Natural selection on plasticity of thermal traits in a highly seasonal environment

Leonardo D. Bacigalupe[1*], Aura M. Barria[1], Avia Gonzalez-Mendez[1], Manuel Ruiz-Aravena[2] Juan D. Gaitan-Espitia[3,4], Mark Trinder[5] and Barry Sinervo[6*]

[1]Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

[2] School of Natural Sciences, College of Sciences and Engineering, University of Tasmania, Hobart, Tasmania, Australia

[3] CSIRO Oceans and Atmosphere, GPO Box 1538, Hobart 7001, TAS, Australia

[4]The Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, China

[5] MacArthur Green, 95 South Woodside Road, Glasgow, UK

[6]Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, CA, 95064, USA

*Both authors contributed equally to this work

Correspondence should be addressed to L.D.B (+5663 2293567, lbacigal@gmail.com)

Abstract

For ectothermic species with broad geographical distributions, latitudinal/altitudinal variation in environmental temperatures (averages and extremes) are expected to shape the evolution of physiological tolerances and the acclimation capacity (i.e., degree of phenotypic plasticity) of natural populations. This can create geographical gradients of selection in which environments with greater thermal variability (e.g., seasonality) tend to favour individuals that maximize performance across a broader range of temperatures compared to more stable environments. Although thermal acclimation capacity plays a fundamental role in this context, it is unknown whether natural selection targets this trait in natural populations. Here we addressed such an important gap in our knowledge by measuring survival, through mark recapture integrated into an information-theoretic approach, as a function of the plasticity of critical thermal limits for activity, behavioural thermal preference and the thermal sensitivity of metabolism in the northernmost population of the four-eyed frog *Pleurodema thaul*. Overall, our results indicate that thermal acclimation in this population is not being targeted by directional selection, although there are signals of selection on other traits. In particular, we found positive directional selection on body size and negative directional selection on all physiological traits: higher tolerance is being favourably selected during the cooler periods of the year, while higher tolerance and preference is being selected against, which suggests that extreme hot temperatures favour individuals that might be able to avoid hot microhabitats.

Keywords: Amphibians, natural selection, physiological plasticity, acclimation, *Pleurodema thaul*, Atacama Desert

Introduction

It is well known that environmental temperature (T_a) is the abiotic factor with major incidence in the evolution, ecology and physiology of most of the biodiversity in the planet (Angilletta 2009 and references therein). The effects of T_a are particularly relevant for ectotherms as their body temperature depends on T_a and therefore any change in T_a affects their fitness and performance (e.g. behaviour, growth, reproduction, metabolism). This relationship between performance and temperature has been described by a thermal performance curve (TPC) (Huey & Berrigan 2001; Angilletta 2009) which has often been used to describe the thermal ecology and evolution of ectotherms (Gilchrist 1995; Huey & Kingsolver 1989), their phenotypic plasticity (Schulte et al. 2011), and to predict their responses to climate change (Clusella-Trullas et al. 2011; Sinclair et al. 2016). The TPC is best captured by three parameters: a minimum critical temperature (CT_{Min}), which represents T_a below which performance is minimum; a maximum critical temperature (CT_{Max}), which represents T_a above which performance is also minimum and an optimum temperature (T_{Opt}), which represents T_a at which performance is maximum. Most of these parameters can exhibit geographic variation depending on the particular environmental context (e.g., local climate) and genetic background of populations (Gilchrist 1996; Kingsolver et al. 2004; Latimer et al. 2011). Furthermore, this geographic variation has the potential to create gradients of selection for TPCs across the species distribution (Kingsolver & Gomulkiewicz 2003) shaping thermal sensitivities, tolerances and thermal acclimation capacities (i.e., thermal plasticity) of local populations (Seebacher et al. 2012; Gaitan-Espitia et al. 2014).

Different climate-related hypotheses have been proposed to explain how physiological tolerances, capacities and their plasticity affect the distributional ranges of species (Bozinovic et

al. 2011). One of them, the climate variability hypothesis (CVH), offers a powerful conceptual framework to explore the interactions between environmental variability and physiological performance of ectotherms (e.g., Gaitan-Espitia et al. 2013; 2014). The CVH predicts that organisms inhabiting more variable environments should have broader ranges of environmental tolerance and/or greater physiological plasticity that enable them to cope with the fluctuating environmental conditions (e.g., seasonality) (Ghalambor et al. 2006; Gaitan-Espitia et al. 2017). In agreement with this hypothesis, other theoretical models have explored the evolutionary mechanisms underlying local thermal adaptation across heterogeneous environments (e.g., Generalist-Specialist models). For instance, the model developed by Lynch and Gabriel (1987), predicts that temporal environmental heterogeneity selects for more broadly adapted individuals, whereas in more constant environments the model developed by Gilchrist (1995), predicts that selection should favor thermal specialists with narrow performance breadth. The mechanistic understanding of these conceptual frameworks has improved with recent studies showing how in thermally variable environments directional selection acts on TPC's parameters, despite the ability of ectotherms to thermoregulate behaviorally (Buckley et al. 2015), favoring organisms that maximize performance across a broader range of temperatures (Logan et al. 2014). Notwithstanding this progress, whether natural selection targets thermal acclimation capacity (i.e., plasticity) itself in natural populations remains unknown.

In addition to increasing mean temperatures, it is known that climate change is changing the frequency and intensity of extreme temperatures and events (Rahmstorf & Coumou 2011; Wang & Dillon 2014; Vazquez et al. 2016). This, in turn, suggests that both averages and variances will have an important impact on different performance related traits (e.g. Lardies et al. 2014; Vasseur et al. 2014; Bartheld et al. 2017). Nevertheless, we still do not know whether

92 selection might also target traits as a function of those extremes. In this context, populations
93 inhabiting highly seasonal environments characterized also by daily extreme temperatures,
94 provide a natural laboratory to evaluate the role of natural selection on the plasticity of critical
95 thermal limits and preferences. We addressed such important gaps in our knowledge by
96 measuring for the first time survival as a function of the plasticity of thermal critical
97 temperatures (CT_{Max} and CT_{Min}), preferred temperature (T_{Pref}) and thermal sensitivity of
98 metabolism (Q_{10}; the magnitude of change in metabolic rate for a 10ºC change in T_a) after
99 acclimation to 10°C and 20°C in the northernmost population of the four-eyed frog *Pleurodema*
100 *thaul*. We tested four predictions regarding phenotypic selection and plasticity that built up from
101 previous findings showing that acclimation to warmer temperatures produces an increase in the
102 upper but not in the lower limits of the thermal performance curve (Ruiz-Aravena et al. 2014)
103 (Fig. 1). First, the high seasonality should have selected for plasticity in TPC parameters and
104 therefore, the plasticity itself should not currently be under directional selection. Second, if daily
105 high extreme temperatures were frequent, then it would be expected positive directional selection
106 on CT_{max} when warm as well as cold-acclimated. Third, if daily low extremes were frequent then
107 it would be expected negative directional selection on CT_{min} during the cooler periods of the
108 year. Finally, as energy inputs are limited, the energetic definition of fitness indicates that
109 individuals with higher maintenance costs (i.e. resting metabolic rate) would have less energy
110 available to allocate to growth, reproduction and/or performance. The main prediction of this
111 principle is that natural selection should maximize the residual available energy, and therefore,
112 higher maintenance costs would be associated with lower fitness if no compensations in other
113 functions were available (Bacigalupe & Bozinovic 2002; Artacho & Nespolo 2009). Thus, our

114 final prediction is that Q_{10} is not under directional selection, which means that the energetic
115 expenditure does not change with acclimation.
116
117 **METHODS**
118 ***Study organism and laboratory maintenance***
119 Eighty-three adults individuals of *P. thaul* were captured during September 2012 on two
120 small ponds at Carrera Pinto (27º06'40.2'' S, 69º53'44.3'' W; 2,000 m.a.s.l.), a small oasis in the
121 Atacama Desert that is known to be the northernmost population of the species (Correa et al.
122 2007). In both ponds, we performed an exhaustive search across microhabitats (below rocks, in
123 the vegetation and in the water). All individuals were transported to the laboratory (Universidad
124 Austral de Chile, Valdivia) within 2 – 3 days of capture. Following capture all animals were
125 marked by toe clipping and maintained in the laboratory for one month at a temperature of 20º ±
126 2ºC and with a photoperiod 12D:12L. Animals were housed (N = 5) in terrariums (length x width
127 x height: 40 x 20 x 20 cm) provided with a cover of moss and vegetation and a small recipient
128 filled with water. Individuals were fed once a week with mealworms (*Tenebrio sp*. larvae) and
129 Mazuri® gel diets.
130
131 ***Acclimation and thermal traits***
132 After one month at maintenance conditions, in a split cross design half the frogs were
133 acclimated to either 10°C or 20°C for two weeks before measuring thermal traits. Frogs were
134 randomly assigned to the first acclimation temperature using a coin. Next they were acclimated
135 to the other temperature and again measured thermal traits. We chose these acclimation
136 temperatures because they are close to the mean minimum temperatures during the breeding

137 season (August - October, 10ºC) and to the mean temperatures during the active period of the
138 species (20ºC) at Carrera Pinto (www.cr2.cl). None of the investigators were blinded to the
139 group allocation during the experiments.

140 Critical temperatures were determined as the environmental temperature at which an
141 individual was unable to achieve an upright position within 1 minute (Ruiz-Aravena et al. 2014).
142 Each individual was placed in a small chamber inside a thermo-regulated bath (WRC-P8,
143 Daihan, Korea) at 30°C (CT_{Max}) and 5ºC (CT_{Min}) for 15 minutes, after which the bath
144 temperature was increased (or decreased) at a rate of 0.8ºC per minute (Rezende et al. 2011).
145 Every minute or at every change in 1ºC, the chamber was turned upside down and we observed if
146 the animal was able to return to the upright position. When an animal was unable to achieve an
147 upright position within 1 minute it was allowed to recover at ambient temperature (CT_{Min}) or for
148 30 minutes in a box with ice packs (CT_{Max}). Body mass (a proxy of body size) was obtained
149 before each trial using a Shimadzu TX323L electronic balance.

150 Preferred temperature (T_{Pref}) was determined simultaneously for five individuals in five
151 open-top terraria (length x width x height: 85 x 12 x 30 cm). Each terrarium had a thermal
152 gradient between 10ºC and 30ºC produced by an infrared lamp overhead (250 W) on one end,
153 and ice packs on the other. The organic gardening soil was moisturized at the beginning of each
154 trial to prevent the desiccation of the frogs. Five individuals were placed at the centre of each one
155 of the terraria and 45 minutes later we registered T_{Pref} as the dorsal body temperature (T_b) using a
156 UEi INF155 Scout1 infrared thermometer. Dorsal and cloacal T_b are highly associated (r_P =
157 0.99) (see Ruiz-Aravena et al. 2014 for details). Body mass was obtained before each trial using
158 a Shimadzu TX323L electronic balance.

159 Standard metabolic rate, measured through oxygen consumption at 20°C and 30°C was
160 measured continuously using an infrared O_2 - CO_2 analyzer (LI-COR LI6262, Lincoln, NV,
161 USA). The analyzer was calibrated periodically against a precision gas mixture. Although there
162 was almost no difference between calibrations, baseline measurements were performed before
163 and after each recording. Flow rates of CO2 – free air was maintained at 100 ml min^{-1} ± 1% by a
164 Sierra mass flow controller (Henderson, NV, USA). We used cylindrical metabolic chambers (60
165 ml), covered by metal paper. O_2 consumption was recorded during 45 minutes per individual.
166 Each record was automatically transformed by a macro program recorded in the ExpeData
167 software (Sable Systems), to (1) transform the measure from % to mlO_2 min^{-1}, taking into
168 account the flow rate and (2) to eliminate the first 5 min of recordings. For each individual, the
169 metabolic sensitivity (Q_{10}) was calculated as the ratio between metabolic rate measured at 30ºC
170 and metabolic rate measured at 20ºC.
171
172 ***Selection on thermal traits***
173 After experiments, all frogs were put back to 20ºC for at least one month before releasing
174 them. Marked frogs were released at Carrera Pinto in April 2013 and their survival was
175 monitored on three separate recapture efforts (13th October 2013, 13th June and 9th September
176 2014). As the desert surrounds these two small ponds dispersal was not a concern.
177 The relationship between trait plasticity and survival was analyzed using a Cormack-Jolly-Seber
178 (CJS) framework in Program MARK. An overall goodness of fit test was run using U-Care to
179 ensure the data were consistent with the assumed structure of the CJS model and to obtain a
180 value for the over dispersion parameter (c-hat). The time interval between capture occasions (as
181 a fraction of 1 year and considering also the original capture event) was included in the analysis

182 to accommodate the unequal intervals. The resulting resighting and survival estimates were
183 therefore corrected to annual estimates. Survival and resighting parameters were obtained in a
184 two-stage process. First, the best-fit resighting model was identified from three candidate models
185 (constant, time dependent and a linear trend). The fit of the three candidate resighting models
186 was compared using survival modeled as both a constant rate and also as a time-dependent rate,
187 to ensure that selection of the best-fit resighting model was not influenced by choice of survival
188 model. Once the best-fit resighting model had been identified (using AICc) this was then
189 retained for all candidate models. A model selection and an information-theoretic approach
190 (Burnham & Anderson 2003) was employed to contrast the adequacy of different working
191 hypotheses (the candidate models) of selection on trait plasticity. The number of candidate
192 models was kept to a minimum to minimize the likelihood of spurious results (Burnham &
193 Anderson 2003; Lucaks et al. 2010). Body mass showed a positive relationship with CT_{Max_20} (r_P
194 = 0.47) and with T_{Pref_10} (r_P = 0.24) and was not associated with any other trait (results not
195 shown). Therefore, we tested only for a model with body mass and models with directional
196 selection for each trait separately and also for correlational selection (interaction of trait
197 combinations) among the same trait at both acclimation temperatures, which indicates plasticity.
198 Body mass was included as a covariate in the case of CT_{Max_20} and T_{Pref_10} (Table 1). All analyses
199 were performed in R version 3.1.3 employing package RMark (Laake 2013). No transformation
200 was required to meet assumptions of statistical tests. Survival in relation to each covariate was
201 obtained as the model averaged value across all candidate models weighted by individual model
202 probability (Table 1).
203
204 **RESULTS**

All measured traits including critical thermal limits (CT_{Max}, CT_{Min}), thermal preference (T_{Pref}) and sensitivity of metabolic rate to temperature (Q_{10}) showed high variance among individuals (Fig. 2). In addition, for all traits some individuals shifted their thermal traits to higher values when acclimated to high temperatures, but other individuals showed the reverse response, that is their traits shifted to lower values after acclimation at higher temperatures (Fig. 3).

Only five out of 28 correlations between physiological traits were statistically significant, and these involved mostly critical thermal limits. In particular CT_{Max_20} was negatively correlated with CT_{Min_10} (r_P = -0.57) and CT_{Max_10} (r_P = -0.41) whilst it was positively correlated with Q_{10_20} (r_P = 0.26). Additionally, CT_{Max_10} was positively correlated with CT_{Min_10} (r_P = 0.31) and negatively correlated with CT_{Min_20} (r_P = -0.25).

The overall goodness of fit measure for the CJS model indicated a moderate level of over-dispersion (c-hat = 2.65, P = 0.103), however with only 3 recapture occasions it was not possible to identify an alternative starting model and the basic CJS model was adopted as the basis for subsequent model fitting, with unexplained over-dispersion controlled using the c-hat adjustment. A constant resighting rate was the best-fit model irrespective of whether survival was modeled as a constant or time dependent rate (Table 1). Consequently, the constant rate-resighting model was retained for subsequent modeling of survival. The model selection procedure indicated that from the 13 candidate models tested, there was not a single best-fit one (Table 1). In particular, the model containing only CT_{Min_10} was the one with the most support (Akaike weight of 0.153). Models including only directional selection on single traits still had some support, with a cumulative Akaike weight of almost 70%. Models including correlational

227 selection (i.e. plasticity) showed rather weak empirical support (Table 1). Survival in relation to
228 each covariate is presented in Fig. 4.
229
230 **DISCUSSION**
231 To understand how organisms adapt to highly fluctuating environments and whether they
232 will be able to adaptively respond to current climate change, we need to evaluate whether
233 selection in nature targets plasticity itself. Populations inhabiting highly seasonal environments
234 that also experience daily extreme temperatures, provide excellent opportunities to test
235 predictions of the fitness consequences of such thermal variation on the plasticity of critical
236 thermal limits and preferences. Here, to the best of our knowledge for the first time, we studied
237 natural selection on thermal acclimation capacity of performance (CT_{Max} and CT_{Min}), metabolism
238 (Q_{10}) and behaviour (T_{Pref}). Our results indicate that thermal acclimation in this population is not
239 being targeted by directional selection, although there are signals of selection on individual traits.
240 Furthermore, survival decreased as values of most of the traits increased in both warm and cold
241 acclimated conditions (Fig. 4).
242 Some theoretical models of thermal adaptation across heterogeneous environments (e.g.,
243 Climate variability hypothesis, generalist-specialist models), suggest that temporal
244 environmental heterogeneity selects for more broadly adapted individuals (Lynch and Gabriel,
245 1987; Gilchrist 1995), favoring increased plasticity particularly regarding thermal tolerance traits
246 (Gunderson & Stillman 2015). Based on these models we predicted that the high seasonality
247 should have already selected for plasticity in thermal traits and therefore, the plasticity itself
248 should not currently be under directional selection. Our prediction turned out to be correct as
249 models including plasticity showed relatively weak support. Although it is important to mention

that strong support for the simpler models may in part be due to the relatively high value of c-hat, which penalizes models on the basis of parameter number. Furthermore, the relatively small sample size (N = 83) and the few (n = 3) recaptures we carried out prevented us from evaluating whether stabilizing selection might be operating, which might be the case if this population has reached, as we assumed, an optimum for the evaluated traits.

Frogs of *P. tahul* in the Atacama Desert (the northernmost population of this species) are exposed to large daily and seasonal oscillations in environmental temperatures. The isothermality (i.e., ratio between daily and annual thermal ranges; O'Donnell & Ignizio 2012) experienced by this population (0.65) is ca. 15% higher compared to a population 2,000 km south (0.52), which experiences narrower daily environmental temperatures at the center of the species' distribution (Barria & Bacigalupe 2017). This means that the studied population experiences a daily variation that is almost 65% of its seasonal variation. The high daily variation together with the fact that climate change is already changing the frequency and intensity of extreme temperatures (Rahmstorf & Coumou 2011; Wang & Dillon 2014; Vasquez et al. 2017) made us wonder whether selection in nature might also target traits as a function of daily extremes. As the cooler end of the thermal performance curve did not change trough acclimation to warmer temperatures (Ruiz-Aravena et al. 2014) we predicted negative directional selection on CT_{min} during the cooler but not the warmer periods of the year. Our results support this prediction, as survival decreased as CT_{min} increased (i.e. less tolerance to cold) when cold-acclimated, which was the most supported model (Table 1). Nevertheless, survival also decreased, albeit very slightly, as CT_{min} increased when warm-acclimated, which suggests that lower temperature extremes might likewise be common during the warm periods of the year.

272 Although acclimation produced an increase in the upper limits of the thermal
273 performance curve in this population (Ruiz-Aravena et al. 2014), we predicted positive
274 directional selection on CT_{max} when warm as well as cold-acclimated if daily high extreme
275 temperatures were frequent. Our results do not support this prediction: survival decreased as
276 CT_{max} increased under warm as well as under cold-acclimated conditions. This suggests that
277 selection might favours individuals that avoid hot microhabitats, possibly by means of behaviour
278 (Ruiz-Aravena et al. 2014). Indeed, behavioural thermoregulation has been proposed as one key
279 factor that prevents an evolutionary response to selection to raising temperatures (Kearney et al.
280 2009; Huey et al. 2012; Buckley et al. 2015). The fact that CT_{Max_20} was negatively correlated
281 with CT_{Min_10} indicates that individuals with higher cold tolerance might be the ones avoiding hot
282 microhabitats, which opens very interesting questions for further research. Overall, our results
283 suggest that selection seems to be operating differently with respect to cold versus hot thermal
284 extremes. In the first case, higher tolerance is being favourably selected during the cooler periods
285 of the year. In the second case, higher tolerance is being selected against, which suggests that
286 extreme hot temperatures might be selecting for behavioural patterns to regulate body
287 temperature.
288 Regarding the sensitivity of metabolism to temperature (Q_{10}) we predicted that Q_{10} is not
289 under directional selection, which means that the energetic expenditure does not change with
290 acclimation. Our results (partially) supported that prediction as the rate at which survival
291 changed with changes in Q_{10} was very small (Fig. 4), although the models with Q_{10} still showed
292 some support (Table 1). Finally, we also predicted no directional selection on T_{Pref} as we have
293 previously shown that acclimation to warmer temperatures produced an increase in this trait
294 (Ruiz-Aravena et al. 2014). Nevertheless, we found that survival decreased, although at a very

295 low rate, as T_{Pref} increased, which further suggest that selection favours those individuals that are
296 able to avoid hot microhabitats.

297 Selection favored bigger individuals, something that have been previously reported in the
298 literature (Aubin-Horth et al. 2005; Iida & Fujisaki 2007; Crosby & Latta 2013; Delaney &
299 Warner 2017). This is somewhat unsurprising, given that body mass is known to be positively
300 associated with several physiological traits that enhance performance (Castellano et al. 1999;
301 Madsen & Shine 2000; Hurlbert et al. 2008; Shepherd et al. 2008; Luna et al. 2009) including
302 plasticity itself (Whitman & Ananthakrishnan 2009). Furthermore, larger individuals showed
303 higher values of CT_{Max_20}. This might seem puzzling as we also shown that survival decreased in
304 individuals with higher CT_{Max_20}. Although this population inhabits two highly isolated ponds
305 where the presence of competitors (anurans) has not been observed, there might be a risk of
306 predation by herons (*L.D.B. personal observation*), and thus, a positive selection for body size
307 might explain it. Nevertheless, further experimental work is needed to evaluate this possibility.

308 It is important to mention that we have measured plasticity in just one life stage. It is
309 likely that other ecological and physiological traits might also be plastic, and their responses to
310 acclimation might be different and they might even be different between different life stages.
311 However, we still consider our results show a strong signal and provide the first evidence that
312 phenotypic plasticity is not being an actual target of selection in nature and that daily climate
313 extremes might be selecting for higher tolerance. Nevertheless, further work including multiple
314 traits and life stages might help unify trends into further generic hypotheses to clarify the role of
315 plasticity in the viability of ectotherm populations in natural conditions.

316

317

Acknowledgements. We thank Nadia Aubin-Horth, Wolf Blanckenhorn, Dries Bonte, Ray Huey and Michael Logan for highly valuable comments on a previous version on the manuscript.

Competing interests. We declare we have no competing interests

Author Contributions. L.D.B conceptualized the study, designed the experimental procedures and carried out the experiment with A.M.B., A.G.M., M.R.A. and J.D.G.E; B.S., M.T. and L.D. B. analyzed the data and L.D.B., B.S. and J.D.G. wrote the paper with input from A.M.B and M.R.A.

Funding. Leonardo Bacigalupe acknowledges funding from FONDECYT grant 1150029. Barry Sinervo was supported by a Macrosystems grant (EF-1241848) from NSF. Aura Barria and Manuel Ruiz-Aravena were supported by a CONICYT Doctoral Fellowship.

Ethics. This study did not involve endangered or protected species and was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Comisión Nacional de Investigación Científica y Tecnológica de Chile (CONICYT). All experiments were conducted according to current Chilean law. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Universidad Austral de Chile.

References

Angilletta, M. J. 2009. Thermal Adaptation: a theoretical and empirical synthesis. Oxford, UK: Oxford University press.

Artacho, P. & Nespolo, R. F. 2009 Natural selection reduces energy metabolism in the garden snail, Helix aspersa (*Cornu aspersum*). *Evolution* **63**, 1044–50.

Aubin-Horth, N., Ryan, D. A. J., Good, S. P. & Dodson, J. J. 2005 Balancing selection on size: Effects on the incidence of an alternative reproductive tactic. *Evol. Ecol. Res.* **7**, 1171–1182.

Bacigalupe, L. D. & Bozinovic, F. 2002 Design, limitations and sustained metabolic rate: lessons from small mammals. *J. Exp. Biol.* **205**, 2963–2970.

Barria, A. M. & Bacigalupe, L. D. 2017 Intraspecific geographic variation in thermal limits and acclimatory capacity in a wide distributed endemic frog. *J. Therm. Biol.* **69**, 254–260.

Bartheld, J. L., Artacho, J. P. & Bacigalupe, L. D. 2017. Thermal performance curves under daily thermal fluctuation: A study in helmeted water toad tadpoles. *J. Therm. Biol* 70, 80-85.

Bozinovic, F., Calosi, P. & Spicer, J. I. 2011. Physiological correlates of geographic range in animals. *Annu. Rev. Ecol. Evol. Syst.* **42**, 155-179.

Buckley, L. B., Ehrenberger, J. C. & Angilletta, M. J. 2015. Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. *Funct. Ecol.* **29**, 1038–1047.

Burnham, K. P. & Anderson, D. R. 2003 *Model selection and multimodel inference: a practical information-theoretic approach.* Springer Science & Business Media.

Castellano, S., Rosso, A., Doglio, S. & Giacoma, C. 1999. Body size and calling variation in the green toad (*Bufo viridis*). *J. Zool.* **248**, 83–90.

Clusella-Trullas, S., Blackburn, T. M. & Chown, S. L. 2011. Climatic Predictors of Temperature Performance Curve Parameters in Ectotherms Imply Complex Responses to Climate Change. *Am. Nat.* **177**, 738-751.

Correa, C., Sallaberry, M., Gonzalez, B., Soto, E. & Mendez, M. 2007 Amphibia, Anura, Leiuperidae, Pleurodema thaul: Latitudinal and altitudinal distribution extension in Chile. *Check List* **3**, 267–270

Crosby, K. & Latta, R. G. 2013 A test of the reproductive economy hypothesis in plants: More offspring per capita come from large (not small) parents in *Avena barbata*. *Evol. Ecol.* **27**, 193–203.

Delaney, D. M. & Warner, D. A. 2017 Effects of age- and sex-specific density on behaviour and survival in a territorial lizard (*Anolis sagrei*). *Anim. Behav.* **129**, 31–41.

Gaitan-Espitia, J. D., Arias, M. B., Lardies, M. A. & Nespolo, R. F. 2013. Variation in thermal sensitivity and thermal tolerances in an invasive species across a climatic gradient: lessons from the land snail *Cornu aspersum*. *PLoS One*, *8*(8), e70662

Gaitan-Espitia, J. D., Bacigalupe, L. D., Opitz, T, Lagos, N. A., Timmermann, T. & Lardies, M. A. 2014. Geographic variation in thermal physiological performance of the intertidal crab *Petrolisthes violaceus* along a latitudinal gradient. *J. Exp. Biol.* **217**, 4379-4386.

Gaitan-Espitia, J. D., Marshall, D. J., Dupont, S., Bacigalupe, L. D., Bodrossy, L. & Hobday, A. J. 2017. Geographical gradients in selection can reveal genetic constraints for evolutionary responses to ocean acidification. *Biol. Lett.* **13**, 20160784.

Ghalambor, C. K., Huey, R. B., Martin, P. R., Tewksbury, J. T. & Wang, G. 2006. Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr. Comp. Biol.* **46**, 5–17.

Gilchrist, G. W. 1995. Specialists and generalists in changing environments. 1. Fitness landscapes of thermal sensitivity. *Am. Nat.***146**:252–270.

Gilchrist, G. W. 1996. A quantitative genetic analysis of thermal sensitivity in the locomotor performance curve of *Aphidius ervi*. *Evolution* **50**, 1560–1572.

Gunderson, A. R. & Stillman, J. H. 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proc. *R. Soc. B* **282**, 20150401

Huey, R. B. & Kingsolver, J. G. 1989. Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol.* **4**, 131-135.

Huey, R. B. & Berrigan, D. 1996 Testing evolutionary hypotheses of acclimation. In *Phenotypic and evolutionary adaptation to temperature* (eds A. Johnston & A. Bennett), pp. 205–237. Cambridge University Press

Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M. & Williams, S. E. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philos. Trans. R. Soc. B Biol. Sci.* **367**, 1665–1679.

Hurlbert, A. H., Ballantyne IV, F. & Powell, S. 2008 Shaking a leg and hot to trot: The effects of body size and temperature on running speed in ants. *Ecol. Entomol.* **33**, 144–154.

Iida, H. & Fujisaki, K. 2007 Seasonal changes in resource allocation within an individual offspring of the wolf spider, Pardosa pseudoannulata (Araneae: Lycosidae). *Physiol. Entomol.* **32**, 81–86.

Kingsolver, J. G. & Gomulkiewicz, R. 2003. Environmental Variation and Selection on

Performance Curves. *Integr. Comp. Biol.* **43**, 470–477.

Kearney, M., Shine, R. & Porter, W. P. 2009 The potential for behavioral thermoregulation to buffer 'cold-blooded' animals against climate warming. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 3835–3840.

Kingsolver, J. G., Ragland, G. J. & Shlichta, J. G. 2004. Quantitative genetics of continuous reaction norms: thermal sensitivity of caterpillar growth rates. *Evolution* **58,**1521-1529.

Laake, J. 2013 RMark: an R interface for analysis of capture–recapture data with MARK. In *AFSC Processed Rep 2013-01*, pp. 25. Seattle, Washington, USA.: Alaska Fisheries Science Center, NOAA, National Marine Fisheries Service

Lardies, M. A., Arias, M. B., Poupin, M. J. & Bacigalupe, L. D. 2014. Heritability of *hsp70* expression in the beetle *Tenebrio molitor*: Ontogenetic and environmental effects. *J. Insect Physiol.* **67**, 70-75.

Latimer, C. A. L., Wilson, R. S. & Chenoweth, S. F. 2011. Quantitative genetic variation for thermal performance curves within and among natural populations of *Drosophila serrata*. *J. Evol. Biol.* **24**, 965–975.

Logan, M. L., Cox, R. M. & Calsbeek, R. 2014 Natural selection on thermal performance in a novel thermal environment. *Proc. Natl. Acad. Sci. U. S. A.* **39**, 14165-14169.

Lukacs, P., Burnham, K. & Anderson, D. 2010 Model selection bias and Freedman's paradox. *Ann. Inst. Stat. Math.* **62**, 117–125

Luna, F., Antenucci, C. D. & Bozinovic, F. 2009 Comparative energetics of the subterranean Ctenomys rodents: breaking patterns. *Physiol. Biochem. Zool.* **82**, 226–35.

Lynch, M., and W. Gabriel. 1987. Environmental tolerance. *Am. Nat.* **129**:283–303.

Madsen, T. & Shine, R. 2000 Energy versus risk: costs of reproduction in free-ranging pythons in tropical Australia. *Austral Ecol.* **25**, 670–675.

O'Donnell, M. S. & Ignizio, D. A. 2012. Bioclimatic predictors for supporting ecological applications in the conterminous United States. Geological Survey Data Series 691.

Schulte, P. M., Healy, T. M. & Fangue, N. A. 2011.Thermal Performance Curves, Phenotypic Plasticity, and the Time Scales of Temperature Exposure. *Integr. Comp. Biol.* **51**, 691–702.

Rahmstorf, S. & D. Coumou. 2011. Increase of extreme events in a warming world. *Proc. Natl. Acad. Sci. U. S. A.* **108**,17905-17909.

Rezende, E. L., Tejedo, M. & Santos, M. 2011 Estimating the adaptive potential of critical

thermal limits: methodological problems and evolutionary implications. *Funct. Ecol.* **25**, 111–121.

Ruiz-Aravena, M., Gonzalez-Mendez, A., Estay, S. A., Gaitán-Espitia, J. D., Barria-Oyarzo, I., Bartheld, J. L. & Bacigalupe, L. D. 2014 Impact of global warming at the range margins: phenotypic plasticity and behavioral thermoregulation will buffer an endemic amphibian. *Ecol. Evol.* **4**, 4467–4475.

Seebacher, F., Holmes, S., Roosen, N. J., Nouvian, M., Wilson, R. S. & Ward, A. J. W. 2012. Capacity for thermal acclimation differs between populations and phylogenetic lineages within a species. *Funct. Ecol.* **26**, 1418–1428.

Shepherd, B. L., Prange, H. D. & Moczek, A. P. 2008. Some like it hot: Body and weapon size affect thermoregulation in horned beetles. *J. Insect Physiol.* **54**, 604–611.

Sinclair, B. J., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., Slotsbo, S., Dong, Y., Harley, C. D. G., Marshall, D. J., Helmuth, B. S. & Huey, R. B. 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecol. Lett.* **19**, 1372–1385.

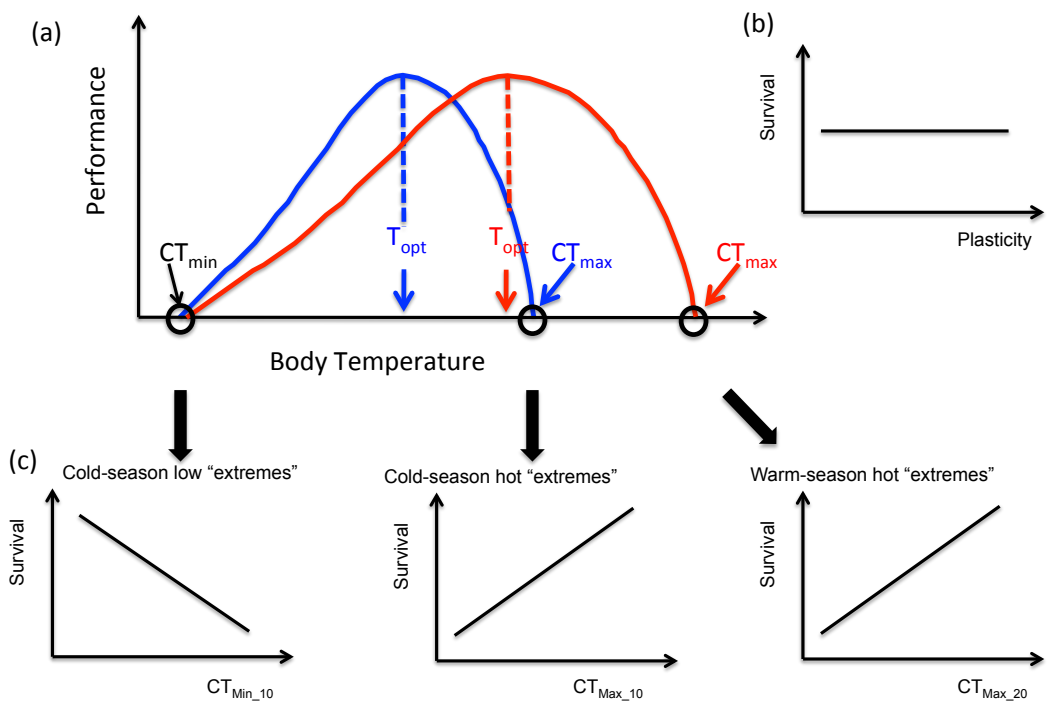
Vasseur, D. A., DeLong, J. P., Gilbert, B., Greig, H. S., Harley, C. D. G., McCann, K. S., Savage, V., Tunney, T. D. & O'Connor, M. I. 2014. Increased temperature variation poses a greater risk to species than climate warming. *Proc. R. Soc. B.* **281**, 20132612.

Vázquez, D. P., Gianoli, E., Morris, W. F. & Bozinovic, F. 2017. Ecological and evolutionary impacts of changing climatic variability. *Biol. Rev.* **92**, 22–42.

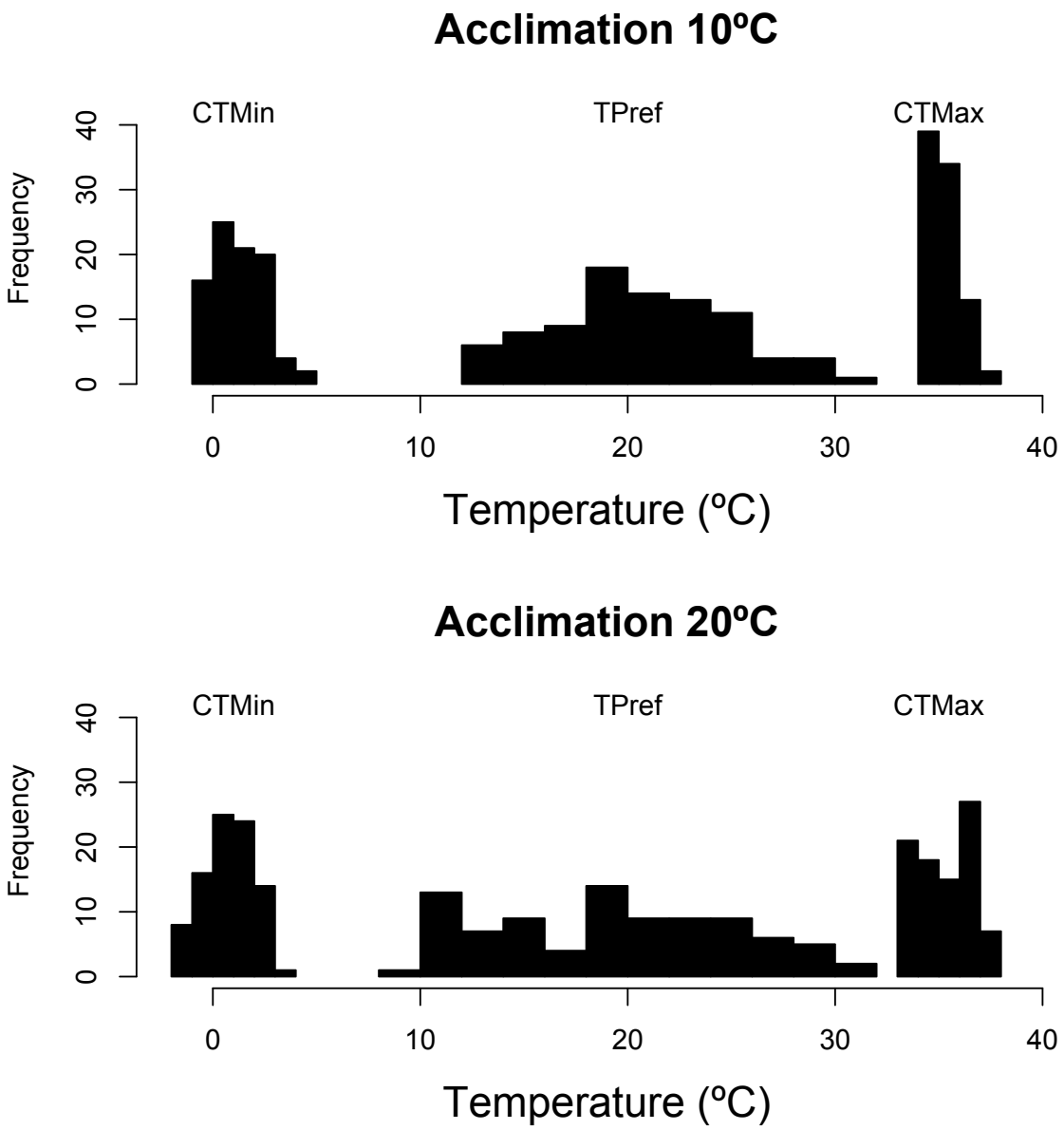
Wang, G. & Dillon, M. E. 2014. Recent geographic convergence in diurnal and annual temperature cycling flattens global thermal profiles. *Nature Climate Change* **4**, 988-992.

Whitman, D. & Ananthakrishnan, T. 2009 *Phenotypic Plasticity of Insects: Mechanisms and Consequences*. Enfield: Science Publishers.

Figure 1. Graphical representation of the predictions tested in this study. (a) Predictions built up from findings showing that acclimation to warmer temperatures produces an increase in the upper but not in the lower limits of the thermal performance curve (Ruiz-Aravena et al. 2014). (b) The high seasonality should have selected for plasticity and therefore, plasticity of all traits should not currently be under directional selection. (c) If daily low extremes are frequent, we expect negative directional selection on CT_{Min} during the cooler periods of the year (left panel). If daily high extreme temperatures are frequent, we expect positive directional selection on CT_{Max} during the warmer periods (right panel) as well as the cooler periods of the year (middle panel). We predict no directional selection on T_{Pref} and Q_{10} at both acclimation temperatures and on CT_{Min} when warm acclimated. Cold acclimated is indicated by a _10 subscript while warm acclimated is indicated by a _20 subscript.



522 **Figure 2.** Frequency distribution of CT_{Min}, T_{Pref} and CT_{Max} of the four-eyed frog when
523 acclimated to 10ºC and 20ºC.



524

525

Figure 3. Individual plasticity in CT_{Min}, T_{Pref}, CT_{Max} and Q_{10} to 10 and 20ºC acclimation treatments. Each line represents the individual value of the specific traits at each temperature. For CT_{Min} and CT_{Max} the width of the line is directly proportional to the number of individuals that showed that specific response.

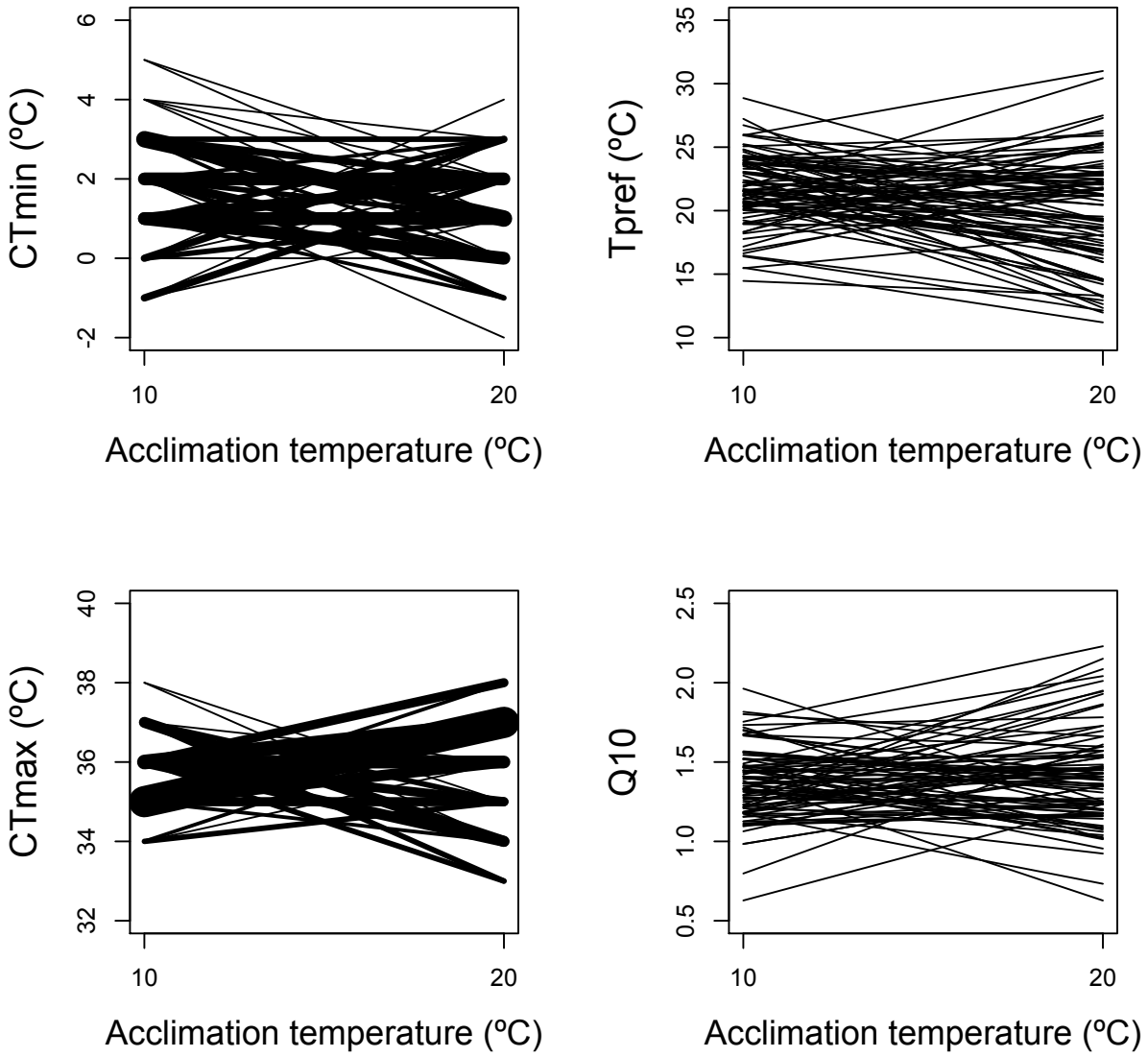


Figure 4. Survival estimates of simple directional selection sorted by model probabilities. *w*: Akaike weights of the model.

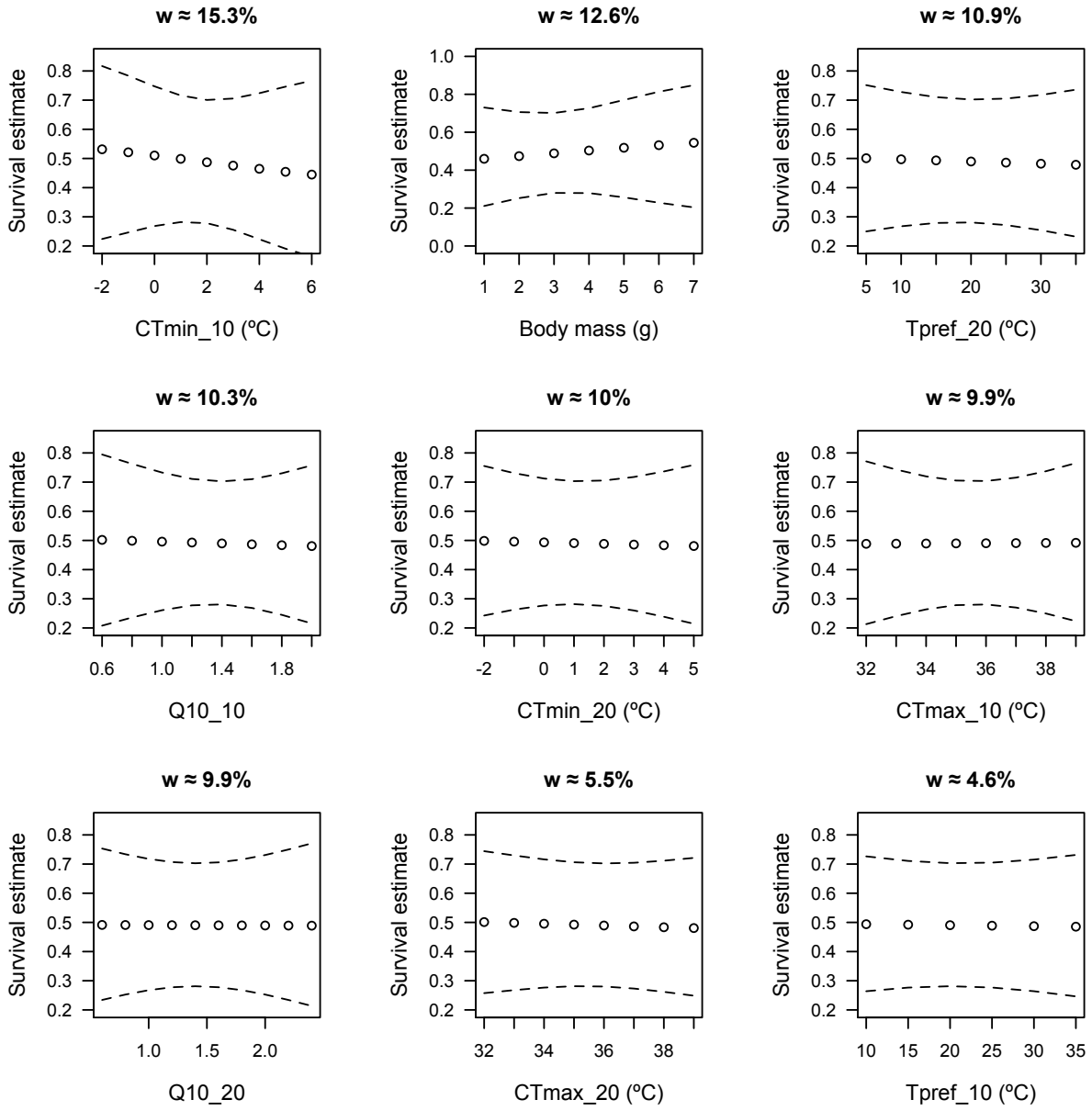


Table 1. Candidate models ordered accordingly to their Akaike weights. Single term models represent directional (e.g. CT_{Max}) and correlational selection represents plasticity (e.g. CT_{Max_10} * CT_{Max_20}). CT_{Min} = minimum critical temperature; CT_{Max} = maximum critical temperature; T_{Pref} = preferred temperature; Q_{10} = thermal sensitivity of metabolism; MB = body mass. Cold acclimated is indicated by a _10 subscript while warm acclimated is indicated by a _20 subscript.

Models		K	AICc	ΔAICc	w_i
1	CT_{Min_10}	3	131	0	0.153
2	MB	3	131.78	0.38	0.126
3	T_{Pref_20}	3	121.08	0.68	0.109
4	Q_{10_10}	3	132.18	0.78	0.103
5	CT_{Min_20}	3	132.25	0.85	0.100
6	CT_{Max_10}	3	132.26	0.86	0.099
7	Q_{10_20}	3	132.26	0.86	0.099
8	$CT_{Min_10} + CT_{Min_20} + CT_{Min_10} * CT_{Min_20}$	5	133.38	1.98	0.057
9	$MB + CT_{Max_20}$	4	133.44	2.04	0.055
10	$MB + T_{Pref_10}$	4	133.82	2.42	0.046
11	$Q_{10_10} + Q_{10_20} + Q_{10_10} * Q_{10_20}$	5	134.17	2.77	0.038
12	$MB + T_{Pref_10} + T_{Pref_20} + T_{Pref_10} * T_{Pref_20}$	6	137.16	5.76	0.009
13	$MB + CT_{Max_10} + CT_{Max_20} + CT_{Max_10} * CT_{Max_20}$	6	137.62	6.22	0.007

K = number of parameters.
AICc: AIC values corrected for small sample sizes.
w_i: Akaike weights.

Figure 4. Survival estimates of simple directional selection sorted by model probabilities.

w: Akaike weights of the model.

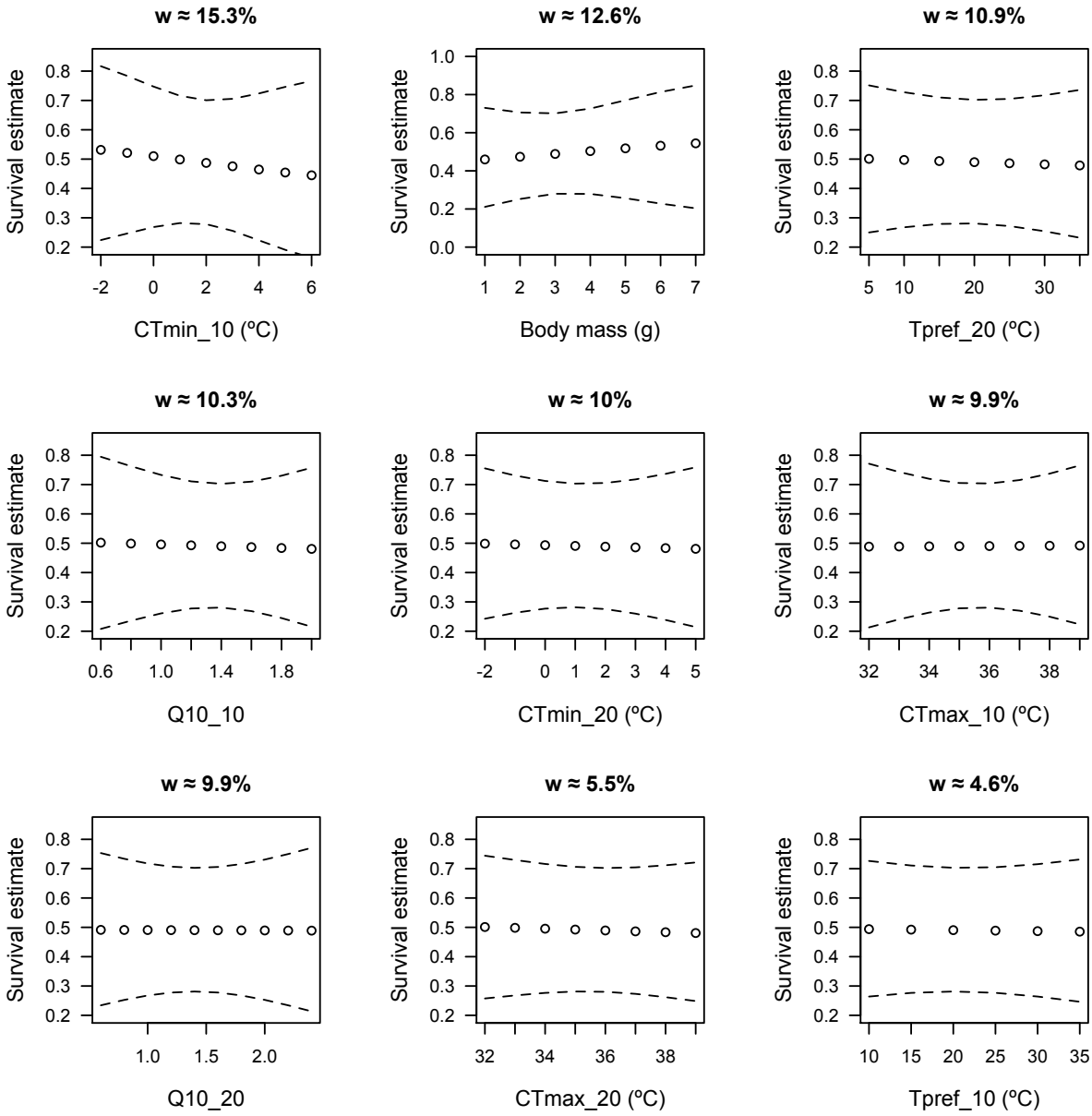


Figure 3. Individual plasticity in CT_{Min}, T_{Pref}, CT_{Max} and Q_{10} to 10 and 20ºC acclimation treatments. Each line represents the individual value of the specific traits at each temperature. For CT_{Min} and CT_{Max} the width of the line is directly proportional to the number of individuals that showed that specific response.

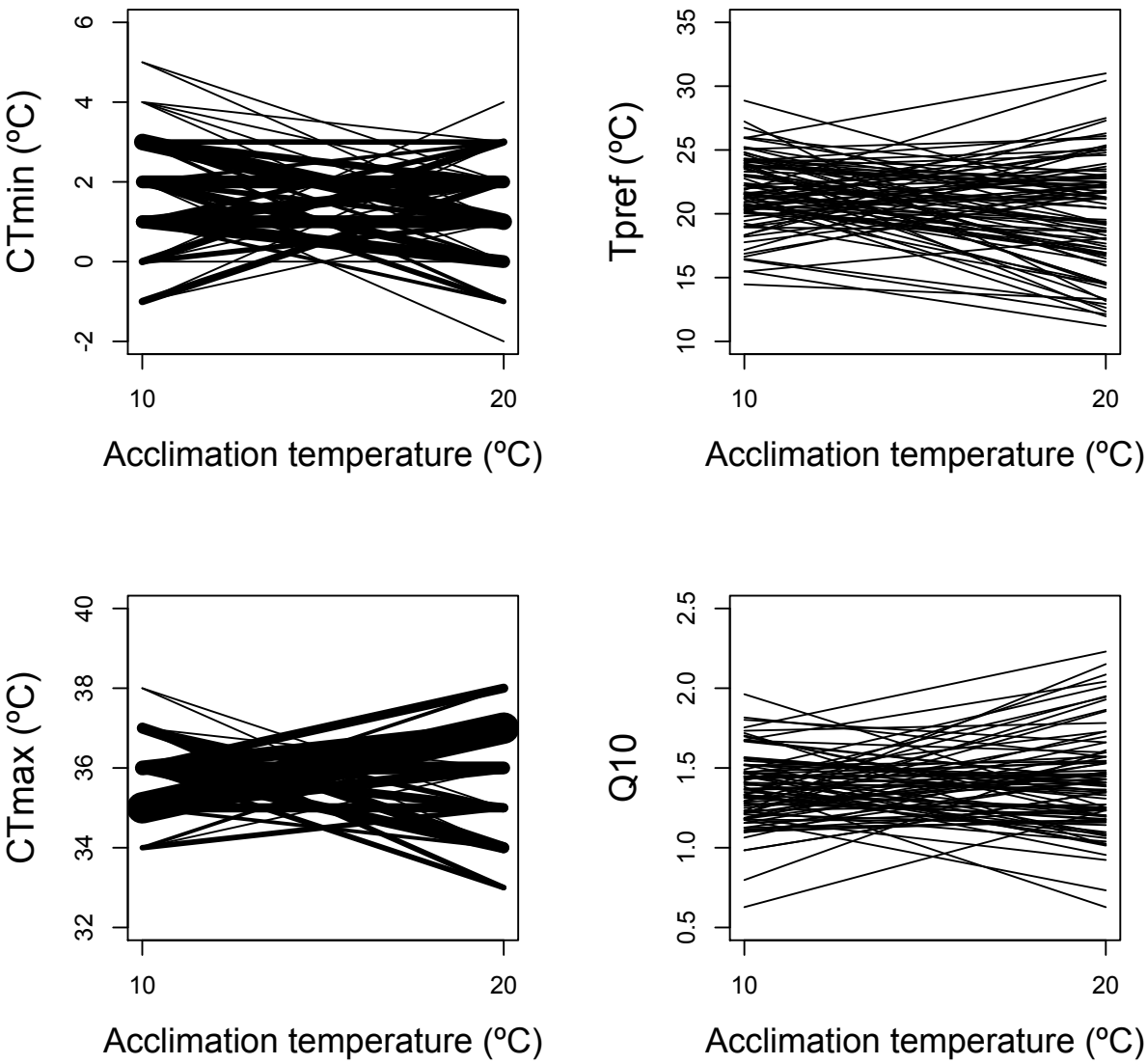


Figure 2. Frequency distribution of CT_{Min}, T_{Pref} and CT_{Max} of the four-eyed frog when acclimated to 10ºC and 20ºC.

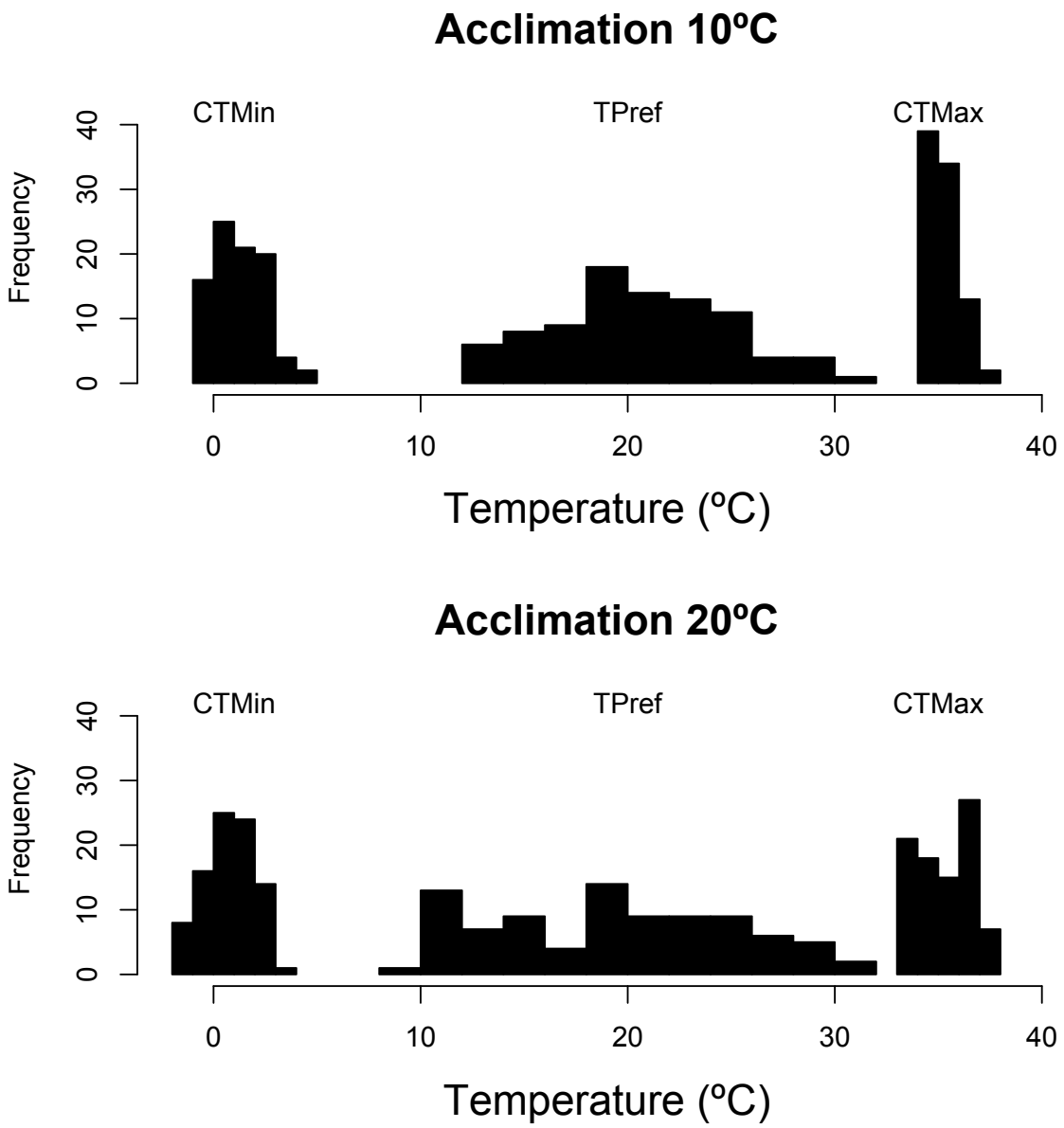
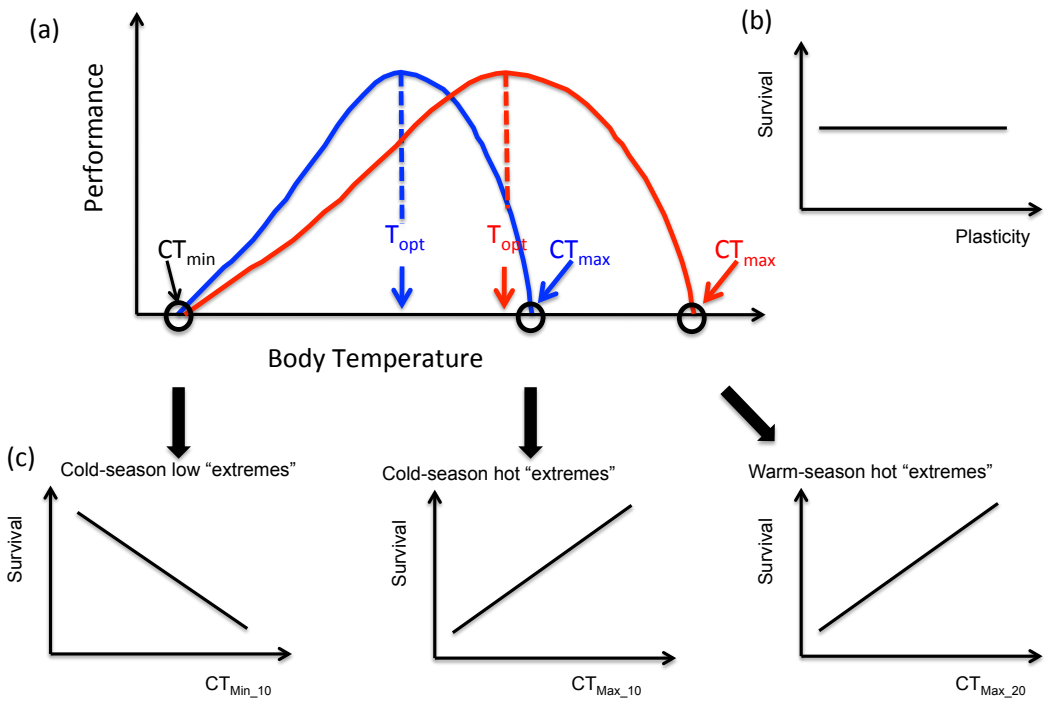



Figure 1. Graphical representation of the predictions tested in this study. (a) Predictions built up from findings showing that acclimation to warmer temperatures produces an increase in the upper but not in the lower limits of the thermal performance curve (Ruiz-Aravena et al. 2014). (b) The high seasonality should have selected for plasticity and therefore, plasticity of all traits should not currently be under directional selection. (c) If daily low extremes are frequent, we expect negative directional selection on CT_{Min} during the cooler periods of the year (left panel). If daily high extreme temperatures are frequent, we expect positive directional selection on CT_{Max} during the warmer periods (right panel) as well as the cooler periods of the year (middle panel). We predict no directional selection on T_{Pref} and Q_{10} at both acclimation temperatures and on CT_{Min} when warm acclimated. Cold acclimated is indicated by a _10 subscript while warm acclimated is indicated by a _20 subscript.