High temperatures disrupt *Artemia franciscana* mating patterns and impact sexual selection intensity

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**ABSTRACT**

Temperature plays a critical role in survival and reproduction, especially in ectotherms. Therefore, it is important to understand the mechanisms influencing life history traits and reproductive behaviours in order to predict climate change impacts on species' occurrence and performance. Here, we used the crustacean *Artemia franciscana* to investigate the potential impacts of temperature on life history traits, mating patterns and intensity of sexual selection. We reared *A. franciscana* at three temperatures 20°C, 25°C, and 30°C and measured life history traits such as growth, mortality or development of sexual traits. Our observations confirmed a clear link between life history traits and temperature, with advanced sexual maturity and increased mortality rates following temperature rises. Also, we found that mating is size assortative close to the ideal developmental temperature. Nevertheless, when temperatures deviate from the optimum, mating patterns were altered. Although selection intensity for females remained similar at all tested temperatures, as males preferentially mated with the larger females, size assortative mating disappeared at the highest temperature. Overall, our results highlight the potential for a temperature-dependent disruption of *A. franciscana* mating patterns. This disruption is especially pronounced under high temperatures as reproduction becomes progressively more random, thus entailing a relaxation of sexual selection intensity.

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1. Introduction

Occurring at an exceptional rate, climate change represents an immense threat to global biodiversity (Bellard et al., 2012). Organisms in all ecosystems are increasingly challenged to adapt to the rapidly shifting conditions and evident alterations in natural populations' geographical distribution and phenotype are being continually documented (Parmesan and Yohe, 2003; Hoffmann and Sgro, 2011). As temperature assumes a critical role in survival and reproduction, especially in ectotherms, one must unavoidably understand the mechanisms supporting current distributional patterns. This way, we will be able to better predict climate change impacts on species' occurrence and performance.

Populations that are becoming progressively closer to their upper thermal tolerance limits are especially susceptible to local extinctions (Somero, 2010) unless they disperse or adjust to the new conditions through plastic or genetic changes (Hoffmann and Sgro, 2011). However, when sexual reproduction is involved, the potential for local adaptation, under a climate change scenario, will surely depend on the interplay between natural and sexual selection. Both of these processes are able to affect pre- and post-copulatory reproductive traits (Grazer and Martin, 2012). Although temperature can have strong effects on reproduction and, consequently, on population fitness, the influence of climate change on the intensity of sexual selection has received less than the deserved attention (Candolin and Heuschele, 2008). However, there are several reports that highlight the potential impacts of rising temperatures on the expression of sexual selection. For instance, in the wolf spider (*Pardosa australis*), courtship latency and duration, as well as copulatory duration declined with increasing temperatures (Xiaoguo et al., 2009). In the worm pipefish (*Nerophis lunulatus*), populations closer to the species thermal tolerance limits showed enhanced expression of sexual traits and increased investment in reproduction (Monteiro and Lyons, 2012; Monteiro et al., 2017). A latitudinalcline in sexual selection pressure was also observed in medaka (*Oryzias latipes*) (Fujimoto et al., 2015). As temperature regimes can modify the costs and benefits arising from sexual selection, the potential rate of adaptation may ultimately be impacted. Although Candolin and Heuschele (2008) cautiously say that the role of sexual selection in extinction is...
probably minor, they also point out that this subject is far from clear.

Here, we used the crustacean _Artemia franciscana_ to investigate the potential impacts of temperature on the expression of the species mating system. We selected this species due to four main reasons: 1) available knowledge on the species’ biology (Criel and Macrée, 2002), mating system (scramble-competitive polygamy; Allen et al., 2017) and sexual behavior (Tapia et al., 2015); 2) marked sexual dimorphism (e.g. males display strikingly enlarged secondary antennae used as clasping organs during mating), suggesting an active role of sexual selection (either through mate choice, intersexual competition, or both); 3) well-documented tolerance to a wide range of water temperatures (Lenz and Browne, 1991; Medina et al., 2007), as we were especially interested in observing reproductive changes despite high levels of phenotypic plasticity; and finally, 4) the need for additional information on the species potential for adaptation, as _A. franciscana_ is an invasive species in the Iberian Peninsula (Amat et al., 2005; Rodrigues et al., 2012). We hypothesised that temperatures close to the species thermal tolerance limits would impact the expression of sexual characters, the mating system and, consequently, the intensity of sexual selection.

2. Methods

2.1. Origin and excystation

A single batch of _Artemia franciscana_ (diploid, sexual) cysts, from San Francisco Bay (USA), were cultured for our experiments. _Artemia_ were mass-hatched from cysts, according to Amat et al. (2005), in synthetic sea water (35 ppm), and fed daily with live microalgae (Phaeodactylum tricornutum). Nauplii were immediately transferred into nine (1L) experimental glass containers (density of ≈11400 nauplii per jar), that were then randomly assigned to the three temperature treatments described below. Seawater was changed (= 90%) every week and salinity adjustments were performed daily, when necessary. The individuals remained within the same jars up to sexual maturity.

2.2. Temperature treatments

The growth and reproductive performance of _Artemia_ was tested at three distinct temperatures (20 °C, 25 °C and 30 °C), at a constant salinity of 35 ppm (synthetic seawater, Tropic Marin). These temperatures were selected based on the results from Browne et al. (1988) that showed _A. franciscana_ to be well adapted to mid temperatures (24 °C) while still tolerating higher values (24-30 °C). For each temperature, one large glass aquaria served as a thermostatically controlled water bath (temperatures were maintained within ± 1 °C), housing three glass containers containing 900 mL of saltwater and an aeration system to maintain oxygenation. Water temperature and salinity were registered daily in each of the 9 jars. During the experiments, the natural photoperiod was approximately 14:1:10D.

At least 10 individuals from each jar (30 individuals per temperature) were regularly photographed to record body length, up to sexual maturity. Nauplii were initially photographed under a Zeiss Axio Scope A1 microscope but, as individuals grew larger, we switched to a handheld USB digital microscope. Size measurements were first conducted in the software Zen (Zeiss) and then in ImageJ (version 1.50i). One of the 20 °C replicate jars was discarded early on due to a handling accident. As there were no differences in size among replicates (One-way ANOVA with size data from day 36; 20 °C: F(1,38) = 0.520, P = 0.475; 25 °C: F(2,57) = 0.859, P = 0.429; 30 °C: F(2,57) = 2.639, P = 0.080), we opted to pool data within temperatures.

Sexual maturity was determined, after the development of secondary sexual traits, by the observation of couples in riding position. These couples were removed from the jars to smaller individual containers (150 ml plastic cups) and, once the riding position ended, we individually photographed the male (to record body length, interorbital distance, and the length and width of the secondary antennae) and the female (to record body length, interorbital distance and brood pouch area). Individuals were first anesthetized using chlorofom saturated water prior to photographs (Amat, 1980). As the duration of the riding position can vary from days to hours (Amat et al., 2007; Anufrieva and Shadrin, 2014), we sometimes missed male-female interactions and later found females already carrying eggs in the brood pouch. These females were also photographed, as mentioned above, even though we had no phenotypic information on the mating male. The swimming velocity of adult individuals was analyzed in short videos of adult _A. franciscana_ temporarily transferred to small Petri dishes (5 cm diameter).

2.3. Statistical analysis

We used the R package Growthcurver to compute _A. franciscana_ growth curve metrics (e.g., growth rate: r; carrying capacity: k), from hatching up to day 37 (near the onset of sexual maturity at 30 °C), using the logistic equation (Sproulske and Wagner, 2016). The developmental speed of selected traits was analyzed using Analysis of Covariance (ANCOVA), with body length used as covariate. The strength of selection on body size (Shuster and Wade, 2003) was assessed using the standardized selection differential for males and females (Smales and Sfemales), in the three selected temperatures (selection differential divided by the standard phenotypic deviation of the trait, where the selection differential is the difference in the mean of the selected individuals in amplexus from the mean of the population).

The software Tracker (v 4.9.8) was used to calculate individual average swimming velocities, in males and females from the three selected temperatures. Individual velocity (cm/s) and body length of 78 individuals (33 females and 45 males) were registered and used to estimate the probability of observing size assortative mating due solely to swimming velocity (i.e. without mate choice directed to any other variable), at the three selected temperatures (20 °C: N = 20; 25 °C: N = 28; 30 °C: N = 30). We simulated, in R, 1000 hypothetical populations per temperature, each comprising 25 couples whose individuals (males and females) derive from a replication, with replacement, of the actually measured individuals. Within each simulated population, we then selected from the randomly formed couples, those showing a difference in velocity between the sexes lower than 0.5 cm/s (average standard deviation of velocity in the measured groups = 0.491 cm/s). If swimming speed was, hypothetically, the only character responsible for couple formation, then these couples formed by individuals with comparable speeds would have the largest probability of breeding. Within each selected couple, we then replaced individual swimming velocity for body length and looked for significant correlations between male and female size (i.e. a clear pattern of size assortative mating) within each simulated population. We assessed the probability (%) of finding non-random mating by dividing the number of theoretical populations found with size assortative mating by 1000, multiplied by 100. We replicated this procedure 10 consecutive times (overall, we conducted 10,000 simulations per temperature) to estimate average and standard deviation of our simulated probability estimates.

Statistical analyses were conducted in R, Statistica (v13) and GraphPad Prism (v6). For parametric tests, assumptions were met (e.g. homogeneity of variances) with the exception of the ANOVA on female body length (two factors: reproductive status and temperature). Nevertheless, as there was no significant correlation between averages and standard deviations, we proceeded with the analysis, as advocated by Lindman (1974). In ANOVAs and ANCOVAs, temperature was coded as a random factor.

3. Results

3.1. Temperature effects in growth, sexual maturity and life span

_Artemia_ grew faster as temperature increased (Fig. 1), retaining
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