

2011 Biological Monitoring Project Plan:
Examination of Seagrasses Adjacent to
EcoElectrica's Pier, Penuelas, PR.

By

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INTRODUCTION

Ecological Basis of the Importance of Seagrasses in Coastal Marine Systems

The importance of seagrass meadows have been summarized in more recent times by Hemminga and Duarte (2000) and an extensive review was assembled by Larkum, Orth and Duarte (2006). Overall seagrass meadows enhance bio- and habitat diversity while supporting production of marine resources. Garcia-Rios (2001) illustrates the numerous groups of organisms associated with seagrass, including coelenterates, bryozoans, ciliates, flagellates, sarcodines, foraminifera, crustaceans, fishes, echinoderms, mollusks, and algae. *Thalassia* beds in the Caribbean have been found to sustain a variety of macrofaunal species, including polychaetes (20), crustaceans (39), mollusks (61) and fishes (41) (Greenway, 1995; in Hemminga and Duarte, 2000). Their proximity to other marine systems such as mangroves and reefs facilitates trophic transfers and cross-habitat utilization by fishes and invertebrates (Orth, et al. 2007). The importance of seagrasses as habitats for juvenile fish have been confirmed (Aguilera-Perera, 2004) and which could be attributed to post settlement of fish species due to the increase of prey in seagrass habitats (Jenkins and Hamer (2001). Seagrasses also provide a direct food source to specially managed species such as green sea turtles (*Chelonia mydas*), and manatees (*Trichechus manatus*).

Other functions of importance include stabilization of bottom sediments, helping minimize turbidity levels due to bottom re-suspension while trapping excess nutrients when available improving in this way water quality. These stabilization or “buffering” capacity of seagrass meadows decreases the possibility of the formation of large blooms of planktonic algae and helps in trapping atmospheric CO₂ and water nutrients into more refractory sedimentary material (Romero et al 2006, thus being an important global carbon and nutrient sink. Overall, the seagrasses contribute significantly to ecosystem service values higher than saltmarsh/mangroves and coral reefs (Constanza et al 1997).

Monitoring Perspective

EcoElectrica LP, operates a natural gas cogeneration facilities in in the outskirts of Guayanilla Bay. This operation requires the use of approximately 20 MGD while producing >500MW. These activities are covered by 40 C.F.R. Subpart M—Ocean Discharge Criteria and Sections 316(b) and 403 of the Clean Water Act in relation to “the effect of disposal of pollutants on human health or welfare, including but not limited to plankton, fish, shellfish, wildlife, shorelines, and beaches”.

The main questions related to the seagrass communities examined in EcoElectrica’s 2011 Biological Monitoring Program Plan (2011 BMPP) can be stated as:

1. Are there significant differences in the biological composition of species between the area close to the discharge and intake sites of the cooling water system (CWS)?
2. Are the differences, if present, probably linked to the long-term operation of the facilities?

The specific purpose of this work, in relation to the is to quantify:

1. patterns of percent seagrass cover at locations adjacent to the Intake (Int) and Outfall (Out) of EcoElectrica’s CWS that will provide up to date information related to the seagrass large scale spatial studies component;
2. patterns of biomass and productivity distribution at the Int and Out locations to supplement information related to the seagrass small scale spatial studies.

Methods

Study area

Figure 1 shows the location of the study area and the location of the main sampling stations. These stations were located based on previous studies, including transects for evaluation of seagrass coverage and productivity.

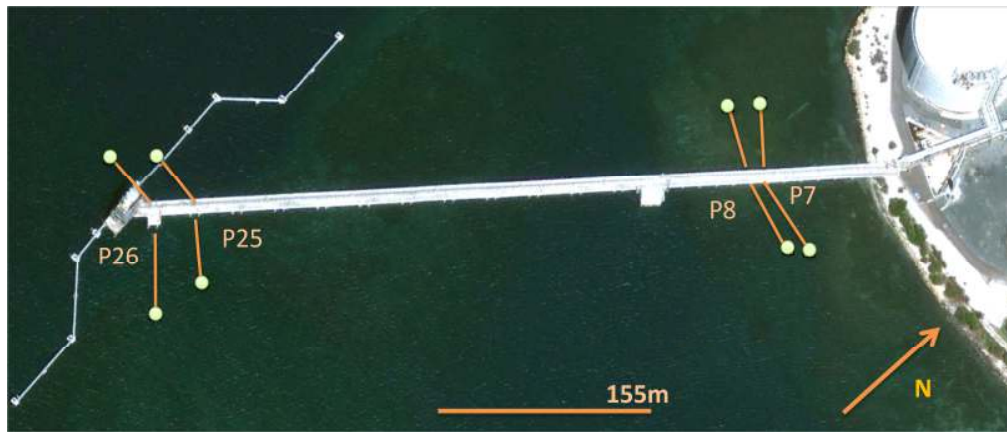


Figure 1. Location of study transects close to the intake (P7 and 8) and outfall (P25 and 26) of EcoElectrica's pier.

Location of transects- A series of 50m long transects perpendicular to both sides of the pier established according to Vicente (2001). In addition the area under the pier will also be examined. The transects were located at bents 7, 8, 25 and 26 (Figure 1).

Seagrass Coverage

The coverage of seagrass was examined using video transects and still photography. Each transect was established by inserting metal rods into the bottom to serve as anchors for a stretched nylon rope marked every 0.5m. The

end of the transect was marked using a temporary surface buoy to establish its location by using a differential geographic positioning system (DGPS) unit. Video was taken at 1-2 m from the bottom at a fixed magnification on a seaward direction from the pier. Photos were taken on the way back to the pier. The marks on the rope served as distance scale for the determination of areal coverage. The following was determined:

- Total seagrass coverage and distribution along transects
Two different approaches were used to evaluate seagrass distribution along the transects. The first approach used video footage along each transect to determine the presence and absence of seagrasses every meter. The second approach, estimated the percent coverage of seagrass from photos taken within 0.25ft² quadrats positioned every 2.5m along each transect. The seagrass coverage was estimated as the percentage of 20 randomly selected points falling on seagrass leaves using CPCe 4.1 (Kohler and Gil (2006) software.
- Seagrass species composition
The presence and absence of seagrass species along the transects was determined based on photography and video images complemented by personal observations.

Seagrass standing crop and growth

Growth estimates were conducted based on Tomasco and Dawes (1990), Tomasko and Hall (1999) and Vicente (2010). In short, four metal squares (quadrant) (0.25ft²=0.023m²) were fixed in seagrass beds along the transects. The location was marked using subsurface buoys suspended at 2m over the bottom. A total of 4 replicates in two transects were examined.

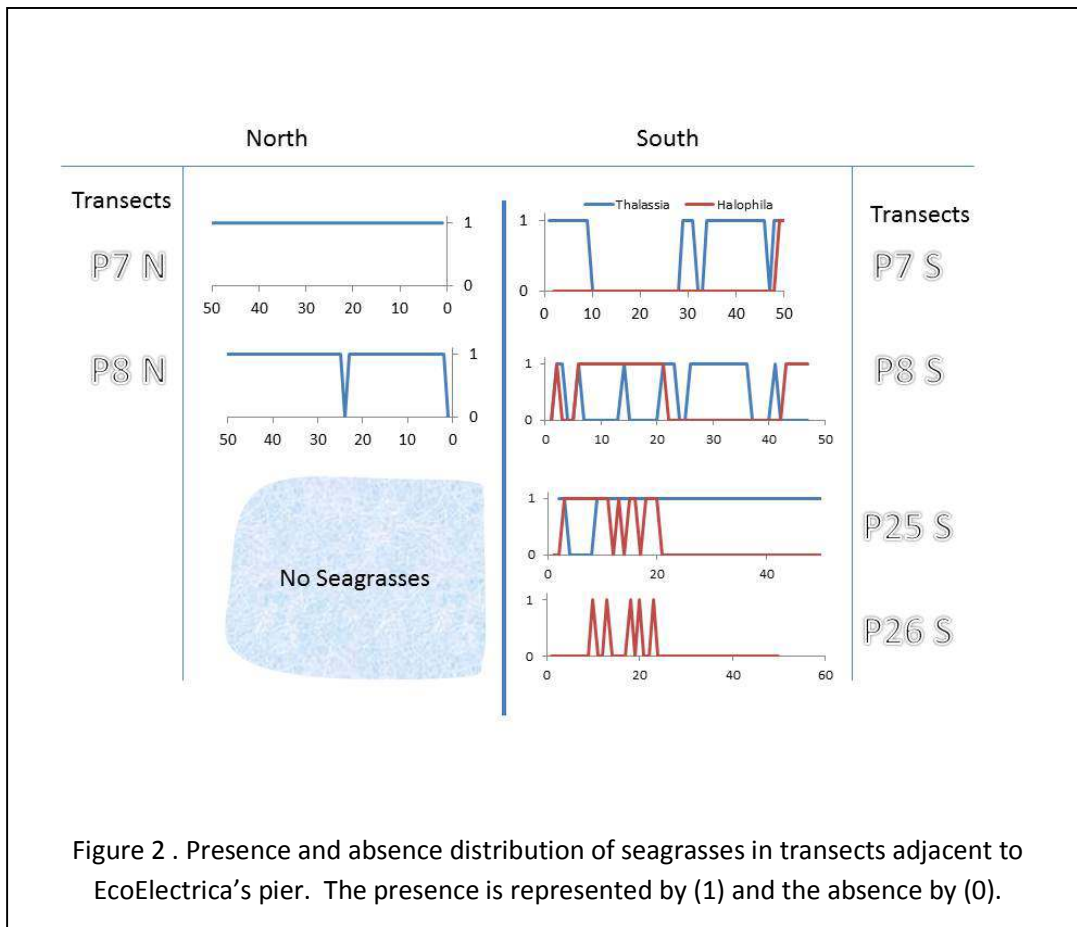
The following estimates were conducted for *Thalassia testudinum* within each square:

- *Leaf Length*: These measurements were conducted for leaves collected for the leaf production estimates. A total of 10 haphazardly selected leaves from each quadrat were used to calculate central and error metrics (i.e. average and SD). A plastic ruler with mm scale was used to measure leaf length from the non pigmented portion of the leaf (surrounded by the bundle sheath) to the tip.
- *Growth*: a hypodermic needle (#25) was inserted through the center of each blade bundle at about 1cm above the blade sheath of the oldest intact leaf. Newly produced leaf material was identified by the displacement of the needle marks of younger blades above the previous mark after 7-10 days. After the selected period of time, all leaf bundles were cut just above the stems and transported to the laboratory in plastic bags within coolers. Once in the lab, new growth material, based on the hypodermic needle mark, was separated from the rest of the material (the older material was not discarded as it was used for the calculation of above ground standing crop), dried and weighted.
- *Above Ground Standing Crop*: This estimate was based on the sum of all leaf material collected from each quadrat. It is the sum of new leaf material plus the older material.

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Results and Discussion

The distribution of seagrasses based on presence and absence (Fig. 2) demonstrates large variability throughout the study area. The north-eastern portion showed consistently high cover (transects (P7N and P8N) of *T. testudinum*. Some locations within this general area contained much reduced



seagrass cover related to sparse blowouts or proximity to the pier. In contrast the southeastern transects (P7S and P8S) evidence a significant patchiness of the two species of seagrasses observed (*T. testudinum* and *Halophila* sp.). The western section of the pier showed a marked decrease of seagrass cover, where the southern portion seem to consist of a transition area towards deeper water where the dominance of *T. testudinum* declines and is replaced by some patches of *Halophila* sp. No seagrasses were observed in the north-west area.

The percent seagrass coverage confirms the general pattern observed above (Fig 3). In general the seagrass coverage at P7N and P8N was frequently close to 80-90% with some spatial drops down to 0 percent coverage. As mentioned above, this fluctuation is the result of the blowouts (more prominent in P8N) and a decrease closer to the pier. Some of the lower covers were also associated to the presence of coral rubble. The overall lower values observed at the southeastern transects was due to the presence of muddy bottoms and low coral cover reef outcrops. The seagrass covers at the southwestern transect south of P25 reached a maximum of about 50 Percent and while a cover of up to 30% was rare and only due to *Halophila* at P26. This area was dominated by other submerged vegetation (fleshy and calcareous algae) interspersed among bare sandy sediments.

Standing Corp and Productivity

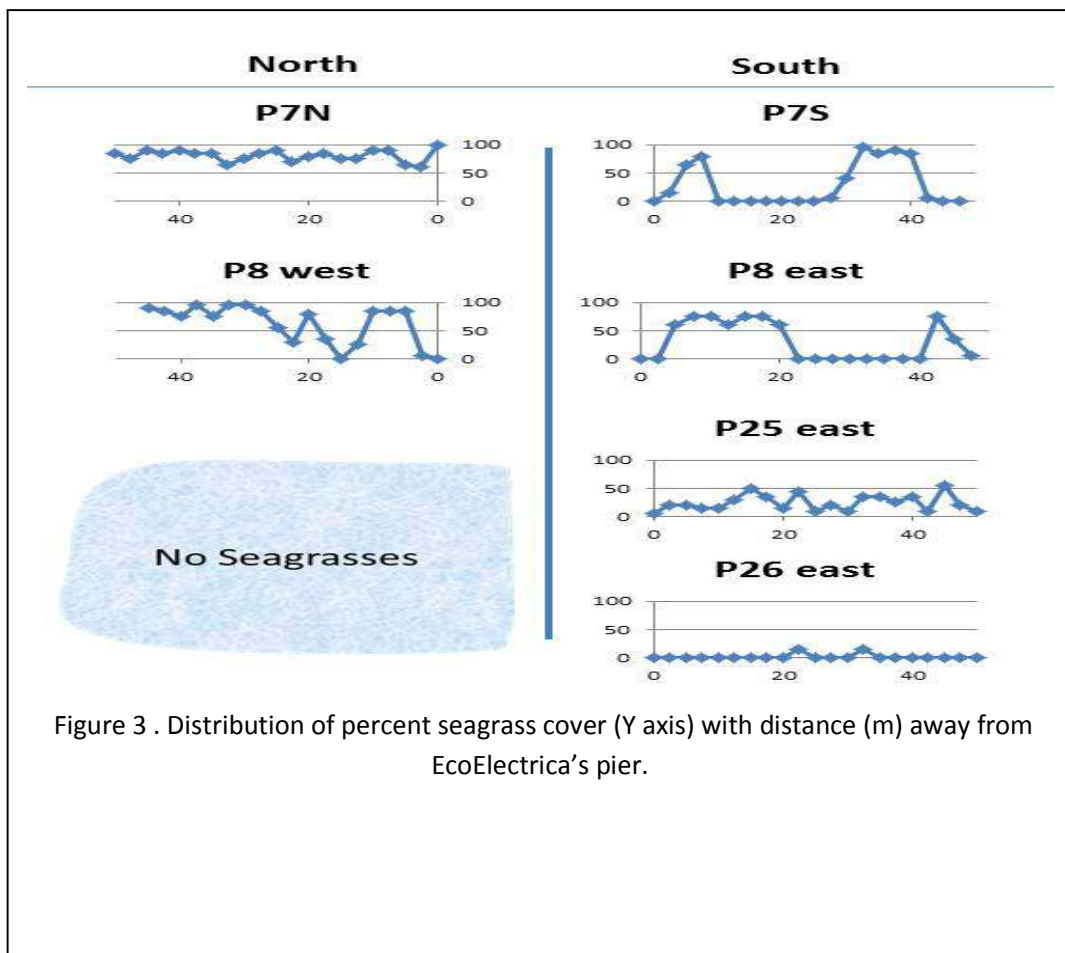
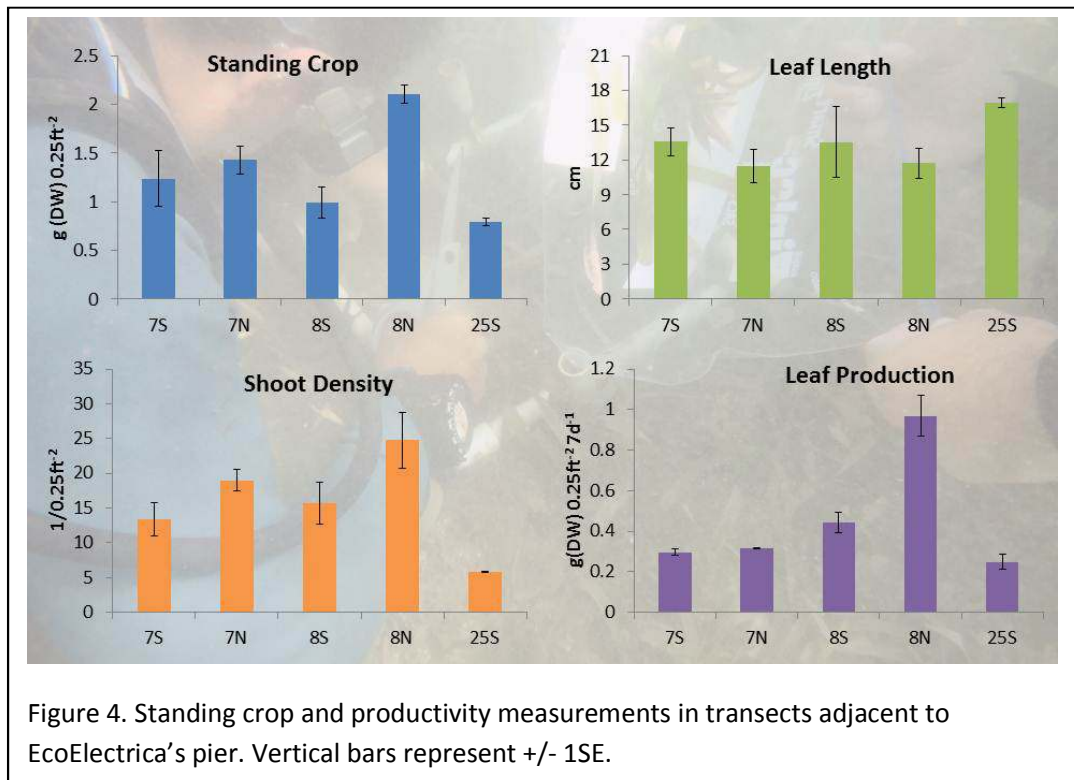


Figure 3 . Distribution of percent seagrass cover (Y axis) with distance (m) away from EcoElectrica's pier.

The different measures of standing crop and production are depicted in Figure 4. Following the patterns observed with seagrass cover; the maximum dry weight, shoot density and production was observed in the north eastern area (Transect 8N). However, the average leaf length was slightly longer in the southwestern transects (25S).



Comparisons With Previous Years

Figure 5 shows seagrass % cover average for the present year and previous years. The data from previous years was derived from Vicente (2010). Nested Analysis of Variance indicate statistical significant differences ($P < 0.05$) between the intake and outfall and between north and south areas. No significant difference was found among the different years (Table 1). These differences suggest the standing crop of seagrasses is in a steady state condition that represented by a heterogeneous distribution to the south that decreases towards the west, closer to the outfall. These distributions may be attributed to several factors, including a decrease in light availability and higher exposure to turbulent waters (more exposed to water currents and waves) to the south.

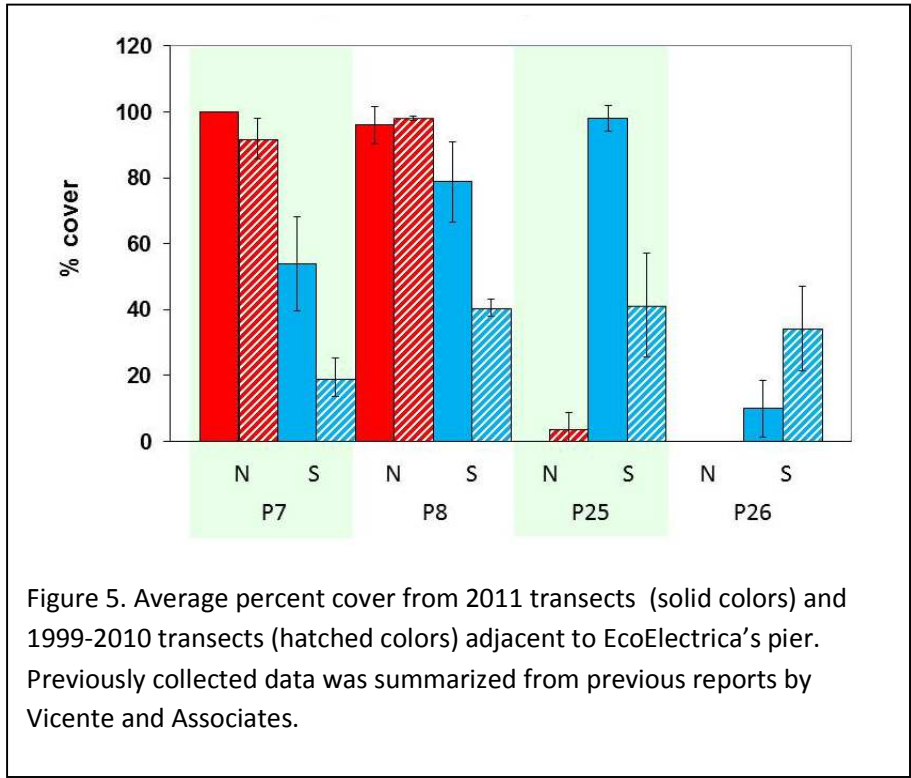
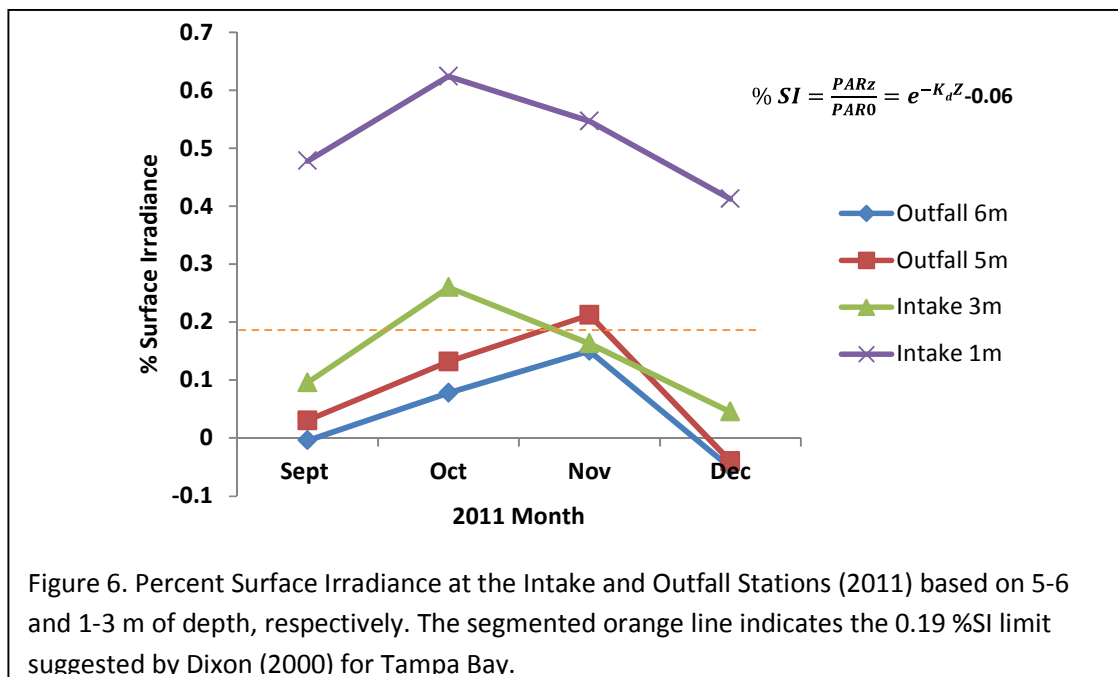


Table 1. Statistical differences (Nested ANOVA; StatistiXL Ver 1.8) of seagrass cover among years, intake vs. outfall sites and north vs. south areas.

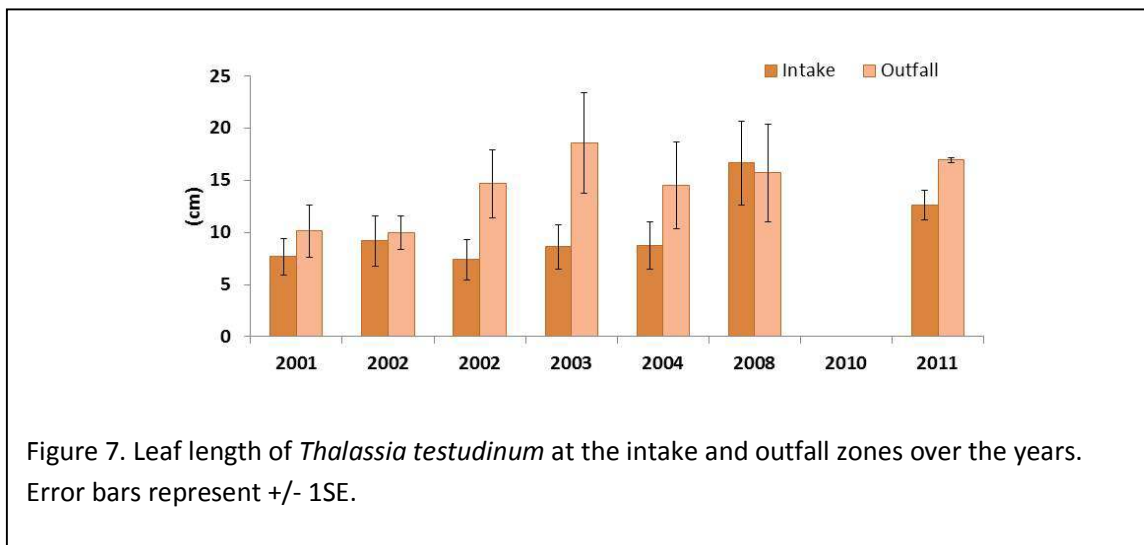
Overall test of model for Y=%cover					
Source	Type III SS	Df	Mean Sq.	F	Prob.
Model	79589.031	31	2567.388	10.265	0.000
Error	8003.905	32	250.122		
Total	87592.937	63			
Tests of effects for Y=%cover					
Source	Type III SS	Df	Mean Sq.	F	Prob.
year	2141.833	7	305.976	1.223	0.319
Zone	30907.383	1	30907.383	123.569	0.000
N/S	1294.949	1	1294.949	5.177	0.030
year*Zone	1027.955	7	146.851	0.587	0.761
year*N/S	1968.650	7	281.236	1.124	0.373
Zone*N/S	41665.138	1	41665.138	166.579	0.000
year*Zone *W/E	583.125	7	83.304	0.333	0.933

Red= statistically significant differences at P=0.05; Zone= Intake/Outfall

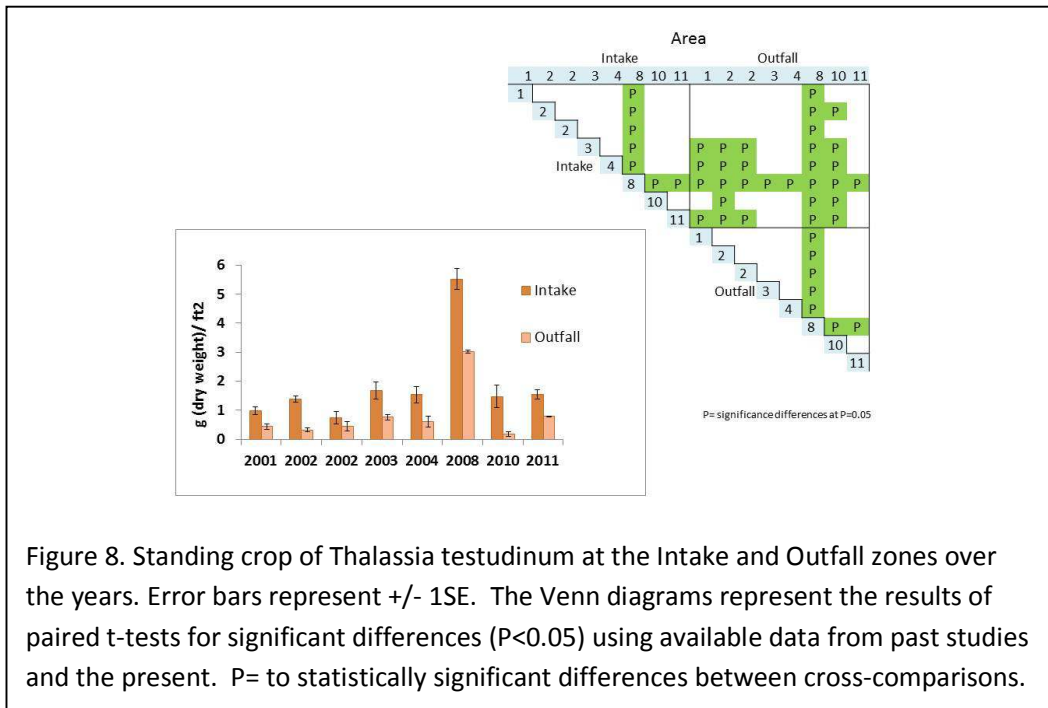
The bathymetry in the area is an important factor controlling the distribution of seagrasses. For instance, the north eastern portion of the study area is shallow (1-1.5 m) and favors the penetration of enough light to sustain seagrass productivity. In contrast the northwestern area can be 17-18 m deep which diminishes the availability of light for the development of seagrass stands. Although the depth to the south of EcoElectrica pier is intermediate (2-6 m) the combination of further light attenuation by turbidity currents derived from river inputs and more exposure to water currents diminish availability of light over the long term and may in part explain the lesser coverage and more patchy distribution of seagrasses. The expected light levels at the different sites were examined using the photosynthetically active radiation attenuation coefficient ($K_{d_{PAR}}$) estimates derived during this year's water quality assessment work (Otero 2012). These attenuation coefficients varied from 0.33 -0.78 m^{-1} depending on climate conditions. The % surface irradiance (%SI) was calculated by subtracting 6% from the in water attenuation to include the atmosphere/water interphase reflectance effects as in Dixon (2000). Dixon (2000) suggested that a %SI of ca. 19% elicited a shading response of *Thalassia testudinum* under the conditions of Tampa Bay. We used that value as an index to evaluate the presence of effects induced by light limitation on *Thalassia* at the study sites close to the intake and outfall. Figure 6. shows the range of %SI at those sites from Sept-Dec 2011. For



these estimates we used depths of 1-3 meters at the intake and 5-6 meters at the outfall seagrass beds to evaluate the higher and lower irradiance levels. These estimates indicate that shallow *Thalassia* beds near the intake sites are not prone to effects of light inhibition while those at the slightly deeper end are exposed to conditions of light limitation. The depth increase from 3 to >5 meters exposes seagrasses at the outfall region to more extreme light limitation conditions and explains the percent area coverage at the latter zone. Biomass and productivity data was also compared with previous records. Figures 7-10 represent leaf length, leaf biomass, shoot density, and leaf production from 2001 to 2011. Multiple t-test comparisons were conducted to evaluate differences among yearly measurements within the seagrass stands close to intake and outfall areas. No statistically significant differences were found relative to leaf lengths.



Leaf biomass (dry wt) showed a statistically significant increase for both the intake and outfall during 2008 (Fig 8). Apart from the 2008 intake biomass peak, comparisons within intake and outfall estimates were not significantly different suggesting an overall stability towards values of 0.5-1.5 g (DW)* ft⁻². Intra year cross comparison between intake and outfall regions showed significant differences only during 2008 and 2010. Overall, the data indicates sustained differences between intake and outfall areas which became more pronounced after 2003.



The differences between outfall and intake areas can be more clearly observed using shoot density estimates. Figure 9, indicate a significant increase of shoot density at the intake area after 2008 which is manifested by the dominant

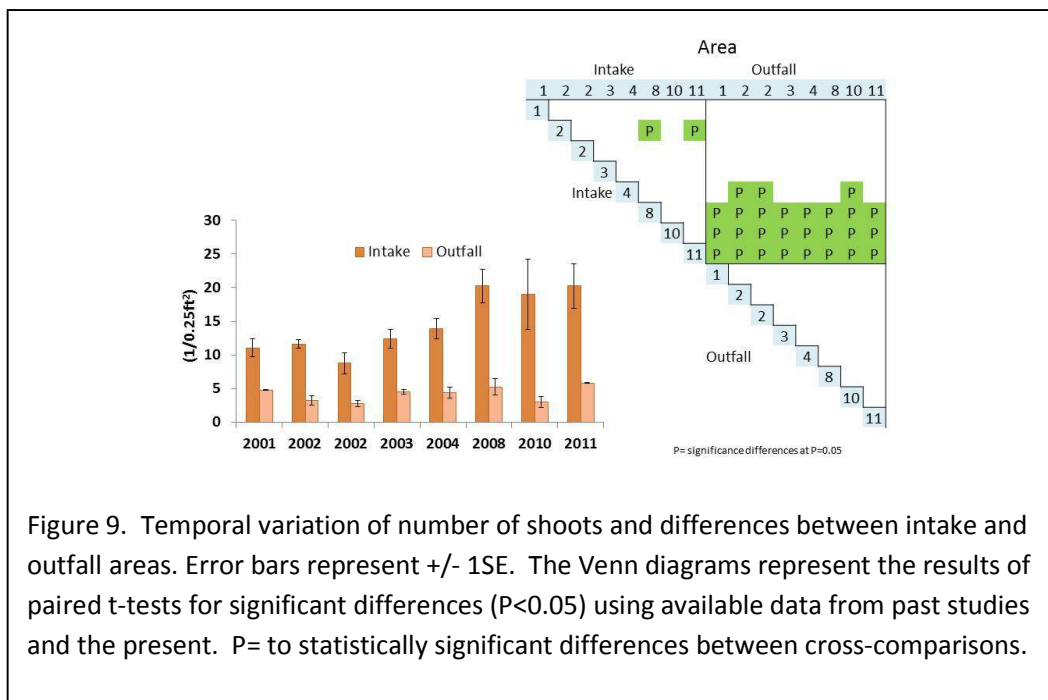


Table 2. Summary of Various *Thalassia testudinum* Parameters From Previous and the Present Study.

Reference	Shoot density	Above ground biomass	Blade Length	Leaf Production	Turn over
	#/m ²	g/m ²	cm	g/m ² /d	%/day
Dixon, 2000	36-109	4.5-22.2	7.9-19.4	<0.1-0.702	0.21-3.1
Tomasco and Lapointe, 1991	106-853	20.5-105.5	-	0.3-1.4	1.1-2.8
Gacia 1999	390-840	197-328	-	2.0-5.1	0.8-2.0
Martinez -Daranas et al 2009	616.7	75.6	13.8	2.1	2.8
Vicente and Associates (2010)	120-873	13.8-238	7.4-17.2	0.6-2.9	3.2-7.1
This study	250-1067	34-91	11.4-16.9	1.5-6.0	4.1-6.6

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Conclusions

- The distribution of seagrasses in the vicinity of EcoElectrica's pier is spatially variable. The north eastern shallower near coastal area can be described as a *monoculture* of *Thalassia testudinum*. The northwestern zone lacks seagrass cover while the southern is characterized by the patchy distribution of turtle grass and *Halophila* sp. No *Halodule wrightii* or *Cymodocea filiforme* were observed.
- Biomass metrics showed higher standing crop at the north eastern *Thalassia* bed stations than that at the other stations while shoot density was only moderately higher at the former location. Overall, the average leaf was only slightly longer near the Outfall region (transect 25S). Leaf production showed a prominent peak associated to the northeastern *Thalassia* beds, which was 2.5-4 times higher than at other locations.
- The cover, biomass and productivity distribution of seagrasses seems to be sharply controlled by bathymetry and the light attenuating character of the fluctuating composition of water substances. The use of water quality data allowed the estimation of % surface irradiances that suggest light limitation as a dominant factor affecting the distribution and growth of seagrasses in the area.
- Comparisons with previous work (Vicente and Associates, 2010) suggest a stable seagrass distribution (%cover) over the years (not statistically significant differences) while the spatial differences (outfall vs intake and north to south) constituted most of the observed differences). This was also the case for biomass and productivity. However, there were instances in which inter-year changes within zones (intake or outfall) were observed. Year 2008 marked an increase in turtlegrass biomass. A shift in the proportion of *Thalassia* shoots at the intake and the outfall was caused by a significant increase of shoots at the intake region. This divergence was also observed for productivity. This pattern suggests an expansion of *Thalassia*

cover on southeastern area (P7-8) over time that should be examined in the future.

- Overall, our data suggests a diverse setting of seagrass habitats comparable with other studies. However, productivity and turnover had the potential to reach higher values than other reported in the Caribbean region probably due to latitudinal and location specific characteristics.
- At present no evidence for a significant change in seagrass cover can be attributed to the operation of EcoElectrica's pier and cooling water system.

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