

2 **Irresistible ants: exposure to novel toxic prey increases**  
3 **consumption over multiple temporal scales**

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8 **Abstract** As species become increasingly exposed to  
9 novel challenges, it is critical to understand how evolu-  
10 tionary (i.e., generational) and plastic (i.e., within life-  
11 time) responses work together to determine a species' fate  
12 or predict its distribution. The introduction of non-native  
13 species imposes novel pressures on the native species that  
14 they encounter. Understanding how native species exposed  
15 to toxic or distasteful invaders change their feeding behav-  
16 ior can provide insight into their ability to cope with these  
17 novel threats as well as broader questions about the evolu-  
18 tion of this behavior. We demonstrated that native eastern  
19 fence lizards do not avoid consuming invasive fire ants fol-  
20 lowing repeated exposure to this toxic prey. Rather fence  
21 lizards increased their consumption of these ants following  
22 exposure on three different temporal scales. Lizards ate  
23 more fire ants when they were exposed to this toxic prey  
24 over successive days. Lizards consumed more fire ants if  
25 they had been exposed to fire ants as juveniles 6 months  
26 earlier. Finally, lizards from populations exposed to fire  
27 ants over multiple generations consumed more fire ants  
28 than those from fire ant-free areas. These results suggest  
29 that the potentially lethal consumption of fire ants may

carry benefits resulting in selection for this behavior, and  
learning that persists long after initial exposure. Future  
research on the response of native predators to venomous  
prey over multiple temporal scales will be valuable  
in determining the long-term effects of invasion by these  
novel threats.

**Keywords** Foraging · Learning · Lizard · Predation ·  
Repeated exposure

**Introduction**

Environmental change can have both immediate and  
long-term effects on species whose response is influenced  
by factors such as the duration, timing (i.e., life stage or  
time passed), and intensity of exposure to given environ-  
mental stimuli (Snyder and Evans 2006; Sax et al. 2007;  
Sinervo et al. 2010). For instance, organisms alter habitat  
use based on intensity and duration of predator cues (e.g.,  
Turner 1997), and stimuli experienced early in life can  
influence how an organism responds to events as an adult  
(Anisman et al. 1998; McCormick and Green 2013). How  
these factors dictate a population's response to environ-  
mental change is of fundamental evolutionary significance;  
however, how these factors work together remains poorly  
understood.

Organisms can respond to change within their lifetime  
[plasticity, including learning (West-Eberhard 1989)] and  
across generations [transgenerational transfer of traits via  
maternal effects, epigenetics, or genetics (Mousseau and  
Fox 1998; Jones and Takai 2001)]. For instance, an indi-  
vidual that has previously experienced a novel environ-  
ment may exhibit a greater response to that stimulus than  
a naïve individual. This response may be learned from a

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61 single exposure or from multiple exposures within the ani- 112  
 62 mal's lifetime (Rogers 1978; Suboski 1992; Chivers and 113  
 63 Smith 1994). As opposed to learned responses within an 114  
 64 animal's lifetime, responses may be inherited (genetically 115  
 65 or maternally) over multiple generations, resulting in indi- 116  
 66 viduals from exposed populations exhibiting a different 117  
 67 response from birth than individuals from naïve popula- 118  
 68 tions (Sax et al. 2007; Love et al. 2013). Responses within 119  
 69 lifetimes and across generations may also interact, with the 120  
 70 ability to learn being selected for [i.e., resulting in faster 121  
 71 learning (Nussey et al. 2005)]. Here, we examine how the 122  
 72 duration and intensity of a novel threat affect an organism's 123  
 73 behavioral response to that threat. We also examine how 124  
 74 long this response persists following exposure to the threat. 125  
 75 We investigate this using a system of native fence lizards 126  
 76 exposed to invasive toxic fire ants that act as novel predator 127  
 77 and stressor (Langkilde 2009; Graham et al. 2012).

78 The red imported fire ant (hereafter, "fire ant"), *Sole- 127  
 79 nopsis invicta*, is a venomous ant native to central South 128  
 80 America (Allen et al. 1974). It has been widely introduced 129  
 81 across the globe, and the resulting ecological and eco- 130  
 82 nomic consequences have been the cause of great concern 131  
 83 for the better part of a century (Banks et al. 1990; Callcott 132  
 84 and Collins 1996). Fire ants were introduced to the USA 133  
 85 in the 1930s via Port Mobile, Alabama, and now occupy 134  
 86 13 states in the southern part of the country. These veno- 135  
 87 mous, predatory invaders often dominate ant communities 136  
 88 within their invasive range due to their aggressive forag- 137  
 89 ing behavior (Holway et al. 2002). Mammals, birds, and 138  
 90 herpetofauna are all susceptible to negative (potentially 139  
 91 population-level) impacts from fire ant invasion (reviewed 140  
 92 in Allen et al. 2004). Most research on the impacts of fire 141  
 93 ant invasion on native wildlife has focused on the impact of 142  
 94 fire ants as novel predators (Tschinkel 2006). Fire ants can 143  
 95 swarm comparatively large vertebrates, paralyze, and kill 144  
 96 them with venomous stings (Wojcik et al. 2001; Allen et al. 145  
 97 2004; Langkilde 2009). However, fire ants also envenomate 146  
 98 and kill native species that attempt to eat them (Webb and 147  
 99 Henke 2003; Boronow and Langkilde 2010), but the impact 148  
 100 of fire ants as a toxic prey species has been largely ignored 149  
 101 (but see Langkilde and Freidenfelds 2010; Robbins and 150  
 102 Langkilde 2012).

103 The eastern fence lizard (hereafter, "fence lizard"), *Scel- 151  
 104 latorus undulatus*, occurs both within areas containing 152  
 105 fire ants and in areas where fire ants have not yet invaded 153  
 106 (Conant and Collins 1998; Regulations CoF 2015). This 154  
 107 has facilitated research on the effects of fire ant invasion on 155  
 108 this native species. Fire ant-invaded populations of fence 156  
 109 lizards appear to have adapted rapidly (within  $\approx 38$  genera- 157  
 110 tions) to pressure exerted by fire ants, displaying changes 158  
 111 in behavior and morphology that decrease the lizards' 159  
 160  
 161  
 162

vulnerability to fire ant attack (Langkilde 2009). The rap- 112  
 idly adapted response of fence lizards to fire ants is likely 113  
 the result of novel pressure exerted by fire ants' highly toxic 114  
 venom and aggressive behavior (Langkilde 2009). Fence 115  
 lizards can be envenomated both during predatory attacks 116  
 by fire ants and while attempting to eat fire ants (Robbins 117  
 and Langkilde 2012). Both types of interactions can prove 118  
 lethal for the fence lizards. As few as 12 fire ants can kill 119  
 adult lizards within a minute (Langkilde 2009), and juve- 120  
 niles succumb to attack by even fewer fire ants and can be 121  
 killed after eating as few as three fire ants (Langkilde and 122  
 Freidenfelds 2010). Additionally, encounters with fire ants 123  
 (both fire ant attack and consumption) that are not imme- 124  
 diately lethal can cause delayed mortality of juvenile fence 125  
 lizards (Langkilde and Freidenfelds 2010).

127 Considering the significant mortality that fence lizards 128  
 129 can suffer following envenomation while consuming fire 130  
 131 ants, there should be significant pressure on these lizards 132  
 133 to display aversion behavior (learned or inherent) fol- 134  
 135 lowing exposure to fire ants. Interestingly, Robbins et al. 136  
 137 (2013) found that the propensity of juvenile fence lizards 138  
 139 to consume fire ants increased when lizards were offered 140  
 141 a single ant per day over the course of 1 week. How- 142  
 143 ever, these results may not be representative of the inter- 144  
 145 actions that occur in the field. Wild lizards living in fire 146  
 147 ant-invaded sites likely encounter fire ants far more fre- 148  
 149 quently than once per day, resulting in more stings and a 150  
 151 more intense negative stimulus, which may promote aver- 152  
 153 sion behavior. Indeed we have observed fence lizards eat- 154  
 155 ing up to six fire ants per encounter in staged semi-natural 156  
 157 interactions (Robbins and Langkilde 2012; Robbins et al. 158  
 159 2013), and fence lizards encounter fire ants frequently in 160  
 161 the field [every 132 s ( $\pm 29$  SE) (Freidenfelds et al. 2012)]. 162  
 Here, we explore whether fence lizards display aversion 163  
 behavior when exposed to ecologically relevant numbers 164  
 of fire ants. We investigate how differences in stimulus 165  
 intensity (number of fire ants per exposure) and temporal 166  
 scales (across generations and following distant and recent 167  
 past exposure to fire ants) impact this species' behavioral 168  
 response to this evolutionarily novel stimulus. We hypoth- 169  
 esize that (1) fence lizards will display stronger learned 170  
 aversion behavior by decreasing their consumption of fire 171  
 ants over time if exposed to greater, ecologically relevant 172  
 numbers of ants; and (2) fence lizards with previous expo- 173  
 sure to fire ants will be more averse to eating fire ants than 174  
 relatively naïve lizards. This latter hypothesis should hold 175  
 true for exposure both across generations and within the 176  
 lifetime of individual lizards, resulting in lizards from fire 177  
 ant-invaded sites displaying inherent aversion of fire ants 178  
 in addition to any behavior learned following exposure 179  
 within their lifetimes.

163 **Materials and methods**

164 We conducted a feeding experiment to examine the effects  
165 of stimulus strength and history of exposure to fire ants on  
166 consumption of these toxic prey by fence lizards. Decreased  
167 rates of consumption of fire ants with successive exposure  
168 would indicate aversion behavior. To test for the effect of  
169 stimulus strength, we offered lizards fewer (four) or greater  
170 (ten) numbers of fire ants per day. To examine the effects of  
171 previous exposure to fire ants, we compared the rates of fire  
172 ant consumption by subadult lizards exposed to these toxic  
173 ants on different temporal scales: (1) lizards from popula-  
174 tions that have coexisted with invasive fire ants for multiple  
175 ( $\approx 38$ ) generations (multigenerational exposure), (2) lizards  
176 that were experimentally exposed to fire ants for 2 weeks as  
177 juveniles (distant past exposure within a lifetime), and (3)  
178 lizards that had been exposed to fire ants in the preceding  
179 days (recent exposure within a lifetime).

180 Our experimental approach was designed to examine  
181 fence lizard foraging behavior on fire ants. Lizards typi-  
182 cally encounter fire ants in low numbers in the field (Frei-  
183 denfelds et al. 2012), and consumption of fire ants under  
184 these conditions is consistent with foraging behavior (T. L.,  
185 unpublished data). However, when lizards encounter large  
186 numbers of fire ants in the field (e.g., on mounds or for-  
187 aging trails), they are quickly attacked by the ants. Lizards  
188 may consume fire ants in such situations; however, in this  
189 context consumption appears to be a defensive rather than  
190 foraging behavior (Robbins and Langkilde 2012). Similar  
191 anti-predatory consumption of fire ants is also observed  
192 in horned lizards (Webb and Henke 2003). To avoid con-  
193 founding differences in the consumption of fire ants in  
194 these two contexts (Robbins and Langkilde 2012; Robbins  
195 et al. 2013), we chose numbers of fire ants that elicited for-  
196 aging, and not defensive, consumption in fence lizards dur-  
197 ing trials.

198 **Multigenerational exposure**

199 We tested for the effects of multigenerational exposure to  
200 fire ants on the consumption of these toxic prey by fence  
201 lizards by comparing lizards from fire ant-invaded and  
202 uninvaded populations, and how these were affected by  
203 repeated exposure to fire ants. We collected juvenile fence  
204 lizards by hand between 3 July 2012 and 9 August 2012 at  
205 four sites, two of which had been invaded by fire ants and  
206 two of which remain uninvaded, respectively (invaded—  
207 Blackwater River State Forest, Santa Rosa County, FL,  
208 30.94°N, 86.82°W, and Geneva State Forest in Geneva Co.,  
209 AL, 31.12°N, 86.16°W; uninvaded—Edgar Evins State  
210 Park, Dekalb County, TN, 36.08°N, 85.83°W, and Standing  
211 Stone State Park, Overton County, TN, 36.47°N, 85.42°W).

**Distant past exposure**

212

213 To test whether relatively short-term exposure of juveniles  
214 to fire ants would have a lasting effect on their propen-  
215 sity to eat these ants, we exposed half of our individuals  
216 to fire ants as juveniles, approximately 6 months prior to  
217 the feeding trial (described in the next section). After cap-  
218 ture, the lizards were transferred to the Solon Dixon For-  
219 estry Education Center in Covington County, Alabama  
220 (31.16°N, 86.70°W) and housed individually in tubs  
221 [30 × 20 × 25 cm, length × width × height ( $L \times W \times H$ )].  
222 Crickets (*Acheta domesticus*) and water were provided  
223 ad libitum. Tubers were furnished with a refuge, water dish,  
224 and paper towels as substrate, and had a heat lamp posi-  
225 tioned at one end to create a temperature gradient that  
226 allowed lizards to thermoregulate. Beginning on 26 July  
227 2012, groups of ten lizards were placed into one of four  
228 520-m<sup>2</sup> outdoor enclosures constructed of aluminum  
229 sheeting. Fire ants occurred at normal densities in half of  
230 the enclosures, while fire ants were removed from other  
231 enclosures daily using a treatment of boiling water to kill  
232 fire ant colonies within the enclosures and in surrounding  
233 areas (Tschinkel and King 2007). Lizards were recaptured  
234 and removed from the enclosures after 2–3 weeks, and the  
235 effect of this (distant past) experience on the lizards' con-  
236 sumption of fire ants 6 months later was quantified.

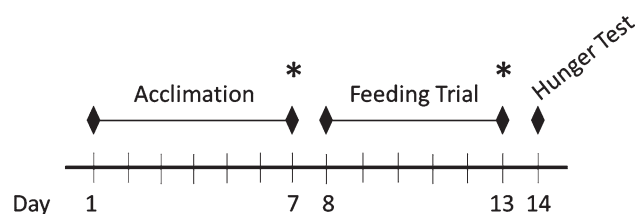
**Feeding trial: recent past exposure and evaluation of fire ant consumption**

237

238

239 The feeding experiment represents a recent exposure of  
240 fence lizards to fire ants and allowed us to assess the effect  
241 of multigenerational, distant and recent past exposure  
242 of lizards to fire ants on their consumption of these ants.  
243 Following the distant past-exposure treatments, we trans-  
244 ported the lizards to our laboratory at the Pennsylvania  
245 State University. Here, they were communally housed for  
246 approximately 6 months, with two to five lizards per tub  
247 (60 × 42 × 30 cm,  $L \times W \times H$ ), furnished as described  
248 earlier. Crickets and water were provided ad libitum. To  
249 commence the feeding experiment lizards were transferred  
250 to individual tubs (30 × 20 × 25 cm,  $L \times W \times H$ ), fur-  
251 nished as described earlier, and assigned to one of two  
252 stimulus treatments, which consisted of us offering either  
253 four (low stimulus) fire ants or ten (high stimulus) fire ants  
254 per day for 6 days. The lizards were evenly distributed  
255 within stimulus treatments with regard to their population  
256 of origin, enclosure treatment, size, and sex (Webb and  
257 Henke 2003).

258 Lizards were acclimated to the experimental feeding  
259 routine for 6 days prior to the beginning of the trial by  
260 feeding them four or eight 0.5-cm crickets, according to



**Fig. 1** A timeline of events during the feeding experiment, which occurred approximately 6 months after the enclosure treatments. Asterisks indicate days when lizards were offered 20 crickets to ensure that they were satiated for the following day's trials

261 their stimulus treatment group, to simulate ant-sized prey  
 262 (Fig. 1). On day 7, we provided all lizards with twenty  
 263 1-cm crickets to ensure that they were satiated before the  
 264 beginning of the trial period to help offset any effects of  
 265 the previous differential feeding regime. Prior to feeding on  
 266 day 7 we recorded the mass and snout-vent length (SVL) of  
 267 all lizards used in the experiment.

268 After acclimation, we started the feeding trial by offer-  
 269 ing lizards four or ten fire ants, according to their stimulus  
 270 treatment (days 8–13; Fig. 1). After 30 min we recorded  
 271 the number of fire ants each lizard had consumed. We then  
 272 removed any remaining fire ants and gave each lizard two  
 273 1-cm crickets as a subsistence diet. The 1-cm crickets were  
 274 left in the tubs overnight, and any that remained the fol-  
 275 lowing morning were removed immediately prior to again  
 276 offering the lizards fire ants to eat. Water dishes and ref-  
 277 uges were removed from the tubs during each daily feed-  
 278 ing. The paper towel substrate was removed for the dura-  
 279 tion of the experiment to ensure prey did not hide and to  
 280 facilitate scoring.

281 To determine if increasing hunger played a role in the  
 282 rate of consumption of fire ants across the experiment, on  
 283 the last day of the feeding experiment (day 13) and after the  
 284 fire ant feeding was completed, we fed all lizards to satia-  
 285 tion by providing them twenty 1-cm crickets. The follow-  
 286 ing day (day 14), we offered all lizards (regardless of treat-  
 287 ment group) ten fire ants in order to determine if satiation  
 288 affected their rate of fire ant consumption (i.e., were any  
 289 changes in ant consumption over the trial due to increasing  
 290 hunger).

## 291 Data analysis

292 We tested factors influencing consumption of fire ants by  
 293 fence lizards during the feeding experiment using repeated-  
 294 measures ANOVA. We included percent of ants eaten on  
 295 successive days (days 8–13) as the repeated dependent  
 296 variable, and fire ant-invasion status of the source popu-  
 297 lations (multigenerational exposure), exposure of lizards  
 298 to fire ants as juveniles in enclosures (distant past expo-  
 299 sure), and stimulus treatment (low vs. high number of fire

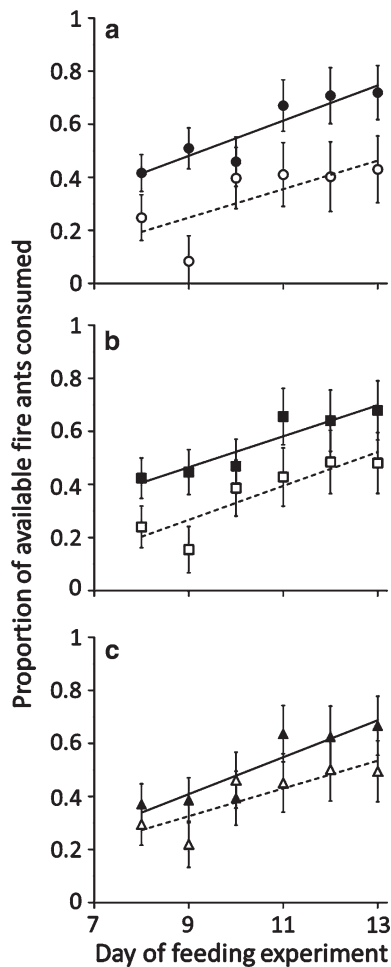
ants) during the feeding experiment as factors. Interactions  
 between these main factors were non-significant ( $P > 0.30$ )  
 and were thus excluded from the final model. Site (nested  
 within invasion status), enclosure identity, sex, mass, and  
 SVL were included as control variables, but were omitted  
 from the final model as they did not significantly explain  
 variation in consumption behavior ( $P > 0.31$ ). We analyzed  
 the proportion of available ants consumed (as opposed to  
 number of ants consumed) to facilitate testing of factors  
 within which lizards were offered a different number of  
 ants based on stimulus treatment (i.e., lizards within a sin-  
 gle invasion status were fed either four or ten ants).

We tested for effects of hunger on fence lizards' con-  
 sumption of fire ants by examining differences between  
 the last day of the feeding experiment (day 13) and the fol-  
 lowing day (after being fed to satiation, day 14). A signifi-  
 cant decrease in fire ant consumption following being fed  
 to satiation would suggest that hunger was driving fire ant  
 consumption. We analyzed hunger effects using repeated-  
 measures ANOVA with number of ants consumed before  
 versus after satiation (day 13 and 14) as the repeated  
 dependent variable, and stimulus treatment (low vs. high  
 stimulus) as the factor. Site (nested within invasion status),  
 enclosure identity, sex, mass, and SVL were included as  
 control variables, but were omitted from the final model  
 as they did not significantly explain variation in consump-  
 tion behavior ( $P > 0.09$ ). In the hunger test we analyzed  
 the total number of ants eaten per lizard (as opposed to  
 percentage of offered ants consumed) on the successive  
 days as the dependent variable. The reasoning for this was  
 that individual lizards in the low stimulus treatment were  
 offered a different number of ants before (four ants) and  
 after (ten ants) satiation, making testing changes in propor-  
 tion of consumed ants uninformative since a lizard that did  
 not change its consumption (i.e., ate the same number of  
 ants on successive days) would eat a different proportion of  
 the ants offered.

Percent of ants eaten was arcsine transformed to meet  
 model assumptions. All statistical analyses were performed  
 using JMP (version 12.1; SAS Institute, Cary, NC) with  
 $\alpha = 0.05$ .

## Results

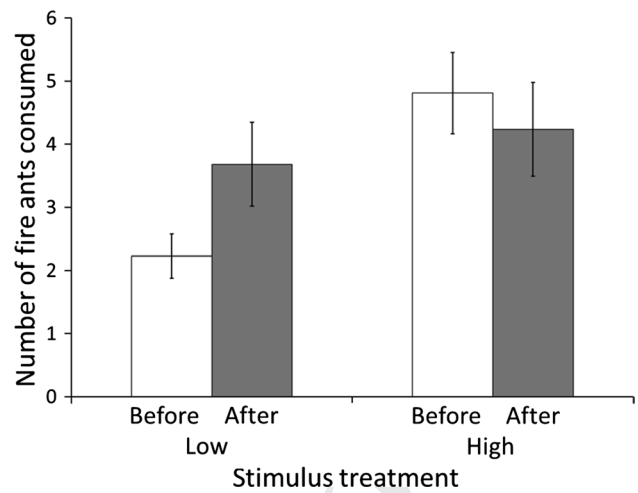
Recent past exposure of lizards to fire ants (previous days)  
 did affect consumption of these toxic prey. However, rather  
 than avoiding eating fire ants with repeated exposure, liz-  
 ards consumed an increasing proportion of fire ants over the  
 course of the feeding experiment (day of trial,  $F_{5,35} = 3.69$ ,  
 $P < 0.01$ ; Fig. 2). Neither multigenerational exposure to fire  
 ants (invasion status of the source population) or strength  
 of the stimulus treatment (four vs. ten fire ants) affected



**Fig. 2** Consumption of fire ants (*Solenopsis invicta*) by fence lizards (*Sceloporus undulatus*) during the 6-day feeding experiment. **a** Lizards originally from fire ant-invaded populations (*solid circles*) consumed more ants over the course of the experiment than did lizards from uninvaded (*open circles*) populations ( $F_{1,39} = 7.02, P = 0.01$ ). **b** Lizards that had been exposed to fire ants as juveniles in outdoor enclosures 6 months prior (*solid squares*) consumed more fire ants during the experiment than lizards that had not been exposed (*open squares*;  $F_{1,39} = 4.07, P = 0.05$ ). **c** Lizards that were presented with a high (ten fire ants per day; *open triangles*) versus low (four fire ants per day; *solid triangles*) stimulus intensity did not consume a significantly different proportion of ants ( $F_{1,39} = 1.41, P = 0.24$ ). Points represent mean  $\pm 1$  SE

350 the rate of change in fire ant consumption over time (day  
351 of trial  $\times$  invasion status,  $F_{5,35} = 2.12, P = 0.09$ ; day of  
352 trial  $\times$  stimulus treatment,  $F_{5,35} = 1.22, P = 0.32$ ).

353 Prior exposure to fire ants (multigenerational and distant  
354 past) affected the overall propensity of lizards to eat  
355 fire ants (Fig. 2). Lizards originally captured from fire ant-  
356 invaded sites, or exposed to fire ants as juveniles in out-  
357 door enclosures 6 months earlier, ate more ants during the  
358 feeding experiment than did lizards from uninvaded sites  
359 or those placed in fire ant-free enclosures (invasion status,  
360  $F_{1,39} = 7.02, P = 0.01$ ; enclosure treatment,  $F_{1,39} = 4.07,$



**Fig. 3** Fire ant consumption by lizards presented with low (four fire ants per day) and high (ten fire ants per day) intensity stimuli before (day 13; *dark bar*) and after (day 14; *light bar*) being fed crickets to satiation. Neither group of lizards significantly decreased their consumption of fire ants following satiation ( $F_{1,41} = 1.09, P = 0.30$ ). Bars represent mean  $\pm 1$  SE

$P = 0.05$ ; Fig. 2a, b). Stimulus intensity (number of fire ants offered) did not affect the overall consumption of fire ants by the lizards ( $F_{1,39} = 1.41, P = 0.24$ ; Fig. 2c).

We observed no significant decrease in the number of ants consumed following lizards being fed to satiation (day 13 vs. 14,  $F_{1,41} = 1.09, P = 0.30$ ). Conversely, lizards consumed a greater number of ants following satiation (Fig. 3). However, this is likely due to increased opportunity as lizards in the low stimulus (four ant) treatment were given ten ants after satiation and ate more accordingly, whereas those in the high stimulus treatment (given ten ants before and after satiation) did not change their ant consumption (day 13 vs. 14  $\times$  stimulus treatment,  $F_{1,41} = 5.75, P = 0.02$ ; stimulus treatment:  $F_{1,41} = 4.27, P = 0.05$ ; Fig. 3).

## Discussion

Lizards did not exhibit aversion learning in response to repeated exposure to fire ants, a toxic prey, even when exposure was high and potentially lethal (Langkilde and Freidenfelds 2010). This species and other lizards can distinguish different ant species (Suarez et al. 2000; Thawley, unpublished data), and are capable of memory retention over 14 weeks (the duration of our distant past exposure) (Benes 1969), suggesting that fence lizards should have been able to learn to avoid fire ants. The fact that lizards' consumption of fire ants increased over multiple temporal scales shows that they were indeed responding to repeated exposure, but surprisingly by increasing their consumption of this toxic prey (Fig. 2). Prior exposure did not, however,

389 affect the rate at which lizards increased their consumption  
390 of fire ants with repeated exposure (Fig. 2); that is, lizards  
391 with prior exposure to fire ants were not more likely to  
392 learn or acquire a taste for fire ants.

393 Recent past exposure to fire ants (in previous days)  
394 increased the propensity of lizards to eat fire ants as they  
395 ate successively more fire ants during our 6-day feeding  
396 experiment. This was unlikely due to increased hunger dur-  
397 ing the experiment since feeding the lizards to satiation did  
398 not decrease the number of fire ants they consumed. Expo-  
399 sure of juvenile lizards to fire ants in outdoor enclosures  
400 increased consumption of fire ants 6 months later (distant  
401 past exposure), suggesting that lizards retained memory  
402 of this experience (Benes 1969) or that the fire ants had  
403 some other lasting physiological effect on the lizards.  
404 Finally, lizards from populations that had been exposed  
405 to red imported fire ants for multiple (38) generations ate  
406 more fire ants than did those from uninvaded populations.  
407 This could suggest maternal and/or inherited transfer of  
408 this behavior. However, these lizards were collected from  
409 the field as juveniles so invasion status is potentially con-  
410 founded with early exposure to fire ants (juveniles from  
411 fire ant-invaded sites likely encountered these ants prior to  
412 being collected, whereas those from uninvaded sites would  
413 not have). It should be noted that the “invaded” popula-  
414 tions used in this study are within both the invasive range  
415 of *Solenopsis invicta* and the historic distribution of the  
416 native fire ants *Solenopsis geminata* and *Solenopsis xyloni*  
417 (Tschinkel 2006). While *S. invicta* likely impose a stronger  
418 selective pressure than the native fire ants due to their more  
419 potent venom (Tschinkel 2006), higher densities (Porter  
420 et al. 1988), and more aggressive attack than native fire ant  
421 species (Lai et al. 2015), it is possible that the prior expo-  
422 sure to native fire ants contributes to the observed differ-  
423 ences between invaded and uninvaded populations.

424 The results of this study expand upon previous research  
425 in this system in several ways:

- 426 1. Lizards increase their consumption of fire ants with  
427 repeated exposure both when exposure is high and  
428 potentially lethal (this study) and when exposure is low  
429 and sublethal (as per Robbins et al. 2013). This find-  
430 ing suggests that, in free-ranging lizards, the increased  
431 consumption of fire ants, and lack of aversion learning,  
432 is likely to occur when ants are at both low densities  
433 (e.g., when the first few invading ants arrive at a site)  
434 and at higher densities [e.g., once ants have become  
435 fully established (Wojcik 1994)].
- 436 2. Lifetime (e.g., learning, plasticity) and cross-genera-  
437 tional effects (e.g., maternal effects, selection) both  
438 contribute to determining consumption of fire ants, the  
439 rate of which increases both at the population level [an  
440 increasing proportion of lizards in a population con-

441 sumes fire ants (Robbins et al. 2013)] and at the level  
442 of the individual lizard [individual lizards will eat more  
443 fire ants (this study)] with repeated exposure to this  
444 toxic prey.

- 445 3. Effects of exposure to fire ants as juveniles can persist  
446 into sub-adulthood. Lizards that were exposed to fire  
447 ants for 2 weeks as juveniles (in enclosures) consumed  
448 more of these ants as subadults (during the feeding  
449 experiment) than did lizards from ant-free enclosures,  
450 even after spending 6 months in captivity with no  
451 exposure to fire ants. Thus the behavioral changes in  
452 fire ant consumption are persistent even after relatively  
453 short-term exposures to fire ants early in life.

454 These behavioral changes in the lizards could have  
455 evolutionary significance by changing phenotypic varia-  
456 tion within populations, and thus opportunity for selection  
457 (Stearns 1992; Roff 2002).

458 Given the possibly severe consequences of eating fire  
459 ants, it is interesting to consider why we saw this increase  
460 in fire ant consumption by fence lizards. Several possibili-  
461 ties exist, including:

- 462 1. With experience, lizards might become more adept at  
463 consuming fire ants without being envenomated and  
464 could therefore safely consume more of these ants.  
465 Within a lifetime, lizards could learn to modify their  
466 feeding behavior in order to prevent fire ant stings.  
467 Across generations, lizards could inherit characteristics  
468 that could allow them to consume these venomous ants  
469 without injury or envenomation (e.g., Sherbrooke and  
470 Schwenk 2008; Cushing 2012), for example, by imme-  
471 diately biting and killing ants before they can attach to  
472 and sting the inside of the mouth (Robbins et al. 2013).
- 473 2. Lizards could also negate the costs of envenomation  
474 through resistance to fire ant venom (e.g., Schmidt  
475 et al. 1989). Because we observed increases in fire ant  
476 consumption both on multigenerational and within-  
477 lifetime scales, if toxin resistance is acquired it does  
478 not appear to be acquired only across generations.  
479 However, there is no evidence of increased resistance  
480 to fire ant toxin by fence lizards following fire ant inva-  
481 sion [measured as effects on blood cell lysis and right-  
482 ing ability (Goldy-Brown, unpublished data; Boronow  
483 and Langkilde 2010)]. Further research should examine  
484 if, with experience, lizards are indeed able to avoid ant  
485 stings during feeding or mitigate any negative effects  
486 of envenomation through other physiological means.
- 487 3. Consuming fire ants could be an anti-predator response  
488 (Robbins and Langkilde 2012). Lizards will flee from  
489 fire ants when they encounter them at sufficient den-  
490 sity, such as on a mound. However, consuming fire  
491 ants when they are encountered at low density (such as

- 492 foraging ants) may prevent further recruitment of addi-  
 493 tional ants to their location (see Webb and Henke 2003;  
 494 Freidenfelds et al. 2012).
- 495 4. The benefits of fire ants as a novel food resource may  
 496 outweigh the costs associated with envenomation  
 497 (Robbins et al. 2013). Where they are present, fire ants  
 498 are often highly abundant and fundamentally alter the  
 499 native arthropod communities (Porter and Savignano  
 500 1990). The possibility of prey switching by fence liz-  
 501 zards, potentially including increased consumption of  
 502 fire ants, should be investigated.
- 503 5. Lizards may actually be developing a preference for  
 504 fire ants (Robbins et al. 2013). Although rare (Rozin  
 505 et al. 1979), animals can become “addicted” to unpal-  
 506 atable or irritant substances [e.g., humans exposed to  
 507 chili pepper (Rozin and Schiller 1980)]. For instance,  
 508 there is anecdotal evidence that dogs lick toxic inva-  
 509 sive cane toads to get high (Hero et al. 2005), and  
 510 may repeatedly lick cane toads after first exposure  
 511 (J. Cochran, personal communication). Examining  
 512 whether fire ants induce a “pleasurable” effect, such  
 513 as the release of endorphins or dopamine, would be  
 514 informative in this regard (Sharma and Verma 2014).
- 515 6. The toxin of fire ants might be of some anti-predator  
 516 value to the lizard. Dendrobatid frogs and Asian keel-  
 517 back snakes sequester defensive toxins from ants  
 518 (Saporito et al. 2004) and toads (Hutchinson et al.  
 519 2007), respectively. The possibility that fence lizards  
 520 sequester fire ant venom to defend themselves against  
 521 predators would be an interesting avenue of future  
 522 research (Savitzky et al. 2012).

523 Understanding the ecological and evolutionary con-  
 524 sequences of invasive species, including those that act as  
 525 novel palatable or toxic prey, is important for predicting  
 526 and managing this increasing environmental perturbation.  
 527 Some taxa display innate abilities to avoid toxic prey spe-  
 528 cies with which they have coevolved (Smith 1975), as well  
 529 as the ability to change feeding behavior when subjected  
 530 to negative stimuli (Susswein et al. 1986; Crossland 2001;  
 531 Somaweera et al. 2011). We demonstrate that eastern fence  
 532 lizards do not avoid consuming fire ants following expo-  
 533 sure and potential envenomation. In fact, we found that  
 534 fence lizards tend to increase their consumption of venom-  
 535 ous fire ants with repeated exposure. This trend is demon-  
 536 strable over multiple temporal scales. Future research on  
 537 the response of other native predators to venomous prey,  
 538 over multiple temporal scales, will be valuable in deter-  
 539 mining the long-term effects of invasion and the evolution of  
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