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MATING BEHAVIOUR AND GENERAL SPAWNING PATTERNS OF THE SOUTHERN DUMPLING SQUID *EUPRYMNA TASMANICA* (SEPIOLIDAE): A LABORATORY STUDY

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ABSTRACT

We provide the first detailed description of mating behaviour and multiple mating in the southern dumpling squid, *Euprymna tasmanica* (Pfeffer, 1884) in the laboratory, as well as details on their general spawning patterns. We found that male *E. tasmanica* increase the number of 'pumps' (mantle contractions) when mating with females that had recently mated, showing that males are able to determine the recent mating history of females. We also found that at the conclusion of mating, the male's hectocotylus was enlarged. To our knowledge, neither had previously been described in sepiolid squid. *Euprymna tasmanica* females lay multiple clutches over a large proportion of their lifespan, from 1 to 121 d. There was a considerable variation in the number of eggs produced per female in captivity, ranging from 6 to 646 eggs, and in the number of eggs per clutch, from 6 to 163 eggs. Egg number per clutch declined significantly over the spawning period. Larger females produced larger hatchlings, and egg mass and hatchling mass were significantly correlated. At higher ambient water temperatures the rate at which females produced clutches increased and the size of the eggs laid decreased.

INTRODUCTION

In all cephalopod species studied to date, both sexes have been found to mate multiple times, resulting in multiple paternity within clutches (e.g. Naud *et al.*, 2005; Van Camp *et al.*, 2005; Voight & Feldheim, 2009). Consequently, sperm competition and sexual selection are likely to play an important role in cephalopod copulatory behaviour and fecundity. Investigating the behaviours of multiple mating and general spawning patterns (details relating to eggs spawned by the female, not including ovulation cycle and oocycte development) may offer important insights into these processes. Multiple mating behaviour and general spawning patterns can be challenging to quantify based on field studies, because of the difficulty of observing successive matings, recording total reproductive output (as opposed to just one reproductive bout or clutch) and identifying hatchlings to species level. Therefore, laboratory-based studies are more informative for measuring these important life-history variables. Here, we present the results of a laboratory-based study describing the mating behaviour over two successive matings and spawning pattern in the sepiolid squid *Euprymna tasmanica* (Pfeffer, 1884).

Sepiolid squid are small-bodied and benthic and are useful model organisms as they are more easily maintained in laboratory conditions than pelagic or larger-bodied cephalopods (Boletzky & Boletzky, 1971). Copulation has been observed in numerous sepiolids: in *Euprymna* (Singley, 1983; Hanlon *et al.*, 1997; Nabhitabhata *et al.*, 2005), *Sepiola* (Racovitza, 1894; Boletzky, 1983; Rodrigues, *et al.*, 2009), *Sepietta* (Bergstrom & Summers, 1983) and *Rossia* (Racovitza, 1894), as summarized by Rodrigues *et al.* (2009). For most sepiolids, there is no courtship (Bergstrom & Summers, 1983; Singley, 1983; Nabhitabhata *et al.*, 2005; Rodrigues *et al.*, 2009) and mating occurs in a 'male-to-female neck' position (Boletzky, 1983; Singley, 1983; Hanlon *et al.*, 1997; Nabhitabhata *et al.*, 2005; Rodrigues *et al.*, 2009). Mating duration varies both within and between species, from 8 min recorded in *Sepiola rondeletii* (Racovitza, 1894) to up to 80 min recorded in *S. atlantica* and *Euprymna scolopes* (Singley, 1983; Rodrigues *et al.*, 2009). The reproductive period of most sepiolids is aseasonal (Jereb, Mazzola & Di Stephano, 1997) and spawning patterns have been described for many species, summarized by Rodrigues *et al.* (2011). Females generally produce numerous eggs in a series of clutches, producing in total between 50 and 200 eggs per female (Mangold, 1987). However, potential fecundity (which includes oocytes in the ovary and ripe eggs in oviduct) of up to 931 eggs has been recorded in *Rossia macrosoma* (Salman & Onsoy, 2010).

Euprymna tasmanica is a small, semisolitary, nocturnal and benthic squid native to southern Australia (Norman & Lu,

1997). Females have a highly pocketed spermatheca, adjacent to the single oviduct opening within the mantle cavity, used for sperm storage (Norman & Lu, 1997). Egg capsules are round, orange and laid in clusters, with on average 95.4 ± 40.1 eggs laid in the first clutch (Sinn, Apiolaza & Moltschaniwskyj, 2006). In the field we found egg clutches laid at the bottom of pier pylons hidden amongst seaweed (Z.E. Squires, unpublished), but eggs are presumably laid attached to any hard substrate. Hatchlings, juveniles and adults can be found in the field all year round. *Euprymna tasmanica* makes a good model for reproductive studies as it is short lived, living for *c.* 5–8 months (Sinn & Moltschaniwskyj, 2005), and comparatively easily maintained in laboratory conditions (Sinn & Moltschaniwskyj, 2005; Moltschaniwskyj & Johnston, 2006; Squires *et al.*, 2012).

In this study we tested whether the mating behaviour of *E. tasmanica* changes over two successive laboratory matings and, if so, whether the differences are attributable to male and/or female mating history (i.e. whether it was their first or second laboratory mating) or their absolute and relative mass. We also describe the general spawning pattern for this species. To do this we measured a suite of reproductive variables and reproductive success, and assessed the influence of female mass and ambient water temperature at mating on reproductive variables. We discuss our results in relation to general patterns found in other sepiolid species.

MATERIAL AND METHODS

Squid culture

We collected 119 *Euprymna tasmanica* using SCUBA, from St Leonards, Victoria, Australia (38°10.81′S, 144°44.60′E). Squid were collected in water less than 5 m deep throughout 2009. Squid were then transported to the Victorian Marine Sciences Consortium facilities and held in individual aquaria (length × width × depth = 30 × 25 × 30 cm, volume = 22.5 l). Each aquarium contained a layer of sand and a length of PVC pipe as refuge. Seawater was aerated and pumped at a flow rate of 4 l per min directly from Port Phillip Bay in an open-water system. Water was kept at natural ambient temperature (13–21°C), which allowed us to assess the effects of temperature on reproductive output, and squid were checked for eggs every second day after collection. Artificial aquarium lights were maintained on a reversed 12 h day:12 h night cycle. Squid were fed live *Palaemon* sp. shrimp *ad libitum*. As we were conducting a larger experiment on the effects of multiple mating on female fitness (Squires *et al.*, 2012), we required females with a similar mating history (ideally virgins). However, taking into account the lengths of time sperm can be stored in some cephalopods (Rodrigues, Guerra & Troncoso, 2011), it is very difficult to collect virgin females from the wild. Therefore, we conducted a pilot trial in an attempt to control for females with different mating histories. We found that females collected from the wild generally laid viable eggs within a median of 13 d in captivity ($n = 11$, range = 4–7 d). Therefore, we used females that had not laid any eggs by day 28 for the mating behaviour experiment. If these females were not virgins, this method at least ensured that females were not ready to lay eggs from stored sperm and standardized, as best we could, variation in female mating history.

Sexually mature female ($n = 45$) and male ($n = 45$) squid, identified by their visible gonads, were blotted and weighed (to 0.1 mg accuracy) and size matched (pairs were within 5 g of each other). As part of a larger experiment (Squires *et al.*, 2012), females were mated once ($n = 18$) or twice—either with the same male twice ($n = 13$) or with two different males ($n = 14$), with 1 d between copulations to allow for a rest period. Male squid were also given 1 d of rest between matings. Male squid were placed in a small aquarium (10 × 10 × 11.5 cm; volume = 1.5 l) for an acclimation time of 10 min. The female was then introduced and the copulation and mating behaviours were video recorded. We quantified latency to mate, duration of copulation, the number of 'pumps' performed (see mating description in Results) and timing of spermatophore transfer as the measures of mating behaviours that are most likely to vary with mating history (number of matings and number of partners). Visual displays did not appear to be important in this species when mating under laboratory conditions, as very little colour change occurred and no courtship was observed. If mating did not occur within 30 min (21 out of 72 trials), females were disturbed from the tank bottom, as males more readily mated with females while in the water column. However, these trials were removed from analysis of mating behaviours. If mating still did not occur ($n = 16$) different squid were chosen. All trials were conducted under red light and mating behaviours were measured from video footage to minimize disturbance.

After mating, squid were returned to their holding tanks and the females left to lay eggs until senescence. Clutches were laid within PVC pipe or in the corner of aquaria below the waterline. All clutches were removed, blotted and weighed (to 0.1 mg accuracy) and the number of eggs counted. We put clutches into separate cylindrical containers that allowed water flow (diameter × depth = 8 × 10 cm, volume = 0.5 l), in an open water system, covered with shade cloth and kept at a constant temperature (19 ± 1°C) to develop. Clutches were inspected for hatchlings every second day to calculate their developmental time (i.e. time to hatching), to count the absolute number and to weigh hatchlings (after blotting, to 0.1 mg accuracy). For weighing, we anaesthetized hatchlings using ethanol. If more than 10 hatchlings emerged from the same clutch on the same day, a subset of 10 randomly selected hatchlings was weighed. In addition to clutch number, egg number, egg mass (clutch mass divided by egg number), hatchling number, hatchling development time and hatchling mass, we also recorded latency to lay (number of days between mating and laying the first clutch), interclutch interval (average number of days between successive clutches), hatching success (proportion hatched to number laid) and hatching synchrony (the last hatch day minus the first hatch day).

Analysis of mating behaviour

Statistical analyses were performed in R (R Development Core Team, 2011). All data were checked for normality and homogeneity of variances using visual inspection of residual plots. Latency to mate data were arcsine transformed to meet the normality assumption. Results are presented as means and standard errors (SE).

First, we tested whether mating behaviours (duration of copulation, latency to mate, number of pumps performed by the male) differed among females that had mated once ($n = 18$), females that had mated twice with the same male ($n = 13$) and those that had mated with two different males ($n = 14$). As there were no differences among the groups (see Results), we pooled all three datasets for descriptive statistics of mating behaviours and pooled the two twice-mated groups to investigate the effects of multiple mating on behaviour. We included female ID as a random factor to account for repeat measures for those females mated twice. We also tested for a relationship between each mating behaviour and temperature. As temperature affected mating duration (see Results), we included the residuals of the relationship between temperature and mating duration in models assessing the effect of mating order and mass on the duration of copulation.

To examine the effect of mating history (whether it was the first or second mating in the laboratory) on copulatory behaviour, we ran ANOVA models with the duration of copulation, latency to mate and the number of pumps performed by the male as the dependent variables, and mating history (from both the male and female perspectives) as predictor variables. We were also interested to see if squid mass influenced mating behaviours. For this, we ran linear mixed models with male mass, female mass and the difference in mass between the female and the male as the predictor variables.

Analysis of spawning patterns

To investigate whether reproductive output changed over the whole laying period for successfully reproducing females ($n = 39$), we used a linear mixed effects model with female ID as a random factor to account for repeated measures from the same female. For all hatchling mass data, clutch ID and female ID were included as random factors to account for repeated measures from the same female and from the same clutch.

To explore the effects of female mass and ambient temperature on reproductive output, we ran linear regression models. We also ran linear regression models to explore the relationships between egg mass and hatchling mass, and hatchling mass and development time. Temperatures were recorded throughout the experiment; however, we used the median temperature from the first clutch lay date to the last clutch lay date per female. For lay latency data we used the temperature at mating. Because hatchlings were relocated into a constant temperature environment, we could not assess the effects of temperature on hatchling development time or hatching success.

RESULTS

Mating behaviour

There was no obvious courtship behaviour between male and female *Euprymna tasmanica*. There was no difference in duration of copulation ($F_{2,48} = 2.773$, $P = 0.073$), latency to mate ($F_{2,48} = 2.330$, $P = 0.108$) or number of pumps performed by the male ($F_{2,48} = 2.693$, $P = 0.078$) among the three groups of mating females (those that had mated once, females that had mated twice with the same male and those that had mated with two different males). We therefore pooled these groups for descriptive statistics of general mating behaviours. Based on 51 observed matings, copulation began within 4.68 ± 0.81 min of the female being introduced (range 0.03–25.42 min), with males catching the female and wrapping their arms around her. Males then performed two to three vigorous mantle contractions (defined here as 'pumps') and manipulated the female into a 'male-to-female neck' position (Fig. 1A). The male inserted his hectocotylus (first left arm) into the female's mantle cavity and performed a series of weaker pumps with the siphon directed into the female's mantle cavity. Overall, males performed 16.30 ± 1.63 pumps (the original pumps plus the subsequent weaker pumps) per copulation (range 2–59). The male then produced a bundle of spermatophores (Fig. 1B) and transferred these to his hectocotylized arm. Spermatophore transfer occurred 4.31 ± 0.68 min (range 0.52–26.95 min) from initiation of mating. The video of mating *E. tasmanica* (Supplementary Material) shows an example of these stages of mating behaviour (for timing of each stage, see caption).

The pair then remained united in this position, but relatively inactive, until the end of mating. Mating duration was 91.77 ±

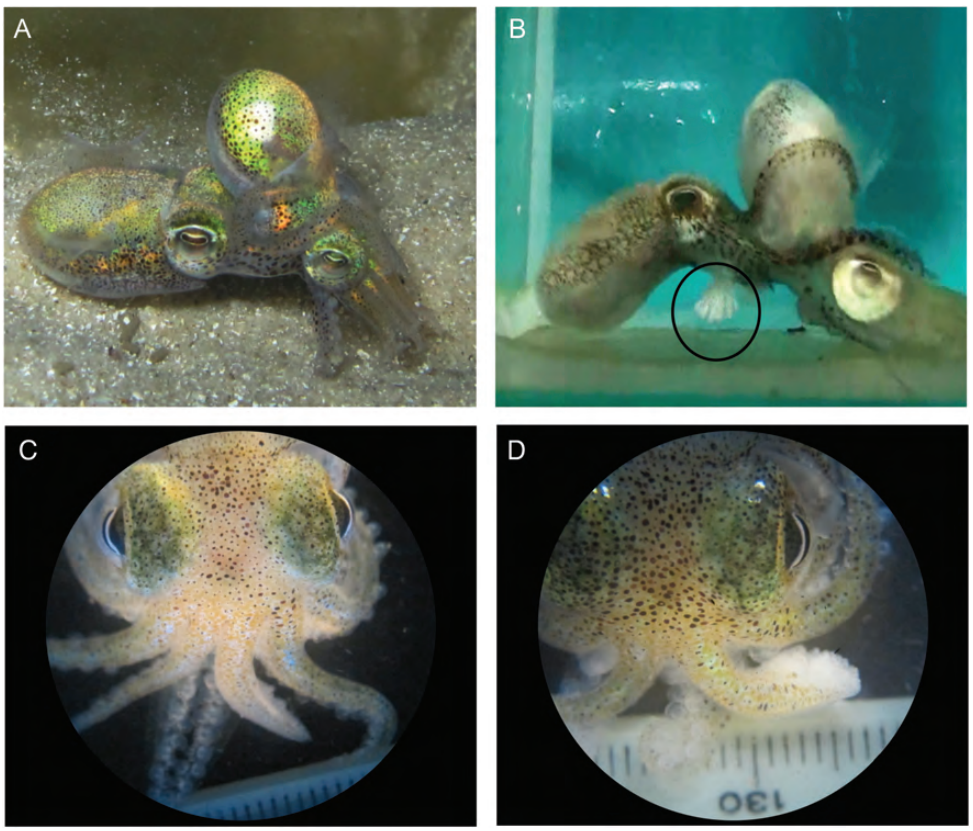

Figure 1. A. Mating pair of *Euprymna tasmanica* in the field (male left). **B.** Male (left) transferring bundle of spermatophores (circled) in a laboratory mating. **C, D**. Hectocotylus of male *E. tasmanica* before (**C**) and after (**D**) mating. (Photos: Z.E. Squires).

4.17 min (range 45–184 min). At the conclusion of mating we observed that the male's hectocotylus was enlarged, in some cases to nearly twice its original width (Fig. 1C, D). Finally, the male would perform two or three forceful movements to disengage with the female and remove his hectocotylus.

Effects of mating history and mass on mating

Temperature did not significantly influence latency to mate ($R^2 = -0.008$, $F_{1,49} = 0.598$, $P = 0.443$) or the number of pumps performed ($R^2 = 0.015$, $F_{1,49} = 1.75$, $P = 0.192$). It did, however, affect mating duration ($R^2 = 0.099$, $F_{1,49} = 6.485$, $P = 0.014$), with shorter durations at higher temperatures.

For the multiply mating females, female mating history (whether it was her first or second mating in the laboratory) did not affect duration of copulation ($F_{1,35} = 0.005$, $P = 0.945$) or latency to mate ($F_{1,35} = 0.042$, $P = 0.838$). It did, however, significantly affect the number of pumps performed, with males nearly tripling the number of pumps in the female's second mating ($F_{1,35} = 21.08$, $P < 0.001$; Fig. 2). This effect was the same regardless of whether females had mated twice with the same male or with two different males (i.e. there was no significant interaction between mating group and female mate history, for the number of pumps performed, $F_{1,34} = 0.025$, $P = 0.876$). Male mating history (whether it was his first or second mating) did not significantly affect any measured mating behaviour (duration of copulation: $F_{1,35} = 3.787$, $P = 0.060$; latency to mate: $F_{1,35} = 0.469$, $P = 0.498$; number of pumps: $F_{1,35} = 0.360$, $P = 0.552$).

Female mass had no effect on duration of copulation ($F_{1,49} = -0.157$, $P = 0.877$), latency to mate ($F_{1,49} = -0.611$, $P = 0.545$) or the number of pumps performed by the male ($F_{1,49} = 0.627$, $P = 0.535$). Male mass did not significantly influence duration of copulation ($F_{1,49} = -0.096$, $P = 0.925$), latency to mate ($F_{1,49} = -1.888$, $P = 0.079$) or number of pumps performed ($F_{1,49} = 0.439$, $P = 0.439$). Likewise, the difference in mass between the male and the female did not affect duration of copulation ($F_{1,49} = 0.075$, $P = 0.941$), latency to mate: $F_{1,49} = -1.269$, $P = 0.224$) or number of pumps performed by males ($F_{1,49} = -0.236$, $P = 0.816$).

Spawning patterns

Table 1 provides fecundity measures for successfully reproducing females ($n = 39$). Table 2 presents results for tests of the influence of female mass (mean 6.71 ± 0.42 g, range 3.23–14.03 g) and temperature (mean 17.42 ± 0.38, range 13–21°C) on reproductive measures. Temperature showed a significant negative relationship with egg mass, smaller eggs being produced at higher temperatures, and with average clutch interval and lay latency, eggs being produced faster at higher temperatures (Table 2). There was also a trend for female mass to significantly positively correlate with egg mass, and a significant influence of female mass on hatchling mass (Table 2).

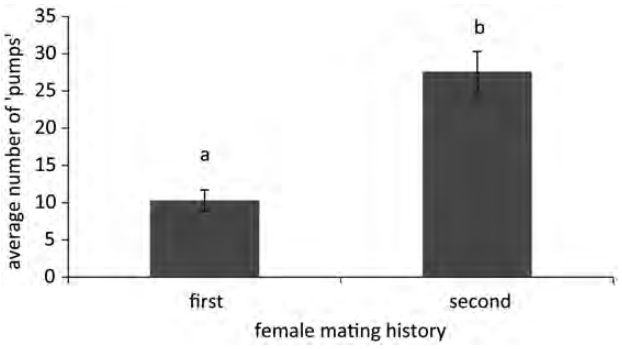

Figure 2. Average number of 'pumps' given by male *Euprymna tasmanica* during copulation with females for whom it was their first mating *versus* females for whom it was their second mating.

Both egg number per clutch and egg mass declined over the laying span, from the first clutch to the last clutch (egg number per clutch: $t_{39,\ 167} = -4.67$, $P < 0.001$, Fig. 3; egg mass: $t_{27,\ 92} = -3.25$, $P = 0.002$) and the first clutch had significantly more eggs than subsequent clutches (first clutch 65.64 ± 4.99, subsequent clutches 39.50 ± 1.58, $t_{1,\ 70} = 4.99$, $P < 0.001$). Reproduction represents a large investment; the total mass of eggs produced over a female's lifetime in the laboratory was on average 2.53 ± 0.32 times its own body mass (range 0.04–9.37 times).

Individual egg mass was positively correlated with individual hatchling mass ($R^2 = 0.079$, $F_{1,118} = 11.15$, $P = 0.001$). There was also a significant positive relationship between hatchling mass and development time ($R^2 = 0.019$, $F_{1,3387} = 64.90$, $P < 0.001$). Some eggs appeared opaque and white, and some females laid unformed irregular eggs throughout the spawning period, none of which developed.

DISCUSSION

Mating behaviour

Generally, the mating behaviour of *Euprymna tasmanica* is similar to that previously reported for other members of the Sepiolidae (Moynihan, 1983; Singley, 1983; Hanlon *et al.*, 1997). Nabhitabhata *et al.* (2005) and Rodrigues *et al.* (2009) have described five general stages of copulatory behaviour in bobtail squids (in *E. hyllebergi* and *Sepiola atlantica*, respectively): (1) female in water column; (2) male approaches and grabs female; (3) male moves into position (male-to-female neck); (4) male

Table 1. Reproductive output in female *Euprymna tasmanica* mated in the laboratory

Variable	Laboratory-mated females ($n = 39$)	
	Mean ± standard error	Range
Lay span (d)	46.61 ± 5.06	0–121
Number of clutches	5.39 ± 0.45	1–13
Number of eggs per clutch ($n = 207$)	47.66 ± 3.85	6–163
Total number of eggs	245.13 ± 23.98	6–646
Egg mass (g)	0.0626 ± 0.0033	0.0267–0.1003
Lay latency (d)	18.23 ± 1.67	1–62
Lay span (last lay day minus first lay day)	44.44 ± 4.99	1–121
Inter-clutch interval (days between each clutch)	10.10 ± 0.54	2.5–16.6
Hatching synchronicity (last hatch day minus first hatch day)	9.01 ± 0.72	0–2.45
Egg development time (d at 20 ± 1°C)	38.04 ± 0.87	26–55
Proportion hatched (%)	0.60 ± 0.04	0–0.96
Hatchling mass (g) ($n = 3810$)	0.0133 ± 0.0006	0.0081–0.0187
Adult survival in captivity (d)	94 ± 4.843	35–144
Adult survival after last clutch (d)	5 ± 0.78	0–15

Table 2. Effects of female mass and temperature on reproductive variables of *Euprymna tasmanica*

Dependent variable	Overall model and individual variables	*n*	R^2	Coefficient ± SE	*P*
Clutch number	Overall model	38	−0.037	–	0.731
	Female mass	–	–	−0.192 ± 0.246	0.441
	Temperature	–	–	−0.154 ± 0.240	0.525
Average clutch interval	Overall model	35	0.367	–	0.001*
	Female mass	–	–	0.037 ± 0.229	0.875
	Temperature	–	–	−0.717 ± 0.214	0.002*
Egg number total	Overall model	38	−0.044	–	0.826
	Female mass	–	–	−7.218 ± 12.227	0.559
	Temperature	–	–	−6.406 ± 11.929	0.595
Average egg mass	Overall model	35	0.533	–	$1.323e^{-06}$*
	Female mass	–	–	0.002 ± 0.001	0.060
	Temperature	–	–	−0.004 ± 0.001	0.003*
Lay latency	Overall model	38	0.069	–	0.105
	Female mass	–	–	−0.041 ± 0.893	0.252
	Temperature	–	–	−1.883 ± 0.872	0.038*
Average development time	Overall model	38	0.051	–	0.096
	Female mass	–	–	0.569 ± 0.332	0.096
Proportion hatched	Overall model	38	0.027	–	0.159
	Female mass	–	–	−0.023 ± 0.016	0.159
Hatchling mass (clutch ID/female mass)	Female mass	27	–	0.001 ± 0.001	0.046*

*$P < 0.05$. Note: temperature was not included in the models for development time, proportion hatched and hatchling mass as eggs were kept at constant temperature (see Methods).

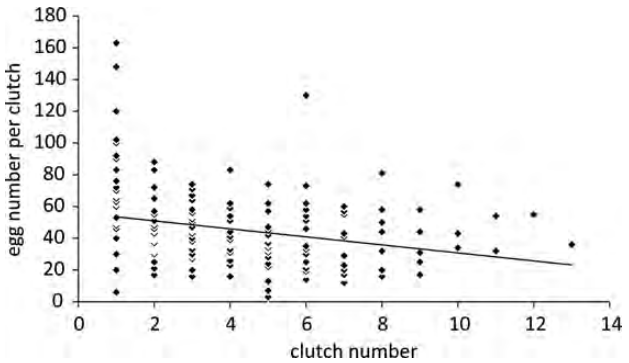

Figure 3. The average number of eggs per clutch decreases as clutch number increases in *Euprymna tasmanica*.

inserts the hectocotylus; (5) the pair moves to the substrate. However, the mating behaviour of *E. tasmanica* differs in two respects that have potentially important implications for sperm competition. These are the pumping behaviour and the enlargement of the hectocotylus. They have not previously been reported in sepiolid squid, but are known in other cephalopods. For example, the pumping (or flushing) behaviour has been recorded in *Sepia apama* (Hall & Hanlon, 2002), *S. lycidas* (Wada *et al*., 2005) and *S. officinalis* (Hanlon, Ament & Gabr, 1999). In these species it has been suggested that pumping functions to remove sperm, whereby jets of water directed into the female's sperm-storage organ help to dislodge spermatophores from previous mates. Debris flushed out by this behaviour was confirmed to contain live sperm in *S. lycidas* (Wada *et al*., 2005) and bits of spermatangia in *S. officinalis* (Hanlon *et al*., 1999). However, in *S. officinalis* it was found not to be very effective, removing sperm only during a short window after a previous mating (Hanlon *et al*., 1999).

We saw no direct evidence that the pumping behaviour removed sperm in *E. tasmanica*, as the sperm are opaque white and presumably easy to see, although we cannot discount this possibility. Another function of the pumping behaviour may be to remove accessory seminal fluids (which are ubiquitous in the animal kingdom; Wolfner, 2009) contained in spermatophores. This fluid may remain around the entrance to the spermatheca once spermatophores are everted. If accessory seminal fluids have detrimental effects on rival sperm, as in other animal groups (den Boer, Baer & Boomsma, 2010), males may benefit by removing seminal fluids of previous males. However, we found that males increased the pumping behaviour in the second mating regardless of the identity of the first mating male (i.e. they pumped more even if their own sperm was in the female's spermatheca). This suggests that males of *E. tasmanica* are not able to distinguish their own from rival sperm, and/or that they cannot recognize females. If the likelihood of encountering the same female is low, then there may be no selection for males to recognize previous mates, which could explain this result. In contrast, male *S. lycidas* do seem to have the ability to recognize previous mates, because they increased sperm removal when the female spermatheca contained rival, but not their own, sperm (Wada *et al*., 2010).

Regardless of the function, our results suggest that males are able to determine the mating history of the female and to alter the number of pumps accordingly. We have not determined experimentally the mechanism by which this occurs, but suggest that it has a chemo-tactile basis. Chemosensory responses to contact pheromones have been demonstrated in other cephalopods (Buresch *et al*., 2003). The hectocoylus of *E. tasmanica* has a concentration of palisade suckers distally (Norman & Lu, 1997). If these suckers have chemosensory abilities, males may use them to determine the presence of sperm (or accessory seminal fluids) within the spermatheca of a female.

The enlarged postmating hectocotylus in *E. tasmanica* could serve several functions. Without histological investigation we

cannot determine the mechanism of this enlargement and whether it could be caused by erectile tissue. Thompson & Voight (2003) were the first to discover erectile tissue in any invertebrate animal, in the cephalopod *Octopus bimaculoides*. They suggested that erectile tissue may have evolved in *O. bimaculoides* in response to opposing selective forces favouring a large transfer organ to transfer larger spermatophores on the one hand and a smaller, less conspicuous organ on the other. In *E. tasmanica* enlargement of the hectocotylus could also serve to open the highly pocketed seminal receptacle (Norman & Lu, 1997), giving the male access to various pockets, which could enhance female sperm storage. It may also enhance the male's ability to efficiently flush out the seminal receptacle. Alternatively, it could serve to hold the female in place during mating. At the conclusion of copulation the male apparently must forcibly detach himself from the female, perhaps to dislodge an enlarged hectocotylus from her seminal receptacle. If this injures the female, it may have important implications for sexual conflict, the female's subsequent decisions regarding optimal mating frequency and could incur additional costs of mating in this species (Franklin, Squires & Stuart-Fox, 2012).

Mating in *E. tasmanica* lasts substantially longer (by up to 82 min) than in the sepiolid squids *Sepiola rondeletii* and *Rossia macrosoma* (Racovitza, 1894). There was also substantial variation in the duration of copulation among mating pairs (range 45–184 min) and this is comparable with ranges previously recorded for *E. scolopes* (45–80 min by Singley, 1983, and 30–50 min by Hanlon *et al.*, 1997). In mating pairs that were forcibly separated during mating (Z.E. Squires, unpubl.), uneverted spermatophores were still attached to the hectocotylus, suggesting that the extended duration may relate to spermatophore eversion times and the complex process of evagination (Hoving *et al.*, 2009; Marian, 2012a, b). Mating duration was shorter at higher temperatures, which could hasten the eversion process.

Spawning patterns

Euprymna tasmanica females laid multiple egg clutches over a long period of time; some females laid eggs over 4 months. The average lay span (46.61 ± 5.06 d), from their first clutch to their last clutch, is longer than previously recorded in other sepiolid squids under laboratory conditions (1–61 d recorded in *Sepiola affinis* by Gabel-Deickert, 1995; 3–30 d in *S. atlantica* by Rodrigues *et al.*, 2011). This is perhaps because the average number of days between each clutch is also longer, at least compared with *S. atlantica* (Rodrigues *et al.*, 2011). The number of clutches produced falls within the range previously reported in that species (Rodrigues *et al.*, 2011). Clutch size is comparable with those of other Sepiolidae (Bello & Deickert, 2003), as are the total number of eggs produced, with a mean of 245.13 ± 23.98 in *E. tasmanica* and a range of 50–250 eggs recorded for *E. scolopes* (Hanlon *et al.*, 1997), 108–464 eggs for *E. hyllebergi* (Nabhitabhata *et al.*, 2005)—although some *E. tasmanica* laid up to 646 eggs. When compared with potential fecundity (oocytes in the ovary and ripe eggs in oviduct) recorded in other Sepiolidae, the maximum number of laid eggs reported here for *E. tasmanica* is within the range reported for *R. macrosoma* (382–837 oocytes), *R. pacifica* (300–1300 oocytes), *Neorossia caroli jeannea* (*c.* 200–800 oocytes) (Laptikhovsky *et al.*, 2008) and *R. macrosoma* (126–931 oocytes) (Salman & Onsoy, 2010).

Like other Sepiolidae such as *S. atlantica* (Rodrigues *et al.*, 2011), *E. tasmanica* spawn more than once (Rocha *et al.*, 2001) and deposit eggs in discrete clutches at different times and locations. There is also substantial variation in the number of eggs per clutch and in the total number of eggs each female produces, perhaps representing the potential for plasticity in these traits.

Steer *et al.* (2004) found no correlation between egg volume and hatchling mass, or egg volume and female mass, in a laboratory study on a Tasmanian population of *E. tasmanica*. In contrast, we found a significant relationship between egg mass and hatchling mass (although with a low R^2 value) and a trend for a positive effect of female mass on egg mass. Steer *et al.* (2004) measured egg volume rather than mass and this alternate method of quantifying egg size may explain the different results. However, Rodrigues *et al.* (2011) found a significant correlation between egg volume and hatchling mantle length in *S. atlantica*. A significant positive correlation between female mass and individual egg mass has also been found in many sepiolid species including *S. affinis*, *S. rondeletii*, *S. intermedia*, *Sepietta obscura* and *Sepietta oweniana* (Gabel-Deickert, 1995). Rodrigues, Guerra & Troncoso (2012) also found a positive correlation between female mantle length in *S. atlantica* and ripe oocyte mass. However, it may be that yolk concentration and composition of the egg are more important (albeit more difficult to determine) measures of egg quality than egg size (Steer *et al.*, 2004).

Additionally, temperature also affects egg size (smaller eggs are produced at higher temperatures) and the rate of egg laying (eggs are laid faster at higher temperatures). This is probably due to the effects of temperature on metabolism in squid, such that females that grow faster at higher temperatures (Andre *et al.*, 2009) may have more available resources for producing eggs and can therefore produce eggs at a faster rate. The low hatching success recorded in this study was similar to that in another laboratory study of *E. tasmanica* (Sinn *et al.*, 2006). Other studies have recorded much higher hatching successes in other species, for example up to 98.5–100% in *S. atlantica* (Rodrigues *et al.*, 2011).

In conclusion, this study has identified behaviours in *E. tasmanica*, some of which are novel for a sepiolid squid, and which potentially have important implications for sperm competition and sexual selection in this species. Future studies on multiple mating will be fruitful avenues for investigation.

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