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Evidence of Direct Particle Trapping by a Tropical Seagrass Meadow

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ABSTRACT: The capacity of seagrass canopies to directly retain sestonic particles was tested by quantifying the rate at which suspended fluorescent tracer particles were retained within a tropical Philippine seagrass meadow and by examining whether the test particles lost from the water column were later bound to seagrass leaves or inside epibionts. The particle loss rates in the presence of seagrass canopies were up to 4 times higher than those in unvegetated and plankton controls. The seagrass canopies trapped particles with a maximum rate of 0.73 (± 0.24) h^{-1}. As much as 5% of the particles trapped by the seagrass leaves were physically adhered to the leaf surfaces following rinsing. Particles were also observed to be ingested by protozoa (ciliates and amoeba-like organisms), residing on the surface of the leaves, and may be the dominant particle trapping mechanism by seagrass leaves. These processes should provide an efficient mechanism for the transfer of planktonic production to the benthos, adding to the high organic carbon input maintained by the high production of the seagrass themselves.

Introduction

The interaction between seagrass meadows and the water column is a key component of the functioning of seagrass ecosystems (Giesen et al. 1990; Sand-Jensen and Borum 1991; Zimmerman et al. 1991; Dennison et al. 1993). Research has provided ample evidence that sestonic particles affect seagrasses through shading, thereby reducing their production and, if excessive, leading to their decline (Cambridge and McComb 1986; Duarte 1995). The effect of seagrasses on seston is largely due to indirect effects, derived from the reduction of turbulence and flow by the plant canopy (Worcester 1995; Koch 1996; Gacia et al. 1999). Seagrass canopies attenuate energy and turbulence intensity (Gambi et al. 1990; Ackerman and Okubo 1993), promoting sedimentation and reducing resuspension (Ward et al. 1984; Gacia et al. 1999; Terrados and Duarte 2000; Gacia and Duarte 2001) and providing a mechanism to explain high particle trapping within seagrass beds. The possibility of direct particle trapping by the seagrass canopy has not been addressed.

Seagrass leaves are substrates for a vast amount of epiphytes, which may release exopolymeric substances (polysaccharides) that are adhesive and can physically bind sestonic particles. The leaves provide a habitat for a diverse range of motile and sessile epifauna (e.g., ascidians, Lemmens et al. 1996; ciliates, Lubel and Murillo 1999) capable of suspension feeding on planktonic organisms, which can actively retain sestonic particles. We tested the capacity of seagrass canopies to directly retain sestonic particles by quantifying the rate at which suspended tracer particles are retained by the canopy of a tropical Philippine seagrass canopies using biological (fluorescently-labelled phytoplankton cells, Sherr and Sherr 1993a) and inert (fluorescent beads) tracer particles (of 1, 3, and 15 μm in diameter). We provide an indication of the importance of passive versus active trapping processes by examining whether the test particles lost from the water column are found bound to seagrass leaves or inside epibionts, respectively.

Methods

The study was conducted in a shallow multispecific seagrass meadow located in the reef lagoon in Silaqui (16°27′03″N, 119°55′35″E), Cape Bolinao, Pangasinan, Northwest Philippines. The meadow which is dominated by *Thalassia hemprichii* (Ehrenb.) Aschers., *Enhalus acoroides* (L.f.) Royle, and *Cymodocea rotundata* Ehrenb. & Hempr. ex Aschers. has been studied extensively (Vermaat et al. 1995; Agawin et al. 1996, 2001; Duarte et al. 1997). Two enclosure experiments were conducted in April 1999. Each experiment consisted of incubations of parcels of the meadow enclosed within PVC rings of 10.2 cm inner diameter supporting a polyethelene plastic chamber, with a septum sampling port, of a height of 30 cm. The chambers enclosed 5 shoots of *T. hemprichii* in a volume of approximately 2.4 L of water. In each experiment, duplicate (experiment 1) to triplicate (experiment 2) chambers were established enclosing seagrasses (*T. hempri-*

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chii), and unvegetated patches created by clipping the *T. hemprichii* leaves initially present. Plankton controls were also included by enclosing seawater within the same type of bag used in the benthic chambers.

A known amount of fluorescently-labelled tracer particles was injected inside the chambers through the septum port, and carefully mixed with the seawater inside the chambers by gently tapping the bags. The tracers used were phytoplankton cells (*Thalassiosira* sp. and *Chlorella* sp. of 15 and 3 μm size, respectively) labelled with a yellow-green fluorescing dye DTAF-5-(4′6-dichlorotriazin-2-yl) aminofluorescein using the method described in Sherr and Sherr (1993a). Inert latex fluorescent beads (1 μm size, Polyscience 17154) were also used. Final concentrations of the tracers in the chambers were approximately 500 cells ml^{-1} for *Thalassiosira* sp., 1,000 cells ml^{-1} for *Chlorella* sp., and 6,000 1-μm beads ml^{-1}. Duplicate chambers with seagrasses, unvegetated sediments, and plankton controls were also incubated free of tracers to determine the net growth rate of naturally occurring picophytoplankton cells (size 1 μm).

Subsamples (duplicates of 5 ml) of water were collected through the septum port of each chamber at 0 h, 1.5 to 2 h, and 24 h after tracer additions. The samples were quickly fixed with glutaraldehyde (1% final concentration) and frozen in liquid nitrogen until determination of the abundance of tracers and naturally occurring picophytoplankton cells with a Facscalibur (Becton-Dickinson) flow cytometer according to population fluorescence and light scatter characteristics as reported in Vaulot et al. (1990). The disappearance rate of the fluorescent tracers from suspension and the net growth rates of the naturally occurring picophytoplankton cells were calculated as $(\ln N_t - \ln N_0)/\text{time}$ where N_0 is the initial cell abundance and N_t is the abundance of cells after a time period t. The disappearance rates of tracers in each treatment were calculated during the first 2 h of the experiment.

The two experiments were combined to have $n = 5$ for each treatment at each sampling time (Experiment 1: $n = 2$, Experiment 2: $n = 3$) since the two experiments gave similar results (as determined by *t*-tests). By combining the experiments, the results have greater statistical significance, and the graphical comparisons are simplified. Analysis of covariance (ANCOVA) was used to test the differences between the relative abundance of tracers among treatments (+seagrasses, −seagrasses, and plankton controls) with time (as covariate). Post hoc comparisons among treatments at each sampling time were conducted using Tukey's highest-significant-difference means test (Sokal and Rohlf 1981). Normality of data was tested using Kolmogorov-Smirnov test (Sokal and Rohlf 1981). The trapping rate of the tracers by the seagrass canopy was estimated, assuming the particle trapping to be additive, as the difference between the disappearance rate of the tracers in chambers with seagrasses and chambers without seagrasses (unvegetated patch).

The leaves of *T. hemprichii* were harvested from the chambers after 24 h of incubation, and fixed with Lugol's solution:formalin:sodium thiosulfate (Sherr and Sherr 1993b) to inspect them through epifluorescent microscopy to assess where the particles end up. Before the microscopic inspection, the second oldest leaves were cut into 3-cm pieces and gently washed three times with filtered seawater. The second oldest leaves were chosen because they were not as heavily epiphitized as the oldest leaves which interfere, because of intense background fluorescence with epifluorescent microscopy. Drops of DAPI (4,6-diamidino-2-phenylindole) 10 μg ml^{-1} concentration were placed on the leaves to stain heterotrophic epibionts and left for 10 to 15 min prior to epifluorescent microscopic examination. Leaf surfaces were examined to detect particles attached to their surfaces and those contained inside the many protozoans associated with the leaves. Counts of particles attached to these leaves represent conservative estimates of trapping by the canopy, since more particles should be attached to the more epiphytized leaves. The microscopic search was done systematically (in zigzag fashion on the leaf surface) in at least 200 fields at 320×, so that about 16% of the total leaf area was examined. The tracers attached to the blade surfaces were counted in at least 100 fields at 100×. The total number of tracers found tightly bound on the seagrass leaves (after rinsing) in the chambers were estimated as the average number of tracers in the microscopic field multiplied by the number of fields to cover the total leaf area surface (double sided) of *T. hemprichii* shoots inside the chambers (*T. hemprichii* shoot leaf area [single side] = 2,656 ± 2.32 mm^2).

Results and Discussion

The relative abundance of tracers differed significantly (ANCOVA, $n = 45$, $p < 0.05$) among the experimental treatments with time (Fig. 1). The disappearance rates of fluorescent tracers in chambers with *T. hemprichii* leaves were up to four times faster than in unvegetated and plankton controls (Fig. 1, Table 1). The loss rates of tracers in unvegetated and plankton controls did not differ significantly from each other (Tukey's multiple comparison test, $p > 0.05$). After 24 h, there were few tracers left inside the chambers with and without

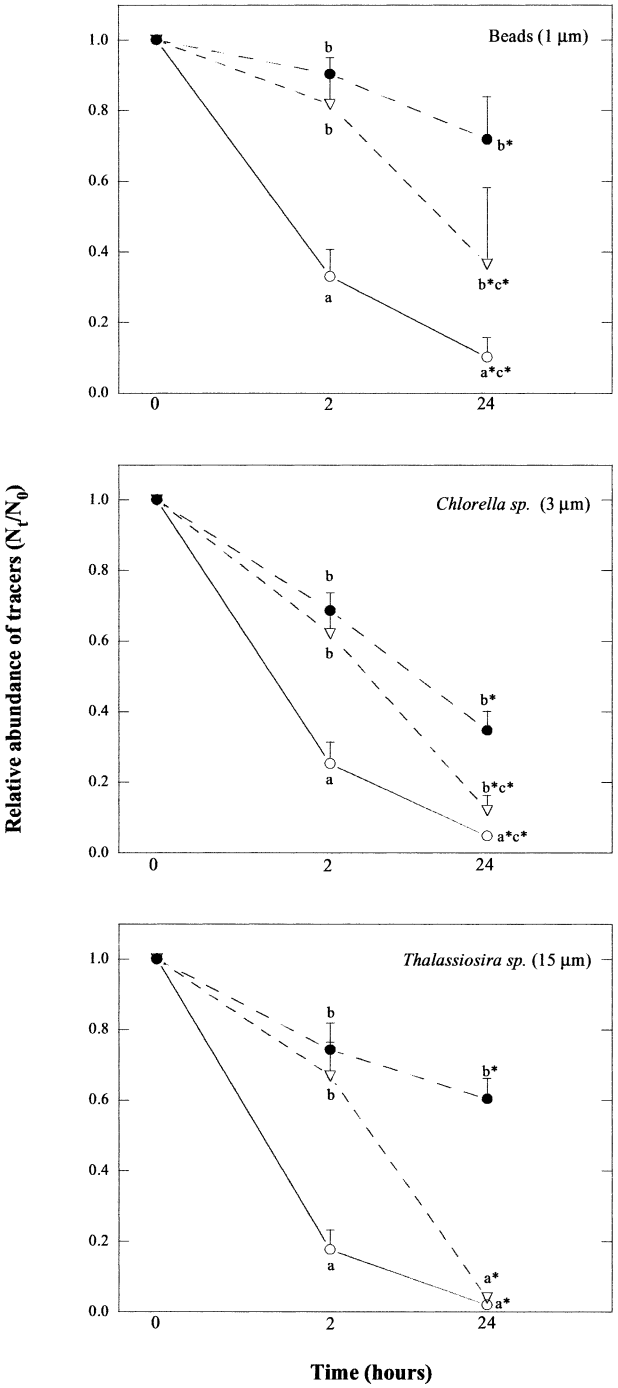

Fig. 1. The relative abundance (mean ± SE, n = 5) of suspended fluorescent tracers in chambers containing shoots of *Thalassia hemprichii* (○), unvegetated chambers (∇), and the water column only (●) in Silaqui, Cape Bolinao. Different letters denote significant ($p < 0.05$) differences among treatments in each sampling period (Tukey's multiple comparison test for each sampling period). * indicate results of Tukey's test for t = 24 h.

the seagrasses (Fig. 1). Seston particles were rapidly trapped by the seagrass canopies, clearing most of the particles within 2 h (Table 1). Although the loss rates among the different tracer particles during the first 2 h of the experiment were not significantly different from each other (ANOVA, $p > 0.05$), micro-sized particles (15 μm) tended to be trapped faster by the canopies than pico-sized particles (1–3 μm; Table 1). The net growth rates of natural picophytoplankton (~ 1 μm) following 2 h of incubation in the presence of seagrass leaves were negative indicating net loss of picophytoplankton (Fig. 2). Net growth rates of natural picophytoplankton populations remained positive in the chambers without seagrass leaves and in the plankton controls (Fig. 2).

Examination of the *T. hemprichii* leaves by epifluorescent microscopy revealed many trapped particles bound to seagrass surfaces. Trapped particles were also observed inside protozoa (ciliates and amoeba-like organisms). After 24 h of incubation, the total number of tracer particles firmly adhered to the leaf surfaces inside the chambers were estimated to be on average 1.7×10^5, 1.6×10^4, and 1.2×10^4 for fluorescent beads, *Chlorella* sp., and *Thalassiosira* sp. tracer particles, respectively. Provided that the trapping rates by the seagrass canopy averaged 1.1, 0.5, and 0.24 d^{-1} (using the data for all sampling times 0, 2, and 24 h) for fluorescent beads, *Chlorella* sp., and *Thalassiosira* sp. tracer particles, respectively, only 1%, 2%, and 5% of the fluorescent beads, *Chlorella* sp. and *Thalassiosira* sp. tracer particles, respectively, were found firmly physically adhered to the seagrass leaves after rinsing.

These results provide evidence that seagrass canopies are capable of substantial direct particle trapping and affect seston not only indirectly through the effects of reduction of flow. The direct trapping of sestonic particles by seagrass canopies occurs through both passive trapping, involving the attachment of particles to leaf surfaces, and active processes, involving particle ingestion by phagotrophic protozoans and filtration by suspension feeders on the seagrass leaves. Although the relative importance of each of these two mechanisms could not be ascertained directly in this study, particles adhered firmly on young seagrass leaves, following rinsing, ranged from 1% to 5% of the particles trapped by the seagrass canopy, suggesting that particle ingestion by phagotrophic protozoans and filtration by suspension feeders on the seagrass leaves may be the dominant trapping mechanism. Seagrass canopies increase the effective benthic surface by as much as 10 fold (Hemminga and Duarte 2000), thereby increasing the surface available for sediment deposition and the probability

TABLE 1. Mean (± SE, n = 5) disappearance rates (h^{-1}) of tracers during the first 2 h of the experiment in the chambers with and without seagrasses and in the water column. Different letters in superscript indicate significant differences ($p < 0.05$) among treatments (Tukey's multiple comparison test). Mean (± SE) trapping rates (h^{-1}) of tracers by the seagrass canopy calculated during the first 2 h of the experiment are also presented.

Tracer Disappearance Rates In	Beads (1 μm)	*Chlorella* sp. (3 μm)	*Thalassiosira* sp. (15 μm)
+ Seagrasses chamber	0.59 (±0.12)[a]	0.74 (±0.15)[a]	0.95 (±0.17)[a]
− Seagrasses chamber	0.15 (±0.05)[b]	0.24 (±0.07)[b]	0.22 (±0.07)[b]
Plankton control	0.06 (±0.03)[bc]	0.20 (±0.04)[bc]	0.16 (±0.05)[bc]
Trapping rates by the seagrass canopy	0.44 (±0.17)	0.5 (±0.24)	0.73 (±0.24)

of contact and subsequent trapping of sestonic particles on the seagrass leaves.

Our results show that seagrass canopies are able to trap as much as 70% of the suspended particles present within the canopy in less than an hour. These results suggest that the canopy of the seagrass meadow studied, should be able to remove the entire particle load of the shallow waters (average depth of 1.5 m) of the Silaqui reef lagoon within each tidal cycle. The rates presented may differ in actual field conditions since water turbulence within the chambers are lower than under field conditions, reducing particle contact rates within the canopy and underestimating the trapping rates by the seagrass leaves. High flow rates in actual field conditions may cause resuspension, washing particles out of the meadow such that the trapping rates by seagrass leaves inside the experimental chamber may also be overestimated.

The natural picophytoplankton populations were also trapped directly by seagrass leaves resulting in negative net rates of population growth in the presence of the seagrass canopy. The apparent high removal of natural picophytoplankton from the water column by the seagrass leaves may explain why the biomass of picophytoplankton remained low although their growth rates were high (up to 1.3 d^{-1}, Agawin et al. in press) in the study area. Our results suggest that this mechanism is an important loss process for picophytoplankton in the shallow waters of this tropical area.

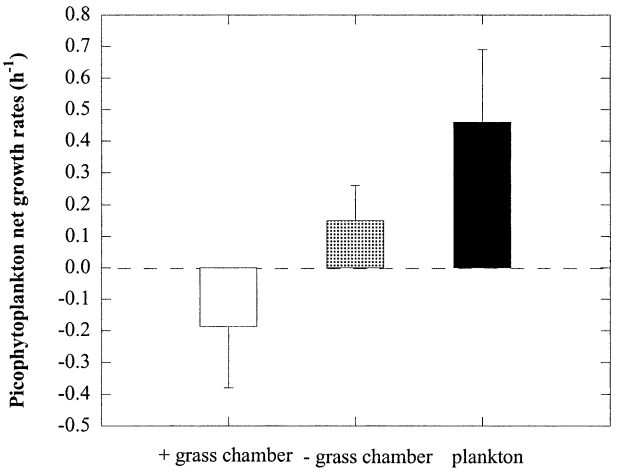

Fig. 2. Mean (± SE, n = 5) net growth rates of natural picophytoplankton in the chambers with and without shoots of *Thalassia hemprichii* (open and dotted bars, respectively) and chambers enclosing the water column only (solid bar) in Silaqui, Cape Bolinao.

The active process of trapping phytoplankton from the water column by epifaunal suspension feeders and phagotrophs on the leaves may be an important loss process in controlling phytoplankton biomass and seston loads in shallow water columns. In seagrass meadows in Western Australia, epifaunal suspension feeders on seagrass leaves (hydrozoids, bryozoans, spirorbirds, barnacles, and amphipods) can attain substantial biomasses and are potentially capable of filtering the overlying water column daily, and may partially control local densities of suspended organic matter (Lemmens et al. 1996). This suggests that seagrass canopies play a major role in the flow of carbon between the water column and the benthic region in shallow coastal ecosystems, since they may trap most of the planktonic primary production.

The canopy of the tropical Philippine seagrass meadow studied serves as an important trap for sestonic particles over a broad size range. The direct processes by which seagrass canopies affect sestonic particles include both passive (particles sticking to the leaf surfaces) and active (particles ingested by the epiphytic fauna) processes. We suspect that these processes operate in seagrass meadows elsewhere, providing an efficient mechanism for transfer of planktonic production to the benthos. The complex and productive food webs associated with seagrass meadows benefit from both the transfer of seagrass-derived carbon and the facilitation of the transfer of planktonic primary production derived from particle trapping by the canopy.

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