Genomic data detect corresponding signatures of population size change on an ecological time scale in two salamander species

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Abstract

Understanding the demography of species over recent history (e.g., < 100 years) is critical in studies of ecology and evolution, but records of population history are rarely available. Surveying genetic variation is a potential alternative to census-based estimates of population size, and can yield insight into the demography of a population. However, to assess the performance of genetic methods it is important to compare their estimates of population history to known demography. Here, we leveraged the exceptional resources from a wetland with 37 years of amphibian mark-recapture data to study the utility of genetically-based demographic inference on salamander species with documented population declines (Ambystoma talpoideum) and expansions (A. opacum); patterns that have been shown to be correlated with changes in wetland hydroperiod. We generated ddRAD data from two temporally sampled populations of A. opacum (1993, 2013) and A. talpoideum (1984, 2011) and used coalescent-based demographic inference to compare alternate evolutionary models. For both species, demographic model inference supported population size changes that corroborated mark-recapture data. Parameter estimation in A. talpoideum was robust to our variations in analytical approach, while estimates for A. opacum were highly inconsistent, tempering our confidence in detecting a demographic trend in this species. Overall, our robust results in A. talpoideum suggest that genome-based demographic inference has utility on an ecological scale, but researchers should also be cognizant that these methods may not work in all systems and evolutionary scenarios. Demographic inference may be an important tool for population monitoring and conservation management planning.
Introduction

Detecting shifts in demography within populations over recent time scales (e.g., < 100 years) is important for understanding evolutionary responses to intrinsic and extrinsic factors. This knowledge is essential to identify the biological, ecological, or environmental drivers of population-size change, and to devise informed conservation management plans when needed. Surveying genetic variation can potentially provide an effective alternative to field-intensive census-based estimates of population size, yielding insight into the demographic history of a population, including migration events, population structure, expansions, and bottlenecks (Schwartz et al., 2007; Gutenkunst et al., 2009; Steiner et al., 2013). However, most studies incorporating genetically based demographic inference have estimated events at historical time scales (i.e., thousands of generations; see Moura et al., 2014; Lanier et al., 2015; Papadopoulou & Knowles, 2015), and not at an ecological time scale (tens of generations or fewer; but see Lozier 2014; McCoy et al., 2014).

Understanding demographic changes at an ecological time scale is particularly important in the conservation of species impacted by recent climate and environmental change. Climate change is predicted to have devastating impacts on ecosystems and communities in the upcoming century, affecting population demography and community dynamics (Parry et al., 2007). An important step towards understanding future impacts of climate change on species is assessing current population responses to environmental variables (Blois et al., 2013; Lanier et al., 2015). Demography and genetic diversity are essential parameters in population health and viability, and the surveying of genetic variation combined with new demographic inference techniques can potentially yield important insights in rapidly changing ecosystems (Shafer et al., 2015).
However, there is a lack of research validating genetically-based demographic parameter estimates with detailed census-based demographic information at an ecological time scale (but see McCoy et al., 2014). Previous studies attempting to estimate recent demographic history with genetic data have been limited by the lack of temporally sampled individuals or small sample sizes, the sole use of mitochondrial DNA data or small numbers of microsatellite loci, or the indirect validation of demography using fossil evidence or simulations (Glenn, 1999; Weber, 2000). The combination of advances in sequencing technology producing large genomic datasets and the development of parameterized demographic inference models may now provide the opportunity to accurately estimate demographic parameters with smaller sample sizes and degraded museum samples (Bi et al., 2013; McCoy et al., 2014; Robinson et al., 2014). At the leading edge of these new opportunities is the need to directly validate the use of genomically-based demographic parameter estimation with known demography. However, comprehensive historic population census data are rarely available, especially in combination with documented ecological change.

Rainbow Bay (RB), an ephemeral wetland in south central South Carolina (Fig. 1), provides a unique opportunity to assess the accuracy of genetically-based demographic inferences in comparison to results based on long-term capture-mark-recapture data (CMR). Rainbow Bay was completely encircled by a drift fence in 1978, with amphibians and reptiles entering and leaving the wetland censused daily since initiation. This continuous sampling has provided a valuable time series over 37 years that has been used to document recent population expansion and decline events for multiple amphibian species. Furthermore, climatological and ecological conditions have changed significantly across this time period and have been shown to be correlated with the changing amphibian community dynamics at

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RB (Todd et al., 2010). This extensive knowledge base and the resources available at RB provide an ideal opportunity to assess the ability of genetically-based demographic inference to provide informative estimates of population demographic shifts over a very recent time scale.

Here, we focus on two salamander species exhibiting contrasting population growth trends based on CMR census data: the marbled salamander (Ambystoma opacum), which colonized the RB wetland in 1980 and expanded steadily since then, and the mole salamander (A. talpoideum), which had an established large population (>1000 females) at RB when monitoring began, and has steadily declined towards local extinction over the 37-year period (Daszak et al., 2005). These population trajectories at RB have been correlated with increased drought and shortened hydroperiod — the length of time a wetland holds water — and they illustrate the rapid response of populations to environmental change (Daszak et al., 2005).

Genetic samples have also been collected over this time period, and offer a unique opportunity to examine temporal genetic diversity. Previous work at RB employing temporal genetic sampling and microsatellite genotyping to estimate effective population size ($N_e$) and potential demographic changes for these two species failed to detect any correlation between genetic summary statistics and CMR-based census-size estimates (Nunziata et al., 2015). The increased power offered by large numbers of single nucleotide polymorphism (SNP) loci coupled with recently developed demographic inference methods may yield greater power and precision to detect these population trends at RB (Morin et al., 2009, Smouse, 2010).

The goal of this study was to compare observed climate-driven demographic trends to those inferred from analyses of genome-scale SNP data. We leveraged the multi-decade CMR data at RB and genetic samples from two temporally spaced sampling years for both A. opacum and A. talpoideum to address the following questions: (1) can recent population
growth or decline be detected using genome-wide SNP data and coalescent-based demographic inference, and (2) do non-temporally sampled data have similar power as temporal samples to detect recent population trends. Our study complements a larger body of work at this long-term study site, which has provided unique insight into amphibian population dynamics and their response to a changing climate (Pechmann et al., 1991; Daszak et al., 2005; Todd et al., 2011). Results of this study are informative in elucidating experimental design and considerations when applying demographic inference to non-model species of conservation concern, which may show rapid population-level impacts from climate or environmental change.

Methods

Population Sampling and Molecular Methods

Rainbow Bay is a seasonal wetland on the US Department of Energy’s 780-km² Savannah River Site on the Upper Coastal Plain of South Carolina (Fig. 1). It is completely encircled by a drift fence with pitfall traps, and has been censused daily for amphibians entering and leaving since the fall of 1978 (Gibbons and Semlitsch, 1982; Pechmann et al., 1991). Ambystomatid salamanders exhibit geographic structuring associated with wetlands, with breeding site fidelity (Gamble et al., 2007). This, coupled with a lack of detected population structure for both species at RB using genetic data (see Supporting Information), and detection of strong genetic structure between *A. opacum* at RB with a neighboring wetland (Nunziata et al. 2015), leads us to consider each species as a single breeding population.
We sampled tissue from twelve *A. opacum* in both 1993 and 2013, and 24 *A. talpoideum* in both 1984 and 2011. Although sample size is lower for *A. opacum*, a large number of SNP markers can provide insight into population history with limited individuals (Brumfield *et al.*, 2003; Moura *et al.*, 2014; Lanier *et al.*, 2015). All sampled individuals were metamorphic juveniles collected at the drift fence as they exited the wetland. For both species, tissues from the first sampling years were taken from individuals found dead in pitfall traps that were preserved as whole animals at -70°C. For both species, samples from the later sampling years were taken from live individuals collected in traps and tail-clipped prior to release.

Whole genomic DNA was extracted using a Qiagen® DNEasy Blood and Tissue Kit, following protocols recommended by the manufacturers. We followed the ddRAD library preparation protocol outlined in Peterson *et al.* (2012), a method that has not yet been performed in large-genome species. Ambystomatid salamanders have genomes ~10x larger than the human genome, with recent estimates at ~32 Gb (Keinath *et al.*, 2015). To account for the large genome size, the ddRAD protocol was modified from the suggested initial amount of 200-500 ng of genomic DNA per individual to instead begin with 3 μg of genomic DNA per individual. Briefly, individual in-line and indexing Illumina sequencing oligonucleotides were ligated onto DNA fragments following restriction digest with *Eco*RI and *Sph*I (Peterson *et al.*, 2012). After individual library preparation, all *A. opacum* samples from both years were pooled, while two library pools were prepared for *A. talpoideum*, one for each sampling year. All three library pools were size-selected between 338 and 414 base pairs using a Pippin Prep (Sage Science) machine, and then amplified using High-Fidelity DNA Polymerase (Bio-Rad) with 12 cycles. The three pooled ddRAD libraries were sequenced separately on three lanes of an Illumina HiSeq 2500 at Florida State University’s...
College of Medicine using 150 base-pair paired-end reads. Our first round of sequencing was performed on the *A. opacum* libraries and to increase sequence diversity in the initial portions of sequence reads we used an additional 30% spike in of PhiX control library. Subsequent to the *A. opacum* sequencing, a 1% PhiX spike in was found to be sufficient, and this level was used for both lanes of *A. talpoideum* sequencing.

**Quality Filtering and Variant Detection**

Sequences were subjected to standard Illumina chastity filtering and then assigned to individuals based on index sequences using STACKS v1.21 (Catchen et al., 2011, 2013). Reads were trimmed to remove adaptors and restriction enzyme recognition sites. STACKS was used to identify RAD loci and call SNPs in forward reads only. We set the minimum depth of identical reads to 4 (m = 4), with additional aligned reads having a maximum number of 4 mismatches (M = 4), and we allowed for a maximum of 15% missing data (across individuals) per locus. To account for potential paralogs, we filtered for highly repetitive stacks that exceeded the expected number of reads given the average depth of coverage (-t option in STACKS). To avoid inclusion of linked non-independent SNPs, only a single SNP was allowed per RAD locus.

Exclusion of missing data can potentially bias demographic inference (Huang and Knowles 2014); however, alleles present at very low frequencies could be the result of sequencing error. Therefore, we analyzed nuclear diversity under different filtering conditions to investigate the sensitivity of our results using an approach similar to Lozier (2014). The following data filtering scenarios were performed: (1) all loci with a maximum of 15% missing data per locus (as discussed above), (2) all loci with a maximum of 5% missing data per locus, (3) all loci with a minor allele frequency cutoff of 0.05 and a maximum of 5%
missing data per locus, and (4) exclusion of the two individuals from each year with the
lowest mean coverage per RAD locus, a minor allele frequency cutoff of 0.05, and a
maximum of 5% missing data per locus (Table 1). For all filtering scenarios, we ensured that
the two sample years were directly comparable by retaining only sites that passed all filter
parameters for both sampled years.

Genotype-Based Population Genetic Analyses

For both species, nucleotide diversity estimates were calculated separately for each
sampled year and were based on individual SNPs. We estimated observed heterozygosity
(Het_{obs}) and Wright’s F-statistic (F_{IS}), as calculated in the POPULATIONS program in
STACKS. To examine differentiation among sampled years, we also used POPULATIONS to
calculate pairwise F_{ST} between years. Nucleotide diversity (\pi) estimates were generated using
the program vcftools v0.1.12b (Danecek et al., 2011), and confidence intervals (CIs) were
generated by bootstrapping per-SNP \pi estimates 10,000 times in the R package boot v1.3 (R
Core Team, 2012; Canty & Ripley, 2013). To test for a genomic signal of population
expansion or decline, we calculated Tajima’s D in the program \partiala\partiali v1.6.3 (Gutenkunst et al.,
2009). Estimates of N_e were generated for each year using the linkage disequilibrium method
implemented in N_eEstimator v2.01 (Do et al., 2014). This method measures the deviation
from the expected genotype frequency based on allele frequencies in the population, which
increases in small populations due to drift (Hill, 1981).

SNP markers were screened for temporal outliers between the two sampling years for
each species using the method of Beaumont & Balding (2004) implemented in BayeScan v2.1
(Foll & Gaggiotti, 2008). This method measures the discord between global and population-
specific, or in this case, year-specific, allele frequencies. Although this method was designed

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to identify outlier loci between populations, the method has proved useful for detecting outliers between temporal samples (Therkildsen et al., 2013; See Methods and Results S1 for detailed Methods and Results).

**Genotype-Free Population Genetic Analyses**

Sequencing error and low-coverage sequences can cause incorrect genotyping when heterozygotes are incorrectly inferred as homozygotes, and vice-versa (Johnson & Slatkin, 2008). Genotyping error causes biased estimates of allele frequencies, and therefore a misrepresentation of the site frequency spectrum (SFS). To minimize these biases and serve as a complementary analysis to STACKS genotyping, we employed a likelihood-based variant detection method in ANGSD v0.700 (Nielsen et al., 2012, Korneliussen et al., 2014). The variant detection method estimates genotype likelihoods — which account for sequencing error, coverage, and alignment quality — and can be used directly in analyses instead of SNP calls. Briefly, we used the STACKS-based cstacks program to generate a consensus sequence from the highest coverage individual for both species (A. opacum: 1993_3 = 37.18x mean coverage; A. talpoideum: 2011_7 = 49.27x mean coverage). These were used as ‘RAD reference’ genomes in BWA v0.7.10 (Li & Durbin, 2009) to create alignments for other individuals. Year-specific maximum-likelihood SFSs were estimated for both species using genotype-likelihood estimation (-GL 1) in ANGSD, excluding loci missing from more than one individual and allowing a minimum base quality score of 20. The genotype-free SFS was optimized with an Expectation Maximization (EM) approach, and included only sites that were present in both sampled years so that the data sets were comparable. We calculated genotype-free genetic diversity statistics directly using the likelihood-estimated SFS, including Watterson’s theta (θ), π, and Tajima’s D.
Demographic Modeling

The influence of recent population size changes on SNP diversity was investigated with a simulation-based approach using the genotype-based SFS, implemented in fastsimcoal2 (fsc2; Excoffier et al., 2013). For both species, we generated the observed folded joint-SFS using SNP data assembled with data filter 1 discussed above, and excluding monomorphic sites (see “removeZeroSFS” option in fsc2) and any outlier loci detected with BayeScan. In order to reduce bias with allele frequency estimates, we removed all missing data and included loci found in a minimum of 10 individuals per year for A. opacum and 18 individuals per year for A. talpoideum to maximize the number of SNPs and individuals included in SFS with complete data. We simulated SFS for six basic demographic models (Fig. 2), including a model based on species-specific demographic history as estimated through CMR data (Model 1b for both species), and fit these simulated SFS to our observed SFS to generate the likelihood of the data under each demographic model. The models tested included three major models, each with two submodels: (1) RB was founded by a source population and subsequently underwent exponential population growth (positive or negative) between founding and the first sampling event \((N_e^{1984} \text{ or } N_e^{1993})\), and between both sampling events using the following equation, where \(N\) is current \(N_e\), \(N_0\) is historical \(N_e\), \(r\) is growth rate, and \(t\) is time in generations:

\[
N = N_0e^{rt}
\]

with (1b) or without (1a) migration with the source population, (2) an ancestral population founded RB and another source population with no subsequent exponential growth with (2b) or without (2a) migration with the source population, and (3) RB is a long-standing population that underwent a bottleneck in the past and has subsequently experienced population growth as estimated by the equation above (3a) or did not experience exponential growth. 

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growth (3b). All models were designed following Excoffier *et al.* (2013; full code available on Dryad). We include an unsampled source population as a source of migrations with RB, which if unaccounted for can lead to spurious signatures of demographic events (Excoffier *et al.* 2013; Malaspinas *et al.* 2016). Although these models do not encompass all possible evolutionary scenarios, they were chosen to represent the range of likely alternate evolutionary models that potentially underlie the history of these two species at RB.

Parameters estimated from models included $N_e$ of the sister population to RB ($N_e$ Source), $N_e$ at the time of sampling (A. *opacum*: $N_e^{1993}$ and $N_e^{2013}$; A. *talpoideum*: $N_e^{1984}$ and $N_e^{2011}$), immigration rates from the sister population (MIG), and the time of initiation of population size change ($T_{DIV}$). We assumed a generation time of two years for both species when converting estimates to years, based on age at first reproduction (Scott, 1994). Defined parameter ranges were uniformly distributed with $N_e$ ranging from 10 to 10,000 and $T$ from 10 to 10,000. A total of 100,000 simulations were performed to estimate the SFS, with a minimum and maximum of 10 and 100 loops (ECM cycles), respectively. The stopping criterion, defined as the minimum relative difference in parameters between two iterations, was set to 0.001. A total of 50 replicate runs were performed per model and the overall maximum likelihood (ML) was retained. The relative likelihood across compared models was generated using Akaike Information Criteria (AIC) as outlined in Excoffier *et al.* (2013). Confidence intervals for parameters in the best-supported model were obtained with a parametric bootstrap approach by simulating 100 SFS from the ML point estimates.

For each model, because we did not include monomorphic sites in the SFS or mutation rate, we fixed the time of historical sampling with other parameters estimated directly from the SFS. To assess bias in scaling estimates from this parameter, we re-estimated parameters for the ML model by fixing the current $N_e$ (A. *opacum*: $N_e^{2013}$; A. *talpoideum*: $N_e^{2011}$) using
estimates from extensive CMR data detailed in Nunziata et al. (2015). Briefly, the effective number of breeders (N_b) was calculated for each year using the equation N_b = 4N_mN_f/(N_m+N_f), where N_m and N_f are the annual census estimates of adult males and females, respectively (Hedrick, 2011). The contemporary N_e was estimated as the harmonic mean of the single-generation N_e estimates from each year since 1978 (estimated as 117 for A.opacum in 2013, 154 for A. talpoideum in 2011). To assess the impact of non-temporal sampling on model choice and parameter estimation, we also performed demographic inference for A. talpoideum using an SFS constructed exclusively from 2011 SNP data with N_e^{2011} fixed from CMR data. We performed initial demographic inference with single-season 2013 data for A. opacum, but results were largely inconsistent between model runs, and we do not present these results.

To test the impact of reduced sample size in A. opacum on parameter estimates from the SFS, we constructed the SFS for A. talpoideum using 12 randomly chosen individuals from each year using criteria outlined above for A. opacum. We then re-estimated parameters for the ML model using this reduced SFS. To estimate recent population size trends, we calculated lambda (λ) as the slope of a linear regression of the log transformation of the current and historical N_e estimates from the best-fitting demographic model. We compared these to empirical estimates of λ, calculated using estimates of N_b from census data for genetic sampling years, as calculated in Nunziata et al. (2015).

Results

ddRADseq Data

Total reads generated for each of the three library pools ranged from 180 to 270 million reads (Table S1). Initial processing of all individuals resulted in 538,628 ddRAD loci with 230,986 SNPs for A. opacum, and 845,433 ddRAD loci with 585,025 SNPs for A.
Using default filtering (filter 1), the mean depth of sequencing coverage was 20.11 (5.67 SD) for *A. opacum*, and 28.48 (8.27 SD) for *A. talpoideum* (Table S1). No outlier SNPs were detected for either species consistently across runs using BayeScan (Methods and Results S1).

**Genotype-based Diversity Statistics**

Genetic diversity estimates were similar between years for both species (Table 1). Estimates of \( \pi \) in *A. opacum* had CIs that were largely overlapping between years and were similar for all filtering scenarios (Table 1). Estimates of \( \pi \) in *A. talpoideum* were slightly higher in 2011 than 1984 (Table 1). Between species, \( \pi \) was slightly higher in *A. opacum* for all filtering scenarios and in both sampled years, which was driven by a larger proportion of intermediate frequency SNPs in *A. opacum* (Fig. S1). When a minor allele frequency cutoff was applied (Filter 3), \( \pi \) estimates increased for both species due to the exclusion of rare alleles from calculations. Both species had minimal changes in genomic diversity over the studied time spans, as revealed by Tajima’s D estimates (Table S3) and between-year F\(_{ST}\) (0.029 and 0.036 for *A. opacum* and *A. talpoideum*, respectively). For both species, estimates of \( N_e \) using NeEstimator reached infinity for each year, indicating that the signal of drift cannot be distinguished from sampling error to accurately generate \( N_e \) estimates (Waples & Do, 2010).

**Genotype-free Diversity Statistics**

After quality filtering, *A. opacum* had a total of 236,048 ddRAD loci and *A. talpoideum* had a total of 224,757 ddRAD loci. Site frequency spectra were correlated between years, and were similar in appearance to the genotype-based SFS, although they
included a lower number of rare alleles (Fig. S2). For *A. opacum*, there was a broader
distribution of per site \( \theta \) estimates in 1993 than in 2013 (Fig. 3a). Mean \( \pi \) was 2.51E-07 and
1.19E-05 in 1993 and 2013, and the distribution of per site \( \pi \) estimates were largely
overlapping between years (Fig. 3a). For *A. talpoideum*, mean \( \pi \) was 4.82E-14 and 7.46E-15
in 1984 and 2011, respectively, and distributions of per site \( \theta \) and \( \pi \) were largely overlapping
between years (Fig. 3b). These results also indicated slightly higher nucleotide diversity in *A.
opacum* than in *A. talpoideum*. These and Tajima’s D estimates (Table S3) were similar to
genotype-based analyses and collectively indicate minimal changes in genomic diversity over
these time spans. Differences in Tajima’s D observed between genotype-based and genotype-
free calculations were likely due to differences in filtering between datasets, with no
individual calculation indicating a significant departure from neutral expectations (i.e., < -2 or
> 2).

**Demographic Modeling**

For *A. opacum*, analyses of temporally-sampled data supported Model 1a (Fig. 2) as
the best fitting model with a relative likelihood of 0.961 (Table S4), which is consistent with
the interpretation that RB was founded by a source population, and has undergone expansion
until the present (Tables 2, S5). However, confidence intervals for estimates of \( N_e^{1993} \) and
\( N_e^{2013} \) were broad (Table 2), and the range of parametric bootstraps were overlapping between
years (Fig. 4). When \( N_e^{2013} \) was fixed using a CMR-based estimate, ML estimates of \( N_e \)
differed by an order of magnitude from unconstrained estimates and were not within the 95%
CIs (Table 2).
For *A. talpoideum*, analyses of temporally-sampled data supported Model 1b (Fig. 2) as the best fitting model with a relative likelihood of 1.0 (Table S6), consistent with the interpretation that RB was founded by a source population and has undergone population decline, with asymmetrical migration with the source population (Tables 2, S7). Confidence intervals for all Ne estimates encompassed point estimates, and alternate analyses using either a fixed Ne\textsuperscript{2011}, or a reduced-sample SFS (n = 12 individuals per year) provided Ne estimates on the same order of magnitude as other estimates (Table 2). The range of Ne estimates from parametric bootstraps was non-overlapping between sample years (Fig. 4). Confidence intervals for migration estimates were broad and varied among the alternate methods of parameter estimation (i.e., using a fixed Ne\textsuperscript{2011} and using a reduced-size SFS; Table 2). The best fitting model for *A. talpoideum* using the single-year SFS from 2011 supported RB being founded by a source population with subsequent population decline (Model 1a; Tables S8, S9).

In both species, estimates of λ using SFS-based genetic estimates and CMR estimates showed similar population size trajectories (Fig. 5). *Ambystoma opacum* had genetic and CMR λ estimates of 0.0417 and 0.0277, respectively. *Ambystoma talpoideum* had genetic and CMR λ estimates of -0.0637 and -0.0481, respectively.

Discussion

We leveraged temporal genomic data from a well-studied natural wetland community with 37 years of CMR data to perform demographic inference on species with documented population declines (*A. talpoideum*) and expansions (*A. opacum*) in response to changes in wetland hydroperiod. Our results provide one of the first demonstrations that coalescent-based demographic model inference based on genome-wide SNP data can potentially be informative.
in detecting trends in population size change on an ecological time scale (also see McCoy et al. 2014). For both species, model selection strongly supported histories of recent exponential population size change, with $N_e$ estimates in *A. talpoideum* producing tight confidence intervals in both sampling years that identified a clear decrease in population size, consistent with well-documented trends from CMR data. In *A. opacum*, while the SNP-based point estimates of $N_e$ produced a pattern of population growth that matched the known population expansion at RB, confidence intervals for $N_e$ estimates were broad and overlapping, tempering our confidence in detecting a demographic trend in this species. Overall, we believe the strength of our model selection results, coupled with robust parameter estimation in *A. talpoideum*, point to the potential utility of demographic inference for examining population responses on an ecological scale. We caution, however, that this may not serve as a panacea for the study of all populations, as particular evolutionary scenarios and unknown factors may challenge the recovery of robust results. We further elaborate on these issues and provide basic pragmatic advice in the following sections.

**Demographic Modeling**

For both species, SFS-based demographic inference strongly supported models of population size change, and produced similar population trajectories to census-based estimates (Fig. 5). In general, a high degree of certainty was found in the $N_e$ parameter estimates from *A. talpoideum* based on bootstrap CIs and these results were robust to the use of alternate inference procedures (models using fixed $N_e$ vs. free $N_e$), all suggesting that the SNP data were informative in elucidating population trends on an ecological time scale. The robust results in *A. talpoideum* may reflect its lower genetic diversity and the progressively increasing bottleneck (based on field data), which may have led to more pronounced signals
of recent allele frequency shifts, and more precise parameter estimates. In the only other study that we are aware of to employ SNP data and coalescent-based demographic inference on an ecological time scale, McCoy et al. (2014) were able to detect a known bottleneck event in an introduced insect population, further suggesting that histories featuring a decrease in $N_e$ may be particularly well recovered using these methods.

However, while our results point to the potential for genetically-based detection of general population trends on an ecological scale, we also note a number of challenges in the recovery of robust results. Most importantly, we highlight the difficulty here with parameter estimation. This was particularly true for estimates of migration in *A. talpoideum*, all of which produced high levels of uncertainty. It is also noteworthy that the best-fitting model for *A. opacum* did not reflect migration, which is apparent from CMR data and metapopulation structuring (Kinkead *et al*., 2007). This may be the result of sampling that was limited to a single population, without incorporating genetic data from potential source populations.

Parameter estimation in *A. opacum* further demonstrates the difficulty in demographic inference under some evolutionary scenarios. Bootstrap CIs for *A. opacum* strongly suggested that all parameter estimates carry uncertainty from the SFS-based inference procedure. Across the six tested demographic models for *A. opacum*, $N_e$-based population trajectories alternated between expansion and decline. In currently large populations that have undergone a recent expansion, like *A. opacum*, large sample sizes are likely needed to observe the rare recent variants that will be needed for SFS-based signatures of population expansion (Fu, 1997; Gravel *et al*., 2011; Robinson *et al*., 2014). This will be an issue with any demographic inference procedure employing coalescent modeling, where expansions cause an increase in deeper coalescent events in a genealogy (Kim *et al*., 2015). Ultimately, our detection of a population expansion in *A. opacum* may represent a chance correlation of genetic and census
population size trajectory. It is also possible the uncertainty in parameter estimates in *A. opacum* may be a result of poor model fit (Thomé and Carstens 2016), or that parameter estimates are reflecting patterns of an overall large and stable metapopulation.

Because a nuclear mutation rate is not known for our species (or salamanders in general), we did not include the mutation rate in our models, and instead scaled free parameters by fixing the time of historical sampling, which may create a bias as a result of the small time frame between sampling events. To address this possible bias, we re-estimated parameters using the ML model and fixed $N_e$ of the most recent sample year using estimates from CMR data. Scaling parameters from demographically estimated $N_e$ also carries bias, as we are uncertain of the true $N_e$ incorporating population structure, reproductive success, and clutch survival; but, this has been used in a similar demographic inference study without apparent bias (McCoy *et al.*, 2014). We found largely different parameter estimates between alternate scaling procedures for *A. opacum*, but similar $N_e$ parameter estimates for *A. talpoideum*, further suggesting uncertainty in *A. opacum* parameter estimates and relatively robust estimates in *A. talpoideum*. This indicates that scaling parameter estimates with temporal sampling may be a feasible option for non-model species to approximate population trajectory, but should be tested against alternate scaling strategies for robustness.

In most cases, researchers will have no *a priori* knowledge of the demographic history of a population to guide sampling designs, scale parameters, or determine the overall applicability of coalescent-based demographic inference on an ecological time scale. However, this procedure may still be of broad use in conservation studies. First, we note that many population genetic studies are focused on species of conservation concern, where populations are typically small and recently bottlenecked, or are known to be decreasing in size. These situations are the most likely to create apparent signatures in the SFS that can be
elucidated at an ecological time scale (as shown here in *A. talpoideum* and in McCoy *et al.*, 2014). Ultimately, the application of coalescent modeling to detect recent population declines will depend on the length and severity of the bottleneck, as the rate of coalescence will vary in turn, with smaller population having more coalescent events in the recent past (Hein *et al.*, 2005; Johnson *et al.*, 2007). Second, researchers should be aware of the difficulties associated with these procedures, particularly when underlying evolutionary scenarios are likely to challenge their implementation. Demographic inference analyses should incorporate thorough attempts at parameter estimation, including the assessment of confidence intervals, using the robustness of their results as a proxy for the informativeness of their data. Finally, as many researchers will not have access to temporally sampled populations, we evaluated model choice and parameter estimates using only contemporary samples for *A. talpoideum*. This analysis resulted in an alternate choice of the best model (model 1a) than when using the joint-SFS, but still supported a decline in $N_e$ from 1984 to 2011. However, relative likelihoods of tested models were much more evenly distributed across models using the single-SFS than when using the joint-SFS from both years, where likelihoods of poorer-fitting models were almost zero. These results demonstrate that the addition of temporal samples, even over a short number of generations, can greatly improve model choice, providing direct information on the magnitude of allele frequency shifts over the sampling time frame (Ramakrishnan *et al.*, 2005). Museum tissue collections and personal collections may serve as unique resources for estimating current responses of species to climate change and reconstructing population history (Bi *et al.*, 2013).
**Genetic Diversity**

*Ambystoma opacum* generally had higher genetic diversity than *A. talpoideum* using both genotype-based and genotype-free estimates, with non-overlapping CIs for estimates of nucleotide diversity for all filtering scenarios. Similar results were seen in the previous study employing microsatellite markers, providing additional support that these differences are real (Nunziata et al., 2015). Diversity differences might be due to a long-term decline of the *A. talpoideum* population at RB, as detected from field data and demographic inference. However, even historical samples of *A. talpoideum*, when population size was large, had lower genetic diversity than *A. opacum*. Overall genetic diversity differences between these species may be the result of lineage history; *Ambystoma opacum* is more broadly distributed than *A. talpoideum* (Petranka, 1998), and may exhibit higher genetic diversity due to this broader range or differences in life-history characteristics such as metapopulation structure (Leffler et al., 2012; Romiguier et al., 2014).

We observed minimal changes in temporal genomic diversity for both species with no correlation with demographic size change, highlighting the utility of demographic inference procedures to reveal patterns in genomic diversity that may not be apparent from summary statistics. This result is not surprising given the time-frame of our sampling, which was likely too short to produce large fluctuations in nucleotide diversity (Bi et al., 2013; Lozier, 2014). Diversity results were largely similar in genotype-free analyses and across filtering scenarios suggesting biases in data filtering were minor. Similar to our previous study involving microsatellites (Nunziata et al., 2015), \( N_e \) estimates from linkage disequilibrium estimates reached infinity. Both of our study species likely exist in metapopulations with migration, which is not accounted for in the linkage disequilibrium model of \( N_e \) and may confound results (Waples & Do, 2010). Sequence-based demographic inference estimates parameters
under more realistic population models, incorporating migration and variable population size, and are more likely to capture important information about population history relative to summary statistics (Drummond et al., 2005).

Conservation Concerns

The \( N_e \) estimates for *A. talpoideum* using both demographic inference and CMR data are at the lower bound of suggested \( N_e \) for maintenance of evolutionary potential of a population (Franklin & Frankham, 1998). However, RB likely exists in a larger metapopulation characterized by local extinction of some breeding wetlands and continued occupancy and population stability of others (Marsh & Trenham, 2001). Because our study focused at a single breeding wetland, we cannot infer if the \( N_e \) of the entire metapopulation is in decline or if the observed \( N_e \) decline at RB is part of a source-sink dynamic that is typical of amphibian populations. Increased droughts at RB from 1978-2004 were negatively correlated with census size in *A. talpoideum*, a species adapted to wetlands with long hydroperiods (Daszak et al., 2005). Drought is possibly impacting surrounding wetlands similarly, causing additional local extinctions of *A. talpoideum* populations, and negatively impacting metapopulation persistence. Walls et al. (2013) found an increase in local extinction rate of *A. talpoideum* at several wetlands within the southeastern Coastal Plain, likely due to increased intensity of drought. The demographic inference procedure employed in fsc2 allowed us to model an unsampled source population representing the metapopulation of wetlands RB exists within. The results indicate an overall large and stable source population; however, this has not been validated with census or genetic data. Future work will focus on sampling wetlands at multiple spatial scales surrounding RB and performing similar demographic inference.
ddRAD in Large Genome Species

The generation of large-scale SNP datasets from ddRAD sequencing is relatively inexpensive, straight-forward, and has been validated in numerous studies on small-genome (<20 Gb) taxa (e.g., Lozier, 2014; Lanier et al., 2015; Papadopoulou et al., 2015). However, this method has yet to be validated in species with larger genomes. In this study, we demonstrate the use of this procedure in two salamander species with ~32 Gb genomes, and show the scalability of the method with little knowledge of the genomic architecture of the study taxa. For both A. opacum and A. talpoideum we were able to generate thousands of SNP loci that passed filtering parameters, even with a stringent missing-data threshold of 95% of individuals across loci. The development of RADseq methods and their applicability to species with large and complex genomes is an important step forward in marker development that carries the advantages of de novo marker discovery and the removal of ascertainment bias in large-genome species where marker development has been formidable in the past (Garner, 2002; Poland et al., 2012).

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References


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Data Accessibility

SNP genotypes in vcf format, fastsimcoal2 input files: Dryad doi:10.5061/dryad.q74p1

Author Contributions

S. O. N., D. E. S, S. L. L., and D. W. W. designed the study; D. E. S. and S. L. L. collected most of the samples; S. O. N. conducted Illumina Library Prep; S. O. N. and D. W. W. analyzed the data; all authors contributed to the manuscript.

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### Tables

Table 1. Filter properties and genetic diversity statistics calculated for *A. opacum* and *A. talpoideum* juveniles at Rainbow Bay. Included are the total number of SNPs (N), the average observed heterozygosity per locus (Het\textsubscript{obs}), Wright’s inbreeding coefficient (F\textsubscript{IS}), and the average nucleotide diversity (\(\pi\)) with 95% confidence intervals obtained by bootstrapping 10,000 times.

<table>
<thead>
<tr>
<th>Species</th>
<th>Filter</th>
<th>Missing Data Allowed</th>
<th>Minimum MAF</th>
<th>Year</th>
<th># of individuals</th>
<th>N</th>
<th>Het\textsubscript{obs}</th>
<th>F\textsubscript{IS}</th>
<th>(\pi)</th>
<th>(\pi) lower 95% CI</th>
<th>(\pi) upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. opacum</em></td>
<td>1</td>
<td>15%</td>
<td>&gt; 0</td>
<td>1993</td>
<td>12</td>
<td>40326</td>
<td>0.2458</td>
<td>0.0111</td>
<td>0.2492</td>
<td>0.2475</td>
<td>0.2509</td>
</tr>
<tr>
<td><em>A. opacum</em></td>
<td>2</td>
<td>5%</td>
<td>&gt; 0</td>
<td>1993</td>
<td>12</td>
<td>15740</td>
<td>0.2121</td>
<td>0.002</td>
<td>0.2117</td>
<td>0.2090</td>
<td>0.2144</td>
</tr>
<tr>
<td><em>A. opacum</em></td>
<td>3</td>
<td>5%</td>
<td>&gt; 0.05</td>
<td>1993</td>
<td>12</td>
<td>10555</td>
<td>0.2979</td>
<td>0.0045</td>
<td>0.2972</td>
<td>0.2943</td>
<td>0.3001</td>
</tr>
<tr>
<td><em>A. opacum</em></td>
<td>4</td>
<td>5%</td>
<td>&gt; 0</td>
<td>1993</td>
<td>10</td>
<td>24793</td>
<td>0.2373</td>
<td>-0.007</td>
<td>0.2339</td>
<td>0.2317</td>
<td>0.2361</td>
</tr>
<tr>
<td><em>A. talpoideum</em></td>
<td>1</td>
<td>15%</td>
<td>&gt; 0</td>
<td>1984</td>
<td>24</td>
<td>45027</td>
<td>0.1718</td>
<td>0.0615</td>
<td>0.1905</td>
<td>0.1889</td>
<td>0.1921</td>
</tr>
<tr>
<td><em>A. talpoideum</em></td>
<td>2</td>
<td>5%</td>
<td>&gt; 0</td>
<td>1984</td>
<td>24</td>
<td>28644</td>
<td>0.1649</td>
<td>0.0306</td>
<td>0.1737</td>
<td>0.1717</td>
<td>0.1756</td>
</tr>
<tr>
<td><em>A. talpoideum</em></td>
<td>3</td>
<td>5%</td>
<td>&gt; 0.05</td>
<td>1984</td>
<td>24</td>
<td>23132</td>
<td>0.2497</td>
<td>0.0465</td>
<td>0.2633</td>
<td>0.2612</td>
<td>0.2654</td>
</tr>
<tr>
<td><em>A. talpoideum</em></td>
<td>4</td>
<td>5%</td>
<td>&gt; 0</td>
<td>1984</td>
<td>22</td>
<td>31497</td>
<td>0.1713</td>
<td>0.0396</td>
<td>0.1835</td>
<td>0.1816</td>
<td>0.1854</td>
</tr>
</tbody>
</table>

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Table 2. Maximum-likelihood (ML) demographic parameter estimates and confidence intervals (CIs) for *A. talpoideum* and *A. opacum* under the best-fitting model (*A. talpoideum*: Model1b; *A. opacum*: Model1a) from fastsimcoal2. Parameter estimates from an alternate analysis that fixed $N_e^{2011}$ and $N_e^{2013}$ (in bold) and with *A. talpoideum* SFS constructed from 12 individuals for each year, are also presented, but CIs are based off of original ML estimates. CIs were obtained by parametric bootstrapping.

<table>
<thead>
<tr>
<th>Species</th>
<th>Parameter</th>
<th>ML Estimate</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
<th>ML $N_e$ Fixed</th>
<th>ML n = 12</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. opacum</em></td>
<td>$N_e^{Source}$</td>
<td>5,613</td>
<td>2,36</td>
<td>28,4</td>
<td>22,9</td>
<td>-</td>
<td>Individuals</td>
</tr>
<tr>
<td><em>A. opacum</em></td>
<td>$N_e^{2013}$</td>
<td>17,74</td>
<td>11,4</td>
<td>41,1</td>
<td>117</td>
<td>-</td>
<td>Individuals</td>
</tr>
<tr>
<td><em>A. opacum</em></td>
<td>$N_e^{1993}$</td>
<td>2,596</td>
<td>451</td>
<td>44,7</td>
<td>46,8</td>
<td>-</td>
<td>Individuals</td>
</tr>
<tr>
<td><em>A. opacum</em></td>
<td>$N_e^{Out}$</td>
<td>228</td>
<td>50</td>
<td>7</td>
<td>412</td>
<td>-</td>
<td>Years before 2013</td>
</tr>
<tr>
<td><em>A. talpoideum</em></td>
<td>$T_{DIV}$</td>
<td>416</td>
<td>1,17</td>
<td>10,8</td>
<td>1,54</td>
<td>4,39</td>
<td>Individuals</td>
</tr>
<tr>
<td><em>A. talpoideum</em></td>
<td>$N_e^{Source}$</td>
<td>3,742</td>
<td>17</td>
<td>35</td>
<td>154</td>
<td>25</td>
<td>Individuals</td>
</tr>
<tr>
<td><em>A. talpoideum</em></td>
<td>$N_e^{2011}$</td>
<td>26</td>
<td>187</td>
<td>1,74</td>
<td>421</td>
<td>995</td>
<td>Individuals</td>
</tr>
<tr>
<td><em>A. talpoideum</em></td>
<td>$N_e^{1984}$</td>
<td>508</td>
<td>34,31</td>
<td>5,11</td>
<td>39,9</td>
<td>18,6</td>
<td>Individuals</td>
</tr>
<tr>
<td><em>A. talpoideum</em></td>
<td>$N_e^{Out}$</td>
<td>34,31</td>
<td>0</td>
<td>4</td>
<td>35</td>
<td>0</td>
<td>Individuals</td>
</tr>
<tr>
<td><em>A. talpoideum</em></td>
<td>$T_{DIV}$</td>
<td>4,806</td>
<td>2,20</td>
<td>5,72</td>
<td>4,21</td>
<td>4,73</td>
<td>Years before 2011</td>
</tr>
<tr>
<td><em>A. talpoideum</em></td>
<td>MIG into RB2011 from Source</td>
<td>2.54</td>
<td>6</td>
<td>4</td>
<td>0.03</td>
<td>0.66</td>
<td>Individuals/Generation</td>
</tr>
<tr>
<td><em>A. talpoideum</em></td>
<td>MIG into RB1984 from Source</td>
<td>1.28</td>
<td>0.00</td>
<td>7</td>
<td>0.35</td>
<td>0.31</td>
<td>Individuals/Generation</td>
</tr>
<tr>
<td><em>A. talpoideum</em></td>
<td>MIG into Source from RB2011</td>
<td>5.91</td>
<td>1.19</td>
<td>8.32</td>
<td>1.35</td>
<td>9.02</td>
<td>Individuals/Generation</td>
</tr>
</tbody>
</table>
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Figure 2. All tested demographic models for *A. opacum* (*A. opa*) and *A. talpoideum* (*A. tal*).

(1a) Rainbow Bay (RB) was founded by a source population and subsequently underwent exponential population growth. (1b) RB was founded by a source population and subsequently underwent exponential population growth, with asymmetrical migration with its source.

(2a) An ancestral population founded RB and a source population with population size remaining constant. (2b) An ancestral population founded RB and a source population, with population size remaining constant, and asymmetrical migration with source.

(3a) RB is a long-standing population that underwent a bottleneck in the past and has subsequently experienced population growth. (3b) RB is a long-standing population that underwent a
bottleneck in the past and has subsequently maintained a stable population size. Parameters estimated from models included $N_e$ of the sister population to RB ($N_e \text{Source}$), $N_e$ at the time of sampling ($A. \text{opacum: } N_e^{1993}$ and $N_e^{2013}$; $A. \text{talpoideum: } N_e^{1984}$ and $N_e^{2011}$), migration rates ($M_1$-$M_4$), and the time of initiation of population size change ($T_{\text{DIV}}$).

Figure 3. Distribution of genome-wide per-site average pairwise nucleotide diversity ($\pi$) and Watterson's theta ($\theta$) for populations of (a) $A. \text{opacum}$ from 1993 and 2013, and (b) $A. \text{talpoideum}$ from 1984 and 2011 at Rainbow Bay. Historic samples are represented in pink, contemporary samples in blue, and the overlap between historic and contemporary samples in purple.
Figure 4. Boxplots showing the median, interquartile ranges, and overall ranges of log $N_e$ estimates for 100 parametric bootstraps for (a) *A. opacum* and (b) *A. talpoideum*. Maximum likelihood point estimates under the best-fitting model (*A. talpoideum*: Model1b; *A. opacum*: Model1a) from fastsimcoal2 are shown as red dots.
Figure 5. Comparisons of estimates of the log of effective number of breeder ($N_b$) as calculated from capture-mark-recapture (CMR) data (squares), and the log of $N_e$ for the maximum likelihood model from fastsimcoal2 (circles) for (a) *A. opacum* and (b) *A. talpoideum* at Rainbow Bay. The slopes of these linear regressions were used as estimates of $\lambda$.  

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