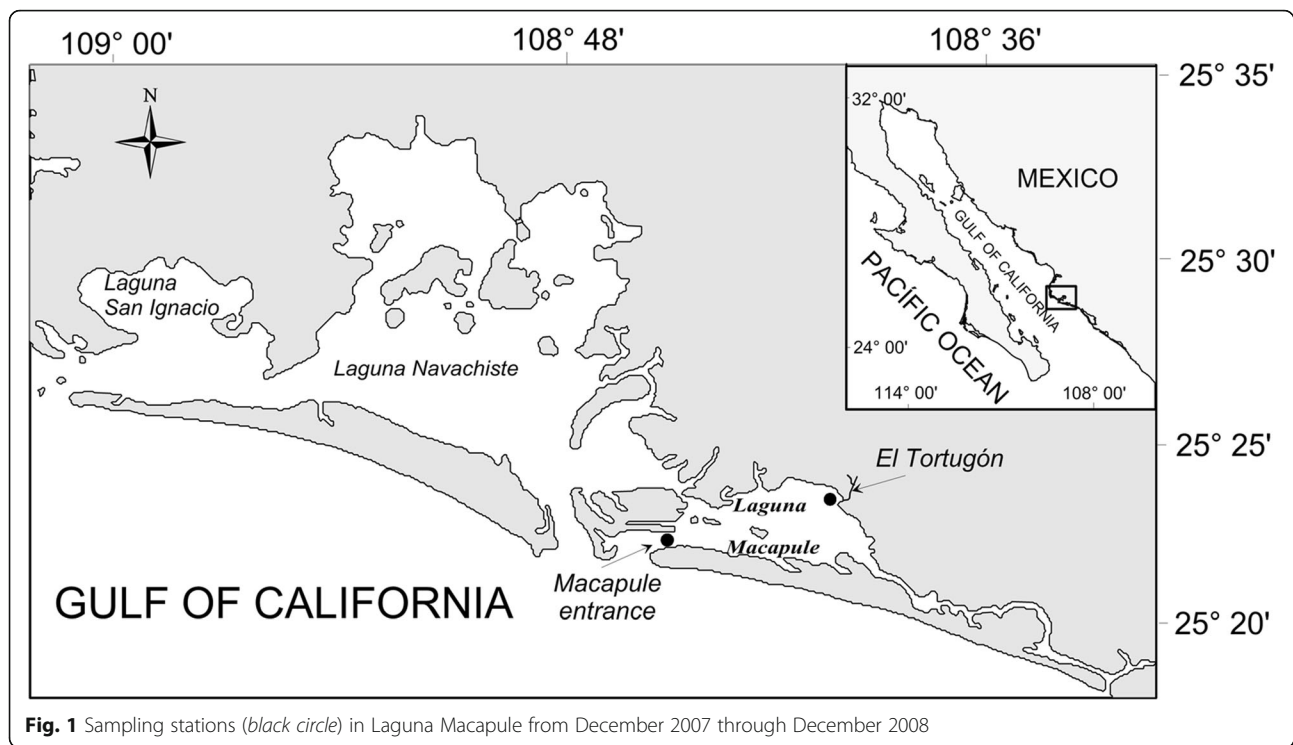
Martínez-López *et al. Ecological Processes* (2017) 6:6

Page 3 of 11

Fig. 1 Sampling stations (*black circle*) in Laguna Macapule from December 2007 through December 2008

have a strong semi-diurnal component with two unequal flood and ebb cycles every 24.84 h. Tidal amplitude is about 1.08 m (Martínez-López et al 2007). Sea surface temperature (SST) was measured with a multiparameter analyzer (Model U-10, Horiba, Kyoto, Japan). Water samples were taken with a segmented pipe sampler (Sutherland et al. 1992) for integrated profiling of the upper 3 m of the water column. The samples were filtered (Whatman GF/F, pore size 0.7 μm) and frozen for later assays of dissolved nutrients. Dissolved inorganic phosphorus (DIP) and urea were assayed according to Strickland and Parsons (1972) and Mulvenna and Savidge (1992), respectively. Ammonium (NH_4^+) was assayed according to Solórzano (1969). Wind speed data were obtained from a meteorological station run by the federal agency Conagua located in the city of Guasave (25° 33′ 45″ N, 108° 27′ 40″ W), which is approximately 27 km from the lagoon.

Triplicate samples were collected in sterile glass bottles to quantify APP (phytoplankton <2 μm), autotrophic nanoplankton, and heterotrophic nanoplankton (2–20 μm). These samples were preserved with 1% phosphate-buffered glutaraldehyde. For APP, unstained subsamples (2–6 mL) were filtered through 0.2-μm polycarbonate membranes (MacIsaac and Stockner 1993). Autotrophic nanoplankton and heterotrophic nanoplankton subsamples (10–17 mL) were filtered through 0.8-μm polycarbonate filters and counted after primulin staining (Caron 1983). Both filters were mounted on microscope slides with non-fluorescent

oil, and abundance was determined by epifluorescence microscopy equipped with a Hg 100-W lamp, blue-light excitation filter cube (exciter BP460 to 490 nm, dichroic mirror 505 nm, barrier 515 nm) and bright line multiband filter cube (exciter BP360 to 730 nm, dichroic mirror 400 nm, barrier 420 nm). At least 200 cells were counted from random fields at ×1000 magnifications in each of three replicate slides. In the case of blooms of autotrophic nanoplankton (>1 × 10⁶ cells L⁻¹), the organisms were identified to class level, with additional observations of water samples preserved with 1% lugol under an inverted microscope (Lund et al. 1958). Data on heterotrophic ciliates and microzooplanktonic organisms, such as microcrustaceans, were collected to assess their impact on the APP. Abundance was estimated with the Utermöhl technique (Lund et al. 1958). Samples preserved with 1% lugol (final concentration) were allowed to settle for at least 24 h in sedimentation chambers (10 cm³), followed by counts at ×200 and ×400 magnification under an inverted microscope (Olympus CKX41, Olympic America, Center Valley, PA).

Statistical analysis

The combined data from both sites and the data from each site were tested for normal distribution, using the Kolmogorov–Smirnov test (Smith et al. 1998). Distribution of many variables differed significantly ($p < 0.05$); therefore, relationships between biological data (abundance of autotrophic nanoplankton, heterotrophic nanoplankton, and

Martínez-López *et al. Ecological Processes* (2017) 6:6

Page 4 of 11

microzooplankton) and various environmental variables (water temperature, temperature, NH_4^+, DIP, and urea) were determined using Spearman's rank correlations analysis (Zar 1999). Statistical differences between environmental variables and abundance of plankton at the two sites were tested with the Wilcoxon method for paired samples (Zar 1999). Monthly abundances of APP were compared, using the Kruskal–Wallis test. Significance was set at $p < 0.05$. Statistical analyses were performed with Statistica 8.0 computer software (StatSoft, Tulsa, OK, http://statistica.io/products/).

Results

Sea surface temperature in 2008 increased from 17 °C in January to remain above 28 °C from June through October, with a peak in July of 32 °C (Fig. 2a). Significant temperature differences were noted between months at both sites (Kruskal–Wallis $H = 24.11$; $p = 0.019$) with slightly higher temperatures (~1.5 °C) at El Tortugón than at the entrance (Wilcoxon $Z = 2.03$; $p = 0.04$). Fluctuations of NH_4^+ were greatest at the entrance, but similar seasonal trends prevailed at both stations (Wilcoxon $Z = 0.02$; $p = 0.81$), with higher concentrations during the cold months, while N:P (sum of dissolved inorganic nitrogen to dissolved inorganic phosphorus) ratio showed values ~4, during spring and up to 5 in early autumn 2008 (Fig. 2b). The ratio values were lower than 4 for the remaining months. At the entrance, concentrations of DIP above 1.5 μM were recorded from April through July, with a peak in May. Low concentrations (mean = 0.9 μM) prevailed for the remainder of the year. At El Tortugón, higher concentrations than the entrance were encountered from May through December, with a peak in June (Fig. 2c). No statistical differences were found between monthly concentrations at either station (Kruskal–Wallis $H = 18.11$; $p = 0.11$). An additional spike in phosphorus was registered in November at El Tortugón (Fig. 2c). Overall, large variations in concentrations of urea occurred at both sites (Wilcoxon $Z = 2.20$; $p = 0.028$) and followed the same trend. Concentrations were generally higher (<35 μM) at the entrance to the lagoon from April through November, but with extreme monthly variability (Fig. 2d).

With the exception of May, >90% of all phytoplankton abundance belonged to the APP (<2 μm). Autotrophic nanoplankton (2–20 μm) rarely exceeded 10% and microphytoplankton (20–200 μm) was around 0.34% of total abundance (Table 1). During most winter months, the concentration of the APP fraction was smaller than the other two fractions. The entrance had higher abundances of microphytoplankton than the other station from February through May (Table 1). Autotrophic nanoplankton included Raphidophyta, Prasinophyta, Cryptophyta, Euglenophyta, and small Bacillariophyta
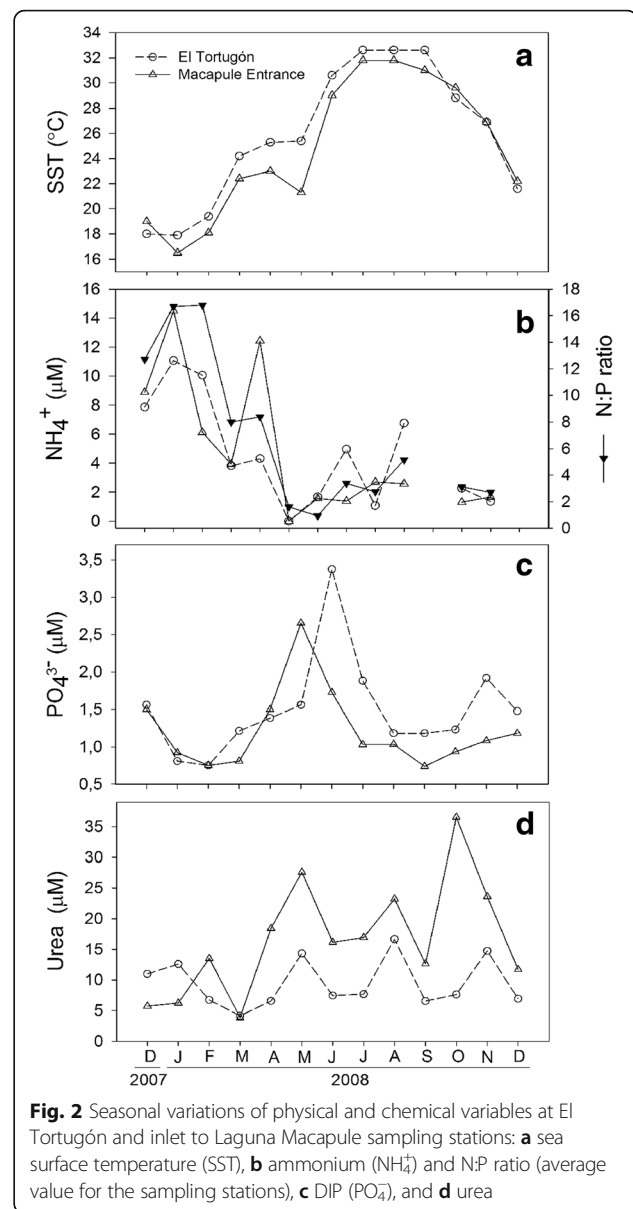

Fig. 2 Seasonal variations of physical and chemical variables at El Tortugón and inlet to Laguna Macapule sampling stations: **a** sea surface temperature (SST), **b** ammonium (NH_4^+) and N:P ratio (average value for the sampling stations), **c** DIP (PO_4^-), and **d** urea

(diatoms). Blooms of Raphidophyta and Prasinophyta appeared in March, July, and October.

The APP was largely composed of coccoidal cyanobacteria. Eukaryotic picophytoplankton was occasionally detected in July and October. Abundances of APP ranged from 15×10^6 to 620×10^6 cells L^{-1}. The trend shows highest densities from April through November. Abundance of APP at the lagoon entrance was lower than at El Tortugón during the warmer months (Wilcoxon $Z = 2.48$; $p = 0.013$). Variability of APP at El Tortugón was extreme, with marked decline during the rainy season from July through September (Fig. 3a), when the SST ranged from 30 to 32 °C.

Martínez-López *et al. Ecological Processes* (2017) 6:6

Page 5 of 11

Table 1 Abundance and biomass contribution of the three phytoplankton size classes at two sampling stations in Laguna Macapule during cold and warm months

El Tortugón				
	Cold months		Warm months	
Phytoplankton size fraction	Average abundance, $\times 10^6$ cells L^{-1} (SD)	Relative biomass (%)	Average abundance, $\times 10^6$ cells L^{-1} (SD)	Relative biomass (%)
0.2–2 µm	164.26 (207.72)	11.70	374.64 (169.16)	28.11
2–20 µm	7.86 (2.94)	10.20	12.0 (9.05)	0.89
20–200 µm	0.21 (0.11)	78.10	0.64 (0.54)	71.00
Macapule entrance				
0.2–2 µm	84.06 (50.84)	6.54	148.64 (30.48)	7.92
2–20 µm	4.33 (1.85)	4.17	4.66 (3.41)	0.44
20–200 µm	0.96 (0.62)	89.29	0.60 (0.20)	91.62
Total average				
0.2–2 µm	124.16 (151.12)	9.07	261.65 (165.41)	17.98
2–20 µm	6.09 (2.99)	10.19	8.33 (7.57)	0.67
20–200 µm	0.59 (0.58)	78.19	0.62 (0.39)	81.35

Densities of heterotrophic nanoflagellates showed a distinctly different pattern from December through March than the other categories of microorganisms. Their abundance peaked at 7.9×10^6 cells L^{-1} in December, declining afterward through June and then remained low (1.44×10^6 cells L^{-1} and 0.34×10^6) until the end of the study (Fig. 3b). Significant statistical differences (Wilcoxon $Z = 3.04$; $p = 0.002$) in density occurred between sampling stations.
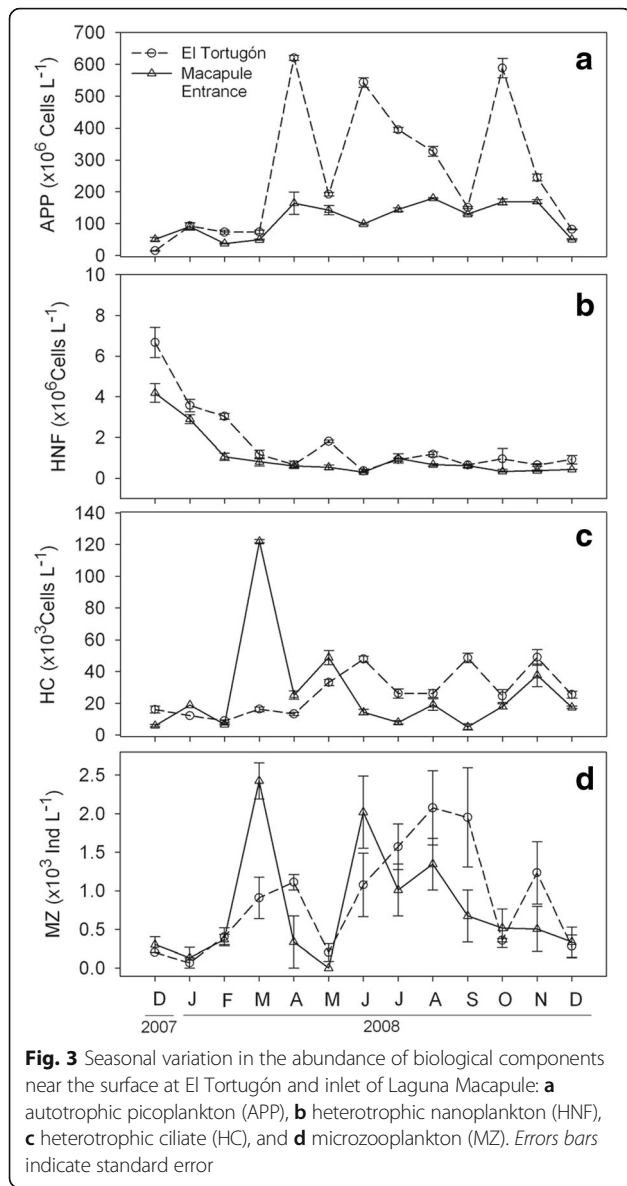
Microzooplankton included small crustacean nauplii, copepods, rotifers, and heterotrophic ciliates (HC). Heterotrophic ciliates, mainly composed of oligotrichs, were the main component of the microzooplankton community. There were no statistical differences between sites (Wilcoxon $Z = 0.87$; $p = 0.38$). Average abundance at both sites was 26.7×10^3 cells L^{-1}. Abundance at the entrance spiked to 120×10^3 cells L^{-1} in March. By June, abundance was less than at El Tortugón and remained low for the rest of the year (Fig. 3c).

The remaining components of the microzooplankton were <12.3% of the total microzooplankton and did not show significant differences between the sites (Wilcoxon $Z = 0.38$; $p = 0.70$). Seasonal densities were lower and followed the same trend during the cold months. In May, their abundance collapsed at both sites, but rebounded in the warm months. Abundance of microzooplankton peaked in June at the entrance, followed by a peak in August and September at El Tortugón (Fig. 3d), which coincided with the highest values of precipitation and relative biomass of autotrophic microplankton (Fig. 4a, b). Rough estimates of APP biomass in Macapule lagoon varied from 2 to 63% of the phytoplankton groups and also showed a similar trend to abundance,

with their highest contribution (>20%) taking place from March to July. Their maximum value was recorded in April at El Tortugón (Fig. 4). A minor participation of APP biomass (<20%) was observed at the Macapule entrance, while ANF exhibited the highest values (0.1–20%) from December 2007 to February 2008 at both sites. The combined data set (Laguna Macapule in Table 2) revealed significant positive relationships between the APP and temperature, DIP ($p < 0.1$; Table 2), and heterotrophic ciliates and microzooplankton ($p < 0.1$; Table 2). The data set from each site only showed positive relationship with SST (Spearman $\rho = 0.59$ and 0.66 for the runoff channel and entrance, respectively; $p = 0.05$) and there was positive correlation with urea at the entrance to the lagoon (Spearman $\rho = 0.78$; $p < 0.05$) and negative correlation with heterotrophic nanoflagellates in the runoff channel (Spearman $\rho = 0.57$; $p < 0.05$).

Discussion

Our results confirmed that APP was numerically the dominant fraction of phytoplankton in this subtropical lagoon, paralleling other studies that have detected this dominance in other subtropical and tropical coastal systems (e.g., Phlips et al. 1999; Agawin et al. 1998; Chang et al. 2003) and in eutrophic environments (Murrell and Caffrey 2005; Cai et al. 2007; Gaulke et al. 2010). Rough estimates of APP biomass in Laguna Macapule varied from 0.9 to 63% of the phytoplankton groups, with its highest contribution (>20%) occurring during March–July; this places AAP as the second largest contributor to the total phytoplankton biomass being surpassed only by the autotrophic microplankton contribution. Estimated abundances levels ($15–620 \times 10^6$ cells L^{-1}) are of

Martínez-López *et al. Ecological Processes* (2017) 6:6

Page 6 of 11

Fig. 3 Seasonal variation in the abundance of biological components near the surface at El Tortugón and inlet of Laguna Macapule: **a** autotrophic picoplankton (APP), **b** heterotrophic nanoplankton (HNF), **c** heterotrophic ciliate (HC), and **d** microzooplankton (MZ). *Errors bars* indicate standard error

Fig. 4 Seasonal variation in **a** precipitation and the relative biomass of the size-fraction phytoplankton groups (autotrophic picoplankton (APP); autotrophic nanoplankton (ANF); and autotrophic microplankton (AM)) near the surface at **b** El Tortugón and **c** inlet of Laguna Macapule

the same order of magnitude as reported values for global abundances of marine cyanobacteria with the maximum value being two times greater than the maximum value reported for open waters (Flombaum et al. 2013) and four times greater than those values reported for open waters in Gulf of California's northern region (Díaz-Hernández and Maske 2000). Considering the global quantitative distribution of *Prochlorococcus* and *Synechococcus*, the most probable scenario is that the latter would be dominant in Laguna Macapule (Flombaum et al. 2013) similar to results from other marine systems (e.g., Palenik et al. 2006; Zwirglmaier et al. 2008; Taylor et al. 2012). Noticeable differences in abundance and biomass among phytoplankton size classes
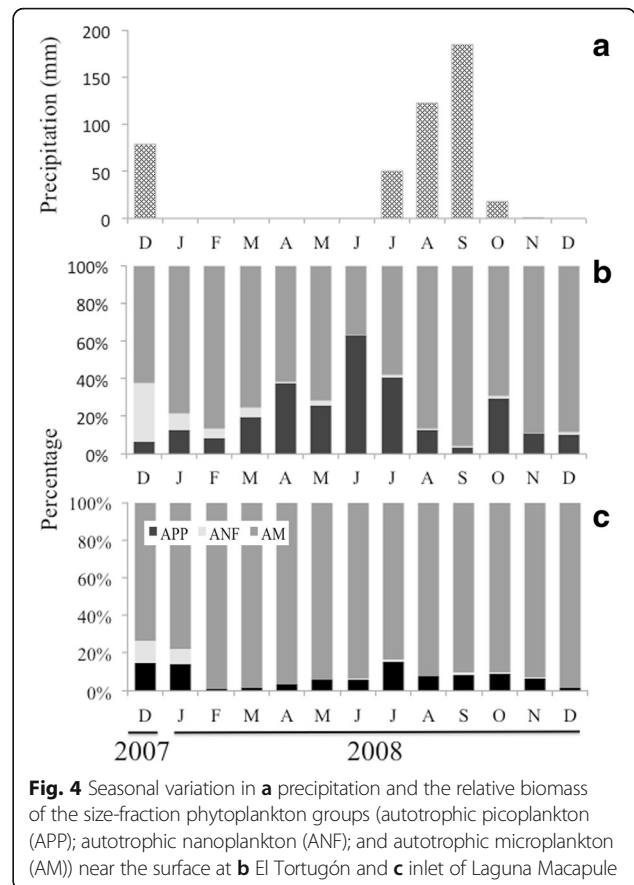
suggest that APP has a competitive advantage over the nanoplankton, in the eutrophic Laguna Macapule, which is opposite to that which was observed in non-impacted lagoons of the Gulf of California (Verdugo-Díaz et al. 2010).

Various studies have proposed diverse mechanisms to account for the success of APP. Some have assigned temperature and grazing (Agawin et al. 1998; Chiang et al. 2002; Chang et al. 2003), while others have assigned nutrient inputs (Murrel and Lores 2004; Barber and Hiscock 2006; Barber 2007) as controlling factors. Our results show that all these mechanisms are important. We found a positive relationship of APP abundance to temperature, DIP (at Laguna Macapule) and urea (at Macapule entrance) and a negative relationship to heterotrophic nanoflagellates (HNF) abundance (Table 2), which suggests that grazing could be a potential regulator. However, the functional relationships are complex and are not readily explained (e.g., the differences between December/February 2007 and December 2008). Thus, a different experimental design is necessary in future studies to address these questions. Moreover, genetic analyses should be useful in discerning complex species compositions and ecological interactions in the communities.

Table 2 Spearman rank correlation coefficients between autotrophic picoplankton (APP), environmental components of temperature, ammonia, dissolved inorganic phosphorus (DIP), N:P ratio, and urea and heterotrophic components of nanoplankton (HNF), ciliates (HC), and microzooplankton (MZ), based on total data (two sampling locations) from Laguna Macapule (n = 26) and each sampling location (n = 13)

Location	Environmental variables					Biological variables		
	SST (°C)	Ammonia (μM)	DIP (μM)	Urea (μM)	N:P ratio	HNF (cells L^{-1})	HC (cells L^{-1})	MZ (ind L^{-1})
Laguna Macapule (combined data set)	0.67*	−0.29	0.38**	0.31	−0.44*	−0.34**	0.40*	0.36**
El Tortugón	0.64*	−0.33	0.33	0.13	−0.44	−0.60*	0.34	0.44
Macapule entrance	0.68*	−0.35	0.24	0.80*	−0.40	−0.42	0.29	0.24

*Significant at $p < 0.05$; **significant at $p < 0.1$

The seasonality of APP, at both sites, presented a similarity in the general tendencies. The natural forcing of SST is the predominant factor in driving seasonal variability of APP in the lagoon. However, even if this dominant driver was discounted, clear differences remained in APP abundance and physicochemical variables between the two sampling stations utilizing a seasonal referential time frame. These differences likely are a consequence of a synergistic mixture of natural and anthropogenic forcing, manifesting in a complex mosaic that is difficult to reduce to independent variables (Bratbak et al. 2011).

Ecologically, many factors contribute to the growth of one species or functional group over another, including biologic interactions. Growth and uptake of nutrients by APP occurred when temperatures were >12 °C, which demonstrates the determinative pressure exerted by SST (Sakamoto and Bryant 1998, Chang et al. 2003; Gaulke et al. 2010). Winter densities of APP were relatively low, despite temperatures above 16 °C and high inorganic nutrient content. This suggests that other variables, such as grazing or nutrient competition with other photoautotrophs, also regulated the abundance of APP at Laguna Macapule. Grazing is consistent with the negative correlation between heterotrophic nanoplankton and abundance of APP. Heterotrophic nanoplankton are the main consumers of heterotrophic prokaryotes (bacteria and archaea) and APP (Rassoulzadegan et al. 1988; Caron et al. 1991; Detmer et al. 1993), but the latter likely are more susceptible to predation due to their larger size (Christaki et al. 1998).

In summer, microzooplankton exhibits a high growth rate in coastal environments (Urrutxurtu et al. 2003; Bojanic et al. 2006; Sakka-Hlaili et al. 2007). In Laguna Macapule, microzooplankton, including ciliates, and abundance of APP followed similar trends. These suggest that APP was not regulated by grazing pressure from this group, which is supported by the positive correlation between most zooplankton components and APP abundance. Thus, it is probable that this pressure influenced the heterotrophic nanoplankton, explaining reduction in their abundance from March until the end

of the year. This is consistent with observations in other systems (Bojanic et al. 2006; Pagano et al. 2006; Toshikazu and Chieko 2007). Interestingly, dominance of heterotrophic ciliate grazers during this period suggests that they are the principal link that transfers microbial biomass to higher trophic levels. Their densities are comparable to maximum numbers found in other eutrophic coastal systems (e.g., Sanders 1987; Montagnes et al. 1988; Xu et al. 2011). However, specific studies on grazing are needed to assess this hypothesis.

Seasonal variability of concentrations of nutrient was multifaceted, with levels of NH_4^+, DIP, and urea having similar patterns. The variety of possible natural and anthropogenic nutrient sources makes it unusually difficult to isolate and determine their independent contributions to water quality. Peak concentrations of NH_4^+ occur during autumn/winter, concurrent with fertilizer input from regional agricultural activity; this previously was reported in nearby coastal lagoons, such as the Topolobampo system (Ayala-Rodríguez 2008) and the San Ignacio–Navachiste–Macapule complex (Escobedo-Urías et al. 1999; Poot-Delgado 2006). These nutrients are transported to the lagoon by runoff drains (Magaña-Álvarez 2004; Poot-Delgado 2006; Martínez-López et al. 2007; Escobedo-Urías 2010) and by atmospheric transport (Escobedo-Urías 2010). Analysis results of NH_4^+ data revealed an unexpected lack of statistical correlation between APP and ammonia. Absence of correlation does not exclude the possibility of efficient use by APP that was not revealed due to data limitations imposed by a monthly sampling regime. Additionally, during autumn/winter, entrainment of enriched-nutrient water from coastal upwelling occurs (Escobedo-Urías et al. 2007). Thus, high concentrations of nitrate, concurrent with low concentrations of SiO_4 and high abundance of diatoms (up to 1.4×10^6 cell L^{-1}) were recorded at the inlet to Laguna Macapule (Hakspiel-Segura 2009), which suggests that upwelling events influenced conditions inside the lagoon. Year-to-year climate variability related to El Niño–La Niña events can affect this region. During the 2008 La Niña event, stronger northwest winds intensified coastal upwelling off Laguna Macapule, augmenting the

Martínez-López et al. Ecological Processes (2017) 6:6

Page 8 of 11

potentially favorable situation for the incursion of nutrient-rich water into the lagoon system (Martínez-López et al. 2008). Under these conditions, diatoms may represent an efficient competitive vector for nutrients, acting (in concert with grazing) to limit the APP.

Abundances of APP began to increase in April at both sampling stations (temperatures >22 °C). At the El Tortugón site, APP was about three times higher than the highest abundances at the entrance, suggesting a response to maximum nutrient loading related to agricultural activity during this period (Escobedo-Urías 2010). During the summer rainy season, a twofold APP decline was noted simultaneously with a diatom increase (Hakspiel-Segura 2009). The decline of APP and an increase of diatoms observed at El Tortugón suggest that episodic nutrient input provides diatoms with a nutrient uptake advantage. This agrees with observations in similar ecosystems, where shifts from picoplankton to diatoms prevail in phytoplankton communities after an influx of nutrients (Šilović et al. 2012). Observation of the effects of heavy rainfall and subsequent runoff, with resultant nutrient input, utilized by fast-growing diatoms in domination over other phytoplankton groups (Martínez-López et al. 2007) support this concept.

Levels of DIP in the lagoon did not show the same seasonal trend reported in 2002 and 2005 (Magaña-Álvarez 2004; Poot-Delgado 2006), when the highest concentrations were recorded during the autumn/winter season or were associated with the summer rainy season. The peak of DIP at the entrance of Laguna Macapule preceded that at El Tortugón before the rainy season, suggesting that diatom blooms controlled phosphorus levels, subsequent input of organic matter derived from the diatoms, and fast regeneration of phosphorus compounds.

A similar situation was observed during an intense bloom of the diatom Asterionella glacialis off the coast of Kalpakkam, Tamil Nadu, India, with decrease in nitrate and silicate and increase in phosphate (Satpathy and Nair 1996). Higher DIP during warming of the water column, related to a mineralization process, is common in estuaries and shallow coastal lagoons (Morris et al. 1981; Pant and Reddy 2001; van der Zee and Chou 2004).

The phosphorus peak that occurred at the mouth of El Tortugón channel may be related to its shallow depth. Rapid mineralization of phosphorus in the sediment could account for the magnitude of the peak in June. Strong winds in June likely lifted phosphorus from the bottom of the water column, reflected in a reduced Secchi depth and in positive correlation with phosphorous (Spearman; $p < 0.05$) (Fig. 5). Lower N:P ratios (Hakspiel-Segura 2009) in May than June support this. Intense reducing conditions often develop in sediments during warm months, as well as enhanced phosphatase activity in the water column (Kobori and Taga 1979; Hoppe 2003) when temperature and microbial activity are higher (Lai and Lam 2008). This in turn increased the content of DIP, stimulating APP growth rates (Timmermans et al. 2005). Based on this pattern, we suggest that the subsequent rapid decline in DIP in Laguna Macapule came from uptake by APP and small phytoplankton that proliferate in the warm months (Poot-Delgado 2006).

Whether APP has a preference for a specific type of nitrogen is not clear. Harrison et al. (1996) found that picophytoplankton prefer NH_4^+, whereas Glover et al. (1988) and Bird and Wyman (2003) demonstrated that they will consume nitrate and nitrite in the absence of other nitrogen sources. Other studies showed APP
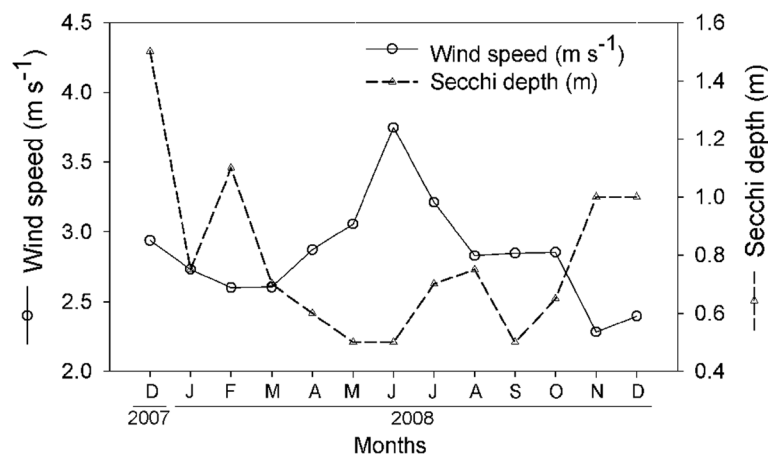

Fig. 5 Monthly average wind speed data from the meteorological stations at El Tortugón and CONAGUA and transparency depth in Laguna Macapule from December 2007 through December 2008

Martínez-López *et al. Ecological Processes* (2017) 6:6

Page 9 of 11

uptake of nitrogen and phosphorus from a variety of inorganic and organic compounds (Bjorkman and Karl 1994; Zubkov et al. 2003). Therefore, when NH_4^+ is insufficient to meet N demand, APP utilizes urea as an organic nitrogen source after internal biological hydrolyzation to NH_4^+ using urease (Berns et al. 1966; Collier et al. 1999). This is more readily accomplished than uptake of oxidized forms of nitrogen (Solomon et al. 2010 and references therein). In Laguna Macapule, urea was more prevalent than dissolved inorganic forms of nitrogen during spring-early autumn, suggesting that it served as a primary source of nitrogen when inorganic nitrogen was limited (Poot-Delgado 2006). It seems clear that urea may be an important player in primary production of this highly impacted lagoon, under limited inorganic nitrogen conditions.

Spatial differences in urea distribution defined that APP was positively correlated with urea only at the entrance to the lagoon (Spearman; $p < 0.05$). At this site during spring-early autumn, higher concentrations of urea also correlated with higher abundances of microzooplankton. This indicates that zooplankton excretion was the urea source (L'Helguen et al. 2005). However, urea generally is present in marine waters at levels below 1 μM-N (Solomon et al. 2010), which is substantially lower than observed (up to 35 μM-N), suggesting that this organic nitrogen derives from an external source and needs to be accounted for with seasonal variability of nutrient concentrations.

Urea concentrations at Laguna Macapule were comparable to other coastal systems where agricultural (Glibert et al. 2006) or other organic impacts are present (Gutiérrez-Mendieta et al. 1998). Higher values from March through October point to effluent discharge from shrimp farms. Urea is used as a nitrogen fertilizer (Lyle-Fritch et al. 2006) to stimulate plankton production in the ponds, as well as being produced by shrimp excretion. Water exchange is a dominant factor in shrimp production from March through October, when discharge from >5000 ha of ponds (Páez-Osuna et al. 2007) inputs large volumes of shrimp excretion and feed waste into the ecosystem (Páez-Osuna et al. 2003). Other sources could be municipal wastes from the city of Guasave (~80,000 inhabitants) or agriculture where urea-based herbicides or fertilizers are used. Shrimp farms occur along the margin of the lagoon, and the effluent enters the lagoon through several drains. However, the channel at El Tortugón contains only a limited discharge related to aquaculture activities. Thus, lagoon's internal circulation predominantly concentrates and transports urea toward the lagoon inlet (Escobedo-Urías com. pers.) where lower abundances of APP are present in comparison to El Tortugón, explaining the strong presence of urea at the entrance of the lagoon. This situation is contrary to the original supposition that this site was less impacted.

The paradigm of inorganic N being the limiting nutrient in marine ecosystems apparently is not applicable in the Laguna Macapule ecosystem since significantly negative correlation was observed between APP and N:P (Spearman; $p < 0.05$). This could be explained by their relatively high affinity to low NID concentrations (Agawin et al. 2000) or more likely because it appears that the APP are using organic nitrogen to overcome limiting conditions (N:P = ~4) under dissolved inorganic nitrogen concentrations under 1.5 μM. Here, urea seems to be, at least for APP, a crucial nitrogen source. Urea is one of the regenerated forms of nitrogen, thus nutrient availability (internal biogeochemical rates), not concentration, is more relevant to limitations of net ecosystem production (Smith 1984). Further investigation is needed to assess the relative importance of biogeochemical rates and biological interactions in this impacted lagoon. From the perspective of this study, our results suggest, in agreement with other research teams (Souchu et al. 2010), that a total nutrient-based approach including dissolved organic nitrogen likely constitutes the most appropriate approach for future studies in this region and similar ones around the world.

Conclusions

Autotrophic picoplankton were described and quantified over a complete annual cycle for the first time in the Gulf of California. APP was the numerically dominant and the second greatest contributor to biomass of the phytoplankton fractions throughout the year, reaching peak levels in spring–early autumn (up to ~0.6×10^9 cells L^{-1}). The interplay between natural (temperature and grazing) and anthropogenic forcing's contributed to delineate the seasonality of APP, thereby promoting high densities in the spring–early autumn. The shift to low abundances of the APP may also result from zooplankton grazing or nutrient competition. Therefore, microphytoplankton blooms occur in winter when they outcompete APP for nutrient resources. Statistical relationships between APP and small-size zooplankton groups suggest that a considerable part of the pool of organic matter is retained in the microbial loop, instead of passing through to the productive herbivorous trophic web. Positive relationship with urea (bottom-up control) from aquaculture appears to contribute to maintaining high densities of APP in spring–early autumn. Increase in abundance of APP with a N:P = ~4 supports that urea is the sustaining nutrient source for this small size autotrophic component. Thus, a total nutrient-based approach likely constitutes the most suitable tool for establishing nitrogen limitation of biological production in this ecosystem as well as for improving criteria for management or restoration. Further investigation is needed to assess the relative importance of biogeochemical rates and biological interactions in this impacted lagoon.

Authors' contributions
AML was involved in the framing hypotheses and experimental design, data interpretation, and manuscript preparation. CHS participated in the design of the study and carried out laboratory quantifications of microorganisms as well as data analysis and participated in the draft manuscript preparation. DEU carried out the fieldwork of the study and laboratory nutrients analysis, and BGA participated in the laboratory quantifications of microorganisms; both helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Centro Interdisciplinario de Ciencias Marinas-IPN (CICIMAR), Av. IPN s/n, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23096, Mexico. [2]Centro de Interdisciplinario de Investigacion para el Desarrollo Integral Regional, (CIIDIR–Unidad Sinaloa), Bulevar Juan de Dios Bátiz Paredes 250, Col. San Joachin, Guasave, Sinaloa 81101, Mexico.

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Martínez-López *et al. Ecological Processes* (2017) 6:6

Page 11 of 11

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