Importance of Nutrients (N, P, and $\text{NO}_3 + \text{NO}_2$) in Growth of the Surfgrass, *Phyllospadix iwatensis* Makino

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Abstract: Indoor experimental transplanting of *Phyllospadix iwatensis* to three aquaria under conditions of 0 g (aq-1), 4 g (aq-2), and 8 g (aq-3) biweekly nutrient enrichment was conducted in order to quantify the role of nutrients in seagrass production. Nutrient concentrations (total N, total P, and $\text{NO}_3 + \text{NO}_2$) in overlying sea water, porewater, and sediment were analyzed using standard chemical methods. Seagrass shoot and rhizome production was measured by a thread marking technique. The growth rates of leaf and rhizome were constant throughout the course of the experiment in aq-1, showed an acceleration in aq-2, and attained their highest levels in aq-3 due to the different nutrient conditions. Similarly, shoot density, inflorescence production, reproductive effort, leaf area index (LAI), C, N, and P contents, photosynthetic pigment, and biomass were also varied greatly with nutrient level, in all cases with concentrations or rates remaining constant in aq-1, increasing or accelerating in aq-2, and reaching their highest levels in aq-3. These results show that nutrients are a strong factor influencing *Phyllospadix iwatensis* production.

Key words: aquarium, nutrient enrichment, growth rate, LAI, nitrogen, phosphorus, surfgrass, *Phyllospadix iwatensis*

INTRODUCTION

Seagrass is known as a "canary of the sea" for its ability to signal changes in marine environments, and its study is important to marine ecology. There have been many cases of seagrass die-off due, indirectly, to the activities of farmers and developers (Finkel 1997). Studies of the ecophysiology of seagrasses and their role in marine ecosystems (Hillman et al. 1995; Pedersen et al. 1997; Moriarty & Boon 1989; McRoy 1974; Thursby 1984; Thursby & Harlin 1984) have obtained contradictory results concerning the role of nutrient concentrations in seagrass growth. Some claim

that nutrients have no effect while others conclude that nutrients might have some function in promoting seagrass growth. Such discrepancies have arisen because the influences of nutrients and physical factors on seagrass growth have only been examined using traditional methods in field experiments, which are limited in precision. Sediment/water nutrients and their role in seagrass growth have not been studied in bench-scale experiments as well.

Measurements of seagrass production that rely on punching holes or stapling clips may have led to incorrect estimates of the importance of nutrients for seagrass growth since these procedures cause injury to the plant. An injured plant cannot grow properly due to physiological problems inside its leaf cells. The most obvious problem in experimentally adding nutrients to natural seagrass beds is that the nutrients become diluted and swept out of the experimentally enriched area

through tidal action. Thus, the precise role of nutrients in seagrass production has remained in question during the past few decades. In fact, these problems with experimental procedure cannot be remedied until more precise methods for determining the role of nutrients on seagrass growth are established. The present work, therefore, is a step towards overcoming these problems by modifying traditional methods to gain a better understanding of how nutrient parameters affect seagrass production. In the present study, instead of natural seagrass beds, transplanted surfgrass, *Phyllospadix iwatensis* Makino, was used in aquaria beds. Biomass production was measured by thread markers instead of punching holes or stapling clips.

MATERIALS AND METHODS

Culture techniques

Surfgrass (*Phyllospadix iwatensis*), sea water, and sediment were collected from just below lowest low tidal level (LLL) of the Aikap coast, Pacific Ocean side of eastern Hokkaido, Japan, on March 10, 1997, and then transported to the laboratory. In the laboratory, epiphytes were removed from the surfgrass by using

razor blades because epiphytes can transfer nutrients from seagrasses (McRoy & Goering 1974). Older leaves were removed and the surfgrass was rinsed with centrifuged sea water. The surfgrass was then prepared for transplanting into aquaria.

Three aquaria were prepared for this experiment. Each aquarium (ca. 1.2 m³) has six artificial beds which each consists of a stone within a basin. The six beds separated into two parts: (1) deep sediment level with 10 cm of sediment on a stone surface, and (2) shallow sediment level with 2 cm of sediment on a stone surface. These sediment depths were chosen because *P. iwatensis* was collected from both habitats. For each part of the aquarium, three basins, i.e., a total of six artificial beds were prepared (Fig. 1). Before transplanting, the walls of the aquaria were cleaned with hot water to prevent epiphytic growth and the aquaria were filled with sea water. Ten shoots sprouted from a rhizome were transplanted into each artificial bed for a total of 60 shoots per aquarium. All plants were selected to be of similar size and shape. A rhizome with ten shoots were fixed to a flat stone with rubber bands in each artificial bed. Lighting in each aquarium (50 $\mu\text{E m}^{-2} \text{s}^{-1}$ quantum irradiance at the surface) was supplied by ten FLR40SW fluorescent tubes, with a 12L/12D photoperiod controlled by an automatic timer. Nutrients were supplied using a commercial fertilizer with NH₄, PO₄, and NO₃+NO₂ components. No commercial fertilizer was added to the first aquarium (aq-1; control). Four grams of commercial fertilizer were added biweekly to each seagrass bed of the second aquarium (aq-2; experiment), and 8 g was added biweekly to each bed in the third aquarium (aq-3; experiment). Nutrient addition was done by burying a stick ampoule of fertilizer in the sediment. The water temperature in the aquaria was not controlled, so the temperature increased with room temperature during the experiment. Room temperature from March to June ranged from 0.5°C to 10.5°C.

Fig. 1. Experimental aquaria (aq-1~3) and artificial surfgrass beds in the experiment.

Nutrient pools in geo-water environments of the surfgrass

Overlying sea water samples from each aquarium were collected biweekly and stored in plastic bags in a refrigerator. A 50 ml syringe was used to collect porewater from the sediment of each bed, which was then stored in plastic bags in a refrigerator. Sediment was collected biweekly from each artificial seagrass bed using a stainless steel spoon and then also stored in plastic bags in a refrigerator. Ammonium is very sensitive to

contamination; therefore, to avoid mixing atmospheric ammonia within the samples, analyses were completed soon after collection. Sea water and porewater were purified separately by using an ultracentrifuge (Hitachi 55P-72) at 12,000 rpm for 20 min at 2°C. The samples were then prepared for analysis using a modified phenol-hypochlorite method for total nitrogen (Patricia et al. 1988) and the method of Strickland & Parsons (1968) for P and $\text{NO}_3 + \text{NO}_2$. The sample sediments were mixed separately into homogeneous slurries and then subsamples were taken to determine adsorbed N, P, and $\text{NO}_3 + \text{NO}_2$. Ten grams of sediment were mixed with 2N KCl solution, diluted in volume 10:1, and shaken for approximately one hour. The samples were then centrifuged at 2,500 rpm for ten min and analyzed for N, P, and $\text{NO}_3 + \text{NO}_2$ by using method of Udy & Dennison (1996).

Surfgrass production

To measure surfgrass production, a modified marking technique was used because punching holes or stapling clips wounds plants and causes a reduction in biomass production. Therefore, instead, threads were tied around the bases of shoots. Then every seven days the distance between the thread and a leaf tip was measured, and the successive differences estimated as leaf production. The same procedure was used to measure rhizome production. A thread was tied at a terminal node of the rhizome and elongation of the rhizome from the marked node was considered to represent rhizome production. The following equations were used to calculate shoot and rhizome production:

$$P_{lf} = (M_{lf} + N_{lf}) / t$$

$$P_{rh} = (M_{rh} + N_{rh}) / t$$

where P_{lf} = production in a shoot, M_{lf} = elongation from the marked point, N_{lf} = length of new leaves if any, t = time, P_{rh} = production of rhizome, M_{rh} = length of rhizome elongation from the marked node, N_{rh} = length of new rhizomes if any.

The numbers of shoots, rhizome tips, and inflorescences in each aquarium were counted biweekly. Reproductive effort, which is the ratio of the reproductive parts to total biomass of the plants, was also measured biweekly. Leaf area index (LAI) was estimated by calculating leaf area and dividing it by the surface area of the experimental bed.

Photosynthetic pigments

Chlorophyll *a* and *b* pigments were measured every 15 days in each aquarium by collecting 1 g of the second youngest leaf of each shoot (most physiologically active; Sand-Jensen 1965; Mukai et al. 1979). The leaves were then ground in a mortar with acetone, poured into 2 ml test tubes, centrifuged at 3,000 rpm (Tomy MC-15A microcentrifuge) for 15 minutes, and chlorophyll concentrations were determined using a spectrophotometer (Shimazu; UV-260).

Nutrient content in surfgrass

Plant material was collected from each experimental bed of the three aquaria biweekly during the experiment, portioned into above- and below-ground parts, dried at 80°C for 48 hrs, cooled in a dessicator, and ground in a mortar. Five hundred micrograms of each sample from both deep and shallow sediment parts were taken and analyzed for C and N concentrations using a CHN autoanalyser (Yanaco; MT-5). Phosphorus concentrations were measured by the methods of Short (1990).

Total biomass

At the end of the experiments the wet biomass of all 60 plants was measured from each aquarium. The wet weights were then roughly converted into dry biomass (10:1 wet/dry biomass; McRoy 1970). On the other hand, the averaged shoot biomass was calculated from several shoots measurement for field samples at the beginning of the experiment.

RESULTS

Nutrients in porewater and sea water

Concentration of nutrients (total-N, P, and $\text{NO}_3 + \text{NO}_2$) in sea water, in porewater and adsorbed onto sediment varied significantly between aquaria during the experimental period. Nutrient concentrations in aq-1 remained constant, those in aq-2 gradually increased, and those in aq-3 increased to the highest in the three aquaria (Table 1). Variation of nutrient concentrations was due to the biweekly addition of fertilizer in the experimental beds in aq-2 and aq-3. Four grams nutrient addition initiated the gradual increase of the nutrient pool in aq-2 during the experiment period, 8 g nutrient addition in aq-3 initiated the highest concentration of

Table 1. Nutrient concentrations in sea water (Sw), porewater in shallow sediment (Pss), porewater in deep sediment (Pds), shallow sediment (Ss), and deep sediment (Ds) within the three different aquaria between March 4, 1997, and June 16, 1997. No nutrients were added to aquarium-1, 4 g of commercial fertilizer was added to aquarium-2, and 8 g of commercial fertilizer was added to aquarium-3 in the *Phyllospadix iwatensis* beds.

Date	Aquarium 1					Aquarium 2					Aquarium 3				
	Sw	Pss	Pds	Ss	Ds	Sw	Pss	Pds	Ss	Ds	Sw	Pss	Pds	Ss	Ds
N (μ g/l)															
03.04.97	0.593	0.945	1.267	6.453	13.876	0.595	0.975	1.197	6.450	13.870	0.588	0.950	1.260	6.560	13.985
03.19.97	0.585	0.952	1.213	6.540	13.670	3.675	8.593	15.450	27.561	89.567	6.453	9.897	19.964	47.867	119.787
04.03.97	0.590	0.950	1.200	6.565	13.880	8.345	12.980	21.993	53.718	133.302	13.533	11.543	25.994	69.256	155.961
04.18.97	0.592	0.943	1.231	6.500	13.875	11.856	15.625	26.993	93.750	155.962	18.342	14.958	29.980	89.745	181.350
05.01.97	0.588	0.944	1.250	6.543	13.867	12.320	17.450	30.980	99.177	185.961	21.765	16.460	36.916	98.790	221.478
05.16.97	0.591	0.946	1.266	6.467	13.867	12.896	18.560	36.453	102.219	221.925	22.564	18.897	45.825	113.382	274.950
06.01.97	0.587	0.945	1.267	6.460	13.560	14.215	19.786	40.342	113.256	242.718	23.876	22.670	53.542	136.068	321.252
06.16.97	0.592	0.944	1.265	6.455	13.776	16.876	21.654	44.786	119.675	278.052	14.764	24.785	60.153	148.710	360.918
P (μ g/l)															
03.04.97	0.035	0.102	0.750	1.756	3.750	0.035	0.110	0.756	1.770	3.745	0.035	0.108	0.755	1.766	3.756
03.19.97	0.035	0.110	0.756	1.760	3.745	0.765	1.320	2.425	16.600	21.225	1.975	3.270	4.270	16.350	28.980
04.03.97	0.035	0.105	0.755	1.765	3.744	2.335	3.945	4.245	19.725	27.867	3.770	5.750	7.110	28.750	35.550
04.18.97	0.035	0.116	0.762	1.770	3.752	3.335	4.285	6.310	21.425	31.550	4.985	6.420	9.120	32.100	45.600
05.01.97	0.035	0.115	0.751	1.750	3.750	3.980	4.980	7.195	24.425	35.975	6.160	6.870	9.345	34.350	48.725
05.16.97	0.036	0.110	0.750	1.763	3.755	4.575	5.558	8.370	27.790	36.850	6.965	7.240	10.034	36.200	50.170
06.01.97	0.035	0.104	0.765	1.759	3.745	5.342	6.730	9.570	33.650	47.850	8.700	10.360	12.590	51.825	62.950
06.16.97	0.035	0.103	0.754	1.755	3.752	6.215	7.575	10.835	37.875	54.175	10.145	12.142	16.145	60.725	80.725
NO ₃ + NO ₂ (μ g/l)															
03.04.97	0.018	0.068	0.174	0.278	0.523	0.017	0.068	0.173	0.267	0.532	0.018	0.067	0.174	0.270	0.533
03.19.97	0.018	0.068	0.174	0.275	0.527	0.128	0.347	0.734	1.456	2.876	0.641	0.726	0.921	2.876	3.980
04.03.97	0.018	0.068	0.170	0.270	0.530	0.168	0.687	0.985	1.986	3.453	0.933	1.046	1.765	2.978	4.356
04.18.97	0.018	0.068	0.018	0.277	0.525	0.523	0.962	1.379	2.234	4.234	1.564	1.743	2.190	3.345	4.765
05.01.97	0.017	0.068	0.017	0.276	0.533	1.345	1.037	1.658	2.654	4.867	1.965	2.394	2.768	3.870	4.890
05.16.97	0.018	0.068	0.018	0.278	0.531	1.499	1.180	1.875	2.986	5.670	2.163	2.522	3.013	4.345	5.324
06.01.97	0.018	0.068	0.018	0.277	0.534	1.783	1.690	1.950	3.345	5.967	2.256	2.618	3.553	4.876	5.760
06.16.97	0.018	0.069	0.017	0.278	0.523	1.822	1.808	2.543	3.867	6.870	2.386	2.987	3.967	4.975	6.013

Fig. 2. Relationships between total nitrogen concentrations in sea water and porewater in three aquaria and six treatments in the experiment.

nutrients, and nutrients remained constant in aq-1 because no nutrients were added entire during the experimental period.

The aquarium with no nutrients added, aq-1, showed very little variation in nutrient concentrations in porewater and overlying sea water, and also little flux between them (Table 1). In the aquarium with 4 g of nutrients added periodically, aq-2, however, the higher the nitrogen concentration in the porewater, the higher the nitrogen concentration in the overlying sea water became (Fig. 2). This relationship was linear, but different regression lines characterized the deep-sediment experimental plots and shallow-sediment ones ($r^2 = 0.9564$ and $r^2 = 0.9674$, respectively).

In the aquarium with 8 g of nutrients added periodically, aq-3, the nutrient concentration relationship

between the two water fractions was different than in aq-2. The relationships both showed saturation curves (Fig. 2), but the levels of nitrogen concentration in the porewater were quite different between deep- and shallow-sediment beds. These results suggest that, (1) in intermediate nutrient concentrations, a high concentration in the porewater can linearly accelerate the flux into sea water, and (2) in extremely high concentrations, this acceleration proceeded very quickly, but total nitrogen concentration in the overlying sea water

saturated at a level of about $24 \mu\text{g l}^{-1}$, even though the nutrient level in the porewater was varied (Fig. 2).

Surfgrass production

Shoot and rhizome production of the surfgrass showed notable variation depending on nutrient concentration (Fig. 3). In aq-1, production rates were nearly constant even though temperature was not constant during the entire experiment. In aq-2, the rate of leaf production

Fig. 3. Biomass production of *Phyllospadix iwatensis* among three aquaria between March 4, 1997, and June 16, 1997: (a) leaf production in deep sediment; (b) leaf production in shallow sediment; (c) rhizome production in deep sediment; (d) rhizome production in shallow sediment. No nutrient was added to aq-1, 4 g of commercial fertilizer was added to aq-2, and 8 g of commercial fertilizer was added to aq-3. Vertical bars represent standard deviations among plants.

gradually increased throughout the experiment as 4 g of fertilizer was added periodically. In aq-3, seagrass production rates increased to the highest of all as 8 g of nutrients were added periodically. However, production in aq-3 remained constant after May 16. Both the initial increase and the leveling off were consistent with the nutrient situation in the overlying sea water.

There was also variation in seagrass production between the two sediment depths. Higher production was found in the deep sediment than in the shallow sedi-

ment. The deep sediment contained higher concentrations of nutrients than the shallow sediment (Fig. 3).

Rates of increase in shoot density and inflorescence production were highest in aq-3, a bit lower in aq-2, and steady in aq-1, and the same was true for leaf/rhizome production (Fig. 4). Sexual reproductive effort and leaf area index also greatly varied depending on nutrient concentration (Fig. 5), following the same pattern.

Fig. 4. Shoot and inflorescence production of *Phyllospadix iwatensis* between March 4, 1997, and June 16, 1997, in the three aquaria: (a) shoot density in deep sediment; (b) shoot density in shallow sediment; (c) inflorescence production in deep sediment; (d) inflorescence production in shallow sediment. Vertical bars represent standard deviations among plants.

Photosynthetic pigments

As with surfgrass production, chlorophyll *a* and *b* concentrations were constant in plants in aq-1, higher in aq-2, and highest in aq-3. However, in aq-3, chlorophyll concentrations as well as production remained constant after May 16 (Fig. 6).

Nutrient content of the surfgrass

The nutrient content (C, N, and P) in the above- and below-ground parts of the surfgrass, which reflected

nutrient uptake from sea water and porewater and its translocation through the entire plant to meet internal nutrient demands, was greatly affected by ambient nutrient concentrations after March 19. Nutrient concentrations in the aquaria promoted changes in the C, N, and P contents of the plants. Later nutrient concentrations were constant after May 16 even in aq-3 (Table 2).

Total biomass

Surfgrass biomass at the end of the experiment was

Fig. 5. Reproductive effort of *Phyllospadix iwatensis* in the experiment: (a) in deep sediment; (b) in shallow sediment; and leaf area index (LAI): (c) in deep sediment; (d) in shallow sediment, between March 4, 1997, and June 16, 1997, in the aquaria. Vertical bars represent standard deviations among plants.

Fig. 6. Photosynthetic pigment (chlorophyll *a*) of *Phyllospadix iwatensis* in the aquaria: (a) from deep sediment surfgrass beds; (c) from shallow sediment surfgrass beds; and chlorophyll *a* content; (b) from deep sediment; (d) from shallow sediment. Vertical bars represent standard deviations among plants.

greatly affected by nutrient additions to the aquaria. Biomass was much higher at the end of the experiment than at the beginning in both aq-2 and aq-3 (Fig. 7). However, biomass remained nearly constant in aq-1 throughout the experiment.

DISCUSSION

Primary production by seagrasses is controlled by two important factors, light and nutrients. There are many reports showing that nutrient enrichment in the sediment / water-column promotes primary production

(Williams & Ruckelshaus 1993; Kenworthy & Fonseca 1992). On the other hand, enrichment of ambient water by nitrate reduced growth and survival of *Zostera marina* (Burkholder et al. 1992). Moreover, some reports have suggested that nutrient concentrations do not greatly influence seagrass production (Bulthuis & Woelkerling 1981; Hillman et al. 1989; Powell et al. 1989; Duarte 1990; Perez et al. 1991; Murray et al. 1992; Bulthuis et al. 1992; Erftemeijer & Middelburg 1993; Duarte 1995). Most of them have focused on light intensity, temperature, water transparency, and seasonal conditions as likely factors influencing seagrass growth. In fact, they could not clarify how these

Table 2. Elemental content (carbon: C, nitrogen: N, and phosphorus: P) in above- and below-ground biomass of *Phyllospadix iwatensis*.

Date	Aquarium 1			Aquarium 2			Aquarium 3		
	%C	%N	%P	%C	%N	%P	%C	%N	%P
Above-ground parts									
03.04.97	25.440	0.760	0.280	25.450	0.750	0.280	25.440	0.760	0.277
03.19.97	25.510	0.750	0.270	28.650	0.830	0.380	30.670	1.534	0.460
04.03.97	25.452	0.766	0.276	29.670	0.922	0.466	31.237	1.985	0.667
04.18.97	25.400	0.755	0.275	30.047	1.056	0.582	31.890	2.760	0.786
05.01.97	25.438	0.753	0.278	30.783	1.450	0.721	33.650	3.904	0.984
05.16.97	25.440	0.762	0.276	31.176	1.760	0.777	33.345	3.982	1.680
06.01.97	25.451	0.764	0.272	32.946	1.860	0.898	33.560	3.915	1.990
06.16.97	25.460	0.765	0.275	33.450	1.900	0.932	33.540	3.920	2.010
Below-ground parts									
03.04.97	23.650	0.650	0.250	24.540	0.660	0.269	24.660	0.650	0.270
03.19.97	23.720	0.643	0.250	26.930	0.784	0.360	28.760	1.330	0.414
04.03.97	23.510	0.683	0.257	27.710	0.894	0.442	29.290	1.769	0.593
04.18.97	23.440	0.701	0.254	28.980	0.978	0.485	30.196	2.387	0.715
05.01.97	23.570	0.695	0.253	29.890	1.036	0.654	31.550	3.018	0.895
05.16.97	23.330	0.685	0.246	30.032	1.480	0.720	31.451	3.023	1.350
06.01.97	23.450	0.695	0.254	31.675	1.775	0.792	31.430	3.015	1.620
06.16.97	23.440	0.694	0.250	31.860	1.840	0.856	31.450	3.103	1.632

Fig. 7. Total biomass variation of the plant *Phyllospadix iwatensis* on a calculated dry weight basis upon planting in the experimental aquaria on March 4, 1997, and at the conclusion at the experiment on June 16, 1997. Vertical bars represent standard deviations among plants.

Fig. 8. Surfgrass production related to total nitrogen concentration of porewater in the three aquaria (aq-1~3) and six treatments (deep versus shallow sediment in each aquarium) in the experiment.

physical parameters influence growth independently without also affecting nutrient concentrations.

Wear et al. (1999) reported significant effects of water-column enrichment on seagrass-epiphyte complexes, mainly through primary production of epiphytic microalgae. The relative contribution of epiphytes to the total primary production in the complex increased remarkably with water-column enrichment. Also, primary production by seagrass leaves increased significantly with water-column enrichment ($\times 1.4$), while that of epiphytes increased two times over that of the control. The epiphyte biomass increased in enriched water, whereas the biomass of seagrass blades did not increase (Tomasko & Lapointe 1991; Neckles et al. 1993; Williams & Ruckelshaus 1993; Coleman & Burkholder 1994; Wear et al. 1999). Sometimes biomass increase of epiphytes leads to reduction of light reaching the seagrass (Silberstein et al. 1986; Pedersen &

Fig. 9. Surfgrass production in different concentrations of total nitrogen in sea water in three aquaria (aq-1~3) and six treatments (deep versus shallow sediment in each aquarium) in the experiment.

Borum 1992).

Effects of sediment enrichment have been studied mainly in field experiments involving either nutrient addition (Kenworthy & Fonseca 1992; Lent et al. 1995) or natural eutrophication (McMahon & Walker 1998). It seems likely that the effects of enrichment were diluted due to various unknown factors, e.g. water flow, wave action, epiphytism, epifaunal effects, bacterial activity, etc. Nevertheless, most studies have shown a significant promotion of primary production. Laboratory experiments involving sediment enrichment have the advantage of avoiding these factors, although there is some risk of epiphytic effects.

Seagrasses take up nutrients mainly by their roots, just as terrestrial plants do (Iizumi & Hattori 1982). This means that a high nutrient concentration in the sediment can promote a higher rate of uptake nutrient, which in turn leads to a high growth rate of seagrass leaves. Under conditions of sea water eutrophication, on the other hand, seagrass biomass does not increase, due to the great abundance of epiphytes, although it may increase somewhat in mildly eutrophic conditions

(Lukatelich et al. 1987; Shepherd et al. 1989).

In natural marine sediments of shallow waters, nutrient fluxes between the sediment and the overlying sea water vary considerably, depending on nutrient concentration, activity of microorganisms (Klump & Martens 1981; Nixon et al. 1976; and many others), water flow, and so on. When nutrient concentrations in sediment porewater are rather high, nutrient flux is directed from the porewater to the overlying sea water by physical diffusion, and a higher concentration of nutrients in the porewater accelerates the rate of flux.

In the present experimental aquaria, the nutrient concentration of the porewater affected leaf growth rate in a sigmoid manner (Fig. 8). This suggests that the surfgrass is nutrient-limited, since nutrient addition promoted leaf production. The aquarium with high nutrient enrichment showed a higher production of seagrass than in the low nutrient enrichment aquarium (Fig. 3). Nutrient uptake from a constant nutrient concentration (aq-1) led to a steady production rate. The sigmoid relationship between nutrients and growth rate also suggests that very high nutrient concentration caused the suppression of nutrient uptake by the plants, and thus, a constant but high production rate. However, the fluctuation of growth rate with time was obtained through different processes in each aquarium, so the acceleration of leaf growth rate in aq-3 was higher than in aq-2. On the other hand, sediment thickness had an effect on the timing of the growth rate fluctuations.

The surfgrass *Phyllospadix iwatensis* grows on rock surfaces and blocks water flow, accumulating sediment around its root system. The degree of sediment accumulation is influential in accumulating nutrients within the sediment and in affecting nutrient flux between the sediment and the water. Accordingly, sediment thickness may affect the growth rate of the surfgrass through nutrient dynamics in both the porewater and the overlying sea water. In the present study, the production of *P. iwatensis* was investigated in two thicknesses of sediment at three different nutrient levels, modelling the field conditions of *P. iwatensis*, which grows on rock surfaces with various amounts of sediment. The ultimate growth rate was not different significantly between the two sediment thicknesses, although with less sediment, a high growth rate was attained in a shorter time than with more sediment (Fig. 8). This means that in the latter situation, the sediment can store much nutrient and postpone the flux increase into the water above.

On the other hand, nitrogen concentrations in sea water showed different influences on leaf growth rates,

that is, in higher concentrations of nitrogen in sea water than ca. $15 \mu\text{g } \ell^{-1}$ leaf growth rate were not proportional to nitrogen concentration (Fig. 9). Rather the nitrogen concentration in sea water would be resulted from sediment enrichment by dilution. The reversal results between aq-2 and aq-3 in growth rate are suggesting the influence of epiphytism in high concentration of nitrogen in sea water.

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REFERENCES

- Bulthuis, D. A. and W. J. Woelkerling 1981. Effects of *in situ* nitrogen and phosphorus enrichment of the sediment on the seagrass *Heterozostera tasmanica* (Matens ex Ascher.) den Hartog in Western Port, Victoria, Australia. *Journal of Experimental Marine Biology and Ecology*, 37: 193-207.
- Bulthuis, D. A., D. Axelrad and M. J. Mickelson 1992. Growth of the seagrass *Heterozostera tasmanica* limited by nitrogen in Port Phillip Bay, Australia. *Marine Ecology Progress Series*, 89: 269-275.
- Burkholder, J. M., K. M. Mason and H. B. Glasgow 1992. Water-column nitrate enrichment promotes decline of eelgrass *Zostera marina*: evidence from seasonal mesocosm experiments. *Marine Ecology Progress Series*, 81: 163-178.
- Coleman, V. L. and J. M. Burkholder 1994. Community structure and productivity of epiphytic microalgae on eelgrass (*Zostera marina* L.) under water-column nitrate enrichment. *Journal of Experimental Marine Biology and Ecology*, 179: 29-48.
- Duarte, C. M. 1990. Seagrass nutrient content. *Marine Ecology Progress Series*, 120: 203-210.
- Duarte, C. M. 1995. Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia*, 41: 87-112.
- Erftemeijer, P. L. A. and J. Middelburg 1993. Factors limiting growth and production of tropical seagrasses: nutrient dynamics in Indonesian seagrass beds. *Ph.D.*

- dissertation, Nijmegen Catholic University.
- Finkel, E. 1997. Marine science; Australia enters deep water in devising management plan. *Science*, 277: 1428-1429.
- Hillman, K., D. I. Walker, A. W. D. Larkum and A. J. McComb 1989. Productivity and nutrient limitation. In: *Biology of Seagrasses*, Larkum A. W. D., A. J. McComb and S. A. Shepherd (eds.), Elsevier, Amsterdam, pp. 637-685.
- Hillman, K., A. J. McComb and D. I. Walker 1995. The distribution, biomass and primary production of the seagrass *Halophila ovalis* in the Swan/Canning Estuary, Western Australia. *Aquatic Botany*, 51: 1-54.
- Iizumi, H. and A. Hattori 1982. Growth and organic production of eelgrass (*Zostera marina* L.) in temperate waters of the Pacific coast of Japan. III. The kinetics of nitrogen uptake. *Aquatic Botany*, 12: 245-256.
- Kenworthy, W. J. and M. S. Fonseca 1992. The use of fertilizer to enhance growth of transplanted seagrasses *Zostera marina* L. and *Halodule wrightii* Aschers. *Journal of Experimental Marine Biology and Ecology*, 163: 141-161.
- Klump, J. V. and C. S. Martens 1981. Biogeochemical cycling in an organic rich coastal marine basin. II. Nutrient sediment-water exchange processes. *Geochimica et Cosmochimica Acta*, 45: 101-121.
- Lent, F. van, J. M. Verschuure and M. L. J. van Veghel 1995. Comparative study on populations of *Zostera marina* L. (eelgrass): *in situ* nitrogen enrichment and light manipulation. *Journal of Experimental Marine Biology and Ecology*, 185: 55-76.
- Lukatelich, R. J., N. J. Schofield and A. J. McComb 1987. Nutrient loading and macrophyte growth in Wilson Inlet, bar-built southwestern Australian estuary. *Estuarine, Coastal and Shelf Science*, 24: 141-165.
- McMahon, K. and D. I. Walker 1998. Fate of seasonal, terrestrial nutrient inputs to a shallow seagrass dominated embayment. *Estuarine, Coastal and Shelf Science*, 46: 15-25.
- McRoy, C. P. 1970. Standing stocks and other features of eelgrass (*Z. marina*) populations on the coast of Alaska. *Journal of the Fisheries Research Board of Canada*, 27: 1811-1821.
- McRoy, C. P. 1974. Seagrass productivity: Carbon uptake experiments in eelgrass, *Zostera marina*. *Aquaculture*, 4: 131-137.
- McRoy, C. P. and J. J. Goering 1974. Nutrient transfer between the seagrass *Z. marina* and its epiphytes. *Nature*, 248: 173-174.
- Moriarty, D. J. W. and P. I. Boon 1989. Interactions of seagrasses with sediment and water. In: *Biology of Seagrasses*, Larkum A. W. D., A. J. McComb and S. A. Shepherd (eds.), Elsevier, Amsterdam, pp. 635-685.
- Mukai, H., K. Aioi, I. Koike, H. Iizumi, M. Ohtsu and A. Hattori 1979. Growth and organic production of eelgrass (*Zostera marina* L.) in temperate waters of the Pacific coast of Japan. I. Growth analysis in spring-summer. *Aquatic Botany*, 7: 47-56.
- Murrey, L., W. Dennison and M. Kemp 1992. Nitrogen versus phosphorus limitation for growth of an estuarine population of eelgrass *Z. marina*. *Aquatic Botany*, 44: 83-100.
- Neckles, H. A., R. L. Wetzel and R. J. Orth 1993. Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Zostera marina* L.) dynamics. *Oecologia*, 93: 285-295.
- Nixon, S. W., C. A. Oviatt, J. Garber and V. Lee 1976. Diel metabolism and nutrient dynamics in a salt marsh embayment. *Ecology*, 57: 740-750.
- Pedersen, M. F. and J. Borum 1992. Nitrogen dynamics of eelgrass *Zostera marina* during a late summer period of high growth and low nutrient availability. *Marine Ecology Progress Series*, 80: 65-73.
- Pedersen, M. F., C. M. Duarte and J. Cebriab 1997. Rate of changes in organic matter and nutrient stocks during seagrass *Cymodocea nodosa* colonization and stand development. *Marine Ecology Progress Series*, 159: 29-36.
- Perez, M., J. Romaro, C. M. Duarte and K. Sand-Jensen 1991. Phosphorus limitation of *Cymodocea nodosa* growth. *Marine Biology*, 109: 129-133.
- Powell, G., J. Kenworthy and J. Fourqurean 1989. Experimental evidence for nutrient limitation of seagrass growth in a tropical estuary with restricted circulation. *Bulletin of Marine Science*, 44: 324-340.
- Sand-Jensen, K. 1975. Biomass, net production and growth dynamics in an eelgrass (*Zostera marina* L.) population in Vellerup Vig, Denmark. *Ophelia*, 14: 185-201.
- Shepherd, S. A., A. J. McComb, D. A. Bulthuis, U. Neverauskas, D. A. Steffensen and R. West 1989. Decline of seagrasses. In: *Biology of Seagrasses*, Larkum A. W. D., A. J. McComb and S. A. Shepherd (eds.), Elsevier, Amsterdam, pp. 346-398.
- Short, F. T. 1990. Primary elemental constituents. In: *Seagrass Research Methods*. Phillips, R. C. and C. P. McRoy (eds.), UNESCO, Paris, pp. 105-109.
- Silberstein, K., A. W. Chiffings and A. J. McComb 1986. The loss of seagrass in Cockburn Sound, Western Australia. III. The effect of epiphytes on productivity of *Posidonia australis* Hook, F. *Aquatic Botany*, 24: 355-371.
- Strickland, J. D. H. and T. R. Parsons 1968. A practical

- handbook of seawater analysis. *Fisheries Research Board of Canada, Bulletin*, 167, pp.1-311.
- Thursby, G. B. 1984. Root-exuded oxygen in the aquatic angiosperm *Ruppia maritima*. *Marine Ecology Progress Series*, 16: 303-305.
- Thursby, G. B. and M. M. Harlin 1984. Interaction of leaves and roots of *Ruppia maritima* in the uptake of phosphate, ammonia and nitrate. *Marine Biology*, 83: 61-67.
- Tomasko, D. A. and B. E. Lapointe 1991. Productivity and biomass of *Thalassia testudinum* as related to water column nutrient availability and epiphyte levels: field observations and experimental studies. *Marine Ecology Progress Series*, 75: 9-17.
- Udy, J. W. and W. C. Dennison 1996. Estimating nutrient availability in seagrass sediments. In: *Seagrass Biology: Proceedings of an International Workshop*, Kuo, J., R. C. Phillips, D. I. Walker and H. Kirkman (eds.), Sciences, University of Western Australia, Perth, pp. 163-172.
- Wear, D. J., M. J. Sullivan, A. D. Moore and D. F. Millie 1999. Effects of water-column enrichment on the production dynamics of three seagrass species and their epiphytic algae. *Marine Ecology Progress Series*, 179: 201-213.
- Williams, S. L. and M. H. Ruchelshaus 1993. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. *Ecology*, 74: 904-918.