Isotopic evidence of subtle nutrient enrichment in mangrove habitats of Golfo Dulce, Costa Rica

Running head: Mangrove nutrient enrichment in Costa Rica

Jimena Samper-Villarreal [a,b]*, Jorge Cortés [b,c] and Nicholas V.C. Polunin [a]

[a] School of Natural & Environmental Sciences, University of Newcastle upon Tyne, NE1 7RU, UK

[b] Centro de Investigación en Ciencias del Mar y Limnología. Ciudad de la Investigación, Universidad de Costa Rica. 11501-2060. San José, Costa Rica

[c] Escuela de Biología, Universidad de Costa Rica. 11501-2060. San José, Costa Rica

* Corresponding author: jimena.sampervillarreal@ucr.ac.cr

Abstract: Mangroves are of great ecological and socioeconomic importance, yet they are under threat from urban development on the southern Pacific coast of Costa Rica. To test for possible nutrient-related impacts, we compared water-column nutrient concentrations, C and N stable isotope values and other environmental variables between mangroves with known sewage loading (three 'nutrient loaded' locations) and those without such loading (three 'reference' locations). Instantaneous nutrient concentrations were low at all locations, Secchi depth was greater at reference locations, and chlorophyll concentrations were higher at nutrient loaded mangroves. Suspended matter did not vary between reference and nutrient loaded mangroves, and nor did bivalve and algal $\delta^{13}C$ and $\delta^{15}N$ values. Enrichment of $\delta^{15}N$ and $\delta^{13}C$ of red mangrove leaves at the nutrient loaded locations is attributed to pulsed inputs of materials that were not detected in the instantaneous nutrient data. We provide evidence of isotopic enrichment at nutrient loaded locations from mangrove material and recommend that adequate waste water treatment be carried out on all anthropogenic discharges into this vulnerable marine system.

Key words: Stable isotopes, macroalgae, benthic filter feeders, *Anadara*, *Rhizophora*, *Bostrychia*.

1. Introduction

Mangrove habitats can act as filters for eutrophication and lessen the impacts of nutrient loading on adjacent coastal habitats such as seagrasses and coral reefs (Bouillon, Moens, & Dehairs, 2004; Kathiresan & Bingham, 2001; Rodelli, Gearing, Gearing, Marshall, & Sasekumar, 1984). Mangrove forests are highly productive tropical coastal ecosystems (Duarte & Cebrian, 1996), which can serve as important nursery habitats, stabilize sediments, buffer against tsunamis and storms, store carbon, and provide food and resources for coastal communities (Donato et al., 2011; Hogarth, 1999; Tomlinson, 1994), but increased human population and catchment development threaten these coastal ecosystems (Duke et al., 2007). Mangrove forests are vulnerable habitats which endure a multitude of anthropogenic stresses and continue to decline worldwide (Duke et al., 2007; Valiela, Bowen, & York, 2001). Sewage discharge in particular can reduce water quality, generate anoxic conditions, and increase water-column pathogens, and thus impact these habitats (Lapointe & Clark, 1992; Lapointe, O'Connell, & Garrett, 1990).

In Costa Rica, 99.9% of mangroves are located on the Pacific coast, and the most extensive mangrove forests occur in the southern sector (Cortés, 2016; Cortés & Wehrtmann, 2009; Jiménez & Soto, 1985). Golfo Dulce on that coast is an environment with relatively low nutrient concentrations (Córdoba-Muñoz & Vargas-Zamora, 1996; Morales-Ramírez, Acuña-González, Lizano, Alfaro, & Gómez, 2015; Wolff, Hartmann, & Koch, 1996) and abundant unlogged forest (Quesada-Alpízar & Cortés, 2006). Golfo Dulce is known for its beauty, habitats, and marine megafauna (Chacón-Chaverri, Martínez-Cascante, Rojas, & Fonseca, 2015; Morales-Ramírez et al., 2015), and its mangroves are nursery grounds for commercially important shrimp (Jesse, 1996) and mud cockle (*Anadara tuberculosa* and *Anadara similis*) for human consumption (Silva & Carrillo, 2004; Silva Benavides & Carrión, 2001; Stern-Pirlot & Wolff, 2006). However, there is increasing pressure from urban and tourism development, including nutrient input, destructive fishing, aquaculture projects, deforestation, and land runoff (Cortés, 1990; González-Chen, 2009; Loaiza, 2007; Morales-Ramírez et al., 2015; Quesada-Alpízar & Cortés, 2006). Golfo Dulce is particularly sensitive to pollution, given its long water residence times due to its fjord-like bathymetry and limited water

circulation (Hebbeln & Cortés, 2001; Morales-Ramírez et al., 2015). Nutrient enrichment and other stress factors may be affecting the Golfo Dulce mangroves; these include high metal concentrations, PCB pollution (García-Céspedes, Acuña-González, & Vargas-Zamora, 2004; Spongberg, 2004; Spongberg & Davis, 1998) and coliform contamination from raw sewage discharge in Golfito Bay (García, Acuña-González, Vargas-Zamora, & García-Céspedes, 2006). Timely identification of nutrient enrichment is needed for management of this vulnerable system (Morales-Ramírez et al., 2015), however, the evidence for any impacts is circumstantial; nutrient enrichment in Golfo Dulce is considered to be low or negligible (Morales-Ramírez et al., 2015; Silva & Acuña-González, 2006) and instantaneous measurements may be poor indicators of low level or transitory nutrient inputs.

Stable isotope data (notably $\delta^{13}C$ and $\delta^{15}N$) can provide useful information on food-web structure (Fry, 2006; Peterson & Fry, 1987; Post, 2002), and $\delta^{15}N$ can also indicate anthropogenic nutrient loading (Costanzo, O'Donohue, Dennison, Loneragan, & Thomas, 2001; Costanzo, Udy, Longstaff, & Jones, 2005; Piola, Moore, & Suthers, 2006; Risk & Erdmann, 2000; Rogers, 1999; Teichberg et al., 2010; Udy & Dennison, 1997). The stable isotope signatures of organisms are subject to tissue turnover effects, but have the benefit of time integration such that they may help detect chronic low-level impacts, including pulsed nutrient runoff events. Mangrove macroalgae have an isotopic turnover rate of as little as four days (Costanzo, O'Donohue, & Dennison, 2003; Costanzo et al., 2005), while marine bivalves can show isotopic variation in weeks to months (Lefebvre, Harma, & Blin, 2009; Paulet, Lorrain, Richard, & Pouvreau, 2006; Piola et al., 2006). In contrast, isotopic turnover rates of mangrove trees occur at yearly timescales, which are linked to their slower growth and thus tissue turnover (Costanzo et al., 2003; Pitt, Connolly, & Maxwell, 2009).

Here, we assess potential anthropogenic nutrient enrichment in mangroves of Golfo Dulce, Costa Rica. We hypothesise that $\delta^{15}N$ values in mangrove organisms (trees, bivalves and algae) are higher in mangroves with sewage inputs ('nutrient-loaded') compared to those without them ('reference') indicating subtle, but long-term nutrient enrichment.

2. Methodology

2.1. Study sites

The study was carried out in Golfo Dulce (southern Pacific coast of Costa Rica; Figure 1), which is ca. 20 km long, with maximum depths of 200 m and a 60 m depth sill at its entrance (Hebbeln & Cortés, 2001; Morales-Ramírez et al., 2015; Quesada-Alpízar & Cortés, 2006; Richards, Anderson, & Cline, 1971). Golfo Dulce is considered to be a fjord-like embayment given its shallow entrance, steep borders and deep waters; this leads to limited water circulation and anoxic conditions at depth (Morales-Ramírez et al., 2015). The water column of Golfo Dulce is low in nutrient concentrations (Morales-Ramírez et al., 2015; Silva & Acuña-González, 2006), however, the human population around Golfo Dulce, increased by 9% and 14% in Osa and Golfito districts respectively between the years 2000 and 2009 (data of the Instituto Nacional de Estadística y Censo (INEC) available at http://www.inec.go.cr/estadisticas-vitales). There is no waste water treatment at any location near Golfo Dulce, and raw sewage is directly discharged into the gulf.

To test our hypothesis of point based nutrient loading, six mangrove forest locations were selected based on distance from human settlements with varying population size and nutrient inputs from untreated sewage. Mangroves were considered *a priori* to be 1) minimally impacted (low or negligible sewage input; hereafter 'reference' mangroves; or 2) exposed to untreated sewage discharge (hereafter 'nutrient loaded'). Three reference mangroves (Rincón, Esquinas, Conte), and three nutrient loaded mangroves (Puerto Jiménez, Depósito, Purruja) were sampled in 2009 (Table 1). Reference mangroves were associated with longer and wider rivers that pass mostly through pristine rainforest areas, and through some land used for agriculture. Nutrient loaded mangroves were associated with shorter rivers directly adjacent to urban settlements (Table 1). The mangrove at Puerto Jiménez is located adjacent to Puerto Jiménez town and receives untreated effluents that are discharged into the river. The Depósito and Purruja mangroves lie within a small embayment (Golfito Bay) that receives raw sewage discharge (García et al., 2006) and effluents from the towns of Golfito and Purruja respectively.

A 400 m coastal section along the shoreline was selected at each mangrove, allowing access during both low and high tide. At the shoreline, mangroves were dominated by one species of mangrove tree, the 'red mangrove' (*Rhizophora mangle*), with canopy heights commonly of ca. 5 m at the outer edge. Each section was divided into 20 m sectors to randomly select sites for biological and water sampling.

2.2. Water quality

2.2.1. Sample collection: Water samples were collected at high tide at all six locations in 2009 on two separate dates: April 16-18 considered to represent dry season conditions, and during May 22-24 considered to represent rainy conditions (IMN, 2009; Morales-Ramírez et al., 2015). Water samples were collected within 50-200 m of the shoreline. Six sites of each coastal section were chosen randomly. At each site we: 1) measured Secchi disk depth (m) three times; 2) collected one 1.8 L subsurface water sample at 10 cm depth in a dark plastic bottle for nutrient and suspended sediment determination; and 3) took one 20 mL subsurface water sample for salinity determination using a hand refractometer (ATAGO ATC). Samples were kept on ice until further processing. Each 1.8 L water sample was well mixed before separating into two subsamples for filtration through glass microfiber filters (ALBET FVC 047) using a vacuum pump (Welch Vacuum 2522B-01). One litre was used for chlorophyll a determination and nutrient analysis, and ca. 800 mL for suspended matter using dried pre-weighed filters. Filtered water samples for nutrient analyses were stored in high-density black polyethylene 1 L bottles. Filters and filtered water samples were kept frozen until processed in the laboratory.

2.2.2. Total suspended solids and chlorophyll a analyses: Pre-weighed filters for determination of suspended matter were dried at 60˚C and re-weighed (Sartorius 2842). Weight variation per filter was then related to the total volume of water filtered per sample to determine suspended matter (mg L^{-1}). Filters for chlorophyll determination were placed in 10 mL of acetone 90 % in plastic laboratory tubes with screw caps, and fitted with removable cardboard covers inhibiting light penetration. Filters were macerated with a fine metal rod. Samples were refrigerated for 20 hours, after which 2 mL of acetone 90 % were added and the sample homogenized. Samples were precipitated using a centrifuge for 10 minutes (IEC Clinical Centrifuge

at 5000 rpm). To calculate chlorophyll concentration, the supernatant of each sample was analyzed in a UV visible spectrophotometer (Shimadzu, UV-1700 Pharma Spec), at 630, 645, 663, 665, and 750 nm wavelengths (Strickland & Parsons, 1972).

2.2.3. Nutrient analyses: Previously filtered and frozen water samples were thawed overnight prior to nutrient analysis. Concentrations (μmol L^{-1}) of ammonium, nitrite, nitrate, phosphate, and silicate were determined following the methodology modified for 10 mL samples (Strickland & Parsons, 1972). On the basis of three subsamples per filtered sample the uncertainty of the analysis was estimated (in μmol L^{-1}) as ±2.21 (ammonium), ±0.07 (nitrite), ±0.40 (nitrate), ±0.06 (phosphate), and ±1.78 (silicate). The average of the three replicates per sample was used for statistical analysis.

2.3. Biological sampling

Samples were collected between April 28 and May 1, 2009. Because the relevant biota were not found in all six mangroves, biological samples were collected at only four mangrove locations (two reference mangroves: Rincón and Esquinas; and two nutrient loaded mangroves: Puerto Jiménez and Depósito). Puerto Jiménez mangrove has a narrow marine border due to increased urban development, and to maintain comparability among mangroves, samples were collected in random sectors in the 400 m area near the entrance of the mangrove channel. At each mangrove shoreline five sites were selected randomly from the 20 m sectors; except for Puerto Jiménez where only four sites were selected due to mangrove characteristics. Samples of mangrove leaves (*R. mangle*), epiphytic macroalgae growing on *R. mangle* prop roots (*Bostrychia calliptera*), and bivalves (*A. tuberculosa*) were collected for carbon and nitrogen stable isotope analysis.

***2.3.1. Algae*:** Various samples of the consortium of macroalgal species that grow epiphytic on *R. mangle* mangrove prop roots were collected at each site. A macroalgal sample refers to algae collected from one mangrove prop root. Samples were placed in airtight plastic containers to maintain moisture, kept on ice in the field, and kept frozen until final processing (2 - 4 weeks). Each sample was thawed at room temperature and cleansed of sediment (using freshwater and a plastic mesh coffee filter). The red macroalga *B. calliptera* was the most abundant alga at the greatest number of sites; therefore, material of this species was separated

149 from individual samples for stable isotope analysis. Algal epiphytes on *B. calliptera* thalli, associated
150 organisms, and point of attachment to the mangrove root were carefully removed with forceps under a
151 dissecting microscope. Samples were then placed in 20 mL plastic containers filled with freshwater at room
152 temperature and placed in an ultrasonic bath (Fisher Scientific FS-14) at 5 min intervals to cleanse adhered
153 sediment and rinsed repeatedly until the water came out clear. Samples were then dried at 60˚C for 72 hours.
154 ***2.3.2. Bivalves*:** Benthic bivalves (*A. tuberculosa*) were collected among *R. mangle* prop root sediment at
155 each site within each mangrove, within 5 m of the shoreline. Samples were kept on ice in the field. Maximum
156 length of shells was measured with a steel vernier calliper. The adductor muscle of each bivalve was
157 dissected and kept frozen until drying at 60˚C for 72 hours. At Rincón, bivalves were uncommon at the
158 randomly selected sites, therefore a nearby site within this mangrove with abundant bivalves was sampled on
159 May 2, 2009.
160 ***2.3.3. Mangroves*:** At each of the five sites within each mangrove three leaves from adult *R. mangle* trees
161 were collected. Care was taken in the field to select healthy leaves of similar size, that were fully developed,
162 and without noticeable parasites, discolouration or herbivory. Samples were kept inside plastic bags on ice
163 for transport. Leaf blades were cleaned with a damp cotton cloth and petioles removed, then dried at 60˚C for
164 72 hours. Maximum length and width of each leaf were measured.
165 ***2.4. Stable isotope analysis***
166 At each of the four mangrove locations, 10 samples of leaves and bivalve adductor muscle were analysed, for
167 a total of 40 samples of each. Seven samples of algae were analysed per mangrove, for a total of 28 algal
168 samples. Mangrove leaves were ground in an agate mortar and pestle prior to weighing. Dried biological
169 material was weighed in silver capsules (mangrove leaves: 3.2 ± 0.1 mg; algae: 1.5 ± 0.1 mg; and bivalves:
170 0.5 ± 0.0 mg). To ensure removal of any carbonate material remaining in algal samples prior to stable isotope
171 analysis, 5 % HCl was added to the capsule in which each algal sample was weighed, then allowed to
172 evaporate at 70˚C on a hotplate inside a fume cupboard. Carbon and nitrogen elemental analysis was
173 performed using an Elemental Analyser (CE Instruments NA2500) and an isotope ratio mass spectrometer

(VG Isogas Prism III) (both instruments interfaced with a VG Dual Reference Gas Box and a VG Diluter). Samples were analysed against the marine sediment standard PACS-2 by the National Research Council of Canada (NRCC) with values of: 5.213 ‰ $\delta^{15}N$ and -22.227 ‰ $\delta^{13}C$. Elemental compositions were standardized by comparison with acetanilide standard (C 71.09 %, N 10.36 %). The standard deviation (SD) was ± 0.11 ‰ for $\delta^{13}C$ and ± 0.10 ‰ for $\delta^{15}N$ (n=17 PACS-2 standard analyses). Stable isotope analysis was carried out in the Grant Institute at Edinburgh University. Elemental analysis error (PACS-2 elemental ratio SD as a percentage of the mean elemental ratios) was 1.6 % for C and 5.4 % for N.

2.5. Statistical analysis

One way Analysis of Variance (ANOVA), nested when appropriate, and correlations between variables were carried out in the statistical program R (v2.13.0) (R Development Core Team, 2012). Mangrove and corresponding treatment (reference and nutrient loaded) were factors, with response variables being water quality parameters (two visits) and biological data. Data were tested for normality and heteroscedasticity. Outliers were removed from the analysis only when necessary due to lack of effectiveness from transformations, yet results proved to be the same with or without outliers. Non-parametric Kruskal Wallis analyses were carried out when transformation of data or removal of outliers were unsuccessful.

3. Results

3.1. Water quality

3.1.1. Water transparency: Chlorophyll concentration was higher in nutrient loaded mangroves ($p<0.001$) (Figure 2a). Secchi depth was greater in reference mangroves than in nutrient loaded mangroves ($p<0.001$), which did not vary seasonally ($p>0.05$) (Figure 2b). Suspended matter did not vary between reference and nutrient loaded mangroves ($p>0.05$) (Figure 2c). Both chlorophyll and suspended matter varied between sampling dates ($p<0.001$), where Jiménez and reference mangroves showed a similar trend of lower chlorophyll and suspended matter in the rainy season (Figure 2a,c).

***3.1.2. Nutrient concentrations and salinity*:** Salinity did not vary between reference and nutrient loaded mangroves ($p>0.05$) or by sampling date ($p>0.05$) (Figure 2d). Nitrite concentrations at reference mangroves

199 did not differ from those at nutrient loaded locations ($p>0.05$). Ammonium and nitrate were not included in
200 the statistical analysis as detected concentrations were at the limits of reliable detection (ammonium=1.18;
201 nitrate=0.26). Phosphate and silicate concentrations varied between reference and nutrient loaded mangroves
202 ($p<0.05$). Neither one showed variation within mangroves between sampling dates (both $p>0.05$, Table 2).

203 ***3.2.* $\delta^{15}N$ and $\delta^{13}C$ isotopic values**

204 ***3.2.1. Macroalgae:*** There was no variation in stable isotope composition or C:N of algae between reference
205 and nutrient loaded mangroves ($p>0.05$) (Figure 3), although algal C:N was slightly lower in more $\delta^{15}N$
206 enriched algal samples ($p<0.05$, Table 3).

207 ***3.2.2. Bivalves:*** Bivalve $\delta^{13}C$ was slightly depleted in reference than in nutrient loaded mangroves ($p<0.001$;
208 Figure 3), however bivalve $\delta^{15}N$ and C:N did not vary between reference and nutrient loaded mangroves
209 ($p>0.05$, Figure 3, Table 3). Bivalve isotopic data varied among mangrove locations ($\delta^{15}N$ & $\delta^{13}C$ $p<0.001$;
210 and C:N $p<0.05$). Length of benthic bivalves varied among all locations ($p<0.001$) but showed no variation
211 between reference and nutrient loaded locations ($p>0.05$). Longer bivalves were found at Escondido
212 (5.47±0.17 cm) and Depósito (5.04±0.06cm), and the bivalves were shorter at Puerto Jiménez (4.18±0.28cm)
213 and Rincón (3.79±0.48 cm). No correlations were found for bivalve isotopic results or with bivalve length.

214 ***3.2.3. Mangroves:*** Leaf stable isotope composition varied between reference and nutrient loaded mangroves.
215 $\delta^{15}N$ values were enriched by up to 3 ‰ at nutrient loaded mangroves ($p<0.001$). These mangroves also had
216 enriched $\delta^{13}C$ values ($p<0.001$) and a narrower range in C:N ($p<0.001$, Figure 3, Table 3).

217 **4. Discussion**

218 Here, we provide isotopic evidence of subtle nutrient enrichment in mangrove habitats of Golfo Dulce
219 (southern Pacific coast of Costa Rica), despite limited evidence of nutrient loading from water quality
220 analyses. These findings are suggestive of chronic low-level nutrient loading in this gulf. This isotopic
221 information is important for understanding the state of the mangrove ecosystem, and at the same time
222 providing mangrove, bivalve, and algal stable-isotope data for future studies.

Despite the lack of waste-water treatment and the fact that raw sewage flows directly into Golfo Dulce, previous water quality measurements in this gulf have not found evidence of eutrophication, and this was the case in the present study. The nutrient concentrations in the water column at Golfo Dulce can be considered to be low (Morales-Ramírez et al., 2015; Silva & Acuña-González, 2006) and similar to those of the Gulf of Chiriquí (Pacific coast of Panama) where the highest values of nitrate were 0.75 µmol L^{-1} and of phosphate were 0.24 µmol L^{-1} (D´Croz & O´Dea, 2007). The Gulf of Panama (Pacific coast of Panama, an upwelling region) had slightly higher nutrient concentrations with phosphate reported up to 1.2 µmol L^{-1} and nitrate up to 14.4 µmol L^{-1}. Nutrient concentrations in Golfo de Nicoya, further north on the Pacific coast of Costa Rica, are higher than in Golfo Dulce, with nitrate reported as high as 10.3 µmol L^{-1}, nitrite up to 2.7 µmol L^{-1} and phosphate as high as 3.6 µmol L^{-1} (Palter, León Coto, & Ballestero, 2007). However, nutrient loading is thought to be occurring in Golfo Dulce given increasing anthropogenic pressure (Cortés, 1990; González-Chen, 2009; Loaiza, 2007; Morales-Ramírez et al., 2015; Quesada-Alpízar & Cortés, 2006) and there is evidence of higher coliform bacterial concentrations in the Golfito embayment (García et al., 2006). The Purruja estuary, the location of one of our nutrient loaded mangroves, is not considered to be highly contaminated despite the presence of polluted areas near sewage input (García et al., 2006); this is attributed to dilution and biological uptake of increased nutrients (Silva & Acuña-González, 2006). Nutrient concentration as indicated by traditional chemical analyses may therefore not have the sensitivity needed to identify nutrient loading at low concentrations or where inputs are pulsed, with the potential dilution of nutrient inputs at current loading rates.

Traditional chemical analyses of nutrient concentration have limited capacity for the detection of biologically significant pulsed nutrient inputs to the system (Costanzo et al., 2001; Costanzo et al., 2005; Gartner, Lavery, & Smit, 2002). There is high precipitation during the rainy season in the area, with approximately 5000 mm yr^{-1} (IMN, 2009), which could reduce measured nutrient concentrations and affect perception of the actual nutrient inputs at the mangrove locations during the rainier periods. Nutrient concentrations may be further diluted when sampling at high tide. Water column nutrient concentrations were

sampled on only two occasions at high tide, and may therefore not be representative of the predominant concentrations in the water column throughout the year; however, studies over longer time periods in the gulf have come up with similar findings (Morales-Ramírez et al., 2015). It is also possible that other marine primary producers are consuming nutrients from the water column that were not sampled as part of this study, such as benthic macroalgae which can be seasonal on the Pacific coast of Costa Rica (Cortés, Samper-Villarreal, & Bernecker, 2014) or seagrasses (Samper-Villarreal, Bourg, Sibaja-Cordero, & Cortés, 2014; Samper-Villarreal, Van Tussenbroek, & Cortés, 2018).

In contrast to low nutrient concentrations, water transparency and chlorophyll concentration both revealed diminished water quality at nutrient loaded mangroves. Nutrient loaded mangroves had higher chlorophyll concentrations and lower Secchi depths than reference mangroves. As phytoplankton is nutrient (primarily nitrogen) limited in the Eastern Tropical Pacific (Pennington et al., 2006), an increase in chlorophyll concentrations at nutrient loaded locations may indicate an increase in phytoplankton productivity, which may be depleting nutrients from the water column. At nutrient loaded locations Secchi depth was similar to the limited water transparency found in Golfo de Nicoya (Palter et al., 2007). At Golfo Dulce, the input of nutrients from raw sewage may therefore be either sufficiently diluted, as to not be detectable by chemical analyses, or be at a level that it is biologically assimilated by the biota leading to low nutrient concentrations in the water column. Studies on phytoplankton nutrient uptake and growth rate should be carried out in Golfo Dulce.

Mangrove leaf isotopic values showed enriched $\delta^{15}N$ at nutrient loaded mangroves, while algae and bivalves showed no variation between reference and nutrient loaded mangroves. The most likely explanation for this is the difference in the tissue turnover rate among these three organisms. Mangroves are considered to be good long term (years) indicators of nutrient loading (Costanzo et al., 2003; Pitt et al., 2009), obtaining their nutrients from interstitial water which can accumulate pulsed nutrient inputs (Hogarth, 1999; Kathiresan & Bingham, 2001; Tomlinson, 1994). *Rhizophora* trees produce a new leaf only approximately every 100 days (Farnsworth, Ellison, & Gong, 1996). Algae have high turnover rates because they obtain their nutrients

directly from the water column (Costanzo, O'Donohue, & Dennison, 2000; Costanzo et al., 2003; Fertig et al., 2009; Gartner et al., 2002) and can show enriched $\delta^{15}N$ values within days of exposure to greater nutrient concentrations (Costanzo et al., 2005; Gartner et al., 2002; Savage & Elmgren, 2004). Bivalves obtain their nutrients indirectly from the water column and have a turnover rate of several weeks to months (Fertig et al., 2009; Piola et al., 2006). Lack of variation of bivalve $\delta^{15}N$ between reference and nutrient loaded mangroves could have resulted from the selection of muscle tissue for this analysis, as some tissues of benthic invertebrates are more sensitive indicators of sewage effluent than others (Piola et al., 2006). Lack of variation in algal and bivalve isotopic data between reference and nutrient loaded locations may actually be indicative of prevailing water column conditions during the dry season prior to sample collection. Isotopic analysis of bivalves and algae at different seasons may provide greater clarity on this topic. However, algae $\delta^{15}N$ at low nutrient concentrations can be very low (Costanzo et al., 2001; Costanzo et al., 2005), and on this occasion all algae stable isotope values were similar to those of mangroves at nutrient loaded mangrove locations. This lack of isotopic variation in the bivalve and alga between nutrient loaded and reference locations might indicate that the algae and bivalves have been subject to nutrient loaded conditions at all locations in recent times.

Other possible causes of isotopic variation in mangrove leaves between reference and nutrient loaded mangroves include potential isotopic variation in soil enrichment factors and nutrient sources, which were not sampled as part of this study. Deposition of bloom phytoplankton or suspended matter can occur due to land use in river catchments in Golfo Dulce (Hebbeln & Cortés, 2001). $\delta^{15}N$ from sewage derived organic matter in California goes from 1.8‰ (Van Dover, Grassle, Fry, Garritt, & Starczak, 1992) to 5.6‰ (Kwak & Zedler, 1997), with sewage sources in Golfo Dulce potentially at the higher level of $\delta^{15}N$ from this range. Limited water circulation between mangroves and seasonal deep-water upwelling (Morales-Ramírez et al., 2015; Quesada-Alpízar & Morales-Ramírez, 2004; Svendsen et al., 2006) may also affect isotopic values in Golfo Dulce, which is anoxic in its deepest areas as upwelling is thought to alter marine $\delta^{15}N$ (Risk &

297 Erdmann, 2000; R. Sweeney, Kalil, & Kaplan, 1980; R. E. Sweeney & Kaplan, 1980). Overall knowledge of
298 water circulation in Golfo Dulce is currently limited and the assumption that there are no cumulative impacts
299 at mangrove locations should be reassessed once more detailed information is available.

300 **4.1. Summary and conclusion**

301 Nutrient concentrations determined by traditional chemical water analysis have not been able to
302 identify nutrient loading in Golfo Dulce, despite evidence of punctual increased coliforms and the overall
303 knowledge that untreated sewage is being delivered to the gulf at increasing rates. Our findings support the
304 notion that nutrient concentrations in the water column are low, however, chlorophyll concentration and
305 water transparency reveal diminished water quality at nutrient loaded mangroves. $\delta^{15}N$ of mangrove leaves
306 was enriched at nutrient loaded locations, although algal and bivalve isotopic values did not reveal variations
307 between reference and nutrient loaded mangroves. Nevertheless, the algal isotope values were similar to
308 those of the mangrove leaves at nutrient loaded locations, potentially indicating increased nutrient conditions
309 at all mangroves at the time of sampling. Future evidence of nutrient loading should focus on both isotopic
310 and traditional chemical water quality assessments. At the time of this study nutrient inputs into Golfo Dulce
311 are considered to be at levels that were readily diluted or consumed by the biota, and thus undetectable with
312 traditional water quality techniques. We provide clear evidence however, of isotopic enrichment at nutrient
313 loaded locations from mangrove material and recommend that adequate waste water treatment be carried out
314 on all anthropogenic discharges into this vulnerable marine system.

315

316 **5. Acknowledgements**

317 This study was partially funded by the School of Marine Science and Technology at Newcastle University.
318 Many thanks to all that helped in the field, particularly J.M. Gudiño. CIMAR at the Universidad de Costa
319 Rica carried out nutrient analysis and provided equipment, with greatly appreciated help from J. Acuña and
320 E. Gómez. Thanks to A. Tudhope and C. Chilcott for stable isotope analysis at the Grant Institute, Edinburgh

University. Supported from the IsoNet network project 808-B6-774 at the University of Costa Rica is acknowledged.

6. References

Bouillon, S., Moens, T., & Dehairs, F. (2004). Carbon sources supporting benthic mineralization in mangrove and adjacent seagrass sediments (Gazi Bay, Kenya). *Biogeosciences Discussions, 1*, 311-333.

Chacón-Chaverri, D., Martínez-Cascante, D. A., Rojas, D., & Fonseca, L. G. (2015). Golfo Dulce, Costa Rica, un área importante de alimentación para la tortuga carey del Pacífico Oriental (*Eretmochelys imbricata*). *Revista de Biología Tropical, 63 (Suppl. 1)*, 351-362.

Córdoba-Muñoz, R., & Vargas-Zamora, J. A. (1996). Temperature, salinity, oxygen, and nutrient profiles at a 200 m deep station in Golfo Dulce, Pacific coast of Costa Rica. *Revista de Biología Tropical, 44 (Suppl. 3)*, 233-236.

Cortés, J. (1990). The coral reefs of Golfo Dulce, Costa Rica: distribution and community structure. *Atoll Research Bulletin, 344*, 1-37.

Cortés, J. (2016). The Pacific coastal and marine ecosystems. In M. Kappelle (Ed.), *Costa Rican Ecosystems* (pp. 97-138). Chicago and London: University of Chicago Press.

Cortés, J., Samper-Villarreal, J., & Bernecker, A. (2014). Seasonal phenology of *Sargassum liebmannii* J. Agardh (Fucales, Heterokontophyta) in an upwelling area of the Eastern Tropical Pacific. *Aquatic Botany, 119*, 105-110.

Cortés, J., & Wehrtmann, I. S. (2009). Diversity of marine habitats of the Caribbean and Pacific of Costa Rica. In I. S. Wehrtmann & J. Cortés (Eds.), *Marine Biodiversity of Costa Rica, Central America* (pp. 1-45). Berlin: Springer + Business Media B.V.

Costanzo, S. D., O'Donohue, M. J., & Dennison, W. C. (2000). *Gracilaria edulis* (Rhodophyta) as a biological indicator of pulsed nutrients in oligotrophic waters. *Journal of Phycology, 36*, 680-685.

Costanzo, S. D., O'Donohue, M., Dennison, W., Loneragan, N., & Thomas, M. (2001). A new approach for detecting and mapping sewage impacts. *Marine Pollution Bulletin, 42*, 149-156.

Costanzo, S. D., O'Donohue, M. J., & Dennison, W. C. (2003). Assessing the seasonal influence of sewage and agricultural nutrient inputs in a subtropical river estuary. *Estuaries and Coasts, 26*, 857-865.

Costanzo, S. D., Udy, J., Longstaff, B., & Jones, A. (2005). Using nitrogen stable isotope ratios ($\delta^{15}N$) of macroalgae to determine the effectiveness of sewage upgrades: changes in the extent of sewage plumes over four years in Moreton Bay, Australia. *Marine Pollution Bulletin, 51*, 212-217.

D´Croz, L., & O´Dea, A. (2007). Variability in upwelling along the Pacific shelf of Panama and implications for the ditritubion of nutrients and chlorophyll. *Estuarine, Coastal and Shelf Science, 73*, 325-340.

Donato, D. C., Kauffman, J. B., Murdiyarso, D., Kurnianto, S., Stidham, M., & Kanninen, M. (2011). Mangroves among the most carbon-rich forests in the tropics. *Nature Geoscience, 4*, 293-297.

Duarte, C. M., & Cebrian, J. (1996). The fate of marine autotrophic production. *Limnology and Oceanography, 41*, 1758-1766.

Duke, N. C., Meynecke, J.-O., Dittmann, S., Ellison, A. M., Anger, K., Berger, U., . . . Dahdough-Guebas, F. (2007). A world without mangroves? *Science, 317*, 41-42.

Farnsworth, E., Ellison, A., & Gong, W. (1996). Elevated CO_2 alters anatomy, physiology, growth, and reproduction of red mangrove (*Rhizophora mangle* L.). *Oecologia, 108*, 599-609.

Fertig, B., Carruthers, T., Dennison, W., Jones, A., Pantus, F., & Longstaff, B. (2009). Oyster and macroalgae bioindicators detect elevated $\delta^{15}N$ in Maryland's Coastal Bays. *Estuaries and Coasts, 32*, 773-786.

Fry, B. (2006). *Stable isotope ecology*. Louisiana, USA: Springer.

García-Céspedes, J., Acuña-González, J., & Vargas-Zamora, J. A. (2004). Metales traza en sedimentos costeros de Costa Rica. *Revista de Biología Tropical, 52 (Suppl.2)*, 51-60.

García, V., Acuña-González, J., Vargas-Zamora, J. A., & García-Céspedes, J. (2006). Calidad bacteriológica y desechos sólidos en cinco ambientes costeros de Costa Rica. *Revista de Biología Tropical, 54 (Suppl.1)*, 35-48.

Gartner, A., Lavery, P., & Smit, A. (2002). Use of $\delta^{15}N$ signatures of different functional forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage dispersal. *Marine Ecology Progress Series, 235*, 63-73.

González-Chen, I. (2009). Vecinos de Golfo Dulce temerosos por impacto de granjas atuneras. *Informa-tico*. Retrieved from http://www.informa-tico.com

Hebbeln, D., & Cortés, J. (2001). Sedimentation in a tropical fjord: Golfo Dulce, Costa Rica. *Geo-Marine Letters, 20*, 142-148.

Hogarth, P. (1999). *The biology of mangroves*. Oxford: Oxford University Press.

IMN. (2009). *Meteorological data for Río Claro, Golfito*. Retrieved from: http://www.imn.ac.cr

Jesse, S. (1996). Demersal crustacean assemblages along the Pacific coast of Costa Rica: a quantitative and multivariate assessment based on the Victor Hensen Costa Rica Expedition (1993/1994). *Revista de Biologia Tropical, 44 (Suppl. 3)*, 115-134.

Jiménez, J. A., & Soto, R. (1985). Patrones regionales en la estructura y composición florística de los manglares de la costa Pacífica de Costa Rica. *Revista de Biología Tropical, 33*, 25-37.

Kathiresan, K., & Bingham, B. L. (2001). Biology of mangroves and mangrove ecosystems. *Advances in Marine Biology, 40*, 81-251.

Kwak, T. J., & Zedler, J. B. (1997). Food web analysis of southern California coastal wetlands using multiple stable isotopes. *Oecologia, 110*, 262-277.

Lapointe, B. E., & Clark, M. W. (1992). Nutrient inputs from the watershed and coastal eutrophication in the Florida Keys. *Estuaries and Coasts, 15*, 465-476.

Lapointe, B. E., O'Connell, J. D., & Garrett, G. S. (1990). Nutrient couplings between on-site sewage disposal systems, groundwaters, and nearshore surface waters of the Florida Keys. *Biogeochemistry, 10*, 289-307.

Lefebvre, S., Harma, C., & Blin, J.-L. (2009). Trophic typology of coastal ecosystems based on $\delta^{13}C$ and $\delta^{15}N$ ratios in an opportunistic suspension feeder. *Marine Ecology Progress Series, 390*, 27-37.

Loaiza, V. (2007). Sala IV frena plan para montar granjas de atún en Golfito. *La Nación*. Retrieved from http://www.nacion.com

Morales-Ramírez, Á., Acuña-González, J., Lizano, O., Alfaro, E., & Gómez, E. (2015). Rasgos oceanográficos en el Golfo Dulce, Pacífico de Costa Rica: una revisión para la toma de decisiones en conservación marina. *Revista de Biología Tropical, 63 (Suppl.1)*, 131-160.

Palter, J., León Coto, S., & Ballestero, D. (2007). The distribution of nutrients, dissolved oxygen and chlorophyll a in the upper Gulf of Nicoya, Costa Rica, a tropical estuary. *Revista de Biología Tropical, 55*, 427-436.

Paulet, Y.-M., Lorrain, A., Richard, J., & Pouvreau, S. (2006). Experimental shift in diet $\delta^{13}C$: a potential tool for ecophysiological studies in marine bivalves. *Organic Geochemistry, 37*, 1359-1370.

Pennington, J. T., Mahoney, K. L., Kuwahara, V. S., Kolber, D. D., Calienes, R., & Chavez, F. P. (2006). Primary production in the eastern tropical Pacific: A review. *Progress in Oceanography, 69*, 285-317.

Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics, 18*, 293-320.

Piola, R. F., Moore, S. K., & Suthers, I. M. (2006). Carbon and nitrogen stable isotope analysis of three types of oyster tissue in an impacted estuary. *Estuarine, Coastal and Shelf Science, 66*, 255-266.

Pitt, K. A., Connolly, R. M., & Maxwell, P. (2009). Redistribution of sewage-nitrogen in estuarine food webs following sewage treatment upgrades. *Marine Pollution Bulletin, 58*, 573-580.

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology, 83*, 703-718.

Quesada-Alpízar, M. A., & Cortés, J. (2006). Los ecosistemas marinos del Pacífico sur de Costa Rica: estado del conocimiento y perspectivas de manejo. *Revista de Biología Tropical, 54 (Suppl.1)*, 101-145.

Quesada-Alpízar, M. A., & Morales-Ramírez, A. (2004). Comportamiento de las masas de agua en el Golfo Dulce, Costa Rica durante El Niño (1997-1998). *Revista de Biología Tropical, 52 (Suppl.2)*, 95-103.

R Development Core Team. (2012). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. Retrieved from http://www.R-project.org/

Richards, F. A., Anderson, J. J., & Cline, J. D. (1971). Chemical and physical observations in Golfo Dulce, an anoxic basin on the Pacific coast of Costa Rica. *Limnology and Oceanography, 16*, 43-50.

Risk, M. J., & Erdmann, M. V. (2000). Isotopic composition of nitrogen in stomatopod (Crustacea) tissues as an indicator of human sewage impacts on Indonesian coral reefs. *Marine Pollution Bulletin, 40*, 50-58.

Rodelli, M., Gearing, J., Gearing, P., Marshall, N., & Sasekumar, A. (1984). Stable isotope ratio as a tracer of mangrove carbon in Malaysian ecosystems. *Oecologia, 61*, 326-333.

Rogers, K. M. (1999). Effects of sewage contamination on macro-algae and shellfish at Moa point, New Zealand using stable carbon and nitrogen isotopes. *New Zealand Journal of Marine and Freshwater Research, 33*, 181-188.

Samper-Villarreal, J., Bourg, A., Sibaja-Cordero, J. A., & Cortés, J. (2014). Presence of a *Halophila baillonii* Asch. (Hydrocharitaceae) seagrass meadow and associated macrofauna on the Pacific Coast of Costa Rica. *Pacific Science, 68*, 435-444.

Samper-Villarreal, J., Van Tussenbroek, B. I., & Cortés, J. (2018). Seagrasses of Costa Rica: from the mighty Caribbean to the dynamic meadows of the Eastern Tropical Pacific. *Revista de Biología Tropical, 66 (Suppl. 1)*, In Press.

Savage, C., & Elmgren, R. (2004). Macroalgal (*Fucus vesiculosus*) $\delta^{15}N$ values trace decrease in sewage influence. *Ecological Applications, 14*, 517-526.

Silva, A. M., & Acuña-González, J. (2006). Caracterización físico-química de dos estuarios en la bahía de Golfito, Golfo Dulce, Pacífico de Costa Rica. *Revista de Biología Tropical, 54 (Suppl.1)*, 241-256.

Silva, A. M., & Carrillo, N. N. (2004). El manglar de Purruja, Golfito, Costa Rica: un modelo para su manejo. *Revista de Biología Tropical, 52 (Suppl. 2)*, 195-201.

Silva Benavides, A. M., & Carrión, R. B. (2001). Abundancia y morfometría de *Anadara tuberculosa* y *A. similis* (Mollusca: Bivalvia) en el manglar de Purruja, Golfo Dulce, Costa Rica. *Revista de Biología Tropical, 49 (Suppl. 2)*, 315-320.

Spongberg, A. L. (2004). PCB contamination in marine sediments from Golfo Dulce, Pacific coast of Costa Rica. *Revista de Biología Tropical, 52 (Suppl.2)*, 23-32.

Spongberg, A. L., & Davis, P. (1998). Organochlorinated pesticide contaminants in Golfo Dulce, Costa Rica. *Revista de Biología Tropical, 46 (Suppl. 6)*, 111-124.

Stern-Pirlot, A., & Wolff, M. (2006). Population dynamics and fisheries potential of *Anadara tuberculosa* (Bivalvia: Arcidae) along the Pacific coast of Costa Rica. *Revista de Biología Tropical, 54 (Suppl.1)*, 87-99.

Strickland, J. D., & Parsons, T. R. (1972). *A Practical Handbook of Seawater Analysis* (Vol. Bulletin 167). Ottawa, Canada: Fisheries Research Board of Canada.

Svendsen, H., Rosland, R., Myking, S., Vargas, J. A., Lizano, O. G., & Alfaro, E. J. (2006). A physical-oceanographic study of Golfo Dulce, Costa Rica. *Revista de Biología Tropical, 54 (Suppl. 1)*, 147-170.

Sweeney, R., Kalil, E., & Kaplan, I. (1980). Characterisation of domestic and industrial sewage in Southern California coastal sediments using nitrogen, carbon, sulphur and uranium tracers. *Marine Environmental Research, 3*, 225-243.

Sweeney, R. E., & Kaplan, I. (1980). Tracing flocculent industrial and domestic sewage transport on San Pedro Shelf, southern California, by nitrogen and sulphur isotope ratios. *Marine Environmental Research, 3*, 215-224.

Teichberg, M., Fox, S. E., Olsen, Y. S., Valiela, I., Martinetto, P., Iribarne, O., . . . Tagliapietra, D. (2010). Eutrophication and macroalgal blooms in temperate and tropical coastal waters: nutrient enrichment experiments with *Ulva* spp. *Global Change Biology, 16*, 2624-2637.

Tomlinson, P. B. (1994). *The botany of mangroves*. Cambridge: Cambridge University Press.

Udy, J. W., & Dennison, W. C. (1997). Physiological responses of seagrasses used to identify anthropogenic nutrient inputs. *Marine and Freshwater Research, 48*, 605-614.

Valiela, I., Bowen, J. L., & York, J. K. (2001). Mangrove forests: one of the world's threatened major tropical environments. *Bioscience, 51*, 807-815.

Van Dover, C. L., Grassle, J., Fry, B., Garritt, R. H., & Starczak, V. R. (1992). Stable isotope evidence for entry of sewage-derived organic material into a deep-sea food web. *Nature, 360*, 153.

Wolff, M., Hartmann, H. J., & Koch, V. (1996). A pilot trophic model for Golfo Dulce, a fjord-like tropical embayment, Costa Rica. *Revista de Biologia Tropical, 44 (Suppl. 3)*, 215-231.

480 **Figure legends**

481

482 **Figure 1.** Study Sites: reference (Conte, Rincón, and Esquinas) and nutrient loaded mangrove (Jiménez,
483 Depósito, and Purruja) locations in Golfo Dulce, southern Pacific of Costa Rica (star).

484

485 **Figure 2.** Water column suspended sediment (a), chlorophyll concentration (b), Secchi depth (c), and salinity
486 (d) (mean ±SE) at three reference mangroves (R) and three nutrient loaded mangroves (N) in Golfo Dulce,
487 southern Pacific of Costa Rica. Sampling I = April 2009, Sampling II = May 2009.

488

489 **Figure 3.** $\delta^{13}C$ and $\delta^{15}N$ values of algae (*Bostrychia calliptera*), bivalves (*Anadara tuberculosa*), and
490 mangroves (*Rhizophora mangle*) at three reference mangroves (R) and three nutrient loaded mangroves (N)
491 in Golfo Dulce, southern Pacific of Costa Rica.

492

493

494

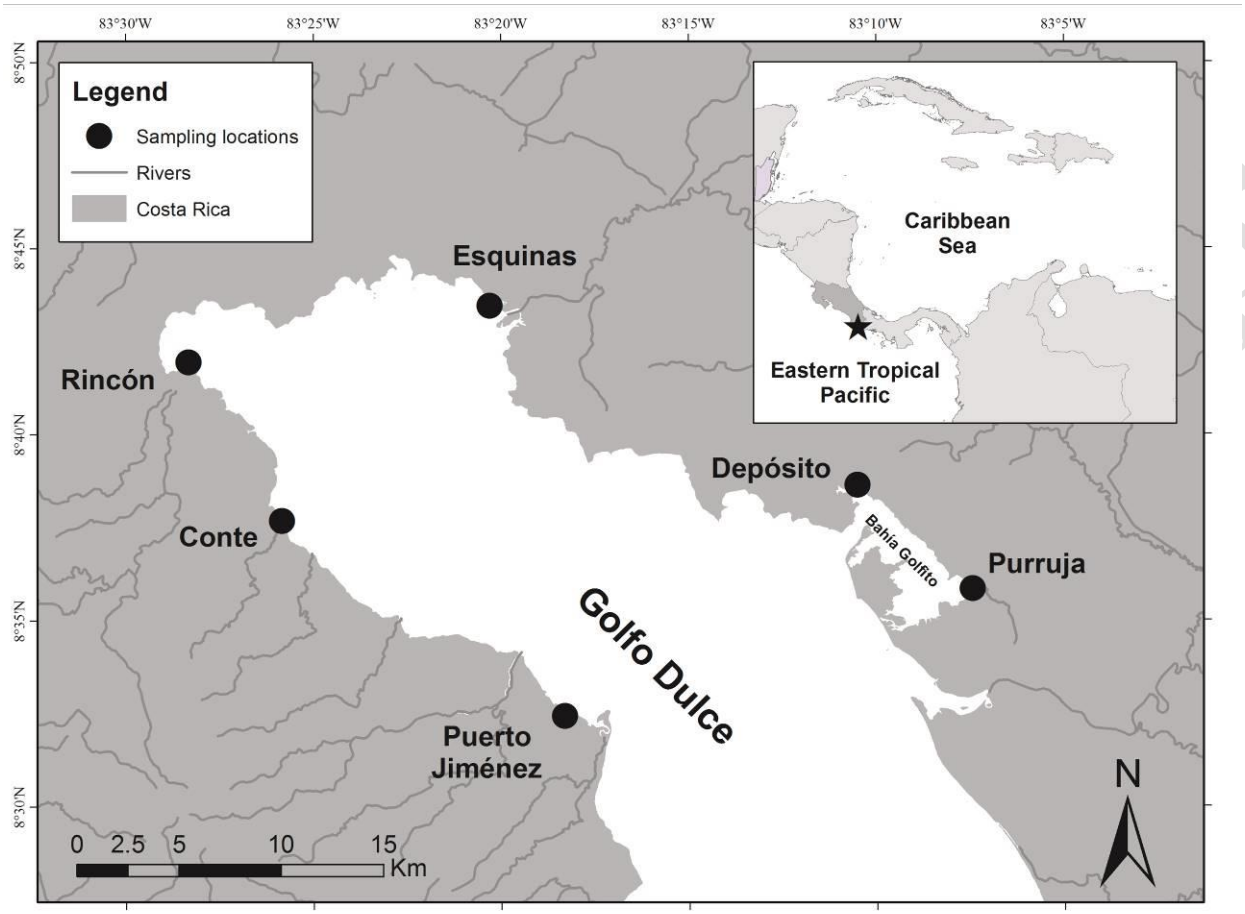

Figure 1. Study site. Golfo Dulce, Southern Pacific coast of Costa Rica.

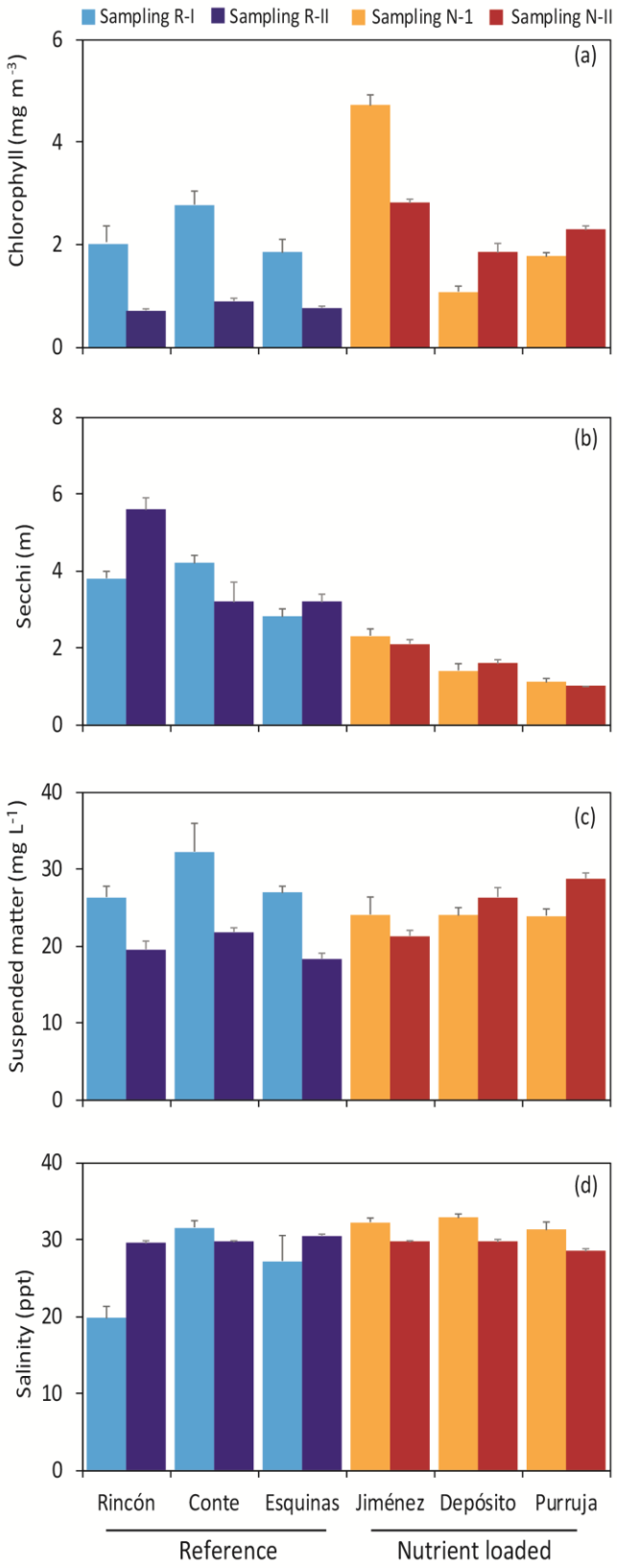

Figure 2. Variation in water quality between reference and nutrient loaded mangroves on two sampling dates.

497

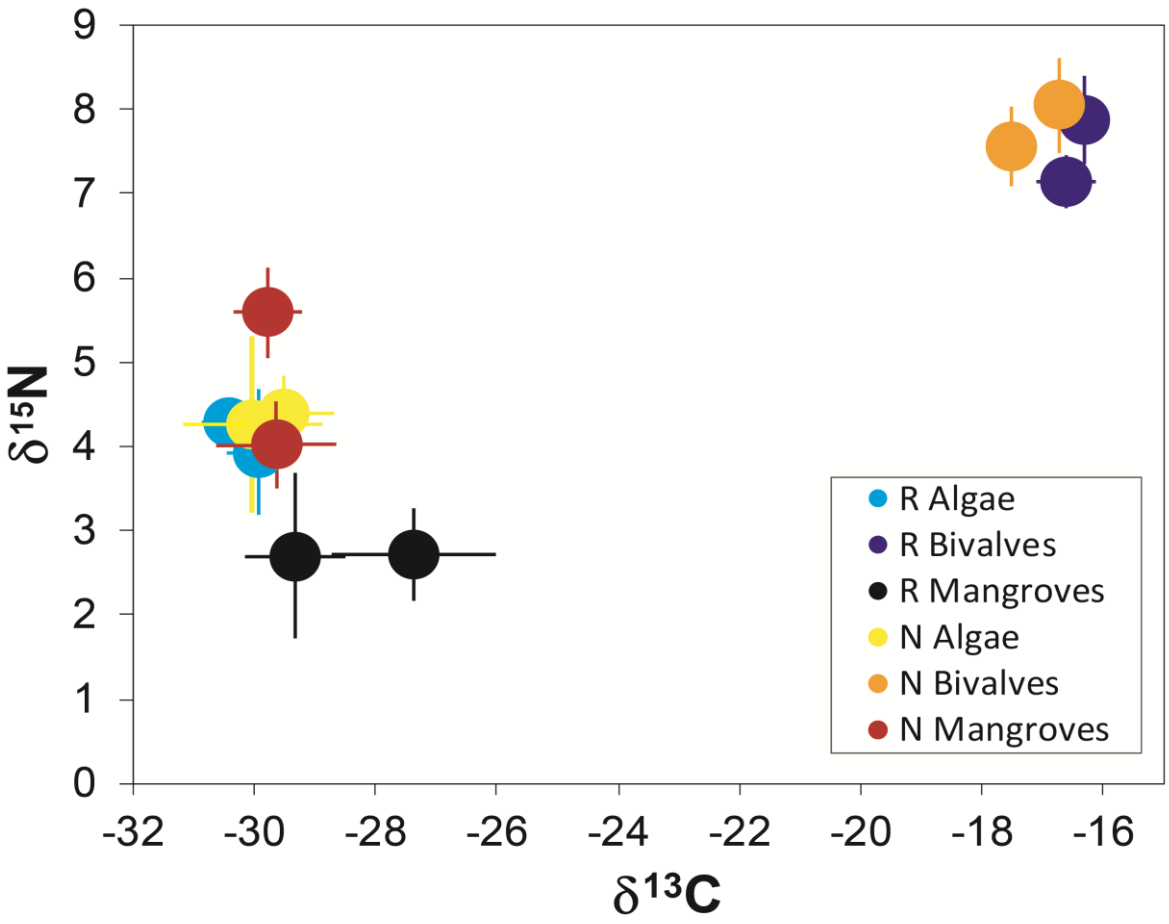

Figure 3. Stable isotope values for mangroves, bivalves and algae in reference and nutrient loaded mangroves.

Table 1. General characteristics of six mangrove habitats in Golfo Dulce, southern Pacific coast of Costa Rica. **Note.** Population source: Instituto Nacional de Estadística y Censo (INEC), Costa Rica (2000 & 2008). Vegetation and river length source: Instituto Geográfico Nacional de Costa Rica map of the area. * Barrigones population was estimated from village size given lack of available data.

Condition	Mangrove	Nearest Town	Population	Characteristics	River	River Length (km)
Reference	Rincón	Rincón	145	Untreated sewage effluent minimal, forest & agriculture	Rincón	27.3
Reference	Conte	Barrigones	~ 100*	Untreated sewage effluent minimal, forest & agriculture	Conte	11.1
Reference	Esquinas	Riyito	66	Untreated sewage effluent minimal, forest	Esquinas	23.0
Nutrient loaded	Jiménez	Puerto Jiménez	7 238	Untreated sewage effluent high	Town estuary	0.8
Nutrient loaded	Depósito	Golfito	13 056	Untreated sewage effluent high	Cañaza	3.4
Nutrient loaded	Purruja	Purruja	436	Untreated sewage effluent high	Purruja	2.4

Table 2. Nutrient concentration (mean ±SE) at six mangrove habitats in Golfo Dulce, southern Pacific coast of Costa Rica. Sampling SI = April 2009, SII = May 2009. n=6. **Note.** Minimum detection levels (µmol/l): phosphate=0.03; silicate=0.78; ammonium=1.18; nitrite=0.03 & nitrate=0.26.

Condition	Mangrove	Sampling	Nutrient Concentration (µmol L^{-1})				
			Phosphate (PO_4^{3-})	Silicate (SiO_4^{2-})	Ammonium (NH_4^+)	Nitrite (NO_2^-)	Nitrate (NO_3^-)
Reference	Rincón	SI	0.28 ±0.07	53.91 ± 11.04	< 1.27	0.052 ± 0.02	< 0.26
		SII	0.19 ± 0.02	16.76 ± 2.26	< 1.18	< 0.03	< 0.26
Reference	Conte	SI	0.40 ± 0.11	13.69 ± 11.59	< 1.27	0.052 ± 0.01	< 0.26
		SII	0.09 ± 0.01	6.42 ± 0.49	< 1.18	< 0.03	< 0.26
Reference	Esquinas	SI	0.10 ± 0.02	8.51 ± 5.00	< 1.27	0.053 ± 0.01	< 0.26
		SII	0.17 ± 0.02	2.29 ± 0.16	< 1.18	< 0.03	< 0.26
Nutrient loaded	Jiménez	SI	0.10 ± 0.02	3.42 ± 0.89	< 1.27	0.063 ± 0.02	< 0.26
		SII	0.09 ± 0.02	4.70 ± 1.00	< 1.18	< 0.03	< 0.26
Nutrient loaded	Depósito	SI	0.22 ± 0.04	23.67 ± 5.45	< 1.27	0.045 ± 0.01	< 0.26
		SII	0.28 ± 0.03	14.21 ± 3.62	< 1.18	< 0.03	< 0.26
Nutrient loaded	Purruja	SI	0.28 ± 0.05	33.40 ± 8.10	< 1.27	0.054 ± 0.01	< 0.26
		SII	0.15 ± 0.01	20.53 ± 0.52	< 1.18	< 0.03	< 0.26

Table 3. Carbon (%), nitrogen (%) and carbon to nitrogen ratios (mean ± SD) for mangrove leaves (n=10), bivalves (n=10) and mangrove macroalgae (n=7) at four mangrove locations in Golfo Dulce, southern Pacific of Costa Rica. (R): Reference mangroves. (N): Nutrient loaded mangroves. * Algae were acidified for inorganic carbon removal.

	Mangrove leaves			Bivalves			Algae*		
	C%	N%	C:N	C%	N%	C:N	C%	N%	C:N
Rincón (R)	44.1 ± 1.2	1.1 ± 0.2	40.3 ± 6.1	46.1 ± 2.4	15.1 ± 1.1	3.1 ± 0.2	33.6 ± 2.9	3.6 ± 0.6	9.4 ± 1.1
Esquinas (R)	45.1 ± 0.6	1.3 ± 0.1	34.5 ± 3.4	41.7 ± 4.0	13.5 ± 1.5	3.1 ± 0.1	31.9 ± 3.9	3.4 ± 0.5	9.5 ± 1.2
Jiménez (N)	43.4 ± 1.6	1.1 ± 0.2	39.0 ± 5.7	43.1 ± 5.0	13.8 ± 1.8	3.1 ± 0.1	30.1 ± 3.5	3.3 ± 0.4	9.2 ± 0.8
Depósito (N)	43.4 ± 1.3	1.2 ± 0.1	36.3 ± 3.3	43.6 ± 1.6	15.6 ± 1.3	2.8 ± 0.2	29.1 ± 2.6	3.4 ± 0.4	8.6 ± 1.0