Measurement of DOC release and analysis

Methods

In situ measurements of DOC release

We estimated rates of DOC release by *Macrocystis* using short-term (2-4 hours) in situ incubations of entire blades and portions of stipes in the kelp forest at Mohawk Reef near Santa Barbara, California, USA (34° 23.660' N, 119° 43.800' W). Incubations of blades were conducted within 2 m of the sea surface at midday on eight dates between October 2007 and August 2011 to investigate the effects of blade growth stage, nutrient status (as indicated by C:N ratio determined using an Exeter Analytical CE-440 CHN/O/S elemental analyzer), epiphyte load, sea surface irradiance, and sea surface temperature on rates of DOC release from intact blades. The DOC produced by giant kelp may include active exudation as well as passive diffusion associated with tissue degradation and senescence. Because our in situ measurements did not distinguish between these processes we refer to them collectively as DOC release.

Blades were identified as growing (young blades located < 2 m from the tip of a growing frond), mature (older and larger robust blades located > 2 m from the tip of a growing frond), and senescent (eroded blades with < 50% of their initial blade margin intact). For each sampling date incubations were performed on 10-15 blades distributed across the three growth stages of blades with each blade being selected from a distinct kelp individual. Giant kelp blades in the Santa Barbara region are commonly encrusted with the bryozoan *Membranipora serrilamella* (Arkema 2009). A visual estimate of the percent cover of *M. serrilamella* on each blade was recorded and used to evaluate whether the amount of DOC produced by blades was related to epiphyte load, as previously suggested by Frankboner and deBurgh (1977). To evaluate the effects of tissue type

(blade vs. stipe) and time of day (day vs. night) on DOC release by *Macrocystis*, we measured the amount of DOC released from 15 mature blades and portions of 15 intact stipes at Mohawk Reef during midday and near midnight in August 2011. During the entire study, photosynthetically active radiation (PAR) at the sea surface was recorded once per min using an integrated spherical PAR sensor and data logger (MKV-L; Alec Electronics, Kobe, Japan) mounted above the sea surface on a vertical spar buoy moored in the kelp forest. Similarly, temperature was recorded every 10 min using automated loggers (Stowaway Onset tidbits; Onset Computer, Bourne, Massachusetts, USA) mounted to the spar buoy 1-2 m below the sea surface. Because temperature and nutrients are closely related in the study region (McPhee-Shaw et al. 2007), temperature also provided an estimate of the concentrations of nutrients in ambient seawater during the incubations. Irradiance (in units of μmol photons $m^{-2} s^{-1}$) and temperature (degrees Celsius) were averaged over each incubation to obtain mean values for each variable.

Incubations of intact blades were performed in clear plastic bags. The bags were 11 cm x 66 cm and constructed of 6 mm polyethylene. A two-piece threaded nylon barbed fitting was inserted through each bag as a sampling port with Teflon coated silicone septa on each side of the polyethylene to ensure a gas and water tight seal. Divers gently slipped a bag over each blade and sealed the bag at the base of the pneumatocyst where it attaches to the stipe using a nylon cable tie (Fig. 1a). Using this technique the volume of the water sealed within each bag varied among blades (generally between 0.3 – 1.2 L). A second type of bag was constructed to sample DOC produced by stipes. For this we adapted a 3.8 L Ziploc polyethylene bag cut open on the two opposing sides (perpendicular to the zippered opening) with a built in sampling port as described above (Fig. 1b). An opened bag was zip locked around a portion of stipe extending between two blades and each end of the bag was then sealed around the stipe using a nylon

cable-tie. Tests in the laboratory and field using rhodamine dye showed no detectable leakage from either type of bag. Samples were drawn with 60 mL polypropylene/polyethylene syringe (no black rubber plunger tip) using luer-lock syringe adaptors fitted to silicone tubing that was attached to the sampling ports (Fig. 1). All bags, sampling ports, tubing, and syringes were bathed in a 5% HCl solution, flushed with Nanopure water and dried prior to use in the field to minimize the leaching of organic material from bags during incubations.

Two replicate 60 mL syringe samples were extracted from each bag at the beginning and end of the incubation and the time of sample collection was recorded. Upon collection the syringes (with sample) were put into Ziploc bags, placed on ice in an insulated cooler and transported to the laboratory. The volume of water and mass of kelp tissue within each bag was determined at the end of the incubation and used to estimate the mass of DOC produced per dry mass of kelp tissue. Kelp tissue was returned to the laboratory where it was weighed wet then dried at 60^{0} C for three days to determine dry mass.

Upon return to the laboratory the contents of each syringe were gently filtered through a GF/F filter (0.7μm; pre-combusted at 450°C for a minimum of 4 h) housed in an acid-cleaned polycarbonate cartridge, collected into 40 mL combusted EPA vials, and stored frozen (-20°C) until analyzed.

DOC analyses

DOC concentrations were determined via high temperature combustion using a Shimadzu TOC-V. The operating conditions of the Shimadzu TOC-V were slightly modified from the manufacturer's model system according to Carlson et al. (2010). CO_2 free carrier gas was produced with a Whatman gas generator. Sample was drawn into a 5 mL injection syringe, acidified with 2M HCL (1.5%) and sparged for 1.5 minutes with CO_2 free gas. Three to five

replicate 100 µL aliquots of sample were injected into the combustion tube heated to 680°C. The resulting gas stream was passed through several water and halide traps, the CO_2 in the carrier gas was analyzed with a non-dispersive infrared detector and the resulting peak area was integrated with Shimadzu chromatographic software. Extensive conditioning of the combustion tube with repeated injections of low carbon water (LCW) and deep seawater was done to minimize the machine blanks. After conditioning, the system blank was assessed with UV oxidized low carbon water. The system response was standardized daily with a four-point calibration curve of potassium hydrogen phthalate solution in LCW. All samples were systematically referenced against low carbon deep ocean reference water (>2600 m) and surface sea water every six to eight analyses (Carlson et al. 2010). The standard deviation of the deep and surface references analyzed throughout a run generally had a coefficient of variation ranging between 1-3% over the three to seven independent analyses (number of references depended on the number of samples in the run). Daily reference waters were calibrated with DOC consensus reference water provided by D. Hansell, University of Miami (Hansell 2005). The DOC concentrations were multiplied by the volume of each incubation bag to determine the grams of DOC produced during each incubation.

DOC release normalized by the mass of kelp tissue (in units of mg C (g dry mass)$^{-1}$ h^{-1}) was calculated as:

$$\text{DOC release} = (Cf - C0)\ VTM$$

Where C_f is the final concentration of DOC (in units of mg C L^{-1}) measured as the mean of two syringe samples taken at the end of the incubation, C_0 is the initial concentration of DOC (in units of mg C L^{-1}) measured as the mean of two syringe samples taken at the beginning of the incubation, V is the volume (L) of water measured at the end of the incubation + volume (L) of

the two syringe samples taken at the end of the incubation, T is incubation time (h) and M is dry mass (g) of kelp tissue used in the incubations.

Literature Cited

Arkema, K. K. 2009. Flow-mediated feeding in the field: consequences for the performance and abundance of a sessile marine invertebrate. Mar. Ecol. Prog. Ser. **388:**207-220.

Fankboner, P. V., and M. E. de Burgh. 1977. Diurnal exudation of ^{14}C-labelled compounds by the large kelp *Macrocystis integrifolia* Bory. J. Exp. Mar. Bio. Ecol. **28:**151-162.

McPhee-Shaw, E. E., D. A. Siegel, L. Washburn, M. A. Brzezinski, and J. L. Jones. 2007. Mechanisms for nutrient delivery to the inner shelf: Observations from the Santa Barbara Channel. Limnol. Oceanogr. **52**:1748-1766.