SBC LTER Food web Studies Using Stable Isotopes
Monthly Water and Kelp Sampling
Field and Laboratory Protocol

The SBC LTER is using a time series of measurements at three core reef sites to examine the transport of nutrients and other constituents to and from the reef ecosystem. The three permanent reef sites are being monitored both by monthly sampling from small boats and continuously sampling instrumented moorings. The principal goal of these observations is to establish baseline data for detecting key events that can affect the reef ecosystem. These include the prevalence of freshwater plumes at each reef site, the flux of nutrients to the macrophytes, and the character and flux of particulate material that fuel the sessile invertebrate community.

Field data collection methods: Monthly sampling

In November 2000, we initiated monthly sampling of water properties at two reef sites (Carpinteria Reef and Naples Reef) and began additional sampling at a third reef site (Arroyo Quemado) in March 2001. Sampling at the three reefs is conducted with small research boat. Three sampling stations at each site are monitored; I, located inshore of each reef, R, located immediately adjacent to each reef approximately halfway between the surf zone and the reef, and O, located approximately one kilometer offshore of each reef.

At each station, a CTD (Seabird SBE19 or SBE-19+; http://www.seabird.com) equipped with a chlorophyll fluorometer (Wetlabs Eco-ASL; http://www.wetlabs.com/) and backscatter meter (Wetlabs Eco-VSF-custom 1 angle) and transmissometer (Wetlabs Cstar) is lowered and data on temperature, salinity, chlorophyll, and suspended sediments are recorded at intervals of 4 Hertz (2 Hertz for the SBE 19) throughout the water column. Pumped water samples at fixed depth stations of 1, 5, 10 and 20 m (depending on water depth) are collected using a modified bilge pump mechanism attached to a long polypropylene hose. Water from each depth is collected in triple-rinsed 4-liter polypropylene carboys and stored in large coolers on the boat for transport to the laboratory. At each sampling depth, the hose is allowed to pump for ~5 minutes prior to water collection to allow the hose to be rinsed with a volume of sample water equal to 3x the hose volume.

Beginning in 2000, we began collecting kelp tissue samples from the monthly water sampling sites. Single *Macrocystis* blades (20 total blades; 10 “New” and 10 “Old”) are collected from individual fronds at the time of the monthly water sampling monitoring.

The ten “New” blades are collected approximately 1 meter from the growing tip of a surface-reaching *Macrocystis pyrifera* frond. The ten “Old” blades are collected from old and/or partially degraded *Macrocystis pyrifera* fronds. “Old” blades are differentiated by the tissue being dark in color, slightly to heavily epiphytized by bryozoans and slightly to heavily degraded in tissue quality.

The "New" and "Old" blades are placed in labeled bags in the field and transported to the lab in coolers. The blades are then held in running seawater until processing (usually 2-48 hours after collection).

Protocol Change History

In October 2002, we modified our field protocol to include filtering a portion of each water sample for dissolved nutrients directly on the boat at the time of collection, rather than in the laboratory.

In November 2002, preliminary data analysis showed no significant difference between the three sampling stations at Naples and Carpinteria. At that time, modified our protocol to perform a CTD cast at all stations at these two sites but to discontinue collecting water samples at the Inshore and Offshore stations at sites Naples and Carpinteria.

In February 2003, we modified our sampling technique to include a CTD-rosette package that simultaneously collects water temperature, salinity, chlorophyll, and suspended sediments data via the CTD as well as uses attached Niskin bottles to collect the fixed depth water samples.

Laboratory Sample Processing

All water samples are filtered within hours of collection and filters or filtrates are stored frozen. Both water and kelp samples are analyzed by the UCSB Marine Science Institute Analytical Laboratory (http://www.msi.ucsb.edu/analab/analabtexts/nalab.htm). Water samples are analyzed for nutrient concentrations (nitrate, silicate, and phosphate). Samples of particulate mater are collected at each depth and analyzed for carbon and nitrogen isotopes, particulate organic carbon, organic nitrogen, and silica concentrations, and chlorophyll concentration.

Below are the laboratory protocols for monthly water sample C & N isotope filtering and monthly C& N isotope composition of *Macrocystis pyrifera* tissue.

C & N Isotope Water Filtration Procedure

Materials required:

- square 1000ml PP bottle for measuring sample volume
- pre-combusted 25mm GF/F filters – 450^{o}C for 5 hours
- 25mm screw together filtration towers
- forceps
- pre-combusted foil pouches – 450^{o}C for 5 hours
- Sharpie, tape and stick on coloured dots

Sample Collection:

- Use a square 1000ml PP bottle to measure sample.
- Rinse bottle and cap 3 times with water from the 4L carboy.
- Fill bottle to brim and cap – this volume is 1240ml.
- Filter samples immediately.

Sample filtering:

Blank Preparation:

- Place a blank pre-combusted filter (25mm GF/G) in a pre-combusted foil packet. Label the packet using the date and "BLANK". Do a blank for every set of samples.

Sample Preparation:

- Rinse 25mm filter towers with Nanopure and shake to remove excess water. Do this before every sample.
- Place filter on base with tweezers; do not use fingers.
- Carefully place tower on base, do not crease filter, and twist together.
- Shake sample container, then pour seawater sample into filter funnel. If the sample bottle is filled to the rim, samples are usually the same volume.
- Turn vacuum pump on; vacuum should be around 10 mm Hg. Pump only until filter dries, do not maintain vacuum on dry filters.
- Using forceps, carefully fold filter in half and slide into foil pouch, fold pouch shut.
- Label foil pouch as per labeling protocol.
- Keep foil pouches in freezer until >60 have accumulated and they can be submitted for analysis.

Pre-submission preparation:

- Use new clean 20 ml glass scintillation vials for sample submissions – if the vials are new right out of the box they do not need to be pre-combusted.
- Thaw frozen filters in foil packets and allow to dry in 65^{o}C oven overnight.
- Transfer filters to scintillation vials – be sure to label vials the same as foil packets.
- Place all vials in a deep Pyrex dish with lid, put dish in fume hood.
- Place a 25ml beaker containing ~20ml of concentrated HCl in the centre of the pyrex dish and put the lid on. Allow to sit fuming overnight.
- Remove HCl and place Pyrex dish containing scintillation vials in 65^{o}C drying oven.
- When dry, remove from oven, cap with dry acid-washed lids and submit to the MSI analytical lab for analysis.

C & N Isotope *Macrocystis* Tissue Processing Procedure

Coring

A sample core is obtained from each collected blade. Before coring, the blade is removed from the plastic bag and cleaned of all epiphytes (bryozoans etc.) and other foreign debris. The blade is placed on a wooden surface and a 2.9 cm diameter core is collected near the pneumatocyst end of the blade using a sharpened stainless steel pipe. Each core is briefly submerged in a 10% HCl solution, then immediately submerged in De-Ionized water, patted dry and then added to a composite sample for "New" and "Old" blades for the particular date and site of collection. The composite "New" and "Old" cores are placed in separate plastic weigh boats labeled with the tissue condition ("New" or "Old"), number of core samples included, and the date and site information.

Drying

All samples are then dried in a 65^{0} C drying oven for a period of 2-5 days until the plant material is completely dry.

Grinding

When the samples are dry, they are removed from the drying oven and each sample is ground into a fine, uniform powder in a mortar and pestle. The ground composite sample

is then transferred to a 1.5 mL centifruge vial and is labeled with the date, site, number of tissue samples and tissue condition information. The mortar and pestle are wiped out between composite samples with a dry Kimwipe tissue.

Labeling

Each composite sample is then assigned and labeled with a consecutive number for sample tracking at the UCSB Marine Science Institute (MSI) Analytical Lab. Consecutive numbers are assigned using the numerical order of date and site of collection. The consecutive number assigned includes an "I" to distinguish the sample as an Isotope analysis sample. The consecutive sample number is entered into the spreadsheet titled "Kelp_Stable_Isotopes.xls" in the "Monthly Kelp Stable Isotopes" folder.

Storage

After the samples are dried, ground and assigned consecutive sample numbers, they are be stored in sealed jars with moisture-retarding silica crystals for short-term storage before analysis. The samples may also be stored in the 65^0 C drying oven.

Sample submission

Samples should be submitted to the MSI Analytical lab in batches of at least 10-20. A submission cover sheet must accompany the samples to the lab. The cover sheet includes the name, phone number and email of the PI and submission contact person, the total number of samples and consecutive sample numbers included, the analysis to be performed and the recharge number for payment. Examples of previous coversheets can be found in the file "Kelp_Isotope_Submission_logs.xls" in the "Submission logs" folder. An electronic copy of each submission coversheet must be saved in the "Kelp_Isotope_Submission_logs.xls" file.

Data Reporting

The MSI Analytical lab emails the data in ascii or excel format to the email address provided on the sample submission sheet. These data are then incorporated into the appropriate datasets.