# Eelgrass (Zostera marina) physiological and morphological traits measured between March of 2013 and August of 2014 using eelgrass collected in Bodega Bay, CA in May of 2012

**Website**: https://www.bco-dmo.org/dataset/725483
**Data Type**: Other Field Results
**Version**: 2
**Version Date**: 2018-06-28

**Project**
» Connecting genetic diversity to ecosystem functioning: links between genetic diversity, relatedness and trait variation in a seagrass community (Genetic Div to Ecosys Functioning)

| Contributors | Affiliation | Role |
|---|---|---|
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**Abstract**
This dataset contains physiological and morphological traits of Zostera marina genotypes collected in Bodega Bay CA in May of 2012 and grown in a common garden at the Bodega Marine Laboratory prior to trait measurements. Trait measurements were conducted from March of 2013 to August of 2014. A separate common garden of replicate shoots was established (N=5 individuals per genotype) in May 2014. Growth measurements and shoot morphology were taken in July of 2014 from the common garden started in May 2014. Other traits were measured on shoots harvested from the original common garden. These results and methodology were published in Abbott et al., 2017 and Abbot et al., in press.

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## Coverage

**Spatial Extent**: **N**:38.3337 **E**:-122.9406 **S**:38.305 **W**:-122.9574
**Temporal Extent**: 2013-03 - 2014-08

## Dataset Description

Site information for transects conducted in Bodega Bay in 2012 can be found in the dataset "Eelgrass transect information" https://www.bco-dmo.org/dataset/725435.

### Methods & Sampling

Methods below are excerpts from Abbot et al., in press.

In May 2012 we collected 20 ramets harvested at 2 m intervals along a 40 m transect at each of three tidal heights (high intertidal, low intertidal, and subtidal) at five sites within Bodega Harbor, CA. The five eelgrass

collection sites are distributed throughout the harbor, between 0.45 and 3.2 km apart. We transported the 260 eelgrass ramets collected from these sites to the Bodega Marine Laboratory (~ 2-4 km), where we trimmed the ramets to a single shoot with 3 cm of rhizome and 30 cm of leaf length and planted them in 11.4 cm diameter by 9.5 cm high plastic flowerpots. We placed all pots in a single common garden flow-through seawater tank; we randomly assigned the pots to an initial position and rotated the pot position weekly. We collected leaf clips from each ramet for genetic analysis. We delineated genotypes and estimated relatedness using 11 microsatellite loci selected from a pool of >30 loci developed specifically for Zostera marina. We identified a total of 219 unique genotypes from the 260 ramets we collected. From this pool we selected 40 genotypes to ensure that we included (1) a wide range of pairwise relatedness values and (2) genotypes from all tidal heights and sites. We transplanted these 40 genotypes into 3.79 L plastic flowerpots and grew them in an outdoor common garden flow-through seawater tank for the duration of trait measurements. We rotated pot position weekly to avoid position effects. The common garden tank was 4.5 m long and 1 m wide and held approximately 3800 L of seawater. Seawater flowed into the tank via 10 inflow valves that were distributed along the length of the tank with a combined seawater flow rate of approximately 16 L per min. We then measured a range of performance and resource-acquisition traits for these 40 genotypes. We measured these traits only on new shoots produced while the plant was in the common garden, and not until shoots had acclimated to the common garden conditions for at least six months and had produced a minimum of three new shoots.

Morphology and production

We harvested five shoots of each genotype from the primary common garden, standardized each to 3 cm of rhizome and 30 cm shoot length, and planted them individually in 11.4 cm diameter by 9.5 cm high plastic flowerpots. The pots were placed in a second identical outdoor common garden in May at the beginning of the growing season. We started a second common garden so that we could retain the original clones in the common garden, while also destructively harvesting shoots for growth and morphological measurements. After a growth period of 10 weeks, we harvested the plants and measured shoot width and length, number of leaves, total rhizome length, rhizome diameter, maximum root length, new shoots produced, and the biomasses of roots, rhizomes, new shoots, and the terminal shoots. One week prior to harvesting plants we punched holes in terminal shoots to measure leaf growth rate (Williams and Ruckelshaus 1993).

Nutrient uptake rate

We quantified biomass-specific leaf nitrate uptake and root/rhizome ammonium uptake rates using two-compartment chambers similar to the design in Terrados and Williams (1997). Eelgrass plants can absorb multiple forms of nitrogen in all their tissues; however, because nitrate is most available in the water column and ammonium in the sediments we measured nitrate uptake in leaf shoots and ammonium uptake in roots/rhizomes at ambient concentrations. Eelgrass shoots were collected from the primary common garden of 40 genotypes, cleaned of all sediment, epiphytes, and invertebrates, and their rhizomes were cut to 3 cm the day prior to allow wound healing. The roots and rhizomes of each shoot were compartmentalized from the leaf shoots by inserting the shoot through a slit in a watertight rubber stopper that was then inserted into a 40 mL opaque plastic chamber filled with 35 mL of nitrogen-free artificial seawater spiked with ammonium to 100 M using a 5M NH4-N stock solution prepared with ammonium sulfate. The root/rhizome chambers with shoots inserted were then placed into 2 L clear acrylic cylindrical chambers filled with 1 L of nitrogen-free artificial seawater spiked with nitrate to 40 M using a 2 M NO3-N stock solution prepared with sodium nitrate. Sixteen acrylic chambers were seated in a water bath with water circulating through a chiller to keep chamber seawater temperature between 10-12C (Bracken et al. 2011). To provide sufficient water flow to prevent mass transfer limitation of uptake, we attached submersible pumps to each chamber via inflow and outflow pipes. Full spectrum quartz halite lamps surrounding the water bath provided the chambers with photosynthesis-saturating light (~700 mol photons m-2s-1). We took water samples from the root/rhizome chambers and shoot chambers prior to the start of the experiment, then sampled the shoot chambers every hour for 4 hours, at which time we detached the root/rhizome chambers from the shoot chamber, removed the shoots and took a final sample. We analyzed the shoot chamber samples (nitrate) using a Lachat 8000 series flow injection auto-analyzer and root/rhizome chamber samples (ammonium) using a Beckman Coulter DU640 spectrophotometer (Koroleff 1976). After removing plants from the chambers, we divided them into shoots, roots, and rhizomes, and dried them at 60C for at least 48 hours and then weighed each for biomass-specific uptake rate. We ran uptake trials with between 10-14 genotypes per day and measured all genotypes each week for nine weeks. Within a week eelgrass genotypes were randomly assigned a day and position in the water bath. Some genotypes did not yield enough shoots in the common garden for the full set of nine replicates (one genotype had only enough shoots for three replicates, but most had seven to nine successful replicates).

Leaf phenolic content

We analyzed total phenolic content using approximately 4 mg of dried, ground leaf material from each genotype (pooled from 3 leaves) following a modified Folin-Ciocalteu method (see Bolser et al. 1998). We extracted phenolics with 2 mL of 80% methanol for 24 hours, and then quantified them with a spectrophotometer using caffeic acid as a standard. Ferulic and caffeic acids are two of the most abundant phenolics in Zostera marina (Quackenbush et al. 1986; Vergeer and Develi 1997), and previous work showed that caffeic, ferulic, or gallic acids standards for eelgrass phenolic content from shoots collected in Bodega Bay produced similar results (Tomas et al. 2011).

Photosynthetic rate

We evaluated the photosynthetic performance of each genotype using a Diving-PAM® (Pulse Amplitude Modulated) fluorometer (Walz, Germany) to measure maximum quantum yield (potential photosynthetic efficiency, FV/Fm) and rapid light curves (RLC), which determine the effective quantum yield as a function of irradiance and can be used to assess light adaptation (Ralph and Gademann 2005, Williams et al. 2009). First, we dark-acclimated the outer leaves of each shoot for 30 minutes by placing a Waltz 4 mm opaque leaf clip 20 cm from the sediment surface on a leaf cleaned of epiphytes, then we immediately took maximum quantum yield and rapid light curve (RLC) measurements. RLCs comprised eight incremental steps of actinic light irradiance from 30 to 1129 PAR (mol photons m-2s-1), and the resulting yield measurements were converted into a relative electron transport rate (rETR) using the following equation:

rETR = F/Fm *PAR*0.5*AF,

where F/Fm is the effective quantum yield, F is the difference between background fluorescence F and F at each PAR increment, Fm is the maximum fluorescence, 0.5 assumes that photons absorbed are equally distributed between photosystems I and II (Genty et al. 1989), and AF is the standard absorption factor (0.55) for seagrasses (Durako 2007). To compare RLCs among genotypes we used curve fitting methods outlined in Ralph and Gademann (2005) to estimate characteristic parameters for each curve including: , initial slope of the curve (rate of increase in photosynthesis with increasing light in light-limited region of the RLC or photosynthetic efficiency); , slope of the curve where yield declines (strength of photoinhibition); and Ps, the maximum potential rETR. We fit each curve to a double exponential decay function (Platt et al. 1980) using the “nls” function in the stats package in R 3.0.2.

**Data Processing Description**

<p>BCO-DMO Data Manager Processing Notes:
* added a conventional header with dataset name, PI name, version date
* modified parameter names to conform with BCO-DMO naming conventions
* All numeric values with more than three decimal places to three decimal places
* data version 2 (2018-06-28) replaces data version 1 (2018-01-31). Data version 2 includes a new parameter Pam_b, and removes "Flag" which was for internal use and not meaningful for re-use of the data since it was replaced by genotype identifier. Previously documented processing steps took place for both data version 1 and data version 2.
* rounded decimal places based on feedback from the data contributor. All numeric values with more than three decimal places to three decimal places except the following:
* rounded to one decimal place: shoots, width, rhiz_D
* rounded to two decimals: length, term_rhiz, new_rhiz, max_root, tot_rhiz

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## Data Files

| File |
|---|
| **EelgrassTraits.csv** (Comma Separated Values (.csv), 7.17 KB) MD5:b26c1d8a98b8be512d4af5b36ea6df19 Primary data file for dataset ID 725483 |

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## Related Publications


Abbott, J. M., DuBois, K., Grosberg, R. K., Williams, S. L., & Stachowicz, J. J. (2018). Genetic distance predicts trait differentiation at the subpopulation but not the individual level in eelgrass, Zostera marina. Ecology and Evolution, 8(15), 7476–7489. Portico. https://doi.org/10.1002/ece3.4260
*Results*
,
*Methods*

Abbott, J. M., Grosberg, R. K., Williams, S. L., & Stachowicz, J. J. (2017). Multiple dimensions of intraspecific diversity affect biomass of eelgrass and its associated community. Ecology, 98(12), 3152–3164. doi:10.1002/ecy.2037
*Results*
,
*Methods*

Bracken, M. E. S., Jones, E., & Williams, S. L. (2011). Herbivores, tidal elevation, and species richness simultaneously mediate nitrate uptake by seaweed assemblages. Ecology, 92(5), 1083–1093. doi:10.1890/10-1374.1
*Methods*

Ralph, P. J., & Gademann, R. (2005). Rapid light curves: A powerful tool to assess photosynthetic activity. Aquatic Botany, 82(3), 222–237. doi:10.1016/j.aquabot.2005.02.006
*Methods*

Terrados, J., & Williams, S. (1997). Leaf versus root nitrogen uptake by the surfgrass Phyllospadix torreyi. Marine Ecology Progress Series, 149, 267–277. doi:10.3354/meps149267
*Methods*

Williams, S. L., & Ruckelshaus, M. H. (1993). Effects of Nitrogen Availability and Herbivory on Eelgrass (Zostera Marina) and Epiphytes. Ecology, 74(3), 904–918. doi:10.2307/1940815
*Methods*


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## Parameters

| Parameter | Description | Units |
|---|---|---|
| Geno | eelgrass (Zostera marina) genotype identifier | unitless |
| Site | Site code (MM=mason's marina, WP = westside park, CC = Campbell Cove, DP = Doran Park, J = jetty | unitless |
| Height | Tidal height (HI = high intertidal, LI = low intertidal, S = subtidal) | unitless |
| Pam_a | alpha parameter (rise) for exponential decay function fit to each rapid light curve (RLC). It is the initial slope of the RLC. See Ralph and Gadelmann, 2005. | unitless |
| Pam_b | beta parameter (fall) for exponential decay function fit to each rapid light curve (RLC). It is the slope of the curve where yield declines (strength of photoinhibition). See Ralph and Gadelmann, 2005. | unitless |

| | | |
|---|---|---|
| Pam_ps | saturation coefficient for exponential decay function fit to each rapid light curve (RLC). It is the scaling factor that determines the height of the RLC. See Ralph and Gadelmann, 2005. | unitless |
| Pam_etrmax | the maximum potential relative Electron Transport Rate (ETR) | unitless |
| ammon | biomass-specific ammonium uptake rate in roots/rhizomes | micromoles per gram per hour (µmol/g/hr) |
| nitrate | biomass-specific nitrate uptake rate in leaves | micromoles per gram per hour (µmol/g/hr) |
| ammon_res | residuals of ammonium uptake rate by sampling week (significant week effect) | micromoles per gram per hour (µmol/g/hr) |
| nitrate_res | residuals of nitrate uptake rate by sampling week (significant week effect) | micromoles per gram per hour (µmol/g/hr) |
| phenolics | Phenolic content | percent (dry mass) |
| TERM | biomass of terminal shoot | grams (g) |
| RHIZ | biomass of rhizome | grams (g) |
| ROOT | biomass of roots | grams (g) |
| NEW | biomass of newly produced shoots | grams (g) |
| GROWTH | biomass of growth (segment of leaf between punches) | grams (g) |
| Tot_below | total belowground biomass | grams (g) |
| Tot_term | total terminal shoot biomass (terminal shoot + growth) | grams (g) |
| Total | total biomass of plant | grams (g) |
| Tot_above | total aboveground biomas (terminal+growth+new shoots) | grams (g) |
| GrowthXwidth_dayean | area in cm of growth per shoot per day | centimeters per day (cm/day) |

| | | |
|---|---|---|
| Tot_Growth_dayean | length in cm of growth per shoot per daty | centimeters per day (cm/day) |
| shoots | number of shoots | per individual |
| width | width of terminal shoot | millimeters (mm) |
| length | length of terminal shoot | centimeters (cm) |
| term_rhiz | length of terminal rhizome | centimeters (cm) |
| new_rhiz | total length of rhiz of new shoots | centimeters (cm) |
| rhiz_D | rhizome diameter | millimeters (mm) |
| max_root | max root length | centimeters (cm) |
| tot_rhiz | total rhizome length (new+terminal) | centimeters (cm) |
| ratio | ratio of above to belowground biomass | dimensionless |

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## Deployments

**Stachowicz_Eelgrass_Transects_2012**

| | |
|---|---|
| **Website** | https://www.bco-dmo.org/deployment/725439 |
| **Platform** | Bodega Harbor |

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## Project Information

**Connecting genetic diversity to ecosystem functioning: links between genetic diversity, relatedness and trait variation in a seagrass community (Genetic Div to Ecosys Functioning)**

There is growing evidence that genetic variation within and among populations of key species plays an important role in marine ecosystem processes. Several experiments provide compelling evidence that the number of genotypes in an assemblage (genotypic richness) can influence critical ecosystem functions including productivity, resistance to disturbance and invasion or colonization success. However, these studies use only the number of genotypes as a measure of genetic diversity. Recent analyses of species diversity experiments show that phylogenetic diversity may be a more reliable predictor of ecosystem functioning than simply the number of species. However, such approaches have not yet been applied to understanding the effects of genetics on ecosystem functioning. While genetic relatedness within a species holds the potential to

predict the outcome of intraspecific interactions, and the functioning of ecosystems that depend on those species, we currently have few data to assess the shape or strength of this relationship. The investigators will build on their own previous work, and that of others, in eelgrass (*Zostera marina*) ecosystems showing strong effects of genotypic richness on a spectrum of critical ecosystem processes. The investigators will ask whether genotypic richness, or - as in studies at the level of species diversity - genetic relatedness/distance better predicts ecosystem functioning? If genetic relatedness measures are better predictors, then what mechanisms underlie this relationship? Can genetic relatedness predict ecological relatedness?

Although the current focus is on eelgrass, the research should be applicable to many systems. The project will assess the relationship between genetic relatedness and phenotypic distinctiveness of a key marine foundation species and use manipulative experiments to test the relative importance of the number of genotypes in an assemblage vs. their genetic relatedness and trait diversity for ecosystem functioning. Specifically, experiments will:
(1) characterize the relationship between genetic relatedness and trait similarity among individual genotypes of eelgrass, including responses to experimental warming;
(2) compare the effects of genetic relatedness and trait similarity among genotypes on the outcome of intraspecific competitive interactions; and
(3) test the relative effect of genetic relatedness vs. number of genotypes of eelgrass on the growth of eelgrass, its associated ecosystem functions it (e.g., primary production, nutrient dynamics, trophic transfer, habitat provision, and detrital production and decomposition).

Seagrass ecosystems provide important services to coastal regions including primary production, nutrient cycling, habitat for fisheries species, and erosion control. Previous studies have shown these services can be compromised by reduction in the numbers of species of grazers or genotypes, but this study will allow a more predictive approach to diversity loss by integrating the effects of multiple components of diversity and clarifying the extent to which diversity effects can be predicted by the genetic or ecological uniqueness of component genotypes.

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## Funding


| Funding Source | Award |
|---|---|
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1234345 |


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