Materials and methods

Sample collection and preparation

Actively growing *Macrocystis pyrifera* blades that were relatively free of epiphytes were collected during the nitrate replete spring (May) and the nitrate-deplete summer (September) from natural populations near Santa Barbara, California for use in incubation experiments of surge uptake. Five replicate experiments were conducted in both spring and summer 2019. For each experiment a single blade located ~2 m from the distal end of a growing frond was collected from 30 unique haphazardly chosen plants. Blades were placed in an insulated cooler upon collection and returned to the laboratory at the University of California Santa Barbara where they were placed in a tank with constantly flowing sand-filtered seawater illuminated with full spectrum LED lamps emitting 100-200 μmol m^{-2} s^{-1} on a 12:12 hour day:night cycle for 1-2 days prior to each experiment.

Experimental design

Nitrogen uptake by *Macrocystis pyrifera* was measured in incubation experiments exposing entire blades to an isotopically labelled form (^{15}N) of one of three nitrogen sources (nitrate, ammonium or urea) for pre-determined amounts of time. In each replicate experiment, single blades were placed in 1 L trays filled with nitrogen-depleted filtered sea water. Nitrogen depleted sea water was obtained by inoculating translucent carboys of sand-filtered seawater with phytoplankton-enriched seawater and placing the carboys in ambient daylight (~ 30 μmol m^{-2} s^{-1}) at room temperature. After a minimum of four weeks, upon which nitrate plus nitrite concentrations were undetectable, the seawater was coarse filtered (10 μm) and then fine filtered (0.2 μm) using a gravitational filtration system, and stored at ambient seawater temperature until used in an experiment.

Each incubation tray was aerated with an aquarium pump and air stone to maintain constant water movement. Experimental pulses of nitrogen were simulated by introducing one of the three nitrogen isotope tracers into an incubation tray filled with 1 L of nitrogen-depleted seawater to achieve an initial concentration of 10 μM nitrogen prior to adding the kelp blade. A concentration of 10 μM nitrogen is representative of nutrient pulses delivered to giant kelp in southern California by internal waves (Brzezinski et al. 2013) and excretion (Bray et al. 1986). The length of exposure to labeled nitrogen was varied to determine how the rate of uptake

changed as a function of the duration of the simulated pulse. Because previous studies have shown that surge uptake in macroalgae typically diminishes after 15-30 minutes (D'Elia and DeBoer 1978; Rosenberg et al. 1984; Thomas and Harrison 1987; Dy and Yap 2001) we tested exposure times of 1, 5, 15, and 45 minutes. Three replicate incubations of the four exposure times were done for each nitrogen form, resulting in simultaneous incubations of 36 unique blades for each of the ten replicate experiments (five in spring and five summer). All experiments were done under conditions of saturating irradiance, >170 μmol photons m^{-2} s^{-1}, to eliminate light as a potentially confounding factor influencing the rate of nitrogen uptake (Colombo-Pallotta et al. 2006).

Blades were removed from the incubation trays at the end of the designated exposure time, and cleaned of any remaining tracer and potential epiphytes by rinsing in 10% HCl and then deionized water. Each blade was blotted and weighed damp, and then dried overnight at 60°C to obtain dry weight. Dried tissue was ground to a powder and subsamples were analyzed using an elemental analyzer isotope ratio mass spectrometer (EA-IRMS) to determine the atom % ^{15}N and the percent weight nitrogen (% N) of each blade, which was used to calculate uptake rates for each incubation treatment. For each experiment, five control blades, collected at the same time as the experimental blades, but not incubated in isotopically labeled ^{15}N, were processed and analyzed for ^{15}N to obtain an estimate of the initial nitrogen isotope composition and total nitrogen content (as a percent dry weight) of experimental blades prior to incubation.

Evaluating the physiological capacity for surge uptake

The uptake rate of nitrogen for nutrient pulses with different durations (V) in units of inverse time was calculated using a modified version of an equation by Legendre and Gosselin (1996):

Equation 1: $$V = \frac{(n_t - n_0)}{t(d_s - d_0)}$$

where n_t is the atom % ^{15}N in the blade after incubation; n_0 is the average concentration of isotope in the control blades; d_s is the atom % ^{15}N of nitrogen in the seawater at the start of the incubation; d_0 is the atom % ^{15}N in the nitrate pool before isotope tracer was added; and t is the length of the incubation in minutes.

Values of V calculated for pulses of different duration using Equation (1) were compared to assess how the physiological uptake of different forms of nitrogen changed over time in response to exposure to a high-concentration pulse. A caveat of this calculation is that uptake is averaged

over the total duration of the nitrogen pulse and thus does not provide instantaneous rates of uptake rate at any given point in time. To provide insight into the temporal evolution of uptake over a pulse lasting 45 minutes, average values of nitrogen uptake for specific time intervals during a 45-minute pulse (V_I) were calculated as:

Equation 2: $$V_I = \frac{(V_f t_f) - (V_i t_i)}{t_{f-} t_i}$$

where V_i and V_f are the uptake rates measured at the start and end of a specific time interval and t_i and t_f are the durations of the time intervals over which V_i and V_f were calculated. Using Equation (2) we calculated values of V_I for time intervals of 0-1 minute, 1-5 minutes, 5-15 minutes, and 15-45 minutes for each of the three nitrogen forms.

Assessing the ecological relevance of uptake rates

The ecological implication of the uptake response to pulsed nitrogen was assessed by examining the biomass-normalized uptake rate ρ [μg N (g dw)$^{-1}$ min^{-1}] using Equation (3):

Equation 3: $$\rho = V\ (\frac{m_N}{60 m_{dw}})$$

where m_N is the mass of isotope in the subsample taken for isotope analysis (μg) and m_{dw} is the dried weight subsample (g) (Legendre and Gosselin 1997).

Values of ρ were used in combination with estimates of daily nitrogen uptake by *Macrocystis pyrifera* to evaluate the likelihood that nitrogen uptake during pulses, and surge uptake in particular, make a significant contribution to giant kelp's nitrogen demand. For this purpose, we estimated the number of pulses of a given concentration and duration that would be needed to meet the nitrogen demand of giant kelp in spring and summer. Estimates of the average daily nitrogen demand in spring and summer were derived from measurements of net primary production by giant kelp (in units of g kelp N produced m^{-2}d^{-1}) and standing biomass (in units of g dry mass m^{-2}) of *Macrocystis pyrifera* obtained from a 15-y monthly time series of three kelp forests in the Santa Barbara Channel collected by the Santa Barbara Long Term Ecological Research program (Rassweiler et al. 2018).

Examination of nitrogen status as a driver of surge uptake

Previous studies have found surge uptake by macroalgae to be more pronounced in specimens previously starved of nitrogen (D'Elia and DeBoer 1978; Rosenberg et al. 1984;

Brinkhuis et al. 1989). Thus, we estimated the percent nitrogen of experimental blades to account for variation in surge uptake due to differences in nitrogen status. Because it was not possible to measure the nitrogen content of a blade prior to an experiment without damaging it, the percent nitrogen of a blade at the start of incubation (N_{t0}) was calculated as

Equation 4: $$N_{t0} = m_N - \frac{\rho t m_{dw}}{m_{dw} - 15\rho t m_{dw}}$$

where m_N is the mass of nitrogen present at the end of the experiments (μg) as measured by the EA-IRMS.