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**Thermal constraints on embryonic development as a proximate cause for
elevational range limits in two Mediterranean lacertid lizards**

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25 Local adaptation and range restrictions in alpine environments are central topics in
26 biogeographic research with important implications for predicting impacts of global
27 climate change on organisms. Temperature is strongly coupled to elevation and greatly
28 affects life history traits of oviparous reptiles in mountain environments. Thus, species
29 may encounter barriers for expanding their ranges if they are unable to adapt to the
30 changing thermal conditions encountered along elevational gradients. We sought to
31 determine whether thermal requirements for embryonic development provide a
32 plausible explanation for elevational range limits of two species of lacertid lizards that
33 have complementary elevational ranges in a Mediterranean mountain range
34 (*Psammodromus algirus* is found at elevations below 1,600 m and *Iberolacerta cyreni*
35 is found at elevations above 1,600 m). We combined experimental incubation of eggs in
36 the laboratory with modelled estimates of nest temperature in the field. In both species,
37 increasing temperature accelerated development and produced earlier hatching dates.
38 The species associated with warmer environments (*P. algirus*) experienced an excessive
39 hatching delay under the lowest incubation temperature. Moreover, newborns from eggs
40 incubated at low temperatures showed poor body condition and very slow rates of
41 postnatal growth. In contrast, eggs of the strictly alpine species *I. cyreni* exhibited
42 shorter incubation periods than *P. algirus* that allowed hatching before the end of the
43 active season even under low incubation temperatures. This was countered by lower
44 reproductive success at higher temperatures, due to lower hatching rates and higher
45 incidence of abnormal phenotypes. Elevational range limits of both species coincided
46 well with threshold temperatures for deleterious effects on embryonic development. We
47 suggest that incubation temperature is a major ecophysiological factor determining the
48 elevational range limits of these oviparous lizards with predictable consequences for
49 mountain distributions under future warmer climates.

50 **Introduction**

51

52 Geographic range boundaries of species are governed by environmental conditions and
53 extinction-dispersal dynamics (Brown et al. 1996). Determining whether expansion
54 beyond range boundaries is impeded by limits to physiological tolerances has important
55 implications for predicting the response of species to ecological changes generated by
56 climate change (e.g. Lee et al. 2009). Elevational gradients offer unique opportunities to
57 deduce mechanisms responsible for geographic range limits as they encompass clinal
58 variation in ecological conditions (i.e., temperature, precipitation, vegetation type) to
59 which organisms have to adjust in order to persist.

60 Ectotherms are suitable models to explore local adaptation and range restrictions
61 in alpine environments because of their dependence on environmental temperature
62 (Blanckenhorn 1998, Bird and Hodkinson 2005). In particular, lizards have often been
63 used to assess ecophysiological and behavioural responses to elevation (Van Damme et
64 al. 1989, Iraeta et al. 2006). Some species have been found to prosper exclusively at
65 high elevations, such as alpine habitats, despite the challenges that cool environments
66 pose for lizards (Carranza et al. 2004). Specialisation is thought to be a consequence of
67 evolutionary adaptation in life histories and/or physiological traits in response to past
68 episodes of geographical isolation driven by historical fluctuations in climate (Avisé et
69 al. 1998). Therefore, if adaptations that have allowed long-term persistence of species in
70 mountains involve traits that are crucial for survivorship in cold environments, they can
71 also affect current distributional limits.

72 Temperature is one of the major environmental factors that varies with elevation
73 and greatly affects life history traits of oviparous lizards. Cool environments found in
74 mountains shorten the active season available for lizards, which places specific demands

75 on reproductive strategies (Iraeta et al. 2006, Warner and Shine 2007). Low
76 temperatures prolong, and may even prevent, the successful incubation of eggs (Shine
77 2002) and can limit foraging time prior to hibernation. In response, lizards may fine-
78 tune hatching dates to enhance the performance and survival of juveniles (Braña and Ji
79 2000, Shine and Olsson 2003). Incubation temperature can also directly affect egg
80 development. Low temperatures can result in hatchlings with reduced performance and
81 fitness (Qualls and Andrews 1999) and temperatures that are too high can be
82 detrimental for development (Ji et al. 2002, Andrews 2008, Braña and Ji 2000). Thus,
83 embryonic development must be optimized in different species according to the
84 environments to which they are exposed.

85 Mechanistic models that link physiology of species and the environmental
86 factors limiting distributions provide a valuable tool to generate more realistic
87 assessments of the likely response of species to contemporary climate change (Kearney
88 and Porter 2004, Buckley 2008). These assessments can then be used to develop
89 management strategies that minimise the impacts of climate change (Mitchell et al.
90 2008). Few studies have incorporated thermal requirements of eggs and embryos into
91 mechanistic models. This is despite recognition that climatic effects on egg
92 development may be equally important as effects on adult stages in determining range
93 limits (Lourdais et al. 2004, Parker and Andrews 2007). Empirical data that link
94 incubation temperature with reproductive success are needed to test specific hypotheses
95 about the role of reproductive physiology of lizards in shaping their range limits.
96 Experimental approaches can be very useful for quantifying physiological sensitivity to
97 incubation temperature (Braña and Ji 2000, Andrews 2008), but the results are best
98 interpreted in the context of environmental exposure directly measured in the field
99 (Williams et al. 2008).

100 Our goal was to determine whether thermal requirements of embryonic
101 development provide a plausible explanation for distribution limits of two species of
102 lacertid lizards that have mutually exclusive, almost complementary elevational ranges
103 in the Sistema Central Mountain Range, Spain. More specifically, we addressed the
104 following questions: 1) does incubation temperature affect lizard reproductive success
105 through its effects on egg development (i.e., by affecting hatching success or causing
106 abnormal hatchling phenotypes) and/or by modifying hatching dates and growth rates of
107 juveniles; 2) if affirmative, do our study species respond similarly to variations in
108 incubation temperature; and 3) to what extent do elevational ranges of lizards coincide
109 with limits predicted from incubation experiments and actual temperature variation in
110 probable nesting sites in the field?

111

112 **Materials and methods**

113

114 Study species and study area

115 The two species examined in this study are distantly related (Arnold et al. 2007),
116 heliothermic, actively foraging, diurnal lacertid lizards found in the Sistema Central
117 Mountain Range. The species have different elevational ranges and are associated with
118 different habitats, although in the study area their ranges are separated by a short
119 distance (ca. 5 km). The Rock lizard *Iberolacerta cyreni* (mean adult snout vent length
120 [SVL] = 68.1 mm males, 70.2 mm females; range = 65-80 mm) is an alpine species
121 endemic to the Sistema Central, and it is only found above 1,600 m, preferring rocky
122 outcrops and mixed rock-shrub formations (Monasterio et al. 2010). The Large
123 Psammodromus *Psammodromus algirus* (mean adult SVL = 75 mm males, 75.9 mm
124 females; range = 65-90 mm) inhabits shrub and forested habitats throughout the entire

125 Iberian Peninsula, south-eastern coastal region of France and north-west Africa (Díaz
126 and Carrascal 1991). This species is linked to warmer habitats and is absent from the
127 highest elevations, reaching its upper elevational limit in the Sistema Central at *ca* 1,600
128 m.

129 Field work was conducted in the Sierra de Guadarrama, which experiences cold
130 wet winters and short dry summers. The mountain base (1,200-1,700 m a.s.l.) is covered
131 with deciduous Pyrenean oak (*Quercus pyrenaica*) forests, which is progressively
132 replaced by Scots pine (*Pinus sylvestris*) forest at higher elevations. Forest occurs from
133 1,500 to 2,100 m a.s.l., gradually becoming less dense at higher elevations until it is
134 dominated by a mosaic of dense mixed-shrub formations of perennial *Juniperus*
135 *communis* and *Cytisus oromediterraneus* interspersed with small meadows of *Festuca*
136 and other grasses. Alpine areas above the tree line (1,700-2,300 m a.s.l.) are also
137 characterized by extensive patches of large granite rocks and scree interspersed among
138 shrub formations.

139

140 Collection and husbandry of adults, eggs and juveniles

141 Between May and June 2008, we noosed 40 adult lizards (*P. algirus*: 15 females and 5
142 males; *I. cyreni*: 14 females and 6 males) that were transported to the lab (Department
143 of Zoology, Universidad Complutense de Madrid) on the day of capture. Lizards were
144 housed in white opaque wall terraria (40 × 60 × 30 cm; 2 females per cage). A single
145 male was placed in each of a subset of cages where we could not confirm the presence
146 of oviductal eggs in females at the time of capture). Cages were covered by a green net
147 (0.5-cm mesh) that prevented escape. The mesh provided a shrubby-like shelter while
148 still allowing daylight to enter the cages. Terraria were filled with moistened earth
149 averaging 10 cm in depth, covered by the appropriate vegetation type according to the

150 species inhabiting it. A 60 W lamp suspended over one end of the cage created a
151 photothermal gradient (ca. 25-50°C) allowing thermoregulation within the preferred
152 temperature range (Díaz and Cabezas-Díaz 2004). UV light was also provided. An
153 earthenware tile (ca. 10 × 15 cm) and a thin section of fallen wood provided additional
154 shade and shelter. Lizards were fed crickets (*Acheta domesticus*) and mealworms
155 (*Tenebrio molitor*), dusted with a commercial vitamin and calcium supplement
156 delivered according to manufacturer's recommendations. All cages were watered *ad*
157 *libitum*.

158 Gravid females were monitored daily to detect changes in body shape or body
159 mass that might indicate egg-laying. When it was clear that a female had laid, eggs
160 where located, removed from the cage, counted, weighed and individually placed in 150
161 ml plastic cups filled with ca. 35 g of moistened vermiculite (10 g vermiculite: 8 g
162 water, which is equivalent to ca. – 200 kPa; Tracy 1980). Eggs were completely covered
163 by the vermiculite, and the jars were closed with a tightly fitting screw top to minimize
164 evaporative water loss. Eggs of the same clutch were distributed evenly over three
165 incubators (MMM Friocell™, Germany) set at constant temperatures of 24, 28 and 32
166 °C. Searches for new hatchlings were conducted daily toward the end of the incubation
167 period. Hatchlings were weighed, measured (snout-vent length, SVL), and individually
168 marked by toe clipping. The incubation period (in days) was calculated as the time
169 elapsed between egg deposition and hatching. After females laid their eggs, all lizards
170 were released at their site of capture.

171 We reared newborns (N = 104) within individual plastic terraria (265 mm length
172 x 162 mm width x 150 mm height) that offered rock and sand substrates. A 60W lamp
173 25 cm above the rock acted as a heat source allowing lizards to bask and UV light was
174 also provided. Juveniles were fed and watered as per adults. To monitor growth of

175 juveniles, we measured (to the nearest mm) and weighed (to the nearest mg) all lizards
176 every four days for a total of 28 days. Size and mass specific growth of juveniles in
177 their first 4 weeks of life was estimated using the formula

$$178 \text{ growth rate} = \ln(S \text{ at day 28} / S \text{ at hatching}) / 28,$$

179 where S is the SVL or weight value. These estimates reflect the proportionate increase
180 in size or mass on a per-day basis (Sinervo 1990). At the completion of the experiment
181 all juveniles were released at their mother's site of capture.

182

183 Presence/absence data

184 We conducted intensive field sampling of the two species throughout the Sierra de
185 Guadarrama. Standardised surveys were conducted between 2006 and 2009 on sunny
186 days during the lizards' active season (i.e. May to September). We recorded all lizards
187 observed along 200 m transects. We supplemented census data with capture records
188 from other studies we carried out in the Sierra de Gredos, which is also part of the
189 Sistema Central. Additionally, we used presence data of the two species in 1x1 km
190 UTM grid cells from the 'Amphibian and reptile database' provided by the Asociación
191 Herpetológica Española for the whole Sistema Central Mountain Range.

192

193 Incubation temperatures available in the field and temperature surfaces

194 Our analysis required incubation temperature to be estimated for an extensive set of
195 geographic locations in the Sierra de Guadarrama where lizards have been found to be
196 present. This was achieved by interpolating point estimates of soil temperature directly
197 quantified in the field. We sampled representative habitats and environments spread
198 throughout the mountain range between 1,200 and 2,300 m a.s.l. Sampling covered an
199 area of ca. 200 km² encompassing all the major alpine and subalpine habitat types in the

200 region. Sampled habitats included rock outcrops with mixed-shrub formations,
201 transition and pure pine forests, and oak forests. Our sampling design incorporated
202 different combinations of elevation, cardinal orientation, slope, and habitat structure.

203 Unfortunately, information on nesting location is lacking for these species.
204 Therefore, at each location, we quantified soil temperature for a range of plausible
205 nesting microhabitats. Thermochron Ibutton temperature loggers (N = 119) were placed
206 at 5 cm depth in soil under three different levels of exposure: bare ground, below rocks
207 with a diameter of ca. 30 cm, and in the centre of shrubs with a diameter of 80-100 cm.
208 Bare and rock exposures were located in open areas. Temperature was recorded every
209 hour (N = 214,848 records across all sites) during the breeding season when eggs were
210 incubated in the wild (late June – early September) and used to estimate the average
211 ground temperature at each location and exposure level (20 June-15 of September;
212 average duration of data collection for all loggers was 79 days \pm 1 day). In total, we
213 obtained 32 estimates of temperature for bare ground, 39 for under rock, and 37 for
214 under shrub (N = 108 loggers).

215 Since the final objective was to estimate average daily soil temperature across
216 the breeding season at locations sampled for lizards (i.e. where direct measurements of
217 soil temperature were not always available), we generated continuous temperature
218 surfaces. This was achieved using field estimates of temperature from loggers
219 previously described and spatial information on independent variables that can
220 potentially serve to modify local temperatures. To predict average daily soil
221 temperatures we derived separate linear regression models for each exposure level,
222 using the formula

223 $\text{Temperature}_{\text{soil}} = a + bE + cS + dC,$

224 where E is elevation, S is solar radiation and C is canopy cover. For a given location,
225 soil temperature values were obtained from the logger measurements, and data on each
226 of the independent variables were extracted from GIS layers as follows: *Elevation* –
227 Elevation was generated from a 90 m resolution digital elevation model (STRM 90m
228 DEM; available from CGIAR-CSI, Jarvis et al. 2008). *Solar radiation* - Solar irradiation
229 (Wh.m⁻².day⁻¹) under clear sky conditions was estimated across the region for the
230 middle day of June, July, August and September using the 90 m resolution DEM and
231 the analysis package *r.sun* (Neteler and Mitasova 2004) in GRASS GIS. Solar
232 irradiation was derived from the sum of all three components of global radiation (beam,
233 diffuse and reflected) calculated at half hourly time steps. The command *r.sun*
234 specifically included the shadowing effect of topography which has the potential to
235 ameliorate exposure to solar radiation in mountainous regions. Monthly estimates of
236 solar radiation were averaged to yield a single estimate of exposure for the time period
237 when eggs are incubated in the wild. *Canopy cover* - Canopy cover was derived from
238 the digitalized vegetation map of Spain (Mapa Forestal de España 1:200.000, provided
239 by the Ministerio de Medio Ambiente y Medio Rural y Marino), that quantified
240 variation in the percentage of tree cover in the study area.

241 Once multiple regression models were obtained, they were extrapolated to the
242 whole study area to generate continuous temperature surfaces for each level of
243 exposure. Data manipulation and regression analyses were performed in ArcGIS and R
244 (<http://www.R-project.org>).

245 We then overlaid occupied locations for both lizard species on each temperature
246 surface and extracted the predicted soil temperatures at these locations. The same
247 process was repeated for a background set of available locations. We used a ‘target-
248 group’ background that was simply a collation of all presence locations of any lizard

249 species in our data set (i.e., locations known to have been sampled for lizards). The
250 ‘target-group’ approach is preferred to a random background because it is better able to
251 cope with spatial bias in sampling effort (Phillips et al. 2009).

252

253 **Results**

254

255 Hatching success

256 We incubated a total of 138 eggs of the two species at three temperatures (Fig. 1). A
257 log-linear analysis of frequencies, with hatching success as the response categorical
258 variable, and species and temperature as the design factors, indicated that the effect of
259 temperature on hatching success differed between the two species ($\chi^2_2 = 6.11$, d.f. = 2,
260 $P = 0.047$). Hatching success of *I. cyreni* was lower at the highest experimental
261 temperature (Fig. 1; lower [24 + 28°C] vs. higher [32°C] temperatures: $\chi^2_1 = 5.25$, $P =$
262 0.022). Temperature had no effect on hatching success in *P. algirus* ($\chi^2_1 = 1.75$, $P =$
263 0.186).

264

265

266 Maternal investment

267 Species differed in mean egg mass (ANCOVA with SVL as the covariate; species
268 effect: $F_{1,19} = 16.66$, $P < 0.001$) with *I. cyreni* laying heavier eggs (mean egg mass \pm 1
269 SE = 0.46 ± 0.02 g) than *P. algirus* (0.36 ± 0.02 g). Clutch size was slightly larger for *P.*
270 *algirus* (6.66 ± 0.41 eggs) than for *I. cyreni* (6.60 ± 0.45 eggs; ANCOVA with SVL as
271 the covariate; species effect: $F_{1,19} = 4.492$, $P = 0.047$). *Iberolacerta cyreni* laid more and
272 larger eggs than *P. algirus*, and its overall reproductive investment (i.e., clutch mass:
273 3.04 ± 0.16 g or 36.5 % of female body mass) was markedly higher than that of *P.*

274 *algirus* (2.40 ± 0.14 g or 24.4 % of female body mass; ANCOVA with SVL as the
275 covariate; species effect: $F_{1,19} = 28.95$, $P < 0.0001$).

276

277 Incubation period

278 Incubation time differed greatly among species and temperatures (two-way ANOVA of
279 data shown in Fig. 2: temperature: $F_{2,101} = 7853.54$; species: $F_{1,101} = 22684.30$; species
280 x temperature: $F_{2,101} = 1202.01$; all P 's < 0.001). Lower temperatures resulted in longer
281 incubation periods, with eggs reared at 24°C taking 1.79 times longer to incubate than
282 those reared at 32°C, and incubation times were 54% shorter for *I. cyreni* than for *P.*
283 *algirus*. The significant interaction between species and temperature indicated that the
284 intensity of the temperature affect on incubation times differed between both species
285 (Fig. 2); *P. algirus* was more strongly affected by changes in incubation temperature
286 than *I. cyreni*.

287 Species also differed in their laying dates (ANOVA: species: $F_{1,151} = 10503.41$,
288 $p < 0.001$; mean laying dates were the 24th of June and 10th of July for *P. algirus* and *I.*
289 *cyreni*, respectively). Hatching date differed between species and temperatures (two-
290 way ANOVA of data shown in Figure 3: temperature: $F_{2,101} = 7946.46$, $p < 0.001$;
291 species: $F_{1,101} = 4816.63$, $p < 0.001$; species x temperature: $F_{2,101} = 1131.99$, $p <$
292 0.001). Higher incubation temperatures resulted in earlier hatching dates (mean hatching
293 dates were the 7th and 16th of August, and 5th of September for 32°C, 28°C and 24°C,
294 respectively) and *I. cyreni* hatched earlier (13th of August), despite having a later mean
295 laying date than *P. algirus* (26th of August). Again, the significant interaction showed
296 that the effect of temperature on laying date was more pronounced for *P. algirus* (mean
297 difference of 40 days between 24°C and 32° C) than for *I. cyreni* (mean difference of 18
298 days between 24°C and 32° C).

299

300 Viability of hatchlings

301 Nine *I. cyreni* neonates were born with abnormalities characterized by immobility of
302 one or two of the forelimbs or an inability to coordinate head movements that hindered
303 feeding and ultimately resulted in mortality. Severity of abnormalities varied among
304 individuals; in some cases, abnormalities were only detected when we observed
305 difficulty feeding of some juveniles. Only one affected lizard (with an immobile
306 forelimb) survived throughout the experiment. The remainder died before the end of the
307 experiment and were classified as non viable juveniles. Unaffected juveniles displayed a
308 normal morphology and feeding behaviour and stayed healthy throughout the
309 experiment. Abnormalities were observed in five of the ten *I. cyreni* clutches. Visual
310 inspection of data suggested a negative effect of incubation temperature on offspring
311 viability (Fig. 1). We estimated for each temperature treatment the proportion of viable
312 juveniles out of the total amount of incubated eggs. The significant result ($\chi^2_2 = 7.544$,
313 $P = 0.023$) confirmed that increased incubation temperatures had a negative effect on
314 juvenile survival in this species (78, 63, and 38% viable juveniles at 24, 28, and 32°C,
315 respectively).

316

317 Size, body condition and growth rates of juveniles

318 Body size (SVL) at hatching was larger for *I. cyreni* than for *P. algirus*. Increased
319 incubation temperature produced smaller juveniles in both species (Table 1 and Fig. 4).
320 Body condition at hatching (residuals of log-body mass on log-SVL) did not differ
321 between species, and the main effect of temperature on hatchling body condition was
322 marginally non-significant. However, the interaction between species and temperature
323 was significant (Table 1). Body condition of *I. cyreni* neonates decreased considerably

324 at higher incubation temperatures. The opposite trend was apparent for *P. algirus* (Fig.
325 4).

326 At the end of the experiment (i.e., at an age of 28 days), body condition differed
327 between species and incubation temperatures (Table 1). In both species, juveniles had a
328 better body condition when they came from eggs incubated at higher temperatures, but
329 once again the negative effect of incubation at low temperature was much more
330 pronounced for *P. algirus* than for *I. cyreni* (Fig. 4).

331 Size and mass specific growth rates also differed between species and incubation
332 temperatures (Table 2 and Figure 5). Overall, SVL-specific growth rate was highest for
333 *P. algirus* at an intermediate (28°C) incubation temperature; in *I. cyreni*, growth rate
334 was largely independent of incubation temperature. Mass-specific growth rate differed
335 between species and temperatures, being larger for *I. cyreni* than for *P. algirus* and
336 increasing at higher temperatures. In addition, there was a significant species x
337 temperature interaction (Table 2); *P. algirus* was much more affected by low
338 temperatures, with hatchlings incubated at 24 °C exhibiting very low mass-specific
339 growth rates (Fig. 5).

340

341 Characterization of incubation temperature in the field

342 Linear regression models explained between 63 and 68% of the variation in soil
343 temperature, depending on the level of microhabitat exposure. Soil temperature on bare
344 ground ($R^2 = 0.68$, $F_{3,28} = 19.90$, $p < 0.0001$) was negatively correlated with elevation
345 ($\beta = - 0.86$, $p < 0.001$) and canopy cover ($\beta = - 0.37$, $p < 0.012$), but positively
346 associated with solar radiation ($\beta = 0.35$, $p < 0.003$). The same pattern was obtained for
347 soil temperature under rocks ($R^2 = 0.68$, $F_{3,33} = 19.90$, $p < 0.0001$; elevation: $\beta = - 0.86$,
348 $p < 0.0001$; canopy cover: $\beta = - 0.30$, $p < 0.025$; solar radiation: $\beta = 0.31$, $p = 0.004$).

349 However, the predictive model for soil temperature under shrubs ($R^2 = 0.63$, $F_{3,32} =$
350 17.87, $p < 0.0001$) included only the effects of elevation ($\beta = -0.63$, $p < 0.0001$) and
351 solar radiation ($\beta = 0.36$, $p = 0.004$), possibly because shrub cover negated the potential
352 effects of canopy cover.

353 Predicted soil temperatures during the breeding season at occupied locations
354 were consistently lower in microhabitats sheltered by shrubs and higher in rocky or bare
355 ground situations (Table 3). Soil temperatures under different levels of exposure ranged
356 from 20.02 to 27.02°C at locations occupied by *I. cyreni* and 27.02 to 35.99 °C at
357 locations occupied by *P. algirus*. Overall, however, soil temperatures were strongly
358 biased toward cool temperatures for *I. cyreni* (below 24°C) and the warm temperatures
359 for *P. algirus* (above 24°C except for microhabitats sheltered by shrubs, Table 3, Figure
360 6).

361

362 **Discussion**

363

364 Our results support a direct connection between elevational range limits and conditions
365 required for embryonic development in two species of lizards. Both species showed a
366 strong response to incubation temperature with higher temperatures leading to earlier
367 hatching dates. This is consistent with studies elsewhere (Packard and Packard 1988,
368 Van Damme et al. 1992). The species associated with warmer environments (*P. algirus*)
369 experienced an excessive hatching delay under low incubation temperatures that would
370 have prevented successful emergence during the active season. In contrast, the species
371 linked to cold habitats (*I. cyreni*) showed shorter incubation periods at all temperatures,
372 an obviously advantageous trait in an environment where the active season is limited,
373 but exhibited reduced juvenile viability at high temperatures. In the following section,

374 we provide a detailed discussion of the ecophysiological mechanisms that can restrict
375 lizard distribution.

376

377 **Life history strategies and incubation temperature dependence: mechanisms of**
378 **range restriction**

379 *Incubation time and emergence timing: the challenge of alpine environments*

380 It is common knowledge that prolonged incubation under cool conditions can delay
381 hatching, reduce hatching success, and produce low-quality hatchling phenotypes
382 (Shine 2002, Braña and Ji 2000). Our experimental results revealed a strong effect of
383 temperature on incubation time and hatching date in both species. However, the lower
384 elevation species was disproportionately affected. *Psammodromus algirus* incubated
385 under cold regimes were born with a reduced body condition and too late in the active
386 season. This is despite some evidence that this species can adjust life history traits to
387 reduce incubation periods at higher latitudes (Díaz et al. 2007) or altitudes (Iraeta et al.
388 2006). Low temperatures delayed hatching until mid-September which is considerably
389 later than documented hatching dates for the species in the study area (i.e., second week
390 of August, Iraeta et al. 2006). In addition, juveniles showed very low activity rates and
391 grew at a strikingly slow rate during the first four weeks of their life, which led to a very
392 poor body condition which can compromise their overwinter survival (Civantos and
393 Forsman 2000).

394 In contrast, even the lowest temperature used in the experiment resulted in
395 adequate hatching dates for *I. cyreni* - the species inhabiting colder environments in the
396 study. Neither high nor low experimental incubation temperatures seemed to limit
397 hatching time for this species. It has been proposed that, because the thermal regimes of
398 cold nest sites are less favourable to embryogenesis than those experienced by oviductal

399 eggs, females could choose to retain their eggs and lay them in a more advanced
400 developmental stage (Qualls and Shine 1998, Braña and Ji 2007). Unfortunately, we
401 currently lack data on development stage at oviposition to assess whether this
402 phenomenon is contributing to the disparity in hatchling dates observed in our study.
403

404 *High incubation temperatures and alpine confinement: costs on hatching success and*
405 *viability*

406 Increased incubation temperatures reduced hatching success and produced abnormal
407 phenotypes of *I. cyreni* that died before the completion of the experiment, reducing the
408 overall reproductive success at higher temperatures. In reptiles, early events of
409 embryogenesis are involved in tissue differentiation and organogenesis to ensure the
410 production of viable phenotypes, whereas late development is characterized by growth
411 in size and physiological changes (Deeming and Ferguson 1991). Nevertheless, it has
412 also been demonstrated that temperature variation during the external incubation period
413 can affect some morphological features of hatchling lizards (Braña and Ji 2007). This
414 could be the case for *I. cyreni*, in which exposure to high temperatures during the post-
415 laying period of egg development imposed severe costs on the viability of offspring.

416 The lethal effect of high temperatures on embryos has been studied in some species, and
417 the upper threshold seems to be relatively lower in lizards inhabiting cold habitats (as it
418 is the case for *I. cyreni*) than in lizards occupying warmer areas (Ji et al. 2002, Lu et al.
419 2006). Although it is known that too high incubation temperatures generate deformities
420 in embryos (Ji et al. 2002), there is a lack of studies documenting temperature-induced
421 phenotypic abnormalities in newborns. Thus, we hope that our results will help draw
422 attention to this potentially interesting thermal effect on the development of reptilian
423 hatchlings.

424

425 **Importance of incubation temperature in constraining distribution limits**

426

427 The temperature value of 24°C was a critical threshold in this study for the following
428 reasons: 1) it was the experimental temperature under which the reproduction of *P.*
429 *algirus* was time-constrained due to overextended incubation; and 2) it was the
430 incubation treatment above which hatchlings of *I. cyreni* exhibited abnormal
431 phenotypes. This threshold value, and associated constraints on embryonic
432 development, coincided remarkably well with predicted soil temperatures at potential
433 nesting sites for both species. That is, soil temperatures at sites occupied by *P. algirus*
434 and *I. cyreni* were typically above and below, respectively, the threshold temperature
435 providing strong support for the hypothesis that incubation temperature is acting to
436 constrain range limits. There were, however, some anomalies in the match between
437 embryonic development and local temperature that arose directly from differences in
438 microhabitat exposure. Shrubs provided microhabitats that were substantially cooler
439 than bare ground or rocks located in open areas. Our predictions suggest that shrubby
440 microhabitats within a large portion of the range of *P. algirus* would be too cold to
441 facilitate complete embryonic development within the activity period. This would
442 suggest that there may be strong selection acting on gravid females to avoid laying eggs
443 in areas densely covered by vegetation. Indeed, nest site selection to ameliorate local
444 exposure to deleterious conditions has been demonstrated in lizards elsewhere (Doody
445 2009, Warner and Shine 2007). For *I. cyreni*, there is a striking reduction in the
446 frequency of occupied locations as temperatures approach 24°C at potential oviposition
447 sites on bare ground and under rock. However, similar temperatures in shrub
448 microhabitats extend to lower elevations, but are apparently not used by this species.

449 This suggests that there may be some sort of avoidance on the part of *I. cyreni* for
450 laying eggs in this type of microhabitat. Unfortunately, despite several years of
451 intensive investigation, no nests of either species have been located in the field. Thus, it
452 is not yet possible to evaluate microhabitat based nest site avoidance.

453 A limitation of this study is that temperatures chosen for the laboratory
454 experiment only spanned a subset of the temperatures experienced by lizards in the
455 field. Nevertheless, it is remarkable how well approximate thresholds for physiological
456 development documented in the laboratory matched predicted temperatures within
457 range limits. The case of *I. cyreni*, the alpine endemic, is especially illustrative. Several
458 potential mechanisms underlying its lower elevational limit have been proposed
459 (Monasterio et al. 2010), but a proximate cause has not been substantiated. Neither
460 competition with wall lizards (Monasterio et al. 2010) nor thermal quality of the habitat
461 for adults (Monasterio et al. 2009) provide an adequate explanation for the distribution
462 pattern of *I. cyreni* in this mountain range, although thermal constraints linked with the
463 scarcity of appropriate refuges may partially explain the barrier effect caused by the
464 pine-forest vegetation belt (Monasterio et al. 2009). Temperatures examined in the
465 experiment reported here showed a reduction in overall viability of hatchlings at
466 incubation temperatures equal or higher (at least for bare ground and rock substrates)
467 than those found at the lower elevational range limit of *I. cyreni*. The ecophysiological
468 adaptations to alpine environments, attained after long-term persistence in mountains
469 (Carranza et al. 2004), may therefore prevent these lizards from colonizing lower
470 elevations. On the other hand, *P. algirus*, which is linked to warmer habitats than *I.*
471 *cyreni*, was generally found above the predicted temperature threshold. Our findings
472 corroborate previous studies in which low temperatures at available nesting sites were

473 found to limit the distribution ranges of other temperate lizards (Hare et al. 2004, Parker
474 and Andrews 2007).

475

476 **Implications for global warming**

477

478 Regional temperatures in Mediterranean mountains are projected to rise considerably
479 over the coming century (Nogués-Bravo et al. 2007). This is expected to have global
480 effects on component ecosystems, with temperature specialists being particularly
481 vulnerable (Ghalambor et al. 2006). We have described a mechanism whereby ground
482 temperature at incubation sites may determine the range limits of two lizard species in
483 the Sierra de Guadarrama. From our results, we predict that contemporary climate
484 warming will result in an upward elevational shift and reduction in range size in *I.*
485 *cyreni*, which is already listed as Endangered in recent IUCN Red Lists because of its
486 limited extent of occurrence and acute fragmentation of its range. Theoretically, rock
487 lizards could compensate for temperature changes by modifying their nesting behaviour
488 (e.g., laying eggs under shrubs), as has been suggested for other species (Doody et al.
489 2006, Telemeco et al. 2009). However, the species does not appear to take advantage of
490 existing, thermally suitable shrub habitat beyond its current lower elevational range
491 limit. This suggests a low capacity to ameliorate the negative effects of warm
492 temperatures by actively selecting cooler microhabitats. Conversely, we predict a
493 population expansion of the lowland species *P. algirus* towards higher-elevation
494 habitats as warmer temperatures and longer activity periods favour embryonic
495 development.

496 Information on the phenotypic plasticity of embryonic development in *I. cyreni*,
497 for example populations living at different elevations, would help to distinguish

498 between environmental and population-specific (e.g., genetic) sources of geographical
499 variation (Bronikowski 2000, Iraeta et al. 2006). We recommend assessing the levels of
500 gene flow among central populations, well adapted to alpine conditions, and marginal
501 ones located at lower elevations. This would help clarify the mechanisms preventing
502 local adaptation beyond range limits, and to discern if the mutational input from central
503 into marginal populations is limiting the expansion of locally beneficial alleles, thus
504 hampering adaptation and restricting their potential distribution range (Bridle and Vines
505 2006).

506

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516 **References**

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518 Andrews, R.M. 2008. Effects of incubation temperature on growth and performance of
519 the veiled chameleon (*Chamaeleo calyptratus*). - J. Exp. Zool. 309A : 435–446.
520 Arnold, E.N. et al. 2007. Systematics of the Palearctic and Oriental lizard tribe
521 Lacertini (Squamata: Lacertidae: Lacertinae), with descriptions of eight new
522 genera. - Zootaxa 1430: 1-86.

- 523 Avise, J.C. et al. 1998. Speciation durations and Pleistocene effects on vertebrate
524 phylogeography. - Proc. R. Soc. B 265: 1707-1712.
- 525 Bird, J.M. and Hodkinson, I.D. 2005. What limits the altitudinal distribution of
526 *Craspedolepta* species (*Sternorrhyncha: Psylloidea*) on fireweed? - Ecol.
527 Entomol. 30 510-520.
- 528 Blanckenhorn, W.U. 1998. Altitudinal differentiation in the diapause response of two
529 species of dung flies. - Ecol. Entomol 23: 1-8
- 530 Braña, F. and Ji, X. 2000. Influence of incubation temperature on morphology,
531 locomotor performance, and early growth of hatchling wall lizards (*Podarcis*
532 *muralis*). - J. Exp. Zool. 286 4: 422-433.
- 533 Braña, F. and Ji, X. 2007. The selective basis for increased egg retention: early
534 incubation temperature determines hatchling phenotype in wall lizards (*Podarcis*
535 *muralis*).- Biol. J. Linn. Soc. 92: 441-447.
- 536 Bridle, J.R. and Vines, T. 2006. Limits to evolution at range margins: when and why
537 does adaptation fail? - Trends Ecol. Evol. 22: 140-147.
- 538 Bronikowski, A.M. 2000. Experimental evidence for the adaptive evolution of growth
539 rate in the garter snake (*Thamnophis elegans*). - Evolution 54: 1760–1767.
- 540 Brown, J. H. et al. 1996. The geographic range: size, shape, boundaries, and internal
541 structure. – Annu. Rev. Ecol. Syst. 27:597-623.
- 542 Buckley, L. 2008. Linking traits to energetics and population dynamics to predict lizard
543 ranges in changing environments. - Am. Nat. 171: E1–E19.
- 544 Carranza, S. et al. 2004. DNA phylogeny of *Lacerta (Iberolacerta)* and other lacertine
545 lizards (Reptilia: Lacertidae): did competition cause long-term mountain
546 restriction? - Systematics and Biodiversity 2: 57–77.

- 547 Civantos, E. and Forsman, A. 2000. Determinants of survival in juvenile
548 *Psammodromus algirus* lizards. - *Oecologia*, 124: 64-72.
- 549 Deeming, D.C. and Ferguson, M.W.J. 1991. Physiological effects of incubation
550 temperature on embryonic development in reptiles and birds. - In: Deeming DC,
551 Ferguson MWJ (eds). *Egg incubation: its effects on embryonic development in*
552 *birds and reptiles*. Cambridge: Cambridge University Press, pp 147–171.
- 553 Díaz, J.A. and Carrascal, L.M. 1991. Regional distribution of a Mediterranean lizard:
554 influence of habitat cues and prey abundance. – *J. Biogeogr.* 18:291–297.
- 555 Díaz, J.A. and Cabezas-Díaz, S. 2004. Seasonal variation in the contribution of different
556 behavioural mechanisms to lizard thermoregulation. - *Funct. Ecol.* 18, 867-875.
- 557 Díaz, J. A. et al. 2007. Reproductive performance of a lacertid lizard at the core and the
558 periphery of the species' range. - *Biol. J. Linn. Soc.* 92: 87-96.
- 559 Doody, J. S. 2009. Superficial lizards in cold climates: Nest site choice along an
560 elevational gradient. - *Austral Ecol.* 34: 773–779.
- 561 Doody, J.S. et al. 2006. Nest site choice compensates for climate effects on sex ratios in
562 a lizard with environmental sex determination. - *Evol. Ecol.* 20: 307-330.
- 563 Ghalambor, C. et al. 2006. Are mountain passes higher in the tropics? Janzen's
564 hypothesis revisited. - *Integr. Comp. Biol.* 46: 5-17.
- 565 Hare, K.M. et al. 2004. Size, growth, and survival are reduced at cool incubation
566 temperatures in the temperate lizard *Oligosoma suteri* (Lacertilia : Scincidae). –
567 *Copeia* 2: 383-390.
- 568 Jarvis, A. et al. 2008. Hole-filled seamless SRTM data V4, International Centre for
569 Tropical Agriculture (CIAT), available from <http://srtm.csi.cgiar.org>.
- 570 Ji, X. et al. 2002. Influence of incubation temperature on hatching success, energy
571 expenditure for embryonic development, and size and morphology of hatchlings

572 in the oriental garden lizard, *Calotes versicolor* (Agamidae). - J. Exp. Zool. 292:
573 649-659.

574 Iraeta, P. et al. 2006. Mediterranean hatchling lizards grow faster at higher altitude: a
575 reciprocal transplant experiment. - Funct. Ecol. 20: 865-872.

576 Kearney, M. and Porter, W.P. 2004. Mapping the fundamental niche: physiology,
577 climate, and the distribution of a nocturnal lizard. - Ecology 85: 3119-3131.

578 Lee, J.E. et al 2009. Physiological tolerances account for range limits and abundance
579 structure in an invasive slug. - Proc. R. Soc. B 276: 1459-1468.

580 Lourdais, O. et al. 2004. Climate affects embryonic development in a viviparous snake,
581 *Vipera aspis*. - Oikos 104: 551/560.

582 Lu, H.L. et al. 2006. Relatively low upper threshold temperature in lizards from cool
583 habitats. - J. Therm. Biol. 31: 256-261.

584 Mitchell, N.J. et al. 2008. Predicting the fate of a living fossil: how will global warming
585 affect sex determination and hatching phenology in tuatara? - Proc. R. Soc.
586 Lond. B 275: 2185-2193.

587 Monasterio, C. et al. 2009. The effects of thermal biology and refuge availability on the
588 restricted distribution of an alpine lizard. - J. Biogeogr. 36: 1673-1684.

589 Monasterio, C. et al. 2010. Altitude and rock cover explain the distribution and
590 abundance of a Mediterranean alpine lizard. - J. Herpetol. 44:158-163.

591 Monasterio, C. et al. 2010. Competition with wall lizards does not explain the alpine
592 confinement of Iberian rock lizards: an experimental approach. - Zoology 113:
593 275-282.

594 Neteler, M. and Mitasova, H. 2004. Open Source GIS: A GRASS GIS Approach. -
595 Kluwer Academic Publishers, Boston.

- 596 Nogués-Bravo, D. et al. 2007. Exposure : global mountain systems to climate change. -
597 Global Environ. Chang. 17: 420-428.
- 598 Packard, G.F and Packard, M.J. 1988. The physiological ecology of reptilian eggs and
599 embryos. - In: Gans, C., Huey, R.B. (Eds.). Biology of the Reptilia. Vol. 16,
600 Ecology (B). Academic Press, London.
- 601 Parker, S.L. and Andrews, R.M. 2007. Incubation temperature and phenotypic traits of
602 *Sceloporus undulatus*: implications for the northern limits of distribution. -
603 *Oecologia*, 151: 218-231.
- 604 Phillips, S. J. et. al. 2009. Sample selection bias and presence-only distribution models:
605 implications for background and pseudo-absence data. *Ecological Applications*,
606 19: 181-197.
- 607 Qualls, F.J. and Shine, R. 1998. Geographic variation in lizard phenotypes: importance
608 of the incubation environment. - *Biol. J. Linn. Soc.* 64: 477-491.
- 609 Qualls, C.P. and Andrews, R.M. 1999. Cold climates and the evolution of viviparity in
610 reptiles: cold incubation temperatures produce poor-quality offspring in the
611 lizard, *Sceloporus virgatus*. - *Biol. J. Linn. Soc.* 67: 353-376.
- 612 Shine, R. 2002. Eggs in autumn: responses to declining incubation temperatures by the
613 eggs of montane lizards. - *Biol. J. Linn. Soc.* 76 :71-77.
- 614 Shine, R. and Olsson, M. 2003. When to be born? Prolonged pregnancy or incubation
615 enhances locomotor performance in neonatal lizards (Scincidae). - *J. Evolution.*
616 *Biol.* 16: 823-32.
- 617 Sinervo, B. 1990. The evolution of maternal investment in lizards: an experimental and
618 comparative analysis of egg size and its effects on offspring performance. -
619 *Evolution* 44:279-294.

620 Telemeco, R.S. et al. 2009. Nesting lizards (*Bassiana duperreyi*) compensate partly, but
621 not completely, for climate change. - Ecology 90: 17-22.

622 Warner, D.A. and Shine, R. 2007. Fitness of juvenile lizards depends on seasonal timing
623 of hatching, not offspring body size. - Oecologia, 154: 65-73.

624 Williams, S.E. et al. 2008. Towards an integrated framework for assessing the
625 vulnerability of species to climate change. - PLoS Biology 6: e325.

626 Van Damme, R. et al. 1989. Altitudinal variation of the thermal biology and running
627 performance in the lizard *Podarcis tiliguerta*. - Oecologia, 80: 516-527.

628 Van Damme, R. et al. 1992. Incubation temperature differentially affects hatching time,
629 egg survival, and hatching performance in the lizard *Podarcis muralis*. -
630 Herpetologica 48: 220–228.

631

632 Table 1. Two-way ANOVAs for the effects of species (*I. cyreni* vs *P. algirus*),
 633 incubation temperature (24, 28, or 32°C), and their interaction, on body size (SVL) and
 634 body condition (residuals of log-mass on log-SVL) at hatching and 28 days after
 635 hatching.

636

	Hatchling SVL			Body condition at hatching			Final body condition		
	df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>
Species	1	16.27	<0.001	1	0.02	0.888	1	48.21	<0.001
Temperature	2	5.40	0.006	2	2.90	0.060	2	50.19	<0.001
Species* Temperature	2	0.80	0.452	2	11.59	<0.001	2	19.08	<0.001
Error	100			100			79		

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Table 2. Two-way ANOVAs for the effects of species (*I. cyreni* vs *P. algirus*), incubation temperature (24, 28, or 32°C), and their interaction, on size-specific and mass-specific growth rates of juveniles during their first four weeks of life.

	Size-specific growth rate			Mass-specific growth rate		
	df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>
Species	1	0.88	0.350	1	30.25	<0.001
Temperature	2	3.58	0.032	2	35.29	<0.001
Species* Temperature	2	4.33	0.016	2	10.72	<0.001
Error	79			79		

Table 3. Predicted breeding season soil temperatures at 5 cm depth under three levels of microhabitat exposure at occupied localities in the Sierra de Guadarrama.

	Exposure	Mean	SE	Minimum	Maximum
All areas (N = 162)	bare	23.45	0.41	9.94	35.99
	rock	22.26	0.38	9.89	33.86
	shrub	18.68	0.29	7.89	27.02
<i>I. cyreni</i> (N = 84)	bare	19.21	0.29	9.94	25.30
	rock	18.29	0.26	9.89	23.84
	shrub	15.72	0.22	7.89	20.02
<i>P. algirus</i> (N = 78)	bare	28.01	0.35	21.90	35.99
	rock	26.53	0.32	21.06	33.86
	shrub	21.86	0.23	17.56	27.02

Figure captions

Figure 1. Hatching success of *I. cyreni* and *P. algirus* at different incubation temperatures.

Figure 2. Incubation period of *I. cyreni* and *P. algirus* at different temperatures.

Figure 3. Hatching dates of *I. cyreni* and *P. algirus* at different incubation temperatures.

Figure 4. Size (snout-vent length) at hatching, body condition at hatching (residuals of log-body mass on log-SVL), and body condition at four weeks of life of *I. cyreni* and *P. algirus* at different incubation temperatures.

Figure 5. Size and mass specific growth rates of *I. cyreni* and *P. algirus* at different incubation temperatures. See methods for details.

Figure 6. Predicted soil temperatures at locations occupied by *I. cyreni* and *P. algirus* (shaded bars) relative to a 'target-group' background of available temperatures (unshaded bars) under three levels of microhabitat exposure during the breeding season. Arrows indicate the threshold temperature value of 24°C (see text for an explanation).

Fig. 1

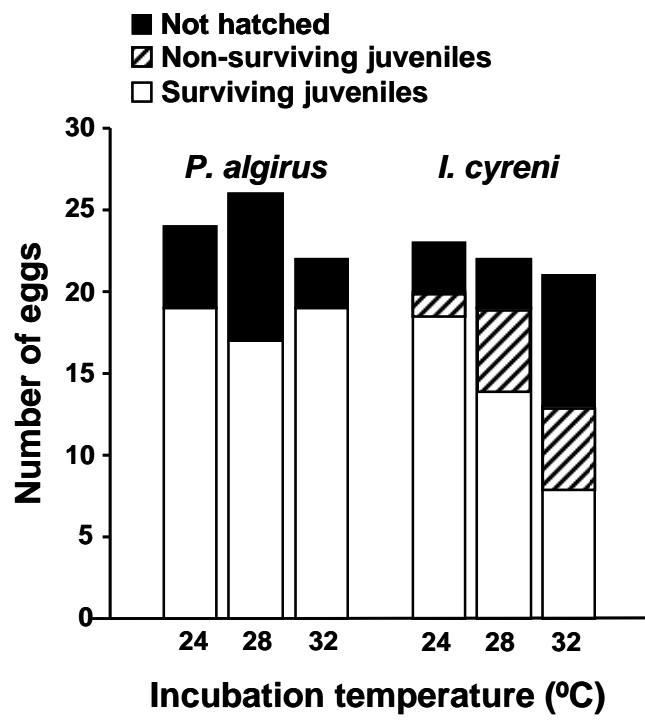


Fig. 2

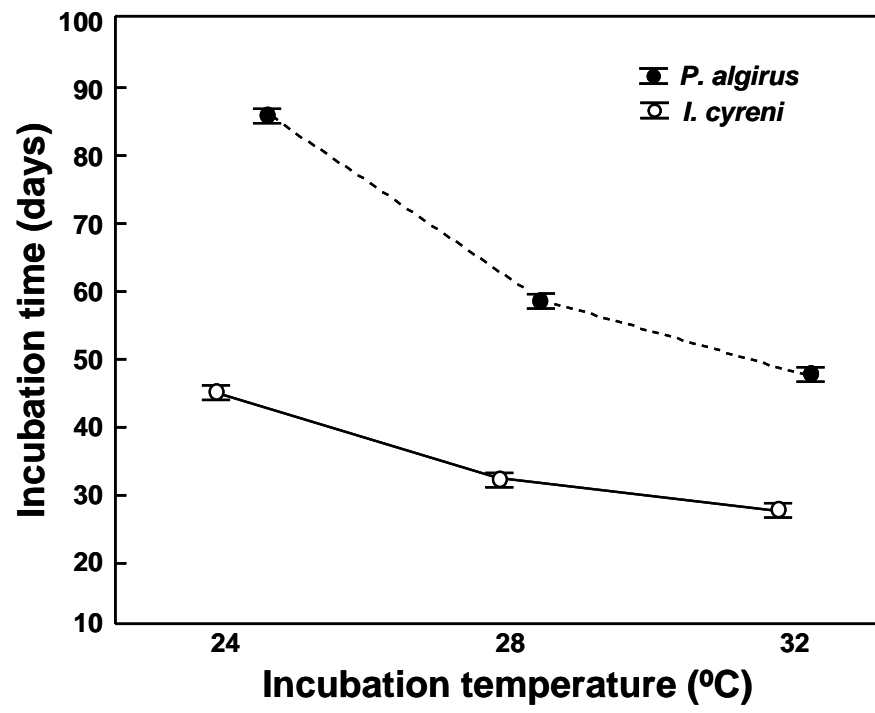


Fig. 3

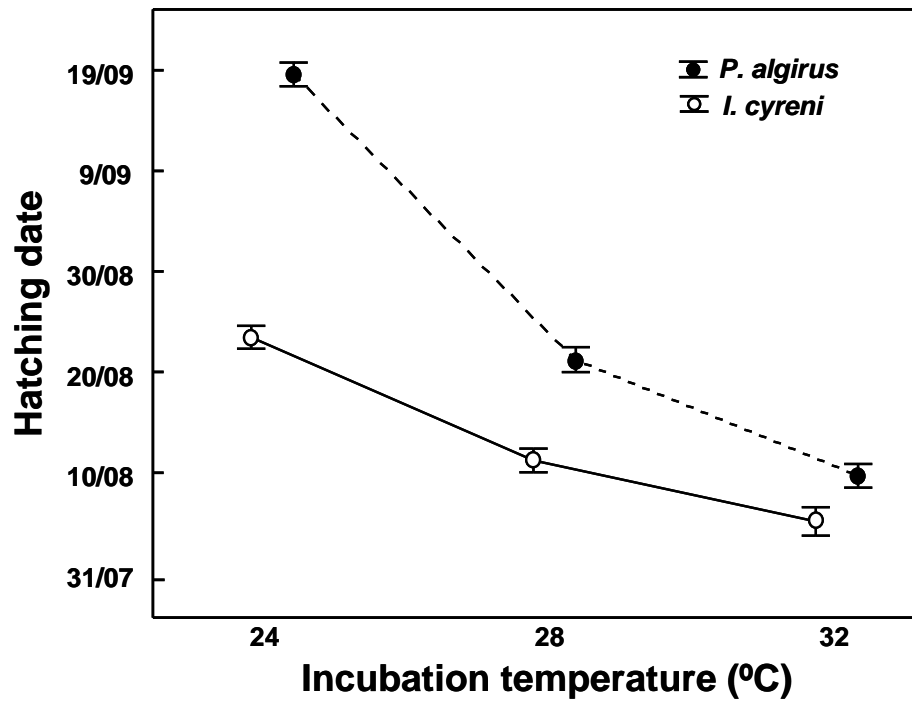


Fig. 4

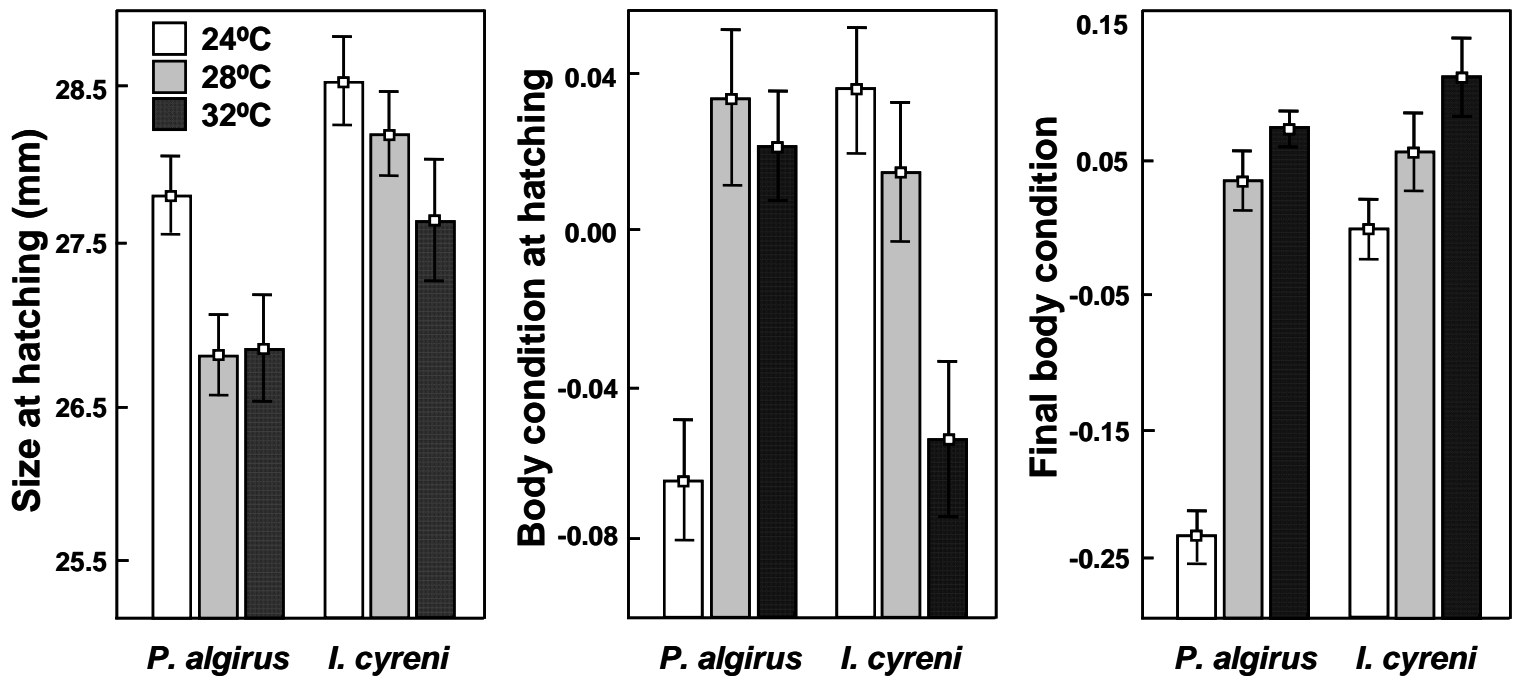


Fig. 5

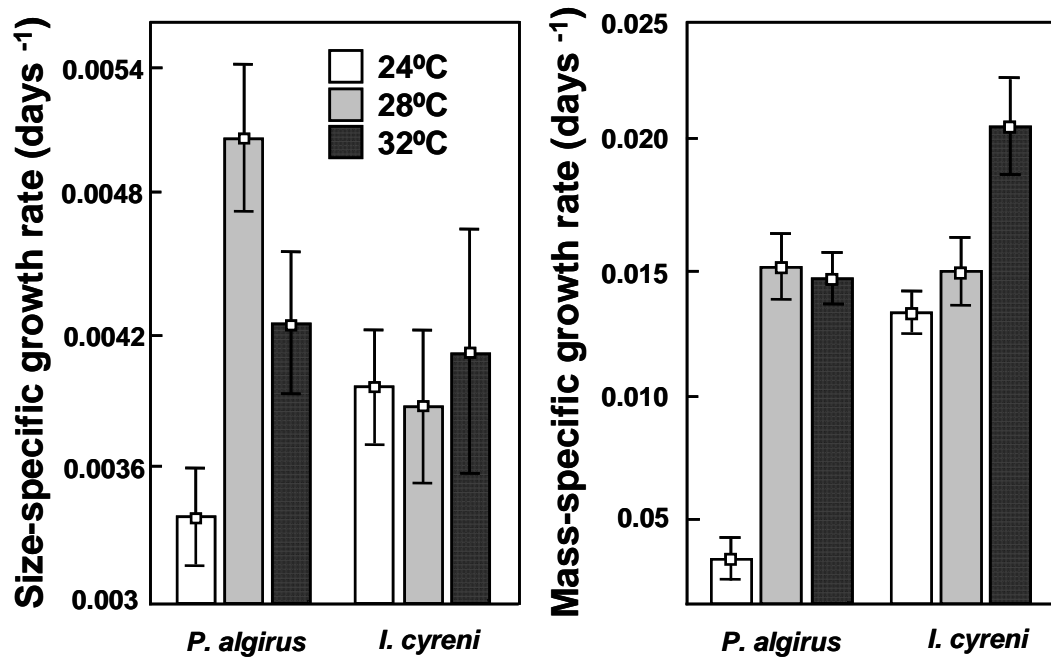


Fig. 6

