Evol Ecol (2014) 28:521–533
DOI 10.1007/s10682-014-9692-0

ORIGINAL PAPER

Rapid evolution of sperm length in response to increased temperature in an ectothermic fish

Ross D. Breckels · Bryan D. Neff

Received: 27 August 2013 / Accepted: 10 February 2014 / Published online: 16 February 2014

Abstract The Intergovernmental Panel on Climate Change predicts an average global temperature increase of 1.8–4.0 °C by 2100. Tropical ectotherms are expected to be particularly sensitive to this temperature increase because they live close to their thermal limits. We investigated the phenotypic plasticity and evolutionary response of sperm traits in guppies (*Poecilia reticulata*) to increased temperatures after 6, 18, and 24 months. Guppies with evolution temperatures of 25 °C (control) or 28 °C were reared in either 25 or 28 °C in a 2 × 2 common garden design. The plastic response to increased temperature was a decreased sperm length, velocity, and path linearity. The evolutionary response was a subsequent increase in sperm length, resulting in complete compensation after just 6 months (at most four generations) in 28 °C water. Sperm velocity and linearity showed no sign of evolution even after 24 months. This study provides evidence that some reproductive traits can respond via rapid evolution to the temperature increase associated with climate change.

Keywords Phenotypic plasticity · Genetic response · Sperm length · Sperm velocity · Guppy · Global warming

Introduction

Changes in the environment can have marked effects on organisms (e.g. Endler 1980; West and Packer 2002), with temperature being one of the most ubiquitous environmental

Electronic supplementary material The online version of this article (doi:10.1007/s10682-014-9692-0) contains supplementary material, which is available to authorized users.

R. D. Breckels · 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: rbreckels@gmail.com

conditions with broad impacts on virtually all species (Dorts et al. 2012). The average global air temperature is projected to increase from current levels, which are already 0.74 °C higher than the beginning of the twentieth century, by 1.8–4.0 °C by the year 2100 (IPCC 2007). Thus there is concern about the potential impact of this warming on species composition and ecosystem health. Indeed, the impacts of global warming have been documented, with some populations already experiencing range shifts, altered phenologies, or even extinctions (reviewed in Parmesan and Yohe 2003; Parmesan 2006; Angilletta 2009). Generally, organisms will have to respond to the projected warming by dispersal to a more appropriate climate or by phenotypic or genetic responses to higher temperatures (Fuller et al. 2010). However, many species, especially aquatic organisms, will face barriers to dispersal such as dams and waterfalls. For those species, phenotypic or genetic responses will be essential in order for them to persist.

Species have the capacity to adapt to a warmer environment by phenotypic resilience or evolutionary adaptation. Phenotypic resilience includes phenotypic plasticity, the ability of a genotype to display different phenotypes in different environments, and canalization, the ability of a genotype to display the same phenotype in different environments. However, phenotypic responses may incur a cost (reviewed in DeWitt et al. 1998); for example, plastic responses might consume energy that could otherwise be used for other somatic processes, such as growth or reproduction. Additionally, the extent of phenotypic responses is often limited (reviewed in DeWitt et al. 1998), and many species, such as tropical ectotherms, are living close to their thermal tolerance limit so there is little capacity for plasticity at elevated temperatures (Stillman 2003; Deutsch et al. 2008). Genetic adaptations to temperature can occur via natural selection acting on either phenological mechanisms or thermal physiology (Angilletta 2009). There are a number of examples of species showing genetic-based adaptations in phenology (e.g. Réale et al. 2003; reviewed in Bradshaw and Holzapfel 2006). However, relatively little is known about rapid evolutionary adaptations of thermal physiology (Leal and Gunderson 2012), especially in vertebrates. Hendry et al. (1998, 2000) provides one of the few empirical examples of a vertebrate, the newly diverged Lake Washington sockeye salmon (*Oncorhynchus nerka*), showing rapid adaptation in thermal tolerance and body shape after only 9–14 generations. This result provides evidence that vertebrate species can adapt rapidly via evolutionary responses to increased temperature.

The effects of temperature in developmental and life history traits have been well documented, yet less is known about reproduction, particularly reproductive morphology, despite these latter traits being crucial to population health and persistence (Angilletta 2009; Berger et al. 2011). In males, sperm length and velocity have been linked to fertilization success, particularly under competition (reviewed in Snook 2005; Simmons and Fitzpatrick 2012; but see Humphries et al. 2008). In many polygamous mating systems, longer sperm may have an advantage in both sperm competition and female cryptic choice because they have higher velocity and longevity (Gomendio and Roldan 1991; Parker 1993), and in internal fertilizers, they have the ability to displace smaller sperm from the female reproductive tract (Lüpold et al. 2012). Higher sperm velocity in itself has also been shown to result in greater fertilization success in guppies and other fishes (e.g. Gage et al. 2004; Casselman et al. 2006; Gasparini et al. 2010; Boschetto et al. 2011). Sperm traits have been shown to be sensitive to changes in temperature, with even slight increases in temperature resulting in reduced sperm numbers (Zeh et al. 2012), longevity (Binet and Doyle 2013), motility (Williot et al. 2000), length (Adriaenssens et al. 2012; Breckels and Neff 2013), and velocity (Breckels and Neff 2013; but see Adriaenssens et al. 2012). Such studies have led Zeh et al. (2012) to claim that reproduction is a potential "Achilles' heel"

for many species in the face of global warming. Certainly, more studies are needed to examine the plastic and genetic responses in reproductive traits to increases in temperature.

Here, we use Trinidadian guppies (*Poecilia reticulata*, Peters 1860) as a model ectotherm to detail the effects of long-term exposure to increased temperature, as projected for the end of the twenty first century. Guppies are a small, live bearing fish, native to north-eastern South America and the Caribbean, that inhabit small freshwater streams (Houde 1997). They tend to be highly polyandrous with males experiencing high levels of sperm competition. Males mature at approximately 7 weeks of age or younger (Reznick et al. 2001). Guppies have overlapping generations; as such generation time has been estimated between 1.5 and 6.9 months (e.g. Endler 1980; Reznick et al. 1997). Over the past six decades, Trinidad has experienced a mean air temperature increase of 1.5 °C (Singh 1997), and is set to increase by a further 1.0–3.5 °C by the end of the twenty first century (Water Resources Agency 2001). This projected increase in air temperature will result in similar increases in stream and small river water temperatures (Stefan and Preudhomme 1993; Caissie et al. 2001; Kaushal et al. 2010). In Trinidad, the current mean daily air temperature is 27.7 °C with daily fluctuations of up to 8.4 °C (max–min daily temperature, calculated between January 1992 and December 2012; weatheronline.co.uk). Mean river water temperatures are approximately 25 °C and fluctuate between 20 and 28 °C (Alkins-Koo 2000). Although guppies periodically experience temperatures of 28 °C, we have previously shown that prolonged exposure to 28 °C affects sperm traits (Breckels and Neff 2013). Thus multi-generational exposure to increased temperature could negatively affect reproduction. Additionally, geographical barriers, such as waterfalls and oceans, mean that natural dispersal is unfeasible. Therefore guppies, like many other species, will have to rely on phenotypic plasticity or evolutionary adaptation in order to respond to a warming environment.

Specifically, we have previously shown that exposure to elevated temperatures during development results in decreased sperm length, velocity, and path linearity (Breckels and Neff 2013). However, this previous study measured only the initial plastic response and thus could not address the multi-generational, evolutionary response. In the present study, we exposed guppies to elevated temperatures for 24 months (approximately eight generations) to evaluate the scope of the genetic response. Our objective was to examine whether sperm length, velocity, or path linearity would respond genetically and if that response was compensatory (returned to baseline levels). These sperm traits typically show high levels of heritability in fish and other taxa (e.g. Simmons and Moore 2009; Evans 2011). We predicted that a genetic response would occur as a consequence of selection acting on individuals with favourable traits, resulting in partial or full compensation.

Methods

Experiments were performed following the Canadian Council of Animal Care's guidelines and were approved by the University of Western Ontario. Guppies used in this experiment were descendants of fish caught from the Paria River, Trinidad in 2003. Guppies were held in the Freshwater Ecology Research Facility room at the University of Western Ontario in tanks lined with bottom layers of gravel and artificial plants to provide cover. Fish were kept on a 12:12 h light–dark cycle with the water temperature set to 25 °C, using internal heaters, to simulate current natural conditions (Alkins-Koo 2000). Fish were fed twice daily, once with Tetramin® flake food and once with brine shrimp.

As outlined in Breckels et al. (2014), on May 1st 2010, six, 250 l experimental tanks ('evolution populations') were seeded with 55 adult fish (25 males and 30 females). The initial water temperature in all six evolution populations was set to 25 °C. The temperature in three of these evolution populations was raised gradually, at a rate of 1 °C every 45 days, up to 28 °C (N = 3; range = 9.6 °C; SD: ±1.2 °C) to simulate average levels of global warming by the end of the century (IPCC 2007). The three other evolution populations remained at 25 °C (N = 3; range = 4.0 °C; SD: ±0.5 °C) throughout the experiment and acted as controls. To produce families for the common garden treatments, 6, 18, and 24 months after the evolution populations were seeded, eight pregnant females (evident from enlarged abdomens and darker anal regions; Houde 1997) were removed from each evolution population and put into separate, individual 10 l rearing tanks with the water set at the same temperature as the evolution population that the female had come from (i.e. if the female came from an evolution population set at 25 °C she was put into a rearing tank with the water set at 25 °C). Females were allowed to give birth, after which they were returned to their original evolution population, leaving only their offspring in the rearing tanks.

Next, we created four treatments in a common garden experimental design by switching the water temperature in four of the eight rearing tanks, 24 h after the first offspring was born, to that of the alternate evolution populations: (1) *25-25* (*control*), fish that had an evolution population and rearing temperature of 25 °C; (2) *25-28*, fish that had an evolution temperature of 25 °C but a rearing temperature of 28 °C; (3) *28-28*, fish that had an evolution population and rearing temperature of 28 °C; and (4) *28-25*, fish that had an evolution temperature of 28 °C but a rearing temperature of 25 °C (see supplementary material). There were a total of 12 tanks in each treatment, four from each of the three different evolution populations with the corresponding temperature. From the offspring in these rearing tanks (i.e. the F_1 generation) we estimated sperm traits as detailed below.

In addition, for the 18 month trial, a breeding design was used to generate an F_2 generation (F_2) of the *28-25* treatment in order to determine whether a perceived genetic response was actually due to maternal effects (i.e. any non-genetic information passed from mother to offspring resulting in an altered phenotype). The breeding design used fish from the four rearing tanks of each evolution population (12 rearing tanks total). Males and females from the *28-25* treatment were separated into individual rearing tanks before they became sexually mature. After approximately 4 months, males and females were paired in a design that ensured brothers and sisters were not mated. The design involved introducing pairing males from the first rearing tank to females from the second rearing tank, males from the second rearing tank to females from the third rearing tank, males from the third rearing tank to females from the fourth rearing tank, and males from the fourth rearing tank to females from the first rearing tank. The pairs were given 3 days to copulate and then the males were removed. When the females gave birth, the offspring were removed and put into separate rearing tanks. The water temperature remained at 25 °C and sperm traits were examined in the offspring as detailed below.

Sperm characteristics

When fish were 3 months of age (mean age in days ± SD: 96 ± 10; N = 233; range = 53 days), males were removed from their rearing tanks and put into individual isolation tanks for 3 days to ensure full sperm reserves (Pilastro et al. 2002), with the water temperature set to the same as they had been reared in. We then anaesthetized the males using MS-222. Males were photographed next to a ruler, which acted as a scale, and

images were later analysed using NIH ImageJ software (http://rsbweb.nih.gov/ij) to calculate each male's total body length. Next, each male was placed under a dissection microscope with their gonopodium swung forward. Forty microlitres of sperm extender medium (207 mM NaCl, 5.4 mM KCl, 1.3 mM $CaCl_2$, 0.49 mM $MgSO_4$, 10 mM Tris, pH 7.5) held at 25 °C was applied to the base of the gonopodium (Evans 2009). Sperm bundles were released into the extender medium by gently applying pressure to the side of the abdomen, anterior to the base of the gonopodium. The sperm was then activated using 40 µl of 150 mM KCl solution with 2 mg/l bovine serum albumin (BSA, which prevents sperm from sticking to the slide) also held at 25 °C. In order to assess sperm velocity, immediately after activation (approximately 15 s), a 15 µl sample of the sperm solution was placed in a 2X-CEL sperm analysis chamber (80 µm depth; Hamilton Thorne, Beverly, MA, USA) and put under a microscope at 200× magnification. Digital images were recorded using an SI-C400N video camera attached to the microscope (Costar Imaging, Lakewood, CA, USA). Following the methods outlined in Breckels and Neff (2010), we extracted images at ten frames per second from the recorded video and determined the two-dimensional co-ordinates using ImageJ. We used the Pythagorean Theorem to calculate the sum of the distance travelled in µm by each sperm cell between each of the 11 individual frames that make up 1 s. The total distance travelled by the sperm was used to calculate the velocity (µm s^{-1}). We then calculated sperm path linearity as the displacement (i.e. distance between the start and end point after 1 s) divided by total distance travelled. A value of 0 represents a sperm that started and ended in the same place whereas a value of 1 represents a sperm that move in a straight line (see Stoltz and Neff 2006 and Kime et al. 2001). Ten sperm per individual were used to calculate mean velocity and path linearity.

To assess sperm length, 20 µl of the sperm solution was put onto a slide and a cover slip was placed on top. The slide was placed under a microscope at 400× magnification. Digital images were analysed using UTHSCSA Image Tool software v. 3.0 (http://compdent.uthscsa.edu/dig/itdesc.html). We measured the tail length (including the flagellum and mid-piece) of 30 sperm per male. We did not measure sperm number here because of logistical constraints, but previous analysis revealed no change in numbers with increased temperature (sperm count ± SD; 25 °C: $2.2 \times 10^6 \pm 0.8 \times 10^6$; 28 °C: $2.5 \times 10^6 \pm 1.0 \times 10^6$; $t_{17} = 0.87$, $p = 0.40$; unpublished data).

Statistical analysis

All presented p values are two-tailed probabilities and all statistical analyses were performed using IBM SPSS v. 20 (SPSS Inc., Chicago, IL, USA). Metrics from individuals within the same family were averaged in order to get family means which were used for all statistical analyses. Sperm path linearity was transformed using a logit transformation to normalize the data. All other variables were normality distributed according to Kolmonov–Smirnov tests ($p > 0.37$ for all). General linear mixed models (GLMMs) were performed on family means of body length, sperm length, VCL, and path linearity. We included time point (6, 18, or 24 months), and evolution and rearing temperatures as fixed factors and evolution population identification nested within population temperature as a random factor in all tests. Any non-significant interactions between the fixed factors were removed from the analysis. To control for body size, we performed a GLMM of log(mean sperm length) using time point, evolution and rearing temperatures as fixed factors, evolution population identification nested within evolution temperature as a random factor, and log(body length) as a covariate. Finally, for the 18 month trial, we also used a one-way

ANOVA and a subsequent Tukey's post hoc test to compare body length, sperm length, velocity, and path linearity among the *control* (*25-25*), *28-28*, *28-25*, and F_2 treatments.

Variation in sperm length, velocity, or path linearity due to rearing temperature, would suggest a phenotypic plastic response. Variation due to evolution temperature suggests either a genetic response or maternal environmental effects. If this latter variation persists in the F_2 treatment, a genetic response is indicated.

Results

A total of 92 families were used across the three time periods (Table 1). This number is lower than the maximum expected of 144 families because some females did not give birth (N = 23; the 28 °C evolution populations produced fewer broods than the 25 °C evolution populations; binomial test, $p = 0.03$; see Breckels et al. 2014 for a full analysis of these results), females gave birth to female only broods or males in the brood died before sperm analysis was conducted (N = 26; binomial test, $p = 1.00$), or no sperm could be taken from males in a family (N = 3; binomial test, $p = 1.00$). There was no effect of time, evolution temperature, or rearing temperature on body length ($F_{2,81} = 1.5$, $p = 0.23$; $F_{1,4.3} = 0.4$, $p = 0.54$; and $F_{1,81} = 2.0$, $p = 0.16$, respectively; Table 1). Similarly, there was no significant difference in male length in the F_2 treatment and the *control*, *28-28*, and *28-25* after 18 months ($F_{3,28} = 1.0$, $p = 0.39$).

Sperm length

There was a significant effect of time, evolution temperature, and rearing temperature on sperm length over the three time periods (Table 2; Fig. 1a–c). Across the three sampling times, fish reared at 28 °C (*25-28* and *28-28*) produced sperm that were about 3.5 % shorter than fish from the corresponding evolution populations but were reared at 25 °C

Table 1 Metrics for the families used in analyses of sperm characteristics in the guppy (*P. reticulata*)

Variable	Treatments				
	Control	*25-28*	*28-28*	*28-25*	F_2
6 months					
No. families	10	9	9	4	
Males per family	1–10	1–5	1–5	2–7	
Body length (mm)	15.0 ± 0.06	14.8 ± 0.10	14.0 ± 0.07	14.0 ± 0.27	
18 months					
No. families	9	7	9	7	7
Males per family	1–5	1–5	1–9	1–3	1–4
Body length (mm)	15.4 ± 0.06	14.6 ± 0.10	14.8 ± 0.06	14.9 ± 0.10	15.1 ± 0.06
24 months					
No. families	9	10	5	4	
Males per family	1–4	1–4	1–2	1–4	
Body length (mm)	14.9 ± 0.07	1.44 ± 0.07	15.2 ± 0.17	15.0 ± 0.17	

N.B. evolution population and rearing temperatures were either 25 or 28 °C, in a 2 × 2 design (see text). Means are plus or minus the 95 % confidence interval. Numbers of families represent those families that were used in the analysis (see text)

(*control* and *28-25*). Conversely, all treatments with fish from the 28 °C evolution populations (*28-28* and *28-25*) had sperm that were over 4 % longer than fish from the 25 °C evolution populations with the corresponding rearing temperature (*25-28* and *control*). When body length was included as a covariate, it was not significant ($F_{1,81} = 3.6$, $p = 0.06$), while the initial effects of time point, and evolution and rearing temperature all remained significant ($p < 0.01$ for all). After 18 months, males from the *28-25* and F_2 treatments had sperm that were similar in length but significantly longer than the *control* and *28-28* treatments ($F_{3,28} = 31.5$, $p < 0.01$; Fig. 2A).

Sperm velocity

There was a significant effect of rearing temperature on sperm velocity (Table 2; Fig. 1d–f). Treatments where fish were reared at 28 °C produced sperm that were 11.5–12.4 % slower than fish from the same evolution populations but reared at 25 °C. There was no effect of time or evolution temperature on velocity (Table 2). Similarly, there was no significant difference between the F_2 treatment and the *control*, *28-28*, and *28-25* after 18 months ($F_{3,26} = 0.9$, $p = 0.45$; Fig. 2B).

Sperm path linearity

There was a significant effect of rearing temperature on sperm path linearity (Table 2). Treatments where fish were reared at 28 °C (*25-28* and *28-28*) produced sperm that travelled about 2 % less linearly than fish from the same populations but reared at 25 °C (*control* and *28-25*; Fig. 1g–i). There was no effect of time or evolution temperature on sperm path linearity (Table 2). There was a significant difference between the *control*, *28-28*, *28-25*, and F_2 treatments at 18 months in sperm path linearity, with the *control* displaying significantly greater path linearity than the *28-28* treatment ($F_{3,26} = 4.8$, $p = 0.01$; Fig. 2C).

Discussion

Previous research shows that reproduction in species could be significantly affected by climate change (e.g. Zeh et al. 2012; Breckels and Neff 2013). Here we also found that rearing temperature had a significant effect on all sperm traits that we measured; the phenotypically plastic response to increased temperature was decreased sperm length, velocity, and path linearity. These traits may be critical for male competitiveness during reproduction (Simmons and Fitzpatrick 2012). Our results corroborate other studies that

Table 2 General linear mixed model results of sperm traits in families of guppies (*P. reticulata*)

Factor	Sperm length	Velocity	Path linearity
Time	$F_{2,81} = 85.6$, $p < 0.01$	$F_{2,75} = 2.2$, $p = 0.12$	$F_{2,75} = 1.4$, $p = 0.24$
Population temperature	$F_{1,4.8} = 157$, $p < 0.01$	$F_{1,6.6} = 0.5$, $p = 0.49$	$F_{1,4.6} = 0.2$, $p = 0.57$
Rearing temperature	$F_{1,81} = 195$, $p < 0.01$	$F_{1,75} = 120$, $p < 0.01$	$F_{1,75} = 17.5$, $p < 0.01$
Random factor	$F_{4,81} = 1.5$, $p = 0.22$	$F_{4,75} = 0.5$, $p = 0.75$	$F_{4,75} = 2.0$, $p = 0.11$

N.B. Evolution population and rearing temperatures were either 25 or 28 °C, in a 2 × 2 design (see text). Time denotes the three sampling periods of 6, 18, and 24 months. The random factor was evolution population identification nested within evolution temperature

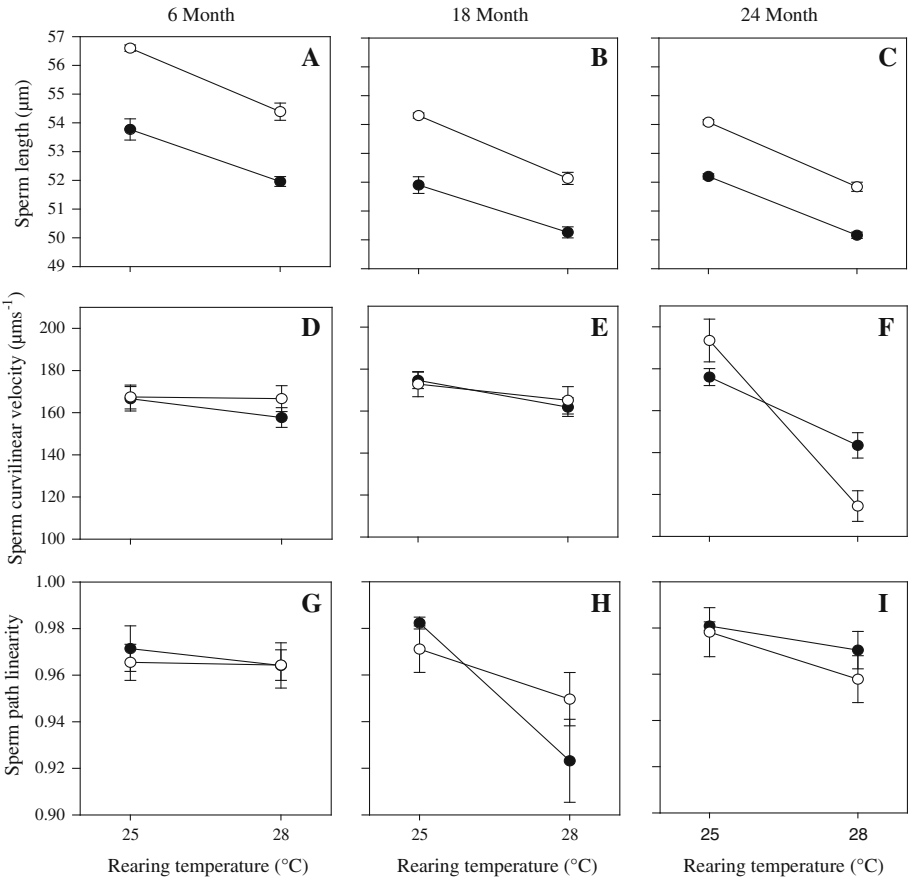

Fig. 1 The effects of evolution population and rearing temperatures on sperm traits in the guppy (*P. reticulata*). Offspring from the three evolution populations at 25 °C (*black circles*) or 28 °C (*open circles*) were sampled at 6, 18, and 24 months and reared at either 25 or 28 °C. Shown are means (±SE) for sperm length (**a**–**c**), velocity (**d**–**f**), and path linearity (**g**–**i**). *Solid* and *dotted lines* represent the means of all the populations with an evolution temperature of 25 and 28 °C, respectively, but reared at the two different temperatures

have similarly shown that an increase in temperature leads to a decrease in sperm length (e.g. Adriaenssens et al. 2012) and velocity (e.g. Beirão et al. 2011; Lahnsteiner and Mansour 2012). Some of those studies suggest that even small changes in temperature can elicit a stress response and negatively affect reproduction. Collectively, these studies suggest that the projected increase in temperature due to climate change could be detrimental to ectotherms, at least in the short term, because it may reduce sperm quality and performance, and consequently, reproductive success and potentially population viability.

Given the potentially negative plastic response observed, sperm traits must instead respond genetically via natural selection to overcome the effect of increased temperature. Sperm traits in several species have been shown to be highly heritable (e.g. Simmons and Moore 2009 and references therein), including in an introduced population of the guppy (Evans 2011), so these traits should have the potential to evolve rapidly. Here, male guppies from the 28 °C evolution populations showed an evolved response with sperm

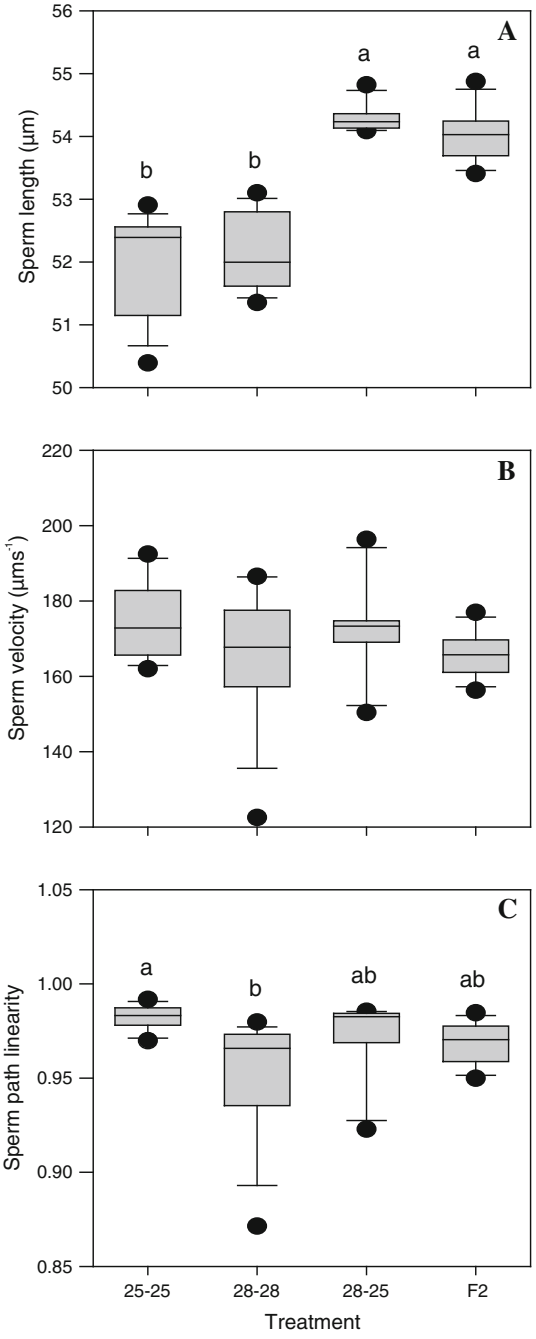

Fig. 2 The effects of temperature treatments after 18 months on sperm traits in the guppy (*P. reticulata*). Treatments include fish from a 25 or 28 °C evolution population (*25-25* and *28-28*, respectively), fish that were from a 28 °C evolution population but reared at 25 °C (*28-25*), and the F_2 offspring of the fish that were from a 28 °C evolution population but reared at 25 °C for two generations. *Boxes* denote the 25th, 50th, and 75th percentiles, whiskers denote 10th and 90th percentile, and the dots denote the 5th and 95th percentiles for sperm length (**A**), velocity (**B**), and path linearity (**C**). Treatments with *different letters* are significantly different ($p < 0.05$) according to a Tukey's b HSD test

length returning to the same size as the 25 °C evolution populations after only 6 months (a maximum of four generations; e.g. Endler 1980). The F_2 offspring (i.e. fish from the 28 °C evolution populations reared at 25 °C for two generations) had sperm lengths similar to the 28 °C evolution populations reared at 25 °C, indicating that the response to the increased

temperature was indeed genetic. Interestingly, we also found that sperm length decreased across all treatments with time. This result might reflect a reduction in the intensity of sexual selection resulting from the reduction in density when the evolution populations were first established (as compared to the density of the original stock tank; e.g. Emlen and Oring 1977). Nevertheless, male fish from the 28 °C evolution populations always produced sperm that were longer than fish from the 25 °C evolution populations, when males were reared at the same temperature (also see Blanckenhorn and Hellriegel 2002). Sperm velocity and path linearity, on the other hand, showed no sign of an evolved response even after 24 months. Our study, however, did not investigate the potential for evolutionary changes in plasticity (the reaction norm) across populations. Such an analysis could be achieved by determining sperm velocity (or path linearity) across a range of temperatures for each evolution population. Thus, at least in guppies, sperm length may play a more important part in reproductive success than previously thought (see Boschetto et al. 2011).

In several species, it has been documented that sperm length co-evolves with different aspects of females' reproductive tract (e.g. Briskie and Montgomerie 1992; Presgraves et al. 1999; Pitnick et al. 1999; Morrow and Gage 2000; Miller and Pitnick 2002). For example, in *Drosophila melanogaster*, females that were artificially selected to have longer sperm storage organs preferentially used longer sperm for fertilization, and consequently males evolved longer sperm (Miller and Pitnick 2002). There was no difference in our evolution populations of guppies in female body length between fish that were acclimated to 25 or 28 °C (Breckels and Neff 2013) or after 6 months in the evolution populations (data not shown). Assuming that body length is an indicator of female reproductive tract length or sperm storage organ size (micropockets in guppies, Kobayashi and Iwamatsu 2002), females from the two evolution temperatures should not differ in those traits. Consequently, female reproductive morphology might impose strong stabilizing selection and influence the evolutionary response in sperm length. Interestingly, the *28-25* treatment males produced significantly longer sperm than either the 25 or the 28 °C evolution population males. It remains to be seen if those males gain higher reproductive success than the 25 or 28 °C evolution population males or whether their sperm are in fact too long and selected against via the females' reproductive tract. Certainly, investigating the role that female reproductive tract length and the micropockets play on selection of sperm traits in guppies, and its potential to explain the compensatory response we observed on sperm length, is an exciting area for future research.

Understanding the genetic covariance between traits is fundamental because it can determine the response of the traits to selection (Lynch and Walsh 1998). At the phenotypic level, sperm length is often correlated with sperm velocity (e.g. Gomendio and Roldan 1991; Malo et al. 2006; Fitzpatrick et al. 2009; but see Humphries et al. 2008). However, little is known about the genetic covariance between these two traits (for exceptions see Mossman et al. 2009 and Evans 2011). Our results suggest that there is minimal genetic covariance between sperm length and velocity as length responded to our temperature treatment independent of velocity. Furthermore, our results may indicate that the traditional kinematics associated with sperm length and velocity can be disassociated, perhaps mediated by changes in sperm energetics (e.g. Burness et al. 2005).

In conclusion, the results of our study show that the short-term effects of the increased temperature predicted for the end of the century could have negative impacts for reproduction in a tropical ectotherm. However, we found evidence of an evolved response in sperm length after only 6 months or a maximum of four generations. This genetic response indicates that guppies can respond to climate warming via rapid evolution, at least for some reproductive traits.

Acknowledgments We thank J. Hung and M. Lau for their help with conducting sperm video analyses and S. Garner, T. Hain, N. Muñoz, C. Partridge, and three anonymous reviewers for providing comment that improved the manuscript. This research was funded by Natural Sciences and Engineering Research Council of Canada.

References

Adriaenssens B, van Damme R, Seebacher F, Wilson RS (2012) Sex cells in changing environments: can organisms adjust the physiological function of gametes to different temperatures? Glob Chang Biol 18:1797–1803

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

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

Beirão J, Soares F, Herráez MP, Dinis MT, Cabrita E (2011) Changes in *Solea senegalensis* sperm quality throughout the year. Anim Reprod Sci 126:122–129

Berger D, Bauerfeind SS, Blanckenhorn WU, Schäfer MA (2011) High temperatures reveal cryptic genetic variance in a polymorphic female sperm storage organ. Evolution 65:2830–2842

Binet MT, Doyle CJ (2013) Effect of near-future seawater temperature rises on sea urchin sperm longevity. Mar Freshw Res 64:1–9

Blanckenhorn WU, Hellriegel B (2002) Against Bergmann's rule: fly sperm increases with temperature. Ecol Lett 5:7–10

Boschetto C, Gasparini C, Pilastro A (2011) Sperm number and velocity affect sperm competition success in the guppy (*Poecilia reticulata*). Behav Ecol Sociobiol 65:813–821

Bradshaw WE, Holzapfel CM (2006) Climate change—evolutionary response to rapid climate change. Science 312:1477–1478

Breckels RD, Neff BD (2010) Pollution-induced behavioural effects in the brown bullhead (*Ameiurus nebulosus*). Ecotoxicology 19:1337–1346

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

Breckels RD, Garner SR, Neff BD (2014) Rapid evolution in response to increased temperature maintains population viability despite genetic erosion in a tropical ectotherm. Evol Ecol 28:141–155

Briskie JV, Montgomerie R (1992) Sperm size and sperm competition in birds. Proc R Soc B 247:89–95

Burness G, Moyes CD, Montgomerie R (2005) Motility, ATP levels and metabolic enzyme activity of sperm from bluegill (*Lepomis macrochirus*). Comp Biochem Physiol 140:11–17

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

Casselman SJ, Schulte-Hostedde AI, Montgomerie R (2006) Sperm quality influences male fertilization success in walleye (*Sander vitreus*). Can J Fish Aquat Sci 63:2119–2125

Deutsch CS, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR (2008) Impacts of climate warming on terrestrial ectotherms across latitude. PNAS 105:6668–6672

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

Dorts J, Grenouillet G, Douxfils J, Mandiki SNM, Milla S, Silvestre F, Kestemont P (2012) Evidence that elevated water temperature affects the reproductive physiology of the European bullhead *Cottus gobio*. Fish Physiol Biochem 38:389–399

Emlen ST, Oring LW (1977) Sexual selection, and the evolution of mating systems. Science 197:215–223

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

Evans JP (2009) No evidence for sperm priming responses under varying sperm competition risk or intensity in guppies. Naturwissenschaften 96:771–779

Evans JP (2011) Patterns of genetic variation and covariation in ejaculate traits reveal potential evolutionary constraints in guppies. Heredity 106:869–875

Fitzpatrick JL, Montgomerie R, Desjardins JK, Stiver KA, Kolm N, Balshine S (2009) Female promiscuity promotes the evolution of faster sperm in cichlid fishes. PNAS 106:1128–1132

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

Gage MJG, Macfarlane CP, Yeates S, Ward RG, Searle JB, Parker GA (2004) Spermatozoal traits and sperm competition in *Atlantic salmon*: relative sperm velocity is the primary determinant of fertilization success. Curr Biol 14:44–47

Gasparini C, Marino IAM, Boschetto C, Pilastro A (2010) Effect of male age on sperm traits and sperm competition success in the guppy (*Poecilia reticulata*). J Evol Biol 23:124–135

Gomendio M, Roldan ERS (1991) Sperm competition influences sperm size in mammals. Proc R Soc B 243:181–185

Hendry AP, Hensleigh JE, Reisenbichler RR (1998) Incubation temperature, developmental biology, and the divergence of sockeye salmon (*Oncorhynchus nerka*) within Lake Washington. Can J Fish Aquat Sci 55:1387–1394

Hendry AP, Wenburg JK, Bentzen P, Volk EC, Quinn TP (2000) Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science 290:516–518

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

Humphries S, Evans JP, Simmons LW (2008) Sperm competition: linking form to function. BMC Evol Biol 8:319

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

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

Kime DE, Van Look KJW, McAllister BG, Huyskens G, Rurangwa E, Ollevier F (2001) Computer-assisted sperm analysis (CASA) as a tool for monitoring sperm quality in fish. Comp Biochem Physiol C 130:425–433

Kobayashi H, Iwamatsu T (2002) Fine structure of the storage micropocket of spermatozoa in the ovary of the guppy *Poecilia reticulata*. Zool Sci 19:545–555

Lahnsteiner F, Mansour N (2012) The effect of temperature on sperm motility and enzymatic activity in brown trout *Salmo trutta*, burbot *Lota lota* and grayling *Thymallus thymallus*. J Fish Biol 81:197–209

Leal M, Gunderson AR (2012) Rapid change in thermal tolerance of a tropical lizard. Am Nat 180:815–822

Lüpold S, Manier MK, Berben KS, Smith KJ, Daley BD, Buckley SH, Belote JM, Pitnick S (2012) How multiple ejaculate traits determine competitive fertilization success in *Drosophila melanogaster*. Curr Biol 22:1667–1672

Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates Inc, Sunderland

Malo AF, Gomendio M, Garde J, Lang-Lenton B, Soler AJ, Roldan ERS (2006) Sperm design and sperm function. Biol Lett 2:246–249

Miller GT, Pitnick S (2002) Sperm-female coevolution in *Drosophila*. Science 298:1230–1233

Morrow EH, Gage MJG (2000) The evolution of sperm length in moths. Proc R Soc B 267:307–313

Mossman J, Slate J, Humphries S, Birkhead T (2009) Sperm morphology and velocity are genetically codetermined in the zebra finch. Evolution 63:2730–2737

Parker GA (1993) Sperm competition games: sperm size and number under adult control. Proc R Soc B 253:245–254

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annu Rev Ecol Evol Syst 37:637–669

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42

Pilastro A, Evans JP, Sartorelli S, Bisazza A (2002) Male phenotype predicts insemination success in guppies. Proc R Soc B 269:1325–1330

Pitnick S, Markow T, Spicer GS (1999) Evolution of multiple kinds of female sperm-storage organs in Drosophila. Evolution 53:1804–1822

Presgraves DC, Baker RH, Wilkinson GS (1999) Coevolution of sperm and female reproductive tract morphology in stalk-eyed flies. Proc R Soc B 266:1041–1047

Réale D, Berteaux D, McAdam AG, Boutin S (2003) Lifetime selection on heritable life-history traits in a natural population of red squirrels. Evolution 57:2416–2423

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

Simmons LW, Fitzpatrick JL (2012) Sperm wars and the evolution of male fertility. Reproduction 144:519–534

Simmons LW, Moore AJ (2009) Evolutionary quantitative genetics of sperm. In: Birkhead TR, Hosken DJ, Pitnick S (eds) Sperm biology: an evolutionary perspective. Elsevier, Oxford, pp 405–434

Singh B (1997) Climate-related global changes in the southern Caribbean: Trinidad and Tobago. Glob Planet Chang 15:93–111

Snook R (2005) Sperm in competition: not playing by the numbers. Trends Ecol Evol 20:45–63
Stefan HG, Preudhomme EB (1993) Stream temperature estimation from air–temperature. J Am Water Resour Assoc 29:27–45
Stillman JH (2003) Acclimation capacity underlies susceptibility to climate change. Science 301:65
Stoltz JA, Neff BD (2006) Sperm competition in a fish with external fertilization: the contribution of sperm number, speed and length. J Evol Biol 19:1873–1881
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
West PM, Packer C (2002) Sexual selection, temperature, and the lion's mane. Science 297:1339–1343
Williot P, Kopeika EF, Goncharov BF (2000) Influence of testis state, temperature and delay in semen collection on spermatozoa motility in the cultured Siberian sturgeon (*Acipenser baeri* Brandt). Aquaculture 189:53–61
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 Chang Biol 18:1833–1842