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RESEARCH ARTICLE

The effects of elevated temperature on the sexual traits, immunology and survivorship of a tropical ectotherm

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SUMMARY

In 2007, the Intergovernmental Panel on Climate Change projected an average global air temperature increase of 1.1–6.4°C by the end of the 21st century. Although the tropics are predicted to experience less extreme temperature increases than regions of higher latitude, tropical ectotherms live close to their thermal limits, and are thus particularly vulnerable to increases in temperature. In this study, we examined how predicted patterns of global warming will affect survival and sexual traits in the Trinidadian guppy (*Poecilia reticulata*). Guppies were exposed from birth to one of four temperature treatments: 23, 25 (control), 28 or 30°C. We measured brood survival and, at sexual maturity, male ornamentation, sperm traits and immune response. Our results show that increases in temperature result in guppies that have shorter, slower sperm but that there is an optimum temperature for ornamental hue at 28°C. Given the importance of sperm quality for reproduction, these results suggest population viability could be affected by warming. However, we found no difference in brood survival or immune response to a novel antigen across the treatments, indicating that survival may not be as vulnerable as previously thought. Overall, our data suggest that male sexual traits, and in particular sperm performance, are more sensitive than survival to a warming environment.

Key words: global warming, phenotypic plasticity, *Poecilia reticulata*, survival, sperm, ornamentation, immune response.

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INTRODUCTION

One of the most ubiquitous environmental conditions that broadly impacts organisms is temperature (Dorts et al., 2012). In 2007, the Intergovernmental Panel on Climate Change projected an average global air temperature increase of 1.1–6.4°C by the end of the 21st century (IPCC, 2007), potentially exceeding the rate of warming at any point in the fossil record (Allan et al., 2005). In particular, global warming may have a significant physiological impact on tropical ectotherms because these species tend to be adapted to fairly constant external temperatures and have a narrow temperature performance breadth (Deutsch et al., 2008; Angilletta, 2009; Dillon et al., 2010). Additionally, the effects of global warming may be exacerbated because many ectotherms live in warm environments and are closer to their thermal tolerance limit (Stillman, 2003). Thus, even a small increase in temperature is likely to have a serious negative impact on their physiological processes. The projected increase in temperature is also likely to have ecological impacts, including reduced food availability, which can be confounded by thermally induced increases in the metabolic rate of ectotherms. Consequently, less energy may be available for other important functions, including reproduction, potentially altering the demographics of populations (Deutsch et al., 2008; Daufresne et al., 2009; Dillon et al., 2010).

The biological impacts of climate change have already been documented (Angilletta, 2009); for example, the level of warming has caused many species to shift their ranges or alter their phenology (reviewed in Parmesan, 2006). Indeed, almost 60% of nearly 1600 species studied have exhibited a shift in their ranges or phenology over the past 20–140 years, predominantly in the direction expected from climate change (Parmesan and Yohe, 2003). However, for many species there are barriers to dispersal such as mountain ranges or dams. These organisms will instead have to cope by adapting to the increased temperature or face extinction (Fuller et al., 2010). Organisms can adapt genetically or rely on phenotypic plasticity to cope in the warmer environment. Phenotypic plasticity is the first response to climate change and will be the only response for many long-lived species (Bradshaw and Holzapfel, 2006; Fuller et al., 2010). Therefore, understanding the extent of plasticity is of crucial importance to better understand the fate of organisms in warming environments (Somero, 2010).

Sexual traits including sperm performance are key determinants of male reproductive success but exposure to elevated temperatures has the capacity to alter these traits (Alavi and Cosson, 2005; Dorts et al., 2012). Increased temperatures have been shown to result in decreased sperm motility (e.g. Williot et al., 2000), decreased sperm number (e.g. Zeh et al., 2012) and, in one study, increased sperm length (e.g. Blanckenhorn and Hellriegel, 2002); most other stressors have, in contrast, been shown to lead to decreased sperm length (e.g. Dey et al., 2009; Immler et al., 2010). These changes subsequently can have a significant impact on male reproductive success (Billard, 1978; Stoss, 1983; Gage et al., 2004; Alavi and Cosson, 2005). In addition, temperature may affect secondary sexual characters that are important sexual traits because they act as an honest signal of male quality and aid females in choosing mates (Kortet et al., 2004). Borg found that the decline of secondary sexual characters during the summer is accelerated by high temperatures in the three-spined stickleback, *Gasterosteus aculeatus* (Borg, 1982). Furthermore, Brian et al. found that an optimum temperature for male secondary sexual characteristics exists in the fathead minnow (*Pimephales promelas*) (Brian et al., 2011). Therefore,

temperature may have the potential to affect both pre- and post-copulatory processes during reproduction.

A rise in temperature is also predicted to result in an increase in the transmission, growth rate and virulence of parasites and pathogens (Harvell et al., 2002; Marcogliese, 2008; Harvell et al., 2009; Dang et al., 2012). The immune system is highly sophisticated and has evolved to defend hosts against the debilitating effects of pathogens and parasites (Møller and Saino, 2004). However, variation in temperature can have marked effects on immunological function and effectiveness: increased temperatures can affect the antibacterial activity, antimicrobial activity and parasite resistance of a host (e.g. Collazos et al., 1996; Lamková et al., 2007; Dang et al., 2012). Indeed, Collazos et al. found that the immune response to phytohaemagglutinin (PHA) is compromised at higher temperatures in the tench, *Tinca tinca* (Collazos et al., 1996). PHA, a protein derived from red kidney beans, is commonly used as a novel antigen to test T-cell proliferation (e.g. Collazos et al., 1996; Bayyari et al., 1997; Ardia and Clotfelter, 2006). The PHA-induced immune response has also been linked directly to parasite resistance (Bayyari et al., 1997). Exposure to PHA thereby provides a simple but effective test of an organism's innate immune response.

The projected change in air temperature will also result in a change in water temperature (e.g. Stefan and Preudhomme, 1993; Caissie et al., 2001). The magnitude of the change in water temperature, however, will depend upon several factors including the location and volume of the water body. Small, shallow streams are likely to experience similar changes to air temperature, whereas large water bodies, such as oceans, will take longer to respond (Ficke et al., 2007). Indeed, long-term increases in river and stream water temperature are strongly correlated to long-term increases in air temperature (Kaushal et al., 2010). Consequently, global warming will be more problematic for obligate freshwater organisms. For fish, this problem is further compounded because of their poikilothermic nature whereby their basic physiology is directly dependent on the temperature of their environment. Given the potential negative impacts that global warming might have, studies addressing the short- and long-term effects of the increased temperature are needed.

Here, we used the Trinidadian guppy (*Poecilia reticulata*, Peters 1860) as a model poikilothermic fish to examine the effects of increased temperature, as projected for 2100. Guppies are a small, polygamous, live-bearing fish native to north-eastern South America and the Caribbean. They inhabit small freshwater streams and pools that flow through lowland and montane rain forests (Houde, 1997). Currently, the mean air temperature in Trinidad is 27.7°C and fluctuates by 2.0°C annually between the coldest months (January and February, 26.5°C) and the warmest month (May, 28.5°C), while the diel temperature fluctuates by ~8.4°C (mean values calculated between January 1992 and December 2012; weatheronline.co.uk). Because of the physical nature of water, short-term temperature variations in water are usually smaller than short-term temperature variations in air (Caissie et al., 2001; Kaushal et al., 2010). The mean water temperature of rivers in Trinidad is ~25°C and ranges between 20 and 28°C (Alkins-Koo, 2000). Over the past 60 years, Trinidad has experienced a mean rise in air temperature of 1.5°C (Singh, 1997), and the temperature is projected to increase by 1.0–3.5°C by the end of the 21st century (Water Resources Agency, 2001). However, variation in temperature is set to decrease as night-time and winter temperatures are projected to increase more than daytime and summer temperatures (IPCC, 2007). Geographical barriers, such as waterfalls and oceans, mean that natural dispersal for individuals within Trinidadian streams is unfeasible. Therefore guppies, like many other poikilotherms, will largely have to rely on phenotypic plasticity in order to respond to global warming.

The objective of this study was to assess brood survival and to detail the phenotypic plasticity of sperm length, sperm velocity, male ornamentation and immune response in guppies exposed to increased temperatures. We exposed guppies from birth to one of four temperature treatments: 23°C to represent a cooler climate, 25°C (control), and 28 or 30°C to represent average or upper projected temperatures for the year 2100, respectively. We hypothesized that there would be an effect of increased temperature on survivorship and reproductive traits. We predicted that exposure to increased temperatures would result in decreased brood survival, sperm length, sperm velocity, male ornament quality and immune response.

MATERIALS AND METHODS

Experiments were conducted following ethical guidelines as implemented by the Canadian Council of Animal Care and were approved by the Animal Use Subcommittee at the University of Western Ontario. Guppies used in this experiment were descendants of fish that were collected in 2003 from a tributary of the Paria River in the Northern Range, Trinidad (10°44′42″N; 61°15′42″W). All guppies were kept at a constant temperature of 25±0.6°C to represent natural conditions (Alkins-Koo, 2000). Pregnant females were put into individual 10 l tanks until they gave birth. The number of offspring at birth and again after 3 months was recorded in order to get an estimate of brood survival. Approximately 24 h after the females gave birth (allowing time for the birth of the entire brood) they were removed from the tanks so only the offspring remained. The temperature in the tanks was then set to one of four temperatures: 30°C to represent the upper range of future climate predictions for the end of the century, 28°C to represent average future climate predictions for the end of the century, 25°C to act as a control and 23°C to represent a cooler climate.

Sperm analysis

At 3 months of age (mean ± s.d. age: 95.8±7.0 days), a subset of males were removed from their tanks and put into individual isolation chambers set at the temperature in which they were acclimated for 3 days to ensure full sperm reserves (Pilastro et al., 2002). Males were then anaesthetized with MS-222 and 'pat-dried' to remove all excess MS-222 from their skin. The males were placed under a dissection microscope with their gonopodium swung forward and 40 µl of sperm extender medium (207 mmol l^{-1} NaCl, 5.4 mmol l^{-1} KCl, 1.3 mmol l^{-1} $CaCl_2$, 0.49 mmol l^{-1} $MgSO_4$, 10 mmol l^{-1} Tris, pH 7.5) was added to the base of the gonopodium (Evans, 2009). Gentle pressure was applied to the side of the abdomen, anterior to the base of the gonopodium, to release all sperm bundles into the extender medium. The sperm was then activated using 40 µl of 150 mmol l^{-1} KCl solution with 2 mg l^{-1} BSA, which helps to prevent sperm from sticking to the slide. Two 15 µl aliquots of the sperm solution were immediately placed in a 2X-CEL sperm analysis chamber (Hamilton Thorne, Beverly, MA, USA) and put under a microscope. Digital images were recorded using an SI-C400N microscope video camera (Costar Imaging, Lakewood, CA, USA) for velocity analysis. Following methods outlined previously (Breckels and Neff, 2010), we extracted images from the recorded video at 10 frames s^{-1} and determined the two-dimensional coordinates using NIH ImageJ software (http://rsbweb.nih.gov/ij). Using the Pythagorean theorem, the distance travelled (µm) by a sperm cell in 1 s was calculated as the sum of the distances travelled between the 11 consecutive frames in that second. The total distance travelled by each sperm in 1 s is called the curvilinear velocity (V_{CL},

$\mu m\,s^{-1}$). We then calculated the straight line velocity (V_{SL}) of the sperm by determining the distance travelled between the first and the last of the 11 consecutive frames. Finally, we calculated the path linearity by dividing the V_{SL} by the V_{CL}. A path linearity value of 0 represents a sperm that started and ended at the same point whereas a value of 1 represents a sperm that travelled in a straight line (see Stoltz and Neff, 2006; Kime et al., 2001). We measured the V_{CL}, V_{SL} and path linearity of 10 sperm per individual.

Next, a 20 µl aliquot of the sperm solution was put onto a slide and covered with a coverslip. The slide was viewed under a microscope at 400× magnification and digital images were taken. Images were analysed in UTHSCSA Image Tool software v. 3.0 (http://compdent.uthscsa.edu/dig/itdesc.html). The tail length, including flagellum and mid-piece, of 30 sperm per male was measured.

Ornament analysis

Female guppies tend to respond favourably to males with larger and more intense orange spots on their body (Kodric-Brown, 1985; Kodric-Brown, 1989; Houde, 1997). Thus, we examined the impact of temperature on both orange spot area and colour intensity. At the same time as the sperm analysis measurements, a photograph was taken of each guppy on a white background with a dark blue paint chip and a ruler, which acted as a scale. Images were then analysed using ImageJ in order to calculate the length of each fish and the proportion of orange on their bodies. For length measurements, fish were measured from the tip of the snout to the end of the caudal peduncle. For the proportion of orange measurements, the outline of the fish was traced in order to get an estimate of the area. Then, each orange spot on the body of the fish was traced and summed to obtain total orange cover. All measurements were repeated three times and then averaged. The value was then divided by the mean fish area to express the cover as a proportion of body size.

To measure the hue, saturation and brightness (HSB) of the orange pigmentation, pictures were analysed using Adobe Photoshop CS3 (San Jose, CA, USA). Each photograph was standardized for lighting conditions (see Villafuerte and Negro, 1998) by recording mean values of red, green and blue (RGB) for the light background and the dark paint chip. Next, the mean RGB values were recorded for the orange pigmentation on the guppies and standardized. From these values we were able to calculate the standardized HSB values for each guppy (Villafuerte and Negro, 1998).

Immune response

To evaluate the immune response, a separate subset of fish from each temperature treatment were injected with PHA and their swelling response was recorded. The PHA swelling response provides a measure of T-cell proliferation and has also been linked to parasite resistance (Bayyari et al., 1997; Ardia and Clotfelter, 2006). After roughly 8 months of age (mean ± s.d. age: 236±43 days), both male and female guppies were anaesthetized using MS-222 and length measurements were taken as detailed above. Next, the guppies were placed under a dissection microscope and the width of the caudal peduncle, in line with the end of the dorsal fin, was measured independently three times for accuracy with a digital calliper (0.01 mm accuracy). The guppies were then injected in the same area with 4 µg PHA, in 2 µl phosphate-buffered saline (PBS) using a 10 µl, 26 gauge syringe (Hamilton Company, Reno, NV, USA). Another subset of guppies, reared at 25°C, were either injected with the needle only or received a dose of PBS without PHA and acted as control groups. The guppies were then put in isolation chambers to avoid contact with other fish, with the temperature set to the temperature that they had been acclimated to, for 24 h. The fish were then anaesthetized again and the caudal peduncle was re-measured as above to determine the swelling response. The immune response of each individual was recorded as the difference in swelling between post- and pre-injection.

Statistical analyses

All statistical analyses were performed using SPSS v. 20 (SPSS Inc., Chicago, IL, USA) or Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA) and all presented *P*-values are two-tailed probabilities. Brood survival, orange cover and sperm path linearity were transformed using logit transformations. A one-way ANOVA was performed to compare brood survival among the four temperature treatments. General linear mixed models (GLMMs) were performed to compare male body length, sperm length, V_{CL}, V_{SL}, path linearity, orange cover, HSB and immune response among the four temperature treatments. Family identification (ID) was included as a random factor and body length was included as a covariate for all tests. Because there was variation in the age of the fish tested in the immune response trials and because we used both sexes, we included sex as an additional fixed factor and age as a covariate. For *post hoc* analysis we used a Tukey's *b*-test. Finally, we preformed linear contrast analyses for the four different sperm traits in order to determine whether there was a linear relationship with temperature.

RESULTS

The number of families reared at 23, 25, 28 and 30°C was 13, 21, 12 and 21, producing mean brood sizes of 6.7, 4.9, 4.8 and 5.5 offspring, respectively. There was no difference in brood survival among the four temperature treatments (mean ± s.d. brood survival: 23°C, 0.81±0.21; 25°C, 0.72±0.29; 28°C, 0.94±0.12; and 30°C, 0.74±0.28; $F_{3,63}$=1.4, P=0.258). A total of 82 fish were used for the sperm trials and ornament analysis (23°C, N=11; 25°C, N=27; 28°C, N=19; and 30°C, N=25). Family ID had a significant effect on male body length at 3 months of age ($F_{13,65}$=2.0, P=0.040) and there was also a significant effect of temperature ($F_{3,65}$=5.0, P=0.003). Interestingly, males in the 23 and 28°C treatments were significantly longer than those in the 25 and 30°C treatments (mean ± s.e.m. length: 23°C, 15.2±0.6 mm; 25°C, 13.9±0.3 mm; 28°C, 15.1±0.4 mm; 30°C, 13.9±0.2 mm).

Sperm analysis

Male body length had no effect on sperm length, V_{CL} or V_{SL}, and neither body length nor family ID had an effect on sperm path linearity across the four treatments (P>0.05 for all). However, family ID had a positive effect on sperm length, V_{CL} and V_{SL} ($F_{13,64}$=2.3, P=0.008; $F_{13,59}$=1.9, P=0.047; and $F_{13,59}$=2.2, P=0.023, respectively). There was a significant decrease in average sperm length with increasing temperature ($F_{3,64}$=38.3, P<0.001), with the 30°C acclimated fish producing significantly shorter sperm than the 28°C acclimated fish, which in turn produced significantly shorter sperm than both the 23 and 25°C acclimated fish (Fig. 1A). Similarly, there was a significant decrease in V_{CL} and V_{SL} with increasing temperature ($F_{3,59}$=7.8, P<0.001 and $F_{3,59}$=8.0, P<0.001, respectively), with 30°C acclimated fish showing significantly decreased V_{CL} and V_{SL} compared with those of fish from the other three temperatures (Fig. 1B,C). Sperm path linearity also decreased significantly with increasing temperature ($F_{3,59}$=3.8, P=0.015; Fig. 1D), with the 23°C acclimated fish displaying a greater path linearity than both the 28 and 30°C acclimated fish. Additionally,

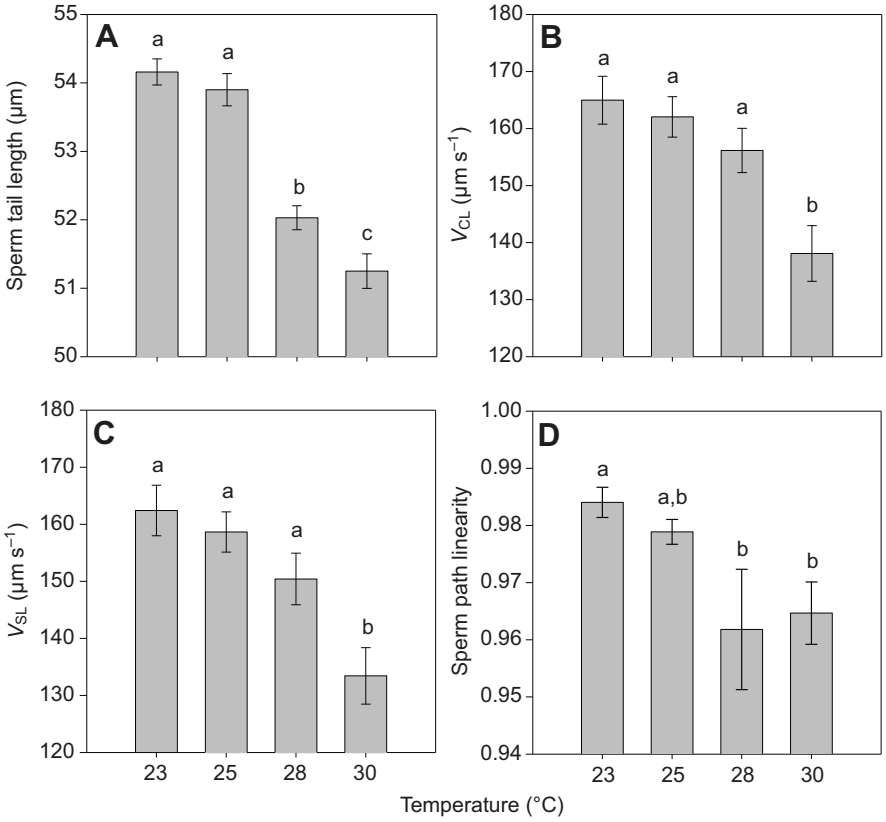

Fig. 1. Sperm measurements of guppies (*Poecilia reticulata*) reared from birth at one of four temperatures. Shown are means ± s.e.m. for (A) sperm length, (B) curvilinear velocity V_{CL}, (C) straight line velocity V_{SL} and (D) path linearity. Error bars with different letters are significantly different (P<0.05) according to a Tukey's *b* HSD test.

sperm length, V_{CL}, V_{SL} and path linearity all declined linearly with increasing temperature ($F_{1,78}$=75.9, P<0.001; $F_{1,73}$=15.7, P<0.001; $F_{1,73}$=17.7, P<0.001; and $F_{1,73}$=12.0, P=0.001, respectively).

Ornament analysis

There was no effect of family ID on orange cover, saturation or brightness, nor was there an effect of body length on orange cover, hue or saturation (P>0.005 for all). There was also no effect of temperature on orange cover or saturation (mean ± s.e.m. orange cover: 23°C, 5.2±0.6%; 25°C, 6.3±0.5%; 28°C, 5.6±0.6%; and 30°C, 6.3±0.6%; $F_{3,64}$=0.7, P=0.548; mean ± s.e.m. saturation: 23°C, 0.87±0.01; 25°C, 0.91±0.02; 28°C, 0.82±0.03; and 30°C, 0.86±0.02; $F_{3,64}$=2.0, P=0.120). There was, however, an effect of family ID ($F_{3,64}$=2.9, P=0.002) and temperature on hue ($F_{3,64}$=17.5, P<0.001; Fig. 2), with the 28°C fish displaying a significantly greater hue than fish from all other treatments. The 25°C fish displayed a significantly greater hue than the 30°C fish, whereas the 23°C fish did not show a significant difference in hue from either the 25 or 30°C fish. There was an effect of body length on brightness ($F_{1,64}$=7.2, P=0.009), but temperature had no effect (mean ± s.e.m. brightness: 23°C, 0.45±0.02; 25°C, 0.45±0.01; 28°C, 0.44±0.01; and 30°C, 0.41±0.01; $F_{3,64}$=1.0, P=0.487).

Immune response analysis

A total of 156 fish were used from 65 families in the immune response trials (control, N=13; PBS control, N=10; 23°C, N=38; 25°C, N=35; 28°C, N=27; and 30°C, N=33). Age, length and family ID had no effect on PHA swelling response (P>0.05 for all). Although there was a significant increase in PHA swelling response between the two controls and the four temperature treatments ($F_{5,121}$=4.4, P=0.001; Fig. 3), there was no difference in swelling response among the four temperature treatments. Additionally, males produced a significantly larger swelling response than did females ($F_{1,121}$=4.8, P=0.031).

DISCUSSION

Climate change, particularly the increased temperature predicted for the end of the century, has the potential to alter many life history traits, including juvenile survival (e.g. Zeh et al., 2012) (reviewed in Pepin, 1991). Although temperature can impact many aspects of natural ecosystems (reviewed in Ficke et al., 2007; IPCC, 2007), establishing its direct effect on physiology and survival is a crucial first step in discerning the impact of climate change on natural populations. A previous study suggested that guppies have lower juvenile survival rates at temperatures of 29°C and above (Karayucel et al., 2008). However, in our study we found no difference among temperature treatments in terms of brood survival. This discrepancy may be because the other study (Karayucel et al., 2008) used commercial aquarium fish that had been selectively bred for their elaborate pigmentation and fins (Karayucel et al., 2006), whereas we used guppies caught from the wild and maintained in a large stock population without any artificial selection. Taken together, our study and that of Karayucel and colleagues (Karayucel et al., 2008) suggest that the elaboration of sexual ornaments affects survival, particularly in warmer environments, indicating that they are costly (Andersson, 1994). The discrepancy between the two studies may also reflect differences in genomic diversity, as aquarium guppies tend to be highly inbred as a result of selective breeding whereas wild-caught guppies have a much higher level of genetic variation (e.g. Bleakley et al., 2008). Thus, wild-caught guppies could potentially have broader thermal limits than aquarium fish, allowing them to survive at higher temperatures. Regardless, we found no evidence to suggest that temperature increases as predicted for the end of the century will have a significant effect on brood survival in guppies.

Secondary sexual characters influence female mate choice because they can act as an honest signal of male quality (Andersson, 1994). Brian and colleagues found that there was an optimum

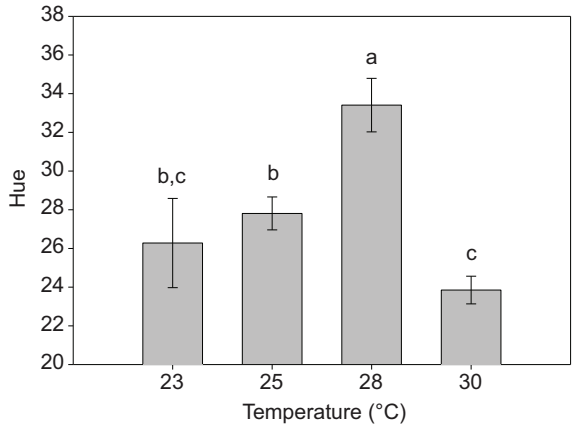

Fig. 2. Ornament hue of *P. reticulata* reared from birth at one of four temperatures. Shown are means ± s.e.m. Error bars with different letters are significantly different (P<0.05) according to a Tukey's *b* HSD test.

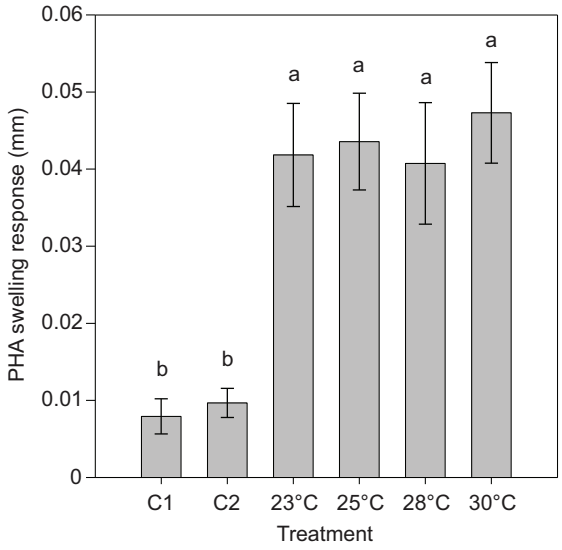

Fig. 3. Phytohaemagglutinin (PHA) swelling response of *P. reticulata* controls (C1, needle only; C2, phosphate-buffered saline injection) and those reared from birth at one of four temperatures. Shown are means ± s.e.m. Error bars with different letters are significantly different (P<0.05) according to a Tukey's *b* HSD test.

temperature for ornamentation in fathead minnows that was slightly higher than the native temperature (Brian et al., 2011). Our results show that ornament hue was highest at 28°C, higher than the mean natural temperature of 25°C. While it has been documented that hue is an important factor in female mate choice for many species [e.g. Chinook salmon, *Oncorhynchus tshawytscha* (Neff et al., 2008) and the blue crab, *Callinectes sapidus* (Baldwin and Johnsen, 2009)], its role in mate choice for guppies is less well known. One study at least suggests that female guppies instead prioritize the area of orange and colour saturation over hue (Karino et al., 2010), yet we found no effect of temperature on those two aspects of ornamentation. It is conceivable that ornamentation traits subject to intense sexual selection become canalized from environmental stressors such as the increased temperature in our study. This then brings into question whether the signals are, in fact, honest. Indeed, Candolin found that the condition of male three-spined sticklebacks displayed a curvilinear relationship with ornament quality; males of both good and poor condition had larger ornaments than males of intermediate condition (Candolin, 1999). Our results show that male guppies reared at higher temperatures had lower quality sperm (a key component of fertility in the guppy) (e.g. Boschetto et al., 2011) but their ornament, as measured by orange colour and saturation, was unaffected, suggesting that these aspects of the secondary sexual character may not be honest signals of quality.

Zeh and colleagues have claimed that the 'Achilles' heel' for tropical ectotherms will be reproduction in a warming climate (Zeh et al., 2012). They (Zeh et al., 2012) found that with slight increases in temperature (3.5°C), male neotropical pseudoscorpions, *Cordylochernes scorpioides*, produced half the sperm load of controls, and females failed to reproduce at all. Lahnsteiner and Mansour similarly found that sperm velocity decreased in both brown trout, *Salmo trutta*, and burbot, *Lota lota*, as temperature increased across a biologically relevant range (Lahnsteiner and Mansour, 2012). We found that increased temperature significantly decreased sperm length, V_{CL} and V_{SL}. Sperm length and velocity are key determinants of fertilization in many ectotherms (Billard, 1978; Stoss, 1983; Gage et al., 2004; Alavi and Cosson, 2005). Indeed, a positive relationship has been found for sperm velocity and fertilization success in internally or externally fertilizing fish (Gage et al., 2004; Casselman et al., 2006; Gasparini et al., 2010). Additionally, sperm length is often positively correlated to sperm velocity (e.g. Gomendio and Roldan, 1991; Malo et al., 2006; Fitzpatrick et al., 2009). Thus, our results indicate that reproduction could be compromised in a warmer environment, supporting the claim that reproduction is the Achilles' heel for tropical ectotherms (Zeh et al., 2012).

Many studies suggest that global warming has the potential to negatively affect the immune system (e.g. Collazos et al., 1996; Dang et al., 2012). Indeed, Collazos and colleagues found that seasonal variation in temperature affects the immune response to PHA in the tench, with the increased summer temperatures experienced by the fish causing a decreased immunological response compared with winter temperatures (Collazos et al., 1996). However, Le Morvan-Rocher and colleagues found no effect of increased temperature on the PHA response in carp, *Cyprinus carpio* (Le Morvan-Rocher et al., 1995). Our results agree with those of Le Morvan-Rocher and colleagues (Le Morvan-Rocher et al., 1995) as we found no evidence of a reduced PHA swelling response at increased temperatures. This apparent difference in results from those of Collazos and colleagues (Collazos et al., 1996) may reflect the experimental manipulation of our studies whereas Collazos and colleagues studied the effects of natural, seasonal variation. Tench breed in the summer so their immune system may be downregulated during this period as resources are shifted to reproduction (e.g. Fedorka et al., 2004; Whitton, 1982; Moret and Schmid-Hempel, 2000). Regardless, our results suggest that the innate immune system of guppies may be able to cope with the projected temperature increase for the end of the century, at least as measured by the swelling response to a novel antigen.

In our study, we found that increased temperatures affected some sexual traits (sperm characteristics and ornament hue) but not aspects of immune function or survival. It is possible that, at the elevated temperatures, guppies channel resources to upregulate their immune system, which then leaves their reproductive system more susceptible to immunological attack (Folstad and Sharstein, 1997). Indeed, the immunocompetence handicap hypothesis (Folstad and

Karter, 1992) states that sperm cells are considered non-self and are therefore subject to attack from the immune system (reviewed in Kosuda and Bigazzi, 1987). To counter attacks on sperm cells, males can release elevated levels of gonadal androgens, which act to downregulate the immune system (Folstad and Sharstein, 1997). We did not, however, directly measure immune cell proliferation or circulating androgen levels in our fish and therefore cannot confirm whether our data support the immunocompetence handicap hypothesis. Our results might also just reflect a trade-off between reproduction and immunity (and potentially other life history traits) with the latter taking precedence over reproductive traits in guppies that are thermally stressed.

In conclusion, the results of our study suggest that the temperature rise predicted by the end of the century has no effect on immunity or survival in the guppy. Conversely, the increased temperature could have a significant impact on reproduction in this fish. We found that increased temperatures resulted in decreased sperm length and motility, which are key aspects of fertility. Our study thereby indicates that key sexual traits are more sensitive to elevated temperatures than are traits linked to survival. Future work might emphasize long-term experiments that examine potential maternal environmental effects (e.g. McAdam et al., 2002), epigenetic effects (e.g. Miller et al., 2012) and genetic adaptations (e.g. Réale et al., 2003) that could all help to ameliorate the negative impacts of climate change.

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AUTHOR CONTRIBUTIONS

R.D.B and B.D.N. conceived and designed the experiment, conducted the data analysis, and wrote the paper. R.D.B. performed the experiments and data collection.

COMPETING INTERESTS

No competing interests declared.

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REFERENCES

Alavi, S. M. H. and Cosson, J. (2005). Sperm motility in fishes. I. Effects of temperature and pH: a review. *Cell Biol. Int.* **29**, 101-110.
Alkins-Koo, M. (2000). Reproductive timing of fishes in a tropical intermittent stream. *Environ. Biol. Fishes* **57**, 49-66.
Allan, J. D., Palmer, M. and Poff, N. L. (2005). Climate change and freshwater ecosystems. In *Climate Change and Biodiversity* (ed. T. E. Lovejoy and L. Hannah), pp. 274-290. New Haven, CT: Yale University Press.
Andersson, M. B. (1994). *Sexual Selection*. Princeton, NJ: Princeton University Press.
Angilletta, M. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford: Oxford University Press.
Ardia, D. R. and Clotfelter, E. D. (2006). The novel application of an immunological technique reveals the immunosuppressive effect of phytoestrogens in *Betta splendens*. *J. Fish Biol.* **68**, 144-149.
Baldwin, J. and Johnsen, S. (2009). The importance of color in mate choice of the blue crab *Callinectes sapidus*. *J. Exp. Biol.* **212**, 3762-3768.
Bayyari, G. R., Huff, W. E., Balog, J. M. and Rath, N. C. (1997). Variation in toe-web response of turkey poults to phytohemagglutinin-P and their resistance to *Escherichia coli* challenge. *Poult. Sci.* **76**, 791-797.
Billard, R. (1978). Changes in structure and fertilizing ability of marine and freshwater fish spermatozoa diluted in media of various salinities. *Aquaculture* **14**, 187-198.
Blanckenhorn, W. U. and Hellriegel, B. (2002). Against Bergmann's rule: fly sperm increases with temperature. *Ecol. Lett.* **5**, 7-10.
Bleakley, B. H., Eklund, A. C. and Brodie, E. D. (2008). Are designer guppies inbred? Microsatellite variation in five strains of ornamental guppies, *Poecilia reticulata*, used for behavioral research. *Zebrafish* **5**, 39-48.
Borg, B. (1982). Seasonal effects of photoperiod and temperature on spermatogenesis and male secondary sexual characters in the 3-spined stickleback, *Gasterosteus aculeatus* L. *Can. J. Zool.* **60**, 3377-3386.
Boschetto, C., Gasparini, C. and Pilastro, A. (2011). Sperm number and velocity affect sperm competition success in the guppy (*Poecilia reticulata*). *Behav. Ecol. Sociobiol.* **65**, 813-821.
Bradshaw, W. E. and Holzapfel, C. M. (2006). Climate change. Evolutionary response to rapid climate change. *Science* **312**, 1477-1478.
Breckels, R. D. and Neff, B. D. (2010). Pollution-induced behavioural effects in the brown bullhead (*Ameiurus nebulosus*). *Ecotoxicology* **19**, 1337-1346.
Brian, J. V., Beresford, N., Margiotta-Casaluci, L. and Sumpter, J. P. (2011). Preliminary data on the influence of rearing temperature on the growth and reproductive status of fathead minnows *Pimephales promelas*. *J. Fish Biol.* **79**, 80-88.
Caissie, D., El-Jabi, N. and Satish, M. G. (2001). Modelling of maximum daily water temperatures in a small stream using air temperatures. *J. Hydrol.* **251**, 14-28.
Candolin, U. (1999). The relationship between signal quality and physical condition: is sexual signalling honest in the three-spined stickleback? *Anim. Behav.* **58**, 1261-1267.
Casselman, S. J., Schulte-Hostedde, A. I. and Montgomerie, R. (2006). Sperm quality influences male fertilization success in walleye (*Sander vitreus*). *Can. J. Fish. Aquat. Sci.* **63**, 2119-2125.
Collazos, M. E., Barriga, C. and Rincon, E. O. (1996). Seasonal variations in the immune system of the tench, *Tinca tinca* (Cyprinidae): proliferative response of lymphocytes induced by mitogens. *J. Comp. Physiol. B* **165**, 592-595.
Dang, V. T., Speck, P. and Benkendorff, K. (2012). Influence of elevated temperatures on the immune response of abalone, *Haliotis rubra*. *Fish Shellfish Immunol.* **32**, 732-740.
Daufresne, M., Lengfellner, K. and Sommer, U. (2009). Global warming benefits the small in aquatic ecosystems. *Proc. Natl. Acad. Sci. USA* **106**, 12788-12793.
Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C. and Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. USA* **105**, 6668-6672.
Dey, S., Kharbuli, S. M., Chakraborty, R., Bhattacharyya, S. P. and Goswami, U. C. (2009). Toxic effect of environmental acid-stress on the sperm of a hill-stream fish *Devario aequipinnatus*: a scanning electron microscopic evaluation. *Microsc. Res. Tech.* **72**, 76-78.
Dillon, M. E., Wang, G. and Huey, R. B. (2010). Global metabolic impacts of recent climate warming. *Nature* **467**, 704-706.
Dorts, J., Grenouillet, G., Douxfils, J., Mandiki, S. N. M., Milla, S., Silvestre, F. and Kestemont, P. (2012). Evidence that elevated water temperature affects the reproductive physiology of the European bullhead *Cottus gobio*. *Fish Physiol. Biochem.* **38**, 389-399.
Evans, J. P. (2009). No evidence for sperm priming responses under varying sperm competition risk or intensity in guppies. *Naturwissenschaften* **96**, 771-779.
Fedorka, K. M., Zuk, M. and Mousseau, T. A. (2004). Immune suppression and the cost of reproduction in the ground cricket, *Allonemobius socius*. *Evolution* **58**, 2478-2485.
Ficke, A. D., Myrick, C. A. and Hansen, L. J. (2007). Potential impacts of global climate change on freshwater fisheries. *Rev. Fish Biol. Fish.* **17**, 581-613.
Fitzpatrick, J. L., Montgomerie, R., Desjardins, J. K., Stiver, K. A., Kolm, N. and Balshine, S. (2009). Female promiscuity promotes the evolution of faster sperm in cichlid fishes. *Proc. Natl. Acad. Sci. USA* **106**, 1128-1132.
Folstad, I. and Karter, A. J. (1992). Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* **139**, 603-622.
Folstad, I. and Sharstein, F. (1997). Is male germ line control creating avenues for female choice? *Behav. Ecol.* **8**, 109-112.
Fuller, A., Dawson, T., Helmuth, B., Hetem, R. S., Mitchell, D. and Maloney, S. K. (2010). Physiological mechanisms in coping with climate change. *Physiol. Biochem. Zool.* **83**, 713-720.
Gage, M. J. G., Macfarlane, C. P., Yeates, S., Ward, R. G., Searle, J. B. and Parker, G. A. (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., Simmons, L. W., Beveridge, M. and Evans, J. P. (2010). Sperm swimming velocity predicts competitive fertilization success in the green swordtail *Xiphophorus helleri*. *PLoS ONE* **5**, e12146.
Gomendio, M. and Roldan, E. R. S. (1991). Sperm competition influences sperm size in mammals. *Proc. R. Soc. Lond. B* **243**, 181-185.
Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S. and Samuel, M. D. (2002). Climate warming and disease risks for terrestrial and marine biota. *Science* **296**, 2158-2162.
Harvell, D., Altizer, S., Cattadori, I. M., Harrington, L. and Weil, E. (2009). Climate change and wildlife diseases: when does the host matter the most? *Ecology* **90**, 912-920.
Houde, A. E. (1997). *Sex, Colour and Mate Choice in Guppies*. Princeton, NJ: Princeton University Press.
Immler, S., Pryke, S. R., Birkhead, T. R. and Griffith, S. C. (2010). Pronounced within-individual plasticity in sperm morphometry across social environments. *Evolution* **64**, 1634-1643.
IPCC (2007). *Climate Change 2007: Synthesis Report*. Cambridge: Cambridge University Press.
Karayucel, I., Orhan, A. K. and Karayucel, S. (2006). Effect of temperature on sex ratio in guppy *Poecilia reticulata* (Peters 1860). *Aquacult. Res.* **37**, 139-150.
Karayucel, I., Orhan, A. K. and Karayucel, 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.
Karino, K., Shimada, Y., Kudo, H. and Sato, A. (2010). Relative importance of the area and intensity of the orange spots of male guppies *Poecilia reticulata* as mating traits preferred by females. *J. Fish Biol.* **77**, 299-307.

Kaushal, S. S., Likens, G. E., Jaworski, N. A., Pace, M. L., Sides, A. M., Seekell, D., Belt, K. T., Secor, D. H. and Wingate, R. L. (2010). Rising stream and river temperatures in the United States. *Front. Ecol. Environ* **8**, 461-466.
Kime, D. E., Van Look, K. J. W., McAllister, B. G., Huyskens, G., Rurangwa, E. and Ollevier, F. (2001). Computer-assisted sperm analysis (CASA) as a tool for monitoring sperm quality in fish. *Comp. Biochem. Physiol.* **130C**, 425-433.
Kodric-Brown, A. (1985). Female preference and sexual selection for male colouration in the guppy (*Poecilia reticulata*). *Behav. Ecol. Sociobiol.* **17**, 199-206.
Kodric-Brown, A. (1989). Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behav. Ecol. Sociobiol.* **25**, 393-401.
Kortet, R., Vainikka, A., Rantala, M. J. and Taskinen, J. (2004). Sperm quality, secondary sexual characters and parasitism in roach (*Rutilus rutilus* L.). *Biol. J. Linn. Soc. Lond.* **81**, 111-117.
Kosuda, L. L. and Bigazzi, P. E. (1987). Animal models of testes autoimmunity. In *Immunology of the Male Reproductive System* (ed. P. E. Bigazzi), pp. 253-352. New York, NY: Marcel Dekker.
Lahnsteiner, F. and 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.
Lamková, K., Simková, A., Palíková, M., Jurajda, P. and Lojek, A. (2007). Seasonal changes of immunocompetence and parasitism in chub (*Leuciscus cephalus*), a freshwater cyprinid fish. *Parasitol. Res.* **101**, 775-789.
Le Morvan-Rocher, C., Troutaud, D. and Deschaux, P. (1995). Effects of temperature on carp leukocyte mitogen-induced proliferation and nonspecific cytotoxic activity. *Dev. Comp. Immunol.* **19**, 87-95.
Malo, A. F., Gomendio, M., Garde, J., Lang-Lenton, B., Soler, A. J. and Roldan, E. R. S. (2006). Sperm design and sperm function. *Biol. Lett.* **2**, 246-249.
Marcogliese, D. J. (2008). The impact of climate change on the parasites and infectious diseases of aquatic animals. *Rev. Sci. Tech.* **27**, 467-484.
McAdam, A. G., Boutin, S., Réale, D. and Berteaux, D. (2002). Maternal effects and the potential for evolution in a natural population of animals. *Evolution* **56**, 846-851.
Miller, G. M., Watson, S. A., Donelson, J. M., McCormick, M. I. and Munday, P. L. (2012). Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. *Nat. Clim. Chang.* **2**, 858-861.
Møller, A. P. and Saino, N. (2004). Immune response and survival. *Oikos* **104**, 299-304.
Moret, Y. and Schmid-Hempel, P. (2000). Survival for immunity: the price of immune system activation for bumblebee workers. *Science* **290**, 1166-1168.
Neff, B. D., Garner, S. R., Heath, J. W. and Heath, D. D. (2008). The MHC and non-random mating in a captive population of Chinook salmon. *Heredity* **101**, 175-185.
Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Syst.* **37**, 637-669.
Parmesan, C. and Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37-42.
Pepin, P. (1991). Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. *Can. J. Fish. Aquat. Sci.* **48**, 503-518.
Pilastro, A., Evans, J. P., Sartorelli, S. and Bisazza, A. (2002). Male phenotype predicts insemination success in guppies. *Proc. Biol. Sci.* **269**, 1325-1330.
Réale, D., Berteaux, D., McAdam, A. G. and Boutin, S. (2003). Lifetime selection on heritable life-history traits in a natural population of red squirrels. *Evolution* **57**, 2416-2423.
Singh, B. (1997). Climate-related global changes in the southern Caribbean: Trinidad and Tobago. *Global Planet. Change* **15**, 93-111.
Somero, G. N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J. Exp. Biol.* **213**, 912-920.
Stefan, H. G. and Preudhomme, E. B. (1993). Stream temperature estimation from air-temperature. *J. Am. Water Resour. Assoc.* **29**, 27-45.
Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science* **301**, 65.
Stoltz, J. A. and Neff, B. D. (2006). Sperm competition in a fish with external fertilization: the contribution of sperm number, speed and length. *J. Evol. Biol.* **19**, 1873-1881.
Stoss, J. (1983). Fish gamete preservation and spermatozoan physiology. *Fish Physiol.* **9**, 305-350.
Villafuerte, R. and Negro, J. J. (1998). Digital imaging for colour measurement in ecological research. *Ecol. Lett.* **1**, 151-154.
Water Resources Agency (2001). *National Report on Integrating the Management of Watersheds and Coastal Areas in Trinidad and Tobago*. Port of Spain, Trinidad: Ministry of Public Utilities and the Environment.
Whitton, B. (1982). *Rivers, Lakes and Marshes*. London: Hodder and Staughton.
Williot, P., Kopeika, E. F. and Goncharov, B. F. (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, J. A., Bonilla, M. M., Su, E. J., Padua, M. V., Anderson, R. V., Kaur, D., Yang, D. S. and Zeh, D. W. (2012). Degrees of disruption: projected temperature increase has catastrophic consequences for reproduction in a tropical ectotherm. *Glob. Change Biol.* **18**, 1833-1842.