Size fractionated phytoplankton biomass and net metabolism along a tropical estuarine gradient.

Authors:

Sara Soria-Píriz [1*], Emilio García-Robledo[1,a], Sokratis Papaspyrou [1,b], Virginia Aguilar [1], Isabel Seguro[1,c], Jenaro Acuña, [2,4], Álvaro Morales [2,3], Alfonso Corzo[1].

1. Departamento de Biología, Universidad de Cádiz, Polígono Rio San Pedro s/n, 11510 Puerto Real, Spain
2. Centro de Investigación en Ciencias del Mar y Limnología (CIMAR), P.O. Box 2060 San Pedro de Montes de Oca, Costa Rica.
3. Escuela de Biología, Universidad de Costa Rica. P.O. Box 2060 San Pedro de Montes de Oca, Costa Rica
4. Escuela de Química, Universidad de Costa Rica. P.O. Box 2060 San Pedro de Montes de Oca, Costa Rica

Present address:

a. Microbiology Section, Department of Biosciences. Aarhus University. Ny Munkegade 116, DK-8000, Aarhus, Denmark
b. Laboratorio de Microbiología y Genética, Departamento de Biomedicina, Biotecnología y Salud Pública, Facultad de Ciencias, Universidad de Cádiz,11510 Puerto Real, Spain.
c. Centre for Ocean and Atmospheric Sciences, School of Environmental Sciences, University of East Anglia, Norwich, NR4 7TJ, United Kingdom

* corresponding author: Sara Soria Píriz

Departamento de Biología
Universidad de Cádiz
Pol. Río San Pedro s/n
11510 - Puerto Real
Spain
Tel. 34-956-016774
e-mail: s.soria.piriz@gmail.com

Running head: *plankton net metabolism in a tropical estuary*

Key words: *primary production, tropical estuary, phytoplankton community, size fractionation, estuarine gradient.*

Abstract

Size structure of phytoplankton determines to a large degree the trophic interactions in oceanic and coastal waters and eventually the destiny of its biomass. Although, tropical estuarine systems are some of the most productive systems worldwide compared to temperate systems, little is known on phytoplankton biomass size fractions, their contribution to net metabolism, or the ecological factors driving phytoplankton size distribution in tropical estuaries. Hence, we measured the size-fractionated biomass and net metabolism of the plankton community along a salinity and nutrient gradient in the Gulf of Nicoya estuary (Costa Rica), during the dry season. Respiration (23.6 mmol O_2 m^{-3} h^{-1}) was highest at the estuary head, whereas maximum net primary production (23.1 mmol O_2 m^{-3} h^{-1}) was observed in the middle of the estuary, coinciding with the chlorophyll a maximum (15.9 mg m^{-3}). Thus, only the middle section of the estuary was autotrophic (2.9 g C m^{-2} d^{-1}), with the rest of the estuary being net heterotrophic. Regression analysis identified light availability, and not nutrients, as the principal factor limiting primary production in the estuary due to increased turbidity. The changes in net metabolism along the estuary were also reflected in the phytoplankton's size structure. Although micro- and picophytoplankton were the most productive fractions overall, in the middle section of the estuary nanophytoplankton dominated primary production, chlorophyll, and autotrophic biomass. These changes along the estuarine gradient will directly affect higher trophic levels and have strong functional implications at the estuary scale.

Introduction

Estuaries are transitional systems providing important ecosystem services such as fisheries maintenance, nutrient cycling, water supply and purification, and recreation (Costanza et al. 1997; Barbier et al. 2011). However, estuaries are under serious threat worldwide due to anthropogenic activities, such as pollution, deforestation, and urbanization amongst others. These activities affect ecological and biogeochemical processes in estuaries, altering the structure of the estuarine food webs from phytoplanktonic primary producers to macroorganisms, including shellfish and fish species of economic interest (Bianchi 2007; Burford et al. 2008; Blaber 2013). Tropical estuaries are being particularly affected as they are often located in developing or recently industrialized countries, with usually high population growth rates (Alongi 2002; Barbier et al. 2011).

Approximately 50 % of total production in estuaries is due to pelagic primary producers (Meyercordt et al. 1999; Underwood and Kromkamp 1999) which span a wide range of size classes across several taxonomic groups i.e. cyanobacteria, diatoms, dinoflagellates, and chlorophytes (Devassy and Goes 1988; Sin et al. 2000; Huang et al. 2004). The abundance and relative contribution of each size class and taxonomic group, and consequently their contribution to planktonic primary production, are affected by changes in the abiotic and biotic environmental factors (e.g. light limitation, mixing, shelf water intrusions) (Lancelot and Muylaert 2011). In addition, the relative contribution of the different phytoplankton size classes to total planktonic community determines the functioning of the ecosystem due to the influence of cell size on growth rates, trophic interactions, sinking and resuspension rates, and overall benthic-pelagic

coupling (Malone 1980; Goldman 1988; De Madariaga et al. 1989; Cermeño et al. 2006).

Primary production rates in tropical estuaries are typically much higher than in temperate ones (Nittrouer et al. 1995; Cloern et al. 2014). These high production rates are due to a comparatively higher nutrient availability, irradiance, and temperature year-round (Nittrouer et al. 1995). Nonetheless, increased turbidity due to high inputs of suspended solids from rivers and sediment resuspension has been shown to limit primary production in many tropical estuaries (Cloern 1987; Fichez et al. 1992; Nittrouer et al. 1995; Burford et al. 2008). Despite the high phytoplankton productivity in tropical estuaries, many important aspects of phytoplankton ecology have been poorly studied compared with temperate ones (Bianchi 2007; Burford et al. 2008; Rochelle-Newall et al. 2011; Cloern et al. 2014). Only in a few cases is there information available on phytoplankton biomass size classes in tropical or subtropical estuaries (Sin et al. 2000; Li et al. 2013; Zhang et al. 2013). As far as we know, no information exists on the contribution of these size classes to primary production and net metabolism. This lack of information affects seriously to 1) the implementation of scientific-based management and conservation practices in these valuable ecosystems at local or regional levels and 2) the capacity of scientists to evaluate quantitatively the contribution of subtropical and tropical estuaries to global biogeochemical cycles (Cloern et al. 2014).

The Gulf of Nicoya is one of the most productive estuaries in the world (Gocke et al. 1990; Córdoba-Muñoz 1998; Gocke et al. 2001a and 2001b; Cloern et al. 2014) and represents a model system for the estuaries of Central America. This gulf is a tropical estuary of about 80 km length from the Tempisque River down to the Pacific Ocean, clearly divided into an inner and outer basin with strong differences in

bathymetry and hydrographic conditions (Peterson 1958; Voorhis et al. 1983). Tempisque River freshwater discharges to the inner basin are high and with a clear seasonality. Nine-year period averages show discharges up to 390 $m^3 s^{-1}$ during the rainy (May – November) and 162 $m^3 s^{-1}$ during the dry (December – April) (Kress et al. 2002). The seasonal changes in river discharges largely control hydrodynamic characteristics of the inner basin of the Gulf of Nicoya, resulting in the estuary being partially stratified during the rainy season and fully mixed during the dry season (Kress et al. 2002; Palter et al. 2007; Seguro et al. 2015).

The few existing studies on phytoplankton in the Gulf of Nicoya, dealing mainly with large nanophytoplankton and microphytoplankton, showed a dominance of diatoms and dinoflagellates in these size classes, and the existence of clear changes in the abundance of microphytoplankton along the riverine-marine gradient in the estuary (Hargraves and Víquez, 1985; Brugnoli-Olivera and Morales-Ramírez 2001 and 2008; Seguro et al. 2015). The importance of the different size fractions, pico-, nano-, and microphytoplankton for the standing stocks of phytoplankton and their relative contribution to total primary production and net metabolism in the water column were investigated along the riverine-marine gradient in the estuary of the Gulf of Nicoya. In addition, the environmental factors which are likely controlling primary production and phytoplankton size distribution in the inner part of the Gulf of Nicoya were measured in order to explain the changes in total and size fractionated community net metabolism and autotrophic biomass along the gradient in the environmental conditions along this tropical estuary.

Materials and Methods

Study site and sampling

147 The inner part of the Gulf of Nicoya extends from the Tempisque River mouth
148 down to near the Puntarenas channel (Fig. 1). It is a shallow area (< 20 m) with
149 extensive tidal flats surrounded mainly by mangroves. Tides are semidiurnal with mean
150 amplitude of 2.5 m (MIO-CIMAR 2012).
151 Five stations, one station per day, were sampled along the inner Gulf of Nicoya
152 during the dry-season in 2012 (14th - 18th April). The innermost station was located
153 near the Amistad Bridge, close to the Tempisque River mouth (Station 1) and the most
154 marine station (Station 5) close to the Caballo Island (Fig.1). Water column temperature
155 (ºC) and salinity profiles (psu) were measured using a multiparameter probe (YSI
156 6600). Photosynthetically active irradiance profiles (PAR) were measured using a
157 radiometer (LiCor 250A with a spherical sensor). Based on the registered light profiles
158 and depending on the station's maximum water depth, 3 to 4 depths were selected
159 within the 1 to 100 % range of incident irradiance plus an additional depth, 1 m from the
160 bottom. Water from each depth was collected using a 10 L Niskin bottle and was used
161 for the determination of chlorophyll *a* concentration (Chl *a*), total suspended material
162 (TSS), particulate organic carbon content (POC), inorganic nutrients and for in situ
163 incubations to measure net production and respiration rates of the whole community.
164 While onboard, all samples were stored on ice and darkness until further analysis in the
165 laboratory. In addition, 30 L of surface water were carried each day to the laboratory in
166 Estación Nacional de Ciencias Marino-Costeras (ECMAR, Universidad Nacional de
167 Costa Rica) for fractionation trough successive filtration and incubations as described
168 below.
169
170 **Chlorophyll, total suspended material, and organic matter**

Five water samples (110 – 550 mL) (which n = 3 per Chl *a*, n = 1 per TSS and n = 1 per POC) from each depth and station were filtered through pre-combusted Whatman GF/F glass fiber (0.7 μm nominal pore size) filters. For the determination of Chl *a*, filters were placed in individual tubes with 4 mL of methanol at 4 °C for 12 hours. Tubes were then centrifuged (3000 rpm, 5 min) and the absorbance of the extract were measured on a UNICAM UV/Vis spectrometer. Chl *a* concentration was calculated according to Ritchie (2008). Filters for TSS determination were dried at 60 °C for 24 h and weighed. POC content was determined on an elemental analyzer (LECO CHNS 932) on dried and weighed filters previously.

Inorganic nutrients

Samples for inorganic nutrients (n = 3 per depth) were filtered through a glass fiber 0.7 μm filter (Fisherbrand®) in polyethylene vials and stored in darkness at -20º C until analysed manually. Ammonium (NH_4^+) was determined according to Bower and Holm-Hansen (1980), phosphate (PO_4^{3-}) and silicate (SiO_4^{4-}) according to Grasshoff et al. (1999) and nitrate (NO_3^-) and nitrite (NO_2^-) according to García-Robledo et al. (2014). Spectrophotometric measurements were done using an UV 1700 Pharmaspec Shimadzu spectrophotometer.

Salinity can be used as a conservative property to calculate the degree of mixing along the estuary. The mixing of end members such as fresh water from the river and modified marine water from the lower gulf, can be calculated and used to analyze if the distribution of a nutrient along the estuary was conservative or to detected nutrient consumption or additional nutrient sources. Similarly to salinity, conservative nutrient distribution along the estuary could be explained only by mixing if no biological

processes were modifying their concentration. Non-conservative nutrient distributions are interpreted either as consumption when measured concentrations are lower than those expected from conservative mixing or as the existence of an additional nutrient source if measured concentrations are higher than the ones expected by mixing. Nutrient concentration for each end member was calculated from the regression lines between nutrients and salinity (Table 1). This information was used to calculate the theoretical concentration of every nutrient, assuming a conservative behavior identical to salinity, as a result of the mixing of both end members for a given salinity according to following equations:

$$S_i = V_i^R S^R + V_i^M S^M \quad (1)$$

$$V_i^R + V_i^M = 1 \quad (2)$$

$$V_i^M = (Si - (V_i^R S^R))/S^M \quad (3)$$

$$V_i^R = 1 - V_i^M \quad (4)$$

$$C_i = V_i^R C^R + V_i^M C^M \quad (5)$$

Where, S_i was the salinity at a given *i* position in the estuary, V^R_i and V^M_i are the volume fractions of the river and marine end members respectively in a liter of water of a given salinity S_i. C_i is the concentration of any given compound at the *i* position resulting from mixing alone, which can be calculated from the concentration in the river and marine end members, C^R and C^M respectively, and the corresponding volume fractions assuming conservative behavior (Boyle et al. 1974; Fisher et al. 1988; Yin et al. 1995).

In situ measurements of planktonic net production and respiration

Water from the selected depths was used to fill three transparent and three dark Winkler bottles and were closed and incubated in situ at the corresponding depth for 1.5

- 3 hours. Short incubation times were chosen to avoid any bottle effect given 1) the high productivity in the gulf (Córdoba-Muñoz 1998; Gocke 2001a and b) and 2) our preliminary tests before the sampling cruises where we obtained a significant change (consumption or production) in the oxygen (O_2) concentration in incubation bottles with water samples from the gulf measured continuously with O_2 microsensors. Samples for the measurement of initial (n = 3 per depth) and final (n = 2 per Winkler bottle) O_2 concentrations were taken in 12 mL Exetainer tubes (Labco, UK) and fixed with the Winkler reagents on board. O_2 samples were analyzed according to Labasque et al. (2004) on a SHIMADZU PharmaSpec/UV-1700/UV-VISIBLE spectrophotometer. The volumetric dark respiration rate (R) was measured as the O_2 consumption in the dark bottles and the volumetric net primary production rate (Pn) from the O_2 changes (positive or negative) in the transparent bottles (Gaarder and Gran 1927). Daily depth integrated of net plankton community production (P_n^d), gross production (P_g^d) and respiration (R^d) rates for the photic layer were calculated from the integrated volumetric rates according to the following equations:

$$P_g^d = P_n^d + R^d \quad (6)$$

$$P_n^d = (\alpha Pn) - (\beta R) \quad (7)$$

$$R^d = (\alpha + \beta)R = 24R \quad (8)$$

The terms α and β represent the local daily light and dark periods in hours at the sampling dates (12.35 and 11.65 h, respectively).

Size fractionated metabolism, chlorophyll, and organic matter

Size fractionation was carried out by two consecutive filtrations through 20 and 2 μm nylon filters (47 mm diameter, Millipore®) using only surface water (0.5 m depth) from every sampling station. P_n and R were measured for each of the following fractions in triplicate: 1) 300 mL of an unfiltered water subsample, 2) 300 mL of a water subsample filtered by 20 μm nylon filter and 3) 300 mL of water subsample filtered by 2 μm nylon filter. All fractions were incubated in light at 530 μmol photons m^{-2} s^{-1} and in darkness to measure P_n and R rates respectively. Incubations were performed in 300 mL Winkler bottles with a magnetic stirrer to ensure internal turbulence and mixing. Bottles were sealed with rubber stoppers holding a 50 μm tip O_2 microsensor (UNISENSE®, Denmark), allowing the continuous measurement of O_2 with time. P_n and R rates were calculated as the time evolution (30 minutes per incubation) of the O_2 concentration. O_2 microsensors have been used in previous studies to measure continuously planktonic respiration (Briand et al. 2004; García-Martín et al. 2011). Microplankton P_n and R were calculated from the differences between the rates measured for the whole community minus the rates measured for the < 20 μm fraction ($\boldsymbol{P_{n\ micro}} = P_{n\ whole} - P_{n\ <20\mu m}$, $\boldsymbol{R_{micro}} = R_{whole} - R_{<20\mu m}$). Nanoplankton contribution was calculated as the rates measured in the < 20μm fraction minus those in < 2 μm fraction ($\boldsymbol{P_{n\ nano}} = P_{n\ <20\mu m} - P_{n\ <2\mu m}$, $\boldsymbol{R_{nano}} = R_{<20\mu m} - R_{d\ <2\mu m}$). Picoplankton rates were directly measured in the < 2μm fraction ($\boldsymbol{P_{n\ pico}} = P_{n\ <2\mu m}$, $\boldsymbol{R_{pico}} = R_{<2\mu m}$).

Once the incubation finished, two water samples of each Winkler bottle, one per Chl *a* and one per POC, were filtered through pre-combusted glass fiber filters (0.7 μm nominal pore size, 47 mm diameter, Whatman GF/F) in each size fraction and in the total community, using the methods previously described. The same calculation

procedure described above for P_n and R was used to determine Chl *a* and POC in the micro-, nano- and picoplankton size fractions.

Phytoplankton abundance, biovolume and biomass

Unfiltered samples (n = 2) of in situ surface water (20 cm depth) were taken for the measurements of prokaryotic and eukaryotic pico- (0.2 – 2 μm) and nanophytoplankton (2 – 20 μm) abundance. Samples were fixed using glutaraldehyde (1% final concentration) and stored at - 80 ºC until been analyzed by flow cytometry in the laboratory. Microphytoplankton (fraction >10 μm) was concentrated by filtering 4-8 L of surface water through a 10 μm mesh. The samples were preserved with formaldehyde (4 % final concentration) and stored in dark bottles for later analysis.

Analyses of pico- and nanophytoplankton abundances were carried out on a Dako CyAnTM ADP (Beckman Coulter™) flow cytometer using fluorescent microspheres (1.1 μm, Ex/Em: 430/465 nm, FluoSpheres® Molecular Probes Inc.™) as standard. Side Scattered Light (SSC), red fluorescence from Chl *a*, and orange fluorescence from phycobiliproteins were used to characterize each population (Corzo et al. 1999; Gasol and del Giorgio 2000; Marie et al. 2005). The relationship between cell size and SSC was calibrated using reference microspheres of known sizes ranging from 0.49 to 9.9 µm (FluoSpheres® Molecular Probes Inc.™). Thereby, biovolumes (μm^3/cell) were calculated assuming cells as spheres. The abundance (cell mL^{-1}) of microphytoplankton was determined by the inverted microscopy technique on a Nikon Eclipse Ti-U microscope. Biovolume (μm^3/cell) was calculated considering the cell shape of each species according to different geometric forms following Hillebrand et al. (1999).

Based on the calculated biovolume, the carbon biomass was then determined for the picophytoplankton (*Prochlorococcus*, *Synechococcus* and *Picoeukaryotes*), nanophytoplankton (*Nanoeukaryotes*) (V. Aguilar, unpubl.) and the 14 most abundant microphytoplankton species (representing more than 75 % of the total at each station): *Actinoptychus undulatus, Corataulina dentata, Chaetoceros curvisetus, Chaetoceros subtilis var. abnormis, Cylindrotheca closterium, Cyclotella spp., Guinardia striata, Paralia sulcata, Prorocentrum minimum, Protoperidinium pallidum, Scenedesmus opoliensis, Strobilidium spp., Thalassionema nitzschioides and Thalassiosira spp*.) (Seguro et al. 2015).

There is significant uncertainty over carbon conversion factors for prokaryotic picophytoplankton derived from uncertainties in both, size and carbon density estimates (DuRand et al. 2001; Shalapyonok et al. 2001). In this study, a conversion factor of 0.235 pg C μm^{-3} was used for prokaryotic phytoplankton, which is an average of CHN (Carbon: Hydrogen: Nitrogen: ratio) measurements for the cyanobacteria of interest: *Synechococcus sp*. and *Prochlorococcus sp.*, as determined by other studies (Shalapyonok et al. 2001; Worden et al. 2004).

The biomass (μg C L^{-1}) of the prokaryotic phytoplankton (*Synechococcus* and *Prochlorococcus*), *Picoeukaryotes*, *Nanoeukaryotes* and microphytoplankton community was calculated using the equations 9 and 10 according to Strickland (1970).

$$Log\ C_{\left(\frac{pg}{cell}\right)} = 0.76 Log V_{(\mu m^3)} - 0.29^{(*)} \qquad (9)$$

$$Log C_{\left(\frac{pg}{cell}\right)} = 0.94 Log V_{(\mu m^3)} - 0.60^{(**)} \qquad (10)$$

(*) for diatoms

(**) for all other cells

Statistical methods

Simple and multiple linear correlation and regression analyses were used to test statistical significance of covariation between different variables and to estimate river and marine end-member nutrient concentrations. The relationship between P_n and the product between the concentration of Chl *a* and the ratio between incident irradiance and the extinction coefficient was tested using linear regression (Cole and Cloern 1984, 1987). In an attempt to increase the explained variability of net production we progressively included the concentration of different inorganic nutrients (NO_3^-, PO_4^{3}, SiO_4^{4-}) and temperature in a statistical model of stepwise multiple regression (PRIMER). Linear correlation between fractionated Chl *a* and total Chl *a* concentrations were tested for surface water samples (n = 15). Since the relationships between any given nutrient and salinity were not linear at the estuary scale, two separate linear regressions were used to estimate the river and marine end member nutrient concentrations more accurately, one for the river end (Stations 1 and 2, n = 7) and another for the marine end (Stations 3 to 5, n =19).

Results

Hydrographic conditions and inorganic nutrients

Physicochemical variables were strongly influenced by the Tempisque River water discharge, showing in general a gradient along the estuary (Fig. 2). Salinity increased progressively from the river to the more marine stations, whereas temperature presented a maximum centered in surface waters in Station 3 (Figs. 2A and B). The vertical profiles of temperature and salinity indicated complete vertical mixing closer to the river (Stations 1 and 2) and a certain degree of stratification with minimal gradients

for temperature in the more marine stations (Fig. 2B). NO_3^-, NO_2^-, PO_4^{3-} and SiO_4^{4-} concentrations were generally highest at the innermost stations (Stations 1 and 2), decreasing progressively towards the marine end. NO_2^- showed a clear maximum in Station 2, whereas no clear patterns were observed for NH_4^+, which was the least abundant of all inorganic nutrients measured.

Comparison of the observed nutrient concentration with the theoretical one derived from the mixing model (Figure 3) indicates: 1) that most of the decrease in NO_3^-, PO_4^{3-} and SiO_4^{4-} along the estuary is due to dilution, 2) an additional source of NO_3^- and PO_4^{3-} seems to exist between Stations 1 and 2 and 3) the large deviation of bottom concentrations from the theoretical ones suggests that the sediment is an additional source of nutrients.

Total suspended material increased from the river towards Station 2 and 3, where the maximum was found and decreased thereafter towards the sea. Photic layer was < 1m at Station 1, increasing progressively with increasing distance from the river down to 10 m depth at Station 5 (Fig. 4A).

Chlorophyll, organic carbon and phytoplankton biomass

Total Chl *a* concentration showed the highest value in the middle of the estuary at 2 m depth (Station 3) and the lowest in the surface water of Station 5 (Fig. 4A). Fractionated chlorophyll, measured only in surface water samples, showed that nanoplankton (2 – 20 μm) was the dominant fraction of Chl *a* throughout the estuary representing 51 - 78 % of total Chl *a* (Fig. 5A). Picoplankton (< 2 μm) and microplankton (> 20 μm) chlorophyll fractions represented up to a maximum of 31% (Station 2) and 37% (Stations 4), respectively. Microplankton was more abundant in the

more marine areas of the estuary, while picoplankton did not show any clear pattern along the estuary (Fig. 5A).

Particulate organic carbon concentration had very similar pattern to total Chl *a*, both along the estuary and with depth with high values at the intermediate Station 3 (Fig. 4B). However, the highest POC concentration was measured at Station 1, near the river mouth (Fig. 4B). POC size fractionation of surface samples showed that pico-particles (< 2 μm) represented the main fraction of the total POC, accounting for 54 to 86 % of the total (Fig. 5B). Nano-particles (2 – 20 μm) accounted for almost 50 % of the total POC at Station 1, but did not exceed 30 % at the remaining stations (Fig. 5B). Micro-particles (> 20 μm) represented less than 5 % of the total POC. The relative contribution of nano- and micro-particles to the total Chl *a* was comparatively larger than to the total POC, however the pico-particles fraction was considerably depleted in Chl *a* with respect to POC (Fig. 6B).

Estimated phytoplankton biomass ranged between 600 and 1600 μg C L^{-1} and showed a spatial distribution along the estuary similar to Chl *a*; a maximum at Station 3 and a minimum at Station 2 (Fig. 5C). Direct counts of phytoplankton confirmed the considerable contribution of nanophytoplankton (always > 92%) to the total autotrophic biomass compared to microplankton (1.3 – 6.8 %) and picoplankton (0.2 - 1.1%) (Fig. 5C). The contribution of pico- and microplankton to the total autotrophic biomass was similar to the one for total Chl *a*. However nanoplankton had a higher contribution to total biomass than Chl *a* (Fig. 6A).

Total and size-fractionated net production and respiration rates

Total P_n rates, determined by in situ incubations, presented a maximum in the middle of the estuary, being the highest total P_n rates (23.1 mmol O_2 m^{-3} h^{-1}) measured

392 at 2 m depth in Station 3 (Fig. 7A). In contrast, the entire water column had negative P_n
393 rates at Station 1. Compensation depth ($P_n = 0$) increased from Station 1 to Station 3,
394 and decreased again at Station 4 (Fig. 7A). *R* rates were highest (23.6 mmol O_2 m^{-3} h^{-1})
395 in the surface at Station 1, decreasing with the distance from the river and with the
396 depth in each station (Fig. 7B). O_2 in the water column was subsaturated in the riverine
397 station and in the bottom layer along the estuary and oversaturated in the surface water
398 from Station 2 seawards (Fig. 7C).

399 Net production in estuaries has been previously related to a composite parameter
400 calculated as the product between the Chl *a* concentration and the ratio between the
401 incident irradiance (I_o) and the extinction coefficient (k) (Cole and Cloern 1984, 1987).
402 The application of this empirical model to our data produced a significant linear
403 correlation ($P_n = 0.0016[\text{Chl } a\ [I_o/k]] - 9.1623$, $r = 0.540$, $p = 0.021$, $n = 18$), with P_n
404 expressed in mmol O_2 m^{-3} h^{-1}, Chl *a* in mg m^{-3}, I_o in μmol m^{-2} s^{-1} and k in m^{-1}. However,
405 this composite parameter that accounts for the Chl *a* concentration and the light
406 availability only explains about half of the variability of P_n along the estuary. Attempts
407 to increase the explained P_n variation by including the concentration of different
408 inorganic nutrients (NO_3^-, PO_4^{-3}, SiO_4^{4-}) and temperature in a statistical model of
409 stepwise multiple regression did not increase the percentage of P_n explained variation
410 (results not shown).
411
412 The relative contribution of different planktonic size classes to pelagic primary
413 production and respiration in the inner part of the gulf changed along the estuary (Fig.
414 8). The picoplankton fraction accounted from 40 to 60 % of the net community
415 production in the inner basin except at Station 3, where its contribution was lower, i.e.
416 20 %. The contribution of picoplankton to net metabolism of the pelagic community

was comparatively higher than its contribution in terms of Chl *a* (Fig. 8C). In general, the picoplankton fraction showed the highest *R* rates and accounted for almost 50 ± 4 % of the total respiration at all stations (Fig. 8B). The importance of picoplankton contribution to total *R* agrees well with the importance of this size class in terms of POC (Fig. 5B, Fig. 8D). Nanoplankton had a high contribution to the total P_n at Stations 3 and 4, being up to 42 % of the P_n at Station 3. However, surprisingly nano-phytoplankton contributed very little to the total P_n in surface samples in the rest of the stations (Fig. 8A). The contribution of nanoplankton to the total Chl *a*, was comparatively higher than to P_n along the estuary (Fig. 8C). The contribution of microplankton to the total community net production was maximum at the most marine station (about 57 %) and in general represented a higher contribution to P_n than to Chl *a* along the estuary (Fig. 8C). Microplankton respiration was the second most important contributor to total community respiration, accounting for up to 30 ± 15 % (Fig. 8B) and its contribution to total *R* was comparatively higher than to POC (Fig. 8D).

Photic layer net ecosystem production

Daily depth integrated net metabolism for the photic layer along the estuarine gradient was calculated from volumetric rates and the duration of local day and night periods (Fig. 9). P_g^d (1.5 – 7.2 g C m^{-2} d^{-1}) and R^d rates (4.3 – 8.9 g C $m^{-2}d^{-1}$) changed along the estuary, showing both of them maxima values at Station 3, where the maxima in Chl *a* and P_n were measured as well (Figs. 4A and 7A). P_n^d was only positive in this sampling station along the estuary (2.9 g C m^{-2} d^{-1}) being the photic layer in the rest of the estuary net heterotrophic (Fig. 9).

Discussion

The influence of Tempisque River on phytoplankton

In the inner basin of the Gulf of Nicoya, during the dry season, tidal and residual currents have enough energy to mix the water column between Station 1 and 2, where the maximum horizontal salinity gradient was observed (Fig. 2). Nonetheless, in the middle of the estuary (Station 3 and 4), a certain degree of stratification was observed due to the presence of a warm water mass centered around Station 3 (Fig. 2B), likely due to warmer water discharge from the Abangares River (Lizano and Vargas 1993).

Previous studies have reported that during the dry season, the Tempisque River contributes less to the nutrient budget of the inner Gulf of Nicoya due to its lower water flow (Voorhis et al. 1983; Chaves and Birkicht 1996; Palter et al. 2007). Nonetheless, our results show that the Tempisque River is a considerable source of inorganic nutrients even during the dry season (Fig. 2 C, F and G). NO_3^-, PO_4^{3-}, and SiO_4^{4-} concentrations at the more riverine stations (Stations 1 and 2) were higher than those reported previously for the Gulf of Nicoya (Palter et al. 2007) and other tropical estuaries (Rochelle-Newall et al. 2011; Burford et al. 2012; Pamplona et al. 2013). Mixing calculations using salinity as a conservative property clearly show that the decrease in SiO_4^{4-} was mainly due to dilution by mixing with seawater of lower nutrient concentrations (Fig. 3, Table 1). The Tempisque River discharged large amounts of SiO_4^{4-} to the estuary (>900 μM), resulting in SiO_4^{4-} being always in stoichiometric excess with respect to total inorganic N and P. Dilution by mixing was also evident as a general decrease of NO_3^- and PO_4^{3-} concentrations along the estuary. However, concentrations of NO_3^- at Station 2, and PO_4^{3-} at Stations 2 and 3 were higher than those predicted by conservative dilution. This suggests the existence of additional sources of both nutrients, either by remineralization in the water column and the sediment, or lateral transport from surrounding mangroves, which are abundant around the inner part

of the Gulf of Nicoya. The sediment seems to play an important role as a source of nutrients (NO_3^-, NO_2^- and PO_4^{3-}) to the water column, since nutrient concentrations close to the sediment were generally higher than those at the water column surface (Fig. 2C, D and F) and they showed a wider deviation from theoretical values, assuming conservative mixing (Fig. 3). Such an important input of regenerated nutrients from the sediment to the water column, driven by biological or physical mechanisms, has been reported previously in other estuaries as well (Fisher et al. 1982; Cowan and Boynton 1996; Corbett 2010).

In addition to inorganic nutrients, fresh water discharge of the Tempisque River supplies high levels of allochthonous dissolved and particulate matter, which has a strong influence on turbidity in the inner gulf (Gocke et al. 2001; Kress et al. 2002; Palter et al. 2007; Seguro et al. 2015). TSS presented highest values at Stations 2 and 3 likely due to flocculation of dissolved organic matter favored by the freshwater and marine water mixing (Bell et al. 2000; Thill et al. 2001; Verney et al. 2009) and an increase in phytoplankton biomass (Fig. 4). The relatively high concentrations of nutrients and the very shallow photic layer suggest that primary production in the inner basin of the Gulf of Nicoya was likely more limited by light availability than by inorganic nutrients as shown in other estuaries (Cloern 1987; Fichez et al. 1992; Nittrouer et al. 1995; Burford et al. 2008).

Phytoplankton spatial distribution and size structure

The maximum of total Chl *a* recorded in the middle of the inner gulf in the dry season (Fig. 4A) was also observed during the rainy season (Seguro et al. 2015). The Chl *a* range measured here is in concordance to that found previously in the Gulf of

492 Nicoya (Kress et al. 2002; Palter et al. 2007) and in other tropical and subtropical
493 estuaries (Burford et al. 2012; Rochelle-Newall et al. 2011; Li et al. 2013). The Chl *a*
494 peak after the maximum salinity gradient is a typical feature in many estuaries (Cloern
495 1987; Humborg et al. 1997). The maximum in phytoplankton biomass in Station 3 also
496 likely explains the maximum observed in POC as well (Fig. 4B). In contrast, in the most
497 riverine station, a large fraction of the high POC concentration measured was of detrital
498 origin, since the input of total Chl *a* concentration with the riverine water was
499 proportionally lower (Fig. 4A).
500
501 The relative importance of the different size classes in terms of Chl *a*, POC and
502 autotrophic biomass (C units) was not fully coincident (Fig. 5, Table 2). Nonetheless,
503 the dominance of nanoplanktonic fraction was confirmed in terms of Chl *a* and of
504 autotrophic C units; nanoplankton contributed more than 61 and 95 %, respectively.
505 This is in agreement with the relative importance of nanoplankton with respect to
506 micro- and picoplankton reported for temperate estuaries (Iriarte and Purdie 1994;
507 Pinckney et al. 1998; Sin et al. 2000; Thomas et al. 2005; Madhu et al. 2010).
508 Surprisingly, nanoplanktonic POC was only about 25 % of total POC, being POC
509 largely abundant in the picoplankton size fraction (72.9 ± 13.1 %), which likely suggest
510 a higher relative contribution of either detritus or heterotrophs to the pico-particle size
511 class. The cell carbon content of bacterioplankton ($3 \times 10^5 - 2 \times 10^6$ cell mL^{-1}, V.
512 Aguilar, unpubl.data) might explain the important contribution of picoplankton size
513 fraction to total POC but not to total Chl *a* and total autotrophic C. Moreover, Chl *a*
514 content in the picoplankton fraction might have been underestimated in our study since
515 the complete extraction of Chl *a* from picocyanobacteria is usually difficult, typically
516 requiring mechanical disruption of cells (Stauffer et al. 1979, Joint and Pomeroy 1986,

Howard and Joint 1989). An estimation of Chl *a* content in picoplankton of our samples can be made based on *Prochlorococcus* and *Synechococcus* abundances measured by flow cytometry during the same cruise (V. Aguilar, unpubl.). Assuming a Chl *a* content per *Synechococcus* cell of 1.9×10^{-11} mg Chl *a* $cell^{-1}$ (Collier et al. 1994) and the same amount of divinyl Chl *a* per *Prochlorococcus* cell, Chl *a* concentration in the picoplankton fraction would be between 2.5 and 4.4 times higher than that one extracted and measured spectrophotometrically here.

The concentration of Chl *a* in larger size fractions in marine and freshwater systems has been shown to be related to the trophic state of the system, which increased as the total Chl *a* increases both in space and seasonally (Chisholm 1992; Iriarte and Purdie 1994; Bell and Kalff 2001). In addition, high nutrient concentrations seem to also favor the increase in biomass and primary production of larger phytoplankton (Chisholm 1992; Agawin et al. 2000). In the inner part of the gulf, total Chl *a* increased as the fraction of microplankton Chl *a* increased ($[\text{Chl } a_{\text{total}}] = 1.112 \text{ Chl } a_{\text{micro}} + 4.076$, $r = 0.463$, $p = 0.0263$, $n = 15$) (Fig. 6). Our results thus mean that the patterns frequently observed in temperate estuaries also apply to tropical estuaries such as the Gulf of Nicoya, i.e. an increase in autotrophic biomass is accompanied by an increase in the relative concentration of microphytoplankton in terms of both biomass and Chl *a*.

The observed changes in the size structure of phytoplankton community along the estuarine gradient most likely have strong functional implications at the estuary scale. Large-sized phytoplankton are more likely to transfer organic matter to higher trophic levels through short, herbivore-based food chains, whereas communities dominated by small-sized phytoplankton are characterized by complex microbial food webs that favor the recycling of organic matter within the system (Ryther 1969, Cushing

1989). The change in the relative importance of pico-, nano-, and microplankton along the estuarine gradient is probably driven by a combination of bottom-up or top-down mechanisms such as light availability, residence time, nutrients, and grazing (Chisholm 1992; Geider et al. 1997; Irwing et al. 2006; Lancelot and Muylaert 2011; Banse 1982; Kiørboe 1993). The relatively small biomass of picophytoplankton in the inner basin of the Gulf of Nicoya and other estuarine systems (Iriarte and Purdie 1994; Pinckney et al. 1998; Sin et al. 2000; Thomas et al. 2005) suggests that heterotrophic protists (microzooplankton) would consume a lower proportion of total primary production than in the open ocean and would therefore graze mainly on heterotrophic bacteria (Thingstad and Rassoulzadegan 1999; Landry and Calbet 2004). In addition, the abundance of nanophytoplankton in the inner part of the Gulf of Nicoya suggests that a higher percentage of phytoplankton biomass is consumed by large metazoans grazers (Thingstad and Rassoulzadegan 1999), with copepods being the dominant group of phytoplankton metazoan grazers in the Gulf of Nicoya (Brugnoli-Olivera and Morales-Ramírez 2008).

Zonation of net metabolism along the estuary

Seasonal changes in the water column stability due to differences in river flow are a common feature of many tropical estuaries and are known to affect phytoplankton abundance and primary production (Ram et al. 2003; Costa et al. 2009; Burford et al. 2012). The spatial distribution of P_n matches well the observed pattern in phytoplankton abundance in the inner basin of the Gulf of Nicoya (Figs. 4A and 7A). P_g^d along the inner gulf ranged from 120 to 580 mg C m^{-2} h^{-1}, being in general higher than those measured in previous studies (Gocke et al. 1990; Córdoba-Muñoz 1998; Gocke et al. 2001a, b). Our results confirm that the inner Gulf of Nicoya is one of the

most productive estuaries worldwide (Cloern et al. 2014). Primary production in this estuarine gradient seems to be limited by light availability due to high turbidity as suggested by the relation between P_n and I_o/k (Cole and Cloern 1984, 1987) and the existence of a mixed layer deeper that than the photic layer (Fig. 2A, Fig 4A). However light availability explained less than half of the variation in P_n in the inner gulf and the inclusion of nutrients in a stepwise multiple regression analysis did not increase the % of variance explained. This result confirms the small importance nutrients have as drivers of primary production variability in the inner basin of the Gulf of Nicoya, and point out to the need of identifying other ecological drivers, which together with light availability, might explain the observed spatial pattern of net primary production in this inner part.

The inner basin of the Gulf of Nicoya can be divided into three different zones (*Zone 1*, *Zone 2* and *Zone 3*) based on daily integrated rates of organic carbon production and consumption (Fig. 9). In *Zone 1* (Stations 1 and 2), the most riverine area, mixing depth exceeds the euphotic depth. Therefore, cells spend considerable time in the dark (Grobbelaar 1995; Domingues et al. 2011) and primary production is most likely limited by light. Allochthonous organic matter (Fig. 4B) contributed to the observed maximum in planktonic microbial respiration rate (Fig. 7B) and the strong oxygen undersaturation in the water column in Station 1 (Fig 7C). Therefore net microbial plankton community production was negative, resulting in a daily net heterotrophic metabolism (P: R < 1) for the photic layer (Fig. 9). The phytoplankton community in this zone was clearly dominated by nanoplankton in terms of both Chl *a* and C units. However, pico- and microphytoplankton contributed comparatively more than nanophytoplankton to the net microbial plankton community production. An uncoupling between primary production and phytoplankton biomass, estimated by Chl

592 *a*, has been reported previously for oceanic waters (Malone et al. 1993; Marañón et al.
593 2003). This is typically explained either as a consequence of top-down control of
594 phytoplankton community by grazers (Banse 1995) or by physiological changes that
595 affect photosynthetic efficiency and photosynthesis: Chl *a* ratios in response to
596 environmental factors (e.g. light availability, temperature, nutrients) (Chisholm 1992;
597 Geider et al. 1997; Cermeño et al. 2005).

598 *Zone 2* was located in the middle of the estuarine gradient (Station 3), where
599 maxima values of primary production, Chl *a*, and POC were registered (Fig. 4, Fig. 7).
600 Contrary to *Zone 1*, and despite the high daily respiration rates, daily integrated net
601 community production was positive, indicating the existence of a net autotrophic
602 microbial community (P: R > 1) in the photic layer (Fig. 9). The high photosynthesis
603 rate in this zone led to a strong O_2 oversaturation during the day (Fig. 7C). This high net
604 primary production is likely due to a combination of factors among which the decrease
605 in turbidity (Seguro et al. 2015) and the corresponding increase in depth of the euphotic
606 layer under conditions of high nutrient availability is particularly important (Fig. 2, 4).
607 Similar effects have been reported in other systems (Cole and Cloern 1984, 1987;
608 Cloern 1987). NO_3^- concentration in the upper water layers was below the theoretical
609 value calculated by the mixing model suggesting a strong consumption rate in the upper
610 photic layer and likely an important nutrient contribution from the sediment (Fig. 3) in
611 this zone. In addition, the stability of the water column was highest at this zone due to a
612 certain degree of thermohaline stratification located around Station 3 (Fig. 2B). This is
613 probably due to a decrease in water velocity of the incoming freshwater and a higher
614 water residence time r in this area (Voorhis et al. 1983). Therefore, it would be expected
615 phytoplankton also experiences a higher residence time in the well illuminated upper
616 layer of the water column in this zone of the estuary, leading to an integrated positive

net production (Sverdrup 1953; Lancelot and Muylaert 2011). In Zone 2, the nanoplankton size class dominated the phytoplankton community in terms of both biomass and Chl *a*, same as in *Zone 1* (Fig. 5). However, in Zone 2, nanoplankton was highly active contributing >40% of net primary production, followed by microplankton (Fig. 8). In contrast, in Zone 2, the contribution of picoplankton in terms of net primary production, Chl *a* and biomass was the lowest throughout the estuarine gradient. High nutrient concentration, light availability, and residence time have been shown to favor larger cells and could explain the shift in the size structure of primary production towards higher cell sizes in the middle section of the inner gulf (Chisholm 1992; Geider et al. 1997; Agawin et al. 2000; Lancelot and Muylaert 2011). Independently of what environmental factors are responsible for the change in size structure of primary production in this area, this probably has wider implications for the planktonic trophic web in the estuarine gradient. The trophic chain would be expected to be shorter and a higher proportion of fixed carbon would be consumed directly by large metazoans grazers (Thingstad and Rassoulzadegan 1999).

Finally, in *Zone 3* (Stations 4 & 5), net daily integrated primary production showed negative values with the microbial pelagic community being again net heterotrophic (P:R ratio <1) (Fig. 9). In this zone, although the euphotic layer depth was the highest (Fig. 4A), the thermohaline stratification observed at Station 3 had disappeared (Fig. 2A) resulting in a mixing layer deeper than the compensation layer. Thus, phytoplankton most likely received a lower daily irradiance dose compared to Zone 2. In addition, nutrient limitation in *Zone 3* might also contribute to the decrease in primary production. Concentrations of NO_3^-, PO_4^{3-}, and SiO_4^{4-} in *Zone 3* were the lowest along the estuarine gradient and below the theoretical values calculated from the conservative mixing model (Fig. 3). Although the microbial planktonic community in

642 *Zone 3* was net heterotrophic with negative daily production rates similar to those of
643 *Zone 1*, the relative contribution of allochthonous and autochthonous organic carbon in
644 each of these zones is likely to be very different. Respiration in *Zone 3* is likely
645 primarily dependent on autochthonous organic carbon production within this inner
646 basin, whereas the contrary occurs in *Zone 1*; in the latter, respiration rate was likely
647 supported by the input of allochthonous organic matter discharged from the Tempisque
648 River in the Gulf of Nicoya (Seguro et al. 2015). The contribution of nanoplankton size
649 class to total Chl *a* and biomass was the lowest in *Zone 3* and seems to decrease
650 seawards along the estuarine gradient (Fig. 5C) same as in other estuaries (Sin et al.
651 2000). Similarly, the contribution of nanophytoplankton to net community production
652 decreased toward the most marine station, being replaced by micro- and
653 picophytoplankton (Fig. 8A). This shift in the size distribution of both phytoplankton
654 biomass and primary production would increase further the direct transfer of primary
655 production from microphytoplankton to even larger herbivorous grazers than in *Zone 2*.
656 The size structure of the phytoplankton community changed considerably along
657 the estuarine gradient in the Gulf of Nicoya, both in terms of standing stock (biomass)
658 and net metabolism (primary production and respiration). In addition, phytoplankton
659 biomass and net metabolism size distributions were partially uncoupled along the
660 estuarine gradient. However, information on what environmental factors, including
661 bottom-up and top-down drivers, explain the observed patterns in phytoplankton size
662 structure in the inner part of the Gulf of Nicoya and others tropical estuaries is still
663 lacking. Bridging this gap of knowledge is critical because such shifts in size
664 distribution of primary production and phytoplankton biomass along an estuarine
665 gradient are likely to have major implications for the entire trophic network and
666 biogeochemical cycling in these highly productive systems.

667

668

669

670

671 **References**

672 Agawin, N.S.R., C.M. Duarte, and S. Agustí. 2000. Nutrient and temperature control of
673 the contribution of picoplankton to phytoplankton biomass and production.
674 Limnol. Oceanogr. 45: 591–600. doi: 10.2307/2670836

675 Alongi, D.M. 2002. Present state and future of the world's mangrove forests. Environ.
676 Conserv. 29: 331–349. doi:10.1017/S0376892902000231

677 Banse, K. 1982. Cell volume, maximal growth rates of unicellular algae and ciliates and
678 the role of ciliates in the marine pelagial. Limnol. Oceanogr. 27: 1059–1071.
679 doi: 10.4319/lo.1982.27.6.1059

680 Banse, K. 1995. Community response to IRONEX. Nature 375: 112.
681 doi:10.1038/375112a0

682 Barbier, E.B., S.D. Hacker, C. Kennedy, E.W. Koch, A.C. Stier, and B.R. Silliman.
683 2011. The value of estuarine and coastal ecosystem services. Ecol. Monogr. 81:
684 169–193. doi: 10.1890/10-1510.1

685 Bell, R., M. Green, T. Hume, and R. Gorman. 2000. What Regulates Sedimentation in
686 Estuaries? Water Atmos. 8:13–16.

687 Bell, T., and L. Kalff. 2001. The contribution of picophytoplankton in marine and
688 freshwater systems of different trophic status and depth. Limnol. Oceanogr. 46:
689 1243–1248. doi: 10.4319/lo.2001.46.5.1243

690 Bianchi, T., 2007. Biogeochemistry of Estuaries. Oxford University Press, New York.

691 Blaber, S.J.M. 2013. Fishes and fisheries in tropical estuaries, the last 10 years. Estuar.,
692 Coast. Shelf Sci. 135: 57–65. doi:10.1016/j.ecss.2012.11.002
693 Bower, C.E., and T. Holm-Hansen. 1980. A salicylate-hypochlorite method for
694 determining ammonia in seawater. Can. J. Fish. Aquat. Sci. 37: 794–798.
695 Boyle, E., R. Collier, A.T. Dengler, J.M. Edmond, A.C. Ng, and R.F. Stallard. 1974. On
696 the chemical mass-balance in estuaries. Geochim. Cosmochim. Acta 38: 1719–
697 1728. doi:10.1016/0016-7037(74)90188-4
698 Briand, E., O. Pringault, S. Jacquet, and J.P Torréton. 2004. The use of oxygen
699 microprobes to measure bacterial respiration for determining bacterioplankton
700 growth efficiency. Limnol. Oceanogr. Methods 2: 406–416.
701 Brugnoli-Olivera, E., and A. Morales-Ramírez. 2001. La comunidad fitoplanctónica de
702 Punta Morales, Golfo de Nicoya, Costa Rica. Rev. Biol. Trop. 49: 11–17.
703 Brugnoli-Olivera, E., and A. Morales-Ramírez. 2008. Trophic planktonic dynamics in a
704 tropical estuary, Gulf of Nicoya, Pacific coast of Costa Rica during El Niño
705 1997 event. Rev. Biol. Mar. Oceanogr. 43: 75–89.
706 Burford, M.A., D.M. Alongi, A.D. McKinnon, and L.J. Trott. 2008. Primary production
707 and nutrients in a tropical macrotidal estuary, Darwin Harbour, Australia.
708 Estuar. Coast. Shelf Sci. 79: 440–448. doi:10.1016/j.ecss.2008.04.018
709 Burford, M.A., I.T. Webster, A.T Revill, R. Kenyon, AM. Whittle, and G. Curwen.
710 2012. Controls on phytoplankton productivity in a wet-dry tropical estuary.
711 Estuar. Coast. Shelf Sci. 113: 141–151. doi:10.1016/j.ecss.2012.07.017
712 Cermeño, P., E. Marañón, V. Pérez, P. Serret, E. Fernández, and C.G. Castro. 2006.
713 Phytoplankton size structure and primary production in a highly dynamic
714 coastal ecosystem Ría de Vigo, NW-Spain, Seasonal and short-time scale

715 variability. Estuar. Coast. Shelf Sci. 671: 251–266.
716 doi:10.1016/j.ecss.2005.11.027
717 Chaves, J., and M. Birkicht. 1996. Equatorial subsurface water and the nutrient
718 seasonality distribution of the Gulf of Nicoya, Costa Rica. Rev. Biol. Trop. 44:
719 41–48.
720 Chisholm, S.W. 1992. Phytoplankton size. In Falkowski, P.G.,and Woodhead, A.D., ed.
721 Primary Productivity and Biogeochemical Cycles in the Sea. Plenum press,
722 New York.
723 Cloern, J.E. 1987. Turbidity as a control on phytoplankton biomass and productivity in
724 estuaries. Cont. Shelf Res. 7: 1367–1381. doi:10.1016/0278-4343(87)90042-2
725 Cloern, J.E., S.Q. Foster, and A.E. Kleckner. 2014. Review: phytoplankton primary
726 production in the world's estuarine-coastal ecosystems. Biogeosciences 11:
727 2477–2501. doi:10.5194/bg-11-2477-2014
728 Cole, B.E. and J.E. Cloern. 1987. An empirical model for estimating phytoplankton
729 productivity in estuaries. Mar. Ecol. Prog. Ser. 36: 299–305. doi:
730 10.3354/meps036299
731 Cole, B.E. and J.E. Cloern. 1984. Significance of biomass and light availability to
732 phytoplankton productivity in San Francisco Bay. Mar. Ecol. Prog. Ser. 17:
733 15–24. doi: 10.3354/meps017015
734 Collier J.L., S.K. Herbert, D. C. Fork, and A. R. Grossman 1994. Changes in the
735 cyanobacterial photosynthetic apparatus during acclimation to macronutrient
736 deprivation. Photosynth Res. 42: 173-183. doi: 10.1007/BF00018260
737 Corbett, D.R. 2010. Resuspension and estuarine nutrient cycling: insights from the
738 Neuse River Estuary. Biogeosciences 7: 3289 – 3300. doi:10.5194/bg-7-3289-
739 2010

740 Córdoba-Muñoz, R. 1998. Primary productivity in the water column of Estero de
741 Morales, a mangrove system in the Gulf of Nicoya, Costa Rica. Rev. Biol.
742 Trop. 46: 257–262.
743 Corzo, A., F. Jiménez-Gómez, F.J.L. Gordillo, R. García-Ruíz, and F.X. Niell. 1999.
744 Synechococcus and Prochlorocccus-like populations detected by flow
745 cytometry in a eutrophic reservoir in summer. J. Plankton Res. 21: 1575–1581.
746 doi: 10.1093/plankt/21.8.1575
747 Costa, L.S., V.L.M. Huszar, and A.R. Ovalle. 2009. Phytoplankton functional groups in
748 a tropical estuary: hydrological control and nutrient limitation. Estuaries
749 Coasts 32: 508–521. doi: 10.1007/s12237-009-9142-3
750 Costanza, R., R. d'Arge, R. de Groot and others. 1997. The value of the world's
751 ecosystem services and natural capital. Nature 387: 253–260.
752 doi:10.1038/387253a0
753 Cowan, J.L.W., and W.R. Boynton. 1996. Sediment-water oxygen and nutrient
754 exchanges along the longitudinal axis of Chesapeake Bay: seasonal patterns,
755 controlling factors and ecological significance. Estuaries Coasts 19: 562–58.
756 doi: 10.2307/1352518
757 Cushing, D.H. 1989. A difference in structure between ecosystems in strongly stratified
758 waters and in those that are only weakly stratified. J. Plankton Res. 11: 1-13.
759 doi: 10.1093/plankt/11.1.1
760 De Madariaga, I., and E. Orive. 1989. Spatio-temporal variations of size-fractionated
761 primary production in the Gernika estuary. J. Exp. Mar. Biol. Ecol. 127: 273–
762 288. doi: 10.1016/0022-0981(89)90079-8

763 Devassy, V.I., and J.I. Goes. 1988. Phytoplankton Community Structure and Succession
764 in a Tropical Estuarine Complex (Central West Coast of India). Estuar. Coast.
765 Shelf Sci. 27: 671–685. doi: 10.1016/0272-7714(88)90074-1
766 Domingues, R.B., T.P. Anselmo, A.B. Barbosa, U. Sommer, and H.M. Galvão. 2011.
767 Light as a driver of phytoplankton growth and production in the freshwater tidal
768 zone of a turbid estuary. Estuar. Coast. Shelf Sci. 91: 526–535. doi:
769 10.1016/j.ecss.2010.12.008
770 DuRand, M.D., R.J. Olson, and S.W. Chisholm. 2001. Phytoplankton population
771 dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. Deep
772 Sea Res. Part II: Top. Stud. Oceanogr. 48: 1983–2003. doi:10.1016/S0967-
773 0645(00)00166-1
774 Fichez, R., T.D. Jickells, and H.M. Edmunds. 1992. Algal blooms in the high turbidity,
775 a result of the conflicting consequences of turbulence on nutrient cycling in a
776 shallow water estuary. Estuar. Coast. Shelf Sci .35: 577–592.
777 doi:10.1016/S0272-7714(05)80040-X
778 Fisher, T.R., P.R. Carlson, and R.T. Barber. 1982. Sediment nutrient regeneration in
779 three North Carolina estuaries. Estuar. Coast. Shelf Sci. 14: 101–116. doi:
780 10.1016/S0302-3524(82)80069-8
781 Fisher, T.R., L.W. Harding, and L.G. Ward. 1988. Phytoplankton, nutrients and
782 turbidity in the Chesapeake, Delaware and Hudson estuaries. Estuar. Coast.
783 Shelf Sci. 27: 61–93. doi: 10.1016/0272-7714(88)90032-7
784 Gaarder, T., and H.H. Gran. 1927. Investigation on the production of plankton in the
785 Oslo-Fjord. Rapp. P.-v. Réun. Cons. Int. Explor. Mer 42:1–48.
786 García-Martín, E.E., P. Serret, and M. Pérez-Lorenzo. 2011. Testing potential bias in
787 marine plankton respiration rates by dark bottle incubations in the NW Iberian

788 shelf: incubation time and bottle volume. Cont. Shelf Res. 31: 496–506.
789 doi:10.1016/j.csr.2010.07.006
790 García-Robledo, E., A. Corzo, and S. Papaspyrou. 2014. A fast and direct
791 spectrophotometric method for the sequential determination of nitrate and nitrite
792 at low concentrations in small volumes. Mar. Chem. 162: 30–36.
793 doi:10.1016/j.marchem.2014.03.002
794 Gasol, J.M., and P. del Giorgio. 2000. Using flow cytometry for counting natural
795 planktonic bacteria and understanding the structure of planktonic bacterial
796 communities. Sci. Mar. 64: 197–224.
797 Geider, R.J., H.L. MacIntyre, and T.M. Kana. 1997. Dynamic model of phytoplankton
798 growth and acclimation: responses of the balanced growth rate and the
799 chlorophyll a: carbon ratio to light, nutrient-limitation and temperature. Mar.
800 Ecol. Prog. Ser. 148:187–200. doi: 10.3354/meps148187
801 Gocke, K., J. Cortés, and C. Villalobos. 1990. Effects of red tides on oxygen
802 concentration and distribution in the Golfo de Nicoya, Costa Rica. Rev. Biol.
803 Trop. 38: 401–407.
804 Gocke, K., J. Cortés, and M.M. Murillo. 2001a. The annual cycle of primary
805 productivity in a tropical estuary, the inner regions of the Golfo de Nicoya,
806 Costa Rica. Rev. Biol. Trop. 49: 289–306.
807 Gocke, K., J. Cortés, and M.M. Murillo. 2001b. Planktonic primary production in a
808 tidally influenced mangrove forest on the Pacific coast of Costa Rica. Rev. Biol.
809 Trop. 49: 279–288.
810 Goldman, C.R., 1988. Primary productivity, nutrients, and transparency during the early
811 onset of eutrophication in ultra-oligotrophic Lake Tahoe, California-Nevada.
812 Limnol. Oceanogr. 33: 1321–1333. doi: 10.4319/lo.1988.33.6.1321.

813 Grasshoff K., K. KremLing, and M. Ehrhardt. 1999. Frontmatter, in Methods of
814 Seawater Analysis, 3rd ed. Wiley-VCH Verlag GmbH, Weinheim, Germany.
815 Grobbelaar, J.U. 1995. Phytoplankton productivity in turbid waters. J. Plankton Res. 7:
816 653–663. doi: 10.1093/plankt/7.5.653
817 Hargraves, P.E., and R. Víquez. 1985. Spatial and temporal distribution of
818 phytoplankton in the Gulf of Nicoya, Costa Rica. Bull. Mar. Sci. 37: 557–585.
819 Hillebrand, H., C.D. Dürselen, D. Kirschtel, T. Zohary, and U. Pollingher. 1999.
820 Biovolume calculation for pelagic and benthic microalgae. J. Phycol. 35: 403–
821 424. doi: 10.1046/j.1529-8817.1999.3520403.x
822 Howard, K. M., and L. R. Joint. 1989. Physiological ecology of picoplankton in the
823 North Sea. Mar. Biol. 102: 275-281. doi: 10.1007/BF00428289
824 Huang, L., W. Jian, X. Song, X. Huang, S. Liu, P. Qian, K. Yin, and M. Wu. 2004.
825 Species diversity and distribution for phytoplankton of the Pearl River Estuary
826 during rainy and dry seasons. Mar. Pollut. Bull. 49: 588–596.
827 doi:10.1016/j.marpolbul.2004.03.015
828 Humborg, C., V. Ittekkot, A. Cociasu, and B.V. Bodungen. 1997. Effect of Danube
829 River dam on Black Sea biogeochemistry and ecosystem structure. Nature. 386:
830 385–8. doi:10.1038/386385a0
831 Iriarte, A., and D. A. Purdie. 1994. Size distribution of chlorophyll a biomass and
832 primary production in a temperate estuary (Southampton Water): the
833 contribution of photosynthetic picoplankton. Mar. Ecol. Prog. Ser. 115: 283–
834 297. doi: 10.3354/meps115283
835 Irwin, A.J., Z.V. Finkel, O.M.E. Schofield, and P.G. Falkowski. 2006. Scaling-up from
836 nutrient physiology to the size-structure of phytoplankton communities. J.
837 Plankton Res. 28: 459–471. doi: 10.1093/plankt/fbi148

838 Joint, I. R., and A. J. Pomroy. 1986. Photosynthetic characteristics of nanoplankton and
839 picoplankton from the surface mixed layer. Mar. Biol. 92: 465-474. doi:
840 10.1007/BF00392506
841 Kiørboe, T. 1993. Turbulence, phytoplankton cell size, and the structure of pelagic food
842 webs. Adv. Mar. Biol. 29:1–72. doi: 10.1016/S0065-2881(08)60129-7
843 Kress, N., S. Brenner, S. León Coto, C.L. Brenes, and C. Arroyo. 2002. Horizontal
844 transport and seasonal distribution of nutrients, dissolved oxygen and
845 chlorophyll-a in the Gulf of Nicoya, Costa Rica: a tropical estuary. Cont. Shelf
846 Res. 22: 51– 66. doi: 10.1016/S0278-4343(01)00064-4
847 Labasque, T., C. Chaumery, A. Aminot, and G. Kergoat. 2004. Spectrophotometric
848 Winkler determination of dissolved oxygen: re-examination of critical factors
849 and reliability. Mar. Chem. 88: 53 – 60. doi: 10.1016/S0304-4203(04)00058-1.
850 Lancelot, C., and K. Muylaert. 2011. Trends in Estuarine Phytoplankton Ecology.
851 Treatise on Estuarine and Coastal Science 7: 5–16. doi:10.1016/B978-0-12-
852 374711-2.00703-8
853 Landry, M. R. and A. Calbet. 2004. Microzooplankton production in the oceans. ICES
854 J. Mar.Sci. 61: 501–507. doi: 10.1016/j.icesjms.2004.03.011
855 Li, L., L. SongHui, J. Tao, and L. Xia. 2013. Seasonal variation of size-fractionated
856 phytoplankton in the Pearl River Estuary. Chinese Sci. Bull. 58: 2303–2314. doi:
857 10.1007/s11434-013-5823-1
858 Lizano, O.G., and J.A. Vargas. 1993. Distribución espacio-temporal de temperatura y
859 salinidad en la parte interna del Golfo de Nicoya. Tecnol. Marcha. 12: 3–16.
860 Madhu, N.V., R. Jyothibabu, and K.K. Balachandran. 2010. Monsoon-induced changes
861 in the size-fractionated phytoplankton biomass and production rate in the

862 estuarine and coastal waters of southwest coast of India. Environ. Monit.
863 Assess.166: 521–528. doi: 10.1007/s10661-009-1020-8
864 Malone, T.C., 1980. Algal size. In I. Morris, ed. The physiological ecology of
865 phytoplankton. University of California Press, Berkeley.
866 Malone, T.C., S.E. Pike, and D.J. Conle. 1993. Transient variations in phytoplankton
867 productivity at the JGOFS Bermuda time series station. Deep Sea Res. Part I:
868 Oceanogr. Res. Pap. 40: 903–924. doi:10.1016/0967-0637(93)90080-M
869 Marañón, E., M.J. Behrenfield, N. González, B. Mourino, and M. Zubvok. 2003. High
870 variability of primary production in oligotrophic waters of the Atlantic Ocean:
871 Uncoupling from phytoplankton biomass and size structure. Mar. Ecol. Prog .Ser.
872 257: 1–11. doi:10.3354/meps257001
873 Marie, D., Simon N., and Vaulot D. 2005. Phytoplankton cell counting by flow
874 cytometry. In Andersen R.A., ed. Algal Culturing Techniques. Academic Press,
875 London.
876 Meyercordt, J., S. Gerbersdorf, and L.A. Meyer-Reil. 1999. Significance of pelagic and
877 benthic primary production in two shallow coastal lagoons of different degrees
878 of eutrophication in the southern Baltic Sea. Aquat. Microb. Ecol. 20: 273–284.
879 doi: 10.3354/ame020273
880 Nittrouer, C., G.J. Brunskill, and A.G. Figueiredo. 1995. Importance of tropical coastal
881 environments. Geo-Mar. Lett. 15: 121–126.
882 Palter, J., S. León Coto, and D. Ballestero. 2007. The distribution of nutrients, dissolved
883 oxygen and chlorophyll a in the upper Gulf of Nicoya. Costa Rica, a tropical
884 estuary. Rev. Biol. Trop. 55: 427–436.
885 Pamplona, F.C, E.T. Paes, and A. Nepomuceno. 2013. Nutrients fluctuations in the
886 Quatipuru River: A macrotidal estuarine mangrove system in the Brazilian

887 Amazonian basin. Estuar. Coast. Shelf Sci. 133: 273–284. doi:
888 10.1016/j.ecss.2013.09.010
889 Peterson, C.L. 1958. The physical oceanography of the Gulf of Nicoya, Costa Rica, a
890 tropical estuary. Bull. Inter. Am. Trop. Tuna Comm 4: 137–216.
891 Pinckney, J.L., H.W. Paerl, M.B. Harrington, and K.E. Howe. 1998. Annual cycles of
892 phytoplankton community-structure and bloom dynamics in the Neuse River
893 Estuary, North Carolina. Mar. Biol. 131: 371–381. doi: 10.1007/s002270050330
894 Ram, A.S.P., S. Nair, and D. Chandramohan. 2003. Bacterial growth efficiency in the
895 tropical estuarine and coastal waters of Goa, southwest coast of India. Microb.
896 Ecol. 45: 88–96.
897 Ritchie, R.J. 2008. Universal chlorophyll equations for estimating chlorophylls a, b, c,
898 and d and total chlorophylls in natural assemblages of photosynthetic organisms
899 using acetone, methanol, or ethanol solvents. Photosynthetica 46: 115–126. doi:
900 10.1007/s11099-008-0019-7
901 Rochelle-Newall, E.J., V.T. Chu, O. Pringault, D. Amouroux, R. Arfi, Y. Bettarel, and
902 J. P. Torréton. 2011. Phytoplankton distribution and productivity in a highly
903 turbid, tropical coastal system (Bach Dang Estuary, Vietnam). Mar. Pollut. Bull.
904 62: 2317–2329. doi:10.1016/j.marpolbul.2011.08.044
905 Ryther, J.H. 1969. Photosynthesis and fish production in the sea. Science 166:72-76.
906 doi: 10.1126/science.166.3901.72
907 Seguro, I., C.M. García, S. Papaspyrou, and others. 2015. Seasonal changes of the
908 microplankton community along a tropical estuary. Reg. Stud. Mar. Sci. 2: 189-
909 202. doi:10.1016/j.rsma.2015.10.006
910 Shalapyonok, A., R.J. Olson, and L.S. Shalapyonok. 2001. Arabian Sea phytoplankton
911 during southwest and northeast monsoons 1995: composition, size structure and

biomass from individual cell properties measured by flow cytometry. Deep Sea Res. Part II: Top. Stud. Oceanogr. 48: 1231–1261. doi: 10.1016/S0967-0645(00)00137-5

Sin, Y., R.L. Wetzel, and I.C. Anderson. 2000. Seasonal variations of size-fractionated phytoplankton along the salinity gradient in the York River estuary, Virginia (USA). J. Plankton Res. 22: 1945–1960. doi: 10.1093/plankt/22.10.1945

Stauffer, R. E., G. F. Lee, and D. E. Armstrong. 1979. Estimating chlorophyll extraction biases. J. Fish. Res. Bd Can. 36:152-157. doi: 10.1139/f79-024

Strickland, J.D.H. 1970. The Ecology of the Plankton Off La Jolla, California, in the Period April Through September, 1967. Bull. Scripps Inst. Oceanogr.

Sverdrup, H.U. 1953. On Conditions for the Vernal Blooming of Phytoplankton. J. Cons. Int. Explor. Mer 18: 287–295. doi: 10.1093/icesjms/18.3.287

Thill, A., S. Moustier, J.M. Garnier, C. Estournel, J.J. Naudin, and J.Y. Bottero. 2001. Evolution of particle size and concentration in the Rhone river mixing zone: influence of salt flocculation. Cont. Shelf Res. 21: 2127–2140. doi: 10.1016/S0278-4343(01)00047-4

Thingstad, T.F., and F. Rassoulzadegan. 1999. Conceptual models for the biogeochemical role of the photic zone microbial food web, with particular reference to the Mediterranean Sea. Prog. Oceanogr. 44: 271–286. doi:10.1016/S0079-6611(99)00029-4

Thomas, C.M., R. Perissinotto, and I. Kibirige. 2005. Phytoplankton biomass and size structure in two South African eutrophic, temporarily open/closed estuaries. Estuar. Coast. Shelf Sci.65: 223–238. doi:10.1016/j.ecss.2005.05.015.

Underwood, G.J.C., and J. Kromkamp. 1999. Primary Production by Phytoplankton and Microphytobenthos in Estuaries. Adv. Ecol. Res. 29: 93–153. doi: 10.1016/S0065-2504(08)60192-0

Verney, R., R. Lafite, and J.C. Brun-Cottan. 2009. Flocculation Potential of Estuarine Particles: The Importance of Environmental Factors and of the Spatial and Seasonal Variability of Suspended Particulate Matter. Estuaries Coasts 324: 678–693. doi: 10.1007/s12237-009-9160-1

Voorhis, A., C.E. Epifanio, D. Maurer, A.I. Dittel, and J.A. Vargas. 1983. The estuarine character of the Gulf of Nicoya and embayment on the Pacific coast of Central America. Hydrobiologia 99: 225–237. doi: 10.1007/BF00008774

Worden, A.Z., J.K. Nolan, and B. Palenik. 2004. Assessing the dynamics and ecology of marine picophytoplankton, the importance of the eukaryotic component. Limnol. Oceanogr. 49: 168–179.

Yin, K., P.J. Harrison, S. Pond, and R.J. Beamish. 1995. Entrainment of nitrate in the Fraser River Estuary and its biological implications. Estuar. Coast. Shelf Sci. 40: 505–528. doi:10.1006/ecss.1995.0036

Zhang, X., Z. Shi, X. Huang, F. Ye, and Q. Liu. 2013. Phytoplankton abundance and size-fractionated structure in three contrasting periods in the Pear River Estuary. J. Mar. Res. 71: 187–210. doi: 10.1357/002224013807719464

Acknowledgments

This study was funded by projects C/023621/09, D/031020/10, and A1/037457/11 from Spanish Agency for International Development and Cooperation (AECID), the projects CTM2013-43857-R and P11-RNM-7199 from the Spanish Ministry of Economy and Competitiveness and Andalusian Regional Government and supported by the project

808-B3-127 at the University of Costa Rica (UCR). We thank all colleagues and the staff from UCA and CIMAR-UCR that contributed to the realization of this study for their personal and logistic support.

Figure Legends

Fig. 1 The inner basin of the Gulf of Nicoya. Map of the study area and the sampling stations.

Fig. 2 Vertical distributions of (**a**) salinity, (**b**) temperature (ºC); (**c**) nitrate (NO_3^-), (**d**) nitrite (NO_2^-), (**e**) ammonium (NH_4^+), (**f**) phosphate (PO_4^{3-}) and (**g**) silicate (SiO_4^{4-}) concentrations (μmol L^{-1}), and (**h**) total Suspended material (TSS) (g m^{-3}) along the sampled area. Data are means of n = 3 for inorganic nutrients and n = 1 for TSS.

Fig. 3 Observed (○ and ●) and calculated (—●—) nutrient concentrations from a mixing model using salinity as a conservative property along the study are. The concentrations of nutrients were measured at different depth in the water column, the bottom water samples have been marked with a different symbol (large grey circle). The calculated nutrient concentrations are presented as the water column mean ± standard error for simplicity. (**a**) nitrate (NO_3^-), (**b**) nitrite (NO_2^-), (**c**) ammonium (NH_4^+), (**d**) phosphate (PO_4^{3-}) and (**e**) silicate (SiO_4^{4-}) concentrations (μM).

Fig. 4 Vertical distribution of (**a**) chlorophyll *a* (Chl *a)* (mg m^{-3}), and (**b**) particulate organic carbon (POC) (g m^{-3}) along the study area. White circles line represent photic layer depth (m). Data are means of n = 3 for Chl *a*, and n = 1 for POC.

Fig. 5 Total concentration (▭) of (**a**) chlorophyll a (Chl *a*) (mg m^{-3}) (n = 3), (**b**) particulate organic carbon (POC) (g m^{-3}) (n = 3), and (**c**) biomass of phytoplankton (mg

C m^{-3}) (n = 2), and their relative contribution (%) of pico- (< 2 μm, ●), nano- (2 - 20 μm, ■), and microplankton (> 20 μm, □) along the sampling stations.

Fig. 6 Relative contribution (%) of pico- (< 2 μm, ●), nano- (2 - 20 μm, ■), and microplankton (> 20 μm, □) in terms of (**a**) chlorophyll a (Chl *a*) versus autotrophic biomass in carbon (C) units, and (**b**) Chl *a* versus particulate organic carbon (POC) along the estuarine gradient. Diagonal lines represent identical contribution to both variables. S1 to S5 represent the sampling stations.

Fig. 7 Vertical distribution of (**a**) volumetric net primary production rates (P_n), (**b**) volumetric dark respiration rates (R) (mmol O_2 m^{-3} h^{-1}) and (**c**) % of oxygen (O_2) saturation along the studied transect.

Fig. 8 Relative contribution (%) of pico- (< 2 μm, ●), nano- (2 - 20 μm, ■), and microplankton (> 20 μm, □) in terms of (**a**) volumetric net primary production rates (P_n) and (**b**) volumetric dark respiration rates (R). Data are means of n = 3. Relative contribution (%) of pico- (< 2 μm, ●), nano- (2 - 20 μm, ■), and microplankton (> 20 μm, □) of (**c**) P_n versus Chlorophyll a (Chl *a*), and (**d**) R versus particulate organic carbon (POC). Diagonal lines represent identical contribution to both variables. S1 to S5 represent the sampling stations.

Fig. 9 Daily depth integrated of gross production rates (P_g^d, ●) and daily depth integrated respiration rates (R^d, ●) (g C m^{-2} d^{-1}) in the photic layer along the estuarine gradient. Grey area represents the region where the microbial community production in the photic layer was positive.