Rapid, but limited, zooplankton adaptation to simultaneous warming and acidification

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Abstract: Predicting the response of marine metazoans to climate change is hampered by a lack of studies on evolutionary adaptation, particularly to combined warming and acidification. To test whether the ubiquitous marine copepod *Acartia tonsa* can adapt to warmer and acidified conditions, we tracked five fitness-relevant life-history traits for 25 generations (~ 1 year) with a 2 × 2 factorial design of temperature (18°C, 22°C) and $p\text{CO}_2$ (400, 2000 μatm). Initially, combined warm, acidic conditions decreased egg production and hatching frequency, resulting in a 56% reduction in population fitness (net reproductive rate). However, both traits recovered after three generations and average fitness was reduced by only 9% thereafter, indicating rapid adaptation. Antagonistic interactions between warming and acidification in later generations decreased survival, thereby limiting full evolutionary rescue. Our results suggest that interactions between warming and acidification constrain evolutionary rescue and add complexity to predictions of metazoan populations' response to climate change.

Global mean temperatures are predicted to increase ~ 3-5°C[1], with projected CO_2 levels expected to exceed 1000 μatm by the end of this century[1], and 2000 μatm by 2300[2]. In coastal regions, the projected warming and acidification (increased CO_2 with a concomitant pH decrease) will likely be more extreme and result in novel environments for a majority of extant marine species[3,4]. Predicting how biota respond to this rapid global change is a crucial yet formidable scientific challenge, particularly whether populations can persist at similar levels and phenotypes in the future[5]. Organisms can respond to novel environments through phenotypic plasticity or genetic adaptation, both of which can mitigate the deleterious effects of climate change[5–9]. In particular, long-term experimental evolution studies are a powerful tool to examine the role of adaptation in mitigating the effects climate change on biota and to explore whether populations can evolve fast enough to keep pace[10].

Performance typically decreases sharply when temperatures increase beyond the optimum[11]. Thus, small temperature increases relative to the optimum can have disproportional deleterious effects on performance. In addition, marine metazoans require the mobilization of energy-demanding acid-base regulatory processes to counteract internal pH reduction in order to maintain homeostasis under high CO_2 conditions. This may result in increased metabolic costs at the expense of energy allocated to growth and reproduction, even for non-calcifying metazoans[12–14]. Although factorial assessments of species sensitivities to ocean warming and acidification have increased rapidly over the past years, multi-generational studies on metazoan populations responding to future simultaneous warming and acidification are rare and based on fitness proxies[15,16]. Here, using both improvement in performance and absolute fitness, we demonstrate evolutionary adaptation of a metazoan population to combined warming and acidification, as well as evolutionary rescue – evolution occurring sufficiently fast to allow population recovery before extirpation[17–19].

We used an experimental evolution approach to test whether a marine zooplankter, the copepod *Acartia tonsa,* can adapt to environments created by warming, acidification, and greenhouse (combined warming and acidification) conditions, and to identify the functional traits under selection. Copepods are the most abundant metazoans on the planet, surpassing insects and nematode worms in number[20,21]. Because copepods link primary producers and other microbes to upper trophic levels, they are key determinants of fisheries productivity[22,23] and are important mediators of marine biogeochemical cycles[24]. *Acartia tonsa* is a foundational species, which is distributed worldwide and dominates estuarine systems from tropical to temperate regions[25], and is a main prey item of forage fish[26]; thus making this species an important zooplankton model.

Experimental setup

Our study measured five fitness-relevant life-history traits (survival, egg production rate (EPR), egg hatching frequency (HF), development time, and sex ratio) for 25 generations in a 2 × 2 factorial design of CO_2 and temperature. A population of *Acartia tonsa* was collected from Long Island Sound (41.3°N, 72.0°W; Groton, CT, USA) and kept under standard laboratory conditions (see methods) for at least three generations prior to the experiment. Four lines of the population, representing the 2 × 2 factorial conditions, were established with four replicates of each condition. The ambient temperature was 18°C (± 0.38 SD, N = 168), which approximates the species' thermal optimum for recruitment (egg production rate × egg hatching success) in the

Northwest Atlantic (see methods). The target ambient $p\mathrm{CO_2}$ concentration was 400 µatm (pH = 8.2 ± 0.1 SD, N = 166; $p\mathrm{CO_2}$ = 382 ± 36 µatm SD, N = 18). The high temperature was 22°C (± 0.33 SD, N = 169) and the target high $p\mathrm{CO_2}$ concentration was 2000 µatm (pH = 7.5 ± 0.08 SD, N = 169; $p\mathrm{CO_2}$ = 2344 ± 208 µatm SD, N = 18), corresponding to open ocean predictions for the year 2100 (RCP 8.5)[1] and the year 2300[2].

Evidence for adaptation to warming and acidification

At the outset of the experiment, in three of the future conditions (warming, acidification, and greenhouse), reductions in egg production rate (EPR) and hatching frequency (HF) were evident relative to ambient conditions: warming (EPR: $p < 0.0001$; HF: $p < 0.01$; t-test), acidification (EPR: $p < 0.0001$; HF: $p = 0.19$; t-test), and greenhouse conditions (EPR: < 0.0001; HF: $p < 0.0001$; t-test; Fig. 1). The decrease was strongest under greenhouse conditions, particularly for HF, indicating synergistic deleterious effects of temperature and $\mathrm{CO_2}$. Significant interactions of temperature and $\mathrm{CO_2}$ on HF were evident across all generations ($p < 0.0001$; three-way ANOVA). After the first generation, EPR decreased under ambient ($p < 0.0001$; GAM ANOVA) and acidified conditions ($p < 0.0001$; GAM ANOVA), but partially recovered in later generations. Meanwhile, HF remained stable (Ambient GAM ANOVA: $p = 0.14$; Acidification GAM ANOVA: $p = 0.064$; Fig. 1). Under warming, EPR decreased with generation ($p < 0.0001$; GAM ANOVA), but did not recover, while HF increased ($p < 0.0001$; GAM ANOVA; Fig. 1C), suggesting some degree of adaptation (improved HF). By contrast, under greenhouse conditions, EPR increased by 50% ($p < 0.02$; GAM ANOVA) and HF doubled ($p < 0.0001$; GAM ANOVA; Fig. 1D) by generation 3, and the improvements were maintained until generation 25; These changes yielded significant effects on fitness (Table 1) and are consistent with rapid adaptation.

Survival from freshly hatched nauplii to reproductively mature adults was independent of treatment ($p > 0.05$, two-way ANOVA) for the first 12 generations for all treatments (Fig. 2). By generation 15, however, survival decreased by 30% under warming ($p = 0.02$, t-test) and greenhouse conditions ($p = 0.01$, t-test) relative to ambient conditions. Acidification elicited no decrease in survival ($p > 0.1$, one-way ANOVA). Although survival recovered in the warming treatment by generation 25, survival decreased under greenhouse conditions by an additional 50% relative to generation 15 for the same treatment ($p < 0.001$, t-test), demonstrating an antagonistic interaction between temperature and acidification in later generations[27]. Traits under selection are expected to increase towards phenotypic optima[28–30], thus survival does not appear to be a trait under selection for adaptation to warming, acidification or greenhouse conditions. Elevated temperatures resulted in faster development in both the warming and greenhouse treatments (Supplementary Fig. 1) with 22-24% shorter development times than at ambient temperature ($p < 0.0001$; two-way ANOVA). Elevated CO_2, in contrast, resulted in 5-6% slower development times than ambient CO_2 across all generations ($p < 0.0001$; two-way ANOVA). Thus, warming and acidification acted antagonistically on development time. Finally, the sex ratio remained unchanged at ~ 1:1 ratio for three of the four treatments (Supplementary Fig. 2). Under greenhouse conditions, the proportion of females decreased across generations, with a significantly lower proportion in generation 25 than generation 0 ($p < 0.01$; Tukey HSD).

To understand adaptation not just in terms of individual traits, but with respect to population fitness, we integrated all measured traits (survival, EPR, HF, development time, and sex ratio) to estimate the net reproductive rate, lambda[31], $\lambda = e^{r\tau t}$, where r is the intrinsic population growth rate (generation^{-1}) and τ is generation time (Fig. 3). Thus, λ is the number of individuals produced per individual in a generation. In generation 0, greenhouse conditions resulted in a 55% reduction in λ relative to ambient conditions, while warming and acidification resulted in 23% and 13% reductions relative to ambient conditions, respectively ($p < 0.01$, t-test; Fig. 3). However, by generation 3, λ in greenhouse conditions had improved by 120% relative to generation 0 ($p < 0.0001$; Tukey HSD; Fig. 3) and recovered to levels equal to ambient conditions ($p = 1.0$; Tukey HSD; Fig. 3), mainly because of improved hatching frequency (Fig. 1). The λ-frequency distribution shows an inflation of zero values (Supplementary Fig. 3), due primarily to the high abundance of mate pairs with low HF at generation 0, particularly under greenhouse conditions. In later generations, as HF increased so did the probability of non-zero λ, and this was particularly evident under greenhouse conditions (Supplementary Fig. 4). Thus, as generations progressed, selection under greenhouse conditions removed members of the population with low fitness. Lambda remained high in greenhouse conditions until generation 15, after which there was a 19% reduction ($p < 0.05$, t-test; Fig. 3). This decrease was driven by reduced survival (Fig. 2), which suggests an inability of *Acartia tonsa* to maintain multiple optimal phenotypes, i.e., high hatching frequency and high survivorship, under greenhouse conditions. While λ was 22% lower under greenhouse than ambient conditions by generation 25, it was still substantially higher than in generation 0 ($p < 0.0001$, t-test; Fig. 3), still consistent with adaptation over time. In accord, significant effects of generation on λ were evident under warming ($p < 0.04$; ANOVA) and greenhouse ($p < 0.001$; ANOVA) conditions, but not under ambient ($p = 0.681$; ANOVA) or acidified ($p = 0.212$; ANOVA) conditions (Table 1). The latter suggests that acidification alone was not a strong selective force in our study.

Adaptation is evident when performance increases towards optimal phenotypes over time[28–30]. Here, we observed improved performance and fitness under warming and greenhouse conditions, but with important differences between the treatments: fitness was fully recovered under warming, but not under greenhouse conditions. *Acartia tonsa* exhibits strong tolerance to high temperatures, consistent with its seasonal dominance in the summer and wide-ranging latitudinal distribution[25,32]. However, the combination of elevated temperature and CO_2 are known to affect ectotherm resource partitioning and energy distribution[12,33], which might account for the limited recovery under greenhouse conditions. During adaptation, multiple phenotypes are expected to reach optimal levels of performance concurrently to yield the maximum possible fitness for a particular environment. We hypothesize that under greenhouse conditions copepods could not sustain multiple optimized phenotypes, as evidenced by the observed reduction in survival following HF and EPR recovered to levels equal to or greater than those of ambient conditions. Under warming, copepods improved both EPR and HF (Fig. 1) and maintained high survival (Fig. 2) across generations to yield the highest λ level by generation 25 (Fig. 3). By contrast, under greenhouse conditions the improvements in EPR and HF across generations yielded the highest λ values between generations 3 and 12, but decreases in survival reduced λ afterwards. These results are consistent with adaptation via selection, while simultaneously allowing persistence of maladaptive traits[29] under greenhouse conditions.

Genetic drift affects global, genome-wide patterns of genetic diversity, while selective processes affect specific regions of the genome[34]. We tested for signatures of genetic drift caused by potential bottlenecks by exploring patterns of nucleotide diversity (π) among treatments using single nucleotide polymorphisms identified with pooled sequencing of genomic DNA from each replicate of each treatment at generation 25. We found that global levels of nucleotide diversity were equivalent across treatment groups (Supplemental Figure 5; Wilcoxon Rank Sum test, p > 0.05; average π across treatments: Ambient: 0.0135 ± 0.006; Acidification: 0.0130 ± 0.006; Warming: 0.0134 ± 0.006; Greenhouse: 0.0138 ± 0.006), revealing no evidence of genetic bottlenecks or drift despite 25 generations of experimental evolution.

Traits affecting fitness

To assess the contribution of each life-history trait to adaptation, we quantified the strength of selection via the standardized linear selection coefficient of the fitness landscape[35]. High linear coefficients of selection for a given fitness landscape suggest that a particular trait is under selection, though not necessarily determining fitness. The coefficients are calculated from multiple regression models of relative λ against all changing life-history traits (see methods). We found that HF, but not EPR or survival, was under selection and had a positive impact on fitness. Specifically, relative λ increased as a function of HF with standardized linear selection coefficients (β) of 0.91 (ambient), 0.94 (acidification), 0.96 (warming), and 0.78 (greenhouse) at generation 0 ($p < 0.0001$, ANOVA; Fig. 4A). In addition, β_{HF} was significantly different for all treatments between generations 0 and 25 ($p < 0.0001$, ANOVA), indicating changing degrees of selection on HF for all treatments-between the first and last generations. By contrast, neither EPR or survival were significant at generation 0 (Fig. 4B, C; $p > 0.1$, ANOVA) suggesting that selection acted in favor of higher HF, but not towards higher survival or EPR for the warming and greenhouse treatments. By generation 25, the impact of HF on relative fitness decreased relative to generation 0 by 88% and 10% for the warming and greenhouse treatments, respectively. Such decreases corresponded to a shift in phenotype distribution towards higher HF and suggested that selection had relaxed by generation 25 as HF within the population reached the phenotypic optimum (Fig. 4A). Finally, despite the significant decrease in sex ratio over the 25 generations in the greenhouse treatment (Supplementary Fig. 2), sex ratio had no effect on relative fitness between generations 0 and 25 ($p = 1.0$, ANOVA), suggesting this trait was not under selection.

We also employed path analysis on structural equation models (SEMs) to identify the hierarchical interactions of all life-history traits and the traits' effects on fitness[36,37]. High correlation coefficients of SEMs indicate causality of a given trait's effect towards fitness. The path analysis revealed that HF had the largest effect on fitness of all life-history traits across treatments, with the exception of the warming treatment at generation 25, and was significant at both generations 0 and 25 (Table 2). Taken together, we conclude that selection on HF both under warming and greenhouse conditions was the critical factor in adaptation to those environments.

Implications

Our 25-generation study showing both improved trait performance and population fitness across generations constitutes the first unequivocal demonstration of rapid evolutionary adaptation and evolutionary rescue of a metazoan to both warming and greenhouse conditions based on extant standing genetic variation; previous work has been limited to phytoplankton[34] or to documenting metazoan traits for limited generations[15,16]. Failing to account for this adaptation overestimates future population vulnerability[38,39]. For example, using the observed deleterious effects at generation 0 to predict future vulnerability would have estimated a fitness reduction for the greenhouse treatment relative to ambient treatment of 56% (Fig. 3). However, accounting for evolutionary rescue (generations 3-25) resulted in an average fitness reduction of only 9%. At the same time, our results suggest that full evolutionary rescue under greenhouse conditions was not achieved (Fig. 3). This result is consistent with the hypothesis that adaptation to stress comes at a cost and that full recovery of populations to future climate conditions based on extant genetic variation is limited[40]. The limited evolutionary rescue we observed also has consequences for future oceanic systems under greenhouse conditions. Because adaptation to greenhouse conditions appears to be costly even under the food replete conditions of our study, it is unclear to what extent evolutionary rescue would occur under scenarios that predict lower resource availability for zooplankton under climate change[39]. Limited evolutionary rescue, in turn, would reduce prey availability for fish, thereby negatively affecting fish production in a greenhouse world[39]. Finally, since copepods are a major vector of carbon transfer from the surface waters to the sea floor[24], limited evolutionary rescue would also reduce the efficiency of the biological carbon pump, with a concomitant lower drawdown of CO_2 from near surface waters.

Our study highlights the need for global change studies to focus on population fitness, rather than individual traits, as the adaptation currency[23,38]. Using individual traits such as survival would have led to the erroneous conclusion of no adaptation under any treatment. Likewise, using EPR and HF alone would have missed the fitness decrease after generation 12 under greenhouse conditions. Further, the tradeoffs of EPR and HF vs survival after generation 12 (Fig. 1 and 2) partly explain the limited evolutionary rescue under greenhouse conditions (Fig. 3).

The shifting interactive effects of combined temperature and acidification through time highlight the need to evaluate long-term evolutionary studies with multiple stressors and underline the complexity that accompanies predicting organism responses to climate change[27]. In our study, adaptation to acidification alone, as has been suggested previously for *Acartia tonsa*[41], was not evident; thus suggesting that adaptation to greenhouse conditions is driven by temperature. Assuming additive effects of warming and acidification should have led to full evolutionary rescue under greenhouse conditions, which was not observed (Fig. 3). Instead, warming and acidification had synergistic deleterious effects on fitness on generation 0, but antagonistic effects on generation 25. We suggest that the limited evolutionary rescue under greenhouse conditions in our study must have arisen from an antagonistic interaction between warming and acidification, as has been hypothesized earlier[16]. In summary, since warming and acidification are occurring simultaneously, even crude predictions of population performance under climate change should consider non-additive effects of temperature and CO_2 interactions on population fitness.

Conclusions

We provide the first direct evidence for metazoan evolutionary adaptation to simultaneous warming and acidification by explicitly assessing changes in population fitness based on a comprehensive suite of fitness-determining traits. Rapid adaptation is evident in the fitness improvements for animals exposed to warming and acidification after a few generations. The strength of selection coefficients and the path analysis suggest the key trait under selection affecting fitness in our study was egg hatching frequency. Evolutionary rescue was limited by the interaction of warming and acidification. The nature of such interaction switched from synergistic at the beginning to antagonistic at the end of the experiment, adding complexity to predictions of population adaptation to climate change.

Methods

a) Copepod culturing and maintenance

Copepods were collected in June of 2016 from Esker Point Beach in Groton, CT, USA (41.320725˚N, 72.001643˚W) and raised for at least three generations as stock cultures prior to the start of transgenerational adaptation to limit maternal effects[42]. Stock cultures were split evenly into eight groups of 160 females and 80 males. Four of these eight groups were acclimatized to high temperature at 1 degree Celsius per day and used to seed the two high temperature treatments (warming and greenhouse). The other four groups remained at ambient temperature and were used to seed the ambient and acidification treatments. After temperature acclimatization, groups of stock cultures seeded the parental (F0) individuals for two days. Stock culture groups yielded an average of 7,173 eggs per group to produce approximately 57,000 parental (F0) eggs. Resulting parental eggs and N1 nauplii were acclimated to one of four experimental treatments over the entire F0 generation: 1) AM – Ambient temperature (18˚C), Ambient CO_2 (~400 µatm CO_2, pH ~8.2); 2) AH – Ambient temperature, High CO_2 (~2000 µatm CO_2, pH ~7.5); 3) HA – High temperature (22˚C), Ambient CO_2; 4) GH (Greenhouse) – High temperature, High CO_2. Treatment replicates were derived from the stock culture groups (i.e. stock culture group 1 after high temperature acclimatization seeded the F0 for warming replicate 1 and greenhouse replicate 1). The ambient temperature was chosen from a Gaussian fit-model[43] for optimal recruitment (egg production × egg hatching frequency) vs temperature derived from *Acartia tonsa* populations from Casco Bay (Gulf of Maine), Long Island Sound, and Chesapeake Bay (USA), $R = 11.1e^{-0.5\left(\frac{|T-17.7|}{6.4}\right)^2}$ where R is recruitment (egg production rate X hatching success) , T is temperature (˚C), 17.7 is the optimal temperature and 6.4 is the standard deviation around the optimal temperature (N = 54, $r^2 = 0.42$; our own unpublished data). Each treatment was kept in a separate temperature-controlled incubator (Thermo FisherScientific® Isotemp™; Waltham, MA, USA) and split into four replicate 10L culture containers (Cambro; Huntington Beach, CA, USA). Copepods were fed every 48-72 hours at food-replete concentrations (≥800 µg Carbon/L) consisting of equal proportions of the phytoplankters *Tetraselmis* sp., *Rhodomonas* sp., and *Thalassiosira weissflogii*, following long-standing copepod culture protocols in our lab[44]. The phytoplankton fed to copepods was deliberately raised under ambient conditions for the entire length of the experiment to avoid confounding effects of possible changes in food quality due to the different temperature and CO_2 among treatments.

Elevated CO_2 levels were achieved with gas proportioners (Cole-Parmer; Vernon Hills, IL, USA) mixing laboratory air with 100% bone dry CO_2 that was delivered continuously to the bottom of each replicate culture. For small volume life-history trait experiments (sections b-d), CO_2-mixed air was fed into custom plexiglass enclosures within each temperature-controlled incubator to allow for passive diffusion of CO_2 into seawater. Target pH values were monitored using a handheld pH probe (Orion Ross Ultra pH/ATC Triode with Orion Star A121 pH Portable Meter (Thermo FisherScientific®; Waltham, MA, USA). Temperature and pH were monitored to ensure that small-volume experiments in the plexiglass enclosures matched those of bulk cultures. To counteract metabolic CO_2 accumulation, control CO_2 conditions were achieved by forcing compressed ambient air through a series of CO_2-stripping units containing granular soda lime (AirGas®; Waterford, CT, USA) and a particle filter (1 µm), and then to each culture container via airstone. Continuous bubbling maintained dissolved oxygen levels at >8 mg/L. Actual pCO_2 conditions were calculated based on measurements of salinity, temperature, pH, and total alkalinity (A_T; Supplementary Table 1). Total alkalinity was measured in triplicates three times over the course of the experiment using endpoint titration (G20 Potentiometric Titrator; Mettler Toledo®)[45].

Because treatments were housed in separate incubators, incubator-specific effects are theoretically possible[43], but unlikely[46,47] given that incubators were held under identical ambient conditions except for the constant temperature and CO_2 conditions that were meticulously monitored, and verified by independent measures throughout the experiment (Supplementary Table 2).

b) Egg production rate and hatching frequency

Egg production rate (EPR), hatching frequency (HF), survival, and development time (see below) were assessed at generations 0, 3, 6, 9, 12, 15, and 25. For each replicate culture within a treatment, 10 pairs of newly developed males and females were placed into 20 mL petri dishes for 48 hours (n = 280 per treatment). The dishes were housed in custom-made, airtight, plexiglass enclosures whose atmosphere was controlled to the appropriate CO_2 concentration (see *copepod culturing and maintenance* section). There was one enclosure per temperature-controlled incubator. After the 48-h egg laying period, adults were checked for survival and removed from the petri dishes. Eggs were left in the dishes for an additional 72 hours to allow for egg hatching and their contents preserved with non-acid Lugol's solution. Dishes with dead males were used for EPR, but not HF, since fertilization could not be assumed. Dishes with dead females were discarded. EPR was calculated as $\frac{E_u+E_h}{t}$ where E_u represents unhatched eggs, E_h represents hatched eggs (nauplii), and t represents egg laying time. Hatching frequency was calculated as $\frac{E_h}{E_u+E_h}$.

c) Survival

Survival was measured from nauplius 1 (N1) stage to copepodid 6 (adult) stage. For a given generation, all adults from the previous generation were removed from the culture and allowed to lay eggs in food-replete media for 48 hours. Resulting nauplii were chosen for tracking survival. Unhatched eggs and any nauplii not chosen for survival analysis were returned to their respective replicate cultures for continued population maintenance. To measure survival for all generations where life-history traits were evaluated, three 250-mL beakers for each replicate culture were supplied with 25 randomly chosen N1 nauplii each and housed in the plexiglass enclosure described above (n = 21 per treatment). Copepods were checked every 48-72 hours. The number of dead, live, and missing copepods were logged for each beaker along with general stage (i.e. nauplius, copepodite, adult female, or adult male). Day specific survival (l_x) was calculated as ${n_x}/{n_i}$ where n_x represents the number of live individuals on day x, and n_i represents initial individuals. Nauplii were grown with media at levels of 500 µg C/L to prevent overgrowth of phytoplankton and allow for adequate nauplii grazing. Following the naupliar stages, copepods were grown with food-replete (800 µg C/L) media as described earlier. Food was replaced with fresh media on monitoring days. Average survival was calculated per each replicate culture at each generation measured. Differences in day-specific survival between replicates and treatments was assessed using the 'survival' package in R[48].

d) Development time

To calculate development time (time from N1 to adulthood) we recorded the number of days at which adults matured during the survival experiments and averaged the observations.

e) Sex ratio

Sex ratio was calculated based on the number of surviving adult females relative to surviving adult males in survival experiments.

f) Fitness

The population net reproductive rate, λ, was calculated as the dominant eigenvalue of an assembled projected age-structured Leslie Matrix constructed from survival and fecundity data[31]. Briefly, day-specific probabilities of survival are calculated from day-specific survival as $P_x = \frac{l_x}{l_{x-1}}$ where l_x represents the individuals on day x and l_{x-1} represents the number of individuals on day $x-1$. Probabilities of survival on day 1 are assumed to be 100% or a proportion of 1. Per capita EPR and HF are calculated as described above, with fecundity rates equaling the product of EPR and HF. Because only females produce offspring, total fecundity rates must be scaled to the sex ratio (proportion of females:males). To account for differences in individual development time for each treatment, fecundity rates are assigned to all days after the first matured adult is observed. We assume that surviving individuals represented by the survival experiments are equally as likely to experience any of the fecundity values observed in EPR experiments. Therefore, each mate-pair fecundity rate was paired with each survival beaker to construct a matrix. This yields a maximum of 120 matrices per treatment per generation (3 survival beakers × 4 replicate cultures × 10 mate pairs).

g) Statistical analyses of life history traits

Statistical analyses were computed using the software package R (v 4.0.2)[49]. To examine effects of generation on changing life-history traits, we created trait-specific generalized additive models (GAMs) smoothed across generations for each treatment[50,51]. To evaluate differences between life-history traits, we created separate linear mixed models with replicates as random effects and used post-hoc t-tests and Tukey HSD tests to compare life-history trait values that were significantly different from other treatments at each generation ($\alpha < 0.05$). Analysis of fitness (λ) calculations also included estimations with a zero-inflated generalized linear mixed effects model with generation and treatment as fixed effects, and replicates as random effects. A low intra-class correlation coefficient suggests no predictive effect of random variables. For the model constructed for fitness over generations, the variance due to replicates within a treatment is very low (ICC = 0.08; Table 1) and does not affect the model results. To estimate the predicted probabilities of $\lambda = 0$ across generations in Supplementary Fig. 4, we converted λ values to either 0 or 1 representing λ values of 0 and >0 respectively. We used the binomial-converted λ data to fit a linear mixed effects model against generation and treatment with replicates included as random effects. To evaluate individual effects of temperature, pH, or generation on life-history traits, we constructed a third linear model that was tested with a three-way analysis of variance.

h) Selection coefficients and path analysis

Linear selection coefficients (β) were evaluated by creating multiple linear regression models of relative fitness ($\lambda/_{\bar{\lambda}}$) against survival, egg production rate, hatching frequency, and sex ratio at both F0 and F25 for each treatment and calculating the partial regression coefficient estimate for each trait[52]. The path analysis was completed by creating structural equation models[36,37] of relative fitness against survival, egg production rate, hatching frequency, and sex ratio at both F0 and F25 for each treatment using the 'lavaan' and 'semPlot' packages in R[53,54]. Development time was omitted from the models because the lack of variance within a replicate violated assumptions of the model.

i) Genetic Diversity

To quantify genetic diversity across the genome, capture probes were designed to target both coding and regulatory regions across the genome. For coding regions, we chose the highest quality probe falling within the region. Regulatory probes were set within 1,000 bp upstream of the transcription start site. Genomic DNA was shipped to Rapid Genomics (Gainesvill, FL, USA) for library preparation and was captured with the 32,413 probes (21,311 coding, 11,102 regulatory). Enriched libraries were sequenced on a HiSeq 4000 with 150 bp paired end reads. Raw data were trimmed for quality and adapter contamination with Trimmomatic v0.36[55]. Trimmed reads were mapped to the *A. tonsa* reference genome[56] with BWA-MEM[57]. SAMTOOLs[58] was used to generate a pileup for each sample, from which genetic diversity (π) was estimated with Popoolation[59]. We identified 1,450 100bp windows in 704 unique scaffolds across the genome that were present across all samples. We estimated π in 100bp sliding windows with a 100bp step size. Each position required a minimum coverage of 30×, max coverage of 1000× (to avoid mapping errors), and at least 0.5 of the window meeting these thresholds. Resulting windows were required to be sequenced across all samples. To take into account the independent replicates within each treatment, we used pairwise Wilcoxon Rank Sum tests with a Holm correction for multiple testing. Genomic data is deposited in GenBank: BioProject number PRJNA590963. All statistics were performed in R[49].

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Figure Legends:

Figure 1 – **Fecundity for transgenerational study.** Egg production rate (EPR, histograms, left Y axis) and egg hatching frequency (HF, lines, right Y axis) during the transgenerational experiment. **A**) Blue (top left) – ambient temperature (18 °C) and CO_2 (400 μatm); **B**) Green (top right) – ambient temperature and high CO_2 (2000 μatm); **C**) Orange (bottom left) – high temperature (22 °C) and ambient CO_2; **D**) Red (bottom right) – high temperature and high CO_2. Error bars represent 95% confidence intervals. Probability (p) values for EPR and HF for each panel are derived from the effect of generation on each trait taken from generalized additive models. Letters represent statistically similar groupings for post-hoc t-test comparison.

Figure 2 – Survival for transgenerational study. Mean survival is for nauplius stage 1 to adult stage. Error bars represent 95% confidence intervals. Asterisks indicate significant decrease ($p < 0.001$). Treatment colors are the same as histograms in Fig. 1. Treatment curves are offset for clarity.

Figure 3 – Fitness. Mean fitness values, λ, calculated for the transgenerational study. Error bars represent 95% confidence intervals. Treatment colors are the same as histograms in Fig. 1. Treatment curves are offset for clarity.

Figure 4 – Fitness landscapes. Frequency distributions (left axes) and relative fitness landscape linear models (right axes) for **A**) Hatching Frequency, **B**) Egg production rate, and **C**) Survival at

F0 (black) and F25 (red). Fitness landscapes estimate the correlations between changing quantitative phenotypes and changing relative fitness. Shading around lines represents standard error.

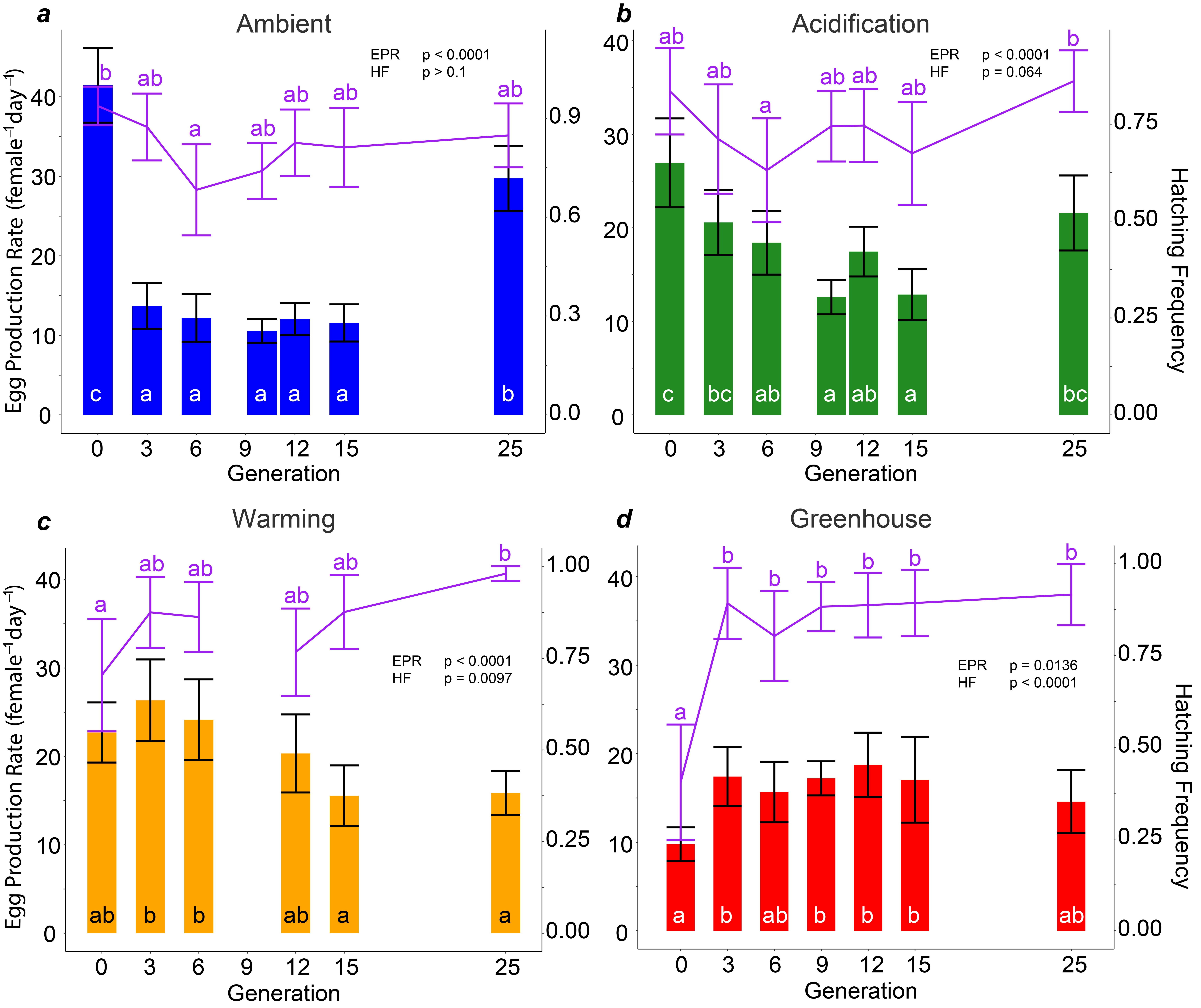
Tables:

Table 1 – Generational effects on fitness. Zero-inflated model results of generation and treatment on fitness, with replicates as random effects. Count model results represents when λ=0 values are omitted. Control treatment is the reference intercept for the model. Interactive effect of generation on ambient conditions is noted as just "Generation" for each model results.

Predictors	*Estimates*	*CI*	*P*
Count model			
(Intercept)	1.19	1.16-1.23	**<0.001**
Generation	-0.00	-0.00 – -0.00	**0.001**
Acidification	0.01	-0.04 – 0.06	0.817
Warming	0.04	-0.01 – 0.09	0.115
Greenhouse	0.13	0.07 – 0.18	**<0.001**
Generation × Acidification	0.00	-0.00 – 0.00	0.951
Generation × Warming	0.00	0.00 – 0.00	**0.009**
Generation × Greenhouse	-0.01	-0.01 – -0.01	**<0.001**
Zero-inflated model			
(Intercept)	-1.97	-2.62 – -1.32	**<0.001**
Generation	-0.01	-0.04 – 0.03	0.681
Acidification	0.55	-0.36 – 1.46	0.233
Warming	1.27	0.39 – 2.16	**0.005**
Greenhouse	1.54	0.65 – 2.44	**0.001**
Generation × Acidification	-0.03	-0.07 – 0.02	0.212
Generation × Warming	-0.04	-0.09 – -0.00	**0.038**
Generation × Greenhouse	-0.19	-0.25 – -0.13	**<0.001**
Random effects			
σ^2	0.01		
$\tau_{00\ Replicate}$	0.00		
Intra-correlation coefficient	0.08		
N Replicate	16		
Observations	2867		
Marginal R^2*/Conditional* R^2	0.230/0.293		

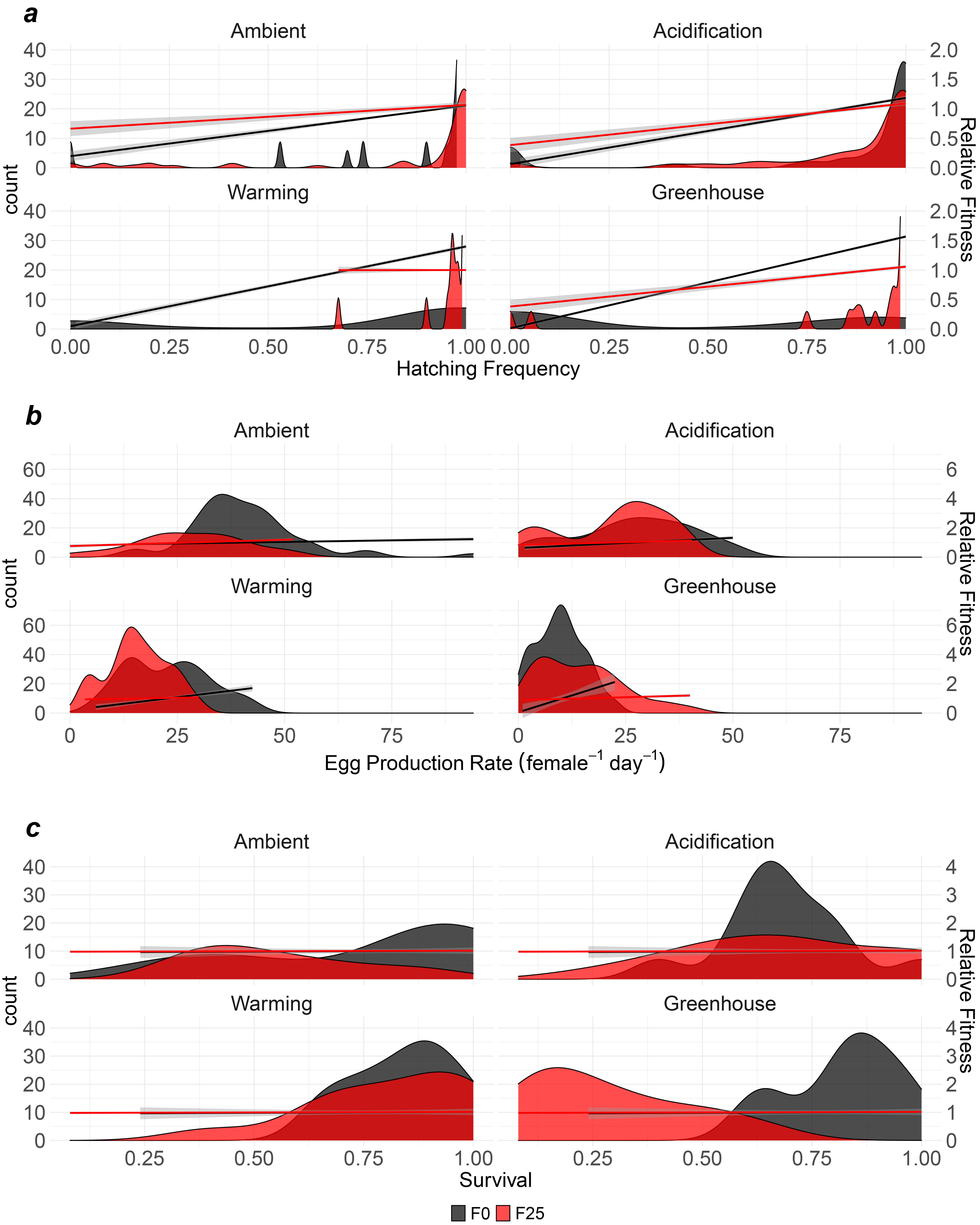
Table 2 – Path analysis and effects on lambda. Standardized parameter estimates from structural equation models for each trait's effect on lambda at both F0 (gray bars) and F25 (white bars) for each treatment. The highest parameter estimate for each treatment at each generation is highlighted in bold.

	Control	**Acidification**	**Warming**	**Greenhouse**
Survival	0.12	-0.09	0.03	0.08
	-0.09	0.13	0.72	0.33
EPR	-0.07	0.04	0.03	0.10
	0.34	0.25	**0.86**	0.23
HF	**0.91**	**0.94**	**0.96**	**0.78**
	0.53	**0.65**	0.11	**0.70**
Sex ratio	0.12	-0.15	0.09	0.21
	-0.39	0.37	0.37	-0.27


a
Ambient
EPR p < 0.0001
HF p > 0.1
Egg Production Rate (female⁻¹day⁻¹)
0
10
20
30
40
0.0
0.3
0.6
0.9
b
ab
a
ab
ab
ab
ab
c
a
a
a
a
a
b
0
3
6
9
12
15
25
Generation
b
Acidification
EPR p < 0.0001
HF p = 0.064
0
10
20
30
40
0.00
0.25
0.50
0.75
Hatching Frequency
ab
ab
a
ab
ab
ab
b
c
bc
ab
a
ab
a
bc
0
3
6
9
12
15
25
Generation
c
Warming
EPR p < 0.0001
HF p = 0.0097
Egg Production Rate (female⁻¹day⁻¹)
0
10
20
30
40
0.00
0.25
0.50
0.75
1.00
a
ab
ab
ab
ab
b
ab
b
b
ab
a
a
0
3
6
9
12
15
25
Generation
d
Greenhouse
EPR p = 0.0136
HF p < 0.0001
0
10
20
30
40
0.00
0.25
0.50
0.75
1.00
Hatching Frequency
a
b
b
b
b
b
b
a
b
ab
b
b
b
ab
0
3
6
9
12
15
25
Generation

Survival
0.75
0.50
0.25
Generation
0
3
6
9
12
15
25
*
*
*

Population Fitness (λ)
1.2
1.0
0.8
0.6
0.4
0
3
6
9
12
15
25
Generation


a
Ambient
Acidification
Warming
Greenhouse
count
Relative Fitness
40
30
20
10
0
2.0
1.5
1.0
0.5
0.0
0.00
0.25
0.50
0.75
1.00
Hatching Frequency
b
Ambient
Acidification
Warming
Greenhouse
count
Relative Fitness
60
40
20
0
6
4
2
0
0
25
50
75
Egg Production Rate (female^{-1} day^{-1})
c
Ambient
Acidification
Warming
Greenhouse
count
Relative Fitness
40
30
20
10
0
4
3
2
1
0
0.25
0.50
0.75
1.00
Survival
F0
F25