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## Investment of both essential fatty and amino acids to immunity varies depending on reproductive stage

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### Abstract

Trade-offs among the key life history traits of reproduction and immunity have been widely documented. However, the currency in use is not well-understood. We investigated how reproducing female side-blotched lizards, *Uta stansburiana*, allocate lipids versus proteins when given an immune challenge. We tested whether lizards would invest more in reproduction or immunity depending on reproductive stage. Females were given stable isotopes (<sup>15</sup>N-leucine and <sup>13</sup>C-1-palmitic acid), maintained on a regular diet and given either a cutaneous biopsy or a sham biopsy (control). Stable isotopes were monitored and analyzed in feces and uric acid, skin biopsies, eggs, and toe clips. We found that lizards deposited both proteins and lipids into their healing wounds (immune challenged), skin (control), and eggs (all) and that catabolism of proteins exceeded incorporation into tissue during wound healing. Specifically, we found that healed biopsies of wounded animals had more leucine and palmitic acid than the non-regrown skin biopsies taken from unwounded control animals. Lizards earlier in reproduction invested relatively more labeled proteins into healing their wound tissue, but not into unwounded skin of control animals. Thus, reproduction is sometimes favored over self-maintenance, but only in later reproductive stages. Finally, we documented positive relationships among the amount of palmitic acid deposited in the eggs, the amount of food eaten, and the amount of palmitic acid excreted, suggesting higher turnover rates of lipids in lizards investing highly in their eggs.

### INTRODUCTION

When an organism is forced to choose between repairing itself and investing in offspring, it is often unclear which will take precedence. Animals allocate limited resources to provide for various physiological processes, such as immune response and reproduction. When resources are severely limited, some processes are neglected and others are favored. The conflicting needs of immune response and reproduction are of particular interest. When female tree lizards have restricted access to food, wounded females have smaller follicles than healthy females (French et al., 2007), suggesting that in some cases immune response, as measured by wound healing, takes precedence over reproduction. However, other studies suggest that this prioritization is

flexible (Nordling et al., 1998; Rödel et al., 2016). Field studies show that when females are at the height of reproductive investment (i.e. vitellogenesis), they heal wounds slower than females in other reproductive phases (French and Moore, 2007). Similarly, laboratory experiments on reproductive males also indicated suppressed wound recovery and immune function (Adamo et al., 2001; French and Moore, 2007; McKean and Nunnery, 2001). These findings suggest that males and females in **more resource-demanding** reproductive states give priority to reproduction, **at least** under certain circumstances, **but these circumstances remain poorly understood with respect to variation across species and reproductive modes as well as within species (Saad and Elridi, 1984; Gustafsson et al., 1994; Christe et al., 2011; Marzal et al., 2007).**

Previous studies have measured relative changes in follicle size for reproductive investment and healing of a small cutaneous wound to estimate immunological investment. While these measures provide the functional response of these two systems respectively, they do not measure resource investment directly. Using stable isotope tracers allows for a non-lethal comparison using common units to follow the “fates” of ingested nutrients and compare resource investment among fundamentally different sink pools within the bodies of living vertebrates (McCue, 2011). Nutrient use (Khalilieh et al., 2012; Cherel et al., 2005) and allocation of resources to reproduction (Hobson, 2006; O'Brien et al., 2000) have been studied separately using stable isotope tracers in organisms as diverse as hawkmoths and birds, but few studies have compared rates of isotope deposition into tissues or organs that compete for common resources (Brace et al., 2015; Durso and French, 2018), and no studies have attempted to use multiple tracers to simultaneously track the fates of different biomolecules.

Here, we introduced a solution of 98%  $^{15}\text{N}$ -leucine to investigate protein allocation and 98%  $^{13}\text{C}$ -1-palmitic acid to investigate lipid allocation, respectively, into female lizards to simultaneously track their fates within the body. We gave small cutaneous or sham (control) biopsies to vitellogenic female side-blotched lizards (*Uta stansburiana*). We simultaneously measured their investment of both an essential amino acid (leucine) and a prevalent fatty acid (palmitic acid), each bearing a unique isotopic label, into their developing follicles and into their healing wounds. We expected that lizards given a wound challenge would invest more into their wounds than the unwounded skin of unwounded animals. Also, based on the short lifespan of side-blotched lizards, we predicted that relative investment would vary depending on stage of reproduction. Specifically, we expected that reproduction would take precedence over self-maintenance in females in later stages of reproduction that had already invested in their clutch, whereas we expected that females in earlier reproductive stages would invest relatively more in self-maintenance (i.e. wound healing).

## METHODS

### *Experimental Design*

We used stable isotope tracers to compare investment in wound healing versus reproduction. After introducing labeled stable-isotope-containing compounds into an

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3 animal, tissues were analyzed to measure the ratio of stable isotopes. High relative  
4 abundance of introduced isotope in newly synthesized tissue indicated recent tissue  
5 growth. By comparing isotope content of tissues involved in wound healing and  
6 integrative immune response (i.e. scab tissue) with that of tissues geared toward  
7 reproductive output (i.e. egg tissue), we quantified resource allocation of **immune-**  
8 **challenged** organisms using a single unit of measure (i.e. micrograms of nitrogen).  
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10  
11 We collected 50 female side-blotched lizards (*Uta stansburiana*) from a single site in St.  
12 George, Utah in May 2016. **They ranged in snout-vent length (SVL) from 41 to 53 mm**  
13 **(mean  $\pm$  SEM =  $47 \pm 0.3$  mm, interquartile range = 45.5 – 48.9 mm) and in mass from**  
14 **1.9 to 5.1 g (mean  $\pm$  SEM =  $3.0 \pm 0.09$  g, interquartile range = 2.7 – 3.4 g).** The mass of  
15 food consumed by the lizards was monitored from day 1 of the study until day 18, and  
16 final tissue samples were collected from the lizards on day 20. On day 3, each lizard  
17 was gavaged with 50 $\mu$ L of 1.5mg/mL Ringer's solution containing 98% <sup>15</sup>N-leucine and  
18 98% <sup>13</sup>C-1-palmitic acid. Three lizards partially regurgitated the isotope mixture, and  
19 were therefore excluded from the study.  
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23 The remaining 47 lizards were randomly assigned to one of two treatment groups,  
24 **which did not differ significantly in SVL or mass ( $p > 0.3$ ).** On the dorsal side of the lizard  
25 between the hind legs, an initial 3.5cm biopsy was collected from the first treatment  
26 group of 23 lizards and the remaining 24 lizards received a sham biopsy on day 4  
27 (Neuman-Lee and French, 2014; French et al., 2006). On days 19 and 20 all animals  
28 received a final biopsy (the biopsy removed the regrown tissue from the previously  
29 wounded group and the control group received an initial biopsy in the same location as  
30 the wounded group). All animals were anesthetized using isoflurane for both initial and  
31 final procedures.  
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34 Digital photographs of all lizards were taken using a Pentax K-x digital camera every 3  
35 to 4 days. Images were blindly analyzed using image analysis software (Image J, NIH  
36 Imaging). The area of the wound was calculated at each time point for all individuals  
37 and the percent of the wound healed between time points was calculated (Neuman-Lee  
38 and French, 2014).  
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41 The lizards were fed a maintenance diet daily over the course of the study, with the  
42 exceptions being the days prior to gavage and surgery. Lizards were given between  
43 0.08g and 0.16g of crickets each day. Fresh crickets were given to the lizards each day.  
44 At the beginning of the day, the crickets from the previous day were removed and  
45 weighed, after which fresh crickets were weighed and introduced into the lizards' cages.  
46 By calculating the difference between the mass of crickets put into the cages and the  
47 mass of crickets removed, the mass of food consumed by each lizard was calculated.  
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50 The lizards' cages were monitored daily for the presence of eggs. **Eighteen** lizards laid  
51 eggs (**9** lizards from the biopsy group, **9** lizards from the control group) over the course  
52 of the experiment. **Eggs from six individuals (2 from the biopsy group and 4 from the**  
53 **control group) were dissected out after their deaths at various points throughout the**  
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experiment and their isotopic ratios were measured, but these are excluded from most statistical analyses below for lack of replication.

On day 3 (prior to the treatment with labeled isotopes), one partial toe clip was collected from each lizard to serve as an indicator of the background levels of carbon and nitrogen isotopes. On day 18 a second toe clip was collected from each lizard, and on day 19 a baseline blood sample was collected within 3 minutes of removing the animals from their cages. Uric acid and feces were collected from each lizard's cage on day 20 (picked up using tweezers from the bottom of each cage). All samples were stored in a drying oven and dried to a constant mass before being processed.

After the study was finished, the toes, biopsies, eggs, uric acid, and feces were processed by folding 0.5 to 2.0mg of each sample into a clean tin capsule (5x9mm; Costech Analytical, Valencia, California, USA). Samples were analyzed for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  using continuous-flow direct-combustion and isotope ratio mass spectrometry (Europa Scientific ANCA-2020; PDZ, Crewe, England).

### *Data Transformation & Statistical Analysis*

We calculated the amount of excess  $^{15}\text{N}$  and  $^{13}\text{C}$  in each tissue by subtracting the background abundance of these isotopes measured by Durso and French (2018) and using additional unpublished data. We calculated the rate of deposition into each tissue following Stark (2000).

We used multivariate regression (function "lm" in R, version 3.3.3) to examine the effect of our wound treatment and the amount of food eaten on the rates of leucine and palmitic acid deposition into eggs, wounds, feces, and uric acid. We removed 4 lizards from our statistical analysis either because they had an abnormally high body mass or ate a very large number of crickets, conditions that seem to have caused abnormalities in isotope concentration in tissues. Figures were made using ggplot2 v. 2.2.1 (Wickham, 2009).

## **RESULTS**

We found a positive effect of the amount of food eaten on the amount of  $^{13}\text{C}$ -1-palmitic acid deposited in the eggs and the amount of  $^{13}\text{C}$ -1-palmitic acid excreted in uric acid (Table 1). Food intake did not affect other nutrient deposition rates (Table 1; Fig. 1). The slopes of the relationships between deposition rate and food intake were not significantly different from zero for either eggs or scabs and either  $^{15}\text{N}$ -leucine or  $^{13}\text{C}$ -1-palmitic acid ( $p > 0.60$ ).

The wounding treatment did not affect the total amount of food eaten by lizards (sham treatment  $1.17 \pm 0.17$  g, wound treatment  $1.30 \pm 0.24$  g;  $p = 0.59$ ,  $R^2 = 0.006$ ). There were no significant relationships between the amount of isotope in scabs or eggs and the rate of wound healing as measured by photography. Wounded lizards excreted less  $^{13}\text{C}$ -1-palmitic acid than control lizards (Table 1). There was also no relationship

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3 between relative investment of  $^{15}\text{N}$ -leucine and  $^{13}\text{C}$ -1-palmitic acid into eggs and wound  
4 healing (Fig. 2). These relationships were not significant ( $p=0.90$  for leucine,  $p = 0.47$  for  
5 palmitic acid), nor were there significant interactions with wounding treatment ( $p = 0.78$   
6 for leucine,  $p = 0.55$  for palmitic acid).  
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9 A limitation is that only approximately half the lizards in our study laid eggs, which  
10 reduced our sample size considerably. However, comparisons between lizards that laid  
11 eggs and those that did not revealed no significant differences in the amount of either  
12 isotope in skin biopsies (Fig. 3A&B; Table S1), in food ingestion (Table S1), or in wound  
13 healing rate (Table S1). Ultrasound data show that individuals that did not lay eggs but  
14 survived to the end of the experiment (11 lizards from the biopsy group, 10 lizards from  
15 the control group) had significantly more egg tissue remaining in the ovary (mean  $\pm$   
16 SEM =  $0.14 \pm 0.02$  mg, interquartile range 0.7 – 0.22 mg) than individuals that had laid  
17 eggs and were starting on a new clutch (essentially 0;  $N = 9$  from each group,  $t = -8.5$ ,  $p$   
18  $< 0.0001$ ,  $R^2 = 0.63$ ; Fig. 3C; Table S1). Eggs were not dissected from any surviving  
19 individuals that did not lay during the 18-day experiment. These individuals had  
20 significantly lower total clutch mass at the beginning of the experiment (mean  $\pm$  SEM =  
21  $0.19 \pm 0.03$  mg, interquartile range 0.09 – 0.24 mg) compared to individuals that laid  
22 eggs (mean  $\pm$  SEM =  $0.60 \pm 0.07$  mg, interquartile range 0.36 – 0.79 mg;  $t = 5.3$ ,  $p$   
23  $< 0.0001$ ,  $R^2 = 0.40$ ; Fig. 3D; Table S1), and were significantly smaller (mean  $\pm$  SEM SVL  
24 =  $46 \pm 0.5$  mm) than individuals that laid eggs (mean  $\pm$  SEM SVL =  $48 \pm 0.4$  mm;  $p$   
25 =  $0.05$ ,  $R^2 = 0.11$ ; Fig. 3E; Table S1). This is likely due to their being earlier in the  
26 reproductive process compared to those that laid. Comparing ultrasound data to date of  
27 laying revealed that there was a strong negative linear relationship between follicle size  
28 & date of laying ( $-0.04$  mg per day;  $p < 0.001$ ,  $R^2 = 0.51$ ; Fig. S1), such that animals  
29 earlier in reproduction took longer to lay, as would be expected.  
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34 Compared to the non-regrown skin biopsies taken at the end of the experiment from  
35 unwounded control animals, the healed biopsies of wounded animals had  $3.4\text{e-}6 \pm 1.1\text{e-}$   
36  $6\text{mg}$  more  $^{15}\text{N}$ -leucine ( $F_{1,35} = 9.41$ ,  $p = 0.004$ ,  $R^2 = 0.19$ , one outlier removed; Fig. 4A)  
37 and  $2.14\text{e-}5 \pm 4.0\text{e-}6\text{mg}$  more  $^{13}\text{C}$ -1-palmitic acid ( $F_{1,35} = 29.16$ ,  $p < 0.001$ ,  $R^2 = 0.45$ ,  
38 one outlier removed; Fig. 4B), after correcting for differing stoichiometry (Fig. 5). In  
39 contrast, estimates of the effect of wounding on the amount of isotope found in eggs  
40 were negative, but not statistically significant (for leucine,  $-9.9\text{e-}7 \pm 2.1\text{e-}6\text{mg}$ ;  $F_{1,22} =$   
41  $0.23$ ,  $p = 0.63$ ,  $R^2 = 0.01$ ; Fig. 4C; for palmitic acid,  $-2.6\text{e-}5 \pm 3.7\text{e-}5\text{mg}$ ;  $F_{1,11} = 0.49$ ,  $p =$   
42  $0.49$ ,  $R^2 = 0.02$ ; Fig. 4D), even when up to 4 outliers were removed (not shown). Three  
43 of the four outliers were the individuals who consumed the most food, and the fourth  
44 was among the top 20% of individuals by amount of food eaten.  
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48 The number of days until the eggs were laid (which corresponds to the stage of  
49 reproduction at the beginning of the experiment) interacted with the wound treatment to  
50 influence the amount of recovered label in the end-of-experiment biopsies not for  $^{15}\text{N}$ -  
51 leucine ( $F_{3,11} = 3.8$ ,  $p = 0.14$ ;  $R^2 = 0.37$ ; Fig. 6A) but for  $^{13}\text{C}$ -1-palmitic acid ( $F_{3,11} = 17.1$ ,  
52  $p < 0.001$ ;  $R^2 = 0.78$ ; Fig. 6B).  
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## 54 DISCUSSION

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4 We found significantly more of both tracers in healed wounds than in regular skin from  
5 control animals that did not receive a biopsy (Fig. 4 A&B). We also found highly altered  
6 stoichiometry of intact skin, scabs, and healed wounds (Fig. 5), and that investment into  
7 healing was dependent on reproductive state (Fig. 6). Isotope incorporation, excretion,  
8 and egestion rates were primarily driven by metabolism and were largely independent of  
9 wounding and wound healing rate, with the notable exception of fatty acid excretion  
10 being reduced in wounded animals. Both leucine ( $^{15}\text{N}$ -labeled) and palmitic acid ( $^{13}\text{C}$ -  
11 labeled) tracers for the most part behaved in a similar way (Fig. 4). **Although we did not**  
12 **document significant decreases in label allocation to eggs in wounded animals, the**  
13 **larger absolute size of clutches of eggs (18-398 mg) may mean that they take longer**  
14 **and/or need a larger effect size in order for significant differences in label incorporation**  
15 **to appear, relative to healed wound tissue (0.01-1.17 mg).** However, we did observe  
16 increased fatty acid deposition in the eggs of animals that ingested more food,  
17 suggesting that these animals had overall higher lipid turnover rates. Finally, in absolute  
18 terms, our rate estimates were lower than those of Durso and French (2018) and of  
19 Brace et al. (2015) for  $^{13}\text{C}$ -leucine to female *Anolis sagrei* gonads.  
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### 24 *Wound Healing Dynamics*

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26 We found that healing wound tissue 15-16 days post-wounding contained more of both  
27 labeled compounds than skin from control animals that did not initially receive a biopsy  
28 (Fig. 4), despite their altered stoichiometry (Fig. 5). The fact that both isotopes are  
29 higher in healing wounds relative to skin demonstrates active deposition into immune  
30 and re-epithelization response of both lipids and proteins. Our finding that some non-  
31 wounded lizards also had high levels of labeled nitrogen in their skin & muscle tissue  
32 suggests that individual variation is high and could be attributed to variation in  
33 unquantified lipid stores at the beginning of the experiment, terminal investment  
34 thresholds (Duffield et al., 2017), or other factors. Of course, low levels of labeled  
35 isotopes in the initial biopsy of experimental animals are expected, because the heavy  
36 isotopes we used as labels are not common in nature.  
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40 Even though the total nitrogen content (both  $^{15}\text{N}$  and  $^{14}\text{N}$  isotopes combined, including  
41 all  $^{15}\text{N}$  label) of healed wound biopsies is much smaller than that of healthy tissue, a  
42 relatively high percentage of this nitrogen came from our label treatment. In general,  
43 protein catabolism is increased after trauma, and skeletal muscle is preferentially  
44 catabolized in humans (Simsek et al., 2014). Wounded lizards in our study built scabs  
45 using ingested amino acids at a rate 2.8x that of allocation to healthy tissue (healthy  
46 skin biopsies), and built new tissue (healed wound biopsies) using ingested amino acids  
47 at a rate 3.8x that of allocation to healthy tissue (healthy skin biopsies). Because the  
48 amount of label given was miniscule compared to the size of the existing pools with  
49 natural-abundance isotope ratios within the body, the absolute values of these rates are  
50 still very small. Finally, distinguishing between proteins involved in tissue reconstruction  
51 and immune proteins recruited to fight infection at the site of the wound is not possible  
52 using our technique, although looking at differential timing in future studies could  
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3 provide evidence, whereby the immunological response precedes the longer  
4 reconstruction phase (Martin, 1997).  
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7 Finally, wounded animals excreted less palmitic acid than unwounded ones (Table 1),  
8 suggesting that they were retaining more lipids than control animals. It may be that  
9 these animals were incorporating more lipids into healing wounds and thus less was  
10 being excreted. This idea is also supported by the greater overall amount of labeled  
11 compounds in the wound tissue relative to the skin of unwounded controls (Fig. 4A&B).  
12 This finding may also partially explain stoichiometric differences among tissues (Fig. 5).  
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### 14 *Stoichiometry*

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17 When examining ratios of total nitrogen to total carbon (both isotopes combined), we  
18 found that healed wounds and scabs contained about half as much nitrogen and about  
19 10% more carbon by mass as unwounded tissue, corresponding to an average total  
20 C:N ratio of ~3:1 in healthy tissue, ~4:1 in scabs, and ~5:1 in healed wounds (Fig. 5).  
21 Scabs and especially healed wounds were more variable than healthy tissue, with C:N  
22 ratios of healed wounds ranging from 3.8:1 to 6.9:1. We interpret this as evidence for a  
23 lower overall concentration of protein in scabs and healed wounds compared to healthy  
24 tissue, despite the above evidence that recently-ingested protein is preferentially  
25 recruited to the wound site (Fig. 4A), which could also be explained by relatively greater  
26 relative lipid investment at wound sites, driving the C:N ratio up (Table 1; Fig. 5).  
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30 Following injury, animals generally metabolize a greater percentage of available amino  
31 acids for energy (Demling and DeSanti, 2000; Simsek et al., 2014). While protein  
32 production also increases following trauma, the increase in amino acid catabolism  
33 outpaces the increase in anabolism, resulting in a net loss of nitrogen from the body  
34 (O'Donnel et al., 1976). Both of these processes may result in a relatively lower amount  
35 of ingested nitrogen being incorporated into new tissues than carbon, with nitrogen  
36 instead being metabolized and otherwise lost (e.g. excreted) from the body. Young and  
37 Huang (1969) explained their finding that rats with fractured femurs incorporated less  
38 labeled carbon into tissue surrounding the injury than non-wounded rats by suggesting  
39 that local severe electrolyte change related to the traumatic injury may have inhibited  
40 the uptake of leucine into the muscle tissue near the injury site. Ryan et al. (1974)  
41 speculated that infection may lead to a decrease in fat mobilization in rats, leading to  
42 the necessity of amino acid combustion to compensate for reduced metabolic fuel. A  
43 slightly more recent study (Askanazi et al., 1980) suggests that proteins are broken  
44 down and transported to the liver for hepatic protein synthesis. Yu et al. (1988) found  
45 that supplementation of branched chain amino acids in burn patients did not result in an  
46 increase of leucine uptake in protein synthesis, but rather resulted in increased leucine  
47 oxidation for energy. Thus there are at least three hypotheses for this phenomenon: that  
48 leucine is directly incorporated into the wound site during local protein synthesis; that  
49 leucine is locally catabolized for energy; or that amino acids are broken down and  
50 transported to the liver for hepatic synthesis. Our results show an overall reduction in  
51 protein in wounded lizards, which supports the last of these three hypotheses.  
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### Reproductive stage

We found support for our hypothesis that the stage of reproduction affected investment into healing. Specifically, animals earlier in reproduction (laid eggs on a later date) invested relatively more labeled proteins and lipids into healing their wound tissue (Fig. 6). That this same relationship was not apparent in the unwounded skin of control animals suggested that without the wounding challenge, investment into skin maintenance did not change over time with reproduction. This finding was also supported by the findings of Durso and French (2018), who found that earlier reproductive stages increased the absolute and relative allocation of protein to self-maintenance. This is consistent with a study in Texas field crickets (*Gryllus texensis*), whereby researchers found that immune-challenged crickets had decreased protein deposition into eggs (Stahlschmidt et al., 2013). Similarly, female Wellington tree wetas (*Hemideina crassidens*) that were immune-challenged laid fewer eggs than non-immune challenged females, and their eggs had lower protein content (Kelly, 2011). The general pattern was similar for protein and lipid investment, although not statistically significant for proteins (Fig. 6B), potentially suggesting that lipids may be a more limiting resource than proteins, although our ability to draw such conclusions is hindered by our small sample size. **Because our study lasted only 18 days, compared to an average gestation period of 48 days (Tinkle, 1967), individual variation in timing of reproduction was probably the most important source of variation in our experiment.**

Studies have shown that various animals will prioritize self-maintenance over reproductive investment when faced with a stressor, particularly in species with relatively short lifespans. For example, burying beetles (*Nicrophorus vespilloides*) that were wounded during breeding had reduced reproductive output (Reavey et al., 2014), and immune challenged Mallee dragons (*Ctenophorus fordi*) showed decreased reproductive investment (i.e. egg mass) (Uller et al., 2006). Similarly, in female dung beetles (*Euoniticellus intermedius*), activation of the immune system causes resources to be diverted away from reproduction (Reaney and Knell, 2010). Immune activation also results in lower circulating levels of testosterone in male Cape ground squirrels (*Xerus inauris*) (O'Brien et al., 2018), and in lower sperm swimming velocity in Great Tits (*Parus major*), impairing reproductive ability (Losdat et al., 2011). **In iteroparous species, including *U. stansburiana*, reproductive costs of immune challenges may be observed in later clutches or litters rather than in the immediate reproductive event (e.g. Marzal et al., 2007), something we do not have the data to assess in the current study.**

We also found positive relationships among the amount of palmitic acid deposited in the eggs, the amount of food eaten, and the amount of palmitic acid excreted (Table 1). This suggests higher turnover rates of lipids in animals investing highly in their eggs. Elevated turnover rates in females investing heavily in their eggs make sense, as pregnancy is known to have high metabolic costs in all animals (Lourdais et al., 2002; Van Dyke and Beaupre, 2011; Sparling et al., 2006). Although metabolic rate was not **measured directly** in this study, reproduction, food consumption, and digestion are **known to be directly related to metabolic rate, and metabolic rates decrease following immune challenges in this species** (Smith et al., 2017; Plasman et al., 2019). **Egg**

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3 formation increases resting metabolic rate by 22% in female zebra finches (*Taeniopygia*  
4 *guttata*; Vézina and Williams, 2005) and by 27% in female great tits (*Parus major*;  
5 Nilsson and Råberg, 2001). Studies in reptiles are few, but both male and female  
6 Argentine Black-and-White Tegus (*Tupinambis merianae*) are known to behaviorally  
7 maintain metabolic rates two to three times higher during the reproductive season via  
8 seasonal endothermy (Tattersall et al., 2016). Durso & French (2018) did not find that  
9 food restriction had a significant effect on allocation of leucine to either reproduction or  
10 wound-healing. Our data suggest that metabolic activity was likely the primary driving  
11 force determining the destination of our isotope label.  
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### 14 *Conclusions*

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17 In conclusion, our study showed that animals invest both lipids and proteins into healing  
18 tissues, and that this investment varies depending on the stage of reproduction, at least  
19 for lipid. By using a stable isotope label, we were able to put these important life history  
20 processes onto a common scale and provide quantitative estimates of the effect of a  
21 highly relevant wound challenge on relative investment into eggs and into wound  
22 healing.  
23

### 24 **ACKNOWLEDGMENTS**

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## References

- Adamo SA, Jensen M, Younger M. 2001. Changes in lifetime immunocompetence in male and female *Gryllus texensis* (formerly *G. integer*): trade-offs between immunity and reproduction. *Animal Behaviour* 62:417-425.
- Askanazi J, Carpentier YA, Michelsen CB, Elwyn DH, Furst P, Kantrowitz LR, Gump FE, Kinney JM. 1980. Muscle and plasma amino acids following injury: influence of intercurrent infection. *Annals of Surgery* 192:78-85.
- Brace AJ, Sheikali S, Martin LB. 2015. Highway to the danger zone: exposure-dependent costs of immunity in a vertebrate ectotherm. *Functional Ecology* 29:924-930.
- Cherel Y, Hobson KA, Hassani S. 2005. Isotopic discrimination between food and blood and feathers of captive penguins: implications for dietary studies in the wild. *Physiological and Biochemical Zoology* 78:106-115.
- Christe P, Glaizot O, Strepparava N, Devevey G, Fumagalli L. 2011. Twofold cost of reproduction: an increase in parental effort leads to higher malarial parasitaemia and to a decrease in resistance to oxidative stress. *Proceedings of the Royal Society B: Biological Sciences* 279:1142-1149.
- Demling R, DeSanti L. 2000. The stress response to injury and infection: role of nutritional support. *Wounds* 12:3-14.
- Duffield KR, Bowers EK, Sakaluk SK, Sadd BM. 2017. A dynamic threshold model for terminal investment. *Behavioral Ecology and Sociobiology* 71:185.
- Durso AM, French SS. 2018. Stable isotope tracers reveal a trade-off between reproduction and immunity in a reptile with competing needs. *Functional Ecology* 32:648-656.
- French SS, Johnston GIH, Moore MC. 2007. Immune activity suppresses reproduction in food-limited female tree lizards (*Urosaurus ornatus*). *Functional Ecology* 21:1115-1122.
- French SS, Matt K, Moore MC. 2006. The effects of stress on wound healing in male tree lizards (*Urosaurus ornatus*). *General and Comparative Endocrinology* 145:128-132.
- French SS, Moore MC. 2007. Immune function varies with reproductive stage and context in female and male tree lizards, *Urosaurus ornatus*. *General and Comparative Endocrinology* 155:148-156.
- Gustafsson L, Nordling D, Andersson MS, Sheldon BC, Qvarnstrom A. 1994. Infectious diseases, reproductive effort and the cost of reproduction in birds. *Philosophical Transactions of the Royal Society B: Biological Sciences* 346:323-331.
- Hobson KA. 2006. Using stable isotopes to quantitatively track endogenous and exogenous nutrient allocations to eggs of birds that travel to breed. *Ardea* 94:359-369.
- Kelly C. 2011. Reproductive and physiological costs of repeated immune challenges in female Wellington tree weta (Orthoptera: Anostostomatidae). *Biological Journal of the Linnean Society* 104:38-46.
- Khalilieh A, McCue MD, Pinshow B. 2012. Physiological responses to food deprivation in the house sparrow, a species not adapted to prolonged fasting. *American Journal of Physiology* 303:R551-R561.
- Losdat S, Richner H, Blount J, Helfenstein F. 2011. Immune activation reduces sperm quality in the Great Tit. *PLoS ONE* 6:e22221.
- Lourdais O, Bonnet X, Doughty P. 2002. Costs of anorexia during pregnancy in a viviparous snake (*Vipera aspis*). *Journal of Experimental Zoology* 292:487-493.
- Martin P. 1997. Wound healing - Aiming for perfect skin regeneration. *Science* 276:75-81.
- Marzal A, Reviriego M, de Lope F, Møller AP. 2007. Fitness costs of an immune response in the house martin (*Delichon urbica*). *Behavioral Ecology and Sociobiology* 61:1573-1580.
- McCue MD. 2011. Tracking the oxidative and nonoxidative fates of isotopically labeled nutrients in animals. *BioScience* 61:217-230.

- 1  
2  
3 McKean KA, Nunney L. 2001. Increased sexual activity reduces male immune function in  
4 *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the*  
5 *United States of America* 98:7904-7909.
- 6 Neuman-Lee LA, French SS. 2014. Wound healing reduces stress-induced immune changes:  
7 evidence for immune prioritization in the side-blotched lizard. *Journal of Comparative*  
8 *Physiology B Biochemical Systemic and Environmental Physiology* 184:623-629.
- 9 Nilsson J-Å, Råberg L. 2001. The resting metabolic cost of egg laying and nestling feeding in  
10 great tits. *Oecologia* 128:187-192.
- 11 Nordling D, Andersson M, Zohari S, Lars G. 1998. Reproductive effort reduces specific immune  
12 response and parasite resistance. *Proceedings of the Royal Society B: Biological*  
13 *Sciences* 265:1291-1298.
- 14 O'Brien DM, Schrag DP, Martinez del Rio C. 2000. Allocation to reproduction in a hawkmoth: a  
15 quantitative analysis using stable carbon isotopes. *Ecology* 81:2822-2831.
- 16 O'Brien K, Waterman J, Anderson W, Bennett N. 2018. Trade-offs between immunity and  
17 testosterone in male African ground squirrels. *Journal of Experimental Biology*  
18 221:jeb177683.
- 19 O'Donnel T, Clowes JG, Blackburn G, Ryan N, Benotti P, Miller J. 1976. Proteolysis associated  
20 with a deficit of peripheral energy fuel substrates in septic man. *Surgery* 80:192-200.
- 21 Plasman M, McCue MD, Reynoso VH, Terblanche JS, Clusella-Trullas S. 2019. Environmental  
22 temperature alters the overall digestive energetics and differentially affects dietary  
23 protein and lipid use in a lizard. *Journal of Experimental Biology* 222:jeb194480.
- 24 Reaney L, Knell R. 2010. Immune activation but not male quality affects female current  
25 reproductive investment in a dung beetle. *Behavioral Ecology* 21:1367-1372.
- 26 Reavey CE, Warnock ND, Vogel H, Cotter SC. 2014. Trade-offs between personal immunity  
27 and reproduction in the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology*  
28 25:415-423.
- 29 Rödel HG, Zapka M, Stefanski V, Holst DV. 2016. Reproductive effort alters immune  
30 parameters measured post-partum in European rabbits under semi-natural conditions.  
31 *Functional Ecology* 30:1800-1809.
- 32 Ryan NT, Blackburn GL, Clowes Jr GH. 1974. Differential tissue sensitivity to elevated  
33 endogenous insulin levels during experimental peritonitis in rats. *Metabolism* 23:1081-  
34 1089.
- 35 Saad AH, El Ridi R. 1984. Corticosteroids and seasonal changes in reptilian immune  
36 responses. *Developmental and Comparative Immunology* 8:945.
- 37 Simsek T, Simsek HU, Canturk NZ. 2014. Response to trauma and metabolic changes:  
38 posttraumatic metabolism. *Turkish Journal of Surgery* 30:153-159.
- 39 Smith GD, Neuman-Lee LA, Webb AC, Angilletta MJ, DeNardo DF, French SS. 2017. Metabolic  
40 responses to different immune challenges and varying resource availability in the side-  
41 blotched lizard (*Uta stansburiana*). *Journal of Comparative Physiology B Biochemical*  
42 *Systemic and Environmental Physiology* 187:1173-1182.
- 43 Sparling C, Speakman J, Fedak M. 2006. Seasonal variation in the metabolic rate and body  
44 composition of female grey seals: Fat conservation prior to high-cost reproduction in a  
45 capital breeder? *Journal of Comparative Physiology B Biochemical Systemic and*  
46 *Environmental Physiology* 176:505-512.
- 47 Stahlschmidt ZR, Rollinson N, Acker M, Adamo SA. 2013. Are all eggs created equal? Food  
48 availability and the fitness trade-off between reproduction and immunity. *Functional*  
49 *Ecology* 27:800-806.
- 50 Stark JM. 2000. Nutrient transformations. In: Sala OE, Jackson RB, Mooney HA, Howarth RW,  
51 editors. *Methods in Ecosystem Science*. New York, NY, USA: Springer. p 214-234.
- 52 Tattersall GJ, Leite CAC, Sanders CE, Cadena V, Andrade DV, Abe AS, Milsom WK. 2016.  
53 Seasonal reproductive endothermy in tegu lizards. *Science Advances* 2:e1500951.
- 54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Tinkle DW. 1967. The life and demography of the side-blotched lizard, *Uta stansburiana*.  
4 *Miscellaneous Publications, University of Michigan Museum of Zoology* 132:1-182.  
5 Uller T, Isaksson C, Olsson M. 2006. Immune challenge reduces reproductive output and  
6 growth in a lizard. *Functional Ecology* 20:873-879.  
7 Van Dyke JU, Beaupre SJ. 2011. Bioenergetic components of reproductive effort in viviparous  
8 snakes: costs of vitellogenesis exceed costs of pregnancy. *Comparative Biochemistry*  
9 *and Physiology Part A Molecular & Integrative Physiology* 160:504-515.  
10 Vézina F, Williams TD. 2005. The metabolic cost of egg production is repeatable. *Journal of*  
11 *Experimental Biology* 208:2533-2538.  
12 Wickham H. 2009. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag.  
13 Young VR, Huang PC. 1969. In vivo uptake of [<sup>14</sup>C]leucine by skeletal muscle ribosomes after  
14 injury in rats fed two levels of protein. *British Journal of Nutrition* 23:271-280.  
15 Yu Y-M, Wagner DA, Walesreswski J, Burke JF, Young VR. 1988. A kinetic study of leucine  
16 metabolism in severely burned patients. Comparison between a conventional and  
17 branched-chain amino acid-enriched nutritional therapy. *Annals of Surgery* 207:421-429.  
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Table 1: Summary of multivariate linear regression models examining the main effects of wounding and food eaten on  $^{15}\text{N}$ -leucine and  $^{13}\text{C}$ -1-palmitic acid deposition rates (mg/day) into four sink pools (egg, healed wound/biopsy, uric acid [excretion], and feces [egestion]).

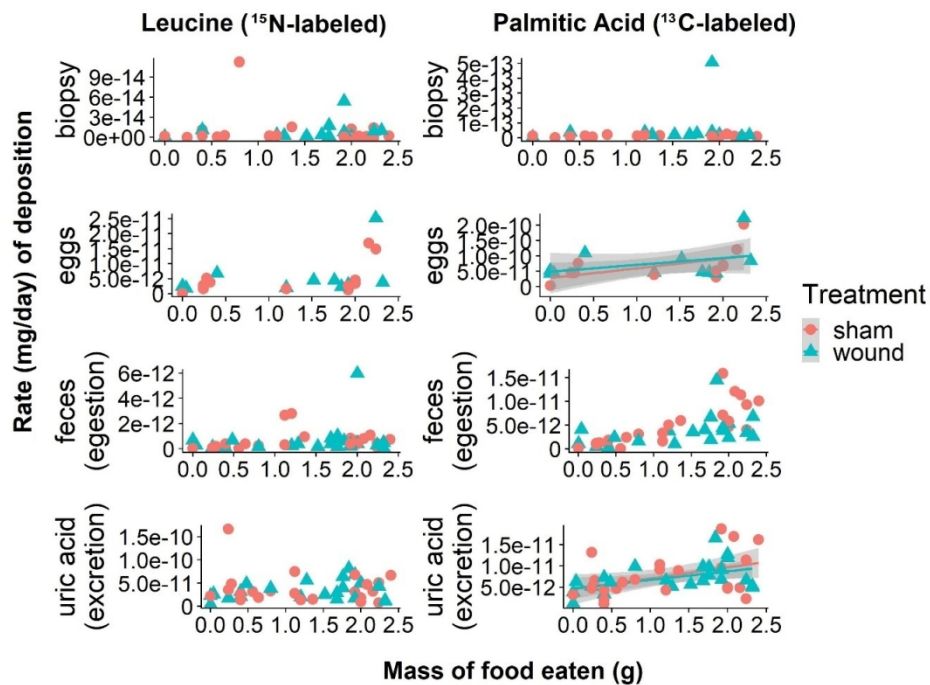
		Response Variable								
Measurement		$^{15}\text{N}$ -Leucine Deposition Rate (mg/day)				$^{13}\text{C}$ -1-Palmitic Acid Deposition Rate (mg/day)				
Sink Pool		Egg	Biopsy	Uric acid (excretion)	Feces (egestion)	Egg	Biopsy	Uric acid (excretion)	Feces (egestion)	
Explanatory Variable	Intercept	Estimate	-2.43e-12	-1.94e-15	5.31e-12	7.75e-11	-4.21e-12	4.14e-15	1.14e-12	7.44e-12
		$\pm$ SE	12 $\pm$ 4.52e-12	$\pm$ 9.13e-15	$\pm$ 4.82e-13	$\pm$ 2.55e-11	$\pm$ 3.73e-11	$\pm$ 8.65e-14	$\pm$ 2.12e-12	$\pm$ 2.91e-12
		(t-value) P	(0.11) 0.91	(1.18) 0.26	(-1.17) 0.27	(-0.65) 0.53	(0.15) 0.88	(1.31) 0.21	(-2.26) <b>0.04*</b>	(-0.86) 0.40
	Wound treatment	Estimate	4.13e-13	-8.59e-15	-4.49e-13	-1.32e-11	4.37e-12	9.05e-14	-3.82e-12	-2.02e-12
		$\pm$ SE	$\pm$ 3.61e-12	$\pm$ 7.30e-15	$\pm$ 3.85e-13	$\pm$ 2.04e-11	$\pm$ 2.98e-11	$\pm$ 6.91e-14	$\pm$ 1.69e-12	$\pm$ 2.33e-12
		(t-value) P	(1.95) 0.08	(0.70) 0.50	(0.49) 0.63	(-1.44) 0.18	(2.28) <b>0.04*</b>	(0.10) 0.92	(3.01) <b>0.01*</b>	(0.34) 0.74
	Amount of food eaten (g)	Estimate	5.04e-12	3.64e-15	1.36e-13	-2.09e-11	4.88e-11	5.03e-15	3.65e-12	5.58e-13
		$\pm$ SE	$\pm$ 2.58e-12	$\pm$ 5.23e-15	$\pm$ 2.76e-13	$\pm$ 1.46e-11	$\pm$ 2.14e-11	$\pm$ 4.95e-14	$\pm$ 1.21e-12	$\pm$ 1.67e-12
		(t-value) P	(1.95) 0.08	(0.70) 0.50	(0.49) 0.63	(-1.44) 0.18	(2.28) <b>0.04*</b>	(0.10) 0.92	(3.01) <b>0.01*</b>	(0.34) 0.74
	Univariate Model Summary	Multiple R <sup>2</sup>	0.26	0.16	0.11	0.20	0.32	0.14	0.50	0.06
		Adjusted R <sup>2</sup>	0.13	0.02	-0.04	0.07	0.21	-0.01	0.41	-0.10
		(F <sub>DF</sub> ) P	(2.05 <sub>2,12</sub> ) 0.17	(1.17 <sub>2,12</sub> ) 0.34	(0.70 <sub>2,12</sub> ) 0.51	(1.52 <sub>2,12</sub> ) 0.26	(2.81 <sub>2,12</sub> ) 0.10	(0.93 <sub>2,12</sub> ) 0.42	(5.88 <sub>2,12</sub> ) 0.02	(0.38 <sub>2,12</sub> ) 0.69

Table S1

There were no differences between females that laid eggs & those that did not in allocation (mg/day) of either isotope to wound healing, feces, or uric acid, and no interactions with wound treatment. There were no differences between females that laid eggs & those that did not in food consumption, but females that laid eggs were larger and heavier than those that did not at the beginning of the experiment.

Element	Response variable	Laid (t / p)	treatment	laid*treatment	Adjusted R <sup>2</sup>
C	Fecal (mg/day)	1.17 / 0.25	-0.53 / 0.60	-0.14 / 0.89	0.01
C	UA (mg/day)	-0.29 / 0.78	0.18 / 0.86	-0.53 / 0.60	-0.04
C	Biopsy (mg/day)	-0.14 / 0.52	0.29 / 0.77	1.13 / 0.27	0.04
N	Fecal (mg/day)	-0.03 / 0.98	0.55 / 0.59	-0.76 / 0.45	-0.04
N	UA (mg/day)	0.66 / 0.51	0.50 / 0.62	-1.17 / 0.25	-0.03
N	Biopsy (mg/day)	-1.17 / 0.25	-0.63 / 0.54	1.01 / 0.32	-0.05
	Wound healing (%; day 5)	1.11 / 0.28	NA	NA	0.01
	Clutch mass (mg; day 1)	3.6 / 0.0008 ***	-0.04 / 0.97	0.74 / 0.47	0.39
	Clutch mass (mg; day 18)	-2.6 / 0.013	0.95 / 0.35	-0.57 / 0.57	0.24
	Food ingestion	0.78 / 0.44	1.03 / 0.31	-0.91 / 0.37	-0.04
	SVL (mm)	2.35 / 0.02 *	1.68 / 0.10	-1.13 / 0.27	0.08
	Mass (g)	2.7 / 0.009 **	0.66 / 0.51	0.24 / 0.81	0.23

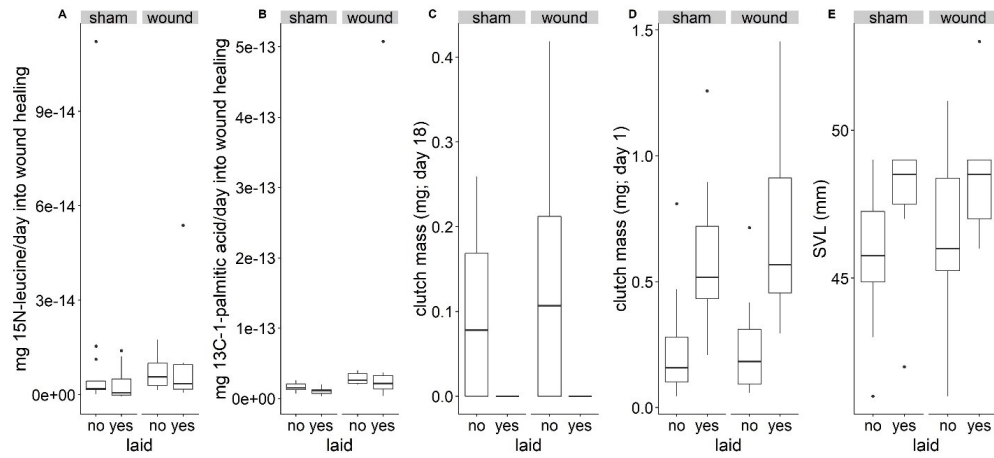




Mass of food ingested by each lizard compared to the rate of isotope incorporation into tissues. The mass of food eaten is plotted against the rates of leucine ( $^{15}\text{N}$ -labeled) and palmitic acid ( $^{13}\text{C}$ -labeled) deposition in skin biopsies (wound healing), eggs, feces (egested), and uric acid (excreted). Wound "healing" measurements from sham animals ( $N=24$ ) come from biopsies of dorsal tissue taken at the end of the experiment (and are directly compared with biopsies of healed wound tissue taken from animals wounded at the beginning of the experiment;  $N = 23$ ). The gray shading represents the 95% confidence level interval for predictions from a linear model ("lm") for significant relationships only.

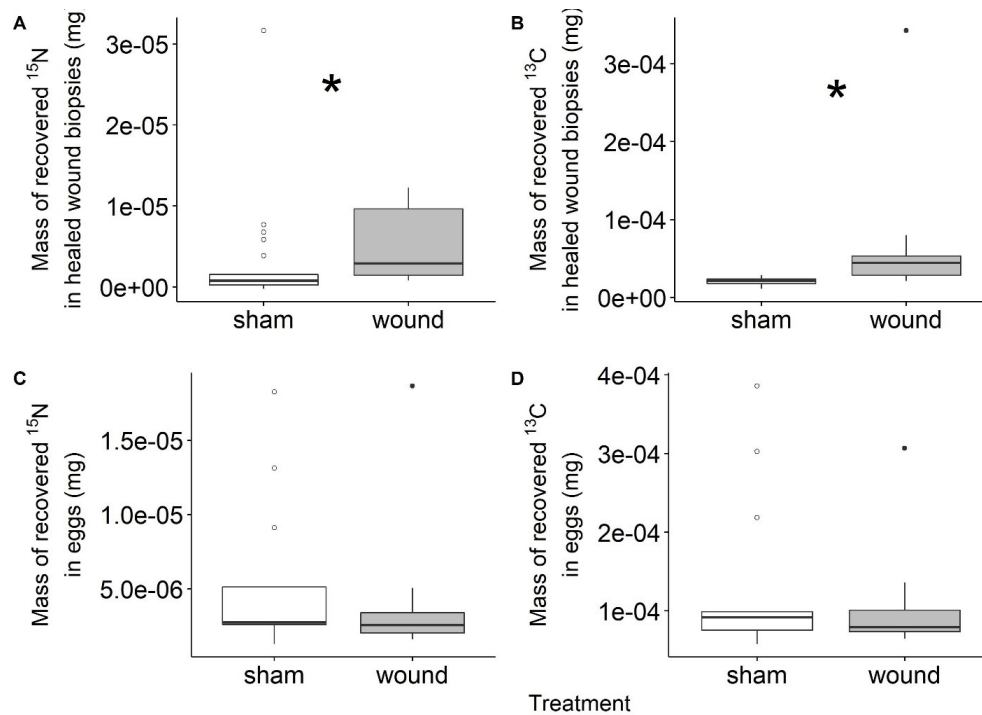
165x121mm (220 x 220 DPI)





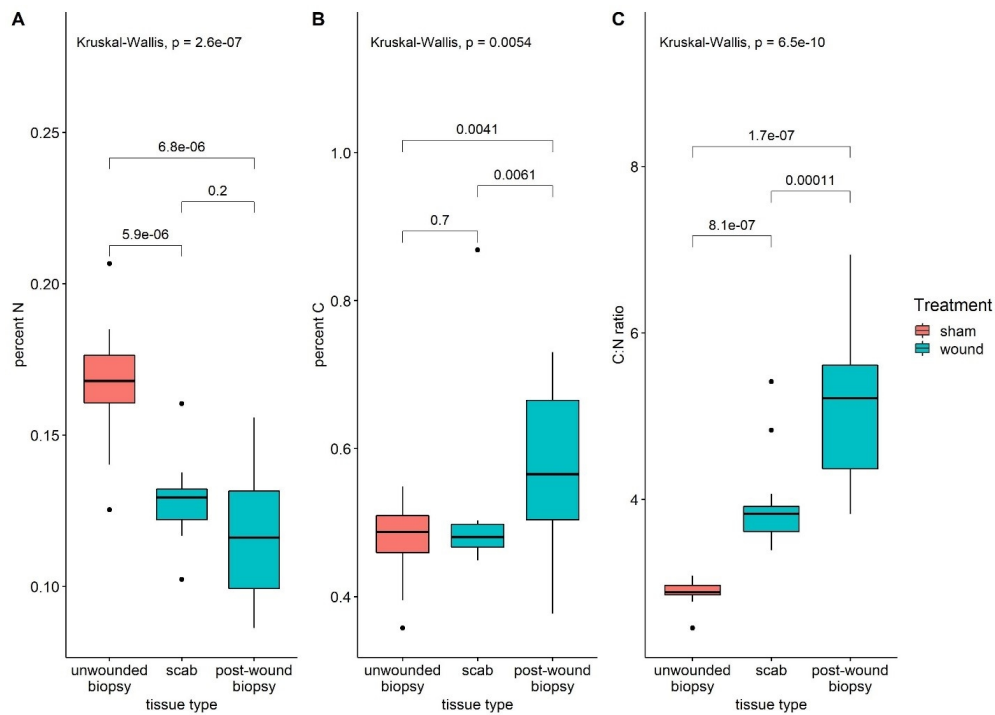
Differences in deposition of A)  $^{15}\text{N}$ -leucine and B)  $^{13}\text{C}$ -1-palmitic acid into wound healing, clutch mass C) before and D) after the experiment, and E) SVL at the beginning of the experiment between individual lizards who laid eggs (N = 9 lizards from the biopsy group, 9 lizards from the control group) and those who did not (N = 11 lizards from the biopsy group, 10 lizards from the control group).

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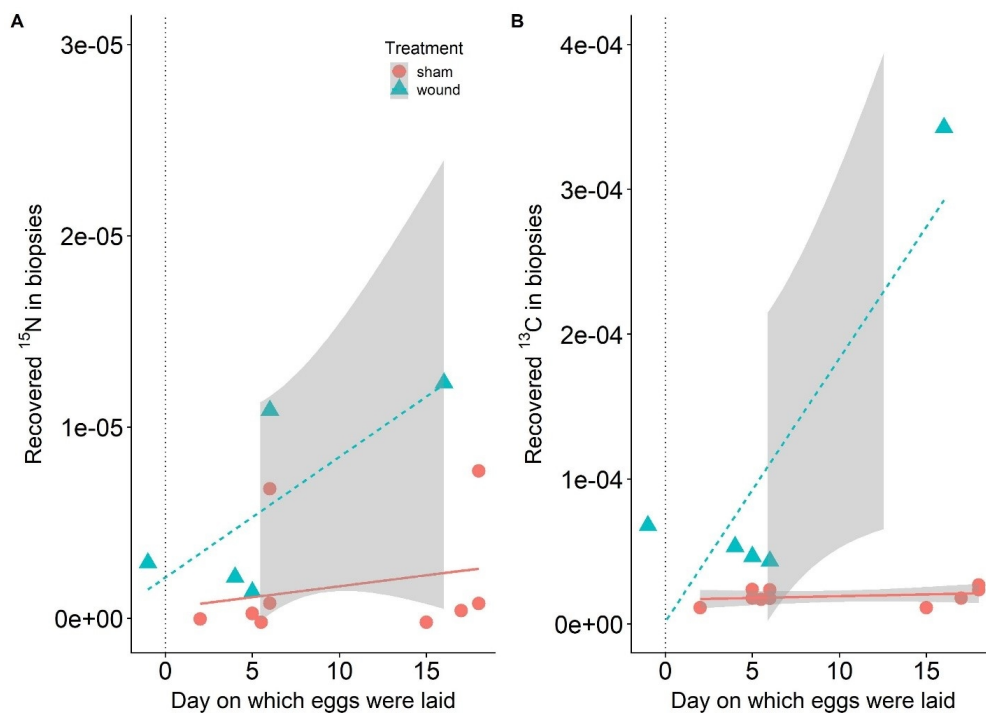
Mass of A)  $^{15}\text{N}$  (leucine) and B)  $^{13}\text{C}$  (palmitic acid) from label in wound biopsies and eggs, by wounding treatment. Wound "healing" measurements from sham animals (N = 21) come from biopsies of dorsal tissue taken at the end of the experiment, in the same location where wound treatment animals (N = 17) were initially wounded. Egg material from 11 lizards from the biopsy group (9 laying eggs and 2 dissected) and 13 lizards from the control group (9 laying eggs and 4 dissected) has here been combined. Note that the y-axes showing nitrogen in A) and C) are one order of magnitude lower than those showing carbon in B) and D), because carbon is more abundant than nitrogen in animal tissues. Significant differences between treatments are shown with a \* in A) and B) after removal of one outlier each. There were no significant differences between treatments in C) or D) even when up to 4 outliers were removed. All outliers are shown (three of the four outliers were the individuals who consumed the most food, and the fourth was among the top 20% of individuals by amount of food eaten).

165x117mm (220 x 220 DPI)



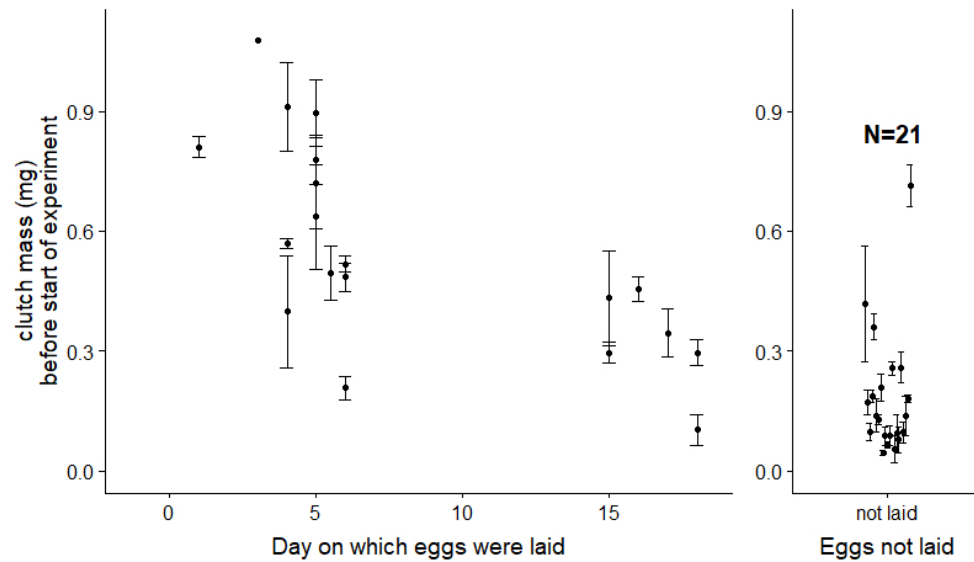
A) Percent N, B) percent C and C) C:N ratio of initial biopsy, scab, and post-wound biopsy. Normal skin (N = 21) contained almost twice as much nitrogen as scabs (N = 14) or healed wounds (N = 17), and healed wounds contained more carbon than normal skin or scabs. These differences are corrected for in the above rates.

165x117mm (220 x 220 DPI)



Relationship between mass of A) <sup>15</sup>N (leucine) and B) <sup>13</sup>C (palmitic acid) from label in wound biopsies and the day on which the eggs were laid, which corresponds to the stage of reproduction at the beginning of the experiment. Wound treatment (N = 5) represented by triangles with dashed lines; control treatment (N = 9) by circles with solid lines. Note that the y-axes showing nitrogen in A) is one order of magnitude lower than that showing carbon in B), because carbon is more abundant than nitrogen in animal tissues.

165x117mm (220 x 220 DPI)



Relationship between A) clutch mass (mg) at the beginning of the experiment and date of laying (N = 19), and B) clutch mass (mg) at the beginning of the experiment for individuals that did not lay eggs (N = 21; x-axis variation ["jitter"] is introduced to better visualize the points). There was a strong negative linear relationship between follicle size & date of laying ( $-0.04$  mg per day;  $p < 0.001$ ,  $R^2 = 0.51$ ). The gray shading represents the 95% confidence level interval for predictions from a linear model ("lm").