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

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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 might be signals of selection on individual traits. According to the most supported models, survival decreased in individuals with less tolerance to cold when cold-acclimated (probably because daily low extremes are frequent during the cooler periods of the year) and increased with body size. However, in both cases, the directional selection estimates were non-significant.

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

70 al. 2011). One of them, the climate variability hypothesis (CVH), offers a powerful conceptual
71 framework to explore the interactions between environmental variability and physiological
72 performance of ectotherms (e.g., Gaitan-Espitia et al. 2013; 2014). The CVH predicts that
73 organisms inhabiting more variable environments should have broader ranges of environmental
74 tolerance and/or greater physiological plasticity that enable them to cope with the fluctuating
75 environmental conditions (e.g., seasonality) (Ghalambor et al. 2006; Gaitan-Espitia et al. 2017).
76 In agreement with this hypothesis, other theoretical models have explored the evolutionary
77 mechanisms underlying local thermal adaptation across heterogeneous environments (e.g.,
78 Generalist-Specialist models). For instance, the model developed by Lynch and Gabriel (1987),
79 predicts that temporal environmental heterogeneity selects for more broadly adapted individuals,
80 whereas in more constant environments the model developed by Gilchrist (1995), predicts that
81 selection should favor thermal specialists with narrow performance breadth. The mechanistic
82 understanding of these conceptual frameworks has improved with recent studies showing how in
83 thermally variable environments directional selection acts on TPC's parameters favoring
84 organisms that maximize performance across a broader range of temperatures (Logan et al. 2014)
85 despite the ability of ectotherms to thermoregulate behaviorally (Buckley et al. 2015).
86 Notwithstanding this progress, whether natural selection targets thermal acclimation capacity
87 (i.e., plasticity) itself in natural populations remains unknown.
88 In addition to increasing mean temperatures, it is known that climate change is changing
89 the frequency and intensity of extreme temperatures and events (Rahmstorf & Coumou 2011;
90 Wang & Dillon 2014; Vazquez et al. 2016). This, in turn, suggests that both averages and
91 variances will have an important impact on different performance related traits (e.g. Lardies et al.

92 2014; Vasseur et al. 2014; Bartheld et al. 2017). Nevertheless, we still do not know whether
93 selection might also target traits as a function of those extremes.
94 In this context, populations inhabiting highly seasonal environments characterized also by
95 daily extreme temperatures, provide a natural laboratory to evaluate the role of natural selection
96 on the plasticity of critical thermal limits and preferences. We addressed such important gaps in
97 our knowledge by measuring for the first time survival as a function of the plasticity of thermal
98 critical temperatures (CT_{Max} and CT_{Min}), preferred temperature (T_{Pref}) and thermal sensitivity of
99 metabolism (Q_{10}; the magnitude of change in metabolic rate for a 10ºC change in T_a) after
100 acclimation to 10°C and 20°C in the northernmost population of the four-eyed frog *Pleurodema*
101 *thaul*. We tested four predictions regarding phenotypic selection and plasticity that built up from
102 previous findings showing that acclimation to warmer temperatures produces an increase in the
103 upper but not in the lower limits of the thermal performance curve (Ruiz-Aravena et al. 2014)
104 (Fig. 1). First, the high seasonality should select for plasticity in TPC parameters and therefore,
105 the plasticity itself should currently be under directional selection. Second, if daily high extreme
106 temperatures were frequent, then it would be expected positive directional selection on CT_{max}
107 when warm as well as cold-acclimated. Third, if daily low extremes were frequent then it would
108 be expected negative directional selection on CT_{min} during the cooler periods of the year. Finally,
109 as energy inputs are limited, the energetic definition of fitness indicates that individuals with
110 higher maintenance costs (i.e. resting metabolic rate) would have less energy available to
111 allocate to growth, reproduction and/or performance. The main prediction of this principle is that
112 natural selection should maximize the residual available energy, and therefore, higher
113 maintenance costs would be associated with lower fitness if no compensations in other functions

114 were available (Bacigalupe & Bozinovic 2002; Artacho & Nespolo 2009). Thus, our final
115 prediction is that Q_{10} is not under directional selection.
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

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

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

Preferred temperature (T_{Pref}) was determined simultaneously for five individuals in five open-top terraria (length x width x height: 85 x 12 x 30 cm). Each terrarium had a thermal gradient between 10ºC and 30ºC produced by an infrared lamp overhead (250 W) on one end, and ice packs on the other. The organic gardening soil was moisturized at the beginning of each trial to prevent the desiccation of the frogs. Five individuals were placed at the centre of each one of the terraria and 45 minutes later we registered T_{Pref} as the dorsal body temperature (T_b) using a UEi INF155 Scout1 infrared thermometer. Dorsal and cloacal T_b are highly associated (r_P = 0.99) (see Ruiz-Aravena et al. 2014 for details). Body mass was obtained before each trial using 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 null model (i.e. neither trait under selection), a model
196 with body mass and models with directional selection for each trait separately and also for
197 correlational selection (interaction of trait combinations) among the same trait at both
198 acclimation temperatures, which indicates plasticity. Body mass was included as a covariate in
199 the case of CT_{Max_20} and T_{Pref_10} (Table 1). All analyses were performed in R version 3.1.3
200 employing package RMark (Laake 2013). No transformation was required to meet assumptions
201 of statistical tests. Survival in relation to each covariate was obtained as the model averaged
202 value across all candidate models weighted by individual model probability (Table 1).
203
204

205 **RESULTS**

206 All measured traits including critical thermal limits (CT_{Max}, CT_{Min}), thermal preference
207 (T_{Pref}) and sensitivity of metabolic rate to temperature (Q_{10}) showed high variance among
208 individuals (Fig. 2). In addition, for all traits some individuals shifted their thermal traits to
209 higher values when acclimated to high temperatures, but other individuals showed the reverse
210 response, that is their traits shifted to lower values after acclimation at higher temperatures (Fig.
211 3).

212 Only five out of 28 correlations between physiological traits were statistically significant,
213 and these involved mostly critical thermal limits. In particular CT_{Max_20} was negatively correlated
214 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}
215 (r_P = 0.26). Additionally, CT_{Max_10} was positively correlated with CT_{Min_10} (r_P = 0.31) and
216 negatively correlated with CT_{Min_20} (r_P = -0.25).

217 The overall goodness of fit measure for the CJS model indicated a moderate level of
218 over-dispersion (c-hat = 2.65, P = 0.103), however with only 3 recapture occasions it was not
219 possible to identify an alternative starting model and the basic CJS model was adopted as the
220 basis for subsequent model fitting, with unexplained over-dispersion controlled using the c-hat
221 adjustment. A constant resighting rate was the best-fit model irrespective of whether survival
222 was modeled as a constant or time dependent rate (Table 1). Consequently, the constant rate-
223 resighting model was retained for subsequent modeling of survival. The model selection
224 procedure indicated that from the 13 candidate models tested, there was not a single best-fit one
225 (Table 1). In particular, the null model was the most supported (Akaike weight of 0.220), whilst
226 models including only directional selection on single traits still had some support, with a
227 cumulative Akaike weight of almost 60% (Table 1). Models including correlational selection

228 (i.e. plasticity) showed rather weak empirical support (Table 1). Overall, survival decreased as
229 values of most of the traits increased in both, warm and cold acclimated conditions (Table 2,
230 Figure 4).
231
232 **DISCUSSION**
233 To understand how organisms adapt to highly fluctuating environments and whether they will be
234 able to adaptively respond to current climate change, we need to evaluate whether selection in
235 nature targets plasticity itself. Populations inhabiting highly seasonal environments that also
236 experience daily extreme temperatures, provide excellent opportunities to test predictions of the
237 fitness consequences of such thermal variation on the plasticity of critical thermal limits and
238 preferences. Here, to the best of our knowledge for the first time, we studied natural selection on
239 thermal acclimation capacity of performance (CT_{Max} and CT_{Min}), metabolism (Q_{10}) and
240 behaviour (T_{Pref}). Our results indicate that thermal acclimation in this population is not being
241 targeted by directional selection, although there might be signals of selection on individual traits.
242 In part, the relatively weak evidence for natural selection on this system might be a consequence
243 of the small sample size we used (N = 83), the few recaptures we carried out (n = 3) and the
244 relatively high value of c-hat in the analyses, which penalizes models on the basis of parameter
245 number. This prevented us not only from evaluating more complex models (i.e. non linear
246 selection) but also resulted in estimates of directional selection with rather large SEs and
247 therefore with 95% confidence intervals that contained the zero in all cases.
248 Some theoretical models of thermal adaptation across heterogeneous environments (e.g.,
249 Climate variability hypothesis, generalist-specialist models), suggest that temporal
250 environmental heterogeneity selects for more broadly adapted individuals (Lynch and Gabriel,

251 1987; Gilchrist 1995), favoring increased plasticity particularly regarding thermal tolerance traits
252 (Gunderson & Stillman 2015). Based on these models we predicted that the high seasonality
253 should select for high plasticity in thermal traits and therefore, the plasticity itself should
254 currently be under directional selection. Our prediction turned out to be incorrect as models
255 including plasticity showed relatively weak support.

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

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 offer support for this prediction: there was a slight
276 trend for survival to decrease as CT_{max} increased under warm as well as under cold-acclimated
277 conditions. However, in both cases estimates were not statistically different from zero.
278 Nevertheless, this might suggests that selection could be favouring individuals that avoid hot
279 microhabitats, possibly by means of behaviour (Ruiz-Aravena et al. 2014). Indeed, behavioural
280 thermoregulation has been proposed as one key factor that prevents an evolutionary response to
281 selection to raising temperatures (Kearney et al. 2009; Huey et al. 2012; Buckley et al. 2015).
282 The fact that CT_{Max_20} was negatively correlated with CT_{Min_10} indicates that individuals with
283 higher cold tolerance might be the ones avoiding hot microhabitats, which opens very interesting
284 questions for further research.

285 Regarding the sensitivity of metabolism to temperature (Q_{10}) we predicted that Q_{10} is not
286 under directional selection. Our results (partially) supported that prediction as the rate at which
287 survival changed with changes in Q_{10} was very small (Fig. 4, Table 2), although the models with
288 Q_{10} still showed some support (Table 1). Finally, we also predicted no directional selection on
289 T_{Pref} as we have previously shown that acclimation to warmer temperatures produced an increase
290 in this trait (Ruiz-Aravena et al. 2014). Nevertheless, we found a non-significant trend showing
291 that survival decreased, although at a very low rate, as T_{Pref} increased, which might suggest that
292 selection favours those individuals that are able to avoid hot microhabitats.

293 Our results indicate a positive trend of survival with body size (although the directional
294 selection estimate was non-significant), something that has been previously reported in the
295 literature (Aubin-Horth et al. 2005; Iida & Fujisaki 2007; Crosby & Latta 2013; Delaney &
296 Warner 2017). This is somewhat unsurprising, given that body mass is known to be positively

297 associated with several physiological traits that enhance performance (Castellano et al. 1999;
298 Madsen & Shine 2000; Hurlbert et al. 2008; Shepherd et al. 2008; Luna et al. 2009) including
299 plasticity itself (Whitman & Ananthakrishnan 2009). Although this population inhabits two
300 highly isolated ponds where the presence of competitors (anurans) has not been observed, there
301 might be a risk of predation by herons (*L.D.B. personal observation*), and thus, a positive
302 selection for body size might explain it. Nevertheless, further experimental work is needed to
303 evaluate this possibility.

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

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Data: data is available for download from the CSIRO Data Access Portal (https://data.csiro.au)

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; M.T., B.S. 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.

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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.

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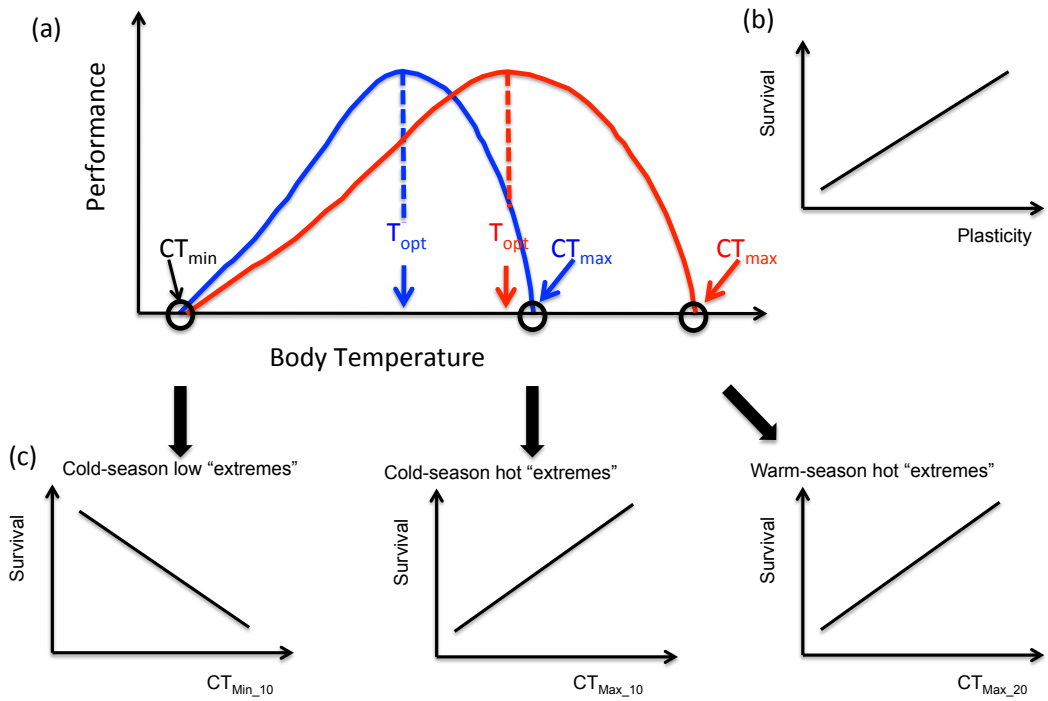
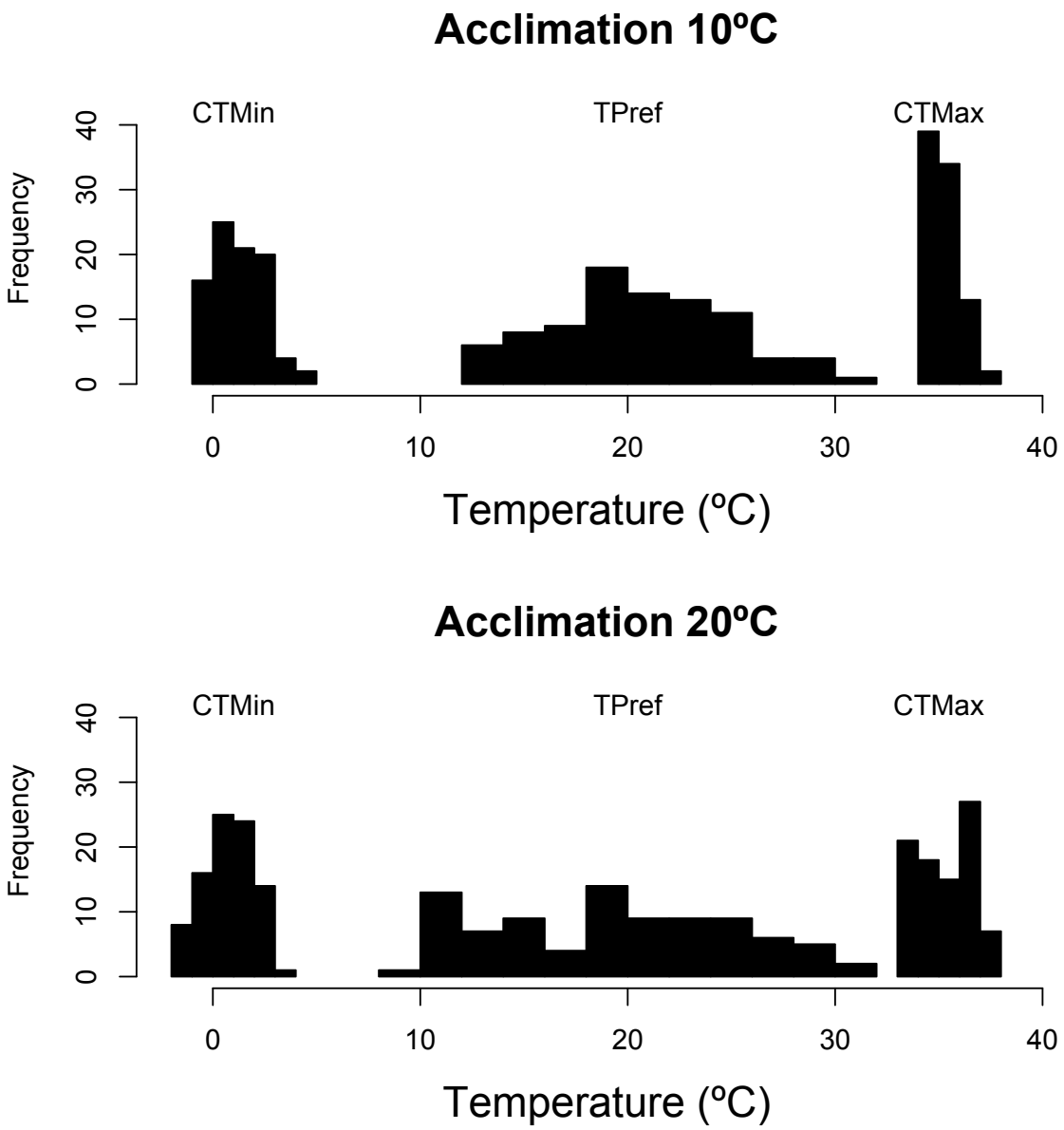
Figure 1. Graphical representation of the predictions tested in this study. (a) Predictions built up from findings showing that acclimation to warmer temperatures produces an increase in the upper but not in the lower limits of the thermal performance curve (Ruiz-Aravena et al. 2014). (b) The high seasonality should have selected for plasticity and therefore, plasticity of all traits should 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.



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



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521 **Figure 3.** Individual plasticity in CT_{Min}, T_{Pref}, CT_{Max} and Q_{10} to 10 and 20ºC acclimation
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523 For CT_{Min} and CT_{Max} the width of the line is directly proportional to the number of individuals
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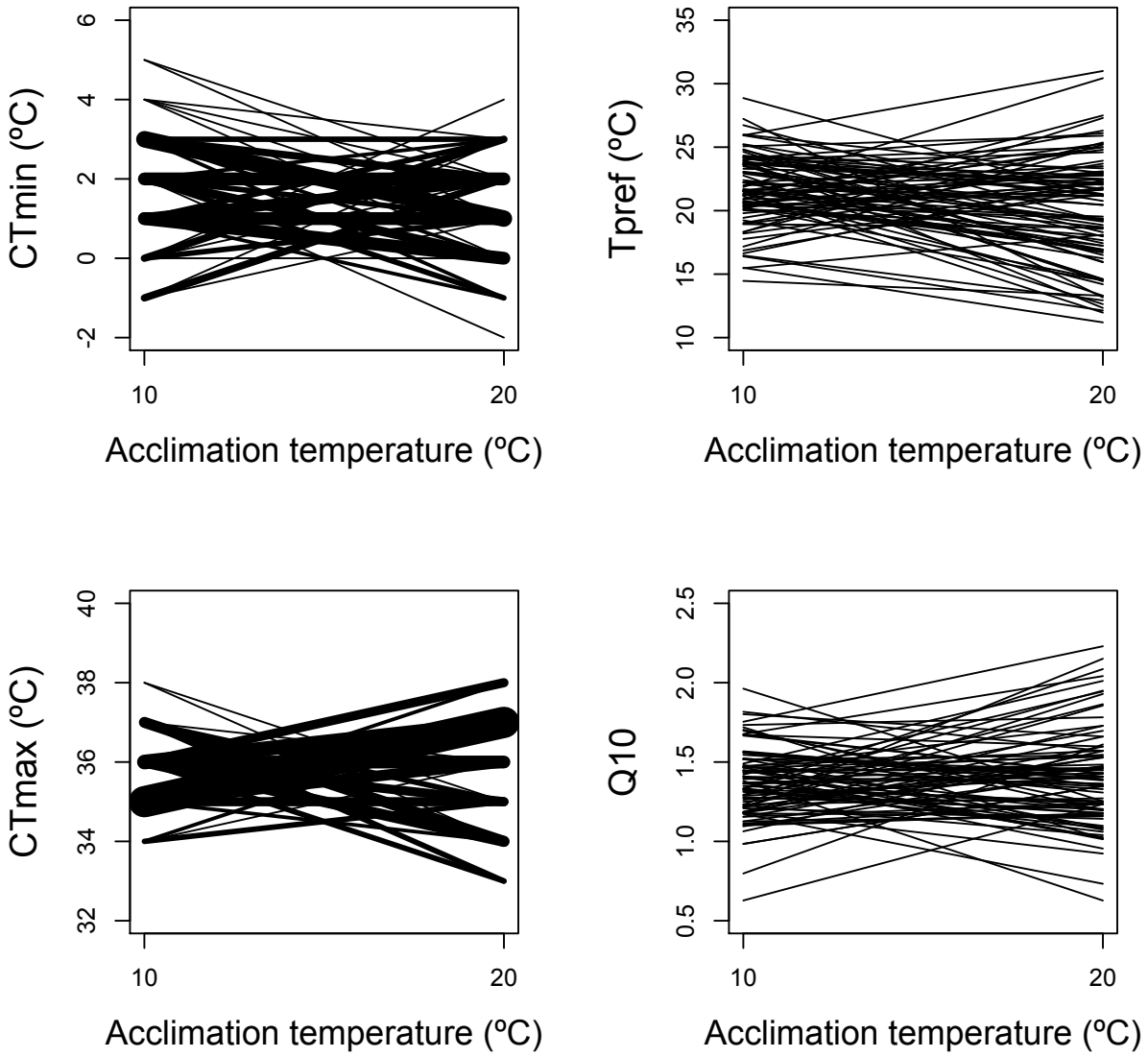

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Figure 4. Survival estimates of simple directional selection sorted by model probabilities. *w*: Akaike weights of the model.

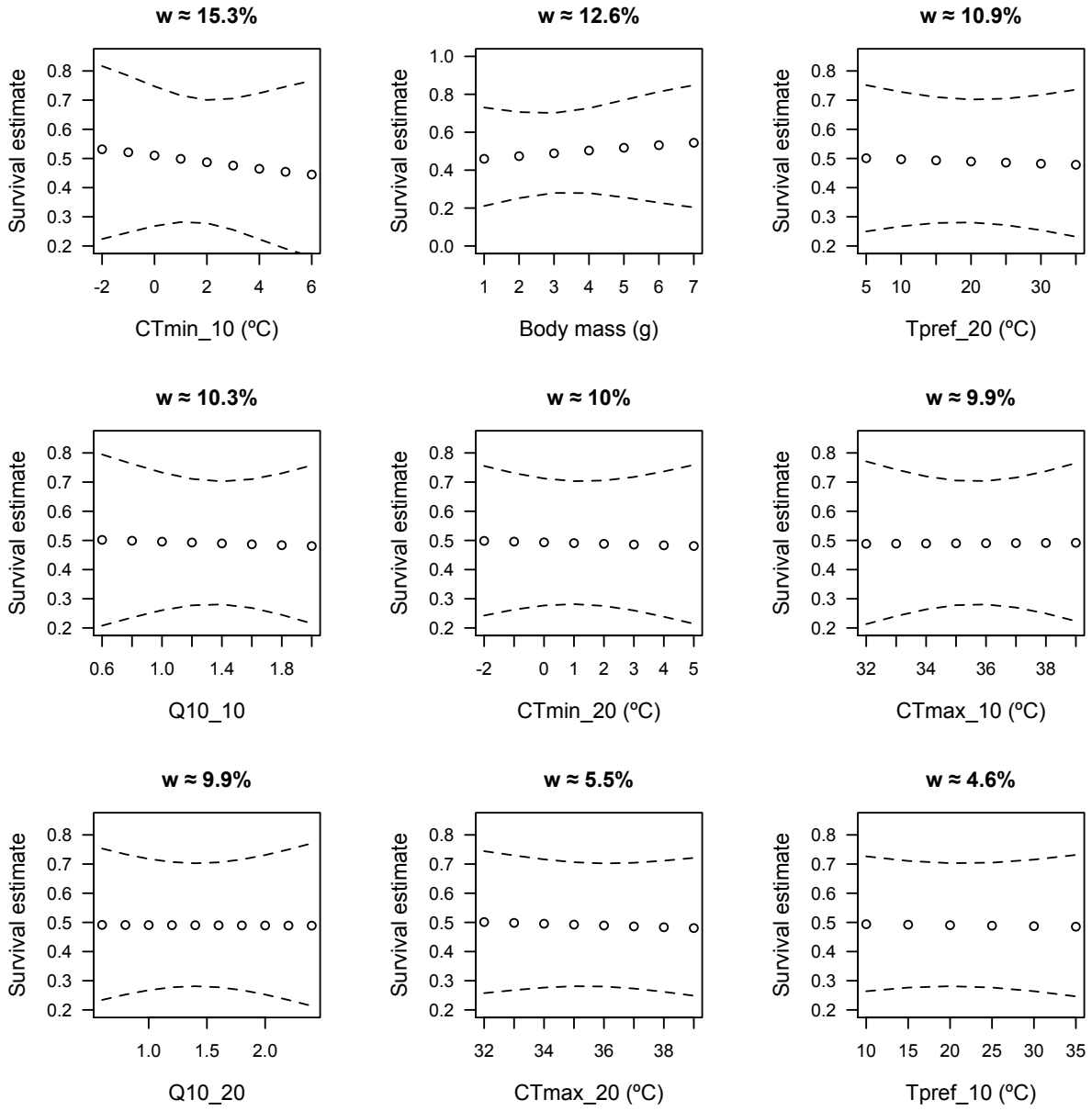


Table 1. Candidate models ordered accordingly to their Akaike weights. Single term models represent directional (e.g. CT_{Max}) and correlational selection represents plasticity (e.g. CT_{Max_10} * CT_{Max_20}). CT_{Min} = minimum critical temperature; CT_{Max} = maximum critical temperature; T_{Pref} = 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.

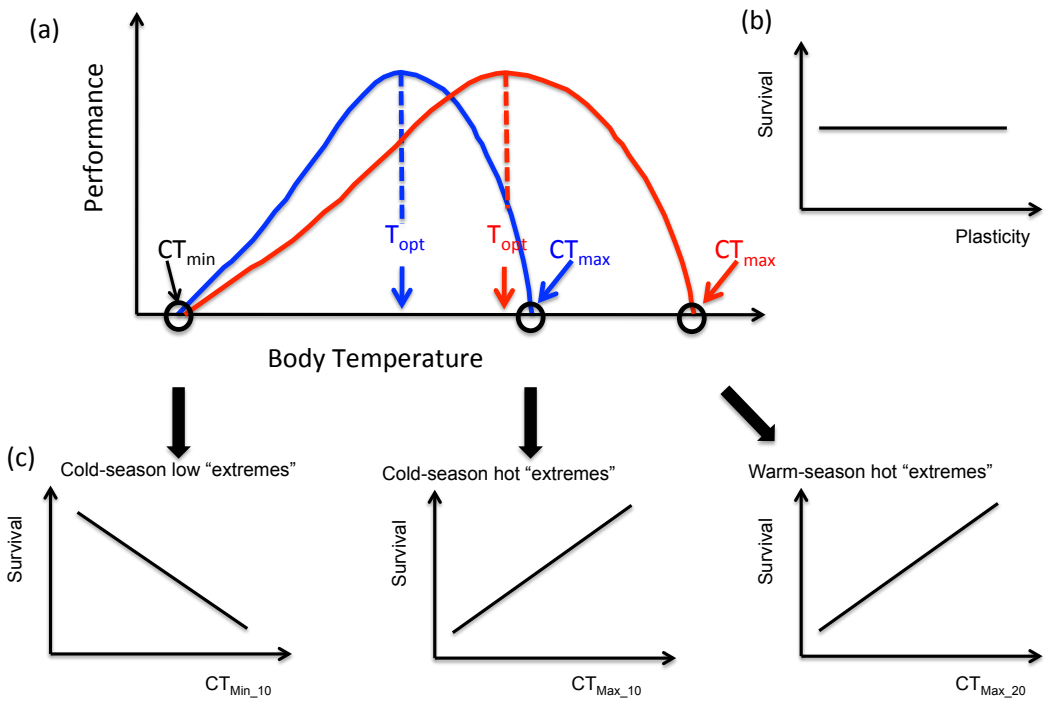
Models		K	AICc	ΔAICc	w_i
1	Null model	2	130.17	0	0.220
2	CT_{Min_10}	3	131.40	1.23	0.119
3	MB	3	131.78	1.61	0.098
4	T_{Pref_20}	3	132.08	1.90	0.085
5	Q_{10_10}	3	132.18	2.01	0.081
6	CT_{Min_20}	3	132.25	2.08	0.078
7	CT_{Max_10}	3	132.26	2.08	0.078
8	Q_{10_20}	3	132.26	2.09	0.077
9	$CT_{Min_10} + CT_{Min_20} + CT_{Min_10} * CT_{Min_20}$	5	133.38	3.21	0.044
10	MB + CT_{Max_20}	4	133.44	3.27	0.043
11	MB + T_{Pref_10}	4	133.82	3.64	0.036
12	$Q_{10_10} + Q_{10_20} + Q_{10_10} * Q_{10_20}$	5	134.17	4.00	0.030
13	MB + $T_{Pref_10} + T_{Pref_20} + T_{Pref_10} * T_{Pref_20}$	6	137.16	6.99	0.007
14	MB + $CT_{Max_10} + CT_{Max_20} + CT_{Max_10} * CT_{Max_20}$	6	137.62	7.45	0.005

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

Table 2. Directional selection estimates from single terms models with their standard errors (SE) and 95% confidence intervals (95% CI). 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.

Trait	Estimate	SE	95% CI
MB	0.209	0.212	-0.206 – 0.625
CT_{Min_10}	-0.248	0.187	-0.616 – 0.119
CT_{Min_20}	-0.030	0.181	-0.384 – 0.324
T_{Pref_10}	-0.025	0.059	-0.140 – 0.090
T_{Pref_20}	-0.026	0.042	-0.109 – 0.056
CT_{Max_10}	0.026	0.257	-0.477 – 0.530
CT_{Max_20}	-0.192	0.195	-0.575 – 0.191
Q_{10_10}	-0.475	1.140	-2.709 – 1.759
Q_{10_20}	-0.048	0.795	-1.607 – 1.510

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.



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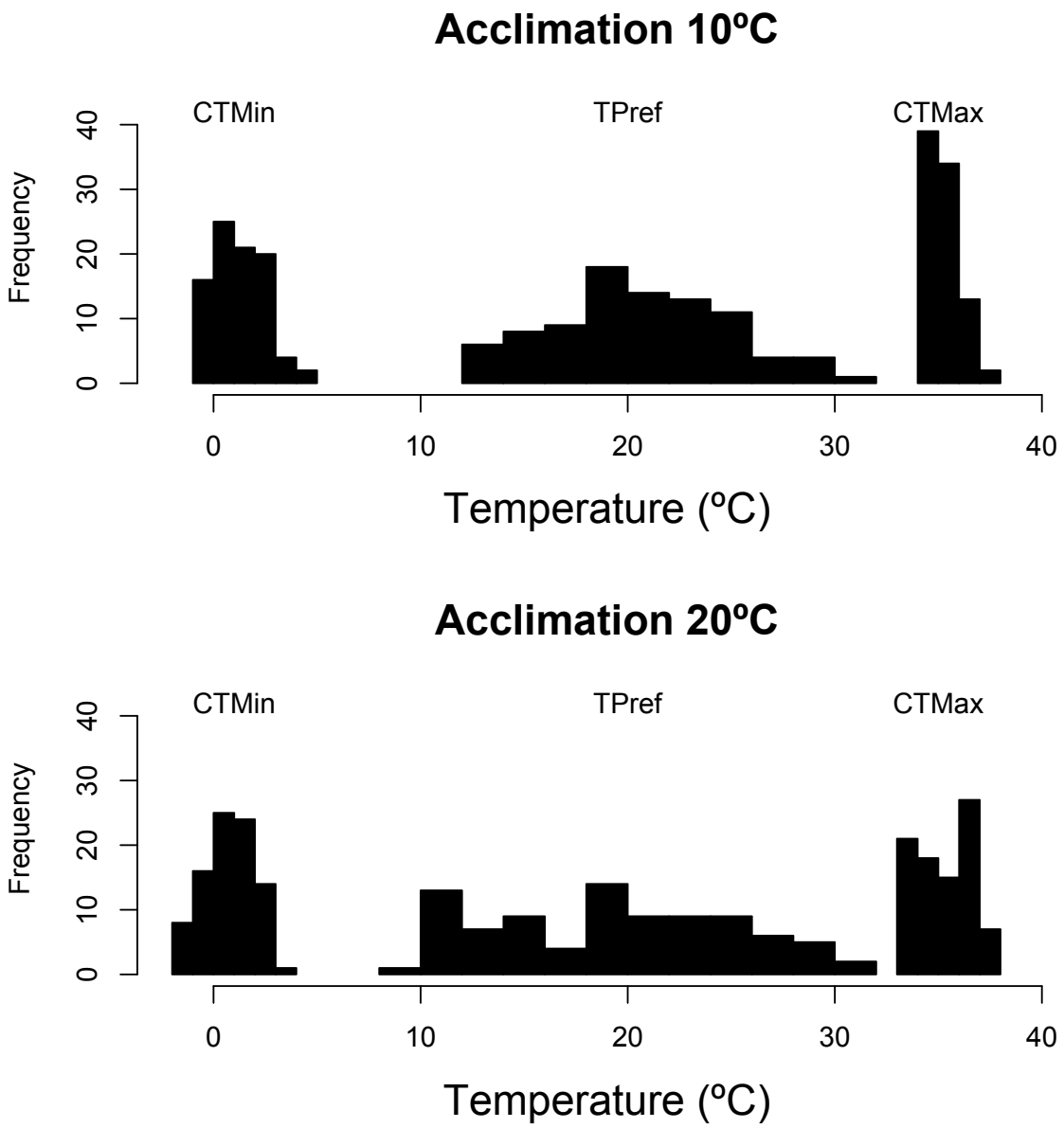

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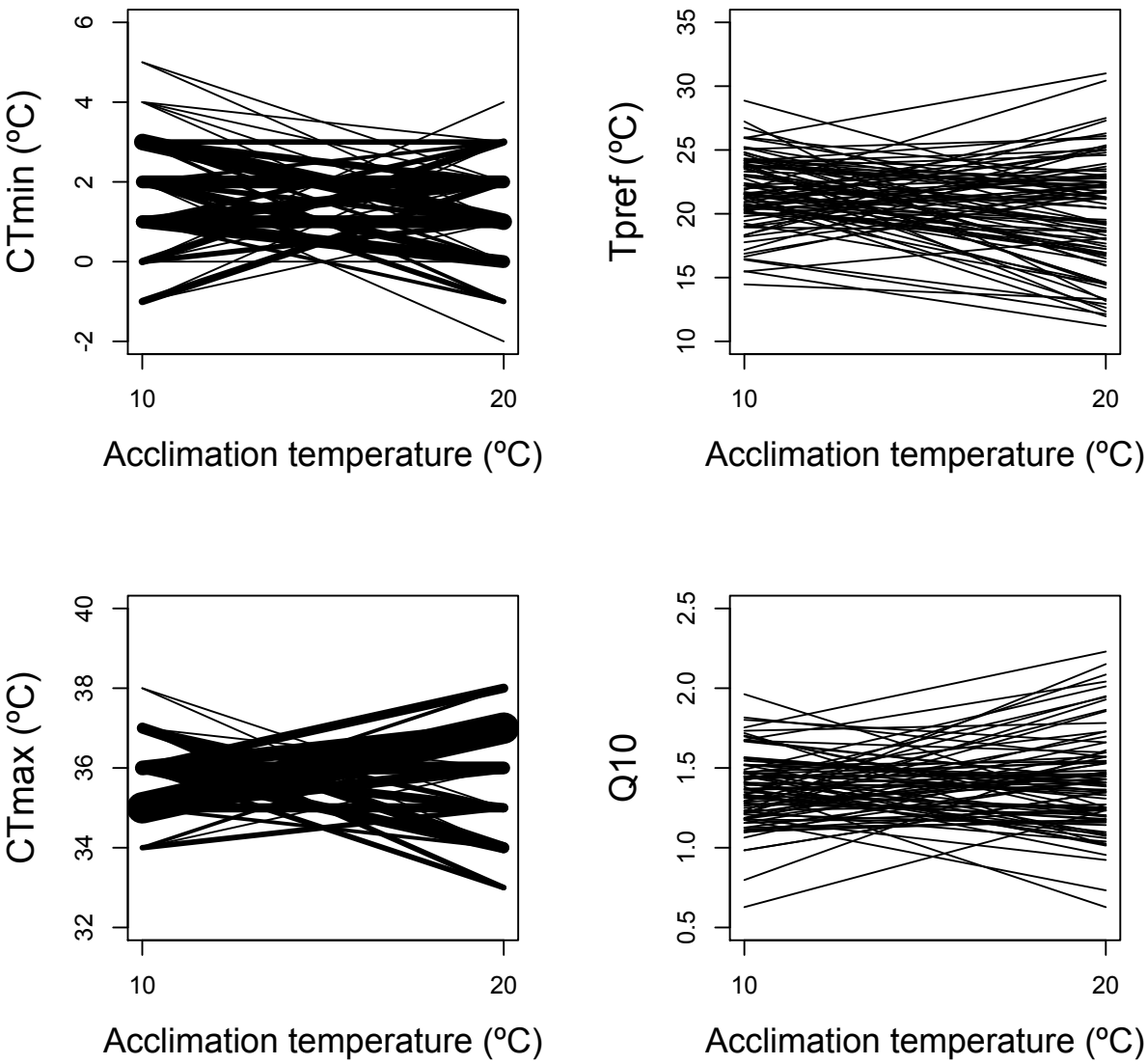

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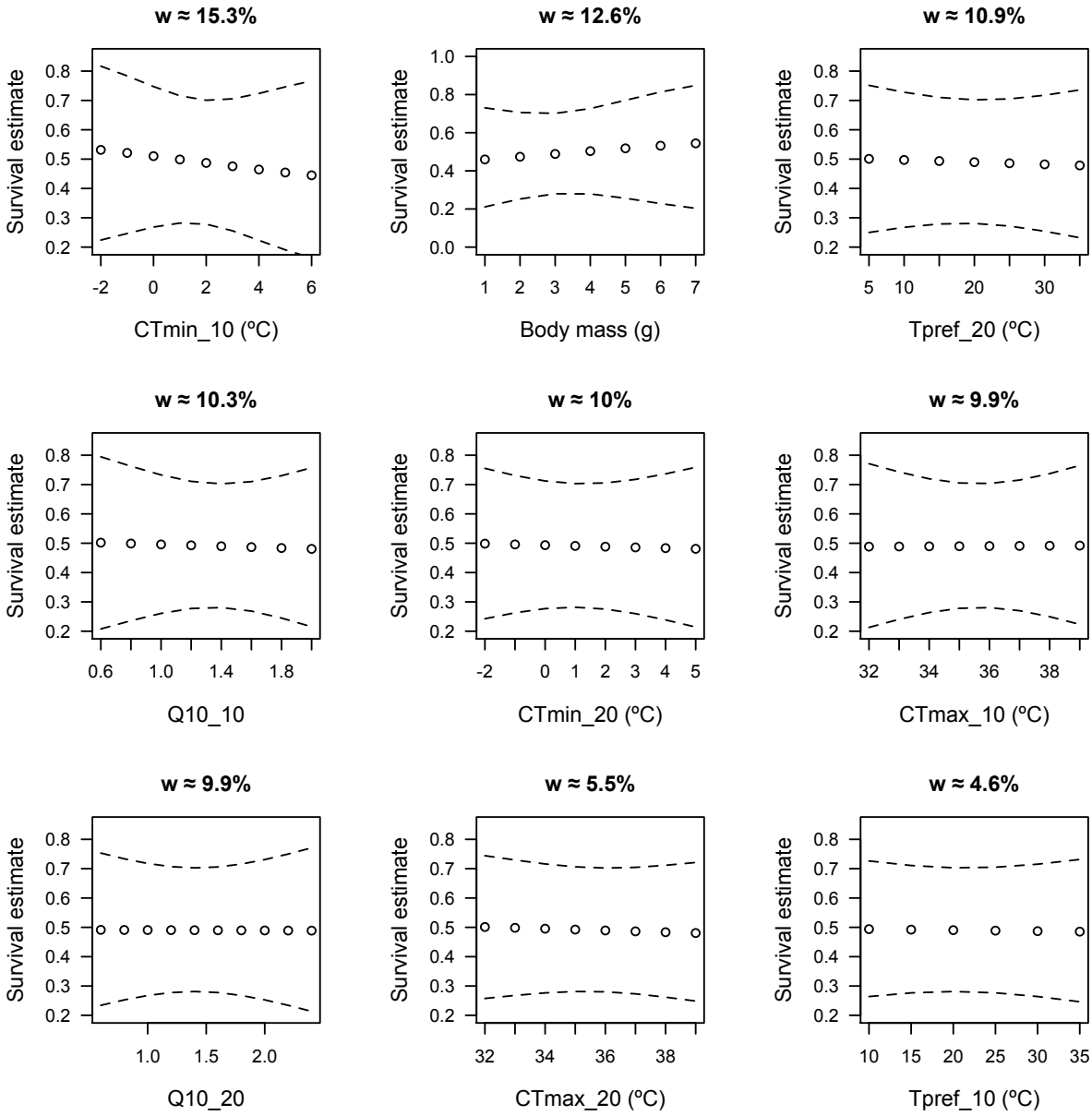
w: Akaike weights of the model.