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Spawning behavior dynamics at communal egg beds in the squid *Doryteuthis* (*Loligo*) *pealeii*☆

Nadav Shashar [a,b,*], Roger T. Hanlon [a]

[a] Marine Resources Center, Marine Biological Laboratory, Woods Hole, MA 02543, USA
[b] Ben Gurion University of the Negev, Department of Life Sciences, Eilat Campus, P.O. Box 653, Beer Sheva 84105, Israel

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ABSTRACT

Spring inshore spawning of the squid *Doryteuthis pealeii* was observed during 136 SCUBA dives over 3 years, and focal behavioral sampling was used to analyze female mate choice and the multiple tactics that males use to achieve paired and extra-pair copulations. *D. pealeii* uses two mating positions, each with different placements and usage of sperm. Near Woods Hole, squids aggregated during the day, and then groups of squids descended to the substrate for reproduction on communal egg beds; the operational sex ratio in these mating arenas was 3.13 M:1 F. Four male mating tactics were observed: *i.* large males acted as paired consorts; *ii.* when unpaired, large males fought consorts or rarely attempted forced copulations with the paired female; *iii.* small males behaved as either surreptitious or *iv.* bold sneakers. In the absence of large males, small males acted as consorts. Paired consorts mated in the male-parallel position and then guarded the female until several egg capsules were laid. Consort males fought other males frequently, winning 90% of fights, and large size was important for winning. Consorts guarded mates, mated frequently, and had 89% mating success. Large lone males often fought consorts but achieved takeovers only 10% of the time and their mating success was 6%. Sneakers did not fight; they mated in the head-to-head position and were not usually challenged by consorts. Collectively, sneakers accounted for 45% of all successful matings due to frequent attempts; average success rate was 29%. Paired, receptive females mated every 14 min, with either consorts or sneakers, and laid egg capsules every 5 min. Results suggest that females exerted direct mate choice by rejecting 55% of all mating attempts; indirect mate choice was evident from multiple sperm sources available to each female and by previously demonstrated multiple paternity in egg cases. This short-lived species demonstrates conditional reproductive tactics with high flexibility.

1. Introduction

In many animals, males exhibit variations in mating behavior that can include physical defense of females by dominant males along with "sneaky" matings by subordinate males (for review see: Andersson, 1994; Dunbar, 1982; Gross, 1996; Shuster and Wade, 2003; Waltz and Wolf, 1984). Gross (1996) highlighted a distinction between true alternative strategies (i.e., two or more discrete behaviors resulting from an underlying genetic polymorphism) and a conditional strategy (i.e., multiple tactics arising from variation in organismal and ecological conditions, not from discrete genetic differences). Within a species, male mating strategy is often conditional, and individual males may shift among behavioral tactics (Gross, 1996; Shuster and Wade, 2003). Such behavioral shifts may be ontogenetic, occurring over an individual's lifetime (Alonzo et al., 2000; Caro and Bateson, 1986), or facultative, where a male varies his tactic based upon his condition relative to that of his rivals (e.g., size, health, and social rank) or upon local ecological factors (e.g., rival density, female density, predation risk).

Different studies have examined determinants of male mating strategies (reviewed in Shuster and Wade, 2003), often indicating the importance of male body size. Previous studies have concentrated on arthropods and vertebrates (Shuster and Wade, 2003). In contrast, the mating strategies of non-arthropod invertebrates remain relatively understudied, as are the determinants of these strategies. This study examines alternative male mating tactics in the Longfin inshore squid *Doryteuthis* (*Loligo*) *pealeii* Lesueur, 1821 (Mollusca: Cephalopoda: Teuthoidea: Loliginidae), and evaluates likely determinants of the tactics. For convenience, we use the term "tactic" to refer to the males' different behavioral suites, without making any assumptions about the genetic bases of these behaviors.

D. pealeii migrates annually from the edge of the continental shelf to near shore spawning grounds in the northeastern USA (Jacobson, 2005). When arriving near shore, schools of squid will pause at selected locations where a complex system of courtship, mating and communal egg laying will arise. After eggs are deposited, squids swim away and no parental care is known to occur. Hence, nearly all sexual selection processes occur on these localized mating grounds. The need to improve

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* Corresponding author. Tel.: +972 8 6304520/531; fax: +972 8 6304538.
E-mail address: nadavsh@bgu.ac.il (N. Shashar).

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our understanding of this mating system led to a series of studies that are revealing a complex set of sexual behaviors involving multiple mates, extra-pair copulations (EPCs), and sperm competition.

Communal spawning arenas, in which many individuals mate and where females lay eggs, appear to be common in the genera *Loligo* and *Doryteuthis* (for review see: Boyle, 1983; Hanlon, 1998). Several examples are available: e.g., *D. pealeii* (Arnold, 1962), *Loligo forbesi*, *Loligo reynaudii* (Hanlon et al., 2002; Sauer et al., 1992, 1997; Shaw and Sauer, 2004), *Loligo bleekeri* (Iwata et al., 2010), and *Doryteuthis opalescens* (Fields, 1965; Hurley, 1977; McGowan, 1954). Similarly, the giant Australian cuttlefish *Sepia apama* is also known to aggregate to spawn (Hall and Hanlon, 2002). Various aspects of reproductive behavior in *D. pealeii* have been reported by Drew (1911), Arnold (1962), Arnold and Williams-Arnold (1977), Griswold and Prezioso (1981), Hanlon (1996, 1998), Hanlon et al. (1997, 1999), Maxwell and Hanlon (2000), King et al. (2003), and Buresch et al. (2003, 2009). *D. pealeii* lays multiple gelatinous egg capsules (egg fingers), each containing 50–400 individual eggs (on average 134; Hanlon et al., 1999; Maxwell and Hanlon, 2000), and attaches these capsules to the substrate at communal sites, often involving dozens of squids (see Griswold and Prezioso, 1981). In captivity, individual females can lay up to 765 egg capsules, often laying at various intervals of a few days or a week, and spanning several weeks (Maxwell and Hanlon, 2000). Total fecundity varies among females, with an average of about 20,000 eggs but reaching 110,000.

Offshore mating is thought to occur before migration because most females that first appear inshore in May arrive with stored sperm in the seminal receptacle (Drew, 1911; Maxwell et al., 1998). Hypothetically, females do not have to mate inshore, but our diving observations indicate that many or perhaps all of them do it. In captivity, females can store viable sperm for at least 40 days, although it appears that stores are limited since many eggs laid after three weeks were not fertilized (Maxwell and Hanlon, 2000). Mechanisms of sperm precedence are not well understood despite controlled behavioral/paternity experiments (Buresch et al., 2001, 2009); simple last-male precedence is apparently not occurring. Similar results have been found by Iwata et al. (2005) in *L. bleekeri*. Furthermore, newly deposited sperm in the mantle cavity of a female is more likely to fertilize more eggs as compared with sperm stored in the seminal receptacle (Buresch et al., 2001). Indeed, multiple mating in *D. pealeii* was reported in both field observations and laboratory trials, leading to multiple paternities within a single egg capsule (Buresch et al., 2001; Hanlon et al., 1997; Maxwell et al., 2000).

In the present study, we report the details of the nearshore mating system based upon three years of in situ observations via SCUBA diving with video. Specifically we examined behavioral tactics leading to mating success, as they are linked to mate choice by female and male squid.

2. Methods

Squids of *D. pealeii* habituate quickly to divers. During the months of May 1996, May 1997, and May–June 1998, 136 SCUBA dives were made on squid spawning grounds by RTH, NS and several volunteers, in Vineyard Sound, off the southern arm of Cape Cod in Massachusetts (lat 41° 35–39′ N, long 70° 02–15′ W). Dives were carried out in all hours of the day from first light till after sunset, typically going to the spawning grounds twice a week. Depths ranged from 3 to 10 m and most sites were within 2 km from shore between Hyannis and Chatham, MA. Spawning squids were found mostly in or near commercial weir traps whose inner pocket (or capture net) dimensions were approximately 20 × 20 m; there were often thousands of squids in these traps, with a portion of them engaged actively in reproductive behavior. In these cases, each weir was visited only once before it was fully fished. Water temperatures ranged from 2 to 13 °C, currents were often strong, and visibility was usually poor and to a human diver was often limited to 3–7 m. Behavioral data were collected using underwater writing slates and video recordings. Artificial illumination was not used even under dim conditions. The dimensions of five spawning arenas were measured with 50 m measuring tapes and depth gages. Video cameras (either analog or digital) in underwater housings provided a total of 16.5 h of recorded behavior. Of these, 302 min was of focal-animal tracking in which 25 mating pairs, three lone large males, and four sneaker males were followed. Analyses of video recordings were conducted with multi-motion playback machines and high-resolution monitors, often examining events field-by-field, providing a time resolution of 1/60 s. These fine-resolution video recordings enabled us to examine in detail complex behavioral scenarios such as reactions to mating attempts.

When analyzing specific questions, we were limited to subsamples of observations on the following data: 94 male–male agonistic bouts, 71 successful matings and 98 unsuccessful mating attempts; successful matings were defined by evidence for actual spermatophore transfer. For example, when examining the reaction of a female to a mating attempt, we were limited to 73 of the 169 successful and unsuccessful attempts since we needed video recordings that could be analyzed field-by-field, in which both female and attempting male were seen continuously from before the beginning of an attempt to after its conclusion. When a size comparison was needed, we were limited to cases in which all animals compared were recorded in the same frame, parallel to each other, and perpendicular to the camera. For animal length, we used images in which squids were seen perpendicular to the camera and to an adjacent object of known size. In many cases we placed a measuring tape near the egg mass and up into the water column. When examining the causation for mating attempt failures, we were limited to 51 recordings in which female, consort male, and attempting male were all seen continuously from before the onset of the attempt to after it was finished. Data were derived from behavioral or from focal animal samplings (*sensu* Martin and Bateson, 1986). When analyzing video for which individual identity may be of relevance (for example, fighting or mating success of a consort), multiple recordings from the same individual (i.e. derived from focal sampling) were omitted. However, when examining fight outcomes of a challenger, we could use data collected while following a consort, as the challengers differed each time.

2.1. Data analyses

Data from the three years of field observations were pooled. Although statistical comparison between mating behaviors in these years was not possible (1996 served for first examination and recordings of the mating system; most information came from 1997 to 1998, whereas in 1998 animal tracking was more narrowly focused), no differences in squid school behavior, arriving time to the egg laying grounds, or overall number of squids in the traps was recorded by the weir trap fishermen, by the US National Marine Fisheries Service (NMFS) or by us.

Results were examined using the nonparametric χ^2 goodness-of-fit test (Sokal and Rohlf, 1995) with equal expected frequency. It is possible that a few squids were observed more than once because we could not keep track of all the squids in a mating arena. Therefore, not all observations are truly independent from each other. However, since the time spent on any dive was short, the field observations were spread over 136 dives, with fewer than 10% repetitive dives on the same day and never more than 2 dives a day at the same location, and since hundreds of squids were in an arena each time, we assume that the number of such repetitive observations was very small.