SHORT COMMUNICATION

Harvestmen are fearful in the light but not the darkness

Júlio M.G. Segovia[1,2], **Gabriel P. Murayama**[1,2] and **Nathalia G. Ximenes**[1,2]: [1]Laboratório de Ecologia Sensorial e Comportamento de Artrópodes, Escola de Artes, Ciências e Humanidades da Universidade de São Paulo, São Paulo, Brazil; [2]Programa de Pós-Graduação em Zoologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil. E-mail: juliodesegovia@gmail.com

Abstract. The defenses of harvestmen encompass nipping with spines, releasing of chemicals, fleeing, bobbing, crypsis, and death feigning. Animals are expected to invest more in death feigning in situations in which they are more vulnerable to predation. For prey species attacked by visually-oriented predators, death feigning should be more frequent in daytime than during nighttime. Here, we used the harvestman *Mischonyx cuspidatus* (Roewer, 1913) (Gonyleptidae) to test whether: (i) individuals perform death feigning during daytime more often than during nighttime; (ii) individual body condition influences the time and frequency of death feigning. We found support for the former prediction, but not for the latter. Our results are in accordance with the hypothesis that among harvestman death feigning would be an advantageous passive defense against visually oriented predators. In this study, for the first time, we demonstrate that harvestmen show differences in death feigning behavior depending on light conditions.

Keywords: Death feigning, defense, Opiliones, thanatosis, tonic immobility.

The "life-dinner" principle postulates that prey are under stronger selective pressure than predators (Dawkins & Krebs 1979). From the perspective of a predator, failing when interacting with potential prey means no more than losing a meal. In turn, from the perspective of a prey, failing in an interaction with a predator usually means losing its life and ultimately losing any future reproductive opportunities. Consequently, in the evolutionary arms-race between predators and prey, prey are expected to be one step ahead of predators (Dawkins & Krebs 1979). As a result, current prey species have many different defensive strategies (Edmunds 1974).

The defensive strategies described for arachnids of the order Opiliones include anachoresis, aposematism, crypsis, mimicry (Gnaspini & Hara 2007), and a hard integument (e.g., Segovia et al. 2015a and references therein). Moreover, after noticing the presence of a predator, harvestmen may actively counter attack by pinching with sharp apophyses on their fourth pair of legs, pedipalps, and chelicerae (e.g., Eisner et al. 2004; Segovia et al. 2015b; Silva et al. 2018) and/or releasing defensive secretions (e.g., Nazareth & Machado 2015; Segovia et al. 2015c). In many harvestman species, however, individuals try to avoid an attack by running away (e.g., Segovia et al. 2015b) or performing passive defenses, such as freezing (e.g., Chelini et al. 2009) and feigning death (e.g., Segovia et al. 2019).

The efficacy of death feigning is related to how prone potential predators are to strike prey when they are moving (Edmunds 1974). Prey performing death feigning could be difficult to find. For example, a harvestman's flexed-leg position may distort its body shape, potentially making it more difficult for predators to find (see Segovia et al. 2019). Death feigning is also expected to evolve in gregarious species because while one individual feigns death, the predators could turn their attention and attack other active conspecifics, which are usually close in gregarious species (Ruxton et al. 2018; see also Miyatake et al. 2009). Regardless of the reasons why predators attack death-feigning prey less often, this defensive behavior is expected to be exhibited more frequently when prey individuals are more vulnerable to predation, such as when prey have a lower probability of escaping and a higher probability of being detected by a predator. Characteristics of both individuals and their environments can affect the probabilities of escape and detection. For example, larger neonate snakes, which have a higher capability of locomotion, spent less time feigning death than

smaller neonates (Gerald 2008; but see Hozumi & Miyatake 2005). Additionally, there is empirical evidence showing that some terrestrial arthropods exhibit lower frequency and spend less time feigning death during nighttime than during daytime, probably because death feigning is more effective against visually-oriented predators (Miyatake 2001; Jones et al. 2011; Watts et al. 2014). However, whether death-feigning behavior varies with individual size and daily cycles of light conditions across the diverse taxa exhibiting such behavior remains unknown.

In this study we tested whether the death feigning behavior of the harvestman *Mischonyx cuspidatus* (Roewer, 1913) (Gonyleptidae) is influenced by time of day. We predicted that individuals would feign death more often during daytime as opposed to nighttime, because during daytime they would be more exposed to visually-oriented predators, such as birds and lizards. We also tested whether size influences death feigning behavior in *M. cuspidatus*. We predicted that smaller individuals would feign death more frequently and for a longer time than their larger counterparts because larger individuals could potentially have an improved capability to escape from predators by fleeing.

Individuals of *M. cuspidatus* are active mostly at night (Pereira et al. 2004). During the day, they usually can be found resting beneath dead tree trunks and rocks (Segovia et al. 2015a; pers. obs.). After being threatened, *M. cuspidatus* can use chemical defense, retaliation, freezing, and death feigning (Segovia et al. 2019). We collected 47 females of *M. cuspidatus* at Parque Ecológico do Tietê, São Paulo City, São Paulo State, Brazil. We housed them individually in plastic containers (base: 13.5 × 9.5 cm; height: 4.5 cm), with a damp sheet of filter paper as substrate. Each container had a moistened cotton ball inside a bottle cap to maintain humidity. Before starting the experiments, conducted in September of 2018, we fed the individuals with dog food (Pedigree Vital Pro ®). We kept the individuals under a natural dark/light cycle (approximately 12:12).

To test the prediction that individuals of *M. cuspidatus* perform death feigning more often during daytime, we used a repeated measures design. Each individual was tested twice, once during daylight hours (between 12–3 pm) and once after dark (6–9 pm). Approximately half of the individuals were tested first during day light, then after dark hours, and vice-versa. To induce death feigning, one experimenter held each individual with hands by its right fourth

Table 1.—Model selection results for a Generalized Linear Mixed-Effects Model (GLMM; binomial distribution) and a Generalized Linear Models (GLM). The GLMM was conducted for death feigning, performed by harvestmen during daytime and nighttime, considering individual size (dorsal scute length). Individuals were considered as random effects in all models. The GLM computed the influence of dorsal scute length on the time spent performing death feigning during daytime trials. A log transformation was applied to the GLM. The most parsimonious models are bolded.

Model	df	AIC	ΔAIC	Deviance	AIC weights
GLMM:					
Death feigning ~ time of the day + (1|ID)	**3**	**74.5441**	**0**	**68.5441**	**0.6192**
Death feigning ~ time of the day + length + (1|ID)	4	76.0617	1.5175	68.0617	0.2853
Death feigning ~ time of the day * length + (1|ID)	5	78.0591	3.5150	68.0591	0.1051
Death feigning ~ 1 + (1|ID)	2	89.7700	15.2259	85.7700	0.0003
Death feigning ~ length + (1|ID)	3	91.3649	16.8208	85.3649	0.0001
GLM:					
log10 (Death feigning duration + 1) ~ length	**3**	**18.3200**	**0**	**2.0230**	**1.0000**
log10 (Death feigning duration + 1) ~ 1	2	187.8712	169.5511	91740	<0.0001

leg femur and released them from a height of approximately 20 cm above the substrate of their individual containers (following the protocol of Segovia et al. 2019). We considered that the individual performed death feigning when the harvestman exhibited a rigid posture with at least 7 of its legs flexed and close to the body after falling on the substrate (see Segovia et al. 2019). We defined the end of death feigning as the moment when the harvestman extended all legs. We considered the individuals that run after being released as nonresponsive.

We video recorded each trial using a Sony Handycam HDXR550V and the night shot mode for the trials performed at dark hours. We kept a weak red light (15 watts) in the room 24 hours per day, allowing us to conduct nocturnal trials without disturbing the animals. After the diurnal and nocturnal trials, we took body measures of all individuals. First, we measured body mass using a digital scale (Shimadzu AUX 320). Then, we preserved all individuals in 70% ethanol and measured the dorsal scute length of each individual using a stereomicroscope Leica M205 C with the software Leica Application Suite V3.7.

We compared the frequency of death feigning between day and night trials using a Generalized Linear Mixed-Effects Model (GLMM), with a binomial distribution. Death feigning was the dependent variable, and the full model was composed by the time of the day and length of the dorsal scute. We considered individuals as random effects. We did not include the variable mass in the model, because dorsal scute length and mass were correlated ($R^2 = 0.252$, df = 45, $P > 0.05$). Thus, they could not be used together. To assess if dorsal scute length influenced the duration of death feigning, we conducted a Generalized Linear Model (GLM), using only data from daytime. We applied log10 (x + 1) transformation this model. We used Akaike Information Criterion to select the best model. We performed the analyses in RStudio (version 1.1.453; R core team 2017) using the package 'lme4' (Bates et al. 2015).

For the frequency of death feigning, the model including time of the day, but not dorsal length, had the lowest AIC value (Table 1). The median of time (in seconds) spent feigning death was 73 s (min. = 15 s; max. = 314 s). More individuals performed death feigning during daytime (15 out of 47) than nighttime trials (1 out of 47) (Table 2). This result is consistent with the hypothesis that performing death feigning during the day would be more advantageous than at night, considering it would help to avoid visually-oriented predators that may find harvestmen during the day (Jones et al. 2011). This hypothesis seems to be plausible especially because *M. cuspidatus'* color usually matches their background (considering human vision spectral sensitivity). Therefore, the combination of death feigning and cryptic coloration would make it more difficult to identify the body form of *M. cuspidatus*. Other studies also showed a difference in the duration performing death feigning when comparing between tests conducted during the day and night (beetles: Miyatake 2001; spiders:

Watts et al. 2014). Herein, we could not test if there was a difference in duration of death feigning between day and night in *M. cuspidatus*, since only one individual engaged in death feigning during nighttime. Therefore, it is possible that *M. cuspidatus* is not flexible in terms of controlling the duration of death feigning. That is, they may not have the ability to perform fine adjustments on the time spent feigning death, but rather only being able to show "yes or no" responses. However, this hypothesis needs further investigation.

Considering the time spent performing death feigning, the model explained by dorsal length had a lower AIC value than the null model (Table 1). However, length does not seem to influence the duration of death feigning at daytime trials (Table 2). This finding contrasts with previous studies. For example, it was found that larger neonate snakes spend less time feigning death than the smaller ones. The author suggests that these results might be related to the fact that bigger snakes have better locomotive capabilities and, ultimately, would be better suited to flee from predators than smaller ones (Gerald 2008). Hozumi & Miyatake (2005) found that heavier beetles tend to spend a longer time feigning death than their lighter counterparts. According to the authors, it may be an adaptive strategy, since smaller beetles tend to have shorter life spans. Therefore, for the small beetles, losing a short amount of time may have a greater impact on their fitness. However, we did not find a relationship between size and the time spent feigning death in *M. cuspidatus*. One possible explanation for this result could be that the size variation present among females of *M. cuspidatus* is irrelevant to their ability to flee from a predator. Thus, the size-specific time spent feigning death might not have been selected in this species. Nonetheless, it remains as a hypothesis to be tested.

Table 2.—Generalized Linear Mixed-Effects Models (binomial distribution) and Generalized Linear Models results. The GLMM, first model, assessed death feigning in harvestmen, considering time of the day and individuals included in models as random effects. The GLM, second model, was log10 (x + 1) transformed and assessed the duration of death feigning performed by harvestmen during the daytime, considering the influence of size (dorsal scute length).

	Estimates	S.E.
Death feigning ~ time of the day + (1|individuals)		
Intercept (daytime)	-0.7577	0.3129
Nighttime	-3.0710	1.0581
log10 (Death feigning duration + 1) ~ length		
Intercept	3.0500	2.0790
Length	-6.4230	10.4750

Finally, we showed that *M. cuspidatus* feign death more often in daylight than in the nighttime. It is in accordance with the hypothesis that this behavior would have been selected by the predatory pressure imposed by visually-oriented diurnal predators. This work includes harvestmen in the list of animals that show differences on death feigning behavior depending on the light conditions.

ACKNOWLEDGMENTS

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