

Effectiveness of maternal egg attendance in an exclusively cave-dwelling harvestman (Arachnida: Opiliones)

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Keywords

cannibalism; clutch size; egg protection; parental care; offspring survival; troglobite; *Phalangodus briareos*; arachnids.

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Editor: Gabriele Uhl

Received 24 August 2016; revised 20 January 2017; accepted 9 February 2017

doi:10.1111/jzo.12452

Abstract

Egg attendance is the most common and phylogenetically widespread form of post-ovipositional care among ectotherms. The main benefit of egg attendance is to enhance offspring survival by preventing or attenuating attacks from natural enemies. In arachnids, there are few experimental studies on the benefits of egg attendance, and they pertain to species living in few types of habitats, mainly forests. To understand how the benefits of egg attendance vary in large geographical scales, we need to include species from poorly explored habitats, such as caves. Here, we describe a case of maternal egg attendance in an exclusive cave-dwelling harvestman, *Phalangodus briareos*. Using a parent removal experiment, we also assessed the benefits of egg attendance. We found that egg attendance improves egg survival because unattended clutches were almost entirely consumed by conspecifics and crickets. When females were maintained on their clutches, egg survival was always high, regardless of female body size. There was a positive effect of clutch size on egg predation, which was higher during nighttime. We suggest that larger clutches emit more chemical cues, attracting more predators in the cave habitat. Finally, we argue that the benefits of egg protection should be higher inside caves when compared to external habitats because food is scarce inside caves and the fauna consists mainly of predators and detritivores.

Introduction

Across taxa, there is great diversity in the ways through which parents enhance the fitness of their offspring, including selection of oviposition sites, preparation of nests, care of eggs inside or outside the parent's body, food provisioning of young before and after birth, and care of offspring after nutritional independence (Smiseth, Kölliker & Royle, 2012). Egg attendance, which occurs when one or both parents remain with the fertilized eggs at the oviposition site until they hatch, is probably the most common and phylogenetically widespread form of post-ovipositional care among ectotherms (Clutton-Brock, 1991). In terrestrial groups, such as frogs and some insects, egg attendance may reduce the risk of dehydration (Delia, Ramírez-Bautista & Summers, 2013; Gilbert, 2014) or flooding (Wyatt, 1986). However, the main benefit attributed to egg attendance in ectotherms is protection against natural enemies. Experimental evidence for terrestrial groups indicates that the absence of the parents usually condemns the eggs to death due to attacks of predators, parasitoids and pathogens (Santos *et al.*, 2017).

Egg attendance has been recorded in species belonging to several arachnid orders, including spiders, harvestmen, mites and camel spiders. In all these orders, parental presence may

prevent or attenuate attacks from natural enemies (Costa, 2006). However, there are only few studies that experimentally tested the protective role of egg attendance in arachnids (Table S1). Additionally, the available studies are concentrated in a few types of habitat, mainly forests (Table S1). Therefore, if we want to understand how the benefits of egg attendance in arthropods vary in large geographical scales in response to biotic and abiotic factors (Santos *et al.*, 2017), we need larger datasets including species from poorly explored places and habitats. Moreover, in order to have truly independent evidence of the benefits of egg attendance, we should focus on species in which this behaviour has evolved independently from the taxa studied so far.

Here, we provide a description of maternal egg attendance in a cave-dwelling harvestman, *Phalangodus briareos* Villarreal & García, 2016 (Opiliones: Cranidae), and experimentally assess the benefits of this behaviour. We tested three hypotheses. (i) The scarcity of food inside caves and the fact that the cave fauna consists mainly of predators and detritivores imply that unprotected eggs are particularly vulnerable in this type of habitat (Machado, 2002). Consequently, the presence of the mother close to the clutch is expected to provide great benefits in terms of egg protection. (ii) Larger egg-tending females should be more effective at defending their eggs than smaller

egg-tending females due to advantages in weaponry and/or strength (e.g. Miller, Rudolph & Zink, 2011). (iii) Because cave-dwellers maintain their circadian rhythms even in the absence of light and are usually more active at night (e.g. Hoenen & Gnaspini, 1999), predation pressure on eggs should be more intense during nighttime. Although maternal egg attendance in harvestmen has already been recorded for four cranaid species (Table S2), this is the first time the effectiveness of this behaviour is investigated in this family. Considering that maternal egg attendance in cranaids evolved independently from other harvestman taxa studied so far (Machado & Warfel, 2006), our findings provide independent evidence of the protective role of this behaviour in arachnids. Moreover, this is the first detailed account on the benefits of maternal egg attendance in an arthropod that spends all its life cycle inside caves (Table S1).

Materials and methods

Study site

We conducted this study in Alsacia cave (6°47'53.53"N, 73°15'26.16"W; 1777 m a.s.l.), in the countryside of Zapatoca, Department of Santander, east Colombian Andes. The mean annual precipitation in Zapatoca is 107 mm and the mean annual temperature is 18.7°C. Inside the cave, environmental conditions are very constant along the year, with mean relative humidity of 89% and mean temperature of 21°C. The cave has a central gallery 1 km long and 5 m wide, and some small secondary galleries. We focused our study on the central gallery, from the entrance up to 700 m inside the cave, because individuals of *P. briareos* are more frequently found in this part of the cave.

Observational data

We visited the cave at 20-day intervals between July and December 2007, once in June 2011, once per month between January and June 2012, and once in January 2016, totalling 39 days of field work. In the visits of 2007–2012, we marked egg-tending females individually with enamel colour paint applied to their hind legs and dorsal scute, which comprises the cephalothorax and the first mesotergal segments. We also marked each oviposition site attaching a plastic tape to the cave wall close to the clutches to investigate whether females switch clutches during the egg-tending period. For each egg-tending female, we measured (to the nearest 0.01 mm) the dorsal scute width, which does not change according to reproductive or nutritional conditions. We also counted the number of eggs in each clutch. In January 2016, we measured (to the nearest 0.01 mm) the diameter of 18 recently laid eggs from one clutch and 14 eggs close to hatching from another clutch to describe how egg volume changes during embryonic development.

To determinate the frequency of egg attendance, we monitored 14 marked females in January 2012. We inspected each egg-tending female six times a day (0800, 1100, 1400, 1700, 2000, and 2300 h) during four consecutive days. We

considered the first four inspections as daytime and the last two inspections as nighttime. At each inspection, we recorded whether the females were present or absent from their clutches. We considered a female as present when she was on the eggs or up to 10 cm away from the eggs; otherwise we considered her absent. Given that harvestmen only detect close-range chemical and tactile stimuli (Willemart & Chelini, 2007), we adopted a distance of 10 cm between egg-tending females and their clutches because it comprises nearly two times the length of the second pair of sensorial legs. Moreover, if egg-tending females were not close (i.e., ≤ 10 cm) to their respective clutches, we never found them further away (from 11 to 100 cm), suggesting that they temporarily deserted their eggs.

To quantify temporary egg desertion during daytime and nighttime, we calculated the percentage of inspections in which we recorded each egg-tending female as absent in relation to the total number of inspections accomplished in each period of the day. We also recorded behavioural interactions between egg-tending females and potential egg predators. To estimate egg mortality under natural conditions, we took photos of the clutches in the beginning and at the end of a 4-day period, and counted the number of eggs per clutch in each of these two moments.

Effectiveness of egg attendance

To quantify the effectiveness of egg attendance against predation, we conducted an experiment during February 2012. We excluded from this experiment all clutches containing dark eggs that were about to hatch because egg hatching during the experiment could lead to an overestimation of egg loss. After eliminating these clutches, we ended up with 16 clutches, which we randomly assigned to two experimental groups. In the 'no care' group, we removed egg-tending females ($n = 8$) from their clutches, leaving the eggs unattended during 4 days. We maintained the removed females alive inside plastic boxes in the laboratory and, after marking and measuring, we returned them to their respective breeding sites at the end of the experiment. In the 'care' group, we removed the egg-tending females ($n = 8$) and, after marking and measuring, we placed them back close to their clutches. In the beginning of the experiment, the median number of eggs per clutch in the no care group was 27.5 (range = 16–35), and in the care group was 29.5 (range = 10–42).

We inspected all experimental clutches six times a day (0800, 1100, 1400, 1700, 2000 and 2300 h) in an attempt to identify egg predators. During the inspections, we also recorded whether females from the care group were present or absent from their clutches following the same criterion presented above. Whenever we encountered predators consuming eggs, we took pictures of them for later identification. Moreover, we photographed each clutch daily, once in the morning (0800 h) and once in the evening (2000 h). We used these photos to count the number of eggs in each clutch during each period of the day, which allowed us to quantify nocturnal (2000–0800 h) and diurnal (0800–2000 h) predation as the total number of eggs that disappeared from each initial clutch.

We also used the photos to identify the position of each egg individually and exclude eggs that were added to the clutches by egg-tending females of the care group during the experiment.

Statistical analyses

To investigate whether fecundity (response variable) increases with female dorsal scute width (predictor variable), we adjusted a generalized linear model (GLM) with negative binomial error distribution. We included in this analysis all egg-tending females ($n = 57$) found in the visits of 2007–2012. To test the effectiveness of egg attendance in providing protection against predators, we adjusted a generalized linear mixed-effects model (GLMM) with negative binomial error distribution. We expected that the number of eggs (response variable) would depend on an interactive effect between two predictor variables: experimental group (care vs. no care) and time (beginning vs. end of the experiment). In this analysis, we included all clutches used in the experiment of effectiveness of egg attendance ($n = 16$). Given that each experimental clutch provided two measures of number of eggs (day 1 and 4), we included clutch identity as a random factor in the model. We implemented these two models in the package `glmmADMB` (Fournier *et al.*, 2012).

To test whether the effectiveness of egg attendance is influenced by female body size, we adjusted a GLM with binomial error distribution. We expected that egg predation would depend on female dorsal scute width. Because variation in the number of consumed eggs was very small, we classified the clutches into two categories as response variable: (0) no egg consumption and (1) at least one egg consumed. In this analysis, we included all females used to quantify the frequency of egg attendance ($n = 14$), and implemented the model using the package `lme4` (Bates *et al.*, 2015).

Finally, we explored the data obtained in the field experiment to test whether the effectiveness of egg attendance in providing protection against predation is influenced by the period of the day (daytime vs. nighttime). Considering that the intensity of egg predation may also be influenced by clutch size, we included the number of eggs in the experimental clutches as a covariate in the analysis. We expected that the number of eggs consumed in each inspection (response variable) would depend on the following predictor variables: period of the day, clutch size, experimental group, and the additive and interactive effects between these variables. To test this, we adjusted several GLMMs with Poisson error distribution (Table S2), and compared their relative fit to the observed data using a model selection approach (Burnham & Anderson, 2002). We included in this analysis all clutches used in the experiment of effectiveness of egg attendance ($n = 16$). Given that each clutch provided repeated measures of number of eggs in the experiment (days 1–4) and that we analysed the data for each day independently, we included the day and clutch identity as crossed random effects. We implemented the models using the package `lme4`, and used the small sample size bias-corrected version of Akaike Information Criterion (AICc) to rank them and calculate their relative weight. We computed

the difference in the AICc between the model with the best fit and all other models using the package `BBMLE` version 1.0.17 (Bolker & R Development Core Team, 2014).

We conducted all statistical analyses in the software `R` 3.2.5 (R Development Core Team, 2015). All mean values presented in the results are followed by standard deviation.

Results

Characterization of egg-tending behaviour

We found egg-tending females in all visits to the cave, indicating that reproductive activity occurs all year long. Females can reproduce more than once because we found four egg-tending females marked in January and February 2012 tending a new clutch in June 2012. We also found one egg-tending female marked in February 2012 tending a new clutch in January 2016. The frequency of egg-tending females varied during the year, and the month with more clutches was February 2012, when we found 18 egg-tending females in the cave.

Egg-tending females were observed prostrated on or close to the eggs (Fig. 1a,b), and none of them switched clutches during the study period. Median number of eggs per clutch was 32 (range = 13–51, $n = 62$ clutches), and eggs were laid directly on the cave walls, in a single layer, and spaced out 0–3 mm within the clutch (Fig. 1a,b). We observed a small amount of mucus under each egg attaching it to the substrate. Mean dorsal scute width of egg-tending females was 9.25 ± 0.37 mm ($n = 53$ females), and there was no relationship between female dorsal scute width and the number of eggs per clutch ($\beta \pm SE = 0.055 \pm 0.106$; $z = 0.51$; $P = 0.607$).

Oviposition was slightly asynchronous, so that 31–95% of the eggs in a clutch were laid in the first 2 days, whereas the remaining eggs were laid up to 5 days later ($n = 4$ females). Embryonic development lasted 60–70 days, and females also remained close to early hatched nymphs for nearly 5 days ($n = 6$ females; Fig. 1b). Recently laid eggs were cream-coloured and had a mean diameter of 2.66 ± 0.10 mm (Fig. 1a). During embryonic development, eggs darkened and became almost fully black before hatching, when mean diameter was 3.13 ± 0.08 mm (Fig. 1b). Mean egg volume increased more than 60%, from 9.92 ± 1.08 mm³ to 16.08 ± 1.27 mm³, during embryonic development.

From the 14 egg-tending females monitored in the field, most spent between 90 and 100% of the inspections close to their eggs (Fig. S1a). Only one egg-tending female remained less than 67% of the inspections close to her eggs (Fig. S1a), suggesting she either died or deserted indefinitely. Although the median percentage of temporary egg desertion was zero during both daytime and nighttime, two females were recorded temporarily absent from their clutches during daytime, and six females were recorded temporarily absent during nighttime (Fig. S1b). The percentage of inspections in which females were temporarily absent from their clutches was 2.5% at daytime and 7.4% at nighttime.

Under non-experimental conditions, 43% of the clutches ($n = 14$) suffered egg loss during 4 days of observations. Egg

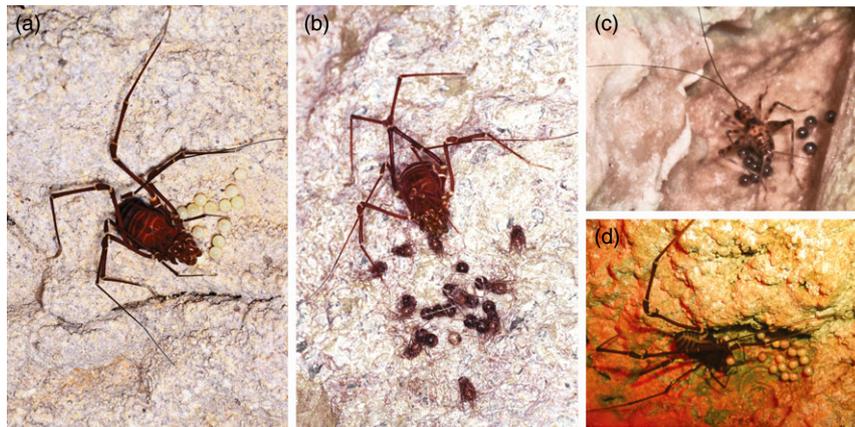


Figure 1 (a) Female of the harvestman *Phalangodus briareos* attending recently laid eggs on the cave wall. (b) Female attending eggs in late stage of embryonic development and recently hatched nymphs. (c) Cave cricket (Phalangopsidae) and (d) conspecific male preying on eggs of unattended clutches. Photos: John A. Uribe.

loss ranged from 1 to 35 (median = 2), and the effectiveness of egg attendance in preventing egg loss was not influenced by female dorsal scute width ($\beta \pm \text{SE} = 2.113 \pm 2.123$; $z = 0.995$; $P = 0.320$). In January 2012, we observed predation events upon eggs of temporarily unattended clutches on two occasions, one by a phalangopsid cave cricket (Fig. 1c) and another by a conspecific male (Fig. 1d). We also observed an egg-tending female attacking and repelling a conspecific male with her second pair of legs.

Effectiveness of egg attendance

There was an interactive effect of experimental group and time on the number of eggs ($\beta \pm \text{SE} = -3.647 \pm 0.686$; $z = -5.320$; $P < 0.001$; Table 1). In the care group, there was no significant reduction in the number of eggs per clutch between the beginning and the end of the experiment (Fig. 2a). However, in the no care group, the number of eggs in the clutches at the end of the experiment was significantly lower than in the beginning (Fig. 2a). In the care group, we identified at least four events of egg loss, which occurred during daytime. In each of these events one or two eggs disappeared from the clutches. Only 1.8% of the eggs in the care group

were lost during the 4-day period of the experiment. Because we observed no egg predation in the clutches of the care group, we cannot rule out the possibility of filial cannibalism.

In the no care group, all clutches were attacked by predators, and six disappeared entirely during the experiment. We estimate that at least 18 predation events occurred in the clutches, 11 during nighttime and seven during daytime. The median number of eggs consumed at each predation event was 9 (range = 1–35 eggs), resulting in 87% of egg loss at the end of the 4-day period (Fig. 2a). We observed predation events on 12 occasions: phalangopsid crickets and conspecific males consumed unattended eggs five times each, and an ovigerous conspecific female and a carabid beetle consumed eggs once each. In one unattended clutch, crickets preyed on 32 eggs in just 3 h.

Two models were equally plausible to explain the number of eggs consumed during the experiment (Table S3). In both models, there was an interaction between clutch size and period of the day, so that the number of eggs consumed increased exponentially with clutch size, and this effect was more intense during nighttime (Fig. 2b). In the best ranked model, there was also an additive effect of the experimental group (Table 2), so that the number of eggs consumed was consistently higher in the no care group, regardless of clutch size and period of the day (Fig. 2b). In the second best ranked model ($\Delta\text{AICc} = 0.4$), in turn, there was an interaction between experimental group and period of the day, so that the number of eggs consumed during nighttime was higher in the no care group. This interaction, however, was not significant (data not shown), and we present here only a summary of the best ranked model (Table 2).

Table 1 Results of generalized linear mixed-effects model for the effect of experimental group (care, $n = 8$ vs. no care, $n = 8$) and time (beginning vs. end of the experiment) on the number of eggs per clutch in a parent removal experiment with the harvestman *Phalangodus briareos*. The asterisk indicates interactive effects between variables. The negative binomial dispersion parameter of the model was 2.444 ± 0.888

Variables	$\beta \pm \text{SE}$	z value	P
Intercept (Care, Beginning)	3.296 ± 0.140	23.590	<0.001
Time (End)	-0.021 ± 0.147	-0.150	0.880
Experimental group (No care)	-0.021 ± 0.197	-0.110	0.910
Time * Experimental group	-3.647 ± 0.686	-5.320	<0.001

Discussion

Here, we described maternal egg attendance and tested the protective role of this behaviour in *P. briareos*, an exclusively cave-dwelling harvestman. Under non-experimental conditions, the frequency of egg attendance was high all day long, and

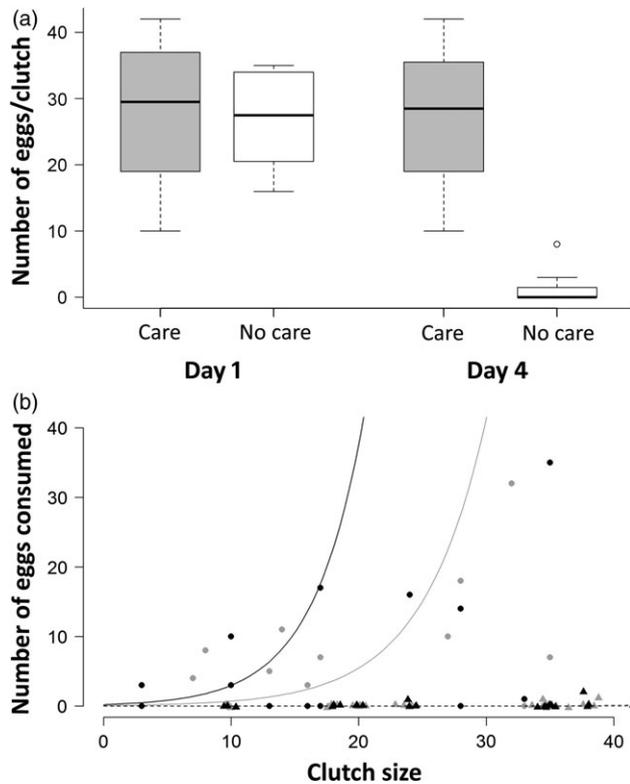


Figure 2 Effect of egg attendance on egg survival in the harvestman *Phalangodus briareos*. (a) Number of eggs in the beginning (day 1) and at the end (day 4) of the experiment for each experimental group: care ($n = 8$) and no care ($n = 8$). In the beginning, there is no difference in the number of eggs between the two groups, but at the end the number of eggs in the no care group is significantly smaller than in the care group (see Table 1). Horizontal lines represent the median, boxes represent the first and third quartile, vertical lines represent the range, and circles represent outliers. (b) The number of eggs consumed depends on clutch size, experimental group and period (daytime vs. nighttime). Circles represent the number of eggs consumed in the no care group, and the continuous lines represent the predicted values for this experimental group. Triangles represent the number of eggs consumed in the care group, and the dashed lines represent the predicted values for this experimental group. Black symbols represent the number of eggs consumed during nighttime, and the black lines represent the predicted values for this period. Grey symbols represent the number of eggs consumed during daytime, and the grey lines represent the predicted values for this period. Note that the grey and black dashed lines are overlapped in the graphic.

egg predation on attended clutches was low. Contrary to our hypothesis, a larger female body size did not provide advantage for defending eggs against predators. However, our field experiment showed that when egg-tending females were absent, eggs suffered intense predation by conspecifics and crickets, which supports our hypothesis that maternal presence improves egg survival. Finally, the number of eggs consumed by predators was positively influenced by clutch size and was higher during nighttime, which supports our hypothesis that

Table 2 Results of the best ranked generalized linear mixed-effects model for the effect of period of the day (daytime vs. nighttime), experimental group (care, $n = 8$ vs. no care, $n = 8$), and clutch size on the number of eggs consumed per clutch in a parent removal experiment with the harvestman *Phalangodus briareos*. The asterisk indicates interactive effects between variables. A complete list of all models is presented in Table S2

Variables	$\beta \pm SE$	z value	P
Intercept (Care, Daytime)	-13.416 ± 2.489	-5.391	<0.001
Period of the day (Nighttime)	0.909 ± 0.478	1.901	0.057
Clutch size	0.202 ± 0.025	8.232	<0.001
Experimental group (No care)	11.077 ± 1.988	5.572	<0.001
Period of the day * Clutch size	0.051 ± 0.019	2.617	0.009

predation is more intense at night, even in a constantly dark habitat. In what follows, we explore how environmental conditions inside caves may influence the reproductive biology of exclusive cave-dwellers.

The harvestman *P. briareos* reproduces all year long, a pattern also found in some arthropods and vertebrates that reproduce inside caves (Howarth, 1980; Poulson, 2001). A recent macroecological study has shown that the length of the breeding season in harvestmen is positively influenced by the number of warm months (i.e., those with mean minimal temperature above 5°C) during the year, and that precipitation plays a secondary positive role in modulating the period adults devote to reproductive activities (Machado *et al.*, 2016). Abiotic conditions inside caves are relatively constant along the year, with temperature similar to local mean annual surface temperature, and humidity near saturation (Howarth, 1980). Therefore, the cave environment offers appropriate conditions for reproduction year-round, so that cave-dwelling arthropods should have longer breeding seasons when compared to their relatives living in the external environment.

Stable abiotic conditions also offer appropriate conditions for the evolution of K-selected traits, including the production of few offspring with large size (Pianka, 1970). In fact, cave species in different taxa usually produce few, but large eggs (Hüppop, 2005). Relative egg size in *P. briareos* is the largest recorded so far among harvestmen with maternal egg attendance (Fig. 3a; Table S3). Regarding clutch size, *P. briareos* females lay a small number of eggs when compared with other harvestman species with maternal egg attendance (Fig. 3b; Table S3), despite the fact they have large bodies (Fig. 3c; Table S3). These findings suggest that *P. briareos* may have evolved K-selected traits that are typical of exclusive cave-dwellers. Based on the data presented in Fig. 3, however, we cannot ascertain whether large eggs and small clutch size in *P. briareos* evolved in response to cave life or are widespread traits in all cranid species, regardless of their habitat type. Thus, a formal test of the hypothesis that relative egg size and clutch size in harvestmen evolved in response to cave life requires analyses controlling for possible phylogenetic effects.

Fecundity has been demonstrated to be related to food intake in many predatory arthropods (Fox & Czesak, 2000). Food inside caves depends mostly on the external environment,

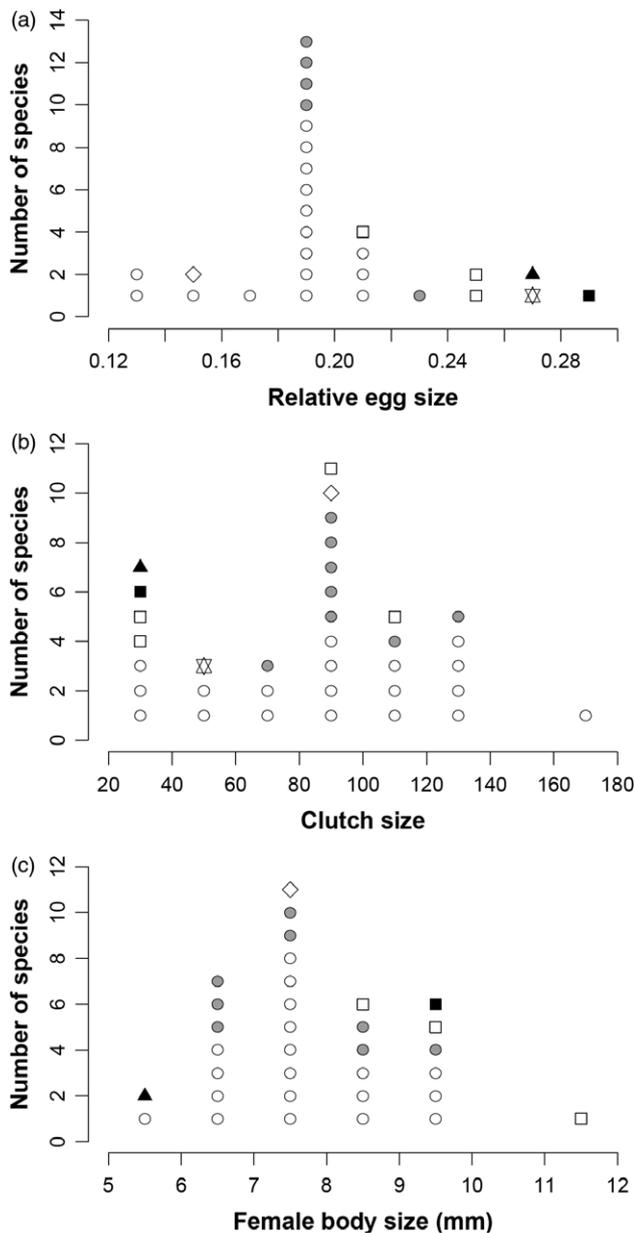


Figure 3 Histograms of (a) relative egg size ($n = 28$ species), i.e. diameter of recently laid eggs divided by female dorsal scute length, (b) clutch size, i.e. the mean number of eggs per clutch ($n = 35$ species), and (c) female body size, i.e. dorsal scute length ($n = 33$ species) for harvestman species with maternal egg attendance (see list in Table S3). Each symbol represents a species and different symbols indicate different families: circles = Gonyleptidae; squares = Cranidae; triangles = Stygnopsidae; diamonds = Cosmetidae; stars = Triaenonychidae. Black symbols represent troglobites, i.e. exclusively cave-dwelling species; grey symbols represent troglaxens, i.e. species that lay eggs inside caves, but forage in the external environment; white symbols represent epigean species, i.e. those that live outside caves. The number of species shown in each panel differs because not all species have complete information on relative egg size, clutch size and female body size.

and it is subject to great seasonal variations (Hervant, 2012). Several harvestmen lay eggs inside caves, but females leave the cave to forage in the external environment (Machado, 2002), where food availability is probably higher. Adults of *P. briareos* spend all their lives inside the cave and females are likely to experience periods of food shortage. Inside caves, therefore, we would expect a great temporal and individual variation in females' nutritional state, leading to a weak relationship between body size and fecundity, as found here for *P. briareos*. Similar information for other cave-dweller arthropods is necessary to test the generality of this pattern.

Large body size in females did not provide advantage for defending the eggs in *P. briareos*, but female presence in the removal experiment greatly reduced egg predation. A recent meta-analysis on the benefits of egg and offspring attendance in terrestrial arthropods has shown that unattended eggs consistently survive less than attended eggs, revealing the importance of the parents in preventing egg mortality imposed by natural enemies (Santos *et al.*, 2017). Intense predation on eggs is one of the so-called 'prime movers' that favour the evolution of parental care in arthropods (Wilson, 1975). Although predation intensity does not explain why egg attendance has evolved in some species, and not in others, it provides an insight into how the benefits of egg attendance vary in different habitats: the higher the predation pressure on eggs, the higher the benefits of egg attendance should be (Santos *et al.*, 2017). In the case of the cave habitat, where food is scarce and the fauna consists mainly of predators and detritivores, the benefits of egg attendance in terms of egg protection should be higher than in external habitats. Do the data obtained here support this prediction?

The experiment conducted here with *P. briareos* lasted 4 days and, during this period, 87% of the eggs in the no care group were consumed by predators. Although there are some studies that experimentally removed parents in harvestmen that reproduce in external environments (Table S1), most of these studies lasted one or 2 weeks, which hampers quantitative comparisons. However, for *Serracutisoma proximum* (Gonyleptidae), whose females care for eggs laid on the vegetation, the authors offer the percentage of egg mortality at 2-day intervals. In this species, the percentage of eggs consumed after 4 days was 42% (Buzatto *et al.*, 2007), indicating that the predation pressure is considerably less intense than that recorded here for *P. briareos*. For other arthropods in which females attend eggs on the vegetation and have no additional line of egg defence (e.g. burrows, mucus or silk coats), such as the chrysomelid beetle *Gonioctena sibirica* and the acanthosomatid bug *Elasmucha dorsalis*, the percentage of eggs consumed exclusively by predators ranged from 42 to 100% after 10 and 15 days of parental removal, respectively (Kudo, Satô & Ôhara, 1989; Kudo & Ishibashi, 1996). Thus, more studies with cavernicolous species are necessary to test whether the benefits of egg attendance are indeed higher in the cave habitat.

There was a positive effect of clutch size on egg predation in *P. briareos*. As far as we known, no previous study with arthropods exhibiting egg attendance has reported this effect. For birds, the Skutch's (1949) hypothesis postulates that provisioning visits increase predation risk because parental activity

alerts predators to nest location. According to this hypothesis, large broods require more provisioning visits, which attract more predators. Contrary to what occurs with birds, in which parents and nestling predators are visually oriented, in the dark cave habitat most species are chemically oriented. This is certainly the case of harvestmen and crickets, which detect food using mostly close-range chemical and/or tactile stimuli (e.g. Lavoie, Helf & Poulson, 2007; Willemart & Chelini, 2007). Considering that conspecifics and crickets, the two most important egg predators reported here for *P. briareos*, are more active during nighttime (pers. obs.), the positive effect of clutch size on the frequency of predator attacks should be high during this period. In fact, this was the pattern we found in the field for experimentally unattended clutches. Thus, we suggest that the larger the clutch size, the greater the amount of chemical cues emitted by the eggs and, consequently, the higher the occurrence of predator attacks in the cave habitat.

In conclusion, we provide evidence on the protective role of egg attendance in a harvestman species in which maternal care has evolved independently from other species studied so far. It is also the first demonstration of the protective role of egg attendance in an exclusively cave-dwelling species. Several features of the reproductive biology of *P. briareos* seem to be associated with cave life, but studies including more species are necessary to shed light on this question. Moreover, female presence seems to be particularly important to deter egg predators in the cave habitat when compared with the external environment. Given that egg attendance is widespread in arachnids and several species live inside caves, the group offers an ideal opportunity to investigate the generality of this pattern and to compare the benefits of egg attendance in a large sample of cavernicolous and epigeal species.

Acknowledgements

We thank John Uribe and Ricardo Prada for helping with fieldwork, Gustavo Requena and Danilo Muniz for helping with statistical analyses, Rodrigo Ferreira and Jesús Cruz-López for sharing photos of harvestmen with maternal care, and Bruno Buzatto, Rogelio Macías-Ordóñez, Gabriele Uhl, and two anonymous reviewers for comments on the manuscript. SGH and GM are supported São Paulo Science Foundation (2012/23135-6, 2015/10448-4) and National Council of Technological and Scientific Development (306550/2014-8).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. (a) Frequency of egg attendance by 14 females of the harvestman *Phalangodus briareos* inspected during four consecutive days. (b) Percentage of temporary desertion of the same egg-tending females during daytime and nighttime.

Table S1. Arachnid species for which the benefits of egg attendance in terms of protection against natural enemies have been experimentally tested in the field.

Table S2. List of all harvestman species with maternal egg attendance. The table includes data on habitat, mean clutch size, mean female body size, and relative egg size.

Table S3. Summary of the models built to investigate the factors influencing the number of eggs consumed in a parent removal experiment with the harvestman *Phalangodus briareos*.