Coral skeletal $\delta^{15}N$ reveals isotopic traces of an agricultural revolution

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Abstract

This study introduces a new method of tracing the history of nutrient loading in coastal oceans via $\delta^{15}N$ analysis of organic nitrogen preserved in the skeleton of the massive *Porites* coral. Four coral cores were collected in Bali, Indonesia, from reefs exposed to high levels of fertilizers in agricultural run-off, from lagoonal corals impacted by sewage, and from a reef located 30 km offshore. Skeletal $\delta^{15}N$ in the agriculturally exposed coral declined from $10.7 \pm 0.4‰$ in 1970–1971, when synthetic fertilizers ($-0.8‰ \pm 0.2‰$) were introduced to Bali, to a depleted "anthropogenic" baseline of $3.5‰ \pm 0.4\%$ in the mid-1990s. $\delta^{15}N$ values were negatively correlated with rainfall, suggesting that marine $\delta^{15}N$ lowers during flood-bourn influxes of waste fertilizers. Reef cores exposed to untreated sewage in terrestrial discharge were enriched (7.8 and $7.3 \pm 0.4‰$), while the offshore core reflected background oceanic signals ($6.2 \pm 0.4‰$). $\delta^{15}N$, N concentration, and C:N systematics indicate that the N isotopic composition of skeletal organic matter was generally well preserved over 30 years. We suggest that skeletal organic $\delta^{15}N$ can serve as a recorder of past nitrogen sources. In Bali, this tracer suggests that the intensification of Western style agricultural practices since 1970 are contributing to the degradation of coastal coral reefs.
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Keywords: *Porites*; Coral; Skeleton; Nitrogen; Isotope; Fertilizers; Wastewater; Indonesia

1. Introduction

Recent evidence points to declining water quality, combined with overexploitation and climate change, as the primary causes of the global decline in coral reefs worldwide (Hoegh-Guldberg, 1999; Hughes et al., 2003; Pandolfi et al., 2003). However it has proven difficult to develop clear links between sediment and nutrient enriched discharge and impacted coastal coral reefs (Larcombe and Woolfe, 1999; McCook, 1999; Haynes and Michalek-Wegner, 2000; Fabricius, 2002; Neil et al., 2002). Isotope geochemistry is useful in this regard. Various geochemical techniques have been used to trace land sourced pollution (Table 1), including sewage, fertilizers, and groundwater, into the tissues of benthic invertebrates, macroalgae and seagrasses (McClelland et al., 1997; Udy et al., 1999; Heikoop et al., 2000b; Costanzo et al., 2001; Risk et al., 2001; Yamamuro et al., 2003). Here we apply this technique to reef corals.

Long lived coral skeletons can provide quantitative in situ baselines of past seawater stable isotopic and trace metal compositions. Studies using coralline aragonite barium to calcium (Ba/Ca) ratios (Alibert et al., 2003; McCulloch et al., 2003) and skeletal

Table 1
Published studies that have used natural variations in nitrogen isotopes (expressed as $\delta^{15}N$) in tissue samples to trace sewage, fertilizer, and wastewater sources into marine ecosystems

Treatment	Region	Sample type	Reference $\delta^{15}N$	Affected $\delta^{15}N$	Tracer duration	Reference
Light	Jamaica, Zanzibar	Coral tissue	1	5	0.5–1 year	Heikoop et al. (1998)
N fertilizer	GBR	Seagrass	1.7	−3.6	Months	Udy et al. (1999)
N fertilizer	GBR	Coral tissue, skeleton	4	1	1 year	Hoegh-Guldberg et al. (2004)
N fixation	Western Pacific	Macrophytes	6.0	2.0	0.5–1 year	Yamamuro et al. (1995)
Sewage	Indo-Pacific	Porites tissue	5.8	7.5	0.5–1 year	Heikoop et al. (2000a)
Sewage	GBR, Indo-Pacific	Porites tissue	4.8	5.5	0.5–1 year	Heikoop et al. (2000b)
Sewage	Indonesia	Stomatopod	7.7	9.4	1 year	Risk and Erdmann (2000)
Sewage	E.Australia	Marine plants	2	10	Months	Costanzo et al. (2001)
Sewage (tertiary)	S. California, US	Water	10.0	2.5	Instant	Sweeney and Kaplan (1980)
Various	Subtropic Pacific	Seagrass	−1.4	5.1	Months	Yamamuro et al. (2003)
Wastewater	GBR	Porites tissue	3.8	5.3	0.5–1 year	Sammarco et al. (1999)
Wastewater	Japan	Macroalgae	5.2	7.8	Months	Umezawa et al. (2002)
Water	Northeast US	Various	4.4	6.7	Instant	McClelland et al. (1997)

accumulations of Mn, Ce and other rare earth elements (REE) (Fallon et al., 2002; Wyndham et al., 2004) have linked upstream changes in catchment basin land use, enhanced terrestrial runoff, and coastal mining operations with degraded coral reefs. McCulloch et al. (2003) demonstrated that since the onset of widespread land clearing after European arrival to Australia (post-1860), the frequency and intensity of coralline Ba concentration peaks in Great Barrier Reef (GBR) corals have increased by five to tenfold. Barium is partitioned into coral carbonate skeletons in proportion with ambient seawater concentrations, which in turn are primarily influenced by Ba desorption from fine-grained suspended particles advected in flood plumes (McCulloch et al., 2003). These findings provided direct evidence that current rates of sediment exports into inshore GBR waters are well beyond historical limits.

Coral records of past marine nitrogen levels and sources would add a valuable proxy to the available suite of geochemical tracers. Studies have indicated that the skeletal organic matrix retained in coral skeletons is stable at century timescales, and is composed primarily of hydrolyzable amino acids and lipids (Goodfriend et al., 1992; Allemand et al., 1998; Ingalls et al., 2003). In the reef corals *Porites lutea* and *Montipora annularis*, the source of inorganic compounds used for synthesis of the coral skeletal matrix has been found to cause more variation in amino acids isolated from the skeleton than does degradation or diagenetic change (Ingalls et al., 2003). These findings suggest that examination of organic material preserved in coral skeletons could provide a means of reconstructing past nutrient concentrations and sources within coral reef waters.

We analyzed the stable isotopic composition of residual nitrogen retained in the skeletal aragonite of the massive reef-building coral *Porites* sp., using coral cores collected from Bali, Indonesia. During biologically med-

iated reactions, the preferential uptake of ^{14}N increases the ratio of ^{15}N to ^{14}N (Minagawa and Wada, 1986; Yamamuro et al., 1995; McClelland et al., 1997). In the case of wastewater from septic systems and untreated sewage, dissolved inorganic nitrogen (DIN) $\delta^{15}N$ (the ratio of ^{15}N to ^{14}N in a sample relative to ratio in atmospheric N) typically exceeds 10 ‰ due to those transformations, including ammonium nitrification and volatilization, and nitrate denitrification into nitrite and ammonia (Heaton, 1986; Minagawa and Wada, 1986; Jordan et al., 1997). Natural marine DIN (mainly dissolved nitrate) ranges between 4‰ and 7‰ (Minagawa and Wada, 1986; Owens, 1987; Altabet et al., 1999). Bacterial N-fixation introduces organic and inorganic nitrogen into coral reef environments at equilibrium with atmospheric N_2, or ∼0‰ (Owens, 1987; Yamamuro et al., 1995). Synthetic N fertilizers are typically depleted in ^{15}N, ranging from −3‰ to +3 ‰ (Heaton, 1986; Kendall, 1998; Udy et al., 1999). In Bali, synthetic nitrogenous fertilizers were introduced to rice farmers in 1970 during the Indonesian Green Revolution (Lansing, 1995; Tomascik et al., 1997; Lansing et al., 2001), and today are applied at average concentrations of 220 kg of urea fertilizer per hectare of rice paddy or "padi sawa".

The studies above suggest that the N isotopic composition of different nitrogenous phases that may enter coastal waters is sufficiently variable that $\delta^{15}N$ in corals may reasonably be expected to record N provenance. We hypothesize that (1) coral skeletal $\delta^{15}N$ exhibits long-term trends and differential enrichments that are indicative of changing nitrogen sources, (2) the introduction and steady increase of fertilizer usage since 1970 in Bali is reflected by a long-term decrease in coral skeletal $\delta^{15}N$, and (3) organic matter in reef skeletons exposed to coastal development and sewage effluent are $\delta^{15}N$ enriched relative to agriculturally exposed and unimpacted sites.

2. Study sites and methods

Bali is located at 113.5 °E, 8.5 °S and covers 2175 square miles. Typical of tropical, equatorial regions, there is seasonal wet/dry variability with summer monsoon rainfall occurring between November and March. Average annual temperatures are in the range of 26–28 °C and average annual precipitation is 1738 mm (NOAA, 1999).

2.1. Study sites

Coral cores were collected from live *Porites* sp. colonies in four distinct regions (Fig. 1). Amed is a crescent-shaped bay characterized by calm waters and year-round agricultural wastewater inputs due to the regulated irrigation of adjacent rice paddies. The Amed coral grew approximately 50 m offshore and 100 m from a small river mouth that delivers agricultural wastewater from rice paddies extending 15 km inland and up the face of Bali's highest volcano, Mt. Agung (3142 m).

The Sanur coral was drilled in the inner lagoon, approximately 250 m offshore. Sanur is a highly developed coastal stretch with over 30 hotels and no primary sewage treatment facilities (Tomascik et al., 1997; Burke et al., 2002). A third core was collected from an eroded coral reef ~1 km off Lovina in north Bali. Lovina is also a popular tourist destination with 3–4 major waterfront hotels. Rice is cultivated 2 km west of Lovina, with pad-

dies extending to within a few hundred meters of the intertidal zone. Finally, the island of Nusa Penida (NP) was selected as an offshore reference site. Located 30 km east of Bali in the deep (up to 2000 m) Lombok Strait, NP is buffered from Bali agricultural and sewage wastewater by virtue of strong mixing with open ocean waters, mediated by energetic (5+ knot) currents. In addition, NP lacks significant local agriculture or development.

2.2. Analytical methods

All cores were collected by SCUBA diving in water depths of 2–7 m (Table 2), using a pneumatic Dotco 750 rpm hand-drill fitted with a 45 cm long diamond-tipped core barrel. The *Porites* specimens selected for coring exhibited regular hemispherical shapes, had intact tissue layers, and showed little or no visible evidence of bite marks, boring bivalves or sponges, algal growth, and other forms of bioerosion. All cores were rinsed with fresh water, air-dried, packaged in PVC casings and returned to Stanford University.

Five-mm thick slabs were cut from the coral cores with a 15 in. diamond blade rotary saw, cleaned in deionized water and dried at 40° C for 24 h. X-radiographs (60 kV, 20 mA, 6 s exposure times) revealed clear annual density bands with minor bioerosion in all cores. Coral growth bands were traced from x-radiographs onto coral slabs, with annual increments confirmed by $\delta^{18}O$
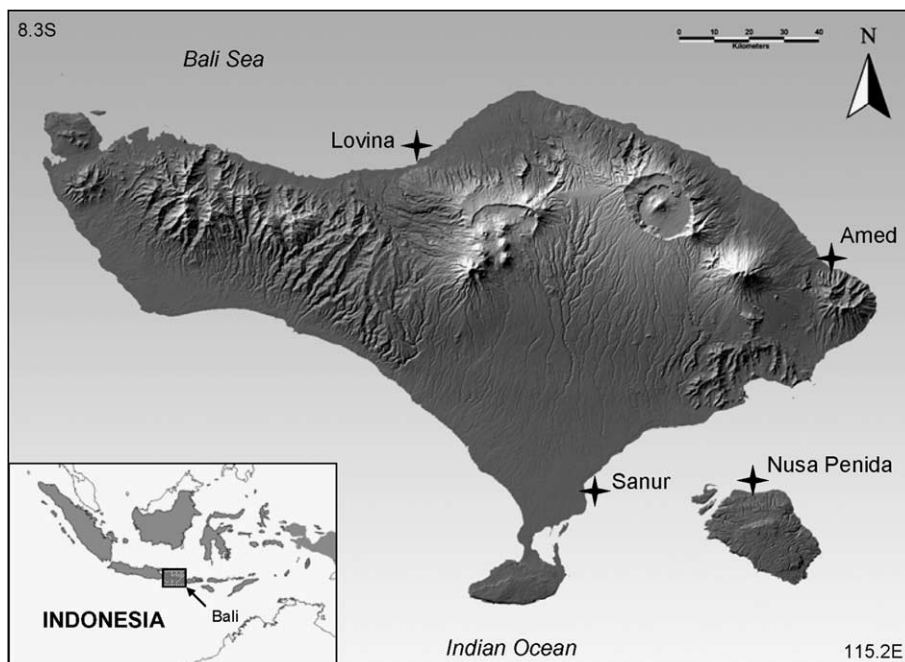

Fig. 1. Coral cores were collected by SCUBA in Bali, Indonesia from live *Porites* corals living in shallow (2–7 m deep) waters. The Amed core is exposed to agricultural and fertilizer waste inputs, the Lovina core to effluent from local agriculture and hotel development, and the Sanur core to continuously discharging septic wastewater. The coral reef at Nusa Penida is located 30 km east of Bali and is buffered from terrestrial runoff by strong ocean currents.

Table 2
Summary of results: The ^{15}N isotopic composition of total skeletal organic matter was analyzed in two *Porites* coral cores from Bali, Indonesia, representing 30 years of growth

Comparison period	Site	Depth	Temp (°C)	Runoff influence	n	$\delta^{15}N$ Trend	$\delta^{15}N$ (‰)		C:N	
						R	Mean	SE	Mean	SE
1993–2001	Sanur	5	26	Sewage	15	–	7.8	0.6	10.1	1.1
	Lovina	5	28	Mixed	22	−0.70**	7.3	0.6	22.2	2.5
	Nusa Penida	4	26	None	14	–	6.2	0.6	12.6	1.8
	Amed	6	28	Fertilizers	31	–	3.9	0.3	12.9	1.1
1970–2000	Sanur	–	–	Sewage	56	–	8.5	0.4	17.0	1.0
	Amed	–	–	Fertilizers	89	−0.46**	4.8	0.3	16.9	0.9

Data was recovered from two other cores (Lovina, Nusa Penida) since 1993.
** Denotes $p < 0.001$.

chronologies (described below; Fig. 2). Sub-annual skeletal samples 30 mm wide by 4.5–6.0 mm long were sectioned with a 20 mm circular saw. Sample resolution ranged from 4 per year to semi-annual (2 per year), depending on annual growth rates. Because nitrogen constitutes from 0.001% to 0.05% of coral aragonite by weight (20.0 ± 9.4 ug N per 800 mg sample) in samples collected below the living tissue layer, large (>750 mg) skeletal samples were needed for $\delta^{15}N$ analysis. To determine instrumental sampling limits, we analyzed a range of skeletal weights, varying from 300 mg to 1500 mg. Ground skeletal samples weighing <750 mg generally produced irregular outlier values indicated by field strengths of <0.3 V, while those weighing 750–800 mg produced reliable $\delta^{15}N$ results in 93% of total CN samples analyzed ($n = 226$).

Skeletal samples were decalcified in dilute (1 N) HCl for 25 min, based on previous findings that coral tissue digested for 5–15 days in both 0.5 and 1 N HCl yielded

identical nitrogen isotopic ratios within measurement error (Heikoop et al., 1998). Digestion in concentrated (3.5N) HCl resulted in $\delta^{15}N$ depletion of up to 1‰. The acid-insoluble residue was glass fiber filtered (0.7 umol L^{-1} pore size), rinsed 2–3 times with 50 ml deionized water, dried at 40 °C for 24 h, and analyzed using a Carlo Erba/ConFlo II elemental analyzer coupled to a Finnigan MAT Delta+ mass spectrometer. Six standards were analyzed with each set of 40 C/N samples. Replicate isotopic and elemental analyses of IAEA-N1 and laboratory grade acetanilide standards yielded an instrumental standard deviation of 0.13‰ for N isotopic composition ($n = 84$) and 0.13% for N content ($n = 42$), while replicate coral samples revealed a coral standard deviation of 0.40‰ ($n = 10$). $\delta^{15}N$ values are reported as the permil variation (‰) of the sample relative to the isotopic ratio of N in air.

2.3. Chronology development

The $\delta^{15}N$ time series were assigned chronologies by matching the sample distance from the top of the core with the age model established using $\delta^{18}O$ profiles and density banding (Fig. 2). Measured $\delta^{18}O$ values closely track gridded historical SST data from Bali (114.5E, 8.5S) (Marion, 2002), providing a means of verifying the chronology assigned by counting annual growth bands. For carbonate $\delta^{18}O$ and $\delta^{13}C$ analyses (used here only as an aid for chronology development), skeletal material was sampled using a small diamond drill along continuous polyp growth tracks at 1 mm intervals, yielding monthly to sub-monthly sample resolution. 65 and 95 µg aliquots of coralline aragonite were acidified in 100% orthophosphoric acid at 70 °C for 470 s using a Finnigan MAT Kiel IV. The resulting CO_2 gas was analyzed in an automated Finnigan MAT 252 mass spectrometer. $\delta^{18}O$ and $\delta^{13}C$ values are reported as the per mil (‰) variation of the sample relative to the Peedee Belemnite (PDB) Limestone Standard. The standard deviation for carbonate isotopic ratios is 0.06‰, based on replicate analyses of the NBS-19 and SLS-1 isotopic standards.
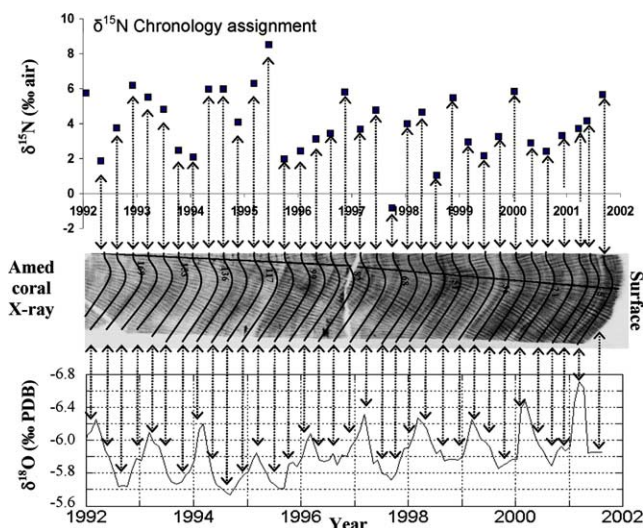

Fig. 2. $\delta^{15}N$ time series were assigned chronologies by matching the sample distance from the top of the core with the age model established using annual density banding (pictured middle) and $\delta^{18}O$ profiles (bottom graph).

Isotopic data was compared with an instrumental precipitation record from Denpasar, Bali, and with a gridded composite rainfall record (Hulme, 1999 monthly precipitation time series, coordinates $7.5S \times 112.5E$ and $7.5S \times 116.5E$- Bali is 8.5S, 113.5E), both of which dated back to 1970. To compare fluorescent bands with precipitation records, coral skeletal slabs were photographed under blacklight and years were assigned as above by matching band distance from the core top with chronologies determined by $\delta^{18}O$ records.

2.4. Statistical analysis

Linear regression analysis was used to assess short (1993–2001) and long-term (1970–2001) coefficients of correlation in $\delta^{15}N$ time series. The magnitude of heavy rainfall months (>250% above mean monthly rainfall) was also correlated with $\delta^{15}N$ data from the closest time increment ($n = 32$) by linear regression. In order to compare the means of raw isotopic values and elemental compositions between all four cores from 1993 to 2001 (dF = 3, $n = 82$) and between the Amed and Sanur corals from 1970 to 2001 (dF = 1, $n = 147$), we used the non-parametric Kruskal-Wallis ANOVA. This non-parametric equivalent of analysis of variance (ANOVA) was applied after determining that the variance of raw data ($\delta^{15}N$, wt %N, C:N) was not homogenous (Levene's test), and that coral isotopic data was not normally distributed (Wilks Shapiro test) within and between all cores (Fig. 3a and b). Significance was assessed using multiple comparisons p-values (2-tailed) generated from the KW ANOVA. All statistical analyses were performed in StatSoft Statistica 6.0.
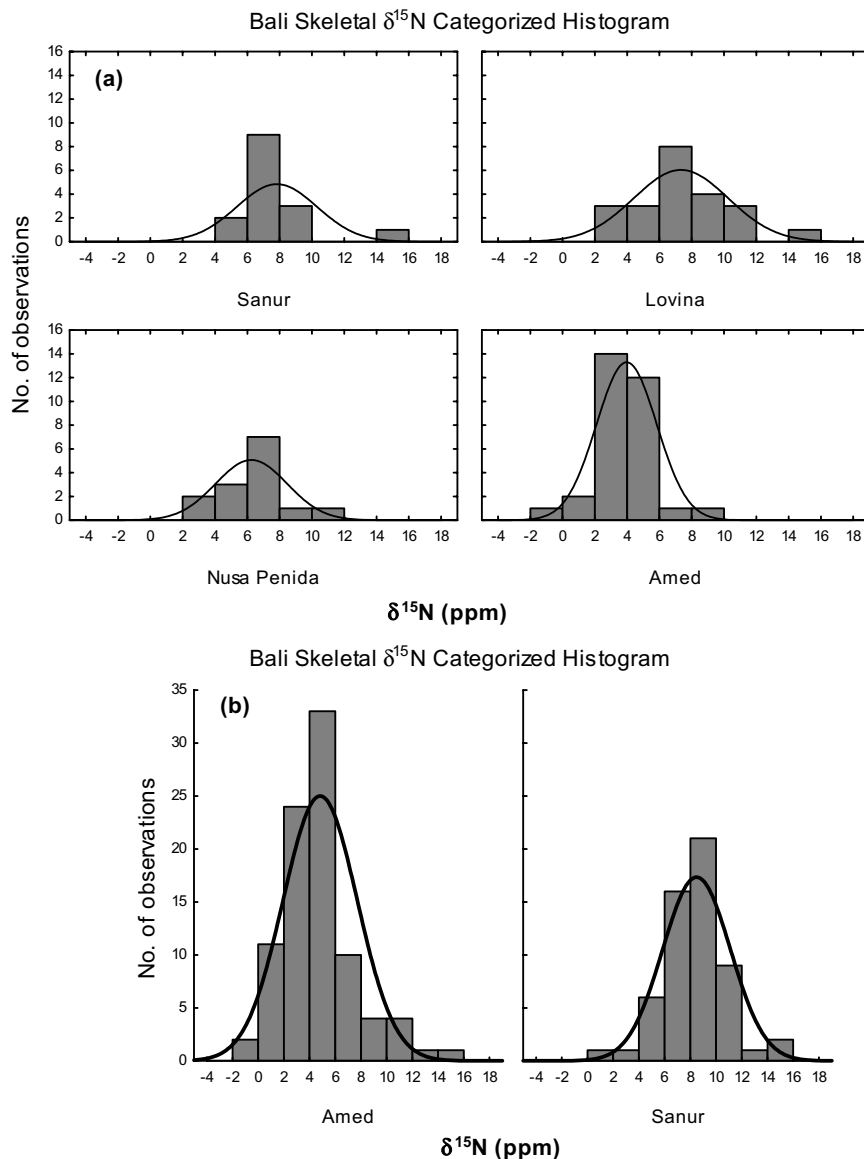

Fig. 3. The distribution of raw isotopic data from Bali skeletal samples between (a) 1993 and 2001, and (b) between 1970 and 2001.

3. Results

Isotopic and elemental data from the Nusa Penida (NP) and Lovina sites span 1993–2001 (Table 3), while the Amed and Sanur data sets extend from 1970 to 2001 (Table 4). Residual nitrogen $\delta^{15}N$ and C/N elemental ratios in *Porites* skeletons both reveal interannual to decadal trends at these sites

Table 3
Average $\delta^{15}N$, C:N, and N concentration data was recovered from all four coral cores for the period 1993–2001

Year	$\delta^{15}N$ (ppm)				C:N				%N (10^{-3})			
	Sanur	Lovina	NP	Amed	Sanur	Lovina	NP	Amed	Sanur	Lovina	NP	Amed
2001	5.6	6.6	6.8	4.5	8.5	9.0	10.4	12.5	15.9	21.3	42.1	45.5
2000	6.7	3.5	7.5	3.6	14.2	9.0	10.3	10.0	9.9	4.6	59.2	3.5
1999	7.7	4.1	7.1	2.8	9.6	13.5	8.8	11.2	6.0	3.2	32.5	2.6
1998	6.1	6.2	7.0	3.8	5.4	19.3	6.3	9.1	8.5	1.8	10.7	2.5
1997	10.1	8.1	5.4	2.5	5.6	22.5	7.9	15.0	7.7	1.6	9.9	2.1
1996	7.4	7.7	3.4	3.7	12.2	44.3	13.1	17.7	5.7	1.4	6.0	3.2
1995	7.1	7.3	7.4	5.6	16.3	35.1	–	11.1	4.4	1.7	3.0	2.6
1994	8.3	10.1	3.4	4.5	10.4	21.9	30.9	13.0	3.2	1.4	3.3	2.8
1993	7.9	15.1	8.6	4.3	8.9	36.0	15.4	16.3	4.1	1.2	3.5	3.9
Mean	7.8	7.3	6.2	3.9	10.1	23.4	12.9	12.9	7.1	3.7	19.8	7.0
n	15	22	14	31	15	24	13	30	15	24	14	31

Table 4
Average isotopic, coral growth, precipitation, and fertilizer consumption data for the years 1970–2001

Year	$\delta^{15}N$ (ppm)		C:N		%N (10^{-3})		Growth (mm)		Precipitation		Fertilizer use
	Sanur	Amed	Sanur	Amed	Sanur	Amed	Sanur	Amed	Wally	UAE	(1000s tons)
2001	5.6	4.5	8.5	12.5	15.9	45.5	–	–	–	–	–
2000	6.7	3.6	14.2	10.0	9.9	3.5	10	16	–	–	–
1999	7.7	2.8	9.6	11.2	6.0	2.6	11	15	–	–	39.9
1998	6.1	3.8	5.4	9.1	8.5	2.5	12	15	71	–	57.8
1997	10.1	2.5	5.6	15.0	7.7	2.1	10	16	47	–	49.3
1996	7.4	3.7	12.2	17.7	5.7	3.2	9	15	175	–	52.5
1995	7.1	5.6	16.3	11.1	4.4	2.6	10	14	185	–	64.9
1994	8.3	4.5	10.4	13.0	3.2	2.8	9	16	103	–	44.5
1993	7.9	4.3	8.9	16.3	4.1	3.9	9	17	106	–	42.1
1992	7.8	3.9	10.0	15.8	2.1	3.0	10	17	138	–	45.8
1991	11.7	3.9	29.9	18.0	1.7	4.1	10	17	111	123	41.9
1990	6.6	1.6	24.5	15.7	1.5	4.1	10	18	133	161	43.5
1989	10.3	2.2	18.1	18.0	1.6	1.6	8	18	178	158	39.7
1988	14.0	5.2	12.3	24.3	1.5	1.9	7	17	138	133	42.8
1987	12.0	0.6	17.7	14.3	1.4	3.0	8	17	100	152	39.6
1986	9.0	5.4	22.5	11.5	1.4	1.5	8	19	342	170	36.9
1985	8.2	4.3	19.2	20.9	1.3	2.0	7	18	127	152	35.1
1984	7.7	4.7	18.4	16.4	1.3	2.6	9	19	205	159	34.7
1983	4.3	8.4	23.3	16.8	1.3	3.1	8	17	177	186	28.3
1982	4.2	2.8	17.2	15.4	1.3	2.3	7	17	59	113	29.2
1981	10.9	3.8	33.5	15.0	1.1	2.2	7	13	167	185	26.9
1980	8.9	5.6	19.8	22.0	1.2	1.6	9	15	142	143	23.0
1979	6.6	5.6	30.3	14.8	1.3	1.8	9	14	122	164	16.7
1978	6.2	6.8	25.6	31.0	1.2	1.8	7	16	203	176	14.8
1977	7.8	6.5	14.5	14.5	1.0	1.4	11	18	122	116	13.1
1976	9.3	5.0	19.4	43.2	1.1	1.3	10	15	82	93	9.5
1975	8.8	9.9	20.0	ND	1.0	0.9	11	14	226	177	9.1
1974	11.3	10.7	22.5	40.1	1.2	0.5	8	16	173	157	8.5
1973	8.8	3.3	13.3	12.0	1.5	0.8	11	18	155	168	8.9
1972	9.3	11.5	14.9	23.3	1.2	0.8	6	16	70	88	6.9
1971	8.7	9.6	21.3	9.7	1.4	0.8	8	16	150	169	5.7
1970	8.3	8.8	14.2	17.0	1.2	0.9	–	–	185	136	5.0
Mean	8.4	5.2	17.3	17.6	3.0	3.5	9.0	16.1	143.1	149.8	31.4
SD	2.2	2.7	7.0	8.0	3.4	7.7	1.5	1.5	59.8	28.0	17.2

Nitrogen content increases markedly in the proteinaceous tissue layer, from ∼0.003% wt N in the skeleton to 0.016% in the Sanur and 0.045% in the Amed tissues.

and differ significantly between cores from different reefs.

3.1. 1993–2001

δ^{15}N values in the Lovina core decline from a 1993–1995 average δ^{15}N of 10.2‰ to a 1999–2001 average of 4.2‰ (Fig. 4b; $R = -0.79$, $n = 22$, $p < 0.001$). δ^{15}N is positively correlated with C:N ($R = 0.44$) and is negatively so with sample nitrogen content (-0.49). None of the other reef sites show a similar trend between 1993 and 2001 (Fig. 4). Between 1993 and 2001, average coral skeletal δ^{15}N varied significantly among all four coral sites ($p < 0.001$, dF = 3, $n = 84$), with δ^{15}N ranging from -0.8‰ (Amed core in 1997) to 15.8‰ (Sanur core in 1997). The 9 year average δ^{15}N of the agriculturally exposed coral from Amed is lower (3.9‰ ± 0.3‰, $n = 31$) than the 9 year averages of each of the Nusa Penida (NP), Sanur, and Lovina corals (Table 3).

C:N ratios of the Sanur, NP, and Amed coral skeletons are similar, ranging from 10.1 to 12.9, while the Lovina coral C:N ratios are significantly higher with a mean of 23.4 (Table 3). The Lovina coral samples also exhibit the lowest average N concentration (0.0037% by weight), which is significantly less than Sanur (0.0071%) and NP (0.0198%). The NP coral has the highest average nitrogen content ($p < 0.001$), as well as the thickest tissue layer ($\sim$12 mm). The combined mean of the three coastal cores' nitrogen concentration was $0.006\% \times 10^{-3}$ N (Table 3).

3.2. 1970–2001

Of the two cores spanning the period 1970–2001 (Table 4), the Amed core is ^{15}N depleted (4.8‰ ± 0.3 ‰, $n = 89$) relative to the Sanur core (8.5‰ ± 0.4 ‰, $n = 56$). Standard deviations were 2.8‰ and 2.5‰ respectively. The Sanur core exhibits no long-term trend in δ^{15}N, whereas the δ^{15}N of the Amed coral declines by -0.17‰/year, from an initial average of 10.7‰ in 1970–1971 to 3.2‰ in 1999–2000 ($R = -0.48$, $n = 85$, $p < 0.001$). This long term decline in δ^{15}N is best represented ($R = -0.53$) by a second order polynomial regression (Fig. 5), suggesting that coral δ^{15}N is perturbed in a non-linear manner.

Average organic nitrogen and carbon concentrations are slightly higher in the Amed core (0.004% N, 0.050% C) than in the Sanur core. The average organic C/N ratios of the Amed (16.9) and Sanur (17.0) corals are similar. The Amed core exhibits a long-term linear decline in C:N ratio ($R = 0.39$, $p < 0.001$, $n = 85$), as does the Sanur coral ($R = 0.39$, $p = 0.002$, $n = 57$) (Fig. 6). Total N concentration ranges from 0.001% in 1970–1980s to 0.072% N within the skeletal tissue layer (Table 4).

Skeletal δ^{15}N in the Amed coral correlates with incidents of heavy rainfall, defined as months in which total rainfall was $>250\%$ above the monthly average (Fig. 7). Linear regression of monthly precipitation and δ^{15}N indicates a significant and inverse relationship ($R = -0.55$, $n = 19$, $p < 0.01$).
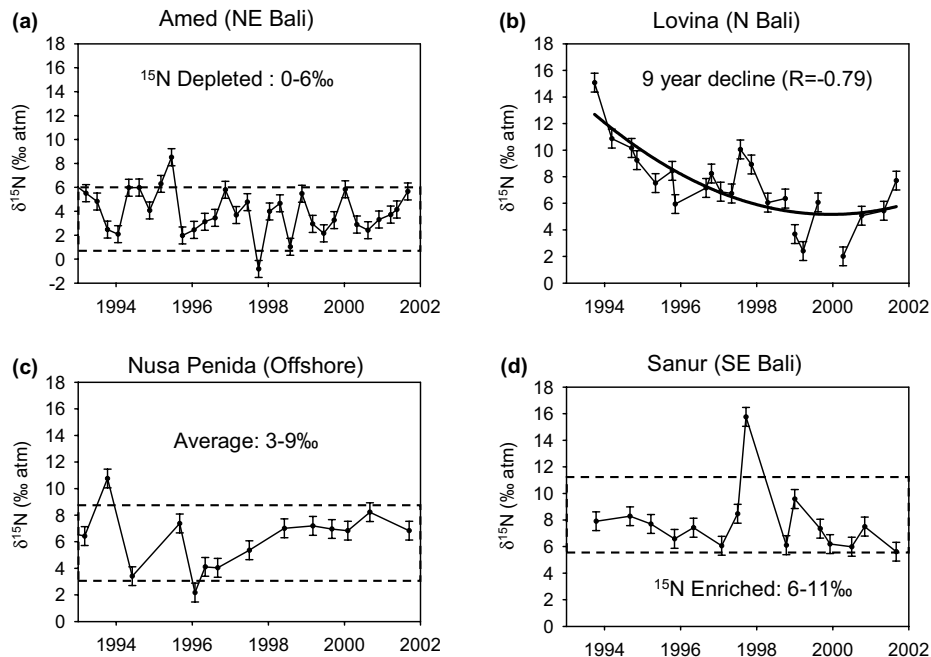

Fig. 4. The agriculturally exposed Amed coral (a) was δ^{15}N depleted between 1993 and 2001, suggesting exposure to fertilizer-derived DIN in wastewater. The rapid δ^{15}N decline in the Lovina (b) coral indicates change in seawater δ^{15}N or cyanobacterial N-fixation. Average δ^{15}N in the offshore coral (c) reflected typical oceanic δ^{15}N fluctuations, while high ^{15}N of the Sanur coral reef (d) indicates the impact of sewage effluent from the many waterfront hotels.
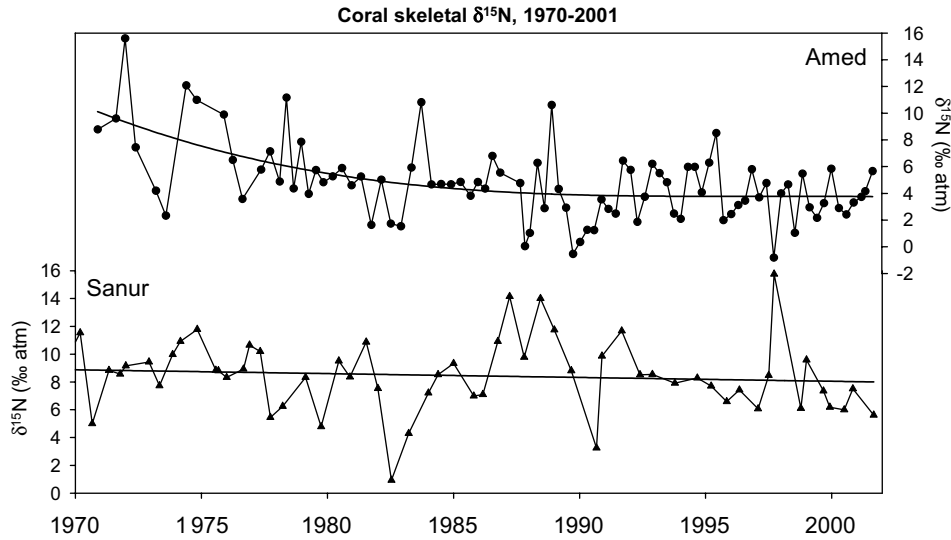
Coral skeletal δ^{15}N, 1970-2001

Fig. 5. Between 1970 and 2001, the Sanur core tends towards heavy δ^{15}N values, indicating exposure to untreated sewage discharge by the waterfront hotels. The progressive depletion of the Amed coral δ^{15}N reflects increasing fertilizer use in regional rice paddies.

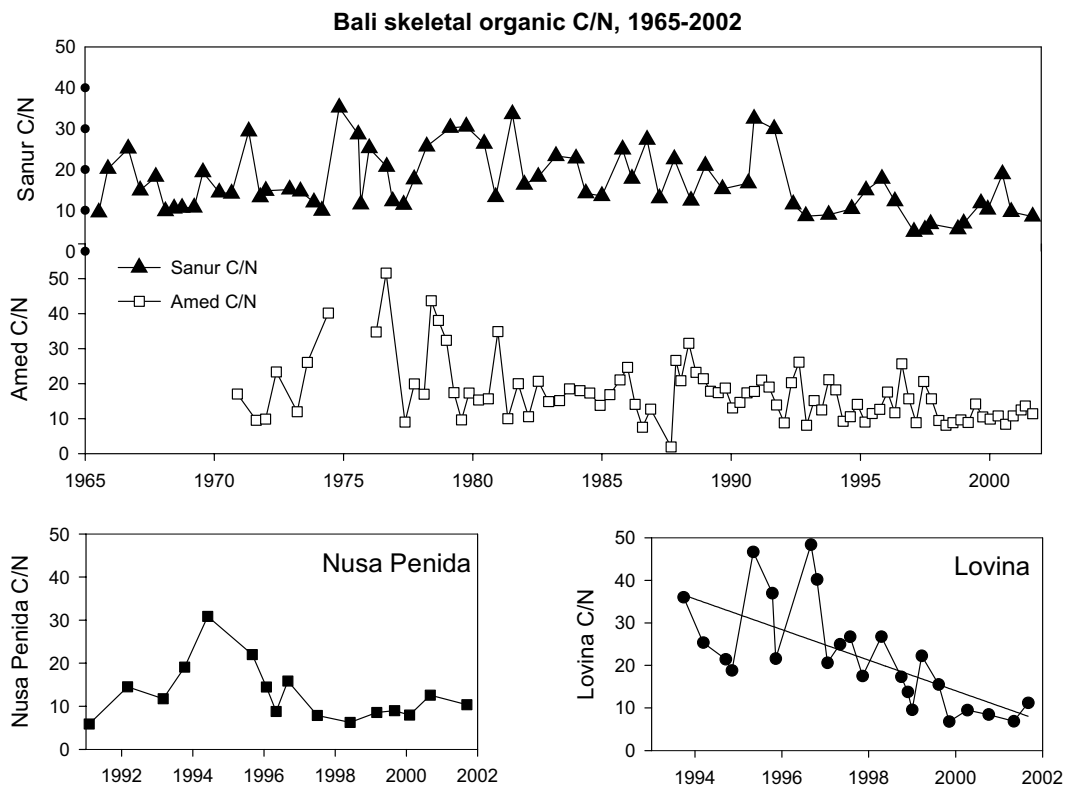**Bali skeletal organic C/N, 1965-2002**

Fig. 6. The long-term decline in CN ratios indicate the higher relative abundance of N-rich proteins and lipids near the coral surface. These are attributable to the coral host, the zooxanthellae, and the sub-surface endolithic algal inhabitants.

4. Discussion

Organic matter preserved within the coral skeleton can include: (1) proteins expressed by corals during biomineralization and skeletal organic matrix formation (Young et al., 1971; Constantz and Weiner, 1988; Alle-mand et al., 1998; Levi et al., 1998), (2) endolithic algae (Le Campion-Alsumard et al., 1995; Fine and Loya, 2002), (3) fungi (Elias and Lee, 1993; Priess et al., 2000), (4) bacteria (Vogel et al., 2000), and (5) particulates incorporated from the water column. A recent study by Ingalls et al. (2003) found that 40–50% of total
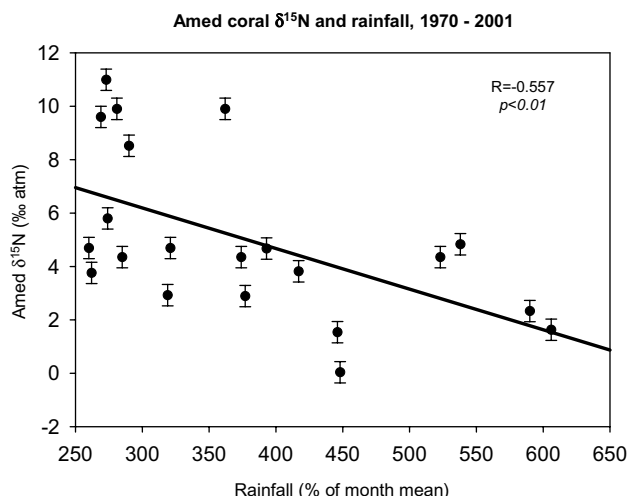
Amed coral δ¹⁵N and rainfall, 1970 - 2001

Fig. 7. Coral $\delta^{15}N$ decreases during prolonged heavy rain periods, indicating flood-bourn influxes of ^{15}N-depleted chemical fertilizer into coastal waters (vertical bars: instrumental precision).

skeletal nitrogen in Red Sea *Porites lutea* corals (spanning 1982–1994) is composed of amino acids, of which aspartic acid, glutamic acid, glycine, and alanine accounted for $\sim$70% of the total amino acid N. The negatively charged aspartic and glutamic acids are primary constituents of the organic matrix found at calcification sites (Marin and Gautret, 1994; Yamashiro and Samata, 1996; Cuif et al., 1999).

The other principal constituent of total skeletal organic matter is the siphonaceous euendolithic algae, notably the chlorophyte *Ostreobium* (Lukas, 1974; Highsmith, 1981; Risk and Muller, 1983). Endolithic algae are the most abundant inhabitants of living *Porites* coral skeletons and play a key role in coral reef primary production (Le Campion-Alsumard et al., 1995; Fine and Loya, 2002). They are efficient oligophotic photosynthesizers that are capable of translocating photoassimilates to the coral host when exposed to less than 1% of surface photosynthetically active radiation (PAR) (Fine and Loya, 2002; Fine et al., 2004). Endolithic inhabitants of *Porites* skeletons are also known net consumers of available O_2, liberating CO_2 and nutrients and driving internal pH regimes (Risk and Muller, 1983). The similar $\delta^{15}N$ values of the tissue and subsurface skeleton commonly inhabited by endolithic algae suggest that the efficient filamentous networks of endoliths preserve seawater $\delta^{15}N$ via the drawdown of internal coral and ambient DIN.

The fidelity with which corals record seawater $\delta^{15}N$ values likely results from rapid N uptake from the water column and assimilation during autotrophy rather than propagation through multiple trophic levels. Allemand et al. (1998) measured ^{14}C-labelled aspartic acid uptake by the branching coral *Stylophora pistillata* and showed that external aspartic and other amino acids are assimi-

lated during skeletal accretion without a detectable lag period. Because seawater DIN is rapidly incorporated into internal coral pools in typically N-limited coral reef waters (Muscatine and Porter, 1977), biological discrimination against the ^{14}N isotope is expected to be minimal (Owens, 1987; McClelland et al., 1997; Heikoop et al., 2000b). Furthermore, the autotrophic status of the coral-algal symbiosis reduces or eliminates the stepwise trophic $\delta^{15}N$ enrichment characteristic of nitrogen in most food webs (Minagawa and Wada, 1986; Yamamuro et al., 1995).

Incorporating previous suggestions that coral tissues are robust indicators of marine $\delta^{15}N$ (Heikoop et al., 2000b; Risk et al., 2001), and that DIN is rapidly partitioned into the skeletal matrix (Allemand et al., 1998; Levi et al., 1998), we now consider the environmental influences and internal coral systematics that could account for the observed $\delta^{15}N$ trends and between-site differences in these four Bali corals. We consider inputs of isotopically distinct new nitrogen into coral reef waters, isotopic fractionation during N incorporation into the coral, and/or alteration of coral skeletal $\delta^{15}N$ via remineralization or diagenesis.

4.1. Fertilizers in agricultural discharge

Our findings show that coral skeletal $\delta^{15}N$ exhibits significant variation between corals from sites exposed to fertilizers versus sewage runoff in Bali coastal waters. The significant $\delta^{15}N$ depletion of the Amed core relative to other cores, both in the 1990s (3.9‰) and from 1970 to 2000 (4.8‰), is consistent with its proximity ($\sim$1/2 km) to the region's major agricultural wastewater discharge creek.

The agricultural valley draining into Amed bay falls within the district of Abang, which contains 717 hectares of terraced rice paddies. The fertilization practices and cropping rotations followed in Abang are similar to those of the Tabanan region, located on the Southwest side of Bali. In Tabanan, farmers apply an average of 226 kg of urea fertilizer per hectare (Wiguna et al., unpublished). High levels of unused fertilizers leach into continuously flowing irrigation ditches that deliver nitrogen rich-wastewater ($\sim$1.2–3.5 umol L^{-1} NO$_3^-$) downstream into coastal estuaries. In the northeast of Bali, Amed bay is the primary recipient of this run-off. Urea fertilizer samples provided by Balinese rice farmers had average $\delta^{15}N$ values of -0.8‰ (±0.2‰).

The annual decrease in $\delta^{15}N$ values between 1970 and 2000 (Fig. 8) is significantly and inversely correlated to total synthetic fertilizer consumption in Bali over the period ($r^2 = 0.48$, $p < 0.001$). Intensive synthetic urea fertilizer application has been practiced in Bali since the early 1970s with the start of the Indonesian Green Revolution (Lansing, 1995; Lansing et al., 2001). This introduced to associated terrestrial and marine
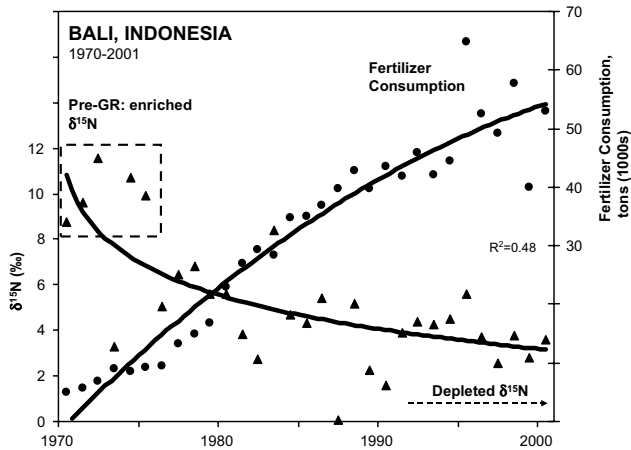
Fig. 8. The introduction of chemical fertilizers ($-0.8\permil$) to Bali in 1970 led to a reduction in average coral $\delta^{15}N$ values, producing an "anthropogenic" baseline of $\sim 3.5\permil$ by the mid-1990s. Prior to the introduction of synthetic fertilizers, high-$\delta^{15}N$ organic manures ($>10\permil$) were used to fertilize rice paddies, evident here as the enriched $\delta^{15}N$ values of 1970–1975.

ecosystems an end member that is $\delta^{15}N$ distinct from the organic manure fertilizers that had been used previously for up to 1000 years (Lansing et al., 2001). Manure, like sewage effluent, is characteristically enriched with $\delta^{15}N$ values generally exceeding $10\permil$ (Kendall, 1998). Its use for centuries as a fertilizer in Bali would account for the high average $\delta^{15}N$ values observed prior to 1975 ($9.0 \pm 2.9\permil$).

Farmers used progressively more synthetic fertilizers until ~ 1995, at which time annual consumption leveled off at $\sim 40,000$ tons (IFA, 2002; Wiguna et al., unpublished). The logarithmic $\delta^{15}N$ decline observed between 1970 and 2000 in the Amed coral closely mirrors the increase in fertilizer usage (Fig. 7). Resolved over five year increments, the rates of fertilizer use increase and $\delta^{15}N$ decrease are closely related ($r^2 = 0.63$, $n = 5$, $p < 0.001$), with maximum change in both factors occurring between 1970 and 1980. The coral organic $\delta^{15}N$ reaches a new "anthropogenic" baseline of $\sim 3.5\permil$ in the early 1990s.

4.2. Floods and elevated source concentration

In order to test whether higher nitrogen source concentrations have a significant effect on coral $\delta^{15}N$, as has previously been suggested in other systems (Jordan et al., 1997; McClelland et al., 1997), we compare $\delta^{15}N$ with periods of significant runoff, as indicated by instrumental rainfall records and fluorescent bands. Fluorescent banding provides an in situ link between nitrogen isotopic records, rainfall, and flood events, and serves as a proxy of terrestrial runoff in *Porites* reef corals (Barnes and Taylor, 2001; Lough et al., 2002).

The fluorescent banding visible in the Amed, Sanur and Lovina corals suggest that those reefs are exposed

to runoff and freshwater influence during each monsoon season (October–March). In Amed, intense fluorescent bands in 1977, 1982, 1987, and 1998 confirm that the heavy rainfall months documented in those same years did produce a significant river water influence on the reef (Fig. 6). Distinct fluorescence is absent in the Nusa Penida (NP) coral, located 30 km off the main island of Bali. It is likely that the deep waters, strong currents and upwelling regime of the Lombok Strait isolates the reefs of NP from Bali freshwater runoff (Tomascik et al., 1997).

The negative correlation between $\delta^{15}N$ and rainfall during high rainfall months ($R = -0.55$, $n = 19$, $p < 0.01$) indicates that flooding lowers the $\delta^{15}N$ value of coastal ocean water at Amed, as expected from a flood-bourne influx of waste fertilizer. The average $\delta^{15}N$ during periods of heavy rain (months with rainfall at $>300\%$ of the mean monthly rainfall, $n = 14$) was $3.7\permil$, compared to $4.7\permil$ over the entire 30 year record.

Two recent manipulative studies also suggest that low $\delta^{15}N$ fertilizers influence tissue $\delta^{15}N$. Coral tissue and skeletal $\delta^{15}N$ were significantly depleted after two years of fertilizer treatments during in-situ experiments at the Great Barrier Reef (Hoegh-Guldberg et al., 2004). *Pocillopora damicornis* cultured in NH_4Cl-treated patch reefs developed ^{15}N depleted tissues and organic matrix constituents as compared to control corals. $\delta^{15}N$ decreased from between $3\permil$ and $4\permil$ to below $1\permil$ in coral tissue, symbiotic dinoflagellate, and coral skeletal compartments (Hoegh-Guldberg et al., 2004). In a separate experiment performed on the Great Barrier Reef, Udy et al. (1999) observed that tissue $\delta^{15}N$ of the seagrasses *Halodule uninervis* and *Syringodium isoetifolium* dropped from 1.7% and 1.3% to $-2.0\permil$ and $-3.6\permil$ after the addition of N fertilizers into coastal waters.

4.3. Sewage in Sanur wastewater

The high skeletal $\delta^{15}N$ values observed in the Sanur coral between 1970 and 2001 (average $8.4 \pm 0.6\permil$, $n = 56$) are consistent with previous reports of elevated $\delta^{15}N$ in sewage affected marine tissues (Heikoop et al., 2000a; Heikoop et al., 2000b; Risk and Erdmann, 2000; Costanzo et al., 2001). In those studies, the tissue $\delta^{15}N$ of sewage impacted Indonesian coral reefs and stomatopods was generally over $7.5\permil$ and $1–5\permil$ higher than reference sites (Heikoop et al., 2000b; Risk and Erdmann, 2000; Costanzo et al., 2001). In 1999, 1.36 million tourists visited Bali and its 104 hotels, at least a quarter of whom stayed in over 30 hotels in Sanur (BPS, 1999). As of 2000, no tertiary sewage treatment facilities existed in Bali or in Indonesia (Risk and Erdmann, 2000; Burke et al., 2002).

Unlike Amed, Sanur coral $\delta^{15}N$ was not significantly correlated with rainfall, an observation that is likely explained by regional topography and hydrodynamics.

While agricultural wastewater in Amed is an explicit N point source and funnels directly from the rising valley of rice paddies into the bay, Sanur is located in the flat southern region of Bali where ground and wastewater are dispersed across a 40 km stretch of coast. Such groundwater discharge constitutes a non-point source of nitrogen (Carpenter et al., 1998; Umezawa et al., 2002), where $\delta^{15}N$ enriched sewage wastewater is mixed with depleted rain and storm water. Effluents discharged into the coastal ocean are buffered by strong ocean tidal flushing in the Sanur Lagoon (Tomascik et al., 1997). Nusa Penida and Sanur are both located within the Lombok Strait and are similarly exposed to upwelling and currents of up to 8 knots in the most exposed channel passages around Nusa Penida (Tomascik et al., 1997; Marion et al., 2003).

4.4. Coral mediated incorporation of nitrogen isotopes

In general, biological processes alter $\delta^{15}N$ via a kinetic affinity for transformations involving the lighter isotope (Owens, 1987). One outcome is a generally observed ∼3.5‰ $\delta^{15}N$ enrichment between adjacent trophic levels in food webs (Minagawa and Wada, 1986; Owens, 1987). Another is coral-algal fractionation under varying light and productivity regimes (Muscatine and Kaplan, 1994; Heikoop et al., 1998). The tissue $\delta^{15}N$ values of 9 coral species collected at 30 and 50 m deep were 0–3‰ lower than surface corals of the same species (Muscatine and Kaplan, 1994; Heikoop et al., 1998). This observed depletion in low light, low productivity regimes is due to coral-algal fractionation of seawater DIN during uptake and assimilation. The relatively low photosynthetic activity of deep water corals compared to surface corals relaxes the diffusive gradient between the coral internal DIN pool and ambient seawater N concentrations, leading to the preferential assimilation of ^{14}N and/or recycling of low $\delta^{15}N$ internal DIN (Muscatine and Kaplan, 1994; Heikoop et al., 1998; Sammarco et al., 1999).

Conversely, N isotopic fractionation is minimal in highly productive, nitrogen-limited conditions, where nutrient-depleted corals consume all available DIN (Muscatine and Kaplan, 1994). All corals selected for this study lived in shallow, clear waters with unrestricted exposure to surface irradiance. Neither between-site nor historical differences in light availability, if any, would explain the significant $\delta^{15}N$ trends observed in this study. Umezawa et al. (2002) similarly concluded that light was not a major determinant of tissue $\delta^{15}N$ in shallow clear waters around Japan.

4.5. Preservation of skeletal organic nitrogen

Despite a significant reduction in N concentration across the junction between the coral polyp layer and the underlying skeleton (Table 3), $\delta^{15}N$ values on either side of each coral are similar ($n = 24$), arguing against significant fractionation of seawater DIN during assimilation. Likewise, Hoegh-Guldberg et al. (2004) reported that differences in the $\delta^{15}N$ of skeletal organic nitrogen and living tissue in the branching Pocillopora damicornis were insignificant.

In the Amed coral, skeletal $\delta^{15}N$ depletion was strongly related to increasing sample nitrogen concentrations ($R = -0.36$, dF = 1,85, $p < 0.001$), but not to CN ratios. Processes that degrade N-rich amino acids, endolithic algal residues, or other skeletal elements would offset the C:N ratios of skeletal organics. Such processes include enzymatic activity by interstitial cyanobacteria (Le Campion-Alsumard et al., 1995) or fungi (Bentis et al., 2000). The lack of such correlations suggests that the long-term $\delta^{15}N$ decline between 1970 and 2001 is best explained by an accumulation of low ^{15}N nitrogenous compounds and not by degradation of skeletal organic nitrogen. In the case of the Lovina coral, the positive correlation between $\delta^{15}N$ and C:N and the negative relationship between skeletal $\delta^{15}N$ and %N suggests that enzymatic degradation or N-fixation by skeleton inhabiting cynobacteria (Le Campion-Alsumard et al., 1995) could account for the sharp isotopic decline between 1993 and 2001 (Fig. 4b).

4.6. Skeletal $\delta^{15}N$ as an environmental recorder

Our findings indicate that nitrogen isotopic signals are well preserved in Porites skeletons for at least several decades, consistent with prior discoveries that organic matter and amino acids are stable for up to hundreds of years in intact coral skeletons (Goodfriend et al., 1992; Allemand et al., 1998; Ingalls et al., 2003). We suggest that the $\delta^{15}N$ of residual organic nitrogen in long-lived Porites skeletons serves as a historical environmental proxy for water quality by aiding in the identification of past variability in nitrogen provenance. This isotopic tracer of chemical fertilizers in coral skeletons suggests that the intensification of Western style agricultural practices since 1970 are contributing to the degradation of coastal coral reefs in Bali.

The expansion of agriculture, pasture lands, urban and port development has been cited as having significantly impacted coastal water quality and coral reef health in tropical oceans around the world (Smith et al., 1981; Bell, 1992; Vitousek et al., 1997; Risk, 1999; Furnas and Mitchell, 2001). Yet interpretation of these changes has generally been limited by the short observation periods and assumptions of what "natural" or pre-impacted baseline conditions were like. $\delta^{15}N$ analysis of organic fractions preserved within the skeleton of Porites corals offers a novel means of exploring long-term changes in nitrogen provenance and dynamics. Such paleoenvironmental records provide a

historical context for present day land-based nutrient sources in coastal oceans, permitting new insights into the extent of longterm anthropogenic pollution stress in coral reef waters. Thus coral skeletal $\delta^{15}N$ offers promise as a management tool by allowing assessment of "baseline" targets for water quality monitoring and remediation programs in countries worldwide.

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