

1 **Assessing the capacity of endemic alpine water beetles to face climate**
2 **change**

3 Running title: Alpine water beetles and climate change

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22 **Abstract**

23 1. Accurate assessments of species' vulnerability to climate change require integrated
24 measurements of its different drivers, including extrinsic (the magnitude and rate of
25 climate change) and intrinsic factors (organisms' sensitivity and adaptive capacity).
26 According to these factors, aquatic insects restricted to alpine ponds may be especially
27 threatened by climate change. However, vulnerability predictions based on such an
28 integrative approach are scarce for alpine pond taxa.

29 2. We combined distributional, climatic data and experimental measurements of heat
30 tolerance and acclimation capacity of two water beetles endemic to Sierra Nevada
31 National Park (Spain) to evaluate different components of their vulnerability to climate
32 change. We estimated: i) changes in climatically suitable habitat under different

33 scenarios of climate change and ii) thermal safety margins (the difference between
34 species upper thermal limits and the maximum temperatures in their current localities),
35 for current and future conditions, and acclimation capacity, as measures of the
36 physiological capacity to persist *in situ*.

37 3. Species distribution models predicted a virtual loss of climatically suitable area under
38 different climate change scenarios. However, both taxa showed heat tolerance limits
39 above the predicted maximum temperatures in their current localities (but no capacity to
40 adjust such limits through acclimation). Therefore, these beetles could have the
41 physiological capacity to deal with warming conditions *in situ*.

42 4. We recommend concentrating conservation efforts in current localities as the most
43 efficient management strategy for both taxa. Our results stress the importance of
44 accounting for physiological tolerances when predicting the vulnerability to climate
45 change in alpine freshwater biota.

46 **Keywords:** Dytiscidae, Coleoptera, *Hydroporus sabaudus sierranevadensis*, *Agabus*
47 *nevadensis*, heat tolerance, acclimation capacity, protected areas, species distribution
48 models, physiology, conservation.

49 **Introduction**

50 Climate-induced extinction is among the major causes of biodiversity loss and it is
51 predicted to accelerate in the future as global temperature rises (Sala *et al.*, 2000; Urban,
52 2015). Designing effective species' conservation strategies is therefore imperative and
53 requires robust assessments of their vulnerability to climate change, considering both
54 extrinsic (exposure) and intrinsic factors (organisms' sensitivity and adaptive capacity).
55 (Williams *et al.*, 2008; Foden *et al.*, 2019). For a given species or population, exposure
56 refers to the magnitude and rate of climate change to which it is exposed in its

57 distribution range. Sensitivity is a function of intrinsic factors such as physiological
58 tolerance, ecological, behavioural and life-history traits and population dynamics. The
59 adaptive capacity is the ability to adjust to climate change *via* evolutionary adaption
60 (associated with genetic diversity and the potential for rapid genetic change),
61 phenotypic and ecological plasticity, as well as the colonization potential for migrating
62 to suitable habitats.

63 Considering such vulnerability parameters, the effects of climate change are expected to
64 be particularly severe for species restricted to the highest altitudinal zones within
65 mountain ranges. There, *exposure* could be magnified because the rate of warming is
66 amplified with elevation, such that high-mountain environments experience more rapid
67 changes in temperature than those at lower elevations (Wang *et al.*, 2014; Pepin *et al.*,
68 2015). Moreover, the insular character of mountain tops, the impossibility for upwards
69 migration and the existence of dispersal barriers hinder organisms' migration to other
70 suitable habitats (Hotaling *et al.*, 2017). High mountain ranges are important post-
71 glacial refugia for cold-adapted species which often lack the capacity to adapt quickly
72 enough to climatic changes (Parmesan, 2006). All these factors together may lead to an
73 important loss of biodiversity (Krajick, 2004; Thuiller *et al.*, 2005; Dirnböck *et al.*,
74 2011) and associated ecosystem services (Palomo, 2017) in these systems.

75 The communities in alpine ponds might be an especially sensitive component of high
76 mountain ecosystems, as the degree of isolation is even higher for the fragmented,
77 patchy aquatic habitats located at the highest elevations. In fact, several trait-based
78 assessments of aquatic insects' vulnerability have pointed out that high-altitudinal
79 species are among the most vulnerable (e.g. Tierno de Figueroa *et al.*, 2010; Rosset &
80 Oertli, 2011; Conti *et al.*, 2014; Hershkovitz *et al.*, 2015). As a fundamental and
81 potentially endangered part of the aquatic biodiversity of high-mountain systems,

82 aquatic invertebrates should be a key element for guiding appropriate conservation
83 strategies in these areas. Aquatic invertebrates have indeed been proposed as “early-
84 response” indicators of global environmental changes (Muhlfeld *et al.*, 2011) and have
85 shown a high indicator value in mountainous systems (e.g. Hodkinson & Jackson, 2005;
86 Guareschi *et al.*, 2012). However, to date, predictions of the effects of climate change in
87 alpine systems are scarce for standing water macroinvertebrates (but see Rosset &
88 Oertli, 2011) in comparison with those from running waters (e.g. Domisch *et al.*, 2011;
89 Muhlfeld *et al.*, 2011; Sauer *et al.*, 2011; Treanor *et al.* 2013; Giersch *et al.*, 2014;
90 Lencioni & Bernabó, 2017) and other groups such as plants (e.g. Guisan & Theurillat,
91 2000; Thuiller *et al.*, 2005; Engler *et al.*, 2009) or vertebrates (e.g. Rehnus *et al.*, 2018;
92 Hof & Allen, 2019). Furthermore, in general such assessments have not accounted for
93 the different components of vulnerability to climate change.

94 Most evaluations of species vulnerability rely on species distribution models (SDM),
95 which allow predicting suitable habitat under future climatic conditions. Under such
96 approach, proxies of exposure (predicted change in climatic conditions) and species
97 sensitivity (usually inferred only from the climatic conditions occupied by the species
98 within their current localities, i.e., the realized niche) are combined. However, these
99 models often underestimate the actual species’ ability to live under climatic conditions
100 outside their current limits (Sánchez-Fernández *et al.*, 2012, 2016). Measurements of
101 the fundamental niche breadth and physiological plasticity could complement SDM
102 providing a more complete estimation of species vulnerability (Williams *et al.*, 2008;
103 Bellard *et al.*, 2012). In this context, Arribas *et al.* (2012) provided a framework for
104 insect vulnerability assessments integrating data on thermal tolerance, acclimation
105 ability, changes in climatically suitable habitat and dispersal capacity to consider both
106 species capacity to maintain present populations and to shift their geographical range

107 (see also Arribas *et al.*, 2017). In aquatic insects, like in other poikilotherm organisms,
108 temperature affects multiple aspects of individual performance and fitness, as
109 embryonic development, growth, metabolism, feeding, locomotion, phenology,
110 behavior, reproductive rate and survivorship (Chown & Nicolson, 2004; Fenoglio *et al.*,
111 2005; Dallas & Ross-Gillespie, 2015). All organisms have a preferred range of
112 temperatures, which is bounded by the thermal tolerance range (i.e. the range over
113 which a species may survive) with upper and lower tolerance limits demarcating the
114 thermal extremes (Dallas & Ross-Gillespie, 2015). Therefore, knowledge on insects'
115 physiological thermal limits and their plasticity to adjust such limits under changing
116 conditions is essential for evaluating potential climate change effects. Such information
117 is particularly relevant for endemic, small range species with low dispersal capacity
118 living in isolated systems like alpine ponds, as they may be doubly threatened by
119 climate change due to limited physiological capacity and habitat loss (Calosi *et al.*,
120 2008a).

121 This study focusses on two alpine water beetles, *Agabus nevadensis* Lindberg, 1939 and
122 *Hydroporus sabaudus sierranevadensis* Shaverdo, 2004 (both belonging to the family
123 Dytiscidae), as models to evaluate the vulnerability to climate change in aquatic insect
124 populations of alpine ponds by using an integrative methodology that combines
125 distributional, climatic data and thermal tolerance experiments. Both beetles are small
126 range taxa endemic to the Sierra Nevada mountain range (Millán *et al.*, 2013), a National
127 Park in Southeast Spain. We used SDM to estimate changes in climatically suitable area
128 in Sierra Nevada under future climate change scenarios, assuming that dispersal outside
129 this mountain system is very unlikely for these microendemic, isolated taxa. We also
130 estimated thermal safety margins (TSM; Deutsch *et al.*, 2008; Huey *et al.*, 2009), as a
131 measure of the degree to which habitat temperature gets close to physiological limits.

132 TSM, together with acclimation capacity (i.e., the plasticity of thermal limits in response
133 to acclimation temperature), provide useful information to evaluate species persistence *in*
134 *situ* in the face of climate change. Finally, we integrated such information on the different
135 components of vulnerability to outline specific conservation strategies for endemic alpine
136 insects according to the framework proposed by Arribas *et al.* (2012, 2017).

137 **Material and methods**

138 *Study area and taxa*

139 The Sierra Nevada mountain range in Spain (36°55'–37°15'N, 2°31'–3°40'W;
140 maximum altitude 3,482 m.a.s.l.) is the southernmost high mountain in Europe and
141 represents one of the areas with the highest values of biodiversity and endemism of the
142 Iberian Peninsula (Molero-Mesa *et al.*, 1992; Millán *et al.*, 2012; Ruano *et al.*, 2013;
143 Mendoza-Fernández *et al.*, 2015). As a high-mountain system located in a
144 Mediterranean climate region, the effects of global warming could be particularly severe
145 in this area (Nogués-Bravo *et al.*, 2008). Sierra Nevada hosts a system of ponds of
146 glacial origin between approximately 2800 and 3050 m.a.s.l. These remote ponds harbor
147 relatively simplified biological communities because of their environmental
148 homogeneity (Morales-Baquerol *et al.*, 1992), but contain highly specific, cold-adapted
149 species assemblages.

150 The aquatic macroinvertebrate community is dominated by water beetles, many of which
151 are Iberian endemisms or microendemisms only present in this area (Millán *et al.*, 2013,
152 2014). This is the case of *A. nevadensis* and *H. s. sierranevadensis*, which are restricted
153 to this system of alpine ponds in Sierra Nevada. Both taxa have been considered as
154 highly endangered according to the criteria for prioritizing species and populations for
155 conservation developed by Abellán *et al.* (2005) and Sánchez-Fernández *et al.* (2008).

156 Larvae and adults of both taxa are strictly aquatic and carnivorous or necrophagous,
157 typically preying on larvae of other macroinvertebrates and preferably found close to
158 vegetated shores. *Agabus nevadensis* is a medium sized species (8.0-8.5 mm). Although
159 its status has been questioned in relation to the very common and widespread *A.*
160 *bipustulatus* (L.) (Ribera *et al.*, 1998), recent allozyme studies of the complex support
161 the hypothesis of reproductive isolation between the species (Drotz *et al.*, 2010), even
162 though *A. nevadensis* is deeply nested within *A. bipustulatus* based on mitochondrial
163 data (Bergsten *et al.*, 2012). *Hydroporus s. sierranevadensis* is a small sized subspecies
164 (3.5-4.0 mm) of the nigrita-group of *Hydroporus* (Shaverdo, 2004).

165 *Distribution and bioclimatic data*

166 We compiled all available distributional data of the two study taxa from the literature,
167 unpublished own data and sampling surveys conducted in Sierra Nevada in September
168 and October 2018 (Fig 1, see also Table S1 in Appendix S1). Literature records with
169 possible problems of reliability or with low spatial resolution were discarded. Nineteen
170 bioclimatic variables were obtained from WORLDCLIM version 1.4
171 (<http://www.worldclim.org>; Hijmans *et al.*, 2005) for present and future scenarios, at 30
172 arc-seconds resolution. We used two future time periods (2050 and 2070), for optimistic
173 and pessimistic Representative Concentration Pathways (RCP), 4.5 and 8.5,
174 respectively, and all the available general circulation models (GCM) available in
175 WORLDCLIM for these RCP and periods (i.e., seventeen).

176 *Heat tolerance and acclimation capacity experiments*

177 Alive adult specimens of *H. s. sierranevadensis* as well as adults and larvae of *A.*
178 *nevadensis* were collected in several ponds in Sierra Nevada (Fig 1, Table S1). We did
179 not included larvae of *H. s. sierranevadensis* in the experiments because of the difficulty

180 of unambiguously identify them, as adults of other *Hydroporus* species were also found
181 in the same ponds. Water temperature and conductivity of each site were monitored
182 with an electrical conductivity meter (Hanna HI98192, Hanna instruments, USA) (data
183 shown in Table S1). As a pre-acclimation phase, specimens were held at a constant
184 temperature of 10°C (ca. the average of the water temperatures measured during
185 collection) and 12:12 light:dark cycle in a climatic chamber (SANYO MLR-351) for 3-
186 4 days prior to experiments. They were fed daily with frozen chironomid larvae.

187 Groups of 7-11 specimens were subjected to two thermal acclimation treatments for one
188 week: 10°C (non-stressful temperature) and 20°C (potentially stressful conditions close
189 to the maximum summer temperatures of the study area). After this acclimation phase,
190 two different physiological endpoints were measured using a dynamic, ramping method
191 (Terblanche *et al.*, 2011) under two different approaches:

192 i) *Upper lethal temperatures* (ULT) were determined underwater at a heating rate of
193 3°C day⁻¹. Survival was monitored every 12 h so that lethal temperatures were recorded
194 with a maximum error of ±1.5°C.

195 ii) *Heat coma temperature* (HCT), the body temperature at the moment of paralysis
196 prior to death, which is preceded by spasmodic movements of legs and antennae, was
197 measured in air by infrared thermography. Heat coma is a typical response used as a
198 critical thermal maximum (CT_{max}) (Vannier, 1994; Lutterschmidt & Hutchison, 1997;
199 Chown & Nicolson, 2004). A heating rate of 1°C min⁻¹ was used, which is a standard
200 rate applied in most thermal tolerance experiments (e.g. Lutterschmidt & Hutchison,
201 1997; Calosi *et al.*, 2010).

202 In both experiments, the heating program started at the temperature at which each group
203 had been acclimated. Controls with a minimum of 5 individuals were kept in aquaria at

204 10°C during the entire duration of the experiments. All adult specimens were conserved
205 in pure ethanol after the experiments and sexed.

206 Additionally, we assessed heat tolerance in larvae (LIII stage) of *A. nevadensis*. In this
207 case, we only measured ULT after acclimation at non-stressful conditions (10°C),
208 because we collected a low number of larvae, and the acclimation duration was
209 shortened to 3 days because larvae of other aquatic beetles have shown limited
210 resistance under laboratory conditions in the long-term (personal observations). For
211 comparison with adults, ULTs were measured on another group of 10 adults acclimated
212 at 10°C for the same time (3 days) than larvae. Additional details on experimental
213 procedures are provided in Appendix S2.1.

214 *Evaluation of species vulnerability under climatic change*

215 *Changes in climatically suitable habitat.* Species Distribution Models (SDM) were run
216 to infer current and future potential distribution of each taxa within our study area. We
217 used the Maxent algorithm (Phillips & Dudík, 2008) as implemented in the *dismo* R
218 package (Hijmans *et al.*, 2011). Maxent is considered to be among the most accurate of
219 SDM algorithms, especially when working with low sample sizes (Wisz *et al.*, 2008).
220 To avoid redundancy and collinearity among bioclimatic variables, from pairs of highly
221 correlated variables (Pearson $r > 0.8$), those with higher variability were retained. This
222 yielded a total of 3 bioclimatic variables: Annual Mean Temperature (Bio1), Mean
223 Diurnal Range (Bio2) and Isothermality (Bio3). To map the projected future habitat
224 suitability, the outputs from the seventeen general circulation models were averaged for
225 each RCP and time period.

226 Model performance was evaluated using the area under the curve of the receiver
227 operator characteristic (AUC). Although SDM accuracy can decline when based on very

228 few records (Wisz *et al.*, 2008), Maxent has been shown to perform well even with
229 sample sizes as low as 5, depending on species' prevalence and species' environmental
230 requirements (Hernandez *et al.*, 2006; Proosdij *et al.*, 2016). Thus, species with small
231 geographic ranges and limited environmental tolerance, as those studied here, can be
232 accurately modelled despite a low number of occurrences because of their reliance on
233 spatially restricted environmental conditions (Hernandez *et al.* 2006; Proosdij *et al.*,
234 2016). Hence, to further assess model performance, significance of AUC values was
235 assessed by comparing our AUC values against those generated from a null model
236 following the approach proposed by Raes & Ter Steege (2007). Details of procedures
237 for model selection and evaluation are shown in Appendix S2.2.

238 In order to obtain binary predictions of suitable habitat for each scenario, we converted
239 the averaged, continuous Maxent outputs into presence/absence predictions using
240 maximum training sensitivity plus specificity thresholding, which has been found to
241 perform well compared with other thresholding methods (Liu *et al.*, 2005).

242 To avoid basing our results solely on a single distribution modelling algorithm, we
243 performed supplementary analyses using the Mahalanobis distance approach (MD;
244 Farber & Kadmon, 2003), which performs well against a variety of other presence-only
245 modelling methods (Tsoar *et al.*, 2007). MD ranks potential sites by their Mahalanobis
246 distance to a vector expressing the mean environmental conditions (the centroid) of all
247 the species' records in the environmental space, and it requires neither absence data nor
248 a definition of study extent. MD analyses were performed on the same variables used
249 with the Maxent analyses using also the *dismo* package. The Mahalanobis distances
250 were transformed into probabilities using a chi-squared distribution (Farber & Kadmon,
251 2003).

252 *Physiological capacity to persist in situ.* We estimated Thermal Safety Margins (TSM)
253 from adult ULT values and the maximum temperature of the warmest month (i.e.,
254 BIO5) in each locality, for current and future scenarios (the seventeen GCM were
255 averaged for each RCP and time period). We used ULT values instead of HCT because
256 the former approach provides a more realistic measurement of heat tolerance limits (i.e.
257 these were measured at a slower heating rate and the potential additional stress caused
258 by experimental procedures was minimum, see Appendix S2.1). Additionally, larvae
259 TSM were estimated for *A. nevadensis*. We also determined the acclimation capacity of
260 each taxon and tested whether the two different experimental approaches (ULT and
261 HCT) provide consistent estimations of such capacity. For this, we evaluated the effect
262 of acclimation temperature on the two endpoints measured. As HCT data showed a
263 normal distribution, we used ANOVA with acclimation temperature and sex as
264 predictors. For ULT, we used non-parametric tests (Kruskal-Wallis) as the data were
265 non-normally distributed. In this case, sex was not included as a predictor because lethal
266 temperatures showed very little inter-individual variation within acclimation treatments.
267 The Kruskal-Wallis test was also used to compare the ULT of larvae and adults of *A.*
268 *nevadensis*.

269 **Results**

270 *Species distribution models*

271 In Maxent models, AUC were high for both taxa (0.993 ± 0.001 for *A. nevadensis* and
272 0.990 ± 0.001 for *H. s. sierranevadensis*). All models were found to significantly
273 outperform the null expectation at $P \ll 0.0001$ (see Fig S1 in Appendix S1). The annual
274 mean temperature was the variable with the highest contribution in both species
275 (72.84 ± 0.85 and $69.49 \pm 1.39\%$ for *A. nevadensis* and *H. s. sierranevadensis*,
276 respectively) followed by isothermality (27.13 ± 0.80 in *A. nevadensis* and $30.50 \pm 1.39\%$

277 in *H. s. sierranevadensis*). Current areas of highest suitability for both species were
278 concentrated at the highest elevations within Sierra Nevada (Figs 2 and 3). The
279 percentage of cells with suitable habitat within the study area for *A. nevadensis* was
280 predicted to decrease from 1.2% (current conditions) to 0.04% in 2050 (RCP 4.5) and to
281 virtually disappear under the other future scenarios tested (Fig 2). Similarly, suitable
282 habitat was predicted to drastically decrease for *H. s. sierranevadensis*, from 1.9%
283 (current conditions) to less than 0.1% in most of the future scenarios and to disappear in
284 2070 (RCP 8.5 scenario) (Fig 3). Results from MD models predicted even a more
285 dramatic loss of suitable habitat for both taxa under the different scenarios and periods
286 (see Figs S2 and S3 in Appendix S1).

287 *Heat tolerance and acclimation capacity*

288 *Hydroporus s. sierranevadensis* adults showed both higher HCT values ($44.82 \pm 0.14^\circ\text{C}$)
289 and ULT ($36.47 \pm 0.37^\circ\text{C}$) than *A. nevadensis* ones (HCT: $42.94 \pm 0.17^\circ\text{C}$, ULT:
290 $34.53 \pm 0.48^\circ\text{C}$). We only found a significant and negative effect of acclimation
291 temperature on the HCT of *A. nevadensis* adults, which were lower in specimens
292 acclimated at 20°C than those at 10°C . In this species, HCT were also significantly
293 lower in males than females in both treatments (Table 1, Fig 4a). ULTs of *A. nevadensis*
294 did not differ between acclimation treatments but tended to be lower in specimens
295 acclimated at 20°C (Fig 4b). These were also significantly lower in larvae
296 ($33.05 \pm 0.64^\circ\text{C}$, $n=10$) than adults ($35.12 \pm 0.25^\circ\text{C}$, $n=9$) acclimated at 10°C for 3 days
297 ($\chi^2 = 5.554$, $P=0.018$). In *H. s. sierranevadensis* neither HCT nor ULTs differed among
298 acclimation treatments or sexes (Table 1), although ULT of specimens acclimated at
299 20°C tended to be lower than those from the 10°C treatment (Fig 4b), as in *A.*
300 *nevadensis*.

301 No mortality was recorded in the control treatments for any of the taxa throughout the
302 entire duration of the experiments.

303 *Thermal safety margins*

304 Average ULT from both acclimation treatments were used to estimate TSM of both
305 taxa, because no significant differences were found between treatments. TSM were
306 wide for both species in current conditions and decreased for the four future scenarios
307 (Tables 2 and 3). The most heat-tolerant species, *H. s. sierranevadensis*, showed higher
308 TSM than *A. nevadensis* for both present and future conditions. In *A. nevadensis*, larvae
309 had lower TSM than adults (see Table S2 in Appendix S1). In general, TSM were
310 similar among the different localities for both species under current conditions, but
311 greater differences were predicted in future scenarios, for example between *Lagunicas*
312 *de la Virgen*, with lower TSMs and *Aguas Verdes*, with higher values (Tables 2, 3 and
313 S2).

314 **Discussion**

315 By combining species distribution models with key thermal physiology traits, we show
316 that the studied alpine insects, despite their little potential for range shifts, have thermal
317 tolerance wide enough to face climate change in their current localities. Although the
318 extent to which these findings are applicable to other alpine freshwater species remains
319 to be investigated, they provide interesting insights on the factors that determine the
320 vulnerability to global warming in alpine freshwater biota, which will be crucial to
321 define strategies directed to dampen its negative effects.

322 *Changes in climatically suitable habitat*

323 SDMs show that the current suitable habitat for both species is confined to the highest,
324 coldest areas within Sierra Nevada mountain range. Based on such narrow current

325 climatic realized niche, both SDMs approaches (Maxent and MD) predict a dramatic
326 loss of suitable habitat in Sierra Nevada under all of the future scenarios of climatic
327 change tested. Current localities of both taxa are predicted to become climatically
328 unsuitable in 2070, and the small proportion of suitable habitat predicted to remain
329 (only for the most proximate and optimistic scenarios) is displaced to the highest
330 elevations within Sierra Nevada. Such results would indicate no possibilities for these
331 beetles neither for range shifts nor to persist in their current populations.

332 Other studies that have evaluated the impact of climate change on a number of
333 mountainous taxa using SDM also predict severe losses of suitable habitat (e.g. Bässler
334 *et al.*, 2010; Domisch *et al.*, 2011; Muhlfeld *et al.*, 2011; Sauer *et al.*, 2011; Rehnus *et*
335 *al.*, 2018). Furthermore, migration to other mountains systems is virtually impossible
336 for species isolated on ‘mountain-top islands’, which have generally evolved low
337 dispersal capacity (Thomas *et al.*, 2004). Such assessments of the vulnerability of
338 mountain biota to climate change have led to general predictions of high extinction risk
339 for mountain-top species (Dirnböck *et al.*, 2011). However, predictions of climate-
340 induced extinction risk might be inaccurate if these are based exclusively on realized
341 climatic niches (as in most SDM) and the species ability to persist *in situ* under different
342 conditions than those currently experienced is ignored, as previous work on
343 subterranean insects has shown (Sánchez-Fernández *et al.*, 2016). Our data may also
344 serve to exemplify this, as despite the fact that the current localities of both beetles are
345 predicted to become climatically unsuitable under climate change according to SDM
346 results, such localities are expected to maintain thermally suitable conditions
347 considering their physiological thermal tolerance (see below). This might potentially
348 enable the studied taxa to cope with warming, to some extent, without the need to
349 migrate.

350 *Physiological capacity to persist in situ*

351 Current maximum summer air temperatures in the localities occupied by the study
352 species range between 23.1-24.5°C and on average, between 2050-2070 and the two
353 RCP considered here, these are forecasted to increase between 3.0-6.5 °C or 2.0-5.0°C
354 in *H. s. sierranevadensis* and *A. nevadensis* localities, respectively. Studies reporting
355 thermal regimes of alpine ponds in Sierra Nevada have shown that maximum surface
356 water temperature closely approaches that of the air during the ice-free period (Sánchez
357 *et al.*, 1989; Rodríguez-Rodríguez *et al.*, 2004). The TSM values obtained here (for
358 adults) show that future maximum temperatures are not likely to exceed lethal limits of
359 the studied taxa. However, among the two taxa, the most heat sensitive *A. nevadensis*
360 could be more compromised in a warming context. It should be noted that we have
361 estimated TSM using thermal tolerance of adults, but larvae of *A. nevadensis* showed
362 lower values, although these were still relatively wide (close or higher than 4°C for the
363 different future scenarios). This suggests that larvae could be a more heat-sensitive
364 stage than adults, as found in an alpine ice-crawler (Schoville *et al.*, 2015) but in
365 contrast to the general trend in terrestrial insects of decline of high-temperature limits
366 into more advanced life stages (Bowler & Terblanche, 2008). Thus, further research
367 should be directed to get experimental data on *H. s. sierranevadensis* larvae in order to
368 obtain a more conservative estimation of TSM for this beetle subspecies. In any case,
369 our results highlight that studying traits of thermal biology for different life stages in
370 aquatic insects could be of crucial importance for enhancing our abilities to predict the
371 fate of particular species and populations under ongoing climate change, in agreement
372 with previous findings for terrestrial insects (Bowler & Terblanche, 2008; Kingsolver *et*
373 *al.*, 2011).

374 According to the TSM values estimated here, it seems that the current populations of the
375 studied taxa could have certain capacity to persist during high-temperature episodes.
376 However, not only absolute thermal tolerance limits, but also thermal plasticity, will be
377 key in determining how ectotherms overcome temperature stress (Chown *et al.*, 2010;
378 Somero, 2010). We did not find positive effects of acclimation temperature with any of
379 the two different approaches used to measure acclimation capacity and, instead,
380 negative effects of acclimation at high temperature were detected in *A. nevadensis*,
381 which showed lower HCT values in the 20°C treatment. In light of such detrimental
382 effects, prolonged exposure to non-lethal but suboptimal temperatures may have
383 sublethal effects on some metabolic or physiological processes in these species (e.g.
384 oxidative stress), which could affect its fitness in the long-term (Monaghan *et al.*, 2009).
385 Overall, and despite of showing relatively wide TSM, the studied taxa appear to have
386 little capacity to adapt to warming conditions via physiological plasticity.

387 Some cautionary considerations need to be taken into account when inferring species
388 persistence capacity from thermal tolerance data. First, thermal limits and plasticity for
389 critical biological functions important for long-term survival or reproduction (e.g.
390 feeding, growth, locomotion, fecundity) may be more constrained than lethal limits.
391 Furthermore, not only the magnitude, but also the frequency and duration of extreme
392 events can dramatically affect physiological, ecological and evolutionary responses
393 (Easterling *et al.*, 2000; Chown *et al.*, 2010). On the other hand, other components of
394 plasticity that could not be explored here, such as developmental plasticity (i.e.
395 phenotypic variation that results from environmentally induced modifications of
396 development and growth; Forsman, 2015), as well as evolutionary adaption, could
397 mitigate the impact of climate change. However, heat tolerance seems to have in general
398 limited capacity for genetic and plastic responses in ectotherms (Gilchrist & Huey,

399 1999; Hoffmann *et al.*, 2013). In fact, plasticity and evolutionary potential might be
400 especially low in small and isolated populations as the taxa studied here, especially if
401 they are exposed to genetic drift, inbreeding and erosion of genetic variation (Fowler &
402 Whitlock, 1999; Skelly *et al.*, 2007). Furthermore, although future temperatures will
403 probably fall within the heat tolerance limits of these species, it is very likely that their
404 current localities will become suitable for species from lower altitudes, with the
405 subsequent changes in the species composition of alpine ponds and in their biotic
406 interactions (Wissinger *et al.*, 2015; Rosset *et al.*, 2010), whose effects on the
407 persistence of the original communities are largely unpredictable.

408 Only a few studies have experimentally measured thermal limits in other alpine
409 invertebrates, and these have shown lower heat tolerance than the taxa studied here (e.g.
410 Treanor *et al.*, 2013; Schoville *et al.*, 2015) or similar UTLs but under shorter exposure
411 times than those applied here (Lencioni & Bernabó, 2017). However, these comparisons
412 should be taken with caution, because different physiological end-points, methods and
413 experimental conditions have been used in each study. Calosi *et al.* (2008a, 2010)
414 showed that within congeneric European diving beetles (*Deronectes* spp.; fam.
415 Dytiscidae), species with lower heat tolerance and hence most at risk under climate
416 change were the most geographically restricted ones, being endemic to Mediterranean
417 mountain systems. In contrast, the HCTs of the endemic taxa studied here were similar
418 to the average value of CT_{max} ($45.5 \pm 1.0^\circ C$, $N=40$) experimentally measured in a range
419 of arthropods at the same heating rate, recently gathered in large global cross-realm
420 multi-taxon dataset (Bennett *et al.*, 2018). Such values were also comparable to those
421 reported for other non-alpine dytiscids (Calosi *et al.*, 2008b, 2010; Bilton & Foster,
422 2016). In particular, those of *A. nevadensis* were similar to the CT_{max} of geographically
423 restricted congeneric species (Calosi *et al.*, 2008b), and *H. s. sierranevadensis* showed

424 higher HCT than its congeneric Palearctic species *H. memnonius* (Bilton & Foster,
425 2016). This suggests that *A. nevadensis* and *H. s. sierranevadensis* may retain, to some
426 degree, tolerance to high temperatures from their widespread, phylogenetically close
427 relatives (*A. bipustulatus* and *H. sabaudus sabaudus* Faubel, respectively).

428 Regarding thermal plasticity, cold-acclimation responses have been relatively well
429 documented in alpine invertebrates (e.g. Block & Zettel, 1980; Bakken, 1985; Schoville
430 *et al.*, 2015) but heat-induced plasticity remains surprisingly unexplored. It is thus
431 difficult to determine to what extent the lack of plasticity in upper thermal limits
432 observed in the studied taxa is generalized among alpine invertebrates. Although
433 experimental data on thermal tolerance are costly to obtain for large sets of species,
434 some of the common difficulties for its measurement are relatively minor for some
435 invertebrates. Many aquatic insects (even rare species) are locally abundant, relatively
436 easy to collect in field and adult specimens generally can be easily maintained at
437 laboratory conditions. Therefore, thermal tolerance data as those obtained here could
438 greatly contribute to our understanding of the effects of climate change on alpine
439 communities.

440 *Conservation implications*

441 Arribas *et al.* (2012) proposed a framework to guide insect conservation strategies on
442 the basis of the evaluation of species persistence and ability to shift their range under
443 climate change (see also Arribas *et al.*, 2017). According to this framework, the
444 concentration of conservation efforts in actual localities (i.e., *in situ* management or
445 conservation at local scale), could be the most efficient and practical strategy for both
446 taxa given that (i) they are confined to the highest, coldest areas of the Sierra Nevada
447 mountain range, with little potential for range shift, and (ii) they would have certain

448 capacity to deal *in situ* with future warming conditions according to physiological
449 experiments. Conservation measures should therefore be focused on the maintenance of
450 current populations and the minimization of other threats. The habitat scale
451 management, with measures to maintain and maximize microclimatic environments and
452 to sustain habitat quality and extension could be fundamental to enhance the persistence
453 of populations in current localities under climatic warming. Similarly, special attention
454 should be given to monitoring their populations, which should consider, when possible,
455 physiological changes in thermal tolerances, phenological adjustments, behavioural
456 thermoregulation changes, such as adjustments in daily activity periods or microhabitat
457 use, quantification of dispersal rates and specially changes in population parameters. It
458 would be also crucial to complement the evaluation of their persistence capacity *in situ*
459 studying the effects of temperature in other important biological traits (e.g. fecundity,
460 life-cycle duration) and focusing, when possible, on the most sensitive stages (larvae).
461 Further studies on metapopulation dynamics, gene flow and connectivity between
462 populations would provide useful additional information to identify those localities of
463 major conservation interest (i.e. those acting as sources or refugees).

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738 **Tables**

739 **Table 1.** Effect of acclimation temperature (T_{acc}) on HCT and ULT in adults of *A.*

740 *nevadensis* and *H. s. sierranevadensis*

| Taxon | Endpoint | Predictor | df | F-value/ χ^2^a | P-value |
|-------------------------------|-----------------|------------------|-----------|---|----------------|
| <i>A. nevadensis</i> | HCT | T _{acc} | 1 | 13.357 | 0.003 |
| | | Sex | 1 | 6.736 | 0.021 |
| <i>H. s. sierranevadensis</i> | ULT | T _{acc} | 1 | 0.005 | 0.942 |
| | HCT | T _{acc} | 1 | 0.004 | 0.948 |
| | | Sex | 1 | 1.257 | 0.272 |
| | ULT | T _{acc} | 1 | 0.729 | 0.393 |

741 ^aF-value is provided for ANOVA analyses (HCT data) and χ^2 for Kruskal-Wallis tests
 742 (ULT data)

743

744 **Table 2.** Current and future thermal safety margins (TSM, °C) for *A. nevadensis* adults
 745 in its current localities (IDs correspond to codes in Figure 1 and Table S1 in Appendix
 746 S1).

| Locality | ID | Current | 2050 | | 2070 | |
|------------------------|----|---------|---------|---------|---------|---------|
| | | | RCP 4.5 | RCP 8.5 | RCP 4.5 | RCP 8.5 |
| Lagunicas de la Virgen | 1 | 10.63 | 7.58 | 6.88 | 6.87 | 5.26 |
| Laguna de Aguas Verdes | 2 | 11.43 | 9.09 | 8.38 | 8.37 | 6.76 |
| Lagunas de Río Seco | 3 | 11.43 | 8.39 | 7.68 | 7.67 | 6.06 |
| Laguna de la Caldera | 4 | 11.23 | 8.19 | 7.49 | 7.49 | 5.88 |
| Laguna Hondera | 5 | 10.73 | 7.69 | 6.99 | 6.99 | 5.38 |
| Laguna del Peñón Negro | 6 | 10.73 | 7.69 | 6.99 | 6.99 | 5.38 |
| Laguna de Borreguiles | 9 | 11.23 | 8.19 | 7.49 | 7.49 | 5.88 |

747

748 **Table 3.** Current and future thermal safety margins (TSM, °C) for *H. s.*
 749 *sierranevadensis* adults in its current localities (IDs correspond to codes in Figure 1 and
 750 Table S1 in Appendix S1)

| Locality | ID | Current | 2050 | | 2070 | |
|------------------------|----|---------|---------|---------|---------|---------|
| | | | RCP 4.5 | RCP 8.5 | RCP 4.5 | RCP 8.5 |
| Lagunicas de la Virgen | 1 | 12.57 | 9.52 | 8.82 | 8.81 | 7.20 |
| Laguna de Aguas Verdes | 2 | 13.37 | 11.03 | 10.32 | 10.31 | 8.70 |
| Lagunas de Río Seco | 3 | 13.37 | 10.33 | 9.62 | 9.61 | 8.00 |
| Laguna de La Caldera | 4 | 13.17 | 10.13 | 9.43 | 9.43 | 7.82 |
| Laguna Hondera | 5 | 12.67 | 9.63 | 8.93 | 8.93 | 7.32 |
| Laguna del Peñón Negro | 6 | 12.67 | 9.63 | 8.93 | 8.93 | 7.32 |
| Laguna de las Yeguas | 7 | 12.57 | 9.52 | 8.82 | 8.81 | 7.20 |
| Laguna de la Mosca | 8 | 12.17 | 9.13 | 8.43 | 8.43 | 6.82 |
| Laguna de Borreguiles | 9 | 13.17 | 10.13 | 9.43 | 9.43 | 7.82 |
| Barranco de San Juan | 10 | 11.97 | 7.88 | 7.14 | 7.16 | 5.52 |

751

752 **Figure legends**

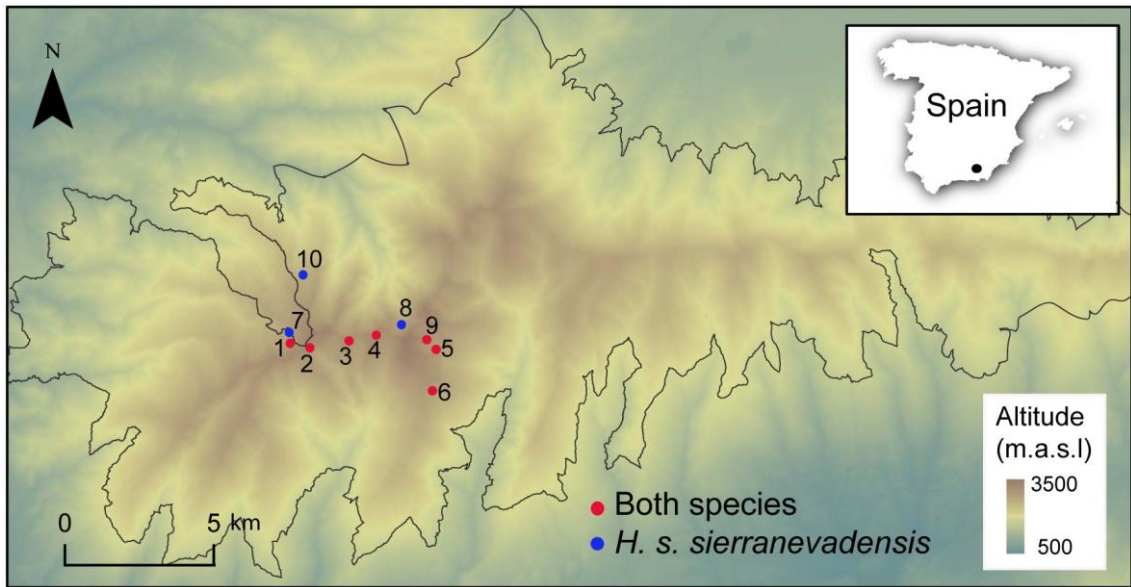
753 **Figure 1** Study area showing the localities of the studied taxa (see Table S1 in
754 Appendix S1 for additional information on each site). The line represents the boundaries
755 of the National Park.

756 **Figure 2** Climatic suitability within the current and future potential distribution of *A.*
757 *nevadensis* estimated with the Maxent method. Maps show averaged values for
758 seventeen general circulation models. Climatic favorability ranges from low (white,
759 light red colours) to high (green). Red dots indicate current localities of *A. nevadensis*
760 and the line represents the boundaries of the National Park.

761 **Figure 3** Climatic suitability within the current and future potential distribution of *H. s.*
762 *sierranevadensis* estimated with the Maxent method. Maps show averaged values for
763 seventeen general circulation models. Climatic favorability ranges from low (white,
764 light red colours) to high (green). Blue dots indicate current localities of *H. s.*
765 *sierranevadensis* and the line represents the boundaries of the National Park.

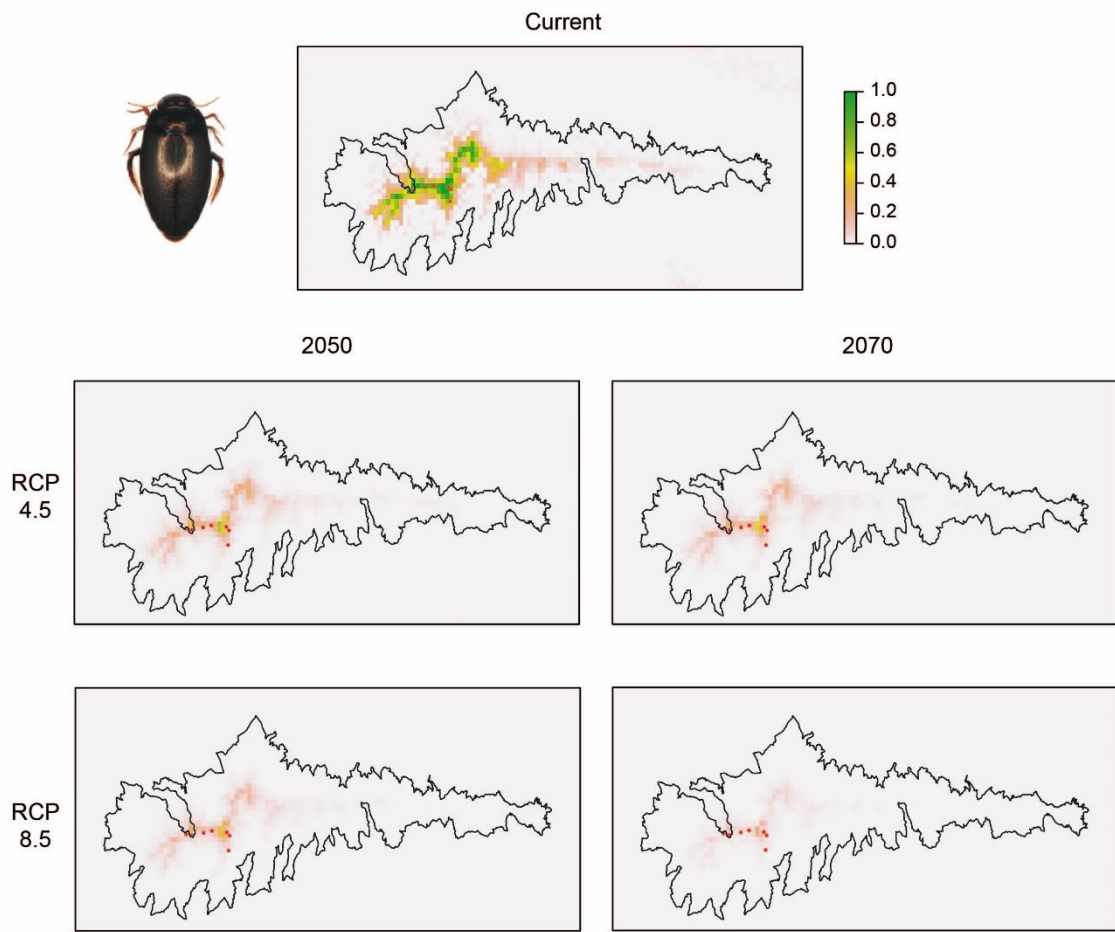
766 **Figure 4** (a) Heat coma temperature and (b) upper lethal temperature measured at a
767 heating rate of $1^{\circ}\text{C min}^{-1}$ and $3^{\circ}\text{C day}^{-1}$, respectively, for adults of *A. nevadensis* and *H.*
768 *s. sierranevadensis*. When significant differences between sexes were found ($p < 0.05$),
769 data from males and females are represented separately.

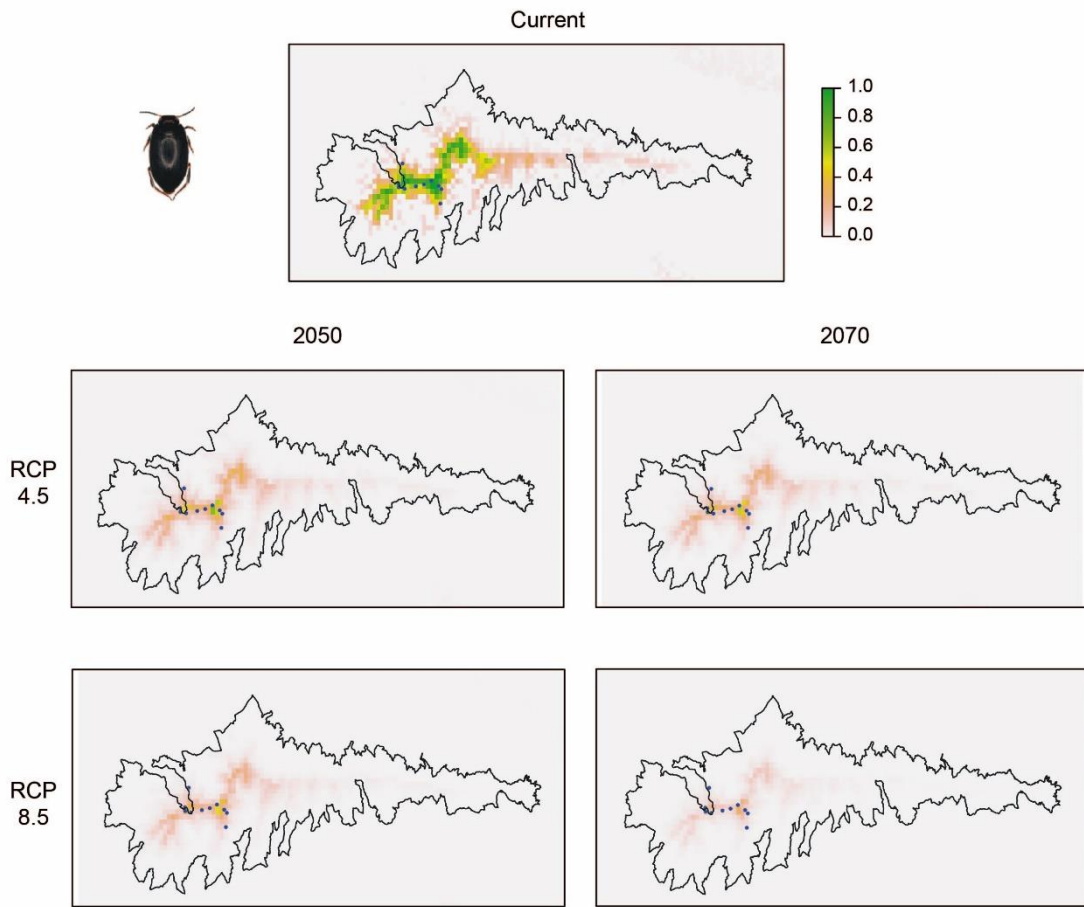
770 **Figure 1**



771

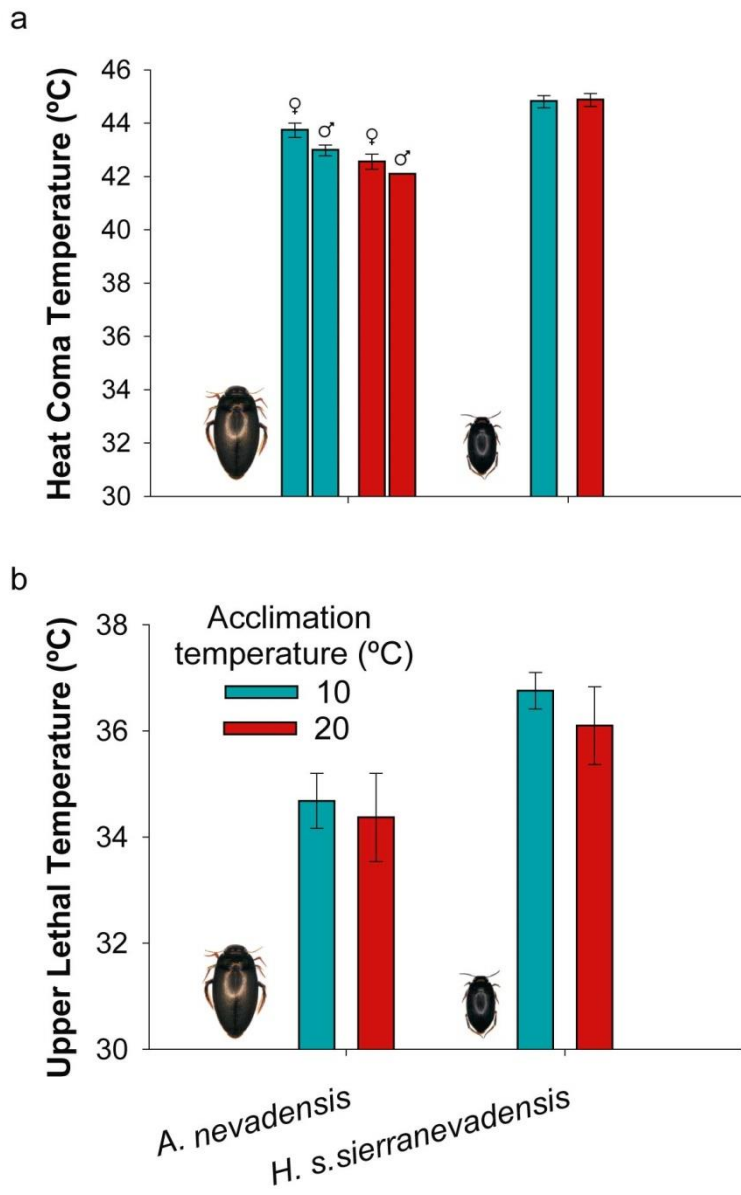
772





777 **Figure 4**

778



779