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**Paper**

Revised impacts of anthropogenic nutrient inputs on coral reefs in Tobago using  
stable isotope analysis

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## ABSTRACT

This study tests the hypothesis that, in the dry season, coral reef ecosystems are increasingly impacted by anthropogenic nutrient inputs with greater proximity to point-source sewage/domestic waste outfalls in Tobago, south east Caribbean. Stable isotope analysis (SIA) measured spatial and trophic variation of nitrogen and carbon stable isotope ratios ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) in tissues from four coral reef organisms at different trophic levels, and from particulate organic matter (POM) from two sewage/domestic waste outfalls. There was significant  $\delta^{15}\text{N}$  fractionation between species across sites ( $p < 0.001$ ), but organisms progressively distant from effluent outfalls showed no significant differences in  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  between sites, apart from at the highest trophic level.  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of POM from one outfall did not differ significantly from estimated natural marine primary production sources, although did differ in a second outfall. However, discharge from the second was too low at this time of year to reach the sea, rendering it unable to contribute to coral reef production sources. Data correlates with another study, showed significant temporal variability in primary production sources. Together, these data indicate either no significant effect of anthropogenic nutrients from these two point sources on Tobago's coral reefs during the dry season, or that SIA is unable to detect differences due to sewage signals not differing from natural background signals. With increasing use of SIA in ecological studies, this demonstrates importance of appropriate use of monitoring tools, and improved understanding of limitations, to avoid misinterpretation and consequent expenditure on ineffective mitigation strategies.

KEY WORDS: Coral reefs • Anthropogenic nutrients •  $\delta^{15}\text{N}$  •  $\delta^{13}\text{C}$  • Coastal management

## INTRODUCTION

Worldwide, anthropogenic inputs are impeding resilience of coral reefs to natural stressors such as natural riverine nutrient inputs, hurricanes and climate change, thereby reducing their capacity to provide ecosystem goods and services (fisheries, tourism, aesthetic and cultural values) that foster sustainable livelihoods (Bellwood et al. 2004). It is therefore increasingly important to monitor effects of anthropogenic nutrient inputs on coral reefs, to assist coastal management authorities in promoting livelihood sustainability.

Coral reefs in Tobago have exhibited accelerated rates of degradation and reduced biodiversity over the past two decades (Lapointe 2003). Previous SIA of macroalgae on reefs close to areas of high population pressure, indicates that nitrogen isotope ratios increase with increasing proximity to two point sources of anthropogenic nutrient inputs, Bon Accord and Buccoo Sewage Plants (Fig. 1) (hereafter 'point sources') (John 1996, Young 1998, Lapointe et al. 2002, Lapointe 2003), and non-point sources (deforestation, agricultural and urban runoff) (Young 1998). However, signatures of river flooding from the Orinoco in Venezuela in skeletally dense bands in radiograph scans of cross-sections of corals indicate influence on Tobago's reefs by seasonal influxes of nutrient-rich, high turbidity freshwater runoff during the wet season (June to December) (Risk et al. 1992). Marine nutrient monitoring tools, such as SIA, are needed to understand and manage this complex combination of localised anthropogenic nutrients superimposed over natural wide scale nutrient fluctuations.

Anthropogenic discharges tend to have higher  $^{15}\text{N} / ^{14}\text{N}$  ratios ( $\delta^{15}\text{N}$ ) (Fry 2006) due to nitrogen transformations that occur prior to, during, or following treatment and discharge of wastewaters. Other potential nitrogen sources in oligotrophic waters of coral reefs have lower  $\delta^{15}\text{N}$  values, derived from microbial nitrogen fixation or fertilisers

(Yamamuro 1995, McClelland et al. 1997, Lapointe 2003, Yamamuro et al. 2003). Carbon in marine organic matter tends to be enriched in  $^{13}\text{C}$  relative to terrestrial organic matter, due to different primary photosynthetic regimes; marine photosynthesis derives carbon from dissolved  $\text{HCO}_3^-$  (hydrogen carbonate) which is about 7‰ enriched compared with atmospheric  $\text{CO}_2$ , the carbon source for land plants (Gagan et al. 1987).  $^{13}\text{C}$ : $^{12}\text{C}$  ratios ( $\delta^{13}\text{C}$ ) in tissues of organisms tend to be weakly enriched in  $^{13}\text{C}$  by about one per thousand (1‰) compared to their diet (DeNiro & Epstein 1978), thus throughout the food web,  $\delta^{13}\text{C}$  remains similar to basal  $\delta^{13}\text{C}$ .  $\delta^{13}\text{C}$  is therefore useful for indicating source carbon to consumers (Harrigan et al. 1989).  $^{15}\text{N}$ : $^{14}\text{N}$  ratios ( $\delta^{15}\text{N}$ ) exhibit stronger diet-consumer fractionation at around 3.4‰ (Minagawa & Wada 1984), and thus increase with trophic distance of consumers from the food base, thereby indicating trophic level (Vander Zanden & Rasmussen 1999).

Due to rapid development, Tobago (Fig.1) is an ideal site to study influence of anthropogenic nutrient inputs on coral reefs in the context of large scale natural nutrient fluctuations (Lapointe et al. 2002). Previous local SIA work to determine effects of discharges on the marine environment is limited, sampling only  $\delta^{15}\text{N}$  in macroalgae from a small area close to sewage outfalls, without quantifying sewage POM isotope values from these outfalls. From this small spatial scale, it was concluded that these anthropogenic effluent point sources contribute to eutrophication, promoting a macroalgal phase shift (Bellwood et al. 2004) affecting Tobago's coral reefs as a whole (Lapointe et al. 2002).  $\delta^{13}\text{C}$  values, which can determine origins of source carbon to consumers (Harrigan et al. 1989), have not been previously used in Tobago, thus a definitive connection between sewage and coral reef production sources remains unsubstantiated. Furthermore, influence of anthropogenic inputs on trophic position of higher organisms across Tobago's coral reefs is unknown, as accurate baseline species from which to determine higher trophic positions have not previously been identified. Baseline data would elucidate the extent to which anthropogenic nutrient inputs supplement production sources across coral reefs on Tobago's west coast, providing an understanding of mechanisms by which anthropogenic nutrients influence coral reef ecosystems in the context of large scale routine natural inputs, thereby equipping coastal managers with improved quantitative information for implementing appropriate and sustainable mitigation strategies in Tobago, as well as at a regional and global scale.

The objectives of this study are to: i) determine whether there are spatial differences in terms of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of primary producers and primary consumers, between sites increasing in distance from point sources, ii) if there are spatial differences, determine whether these are reflected across higher trophic levels, iii) establish the extent to which any spatial differences are accounted for by point sources, and iv) evaluate differences in outcomes of this study and previous SIA work in Tobago in the context of implications for coastal management approaches.

## MATERIALS AND METHODS

**Study area description.** The twin island nation of Trinidad and Tobago is situated in the south east Caribbean. The southern coast of Trinidad is 7km north of Venezuela, South America. Tobago lies at  $\sim 11^\circ\text{N}$  latitude and  $\sim 60^\circ\text{W}$  longitude, 41.6km north east of Trinidad. The prevailing wind is from the west (Kenny 1976).

**Sampling sites.** Surveying took place between 26<sup>th</sup> April and 1<sup>st</sup> June 2007 (late dry season). Samples were collected from six marine sites on Tobago's west coast (Kariwak, Buccoo Reef Outer Channel (BROC), Mount Irvine, Culloden, Little Englishman's Bay (LEB), and Three Sisters Rocks (Sisters)) (Fig.1) situated at roughly equidistant

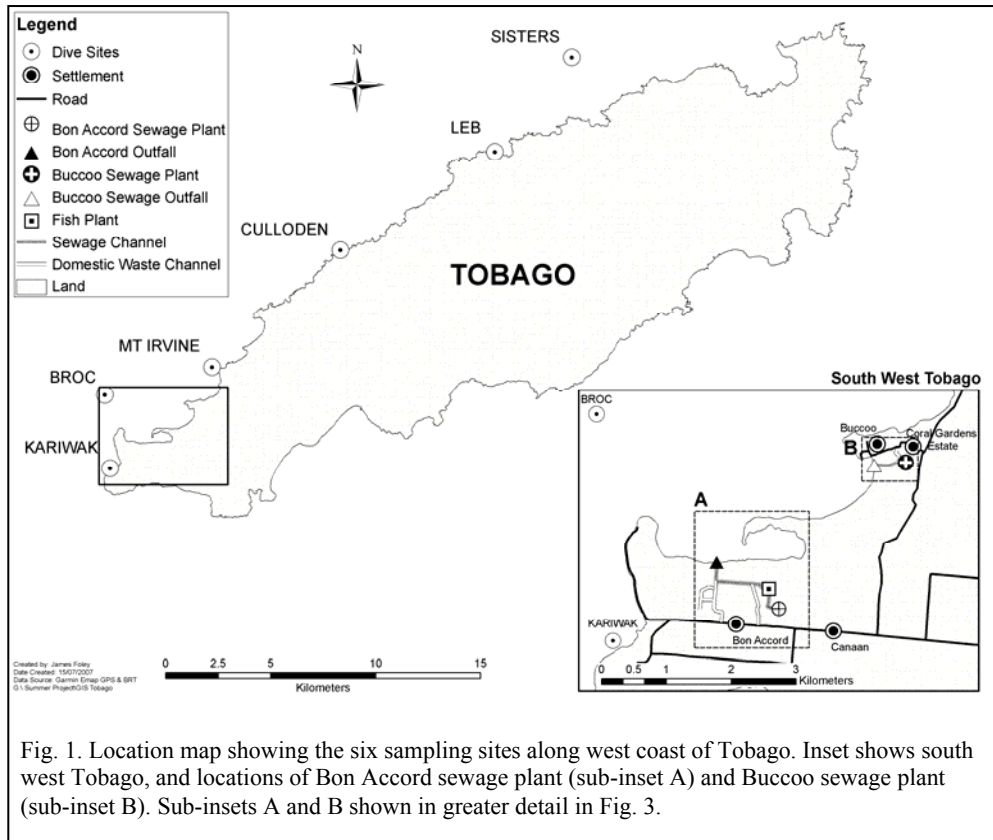


Fig. 1. Location map showing the six sampling sites along west coast of Tobago. Inset shows south west Tobago, and locations of Bon Accord sewage plant (sub-inset A) and Buccoo sewage plant (sub-inset B). Sub-insets A and B shown in greater detail in Fig. 3.

intervals along a reported declining nutrient gradient (Lapointe 2003), north eastwards from two outfalls carrying effluent from Bon Accord and Buccoo sewage plants (BAP and BUP respectively) in south west Tobago, approximately 2.5km apart (Fig. 1, inset). Each plant discharges over 130,000 litres of treated effluent per day (Water & Sewage Authority (WASA), pers. comm.) into sewage channels which are joined by domestic waste channels (Fig. 3: A & B). BAP

effluent drains through a poorly maintained (thickly vegetated) waste stabilising pond. BUP effluent drains several hundred metres to the coast into a large natural vegetated swamp which then drains into the sea. Four marine sampling sites (Kariwak, Mount Irvine, Culloden and LEB) are topographically similar nearshore fringing reefs, with reef surface depth ranging from approximately 5 m shoreward to 14-16 m seaward, thereupon meeting gently seaward-sloping sand. BROCC is a seaward-sloping fore reef on the outer edge of the Buccoo Reef Complex, a marine protected area encompassing a coral reef platform approximately 7km<sup>2</sup> in area (Laydoo 1988), purported to be heavily influenced by anthropogenic nutrient inputs (Lapointe et al. 2002, Lapointe 2003). Sisters comprises three small rocky islets roughly 3km from the west coast of Tobago. Underwater, Sisters was topographically similar to other nearshore fringing reef sites, except for being situated on the east side of the islets, (facing Tobago), thereby deepening in the opposite direction (towards mainland Tobago). Sisters is thought to receive least anthropogenic influence, due to its offshore location and strong currents. Although Kariwak is south of point sources, it is adjacent to a number of hotels, several of which are not connected to the main sewage system, instead discharging raw sewage onto adjoining reefs (Buccoo Reef Trust, personal communication). BROCC, Mt Irvine and Culloden are situated increasingly distant from densely populated south west Tobago, and LEB is adjacent to the sparsely inhabited Tobago Forest Reserve.

**Species selection.** *Dictyota spp* macroalgae were selected to correlate with previous local isotope studies (Lapointe et al. 2002). This species assimilates dissolved inorganic nitrogen (DIN) from seawater (Wallentinus 1984), and therefore may indicate anthropogenic nutrient dispersal. High tissue turnover rates and short life histories prevent primary producers (e.g. *Dictyota*) from providing a reliable time-integrated measure of nutrient inputs, and are therefore poor baseline indicators from which to measure trophic position of consumers (Vander Zanden & Rasmussen 1999). Baselines are required to define trophic position because the pools of nitrogen that support marine food webs

have different  $\delta^{15}\text{N}$  (Owens 1987). This will help to determine if there are spatial differences in  $\delta^{15}\text{N}$  across sites. *Dendostrea frons*, a filter-feeding primary consumer, may assimilate anthropogenic POM if present, and was selected as a more reliable baseline indicator due to larger body size and greater longevity resulting in less seasonality in  $\delta^{15}\text{N}$  signatures (Vander Zanden & Rasmussen 1999). Herbivorous *Stegastes partitus* were selected as a higher trophic level organism, as these are territorial over a small area ( $\sim 1\text{m}^2$ ) (Hixon & Beets 1993), representing site-specific conditions. Invertivorous *Mulloidichthys martinicus* were selected to represent one trophic level above detritivorous invertebrates, and therefore should hold a higher trophic position than herbivorous *S. partitus*. Sewage and waste water POM samples were necessary to determine their contribution to nutrient assimilation of the above species. Without signatures from these sources, it is not possible to quantify connections between anthropogenic inputs and coral reef production sources.

**Field sampling strategy.** Samples were collected along three 20m length ‘transect lines’ in series along 10m depth contours at each site. A ‘transect’ is defined as all three ‘transect lines’ at each site. Water temperature and depth were measured using a Suunto Stinger dive computer (27-28°C at all sites throughout study). Two to four replicates of each species were collected at each site to achieve statistical robustness, one to two from each transect line where possible. Three replicates of *Dictyota spp.* (one from each transect line) were collected and visible inorganic material removed *in situ*. Individual *D. frons* specimens yield only very small amounts of tissue, therefore triplicate sets were collected within 3m of each transect line and pooled to retain sufficient material. Three to four replicates of *S. partitus*, and two to four replicates of *M. martinicus* were caught via spear. Due to abundance of *S. partitus*, all specimens were caught within 3m of respective transect lines. *M. martinicus* were more scarce, therefore specimens caught up to 30m from transects were accepted. It was attempted to control fish for size, however this was only possible for *S. partitus* as *M. martinicus* was scarce at some sites, preventing desired size from being retained on occasions. This is reflected in the heterogeneity of *M. martinicus* results.

**Sample preparation.** On the boat, samples were immediately put on ice. Upon return to land (< 60 min), all samples were frozen to -30°C to prevent isotopic alterations. In the laboratory, *Dictyota spp.* samples were cleaned in distilled water, and remaining material/epibionts removed. *D. frons* specimens were thawed, and bulk soft tissue minus shell weighed to ensure sample comparability before excising the adductor muscle (justification of tissue choice below). Fish were thawed, weighed whole and length measured to ensure sample comparability, and 3-5g of white muscle tissue from under the dorsal fin excised. Adductor muscle for *D. frons*, and white muscle tissue for fish were used because their slow turnover rates result in integrating diet effects over months, thus allowing exclusion of short-term variability effects (Gearing 1991). Immediately after dissection, all tissues were oven-dried at 60°C for 48 hours and ground to fine powder (<100 $\mu\text{m}$ ) to achieve homogeneity. Ground tissue samples were stored in vials in a desiccation unit at -30°C until sample collection and preparation was complete. A 1.0 mg aliquot of each fish and bivalve sample, and a 4.0mg aliquot of each *Dictyota spp.* sample were weighed into individual tin capsules for stable isotope analysis.

**Sewage POM.** Water samples were collected on 1<sup>st</sup> June 2007, in 0.5 l plastic bottles from points in waste water channels downstream from these towards the sea (BA1 and BA2 downstream from BAP, BU1, BU2 and BU3 downstream from BUP) (Fig 3.: A & B). POM was extracted using Whatman GF/F glass filters, oven-dried for 48

hours at 60°C, and placed in a desiccation unit at -30°C to await stable isotope analysis. Basic water quality tests for DIN (ammonium/ammonia ( $\text{NH}_3/\text{NH}_4^+$ ), nitrite ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ )), and phosphate ( $\text{PO}_4$ ) were carried out on the same samples, post-filtration with a Hagen<sup>ltd.</sup> fresh and saltwater aquarium nutrient testing kit. Concentrations are based on visual comparison with colour charts, thus lack precision, but provide a general indication of the association between stable isotopes and water chemistry.

**Isotopic analysis.** Tissue aliquots and POM samples were combusted in an Elemental Analyser (Thermo 1112 Flash) to convert them to a gaseous state, and delivered to a Thermo/Finnigan DELTA V Advantage mass spectrometer via a ConFloIII interface. This yielded raw ratios of heavy to light isotopes in each sample, which were converted to  $\delta$  values in ‰ notation using the formula:  $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where  $X$  is the heavier isotope  $^{13}\text{C}$  or  $^{15}\text{N}$  and  $R$  is  $^{13}\text{C} : ^{12}\text{C}$ , or  $^{15}\text{N} : ^{14}\text{N}$ . Carbon isotope data were calibrated against IAEA standards NBS 19 and NBS 20 and shown as  $\delta^{13}\text{C}$  versus VPDB (Vienna Peedee Belemnite) (Coplen 1996).  $\delta^{15}\text{N}$  values were expressed relative to  $\text{N}_2$  in air. A sample of internal reference material of known  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was used every tenth sample to calibrate the system and compensate for drift with time.

**Lipid correction.** The widely assumed 1‰  $\delta^{13}\text{C}$  trophic fractionation value (DeNiro & Epstein 1978) may be subject to variation due to naturally disproportionate lipid allocation among organism tissues (Sotiropoulos et al. 2004). Therefore a revised lipid-normalisation model (Kiljunen et al. 2006) was tested on fish only (use on invertebrates and primary producers is advised against (Kiljunen et al. 2006)) and full two-way ANOVA rerun on all species'  $\delta^{13}\text{C}$  values to determine if differences between bulk (non lipid-normalised)  $\delta^{13}\text{C}$  and lipid-normalised  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C}'$ ) affect conclusions of the study.

**Statistical analysis.** Data from each species at each site were tested for normality using the Ryan-Joiner method, and in all cases the data were normally distributed. To test for differences across sites and species,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were analysed using full two-way ANOVA with interaction terms (site, species & site  $\times$  species), as this has more power to detect differences than one-way ANOVA. Standard error (SE) was used rather than standard deviation, to provide a size-sensitive indication of data spread due to small replicate numbers.

## RESULTS

Mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  ( $\pm$ SE) for each species and site are shown in Table 1 and plotted in Fig. 2. The average trophic fractionation factor between species was 2.35‰ for  $\delta^{15}\text{N}$  and 0.74‰ for  $\delta^{13}\text{C}$ .

**$\delta^{15}\text{N}$ .** For  $\delta^{15}\text{N}$  values, there was no significant interaction term ( $F_{15,53} = 1.49$ ,  $p > 0.05$ ), therefore two-way ANOVA was re-run excluding the interaction term as this skews the test. There was a small significant difference in  $\delta^{15}\text{N}$  between sites ( $F_{5,68} = 2.87$ ,  $p < 0.05$ ) across the four species, but pairwise comparisons revealed this difference to lie uniquely between BROCC and Sisters ( $p < 0.05$ ). For all other possible pairs of sites, there was no significant difference (all cases  $p > 0.05$ ). There was however a highly significant difference in  $\delta^{15}\text{N}$  between the four species ( $F_{3,68} = 758.25$ ,  $p < 0.001$ ) across all sites (Fig. 2).

**$\delta^{13}\text{C}$ .** The  $\delta^{13}\text{C}$  site-species interaction term was highly significant ( $F_{15,53} = 4.57$ ,  $p < 0.001$ ), therefore pairwise comparisons were run on the interaction term only, showing no significant differences between *Dictyota spp.* across sites ( $p > 0.05$ ) except Culloden *Dictyota spp.*, which was significantly different from *Dictyota spp.* at Sisters, BROCC,

and Mt Irvine (each interaction,  $p < 0.05$ ). There were no significant differences in  $\delta^{13}\text{C}$  between *D. frons* across all sites, nor between *S. partitus* across all sites (all interactions,  $p > 0.05$ ), and no significant differences in  $\delta^{13}\text{C}$  between *M. martinicus* across all sites (all interactions,  $p > 0.05$ ), except for BROC, where *M. martinicus* had significantly different  $\delta^{13}\text{C}$  from this species at all other sites (all cases,  $p < 0.05$ ).

Therefore the null hypothesis, that there is no significant difference in  $\delta^{15}\text{N}$  values at reef sites closer to point sources than those further away, at four different trophic levels is accepted, indicating that during the dry season there is no anthropogenic nutrient gradient away from point sources.

Table 1. Mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values ( $\pm\text{SE}$ ) of the four species collected at each site. Mean C:N ratios were used to calculate lipid-normalised values ( $\delta^{13}\text{C}'$ ) ( $\pm\text{SE}$ ) for fish only, shown in far right hand two columns, using Kiljunen's et al. (2006) revised lipid normalisation model.

Species	Site	n	Mean C:N	Mean $\delta^{15}\text{N}$	SE	Mean $\delta^{13}\text{C}$	SE	Mean $\delta^{13}\text{C}'$	SE
<i>Dictyota spp.</i>	Kariwak	3	17.80	1.77	0.11	-17.05	0.72		
	BROC	3	18.68	2.22	0.50	-15.79	0.86		
	Mt Irvine	3	16.64	2.55	0.03	-16.35	0.55		
	Culloden	3	13.18	2.32	0.24	-19.16	0.28		
	LEB	3	16.58	2.34	0.03	-17.54	1.14		
	Sisters	3	17.92	2.65	0.30	-15.56	1.28		
<i>Dendostrea frons</i>	Kariwak	3	4.17	5.08	0.13	-16.75	0.18		
	BROC	3	4.19	4.66	0.08	-16.40	0.10		
	Mt Irvine	3	4.19	4.74	0.08	-17.38	0.07		
	Culloden	3	4.14	4.83	0.28	-17.20	0.24		
	LEB	3	4.15	4.70	0.06	-17.69	0.20		
	Sisters	3	4.17	4.83	0.11	-17.89	0.17		
<i>Stegastes partitus</i>	Kariwak	4	4.19	8.39	0.50	-16.49	0.09	-14.46	0.12
	BROC	4	4.10	7.61	0.25	-14.98	0.14	-13.07	0.13
	Mt Irvine	4	4.07	7.74	0.32	-15.80	0.14	-13.92	0.15
	Culloden	3	4.00	8.31	0.22	-16.03	0.06	-14.24	0.07
	LEB	4	4.05	8.01	0.13	-15.39	0.19	-13.54	0.18
	Sisters	4	3.94	8.45	0.27	-16.11	0.05	-14.42	0.07
<i>Mulloidichthys martinicus</i>	Kariwak	3	3.92	9.79	0.09	-13.91	0.51	-12.24	0.60
	BROC	3	3.93	8.87	0.49	-11.50	0.61	-9.81	0.61
	Mt Irvine	3	3.98	9.48	0.15	-15.19	0.41	-13.43	0.41
	Culloden	3	3.99	9.71	0.32	-15.27	0.16	-13.49	0.18
	LEB	2	3.99	8.35	0.13	-16.70	0.03	-14.92	0.01
	Sisters	4	3.99	9.78	0.11	-15.54	0.21	-13.77	0.23

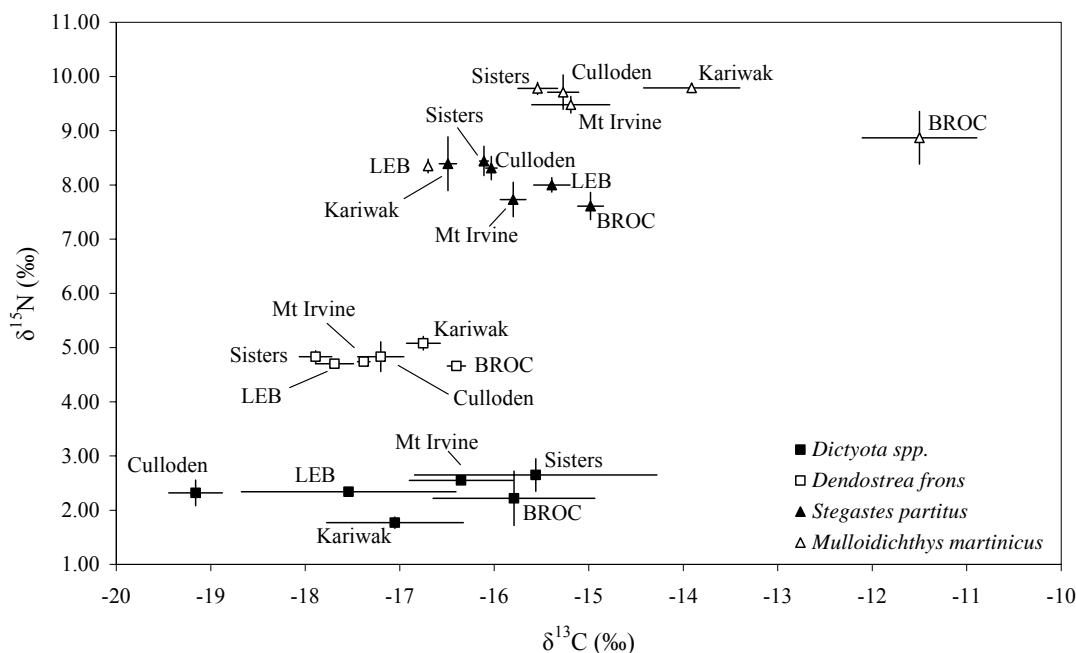


Fig. 2. Average ( $\pm\text{SE}$ )  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios of the four sample species at all six sites (non lipid-normalised).

**Lipid normalisation:** The lipid model shifts all fish species to more enriched  $\delta^{13}\text{C}$  values (Table 1) (average  $\delta^{13}\text{C}$  enrichment due to model: *S. partitus*  $1.86 \pm 0.5\%$  (SE), *M. martinicus*  $1.74 \pm 0.02\%$  (SE)), thus increasing occurrence of significant differences in  $\delta^{13}\text{C}$  fractionation values between fish and non-fish species, but relationships within fish species remain virtually unchanged. This model is based on temperate species, which tend to have higher lipid content (Kiljunen et al. 2006), and therefore it was suspected that the model over-compensates for tropical species. As the model had little effect on relationships among fish, it was discarded because it does not effect overall conclusions of the study.

**Sewage POM.** Sewage POM  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are shown in Fig. 3: A & B, and corresponding water quality data in Table 2. POM  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are plotted to demonstrate position relative to marine species (Appendix Fig. A3). Raw influent  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are similar for both sewage plants (BAP Raw In & BUP Raw In), whilst there is a large difference between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  at seaward ends of each outfall (BA2 & BU3). Effluent leaving BUP (BU2) has become  $\delta^{15}\text{N}$  enriched during treatment. This is explained by the transition in water quality from ammonia to nitrate during treatment. From BU2, this sewage channel meets a domestic waste channel with more depleted  $\delta^{15}\text{N}$  (BU1). At BU3, final outfall  $\delta^{15}\text{N}$  reflects a compromise between both channels ( $5.26 \pm 0.37\%$ ), but  $\delta^{13}\text{C}$  is more depleted than both BU1 and BU2 ( $-27.72 \pm 0.21\%$ ). Conversely, BA1  $\delta^{15}\text{N}$  is depleted in comparison with BAP Raw In, explained by the fact that ammonia-rich BAP raw influent is mostly converted to nitrate during treatment (BAP Effluent, Table 2), but reverts back to ammonia in a poorly maintained stabilisation pond (Fig. 3: A), before reaching BA1. This channel continues past a fish processing plant seen to discharge effluent into this channel (Fig. 3: B), before reaching BA2, the closest sample point to the Bon Accord outfall, where  $\delta^{15}\text{N}$  is  $2.18 \pm 0.17\%$  (lower than at the Buccoo Outfall (BU3)) and  $\delta^{13}\text{C}$  is  $-23.05 \pm 0.03\%$ .

Table 2. Ammonium/ammonia ( $\text{NH}_3/\text{NH}_4^+$ ), nitrite ( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ), and phosphate ( $\text{PO}_4$ ), corresponding with POM isotope values shown in Fig. 3. Note: ‘\*’ =  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values not available. ‘Raw In’ = raw sewage influent.

Sewage POM	$\text{NH}_3/\text{NH}_4^+$ (mg l <sup>-1</sup> )	$\text{NO}_2^-$ (mg l <sup>-1</sup> )	$\text{NO}_3^-$ (mg l <sup>-1</sup> )	$\text{PO}_4$ (mg l <sup>-1</sup> )
BAP Raw In	>8.00	0.00	0	>5.00
BAP Effluent*	0.25	0.25	40	>5.00
BA1	>8.00	0.00	0	>5.00
BA2	4.00	0.25	0	5.00
BUP Raw In	>8.00	0.00	0	>5.00
BUP Effluent*	0.00	0.00	>160	>5.00
BU1	2.00	0.50	10	2.5
BU2	0.25	0.50	80	5
BU3	0.25	0.00	0	3

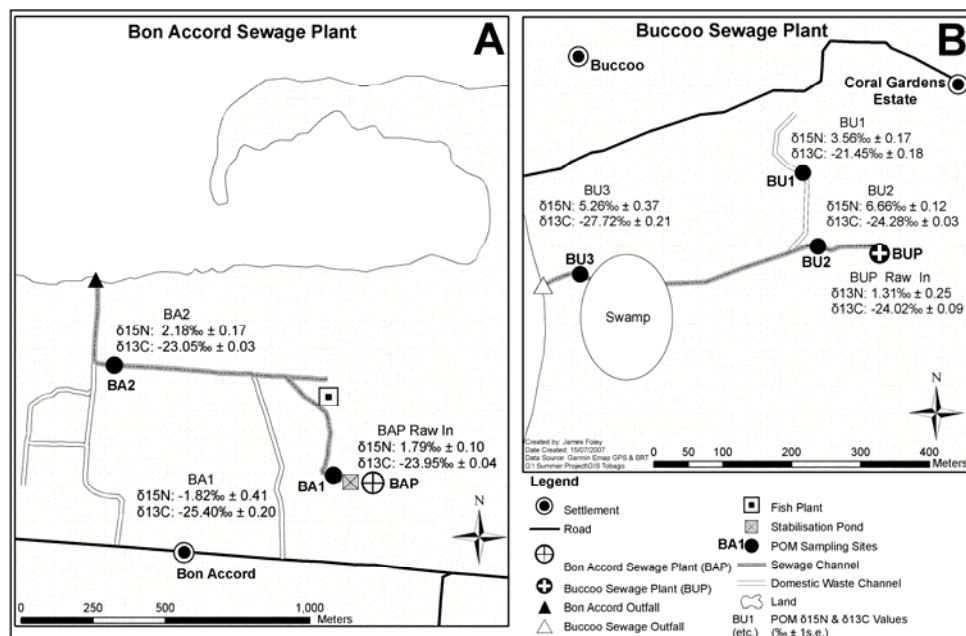


Fig. 3.: Insets A and B (from Fig. 1). A = Bon Accord Sewage plant (BAP), B = Buccoo sewage plant (BUP). Sampling points with POM  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  shown on map. For BUP Raw In, BU3 and BA1  $n = 4$ . All other samples  $n = 5$ . Water quality shown in Table 2.

## DISCUSSION

**$\delta^{13}\text{C}$  site.** Minimal differences in  $\delta^{13}\text{C}$  were found in any species across the six marine sites, suggesting that sewage derived carbon from the point sources is not contributing to organism diet at any site. However, there is greater  $\delta^{13}\text{C}$

variation across sites for *M. martinicus*, possibly due to their greater mobility and foraging distance over *S. partitus*. Gut content analysis would need to be carried out to establish this. There is substantial  $\delta^{13}\text{C}$  variation in *Dictyota spp.* demonstrating inherent variability in primary producers due to seasonality, depth, salinity, light etc. (Mann & Lazier 1996).

**$\delta^{13}\text{C}$  species.** All species at BROC generally exhibit higher  $\delta^{13}\text{C}$  values than any other site (Fig. 2). This  $\delta^{13}\text{C}$  enrichment may indicate a more marine-derived carbon source than at other sites, whereby *Dictyota spp.* at BROC may be deriving carbon from natural dissolved  $\text{HCO}_3^-$  (Gagan et al. 1987), which is then reflected up the food web. This could be due to BROC being further from shore than most other sites (Fig. 1), as  $\delta^{13}\text{C}$  of macrophytes has reflected sampling location in other studies (e.g. Yamamuro et al. 1995). This theory is supported by concurrently enriched  $\delta^{13}\text{C}$  in *Dictyota spp.* at Sisters, situated approximately equidistant from shore. This suggests that any impacts as a result of point source effluents is confined to inshore areas, possibly due to prevailing wind from the west blowing onshore.

**$\delta^{15}\text{N}$  site.** Species at Sisters were marginally more enriched in  $\delta^{15}\text{N}$  (except *D. frons*) than at other sites. This is interesting, as sites closer to point sources were predicted to have higher  $\delta^{15}\text{N}$  than those further away. A large bird colony was seen to inhabit the rocks at this site, and so increased  $\delta^{15}\text{N}$  values could have arisen from guano runoff into adjacent waters, as seen elsewhere (Golovkin 1967). Besides this, there is little difference in  $\delta^{15}\text{N}$  among species at different sites (Fig. 2). *D. frons* is a good baseline species from which to infer trophic positions of consumers as there is little variation in  $\delta^{15}\text{N}$  among individuals at each site (Fig. 2). Assuming sewage POM contributes to their diet, this species should show a  $\delta^{15}\text{N}$  fractionation of sewage POM  $\delta^{15}\text{N}$  plus 3.4‰ (Minagawa & Wada 1984). At Bon Accord outfall, sewage POM from BA2 has a  $\delta^{15}\text{N}$  of  $2.18 \pm 0.17\text{‰}$ . Therefore *D. frons* should have a  $\delta^{15}\text{N}$  value of this plus 3.4‰ (~5.58‰). This is not much higher than observed values (Table 1.), and as natural POM is likely mixed with sewage POM (Shumway et al. 1987), values closer to those observed are expected. However, lack of significant differences in  $\delta^{15}\text{N}$  across sites for this species indicates that sources of productivity driving these systems (or at least  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of these sources) do not differ across sites, suggesting that either there is no sewage POM present, or that SIA cannot differentiate it from natural marine POM.

**$\delta^{15}\text{N}$  species.** Baseline *D. frons*, a primary consumer, can be assumed to have a trophic position of 2, and from this the next higher trophic position at each site can be estimated, and assigned to organisms (Owens 1987). Resultant values from adding 3.4‰ (one trophic level) to mean  $\delta^{15}\text{N}$  values for *D. frons* from each site, closely approximate observed  $\delta^{15}\text{N}$  values for *S. partitus* (e.g. Kariwak *D. frons*  $\delta^{15}\text{N} = 5.08\text{‰}$ , + 3.4 ‰ = 8.48‰, expected value for trophic level 3, Kariwak *S. partitus* observed  $\delta^{15}\text{N} = 8.39\text{‰}$ ), thus *S. partitus* occupies trophic level 3. Assuming that *S. partitus* is a herbivore and *M. martinicus* is an invertivore (Humann & DeLoach 2003), these two species exhibit less trophic separation than expected. It is possible that *S. partitus* is less herbivorous and/or *M. martinicus* is less invertivorous than originally thought. As many herbivorous fish are known to consume protein-rich fish faeces to supplement their diet (Robertson 1982), this may be causing elevated  $\delta^{15}\text{N}$  values in *S. partitus*, since this highly territorial fish is unlikely to sustain its dietary requirements from the small area over which it presides. Goatfish roam over a much larger territory, making the need for dietary supplementation less likely in this species.

Two sites in this study (BROC and Mt Irvine), correlate with previous work carried out at the same time of year (April – June 2001) on *Dictyota spp.* by Lapointe et al. (2002). To gauge temporal variability, findings from both

studies were compared (Appendix Fig. A1, Fig. A2). At BROC, Lapointe et al. (2002) determined  $\delta^{15}\text{N}$  of *Dictyota spp.* to be  $6.4 \pm 0.05\text{‰}$ , compared with  $2.22 \pm 0.50\text{‰}$  in this study, and at Mt Irvine  $\delta^{15}\text{N}$   $5.9 \pm 0.8\text{‰}$  compared with  $2.55 \pm 0.03\text{‰}$  in this study. With their values, primary production sources should have a  $\delta^{15}\text{N}$  value of between 2.5‰ and 3.0‰. In the present study, BA2  $\delta^{15}\text{N}$  is too low ( $2.18 \pm 0.17\text{‰}$ ) and BU3  $\delta^{15}\text{N}$  too high ( $5.26 \pm 0.37\text{‰}$ ) (Fig. 3: A & B) to reasonably be a major contributor to nutrient uptake in macroalgae in Lapointe's et al. (2002) study. This suggests significant temporal variability in natural and/or anthropogenic production sources.

**POM  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .**  $\delta^{13}\text{C}$  of POM from Bon Accord outfall (BA2:  $-23.05 \pm 0.03\text{‰}$ ) is within the range acceptable for interpretation that *Dictyota spp.* ( $\delta^{13}\text{C}$   $-19.16\text{‰}$  to  $-15.56\text{‰}$ ) and *D. frons* ( $\delta^{13}\text{C}$   $-17.89\text{‰}$  to  $-16.40\text{‰}$ ) are using this as a supplement production source. However, BA2 POM  $\delta^{13}\text{C}$  does not differ greatly from reported natural primary sources under similar conditions (e.g. Yamamuro et al. 1992, natural marine POM  $\delta^{13}\text{C}$ :  $-22.0\text{‰}$  from Palau, latitude  $7^\circ\text{N}$ , water temperature  $28^\circ\text{C}$ ), Although Buccoo sewage outfall had more negative  $\delta^{13}\text{C}$  POM ( $-27.72 \pm 0.21\text{‰}$ ) (Fig. 3: B) than Palau values, no discharge was exiting Buccoo outfall (seaward of BU3) throughout the seven weeks of this dry season study (personal observation), preventing it from contributing to production sources on nearby coral reefs. Assuming absorptive capacity of Buccoo swamp (Fig. 3: B) exceeds discharge of anthropogenic nutrients into it during the dry season, only Bon Accord outfall (BA2) can impact coral reefs at this time of year. If POM  $\delta^{13}\text{C}$  between Bon Accord effluent (BA2) and natural primary production sources in Tobago are similar as values from Yamamuro et al. (1992) suggest, SIA is not an appropriate tool for marine trophic food web studies in Tobago, at least in the dry season, as it cannot differentiate between isotopically similar natural and anthropogenic inputs. If discharges increase from BUP beyond the rate of uptake by the swamp for long periods (months) during the wet season, and allow BUP discharge to reach the sea, then the more negative  $\delta^{13}\text{C}$  signature from this outfall could become traceable in a subsequent wet season study, assuming observed  $\delta^{13}\text{C}$  at BU3 remains perennially constant. However, mineralisation of swamp vegetation detritus induces a shift in  $\delta^{13}\text{C}$  to more negative values (Marguiller et al. 1997), and as there are likely to be higher rates of decomposition in the swamp during the dry season when swamp water movement is poor, sewage POM  $\delta^{13}\text{C}$  at BU3 may become less negative during the wet season. Wet season data is therefore needed in order to verify whether there is a difference in POM  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  at the Buccoo outfall.

Lapointe (2003) found macroalgae had higher mean  $\delta^{15}\text{N}$  in the wet season ( $+5.44 \pm 0.72\text{‰}$ ) compared with the dry season ( $+2.88 \pm 0.16\text{‰}$ ), concluding that this indicated greater spatial extent of sewage dispersion in the wet season. Whilst this is not unlikely, BU3 in this study had high  $\delta^{15}\text{N}$  values ( $+5.26 \pm 0.37\text{‰}$ ) (Fig. 3), therefore higher macroalgal  $\delta^{15}\text{N}$  during the wet season could be due to this higher  $\delta^{15}\text{N}$  production source reaching the ocean, rather than there being a greater spatial extent of sewage dispersion in the wet season. Additionally, POM  $\delta^{15}\text{N}$  at BA2 ( $2.18 \pm 0.17\text{‰}$ ) was lower than typical for sewage (Costanzo et al. 2001) so that it cannot be said that there is less impact from sewage during the dry season. This low value could be explained by poor treatment, as BA1 was ammonia-rich (Table 2).

#### CONCLUSIONS & MANAGEMENT RECOMMENDATIONS

Lapointe et al. (2002) pioneered important advances in the application of modern monitoring tools in ecological nutrient impact studies in Tobago, concluding that anthropogenic nutrient inputs may be causing eutrophication of coral reefs. While this is reasonably the case, lack of stable isotopic variation across sites among different trophic

levels found in the present study indicates that spatial extent of these impacts is either smaller than Lapointe et al. (2002) concluded (i.e. not extending beyond the immediate vicinity of the outfalls), or that effluent discharging from BAP could be present across sites, but that its isotopic signatures are not significantly different from background marine POM, thus preventing SIA from differentiating between sources. In either case, the results obtained from SIA in this study did not support the hypothesis that in the dry season, coral reef ecosystems in Tobago are increasingly impacted by anthropogenic nutrient inputs with greater proximity to point-source sewage/domestic waste outfalls.

Studies often do not measure  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of sewage POM, assuming values derived from marine primary producers and primary consumers. However, without temporally and spatially specific sewage POM  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values it is not possible to quantify impacts on coral reef ecosystems from specific point sources. While this study is limited by snapshot sampling it shows that stable isotopes of POM from different point sources can vary greatly depending on treatment, mixing with other runoff channels etc. For the systems analysed here, many more measurements of isotopic ratios are required for a precise statement. This highlights the importance ensuring that use of modern monitoring tools such as SIA is in fact appropriate in all cases, when more traditional (less expensive) approaches such as gut content analysis could have been used. If used appropriately, costs of SIA can be offset by resultant management plans that are better informed on how best to mitigate impacts of anthropogenic nutrient inputs on coral reef ecosystems. With an increasing shift towards modern tools in ecological studies, it is increasingly important that their application is appropriate and comprehensive, and that ecologists and coastal managers have a greater understanding of their limitations to prevent expenditure on ineffective management approaches.

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APPENDIX

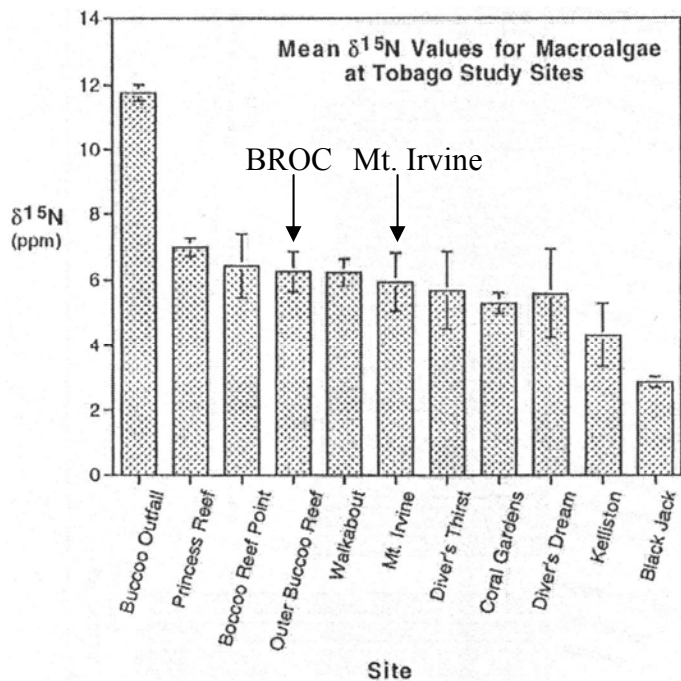


Fig A1. Mean  $\delta^{15}\text{N}$  values for *Dictyota spp.* macroalgae from previous dry season study (Lapointe et al. 2002). Two sites are comparable with this study; BROC ( $\delta^{15}\text{N}$  6.4  $\pm$  0.05‰) and Mt Irvine ( $\delta^{15}\text{N}$  5.9  $\pm$  0.8‰) (shown in Fig. A2).

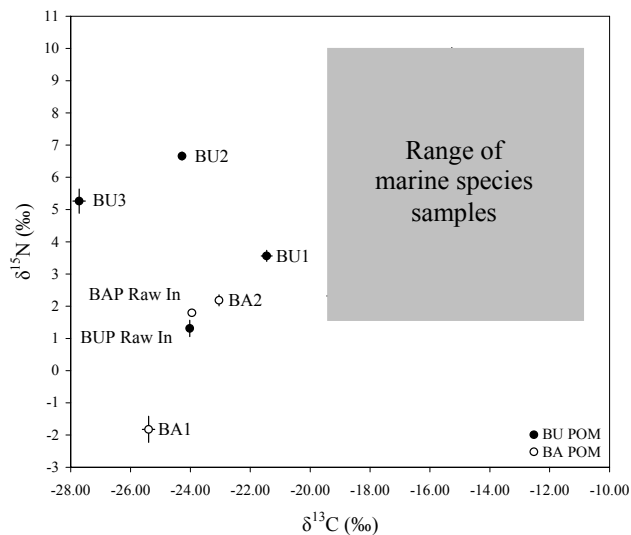


Fig. A3. Average ( $\pm$  SE)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios of Sewage POM from sewage channels at Bon Accord and Buccoo sewage plants and sewage/waste water channels. Range of marine data (shown in Fig. 2.) is shown in grey area.

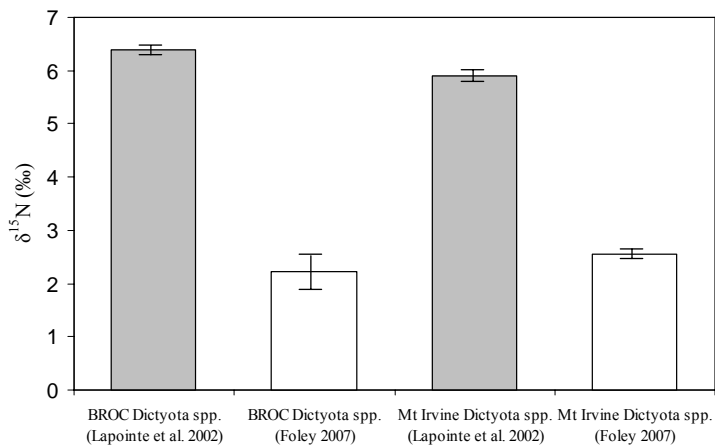


Fig A2. Comparison of  $\delta^{15}\text{N}$  values for *Dictyota spp.* between Lapointe et al. (2002) and Foley (2007).