Methods from Edmunds PJ. (2012) Effect of pCO_2 on the growth, respiration, and photophysiology of massive *Porites* spp. in Moorea, French Polynesia. Marine Biology 159: 2149-2160.

Corals and treatments

Thirty colonies (<=4-cm diameter) of massive Porites spp. were collected from 3 to 4 m depth in the back reef of Moorea on April 13, 2011, taking care to choose colonies that were free of bioeroding Lithophaga bivalves. Corals were returned to the Richard B. Gump South Pacific Research Station and prepared as nubbins (Birkeland 1976) using epoxy (Z-Spar A788) to attach them to plastic tiles. Nubbins were left to recover in a 150-L tank at ~28 oC (ambient seawater temperature when the experiment was conducted) and an irradiance (photosynthetically active radiation, PAR, 400-700 nm) of 537 umol quanta m-2 s-1 (measured with LI-Cor 4-pi quantum sensor LI- 193); the tank was filled with seawater from Cook's Bay that was filtered (50 um) and partially exchanged (20 %) daily. On April 16, 2011, the corals were buoyant weighed (± 1 mg) to evaluate their skeletal weight at the start of the experiment (Davies 1989) and allocated randomly to 6 tanks (5 corals tank-1) representing a contrast of three pCO2 regimes at a single temperature.

The experiment was completed in 6 tanks in a custommade microcosm (Aqua Logic, San Diego, CA). Each tank was independently regulated for temperature, light, and pCO2, and the system configured to contrast three pCO2 regimes in a nested design (2 tanks treatment-1). Tanks were illuminated with 400 W metal halide lamps (True 10,000 K Hamilton Technology) and filled with filtered seawater (50 um) that was partially (20 %) exchanged daily and fully exchanged weekly. The water changes were used, in part, to ensure that the metabolism of the corals did not affect the total alkalinity (TA) of the seawater. Tanks were adjusted to achieve target irradiances of ~550 l mol quanta m-2 s-1 (on a 12:12 h light:dark cycle), a target temperature of 28 oC, and target pCO2 of ambient (~42.6 Pa for seawater in the aquarium system at this location, effectively the control), ~76.0 and ~1.1 Pa. pCO2 treatments mimicked conditions expected to occur, or be exceeded, this century under representative concentration pathway (RCP) scenario RCP8.5 (a high emission scenario); RCP6 (a medium baseline scenario) predicts pCO2 levels of ~63.3 Pa by 2100 (van Vuuren et al. 2011). The three treatments enhanced the capacity to describe the functional relationship between dependent variables and pCO2. The lighting provided a daily, integrated irradiance of PAR (24 mol quanta m-2 day-1) similar to that found where the corals were collected (22 mol quanta m-2 day-1 on 5 May 2009, recorded with a logging cosine PAR sensor [Compact LW, JFE Advantech] at 2-m depth).

pCO2 treatments were established by bubbling ambient air or CO2-enriched air into the tanks. The CO2-enriched air was created with two, independent solenoid-controlled gas regulation systems (Model A352, Qubit Systems) receiving 99 % pure CO2 from a gas cylinder (at 17 kPa) and ambient air from a compressor (103 kPa). Automated mass-flow controllers and a solenoid valve with a variable duty cycle were used to blend air and CO2 in mixing chambers

from which gas was drawn to measure pCO2 with infrared (IR) gas analyzers (S151, Qubit Systems). The IR-gas analyzers were calibrated with certified reference gas (1,000 ppm, Part no. X02 N199CP10CQS3, Airgas West, Northridge, CA), and their output dynamically adjusted the duty cycle of solenoid valves maintaining the desired pCO2. Output pCO2 was logged (in ppm) on a PC running LabPro software (Vernier Software and Technology). The final gas mixtures were pumped (using a Model DOA-P704-AA Gast pump) at ~10-15 L min-1 through tubing split among 4 tanks that received elevated pCO2; air was supplied to the other 2 tanks at a similar rate.

To evaluate the efficacy of the treatments, tanks were monitored for light intensity (PAR), temperature, salinity, and DIC chemistry. Light intensity was recorded approximately daily below the surface of the seawater using a 4-p quantum PAR sensor (LI-193) and meter (Li-1400) (Li-Cor Biosciences), and temperature was recorded daily (but monitored more frequently) with a certified digital thermometer (Fisher Scientific, 15-077-8, ± 0.05 oC). The chemistry of the seawater was analyzed every third day for total alkalinity (TA) using potentiometric titration according to standard operating procedure (SOP) 3b (Dickson et al. 2007); preliminary measurements revealed stable treatments were maintained throughout each day. Titrations were completed with an automatic titrator (Model T50, Mettler-Toledo, Columbus OH) fitted with a DG115-SC pH probe (Mettler-Toledo, Columbus OH) that was 3-point calibrated with pH 4.00, 7.00, and 10.00 NBS buffers (Fisher) and filled with certified acid titrant (~0.1 mol L-1 HCl and 0.6 mol L-1 NaCl, from A. Dickson Laboratory, Scripps Institution of Oceanography). Seawater was analyzed in single samples (~400 mL) drawn from each tank at ~09:00 h. Samples were equilibrated to the laboratory temperature and processed within 3 h for salinity (YSI 3100 Conductivity Meter), pH (using a spectrophotometric method with m-cresole purple dye [SOP 7 (Dickson et al. 2007)]), and potentiometric titration. The results were processed in a Microsoft Excel spreadsheet (Fangue et al. 2010) to determine TA (lmol kg-1), and the accuracy and precision of the determinations using certified reference material (CRM) (Batch 105 from A. Dickson Laboratory, Scripps Institution of Oceanography) with each series of samples. Over the course of the study, CRMs were analyzed with mean accuracy of 0.4 %. TA, pH, salinity, and temperature were used to calculate DIC parameters using CO2SYS (Lewis and Wallace 1998) with the constants of Mehrbach et al. (1973) and pH expressed on the total scale.

The experiment was designed to expose corals to pCO2 treatments beginning on April 16, 2011, but equipment problems delayed the start of pCO2 additions until April 25, 2011; all other conditions were regulated over this period. pCO2 treatments were maintained for 11 days before the experiment was concluded on May 6, 2011.

Response variables

The response of the corals to the treatments was evaluated by measuring aerobic respiration, calcification, and photophysiology. The area of coral tissue and biomass were measured to normalize physiological rates and, in part, because changes in biomass can be a component of

the functional response of corals to differing pCO2 regimes (Fine and Tchernov 2007; Edmunds 2011; Chauvin et al. 2011). Respiration was measured using a confined respirometer (2,127 mL; designed after Patterson et al. 1991), with determinations completed during the day by placing the corals in a darkened chamber and recording O2 depletion after a steady decline began (usually within 15 min). Coral respiration is typically elevated during daylight (Edmunds and Spencer-Davies 1988; Shick 1990), and while it is unknown how long this effect persists after darkness begins, it might persist for a few minutes if caused by hyperoxia, which disappears quickly in darkness (Ku¨hl et al. 1995), or longer (as in Schneider et al. 2009) if caused by the catabolism of carbon from the Symbiodinium. In the present study, logistical constraints required respiration to be measured in darkness during the day, and while this likely resulted in an upward bias in the value recorded (Edmunds and Spencer-Davies 1988; Shick 1990), it more closely approximated light-enhanced dark respiration (LEDR) that has been associated with increases in pCO2 (Crawley et al. 2010). To process the corals for respiration using the single respirometer available, measurements began on May 1, 2011 and were completed by May 5, 2011 (with ~6 corals day-1), using corals drawn randomly from the 6 tanks. After measurements, the corals were returned to their treatment tank until the experiment concluded on May 6, 2011.
Flow in the respirometer was regulated through the DC voltage supplied to a pump (Rule 1,360 L h-1), with the objective of creating turbulent and ecologically relevant water motion. Flow speed was measured by photographing hydrated Artemia spp. eggs (Sebens and Johnson 1991), which revealed a mean flow speed of 12.3 ± 0.5 cm s-1 (± SE, n = 50) in the center of the working section of the respirometer. The partial pressure of O2 in the respirometer was measured using a fiber optic oxygen sensor (Foxy-R, Ocean Optics, Dunedin, FL, USA) attached to a spectrophotometer (USB 2000, Ocean Optics, Dunedin, FL, USA) and operated using a PC running the manufacturers' software (OOIsensors 1.00.08). The sensor was calibrated with a zero solution (sodium sulfite and 0.01 M sodium tetraborate) and an O2 maximum created in water-saturated air. O2 saturation was maintained between 90 and 100 % during trials lasting 15-30 min. Control trials were completed daily (<= 2 days-1) and consisted of a respirometer filled with seawater alone and operated in an identical manner to that described above. For experimental trials, the rate of decline in O2 saturation was determined by fitting a least squares linear regression to the O2 saturation versus time relationships, correcting for O2 fluxes in the controls, and converted to O2 concentration in seawater of a known temperature and salinity using tabulated constants (N. Ramsing and J. Gundersen at http://www.unisense.com [based on Garcia and Gordon 1992]). Respiration was normalized to the area of coral tissue (lmol O2 cm-2 h-1) and biomass (lmol O2 mg-1 h-1) (described below).

To measure calcification, the corals were again buoyant weighed (± 1 mg) on May 6, 2011, and the difference between the initial (from 16 April 2011) and final buoyant weight converted to dry weight using the density of aragonite (2.93 g cm-3, Davies 1989). Dry weight, standardized by time and area (mg cm-2 day-1) and biomass (mg mg-1 day-1), was used as a measure of calcification. To gain insight into the relationship between metabolic costs and calcification, aerobic respiration was translated to energy units using an oxy-joulimetric conversion of 440 J mmol O2 -1 (Elliott and Davison 1975) and normalized to the quantity of CaCO3 deposited

over a comparable period (i.e., J mg-1). While this is not the cost of calcification, reflecting instead a rough estimate of the combined costs of metabolic and chemical work, it should capture shifts in the costs of calcification, which are thought to account for 13-30 % of total energetic costs of corals in shallow (10 m) water (Allemand et al. 2011).

The area of the corals was determined at the conclusion of the experiments using aluminum foil (Marsh 1970) and, thereafter, the corals were fixed in 5 % formalin in seawater. After 24-48 h of fixation, the corals were decalcified in 5 % HCl in seawater until a skeleton-free tissue tunic remained. Forceps were used to remove bioeroding sponges, organic matrix, and the alga Ostreobium spp. from the central void of each tunic, and the tunic rinsed in distilled water and dried to a constant weight at 60 oC. When standardized to area (mg cm-2), this provided a measure of biomass that was downwardly biased by ~10 % due to the effects of formalin and HCl on cnidarian tissue (Davies 1980).

To gain insight into the broader effects of pCO2 on massive Porites spp., the photochemistry of their Symbiodinium was examined through measurements of maximum photochemical efficiency of open RCIIs (Fv/Fm) and effective photochemical efficiency of RCIIs in actinic light (deltaF/F'm), using pulse amplitude modulation (PAM) fluorometry. Fv/Fm is measured after dark adaptation when photochemical quenching is fully relaxed, and it assesses the greatest efficiency with which PSII harvests light and converts it to excited electrons (Maxwell and Johnson 2000; Enriquez and Borowitzka 2010). deltaF/F'm is measured in a light-adapted state, and through its depression relative to Fv/Fm, assesses the extent of non-photochemical quenching (Maxwell and Johnson 2000; Enriquez and Borowitzka 2010). Fv/Fm is determined from (Fm - F')/ Fm, where Fm = maximum fluorescence yield, and F' = fluorescence yield in darkness; deltaF/F'm is determined from (F'm - F0)/F'm , where F'm = maximum fluorescence yield in actinic light, and F0 = fluorescence yield in actinic light. The two measures of photochemical efficiency were used to calculate excitation pressure (Qm) from [1 - deltaF/F'm/(Fv/Fm)] which standardizes deltaF/F'm among samples differing in Fv/Fm (Iglesias-Prieto et al. 2004). Qm scales from 0 to 1, with values close to zero indicating mostly open reaction centers in PSII, and potential light limitation, whereas values close to 1 indicating mostly closed reaction centers, and potentially photoinhibition (Iglesias-Prieto et al. 2004).

deltaF/F'm was measured between 12:00 and 13:00 h on May 5, 2011, and Fv/Fm was measured on the same day between 18:50 and 19:15 h following ~60 min dark adaptation. The Diving PAM was operated with a constant measuring intensity and gain of 6 for all measurements, and was fitted with the 5.5-mm diameter fiber optic cable that had a clear plastic spacer fitted to the tip that held it 10 mm above the coral tissue. deltaF/F'm was measured with the corals in the tanks and beneath the metal halide lamps, and care was taken to ensure the corals were not shaded during this process. For the measurement of Fv/Fm, a preliminary experiment was used to select the duration of dark adaptation following demonstration that Fo and Fm adjusted to stable values following 15-60 min of darkness after being in the light all day.

Statistical analysis

Physical and chemical conditions in the tanks, as well as the responses of the corals, were analyzed with a two factor, nested ANOVA in which treatments (pCO_2 regimes) were a fixed factor, and tanks were a random factor nested in treatments. Where tank effects were not significant (at $P>0.25$), they were dropped from the model (Quinn and Keough 2003) and the analyses repeated as a one-way ANOVA using the corals as replicates. Post hoc analyses were completed with Fishers Least-Significant Difference test, and the normality and homoscedasticity assumptions of ANOVA were explored through graphical analyses of the residuals. Power calculations for select non-significant results were calculated according to Zar 2010).