Evol Ecol (2014) 28:141–155
DOI 10.1007/s10682-013-9668-5

ORIGINAL PAPER

Rapid evolution in response to increased temperature maintains population viability despite genetic erosion in a tropical ectotherm

Ross D. Breckels · Shawn R. Garner · Bryan D. Neff

Received: 25 June 2013 / Accepted: 20 August 2013 / Published online: 30 August 2013

Abstract Climate change is predicted to increase the average global air temperature by up to 4.0 °C by the end of the century. This increased temperature could have negative effects on many life history traits that are closely linked to fitness. Many species will therefore have to adapt to the warmer environment, but life history traits often have limited additive genetic variance. Here, we investigated population demographics and the evolutionary response of life history traits, as well as genetic diversity in guppies (*Poecilia reticulata*), in response to an experimentally increased temperature. There were fewer successful pregnancies, smaller brood sizes, and males matured earlier at a higher temperature as compared to control populations. However, there was no sign of an evolutionary response in these traits after 24 months of exposure to the increased temperature. We also found that population size, brood survivorship, sex ratio, and male length at maturity were unaffected by the increased temperature. Genetic diversity decreased rapidly in the increased temperature populations at a rate equivalent to an effective population size of only one quarter of the controls, revealing a clear signature of selection in response to increased temperature. This genetic erosion, however, could hamper the adaptive potential of the populations to other environmental changes associated with climate change.

Keywords Life history traits · Population demographics · Global warming · Genetic diversity · Effective population size · Temperature-size rule · *Poecilia reticulata*

Electronic supplementary material The online version of this article (doi:10.1007/s10682-013-9668-5) contains supplementary material, which is available to authorized users.

R. D. Breckels · S. R. Garner · B. D. Neff (✉)
Department of Biology, University of Western Ontario, London, ON N6A 5B7, Canada
e-mail: bneff@uwo.ca

R. D. Breckels
e-mail: rbreckel@uwo.ca

Introduction

Global warming is projected to increase the average global temperature by 1.8–4.0 °C by the end of the century, and the rate of warming is expected to become increasingly rapid over the coming decades (IPCC 2007). In response to this warming, many species will have to disperse or adapt, or face the risk of extinction (Fuller et al. 2010). However, for many species, dispersal to new, favourable habitats is unfeasible, so they will have to respond to their current habitat via phenotypic resiliency or via genetic adaptation. Phenotypic resiliency enables individuals to survive in a range of environments and might involve phenotypic plasticity, when an individual displays different phenotypes in different environments, or canalisation, when an individual displays the same phenotype in different environments. New genetic adaptations, on the other hand, can arise from natural selection on favourable traits (including selection for adaptive phenotypic plasticity) and can occur via selection acting on new mutations or standing genetic variation (Barrett and Schluter 2008). Phenotypic resilience is a faster response than genetic adaptation because it can occur in an individual's lifetime. However, there are advantages to genetic adaptation over phenotypic resiliency, as maintaining plasticity or canalisation often incurs a fitness cost and plasticity often is limited in scope (see DeWitt et al. 1998). Additionally, many species, particularly tropical ectotherms, are living close to their thermal limits and will have to rely on genetic adaptation to respond to global warming (Angilletta 2009).

Life history traits are a major determinant of both individual and population fitness and may be an especially important target for selection and genetic adaptation in response to a warming environment (reviewed in Crnokrak and Roff 1995; Roff and Emerson 2006). Life history traits include individual growth rate, age and size at maturity, reproductive investment, such as brood or clutch size, sex ratio, and survivorship (Stearns 1992). Recent evidence indicates that increases in temperature have negative impacts on many of these traits (e.g. guppies, *Poecilia reticulata* Dzikowski et al. 2001; Karayücel et al. 2008; neotropical pseudoscorpions, *Cordylochernes scorpioides* Zeh et al. 2012; and grayling, *Thymallus thymallus* Wedekind et al. 2013). For example, the effects of short-term increases in temperature on life history traits include reduced successful parturition (e.g. Karayücel et al. 2008; Zeh et al. 2012), reduced brood sizes (e.g. Dzikowski et al. 2001; Karayücel et al. 2008), decreased survival (e.g. Zeh et al. 2012), and altered sex ratios resulting from sex-specific differences in survival (e.g. Karayücel et al. 2008; Wedekind et al. 2013). However, because life history traits are so closely linked to fitness, they often have little additive genetic variance and therefore cannot respond to selection, at least until new mutations arise (reviewed in Crnokrak and Roff 1995; Roff and Emerson 2006). On the other hand, more recently there has been evidence for cryptic genetic variation in many traits (reviewed in Gibson and Dworkin 2004), which is expressed as a result of changes in the environment (i.e. genetic variation among individuals in phenotypic plasticity). Thus, some life history traits may show higher levels of additive genetic variance in a warmer environment, which could help species respond to global warming and could facilitate evolutionary adaptation.

Of particular relevance to global warming is the temperature-size rule which is a taxonomically widespread relationship between temperature and life history traits (Atkinson 1994; Angilletta 2009). According to this rule, for ectotherms, age at maturity and size at maturity decrease with increasing temperature (Atkinson 1994). This rule can be largely explained by a direct effect of the environment on physiological processes, which are dependent on the ambient temperature in ectotherms. Increased temperature leads to a phenotypically plastic response of earlier maturation because it causes more rapid cell division and differentiation, and smaller size at maturity when the rate of cell

division and differentiation exceeds the rate of growth (van der Have and de Jong 1996; Angilletta et al. 2004). The specific relationship between size or age at maturation and temperature (i.e. the reaction norm) for a population or species is generally understood to be genetically controlled and influenced by the relationship between size and reproductive success, as well as selection from ecological factors such as predator–prey interactions and competition (Neuheimer and Taggart 2007; Daufresne et al. 2009). Global warming may therefore push populations off their optimum trait value for size or age at maturation until selection can act on new mutations or existing genetic variation in the reaction norm.

Here, we use the Trinidadian guppy (*P. reticulata*, Peters 1860) as a model ectotherm to determine the effects of multi-generational exposure to an elevated temperature. We exposed replicate experimental populations of guppies over 2 years (approximately 8 generations) to the temperature predicted for the end of the century, and measured multiple life history traits and population demographics, as well as levels of genetic diversity. Specifically, we measured population size, the number of successful pregnancies, brood size, brood survivorship, sex ratio, age and length at sexual maturation, and genetic diversity using microsatellite loci. We compared these traits to control experimental populations and partitioned variation between phenotypic plasticity and genetic responses.

Methods

Study species

Guppies inhabit shallow pools in streams and rivers of north-eastern South America and the Caribbean (Houde 1997). Currently, the mean water temperature in Trinidad is approximately 25 °C with annual fluctuations between 20 and 28 °C (Alkins-Koo 2000; Grether et al. 2001). Trinidad is projected to have an average air temperature increase of 1.0–3.5 °C by the end of the century (Water Resources Agency 2001), which will likely result in similar increases in water temperature (e.g. Stefan and Preudhomme 1993; Caissie et al. 2001; Kaushal et al. 2010). The current natural variation in temperature experienced by guppies may mitigate the potential negative effects of global warming, yet they rarely experience temperatures of 28 °C for prolonged periods of time. Thus, the predicted temperature increase for the end of the century could be detrimental to guppies.

Guppies are sexually dimorphic, and males can be differentiated from females after 5–6 weeks as their anal fin develops into a rod like structure known as a gonopodium (Houde 1997). Males are mature when the gonodopial hood extends beyond the main part of the gonopodium, which typically occurs at approximately 7 weeks (49 days) of age or younger (Houde 1997; Reznick et al. 2001). Breeding occurs throughout the year and females have a gestation period of approximately 3–4 weeks (Houde 1997). Generation times in guppies have been estimated to range between 1.5 and 6.9 months (e.g. Endler 1980; Reznick et al. 1997).

Experimental set-up

Fish used in this experiment were descendants of fish collected from a tributary of the Paria River, Trinidad, in 2003. Guppies were held at the Freshwater Ecology Research Facility at the University of Western Ontario in tanks lined with gravel and artificial plants. Fish were kept on a 12:12 h light–dark cycle and fed twice daily, once with Tetramin® flake food and

once with brine shrimp. The water temperature was set to 25 °C to match the current temperature in natural streams (Alkins-Koo 2000; Grether et al. 2001).

On May 1, 2010, six, 250 L tanks, with the water temperature set to 25 °C, were seeded with 55 adult fish (25 males and 30 females). We kept the water temperature in three of the tanks (experimental populations) at 25 °C [±0.5 °C (SD); controls] throughout the experiment. In the other three experimental populations, the temperature was raised to 28 °C [±1.2 °C (SD)] at a rate of 1 °C every 45 days. The temperature in each tank was monitored with HOBO® temperature loggers (Onset Computer Corporation, MA, USA). Population size was counted three times for each population (repeatability $r^2 = 0.994$; $F_{23,71} = 712$, $p < 0.001$) every 6 months up until 24 months.

At 6, 18, and 24 months, eight gravid females (indicated by having darker anal regions and enlarged abdomens; Houde 1997) were removed from each experimental population and placed into individual 10 L rearing tanks until they gave birth. The water temperature was set to the same temperature as each female's original experimental population. Approximately 24 h after a female had given birth she was returned to her original experimental population so that only her offspring remained in the rearing tank. If the female did not give birth within 2 months, she was replaced by a new female from the same experimental population. If the second female did not give birth within 2 months, she was not replaced and no data were collected from that rearing tank.

We created four treatments in a 2 × 2 common garden experimental design by switching the temperature in half of the rearing tanks after the female was removed, and keeping the remaining half of the rearing tanks at the same temperature to act as controls. Thus, we created four treatments (see supplementary material): (1) *control* (25–25), fish from a 25 °C experimental population that were reared at 25 °C; (2) *25–28*, fish from a 25 °C experimental population that were reared at 28 °C; (3) *28–28*, fish from a 28 °C experimental population that were reared at 28 °C; and (4) *28–25*, fish from a 28 °C experimental population that were reared at 25 °C. From the fish in these 48 rearing tanks (8 rearing tanks × 6 experimental populations) we estimated life history traits at each time point (6, 18, and 24 months) as outlined below.

After conducting the 18 month trial, to examine maternal versus genetic effects on the offspring traits, we also generated an F_2 generation of the fish from the *28–25* treatment by using a simple breeding design. Briefly, this design used fish from the four *28–25* rearing tanks from each 28 °C experimental population. Females were separated from the males before maturation. After the life history measurements were completed (approximately 4 months of age), these virgin females were paired with a male from the same experimental population, but a different family to avoid inbreeding. After a 3 day copulation period, the males were removed, leaving just the female in the rearing tank. Once the female had given birth, she too was removed, leaving just the offspring. The water temperature remained at 25 °C throughout, and the life history traits were measured on the offspring (i.e. the F_2 generation).

Demographics and life history traits

We measured the number of successful pregnancies, brood size, brood survivorship, and sex ratio for the broods in each rearing tank. For the male offspring in each brood, we also measured male age and body length at maturation. The number of successful pregnancies was calculated as the number of females that produced a brood within 2 months. Brood size was calculated by counting the number of offspring that each female produced within the first 24 h of birthing her first offspring (females that did not produce broods were not

included in this analysis). Brood survivorship was calculated as the proportion of the offspring born in a rearing tank that survived to 3 months of age. Sex ratio was calculated as the proportion of each brood that were male (determined at 3 months of age when all fish had reached maturity). Male age at maturity was calculated as the number of days from birth until a given male offspring first reached sexual maturity. At maturation, we also measured male body length from the tip of the snout to the end of the caudal peduncle.

Genetic analysis

At the baseline and every 6 months, fin clips were taken from 30 adults per experimental population and stored in 95 % ethanol for microsatellite analysis of genetic diversity. DNA was first isolated from each fish using a proteinase K digestion (Neff et al. 2000). Eight previously described microsatellite loci were then PCR amplified for each individual (*Pr36*, *Pr39*, *Pr80*, *Pr92*, and *Pr171*; Becher et al. 2002 and *Pre8*, *Pre9*, and *Pre17*; Paterson et al. 2005). The resulting microsatellite products were visualised using an ABI 3730S DNA analyzer and manually sized using GENEMAPPER v. 4.0 (Applied Biosystems).

We checked for linkage disequilibrium between pairs of loci using GENEPOP v. 4.1 (Rousset 2008) at each time point, resulting in 840 comparisons; a Bonferroni correction method was used. We checked for the presence of non-amplifying ('null') alleles using MICRO-CHECKER v. 2.2 (van Oosterhout et al. 2004). Null alleles were detected in our data, so we used FREENA (Chapuis and Estoup 2007) to correct the allele frequencies. Next, for the loci without null alleles, we assessed whether each locus from each experimental population at each time point was in Hardy–Weinberg equilibrium (HWE) using GENALEX v. 6.5 (Peakall and Smouse 2012), again applying a Bonferroni correction. Allelic richness was also estimated at these times as the average number of alleles observed at the eight microsatellite loci based on the sample of 30 fish. Finally, we estimated Nei's standard genetic distance between the experimental populations at each time point using GENALEX.

Assessing a signature of selection

We used a simulation approach to determine if declines in allelic richness over the course of the experiment could be explained by genetic drift, given the observed population sizes in each tank. The simulations were run using customized Visual Basic macros for Excel (Microsoft 2007) and are available from the authors upon request. We used the combined allele frequencies across all six tanks at the initial time point, correcting for and incorporating the null alleles, to seed our simulated populations. To mirror our experimental design, we then simulated six replicate populations of 25 males and 30 females with genotypes chosen at random based on the initial allele frequencies. We first modeled the behaviour of each population assuming random mating in each generation, with the parents for each individual chosen at random from all individuals of the appropriate sex in the previous generation. The sex of each offspring was assigned probabilistically based on a 45 % male sex ratio (see results; Table 1). The population size of the simulated populations was altered between generations to match the observed values in the experimental populations. We assumed non-overlapping generations and a 3 month generation time (similar results were obtained with a 6 month generation time). For each simulated population, we sampled 30 individuals at each time point to calculate allelic richness as in the experimental populations. We then repeated this simulation 1,000 times to produce an expected distribution of allelic richness from which the 99 % confidence intervals in the

Table 1 Metrics for the families used in analyses of life history traits in the guppy (*Poecilia reticulata*)

Variable	Treatments				
	Controls	*25–28's*	*28's*	*28–25's*	*F_2's*
6 month					
No. of families	12	12	11	7	
Brood size	6.7 ± 5.8	4.2 ± 2.8	4.9 ± 3.0	6.6 ± 5.9	
Brood survivorship (%)	89.2 ± 13	93.8 ± 12	90.2 ± 23	95.4 ± 6.6	
Sex ratio (% males)	36.4 ± 21	43.2 ± 29	48.1 ± 30	32.6 ± 40	
18 month					
No. of families	12	9	12	8	10
Brood size	4.3 ± 3.4	4.4 ± 2.3	5.6 ± 4.1	4.0 ± 3.0	4.4 ± 2.8
Brood survivorship (%)	87.5 ± 31	90.1 ± 13	84.4 ± 31	100 ± 0.0	86.4 ± 30
Sex ratio (% males)	38.0 ± 28	42.9 ± 33	46.7 ± 22	51.9 ± 29	44.8 ± 38
24 month					
No. of families	11	12	9	7	
Brood size	7.9 ± 4.0	4.5 ± 3.1	2.1 ± 0.8	3.6 ± 2.5	
Brood survivorship (%)	88.4 ± 30	91.0 ± 17	96.3 ± 11	90.5 ± 19	
Sex ratio (% males)	40.5 ± 21	42.6 ± 29	46.3 ± 47	71.0 ± 37	

N.B. Experimental population and rearing temperatures were either 25 or 28 °C, in a 2 × 2 design (see text). Means are plus or minus one standard deviation

25 °C and 28 °C simulated populations could be estimated at each time point. We used 99 % confidence intervals to correct for repeated comparisons at the five time points (0, 6, 12, 18, 24 months). Ultimately, these simulations allowed us to determine if genetic drift could explain the declines in allelic richness that were observed in the 25 and 28 °C experimental populations.

Additionally, when the simulations indicated that genetic drift alone could not explain the decline in allelic richness, we estimated how much smaller the effective population size would need to be relative to the census population size to produce the allelic richness values that we observed at 24 months. We did this by allowing only a fixed proportion of the individuals to breed in any generation. For example, if this proportion was set at 0.5, then only 50 % of the individuals in any generation were included as potential parents in the next generation. The simulations were repeated in 0.05 increments for each value of this proportion between 0.1 and 1.0, from which we selected the proportion that best matched the observed data. We considered the proportion that best fit the data to be the proportion that produced an average allelic richness that was most similar to the observed allelic richness at the 24 month point.

Statistical analysis

The sex ratio and brood survivorship data were transformed using a logit transformation. All other variables were normality distributed (Kolmogorov–Smirnov test; all $p > 0.091$). A log-linear model was performed to compare the prevalence of successful and unsuccessful pregnancies between the two experimental population temperatures across the three time points (6, 18, and 24 months). General linear mixed models (GLMMs) were then used to analyse differences among brood size, sex ratio, brood survivorship, and age and length

at maturity at each time point. For brood size, we included experimental population temperature as a fixed factor and, for all other tests, we included experimental population temperature and rearing temperature as fixed factors. Experimental population replicate number (tank ID) nested within experimental population temperature was included as a random factor for all tests. When significant effects were found, we performed linear contrasts between fish from different population temperatures with the same rearing temperature to determine whether (1) adaptation to high temperature compromises performance at the control temperature (i.e. *25–25* > *28–25*), and (2) adaptation to high temperature increases performance at high temperatures relative to the controls (i.e. *25–28* < *28–28*). Additionally, for the 18 month trial, we used GLMMs to compare brood size among fish born in 25, 28, and the F_2 and to compare the other demographic and life history traits among the *control*, *28*–28, *28*–25, and F_2 treatments. We included treatment as a fixed factor and tank ID as a random factor in the GLMMs.

T tests were performed to compare allelic richness between the 25 and 28 °C experimental populations at the baseline level. Then, repeated measure analysis of variance (ANOVA) tests were performed to compare the estimated population size and allelic richness among experimental populations from different temperatures across all sampling times. One-way ANOVAs were performed at each time point to compare Nei's standard genetic distance among the three pair-wise comparisons treatments: the two intra-temperatures and the inter-temperature experimental population pair-wise comparisons (i.e. all three 25 vs. 25 °C pair-wise comparisons, all three 28 vs. 28 °C pair-wise comparisons, and all nine 25 vs. 28 °C pair-wise comparisons). All statistical analyses were performed using the statistical software packages IBM SPSS v. 20 (SPSS Inc., Chicago, IL, USA) or JMP v. 4 (SAS Inc., Cary, NC, USA).

Results

Demographics and life history traits

Although the experimental populations nearly doubled in size during the experiment, the change in size over time was not significant ($F_{4,16} = 2.5$, $p = 0.086$) and there was no difference in population size between the two temperatures ($F_{4,16} = 0.7$, $p = 0.598$; Fig. 1). There was no difference in the number of successful pregnancies across the three time periods (loglinear model: $\chi^2 = 4.0$, df = 2, $p = 0.135$). However, the proportion of successful pregnancies was greater in the 25 °C (68/88 = 77 %) than the 28 °C (56/100 = 56 %) experimental populations ($\chi^2 = 10.0$, df = 1, $p = 0.002$) and this difference increased over time ($\chi^2 = 6.7$, df = 2, $p = 0.035$). There was no effect of experimental population temperature on mean brood size at the 6 and 18 month time points ($F_{1,13.6} = 0.0$, $p = 0.986$ and $F_{1,17.4} = 0.1$, $p = 0.705$, respectively; Table 1). At the 18 month time point, there also was no difference in brood size among fish from the 25 and 28 °C experimental populations and the F_2 treatment ($F_{2,44} = 0.2$, $p = 0.819$; Table 1). However, at the 24 month time point, females from the 28 °C experimental populations produced approximately half as many offspring as fish from the 25 °C experimental populations ($F_{1,14.3} = 11.6$, $p = 0.004$; Table 1). There was no effect of experimental population or rearing temperature on brood survivorship or sex ratio at any time point (Table 1, 2), nor was there a significant difference between the *control*, *28–28*, *28–25*, and the F_2 treatments in brood survival or sex ratio for the 18 month trial ($F_{3,36} = 0.6$, $p = 0.598$ and $F_{3,35} = 0.5$, $p = 0.706$, respectively).

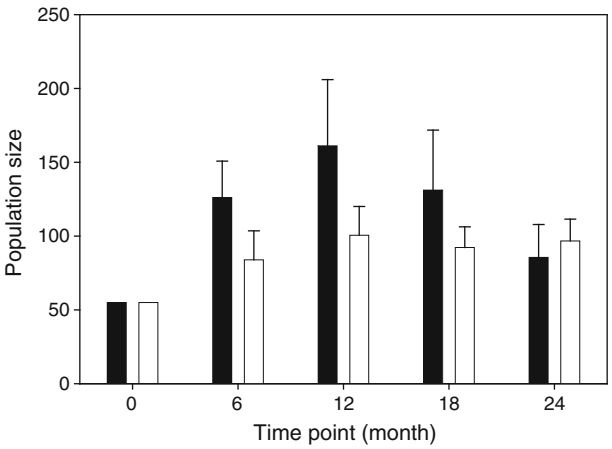

Fig. 1 Size of experimental populations of guppies (*Poecilia reticulata*). Shown are means (±SE) for populations at 25 °C (*black bars*) or 28 °C (*open bars*) over five time points. *Error bars* are based on three replicate populations for each temperature

Males reared at 28 °C matured approximately 7, 8, and 11 days sooner than fish reared at 25 °C at the 6, 18, and 24 month time points, respectively (Table 2; Fig. 2a–c). However, there was no effect of experimental population temperature on male age at maturity. There was also no difference at any time point between the *25–25* and *28–25* treatments (linear contrasts, 6 month: $F_{1,26} = 2.3$, $p = 0.142$; 18 month: $F_{1,19} = 0.9$, $p = 0.34$; 24 month: $F_{1,20} = 1.5$, $p = 0.233$) or between the *25–28* and *28–28* treatments (linear contrast, 6 month: $F_{1,26} = 0.1$, $p = 0.811$; 18 month: $F_{1,19} = 0.5$, $p = 0.481$; 24 month: $F_{1,20} = 1.1$, $p = 0.300$). Age at maturity also did not differ between males from the *control*, *28–28*, *28–25*, and the F_2 treatments at 18 months ($F_{2,26} = 2.4$, $p = 0.108$).

There was no effect of either experimental population or rearing temperature on male body length at maturity at the 6 and the 24 month time points (Table 2; Fig 2d, f). At the 18 month time point, males reared at 28 °C were approximately 0.8 mm shorter at maturity than fish reared at 25 °C (Table 2; Fig. 2e). Males from the 28 °C experimental populations were shorter than fish from the 25 °C experimental populations, but this difference was not statistically significant ($p = 0.053$). Fish from the *28–25* treatment were approximately 1.7 mm and 1.0 mm shorter at maturation than fish from the *25–25* treatment after 6 and 18 months, respectively (6 month: $F_{1,26} = 4.4$, $p = 0.046$; 18 month: $F_{1,19} = 7.1$, $p = 0.015$). However, this effect was not observed after 24 months ($F_{1,20} = 1.0$, $p = 0.322$). There was no difference between the *25–28* and *28–28* treatments in size at maturity at any time point (linear contrast, 6 month: $F_{1,26} = 0.0$, $p = 0.629$; 18 month: $F_{1,19} = 0.1$, $p = 0.766$; 24 month: $F_{1,20} = 0.0$, $p = 0.905$). Additionally, there was no difference in length at maturity between males from the *control*, *28-28*, *28-25*, and the F_2 treatments at 18 months ($F_{2,26} = 1.0$, $p = 0.366$). Consequently, the differences in body length observed between the *control* and *28–25* treatments cannot be explained by a genetic response.

Genetic diversity

After applying a Bonferroni correction, approximately 2 % of the pair-wise comparisons between microsatellite loci showed significant linkage disequilibrium (18 of 840).

Table 2 General linear mixed model results of the life history traits in families of guppies (*Poecilia reticulata*)

Factor	Experimental population temperature	Rearing temperature	Experimental population × rearing temperature	Nested factor
6 month				
Brood survival	$F_{1,4.3} = 0.0$, $p = 0.837$	$F_{1,34} = 1.3$, $p = 0.268$	$F_{1,34} = 0.2$, $p = 0.694$	$F_{4,34} = 1.2$, $p = 0.301$
Sex ratio	$F_{1,4.4} = 0.7$, $p = 0.461$	$F_{1,34} = 1.2$, $p = 0.280$	$F_{1,34} = 0.6$, $p = 0.434$	$F_{4,34} = 1.1$, $p = 0.366$
Age at maturity	$F_{1,4.5} = 0.4$, $p = 0.555$	$F_{1,26} = 10.7$, $p = 0.003$	$F_{1,26} = 1.9$, $p = 0.185$	$F_{4,26} = 3.6$, $p = 0.017$
Length at maturity	$F_{1,5.3} = 3.1$, $p = 0.136$	$F_{1,26} = 0.0$, $p = 0.893$	$F_{1,26} = 2.0$, $p = 0.165$	$F_{4,26} = 1.4$, $p = 0.254$
18 month				
Brood survival	$F_{1,4.5} = 0.3$, $p = 0.615$	$F_{1,33} = 1.4$, $p = 0.243$	$F_{1,33} = 0.2$, $p = 0.634$	$F_{4,33} = 0.7$, $p = 0.599$
Sex ratio	$F_{1,4.1} = 0.4$, $p = 0.585$	$F_{1,31} = 0.1$, $p = 0.803$	$F_{1,31} = 0.1$, $p = 0.794$	$F_{4,31} = 3.0$, $p = 0.033$
Age at maturity	$F_{1,7.6} = 0.0$, $p = 0.829$	$F_{1,19} = 4.6$, $p = 0.045$	$F_{1,19} = 1.6$, $p = 0.219$	$F_{5,19} = 1.4$, $p = 0.276$
Length at maturity	$F_{1,14.9} = 8.3$, $p = 0.011$	$F_{1,19} = 3.9$, $p = 0.063$	$F_{1,19} = 3.6$, $p = 0.072$	$F_{5,19} = 0.9$, $p = 0.860$
24 month				
Brood survival	$F_{1,5.5} = 1.9$, $p = 0.225$	$F_{1,31} = 0.8$, $p = 0.374$	$F_{1,31} = 0.0$, $p = 0.834$	$F_{4,31} = 0.7$, $p = 0.617$
Sex ratio	$F_{1,4.9} = 0.5$, $p = 0.519$	$F_{1,31} = 0.6$, $p = 0.458$	$F_{1,31} = 1.6$, $p = 0.219$	$F_{4,31} = 1.1$, $p = 0.352$
Age at maturity	$F_{1,8.2} = 2.3$, $p = 0.166$	$F_{1,20} = 16.1$, $p < 0.001$	$F_{1,20} = 0.0$, $p = 0.962$	$F_{4,20} = 1.0$, $p = 0.416$
Length at maturity	$F_{1,11.1} = 0.4$, $p = 0.527$	$F_{1,20} = 0.3$, $p = 0.605$	$F_{1,20} = 0.7$, $p = 0.422$	$F_{4,20} = 0.6$, $p = 0.642$

N.B. Experimental population and rearing temperatures were either 25 or 28 °C, in a 2 × 2 design (see text). The nested factor was experimental population replicate identification nested within experimental population temperature

However, the pair-wise comparisons that did show linkage disequilibrium did not include the same pairs of loci across different populations or time points, suggesting that the deviations do not reflect actual linkage between the loci. Only 72 % of the microsatellite loci were in Hardy–Weinberg equilibrium (HWE; 173 out of 240) after controlling for multiple comparisons. However, when excluding loci with null alleles present (four out of eight loci), 92 % of the remaining loci were in HWE (110 out of 120) and there again was no consistent pattern across tanks or time points.

Mean allelic richness did not differ between experimental populations based on temperature at the baseline ($t_4 = 0.1$, $p = 0.923$). Mean allelic richness decreased over time in all tanks ($F_{4,16} = 55.3$, $p < 0.001$), but the 28 °C experimental populations decreased significantly more rapidly than the 25 °C experimental populations ($F_{4,16} = 12.0$, $p < 0.001$; Fig. 3). From our simulation model of genetic drift, we determined that drift alone could explain the decrease in allelic richness in the 25 °C experimental populations (Fig. 3).

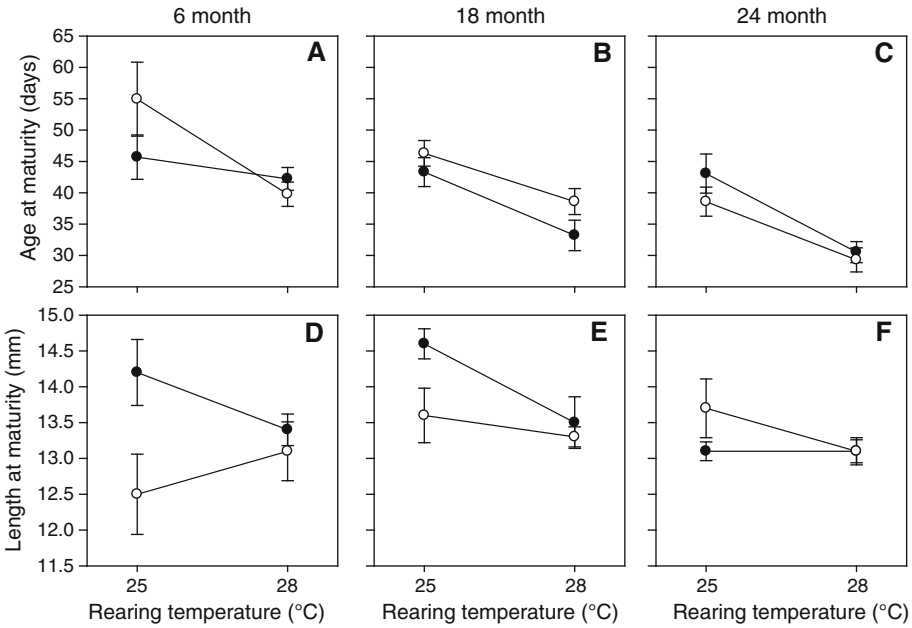

Fig. 2 The effects of experimental population and rearing temperature on life history traits in families of guppies (*Poecilia reticulata*). Offspring from populations at 25 °C (*black circles*) or 28 °C (*open circles*) were sampled at 6, 18, and 24 months and reared at either 25 or 28 °C. Shown are means (±SE) for male age (**a**–**c**), and body length (**d**–**f**) at maturity

However, the 28 °C experimental populations experienced a greater decline in allelic richness than could be explained by drift. We calculated that the 28 °C experimental populations lost allelic richness at a rate equivalent to populations that had an effective size that was only 25 % of the observed size.

There was no significant difference in Nei's standard genetic distance among treatments for the pair-wise comparisons of intra- or inter-temperature experimental populations (i.e. all 25 vs. 25 °C, 28 vs. 28 °C, and 25 vs. 28 °C pair-wise comparisons) at the 0, 6, 12, or 18 month time points ($p > 0.084$ for all). However, at the 24 month time point, the 28 °C experimental populations were significant more diverged from each other than were the 25 °C experimental populations ($F_{2,14} = 4.5$, $p = 0.034$), with the inter-temperature pair-wise comparisons not significantly different from either the 25 or 28 °C pair-wise comparisons ($p > 0.137$; Table 3). This result is consistent with the reduced effective population size and consequently increased genetic drift in the 28 °C experimental populations relative to the 25 °C populations.

Discussion

Global warming is predicted to have a negative impact on population viability in many species (e.g. Karayücel et al. 2008; Zeh et al. 2012). Previous research conducted using guppies acclimated to various temperatures from birth documented reduced offspring survival in water temperatures equal to or higher than 29 °C (Karayücel et al. 2008). As well, Dzikowski et al. (2001) found differential survival between the sexes at higher

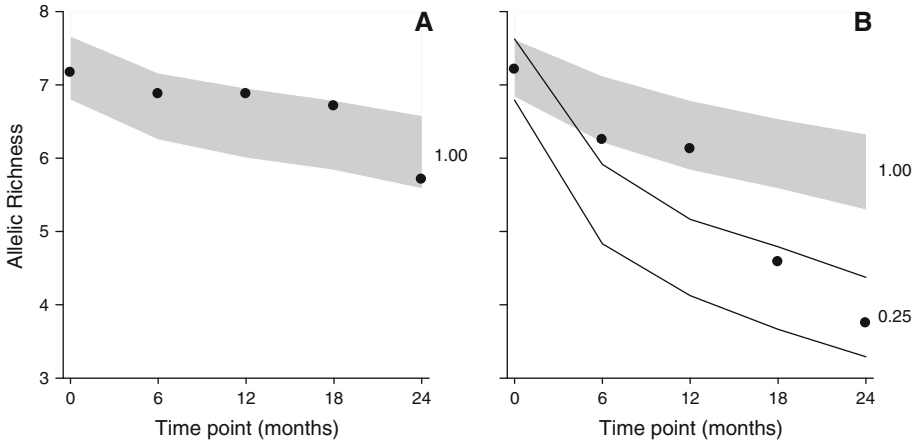

Fig. 3 Allelic richness in experimental populations of guppies (*Poecilia reticulata*). Shown are populations at 25 °C (**a**) and 28 °C (**b**). The *black dots* indicate the average observed allelic richness (mean number of alleles) at each time point. The *shaded section* denotes the 99 % confidence intervals from a simulation that modelled declines in allelic richness based solely on genetic drift. The *solid lines* in panel B denote the 99 % confidence intervals for the simulated population size that best matched the observed declines in allelic richness, with the number to the right of the graph indicating the proportional size (effective size) of the simulated population relative to the observed experimental population

Table 3 Pair-wise population comparisons of Nei's standard genetic distance after 24 months in experimental populations of guppies (*Poecilia reticulata*)

	25 °C			28 °C	
	1	2	3	4	5
25 °C					
2	*0.100*				
3	*0.139*	*0.139*			
28 °C					
4	0.114	0.140	0.136		
5	0.189	0.162	0.192	*0.220*	
6	0.181	0.166	0.182	*0.168*	*0.183*

N.B. Experimental populations 1–3 and 4–6 were held at a constant temperature of 25 and 28 °C, respectively. Italics represent pair-wise comparisons between experimental populations at the same temperature

temperatures in guppies, resulting in a male biased sex ratio. However, we found no difference in population size, sex ratio, or brood survivorship between our control temperature (25 °C) and the elevated temperature predicted by global warming (28 °C), at any of our three sampling time points. Guppies may be able to tolerate 28 °C because they periodically experience temperatures that high in their natural environment (Alkins-Koo 2000). We did find, however, that the performance of offspring from the 28 °C experimental populations was compromised when reared at the control temperature; these offspring were significantly shorter than fish from the control populations after 6 and 18 months. The F_2 fish did not display this effect which implies that it is not a genetic effect but perhaps explained by maternal or developmental effects. Interestingly, after

24 months, there was no longer any evidence of this compromised performance suggesting that fish from the 28 °C populations had become better adapted to the higher temperature. Taken together, these results suggest that, although temperatures up to 28 °C have a limited effect on demographic parameters in guppies, temperatures at or above 29 °C are associated with a significant decrease in survival, particularly for females.

On the other hand, we also found that there were fewer successful pregnancies at 28 °C than at 25 °C and the brood size of the 28 °C females was half that of 25 °C females at the 24 month time period. Zeh et al. (2012) have argued that, in a warming climate, reproduction is likely to be particularly vulnerable for tropical species, and indeed, many studies have documented effects on reproductive traits in response to increases in temperature (e.g. Karayücel et al. 2008; Zeh et al. 2012; Breckels and Neff 2013; Lahnsteiner and Leitner 2013). In our case, the reduced reproductive success could have been the result of dysfunctional sperm as we have previously shown that multiple sperm traits in guppies are negatively affected by increasing temperature (Breckels and Neff 2013). The reduced reproductive success also could be a product of a change in female investment in reproduction (e.g. Zeh et al. 2012), or perhaps a sign of inbreeding depression, as there was a sharp reduction in genetic diversity in the 28 °C experimental populations. Although this reduction in reproductive success did not yet translate into lower population sizes, our results suggest that even if other demographic parameters are unaffected by an increased temperature of 28 °C, reproduction in guppies is compromised. Thus, as suggested by Zeh et al. (2012), reproduction may indeed be the "Achilles' heel" for tropical ectotherms.

According to the temperature-size rule, global warming should result in earlier maturation at a smaller size for ectotherms (Atkinson 1994; Angilletta et al. 2004). Numerous other studies on ectotherms have found that exposure to increased temperature results in a younger age at maturity (e.g. Dhillon and Fox 2004; Zeh et al. 2012). Our results partially support the temperature-size rule in guppies; males showed a plastic response of maturing at a younger age when reared at a higher water temperature. Indeed, this earlier maturation may reduce generation time in the 28 °C experimental populations and maintain population viability despite reduced reproductive performance. Increased temperature should also result in smaller size at maturity (Angilletta et al. 2004; e.g. Dhillon and Fox 2004; Zeh et al. 2012). However, our results did not support this latter prediction as length at maturity did not differ across the two rearing temperatures, suggesting that this trait is canalised. It is possible that guppies have compensating mechanisms to counteract the relationship between higher growth rate and decreased size at maturity, which is likely driven by strong size-dependent predation that favours reaching a threshold size before allocating resources towards reproduction (Reznick and Endler 1982). Overall, guppies exposed to warmer temperatures matured at a younger age as predicted by the temperature-size rule, although their size at maturity was not affected by the increased temperature.

Selection on favourable traits can result in the loss of genetic diversity within experimental populations even if demographics are unaffected (e.g. Santos et al. 2005; Athrey et al. 2007; reviewed in Hoffman and Willi, 2008; Pauls et al. 2013). Here, we found that the multi-generational exposure to an elevated temperature (28 °C) significantly reduced allelic richness compared to the control temperature (25 °C) despite no reduction in population size. As well, our simulation model suggested that the loss of allelic richness was far greater than could be explained by genetic drift alone; relative to the control experimental populations, only about one quarter as many fish from the 28 °C experimental populations were likely contributing their genes to the next generation. The initial deviation observed in allelic richness compared to our simulation model in the 28 °C experimental populations may simply reflect an increased effective population size due to

the initial females used to seed the experimental population being pregnant. Regardless, we did not find any evidence of a genetic response in any life history traits that we measured, which may reflect an absence of additive genetic variance in these traits (see Crnokrak and Roff 1995; Roff and Emerson 2006). We also found no evidence that the microsatellites we used consistently deviated from Hardy–Weinberg equilibrium, indicating that these loci were not linked to genes under selection to the thermal environment. Instead, this signature of selection could be driven by a gene for thermal tolerance, at least one of which may reside on the X chromosome in guppies (Fujio et al. 1990; Nakajima et al. 2009), or possibly selection acting on sperm traits as we have previously documented (Breckels and Neff Unpublished data). Nevertheless, our data clearly show a signature of selection in response to increased temperature, mediated by increased variance in reproductive success among individuals.

Despite showing a clear signature of selection to increased temperature, the future adaptive potential of guppies in the 28 °C experimental populations may nevertheless be compromised. There is mounting evidence that the adaptive potential of populations is hampered by small effective population sizes and reduced genetic diversity after exposure to a stressor (e.g. Athrey et al. 2007; Nowak et al. 2009). Although the experimental populations exposed to elevated temperature in our study maintained similar population sizes as the control experimental populations, they displayed significantly less genetic diversity and consequently lower effective population sizes. The 28 °C experimental populations were also significantly more diverged from each other after 24 months than were the control experimental populations, which was likely a product of increased genetic drift acting on the 28 °C experimental populations in the latter time points. This reduction in diversity may have led to increased inbreeding (e.g. Kristensen et al. 2003; reviewed in Keller and Waller 2002; Frankham et al. 2005), which can result in inbreeding depression and reduced population viability (Charlesworth and Charlesworth 1987). Inbreeding depression might explain the reduced fertility of the female guppies in the elevated temperature populations at the latter time points (see Kristensen et al. 2003; Pitcher et al. 2008). Importantly, the loss of genetic diversity and lower effective population sizes will decrease the chance for further adaptation to other stressors (e.g. Meyer and Di Giulio 2003; Vogt et al. 2010). Thus, although the demographic and life history traits appear unaffected by increased temperature, there was an underlying erosion of genetic variation which will reduce the adaptive capacity of the populations. Given that climate change is predicted to result in multiple stressors, populations may become too genetically impoverished to adapt to all environmental or ecological changes. Certainly more studies examining multiple stressors are needed to fully understand the adaptive capacity of populations to climate change.

Acknowledgments We thank A. Berchtold, L. Crawford, J. Hung, M. Khan, D. Koscinski, and M. Lau for help with genetic analysis. T. Hain, A. Houde, and two anonymous reviewers provided comments on the manuscript. This project was funded by the Natural Sciences and Engineering Research Council of Canada.

References

Alkins-Koo M (2000) Reproductive timing of fishes in a tropical intermittent stream. Environ Biol Fishes 57:49–66

Angilletta MJ (2009) Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press, Oxford

Angilletta MJ, Steury TD, Sears MW (2004) Temperature, growth, and body size in ectotherms: fitting pieces of a life-history puzzle. Integr Comp Biol 44:498–509

Athrey NRG, Leberg PL, Klerks PL (2007) Laboratory culturing and selection for increased resistance to cadmium reduce genetic variation in the least killifish, *Heterandria formosa*. Environ Toxicol Chem 26:1916–1921

Atkinson D (1994) Temperature and organism size: a biological law for ectotherms? Adv Ecol Res 25:1–58

Barrett RDH, Schluter D (2008) Adaptation from standing genetic variation. Trends Ecol Evol 23:38–44

Becher SA, Russel ST, Magurran AE (2002) Isolation and characterization of polymorphic microsatellites in the Trinidadian guppy (*Poecilia reticulata*). Mol Ecol Notes 2:456–458

Breckels RD, Neff BD (2013) The effects of elevated temperature on the sexual traits, immunology, and survivorship of a tropical ectotherm. J Exp Biol 216:2658–2664

Caissie D, El-Jabi N, Satish MG (2001) Modelling of maximum daily water temperatures in a small stream using air temperatures. J Hydrol 251:14–28

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–631

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Ann Rev Ecol Syst 18:237–268

Crnokrak P, Roff DA (1995) Dominance variance: associations with selection and fitness. Heredity 75:530–540

Daufresne M, Lengfellner K, Sommer U (2009) Global warming benefits the small in aquatic ecosystems. PNAS 106:12788–12793

DeWitt TJ, Sih A, Wilson DS (1998) Costs and limitations of phenotypic plasticity. Trends Ecol Evol 13:77–81

Dhillon RS, Fox MG (2004) Growth-independent effects of temperature on age and size at maturity in Japanese medaka (*Oryzias latipes*). Copeia 1:37–45

Dzikowski R, Hulata G, Karplus I, Harpaz S (2001) Effect of temperature and dietary L-carnitine supplementation on reproductive performance of the female guppy (*Poecilia reticulata*). Aquaculture 199:323–332

Endler JA (1980) Natural selection of colour patterns in *Poecilia reticulata*. Evolution 34:76–91

Frankham R, Ballou JD, Briscoe DA (2005) Introduction to conservation genetics. Cambridge University Press, Cambridge

Fujio Y, Nakajima M, Nagahama Y (1990) Detection of a low temperature-resistant gene in the guppy (*Poecilia reticulata*), with reference to sex-linked inheritance. Jap J Genet 65:201–207

Fuller A, Dawson T, Helmuth B, Hetem RS, Mitchell D, Maloney SK (2010) Physiological mechanisms in coping with climate change. Physiol Biochem Zool 83:713–720

Gibson G, Dworkin I (2004) Uncovering cryptic genetic variation. Nat Rev Genet 5:681–690

Grether GF, Millie DF, Bryant MJ, Reznick DN, Mayea W (2001) Forest canopy cover, resource availability, and life history evolution in guppies. Ecology 82:1546–1559

Hoffman AA, Willi Y (2008) Detecting genetic responses to environmental change. Nat Rev Genet 9:421–432

Houde AE (1997) Sex, colour, and mate choice in guppies. Princeton University Press, Princeton, NJ

IPCC (2007) Climate change 2007: synthesis report. Cambridge University Press, Cambridge

Karayücel I, Orhan AK, Karayücel S (2008) Effect of temperature on some reproductive parameters of gravid females and growth of newly hatched fry in guppy, *Poecilia reticulata* (Peters, 1860). J Anim Vet Adv 7:1261–1266

Kaushal SS, Likens GE, Jaworski NA, Pace ML, Sides AM, Seekell D, Belt KT, Secor DH, Wingate RL (2010) Rising stream and river temperatures in the United States. Front Ecol Environ 8:461–466

Keller LF, Waller DW (2002) Inbreeding effects in wild populations. Trends Ecol Evol 17:230–241

Kristensen TN, Dahlgaard J, Loeschcke V (2003) Effects of inbreeding and environmental stress on fitness—using *Drosophila buzzatii* as a model organism. Conserv Genet 4:453–465

Lahnsteiner F, Leitner S (2013) Effect of temperature on gametogenesis and gamete quality in brown trout, *Salmo trutta*. J Exp Zool Part A 319:138–148

Meyer JN, Di Giulio RT (2003) Heritable adaptation and fitness costs in killifish (*Fundulus heteroclitus*) inhabiting a polluted estuary. Ecol Appl 13:490–503

Nakajima M, Fujisawa K, Taniguchi N (2009) Estimation of the mode of inheritance of thermal tolerance in the guppy *Poecilia reticulata*. Fish Sci 75:683–687

Neff BD, Fu P, Gross MR (2000) Microsatellite multiplexing in fish. Trans Am Fish Soc 129:584–593

Neuheimer AB, Taggart CT (2007) The growing degree-day and fish size-at-age: the overlooked metric. Can J Fish Aquat Sci 64:375–385

Nowak C, Vogt C, Pfenninger M, Schwenk K, Oehlmann J, Streit B, Oetken M (2009) Rapid genetic erosion in pollutant-exposed experimental chironomid populations. Environ Pollut 157:881–886

Paterson IG, Crispo E, Kinnison MT, Hendry AP, Bentzen P (2005) Characterization of tetranucleotide microsatellite markers in guppy (*Poecilia reticulata*). Mol Ecol Notes 5:269–271

Pauls SU, Nowak C, Bálint M, Pfenninger M (2013) The impact of global climate change on genetic diversity within populations and species. Mol Ecol 22:925–946

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539

Pitcher TE, Rodd FH, Rowe L (2008) Female choice and relatedness of mates in the guppy (*Poecilia reticulata*). Genetica 134:137–146

Reznick DN, Endler JA (1982) The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution 36:160–177

Reznick DN, Shaw FH, Rodd FH, Shaw RG (1997) Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science 275:1934–1937

Reznick D, Buckwalter G, Groff J, Elder D (2001) The evolution of senescence in natural populations of guppies (*Poecilia reticulata*): a comparative approach. Exp Gerontol 36:791–812

Roff DA, Emerson K (2006) Epistasis and dominance: evidence for differential effects in life-history versus morphological traits. Evolution 60:1981–1990

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Resour 8:103–106

Santos M, Céspedes W, Balanyà J, Trotta V, Calboli FCF, Fontdevila A, Serra L (2005) Temperature-related genetic changes in laboratory populations of *Drosophila subobscura*: evidence against simple climatic-based explanations for latitudinal clines. Am Nat 165:258–273

Stearns SC (1992) The evolution of life histories. Oxford University Press, Ox ford

Stefan HG, Preudhomme EB (1993) Stream temperature estimation from air-temperature. J Am Water Resour Assoc 29:27–45

van der Have TM, de Jong G (1996) Adult size in ectotherms: temperature effects on growth and differentiation. J Theor Biol 183:329–340

van Oosterhout C, Hutchinson WF, Willis DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Vogt C, Heß M, Nowak C, Diogo JD, Oehlmann J, Oeteken M (2010) Effects of cadmium on life cycle parameters in a multigenerational study with *Chironomus riparius* following pre-exposure of populations to two different tributyltin concentrations for several generations. Ecotoxicology 19:1174–1182

Water Resources Agency (2001) National report on integrating the management of watersheds and coastal areas in Trinidad and Tobago. Ministry of Public Utilities and the Environment, Port of Spain

Wedekind C, Evanno G, Székely T Jr, Pompini M, Darbellay O, Cuthruf J (2013) Persistent unequal sex ratio in a population of grayling (Salmonidae) and possible role of temperature increase. Conserv Biol 27:229–234

Zeh JA, Bonilla MM, Su EJ, Padua MV, Anderson RV, Kaur D, Yang DS, Zeh DW (2012) Degrees of disruption: projected temperature increase has catastrophic consequences for reproduction in a tropical ectotherm. Glob Change Biol 18:1833–1842