The following supplements accompany the article

Thermal acclimation has little effect on tadpole resistance to *Batrachochytrium dendrobatidis*

Karie A. Altman*, Thomas R. Raffel

*Corresponding author: karie.altman@pitt.edu

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Supplement 1. Additional figures and tables

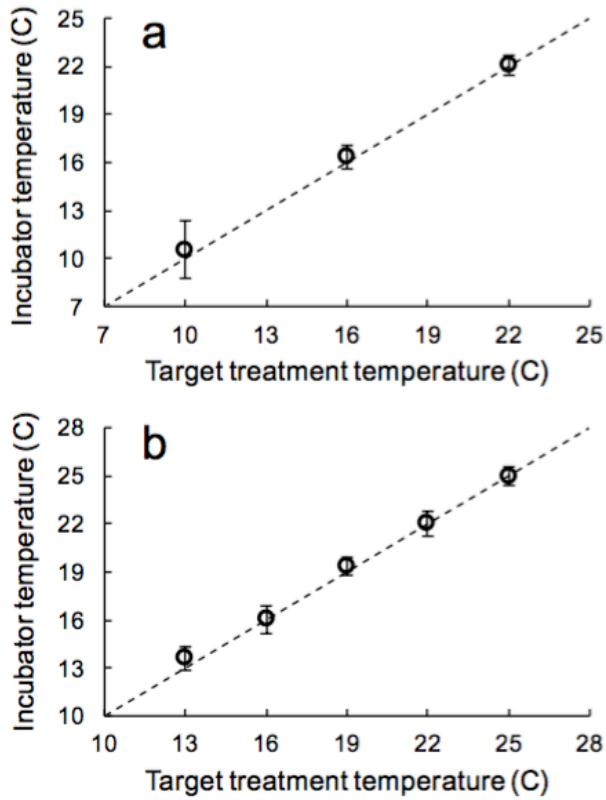

Figure S1. Relationship between target treatment temperatures and the average temperatures recorded in incubators throughout (a) the 2-week acclimation period and (b) the 4 weeks following exposure to *Batrachochytrium dendrobatidis* (Bd). The dashed line shows a 1:1 relationship. Error bars are ± 1 SD to describe variation in temperature readings among individual incubators.

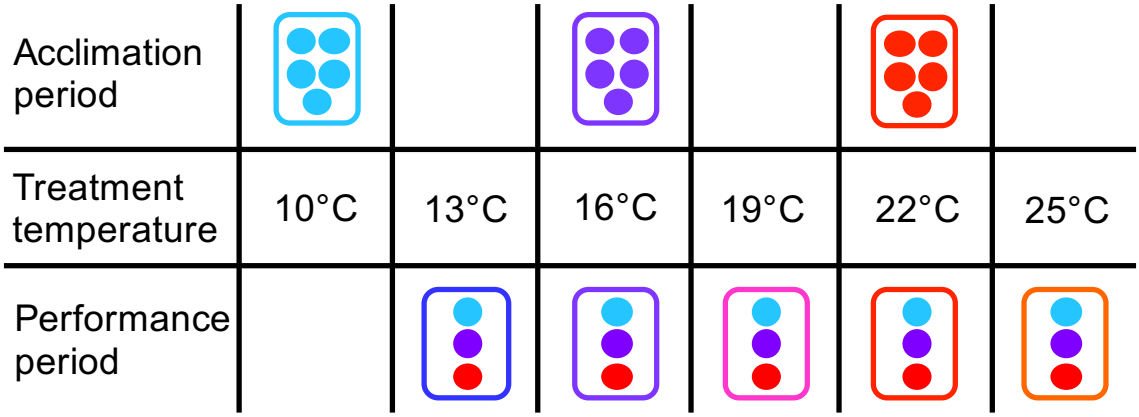

Figure S2. Experimental design. Tadpoles (circles) were acclimated to one of three temperatures (10, 16, or 22°C) within incubators (rounded rectangles) for 14 days. After acclimation, each tadpole was moved to one of five performance temperatures (13, 16, 19, 22, or 25°C), at which it was exposed to *Batrachochytrium dendrobatidis* (Bd) that was acclimated to each performance temperature. Circle color corresponds to the tadpole's acclimation temperature, and the outline of the rounded rectangle corresponds to the tadpole's current temperature during the acclimation (top row) and performance (bottom row) periods.

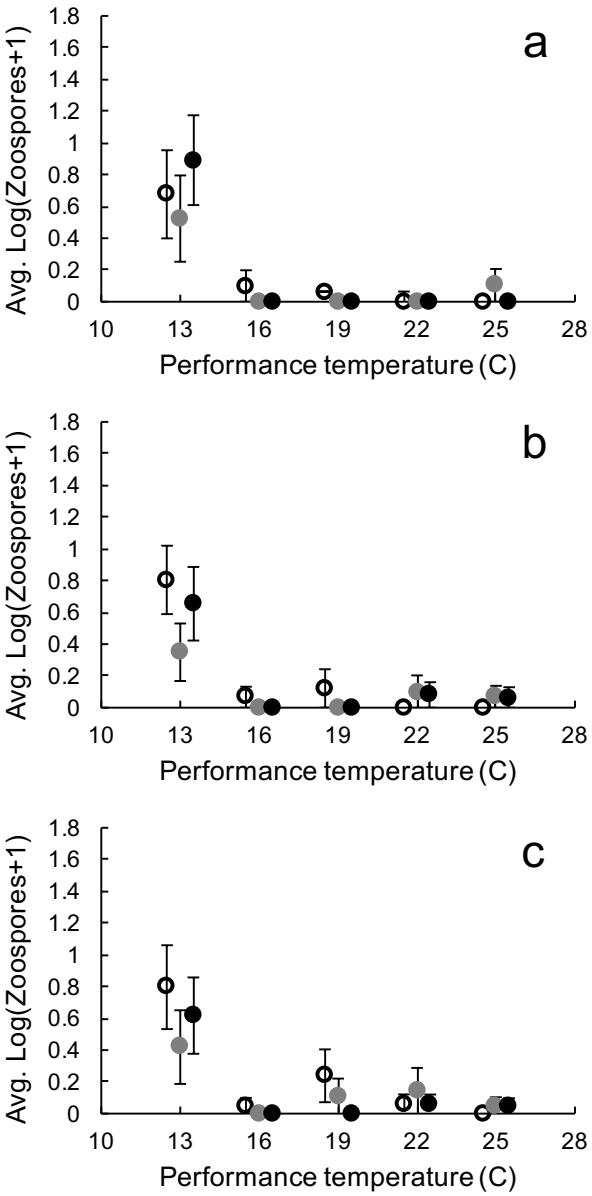

Figure S3. The mean number of Bd zoospores (log-transformed) detected on tadpoles acclimated to 10°C (open circles), 16°C (gray circles), and 22°C (black circles) at each performance temperature (tadpoles were exposed to Bd at the same performance temperatures, but points are offset for clarity). Each panel shows data averaged through each of the swabbing time points: (a) 7-14, (b) 7-21, and (c) 7-28 days post-exposure to Bd. Error bars are ± SE. See Figure 1a for data at the 7-day time point.

Table S1. Number of tadpoles assigned to each acclimation × performance temperature treatment. For cases in which tadpoles died after exposure, the number of tadpoles included in analyses is shown in parentheses. Most of the tadpoles that died during the experiment died within 3 days of Bd or sham exposure (before water had been changed following exposure). These animals were excluded from analyses, because swabs were likely to pick up transient Bd DNA from zoospores that failed to establish in the tadpoles. The only exception to this, indicated by an asterisk in the table below, is one tadpole in the 22°C-acclimation × 19°C-performance temperature treatment, which died in between 7 and 14 days post-exposure. This tadpole was therefore included in the 7-day post-exposure analysis (N = 5 for that temperature treatment at 7 days post-exposure) but was excluded from subsequent analyses (N = 4 for that temperature treatment for 14, 21, and 28 days post-exposure).

Exposure treatment	**Acclimation temperature**	**N per performance temperature**				
		13°C	**16°C**	**19°C**	**22°C**	**25°C**
Bd-exposed	**10°C**	5	6	6	6	5 (4)
	16°C	5 (3)	6	6	5	6
	22°C	5	5	5 (4)*	5	5 (4)
Control	**10°C**	3 (2)	3 (2)	3	3	3
	16°C	3	3	3 (2)	3	3 (2)
	22°C	3 (2)	2	3 (2)	2	3

Table S2. Regression statistics from linear mixed-effects models (function "lmer" in R package "lme4") describing temperature effects on the number of Bd zoospores on each tadpole averaged through each of the final three of the four swab time points: 7-14, 7-21, and 7-28 days (see Table 1 for 7-day results). Results are from the simplest model at each time point ($p < 0.1$ for inclusion in the model). Note that non-significant terms were included in the model if they were part of the experimental design (e.g., acclimation and performance temperatures) or if they were marginal to terms that were significant predictors of Bd load. Mass, tadpole population, and block were also included in all models. All models included a random effect of performance incubator, except when testing for main effects of acclimation temperature. For these model comparisons, acclimation incubator was included as a random effect instead. Block coefficients are reported as "NA", because block was a categorical variable with more than two treatment levels and therefore generated multiple coefficients.

Swab time point	Predictor	Coefficient	df	F	p
7-14 days post-exposure	**Mass**	**2.33×10^{-1}**	**1, 63.9**	**8.35**	**0.005**
	Tadpole population	-6.45×10^{-2}	1, 67.2	0.18	0.672
	Block	NA	2, 25.6	1.37	0.272
	Acclimation temperature	4.20×10^{-4}	1, 33.1	<0.01	0.971
	Performance temperature	**-4.13×10^{-2}**	**1, 25.1**	**11.16**	**0.003**
	Performance temperature^2	**1.22×10^{-2}**	**1, 25.2**	**12.75**	**0.001**
7-21 days post-exposure	Mass	9.71×10^{-2}	1, 65.6	1.44	0.235
	Tadpole population	2.35×10^{-2}	1, 62.1	0.02	0.875
	Block	NA	2, 30.2	0.68	0.516
	Acclimation temperature	-7.32×10^{-3}	1, 28.9	0.52	0.477
	Performance temperature	-1.63×10^{-2}	1, 29.7	0.63	0.435
	Acc. temp. × perf. temp.	**6.45×10^{-3}**	**1, 45.3**	**8.88**	**0.005**
7-28 days post-exposure	**Mass**	**2.93×10^{-1}**	**1, 64.3**	**7.16**	**0.009**
	Tadpole population	-2.02×10^{-1}	1, 67.9	0.94	0.334
	Block	NA	2, 26.4	0.23	0.798
	Acclimation temperature	-2.00×10^{-2}	1, 29.4	1.83	0.186
	Performance temperature	-2.84×10^{-2}	1, 25.9	2.80	0.106

Table S3. Regression statistics from a linear mixed-effects model (function "lmer" in R package "lme4") describing the effects of acclimation and performance temperature on the average number of Bd zoospores detected on tadpoles, including swab time point as a continuous predictor. All tadpoles were included in this analysis (both infected and uninfected). The model included a random effect of tadpole nested under performance incubator, except when testing for main effects of acclimation temperature. For this model comparison, the random effect was tadpole was nested under acclimation incubator instead. Block coefficients are reported as "NA", because block was a categorical variable with more than two treatment levels and therefore generated multiple coefficients.

Predictor	Coefficient	df	F	p
Swab time	4.00×10^{-3}	1, 228.2	1.53	0.217
Mass	$\mathbf{1.21 \times 10^{-1}}$	**1, 69.3**	**5.20**	**0.026**
Tadpole population	-6.22×10^{-2}	1, 65.9	0.40	0.530
Block	NA	2, 28.5	1.06	0.359
Acclimation temperature	-7.05×10^{-3}	1, 28.5	0.96	0.335
Performance temperature	$\mathbf{-3.25 \times 10^{-2}}$	**1, 28.0**	**10.89**	**0.003**
Performance temperature^2	$\mathbf{7.78 \times 10^{-3}}$	**1, 27.0**	**8.07**	**0.008**

Table S4. Regression statistics from a binomial generalized linear mixed-effects model (function "glmer" in R package "lme4") describing the effects of acclimation and performance temperature on whether a tadpole tested positive for Bd at any point throughout the 28 days post-exposure. The model included a random effect of performance incubator, except when testing for a main effect of acclimation temperature. For this model comparison, acclimation incubator was included as a random effect instead. Block coefficients are reported as "NA", because block was a categorical variable with more than two treatment levels and therefore generated multiple coefficients.

Predictor	Coefficient	df	χ^2	p
Mass	2.85×10^{-1}	1	0.46	0.498
Tadpole population	1.11×10^{-1}	1	0.01	0.906
Block	NA	2	2.35	0.309
Acclimation temperature	-4.49×10^{-3}	1	0.01	0.940
Performance temperature	$\mathbf{-1.41 \times 10^{-1}}$	**1**	**4.62**	**0.032**
Performance temperature^2	$\mathbf{3.88 \times 10^{-2}}$	**1**	**3.99**	**0.046**

Table S5. Regression statistics from a linear mixed-effects model (function "lmer" in R package "lme4") describing the effects of acclimation and performance temperature on the average number of Bd zoospores detected only on tadpoles that tested positive for Bd (Uninfected frogs were excluded from this analysis. The number of infected frogs in each temperature treatment is shown in Table S6). The model included a random effect of tadpole nested under performance incubator, except when testing for main effects of acclimation temperature. For this model comparison, the random effect was tadpole was nested under acclimation incubator instead. Block coefficients are reported as "NA", because block was a categorical variable with more than two treatment levels and therefore generated multiple coefficients.

Predictor	**Coefficient**	**df**	**F**	**p**
Swab time	2.25×10^{-2}	1, 14.5	3.16	0.096
Mass	-3.89×10^{-2}	1, 7.3	0.04	0.838
Tadpole population	-2.32×10^{-1}	1, 10.0	0.22	0.646
Block	NA	2, 10.3	0.83	0.462
Acclimation temperature	3.65×10^{-2}	1, 12.8	1.15	0.303
Performance temperature	-4.89×10^{-2}	1, 11.7	2.15	0.169

Table S6. The number of tadpoles in each temperature treatment that became infected with Bd at any point during the 28 days post-exposure and were included in the analysis of infected tadpoles (Table S5). Tadpoles with fewer than one zoospore detected were considered uninfected.

Acclimation	**N infected per performance temperature**				
temperature	**13°C**	**16°C**	**19°C**	**22°C**	**25°C**
10°C	5	1	2	1	0
16°C	2	0	1	1	1
22°C	4	0	0	1	1

Supplement 2. Converting genome equivalents to zoospore equivalents for Bd strain JEL 423

Methods

In-house Bd standards were generated from a lab culture of Bd strain JEL 423, following the protocol of Boyle et al. (2004). A liquid Bd culture (1% tryptone broth) was filtered through a 20-µm nylon mesh (Spectrum Laboratories, Inc., Rancho Dominguez, CA) to remove zoosporangia. The filtrate, which contained only zoospores in broth, was then divided into three subsamples, and the zoospore concentration of each subsample was determined by counting zoospores on a hemocytometer. Each subsample was then diluted to a quantity of 10^6 zoospores in 1 mL broth. Zoospores were pelleted by centrifuging samples at 13,000 rpm for 1 min. After removing the supernatant, zoospore pellets were resuspended in 200 µL PrepMan Ultra DNA extraction buffer (Applied Biosystems, Foster City, CA). Samples were placed in a hot bath at 100°C for 10 min, cooled for 2 min, then centrifuged at 13,000 rpm for 3 min. The supernatant contained Bd DNA at a concentration of 5000 zoospores/µL, and 150 µL was removed from each sample. Five serial dilutions for each of the three original filtrate samples were created to generate concentrations of 0.5, 5, 50, 500, and 5000 zoospores/µL.

Quantitative polymerase chain reaction (qPCR) was run according to the protocol of Kriger et al. (2006) using a Bio-Rad CFX Connect system (Bio-Rad Laboratories, Inc., Hercules, CA). Each in-house zoospore standard was run in duplicate (3 subsamples × 3 5 dilutions/subsample × 2 wells/dilution). A volume of 2 µL of each zoospore standard was added to each well, resulting in target quantities of 1, 10, 100, 1000, and 10,000 zoospores per reaction. A 1:10 dilution series of four concentrations of commercial zoospore standards (Pisces Molecular, Boulder, CO) was also run in duplicate. Commercial standard reaction concentrations ranged from 4.2×10^2 to 4.2×10^5 gene copies per reaction.

Using a standard curve based on the results for the commercial zoospore standards, cycle threshold (C_T) values for the in-house standards were converted to units of gene copies. Next, a regression line was fit to the in-house standard data, using the log(zoospores) as the predictor variable and log(gene copies) as the response variable (Figure S4). If the number of gene copies is directly proportional to the number of zoospores, as expected, then this regression line should have a slope of 1.0. The lowest reaction concentration (1.0 zoospore equivalent) yielded low gene copy values relative to a regression line fitted to the other data, and when included in the overall analysis resulted in a line with a slope significantly less than 1.0 (Figure S4a, $t = 2.70$, $df = 10$, $p = 0.02$). At such a low concentration, it is possible that random sampling effects might have led to low-biased measurements. We therefore excluded the 1.0 zoospore concentration from the gene copy per zoospore calculation. A student's t-test was conducted to confirm that the slope of the subsequent regression line (including data for 10, 100, 1000, and 10,000 zoospores) did not significantly differ from 1.0.

Results and conclusions

The slope of the regression line for gene copies as a function of zoospore number was 1.0153, and the y-intercept was 1.7649 (Figure S4b). The slope did not significantly differ from 1.0 ($t = 0.70$, $df = 10$, $p = 0.5$), indicating that log(gene copies) was directly proportional to log(zoospores). We therefore calculated the mean log(gene copies/zoospore), which is equal to log(gene copies)-log(zoospores). Using this calculation, we concluded that there were 63.5 gene copies per zoospore for Bd strain JEL 423 (95% confidence interval, 56.3-71.7 gene copies per zoospore). We used this value to convert Bd load results from units of gene copies to units of zoospores in in this study.

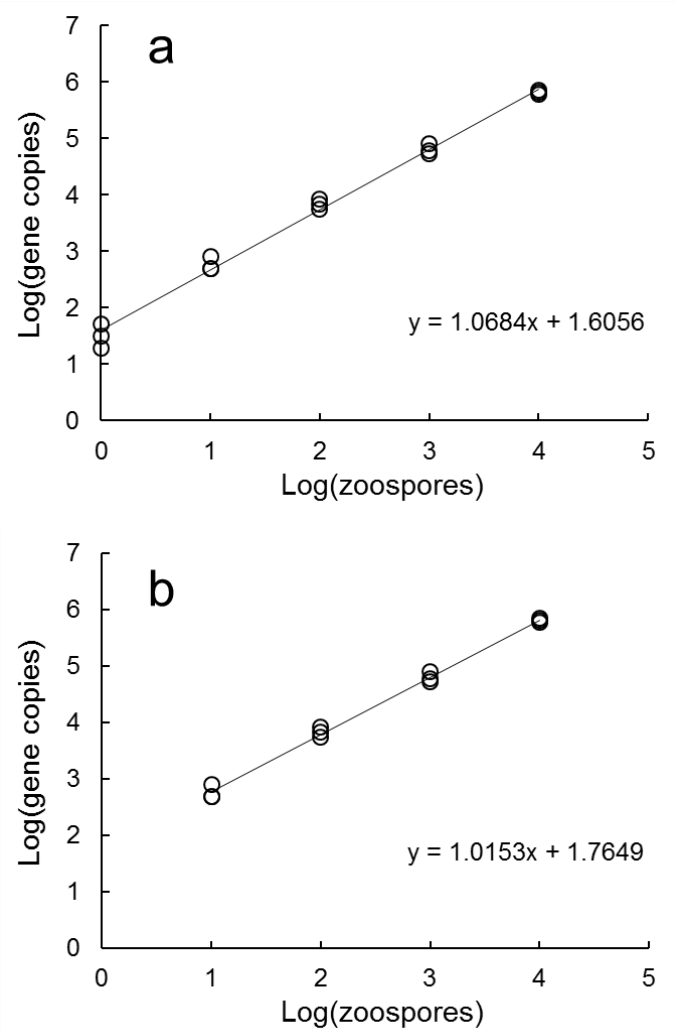

Figure S4. Log-log plots showing gene copies as a function of zoospore number for Bd strain JEL 423 using (a) data from all in-house zoospore standards and (b) data excluding the lowest zoospore standard. The slope of the regression line in panel (b) did not significantly differ from 1.0, indicating that gene copy number and zoospore number were directly proportional, as expected. Therefore, data from the lowest in-house zoospore standard were excluded when calculating the estimated number of gene copies per zoospore.

References

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