**Abstract**

Female investment in large eggs increases the demand for fatty acids, which are allocated for yolk production. Since the biosynthetic pathway leading to fatty acids uses the same precursors used in the formation of polyketides, allocation trade-offs are expected to emerge. Therefore, egg production should constrain the investment in chemical defenses based on polyketides, such as benzoquinones. We tested this hypothesis using the harvestman *Acutisoma longipes*, which produces large eggs and releases benzoquinones as chemical defense. We predicted that the amount of secretion released by ovigerous females (OFs) would be smaller than that of non-ovigerous females (NOF). We also conducted a series of bioassays in the field and in the laboratory to test whether egg production renders OFs more vulnerable to predation. OFs produce less secretion than NOFs, which is congruent with the hypothesis that egg production constrains the investment in chemical defenses. Results of the bioassays show that the secretion released by OFs is less effective in deterring potential predators (ants and spiders) than the secretion released by NOFs. In conclusion, females allocate resources to chemical defenses in a way that preserves a primary biological function related to reproduction. However, the trade-off between egg and secretion production makes OFs vulnerable to predators. We suggest that egg production is a critical moment in the life of harvestman females, representing perhaps the highest cost of reproduction in the group.

**Introduction**

The production of large and heavily yolked eggs is perhaps the most widespread form of parental care among animals [1]. Egg size is related to survival and growth of early hatched young in multiple taxa, including arthropods [2], fishes [3], amphibians [4], and birds [5]. Despite the benefits to the offspring, the production of large eggs may also impose costs to females because reproduction and self-maintenance are two processes that require great investment of energy and resources (review in [6]). Indeed, a negative correlation between survival and female investment in current reproduction is one of the most ubiquitous life-history trade-offs.
reported in the literature [7,8]. This pattern may emerge as a consequence of several different processes, but the allocation trade-off between reproduction and immune function has received the most attention in recent years [9,10]. Experimental evidence of insects, lizards, and birds has consistently shown that increases in the reproductive effort lead to decreases in the immune function and vice-versa (e.g., [11–15]).

Although intensively studied, the immune system is one of the last lines of defense against natural enemies exhibited by animals [16]. Comparatively, few studies have investigated possible trade-offs between reproduction and other types of defense; most of them are focused on plants in which an increase in induced chemical defenses against herbivores promotes a decrease in fruit or seed production ([17, 18]; but see [19]). There is also indirect evidence suggesting a trade-off between reproduction and chemical defenses among some marine animals. In the marine bryozoan *Membranipora membranacea*, for instance, colonies rapidly produce defensive spines in response to cues from a specialized predatory gastropod. In this case, colonies producing spines grow at lower rates than control colonies, and this growth decrease is directly translated into a reduced output of sexual propagules because fecundity is positively related to colony size in bryozoans [20]. In the sponge *Oscarella balibaloi*, the production of secondary metabolites decreases during the period of embryogenesis, suggesting a trade-off between the resources dedicated to reproduction and the production of chemical defenses [21].

Given that chemical defenses are extremely common among many taxonomic groups, and that there is strong evidence showing that these chemical defenses are costly (review in [22]), it is surprising that the trade-off between reproductive investment and the production of chemical weaponry has never been directly addressed in animals. Arthropods are perhaps one of the most tractable animal groups to explore this trade-off for several practical reasons: (a) many species are chemically defended [23], (b) the composition and biosynthetic pathways of many chemical compounds are well-known [24], (c) female investment in egg size presents huge variation among taxa [25], and (d) chemical defenses may be costly, and thus may compete for resources and energy with other life-history traits. Experimental evidence indicates that synthesizing chemical defenses can slow larval growth in holometabolous insects and also promote a reduction in the final size of the adults, which in turn may reduce their reproductive success (see [26] and references therein).

Quinones are polyketides found in the defensive glands of a wide range of arthropod species, including earwigs, cockroaches, termites, grasshoppers, beetles, millipedes, and harvestmen [23]. Despite the great diversity of quinonoid compounds produced by these arthropods, there are only two metabolic pathways for the generation of benzoquinones [27]. In millipedes (Diplopoda) and insects, benzoquinones may be biosynthesized from preformed aromatic rings of amino acids, such as tyrosine, or using acetate or propionate as precursors, suggesting a polyketide origin [27–29, 24]. In harvestmen (Opiliones), however, alkylated benzoquinones seem to be biosynthesized exclusively using acetate and propionate as precursors [27, 30] (Fig 1). According to the Y model of resource allocation, limited resources allocated to reproduction are not available for the soma [6]. Under the perspective of an arthropod female, the investment in large and heavily yolked eggs increases the demand for fatty acids, which are allocated for the production of the vitelline membrane and lipid droplets imbedded in the yolk [31] (Fig 1). Given that the biosynthetic pathways leading to long chain fatty acids are analogous to the formation of polyketides, and that the same precursors are used by both fatty acid synthases and polyketide synthases, allocation trade-offs are expected to emerge [29] (Fig 1).

In this study, we tested the hypothesis that egg production constrains the investment in chemical defenses based on polyketide compounds, such as alkylated benzoquinones, using the harvestman *Acutiosoma longipes* (Gonyleptidae) as study organism (more details in 'Study species' below). Our prediction was that the amount of secretion stored in the glandular sac of
ovigerous females would be smaller than the amount stored by non-ovigerous females. We also conducted a series of bioassays in the field and in the laboratory to test whether egg production renders ovigerous females more vulnerable to predation. Our predictions were: (i) the amount of secretion released by ovigerous females would be less effective in deterring potential predators than the amount released by non-ovigerous females, and (ii) the chemical shield provided by the defensive secretion [32] would last longer in the non-ovigerous than in the ovigerous females.

Methods
Study Species

Individuals of *A. longipes* produce a large amount of defensive secretion composed of two alkylated 1,4-benzoquinones that are released through a pair of exocrine glands located at the anterior margins of the carapace [32] (Fig 1). Although these benzoquinones are highly effective in repelling several invertebrate and vertebrate predators, they are employed only when all other evasive measures were unsuccessful in preventing the predator attack [32, 33], which
suggests that their production is costly. Females also produce 80–200 large yolked eggs that occupy more than 50% of their body volume before oviposition [34, 35] (Fig 1). These eggs are laid on rock walls inside caves and are guarded by the mother until hatching and dispersal of the nymphs [34].

**Collection of Individuals**

We collected individuals of *A. longipes* inside caves at Parque Florestal do Itapetinga (23°15' S; 46°45' W), Atibaia, state of São Paulo, southeastern Brazil, between October 2003 and May 2004 (COTEC permission #41.852/2001). We selected adult females in three phases of their reproductive cycle. (1) Non-ovigerous females (NOFs) were those bearing no egg and that were not guarding eggs. (2) Ovigerous females (OFs) were those bearing mature eggs and that were about to oviposit (Fig 1). These females can be easily recognized because they show free tergites spaced out with the intersegmental membrane clearly visible [35]. (3) Egg-guarding females were those that already oviposited and were guarding their eggs for nearly 15 days. The age of a clutch in harvestman can be easily inferred because eggs change in color and size over the course of the embryonic development [36]. We selected 15 day-old clutches because previous laboratory experiments with other quinone-releasing harvestman species indicate that it is the time requested for starved individuals to recover most of their gland volume (Nazareth et al. unpublished data). In the laboratory, we placed females belonging to each reproductive phase in different terraria (60 x 40 cm base, 35 cm high) containing pieces of cotton wetted with water to maintain the humidity.

To exclude the possibility that the concentration of the secretion differs between females in different reproductive phases, we sampled additional ovigerous and non-ovigerous females in the same locality in May 2014. We did not sample egg-guarding females because our previous collection indicated that there is no difference in the mass of secretion produced by females in this phase and non-ovigerous females (see **Results**).

**Production of Chemical Defenses**

We quantified the mass of defensive secretion released by females in each reproductive phase 24 h after collection in the field. First, we weighed a small piece of cotton wool, seized an individual by hand, and induced the emission of exudate by pressing the cotton wool held by tweezers against the gland openings. We repeated this procedure three times to ensure that the gland sacs were completely depleted, and then weighed the cotton wool again. Since harvestmen usually release water from the mouth before or after the emission of defensive secretion [36], we blocked the mouthparts of the females with another piece of cotton wool when milking them of secretion to avoid that enteric water fluid would mix with the gland exudate. We discarded this piece of cotton wool soaked with water, and used the difference in weigh between the second and the first measurements of the cotton wool soaked with exudate to estimate the mass of secretion released by each female. Finally, we measured the dorsal scute length (DSL) of each female using digital calipers (to nearest 0.01 mm). DSL is a standard estimate of body size in harvestmen because it does not change according to hunger or reproductive phase [37].

To quantify the concentration of benzoquinones released by OFs and NOFs from the second sample, we induced the emission of exudate as described above. We then washed the cotton wool tree times with 500 μl of CH₂Cl₂ to guarantee complete extraction of benzoquinones. We added 1 μl of the obtained solution (solvent and secretion) to 600 ul of CH₂Cl₂ and 1 μl of benzophenona (internal standard), and analyzed the sample by gas chromatography. We used a Shimadzu GC-FID 2014 gas chromatograph coupled with an AOC20i autosampler fitted with a RTX-5 capillary column. The oven temperature was as follow: 40°C (2 min), 5°C min⁻¹
Nitrogen was used as the carrier gas at a linear velocity, column flow, and purge of 18.7 cm s\(^{-1}\), 1 mL min\(^{-1}\) and 3 mL min\(^{-1}\), respectively. Injections of 1 μL were carried out in a splitless mode, during 1 min at 220°C and 32.5 kPa. Temperature, air (20% O\(_2\) in N\(_2\)), and hydrogen flows of detector were set at 250°C, 400 and 40 ml min\(^{-1}\), respectively. We calculated the relative amount of the two benzoquinones contained in each sample by the ratio between the area and mass of the internal standard and the area of the benzoquinones in each sample. We estimated total benzoquinones per sample as the sum of the net quantities of the two benzoquinones present in the mixture released by \textit{A. longipes} females. We quantified benzoquinones from the linear regression equation (\(R^2 = 0.994\)) of a calibration curve constructed for 1,4-benzoquinone, so that all amounts are expressed as 1,4-benzoquinone equivalents.

We used general linear models (GLMs) to compare the mass of secretion and concentration of benzoquinones released by females (response variables with Gaussian error distribution) in different reproductive phases (categorical predictor variable), controlling for the effect of body size (continuous predictor variable). Given that we did not expect any interaction between reproductive phase and body size, our models include only the additive effect of these variables (exploratory analyses showed that the interaction is indeed not significant; data not shown).

Bioassays

We conducted a series of bioassays to test the efficiency of NOF and OF secretions against two groups of potential predators: ants and spiders. Immediately before each trial, we milked a female of \textit{A. longipes} of secretion by seizing it by hand and collecting the exudate with capillary tubes. We never repeated the same female in different trials because repeated milking of the same individual reduces the amount of released secretion.

Tests with Ants

We conducted two bioassays to test the potential effect of the secretion on ants: one in the field to test the repellent potential of NOF and OF secretions, and another in the laboratory to test the effectiveness of NOF and OF secretions as a chemical shield. The field experiment consisted of presenting baits made up of pieces of filter paper (1 cm\(^2\)) embedded with a saturated sugar solution and placed on plastic dishes (5 cm diameter). We randomly distributed 100 baits on the forest floor (5 m from each other) at Parque Florestal do Itapetinga. When ants were feeding at the margin of the bait, we stimulated them discharging a solution in the center of the filter paper with a syringe. The constitution of this solution differed among three experimental groups: (1) glandular secretion of one NOF diluted in 100 μl of water, (2) glandular secretion of one OF diluted in 100 μl of water, and (3) 100 μl of distilled water (control). In the three experimental groups, we counted the number of ants in contact with the bait before and 5 s after presentation of solution.

To analyze the data, we created a repellency index (\(RI\)) given by \(RI = (N_0 - N_5)/N_0\), where \(N_0\) is the number of ants in contact with the bait before and \(N_5\) is the number of ants 5 s after presentation of solution. The \(RI\) indicates the proportion of ants that were repelled from the sugar bait after stimulation, so that when \(RI = 0\) no ant was repelled and when \(RI = 1\) all ants were repelled from the bait. We performed a GLM on the repellency index (response variable with Gaussian error distribution) including ant species and the experimental groups as predictor categorical variables. Given that the response of the workers to the different experimental groups could vary according to the ant species, we included the interaction between these two variables in the model. If egg production constrains the production of chemical defenses, OF secretion should be less efficient in repelling ants. Thus, the reduction in the number of ants in
contact with the baits after stimulation with OF secretion should be intermediate between the control group and those stimulated with NOF secretion.

In the laboratory experiment, we used five colonies of large predatory ants that occur syntopically with *A. longipes*: two colonies of *Odontomachus chelifer* and three of *Pachycondyla striata* (both Ponerinae). We collected all colonies in the field and brought them to the laboratory, where they were placed inside plastic trays (25 x 40 cm). In each tray, we placed two test tubes (2 cm diameter x 15 cm length) containing water trapped behind a cotton plug that were used as nests by the ants. Nearly two months after the colonies were brought to the laboratory, we conducted the experiment of chemical shield. Inside each tray, we presented a glass cover slip (1 x 10 cm) divided in three equal parts randomly designated as treatment 1, treatment 2, and control. Treatments 1 and 2 consisted of a filter paper (1 cm²) wetted with 100 μl of a saturated sugar solution mixed with the glandular secretion of one NOF and one OF, respectively. The control contained only a filter paper (1 cm²) wetted with 100 μl of a saturated sugar solution. We counted the total number of ants feeding on each bait at 2 min-intervals during 40 min after the first contact.

To analyze the data, we performed repeated measures analysis of variance using the number of ants feeding on the baits at each 2 min-interval as response variable and experimental groups as predictors. Degrees of freedom were corrected using the Greenhouse-Geisser procedure to avoid sphericity problems. Given that the number of colonies of each ant species was limited, we did not include species identity in the analysis. If egg production constrains the production of chemical defenses, the chemical shield promoted by OF secretion should last less time than that promoted by NOF secretion for both ant species.

**Test with Spiders**

*T. biocellatus* (Trechaleidae) is a large (2–3 cm body length) wandering spider, abundant in the study site, which is generally found near river margins or in other moist habitats, such as caves [32]. We collected individuals of the species at Parque Florestal do Itapetinga between October 2003 and May 2004, and maintained them in individual cages (20 x 10 cm base, 15 cm high) containing a piece of cotton wetted with water to maintain the humidity. Only subadults and adults of both sexes (n = 60) were used in the experiments, and each individual was starved for 5–6 days before the experiments to bring them to a similar level of hunger.

To test the role of the defensive secretion alone and exclude the interference of other possible defenses, such as spines on legs and pedipalps, we did not offer individuals of *A. longipes* directly to the spiders (following [32]). Rather, we offered individuals of the common cricket *Gryllus gryllus* (nearly 1 cm of body length), which the spiders promptly took as prey. In order to ensure that the crickets were unable to promote injuries to the spiders, we removed the hind legs (armed with several spines) of the crickets just before the experiment. After the cricket was grabbed, we stimulated each spider with one of the following solutions: 1) OF secretion diluted in 100 μl of water (n = 20); 2) NOF secretion diluted in 100 μl of water (n = 20), or 3) 100 μl of distilled water (control). We applied the solutions with a syringe directly to the base of the chelicerae. Spiders that extricated the chelicerae and abandoned the prey within 5 min were scored as respondents (following [38]). We compared the number of spiders that released or not the prey using two Fisher exact tests: one between control and OF secretion, and other between NOF and OF secretions. Given that we performed two analyses using the same dataset, we used the Bonferroni correction to adjust the p values. If egg production constrains the production of chemical defenses, OF secretion should be less efficient in repelling spiders. Thus, the number of crickets released by the spiders stimulated with OF secretion should be lower when compared to the spiders stimulated with NOF secretion.
Results
Production of Chemical Defenses

The mass of defensive secretion released by the females was affected by their reproductive phase, but not by their body size. Egg-guarding females ($n = 12$) and NOFs ($n = 25$) released a similar mass of secretion, which was on average 71.8% greater than the mass of secretion released by OFs ($n = 25$; Fig 2; Table 1). Females produced defensive secretion with similar concentration of benzoquinones, regardless of body size and reproductive stage (mean ± SD): NOFs = 21.03 ± 15.28 μg/mL and OFs = 18.97 ± 12.93 μg/mL (Table 1).

Tests with Ants

In total, 51 baits were visited by workers of seven ant species in the field: three by *Crematogaster* sp. (one for each experimental group), three by *Pachycondyla striata* (one for each experimental group), three by *Pheidole* sp.1 (one for each experimental group), six by *Odontomachus chelifer* (two for each experimental group), nine by *Camponotus* sp. (three for each experimental group), nine by *Pheidole* sp.2 (three for each experimental group), and 18 by *Gnamptogenys* sp. (six for each experimental group). The discharge of defensive secretions induced a marked reduction in the number of ants feeding on the sugar baits, regardless of the ant species (Table 2). Baits treated with NOF secretion, however, had slightly higher repellency than those treated with OF secretion (Fig 3). No significant reduction was observed in the control baits (Fig 3).

In the laboratory bioassay, the number of workers tending control baits increased fast from 0 to 12 min. After this period until the end of the experiment, we observed, on average, five to seven workers tending the baits (Fig 4). No ant was observed tending the baits containing OF secretion during the first 2 min. After 4 min, however, the number of workers tending the baits increased slowly, and from 22 min until the end of the experiment, we observed, on average, one to two workers tending the baits (Fig 4). No ant was observed tending the baits containing NOF secretion during the entire experiment in almost all colonies (Fig 4). In general, there was a significant interaction between time and experimental group ($F_{40, 160} = 4.55; p = 0.002$).

Tests with Spiders

Only one individual of *T. biocellatus* released the prey in the control group ($n = 20$). When individuals were stimulated with OF secretion ($n = 20$), only two released the prey and this frequency does not differ from the control (Fisher exact test, $p = 1.00$). When individuals were stimulated with NOF secretion ($n = 20$), nine released the prey and this frequency is significantly higher than the group stimulated with OF secretion (Fisher exact test, $p = 0.016$).

Discussion

We found that females of the harvestman *A. longipes* bearing mature eggs produce less defensive secretion than females in other reproductive phases, including non-ovigerous and egg-guarding females. Moreover, we found no difference in the concentration of total benzoquinones released by OFs and NOFs, indicating that there is no compensation related to increased concentration of defensive compounds during egg production. These results are congruent with the hypothesis that egg production constrains the investment in chemical defenses based on benzoquinones (Fig 1). Finally, the results of our bioassays clearly indicate that the low amount of secretion released by OFs is less effective in deterring potential predators (ants and spiders) than the high amount released by NOFs. In what follows, we will explore these results in more detail and discuss their implications for our understanding on the costs of reproduction in a chemically defended animal.
During egg production, a large amount of yolk must be stored in the oocytes in a relatively short period of time to provide nutritional supply for the developing embryo [39]. The increased physiological requirement promoted by egg production leads females of many species to intensify their foraging activities [40]. In the fishing spider *Dolomedes triton*, for instance, females switch from a sit-and-wait strategy to more active foraging upon maturation [41,42]. In the harvestman *Serracutisoma spelaeum*, OFs forage more frequently than NOFs, leaving the cave habitat to search for food almost every night [42]. The same pattern seems to occur with *A. longipes* (G. Machado, pers. obs.), and our results suggest that the resources acquired by OFs during this period of intense foraging activity are invested predominantly in egg production, rather than chemical defenses. Histological studies of *A. longipes* support this suggestion, indicating that during the period of egg production there is a marked increase in the lipid content of the fat body [44], an organ that is the source of most part of the yolk received by the ovarian follicles [31].

Fig 2. Mass of defensive secretion (mean ± SE) released by egg-guarding, non-ovigerous, and ovigerous females of the harvestman *Acutisoma longipes*. Different letters indicate significant differences (post-hoc test, p < 0.05).

doi:10.1371/journal.pone.0134908.g002

Table 1. Results of the GLM testing the effect of body size and reproductive phase on the mass of defensive gland secretion and concentration of total benzoquinones released by females of the harvestman *Acutisoma longipes*. Significant p-values are shown in bold.

| Effect                                | DF | MS     | F       | p     |
|---------------------------------------|----|--------|---------|-------|
| Mass of defensive secretion           |    |        |         |       |
| Body size                             | 1  | 35.507 | 2.430   | 0.125 |
| Reproductive phase (egg-guarding, non-ovigerous, and ovigerous females) | 2  | 93.733 | 6.416   | **0.003** |
| Error                                 | 58 | 14.611 |         |       |
| Concentration of total benzoquinones  |    |        |         |       |
| Body size                             | 1  | 0.040  | 0.315   | 0.576 |
| Reproductive phase (non-ovigerous and ovigerous females) | 1  | 0.168  | 1.296   | 0.258 |
| Error                                 | 83 | 0.128  |         |       |

doi:10.1371/journal.pone.0134908.t001
The possible trade-off reported here for *A. longipes* contrasts with the results obtained in a previous study with *Zophobas atratus*, a tenebrionid beetle that produces two alkylated benzoquinones also found in many harvestman species [30]. In this beetle, egg production did not affect the investment in chemical defenses, so that mated females that produced twice as many eggs as virgin females released nearly the same amount of defensive secretions [45]. The authors suggest that individuals do not channel much energy into the production of defensive secretions, and that a marked trade-off between egg and secretion production should be found in species that invest more energy in chemical defenses [45]. We think, however, that the lack of a trade-off reported for *Z. atratus* is related to the high abundance of food provided to the beetles in the laboratory. Trade-offs are more likely to emerge when internal energy reserves are limited [6], and under natural conditions, egg-producing *A. longipes* females are probably food limited because most foraging trips outside the cave habitat are unsuccessful [33]. This may explain why we detected a negative influence of egg production on the total mass of secretion produced by OFs.

**Table 2. Results of the GLM testing the effect of ant species and experimental groups on the repellency index.** The experimental groups were: (1) secretion of one non-ovigerous female of *Acutisoma longipes*, (2) secretion of one ovigerous female of *A. longipes*, and (3) distilled water (control). Significant p-values are shown in bold.

| Effect                      | DF | MS    | F     | p      |
|-----------------------------|----|-------|-------|--------|
| Intercept                   | 1  | 13.109| 601.975| < 0.001|
| Ant species                 | 6  | 0.013 | 0.609 | 0.721  |
| Experimental group          | 2  | 2.664 | 122.322| < 0.001|
| Ant species x Experimental group | 12 | 0.014 | 0.661 | 0.773  |
| Error                       | 30 | 0.022 |       |        |

doi:10.1371/journal.pone.0134908.t002

**Fig 3. Results of the field experiment in which the number of ants tending sugar baits was counted before and after stimulation with one of the three experimental groups: (1) secretion of one non-ovigerous female of *Acutisoma longipes*, (2) secretion of one ovigerous female of *A. longipes*, and (3) distilled water (control).** Different letters indicate significant differences (post-hoc test, p < 0.05).

doi:10.1371/journal.pone.0134908.g003
Egg-guarding females released the same mass of secretion reported for NOFs (Fig 2), indicating that the investment in chemical defenses is resumed after oviposition. Given that females are prevented from foraging while caring for the offspring, and remain stationary on the clutch all day long during the entire period of embryonic development [34], which resources are used to produce benzoquinones? Detailed histological studies of *A. longipes* show that some oocytes are reabsorbed during the period of maternal care [44], and we suggest that the nutrients obtained from the oocytes are the source for the production of benzoquinones during the caring period. The possible translocation of resources between the endpoints of the Y model of resource allocation reinforces the notion that the same precursors can be used to produce both fatty acids and polyketides [29], giving rise to the trade-off we are proposing here between egg and benzoquinone production (Fig 1). The increased production of chemical defenses after oviposition may be particularly important for egg-guarding females, which remain on the clutch for more than one month probably exposed to active-hunting predators [34]. In fact, a long-term field experiment with the harvestman *S. proximum*, which is closely related to *A. longipes*, indicates that the mortality of egg-guarding females is not reduced when compared with females prevented from caring [46], suggesting that females are well-protected against predation during the period of parental care.

Fig 4. Mean (± SE) number of workers of the ants *Odontomachus chelifer* and *Pachycondyla striata* tending three types of baits: (1) sugar solution + secretion of one non-ovigerous female of *Acutisoma longipes*, (2) sugar solution + secretion of one ovigerous female of *A. longipes*, and (3) sugar solution (control). The photo illustrates the experimental setup of an *O. chelifer* colony at 14 min of experiment. doi:10.1371/journal.pone.0134908.g004
The strong irritating properties of benzoquinones are known to repel numerous invertebrate and vertebrate predators (see [23] and references therein). In a previous study with *A. longipes*, the defensive secretion of adult males and NOFs repelled seven ant species, two species of large wandering spiders, and one frog species [32]. Using similar protocols, we showed here that the efficiency of the defensive gland secretion released by OFs is reduced when compared with NOFs. In the field experiment with ants, the repellency index of OF secretion was slightly lower than that of NOF (Fig 3). However, the variance in the repellency index of the secretion released by OFs was much higher, with some values overlapping the values of the control group (Fig 3). In the laboratory experiment, the chemical shield promoted by the OF secretion lasted considerably less time than that of NOF secretion (Fig 4). After 40 min, an average of two ants was tending the baits wetted with OF secretion while no ant was tending the baits wetted with NOF secretion (Fig 4). Finally, spiders stimulated with OF secretion released the crickets 4.5 times less frequently than those stimulated with NOF secretion.

Field studies with several arthropod species, including three neotropical harvestmen, indicate that individuals that are more active during the reproductive period are more frequently captured by ambush predators than sedentary individuals [37, 47–50]. As we mentioned above, females of goniosomatine harvestmen increase their foraging activities during the period of egg production and leave the cave habitat on a daily basis, while males and NOFs may remain stationary inside the cave for three or more days [43, 33]. Therefore, highly vagile OFs are probably under a higher risk of predation than other conspecifics, especially if the egg load also decreases female locomotor ability, as it has already been reported for females of many animal groups (review in [51]). Additionally, the results of our bioassays suggest that the trade-off between egg and benzoquinone production makes OFs particularly vulnerable to predation when compared with NOF. Taken together, these findings support the notion that egg production is a critical moment in the life of harvestman females, representing perhaps the highest cost of reproduction, as also suggested for many bird species [52].

In conclusion, females allocate resources to chemical defenses in a way that preserves a primary biological function related to reproduction. As far as we know, this is the first time this trade-off has been directly demonstrated for animals. In the future, mark-recapture studies should be conducted in the field to access whether mortality rates of OFs are higher than NOFs. Moreover, it would be interesting to investigate whether OFs fed *ad libitum* in the laboratory are able to channel more resources to egg production, so that the trade-off observed under field conditions is somehow attenuated. Finally, a metabolomic approach to the trade-off between egg and benzoquinone production could be valuable to characterize the physiological responses of the females at the biochemical level (see [53]).

**Acknowledgments**

We are grateful to Billy Requena and Augusto K. Machado for helping in the fieldwork, to Eduardo A. Santos for advices on the statistical analyses, and to Rodrigo H. Willemart, Taran Grant, and Luis Schiesari for helpful comments on an early version of the manuscript. The authors are supported by grants from FAPESP (TMN, proc. 11/50800-8; GM, proc. 99/05446-8; 02/00381-0; 12/50229-1) and CNPq.

**Author Contributions**

Conceived and designed the experiments: TMN GM. Performed the experiments: TMN GM. Analyzed the data: TMN GM. Contributed reagents/materials/analysis tools: TMN GM. Wrote the paper: TMN GM.
References

1. Smiseth P, Kölliker M, Royle NJ (2012) What is parental care? In: Royle NJ, Smiseth P, Kölliker M, editors. The Evolution of Parental Care. Oxford (UK): Oxford University Press. p. 1–17.

2. Fox CW, Czesak ME (2000) Evolutionary ecology of progeny size in arthropods. Annu Rev Entomol 45: 341–369. PMID: 10761581

3. Morrongiello JR, Bond NR, Crook DA, Wong BBM (2012) Spatial variation in egg size and egg number reflects trade-offs and bet-hedging in a freshwater fish. J Anim Ecol 81: 806–817. doi: 10.1111/j.1365-2656.2012.01961.x PMID: 22309288

4. Dziminski MA, Vercoe PE, Roberts JD (2009) Variable offspring provisioning and fitness: a direct test in the field. Funct Ecol 23: 164–171.

5. Williams TD. (1994) Intraspecific variation in egg size and egg composition in birds—effects on offspring fitness. Biol Rev 69: 35–59. PMID: 8193216

6. Harshman LG, Zera AJ (2007) The cost of reproduction: the devil in the details. Trends Ecol Evol 22: 80–86. PMID: 17056152

7. Stearns SC (1992) The Evolution of Life Histories. Oxford (UK): Oxford University Press.

8. Roff DA (2002) Life History Evolution. Boston: Sinauer Associates Inc.

9. French SS, Moore MC, Demas GE (2009) Ecological immunology: The organism in context. Integr Comp Biol 49: 246–253. doi: 10.1093/icb/icp032 PMID: 21665817

10. Moreno-García M, Córdoba-Aguilar A, Conde R, Lanz-Mendoza H (2013) Current immunity markers in insect ecological immunology: assumed trade-offs and methodological issues. Bull Entomol Res 103: 127–139. doi: 10.1017/S000748531200048X PMID: 22929006

11. French SS, Johnston GH, Moore MC (2007) Immune activity suppresses reproduction in food-limited female tree lizards, Urosaurus ornatus. Funct Ecol 21: 1115–1122.

12. Knowles SCL, Nakagawa S, Sheldon BC (2009) Elevated reproductive effort increases blood parasitaemia and decreases immune function in birds: a meta-regression approach. Funct Ecol 23: 405–415.

13. Cox RM, Parker EU, Cheney DM, Liebl AL, Martin LB, Calsbeek R (2010) Experimental evidence for physiological costs underlying the trade-off between reproduction and survival. Funct Ecol 24: 1262–1269.

14. Bascuñán-García AP, Lara C, Córdoba-Aguilar A (2010) Immune investment impairs growth, female reproduction and survival in the house cricket, Acheta domesticus. J Insect Physiol 56: 204–211. doi: 10.1016/j.jinsphys.2009.10.005 PMID: 19840805

15. Bazzaz FA, Chiarello NR, Coley PD, Pitelka LF (1987) Allocating resources to reproduction and defense. BioScience 37: 58–67.

16. Stamp N (2003) Out of the quagmire of plant defense hypotheses. Q Rev Biol 78: 23–55. PMID: 12661508

17. Neilson EH, Goodger JOD, Woodrow IE, Møller BL (2013) Plant chemical defense: at what cost? Trends Plant Sci 18: 250–258. doi: 10.1016/j.tplants.2013.01.001 PMID: 23415056

18. Harvell CD (1986) The ecology and evolution of inducible defenses in a marine bryozoan: cues, costs, and consequences. Am Nat 128: 810–823.

19. Ivanisvic J, Thomas OP, Pedel L, Penez N, Ereskovsky AV, Culioli G, Perez T (2011) Biochemical trade-offs: evidence for ecologically linked secondary metabolism of the sponge Oscarella balibaloi. PLoS One 6: e28059. doi: 10.1371/journal.pone.0028059 PMID: 22132209

20. Berenbaum MR (1995) The chemistry of defense: theory and practice. In: Eisner T, Meinwald editors. The Chemistry of Biotic Interaction. Washington: National Academy Press. p. 1–16.

21. Eisner T, Eisner M, Seigler M (2005) Secret Weapons: Defenses of Insects, Spiders, Scorpions, and Other Many-Legged Creatures. Cambridge (MA): Harvard University Press.

22. Morgan ED (2004) Biosynthesis in Insects. Cambridge (UK): Royal Society of Chemistry.

23. Gilbert JDD, Manica A (2010) Parental care trade-offs and life-history relationships in insects. Am Nat 176: 212–226. doi: 10.1086/653661 PMID: 20528469

24. Higginson AD, Delf J, Ruxton GD, Speed MP (2011) Growth and reproductive costs of larval defence in the aposematic lepidopteran Pieris brassicae. J Anim Ecol 80: 384–392. doi: 10.1111/j.1365-2656.2010.01786.x PMID: 21155771
27. Blum MS (1981) Chemical Defenses of Arthropods. New York: Academic Press.
28. Meinwald J, Happ GM, Labows J, Eisner T (1966) Cyclopentanoid terpene biosynthesis in a phasmid insect and in catmint. Science 151: 79. PMID: 5908966
29. Pankewitz F, Hilker M (2008) Polyketides in insects: ecological role of these widespread chemicals and evolutionary aspects of their biogenesis. Biol Rev 83: 209–226. doi: 10.1111/j.1469-185X.2008.00040.x PMID: 18410406
30. Rocha DFO, Wouters FC, Zampieri DS, Brocksom TJ, Machado G, Marsaioli AJ (2013) Harvestman phenols and benzoquinones: characterisation and biosynthetic pathway. Molecules 18: 11429–11451. doi: 10.3390/molecules180911429 PMID: 24043140
31. Trougakos IP, Margaritis LH (2002) Novel morphological and physiological aspects of insect eggs. In: Hilker M, Meiners T, editors. Chemoecology of Insect Eggs and Egg Deposition Oxford (UK): Blackwell Publishing. p. 3–31.
32. Machado G, Carrera PC, Pomini AM, Marsaioli AJ (2005) Chemical defense in harvestmen (Arachnida: Opiliones): do benzoquinone secretions deter invertebrate and vertebrate predators? J Chem Ecol 31: 2519–2539. PMID: 16273426
33. Machado G, Oliveira PS (2000) Daily activity schedule, gregariousness, and defensive behaviour in the neotropical harvestman Goniosoma longipes (Opiliones: Gonylyptidae). J Nat Hist 34: 587–596.
34. Machado G, Oliveira PS (1998) Reproductive biology of the neotropical harvestman Goniosoma longipes (Arachnida: Opiliones: Gonylyptidae): mating and oviposition behaviour, brood mortality, and parental care. J Zool 246: 359–367.
35. Machado G, Macias-Ordóñez R (2007) Reproduction. In: Pinto-da-Rocha R, Machado G, Giribet G, editors. Harvestmen: The Biology of Opiliones. Cambridge (MA): Harvard University Press. p. 414–454.
36. Gnaspini P (2007) Development. In: Pinto-da-Rocha R, Machado G, Giribet G, editors. Harvestmen: The Biology of Opiliones Cambridge (MA): Harvard University Press. p. 455–472.
37. Requena GS, Buzatto BA, Martins EG, Machado G (2012) Paternal care decreases foraging activity and body condition, but does not impose survival costs to caring males in a neotropical arachnid. PLoS One 7: e46701. doi: 10.1371/journal.pone.0046701 PMID: 23071616
38. Eisner T, Morgan RC, Attygalle AB, Smedley SR, Herath KB, Meinwald J (1997) Defensive production of quinoline by a phasmid insect (Oreophoetes peruana). J Exp Biol 200: 2493–2500. PMID: 9366083
39. Bownes M (1986) Expression of the genes coding for vitellogenin (yolk protein). Annu Rev Entomol 31: 507–531.
40. Helfman GS (1990) Mode selection and mode switching in foraging animals. In: Slater PJB, Rosenblatt JS, Beer C, editors. Advances in the Study of Behavior, vol. 19. San Diego: Academic Press. p. 249–298.
41. Kreiter NA, Wise DH (1996) Age-related changes in movement patterns in the fishing spider, Dolomedes triton (Araneae, Pisauridae). J Arachnol 24: 24–33.
42. Kreiter NA, Wise DH (2001) Prey availability limits fecundity and influences the movement pattern of female fishing spiders. Oecologia 127: 417–424.
43. Gnaspini P (1996) Population ecology of Goniosoma spelaeum, a cavernicolous harvestman from south-eastern Brazil (Arachnida: Opiliones: Gonylyptidae). J Zool 239: 417–435.
44. Tomaino-Gomes GA (2008) Morphologic characterization of the ventricle and of the ovary of Acutisoma longipes (Opiliones: Gonylyptidae) during reproductive cicle. PhD Dissertation. Universidade Estadual de Campinas, São Paulo, Brazil.
45. Hill CS, Tschinkel WR (1985) Defensive secretion production in the tenebrionid beetle, Zophobas atratus: effect of age, sex, and milking frequency. J Chem Ecol 11: 1083–1092. doi: 10.1007/BF01020677 PMID: 24310332
46. Buzatto BA, Requena GS, Martins EG, Machado G (2007) Effects of maternal care on the lifetime reproductive success of females in a neotropical harvestman. J Anim Ecol 76: 937–945. PMID: 17714272
47. McCauley DE, Lawson EC (1986) Mating reduces predation on male milkweed beetles. Am Nat 127: 112–117.
48. Polis GA, Barnes JD, Seely MK, Henschel JR, Enders MM (1998) Predation as a major cost of reproduction in Namib desert tenebrionid beetles. Ecology 79: 2560–2566.
49. Buzatto BA, Requena GS, Lourenço RS, Munguía-Steyer R Machado G. (2011) Conditional male dimorphism and alternative reproductive tactics in a Neotropical arachnid (Opiliones). Evolutionary Ecology, v. 25, p. 331–349.
50. Requena GS, Machado G (2015) Lack of costs associated with nest-related behaviors in an arachnid with exclusive paternal care. Oikos 124: 372–380.

51. Magnhagen C (1991) Predation risk as a cost of reproduction. Trends Ecol Evol 6: 183–186. doi: 10.1016/0169-5347(91)90210-O PMID: 21232452

52. Williams TD (2005) Mechanisms underlying the costs of egg production. BioScience 55: 39–48.

53. Bundy JG, Davey MP, Viant MR (2009) Environmental metabolomics: a critical review and future perspectives. Metabolomics 5: 3–21.