SBC LTER Food web Studies Using Stable Isotopes
Benthic organism
Field and Laboratory Protocol

Potentially important food sources to primary consumers on shallow subtidal reefs include phytoplankton-dominated seston, kelp-derived detritus, and for locations adjacent to sources of freshwater runoff, terrestrially-derived POM. We are conducting stable carbon and nitrogen isotope ratio analysis of consumers of varying trophic status to evaluate the relative contribution of these sources to reef food webs. Our research activity has focused characterizing variability in the isotope values of potential food sources (phytoplankton, kelp, and terrestrial POM). This information is needed to evaluate whether these isotopic values differ enough from one another to permit the use of mixing models to estimate the contribution of each source to the reef food web.

Organism collection

In March 2002, SBC LTER initiated annual invertebrate and algae organism collections from four core research sites in the Santa Barbara Channel (Arroyo Quemado 34.4677^0 N 120.1193^0 W, Carpinteria 34.3902^0 N 119.5399^0 W, Mohawk 34.3944^0 N 119.7300^0 W and Naples 34.4239^0 N 119.9503^0 W). Divers use hand tools to collect as many as six individuals of each of the species listed below. Organisms are collected from the subtidal reef at each site, placed in plastic bags or jars and then transferred to coolers or buckets for transport back to the laboratory. Initial processing of samples involves drying tissue immediately (60^0 C drying oven), storing in running seawater overnight, storing in 10% HCl overnight or immediate freezing (-20^0 C freezer). (Table 1). Further processing involves species-specific dissection of organisms (See Laboratory Sample Processing below).

Table 1. Initial organism sample processing procedure.

Group	Genus	Species	Sample process
Deposit feeder	*Parastichopus*	*parvimensis*	Freeze
Grazer	*Strongylocentrotus*	*purpuratus*	Freeze
Grazer	*Lithopoma*	*undosum*	Freeze
Grazer	*Lithopoma*	*gibberosum*	Freeze
Filter feeder	*Crassedoma*	*giganteus*	Freeze
Filter feeder	*Styela*	*montereyensis*	Freeze
Suspension feeder	*Corynactis*	*californica*	Freeze
Suspension feeder	*Ophiothrix*	*spiculata*	10% HCl overnight then freeze
Suspension feeder	*Cucamaria*	*salma*	Freeze
Predator	*Diopatra*	*ornata*	Running seawater overnight then freeze
Predator	*Conus*	*californica*	Freeze
Predator	*Pisaster*	*giganteus*	Process immediately
Predator/Scavenger	*Asterina*	*miniata*	Process immediately
Predator/Scavenger	*Cypraea*	*spadicea*	Freeze
Algae-brown	.	.	Identify species then freeze

Algae-red	.	.	Identify species then freeze
Algae	*Corallina*	*corallina*	10% HCl overnight then freeze

Laboratory Sample Processing

General Overview

The individual species collected for foodweb stable isotope samples each have unique sample processing steps (detailed below). All samples are labeled with date, site, species, replicate number (if applicable), and then dried in a drying oven set at 60^0 C. The samples are then individually ground using a ceramic mortar and pestle. The mortar and pestle is cleaned with 70% EtOH and dried each time the sample species or site changes. If organisms of the same species from the same site are ground consecutively, the mortar and pestle is cleaned by vigorously scrubbing with a Kim wipe tissue between the samples. All other tools used in the sample processing (forceps etc.) are also cleaned whenever the site or species changes. After grinding, the samples are placed in labeled plastic scintillation vials and stored in either the drying oven or in a dessicator to prevent moisture contamination.

Samples are sent to the University of California at Santa Barbara Marine Science Institute Analytical Lab (http://www.msi.ucsb.edu/analab/analabtexts/analab.htm) for isotope analysis.

Data Reporting

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

Species-specific Protocols

Deposit Feeders

Parastichopus parvimensis:

Gut Content: While still frozen, slice along length of cucumber with scalpel. Skin can be peeled away from the frozen insides of the animal. A sample of the gut content can be easily removed from this frozen mass. Sample size: as much as possible. Submerge the sample in 10% HCl until bubbles stop forming. Rinse with DI water. Place in labeled weigh boat. Dry in oven.

Muscle: Remove skin from body mass as described above. The muscle bands run along the insides of the skin. Remove a section of the muscle band by slipping curved forceps between the muscle and the skin and sliding along, separating the muscle. Sample size: as much as possible. Submerge in 10% HCl until bubbles stop forming. Rinse with DI water. Place in labeled weigh boat. Dry in oven.

Grazers

Strongylocentrotus purpuratus:

Lantern tissue: Make sure that you do not sample gut content or any reproductive tissue. The easiest tissue to use is the tissue encircling Aristotle's lantern. Allow the organism to

thaw. Cut around the outer edge of this tissue. Then pull away from Aristotle's lantern with forceps. If you get all of this tissue it should be enough for your sample. Submerge tissue in 10% HCl until bubbles stop forming. Rinse tissue with DI water. Place in labeled weigh boat. Dry in oven.

Lithopoma undosum and *L. gibberosum:*

Gut Content: Crack shell along spiral and remove shell while organism is still frozen. The gut content is readily accessible about one third to one half of the way up the spiral. Remove the gut sample as soon as possible after removal from the freezer (once it thaws it is very difficult). Sample size: as much as possible. Take care to remove all pieces of *Lithopoma* intestinal tissue from the sample. Submerge sample in 10% HCl until bubbles stop forming. Rinse with DI water. Place in labeled weigh boat. Dry in oven.

Foot: Cut a sample of the foot from near the operculum. Sample size: as much as possible. Submerge sample in 10% HCl until bubbles stop forming. Rinse with DI water. Place in labeled weigh boat. Dry in oven.

Filter Feeders

Crassadoma giganteus:

Adductor: Separate the shell halves by slicing the adductor muscle. Cut a sample of flesh from adductor and place it in 10% HCl until bubbles stop forming. Sample size: as much as possible. Rinse the sample with DI water. Place in labeled weigh boat.

Mantle: Select a sample of mantle tissue (I used all the portions that did not include the eyes), and cut it out with the scalpel. Submerge sample in 10% HCl until bubbles stop forming. Rinse sample with DI water. Place in labeled weigh boat. Dry in oven.

Styela montereyensis:

Body wall: Cut stalk off of body. Use scissors to cut from bottom to top of body. Entire insides can be easily pulled away from body wall with forceps, and discarded. Cut a sample of body wall and scrape away all epiphytes (scalpel works best). Sample size: as much as possible. Submerge sample in 10% HCl until bubbles stop forming. Rinse sample with DI water. Place in labeled weigh boat. Dry in oven.

Suspension Feeders

Corynactis californica:

Entire Anemone: Pull anemone from its substrate with forceps. Use a scalpel to cut away any pieces of substrate that stick to the base of the anemone. Sample size: whole anemone. Submerge entire organism in 10% HCl until bubbles stop forming. Rinse with DI water. Place entire organism in labeled weigh boat. Dry in oven.

Ophiothrix spiculata:

Entire star: Submerge entire organism in 10% HCl overnight (12- 14 hours). Rinse with DI water. Place organism in labeled weigh boat. Dry in oven.

Barnacles (any spp.):

Muscle: Place live animal in seawater overnight to allow it to purge its gut content. Remove organism from shell. Cut your sample from the muscle at the base of the organism. Sample size: as much as possible. Submerge sample in 10% HCl until bubbles stop forming. Rinse with DI water. Place in labeled weigh boat. Dry in oven.

Cucamaria salma:

Body wall: Before beginning allow the organism to thaw. Slice along the length of the body and remove guts and internal organs. Cut a sample from the body wall with a scalpel. Sample size: as much as possible. Submerge sample in 10% HCl until bubbles stop forming. Rinse with DI water. Place in labeled weigh boat. Dry in oven.
Muscle: Once the guts and organs are removed from the organism the muscle strips are readily apparent. Collect the muscles by cutting them away from their attachment with a scalpel. Sample size: as much as possible. Submerge the sample in HCl until bubbles stop forming. Rinse sample with DI water. Place in labeled weigh boat. Dry in oven.

Predators

Diopatra ornata:

Entire worm: Place live organism in seawater overnight to allow it to purge its gut contents then place in freezer until time of sample processing. Allow the organism to thaw before beginning sampling. Pull small pieces off of an end of the debris-covered tube until the head or tail of the worm is exposed. Gently pull entire worm from tube with forceps (this cannot be done while sample is frozen). Sample size: entire worm. Submerge entire worm in 10% HCl until bubbles stop forming. Rinse with DI water. Place entire organism in labeled weigh boat. Dry in oven.

Conus californica:

Foot: Allow organism to thaw. Remove operculum from foot by pulling it with forceps. Grasp foot with forceps and gently pull. Entire organism should be easily removed from its shell. Cut organs away from the foot and use the foot as your sample. Sample size: entire foot. Submerge in 10% HCl until bubbles stop forming. Rinse with DI water. Place in labeled weigh boat. Dry in oven.

Pisaster giganteus:

Tube feet: Process immediately upon return to laboratory. Using scalpel, cut tube feet at base along the arms of the star. Sample size: as many as possible. Submerge sample in 10%HCl. Rinse sample with DI water. Place in weigh boat. Dry in oven.

Predator/Scavenger

Asterina miniata:

Body wall: Process immediately upon return to laboratory. Using scalpel, cut tube feet at base along the arms of the star. Sample size: as many tube feet as possible. Submerge sample in 10% HCl until bubbles stop forming. Rinse sample with DI water. Place in labeled weigh boat. Dry in oven.

Cypraea spadicea:

Gut content: While the animal is still frozen use a vise to crack the shell. By removing the crown of the shell you can easily access the gut content. This should be done as soon as possible after removing animal from freezer. Thawed gut content is difficult to remove. Remove gut contents and submerge in 10% HCl until bubbles stop forming (be careful to remove all pieces of *Cypraea* intestine from the sample). Sample size: as much as possible. Rinse with DI water. Place in labeled weigh boat. Dry in oven.

Foot: Remove foot from animal with scalpel; take care not to include the mantle in your sample. Sample size: entire foot. Submerge in 10% HCl until bubbles stop forming. Rinse with DI water. Place in labeled weigh boat. Dry in oven.

Algae

*All non-coralline species: First identify the collected algae. Samples can be frozen until processing. Remove all epiphytes from algal sample. Organisms that are high in CaCO3 such as encrusting bryozoans should still be scraped off manually before submerging the sample in HCl. This ensures that the flesh of the organism is removed as well as the $CaCO_3$ skeleton. Submerge sample in 10% HCl until bubbles stop forming. Rinse with DI water. Place in labeled weigh boat. Dry in oven.

**Corallina corallina*: Select two small (3-4 cm diameter) rocks that are heavily encrusted, and remove all other organisms and algae. It is best to use very hard, dense rocks. Softer rocks often have boring invertebrates inside them. These organisms deteriorate in HCl and will contaminate your sample. Place your rocks in 10% HCl overnight. When the alga is decalcified it can be easily scraped from the rock to produce the sample. Rinse the sample with DI water. Place in labeled weigh boat. Dry in oven.