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May future climate change promote the invasion of the marsh frog? An integrative thermo-physiological study

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Highlighted student paper *Through the study of an overlooked invasive frog species, we show that multi-traits methods improve our understanding of how an invasive ectotherm may benefit from climate change.*

Author contribution statement PP, AH and MD conceived the study; PP acquired the data, did the statistical analysis, and wrote the first draft of the manuscript; MD and AH administrated research grants; All authors contributed equally to the drafts and gave final approval for publication.

1 **Abstract**

2 Climate change and invasive species are two major drivers of biodiversity loss and their
3 interaction may lead to unprecedented further loss. Invasive ectotherms can be expected to
4 tolerate temperature variation because of a broad thermal tolerance and may even benefit from
5 warmer temperatures in their new ranges that better match their thermal preference. Multi-trait
6 studies provide a valuable approach to elucidate the influence of temperature on the invasion
7 process and offer insights into how climatic factors may facilitate or hinder the spread of
8 invasive ectotherms.

9 We here used marsh frogs, *Pelophylax ridibundus*, a species that is invading large areas
10 of Western Europe but whose invasive potential has been underestimated. We measured the
11 maximal and minimal temperatures to sustain physical activity, the preferred temperature, and
12 the thermal dependence of their stamina and jumping performance in relation to the
13 environmental temperatures observed in their invasive range.

14 Our results showed that marsh frogs can withstand body temperatures that cover 100%
15 of the annual temperature variation in the pond they live in and 77% of the observed current
16 annual air temperature variation. Their preferred body temperature and performance optima
17 were higher than the average temperature in their pond and the average air temperature
18 experienced under the shade.

19 These data suggest that invasive marsh frogs may benefit from a warmer climate. Broad
20 thermal tolerances, combined with high thermal preferences and traits maximized at high
21 temperatures, may allow this species to expand their activity period and colonize
22 underexploited shaded habitat, thereby promoting their invasion success.

23 **Keywords** Invasive species · Ectotherm · Temperature · Thermal tolerance · Performance
24 curve

25 **Introduction**

26 Human-induced climate change (Cattiaux et al. 2015; Simmons et al. 2017; IPCC 2022) puts
27 pressure on living organisms, pushing them to their physiological limits (Parmesan and Yohe
28 2003; Warren et al. 2018). Indeed, temperature affects the rates of chemical reactions within an
29 organism and consequently their physiology (Tattersall et al. 2012). Their response to
30 temperature is described by reaction norms that typically have a parabolic shape (Cossins and
31 Bowler 1987; Angilletta 2009) (Fig. 1). Among Metazoa, ectothermy is the most common
32 thermoregulatory mechanism, and because ectotherms rely mostly on environmental
33 temperature, many species are expected to be impacted by climate change (Deutsch et al. 2008;
34 Sinclair et al. 2016; Lotze et al. 2019; Sánchez-Bayo and Wyckhuys 2019). Regardless of their
35 geographical origin, both tropical and temperate species are at risk from global warming
36 (Deutsch et al. 2008) and of the predicted stronger temperature variations (Paaijmans et al.
37 2013; Vasseur et al. 2014; Dillon et al. 2016). This is especially the case for amphibians which
38 are already among the most threatened vertebrates (Stuart et al. 2004; IUCN 2022) and for
39 which climatic change has been invoked as a direct driver of population extinction (Araújo et
40 al. 2006; Wake and Vredenburg 2008; Blaustein et al. 2010). Other drivers of population
41 decline such as invasive species may interact with climate change and precipitate the extinction
42 of threatened populations (Pounds et al. 2006; McMenamin et al. 2008).

43 In many cases invasive species can maintain an activity over a broad range of
44 temperatures and as such may be less impacted or even benefit from the ongoing climatic crisis
45 (Huang et al. 2011; Zerebecki and Sorte 2011; Kelley 2014). Bennett (1987) hypothesized that
46 warmer temperatures maximize locomotor capacity which may benefit dispersal in invasive
47 species. Consequently, the interaction between invasive species and climate change may result
48 in unprecedented risks to native ecosystems (Rahel and Olden 2008; Mainka and Howard
49 2010). Despite the general vulnerability of amphibians to temperature change, some introduced
50 amphibians are able to withstand and quickly adapt to new environmental conditions (Vimercati
51 et al. 2018; Mittan and Zamudio 2019; Araspin et al. 2020). African clawed frogs (*Xenopus*
52 *laevis*) and bullfrogs (*Lithobates catesbeianus*), for example, are two species that have been
53 introduced worldwide (IUCN 2015, 2020). Andersen et al (2021) recently predicted suitable
54 niches on all continents except Antarctica for both species. Johovic et al (2020) also showed
55 that climate change would benefit the spread of bullfrogs. However, most of the model

56 predictions for invasive species are based on occurrence data only. Empirical measurements of
57 not only the thermal limits but also thermal preferences and physiological response to
58 temperature of invasive species need to be gathered and then used to improve these predictions
59 (Ginal et al. 2023).

60 Some traits have been recognized to be associated with the invasive potential of a
61 species (Sakai et al. 2001; Allen et al. 2017). Range expansion, for example, is a stage during
62 invasion that benefits from greater dispersal capacity and survival. Stamina may play an
63 important role during dispersal and is driven by the capacity to maintain prolonged muscular
64 effort and an efficient metabolism. On the other hand, a quick reaction time and explosive
65 locomotor performance may allow a better escape from predators and increase prey capture
66 success. To establish and disperse, invaders also need to overcome abiotic ecological filters,
67 such as temperature (Olyarnik et al. 2009). Indeed, global surface temperature is continuously
68 increasing and a global warming of 2 °C will be exceeded in the 21st century (IPCC 2022). A
69 broad thermal performance breadth would then help invaders to maintain their performance and
70 potentially fitness. By consequence, global warming will result in a shift in environmental
71 temperature that may maximize locomotor performance (Fig. 1). These two adaptations (i.e.
72 large thermal tolerance range and broad thermal performance breadth) combined may allow
73 activity and habitat use of invasive species to be less constrained by daily and annual variations
74 in environmental temperature in addition to increasing their dispersal efficiency and invasive
75 potential.

76 In the present paper, we performed a multi-trait study using, as our model species, marsh
77 frogs. The *Pelophylax* genus is widely distributed in the Palearctic (Lymberakis et al. 2007;
78 Dufresnes and Mazepa 2020), with multiple introductions out of their native range particularly
79 in Western Europe, such as Belgium (Holsbeek and Jooris 2010), Switzerland (Dubey et al.
80 2014) or France (Doniol-Valcroze et al. 2021). These have resulted in the establishment of
81 invasive populations (Dufresnes et al. 2017b). The major threats reported from their invasion
82 is their potential hybridization with native species of the genus (Holsbeek and Jooris 2010) as
83 well as predation on native species (Pille et al. 2021). The invasive range of these animals has
84 increased over the last decades with new areas being rapidly colonized such as the Larzac
85 plateau in southern France where *Pelophylax* frogs were introduced after being historically
86 absent (Dufresnes et al. 2017a; Denoël et al. 2022; Duret et al. 2022). It has been suggested that
87 recent warmer conditions may have enhanced their invasion (Dufresnes et al. 2017a), but this

88 remains untested to date. The Larzac plateau has seen its temperature increased over the last
89 years (Ali et al. 2022). Climate models predict that warming will be more rapid in the region
90 compared to the global average (Kjellström et al. 2018). If this increase in temperature does
91 indeed benefit the invasive capacity of these frogs, then this may have major consequences to
92 the native ecosystem.

93 Specifically, the goals of this study were to evaluate whether warm temperatures such
94 as those arising due to climate change could favour invasive marsh frogs. We expect marsh
95 frogs to show: (i) a broad thermal tolerance providing them with a large period of activity; (ii)
96 a preference for high environmental temperatures that may be higher than the current
97 environmental temperatures; (iii) locomotor capacities that are maximised at high temperatures,
98 i.e. higher than the current environmental temperatures. Lastly, sex differences in thermal traits
99 are not often investigated whereas they may be expected due to the inherent differences in
100 physiology and behaviour between males and females (Bodensteiner et al. 2021). We therefore
101 took the opportunity of this study to test for an effect of sex in each thermal trait previously
102 mentioned.

103 **Materials and methods**

104 **Study organisms**

105 Adult marsh frogs (*Pelophylax ridibundus*) were caught in August 2020 (Critical thermal limits:
106 $n = 40$, 20 for critical minimum and 20 for critical maximum with 10 females and 10 males in
107 each; Stamina: $n = 21$, 8 females and 13 males; Jump force: $n = 25$, 11 females and 14 males)
108 and April 2022 (Preferred temperature: $n = 29$, 11 females and 18 males) at the border of ponds
109 in Larzac (43°51'N – 3°21'E, Hérault, France). Previous molecular analysis confirmed the
110 taxonomic assignment and the invasive status of the marsh frogs in the studied population
111 (Dufresnes et al. 2017a; M. Pabijan, pers. comm.). All individuals were PIT-tagged (Biolog-
112 ID, 134.2 KHz) to allow individual identification. The tag was inserted dorsally under the skin
113 (Christy 1996). The snout-vent length (SVL; from the tip of the snout to the end of cloaca) was
114 measured with callipers after gently laying each frog on a flat surface (mean \pm SE = 68.23 \pm
115 1.91 mm). Each frog was weighed (mean \pm SE = 30.97 \pm 2.61 g) using a digital scale (Ohaus,
116 Brooklyn, NY, USA; precision 0.1 g). Sex was identified by the presence of a vocal sac and
117 nuptial pads on the males' thumbs and later verified through dissection and inspection of the

118 reproductive organs.

119 **Transportation and housing**

120 Frogs were brought inside large coolers filled with 3cm of water to the Function and Evolution
121 (FUNEVOL) laboratory at the Muséum National d'Histoire Naturelle of Paris, France. Frogs
122 were maintained singly in 5L tanks (21.4 x 36.5 x 16.5 cm; water depth: 5 cm) with holes in
123 the lid. Tanks were inclined by placing a 10 cm wood block under one of the sides creating a
124 dry area that occupied one third of the tank. Animals rested for the first week to allow them to
125 acclimate to laboratory conditions. Water in the tanks was renewed weekly. Two adult crickets
126 (*Acheta domestica*) were given twice weekly and additionally after each trial. Night and day
127 periods were set to 12 hours each. The room temperature was set at 24 °C.

128 **Field temperatures**

129 Air temperatures were recorded each 6 minutes and obtained from a meteorological station
130 situated at 6 km from the site from January 2018 to January 2019 (Larvor et al. 2020; Table 1).
131 Water frogs spend a large part of their time at the water-land interface and take shelters in the
132 bottom of ponds, therefore, we incorporated water temperatures with automatic measures each
133 30 minutes from March 2019 to March 2020 with a submersible temperature logger (HOBO
134 U20L-01) placed at a depth of 1.5m in a pond at the center of the invaded area (Table 1).

135 **Critical temperatures (CT)**

136 Each frog was placed individually inside an incubator (Aqualytic-LIEBHERR, TC 256
137 G/256L/2–40 °C) with light in a 5L plastic box (21.4 x 36.5 x 16.5 cm), with 5 mm of water
138 depth to prevent dehydration. Twenty animals (10 males, 10 females) were used to measure
139 minimal critical temperatures and twenty others (10 males, 10 females) were used for maximal
140 critical temperatures to avoid too much stress on the same animals. To establish critical
141 minimum temperature (CT_{min}), the incubator was set at 12 °C and the frogs were kept at that
142 temperature for one hour. Next, the temperature was decreased at a rate of cooling of 2°C each
143 30 minutes until reaching 6°C when we switched to a rate of 1°C each 30 minutes (Fig. 2a). For
144 critical maximal temperature (CT_{max}), frogs were placed in a container placed inside a large
145 cooler filled with water heated by a water heater. Experiments started at the acclimation

146 temperature of 24 °C after which the temperature was increased with a rate of warming of 2°C
147 each 30 minutes until reaching 34°C at which point we switched to a rate of 1°C each 30 minutes
148 (Fig. 2a). For both minimal and maximal critical temperatures, the temperature was increased
149 or decreased by 0.5 °C every 30 minutes whenever animals showed slower righting response
150 (Fig. 2a). Even if the onset of spasms has been considered by some researchers as a more precise
151 measurement of thermal tolerance (Lutterschmidt and Hutchison 1997), we used the loss of
152 righting response instead to avoid excessive stress (Taylor et al. 2021). The lack of a righting
153 response was tested by giving the animal two seconds to recover a normal upright position after
154 having been placed on their back. When the frog was no longer able to right itself during this
155 short time period, its internal body temperature was measured using a thermocouple inserted
156 inside the cloaca (APPA51 thermometer K-type, Appa technology corp, resolution 0.1°C).

157 **Preferred temperature (T_{pref})**

158 The experimental setup consisted of five individual lanes (190 x 20 x 30 cm each) within a
159 wood arena covered with cork. The gradient ranged from approximately 14.5 °C on the side
160 cooled by ice packs to approximately 42 °C on the side heated by light bulbs (60 watts, Repti
161 Basking Lamp Spot ZooMed, SL2-60) (Fig. 2b, Fig. S1). The room temperature was set to 24
162 °C and the average temperature in the lanes was 24 ± 1.73 (SE) °C. Each lane was humidified
163 on its whole length before the trial with a spray bottle filled with tap water. Animals were not
164 fed for three days before trials to avoid digestion effect on thermoregulation (Gatten 1974). One
165 frog was set inside each lane and was left free to thermoregulate for one hour. After one hour
166 we measured the body temperature of each frog and calculated the mean across all individuals
167 which we considered as an estimate of the population thermal preference. Each frog was
168 recorded twice separated by three days. As we did not find significant difference between each
169 recording (paired t -test: $t_{28} = -0.56$, $p = 0.57$, first recording: mean \pm SE = 21.79 ± 0.68 °C,
170 second recording: 22.27 ± 0.66 °C), we used the second recording to avoid potential bias
171 coming from their initial discovery and exploration of the experimental setup. The temperature
172 of the animals was assessed using a thermal camera (FLIR-E6xt 2.1L) and thermal image
173 analysis software (FLIR Tools, version 6.4.18039.1003). Thermal pictures were taken with an
174 incidence angle close to 0°C and at a distance of one meter and an emissivity of 0.97 (Fig. S2).
175 Temperature was also verified by a thermocouple (APPA51 thermometer K-type, Appa
176 technology corp) inserted in the cloaca at the end of the trial. Since there were no significant

177 differences of the methodology (i.e. thermal camera vs thermocouple measurements) on the
178 measures of body temperatures (linear mixed model with method, sex and SVL as covariates
179 on body temperature; methods: estimate = -0.8 , CI = -1.62 to 0.03 , $p = 0.06$; sex: estimate =
180 0.76 , CI = -1.59 to 3.11 , $p = 0.52$; SVL: estimate = -0.08 , CI = -0.29 to 0.12 , $p = 0.42$), we
181 decided to use the thermal camera values because of its instantaneity and to avoid delays created
182 by the cloacal measurements due to the capturing and handling of the animals.

183 **Terrestrial exertion**

184 Frogs were stimulated by hand to move back and forth across a linear track (200 x 40 x 50 cm
185 ; graduated each 10 cm) with a humidified cork bottom providing grip. The time and the
186 distance that frogs were able to continuously move until exhaustion were measured (Herrel and
187 Bonneaud 2012a, b) (Fig. 2c). Time was measured using a stopwatch and distance was based
188 on the number of graduations crossed by the frogs during movement. From these variables, we
189 calculated a composite variable referred to henceforth as mean speed. Exhaustion was defined
190 by the lack of a righting response determined by giving the animal two seconds to recover a
191 normal position after having been placed on its back. After the trial, body temperature was
192 verified using a thermocouple. Frogs were then placed back in their maintenance boxes, given
193 food, and not used for other observations for one week. Trials were repeated twice at each
194 temperature, and the maximum time and distance at each temperature were retained for each
195 individual. To evaluate how exertion changes as a function of temperature (Angilletta 2009)
196 our observations were repeated in random order. Because the thermal optimum for exertion was
197 not clear after testing animals at four temperatures (9,14, 19, 24°C), we ran additional exertion
198 tests at two additional temperatures (4 and 29 °C) providing a better fit of our models.

199 **Jump forces**

200 Maximal jump forces were measured using a piezo-electric force platform (20 x 10 cm, Kistler
201 Squirrel force plate, ± 0.1 N; see Herrel et al. 2014) (Fig. 2d). The platform was connected to a
202 charge amplifier (Kistler – Charge Amplifier type, 9865) and forces were recorded for 60
203 seconds at 500 Hz using the BioWare software (Kistler). A cork surface was glued to the force
204 plate to provide a better grip. Frogs were placed individually on the force plate, allowed to rest
205 a few seconds, and then induced to jump by approaching them. Peak force data were extracted
206 using the Kistler BioWare software and the total resultant force (vector sum of the X-, Y- and

207 Z-forces) was calculated. Three sessions of at least 3 jumps within each session were recorded
208 and only the single best jump was retained and used in the analyses. Tests at four temperatures
209 (14, 24, 28, 33 °C) were enough to determine the thermal optimum. Prior to each test, frogs
210 were placed individually for 3 hours at the desired temperature in an incubator in a box with
211 some water to prevent dehydration.

212 **Statistical analyses**

213 For both one-way analyses of co-variance (ANCOVAs) and generalized additive mixed model
214 (GAMM), model validation was performed by visualising the residuals which had a
215 homogeneous, normal distribution and by plotting the response variable on fitted values. All
216 analyses were done using R version 4.1.2 (R Core Team 2019).

217 Mass and snout vent length (SVL) were significantly correlated ($t_{19} = 12.01$, $R = 0.94$;
218 $p < 0.01$). We therefore used snout-vent length as our covariate in the analyses because mass is
219 dependent on the daily food intake and excretion. Snout-vent length did not differ between
220 males and females ($t_{19} = 0.64$, $p = 0.52$).

221 ANCOVAs were run for CT_{\min} and CT_{\max} to determine the effect of sex on body
222 temperature after controlling for SVL. Another ANCOVA was run with the effect of sex on the
223 preferred temperature with SVL as covariate.

224 Because temperature often has a non-linear effect on biological processes (Huey and
225 Kingsolver 1989; Bulté and Blouin-Demers 2006), we fitted a GAMM (Wood 2011) for each
226 performance trait. GAMM can fit complex non-linear relationships using a smoother function
227 on sections of the data (Wood 2006). They are therefore good tools to estimate thermal
228 performance curves (TPC) and were previously used in the study of reptiles (Vickers et al.
229 2017; Cecchetto et al. 2020). Effective degrees of freedom (edf) were used to quantify the
230 strength of non-linearity. An edf of one being a linear effect, an edf between one and two was
231 considered as a weak non-linear effect and an edf greater than two was a highly non-linear
232 relationship (Zuur et al. 2009). In each model, SVL was used as covariate; temperature as a
233 fixed effect dependent on a smooth function and sex was added as a fixed factor. Because the
234 same frogs were measured at each temperature, we added individual identity as a random term
235 (Wood 2006). For temperature, we used a smoothing parameter (k) that avoids both over
236 smoothing and overfitting biologically incoherent variation. This was based on the models
237 having the highest R -squared values and verifications from the function of the “mgcv” R

238 package (v1.8-35; Wood 2011). F-statistics were reported with the model degrees of freedom
239 (mdf), calculated by adding up the degrees of freedom of each term, and the residual degrees
240 of freedom (rdf) calculated as the difference between the number of observations (n) and the
241 mdf. To create the thermal performance curves, we used the predictions from the statistical
242 models. The thermal optimum and the thermal performance breadth, i.e. the range of
243 temperatures over which an animal can maintain 95, 80 and 50% of its maximal performance,
244 were extracted from the model predictions (Taylor et al. 2021). Note that positive and negative
245 predicted values at 2.8°C and 38.4°C can seem biologically impossible from our values
246 observed at each CT, however these predictions only indicate predicted CTs at colder/warmer
247 temperature (Fig. 4, 5).

248

249 **Results**

250 **Critical temperature**

251 The studied marsh frogs showed a CT_{min} (mean \pm SE) of 2.80 ± 0.21 °C, a CT_{max} of $38.40 \pm$
252 0.09 °C and, an average thermal range ($CT_{max} - CT_{min}$) of 35.60 ± 0.23 ° C (Fig. 3). There was
253 no significant effect of sex (ANCOVA; $F_{1,17} = 0.13$, $p = 0.91$) or SVL ($F_{1,17} = 0.09$, $p = 0.76$)
254 for CT_{min} . However, for CT_{max} the effect of sex was significant ($F_{1,17} = 7.49$, $p = 0.01$) with
255 males having a slight but significantly higher upper thermal tolerance (0.44 °C) than females
256 (Fig. 3). Snout-vent length had no significant effect on CT_{max} ($F_{1,17} = 2.08$, $p = 0.16$).

257 **Preferred temperature**

258 After thermoregulating for an hour, marsh frogs showed an average body temperature of 21.8
259 ± 3.71 °C. There was no significant effect of either sex ($F_{1,26} = 0.12$, $p = 0.73$) or SVL ($F_{1,26} =$
260 0.19 , $p = 0.67$).

261 **Stamina**

262 Based on the adjusted *R*-squared values (GAMM), temperature, SVL and sex together
263 explained 82.8% of variation in the distance travelled by the frogs before exhaustion.
264 Temperature had a significant highly non-linear effect on the distance travelled before
265 exhaustion (edf = 3.91 ; $F_{20,27, 147.77} = 185.23$, $p < 0.01$; Fig. 4a). Predictions from the model fit

266 estimated a thermal optimum (T_{opt}) at 24.32 °C and frogs were able to maintain more than 95,
267 80 and 50 % of their performance over a range of respectively 11.58, 20.58, and 28.06 °C (Fig.
268 4a). Snout-vent length showed a significant highly non-linear effect on the distance travelled
269 before exhaustion ($edf = 4.67$; $F_{20.27, 147.77} = 3.55$, $p < 0.01$). There was no significant effect of
270 sex ($t_{21} = 1.32$, $p = 0.18$).

271 Based on the adjusted R -squared values (GAMM), temperature, SVL and sex together
272 explained 66.4% of variation in time spent moving by the frogs before exhaustion. Temperature
273 showed a significant highly non-linear effect on time spent moving before exhaustion ($edf =$
274 3.92 ; $F_{15.02, 152.98} = 78.10$, $p < 0.01$; Fig. 4b). Predictions from the model fit estimated a thermal
275 optimum at 11.70 °C and frogs were able to maintain more than 95, 80 and 50 % of their
276 performance over a range of 4.82, 10.09, and 24.52 °C, respectively (Fig. 4b). There were no
277 significant effects of SVL ($F_{15.02, 152.98} = 1.15$, $p = 0.28$) and sex ($t_{21} = -0.10$, $p = 0.91$).

278 Based on the adjusted R -squared values (GAMM), temperature, SVL and sex together
279 explained 88.1% of the variation in average speed of the frogs. Temperature showed a
280 significant highly non-linear effect on average speed during the endurance trial ($edf = 3.96$;
281 $F_{16.23, 151.76} = 272.75$, $p < 0.01$; Fig. 4c). Predictions from the model fit estimated a thermal
282 optimum at 27.20 °C and frogs were able to maintain more than 95, 80 and 50 % of their
283 performance over a range of 4.97, 10.58, and 21.88 °C, respectively (Fig. 4c). Snout-vent length
284 showed a significant ($F_{16.23, 151.76} = 1.83$, $p = 0.17$) positive linear effect ($edf = 1$). There was no
285 significant of sex ($t_{21} = 0.75$, $p = 0.45$).

286 **Jump force**

287 Based on the adjusted R -squared values (GAMM), temperature, SVL, and sex together
288 explained 89.2% of variation in jump force of the frogs. Temperature showed a significant
289 ($F_{21.73, 128.26} = 237.37$, $p < 0.01$; Fig. 5) highly non-linear relationship ($edf = 3.97$) with jump
290 force. Predictions from the model fit estimated a thermal optimum at 29.77 °C and frogs were
291 able to maintain more than 95, 80 and 50 % of their performance over a range of 4.53, 10.16,
292 and 26.35 °C, respectively (Fig. 5). Snout-vent-length showed a significant effect ($F_{21.73, 128.26}$
293 $= 5.94$, $p < 0.01$) that was highly non-linear ($edf = 2.67$). However, no significant effects were
294 detected for sex ($t_{25} = 1.08$, $p = 0.28$).

295 **Discussion**

296 Marsh frogs showed a thermal tolerance that covers the entire annual variation of bottom pond
297 temperatures and 76.5% of the annual variation in air temperature, with colder temperature
298 being the only limit (Fig. 6, Table 1). It is important to point out that air temperature is a
299 representation of the environmental temperature in shaded area and that solar radiation can
300 generate surface temperatures far exceeding observed air temperatures under the shade. These
301 results suggest that warmer temperatures may allow invasive marsh frogs to have an increased
302 margin of locomotor activity particularly in shaded conditions or during night time.
303 Nonetheless, even if their upper thermal limit is 5.2°C greater than the current maximal air
304 temperature recorded (Fig. 6), extreme thermal events may limit their fitness before reaching
305 this critical limit. It is also interesting to note that under laboratory conditions marsh frogs try
306 to maintain a body temperature (T_{pref}) that is higher than the average temperature they can
307 experience in shaded areas (Fig. 6, Table 1). The same applies for their thermal performance
308 breadths on distance jumped before exhaustion, average speed, and jump force (Fig. 6, Table
309 1). Our data suggest that warmer air temperature may allow them to better spread across shadier
310 habitats and also to colonize previously unfavourable ponds while continuing to inhabit more
311 exposed ponds (Denoël et al. 2022) due to their ability to bask or dive into the pond to
312 thermoregulate. Lastly, regarding variation between males and females, a slight difference was
313 observed in CT_{max} but not for other traits, suggesting a similar thermal biology for the two
314 sexes.

315 **Marsh frogs, an overlooked invasive species with a broad thermal tolerance**

316 Despite the extent of their invasion over a large part of Western Europe, the invasive potential
317 of the marsh frog is underestimated (Pille et al. 2021). Here we show that their thermal tolerance
318 extends to over 35 °C with an upper thermal limit beyond the maximum water and air
319 temperatures (in shadow) in their natural environment. Similar tolerance ranges were found in
320 the three most invasive amphibians: *Rhinella marina* (Johnson 1972), *Xenopus laevis* (Araspin
321 et al. 2020) and *Lithobates catesbeianus* (Lotshaw 1977) (Suppl. Table S1). The first two
322 species showed thermal tolerance range that are respectively 9% and 37% narrower than the
323 marsh frogs (Suppl. Table S1). The thermal tolerance of *Lithobates catesbeianus* may, however,
324 appear wider than that of marsh frogs (38.55 °C, Suppl. Table S1), yet was measured using the
325 lower lethal temperature instead of the loss of righting response. Spellerberg (1972) evaluated
326 both CT_{min} using the loss of righting response and the lethal minimal temperature on ten species

327 of reptiles and the mean gap (\pm SD) between each measurement was 8.4 ± 2.5 °C. Overall, a
328 broad thermal tolerance may allow invasive ectotherms to maintain a long period of activity
329 year-round, in addition to being able to face daily variations in temperature as well as
330 temperature extremes. We can expect from predicted warming that the environmental
331 temperatures may remain within the thermal tolerance range of invasive ectotherms with a high
332 upper thermal tolerance which may lead to phenological changes (Sheridan et al. 2018).
333 However, the lack of knowledge on the thermal tolerance of native amphibians is a limitation
334 preventing a mechanistic understanding of their respective success. Some data on palmate
335 newts from the studied area highlight that they will have a lower reproductive fitness in warmer
336 temperatures (Galloy and Denoël 2010) and that droughts could impact population sizes in
337 natural populations (Denoël 2006). Moreover, even in the scenario of thermal adaptation of
338 some native species, the increased invasion of habitats due to the high thermal tolerance of
339 marsh frogs would likely lead to multiple situations of habitat overlap with the native species
340 and a consequent increase in predation risk (Pille et al. 2021).

341 **Is warmer better?**

342 A recent study (Denoël et al. 2022) highlighted the invasiveness potential of this species and,
343 more specifically, revealed that sun exposure was one of the pond features associated with their
344 occurrence. Marsh frogs prefer habitats with a high sun exposition to bask under the current
345 climatic conditions. Our data show that marsh frogs try to maintain a body temperature higher
346 than the average air temperature found in a shaded environment. Niche shifts have already been
347 observed at a larger scale in invasive amphibians (Rödder et al 2017; Tingley et al 2014) and
348 our results suggest that warmer climates may facilitate the invasibility of habitats cooler than
349 open landscapes, such as forests, and therefore increasing their overlap with native amphibians
350 in these environments.

351 Each performance trait showed, similarly, a thermal optimum at a temperature that was
352 higher than the mean air temperature in addition to showing broad thermal performance
353 breadths. Warmer climatic conditions may shift the environmental temperature to conditions
354 that will maximize most performance traits of this frog species and therefore its invasion
355 potential. Furthermore, broad performance breadths indicate that this species is able to maintain
356 performance despite daily variations in temperature. This is particularly the case for dispersal,
357 as suggested by the distance moved before exhaustion, where 80% of its maximum capacity

358 covers 58% of the frog's absolute thermal tolerance (Fig. 6). Araspin and co-authors (2020)
359 similarly measured the thermal performance breadth at 80% for exertion (mean \pm SE = 8.25 \pm
360 0.4 °C) in African clawed frogs, *Xenopus laevis*, a worldwide invasive species that was
361 introduced in Western France and that shares the habitat with introduced populations of marsh
362 frogs. Yet this range is 60% narrower, unveiling again the wide performance breadth and
363 invasive potential of marsh frogs.

364 **Thermal performance differs across traits**

365 Given that an increase in temperature accelerates chemical reaction rates (Tattersall et al. 2012)
366 which in turn drive the thermal dependence of muscle contraction (Bennett 1984), it is not
367 surprising that all performance traits were significantly affected by temperature. Average speed
368 during the endurance trial and jump force increase with temperature to reach their optimum at
369 high temperatures and finally drop off near the critical maximal limit of the animal, typical for
370 burst performance traits (Batty and Blaxter 1992; Pinch and Claussen 2003; Araspin et al.
371 2020). Both traits follow the response of chemical reaction rates to an increase in temperature
372 predicting that locomotor capacity is optimized at high temperatures that are not often reached
373 in a shaded environment (Fig. 6). These two traits are essential for predator escape or prey
374 capture, both relevant to the survival of invasive species.

375 Time and distance before exhaustion show, however, different thermal responses
376 compared to one another, and also different from what was observed for burst performance.
377 Because endurance capacity is dependent on oxygen uptake and the metabolic rate of the
378 organism, we expected their thermal performance curves to follow the response observed for
379 enzymatic reactions. James and co-authors (James et al. 2012) also showed that higher
380 temperatures maximize the performance at the muscle level for both burst performance and
381 endurance. Interestingly time and distance before exhaustion were not maximized at high
382 temperatures. This difference across traits needs to be quantified and used in future predictions.
383 Mechanistic models, for example, often use data on a single trait which does not reflect the
384 overall thermal performance of an organism (van Damme et al. 1991; Kellermann et al. 2019).
385 The time spent in movement is maximized at lower temperatures and declines towards the
386 critical maximal limit. This trait being maximal at lower temperature may not reflect the
387 dispersal of the frog, because longer time spent in movement does not mean more distance
388 covered per se. This result seems mostly explained by slower responses and shorter jumps, but

389 this requires further study. Lastly, the distance covered before exhaustion increases from low
390 temperatures to quickly show a broad plateau. This low sensitivity to temperature is reflected
391 through the shape of its thermal performance curve (Fig. 4a, 6), which is typically not observed
392 in other studies on the thermal sensitivity of locomotion (Bennett 1990; Angilletta 2001;
393 Araspin et al. 2020). This thermal response suggests that the distance covered before
394 exhaustion, at least in invasive marsh frogs, is likely determined by a number of physiological
395 factors that each cover different parts of the overall thermal range. In contrast to jump force
396 which is mostly determined by muscle contractile properties, stamina is indeed also driven by
397 the functioning of the cardiovascular and the respiratory systems.

398 **Conclusions**

399 Integrating multiple-trait measures with environmental variables enhances our understanding
400 of the thermal dependence of invasive ectotherms (Ryan and Gunderson 2021; Young et al
401 2022). Broad thermal tolerance and thermal preferences with optima of performance at
402 temperatures higher than the air temperature of their environment were observed for the
403 invasive marsh frogs studied here. As previously suggested (Hellmann et al. 2008), all steps of
404 the invasion process can benefit from an increase of temperature if it leads to more frequent
405 temperatures within their thermal performance breadth and closer to their thermal preference.
406 Our results support this hypothesis with warmer temperatures favouring jumping capacity as
407 well as the distance moved, and the average speed. This can allow animals to cover more
408 distance which can facilitate their spread, including across shaded habitats. Dufresnes and co-
409 authors (2017a) questioned whether the rapid warming of the last two decades may have helped
410 invasive *Pelophylax* to successfully spread and establish in the region. Given that the annual
411 mean air temperature in the region has been increasing by more than 0.05 °C each year since
412 1980 (field meteorological data), we suggest that climate change has indeed favoured the spread
413 of this invasive species, and will continue to do so given the current predictions of a mean
414 warming in the range of 0.9 to 5.6 °C by the end of the 20th century (Ali et al. 2022).

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429 **Availability of data and materials** The datasets generated during and/or analysed during the
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431 **Code availability** The code used for statistical analysis was written in R and is available from
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433 **Declarations**

434 **Conflict of interest** The authors declare that they have no conflict of interest.

435 **Ethical approval** All applicable institutional and/or national guidelines for the care and use of
436 animals were followed.

437 **Consent to participate** Not applicable.

438 **Consent for publication** All authors gave consent.

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Fig. 1 Theoretical representation of a shift of annual temperature distribution (histogram) that better fits a performance curve of an invasive ectotherm. The shift has been increased for readability. The curve, in purple, is bounded by the critical thermal minimum (CT_{min} , blue triangle), and the critical thermal maximum (CT_{max} , red triangle). The range of temperature between these bounds defines the thermal tolerance where the organism can maintain activity (horizontal grey dashed lines). The temperature associated with the maximum value of performance defines the thermal optimum (T_{opt} , red). Finally, the range of temperatures where performance is greater or equal to a desired level (commonly 80% or 95%) defines a performance breadth (T_{pb} , horizontal black dashed line; Angilletta et al., 2002).

Fig. 2: Graphical summary of each set of measurements made during the study on marsh frogs. (a) Critical thermal limits assessed with a thermocouple inserted inside the cloaca; (b) Preferred temperature estimated after one hour within a thermal gradient; (c) Stamina was estimated through the distance and the time travelled until exhaustion; (d) Jump force was measured using a force plate.

Fig. 3: Thermal tolerance in males and females of the invasive marsh frog. Critical thermal minimum (CT_{min}) is shown on the upper panel and the critical thermal maximum (CT_{max}) on the lower panel. A white diamond indicates the mean value and the middle line within the boxplot the median. Females are shown in purple and males in green. To allow a better visualisation of individuals the Y axis scale was rescaled for CT_{max} .

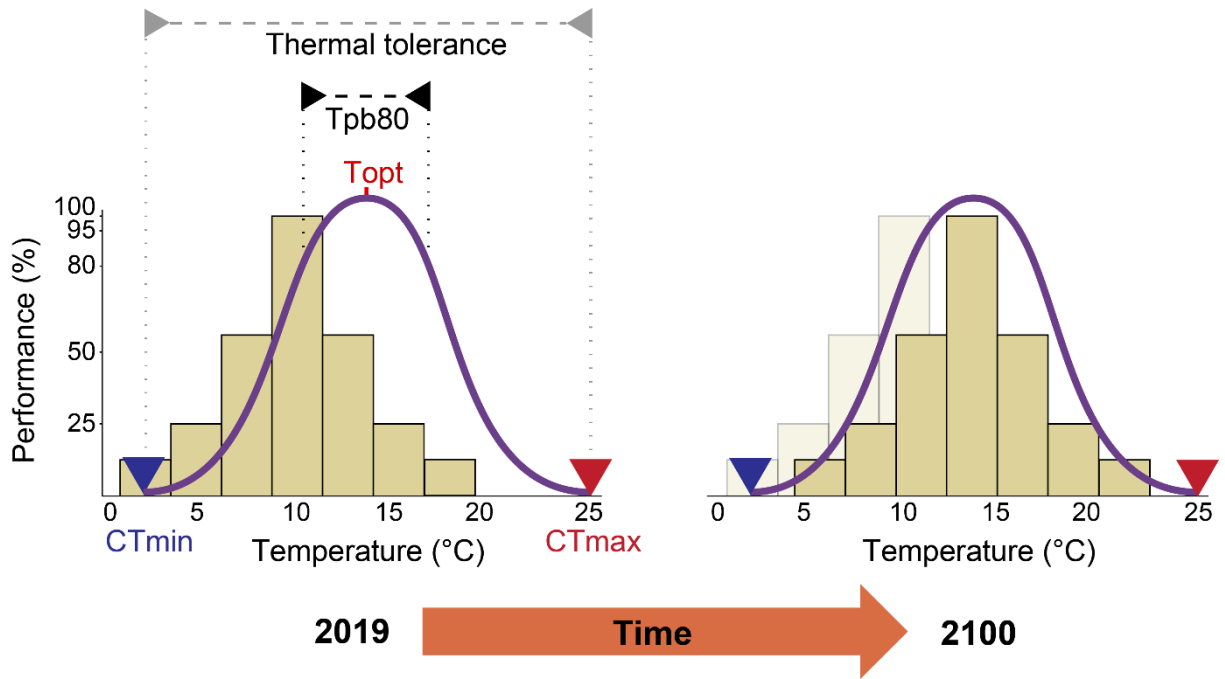
Fig. 4: Thermal performance curves predicted from GAMM models for (a) the distance travelled before exhaustion, (b) the time spent moving before exhaustion, and (c) the average “speed” (i.e. distance divided by time before exhaustion) in invasive marsh frogs. Thermal optimum (T_{opt}) and thermal performance breadths (T_{pb}) at 50, 80 and 95% of the maximal performance are displayed. The uncertainty of the estimate is displayed through a 95% grey confidence band. Data is shown as dots for each individual. Temperature is shown by the color gradient.

Fig. 5: Thermal performance curve predicted from GAMM models on jump force in invasive marsh frogs. Thermal optimum (T_{opt}) and thermal performance breadths (T_{pb}) at 50, 80 and 95% of the maximal performance are displayed. Uncertainty of the estimate is displayed through a 95% grey confidence band. Data is shown as dots for each individual. Temperature is shown

by the color gradient.

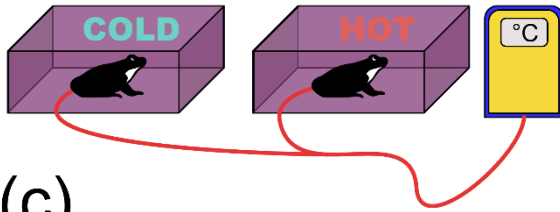
Fig. 6: Graphical summary of the thermal response of the invasive marsh frog (*Pelophylax ridibundus*), to the air temperature during their activity (grey) or during the annual variation (beige). The dotted horizontal red line indicates the 80% thermal performance breadth. The dashed vertical red line indicates the preferred temperature (T_{pref}).

1



2

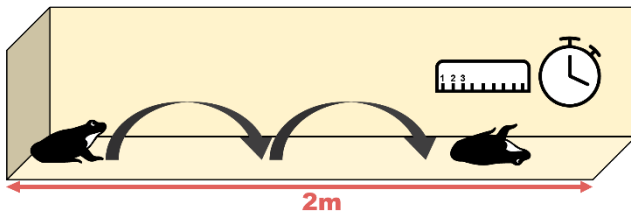
(a) $2^{\circ}\text{C} \rightarrow 1^{\circ}\text{C} \rightarrow 0.5^{\circ}\text{C} / 30\text{min}$



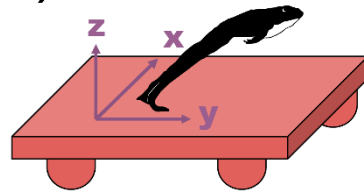
(b)



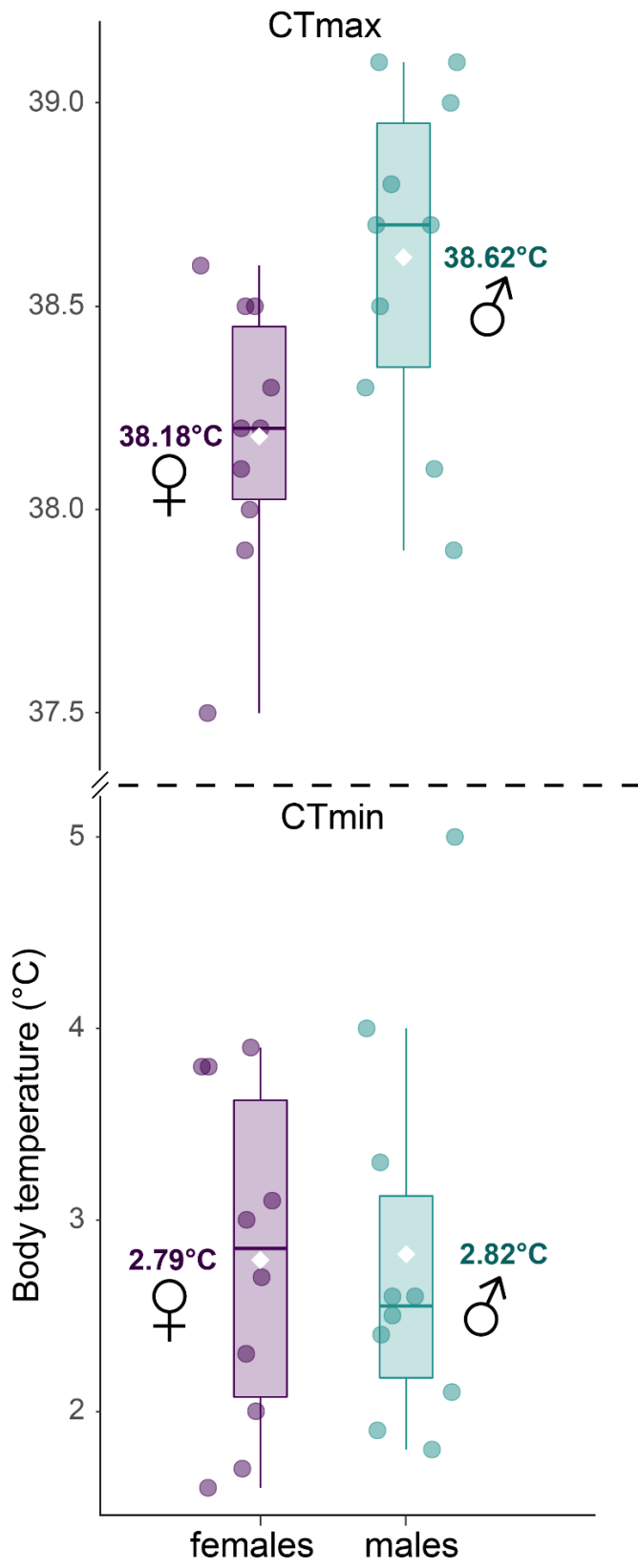
(c)



(d)

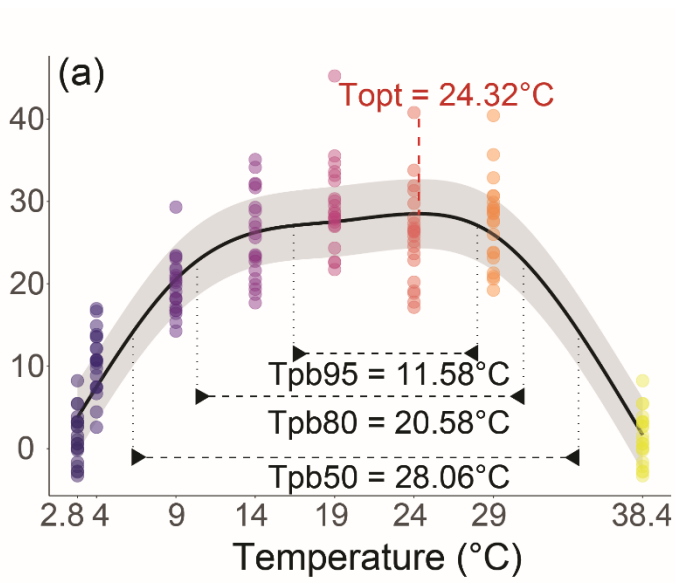


3

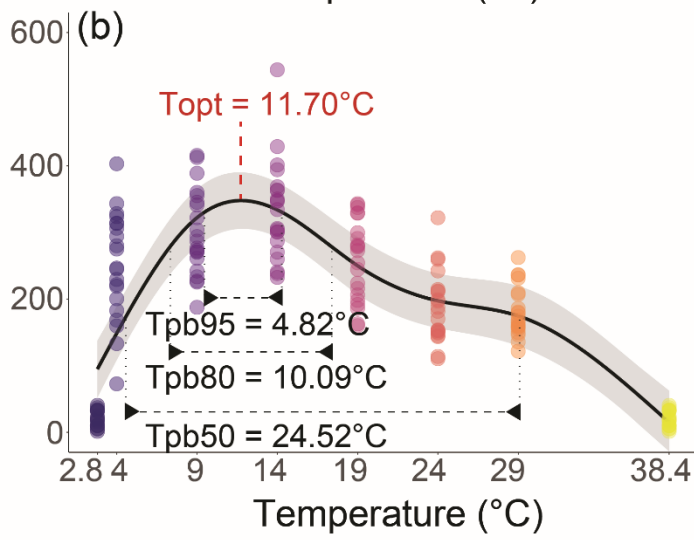


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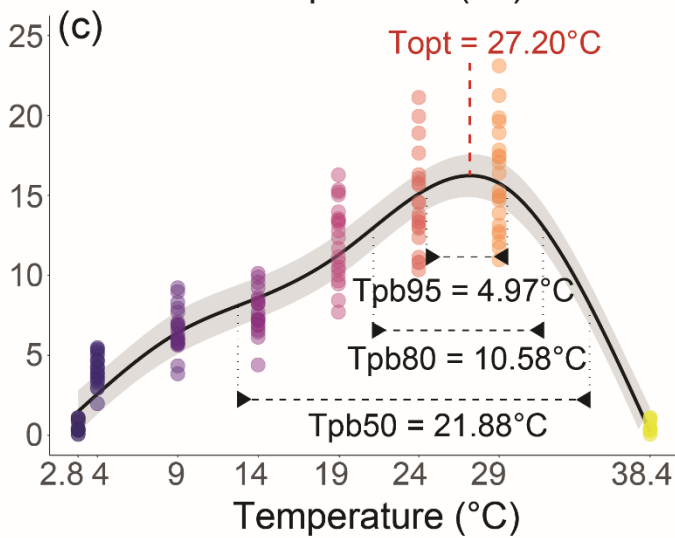
Distance before exhaustion (m)



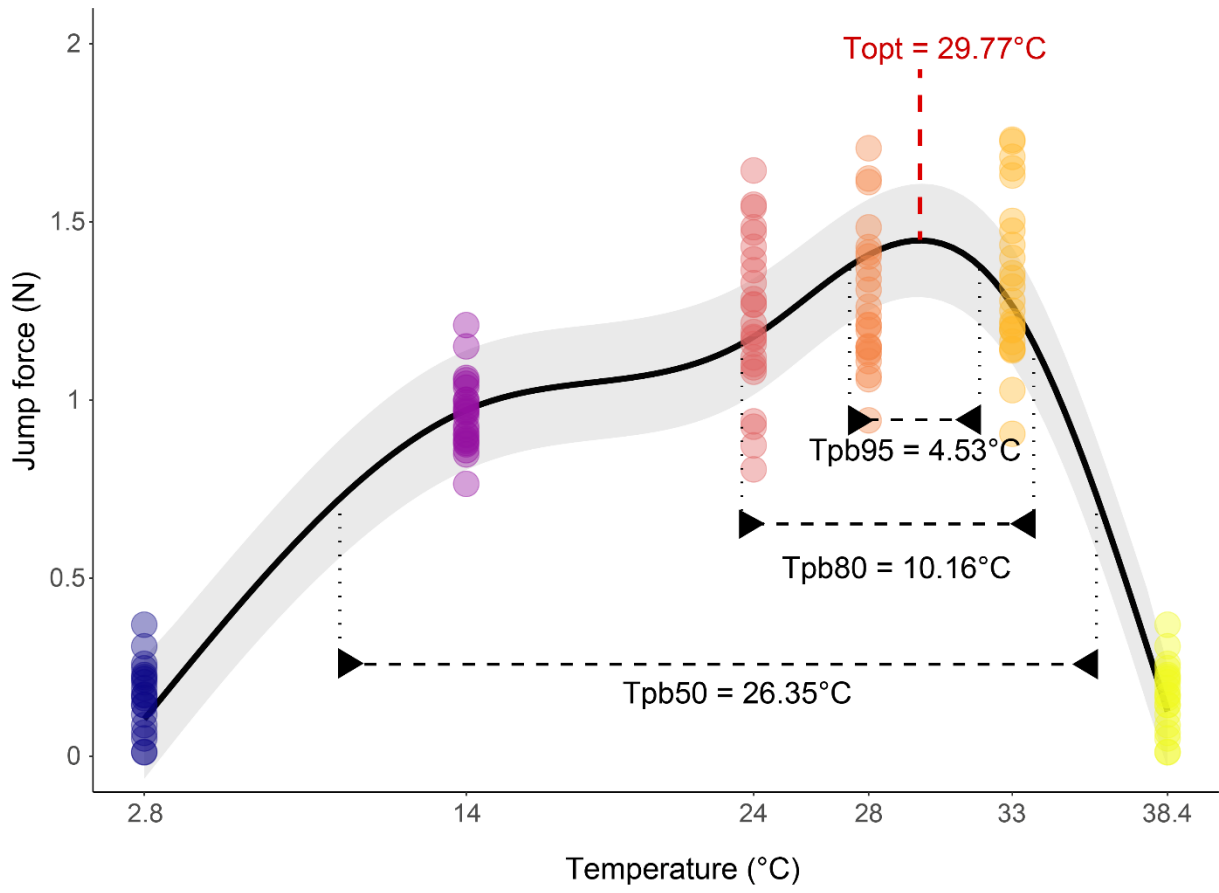
Time before exhaustion (s)



Average speed (cm/s)



5



6

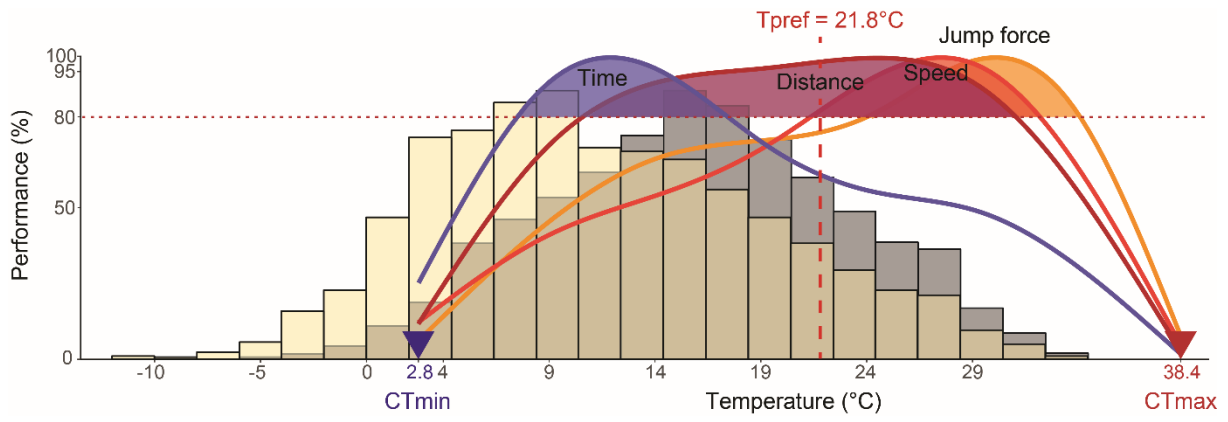


Table 1: Environmental temperature annually and relative to marsh frog period of activity in Larzac, France.

Temperature (°C)		mean ± SE	min	max
Annual	Air	11.17 ± 0.02 °C	-13.5 °C	33.2 °C
	Bottom of the pond	10.73 ± 0.03 °C	5.34 °C	21.37 °C
During period of activity (early spring to late summer)	Air	15.67 ± 0.03 °C	-5.6 °C	33.2 °C
	Bottom of the pond	13.47 ± 0.04 °C	6.97 °C	21.37 °C