

# Mean plot-level responses observed in an experiment conducted at Zeke's Island National Estuarine Research Reserve where abundance of the seaweed *Gracilaria vermiculophylla* was manipulated to assess impact on multiple ecosystem functions

**Website:** <https://www.bco-dmo.org/dataset/716208>

**Data Type:** Other Field Results, experimental

**Version:** 1

**Version Date:** 2017-10-05

## Project

» [Small Grazers, Multiple Stressors and the Proliferation of Fungal Disease in Marine Plant Ecosystems](#) (small grazers facilitating fungal disease)

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## Abstract

These data represent the time-averaged value of each variable measured monthly in each plot over the course of a 10 month experiment carried out on intertidal mud and sandflats located within the Zeke's Island National Estuarine Research Reserve (33.95 N, 77.94 W), North Carolina, USA.

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## Coverage

**Spatial Extent: Lat:33.95 Lon:-77.94**

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## Dataset Description

These data represent the time-averaged value of each variable measured monthly in each plot over the course of a 10 month experiment carried out on intertidal mud and sandflats located within the Zeke's Island National Estuarine Research Reserve (33.95 N, 77.94 W), North Carolina, USA.

The data and experiments are further described in:

Ramus AP, Silliman BR, Thomsen MS, Long ZT (2017) An invasive foundation species enhances multifunctionality in a coastal ecosystem. PNAS 114(32):8580-8585.

doi:[10.1073/pnas.1700353114](https://doi.org/10.1073/pnas.1700353114)

Data are also available in GitHub: <https://github.com/apramus/invFSxfunc>

## Acquisition Description

**For complete methodology, see Ramus et al., 2017 (doi:[10.1073/pnas.1700353114](https://doi.org/10.1073/pnas.1700353114)). In summary:**

The experiment was carried out on intertidal mud and sandflats located within the Zeke's Island National Estuarine Research Reserve (33.95 N, 77.94 W), North Carolina, USA. We manipulated six densities (n = 8 per treatment) of the nonnative seaweed *Gracilaria vermiculophylla* in 48 large 25 square meter plots over a 10-month period. We selected three low-intertidal flats spanning over 1 km in the reserve that differed in area, flow regimes, *Gracilaria* cover, grain size, and proximity to the *Spartina* salt marsh. The three flats represented the continuum of estuarine habitats where *Gracilaria* naturally occurs in this area. We established the 48 plots along the mean low water line at 5-m intervals by adding 3-m steel rebar 1.2 m into the substrate at each plot-corner. Treatments were randomly assigned to the plots to avoid potentially confounding small-scale effects of site (and all plots had only few *Diopatra* tubes). *Gracilaria* was fixed in a plot with metal 'u-pegs' (constructed from clothes hangers) by physically staking handful-sized 'clumps' of loose thalli to the sediment surface. Pegs were flushed with the sediment surface to avoid above-surface experimental artifacts. Our

six treatments were based on the total number of pegs per 25 square meters (arranged in squared grids) as follows: 0 (0×0), 9 (3×3), 36 (6×6), 100 (10×10), 225 (15×15), and 400 (20×20). *Gracilaria* was collected from nearby locations and added to plots in the u-peg grids in August 2013. Treatments were maintained and response variables quantified approximately monthly from September 2013 to June 2014 (treatments were maintained and measured at total of 10 times). For each plot visit we quantified the cover of *Gracilaria* (in 10 randomly placed 0.25 square meter quadrats per plot) and seven ecosystem functions (see next section for detail) before maintaining *Gracilaria* densities (by replenishing u-pegs devoid of *Gracilaria* and manually removing *Gracilaria* from control plots).

To examine the effect of *Gracilaria* on epifauna, we positioned a 0.25 square meter quadrat in the center of each plot and collected all *Gracilaria* and its associated epifauna into a zip-top bag. In the laboratory, *Gracilaria* was rinsed in freshwater and shaken for about 1 min to remove epifauna, which were captured on a 500 micron sieve. Epifauna were identified and enumerated to broad taxonomic groupings (typically family level) under a stereomicroscope (~18x, Nikon SMZ800). For simplicity, all faunal data were standardized to unit area. Taxonomic richness was rescaled to unit area using the species-area relationship and assuming a conservative value of 0.15 for  $z$ .

To quantify whether *Gracilaria* attenuates hydrodynamic forces, we used gypsum dissolution blocks that dissolve at a rate proportional to water velocity and thus represent an integrated proxy for tidal currents and wave exposure. We created gypsum blocks as hemispheres ( $\phi = 6.5$  cm) from dental plaster (Die Keen, Heraeus Kalzer), covered on the bottom with two layers of polyurethane to ensure that an equal surface area would be subject to dissolution. Gypsum blocks were dried at 60 degrees C for a minimum of 24 h before recording the initial mass and deploying one block flush with the substrate surface in the center of each plot for 4 d. Following retrieval, gypsum blocks were dried and reweighed, and the dissolution rate calculated as grams of gypsum dissolved per day. Because lower dissolution rates indicate greater flow reduction, dissolution rates were reflected using the equation  $-\mathbf{fi} + \mathbf{max}(\mathbf{fi})$ , so that greater flow reduction corresponds with a positive contribution to ecosystem functioning.

To examine the effect of *Gracilaria* on sediment stabilization, we marked all corner poles at 20 cm above the substrate surface in August 2013. The distance between the marking and substrate surface was measured with a ruler to the nearest 0.5 cm at the end of each month. We calculated the monthly (30 d) change in height in cm by subtracting the final from initial distance to the substrate (using the average of the 4 corners per plot) and correcting for the time interval between measurements. Accretion and erosion are represented as positive and negative values, respectively.

To assess the effectiveness of *Gracilaria* as a nursery habitat for commercially and recreationally important species, we sampled the entire plot using a 1.2-m high × 6.7-m wide nylon seine net (The Fish Net Company, Jonesville, LA; mesh size = 3.175 mm) during a

falling tide. Upon completion of a pass, we swiftly pulled the net taught, tilted it into a horizontal position, and lifted it from the water into an adjacent boat (R/V Adelaide) in a single motion. Organisms (greater than 1 cm) retained on the boat were identified to the family-level and enumerated before being returned to the water. Abundances were reported per unit area (dividing by 25 square meters) and richness data were rescaled to unit area using the species–area relationship and assuming a conservative value of 0.15 for  $z$ .

To quantify the effect of *Gracilaria* on decomposition processes, standing dead *Spartina* stems were collected from adjacent salt marshes, washed, and dried at 60 degrees C for a minimum of 72 h (until no further weight loss). We pooled multiple stems to achieve an initial mass of 7.0 +/- 0.5 g and placed them inside a mesh litter bag, which was closed and deployed on the sediment surface in the center of each plot. Bags were retrieved just prior to the next treatment maintenance. Remaining stem material was washed, dried, and weighed and decomposition rate was reported as the mass lost in grams per month.

To simplify our analyses and remove temporal autocorrelation, we calculated the average response of each function in each plot using the full 10 month data set (48 plots sampled each month). At the end of the experiment we measured four additional functions (months 8 through 10). Because we did not have seasonal data for these responses they were excluded from the main analysis of multifunction effects.

To sample benthic infauna, triplicate core samples (5-cm diameter, 15-cm depth, volume = 294.5 cubic cm) were taken equidistant along a diagonal transect of each plot on June 25, 2014. The three sediment core samples from each plot were pooled into a ziptop bag. Upon return to the laboratory, the content of each bag were drained and rinsed over a 1-mm mesh sieve to remove fine sediments. Infauna retained on the sieve were preserved in 75% ethanol. The 1-mm mesh-size was chosen to concentrate sampling efforts on juvenile and early life stages of crustaceans, molluscs, and larger polychaete taxa. Infauna were identified and enumerated under a stereomicroscope (~18x, Nikon SMZ800) to families, and, in some cases, phyla. Infaunal data were standardized and rescaled to unit volume (using the reciprocal of 0.8836 L and a conservative  $z$  of 0.15) following the same methods described previously for epifauna and nursery functions.

To evaluate the effect of *Gracilaria* on ray foraging activity, we counted the number of ray holes in each plot on 3 to 4 different days in a given month. We here report the average number of ray holes standardized to unit area (by dividing by 25 square meters) during a given low tide on a single day.

To investigate the association of waterfowl with *Gracilaria*, we delimited the 48 plots into four sites based on spatial proximity (plots 1-12, 13-24, 25-36, and 37-48) and surveyed all waterfowl activity occurring within a site (containing 12 plots) for a 15-min period during low tide. Bird counts were made through binoculars from our research vessel from a distance of

about 100 m to avoid disturbances arising from our presence. We tallied the number of birds initially present, and that became present, within the boundaries of each plot during the observation period. After completing the 15-min observation of a site, we moved to a new vantage point for observing the next 12 plots. Hence, by repeating this procedure at all sites, all 48 plots were sampled with equivalent effort in a ~1 h period. Because measurements were made on 1 to 3 different days in a given month, we present the average number of birds tallied per unit area (by dividing by 25 square meters) per unit time (by multiplying by 4; 15 min x 4 = 60 min = 1 h) of low tide.

## Processing Description

BCO-DMO Processing:

- modified parameter names to conform with BCO-DMO naming conventions.

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## Related Publications

Ramus, A. P., Silliman, B. R., Thomsen, M. S., & Long, Z. T. (2017). An invasive foundation species enhances multifunctionality in a coastal ecosystem. *Proceedings of the National Academy of Sciences*, 114(32), 8580–8585. doi:[10.1073/pnas.1700353114](https://doi.org/10.1073/pnas.1700353114)

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## Parameters

Parameter	Description	Units
Plot	The experimental plot, numbered from West to East	unitless
TrtPeg	The density treatment in total number of u-pegs assigned to each plot	unitless (count)
Gcvr	The average Gracilaria cover (%) maintained in each plot over the course of the experiment	unitless (percent)
Epi	Mean abundance of epifauna	number per square meter (# m <sup>-2</sup> )

EpiRich	Mean richness of epifauna taxa	taxa per square meter (taxa m <sup>-2</sup> )
Dsln	Mean chalk dissolution expressed as mass lost in grams per day	grams per day (g d <sup>-1</sup> )
DslnFlip	Mean reflected chalk dissolution; calculated as an intermediate step and intended to be standardized on a scale of 0-1 when used in any analysis.	grams per day (g d <sup>-1</sup> )
Sed	Mean sediment stabilization expressed as the change in height in cm per month	centimeters per month (cm mo <sup>-1</sup> )
Nrsy	Mean abundance of nursery species	number per square meter (# m <sup>-2</sup> )
NrsyRich	Mean richness of nursery taxa	taxa per square meter (taxa m <sup>-2</sup> )
Dcmp	Mean decomposition of Spartina stems expressed as mass lost per month	grams per month (g mo <sup>-1</sup> )
Infa_sr	Mean abundance of infauna; the suffix "sr" denotes supporting responses only measured near the end of the experiment	number per liter (# L <sup>-1</sup> )
InfaRich_sr	Mean richness of infauna taxa; the suffix "sr" denotes supporting responses only measured near the end of the experiment	taxa per liter (taxa L <sup>-1</sup> )
Rays_sr	Mean number of ray holes; the suffix "sr" denotes supporting responses only measured near the end of the experiment	number per square meter per day (# m <sup>-2</sup> d <sup>-1</sup> )

RaysFlip_sr	Reflected mean number of ray holes; the suffix "sr" denotes supporting responses only measured near the end of the experiment; calculated as an intermediate step and intended to be standardized on a scale of 0-1 when used in any analysis.	number per square meter per day (# m <sup>-2</sup> d <sup>-1</sup> )
Wfwl_sr	Mean abundance of waterfowl; the suffix "sr" denotes supporting responses only measured near the end of the experiment	number per square meter per hour (# m <sup>-2</sup> h <sup>-1</sup> )

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## Instruments

<b>Dataset-specific Instrument Name</b>	stereomicroscope
<b>Generic Instrument Name</b>	Microscope-Optical
<b>Dataset-specific Description</b>	Epifauna were identified and enumerated to broad taxonomic groupings (typically family level) under a stereomicroscope (~18x, Nikon SMZ800).
<b>Generic Instrument Description</b>	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

<b>Dataset-specific Instrument Name</b>	
<b>Generic Instrument Name</b>	Scale
<b>Generic Instrument Description</b>	An instrument used to measure weight or mass.

<b>Dataset-specific Instrument Name</b>	seine net
<b>Generic Instrument Name</b>	Seine Net
<b>Dataset-specific Description</b>	To assess the effectiveness of Gracilaria as a nursery habitat for commercially and recreationally important species, we sampled the entire plot using a 1.2-m high × 6.7-m wide nylon seine net (The Fish Net Company, Jonesville, LA; mesh size = 3.175 mm) during a falling tide. Upon completion of a pass, we swiftly pulled the net taught, tilted it into a horizontal position, and lifted it from the water into an adjacent boat (R/V Adelaide) in a single motion.
<b>Generic Instrument Description</b>	A seine net is a very long net, with or without a bag in the centre, which is set either from the shore or from a boat for surrounding a certain area and is operated with two (long) ropes fixed to its ends (for hauling and herding the fish). Seine nets are operated both in inland and in marine waters. The surrounded and catching area depends on the length of the seine and of the hauling lines. (definition from: fao.org)

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## Deployments

### Silliman\_Gracilaria\_experiments

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/716418">https://www.bco-dmo.org/deployment/716418</a>
<b>Platform</b>	Zeke's Island National Estuarine Research Reserve

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## Project Information

### Small Grazers, Multiple Stressors and the Proliferation of Fungal Disease in Marine Plant Ecosystems (small grazers facilitating fungal disease)

**Coverage:** Coastal Plant Ecosystems in North and South America.

In terrestrial communities, grazer-facilitation of fungal disease in plants has been studied for over a century. Despite the prevalence of this interaction in terrestrial systems, it was not considered relevant to the structure of marine plant communities until the investigator's recent work in salt marshes. By manipulating both grazer and fungal presence, he demonstrated that snail grazing and subsequent fungal infection in live grass led to drastic reductions in plant growth and, at high grazer densities, destruction of canopy. If grazer promotion of fungal disease in marine plants is not limited to marshes (as suggested by preliminary data from a world-wide survey of 4 marine plant ecosystems) then small grazers that take small bites out of plants could be exerting similarly strong, but undetected control over marine plants globally. In addition, since physical stress commonly reduces plant immune responses, intensifying multiple stressors associated with marine global change could intensify and destabilize these unstudied grazer-disease-plant interactions. To test the global generality of this potentially keystone ecological interaction, this project will answer the following questions with a combination of multi-site surveys and manipulations across 4 ecosystems spanning 2 continents: 1) Is grazer facilitation of fungal disease in marine plants a common but overlooked interaction? 2) What is the resultant impact of grazer-facilitated fungal infection on marine plant growth? 3) How do multiple stressors impact the strength of grazer facilitation of fungal disease in marine plants? The work represents a transformative step forward in our understanding of plant-grazer interactions in marine ecosystems as it fills a > 100-year intellectual gap in our understanding of top-down control in marine plant ecosystems: Do small grazers commonly facilitate fungal disease in marine plants and does this interaction suppress plant growth? Evidence for this cryptic, yet powerful mechanism of grazer regulation of marine plants will compel marine ecologists to reevaluate our understanding of top-down control and lead to widespread integration of disease dynamics in marine food web ecology. The consequences of marine plant ecosystem health are far-reaching for humans, since these communities provide many essential services. Results from this study will allow managers to better predict effects of disease and global change on marine plant systems and formulate effective strategies for conservation. To help integrate plant disease dynamics into marine ecology and conservation, the investigator will: (1) produce an edited volume on Food Webs and Disease in Marine Ecosystems and (2) work closely with The Nature Conservancy to incorporate findings into their global marine learning exchanges. In addition, an integrated educational plan will increase student: (1) understanding of disease and food web dynamics in marine ecosystems and (2) consideration of marine science careers.

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## Funding

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1445834</a>
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1056980</a>

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