A Simple, Inexpensive, and Field-Relevant Microcosm Tidal Simulator for Use in Marsh Macrophyte Studies

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Protocol Note

A simple, inexpensive, and field-relevant microcosm tidal simulator for use in marsh macrophyte studies[1]

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- *Premise of the study:* A microcosm unit with tidal simulation was developed to address the challenge of maintaining ecologically relevant tidal regimes while performing controlled greenhouse experiments on smooth cordgrass, *Spartina alterniflora.*
- *Methods and Results:* We designed a simple, inexpensive, easily replicated microcosm unit with tidal simulation and tested whether *S. alterniflora* growth in microcosms with tidal simulation was similar to that of tidally influenced plants in the field on Sapelo Island, Georgia. After three months of exposure to either natural or simulated tidal treatment, plants in microcosms receiving tidal simulation had similar stem density, height, and above- and belowground biomass to plants in field plots.
- *Conclusions:* The tidal simulator developed may provide an inexpensive, effective method for conducting studies on *S. alterniflora* and other tidally influenced plants in controlled settings to be used not only to complement field studies, but also in locations without coastal access.

Key words: biomass; greenhouse; marsh; plant growth; *Spartina alterniflora*; tidal simulation.

Tidal marshes provide valuable ecosystem services including carbon cycling, storm surge protection, and nutrient retention (Bowen and Valiela, 2008; Barbier et al., 2011) largely due to the presence of macrophytes that tolerate regular flooding associated with tides. Cordgrasses in the family Poaceae belonging to the genus *Spartina* Schreb. have high rates of primary production, have extensive root systems that stabilize shorelines, and are capable of both root and foliar uptake of nutrients (Barbier et al., 2011). Furthermore, they are considered foundation species that are tightly linked to the distribution and abundance of salt marsh plants (Bruno, 2000); macroinvertebrates such as fiddler crabs, snails, and mussels; as well as smaller meiofauna that live in the sediment (Bertness and Leonard, 1997). In addition, coastal bird species such as the endangered light-footed clapper rail rely on *Spartina* sp. marshes for nesting habitat (Boyer and Zedler, 1999). Thus, having the ability to study tidal marsh plant responses to a variety of environmental factors including nutrient and contaminant (e.g., oil and heavy metals) concentrations using manipulative experiments is particularly useful (Portnoy and Valiela, 1997; Millward et al., 2004). However, conducting research on tidal marsh plants in a controlled (i.e., greenhouse) setting can be challenging due to difficulties associated with replicating tidal flushing. Establishing tides in salt marsh studies is important because previous studies using permanently inundated plants documented reduced plant growth stemming from problems with flooded soils including altered redox potential (Armstrong et al., 1985) and elevated hydrogen sulfide concentrations (Mendelssohn and McKee, 1988; Portnoy and Valiela, 1997).

Although a number of tidal simulator studies exist in the literature, there are location, cost, and space limitations associated with current designs, as well as a paucity of information regarding relevance to field conditions (Table 1). For example, systems that require seawater are typically restricted to coastal facilities with plumbing infrastructure, such as the drip irrigation design of Boyer and Fong (2005) that piped seawater directly to and from the Redondo Beach, California, Generating Station, or the pumping of bay water into large-volume (2.5-m^3) tanks from the adjacent San Francisco Estuary performed by Cohen et al. (2009). Tidal simulation systems constructed at a distance from coastal seawater supplies can be expensive, elaborate, or both. Gleason et al. (1981) developed an effective but expensive system using programmable circuit boards connected to a series of sensors that transferred water from a 2700-L reservoir to an equally sized experimental unit and then turned off the water pumps once the desired water depth was reached. Previously developed systems that use artificial seawater range from large and recirculating (e.g., 1100-L mesocosms connected to reservoirs; Catallo, 1999) to less expensive versions that discard water after one tidal cycle, creating additional cost associated with water preparation (e.g., Brown et al., 2006) and potentially disposal, particularly if pollutants are used in test solutions. Although existing salt marsh tidal simulation systems have been used to examine responses other than or in addition to plant growth, such as sediment and water biogeochemistry, mercury methylation, and redox potential (Table 1), a major assumption is that the simulated system experiences field-like conditions. However, with the exception

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TABLE 1. Comparison of the current tidal simulator to previously published designs using estimated costs, space required, and whether field testing was conducted.

Source	Plant species present	Measured variable(s)	Space/facilities required	Custom or expensive parts	Field comparison
Current study	*Spartina alterniflora*	Plant height, stem density, above- and belowground biomass	0.18-m^2 area required for each microcosm unit No permanent plumbing required Standard electrical connection for pumps	None	Yes
Catallo (1999)	*Spartina alterniflora*	Soil redox potential	Facility to contain two 1100-L mesocosm units connected to water supply tanks No permanent plumbing required Standard electrical connection for pumps/lights	Custom tidal tanks and remotely timed sputter pumps and artificial lighting	Yes
Boyer and Fong (2005)	*Salicornia virginica*	Plant biomass, tissue and soil total N and ^{15}N, redox potential	0.3-m^2 space required for each microcosm unit Extensive plumbing infrastructure required Standard electrical connection for timer	Permanent seawater connection	No
Cohen et al. (2009)	*Spartina foliosa*	Plant biomass, stem density, interstitial and soil nutrients, N_2 fixation	4.2-m^2 area for each tidal tank Extensive plumbing infrastructure required Standard electrical connection for timer	Permanent seawater connection and tidal tanks	No
Spaulding and Hester (2007)	*Spartina patens*, *Sagittaria lancifolia*, *Panicum hemitomon*	Plant biomass, interstitial nutrients, soil organic matter, redox potential	Large facility to contain 108 200-L mesocosm units connected to four 3000-L water tanks Plumbing infrastructure required Standard electrical connection for water pumps	Custom facility and tidal tanks	No
Gleason et al. (1981)	*Spartina alterniflora*	Method only (no verification data provided)	4.6-m^2 area required for each mesocosm unit Tanks connected by PVC plumbing and water level controlled by programmable circuit boards Standard electrical connection to operate pumps	Custom programmable circuit board and tidal tanks	No
Brown et al. (2006)	*Spartina alterniflora*	Plant biomass; tissue Al, Ca, Fe, Mg, N, P, K; soil redox potential	Space for three 240-L, nine 90-L tanks, and an additional 9-L container per microcosm unit Permanent PVC plumbing required Standard electrical connection to operate pumps	None	No
Ulrich and Sedlak (2010)	*Sarcocornia pacifica*	Water and sediment S, Fe, C, and methylmercury	0.45-m^2 area for each microcosm unit No permanent plumbing required Standard electrical connection for multichanneled peristaltic pump operation	Multichanneled peristaltic pump	No

of Catallo (1999), who specifically compared soil redox measurements in static and tidal mesocosms to those in the field, none of the aforementioned studies directly compared measurements in their tidal simulation systems to those under natural tidal conditions.

The goal of this study was to develop a small, inexpensive, microcosm tidal simulator that could be used at inland locations to conduct ecologically relevant studies of tidal marsh macrophytes. The tidal system presented was constructed with easily obtainable materials and required minimal space and infrastructure compared to previously developed systems. Macrophyte growth in units with tidal simulation was also evaluated against growth in field plots to test the hypothesis that smooth cordgrass *S. alterniflora* Loisel. growth in the tidal simulation system is comparable to growth by plants exposed to a natural tidal regime.

METHODS AND RESULTS

Tidal simulators were constructed using two 18.9-L (5-gal) buckets, one acting as the microcosm and the other as a water reservoir with a lid to prevent evaporation (Fig. 1). Water transfer between the reservoir and microcosm was accomplished using two Tom Aquatics Aqualifter dosing pumps (Premium Aquatics, Indianapolis, Illinois, USA) mounted to the outer lip of the microcosm; one pump transferred water into and the other withdrew water from the microcosm. Aquarium tubing (0.65-cm diameter; Python Pro Quality Air Line Tubing, Premium Aquatics) was secured to the side of each bucket with cable ties and extended from each pump to the bottom of both the microcosm and reservoir buckets. The pumps were then connected to programmable timers (GE 6-Outlet Heavy Duty Outdoor Timers; General Electric, Fairfield, Connecticut, USA) to simulate semidiurnal tides. Every six hours, 11 L of water was transferred from the reservoir to the microcosm to simulate high tide. Then, after six hours elapsed, the water was moved back into the reservoir, completely draining the microcosm from the bottom to establish low tide. Transfer of water from one bucket to the other occurred over a 75-min period. The result was two fixed high and low tides over a 24-h period.

The tidal simulator we designed has space, cost, and infrastructure benefits over previously designed tidal simulators (Table 1). Each fully functioning microcosm unit takes up 5–60% of the space of the other systems (0.18 m^2 compared to 0.3–4.6 m^2). Furthermore, our units are cost effective given that they do not require custom tanks (Spaulding and Hester, 2007), programmable circuit boards (Gleason et al., 1981), or expensive multichanneled peristaltic pumps (Ulrich and Sedlak, 2010). The cost associated with each unit is primarily due to the two dosing pumps (Appendix 1). Furthermore, the system we describe can be used anywhere; it requires neither the plumbing infrastructure nor the

Fig. 1. Tidal simulator microcosm units consisted of one 18.9-L bucket containing a *Spartina alterniflora* sward connected to a second reservoir bucket (with a lid to prevent evaporation) by aquarium tubing attached to two water-lifting pumps. Pump 1 transferred water from the reservoir into the microcosm, and pump 2 transferred the water from the microcosm back into the reservoir (A). Units were connected to an outdoor timer (3) programmed to create tidal exchange every 6 h (B), and aquarium tubing extended from pumps to the bottom of both the microcosm and reservoir buckets to ensure complete transfer of water between them (C).

special facilities required by other systems (Table 1). Finally, growth of tidal marsh macrophytes in our simulators was tested against growth of plants exposed to natural tidal inundation in the field.

Spartina alterniflora growth in the new tidal simulators was compared to growth in field plots exposed to tidal inundation on Sapelo Island, Georgia (31°24′39.33″N, 81°17′8.70″W), from June to August 2012. Sixteen marsh plugs (swards) consisting of between three and 10 *S. alterniflora* ramets with surrounding sediment, rhizomes, and roots (Mendelssohn and McKee, 1988) were collected from a short-form monospecific stand of *S. alterniflora* near Oakland Creek (31°24′42.36″N, 81°17′7.28″W). The variation in ramet number was difficult to avoid given that we attempted to collect swards containing stem densities that were representative of the natural marsh without damaging the plants during the collection process. Swards were collected ~1 m apart to ensure genetic diversity (Richards et al., 2004). Half of the excavated swards ($n = 8$) were placed in 18.9-L buckets that were each impregnated with 12 (10-cm diameter) holes to allow connection to the surrounding sediment and returned to their original location in the marsh. The remaining swards were placed into nursery pots for transfer into the microcosm units, with enough space between the sward and the bucket for the aquarium tubing to reach the bottom of the microcosm without being compressed. Drainage at simulated low tide occurred through holes in the bottom of the pots. The microcosms with tidal simulation were established in an open-sided greenhouse covered with 6-mm clear plastic sheeting. Photosynthetically active radiation (PAR) through the sheeting was reduced by ~36%; therefore, plastic sheeting was also placed at the same height (1.5 m) above each field plot. Water used for the tidal simulators was drawn from a tidal creek adjacent to the site containing the field plots. Each 18.9-L microcosm unit received one *S. alterniflora* sward ($n = 8$).

Plant height and stem density were measured weekly in response to tidal treatment. Plant height was estimated as the mean of the five tallest shoots above 30 cm in each microcosm measured from the sediment surface to the tip

of the longest leaf (Boyer and Zedler, 1998). Stem density was defined as the total number of live shoots per microcosm (Dai and Wiegert, 1996). Above- and belowground *S. alterniflora* biomass were determined only at the conclusion of the experiment due to the destructive nature of the sampling. *Spartina alterniflora* aboveground biomass was collected by clipping shoots at the surface of the sediment (Morris and Haskin, 1990). Belowground biomass was separated from sediment by rinsing through a 2-mm mesh screen. Biomass samples were stored at 4°C until processing (Darby and Turner, 2008). Plant tissues were separated into live and dead categories based on color and turgor pressure according to the methods of Darby and Turner (2008); aboveground tissue was considered live if it was green and turgid, and belowground tissue was considered live if it appeared white, orange, or reddish and turgid. Dead above- and belowground plant tissue was identified by a yellow or brown color and flaccid texture. Following separation, plant tissue was washed free of sediment and dried at 55°C to constant weight (Darby and Turner, 2008). Stem density (m^{-2}) and above- and belowground biomass ($g \cdot m^{-2}$) were standardized to surface area (Morris and Haskin, 1990).

Data were tested for assumptions of normality using the Shapiro–Wilk *W* test and homogeneity of variances using Levene's test. Square root transformations were required only for stem density data to meet the assumptions of parametric tests. Differences in stem density and plant height between field and simulated tidal treatments were analyzed using repeated-measures ANOVA. Differences in above- and belowground biomass between treatments were analyzed at the end of the experiment using *t* tests. All statistical analyses were conducted using JMP 10 (SAS Institute, Carey, North Carolina, USA).

Spartina alterniflora stem density and plant height did not differ between field inundation and tidal simulation treatments (Table 2). Stem density changed over time regardless of treatment, and the observed increase from June to August was consistent with *S. alterniflora* growth over the summer months in southeastern tidal marshes (Morris and Haskin, 1990) (Fig. 2A). A strong trend toward a decrease in plant height over time occurred likely due to the production of new, shorter stems as main culms in the swards senesced (Dai and Wiegert, 1996), but the pattern in plant height was similar in both tidal treatments (Fig. 2B). At the end of the experiment, no effect of tidal treatment was evident on aboveground (2-tailed *t* test for unpaired samples: $t_{14} = 1.09$, $P = 0.30$) or belowground ($t_{14} = 1.24$, $P = 0.24$) biomass. Aboveground biomass averaged 367 ± 42.9 $g \cdot m^{-2}$ vs. 438 ± 48.9 $g \cdot m^{-2}$, and belowground biomass averaged 592 ± 49.1 $g \cdot m^{-2}$ vs. 738 ± 107 $g \cdot m^{-2}$ for field and microcosm simulators, respectively. Belowground to aboveground biomass ratios for field inundation (1.61) and tidally simulated microcosms (1.68) were similar to the ratio of 1.42 previously observed for *S. alterniflora* on Sapelo Island, Georgia, collected during peak biomass (Gross et al., 1991), and was within the range (0.7–14) reported for *S. alterniflora* in a Louisiana salt marsh (Darby and Turner, 2008).

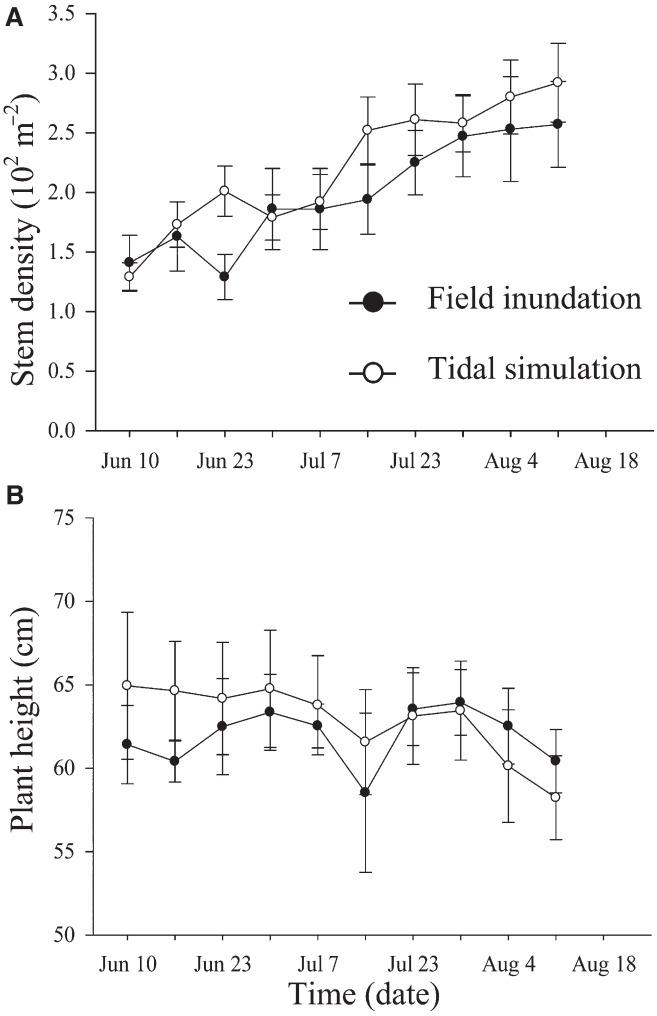

Fig. 2. Average (A) stem density (number of stems × 10^2 m^{-2}) and (B) mean plant height (cm microcosm^{-1}) for *Spartina alterniflora* exposed to either tidal simulation or field inundation from June to August 2012. Error bars are ± one standard error of the mean (SEM) and $n = 8$.

CONCLUSIONS

Simple construction, low cost, and versatility are major advantages of this tidal simulator over existing systems. Using materials found at hardware and aquarium retailers, each tidal simulation unit was constructed for less than US$27 in approximately 20 min, thus a large number of tidal simulator units can be set up within several days, unlike other large tidal simulation systems that use expensive custom tanks or facilities and can take months to set up (Gleason et al., 1981; Spaulding and Hester, 2007; Cohen et al., 2009). Each unit also only takes up 0.18 m^2 (0.3 × 0.6 m), allowing for high levels of experimental replication in a small space. Furthermore, *S. alterniflora* growth in the microcosm tidal simulation system was similar to that of plants exposed to natural tidal regimes; no differences in plant height, stem density, or above- and belowground biomass occurred between tidal treatments, providing ecological relevance to experiments conducted using the simulators.

The system is also highly versatile and can be adapted for a large variety of experimental applications. First, the tidal simulator units can be set up in any environment provided there is a power supply for the pumps and timer; plumbing is not required. Second, due to each unit having its own water reservoir, the set-up is ideal for studies testing the effects of dissolved substances on tidal marsh plant characteristics. While the small reservoir volume and the recirculating nature of the system reduce the amount of solution needed, there is potential for solutions to become depleted of macro- and micronutrients. *Spartina alterniflora* has specific nitrogen, phosphorous, sulfur, magnesium, and iron requirements (Broome et al., 1975) that can be maintained by replacing synthetic seawater solutions at regular intervals or

TABLE 2. Repeated measures ANOVA results for stem density and plant height.

Characteristic	*df*	F	*P*
Stem density			
Tidal treatment	(1,14)	0.85	0.37
Time	(9,126)	19.74	<0.001
Tidal treatment*time	(9,126)	1.78	0.07
Plant height			
Tidal treatment	(1,14)	0.08	0.91
Time	(9,126)	1.87	0.05
Tidal treatment*time	(9,126)	0.93	0.28

adding nutritional supplements such as diluted Hoagland's solution (Merino et al., 2010). Regular water changes may also prevent accumulation of potentially toxic compounds such as sulfide and humic acid that can be flushed from the sediments during drainage at low tide (Portnoy and Valiela, 1997). Furthermore, we recommend that a piece of mesh be used as a filter either at the bottom of the container containing the sward or over the intake end of the aquarium tubing itself to reduce clogging with any sediment that may be flushed into the bucket during low tide. Third, the depth of tidal inundation can be altered by adjusting the height of the return aquarium tubing, which allows testing for the effects of drought or sea level rise. Finally, although we tested the tidal simulator using *S. alterniflora*, it may be useful for applications using other tidally influenced species. For example, previous studies have used tidal simulators for marsh plant species including *Panicum hemitomon* Schult. and *Sagittaria lancifolia* L. (Spaulding and Hester, 2007) and *Sarcocornia pacifica* (Standl.) A. J. Scott (Ulrich and Sedlak, 2010); therefore, use of other plant species with our system merits further investigation. It is also important to note that while the small size of our tidal simulator is optimal for high replication under space constraints, the size may limit its use to research on sediment processes, certain species of plants, infauna, and macroinvertebrates with limited ranges of movement, such as some gastropod species. In contrast, a larger simulator unit would be needed to accommodate highly mobile macroinvertebrates such as crabs, as well as a greater volume of water for filter-feeding bivalves that require a reliable source of food in the water column (Bertness, 1999). However, that we found plant growth in the microcosms with tidal simulation to be similar to that in the field inundation treatment suggests the tidal simulation system presented is a simple, inexpensive, and ecologically relevant way to conduct *S. alterniflora* marsh studies in a greenhouse setting.

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Appendix 1. Materials, costs, and instructions for the construction of the tidal simulator.

A. Suppliers and costs for items needed to create one tidal simulation microcosm unit

Item (no. needed)	Supplier	Cost (U.S. dollars)[a]
18.9-L bucket (2)	Hardware	$4.00
18.9-L bucket lid (1)	Hardware	$1.00
Nursery pot (1)	Nursery	$0.10
Tom Aquatics Aqualifter pumps (2)	Premium Aquatics	$20.00
Airline tubing (3 m)	Premium Aquatics	$0.70
2.54-cm wood screws (2)	Hardware	$0.50
Marine epoxy	Hardware	$0.30
13 × 1.3 cm cable tie (4)	Hardware	$0.10
Total		$26.70

[a] Items for the current study were purchased in 2012.

B. Step-by-step instructions for construction

Step 1. Fasten two 13-cm cable ties into small circles large enough for airline tubing to pass through without being constricted.

Step 2. Use marine epoxy to secure two cable tie loops in each 18.9-L bucket at the desired height. For example, we installed the loops approximately 5 cm above the bottom to hold the airline tubing in place against the bottom of the bucket and ensure complete drainage of water from the unit. Note: Lightly sanding the area before applying marine epoxy will ensure a longer-lasting adhesive bond.

Step 3. To minimize the amount of airline tubing required, insert the two 2.54-cm wood screws into the outer lip of one of the 18.9-L buckets (ca. 5–7 cm apart) directly above the cable tie loops. The screws will be the attachment sites for the Aqualifter pumps.

Step 4. Hang one pump on each screw.

Step 5. Arrange the second 18.9-L bucket next to the first such that the cable tie loops are directly below the Aqualifter pumps hanging from the adjacent bucket.

Step 6. Cut four sections of airline tubing (0.75-m segments should be sufficient) and secure two lines to each pump, making sure that the ends run through the cable tie loops. Note: One Aqualifter pump will draw water from the reservoir into the microcosm, and the other will draw water from the microcosm into the reservoir.