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Cuidado maternal no opilião *Neosadocus maximus*
(Arachnida: Opiliones)

Foto: André Benedito

SÃO PAULO

2011

MARIE CLAIRE CHELINI

**Cuidado maternal no opilião *Neosadocus maximus*
(Arachnida: Opiliones)**

Dissertação apresentada ao Instituto de
Biociências da Universidade de São Paulo,
como parte dos requisitos para obtenção
do título de Mestre em Ciências na área
“Ecologia”.

Orientador: Glauco Machado

SÃO PAULO

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Are these humans the best thing in this savage garden, warring as they have done so long upon one another? Or are they simply an undifferentiated part of it, no more complex ultimately than the crawling centipede or the slinky satin-skinned jaguar or the silent big-eyed frog so very toxic that one touch of his spotted back brings certain death?

[...]

It doesn't matter.

We can't stop ourselves from making beauty. We can't stop the world.

Lestat de Lioncourt

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RESUMO

Muitas formas de cuidado parental aumentam a sobrevivência da prole, ao custo de uma diminuição na capacidade dos pais em investir em proles futuras. Espera-se, portanto, que indivíduos parentais adotem estratégias de cuidado que lhes permitam balancear benefícios imediatos e custos futuros, otimizando seu sucesso reprodutivo total. Nesta dissertação, testamos um potencial custo e um benefício do cuidado maternal do opilião *Neosadocus maximus*, espécie cujas fêmeas desertam suas desovas periodicamente e acrescentam ovos a elas por um período de até duas semanas – características únicas entre opiliões com cuidado maternal. Exploramos também quais fatores permitem a deserção temporária das fêmeas de *N. maximus*. Mais especificamente, testamos as seguintes hipóteses: (1) o cuidado maternal protege os ovos contra predadores, que são mais comuns no período noturno; (2) o cuidado maternal diminui a fecundidade imediata da fêmea; (3) a camada de muco que as fêmeas depositam sobre seus ovos mantém a prole protegida nos momentos de deserção temporária. Nossos resultados apontam que o cuidado maternal é uma proteção eficaz contra o ataque de predadores, sem afetar negativamente a fecundidade imediata das fêmeas. Demonstramos também que a camada de muco mantém os ovos relativamente protegidos na ausência da fêmea guardiã, permitindo que estas se ausentem de suas desovas sem deixar a prole totalmente vulnerável. Sendo assim, a camada de muco que recobre os ovos de *N. maximus* permite que estas fêmeas minimizem os custos fisiológicos associados ao cuidado maternal sem, entretanto, aumentar os custos da deserção em termos de redução de prole provocada por predação.

Palavras-chaves: Deserção temporária, esforço parental; fecundidade; investimento parental; proteção de ovos; reprodução.

ABSTRACT

Many forms of parental care increase offspring survival, at the cost of a decrease in the parental individual's capacity to invest in a future brood. It is expected, therefore, that parental individuals adopt parental strategies that allow them to balance immediate benefits and future costs, optimizing their total reproductive success. In this thesis, we aimed to identify costs and benefits of maternal care in the harvestman *Neosadocus maximus*, whose females periodically desert their broods and add eggs to their clutches for up to two weeks – unique characteristics among harvestmen with maternal care. We also explored which factors allow *N. maximus* females to desert their clutches frequently. We tested the following hypothesis: (1) maternal care protects the eggs against predators, especially at night; (2) maternal care decreases the current female fecundity; (3) the mucus coat covering the eggs protects them against predators even in the absence of the guarding female. Our results indicate that maternal care is an effective protection against egg predators, and does not decrease current female fecundity. We also demonstrated that the mucus coat provides effective protection to the eggs in the absence of the guarding female, allowing them to abandon periodically their clutches without leaving the offspring completely vulnerable to predators. The mucus coat covering *N. maximus* eggs allows these females to minimize the physiological costs of caring, with no severe increase in the cost of deserting in terms of brood reduction by predation.

Key-words: Fecundity; egg protection; maternal effort; parental investment; reproduction, temporary desertion

INTRODUÇÃO GERAL

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Cuidado parental

Cuidado parental é qualquer comportamento exibido por um ou ambos os indivíduos parentais que aumente a sobrevivência e o sucesso reprodutivo da prole (Clutton-Brock, 1991). Mesmo formas simples de cuidado parental, tais como a proteção física dos ovos contra predadores e parasitóides (Tallamy & Wood, 1986), envolvem custos, que são invariavelmente pagos por um ou ambos os pais. De fato, ao cuidar da prole, um indivíduo parental pode diminuir suas chances de copular novamente em uma mesma estação reprodutiva, assim como reduzir sua fecundidade futura e/ou sua longevidade (Clutton-Brock, 1991). Portanto, espera-se que, em espécies iteropáricas, os indivíduos parentais otimizem seu sucesso reprodutivo total adotando estratégias de cuidado que permitam que o investimento em uma prole atual não comprometa o investimento em proles futuras (Gross, 2005).

De forma geral, os custos do cuidado maternal podem ser divididos em duas categorias: (1) custos ecológicos, quando o cuidado à prole expõe a fêmea a riscos aos quais ela não estaria exposta em outras condições e (2) custos fisiológicos, quando a fêmea aloca uma parte importante da energia normalmente investida em seu próprio organismo na produção de ovos e nas atividades parentais (Tallamy & Denno, 1982; Clutton-Brock, 1991). A guarda dos ovos apresenta como custo ecológico mais comum uma maior exposição da fêmea a predadores, seja por ter sua mobilidade reduzida (como nas espécies que carregam os ovos atados ao corpo, veja Reguera & Gomendio, 1999) ou por aumentar as chances de serem atacadas durante a defesa ativa dos ovos (Tallamy & Wood, 1986). O custo fisiológico geralmente associado à guarda de ovos é uma diminuição no tempo de forrageio da fêmea, o

que leva a uma menor disponibilidade de energia para a manutenção de seu organismo e para a produção de novos ovos (Clutton-Brock, 1991; Tallamy & Brown, 1999). Dessa forma, quanto maiores o tempo e a energia investidos por uma fêmea em uma determinada prole, menor sua fecundidade futura (Tallamy & Denno, 1982; Buzatto *et al.*, 2007). Essa diminuição da fecundidade será adaptativa somente se o cuidado maternal aumentar o número de jovens a atingirem a idade reprodutiva e/ou a rapidez com a qual esta maturidade será atingida (Tallamy & Wood, 1986).

Os custos ecológicos e fisiológicos do cuidado maternal podem ser maiores ou menores em diferentes espécies, populações ou até mesmo indivíduos, visto que estão estreitamente relacionados a variáveis ambientais (tais como disponibilidade de alimento e pressão de predação), que não se mantêm constantes no tempo e no espaço (Scott & Traniello, 1990). Sendo assim, fêmeas podem adotar estratégias condicionais, em que a dedicação à prole (em tempo e energia) varia de acordo com as condições ambientais de seu habitat durante o período de maturação dos ovos. Zink (2003), por exemplo, sugere que fêmeas de *Publilia concava* (Hemiptera: Membracidae) optem por guardar sua prole ou abandoná-la em função das condições ambientais nas proximidades de sua desova, tais como a disponibilidade de alimento, abrigo contra predadores e presença de formigas, que adotam e protegem ativamente os ovos desta espécie. De modo geral, entretanto, espera-se que a guarda de ovos, assim como qualquer forma de cuidado maternal, evolua somente em situações em que seus custos sejam menores do que seus benefícios (Clutton-Brock, 1991). Isso pode acontecer quando o cuidado oferece benefícios extremamente altos comparados a custos moderados, ou benefícios moderados comparados a custos extremamente baixos.

Os benefícios da guarda de ovos em artrópodes já foram quantificados experimentalmente para diversos insetos (e.g. Tallamy & Denno, 1981; Kudo & Ishibashi, 1995; Kudo, 2002; Stegmann & Linsenmair, 2002; Zink, 2003), alguns aracnídeos (e.g. Fink, 1986; Gillespie, 1990; Gundermann *et al.*, 1997; Machado & Oliveira, 1998, 2002; Buzatto *et*

al., 2007) e um crustáceo bromelícola (Diesel, 1992). Em todos esses casos a presença da fêmea aumenta a sobrevivência da prole, constituindo uma proteção efetiva contra predação e/ou parasitoidismo. Os custos do cuidado maternal em espécies de artrópodes, em contrapartida, foram menos estudados. Poucos trabalhos com insetos (e.g. Tallamy & Denno, 1982; Stegmann & Linsenmair, 2002; Zink, 2003) e aracnídeos (e.g. Fink, 1986; Gundermann *et al.* 1997; Buzatto *et al.* 2007) quantificaram experimentalmente os custos da guarda de ovos. Todos eles demonstram, no entanto, que o cuidado maternal influencia negativamente a fecundidade global das fêmeas (*lifetime fecundity*).

A guarda de ovos pelas fêmeas está amplamente difundida entre as espécies da ordem Opiliones, tendo evoluído independentemente sete vezes (revisado por Machado & Macías-Ordóñez, 2007). Entretanto, os seus benefícios foram testados experimentalmente em apenas três espécies, todas da família Gonyleptidae: *Acutisoma longipes* (Machado & Oliveira, 1998), *Serracutisoma proximum* (Buzatto *et al.*, 2007) e *Bourguyia trochanteralis* (Machado & Oliveira, 2002). Nessas três espécies demonstrou-se que a presença materna é crucial para a proteção dos ovos contra predadores. Todas elas apresentam estratégias de cuidado semelhantes: a fêmea ovipõe todo o conjunto de ovos de que irá cuidar em um intervalo curto de tempo (1 a 5 dias) e permanece sobre eles não somente até a sua eclosão, mas até a dispersão das ninfas de 1º estágio. Durante este período, as fêmeas não abandonam a desova em momento algum e, portanto, não se alimentam. Esta supressão total do forrageio e imobilidade prolongada podem gerar custos ecológicos e fisiológicos, reduzindo a disponibilidade de energia para a manutenção de seu organismo e para a produção de novos gametas. O único trabalho que investigou custos do cuidado maternal em opiliões usou *S. proximum* como modelo de estudo (Buzatto *et al.*, 2007) e demonstrou que a guarda dos ovos diminui a fecundidade global das fêmeas, porém aumenta seu sucesso reprodutivo total. Isso se deve ao fato de que, nessa espécie, desovas deixadas sem proteção são rapidamente

consumidas por predadores, tornando extremamente baixo o sucesso reprodutivo de uma fêmea que opte por não cuidar dos ovos.

Modelo de estudo

Neosadocus maximus (Gonyleptidae: Gonyleptinae) é uma espécie de opilião que ocorre na Mata Atlântica do sul do Estado de São Paulo (Kury, 2003). Os indivíduos da espécie podem ser encontrados sobre a vegetação e no solo às margens das trilhas próximas a riachos de interior de mata. O período de atividade é predominantemente noturno e os indivíduos se alimentam de pequenos artrópodes (tanto vivos quanto mortos), moluscos e frutos frescos e em decomposição (Willemart *et al.*, 2007). As fêmeas desovam na superfície abaxial de folhas em vegetação rasteira às margens de riachos durante a estação chuvosa (outubro e março) e oferecem cuidado parental aos ovos e ninfas recém-eclodidas. Entretanto, ao contrário de outras espécies de opiliões com cuidado maternal (veja Machado & Macías-Ordóñez, 2007), as fêmeas não ficam sobre os ovos durante todo o período de desenvolvimento embrionário. A frequência de cuidado parece ser maior de noite do que de dia e, nos períodos em que as fêmeas não estão sobre a prole durante o dia, elas podem ser encontradas abrigadas em cavidades naturais no chão, distantes entre 20 e 100 cm da planta em que se encontra a desova. A maior presença das fêmeas sobre suas desovas no período noturno pode estar relacionada a diferenças na pressão de predação exercida sobre os ovos durante o dia e a noite.

Nos períodos em que as fêmeas não estão sobre a prole durante a noite, elas podem ser vistas forrageando no chão. Portanto, ao se ausentarem de suas desovas, fêmeas de *N. maximus* podem estar forrageando a fim de obter recursos energéticos adicionais. Esses recursos energéticos podem permitir que fêmeas que forrageiam mais depositem um maior número de ovos ao longo da estação reprodutiva, reduzindo os custos do cuidado parental (Clutton-Brock, 1991). Provavelmente, a adição de ovos ao longo de um período maior do que

em outras espécies explica porque são encontradas desovas com ovos em diferentes estágios de desenvolvimento, um padrão similar às desovas de espécies com cuidado paternal (Machado & Macías-Ordóñez, 2007, Requena *et al.*, 2009). Não sabemos, entretanto, quais são os custos e quais são os benefícios proporcionados por essa estratégia de cuidado. Não sabemos também se a camada de muco depositada pelas fêmeas ao redor dos ovos após a oviposição possui uma função defensiva contra predadores, assim como foi recentemente documentado para o opilião *Iporangaia pustulosa*, cujos machos cuidam da prole (Requena *et al.*, 2009).

Objetivos gerais

Esta dissertação teve como objetivo geral estudar a forma peculiar de cuidado maternal apresentada pelo opilião *N. maximus*. O trabalho está dividido em dois capítulos redigidos em inglês e em formato de artigos para publicação. No primeiro capítulo, testamos quais são os custos e benefícios associados à deserção temporária das desovas pelas fêmeas de *N. maximus*. Mais especificamente, testamos duas hipóteses: (1) a deserção temporária traz um custo para as fêmeas em termos de uma menor sobrevivência da prole, especialmente durante o período noturno; (2) a deserção temporária traz um benefício para as fêmeas em termos de aumento de fecundidade. Fêmeas que passam menos tempo cuidando de sua prole têm mais oportunidades de forrageio e, conseqüentemente, podem adicionar mais ovos às suas desovas do que fêmeas com menor esforço materno. No segundo capítulo, testamos se o custo de abandonar frequentemente uma desova, em termos de número de ovos perdidos por predação, pode ser amenizado pela camada de muco que as fêmeas depositam sobre seus ovos no momento da oviposição. Este capítulo, portanto, complementa as informações comportamentais apresentadas no primeiro capítulo sobre custos e benefícios da deserção temporária. Na Conclusão Geral, integramos as informações apresentadas nos dois capítulos e discutimos as implicações gerais de todos os resultados obtidos.

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CAPÍTULO 1

CAPÍTULO 1

CUSTOS E BENEFÍCIOS DA DESERÇÃO TEMPORÁRIA DA PROLE NO OPILIÃO

NEOSADOCUS MAXIMUS (ARACHNIDA, OPILIONES)

Deserção da prole é uma estratégia que indivíduos parentais podem adotar a fim de diminuir os custos do cuidado e otimizar seu sucesso reprodutivo futuro. O opilião *Neosadocus maximus* é um bom organismo modelo para estudar custos e benefícios desta estratégia pois, apresenta deserção temporária da prole e acrescenta ovos à desova por algumas semanas. Testamos as seguintes hipóteses: (a) a deserção temporária apresenta um custo para as fêmeas guardiãs, expondo os ovos a um risco maior de predação; (b) a deserção temporária apresenta um benefício para fêmeas guardiãs, aumentando suas oportunidades de forrageio e, conseqüentemente, sua fecundidade. Com observações comportamentais seguidas de análises de GLMMs, mostramos que fêmeas com maior frequência de deserção da prole perdem mais ovos por predação. Em contrapartida, deserções mais frequentes não implicam em uma fecundidade maior, medida como o número de ovos acrescentados à desova atual. Se a deserção temporária da prole não se reverte em uma fecundidade maior da fêmea, por que as fêmeas de *N. maximus* deixam seus ovos expostos a predadores com tanta frequência durante o dia? Sugerimos que as fêmeas guardiãs ajustam seu esforço materno em resposta ao risco de predação, que é mais elevado de noite. Uma explicação não-excludente seria que as fêmeas são obrigadas a desertar temporariamente suas desovas para amenizar os custos fisiológicos do cuidado parental durante o período diurno. A camada de muco que as fêmeas de *N. maximus* depositam sobre seus ovos pode ser uma importante proteção física contra predadores nos períodos em que a fêmea guardiã deserta sua prole.

**Costs and benefits of temporary brood desertion in a Neotropical harvestman
(Arachnida: Opiliones)**

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Running title: *Costs and benefits of temporary brood desertion*

Abstract

Brood desertion is a life-history strategy that parental individuals may adopt to minimize the caring costs and optimize their future reproductive success. *Neosadocus maximus* is a good model organism to study costs and benefits of this strategy since females abandon their clutches periodically and keep adding eggs to their clutches for some weeks. We tested two hypotheses derived from parental care theory: (a) temporary brood desertion presents a cost to guarding females, exposing the offspring to increased mortality; (b) temporary brood desertion presents a benefit to guarding females, increasing their foraging opportunities and, consequently, their fecundity. With behavioral observations followed by GLMM analysis, we showed that females with higher frequency of brood desertion suffer from higher egg mortality. Contrary to our second hypothesis, an increased frequency of brood desertion does not imply in higher fecundity, measured as the number of eggs added to the current clutch. If brood desertion does not increase fecundity, why would *N. maximus* females leave their eggs exposed to predators so frequently during the day? We suggest that guarding females adjust their maternal effort to the predation risk, which is higher at night. A non exclusive explanation is that females are constrained to desert their brood in order to attenuate physiological costs of parental care during daylight hours. The mucus coat deposited by females after oviposition may be important in providing physical protection against predators during temporary brood desertion.

Key-words: Egg protection; fecundity; brood success, brood size, maternal care; parental effort.

Introduction

Parental individuals are expected to maximize their fitness adjusting their expenditure on parental care in relation to two main components: the benefits provided by the increased survival of the current brood and the costs paid in terms of reduction of subsequent reproductive success (Trivers, 1972; Winkler, 1987; Gross, 2005). Theoretical models generally assume that parental effort increases the reproductive success related to the current brood, but also decreases the subsequent reproductive success of parental individuals (reviewed in Stiver & Alonzo, 2010). On the other hand, the allocation of resources for future reproductive events may increase the parents' subsequent reproductive success at the expense of the current brood's success (Clutton-Brock, 1991). Experimental evidence for species exhibiting post-zygotic maternal care has indeed shown that whereas offspring are clearly benefited from protection and provisioning, maternal care usually compromises females' future fecundity (e.g. Altmann & Samuels, 1992; Balshine-Earn, 1995; Hanssen *et al.*, 2005; Buzatto *et al.*, 2007; but see Stiver & Alonzo, 2009 and Gilbert *et al.*, 2010).

Females of several species developed strategies that allow them to avoid the costs of post-zygotic parental care, without leaving the eggs completely vulnerable to predators. These strategies include, for example, egg dumping in the nests of conspecific caring females (e.g. Zink, 2005; Tallamy, 2005), egg hiding (e.g. Tallamy & Schaefer, 1997; Machado & Raimundo, 2001), egg coating with protective substances (e.g. Ang *et al.*, 2008) or even leaving the eggs under the male's protection (e.g. Tallamy, 2000). Brood desertion, be it temporary or permanent, is a life-history strategy that individuals exhibiting post-zygotic parental care may adopt to minimize the caring costs and optimize their future reproductive success (e.g. Balshine-Earn, 1995; Székely & Cuthill, 2000;

Ackerman & Eadie, 2003). The net benefits of desertion depends on the parent's ability to raise another brood in the same reproductive season, on the costs of raising broods to the parent's residual reproductive value, and on the reproductive value of the current brood (Stiver & Alonzo, 2009). The reproductive value of a brood, in turn, may depend on the number of young, their age, and their survival prospects (Trivers, 1972; Clutton-Brock, 1991).

Post-zygotic maternal care is widely distributed among arthropods (Costa, 2006), with many described cases in arachnids of the order Opiliones (Machado & Macías-Ordóñez, 2007). In all harvestman species studied so far, guarding females remain close to their clutches during the entire embryonic development of the eggs, also extending the caring period to the hatched nymphs until they disperse (reviewed in Machado & Macías-Ordóñez, 2007). The benefits derived from maternal care are restricted to offspring defense against egg predators, whereas the only detected cost is a marked reduction in female lifetime fecundity (Buzatto *et al.*, 2007). Unlike other species of harvestmen with maternal care, females of *Neosadocus maximus* (Gonyleptidae) abandon their clutches periodically. Moreover, females also do not deposit all eggs simultaneously and keep adding eggs to their clutches for up to two weeks, another unique behavioral trait among harvestman species with maternal care (but see Buzatto *et al.*, 2011).

Given the general features of the maternal behavior in *N. maximus*, the species is a good model system to understand the costs and benefits of temporary brood desertion in arthropods. Since female presence is important to deter egg predators (e.g. Machado & Oliveira, 1998, 2002; Buzatto *et al.*, 2007), we hypothesize that egg mortality is inversely influenced by the caring frequency. In this way, brood desertion would represent a cost both for the offspring, which is more exposed to mortality, and for the guarding female,

which has her reproductive success reduced by offspring predation. We also hypothesize that the number of eggs added to the current clutch is inversely influenced by the caring frequency because harvestman females do not feed while guarding their eggs (Machado & Macías-Ordóñez, 2007). Therefore, brood desertion would represent a benefit to guarding females, increasing their foraging opportunities and the amount of food that can be converted in eggs (Wheeler, 1996).

Methods

Study species

Neosadocus maximus is a large-bodied Neotropical harvestman whose females care for their eggs until all nymphs have hatched and dispersed, comprising a caring period of nearly 40 days (Fig. 1). However, the time spent by individual females guarding their eggs, is extremely variable, varying from 2 to 98% of the embryonic development period (Fig. 2A). When not caring for their eggs, females may be seen walking on the ground up to 1.5 m away from their eggs (n = 62), hidden in natural cavities or under dead leaves close (up to 50 cm) to their clutches (n = 35) or eating at the vicinity of their clutches (n = 6). Most of the times, though, females are away from their clutches and we are unable to find them because they easily hide on the vegetation or among the leaf litter. Female lays her eggs asynchronously, so that the current clutch contains eggs in several stages of embryonic development coming from different oviposition events (Fig. 1). Moreover, females cover their eggs with a hygroscopic mucus coat (Fig. 1), similar to that described for the harvestman *Iporangaia pustulosa*, whose males care for the eggs (Requena *et al.*, 2009).

Behavioral observations

We conducted this study at Intervalles State Park (24°14'S, 48°04'W), a large Atlantic Forest fragment in the state of São Paulo, southeastern Brazil. This region has a subtropical weather, with a cold–dry season from April to September and a warm–wet season from October to March, which corresponds to the reproductive season of *N. maximus*. We gathered our data in seven field trips regularly spread between October 2009 and February 2010. We conducted our behavioral observations along a 450 m long transect flanked by abundant vegetation and near a stream. This specific site was chosen among several tracks due to the relatively high density of *N. maximus* individuals, especially egg-guarding females. We individually marked all guarding females with enamel paint, and followed them throughout the entire reproductive season.

In each field trip we monitored each clutch hourly, in daily 8 h long observation rounds. We alternated these observation rounds between mornings (0600 to 1400 h), afternoons (1400 to 2200 h) and nights (2200 to 0600 h). Following this scheme, we completed 24 hourly checks on each clutch after three days. Each field trip lasted between eight and 14 days, during which we obtained two to four full 24 h sets of observations. For analytical purposes, we divided each set of 24 hourly checks into two blocks composed of 12 checks each. We considered that all hourly checks made between 0700 and 1800 h were diurnal observations and those made between 1900 and 0600 h were nocturnal observations. In each one of the hourly checks, we registered the position of the guarding female (when she was visible) and her distance to her clutch. We considered that a female was caring for her eggs when she was on the leaf supporting the clutch or up to 5 cm from this leaf. We calculated then the caring frequency during the day and night for each female dividing the number of times that we saw her caring for her eggs in each period by 12.

Throughout the study period, we found a total of 52 clutches and included 46 with at least one complete set of 24 hourly observations in our analyses (see below).

To determine how many eggs were consumed and/or added to each clutch during the day and night, we photographed each clutch at 0700 (beginning of the diurnal observations) and at 1900 (beginning of the nocturnal observations). We counted the eggs in all photos using the software Image Tool. Comparing and superposing subsequent images we were able to identify which eggs were added or consumed. Additionally, we classified the clutches in seven age classes based on the stage of embryonic development of the eggs (loosely based on Gnaspini & Lerche, 2010). From younger to older clutches, the ages were (Fig. 1B): (1) clutches containing only recently laid eggs, which are uniformly white; (2) clutches containing at least some cream colored eggs and with the germ band clearly distinguishable; (3) same as before, but with embryo's eyes visible; (4) same as before, but with intestinal cecum clearly distinguishable; (5) clutches containing at least some grayish eggs with embryos clearly pigmented; (6) clutches with one to 10 eggs already hatched; (7) clutches with more than 10 hatched eggs. We considered these ages as a proxy for the time females had already invested in parental activities and used them as a categorical variable in our analyses.

Predictions and statistical analyses

To investigate the costs (C) of brood desertion, we built four models (plus a null model), associating the number of eggs consumed in each clutch to the female caring frequency and to the period of the day (Table 1). We predicted that (C1) predation pressure on the eggs is higher during the night, when conspecifics and other important egg predators are more active (Buzatto *et al.*, 2007; Cokendolpher & Mitov, 2007; Requena *et*

al., 2009; Chelini & Machado in prep), and that (C2) female presence is an effective protection against egg predation. Regarding the benefits (B) of brood desertion, we built three models (plus a null model) associating the number of eggs added to the clutch to the female caring frequency and to clutch age (Table 1). We predicted that (B1) females that care more will be less able to invest in new eggs due to less foraging opportunities, and that (B2) the number of eggs added to the clutches decreases throughout the caring period because the continuous oviposition would increase the total caring time and, consequently, the costs of maternal care.

We tested all predictions with generalized linear mixed models (GLMMs), using female identity as a random variable. GLMMs are a recently developed statistical tool that provides a more flexible approach for analyzing non-normal data when random effects are present, extending the analytical power of GLMs for analysis of blocked data with count or proportional responses (Bolker *et al.*, 2009). To determine if there was an effect of the female identity on the caring frequency and its effects, we compared a saturated GLM to a saturated GLMM. The best model was the GLMM, allowing us to test the effects of the variables encompassing the variation among females (GLM: AIC = 1295, $k = 6$; GLMM: AIC = 995, $k = 5$). We fitted the data to a Poisson distribution for the models related to the egg mortality and female fecundity, and selected the best of each set of competing models through AIC values comparison, selecting those with the smaller ΔAIC . We considered models with ΔAIC smaller or equal to 2 to be equivalently fitted (Burnham & Anderson, 2002). We ran these analyses with the software R, using the function `glmer` of the `lme4` library package (R Development Core Team, 2009).

Results

Costs of brood desertion: egg mortality

Comparing day and night photographs of the clutches, we identified 68 events of egg predation on 35 clutches – 25 events occurring during the day and 43 occurring at night. Moreover, we witnessed 15 predation events, 10 of which occurred during the day. The number of eggs consumed during the day varied between 1 and 43 and between 1 and 135 at night (Fig. 2B). Our analysis indicates that predation pressure is higher at night, which provides support for our prediction C1. Caring frequency alone did not have an overall influence on the number of eggs consumed. However, the best model indicated that there is an interaction between period of the day and caring frequency (Table 1). This result indicates that maternal care does not influence the number of consumed eggs at night (estimate = -0.12), but has a strong negative influence on the number of eggs consumed during the day (estimate = -8.42), i.e., the higher the caring frequency, the lesser the number of consumed eggs. Our prediction C2, therefore, was corroborated only for the diurnal period, and refuted for the night. Nevertheless, Fig. 2B points that there are several outliers at night, corresponding to massive predation events on individual clutches. To assess the effect of outliers on the results, we re-analyzed our data excluding them (both during the day and at night). The best model was still the one combining the effect of the period and the frequency of caring (Table 1), but this time the caring frequency influences negatively the number of consumed eggs in both periods (estimate = -1.31)

Benefits of brood desertion: increased female fecundity

Thirty two of the 53 females that we followed added eggs to their clutches while caring. Recently laid clutches (age 1) contained from 9 to 295 eggs (mean $\pm$ s.d. =

118.6±68.1 eggs). The number of eggs added in a single day varied between 2 and 73 (mean±s.d. = 3.2±8.2 eggs), and the interval between two oviposition events of the same female varied between one and 14 days. We observed up to eight different oviposition events in a clutch, spaced out by two to three day-intervals. The number of eggs added to a clutch is positively associated with the caring frequency (estimate = 2.32), contrarily to our prediction B1 (Table 1). However, the number of added eggs is negatively associated with the age of the clutch (estimate = -0.18), supporting our prediction B2 (Table 1). The best model describing female fecundity is the one that combines both variables (Table 1).

Discussion

Costs of brood desertion: egg mortality

Our results demonstrate that *N. maximus* females with lower maternal effort (i.e., higher brood desertion) suffer from higher egg mortality, since caring frequency influences negatively the number of eggs consumed in a clutch, especially during the day. Field observations indicate that guarding females are able to repel small-bodied egg predators and, during the day, the most common species observed attacking the clutches were the harvestmen *Promitobates ornatus* (Gonyleptidae) and *Jussara* sp. (Sclerosomatidae), which are considerably smaller than *N. maximus* (Chelini & Machado in prep.). However, females seem to be unable to protect their eggs against predation caused by large-bodied arthropods, such as centipedes (Chilopoda: Scolopendridae) and wetas (Orthoptera: Anostostomatidae), which are active mainly at night (Chelini & Machado in prep.), and were responsible for the four cases of high egg predation identified as outliers (Fig. 2B). When we removed all outliers from our dataset, we found that caring frequency negatively

influences the number of eggs even at night, when the number of attacks is greater and a higher number of eggs is consumed.

Considering that *N. maximus* caring frequency is considerably higher at night, guarding females may be adjusting their maternal effort to the temporal variation in predation pressure. A similar adjustment has already been reported for the membracid *Publilia concava* (Hemiptera), in which females decide whether to care for a brood or abandon it permanently depending on conditions such as food availability, shelter against predators, and presence of tending ants that adopt and actively protect the membracid eggs (Zink, 2003). An experiment with the mouthbrooding cichlid fish *Ctenochromis horei* also demonstrated that females adjust the total time of maternal care to the presence of predators in their close vicinity (Taborsky & Foerster, 2004). These females care longer for their brood when predators are present than when predators are absent. In another cichlid fish, *Cichlasoma nigrofasciatum*, parental individuals decrease their foraging activities and increase their defensive behavior in the presence of a predator (Rangeley & Godin, 1992). These examples and our own data on *N. maximus* provides support for the notion that females are able to adjust their maternal effort to the relative exposure of their offspring to predation risk, which is regarded as the main cost of brood desertion in ectotherms (Clutton-Brock, 1991).

Benefits of brood desertion: increased female fecundity

Contrary to our prediction, an increased frequency of brood desertion in *N. maximus* does not imply in higher fecundity, measured as the number of eggs added to the current clutch. The asynchronous oviposition of this species, therefore, cannot be explained by higher foraging opportunities of guarding females that temporarily abandon their clutches

because females that care the most are also those that add more eggs to their clutches. Both fecundity and caring frequency are likely to be strongly influenced by female nutritional state prior to the each reproductive event, so that the energy acquired before the beginning of oviposition may allow the production of a limited number of eggs – which also explains why the number of eggs added to a clutch decreases throughout the time. Moreover, females in good conditions, which probably have more mature eggs to lay, would then invest as much as possible in the current clutch, while females in bad condition and with few mature eggs, may benefit from or be constrained to more frequent foraging bouts.

It is worth noting, however, that our results do not mean that desertion of the current brood may not have long-term benefits in *N. maximus*. In many species, fecundity benefits of brood desertion are only detected when analyzing female's lifetime reproductive success (see example with harvestmen in Buzatto *et al.*, 2007). Given that *N. maximus* females are iteroparous, live up to three years as adults in the field, and can lay more than one clutch per reproductive season (*pers. obs.*), females with low parental effort in a current clutch could have a greater probability to produce another clutch than females with high parental effort. Although we did not test this hypothesis, we found only five females (less than 10% of the total) producing an additional clutch during the reproductive season. If there was a trade-off between current maternal effort and future fecundity, we would expect a higher number of females producing a second clutch, especially among those exhibiting frequency of brood desertion (Fig. 2A). It seems, therefore, that brood desertion also does not imply in long-term fecundity benefits to *N. maximus* females. In accordance to our findings, data on parental care modes across a phylogeny of 287 insect species indicate that lifetime

fecundity scales differently depending on care mode, being positive under no care and egg-guarding, but negative only under provisioning (Gilbert *et al.*, 2010).

Concluding remarks

If brood desertion does not increase immediate fecundity and if females' ability to raise another brood in the same reproductive season is very low, why would *N. maximus* females leave their eggs exposed to predators so frequently, especially during the day? Above we discussed the possibility that guarding females adjust their maternal effort in response to differences in predation risk throughout the day. Here we propose an alternative, but non exclusive hypothesis according which females would be constrained to brood desertion in order to attenuate physiological costs of parental care, especially during warm days. Indeed, *N. maximus* is known to be particularly photophobic and prefer colder temperatures than the syntopic *Serracutisoma proximum* (Santos, 2003), whose females remain on their clutches all the time (Buzatto *et al.*, 2007). This alternative hypothesis also explains differences in the caring frequency throughout the day because diurnal conditions during the warm season may impose severe physiological stress to *N. maximus*, whose individuals are typically nocturnal, justifying longer and/or more frequent desertions when compared to the night.

Although temporary desertion of *N. maximus* females does impose a cost associated with egg losses, this cost is markedly smaller than for other harvestmen with maternal care — including syntopic species that oviposit in similar microhabitats and whose eggs are exposed to similar predators (see Fig. 2 in Buzatto *et al.*, 2007). We suggest that the mucus coat deposited by *N. maximus* females after oviposition (Fig. 1) may keep their eggs physically protected against predators during temporary brood desertion. Eggs of the

harvestman *Iporangaia pustulosa* are also covered by a hygroscopic mucus coat deposited by females before abandoning them to the males' care (Machado *et al.*, 2004). Using both field and laboratory experiments, Requena *et al.* (2009) demonstrated that this mucus coat hampers egg consumption by potential predators during temporary male desertion, so that *I. pustulosa* clutches are less susceptible to predation than clutches of the syntopic *S. proximum*, whose eggs lack the mucus coat. Although we do not know if the presence of mucus around the eggs is the cause or the consequence of temporary brood desertion in *N. maximus*, the potential physical protection provided by the mucus could attenuate both the costs related to egg loss during female absence, and the physiological costs of maternal care, allowing females to shelter during the day.

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Table 1. Summary of predictor combinations fitted to data on costs and benefits of temporary brood desertion in the harvestman *Neosadocus maximus*. k = number of parameters of the models; M = egg mortality (measured as the number of eggs consumed in a 12 h period); P = period of the day (day or night); CF = caring frequency (measured as the proportion of times a guarding females was found on the clutch in a 24 h period); F = fecundity (measured as the number of eggs added to the clutch in a 24 h period); A = brood age.

Models	Predictions	AIC	K	Δ AIC	AIC weight
Costs of brood desertion: egg mortality (complete dataset)					
M~1	(Null) M is not influenced by P or CF	1644	2	363.35	<0.0001
M~P	M is higher at night	1382	3	101.35	<0.0001
M~CF	M is inversely proportional to CF	1642	3	361.35	<0.0001
M~P+CF	M is inversely proportional to CF and higher at night	1280.7	4	0	0.54
M~CF*P	M is inversely proportional to CF and is influenced by P	1281	4	0.35	0.46
Costs of brood desertion: egg mortality (without outliers)					
M~1	(Null) M is not influenced by P or CF	701.7	2	91.1	<0.0001
M~P	M is higher at night	634.7	2	24.1	<0.0001
M~CF	M is inversely proportional to CF	651.1	3	40.5	<0.0001
M~P+CF	M is inversely proportional to CF and higher at night	630	3	19.4	<0.0001
M~CF*P	M is inversely proportional to CF and is influenced by P	610.6	4	0	0.999933
Benefits of brood desertion: increased female fecundity					
F~1	(Null) F is not influenced by CF or A	1478	2	255	<0.0001
F~CF	F is inversely proportional to CF	1227	3	4	0.12
F~A	F is inversely proportional to A	1475	3	252	<0.0001
F~CF+A	F is influenced b the additive effect of A and CF	1223	4	0	0.88

Figure 1: (A) *Neosadocus maximus* female caring for her eggs on the undersurface of a leaf.

(B) Clutch containing eggs in different stages of embryonic development and recently hatched nymphs. Numbers indicate eggs in each one of the seven age classes and 'N' indicates an early hatched nymph. In both pictures, note the presence of a mucus coat around the eggs.

Figure 2: (A) Caring frequency of the harvestman *Neosadocus maximus*, and (B) number of eggs consumed during the day and the night. Boxes indicate the first and the third quartile, the horizontal lines indicate the median value, and the vertical lines indicate the range. Dots indicate outlier values.

CAPÍTULO 2

CAPÍTULO 2

MÃES AUSENTES OU SUPERPROTETORAS? FÊMEAS COMBINAM DEFESAS FÍSICAS E COMPORTAMENTAIS NA DEFESA DE SEUS OVOS EM UM OPILIÃO COM CUIDADO MATERNAL

Espera-se que defesas não-comportamentais aumentem a sobrevivência da prole em situações em que a pressão de predação exercida sobre a prole é intensa, predadores ou parasitóides especialistas tenham desenvolvido estratégias de contornar a proteção parental ou quando os pais se ausentam com frequência da proximidade de sua prole. Diferentemente de outras espécies de opilião com cuidado maternal, fêmeas de *Neosadocus maximus* desertam periodicamente suas desovas durante o dia e a noite, sem sofrer perdas severas de ovos por predação. Fêmeas desta espécie cobrem seus ovos com uma camada de muco higroscópico que pode mantê-los fisicamente protegidos contra predadores durante os momentos de ausência da fêmea guardiã. Testamos esta hipótese manipulando experimentalmente a camada de muco que envolve os ovos e oferecendo-os a dois potenciais predadores. A camada de muco diminui o número de ovos predados por coespecíficos, sendo ainda mais efetiva contra um predador menor, o opilião *Promitobates ornatus*. Resultados similares foram obtidos com o opilião *Iporangaia pustulosa*, cujos machos guardiões também abandonam com frequência seus ovos previamente recobertos de muco pelas fêmeas. A evolução convergente de uma camada de muco nessas duas espécies pode ser vista como um caráter naturalmente selecionado que aumenta a sobrevivência da prole em espécies com deserção temporária, minimizando os custos do cuidado parental.

Absent or overzealous mothers? Females combine physical and behavioral egg defenses in a harvestman with post-ovipositional maternal care

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Running title: Physical and behavioral egg defenses in harvestmen

Abstract

Non-behavioral offspring defenses are expected to increase offspring survival when brood predation is intense, specialist predators or parasitoids have evolved counterstrategies against maternal behavior, or parents temporarily desert their brood. Unlike other harvestman species with maternal care, females of *Neosadocus maximus* periodically desert their clutches during the day and at night, without severe brood reduction due to egg predation. These females cover their eggs with a hygroscopic mucus coat that may keep their eggs physically protected against predators during temporary brood desertion. We tested this hypothesis experimentally manipulating the presence of mucus around the eggs and offering them to two potential predators. The mucus coat decreased the number of eggs consumed by conspecifics, and was even more effective against a smaller predator, the harvestman *Promitobates ornatus*. Similar results were obtained for the harvestman *Iporangaia pustulosa*, in which caring males also leave the clutches unprotected periodically and females deposit a mucus coat on the eggs. The convergent evolution of a mucus coat on the eggs of these two species may be viewed as a naturally-selected trait that increases the survival of the offspring in species with temporary brood desertion, minimizing the costs of egg caring.

Key words: brood desertion, costs and benefits, *Neosadocus maximus*, Opiliones, parental care.

Introduction

Female reproductive success is strongly influenced by her ability to produce eggs (Trivers, 1972). In species exhibiting post-ovipositional maternal care, this success is also determined by female effectiveness in defending the eggs from natural enemies and sometimes provisioning her offspring before or after eclosion (Clutton-Brock, 1991). While female fecundity depends mostly on the amount of food that is converted in eggs (Wheeler, 1996), offspring protection against predation can be achieved through a great variety of defensive strategies. Although guarding females in many animal groups actively repel egg predators (examples in Clutton-Brock, 1991), some species rely on defensive strategies other than behavioral surveillance to protect the offspring from predation (see Tallamy & Schaeffer, 1997). These strategies range from simply camouflaging the eggs with substrate particles (e.g. Willemart, 2001) to the transference of noxious chemicals to the eggs in order to make them unpalatable to generalist predators (Hilker & Meiners, 2002). Arthropod females may also provide physical barriers that hinder predator access to the embryo, such as a hard extrachorion (Ang *et al.*, 2008) or an abundant mucus coat surrounding the eggs (Requena *et al.*, 2009). Less often, physical defenses may be observed in species already exhibiting post-ovipositional maternal care, so that the offspring is protected against predators by more than one line of defenses. Examples include egg cases attached or not to females' body in cockroaches (e.g. Perry & Nalepa, 2003) and spiders (e.g. Vieira & Romero, 2008) and fecal shields in chrysomelid beetles (e.g. Chaboo, 2007). These additional defenses are expected to be particularly important in increasing offspring survival when predation pressure on eggs is intense, specialist egg predators or parasitoids have evolved counterstrategies against maternal behavior, or females abandon the eggs temporarily.

Harvestmen (Opiliones) were one of the first arthropod groups to invest in post-zygotic parental care (Nazareth & Machado, 2010), with species exhibiting uniparental male and female care (Machado & Macías-Ordóñez, 2007). There are several differences in the

behavioral patterns exhibited by parental individuals of each sex (see discussion in Machado *et al.*, 2004). First, females care for clutches containing eggs in only one stage of embryonic development, while males care for clutches containing eggs in several stages of embryonic development that come from different oviposition events (Fig. 1A). Second, the total time spent by females guarding eggs and newly-hatched nymphs rarely exceeds 60 days, while paternal activities may last up to eight months because females continually add eggs to the clutches. Finally, guarding females remain all the time on their eggs, while guarding males frequently leave their clutches to forage or shelter (Fig. 1C). However, in many harvestman species with paternal care, when males temporarily abandon their clutches, the unattended eggs are still protected by a thick mucus coat that prevents or decrease predation rate (Requena *et al.*, 2009; Fig. 1A). This mucus coat has not been described in any species exhibiting maternal care, and in all species studied so far female presence during the entire brooding period is enough to confer protection against egg predators (e.g. Machado & Oliveira, 1998, 2002; Buzatto *et al.*, 2007).

Neosadocus maximus (Gonyleptidae) is a Neotropical harvestman whose females oviposit on the undersurface of leaves of herbaceous plants and care for their brood until the dispersion of the early hatched nymphs (Fig. 1B). Unlike other harvestman species with maternal care, however, *N. maximus* females do not guard their eggs during the entire period of embryonic development, abandoning their clutches periodically during the day and at night (Fig. 1D). Females also do not deposit all eggs simultaneously and keep adding eggs to their clutches for up to two weeks (Fig. 1B). Female behavior in this species is, therefore, more similar to harvestman species exhibiting paternal care, such as *Iporangaia pustulosa* (Machado *et al.*, 2004; Requena *et al.*, 2009; Fig. 1), than to species exhibiting maternal care. Another striking similarity between *N. maximus* and *I. pustulosa*, which belong to different subfamilies (Kury, 2003), is that females of both species cover their eggs with a hygroscopic mucus coat (Fig. 1A,B). We hypothesize that this mucus coat covering *N. maximus* eggs

provides physical protection against egg predators in the absence of the guarding female, as recently described for *I. pustulosa* (Requena *et al.*, 2009). The independent occurrence of a mucus coat in two harvestman species exhibiting post-zygotic parental care suggests that additional lines of egg defenses are likely to evolve when parental individuals are constrained to temporarily abandon their clutches.

Methods

We conducted our fieldwork at Intervalles State Park (24°14'S, 48°04'W; 800 m above sea level), a large Atlantic Forest fragment in the state of São Paulo, southeastern Brazil. The region's weather is mainly subtropical, with a warm-wet season from October to March and a cold-dry season from April to September. The warm-wet season corresponds to the reproductive season of *N. maximus*, as well as of most harvestman species found in the area (e.g. Gnaspini, 1995; Buzatto *et al.*, 2007; Requena *et al.*, 2009; Zatz *et al.*, 2010). We conducted diurnal and nocturnal rounds of observation in a 450 m long transect flanked by abundant vegetation and near a stream during seven field trips regularly spread between October 2009 and February 2010. These rounds of observation summed up nearly 600 hours of fieldwork, during which we witnessed both diurnal and nocturnal predation events.

Based on preliminary field observations on egg predation, we selected two model predators for our experiment: conspecifics and the harvestman *Promitobates ornatus* (Gonyleptidae), which is very abundant in the study area and is active throughout the day during the warm-wet season (Zatz *et al.*, 2010). We collected 40 individuals of each predator species (20 males and 20 females) far from our transect and brought them to our laboratory. We housed all the collected individuals individually in plastic boxes (17 x 13 cm of base, 11 cm high) with water, leaves for shelter and branches for perching. Climatic conditions in the laboratory were: 21-24 °C, 61-80% air humidity, and a photoperiod of 12 h of light.

Before we began the experiment, we fed all individuals with a small amount of low-protein food (a grain of cooked rice) and allowed them to eat it for 24 h. We then removed all the remaining food and kept the individuals in starvation for five days. After this period, we separated the individuals into two experimental groups composed of 10 females and 10 males of each species: (a) ‘without mucus’, in which we offered to each individual 10 *N. maximus* eggs whose mucus coat had been previously removed by gently rolling them in a clean piece of filter paper; (b) ‘with mucus’, in which we offered to each individual 10 *N. maximus* eggs that were manipulated, but did not have their mucus coat removed. Eggs in both experimental groups were placed on a small clean plastic lid laid on the bottom of each cage. We recorded the number of eggs consumed by each individual once a day during five consecutive days, always around 10 AM. We also conducted *ad libitum* observations at different moments of the day to describe the predatory behavior of the individuals of both experimental groups. After the experiment, we released all the experimental individuals in the field and preserved the remaining eggs for future studies.

To test if the mucus coat has a protective role against both predator species, we used a generalized linear model approach (GLM), adjusting a beta-binomial distribution to control for overdispersion in our data ($\text{mean} \pm \text{sd} = 2.9 \pm 4.2$) (Bolker, 2008). We compared the null model with models including the effect of the experimental group, predator species, and combinations of these two factors (Table 1). We selected the best model through AIC values comparison, selecting the one with the lowest value, unless there were two or more models with ΔAIC smaller or equal to 2. In this case, we considered the models to be equally fitted (Burnham & Anderson, 2002).

Results

We witnessed 15 predation events in the field, 10 of which occurred during the day. The diurnal predators were: (a) one individual of the harvestman *Jussara* sp. (Sclerosomatidae), which was observed preying upon a recently hatched nymph (Fig. 2A); (b) seven individuals of the harvestman *P. ornatus*, including males and females that consumed unprotected eggs (Fig. 2B) and also the mucus coat ($n = 1$); (c) a velvet mite (Trombididae), which was probably consuming only the mucus coat, and (d) a conspecific female (Fig. 2C). The nocturnal predators were: (a) a centipede (Scolopendridae) that ate an entire clutch containing approximately 90 eggs (Fig. 2D); (b) a small non-identified spider; (c) an individual of the weta *Lutosa* sp. (Anostostomatidae), and (d) the same conspecific female that was observed consuming eggs during the day, preying upon the same clutch. This cannibalistic female had her own clutch less than 1 m away from the attacked clutch and preyed upon unprotected eggs while the guarding female was less than 10 cm away, on the ground at the basis of the host plant. Besides the predation events, we also observed *N. maximus* females expelling individuals of *Jussara* sp. ($n = 1$), *P. ornatus* ($n = 3$), and a small non-identified katydid (Tettigoniidae) from their clutches and chasing them away.

In our laboratory experiment, both *N. maximus* and *P. ornatus* ate more eggs without mucus than eggs covered with the mucus coat (Fig. 3). We also observed individuals of both predator species manipulating the mucus coat with their pedipalps, without effectively consuming the eggs. The proportion of eggs consumed was higher for *N. maximus* than for *P. ornatus* in both experimental groups (Fig. 3). Our analysis indicates that the presence of the mucus coat influenced negatively the number of eggs consumed by both predator species (Table 1). The AIC ranking indicates that the two models best fitted to our data are those combining the effects of both predator species and experimental group, but their small ΔAIC does not allow us to determine if these effects are additive or if there is an interaction between them (Table 1).

Discussion

Our field observations indicate that while *N. maximus* females temporarily abandon their clutches, eggs are attacked by several arthropod predators. We witnessed more attacks during the daylight hours, when female assistance is less frequent (Fig. 1D). With few exceptions, however, the number of eggs consumed in each predatory attack was low because some predator species apparently consumed only the mucus coat and did not harm the eggs. Given that all predators that consumed eggs also consumed mucus, and that many individuals consumed only mucus from the clutches, it is unlikely that the mucus contains unpalatable chemical substances. It seems that the mucus is simply a physical barrier that hampers the access of potential predators to the eggs, preventing or decreasing consumption rate so that guarding females may leave their clutches unattended for hours without a significant reduction in egg number. On the other hand, clutches of a syntopic harvestman *Serracutisoma proximum* that also oviposits on the vegetation, but does not cover the eggs with mucus, may be entirely consumed by predators in a few hours if they are left unattended (Buzatto *et al.*, 2007).

The laboratory experiment provides additional support for the fact that the mucus coat of *N. maximus* eggs minimizes predation. The mucus coat decreased the number of eggs consumed by conspecifics, and was even more effective as a protection against the harvestman *P. ornatus* (Fig. 3), which was frequently observed visiting unprotected clutches during the day in the field. For many predatory arthropods, the size of the trophic apparatus has a great influence on the types of prey that can be successfully captured or consumed (Schaefer & Leschen, 1993). Given that individuals of *P. ornatus* have nearly half the size of *N. maximus* and have considerably smaller chelicerae and pedipalps (Fig. 2B,C), the mucus coat may increase manipulation time and make the access to the embryo more difficult, which eventually hinder the predation attack. This is also probably true for other small-bodied predators that are locally abundant on the vegetation, such as the harvestman

Jussara sp., which was only observed consuming an early hatched nymph. By covering their eggs with mucus, *N. maximus* females are then providing an additional line of physical defense to their eggs that is especially important during the daylight hours when females are rarely found guarding their clutches (Fig. 1D).

The results obtained here are very similar to those previously described for the non-phylogenetically related harvestman *I. pustulosa*, in which males care for the eggs (Requena *et al.*, 2009). The convergent evolution of a mucus coat secreted by females of these two harvestman species after oviposition may be viewed as a naturally-selected trait that provides physical protection to the eggs when parental individuals temporarily abandon their clutches. This additional line of protection probably minimizes foraging costs of egg guarding for both parental males and females, allowing them to occasionally feed while caring. Thus, temporary brood desertion for food acquisition could explain why *N. maximus* is the only harvestman species exhibiting maternal care in which females continually add eggs to their clutches. However, we previously demonstrated that caring frequency is positively related to the number of eggs added to the current clutch, suggesting that female nutritional state prior to oviposition has a strong influence on both fecundity and caring frequency (Chelini & Machado, in prep.). We suggest, therefore, that temporary brood desertion in *N. maximus* is a maternal strategy to minimize the physiological costs imposed warm temperatures during the daylight hours, when the dehydration risk is probably higher. In fact, when compared to other syntopic harvestman species, *N. maximus* seems to be particularly vulnerable to high temperatures (Santos, 2003), and during the warm months of the reproductive season, guarding female may be constrained to shelter against unfavorable climatic conditions (Chelini & Machado, in prep.).

Females of the chrysomelid beetle *Cryptocephalus hypochaeridis* also invest in an egg coat that protects their eggs even in the absence of egg guarding behavior (Ang *et al.*, 2008). Laboratory experiments have shown that adding the protective coating was very costly to

females of this species, both in terms of material and energy used. The mass of the hard extrachorion, for instance, is equivalent to half the mass of the egg, and water loss and energy expenditure while coating an egg is equivalent to half what would be lost while laying a further egg (Ang *et al.*, 2008). Even though we did not perform any experiment, we suppose that the costs paid by *N. maximus* females are probably lower than the costs paid by *C. hypochaeridis*. First, the mucus is a highly hygroscopic substance that is deposited in small amount around the eggs just after oviposition (Gnaspini & Lerche, 2010), probably by an accessory gland of the females' reproductive tract. In contact with the air moisture, the mucus absorbs water and grows creating a protective coat. Thus, although the total mass of mucus is probably similar to the mass of the eggs, the initial investment by *N. maximus* females in terms of material is not so high. Second, since egg absorption is a passive process, females probably do not expend energy coating the eggs. Therefore, the mucus coat in harvestmen is likely to be a low-expense non-behavioral maternal strategy that provides an extremely efficient protection to eggs, allowing parental individuals to leave their clutches periodically unattended and minimizes the costs of parental care.

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Table 1. AIC values for the number of eggs consumed (PREDATION) by *Neosadocus maximus* and *Promitobates ornatus* (Species) in a four day long experiment in which the mucus coat that cover *N. maximus* eggs was either removed or left intact (Experimental group).

Model	k	Delta AIC	AIC weight
PREDATION ~ 1	1	16.5	0.0002
PREDATION ~ Experimental group	2	2.4	0.1763
PREDATION ~ Species	2	14.7	0.0004
PREDATION ~ Experimental group + Species	3	0	0.5852
PREDATION ~ Experimental group * Species	3	1.8	0.2379

Figure 1: (A) Male of *Iporangaia pustulosa* (Progonyleptoidellinae) and (B) female of *Neosadocus maximus* (Gonyleptinae) guarding eggs covered by mucus (scale bars = 1 cm). Both species use leaves as oviposition site and their clutches are composed of eggs in different phases of embryonic development indicating that they were not laid at the same time. Dotted lines delimitate recently laid eggs, which are slightly smaller than older eggs, in which it is possible to see leg buttons or the pair of ocelli. Parental individuals of (C) *I. pustulosa* (n = 127) and (D) *N. maximus* (n = 45) temporarily abandon their clutches to forage or shelter. The continuous lines represent the kernel density estimates. Information on caring frequency for *I. pustulosa* was provided by G.S. Requena (unpublished data) and, for *N. maximus*, the data were based on Chelini & Machado (in prep.).

Figure 2: (A) *Neosadocus maximus* nymph being consumed by the harvestman *Jussara* sp. (Sclerosomatidae). (B) *Promitobates ornatus* female preying upon an *N. maximus* egg. (C) *N. maximus* female preying upon a conspecific clutch. (D) *N. maximus* clutch being attacked by a centipede (Scolopendridae). Scale bars = 1 cm.

Figure 3: Mean and standard error of the number of *Neosadocus maximus* eggs consumed by conspecifics and the harvestman *Promitobates ornatus* in a four days long experiment in which the mucus coat that cover the eggs was either removed or left intact.

CONCLUSÃO GERAL

CONCLUSÃO GERAL

Os resultados apresentados no Capítulo 1 demonstram que a deserção temporária de fêmeas de *Neosadocus maximus* apresenta um custo relativo a uma maior perda de ovos por predação e nenhum benefício associado à fecundidade das fêmeas. Em relação aos custos da deserção, observamos que fêmeas que abandonaram suas desovas com maior frequência foram aquelas cujas desovas foram mais predadas. A eficácia da proteção materna está associada ao período do dia: fêmeas guardiãs protegem seus ovos contra predadores pequenos com eficácia similar durante o dia e durante a noite, mas não são capazes de remover artrópodes noturnos grandes como lacraias e grilos-rei. O número de ataques e o número de ovos consumidos por ataque são maiores no período noturno, que é também o período em que as fêmeas exibem uma frequência mais elevada de cuidado. O fato de fêmeas de *N. maximus* cuidarem mais de seus ovos no período em que há maior risco de predação sugere que fêmeas guardiãs podem estar ajustando seu esforço materno à variação temporal no risco de predação.

Nossos resultados também indicam que a deserção da prole em *N. maximus* não se reverte em uma maior fecundidade, avaliada como o número de ovos depositado na desova atual. O padrão de oviposição assincrônico dessa espécie, portanto, não pode ser explicado pelas oportunidades de forrageio de fêmeas que frequentemente desertam suas desovas. É possível que tanto a fecundidade quanto a frequência de cuidado sejam fortemente influenciadas pelo estado nutricional das fêmeas anteriormente a cada evento reprodutivo. Fêmeas em boas condições e, conseqüentemente, com um número maior de ovos maduros, investiriam mais tempo cuidando de sua prole, ao passo que fêmeas em pior condição teriam poucos ovos a acrescentar às suas desovas e seriam obrigadas a, ou se beneficiariam mais em abandonar periodicamente suas desovas para forragear.

Em diversas espécies, os benefícios da deserção da prole somente são detectados após a análise da fecundidade da fêmea ao longo de toda sua vida. Apesar dessa possibilidade não ter sido testada nesta dissertação, apenas cinco fêmeas (menos de 10% do total) produziram uma segunda desova ao longo da estação reprodutiva. Caso houvesse um conflito entre esforço materno presente e fecundidade futura, seria esperado que um número maior de fêmeas produzisse uma segunda desova, especialmente entre aquelas com esforço materno baixo. Ao que parece, portanto, o deserção da prole em *N. maximus* também não implica em um benefício de fecundidade a longo prazo. Mas se a deserção não aumenta a fecundidade das fêmeas e apresenta custos em relação à quantidade de ovos predados, porque *N. maximus* abandona seus ovos periodicamente? Sugirerimos que esta deserção temporária beneficie as fêmeas em termos de uma diminuição nos custos fisiológicos do cuidado maternal. Estudos fisiológicos apontam que *N. maximus* é particularmente fotofóbico e prefere temperaturas mais frescas que o opilião sintópico *Serracutisoma proximum*, cujas fêmeas não desertam suas desovas. É possível, portanto, que fêmeas de *N. maximus* sejam obrigadas a desertar suas desovas com frequência para se abrigar de condições adversas, principalmente durante as horas mais quentes do dia.

Apesar da deserção temporária de *N. maximus* apresentar um custo associado à perda de ovos, este custo é menor do que aquele observado em outras espécies sintópicas de opilião com cuidado maternal que também desova em folhas e cujos ovos são atacados por predadores similares. Os resultados que apresentados no Capítulo 2 indicam que os ovos de *N. maximus* são protegidos contra predadores por uma camada de muco que os recobre, sendo relativamente pouco vulneráveis a predadores mesmo na ausência da fêmea guardiã. De fato, em diversos ataques de predadores observados em campo, apenas a camada de muco foi consumida e os ovos permaneceram intactos. Esses dados foram reforçados por um experimento realizado em laboratório no qual ficou demonstrado que camada de muco de fato diminui o ataque de co-específicos e é ainda mais efetiva como proteção contra o opilião

Promitobates ornatus. Ao cobrirem seus ovos com uma camada de muco, fêmeas de *N. maximus* estão, portanto, provendo uma linha de defesa adicional aos seus ovos que é especialmente importante durante o dia, quando as fêmeas se ausentam mais frequentemente de suas desovas.

Os resultados obtidos no experimento acima são muito similares àqueles obtidos para o opilião *Iporangaia pustulosa*, em que os machos cuidam dos ovos e também abandonam suas desovas com frequência. A evolução convergente de uma camada de muco secretada pelas fêmeas dessas duas espécies de opiliões após a oviposição pode ser vista como um caráter naturalmente selecionado que provê proteção aos ovos quando os indivíduos parentais se ausentam de suas desovas. Essa linha de defesa adicional provavelmente minimiza os custos fisiológicos do cuidado parental tanto para fêmeas como machos guardiões. Sendo assim, a camada de muco que recobre os ovos de *N. maximus* provavelmente permite que estas fêmeas minimizem os custos fisiológicos associados ao cuidado maternal sem, entretanto, aumentar os custos da deserção em termos de redução de prole provocada por predação.