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Does thermal physiology explain the ecological and evolutionary success of invasive species? Lessons from ladybird beetles

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ABSTRACT

Background: Several hypotheses have been proposed to explain invasive species success. Much of the research in this field has been conducted at the ecosystem or community level. Physiological traits are usually ignored, although they may play a role. As invasiveness has been correlated with species range expansion, it has been assumed – but scarcely tested – that species with greater physiological thermotolerances could be more invasive and colonize more habitats.

Aims: We assessed the responses of native (*Eriopis chilensis*) and invasive (*Harmonia axyridis, Hippodamia variegata*) coccinellids to thermal stress in central Chile after a period of acclimation. We tested and compared their thermal tolerances, their acclimation responses, and the consequences of these on fitness.

Results: Upper and lower lethal temperatures were not significantly different among species and no species was able to increase its lethal limits following acclimation. Our results suggest a trade-off between the 'difference between lower and higher thermal tolerances' and 'ability to acclimate'. However, we also observed significant differences in critical temperatures. *Harmonia axyridis* had lower critical thermal maxima than the other species, which is in line with aestivation behaviour seen in the field. In addition, *H. axyridis* has a higher critical thermal minimum than the other two species, which matches its rapid ongoing invasion into the southern (cooler) part of Chile. Similarly, *H. axyridis* has better egg-laying capabilities than the other two species.

Conclusions: Physiological and life-history traits do play a role in the invasion success of these coccinellids. Also, the interplay between physiological and life-history traits is fundamental to any understanding of invasive species success in the recipient community under a global climate change scenario.

Keywords: Coccinellidae, ecological and evolutionary physiology, global climate change, invasive species, thermal tolerance.

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INTRODUCTION

Owing to its impact on physiological processes, temperature is one of the most important abiotic factors determining species distributions (Somero, 2005; Johnston and Bennett, 2008; Bozinovic *et al.*, 2011a). The physiological responses of animals to environmental temperatures are important in the current context of climate change, and allow us to investigate how an increase in ambient temperature can affect the performance and distribution of species. Invasive species are good models to study because they constitute natural experiments in which species are brought to novel environments and challenged to establish themselves therein.

Species introductions into new habitats are a phenomenon of growing ecological and economic concern (Kolar and Lodge, 2001; Perrings *et al.*, 2002; Jaksic and Castro, 2014; Bollen *et al.*, 2016). Transportation, global trade, and tourism, all are a part of globalization, and promote the connectivity of previously isolated ecosystems, thus facilitating the introduction of exotic species (Bollen *et al.*, 2016). For ectotherms in particular, whose body temperature is determined primarily by environmental temperature, understanding how an invading organism may respond to variations in environmental temperature can provide significant insight regarding the patterns and processes of species invasion (Kelley, 2014). Variation in the ability of species to establish and spread has provided clues to underlying ecological attributes correlated with invasiveness (García-Berthou, 2007). Thus, thermal ecology has the potential to provide a mechanistic explanation for the relationship between the physiology of organisms and the environment (Bozinovic and Vásquez, 1999).

There is some evidence that invasive species are more eurythermal than natives, which is to say that they can maintain physiological function over a wide range of temperatures. Unfortunately, the few studies to have compared temperature tolerances between invasive and native non-invasive species have yielded conflicting results (see meta-analysis by Kelley, 2014). Because invasiveness is correlated with species range expansion, it has been assumed that species with greater physiological thermotolerances should be more invasive and thus colonize more habitats (Rejmanek, 2000; Gaston and Spicer, 2001; McMahon, 2002; Braby and Somero, 2006a, 2006b; Calosi *et al.*, 2010; Zerebecki and Sorte, 2011; Kelley, 2014). But this idea has rarely been tested. In the same way that the range of thermal tolerances is important for predicting the presence or absence of an organism in a given environment (Janzen, 1967; Chown and Terblanche, 2006), so also is the ability to acclimate because it reflects tolerance of a wider range of temperatures.

The thermal tolerance and acclimation ability of organisms are aspects of their fundamental physiological niche. Hypothetically, high acclimation ability may promote invasiveness because it aids exotic species to express advantageous phenotypes over a broad range of environments. This ability allows them the potential to acquire a wider range of thermal tolerance (Bale *et al.*, 2000; Terblanche *et al.*, 2005). Thus, organisms can increase their survival in stressful environmental conditions by previous exposure to stressful but nonlethal conditions (Hoffmann, 1995). Also, a limited ability to adjust thermal limits to changes in environmental temperature is likely to affect species' responses to current climate change scenarios (Stillman, 2003; Calosi *et al.*, 2008).

Many hypotheses have been proposed to explain the success of invasive species in recipient communities: genetic diversity of the founding colony (Prentice *et al.*, 2008), environmental range correlations (Sax, 2001), Darwin's naturalization hypothesis (Daehler, 2001), release of natural enemies (Roy *et al.*, 2011), the novel weapon hypothesis (Callaway and Ridenour, 2004), increased competitive ability (Labrie *et al.*, 2006), and invasional meltdown (Grosholz, 2005; Castro *et al.*, 2008), among others. However, physiological and life-history traits are usually ignored even though they may play an important role. Such a lack of attention is paradoxical in the current scenario of climate change and its potential impact on the physiology and life history of organisms (but see Lardies and Bozinovic, 2006, 2008; Folguera *et al.*, 2007).

We must also consider the consequences of life-history traits that may cause differences in thermal tolerance between species. Life-history traits are a critical component of the ability of an organism to colonize and survive in different environments, and are thus considered a proxy of fitness (Ricklefs and Wikelski, 2002). In this sense, life-history traits may be defined as the way organisms allocate time and energy to reproduction, growth, and survival (Ricklefs and Wikelski, 2002). Thus, life-history traits should be a critical component of the ability of an organism to colonize and survive in different environments (Sun and Cheptou, 2012). In fact, geographical range expansion requires genetic variation in life-history traits. Yet most work that has explored thermal limits in terrestrial ectotherms has ignored life-history traits. But Boher *et al.* (2010) asked, what would be the advantage to tolerating extreme temperatures if fertility and viability do not allow the population to persist in extreme conditions?

Insects are one of the most dominant agents of biological invasion worldwide (Keller *et al.*, 2011). To evaluate whether thermal performance might contribute to invasion success, we adopted two invasive (*Hippodamia variegata* and *Harmonia axyridis*) and one native (*Eriopis chilensis*) aphidophagous coccinellids as study models. *Eriopis chilensis* is an endemic species of southern South America and the most common Chilean coccinellid (González, 2014); in Chile, it ranges from 18°32′S to 41°10′S. Little is known about the biology and ecology of *E. chilensis*, and it is apparently being displaced by invasive ladybirds (Grez *et al.*, 2011).

Hippodamia variegata and *Harmonia axyridis* were introduced to Chile as biological control agents (Koch, 2003; Rebolledo *et al.*, 2009; Grez *et al.*, 2012) despite the knowledge that they have had negative side effects – including their impact on native species – where they have been introduced (Alyokhin and Sewell, 2004; Saini, 2004). They have become a plague in fruit production, particularly as a contaminant during wine production (Pickering *et al.*, 2004). They can even cause discomfort to humans because they hibernate inside houses (Nalepa, 2007).

Hippodamia variegata was introduced from South Africa in the 1970s to control aphids in cereal plantations (Zúñiga *et al.*, 1986). Today it is one of the most abundant coccinellid species, and like *E. chilensis* widely distributed and established throughout Chile from 18°32′S to 41°10′S. Grez *et al.* (2012) suggest that it is displacing native coccinellids. *Harmonia axyridis*, native to Asia, was introduced in 1998, but wild populations were not recorded until 2003 (Grez *et al.*, 2010). Again, *H. axyridis* is extensively distributed in Chile; if one includes extreme occurrences, it ranges from 27°30′S to 53°10′S (Grez *et al.*, 2016). Genetic analyses demonstrate that populations in Chile actually originated from eastern North American populations (Lombaert *et al.*, 2014).

We will attempt to explain the differential success of invasive and native coccinellids in central Chile by comparing the lethal thermal limits of invasive and native species and evaluating the ability of lethal limits to respond to acclimation. We will also compare the critical thermal limits of invasive and native coccinellids and will evaluate their ability to change critical limits as a response to acclimation. Furthermore, we will construct thermal tolerance polygons from critical thermal data to estimate the thermal tolerance ranges of the different species. Finally, we assess the relationship between thermal limits and life-history traits.

MATERIALS AND METHODS

Breeding conditions

Adult individuals of the three species studied were collected with entomological nets in *Medicago sativa* (alfalfa) fields around Santiago (33°27′S, 70°38′W) and taken to the laboratory in ventilated cans with alfalfa branches. Individuals were kept in acrylic boxes measuring 60 × 50 × 50 cm at a temperature of 24°C and photoperiod of 12L : 12D. They were fed regularly with aphids, *Acyrthosiphon pisum* (Hemiptera: Aphididae) raised in the laboratory. In all experiments, we used adults of the second generation to avoid maternal and environmental effects.

Acclimation conditions

We chose acclimation temperatures based on the mean minimum, average, and maximum temperatures recorded in a government weather station near the collection area. Thus our three acclimation treatments (and ranges) were as follows: 9 ± 0.5°C, 15 ± 0.5°C, and 26 ± 0.5°C. The acclimation protocol consisted of randomly assigning adults from the second generation for two weeks to the three acclimation treatments at 12L : 12D in climate chambers.

Lethal temperatures

We assessed lethal limits using the LT_{50} method, defined as the temperature at which 50% of individuals could not survive for a specified period (Fry *et al.*, 1942), following the protocol proposed by Bale *et al.* (2000). To determine the upper (*ULT*) and lower (*LLT*) lethal temperatures, we randomly assigned individuals from each acclimation group (see above) to upper or lower lethal temperature tests. We placed at least twelve individuals from each experimental group into glass test tubes, which we wedged into a test-tube rack so that all the tubes were held at the same level. We put the test-tube rack into a programmable water bath with commercial antifreeze (±0.5°C), set initially to the acclimation temperature of individuals. From that temperature, we either cooled each water bath at a rate of 0.5°C per minute to a range of minimum temperatures between −10°C and −14°C, or we heated the bath at a rate of 0.5°C per minute to a range of maximum temperatures between 42°C and 46°C. The final temperature was maintained for 60 minutes. We then returned the ladybird beetles to their acclimation temperature at the same rate, 0.5°C per minute. We added a wet filter paper to each vial in the *ULT* trials to avoid desiccation effects. After each trial, we estimated survival.

Critical temperatures

After the two-week acclimation period (see above), adults from the second generation were removed from each acclimation group and randomly assigned to critical thermal maxima ($CT_{\max}$) and critical thermal minima ($CT_{\min}$) groups for testing. Twelve replicates per acclimation condition and species were used as a minimum for each test. Each individual was submerged in a glass vial in a programmable water bath and allowed to equilibrate for 10 minutes at either 10°C or 28°C (following Terblanche *et al.*, 2005). The ramp rate was set at

0.1°C per minute. We considered the maximum and minimum critical points to be the temperatures at which an individual could not right itself. We put a thermocouple inside one of the vials to measure its ambient temperature. We assumed that because of their small body size, coccinellid body temperatures equalled the ambient temperature of the vial (Klok and Chown, 2003).

Life-history traits

Adults were acclimated for two weeks at one of the three acclimation temperatures (9 ± 0.5°C, 15 ± 0.5°C, and 26 ± 0.5°C) and placed in boxes (see above) at the rearing temperature of 24°C until we observed male–female pairings. We placed these couples onto plates and removed them after egg-laying. We estimated fecundity as the number of eggs per egg batch.

Data analysis

We plotted survival against extreme temperature and obtained the LT_{50} using a probit model. We conducted a logistic regression between body mass and survival to assess the effect of body mass. We estimated confidence intervals for each lethal temperature using the method of Litchfield-Wilcoxon (1949). The effect of acclimation on critical temperature is mathematically reflected by the magnitude of the slope that connects these two variables. We used a factorial design with environmental conditions (acclimation temperature) and genetic component (different species) as fixed factors. We considered a result plastic if the effect of the environmental factor (acclimation temperature) on the trait (thermal tolerance) was statistically significant.

We estimated the thermal tolerance range for each species from the total area defined by a thermal tolerance polygon. We constructed these polygons for each species, choosing as vertices the values estimated for $CT_{\max}$ and $CT_{\min}$ in each acclimation treatment. The area of the polygon expresses the range of temperature tolerance in units of $°C^2$, where a larger area is related to a greater degree of eurythermicity (Beitinger and Bennett, 2000; Ford and Beitinger, 2005; Calosi *et al.*, 2008).

We measured the effect of temperature on fecundity using a factorial design. We considered acclimation temperature and the different species as fixed factors. Before analysis, we tested the assumptions of normality and heteroscedasticity. We conducted statistical analyses using the software Statistica (Statsoft, v.7.0).

RESULTS

Following acclimation, both the upper and lower lethal limits were conserved and did not show differences among species. Moreover, none of the species was able to increase its upper or lower lethal limit (Fig. 1). Although species did not differ in their lethal limits, they did in their critical temperatures (Fig. 2). *Harmonia axyridis* had significantly lower $CT_{\max}$ than both *Eriopis chilensis* and *Hippodamia variegata* ($F = 37.77$, $P < 0.0001$; Table 1). *Harmonia axyridis* also had a higher $CT_{\min}$ (tolerance to lower temperature) than the other two species ($F = 4.14$, $P = 0.017$; Table 1). The invasive *H. axyridis* lost coordinated movement at a lower temperature than the other two species. As with lethal limits, acclimation did not affect any of the species' critical temperature.

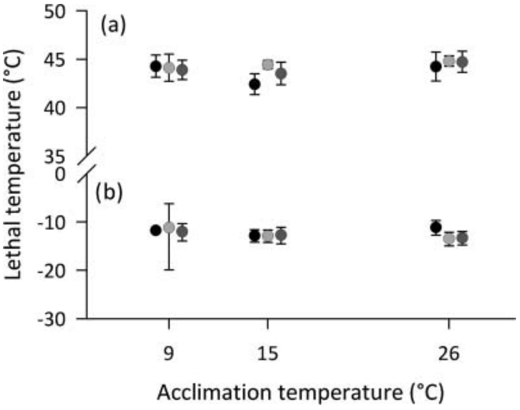

Fig. 1. Effect of acclimation on (a) upper lethal temperature and (b) lower lethal temperature (mean ± 95% confidence intervals) in (from left to right) the native *Eriopis chilensis* and the invasive *Harmonia axyridis* and *Hippodamia variegata.*

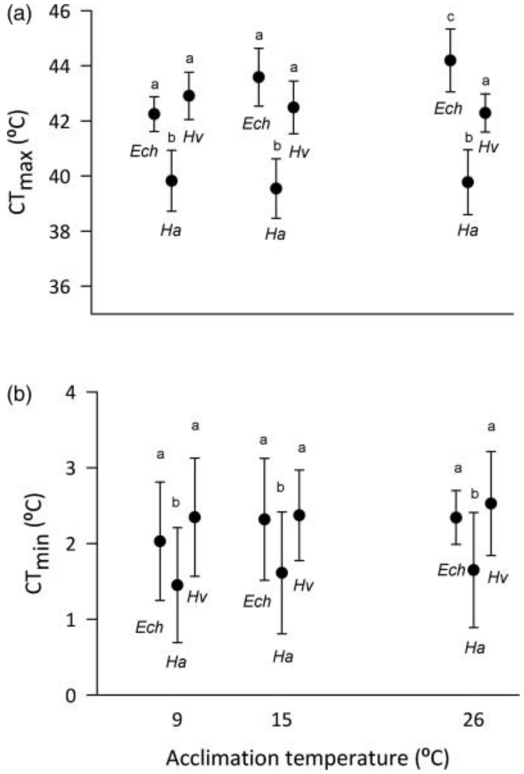

Fig. 2. Effect of acclimation on (a) critical thermal maxima and (b) critical thermal minima (mean ± 95% confidence intervals) in the native *Eriopis chilensis* (*Ech*) and the invasive *Harmonia axyridis (Ha*) and *Hippodamia variegata* (*Hv*). Different letters indicate significant differences.

Table 1. Influence of species identity and acclimation temperature on critical thermal maxima and minima in the native *Eriopis chilensis* and the invasive *Harmonia axyridis* and *Hippodamia variegata*

Source	*df*	SS	*F*	*P*
CT_{max}				
Species	2	429.013	37.777	0.000
Acclimation	2	6.526	0.575	0.564
Species × Acclimation	4	52.067	2.292	0.060
CT_{min}				
Species	2	22.095	4.142	0.017
Acclimation	2	1.688	0.317	0.729
Species × Acclimation	4	0.357	0.033	0.998

The native *E. chilensis* was the only species that responded to acclimation in the CT_{max} test. Species' responses to acclimation differed most in the CT_{max} test. Indeed, statistical rejection of the interaction term may well have been a type II error ($F = 2.29$, $P = 0.06$; Table 1). For all three species, CT_{min} was not influenced by acclimation temperature (CT_{max}: $F = 0.58$, $P = 0.564$; CT_{min}: $F = 0.32$, $P = 0.729$; Table 1). Responses to acclimation did not differ among species in the CT_{min} treatment, as the interaction term was not significant in the model ($P = 0.998$; Table 1). The thermal tolerance polygon areas were similar among species: *E. chilensis* had the largest thermal tolerance polygon (701°C^2), followed by *H. variegata* (681°C^2) and *H. axyridis* (647°C^2). But the polygon area of the invasive *H. axyridis* was displaced downward (Fig. 3). In addition, in all acclimation treatments, the fecundity of *H. axyridis* was higher than that of the other two species (Fig. 4).

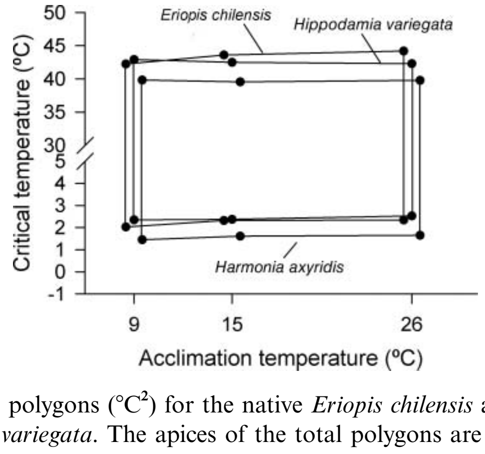

Fig. 3. Thermal tolerance polygons (°C^2) for the native *Eriopis chilensis* and the invasive *Harmonia axyridis* and *Hippodamia variegata*. The apices of the total polygons are set by the critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}) of individuals acclimated at 9°C, 15°C, and 26°C. *Eriopis chilensis* has the largest polygon (701°C^2), followed by *H. variegata* (681°C^2) and *H. axyridis* (647°C^2).

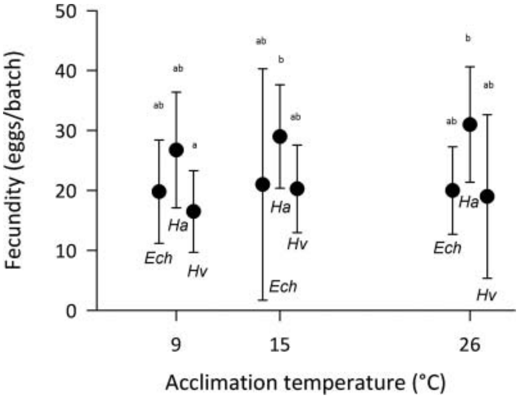

Fig. 4. Fecundity estimated as eggs per batch at the three acclimation temperatures (9°C, 15°C, and 26°C). Different letters indicate significant differences. *Ech*, *Eriopis chilensis*; *Ha*, *Harmonia axyridis*; *Hv*, *Hippodamia variegata*.

DISCUSSION

Understanding the role played by physiological responses to temperature in governing the distribution patterns of species is attracting more attention in the current era of global climate change. Most research about the characteristics that enable the establishment and subsequent expansion of the distribution range of invasive coccinellids is related to intra-guild predation (Burgio *et al.*, 2002; Cottrell, 2004; Felix and Soares, 2004; Grez *et al.*, 2012) and competition for food with native species (Michaud, 2002; Koch, 2003; Soares and Serpa, 2007; Grez *et al.*, 2011, 2012). In an attempt to clarify the causes of the population decline of *Eriopis chilensis* in alfalfa fields in central Chile, Grez *et al.* (2012) found that cannibalism, intra-guild predation, and competition were quite similar in both larvae and adults of native and invasive species, concluding that these biotic factors could not explain the higher relative abundance of invasive coccinellids (Dethier and Hacker, 2005).

As far as we are aware, ours is the first study to assess lethal temperatures and critical temperatures in these species. There were no significant differences in either upper or lower lethal limits among the three species and none of them was able to increase its lethal limits following acclimation. This lack of response supports Stillman's (2003) hypothesis of a trade-off between total thermal range size and the ability to acclimate. Unlike lethal limits, the species studied did show significant differences in critical temperatures. *Harmonia axyridis*, considered a harmful pest all over the world, had lower critical thermal maxima than either of the other two species. Reduced lower critical thermal maxima are in line with the aestivation behaviour observed in the field. The absence of *H. axyridis* in *Medicago sativa* fields in summer correlated with lower $CT_{\max}$. In addition, *H. axyridis* had a higher critical thermal minimum (tolerance to lower temperature) than the other two species, which also matches its rapid ongoing invasion towards the southern (cooler) part of the South American continent (Grez *et al.*, 2016). Given that invasive *H. axyridis* had lower $CT_{\min}$ at all acclimation temperatures compared with the other two species, we think these differences might be even more stark because, as pointed out by Sinclair *et al.* (2015), insects such as walking sticks (Phasmatidae) and beetles (Coleoptera) become immobile when disturbed.

Harmonia axyridis was the only species in our experiments that behaved this way. Thus we recommend assessing $CT_{\min}$ for beetles by driving them to a fixed lower temperature and then measuring their recovery time, instead of assessing the temperature when they cease coordinated movement.

Barahona-Segovia *et al.* (2016) determined preferential temperature in the same species and demonstrated that *H. axyridis* selects lower ambient temperatures than *Eriopis chilensis* and *Hippodamia variegata*, reinforcing our findings that this invader shows better performance at lower temperatures. Overall, there is marked thermal niche overlap among species, which does not support the hypothesis of greater eurythermicity of invasive species. Nevertheless, a decoupling of thermal niches shows a correlative scenario with the rapid ongoing invasion of the harmful *H. axyridis* towards the southern part of Chile. As pointed out by Christiansen *et al.* (2015), ecological physiology provides powerful empirical knowledge that connects theory with natural phenomena and may improve the accuracy of species distribution assessments. It is worth noting that upper lethal limits were close to $CT_{\max}$, whereas lower lethal limits were several degrees lower than $CT_{\min}$ for all acclimation temperatures in the three species. This must reflect the asymmetric relationship between body temperature and organismal performance in ectotherms. Generally, the optimal temperature for performance is many degrees above the lower limit of thermal tolerance but only a few degrees below the upper limit (Bozinovic *et al.*, 2011b). Therefore, near the optimum temperature, variation in body temperature can easily cause animals to exceed their upper thresholds (Deutsch *et al.*, 2008).

The total polygon areas of all three species are larger than those reported for other insect species (Kimura, 2004; Deere *et al.*, 2006). Only a weak, non-significant response to acclimation was evident for both $CT_{\max}$ and $CT_{\min}$. The lack of an acclimation response suggests limited thermal plasticity. Thus, the inability to express a wider thermal range by means of physiological adjustments after a change in environmental temperature suggests hard-wired thermal tolerance levels in these coccinellids, which may change only through adaptive evolution. Their lack of plasticity contrasts with what has been proposed for some other insect species (Deere *et al.*, 2006; Terblanche *et al.*, 2006). A limited ability to adjust thermal limits to changes in environmental temperature is likely to affect species' responses under current global warming scenarios (Stillman, 2003; Somero, 2005). Interestingly, the only species that did respond to acclimation was the native *E. chilensis* (its $CT_{\max}$ rose if it was subjected to a higher acclimation temperature). Thus, although plasticity has been invoked as a likely reason for an invasive species' potential to acclimate to novel environments (Deere *et al.*, 2006; Terblanche *et al.*, 2006; Funk, 2008; Bierbach *et al.*, 2016; Ducatez *et al.*, 2016), we interpret this claim the other way around – an invasive species could perform well in all environments, and a lack of plasticity might be beneficial instead of detrimental (see Chown *et al.*, 2007). Recently, Knapp *et al.* (2018) studied chill coma recovery in *H. axyridis* and reported important changes in recovery through ontogeny, thus concluding that chill coma recovery is not a reliable measure of overwintering success.

Hippodamia axyridis has higher fecundity than either *E. chilensis* or *H. variegata*, so this life-history trait could also be helpful for its ongoing invasion southward. Previous analyses of the biological effects of climate change and invasiveness of exotic species have been correlative. Now we must focus on the underlying causal mechanisms behind the observed effects. Physiologists clearly can contribute to this important discussion (Somero, 2005). Empirical knowledge of environmental preferences and physiological tolerances are particularly valuable for pinpointing potential habitats and invasion routes (core issues for spread models), integrating statistical and mechanistic approaches, and forecasting realistic dispersal scenarios for invasive species in the face of global climate change (Somero, 2005; Christiansen *et al.*, 2015).

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