

1 Short Title: Shifts in temperature and *Batrachochytrium*

2 **Shifts in temperature influence how *Batrachochytrium dendrobatidis* infects**  
3 **amphibian larvae**

4 Paul W. Bradley<sup>1</sup>, Michael D. Brawner<sup>2</sup>, Thomas R. Raffel<sup>3</sup>, Jason R. Rohr<sup>4</sup>, Deanna H.  
5 Olson<sup>5</sup>, and Andrew R. Blaustein<sup>2</sup>

6 <sup>1</sup> Department of Biology, University of San Diego, 5998 Alcalá Park, San Diego CA,  
7 93110, USA.

8 <sup>2</sup> Department of Integrative Biology, 3029 Cordley Hall, Oregon State University,  
9 Corvallis, OR, 97331, USA.

10 <sup>3</sup> Department of Biology, 375 Dodge Hall, Oakland University, Rochester, MI, 48309,  
11 USA.

12 <sup>4</sup> Department of Integrative Biology, University of South Florida, 4202 East Fowler  
13 Avenue, Tampa, FL, 33620, USA.

14 <sup>5</sup> USDA Forest Service, Pacific Northwest Research Station, 3200 SW Jefferson Way,  
15 Corvallis, OR, 97331, USA.

16

17 Corresponding author: paulwilliambradley@gmail.com

18

19 **Author contribution statement**

20 ARB, JRR, and TRR originally formulated the idea. PWB designed the experiment and developed the  
21 methodology. PWB performed the experiment. PWB and MDB performed the molecular analysis. PWB  
22 and TRR performed the statistical analyses. ARB, JRR, TRR, and DHO obtained funding. PWB wrote the  
23 manuscript and other authors provided editorial advice.

24

25 **Abstract:**

26 Many climate change models predict increases in mean temperature, and increases in  
27 frequency and magnitude of temperature fluctuations. These potential shifts may impact  
28 ectotherms in several ways, including how they are affected by disease. Shifts in  
29 temperature may especially affect amphibians, a group with populations that have been  
30 challenged by several pathogens. Because amphibian hosts invest more in immunity at  
31 warmer than cooler temperatures and parasites may acclimate to temperature shifts faster  
32 than hosts (creating lags in optimal host immunity), researchers have hypothesized that a  
33 temperature shift from cold-to-warm might result in increased amphibian sensitivity to  
34 pathogens, whereas a shift from warm-to-cold might result in decreased sensitivity.  
35 Support for components of this climate-variability based hypothesis have been provided  
36 by prior studies of the fungus *Batrachochytrium dendrobatidis* (Bd) that causes the  
37 disease chytridiomycosis in amphibians. We experimentally tested whether temperature  
38 shifts before Bd exposure alter susceptibility to Bd in the larval stage of two amphibian  
39 species – western toads (*Anaxyrus boreas*) and northern red legged frogs (*Rana aurora*).  
40 Both host species harbored elevated Bd infection intensities under constant cold (15° C)  
41 temperature in comparison to constant warm (20° C) temperature. Additionally, both  
42 species experienced an increase in Bd infection abundance when shifted to 20° C from  
43 15° C, compared to a constant 20° C but they experienced a decrease in Bd when shifted  
44 to 15° C from 20° C, compared to a constant 15° C. These results are in contrast to prior  
45 studies of adult amphibians that found increased susceptibility to Bd infection after a  
46 temperature shift in either direction, highlighting the potential for species and stage  
47 differences in the temperature-dependence of chytridiomycosis.

48 **Keywords:** amphibian declines, *Batrachochytrium dendrobatidis*, chytridiomycosis,  
49 climate variability hypothesis, infectious disease, temperature, *Rana aurora*, *Anaxyrus*  
50 *boreas*

51

## 52 **Introduction**

53 Climate change represents one of the greatest challenges to biodiversity and  
54 conservation because it might compromise ecosystem functions worldwide. Changes in  
55 climate have affected plant-animal interactions, predator-prey interactions and disease  
56 dynamics (Lafferty 2009, Rohr *et al.* 2011, Sheldon, Yang & Tewksbury 2011, Garcia *et*  
57 *al.* 2014). Changes to annual or seasonal mean temperatures often are used to predict  
58 climate-change-induced effects on disease risk (Paaijmans, Read & Thomas 2009,  
59 Paaijmans *et al.* 2010). However, many climate change models also predict increases in  
60 the frequency and magnitude of extreme weather events and increases in temperature  
61 variability at monthly to weekly timescales (Easterling *et al.* 2000, Meehl & Tebaldi  
62 2004, Schar *et al.* 2004, Paaijmans *et al.* 2010, Rummukainen 2012). Yet few studies  
63 have investigated how increases in temperature variability affect disease dynamics  
64 despite the likelihood that such variability might differentially affect hosts and pathogens  
65 (Paaijmans *et al.* 2010, Ben-Horin, Lenihan & Lafferty 2012, Raffel *et al.* 2013,  
66 Bannerman & Roitberg 2014, Luis *et al.* 2014, Raffel *et al.* 2015). Ectotherms, such as  
67 amphibians, are particularly sensitive to climate change (Blaustein *et al.* 2010, Lawler *et*  
68 *al.* 2010, Shoo *et al.* 2011, Li, Cohen & Rohr 2013) and are experiencing disease-  
69 associated population declines and extinctions worldwide (Stuart *et al.* 2004, McCallum

70 2007, Rohr *et al.* 2008, Wake 2012), making them an ideal group to investigate the  
71 relationship between temperature shifts and disease risk.

72 Chytridiomycosis is an emerging infectious disease of amphibians caused by the  
73 aquatic chytrid fungal pathogens *Batrachochytrium dendrobatidis* (Bd) and *B.*  
74 *salamandrivorans* (Longcore, Pessier & Nichols 1999, Martel *et al.* 2013). Bd is  
75 widespread globally (Liu, Rohr & Li 2013, Olson *et al.* 2013) and is associated with  
76 worldwide amphibian population declines (Stuart *et al.* 2004, Skerratt *et al.* 2007).  
77 Moreover, models based on IPCC climate futures predict that Bd will shift to higher  
78 latitudes and altitudes due to increased environmental suitability in those regions under  
79 climate change, thus potentially affecting additional amphibian populations (Xie, Olson  
80 & Blaustein 2016).

81 The negative effects of Bd infection are more pronounced in post-metamorphic  
82 stages, often leading to death (Blaustein *et al.* 2005, Garner *et al.* 2009, Gervasi *et al.*  
83 2013, Gervasi *et al.* 2017). In larvae, Bd infection can cause host mortality in some  
84 species (Blaustein *et al.* 2005, Garner *et al.* 2009). However the infection is localized to  
85 keratinized larval mouthparts, (Marantelli *et al.* 2004, McMahon & Rohr 2015) often  
86 resulting in sublethal effects including inhibited foraging capacity, reduced growth and  
87 development, altered predator avoidance, or changes to other behaviors (Han, Bradley &  
88 Blaustein 2008, Venesky, Parris & Storfer 2010, Buck *et al.* 2012, Gervasi *et al.* 2013).  
89 Additionally, larvae of many species are important members of aquatic communities and  
90 alterations to larval feeding have the potential to cascade through the aquatic ecosystem  
91 (Alford 1989, Brönmark, Rundle & Erlandsson 1991, Lamberti *et al.* 1992, Kupferberg  
92 1997).

93           Temperature is considered one of the most important environmental factors  
94 driving chytridiomycosis (Drew, Allen & Allen 2006, Bosch *et al.* 2007, Daskin, Alford  
95 & Puschendorf 2011, Forrest & Schlaepfer 2011, Voyles *et al.* 2017). Bd is non-linearly  
96 sensitive to temperature with an optimal growth range in culture between 17° and 25°  
97 (Piotrowski, Annis & Longcore 2004, Rohr & Raffel 2010, Raffel *et al.* 2013) and a  
98 temperature-dependent generation time of 4 to 10 days (Woodhams *et al.* 2008). The  
99 upper thermal limit for Bd growth in culture is between 25°C and 28°C, with Bd  
100 mortality occurring above 30°C (Longcore, Pessier & Nichols 1999, Piotrowski, Annis &  
101 Longcore 2004). Bd has been shown to be reliably cleared from multiple amphibian  
102 species by extended exposure to 30°C (McMahon *et al.* 2014). Its lower thermal limit is  
103 below 4°C (Piotrowski, Annis & Longcore 2004). Additionally, life history strategies of  
104 the pathogen can be altered by environmental temperature, where colder temperatures can  
105 cause Bd zoosporangia to develop and mature more slowly (Voyles *et al.* 2012), but  
106 produce more and longer-lived zoospores overall (Hyatt *et al.* 2007, Woodhams *et al.*  
107 2008).

108           Because physiologies of both the host and pathogen are strongly influenced by  
109 environmental temperature, climate change has been used to explain several major Bd  
110 outbreaks and amphibian population declines, (reviewed in Li, Cohen & Rohr 2013, Rohr  
111 *et al.* 2013). Yet, the host and pathogen are not expected to share a uniform response to a  
112 given temperature (Brown *et al.* 2004, Paull, LaFonte & Johnson 2012, Rohr *et al.* 2013),  
113 and thermal responses measured in constant-temperature artificial environments might  
114 not reflect organism responses in more realistic variable-temperature environments.  
115 Providing evidence of the lack of a uniform response between Bd and amphibians to

116 temperature shifts, Rohr and Raffel (2010) found a strong correlation between elevated  
117 month-to-month temperature variability and Bd-associated amphibian population  
118 declines of *Atelopus* spp. across Central and South America. Further support of the  
119 relationship between chytridiomycosis and temperature variation has been provided by  
120 laboratory studies. In one study, Cuban treefrogs (*Osteopilus septentrionalis*) displayed  
121 reduced resistance to Bd infection when exposed to random daily temperature  
122 fluctuations or when exposed to a temperature decrease after acclimation to a warmer  
123 temperature (Raffel *et al.* 2013). Similar results were obtained in newts (*Notophthalmus*  
124 *viridescens*) exposed to Bd, except both decreases and increases in temperature were  
125 associated with elevated Bd abundance relative to abundances at constant temperatures  
126 (Raffel *et al.* 2015).

127         The potential for temperature variability to increase disease severity in  
128 amphibians was first postulated by Raffel *et al.* (2006) and has subsequently been  
129 referred to as the “climate variability hypothesis” (Rohr & Raffel 2010). This hypothesis  
130 posits that parasites acclimate to the new temperature more rapidly than their hosts,  
131 leading to lags in host acclimation following a temperature shift that could make hosts  
132 more susceptible to infection (Raffel *et al.* 2013). This hypothesis assumes that: 1)  
133 pathogens acclimate to the new temperature faster than the host because of their  
134 relatively smaller size and higher metabolic rate (Gillooly *et al.* 2001, Raffel *et al.* 2013);  
135 and 2) both host and parasite acclimation responses lead to increased performance at the  
136 new temperature, in accordance with the “beneficial acclimation hypothesis” of thermal  
137 biology (Angilletta 2009). However, Raffel *et al.* (2006) also pointed out potential

138 complexities in acclimation of the ectotherm immune system that might lead to  
139 alternative predictions.

140         According to the “lag effect” hypothesis (Raffel *et al.* 2006), changes in levels of  
141 temperature-dependent immune parameters might simply lag behind environmental  
142 temperature shifts (Fig. 1) because it takes time to produce necessary, or remove  
143 unnecessary, immune cells from the host. For example, amphibians are expected to  
144 require more immune cells at warmer temperatures to fight off faster-growing pathogens  
145 (Maniero & Carey 1997), and lags in production of new immune cells could lead to sub-  
146 optimal immunity following a temperature increase (Raffel *et al.* 2006). Conversely, the  
147 amphibian immune system is expected to be down-regulated following a temperature  
148 decrease (Macela & Romanovsky 1970), with the removal of mature white blood cells  
149 determined by the rate of their respective half-lives (DeSantis & Strauss 1997, Janeway  
150 2008). A lag in this process might lead to a brief period of elevated immune  
151 responsiveness relative to an already cold-acclimated host. Thus, the “lag effect”  
152 hypothesis predicts the opposite effect from the “climate variability hypothesis”  
153 following a temperature decrease, at least on a short timescale. These mechanistic  
154 hypotheses are not mutually exclusive, and it is unclear which effects might be more  
155 important for a given host-parasite combination.

156         We tested the general prediction that an amphibian shifted to a new temperature  
157 before Bd exposure would respond to infection differently than a host already acclimated  
158 to the exposure temperature. We postulated that the direction of the effect would depend  
159 upon the direction of the temperature shift, in accordance with the “lag effect” hypothesis  
160 of Raffel *et al.* (2006). Given the differences in size between the host and the pathogen,

161 and associated physiological process rate differences, we assumed Bd would  
162 physiologically respond to the temperature shift faster than the host, such that an  
163 idealized host-immune response to Bd exposure would temporarily lag behind the  
164 temperature shift. Thus, we predicted that a temperature shift from cold-to-warm would  
165 result in an *increase* in susceptibility to Bd exposure, whereas a temperature shift from  
166 warm-to-cold would result in a *decrease* in susceptibility to Bd exposure. To test these  
167 predictions, we quantified susceptibility to Bd by measuring infection abundance after  
168 exposure to the pathogen.

169

## 170 **Materials and Methods**

171 To examine the how temperature shifts may alter larval amphibian infection  
172 dynamics, we selected two species of amphibian hosts, the northern red legged frog  
173 (*Rana aurora*) and the western toad (*Anaxyrus boreas*). Both species have been observed  
174 in the field with Bd infections (Pearl *et al.* 2007, Muths, Pilliod & Livo 2008, Piovia-  
175 Scott *et al.* 2011) and both species are susceptible to chytridiomycosis (Han, Bradley &  
176 Blaustein 2008, Gervasi *et al.* 2013). To ensure that the animals used in our experiment  
177 were not previously infected with Bd, amphibians were collected as eggs from natural  
178 oviposition sites. Red legged frog eggs were collected from a permanent pond located  
179 near Florence, Oregon, USA (Lincoln County, elevation 12 m; latitude/longitude:  
180 44.088/-124.123) in the Oregon Coast Range on 11-Feb-2012. Western toad eggs were  
181 collected from Little Three Creeks Lake (Deschutes County, elevation 2,000 m;  
182 latitude/longitude: 44.009/-121.643) in the Cascade Range on 9-Jul-2011. Immediately  
183 after collection, eggs were transported to a laboratory at Oregon State University where



184 they were maintained at 14° C, under a 12-12 photoperiod in 40-liter aquaria filled with  
185 dechlorinated water. Upon hatching, larvae were maintained at a density of  
186 approximately 200 individuals per aquarium and fed *ad libitum* a mixture of TetraMin  
187 fish food and ground alfalfa pellets (1:3 ratio by volume). Water was changed every  
188 seven days. The 40-day trials for each species were not run concurrently, but identical  
189 protocols were used for both species and both trials consisted of individuals of identical  
190 larval stage (Gosner stage 26).

### 191 *Acclimation Period*

192 Independent trials for each host species began with a 20-day acclimation period  
193 with 80 (Gosner stage 26) larvae randomly selected, and individually placed into 80  
194 plastic 500-mL containers where they were housed for the duration of the acclimation  
195 period and experiment. Each container was filled with 14° C dechlorinated water and  
196 covered with a lid to help maintain water temperature and limit evaporation. Each  
197 container had 2-mm diameter holes drilled between the water line and the lid to allow air  
198 circulation into the container. Pairs of containers were then placed within 40 individual  
199 temperature-controlled chambers (to ensure independent replication of the temperature  
200 treatments) that were set at 15° C to avoid cold-shocking the larvae. Each temperature-  
201 controlled chamber was independently controlled via its own thermostat and the interior  
202 measured approximately 37 cm deep x 21 cm wide x 13 cm in height. Half of the 40  
203 temperature-controlled chambers were then randomly selected to begin the acclimation  
204 period at 20° C (warm treatment) and the other half were kept at 15° C (cold treatment).  
205 The placement of temperature chambers within the laboratory was randomized, as was  
206 the placement of 500-mL containers within each temperature chamber.

207 *Temperature Shifts*

208           On day 20 of the experiment, half of the temperature chambers in each of the two  
209 acclimation temperatures (15° C and 20° C) were randomly selected to undergo a  
210 temperature shift, either from 20° to 15° C or from 15° C to 20° C. The other half of the  
211 temperature chambers underwent no shift in temperature. Thus, each of the temperature  
212 chambers was subjected to one of four temperature treatments: a constant 15° C (cold)  
213 throughout the experiment; a constant 20° C (warm) throughout the experiment; a  
214 temperature shift from 15° C to 20° C (cold-to-warm); or a temperature shift from 20° C  
215 to 15° C (warm-to-cold).

216 *Bd exposure*

217           On day 24, one of the two 500-mL containers within each temperature-controlled  
218 chamber was randomly selected to undergo a Bd-exposure treatment and the other was  
219 selected as a control. Larvae in the Bd-exposure treatment were exposed to a single  
220 inoculate of Bd strain JEL 274, which was grown in pure culture on 1% tryptone agar in  
221 10-cm diameter Petri dishes. This Bd strain was selected as it is one of the more virulent  
222 strains associated with major amphibian populations declines (Rosenblum *et al.* 2013).  
223 The Petri dishes were inoculated with liquid culture 10 days before the start of the  
224 experiment and incubated at 15° C. To harvest the zoospores, 10 plates were flushed with  
225 15 mL of 15° C dechlorinated water and remained undisturbed for 10 minutes. The plates  
226 were scraped with a rubber spatula to release the zoospores and sporangia adhering to the  
227 agar. The inoculum from each plate was then pooled in a beaker and the number of  
228 moving zoospores was determined using a hemocytometer. After quantifying the  
229 zoospore concentration, the inoculum was diluted to 10,000 zoospores/mL. Individuals in

230 the Bd-exposed treatments were exposed to 10 mL of inoculum transferred into the 500-  
231 mL container housing an individual larva. Control individuals were exposed to 10 mL of  
232 sham inoculum lacking the Bd culture (made from 1% tryptone sterile agar plates  
233 following the same methods), similarly transferred into the 500-mL container housing  
234 each larva. Thus, the individual larva underwent their exposure treatment on day 24, four  
235 days after the water temperature shift for chambers in the two temperature shift  
236 treatments.

237         During the 40-d trial, survival and metamorphic status were checked daily. Water  
238 for each 500-mL container within the temperature chambers was changed every 12 days  
239 and consisted of dechlorinated water of the same temperature (15° C and 20° C).  
240 Individuals that survived until the end of the trial (i.e., day 40) were euthanized in a 2%  
241 solution of MS-222, and then preserved in 95% ethanol. Individuals that reached  
242 metamorphosis (Gosner stage 42: emergence of forelimbs) were euthanized, measured,  
243 and preserved as previously described.

#### 244 *Determining infection status*

245         We used quantitative polymerase chain reaction (qPCR) to determine infection  
246 status and quantify Bd-infection intensity of all individuals in the Bd-exposure  
247 treatments. Additionally, we investigated Bd-infection status in eight randomly selected  
248 control individuals per species. To sample the individuals for Bd, we extracted whole  
249 mouthparts of the larvae using sterile dissection scissors. We conducted qPCR using an  
250 ABI PRISM 7500 sequencer (Applied Biosystems) according to the methods of Boyle *et*  
251 *al.* (2004) except that we used 60 µL of Prepman Ultra (Applied Biosystems, Carlsbad,  
252 California, USA), instead of the 40 µL in the DNA extraction. All samples were run in

253 triplicate and averaged.

#### 254 *Statistical Analyses*

255 Each temperature-controlled chamber was an experimental unit (whole plot) and  
256 the pairs of containers within each chamber acted as subplots. The whole plots were  
257 subjected to one of four temperature regimes consisting of a Bd-exposure temperature  
258 combined with a temperature shift status (constant cold, constant warm, shifted to cold,  
259 and shifted to warm). Further, subplots were subjected to one of two exposure treatments  
260 (Bd exposed and Bd unexposed).

261 Survival was compared between temperature treatments for western toad  
262 larvae with a Cox proportional hazards model (Cox 1972) using TIBCO Spotfire S+  
263 version 8.1. The model consisted of the main effects of the temperature treatment,  
264 temperature shift status (constant versus shifted), and an interaction between the  
265 two variables. Due to losses of western toad larvae prior to the application of the  
266 exposure treatment, we lacked the power to statistically compare survival in  
267 western toad larvae between the Bd exposure treatments

268 Bd infection abundance (Bd genomic equivalents) among temperature treatments  
269 and between host species was analyzed using R version 3.1.1. We used a zero-inflated  
270 negative-binomial generalized linear model (function ‘zeroinf’ in package ‘pscl’) as  
271 described by Raffel *et al.* (2010), which includes a zero-inflation component that models  
272 infection status as a binomial process (binomial distribution with a logit link) and a count  
273 component that models infection intensity as a negative binomial process (negative  
274 binomial distribution with a log link). Our full model investigated the effects of all of the  
275 explanatory variables including host species, exposure temperature, temperature shift

276 status, and all two- and three-way interactions on Bd (*Batrachochytrium dendrobatidis*)  
277 abundance. Interpretation of this analysis required further reduced models to investigate  
278 the effect of exposure temperature and temperature shift for each species (species model)  
279 and the effect of temperature shift for each Bd-exposure temperature and host species  
280 combination (Bd-exposure temperature model).

281

## 282 **Results**

283 Survival differences were not detected between exposure temperatures (Cox,  
284  $Z = -1.099$ ,  $p = 0.27$ ) or temperature shift status (Cox,  $Z = -0.277$ ,  $p = 0.78$ ) in Bd-  
285 exposed western toad larvae. We were unable to detect survival differences in red  
286 legged frog larvae, as only one individual larva experienced mortality after  
287 application of the exposure treatment (Table S1).

288

### 289 *Infection Abundance*

290 We detected a host species by temperature shift interaction ( $\chi^2_1 = 3.83$ ,  $p = 0.050$ ;  
291 Table S2) and a Bd-exposure temperature by temperature shift interaction ( $\chi^2_1 = 7.50$ ,  $p =$   
292  $0.006$ ; Table S2). We investigated these interactions with reduced models to investigate  
293 effects on Bd abundance at the levels of species and exposure temperature.

294 Red legged frog larvae had higher Bd abundance when they were exposed to  
295 infection at 15° C when compared to 20° C ( $\chi^2_1 = 3.88$ ,  $p = 0.049$ ; Fig. 2). The main  
296 effect of temperature shift was marginally significant in the reduced species model  
297 analysis ( $\chi^2_1 = 3.50$ ,  $p = 0.061$ ), but there was a significant effect of temperature shift for  
298 individuals exposed at 20° C in the reduced Bd-exposure model ( $\chi^2_1 = 5.7$ ,  $p = 0.017$ ),

299 with individuals shifted from 15° C to 20° C having higher Bd abundance than red legged  
300 frog larvae experiencing constant 20° C (Fig. 2). In contrast, there was no evidence that a  
301 temperature shift influenced Bd infection when red legged frog larvae were exposed to  
302 Bd at 15° C ( $\chi^2_1 = 0.6$ ,  $p = 0.4$ ; Fig. 2). There was no statistically significant interaction  
303 between exposure temperature and temperature shift for red legged frog larvae ( $\chi^2_1 = 2.4$ ,  
304  $p = 0.13$ ).

305 We detected an interactive effect of exposure temperature and temperature shift  
306 on Bd abundance in western toad larvae ( $\chi^2_1 = 5.2$ ,  $p = 0.023$ ). This was driven by  
307 elevated Bd abundance in individuals under the constant 15° C temperature when  
308 compared to individuals that experienced a temperature shift from 20° to 15° C, but no  
309 evidence of an effect of shifting temperature from 15° C to 20° C (Fig. 2). There were no  
310 main effects of exposure temperature ( $\chi^2_1 = 0.50$ ,  $p = 0.5$ ) or temperature shift ( $\chi^2_1 < 0.01$ ,  
311  $p = 0.9$ ) on Bd abundance in western toad larvae. Further, when investigating the  
312 exposure temperatures individually in the reduced Bd-exposure model, there was no  
313 evidence that a temperature shift influenced Bd infection in western toad larvae after  
314 exposure to Bd at 15° C ( $\chi^2_1 = 3.4$ ,  $p = 0.066$ ) or 20° C ( $\chi^2_1 = 2.5$ ,  $p = 0.11$ ).

315 We failed to find evidence that the two host species differed in response to  
316 exposure to the pathogen, leading us to conclude that general patterns for both species  
317 were similar (Fig. 2). Both species experienced an increase in Bd abundance when  
318 shifted to 20° C compared to a constant 20° C, and both generally experienced a decrease  
319 in Bd abundance when shifted to 15° C compared to a constant 15° C. Additionally, both  
320 host species experienced elevated Bd abundance in the constant 15° C treatment when  
321 compared to the constant 20° treatment.

322 All red legged frog individuals survived until the end of the experiment but a  
323 number of western toad individuals died or metamorphosed earlier (Table S2). We  
324 therefore assessed the possibility that the timing of Bd sampling or the proximity of a  
325 larva to metamorphosis might drive observed patterns of Bd abundance in western toads.  
326 The model for Bd abundance on western toads was not significantly improved by adding  
327 either a variable coding whether individuals were near metamorphosis when sampled ( $\chi^2_1$   
328 = 4.00,  $p = 0.150$ ) or a covariate indicating the sampling date ( $\chi^2_1 = 3.33$ ,  $p = 0.068$ ).  
329 Furthermore, neither variable qualitatively changed the contribution of exposure  
330 temperature or temperature shift status to the model. Therefore, we omitted both  
331 covariates from the final model for western toads.

332

### 333 **Discussion**

334 Our results suggest that Bd infection dynamics in larval amphibians can be  
335 affected by a shift in water temperature before host exposure to the pathogen, and that the  
336 direction of temperature shift determines the outcome of Bd exposure. Similar patterns  
337 were observed for the two host species when comparing individuals exposed to constant  
338 versus shifted temperatures. A shift from the warm temperature to the colder temperature  
339 was associated with a significant decrease in Bd abundance in western toad larvae and no  
340 significant decrease in red legged frog larvae. Likewise, a shift from the cold temperature  
341 to the warmer temperature significantly increased Bd abundance in red legged frog larvae  
342 and had no significant effect in western toad larvae. Importantly, we detected the effects  
343 of temperature shifts despite the host having a four-day head start on acclimating to the  
344 Bd exposure temperature relative to the pathogen. This suggests that we are likely

345 underestimating the strength of these effects and that their magnitudes might have been  
346 larger if the host and pathogen experienced the shifts concurrently.

347 Amphibian species do not all respond similarly to a given Bd exposure. Species-  
348 level differences in host tolerance to Bd infections have been well documented under  
349 controlled laboratory conditions (Searle *et al.* 2011, Gervasi *et al.* 2017). Under natural  
350 conditions, pathogen tolerance within a species may be affected by biotic factors such as  
351 inter- and intra-specific interactions, proximity to metamorphosis, or life stage (Parris &  
352 Cornelius 2004, Rachowicz & Vredenburg 2004, Blaustein *et al.* 2005, McMahon &  
353 Rohr 2015) or abiotic factors such as temperature, season, or resource availability (Berger  
354 *et al.* 2004, Raffel *et al.* 2010). For some susceptible host species, temperature-shift  
355 induced changes in Bd abundance might alter the outcome of infection by either pushing  
356 *Bd* abundance over or under a tolerance threshold. Such changes in relation to pathogen  
357 abundance and pathogen tolerance may result in altering the strength of negative effects  
358 of Bd infection. For example, temperature shifts in synergy with Bd infection may result  
359 in either positive or negative effects on growth and development rates, foraging  
360 efficiency, or predator avoidance (Parris & Cornelius 2004, Parris, Reese & Storfer 2006,  
361 Venesky, Parris & Storfer 2010, Venesky, Wassersug & Parris 2010).

362 We hypothesized that hosts exposed to a shifted temperature would respond to  
363 infection differently than hosts exposed to a constant temperature, and under the  
364 framework of the “lag effect” hypothesis (Raffel *et al.* 2006, Rohr & Raffel 2010), the  
365 direction of the temperature shift would differentially affect infection severity. We  
366 predicted that a temperature shift from cold-to-warm would leave hosts in a temporarily  
367 immune-compromised state and result in an elevated Bd abundance after exposure when



368 compared to hosts exposed to a constant warm temperature. Conversely, we predicted  
369 that a temperature shift from warm-to-cold would provide hosts with a temporarily  
370 elevated-immune responsiveness and result in a decrease in Bd abundance after exposure  
371 when compared to hosts exposed to a constant cold temperature. Our results were  
372 consistent with predictions of the “lag effect” hypothesis, and were generally consistent  
373 with previous studies showing that a shift in temperature influences Bd infection in  
374 postmetamorphic amphibians (Raffel *et al.* 2013, Raffel *et al.* 2015). In particular, our  
375 finding of decreased resistance to infection following a temperature increase (relative to  
376 warm-acclimated individuals) mirrored a laboratory study of post-metamorphic red-  
377 spotted newts (*Notophthalmus viridescens*), where juvenile newts exhibited decreased Bd  
378 resistance following a shift from 15° C to 25° C (Raffel *et al.* 2015). These findings of  
379 fluctuating temperature effects on Bd infection across four anuran taxonomic groups and  
380 life-stages suggest that effects of temperature shifts and Bd-related chytridiomycosis  
381 susceptibility might be widespread within amphibians. However, our finding of increased  
382 resistance to Bd infection following a temperature decrease (relative to cold-acclimated  
383 individuals) was opposite the pattern observed in red-spotted newts and Cuban treefrogs  
384 (Raffel *et al.* 2013, Raffel *et al.* 2015) These contrasting results suggests that there are  
385 important among-taxa or among-stage differences in the underlying mechanisms driving  
386 the effects of temperature fluctuation on Bd infection; whereas our results in pre-  
387 metamorphic life-stage of western toads and red legged frogs are consistent with the “lag  
388 effect” hypothesis, results of similar studies investigating post-metamorphic red-spotted  
389 newts and Cuban treefrogs support the “climate variability hypothesis.”

390           We observed differences in Bd abundance on our two amphibian species at the  
391 two constant temperature treatments. Higher Bd abundances were observed for both host  
392 species under the constant cold temperature treatment compared to the constant warm  
393 temperature treatment. These results are consistent with previous experiments that  
394 showed increased Bd abundance (Raffel *et al.* 2015) and Bd-induced mortality  
395 (Kilpatrick, Briggs & Daszak 2010, Murphy, St-Hilaire & Corn 2011, Raffel *et al.* 2015)  
396 associated with lower temperatures. This is despite Bd growing best in culture at about  
397 23° C, which is much closer to the warm than cold temperature treatments in this  
398 experiment (Piotrowski, Annis & Longcore 2004, Woodhams *et al.* 2008). This might be  
399 because the larval immune response to Bd infection increases with increasing  
400 temperatures at a faster rate than the infectivity or growth rate of Bd (Raffel *et al.* 2013),  
401 or alternatively because of the differences between the growth rate of Bd in culture  
402 compared to the growth rate on host tissue (Venesky *et al.* 2013). Our results provide  
403 further evidence to suggest patterns of Bd growth in culture differ from patterns of Bd  
404 growth on a host and that it is important to assess the host-parasite interaction when  
405 predicting effects of climate and climate change on disease risk.

406           Alternatively, differences in Bd abundance between the two constant temperature  
407 treatments might be due to temperature effects on the pathogen rather than the host  
408 (Woodhams *et al.* 2008, Voyles *et al.* 2012). The Bd was cultured at 15° C; it is possible  
409 that the temperature shift experienced by the pathogen in the warm exposure treatment  
410 caused the depressed Bd abundances observed in both host species compared to the  
411 elevated Bd abundance in the cold exposure temperature treatment. A decrease in  
412 temperature may cause an increase in the number of Bd zoospores released from

413 zoosporangia (Hyatt *et al.* 2007, Woodhams *et al.* 2008), however the effect of a similar  
414 increase in temperature on Bd physiology is unclear.

415         In conclusion, our results provide additional evidence for climate variability  
416 affecting Bd infection in amphibians but suggest important among-taxa differences in the  
417 directionality of these effects. Our finding of increased host resistance to infection  
418 following a temperature decrease is consistent with the “lag effect” hypothesis of Raffel  
419 *et al.* (2006) but contradicts components of the “climate variability hypothesis”, which  
420 has been proposed as an explanation for patterns of Bd-associated amphibian population  
421 declines (Rohr & Raffel 2010, Raffel *et al.* 2013, Raffel *et al.* 2015). Our study highlights  
422 the complexity that temperature plays in determining the outcome of Bd-amphibian  
423 interactions and the role that a fluctuating temperature might play in altering these  
424 interactions. Furthermore, this study increases the diversity of amphibian species and  
425 stages that have been shown to exhibit thermal acclimation effects on disease, and the  
426 broad generality of this pattern across four disparate taxa suggests that fluctuating-  
427 temperature effects on amphibian infection may be widespread. Accurately predicting the  
428 effects of global climate change on infectious diseases, such as chytridiomycosis will  
429 require further understanding of how infectious agents respond to heterogeneity in  
430 temperatures and temperature fluctuations.

431

### 432 **Acknowledgments**

433         All applicable institutional and national guidelines for the care and use of animals  
434 were followed; this research was conducted under Oregon State University IACUC  
435 animal care and use permit 3917. Collection of amphibian eggs was approved by the

436 Oregon Department of Fish and Wildlife (Oregon Scientific Taking Permit #006-12  
437 issued to ARB). We thank S. Bauer, E. Davis, E. Hunt, A. Koosman, B. Meyers, M.  
438 Ouspenskaya, E. Peseke, V. Raffeale, and C. Rains for their help performing the  
439 experiment, K. Boersma for her help with the experimental design, and E. Boersley for  
440 her support and assistance. Additionally we thank J. Spatafora, V. Weis, and the Center  
441 for Genome Research and Biocomputing at Oregon State University for providing  
442 laboratory space for qPCR. This research was supported by grants from the National  
443 Science Foundation (EF-1241889), National Institutes of Health (R01GM109499,  
444 R01TW010286), U.S. Department of Agriculture (NRI 2006-01370, 2009-35102-0543),  
445 and U.S. Environmental Protection Agency (CAREER 83518801) to JRR and NSF grant  
446 IOS 1121529 to TRR. Support was provided by the U.S. Forest Service Pacific  
447 Northwest Research Station, Corvallis, Oregon to DHO.

448

449 Conflict of Interest: The authors declare that they have no conflict of interest.

450

#### 451 **Supporting Information**

452 Additional supporting information may be found in electronic supplementary

453 material for this article.

454 **Literature Cited**

- 455 Alford, R. A. (1989) Variation in predator phenology affects predator performance  
456 and prey community composition. *Ecology*, **70**, 206-219.
- 457 Angilletta, M. J. (2009) *Thermal adaptation a theoretical and empirical synthesis*.  
458 New York, New York. USA.: Oxford University Press.
- 459 Bannerman, J. A. & B. D. Roitberg (2014) Impact of extreme and fluctuating  
460 temperatures on aphid-parasitoid dynamics. *Oikos*, **123**, 89-98.
- 461 Ben-Horin, T., H. S. Lenihan & K. D. Lafferty (2012) Variable intertidal temperature  
462 explains why disease endangers black abalone. *Ecology*, **94**, 161-168.
- 463 Berger, L., R. Speare, H. B. Hines, G. Marantelli, A. D. Hyatt, K. R. McDonald, L. F.  
464 Skerratt, V. Olsen, J. M. Clarke, G. Gillespie, M. Mahony, N. Sheppard, C.  
465 Williams & M. J. Tyler (2004) Effect of season and temperature on mortality  
466 in amphibians due to chytridiomycosis. *Aust. Vet. J.*, **82**, 434-439.
- 467 Blaustein, A. R., S. S. Gervasi, P. T. J. Johnson, J. T. Hoverman, L. K. Belden, P. W.  
468 Bradley & G. Y. Xie (2012) Ecophysiology meets conservation:  
469 understanding the role of disease in amphibian population declines. *Philos.*  
470 *Trans. R. Soc., B*, **367**, 1688-1707.
- 471 Blaustein, A. R., J. M. Romansic, E. A. Scheessele, B. A. Han, A. P. Pessier & J. E.  
472 Longcore (2005) Interspecific variation in susceptibility of frog tadpoles to the  
473 pathogenic fungus *Batrachochytrium dendrobatidis*. *Conserv. Biol.*, **19**, 1460-  
474 1468.

- 475 Blaustein, A. R., S. C. Walls, B. A. Bancroft, J. J. Lawler, C. L. Searle & S. S.  
476 Gervasi (2010) Direct and indirect effects of climate change on amphibian  
477 populations. *Diversity*, **2**, 281-313.
- 478 Bosch, J., L. Carrascal, L. Durán, S. Walker & M. Fisher (2007) Climate change and  
479 outbreaks of amphibian chytridiomycosis in a montane area of central Spain;  
480 is there a link? *Proceedings of the Royal Society B*, **274**, 253.
- 481 Boyle, D. G., D. B. Boyle, V. Olsen, J. A. T. Morgan & A. D. Hyatt (2004) Rapid  
482 quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*)  
483 in amphibian samples using real-time Taqman PCR assay. *Dis. Aquat. Org.*,  
484 **60**, 141-148.
- 485 Brönmark, C., S. D. Rundle & A. Erlandsson (1991) Interactions between freshwater  
486 snails and tadpoles: competition and facilitation. *Oecologia*, **87**, 8-18.
- 487 Brown, J. H., J. F. Gilgooly, A. P. Allen, V. M. Savage & G. B. West (2004) Toward  
488 a metabolic theory of ecology. *Ecology*, **85**, 1771-1789.
- 489 Buck, J. C., E. A. Scheessele, R. A. Relyea & A. R. Blaustein (2012) The effects of  
490 multiple stressors on wetland communities: pesticides, pathogens and  
491 competing amphibians. *Freshwat. Biol.*, **57**, 61-73.
- 492 Daskin, J. H., R. A. Alford & R. Puschendorf (2011) Short-term exposure to warm  
493 microhabitats could explain amphibian persistence with *Batrachochytrium*  
494 *dendrobatidis*. *PLoS ONE*, **6**, e26215.

- 495 DeSantis, D. E. & R. G. Strauss, (1997) Cell biology and disorders of neutrophils. In:  
496 *Clinical Hematology and Fundamentals of Hemostasis*: 265– 282. D. M.  
497 Harmening (Ed.). Davis, Philadelphia, Pennsylvania. USA.
- 498 Drew, A., E. J. Allen & L. J. S. Allen (2006) Analysis of climatic and geographic  
499 factors affecting the presence of chytridiomycosis in Australia. *Dis. Aquat.*  
500 *Org.*, **68**, 245-250.
- 501 Easterling, D. R., G. A. Meehl, C. Parmesan, S. A. Changnon, T. R. Karl & L. O.  
502 Mearns (2000) Climate extremes: observations, modeling, and impacts.  
503 *Science*, **289**, 2068-2074.
- 504 Forrest, M. J. & M. A. Schlaepfer (2011) Nothing a hot bath won't cure: infection  
505 rates of amphibian chytrid fungus correlate negatively with water temperature  
506 under natural field settings. *PLoS ONE*, **6**, e28444.
- 507 Garcia, R. A., M. Cabeza, C. Rahbek & M. B. Araujo (2014) Multiple dimensions of  
508 climate change and their implications for biodiversity. *Science*, **344**, 1247579.
- 509 Garner, T. W. J., S. Walker, J. Bosch, S. Leech, J. M. Rowcliffe, A. A. Cunningham  
510 & M. C. Fisher (2009) Life history tradeoffs influence mortality associated  
511 with the amphibian pathogen *Batrachochytrium dendrobatidis*. *Oikos*, **118**,  
512 783-791.
- 513 Gervasi, S., C. Gondhalekar, D. H. Olson & A. R. Blaustein (2013) Host identity  
514 matters in the amphibian-*Batrachochytrium dendrobatidis* system: fine-scale

- 515 patterns of variation in responses to a multi-host pathogen. *PLoS ONE*, **8**,  
516 e54490.
- 517 Gervasi, S. S., P. R. Stephens, J. Hua, C. L. Searle, G. Y. Xie, J. Urbina, D. H. Olson,  
518 B. A. Bancroft, V. Weis, J. I. Hammond, R. A. Relyea & A. R. Blaustein  
519 (2017) Linking Ecology and Epidemiology to Understand Predictors of Multi-  
520 Host Responses to an Emerging Pathogen, the Amphibian Chytrid Fungus.  
521 *PLoS ONE*, **12**, e0167882.
- 522 Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage & E. L. Charnov (2001)  
523 Effects of size and temperature on metabolic rate. *Science*, **293**, 2248-2251.
- 524 Han, B. A., P. W. Bradley & A. R. Blaustein (2008) Ancient behaviors of larval  
525 amphibians in response to an emerging fungal pathogen, *Batrachochytrium*  
526 *dendrobatidis*. *Behav. Ecol. Sociobiol.*, **63**, 241-250.
- 527 Hyatt, A. D., D. G. Boyle, V. Olsen, D. B. Boyle, L. Berger, D. Obendorf, A. Dalton,  
528 K. Kriger, J. M. Hero, H. Hines, R. Phillott, R. Campbell, G. Marantelli, F.  
529 Gleason & A. Colling (2007) Diagnostic assays and sampling protocols for  
530 the detection of *Batrachochytrium dendrobatidis*. *Dis. Aquat. Org.*, **73**, 175-  
531 192.
- 532 Janeway, C. (2008) *Janeway's Immunobiology*, 7 edn. New York, New York. USA.:  
533 Garland Science.



- 534 Kilpatrick, A. M., C. J. Briggs & P. Daszak (2010) The ecology and impact of  
535 chytridiomycosis: an emerging disease of amphibians. *Trends Ecol. Evol.*, **25**,  
536 109-118.
- 537 Kupferberg, S. J. (1997) The role of larval diet in anuran metamorphosis. *Am. Zool.*,  
538 **37**, 146-159.
- 539 Lafferty, K. D. (2009) The ecology of climate change and infectious diseases.  
540 *Ecology*, **90**, 888-900.
- 541 Lamberti, G., S. Gregory, C. Hawkins, R. Wildman, L. Ashkenas & D. Denicola  
542 (1992) Plant—herbivore interactions in streams near Mount St Helens.  
543 *Freshwat. Biol.*, **27**, 237-247.
- 544 Lawler, J. J., S. L. Shafer, B. A. Bancroft & A. R. Blaustein (2010) Projected climate  
545 impacts for the amphibians of the Western hemisphere. *Conserv. Biol.*, **24**, 38-  
546 50.
- 547 Li, Y., J. M. Cohen & J. R. Rohr (2013) Review and synthesis of the effects of  
548 climate change on amphibians. *Integr Zool*, **8**, 145-161.
- 549 Liu, X., J. R. Rohr & Y. Li (2013) Climate, vegetation, introduced hosts and trade  
550 shape a global wildlife pandemic. *Proceedings of the Royal Society B:*  
551 *Biological Sciences*, **280**, 20122506.
- 552 Longcore, J., A. Pessier & D. Nichols (1999) *Batrachochytrium dendrobatidis* gen. et  
553 sp. nov., a chytrid pathogenic to amphibians. *Mycologia*, **91**, 219-227.

- 554 Luis, A. D., R. J. Douglass, J. N. Mills & O. N. Bjørnstad (2014) Environmental  
555 fluctuations lead to predictability in Sin Nombre hantavirus outbreaks.  
556 *Ecology*, **96**, 1691-1701.
- 557 Macela, A. & A. Romanovsky (1970) The role of temperature in separate stages of  
558 the immune reaction in anurans. *Folia Biologica*, **15**, 157-160.
- 559 Maniero, G. D. & C. Carey (1997) Changes in selected aspects of immune function in  
560 the leopard frog, *Rana pipiens*, associated with exposure to cold. *J. Comp.*  
561 *Physiol., B*, **167**, 256-263.
- 562 Marantelli, G., L. Berger, R. Speare & L. Keegan (2004) Distribution of the  
563 amphibian chytrid *Batrachochytrium dendrobatidis* and keratin during tadpole  
564 development. *Pac. Conserv. Biol.*, **10**, 173-179.
- 565 Martel, A., A. Spitzen-van der Sluijs, M. Blooi, W. Bert, R. Ducatelle, M. C. Fisher,  
566 A. Woeltjes, W. Bosman, K. Chiers, F. Bossuyt & F. Pasmans (2013)  
567 *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis  
568 in amphibians. *Proc. Natl. Acad. Sci. U. S. A.*, **110**, 15325-15329.
- 569 McCallum, M. L. (2007) Amphibian decline or extinction? Current declines dwarf  
570 background extinction rate. *J. Herpetol.*, **41**, 483-491.
- 571 McMahon, T. A. & J. R. Rohr (2015) Transition of chytrid fungus infection from  
572 mouthparts to hind limbs during amphibian metamorphosis. *EcoHealth*, **12**,  
573 188-193.

- 574 McMahon, T. A., B. F. Sears, M. D. Venesky, S. M. Bessler, J. M. Brown, K.  
575 Deutsch, N. T. Halstead, G. Lentz, N. Tenouri, S. Young, D. J. Civitello, N.  
576 Ortega, J. S. Fites, L. K. Reinert, L. A. Rollins-Smith, T. R. Raffel & J. R.  
577 Rohr (2014) Amphibians acquire resistance to live and dead fungus  
578 overcoming fungal immunosuppression. *Nature*, **511**, 224-227.
- 579 Meehl, G. A. & C. Tebaldi (2004) More intense, more frequent, and longer lasting  
580 heat waves in the 21st century. *Science*, **305**, 994-997.
- 581 Murphy, P. J., S. St-Hilaire & P. S. Corn (2011) Temperature, hydric environment,  
582 and prior pathogen exposure alter the experimental severity of  
583 chytridiomycosis in boreal toads. *Dis. Aquat. Org.*, **95**, 31-42.
- 584 Muths, E., D. S. Pilliod & L. J. Livo (2008) Distribution and environmental  
585 limitations of an amphibian pathogen in the Rocky Mountains, USA. *Biol.*  
586 *Conserv.*, **141**, 1484-1492.
- 587 Olson, D. H., D. M. Aanensen, K. L. Ronnenberg, C. I. Powell, S. F. Walker, J.  
588 Bielby, T. W. J. Garner, G. Weaver, M. C. Fisher & T. B. M. Group (2013)  
589 Mapping the global emergence of *Batrachochytrium dendrobatidis*, the  
590 amphibian chytrid fungus. *PLoS ONE*, **8**, e56802.
- 591 Paaijmans, K. P., S. Blanford, A. S. Bell, J. I. Blanford, A. F. Read & M. B. Thomas  
592 (2010) Influence of climate on malaria transmission depends on daily  
593 temperature variation. *Proc. Natl. Acad. Sci. U. S. A.*, **107**, 15135-15139.

- 594 Paaijmans, K. P., A. F. Read & M. B. Thomas (2009) Understanding the link between  
595 malaria risk and climate. *Proc. Natl. Acad. Sci. U. S. A.*, **106**, 13844-13849.
- 596 Parris, M., E. Reese & A. Storfer (2006) Antipredator behavior of chytridiomycosis-  
597 infected northern leopard frog (*Rana pipiens*) tadpoles. *Can J Zool*, **84**, 58-65.
- 598 Parris, M. J. & T. O. Cornelius (2004) Fungal pathogen causes competitive and  
599 developmental stress in larval amphibian communities. *Ecology*, **85**, 3385-  
600 3395.
- 601 Paull, S. H., B. E. LaFonte & P. T. J. Johnson (2012) Temperature-driven shifts in a  
602 host-parasite interaction drive nonlinear changes in disease risk. *Global  
603 Change Biol.*, **18**, 3558-3567.
- 604 Pearl, C. A., E. L. Bull, D. E. Green, J. Bowerman, M. J. Adams, A. Hyatt & W. H.  
605 Wente (2007) Occurrence of the amphibian pathogen *Batrachochytrium  
606 dendrobatidis* in the Pacific Northwest. *J. Herpetol.*, **41**, 145-149.
- 607 Piotrowski, J. S., S. L. Annis & J. E. Longcore (2004) Physiology of  
608 *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians.  
609 *Mycologia*, **96**, 9-15.
- 610 Piovia-Scott, J., K. L. Pope, S. P. Lawler, E. M. Cole & J. E. Foley (2011) Factors  
611 related to the distribution and prevalence of the fungal pathogen  
612 *Batrachochytrium dendrobatidis* in *Rana cascadae* and other amphibians in  
613 the Klamath Mountains. *Biol. Conserv.*, **144**, 2913–2921.

- 614 Rachowicz, L. J. & V. T. Vredenburg (2004) Transmission of *Batrachochytrium*  
615 *dendrobatidis* within and between amphibian life stages. *Dis. Aquat. Org.*, **61**,  
616 75-83.
- 617 Raffel, T. R., N. T. Halstead, T. A. McMahon, A. K. Davis & J. R. Rohr (2015)  
618 Temperature variability and moisture synergistically interact to exacerbate an  
619 epizootic disease. *Proceedings of the Royal Society B: Biological Sciences*,  
620 **282**, 20142039.
- 621 Raffel, T. R., P. J. Michel, E. W. Sites & J. R. Rohr (2010) What drives chytrid  
622 infections in newt populations? Associations with substrate, temperature, and  
623 shade. *EcoHealth*, **7**, 526-536.
- 624 Raffel, T. R., J. R. Rohr, J. M. Kiesecker & P. J. Hudson (2006) Negative effects of  
625 changing temperature on amphibian immunity under field conditions. *Funct.*  
626 *Ecol.*, **20**, 819-828.
- 627 Raffel, T. R., J. M. Romansic, N. T. Halstead, T. A. McMahon, M. D. Venesky & J.  
628 R. Rohr (2013) Disease and thermal acclimation in a more variable and  
629 unpredictable climate. *Nat. Clim. Change*, **3**, 146–151.
- 630 Rohr, J. R., A. P. Dobson, P. T. Johnson, A. M. Kilpatrick, S. H. Paull, T. R. Raffel,  
631 D. Ruiz-Moreno & M. B. Thomas (2011) Frontiers in climate change-disease  
632 research. *Trends Ecol. Evol.*, **26**, 270-277.

- 633 Rohr, J. R. & T. R. Raffel (2010) Linking global climate and temperature variability  
634 to widespread amphibian declines putatively caused by disease. *Proc. Natl.*  
635 *Acad. Sci. U. S. A.*, **107**, 8269-8274.
- 636 Rohr, J. R., T. R. Raffel, A. R. Blaustein, P. T. J. Johnson, S. H. Paull & S. Young  
637 (2013) Using physiology to understand climate-driven changes in disease and  
638 their implications for conservation. *Conserv. Physiol.*, **1**, cot022.
- 639 Rohr, J. R., T. R. Raffel, J. M. Romansic, H. McCallum & P. J. Hudson (2008)  
640 Evaluating the links between climate, disease spread, and amphibian declines.  
641 *Proc. Natl. Acad. Sci. U. S. A.*, **105**, 17436.
- 642 Rosenblum, E. B., T. Y. James, K. R. Zamudio, T. J. Poorten, D. Ilut, D. Rodriguez,  
643 J. M. Eastman, K. Richards-Hrdlicka, S. Joneson, T. S. Jenkinson, J. E.  
644 Longcore, G. Parra Olea, L. F. Toledo, M. L. Arellano, E. M. Medina, S.  
645 Restrepo, S. V. Flechas, L. Berger, C. J. Briggs & J. E. Stajich (2013)  
646 Complex history of the amphibian-killing chytrid fungus revealed with  
647 genome resequencing data. *Proc. Natl. Acad. Sci. U. S. A.*, **110**, 9385-9390.
- 648 Rummukainen, M. (2012) Changes in climate and weather extremes in the 21st  
649 century. *Wiley Interdiscip. Rev.: Clim. Change*, **3**, 115-129.
- 650 Schar, C., P. L. Vidale, D. Luthi, C. Frei, C. Haberli, M. A. Liniger & C. Appenzeller  
651 (2004) The role of increasing temperature variability in European summer  
652 heatwaves. *Nature*, **427**, 332-336.

- 653 Searle, C. L., S. S. Gervasi, J. Hua, J. I. Hammond, R. A. Relyea, D. H. Olson & A.  
654 R. Blaustein (2011) Differential host susceptibility to *Batrachochytrium*  
655 *dendrobatidis*, an emerging amphibian pathogen. *Conserv. Biol.*, **25**, 965-974.
- 656 Sheldon, K. S., S. Yang & J. J. Tewksbury (2011) Climate change and community  
657 disassembly: impacts of warming on tropical and temperate montane  
658 community structure. *Ecol. Lett.*, **14**, 1191-1200.
- 659 Shoo, L. P., D. H. Olson, S. K. McMenamin, K. A. Murray, M. Van Sluys, M. A.  
660 Donnelly, D. Stratford, J. Terhivuo, A. Merino-Viteri, S. M. Herbert, P. J.  
661 Bishop, P. S. Corn, L. Dovey, R. A. Griffiths, K. Lowe, M. Mahony, H.  
662 McCallum, J. D. Shuker, C. Simpkins, L. F. Skerratt, S. E. Williams & J.-M.  
663 Hero (2011) Engineering a future for amphibians under climate change. *J.*  
664 *Appl. Ecol.*, **48**, 487-492.
- 665 Skerratt, L., L. Berger, R. Speare, S. Cashins, K. McDonald, A. Phillott, H. Hines &  
666 N. Kenyon (2007) Spread of chytridiomycosis has caused the rapid global  
667 decline and extinction of frogs. *EcoHealth*, **4**, 125-134.
- 668 Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L.  
669 Fischman & R. W. Waller (2004) Status and trends of amphibian declines and  
670 extinctions worldwide. *Science*, **306**, 1783-1786.
- 671 Venesky, M., M. Parris & A. Storfer (2010) Impacts of *Batrachochytrium*  
672 *dendrobatidis* infection on tadpole foraging performance. *EcoHealth*, **6**, 565-  
673 575.

- 674 Venesky, M. D., T. R. Raffel, T. A. McMahon & J. R. Rohr (2013) Confronting  
675 inconsistencies in the amphibian-chytridiomycosis system: implications for  
676 disease management. *Biol. Rev. Camb. Philos. Soc.*, **89**, 477-483.
- 677 Venesky, M. D., R. J. Wassersug & M. J. Parris (2010) Fungal pathogen changes the  
678 feeding kinematics of larval anurans. *J. Parasitol.*, **96**, 552-557.
- 679 Voyles, J., L. R. Johnson, C. J. Briggs, S. D. Cashins, R. A. Alford, L. Berger, L. F.  
680 Skerratt, R. Speare & E. B. Rosenblum (2012) Temperature alters  
681 reproductive life history patterns in *Batrachochytrium dendrobatidis*, a lethal  
682 pathogen associated with the global loss of amphibians. *Ecology and*  
683 *Evolution*, **2**, 2241-2249.
- 684 Voyles, J., L. R. Johnson, J. Rohr, R. Kelly, C. Barron, D. Miller, J. Minster & E. B.  
685 Rosenblum (2017) Diversity in growth patterns among strains of the lethal  
686 fungal pathogen *Batrachochytrium dendrobatidis* across extended thermal  
687 optima. *Oecologia*, 1-11.
- 688 Wake, D. B. (2012) Facing extinction in real time. *Science*, **335**, 1052-1053.
- 689 Woodhams, D. C., R. A. Alford, C. J. Briggs, M. Johnson & L. A. Rollins-Smith  
690 (2008) Life-history trade-offs influence disease in changing climates:  
691 strategies of an amphibian pathogen. *Ecology*, **89**, 1627-1639.
- 692 Xie, G. Y., D. H. Olson & A. R. Blaustein (2016) Projecting the Global Distribution  
693 of the Emerging Amphibian Fungal Pathogen, *Batrachochytrium*  
694 *dendrobatidis*, Based on IPCC Climate Futures. *PLoS ONE*, **11**, e0160746.



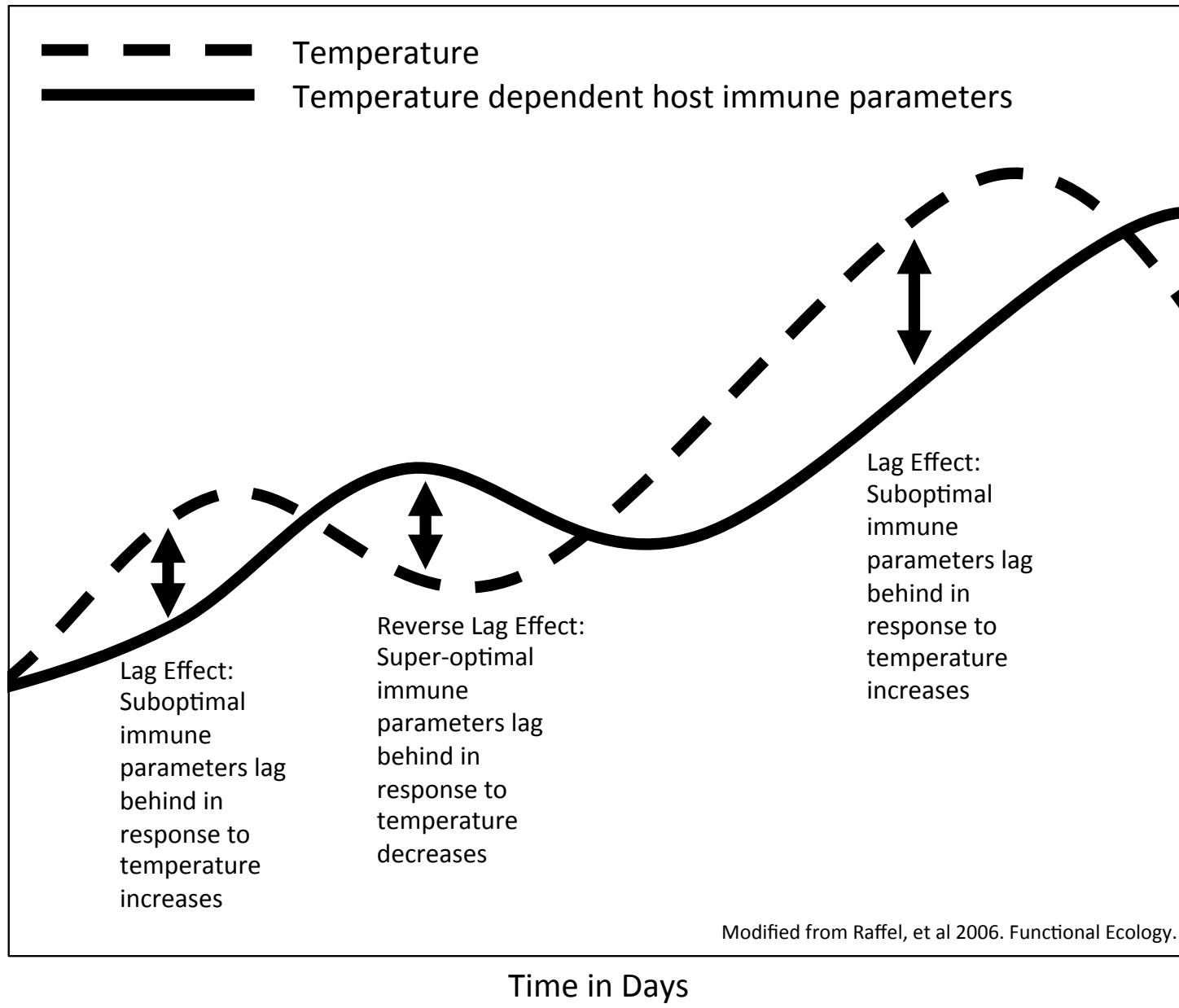
695

696

697 **Fig 1.** Hypothesized lag effect showing the relationship between fluctuating  
698 temperatures (over days to weeks) and the optimal levels of a hypothetical  
699 temperature-dependent host immune parameter. The immune parameter follows and  
700 lags behind temperature changes – resulting in periods of a compromised immune  
701 status after a temperature increase, and resulting in an over-active (or unnecessarily  
702 costly) immune status after a temperature decrease. Modified from Raffel *et al.*  
703 (2006).

704

705 **Fig 2.** Mean *Batrachochytrium dendrobatidis* (Bd) infection abundance ( $\pm$  SE)  
706 measured at death, or at euthanasia 16-days after Bd exposure, in both western toad  
707 (*Anaxyrus boreas*) larvae and red legged frog (*Rana aurora*) larvae from Oregon,  
708 USA, and between the two temperatures at the time of Bd-exposure (cold [15° C]  
709 versus warm [20° C]) and between larvae having experienced either a constant or  
710 shifted temperature. Bd infection abundance is quantified as the log (1 + Bd genomic  
711 equivalents) per excised larval mouthparts of all individuals exposed to the pathogen.  
712



Bd Infection Abundance (Log (1 + genomic equivalents))

