Turf and Foliose Algae Productivity Experiment Protocol

Miller et al. 2009

General Notes:

The study site was Naples Reef, a shale outcrop off the coast of Santa Barbara County ($34^{o}25'$ N, $119^{o}57'$W, see Ebeling et al 1980 for detailed site description). Turf was defined as distinct mats of low-growing algae less than 5cm canopy height. Foliose assemblages were high, bushy canopies generally >10cm high; both assemblages were quite distinct and have been identified as such in past work at this site (Laur and Ebeling 1983). We compared the species composition and metabolism of foliose and turf algal assemblages along 10m long transects placed at 10 to 12m depth in areas characterized by the two algal assemblages. Giant kelp, *Macrocystis pyrifera*, which is known to suppress understory algae through shading (Reed and Foster 1984), was sparse during our study.

Measuring Primary Production and Respiration:

Primary production and respiration of the benthos were measured *in situ* as changes in oxygen in closed chambers that covered $0.1m^2$ of the bottom. Measurements were taken simultaneously in two chambers placed randomly along each of two transects at midday on seven dates during November and December 2006. The chambers were tunnel-shaped, with end walls made of clear rigid acrylic and an open bottom framed by fiberglass-reinforced plastic. Flexible teflon sheeting (Tefzel, DuPont) was stretched over the side walls of the frame and held in place by a nylon gasket. This flexibility permitted wave energy to be transmitted through the walls of the chambers, allowing seaweed inside to oscillate naturally with wave-generated flow (sensu Gust 1977, Malan and McLachlan 1991, Yates and Halley 2003). Chamber volume was ~45 liters, and was measured for each chamber for production calculations. Water circulation, to ensure mixing of oxygen and prevent mass-transfer limitation of algal photosynthesis, was provided with a battery-powered submersible pump (Rule 500 baitwell pump, 1890 liters/hr). The chambers were sealed against the bottom with a weighted flexible plastic skirt. Observations using rhodamine dye indicated that this made a highly effective seal. Self-contained optical probes (D-Opto, ENVCO Inc., New Zealand) logged dissolved oxygen concentration and temperature inside the chambers at a frequency of once per minute.

Chambers were incubated in the light for ~30 minutes, then covered with blackout cloth for a dark incubation of ~20 minutes, which was sufficient in duration to show distinct slopes in oxygen change. Following the dark incubation, insecticide (Triazicide, 0.5% Lambda-Cyhalothrin, United Industries Corp., USA) was injected into the chambers to kill animals, allowing estimation of net primary production (NPP). Insecticide concentration in the chambers was ~1.9 ppm, which laboratory tests showed was sufficient for near-instantaneous death of small crustaceans and polychaete worms. Lambda-Cyhalothrin is a synthetic pyrethroid which has low phytoxicity (Dupont, personal communication) and has been shown to have no affect on algal photosynthesis (Hill et al. 1994, Farmer et al 1995).

To determine whether photosynthetic rates varied due to light levels, we measured bottom irradiance (PAR, 400-700 nm f=1s) during the incubations using logging meters

with hemispherical collectors (MKV-L, Alec Electronics, Japan) mounted on the bottom near the chambers. The collectors were ~10 cm above bottom. PAR data was averaged over incubations for analysis.

After incubations were completed, the benthos in the plots was collected by gently scraping dislodged algae and other material from the bottom and vacuuming it into a fine mesh bag using an airlift (see e.g. Coyer et al 2000). Macroalgae was separated from animals and other material in the laboratory and sorted by taxa. Epiphytes, which were all minute, were not identified. The algae was weighed and dried at 60^{o}C to obtain dry weights. Subsamples of dried tissue for each algal taxon were ground and analyzed for carbon content using an automated organic elemental analyzer (Carlo-Erba Flash EA 1112 series, Thermo-Finnigan Italia, Milano, Italy), and these values were used to convert dry mass to carbon mass. Macroinvertebrates and associated sediment and debris were fixed in formalin for 2 days before being transferred for storage in 70% ethanol. This method of preservation does not significantly affect measurements of biomass (Gaston et al. 1996). These samples were sieved at 0.25mm, split as necessary and sorted under a dissecting microscope. Macrofauna were separated by major taxa (generally phylum), counted, dried at 60^{o}C, weighed, and combusted to obtain ash-free dry weights.