Life and death of a sewage treatment plant recorded in a coral skeleton $\delta^{15}N$ record

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ABSTRACT

We investigated the potential of coral skeleton $\delta^{15}N$ (CS-$\delta^{15}N$) records for tracking anthropogenic-N sources in coral reef ecosystems. We produced a 56 yr-long CS-$\delta^{15}N$ record (1958-2014) from a reef flat in Guam that has been exposed to varying 1) levels of sewage treatment 2) population density, and 3) land use. The CS-$\delta^{15}N$ record reflected the increase of raw sewage discharge resulting from an increase in the watershed population. However, population change and CS-$\delta^{15}N$ variations become decoupled when a secondary treatment plant opened. This study demonstrates the potential of CS-$\delta^{15}N$ records to yield information regarding the history of human impacts on coastal water quality. Yet, we revealed that sewage treatment systems may lead to underestimation of the human-N footprint in CS-$\delta^{15}N$ records. It is thus crucial to obtain baseline $\delta^{15}N$ values from periods of no or limited human influence.

INTRODUCTION

The tremendous biodiversity hosted by coral reefs provides a wealth of ecosystem services to humankind, including protection against coastal erosion, revenues from ecotourism, food security and a natural reservoir for biomedical and industrially valuable compounds (Ferrario et al., 2014; Fisher et al., 2015; Moberg and Folke, 1999). Yet, coral reefs and their associated services are declining due to increased human pressure (Bruno and Selig, 2007; Hughes et al., 2013; Jackson, 1997; Pandolfi et al., 2003). Globally, ocean acidification and climate change are identified as major culprits of coral reef decline (Pandolfi et al., 2011). However, local factors such as overfishing and eutrophication may additively and/or synergistically compound stress (Cinner et al., 2016; Jackson et al., 2014; Wooldridge and Done, 2009). Eutrophication is particularly acute along densely populated tropical shorelines, putting coastal coral reefs under strong anthropogenic pressure (Duprey et al., 2016; Fabricius et al., 2005; Wear and Thurber, 2015).

Decades of research have demonstrated a wide range of deleterious effects from nutrient enrichment on corals, particularly from dissolved inorganic nitrogen - DIN (Bell, 1992; Bell et al., 2014; De’Ath and Fabricius, 2010; Duprey et al., 2016). N-discharge mitigation is thus a critical step toward coral reef conservation. Yet, identifying anthropogenic-N sources affecting reefs remains challenging, and information on human N-footprint over historical time-scale is scarce (Baker et al., 2013). This lack of data compromises our understanding of reef eutrophication and undermines our ability to study N-pollution (Wear and Thurber, 2015). Stable nitrogen isotopes records ($\delta^{15}N$) of long-lived cnidarians: gorgonians, antipatharians and scleractinians provide a powerful tool to identify N derived from anthropogenic sources and to document past changes in N-sources (Baker et al., 2017, 2010; Erler et al., 2016; Marion et al., 2005; Sherwood et al., 2010; Wang et al., 2015; Williams et al., 2007).

The aragonitic skeleton of scleractinians is built upon an organic matrix which is very stable over time and allows the investigation of N source changes over centuries or millennia (Frankowiak et al., 2016; Muscatine et al., 2005; Yamazaki et al., 2013). Yet, the use of corals to reconstruct changes in N sources over time needs further examination as the interpretation of coral skeleton stable nitrogen isotopes ratio (CS-δ^{15}N) for anthropogenic-N remains debated (Baker et al., 2017; Wang et al., 2015). Additionally, CS-δ^{15}N records obtained from eutrophic reefs of the Great Barrier Reef (GBR, Australia) such as: Magnetic Island, Central GBR (Erler et al., 2016), and the agricultural region of Mackay, Southern GBR (Jupiter et al., 2008), differed from land-use reconstructed eutrophication history (deforestation, cattle, fertilizer use, etc.). Reasons for this mismatch remain unclear and may involve complex nutrient cycling dynamics or N-sources mixing in coastal waters due to heterotrophic/autotrophic feeding, and/or dilution of anthropogenic N sources (Erler et al., 2016, 2015; Wang et al., 2015). Consequently, the correlation between CS-δ^{15}N and anthropogenic N-sources needs to be better constrained by investigating records from location with simpler N-dynamics.

Unlike the GBR, which has multiple natural and anthropogenic N sources, reefs in Guam (Marianas, USA), are mostly dominated by sewage-derived N (Burdick et al., 2008; Porter et al., 2005; Redding et al., 2013). The population of Guam (160,000 in.) releases ~100,000 m^3 of sewage per day, of which 92% receives only primary treatment[1] (Guam Waterworks Authority, 2006). For the last two decades, the seven sewage treatment facilities of the island have been

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[1] Primary treatment removes solids from the sewage but does not remove dissolved nutrients (C, N and P).

characterized by chronic failures from poor maintenance and mismanagement. Failures typically lasted months to years, and resulted in the frequent discharge of raw sewage in coastal waters (Guam EPA, 2013). Sewage derived-N has been shown to increase the coral disease severity on Guam's coral reefs (Redding et al., 2013), highlighting the magnitude of sewage pollution on the island. Thus, the well documented history of sewage pollution in Guam makes this location an interesting ground to assess the potential of CS-δ^{15}N to track the anthropogenic N-footprint on historical timescales, particularly with regards to sewage-N discharge.

The Togcha River watershed, located on the east coast of Guam, covers a relatively small area (5.3km^2), providing a tractable system in which to examine anthropogenic N sources affecting the downstream fringing reef (Fig. 1). The Baza Gardens sewage treatment plant (BG-STP), which discharges effluent in the Togcha River, has served the Baza Gardens community since 1975 and the nearby village of Talofofo since 1990 (Lekven and Constantinescu, 2014). The BG-STP was designed as a secondary treatment plant for a service life of 30 years (1975-2005) but is still in use today (Guam EPA, 2013). Inspections over the 2007-2013 period revealed that the structure was in poor condition and functioned improperly, with bacterial (*Escherichia coli* and *Enterococci*) and dissolved nutrient (N and P) concentrations well above the National Pollution Discharge Elimination System limits (Lekven and Constantinescu, 2014). The Togcha River forms a deep groove that cuts through the 500-m wide reef flat, exposing corals growing along the groove to riverine inputs (Myers and Raymundo, 2009). Thus, this site offers the opportunity to assess the impact of varying sewage treatment levels on CS-δ^{15}N: raw sewage before 1975, secondary treatment from 1975 to 2005 and sub-optimal after 2005.

The objectives of this study are 1) to characterize spatial and seasonal variability of anthropogenic N-sources in the Togcha River and reef flat using conventional indicators; i.e., bacterial assays and macroalgae $\delta^{15}N$, 2) to produce a historical CS-$\delta^{15}N$ record from the Togcha reef flat and, 3) to assess the reliability of the CS-$\delta^{15}N$ record to track various sewage treatment phases (i.e., raw sewage, secondary treatment and suboptimal secondary treatment), population and land-use changes.

MATERIALS & METHODS

Study site – Guam is characterized by cooler seawater temperature (27.5°C) and lower rainfall (1,000 mm.month^{-1}) from January through March and warmer seawater (29.0°C) and higher rainfall (3,000 mm.month^{-1}) from June through September. These periods are referred to as dry and wet seasons hereafter. The Togcha River, located on the southeastern coast (Fig. 1a), is 6.2 km long and is intersected by one 1-km-long tributary (Fig. 1b). Potential anthropogenic inputs into the river system include two golf courses built in 1972 and 1993, private septic tank outflows and the Baza Gardens sewage treatment plant (BG-STP). The collective population of the villages served by the BG-STP (Baza Gardens and Talofofo) is 3,070 (Lekven and Constantinescu, 2014). The BG-STP releases 2,300 m^3 of effluent daily into the Togcha River which has undergone primary treatment (solid removal) and secondary treatment (dissolved organic carbon removal). The BG-STP is not designed to remove dissolved nutrients; i.e., DIN, DIP (Lekven and Constantinescu, 2014).

The Togcha reef flat (also known as Ipan reef flat) is used for recreational activities: fishing, swimming. However, the reef presents signs of severe eutrophication along the groove, and in particular near the river mouth (i.e., where the river enters the reef flat). The coral community present a low biodiversity, composed almost exclusively of *Pocillopora damicornis* and massive

Porites sp. colonies heavily infested by the bioeroding worm *Dendropoma sp.*, and outgrown by the fleshy macroalgae *Padina sp.* and cyanobacterial mats (NND, DMB, LJR, *pers. obs.*). Moreover, this reef flat presents the highest coral disease prevalence out of 15 reefs surveyed by Myers and Raymundo (2009), likely in response to the sewage discharge of the Togcha River.

Study design – We first studied the spatial and seasonal extent of current N-pollution in the Togcha River using conventional indicators of sewage pollution; i.e., macroalgae $\delta^{15}N$ and *Enterococci* count (Baker et al., 2007; Cheung et al., 2015; Moynihan et al., 2012). Subsequently, we produced a 56 year-long CS-$\delta^{15}N$ record from a coral core collected on the Togcha reef flat to assess the applicability of this proxy for tracking anthropogenic N-sources through time. We compared CS-$\delta^{15}N$ variations with various sewage treatment phases, as well as population density, land-use, and rainfall during the last 60 years.

Togcha River survey – *Enterococci* is recommended by the World Health Organization (WHO) over other faecal indicators (e.g., faecal coliforms) to characterize the presence of sewage in fresh and marine recreational waters (EPA, 2012; World Health Organization, 2003). Indeed, *Enterococci* has a high tolerance to saline waters and to solar radiations providing a conservative metric of sewage contamination, thus allowing to survey a tropical stream from its source to its brackish rivermouth (Hanes and Fragala, 1967; Sieracki, 1980). However, elevated *Enterococci* concentrations are commonly found on the streams of tropical islands like Guam and Hawai'i due to the persistence of *Enterococci* in the soil under tropical conditions (Denton et al., 2008; Fujioka et al., 1998). To rule out the background population effect, we measured the *Enterococci* concentration at a reference site located directly at the BG-STP outfall (Site 2; Fig. 1b). We assumed that the residence time of the sewage at this place was too short to be contaminated significantly by soil *Enterococci*, thus providing a reliable measurement of the sewage *Enterococci* concentration. Additional samples were taken at five locations: upstream of the STP (Site 1), upstream from the junction with the tributary (Site 3), upstream from the tributary (Site 4), downstream from the junction with the tributary (Site 5) and at the river mouth (Site 6; Fig. 1b). At each location, water surface samples were collected in sterilized 500 ml HDPE (dark) Nalgene bottles. After collection, water was filtered over a sterile membrane, and filters were placed on Difco® *Enterococcus* selective media and incubated. Although conventional methods use an incubation period of 48 hours at 41°C (APHA, 1999), samples were maintained in the dark at ambient air temperature (i.e., 28±1°C) during 4 days, due to field limitation. Colonies were counted using the software ImageJ® and normalized to colony forming units per 100 ml (CFU ml^{-1}). Three replicates filters from each water sample were used to calculate the mean *Enterococci* concentration. The modified protocol used in the present study is less favorable to *Enterococcus* growth, and as a result, all values obtained in this study are conservative estimates of the real *Enterococcus* concentration.

Fringing reef monitoring – The ubiquitous brown algae *Padina sp.* has been commonly used to record DIN-δ^{15}N over the last 15 years (Derse et al., 2007; Umezawa et al., 2002). *P. boryana* samples were collected at seven sites (replicates per site ≥ 3), along a 500 m-long transect stretching from the river mouth toward the reef crest, to assess the seasonal variability in the N sources affecting the reef flat (Fig. 1b). Samples were collected at 1–2 m depth on the northern edge of the river channel at ~80m intervals. A first sampling was conducted in the dry season (January 2014), followed by a second sampling in the wet season (August 2014). *P. boryana* samples were also collected at Pago Bay (August 2014) to characterize the isotopic ratio of oceanic DIN (Fig. 1a). Pago Bay presents a similar setting as the Togcha reef, with an extended reef flat facing East (Fig. 1a). We assumed that this well flushed area receives only limited sewage, and thus, was considered as a reference site where the N-pool is mostly dominated by oceanic DIN. Samples were collected on the reef slope at 200-250 m from the Pago reef crest at 17 m and 27 m depth.

δ^{15}N *analysis* – Algal holdfasts were removed and blades were carefully screened for fouling (invertebrates eggs, algal epibionts, silt), rinsed with de-ionized water and oven dried at 60°C for 24 h. Dried *P. boryana* samples were homogenized into a fine powder using a mortar and pestle. Three milligrams of each sample were weighed into 4x6 mm tin capsules. Samples were then combusted in a EuroVector, model EA3028 elemental analyzer and the resulting gases analyzed in a Nu Instruments, Perspective series, stable isotope ratio mass spectrometer (EA-IRMS) at the University of Hong Kong (HKU). Results are reported as δ^{15}N values relative to atmospheric N_2. The precision of the analysis was determined based on the measurements of an in-house acetanilide standard (ACET). Precision on δ^{15}N measurements is routinely ±0.2‰ at the HKU laboratory.

Coral core – Samples for CS-δ^{15}N analysis were obtained from a colony of massive coral *Porites sp.* Due to field and cost constraints, a single core was collected from a colony located at 13°21’57”N, 144°46’22”E, at 360 m from the river mouth, inside the reef groove (Fig. 1b). The bottom of the colony was located at 6 m depth. A 70-cm-long core (*reference #GMTO14 - HKU coral cores repository*) was drilled using a 55 mm diameter coring bit. In January 14th, 2014 a 33 cm-long section was cored, and on May 4th, 2014 the coring was continued at the exact same point on the colony, resulting in an additional 37-cm-long segment. The hole left by the coring operations was closed between January and May using a concrete plug to avoid the settlement of bioeroders inside the borehole. The plug was definitively sealed with underwater epoxy putty in May 2014. In May 2016, visual observation revealed that the coral tissues had overgrown the concrete plug, indicating that the health of the colony had not been altered by the drilling.

CS-δ^{15}N analysis – The core was cut into two 10-mm thick slabs. X-ray images of the coral slabs were taken at the Ocean Park Veterinary Hospital (Aberdeen, Hong Kong SAR) to reveal the annual density variation of the skeleton. Annual bands were defined as one couplet of a low density and a high density band (Asami et al., 2005). Fifty-six annual growth bands were identified corresponding to the period 1958-2014. The mean extension rate was 12.2±2.2 mm.yr^{-1} (mean±sd; n=56)[2]. Each annual band was ground using a hand drill and a diamond-coated dental burr. Due to effort and cost constraints of CS-δ^{15}N analyses (Sigman et al., 2001; Wang et al., 2014; Weigand et al., 2016), we choose to produce a biennial record. Biennial sampling design was chosen as it allows tracking reliably decadal environmental variation (Goodkin et al., 2005), while optimizing the number of samples to be analyzed. To do so, bands deposited during an even year were selected (n=29) to build the biennial CS-δ^{15}N record. Samples corresponding to odd years 1973 and 1975 were added to the record to increase the temporal resolution around the opening year of the BG-STP. All selected samples (n=31) were homogenized into a fine powder using an acid-cleaned and combusted pestle and mortar inside a biological safety cabinet (type II, class B) to avoid dust contamination. Powdered samples (weight = 20 mg) received an oxidative cleaning using reagent grade sodium hypochlorite for 24 hr, and were analyzed at the Sigman Laboratory, Princeton University (New Jersey, USA). The N of the coral skeleton

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[2] unless stated otherwise, all mean values in the article are given plus/minus one standard deviation

organic matrix was first oxidized into nitrate using potassium persulfate, then nitrate was converted into nitrous oxide using denitrifying bacteria and analyzed for stable nitrogen isotopes (Sigman et al., 2001; Wang et al., 2015). USGS-40 and USGS-41 organic nitrogen standards and an in-house coral skeleton standard were used to ensure the accuracy of the analyses. The precision of the analysis run was ±0.1 ‰ (n=3), based on the in-house coral skeleton standard.

Datasets – Population density and land-use change within the Togcha watershed were assessed from historical aerial photographs (Fig. S1 and S2) and a recent (2000s) geographic information system shapefile layer showing the footprint of buildings in Guam (Fig. 1a). Population density estimates were obtained by counting the number of buildings within the watershed on the aerial photographs using GIS software (QGIS®). We assumed that all buildings identified were single family homes, and the population was calculated for the years 1948, 1966, 1975 and 1990 by multiplying the number of buildings by the average number of individuals per household in Guam (i.e., 3.15 ind.household^{-1} ; Bureau of Statistics and Plans and Office of the Governor, 2012) and dividing by the watershed area (5.3 km^2). Population density estimates are compiled in Table S1.

Rainfall was found to modulate the N-discharge in Guam (Redding et al., 2013). To assess the influence of rainfall on the N-discharge at the study site, a bivariate linear regression test was run between rainfall (composite monthly time series; 1958-2014; n=1,459 data points; source: www.ncdc.noaa.gov), and CS-δ^{15}N by matching the annually averaged rainfall data corresponding to each CS-δ^{15}N data point.

RESULTS

The extent of the sewage pollution within the Togcha watershed was assessed by measuring *Enterococci* concentrations in the river. The *Enterococci* concentration measured at the reference site (i.e., sewage outflow) was 175±23 CFU.100ml^{-1}. This value was considered as a threshold value for sewage contamination. Concentrations measured at the other sites were similar to the value of the sewage effluent (from 110 ± 22 to 205 ± 11 CFU.100ml^{-1}; Fig. 2). All values were above the US EPA threshold of 104 CFU.100ml^{-1} for a single-sample cut-off for safe recreational waters in fresh and marine environment. *Enterococci* measurements from sites that were not affected by the BG-STP effluent (Sites 1 and 4 – Fig. 2) have similarly high *Enterococci* concentrations.

The extent of the sewage pollution on the reef flat was assessed by comparing δ^{15}N of *P. boryana* collected across the reef flat with the values measured on *P. boryana* specimens collected at the reference site (1.9±0.1‰; Fig. 3). The value found at the reference site was in agreement with the δ^{15}N range reported for macroalgae *Caulerpa serrulata* and *Halimeda micronesica* (~ 2-3‰) collected within the same depth range, at Luminao (Guam), which is also minimally impacted by sewage (Redding et al., 2013). Compared with the Pago baseline, the *P. boryana* samples collected along the Togcha reef flat show enriched δ^{15}N values ranging from 4.8 to 10.1‰ (Fig. 3). Strong seasonal variations are observed within 360 meters of the river mouth with δ^{15}N values ranging from 10.1‰ close to the rivermouth and decreasing to 5.0‰ toward the reef crest during the dry season whereas stable δ^{15}N values fluctuating around 5.1±0.3‰ are observed across the reef flat during the wet season.

Between 1945 and 1950 the Togcha water has experienced intense military development, in particular, with the construction of a military rehabilitation camp (Camp Ethridge; Fig. 4d). However, military personnel were assumed to have left the area by 1950 when the camp was shut down. This is supported by the 1966 aerial photograph showing that all military infrastructures had been dismantled by then (Fig. S2). From the 1948 aerial photograph, the non-military population was estimated at 50 inhabitants (10 ind.km^{-2}; Table S1) and we assumed that it remained unchanged until 1958 when the CS-δ^{15}N record begins. The estimated population density in the watershed was ~26 ind.km^{-2} in 1966 (Fig. 4d). Over the period 1958-1966 the mean CS-δ^{15}N was 6.8±0.3‰, with 1958 presenting the lowest value of the record (6.3‰; Fig. 4a).

The largest population increase was observed from 1966 (~26 ind.km^{-2}) to 1975 (~300 ind. km^{-2}; Fig. 4c). This increase was due to the development of Baza Gardens village, which was completed in 1975 (Fig. 4d). From 1958 to 1975 the overall isotopic enrichment rate of the coral skeleton was 0.7‰.10yr^{-1} (R^2=0.77; p<0.001; n=11). In 1975, when the BG-STP opened, the increasing CS-δ^{15}N curved abruptly and plateaued at 7.6±0.2‰ until the 2000's (Fig. 4a). The connection of Talofofo village (located outside the watershed) to the BG-STP in 1990 added an extra ~2,000 individuals to the existing sewage treatment facilities. This increased the population load of BG-STP up to ~3,600 individuals, for an equivalent population density of ~680 ind. km^{-2} within the watershed (Fig. 4c). There was no noticeable change in the CS-δ^{15}N record after the connection of Talofofo village in 1990 (Fig. 4a). From 1990 to 2014, there was no documented change in the population of both Talofofo Village and of Baza Gardens community. However, the last decade presents the highest CS-δ^{15}N values recorded in the 56 years-long record: 8.1‰

(2004), 7.8‰ (2008), 7.8‰ (2010) and 8.0‰ (2014). An exception is noted for the year 1980 which recorded an anomalously high CS-δ^{15}N value (8.0‰). This event could not be match to any change in land use or population change. Bivariate linear regression analyses showed that rainfall has a significant positive, although weak, effect on the CS-δ^{15}N record explaining 15% of the isotopic record variation (R^2=0.15; $p<0.05$; Fig. S3), revealing that the CS-δ^{15}N record is modulated by rainfall.

DISCUSSION

Coral skeleton δ^{15}N (CS-δ^{15}N) records open new opportunities for investigating nutrient cycling over historical time scales and, in particular, to assess the anthropogenic N-footprint on coral reefs. The Togcha watershed offers an interesting system to assess the reliability of the CS-δ^{15}N record in tracking anthropogenic sources by providing a “simpler” system mostly dominated by sewage-N (Redding et al., 2013). Additionally, reconstruction of historical population changes within the Togcha River watershed reveals that the reef flat has been exposed to a dramatic increase in population density over the last 60 years. For instance, the population density in 1958-1966 was <30 ind.km^{-2}, which is below the current world’s average density (50 ind.km^{-2}; www.data.worldbank.org), however, by 1975, the population (300 ind.km^{-2}) was similar to the current population density of Japan (336 ind.km^{-2}, www.stat.go.jp). By 1990, the apparent population density in the Togcha watershed was ~680 ind.km^{-2} and thus, higher than the populous island of Taiwan (650 ind.km^{-2}, http://eng.stat.gov.tw). Such a population increase offers interesting scenarios to benchmark CS-δ^{15}N records as a metric for human pressure.

In a first step, we characterized current footprint of anthropogenic-N on the river and reef flat under a population density of ~680 ind.km^{-2}. The five sites sampled for *Enterococci* along the river presented concentrations as high as the concentration measured directly at the BG-STP outfall, including sites that are not exposed to the outfall (Fig. 2). This suggests that the entire river is contaminated by sewage coming, not only from the BG-STP, but also from septic tank outflows. Additionally, the use of manure as fertilizer by the golf courses may also explain such high *Enterococci* concentration in the river. However, the golf courses within the Togcha watershed do not use manure as a fertilizer, although artificial fertilizers are used occasionally (B. Quichocho, Talofofo Onward Golf Course manager, *Pers. Com.*, 2016). The consistently high macroalgae δ^{15}N values (+3‰ to +8‰ above the 2‰ baseline) found at the rivermouth and reef flat confirmed that the ecosystem is dominated by sewage derived N all year long (typical δ^{15}N of 10 to 20‰; Heaton, 1986; Kendall et al., 2007). However, the macroalgae isotopic data rules out golf courses as major contributors to N discharge since artificial fertilizers have δ^{15}N values close to ~0 ‰ (Heaton, 1986). Thus, the Togcha reef flat is currently affected by sewage-derived N, mainly from the BG-STP and from septic tank outflows, supporting that the high coral disease prevalence reported at this location is likely linked to sewage-N (Myers and Raymundo, 2009).

The macroalgae data also shows that the discharge of sewage-derived N is modulated seasonally over the reef flat. Stable and low δ^{15}N values (~5‰) are found across the reef flat during the wet season whereas a steep decrease from high (~10‰) to lower (~5‰) δ^{15}N values toward the reef crest is observed during the dry season (Fig. 3). Dilution by rainfall during the wet season cannot explain alone the change in the isotopic composition observed in the macroalgae data, unless rainfall increases wet deposition of isotopically light N, as observed in the South China Sea (Jia and Chen, 2010). Atmospheric N deposition remains largely undocumented in Guam and a change of this amplitude in the macroalgae $\delta^{15}N$ data would imply the deposition of unrealistic amounts of N given the size of the watershed (5.3km^2) and the N-discharged by the BT-STP. This pattern is best explained by a stronger dilution of sewage-N ($\delta^{15}N$ >10‰) by oceanic DIN ($\delta^{15}N$ ~2‰). Because Togcha reef flat is facing East, a strong water mixing occurs over the reef flat when the swell comes from the East (Fig. 1). Data from the National Data Buoy Center (NOAA) reveals that the highest number of days with eastern swell occurs during wet season, when prevailing winds blow from the East, whereas dry season is characterized by East-North-East winds and swell that attenuate water mixing over the reef flat (Fig. S4). The trend observed in the macroalgae $\delta^{15}N$ data indicates thus that sewage-N pollution over the reef flat tends to be alleviated during wet season.

Rainfall has been found to be another important factor in modulating anthropogenic N discharge to Guam's reefs (Redding et al., 2013). The 56 year-long CS-$\delta^{15}N$ time series produced in this study allows to evaluate the importance of rainfall in modulating N-cycling dynamic on Togcha fringing reef. Increased rainfall was found to enrich coastal N with heavy nitrogen at a rate of 0.3‰.10^3 mm.month^{-1} (Fig. S3). A similar rainfall-induced isotopic enrichment of the coastal N-pool has been observed on the west coast of Guam, from soft coral *Sinularia polydactyla* $\delta^{15}N$ records, 1.1‰.10^3 mm.month^{-1} (Redding et al., 2013). These observations suggest that rainfall enhances sewage inputs to the coastal area through increased river discharge, overland runoff, septic tank leaching and/or sewage treatment plant overflow. The fact that the sewage discharge into Guam's coastal waters was modulated by rainfall on both the eastern and the western coasts of the island confirms that the island's sewage collection and treatment system is inadequate and poorly maintained, as suggested previously (Burdick et al., 2008; Myers and Raymundo, 2009; Porter et al., 2005; Redding et al., 2013). Yet, rainfall only accounts for 15% of CS-δ^{15}N variations, highlighting the importance of exploring how land-use, population change and sewage treatment have modulated the extent of the human-N footprint on Togcha reef in the recent past.

The CS-δ^{15}N record presented here encompasses the period 1958-2014 and the lowest population density found within the Togcha watershed was at its lowest in 1958-1966 (<30 ind.km^{-2}; Fig. 4c), as such the average CS-δ^{15}N value calculated from record (6.8±0.3‰) was thus set as the environmental baseline for this period. The isotopic baseline defined for the macroalgae (2‰) does not apply here because of the higher trophic position of corals and of internal N recycling occurring within the coral-algal symbiosis (Erler et al., 2015; Wang et al., 2015). However, the baseline value found in this study is similar to the range of CS-δ^{15}N reported for “pristine” locations of the western Pacific region, 4.2-6.6 ‰ (Wang et al., 2016), suggesting that human densities <30 ind.km^{-2} have only a limited N-footprint on the marine environment.

The following decade experienced a dramatic increase in population, i.e. from <30 ind.km^{-2} to ~300 ind.km^{-2}, paralleled by a CS-δ^{15}N enrichment of ~1‰ (Fig. 4). This enrichment likely results from the changes in the human activities within the watershed. The Baza Garden village development took place in the early 1970s, and the Country Club of the Pacific (CCP) opened in 1973 (Fig. 4). There was no sewage treatment system in the area until 1975; thus, the growing population likely progressively increased the amount of untreated sewage discharge to the reef flat, through direct discharge and/or septic tanks outflow. In addition, the construction of the CCP golf club and the Baza Gardens village involved significant land clearing. Although tropical soil δ^{15}N is less enriched than sewage (8-12‰; Martinelli et al., 1999), the washout of isotopically enriched soil-derived N to the reef flat from land clearing activities may have contributed to enrich the CS-δ^{15}N above the baseline (6.8‰). This indicates that the N-sources on a coral reef ecosystem can change rapidly above a population density of 30 ind.km^{-2}. This supports the hypothesis that CS-δ^{15}N is a reliable metric of human pressure on reefs and stresses the fact that isotopic baselines of natural N-sources must be set during period of low population density and ideally prior to human settlement.

In 1975, the BG-STP began operation, treating raw sewage previously discharged in the Togcha River. Simultaneously with the opening of the plant, CS-δ^{15}N values oscillated around 7.6±0.2‰ until the early 2000s (Fig. 4a). Surprisingly, the connection of Talofofo village to the BG-STP network, in 1990, with an equivalent density of ~680 ind.km^{-2}, was not reflected in the CS-δ^{15}N record (Fig. 4c). The decoupling observed between population density and CS-δ^{15}N may involve several processes. First, the extended sewer network built for the BG-STP to include the Talofofo Village redirected all private sewers and septic tanks to a central sewer network (Lekven and Constantinescu, 2014). Discharge of the central sewer network (Talofofo and Baza Gardens) is less sensitive to heavy rainfall, in comparison with independent private sewers and septic tanks that can easily overflow and release raw sewage. Consequently, the central sewer network may regulate sewage discharge by stabilizing and homogenizing the inputs of anthropogenic N to the reef, although the central sewer network does probably not reduce the amount of sewage released to the Togcha River. Stabilized sewage discharge may thus explain the flattening of the CS-δ^{15}N record at the opening of the BG-STP. However, this explanation is insufficient to explain the decoupling observed between population changes and CS-δ^{15}N variations after 1990.

The second mechanism that could be invoked to explain the decoupling observed between population density and CS-δ^{15}N after 1990 is the dominance of sewage derived-N in the reef's N-pool. Although DIN concentration data are lacking to back up this hypothesis, the curbing of the CS-δ^{15}N record at 1 ‰ above the baseline in the mid-1970s may reflect probably the saturation of the reef N-pool with sewage-N above 300 ind.km^{-2} (Fig. 4a). This highlights a potential limit of CS-δ^{15}N to detect anthropogenic-N sources in heavily polluted environments and may explain the mismatch between documented environmental degradation and CS-δ^{15}N records in the GBR (Erler et al., 2016; Jupiter et al., 2008). The discrepancies observed in the GBR CS-δ^{15}N records may suggest that the environment was already saturated with human derived-N long before the GBR coral records started, decoupling land-use/population changes and the CS-δ^{15}N record.

The decade 2004-2014 provides another interesting example of decoupling between population density and CS-δ^{15}N. Years 2004, 2008, 2010 and 2014 present the highest values (≥7.8‰) observed in the record and this enrichment occurred without any known population increase in the watershed. The obsolescence of the BG-STP and of the sewers network diagnosed in 2007 by Lekven and Constantinescu (2014) may have triggered a change in the sewage-δ^{15}N. In their 2014's report they state: "*The aeration section of the WWTP has inadequate mixing to maintain the activated sludge in suspension. As a result, sludge accumulates in the bottom of the aeration section and the system is operating more like a partial-mix aerated lagoon rather than a completely-mixed activated sludge process*". Improper aeration of the sludge leads to the formation of anoxic zones suitable for microbial denitrification (Piña-Ochoa and Álvarez-

Cobelas, 2006; Solomon et al., 2009). Denitrification converts nitrate into isotopically lighter N_2 by selecting preferentially the lighter isotope, inducing a fractionation of the remaining N pool, resulting in an increased sewage-δ^{15}N (Seitzinger et al., 2006; Seitzinger, 1988). The progressive deterioration of the aeration section may have enhanced the denitrification in the system during the last decades, increasing the isotopic value of the N pool in the river and on the reef flat, resulting in a progressive enrichment of the CS-δ^{15}N (Fig. 4). Further work is needed to confirm this hypothesis, notably by characterizing the loss of N due to denitrification by comparing the DIN concentration at the influent and at the effluent of the BG-STP. However, this suggests that CS-δ^{15}N records are a powerful tool to assess the proper functioning of sewage treatment plants over their entire operational life-time.

CONCLUSION

CS-δ^{15}N records appear be very sensitive to small human population density changes above 30 ind.km^{-2}, and to changes in the level of sewage treatment. Thus, CS-δ^{15}N can be used to track the anthropogenic N-footprint in reef ecosystems over time. However, we also report several processes that can attenuate the magnitude of unnatural N sources in CS-δ^{15}N records. We suspect that the dominance of a single anthropogenic N source can saturate the N-pool, and thus the CS-δ^{15}N record, decoupling human impacts from variation in the geochemical record. Natural processes such as water mixing and rainfall may also modulate N-sources on reef environments and must be taken into consideration when tracking anthropogenic N-sources. We recommend that CS-δ^{15}N baselines must be obtained from records pre-dating human development. Although challenging, such baselines may be obtained from the relatively abundant Pliocene and Early Holocene fossil corals for most Pacific and Caribbean reefs

(Cabioch, 2003; Corrège et al., 2000; Duprey et al., 2012).

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FIGURES CAPTIONS

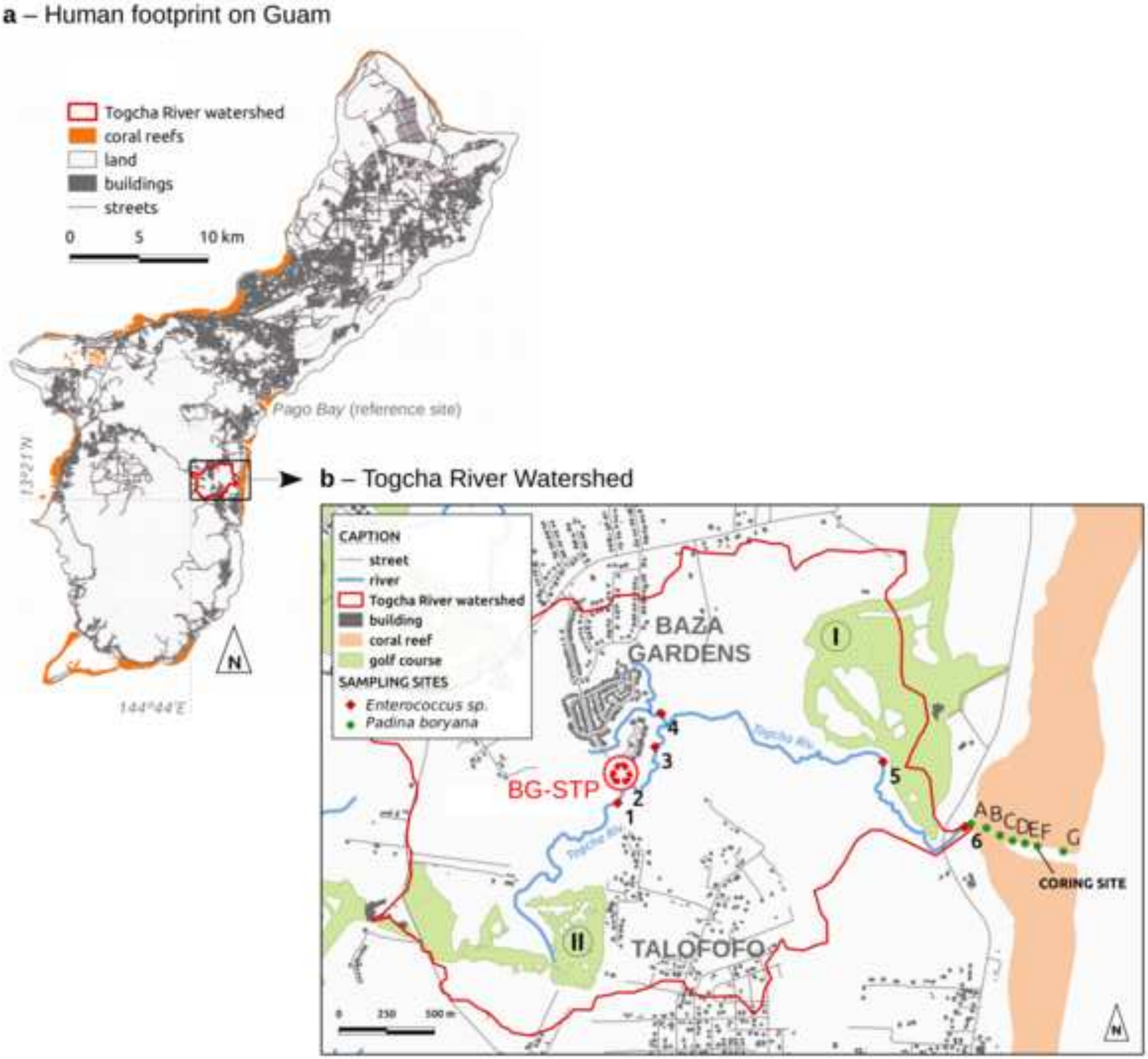
Figure 1: Map of the study site: **a** – Human footprint on Guam and the location of the Togcha River watershed and **b** – detailed map of the watershed showing the potential sources of anthropogenic N: the Baza Gardens Sewage Treatment Plant (BG-STP) and golf courses (I - Pacific Country Club; II - Onward Talofofo Golf Club). Numbers indicate water collection sites for *Enterococci* concentration measurements and letters indicate *Padina boryana* collection sites for isotopic analysis.

Figure 2: *Enterococci* count from sample samples collected in the Togcha River. The number of filters (n) included in the calculation of the mean are indicated below the graph. The dotted line shows the USEPA threshold of 104 CFU.100ml^{-1} for single-sample cut-off for safe recreational waters (fresh or marine).

Figure 3: Stable nitrogen isotopes ratio ($\delta^{15}N$) measured from *Padina boryana* samples collected along a transect stretching from the Togcha river mouth to the reef crest during the dry and the wet season. The blue dot indicates the $\delta^{15}N$ value at the reference site obtained from *P. boryana* samples collected on the reef slope in Pago Bay (Fig. 1a).

Figure 4: Historical records of **a** – coral skeleton $\delta^{15}N$ (CS-$\delta^{15}N$) from core GMTO14. The yellow line shows a 21-years Gaussian smoothing to highlight the long-term trend of the record, **b** –rainfall (composite records, see text for details), **c** – population density of the watershed. The population density is derived from the number of buildings located within the watershed or being connected to the BG-STP (see table S1) **d** – land use change in the watershed.

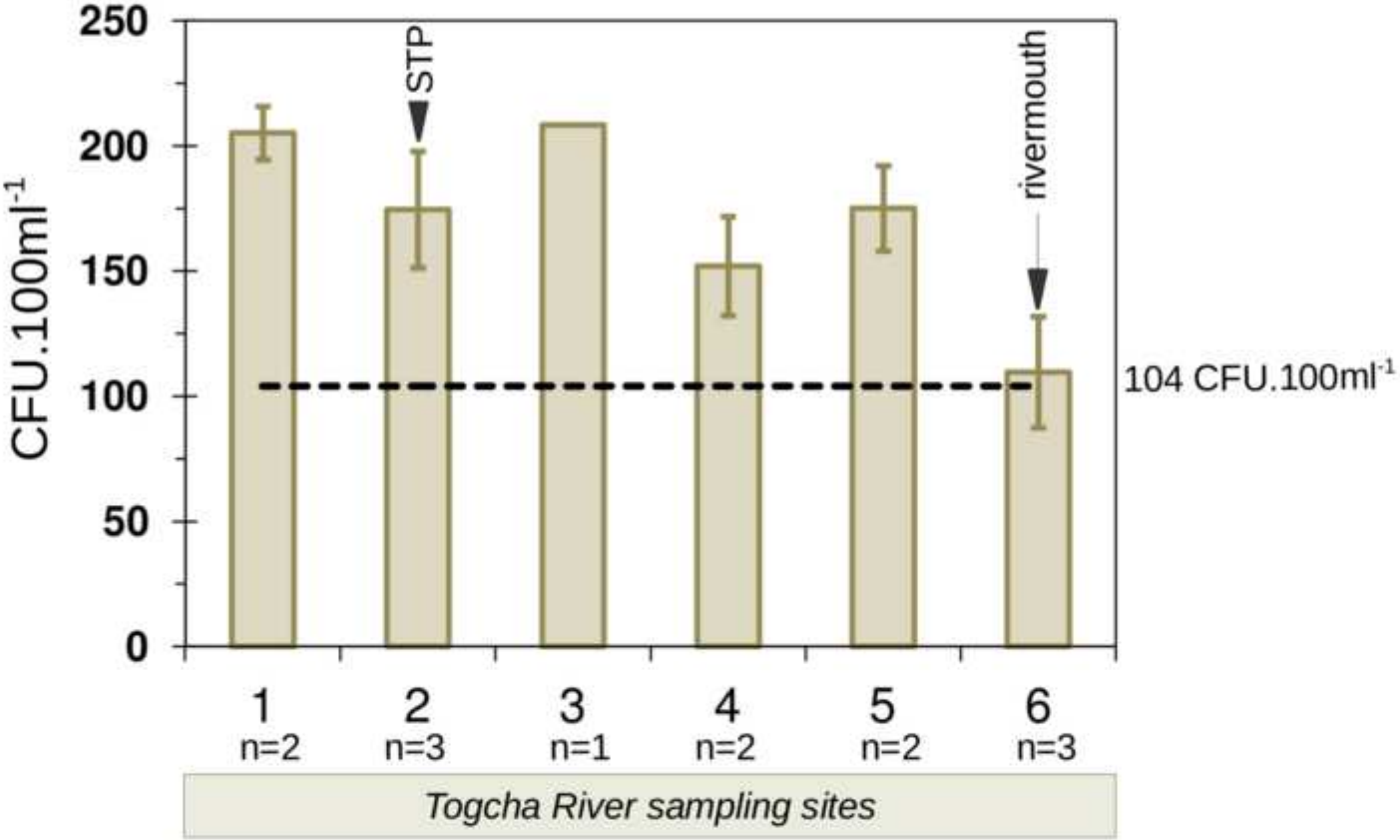
Figure 1



a – Human footprint on Guam

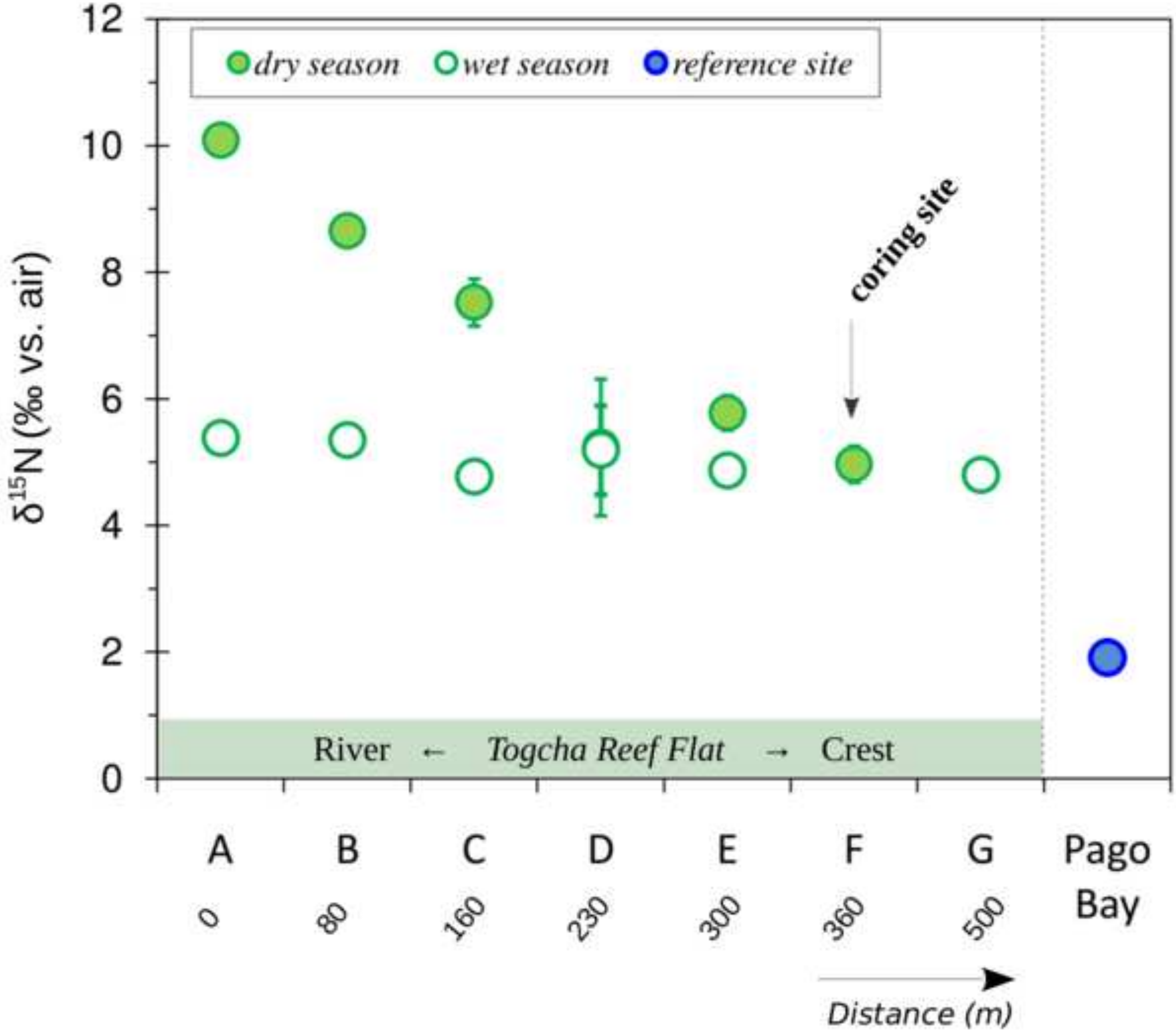
b – Togcha River Watershed

Figure 2



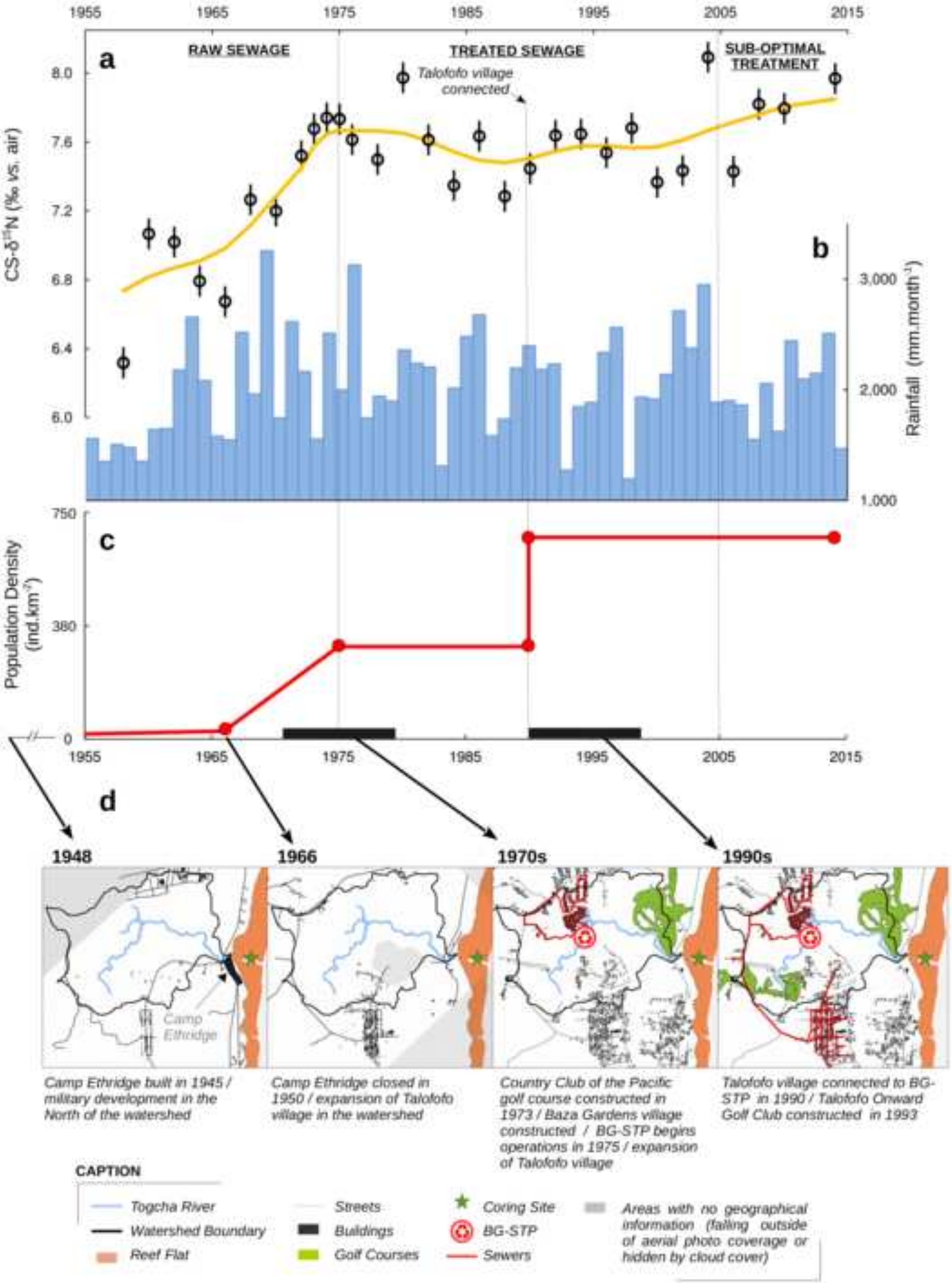
250
200
150
100
50
0
CFU.100ml-1
STP
rivermouth
104 CFU.100ml-1
1
n=2
2
n=3
3
n=1
4
n=2
5
n=2
6
n=3
Togcha River sampling sites

Figure 3



δ15N (‰ vs. air)
12
10
8
6
4
2
0
dry season
wet season
reference site
coring site
River ← Togcha Reef Flat → Crest
A
B
C
D
E
F
G
Pago Bay
0
80
160
230
300
360
500
Distance (m)

Figure 4



Camp Ethridge built in 1945 / military development in the North of the watershed

Camp Ethridge closed in 1950 / expansion of Talofofo village in the watershed

Country Club of the Pacific golf course constructed in 1973 / Baza Gardens village constructed / BG-STP begins operations in 1975 / expansion of Talofofo village

Talofofo village connected to BG-STP in 1990 / Talofofo Onward Golf Club constructed in 1993