

1 **Insect temperature-body size trends common to laboratory,**
2 **latitudinal and seasonal gradients are not found across altitudes**

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4 **Curtis R. Horne^a, Andrew. G. Hirst^{a,b*}, and David Atkinson^c**

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6 ^aSchool of Environmental Sciences, University of Liverpool, Liverpool, L69 3GP, United
7 Kingdom.

8 ^bCentre for Ocean Life, National Institute for Aquatic Resources, Technical University of
9 Denmark, Kavalergården 6, 2920, Charlottenlund, Denmark

10 ^cInstitute of Integrative Biology, University of Liverpool, Liverpool, L69 7ZB, United Kingdom.

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12 **Corresponding author: aghirst@liverpool.ac.uk*

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16

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18 CH, AGH and DA designed the study and wrote the paper. CH collected the data and
19 performed the statistical analyses.

20

21

22 **ABSTRACT**

- 23 1. Body size affects rates of most biological and ecological processes, from individual
24 performance to ecosystem function, and is fundamentally linked to organism fitness.
25 Within species, size at maturity can vary systematically with environmental
26 temperature in the laboratory and across seasons, as well as over latitudinal gradients.
27 Recent meta-analyses have revealed a close match in the magnitude and direction of
28 these size gradients in various arthropod orders, suggesting that these size responses
29 share common drivers.
- 30 2. As with increasing latitude, temperature also decreases with increasing altitude.
31 Although the general direction of body size clines along altitudinal gradients has been
32 examined previously, to our knowledge altitude-body size (A-S) clines have never
33 been synthesised quantitatively, nor compared with temperature-size (T-S) responses
34 measured under controlled laboratory conditions.
- 35 3. Here we quantitatively examine variation in intraspecific A-S clines among 121 insect
36 species from 50 different global locations, representing 12 taxonomic orders. While
37 some taxa were better represented in the literature than others, our analysis reveals
38 extensive variation in the magnitude and direction of A-S clines. Following the
39 assumption that temperature on average declines by 1°C per 150m increase in
40 altitude, order-specific A-S clines in the field appear to deviate from laboratory T-S
41 responses. Specifically, the magnitude of A-S clines and T-S responses are more
42 closely matched in some taxonomic orders (e.g. Diptera) than others (e.g. Orthoptera).
43 These findings contrast with the strong co-variation observed between latitude-size
44 clines and T-S responses, and between laboratory and seasonal T-S responses.
- 45 4. The lack of clear size relationships with elevation, and hence temperature, is likely due
46 to the counteracting effects of other major drivers with altitude, including season length
47 and oxygen partial pressure. Switches in voltinism within species across altitude, and
48 the dispersal of individuals across different elevations, may also obscure trends.

49

50 INTRODUCTION

51 Body size is fundamentally linked to an organism's fitness, and is correlated with vital
52 physiological rates and life history characteristics (Kleiber 1947; Brown *et al.* 2004; Hirst,
53 Glazier & Atkinson 2014). Further, both within and between species, body size has been found
54 to co-vary with a range of environmental conditions (Bergmann 1847; James 1970; Atkinson
55 1994). Changes in the body size of organisms can affect not only the fate of individuals and
56 populations (e.g. by influencing survival, fecundity, population production), but also ecosystem
57 functioning via size-dependent processes (e.g. predator-prey dynamics, food web
58 connectivity) (Peters 1983; Honěk 1993). Thus, understanding and ultimately predicting body
59 size variation with environmental conditions presents a significant ecological challenge of
60 global importance.

61

62 In ectothermic species, intraspecific variation in body size has been shown to strongly
63 correlate with temperature, both in controlled laboratory conditions (Atkinson 1994; Forster,
64 Hirst & Atkinson 2012) and seasonally across the year (Horne *et al.* 2016; Horne, Hirst &
65 Atkinson 2017). Recurrent patterns in body size also occur across latitudes, and have led to
66 prominent biogeographical 'rules', such as Bergmann's rule (across taxa, larger species are
67 found at higher, colder latitudes) (Bergmann 1847), and James' rule (within species, larger
68 individuals are found at higher, colder latitudes) (James 1970). In arthropods, including
69 insects, close parallels were recently identified between phenotypically plastic size responses
70 to temperature measured in the laboratory, and intraspecific changes in body size observed
71 in the field, both across latitudes and seasonally over an annual cycle. Consistent differences
72 in both the magnitude and direction of body size gradients were found among taxonomic
73 orders, between environments (aquatic vs. terrestrial, suggesting an important role for oxygen
74 availability), and between univoltine and multivoltine terrestrial species (likely an evolutionary
75 adaptation to changing season length) (Horne, Hirst & Atkinson 2015; Horne, Hirst & Atkinson
76 2017). This co-variation suggests that these widespread body size phenomena may be driven
77 by similar selective pressures, and that temperature is an important correlate of size

78 responses in the field, despite a number of confounding factors that can also influence body
79 size (e.g. resource availability, mortality risk and competition) (Chown & Gaston 2010).

80

81 As with increasing latitude, temperature commonly declines with increasing altitude. On
82 average, temperature falls by 5.5 to 6.5°C per 1000m increase in elevation (Anslow & Shawn
83 2002), though this of course varies considerably depending on other climatic and topographic
84 influences, including aspect, wind speed and cloud cover amongst others (Hodkinson 2005).

85 We might therefore expect those species that grow to a larger adult size in the cold and with
86 increasing latitude will also exhibit a positive cline in body size with increasing altitude. To our
87 knowledge, most large syntheses of altitude-body size (A-S) clines have predominantly
88 focused on whether body size gradients were negative, positive or non-significant (e.g. Dillon,
89 Frazier & Dudley 2006; Shelomi 2012). Yet, by quantifying and comparing the magnitude of
90 A-S clines between species and among higher taxonomic groups, we can begin to link
91 variation in the strength of these body size gradients with key life history characteristics,
92 habitat and/or functional groups. Furthermore, quantitative data capturing the magnitude of A-
93 S clines allow for a more direct comparison with laboratory temperature-size (T-S) responses.

94

95 To our knowledge, A-S clines and laboratory T-S responses have not previously been
96 compared quantitatively. Such a comparison provides an opportunity to explore the extent to
97 which changes in other environmental parameters might be confounding the effects of
98 temperature across altitude in the field. For example, an increase in altitude is also
99 accompanied by a decline in the partial pressure of atmospheric gases, including oxygen,
100 which decreases near linearly with altitude, such that inspired oxygen pressure at 5500m
101 elevation is 50% of that at sea level (Peacock 1998). The length of the season in which
102 individuals are able to grow also typically declines with increasing altitude: these growth
103 conditions encompass not just appropriate temperatures but also extent of resource
104 availability (Hodkinson 2005) (see Fig. 1 for an illustration of the predicted effects of these key

105 parameters on body size). Other biologically relevant environmental factors that increase with
106 altitude include wind speed, precipitation, and UV radiation (Hodkinson 2005).

107

108 Variation in the direction of A-S clines among species has often been attributed to seasonality
109 and differences in reproductive strategy (Chown & Klok 2003; Shelomi 2012). Multivoltine
110 species are predicted to grow to a larger adult size at higher colder altitudes, in accordance
111 with the temperature-size rule (Atkinson 1994). In contrast, univoltine species are much more
112 dependent on season length and hence on time available for growth (often positively
113 correlated with temperature) (Chown & Gaston 1999; Blanckenhorn & Demont 2004;
114 Kozłowski, Czarnoleski & Danko 2004; Shelomi 2012). Consequently, univoltine species are
115 predicted to reduce their body size with increasing altitude. The extent to which season length
116 decreases with increasing altitude may also influence the magnitude of A-S clines, particularly
117 in univoltine species for which time available for growth is limiting. Increased time constraints
118 with elevation may be especially important in more thermally seasonal environments, such as
119 at higher latitudes, where the decline in season length along altitudinal gradients is much more
120 pronounced than in the tropics (Körner 2000; Chown & Klok 2003). Of course, voltinism itself
121 can vary with environmental conditions, and any switch in reproductive strategy could also
122 affect the magnitude of any apparent size change (Roff 1980; Mousseau & Roff 1989; Zeuss,
123 Brunzel & Brandl 2016).

124

125 The spatial distance covered by an A-S cline and the dispersal or migratory ability of a species
126 may also affect the magnitude of the A-S cline. Latitudinal-size (L-S) clines are typically
127 measured over larger distances than A-S clines, sometimes spanning thousands of
128 kilometres, whole continents, and entire species' ranges (e.g. Hassall 2013). By contrast,
129 altitudinal clines in the size of species are often studied along localized transects (e.g. Smith
130 *et al.* 2000), or in some cases over tens to hundreds of kilometres at most (e.g. Eweleit &
131 Reinhold 2014). Consequently, populations are more likely to become interconnected by
132 individual movement along relatively short-distance elevation gradients (for example by flight

133 and/or passive transport by wind) than along longer-distance latitudinal transects. Therefore,
134 adults collected at one altitude might have developed at another, where environmental
135 conditions were very different. This shift between environmental conditions during ontogeny
136 is more likely for more mobile species with greater dispersal ability, such as winged species
137 capable of flight (Roff & Fairbairn 2007). As a result, A-S clines in more mobile species may
138 be obscured or appear weaker.

139

140 Here we explore these different potential influences on A-S clines by quantifying and
141 examining variation in intraspecific A-S clines in insects, along with a small number of
142 arachnids and Crustacea. We compare the magnitude and direction of A-S clines based on
143 taxonomy, voltinism, flight capability, and environment type (freshwater vs. terrestrial). We
144 then compare these clines to T-S responses measured under controlled laboratory conditions,
145 to determine whether these major size gradients co-vary, and thus to better understand to
146 what extent changes in other environmental parameters might be confounding the effects of
147 temperature on body size across altitude in the field.

148

149 **MATERIALS AND METHODS**

150 To quantitatively describe species-specific altitudinal-size clines, we searched the literature
151 using the Web of Science database (<http://apps.webofknowledge.com/>) and Google Scholar
152 for published field records of adult body size measured at different altitudes, covering at least
153 150m in altitudinal range. Most studies were conducted along individual mountain transects,
154 but occasionally sampling locations were more widely distributed within a local area or region;
155 in these instances, we only accepted studies in which the sampling locations were distributed
156 over less than 5° latitude. The primary search term combinations used were: (“altitud*” OR
157 “elevation” OR “mountain”) AND “size” AND (“arthropod” OR “insect” OR “[<insert taxonomic
158 order or common name of taxon>]”). We also used reference lists from the papers we found
159 to identify additional key literature. Adult size data were collected as lengths, or dry, wet or
160 carbon masses and subsequently standardised to dry mass (mg) using published intra-

161 specific regressions and conversion factors (see Dataset S1 in Supplementary Information).
162 In cases where species-specific regressions were unavailable, regressions for related
163 species, or more general inter-specific regressions were used. All altitudinal measurements
164 were standardized to metres above sea level.

165

166 We derived species-specific slopes of ordinary least-squares (OLS) regressions between In-
167 transformed dry mass (mg) and altitude (m). Slopes were calculated for each sex separately
168 where these data were available. We used this exponential function of body mass as it has
169 consistently been found to be the best for modelling body size gradients with both temperature
170 (in the laboratory and seasonally) and across latitude (Forster, Hirst & Atkinson 2012; Horne,
171 Hirst & Atkinson 2015; Horne, Hirst & Atkinson 2017). Further, this exponential function
172 allowed us to easily compare these different size gradients with one another. Altitudinal-size
173 clines were then transformed into percentage change in dry mass per metre of elevation, using
174 the formula $(\exp^{(\text{slope})} - 1) * 100 = \% \text{ change in mass per m}$ (Forster, Hirst & Atkinson 2012).
175 Thus, a positive cline denotes an increase in body size with increasing altitude, whereas a
176 negative cline denotes a decrease in body size with increasing altitude. In general,
177 temperature decreases with increasing altitude at a rate of between 5.5°C and 6.5°C per
178 1000m (Anslow & Shawn 2002), which equates to approximately 1°C per 150m elevation.
179 Thus, we also calculated the percentage change in dry mass per 150m, using the formula
180 $(\exp^{(\text{slope} \times 150)} - 1) * 100 = \% \text{ change in mass per 150m elevation (or approximately per } ^\circ\text{C)}$. This
181 measure of size change allowed us to more appropriately compare the strength of A-S clines
182 measured in the field with T-S responses (% change in mass per °C) measured in the
183 laboratory.

184

185 **Statistical analyses**

186 All statistical analyses were conducted in R (version 3.4.1) (R Core Team 2014). Using A-S
187 clines (% change in mass per 150m) as the dependent variable, we compared several
188 candidate models to best predict variation in the magnitude and direction of A-S clines based

189 on the Akaike's information criterion (AIC). The following were incorporated as fixed variables
190 in a global linear mixed effects model (using package lme4): environment (freshwater vs.
191 terrestrial), voltinism, categorised here as species that are generally regarded as having ≤ 1
192 generation per year (includes univoltine) or >1 generation per year (multivoltine), flight
193 capability (flying vs. flightless), and \log_{10} -transformed species body mass (calculated at 1500m
194 altitude using species-specific A-S slopes, and representing the approximate mid-point of the
195 altitudinal range across all the studies in our data set). \log_{10} -transformed species body mass
196 was included as a covariate to determine if A-S clines were dependent on the body size of the
197 species. A-S clines from multiple studies of the same species were included in our analyses;
198 thus, given that species have shared evolutionary histories and so are not completely
199 independent, we included levels of taxonomic classification (order, family, and species) as
200 nested (hierarchical) random effects on the intercept in all models to help control for
201 phylogeny, and to account for the disproportionate representation of taxonomic orders in our
202 data set. Given that A-S clines did not differ significantly between males and females, and sex
203 was not reported in all studies, we chose to exclude it as a random effect in the models. A-S
204 clines for multiple species were sometimes measured in similar study locations or regions,
205 and so we also included location (categorized using latitude and longitude) as a random effect
206 on the intercept, to help control for potential geographical clumping of the data. Finally, we
207 accounted for variation in information quality by weighting each A-S cline by the inverse of the
208 variance of its slope estimate (using the 'weights' function in R) (Koricheva, Gurevitch &
209 Mengersen 2013). This helped to account for the fact that A-S clines were derived from data
210 that varied in their goodness of fit between studies and species. We compared all possible
211 combinations of the global model terms using the dredge function in the MuMIn package
212 (Barton 2017); this included an intercept-only model, which contained no independent
213 variables and predicted that the best estimate of A-S clines was the intercept. The best model
214 was identified as that with the lowest small-samples corrected AIC (AICc). Where the
215 difference between a model's AICc and the lowest AICc (i.e. $\Delta AICc$) was <2 , a set of best fit
216 models, rather than a single best model, was assumed. Model averaging was then used to

217 identify the best predictor variables across the top candidate models, and determine their
218 relative importance (computed for each variable as the sum of the Akaike weights from all
219 models in which they appear). In addition to AIC, a series of F tests were used to verify the
220 significance ($p < 0.05$) of each parameter's effect on the strength of the seasonal T-S gradient.

221

222 To compare A-S clines with laboratory controlled T-S responses, we used the data compilation
223 of Horne *et al.* (2015) . For each data set, we first generated single species-specific body size
224 gradients by combining size gradients from multiple studies of the same species into a simple
225 mean. We then averaged these species-specific gradients for each taxonomic order,
226 separated by voltinism, and plotted the resulting order-specific A-S clines against order-
227 specific laboratory T-S responses. We then assessed the extent to which both of these body
228 size gradients co-varied (i.e. whether a 1% increase in body size per 150m altitude = 1%
229 decrease in body size per °C in the laboratory).

230

231 We acknowledge that the use of interspecific length-mass conversions may increase the
232 likelihood of inaccuracy when determining body size gradients, particularly as small deviations
233 in the equation's power term can result in substantial over- or under-estimation of the
234 percentage change in body size. Wherever possible, therefore, we used species-specific
235 length-mass regressions, but sometimes had to rely on family- and order-specific conversions,
236 and authors often employed a variety of equation forms. To reduce risks that uncertainties in
237 these length-to-mass conversions would bias our conclusions, we therefore repeated our
238 analysis using length in place of dry mass to generate a second set of A-S clines (% change
239 in length 150m^{-1}). To do this we used either the original length measurements reported, or
240 calculated the cube-root of mass when this was given.

241

242

243 **RESULTS**

244 We derived a total of 229 altitude-size clines representing 121 species from 12 taxonomic
245 orders. These size clines were recorded at 50 different global locations ranging from 53° South
246 to 66° North, with most locations falling around mid-latitude regions (Fig. 2). The data set
247 contained a near even distribution of negative and positive A-S clines, with 49% of clines
248 showing a decrease in adult body size with increasing altitude. We found that neither of the
249 sexes within species showed a consistently stronger cline in body size with altitude than the
250 other ($F_{1,227}=0.0004$, $p=0.98$; also see Fig. 3), which parallels the lack of sex differences in
251 laboratory T-S responses within arthropod species (Hirst, Horne & Atkinson 2015). Across
252 species, the magnitude of A-S clines varied considerably, with some of the strongest size
253 clines observed in the Coleoptera, Orthoptera and Hymenoptera (see Fig. 3). Approximately
254 69% of the A-S clines in our data set were based on measurements of body length, width or
255 direct measurements of mass, whereas the remaining clines were derived from limb
256 measurements, including femur length and wing length, as well as head width (all of which
257 had been converted to mass). Whether A-S clines were originally derived from whole- or part-
258 body measurements had no significant effect on the magnitude of A-S clines in our data set,
259 ($F_{1,227}=1.99$, $p=0.16$), and this was also the case when A-S clines were calculated using length
260 in place of dry mass ($F_{1,227}=1.88$, $p=0.17$).

261
262 None of the fixed variables included in our global model (environment, voltinism, flight
263 capability and \log_{10} -transformed species body mass) could significantly explain variation in A-
264 S clines. Although the best supported model contained environment type (freshwater vs.
265 terrestrial), voltinism, and \log_{10} -transformed species body mass as independent variables
266 ($R^2=0.26$) (see Table S1 in Supplementary Information for AIC output), 6 alternative models
267 had a $\Delta AICc$ less than 2; this included an intercept-only model, which contained no
268 independent variables and predicted that the best estimate of A-S clines was the intercept,
269 which did not differ significantly from zero (3.9% change in body mass $150m^{-1}$; ± 7.1 95% CI).

270 Coleoptera (-1.5% body mass 150m^{-1} ; ± 4.5 95% CI) and Orthoptera (-0.7% body mass 150m^{-1} ;
271 ± 2.3 95% CI) were among those taxonomic orders to show on average a decrease in body
272 size with increasing altitude, whereas orders such as Diptera (1.2% body mass 150m^{-1} ; ± 2.3
273 95% CI), Hymenoptera (1.8% body mass 150m^{-1} ± 5.2 95% CI) and Lepidoptera (1.1% body
274 mass 150m^{-1} ; ± 1.2 95% CI) tended to show the opposite pattern (Fig. 4). Yet, these patterns
275 were not strong enough for a significant effect of taxonomic order on the magnitude of the A-
276 S cline to be detected ($F_{11,217}=0.80$, $p=0.64$).

277

278 When we categorized species into those which commonly have one generation or fewer per
279 year, or species that have multiple generations per year, the former showed on average a
280 reduction in their body size with increasing altitude (-0.4% body mass 150m^{-1} ; ± 1.4 95% CI),
281 whereas the latter showed a mean increase in body size with increasing altitude (1.0% body
282 mass 150m^{-1} ; ± 2.0 95% CI) (Fig. 5a). However, again these patterns were not strong enough
283 to detect a significant effect of voltinism on the magnitude of the A-S cline ($F_{1,227}=0.66$, $p=0.42$).
284 Similarly, we found no significant effect of flight capability ($F_{1,227}=2.25$, $p=0.14$) (Fig. 5b) or
285 \log_{10} transformed species body mass, when calculated either at 1500m for all species
286 ($F_{1,227}=1.11$, $p=0.29$), or at the mid-altitude of each individual study ($F_{1,227}=0.64$, $p=0.42$). Akin
287 to patterns observed in temperature-size (laboratory and seasonal) and latitude-size
288 gradients, on average freshwater species exhibited stronger positive A-S clines (4.8% body
289 mass 150m^{-1} ; ± 4.0 95% CI) relative to terrestrial species (-0.1% body mass 150m^{-1} ; ± 1.2 95%
290 CI), though again this effect was not significant ($F_{1,227}=2.36$, $p=0.13$) (Fig. 5c).

291

292 We tested the robustness of our conclusion that none of the fixed parameters significantly
293 affected the strength of the A-S cline. First, we responded to the more variable strengths of A-
294 S clines observed over relatively small altitudinal ranges by re-running analyses only for A-S
295 clines measured over $\geq 500\text{m}$ altitudinal range (determined by the point at which variation in
296 the magnitude of A-S clines appeared to stabilize; see Figure S1). Again, none of the fixed
297 variables included in our global model could significantly explain variation in A-S clines.

298 Second, re-analysis using A-S clines calculated from original length measurements, in place
299 of dry mass, also revealed no significant effects of any of the fixed variables. The findings from
300 this approach are summarised in more detail in the Supplementary Information.

301

302 Finally, we examined whether A-S clines were similar in direction and magnitude to
303 temperature-size responses measured under controlled laboratory conditions (at the level of
304 taxonomic order, given that A-S and T-S data sets largely contained different species). The
305 plot of order-specific A-S clines (% change in dry mass per 150m) against laboratory T-S
306 responses (% change in dry mass per °C), taken from the comprehensive data set of Horne
307 *et al.* (2015), is shown in Figure 6. Given the low correlation coefficient ($r^2=0.24$) between A-
308 S clines measured in the field and T-S responses measured in the laboratory, following Smith
309 (2009), we chose not to fit an RMA regression through the data. However, from visual
310 inspection we might ascertain from Figure 6 that temperature is a more important driver of
311 body size change in some taxa compared to others. These comparisons largely test the
312 degree to which A-S clines and laboratory T-S responses are influenced by temperature
313 gradients. However, even when comparing order-specific A-S clines with latitudinal-size
314 clines, which capture variation both in season length and temperature, we did not observe a
315 significant correlation (RMA slope= 0.50 ± 0.50 95% CI, $r^2=0.01$). This leads us to suggest that
316 the confounding factors affecting the direction and magnitude of body size clines across
317 altitudes are much stronger than those across latitudes, particularly given the strong co-
318 variation between L-S clines and laboratory T-S responses (Horne, Hirst & Atkinson 2015).

319

320 **DISCUSSION**

321 Our synthesis of A-S clines in insects reveals widespread variation not just in the direction of
322 these body size gradients, but also in their magnitude. The lack of statistical support for the
323 effects of our model parameters [environment (aquatic vs. terrestrial), voltinism, flight
324 capability and species body mass], and the large amount of unexplained variation in A-S

325 clines, suggests that other environmental factors or life history traits not captured by our model
326 are influencing altitudinal clines in body size.

327

328 We suggest that genotype mixing is more likely along short-distance elevation gradients
329 (compared to longer-distance latitudinal gradients), and thus A-S clines may result primarily
330 from phenotypic plasticity, as is observed in laboratory T-S responses, rather than genetic
331 variation (Keller *et al.* 2013). Thus, if temperature is a major correlate of body size variation
332 with altitude, we might expect a particularly close match between A-S clines and plastic
333 laboratory T-S responses. However, although A-S clines observed at the level of taxonomic
334 order were generally similar in direction to temperature-body size responses measured under
335 controlled laboratory conditions (falling within the non-shaded areas in Fig. 6), they did not
336 significantly correlate in magnitude.

337

338 The lack of correlation between A-S clines and T-S responses is in contrast to the strong
339 correlations observed between L-S clines and T-S responses (Horne, Hirst & Atkinson 2015),
340 and also between laboratory and seasonal T-S responses (Horne, Hirst & Atkinson 2017),
341 which did not differ significantly from a 1:1 relationship on average. We note however, that the
342 A-S and T-S data sets largely contain different species, with some taxonomic orders better
343 represented than others in our data set. We also re-emphasize that the extent to which these
344 two body size gradients co-vary is based upon the assumption that temperature on average
345 declines by 1°C per 150m increase in altitude, which does not capture the variation between
346 study locations (Hodkinson 2005).

347

348 Another counter argument to our conclusion that the environmental parameters confounding
349 the effects of temperature are stronger across altitudes than across latitudes, is that the strong
350 correlation observed between L-S clines and T-S responses (Horne, Hirst & Atkinson 2015) is
351 due to the inclusion of additional taxonomic orders, particularly aquatic crustaceans. Yet, even
352 when those orders not present in the A-S data set are excluded from the data of Horne, Hirst

353 and Atkinson (2015), we still observe a significant negative correlation (inferred from 95% CIs)
354 between order-specific laboratory T-S responses and L-S clines (RMA slope= -0.55 ± 0.27 95%
355 CI) which explains much of the variability in the data ($r^2=0.88$). Therefore, we suggest that the
356 mismatch in the magnitude of A-S clines and laboratory T-S responses arises from other
357 environmental parameters confounding the effects of temperature on the magnitude of A-S
358 clines.

359

360 In previous studies (Chown & Klok 2003; Shelomi 2012), differences in the direction of A-S
361 clines were observed between taxa with different reproductive strategies (i.e. voltinism), which
362 corresponds with intraspecific body size patterns observed across latitudes, and also with T-
363 S responses measured in the laboratory (Chown & Gaston 1999; Blanckenhorn & Demont
364 2004; Horne, Hirst & Atkinson 2015). Season length generally declines at higher elevations,
365 and unlike species with multiple generations per year, adult sizes of univoltine species are
366 likely to be much more sensitive to changes in seasonality: as season length decreases, so
367 does time available for growth and reproduction, which imposes limits on size at maturity; thus,
368 the maximisation of fitness may come from utilising as much of the available season length as
369 possible. In contrast, generation time in multivoltine species is relatively short and
370 consequently adult size in each generation is less sensitive to changes in season length;
371 instead, size at maturity in multivoltine species is predicted to be more dependent on changes
372 in temperature (Chown & Gaston 1999; Kozłowski, Czarnoleski & Danko 2004). Yet here, we
373 found no significant effect of voltinism on A-S clines.

374

375 Voltinism itself can vary with environmental conditions within a species (Zeuss, Brunzel &
376 Brandl 2016), and this has the potential to obscure body size gradients in the field. It is
377 plausible that species with longer generation times may switch between a bivoltine and
378 univoltine life cycle at higher altitudes, for example, allowing more time for growth despite a
379 decrease in season length, thus weakening any negative cline in body size. Such a switch in
380 voltinism is predicted to result in a 'saw-tooth' body size cline (Roff 1980), and consequently

381 the slope of size change across the entire altitudinal gradient would appear shallower.
382 Similarly, although multivoltine species with very short generation times are predicted to
383 increase in size at higher colder altitudes, in species with just two generations per year,
384 variation in size at maturity may be more strongly dependent on season length than
385 temperature. Thus, these species may be more likely to exhibit a negative A-S cline. Of
386 course, the extent to which season length decreases with altitude can vary between study
387 locations, influencing the magnitude of A-S clines; for example, Chown and Klok (2003) found
388 opposing A-S clines in weevil species sampled from two regions that differed in their
389 seasonality. Although altitudinal variation in seasonality is often more pronounced at higher
390 latitudes (Körner 2000), latitude is only a proxy for seasonality, corresponding with relatively
391 broad changes in environmental conditions. We lack the high-resolution climate data,
392 including changes in season length and resource availability with altitude, that would provide
393 a much more robust test of this hypothesis. This lack of high-resolution data is particularly
394 important for mountainous regions, as fine-scale variation in environmental conditions due to
395 local topography can result in microclimates that differ from surrounding regions (Suggitt *et al.*
396 2011).

397

398 We also hypothesised that a reduction in oxygen partial pressure (i.e. a reduction in oxygen
399 availability per unit volume of air) with altitude might limit energy available for growth if species
400 cannot increase their air intake, confounding the effects of temperature on body size (Peacock
401 1998). Indeed, under experimental conditions, lower proportional oxygen concentrations have
402 been shown to lead to a reduction in size-at-stage (Frazier, Woods & Harrison 2001; Peck &
403 Maddrell 2005; Atkinson, Morley & Hughes 2006; Walczyńska *et al.* 2015). This might act to
404 weaken any potential increase in adult body size at higher colder altitudes, or even exacerbate
405 body size reduction in those species that already grow to a smaller size with decreasing
406 season length. In aquatic species, a greater increase in oxygen demand than supply with
407 warming has also been proposed as an important driver of body size reduction (Atkinson,
408 Morley & Hughes 2006), with species developing in water exhibiting particularly strong

409 reductions in size with warming and towards the equator compared to those on land (Forster,
410 Hirst & Atkinson 2012; Horne, Hirst & Atkinson 2015; Horne, Hirst & Atkinson 2017). These
411 patterns would correspond with an increase in size at higher colder altitudes, and although not
412 significant, on average aquatic species did show stronger positive A-S clines relative to
413 terrestrial species. Obtaining data for freshwater species proved extremely difficult, with many
414 studies having explored inter- as opposed to intra-specific patterns in body size.
415 Consequently, species from aquatic environments contribute only a very small proportion of
416 the data analysed here. A-S clines for aquatic species represent a significant gap in the
417 literature, and we would strongly encourage field studies to incorporate such measurements
418 in the future. We caution, however, that many insects lay their eggs upstream, which can then
419 drift downstream, making it difficult to know exactly where they developed.

420

421 Of the taxonomic orders better represented in our data set, Orthopterans exhibited a
422 particularly weak A-S cline relative to their T-S response. Although overall we found no
423 significant effect of flight ability on the magnitude of the A-S cline, Orthoptera are
424 hemimetabolous, producing nymphs that resemble the adult phase (Daly, Doyen & Ehrlich
425 1978). Although nymphs cannot fly, the increased mobility of individuals during ontogeny (c.f.
426 relatively sedentary larvae in holometabolous taxa, such as Diptera) could potentially obscure
427 A-S clines observed in this taxon. In a study of the bush cricket *Pholidoptera griseoptera*,
428 juveniles and imagos exhibited equally good dispersal ability (Diekötter *et al.* 2005), whilst
429 Alexander (1964) reported collecting many species of montane grasshopper as much as
430 several thousand feet above their normal breeding range. Consequently, altitude at time of
431 collection may not necessarily resemble altitude during ontogeny in these more mobile
432 species, increasing the likelihood of dispersal and/or migration between sampling locations.
433 Even when stricter screening criteria excluded clines covering less than 500m in altitude,
434 potentially removing some of the noise in the data caused by the movement of individuals
435 between nearby sampling locations, the estimated average A-S cline for univoltine Orthoptera
436 was still half that of their laboratory T-S response. One alternative way to examine the effect

437 of dispersal on A-S clines would be to measure body size in individuals grown in field
438 enclosures at different elevations, and which hence would not be able to disperse across
439 altitudes. Such an approach helps exclude not just the effect of dispersive individuals, but also
440 other confounding biotic factors, such as certain types of predation and competition (e.g. Joern
441 & Klucas 1993; Blanckenhorn 1998; Darr *et al.* 2016). However, lack of such data prevented
442 a quantitative investigation of species with highly mobile juveniles reared inside enclosures
443 throughout their ontogeny at natural rather than manipulated densities, and which would allow
444 body size to be compared within versus outside enclosures across a suitable altitudinal range.

445

446 Given that altitudinal environmental changes can differ substantially between study locations,
447 currently it seems that only broad predictions can be made about the direction of A-S clines.
448 Although altitude correlates with average temperature, it also correlates with many other
449 environmental parameters, and disentangling their effects is challenging. For example, a
450 reduction in the partial pressure of respiratory gases with altitude not only reduces oxygen
451 availability, but also serves to reduce overall air density (Hodkinson 2005), which can be
452 particularly problematic for active fliers. Selection in these species may favour larger wings
453 with greater surface area to maintain flight performance at higher altitudes, or alternatively,
454 smaller body mass to reduce wing loading (Hodkinson 2005). A recent study also showed that,
455 on average, predation risk for insects increases with decreasing latitude and elevation (Roslin
456 *et al.* 2017), and greater predation risk at lower altitudes may select for earlier maturation at a
457 smaller adult size. To more accurately predict the magnitude of intraspecific body size clines,
458 a detailed understanding of the life history of the species or taxonomic group in question,
459 coupled with fine scale information on local environmental conditions and topography, should
460 be provided on a case by case basis. Nevertheless, by comparing A-S clines with laboratory
461 T-S responses, in which many confounding variables are controlled, we can begin to postulate
462 which environmental factors and/or life history traits are important in driving variation in A-S
463 clines between different taxonomic groups. Although we observe a relatively close match in
464 the magnitude of A-S clines and laboratory T-S responses in some taxa (e.g. Diptera,

465 Coleoptera, Hymenoptera), suggesting a particularly important role for temperature,
466 deviations away from a 1:1 relationship in other taxa (e.g. Orthoptera) may indicate that other
467 environmental variables confound the effects of temperature on body size along elevation
468 gradients.

469

470 These observations may be particularly informative for predicting effects on body size of
471 terrestrial ectotherms that are shifting their altitudinal distribution with climate warming (Chen
472 *et al.* 2011). These range shifts may enable species to track favourable thermal environments,
473 thereby negating any potential effects of warming on body size; yet, warming of high-altitude
474 environments will not be accompanied by similar changes in other environmental parameters
475 (e.g. oxygen partial pressure, photoperiod). Therefore, in those species whose adult size is
476 influenced more by these other variables, altitudinal range shifts may still be accompanied by
477 significant changes in the size of species.

478

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613

614 **SUPPORTING INFORMATION**

615 Additional supporting information may be found in the online version of this article.

616

617 **Data Set S1.xlsx:** Raw data used in this study.

618 **Supplementary Information.pdf:** Table S1, Table S2, Figure S1 and additional length-
619 based analyses.

620

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628 **FIGURE LEGENDS**

629

630 **Figure 1.** The predicted effects of i) decreasing temperature, ii) decreasing oxygen partial
631 pressure and iii) decreasing season length with increasing altitude on size at maturity in
632 insects. Body size is predicted to increase with decreasing temperature, following the
633 temperature-size rule, particularly in multivoltine species. However, a decrease in the partial
634 pressure of oxygen at higher altitudes may reduce the available energy for growth, acting in
635 the opposite direction to constrain body size. Similarly, shorter season length is predicted to
636 decrease body size at higher altitude in univoltine species, as resource availability and time
637 available for growth is reduced.

638

639 **Figure 2.** World map indicating the location of studies (n=50) from which altitudinal-size clines
640 were recorded.

641

642 **Figure 3.** Altitudinal-size clines (% change in body mass per 150m) of individual species,
643 including both males and females, categorized by taxonomic order (Amphipoda (A), Araneae
644 (Ar), Coleoptera (Col), Diptera (Dip), Hemiptera (He), Hymenoptera (Hym), Isopoda (I),
645 Lepidoptera (Lep), Orthoptera (Orth), Plecoptera (Plec), Trichoptera (T) and Trombidiformes
646 (Tr)). Species within each order are also categorized by voltinism: one generation or less per
647 year (unshaded background), multiple generations per year (shaded background). Dashed
648 horizontal line indicates no change in body size with altitude. Dashed vertical lines divide
649 taxonomic orders.

650

651 **Figure 4.** A-S clines ($\pm 95\%$ CI), averaged by taxonomic order and by voltinism (one generation
652 or less per year (≤ 1), multiple generations per year (> 1)). The number of species within each
653 order is given in brackets. Dashed horizontal line indicates no change in body size with altitude
654 There is no significant effect of taxonomic order on the strength of the A-S cline.

655

656 **Figure 5.** Mean A-S clines ($\pm 95\%$ CI) in (A) species with one generation or less per year vs.
657 those with multiple generations per year, (B) flightless vs. flying species, and (C) freshwater
658 vs. terrestrial species. The number of A-S clines are given in brackets. Dashed horizontal line
659 indicates no change in body size with altitude. Shared letters above data points indicate no
660 significant difference.

661

662 **Figure 6.** A comparison of order-specific altitudinal-size clines (% change in body mass per
663 150m \pm SE) with laboratory temperature-size responses (% change in body mass per $^{\circ}\text{C} \pm$ SE)
664 for species with one generation or less per year (≤ 1 ; open symbols) and multiple generations
665 per year (> 1 ; shaded symbols). Dashed line indicates a 1:1 relationship. Taxonomic orders
666 which fall within the shaded quadrants exhibit a mismatch in the direction of their mean A-S
667 cline and T-S gradient.

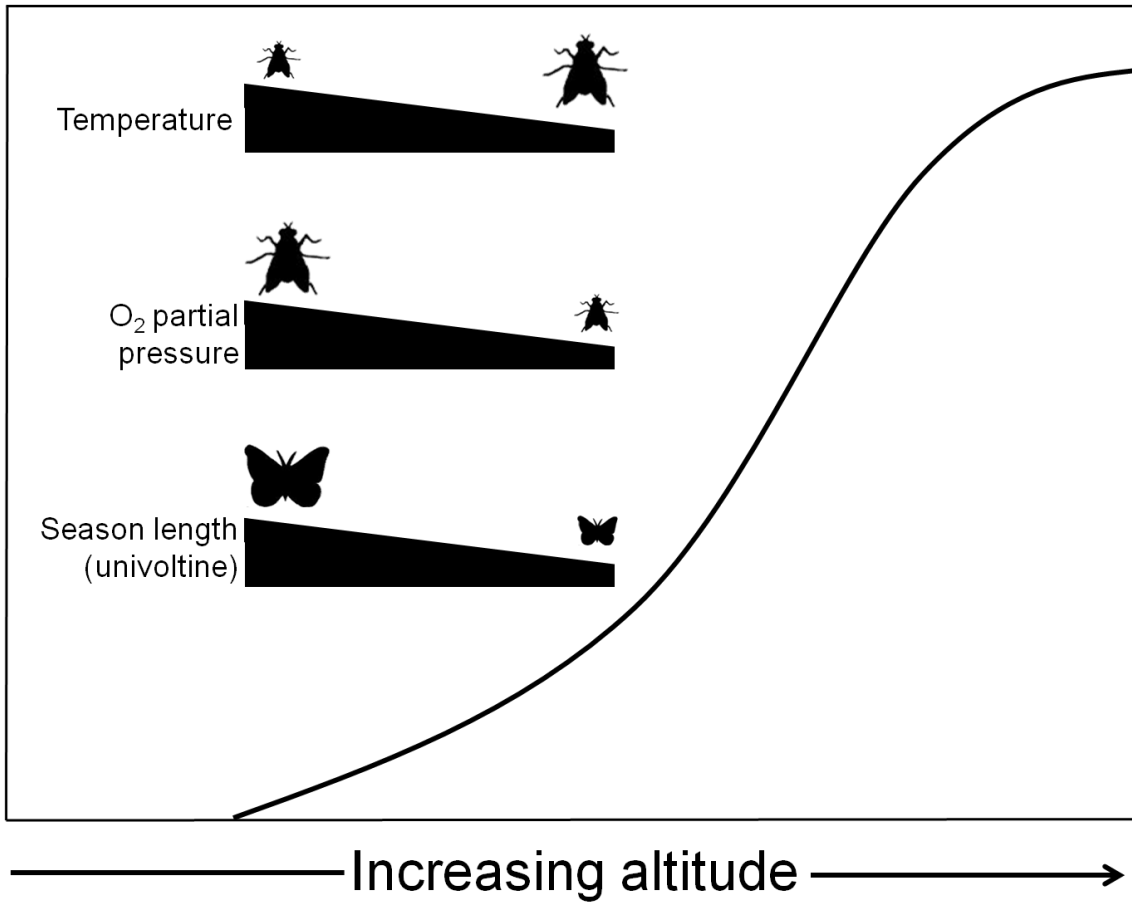


Figure 1

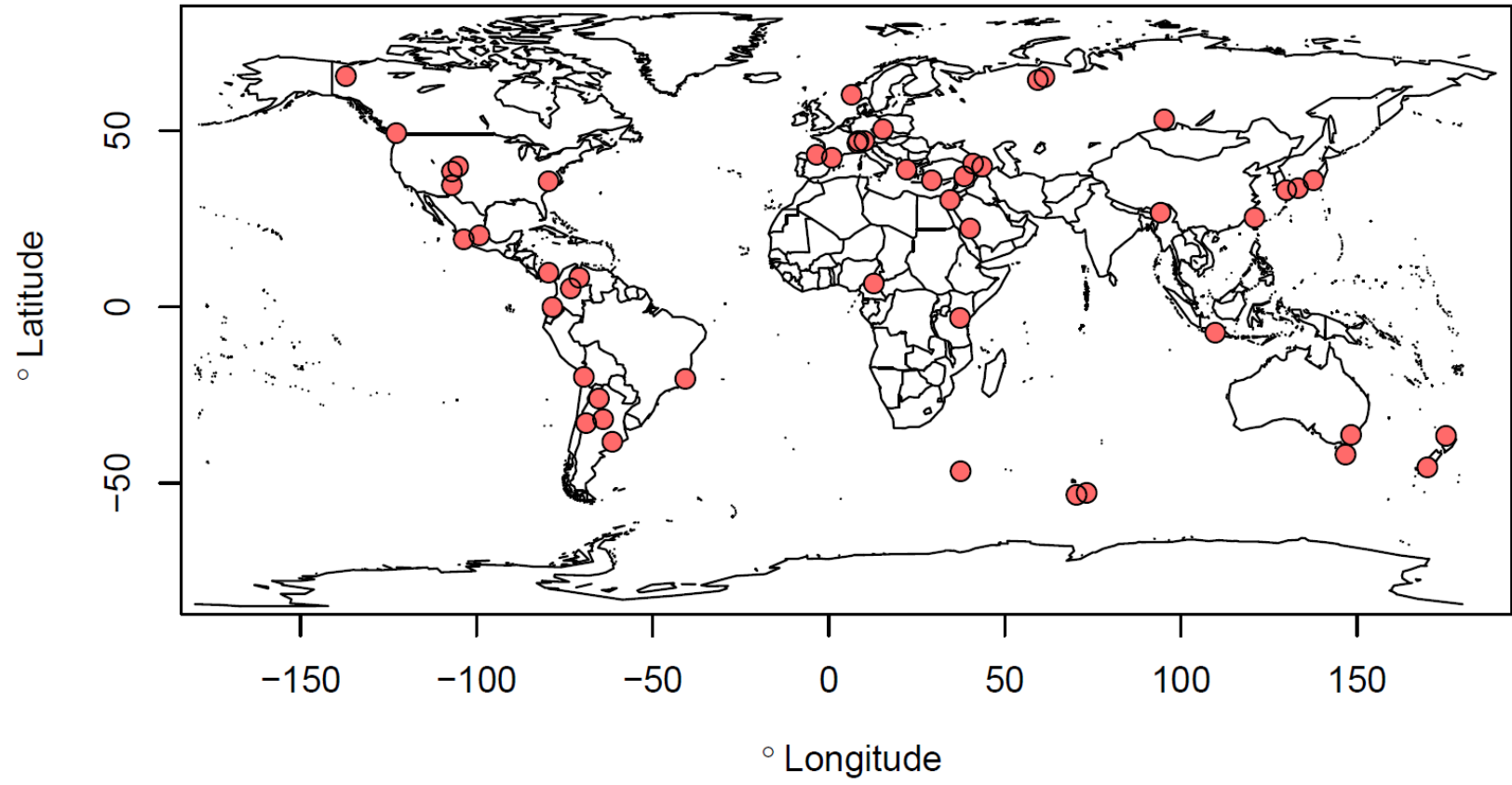


Figure 2

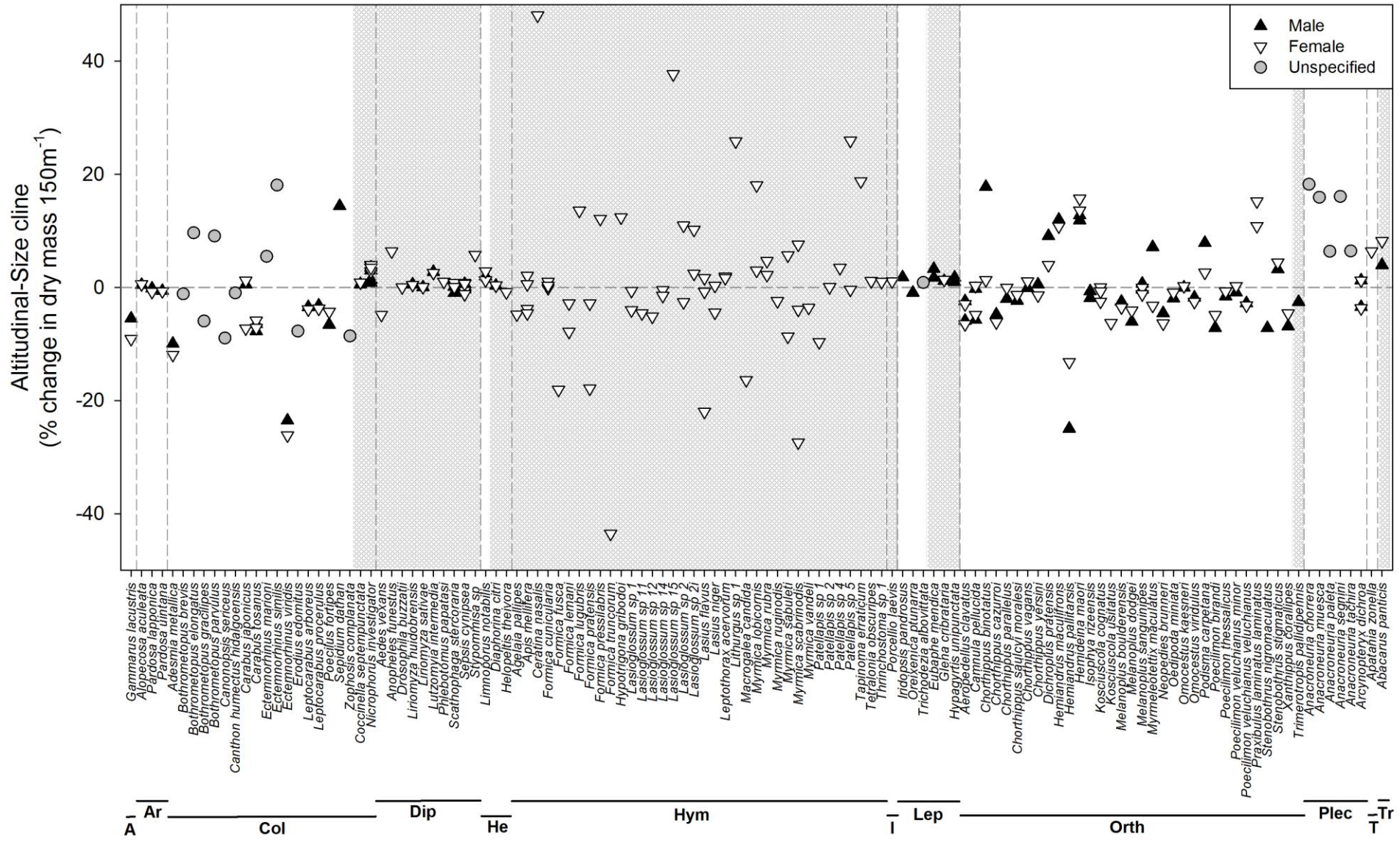


Figure 3

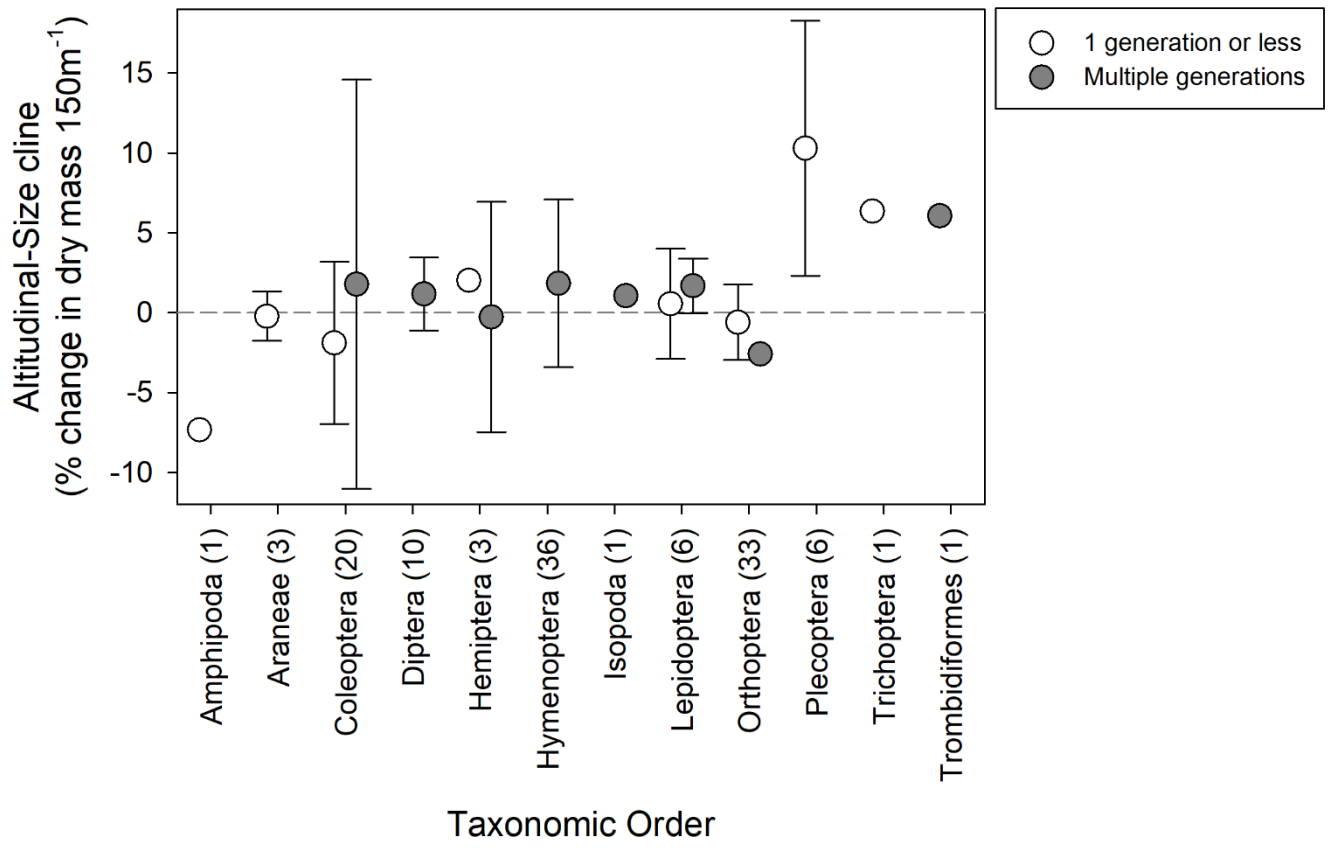


Figure 4

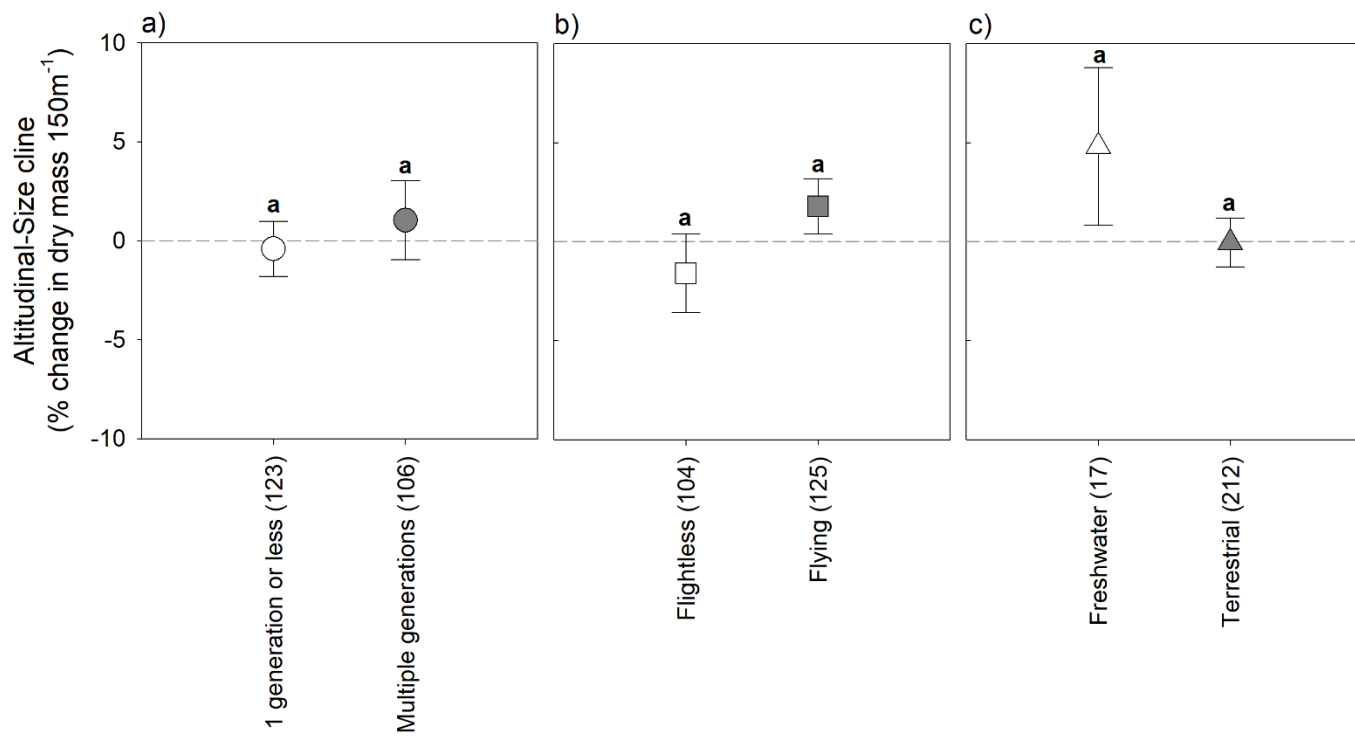


Figure 5

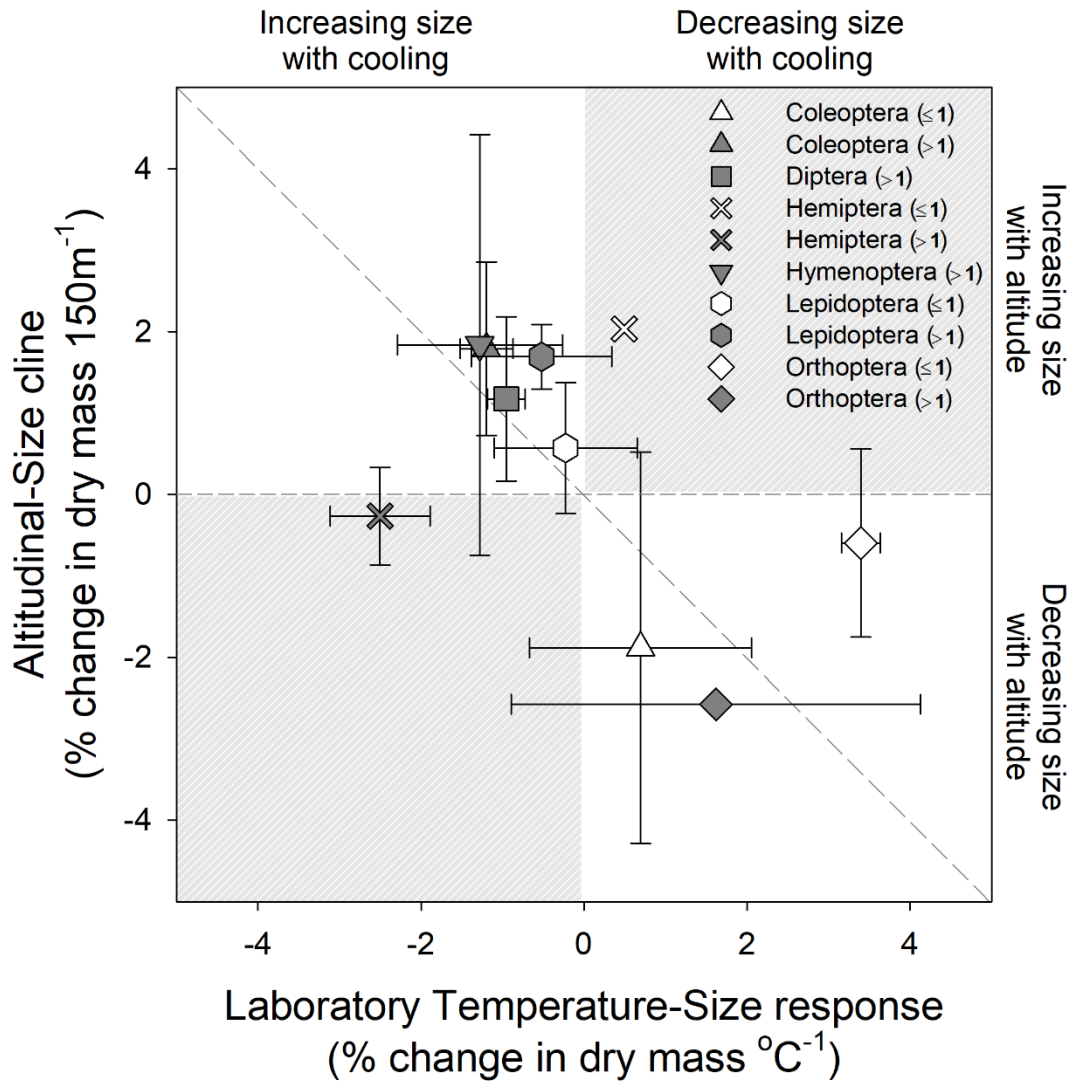


Figure 6