

SPATIAL AND SEASONAL VARIABILITY OF *THALASSIA TESTUDINUM* IN NUEVITAS BAY, CUBA

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ABSTRACT

A study was carried out on biomass, shoot density and leaf production variability in three *Thalassia testudinum* meadows under different environmental characteristics in Nuevitas Bay, Cuba, in different seasons. The first site has muddy-sandy sediments and it is affected by bottom trawl fishing; the second has sandy sediments and it is affected by waste-water discharges, and the third has sandy-muddy sediments and no human impacts are present. Leaf, rhizome, and root biomass, daily production of leaves, density of short shoots, and length and width of leaves were estimated five times in a year. Seasonal variations were observed, with higher values of leaf and rhizome biomass, short shoot density, and daily production of leaves occurring in spring and summer. Spatial differences seem to be related to the environmental characteristics of each site: Leaf daily production, leaf biomass and leaf length were lower, and root biomass was higher in the site where sediments are impacted by fishing with bottom trawls; short shoot density and rhizome biomass were higher in the nonaffected site. Lower values of root biomass appeared where waste-water discharges occurred.

Keywords: Biomass, shoot density, leaf production, seasonal variation, *Thalassia testudinum*, bottom-trawl fisheries, sewage.

RESUMEN

Se realizó un estudio de la variación de la biomasa, la densidad de vástagos y la producción de hojas en tres praderas de *Thalassia testudinum* con diferentes características ambientales en la bahía de Nuevitas, Cuba, en diferentes épocas del año. El primer sitio tiene sedimentos fango-arenosos y está afectado por pesquería de arrastre; en el segundo, el sedimento es arenoso y se vierten aguas residuales en su cercanía, y el tercero, con sedimentos arenoso-fangosos, no está afectado por impactos antropogénicos. La biomasa de hojas, rizomas y raíces, la producción diaria de hojas, la densidad de vástagos, y el largo y ancho de las hojas fueron estimadas cinco veces en un año. Se observaron variaciones estacionales, con los valores más altos de biomasa de hojas y de rizomas, densidad de vástagos y de la producción diaria de hojas en primavera y verano. Las variaciones espaciales parecen estar relacionadas con las características de cada sitio: La producción diaria de hojas, la biomasa de hojas y su longitud fueron menores, y la biomasa de raíces mayor, donde los sedimentos están impactados por la pesquería con arrastre; la densidad de vástagos y la biomasa de rizomas fueron mayores en el sitio no afectado por ninguna acción antrópica. Los menores valores de la biomasa de raíces aparecieron donde se realiza la descarga de residuales.

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Palabras claves: Biomasa, densidad de vástagos, producción foliar, variaciones estacionales, *Thalassia testudinum*, pesquería de arrastre, aguas residuales.

INTRODUCTION

Seagrass meadows world-wide are composed by a small number of marine angiosperm species. They are highly productive ecosystems, comparable to most efficient ecosystems on Earth (Duarte & Chiscano, 1999). They are clonal plants that colonize space successfully through the replication of ramets as a result of extension of the rhizome (Duarte *et al.* 2006). Plasticity in the growth-form of seagrasses is an important component of their capacity to adapt to the heterogeneity of resources and is a result of physiological changes in response to environmental conditions. Therefore, certain seagrass characteristics such as biomass and shoot density can be used as an indicator of diverse impacts on their growth (Kirkman, 1996).

Moreover, water-column nutrient concentrations themselves tend to be poor indicators of nutrient status because water column nutrients (phosphorus and nitrogen) can have turnover rates of less than 10 min. For that reason, traditional water-quality monitoring programs can fail to detect nutrient induced declines in seagrass health, and seagrass meadows should be monitored directly to determine status and/or trends in the health of estuarine environments (Tomasko *et al.* 1996). For using seagrass characteristics as indicators in

monitoring, it is necessary to understand their variability.

Seasonal variability of abundance, productivity and morphometric characteristics in seagrass meadows has been documented in temperate meadows (i.e. Gobert *et al.* 2006; Moore & Short, 2006). Although tropical habitats seems to be more stable than temperate ones concerning to temperature and light regimes, tropical seagrasses also experience differences according to season (Gacia, 1999; Martínez-Daranas *et al.* 2005a; van Tussenbroek *et al.* 2006).

Many authors have documented the disturbance through human activity in seagrass habitats as rates of declining are about 1 to 2% yr⁻¹ on a global scale for several decades, and these rates seems to be accelerating during the last years placing them among the most vulnerable ecosystems in the planet (Fourqurean & Robblee, 1999; Orth *et al.* 2006). This decline has multiple causes where eutrophication (Short & Wyllie-Echeverría, 1996; Borum *et al.* 2004; Orth *et al.* 2006; Ralph *et al.* 2006) and physical actions due to human activities, as dredging, boating and bottom trawl fisheries, are among the most impacting factors worldwide (Leriche *et al.* 2006; Erftmeijer & Lewis 2006; Gillanders 2006; Badalamenti 2006).

Thrush & Dayton (2002) summarizes the deleterious effects of physical actions as trawling and dredging on marine benthic habitats as impacts on community structure and physical

changes in habitat structure, along with functional changes to seafloor ecosystems. Among the long-term physical effects of bottom fishing are: the homogenization of the substratum that will affect the survivorship of juvenile life stages of species (including some of the target species of fisheries); consequently, the reduction of species diversity; the disturbance of the seafloor, enhancing the upward flux of nutrients by releasing pore-water nutrients with potentially ecosystem-wide consequences on phytoplankton growth. All these changes lead to changes in the functional roles played by organisms, communities, and ecosystems, making unable to fulfil their natural roles in community and ecosystem function.

In Nuevitas Bay, as in many coastal zones around the world, different socioeconomic uses of the coastal zone are overlapped, such as: industry wastes, port, fishing, tourism and recreational activities. This bay is surrounded by a border of nearly 2 000 m of *Rhizophora mangle*, which is included in the protected area "Ballenatos Keys and Nuevitas Bay Mangrove Swamp Fauna Refuge," where the manatee *Trichechus manatus* and the crocodile *Crocodilus acutus* have their habitat (Alcolado *et al.* 1999). In the other hand, this area also receives 1 128 t of organic load in DBO_d terms, as well as waste waters enriched with heavy metals and inorganic nitrogen from several industries (power plant, factories of fertilizer, cement and wires), port activities and direct untreated waste waters from a city with

more than 46 000 inhabitants (Montalvo *et al.* 2007). An early study on seagrasses from Nuevitas Bay revealed a relationship between loss of dry biomass and species richness decline, with seawater turbidity in areas of this bay (Martínez-Daranas *et al.* 1996).

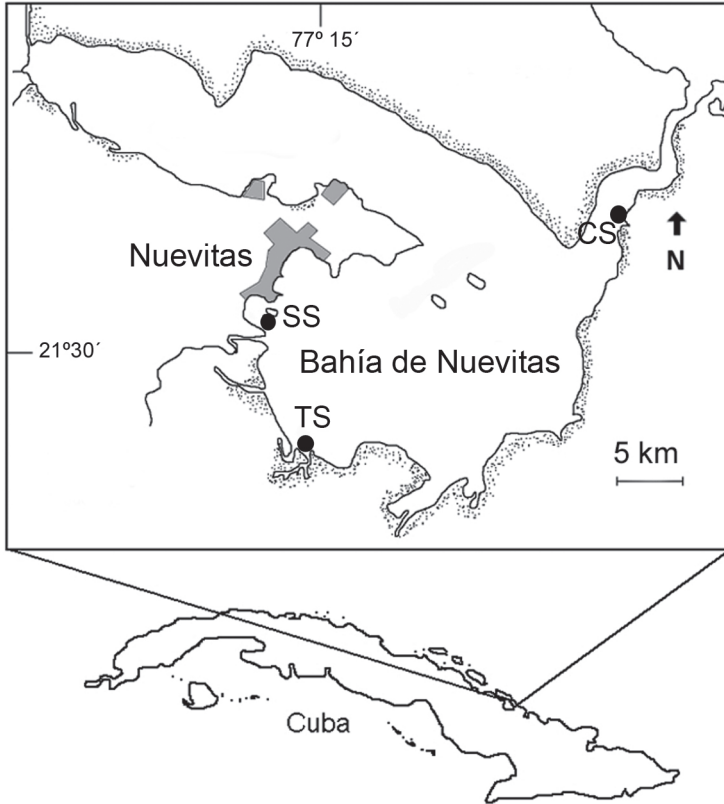
The objective of this work was to study the possible seasonal variability of some characteristics of *Thalassia testudinum* Banks ex König in three areas of Nuevitas Bay, and to evaluate the impact of different anthropogenic factors such as bottom-trawl fisheries and sewage, on these seagrass meadows.

MATERIALS AND METHODS

Study site

Nuevitas Bay is located in the North-eastern Cuban shelf and it is subdivided into two lobes (Figure 1): a shallow Northern lobe called Mayanabo Inlet, where seagrasses are poorly developed, and the Southern lobe (properly Nuevitas Bay), where *T. testudinum* meadows cover the bottom. Other species of seagrasses (*Syringodium filiforme*, *Halodule wrightii*, *Halophila decipiens* and *Halophila engelmanni*) and macroalgae (mainly Bryopsidales, Chlorophyta) are frequently found in this bay (Martínez-Daranas *et al.* 1996). Commercial fishing with bottom trawls and the spill of domestic and industrial wastes from Nuevitas city are threats to marine ecosystems in this bay.

Figure 1



Geographical location of Nuevitás Bay and sampling sites. Legend: SS = sewage site; TS = trawling site; CS = control site.

Three sites were selected (Figure 1) to study the spatial and seasonal variability in the following parameters: biomass, density, leaf length and production of seagrass *T. testudinum* in monospecific stands 2 m deep. The first site (identified as sewage site or SS) is near to industrial (wire and electrode factory) and urban sewage drains, with a muddy-sandy substrate. The second site (identified as trawling site or TS) is located about 6 km South-southeast of site 1, and it

is exposed to trawling fisheries, with sandy sediments. The third site (considered as control site or CS) is far away from the disturbance sources, near the bay's entrance channel, with constant exchange with clean seawater, and sandy-muddy sediments.

Nuevitás Bay seawater has been classified in general as mesotrophic to eutrophic, according to nutrients and phytoplankton (Montalvo *et al.* 2007). Based on different studies undertaken from 1994 to 2005, these

authors considered that in the area where the sewage site (SS) is located, the quality of water and sediments is worse than in the trawling site (TS). The best water quality was found around the control site (CS).

Sampling and analysis

Samplings were done in February, August and October 2001, and January and April 2002 at each site. The above- and belowground plant sections were harvested using a PVC core of 15 cm of internal diameter that was introduced up to approximately 15 cm in the sediment, using four sampling units each time, following the methodology of CARICOMP (2001). Sediment was removed with running water and calcareous epiphytes were eliminated with 5% HCl. Collected material was separated in leaves, vertical and horizontal rhizomes, and roots. Each tissue group was placed in aluminium foils, previously weighed, and dried at 70°C for 24 h., until a constant weight was reached.

The daily production of leaves was estimated using the Zieman (1975) method, marking the leaves with a hypodermic needle and using twelve sampling units with 10 x 25 cm quadrates, leaving them for 8-12 days before collecting the leaves. At this time, the density of short shoots was estimated.

Fifteen short shoots were randomly taken in each site and the most developed and complete leaf

of each shoot was used to calculate the length of leaves with a ruler. The width of leaves was estimated using a binocular microscope.

Data normality and homogeneity of variance were tested (Zar, 1996) and the variables that did not match these assumptions were log transformed. A Two-Factor ANOVA was applied to the measured variables and the Newman-Keuls (SNK) *post-hoc* comparison test was performed when significant differences existed. All the statistical tests and graphs were carried out using Statistica 6.0 (© Statsoft Inc., 1984-1995) with a 5% probability.

RESULTS

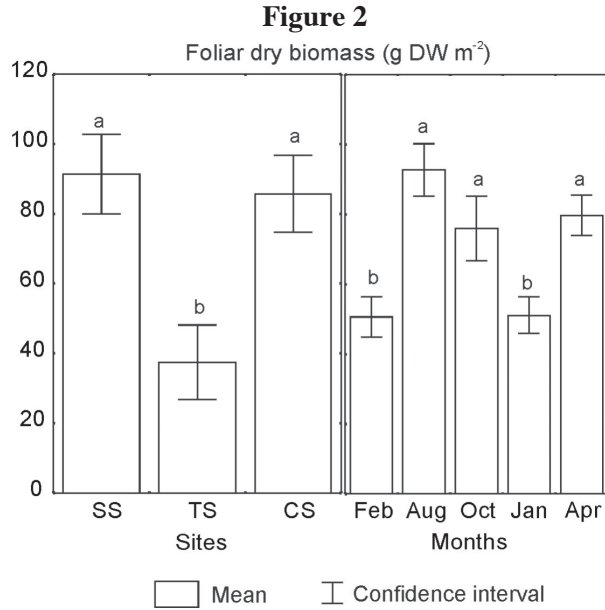
Seasonal trends were similar for the three sampling sites with respect to leaf, rhizomes and root biomass, as well as for short shoot density and leaf length for *T. testudinum*, as the F-value for the interaction of these variables were not significant (Table 1).

Leaf biomass of this species showed lower means in TS and in the winter months (February 2001 and January 2002; Table 1; Figure 2). Vertical rhizomes biomass was lower in TS and higher in CS, and it was lower in winter months than in the others (Table 1; Figure 3). Horizontal rhizomes showed the same spatial and seasonal differences as the vertical ones. Root biomass showed significant differences only among sites, lower in SS and higher in TS (Table 1; Figure 4).

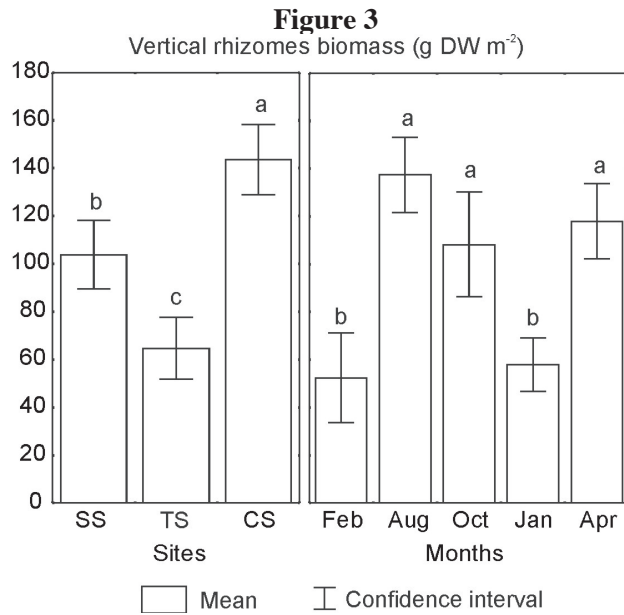
Table 1

Mean and confidence intervals (C.I.) for leaf length and width (n = 225), dry biomass of leaves, vertical rhizomes, horizontal rhizomes and roots (n = 60), short shoot density (n = 60) and daily leaf production (n = 180) of *Thalassia testudinum* in three sites in Nuevitas Bay; F = values resulting from two-factor ANOVA among sites, months and the interaction; df = degrees of freedom (Error df = 45); * p < .05; ** p < .01.

	Mean	C.I.	F		
			Sites (df = 2)	Months (df = 4)	Interaction (df = 8)
Leaf length (cm)	13.8	4.7	181.19**	259.45**	10.32
Leaf width (mm)	8.1	1.0	35.05	27.66	4.82
Leaf biomass (g DW m ⁻²)	75.6	19.3	6.17*	4.06**	1.92
Vertical rhizome biomass (g DW m ⁻²)	104.6	27.9	14.82**	4.79*	1.20
Horizontal rhizome biomass (g DW m ⁻²)	159.6	46.5	7.86*	6.34**	0.544
Root biomass (g DW m ⁻²)	208.0	27.9	10.96**	1.17	3.03
Density (short-shoots m ⁻²)	616.7	63.5	8.71*	10.80*	0.60
Daily leaf production (g DW m ⁻² d ⁻¹)	2.08	0.48	19.68**	8.01**	2.18*

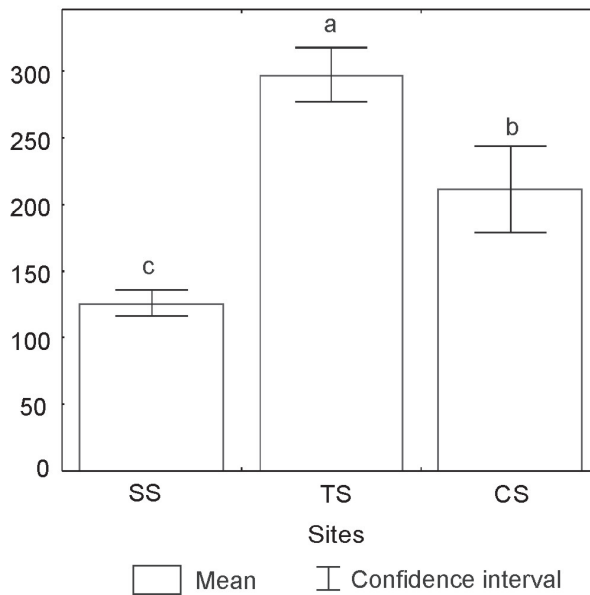


Thalassia testudinum leaf dry biomass per sites and months. Different letter designations indicate significant differences among means at $p > .05$ as judged by SNK post hoc comparisons. Legend similar to Figure 1.



Thalassia testudinum vertical rhizomes dry biomass per sites and months. Legend similar to Figure 1.

Figure 4
Roots biomass (g DW m⁻²)



Thalassia testudinum root dry biomass per sites. Legend similar to Figure 1.

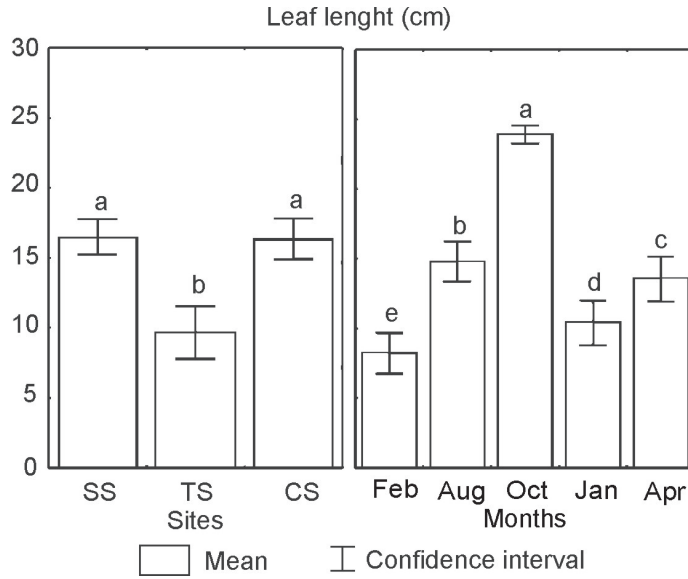
Leaf width did not show differences (Table 1). Lower values of leaf length were found at TS, and among months, it was lower in winter, while in October 2001 it presented the highest value (Figure 5).

Daily production of leaves showed different trends among the three sites, months, and the interaction of the two factors (Table 1).

Higher values were generally found in CS, although they were higher in April 2002 for all sites, as well as in August 2001 in SS (Figure 6). Lower production was detected in February 2001 in SS, August 2001 in CS and during winter months in TS.

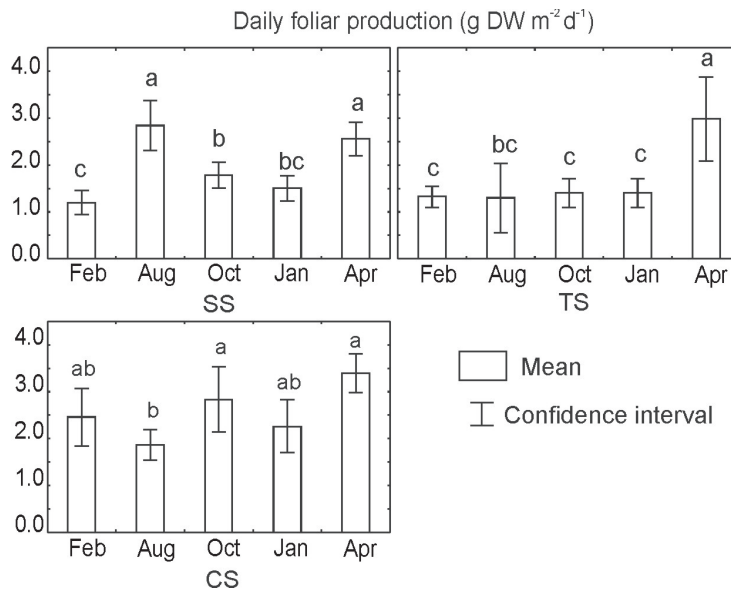
Short shoot density was higher in CS and lower in TS. Minimum density was found in January 2002 and maximum in April 2002 for all sites (Table 1, Figure 7).

Figure 5

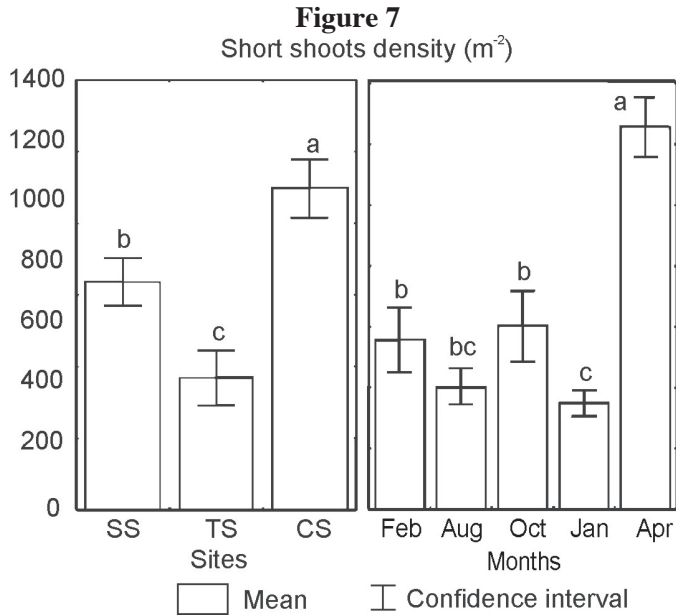


Thalassia testudinum leaf length per sites and months. Legend similar to Figure 1.

Figure 6



Thalassia testudinum leaf daily production per sites and months. Legend similar to Figure 1.



Thalassia testudinum short-shoot density per sites and months. Legend similar to Figure 1.

DISCUSSION

Temporal variations

Seasonal variations in tropical areas are weaker than in temperate regions; but fluctuations in temperature, radiation, rain, and storms can explain seasonal changes in production in the tropics (Hemminga & Duarte, 2000; Zieman, 1975). In the North coast of Cuba, higher values of *T. testudinum* leaf biomass and short shoot density were found in spring (March-April; Buesa, 1974; Jiménez & Alcolado, 1989; Martínez-Daranas *et al.* 2005a), and higher leaf production in summer (June; Martínez-Daranas *et al.* 2005a). In the Indian River, Florida, which is

located at a higher latitude than Cuba, *T. testudinum* leaf biomass and short shoot density were higher in mid-summer (July) and lower by the end of the winter (Gacia, 1999).

In Nuevitas bay, *T. testudinum* leaf and rhizome biomass as well as leaf length presented lower values in colder months (February 2001 and January 2002). As a result, total biomass of *T. testudinum* was lower in winter. The whole growth of the plant and the accumulation of reserves in the rhizomes depend on the photosynthetic activity in the leaves (Hemminga & Duarte, 2000; Marbá & Duarte, 1998). Therefore any factor that causes a reduction of photosynthesis will have a negative incidence

on non-photosynthetic biomass (root and rhizomes) biomass.

Variations in leaf biomass are the result of the leaf area, the short shoot density and the number of leaves. In this study, leaf width did not show differences. Leaves were shorter in winter probably due to the effects of cold fronts that caused the detachment and breaking of leaves, and the lower production of tissues induced by low temperatures. The increment in length from August to October can be the result of aging leaves. This result coincides with Agawin *et al* (2001) for *T. hemprichii* in the Philippines, and with Durako & Moffler (1985) for *T. testudinum* in Tampa Bay. The density of short shoots showed highest values in spring (April 2002), and the lowest in January 2002; but not so low in February 2001. Thus, the highest values of leaf biomass must be mainly the result of leaf length in October 2001 and of density of short shoots in April 2002.

Similar seasonal patterns for above-ground blade dynamics, blade length, and number of blades per shoot, as well as for the contribution of *T. testudinum* blades to total plant biomass have been found in Puerto Morelos, Mexico, with higher values in summer (van Tussenbroek, 1995, 1998). This author did not find variations for rhizome weight per unit length between summer and winter, what might indicate that this seagrass bed has a positive carbon balance throughout the year (van Tussenbroek, 1998).

Changes in rhizome biomass observed in this work can be due to variations in the storage of carbohydrate reserves (Larkum *et al* 2006). The storage capacity of horizontal rhizomes is usually higher than that of the vertical ones, but both change during the seasons (Hemminga & Duarte, 2000). In the Gulf of Mexico, Lee & Dunton (1997) found that non-structural carbohydrates of *T. testudinum* in Laguna Madre (Texas, USA) increased in summer, and that this species required an annual quantum flux in excess of 1628 mol m^{-2} in order to maintain a positive carbon balance, which corresponded with 14% of the surface irradiance. Tomasko & Dawes (1989) experienced with *T. testudinum* shaded shoots and concluded also that the light environment is an important factor in the carbon balance and in the translocation of resources from rhizomes to leaves and meristematic tissues.

The daily production also shows temporal variations and differences among sites. Daily leaf production increased in April 2002 for the three studied sites, similar to other zones in Cuba (Martínez-Daranas *et al.* 2005a). Lower values were found mostly in winter for SS and TS; but lower values were also found in August 2001 for CS and in October 2001 for SS. These differences cannot be explained at this time and may be due to other factors not analyzed in this study.

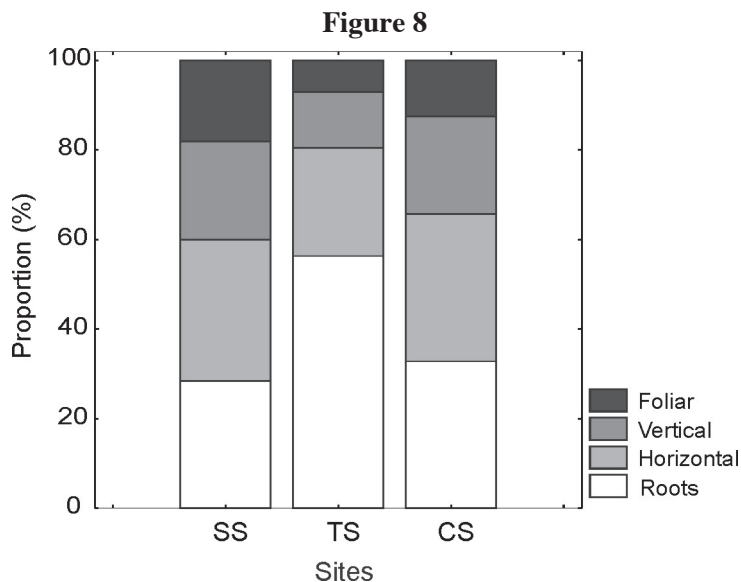
Seasonal differences in several characteristics of *T. testudinum* as leaf production, biomass or short shoot density have been found in several studies (i.e. Buesa, 1974; Durako & Moffler, 1985; Jiménez & Alcolado, 1989; van Tussenbroek, 1995, 1998; Gacia, 1999; Martínez-Daranas *et al.* 2005a; this study). Therefore, seasonal variability must be considered for detecting reliable differences among locations or dates in monitoring seagrass health.

Spatial variations

Leaf and rhizome biomass, leaf length and density of short shoots were lower in the site affected by bottom trawl fisheries (TS). These effects could be due to physical damage on plants and on sediments caused by the use of fishing gears in the area. Similarly, Martínez-Daranas *et al.* (2005b) found lower values of macroalgal relative abundance and species heterogeneity (H') in TS, when comparing with the other two sites. Root biomass was higher in TS site as well as the proportion of root-biomass: total-biomass when it is compared with the two other sites (Figure 8). A higher development of roots could help the plant get nutrients from the pore water and fasten better to the sand, coinciding with the results of Dawes & Lawrence (1979) and Kimbel & Carpenter (1981). Bottom trawl fisheries stopped in February 2002 and two months later, in April 2002, leaf pro-

duction increased significantly, thus supporting the idea that these actions affected *T. testudinum* meadow at this site, and strengthening the criteria that *T. testudinum* has a high tolerance to burial and erosion of sediments (Cabaço *et al.* 2008). Those results coincide with González-Correa *et al.* (2005) who assessed the recovery capacity of meadows of the Mediterranean seagrass *Posidonia oceanica* in an area affected by illegal trawling and they found that, as in this case, the vegetative growth was positive in both impacted and control meadows, but growth rates were lower in the bottom trawl fisheries site than in control one. This sort of fishery explains the lower values of rhizome biomass due to photosynthetic tissue loss and sediments resuspension, reducing light availability (Pascualini *et al.* 1999; González-Correa *et al.* 2005). Fishing can also disturb sediments, causing the loss of nutrients and hydrogen sulphide in the pore water as well as minor particles of sediments, changing their characteristics (Thrush & Dayton, 2002; Gillanders, 2006).

The presence of finer sediments in sites nonaffected by bottom trawling can be explained also because seagrasses usually lead to increased net sedimentation rates and reduction of the grain size (Bos *et al.* 2007). Higher silt content in dense seagrass beds is the result of waves and currents attenuation and sediments stabilization by seagrass leaves (Koch *et al.* 2006).



Components of the total dry biomass of *Thalassia testudinum* per sites. Legend similar to Figure 1; Horizontal = horizontal rhizomes; Vertical = vertical rhizomes.

In studied sites, differences were not detected in leaf biomass and leaf length between SS (site affected by waste water) and CS (control site), but rhizome and total biomass, as well as short shoot density were lower in the first than in the latter. Daily leaf production was also generally lower in SS, except in August 2001 and in April 2002. This can be due to the influence of nutrients that could enhance macroalgae development, reducing light availability. Martínez-Daranas *et al* (2005b) found the highest values of macroalgae abundance and diversity in this site, in the same period, with many opportunistic species such as Cyanobacteria, filamentous (Ceramiaceae, Ectocarpales and Sphacelariales), foliose (Ulvaceae, Dictyotaceae) and fleshy macroalgae

(Gracilariaceae) growing in this seagrass meadow and as epiphytes.

Many authors state that, as nutrient inputs raise, micro and macroalgae, and phytoplankton biomass in the water column increase, reducing the light available for photosynthesis which may result in a negative carbon balance (Hemminga & Duarte, 2000; Lapointe, 1989; Tomasko & Lapointe, 1991). The capacity to store carbohydrate reserves in the rhizomes allows seagrasses to survive unfavourable periods, such as shading associated with sporadic algal blooms; but these reserves cannot prevent mortality when light reduction is imposed over long time spans. Light reduction is the most important factor responsible for seagrass decline in eutrophic waters, because

sediments can become anoxic; therefore, meristematic tissues of *Thalassia* can be affected if eutrophication lasts for long periods. The internal transport of oxygen in seagrasses is predominantly unidirectional from photosynthetic tissues to rhizomes to roots driven, and insufficient oxygen supply to seagrass meristems and roots may have severe implications for seagrass growth and survival. Tissue anoxia impairs growth of roots, nutrient uptake and translocation of nutrients and carbohydrates, and the disappearance of the oxic microshield around roots and rhizomes, normally provided by the radial oxygen loss, allows the invasion of reduced phytochemicals, such as sulphides, from the

sediment to the plant tissues (Borum *et al.* 2006).

Unpublished data obtained in the study area in April 2002 by the Laboratory of Chemistry at the Instituto de Oceanología (José F. Montalvo, personal communication) showed that in SS the values of BOD₅ and ammonium in water, as well as the content of organic carbon, total phosphorus and hydrogen sulphide in sediments, were higher than in the other two sites (Table 2). Lower values of root biomass were found in SS, the site impacted by waste waters, where the sediment is muddy and total phosphorus concentration in sediment was higher than in the other two sites (Table 2).

Table 2

Quality of seawater and sediments in the three sites. CS = Control site; SS = sewage site; TS = trawling site; BOD₅ = seawater biochemical oxygen demand, NH₄ = seawater concentration of ammonia; TOC = total organic carbon in sediments; TPS = total phosphorus in sediments; H₂S = hydrogen sulphide in sediments.

Site	BOD ₅ (mg L ⁻¹)	NH ₄ (μM)	TOC (%)	TPS (%)	H ₂ S (μg L ⁻¹)
CS	0.48	0.20	0.40	0.05	0.46
SS	1.37	3.30	2.58	0.09	11.93
TS	0.06	0.68	0.61	0.03	2.62

CS presented the highest mean values of rhizomes and total biomass, short shoot density and daily leaf production. This site is more remote and pristine, far away from pollution sources and fishing gears are not

used. The abundance and diversity of macroalgae in this site was between SS and TS and mainly dominated by calcareous species, such as Bryopsidales (*Halimeda*, *Udotea*, *Caulerpa* and *Penicillus*) and abundant coralline red

algae (Martínez-Daranas *et al.* 2005b). According to Lapointe *et al.* (1994) and Littler & Littler (2007), calcareous species have slow growth rates and are characteristic of areas with low level of nutrients, indicating a stable and constant condition.

Conclusions

Variations found in the studied parameters for *T. testudinum* in Nuevitas Bay are the outcome of morphologic and physiologic responses to natural environmental conditions. Seasonal changes in light and temperature explain its seasonal differences in biomass, density and productivity. Physical impacts as light reduction induced by eutrophication, or direct physical damage by fishing gears, affect the foliar production, biomass and short shoots density of *T. testudinum*. According to these results, monitoring biomass and density of seagrasses in Nuevitas bay will be helpful to monitor effects of anthropogenic actions, considering seasonal variations.

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