

Original Article

Strategic male mate choice minimizes ejaculate consumption

Benjamin J. Wegener,^a Devi M. Stuart-Fox,^b Mark D. Norman,^c and Bob B.M. Wong^a

^aSchool of Biological Sciences, Monash University, Wellington Road, Clayton VIC 3800, Australia,

^bDepartment of Zoology, Melbourne University, Melbourne VIC 3010, Australia, and ^cMuseum Victoria Melbourne VIC 3001, Australia

Reproduction can be costly for males, particularly when they provide nutrient-rich ejaculates in the form of spermatophores or secondary ejaculatory components. These costs may further increase if females reject or consume a male's ejaculate rather than using it for egg fertilization. Males should, therefore, attempt to minimize these costs to maximize their reproductive payoffs. We examined male reproductive investment toward virgin females in the Southern Bottletail Squid, *Sepiadarium austrinum*. Specifically, we assessed whether the presence of unfertilized eggs inside females influenced male reproductive investment, whether females removed spermatophores postcopulation, and what phenotypic characteristics influenced spermatophore removal. We found that males were more likely to attempt mating with females that were proportionally larger, but mated for longer and were more likely to transfer spermatophores to egg-carrying females. Females consistently ate spermatophores transferred by males, with the smallest females eating the most. However, males did not vary spermatophore investment according to female size or the associated risk of spermatophore consumption. *Key words:* ejaculate consumption, mating costs, sexual conflict, strategic investment. [*Behav Ecol*]

INTRODUCTION

Sexual reproduction is costly. As a result, both sexes often use a variety of mating tactics to reduce their reproductive costs (Arnqvist and Rowe 2005). Research, to date, has traditionally centered on how females attempt to mitigate the cost of mating, due to their often comparatively higher gametic and parental investments (e.g., McNamara et al. 2008; Nilsen et al. 2010). However, male strategies that reduce mating costs have received far less attention. To minimize such costs, male reproductive effort should be directed toward females that will provide the greatest fitness payoffs (Wedell et al. 2002). However, accurate mate evaluation is often difficult given the many, variable factors that influence female fitness such as reproductive history (Nilsen et al. 2010; delBarco-Trillo 2011), parasite load (Partridge et al. 2009), age (Moore and Moore 2001), and season (Milner et al. 2010). If morphological traits are indicative of female fitness, males may benefit from assessing these to guide their reproductive decisions (Luttbegg 2002; Kelly and Jennions 2011).

For males in many species, mating costs increase dramatically when they produce a nutrient-rich ejaculate for the female (e.g., spermatophores or seminal proteins) (Hunt et al. 2004; McNamara et al. 2008). Females may use these ejaculates for mate assessment, with only the sperm from high-quality suitors being used to fertilize their eggs (Cameron et al. 2007; Wigby et al. 2009). Although extremely rare, females may also consume these ejaculates, rather than using them for egg fertilization. Such behaviors can indirectly benefit the male by increasing female fecundity. For example, partial ejaculate consumption in the carrion fly *Prochyliza*

xanthostoma plays an important role in stimulating female reproduction and increasing male reproductive success (Bonduriansky et al. 2005). However, it remains unclear how male reproductive strategies vary when ejaculate rejection or consumption occurs (Pizzari and Birkhead 2000; Wedell et al. 2008; Brunel and Rull 2010).

The Southern Bottletail Squid (*Sepiadarium austrinum*, Figure S1 Supplementary Material) is an annual species endemic to the southern oceans of Australia. Like all cephalopods studied to date (Hanlon and Messenger 1999), this species appears polygamous with individuals readily mating multiple times in the laboratory. Females store multiple spermatophores in a buccal cavity (an external fleshy invagination ventral to the mouth), over which the females pass eggs to effect external fertilization. The pouch is open to the surrounding seawater and provides the opportunity for males to remove the spermatophores of rivals during copulation. Sperm depletion is another potential risk for males, with each mating typically resulting in the transfer of up to 60% of spermatophore stocks to the female (Wegener BJ, unpublished data). Males aggressively initiate mating by lunging at the female without any obvious courtship behavior. There is also no visible evidence of females initiating mating, with females consuming spermatophores both during and postcopulation (Wegener BJ, personal observation). Spermatophores in this species do not contain a nutrient body for female consumption (cf. Wedell et al. 2008). Instead, spermatophores evert on transfer to the female to form a single sperm mass (spermatangia) that attaches to the buccal cavity via a sticky cement body. Spermatangia are small (~1 mm in diameter), with females being able to store dozens in their buccal cavity. Females are able to remove these postcopulation using their beak-like mouths. These attributes make the Southern Bottletail squid an excellent model for testing predictions of strategic male investment when mating with females of variable quality.

Address correspondence to B.J. Wegener. E-mail: benjamin.wegener@monash.edu.

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The current study set out to examine the following questions: 1) Do males vary their reproductive investment in relation to female reproductive status or size? 2) How prevalent is female spermatophore consumption? 3) What factors influence female spermatophore consumption?

MATERIALS AND METHODS

Squid collection and housing

Juvenile squid were collected by SCUBA in Port Phillip Bay, Australia between April and May 2009. Consistent with other species with no generation overlap (Begon et al. 2006), the hatching time and maturation rates in this squid are highly synchronized resulting in similarly aged individuals year-round. Males and females were housed individually in 5-L tanks and fed a diet of amphipods and *Palaemonetes* shrimp ad libitum. All tanks were connected to a flow-through marine system that provided continuous, fresh seawater.

Experimental procedures

Virgin females were categorized according to the presence or absence of eggs in their mantle cavity and were considered gravid when unfertilized eggs were present.

To determine if males were strategic in their reproductive investment, we recorded the behavior of virgin males when presented with either a gravid or nongravid female. Each male was placed inside a 1-L aquarium with freshly circulating seawater and given 10 min to acclimate. Thereafter, a single female was added to the tank and mating duration recorded. Males were given 20 min to initiate mating, after which time the mating was considered unsuccessful. After mating, the female was immediately removed and kept in isolation for 1 h to allow her to consume any spermatophores. The 1-h time period was chosen because preliminary behavioral observations showed no spermatophore consumption occurring after this time. Visual confirmation of spermatophore consumption was possible as individuals are transparent, allowing for clear viewing of the esophagus and digestive tract. After 1 h, each individual was blotted on paper toweling to remove excess water and weighed to the nearest 0.001 g (as a measure of body size). Females were euthanized in a 5% $MgCl_2$ seawater solution and their buccal cavities and stomachs dissected to determine the number of spermatophores transferred during mating, as well as any that may have been consumed.

Measures of male reproductive investment were as follows: mating attempted (yes/no), successful spermatophore transfer (yes/no), mating duration, and number of spermatophores transferred. We also recorded the number of spermatophores eaten, proportion eaten, and number stored in the female buccal cavity.

To test whether males differed in reproductive investment toward gravid and nongravid females, we used a generalized linear mixed model (GLMM) with stepwise selection and, depending on the variable, either a binomial or Poisson error distribution (PROC GLIMMIX, SAS v9.2). Treatment (gravid vs. nongravid), male size, female size, and size ratio (male/female weight) were predictor variables and each measure of male reproductive investment was the dependent variable. As R^2 values comparing size ratio with male and female size showed nonsignificant levels of collinearity ($R^2 = 0.43$ and 0.72 , respectively), these were considered independent in the model (Zar 2010). As all but 3 successful matings were with gravid females (see Results), we also tested whether males varied the number of spermatophores transferred in relation to female size, male size, and size ratio for gravid females only. To identify factors influencing female spermatophore

consumption and storage, we used a GLMM as above with the same predictor variables.

RESULTS

Effects of size and female reproductive condition on male investment

The only factor determining male mating attempts was size ratio ($F_{1,40} = 9.42$, $P = 0.004$, $n = 42$, Figure 1a) with males more likely to attempt mating with females that were proportionally larger. However, males mated longer ($F_{1,27} = 12.29$, $P = 0.002$, $n = 29$, Figure 1b) and were more likely to successfully transfer spermatophores ($F_{1,27} = 41.28$, $P < 0.0001$, $n = 29$) with gravid females. All but 1 mating attempt with gravid females (i.e., 18 out of 19) resulted in successful spermatophore transfer (compared with 3 out of 10 for nongravid females). We found no difference in the number of spermatophores transferred to gravid versus nongravid females in successful matings; however, this is likely due to low mating success in the nongravid treatment. Among gravid females, there was no effect of male size, female size, or size ratio on the number of spermatophores transferred by males (Table S2; full model results are given for all analyses in Supplementary Material).

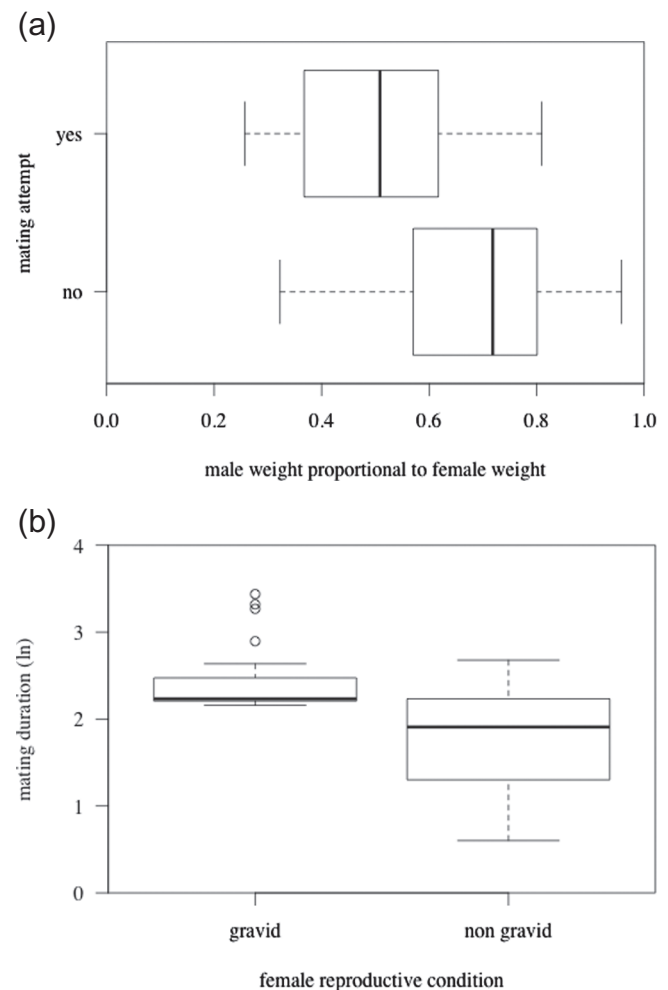


Figure 1
(a) Effects of male-to-female weight ratio on male mating attempts toward females. (b) Mating duration when male is paired with gravid versus nongravid females.

Female spermatophore consumption

In all successful matings, females ate a portion of the spermatophores transferred to them during copulation regardless of their reproductive condition (mean \pm SE, no. of spermatophores transferred = 28.13 ± 1.82 ; eaten by gravid females = 8.28 ± 1.05 , nongravid females = 7.33 ± 0.38 ; $F_{1,19} = 0.28$, $P = 0.602$). Smaller females ate proportionally more spermatophores than their larger counterparts (linear regression: proportion of spermatophores eaten: all successful matings $R^2 = 0.33$, $P = 0.006$; gravid treatment matings $R^2 = 0.327$, $P = 0.013$; Figure 2). Females did not appear to conserve a minimum number of spermatophores for future fertilizations when eating spermatophores (number of spermatophores after consumption: minimum = 8, maximum = 35, range = 27), with no increase in consumption when males invested heavily (controlled for female size: $F_{1,15} = 3.53$, $P = 0.079$). Male size had no effect on female spermatophore consumption (linear regression: $R^2 = 0.023$, $P = 0.632$).

DISCUSSION

We found that male bottletail squid prefer to attempt mating with proportionally larger females, regardless of female reproductive condition. Previous studies have suggested that male squid are opportunistic breeders, with individuals often employing sneaking and forced mating tactics to secure copulations (Hall and Hanlon 2002; van Camp et al. 2005; Huffard et al. 2008). Our findings demonstrate that male squid can be highly strategic when evaluating potential mates. Although male mating attempts were determined by relative female size, mating success was determined by female reproductive condition, with males mating for longer and being more likely to transfer spermatophores to gravid females. Interestingly, sperm storage appears to be short term in this species (Wegener BJ, Stuart-Fox DM, Norman MD, Wong BBM, unpublished data). As a result, copulating nongravid females may be unable to produce mature eggs in time for fertilization, with males losing their reproductive investment in such cases.

Male preference for relatively larger mates when attempting copulation may suggest female size is positively related to

fitness potential in this species. However, female size was also inversely related to spermatophore consumption. All females consumed at least some of the spermatophores received. However, the smallest females consumed the most. As such, the preference shown by males for relatively larger mates could be a strategy to minimize their exposure to female spermatophore consumption, with males using relative size as an indication of female reproductive condition. The fact that males did not simply attempt matings with the largest females may also be an indication of size assortative mating in this species. Interestingly, males did not reduce their spermatophore investment when mating with smaller females, even though males might be expected to avoid postcopulatory ejaculate consumption if it results in a net decrease in male fitness (e.g., Bonduriansky et al. 2005). Similarly, males should invest less reproductive effort toward smaller females if those matings result in lower fitness payoffs (Stuart-Fox and Whiting 2005). Why, then, did males not decrease their spermatophore investment when mating with smaller females?

Increased spermatophore consumption by smaller females may result in fitness benefits for males, particularly if the nutrients gained from such behavior boost offspring quality or quantity (Gwynne 2008). Ejaculatory consumption is known to increase female fecundity in several other taxa including carrion flies (Bonduriansky et al. 2005) and crickets (Simmons 1990). Whether similar fitness benefits are found in cephalopods is unknown.

Mating history may also influence male reproductive effort. In the hide beetle, *Dermestes maculatus*, virgin males invest heavily in their first copulation regardless of female quality (Jones and Elgar 2004). This may also be the case for bottletail squid because we tested only virgin males. Future studies should examine whether male squid adjust their spermatophore investment based on their own mating history. More generally, the positive size–fecundity relationship found in many species is often used to explain why males typically prefer to mate with larger females (e.g., Reading and Backwell 2007). Our study suggests that such preferences could also be influenced by additional factors, such as female spermatophore consumption, not traditionally associated with reproductive potential.

This small benthic squid possesses the unique combination of external sperm reception and egg fertilization, a

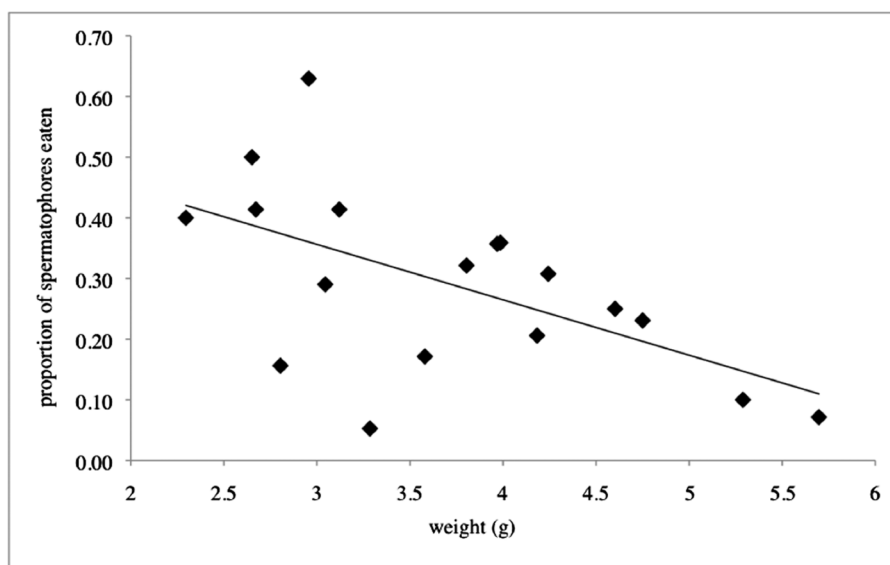


Figure 2
Proportion of spermatophores eaten by gravid females.

transparent body (enabling observation of spermatophore consumption) and easily instigated and observed mating behavior. As such, it makes an excellent model for investigating the intricacies and complexities of the often hidden realm of animal reproductive strategies.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>

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