Herpetological Conservation and Biology 13(2):479–487.
Submitted: 15 September 2015; Accepted: 14 July 2018; Published: 31 August 2018.

IMPACT OF LAYING DATE AND FIRE ANTS ON HATCHLINGS OF *CHELONOIDIS PORTERI* ON SANTA CRUZ ISLAND, GALÁPAGOS, ECUADOR

NINA WAUTERS[1,2], WOUTER DEKONINCK[2], ZOLTAN T. NAGY[3], AND DENIS FOURNIER[1,4]

[1]Evolutionary Biology and Ecology, Université libre de Bruxelles, Brussels, Belgium
[2]Royal Belgian Institute of Natural Sciences, Brussels, Belgium
[3]Taxonomy and Phylogeny, Royal Belgian Institute of Natural Sciences, Brussels, Belgium
[4]Corresponding author, e-mail: Denis.Fournier@ulb.ac.be

***Abstract.—Chelonoidis* land tortoises are iconic species endemic to the Galápagos Islands of Ecuador. Their populations have been dramatically reduced by human activities in the last three centuries, including indirect effects such as the introduction of invasive species. We investigated the mortality of eggs and hatchlings in 48 nests of *Chelonoidis porteri* on Santa Cruz Island with regard to various mortality causes such as the occurrence of fire ants and the date of laying. The average mortality rate was 0.56. Tropical Fire Ants (*Solenopsis geminata*) were present within 1 m of 75% of the *C. porteri* nests, and we encountered fire ants in 12.5% of excavated nests. We found no relationship between Tropical Fire Ant abundance and *C. porteri* egg and hatchling survivorship. We observed no signs of mold inside the nests. We determined that early deposition dates were associated with lower clutch survival and identified egg development as the critical life stage. Finally, we discuss the potential impacts of fire ants and climate change on tortoise survival and reproduction and stress the importance of taking these factors into account for the conservation of the endemic land tortoises of the Galápagos.**

Key Words.—biodiversity; biological invasions; conservation efforts; land tortoises; *Solenopsis geminata*; Testudines

INTRODUCTION

Giant land tortoises of the genus *Chelonoidis* (formerly *Geochelone nigra*) are iconic species of the Galápagos Islands, Ecuador. They are native to the Galápagos and occur on all of the major islands, represented by 11 extant and five extinct species (Van Denburgh 1914; Poulakakis et al. 2012; Poulakakis et al. 2015; http://www.reptile-database.org/). Galápagos tortoises are important ecosystem engineers (Gibbs et al. 2010; Froyd et al. 2014), as they are the only native large-bodied herbivores on the archipelago and are also important seed dispersers (Blake et al. 2013b). However, over the last three centuries, Galápagos Tortoises have been harvested for their meat and oil (Townsend 1925; MacFarland et al. 1974a). Moreover, the introduction of alien mammalian species, which can serve as either predators or competitors (e.g., pigs, rats, cats, dogs, goats, and donkeys), has led to a further decline of many of the endemic *Chelonoidis* populations. Populations have declined from 100,000–250,000 individuals before human colonization in the 16th Century to fewer than 8,000–14,000 in the 1970s. During this period, two species of *Chelonoidis* went extinct (Townsend 1925; MacFarland et al. 1974a; Swingland and Klemens 1989; Jackson 1994). In 1965, Galápagos National Park launched a conservation program for giant tortoises, which included captive breeding and rearing, repatriation, and protection of nesting areas (Perry 1970; Márquez et al. 1987; Cayot 2008), and non-native mammal control (Cruz et al. 2005; Carrion et al. 2007; Cruz et al. 2009; Rivera-Parra et al. 2012; Márquez et al. 2013). The program has been a success, with more than 4,049 tortoises belonging to eight populations from six islands repatriated by 2008 (Cayot 2008).

Invasive fire ants, Little Fire Ant (*Wasmannia auropunctata*) and Tropical Fire Ant (*Solenopsis geminata*), first recorded in the Galápagos Islands at the beginning of the 20th Century (Williams and Whelan 1991; Williams and Whelan 1992), can potentially threaten long-term survival of reptiles and birds (Roque-Albelo and Causton 1999; Roque-Albelo et al. 2000; Causton and Sevilla 2008). More specifically, observations were made of juvenile *Chelonoidis* tortoises stung by *S. geminata* (Williams and Whelan 1991), as well as depredated eggs and hatchlings inside nests (Snell et al. 2002; Tye et al. 2002; Cayot 2008; Blake et al. 2013b). *Solenopsis geminata* is a well-known tramp ant species and is considered one of the most damaging and widespread introduced ant species (Holway et al. 2002). In the Galápagos Islands, the species has been recorded in seven islands and 11 islets (Wauters et al. 2014) and belongs to a small group of invertebrates having a significant impact on Galápagos wildlife, including reptiles, birds, and invertebrates (e.g., Causton et al. 2006; Herrera and Causton 2008; Wauters et al. 2016; Wauters et al. 2017).

Here, we present a preliminary study to assess survival of *Chelonoidis porteri* within the egg and immediately after hatching, when the young stay inside their burrows and are particularly vulnerable to predators. The incubation period of *C. porteri* extends between June to March and the incubation time lasts from 110–240 d, depending upon the temperature and the humidity in the nest. The egg-laying period occurs from June to December and the hatching period from December to March (Swingland and Klemens 1989; Blake et al. 2013a). The hatchlings may remain in the egg for a day or longer after pipping has occurred. After hatching, hatchlings stay inside the nests for several days (MacFarland et al. 1974b; Swingland and Klemens 1989). They are nourished by their yolk sac that protrudes from the lower shell, which provides the much-needed energy for the tortoises after they emerge from the burrow (Vitt and Caldwell 2013). The burrow allows hatchlings to stay underground, safely away from some predators, but the external yolk sac causes the tortoise to be relatively immobile, increasing its susceptibility to predation. In a protected nesting area on Santa Cruz Island, we estimated the proportion of damaged eggs and the mortality rate of hatchlings and investigated whether overall mortality of clutches was associated with the deposition date of eggs in the burrows and/or the presence of *S. geminata*.

Materials and Methods

Our research was conducted in the protected nesting area of La Torta (Lat. -0.747637, Long. -90.341141), on Santa Cruz Island, Ecuador. La Torta is located in the arid zone, where the dominant plants are deciduous trees and shrubs such as *Bursera* spp. and cacti such as *Opuntia* spp. and *Jasminocereus* spp. Galápagos National Park wardens protected each new nest from mammalian predators using metallic mesh fixed by heavy rocks and visited the area weekly during the incubation period (i.e., from June to December). The meshing allows hatchlings to pass through and leave the nest cages. At the end of the hatching period in March, wardens excavated marked nests to facilitate the emergence of hatchlings from underground and surveyed the tortoises on the site.

We examined 48 tortoise nests sharing similar environmental conditions (i.e., sunshine, rainfall, temperature) between 3–28 March 2012. We excavated the tortoise nests and counted and released live tortoises; we then estimated the number of individuals that had already left the nest prior to the experiment by counting empty eggshells. We also counted damaged eggs (i.e., eggs with some cracks in the outer calcified shell, or eggs likely broken by the female tortoise when she covered up the hole), and dead hatchlings.

We considered the proportion of damaged eggs and the mortality of hatchlings separately, as potential threats during each period differ. We estimated the overall mortality of clutches as the proportion of dead tortoises at the excavation time. We conducted all fieldwork under the supervision of Galápagos National Park wardens.

We investigated the possible causes of mortality by looking for signs of the presence of fire ants and fungus growth inside the nests. To assess the fire ant abundance near the nests, we applied peanut butter (approximately 2 g) to a 10 × 10 cm piece of cardboard on the soil surface 1 m away from of each nest. All baits were set between 1100 and 1330 and after 1 h, we collected the baits and sealed them into a Ziploc® bag. We then killed, identified, and counted the ants. To estimate the ant activity around the nests, and identify other potentially damaging invertebrate species, we set up one pitfall trap (i.e., a plastic cup; diameter 6.5 cm, depth 10 cm) of water and detergent buried into the ground, at the same level as the ground surface next to 10 of the 48 nests and left them undisturbed for one week. We identified and counted the invertebrates captured in the traps. As Galápagos National Park wardens visited the area regularly during the incubation period, we used the protection date as a proxy of the deposition date of eggs in the burrows.

We used generalized linear models (GLMs) to assess whether mortality of tortoises was related to *S. geminata* abundance, deposition date of eggs, and/or clutch size. We used the Akaike information criterion (AIC) as an estimator of the relative quality of statistical models (Burnham and Anderson 2002), and performed model selection using the package AICcmodavg (Mazerolle 2016). We performed all statistical analyses using R (version 3.4.2, https://www.r-project.org) and RStudio (version 1.1).

Results

The period of deposition of eggs greatly differed between nests and ranged from August to December (Table 1). We observed 126 damaged eggs and 115 hatchlings in 48 nests and deduced from remaining empty eggs that 73 tortoises had left the burrows prior to surveys (mean clutch size = 6.54 ± [SD] 1.68; range, 3–11). Nearly 40% of total laid eggs (126 of 314) were damaged and 22.3% of hatchlings (42 of 188) were dead at the time of our survey with the mean number of damaged eggs per nest = 2.63 ± 1.99 (range, 0–7) and the mean number of dead hatchlings per nest = 0.88 ±1.25 (range, 0–4; Table 1). The mean proportion of

Table 1. Composition of *Chelonoidis porteri* nests opening in La Torta, Santa Cruz Island, Galápagos. Displayed are the dates of the discovery of the nests and the dates of their excavation, the number of damaged eggs, the number of tortoises released by Galápagos National Park wardens, the number of tortoises that have left the nest before its excavation, the number of dead hatchlings, the clutch size and the abundance of Tropical Fire Ants at baits (baits were placed 1 m away from of each nest). An asterisk (*) in the ants abundance column indicates a mild infestation by fire ants inside the nests (< 100 workers); a double asterisk (**) indicates a heavy infestation (> 100 workers).

Nest	Protection date	Excavation date	Damaged eggs	Live hatchlings	Tortoises having left the nest	Dead hatchlings	Clutch size	Ants abundance
1_25	28 October 2011	6 March 2012	0	6	0	0	6	0
1_57	7 September 2011	6 March 2012	2	5	0	0	7	0
2_56	7 September 2011	6 March 2012	3	0	0	3	6	3
2_123	16 November 2011	6 March 2012	4	0	0	1	5	13
3_52	7 September 2011	6 March 2012	2	0	5	0	7	122
3_54	7 September 2011	6 March 2012	2	0	4	0	6	163
3_81	6 October 2011	6 March 2012	1	0	6	0	7	204
3_82	6 October 2011	6 March 2012	1	0	7	0	8	0
3_139	16 November 2011	6 March 2012	0	7	0	2	9	183
3_140	16 November 2011	6 March 2012	6	0	0	0	6	251
4_50	30 August 2011	6 March 2012	3	4	0	2	9	0
4_51	30 August 2011	6 March 2012	7	2	0	0	9	278
4_92	6 October 2011	6 March 2012	2	2	0	4	8	42
4_93	6 October 2011	6 March 2012	2	0	4	0	6	96
4_94	6 October 2011	6 March 2012	2	0	0	1	3	179
4_95	6 October 2011	6 March 2012	3	3	0	1	7	195
4_102	6 October 2011	6 March 2012	6	0	0	0	6	274
5_174	27 December 2011	14 March 2012	0	0	4	0	4	109*
5_177	27 December 2011	14 March 2012	1	0	3	0	4	229*
6_31	30 August 2011	14 March 2012	3	0	0	4	7	0**
6_176	27 December 2011	14 March 2012	6	5	0	0	11	0
6_178	27 December 2011	14 March 2012	0	0	6	0	6	431
7_170	27 December 2011	14 March 2012	0	0	5	1	6	96
7_171	27 December 2011	14 March 2012	1	5	0	0	6	96
7_173	27 December 2011	14 March 2012	7	0	0	0	7	1
8_32	30 August 2011	14 March 2012	4	0	0	2	6	127
9_179	27 December 2011	14 March 2012	2	5	0	0	7	112
9_180	27 December 2011	14 March 2012	1	6	0	0	7	116*
10_181	27 December 2011	14 March 2012	3	0	0	2	5	0**
10_182	27 December 2011	14 March 2012	1	0	4	0	5	112
10_183	27 December 2011	14 March 2012	1	6	0	2	9	0
10_184	27 December 2011	14 March 2012	1	0	0	3	4	0
11_161	27 December 2011	28 March 2012	3	2	0	0	5	256
11_162	27 December 2011	28 March 2012	3	2	0	0	5	256
11_164	27 December 2011	28 March 2012	5	0	0	2	7	202
11_165	27 December 2011	28 March 2012	2	0	0	3	5	202
12_73	6 October 2011	19 March 2012	4	0	0	2	6	289
12_75	6 October 2011	19 March 2012	5	0	0	0	5	141

Table 1 (continued). Composition of *Chelonoidis porteri* nests opening in La Torta, Santa Cruz Island, Galápagos. Displayed are the dates of the discovery of the nests and the dates of their excavation, the number of damaged eggs, the number of tortoises released by Galápagos National Park wardens, the number of tortoises that have left the nest before its excavation, the number of dead hatchlings, the clutch size and the abundance of Tropical Fire Ants at baits (baits were placed 1 m away from of each nest). An asterisk (*) in the ants abundance column indicates a mild infestation by fire ants inside the nests (< 100 workers); a double asterisk (**) indicates a heavy infestation (> 100 workers).

Nest	Protection date	Excavation date	Damaged eggs	Live hatchlings	Tortoises having left the nest	Dead hatchlings	Clutch size	Ants abundance
12_76	6 October 2011	19 March 2012	3	1	0	3	7	288
12_78	6 October 2011	19 March 2012	3	6	0	0	9	249
13_167	27 December 2011	28 March 2012	5	0	5	0	10	0
13_169	27 December 2011	28 March 2012	0	0	7	0	7	0
14_79	6 October 2011	19 March 2012	4	0	0	1	5	29
14_80	6 October 2011	28 March 2012	5	0	0	0	5	29
16_172	27 December 2011	14 March 2012	4	0	0	3	7	272**
17_159	27 December 2011	28 March 2012	3	6	0	0	9	135
18_166	27 December 2011	28 March 2012	0	0	6	0	6	57
18_168	27 December 2011	28 March 2012	0	0	7	0	7	361

damaged eggs per nest and dead hatchlings per nest were 0.41 ± 0.31 (range, 0–1) and 0.34 ± 0.45 (range, 0–1), respectively. The overall mortality rate (from laying to hatchlings) per nest was 0.56 ± 0.39 (range, 0–1; Table 1).

We observed no sign of fungal infections. However, *S. geminata* was present within 1 m of 75% of nests (36 of 48). Worker ants of this species generally forage within 15 m of their nest (Levins et al. 1973; Perfecto 1994), and foraging may occur underground (Chang and Ota 1976). Around the baits, field experiments revealed that *S. geminata* was the only ant species present after one hour. *Solenopsis geminata* ants recruit rapidly, numerically dominate, and aggressively exclude from baits other ant species (Wauters et al. 2014).

The abundance of *S. geminata* varied from 0 to 341 individuals (mean = 129.12 ± 115.27; median = 114) per baited site. Worker ants of *S. geminata* were present inside six nests, three of which were mildly infested (i.e., presence of a few ants only), and three were heavily infested (i.e., hundreds of ants). In the three heavily infested nests, we found dead hatchlings (two to four individuals per nest) and three to four damaged eggs. Some dead tortoises presented injuries consistent with *S. geminata* attacks such as damaged gnawed skin.

We collected *S. geminata* in all 10 pitfall traps [mean individuals per trap = 49.00 ± 43.20 (range, 5–124)]. We also identified four other ant species, constituting only 28 (5.41%) of 518 individuals collected: *Brachymyrmex heeri* (15 individuals in four traps), *Camponotus zonatus* (seven individuals in three traps), *Odontomachus bauri* (three individuals in three traps) and *Nylanderia steinheili* (three individuals in three traps). These four ant species, although introduced, do not *a priori* constitute a threat to the tortoises, as they have never been observed attacking vertebrates in the Galápagos Islands, nor, to our knowledge, elsewhere (unpubl. data). Except for a small number of dipterans, we did not find any other invertebrates in pitfall traps.

Overall mortality rate of tortoises (from laying to hatchlings) was best explained by a model including the date of deposition, the clutch size, and the interaction between these two factors (lowest AIC value and an Akaike's weight of 0.91; Table 2). Ant presence inside the burrows did not influence overall mortality rate (Table 2). Overall mortality rate was significantly negatively associated both with the earliest dates of deposition (estimated model coefficient = -0.04, $P < 0.001$) and with the total number of laid eggs (estimated model coefficient = -68.79, $P < 0.001$; Table 3). More specifically, the deposition date and the total number of laid eggs significantly affected the pre-hatching mortality (i.e., impacts eggs; deposition date: estimated model coefficients = -0.010, $P = 0.018$; clutch size: estimated model coefficients = -0.181, $P = 0.047$) but not the post-hatching mortality (i.e., mortality of hatchlings; all $P > 0.079$; Table 3).

Discussion

Tortoise mortality.—We estimated mortality rate of eggs for *C. porteri* in La Torta, Santa Cruz Island to be 55.6%. We found a negative association between the early date of egg laying and the number of damaged eggs. The time spent in the burrows is directly linked with temperature (Swingland and Klemens 1989; Deeming and Ferguson 2004). Clutches laid early in the cool season will experience cooler temperatures and need longer incubation periods, while eggs laid late in the season will experience warmer temperatures and require a shorter incubation period, consistent with the degree-day model (MacFarland et al. 1974b; Deeming

Table 2. Results of generalized linear model selection showing Akaike information criterion (AIC), the delta AIC to the most likely model (Delta *i*), the Akaike weights (AIC Wt), the cumulative Akaike weights (Cum.Wt) and the log-likelihood (LL) of each model. Abbreviations are AA = ants abundance, D1 = date of the discovery of the nests (a proxy of the deposition date of eggs in the burrows), and CS = clutch size.

Models	AIC	Delta *i*	AIC Wt	Cum.Wt	LL
Mortality of tortoises = D1 * CS	260.17	0	0.91	0.91	-126.09
Mortality of tortoises = D1 + CS	265.55	5.38	0.06	0.97	-129.78
Mortality of tortoises = AA + D1 + CS	267.46	7.29	0.02	0.99	-129.73
Mortality of tortoises = D1	270.69	10.52	0	1	-133.35
Mortality of tortoises = AA * D1	272.58	12.41	0	1	-132.29
Mortality of tortoises = AA + D1	272.67	12.49	0	1	-133.33
Mortality of tortoises = CS	276.10	15.93	0	1	-136.05
Mortality of tortoises = AA + CS	277.97	17.79	0	1	-135.98
Mortality of tortoises = AA * CS	279.77	19.60	0	1	-135.89
Mortality of tortoises = AA	282.75	22.57	0	1	-139.37

and Ferguson 2004; Du et al. 2009). Usually, cool temperatures coupled with long incubation times are associated with greater survival and greater body size in hatchlings because the cooler temperatures prevent the eggs from desiccating and overheating (Van Damme et al. 1992, Vitt and Caldwell 2013). However, long incubation times can also be deleterious because the eggs are more vulnerable to other causes of mortality, such as predators, flooding, and fungal infections (Vitt and Caldwell 2013). As we did not observe fungal infections, and because our nests were protected against mammalian predation, egg loss in this study could be caused by excessive humidity (Van Damme et al. 1992; Deeming and Ferguson 2004). Although a certain amount of humidity is necessary for the embryos during the early incubation stages to protect them from drying out, the drying of embryos is necessary at a later stage of development to initiate air breathing (Deeming and Ferguson 2004). The nesting area was generally arid but water puddles were common due the clay content of the soil, potentially temporarily flooding some of the tortoise nests.

Table 3. Generalized linear model coefficients of the most likely model of mortality of tortoises (= D1×CS). Abbreviations are as in Table 2. Given are the estimated model coefficients (coef.), their standard error (SE) and the *P*-value. A negative model coefficient indicates a negative relationship.

	Coef.	SE	*P*-value
Overall mortality rate			
D1	-0.041	0.012	0.001
CS	-68.794	25.713	0.007
D1×CS	0.004	0.002	0.008
Pre-hatching mortality			
D1	-0.010	0.004	0.018
CS	-18.130	8.839	0.0463
D1×CS	0.001	0.001	0.0467
Post-hatching mortality			
D1	-0.011	0.006	0.0789
CS	-19.150	13.030	0.1499
D1×CS	0.001	0.001	0.1517

Another factor potentially influencing the mortality of tortoises could be linked to climate change. Bell et al. (2013) show that global warming in the tropics can reduce hatchling viability and performances of ectothermic oviparous reptiles. Measurements of sea surface temperature, air temperature, and rainfall since 1965 (http://www.darwinfoundation.org/datazone/climate/) show no recent warming in the Galápagos Islands (Trueman and d'Ozouville 2010; Wolff 2010). However, climate models predict longer and more common El Niño and La Niña events with increased rainfall (Grant and Grant 1992; Snell et al. 2002; Sachs and Ladd 2010). Because extreme temperatures are more informative of the thermal environment than average temperatures (Camacho et al. 2015), this increase of extreme climatic events can also have detrimental effects on tortoises eggs and hatchlings survival (Santidrián Tomillo et al. 2012). The last two strong El Niño events in 1982–83 and 1997–98 caused: (1) high mortality of *Chelonoidis* tortoises falling down in ravines; (2) drowning in floods; and/or (3) decreased their reproductive output (Snell et al. 2002). The nests were rendered unviable because increased vegetation and wet soils reduced incubation temperatures, favored mold, and impeded gas exchange (Márquez et al. 2008; Larrea and Di Carlo 2011). These events also seemed to favor fire ants, which attacked and killed more hatchlings during the El Niño years (Trueman and d'Ozouville 2010).

MacFarland et al. (1974 a,b) observed hatching rates of *C. porteri* in 1969–1970, 1970–1971, and 1971–1972 of 84.6%, 73.2%, and 75.6%, respectively. They did not mention the name of their study site on Santa Cruz, nor if they used cages to protect nests against mammalian predators. Given that a slight change in microhabitat can influence the incubation temperature and the mortality rate of eggs (Deeming and Ferguson 2004), the difference with our results could be explained by a difference in habitat type and/or methodology.

Influence of predation.—A recent study was conducted at a different site on Santa Cruz Island (El Chato) in a more humid area (Blake et al. 2013a). In this study, despite the mesh and rocks around the nests, feral pigs preyed upon 57% of nests of the protected area, and trampling and/or predation by donkeys, pigs, and *S. geminata* destroyed an additional 14% of nests. During our survey, we did not observe pigs or donkeys on our study site, which is separated by < 14 km from the El Chato study site. The harsh habitat conditions of La Torta (hot weather, rocky soil, vegetation composed of sclerophyllic plants and cacti) compared with the more humid site of El Chato, combined with the remoteness of La Torta, prevents pigs and donkeys from colonizing the area. Other vertebrates such as rats or cats might also be present in La Torta, but the meshes and heavy rocks did not show signs of deterioration and may be sufficient to keep these predators from destroying nests.

Fire ants are abundant on La Torta, and readily attack vertebrate intruders, including humans (Wheeler 1910; pers. obs.). Although we found no relationship between *S. geminata* abundance and tortoise mortality, the presence of fire ants inside some of the nests and the feeding on dead individuals confirm that *S. geminata* are able to enter nests and potentially cause damage to hatchlings. We were not able, however, to determine whether *S. geminata* found inside nests caused the death of hatchlings or were only scavenging on their bodies. Márquez et al. (2004) suggested that *S. geminata* workers might perforate *Chelonoidis* eggs and feed on the embryos. It is unlikely that *S. geminata* were able to enter the intact eggs, given the hard and thick shell of *Chelonoidis* eggs (Swingland and Klemens 1989), but fire ants could attack cracked eggs or hatchlings right after breaking the shell, when they are the most vulnerable. Others have observed fire ants preying upon cracked eggs and hatchlings (Snell et al. 2002; Blake et al. 2013a; Galápagos National Park wardens, pers. comm.), and attacking the skin of juvenile and adult tortoises (Williams and Whelan 1991; Márquez et al. 2004).

Another species of fire ant closely related to *S. geminata* is the Red Imported Fire Ant (*S. invicta*), which is capable of penetrating the eggs of several reptile species including the Loggerhead Sea Turtle (*Caretta caretta*; Diffie et al. 2010). However, *S. invicta* workers are not able to penetrate intact eggshells of the Florida Red-bellied Turtle, *Pseudomys nelsoni* (Allen et al. 2004), or of the Common Snapping Turtle, *Chelydra serpentina* (Conners 1998); (Diffie et al. 2010). Similarly, *S. invicta* workers could not penetrate Florida Softshell (*Apalone ferox*) or Musk Turtle (*Sternotherus odoratus*) eggs (Diffie et al. 2010), two species that have hard thick shelled eggs, similar to those of *Chelonoidis*.

The apparent contradiction between the previously observed impacts of *S. geminata* and the results of our study may be explained by the use of baits to estimate ant abundances. Although widely used (Gotelli et al. 2011), these baits may present biases when estimating ant abundance. Even if workers of *S. geminata* are active 24 h a day (Wauters et al. 2014), with a relative constant number of individuals, we cannot exclude that the abundance of ants may have been different at another time than that of our observations. Moreover, as all baiting experiments were conducted mid-day, it is possible that the heat had a negative impact on ant activity on the surface, and that ants were mostly confined to underground trails instead. For example, on two occasions, we observed *S. geminata* inside tortoise nests but found no ants at the bait stations. A differential exposure of the baits to the sun among traps may not reflect actual abundance of ants locally. The discrepancy could also be explained by a saturation of the site by *S. geminata*. *Solenopsis geminata* are so abundant that all nests go through the same stress caused by these ants and no correlation can be found between ant abundance and mortality. Finally, the loss of evidence of *S. geminata* predation (or other causes of mortality) may stem for the fact that the nests were not evaluated shortly after individuals hatched for all nests. Indeed, to avoid any repetitive and unnecessary disturbances, Galápagos National Park wardens recommended that we waited until March before excavating nests, but some tortoises could have hatched and been predated inside the nests earlier in the season.

Perspectives and recommendations.—To protect this iconic *Chelonoidis* species, we recommend close monitoring of La Torta. *Solenopsis geminata* are widespread on the archipelago and have been spreading in recent decades (Wauters et al. 2014; Wauters et al. 2016), including to tortoise nesting areas (Márquez et al. 2004; Márquez et al. 2008). As they have been reported attacking *Chelonoidis* tortoises on several occasions, we recommend close monitoring of *S. geminata* populations. For example, ant baits could be used at dusk or dawn to prevent heat interfering with ant activity. Moreover, we also recommend monitoring areas at different times during the incubation period, with and without nests to see if ants show spatial correlation to active nests. To precisely determine if the *S. geminata* impact the giant tortoise *C. porteri*, it could be useful to conduct a similar experiment by comparing the mortality of tortoises within a same population in zones infested by *S. geminata* and in areas without *S. geminata*. We also recommend documenting the presence of *S. geminata* inside turtle nests or their predation on hatchlings when excavating the nests. Fire ant eradications using insecticides that have minimal impacts on vertebrates could be used as a preventative measure on *Chelonoidis* nesting sites (Abedrabbo 1994; Causton et al. 2012). Finally, as climate change has been shown to have negative impacts on *Chelonoidis* tortoise survival and reproduction, weather data and nest temperatures should be included in future tortoises survival studies.

Acknowledgments.—This work was supported by several grants from the Belgian FRS-FNRS (Fonds National pour la Recherche Scientifique). We would like to thank the Charles Darwin Research Station (CDRS) and the Galápagos National Park (GNP) for providing permits (PR.CDS.ACI.PO1.R01 – PC-23-12) and field support, the Belgian Worktable for financial and logistic support at the CDRS, the Léopold III Founding and the Entomology Department of RBINS for their financial supports. Thanks to all the GNP rangers for their help in the field and Washington Tapia and Kleber Aguilar for logistic support. This project is part of the 'Control of invasive invertebrates' project of the Charles Darwin foundation. This publication is contribution number 2182 of the Charles Darwin Foundation for the Galapagos Islands.

LITERATURE CITED

Abedrabbo, S. 1994. Control of the Little Fire Ant, *Wasmannia auropunctata*, on Santa Fe Island in the Galapagos Islands. Pp. 219–227 *In* Exotic Ants: Biology, Impact, and Control of Introduced Species. Williams, D.F. (Ed.). Westview Press, Boulder, Colorado, USA.

Allen, C.R., D.M. Epperson, and A.S. Garmestani. 2004. Red Imported Fire Ant impacts on wildlife: a decade of research. American Midland Naturalist 152:88–103.

Bell, K., S. Blomberg, and L. Schwarzkopf. 2013. Detrimental influence on performance of high temperature incubation in a tropical reptile: is cooler better in the tropics? Oecologia 171:83–91.

Blake, S., J. Gibbs, F. Cabrera, W. Tapia, and M. Wikelski. 2013a. Long distance migration of Galapagos tortoises: the importance of nesting and nest sites. National Geographic Society, Global Exploration Fund, Washington D.C., USA.

Blake, S., C.B. Yackulic, F. Cabrera, W. Tapia, J.P. Gibbs, F. Kümmeth, and M. Wikelski. 2013b. Vegetation dynamics drive segregation by body size in Galapagos tortoises migrating across altitudinal gradients. Journal of Animal Ecology 82:310–321.

Burnham K.P., and D.R. Anderson. 2002. Model Selection and Multimodel Inference. Springer-Verlag, New York, New York, USA.

Camacho, A., M. Trefaut Rodrigues, and C.A. Navas. 2015. Extreme operative temperatures are better descriptors of the thermal environment than mean temperatures. Journal of Thermal Biology 49–50:106–111.

Carrion, V., C.J. Donlan, K. Campbell, C. Lavoie, and F. Cruz. 2007. Feral Donkey (*Equus asinus*) eradications in the Galápagos. Biodiversity and Conservation 16:437–445.

Causton, C.E., and C.R. Sevilla. 2008. Latest records of introduced invertebrates in Galápagos and measures to control them. Charles Darwin Foundation (FCD), Galapagos National Park (PNG), and Instituto Nacional Galápagos (INGALA), Puerto Ayora, Galápagos, Ecuador.

Causton, C.E., S.B. Peck, B.J. Sinclair, L. Roque-Albelo, C.J. Hodgson, and B. Landry. 2006. Alien insects: threats and implications for conservation of Galápagos Islands. Annals of the Entomological Society of America 99:121–143.

Causton, C.E., C. Sevilla, W. Cabrera, A. Carrion, and V. Carrion. 2012. Evaluación del Manejo de Hormigas Invasoras Galápagos. Fundación Charles Darwin, Dirección Parque Nacional Galápagos, Island Conservation, Puerto Ayora, Islas Galápagos, Ecuador.

Cayot, L.J. 2008. The restoration of giant tortoise and land iguana populations in Galápagos. Notícias de Galápagos 65:39–42.

Chang, V.C.S., and A.K. Ota. 1976. Fire ant damage to polyethylene tubing used in drip irrigation systems. Journal of Economic Entomology 69:447–450.

Conners, J.S. 1998. *Chelydra serpentina* (Common snapping turtle). Predation. Herpetological Review 29:235.

Cruz, F., V. Carrion, K.J. Campbell, C. Lavoie, and C.J. Donlan. 2009. Bio-economics of large-scale eradication of feral goats from Santiago Island, Galápagos. Journal of Wildlife Management 73:191–200.

Cruz, F., C. Josh Donlan, K. Campbell, and V. Carrion. 2005. Conservation action in the Galàpagos: Feral Pig (*Sus scrofa*) eradication from Santiago Island. Biological Conservation 121:473–478.

Deeming, D.C., and M.W.J. Ferguson. (Eds.). 2004. Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles. Cambridge University Press, Cambridge, UK.

Diffie, S., J. Miller, and K. Murray. 2010. Laboratory observations of Red Imported Fire Ant (Hymenoptera: Formicidae) predation on reptilian and avian eggs. Journal of Herpetology 44:294–296.

Du, W.G., R.S. Radder, B. Sun, and R. Shine. 2009. Determinants of incubation period: do reptilian embryos hatch after a fixed total number of heart beats? Journal of Experimental Biology 212:1302–1306.

Froyd, C.A., E.E.D. Coffey, W.O. van der Knaap, J.F.N. van Leeuwen, A. Tye, K.J. Willis, and D. Sax. 2014. The ecological consequences of megafaunal loss: giant tortoises and wetland biodiversity. Ecology Letters 17:144–154.

Gibbs, J.P., E.J. Sterling, and F.J. Zabala. 2010. Giant tortoises as ecological engineers: a long-term quasi-experiment in the Galápagos Islands. Biotropica 42:208–214.

Gotelli, N.J., A.M. Ellison, R.R. Dunn, and N.J. Sanders. 2011. Counting ants (Hymenoptera: Formicidae): biodiversity sampling and statistical analysis for myrmecologists. Myrmecological News 15:13–19.

Grant, P.R., and R.B. Grant. 1992. Global warming and the Galápagos. Notícias de Galápagos 51:14–16.

Herrera, H.W., and C.E. Causton. 2008. Distribution of fire ants *Solenopsis geminata* and *Wasmannia auropunctata* (Hymenoptera: Formicidae) in the Galapagos Islands. Galapagos Research 65:11–14.

Holway, D., L. Lach, A.V. Suarez, N.D. Tsutsui, and T.J. Case. 2002. The causes and consequences of ant invasions. Annual Review of Ecology and Systematics 33:181–233.

Jackson, M. 1994. Galápagos: a Natural History. University of Calgary Press, Calgary, Alberta, Canada.

Larrea, I., and G. Di Carlo. (Eds.). 2011. Climate Change Vulnerability Assessment of the Galápagos Islands. World Wildlife Fund and Conservation International, USA, Quito, Ecuador.

Levins, R., M.L. Pressick, and H. Heatwole. 1973. Coexistence patterns in insular ants. American Scientist 61:463–472.

MacFarland, C.G., J. Villa, and B. Toro. 1974a. The Galápagos Giant Tortoises (*Geochelone elephantopus*) Part I: Status of the surviving populations. Biological Conservation 6:118–133.

MacFarland, C.G., J. Villa, and B. Toro. 1974b. The Galápagos Giant Tortoises (*Geochelone elephantopus*) Part II: Conservation methods. Biological Conservation 6:198–212.

Márquez, C., F. Cepeda, F. Llerena, M. Wilson, and S. Rea. 1987. The Giant Tortoise conservation program. Notícias de Galápagos 45:17–18.

Márquez, C., J.P. Gibbs, V. Carrión, S. Naranjo, and A. Llerena. 2013. Population response of Giant Galápagos Tortoises to feral goat removal. Restoration Ecology 21:181–185.

Márquez, C., D.A. Wiedenfeld, S. Naranjo, and W. Llerena. 2008. 1997-8 El Niño and the Galapagos Tortoises *Geochelone vandenburghi* on Alcedo Volcano, Galapagos. Galapagos Research 65:7–10.

Márquez, C., D. Wiedenfeld, H. Snell, T. Fritts, M.F. Belen, C. MacFarland, W. Tapia, and S. Naranjo. 2004. Estado actual de las poblaciones de tortugas terrestres gigantes (*Geochelone* spp., Chelonia: Testitudinae) en las Islas Galápagos. Ecología Aplicada 3:98–111.

Mazerolle, M.J. 2016. AICcmodavg: Model Selection and Multimodel Inference Based on (Q)AIC(c). http://cran.r- project.org/package=AICcmodavg.

Perfecto, I. 1994. Foraging behavior as a determinant of asymmetric competitive interaction between two ant species in a tropical agroecosystem. Oecologia 98:184–192.

Perry, R. 1970. Tortoise rearing in the Galapagos Islands. Notícias de Galápagos 15–16:3–7.

Poulakakis, N., D.L. Edwards, Y. Chiari, R.C. Garrick, M.A. Russello, E. Benavides, G.J. Watkins-Colwell, S. Glaberman, W. Tapia, J.P. Gibbs, et al. 2015. Description of a new Galapagos giant tortoise species (*Chelonoidis*; Testudines: Testudinidae) from Cerro Fatal on Santa Cruz Island. PLoS ONE 10:e0138779. https://doi.org/10.1371/journal.pone.0138779.

Poulakakis, N., M. Russello, D. Geist, and A. Caccone. 2012. Unravelling the peculiarities of island life: vicariance, dispersal and the diversification of the extinct and extant giant Galápagos tortoises. Molecular Ecology 21:160–173.

Rivera-Parra, J.L., K.M. Levenstein, J.C. Bednarz, F.H. Vargas, V. Carrion, and P.G. Parker. 2012. Implications of goat eradication on the survivorship of the Galapagos Hawk. Journal of Wildlife Management 76:1197–1204.

Roque-Albelo, L., and C. Causton. 1999. El Niño and introduced insects in the Galápagos Islands: different dispersal strategies, similar effects. Notícias de Galápagos 60:30–36.

Roque-Albelo, L., C.E. Causton, and A. Mieles. 2000. The ants of Marchena island, twelve years after the introduction of the Little Fire Ant, *Wasmannia auropunctata*. Notícias de Galápagos 61:17–20.

Sachs, J.P., and S.N. Ladd. 2010. Climate and oceanography of the Galapagos in the 21st Century: expected changes and research needs. Galapagos Research 67:50–54.

Santidrián Tomillo, P., V.S. Saba, G.S. Blanco, C.A. Stock, F.V. Paladino, and J.R. Spotila. 2012. Climate driven egg and hatchling mortality threatens survival of Eastern Pacific Leatherback Turtles. PLoS ONE 7:e37602. https://doi.org/10.1371/journal.pone.0037602.

Snell, H.L., A. Tye, C.E. Causton, and R. Bensted-Smith. 2002. The status of and threats to terrestrial biodiversity. Pp. 30–47 *In* A Biodiversity Vision for the Galapagos Islands: Based on an International Workshop of Conservation Biologists. Bensted-Smith, R. (Ed.). Charles Darwin Foundation, World Wildlife Fund, Puerto Ayora, Ecuador.

Swingland, I.R., and M.W. Klemens. (Eds.). 1989. The Conservation Biology of Tortoises. International Union for Conservation of Nature - the World Conservation Union, Gland, Switzerland.

Townsend, C.H. 1925. The Galapagos tortoises in their relation to the Whaling Industry - A study of old logbooks. Zoologica 4:55–135.

Trueman, M., and N. d'Ozouville. 2010. Characterizing the Galapagos terrestrial climate in the face of global climate change. Galapagos Research 67:26–37.

Tye, A., H.L. Snell, S.B. Peck, and H. Adsersen. 2002. Outstanding terrestrial features of the Galapagos archipelago. Pp. 12–23 *In* A Biodiversity Vision for the Galapagos Islands: Based on an International Workshop of Conservation Biologists. Bensted-Smith, R. (Ed.). Charles Darwin Foundation, World Wildlife Fund, Puerto Ayora, Ecuador.

Van Damme, R., D. Bauwens, F. Braña, and R. Verheyen. 1992. Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. Herpetologica 48:220–228.

Van Denburgh, J. 1914. The gigantic land tortoises of the Galápagos archipelago. Proceedings of the California Academy of Sciences 2:203–374.

Vitt, L., and J. Caldwell. 2013. Herpetology - An Introductory Biology of Amphibians and Reptiles. Academic Press, New York, New York, USA.

Wauters, N., W. Dekoninck, F. Hendrickx, H.W. Herrera, and D. Fournier. 2016. Habitat association and coexistence of endemic and introduced ant species in Galápagos Islands. Ecological Entomology 41:40–50.

Wauters, N., W. Dekoninck, H. Herrera, and D. Fournier. 2014. Distribution, behavioral dominance and potential impacts on endemic fauna of the Tropical Fire Ant *Solenopsis geminata* (Hymenoptera: Myrmicinae) in the Galápagos archipelago. Pan-Pacific Entomologist 90:205–220.

Wauters, N., D. Fournier, and W. Dekoninck. 2017. Do agricultural zones on Santa Cruz Island, Galápagos, host native and endemic arthropods? Pan-Pacific Entomologist 93:141–158.

Wheeler, W.M. 1910. Ants: Their Structure, Development and Behavior. Columbia University Press, New York, New York, USA.

Williams, D.F., and P. Whelan. 1991. Polygynous colonies of *Solenopsis geminata* (Hymenoptera: Formicidae) in the Galapagos Islands. Florida Entomologist 74:368–371.

Williams, D.F., and P.M. Whelan. 1992. Bait attraction of the introduced pest ant, *Wasmannia auropunctata* (Hymenoptera: Formicidae) in the Galápagos Islands. Journal of Entomological Science 27:29–34.

Wolff, M. 2010. Galapagos does not show recent warming but increased seasonality. Notícias de Galápagos 67:38–44.

Nina Wauters was a Ph.D. student at the Université libre de Bruxelles, Brussels, Belgium, who worked on the impact of the invasive ant *Solenopsis geminata* on the endemic fauna of the Galápagos Islands. She obtained her doctorate under the supervision of Drs. Denis Fournier and Wouter Dekoninck in May 2015. (Photographer unknown).

Zoltan T. Nagy was a Post-Doctoral student at the Royal Belgian Institute of Natural Sciences, Brussels, Belgium. Zoltan is a Zoologist and Evolutionary Biologist interested in herpetology in Africa, Madagascar, and Europe. (Photographer unknown).

Wouter Dekoninck is a member of the Scientific Heritage Service and is the Curator of the entomological collection and working as Curator of databases of several insect families. He was involved in several projects and publications using data and specimens from the Royal Belgian Institute of Natural Sciences collection. His main area of expertise is taxonomy, ecology, and molecular studies on Hymenoptera, Formicidae, and ecology of Coleoptera and Carabidae. At the national level he is involved in several projects to obtain knowledge on insect distribution and protection of threatened habitats/species and since 2009 he started a project on evolutionary ecology and biogeography of ants on Galápagos. (Photographed by Wouter Dekoninck).

Denis Fournier is a Research Associate of the Belgian FRS-FNRS at the Evolutionary Biology & Ecology lab (Université libre de Bruxelles, Belgium). He works on various aspects of evolutionary ecology; current investigations including sex and clonality in ants and termites. He is interested in the why and how of this unusual strategy and he has studied the evolution of life-history traits in social insects where he investigated how several factors of the breeding system affect the colony genetic structure, the reproductive conflicts, the dispersal strategies, and the level of inter-nest aggression or the success of biological invasions. (Photographed by Denis Fournier).