Title: Evolutionary rescue and adaptive reversal allowed the persistence of freshwater copepods during historical lake acidification and pH recovery

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Abstract

The persistence of populations facing severe environmental disturbance can be enabled by natural selection on heritable phenotypic variation – a process known as evolutionary rescue. Examples of evolutionary rescue have been documented in wild populations. Still, few studies have investigated the combination of factors (demographic, genetic, and environmental) that facilitate the rescue of populations in complex natural settings. This is important because the interplay of these factors in wild populations can cause uncertainty about the long-term outcome of evolutionary rescue: the persistence of rescued populations could potentially be threatened during environmental recovery due to a loss of genetic diversity from strong selection and demographic effects and reduced fitness in the recovered environment. Here, we used copepod resting eggs of *Leptodiaptomus minutus* from three time periods of lake ecological history, spanning ≈200 generations in two populations impacted by historical acidification. Whole genome sequencing of the resting eggs allowed us to uncover allele frequency shifts associated with the acidification followed by pH recovery. We used a resurrection ecology approach to retrace adaptive shifts concomitant with environmental transitions. Resurrected copepods from the pre-acidification period showed sensitivity to acidity, while individuals from the acidification period were adapted to acidic pH and showed high fitness overall. This tolerance was subsequently lost during pH recovery. We found evidence of rapid directional selection in genome scans, consistent with the results of the phenotypic assays. Significant changes in allele frequencies of outlier loci during acidification were followed by a reversal in the recovered populations, implying that the loss of acid tolerance could be due to an adaptive reversal. Demographic models indicated a demographic decline during the acidification process followed by population recovery, suggesting that selection led to evolutionary rescue. By employing temporal genomic data and resurrection ecology experiments in lake ecosystems that have been impacted by historical human disturbance,

this study fills a critical knowledge gap about the long-term implications of evolutionary rescue in the wild.

Introduction

Understanding and predicting adaptive responses to rapid anthropogenic impacts on the environment are important goals in evolutionary biology (1–3). The process of evolutionary rescue (i.e., an increase in the frequency of adaptive alleles following an initial population decline that allows demographic recovery) has been the focus of several recent studies because of its potential to allow populations to avoid extinction from sudden environmental degradation (4–9). The general predictions of evolutionary rescue were first demonstrated empirically with yeast in the laboratory (10). Factors that influence the probability of evolutionary rescue have been explored theoretically and experimentally (6), such as the role of new mutations versus standing variation (11), starting population size (7), the strength of environmental disturbance (12), and the capacity for phenotypic plasticity (13). Examples of evolutionary rescue have also been hinted at in wild populations (6,14,15). For instance, field crickets are hypothesized to have escaped high mortality rates from invasive parasitoid flies via a single gene mutation that suppresses the sexual song of males, which attracted the flies (16). Despite the potential short-term benefits of evolutionary rescue, most studies that have addressed the factors affecting evolutionary rescue have been laboratory experiments and theoretical modeling, and validation of the role of these factors and their interaction under natural conditions is still lacking (8). This knowledge gap may be due to the difficulty of documenting the interplay between demographic, genetic, and environmental factors during an evolutionary rescue event, particularly in complex, natural settings where many ecological and evolutionary processes act concurrently (6).

An additional knowledge gap is that the long-term stability of evolutionary rescue in the wild is largely unknown, likely due to the intensive requirements of long-term monitoring of natural populations following an environmental disturbance (6). Although evolutionary rescue has been proposed as a conservation measure for populations facing extinction (17), laboratory experiments and observations in the field suggest that it could potentially have detrimental effects over the long term (18). Indeed, the long-term benefits of evolutionary rescue could potentially be mitigated by two effects: 1) a loss of genetic diversity due to strong selection on adaptive alleles and demographic stochasticity, thereby reducing the ability of populations to adapt to future disturbances (18) and 2) when ecosystems eventually recover or move toward another equilibrium,

the phenotypic trait value that was selected for during the disturbance may be distant from the adaptive peak in the restored environmental conditions (unpredictable evolutionary directions: Grant & Grant, 2002; lags in adaptive reversals: Derry & Arnott, 2007; evolutionary traps: Carlson et al., 2014). However, environmental recovery may also lead to positive outcomes such as an adaptive reversal (i.e., a reversal in the direction of selection resulting in alleles and phenotypes reverting to their ancestral state; Chaturvedi et al., 2021) due to the relaxation of selection (22). The presence of an adaptive reversal following an evolutionary rescue event would imply that the latter does not necessarily prevent future adaptations. The maintenance of standing genetic variation is likely a key factor allowing adaptive reversal in fluctuating environments (21,23,24). It is thus critical to study population demography, genetics, and environmental characteristics jointly during and after evolutionary rescue in the wild, as this combination of factors may be especially important in determining the outcome of evolutionary rescue (8).

Resurrection ecology provides a powerful toolkit to explore how environmental conditions impact demography and genetics factors during evolutionary rescue over long time periods (25,26). Many aquatic organisms produce resting stages as part of their life cycle, which can accumulate in sediments over time and form ‘seed banks’(27). These resting eggs can be hatched in the lab after being exposed to the proper hatching cues and reared in different environmental conditions in a common garden or reciprocal transplant experimental setup (25,28). With a combination of resurrection ecology and genomics, these resting eggs can be used to retrace the evolutionary trajectories of populations relative to environmental disturbances (26,29,30). The synthesis of these two methods offers a powerful approach to addressing evolutionary questions because it has the potential to illuminate past phenotypic and genetic changes that occurred in a population (25,30). Resurrection ecology has been used extensively in the model species of the genus *Daphnia* (*Daphnia magna* and *Daphnia pulicularia*), for instance, to study adaptive responses to changes in predation regimes (21,31) or temperature (32). However, this approach has been comparatively less developed for other aquatic organisms (29), although some species such as diatoms (29) (Burge et al., 2018), brine shrimp (33) and copepods (34) are emerging as new study systems for resurrection ecology studies. Adding whole-genome sequencing approaches to this research will yield important new insights into the genetic mechanisms underlying adaptive responses to historical environmental change (30,35). Genomics tools can document (adaptive)

allele frequency changes over time (e.g.; Brennan et al., 2022) and reconstruct demographic history through modeling (e.g.; Matz et al., 2018).

The ecological history of disturbance and recovery in the lakes of Killarney Park, ON (Canada) provides a valuable study system for testing for evolutionary rescue and its potential effects on population resilience in aquatic organisms. Many lakes in this region were acidified in the 1960s as a result of metal mining emissions but have undergone chemical recovery in water pH over the last several decades following government controls on emissions in the 1970s (38). Lake acidification resulted in high mortality rates and local extinctions of fish (39), zooplankton (40), and phytoplankton (41). However, a species of a calanoid copepod, *Leptodiaptomus minutus*, developed resistance to acidic pH and subsequently became dominant in zooplankton communities across this acidified landscape (34,40,42). Resurrected nauplii from resting eggs of *L. minutus* dated from the pre-industrial period (i.e., unexposed to acidity) showed variable survival to acidity in contrast with the consistently high survival of resurrected individuals from the acidification period. However, this was then followed by a return to lower but variable survival to acidic pH during the recovery period (20,34). The sensitivity of unexposed individuals suggests that the acute acidification could have resulted in high levels of mortality, which was the case in a whole lake acidification experiment in which the population of *L. minutus* crashed until extinction (43). While many Killarney lakes have undergone progressive pH recovery over the last several decades, biological recovery has been slower and zooplankton communities have not returned to their initial composition and abundance (42).

This study aimed to provide evidence for and evaluate the factors underlying evolutionary rescue and adaptive reversal of copepod populations in two pH-recovered lakes with a history of acute acidification. We tested for evidence of the selection of acid-tolerant phenotypes and alleles during the lake acidification, followed by the selection of acid-sensitive phenotypes and alleles during the lake pH recovery, by using resting eggs sampled from sediment cores at three time points of lake ecological history (pre-acidification, acidification, and recovered). Our approach is two-fold: First, we conducted a laboratory reciprocal transplant that employed resurrected *L. minutus* individuals to measure survival, fecundity, and development time at two pH treatments (acidic and neutral pH). Second, we analyzed temporal changes in allele frequencies based on whole genome sequencing (WGS) across the time series. At the phenotypic level, resurrected individuals from the pre-industrial period should show a variable (but on average low) survival at

acidic pH. Conversely, resurrected individuals from the acidification period should have high survival at acidic pH. The return to variable (low) survival of *L. minutus* to acidic pH during the recovery period (20,34) implies that some acid-sensitive phenotypes could have either persisted during the acidification or been reintroduced to the population through immigration from other lakes or the resting egg bank. Acid resistance (survival) could incur a fitness cost (trade-off) in neutral pH conditions, for example, due to antagonistic pleiotropy (22,44).

If the rise and fall of acid tolerance (indicated by high survival rates and its correlated trait fecundity) represents evolutionary rescue followed by adaptive reversal, we expect that resurrected individuals from the acidification period should have lower fitness at neutral pH than at acidic pH (i.e., a fitness trade-off) and that copepods from the recovery period should have lower fitness at acidic pH. At the genetic level, in the case of an adaptive reversal, we expect to observe signatures of directional selection between the pre-acidification and the acute phase of lake acidification, followed by a reversal in the trajectory of allele frequency change between the period of acidification and pH recovery. The potential demographic effects of the initial low acid tolerance, combined with the development of acid tolerance over time and the high density of *L. minutus* during the acidification period (40,45), hints at the potential U-shaped demographic signature of evolutionary rescue (4). At the genomic level, we should thus observe a pattern of reduction in effective population size during the acidification phase followed by a rebound. Our study leverages historical data and samples to document a valuable example of evolutionary rescue and its long-term consequences in nature. By combining resurrection ecology with WGS, we were able to characterize the interaction between demography, the genetics underlying rapid adaptation, and environmental selection that promoted the evolutionary rescue of populations from acute acidification as well as adaptive reversal during recovery.

Methods

1. Study sites, sampling, and physicochemical data collection

Killarney Park, Ontario, Canada (46°01′ N, 81°24′ W) contains over 600 boreal lakes from the Canadian Shield geological region with pH ranging from 4.3 to 7. This wide range in pH among lakes is the result of natural pH variation, as well as anthropogenically induced acidification associated with the long-range transport of acid deposition from SO_2 emissions from nearby metal

mining smelters during the mid-1900s (38). Subsequent emission reductions in the mid-1970s led to differences in pH recovery trajectories between lakes due to differences in acid buffering capacities originating from the geological properties of their watersheds (46).

We collected sediment cores from two lakes that acidified and are now at different stages of pH recovery: George (N46°01.7960 W81°23.8310) for which the pH was recovered to circumneutral pH in 1994, and Lumsden (N46°01.5240 W81°25.9730) which is still recovering (pH < 6; 46). These two lakes were also impacted differently by the acidification (Fig. S1B), with a lower and more progressive drop in pH for George, and a faster, stronger acidification in Lumsden (46). We sampled 53 sediment cores from each lake during July 2018 and June-July 2019 from the deepest point of each lake. We divided sediment cores into 1 cm-thick sections on-site using a custom-made sediment core extruder and then stored each sediment section in airtight Whirl-pack® bags that were refrigerated in the dark until analysis. We isolated resting eggs of *Leptodiaptomus minutus* from the sediment cores of each lake, representing different periods of ecological history (pre-acidification, acidification, and recovery). The exception was that we were unable to obtain viable *L. minutus* resting eggs from the pre-acidification period of George Lake due to low resting egg density.

We conducted ^{210}Pb dating on one sediment core per lake at the Geotop Center (Université du Québec à Montréal). For each section, we weighed the sediments before (wet weight) and after drying (dry weight) to obtain the dry bulk density ρ. For each section, 100 mg of dry weight was isolated, and alpha-particle radiation activity was counted for 48 hours in an alpha counter. We then used the rplum R package v0.5.1 to calculate ^{210}Pb dates for core intervals (47), setting the decimal date to 2019.477, with 5,000 simulations and retaining three levels of supported ^{210}Pb activity. ^{210}Pb dates output by rplum for the sediment cores collected from each lake and the temporal sections selected for the resurrection and genomics experiments are presented in Figure S1A. We used a YSI Pro Plus multiparameter probe in situ (model 10102030; Yellow Springs Inc.) in 2018 and collected water samples in 2019 to obtain physicochemical properties for George and Lumsden lakes (surface temperature, pH, conductivity, dissolved organic carbon, total phosphorus, total nitrogen). Details of the analytical methods for water chemistry and physicochemical parameters of the lakes can be found in the supplementary methods and table S1.

2. <u>Resurrection ecology experiment</u>

From the resting eggs recovered from the lake cores, we applied a resurrection ecology experimental approach in which F_1-generation copepods were hatched from resting eggs originating from three time periods (pre-acidification, acidification, and recovery) and exposed to neutral (pH 6.5) and acidic pH (4.5) to measure survival, fecundity, and development time throughout their lifetime (~1 month, Fig.1). The copepod mothers who produced the eggs in the lake were considered F_0-generation copepods. We collected their resting eggs; these eggs remained dormant in the lake, arrested in time, and hatched for the experiment, making them F_1 generation. No maternal effects were standardized in this experiment as we were unable to obtain enough hatchlings from the reproduction of the F1 individuals to carry on the experiment to the F_2 generation. We used two different methods to hatch the resting eggs as the older eggs (pre-acidification and acidification periods) could not be hatched directly (34). The first method was implemented for the eggs corresponding to the recovery period (from the past 10-20 years). Following the method in Hairston & Kearns (28), we added the equivalent of 1 cm of the core sediment to the bottom of a plastic tray and then covered the eggs with 1-2 cm of COMBO culture medium (48) prepared in the laboratory and pH adjusted to 4.5 or 6.5 with 0.02M sulfuric acid. In the second method, used for eggs corresponding to the pre-acidification and acidification periods, we separated the eggs from the sediments for each time period (acidification and recovery) with a sugar flotation method modified from (49). We then stored the isolated resting eggs from the acidification and pre-acidification periods in distilled water in a dark refrigerator at 4°C. Prior to exposure to hatching signals (luminosity, temperature, and oxygenation), we stored the eggs from the acidification period for 2 weeks to 1 month under the above conditions and the eggs from the pre-acidification period for 2 months following Derry et al. (34). This is necessary for resting eggs that have been buried for longer periods in lake history, as more time is required to stimulate them to hatch (28).

After these storage periods, we transferred all the resting eggs to a hatching beaker containing 200 mL of COMBO medium. For both methods, resting eggs from several sediment core sections had to be combined in the beakers and hatching trays presented to obtain enough hatchlings for the resurrection experiment, but we did sediments from different time periods or lakes. We exposed resting eggs to the following hatching signals: 16h:8h light:dark cycle, 20°C, and oxygenation (34,50). We kept the hatching trays and beakers in a Thermo Scientific Precision Model 818 Incubator growth chamber and examined them regularly for the presence of nauplii.

We verified the identity of nauplii using the list of species present in our study lakes (Suenaga, 2018) as well as a key from the Department of Biological Sciences at the University of New Hampshire (51). We removed a few nauplii from the beakers and datasets that were misidentified (N = 5 cyclopoid copepods and N = 1 calanoid copepod: *Epischura lacustris*).

We transferred hatchling nauplii (≈ 20 individuals per replicate) to beakers with 200 ml of COMBO medium adjusted to either an acidic pH of 4.5 or a circumneutral pH of 6.5. We changed the culture medium regularly (once to three times a week). We fed the copepods with a mixture of 1 mL of brown algae (*Cryptomonas sp.* CPCC 336) and 1 mL of green algae (*Chlamydomonas sp.* CPCC 243) cultured at a stationary phase. We cultured the green algae in a modified Bold's Basal medium (Stein, 1973), and the brown algae in COMBO adjusted to pH 6.5, in the growth chambers containing the copepod cultures at 16h:8h light:dark cycle and 20°C. We noticed algal growth in the copepod cultures after 2 days, indicating sufficient food abundance. We observed copepods in each replicate during water changes to record 1) their survival, 2) the developmental stage, and 3) the number of eggs or directly hatched nauplii produced throughout their lifetime (from hatching to metamorphosis and from metamorphosis to sexual maturity). We measured the survival of copepods by counting the number of living and dead individuals. We obtained the developmental stage of copepods by counting the number of individuals observed at each life stage: nauplii, copepodites, and adult copepods. Their fecundity was measured by counting the number of eggs or nauplii produced relative to the number of adult females present in the culture.

3. Analysis of life-history datasets

We analyzed survival, fecundity, and development times with generalized linear mixed effect models (GLMM), generalized linear models (GLM), and t-tests. We modeled survival for Lumsden and George lakes with GLMMs using a binomial distribution and logit link function. We modeled fecundity with GLMs using a quasi-Poisson distribution to account for overdispersion. For development time, as the number of replicates was low for George Lake, we used different statistical tests for the two lakes. For Lumsden, we used a GLM with a Gamma distribution and a log link function, and for George, a t-test pooling data over the pH treatment (testing for the effect of time period). For the t-test, the assumption of normality was verified with Shapiro-Wilk tests and the equality of variance with Bartlett's tests. We checked all GLMM and GLM models for overdispersion using the overdisp_fun function from https://bbolker.github.io. To evaluate the

fixed effect on survival in our GLMMs, we used an AIC approach corrected for small sample size (AICc) with the ΔAIC criterion from the R package 'bbmle'. We kept the effects when ΔAIC > 2. To evaluate the fixed effect on fecundity in our GLMs, we used an analysis of deviance with an F approximation due to the overdispersion. For the development time from Lumsden, we used the likelihood ratio test to examine the fixed effects. The fixed effect coefficients of the GLMs and GLMMs and their confidence intervals were converted to odds ratios (survival), incident rate ratios (fecundity), and fold change (development time) using an exponential function.

4. Whole genome sequencing and SNP calling

We applied a pool-seq approach to the sequencing of temporal samples from the two lakes (Lumsden: pre-acidification, acidification, recovery; George: acidification, recovery). First, we separated the eggs from the sediment with the sugar flotation method described above. Each pool consisted of 200 eggs of *L. minutus* (except for Lumsden pre-acidification: 155 eggs) that were isolated in 70% ethanol after identification under high-resolution stereomicroscopes (Olympus). The resting eggs were first crushed with a plastic pestle after being flash-frozen with liquid nitrogen. We then extracted DNA from the crushed eggs with the QIAGEN MagAttract HMW kit, following the manufacturer's instructions. DNA quality control (QC) was done with the Agilent gDNA 165 kb kit on the Femto pulse system. Libraries were prepared for Illumina sequencing with the Lucigen NxSeq AmpFREE kit and then had QC performed, then PCR enriched for 6 cycles followed by a final QC. The libraries were then sequenced on an Illumina NovaSeq6000 Sprime v1.5 to generate 150 bp paired-end reads. Quality control, library preparation, and sequencing were conducted at the McGill Genome Center. We trimmed reads with fastp (52) with a minimum base quality score of 20 in a sliding window starting at the 3' end and a minimum length of 50 bp. As a draft genome for *L. minutus* or a closely related species was unavailable, we applied the reference-free SNP discovery method from discosnp++ (53). This method uses a de Bruijn graph analysis of k-mers, produces SNP datasets equivalent to reference-based approaches (53), and has been previously used for a copepod genomic dataset (54). We ran discosnp++ with the branching strategy (option -b 1), which allows a good compromise between precision and recall (53), extending polymorphism with left and right unitigs (only one SNP per contig) and a minimum coverage of 5 per pool. We converted the vcf file output by discosnp++ into a "pooldata" object with the poolfstat package in R (55), with a haploid pool size of 400 for all populations (except for

the size of 310 for Lumsden pre-acidification), minimum coverage of five per pool, maximum coverage of 300, a minimum read count per base of two, a minimal minor allele frequency of 0.0032 (to remove singletons) and removing indels. This generated a dataset of 4,211,276 biallelic SNPs after filtering.

5. Detecting signals of selection

To assess the presence of putative loci under selection during the acidification and pH recovery time periods, we used two approaches: 1) an F_{ST} scan for loci showing exceptional differentiation between time periods, and 2) an environmental association analysis. We conducted the F_{ST} scan approach with poolfstat to detect outlier SNPs showing large differences in allele frequency between time points. SNP-specific pairwise F_{ST} values were obtained with the compute.pairwiseFST function using the ANOVA method (default) and the same parameters as described in the filtering step above. We identified outliers SNPs as those included within the 95% percentile (top 5%) of the distribution of pairwise F_{ST} values across all temporal comparisons and lakes. Because genomic scans using pairwise F_{ST} do not consider population structure and demographic processes, we also used the hierarchical models implemented in Baypass (56), with the core model as an outlier analysis and the auxiliary (Aux) model as an environmental association analysis. To avoid computing limitations, we divided the poolfstat dataset into four pseudo-independent datasets by converting the pooldata object to four Baypass input files, using the "thinning" subsampling method with a sub-sample size of 763,496 SNPs. We ran both the core and auxiliary models with default parameters. We first used the core model to estimate the scaled covariance matrix of population allele frequencies Ω (Fig. S2), which reflects population demography and is then explicitly accounted for during the detection of outlier SNPs. This model also allows the estimation of the XtX statistics, which is equivalent to SNP-specific F_{ST} and is based on the variance in allele frequencies between all populations (57), accounting for Ω and derived p-values under a χ^2 distribution with 5 degrees of freedom (N = 5 pools, bilateral test). We confirmed that the p-values behaved well based on the shape of their histogram (Fig. S3; 60). We selected the aux model to detect SNPs significantly associated with the acidification (coded as a binary covariable; -1: pre-acidification and recovery, 1: acidification) in both the Lumsden and George datasets, as it is suitable for analyses of time samples such as experimental evolution datasets (see Baypass manual). This model computes the regression coefficient β_{ik} of the

association between the SNP allele frequencies and the covariable (here positive and negative association with the acidification), as well as an associated Bayesian auxiliary variable δ_{ik} from which a Bayes factor BF_{mc} is derived. We considered SNPs to be significantly associated with the acidification covariable when their BF_{mc} > 20 dB (Jeffrey's rule for "decisive evidence"; 58). The acidification covariable was standardized to $\hat{\mu} = 0$ and $\widehat{\alpha^2} = 1$ as recommended. We compared the four Ω matrices visually to ensure that the results were concordant between sub-datasets, then we combined the statistics estimated for each SNP.

6. <u>Population structure and demography.</u>

We first estimated population structure with the core model from Baypass. We then compared results of the core model to a genome-wide pairwise F_{ST} matrix estimated with the poolfstat package, using the same parameters as described above. To evaluate the potential effect of demography on genetic diversity levels, we also estimated observed heterozygosity for each temporal sample with poolfstat. To assess the possibility of evolutionary rescue (a demographic decline during acidification followed by population recovery due to adaptation), we used the diffusion approximation approach from δaδi (59). We selected the recovery temporal samples of Lumsden and George for our dataset and analyzed each population separately (1D model). The most complex model ("bottlegrowth") has a U-shaped demography as described above (Fig. 4A), with an ancestral effective population size *Nref*, then a genetic bottleneck at time T_B (scaled time between the bottleneck and present) corresponding to a decrease in effective population size to an effective population of size *NuB*, followed by exponential recovery in N_e to reach the effective population size *NuF*. As we knew the approximate time of the potential bottleneck (Lumsden = 1976, set as 42 years before sampling; George = 1972, set as 46 years before sampling; with two generations per year; 62), T_B was defined as a fixed parameter, and the parameter $\theta = 4\mu L$ as an explicit parameter, for all models except the neutral model. We defined θ with μ the mutation rate of 2.64×10^{-9} substitutions per site per year from the snapping shrimp *Alpheus spp.* (61) and *L* the effective sequenced length of 319,822,866 bp for Lumsden and 334,764,074 bp for George, calculated as L ≈ total length of sequence analyzed × SNPs retained for use in δaδi/total SNPs in analyzed sequence.

We also analyzed three simpler models, including two nested models: a) genetic bottleneck without recovery ("two epochs" model), b) growth starting at T_B, and c) neutral (Fig. S3). To

generate an allele frequency table, we used the summary_yij_ypij.out file output from Baypass with the Core model and selected the M_P column corresponding to the mean of the posterior distribution of the α_{ij} parameter, which is closely related to the allele frequency of the reference allele (chosen arbitrarily). We filtered out SNPs that were detected as outliers (putatively under selection) in the Baypass (core and aux models) and F_{ST} scan analyses, and SNPs with an allele frequency < 0.0025, which corresponds to an allele count of 0 and is uninformative in δaδi. The filtering retained 3,681,690 SNPs for Lumsden and 3,853,688 SNPs for George. To transform the allele frequency data into the input format of δaδi, we used the dadi_input_pools function from the genomalicious R package (62) applying the "probs" parameter in the methodSFS option. As we lacked information on the ancestral allele state, we inferred the folded SFS with δaδi. We used the default local optimizer on the log of parameters and optimized the parameters for each model until convergence was reached (three runs falling within 1% of the best likelihood). We compared our nested models (bottlegrowth and growth) with an adjusted likelihood ratio test, and the two remaining models (two epochs and neutral) based on the large differences in the likelihoods and residuals between these models and the model showing the highest likelihood (growth). As we obtained unlikely results during the conversion of parameters of best model for each population, possibly due to imprecise mutation rates from a distantly related species, we do not report the results of parameter conversion. To obtain the uncertainties on the parameters while accounting for the effect of linkage, we used bootstrapping and the Godambe Information Matrix approach (63). For this, we generated 100 bootstrapped datasets with a chunk size of 1×10^5 bp. Note that due to the SNPs being called from contigs in the reference-free SNP discovery approach, the bootstrapping was executed with individual SNPs, which possibly led to the underestimation of uncertainties.

Results

1. Resurrection ecology experiment: Adaptive shifts in life-history traits following acidification and pH recovery.

The resurrection ecology experiment revealed population life history traits shifts (lifetime survival, development time, and fecundity) that were consistent with the adaptation of *L. minutus* to lake acidification, followed by a reversal during recovery (Fig. 2, Tables 1 and 2). For the survival of copepods from nauplii to adulthood (lifetime survival) in both Lumsden and George

lakes (Fig. 2A), we found significantly greater survival rates of the resurrected individuals from the acidification period than from the recovery period. Specifically, for Lumsden, there were no significant differences in survival between individuals from the pre-acidification and recovery periods. These results hold regardless of pH (non-significant interaction). Although this interaction was not significant, survival was also significantly higher at neutral pH compared to acidic pH. We found similar results, albeit less pronounced, for early survival (from hatching into nauplii to metamorphosis into copepodid), except for the effect of pH, which was not significant (Fig. S4A, tables 2 and 3). Copepods from the acidification period developed significantly faster to adulthood compared to the pre-acidification period for Lumsden, and for both Lumsden and George, there was no difference in the development time between acidification and recovery individuals (Fig. 2C, Tables 1 and 2). The pH treatment and the interaction between time period and pH did not affect development time to adulthood for both lakes (Table 1). The results were similar for early development (Fig. S4B) except for a significant difference between acidification and recovery for Lumsden (Tables 1 and 2). Female copepods from Lumsden were significantly more fecund during the acidification period than during the pre-acidification and recovery periods, but the pH and the interaction between time period and pH did not affect their fecundity (Fig. 2B, Tables 1 and 2). However, George Lake female fecundity was only marginally affected by time period and the time period × pH interaction. To summarize, our experiment suggests there was an acquisition of acid tolerance during the acidification period followed by a loss of tolerance, shown through differences in survival and fecundity. The increase in acid tolerance was accompanied by faster development, but this change in development rate did not reverse to pre-acidification levels following pH recovery. These results were consistent across both lakes despite having experienced different strengths of historical acidification.

2. <u>Population genomics: demography, population structure, and rapid genetic adaptation to acidification and lake pH recovery</u>

Using the recovery time period samples from George and Lumsden lakes, we found indications of significant declines in their effective population sizes during the acidification period. The best models based on the highest likelihood for both populations were the models including population growth at a known fixed time in the past corresponding to the acidification event. (Fig. 3C and D, Table S2). However, for Lumsden Lake, the likelihood ratio test led to the rejection of

the nested model of growth (Fig. S5, Table S2), favoring the more complex model of bottleneck followed by growth (Figure 3A and B, Table S2, adjusted D-statistic = 5.29, p-value = 0.02), whereas the nested growth model was favored for George Lake (adjusted D-statistic = 1.12, p-value = 0.29). For the latter, the small nu parameter value obtained for the growth model implies a population decline toward a very small effective population size (Table S2). Our analyses suggest that the ancestral effective population sizes were possibly quite large, with estimated θ values of 2.9×10^6 for George and 1.5×10^6 for Lumsden (see table S2; $\theta = 4N_{ref}\mu L$, with N_{ref} the ancestral effective population sizes, μ the mutation rate, and L the effective sequenced length). In contrast, the decrease in effective population size was likely very pronounced, with very low inferred values for the ratio of contemporary effective population size to ancestral population size in George Lake (*nu*: 3.5×10^{-5}, table S2), and for the ratio of N_e after the genetic bottleneck to the ancient population size in Lumsden Lake (*nuB*: 2.3×10^{-5}, table S2). These parameter values should be interpreted with caution given the high uncertainty around the parameter estimates (Table S2). This demographic decline was accompanied by a decrease in heterozygosity in Lumsden from 0.30 to 0.28, followed by a smaller increase to 0.29 during the recovery phase. Conversely, for George, we did not find a change in heterozygosity during the recovery (observed heterozygosity = 0.28 for both the acidification and recovery time periods). We found that pairwise F_{ST} values were higher between lakes across time periods (moderate, 0.09 on average) than between time periods within lakes (low, 0.03 on average). For Lumsden, genetic differentiation was slightly lower between the pre-acidification and the recovery periods than between the acidification and the pre-acidification or recovery (Table S3).

The genome scan of pairwise F_{ST} values detected outlier loci in temporal comparisons of both study lakes (Lumsden: pre-acidification vs acidification and acidification vs recovery, George: acidification vs recovery). The 95% percentile (top 5%) of pairwise F_{ST} values across all time comparisons for both lakes was $F_{ST} > 0.29$, which was used as a threshold to detect outlier SNPs. For Lumsden, we found 165,087 outlier SNPs in the pre-acidification vs acidification comparison (≈ 4% of the dataset), and 232,515 outliers in the acidification vs recovery comparison (≈ 6% of the dataset). For George, we found 157,597 outlier SNPs in the acidification vs recovery comparison (≈ 4% of the dataset). We also found 19,525 outlier SNPs in common between the two-time comparisons for Lumsden in the F_{ST} genome scan and significantly associated with the acidification in the Baypass Aux model (BFmc > 20). Remarkably, all of these outliers showed a

reversal in allele frequencies during the recovery period (Fig. 4A). These SNPs are considered candidates for directional selection during the acidification followed by an adaptive reversal during recovery. We identified 78,864 SNPs that were outliers in the Lumsden F_{ST} scan for the pre-acidification to acidification comparison, but not for the acidification to recovery comparison, and not significantly associated with the acidification in Baypass (BFmc < 20, ≈ 2% of the dataset, Fig. 4B). The remaining 141,365 Lumsden outlier SNPs were not significantly differentiated in the F_{ST} scan for the pre-acidification to the acidification comparison, but were significant for the acidification to recovery comparison, and not significant in Baypass (BFmc < 20, ≈ 3% of the dataset, Fig. 4C). We also found 5,907 outlier SNPs in common in the F_{ST} scan for the acidification to recovery temporal comparisons in both Lumsden and George lakes and significant in Baypass (BFmc > 20, ≈ 0.1% of the dataset, 1% of the total outliers across methods), and 100% of these outliers had allele frequency changes in the same direction for this time period transition. These SNPs are considered candidates for parallel selection from the increase in pH during the recovery phase.

Using the core model in Baypass as our outlier analysis, we found 36,110 outlier SNPs with p-value < 0.001, which represented ≈1% of the dataset (Fig. S6 and S7). The XtX estimate is based on the variance in allele frequencies across populations, thus, in the present case, outlier SNPs are significantly differentiated considering both the populations (Lumsden and George) and temporal samples (pre-acidification, acidification, and recovery). For the Aux model testing the association between SNP allele frequencies and acidification, we found 176,744 outlier SNPs significantly associated with acidification (BFmc > 20; ≈ 4% of the dataset). These outlier SNPs have allele frequencies both negatively and positively associated with acidification (Fig. 4, Fig. S8), and some showed a strong association, with their correlation coefficient $|\beta_i| > 0.2$ (33,279 outliers, Fig. S8). Finally, we found outliers in common between the F_{ST} scan and the auxiliary model in Baypass (Fig. S7): 64,767 outliers for Lumsden (using the SNPs that are outliers in the F_{ST} scan in at least one temporal comparison) and 43,249 outliers for George (Fig. 4E).

Discussion

Understanding the factors that determine the long-term success of evolutionary rescue is crucial for predicting populations' responses to ongoing environmental change (6), but knowledge of how demographic, genetic, and environmental mechanisms interact as this process unfolds in

complex natural ecosystems is lacking. Here, we investigated the possibility of evolutionary rescue in freshwater calanoid copepod (*Leptodiaptomus minutus*) populations that were historically exposed to acute lake acidification, followed by an adaptive reversal during lake pH recovery. We combined a resurrection ecology experiment with whole-genome data spanning ≈ 200 generations in two lakes with histories of acidification. We found adaptive shifts during acidification, with increased acid tolerance and fecundity as well as faster growth rate, which were probably mediated by directional selection at the genomic level. We also found evidence for a genetic bottleneck from lake acidification followed by high population density and a potential recovery of the effective population size, suggestive of evolutionary rescue. Resurrected copepods from the recovery period showed reduced acid tolerance and fecundity, accompanied by a reversal in allele frequency at candidate loci. Overall, our results support the possibility of evolutionary rescue in these wild populations and help shed light on the factors (demographic, genetic, and environmental) that likely promoted the rescue.

1. Contemporary adaptation to acute historical acidification

Using a resurrection ecology experimental approach, we detected shifts in life-history traits between the pre-acidification and acidification time periods in both study lakes. We documented an increase in lifetime survival and fecundity as well as faster development rates overall (across pH treatments) for copepods from the acidification period. As our resurrection experiment was conducted within a single generation and thus could not account for maternal effects, we acknowledge that our experimental setup did not allow us to disentangle the influence of genetic versus plastic and maternal effects on the life-history traits. Apart from survival, for which the effect of pH was significant, fecundity and development time showed flat reaction norms across pH treatments, with most differences occurring between individuals from distinct time periods. Considering that the individuals from different time periods correspond to distinct genotypes within the same continuous population, this implies that we did not find indications of genotype × environment interactions for these traits. This suggests that plasticity may play a limited role in changes in fecundity and development as a response to acidity. There were some signs of initial intraspecific variation in acid tolerance, as a few individuals from the pre-acidification period from Lumsden Lake were able to survive at acidic pH until sexual maturity. These acid-tolerant phenotypes could have been positively selected during the process of acidification, leading to

adaptation to acidic pH. Our experiment also revealed an important concomitant decrease in development time following the acidification for both early and late development stages. Our results are consistent with adaptation to historical lake acidification leading toward a faster pace of life, with higher survival, fecundity, and increased growth rates, which have also been reported in response to stressful environments, such as urbanization (64). However, the increased survival and fecundity we found in our experiments for the individuals from the acidification period contrast with studies of marine copepods, which have reported few selective effects of acidity in response to ocean acidification (65,66). Given these contradictions, and apart from a few relevant examples (36,66,67), studies examining the full scope of responses to acidification (phenotypic, genetic, and demographic) are still lacking and will require further investigation (68).

The adaptive shifts in life-history traits that we detected were accompanied by significant allele frequency changes between the pre-acidification and the acidification periods. This implies that the life-history shifts observed between these two time periods could be due in part to shifts in adaptive allele frequency, resulting from directional selection due to acidification. As these allele frequency changes were both positively and negatively associated with lake acidification, and we do not know the ancestral allele states, adaptation could have occurred through positive selection, purifying selection, or a combination of both (69,70). The high number of outlier SNPs detected with our two genome scan analyses also suggests that linked selection could have played a role in structuring genomic responses in these populations (71). Based on the number of generations between the pre-acidification and acidification periods and the presence of a few acid-resistant copepods initially, we hypothesize that selection acted on standing variation rather than on *de novo* mutation (soft sweeps; 74).

Leptodiaptomus minutus does not currently have an annotated genome, and thus we were unable to conduct functional analyses of the SNPs putatively under selection from acidification. Possible functions under selection to explore in future studies could be related to osmoregulation and homeostasis, as these physiological functions have consistently been identified as a mechanism of response to acidification in copepods (36,67,73–75). Regulation of diapause (76,77) and lipid metabolism (78) are also of interest as they tend to be favored in stressful environments. Taken together, the changes in life-history traits (survival, fecundity, and development) as well as significant shifts in the frequency of putatively adaptive alleles provide support for the occurrence of rapid adaptive evolution due to historical changes in pH.

2. Demographic consequences of acidification and evolutionary rescue

The universal increase in survival, fecundity, and growth rates that we observed in copepods that experienced lake acidification suggests that individuals from this time period have higher fitness than individuals from the pre-acidification period. During the initial process of acidification, the lower survival and fecundity at acidic pH, combined with a slow development rate, likely had important demographic effects, potentially triggering a population decline. This was indeed confirmed with our demographic analysis, in which the best model for Lumsden Lake included a genetic bottleneck and a population decline for George Lake after the acidification. This decline resulted in a moderate reduction in genetic diversity during the acidification period for Lumsden. The population decline due to acidification was likely followed by a population rebound, as *L. minutus* populations were documented to be at high density in acidified lakes by 1972-1973 (8 ind./L in George, 17 ind./L in Lumsden; 46). The genetic bottleneck and N_e decline detected in our study populations during the acidification, in conjunction with a probable population recovery and an increase in the frequency of adaptive phenotypes and putative alleles, indicate that our study populations likely avoided local extinction (e.g., 44) through evolutionary rescue.

This rescue could have been facilitated by several factors: large initial population size, standing genetic variation, and gradual rate of environmental change. Population size was probably large initially, as the model parameters suggest a high ancestral effective population size prior to the acidification. Larger population sizes have been shown to increase the likelihood of evolutionary rescue (7). It is also likely that the evolutionary rescue was fuelled by adaptation from standing genetic variation rather than from new mutations, as acid-tolerant phenotypes appeared to be present in the populations during the pre-acidification period. Theoretical models suggest that adaptation from standing variation can require less time and a smaller initial population size for evolutionary rescue to occur (11). A recent study highlighted the role of standing genetic variation in the evolutionary rescue of bats from white-nose syndrome (15). It has also been suggested that evolutionary rescue from high levels of environmental stress should be driven by large-effect mutations that are present as standing genetic variation (12), which might be the case in our study system and will require further investigation. The populations from both of our study lakes showed similar responses despite the different trajectories during acidification (much faster and steeper for Lumsden Lake; 81). These results confirm that the rate of acidification that the lakes in the Killarney Park region experienced (gradual or abrupt) represented a very stressful

environmental event for aquatic organisms, resulting in a genetic bottleneck or N_e decline in *L. minutus*, which was one of the most tolerant zooplankton species in this region (34,40). This is consistent with the high extirpation rate recorded at the community level for other zooplankton species and aquatic taxa (39,40).

Despite evidence for evolutionary rescue in *L. minutus* populations, our demographic analyses highlight the potential negative consequences of this process. Indeed, the strong selection that occurred during evolutionary rescue appears to be associated with a genetic bottleneck and a decline in effective population size. Even though N_e seems to have recovered in Lumsden Lake during the following environmental recovery and increased population abundance, the best model for George suggests that N_e did not recover in this population. This is consistent with a previous study that found that evolutionary rescue led to a reduction of genetic diversity due to strong selection and the drift occurring at reduced population sizes (18). Prior experimental studies of evolutionary rescue have also shown demographic and genetic bottlenecks with large reductions in effective population size, followed by limited N_e recovery (9). This suggests that the short-term benefits of evolutionary rescue might be accompanied by detrimental effects over the long term.

3. <u>Evidence for an adaptive reversal during lake pH recovery</u>

The results of the resurrection experiment indicated a loss of acid tolerance (survival and fecundity) in individuals from the recovery period for both lakes. In the case of an adaptive reversal, we might expect to find a trade-off between survival under acid stress and survival or fecundity at neutral pH for the copepods from the acidification period, for example, due to antagonistic pleiotropy (22,44). This would lead to the selection of acid-sensitive phenotypes during pH recovery in the lakes. This pattern has been previously shown in populations of *L. minutus* from acidic lakes and ponds (20,80,81). Surprisingly, we did not find indications of such a trade-off in individuals from the acidification period, which showed high fitness across neutral and acidic pH treatments. This pattern could be due to unmeasured maternal effects that had positive effects on copepod fitness from the acidification period, possibly masking the expected trade-off. Although we did not quantify lipid content or fatty acid profiles, we observed that eggs and nauplii from the acidification time period contained more pronounced lipid droplets with red carotenoid pigments compared to the two other time periods, a trend that continued to adulthood. This coloration can be indicative of the accumulation of fatty acids from their algal diet (82), which

are known to affect egg production (83), survival (84), and overall individual fitness (85). Changes in the species composition and biomass of phytoplankton were documented during the acidification and recovery period (41,86), and the important reduction in zooplankton diversity during the acidification (40) could have led to reduced competition and facilitated the accumulation of fatty acids. Additionally, our results are based on a laboratory experiment in a simplified environment, whereas *L. minutus* fitness might have been affected by ecological interactions such as increased competition and predation during the progressive ecological and chemical recovery (42). Finally, fitness trade-offs from local adaptation are not always detected (87). This might be the case in the results presented here due to the complexity of the study system and its inherent stochasticity, which could have masked these effects.

Despite the apparent absence of a fitness trade-off for acid tolerance, we did find signs of an evolutionary reversal at the genomic level. Indeed, a large number of Lumsden outliers which were significant for both temporal comparisons in the F_{ST} scan (pre-acidification to acidification and acidification to recovery) were also significant in the Baypass Aux model, and all of these outliers showed a reversal in the direction of allele frequency change (either positive or negative) during recovery. Our results thus indicate that the reversal in acid tolerance at the phenotypic level is at least in part due to a reversal in adaptive allele frequency at the genomic level. A similar adaptive reversal at both the phenotypic and genomic levels has previously been shown in *Daphnia magna* in response to shifts in predation levels (21). Interestingly, the observed site frequency spectrum in both populations (Fig. 3) indicates an excess of intermediate frequency alleles, which can be the result of balancing selection (88). During the filtering of SNPs for demographic analyses with δaδi, we removed SNPs that were outliers in the genome scan analyses, but given that the SNPs were discovered from contigs, we did not control for linkage disequilibrium. This implies that the SNPs included in the dataset for the demographic analyses could be in LD with SNPs under balancing selection, which could be the potential result of the adaptive reversal.

In contrast to survival and fecundity, development time showed no sign of reverting to pre-acidification trait values or being responsive to pH. This could be because the developmental rate has responded to a selection pressure that we did not measure in the present study. This would mean that the acceleration of the developmental rate during the acidification period and persistence during recovery does not result from adaptation to acidity but rather from a distinct selection pressure occurring on a similar timescale. A potential cause for the acceleration of development

times is temperature increase linked to climate change, which has been documented in the region of Killarney (89). A previous study on marine copepods found that selection from increased temperature resulted in faster growth rates, whereas acidification slowed down development (66). Ecological monitoring has also revealed that a population of *Leptodiaptomus ashlandi* shifted from one to two generations per year concurrently with increasing temperatures, implying faster growth rates due to climate warming (90). Our results thus indicate that the reversal of life-history traits (fecundity, survival) is due in part to directional selection, whereas development time may be responding to other sources of selection and therefore did not experience a reversal.

4. Consequences of evolutionary rescue and future trends

George and Lumsden lakes are not at the same stage of ecological recovery from historical lake acidification, but we nonetheless found comparable phenotypic responses over time between the two populations, which is suggestive of parallelism in life-history trait shifts (91). However, we found a lower proportion of parallel SNPs at the genomic level (1% of the outliers) compared to what has been found in other study systems (91–94). The low genomic parallelism between lakes could be due to differences in the extent of historical acidification and pH recovery trajectories (45,79). Finally, our results suggest that both populations went through a similar pattern of evolutionary rescue followed by an adaptive reversal, implying that these processes may have been repeated in other *L. minutus* populations with similar ecological histories of lake acidification and pH recovery. Adaptive reversals were indeed documented in other populations of the region from historically acidified lakes (20). This implies that evolutionary rescue and adaptive reversal could be repeatable and predictable in this study system. These processes might not be highly repeatable at the SNP level, but they could be at the gene or functional level due to redundancy (91,95).

Although *Leptodiaptomus minutus* populations were sufficiently resilient to undergo evolutionary rescue and adaptive reversals from historical acidification and lake pH recovery, this might not be repeatable under future threats. Acidified lakes in the Killarney, Ontario (Canada) region have not reverted to their original ecological state (42), and additional stressors relevant to copepod biology appeared during the same time period as the acidification. A potential selection pressure that arose before the acidification period and continued during the pH recovery period is climate change (89), which is known to be a potent selection pressure in marine copepods

(36,66,67,70). The reduced effective population size found in one of the *L. minutus* populations suggests that their present and future adaptive potential could potentially be reduced (Blanquart et al., 2012). Decreases in *L. minutus* populations were recently reported across several lake ecosystems (Laurentian Great Lakes, Adirondack Park and Killarney Provincial Park) along with ongoing physicochemical and biotic changes (shifts in productivity, browning, predation, and competition levels; 98–100), highlighting the need for an assessment of the adaptive potential to climate change and other stressors in ecologically significant zooplankton, such as freshwater calanoid copepods.

Conclusions

Our study fills a critical gap in the potential for evolutionary rescue in nature and on the demographic, genetic, and environmental factors that promote the rescue, which combined role is still poorly understood in natural populations. We assessed the long-term consequences of evolutionary rescue on the resilience of populations to future environmental changes by showing that evolutionary rescue did not prevent an adaptive reversal during environmental recovery, implying that the loss of genetic diversity that occurs during evolutionary rescue does not necessarily prevent future adaptation.

Author contributions:

MS, AMD, and RDHB designed the study. MS, MSM and AMD collected the field samples. MS and MSM conducted the laboratory experiment. MS performed the DNA extractions, generated the pool-seq processing pipeline, and ran the bioinformatic and statistical analyses, with input from AMD and RDHB. The article was written by MS, with input from AMD and RDHB. All authors contributed to the review and editing. AMD and RDHB funded the study.

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Data Accessibility

The raw sequencing reads have been deposited in the National Center for Biotechnology Information Sequence Read Archive SRA repository (BioProject PRJNA1229077) and the accompanying metadata are also stored in the SRA (BioProject PRJNA1229077), using the Eukaryotic water MIxS package. The scripts and input data have been uploaded to the Dryad Digital Repository (DOI: 10.5061/dryad.qbzkh18v6).

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Figures and table legends:

Figure 1: Location of the sampled lakes and experimental design of the resurrection ecology experiment. Top: George and Lumsden are located in Killarney Provincial Park ON, Canada. Bottom left: Schematic representation of the resurrection experiment and the temporal genomics. Bottom right: Steps and details of the resurrection experiment.

Figure 2: Observed shifts in life-history traits in freshwater copepods from different ecological periods (pre-acidification, acidification, recovery) in two historically acidified lakes as a response to two levels of pH treatment as revealed by the resurrection ecology experiment. (A) Lifetime survival, **(B)** development time from birth to adulthood, **(C)** Fecundity (number of eggs or nauplii per female). Each dot represents a measurement for one replicate, and the black bars indicate the mean response for each treatment.

Figure 3: Results of the best demographic model tested with δaδi. (A) Visual 1D demographic model of Lumsden Lake, with a genetic bottleneck at the time of acidification T_B, followed by exponential recovery. NuB and NuF are the effective population sizes after the bottlenecks and during the recovery, respectively. T_B is the known scaled time between the bottleneck and present **(B)** Top: Folded site frequency spectrum (SFS) for a sample size of 400 (two times the number of individuals) for the observed data (blue line) and the model (red line), showing the logarithm of the number of sites (y-axis) as a function of a given read count (x-axis). Bottom: residuals of the normalized differences between the observed data and the model. **(C)** Visual 1D demographic model of George Lake, with a growth event starting at the time of acidification T_B. Nu is the ratio of contemporary effective population size to ancestral N_e. **(D)** Folded SFS (top) and residuals between the observed data and the model for George Lake (bottom).

Figure 4: Allele frequency changes of outliers detected with the FST scan and Baypass. SNPs are significantly associated with lake acidification in the Baypass Aux model if BFmc > 20, and not associated when BFmc < 20. The red and blue colors indicate positive and negative associations with acidification in Baypass (regression coefficient $\beta ik > 0$ and $\beta ik < 0$ respectively). Plots show a random subset of 500 SNPs from the category of outlier and non-outlier SNPs described for Lumsden and George Lake copepods. **(A)** Lumsden SNPs significant in the

F_{ST} scan for both temporal comparisons and significant in Baypass, **(B)** Lumsden SNPs significant in the F_{ST} scan for the pre-acidification to acidification comparison but not for the acidification to recovery comparison, and not significant in Baypass, **(C)** Lumsden SNPs not significant in the F_{ST} scan for the pre-acidification to acidification comparison but significant for the acidification to recovery comparison, and not significant in Baypass, **(D)** Lumsden non-outlier SNPs: non-significant in the F_{ST} scan for both time comparisons and not significant in Baypass, **(E)** George outlier SNPs: significant in the F_{ST} scan and Baypass **(F)** George non-outlier SNPs: non-significant in the F_{ST} scan and Baypass.

Table 1: Results of the analysis of survival, fecundity, and development times with generalized linear mixed effect models (GLMM), generalized linear models (GLM), and t-tests. Lifetime survival indicates survival from hatching to adulthood and early survival from hatching to copepodid stage. For the GLMMs, a ΔAIC > 2 indicates that the effect is significant. Significant fixed effects are indicated in bold.

Table 2: Fixed effect coefficients of the GLMs and GLMMs and their confidence intervals after conversion with an exponential function for each response variable and lake. The converted coefficients are given as the first level compared to the second (reference) level. The relationship is significant if the converted coefficients do not include 1 (in bold), is positive if > 1, and negative if < 1. When the converted coefficient is < 1, the inverse is given in parentheses and indicates how much less likely an event is to be observed. For the t-tests results (development time for George), the estimate represents the difference (in days) between time periods.

Figure 1:

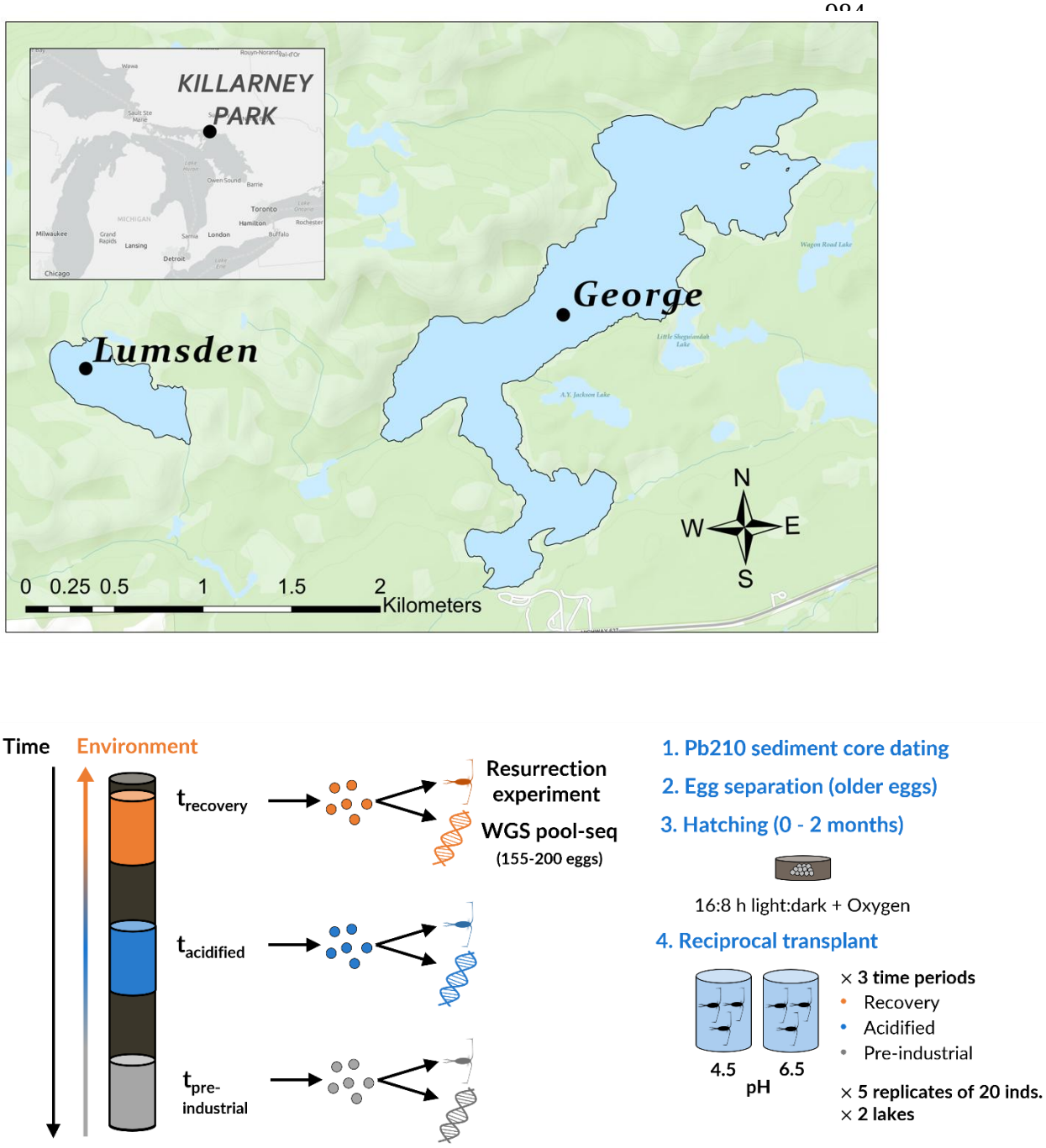
KILLARNEY PARK
George
Lumsden
N
W
E
S
0 0.25 0.5 1 1.5 2
Kilometers
Time
Environment
t recovery
t acidified
t pre-industrial
Resurrection experiment
WGS pool-seq
(155-200 eggs)
1. Pb210 sediment core dating
2. Egg separation (older eggs)
3. Hatching (0 - 2 months)
16:8 h light:dark + Oxygen
4. Reciprocal transplant
4.5
6.5
pH
× 3 time periods
Recovery
Acidified
Pre-industrial
× 5 replicates of 20 inds.
× 2 lakes

Figure 2:

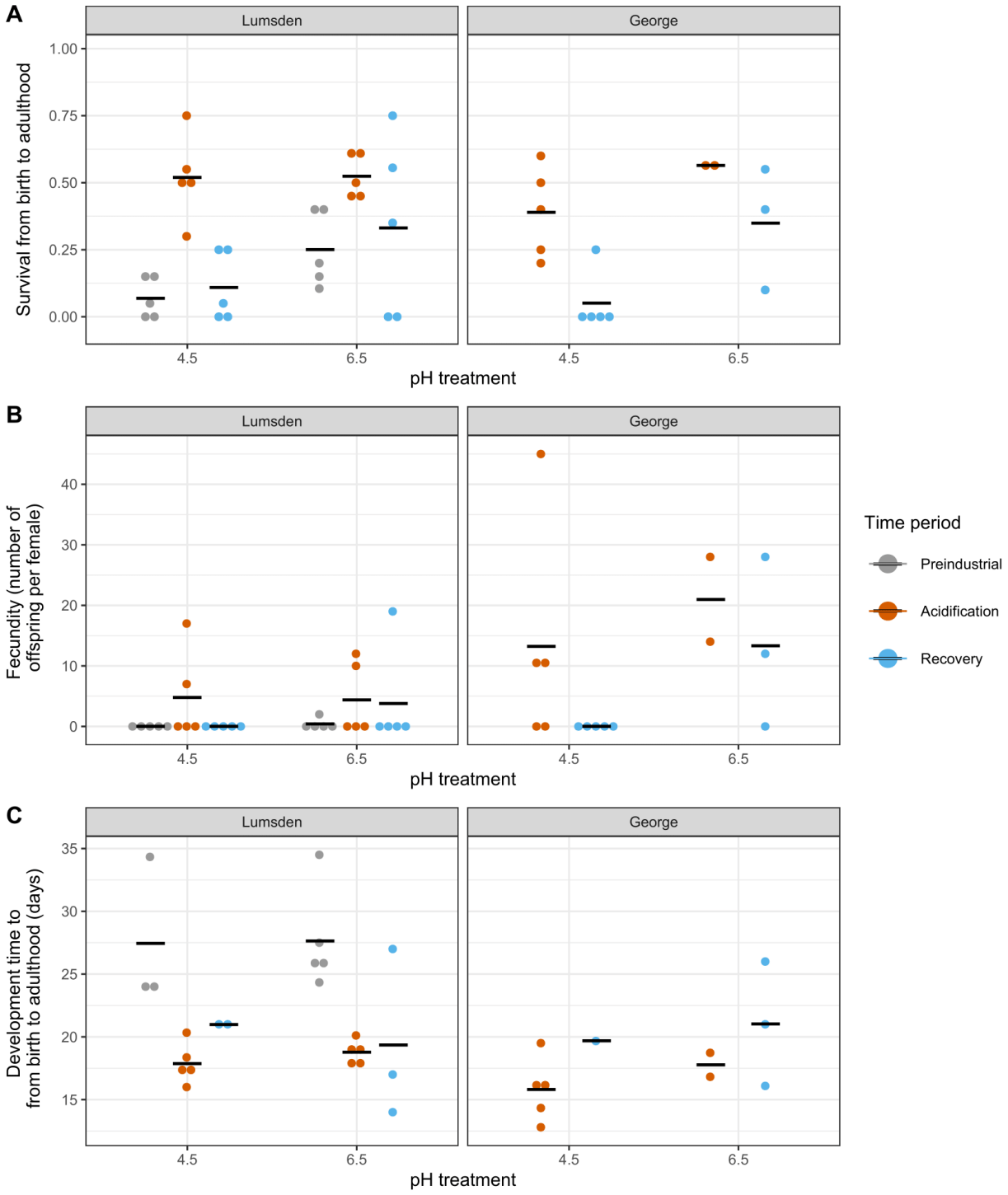
A
Lumsden
George
Survival from birth to adulthood
1.00
0.75
0.50
0.25
0.00
4.5
6.5
pH treatment
B
Fecundity (number of offspring per female)
40
30
20
10
0
C
Development time to from birth to adulthood (days)
35
30
25
20
15
Time period
Preindustrial
Acidification
Recovery

Figure 3:

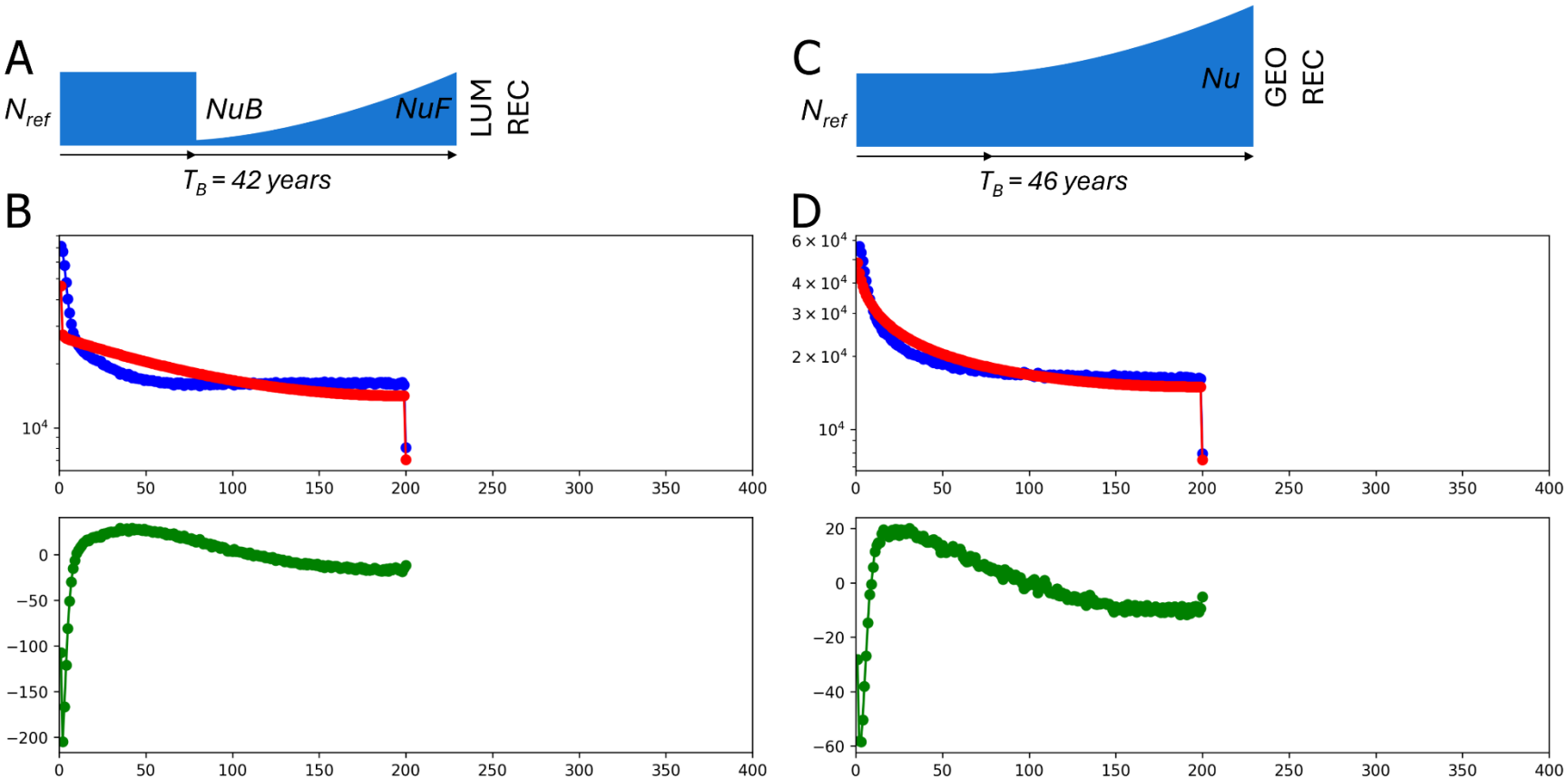
A
N_{ref}
NuB
NuF
LUM
REC
T_B = 42 years
B
C
N_{ref}
Nu
GEO
REC
T_B = 46 years
D

Figure 4:

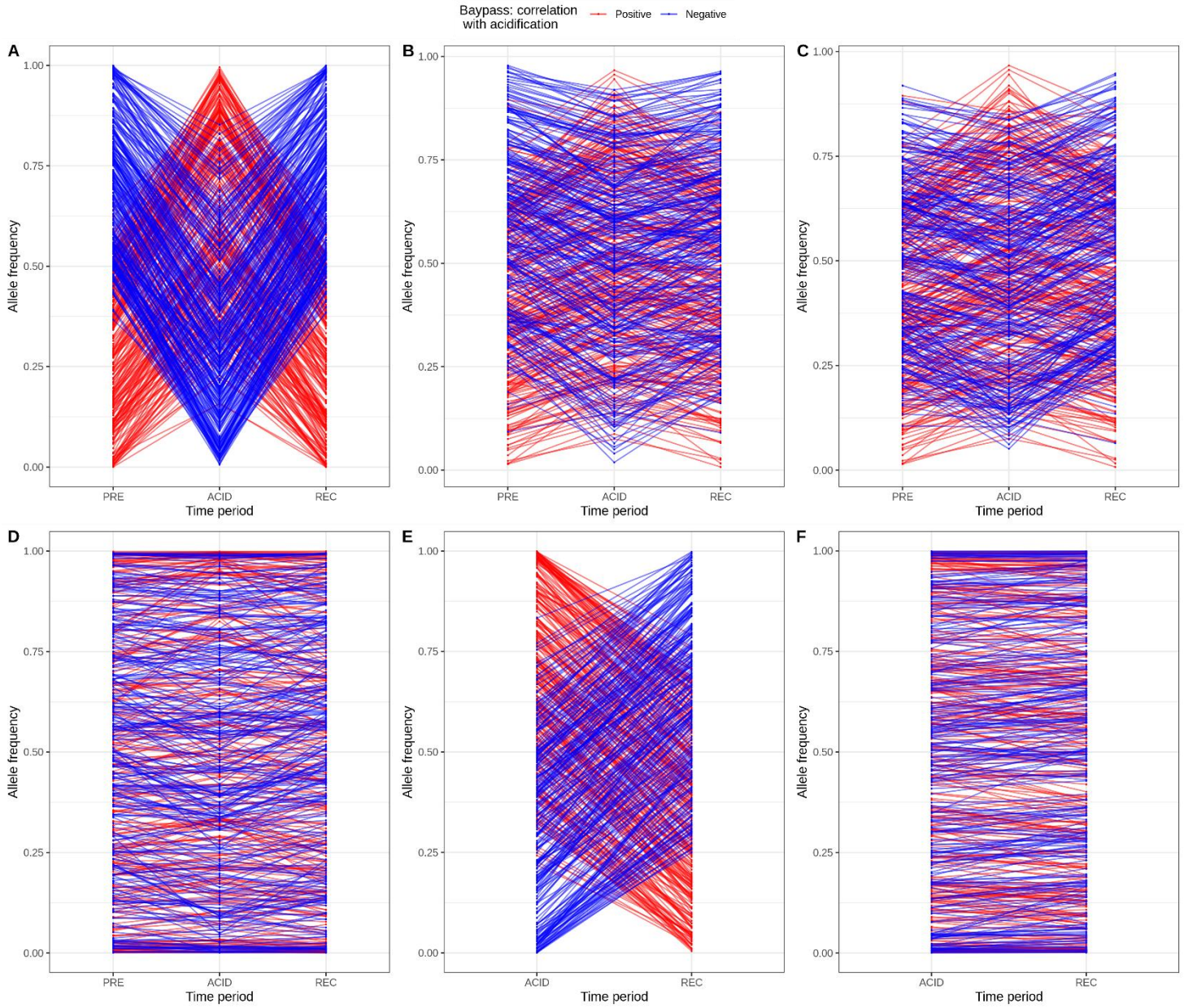
Baypass: correlation with acidification
Positive
Negative
A
B
C
D
E
F
Allele frequency
1.00
0.75
0.50
0.25
0.00
PRE
ACID
REC
Time period

Table 1:

Response variable	Lake	Type model	Test	Fixed effects	df	p-value ΔAIC
Lifetime survival	Lumsden	GLMM binomial logit	AICc	Time period × pH	5	-2.95
				Time period	3	**10.83**
				pH	4	**2.01**
Early survival	Lumsden	GLMM binomial logit	AICc	Time period × pH	5	-5.9
				Time period	3	**8.41**
				pH	4	-0.90
Lifetime survival	George	GLMM binomial logit	AICc	Time period × pH	4	-1.87
				Time period	3	**7.55**
				pH	3	**3.48**
Early survival	George	GLMM binomial logit	AICc	Time period × pH	4	- 4.44
				Time period	3	**5.94**
				pH	3	-3.23
Fecundity	Lumsden	GLM quasi-poisson	Quasi-F	Time period × pH	24	0.20
				Time period	26	**0.04**
				pH	28	0.38
Fecundity	George	GLM quasi-poisson	Quasi-F	Time period × pH	11	0.08
				Time period	12	0.07
				pH	13	0.15
Lifetime development	Lumsden	GLM gamma log	LRT	Time period × pH	17	0.78
				Time period	20	$\mathbf{5.59\times 10^{-7}}$
				pH	19	0.93
Early development	Lumsden	GLM gamma log	LRT	Time period × pH	22	0.16
				Time period	25	**0.002**
				pH	24	0.39
Lifetime development	George	-	t-test	Time period	4	0.121
Early development	George	-	t-test	Time period	12	0.07

Table 2:

Response variable	Lake	Fixed effect coefficients	Comparison	Estimate	Lower limit	Upper limit
Lifetime survival	Lumsden	Odds ratio	Acidification vs pre-acidification	**7.98**	2.96	21.51
			Recovery vs pre-acidification	1.31	0.46	3.72
			Neutral pH vs acidic pH	**2.59**	1.14	5.87
Early survival	Lumsden	Odds ratio	Acidification vs pre-acidification	**3.36**	1.42	7.97
			Recovery vs pre-acidification	0.59 (1.69)	0.25	1.41
			Neutral pH vs acidic pH	1.70	0.84	3.45
Lifetime survival	George	Odds ratio	Recovery vs acidification	**0.12 (8.33)**	0.04	0.40
			Neutral pH vs acidic pH	**5.35**	1.66	17.19
Early survival	George	Odds ratio	Recovery vs acidification	**0.27 (3.70)**	0.11	0.67
			Neutral pH vs acidic pH	1.45	0.57	3.75
Fecundity	Lumsden	Incident rate ratios	Acidification vs pre-acidification	**23.0**	1.32	$\mathbf{9.30\times 10^5}$
			Recovery vs pre-acidification	9.5	0.36	$\mathbf{3.96\times 10^5}$
Fecundity	George	Incident rate ratios	Recovery vs acidification	0.32 (3.13)	0.05	1.41
Lifetime development	Lumsden	Fold change	Acidification vs pre-acidification	**0.67 (1.49)**	0.58	0.77
			Recovery vs pre-acidification	**0.73 (1.36)**	0.61	0.86
Early development	Lumsden	Fold change	Acidification vs pre-acidification	**0.82 (1.22)**	0.72	0.93
			Recovery vs pre-acidification	0.98 (1.02)	0.87	1.13
Lifetime development	George	-	Recovery vs acidification	4.34	-	-
Early development	George	-	Recovery vs acidification	1.68	-	-