Living Shorelines Project: SF State Progress Report

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Summary

Over the past two years, since installation of the Living Shorelines: Nearshore Linkages Project in late July and August 2012, the SF State team has conducted quarterly monitoring of eelgrass establishment and use of the restored habitat (oyster reef, eelgrass, both alone and together) by epifaunal invertebrates and fishes, and has made a variety of water quality measures.

After replanting eelgrass in April 2013 (as the original late-summer planting in 2012 did not succeed), plants at the larger scale project at San Rafael have performed well, reaching 50% of planted densities by July 2013 and 124% by July 2014. Plant heights are comparable to those in natural beds (tallest shoots 150-190 cm in spring and summer 2014). A trend appears to be developing of lower overall densities and heights in the eelgrass + oyster plot compared to the eelgrass-only plot, possibly due to abrasion of plants against the oyster shells. Plants originating from Point Molate tended to produce higher densities than those from Point San Pablo, perhaps due to better matching of site conditions between the Point Molate and San Rafael sites (finer sediments than Point San Pablo). The two donors are now very difficult to distinguish due to overlapping clonal growth and we will not be able to track them separately on future sampling dates. Stable isotopic signatures of eelgrass appeared different with oyster reef present in an early comparison; a detailed collection of tissues to evaluate isotopic signatures that can indicate food web relationships was conducted in summer 2014 in collaboration with the project's oyster team at UC Davis, and analysis is underway. Trapping with minnow and oval traps indicated an early response of species reliant on physical structure, including bay shrimp and Dungeness crab. These patterns have persisted and additional species have been trapped in plots with oyster reef and eelgrass present (e.g., red rock crabs and red crabs). Suction sampling of epibenthic invertebrates indicates that community composition is distinct in the structured habitats relative to the controls and pre-construction conditions, and eelgrass + oyster invertebrate communities are intermediate between those in the eelgrass-only and oyster-only plots. Similarly, freshwater dips of eelgrass shoots to assess epifauna communities show differences when oyster reef is present along with eelgrass. Epifauna assemblages on eelgrass at the San Rafael site have not converged with those at natural comparison sites Point Molate and Keller Beach (the closest two natural beds, just across the bay), with two native species known to remove epiphytes notably absent (the isopod *Idotea resecata* and the sea hare *Phyllaplysia taylori*) at the restored site. Trapping of fish showed much overlap in species composition among the treatments; however, black surfperch and bay pipefish had greater association with eelgrass habitat and Pacific staghorn sculpin with oyster reef. Seining results indicated early recruitment to eelgrass by bay pipefish (within one month of the April 2013 replant), and that eelgrass presence increased the occurrence of certain fish species among oyster reef structures (bay pipefish, shiner surfperch, and saddleback gunnel). Acoustic monitoring to detect tagged fish showed that individuals of several species visited the site, including two white sturgeon, a green sturgeon (threatened species), a leopard shark, a steelhead smolt, and a striped bass. Positional analysis currently underway will help to determine the degree to which the fish were lingering at the site and whether they exhibited preferences among the treatments.

Although plants at the smaller scale project at Hayward (offshore of the Eden Landing Ecological Reserve) reached 75% of planted densities by July 2013 (after a May 2013 replant) and survived through the fall months, major declines occurred during the next winter and only two shoots remained by summer 2014 across the ten small plots. Eelgrass was always shorter at Hayward (~70 cm) than San Rafael perhaps due to shallower site conditions at the former. Plants at this site had high densities of the Atlantic mud snail (both adults and eggs) on their leaves and may have experienced substantial sediment movement and burial; either or both could have contributed to the observed eelgrass mortality. Trapping results showed that shore crab abundances have increased within the treatment

area relative to controls and pre-project conditions, and suction sampling of epibenthic invertebrates indicates that the oyster shell reefs are developing a distinct community relative to the control area and baseline conditions.

Water quality data showed differences between the San Rafael and Hayward site, with the latter frequently warmer and more saline, although dissolved oxygen, chlorophyll-a, and light attenuation were generally similar. We did not detect variation in water quality measures among treatments at either site. Onset HOBO temperature/conductivity loggers provided continuous data for temperature that should be useful for exploring seasonal patterns in response variables for multiple groups within the project team; however, the manufacturer announced early on that the conductivity sensors were defective. We have included the plots of conductivity data here even though many periods show obvious errors (fluctuations of 20+ salinity units on a daily time scale); however, it is possible that with comparison to other conductivity sensors nearby some of these data will be deemed usable.

Introduction

This report summarizes the methods and results of activity to date for the San Francisco Bay Living Shorelines: Near-Shore Linkages Project at the The Nature Conservancy ('TNC') site in San Rafael Bay and the Eden Landing Ecological Reserve ('ELER') site in south San Francisco Bay, near Hayward (Figures 1 and 2). Following pre-installation site assessments, San Francisco State University scientists transplanted vegetative eelgrass shoots at both TNC and ELER and conducted buoy-deployed seeding at TNC during the summer of 2012. After limited success of these transplants, perhaps due to plantings being carried out late in the growing season, SFSU repeated transplanting of vegetative shoots at both sites during the spring of 2013. The SF State team is monitoring the effectiveness of this restoration on establishment of eelgrass, alone and in combination with oyster settlement substrate (see experimental design, Figures 1 and 2). We have also been working to monitor fish and invertebrate assemblages both before and after project installation as an indicator of the impacts of eelgrass and oyster substrate elements on local wildlife communities and abundances. In addition, we measure a number of other abiotic and biotic conditions in an effort to distinguish the effects of the experimental treatments.

Section 1: Methods

1. 1. Eelgrass Planting: San Rafael

1.1.1 Vegetative Shoots

In July 2012 we transplanted eelgrass to the San Rafael site ('TNC') in the two large plots indicated in Figure 1. A total of 1152 vegetative shoots were collected, 576 from Point Molate ('PM'), and 576 from Point San Pablo ('PSP'), and returned to the Romberg Tiburon Center. The plants were dipped in freshwater (three times for 1 minute) to remove as many invasive invertebrates as possible (Carr et al. 2011), and were then attached to bamboo stakes with twist-ties and burlap (to protect the shoots from abrasion) following a protocol developed by team member Stephanie Kirakopolos (see Boyer and Wyllie-Echeverria 2010). The plants were then stored in flat rectangular tanks in running bay water overnight. The eelgrass shoots were planted at TNC, in a dice formation (5 positions, as in the number five on a die) with 24 plants in each 1.5m x 1.5m unit (four patches of 5 plants in a 0.25-m^2

quadrat, and one center patch of 4; see Figure 3). A total of 48 units of this configuration were planted at the site; 24 were planted in the eelgrass only plot (E) in three rows of 8 units, and the same in between units of oyster shell bags (the eelgrass + oyster plot = E+O). Following the poor success of these transplants, we repeated the transplant effort using this same protocol in April 2013.

1.1.2. Flowering Shoots

In conjunction with vegetative shoot collection in 2012, 740 flowering shoots were collected from the PSP donor (PM had no flowering shoots available). These shoots were placed into mesh bags (15 per bag) and held in tanks of running baywater at the Romberg Tiburon Center. Mesh bags were dipped in freshwater repeatedly to remove epifauna and were attached along with a buoy and rope to the PVC stakes within each eelgrass unit (making a "seed buoy"; Pickerell et al. 2005), approximately two weeks after the vegetative shoots were planted. An extra 20 flowering shoots were collected from each donor site, to be used as a reference for recording flowering stage and seed drop within the eelgrass units. We assessed the site for seedlings prior to the second transplanting effort in spring 2013 and did not observe any new shoots, which indicates the seed bouys did not succeed in establishing seedlings in the plots.

1.2 Eelgrass Planting: Hayward

In August 2012, we collected 200 vegetative eelgrass shoots, 100 from the shoreline adjacent to Bay Farm Island (BFI) in Alameda and 100 from eelgrass patches at Eden Landing Ecological Reserve (ELER) offshore of our study site. These shoots were dipped in freshwater (brought to the field in in tubs) and attached in the field to their bamboo stakes as described above. The vegetative shoots were planted in sets of 20, with five plants in a 0.25-m^2 quadrat, within 1m x 1m units (see Figure 3). Two eelgrass units (n = 40 plants) were planted within each of the five blocks at ELER, one with eelgrass only, and one directly adjacent to an oyster shell bag mound. Seed buoys were not used at this site, as flowering shoots were not available from either donor site. After the original transplant did not succeed (see results), we repeated the transplanting effort at Hayward in May 2013.

1.3 Eelgrass Monitoring

The eelgrass transplanted in July 2012 was monitored in November 2012 and January 2013. This monitoring included densities, shoot heights, epiphyte load, epifauna abundance and diversity, and elemental and isotopic analysis of eelgrass and epiphyte tissues. Density and shoot height monitoring were conducted concurrently, and eelgrass collections were made immediately after these measurements. After the second transplant effort at each site in spring of 2013, we have conducted quarterly monitoring, using the same protocol as before.

1.3.1 Densities

All shoots within each eelgrass patch were counted to give a total shoot density per donor and treatment plot. The number of shoots per genet was also recorded during early monitoring when plants could still be distinguished. Shoot location in relation to bamboo stakes indicated which donor the shoot had originated from and the total number of shoots, including any that had emerged from clonal growth, providing the total shoot number. Additionally, the number of flowering shoots was recorded, and height and the stage of flower or fruit development on spathes, according to de Cock (1980).

In addition to shoot densities, in fall 2012, rhizomes in four of the eelgrass units were assessed by gently digging under the sediment around the bamboo stakes where shoots had previously been planted. This helped to interpret whether missing shoots retained rhizome structure that might still recover.

1.3.2 Heights

The height of the tallest vegetative shoot within each eelgrass patch (and of flowering shoots, if present) was measured to the nearest centimeter, from the sediment to the top, with the plant extended fully upright.

1.3.3 Epifauna, Epiphytes, and Plant Elemental and Isotopic Analyses

Eelgrass collections have been made quarterly to assess epifauna abundance, epiphytic loading, and nutrient and stable isotope composition of the eelgrass and epiphyte tissues. We did not collect any samples in January 2013 as there were very few shoots present at either site.

At TNC, an "exclusion zone" was established within each treatment plot area (Figure 1) to reduce sediment disturbance during monitoring. We did not include this area in our epifauna/epiphyte/nutrient analysis (although we did include it in our density counts described above, by floating over the area on boogie boards when water was over the sediment).

In fall 2012, two portions of selected shoots were collected from both sites, including the top 10 cm of the second and fourth most interior leaves ('leaf 4 and leaf 2'). This subsampling allowed us to avoid removing the entire shoot, thus enabling shoots to continue to grow. At Eden Landing, a total of 29 portions of leaves were collected, including 13 'leaf 4' samples (seven from ELER and six from BFI) and 16 (eight from ELER and eight from BFI) 'leaf 2' samples. At TNC, only three 'leaf 4' (all from PM patches) and three 'leaf 2' samples (also all from PM patches) were collected from the eelgrass-only plot due to low shoot densities. A further 20 'leaf 4' (10 from each donor) and 18 (11 from PSP, and seven from PM patches) 'leaf 2' samples were collected from the eelgrass +oyster plots.

However, in summer 2013, and subsequent quarterly monitoring events, whole shoots were collected due to higher eelgrass densities. Two shoots were collected from each unit of eelgrass (one unit = a patch of 24 plants at TNC and of 20 plants at Eden Landing, see figure 3) at both sites (except those in the exclusion zones at TNC), one from each donor, giving a total of 77 shoots from San Rafael and 20 shoots from Eden Landing. Samples were kept cold after collection and taken back to the laboratory for processing over the following three days. Therefore, all epiphyte and epifaunal assessments from summer 2013 and onward are in relation to the whole eelgrass shoot compared to just leaf 4 in 2012.

To assess epifaunal communities, each leaf 4 sample (in fall 2012) or whole shoot (in summer 2013 and on) was placed onto a 500 μm sieve and subjected to three 1-minute freshwater dips to remove clinging epifauna. Invertebrates removed during the freshwater dips were preserved in 70% ethanol and are identified to the lowest possible taxon according to Carlton (2007), and enumerated (per shoot or sub-sample).

Then, to assess epiphytic loading, leaf 4 of the shoot samples was then gently rinsed again in bay water in a flat-bottomed tray to remove any loose sediment. Using a microscope slide, each sample was scraped into a flat bottom tray until all epiphytes had been visibly removed. The epiphytes were then transferred from the tray (and any from the collection bags) to a pre-weighed microfilter glass fiber filter using a GAST vacuum pressure pump, and filters with epiphytes dried in a 65° C oven for

48 hours to determine dry weights. Leaf 4 or whole shoot samples, with all visible epiphytes removed, were blotted dry and weighed, then dried in a 65° C oven for 48 hours.

To assess %C, %N, %S and C:N of eelgrass as well as the carbon, nitrogen and sulphur stable isotope composition ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) the second youngest leaf ("leaf 2") was removed from each shoot, rinsed in DI water, blotted dry and weighed in a new weigh dish. The leaf 2 samples were then also dried in a 65° C oven for 48 hours. Dry weights of whole shoots and of leaf 2 sub-samples were then recorded. The dried eelgrass tissue and epiphytes samples collected and processed as described above were ground with a mortar and pestle, packed into tin capsules and stored in a desiccator until analysis. Stable isotope analysis and data analysis are described in more details in section 1.7.3 and 1.7.4.

1.4. Invertebrate Monitoring

1.4.1 Minnow and Collapsible Traps

Six pre-construction monitoring plots (spaced approximately 66 m apart) were established along a 330-m sampling transect located 250 m offshore at each site (San Rafael and Hayward), to span the length of the large-scale restoration treatments (n = 6). Plots were sampled once during each of the four quarterly rounds (October 2011, January, April, and July 2012) prior to the treatment implementation in July/August 2012.

At the San Rafael (TNC) site, three post-treatment monitoring plots were established in July 2012 within each of the four treatments (n = 12) distributed evenly within the 330 x 32 m sampling transect (control, eelgrass, oyster and eelgrass plus oyster) ('TNC Treatment Area'). In addition, 12 sampling plots were established in an adjacent 330 x 32 m area ('TNC Control Area') located approximately 20 m directly south of the TNC Treatment Area.

At the Hayward (ELER) site, six post-treatment monitoring plots were established in July 2012, one near each set of trial elements and one at the north end of the entire treatment area ('Eden Treatment Area'). An additional six control plots were established approximately 30 m north of the Eden Treatment Area ('Eden Control Area').

Post-treatment plots were sampled quarterly during eight post-treatment surveys in October 2012, January, April, and July/August, October 2013, and January, April, and July 2014. All plots were sampled within a two-week period during each sampling round. Sampling rounds consisted of three methods: 1) suction sampling (described in Section 1.4.2), 2) minnow traps, and 3) collapsible multi-species traps. All data (including date and time) are recorded on standardized data sheets, and data are entered into an electronic database file.

One minnow trap and one collapsible oval trap (without escape pot) are attached by rope to make one 'two-section' trap array per plot. Each trap is baited with a uniform combination of fish-based bait (1 squid plus 3 anchovies) suspended in mesh bags. Trap arrays are weighted with one half brick, attached to a labeled scientific buoy and deployed for 24 hours within each plot. Upon retrieval, all specimens are immediately identified, sexed (if possible), and measured (carapace width, or body length and body + tail length) in a wet tray to minimize harm. All trap catch is released live immediately after processing, or preserved in ethanol if additional steps are required for identification.

1.4.2 Epifauna by Suction

Suction sampling methods are adapted from previous invertebrate surveys within eelgrass beds conducted by the Boyer Lab. A hand-held, battery-operated aquarium gravel vacuum with a modified opening of approximately 10 mm is used to sample the epibenthic aquatic invertebrates and post-larval crabs (<10 mm) in one 0.5 x 0.25 m quadrat within each plot during low tides (<1.0 m). Suction samples are collected as pairs, with one sample collected from the vertical structure (eelgrass or oyster) or water column (control plot), and one sample collected from the epibenthic layer (either between the eelgrass plants, at the base of the oyster reef, or the mudflat of the control plot).

At the San Rafael site, six paired suction samples are collected from each single treatment (eelgrass only and oyster only) and each of the two control plots. An additional 12 paired suction samples are collected from the eelgrass plus oyster treatment (6 samples for both). Therefore, there are a total of 36 paired samples.

Sampling locations are selected using a random number generator, with each unit per plot being assigned a number from one to 24. Quarterly samples are each collected from a different quarter-section within the unit to avoid disproportionately sampling within a unit. If the selected quarter-section is damaged or otherwise unable to be sampled, then a secondary protocol is implemented to select the next appropriate sampling location.

At the Hayward site, paired samples are collected within every eelgrass unit (n = 5), oyster unit (n=5), eelgrass portion of the eelgrass plus oyster units (n = 5), and oyster portion of the eelgrass plus oyster units (n=5), for a total of 20 paired samples. Additionally, 5 paired samples are collected in the control area.

A section of fine mesh pantyhose is connected to the output of the vacuum, allowing all water to pass through while trapping fine sediments and invertebrates. The mesh is then removed from the output and placed in ethanol to preserve the sample in the field. In the lab the sample is washed through a series of fine sieves (500 µm) to remove the sediment and isolate the invertebrates. The invertebrate sample is then split to ½ using a professional grade sample splitter. Invertebrates are then sorted to the most appropriate taxonomic level and counted under a light microscope.

1.4.3 Epifauna on Eelgrass Shoots

Freshwater dips of eelgrass shoots are another method used to compare epifauna present on the eelgrass when grown alone or in combination with the oyster reefs. Methods are described in section 1.3.3. In addition to the freshwater dips of eelgrass from the San Rafael site, shoots were also collected from the nearest natural eelgrass beds, at Keller Beach in Point Richmond, and at Point Molate, Richmond and subjected to the same protocols to enable comparisons of development of the invertebrate community in the restored site.

1.5 Fish Monitoring

1.5.1 Minnow and Collapsible Traps

Fish are monitored quarterly using the same sampling array and gear as described for invertebrates in Section 1.4.1. Fish captured are identified, measured, and released.

1.5.2. Seining

In May, August and October of 2013 and 2014, seining was carried out at The Nature Conservancy site at San Rafael, and at the natural eelgrass beds at Keller Beach and Point Molate. During an incoming tide, when water reached at least 2 feet in depth, 3 seine transects per treatment plot at TNC and at the whole site at the two natural beds were conducted.

Two people held a seine (1m tall by 2.8 m wide) just above the sediment, at an approximate 45° angle in the water column. A third person stood 30m from the seine, and the seine was swept through the water column along a 30m transect, with the third person preventing animals trapped from swimming back out. Two transects were swept on the eastern length of each plot at TNC, one directly east of the plot and one just beyond the first. A third transect was swept along the western length of each plot to give a total of 12 seine sweeps at the site.

At the end of each transect, the seine was lifted out of the water and all animals caught were enumerated, identified to species and measured. The first 10 individuals of each species were measured, and any remaining were counted but not measured to save time in the field. If any identifications were uncertain then photographs were taken, and/or vouchers were brought back to the lab for verification.

1.5.3. Acoustic Monitoring

1st Year of Acoustic Telemetry – December 22, 2012 through April 30, 2013. An array of 27 Vemco VR2W acoustic receivers was installed at the TNC site to continuously detect the presence and position of any acoustically tagged fish that visited the site during the winter and spring seasons. The array consisted of twenty three receivers that detect 69 kilohertz tagged fish (sturgeons, steelhead, and striped bass) and four receivers that detect 180 kilohertz tagged fish (Chinook salmon). Seven Vemco timing synchronization transmitters were installed at the site to enable precise positioning of visiting tagged fish within each plot.

For comparison, two 69 kilohertz acoustic receivers were deployed during this same period approximately one mile south along the San Rafael Bay shoreline at a smaller oyster/eelgrass restoration project site on subtidal land owned by the Marin Rod and Gun Club.

On April 30, 2013 all receivers were retrieved from the sites. Data was downloaded and analyzed with the VUE software program at SFSU and the positioning data was sent to Vemco for further analysis.

2nd Year of Acoustic Telemetry – December 13, 2013 through June 17, 2014. For our second year of acoustic fish telemetry, we made several changes to the design of the receiver array, increased the duration that the receivers were deployed and added a second smaller nearby comparison site.

We discontinued the use of 180 kilohertz to concentrate on the 69 kilohertz system. We consulted with Vemco, the manufacturer of the acoustic receivers, and based on the Year 1 detection data we determined that we could spread the receivers further apart to expand our range of detection at the site without compromising the ability to determine precise positioning of fish. On December 13, 2013 our team repositioned the 69 kilohertz receivers and two of the synchronization tags. All receivers were retrieved in early February, data was downloaded and the system was confirmed as working properly for fish positioning. All receivers were reinstalled and deployed until June 17, 2014.

1.6 Water Quality Monitoring

1.6.1 Continuous Temperature and Salinity

We deployed Onset HOBO U24-002 conductivity/temperature (CT) data loggers to collect continuous data on salinity and temperature at both the ELER and TNC sites. In November 2012, a total of five CT sensors were placed at the TNC site, one in each of the large-scale plots and one in the large control area (Figure 1). Two days later, five CT sensors were placed at the ELER site, four spaced along the small-scale substrate elements installed in 2012 and one in the large control area outside the substrate element project area (Figure 2). Each CT logger was attached vertically to a 5ft x 4in fiber reinforced plastic rectangular stake via 3/16" stainless steel screws with eyelets, with the sensor approximately three inches from the top of the stake. Copper mesh was used to cover the sensor panel to deter biofouling. The loggers were deployed so that the base is approximately six inches from the sediment. This mooring minimizes CT logger contact against the stakes and reduces the potential for sediment loading on the sensors. Each logger was deployed on the shore side of each oyster structure or eelgrass unit (exact locations will be mapped with GPS), with the sensor facing the shore at both sites. At TNC, the logger in each patch is located just west of the most southern unit in the eastern row.

These sensors record conductivity and temperature continuously and are cleaned when the data are downloaded in the field using a waterproof shuttle. Cleaning and data downloads have been taken every six weeks since the beginning of December 2012.

In April 2013, Onset released a product performance notification about the U24-002 data logger we are using at the sites, stating that the conductivity sensors are not functioning accurately. Due to this technical problem with the loggers, and after looking at the salinity data downloaded from them, we are unsure if we can use all of the salinity data. We can, however, still use the temperature data. Additionally, over time the sensors themselves have become less robust with frequent drying at low tide and biofouling by algae and invertebrates. This has reduced the amount of data available for use, also. Due to these problems, we now take the CT loggers out of the field when downloading the data, to properly clean and re-launch them effectively.

1.6.2. Quarterly Temperature, Salinity, and Dissolved Oxygen

In addition to the CT loggers, we have been measuring salinity, conductivity, temperature and dissolved oxygen every quarter using a handheld YSI 85 instrument. These measurements are made once in each of the treatment plots and in the large control area at TNC, and once in the three most northern plots and the control area at Eden Landing. Measurements are made while holding the sensor just below the surface of the water.

1.6.2. Water Column Chlorophyll-a

We collect chlorophyll a data to determine if treatments influence phytoplankton abundance, thus potentially affecting competition for light and nutrients with eelgrass. Post-construction chlorophyll monitoring commenced in October 2012 and collections have been taken every quarter since then. 15 water column samples are collected from TNC, three from each treatment plot and three from the large control area. Six water column samples are collected from Eden Landing, three from the southern treatment blocks and three from the control area.

Collection vials are acid washed to sterilize before collection. In the field, each vial is rinsed with bay water before being submerged to just below the surface, upturned to remove air bubbles, and then capped while still under the water. The vial is immediately placed in a cooler on ice to keep cold and dark.

Back at the Romberg Tiburon Center, chlorophyll extractions follow the method of Arar and Collins (1992) followed by fluorometry analyses as described by Smith et al. (1981). The Turner Designs model 10 fluorometer used in this study is calibrated annually with a Turner primary (chlorophyll) standard that is serially diluted to obtain a standard curve and coefficients. This fluorometer is occasionally (approximately every other year) cross-calibrated with other fluorometers at RTC (e.g., RTC joint-use Turner Designs 10AU bench top fluorometer).

1.6.3. Light Attenuation

Photosynthetically active radiation (PAR) has been measured quarterly just below the surface and at one-meter depth using a Li-Cor underwater spherical PAR sensor. Measuring light at two depths permits calculations of light attenuation through the water column, which can then be compared among treatments and with other such measures for San Francisco Bay (Zimmerman et al. 1991, Merkel and Associates 2005).

One measure within each large-scale treatment plot were taken at TNC on each sampling date. One measure in each of three of the blocks of the small-scale substrate element experiment at ELER North were also taken, along with an additional three replicate measures in the control plot at each sampling date.

Unfortunately, we were unable to measure PAR in spring and summer 2014, due to the LI-COR PAR sensor malfunctioning. We will replace the equipment in the fall of 2014 to continue our monitoring.

In order to calculate the degree of attenuation, we followed the Bouger-Lambert law which uses the following equation:

$$K(d)=(1/Z_2-Z_1)\ \ln((Ed(Z_1))/(Ed(Z_2)))$$

Where, K(d) = the light attenuation coefficient (higher values indicate less light penetration through the water column), Z_1 = depth 1 (surface = 0m), Z_2 = depth 2 (1m), $Ed(Z_1)$ = PAR reading at depth 1, $Ed(Z_2)$ = PAR reading at depth 2.

1.7 Food Web Interactions

1.7.1. Preliminary Experiment

In May 2014, we conducted a preliminary experiment to be able to refine our initial sampling strategy and study design. The objectives were:

- to determine the appropriate sample mass to use for triple isotope analysis (C,N,S) for the different types of samples
- to evaluate if microphytobenthos (MPB), Particulate Organic Matter (POM), and Sedimentary Organic Matter (SOM) differed significantly in their elemental and stable isotope composition (number of samples and collection/processing methods as described below)

- to assess the effectiveness of carbonate removal from POM and SOM through acidification (necessary in carbonates-rich environments like estuarine ecosystems), and its potential unwanted impact on nitrogen and/or sulphur content and isotopic ratios (using a subset of samples)
- to verify if it was necessary to collect SOM and POM samples from each of the treatments (3 sediment cores and 3 1L bottles of water collected in each treatment plot)
- to assess if collecting POM samples during the flow or ebb would have an impact on their elemental and isotope composition: POM sampling (3 replicates/treatment) was repeated 2h before and 2h after low tide.

The sample preparation and analysis turn-over times only allowed us to receive analyze data for a subset of samples from this preliminary experiment before the next sampling sessions (June and July 2014). Those preliminary results are presented in section 2. Our sampling strategy (as described in 1.7.2) has been adjusted accordingly to keep analytical costs as low as possible.

1.7.2. Sample Collection and Processing

All of the potential organic matter sources likely to fuel the food web at TNC were collected on three occasions over 3 months (May, June and July 2014) before collection of the higher trophic levels. This should allow us to document and eventually account for any temporal variation of the stable isotope composition of these sources over a period matching the consumer tissues isotopic turnover rate (integration time may vary from a few weeks to a few months for muscle tissues of larger, slow growing organisms). Whenever possible, 3 to 5 replicates of each source or consumer in each treatment at each date were collected and prepared for analysis. For some consumer species of particular interest or concern (oysters), up to 10 samples were prepared (and many more back-up samples were archived for later use if necessary) for use in stable isotope mixing models. This should allow for better estimation of the likely percentage contribution of the different sources to their diet.

SOM (Sedimentary Organic matter): Three sediment cores (4cm diameter, 5cm deep) per treatment were collected using PVC corers and either stored frozen at -20°C or directly homogenized and dried at 55°C until further processing. Dried sediments were then ground thoroughly using a mortar and pestle and sieved on a 250µm screen to remove large particles. Carbonates were removed from a 100mg subsample from each replicate by successively adding 1mL HCl 0.5N, evaporating the acid overnight and repeating the operation 2 times with Milli Q water to rinse the subsample.

POM (Particulate Organic Matter): Six replicate water samples were collected every month across the whole experimental setup (1 North, 1 South, and one in each treatment plot) approximately 2 to 3 hours after low tide during the flow by filling 1L Nalgene bottles (previously acid-washed and rinsed 3 times with site water) with subsurface water. The bottles were kept on ice in the dark (in a cooler) until further processing. Back in the lab (within 12h after collection) the water samples were filtered on pre-combusted Whatman GF/F filters (4h at 450°C) and the volume filters was recorded. Two filters were prepared from each 1L bottle and dried at 55°C. Carbonates were removed from replicate samples by placing the filters in a glass desiccator under a vacuum with a beaker of fuming HCl (12N) for 4hours. Dried particulate matter was later scraped out of the filters and packed into tin capsules for analysis.

MPB (Microphytobenthos): MPB was collected at low tide by scraping the surface of the sediment (1-2mm) with a spatula where the biofilm appeared the most concentrated. It was later extracted from the

sediments and concentrated using its migratory behavior, retrieved in filtered water from the site and dried at 55°C.

Eelgrass, epiphytes, algae, sponges and bryozoans: Eelgrass leaves 2 and epiphytes were collected and processed following the protocol described for nutrient and isotope monitoring. Algae, sponges and bryozoans were carefully cleaned in DI water, then dried at 55°C and ground using a mortar and pestle.

Consumers: Crabs, polychaetes, nudibranchs, mussels, oysters, corophiums, other amphipods and isopods were collected from each of the treatments, allowed to clear their guts in filtered water from the site or artificial seawater overnight and frozen at -20°C until further processing. They were then identified at the lowest possible taxonomic level, carefully cleaned in DI water and either dried, ground and packed directly into tin capsules (smaller invertebrates like corophiums, 1 to 40 individuals), or dissected first to extract muscle tissues when possible (larger organisms such as crabs, mussels, and oysters).

1.7.3 Stable Isotope Analysis

The samples were analyzed by Continuous-Flow Isotope Ratio Mass Spectrometry (CF-IRMS) at the Center for Stable Isotope Biogeochemistry at the University of California, Berkeley using an IsoPrime 100 mass spectrometer (Isoprime, Cheadle, UK) interfaced with a CHNOS Elemental Analyzer (vario ISOTOPE cube, Elementar, Hanau, Germany). Stable isotope ratios were expressed as parts per mil (‰) in the standard δ notation relative to Vienna PeeDee Belemnite (V-PDB), atmospheric N2, and Vienna Canyon Diablo Troilite (V-CDT) for C, N and S respectively:

$$\delta X(‰) = [(R\ sample / R\ standard)-1] \times 103$$

Replicate analyses of international standards (NIST-1577b bovine liver, fishmeal and spirulina) gave long-term analytical errors (expressed as standard deviation) of less than 0.1‰ for C, 0.15‰ for N and 0.4‰ for S.

1.7.4 Data Analysis

Elemental and isotopic data were analyzed and graphics were created using the R software (R development Core Team, 2013). Since we were more interested by the source and range of variation in the data rather than “point” values, boxplots were most of the time used as a powerful way to summarize the information through quartiles (Figure 5): a box is drawn around the median from the lower hinge (Q1) to the upper hinge (Q3), with “whiskers” extending to the most extreme data point that is no less than Q1-1.5×IQR (Inter-Quartile Range), or not greater than Q3+1.5×IQR. Data points outside of this range are depicted as outliers (dots).

Normality and homoscedasticity were assessed through a combination of visual methods (qqplots) and tests (Shapiro-Wilk, d’Agostino). When the assumptions for the use of parametric tests were not met, data were either transformed using a modified BoxCox procedure, or analyzed using non parametric tests. For instance, after transformation of the initial dataset, differences between sites, treatments and donors inn the elemental and stable isotope composition of eelgrass leaves and epiphytes were investigated using a nested, fixed factor ANOVA. Acidified (carbonate removal) and non-acidified POM and SOM samples were compared using Wilcoxon signed-rank tests for paired samples. POM samples collected during the ebb and flow were compared using Mann-Whitney U-tests. Differences between POM, SOM and MPB were investigated using Kruskal-Wallis tests.

When all of the stable isotope data will be available, graphical (biplots) and statistical methods (Bayesian stable isotope mixing models) will be used to reconstruct the food web, characterize the diet of some consumers and delineate the trophic relationships between the different organisms.

SECTION 2. Results and Discussion

2.1 Eelgrass

2.1.1. Densities

In fall 2012 and winter 2013, densities of surviving eelgrass shoots were found to be very low at both sites. At TNC, only four shoots were observed in the eelgrass only (E) plot (Figure 6). Of these four shoots, three originated from PM and one from PSP. In addition, one flowering shoot from the PM donor site was observed. In the eelgrass + oyster (E+O) plot, a total of 55 vegetative shoots were present (including shoots emerging from clonal growth), 34 from PM and 21 from PSP, no flowering shoots were observed here.

In winter 2013, densities dropped again with no shoots remaining in the E plot and only 35 vegetative shoots observed in the E+O treatment plot, 24 from PM and 12 from PSP.

Additionally, in the four eelgrass units in the E plot that were assessed for rhizome presence in fall 2012, rhizomes were observed at the base of most stakes (which previously had eelgrass shoots attached), indicating a die off of above-sediment eelgrass biomass. Most shoots were missing from a point below where the meristematic tissue would have been, so regrowth from these rhizomes was not expected. No seedlings were seen in spring 2013 in either of the treatment plots.

Overall, after the first round of transplants, in both treatment plots, PM plants seemed to fare better, and higher plant densities were seen in the E+O treatment than the E treatment in both fall 2012 and winter 2013.

After the repeated transplanting effort in spring 2013, 245 vegetative shoots were observed in the eelgrass only (E) plot (Figure 6) in July 2013. Of these shoots, 151 originated from PM and 94 from PSP. In addition, 31 flowering shoots were observed, 25 from PM and 6 from the PSP donor site. In the eelgrass + oyster (E+O) plot, a total of 220 vegetative shoots were observed. Of these shoots, 128 originated from PM and 92 from PSP. Additionally, 18 flowering shoots were present, 11 from PM and 7 from PSP.

Like the 2012 transplants, in both treatment plots more plants originating from PM were observed. There was a trend of slightly higher density in the E treatment than the E+O treatment (Figure 5).

In fall 2013, there was a very slight decrease in eelgrass density to 242 vegetative shoots in the E treatment (144 PM [+3 flowering shoots], and 98 PSP) but an increase in density in the E+O treatment to 335 vegetative shoots (201 PM plants [+6 flowering shoots], and 134 PSP plants [2 flowering shoots]).

By winter (January) 2014, densities had increased in both treatments, despite some presumed losses due to flowering shoots and other seasonal changes. No flowering shoots were present in winter 2014. There were 602 plants (406 PM and 196 PSP) in the E treatment, and 512 plants (286 PM and 226 PSP) in the E+O treatment.

By spring 2014, densities in the E treatment had increased to 790 plants (534 PM and 256 PSP), and there were also 66 flowering shoots present (35 PM, 31 PSP). However the number of plants in the E+O treatment had dropped to 464 plants (263 PM and 201 PSP), with 35 flowering shoots (20 PM, 15 PSP).

In our most recent monitoring effort in summer 2014, eelgrass densities had increased in the E treatment to 960 vegetative shoots in the E treatment (592 PM, 368 PSP), along with 37 flowering shoots (23 PM and 14 PSP). Eelgrass density had remained relatively constant in the E+O plot at 465 vegetative shoots (232 PM, 233 PSP) and 3 flowering shoots.

In fall 2012 a total of 58 vegetative shoots were observed at Eden Landing (Figure 6), which consisted of 31 from the ELER donor site and 27 from BFI. Three flowering shoots were also observed from the ELER donor site, and one from BFI. Eelgrass units in blocks 3 and 5 (central and most northern block respectively) had the highest densities, with 15 and 30 surviving shoots, respectively (data not shown).

In winter 2013, we saw a slight decrease in vegetative shoot density with a total of 47 shoots observed. 32 of these shoots originated from BFI (a slight increase from the fall 2012 BFI shoot density) and 15 from ELER. No flowering shoots were observed.

After the repeated transplanting effort in spring 2013, a total of 163 vegetative shoots were observed in the plots (Figure 6) in July 2013, which consisted of 85 from the ELER donor site and 76 from BFI. There seems to be little difference in densities between plants from the two donors. Two flowering shoots were also observed: one from the ELER donor site, and one from BFI. There seems to be a trend of slightly higher eelgrass numbers in plots at the most northern blocks versus those in southern plots (data not shown).

Like the 2012 transplants, some eelgrass shoots seemed to be growing away from any bamboo stakes. This suggests some clonal growth has occurred and therefore we hoped further shoots would establish from transplanted parent plants, to encourage a higher eelgrass density.

In fall 2013, 155 vegetative shoots were present at Eden Landing, (65 BFI [+1 flower], 4 EL [+4 flowers]).

By January 2014, no eelgrass had survived at Eden Landing. We are not certain of the cause of the sudden demise of the plants, but it is likely due to a combination of factors. It was noted that the sediment seemed very transient in texture (varying dramatically in short time periods from high sand to high clay content), and many of the bamboo stakes were buried, which indicates high sediment movement and accumulation. Also the very large numbers of *Ilyanassa obsoleta* at the site, which lay their eggs on the eelgrass blades, may have prevented the leaves from reaching up into the water column to receive light during higher tides. We currently have no plans to replant at this site.

Overall, the transplantation effort carried out in spring and monitored in summer 2013 was far more successful than the 2012 transplants. We can calculate an establishment success indicator by comparing the number of shoots originally planted in spring 2013 in each plot with the number of shoots (including clonal growth) present in summer 2013 and summer 2014. In summer 2013, in the E treatment plot at TNC, shoot densities averaged 53.9% of planted densities for the Point Molate donor and 44% for Point San Pablo. In the E+O plot, densities were on average 49.2% of planted densities for PM plants and 49% for PSP. In summer 2014, in the E treatment plot at TNC, shoot densities averaged 201% of planted densities for the Point Molate donor and 127% for Point San Pablo (167%

overall for the eelgrass plot). In the E+O plot, densities were on average 81% of planted densities for both PM and PSP plants (81% overall for the eelgrass + oyster plot). Densities across both treatment plots and donors were 124% of the planted densities.

Looking at Eden Landing survival, in winter 2013 only 47 shoots remained compared to the 200 planted in summer 2012. In summer 2013, however, 163 shoots were present compared to 200 transplanted in spring 2013. Thus, shoot densities were 76% of planted densities for the Bay Farm Island donor and 85% for the ELER donor before the plants disappeared.

2.1.2. Heights

All mean heights refer to the mean tallest height, as the tallest vegetative or flowering shoot in each patch was selected to be measured.

In fall 2012, the mean maximum height of vegetative eelgrass shoots across all plots at TNC was 97.2cm (n=67). In the E plot, mean maximum height of PM plants was 110cm (n=3), and of PSP plants was 53.2cm (n=1). In the E+O plot, the mean maximum height of PM plants was 111.1cm (n=18) and of PSP plants was 114.5 cm (n=15, Figure 7).

In winter 2013, the mean maximum height of vegetative shoots at TNC was 34cm (n=18). No shoots were present to measure in the E treatment. The mean maximum height of PM plants in the E+O plot was 32.9cm (n=12) and of PSP plants was 35.14cm (n=6).

After the replant in spring 2013, the mean maximum height of vegetative eelgrass shoots across all plots in July 2013 at TNC was 125.2cm (n=195). In the E plot, maximum height of PM plants was 127.2cm (n=52), and of PSP plants was 117.4cm (n=48). In the E+O plot, the maximum height of PM plants was 129.3cm (n=55) and of PSP plants was 126.6 cm (n=40) (figure 8).

In fall 2013 shoot heights had increased in both treatment plots to an overall mean of 166.2cm (n=122). In the E plot, the mean max heights of PM and PSP plants were 162.1cm (n=22), and 159.1cm (n=24) respectively. In the E+O plot the mean max heights of PM and PSP plants were 172cm (n= 36) and 171.5cm (n=36) respectively.

In winter 2014, the mean maximum shoot height had reduced by around 50% to 81cm (n=163) across all treatments. In the E plot, the mean maximum height of PM and PSP plants was 85.5cm (n=39) and 76.8cm (n=41) respectively. In the E+O plot the mean maximum height of PM and PSP plants was 76.8cm (n=47) and 81.6cm (n=30) respectively.

In spring 2014, the mean maximum shoot height across all treatments had increased to 157.6cm (n=137). In the E plot the mean shoot height of PM and PSP plants was 188.9cm (n=43) and 187.6cm (n=45) respectively. In the E+O plot, the mean shoot height of PM and PSP plants was less than the EG plot, at 160.2cm (n=39) and 157cm (n=41).

In our most recent monitoring effort in summer 2014, the mean maximum height of eelgrass across all treatments had reduced since spring to 157.6cm (n=137). In the E plot, the mean maximum height of PM and PSP plants was 165.8cm (36) and 166.1cm (n=31) respectively. In the E+O plot the mean maximum height of PM and PSP plants was 150cm (n=29) and 148.3cm (n=36) respectively.

There does not seem to be much variation in height of plants from different donors and treatments at TNC, except a slight trend currently of smaller plants in the E+O plot. This could be due to scouring of the leaves from the shellbag mounds leading to breaking, which has been noticed on some plants.

In fall 2012, the mean maximum height of the tallest eelgrass shoots at ELER was 64.8cm (n=34). The mean maximum height of shoots from the ELER donor site was 61.2cm (n=20), and from the BFI donor was 69.4 cm (n=16, Figure 8).

In winter 2013, the mean maximum height dropped to 32.6cm (n=23). The mean tallest height of shoots from BFI was 32.1cm (n=13) and of those from ELER was 33.2cm (n=10).

After repeating the planting effort in spring 2013, the mean maximum height of eelgrass in July 2013 at ELER was 68.5cm (n=40). The mean maximum height of shoots from the ELER donor site was 70.4cm (n=20), and from the BFI donor site was 66.6 cm (n=20, Figure 8).

In fall 2013, the mean maximum height of plants across all plots was 88.8cm (n=15). Of these, the mean for BFI plants was 85.4cm (n=4) and the mean for ELER plants was 90.2cm (n=10).

The maximum eelgrass shoot height seemed not to vary between the donors at ELER, but overall the tallest plants from this site were on average quite significantly smaller than those at TNC. The high density of *Ilyanassa obsoleta* (an invasive snail species) adults and eggs on the plants weigh them down and may limit the plants' ability to extend into surface waters with greater light. In addition, the shallower depth of the ELER site may negatively influence plant heights.

2.1.3 Epiphytes

Summer 2013 epiphytic loading data represent the loads collected from all leaves, and so they cannot be compared directly to the loading recorded from the 2012 transplants, when only a single, older leaf was collected. Epiphyte load is expressed as a ratio of epiphyte biomass, to eelgrass biomass in grams, with higher ratios indicating a higher load.

At the TNC site in fall 2012, only 3 samples were collected from the eelgrass plot (to preserve remaining shoots), thus epiphyte loading between the eelgrass and eelgrass + oyster treatment could not be compared. There was a trend toward higher epiphyte loads on Point Molate plants compared to Point San Pablo plants within the E+O plot (Table 1, Figure 9).

In summer 2013, there was a trend of higher epiphyte loading in the E+O plot than the E plot (Figure 10, Table 1), but little difference between donors in either plot. Epiphytic loading remained at a similar level relative to eelgrass biomass until our most recent monitoring effort in summer 2014 when we saw an increase in epiphytes in both treatment plots.

Table 1- Average biomass (g) of dry eelgrass, dry epiphyte [plus 95% confidence intervals (95% CI)] and average dry mass of epiphytes per 1g of dry eelgrass, by donor and treatment at TNC. Data are from the fall 2012 and summer 2013 samples. Note that Fall 2012 sampling was of a single, older leaf per sampled shoot, while summer 2013 and on was of whole shoots; thus, comparisons should be made among treatments within dates only.

Treatment	Donor	Fall 2012			Summer 2013		
		Average dry mass of eelgrass leaf #4 (g) [95% CI]	Average dry mass of epiphyte (g) [95% CI]	Average dry mass of epiphyte per g of dry eelgrass tissue (g)	Average dry mass of eelgrass shoot (g) [95% CI]	Average dry mass of epiphyte (g) [95% CI]	Average dry mass of epiphyte per g of dry eelgrass tissue (g)
Eelgrass	PM	0.34[+/- 0.15]	0.79[+/- 0.64]	2.31	1.8 [+/- 0.81]	0.52 [+/-0.08]	0.29
	PSP	N/A	N/A	N/A	1.3 [+/-0.77]	0.47 [+/-0.06]	0.36
	Both donors	N/A	N/A	N/A	1.5 [+/-0.81]	0.49 [+/-0.05]	0.33

Eelgrass +Oyster	**PM**	0.14[+/- 0.07]	0.35 [+/- 0.14]	2.45	1.3 [+/-0.59]	0.76 [+/-0.45]	0.61
	PSP	0.14[+/- 0.06]	0.20 [+/- 0.06]	1.44	1.4 [+/-0.86]	0.94 [+/-0.51]	0.65
	Both donors	0.14[+/- 0.10]	0.27[+/- 0.21]	1.95	1.4 [+/-0.74]	0.85 [+/-0.51]	0.63
	All treatments	0.17[+/- 0.11]	0.34[+/- 0.07]	2.05	1.4 [+/-0.77]	0. 67[+/-0.19]	0.46

Treatment	**Donor**	Fall 2013			Spring 2014		
		Average dry mass of eelgrass shoot (g) [95% CI]	**Average dry mass of epiphyte (g) [95% CI]**	**Average dry mass of epiphyte per g of dry eelgrass tissue (g)**	**Average dry mass of eelgrass shoot (g) [95% CI]**	**Average dry mass of epiphyte (g) [95% CI]**	**Average dry mass of epiphyte per g of dry eelgrass tissue (g)**
Eelgrass	**PM**	1.74 [+/- 0.29]	0.45 [+/- 0.13]	0.256083405	2.62 [+/-0.43]	1.49 [+/- 0.44]	0.567749733
	PSP	1.7 [+/- 0.43]	0.61 [+/- 0.25]	0.359829509	3.03 [+/-0.61]	1.85 [+/- 0.62]	0.611440322
	Both donors	1.72 [+/- 0.24]	0.52 [+/- 0.13]	0.301564964	2.11 [+/- 0.56]	1.05 [+/- 0.53]	0.499751155
Eelgrass +Oyster	**PM**	1.68 [+/- 0.27]	0.56 [+/- 0.17]	0.333304819	1.99 [+/- 0.76]	1.81 [+/- 0.29]	0.910078295
	PSP	1.61 [+/- 0.19]	0.71 [+/- 0.22]	0.439275432	2.15 [+/- 0.43]	1.36 [+/- 0.46]	0.63430278
	Both donors	1.65 [+/- 0.16]	0.64 [+/- 0.14]	0.386558945	2.07 [+/- 0.60]	1.5 [+/- 0.34]	1.256833274
	All treatments	1.68 [+/- 0.29]	0.58 [+/- 0.19]	0.342467645	2.33 [+/-0.70]	1.64 [+/- 0.28]	0.703487943

Treatment	**Donor**	Summer 2014		
		Average dry mass of eelgrass shoot (g) [95% CI]	**Average dry mass of epiphyte (g) [95% CI]**	**Average dry mass of epiphyte per g of dry eelgrass tissue (g)**
Eelgrass	**PM**	2.54 [+/- 0.31]	3.34 [+/- 0.63]	1.313290238
	PSP	2.4 [+/- 0.46]	3.56 [+/- 1.02]	1.483633698
	Both donors	2.72 [+/- 0.41]	3.11 [+/- 0.74]	1.141685951
Eelgrass +Oyster	**PM**	2.06 [+/- 0.26]	2.43 [+/- 0.50]	1.182236406
	PSP	2.09[+/- 0.42]	2.11 [+/- 0.63]	1.011886092
	Both donors	2.03 [+/- 0.32]	2.77 [+/- 0.78]	1.369543045
	All treatments	2.29 [+/- 0.21]	2.88 [+/- 0.42]	1.263329457

For the Eden Landing site, epiphytic loads were lower in summer 2013 compared to fall 2012 also (Table 2, Figure 11). Overall, though, the average dry epiphyte mass was very similar in both years. Compared to the TNC site, there was not a noticeable difference in epiphyte loads at Eden Landing.

Table 2 - Epiphyte: eelgrass biomass (g) ratios by donor at Eden Landing, Hayward.

Donor	Fall 2012			Summer 2013		
	Average dry mass of eelgrass shoot (g) [95% CI]	Average dry mass of epiphyte (g) [95% CI]	Average dry mass of epiphyte per g of dry eelgrass tissue (g)	Average dry mass of eelgrass shoot (g) [95% CI]	Average dry mass of epiphyte (g) [95% CI]	Average dry mass of epiphyte per g of dry eelgrass tissue (g)
BFI	0.13[+/-0.02]	0.06[+/-0.02]	0.44	0.52 [+/- 0.17]	0.17 [+/-0.01]	0.34
ELER	0.15[+/-0.05]	0.05[+/-0.01]	0.31	0.41[+/-0.13]	0.18[+/-0.01]	0.44
Both donors	0.14 [+/-0.03]	0.05[+/-0.01]	0.37	0.46[+/-0.11]	0.18[+/-0.01]	0.39

2.1.4. Elemental and Isotopic Composition Analysis

The stable isotopes $\delta^{13}C$ and $\delta^{15}N$ from fall 2012 leaf 2 samples are plotted in Figure 12. There appears to be a separation of leaf stable isotope composition with EL and BFI donors plotting similarly to each other, and the PM and PSP plants at TNC plotting separately from the ELER site. The signature of the E+O samples at the TNC site appears to plot separately from the EG only samples, a pattern that we intend to explore further in additional samples from later quarters.

Summer 2013 data are plotted in Figures 13 to 16. The largest differences in eelgrass elemental and isotopic composition are observed between sites (nested ANOVA, Table 3), with samples from Eden Landing being consistently enriched in ^{15}N and slightly depleted in ^{13}C (Figures 13 and 14). They also have higher C content and C/N ratios and lower N and S contents than TNC samples (Figure 13). Significant but quite small treatment effects were only observed for C and S content and $\delta^{13}C$, while we did not observe any difference imputable to the initial donor bed (Table 3). All of the eelgrass samples from TNC had relatively homogeneous stable C and N isotopic composition. Local sources of N nutrients, potentially from anthropic origin are likely responsible for the ^{15}N-enriched eelgrass tissues at Eden landing and may provide an explanation for the apparently poorer N incorporation at Eden (significantly lower N content).

Similarly, most of the differences observed in the epiphytes elemental and stable isotope composition were between the 2 sites, with Eden landing epiphytes being enriched in ^{13}C and ^{34}S compared to the one from TNC, and exhibiting higher C and S content overall (Figures 15 and 16). As for the eelgrass shoot, their N content however was significantly lower and their C/N ratio higher (particularly for epiphytes growing on eelgrass originally from BFI, Table 4). Although this time, their $\delta^{15}N$ values do not seem significantly higher than the one measured at TNC.

Table 3: Fixed factor - nested ANOVA results for eelgrass (leaf 2). Significant differences highlighted in grey.

		C (%)	N (%)	S (%)	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	C/N
Site Effect	*Df*	1	1	1	1	1	1	1
	F	7.832	15.326	21.677	10.647	60.381	0.014	27.426
	p value	0.006	0.000	0.000	0.002	0.000	0.908	0.000
Interaction Site:Treatment	*Df*	1	1	1	1	1	1	1
	F	7.608	0.001	21.047	5.499	3.159	0.05	1.483
	p value	0.007	0.976	0.000	0.022	0.079	0.824	0.227
Interaction Treatment:Donor	*Df*	3	3	3	3	3	3	3
	F	0.447	1.338	1.441	2.565	0.315	0.292	1.009
	p value	0.72	0.268	0.237	0.061	0.814	0.831	0.393

Table 4: Fixed factor - nested ANOVA results for epiphytes. Significant differences highlighted in grey.

		C (%)	N (%)	S (%)	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	C/N
Site Effect	*Df*	1	1	1	1	1	1	1
	F	7.82	3.436	5.192	22.023	0.244	11.546	76.037
	p value	0.006	0.066	0.025	0	0.622	0.001	0
Interaction Site:Treatment	*Df*	1	1	1	1	1	1	1
	F	0.272	0.251	0.939	2.977	5.311	1.444	0.466
	p value	0.603	0.617	0.335	0.087	0.023	0.232	0.496
Interraction Treatment:Donor	*Df*	3	3	3	3	3	3	3
	F	2.482	5.424	0.759	1.752	0.539	0.425	0.736
	p value	0.065	0.002	0.52	0.161	0.656	0.736	0.533

2.2. Epibenthic Invertebrates

2.2.1. Minnow and Collapsible Traps

TNC Species Richness

During the baseline sampling prior to treatment, shrimp (*Crangon* sp.) and two crab species, Dungeness crab (*Metacarcinus magister*) and the Oregon shore crab (*Hemigrapsus oregonensis*) were the only invertebrate taxa detected during trapping. Post-treatment, four additional crab species were also detected, European green crab (*Cancer maeanus*), red rock crab (*Cancer productus*), Pacific rock crab (*Romaleon antennarium*), and the northern kelp crab (*Pugettia producta*).

Although these additional species were observed during trapping post-treatment, mean species richness did not significantly increase between baseline (Year 1: 2011-12) and treatment sample periods (Year 2: 2012-13 and Year 3: 2013-14; $p=0.06$, Figure 17). Additionally, no difference was observed between treatments (baseline, control, eelgrass, oyster, and eelgrass + oyster) across sample periods ($p=0.16$; Figure 18).

TNC Abundance

Total invertebrate abundance was not significantly impacted by treatment ($p>0.05$, Kruskal-Wallis MC). Figure 19 illustrates mean abundance of the site during baseline and treatment sample periods.

However, individual species did respond to treatment across sampling periods (Figure 20). Shrimp, the shore crab, and the Pacific rock crab, all showed increases in abundances within some treatment areas as compared to baseline conditions. However the difference between the control plots and treatment plots were not significant. Shrimp abundances decreased from pre-treatment conditions within the eelgrass, oyster, and combination plots ($p<0.01$),with no difference observed between the pre-treatment and control plots. Shore crab abundances increased from pre-treatment conditions in both the combination and oyster plots ($p<0.01$), with no differences observed between the pre-treatment and control or eelgrass. Pacific rock crab increased from pre-treatment conditions in the oyster plots ($p<0.01$). No responses were observed within the remaining crab species.

Eden Species Richness

At ELER, two taxa were observed prior to project implementation. These include the yellow shore crab and the eastern mud snail (*Illyanassa obsoleta*). After project implementation, three additional invertebrate taxa was observed on site, Dungeness crab, Eastern green crab, and shrimp species. Species richness increased sitewide from baseline monitoring (Year 1) to treatment monitoring (Years 2 and 3), as shown in Figure 21. Additionally, a significant difference was observed between the treatment area and both the baseline and control ($p<0.05$), as shown in Figure 22.

Eden Abundance

An increase in abundance was observed between baseline (Year 1) and control and treatment (Years 2 and 3) ($p<0.05$), but no difference was observed between the control and treatment. However, if the most abundant species, the Eastern mud snail, is removed from the analyses, a pattern of increased abundance among the remaining taxa is evident in the treatment plot, as compared to both the baseline and control plots ($p<0.05$). No difference is observed between the control and baseline conditions ($p<0.05$) (see Figure 23).

Invertebrate abundance within the Eden traps was overwhelmingly dominated by the Eastern mud snail, which seasonally measured in the hundreds per trap, an order of magnitude more than other species present. Eastern mud snail abundance increased from baseline to control and treatment, but no difference was observed between control and treatment (see Figure 24). It is possible that this annual variation of the highly abundant species is masking increases in abundance of other taxa. Shore crab abundances increased significantly within the treatment area ($p<0.05$), as compared to both the baseline and the control. No difference was observed between the baseline and control. This increase suggests that shore crab could be responding positively to the treatment at Eden, rather than simply displaying annual variation (see Figure 24). Unlike at the TNC site, shrimp did not respond to treatment at Eden, possibly due to very low overall abundances.

2.2.2. Epifauna by Suction

Baseline (pre-project) invertebrate sampling was conducted quarterly at ELER and TNC in Fall 2011, Winter 2012, Spring 2012, and Summer 2012. Post-treatment monitoring was conducted quarterly in Summer 2013, Fall 2013, Winter 2014, and Spring 2014.

TNC Community Assemblage: Overall Restoration Treatment Enhancements

Correspondence analyses show that overall, the baseline, control, and large control communities (all are mudflat with water column) are similar with extensive overlap. These communities vary from the eelgrass and oyster communities (including eelgrass and oyster from both single and combination plots), which exhibit more overlap with each other than the control plots. Figure 25 displays species and treatments graphed over three factors, with total a cumulative Eigen value of 37.9% (explanation of variation). This pattern suggests that the oyster and eelgrass treatments are supporting different invertebrate communities than the control and baseline communities. This shift is even more evident when assessed within individual seasons (Figures 26-29).

The community similarities between eelgrass and oyster, and their difference between the controls, are especially evident during the summer (Figure 26). During this season, the eelgrass, oyster, control and large control all overlap to some degree with the baseline conditions. However, it is evident that after this year of treatment, the eelgrass and oyster communities have become separate from the controls (three factors displayed with cumulative Eigenvalue of 65.5%). During the fall and winter samples, there is a continued separation of communities, with the eelgrass and oyster showing more differentiation from other (Figures 27 and 28). During these seasons, the controls and baseline communities are strongly similar, but show very little similarity to the eelgrass and oyster communities (Fall: Eigenvalue of 50.9%, Winter Eigenvalue of 51.2%). During the spring, the eelgrass and oyster communities still vary from the controls, but exhibit more overlap than during the other seasons (Eigenvalue 56.5%; Figure 29). It also appears that the baseline community is less similar to all the other post-treatment communities than in any other season, suggesting annual variation across the site, regardless of treatment.

TNC Community Assemblage: Specific Restoration Treatment Enhancements

The previous figures show that eelgrass and oyster structures support invertebrate communities that vary from baseline and control communities, and that overall baseline and control communities are similar. At the TNC site, eelgrass and oysters were planted in single species plots, as well as in a combined plot. These plots were sampled separately to determine whether or not combining eelgrass and oyster habitats supports invertebrate assemblages that differ from single species habitats. The following figures display invertebrate communities separated by individual and combined plots within

the treatment site (Black: Control, Red: Eelgrass, Dark Blue: Oyster, Green: Eelgrass Combo, and Light Blue:Oyster Combo), sampled post-treatment (Summer 2013, Fall 2013, Winter 2014 , and Spring 2014).

Figure 30 displays the community assemblages within specific restoration treatments during the post-treatment sampling year. When all seasons are combined, there is a large amount of overlap between treatments. However, Eelgrass and Eelgrass Combo appear to more closely resemble each other, whereas Oyster and Oyster Combo appear to be more closely related. All treatments overlap partially with the Control. Community differentiations are more evident in individual seasons than across the entire year.

During the post-treatment summer (2013), all treatment communities are separate from the control (Figure 31). For factors 1 and 2, we see a strong overlap between all treatment communities (cumulative 52.5% Eigenvalue), but see some separation between eelgrass and oyster communities with factor 3, with combination communities situated between the two individual communities (factor 3: 12.8% Eigenvalue). This suggests that combination communities may be supporting species assemblages that combine parts of both eelgrass and oyster communities.

During the fall (2013), the eelgrass and eelgrass combination communities appear to vary from each other more than the oyster and oyster combination communities, and also overlap very little with the oyster communities (Eigenvalue 52.4%; Figure 32). This pattern is similar in the winter (2014), though the oyster combination community does overlap with the eelgrass combination community through all three factors (Eigenvalue 55.1%; Figure 33). For both seasons, specific taxa such as caprellids and *Ampithoe valida* appear to persist mainly in eelgrass and eelgrass combination communities which is likely responsible to shifting the composition away from oyster and control communities.

During the winter (2014), the eelgrass overlaps most strongly with the control with the eelgrass combination assemblage more resembles the oyster combination (factors 1:3 Eigenvalue 50.4%) and oyster plots (factors 1:2) (Figure 37). This could be due to seasonal variation of eelgrass specific invertebrates, or the life stage of the eelgrass. It could also suggest that the oyster habitat supports a more consistent invertebrate community throughout the year than eelgrass alone, and that some of this community spills over into eelgrass habitat planted in combination with oysters.

Eden Community Assemblage: Overall Restoration Treatment Enhancements

An overall correspondence analysis assessing invertebrate assemblage across seasons, including baseline and post-treatment conditions, shows that the eelgrass and control communities are very similar to the baseline invertebrate communities (Figure 35). This suggests that the invertebrate communities did not vary greatly between pre-treatment (baseline), and post-treatment (control) years. This also suggests that the eelgrass treatment did support a separate community from the control at Eden. However, the oyster treatment does appear to support a community that varies from the baseline and control (factors 1:3 Eigenvalue 55.1%). It also appears that certain taxa, such as isopods, are more strongly associated with the oyster treatment than the eelgrass or control.

Seasonal assessments suggest a similar pattern (Figures 36-38), though eelgrass communities appear to be slightly more differentiated from the control in Summer 2013, potentially due to the presence of *Ampithoe valida* (Figure 36). The eelgrass was gone from the Eden during the Fall 2013 and Winter 2014 surveys (Figures 37-38). During these months the oyster communities appear to progressively

resemble the control communities, though they are continuing to support specific taxa more than the control, including isopods, caprellids, *A. valida*, and *Corbula amurensis*.

2.2.3 Epifauna on Eelgrass Shoots

Epifaunal invertebrates were collected from the TNC eelgrass restoration plots, as well as Keller Beach and Point Molate natural populations. Point Molate was one of two source populations for eelgrass that was transplanted to the TNC restoration plots. Correspondence analyses of these communities can help display how similar the restored communities are to natural communities, as well as whether or not restored communities maintained similarities to their source populations (though the shoots were stripped of invertebrates before they were transplanted through freshwater rinses).

Figure 39 suggests that the Spring 2014 TNC restored populations ('Eelgrass': eelgrass only plots and 'Combo': eelgrass + oyster combination plots) are more similar to each other than they are to the natural populations ('Natural': Keller Beach and Point Molate). Specific taxa appear to be more closely associated with the natural populations than the restored, including *Idotea resecata* and *Phyllaplysia taylori. B*oth these native species are frequently associated with eelgrass, but have not yet colonized the restored populations to levels that resemble the sampled natural populations.

By removing *I. resecata* from the correspondence analysis, we are able to assess the impacts of less influential indicator taxa on specific population assemblages (Figure 40). This figure suggests that the restored populations, Eelgrass and Combo, do appear to be somewhat differentiated from each other in addition to varying from the natural populations.

These two taxa specifically appear to be correlated with the Point Molate natural populations (PM-N), but do not appear to be supported by the Point Molate sourced plants (PM-R) at the TNC restored site (Figure 41). Additionally, variation between natural populations is shown by the separation of the Keller Beach natural population (KB-N) and the Point Molate natural population. This figure also shows that the TNC restored populations are supporting very similar assemblages. These differentiations by location are more evident when you remove *I. resecata* from the analysis, as shown in Figure 42. This differentiation of communities by the three sample locations (KB, PM, and TNC) suggests that invertebrate communities may be influenced by location of eelgrass site within San Francisco Bay.

2.3. Fish

2.3.1. Minnow and Collapsible Traps

At TNC, a total of 11 individual fish of 5 species were observed during the four quarterly monitoring periods prior to project implementation. During the eight quarterly monitoring periods post-treatment, 42 individuals of 8 species were detected (Table 5). Fish abundance detected during trapping was low, and it is difficult to determine trends from this dataset.

Table 5 – Abundance of fish taxa detected during trapping at TNC prior to project implementation (October 2011- July 2012) and post-treatment (October 2012 - July 2014).

Year 1: Baseline	**Quarter 1 Oct'11**				**Quarter 2 Jan '12**				**Quarter 3 Apr '12**				**Quarter 4 Jul '12**			
	Baseline				**Baseline**				**Baseline**				**Baseline**			
Bay Pipefish																
Jacksmelt											1				5	
Leopard Shark			1													
Pacific Staghorn Sculpin											1					
Shimofuri Goby											2					
Shiner Surfperch											1					
Black Surfperch																
Year 1 Total	**1**				**0**				**5**				**5**			
Year 2: Post-Treatment	**Quarter 1 Oct '12**				**Quarter 2 Jan '13**				**Quarter 3 Apr '13**				**Quarter 4 Jul '13**			
	O	**E**	**OE**	**C**	**O**	**E**	**OE**	**C**	**O**	**E**	**OE**	**C**	**O**	**E**	**OE**	**C**
Bay Pipefish	1									1	1					
Jacksmelt					1			1				1	1			
Leopard Shark												2	1			
Pacific Staghorn Sculpin																
Chameleon Goby					1											
Shimofuri Goby											2	2			1	
Shiner Surfperch																
Black Surfperch							1					1			1	1
Year 2 Total	**1**	**0**	**0**	**0**	**2**	**0**	**1**	**1**	**0**	**1**	**3**	**6**	**2**	**0**	**2**	**1**
Year 3: Post-Treatment	**Quarter 1 Oct '13**				**Quarter 2 Jan '14**				**Quarter 3 Apr '14**				**Quarter 4 Jul '14**			
	O	**E**	**OE**	**C**	**O**	**E**	**OE**	**C**	**O**	**E**	**OE**	**C**	**O**	**E**	**OE**	**C**
Bay Pipefish		1				1					1					
Jacksmelt														1		
Leopard Shark				1				1			2	1	1			
Pacific Staghorn Sculpin									1							
Chameleon Goby																1
Shimofuri Goby			2				2									
Shiner Surfperch												2				
Black Surfperch		2				2										
Year 3 Total	**0**	**3**	**2**	**1**	**0**	**3**	**2**	**1**	**1**	**0**	**3**	**3**	**1**	**1**	**0**	**1**

O= Oyster Treatment; E= Eelgrass Treatment; OE= Combination Oyster plus Eelgrass Treatment; C= Control (both plot control and site control combined)

At ELER, a total of 14 individual fish of 5 species were observed during the quarterly monitoring prior to project implementation (Table 6). During post-treatment quarterly monitoring, 30 individuals of 4 species were detected, which is fairly consistent with baseline levels. One new species was

detected during the post-project monitoring, a sand dab. Fish detection levels at Eden Landing are very low, and therefore pose difficulty deriving statistically significant conclusions.

Table 6 – Abundance of fish taxa detected during trapping at Eden Landing prior to project implementation (October 2011- July 2012) and post-treatment (October 2012 - July 2014).

Treatment	Baseline				Post-Treatment															
Date	Oct '11	Jan '12	Apr '12	Jul '12	Oct '12		Jan '13		Apr '13		Jul '13		Oct '13		Jan '14		Apr '14		Jul '14	
	B	B	B	B	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
Barred Surfperch			1		1	2														
Leopard Shark*	9				4	3			1			2	1		1	3			6	1
Pacific Staghorn Sculpin	1																			
Sevengill Shark	2																			
Topsmelt	1																			
Jacksmelt																			1	
Sand dab										1										
Total	**13**	**0**	**14**	**0**	**8**	**5**	**0**	**0**	**1**	**1**	**0**	**2**	**1**	**0**	**1**	**3**	**0**	**0**	**7**	**1**

*From quarters 2-4, traps were modified to exclude large sharks
T= Treatment; C= Control

2.3.2. Seining

Table 7 shows all species caught so far by seining at TNC. The mean number of fish and invertebrates caught, and the contributions each species made to that mean count per 30m seine transect, per treatment is shown for May 2013 in Figure 43. The control area at TNC caught the highest number of individuals (mean = 53.67 per 30m seine) in May 2013, but had the lowest diversity (3 species), with *Atherinopsis californiensis* (Jacksmelt) contributing the most to this mean count (49.67 mean individuals). The highest diversity (and second highest mean count per seine of 19) was observed in the eelgrass treatment, with 8 species (mean of 6 individuals). The next highest mean count was observed in the eelgrass and oyster combined treatment (mean of 8.67 individuals, 5 species). The lowest mean count per seine was seen in the oyster only treatment (4.33 individuals, 4 species). The Jacksmelt contributed the most to the mean seine count in the eelgrass and eelgrass+oyster treatments, but was not seen in the oyster treatment where the most commonly caught individual was the Bay shrimp (*Crangon franciscorum)*.

The mean number of fish and invertebrates caught, and the contributions each species made to that mean count per 30m seine transect, per treatment is shown for August 2013 in Figure 44. The control area caught the highest number of individuals again (mean = 39.00 per 30m seine), with *Leptocottus armatus* (Pacific Staghorn Sculpin) contributing the most to this mean count (8.67 mean individuals) and 8 species observed in total, which is higher than the diversity in May 2013. The second highest mean count per seine was observed in the eelgrass and oyster combined treatment (mean of 32.67 individuals), and a slightly higher number of species caught (9 species) than the control and the May

2013 seining for the eelgrass + oyster treatment. The most commonly seen species in this treatment was the Bay shrimp again (*Crangon franciscorum,* mean = 12.33). The next highest mean count was observed in the eelgrass treatment (mean = 10 individuals), with 5 species observed (less than May 2013) and the most commonly seen species being the Bay shrimp again (mean = 6.33*)*. The lowest mean count per seine and diversity, as with the May 2013 seine results, was seen in the oyster only treatment (3.67 individuals, 3 species). The Bay Goby, (*Lepidogobius lepidus*) contributed the most to the mean seine count in this treatment (mean = 3).

The results from these seine efforts indicate a lower abundance but higher diversity in some treatments in August 2013 compared to May 2013.

In May 2014, the eelgrass plot saw the higher abundance (mean of 17.7 individuals, and 8 different species, (Figure 45). The oyster plot had the next highest mean abundance (mean of 14 individuals and 6 species). The control plot saw the third highest abundance of individuals (mean of 10 individuals, 6 species). The eelgrass+oyster plot had the lowest abundance (mean of 9.3 individuals, 8 species) of all plots. The most common species caught in all treatments was the bay shrimp, and second common was the Oriental shrimp. Two bay pipefish were caught in the eelgrass plot, which is a good indicator of high quality habitat.

In July 2014, the oyster plot had the highest abundance of individuals (mean of 24 individuals, 8 species, Figure 46). Next abundant was the eelgrass+oyster plot (mean of 14.3, 6 species). The eelgrass plot had the third highest abundance (mean of 8.6 individuals, 3 species) and the control plot had the lowest abundance (mean of 8, 5 species). The most frequently caught species in July 2014 was this time the Oriental Shrimp.

Overall, the seining data show high variation in species richness and abundance across treatments, but a few patterns are worth noting. Bay pipefish were found in the eelgrass within a month of the replanting in April 2013, indicating they were able to quickly recruit to the new habitat. In addition, presence of eelgrass along with oyster reefs (E+O treatment) allowed additional species to be present beyond those found with oyster reefs only, namely bay pipefish, saddleback gunnel, and shiner surfperch. Hence, the seining data suggest that eelgrass planting among oyster reefs will increase the suite of native fish species presence in restoration projects such as this.

Table 7 – List of all species caught by seining so far at TNC.

	Common Name	Latin Name
Fish	Bay Goby	*Lepidogobius lepidus*
	Jacksmelt	*Atherinopsis californiensis*
	Bay Pipefish	*Syngnathus leptorhynchus*
	Pacific Staghorn Sculpin	*Leptocottus armatus*
	Saddleback Gunnel	*Pholis ornata*
	Shiner Surf Perch	*Cymatogaster aggregata*
Crabs	Dungeness Crab	*Metacarcinus magister*
	Bay Shore Crab	*Hemigrapsus oregonensis*
	Slender Crab	*Metacarcinus gracilis*
Shrimp	Bay Shrimp	*Crangon franciscorum*
	Oriental Shrimp	*Palaemon macrodactylus*
	Unknown Shrimp spp.	*shrimp spp. TBD*
Snails	New Zealand Sea Slug	*Philine auriformis*

2.3.3. Acoustic Monitoring

1st Year of Acoustic Telemetry – December 22, 2012 through April 30, 2013. Results from the 69 kilohertz acoustic receivers showed that two White Sturgeons and one out-migrating Steelhead smolt were detected at the TNC site. No fish were detected by the 180 kilohertz receivers.

Sturgeon #47835 - Tagged in 2010, this sturgeon visited the TNC site on March 23, 2013. Almost all detections over the 38 minute visit were recorded by receivers within the oyster shell bag mounds, likely indicating that this fish preferred the areas with structure over the nearby mudflat.

White Sturgeon #56431 – Tagged in 2011, this fish was detected on Feb 20, 2013 at both the TNC site and the smaller Marin Rod and Gun Club. The fish was detected for over 10 hours at the Marin Rod and Gun Club reef, with one brief trip to the Oyster only treatment area of the Living Shorelines reef within that 10 hour period.

Steelhead # 8007 – This smolt from the Nimbus Hatchery was released into the American River on Feb 14, 2013 and visited our site on March 6, 2013. Positioning analysis shows that the fish changed direction from its outward migration path and wandered through all treatment areas over the course of 37 minutes before passing under the Richmond- San Rafael Bridge later that day.

2nd Year of Acoustic Telemetry – December 13, 2013 through June 17, 2014. Preliminary review of the Year 2 data shows that four tagged fish may have been detected at the TNC site with enough detections for fine scale positioning analysis. One of these fish is a Green Sturgeon, listed as a federally-threatened species since 2006. The sturgeon, which was, tagged in Gray's Harbor, Washington, swam through our restoration site on February 23, 2014, likely on its way to spawn in the Sacramento River. Another fish that traveled far to visit our site was a Leopard Shark which was tagged in Elkhorn Slough in early summer 2013. This shark was detected at our site over a two week period beginning May 23, 2014, possibly indicating successful foraging. A tagged Striped Bass was detected on March 11, 22014. We are currently in the process of identifying the fourth fish through collaborations in the California Fish Tracking Consortium. The full data analysis and positioning results for all Year 2 detections is underway.

Nearby at Marin Rod and Gun club our receivers detected three Steelhead smolts passing by on their migration out of the Estuary.

New for Year 2 we deployed three 69 kilohertz receivers almost directly across the Bay at similar depths. Two receivers were deployed attached to pilings on the City of Richmond's Point Molate Pier and one was deployed within bed of eelgrass. Results from these receivers will be included in our next report.

In summary, two years of acoustic fish telemetry monitoring provide evidence that fish of special concern, Sturgeons and Salmonids, are occasionally present at the TNC site. One of the goals of this restoration project is provide improved habitat quality for these species.

2.4. Water Quality

2.4.1 Continuous Temperature and Salinity

Figures 47 to 74 show the monthly average temperature and salinities at TNC since November 2012, and Figures 75-119 show the monthly average temperature and salinities at ELER since November 2012. The continuous temperature data should be useful in exploring seasonal patterns in response

variables measured by multiple groups within the project team. As noted in the methods, Onset announced early on that the conductivity sensors were defective, and this is obvious on many of the plots with large (20+ salinity units) fluctuations on a daily or tidal basis. However, it is possible with comparison to the sonde deployed for periods of time by the physical team (ESA) or other sondes within the vicinity of each project site, that some of the data will be deemed usable.

2.4.2. Quarterly Temperature, Salinity, and Dissolved Oxygen

Temperature (°C), dissolved oxygen (% and mg/l), conductivity (ms), and salinity (ppt) of the water column were taken every quarter from January 2013 at both sites. These readings help indicate any seasonal and inter-treatment changes in water quality and are shown in Tables 8 and 9 for the San Rafael and Hayward site, respectively.

There were no obvious differences between treatments at either site, but ELER was frequently more saline and warmer than TNC.

Table 8 - Temperature (Temp, °C), dissolved oxygen (DO, % and mg/l), conductivity (ms) and salinity (ppt) at The Nature Conservancy site, San Rafael. Due to equipment malfunction, we were unable to take readings in the large control during April 2013.

	Jan-13				
	Oyster	Eelgrass	Eelgrass+Oyster	Small Control	Large Control
Temp (°C)	11.3 C	10.9 C	10.9 C	10.9 C	10.9 C
DO %	94.8	93.5	88.9	90	86.1
DO mg/l	9.05	8.95	8.5	8.59	8.22
Salinity	22.5	22.9	23	22.9	23
	Apr-13				
	Oyster	Eelgrass	Eelgrass+Oyster	Small Control	Large Control
Temp (°C)	18.2	18.2	17.9	18.2	Not taken
DO %	85.2	90.6	83.9	78.2	
DO mg/l	6.962	7.22	6.78	6.53	
Conductivity (ms)	32.26	32.45	32.2	33.03	
Salinity (ppt)	23.6	23.6	23.6	23.6	
	Jul-13				
	Oyster	Eelgrass	Eelgrass+Oyster	Small Control	Large Control
Temp (°C)	24.1	23.6	24.1	24.41	25
DO %	90.2	87.6	92	85.2	91.3
DO mg/l	7.34	7.14	8.12	6.78	7.48
Conductivity (ms)	33.1	32.98	33.45	33.15	32.95
Salinity (ppt)	24.2	24.2	24.3	24.2	24.2
	Oct-13				
	Oyster	Eelgrass	Eelgrass+Oyster	Small Control	Large Control
Temp (°C)	28.1	28.2	28.2	28	28.2
DO %	101.3	100	98.5	97.1	99.6
DO mg/l	8.71	8.48	8.25	8.42	8.46
Conductivity (ms)	35.42	35.23	35.27	35.19	35.6
Salinity (ppt)	15.1	15	14.9	14.9	15
	Jan-14				
	Oyster	Eelgrass	Eelgrass+Oyster	Small	Large

				Control	**Control**
Temp (°C)	**11.4**	**11.4**	**11.4**	**11.3**	**11.7**
DO %	**127.6**	**132.5**	**132.6**	**125.9**	**124.7**
DO mg/l	**10.76**	**16.11**	**12.11**	**11.61**	**11.49**
Conductivity (ms)	**31.8**	**31.4**	**31.8**	**42.8**	**31.93**
Salinity (ppt)	**27.5**	**27.7**	**27.7**	**27.4**	**27.5**
	Apr-14				
	Oyster	**Eelgrass**	**Eelgrass+Oyster**	**Small Control**	**Large Control**
Temp (°C)	**20.3**	**20.9**	**20.4**	**21.5**	**21.4**
DO %	**123.1**	**127.4**	**126.3**	**124.2**	**123.2**
DO mg/l	**9.16**	**9.75**	**9.54**	**8.98**	**9.45**
Conductivity (ms)	**36.5**	**35.1**	**36.2**	**36.7**	**36.2**
Salinity (ppt)	**26.4**	**25.9**	**26.3**	**26.7**	**26.5**
	Jul-14				
	Oyster	**Eelgrass**	**Eelgrass+Oyster**	**Small Control**	**Large Control**
Temp (°C)	**21.1**	**21.1**	**20.8**	**20.8**	**20.7**
DO %	**77.8**	**79.4**	**79.4**	**78.3**	**78.7**
DO mg/l	**7.38**	**5.97**	**5.88**	**5.79**	**6.14**
Conductivity (ms)	**42.71**	**42.61**	**42.23**	**42.17**	**41.99**
Salinity (ppt)	**29.9**	**46.02**	**29.8**	**29.8**	**29.6**

Table 9 - Temperature (Temp, °C), dissolved oxygen (DO, % and mg/l), conductivity (ms) and salinity (ppt) measured from treatment plots 1, 2 and 3 (1 being the most northern) and the control area of Eden Landing Ecological Reserve site, in Hayward.

	January 2013			
	Plot 1	Plot 2	Plot 3	Control Area
Temp (°C)	12.4	12.1	12.3	12.2
DO (%)	100.9	101.4	100.6	103.3
DO (mg/l)	9.95	9.95	9.8	9.55
Conductivity (ms)	26.54	26.57	26.65	26.97
Salinity (ppt)	22.2	22.2	22.3	22.3
	April 2013			
	Plot 1	Plot 2	Plot 3	Control Area
Temp (°C)	18.6	20.3	19.2	19.5
DO (%)	93.4	91.5	90.1	92.5
DO (mg/l)	7.21	7.15	7.05	7.24
Conductivity (ms)	31.79	35.8	33.2	32.5
Salinity (ppt)	23	25.1	24	23

	July 2013			
	Plot 1	Plot 2	Plot 3	Control Area
Temp (°C)	24.8	24.9	25.1	25.3
DO (%)	91.4	90.3	88.6	89.7
DO (mg/l)	7.09	7.06	6.98	7.01
Conductivity (ms)	34.8	33.6	32.9	33.5
Salinity (ppt)	24	25	24.9	25
	October 2013			
	Plot 1	Plot 2	Plot 3	Control Area
Temp (°C)	26.1	27.2	27.1	26.9
DO (%)	96.2	94.3	95.8	96.3
DO (mg/l)	8.61	8.42	8.53	8.63
Conductivity (ms)				
Salinity (ppt)	18.6	19.3	17.5	16.3
	January 2014			
	Plot 1	Plot 2	Plot 3	Control Area
Temp (°C)	13.6	13.9	13.4	13.7
DO (%)	100.1	99.8	101.2	98.9
DO (mg/l)	8.65	8.57	8.71	8.41
Conductivity (ms)	45.62	46.14	45.24	45.23
Salinity (ppt)	29.6	29.8	29.4	29.3
	April 2014			
	Plot 1	Plot 2	Plot 3	Control Area
Temp (°C)	16.4	16.2	16.7	15.9
DO (%)	88.6	89.5	91.3	88.4
DO (mg/l)	7.3	7.51	7.79	7.24
Conductivity (ms)	43.7	42.8	43.7	42.9
Salinity (ppt)	28.3	28.1	28.3	28.2
	July 2014			
	Plot 1	Plot 2	Plot 3	Control Area

Temp (°C)	26.4	26.5	26.4	26.3
DO (%)	90.2	87.5	88.3	90.1
DO (mg/l)	6.35	6.16	6.21	6.34
Conductivity (ms)	34.8	33.6	32.9	33.5
Salinity (ppt)	24.7	24.7	24.8	24.9

2.4.3 Water Column Chlorophyll-a

Initially, samples from the ELER showed higher Chlorophyll-a concentrations (Table 11) than those from TNC (Table 10) (an average of 15.1 ug/L at ELER and 7.5 ug/L at TNC) (Figures 120-121), perhaps indicating somewhat higher phytoplankton abundance at ELER. However, over time the Chlorophyll-a levels at ELER dropped, and were at similar levels at both sites. In 2013, there was a large increase in Chl-a at TNC in fall, and the Chl-a levels in summer 2014 had increased in most treatments so this may be a recurring pattern of phytoplankton blooms in the fall following increasing water temperatures during the summer months.

Table 10 – Chlorophyll-a extractions from water column samples in treatment and control areas at both ELER and TNC.

Year	Season	Plot	Average Chlorophyll (ug/l)	Standard Deviation	95% CI
		Oyster	7.98	1.24	1.41
		Eelgrass	7.81	2.14	2.42
		Eelgrass+Oyster	6.75	0.44	0.50
		Small Control	9.51	2.12	2.40
2012	Fall	Large Control	5.77	1.06	1.20
		Oyster	5.92	1.00	1.13
		Eelgrass	2.64	0.53	0.59
		Eelgrass+Oyster	6.71	0.14	0.16
		Small Control	3.17	0.63	0.71
	Winter	Large Control	3.84	1.26	1.43
		Oyster	3.44	0.33	0.37
		Eelgrass	2.31	0.66	0.75
		Eelgrass+Oyster	3.42	1.03	1.16
		Small Control	2.34	0.11	0.13
	Spring	Large Control	2.82	1.39	1.57
		Oyster	5.93	1.05	1.19
		Eelgrass	3.17	0.74	0.83
		Eelgrass+Oyster	4.69	0.59	0.67
		Small Control	3.73	2.36	2.67
	Summer	Large Control	3.35	0.34	0.39
		Oyster	13.88	5.88	6.65
		Eelgrass	9.58	1.99	2.25
		Eelgrass+Oyster	12.28	1.46	1.65
		Small Control	8.16	7.96	9.00
2013	Fall	Large Control	12.37	1.26	1.43
		Oyster	1.13	0.21	0.24
2014	Winter	Eelgrass	1.08	0.43	0.49

		Eelgrass+Oyster	1.10	0.92	1.04
		Small Control	1.15	0.23	0.26
		Large Control	1.21	0.47	0.53
		Oyster	0.68	0.77	0.87
		Eelgrass	0.57	0.59	0.67
		Eelgrass+Oyster	1.10	0.92	1.04
		Small Control	1.15	0.23	0.26
	Spring	Large Control	1.21	0.47	0.53
		Oyster	5.24	0.18	0.21
		Eelgrass	4.24	2.81	3.18
		Eelgrass+Oyster	2.86	1.68	1.90
		Small Control	1.67	0.89	1.01
	Summer	Large Control	3.42	2.86	3.23

Table 11 – Chlorophyll-a extractions from water column samples in treatment plots and control areas at ELER

Year	Season	Treatment	Average Chlorophyll (ug/l)	Standard Deviation	95% CI
2012	Fall		13.41	3.61	4.09
	Winter		10.63	0.42	0.479
	Spring		3.60	0.65	0.74
	Summer		8.36	0.67	0.76
2013	Fall		3.18	0.32	0.36
	Winter		6.10	3.30	3.73
	Spring		2.82	0.65	0.74
2014	Summer	Plots 1-5	4.53	4.2	4.76
2012	Fall		16.80	1.06	1.20
	Winter		8.31	1.96	2.22
	Spring		3.95	0.44	0.50
	Summer		5.912	2.29	2.59
2013	Fall		4.12	1.43	1.62
	Winter		3.96	1.99	2.25
	Spring		2.96	1.13	1.28
2014	Summer	Control	5.24	1.57	1.78

2.4.4 Light Attenuation

Tables 12 and 13 below show the PAR readings at both sites since October 2012. We used these data to calculate the attenuation in the water column, which then indicates the amount of light reaching organisms at different depths. Figures 122-123 show the light attenuation coefficients in each season at TNC and ELER, respectively. Due to equipment malfunction, there were no readings taken in the large control at TNC in April, or in two of the control area sample positions at TNC.

There were no clear differences in light attenuation between treatments at TNC, but there were seasonal changes in turbidity of the water column. At ELER, there was more variation among treatments in light attenuation (Figure 123), but no consistent patterns by treatment over time.

Table 12 – PAR measured at the Nature Conservancy site in San Rafael every quarter since October 2012. Units are micromoles of photons per second per square meter (µmol s^{-1} m^{-2}).

Treatment	Depth	Fall 2012	Winter 2013	Spring 2013	Summer 2013	Fall 2013	Winter 2014
	Air	1079	2731	2844	2857	2541	2676
	Surface	498	1776	2123	2141	1895	1894
OY	1m	148	430	114	153	111	765
	Air	1172	2607	2981	2647	2612	2676
	Surface	502	1789	2570	2003	1956	1738
EG	1m	125	341	174	125	102	706
	Air	1853	2643	2964	2956	2654	2676
	Surface	783	1489	2107	2114	1745	1519
OE	1m	215	341	71	124	96	638
	Air	2012	2675	2942	2884	2457	2676
	Surface	1101	1425	2249	2145	2003	1719
SC	1m	282	409	73	175	104	541
	Air	1030	2642		2965	2584	2652
	Surface	443	1796	Not	2101	1895	1756
LC	1m	125	325	taken	153	112	497

Table 13 - PAR measured at Eden Landing, Hayward every quarter since October 2012. Units are micromoles of photons per second per square meter (µmol s^{-1} m^{-2}).

Treatment	Depth	Fall 2012	Winter 2013	Spring 2013	Summer 2013	Fall 2013	Winter 2014
	Air	2475	2587	2966	2958	2241	2658
	Surface	2020	1283	1986	1874	1965	1452
Block 1	1m	105	233	314	204	168	78
	Air	2568	3000	3958	2485	2547	2475
	Surface	1980	2182	2008	1564	1354	1452
Block 2	1m	211	123	163	125	114	136
	Air	2486	3981	3457	2457	2664	2457
	Surface	2018	2056	1658	1658	1236	1356
Block 3	1m	273	554	199	124	148	102
	Air	2555	3970	3015	2145	2688	2589
	Surface	1995	1850	1895	1124	1457	1356
Control 1	1m	173	112	96	89.3	116	117
	Air	2645		3742	2547	2469	2569
	Surface	2050		2561	1548	1655	1458
Control 2	1m	202	Not	189	102	187	154
	Air	2640	taken	3566	3002	2589	2569
	Surface	1836		2154	1758	1455	1547
Control 3	1m	269		158	354	124	105

2.5 Food Web Interactions

As of September 2014, all of the samples have been collected and 95% of them have been processed and prepared for analysis. Some are already out for analysis at the UC Berkeley stable isotope facility

with more to follow shortly. Data analysis will occur shortly after reception of the data, and results concerning the food web component can be expected by early 2015.

We present here some preliminary results and methodological considerations based on the "sources" sampling that took place in May 2014.

Carbonate removal effect: As expected, carbonate removal from the SOM and POM samples proved useful, with significant differences detected between the $\delta^{13}C$ values of acidified and non-acidified samples (up to ~1‰ for SOM, Fig. 124). Although not quite as large, significant differences in $\delta^{15}N$ were also detected for sediment samples, highlighting the necessity to conduct a separate analysis on non-acidified samples for N and probably S (unfortunately we did not have enough data to assess the impact of acidification on S). The impact of acidification appeared slightly less acute on POM samples, probably due to the less aggressive acid-fumes technique. But despite the absence of significant differences in $\delta^{15}N$ (likely due to the small sample size and lack of power of the test), we cannot definitely rule out unwanted effects (see Fig. 125) and will keep analyzing separate unacidified replicates for N and S.

Tide and Treatment Effect on POM: Although a more complete set of samples was initially collected, only a limited number of these samples have been analyzed so far due to unexpected difficulties in finding the appropriate mass to submit for analysis (for a given mass, some samples would quickly saturate in S while some other would be too low in N). While it (logically) seems that POM composition could vary with the tide (different water masses sampled), our small sample size only allowed us to detect significant differences in C content (Fig. 126). Similarly, most of the POM samples did not differ significantly between treatment, while the 3 replicates from the same treatment sometimes exhibited variations of equivalent or even higher magnitude (Fig. 127, only samples collected during the flow used here). Although a detailed biogeochemistry study aiming at characterizing the POM dynamic over a tide cycle would be interesting, it is not the focus of the current project. Since water masses (and thus POM) are constantly flowing across all treatments through the whole experimental setup, we decided to limit analytical costs and adjust the sampling strategy to collect only 6 replicates overall, only during the flow (water masses in which the oysters and other organisms are likely to feed).

Treatment Effect on SOM: Although once again the small sample size for the preliminary experiment did not allow for high power when testing for differences between treatments (no significant difference detected at $\alpha=0.05$), the two oyster plots seem to have higher sulfur content sediments (2-3 times higher in E+O than E and 10 times higher than the control plot, Fig. 128). The stable isotope composition of C and N does not appear to vary much between treatments (<0.5‰ overall for both C and N). More samples will be analyzed soon and will help us eventually confirm those observations.

Differences between POM, SOM and MPB: Although previous work in other locations in the San Francisco Bay had found no difference between the stable isotope composition of sediments, benthic microalgae and suspended particulate organic matter (Levin et al., 2006), microphytobenthos in our study appeared extremely different from SOM and POM (Fig. 129, 130). It logically had much higher C and N and S content, but was also extremely ^{13}C-, ^{15}N- and ^{34}S-enriched (-16‰ to -17‰ compared to -23‰ to -24‰; 13‰ vs 7-8‰; and 15-20‰ vs -15‰, respectively). A likely explanation for this isotopic enrichment in ^{13}C, ^{15}N and ^{34}S could lie in the decrease in fractionation due to boundary-layer effects (France, 1995). Differences between SOM and POM were often smaller, at least in their stable isotope composition.

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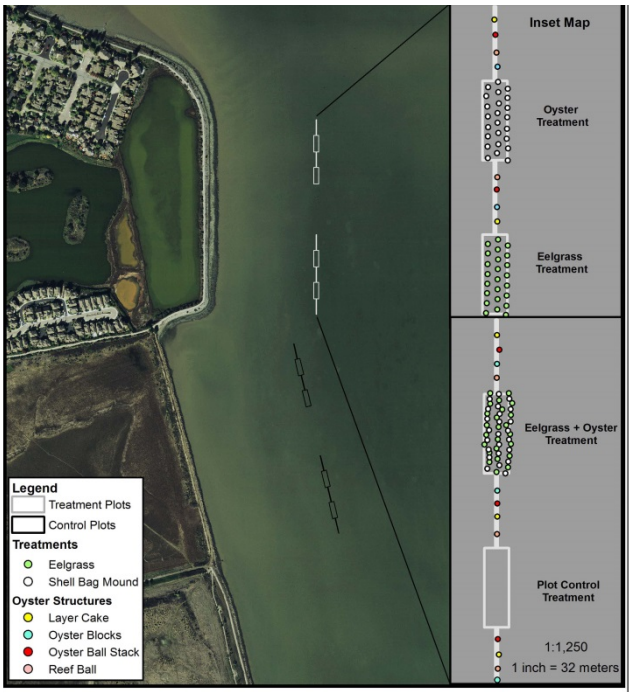


Figure 1. Map showing the location and orientation of plots at The Nature Conservancy (TNC) site in San Rafael Bay.

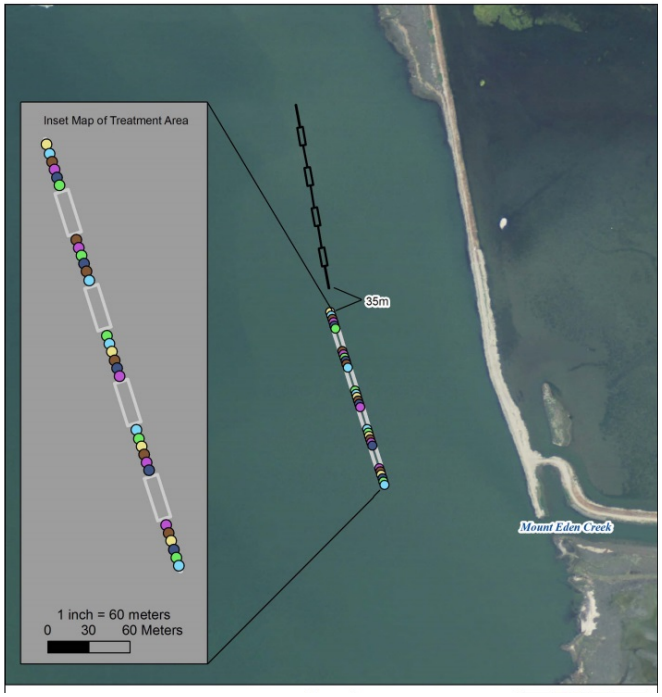


Figure 2. Map showing the location and orientation of plots at the Eden Landing Ecological Reserve (ELER) site in Hayward.

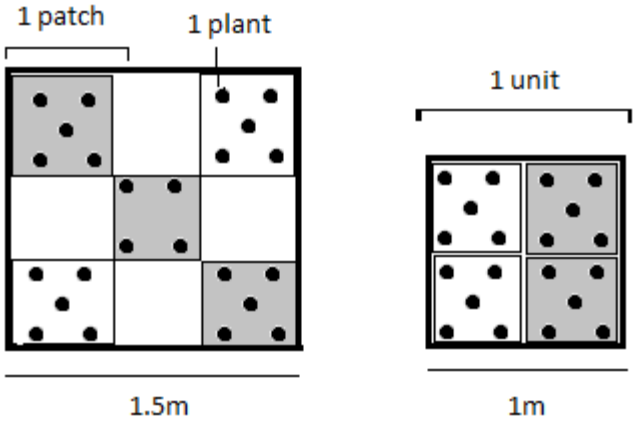
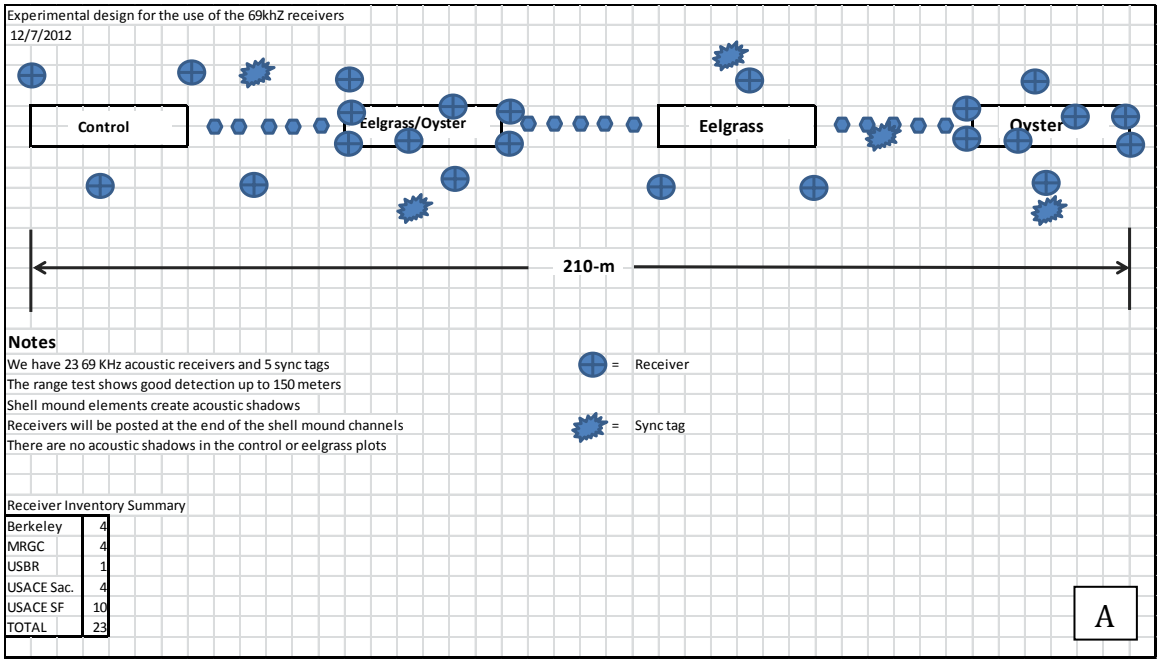


Figure 3. Planting schematic for eelgrass units at TNC (left) and ELER (right). Shading indicates the two different donors. The central patch of eelgrass at TNC alternated between Point Molate (PM) and Point San Pablo (PSP) to provide 12 PM-dominated and 12 PSP-dominated units in each plot (24 total in both the Eelgrass and Eelgrass+Oyster treatment plots). Patches are numbered 1 to 5, ascending in a clockwise direction with 5 being the patch in the center. Each ELER unit contained 10 plants from Bay Farm Island and 10 from ELER (20 total).



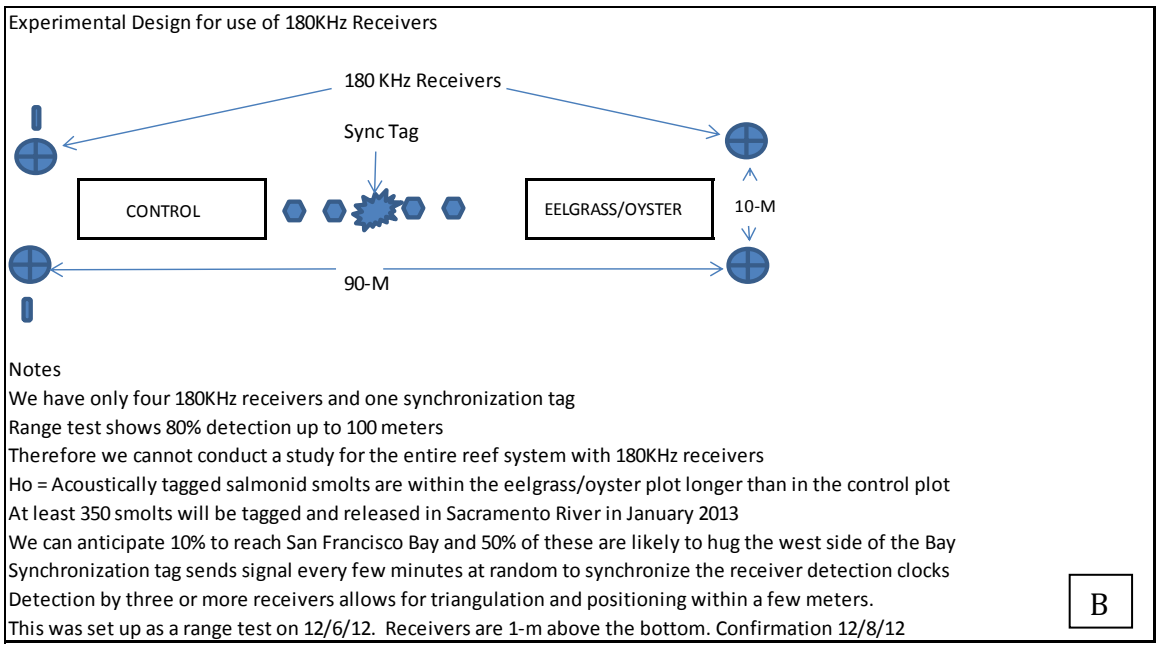


Figure 4. A) Schematic of the locations of 27 Vemco acoustic receivers for fish monitoring at the TNC site, and B) Detailed schematic of the design of sync tag and acoustic receiver layouts between the Eelgrass+Oyster treatment and control. Drawings courtesy of Bud Abbott.

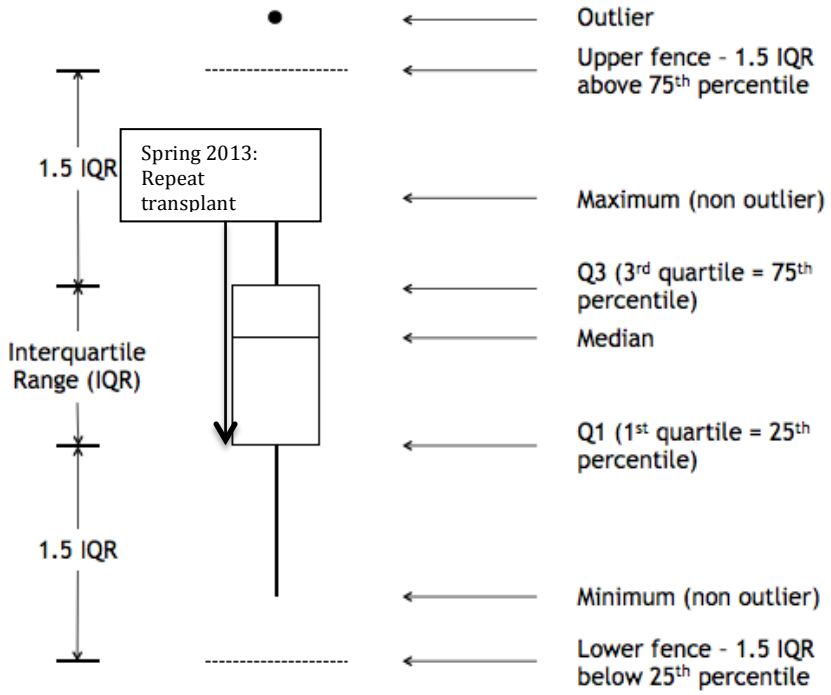


Figure 5. Distribution of data within a boxplot, for reference when reading the isotope and invertebrate figures that display data in this manner.

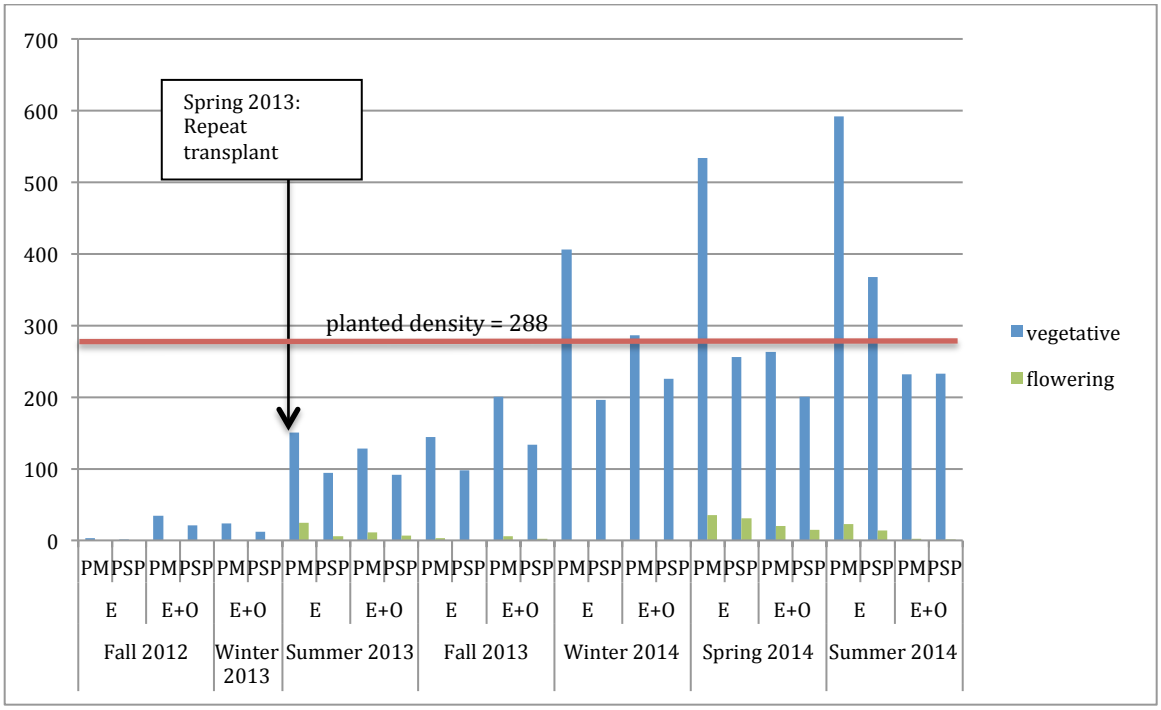


Figure 5. Total number of vegetative and flowering eelgrass shoots present, per donor and treatment plot at TNC site, quarterly through summer 2014. E = eelgrass plot, E+O = eelgrass and oyster plot, PM = plants from the Point Molate donor site and PSP = plants from the Point San Pablo donor site.

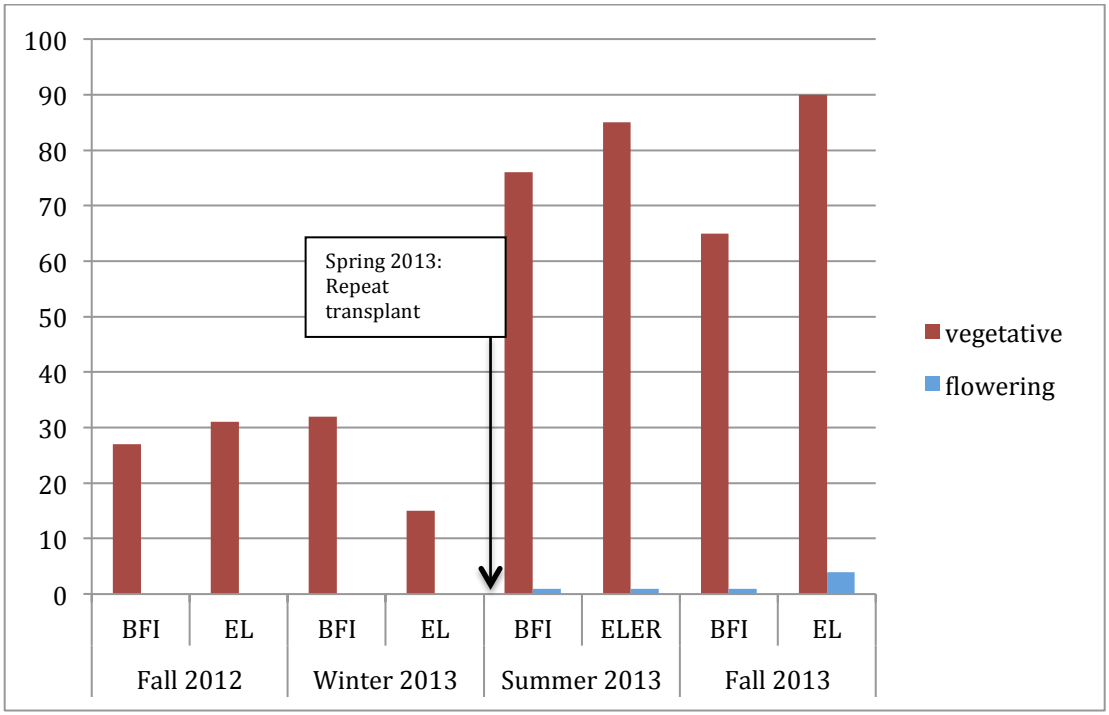


Figure 6. Number of vegetative and flowering shoots by donor at ELER site, Hayward, quarterly through fall 2013 (after which plants were essentially gone). BFI = plants from the Bay Farm Island donor site and ELER = plants from the Eden Landing Ecological Reserve site.

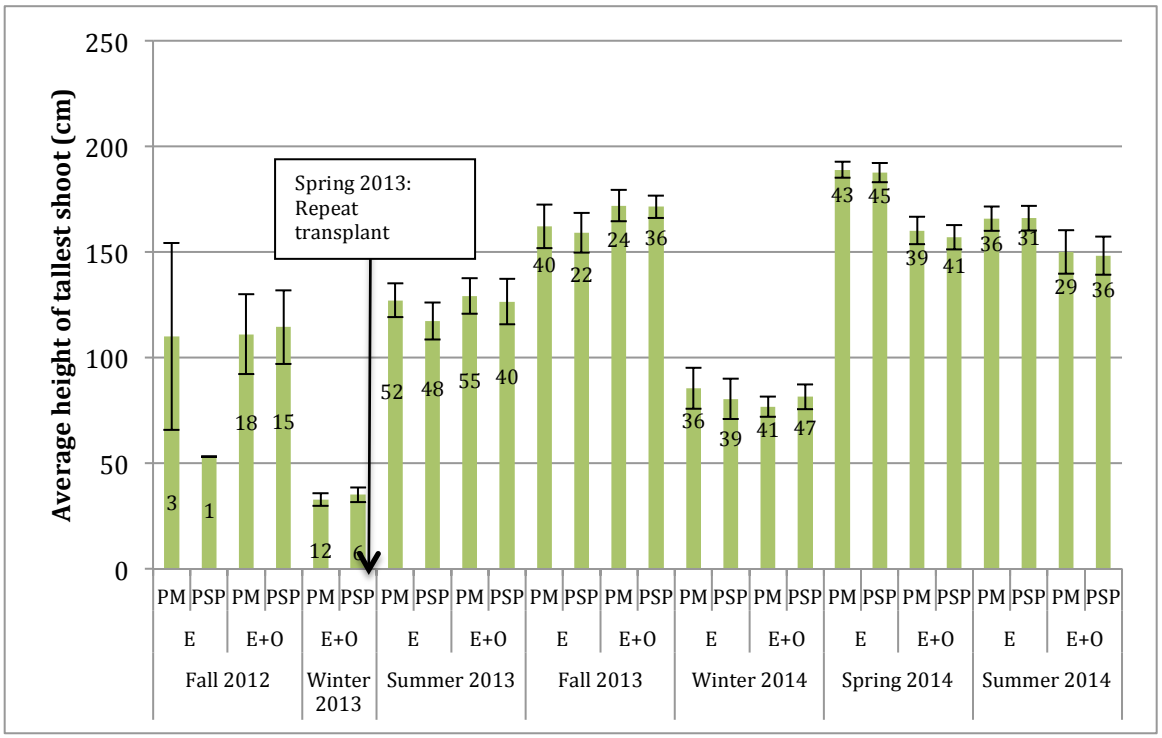


Figure 7. Average height of the tallest eelgrass shoot in each patch, by donor and treatment plots at The Nature Conservancy site at San Rafael in each quarterly monitoring effort. E = eelgrass only plot, E+O = eelgrass and oyster substrate plot, PM = plants from the Point Molate donor site and PSP = plants from the Point San Pablo donor site. Numbers on columns indicate the sample size. Error bars = 95% confidence intervals.

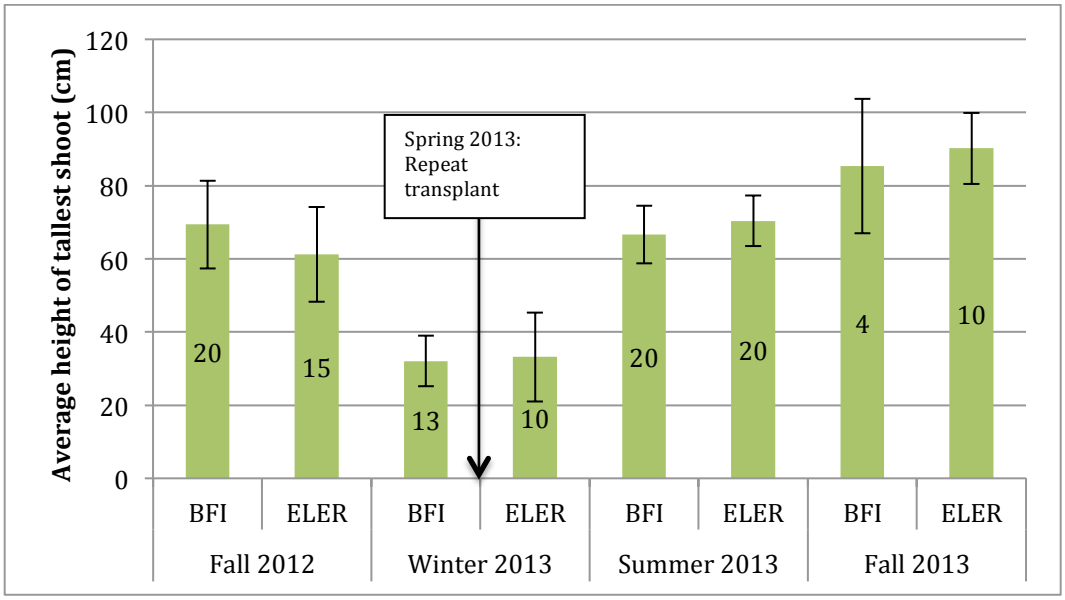


Figure 8. Average height of the tallest eelgrass shoot in each patch, by donor at Eden Landing, Hayward. BFI = plants from the Bay Farm Island donor site and ELER = plants from the Eden Landing Ecological

Reserve site in fall 2012, winter 2013 and summer 2013. Numbers in columns indicate the sample size. Error bars = 95% confidence interval.

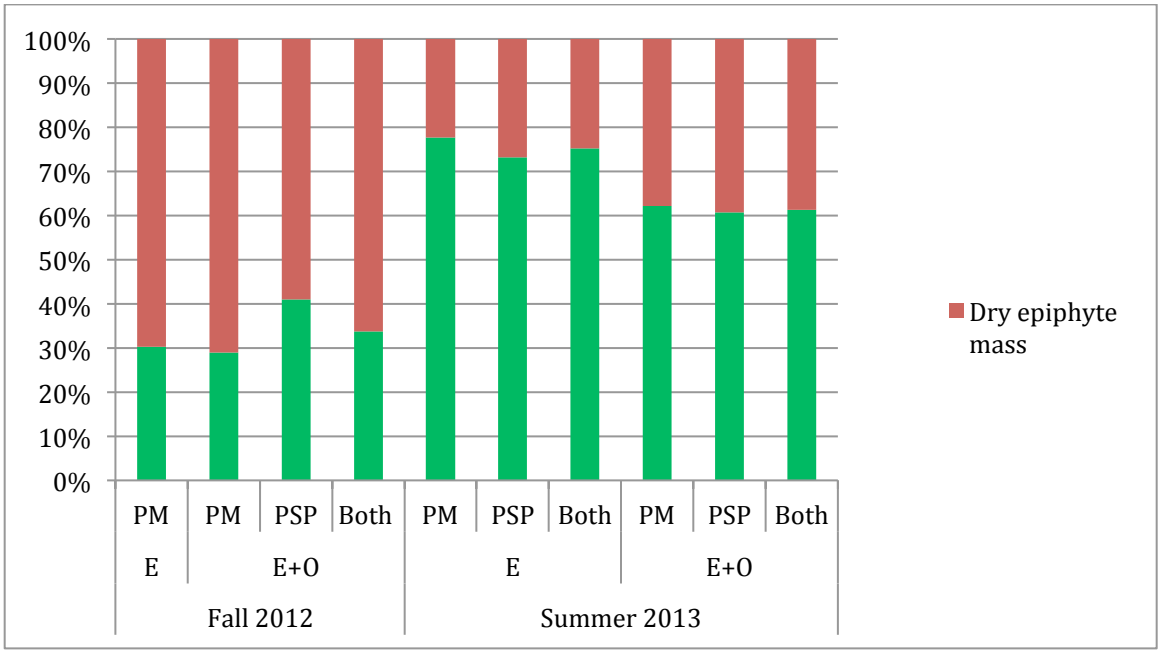


Figure 9. Mean epiphyte load, shown as a percent of dry mass, collected at The Nature Conservancy site in fall 2012 and summer 2013. E = eelgrass plot, E+O = eelgrass + oyster plot, PM = plants from Point Molate and PSP = plants from Point San Pablo. Fall 2012 samples included only leaf #4 to avoid removing whole plants when there were few. Leaf #4 is an older leaf and likely to have proportionally greater epiphyte biomass per g of eelgrass biomass than would whole shoots, as were collected in summer 2013.

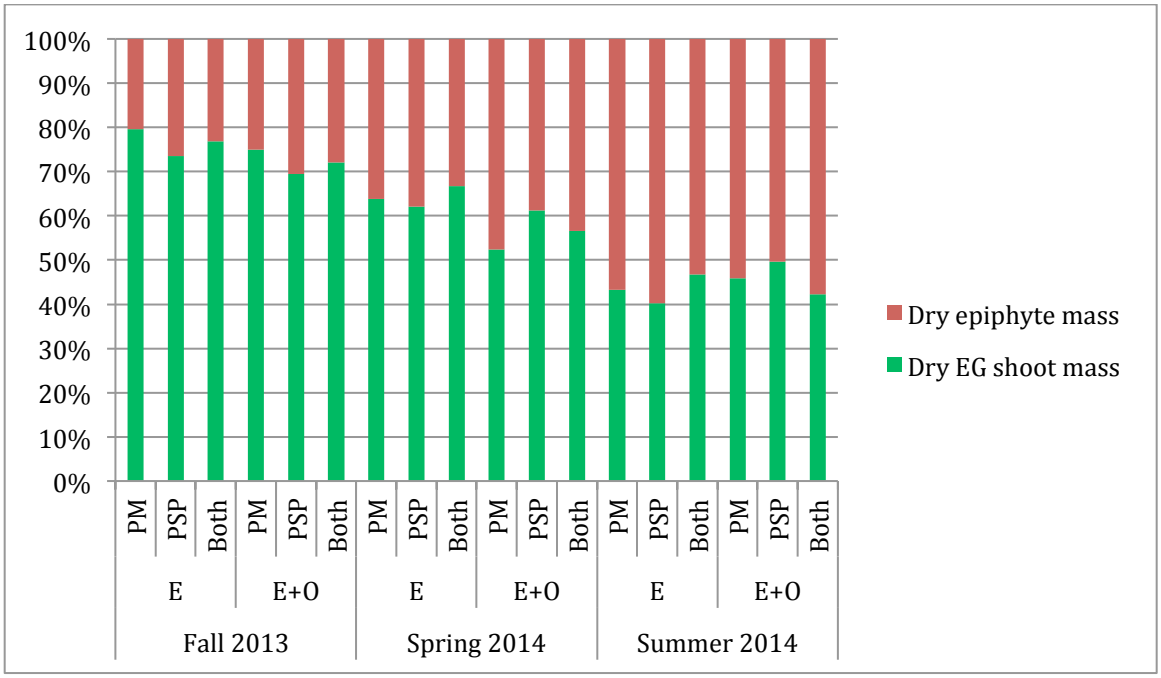


Figure 10. Mean epiphyte load, shown as a percent of dry mass, collected at The Nature Conservancy site in fall 2013, spring 2014 and summer 2014. E = eelgrass only plot, E+O = eelgrass + oyster substrate plot, PM = plants from Point Molate and PSP = plants from Point San Pablo.

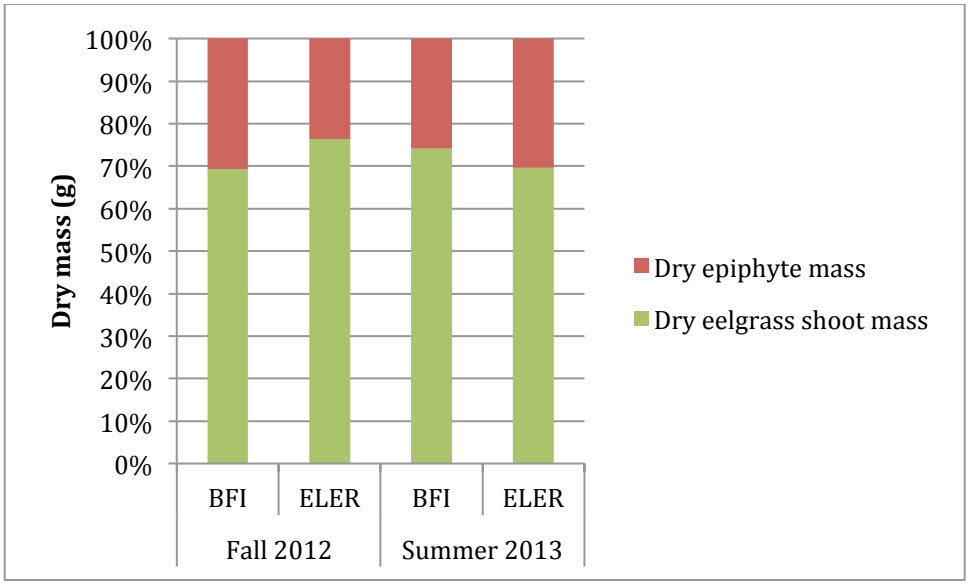


Figure 11. Mean epiphyte load, shown as a proportion of the total sample weight from samples at Eden Landing, Hayward, in fall 2012 and summer 2013. BFI = plants from Bay Farm Island and ELER = plants from the Eden Landing Ecological Reserve. Fall 2012 samples included only leaf #4 to avoid removing whole plants when there were few. Leaf #4 is an older leaf and likely to have proportionally greater epiphyte biomass per g of eelgrass biomass than would whole shoots, as were collected in summer 2013.

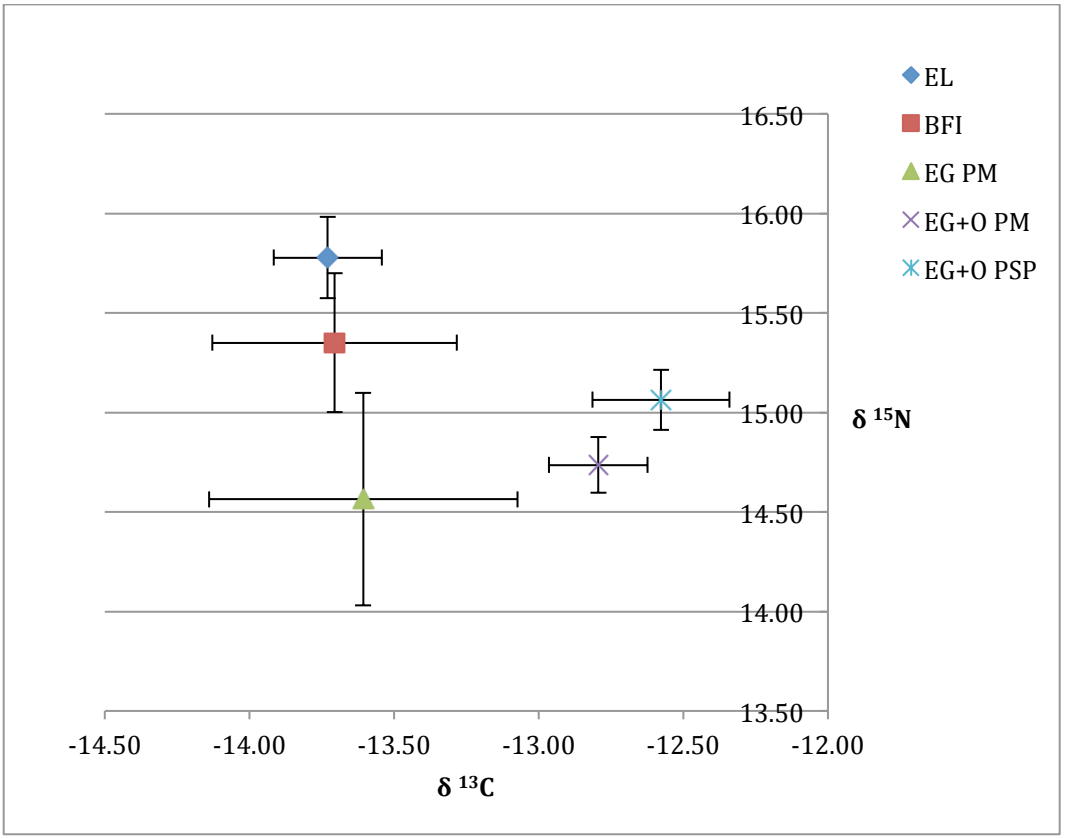


Figure 12. $\delta^{13}C$ plotted against $\delta^{15}N$ for leaf 2 subsamples, collected in fall 2012. Error bars = 95% confidence intervals.

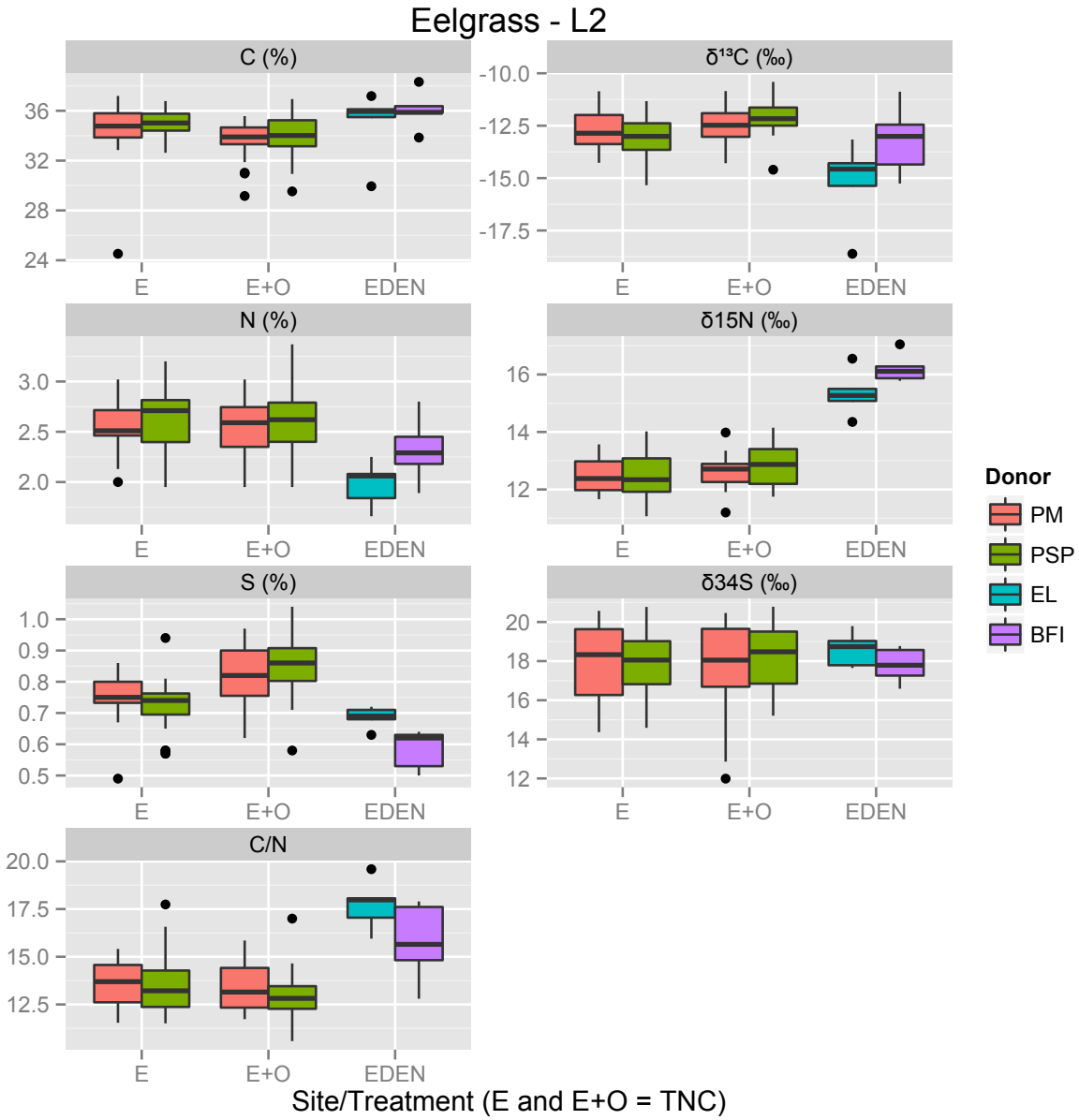


Figure 13. Site/treatment and donor effect on eelgrass (leaf 2) elemental and isotopic composition, summer 2013. EDEN = Eden Landing, E and E+O = TNC; C, N and S = carbon, nitrogen or sulphur content (%); C/N = carbon to nitrogen ratio; $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ = carbon, nitrogen and sulphur isotopic composition (‰).

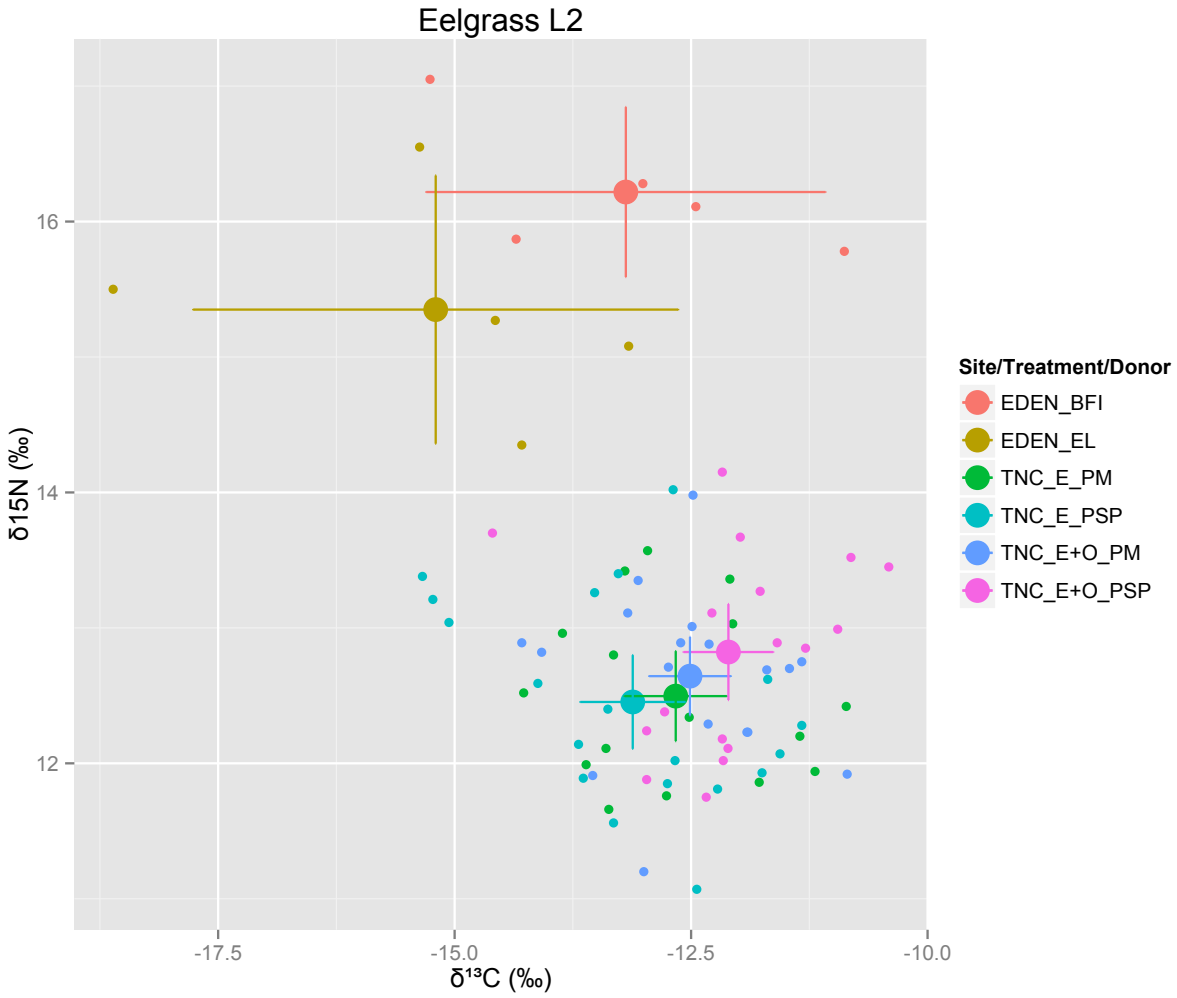


Figure 14. Carbon and Nitrogen stable isotope biplot for Eelgrass (leaf 2) at TNC and EDEN, summer 2013. Colors depict site, treatment and donor when applicable. Large dots = mean ± 95% confidence interval, small dots = individual samples.

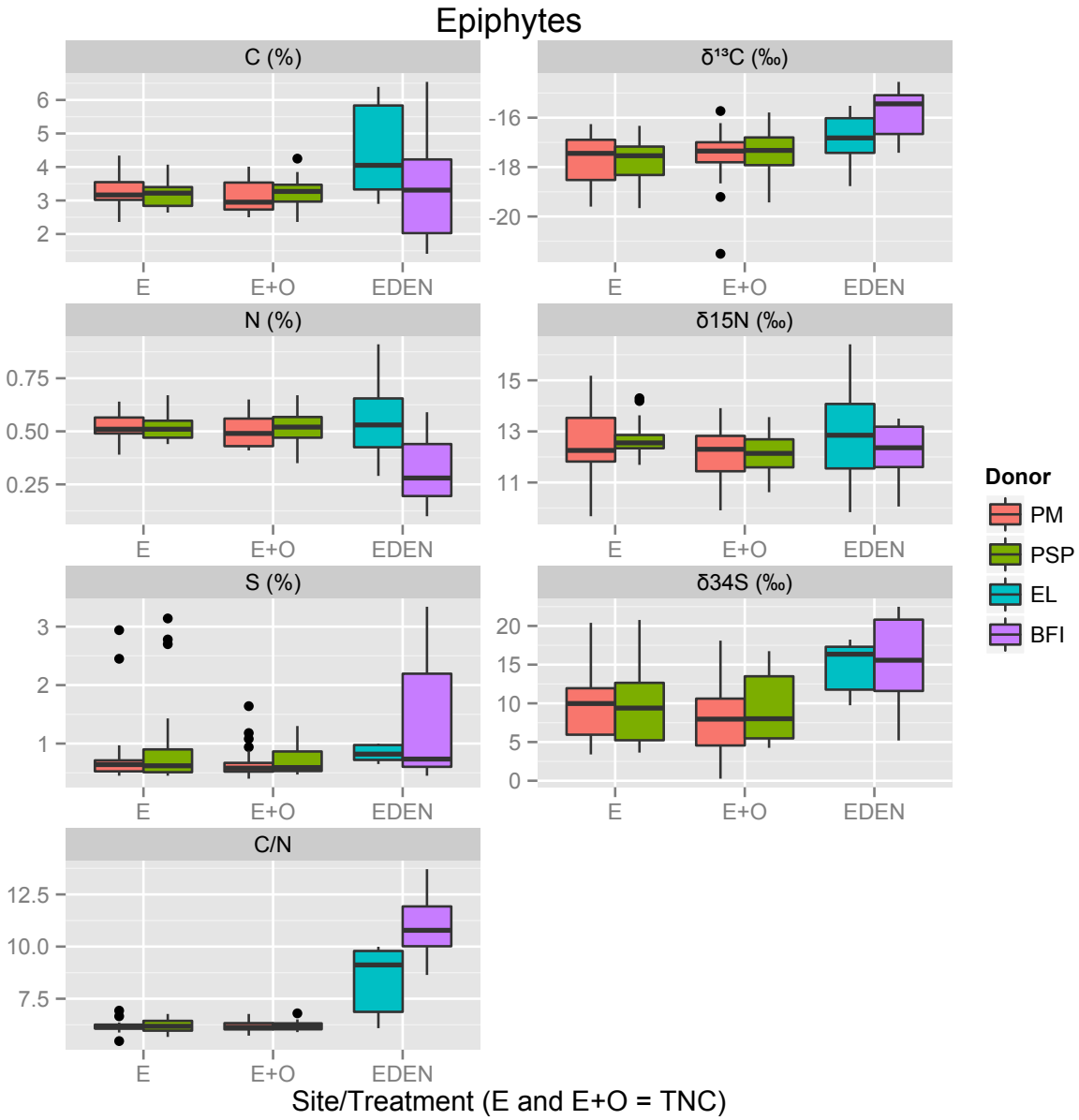


Figure 15. Site/treatment and donor effect on eelgrass epiphytes elemental and isotopic composition, summer 2013. EDEN = Eden Landing, E and E+O = TNC; C, N and S = carbon, nitrogen or sulphur content (%); C/N = carbon to nitrogen ratio; $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ = carbon, nitrogen and sulphur isotopic composition (‰).

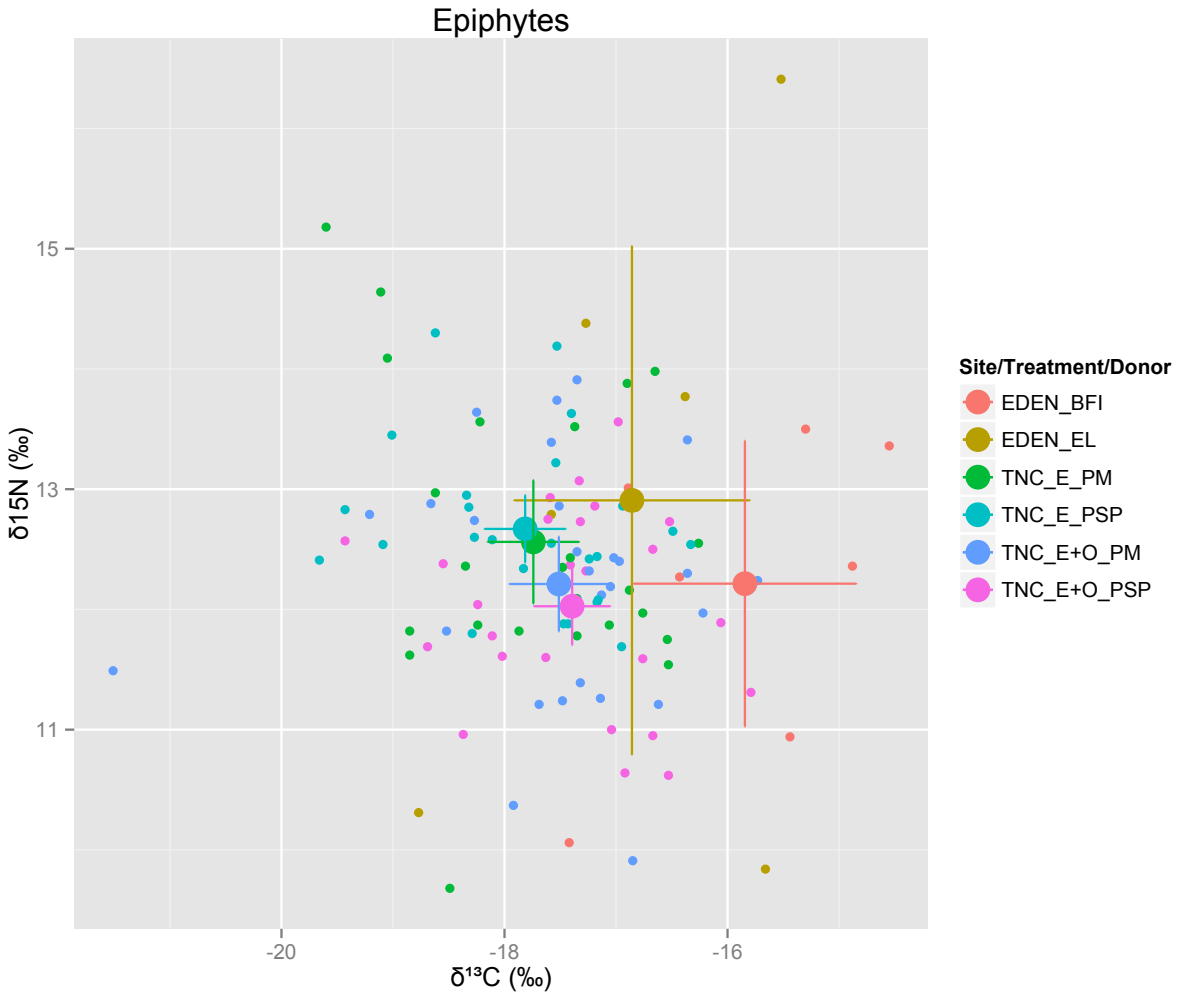


Figure 16. Carbon and Nitrogen stable isotope biplot for eelgrass epiphytes at TNC and EDEN, summer 2013. Colors depict site, treatment and donor when applicable. Large dots = mean ± 95% confidence interval, small dots = individual samples.

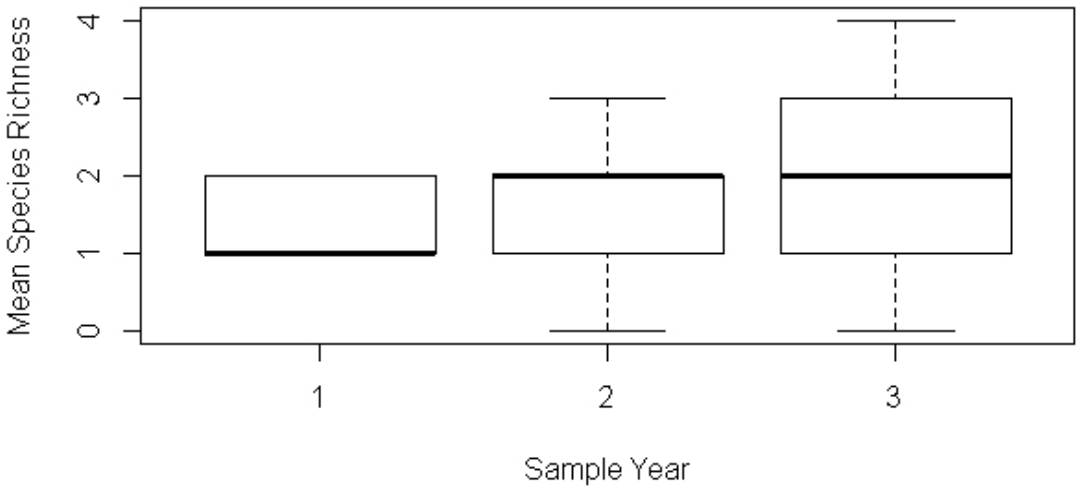


Figure 17. Mean species richness of baseline and post treatment sample years, p>0.05 Kruskal Wallis MC.

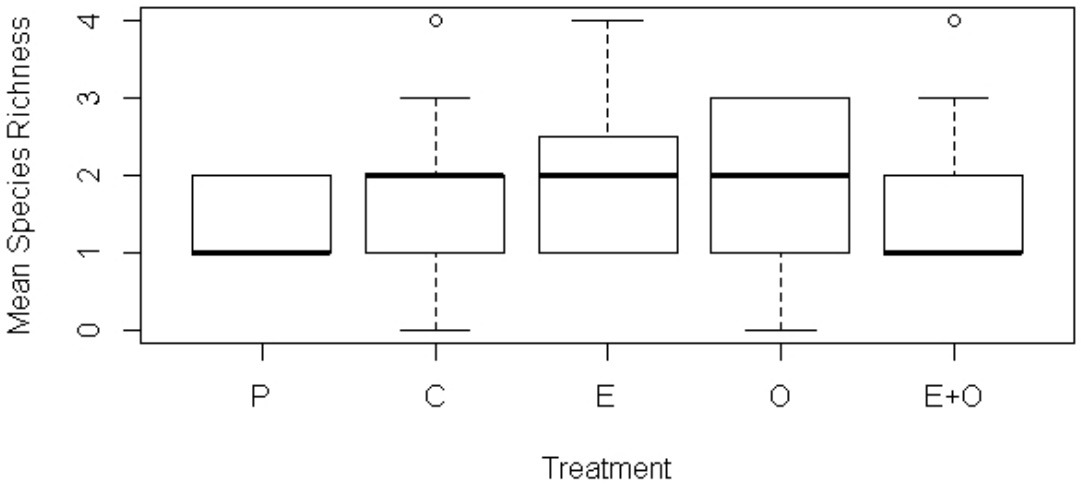


Figure 18. Mean species richness of baseline and treatment plots across all sample years, p>0.05 Kruskal Wallis MC.

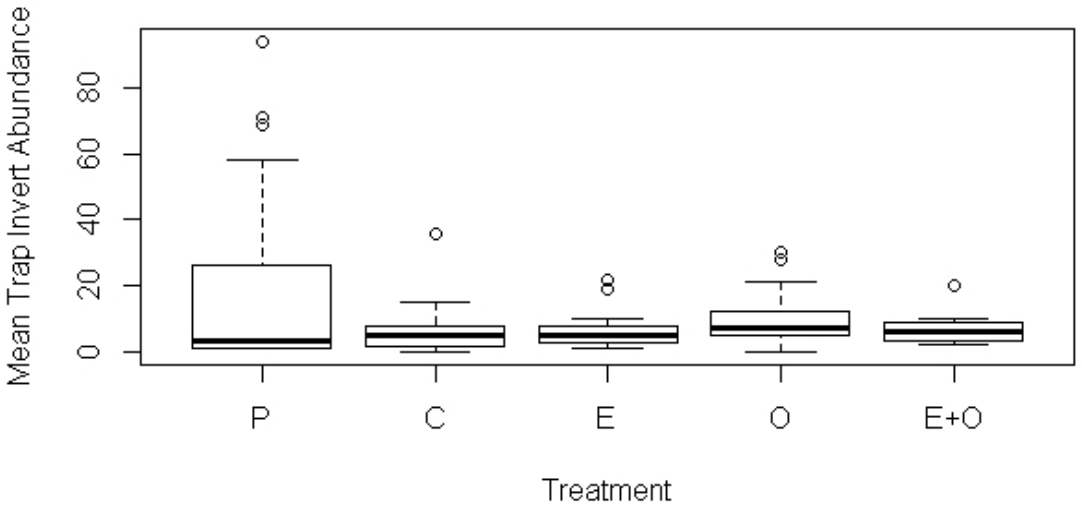


Figure 19. Mean abundance of baseline and treatment plots across all sample years, p>0.05 Kruskal-Wallis MC.

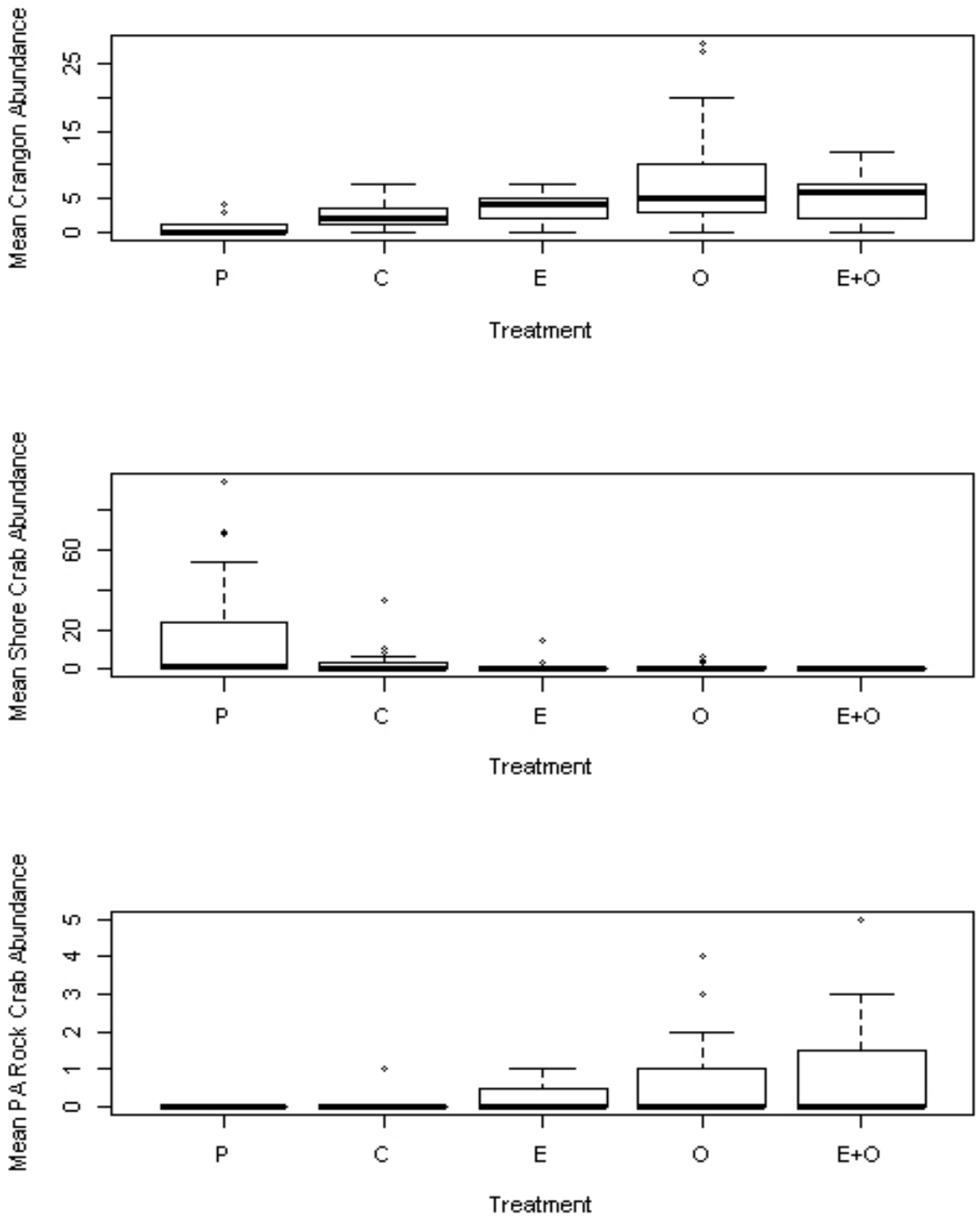


Figure 20. Responses of individual taxa to treatment across sampling periods.
*Significant differences from pre-treatment baseline ($p<0.01$, Kruskal-Wallis multiple comparison test)

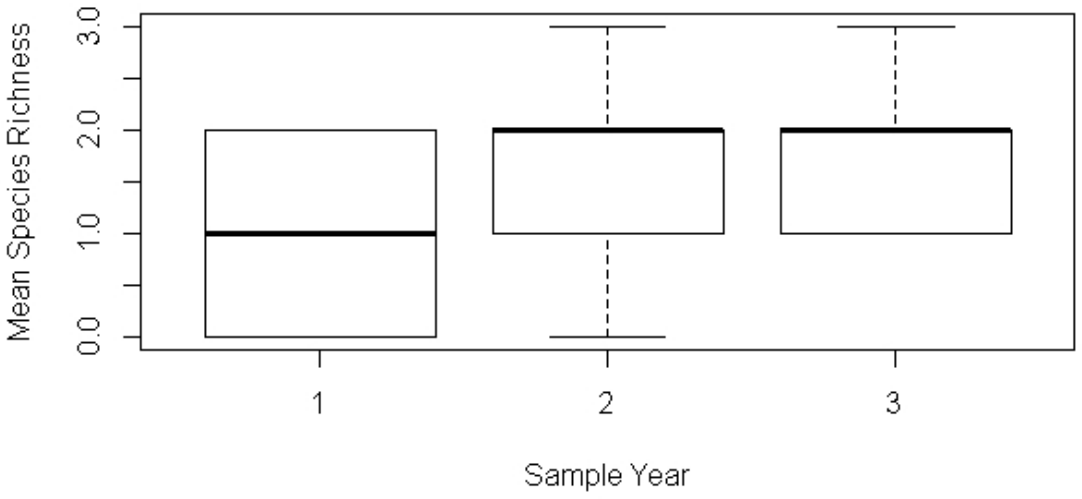


Figure 21. Mean species richness increased across the ELER site from baseline (Year 1) through treatment (Years 2 and 3) $p<0.01$ Kruskal Wallis.

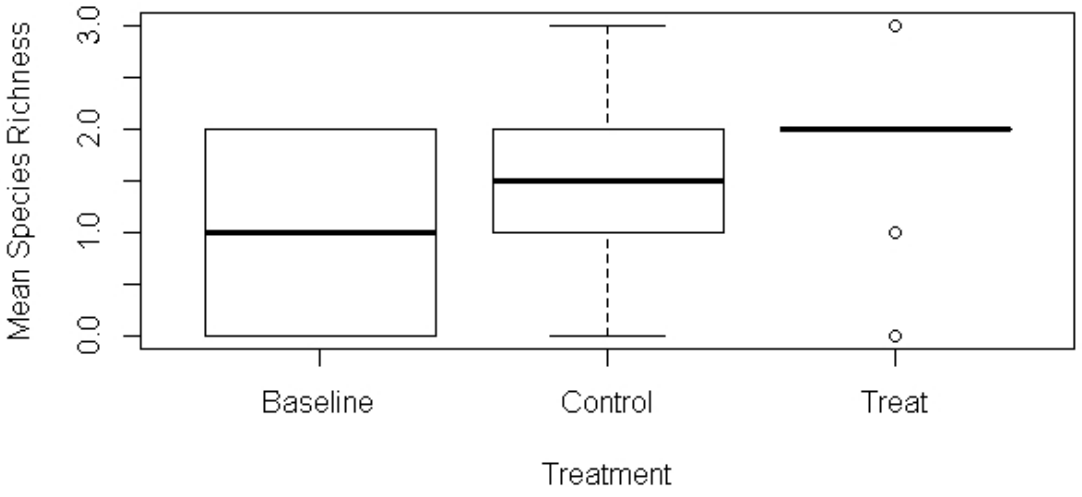


Figure 22. Mean species richness increased in the treatment, differing from baseline and control ($p<0.05$ Kruskal Wallis MC). No difference observed between baseline and control.

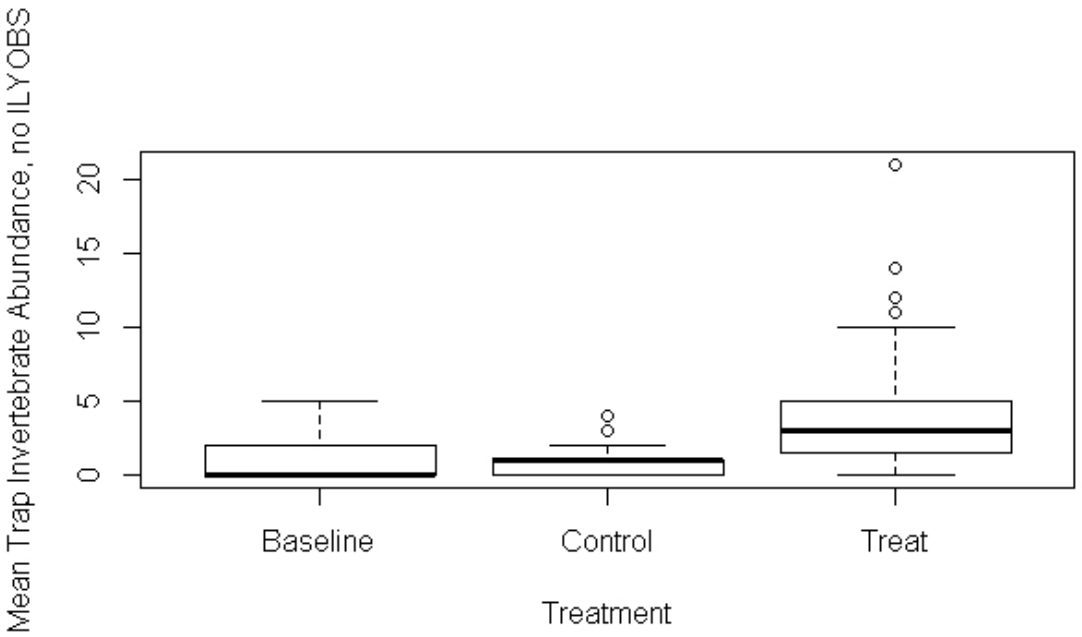


Figure 23. Total Eden abundance increased in the treatment area, as compared to both the baseline and control conditions ($p<0.05$ Kruskal Wallis). However, this increase is only evident when the highly abundant Eastern mud snaol (ILYOBS) is removed.

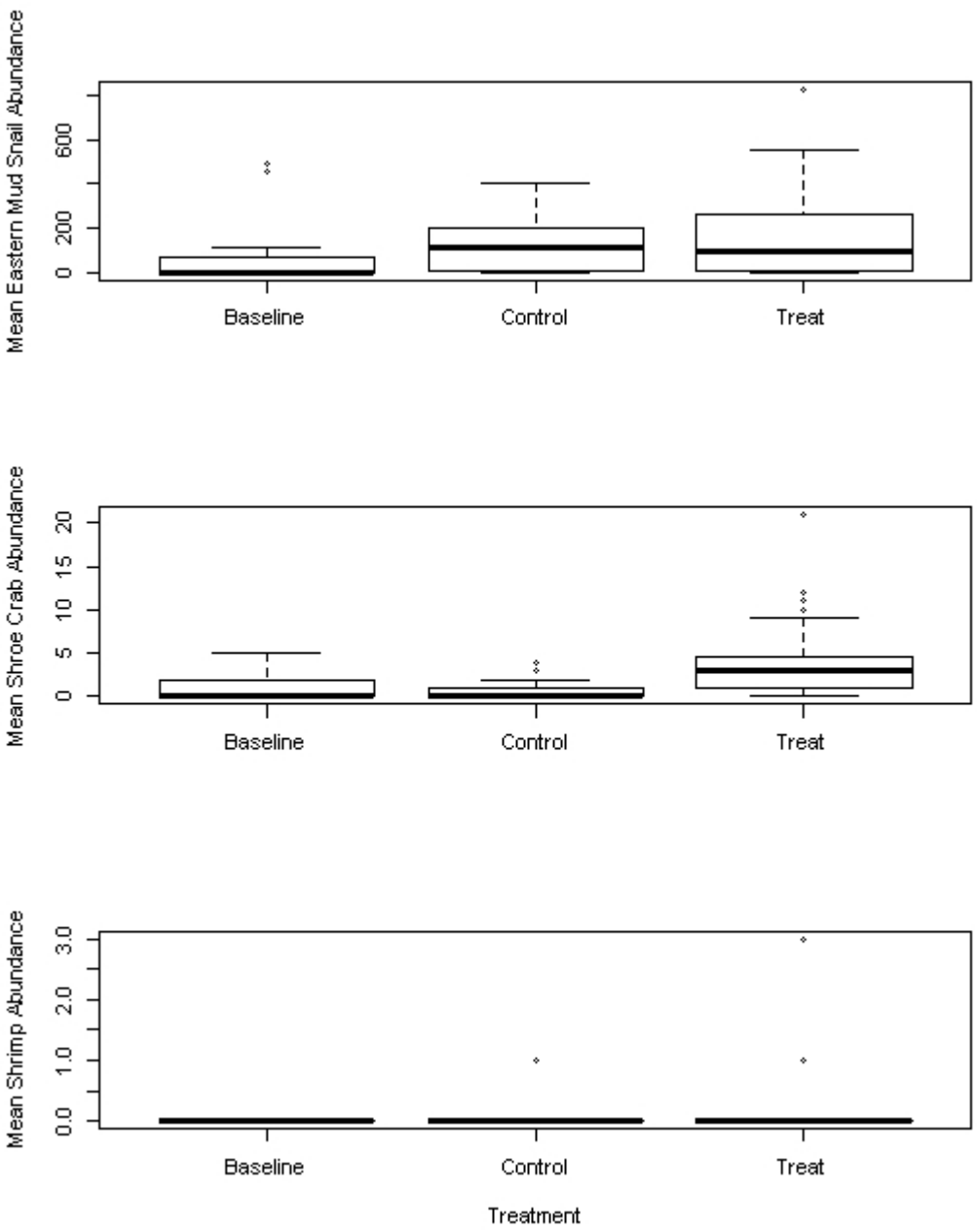


Figure 24. Mean trap abundances of taxa at Eden across treatments.

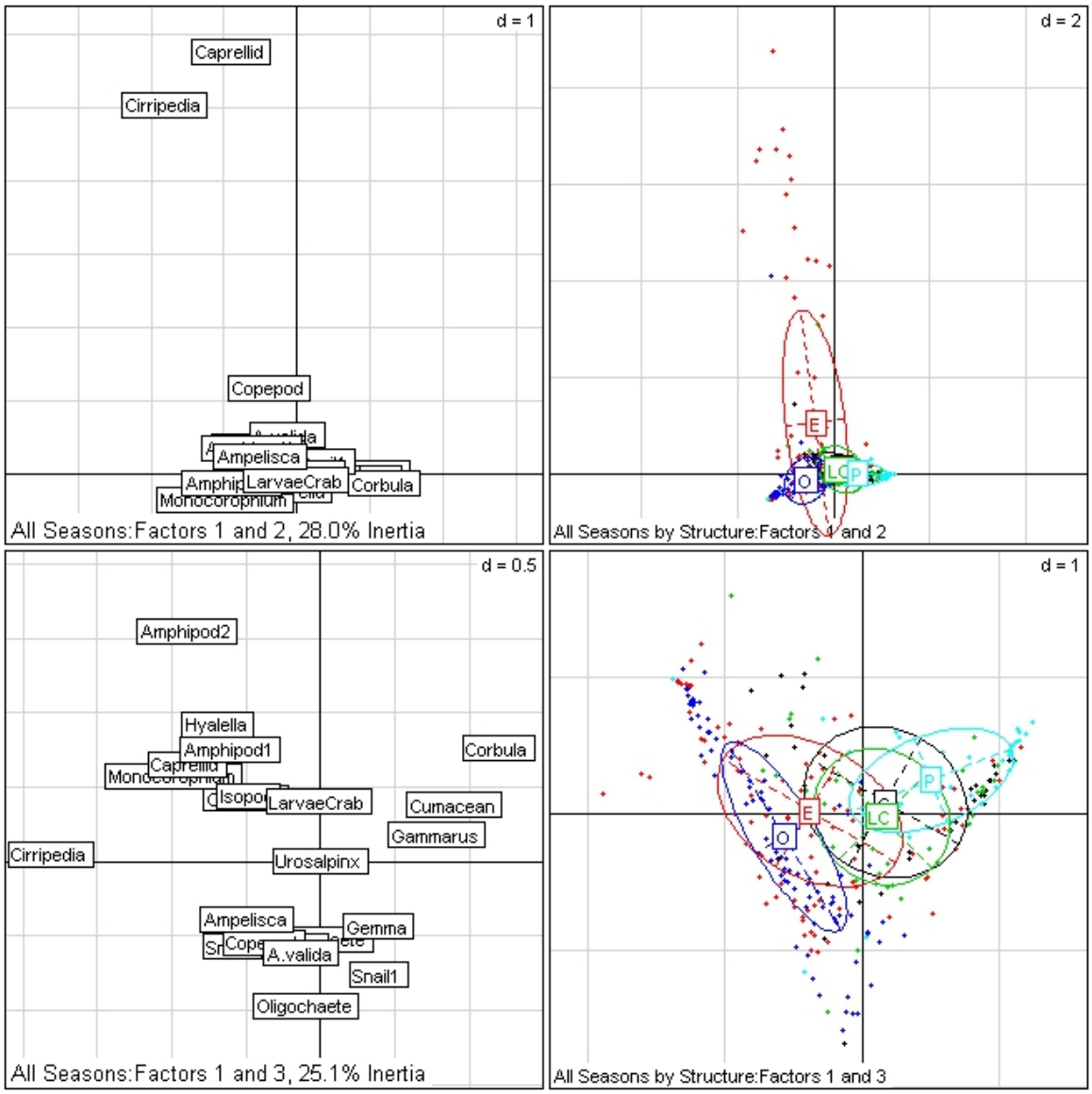


Figure 25. Correspondence analysis of TNC suction samples, all seasons combined. Factors 1, 2, and 3 total 37.9% of variation.

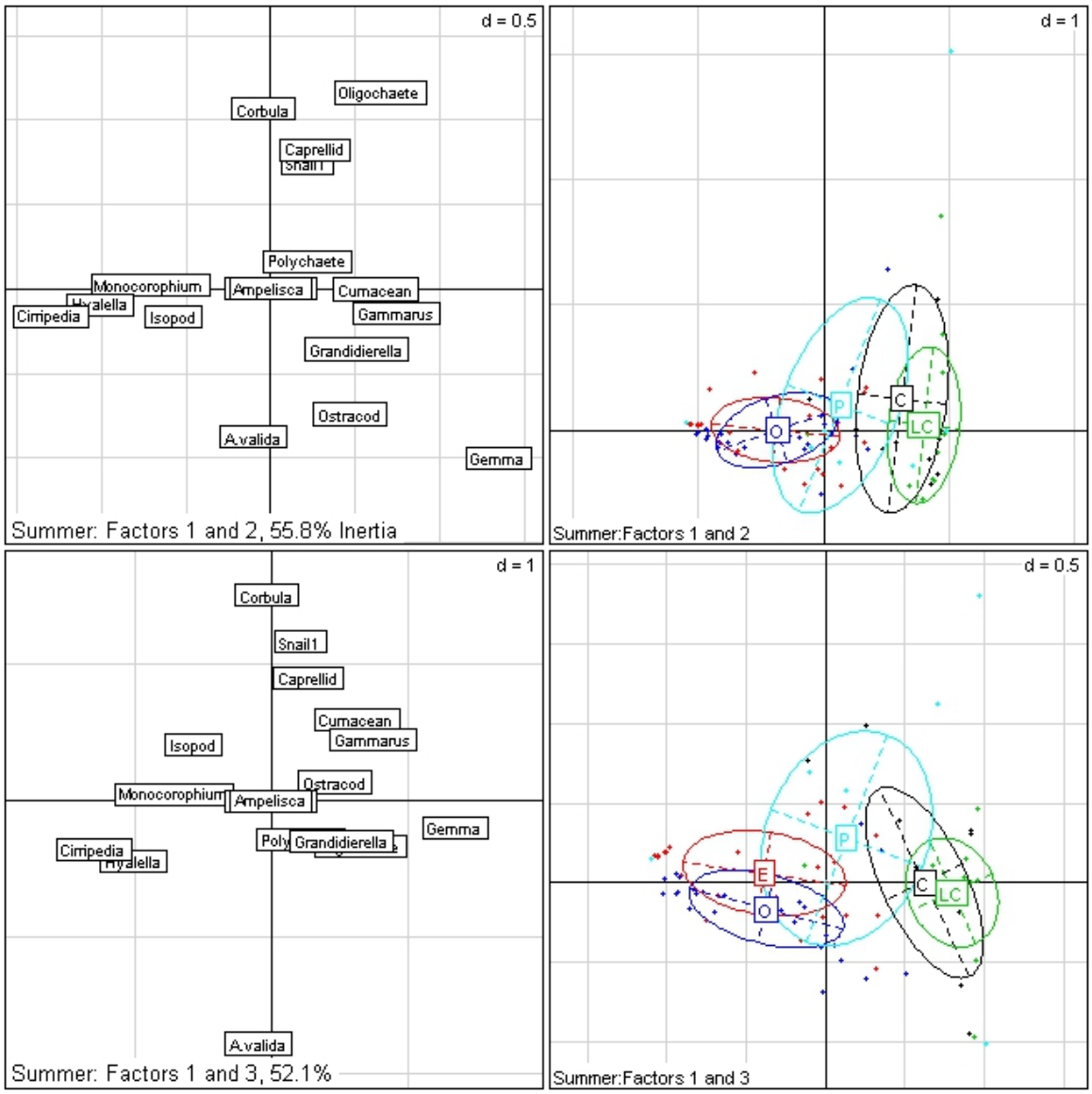


Figure 26. Correspondence analysis of TNC suction samples collected during Summer 2011 (Baseline) and Summer 2013 (eelgrass, oyster, plot control, site control). Actual eigenvalues; 55.1% and 51.6%.

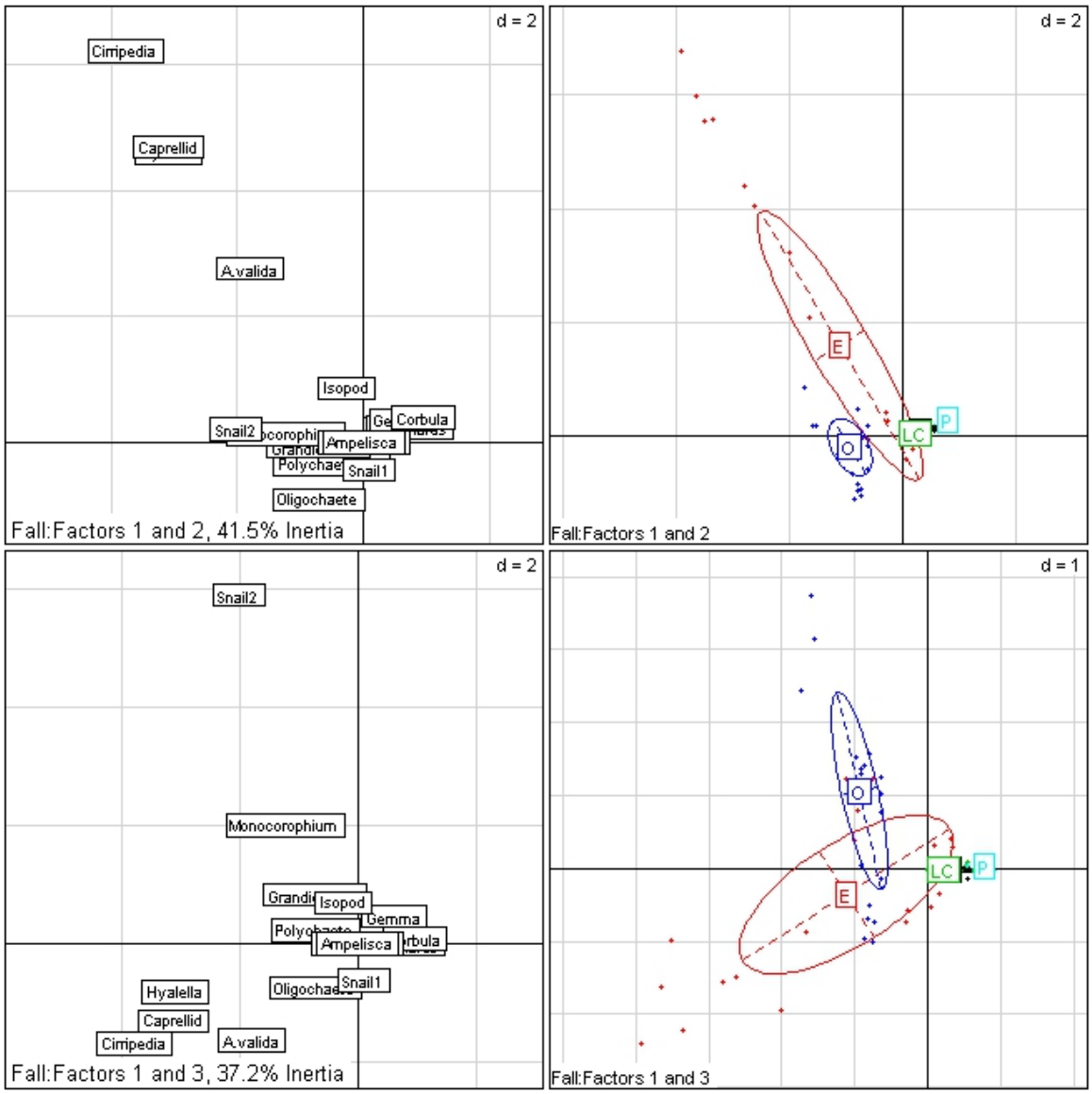


Figure 27. Correspondence analysis of TNC suction samples collected during Fall 2011 (Baseline) and Fall 2013 (eelgrass, oyster, plot control, site control).
Actual Eigen: 38.4% and 34.9%.

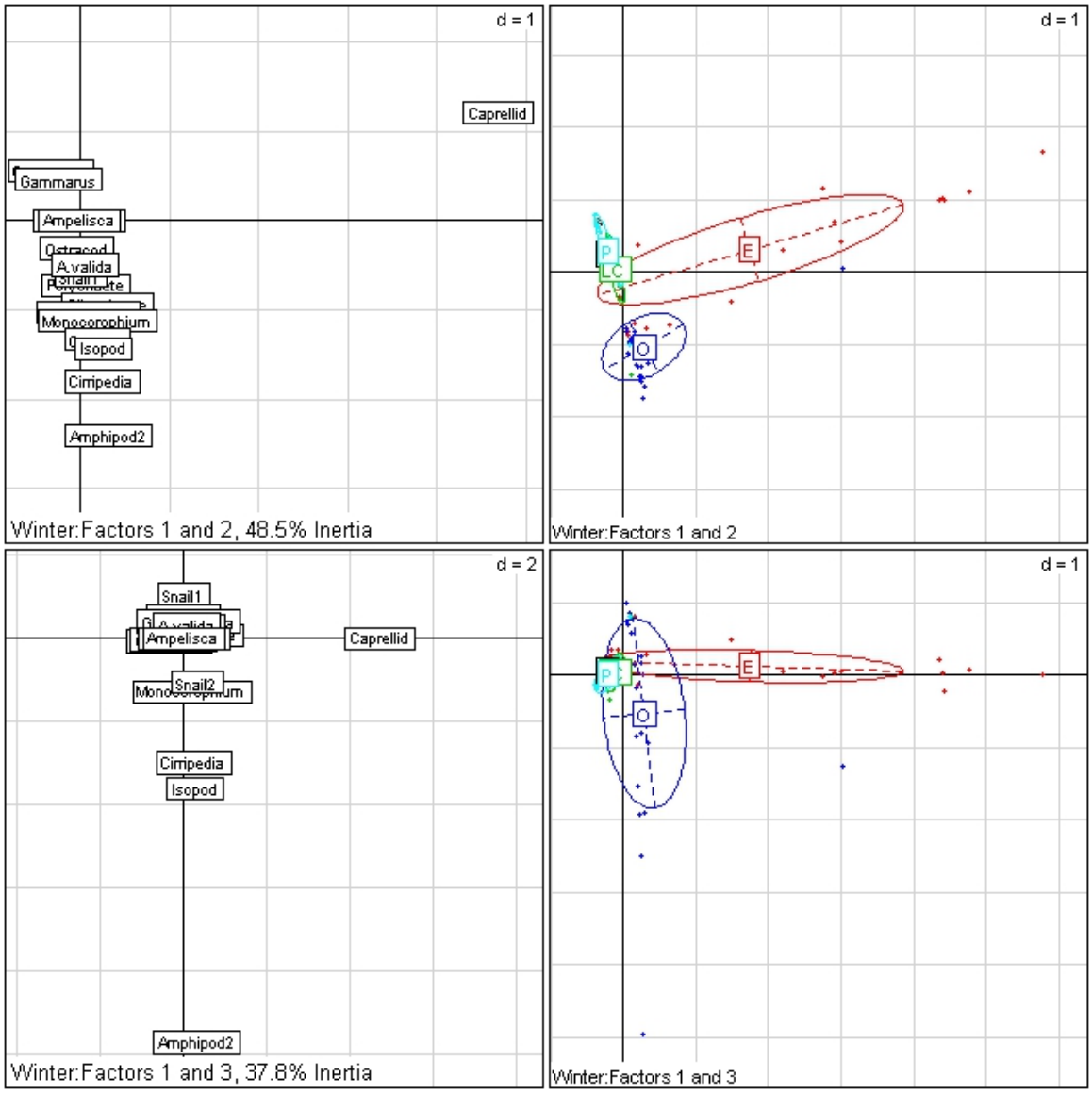


Figure 28. Correspondence analysis of TNC suction samples collected during Winter 2012 (Baseline) and Winter 2014 (eelgrass, oyster, plot control, site control).
Actual Eigen: 40.6% and 35.9%.

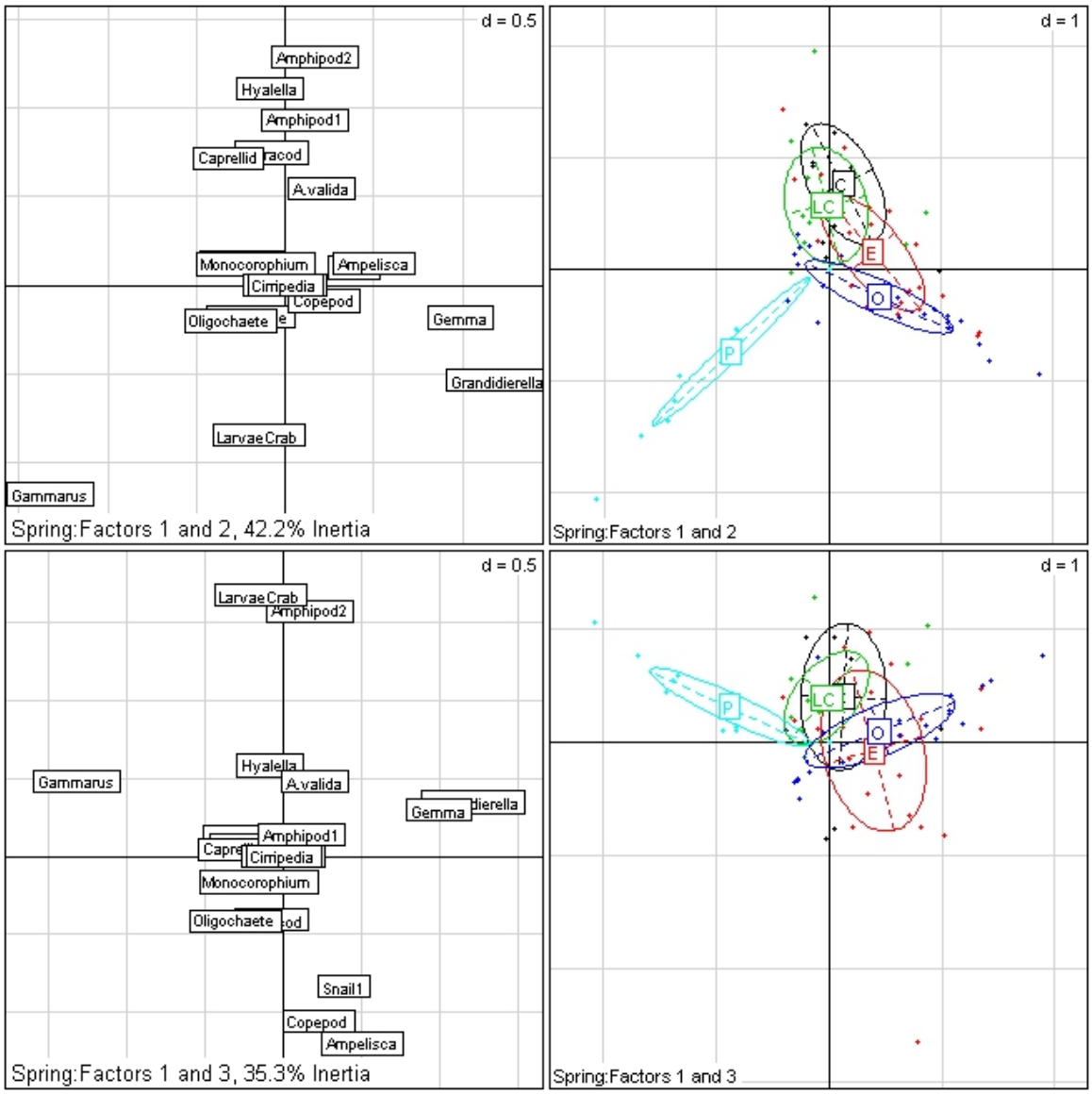


Figure 29. Correspondence analysis of TNC suction samples collected during Spring 2012 (Baseline) and Spring 2014 (eelgrass, oyster, plot control, site control).
Actual Eigen: 43.7% and 36.3%.

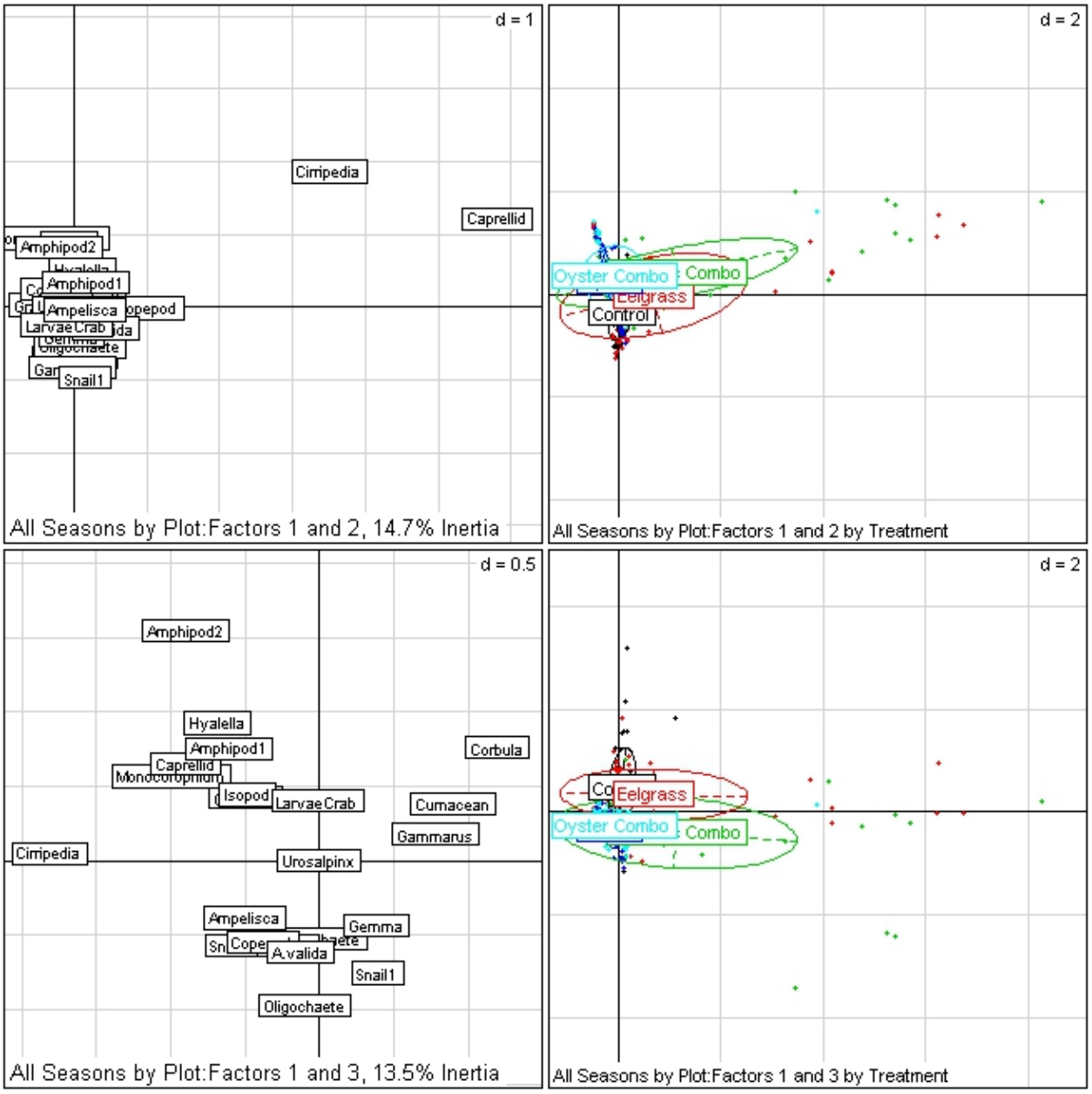


Figure 30. Correspondence analysis of TNC suction samples collected within the treatment site during all four sampling periods combined from Summer 2013-Spring 2014 (oyster, eelgrass, oyster combo, eelgrass combo, and plot control). (All overlap, some possible differentiation within species).

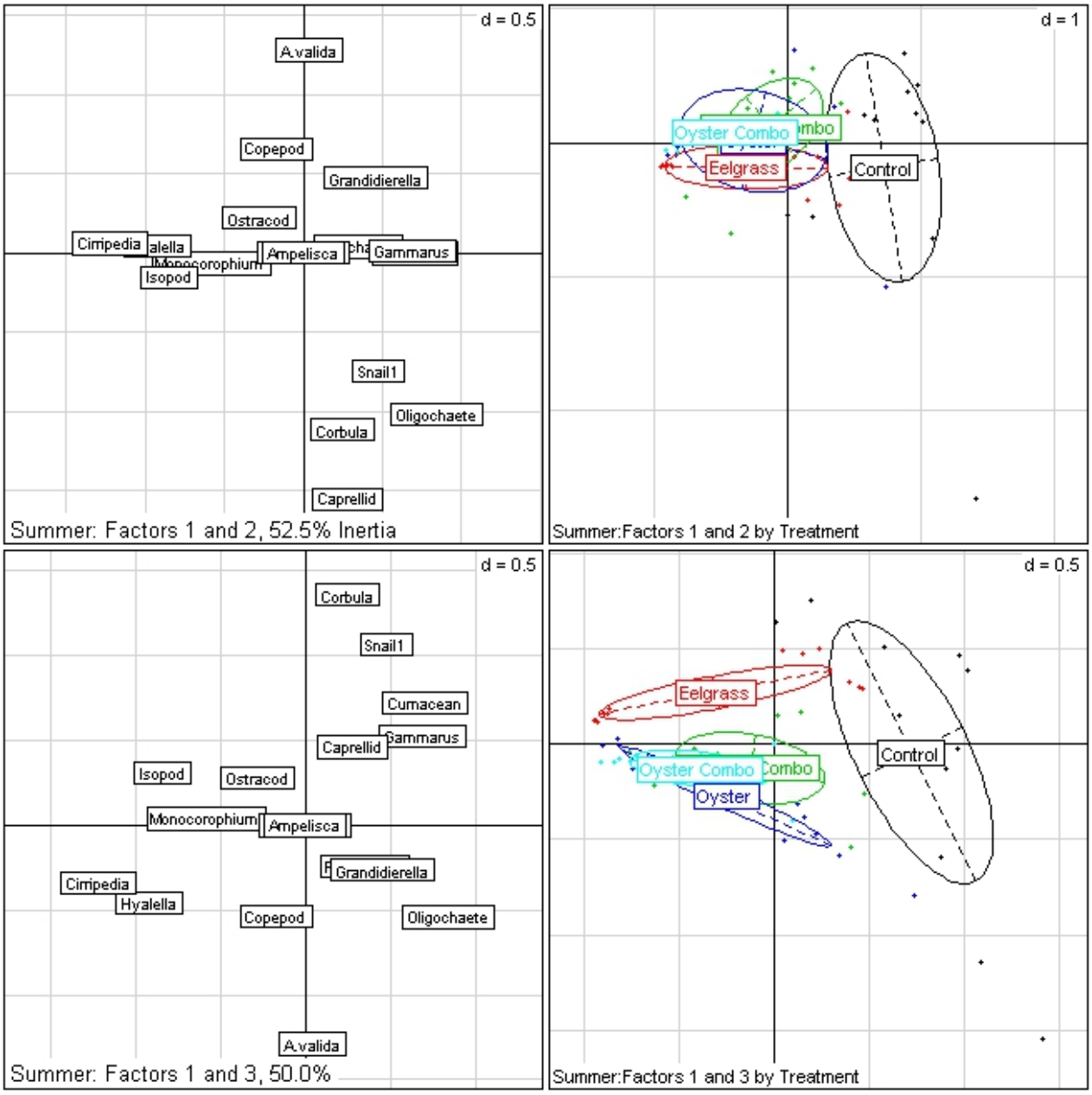


Figure 31. Correspondence analysis of TNC suction samples collected within the treatment site during Summer 2013 (oyster, eelgrass, oyster combo, eelgrass combo, and plot control). Cumulative Eigen value of for factors 1:3 of 65.2%.

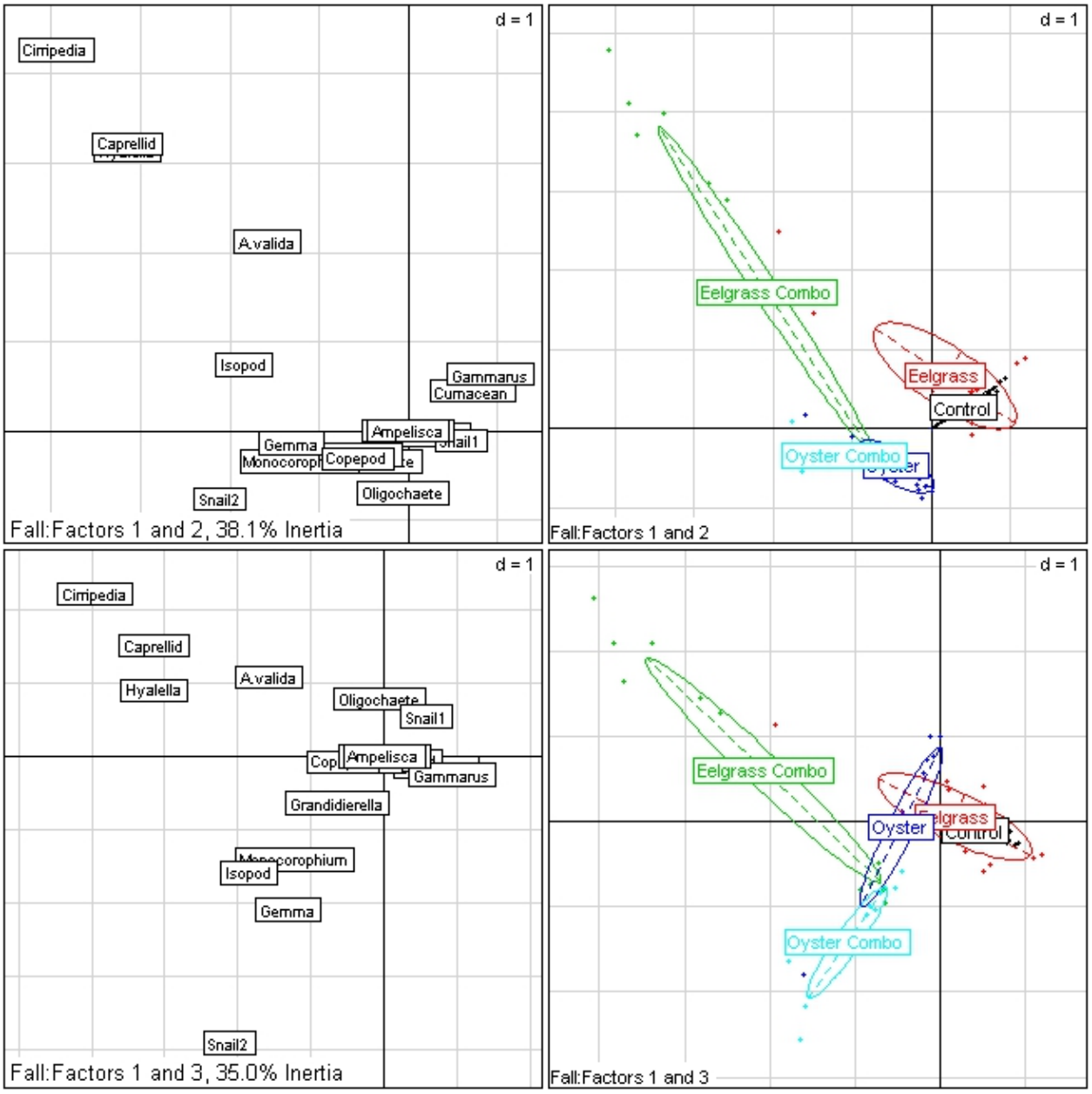


Figure 32. Correspondence analysis of TNC suction samples collected within the treatment site during Fall 2013 (oyster, eelgrass, oyster combo, eelgrass combo, and plot control). Cumulative Eigen value 52.4% for factors 1:3.

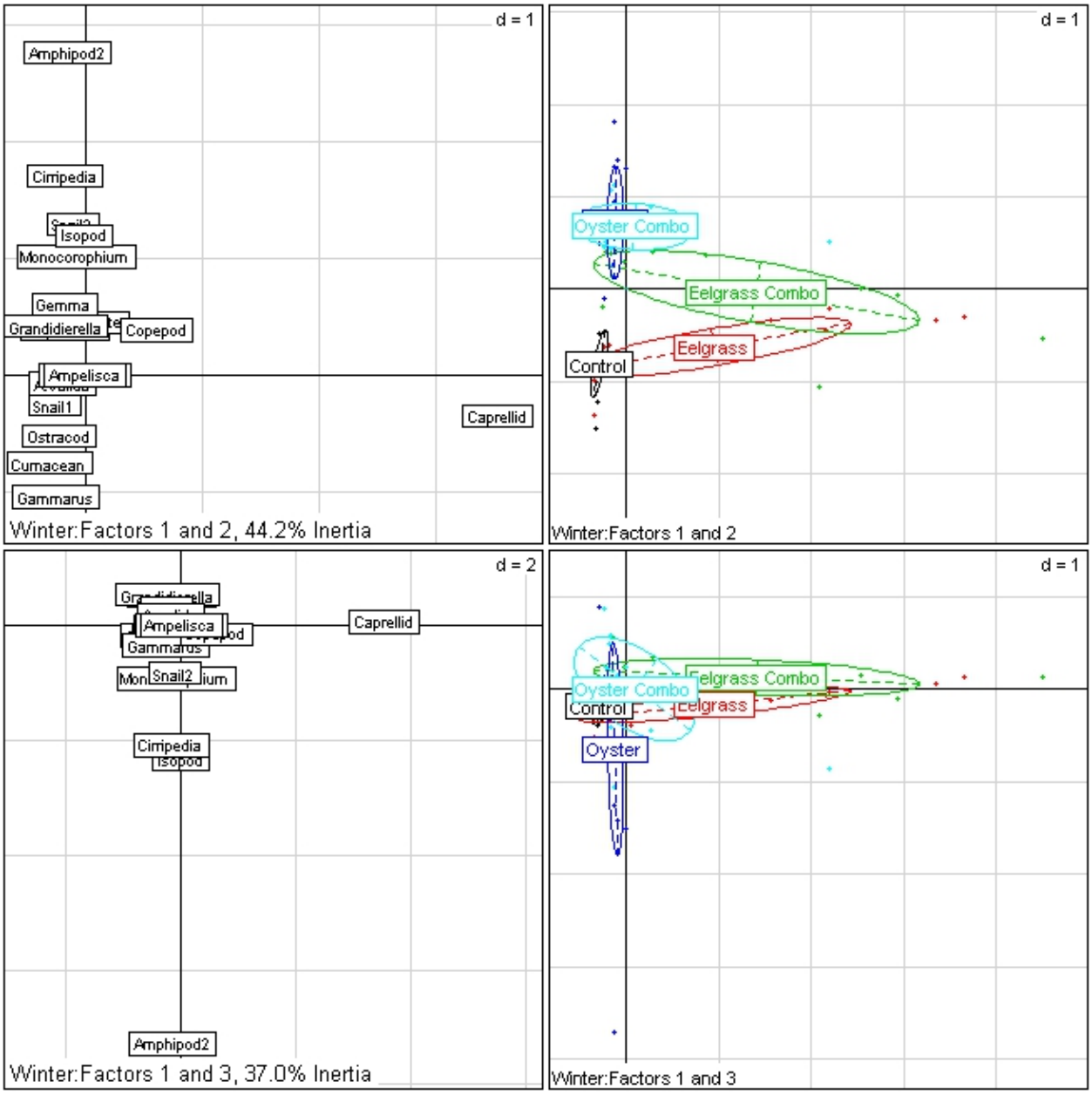


Figure 33. Correspondence analysis of TNC suction samples collected within the treatment site during Winter 2014 (oyster, eelgrass, oyster combo, eelgrass combo, and plot control). (Eelgrass and eelgrass combo more close than oyster and oyster combo)

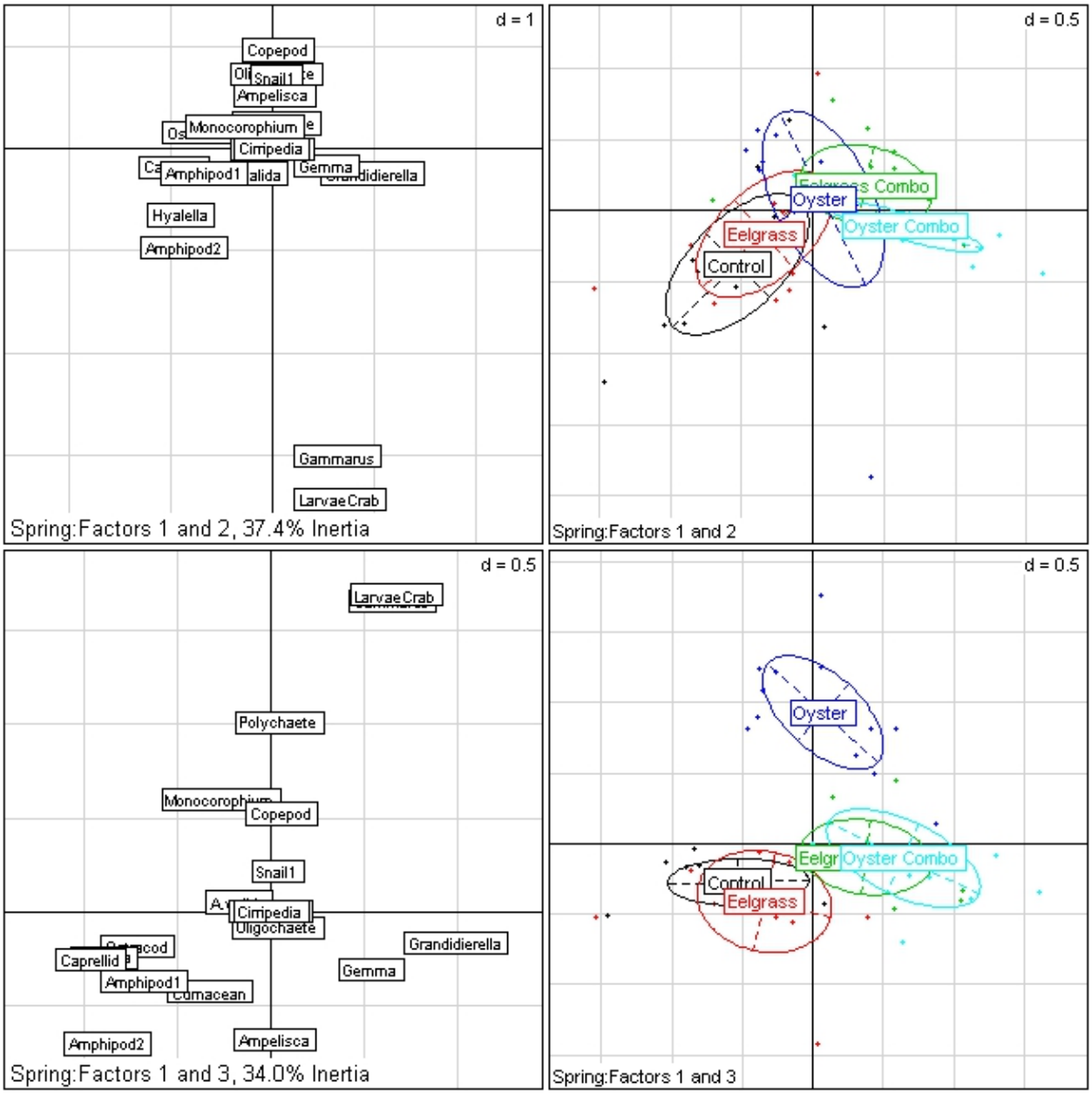


Figure 34. Correspondence analysis of TNC suction samples collected within the treatment site during Spring 2014, factors 1:3 Eigen 50.4%.

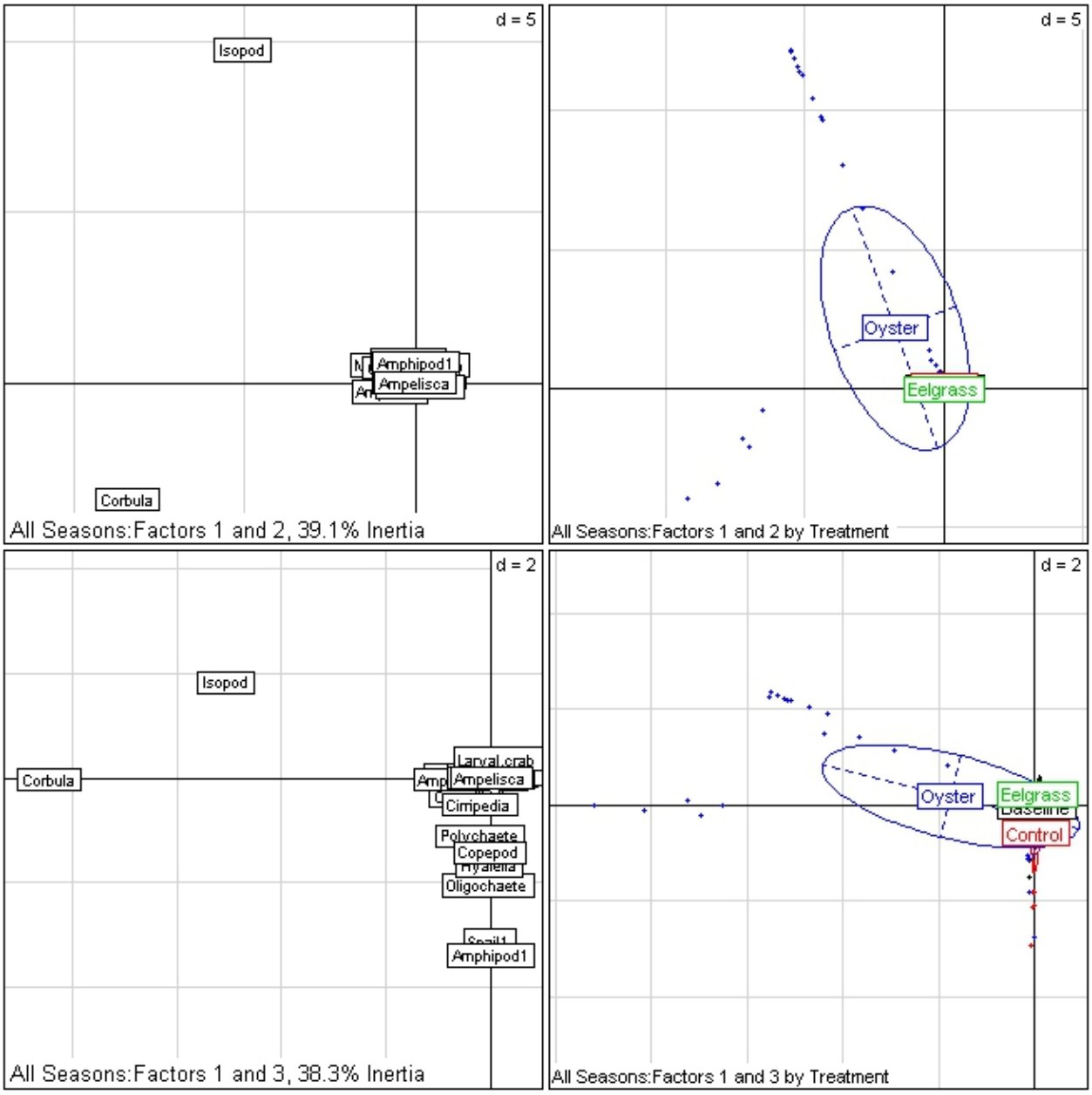


Figure 35. Correspondence analysis of Eden suction samples, all seasons combined. Factors 1, 2, and 3 total 55.1% of variation.

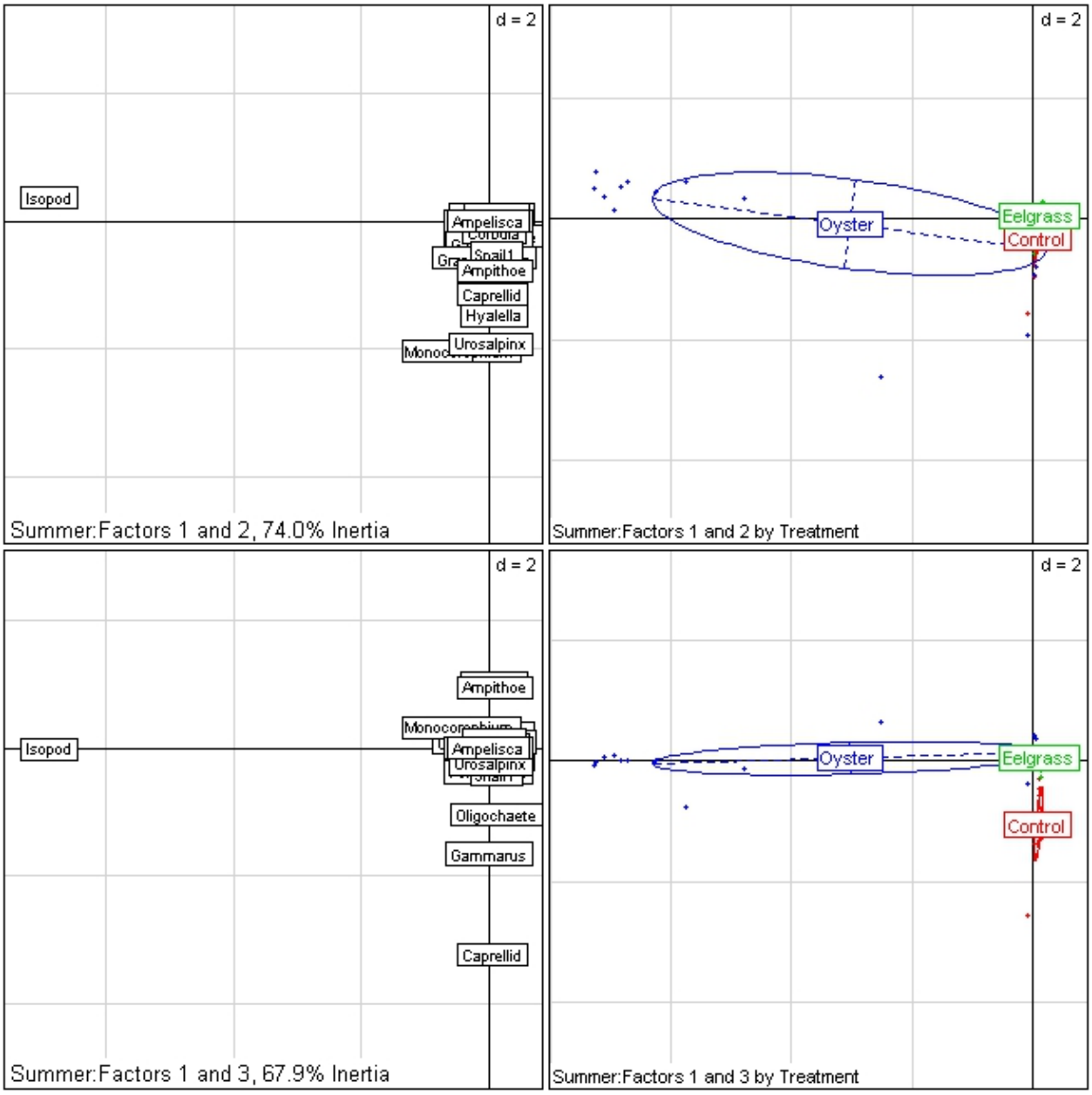


Figure 36. Correspondence analysis of Eden suction samples, Summer. Factors 1, 2, and 3 total 80.4% of variation.

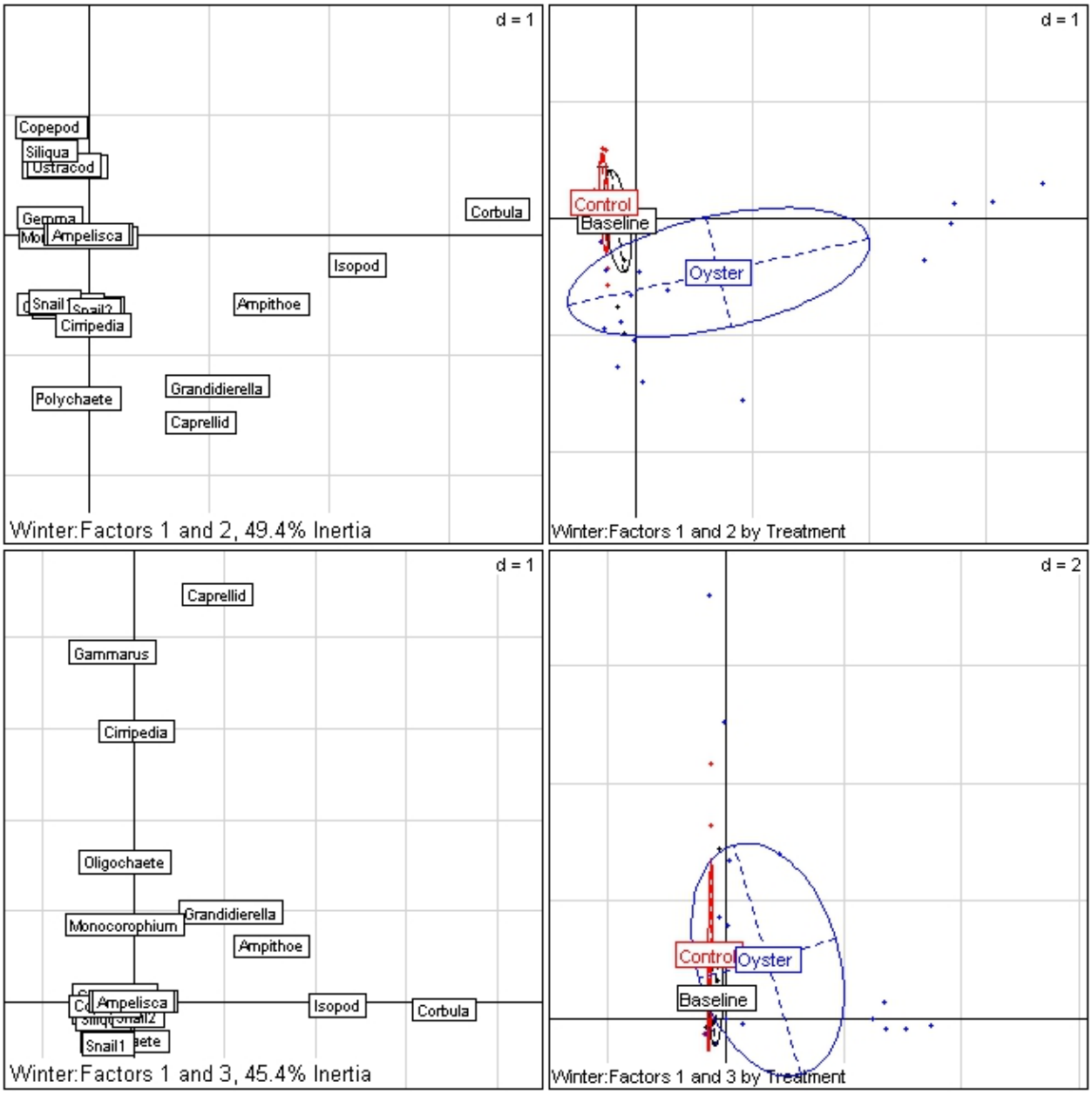


Figure 37. Correspondence analysis of Eden suction samples, Winter. Factors 1, 2, and 3 total 61.8% of variation.

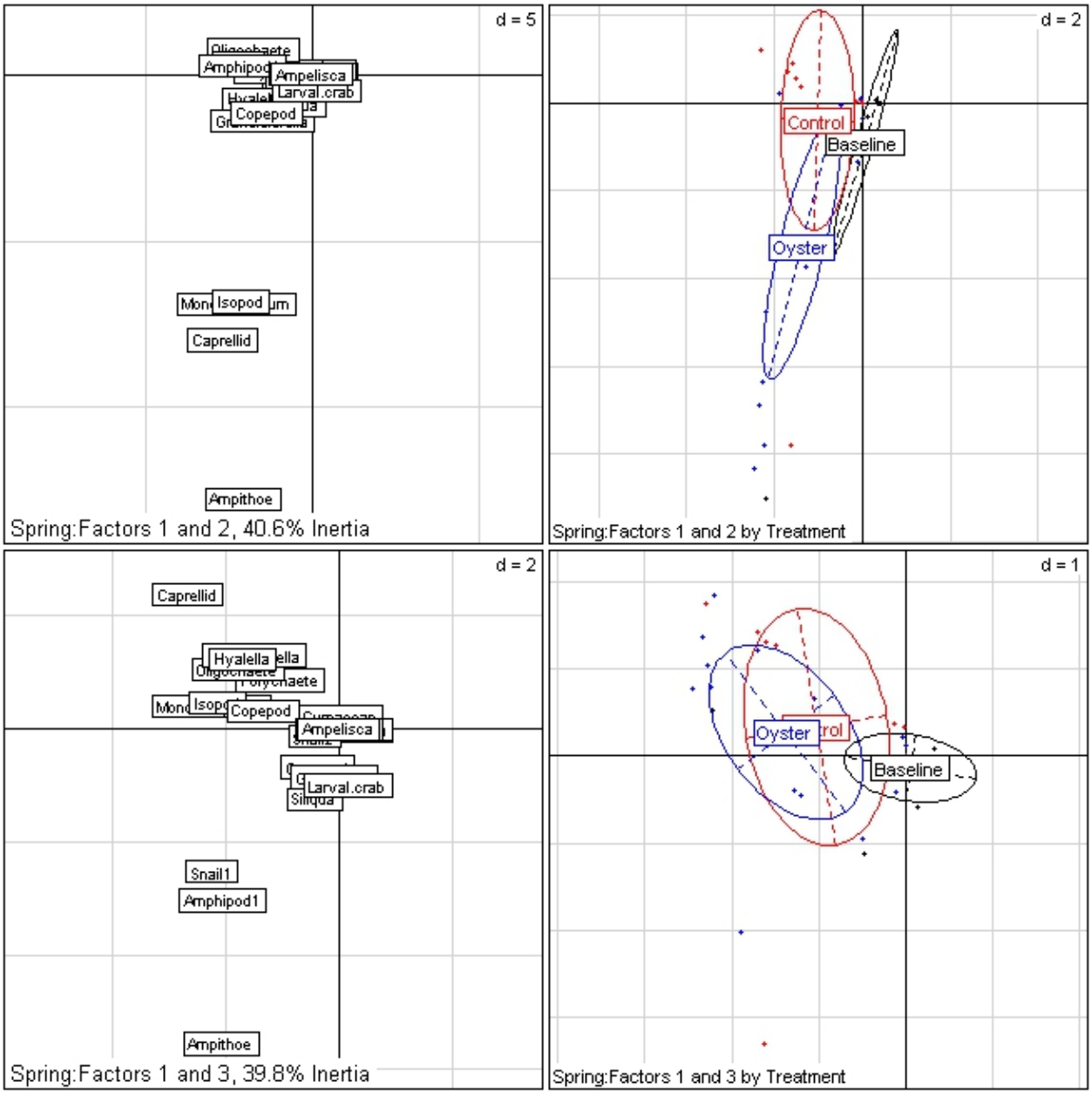


Figure 38. Correspondence analysis of Eden suction samples, Winter. Factors 1, 2, and 3 total 55.3% of variation.

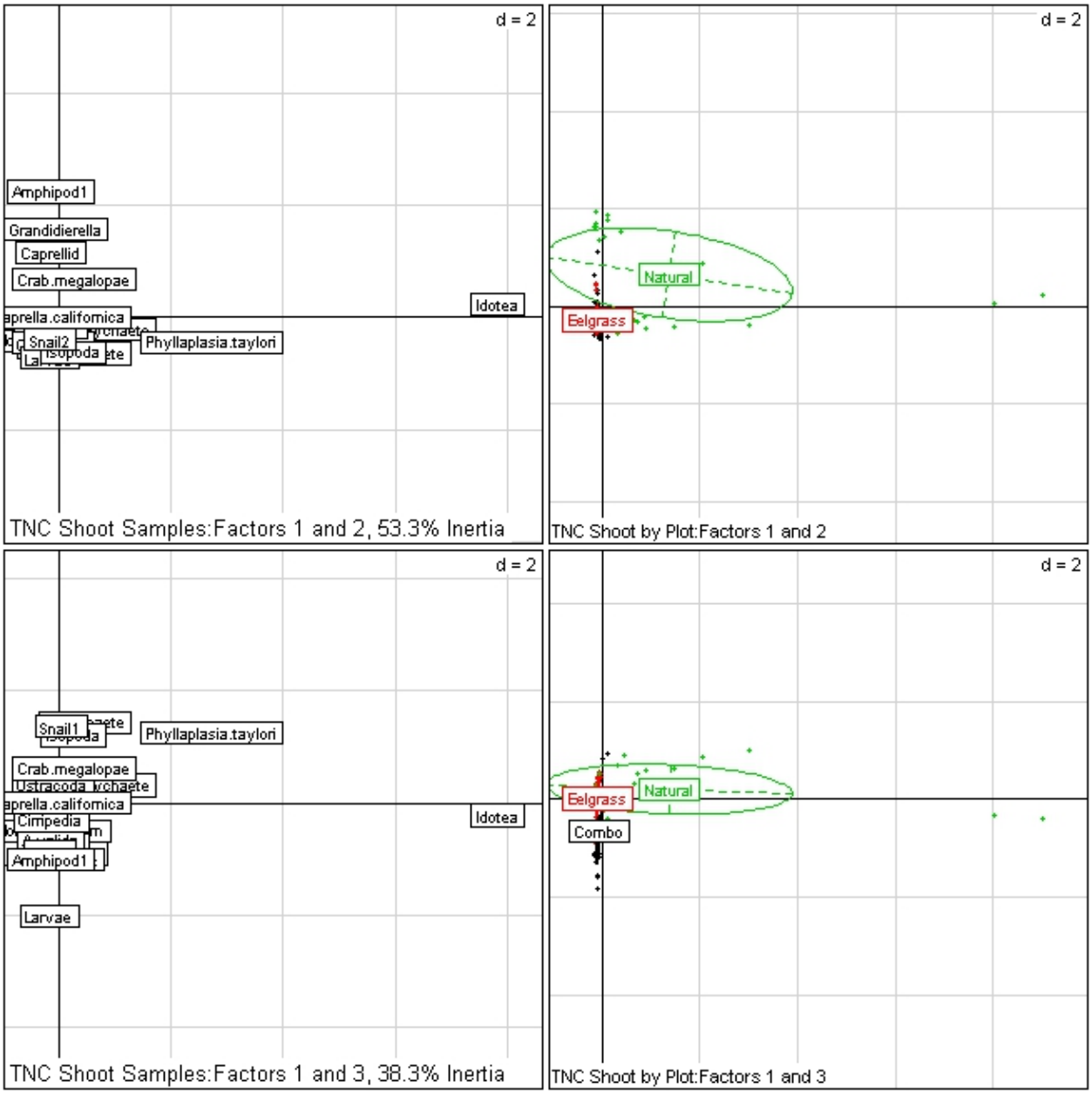


Figure 39. Correspondence analysis of shoot samples collected during Spring 2014 at TNC, Keller Beach, and Point Molate shown by Plot
Eelgrass = TNC eelgrass plot
Combo= TNC eelgrass + oyster plot
Natural = Point Molate and Keller Beach natural populations

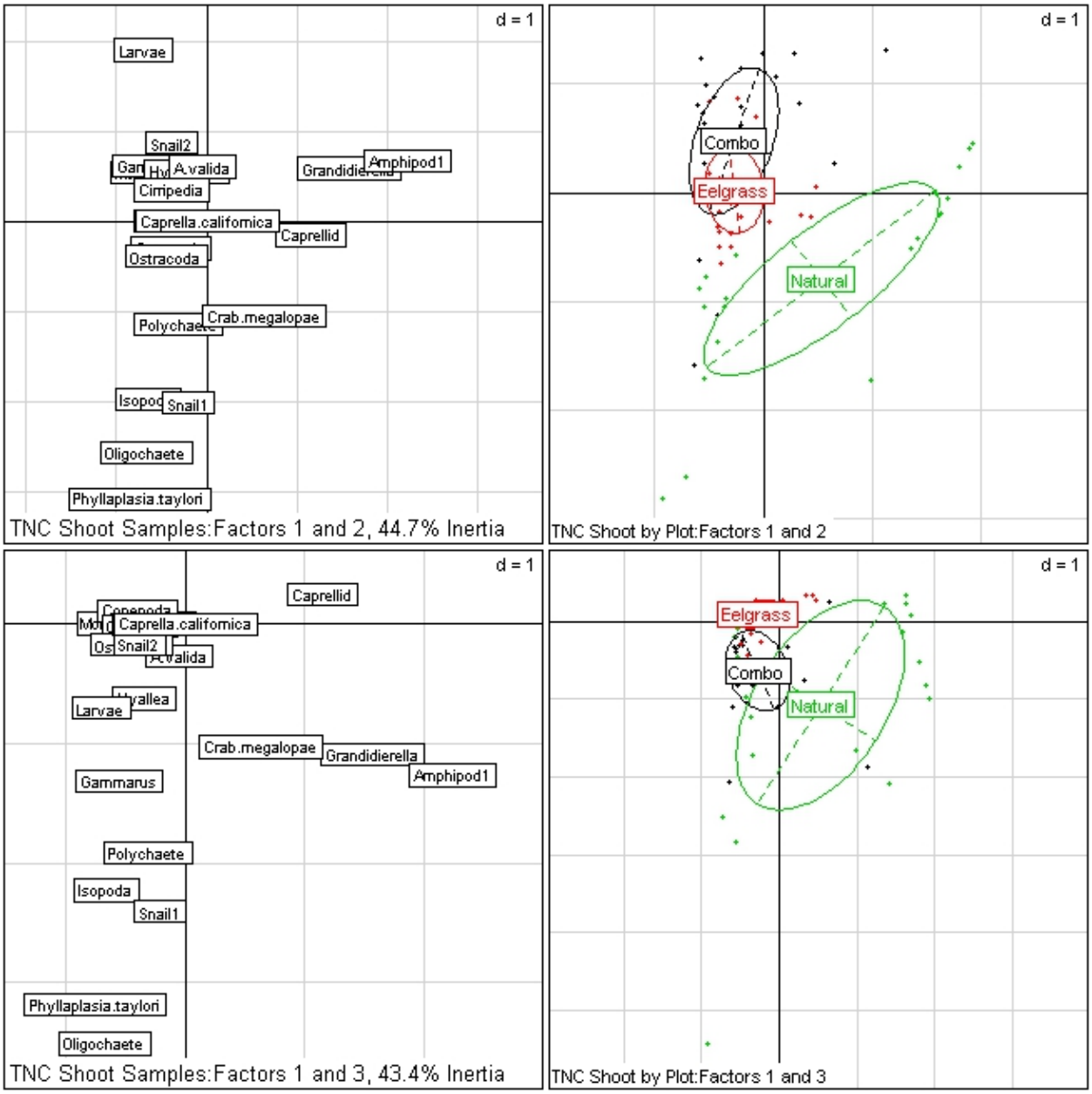


Figure 40. Correspondence analysis of shoot samples collected during Spring 2014 at TNC, Keller Beach, and Point Molate shown by Plot- with Idotea removed to spread results
Eelgrass = TNC eelgrass plot
Combo= TNC eelgrass + oyster plot
Natural = Point Molate and Keller Beach natural populations

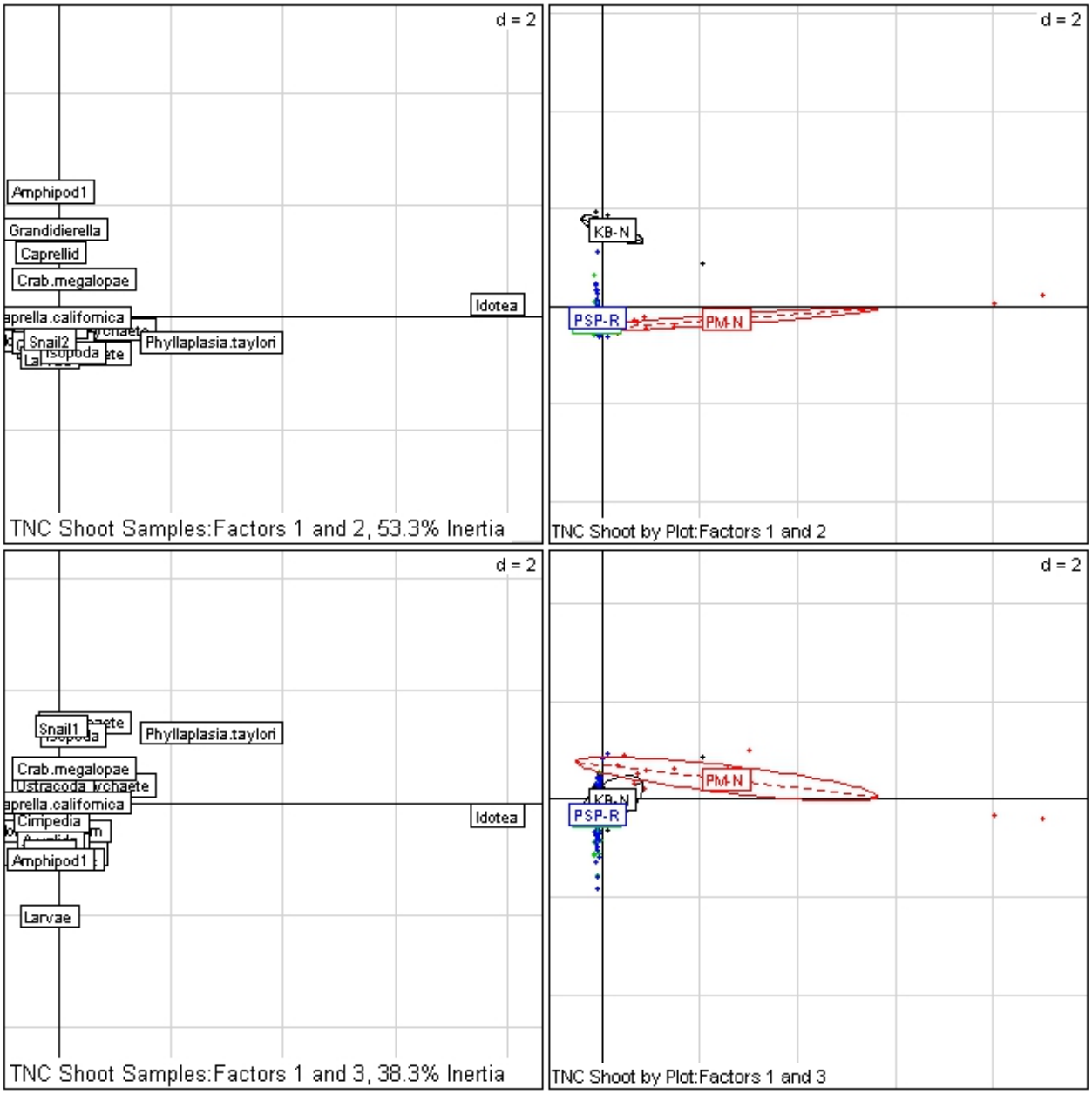


Figure 41. Correspondence analysis of shoot samples collected during Spring 2014 at TNC, Keller Beach, and Point Molate- shown by source

KB-N= Keller Beach natural population

PM-N= Point Molate natural population

PSP-R= TNC restored population sourced from Point San Pablo

PM-R= TNC restored population sourced from Point Molate

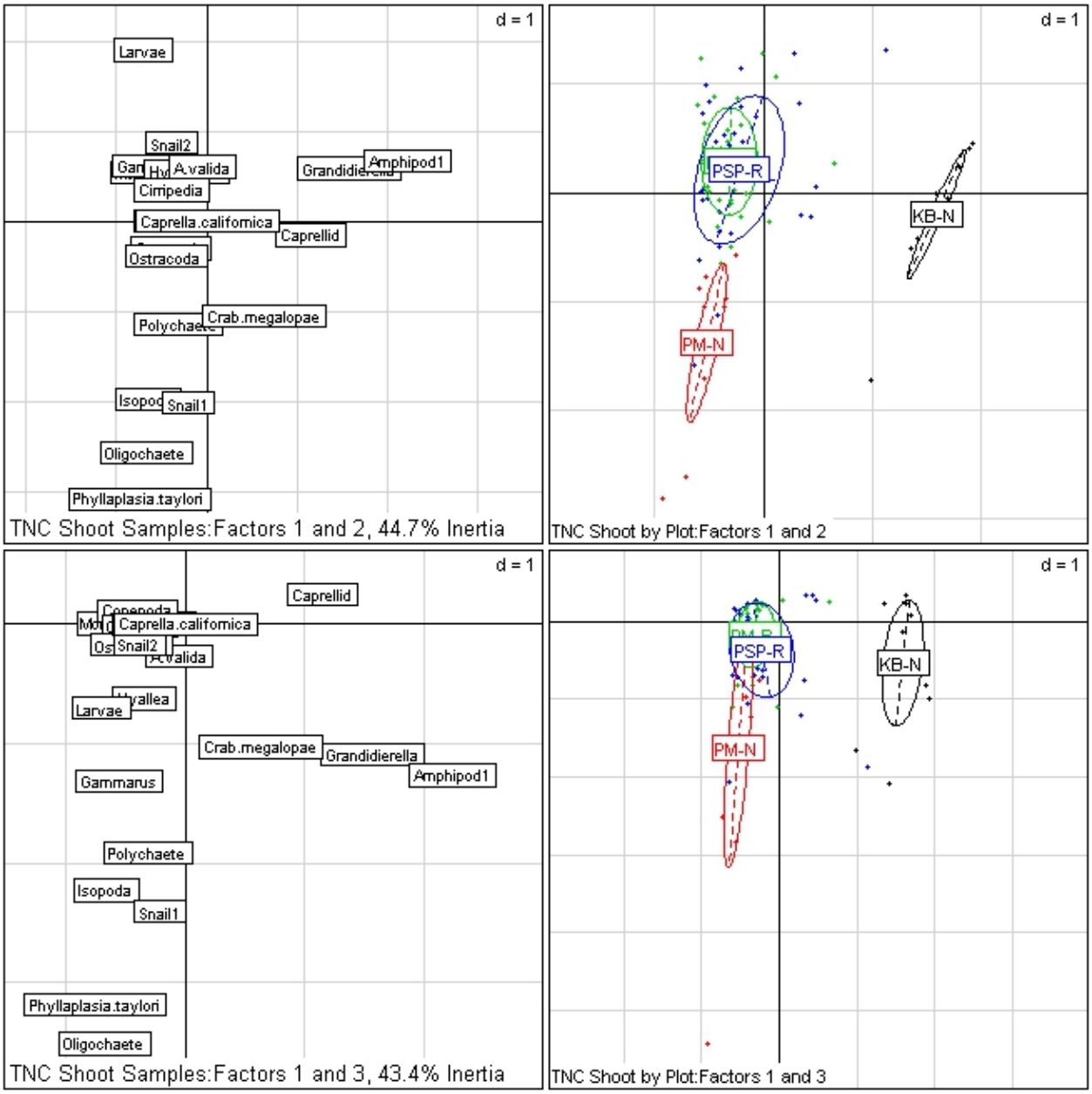


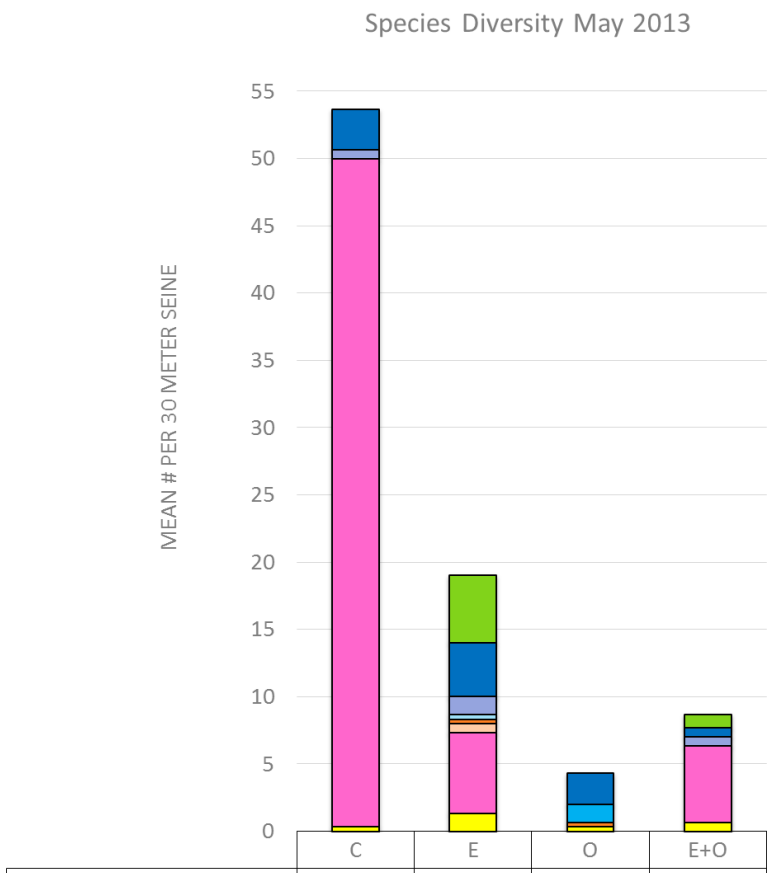
Figure 42. Correspondence analysis of shoot samples collected during Spring 2014 at TNC, Keller Beach, and Point Molate- shown by source, with Idotea removed to spread results

KB-N= Keller Beach natural population

PM-N= Point Molate natural population

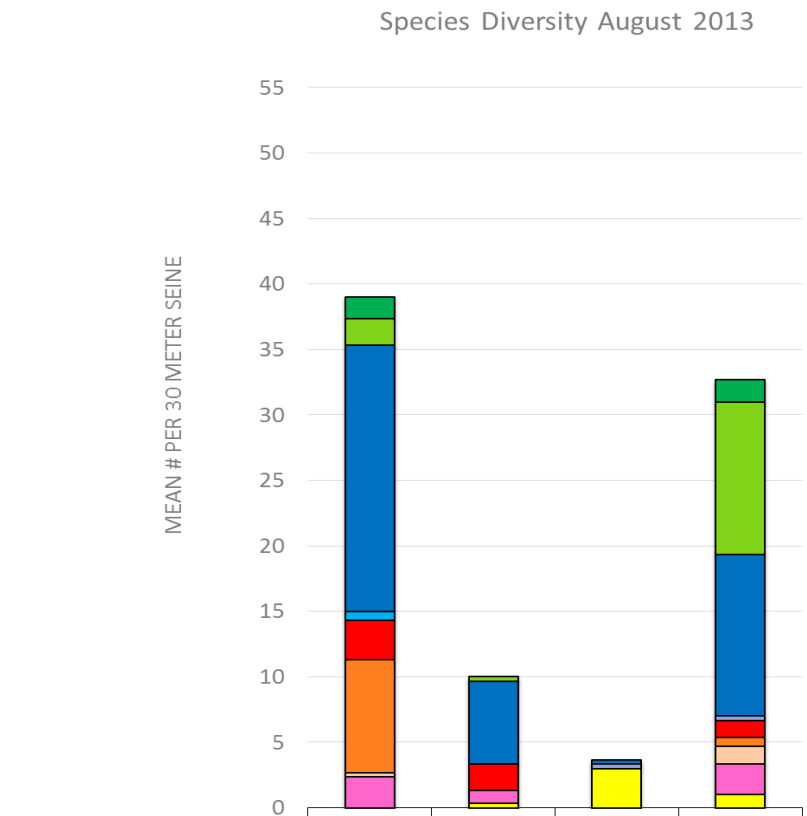
PSP-R= TNC restored population sourced from Point San Pablo

PM-R= TNC restored population sourced from Point Molate



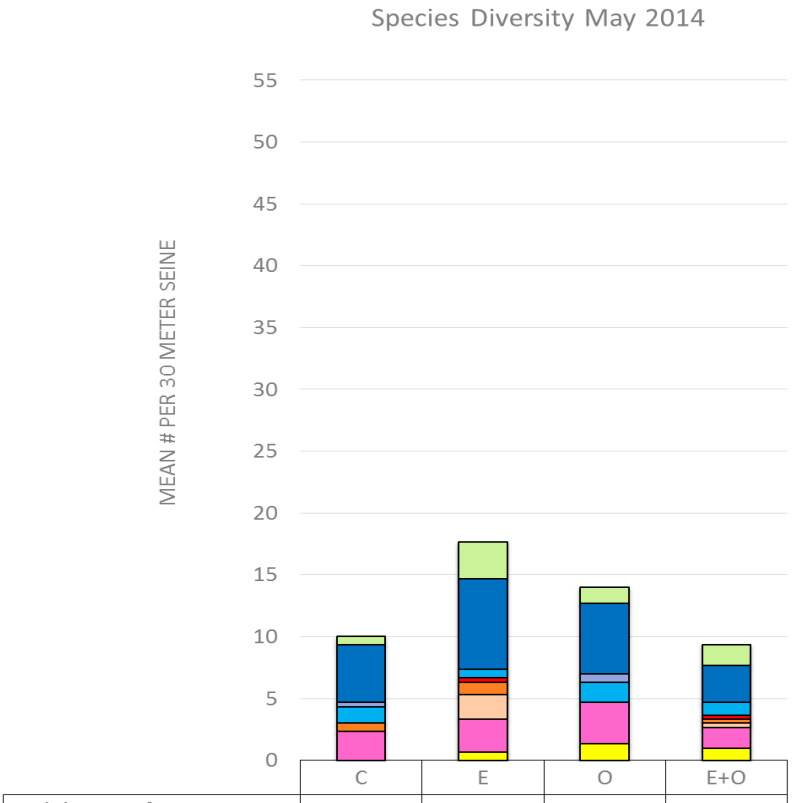
	C	E	O	E+O
Philine auriformis	0.00	0.00	0.00	0.00
shrimp spp. TBD	0.00	5.00	0.00	1.00
Palaemon macrodactylus	0.00	0.00	0.00	0.00
Crangon franciscorum	3.00	4.00	2.33	0.67
Metacarcinus gracilis	0.67	1.33	0.00	0.67
Hemigrapsus oregonensis	0.00	0.00	1.33	0.00
Metacarcinus magister	0.00	0.33	0.00	0.00
Cymatogaster aggregata	0.00	0.00	0.00	0.00
Pholis ornata	0.00	0.00	0.00	0.00
Leptocottus armatus	0.00	0.33	0.33	0.00
Syngnathus leptorhynchus	0.00	0.67	0.00	0.00
Atherinopsis californiensis	49.67	6.00	0.00	5.67
Lepidogobius lepidus	0.33	1.33	0.33	0.67

Figure 43. Mean number of individuals caught per seine at TNC in May 2013. C = Control, E = Eelgrass plot, O = Oyster plot, E+O = Eelgrass and Oyster plot.



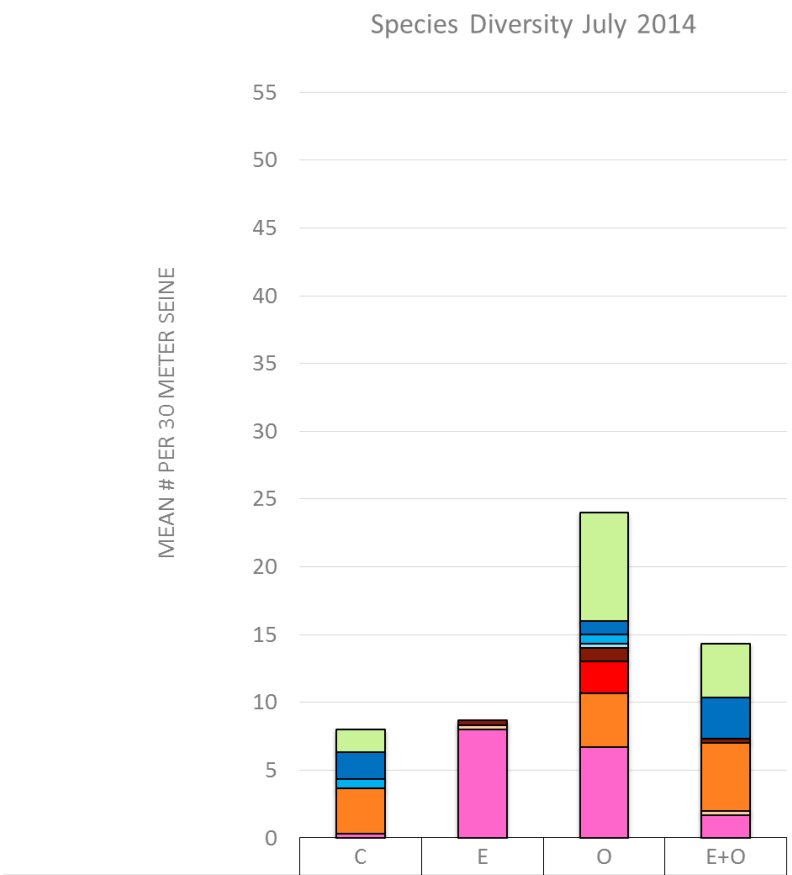
	C	E	O	E+O
Philine auriformis	1.67	0.00	0.00	1.67
shrimp spp. TBD	2.00	0.33	0.00	11.67
Palaemon macrodactylus	0.00	0.00	0.00	0.00
Crangon franciscorum	20.33	6.33	0.33	12.33
Metacarcinus gracilis	0.00	0.00	0.33	0.33
Hemigrapsus oregonensis	0.67	0.00	0.00	0.00
Metacarcinus magister	0.00	0.00	0.00	0.00
Cymatogaster aggregata	0.00	0.00	0.00	0.00
Pholis ornata	3.00	2.00	0.00	1.33
Leptocottus armatus	8.67	0.00	0.00	0.67
Syngnathus leptorhynchus	0.33	0.00	0.00	1.33
Atherinopsis californiensis	2.33	1.00	0.00	2.33
Lepidogobius lepidus	0.00	0.33	3.00	1.00

Figure 44. Mean number of individuals caught per seine at TNC in August 2013. C = Control, E = Eelgrass plot, O = Oyster plot, E+O = Eelgrass and Oyster plot.



	C	E	O	E+O
Philine auriformis	0	0	0	0
shrimp spp. TBD	0	0	0	0
Palaemon macrodactylus	0.667	3	1.333	1.667
Crangon franciscorum	4.667	7.333	5.667	3
Metacarcinus gracilis	0.333	0	0.667	0
Hemigrapsus oregonensis	1.333	0.667	1.667	1
Metacarcinus magister	0	0	0	0
Cymatogaster aggregata	0	0	0	0
Pholis ornata	0	0.333	0	0.333
Leptocottus armatus	0.667	1	0	0.333
Syngnathus leptorhynchus	0	2	0	0.333
Atherinopsis californiensis	2.333	2.667	3.333	1.667
Lepidogobius lepidus	0	0.667	1.333	1

Figure 45. Mean number of individuals caught per seine at TNC in May 2014. C = Control, E = Eelgrass plot, O = Oyster plot, E+O = Eelgrass and Oyster plot.



	C	E	O	E+O
Philine auriformis	0	0	0	0
shrimp spp. TBD	0	0	0	0
Palaemon macrodactylus	1.667	0	8	4
Crangon franciscorum	2	0	1	3
Metacarcinus gracilis	0	0	0	0
Hemigrapsus oregonensis	0.667	0	0.667	0
Metacarcinus magister	0	0	0.333	0
Cymatogaster aggregata	0	0.333	1	0.333
Pholis ornata	0	0	2.333	0
Leptocottus armatus	3.333	0	4	5
Syngnathus leptorhynchus	0	0.333	0	0.333
Atherinopsis californiensis	0.333	8	6.667	1.667
Lepidogobius lepidus	0	0	0	0

Figure 46. Mean number of individuals caught per seine at TNC in July 2014. C = Control, E = Eelgrass plot, O = Oyster plot, E+O = Eelgrass and Oyster plot.

N.B. In the following figures, all temperatures are in degrees centigrade. For figures 47-74: OY= Oyster plot, EG= Eelgrass plot, EO = Eelgrass+Oyster plot, SC = Small (plot) control, LC = Large (whole site) control.

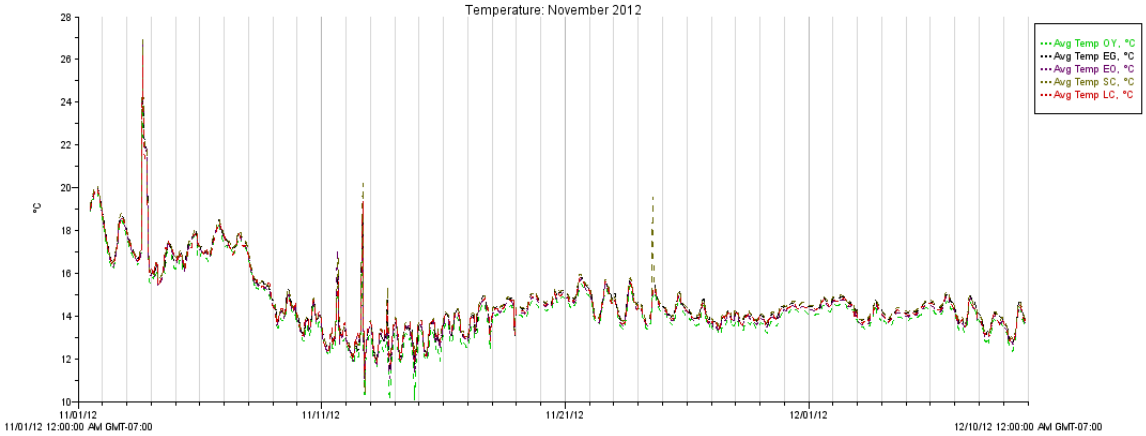


Figure 47: Average hourly temperature at TNC in November 2012

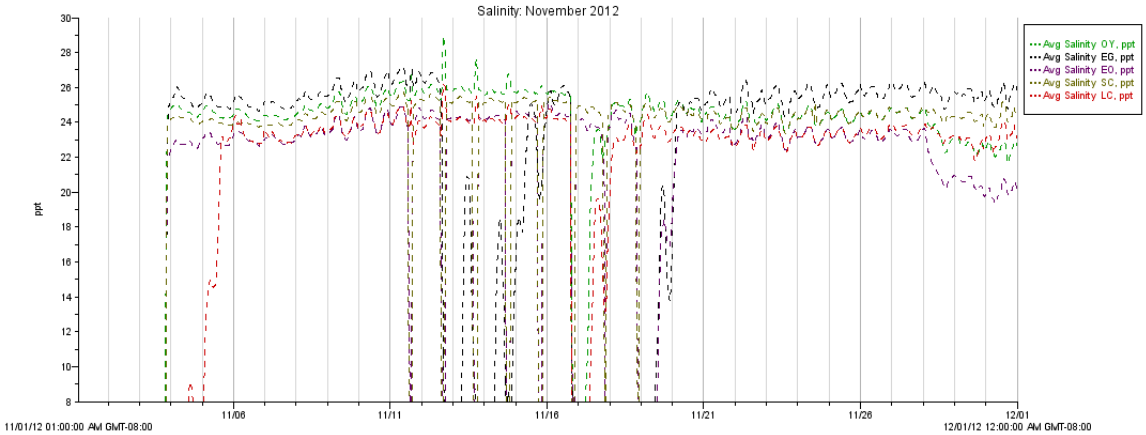


Figure 48: Average hourly salinity at TNC in November 2012

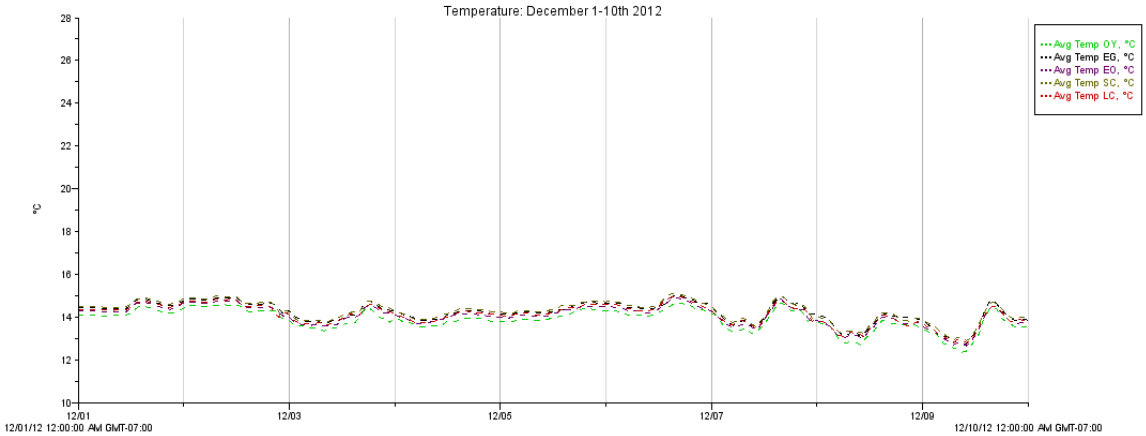


Figure 49: Average hourly temperature at TNC in December 1-10th 2012

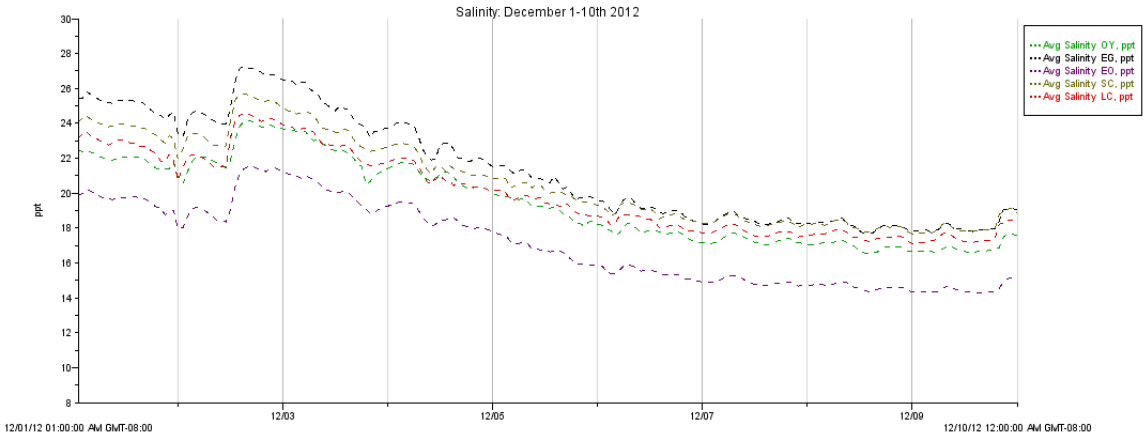


Figure 50: Average hourly salinity at TNC in December 1-10th 2012

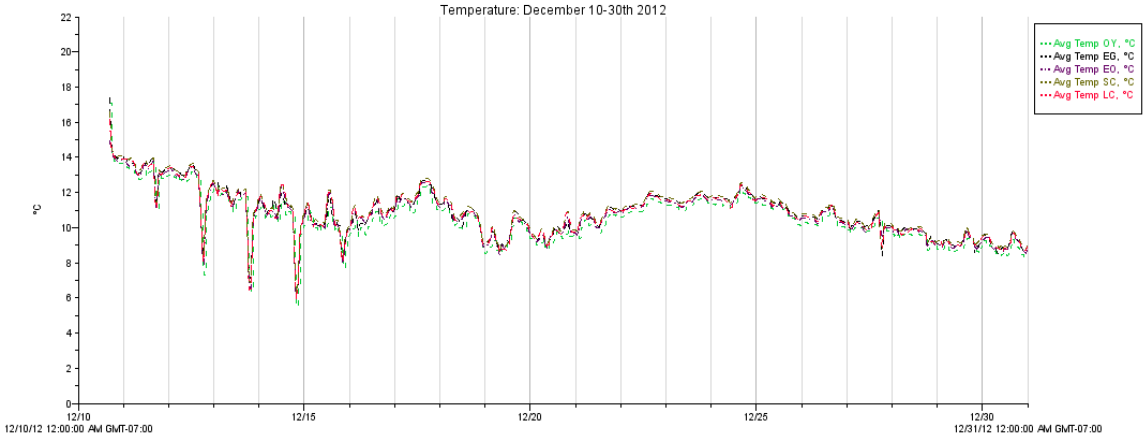


Figure 51: Average hourly temperature at TNC in December 10-30sth2012

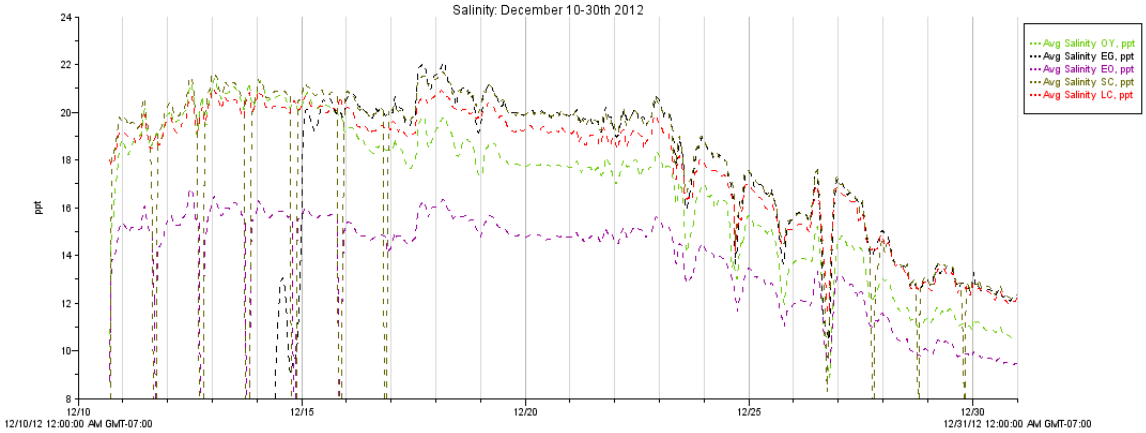


Figure 52: Average hourly salinity at TNC in December 10-30th 2012

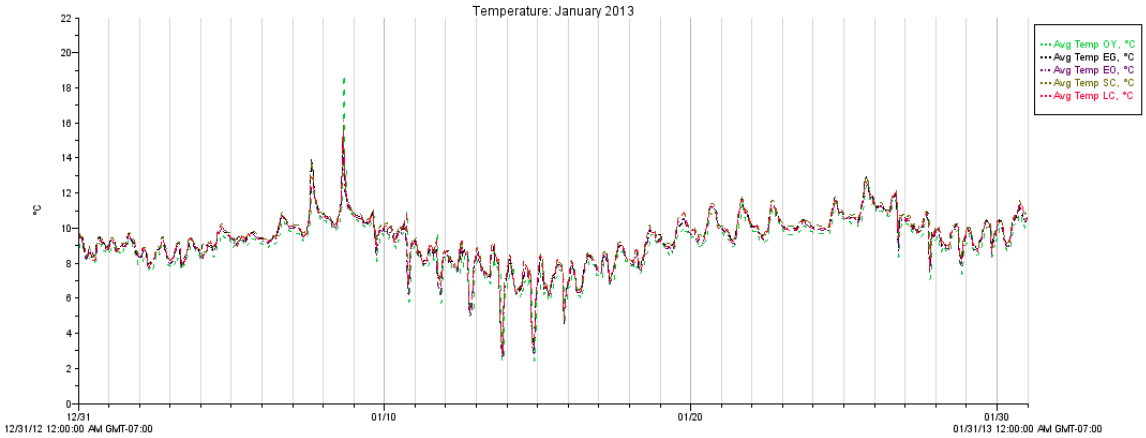


Figure 53: Average hourly temperature at TNC in January 2013

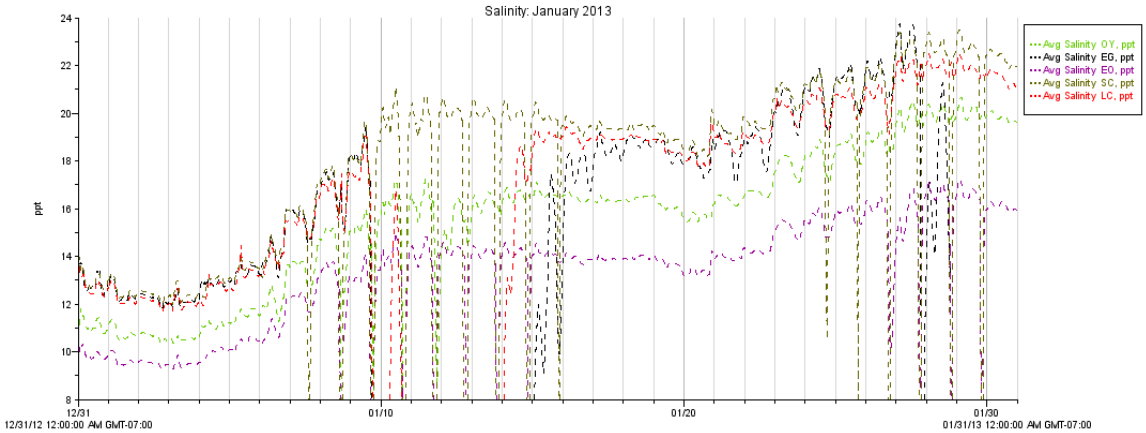


Figure 54: Average hourly salinity at TNC in January 2013

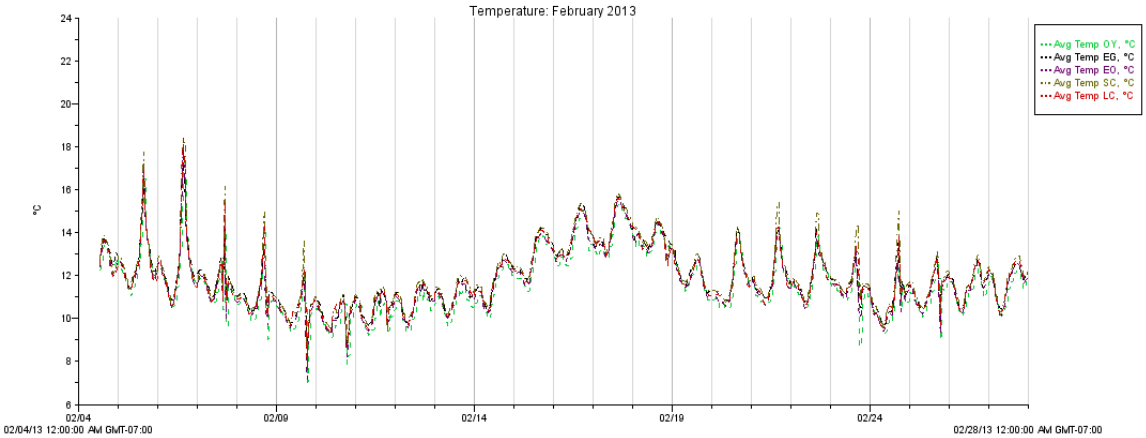


Figure 55: Average hourly temperature at TNC in February 2013

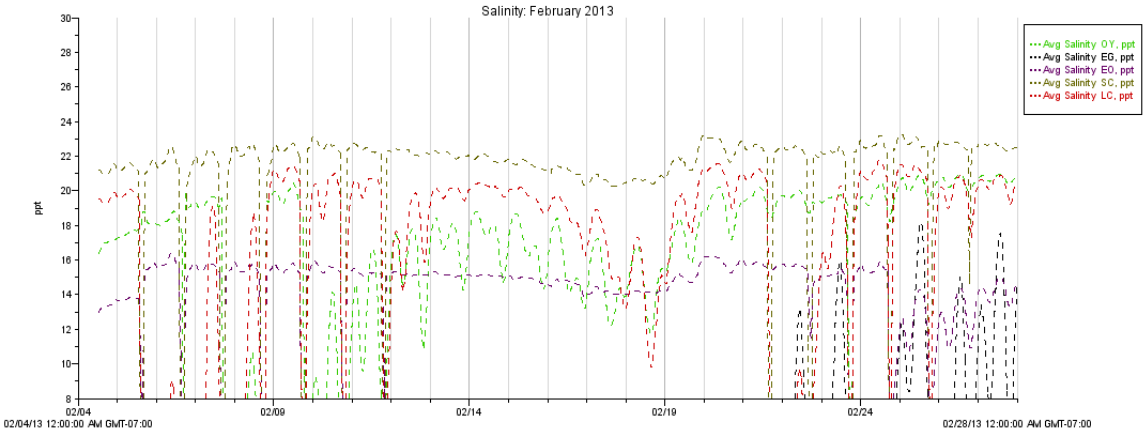


Figure 56: Average hourly salinity at TNC in February 2013

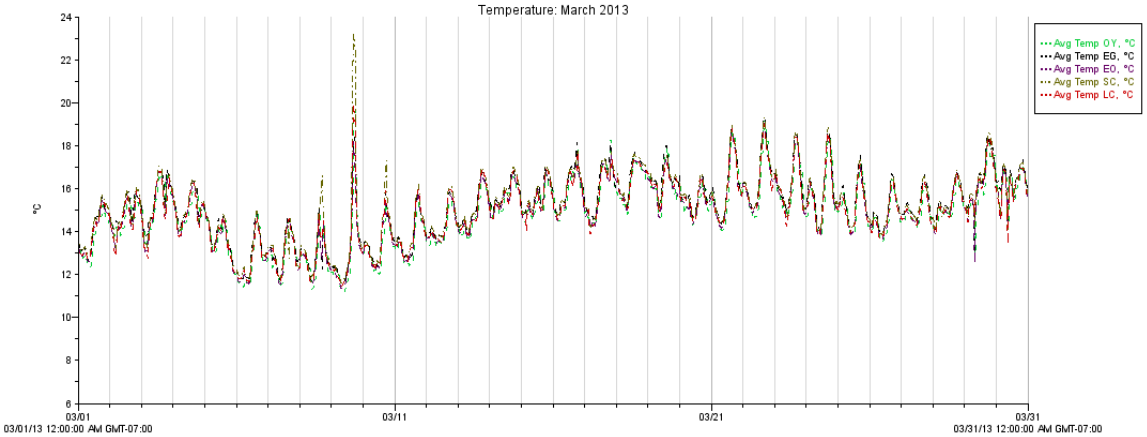


Figure 57: Average hourly temperature at TNC in March 2013

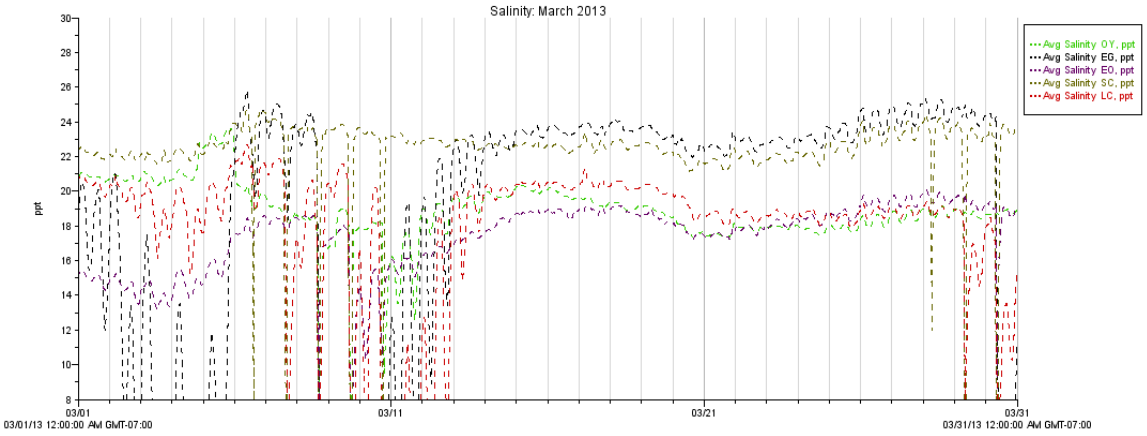


Figure 58: Average hourly salinity at TNC in March 2013

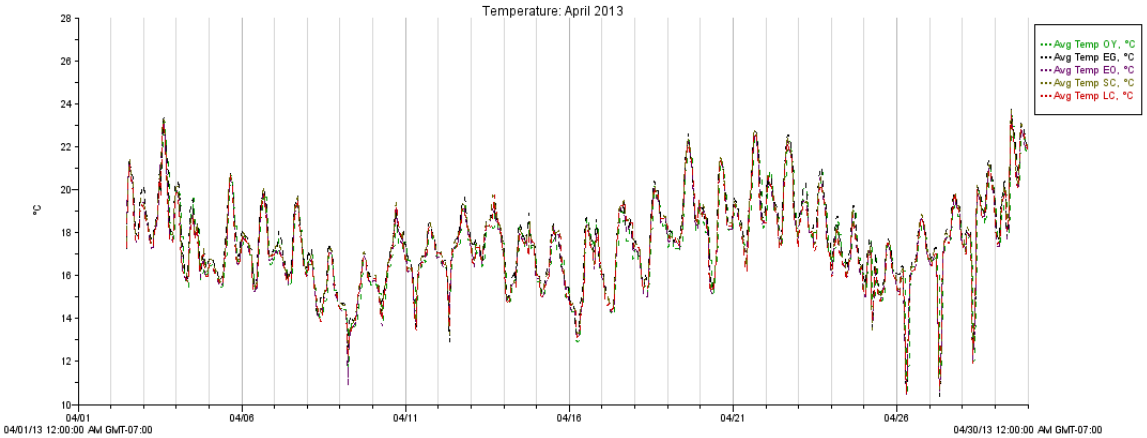


Figure 59: Average hourly temperature at TNC in April

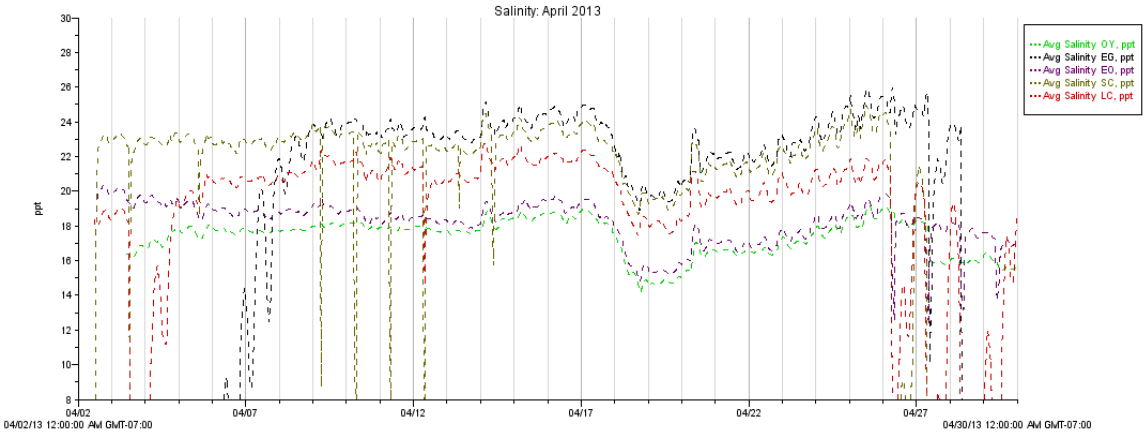


Figure 60: Average hourly salinity at TNC in April 2013

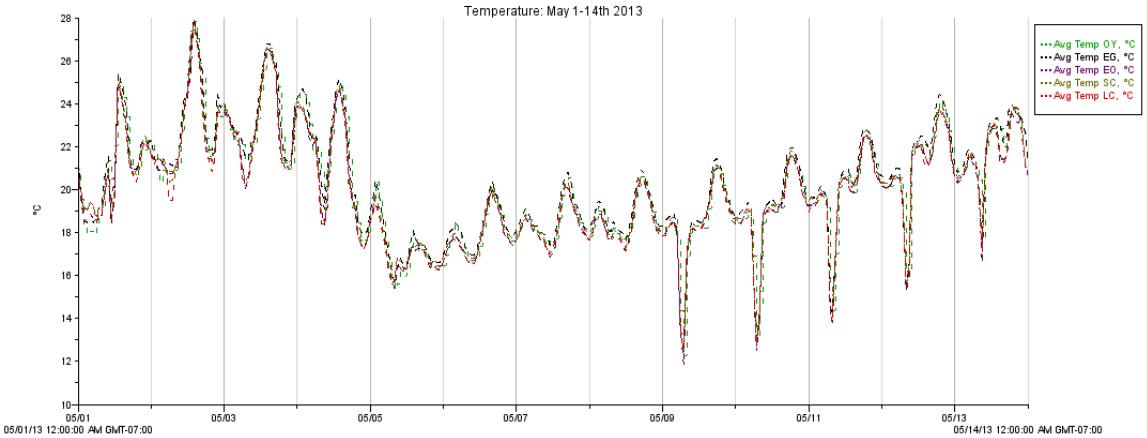


Figure 61: Average hourly temperature at TNC in May1-14th 2013

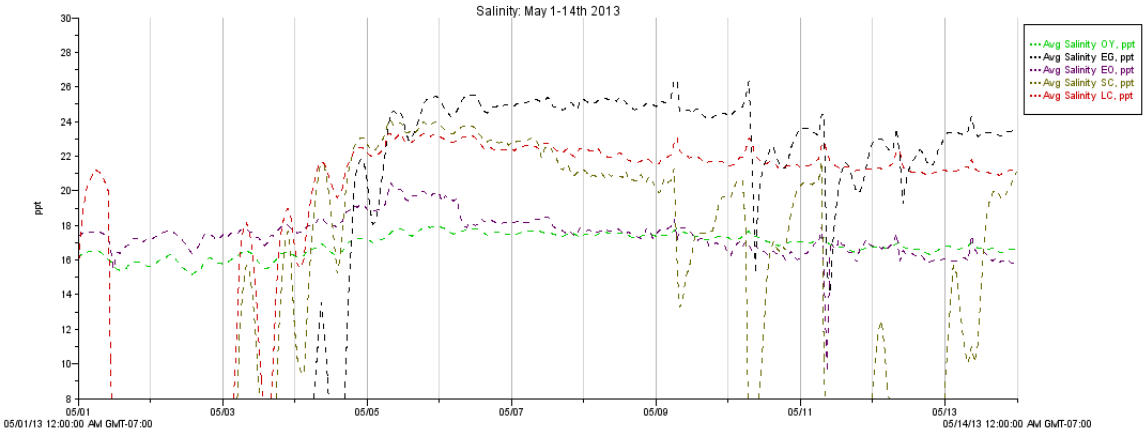


Figure 62: Average hourly salinity at TNC in May 1-14th 2013

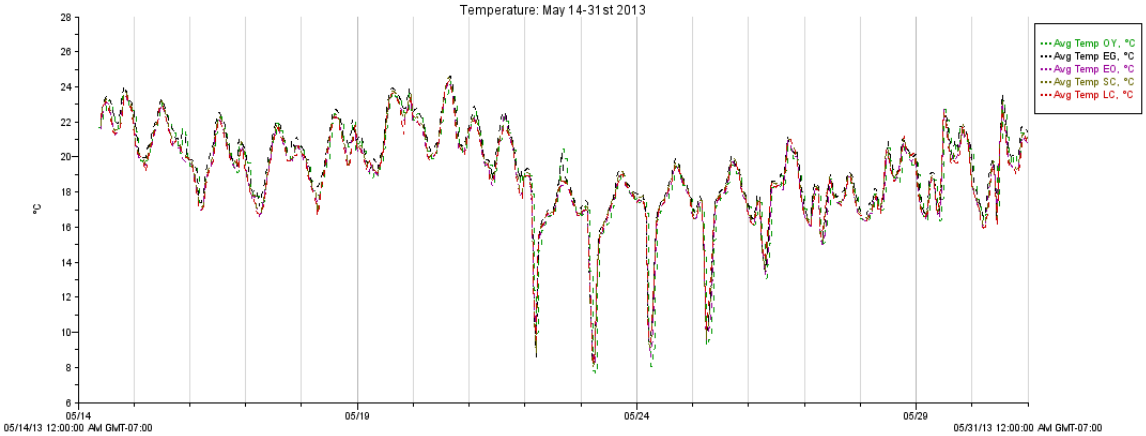


Figure 63: Average hourly temperature at TNC in May 14-31st 2013

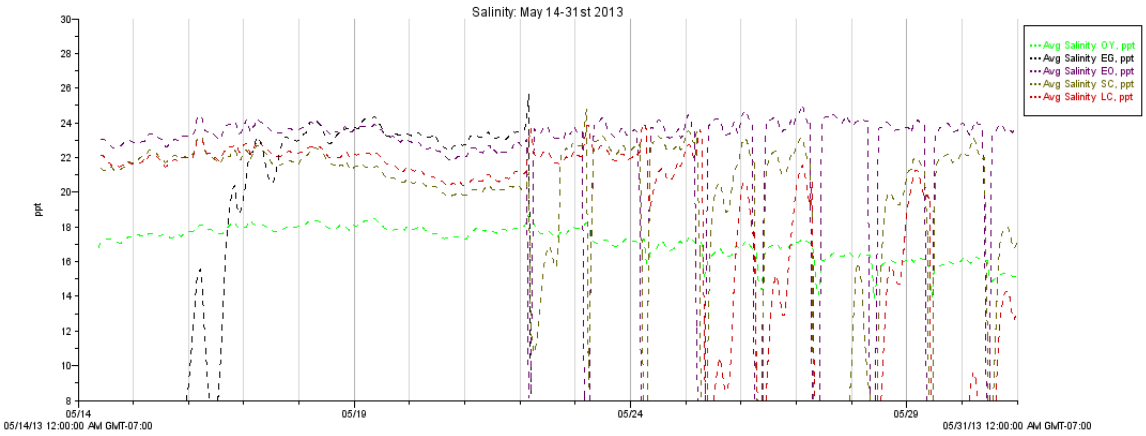


Figure 64: Average hourly salinity at TNC in May 14-31st 2013

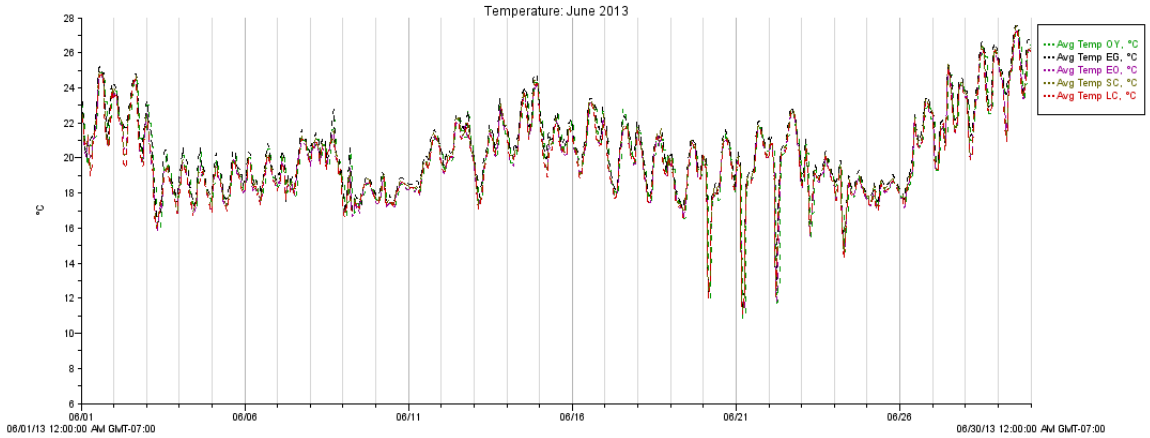


Figure 65: Average hourly temperature at TNC in June 2013

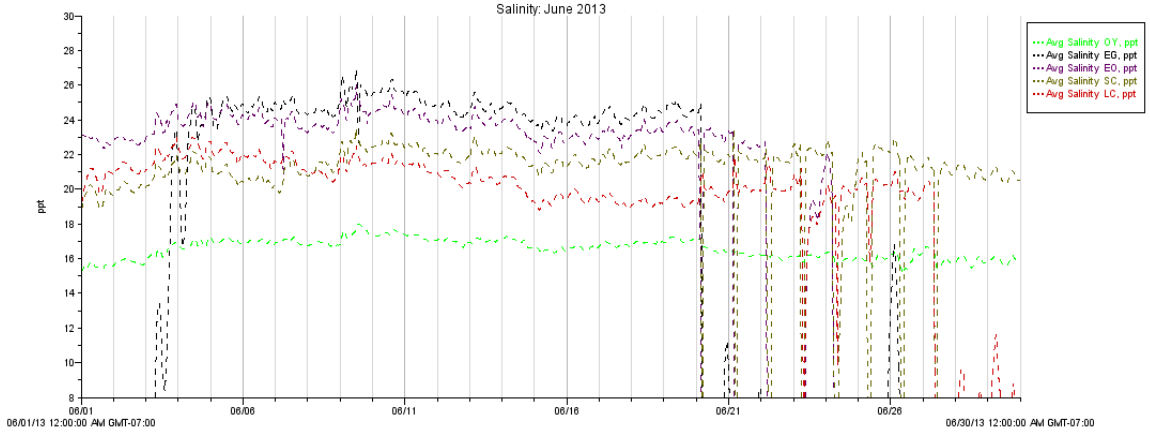


Figure 66: Average hourly salinity at TNC in June 2013

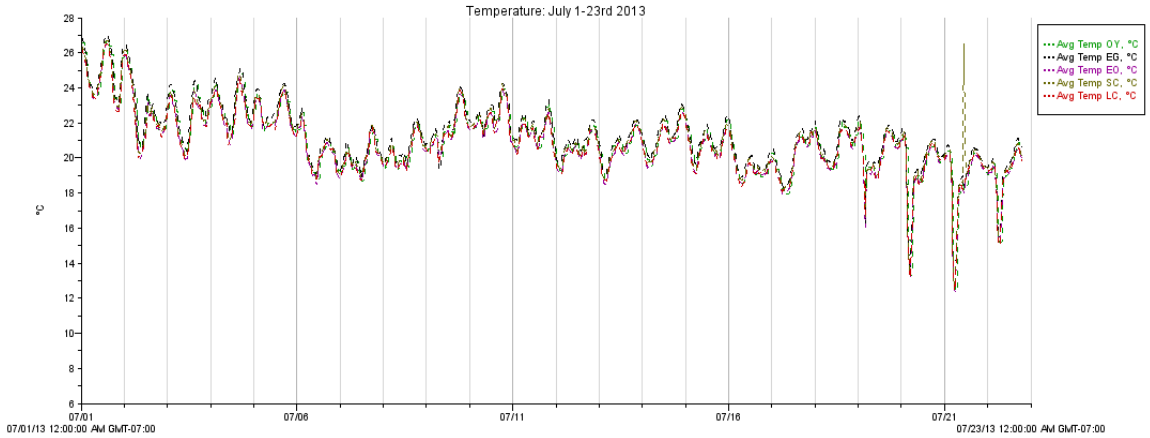


Figure 67: Average hourly temperature at TNC in July 1-23rd 2013

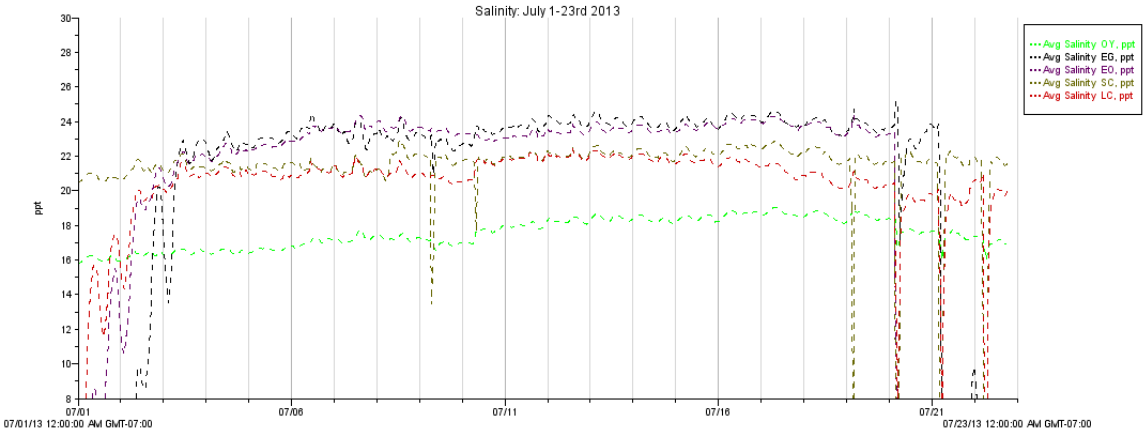


Figure 68: Average hourly salinity at TNC in July 1-23rd 2013

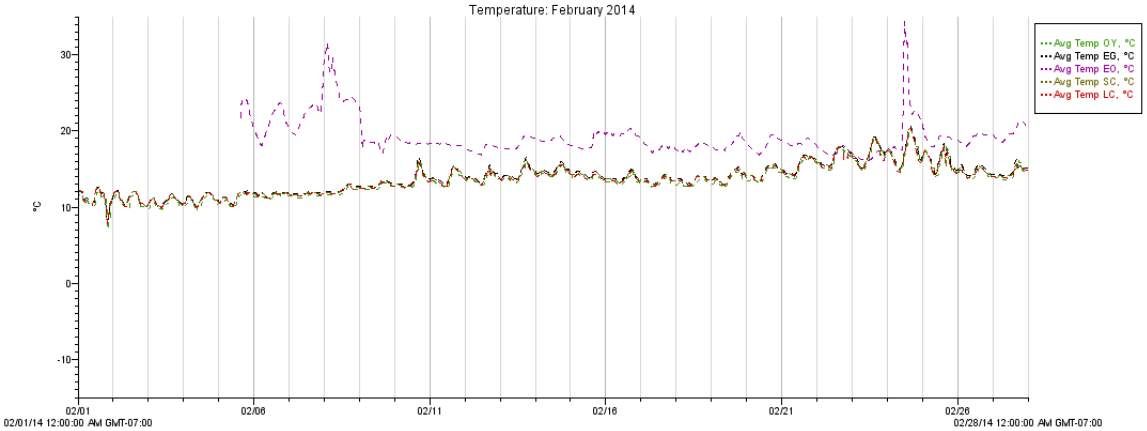


Figure 69: Average hourly temperature at TNC in February 2014

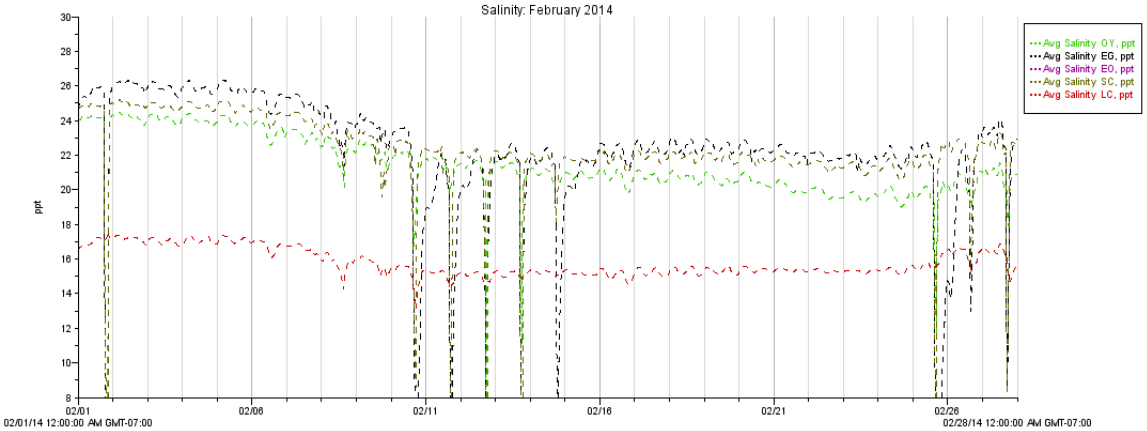


Figure 70: Average hourly salinity at TNC in February 2014

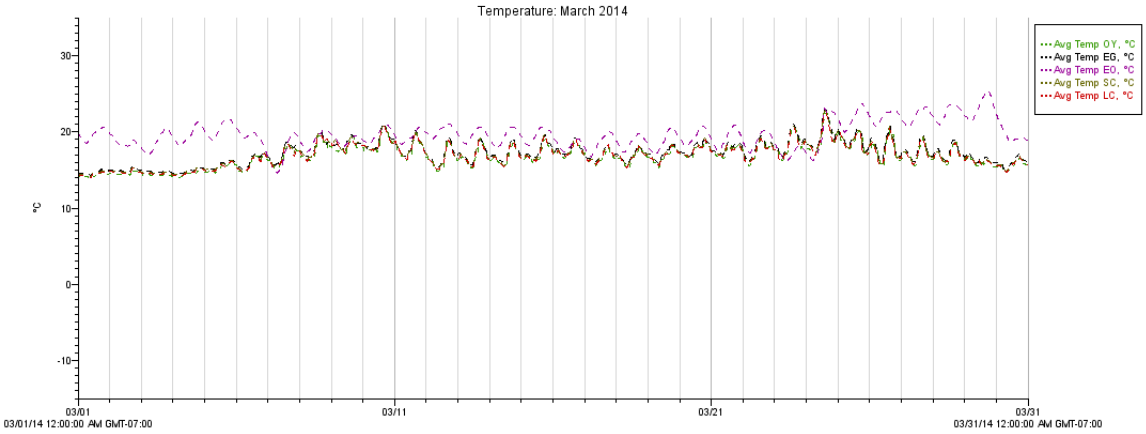


Figure 71: Average hourly temperature at TNC in March 2014

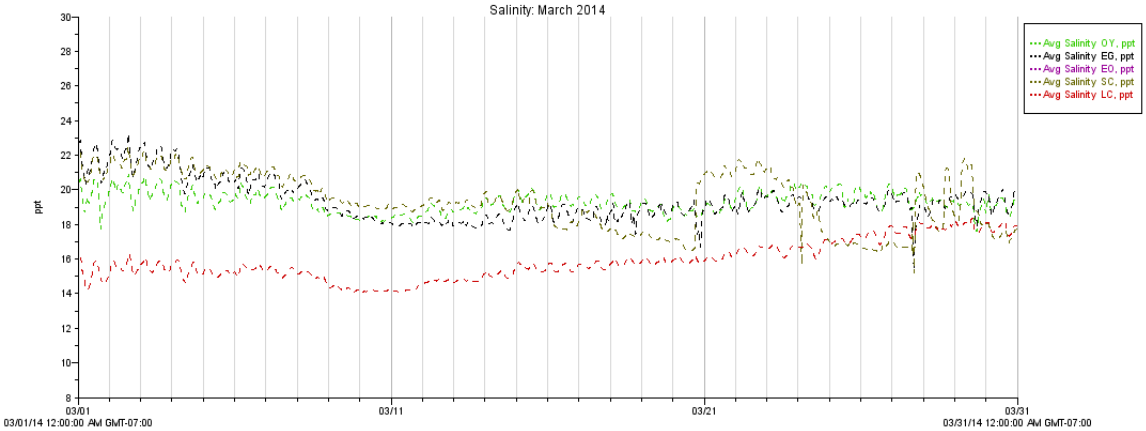


Figure 72: Average hourly salinity at TNC in March 2014

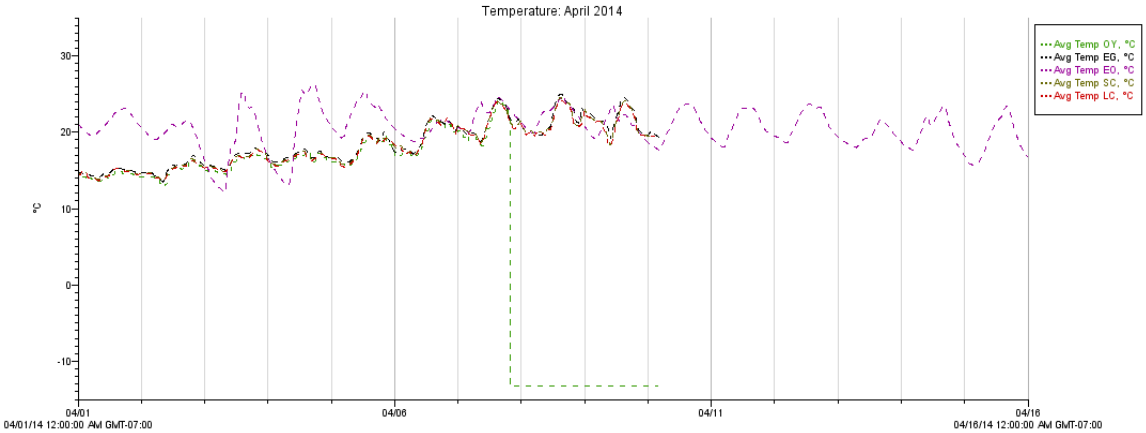


Figure 73: Average hourly salinity at TNC in April 2014

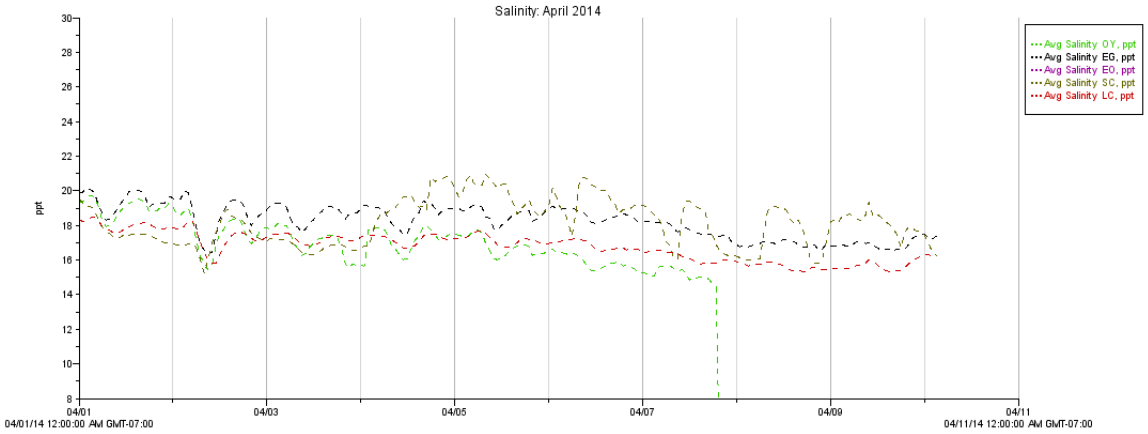


Figure 74: Average hourly salinity at TNC in April 2014

For figures 75 - 119: P1 OY block = Plot 1 (most northern), logger is adjacent to Oyster castle block, P2 EO = Plot 2 logger is adjacent to eelgrass+oyster combination, P3 EG = Plot 3 logger is adjacent to the eelgrass only unit, P5 OY ball = Plot 5 (most southern) logger is adjacent to the oyster reef ball, control = whole project control, north of the plots.

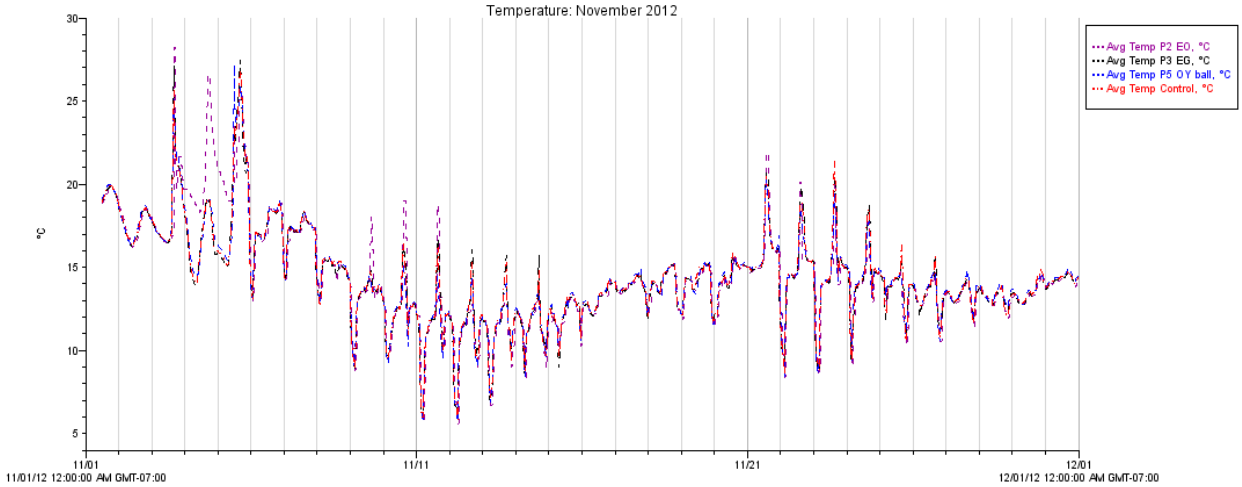


Figure 75: Average hourly temperature at ELER in November 2012

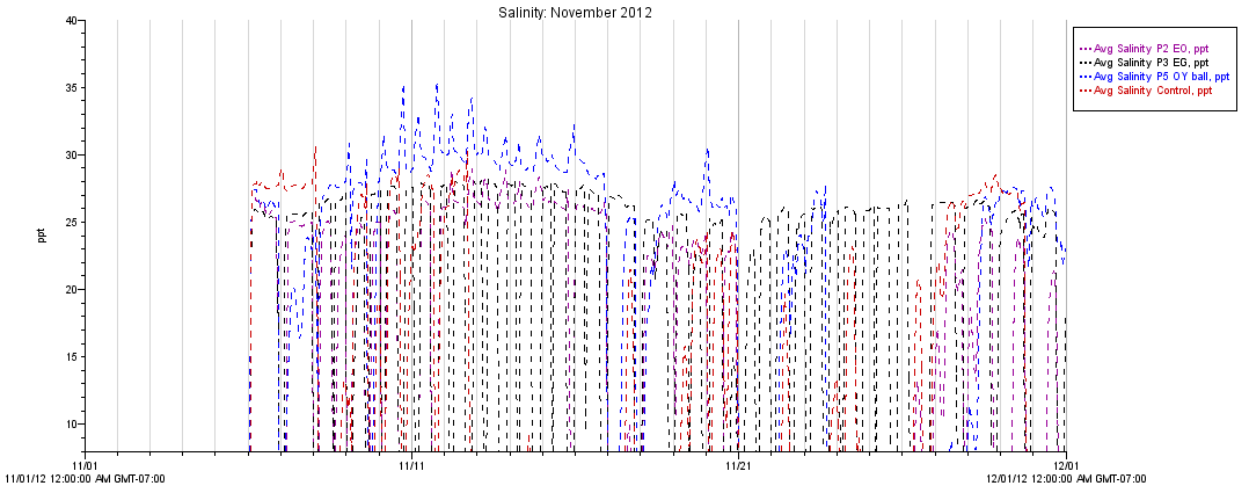


Figure 76: Average hourly salinity at ELER in November 2012

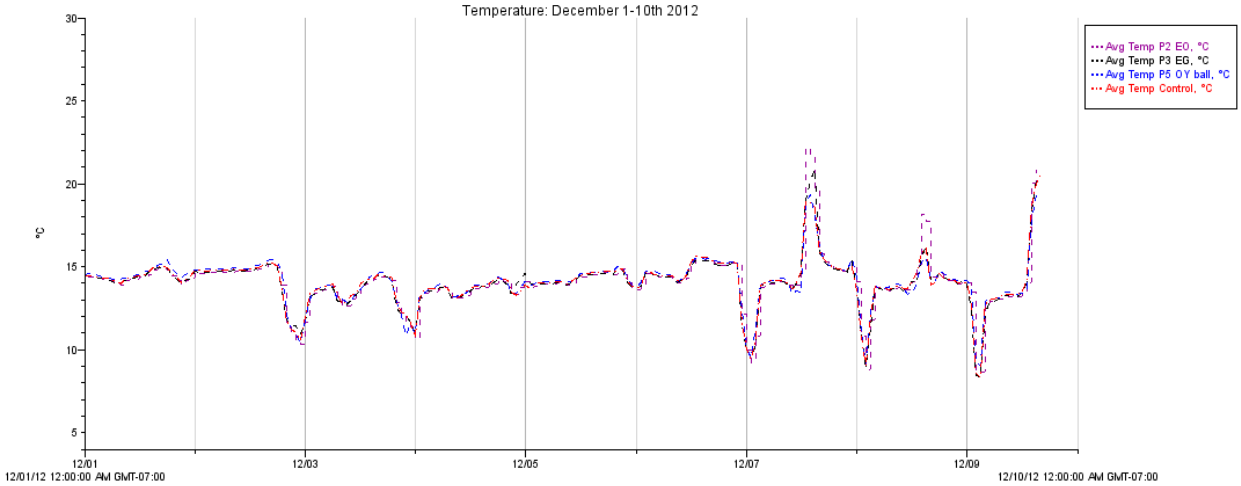


Figure 77: Average hourly temperature at ELER in December 1-10th 2012

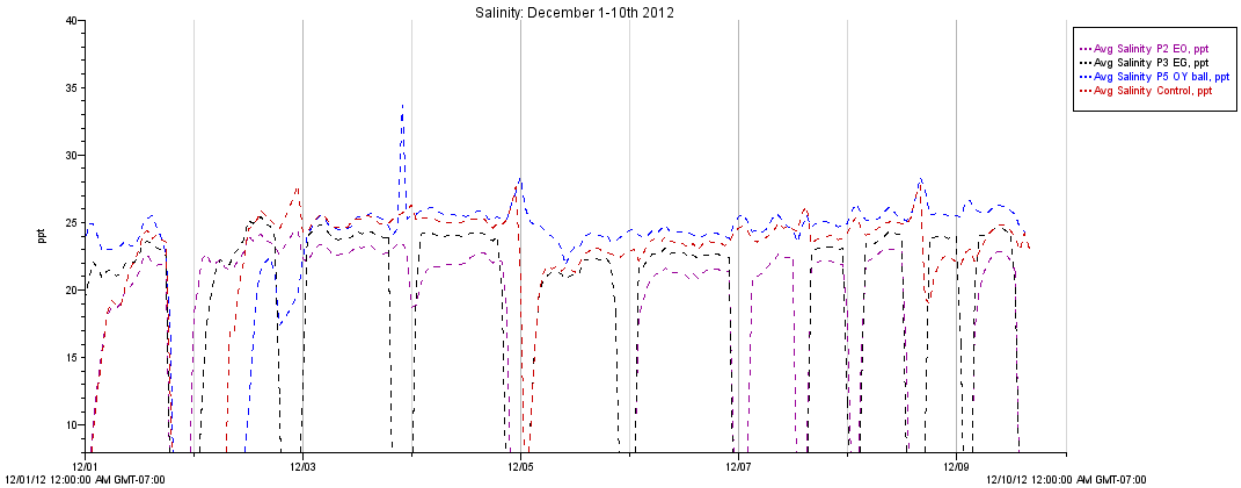


Figure 78: Average hourly salinity at ELER in December 1-10th 2012

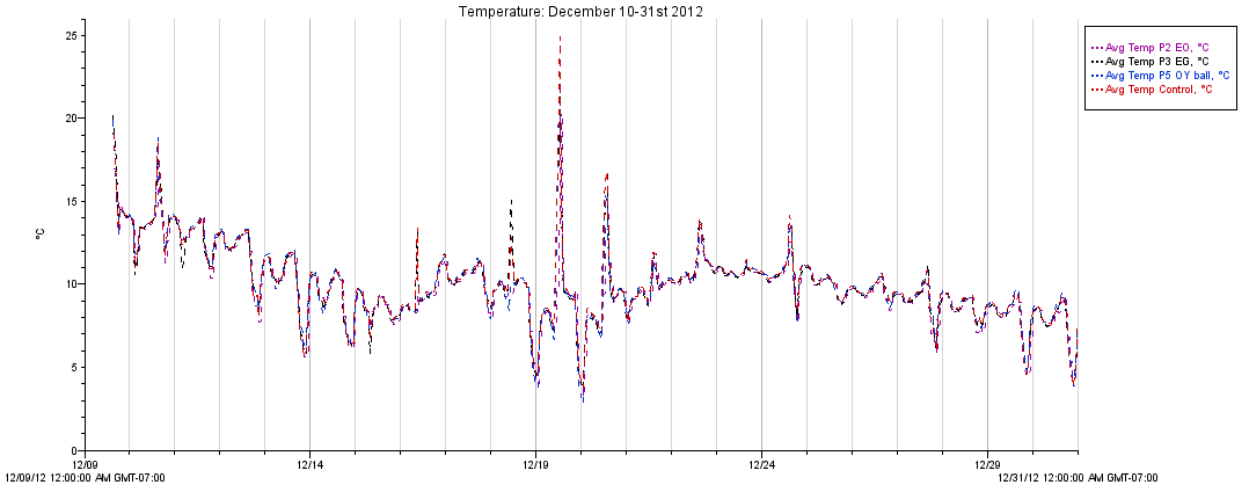


Figure 79: Average hourly temperature at ELER in December 10-31st 2012

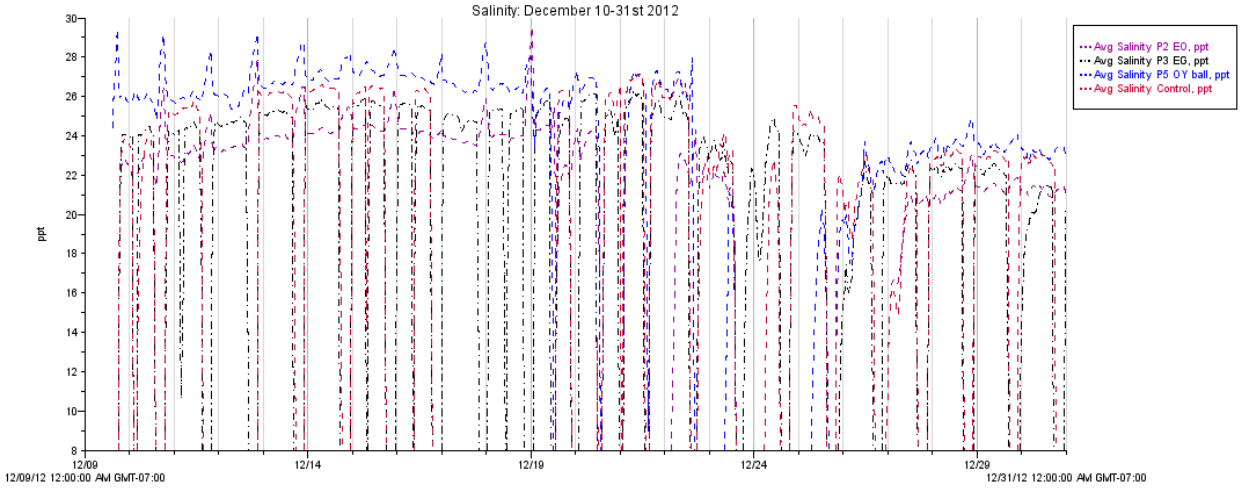


Figure 80: Average hourly salinity at ELER in December 10-31st 2012

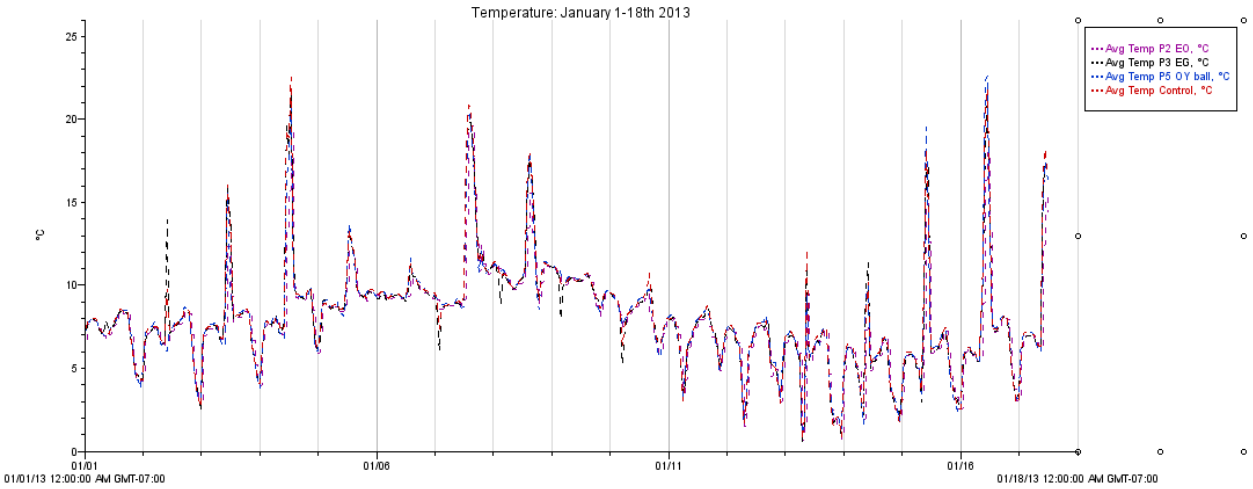


Figure 81: Average hourly temperature at ELER in January 1-18th 2013

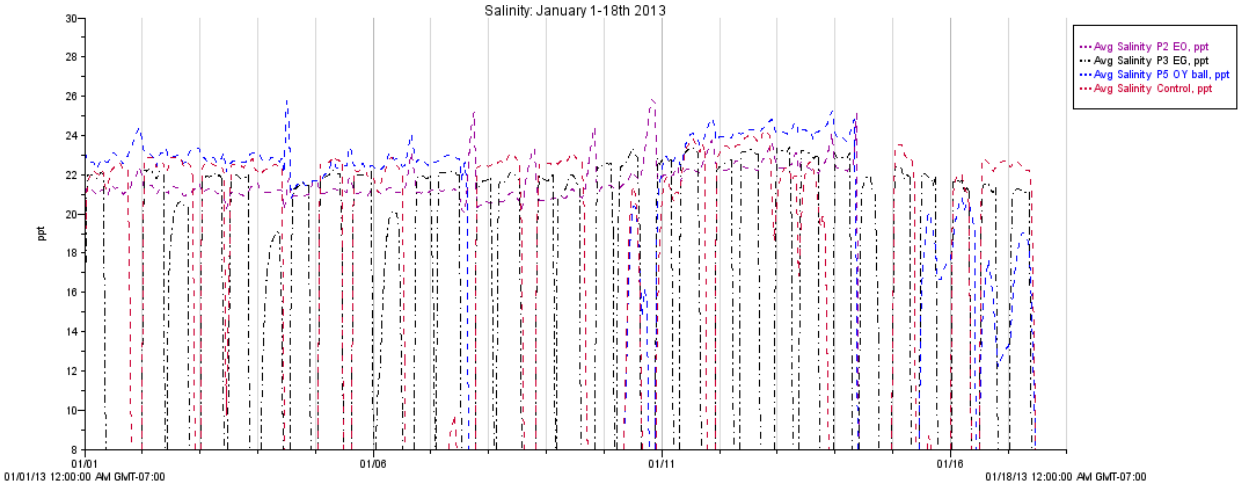


Figure 82: Average hourly salinity at ELER in January 1-18th 2013

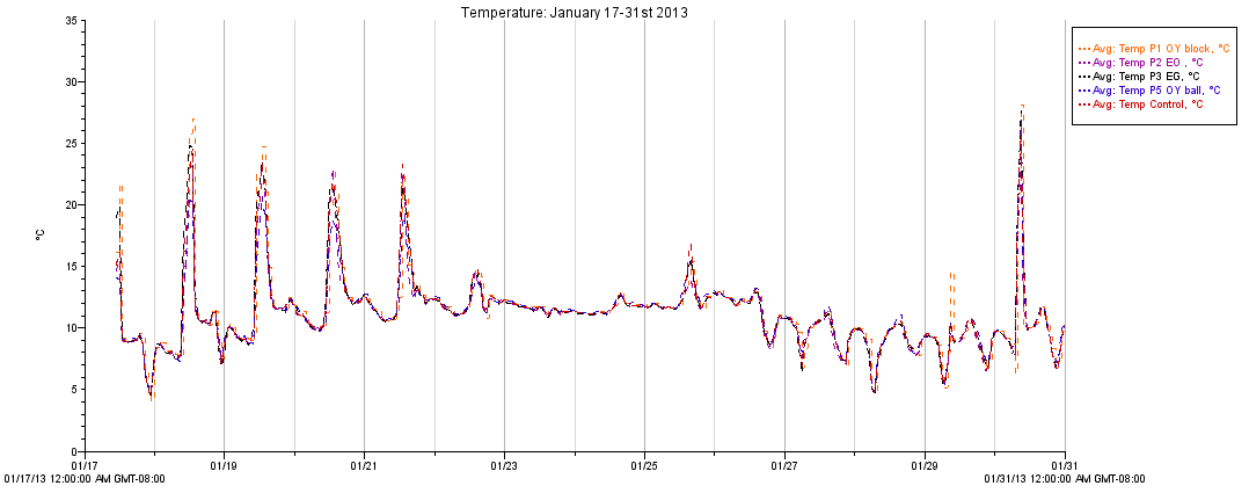


Figure 83: Average hourly temperature at ELER in January 17-31st 2013

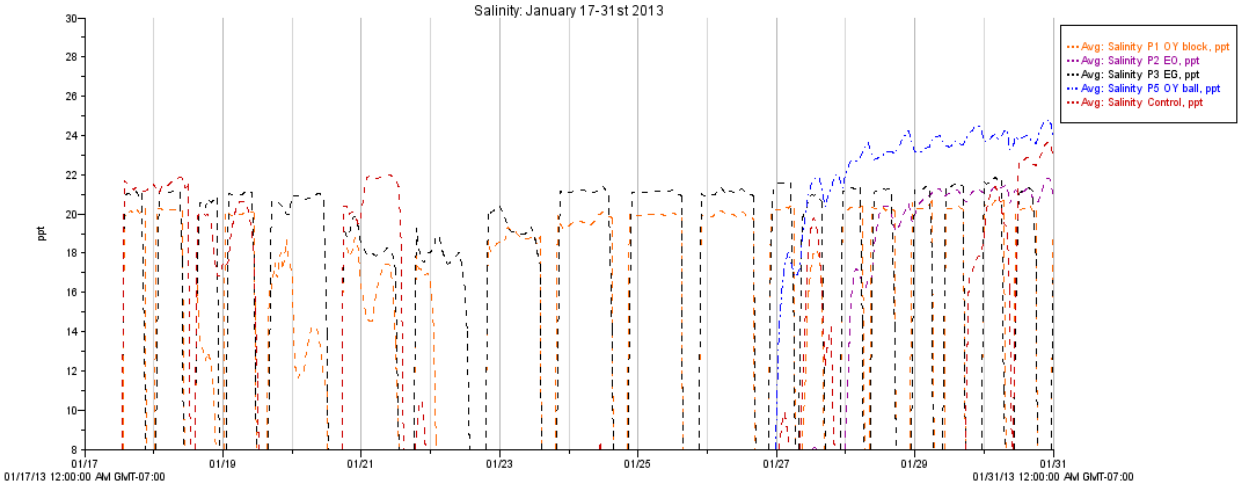


Figure 84: Average hourly salinity at ELER in January 17-31st 2013

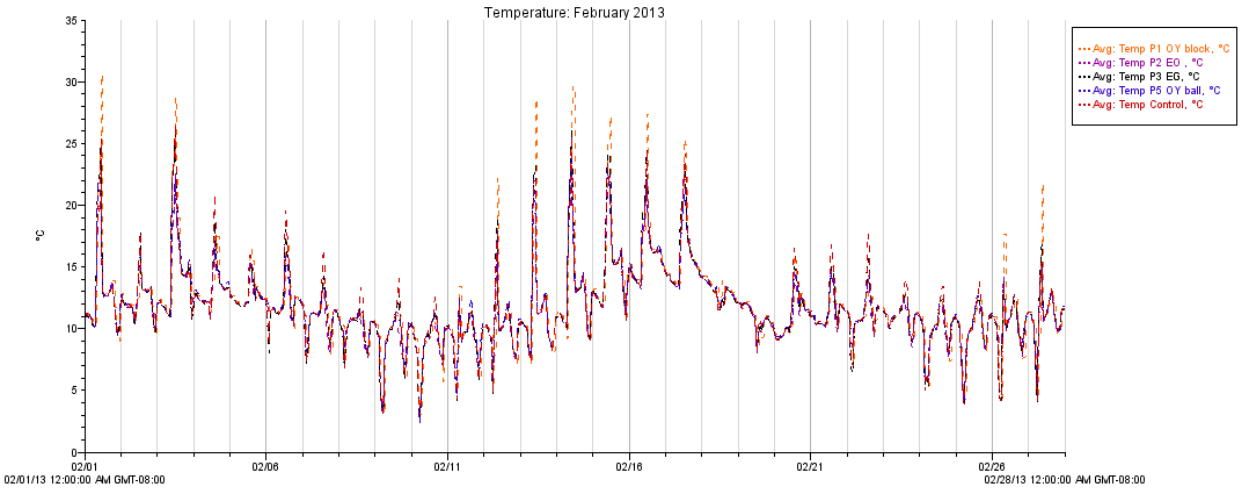


Figure 85: Average hourly temperature at ELER in February 2013

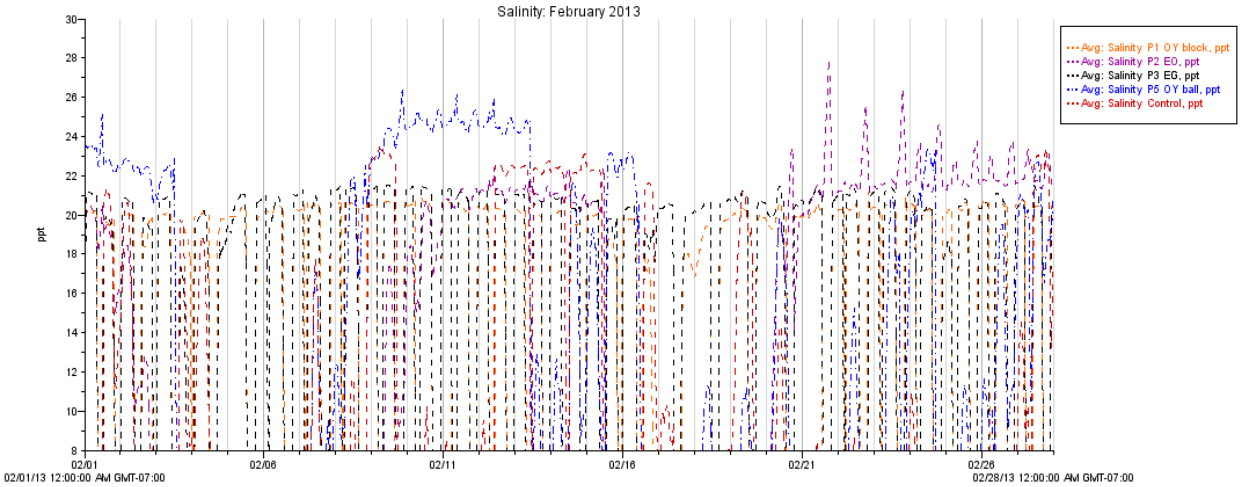


Figure 86: Average hourly salinity at ELER in February 2013

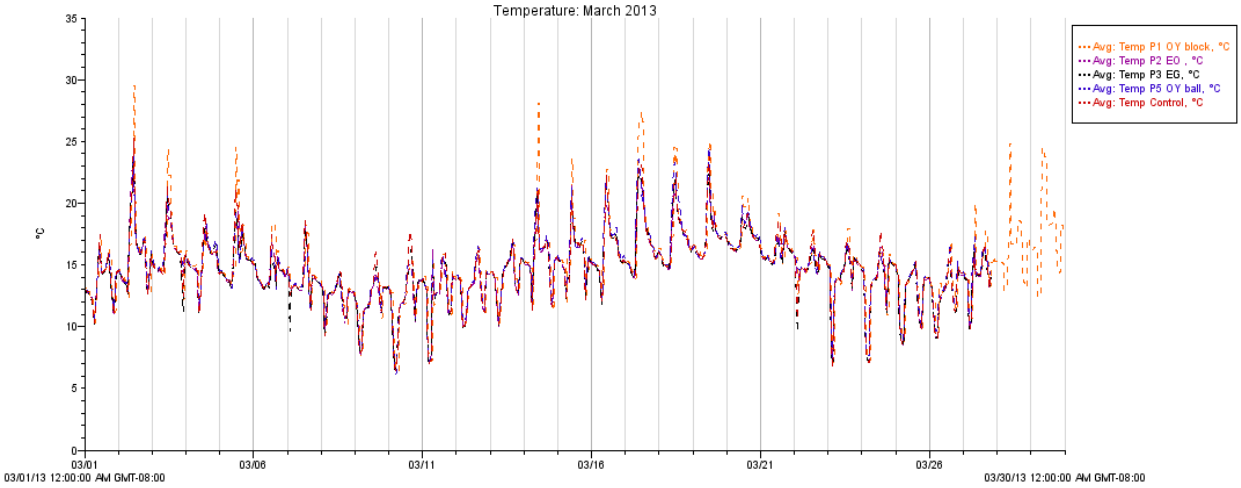


Figure 87: Average hourly temperature at ELER in March 2013

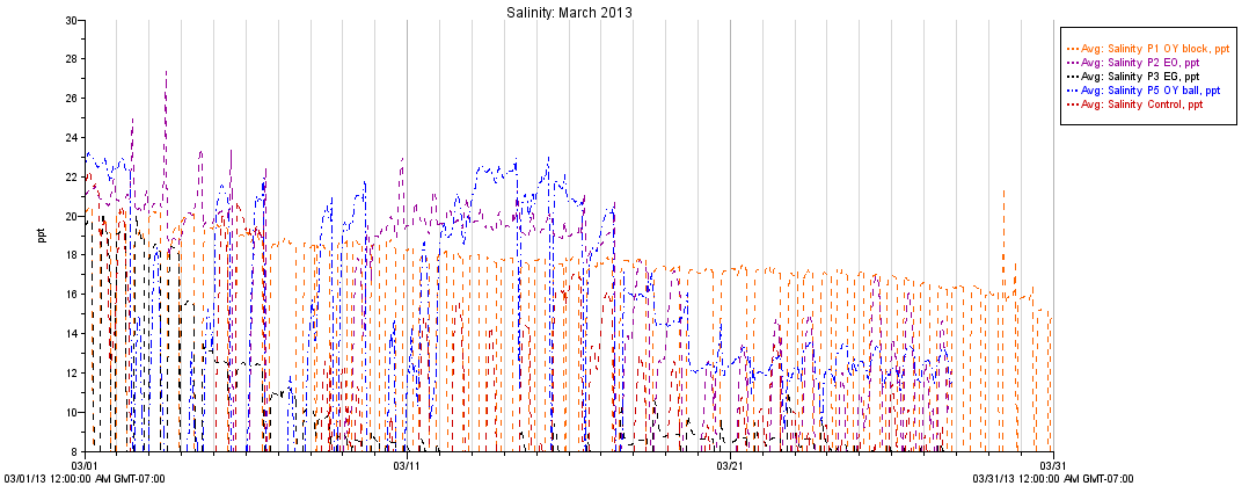


Figure 88: Average hourly salinity at ELER in March 2013

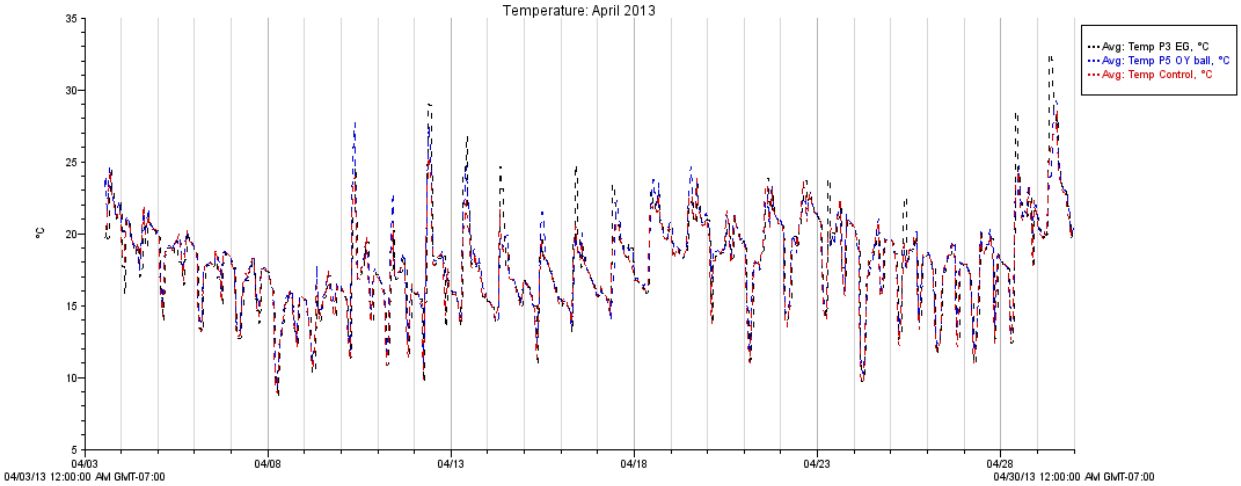


Figure 89: Average hourly temperature at ELER in April 2013

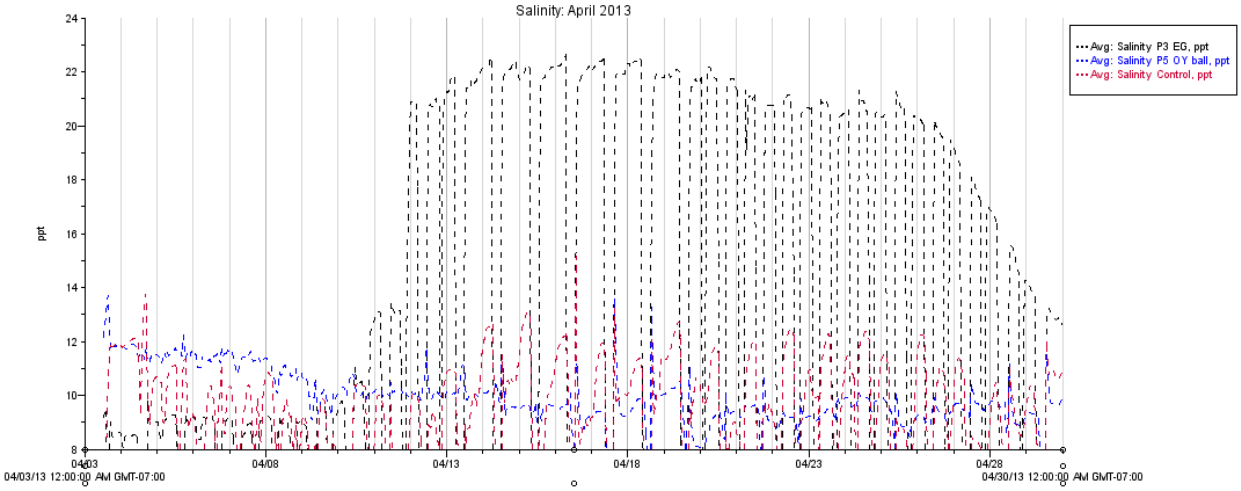


Figure 90: Average hourly salinity at ELER in April 2013

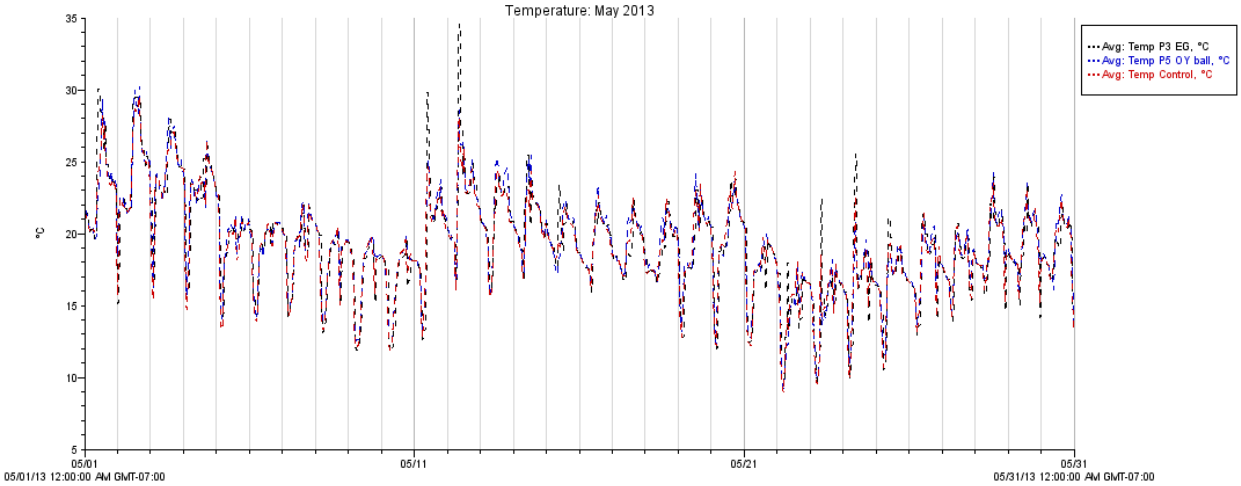


Figure 91: Average hourly temperature at ELER in May 2013

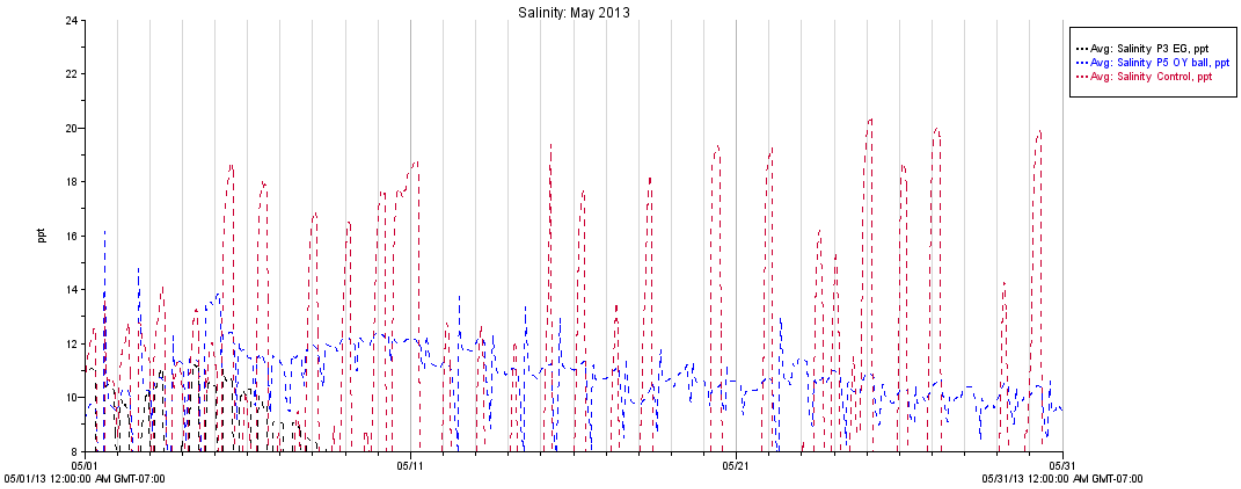


Figure 92: Average hourly salinity at ELER in May 2013

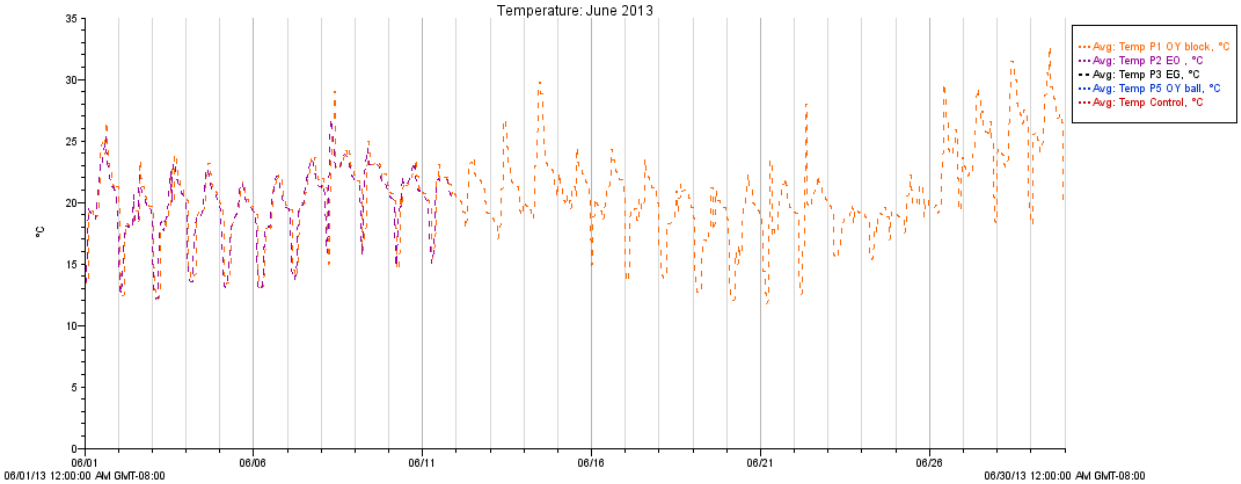


Figure 93: Average hourly temperature at ELER in June 2013

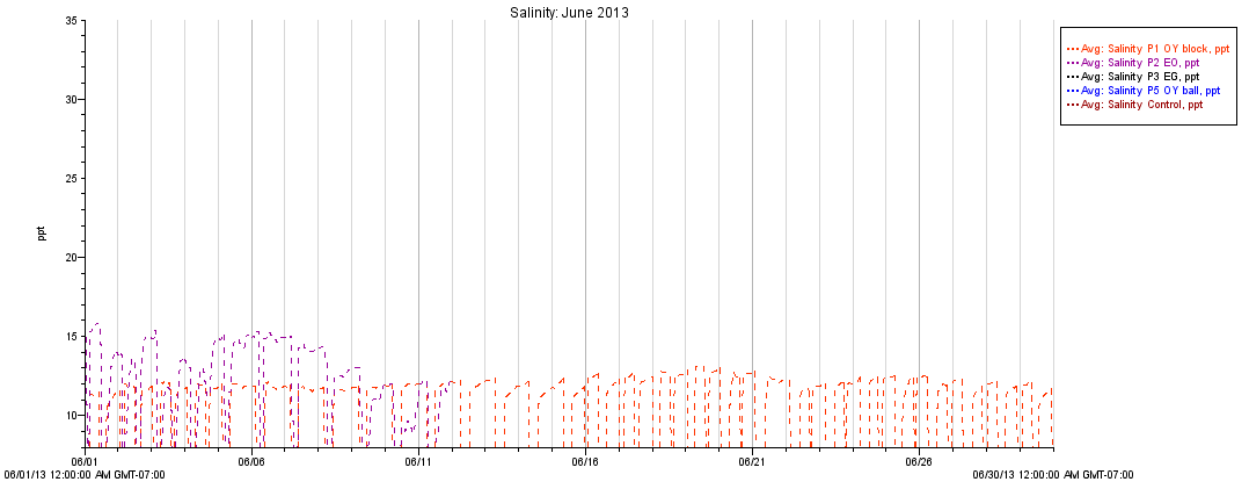


Figure 94: Average hourly salinity at ELER in June 2013

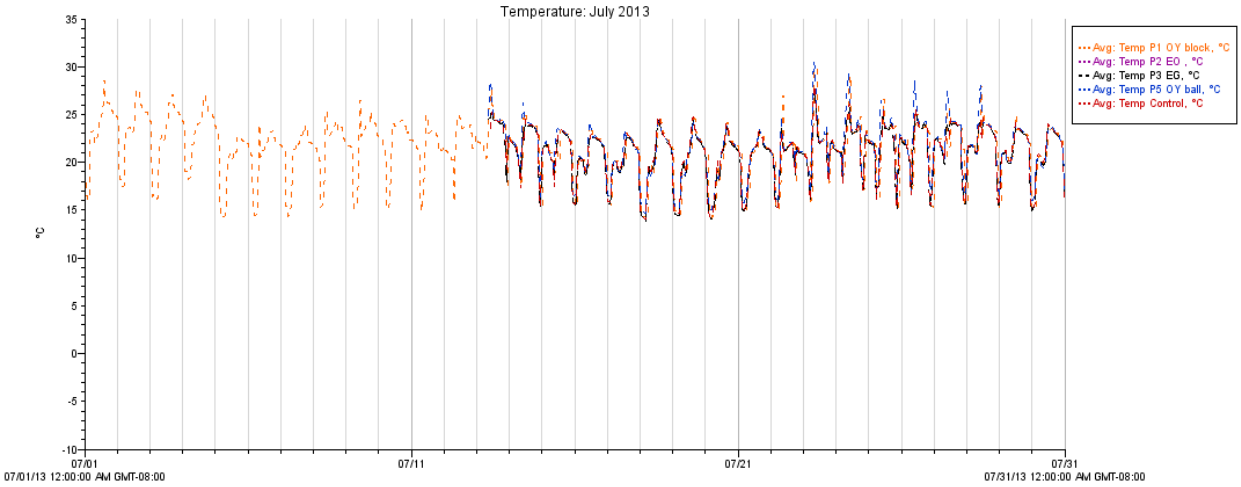


Figure 95: Average hourly temperature at ELER in July 2013

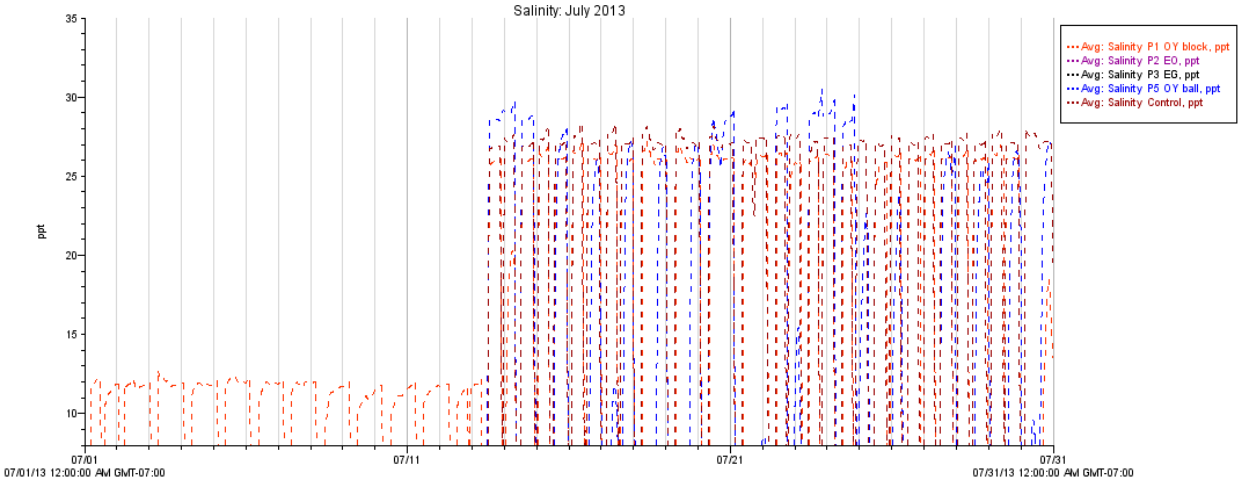


Figure 96: Average hourly salinity at ELER in July 2013

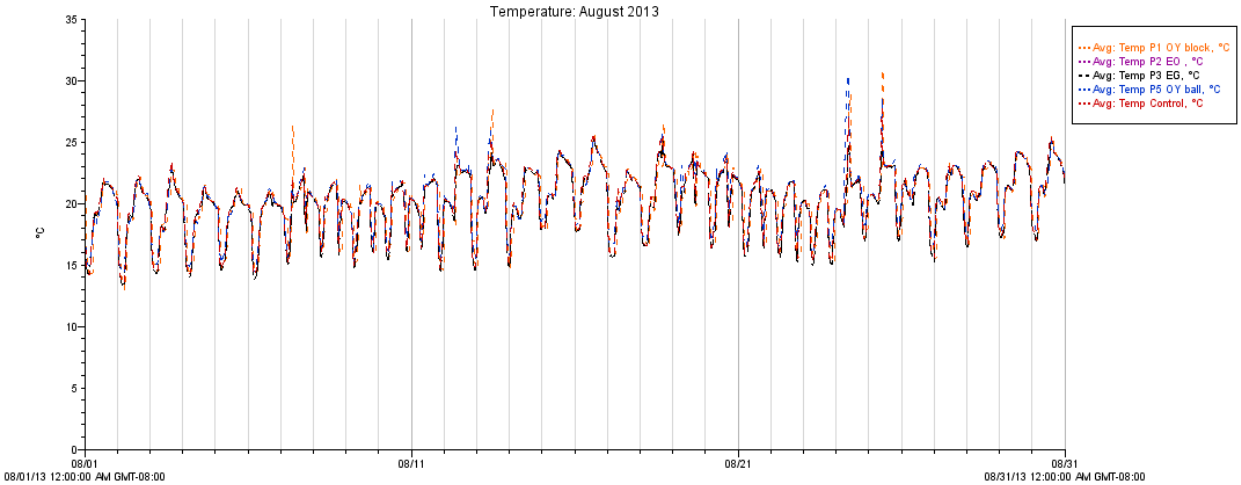


Figure 96: Average hourly temperature at ELER in August 2013

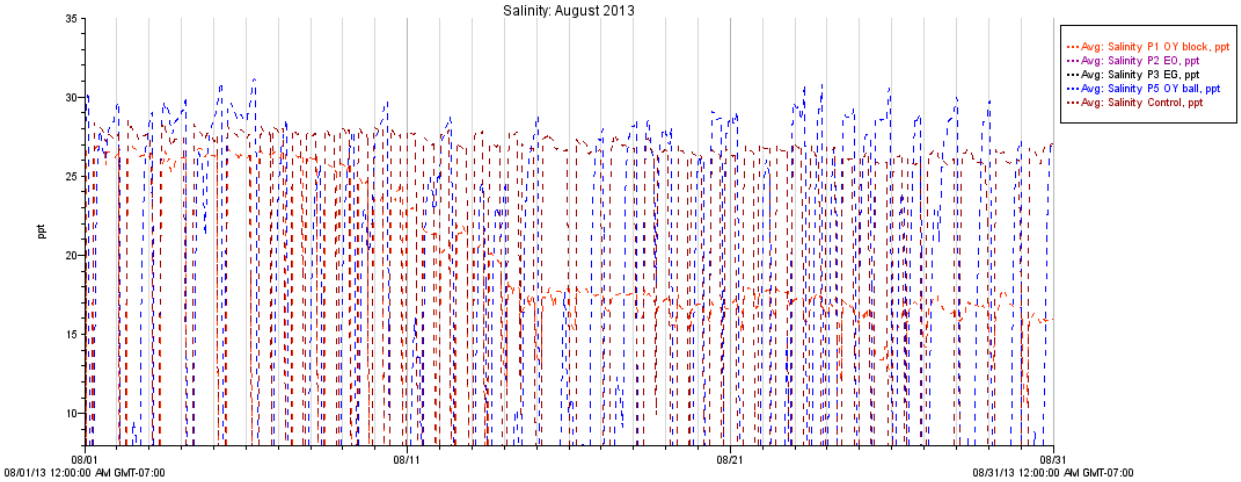


Figure 97: Average hourly salinity at ELER in August 2013

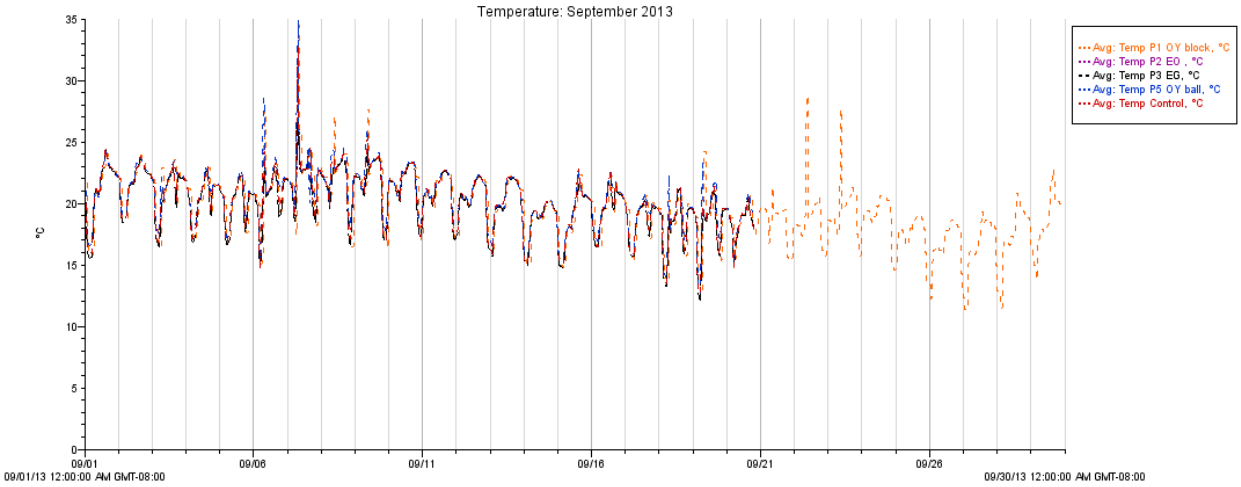


Figure 98: Average hourly temperature at ELER in September 2013

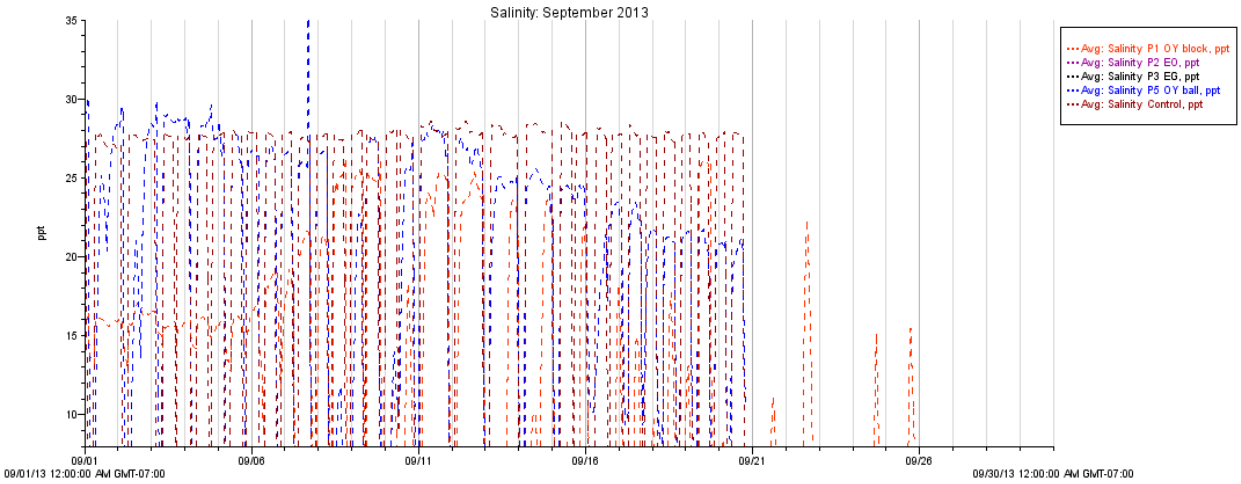


Figure 99: Average hourly salinity at ELER in September 2013

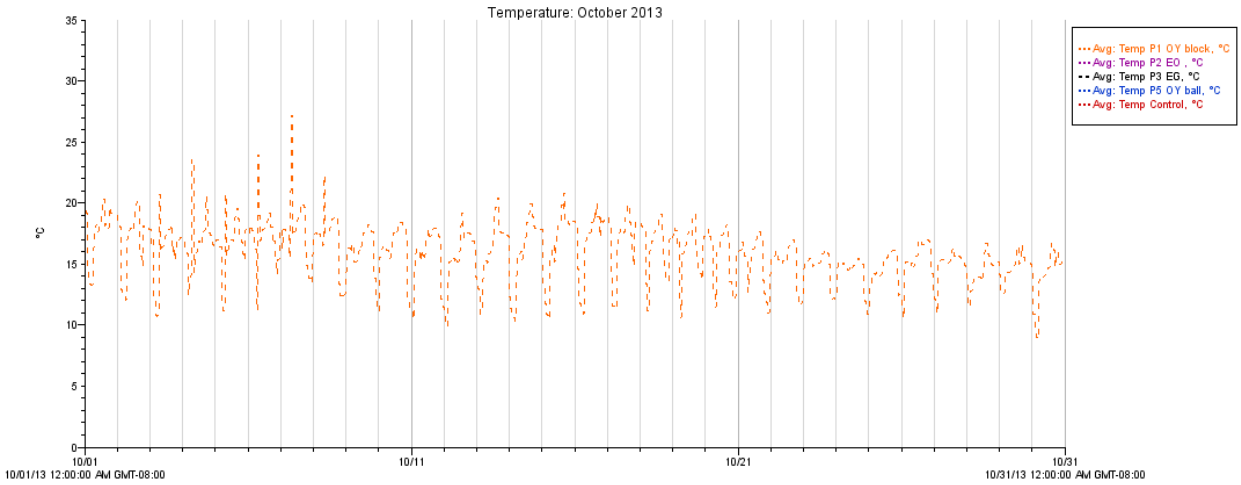


Figure 100: Average hourly temperature at ELER in October 2013

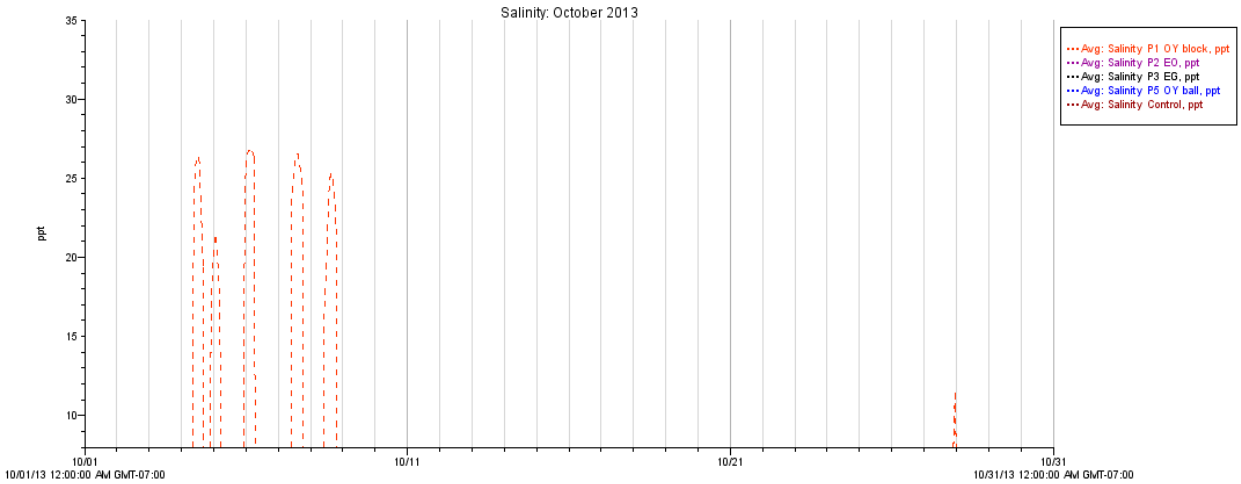


Figure 101: Average hourly salinity at ELER in October 2013

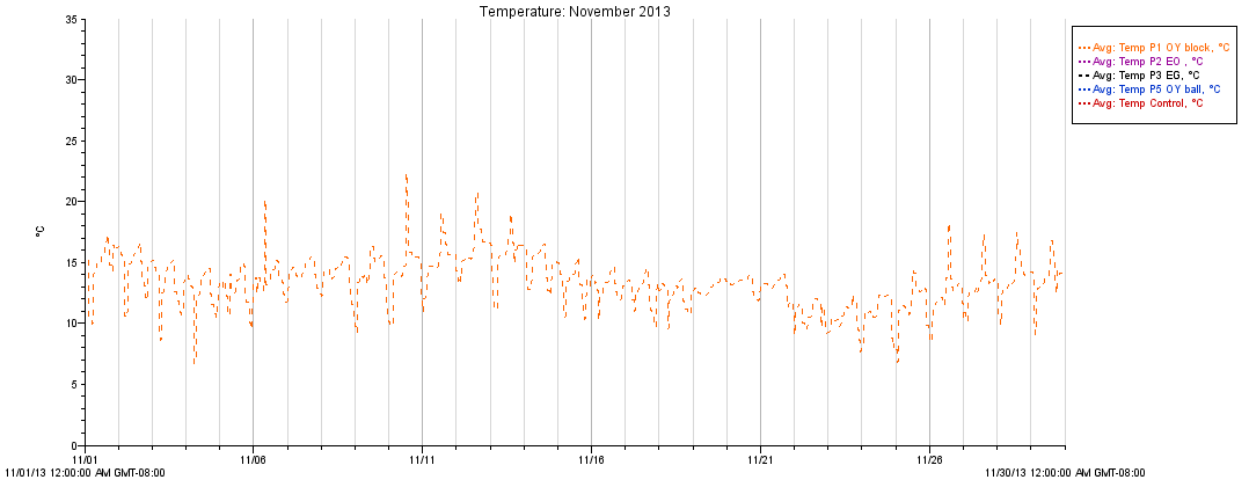


Figure 102: Average hourly temperature at ELER in November 2013

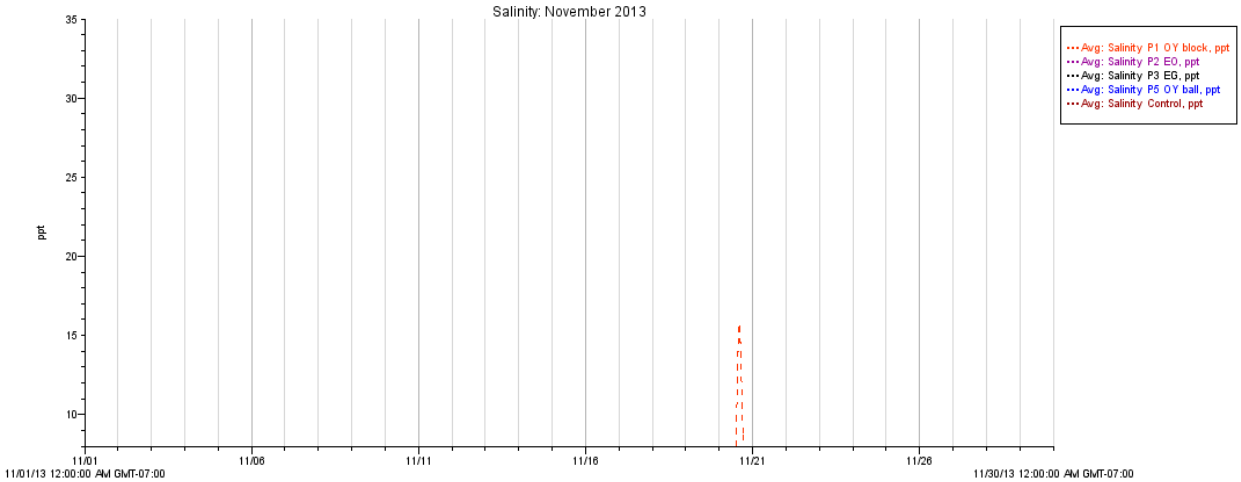


Figure 103: Average hourly salinity at ELER in November 2013

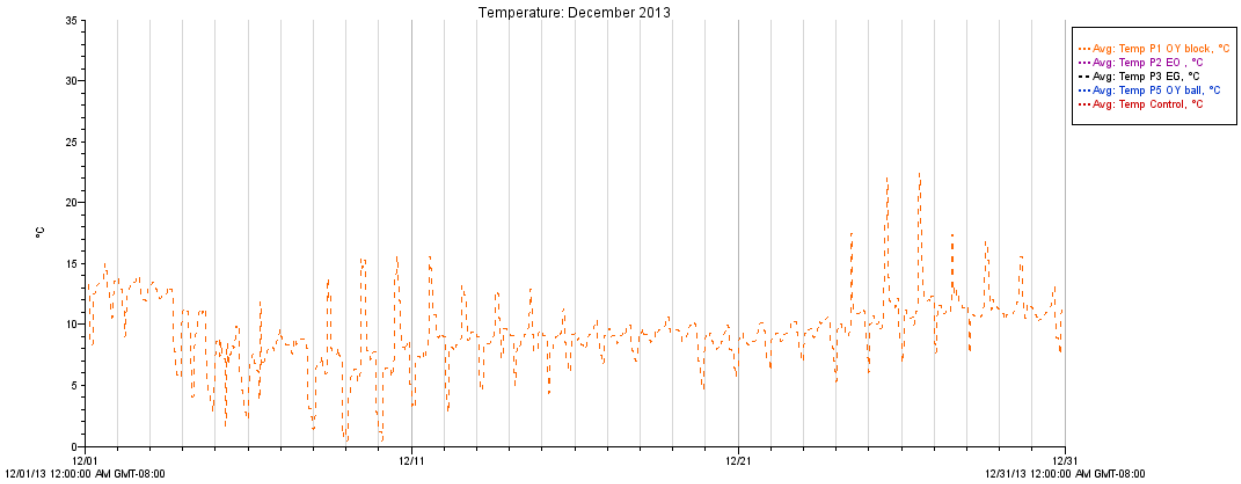


Figure 104: Average hourly temperature at ELER in December 2013

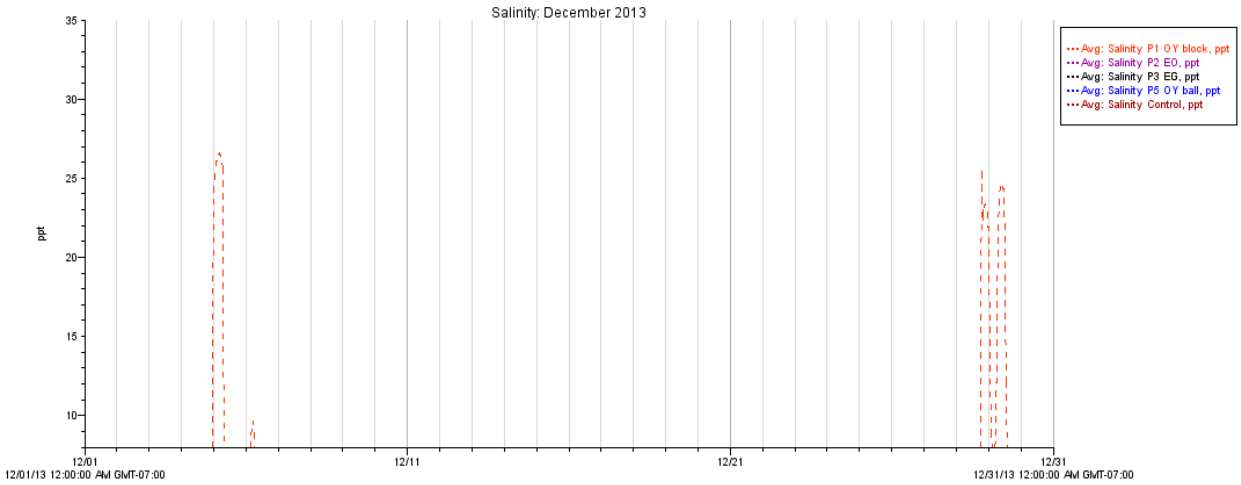


Figure 105: Average hourly temperature at ELER in December 2013

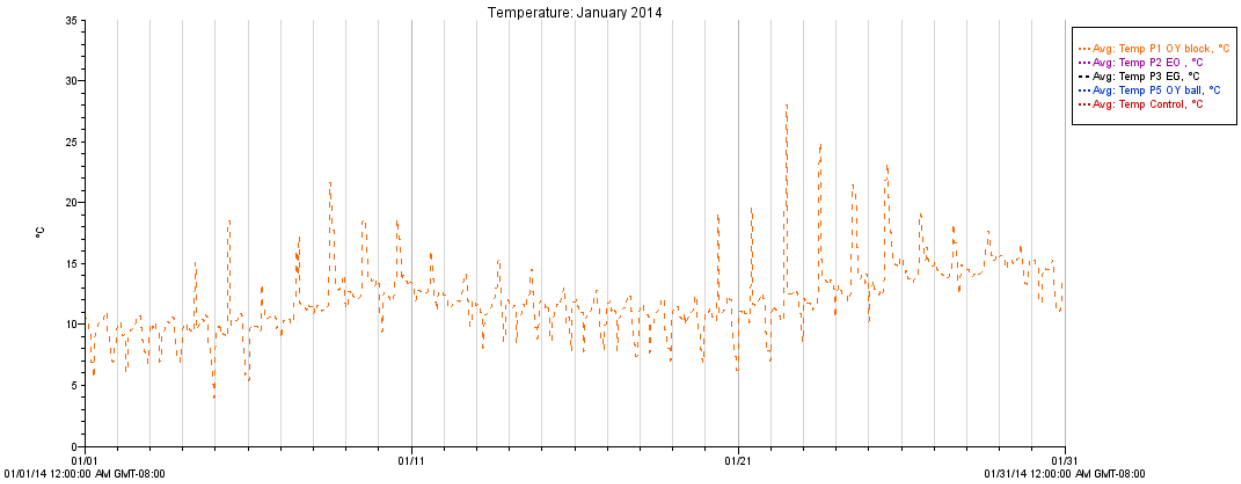


Figure 106: Average hourly temperature at ELER in January 2014

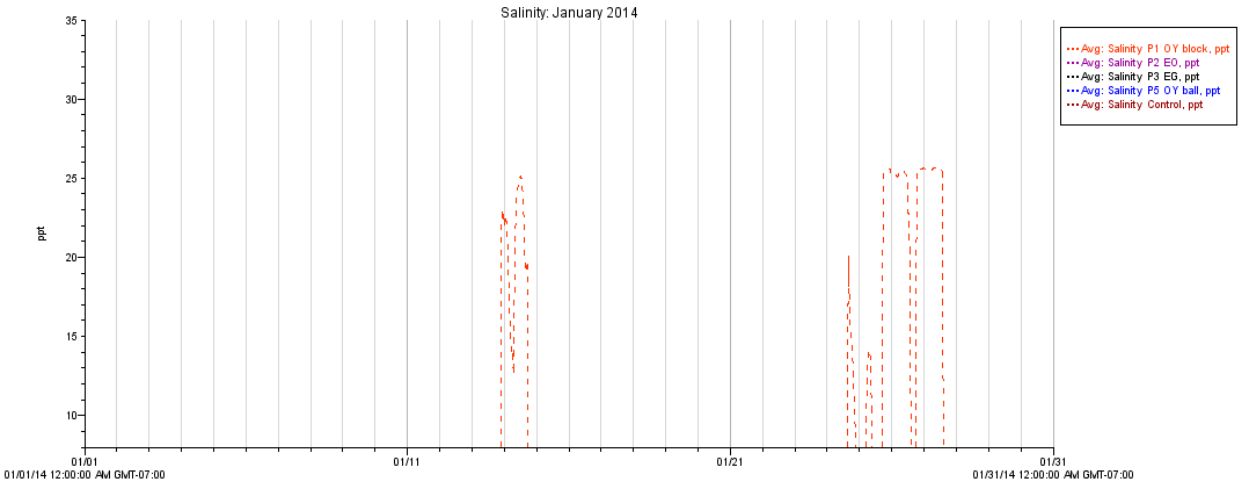


Figure 107: Average hourly salinity at ELER in January 2014

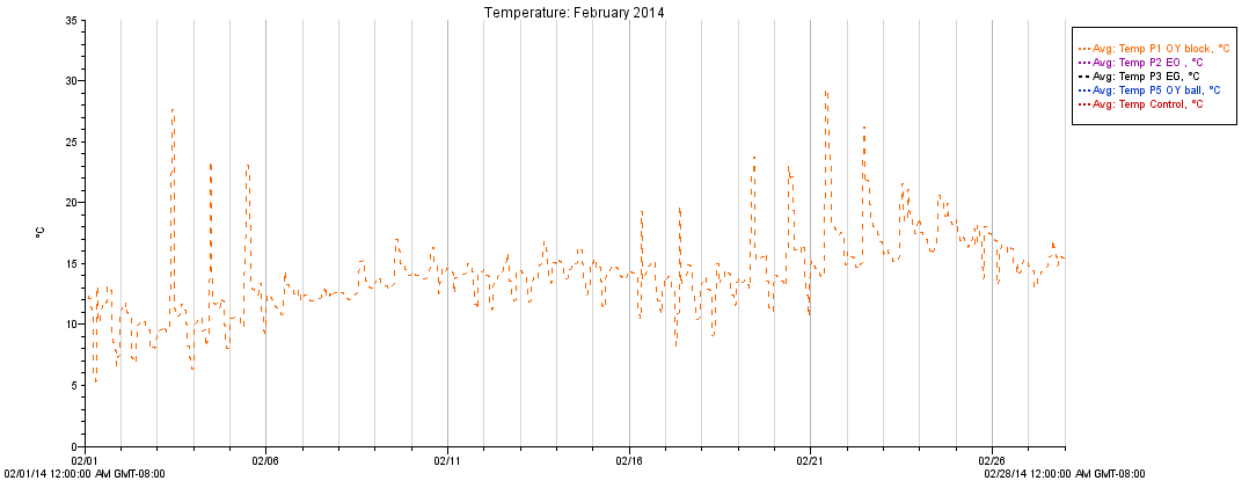


Figure 108: Average hourly temperature at ELER in February 2014

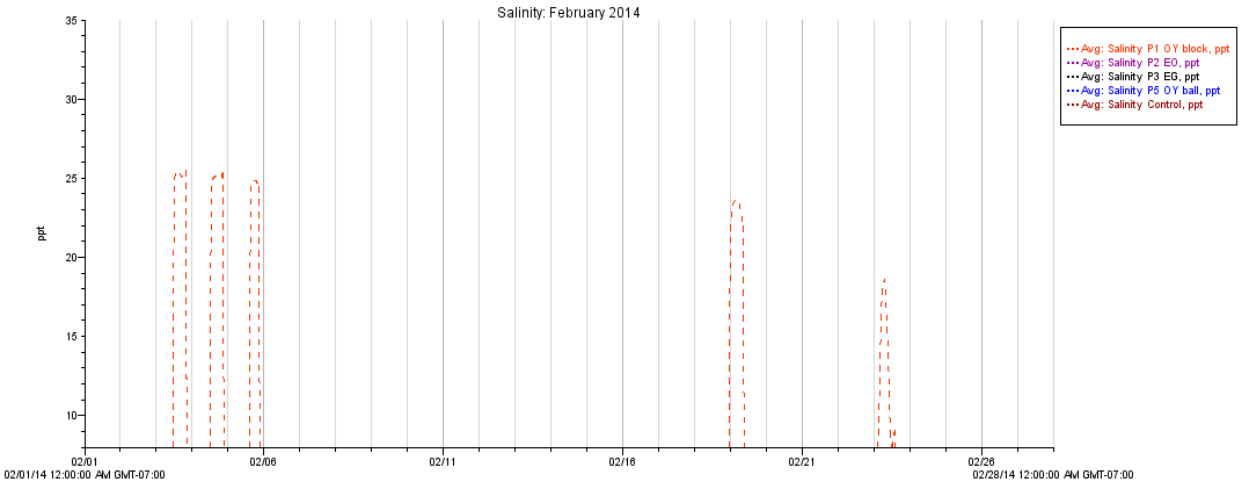


Figure 109: Average hourly salinity at ELER in February 2014

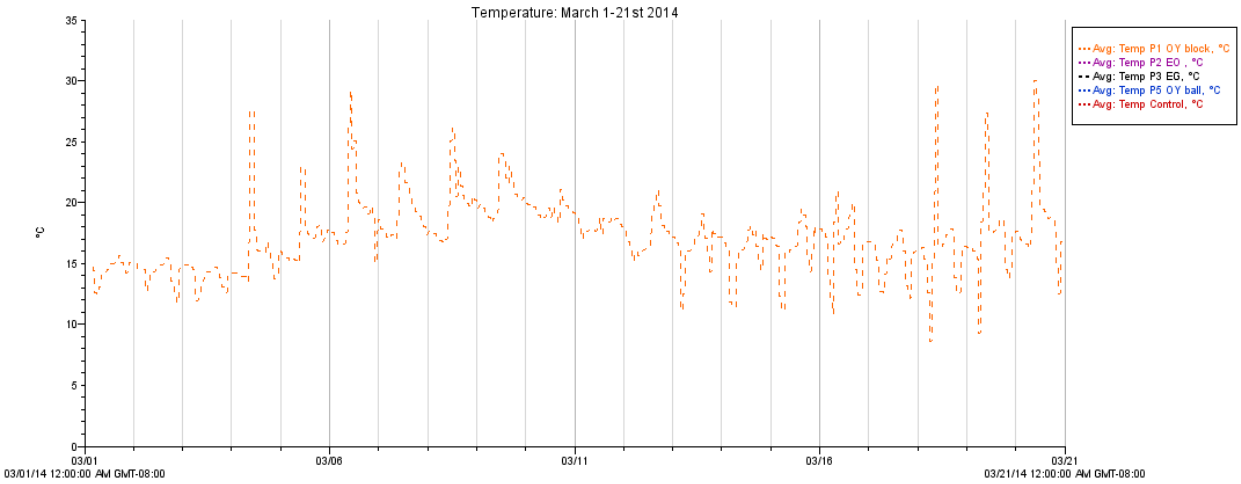


Figure 110: Average hourly temperature at ELER in March 1-21st 2014

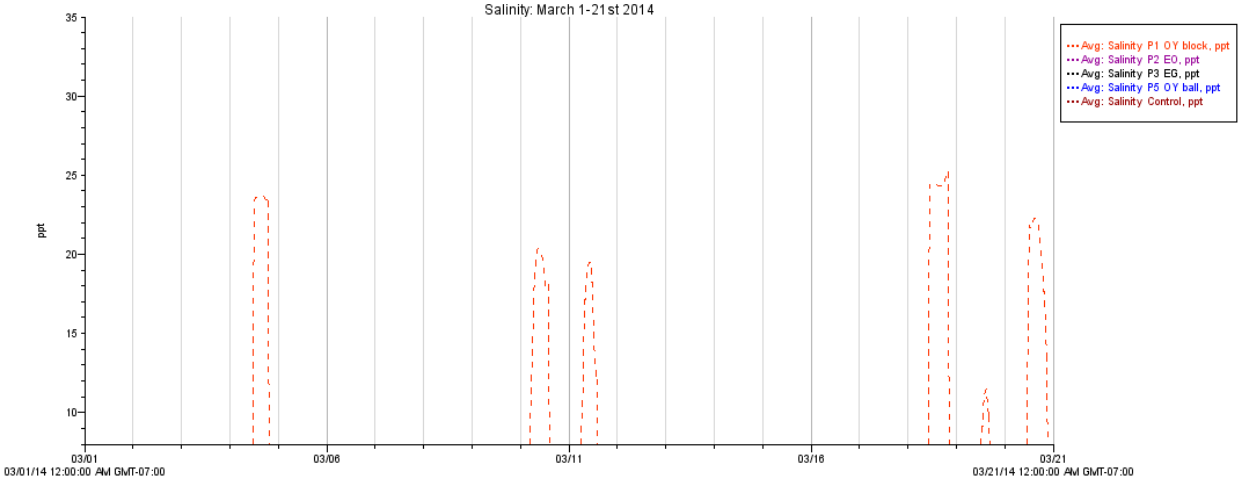


Figure 111: Average hourly salinity at ELER in March 1-21st 2014

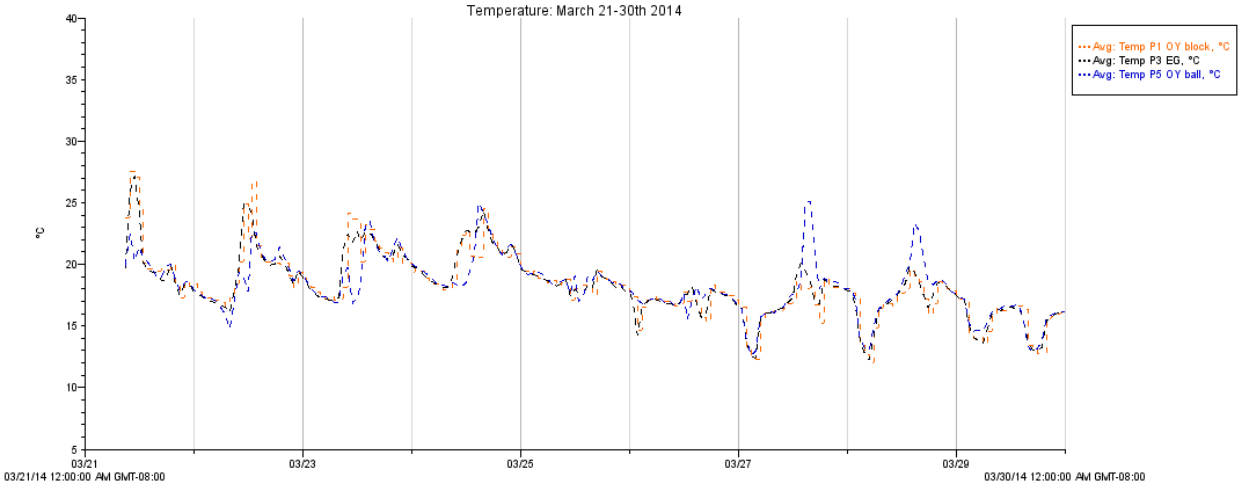


Figure 112: Average hourly temperature at ELER in March 21-31st 2014

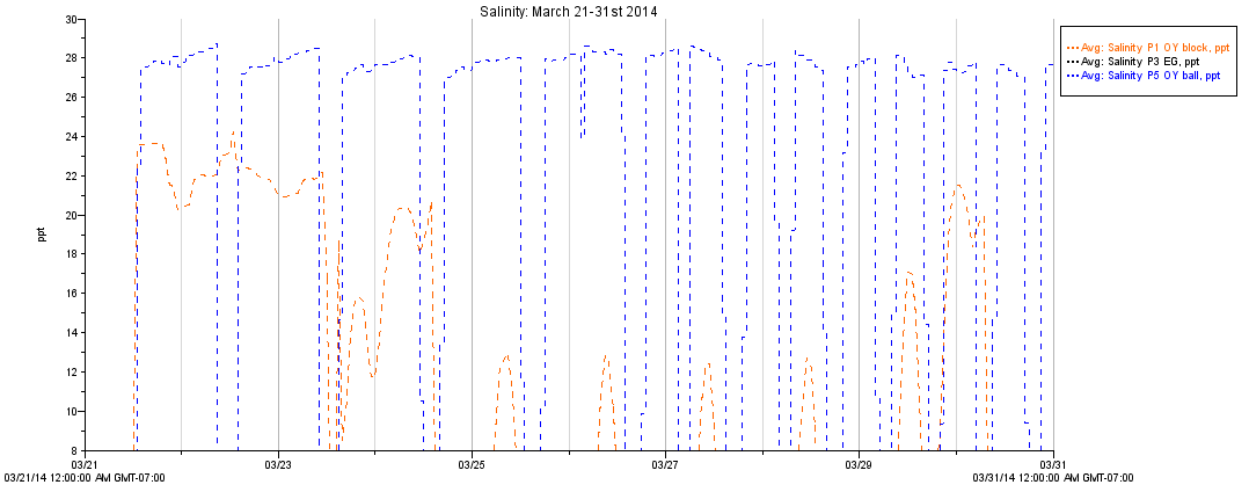


Figure 113: Average hourly salinity at ELER in March 21-31st 2014

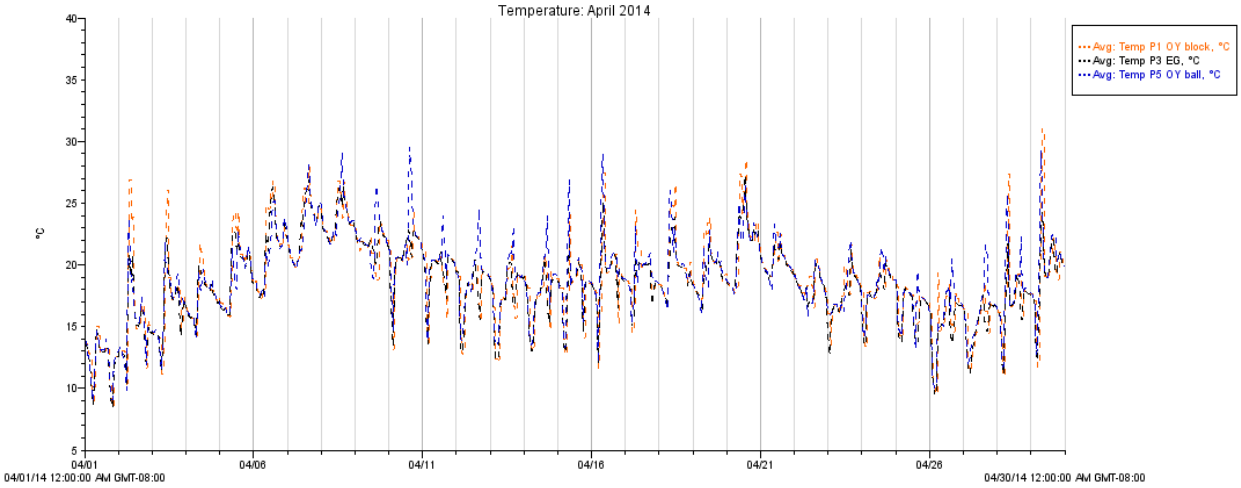


Figure 114: Average hourly temperature at ELER in April 2014

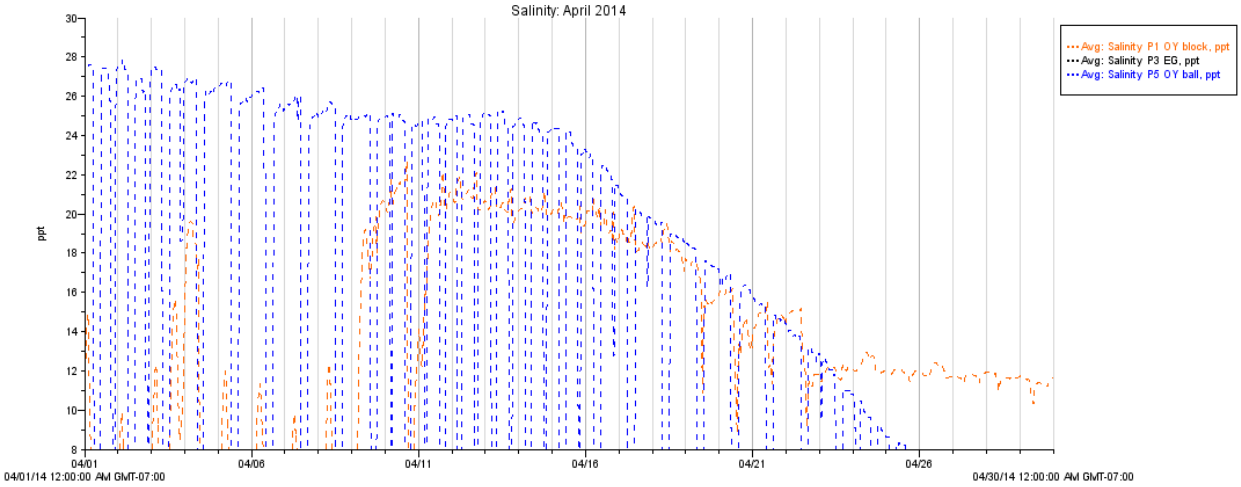


Figure 115: Average hourly salinity at ELER in April 2014

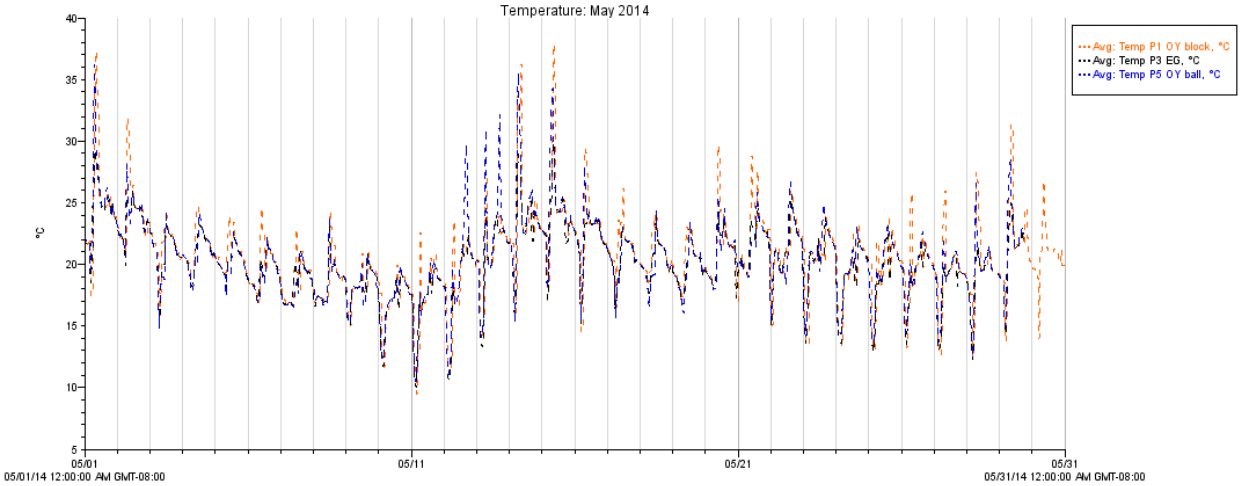


Figure 116: Average hourly temperature at ELER in May 2014

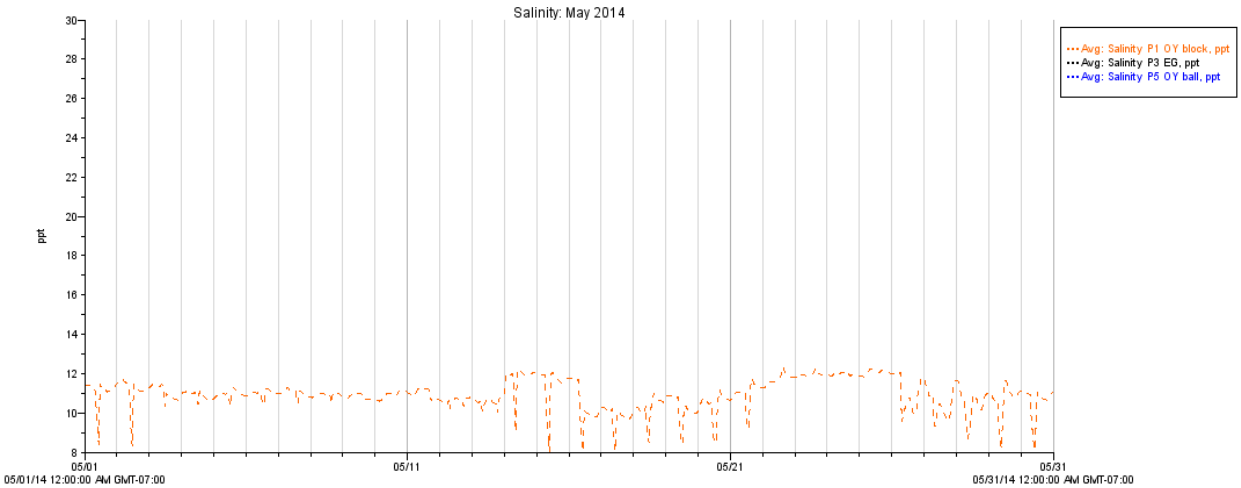


Figure 117: Average hourly salinity at ELER in May 2014

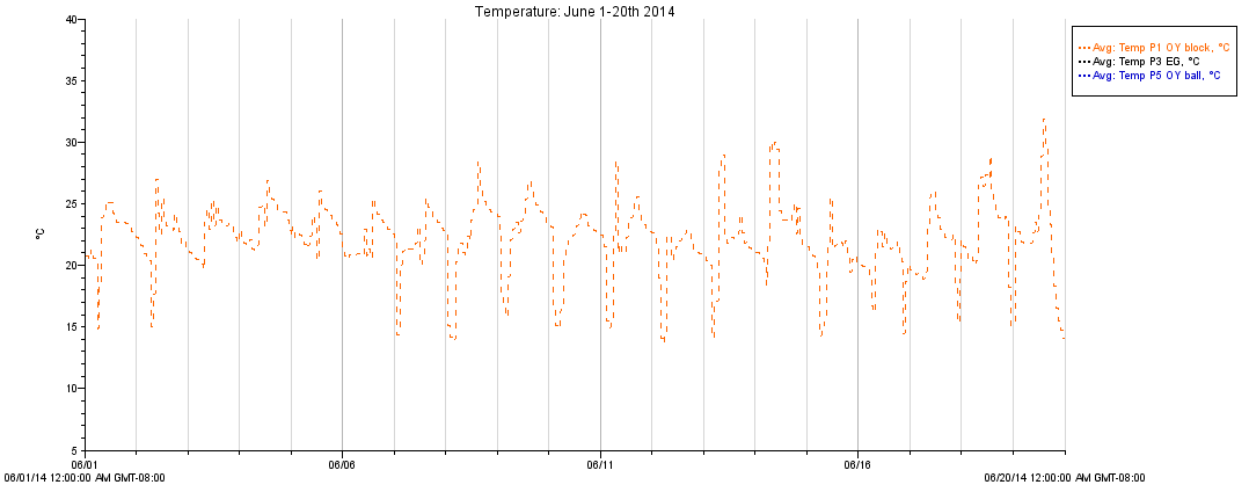


Figure 118: Average hourly temperature at ELER in June 1-20th 2014

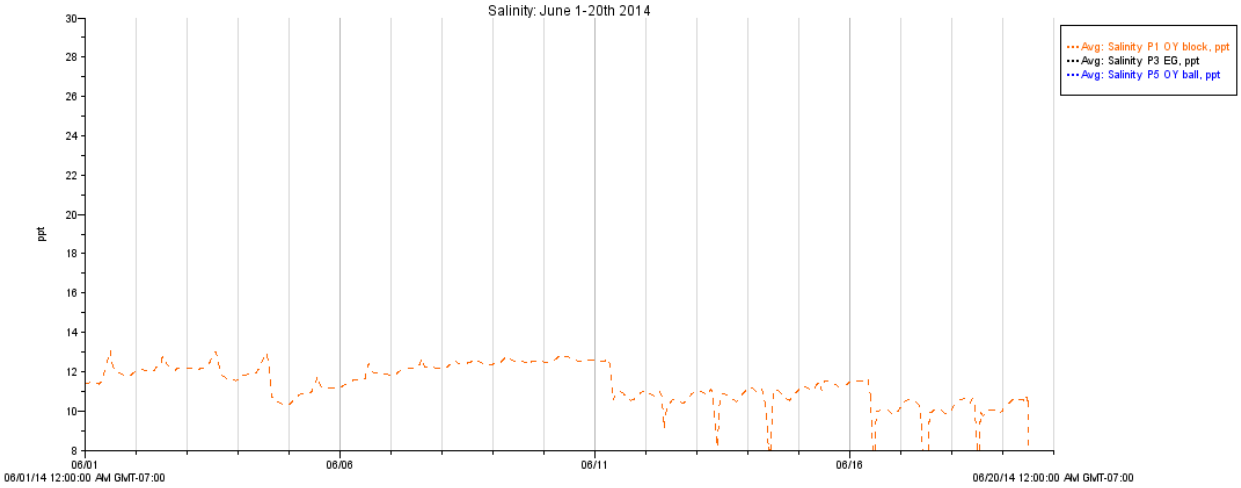


Figure 119: Average hourly salinity at ELER in June 1-20th 2014

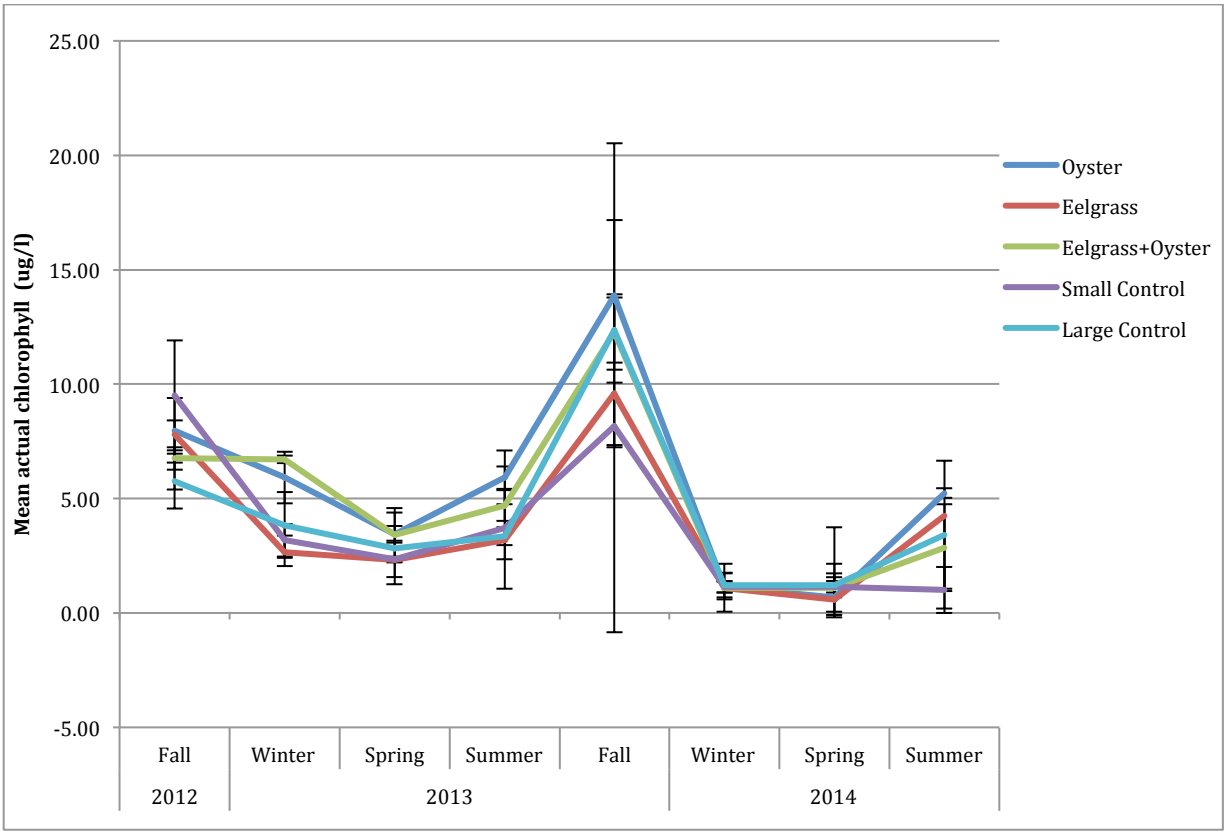


Figure 120: Average actual chlorophyll (ug/l) of water column samples in each treatment for every quarter collected at TNC. Error bars = 95% CI

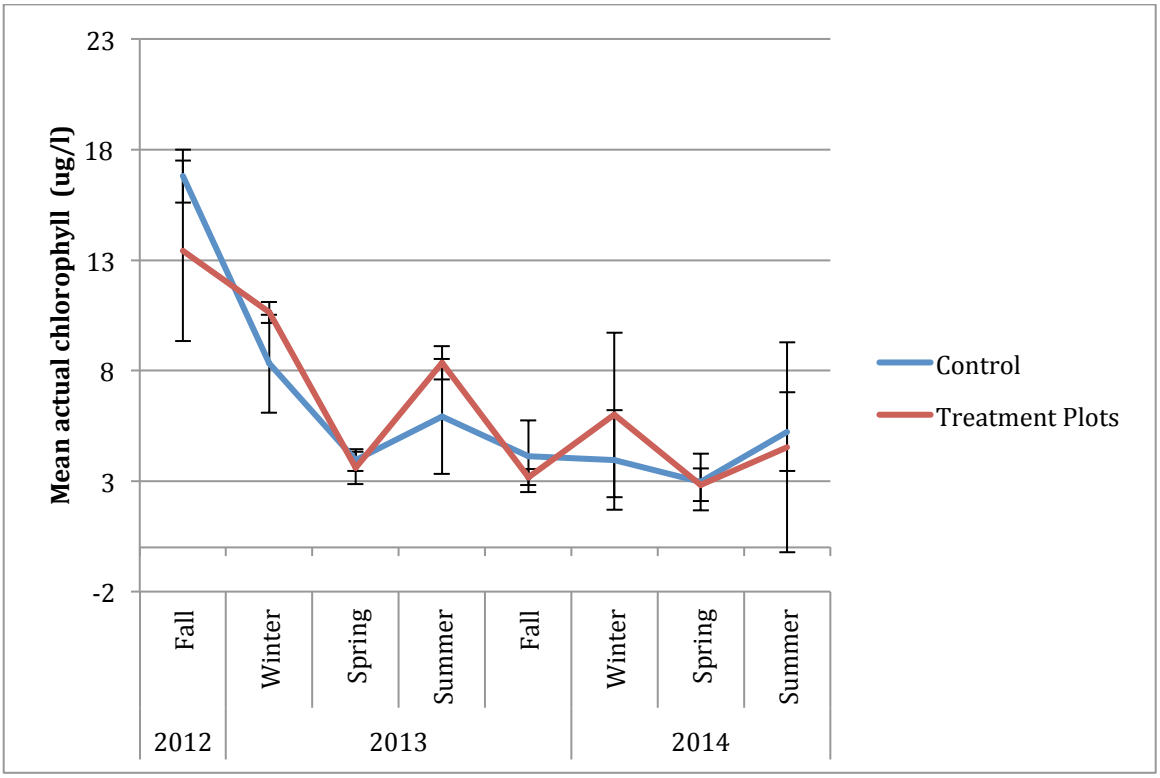


Figure 121: Average actual chlorophyll (ug/l) of water column samples in the control and treatment plots for every quarter collected at ELER. Error bars = 95% CI

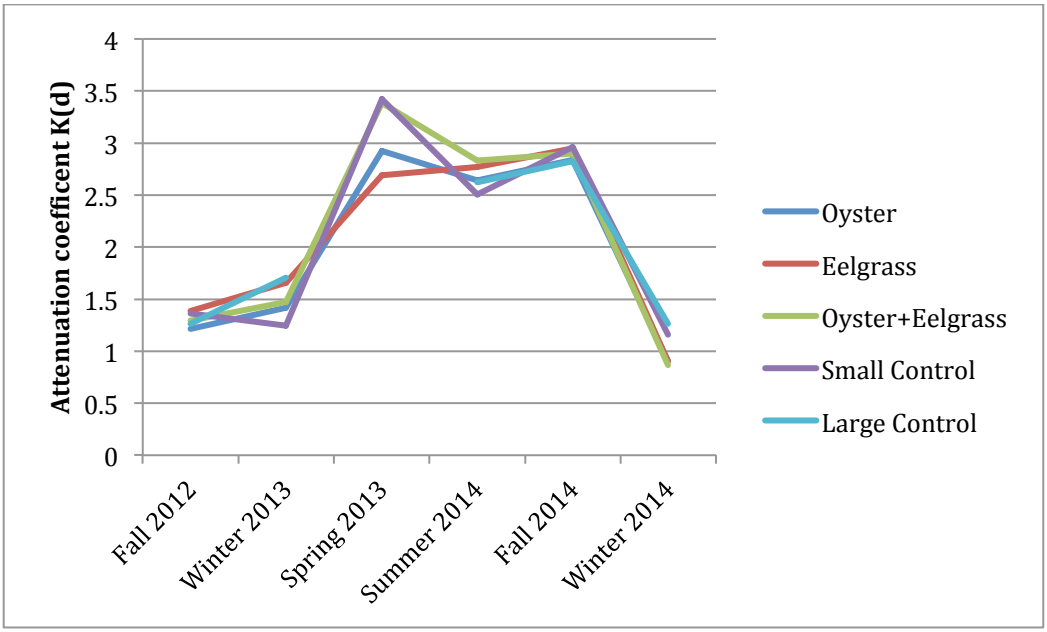


Figure 122 – Light attenuation coefficient of the water column in each treatment at TNC, every quarter. N.B. a higher attenuation coefficient indicates less light is penetrating through the water column / higher turbidity.

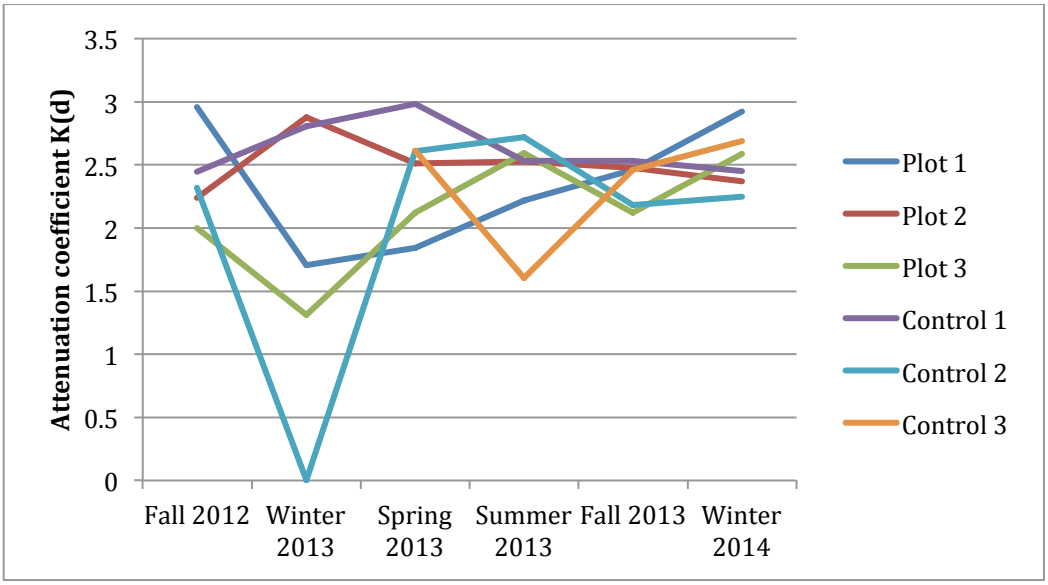


Figure 123 – Light attenuation coefficient of the water column in each treatment at ELER, every quarter. N.B. a higher attenuation coefficient indicates less light is penetrating through the water column / higher turbidity.

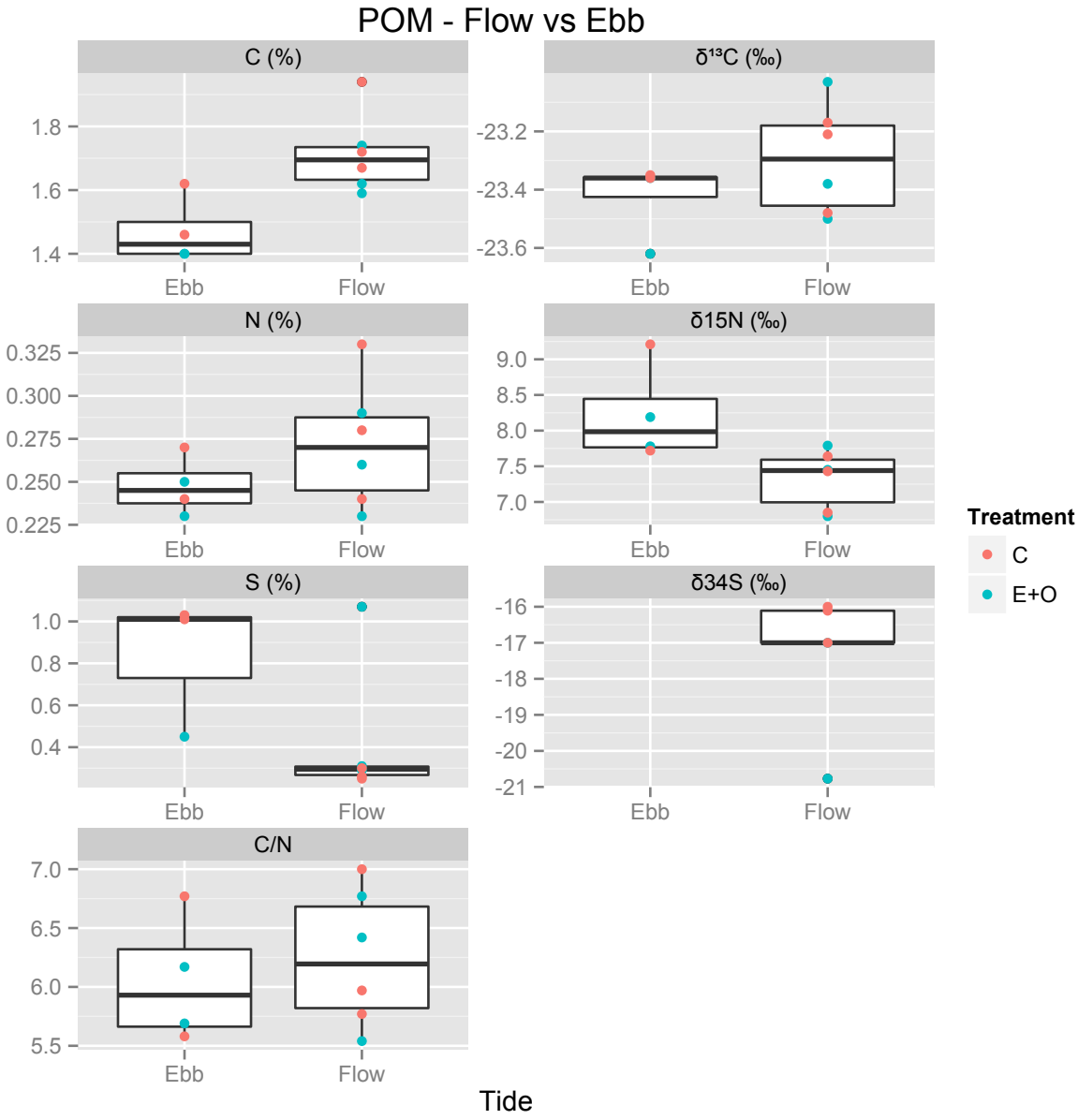


Fig 124: Effect of tide level (ebb vs flow) on the elemental and isotopic composition of Particulate Organic Matter (POM): C, N and S = carbon, nitrogen or sulphur content (%); C/N = carbon to nitrogen ratio; $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ = carbon, nitrogen and sulphur isotopic composition (‰). Boxplots depict overall distribution of available data, colored dots = individual samples and treatment

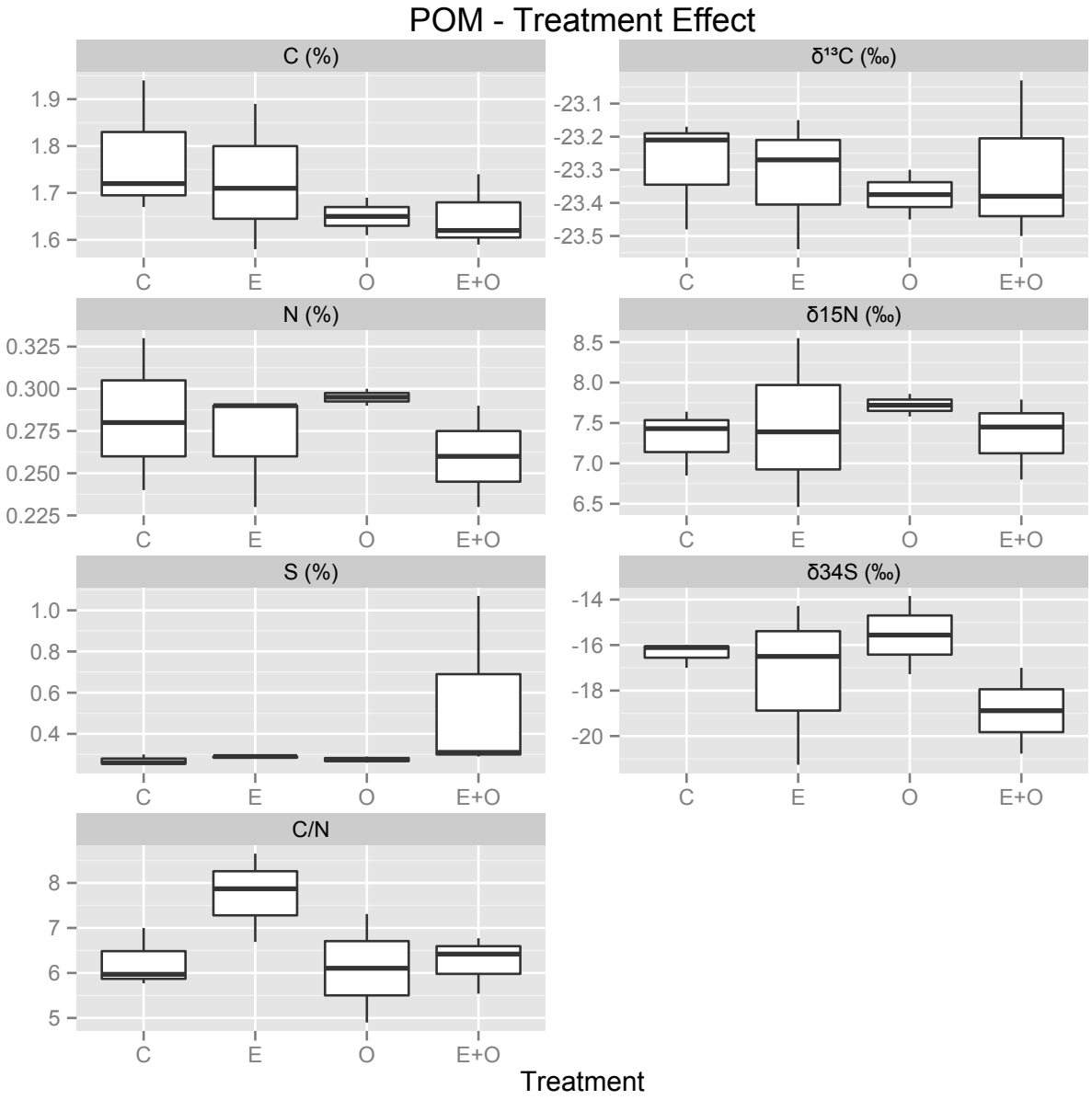


Figure 125. Treatment effect on Particulate Organic Matter (POM) elemental and isotopic composition: C, N and S = carbon, nitrogen or sulphur content (%); C/N = carbon to nitrogen ratio; $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ = carbon, nitrogen and sulphur isotopic composition (‰)

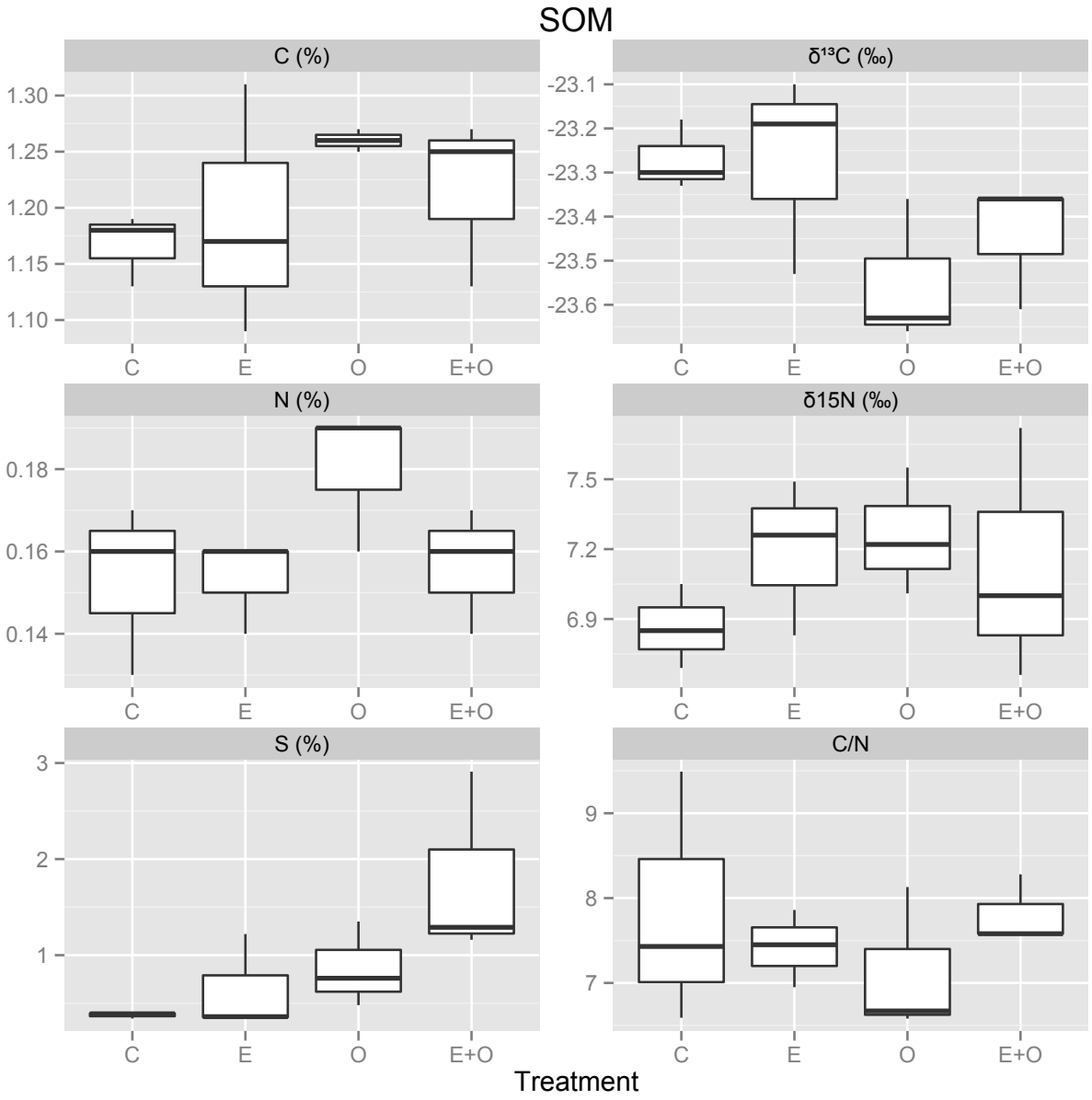


Fig 126: Treatment effect on Sedimentary Organic Matter (SOM) elemental and isotopic composition: C, N and S = carbon, nitrogen or sulphur content (%); C/N = carbon to nitrogen ratio; $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ = carbon, nitrogen and sulphur isotopic composition (‰)

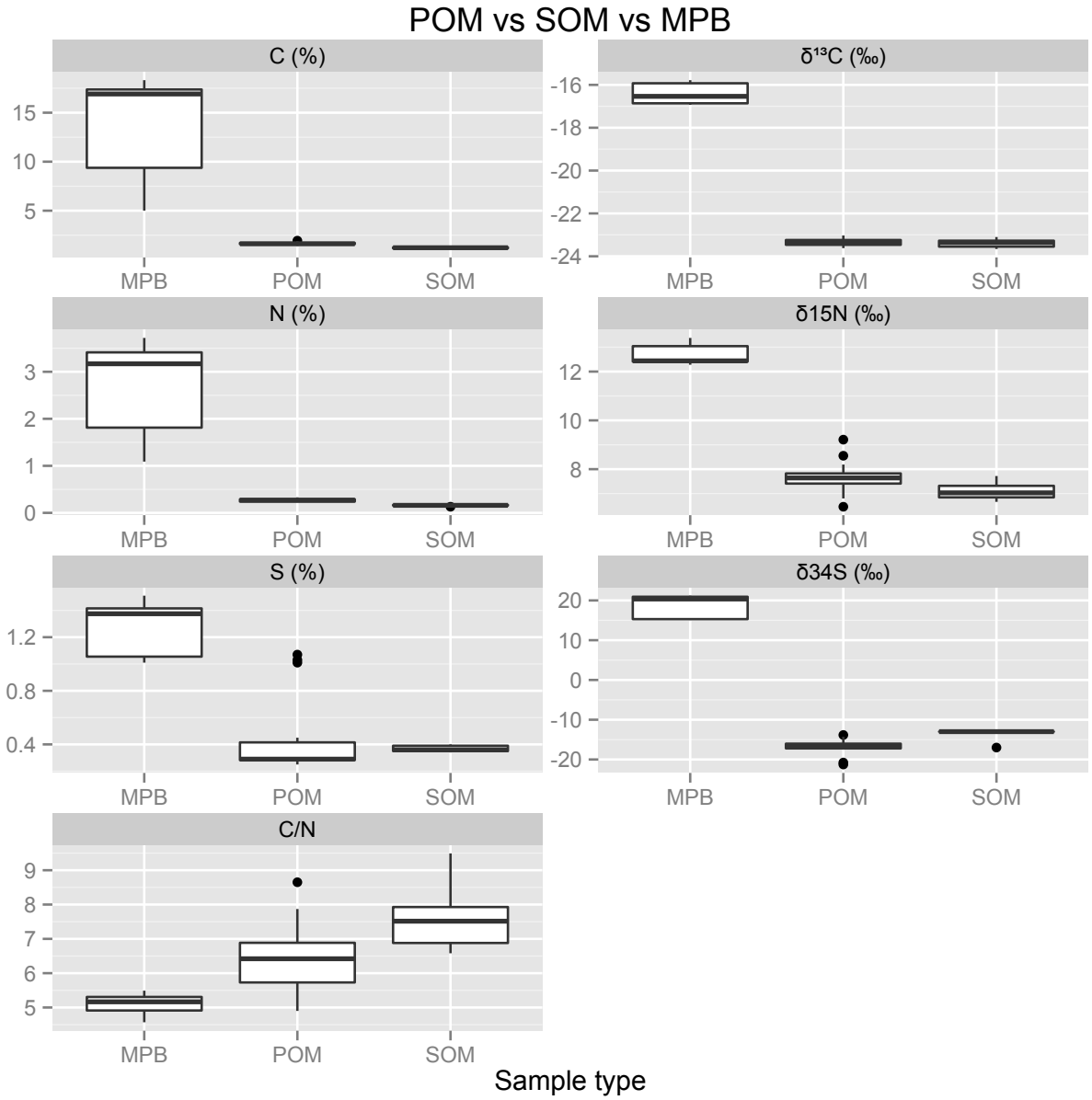


Fig 127: Microphytobenthos (MPB), Particulate Organic Matter (POM) and Sedimentary Organic Matter (SOM) elemental and isotopic composition: C, N and S = carbon, nitrogen or sulphur content (%); C/N = carbon to nitrogen ratio; $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ = carbon, nitrogen and sulphur isotopic composition (‰)

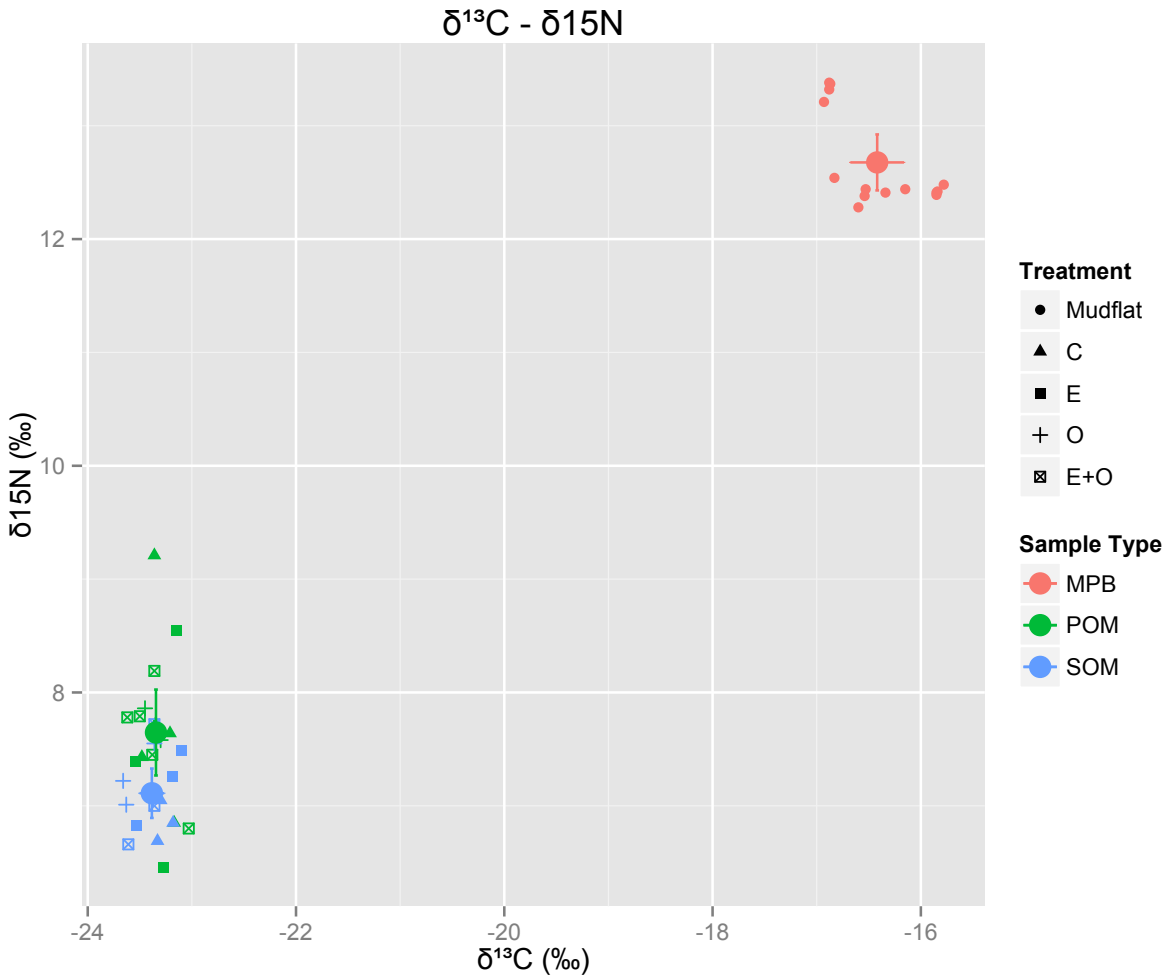


Fig 128: Carbon and Nitrogen stable isotope biplot for microphytobenthos (MPB), Particulate Organic Matter (POM) and Sedimentary Organic Matter (SOM). Colors = sample type. Symbols = treatment. Large dots = mean ± 95% confidence interval

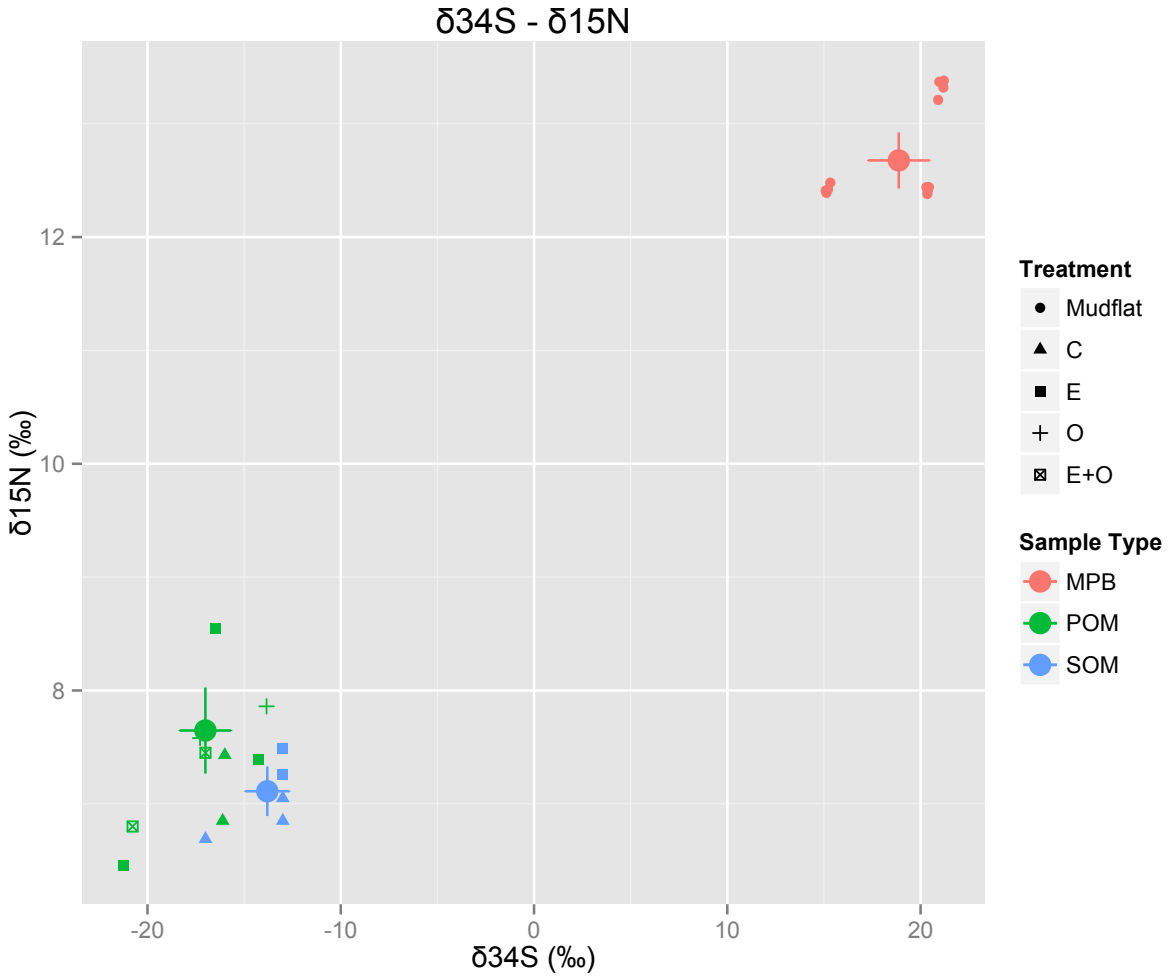


Fig 129: Sulphur and Nitrogen stable isotope biplot for microphytobenthos (MPB), Particulate Organic Matter (POM) and Sedimentary Organic Matter (SOM). Colors = sample type. Symbols = treatment. Large dots = mean ± 95% confidence interval

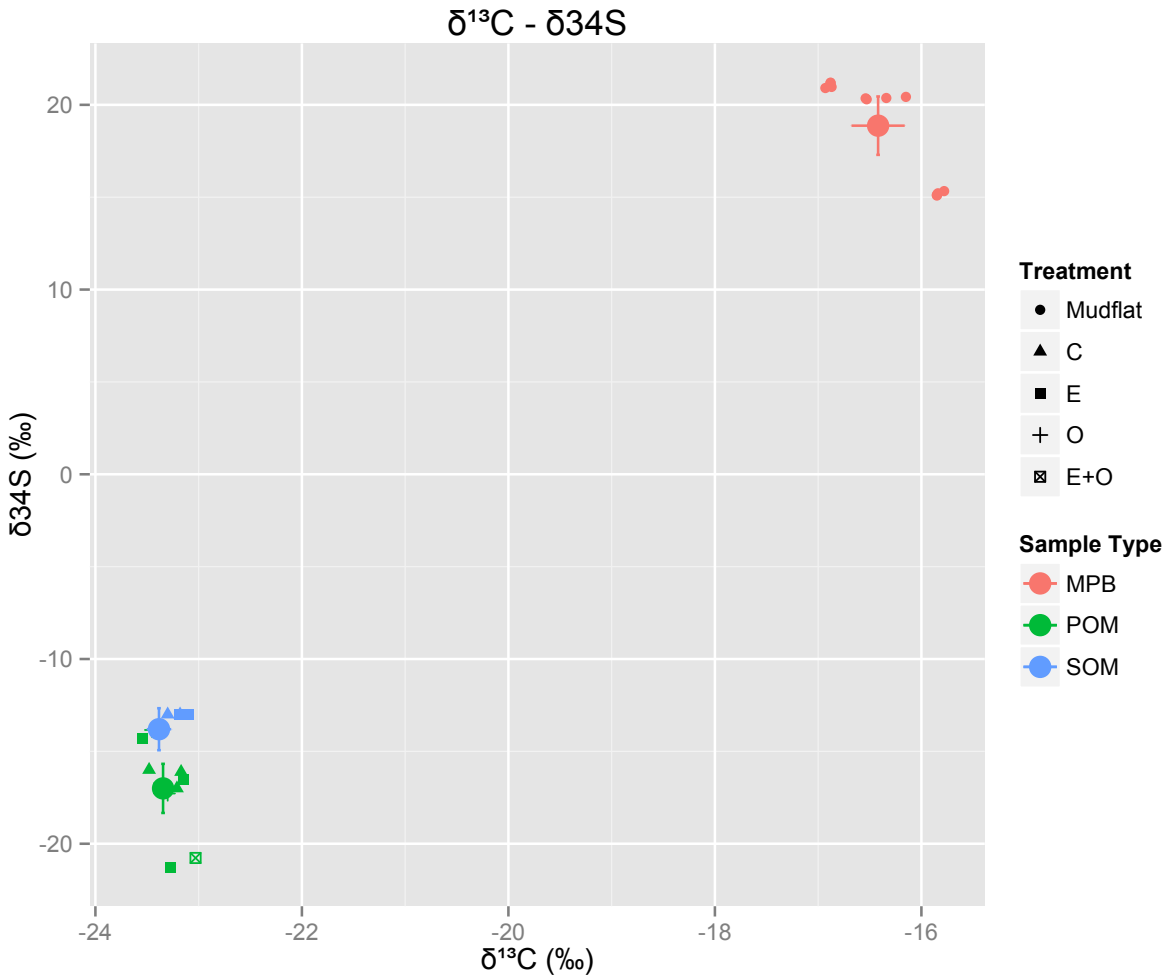


Figure 130: Carbon and sulphur stable isotope biplot for microphytobenthos (MPB), Particulate Organic Matter (POM) and Sedimentary Organic Matter (SOM). Colors = sample type. Symbols = treatment. Large dots = mean ± 95% confidence interval.