Seasonal changes of the microplankton community along a tropical estuary

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Abstract

Microplankton organisms are an important link in the transfer of matter and energy between the benthic-pelagic microbial food web and higher trophic levels in estuaries. Although tropical estuaries are among the most productive aquatic systems globally, information on the spatial and seasonal dynamics of microplankton in such systems is scarce. In order to identify which variables control microplankton abundance and community structure a number of environmental variables were measured along the tropical Gulf of Nicoya (Costa Rica) during the rainy and dry seasons (2011-2012). The Tempisque River was a major source of nutrients and turbidity and thus imposing a clear gradient along the estuary. Chlorophyll *a* (chl *a*) concentration was highest in the middle of the estuary (2.7-20 mg m⁻³), where turbidity decreased. The microplankton comprised mainly diatoms (88%) and dinoflagellates (8%). Multivariate analysis revealed five different microplankton assemblages associated with a seasonal and riverine-marine gradient, and supporting an ecotone model at the estuary head that shifts to an ecocline model for the rest of the estuary. Our results suggest that primary producers in the estuary were mainly limited by light rather than nutrients.

Keywords: Phytoplankton, biogeochemistry, tropical estuary, salinity, turbidity, diversity.

1. Introduction

Estuaries are among the most productive aquatic systems. These areas are important resources for the human population, being sometimes their only source of income (i.e. fishing). Estuaries are highly dynamic systems, undergoing from daily tidal changes to seasonal changes. This variability in time and space makes estuaries highly heterogeneous and complex ecosystems able to sustain high biodiversity levels (Attrill and Rundle 2002, Muylaert *et al.* 2009). Phytoplankton is a key constituent of estuaries, being a primary producer that highly influences water quality (i.e. eutrophication). In estuaries, seasonal phytoplankton dynamics are mainly determined by large variations in irradiance, temperature, nutrients, and river runoff (Hitchcock and Smayda 1977, Cloern 1987, Alpine and Cloern 1988, Underwood and Kromkamp 1999, Eyre and Balls 1999). In general, tropical estuaries are characterized by higher temperature and irradiance levels year round, and larger variations in river runoff, than temperate estuaries (Nittrouer *et al.* 1995, Eyre and Balls 1999). These characteristics lead to high primary productivity rates, with some tropical estuaries being among the most productive aquatic systems globally (Cloern *et al.* 2013). Despite the importance of tropical estuaries in terms of primary production, current information on the structure, abundance and ecology of the phytoplankton and the factors driving its dynamics in such systems is scarce compared to that for temperate ones (Costa *et al.* 2009, Cloern and Jassby 2010, Cloern *et al.* 2013).

Microplankton are a critical key in the pelagic community of aquatic ecosystems. Microphotoautotrophic primary production is readily transferred to large zooplankton and small fishes (Azam *et al.* 1983, Costalago *et al.* 2012), whereas microzooplankton not only graze on microphytoplankton and autotrophic and heterotrophic nanoplankton, but also act as prey to higher trophic levels (Azam *et al.* 1983, Di Poi *et al.* 2013). Therefore, microplankton integrate the matter and energy flowing through different pathways along the microbial food web and channels it to higher trophic levels (Calbet and Landry 2004, Calbet and Saiz 2005). Microplankton in estuaries also play a major role in benthic-pelagic coupling through consumption by bivalves and other benthic filter feeders (Officer *et al.* 1982, Werner and Hollibaugh 1993, Greene *et al.* 2011). In addition, benthic diatoms are frequently resuspended

to the water column, thus contributing to pelagic primary production (Shaffer and Sullivan 1988, Jonge and Beusekom 1995). The increasing human pressure on tropical estuaries over the last decades has had a major impact on their ecology and their ecosystem services (Bianchi 2007, Barbier *et al.* 2011, Blaber 2013). Changes in environmental variables due to climate change can also affect phytoplankton communities' structure, zooplankton and fish (Vezzulli *et al.* 2012). Therefore, understanding seasonal variability and which variables influence the structure and function of microplankton is necessary to be able to predict their future response to anthropogenic and climate changes.

The Gulf of Nicoya, located in the Pacific Coast of Costa Rica, is a highly productive estuarine system (Peterson 1960, Gocke *et al.* 2001, Cloern *et al.* 2013) supporting important fisheries, particularly in its inner basin (Vargas 1995, Palter *et al.* 2007). The hydrographic structure and dynamics of the water column in the inner basin of Nicoya Gulf are strongly influenced by seasonal changes in freshwater flow from the Tempisque River (Lizano 1998, Brenes *et al.* 2001). These seasonal changes in the river runoff are likely to affect the biological community and the flow of matter and energy in the estuarine ecosystem, including water column productivity and phytoplankton taxonomic composition (Sin *et al.* 2000, Smayda and Reynolds 2003, Costa *et al.* 2009, Cloern *et al.* 2013). Changes in the species that dominate the estuary can lead to harmful algal blooms, such as those already reported in the Gulf of Nicoya (Hargraves and Vázquez 1995, Vargas and Freer 2004, Vargas *et al.* 2008). In the inner basin of Nicoya Gulf, important changes in inorganic nutrients, oxygen, and chl *a* concentrations have been reported along the river - sea gradient (Kress *et al.* 2002, Palter *et al.* 2007). However, similar to other tropical estuaries, there is scarce information on how the different components of the planktonic community respond to seasonal changes in the environmental conditions (Cloern and Jassby 2010, Cloern *et al.* 2013). Previous studies in the Gulf of Nicoya provided a general taxonomic description of the microphytoplankton community, showing that the community composition differs from that in other areas of the Pacific Central America coast (Hargraves and Viquez 1981, 1985). Changes in phytoplankton taxonomic composition together with the physicochemical properties and chl *a* concentrations, have been studied in only a single

point in the inner gulf (Brugnoli-Olivera and Morales-Ramírez (1999, 2001, and 2008). Therefore, as yet, no single study has considered all the environmental variables, phytoplankton abundance and taxonomy during the two tropical seasons along the inner Gulf, and therefore established the possible drivers of the tropical estuarine phytoplankton composition. In addition, understanding spatial and seasonal dynamics of microplankton in tropical and subtropical estuaries is essential to evaluate the contribution of estuaries to global biogeochemical cycles; however, such studies are largely biased toward temperate European and North American estuaries, with warmer estuaries being largely under-represented (Cloern and Jassby 2010, Cloern *et al.* 2013).

Here we study the abundance and composition of the microplankton community in the inner part of a tropical estuary, the Gulf of Nicoya, where changes in the main river runoff was expected to be the greatest influence. We hypothesized that there is a seasonal shift of the microplankton community and question whether it fits an ecotone or ecocline model along the river-sea gradient. We examine patterns of variability and potential environmental factors influencing microplankton abundance and species composition in tropical estuaries as model of gradient aquatic ecosystems. The results provide new insight into the relationship between species and environmental variables in tropical estuaries in order to identify management strategies that protect these important coastal ecosystems.

2. Material and methods

2.1. Study area

The Gulf of Nicoya is a mid-sized tropical estuary located in the northwest Pacific coast of Costa Rica (10°N 85°W) (Fig 1). The estuary is 80 km long from the mouth of the Tempisque River to the Pacific Ocean and 50 km wide at its southernmost extension, having a total surface area of 1543 km². Based on its bathymetry, the gulf can be divided into two regions: the inner gulf, from the river mouth to the Puntarenas Channel, with a maximum water depth of ~20 m, and the external gulf, with depths down to ~200 m (Fig. 1). Sand and rocky cliffs border the external gulf, whereas mangroves border the inner gulf (Voorhis *et al.* 1983, Palter *et al.* 2007).

Tides are semidiurnal with mean amplitude of 2.28 m (Lizano 2006) and the gulf is classified as a mesotidal estuary (Monbet 1992). Freshwater inputs from the Tempisque River produce intense salinity gradients in the inner gulf; mean discharge rates range between 333.3 and 157.9 m³ s⁻¹ during the rainy (May - November) and dry seasons (December - April), respectively (Kress *et al.* 2002). The mean values of the sampled months were inside normal historical conditions.

2.2. Sampling and sample processing

Samples were collected in late July 2011, during the rainy season, and in mid April 2012, during the dry season. Five sampling stations were selected along the inner gulf, starting from the mouth of the estuary (Station 1) to the middle of the channel, between Isla Caballo and the eastern coast (Station 5) (Fig. 1). Both sampling cruises started from Station 1 at high tide and followed the ebb tide flow to avoid biases due to the tidal cycle. Temperature, salinity and turbidity were measured along the entire water column using a YSI 6600 multiparameter sonde. Quantum Scalar Irradiance (QSI) was measured with a LI-COR 250A radiometer equipped with a LI-193 Underwater Spherical Quantum Photosynthetic Active Radiation (PAR) sensor. The depth of the euphotic layer (1% of incident light) and the coefficient of light extinction were calculated from the model of Beer-Lambert.

Water samples were collected with a 4.2 L Van Dorn bottle from the surface layer (0 - 1 m depth) and one meter under the halocline in the rainy season or one meter above the bottom in the dry season, when the water column was mixed. Water samples for chl *a* analysis and nutrients, ammonium (NH₄⁺), nitrite (NO₂⁻), nitrate (NO₃⁻), silicate (SiO₄⁴⁻) and phosphate (PO₄³⁻) were stored in polyethylene bottles until further processing at the Punta Morales Marine Station (National University of Costa Rica). For chl *a* extraction, a known volume of water was filtered through Whatman GF/F filters. Filters were placed in tubes with 100% methanol (buffered with MgCO₃) to extract pigments overnight at 4°C in darkness. Tubes were then centrifuged and the absorption spectrum (400-800nm) of the extract was measured on a Shimadzu UV-1700 spectrophotometer. Pigments concentration was estimated according to

Ritchie (2008). Nutrient samples were filtered through Whatman GF/F (0.7 μ m pore size) filters and stored frozen at -20°C until analysed. Nutrient concentration was determined on a ZUZI 4211/50 spectrophotometer following standard protocols: nitrite and nitrate (Garcia-Robledo *et al.* 2014), ammonium (Bower and Holm-Hansen 1980), and silicate and phosphate (Grasshoff 1999). For the determination of CDOM, samples were filtered *in situ* through double filters, a Whatman GF/F pre-filter and a Millipore 0.2 μ m pore size cellulose acetate filter, in combusted glass vials. Absorbance was measured on a Shimadzu UV - 1700 spectrophotometer with a 10 cm quartz cell in the spectral range 400 - 700 nm. The absorption at 440 nm was used as a measure of CDOM concentration and the spectral region 400 - 440 nm for the calculation of the CDOM slope. All samples were kept on ice during sampling, for no more than six hours until arrived to the lab.

To ensure the presence of large cells and minimize the sampling bias, water samples (4 – 8L) for microplankton identification and abundance were filtered through a 10 μ m mesh, extracted by spraying the mesh carefully with pressurized water and kept in amber bottles with formalin 4%. Species identification and counting was made using an inverted microscope (Nikon Eclipse Ti-U) following the technique of Utermöhl (1958); each sample was randomly resampled to reach at least a total of 300 individuals counted per sample, thus assuming a maximum error of 11,5% (Lund *et al.* 1958). The taxonomic identification was based mainly on Peragallo (1897-1908), Hustedt (1930), Lebour (1930), Cupp (1943), Dodge (1985), Tracana (1985), Ricard (1987), Round (1990), Tomas (1997), Hoppenrath (2009), Larink (2006).

2.3. Satellite imagery

Satellite imagery is a powerful tool that provide a quasi-synoptic measurements of chl *a* and sea surface temperature (SST). Here we processed monthly climatology images from 2002 to 2011, with a spatial resolution of 4x4 km, to produce climatological seasonal images. This allows us to assess, which are the regional conditions and evaluate if the sampling period corresponded to standard conditions of the Gulf.

The images used came from the Aqua-MODIS satellite were downloaded from the server (<http://oceancolor.gsfc.nasa.gov>) and processed using Matlab toolbox hdfreader and projected to Mercator scale by the toolbox M_Map written by Pawlowicz (<http://www.eos.ubc.ca/~rich/map.html>).

2.4. Statistical analysis

A Bray-Curtis similarity matrix of square root transformed phytoplankton abundance was first calculated, followed by a cluster analysis based on group averages. Significant groups at a $p < 0.05$ level were determined by the SIMPROF routine. Then a non-metric multidimensional scaling (nMDS) ordination plot was constructed using the same matrix. SIMPROF groups were indicated on the same plot. Spearman correlations between the environmental variables and the axes of the phytoplankton community ordination plot were drawn. Multivariate analyses were performed using the statistical package PRIMER 6 (Plymouth Marine Laboratory, UK).

To obtain the phytoplankton variability along the sampling site the Shannon diversity index was calculated according to Shannon and Weaver (1963) using the following equation: $H = -\sum_{i=1}^S p_i \ln(p_i)$. Diversity values for the groups obtained from the SIMPROF routine during cluster analysis are also shown.

Biomass of microplankton was calculated for the surface water averaged along the five stations in both seasons. Abundance data was first transformed to microplankton biovolume from mean size of most abundant taxa (Hillebrand *et al.*, 1999, López-Fuerte and Siqueiros-Beltrones 2006) and then to biomass ($\mu\text{g C L}^{-1}$) using conversion factors (Eppley *et al.* 1970, Leblanc *et al.* 2012).

3. Results

3.1. Characterization of study area by remote sensing

Analysis of remote sensing images for SST and chl *a* registered over the last 10 years and averaged for the dry and the rainy seasons were processed to obtain the typical conditions of the Gulf with respect to the rest of the Pacific coast of Costa Rica (Fig. 2). During the rainy season, the inner gulf is warmer than adjacent oceanic and coastal areas (Fig. 2a), whereas during the dry season it tends to be cooler than the southwest coast of Costa Rica (Fig. 2b). Waters inside the estuary have a difference of $\sim 1.5^{\circ}\text{C}$ between seasons. Chl *a* concentration in the Gulf of Nicoya was about five times higher than in any adjacent coastal and oceanic areas at all times despite evident differences in SST between seasons (Fig. 2c, d). Mean chl *a* values in the rainy season reached $\sim 18 \text{ mg m}^{-3}$. These images confirm the high and constant productivity of this tropical estuary compared with the rest of the Costa Rican coast.

3.2. Environmental variables in the inner Gulf of Nicoya

A clear salinity gradient was present along the studied transect during both seasons, being slightly steeper during the rainy season (Fig. 3a, b). In addition, during the rainy season, a halocline developed between 1-2 m depth at all sampling stations, being more pronounced in the middle of the estuary (Fig. 3a). No halocline was observed during the dry season (Fig. 3b). The information from rainfall stations in the basin of the Tempisque river indicate that rainfall during the sampling months was $\sim 200 \text{ mm}$ in rainy season, $\sim 100 \text{ mm}$ in dry season (INM 2009). These values are within the normal range in the area. Temperature patterns were similar in both seasons, reaching maximum values at the surface of intermediate stations in the rainy season, where higher thermal stratification was observed (Fig 3c).

Turbidity decreased towards the estuary mouth in both seasons (Fig. 4). However, the strongly mixed water column in the dry season was more turbid throughout the entire estuary compared to the rainy season, especially in subsurface layers, with the highest values being observed in Station 1 (Fig. 4). In accordance to this, the depth of the euphotic layer (1% of incident light) increased from Station 1 ($\sim 1 \text{ m}$ in both seasons) to Station 5 (12 m and 4.5 m, in rainy and dry seasons, respectively), being deeper than the halocline during the stratified rainy season (Fig. 3a). Whereas turbidity is the measurement of attenuation of light due to particulate

matter, CDOM is also responsible of the depth of the euphotic layer because reduces the light intensity and absorbs from ultraviolet to blue lights. According to turbidity, CDOM decreased from the riverine stations seawards, but interesting, was higher during the rainy season than in the dry season (Fig. 4).

Strong nutrient concentration gradients existed for NO_3^- , PO_4^{3-} and SiO_4^{4-} along the estuary in both seasons (Fig. 5). Concentration of NO_3^- decreased from 18.5 μM (rainy) and 33.2 μM (dry) at the estuary head to less than 7.4 μM on average at the marine end. No significant differences were found between surface and bottom water samples, especially in the dry season; however, a depletion of surface NO_3^- at the marine Stations 4 and 5 in the rainy season was evident. The NO_3^- versus salinity plot shows a non-conservative decrease of NO_3^- from Station 2 in the dry season and from Station 3 in the rainy season. PO_4^{3-} concentration showed a similar pattern to NO_3^- , decreasing from 5.6 μM (rainy) and 4.8 μM (dry) at the river to 1.5 μM at Station 5. Differences with depth were also generally small, with the exception of Station 1 during the rainy season. PO_4^{3-} concentrations remained constant during the dry season up to Station 2 over a large range of salinities (~ 10 to > 30), decreasing strongly and non-conservatively towards the sea. However, PO_4^{3-} changes with salinity were more conservative in the rainy station. NO_2^- concentrations changed non-conservatively along the estuary with highest values ($\sim 3 \mu\text{M}$) in the middle of estuary at intermediate salinities in both seasons. SiO_4^{4-} concentrations were very high ($> 1000 \mu\text{M}$) near the river, decreasing conservatively according to salinity along the estuary to concentrations lower than 200 μM at Station 5. NH_4^+ concentrations were generally low ($\sim 2 \mu\text{M}$) in both seasons, especially for the dry season. NH_4^+ concentrations in bottom waters decreased from the estuary head to the estuary mouth during the rainy season and the inverse trend occurred during the dry season. Although no clear spatial trend was observed for surface NH_4^+ concentrations.

Overall, when considering the seasons, concentrations of NO_x and PO_4^{3-} were higher during the dry season, whereas the opposite was true for SiO_4^{4-} .

3.3. Chl *a* and microplankton abundance

Chl *a* spatial patterns and concentration levels differed between seasons (Fig. 6). During the rainy season, chl *a* concentrations in the bottom layer was nearly constant ($\approx 5 \text{ mg m}^{-3}$), whereas in the surface layer it was highest at the intermediate stations ($\sim 20 \text{ mg m}^{-3}$) decreasing toward the sea. In the dry season, chl *a* concentrations in the surface and bottom layers showed inverse patterns along the estuary; concentrations increased from the river toward the estuary mouth in the surface layer, whereas in the bottom water, chl *a* maxima occurred in the shallower riverine and intermediate stations (Stations 1 - 3).

Total abundance of microplankton in the Gulf was between 25-1017 ind mL^{-1} . During the rainy season, abundance in both surface and bottom layers increased from river to sea, following the opposite trend as chl *a* from the middle of the estuary toward the sea (Fig. 6). In contrast, during the dry season, total abundance followed the trend observed for chl *a*; abundance in the bottom layer decreased from the estuary head to the estuary mouth, and increased in the surface. Disagreements between chl *a* and microphytoplankton abundance trends in the surface during the rainy season are likely due to the lesser abundance of microphytoplankton (9.4% of total biomass), compared to the almost double microphytoplankton representation in the dry season (Table 2).

3.4. Microplankton diversity and taxonomic groups

In total 146 microplanktonic taxa were identified (>95% to species level) in the inner basin of the Gulf of Nicoya over the seasons (Online Resource A). Shannon diversity index (*H*) for microplankton changed between stations and ranged from 1.70 to 2.90 (Table 1). Diversity index values obtained during the rainy season were generally higher than those obtained in the dry season. In the rainy season, diversity index did not show any clear trend along the estuary or with depth; during the dry season, on the other hand, diversity values were generally higher in surface water and towards the marine stations.

Diatoms were the dominant group of microplankton in both seasons (73% of total abundance for centric diatoms and 15% for pennate diatoms). The second most abundant group was dinoflagellates (7.5-8.5%). Other groups such as tintinnids, Chlorophyta, ciliates,

Haptophyta, Cyanobacteria, and Euglenophyta, represented all together less than 3% of the total abundance (Fig. 7).

Centric diatoms abundance was one order of magnitude higher than that of pennate diatoms in estuary head (898 ind mL⁻¹ and 90 ind mL⁻¹, respectively). The centric diatoms *Cerataulina dentata* and *Thalassiosira* spp. were the most abundant taxa during the rainy season and *Cyclotella* spp. during the dry season. The pennates *Thalassionema nitzschioides* and *Cylindrotheca closterium* were the species with the highest abundance during both seasons.

Dinoflagellates were more abundant in the surface layer, increasing toward the sea in both seasons and reaching 165 ind mL⁻¹ in Station 5 during dry season. The heterotrophic *Protoperidinium* spp. (16% of dinoflagellates) and autotrophic *Prorocentrum minimum* (61% of dinoflagellates) were the most abundant species in rainy and dry season, respectively.

Chlorophytes abundance was low, with the highest abundance observed at the estuary head (5 ind mL⁻¹). The freshwater genus *Scenedesmus* spp. was the most abundant genus in both seasons. Tintinnids were patchily present and only in low abundances (>8 ind mL⁻¹). *Codonellopsis* spp. was the most abundant genus. From the rest of the groups mentioned above, the most representative were the ciliate *Strobilidium* spp. (7 ind mL⁻¹) and flagellate *Ebria tripartita* (7 ind mL⁻¹), both during the rainy season (Online Resource B).

3.5. Microplankton assemblages and their relationship with environmental variables

The nMDS ordination obtained from the microplankton community data showed a clear spatial gradient on the horizontal axis separating the stations from the estuary head (Station 1) to the estuary mouth (Station 5) (Fig. 8A). On the vertical axis, stations from the rainy and dry seasons were also clearly separated. SIMPROF analysis of the group averages dendrogram plot identified five significant groups or assemblages in the microplankton community (DI, DII, RI, RII, RIII). DI includes the surface and bottom samples in Station 1 during dry season. DII consists of the remaining samples along the estuary during dry season. Samples at Station 1 during the rainy season are named RI, whereas RII comprises surface samples at Stations 2 and 3 during the rainy season. Finally, RIII includes the remaining rainy season samples, namely

surface layer samples at Stations 4 and 5, and bottom layer samples from Station 2 to Station 5 (see Online Resource B for detailed taxonomic composition of groups). According with the geographical separation obtained with the nMDS, the Shannon diversity index showed the lowest H values in the estuary head groups (DI & RI) as well as seasonal differences with the highest diversity in the rainy season.

High correlations of environmental variables were found with the axes of the ordination plot. Turbidity, NO_3^- and PO_4^{3-} concentrations showed high correlation ($p > 0.7$) with the first axis, the horizontal one, separating the inner most station from outer-marine stations. A lower correlation ($p > 0.5$) was shown by CDOM slope, CDOM concentration and SiO_4^{4-} . With respect to the second axis, which separates samples for rainy and dry seasons, high correlations ($p > 0.5$) were found with salinity, SiO_4^{4-} and CDOM (Fig. 8a).

The taxa that showed the highest correlations with the horizontal axis of the ordination plot were *Scenedesmus opoliensis* associated to riverine waters, especially in the rainy season, and *Chaetoceros curvisetus* and *Cerataulina dentata*, which increased toward marine stations in the rainy season. The species that showed the largest variations between seasons, *Cyclotella* spp., *Thalassiosira* spp., and *Thalassionema nitzschioides*, were more representative during the dry season, whereas the opposite was true for *Protoperidinium pallidum* (Fig. 8b).

4. Discussion

4.1. The importance of the Tempisque River

The Tempisque River discharges a large volume of fresh and relatively warm water to the inner gulf, approximately 2 times higher in the rainy season than in the dry season (Kress *et al.* 2002). The seasonal difference in river runoff is a major driver of change in the water column's physical structure of tropical estuaries, with wide implications for phytoplankton abundance and primary production (Ram *et al.* 2003 Costa *et al.* 2009, Burford *et al.* 2012). In the dry season, when the fresh water flow is lower, tidal currents mix the water column (well-mixed estuary phase). In contrast, during the rainy season, tidal mixing was insufficient to fully

mix the water column, shifting the estuarine physical dynamics to a phase of partially stratified estuary, as found previously here (Brenes *et al.* 2001, Lizano 2006) and in other tropical estuaries (Eyre 1998, Eyre and Balls 1999, Boonphakdee and Fujiwara 2008).

The Tempisque River discharges a large amount of particulate and dissolved organic and inorganic matter to the estuary. Intense longitudinal gradients of turbidity, CDOM and nutrients were observed from the riverine to the marine end of the inner estuary. Turbidity was highest in the dry season, contrary to what has been observed in other estuaries (Cravo *et al.* 2006, Lund-Hansen *et al.* 2010), while CDOM showed the inverse seasonal variation, being higher in the rainy season. CDOM can increase due to the river discharge and resuspension of bottom sediments. Here, the higher values during the rainy season confirm the hypothesis that the influence of the river prevails over the tidal force. Although the total amount of CDOM decreased seawards, the slope of CDOM absorption spectra increased (results not shown) showing a progressive decrease of the more refractory allochthonous component from terrestrial and mangrove origin along the estuary (Stedmon and Markager 2003, Galgani *et al.* 2011). Higher concentration of CDOM during rainy season has been reported in other subtropical and tropical estuaries as well (Gallegos 2005, Lund-Hansen *et al.* 2010), although there are reports where no clear seasonal differences were found (Maie *et al.* 2006). Overall, concentrations of CDOM reported here are higher than those reported in estuaries from different latitudes (Lund-Hansen 2004, Lund-Hansen *et al.* 2010, Maie *et al.* 2006). These spatial patterns and their seasonal changes reinforce the importance of the Tempisque River as a source of allochthonous particulate and dissolved matter for the Gulf of Nicoya.

The most abundant inorganic nutrients concentrations, namely NO_3^- , PO_4^{3-} and SiO_4^{4-} , decreased downstream suggesting that the Tempisque River was an important source of inorganic nutrients in both seasons. NO_3^- and PO_4^{3-} were lower during the rainy season, suggesting a dilution by precipitation of the last two nutrients. At the same time, rain would increase the SiO_4^{4-} input from stronger runoff effect during the rainy season. However, the range of NO_3^- , PO_4^{3-} and SiO_4^{4-} concentrations measured in this study at the most riverine stations

were higher than concentrations reported previously for the Gulf of Nicoya (Kress *et al.* 2002, Brugnoli-Olivera and Morales-Ramírez 2008 and Palter *et al.* 2007) and other tropical estuaries (Rochelle-Newall *et al.* 2011, Burford *et al.* 2012, Pamplona *et al.* 2013). This increase in nutrients concentration could be due to anthropogenic inputs (expansion in agriculture, industrial waste and untreated sewage), affecting the water sources that discharge directly into the Gulf of Nicoya (Bower 2013). The generally low N:P stoichiometric ratio observed in the water column, which decreased from the river (~ 6:1) to (~ 2:1) seawards, indicate N limitation, but as concentrations measured were high, it is not relevant. Moreover, concentrations were higher than typical half-saturation constants of nutrient uptake for natural phytoplankton communities and diatom species, meaning that nutrient limitation will be only expected for some species in the surface layer of the outer stations (Fisher *et al.* 1988).

Silicate was always in stoichiometric excess over N and P and conservative with respect to salinity, favouring the observed dominance of diatoms (88%) in the microplankton community. Tropical estuaries dominated by diatom communities seem to be a common occurrence (Devassy and Goes 1988, Jennerjahn *et al.* 2004, Madhu *et al.* 2007, Costa *et al.* 2009, Canini *et al.* 2013). Dinoflagellates represented the second most abundant group (8%) after diatoms as is often found in tropical estuaries (Sidik *et al.* 2008, Costa *et al.* 2009, Biswas *et al.* 2010 Canini *et al.* 2013). Dinoflagellates were more abundant in the warmest season in a Philippine mangrove estuary (Canini *et al.* 2013), and they were more abundant as well during the dry season and toward the mouth of the Marapanin tropical estuary in Brazil (Santana *et al.* 2010). In the Gulf of Nicoya, dinoflagellates abundance generally increased towards marine stations showing higher abundance during the dry season. Many dinoflagellates, including toxic species, have a better ability to obtain nutrients than diatoms under non-Redfield ratios, explaining why diatoms and dinoflagellates follow opposite trends in distribution along the estuary (Glibert *et al.* 2011). The most abundant specie was *Prorocentrum minimum* in the surface of the most marine station during the dry season. *P. minimum* causes harmful algal blooms in many estuaries and coastal environments, including the Pacific coast of Costa Rica,

due to increasing coastal eutrophication (Hargraves and Viquez 1985, Heil *et al.* 2005, Vargas *et al.* 2008).

Other nutrients, NO_3^- , NO_2^- and PO_4^{3-} , clearly changed in a non-conservative way with salinity along the estuary. The strong increase in the slope of NO_3^- from the middle of the estuary most likely indicates a strong increase in NO_3^- consumption by phytoplankton. At the same time there is a NO_2^- maximum that, in oxic environments, usually indicates an interrelated mechanism of excess in assimilatory nitrate reduction rate by phytoplankton below the photic zone, and nitrification by bacteria in surface layers (Lomas and Lipschultz 2006). The relationship of PO_4^{3-} versus salinity, which is particularly evident in the dry season, indicates the existence of a source of this nutrient in Station 3, probably due to sewage waters. The combination of a new source of nutrients at this point, together with less turbid waters than in the estuary head, clearly trigger the observed chl *a* maximums. This study shows an increase in nutrients over those observed in past studies, probably due to an increase in inappropriate agricultural practises, untreated sewage and industrial waste (Bower 2013), highlighting the necessity of biogeochemical studies in tropical estuaries to predict the response of an evident increase in nutrients input.

The changes in turbidity, CDOM and inorganic nutrients along the estuary and between seasons described above confirm the high heterogeneity of this gradient ecosystem, which likely favours the development of different microplankton assemblages along the estuary. Despite differences in the relative contribution of freshwater and marine sources to nutrient budgets (Kress *et al.* 2002, Palter *et al.* 2007), the relatively constant input of nutrients from the Tempisque River could explain the continuous high standing stocks of chl *a*. Satellite images show that the Gulf of Nicoya has regularly high chl *a* values compared to the rest of the Pacific coast of Costa Rica, regardless of season. Our discrete sampling of chl *a* agreed with the average values from the satellite images for each season, showing that the sampling period represents the typical seasonal conditions in the Gulf. The range of chl *a* concentrations (2.7 - 20 mg m^{-3}) measured in our study was similar to those reported previously for the Gulf of Nicoya (Kress *et al.* 2002, Palter *et al.* 2007) and other tropical estuaries with diverse nutrient

loads (Burford *et al.* 2012, Rochelle-Newall *et al.* 2011). These chl *a* lie around the middle concentrations of the annual mean chl *a* concentrations (0.2 - 50 mg m⁻³) of a data set of 84 estuaries worldwide (Cloern and Jassby 2010). In the Gulf of Nicoya, the range of chl *a* concentration was similar in both seasons, although highest values were found in the surface layer in the rainy period. The pattern and amplitude of seasonal changes in phytoplankton biomass in estuaries seems to be site-dependent. Higher chl *a* concentrations during the rainy season, or after episodic increased flow events, is a common feature in tropical and subtropical estuaries (Le *et al.* 2013, Cloern 1991, Adolf *et al.* 2006, Abreu *et al.* 2009). The higher phytoplankton biomass during the rainy season has been attributed to higher nutrient inputs and a stronger stratification in the water column due to the increased fluvial discharge (Cloern *et al.* 2013). Nonetheless, other studies show that high riverine runoff could increase turbidity and decrease water residence time. This could favour a decrease in primary production and the flushing of phytoplankton biomass out of the estuary, resulting in lower chl *a* concentration (Sin *et al.* 2000, Ram *et al.* 2003, Costa *et al.* 2009, Burford *et al.* 2012). However, we found that in this middle station as the estuary becomes wider, the current decreases and accumulation of phytoplankton is favoured. Additionally, we found less turbidity during the rainy season, which likely favoured primary production. The presence of a chl *a* peak in the middle of the inner part of the Gulf of Nicoya, after the salt wedge, is a typical feature of stratified and partially stratified estuaries (Voorhis *et al.* 1983, Collins and Williams 1981, Humborg *et al.* 1997, Cloern *et al.* 2013). This happens because while turbidity decreases along the estuary, inorganic nutrients are still available in high concentrations, stimulating phytoplankton production and growth (Sverdrup 1953).

During the dry season, the higher chl *a* and microplankton abundance at the bottom layer coincident with turbidity maxima could be explained by a combination of physical accumulation and river-marine inputs of both particles and cells (Lancelot and Muylaert 2011). Except on this occasion, microplankton abundance in the Gulf of Nicoya generally increased towards the sea.

Microplankton spatial distribution patterns may vary between sites and seasons widely. For example, in the York River estuary, microplankton abundance increased seaward and was correlated with river discharge (Sin *et al.* 2000), whereas in an Indian tropical estuary, microphytoplankton abundance did not show any clear spatial trend in any season (Madhu *et al.* 2007). In the Gulf of Nicoya, microplankton abundance along the estuary did not generally coincide with chl *a* patterns. One explanation for this discrepancy is the fact that phytoplankton biomass in the Gulf was dominated by nanoplankton in both seasons, while estimated microplankton biomass contributed only 9.4% and 16.8 % of the total in the rainy and dry seasons, respectively (Table 2). Furthermore, direct measurements of chl *a* concentration in the microplankton fraction did not exceed 6.8 % of total chl *a* in a parallel size-fractionation study during the same sampling campaign (Soria 2011). In this estuary, we observed that higher river flow supports better nanoplankton populations, which are able to maintain their biomass, even in high flushing conditions, due to the ability of these cells to grow fast (Costa *et al.* 2009).

4.2. Ecological drivers of microplankton assemblages

Taxonomic analysis of microplankton revealed 146 taxa in the Gulf of Nicoya, when both seasons are considered (Online Resource A), 52 of them being new taxa for the Pacific coast of Costa Rica (Viquez and Hargraves 2009). This is a relatively high number of species compared with the 60 species of diatoms and 6 of dinoflagellates reported in other estuaries on the same Costa Rican coast (Morales-Ramírez 2011). The species richness in the Gulf of Nicoya was high compared with other river dominated tropical estuaries where the number of species found was between 61 - 129 species (Madhu *et al.* 2007, Canini *et al.* 2013, Nursuhayati *et al.* 2013, Santiago *et al.* 2010).

The nMDS ordination analysis showed clear changes in the taxonomic composition and relative abundance of different taxa in the microplankton community along the estuary and with seasons. Changes in the phytoplankton community using multivariate techniques have also been observed in other temperate and tropical estuaries (Devassy and Goes 1988, Lassen *et al.* 2004, Santiago *et al.* 2010, Matos *et al.* 2011, Lawrenz *et al.* 2012, Canini *et al.* 2013). SIMPROF

analysis identified five statistically significant groups of microplankton samples, two during the dry season and three during the rainy season. The innermost Station 1, exhibited characteristic associations of microplankton species (DI and RI) for each season, that differed significantly in taxonomy and abundance from the rest of the estuary. Estuaries are a transition zone and can be differentiated into two main types of boundary model: ecotone, when the transition is a narrow ecological zone that separates two relative homogenous zones, and ecocline when the transition is gradual (Atrill and Rundle 2002, Quinlan and Philips 2007, Muylaert *et al.* 2009). Most studies using multivariate ordination techniques applied to the phytoplankton community in estuaries clearly separate a riverine assemblage from the rest of the estuarine community (Quinlan and Philips 2007, Matos *et al.* 2011, Canini *et al.* 2013). Our results seem to support both: the “ecotone model” since the community in the estuary head was clearly separated in the nMDS ordination plot from the rest, and the “ecocline model” since microplankton assemblages presented higher similarities for the rest of the estuary.

DI and RI consist of mainly estuarine-marine microplankton species that reach Station 1 with the incoming bottom marine current (*Cyclotella* spp., *Thalassiosira* spp., *Paralia sulcata*, *Thalassionema nitzschioides*) and are mixed with some riverine species (e.g. *Scenedesmus opoliensis*), especially in the rainy season. The occurrence of freshwater species in the Gulf of Nicoya was low compared to others estuaries (Costa *et al.* 2009). Major differences between DI and RI (i.e. between seasons) were mainly due to the higher abundance of centric and pennate diatoms during the dry season (Santiago *et al.* 2010). Pennate and centric diatoms were more abundant in the dry season, particularly in the bottom water, where turbidity was higher. The benthic species (i.e. *T. nitzschioides*) found in the most turbid waters confirmed the effect of hydrodynamic driven accumulation of resuspended sediment and cells in the estuary head due to inward bottom tidal currents.

During the dry season, the rest of the estuary (DII) was characterised by the marine species *Cyclotella* spp., *Thalassiosira* spp, and *T. nitzschioides*. The higher mixing of the water column in the estuary in the dry season (Brenes *et al.* 2001) was likely the main reason for this uniformity in the microplankton community along the estuary. In contrast, during the rainy

season, the more complex hydrographic conditions characterised by a stratification in the water column supported a higher degree of habitat heterogeneity that explains the existence of two microplanktonic assemblages in the rest of the estuary (RII and RIII). RII included only surface samples from Stations 2 and 3, characterised by intermediate salinities (between 16 and 22). The microplankton assemblage of RII coincides with the stations where the chl *a* maximum was found in the rainy season (Station 3), with most of this chl *a* coming from autotrophic nanoplankton. Nanoplankton and picoplankton form the basic food source of the heterotrophic dinoflagellate *Protoperidinium pallidum*, and the oligotrichous ciliate *Strobilidium sp.* (Rassoulzadegan *et al.* 1988), which, together with the centric diatom *Ceratulina dentata*, characterised the microplankton community of RII. Finally, RIII, which included samples from the bottom water in Stations 2 and 3 and from both depths in Station 4 and 5, was characterised by the most typically marine species. Centric diatom abundance increased towards the sea because brackish marine water mixes continuously with the overlaying fresher water due to the incoming tidal and residual currents. This mixing seeds the surface layer of the estuary head with marine phytoplanktonic cells where, even with less nutrient, the occurrence of a mixed layer shallower than the photic layer favours diatom growth (Alpine and Cloern 1988).

Phytoplankton diversity changes seasonally and spatially along tropical estuaries, being higher during the monsoon period and increasing towards the sea (Jalal *et al.* 2011, Canini *et al.* 2013). This spatial pattern agrees with the higher species richness at high salinity of the Renane diagram (Attrill and Rundle 2002) was also observed for the Gulf of Nicoya. We found higher diversity during the rainy season and in the more marine samples (RII and RIII) and in particular in Station 3 which coincides with the chl *a* maximum. Following the same predictions of the Renane diagram with a minimum in diversity expected at a salinity of about 7, the estuary head groups DI and RI at salinities <12 and < 8 in the rainy and dry seasons respectively presented the lowest diversity. However, it should be noted that despite the high number of taxa we observed, diversity values in the inner Gulf of Nicoya are generally low compared to other tropical estuaries (Maurer and Vargas 1984).

As we have seen so far, the drivers that control primary producers in tropical estuaries do not follow a simple model. Microplankton community composition in tropical estuaries has been shown to change in response to different abiotic variables such as freshwater flow (Jalal *et al.* 2011); total suspended solid, salinity and nitrate concentration (Canini *et al.* 2013); salinity, light and nutrients (Lassen *et al.* 2004); salinity, light, temperature and nutrients (Quinlan and Philips 2007); and heavy metal concentrations (Rochelle-Newall *et al.* 2011). In our area, the correlation between the axes of the nMDS ordination plot with the environmental variables helps to identify which ones better explain the observed group ordination. In the Gulf of Nicoya, nutrients concentration, turbidity, and CDOM were correlated with the first axis separating the riverine (DI and RI) and more marine communities (DII, RII and RIII). The vertical axis separated samples from the dry and rainy seasons and was mainly correlated with salinity, CDOM, silicate and ammonium. Therefore, this study and the scarce available literature linking phytoplankton community composition to changes in environmental variables in tropical estuaries reinforce the importance of changes in freshwater flow, both spatially and seasonally for the phytoplankton community (Lancelot and Muylaert 2011, Cloern *et al.* 2013). These changes in freshwater flow affect a number of physical and physicochemical variables, like water column stability, residence time, salinity, turbidity, nutrient availability etc., which in turn can promote or suppress biomass and production of specific phytoplanktonic groups (Sin *et al.* 2000, Cloern *et al.* 2013).

5. Conclusion

Our results confirmed both the seasonal shift of microphytoplankton communities and the existence of a strong riverine-marine gradient. Different microplankton assemblages appear to be driven by seasonal river runoff changes, with specific taxa characterizing each group. While assemblages transitions in the estuary head fit with an ecotone model, these shift to an ecocline model for the rest of the estuary. Our analysis showed that the main ecological driver of microplankton abundance and composition was the light availability (turbidity). Diatoms, followed by dinoflagellates, dominated microplankton species composition, of which 52 are

new citations for the Pacific coast of Costa Rica. The most abundant dinoflagellate was the toxic *Prorocentrum minimum*. The present study increases our understanding of microphytoplankton interactions in tropical estuaries and contributes an important dataset that will aid predictions of the anthropogenic effects on these little studied but very productive tropical ecosystems.

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Figures

Figure 1. I. Seguro et al. 2015

Figure 2. I. Seguro et al. 2015

Figure 3. I. Seguro et al. 2015

Figure 4. I. Seguro et al. 2015

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Figure 8. I. Seguro et al. 2015

Figure Captions

Fig. 1 Map of the Gulf of Nicoya indicating the five stations sampled in the rainy and dry seasons.

Fig. 2 Sea surface temperature (SST) in °C (a & b) and (c & d) chlorophyll *a* (chl *a*) in mg m⁻³ (logarithmic scale) (c & d) along the Pacific Coast of Costa Rica during the rainy and dry season averaged for the period 2002-2011.

Fig. 3 Cross section contour plots of salinity and temperature (°C) along in the inner Gulf of Nicoya versus distance from the river in the rainy and dry season. Crosses represent sampled depths and discontinuous line the limit of the euphotic zone.

Fig. 4 Temporal and spatial variation in turbidity (NTU) and CDOM (m⁻¹) along the five stations in the inner Gulf of Nicoya for the surface and bottom layers in the rainy and dry season.

Fig. 5 Temporal and spatial variation of NO₃⁻, NO₂⁻, NH₄⁺, PO₄³⁻ and SiO₃³⁻ concentrations (μM) along the five stations in the inner Gulf of Nicoya for the surface and bottom layers during the rainy and dry season. The nutrient vs salinity relationship is presented as inserted plots.

Fig. 6 Temporal and spatial variation of chl *a* (mg m⁻³) and microplankton abundance (ind mL⁻¹) along the five stations in the inner Gulf of Nicoya for the surface and bottom layers during the rainy and dry season. Distance is calculated starting from the most riverine station.

Fig. 7 Temporal and spatial variation of main taxonomic groups. Abundance (ind mL⁻¹) along the five stations in the inner Gulf of Nicoya for the surface and bottom layers during the rainy and dry season. Distance is calculated starting from the most riverine station.

Fig. 8 Log (x+1) transformed microplankton abundance data along the five stations in the inner Gulf of Nicoya for the surface and bottom layers during the rainy and dry season. (Stress: 0.11). Circles indicate groups (Group I-V) at a similarity level of 48% derived from cluster analysis. Vectors indicate direction and relative magnitude of Spearman correlation with the plot axes of (a) the environmental variables > 0.5 and (b) the species abundance (> 0.7).

Tables

Table 1 Shannon diversity index along the five stations in the inner Gulf of Nicoya for the surface and bottom layers in the rainy and dry season. Diversity values for the five assemblages (DI, DII, RI, RII and RIII) obtained from the SIMPROF routine during cluster analysis are also shown. Shannon diversity index was calculated according to Shannon and Weaver (1963) using the following equation:

$$H = -\sum_{i=1}^S p_i \ln(p_i)$$

	1	2	3	4	5
<i>Rainy season</i>					
Surface	2.40	2.20	2.80	2.44	1.97
Bottom	2.32	2.45	2.36	2.90	2.56
<i>Dry season</i>					
Surface	1.91	2.22	2.30	2.75	2.45
Bottom	1.70	1.86	1.72	2.15	2.60
Group	DI	DII	RI	RII	RIII
	1.77	2.54	2.40	2.77	2.77

Table 2 Biomass and percentage of pico, nano and microplankton in the surface water averaged along the five stations in the inner Gulf of Nicoya in the rainy and dry season. Abundance data were first transformed to microplankton biovolume from mean size of most abundant taxa (Hillebrand *et al.*, 1999, López-Fuerte and Siqueiros-Beltrones 2006) and then to biomass ($\mu\text{g C L}^{-1}$) using conversion factors (Eppley *et al.* 1970, Leblanc *et al.* 2012). Similarly, autotrophic picoplankton and nanoplankton biomasses were calculated from measured abundance and size of both communities using flow cytometry in a complementary study (Aguilar *et al.* in prep.). Picoplankton and nanoplankton abundance and size were transformed from biovolume to biomass, in C units, using available conversion factors (DuRand *et al.* 2001, Shalapyonok *et al.* 2001, Worden *et al.* 2004)

<i>Rainy season</i>		Picoplankton	Nanoplankton	Microplankton
Biomass ($\mu\text{g C L}^{-1}$)	$\bar{x} \pm \text{SE}$	14.32 ± 6.14	1642.79 ± 369.77	171.9
%		0,78	89.81	9,4
<i>Dry season</i>				
Biomass ($\mu\text{g C L}^{-1}$)	$\bar{x} \pm \text{SE}$	5.26 ± 1.33	1019.43 ± 179.76	207.8
%		0.43	82.7	16.9