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

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Ocean acidification (OA) poses a major threat to marine ecosystems and shellfish aquaculture. A promising mitigation strategy is the identification and breeding of shellfish varieties exhibiting resilience to acidification stress. We experimentally compared the effects of OA on two populations of red abalone (Haliotis rufescens), a marine mollusc important to fisheries and global aquaculture. Results from our experiments simulating captive aquaculture conditions demonstrated that abalone sourced from a strong upwelling region were tolerant of ongoing OA, whereas a captive-raised population sourced from a region of weaker upwelling exhibited significant mortality and vulnerability to OA. This difference was linked to population-specific variation in the maternal provisioning of lipids to offspring, with a positive correlation between lipid concentrations and survival under OA. This relationship also persisted in experiments on second-generation animals, and larval lipid consumption rates varied among paternal crosses, which is consistent with the presence of genetic variation for physiological traits relevant for OA survival. Across experimental trials, growth rates differed among family lineages, and the highest mortality under OA occurred in the fastest growing crosses. Identifying traits that convey resilience to OA is critical to the continued success of abalone and other shellfish production, and these mitigation efforts should be incorporated into breeding programs for commercial and restoration aquaculture.

Significance

The pH of the global ocean is decreasing due to the absorption of anthropogenically emitted atmospheric carbon dioxide (CO₂), a process termed ocean acidification (OA) (1, 2). This process negatively impacts the growth, calcification, reproduction, and survival of calcifying shellfish (3–8). Additional acidification over the coming decades (9) threatens the long-term economic viability of commercial mollusk shellfish production (10), which is valued globally at more than 19 billion USD (11) and is considered a more sustainable alternative to terrestrial animal protein (12, 13).

The commercial production of molluscs requires the culturing of diverse life history phases, including larval and juvenile stages (i.e., “seed production”) (14). These life history phases are particularly sensitive to OA impacts (4–8). Significant uncertainty remains regarding how these sensitivities will affect shellfish production, especially since commercially cultured taxa show different vulnerabilities to OA (15). Variation in responses to OA is likely a complex function of differences in natural selection regimes (15–17), sensitivities among different early-life history stages (18), and in the nature and strength of trans-generational effects (19). As such, there is a critical need to understand how, and in which contexts, these factors contribute to OA impacts on molluscan seed production.

Red abalone (Haliotis rufescens) is a popular seafood and culturally iconic marine gastropod native to the west coast of North America (20, 21). Once abundant populations of this species supported both commercial and recreational fisheries, but with recent widespread population collapses (22, 23), commercial aquaculture now serves as the only source for abalone in the United States. OA poses a broad threat to the sustainable commercial and restoration aquaculture of this and other abalone species worldwide (5, 24–29). During early life, the non-feeding (lecithotrophic) larvae of abalone depend on maternally provisioned energy reserves, much of which occurs in the form of yolk lipids (30), which support numerous physiological processes (31). Yolk lipids remaining at the end of the swimming phase likely play a critical role in determining how OA affects abalone seed success after larval settlement.

Along the west coast of North America, acidification rates are among the highest in the global surface ocean (32). Regional wind and currents drive coastal upwelling of deep-oceanic, low pH waters during spring and summer months (33), thereby

climate resilience | aquaculture | genetic variation | global environmental change | lipid regulation

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periodically exposing coastal habitats (including regions of aquaculture activity) to extremely low pH (34, 35). Ocean circulation models predict that the intensity, frequency, and duration of these events will increase rapidly in the coming decades, outpacing many other regions (35–37), highlighting the need for industry adaptation and management options for this region in particular. Moreover, upwelling intensity is not uniform along the coast (34), and the resulting mosaic of strong and weak upwelling regions might have selected for variable sensitivities to OA in resident populations (38).

In this work, we sought to identify abalone traits that affect resilience to OA and that may be harnessed to support continuing productive aquaculture in the face of OA. Our experiments contrasted the responses to OA of early-stage abalone from two populations in California: a low pH, strong upwelling zone population (Van Damme State Park, CA; VD) and a higher pH, weaker upwelling zone population (Santa Barbara, CA; SB) (39). VD animals were wild-collected, whereas SB animals were aquaculture-raised, fourth-generation descendants of wild-collected abalone from the Santa Barbara Channel. We hypothesized that VD animals would be resilient to the effects of OA, given their historical exposure to low pH conditions. Families from each population were created and cultured from embryos to 3 mo of age (SI Appendix, Fig. S1) in an experimental CO2 manipulation apparatus (38). Experiments focused on survival and growth in two life history stages that encompass the period of highest mortality for red abalone (40): the swimming, larval phase (lasting 7 d post-fertilization, or “DPF”) through the juvenile post-settlement stage (97 DPF). After this critical bottleneck, mortality rates steeply decline to nominal levels during the market grow-out period (typically 3–4 y). Survival and growth were therefore assessed during this critical initial developmental window. Total lipid content of red abalone larvae was also quantified at 4 DPF, 7 DPF, and in newly settled spat (10 DPF) from random samples of individuals (see Materials and Methods and SI Appendix for full methodology).

Results
Lipid Concentrations and Relationship to Survival. Population and CO2 treatment did not affect larval survival during the initial swimming phase, while lipid provisioning and utilization strategies varied significantly between populations. During the larval phase at both 4 DPF and 7 DPF, F1-generation animals from SB (hereafter SB-F1) showed very low lipid concentrations (Fig. 1A). In contrast, VD larvae exhibited lipid concentrations that were up to 29× greater than SB-F1 (Fig. 1A). Lipid concentrations were greater in VD-F1 than SB-F1 for all pairwise combinations (P < 0.0001). CO2 did not affect larval lipid concentration in either population. Immediately post-settlement (10 DPF), VD-F1 abalone exhibited over 3× the lipid concentration of SB-F1 animals under low CO2 and 4× the lipid concentration of SB-F1 animals under high CO2 (all P < 0.0001, Fig. 1A), indicating that this difference in provisioning carried over from swimming larvae into the settled, early-juvenile, feeding phase. No differences were observed between CO2 treatments within populations. When aggregated across F1 experiments, post-settlement larval lipid concentration at 10 DPF was a significant predictor of survival at 10 DPF (R = 0.031), 28 DPF (P = 0.0083), and 97 DPF (P = 0.026) under low CO2, but was not a significant predictor at any time point under low CO2 (Fig. 1B), highlighting the strong relationship between energy reserves during earliest development and eventual survival success for abalone under OA.

We examined the persistence of these differences into the next generation by raising SB-F1 abalone to maturity and spawning them. In the resulting F2-generation larvae (SB-F2), we observed differences in lipid metabolism at the level of individual fathers (Fig. 2A). This metabolic variation was associated with differential susceptibility to mortality under high CO2 (Fig. 2B). As

![Fig. 1. Population differences in larval lipid concentration and relationship to survival in red abalone. (A) Box plots of lipid concentrations assessed across developmental time points indicate that abalone sourced from an upwelling region at VD exhibit significantly elevated larval lipid concentrations throughout the larval phase at 4 d post-fertilization (DPF), 7 DPF, and post-settlement at 10 DPF as compared to larvae from SB (all P < 0.0001). Shared letters indicate group means that are not significantly different based on means comparisons of least squares group estimates assessed separately at each time point (P > 0.05). (B) Under high CO2, post-settlement lipid concentration was a significant predictor of survival across post-settlement time points while this trend was not observed under low CO2. Shaded areas surrounding regression slopes represent 95% confidence bands. Lipid data presented taken from combined results on VD-F1 and SB-F1 animals.](www.pnas.org/doi/10.1073/pnas.2006910117)
sensitivity to OA than others, whereas all VD-F2 families were equally resilient to OA (P = 0.0001; SI Appendix, Fig. S4). At 97 DPF, mean survival was reduced across SB-F1 paternal families under high CO2 compared to low CO2 (P = 0.0025), with survival reduced by 67% in the most affected family (family SB-F1-E, Fig. 4A, P = 0.0038). In contrast, no declines in survival were observed among maternal VD-F1 families under high CO2, indicating that this population was well adapted to high CO2 conditions.

While VD-F1 abalone experienced less mortality under high-CO2 conditions, these animals grew more slowly than the SB-F1 population (Fig. 5). We observed negative density dependence in both populations, where the average size of animals in experimental units was negatively associated with the number of survivors in those units (P < 0.0001). However, this relationship varied by population (population × density, P = 0.0003), with a dampened influence on size in VD-F1 compared to SB-F1 (all VD-F1 vs. SB-F1 pairwise comparisons, P < 0.0001) indicating a substantially lower effect of density on growth in the VD-F1 population.

In F2-generation abalone, SB-F2 paternal family lineages showed significant reductions in survival under high CO2 at 97 DPF (Fig. 4B, P < 0.0001). These SB-F2 lineages were descended survival to 28 DPF and 97 DPF, as observed in the aggregate SB-F1-generation response, whereas, under low CO2, no relationship was observed (SI Appendix, Fig. S2). This suggests that larval lipid metabolism in the SB population is a potentially heritable trait, which varies among individual paternal lineages, leading to differential survival outcomes under high CO2 stress.

Differences in Survival and Growth between Populations and Among Families. Post-settlement survival in SB-F1 animals was significantly impacted by OA, whereas VD-F1 showed resilience to this stressor. Relative to low CO2, SB-F1 abalone exhibited declines in survival under high CO2 at all time points, with mean reductions of 33% at 10 DPF (P = 0.0047), 50% at 28 DPF (P = 0.0008) and 43% at 97 DPF (P = 0.0276) (Fig. 3). Post-settlement survival of VD-F1 animals did not differ between high and low CO2 at any time point. Post-settlement survival was affected by CO2 (P = 0.0005) and population (P = 0.0353), with significant interactions of population × CO2 (P = 0.0391), CO2 × time (P = 0.0019), population × time (P = 0.0212), and suggestive evidence of a three-way interaction of CO2 × population × time (P = 0.0588).

Within the SB-F1 population, some maternal families were more sensitive to OA than others, whereas all VD-F2 families were equally resilient to OA (P < 0.0001; SI Appendix, Fig. S4). At 97 DPF, mean survival was reduced across SB-F1 maternal families under high CO2 compared to low CO2 (P = 0.0025), with survival reduced by 67% in the most affected family (family SB-F1-E, Fig. 4A, P = 0.0038). In contrast, no declines in survival were observed among maternal VD-F1 families under high CO2, indicating that this population was well adapted to high CO2 conditions.

In F2-generation abalone, SB-F2 paternal family lineages showed significant reductions in survival under high CO2 at 97 DPF (Fig. 4B, P < 0.0001). These SB-F2 lineages were descended
from family SB-E-F1 reared in low CO2 conditions and exhibited a comparable mortality response to the cumulative impact observed in SB-E-F1 in the prior generation (Fig. 4). Particularly large declines in survival were observed in paternal families SB-A-F2 (73%, \( P < 0.0001 \)) and SB-C-F2 (80%, \( P < 0.0001 \)). Post-settlement survival of SB-F2 was affected by CO2 (\( P = 0.0221 \)), paternal identity (\( P < 0.0001 \)), and the interaction terms of time \( \times \) CO2 (\( P < 0.0001 \)), time \( \times \) paternal identity (\( P < 0.0001 \)), CO2 \( \times \) paternal identity (\( P < 0.0001 \)), and the three-way interaction of paternal identity \( \times \) time \( \times \) CO2 (\( P < 0.0001 \)), demonstrating that individual paternal SB-F2 lines were differentially susceptible to OA stress.

No difference in the size of survivors was observed among CO2 treatments or family groups in the SB-F2 generation. However, we observed a 42% reduction in the average CaCO3 shell mass in SB-F2 generation abalone cultured under high CO2 compared to those raised under low CO2 (\( P = 0.0039 \)), as well as variation in this response by paternal family (paternal family \( \times \) CO2 interaction, \( P = 0.0409 \)). The average organic mass of these animals was 27% lower under high CO2 (\( P = 0.085 \)), and there was suggestive evidence of a paternal family \( \times \) CO2 interaction (\( P = 0.0627 \)). When shell areas were regressed against either CaCO3 mass or organic mass under high and low CO2, we observed differences in the slopes of these relationships (CaCO3, \( P < 0.0001 \); organic, \( P = 0.0129 \)), where slopes were shallower under high CO2. This indicated that, while SB-F2 animals superficially achieved equivalent sizes under high and low CO2, juveniles raised under high CO2 were significantly lighter and altered in their compositions (SI Appendix, Fig. S5).

Tradeoffs Underlying Sensitivity to OA. When contrasting the growth performance of all maternal F1 family groups tested, we observed a negative relationship between the average size attained by family crosses under low CO2 and the survival of that same family under high CO2 (\( P = 0.0338 \), Fig. 6). This result is consistent with a compensatory tradeoff, where faster growing lineages under low CO2 conditions are those most likely to experience elevated mortality under high CO2.

Discussion

In this multigenerational study of OA effects on abalone, we demonstrated that mortality under OA is strongly correlated with differences in lipid provisioning and metabolism and that variation in traits associated with lipid regulation are observed at population, maternal, and paternal genetic levels. Low lipid concentrations were linked to significant mortality and vulnerability to high CO2 in animals sourced from the weaker upwelling environment in Santa Barbara, and this pattern persisted in F2-generation abalone. These results suggest that the negative effects of OA on red abalone populations are mediated in part by
We observed that these starting resources boosted survival under OA conditions, as maternally provisioned energy reserves likely serve as a critical pacts of OA on the digestibility and energetic value of diatoms, with resulting declines in the fatty acid composition, growth, and (SFA) and monounsaturated fatty acids (44). Recent work sug-
ing marine molluscs (43), with PUFA being the most digestible essential to the maintenance of metabolic functions in develop-
ted to counteract the negative effects of OA on the SB pop-
tured benthic diatoms. However, our results suggest that the energy and nutrition provided by this food source was insuffi-
ticated to counteract the negative effects of OA observed during post-larval growth in this population (Figs. 1 and 3). Despite this advantage, increased survival under OA appears to be associated with a sharply reduced growth rate (Fig. 5). VD animals were 70% and 80% smaller at 3 mo of age under low and high CO2, respec-
ally, as compared to SB crosses raised at equivalent densities. Given that aquaculture operators typically select for faster growing animals with quicker times to market to reduce stock loads, this outcome may reflect farm selection for rapid growers in the SB population. When the average size attained by F1 maternal family crosses under low CO2 is regressed against the mortality response observed by those same family groups under high CO2, a clear tradeoff appears to operate where the fastest growing families are those most susceptible to mortality under high CO2 (Fig. 6). This growth/OA-susceptibility tradeoff has been postulated for wild populations of the oyster Ostrea lurida, where slow shell building may lessen the energetic burden of acidification (46). Similarly, in studies of the purple sea urchin Stronglylocentrotus purpuratus, reallocation of energy under a low basal metabolic rate allowed for tolerance of OA stress (47).

It is tempting to assume that rapidly growing SB lineages that survive early OA stress will maintain these growth rates to adulthood. However, this conclusion does not account for our results that indicated at the scale of populations within a single species. Our evidence suggests that in the case of abalone, traits enabling rapid growth show a negative correlation with traits needed for OA resistance during early development, in contrast to findings from commercially selected oysters in Australia (17). While it is unknown as to whether this relationship may hold true for other wild upwelling/nonupwelling populations, by selecting for rapid growers, aquaculture operators may inadvertently be selecting for genotypes vulnerable to OA. This relationship could have significant implications for continuing seed production success and the viability of abalone aquaculture.

Evolutionary differences arising from the interaction of this stressor with underlying genetic variation for lipid regulation.

Unlike in other marine molluscs with planktotrophic (feeding) larvae (7, 41), our results indicate that abalone larvae exposed to high CO2 experienced delayed impacts, as there were no differ-
ces in survival among treatments or populations during the larval swimming phase. However, clear differences in lipid metabolism were observed between populations during the swim-
ing phase. These differences strongly structured eventual survival (Fig. 1F), even at the fine scale of individual F2 par-
ners (Fig. 2), highlighting the importance of differential maternal provisioning and heritable variation in lipid metabolism as critical factors influencing vulnerability to OA. These results em-
phasize the importance of carryover effects of larval condition on juvenile performance (4, 42), an increasingly vital consideration for abalone and other shellfish producers under OA.

After settlement, energy should not be limiting for commerci-
cially raised abalone, which are typically provided a diet of cul-
tured benthic diatoms. However, our results suggest that the energy and nutrition provided by this food source was insuffi-
cient to counteract the negative effects of OA on the SB pop-
ulation post-settlement. Polyunsaturated fatty acids (PUFA) are essential to the maintenance of metabolic functions in developing marine molluscs (43), with PUFA being the most digestible lipid form for abalone as compared to saturated fatty acids (SFA) and monounsaturated fatty acids (44). Recent work sug-
ests that OA can reduce the ratio of PUFA:SFA in diatoms, with resulting declines in the fatty acid composition, growth, and egg production of copepods fed this diet (45). Given these im-
ports of OA on the digestibility and energetic value of diatoms, maternally provisioned energy reserves likely serve as a critical buffer for aquaculture-raised abalone under OA conditions, as we observed that these starting resources boosted survival under OA throughout the early-juvenile phase (97 DPF).

Tradeoffs between Growth and Vulnerability to OA. In our experimental comparisons of animals sourced from a strong vs. weaker upwelling environment, animals from the strong upwelling pop-
ulation (VD) retained a roughly fourfold greater concentration of lipids after completing the larval phase, which may largely explain the resilience to OA observed during post-larval growth in this population (Figs. 1 and 3). Despite this advantage, increased survival under OA appears to be associated with a sharply reduced growth rate (Fig. 5). VD animals were 70% and 80% smaller at 3 mo of age under low and high CO2, respect-
ively, as compared to SB crosses raised at equivalent densities. Given that aquaculture operators typically select for faster growing animals with quicker times to market to reduce stock loads, this outcome may reflect farm selection for rapid growers in the SB population. When the average size attained by F1 maternal family crosses under low CO2 is regressed against the mortality response observed by those same family groups under high CO2, a clear tradeoff appears to operate where the fastest growing families are those most susceptible to mortality under high CO2 (Fig. 6). This growth/OA-susceptibility tradeoff has been postulated for wild populations of the oyster Ostrea lurida, where slow shell building may lessen the energetic burden of acidification (46). Similarly, in studies of the purple sea urchin Stronglylocentrotus purpuratus, reallocation of energy under a low basal metabolic rate allowed for tolerance of OA stress (47).

Authors of this study concluded that heritable variation in met-
abolic rates and linked variation in the ability to allocate ATP could strongly influence resilience to OA and the adaptive potential of populations.

We also demonstrate such variation in abalone, with trait di-
vergence indicated at the scale of populations within a single species. Our evidence suggests that in the case of abalone, traits enabling rapid growth show a negative correlation with traits needed for OA resistance during early development, in contrast to findings from commercially selected oysters in Australia (17). While it is unknown as to whether this relationship may hold true for other wild upwelling/nonupwelling populations, by selecting for rapid growers, aquaculture operators may inadvertently be selecting for genotypes vulnerable to OA. This relationship could have significant implications for continuing seed production success and the viability of abalone aquaculture.

Breeding Programs for OA Resilience. Results from our experiments on SB-F2-generation animals indicate that, during the larval phase, lipid metabolism is potentially a paternally inherited trait. We found that paternal lineages crossed with a common mother exhibited differential drawdown of equivalently provisioned maternal yolk. Greater yolk drawdown during the larval phase had dramatically reduced shell (total CaCO3) and body masses (total organic mass) per unit area under high CO2 (SI Appendix, Fig. S5). As a result, SB-F2 animals raised under high CO2 were lighter and potentially weaker with less mass to withstand envi-
ronmental stress during grow-out (48) and an uncertain long-
term growth and commercial viability. Further investigation of this relationship in OA-tolerant VD animals is necessary to de-
termine the mechanisms responsible for these results and the generality of this effect on abalone growth.

**Fig. 6.** Potential tradeoff between growth rate and mortality in maternal families of red abalone under high CO2. Cumulatively across all maternal F1 families examined, a negative relationship was observed between the average size of crosses attained under low CO2 and the average survival of that cross under high CO2, suggesting that slower growing families are more resilient to the effects of OA.

**SI Appendix**
Materials and Methods

Abalone were cultured under mean conditions of 14.2 °C at either low CO₂ (450 μatm, pH₇.0, ρH₂atm, CO₂ = 2) or high CO₂ (1,080 μatm, pH₂.65, ρH₂atm, CO₂ = 0.98) (SI Appendix, Table S1) characteristic of nonupwelling vs. upwelling pH values experienced along the California coast. Animals were cultured from embryos to 3 to mo of age in an experimental CO₂ manipulation apparatus housed at the University of California, Davis, Bodega Marine Laboratory (38). Survival in experimental replicates was visually quantified across experiments during the larval phase at 4 and 7 DPF and after settlement at 10, 28, and 97 DPF (see SI Appendix, Fig. S1 for outline and phasing of experiments). This time course of early development captured critical phases of metamorphosis from yolk-dependent, swimming larvae to feeding, benthic juveniles. During the larval phase and immediately post-settlement, animals were analyzed for total lipid content. At the conclusion of experiments, abalone were photographed and sized using digital imaging. Surviving SB-F1 abalone were then raised for 616 d and induced to spawn. Experiments were repeated on the resulting SB-F2 animals. At the conclusion of the SB-F2 experiment, size and total organic and carbonate masses of individuals was measured. See SI Appendix for additional methodological details.

Data Availability. Data files underlying the presented analyses are available via the Dryad digital data repository (https://doi.org/10.52533/8BXX8R).

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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-F₁ experiments: 5 February, 2016 - 13 May, 2016, VD-F₁ experiments: 15 July, 2016 - 18 May, 2017, SB F₂ 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 CO₂ 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 F₂ 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 µm 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-F1 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 CO₂ replicates for Van Damme Family C were lost prior to 4 DPF sampling, thus replicates from this maternal lineage were absent in subsequent low CO₂ time point analyses. In the case of all F₁ experiments, 30,000 cleaving embryos
from each maternal family cross were added to larval culture buckets. In the case of SB-F₁ experiments, a single maternal family (SB-F₁-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 CO₂, respectively. In VD experiments, the 1x1 cross from 2016 was replicated in 3 larval culture bucket replicates per CO₂ level, while each maternal family cross from VD 2017 was replicated in 2 culture bucket replicates per CO₂ level.

After being photographed at the completion of experiments, surviving F₁ 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 F₁ family cross (SB-E) which had been carried through the prior generation under low CO₂, thus creating replicated paternal SB-F₂ families for comparison. In the case of SB-F₂ 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 CO₂ 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-F₁ 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-F₂, 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-F₁ experiments, 8 post-settlement chamber replicates were created per larval source bucket, with one culture bucket randomly chosen from SB-F₁-B for use in subsequent experiments, leading to 80 total replicates (40 high CO₂ vs. 40 low CO₂, respectively.) In the case of VD 2016 experiments, 10 post-
settlement chamber replicates were created from a single randomly selected larval culture bucket per CO₂ 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 CO₂ treatment leading to 80 culture replicates (34 low CO₂, 46 high CO₂, respectively). In the case of SB-F₂ 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 CO₂, 76 high CO₂, 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 $p$CO$_2$$_{\text{calc}}$, DIC$_{\text{calc}}$, $\Omega$$_{\text{aragonite}}$ and $\Omega$$_{\text{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 CO₂ treatments during the larval and post-settlement phases of experiments. Partial pressure of CO₂ (pCO₂calc), Ωaragonite, Ωcalcite and total dissolved inorganic carbon (DICcalc) were calculated from the measured values of total alkalinity (TA), pH total (pHₗ), 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 CO₂ | SB-High CO₂ | VD-Low CO₂ | VD-High CO₂ | SB-F. Low CO₂ | SB-F. High CO₂ | 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,B,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,AA |
| TA (µmol Kg⁻¹)     | 2208 +/- 8.46   | 2207 +/- 9.19   | 2181 +/- 55.7   | 2203 +/- 37.7   | 2305 +/- 51.0   | 2310 +/- 58.0   | B,B,B,B,AA |
| DICcalc (µmol Kg⁻¹)| 2032 +/- 13.4   | 2160 +/- 12.7   | 1972 +/- 59.6   | 2150 +/- 45     | 2119 +/- 13.4   | 2284 +/- 12.7   | C,B,C,B,B,A |
| pHₗ               | 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,B,C,D |
| pCO₂calc          | 445 +/- 33.4    | 1114 +/- 85.3   | 344 +/- 46      | 1065 +/- 166    | 442 +/- 10.7    | 1374 +/- 49.8   | B,C,B,C,A |
| Ωaragonite        | 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,B,C,B |
| Ωcalcite          | 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 CO₂ | SB-High CO₂ | VD-Low CO₂ | VD-High CO₂ | SB-F. Low CO₂ | SB-F. High CO₂ | 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,A,B,A,B,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,A,A,AB |
| TA (µmol Kg⁻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 |
| DICcalc (µmol Kg⁻¹)| 2056 +/- 23.4    | 2168 +/- 26.4   | 2033 +/- 63.9   | 2158 +/- 77.2   | 2076 +/- 24.7   | 2197 +/- 24.5   | C,B,D,B,CA |
| pHₗ               | 7.960 +/- 0.038  | 7.640 +/- 0.050 | 8.005 +/- 0.069 | 7.659 +/- 0.061 | 7.999 +/- 0.038 | 7.664 +/- 0.103 | B,C,A,C,A,C |
| pCO₂calc          | 490 +/- 48.3     | 1101 +/- 125    | 434 +/- 82.5    | 1047 +/- 155    | 449 +/- 49     | 1053 +/- 39.9   | B,A,C,A,CA,B |
| Ωaragonite        | 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 |
| Ωcalcite          | 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-F₁ experiments: February 5, 2016 – May 13, 2016; VD-F₁ experiments: July 15, 2016 – May 18, 2017; SB-F₂ 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-F₁ 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 CO₂ treatments.
Fig S4. Family variation in F₁ survival response of red abalone. A maternal family x CO₂ 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 CO₂. 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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