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Influence of submarine springs and wastewater on nutrient dynamics of Caribbean seagrass meadows

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Abstract

The east coast of the Yucatan Peninsula, Mexico, consists of highly permeable limestone, such that surface flow and rivers are absent in this region. Extensive underground cave systems connect sink holes (cenotes) to submarine springs (ojos de aqua), which vent into the seagrass meadows of the adjacent oligotrophic coastal lagoons. This study investigated the potential for these submarine springs to influence nutrient processes within seagrass meadows, by assessing nutrient status of *Thalassia testudinum* meadows in two contrasting coastal lagoons along the north eastern Yucatan peninsula. Tissue nutrient concentrations as well as $\delta^{15}N$ values of *T. testudinum* were surveyed in the Puerto Morelos Reef Lagoon and the Nichupte Lagoon System, Cancun Hotel Zone, during an extended dry period and again following heavy rainfall. After a period of heavy rainfall, *T. testudinum* near submarine springs in Puerto Morelos Reef Lagoon had exceptionally high leaf tissue phosphorus concentrations of $0.38 \pm 0.06\%$. These submarine springs may have been a direct source of phosphorus and/or a source of iron to this very iron limited carbonate system. *Thalassia testudinum* nutrient concentrations suggest that nitrogen loading to the Nichupte Lagoon System is regionally high and has increased over the past decade (mean leaf N: 2.04% N in 1991 to 2.71% N in 2002). Nitrogen content in leaf tissue of *T. testudinum* was significantly higher within the poorly flushed Nichupte Lagoon System ($2.93 \pm 0.12\%$ N) than in the well-flushed Puerto Morelos Reef Lagoon ($1.80 \pm 0.07\%$ N). Stable isotope ratios of nitrogen suggest that this high and increasing nitrogen loading within the Nichupte Lagoon System is a result of wastewater nitrogen ($\delta^{15}N$ 9.06 ± 0.07 in northern Nichupte Lagoon System vs. 1.69 ± 0.07 in Puerto Morelos Reef Lagoon).

Keywords: nutrient sources; sewage; submarine springs; groundwater; eutrophication; *Thalassia testudinum*; $\delta^{15}N$; nitrogen; phosphorus; iron; seagrass; conceptual diagrams

1. Introduction

The north east of the Yucatan Peninsula consists of highly permeable limestone so that rainwater rapidly infiltrates into the aquifer, resulting in the absence of surface drainage or rivers (Merino et al., 1990). This aquifer is visible in numerous sink holes (cenotes) throughout the peninsula. The rainwater eventually passes through an extensive network of underground caves and channels to vent into the marine coastal lagoons through submarine springs and fissures (ojos de agua: Back, 1985). This geology provides the potential for land sourced nutrients to enter the water table and be transported to coastal lagoons kilometers away from their source.

Reef systems along the Mexican Caribbean coast thrive under low nutrient concentrations and variations in

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seagrass growth related to inflows from mangrove lagoons suggest that even slight increases in nutrient input into these areas may cause significant changes in these coastal ecosystems (Lapointe et al., 1992; van Tussenbroek et al., 1996). *Thalassia testudinum* is a sensitive indicator of increasing nutrient loads with measurable increases in productivity and leaf area as well as reduction in shoot density (Green and Webber, 2003) and eventually decline as *T. testudinum* is out-competed by other species (Fourqurean et al., 1995; Fourqurean and Rutten, 2003). Another study in Sarasota Bay (Florida) reported a negative correlation between biomass and density of *T. testudinum* meadows and watershed nitrogen load (Tomasko et al., 1996), suggesting the importance of understanding all potential sources of nutrients into these naturally low nutrient systems.

The importance of porewater nutrients in determining the nutrient status and composition of tropical seagrass meadows is well established (e.g. Fourqurean et al., 1992a). As a result, discussion of nutrient status of tropical seagrass meadows has often focused on sediment characteristics (such as carbonate vs. siliclastic sediments and grain size) and the influence of these characteristics on potential nutrient delivery to seagrasses (Short, 1987; Erftemeijer, 1994; McGlathery et al., 1994). However, the potential for leaf uptake of nutrients from the water column has also been fully recognized, especially in low nutrient situations (Lee and Dunton, 1999; Hemminga and Duarte, 2000; Gras et al., 2003).

This study assessed the potential effect of inputs through submarine springs upon nutrient status of *Thalassia testudinum* in the well flushed Puerto Morelos Lagoon. To provide a context for potential influences, nutrient status of *Thalassia testudinum* was also assessed in a nearby lagoon without submarine springs, the poorly flushed Nichupte Lagoon System.

This paper addresses the following questions:

1. Do submarine springs influence *Thalassia testudinum* nutrient processes in Puerto Morelos Reef Lagoon?
2. In comparison to the nearby Nichupte lagoon, what is the local context for patterns observed adjacent to submarine springs?
3. What is the regional and global context for the nutrient status of Puerto Morelos and Nichupte coastal lagoons?

2. Methods

2.1. Study sites

The reef lagoon in front of Puerto Morelos village (20°51′ N; 86°55′ W, Fig. 1) is delimited on the seaward side by a barrier fringing reef situated 600 to 1800 m from the shore, creating a lagoon from 3-4 m mean depth.

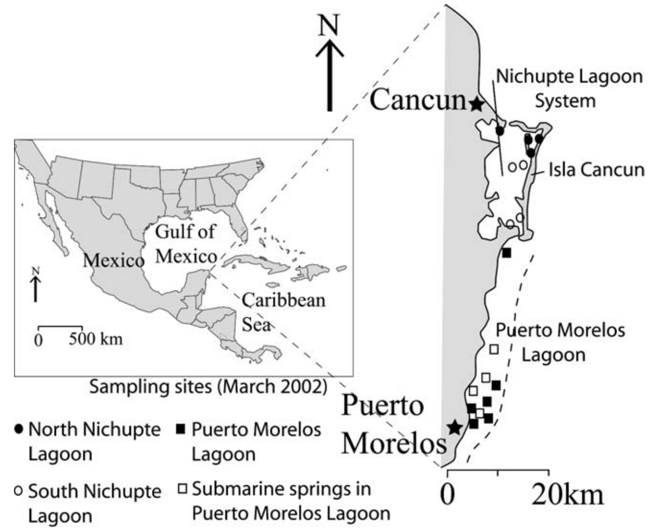

Fig. 1. Map of Yucatan peninsula, Mexican Caribbean, detailing sampling locations. NB: dashed line indicates edge of reef crest.

Inside this, the coastline is bordered by a 50–200 m wide sand barrier isolating the inland mangrove wetlands from the sea. Strong currents ensure good flushing and predominantly well mixed marine conditions in the lagoon. When compared to 18 sites throughout the Caribbean, this lagoon was in the top 30% of the most exposed (storm, current, wind, wave and tide) and the lowest 22% for sediment loading, indicating that terrestrial inputs are low and rapidly dispersed by strong flushing (CARICOMP, 1997). Within the Puerto Morelos Lagoon are numerous submarine springs, which release fresh water into the lagoon after heavy rains. Annual rainfull at Puerto Morelos varied between 815 and 1269 mm between 1993 and 2002, with no clear seasonal pattern (F. Ruíz, CARICOMP data).

Nichupte Lagoon System (86°44′ W, 21°31′ N, Fig. 1), is situated between Cancun townsite on the mainland and the Cancun Hotel Zone which is built on a relic fringing reef known as 'Isla Cancún.' This site consists of a series of shallow lagoons (1.5–2.0 m depth) with a long mean flushing time of up to 1.9 years and is surrounded by mangroves and lowland swamps, resulting in high organic and fine sediment accumulation (Merino et al., 1990, 1992). Water exchange with the open Caribbean Sea occurs through two narrow openings, dredged to 4 m deep, in the extreme north and south of the lagoon (Merino et al., 1992). Freshwater inputs to this and similar lagoons are from rainfall, runoff from adjacent land and groundwater flow (Merino et al., 1990; Herrera-Silveira, 1994).

2.2. Sampling

In March 2002 (during a dry period), *Thalassia testudinum* shoots were sampled from 10 sites throughout Puerto Morelos Reef Lagoon and 8 sites throughout the

Nichupte Lagoon System (Fig. 1). At each site, five *T. testudinum* shoots were haphazardly sampled, with at least 1 m between any two sampled shoots. At the submarine springs in Puerto Morelos Reef Lagoon, the shoots were sampled as close as possible to the freshwater output of the spring (the largest spring had 3–5 m of bare sand around the rock fissure). At these same sites, porewater samples were collected during the same dry period in May 2002. Porewater samples were collected using a sediment sipper of perforated 16 mm diameter PVC pipe with an inner sleeve of 10 μm mesh (Udy and Dennison, 1996), with inlet ports to take an integrated sample from 50 to 150 mm depth. Samples were immediately filtered through 0.45 μm cellulose acetate filters, stored on ice, and transported to the laboratory.

To assess potential effects of inputs through submarine vents, in July 2003, after a period of heavy rain (Table 1), samples were retaken throughout Puerto Morelos Reef Lagoon, both near and away from submarine springs. All of the March 2002 sites were sampled again, 16 additional reef lagoon sites and one additional submarine spring site were also sampled. Surface salinity and temperature was measured directly above, 10 m and 100 m away from submarine springs to assess if flow could be detected (Table 2).

To put potential variation of *Thalassia* tissue nutrients resulting from submarine inputs into context, decadal changes in the nearby Nichupte Lagoon were assessed. In Nichupte Lagoon System, three sites sampled in March 2002 were identical to those sampled by van Tussenbroek et al., 1996 (CN1, CN2, BJ3). These data are mean values of monthly sampling between July 1991 and January 1992.

2.3. Processing

The basal 2 cm of *T. testudinum* leaf tissue above the leaf sheath was removed and scraped of any epiphytes. After drying at 60 °C for 48 h, leaf tissue was ground in a Janke and Kunkel IKA Labortechnik grinder, a subsample was weighed to approximately 3 mg into tin capsules and used for stable isotope analysis. The remaining sample was used for analysis of CHN and P. Stable isotope analyses were carried out on a Europa Hydra 20/20 continuous flow IRMS. Tissue nutrient analyses were run through a CHN analyzer (Fisons NA1500). Dry oxidation, acid hydrolysis extraction followed by a colorimetric analysis was used to determine phosphate concentration of the extract (Fourqurean et al., 1992b). Elemental content of leaf tissue was calculated on a dry weight basis and elemental ratios on a molar basis.

Table 1
Rainfall during and in weeks preceding sampling events

Date	Parameters sampled	Rainfall in week of sampling (mm)	Rainfall in previous two weeks (mm)
March 2002	*T. testudinum* tissue	0	16
May 2002	Porewater	0	2
July 2003	*T. testudinum* tissue	32	168

Table 2
Salinity and temperature near to a large submarine discharge, July 2003 (rain)

Location	Salinity (ppt)	Temperature (°C)
Center of spring	33.3 (0.3)	28.4 (0.3)
10 m south	36.1 (0.1)	30.1 (0.0)
10 m east	36.2 (0.0)	29.8 (0.0)
10 m west	36.3 (0.0)	30.4 (0.0)
100 m south	36.1 (0.1)	30.2 (0.0)
100 m east	36.2 (0.0)	30.0 (0.0)
100 m west	36.2 (0.0)	30.6 (0.1)

2.4. Water analyses

At arrival at the laboratory (within 20 min of collection) the water samples were stored in a freezer at −60 °C. Within 30 days of collection, the samples were transported by air from Cancun to Mexico City, and on arrival they were analyzed with a Skalar (Scanplus System) autoanalyzer to determine concentrations of ammonia, phosphates, nitrates and nitrites.

2.5. Statistics

To assess if *T. testudium* in the vicinity of submarine vents showed a change in nutrient status with flow events, two way ANOVAs (season and proximity to submarine spring) were carried out on %C, %N and %P data. To test for potential variation in %C, %N and %P within the Nichupte Lagoon, *t*-tests were carried out. To provide a regional context for $\delta^{15}N$, a one-way ANOVA was performed between samples collected in north and south Nichupte Lagoon as well as in Puerto Morelos Lagoon, both adjacent to and away from submarine springs. All data were tested for homogeneity of variances using Cochrans' test (Winer, 1971) and in all but one case data were homogeneous. The variances for the two-way analysis on %P was heterogeneous; this was rectified by $\log(x)$ transformation as variances were proportional to the means. Tukey's post hoc test was used to determine groupings in the analysis of $\delta^{15}N$ data (Zar, 1984).

3. Results

3.1. Nutrients in Puerto Morelos Lagoon

Leaf tissue nitrogen concentrations did not vary significantly with season or proximity to submarine

Table 3
Thalassia testudinum leaf tissue nutrient values from Puerto Morelos Lagoon, sampled during a dry season (March 2002) and after a large rain event (July 2003)

	Location	%C (SE)	%N (SE)	%P (SE)	C:N:P	*n*
Dry	Puerto Morelos springs	35.60 (0.24)	2.11 (0.16)	0.18 (0.02)	528:26:1	4
Dry	Puerto Morelos Lagoon	35.25 (0.72)	1.80 (0.07)	0.13 (0.01)	740:32:1	6
Rain	Puerto Morelos springs	39.46 (0.40)	1.85 (0.14)	0.38 (0.06)	275:11:1	3
Rain	Puerto Morelos Lagoon	36.79 (0.37)	1.91 (0.05)	0.14.(0.01)	698:31:1	20

Values are means ± standard error.

springs (Table 3); however, phosphorus concentration was highest near to submarine springs in the wet season (0.38 ± 0.06%) (Table 3). The high tissue phosphorus near the submarine springs resulted in significant season, site and season by site interaction terms ($P < 0.01$, Table 4). Percent carbon in leaf tissue was higher in the rain season and higher at submarine spring sites (Table 3). These differences were all significant ($P < 0.05$, Table 4).

3.2. Nutrients in Nichupte Lagoon

Highest mean concentrations of 2.93% tissue nitrogen were measured in the northern Nichupte Lagoon System, lower in the southern part of this lagoon system (mean of 2.5% N; Table 5) in comparison to the 1.80% N in the Puerto Morelos Reef Lagoon (Table 2). For dry period (February/March 2002) samples, total %N tissue concentrations were significantly different between the sites in the northern and southern sections of Nichupte Lagoon System (Table 5).

Comparison of samples taken in the Nichupte Lagoon System in 1991 and 2002 show an increase in leaf *T. testudinum* total nitrogen within the lagoon from a mean of 2.04% N to 2.71% N (Table 6).

3.3. Natural abundance of nitrogen stable isotopes

Stable nitrogen isotope ($\delta^{15}N$) values within *T. testudinum* leaf tissue decreased from Nichupte Lagoon System to the Puerto Morelos Reef Lagoon, with significant differences between Nichupte north (9.1‰) and Puerto Morelos Lagoon away from submarine springs (1.7‰, Tables 7 and 8).

Table 4
Results of two-way ANOVA on *Thalassia testudinum* leaf tissue nutrient data presented in Table 3

	Source	df	MS	*F*	*p*
%C	Season	1	36.34	15.33	<0.01
	Site	1	11.39	4.80	<0.05
	Season × Site	1	6.72	2.83	0.10
	Error	29	2.37		
%N	Season	1	0.027	0.517	0.48
	Site	1	0.083	1.575	0.22
	Season × Site	1	0.177	3.356	0.08
	Error	29	0.053		
Log %P	Season	1	0.055	54.63	<0.01
	Site	1	0.113	112.00	<0.01
	Season × Site	1	0.045	45.26	<0.01
	Error	29	0.001		

3.4. Porewater nutrient concentrations

Porewater dissolved inorganic nitrogen and phosphate were lower close to submarine springs (2.83 and 1.26 μM, respectively) than in the general lagoon (4.37 and 1.52 μM, respectively; Table 9).

4. Discussion

4.1. Nutrient processes within Puerto Morelos Lagoon

Increases in *Thalassia testudinum* leaf %P near submarine springs in Puerto Morelos Reef Lagoon, especially after rain events (0.38% P; Table 3), provides evidence on how outflow from submarine springs may influence nutrient processes within this reef lagoon (Fig. 2). We propose two possible explanations for the observed trend; either (1) the groundwater contains high concentrations of phosphorus and/or (2) contains iron, which indirectly may increase availability of phosphorus to *T. testudinum* growing in the lagoon.

Groundwater flows into eastern Florida Bay are thought to deliver as much nitrogen and phosphate as surface water flows from the Everglades (Corbett et al., 1999). The potential influence of such groundwater nutrient inputs through diffuse flow and springs has been noted previously in Jamaica, Mexico, U.S. and Kenya (Simmons, 1992; Herrera-Silveira, 1996; Lapointe, 1997; Kamermans et al., 2002). In those studies, however, the

Table 5
Thalassia testudinum leaf tissue nutrient values from Nichupte Lagoon, sampled during a dry season, (March 2002)

Location		%C (SE)	%N (SE)	%P (SE)	C:N:P	*n*
Nichupte north	Dry	33.49 (1.28)	2.93 (0.12)	0.17 (0.01)	541:42:1	4
Nichupte south	Dry	34.35 (0.37)	2.50 (0.18)	0.13 (0.02)	794:50:1	4
	p	0.54	0.09	0.16		

Values are means ± standard error and significance values are for unpaired *t*-tests.

Table 6
Comparison of *Thalassia testudinum* leaf tissue nutrient values from Nichupte Lagoon ($n=3$), between 1991 (van Tussenbroek, 1996; CN1, CN2, BJ3) and 2002 (current study)

	1991, mean (SE)	2002, mean (SE)	*n*
%C	36.87 (1.16)	34.78 (0.12)	3
%N	2.04 (0.14)	2.77 (0.19)	3
%P	0.14 (0.01)	0.12 (0.02)	3
C:N:P	680:32:1	749:51:1	3
C:N	21:1	15:1	3

Values are means ± standard error.

groundwater flows contained high dissolved nitrogen concentrations and were implicated in such effects as the increasing abundance of macroalgae and algal turfs over coral reefs (Lapointe, 1997), or changes in $\delta^{15}N$ ratios (Kamermans et al., 2002). Fertilization experiments in the Puerto Morelos Reef Lagoon, where both nitrogen and phosphorus were added, resulted in elevation of both %N and %P in seagrass tissues (Duarte et al., 1995); therefore, the current elevation of *T. testudinum* tissue phosphorus but not nitrogen suggests that the Puerto Morelos submarine vents are primarily increasing the availability of phosphorus to *T. testudinum*.

Point sources of nutrients, resulting in elevated *T. testudinum* tissue phosphorus, have previously been reported adjacent to bird colonies in tropical carbonate systems (Fourqurean et al., 1992a). Similar to the current study, *T. testudinum* %N values were not significantly different near to or away from the source, but leaf %P was significantly elevated in close proximity to the source. In that study the maximum tissue phosphorus values, 30 m from the bird colony, were 0.161 ± 0.002% P (Fourqurean et al., 1992a), which is considerably lower than the 0.38 ± 0.06% phosphorus measured adjacent to submarine springs after rain in Puerto Morelos Lagoon.

Porewater nutrient concentrations within the Puerto Morelos Reef Lagoon (NH_4^+ 1.2–3.42 μM, DIN 2.8–4.4 μM and PO_4^{3-} 1.0–1.5 μM; Table 9) measured within this study were extremely low compared to global mean values for seagrass meadows of 86 μM (NH_4^+) and 12 μM (PO_4^{3-}) (Hemminga and Duarte, 2000). However, the measured values agree with previous reports of 5.5 ± 1 μM DIN and 2.7 ± 1.1 μM PO_4^{3-} for the Puerto Morelos Reef Lagoon (Duarte et al., 1995). But, while PO_4^{3-} concentrations are comparable to other carbonate tropical systems such as Florida Bay, mean NH_4^+ concentrations are two orders of magnitude lower than Sulawesi, Indonesia (100 μM), Corpus Cristi (87–100 μM) and Florida Bay (100 μM) (Erftemeijer et al., 1994; Lee and Dunton, 1999, 2000; Fourqurean et al., 1992a); and one order of magnitude less than Laguna Madre (26–30 μM; Lee and Dunton, 1999, 2000). Enrichment experiments have previously suggested that *Thalassia testudinum* may be nitrogen limited in porewater NH_4^+ concentrations less than 100 μM (Lee and Dunton, 2000) which is consistent with experiments on other seagrass species (*Zostera marina*; Dennison et al., 1987) and would suggest that nitrogen is possibly highly limiting in the Puerto Morelos Lagoon. Extensive surveys throughout Florida Bay suggest that porewater PO_4^{3-} concentrations < 2 μM are likely to result in phosphorus limitation of seagrass growth (Fourqurean et al., 1992b). However, even with very low porewater NH_4^+ concentrations and low N:P porewater ratio (Table 9), *T. testudinum* leaf tissue did not show evidence of nitrogen limitation (%N > 1.8%, Table 3).

Thalassia testudinum tissue nutrient concentrations within the Puerto Morelos Lagoon away from submarine springs (1.8–1.9% N and 0.14% P; Table 3) are similar to mean values measured from over 500 sites in Florida Bay (1.82% N and 0.11% P; Fourqurean and Zieman, 2002). These concentrations are also comparable to sites classified as mesotrophic in Bailey's Bay, Bermuda (1.89% N and 0.13% P; McGlathery, 1995). In the current study, there was no increase in tissue % N with high flows through submarine springs, however there must be an adequate source of nitrogen to support the tissue concentrations measured in these seagrasses. A potential source is nitrogen fixation within surface sediments. Nitrogen fixation rates of 0.07–3.9 mg N m^{-2} d^{-1} have previously been measured in coarse grained carbonate sediments (O'Neil and Capone, 1989). If this nitrogen were dispersed through the bottom 1 m of the water column, it would raise dissolved inorganic nitrogen by up to 0.3 μM per day.

As there is currently no direct evidence for phosphorus input through submarine vents (absence of water column data), an alternate explanation for the increased tissue % P after rain events is that dissolved iron may be transported to the reef through the groundwater, resulting in greater phosphorus availability in *T. testudinum* meadows. The carbonate sediments of the Caribbean coastline of the Yucatan Peninsula combined with the low rainfall and absence of riverine input result in regionally and globally low marine porewater iron concentrations (< 1 ppm in porewaters) (Duarte et al., 1995). This has led to the suggestion that *T. testudinum* growth in the Puerto Morelos Reef Lagoon may be limited by iron (Duarte et al., 1995).

Table 7
Thalassia testudinum leaf tissue stable isotope values ($\delta^{15}N$; ‰), along north east Yucatan Peninsula

Location	$\delta^{15}N$ (SE)	*N*
Nichupte north	a 9.06 (0.73)	4
Nichupte south	ab 5.49 (0.77)	4
Submarine springs	bc 1.90 (0.81)	4
Puerto Morelos Lagoon	c 1.69 (0.88)	6

Values are means ± standard error, letter codes indicate results of a post hoc Tukey's test.

Table 8
Results of a one-way ANOVA on $\delta^{15}N$ data presented in Table 7

	Source	df	MS	F	P
$\delta^{15}N$	Site	3	57.48	14.75	<0.001
	Error	14	3.90		

Leaf tissue iron concentrations of 60–80 μg Fe g_{dry}^{-1} previously measured in the lagoon are well below the 100 μg Fe g_{dry}^{-1} considered as critical for angiosperms (Duarte et al., 1995). When iron alone was added to *T. testudinum* meadows in Puerto Morelos Reef Lagoon, even recognizing the very low concentrations of porewater ammonium and phosphate, leaf tissue %N remained constant while %P increased from 0.19 ± 0.01 to 0.26 ± 0.01%P (Duarte et al., 1995). This is the same pattern observed after a rain event in the *T. testudinum* close to submarine springs in the current study (Table 2). Another iron addition study in Florida Bay found leaf iron concentration and porewater phosphorus to increase, although *T. testudinum* leaf phosphorus concentrations were not significantly different between iron addition and control treatments (Chambers et al., 2001). However, all treatments in the Chambers et al. (2001) study showed a significant increase in tissue %P over the time of the experiment, suggesting that phosphorus demand may well have been satiated.

4.2. Nutrient processes within Nichupte Lagoon System

In contrast to Puerto Morelos Lagoon, *Thalassia testudinum* leaf tissue from the Nichupte Lagoon System had nitrogen concentrations that were high on a global scale and phosphorus concentrations that were high on a regional scale (Fig. 2). Tissue nitrogen was measured as 2.5–2.9% N (Table 5), which is higher than the mean for Florida Bay (1.8%; Fourqurean and Zieman, 2002), and also higher than the global median which includes (often eutrophic) temperate estuaries of north America and Europe (1.8%; Duarte, 1990). The Nichupte Lagoon values are comparable to concentrations measured in Bermuda for fertilizer addition treatments in a bay already classified as eutrophic (2.81%; McGlathery, 1995). Leaf tissue phosphorus concentrations in Nichupte Lagoon (0.13–0.17% P, Table 3) are lower than global median values (0.2% P; Duarte, 1990) as expected for tropical carbonate sediments, however are higher than mean values for Florida Bay (0.11%; Fourqurean and Zieman, 2002) and comparable to values measured in meadows classified as mesotrophic and eutrophic in Bermuda (0.12–0.19%; McGlathery, 1995). Other indicators such as changes in benthic communities and oxygen flux also indicate eutrophication of the north east corner of the Nichupte Lagoon System (Reyes and Merino, 1991; Merino et al., 1992; van Tussenbroek et al., 1996).

Nutrient content of *T. testudinum* indicates an increase in nitrogen loading within the Nichupte Lagoon System over the past decade (Table 6). Although the season was different between the comparison, nitrogen uptake rate (Lee and Dunton, 1999), calorific value (Dawes, 1986) and production (van Tussenbroek, 1995) of tropical/subtropical *T. testudinum* have all been measured to be higher in summer and fall (June - November) than during spring (March). Therefore if a seasonal component was present in differences between the 1991/1992 and 2002 data, it would result in the 1991/1992 summer/fall samples having higher leaf nutrient content. The 2002 samples were also taken after an extensive dry period (Table 1) such that any sources of nutrients to the lagoon would be at a minimum.

Seagrass and macroalgae, or seagrasses alone have been reported to have median 'Redfield' ratios of 550:30:1 and 474:24:1, respectively (Atkinson and Smith, 1983; Duarte, 1990). The 1991 samples, with an N:P of 32:1 (Table 6) indicate these seagrasses to be either balanced or somewhat elevated in nitrogen and therefore potentially limited by other factors than nutrients (Fourqurean and Rutten, 2003). In a system with potential eutrophication, light limitation is possible and has previously been reported to change C:N ratios in seagrass (Abal et al., 1994; Grice et al., 1996). However, water transparency in the Nichupte Lagoon System is high, except for certain storm-exposed areas near to fringing mangroves and these areas were not included in the present study (van Tussenbroek, unpublished data). Also, consistent light limitation would be expected to result in consistent N:P ratios (at maximum tissue %N and %P) over time, whereas between 1991 and 2002, the N:P ratio of *T. testudinum* in Nichupte Lagoon System increased from 32:1 up to a nitrogen enriched 51:1. In Nichupte Lagoon, C:P ratios have increased from 680:1 to 749:1 while C:N ratios have declined from 21:1 down to 15:1. This suggests that, rather than light limitation resulting in luxury nutrient uptake, ambient nitrogen availability has increased, possibly even reducing tissue phosphorus.

Table 9
Porewater nutrient concentration (μM) from Puerto Morelos Reef Lagoon, away from and adjacent to submarine springs, May 2002

	NH_4^+	NO_3^-	NO_2^-	DIN	PO_4^{3-}	N:P
Puerto Morelos Lagoon	3.42 (0.61)	0.92 (0.19)	0.04 (0.01)	4.37 (0.75)	1.52 (0.06)	2.9:1
Submarine springs	1.20 (0.25)	1.57 (0.80)	0.07 (0.04)	2.83 (0.59)	1.26 (0.12)	2.2:1

Values are means ± standard error.

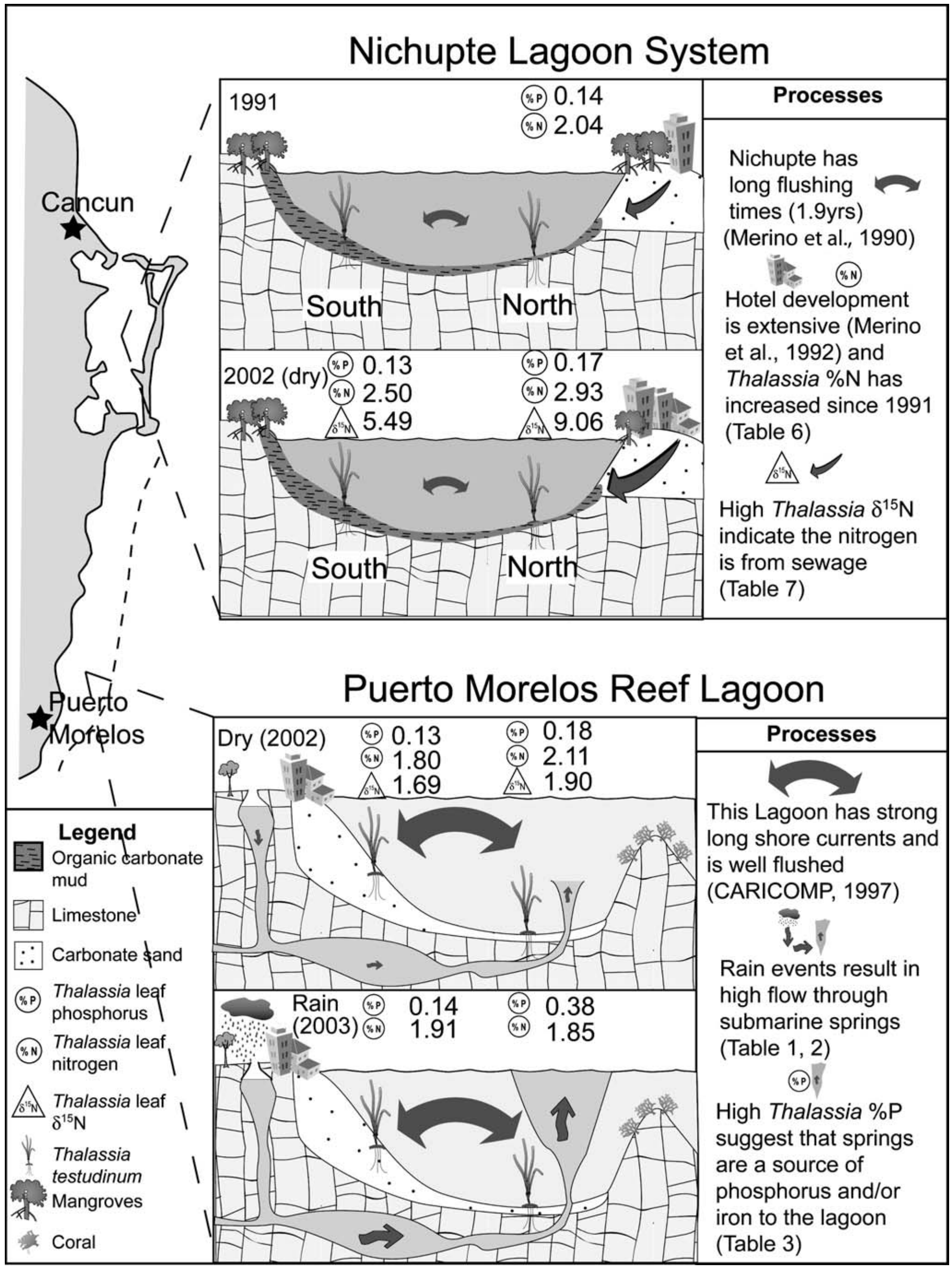

Fig. 2. Conceptual diagram of processes of nutrient input to the Nichupte Lagoon System and the Puerto Morelos Reef Lagoon.

The $\delta^{15}N$ of *T. testudinum* leaves within the Nichupte Lagoon System are consistent with wastewater being a source of nitrogen into the north of the lagoon system. This is the locality of Cancun town as well as the most intense development of the Cancun 'hotel zone.' There is an increasing body of literature that successfully links patterns of $\delta^{15}N$ in seagrasses, corals, mangroves, polychaetes, benthic microalgae and macroalgae to known wastewater or other anthropogenic sources of nitrogen (Lapointe, 1997; Udy and Dennison, 1997; Riera et al., 2000; Jones et al., 2001; Waldron et al., 2001; Gartner et al., 2002; Costanzo et al., 2003). However, other factors such as heterotrophic processing of nitrogen through denitrification can also result in high values of $\delta^{15}N$, a process also favoring removal of ^{14}N. In Tomales Bay (California), nitrogen predominantly enters the bay from the ocean entrance and is trapped within the system and increasingly denitrified, so that *Zostera marina* growing near the ocean has a relatively low $\delta^{15}N$ to that growing at the head of the bay (Fourqurean et al., 1997). This pattern could be erroneously attributed to wastewater inputs at the head of the bay with dilution due to ocean flushing. The Nichupte Lagoon System has two small openings, one in the far north and one in the far south of the system. Both are narrow, resulting in small tidal exchange into the system (Merino et al., 1990). The (non-significant) north to south gradient (from higher to lower) in tissue nutrients (Table 5), as well as the low porewater (Table 9) and tissue nutrient (Table 3) concentrations measured outside Nichupte Lagoon System in the nearby Puerto Morelos Reef Lagoon, suggest that nutrients are not entering Nichupte Lagoon System through these channels. The flushing time within the system (ca. 1.9 years; Merino et al., 1990) also does not have a north-south gradient (as in Tomales Bay; Fourqurean et al., 1997) so the best explanation for the trends in $\delta^{15}N$ within the Nichupte Lagoon System is that nitrogen with a high $\delta^{15}N$ (e.g. wastewater) is being added either directly or through groundwater into the northern region of the Nichupte Lagoon System.

4.3. Summary

This study used the widely distributed seagrass *Thalassia testudinum* to investigate nutrient status and sources into the Puerto Morelos Reef Lagoon and Nichupte Lagoon System of the north east Yucatan Peninsula. The potential influences of the different sources of nutrients were identified (Fig. 2). Submarine springs were found to be a potential cause of nutrient patterns observed in the Puerto Morelos Lagoon by increasing the availability of phosphorus to *T. testudinum* during rain events, either through adding phosphorus and/or iron to meadows. Within the Nichupte Lagoon System, wastewater was inferred to be resulting in the high and increasing nitrogen availability to these seagrass meadows.

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