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Authors for correspondence:
J. Michael Reed
e-mail: michael.reed@tufts.edu
Craig A. Stockwell
e-mail: craig.stockwell@ndsu.edu

[†]These authors contributed equally to this work.

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Evaluating an icon of population persistence: the Devil's Hole pupfish

J. Michael Reed[1,†] and Craig A. Stockwell[2,†]

[1]Department of Biology, Tufts University, Medford, MA 02155, USA
[2]Department of Biological Sciences, North Dakota State University, Fargo, ND 58108, USA

The Devil's Hole pupfish *Cyprinodon diabolis* has iconic status among conservation biologists because it is one of the World's most vulnerable species. Furthermore, *C. diabolis* is the most widely cited example of a persistent, small, isolated vertebrate population; a chronic exception to the rule that small populations do not persist long in isolation. It is widely asserted that this species has persisted in small numbers (less than 400 adults) for 10 000–20 000 years, but this assertion has never been evaluated. Here, we analyse the time series of count data for this species, and we estimate time to coalescence from microsatellite data to evaluate this hypothesis. We conclude that mean time to extinction is approximately 360–2900 years (median 410–1800), with less than a 2.1% probability of persisting 10 000 years. Median times to coalescence varied from 217 to 2530 years, but all five approximations had wide credible intervals. Our analyses suggest that Devil's Hole pupfish colonized this pool well after the Pleistocene Lakes receded, probably within the last few hundred to few thousand years; this could have occurred through human intervention.

1. Introduction

It is widely recognized that small populations are at greater risk of extinction than are larger populations [1]. Small population dynamics are driven by stochastic events, and populations tend to decline to extinction through a variety of feedback mechanisms sometimes referred to as extinction vortices [2–4]. Support for the idea that small populations are unlikely to persist comes from observations of native and recently established populations [5–9], and population viability analyses [10,11]. Despite widespread theoretical and empirical evidence that small, isolated populations of vertebrates should not or do not persist, there are purported counter-examples in the scientific literature [12–15]. Some authors have used such counter-examples to argue that minimum viable population size might be orders of magnitude smaller than concluded from other sources (e.g. [12,16] but see [17]). Thus, it is important to verify the persistence time for small isolated populations. Evaluating persistence times for such populations is also important for understanding the rate of evolutionary divergence. A widely cited example of a persistent, small, isolated vertebrate population is the Devil's Hole pupfish *Cyprinodon diabolis* [13,18–20], and it is on this species that we focus in this paper.

The Devil's Hole pupfish is endemic to a unique cavern-like habitat, Devil's Hole, that opened to the surface about 60 000 years ago [21]. Most researchers have assumed that pupfish were isolated in Devil's Hole 10 000 to 20 000 years ago as the local climate became drier [18]. However, hydrological evidence suggests the lack of a surface connection to Devils Hole [21–23], thus begging the questions of how and when pupfish colonized this habitat. Based on the extent to which this example is cited as a case of persistence in isolation, the Devil's Hole pupfish has reached iconic status. The timing of this colonization event, however, has not been evaluated.

Our goal was to evaluate the claim that the Devil's Hole pupfish has been isolated for 10 000 years or more. Specifically, we made the following assessments: (i) using count data since the 1970s and approximated carrying capacity, we estimated mean time to extinction (MTE); (ii) using this estimate,

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we evaluated the likelihood that this population has persisted for 10 000 years or more; and (iii) we complemented these analyses with genetic analyses of microsatellite data to estimate time of coalescence between *C. diabolis* and a closely related congener.

2. Material and methods

(a) Study system and species

Devil's Hole (36°25′31″ N, 116°17′27″ W) is deep, but Devil's Hole pupfish use only the upper 27 m of the water, with a surface area of about 0.008 ha, and all breeding occurs on a 3×6 m shelf, which is considered the smallest known habitat for any vertebrate species [24] (E. S. Gustafson 1998, unpublished master thesis). The species is 2–3 cm long, breeds once per year and lives little over 1 year [25,26]. Devil's Hole pupfish was listed as endangered because of a population decline coinciding with declining water levels from ground water removal in the late 1960s and early 1970s [27,28]. After listing, regular population monitoring was initiated with censuses conducted twice each year to capture the pre- and post-breeding population sizes [28] (electronic supplementary material, table S1). The population size recovered during the 1970s, and until recently the breeding population size (low yearly counts, pre-breeding) varied from approximately 125 to 310 individuals each year; since then, the population has varied at a smaller population size (electronic supplemental material, table S1). More recently, the population declined sharply [29,30], and intense efforts are underway to assist in population recovery.

(b) Mean time to extinction

Foley [31] presented an equation to estimate MTE from a time series of count data: $\mathrm{MTE} = (2n_0/v_r)(k - n_0/2)$. Here, $n_0 = \ln(N_0)$, the natural log of the population size at time 0, $k = \ln(\text{carrying capacity}, K)$ and v_r = variance in ln-transformed growth rate [var(r), where $r = \ln\lambda = \ln(N_{t+1}/N_t)$]. For this model to be accurate, it assumes long-term $r \approx 0$ and exponential population growth with a ceiling. We also corrected our variance estimate for autocorrelation as per Foley [31] by replacing v_r with v_{re}: $v_{\mathrm{re}} \approx ((1+\rho)/(1-\rho))v_r$, where ρ is the correlation between r_t and r_{t+1}; see [32] for validation of this approach.

We used only the low-count (spring) data for our analyses because the high-count data include a mix of adults and young-of-the-year fish. We analysed two sets of time series of low counts: 1972–2004 and 1972–2014 (electronic supplementary material, table S1). We analysed both time series because there is a significant apparent change-point after the survey in 2004 ($F_{1,40} = 72.0$, $p < 0.0001$), coincident with a trapping accident that killed about one-third of the fish [33]; this change-point might violate model assumptions. For the shorter time series, initial and ending population sizes were approximately the same. We calculated MTE for both time series for four initial population sizes, $N_0 = 100, 200, 300, 400$, and for four different carrying capacities, $K = 300, 400, 450, 500$. We used a perturbation style sensitivity analysis to determine the effects of changing k and v_{re}.

Using approximate mid-points for mean times to extinction (MTE) for the two time series, we determined the probability of persistence across a broad time frame. Foley [31] provided a method to make this calculation assuming that extinction risk occurs at a fixed rate each year and that r is normally distributed. If extinction rate (y) is fixed, then the distribution of extinction times is a negative exponential: $y = 1 - \mathrm{e}^{-x/\mu}$. Here, $\mu = \mathrm{MTE}$; from the above analysis (see Results), we selected $\mu = 2600$ years for the shorter time series and $\mu = 410$ years for the longer series. We then solved for $(1 - y)$, the probability of persistence, across a range of x years (approx. 200–20 000).

(c) When does *Cyprinodon diabolis* reach coalescence with a closely related congener?

Emerging Bayesian computational tools provide another avenue to examine time of isolation for Devil's Hole pupfish. We analysed microsatellite data (provided in [34]) using approximate Bayesian computational analyses (ABC) (DIYABC v. 2.0) [35] to estimate divergence time of *C. diabolis* from the Point of Rocks population of *Cyprinodon nevadensis mionectes*. Earlier genetic work showed that populations of *C. mionectes* are closely related to *C. diabolis* [36,37]. Our scenario also included microsatellite data for the Hoover Dam refuge population of *C. diabolis* that ultimately descended from the Devil's Hole population 5–25 generations earlier ([38]; see the electronic supplemental material, S-A). Coalescence times of Devil's Hole with the Hoover Dam refuge and Devils Hole with the Point of Rocks *C. n. mionectes* population were estimated as were the long-term effective population size (N_{e}) of all three populations.

The dataset included 12 microsatellite loci scored for 19 Devil's Hole pupfish (sampled across three time frames), 70 individuals from the Hoover Dam Refuge population of *C. diabolis* and 53 individuals from a population of *C. n. mionectes* ([29,34]; see the electronic supplemental material, S-A). Our reference table, which consisted of 1 000 000 simulated datasets, formed the basis for parameter estimation. Preliminary runs showed a good fit (simulated not significantly different from observed) was observed for only five of 11 available summary statistics. Thus, each record of the reference table was based on various combinations of those five summary statistics. We ran five sets of approximations, each of which included allele size variance (V) [39] for each population. The approximations included various combinations of the following two-population summary statistics: allele size variance, pairwise F_{ST} [40], Goldstein's $\delta\mu^2$ [41] and shared allele distance [42] (electronic supplementary material, table S2). We used the default generalized stepwise mutation model [43,44] with a mean mutation rate across loci set at 10^{-3} to 10^{-4} per locus per generation. We used uniform priors with the same ranges for population sizes used by Zegers *et al.* [34] (Devil's Hole $N = 2$–500; Hoover Dam *C. diabolis* refuge $N = 2$–500; Point of Rocks population of *C. n. nevadensis* $N = 50$–2000). We also used uniform priors for time of coalescence: 5–25 generations for Hoover Dam refuge with Devils Hole native population (see the electronic supplementary material, S-A) and 75–20 000 generations for *C. diabolis* with *C. n. mionectes* (*C. diabolis* was described in 1930, approx. 75 years before the samples were collected).

3. Results

(a) Mean time to extinction

MTE estimated from the 1972–2004 data across our different starting conditions (K and N_0) was 2370–2918 years, with a midpoint of approximately 2600 years (figure 1). MTE estimated from the 1972–2014 data was much shorter: 365–450 years, with a median of approximately 410 years. For both time series, MTE increased with initial population size although the differences were small (approx. 3.5–7.1%) (figure 1). MTE was more affected by increases in carrying capacity, which increased MTE approximately 15–19%. To further explore the sensitivity of MTE to carrying capacity, we determined K, assuming MTE was equal to 10 000 years. For the 1972–2004 series, we found that if we set $N_0 = 400$, K would have to be approximately 1 100 000 ($\ln(k) = {\sim}14$) for MTE to be approximately 10 000 years. For the longer time series, using the same values for MTE (10 000 years) and N_0 (400), K would

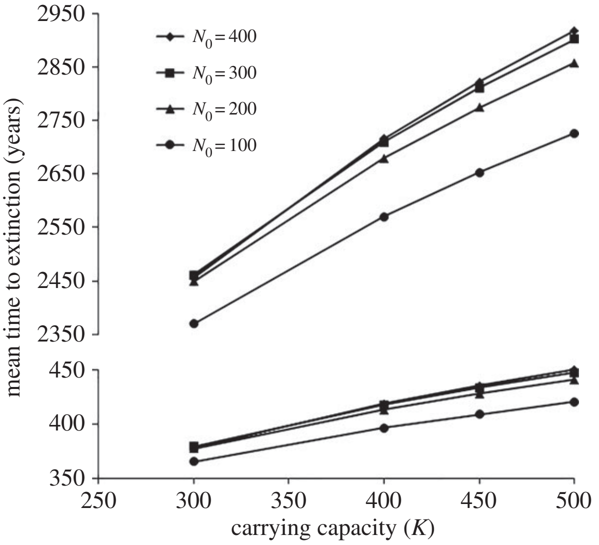

Figure 1. Estimated MTE for four initial population sizes (N_0) and four carrying capacities (K). Estimates are made using two time series from the lower of the two annual population counts: 1972–2004 produced the upper lines and 1972–2014 produced the lower lines.

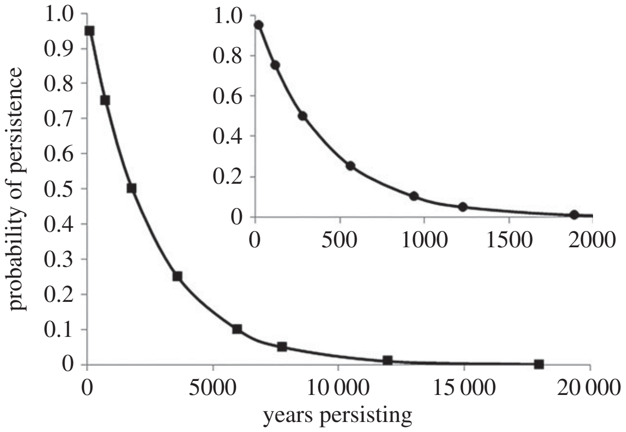

Figure 2. Estimated probability of persistence over time for Devil's Hole pupfish. The larger graph comes from the 1972–2004 time series and an MTE of 2600 years. The smaller graph comes from the 1972–2014 data and MTE = 410 years.

have to be orders of magnitude larger. Both analyses show that K would have to be orders of magnitude larger than the largest fish count for Devil's Hole (late-season count of 553) to have credible likelihood of persisting 10 000 years. We also evaluated how reduced v_{re} may affect persistence time. If we set carrying capacity for the shorter time series at 500 individuals, higher than any of the low-population size counts recorded, and $N_0 = 200$, v_{re} would have to be approximately 0.0035 for MTE to be approximately 10 000 years, almost an order of magnitude smaller (on a log scale) than that observed. Again, v_{re} would have to be much smaller to achieve the same effect with the longer time series.

Using the 1972–2004 data, we estimated that the probability of Devil's Hole pupfish persisting 10 000 years is 2.1% if MTE = 2600 years, and a median time to extinction [$= \mu \times \ln(2)$] of 1800 years (figure 2). We further estimated that there is an approximate 68% probability of a population having persisted 1000 years and that a 95% probability coincides with only 133 years. Using the complete time series, the probabilities for Devil's Hole pupfish persisting 10 000 years and 1000 years is 2×10^{-11} and 8.5%, respectively, while there is a 95% probability of persisting 21 years.

(b) Time to coalescence

The five simulations provided relatively consistent estimates for long-term effective population sizes. All approximations produced high median population sizes for Devil's Hole pupfish (389–483), which were constrained by setting the upper prior at $N_e = 500$. The median effective population sizes for the Hoover Dam refuge of Devil's Hole pupfish were all relatively small, 23–59, whereas the *C. n. mionectes* median population size estimates varied from 194 to 1540 (table 1). Median times of divergence for the Hoover Dam refuge population of *C. diabolis* and the native population of *C. diabolis* varied from 14 to 21 generations, whereas the median times of divergence between *C. diabolis* and *C. n. mionectes* varied from 217 to 2530 generations (=years) (table 1; electronic supplementary material, figure S1).

4. Discussion

Our results suggest that assertions in the literature about the long-term persistence of the Devil's Hole pupfish (see Introduction) have been overestimated and that the persistence of *C. diabolis* to modern times should be viewed more modestly. Based on our analyses, likely persistence time of the isolated population of Devil's Hole pupfish is well short of the 10 000–20 000 years asserted in the literature. Using population size data and its variability, we estimate only a 50% probability of persisting approximately 410–1800 years, depending on which portion of the time series is used; if one wants a higher probability, say 90% chance of persisting, the value drops to 30–275 years. These results were consistent with our analyses of the limited microsatellite data available, which showed median coalescence times of *C. diabolis* with *C. n. mionectes* ranged from a few hundred to a few thousand years. Note that this assessment assumes that observed stochasticity in the time series is representative of population sizes from the past to the present time. As the Devil's Hole pupfish has exhibited a much smaller population size since 2004 [29,30], its likelihood of persistence into the future is at the lower end of our projections because of its current smaller population size. Furthermore, this species should not be used as a general measure of minimum viable population size or as justification for protecting vertebrate populations at sizes that are inadequate for long-term persistence [17].

As the water in Devil's Hole has been isolated from other water sources for 60 000 years [21], we conclude that pupfish colonized this habitat in the last few hundred to few thousand years. This assertion is supported by the observations that Devil's Hole water levels were exceptionally low during the Altithermal (6000 years ago) and that population size is correlated with water level [28]. The water level decline in the early 1970s led to the near extinction of *C. diabolis* [27,28], yet the duration of the water decline during the Altithermal probably lasted much longer [28], suggesting that persistence of *C. diabolis* during the Altithermal seems doubtful.

Our analyses do not speak to the mode of colonization, but others have suggested that colonization could have been through subterranean waters or introduction over dry land [22,28,45]. A subterranean colonization would probably have involved pupfish from the nearest populations of Warm Springs pupfish (*Cyprinodon nevadensis pectoralis*); however, genetic analyses show *C. diabolis* to be more closely related to *C. n. mionectes* [46]. The overland colonization hypothesis is intriguing and infers a provocative sub-hypothesis that Native Americans

Table 1. Median effective population size (N_e) estimates (and 95% credible intervals) and estimated time of divergence (years) from each of five ABC approximations of the posteriors.

effective population size (N_e)					
population	approximation-1	approximation-2	approximation-3	approximation-4	approximation-5
C. diabolis (natural)	478 (399–499)	389 (147–496)	483 (418–500)	394 (151–495)	477 (394–499)
C. diabolis (refuge)	33 (6–312)	24 (5–287)	24 (5–261)	23 (18–278)	59 (7–411)
C. n. mionectes	1510 (902–1950)	1050 (295–1940)	1540 (937–1950)	1080 (299–1930)	194 (65–795)
divergence time between native population of *C. diabolis* and Hoover Dam refuge population					
	16 (6–25)	19 (7–25)	14 (5–25)	19 (7–25)	21 (7–25)
divergence time between native populations of *C. diabolis* and *C. n. mionectes*					
	1780 (447–10 600)	217 (90–815)	2200 (541–12 400)	223 (91–774)	2530 (620–14 400)

may have intentionally or unintentionally introduced pupfish to Devil's Hole; see [47] for a discussion on prehistoric translocation of fishes. In fact, Native Americans used pupfish as a food source in the nearby locations of Death Valley and Owens Valley [48,49], so this hypothesis seems reasonable.

Although Devil's Hole pupfish is renowned for its low level of genetic diversity [36,37], more detailed genetic data would improve estimates of isolation time. Our estimates using Bayesian computational methods [35] to estimate time of coalescence varied from 217 to 2530 generations (years), but the same analyses provided unusually high estimates for the effective population size of the Devil's Hole population. Furthermore, the only approximations that would fit the data used a limited battery of the available summary statistics.

One might argue that isolation might be longer than our predictions because *C. diabolis* is a recognized species that presumably speciated since its isolation in Devils Hole. However, recent observations and experimental work suggest that observed divergence could be due to rapid evolutionary divergence and/or phenotypic plasticity. For instance, artificial refuge populations of *C. diabolis* showed rapid phenotypic divergence from the source population at Devil's Hole [50,51]. Furthermore, the Devil's Hole pupfish traits have been experimentally induced in a congener (*Cyprinodon nevadensis amargosae*) by restricting diet and increasing water temperature to mimic Devil's Hole environmental conditions [52]. In a similar study system, Collyer *et al.* [53] showed rapid body shape evolution for a refuge population of White Sands pupfish. The evolutionary divergence of the refuge population occurred over a 30 year period and exceeded the divergence between two native populations that had been isolated for 3000–5000 years [53,54]. Collectively, these observations show the morphological divergence of *C. diabolis* could have occurred over decades. Thus, morphological divergence *per se* may tell us little about time of isolation.

Molecular analyses using mt-DNA have shown a lack of recriprocal monophylly between *C. diabolis* and the various populations of the two subspecies of *C. nevadensis* [36,46]. These researchers have suggested that incomplete lineage sorting could explain the lack of reciprocal monophyly between *C. nevadensis* and *C. diabolis*. Thus, the mt-DNA molecular data provide little insight regarding time of isolation for *C. diabolis*.

Based on our analyses, we conclude that rather than being an icon of unusual persistence in isolation, the Devil's Hole pupfish has only persisted a few hundred to a few thousand years. Nevertheless, its persistence is still noteworthy as several hundred individuals might not be expected to persist this long (e.g. [10,11,20]). Because of its recent population decline [29,30], its likelihood of persistence (if unmanaged) has declined. Our analyses do not address alternative current management recommendations for the Devil's Hole pupfish but suggest that time might be short for saving this species. This pupfish is a recognized endangered species that is protected by law, and many efforts have been made to protect the species (e.g. [24,27,29,30]). Our results, however, do suggest that assertions in the literature about the long-term persistence of the Devil's Hole pupfish (see Introduction) have been overestimated and that the persistence of *C. diabolis* to modern times should be viewed more modestly. Furthermore, this species should not be used as a general measure of minimum viable population size or as justification for protecting vertebrate populations at sizes that are inadequate for long-term persistence [17].

Data accessibility. Data used in demographic analyses are found in the electronic supplementary material, table S1. The microsatellite data come from [34].

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Supplemental Supporting Information for

Evaluating an icon of population persistence: The Devil's Hole pupfish

J. Michael Reed and Craig A. Stockwell

Supplement A

Methods: When does *C. diabolis* reach coalescence with a closely related congener?

We analyzed microsatellite data (provided in [35]) using Approximate Bayesian Computation (DIYABC v. 2.0; [36]) to estimate divergence of *C. diabolis* from a population of *C. nevadensis mionectes.* The data set included 12 microsatellite loci scored for 19 Devil's Hole pupfish (sampled across three time frames), and 70 individuals from the Hoover Dam Refuge population of *C. diabolis* (one sample) and 55 individuals from the Point of Rocks population of *C. nevadensis mionectes* (one sample). The original Devil's Hole sample included 20 fish from three sample periods, but Zegers et al. [35] reported evidence that one of the fish was of suspect origin, thus we used only 19 individuals.

We conducted a preliminary set of simulations with a data set that was reduced by limiting the *C. diabolis* population to a sample of 10 individuals that were all collected in 1994. We also limited this second set of simulations to 9 loci, because earlier workers showed linkage dis-equilibrium between three pairs of loci [35]. The second set of analyses produced very similar results to the analyses including all 19 *C. diabolis* and all 12 markers, thus we report the analyses using the larger data set.

History of the *C. diabolis* refuges

Since 1972, *C. diabolis* has been managed intensively, in part by establishing refuge populations [39,S1]. Recently, [39] presented the history of 3 refuge populations that were extant in 2002; all three refuge populations were lost by 2007. However, during their existence, these populations were studied and sampled, including genetic sampling of the Point of Rocks and Hoover Dam refuges in 2005 [30,35]. We used genetic data from the Hoover Dam population because its lineage can be traced back to Devils Hole as early as 1980, 25 years prior to the samples examined by [35].

The refuge populations have a complicated history that involved original stocking, periodic bottlenecks (or extinction), often followed by additional supplementation (or restocking) [39,S1]. The School Spring population was originally established in 1980 with 24 *C. diabolis* individuals from Devils Hole, and supplemented with another 6 *C. diabolis* individuals from Devils Hole in 1991 [39] . The Point of Rocks Refuge population was established with pupfish from the School Spring Refuge (16 fish in 1991 and 16 fish in 1992) and was supplemented with *C. diabolis* from Devils Hole in 1994 (6 individuals) and 2006 (12 individuals)

[39]. Finally, The Hoover Dam Refuge was established in 1998 with 10 *C. diabolis* individuals from the School Spring Refuge and 11 *C. diabolis* individuals Point of Rocks Refuge. This population was then supplemented with 20 *C. diabolis* in 2000 (10 individuals from each of the other two refuges).

Although the Point of Rocks population was compromised by admixture with *C .n. mionectes*, the admixture was apparently restricted to this refuge population, as PCA showed that the Hoover population grouped with the native Devils Hole population, and both were distinct from the Point of Rocks Refuge population [30, 35].

Supplemental Reference

S1. Baugh T, Deacon JE. 1988. Evaluation of the role of refugia in conservation efforts for the Devils Hole pupfish, *Cyprinodon diabolis* Wales. Zoo Biol. 7**,** 351-358.

Year	Low Count
1972	127
1973	184
1974	143
1975	148
1976	180
1977	198
1978	219
1979	179
1980	165
1981	237
1982	193
1983	218
1984	143
1985	164
1986	missing
1987	180
1988	236
1989	223
1990	226
1991	215
1992	238
1993	226
1994	310
1995	217
1996	252
1997	164
1998	227
1999	197
2000	181
2001	159
2002	161
2003	124
2004	123
2005	84
2006	38
2007	38
2008	45
2009	70
2010	121
2011	104
2012	63
2013	35
2014	92

Table S1. Survey results for Devil’s Hole pupfish. There are two surveys each year, with a systematic difference between the two because of the pattern of reproductive timing (i.e., higher counts come post reproduction). We used only the low-count data (before breeding) as they were not confounded by that year’s reproduction. Data depicted are those used in the demographic analyses, and they were provided by the U. S. Fish and Wildlife Service.

Table S2. Observed summary statistics compared to simulated summary statistics for the 5 DIY-ABC approximations.

	Observed Summary Statistics[+]			**Approximation-1**			**Approximation-2**		
One Population Summary Statistics									
	C. n. mionectes	*C. diabolis* Hole	*C. diabolis* Refuge	*C. n. mionectes*	*C. diabolis* Hole	*C. diabolis* Refuge	*C. n. mionectes*	*C. diabolis* Hole	*C. diabolis* Refuge
Allele Size Variance	1.513	1.673	1.441	√	√	√	√	√	√
Two Population Summary Statistics									
Allele Size Variance									
C. n. mionectes									
C. diabolis - Hole	2.251			√			√		
C. diabolis - Refuge	2.510	1.597		√	√		√	√	
F_{ST}									
C. n. mionectes									
C. diabolis - Hole	0.117			√*			√*		
C. diabolis - Refuge	0.222	0.156		√*	√		√*	√	
Goldstein's $\delta\mu^2$									
C. n. mionectes									
C. diabolis - Hole	4.223			√			√		
C. diabolis - Refuge	4.200	0.546		√	√*		√	√*	
DAS[1]									
C. n. mionectes									
C. diabolis - Hole	0.038			√					
C. diabolis - Refuge	0.026	0.156		√	√*				

[+] Observed Summary Statistics – to which simulations are compared

*simulated significantly different from observed.

[1] DAS – shared allele distance between populations i and j

Table S2. Continued.

	Approximation-3			**Approximation-4**			**Approximation-5**		
	One Population Summary Statistics								
	C. n. mionectes	*C. diabolis* Hole	*C. diabolis* Refuge	*C. n. mionectes*	*C. diabolis* Hole	*C. diabolis* Refuge	*C. n. mionectes*	*C. diabolis* Hole	*C. diabolis* Refuge
Allele Size Variance	√	√	√	√	√	√	√	√	√
	Two Population Summary Statistics								
Allele Size Variance									
C. n. mionectes									
C. diabolis - Hole	√			√			√		
C. diabolis - Refuge	√	√		√	√		√	√	
F_{ST}									
C. n. mionectes									
C. diabolis - Hole	√*			√*					
C. diabolis - Refuge	√*	√		√*	√				
Goldstein's $\delta\mu^2$									
C. n. mionectes									
C. diabolis - Hole				√					
C. diabolis - Refuge				√	√*				
DAS[1]									
C. n. mionectes									
C. diabolis - Hole	√						√		
C. diabolis - Refuge	√	√*					√	√*	

*simulated significantly different from observed.

[1] DAS – shared allele distance between populations i and j

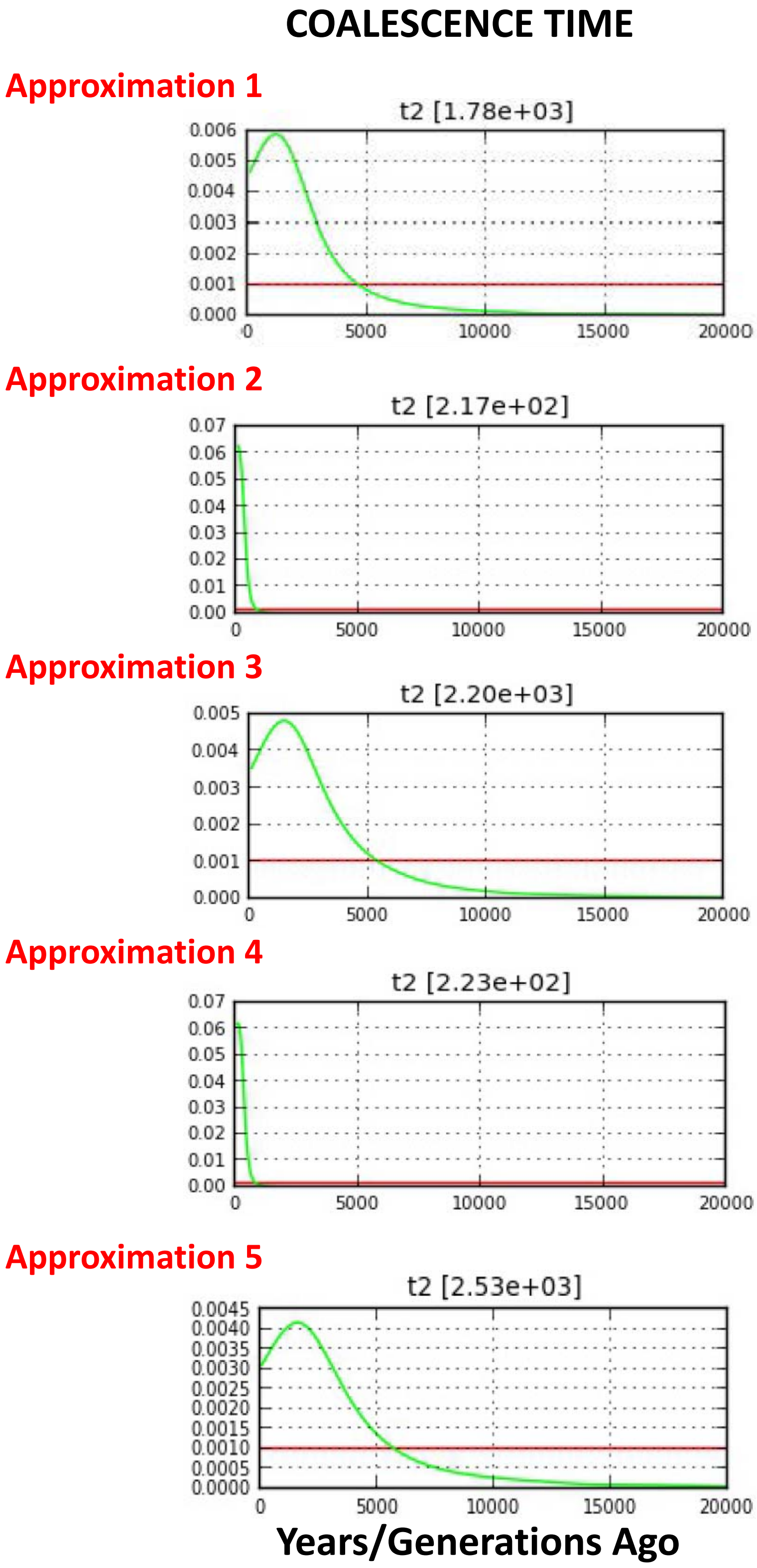

Figure S1
DIYABC posterior probability distributions (green lines) and uniform priors (red lines) coalescence time between *Cyprinodon diabolis* and *C. nevadensis mionectes* for 5 approximations using different combinations of summary statistics; median in brackets (Table S1).