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Testing the hypothesis of greater eurythermality in invasive than in native ladybird species: from physiological performance to life-history strategies

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Abstract. 1. Global warming and biological invasions are important threats to biodiversity. Nonetheless, there is little information on how these factors influence performance or life-history traits of invasive and native species.

2. The effects of temperature on physiological and fitness traits of two invasive alien species (*Harmonia axyridis* and *Hippodamia variegata*) and one native species (*Eriopis chilensis*) of coccinellid were evaluated, testing a model of eurythermality. Eggs of all species were exposed to four temperature treatments (20, 24, 30 and 33 °C). In adult F_2 we measured fecundity, locomotor performance, development time (total and per life stage), survival, and preferred body temperature in a thermal gradient.

3. It was found that *H. axyridis* had comparatively better performance at low temperatures (i.e. 20 °C), while the performance of *H. variegata* and *E. chilensis* did not change with temperature or was better at higher temperatures (30 °C). The standardised Levins index showed that all species are eurythermic. *E. chilensis* had a high niche overlap with the invasive alien ladybird species, rejecting the hypothesis of greater eurythermality of invasive species than native species.

4. Although there were differences in the temperature preferences and in the response of some physiological and life-history traits of ladybirds to temperature, both the native and invasive alien species are eurythermic, contrary to the prediction. The better performance of *H. axyridis* at lower temperatures may result in displacement of its current distribution, and thus not all invasive species will respond favourably to global warming.

Key words. Environmental temperature, *Eriopis chilensis*, *Harmonia axyridis*, *Hippodamia variegata*, invasive alien species.

Introduction

Global warming is expected to increase much faster in the future than in the last century and could be a significant threat to biodiversity (Sala *et al.*, 2000; IPCC, 2013); many organisms are expected to suffer a decrease in fitness and changes in their distribution (Beaumont & Hughes, 2002; Bellard *et al.*, 2012; Lejeusne *et al.*, 2014). Global warming may also interact with other threats, such as invasive alien species (Dukes & Mooney, 1999; Lu *et al.*, 2014). Invasive species are those introduced to a habitat or ecosystem where they are not native and that become established, spread, and cause damage to biodiversity and human welfare (Vitousek *et al.*, 1997; Mainka & Howard, 2010); they may be more successful due to temperature increases, with possible changes in ecological interactions (Rixon *et al.*, 2005; Chown *et al.*, 2007, 2010; Hartley *et al.*, 2010; Hoffman & Todgham, 2010).

There is a need to measure traits in alien invasive and native species to predict the effects of global warming on the performance of these species. The high survival rates and fast growth rates of invasive alien species may help them to succeed in

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competition with native species under global change scenarios (Whitney-Johnson *et al.*, 2005; Mainka & Howard, 2010; Radchuk *et al.*, 2013; Oliveira Ramos *et al.*, 2014). Moreover, with global warming, the physiological performance of native and invasive alien species and the persistence of their populations may also change (Folguera *et al.*, 2009; Bozinovic *et al.*, 2011a,b). Zerebecki and Sorte (2011) proposed the hypothesis of greater invasive eurythermality, suggesting that if an invasive species has a broader range of temperature tolerance than a native species, then an increase in temperature through global warming is likely to have a highly positive impact on the fitness or performance of the invasive alien species in comparison to native species. Although there is strong evidence that invasive alien species may tolerate global warming better than native species (Human & Gordon, 1996; Vilá & Weiner, 2004; Molina-Montenegro *et al.*, 2009), few studies have examined the physiological mechanisms and life-history traits that underlie differences in success (Anacleto *et al.*, 2014; Lejeusne *et al.*, 2014).

An increase in temperature of 2–4 °C has been predicted by the year 2050 due to global warming in agricultural systems of the Mediterranean zone of central Chile (Fuenzalida *et al.*, 2007; Cabré *et al.*, 2010). This could have important effects on the physiology and life-history trait responses of native and alien species of coccinellids living in these systems, which has never been studied. Coccinellids (Coleoptera: Coccinellidae) are an ecologically important group of insects that provide valuable pest control services in agricultural ecosystems (Obrycki & Kring, 1998; Hodek *et al.*, 2012). For this reason, several species have been introduced into many countries worldwide over the last century (Obrycki & Kring, 1998; Evans *et al.*, 2011).

A diverse community of exotic and native species of coccinellids coexists in the Mediterranean habitat in Chile. The most abundant are the exotics *Hippodamia variegata* (Goeze) and *Harmonia axyridis* (Pallas) and the native *Eriopis chilensis* (Germar), all of them aphidophagous species. *Hippodamia variegata*, native to the Palearctic region (Gordon, 1987) is widely distributed in Europe, North America, Middle East, and Africa (Gordon, 1987; Mandour, 2013). It was introduced into Chile from South Africa in the 1970s for biological control of aphids and currently is one of the most abundant species, extensively distributed and established throughout the country; it has been suggested that it is displacing other native coccinellids (Grez *et al.*, 2012). The adults are 4–6 mm in length, with very well-known life-history traits (Wu *et al.*, 2010; Hodek *et al.*, 2012). *Harmonia axyridis* (Pallas) is a large ladybird species (adults, 5.5–8.3 mm), originally from temperate and subtropical regions of east and central Asia, now distributed in large areas in Europe, North and South America and South Africa (Koch, 2003; Koch *et al.*, 2006; Roy & Brown, 2015). It is considered an invasive alien species because of its rapid spread and negative consequences upon biodiversity, agriculture, and people (Koch, 2003; Koch *et al.*, 2006; Osawa, 2011; Roy & Brown, 2015). It was introduced in Chile in 1998, but wild populations were not recorded until 2003 (Grez *et al.*, 2010). Recent genetic analyses demonstrated that populations in Chile originated from eastern North American populations, which correspond to the invasive strain (Lombaert *et al.*, 2014). Finally, *Eriopis chilensis* Hofmann is endemic and the most common Chilean species (González, 2006, 2014). Adults are 4–6 mm in length; the life cycle has been described by different authors (Etchégaray & Barrios, 1979; Gyenge *et al.*, 1998) but little is known about its biology and ecology.

Following the hypothesis of greater eurythermality in invasive than in native species, we propose that under a climate warming scenario the exotic coccinellid species will have some advantages over the native species. We predict that with increased temperature, locomotor performance and life-history parameters (i.e. fecundity, development time, and mortality) will be better in the invasive alien species (*H. axyridis and H. variegata*) than in the native species (*E. chilensis*). We also predict that the thermal niche will be eurythermic in invasive alien species and stenothermic in the native species.

Material and methods

Adults of the three species were collected in alfalfa fields near Santiago, Chile (33°40′S, 70°35′W), and maintained in the laboratory in acrylic cages (60 × 50 × 50 cm) at 24 °C, 70% RH and LD 12:12 h. Animals were fed *ad libitum* with aphids of the species *Acythosiphon pisum* and *Aphis craccivora* reared on plants of *Vicia faba*.

Experimental design

We worked with the F_2 generation in order to remove field biotic and abiotic effects. To avoid overstressing, individuals of each species were separated into three groups and used to study locomotor performance and fecundity (group 1); development time and survival throughout the life cycle (group 2); or thermal preferences at different ambient temperatures (group 3). Each group consisted originally of five pairs of each species copulating in Petri dishes. Females were allowed to lay their eggs for a maximum of 5 days and were fed *ad libitum* with aphids to prevent cannibalism. Eggs from the first two experimental groups were subsequently assigned to different ambient temperatures (20, 24, 30, and 33 °C) and maintained until adult emergence (F_2); however, at the highest temperature, no eggs of any of the three species survived this treatment. The letter 'L' followed by a number represents the four larval instars (i.e. L1, L2, L3 and L4), pre-pupa and pupa instars in all experiments, which were performed in growth chambers with a constant LD 12:12 h photoperiod and 70% RH.

Locomotor performance

Locomotor performance under different ambient temperatures (in °C) was studied following Castañeda *et al.* (2005) and Folguera *et al.* (2009). First we measured rollover speed (RoS; i.e. the time it takes for a ladybird, after being placed upside down, to roll over into the upright position) in individuals reared at different temperatures ($n = 20$ individuals per species and ambient temperature). RoS is considered a measure of performance (Lutterschmidt & Hutchinson, 1997). Each individual was placed upside down in a 9-cm-diameter Petri dish (with smooth surface) for a maximum of 10 min or until it returned to its normal position, at which point the time was recorded.

Thermal preferences

To estimate the thermal preferences in ladybirds we used 30 individuals per species that were reared only at 24 °C (i.e. the temperature of acclimatisation). We built a thermal gradient using an 80 cm long × 5 cm high × 21 cm wide metal plate, covered with a 4 mm layer of transparent plastic. The plate was divided into six separate runways (each 80 cm long and 3 cm wide) using opaque walls 5 cm high. To establish the thermal gradient, we heated one of the extremes of the metal plate with a potentiometer and cooled the other extreme with a coolant. The range of temperatures in the gradient varied from 4 to 50 °C and did not change during the experiments. One adult from each species was deposited in each individual runway, and after 25 min we measured the temperature body preference (T_{bp}) using an infrared thermometer (±0.5 °C, EXTECH Instruments, model 42509, Nashua, New Hampshire, range: −20 to 510 °C).

Fecundity

Females of the first group that emerged at different ambient temperatures were left under the same environmental conditions and fed with aphids *ad libitum* for 15 days until reproductive maturity (Grez *et al.*, 2012). Each female was then mated with a male raised at the same ambient temperature, transferred to a larger Petri dish (9 cm diameter by 1 cm high) and maintained at the same treatment temperature. We followed five females per species and ambient temperature treatment. During the following 5 days we recorded the total number of eggs laid by each female. Eggs were removed from the Petri dishes each day to avoid cannibalism.

Development time and mortality

After hatching, larvae of the second group were separated from unhatched eggs and placed individually in Petri dishes (3.5 cm diameter, 0.5 cm height), which were covered by foam to prevent escape. Individuals were maintained at the different ambient temperatures indicated earlier. Development time was measured as the time to reach each larval stage (L1–L4), pre-pupa, pupa and adult, measured as the number of days that the individual remained in each stage of the life cycle. Larval moult was checked daily by visual inspection. We measured the total mortality of individuals (%) at different stages of the life cycle after eclosion for all species and temperatures, and also the total survival to adulthood. Larvae were fed daily *ad libitum* with aphids *A.pisum* and *A. craccivora* until the pre-pupa period.

Statistical analysis

The effects of ambient temperature and species on locomotor performance, development time (i.e. total and per life cycle stage), and fecundity were tested by two-way ANCOVAS using body mass as covariate, with species and temperature as fixed factors. Normality and homoscedasticity of data were tested using Kolgomorov–Smirnov and Cochran C tests, respectively. When necessary, data were transformed to $\log_{10} x + 1$ to meet the statistical assumptions. When differences were significant ($P < 0.05$), we used the *a posteriori* Tukey test for multiple comparisons. Ladybird survival at different temperatures was estimated by Kaplan–Meier analysis (Kaplan & Meier, 1958), with equations adjusted to a non-linear model. A log-rank test was applied to compare survival rates, and statistics were computed using the survival package in R 2.15.2 (Therneau & Lumley, 2009; R Core Team, 2012).

To determine differences in T_{bp} we followed the analyses of Blandford *et al.* (2009) and Alfaro *et al.* (2013). First, the temperatures chosen by coccinellids were characterised with frequency histograms. We then calculated the thermal niche breadth of each species with Levins index (Jaksic & Marone, 2007), $B = (1/\sum p_i^2)$, where p_i is the proportion of use of temperature i. Furthermore, the standardised Levins index was estimated following Colwell and Futuyma (1971) as $B_{\mathrm{s}} = ((1/\sum p_i^2) - 1/(n - 1))$, where n is the number of temperatures selected in intervals of 1 °C. The index values range from 0 to 1; values <0.6 indicate a selection of few temperatures (stenothermic specialist) and values >0.6 indicate a generalist use of temperatures (eurythermic). Then to assess the similarity in thermal preferences of the species, niche overlap was calculated with Pianka's index: $O_{\mathrm{pq}} = (\sum p_i \times q_i / \sqrt{\sum p_i^2 \times q_i^2})$, where p and q represent two species of ladybirds and i is the ambient temperature. This index varies between 0 and 1, with 0 being no overlap and 1 complete overlap (Pianka, 1973; Alfaro *et al.*, 2013). Additionally, to analyse differences among thermal preference curves we used an F test to compare the curves with a null model (normal distribution), using the GRAPHPAD (GraphPad Software, 2015). To test T_{bp} differences among coccinellid species we used one-way ANOVA, with species as a fixed factor. All ANOVA and ANCOVA analyses were performed using STATISTICA 6.0 (StatSoft Inc., Tulsa, Oklahoma).

Results

Locomotor performance

There were statistically significant effects of species ($F_{2,170} = 21.84$; $P < 0.001$), temperature ($F_{2,170} = 3.61$; $P = 0.023$) and the interaction of species × temperature ($F_{4,170} = 9.05$; $P < 0.001$) on RoS. Body mass did not affect RoS among species ($F_{1,170} = 0.02$; $P = 0.866$). *Harmonia axyridis* took more time to return to the upright position at higher temperatures, while *H. variegata* and *E. chilensis* maintained the same RoS at different temperatures (Fig. 1).

Thermal preferences

Significant differences were found in the T_{bp} of the different species ($F_{2,12} = 14.07$, $P < 0.001$). *Harmonia axyridis* preferred temperatures between 15 and 20 °C, with a thermal range

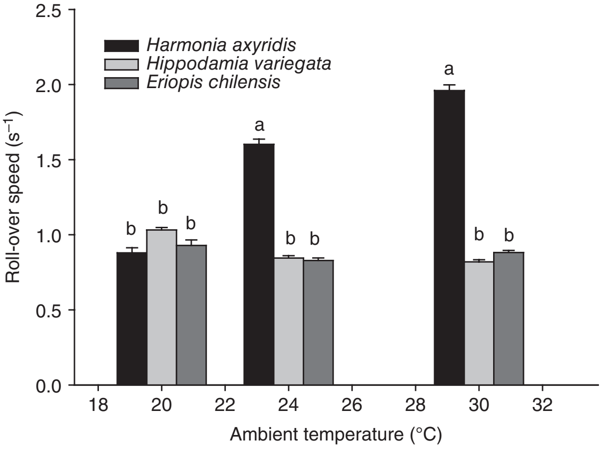

Fig. 1. Average roll-over speed (RoS ± 1 SE) for invasive alien and native species of ladybirds in experimental temperature treatments. Black bars, *Harmonia axyridis*; light grey bars, *Hippodamia variegata*; dark grey bars, *Eriopis chilensis*. The same letters above bars represent homogeneous groups (Tukey; $P > 0.05$).

of 8.6–27.2 °C, while *H. variegata* and *E. chilensis* preferred temperatures between 20 and 30 °C, ranging between 16.8 and 36.2 °C for *H. variegata* and 11.6–39.2 °C for *E. chilensis* (Fig. 2). The thermal niche breadths were similar for the three species, with the Levins index for *H. axyridis*, $B_{\mathrm{Ha}} = 27.67$, for *H. variegata*, $B_{\mathrm{Hv}} = 29.04$, and for *E. chilensis*, $B_{\mathrm{Ec}} = 27.38$; the standardised values were $B_{\mathrm{sHa}} = 0.59$; $B_{\mathrm{sHv}} = 0.62$, and $B_{\mathrm{sEc}} = 0.58$. The thermal niche overlaps among the three species were $\mathrm{O}_{\mathrm{Ha\text{-}Hv}} = 0.47$, $\mathrm{O}_{\mathrm{Hv\text{-}Ec}} = 0.67$, and $\mathrm{O}_{\mathrm{Ec\text{-}Ha}} = 0.88$. The curves of thermal preferences showed significant differences among the three coccinellid species ($F_{6,12} = 4.83$, $P = 0.009$).

Fecundity

Oviposition was significantly affected by temperature ($F_{2,36} = 4.87$; $P = 0.013$) and species × temperature interaction ($F_{4,36} = 4.97$, $P = 0.003$), but not by species ($F_{2,36} = 1.49$, $P = 0.239$). Body mass did not affect fecundity among species ($F_{1,36} = 0.75$, $P = 0.391$). *Harmonia axyridis* had the greatest oviposition (40 ± 5.16 eggs per female per 5 days) compared with *H. variegata* and *E. chilensis* (24.73 ± 4.12 and 26.40 ± 3.61, respectively). The fecundity of *H. variegata* was lowest at 24 °C, while the fecundities of *H. axyridis* and *E. chilensis* were similar at all temperatures (Fig. 3).

Development time

The numbers of eggs reared for this experiment were as follows (for 20, 24 and 30 °C, respectively): for *H. axyridis*, 164, 267, and 169 eggs; for *H. variegata*, 74, 53, and 215 eggs; and for *E. chilensis*, 103, 108 and 163 eggs (depending on the availability of eggs). Body mass did not affect the development time (total or per life cycle stage; see Table 1) among species. Only temperature had significant effects on total development time (i.e. egg to adult; $F_{2,36} = 89.38$, $P < 0.001$); the three species of coccinellids had shorter development time at 30 °C (2.00 ± 0.07 days) and longer at 20 °C (2.38 ± 0.008 days). However, the development time per life cycle stage changed for each species (Table 1). *Harmonia axyridis* was the only species that decreased development time for all stages with an increase in temperature. At 20 °C this species took more time to develop than at 30 °C in all stages with the exception of pre-pupae, which lasted the same time at different temperatures. In *H. variegata* and *E. chilensis*, development time only decreased at higher temperatures in L1 and L2; in the other stages the development times were similar at all temperatures (Table 1).

Survival

The Kaplan–Meier analysis at 20 °C showed that *H. axyridis* had a longer mean survival time (19.98 ± 0.5 days, 95% CI: 18.99–20.97) than *H. variegata* and *E. chilensis* (10.68 ± 0.98 days, 95% CI: 8.75–12.62 and 7.14 ± 0.41 days, 95% CI: 6.31–7.96, respectively). In particular, eggs of *H. axyridis* had low mortality at this temperature compared with the other coccinellids (7.9% vs. 55.4% and 42.3% for *H. variegata* and *E. chilensis*, respectively). The log-rank test demonstrated that the survival curves were significantly different between coccinellids ($\chi_2^2 = 88.35$; $P < 0.001$; Fig. 4a). At 24 °C the mean survival time of *H. axyridis* was also longer (14 ± 0.41 days, 95% CI: 13.17–14.82) than for *H. variegata* (7.81 ± 0.84 days, 95% CI: 6.14–9.47) and *E. chilensis* (8.48 ± 0.65 days, 95% CI: 7.19–9.77), and at this temperature the survival probabilities of coccinellids were significantly different ($\chi_2^2 = 39.00$, $P < 0.001$; Fig. 4b). Mortalities of eggs at this temperature were 18.7%, 60.37% and 56.5% for *H. axyridis*, *H. variegata*, and *E. chilensis*, respectively. However, at 30 °C, *H. axyridis* had a shorter survival time than *H. variegata* (4.11 ± 0.24 days, 95% CI: 3.64–4.59 vs. 7.98 ± 0.36 days, 95% CI: 7.27–8.70, respectively) and *E. chilensis* (6.97 ± 0.38 days, 95% CI: 6.21–7.74). The log-rank test showed significant differences among survival curves ($\chi_2^2 = 26.46$; $P < 0.001$; Fig. 4c), with the eggs of *H. axyridis* and *E. chilensis* having greater mortality (66.3% and 61.6%) than *H. variegata* (42.3%) at this temperature.

Discussion

Biological invasions do, and will, interact with global warming in different ways, altering mechanisms of transport and introduction, modifying climatic constraints on invasive species, changing the distribution of existing invasive species, altering the impact of existing invasive species and the effectiveness of management strategies for these species (Hellman *et al.*, 2008). Theoretically, an invasive alien species should be favoured by increases in temperature because of its broader range of temperature tolerance than native species (Dukes & Mooney, 1999; Deutsch *et al.*, 2008; Dukes, 2011; Zerebecki & Sorte, 2011; Lejeusne *et al.*, 2014). Furthermore, invasive alien species may have other traits, such as phenotypic plasticity, which may confer advantages over native species and aid their invasive process

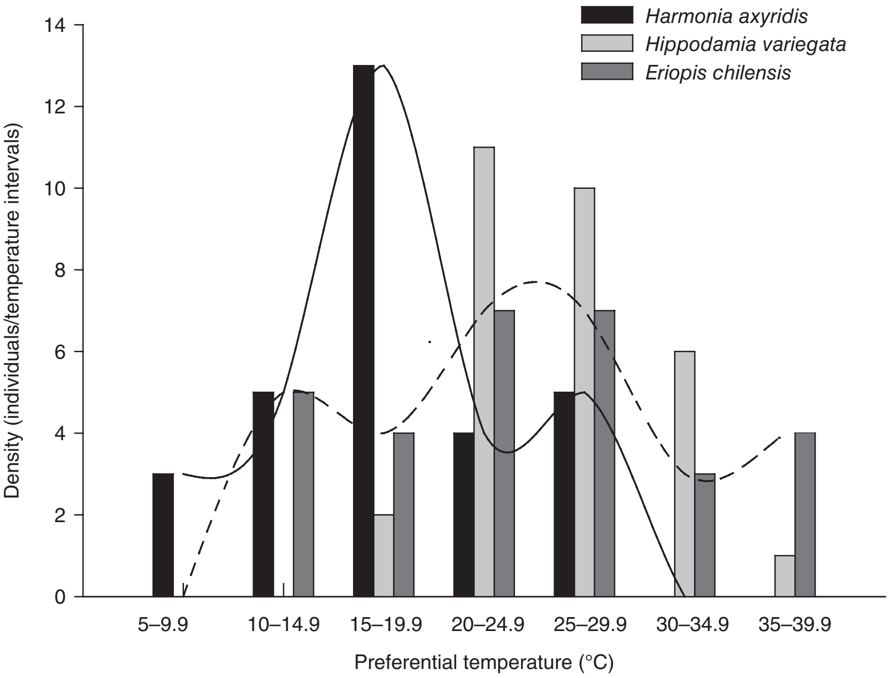

Fig. 2. Thermal preferences of invasive alien and native ladybirds species in the thermal gradient (°C). Curves represent the thermal niche of each species. Black bars and continuous lines represent *Harmonia axyridis*; light grey bars and dotted lines represent *Hippodamia variegata*; dark grey bars and dashed lines represent *Eriopis chilensis*.

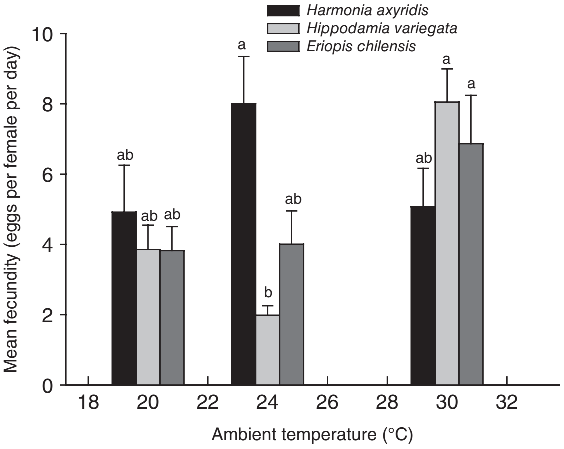

Fig. 3. Mean fecundity (±1 SE) of invasive alien and native ladybirds species at different experimental temperatures. Black bars, *Harmonia axyridis*; light grey bars, *Hippodamia variegata*; dark grey bars, *Eriopis chilensis*. The same letters above bars represent homogeneous groups (Tukey; $P > 0.05$).

in a global warming scenario. Nevertheless, better performance of invasive species under global warming scenarios and the underlying mechanisms are poorly known and have also proved controversial, with some studies supporting this idea and other studies finding no differences between alien and native species in these traits (Janion *et al.*, 2010).

Our study demonstrated that under laboratory conditions native and invasive ladybird species vary differentially in their physiological responses and their life-history traits with ambient temperature. But contrary to our predictions, the invasive *H. axyridis* preferred lower temperatures and showed better performance in some physiological and life-history traits at lower temperatures than the other species studied. Specifically, *H. axyridis* had better locomotor performance and higher survival of eggs at the lowest temperature, in contrast to *H. variegata* and *E. chilensis*, which performed better at higher temperatures. The higher RoS at low temperatures shown by *H. axyridis* could be considered a positive thermal response and thus low temperature might act as a potential selective agent on the performance of this species (Castañeda *et al.*, 2005; Folguera *et al.*, 2009). When individuals take more time to return to the normal position, they may be exposed to high temperatures for long periods, which could provoke cumulative damage in muscles or nerves (Hosler *et al.*, 2000; Glanville & Seebacher, 2006); also under these hot conditions they may express fewer heat-shock proteins (Zerebecki & Sorte, 2011). The higher survival of eggs of *H. axyridis* at low temperatures probably provides a fitness advantage during the cold season (Castañeda *et al.*, 2005). However, the lower survival of eggs at higher temperatures observed in *H. axyridis* and other ectotherms (Huang *et al.*, 2008; Radchuk *et al.*, 2013; Zhang *et al.*, 2013; Oliveira Ramos *et al.*, 2014; Benelli *et al.*, 2015) may limit populations during summer or in hot regions. In agreement with this, a recent review of the worldwide distribution of *H. axyridis* confirms that this species is scarce in tropical countries and in desert regions (A. A. Grez, pers. comm.).

Fecundity was little affected by temperature. This is similar to what has been observed in *E. connexa* (Gyenge *et al.*, 1998) and

Table 1. Development time (mean ± SE) of each immature stage of the three species of ladybirds at different temperatures as well as ANCOVA results.

	Development time (days) ± SE				ANCOVA results			
State	Species	20 °C	24 °C	30 °C	Factors	g.l.	*F*	*P*
					Body mass	1	0.01	0.922
L1	*Harmonia axyridis*	3.1 ± 0.3 a	2.7 ± 0.2 a	1.2 ± 0.1 b	Species	2	1.81	0.178
	Hippodamia variegata	3.1 ± 0.3 a	2.0 ± 0.1 a	1.6 ± 0.1 b	Temperature	2	20.40	< 0.001
	Eriopis chilensis	2.2 ± 0.3 a	2.3 ± 0.4 a	1.4 ± 0.2 b	Species × temperature	4	2.41	0.068
					Body mass	1	2.31	0.137
L2	*Harmonia axyridis*	2.4 ± 0.1 a	1.8 ± 0.1 b	1.2 ± 0.1 b	Species	2	1.61	0.214
	Hippodamia variegata	1.9 ± 0.4 a	1.0 ± 0 b	1.0 ± 0.1 b	Temperature	2	12.83	< 0.001
	Eriopis chilensis	1.5 ± 0.2 a	1.4 ± 0.2 b	0.8 ± 0.1 b	Species × temperature	4	0.47	0.756
					Body mass	1	1.19	0.281
L3	*Harmonia axyridis*	2.7 ± 0.1 a	2.7 ± 0.4 a	1.4 ± 0.1 cd	Species	2	3.18	0.054
	Hippodamia variegata	1.6 ± 0.1 abc	0.8 ± 0.1 bcd	0.7 ± 0.1 d	Temperature	2	15.56	< 0.001
	Eriopis chilensis	1.4 ± 0.1 ab	1.0 ± 0.1 abcd	0.7 ± 0.1 abcd	Species × temperature	4	2.78	0.042
					Body mass	1	0.22	0.638
L4	*Harmonia axyridis*	1.8 ± 0.2a	1.8 ± 0.1 a	0.7 ± 0.03 bc	Species	2	12.70	< 0.001
	Hippodamia variegata	1.2 ± 0.3ab	0.9 ± 0.2 c	0.9 ± 0.1 bc	Temperature	2	14.22	< 0.001
	Eriopis chilensis	1.4 ± 0.1a	0.8 ± 0.03 abc	0.7 ± 0.2 abc	Species × temperature	4	4.72	0.003
					Body mass	1	0.84	0.365
PP	*Harmonia axyridis*	5.1 ± 0.1 a	5.3 ± 0.5 a	3 ± 0.2 ab	Species	2	21.60	< 0.001
	Hippodamia variegata	2.1 ± 0.5 b	1.2 ± 0.1 c	1.9 ± 0.3 bc	Temperature	2	5.69	0.007
	Eriopis chilensis	2.1 ± 0.3 ab	1.6 ± 0.3 bc	1.7 ± 0.5 ab	Species × temperature	4	5.35	< 0.001
					Body mass	1	0.21	0.647
P	*Harmonia axyridis*	1.4 ± 0.1 a	1.1 ± 0.1 ab	0.3 ± 0.04 e	Species	2	5.82	0.006
	Hippodamia variegata	0.8 ± 0.2 bcd	0.7 ± 0.1 cde	0.7 ± 0.06 cde	Temperature	2	22.78	< 0.001
	Eriopis chilensis	0.9 ± 0.1 bc	0.5 ± 0.1 de	0.7 ± 0.1 bcd	Species × temperature	4	14.35	< 0.001

Letters represent significant differences of the Tukey test ($P < 0.05$). L1–L4, larval stages; PP, pre-pupae; P, pupa; g.l., degree freedom.
The numbers of eggs reared for this experiment were as follows (for 20, 24 and 30 °C, respectively): for *H. axyridis*, 164, 267 and 169 eggs; for *H. variegata*, 74, 53 and 215 eggs; and for *E. chilensis*, 103, 108 and 163 eggs (depending on the availability of eggs).

also in *H. axyridis*, for which fecundity did not differ between 20 and 25 °C, although it was greater at 15 °C (Castro *et al.*, 2011), a temperature not included in our study. The only species affected by temperature was *H. variegata*, with the highest oviposition at 30 °C, which suggests that only this species will be favoured in this trait by global warming.

The only variable that was favoured by increased temperature in *H. axyridis* was development time, with all stages developing more rapidly at 30 °C. Rapid development could be advantageous given that individuals reach maturity quickly and could readily colonise several crops, as well as increase predatory activity against the larval stages of other ladybirds (Yasuda *et al.*, 2001; Labrie *et al.*, 2006; Lucas, 2012). Rapid development time might also lead the most vulnerable stages (i.e. eggs, larvae, and pupae) to be less exposed to their own predators (Lucas, 2012) and therefore increase the number of individuals reaching the adult stage. However, rapid development time can also be disadvantageous because it usually results in smaller individuals with lower reproductive capacity (Yasuda *et al.*, 2001; Nedvěd & Honěk, 2012).

Although the three ladybird species differ in temperature preferences, they are all eurythermic, rejecting the hypothesis of greater eurythermality of invasive species proposed by Zerebecki and Sorte (2011). Moreover, the three species have high thermal niche overlap (46–88%), which may explain their current coexistence in central Chile (Grez *et al.*, 2013) even at small spatial scales (i.e. microhabitats). This in turn may result in a high probability of interspecific interactions among these species, such as competition and intraguild predation, a very common phenomenon in ladybirds that could result in interspecific competitive displacement (Molina-Montenegro *et al.*, 2009; Grez *et al.*, 2012; Bahlai *et al.*, 2015; Roy & Brown, 2015). But even though there is high niche overlap among the three species, they differ in their temperature preferences, with *H. axyridis* preferring lower temperatures than *H. variegata* and *E. chilensis*, perhaps increasing the abundance of the latter two species. Therefore the outcome of competition may depend on the thermal environmental conditions and the species' performance in these conditions.

Our results suggest that *H. axyridis* should have higher probability to invade colder places with suitable habitats for its establishment (Koch *et al.*, 2006; Poutsma *et al.*, 2008; Bidinger *et al.*, 2012). This phenomenon is currently occurring in Chile, with *H. axyridis* spreading rapidly from central Chile to the south of the country (www.chinita-arlequin.uchile.cl). Our results also suggest that *E. chilensis* and *H. variegata* should be more frequent in the north-central part of the country, with milder temperatures, which is in fact their current distribution (González, 2006).

It is known that global warming may favour biological invasions, generating changes in ecological parameters such as abundance, distribution, and biological interactions (Hiey & Stevenson, 1979; Chown *et al.*, 2010; Huey *et al.*, 2012). Under a global warming scenario, the thermal conditions in Chile will change, with a 2–4 °C increase in temperature in central and southern

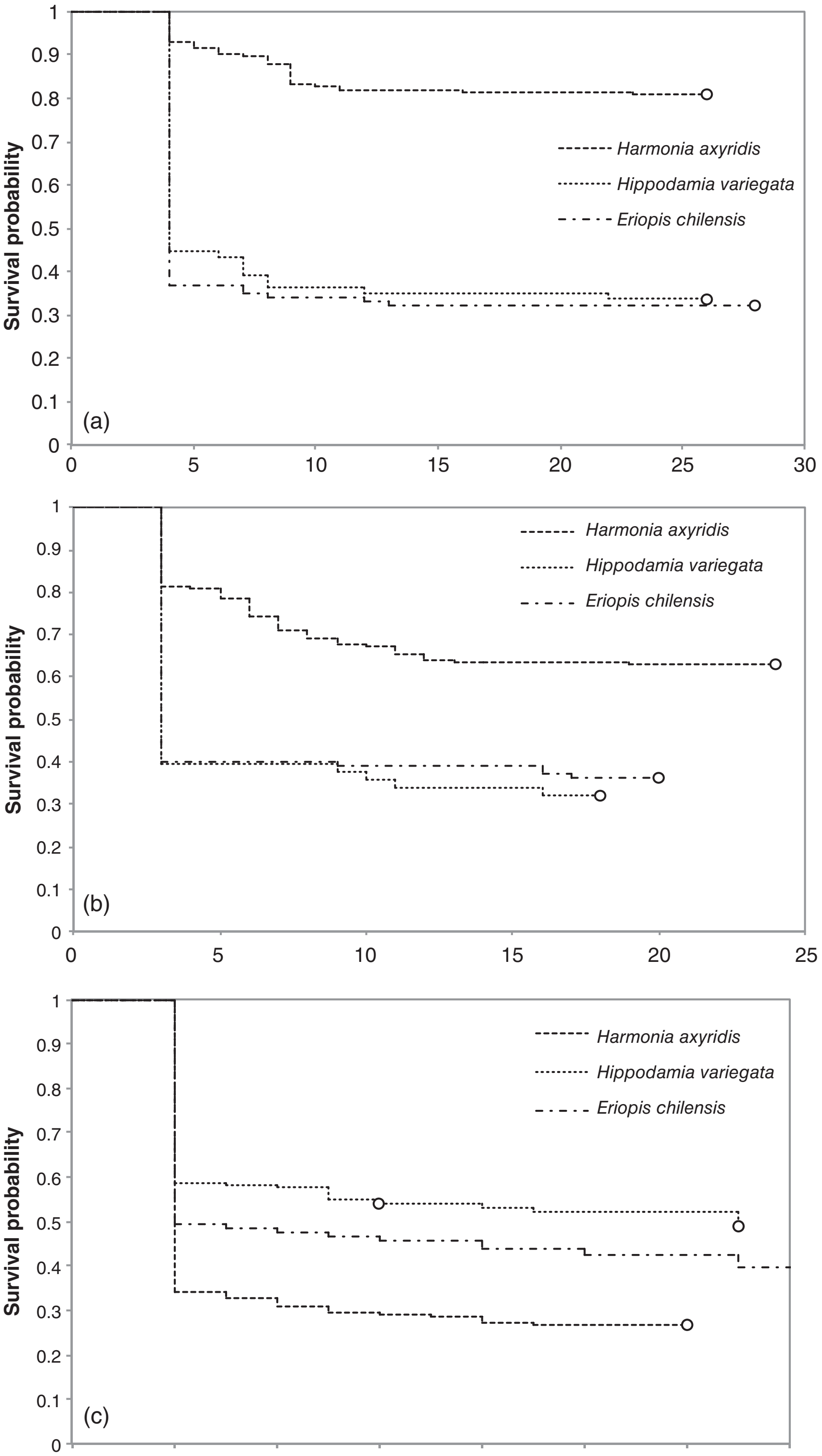

Fig. 4. Kaplan–Meier estimates of survivorship function of invasive alien and native ladybird species at the following temperatures: (a) 20 °C; (b) 24 °C; (c) 30 °C. Dashed lines in all graphics correspond to the invasive alien species, *Harmonia axyridis*; dotted lines to the invasive alien species, *Hippodamia variegata*; and dotted/dashed lines to the native species, *Eriopis chilensis*.

Chile by 2050 (Fuenzalida *et al.*, 2007; Cabré *et al.*, 2010). Our results suggest a stronger effect of global warming on *H. axyridis* than on the other two species, which could result in a change in its current distribution according to the new climatic conditions to areas with lower temperatures. However, this species is widespread across different environments and there could be a potential for plasticity (not tested here) or evolutionary adaptation for persistence under global warming (Urbanski *et al.*, 2012).

In conclusion, our results show that although there are differences in the temperature preferences and in the response of some physiological and life-history traits of ladybirds to temperature, both the native and invasive alien species are eurythermic, contrary to the prediction of Zerebecki and Sorte (2011). But the better performance of *H. axyridis* at lower temperatures may result in a displacement of its current distribution under a global warming scenario. Thus not all invasive species will respond favourably to global warming. Further advances are necessary in predictive models that include the combined effects of average temperature and its variability with physiological and life-history traits in order to compare the differential responses between invasive alien and native species (Bozinovic & Pörtner, 2015).

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