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Supporting Information for "Evolved differences in energy metabolism and growth dictate the impacts of ocean acidification on abalone aquaculture"

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Experimental Overview

Santa Barbara (SB) red abalone used in experiments were sourced from The Cultured Abalone Farm in Goleta, CA. These animals were fourth generation descendants of wild abalone collected from locations throughout the Santa Barbara Channel (34.241943°N, 119.889999°W) in 1994. Van Damme (VD) animals were wild broodstock collected by hand via SCUBA from Van Damme State Park, CA (39.2757°N, 123.8000°W) on 15 June, 2015 by California Department of Fish and Wildlife (CDFW) divers.

Prior to spawnings, broodstock were held under ambient, flow-through seawater and fed *ad libitum* on a mixed diet of locally collected red and brown seaweeds. Abalone were spawned and experiments conducted over three intervals: SB-F_1 experiments 5 February, 2016 - 13 May, 2016, VD-F_1 experiments: 15 July, 2016 - 18 May, 2017, SB F_2 Experiments: 18 Jan, 2018 – 26 April, 2018 (Fig S1). Animals were spawned utilizing the hydrogen peroxide method (1) and raised from embryos to three months of age in an experimental CO_2 manipulation apparatus housed at the Bodega Marine Laboratory (2).

Abalone were cultured in two phases; the initial 7-day swimming larval phase conducted in custom designed PVC larval culture bucket pairs (see 'Larval Culture' for detail) and then for 90 days post-settlement (i.e., 97 total days post-fertilization, or "DPF") in experimental 120 ml culture chambers (Starplex Scientific Leak Buster™). Survivors in replicates were visually counted at 4 DPF and 7 DPF during the larval phase and then post-settlement at 10 DPF, 28 DPF, and 97 DPF. At the conclusion of experiments, surviving animals were photographed under a stereo microscope (Leica M125 with a Leica DC290 camera; Leica Microsystems) to calculate shell areas from digital photography, and in the case of experiments on the F_2 generation, animals were additionally weighed for shell and tissue mass on a microbalance (Sartorius Ultramicro) to determine organic and carbonate mass components (see (2) for additional methodological detail of microbalance methods). In each experiment, total lipid content of red abalone larvae at 4 DPF, 7 DPF and newly settled spat (10 DPF) was determined from a random sample of individuals using a modified spectrophotometric sulfophosphovanillin method (3, 4) (see full methodology below).

Spawning and Fertilization

Abalone were induced to spawn in individual 18 L buckets using three percent hydrogen peroxide/1M TRIS solution in six liters of UV treated seawater. Animals were removed from chemicals upon gamete release, rinsed in UV treated 1 µm filtered seawater and the placed in a new bucket with fresh 1 µm filtered seawater from which subsequent gamete releases were collected. Concentrated sperm was collected directly from respiratory pores and stored on ice in 50mL conical tubes until fertilization. Eggs were rinsed with UV filtered seawater using a 100 mm mesh sieve, quality was assessed under a compound microscope, and eggs were fertilized within one hour of release to maximize fertilization success. Polyspermy was avoided by decanting excess sperm using fresh UV filtered seawater until approximately 5-15 sperm could be observed attached to the envelope of eggs under a compound microscope. After the addition of sperm, at 4 hours post-fertilization, we estimated fertilization success via microscope by assessing the proportion of embryos undergoing normal cleavage over the total number of eggs and embryos in 20 random samples of 200 µL per cross. All fertilizations and subsequent embryonic development prior to the addition of embryos to larval culture buckets was completed in a temperature-controlled room held at 14˚C.

Experiment Phasing

SB-F_1 animals were spawned on 5 February 2016 and five maternal families (SB Families A-E) were created by crossing eggs from individual females with pooled sperm from 5 contributing males, yielding up to 25 genetic families. VD animals were spawned on two occasions; first on 15 July 2016 yielding a cross between a single male and female, and again on 10 February 2017 yielding 3 additional maternal families generated by crossing 3 more females with pooled sperm from 3 additional males resulting in 4 total maternal families (VD Families A-D). Thus, up to 10 VD genetic families (3x3 and 1x1) were produced. Due to human error during larval culture maintenance, low CO_2 replicates for Van Damme Family C were lost prior to 4 DPF sampling, thus replicates from this maternal lineage were absent in subsequent low CO_2 time point analyses. In the case of all F_1 experiments, 30,000 cleaving embryos

from each maternal family cross were added to larval culture buckets. In the case of SB-F_1 experiments, a single maternal family (SB-F_1-B) was replicated in three larval culture buckets; while others (due to lower spawning output) were replicated in a single culture bucket each under high and low CO_2, respectively. In VD experiments, the 1x1 cross from 2016 was replicated in 3 larval culture bucket replicates per CO_2 level, while each maternal family cross from VD 2017 was replicated in 2 culture bucket replicates per CO_2 level.

After being photographed at the completion of experiments, surviving F_1 animals from the SB population were cultured for an additional 616 days at 15˚C under ambient, flowing seawater, and fed *ad libitum* on locally collected red and brown seaweeds. At 713 DPF, these animals were spawned and 5 individual family crosses were created between a single successfully spawning female and 5 males, all derived from a single prior F_1 family cross (SB-E) which had been carried through the prior generation under low CO_2, thus creating replicated paternal SB-F_2 families for comparison. In the case of SB-F_2 experiments, paternal identity served as the unit of replication, and 3000 cleaving embryos from each paternal cross were added to culture buckets, with each family being replicated in two larval buckets per CO_2 level. In all experiments, the crosses created, and the larval culture design implemented, utilized all viable crosses generated during each spawning event, maximizing replication and genetic diversity given the levels of successful spawning achieved at each attempt.

Larval Culture

Experiments were performed in a pre-existing OA culture apparatus (2) modified for larval abalone culture. Pumps continuously delivered treatment seawater held at experimental values to overhead PVC manifolds, which delivered water to larval culture buckets via individual 3.2mm plastic tubing lines, regulated by irrigation drippers (7.57 L h^{-1}, Netafim 2 GPH pressure compensating emitters). Treatment sea water was delivered to the bottoms of custom designed 7-L circular PVC buckets fitted with 100 µM nylon mesh bottoms to retain swimming larvae, modeled after buckets used in commercial operations at The Cultured Abalone Farm. Buckets were connected in pairs by a 1.27 cm PVC bridge at the upper

water line such that sea water directionally flowed from the bottom of one bucket to the surface of the adjacent bucket pair via the PVC bridge. Once added to the buckets, abalone larvae directionally flowed to the adjacent bucket pair over the course of 24 hours, leaving dead and malformed larvae unable to properly swim in the bucket at the head of the flow direction. Buckets were visually inspected for larval transfer completion daily, and once transfers of living buoyant larvae were complete, buckets containing dead abalone were removed for cleaning, where dead abalone waste was carefully removed from the mesh bottoms of buckets by submerging buckets in fresh distilled water to remove dead larvae. Buckets were then dried, filled with treatment sea water, and directional flows were reversed such that larvae passively flowed back into the cleaned bucket. This process was repeated daily over 7 days until larvae were assessed as competent to settle utilizing γ-aminobutyric acid (GABA) competency tests (5). In all experiments, we quantified larval survival at four days post-fertilization (4 DPF) and just prior to settlement (at seven days post-fertilization, 7 DPF), by counting the total number of larvae in 10-15 replicate subsamples of known volume and then extrapolating to generate estimates of total abundance in each larval bucket. For each larval bucket at each survival sample point, 3 replicate samples of 20 larvae each were collected for total lipid quantification and comparisons.

Settlement and Grow Out

In the case of VD and SB-F_1 experiments, at the completion of the larval phase at 7 DPF, 300 animals from each maternal cross were added to experimental chambers each coated with thin layer of commercially produced diatoms (*Navicula* sp. Reed Mariculture LLC.) which had been pre-settled to the bottom surfaces of chambers 24 hours prior to the addition of larvae. In the case of SB-F_2, due to the lower spawning output in this experiment, 90 animals from each paternal cross were added to each chamber. Treatment seawater was then added and brought to a GABA concentration of 1 μM, a standard practice to encourage settlement in commercial abalone aquaculture (5). In the case of SB-F_1 experiments, 8 post-settlement chamber replicates were created per larval source bucket, with one culture bucket randomly chosen from SB-F_1-B for use in subsequent experiments, leading to 80 total replicates (40 high CO_2 vs. 40 low CO_2, respectively.) In the case of VD 2016 experiments, 10 post-

settlement chamber replicates were created from a single randomly selected larval culture bucket per CO_2 level, while in VD 2017 experiments, 6 post-settlement chamber replicates were created from each of the two larval culture bucket replicates per maternal family cross in each CO_2 treatment leading to 80 culture replicates (34 low CO_2, 46 high CO_2, respectively). In the case of SB-F_2 experiments, owing to variable larval phase abundances, 2-13 post-settlement chamber replicates were created per larval culture bucket, resulting in 6-18 total replicates per paternal family (72 low CO_2, 76 high CO_2, respectively). These containers were then sealed and placed in a temperature-controlled incubator held at 14˚C in total darkness for 24 hours. Each chamber possessed a pre-drilled 17 mm diameter hole sealed with parafilm at the 60 mL mark. After the settlement incubation, treatment sea water from these sealed chambers was gently siphoned to the 60 mL mark through a 50 µm filter, and parafilm sealing this hole was gently removed and replaced with a 100 µM mesh filter, held in place by a ring of rigid plastic, allowing for flow-through water movement post-settlement. Parafilm seals and 50 µM filter meshes were carefully inspected under a dissecting microscope, and any non-settled larvae out of water on these surfaces or on the sides of chambers post-siphoning were gently re-added to chambers using a flow of 1 µM UV treated filtered seawater applied through a squeeze bottle.

Experimental chambers containing settled larvae were then added into the flow-through OA apparatus, with each container receiving treatment seawater from sumps regulated through low flow irrigation drippers (1.89 L h^{-1}, Netafim pressure compensating emitters). Water was delivered directly to the bottoms of containers via hard plastic tubing, with delivery tubing oriented 2 mm off the bottoms of the center of containers to encourage upward vertical water flow movement. After the first month of post-settlement culturing activity as animals increased in size, the 100 µM mesh through which water passed in each chamber was replaced with 200 µM mesh. In all experiments, animals were fed *ad libitum* on a diet of *Navicula sp.* which was replenished when diatom layers were visually assessed to thin, or roughly at a monthly replenishment interval. Experiment wide survival in experimental replicates was assessed at 10 DPF, 28 DPF, and 97 DPF by counting all surviving individuals in all experiment units under dissecting microscopes at each time point.

Total Lipid Quantification

Total lipid content of red abalone larvae and newly settled spat was determined using a modified spectrophotometric sulfo-phospho-vanillin method (3, 4). Swimming larvae and newly settled spat were randomly selected from replicates, rinsed with distilled water, pipette-dried, and stored at -80°C until analysis. Larvae and post-settlement juveniles were sampled at time points as described (Fig S1; n=20 individuals/replicate, n=3 replicates per larval bucket/post-settlement treatment group). Samples were transferred from -80°C storage and homogenized in 500 mL of 1:1 chloroform – methanol solution using an ultrasonic probe (Fisher Scientific, CL-18) on ice at 30% amplitude for six-second pulses in three intervals. Homogenate was transferred into glass culture tubes (Fisher #14-376-27) and placed into a test-tube heat block at 95°C until solvent evaporated. Samples were removed from the heat source and once cooled, were eluted by gently mixing in 300 mL 95% sulfuric acid (Fisher A300S-500) and kept on ice. A standard curve was generated by serially diluting 2 mg mL^{-1} triglyceride (TAG Standard – T7531-STD, Pointe Scientific) in 95% sulfuric acid with concentrations ranging from 0 – 200 mg. Standards and samples were both placed into the heat block for ten minutes then allowed to cool on ice. Colorimetric reagent was prepared by mixing vanillin (99%, Sigma-Aldrich Reagent Plus® V1104) in 85% phosphoric acid (A242-500) and distilled water. In new 1.5 mL centrifuge tubes containing 1 mL of vanillin colorimetric reagent, 200 mL of the standard-homogenate mixture was added, vortexed, protected from light, and kept on ice; the process was repeated for each of the sample-homogenate mixtures. In a 96-well flat-bottomed microplate (Corning, CLS3601), 150 mL of sample or standard was transferred in triplicate into wells and absorbance at 540 nm was read at 25°C using a BioTek® Synergy HT microplate spectrophotometer. Sample concentrations were calculated using the equation for a best-fit line. Total lipid content per individual (ng $individual^{-1}$) was determined by multiplying concentrations by a dilution factor corresponding to the number of individuals per replicate.

Monitoring of Seawater Chemistry and Temperature

Seawater sampling and chemical analyses followed previously established methods (6). Throughout each experiment, pH (total scale; pH_T) of seawater in treatment sumps and total alkalinity (TA) was measured on three days each week. pH_T was quantified using spectrophotometric characterization of m-cresol Purple determined using a spectrophotometer (Shimadzu UV-1800, Shimadzu, Kyoto, Japan). Alkalinity samples from our treatment sumps were measured via automated end-point titration using a titrator (Metrohm 809 Titrando), standardized using certified reference material from A. Dickson at Scripps Institute of Oceanography. Hourly measurements of temperature, pH and salinity were further recorded in treatment sumps using multi-parameter water quality sondes (YSI 6920 V2). The pH measurements from these YSI instruments were then calibrated in total scale using sump pH_T measurements determined by spectrophotometer (7). To adjust sump data to experimental chamber conditions, we measured spectrophotometric pH in 3-6 randomly selected buckets/chambers per treatment in each experiment at three intervals during both the larval phase and post-settlement. These data were paired with temperature offsets calculated by averaging temperature differences observed in experimental chambers as compared to sump temperatures as recorded by data loggers (Hobo Tidbit v2, Onset Computer) placed in three randomly selected chambers per treatment group. From these data, small sump to chamber offsets were applied to the pH_T calibrated continuous chemistry records generated by the water quality sondes for each experiment. Adjusted hourly measurements from the continuous sump records were then binned into daily experimental averages for statistical comparison.

Before experiments, we also compared the total alkalinity of sea water in 6-10 randomly selected experimental chambers to total alkalinity values in sumps once per week for 8 weeks during standard system operation. No significant differences were found between and among sumps or containers during these trials, and so alkalinity variation among chambers was assumed to be minimal during our experiment and sump alkalinity values were used for calculations of in-situ conditions. Because pH_T was calculated daily from continuous records, but alkalinity was only sampled 3 times per week, we performed a salinity to alkalinity regression from cumulative experimental data in both high and low CO_2 treatment

sumps (8). These regression relationships were then applied to generate daily alkalinity projection averages from salinity measurements on days when alkalinity was not directly sampled.

From these data, seawater pCO_{2calc}, DIC_{calc}, $\Omega_{aragonite}$ and $\Omega_{calcite}$ were determined using the carbonate system software CO2SYS (9) by defining daily pH, alkalinity, salinity and temperature data as input variables using constants from (10) as refit by Dickson & Millero (11). During some time intervals over the course of experiments, our YSI multi-parameter sondes were deployed for use in other experiments. During these periods, carbonate system parameters were constructed from our regular weekly sampling, rather than continuous daily sonde-derived averages. During these intervals, sump salinity and temperatures were monitored using a multi-parameter instrument (YSI Pro Plus).

Statistical Analyses

The significance of main effects in survival time series data were analyzed under a fully crossed repeated measures mixed model design, with CO_2, population, maternal family and time point modeled as fixed effects and culture bucket/settlement chamber modeled as a random effect, fit utilizing Restricted Maximum Likelihood (REML) as implemented in JMP Pro 15 (SAS Institute). Tukey-Kramer tests were used to assess the significance of group mean differences at individual time points in all cases. When comparing non-time series data, results were analyzed under factorial analysis of variance, fit utilizing standard least squares. Linear regressions were utilized in analyses of density dependence, lipid to survival and size to survival relationships, with pairwise slope comparisons performed under analysis of covariance. In all analyses, outliers were identified and excluded using jackknife distances for each maternal family×CO_2 grouping at the 0.05 level and model factors were reduced ad hoc by removing combinations of factors whose significance fell above a p-value of 0.1. Where applicable, model assumptions were tested using Levene's test for homogeneity of variance, and the Shapiro–Wilk test for normality.

Table S1. Experimental chemistry conditions. Mean carbonate system parameter values, standard deviations and significance of difference between low and high CO_2 treatments during the larval and post-settlement phases of experiments. Partial pressure of CO_2 (pCO_{2calc}), $\Omega_{aragonite}$, $\Omega_{calcite}$ and total dissolved inorganic carbon (DIC_{calc}) were calculated from the measured values of total alkalinity (TA), pH total (pH_T), temperature and salinity. The significance of differences between treatments was assessed using Tukey-Kramer tests. Shared letters indicate treatment groups that were not significantly different (P>0.05).

Sea Water Property Larval Phase	SB-Low CO_2	SB-High CO_2	VD-Low CO_2	VD-High CO_2	SB-F_2 Low CO_2	SB-F_2 High CO_2	Significance Grouping
Temperature (°C)	14.679 +/- 0.046	14.541 +/- 0.091	14.413 +/- 0.260	14.532 +/- 0.276	13.722 +/- 0.186	13.709 +/- 0.018	A,A,A,A,B,B
Salinity	32.483 +/- 0.172	32.203 +/- 0.358	31.786 +/- 1.484	32.277 +/- 1.424	33.323 +/- 0.031	33.347 +/- 0.039	AB,AB,B,AB,A,A
TA (µmol Kg_{sw}^{-1})	2208 +/- 8.45	2207 +/- 9.19	2181 +/- 55.7	2203 +/- 37.7	2305 +/- 51.0	2310 +/- 58.0	B,B,B,B,A,A
DIC_{calc} (µmol Kg_{sw}^{-1})	2032 +/- 13.4	2160 +/- 12.7	1972 +/- 59.6	2150 +/- 45	2119 +/- 13.4	2284 +/- 12.7	C,B,C,B,B,A
pH_T	7.997 +/- 0.027	7.636 +/- 0.029	8.091 +/- 0.045	7.653 +/- 0.059	8.013 +/- 0.010	7.565 +/- 0.006	B,C,A,C,B,D
pCO_{2calc}	445 +/- 33.4	1114 +/- 85.3	344 +/- 46	1065 +/- 166	442 +/- 10.7	1374 +/- 49.8	C,B,C,B,C,A
$\Omega_{aragonite}$	2.01 +/- 0.105	0.95 +/- 0.056	2.34 +/- 0.167	0.98 +/- 0.117	2.13 +/- 0.105	0.84 +/- 0.056	B,C,A,C,B,C
$\Omega_{calcite}$	3.15 +/- 0.105	1.48 +/- 0.056	3.67 +/- 0.167	1.54 +/- 0.117	3.33 +/- 0.085	1.32 +/- 0.018	B,C,A,C,B,C
Sea Water Property Post-settlement	**SB-Low CO_2**	**SB-High CO_2**	**VD-Low CO_2**	**VD-High CO_2**	**SB-F_2 Low CO_2**	**SB-F_2 High CO_2**	**Significance Grouping**
Temperature (°C)	14.230 +/- 0.314	14.074 +/- 0.442	14.202 +/- 0.433	14.216 +/- 0.359	14.199 +/- 0.200	14.146 +/- 0.113	A,B,AB,A,AB,AB
Salinity	32.752 +/- 0.557	32.822 +/- 0.592	33.191 +/- 1.071	33.544 +/- 0.796	33.498 +/- 0.294	33.457 +/- 0.549	C,C,B,A,A,AB
TA (µmol Kg_{sw}^{-1})	2215.4 +/- 21.7	2217.4 +/- 19.9	2213 +/- 63.5	2215 +/- 70.6	2257 +/- 24.2	2256 +/- 22.4	B,B,B,B,A,A
DIC_{calc} (µmol Kg_{sw}^{-1})	2056 +/- 23.4	2168 +/- 26.4	2033 +/- 63.9	2158 +/- 77.2	2076 +/- 24.7	2197 +/- 24.5	C,B,D,B,C,A
pH_T	7.960 +/- 0.038	7.640 +/- 0.050	8.005 +/- 0.069	7.659 +/- 0.061	7.999 +/- 0.038	7.664 +/- 0.013	B,C,A,C,A,C
pCO_{2calc}	490 +/- 48.3	1101 +/- 125	434 +/- 82.5	1047 +/- 155	449 +/- 49	1053 +/- 39.9	B,A,C,A,BC,A
$\Omega_{aragonite}$	1.86 +/- 0.174	0.95 +/- 0.121	2.04 +/- 0.296	1.01 +/- 0.162	2.07 +/- 0.146	1.03 +/- 0.032	B,C,A,C,A,C
$\Omega_{calcite}$	2.91 +/- 0.269	1.49 +/- 0.189	3.19 +/- 0.463	1.58 +/- 0.252	3.23 +/- 0.228	1.62 +/- 0.049	B,C,A,C,A,C

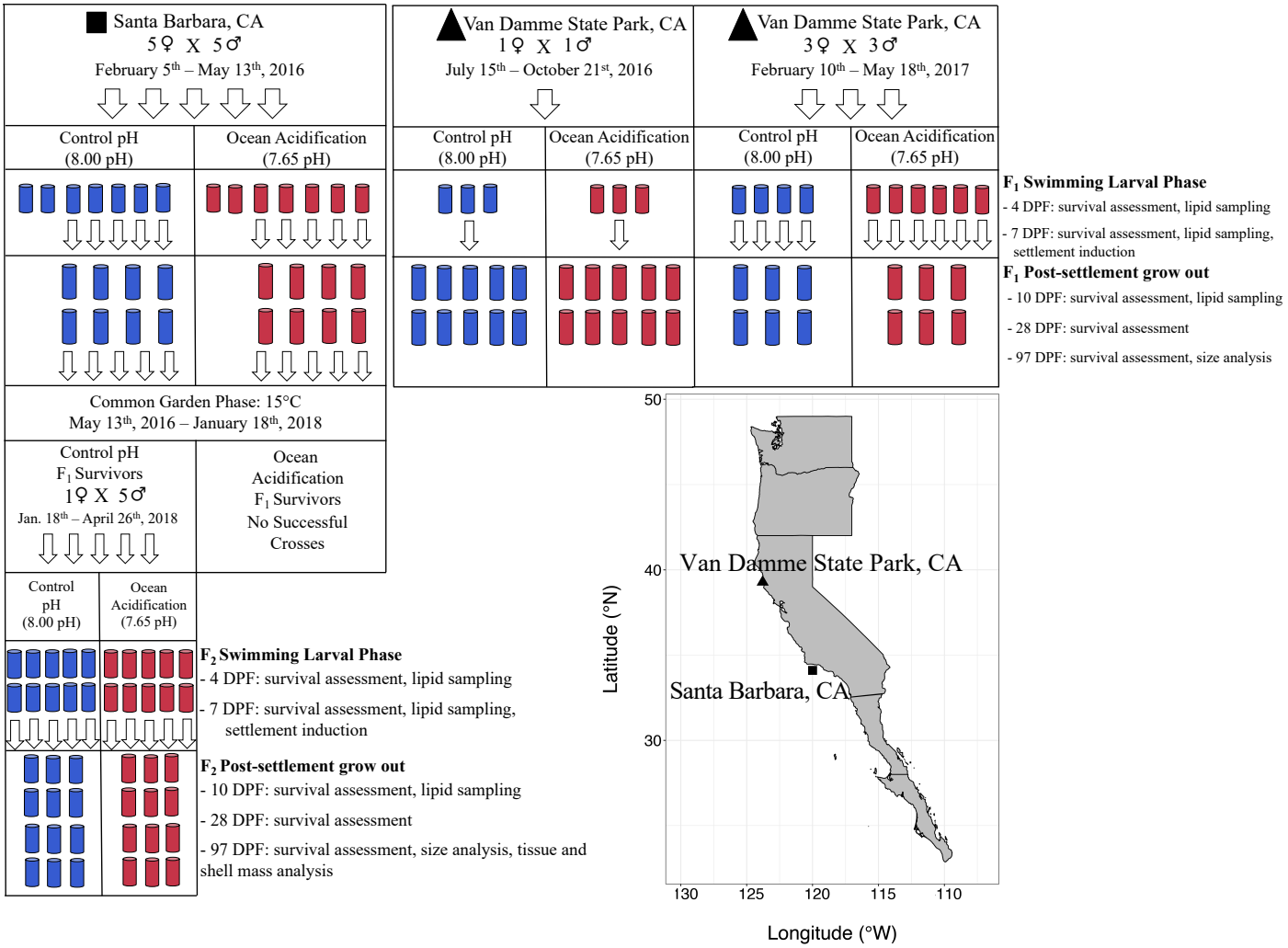

Fig S1. Diagrammatic outline of experiments and broodstock source populations. Abalone were spawned and experiments conducted over three intervals: SB-F_1 experiments: February 5, 2016 – May 13, 2016; VD-F_1 experiments: July 15, 2016 – May 18, 2017; SB-F_2 Experiments: Jan 18, 2018 – April 26, 2018 with survival, lipid assessments and growth measurements carried out at repeated intervals (listed at days post-fertilization intervals, or "DPF"). Experimental data from trials on VD animals were combined for the purposes of statistical comparison to SB-F_1 results.

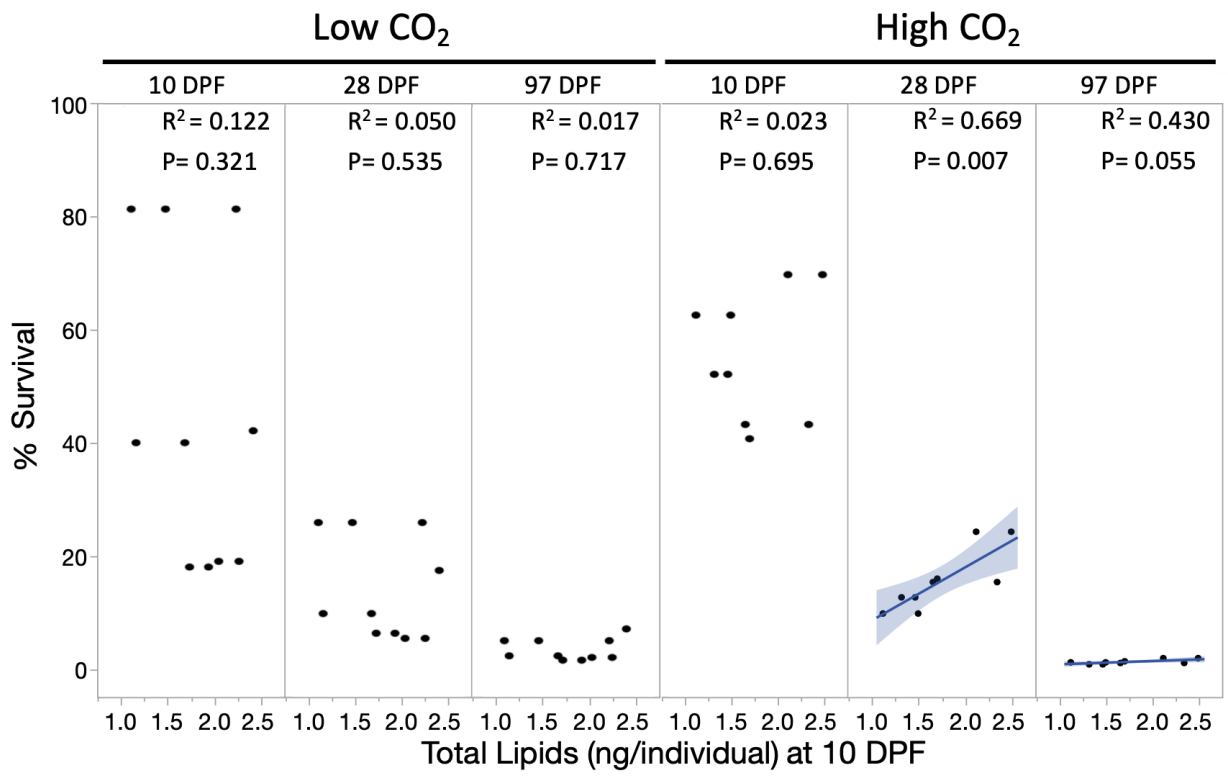

Fig S2. Post-settlement lipid concentration predicts red abalone survival under high CO_2. In experiments on SB-F_2 abalone, post-settlement lipid concentration was a significant predictor of survival at 28 days post-fertilization (DPF) (P=0.007) and at 97 DPF (P=0.055); whereas, under low CO_2 no relationship was detected. Shaded areas surrounding regression slopes represent 95% confidence bands.

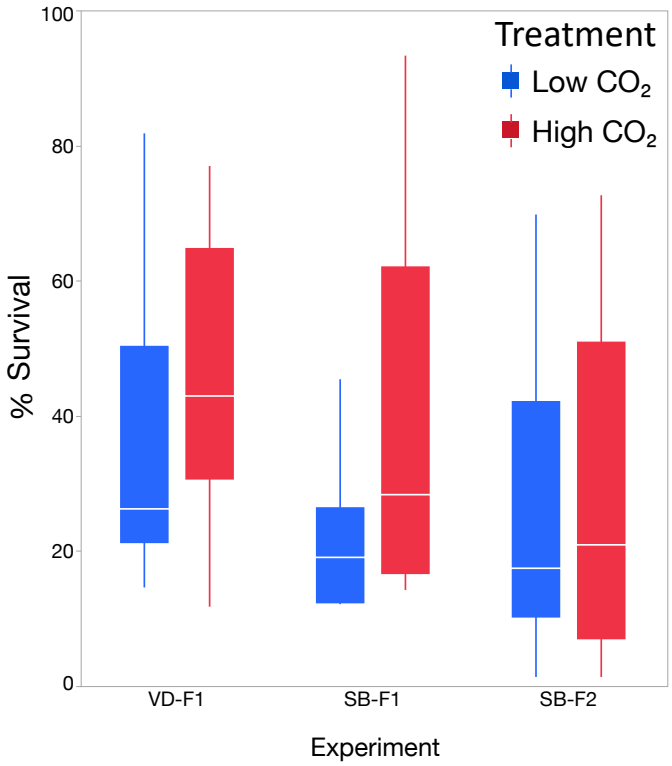

Fig S3. Cumulative larval survival of abalone across experiments. Box plots represent larval survival assessed at 7 days post-fertilization. During the larval phase, survival did not significantly vary between experiments or under contrasting CO_2 treatments.

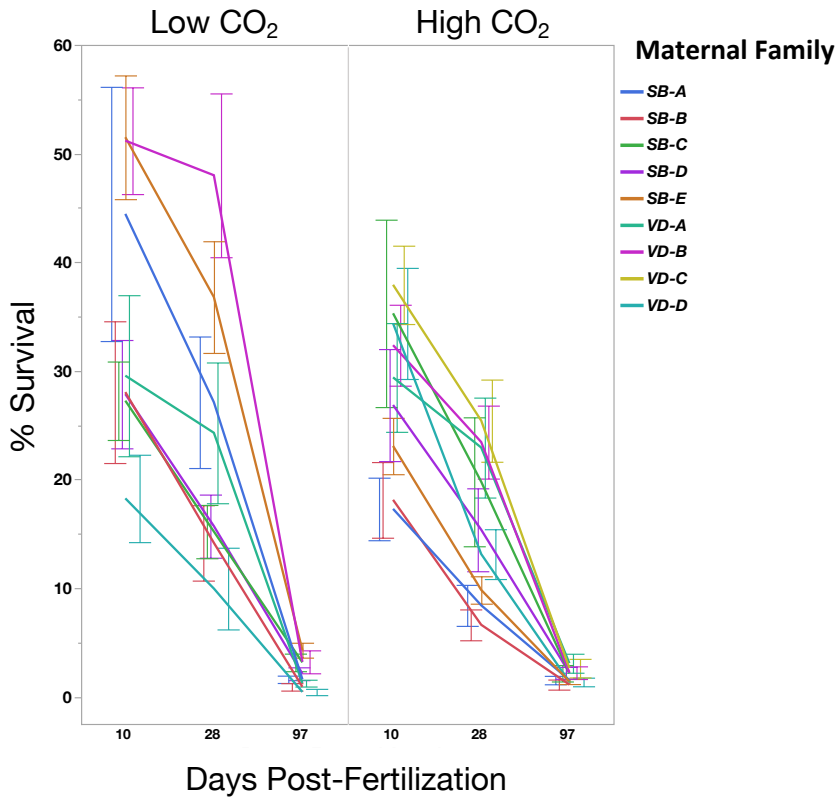

Fig S4. Family variation in F_1 survival response of red abalone. A maternal family x CO_2 x time interaction was observed across experiments ($P<0.0001$) and significant differences were observed between maternal family groups at all time points. While paternal identity in maternal crosses was unknown due to fertilization with pooled sperm from contributing fathers, these results provide strong evidence that underlying maternal genetic variation strongly influences ultimate susceptibility to high CO_2. Error bars are ±1 SE.

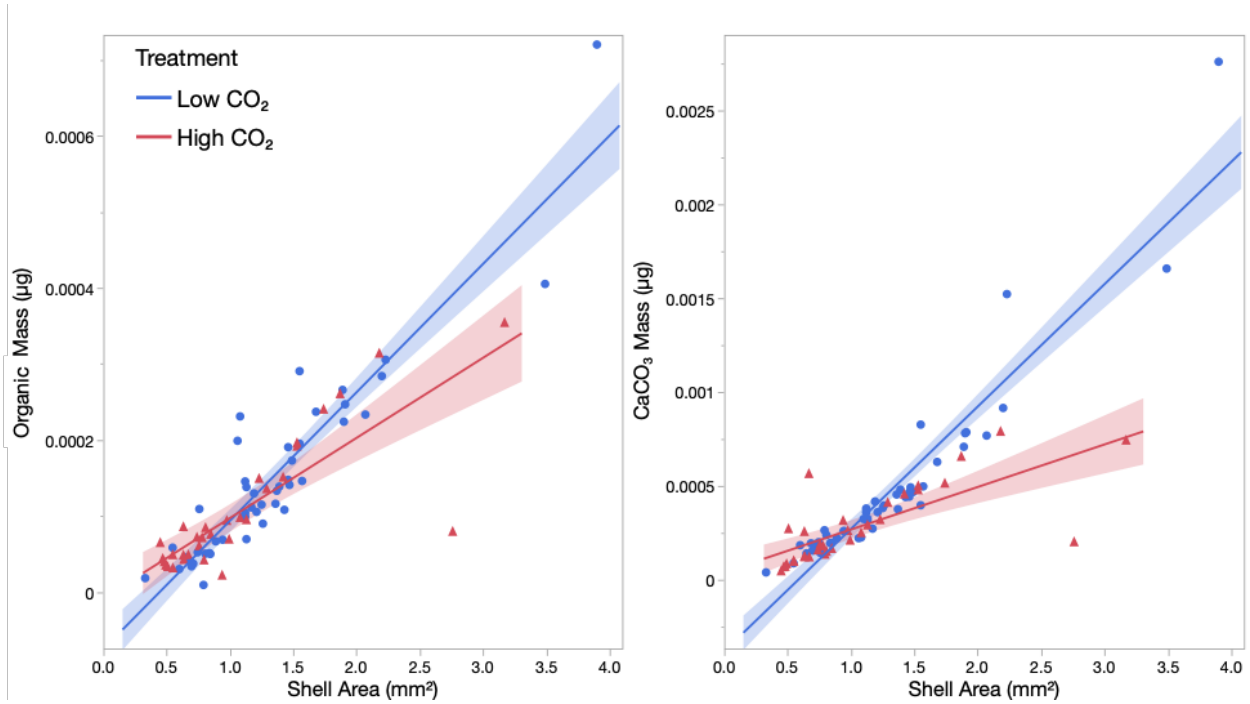

Fig S5. $CaCO_3$ and organic mass response in red abalone raised under low and high CO_2 until 97 days post-fertilization. Under high CO_2, the slopes of the relationships between both $CaCO_3$ and organic mass to total shell area were significantly reduced in F_2 generation abalone from SB ($CaCO_3$ $P<0.0001$, organic mass $P=0.0129$). While F_2 animals superficially reached equivalent sizes between low and high CO_2, this observation suggests that these animals were significantly lighter, and potentially structurally compromised. Red triangles and blue circles are high and low CO_2, respectively, shaded areas surrounding regression slopes represent 95% confidence bands.

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