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REPORT

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Importance of epiphytic cyanobacteria as food sources for heterotrophs in a tropical seagrass bed

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Abstract The natural carbon and nitrogen stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$) of various autotrophs and heterotrophs were measured in a *Syringodium isoetifolium*-dominated seagrass bed at Dravuni Island, Fiji to define carbon and nitrogen sources for heterotrophic organisms in a system where few animals graze directly on seagrass leaves. The organic carbon, nitrogen, and phosphorus content of organisms was also determined. The $\delta^{13}C$ and $\delta^{15}N$ data suggest that herbivorous heterotrophs in this seagrass bed depend significantly on epiphytic cyanobacteria rather than seagrass leaves and its detritus. This can be attributed to relative differences in nitrogen content of those organic materials. The cyanobacteria nitrogen content (3.6–4.8% of DW) is nearly half that of heterotrophs (7.0–8.6% N of DW) while that of *S. isoetifolium* origin (0.6–1.1% N of DW) is less than one third of the cyanobacteria nitrogen content. Phosphorus content was similar among cyanobacteria (0.8–1.1 mg g^{-1}) and *S. isoetifolium* (0.4–1.4 mg g^{-1}). These results suggest that cyanobacteria are important food sources for heterotrophs at the study site, and that inorganic nitrogen released through breakdown of cyanobacteria by heterotrophs may support the continued production of *S. isoetifolium*.

Key words *Syringodium isoetifolium* · Cyanobacteria · Stable isotopes · Nitrogen · Phosphorus

Introduction

Tropical seagrass beds are one of the conspicuous components of coral reef ecosystems. They develop mostly on the soft bottoms of lagoons or in reef-flat zones (Sorokin 1993). Tropical seagrass beds have been recognized as areas of high primary production (Hillman et al. 1989), and their production sometimes exceeds that of seaweeds or corals (Sorokin 1993).

Seagrass beds support the production of various heterotrophs by offering microhabitats (Mellors and Marsh 1993) and food that originates in seagrasses and associated primary producers in the community. Although a few heterotrophs such as herbivorous fishes (Ogden 1980) and dugongs (Preen 1995) graze directly on seagrass leaves, most marine invertebrates do not because they lack the enzymes to hydrolyze refractory structural compounds. Seagrass detritus, which is nutritionally enriched through bacterial decomposition, is, however, a candidate as heterotroph food (Mann 1988). Organic materials of epiphyte origin may be another important food source for heterotrophs (Mateo and Romero 1997).

To evaluate the importance of organic materials originating in seagrasses and from other primary producers as fuel for heterotrophic production, a seagrass bed dominated by *Syringodium isoetifolium* (Ascherson) Dandy at Dravuni Island, Fiji (Fig. 1) was studied. The island is surrounded by Great Astrolabe Reef, and the seagrass bed develops on coralline sediments. The net primary production by seagrass leaves at the site is high (2.5g DW m^{-2} day^{-1}, Aioi and Pollard 1993), but few herbivorous fishes graze directly on living seagrass (Nojima 1994). Tufts of epiphytic cyanobacteria cover the seagrass, particularly the tips of leaves (Fig. 2A), and epiphytic cyanobacteria constitute 29–95% of the above-ground plant wet weight biomass (Mukai and Iijima 1995). Colonial ascidians with symbiotic algae are also conspicuous autotrophs here (Fig. 2B). *Didemnum molle* (Herdman) is one of the most abundant of symbiotic ascidians, and has been found inhabiting *S. isoetifolium* leaves in maximum colony densities of 980 m^{-2} (Nishihira and Suzuki 1994).

The natural differences in $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of consumer organisms have been used to identify

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autotrophic sources of carbon and nitrogen assimilated over time, assuming no ^{13}C enrichment and approximately 3‰ ^{15}N enrichment for each food chain step (Michener and Schell 1994). If there are clear and consistent differences in the isotopic compositions of primary producers, including seagrasses and epiphytic algae, the contribution of each primary producer can be evaluated as a diet for heterotrophs.

Higher concentrations of nitrogen and phosphorus in animals than in plants and/or detritus imply that nitrogen and phosphorus may limit heterotrophic growth (Hatcher 1994). Therefore, in addition to carbon, we measured nitrogen and phosphorus content to assess the relative quality of heterotroph food sources.

Materials and methods

Study site

All samples were collected between December 8–15, 1995, within a seagrass bed at Dravuni Island, Kadavu, Fiji (18°45′S; 178°30′E, area 0.8 km^2, Fig. 1). Nutrient concentrations in surface waters at the bed were low (< 0.017 μM for nitrite, < 0.025 μM for nitrate, < 0.05 μM for ammonium, and 0.19 μM for phosphate, Yamamuro et al. unpublished). The chlorophyll *a* concentration in surface waters was 0.59 μg l^{-1} (Yamamuro et al. 1993).

The seagrass bed occupies depths from 2 m to 5 m, parallel to the beach line, never emerging even at low tide in spring. The bed consists of three seagrass species, *Syringodium isoetifolium*, *Halodule uninervis* (Forsk.) Aschers., and *Halophila ovalis* Hook, but most of the area is monospecific stands of *S. isoetifolium*.

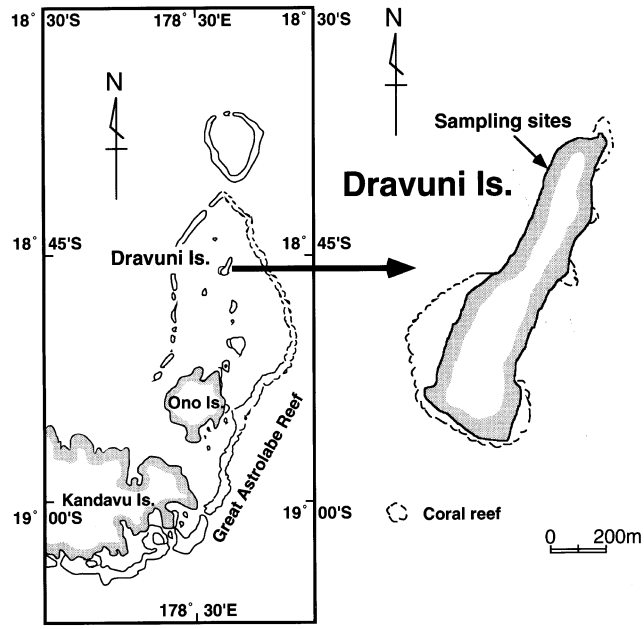

Fig. 1 Study site

Sampling and preparation of autotrophic sources

Particulate organic matter (POM) of bottom water close to the substratum, presumably composed of both phytoplankton and detritus, was sampled by a SCUBA diver using an acid-cleaned plastic bottle at about 3 cm above the sediment in seagrass beds. Water was prefiltered through a 100 μm plankton net to remove zooplankton, then filtered through precombusted (450 °C, 4 h) Whatman GF/F glass fiber filters. Filters were kept frozen until freeze-drying.

Tufts of epiphytic cyanobacteria were collected from the tip of *Syringodium isoetifolium* leaves. Entangled visible materials other than cyanobacteria were removed with forceps in filtered (Whatman GF/F) seawater under a binocular microscope. Cleaned cyanobacteria samples were kept frozen until freeze-drying. Cyanobacteria tufts attached to *Syringodium isoetifolium* leaves were subdivided into three types: type 1, red; type 2, brown; and type 3, brown with longer and thicker tufts than type 2. The species composition of these cyanobacteria was not determined except for type 3, which was made up of an unknown Oscillatoriaceae and *Hydrocoleum cantharidosmum* (Montagne) Gomont.

Seagrasses were cleaned with brushes under a binocular microscope to remove all visible attached materials, rinsed with filtered seawater, and dried at 60 °C to constant weight.

Above-ground parts of *Syringodium isoetifolium* (leaf and vertical rhizome) were categorized into four types: non-senescent young leaf and vertical rhizomes less than 10 cm; senescence type 1: leaf and vertical rhizomes 10 cm or longer without any visible epiphytic organisms; senescence type 2: early senescent leaves and vertical rhizomes 10 cm or longer whose color partly changed to brown and which were partly covered with epiphytic organisms (Fig. 2C); and senescence type 3: late senescent leaves and vertical rhizomes 10 cm or longer whose color was totally brown and which were covered with epiphytic organisms. According to Pollard and Kogure (1993), epiphytic organisms living on senescent leaves are mainly cyanobacteria: *Oscillatoria* spp., *Anabena* or *Aphanizomenon* spp., associated with a few diatoms.

Detritus identifiable as of seagrass origin was separated from surface sediment with forceps under a binocular microscope and dried at 60 °C without cleaning. Macroalgae were cleaned with brushes under a binocular microscope to remove all visible attached materials, rinsed with filtered seawater, and kept frozen until freeze-drying.

Sampling and preparation of heterotrophic consumers

Since the goal of the study was to determine the heterotrophic use of organic matter above the sediment surface, collection of heterotrophs was restricted to above ground in the seagrass bed. Free-living zooplankton were collected with a 10 μm plankton net (mouth 20 cm in diameter) towed for 50 m at 1–2 m depth by a SCUBA diver early at night. The zooplankton sample in the collecting bottle was filtered through a Nucleopore filter (pore size 10 μm), rinsed with deionized water, and dried at 60 °C to constant weight. Dried zooplankton were picked off the filter under a binocular microscope using forceps. The same method did not yield adequate amounts of zooplankton in the daytime.

Two species of epiphytic gammarids inhabit the canopy of the seagrass bed. *Leucothoe* sp. lived in the symbiotic ascidian *Didemnum molle* and *Ampithoe* sp. lived in cyanobacteria tufts. *D. molle* and cyanobacteria tufts were collected by a SCUBA diver, and gammarids were separated from ascidians and tufts under a binocular microscope using forceps. Isolated gammarids were placed on a Nucleopore filter (pore size 10 μm), rinsed with deionized water, and dried at 60 °C to constant weight.

Symbiotic colonical ascidians *Didemnum molle*, were picked from the leaf of *Syringodium isoetifolium*, rinsed with filtered seawater, and kept frozen until freeze-drying. *D. molle* was subdivided into two types – with and without *Leucothoe* sp. For the elemental analysis of

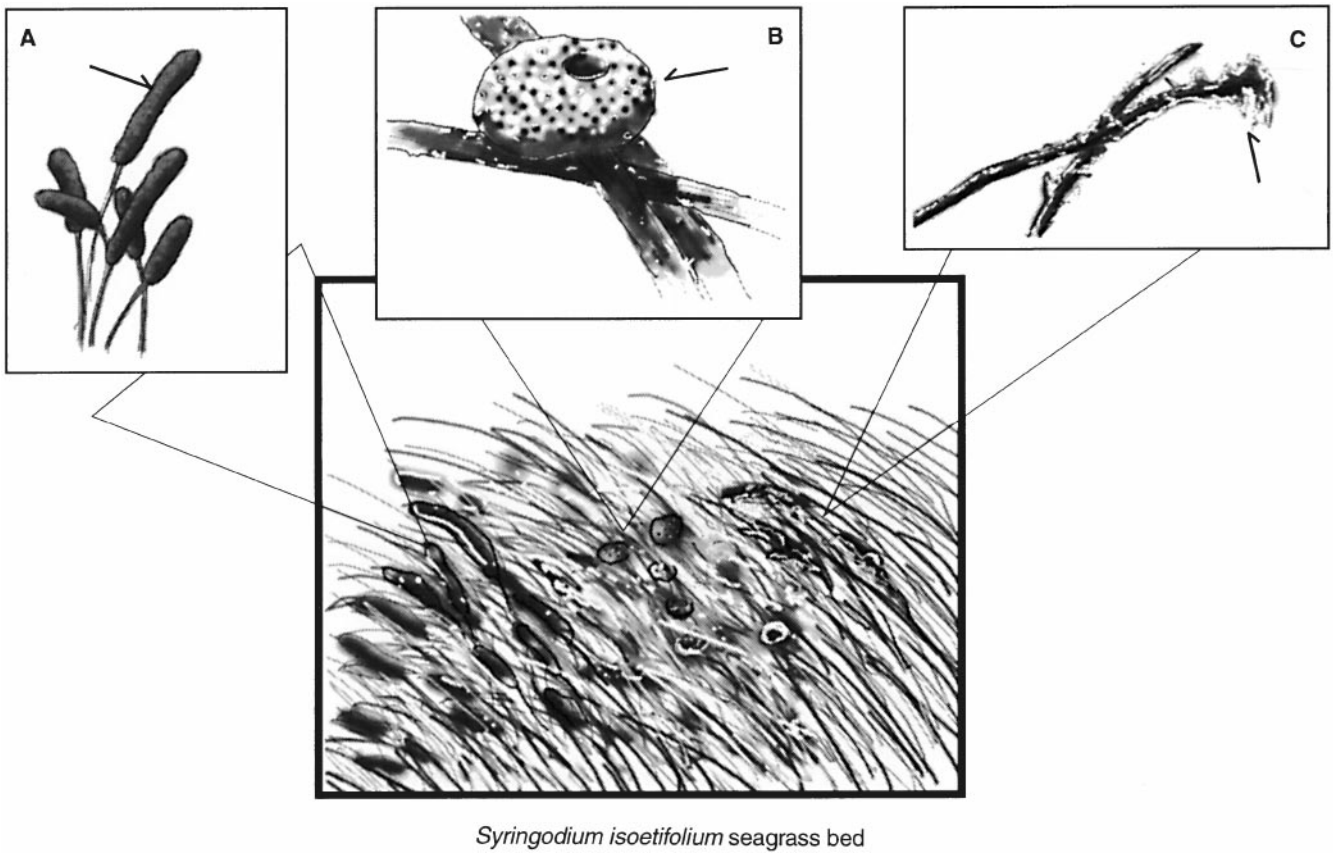

Fig. 2A–C Conspicuous epiphytic autotrophs at the *Syringodium isoetifolium*-dominated seagrass bed. **A** Cyanobacteria tufts; **B** symbiotic ascidian, *Didemnum molle*; **C** filamentous epiphytic organisms

D. molle with *Leucothoe* sp., the sample was prepared after removing *Leucothoe* sp.

Although tropical seagrass beds generally contain abundant epibenthic megafauna such as sea urchins and sea cucumbers, no sea urchins and sea cucumbers were present at the study site. Macrobenthos which may feed on living or senescent autotrophs, including detritus, were not observed in the daytime, so they were collected early at night with a hand net by a SCUBA diver towed over the sediment surface in the seagrass meadow. This sampling collected one species of herbivore (sea hare) and two species of carnivore (mantis shrimp and shrimp). These were rinsed with filtered seawater, and kept frozen until freeze-drying. Free-swimming Gammaridea spp. (length 5–10 mm) were also collected by hand net and classified as epibenthos.

Freeze-dried organisms were powdered and homogenized with an agate mortar and pestle to determine carbon and nitrogen stable isotope ratios and their carbon, nitrogen, and phosphorus content.

Analytical method

To determine stable isotope ratios, organisms were combusted at 1020 °C in an elemental analyzer (Fisons Instruments EA1108) and combustion products (CO_2 and N_2) introduced to an isotope-ratio mass spectrometer (Finigan Mat 252) in a continuous flow using a He carrier. Ratios of $^{13}C:^{12}C$ and $^{15}N:^{14}N$ were expressed relative to the PeeDee Belemnite (PDB) standard for carbon and N_2 in air for nitrogen. Ratios of $^{13}C:^{12}C$ and $^{15}N:^{14}N$ were calculated as:

$$\delta^{13}C,\ \delta^{15}N = \{R(\text{sample})/R(\text{standard}) - 1\} \times 1000\ (‰) \quad (1)$$

where $R = {}^{13}C/{}^{12}C$ or $^{15}N/^{14}N$.

Isotope analysis reproducibility was determined with L-*a*-alanine (SD $\pm$ 0.27 with $n = 33$ for $\delta^{13}C$ and SD $\pm$ 0.15 with $n = 34$ for $\delta^{15}N$). The accuracy was determined using interlaboratory-determined nitroarginine following the method of Minagawa et al. (1984) for $\delta^{13}C$ ($-22.27‰$) and IAEA-N1 for $\delta^{15}N$ (0.54‰). The amount of nitrogen and carbon in the standards and samples were 60–100 µg and 1–2 mg, respectively.

Organic carbon and nitrogen content was determined using a Yanaco MT-5 CHN analyzer following the method of Yamamuro and Kayanne (1995). Phosphorus was determined colorimetrically with the molybdate blue method after samples were digested by autoclaving (121 °C for 3 h) with potassium persulfate (6.7 $g l^{-1}$) and sulfuric acid (0.25N).

Results

Stable isotope ratios

Each primary producer exhibited characteristic isotope values, including several distinct isotopic signatures within individual categories (Table 1 and Fig. 3).

Table 1 Mean, standard deviation (SD), and number of determinations (*n*) of $\delta^{13}C$ and $\delta^{15}N$ values for organisms sampled from the seagrass bed in Dravuni Island, Fiji. Types 1–3 of cyanobacteria and types 1–3 of senescence in *Syringodium isoetifolium* leaf are defined in text

	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
Sample	Mean	SD	n	Mean	SD	n
POM in bottom water	− 11.4	0.5	2	0.9	0.2	3
Cyanobacteria						
Type 1	− 13.2	0.2	2	− 1.2	0.5	2
Type 2	− 13.6	0.6	2	− 1.9	0.1	2
Type 3	− 14.1	0.1	2	− 1.3	0.1	2
Macroalgae						
Cladosiphon okamuranus	− 12.9	0.1	2	1.4	0.1	4
Seagrasses						
Syringodium isoetifolium						
Leaves	− 5.1	0.1	2	− 0.1	0.9	2
Vertical rhizomes	− 4.0	0.0	2	1.3	0.4	4
Horizontal rhizomes	− 3.3	0.1	2	2.2	0.0	2
Root	− 4.1	0.1	2	1.9	0.0	2
Leaves and vertical rhizomes						
Non senescence	− 4.2	0.3	2	1.4	0.4	2
Senescence type 1	− 3.9	0.0	2	1.6	0.3	2
Senescence type 2	− 5.2	0.6	4	0.9	0.5	4
Senescence type 3	− 4.7	0.4	2	1.0	0.1	2
Detritus	− 3.0	0.1	2	2.2	0.2	2
Halodule uninervis						
Leaves and vertical rhizomes	− 8.3	0.2	2	− 0.1	0.3	2
Horizontal rhizomes	− 8.4	0.1	2	3.3	0.2	2
Root	− 8.5	0.7	4	3.4	0.1	2
Free-living zooplankton	− 11.4	0.1	2	3.2	0.0	2
Epibenthic gammarids						
Ampithoe sp.	− 10.5	0.1	2	1.4	0.1	2
Leucothoe sp.	− 10.3	0.4	2	4.3	0.2	2
Gammaridea spp.	− 9.7	0.2	2	1.1	0.0	2
Symbiotic ascidian						
Didemnum molle without *Leucothoe* sp.	− 16.9	0.2	3	0.7	0.2	2
Didemnum molle with *Leucothoe* sp.	− 17.4	0.2	3	0.9	0.0	2
Other macrobenthos						
Stomatopoda sp. (Mantis shrimp)	− 16.2	0.1	2	4.7	0.4	3
Caridea sp. (Shrimp)	− 12.0	0.2	2	3.1	0.0	2
Aplysia sp. (Sea hare)	− 13.8	0.2	2	− 1.0	0.2	2

Seagrasses, including older, senescing plant parts, had the most positive $\delta^{13}C$ values (− 3 to − 9‰). The $\delta^{15}N$ values of both *Syringodium isoetifolium* and *Halodule uninervis* leaves were 0‰. The $\delta^{15}N$ of rhizomes and roots were more positive for *H. uninervis* (3‰) than for *S. isoetifolium* (1 to 2‰). The $\delta^{13}C$ and $\delta^{15}N$ values of seagrass detritus were within the ranges of rhizomes and senescent leaves of *S. isoetifolium*. The mean $\delta^{13}C$ of the cyanobacteria, POM, and macroalgae were between − 14 and − 11‰; the characteristically more-depleted $\delta^{15}N$ of the cyanobacteria distinguished them from the POM and macroalgae (Fig. 3). Colonial ascidians with symbiotic algae had the most depleted mean $\delta^{13}C$ values (− 17‰) among autotrophs, although this included both autotrophic and heterotrophic components of the organism.

Among heterotrophs, the mean $\delta^{13}C$ of epibenthos and zooplankton were the most enriched (− 11 to − 10‰), followed by macrobenthos excluding the symbiotic ascidian (− 16 to − 12‰). *Aplysia* sp. were the most depleted $\delta^{15}N$ (− 1.0‰) and Stomatopoda sp. the most enriched (4.7‰).

Carbon, nitrogen, and phosphorus contents

Organic carbon content ranged from 20–40% of DW for both autotrophs and heterotrophs (Table 2). Among autotrophs, the phosphorus content of aboveground parts was 1–2 mg g^{-1} (Fig. 4), while the nitrogen content was highest for cyanobacteria (3.6–4.8% of DW) compared to other macrophytes (0.99–1.9% of

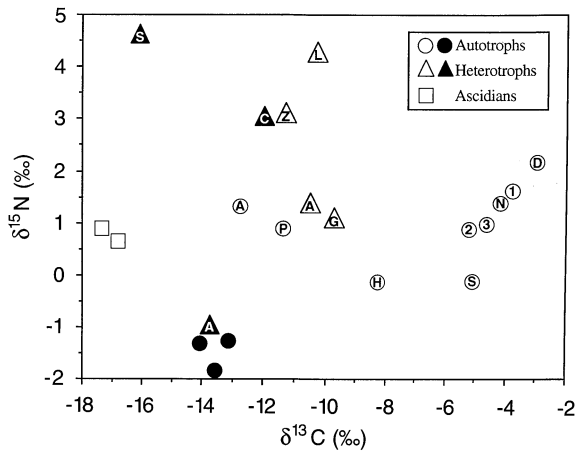

Fig. 3 $\delta^{13}C$ and $\delta^{15}N$ for: cyanobacteria (●) and other autotrophs (○) A: macroalgae, D: seagrass detritus, H: *Halodule uninervis* leaf and vertical rhizome, N: non-senescent *Syringodium isoetifolium* leaf and vertical rhizome, P: particulate organic materials in bottom water, S: *Syringodium isoetifolium* leaf, 1–3: *Syringodium isoetifolium* leaf and vertical rhizome under type 1–3 senescence); macrobenthos (▲ A: *Aplysia* sp., C: Caridea sp., S: Stomatopoda sp.), epibenthos and zooplankton (△ A: *Ampithoe* sp., G: Gammaridea spp., L: *Leucothoe* sp., Z: zooplankton), and symbiotic colonial ascidian, *Didemnum molle* (□)

DW). The nitrogen content of heterotrophs was 5.4–10% of DW and the phosphorus content was 5.0–18 mg g^{-1}.

Senescence tissues increased the carbon and nitrogen content and decreased the phosphorus content (Table 2) of above-ground *Syringodium isoetifolium*. Detritus of *S. isoetifolium* had the lowest carbon, nitrogen, and phosphorus content among all *S. isoetifolium* parts.

Discussion

Characteristics of $\delta^{13}C$ and $\delta^{15}N$ in seagrasses and cyanobacteria

The $\delta^{13}C$ of *Syringodium isoetifolium* leaves ($-5‰$) and the leaves and rhizomes of *Halodule uninervis* ($-8‰$) were within the range of $\delta^{13}C$ previously reported for *S. isoetifolium* (-8.3 to $-3.6‰$) and *H. uninervis* (-13.0 to $-7.8‰$) (Hemminga and Mateo 1996). The leaves of *S. isoetifolium* had more negative $\delta^{13}C$ than the rest of the plant, in agreement with the general trend for photosynthetic tissues to be more depleted in ^{13}C than tissues with a storage function (O'Leary 1981). Such a trend, however, was not clear for *H. uninervis*.

The mean $\delta^{15}N$ of leaves of *Syringodium isoetifolium* and *Halodule uninervis* ($-0.1‰$) was more negative than previously reported for tropical seagrasses: *Halodule wrightii* = 0.4‰ (Wada and Hattori 1991) and 3.9‰ (Macko et al. 1987); *Enhalus acoroides* = 1.6‰ (Bunn et al. 1995); *Thalassia* spp. = 2–5‰ (Yamamuro et al. 1995); and *S. isoetifolium* = 2.9‰ and *H. uninervis* = 2.6‰ (Grice et al. 1996). The mean $\delta^{13}C$ of POM ($-11.4‰$) was similar to that of phytoplankton in the tropics (Deuser et al. 1968), and the $\delta^{15}N$ of cyanobacteria was slightly more negative than that previously reported for tropical cyanobacterial mats (0.0‰, Yamamuro et al. 1995).

Relative importance of autotrophs as food sources for heterotrophs

A comparison of the $\delta^{13}C$ of heterotrophs to that of *Syringodium isoetifolium* (Table 1, Fig. 3) suggests that this seagrass was not the major food source for heterotrophs, because all heterotrophs had more depleted $\delta^{13}C$ values. Detritus, with a similar $\delta^{13}C$ and $\delta^{15}N$ to above-ground *S. isoetifolium*, also did not appear to be a major food for herbivores. Mukai and Iijima (1995) examined the gut contents of the epiphytic gammarid, *Ampithoe* sp., at this study site. Based on microscopic examination, cyanobacteria tufts made up 33% of the *Ampithoe* sp. gut contents while *Syringodium isoetifolium* tissue made up 17%.

Where a mixed diet of two different food sources is suspected the proportion of each source can be calculated using $\delta^{13}C$ and $\delta^{15}N$ values in the following mass balance equation:

$$\delta_m = \delta\text{animal} - \Delta\text{animal-diet} \quad (2)$$

$$\delta_m = f_1 \times \delta_1 + f_2 \times \delta_2 \quad (3)$$

$$f_1 = \frac{\delta_m - \delta_2}{\delta_1 - \delta_2} \quad (4)$$

$$f_2 = 1 - f_1 \quad (5)$$

where δ_m and δanimal are delta values for diet and animal tissue, respectively; Δanimal-diet is an offset due to isotope fractionation between diet and animal tissue; f_1 and f_2 are proportions for source 1 and source 2; and δ_1, δ_2 and δ_m indicate delta values of carbon or nitrogen, for source 1, source 2 and a mixed diet, respectively (Minagawa 1992). For this study, values of Δanimal-diet of 0‰ for $\delta^{13}C$ and 3‰ for $\delta^{15}N$ (Michener and Schell 1994) were adopted. Since the $\delta^{15}N$ of *Ampithoe* sp. was 1.4‰, the $\delta^{15}N$ of its food sources should have been less than $-1.6‰$ ($= 1.4–3.0‰$). The isotope value of type 2 cyanobacteria ($\delta^{13}C = -13.6‰$, $\delta^{15}N = -1.9‰$) suggests that cyanobacteria were a major food source for this amphipod, because it was the only potential food that had a $\delta^{15}N$ lower than $-1.6‰$. Because *S. isoetifolium* tissue in the gut may have been either fresh or senescent, the relative contribution of cyanobacteria and either fresh or senescent, the relative contribution of cyanobacteria and either fresh ($\delta^{13}C = -5.1‰$, $\delta^{15}N = -0.1‰$) or averaged

Table 2 Mean, standard deviation (SD), and number of determinations (*n*) of organic carbon and nitrogen (% of DW) and phosphorous (mg g^{-1}) content for organisms sampled from the seagrass bed in Dravuni Island, Fiji. Types 1–3 of cyanobacteria and types 1–3 of senescence in *Syringodium isoetifolium* leaf are defined in text

	Organic carbon			Nitrogen			Phosphorus		
	Mean	SD	n	Mean	SD	n	Mean	SD	n
Cyanobacteria									
Type 1	34.0	3.3	5	3.56	0.24	4	0.78	0.03	2
Type 2	27.5	0.1	2	3.58	0.42	2	0.91	0.10	2
Type 3	29.4	4.3	2	4.84	0.06	2	1.05	0.10	3
Macroalgae									
Cladosiphon okamuranus	29.6	1.4	4	1.90	0.23	4	1.26	0.20	2
Seagrasses									
Syringodium isoetifolium									
Leaves	23.6	2.4	6	0.99	0.09	5	0.82	0.15	3
Vertical rhizomes	37.4	0.1	2	0.40	0.08	4	0.36	0.06	2
Horizontal rhizomes	42.8	0.6	2	0.46	0.04	2	0.30	0.12	4
Root	24.4	2.6	5	0.93	0.18	5	0.42	0.10	3
Leaves and vertical rhizomes									
Non senescence	19.4	0.2	2	0.55	0.01	2	1.40	0.17	3
Senescence type 1	24.0	1.0	2	0.69	0.02	2	0.98	0.15	3
Senescence type 2	28.6	0.0	2	1.06	0.00	2	0.51	0.07	3
Senescence type 3	29.5	0.5	2	1.10	0.07	2	0.36	0.06	3
Detritus	18.9	1.8	6	0.75	0.12	6	0.29	0.04	2
Halodule uninervis									
Leaves and vertical rhizomes	35.3	2.4	5	1.55	0.13	5	1.98	0.12	2
Horizontal rhizomes	34.2	0.7	4	0.55	0.14	6	1.52	0.08	3
Root	26.1	1.1	4	0.92	0.16	6	0.65	0.04	2
Free-living zooplankton	35.6	1.2	2	9.40	0.11	2	8.06	1.45	2
Epibenthic gammarids									
Ampithoe sp.	38.1	3.7	2	8.06	0.52	2	5.78	0.25	2
Leucothoe sp.	30.6	0.3	2	7.01	0.27	2	8.24	0.63	3
Gammaridea spp.	32.6	2.7	3	7.96	0.64	5	5.67	0.17	3
Symbiotic ascidian									
Didemnum molle without *Leucothoe* sp.	22.9	0.3	2	3.33	0.32	3	2.67	0.38	2
Didemnum molle with *Leucothoe* sp.	22.1	1.5	2	3.53	0.20	5	2.14	0.26	2
Other macrobenthos									
Stomatopoda sp. (Mantis shrimp)	26.6	5.8	2	5.07	0.14	4	17.8		1
Caridea sp. (Shrimp)	36.5	0.7	4	10.2	0.47	5	11.0	0.02	2
Aplysia sp. (Sea hare)	37.2	5.5	2	8.62	0.50	2	4.96	1.02	2

senescent ($\delta^{13}C = -4.6‰$, $\delta^{15}N = 1.2‰$) *S. isoetifolium* leaves was calculated. Calculations using $\delta^{13}C$ suggest that fresh *S. isoetifolium* leaves contributed 36% and senescent 34% of the total food, while solutions using $\delta^{15}N$ suggest 17% and 10% respectively. Calculations using a combination of cyanobacteria and detritus ($\delta^{13}C = -3.0‰$, $\delta^{15}N = 2.2‰$) also suggest the importance of cyanobacteria as a food source (71% using $\delta^{13}C$ and 93% using $\delta^{15}N$). Calculations with combinations of cyanobacteria and other potential sources (POM and macroalgae) failed to give appropriate solutions.

Availability of both $\delta^{13}C$ and $\delta^{15}N$ values enables the calculation of the relative contribution of three food sources. Using the mass balance equation, the proportion of one source is determined analytically as follows:

$$f_1 = \frac{(\delta_m - \delta_3)(\delta'2 - \delta'3) - (\delta_2 - \delta_3)(\delta'm - \delta'3)}{(\delta_1 - \delta_3)(\delta'2 - \delta'3) - (\delta'2 - \delta'3)(\delta_2 - \delta_3)} \quad (6)$$

$$f_2 = \frac{\delta'm - \delta'3 - f_1(\delta'1 - \delta'3)}{\delta'2 - \delta'3} \quad (7)$$

and

$$f_3 = 1 - f_1 - f_2 \quad (8)$$

where δ_1, δ_2, δ_3, and δ_m represent $\delta^{13}C$ of food sources 1, 2, 3, which comprise proportions f_1, f_2, and f_3. $\delta'1$, $\delta'2$. $\delta'3$, and $\delta'm$ indicate $\delta^{15}N$ in the same way (Minagawa 1992). Calculations with combinations of cyanobacteria, fresh, senescent, and detrital *Syringodium isoetifolium* leaves and other potential food sources

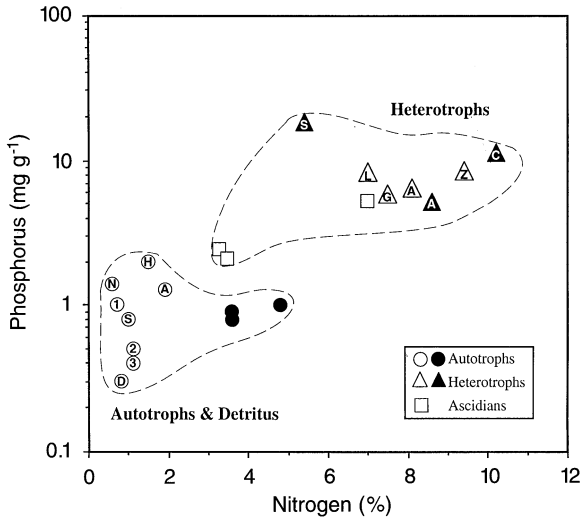

Fig. 4 Nitrogen content (w/w%) versus phosphorus content (mg g^{-1}). Symbols are the same as in Fig. 3

(POM, macroalgae, and detritus) failed to give realistic solutions. Thus, based on stable isotope analysis of seagrass and cyanobacteria alone, the possible contribution of fresh, senescent, and detritus seagrass leaves as a food source of *Ampithoe* sp. is 7–36%, while the contribution of cyanobacteria is 64–93%. These results are in good agreement with gut content analysis results, which suggests that epiphytic cyanobacteria tufts represent a major food source for *Ampithoe* sp.

The diet of Gammaridea spp., whose $\delta^{13}C$ and $\delta^{15}N$ values were similar to those of *Ampithoe* sp., may be similar to that of *Ampithoe* sp. The $\delta^{13}C$ and $\delta^{15}N$ values of the sea hare, *Aplysia* sp., were essentially identical to that of cyanobacteria. Because the gut contents of *Aplysia* sp. were not removed before stable isotope analysis, the *Aplysia* values would include a contribution of these contents. This does not preclude, however, the interpretation that *Aplysia* sp. depends on cyanobacteria rather than seagrass and organic materials of seagrass origin.

The $\delta^{13}C$ and $\delta^{15}N$ values presented here suggest that herbivorous heterotrophs in this seagrass bed depended mainly on the cyanobacteria tufts rather than on *Syringodium isoetifolium* leaves or other organic materials of seagrass origin. I propose that this is due to consumer preference for more nutritious foods, reflected in the higher nitrogen content of cyanobacteria. The nitrogen content of cyanobacteria (3.6–4.8% of DW, Table 2) was closer to that of the heterotrophs than to all other macrophyte (0.99–1.9% of DW), while the phosphorus contents were similar for cyanobacteria (0.8–1.1 mg g^{-1}, Table 2) and macrophytes (0.8–2.0 mg g^{-1}).

POM and detritus of *Syringodium isoetifolium* origin did not appear to be main food sources for the symbiotic ascidian, because the latter was more ^{13}C depleted than POM or detritus of *S. isoetifolium* origin. *Leucothoe* sp. ($\delta^{13}C = -10.3‰$; $\delta^{15}N = 4.3‰$, Table 1) living within the symbiotic ascidian *Didemnum molle* may depend mainly on POM ($\delta^{13}C = -11.4‰$; $\delta^{15}N = 0.9‰$) filtered by *D. molle*. The relative contributions of POM and detritus ($\delta^{13}C = -3.0‰$; $\delta^{15}N = 2.2‰$) as food sources calculated with $\delta^{13}C$ was 87% for POM and 13% for detritus, while solutions calculated with $\delta^{15}N$ were 69% for POM and 31% for detritus. Zooplankton ($\delta^{13}C = -11.4‰$; $\delta^{15}N = 3.2‰$) may also depend on POM. The relative contributions of POM and cyanobacteria ($\delta^{13}C = -13.6‰$, $\delta^{15}N = -1.9‰$) as food sources to zooplankton calculated with $\delta^{13}C$ was 100% for POM, while solutions calculated with $\delta^{15}N$ were 75% for POM and 25% for cyanobacteria.

Thus, for the species analyzed, epiphytic cyanobacteria followed by POM were more prominent sources of organic matter supporting herbivorous heterotrophs than seagrasses in this *Syringodium isoetifolium*-dominated seagrass bed ecosystem. However, some of the POM may have originated from seagrass and its detritus.

Interaction between *Syringodium isoetifolium* and epiphytic cyanobacteria

It is likely that nutrients are lost from a seagrass bed through leaching from living and dead leaves and the export of sloughed leaves and leaf fragments (Hemminga et al. 1991). To facilitate the continuous production of *Syringodium isoetifolium* leaves (2.5g dry wt.m^{-2} day^{-1}, Aioi and Pollard 1993), nutrients lost as plant parts must be replenished. Nutrient concentrations in surface water at the study site in December 1995 were < 0.017 μM for nitrite, < 0.025 μM for nitrate, < 0.05 μM for ammonium, and 0.19 μM for phosphate (Yamamuro et al. unpublished). The low concentration of inorganic nitrogen in the water column of the seagrass bed may have benefited cyanobacteria which fix gaseous nitrogen. The $\delta^{15}N$ of photosynthetic organisms which depend totally on gaseous nitrogen for their nitrogen source are 0 to $-2‰$ (Wada and Hattori 1991; Shearer and Kohl 1993). The $\delta^{15}N$ of the cyanobacteria tufts (-1.9 to $-1.2‰$, Table 1) suggests that all types of cyanobacteria examined depend on gaseous nitrogen as nitrogen source.

Grice et al. (1996) reported that the $\delta^{15}N$ of tropical seagrass leaf tissue was affected by the source of inorganic nitrogen rather than the species of seagrass, and that higher $\delta^{15}N$ was found at more eutrophic sites with high anthropogenic influence. An elevated $\delta^{15}N$ of seagrass leaves was also reported for a temperate estuary with an increased wastewater loading (McClelland et al. 1997). The $\delta^{15}N$ values found in *Syringodium*

isoetifolium and *Halodule uninervis* leaves in this study (Table 1) are the lowest $\delta^{15}N$ for seagrass leaves ever reported suggesting that dissolved inorganic nitrogen supporting seagrasses production at this locality originates from molecular nitrogen fixation. Iizumi (1994) estimated the nitrogen fixed by epiphytic cyanobacteria at the study site to be 113 mg N m^{-2} d^{-1}, more than sufficient to support the seagrass production. High rates of nitrogen fixation (100–200 mg N m^{-2} d^{-1}) by benthic cyanobacteria have also been reported for other tropical and subtropical coastal areas (e.g. Goering and Parker 1972; Wiebe et al. 1975; Hanson and Gundersen 1977). Hanson and Gundersen (1977) reported that in Kaneohe Bay, Hawaii, higher rates of nitrogen fixation were observed in water with less sewage influence. The high nitrogen fixation rates reported for the present study site may be explained by the low concentration of inorganic nitrogen in the water column.

Organic materials of cyanobacterial origin may quickly decompose in the sediment through food-chain processes, and remineralized dissolved inorganic nitrogen with low $\delta^{15}N$ will be subsequently supplied to the seagrasses. Seagrass leaves may also take up dissolved nitrogen (i.e. Stapel et al. 1996) if directly released by epiphytic cyanobacteria.

In conclusion, in the *Syringodium isoetifolium*-dominated seagrass bed on an oligotrophic coral island, *S. isoetifolium* serves as a substratum for attachment and growth of cyanobacteria. Cyanobacteria, which can fix gaseous nitrogen, are consumed by herbivorous heterotrophs, and the supply of remineralized inorganic nitrogen originating from cyanobacteria through excretion and/or break down by heterotrophs, may support the continued production of *S. isoetifolium*. Thus cyanobacteria likely play an important role in the nitrogen cycling within this oligotrophic tropical ecosystem.

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