Ocean warming but not acidification weakens top-down control of an intertidal foundation species

Tena S. Dhayalan[1], Nyssa J. Silbiger[2]

Corresponding author: Tena Dhayalan

(858) 527-5271 - tenadhayalan@gmail.com

Supplemental Methods

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[1] Department of Biology, California State University Northridge, Northridge, California, USA
[2] Uehiro Center for the Advancement of Oceanography, Department of Oceanography, University of Hawai‘i at Manoa, Honolulu, Hawai‘i, USA

SUPPLEMENTAL MATERIALS AND METHODS

Mesocosm system

The mesocosm is a recirculating system where water from aquaria passes through 50 μm filters, four low density filters (Matala, FSM190, 1.7 mm), four high density filters (Matala, FSM365, 0.55 mm), three carbon filters (Aqua Logic, CF28AC), a UV sterilizer (Comet Series 95 Watt Lamp), and a chiller (Aqua Logic Delta Star, DS-4). We replaced approximately 50% of the system's total volume weekly to prevent the accumulation of metabolic waste, reduce evaporation, and maintain total alkalinity (Supplemental Table 1). We spot-checked mesocosm water for nitrate, nitrite, and ammonia levels using an API saltwater master test kit (Mars, Inc). Additionally, we collected water from individual tanks each week in 125 mL, acid-washed Nalgene bottles to track total alkalinity.

Temperature and pH treatments were maintained using a Neptune Apex system. We monitored pH using a 2-point calibrated (7 and 10 pH units NBS) double junction pH probe (Neptune Systems) taking measurements at 10 min increments. These aquaria probes were cross-calibrated with tris-calibrated pH probes once a day using an Orion Star Multiparameter Meter with a ROSS Ultra glass electrode (Thermo Scientific) measuring mV paired with a traceable digital thermometer (FisherBrand™, precision = 0.0001, accuracy = ±0.05°C; SOP 6; (Dickson et al. 2007). We calibrated the glass electrode every three days using a multipoint calibration to a tris standard solution from the Dickson Lab at Scripps Institution of Oceanography (Dickson et al. 2007). We then calculated pH_T from mV and *in situ* temperature measurements using the *seacarb* package in R (Gattuso et al. 2017). pH was manipulated in each aquarium by bubbling in CO_2 and CO_2-free air controlled by solenoids in the Apex system. CO_2-free air was achieved by pumping compressed air (Kaeser Compressors) through carbon dioxide absorbers (VCD4, Altec Air). We recorded temperature using HOBO TidBit Loggers

(HOBO TidBit MX2203, ± 0.2°C, 10 min) and Apex probes in each tank taking measurements every 10 min, which were cross-calibrated daily using a traceable digital thermometer (FisherBrand™). We manipulated the temperature within tanks using heaters (200W or 400W, Hydor) connected to the Apex thermostat system. Water was circulated in the aquaria using a submersible powerhead pump (Hydor Nano Koralina 240 powerhead, 240 GPH).

We mimicked local light and tidal conditions. LED lights (Halo Basic M-110 & Halo Deluxe MX-80) were programmed to a 12:12 photoperiod based on local sunrise and sunset times, with high Kelvin white and blue light (400–480 nm) during the day and only blue light at night. We measured the photosynthetically active radiation (PAR) for each tank weekly using a full-spectrum, underwater quantum Apogee light meter (MQ-510; μmol m^{-2} s^{-1}; accuracy ± 5%) to ensure all organisms received the same light intensity. High and low tides based on semidiurnal tides from the collection site were simulated using a solenoid (Apex Fusion) to decrease outflow during high tides and increase outflow during low tides. Low tide lasted for 2 hours, based on the average exposure time in 2022 at Los Angeles Harbor at a tidal height of 0.3 m above MLLW (CO-OPS 2007), the average height of mussel beds at White Point Beach, CA. In addition to monitoring using flowmeters (Apex Fusion FS-25), we manually checked inflow (10.8 L/h) to each aquarium using a graduated cylinder and timer to ensure high and low tides were consistent.

Respiration and calcification measurements

Organisms were individually placed in respiration chambers with a stir bar (200 rpm), a temperature probe (PreSens Pt100, ± 0.1°C), and oxygen probe (PreSens DP-PSt7, ± 0.05% O_2) taking measurements at a frequency of 1Hz. Oxygen probes were calibrated to oxygen-free water (1 g sodium sulfite and 50 μL cobalt nitrate) and oxygen-saturated water using an air pump and air stone. Due to differences in organism size and metabolic rates, we used different respiration

chambers for mussels and whelks. Both chambers included platforms for securing organisms above the stir bar to prevent obstruction of mixing. For mussels, respiration chambers were 10 × 10 × 11.5 cm with a maximum capacity of 650 mL. For whelks, respiration chambers were 5.5 × 5.5 × 6.5 cm with a maximum capacity of 116 mL.

Respiration chambers were submerged into a temperature-controlled water bath (InkBird ITC-308; ±1°C) maintained by a 200W heater (Hydro) and chiller (Aqua Logic Delta Star, DS-4) and oxygen evolution was measured for one hour in the dark. Only mussels from the control cages (i.e., no predator) were selected for metabolic rates, to reduce the effects of predator presence and ensure all mussels were exposed to treatment conditions for the same period of time. All mussels and whelks from one tank were run in concert over one to two blocks along with one seawater blank (max n = 10 chambers per block), all filled with water from their respective treatment tanks (i.e., at treatment pH and temperature).

To calculate respiration rates, we first deleted the first two minutes of oxygen measurements as organisms adjusted to chamber conditions. Then, oxygen measurements were thinned such that one measurement was retained per 20 seconds to reduce the data processing time (n = ~180 oxygen measurements per individual) while maintaining the trend in oxygen evolution over time. We used repeated local linear regressions on thinned data to calculate oxygen flux rates within incubation chambers using the R package LoLinR (Olito et al. 2017). For mussels that were determined not to be gaping during part of the respiration trials (n=7), closed portions of the trial were cut (< 20 min) to reduce dampening of the respiration slope. We confirmed that there was not a significant difference between respiration rates calculated from 40 min runs compared to one hour runs (t-test: $t_{164} = 1.06$, $P = 0.29$). We accounted for both

chamber seawater volume and blank chamber rates to calculate the biomass-normalized respiration rates.

The calcification rate was determined using the total alkalinity (TA) anomaly technique from the change in water collected before and after the one-hour respiration incubation (Chisholm and Gattuso 1991; Tagliarolo et al. 2013; Ninokawa et al. 2024). Initial TA values were calculated from triplicate samples collected prior to the incubation. We collected all water samples in acid-washed, triple-rinsed Nalgene bottles and immediately fixed the samples with 50 µl of 50% saturated mercuric chloride ($HgCl_2$) in deionized water.

TA was measured using open cell potentiometric titrations following standard operating procedures (SOP 3b; Dickson et al. 2007) using an automatic titrator (Mettler-Toledo T5) with a InMotion Pro-sample carousel and certified HCl titrant (Dickson Lab). We calibrated the titrator pH probe (Mettler-Toledo, DGi-115) daily using NBS buffers (Mettler Toledo) and measured titrator accuracy, which was always less than 0.9% off from a Certified Reference Material (Dickson Lab). Seawater parameters were measured using a tris-calibrated Orion Star Multiparameter Meter with a ROSS Ultra glass electrode (Thermo Scientific), traceable digital thermometer (FisherBrand™, ±0.05°C), and YSI Conductivity/Temp Handheld Meter Pro 2030 (±0.1 ppt). We then used TA, pH_T, temperature, and salinity to calculate carbonate system parameters for the mesocosm system (pCO_2, CO_2, HCO_3^{-2}, CO_3^{-2} , DIC, $\Omega_{aragonite}$, $\Omega_{calcite}$) using the *seacarb* package in R (Gattuso et al. 2017).

SUPPLEMENTAL RESULTS

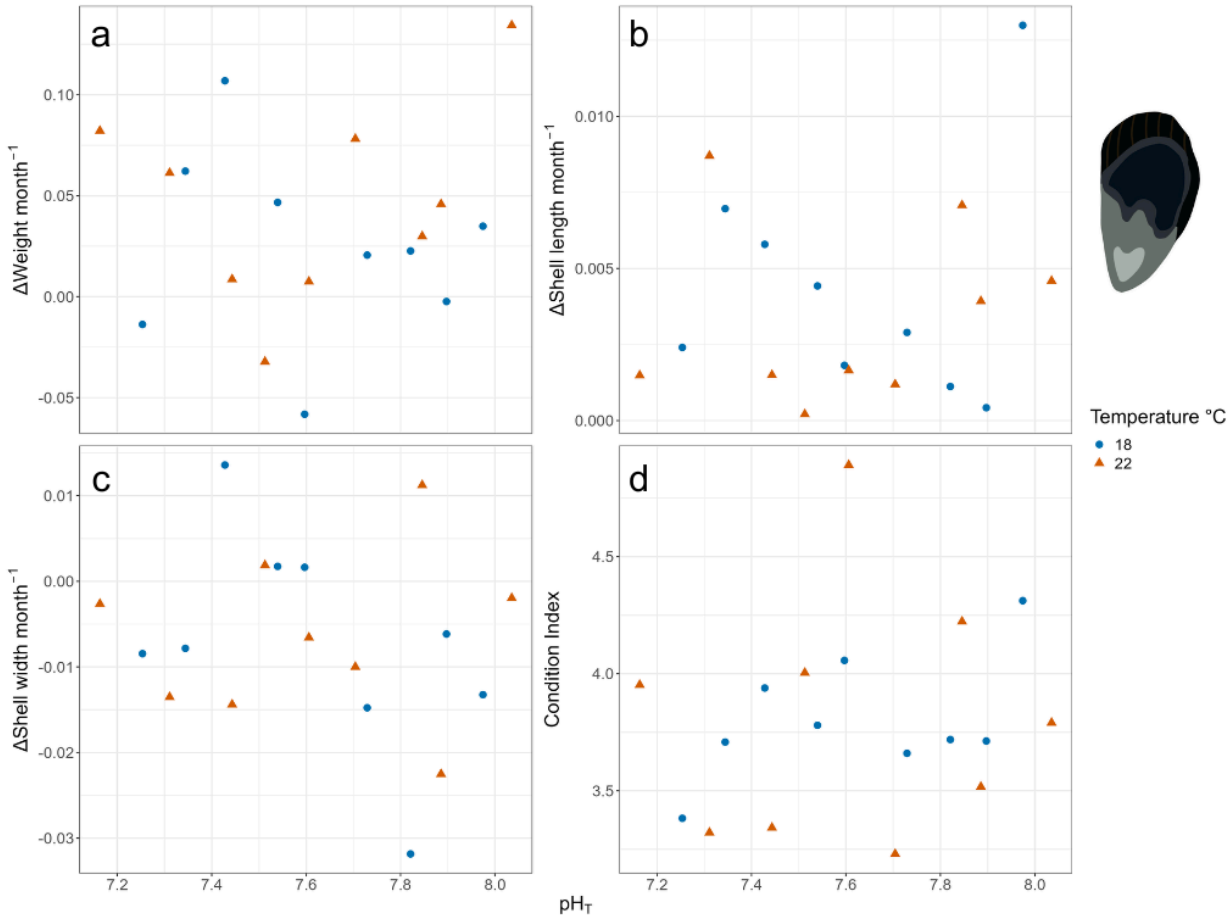
Supplemental Table 1. Carbonate chemistry of all pH × temperature treatments (mean ± SD based on n = 3 samples). Seawater parameters are calculated from total alkalinity (TA; μmol kg^{-1}), pH_T, temperature (T; °C), and salinity (S; psu). Calculated parameters included pCO_2 (μatm), dissolved inorganic carbon (DIC; μmol kg^{-1}), and saturation states of aragonite (Ω_{arg}) and calcite (Ω_{cal}).

pH_T	T	S	pCO_2	DIC	TA	Ω_{arg}	Ω_{cal}
7.14 ± 0.05	21.98 ± 0.12	33.01 ± 0.92	3188.38 ± 374.47	1889.69 ± 30.28	1825.89 ± 26.17	0.36 ± 0.04	0.55 ± 0.07
7.21 ± 0.03	18.08 ± 0.42	32.99 ± 0.88	2565.19 ± 258.78	1853.12 ± 80.47	1800.46 ± 72.44	0.35 ± 0.02	0.55 ± 0.02
7.26 ± 0.09	22.04 ± 0.12	33.02 ± 0.90	2360.95 ± 563.58	1832.33 ± 66.86	1805.79 ± 53.27	0.47 ± 0.09	0.72 ± 0.14
7.30 ± 0.03	18.25 ± 0.49	32.97 ± 0.90	2070.61 ± 211.62	1834.63 ± 75.26	1807.84 ± 67.06	0.44 ± 0.01	0.68 ± 0.02
7.39 ± 0.04	17.82 ± 0.13	32.98 ± 0.90	1714.04 ± 164.18	1831.31 ± 29.13	1824.31 ± 20.09	0.52 ± 0.04	0.81 ± 0.06
7.40 ± 0.03	22.05 ± 0.22	33.01 ± 0.94	1652.59 ± 179.70	1786.65 ± 66.52	1797.88 ± 60.38	0.63 ± 0.04	0.97 ± 0.06
7.45 ± 0.10	22.10 ± 0.22	33.05 ± 0.92	1513.44 ± 420.05	1782.55 ± 89.68	1805.88 ± 67.50	0.71 ± 0.13	1.09 ± 0.19
7.53 ± 0.16	21.97 ± 0.21	32.97 ± 0.94	1288.11 ± 557.00	1770.43 ± 73.77	1815.28 ± 39.40	0.86 ± 0.25	1.32 ± 0.39
7.54 ± 0.03	18.10 ± 0.53	33.00 ± 0.92	1160.45 ± 105.36	1755.66 ± 68.01	1788.71 ± 67.71	0.72 ± 0.05	1.12 ± 0.08
7.59 ± 0.01	18.09 ± 0.20	33.00 ± 0.89	1015.00 ± 62.93	1733.62 ± 69.45	1779.80 ± 66.96	0.80 ± 0.02	1.24 ± 0.02
7.66 ± 0.13	18.02 ± 0.14	32.99 ± 0.90	890.54 ± 324.17	1721.36 ± 90.46	1786.66 ± 66.87	0.94 ± 0.22	1.46 ± 0.35

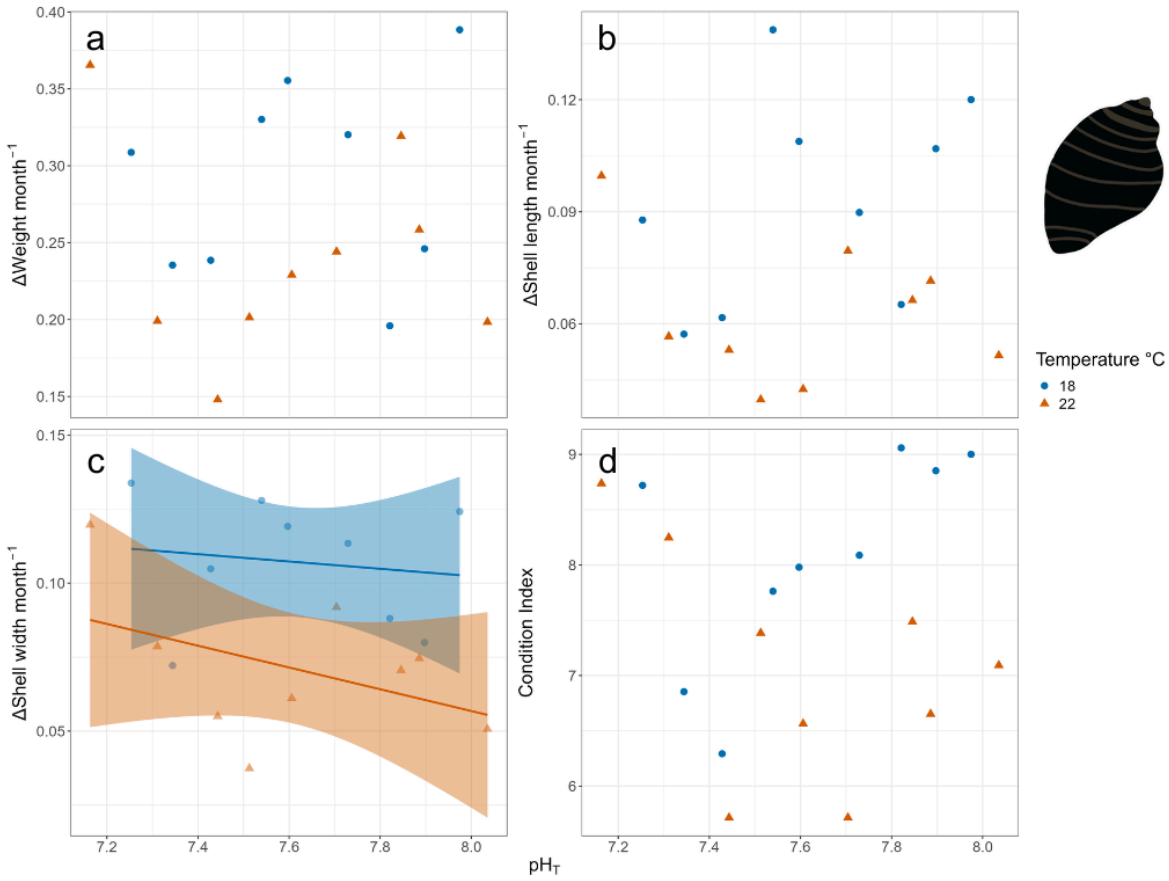
7.67 ± 0.10	22.17 ± 0.20	33.02 ± 0.92	870.19 ± 242.57	1704.82 ± 93.26	1788.83 ± 70.51	1.12 ± 0.19	1.71 ± 0.30
7.83 ± 0.02	18.20 ± 0.17	32.98 ± 0.92	562.57 ± 44.15	1681.94 ± 54.75	1797.23 ± 49.46	1.33 ± 0.02	2.06 ± 0.02
7.85 ± 0.03	22.00 ± 0.15	32.97 ± 0.92	529.39 ± 59.33	1650.44 ± 56.57	1793.06 ± 48.34	1.60 ± 0.09	2.46 ± 0.13
7.86 ± 0.05	18.07 ± 0.45	32.99 ± 0.90	521.06 ± 79.14	1657.96 ± 70.31	1781.37 ± 58.90	1.39 ± 0.09	2.15 ± 0.14
7.86 ± 0.07	22.33 ± 0.65	33.03 ± 0.92	534.63 ± 114.90	1650.69 ± 76.50	1796.21 ± 51.77	1.63 ± 0.19	2.51 ± 0.29
7.97 ± 0.02	18.24 ± 0.40	32.98 ± 0.91	381.88 ± 35.49	1618.56 ± 68.52	1784.11 ± 63.43	1.76 ± 0.01	2.72 ± 0.01
8.00 ± 0.08	21.92 ± 0.16	33.00 ± 0.88	360.53 ± 91.72	1588.64 ± 72.34	1789.30 ± 50.73	2.12 ± 0.30	3.26 ± 0.46

Supplemental Table 2. Control mussel and whelk mortality per tank over the experimental period. Target pH_T and temperatures for each tank are represented here.

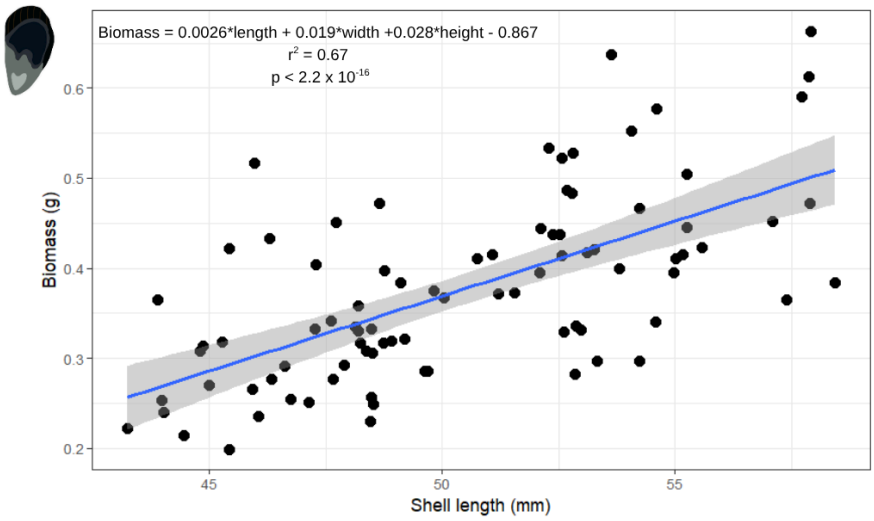
Species	pH_T	Temperature °C	Mortalities	Temperature °C	Mortalities
Mussel	7.2	18	4	22	1
	7.3	18	1	22	2
	7.4	18	0	22	0
	7.5	18	1	22	1
	7.6	18	1	22	1
	7.7	18	1	22	2
	7.8	18	0	22	1
	7.9	18	0	22	1
	8	18	1	22	1
Whelk	7.2	18	0	22	1
	7.3	18	0	22	0
	7.4	18	0	22	0
	7.5	18	0	22	1
	7.6	18	0	22	0
	7.7	18	0	22	0
	7.8	18	0	22	1
	7.9	18	1	22	0
	8.0	18	0	22	0



Supplemental Fig. 1. Average proportional mussel a) change in weight, b) shell length, and c) shell width over the course of one month, and d) condition index across pH_T and temperature treatments, with blue dots representing mussels at 18°C and red triangles at 22°C.



Supplemental Fig. 2. Average proportional whelk a) change in weight, b) shell length, and c) shell width over the course of one month, and d) condition index across pH_T and temperature treatments, with blue dots representing mussels at 18°C and red triangles at 22°C. The trend lines represent the general linear model with change in shell width as a predictor, pH_T as a continuous predictor, and temperature as a factor with two levels, where temperature was a significant predictor ($F_{1,14} = 10.64$ $P = 0.006$).



Supplemental Fig. 3. Mussel biomass predicted by a linear regression with predictors shell length, width, and height (n=86). Where length is the anterior–posterior distance, width is left–right valve distance, and height is the dorsal–ventral distance.

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