

# Journal of The Malacological Society of London Molluscan Studies

*Journal of Molluscan Studies* (2018) **84**: 157–162. doi:10.1093/mollus/eyy002 Advance Access publication date: 14 February 2018

# Spermatophore dimorphism in the chokka squid *Loligo reynaudii* associated with alternative mating tactics

Yoko Iwata<sup>1</sup>, Warwick H. H. Sauer<sup>2</sup>, Noriyosi Sato<sup>3</sup> and Paul W. Shaw<sup>4</sup>

<sup>1</sup>Atmosphere and Ocean Research Institute, University of Tokyo, Chiba, Japan;

<sup>2</sup>Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, South Africa;

<sup>3</sup>Oki Marine Biological Station, Shimane University, Shimane, Japan; and

<sup>4</sup>Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University, Aberystwyth, UK

Correspondence: Y. Iwata; e-mail: iwayou@aori.u-tokyo.ac.jp

(Received 22 September 2017; editorial decision 14 December 2017)

## ABSTRACT

Chokka squid (*Loligo reynaudii*) have characteristic alternative mating tactics: 'consort' males temporarily pair with and guard a female and transfer spermatophores onto her oviduct opening inside the mantle cavity, whereas 'sneaker' males rush towards a mating pair and transfer spermatophores onto the female's buccal membrane near her sperm storage organ. Differences in mating behaviours and their related sperm-storage sites clearly constrain the fertilization process and can drive dimorphism between consort and sneaker males. The presence and character of male dimorphism has not yet been fully examined in this species, but consort males are commonly much larger than sneaker males. We observed clear dimorphism in spermatangia (the sperm mass ejaculated from the spermatophore), consistently associated with the two alternative sperm storage sites on the female's body. Observations of spermatophores stored in the Needham's sac of mature males confirmed that small males produce 'sneaker-type' spermatangia whereas larger males produce 'consort-type' spermatangia, and no individuals possessed both types. Therefore, by association, the mating tactic adopted (including the sperm deposition site used) by individual males can be determined from observation of their spermatangia type, without requiring direct behavioural observation of mating. This ability to infer information about mating tactic will improve our understanding of the reproductive system and mating dynamics in this species.

#### INTRODUCTION

Sexual selection works on male traits not only before mating, to gain access to females through male competition and female mate choice, but also after mating to achieve fertilization of a female's eggs through sperm competition and cryptic female choice (Parker, 1970; Eberhard, 1996). Sperm competition theory predicts that males allocate sperm strategically in each mating, and that males with high sperm competition risk use more sperm in a single mating (Parker, 1993). Males with well-developed secondary sexual traits gain advantage in the competition for access to females, through favourable mating position or order of copulation, whereas less desirable males have to accept a less favourable position and/or order of mating. Such asymmetry in competitiveness can lead to evolution of alternative mating tactics and associated male dimorphism within a species (e.g. Gross, 1996; Emlen, 1997; Oliveira, Taborsky & Brockmann, 2008). Alternative mating tactics may also lead to asymmetry in sperm-competition risk and males may adapt their sperm-allocation pattern and sperm quality in line with the sperm-competition risk (Parker, 1990; Snook, 2005)

Alternative mating tactics are commonly observed among males of loliginid squid species in the genera *Loligo* and *Doryteuthis* (e.g. Hanlon & Messenger, 1996; Sauer et al., 1997; Hanlon, Smale & Sauer, 2002; Iwata, Munehara & Sakurai, 2005; Buresch et al., 2009; Marian, 2012) and males of these species show body-size dimorphism. Large 'consort' males compete with rival males, form a temporary pair with a female, transfer spermatophores into the female's mantle cavity and guard the female until spawning. On the other hand, small 'sneaker' males usually do not fight with other males, but rush toward a mating pair and transfer spermatophores onto the female's buccal membrane. These alternative matings often contribute to multiple paternity within broods and lead to biased fertilization success among males (Hanlon, Maxwell & Shashar, 1997; Shaw & Sauer, 2004; Iwata et al., 2005; Buresch et al., 2009; Naud et al., 2016). Differences among males in mating position and spermatophore placement site result in differences in the fertilization environment and in potential sperm competition, and can lead to strong sexual selection. Divergence of male morphological and physiological adaptations associated with alternative mating tactics has been described in detail in the loliginid squid Heterololigo bleekeri: large males produce long spermatophores, ropelike spermatangia and short-lived small sperm, whereas small males produce short spermatophores, drop-like spermatangia and longlived large sperm that display chemotaxis leading to self-aggregation

© The Author(s) 2018. Published by Oxford University Press on behalf of The Malacological Society of London, all rights reserved. For Permissions, please email: journals.permissions@oup.com

(Iwata & Sakurai, 2007; Iwata *et al.*, 2011; Hirohashi *et al.*, 2013, 2016a, b; Iwata, Sakurai & Shaw, 2015). Similar reproductive trait dimorphism is found in *Doryteuthis plei* (Marian, 2012; Apostólico & Marian, 2017), suggesting that such distinct morphological and physiological traits associated with alternative mating tactics might be common in this group.

Similar to the loliginid squid mentioned above, Chokka squid (Loligo reynaudii), also known as Cape Hope squid, display alternative mating tactics related to body size dimorphism (Sauer et al., 1997; Hanlon et al. 2002) and show multiple paternity within broods (Shaw & Sauer, 2004; Naud et al., 2016). As with H. bleekeri and D. plei, it is expected that alternative mating tactics should lead to morphological divergence among males in L. reynaudii. The fishery on L. reynaudii targets spawning aggregations by jigging, a fishing method that tends to catch large males selectively (Lipinski, 1994); managing such fisheries necessitates a detailed understanding of the reproductive ecology of the species to allow more accurate predictions of possible changes to the population's demography caused by selective fishing pressure. However, the morphological characteristics of consort males and small sneaker males are unknown for this species, as previous studies on the mating system were based on in situ behavioural observations by SCUBA (Smale, Sauer & Roberts, 2001; Hanlon et al., 2002; Shaw & Sauer, 2004; Naud et al., 2016).

In this study, we analysed spermatophore dimorphism hypothesized to be associated with alternative mating tactics in *L. reynaudii* in order to identify individual mating tactic types from a morphological perspective, and to allow a better understanding of population-level reproductive demographics.

### MATERIAL AND METHODS

A total of 145 female and 287 male *Loligo reynaudii* were collected by jigging on the spawning grounds in St Francis Bay, South Africa, in November 2008–2010. All individuals were stored at 4 °C after landing and dissected within 2 d. The maturity stages of all individuals were determined according to the maturity stage indices described by Perez, Aguiar & Oliveira (2002) for *L. plei*.

Females of loliginid squid have a seminal receptacle, a saccate sperm storage organ on the buccal membrane, and store sperm for long period of time (Drew, 1911). Males attach spermatophores around the seminal receptacle by head-to-head mating and also attach spermatophores around the oviduct opening inside the mantle cavity by male-parallel mating (Drew, 1911). For each female, the two sites of spermatophore attachment were examined for the presence of spermatangia (Fig. 1A). If attached spermatangia were present, their morphology was described using a stereomicroscope. If the seminal receptacle was swollen and displayed a distinctive white colour, it was concluded that stored sperm were present.

All males, except for one individual, were fully mature. Sixty of the 286 mature males had only broken spermatophores in their Needham's sac storage site, therefore we used 226 males to examine the morphology of the spermatophore. Mantle length (ML) was recorded for each individual male along with the lengths of ten spermatophores, measured with electric slide calipers, randomly collected from the Needham's sac of each male. In addition, at least five spermatophores per individual were removed from the Needham's sac with forceps, placed on a petri dish filled with filtered seawater and stimulated to induce a spermatophoric reaction. The morphology of the ejaculated spermatangium was subsequently observed under a stereomicroscope.

A logistic regression analysis was performed with spermatangium morphology as the dependent variable and ML as a covariate.

Among males having rope-type spermatangia, it appears that the relationship between ML and spermatophore length (SL)

changes at some point (Fig. 2). Therefore, dimorphism among males with rope-type spermatangia was tested using the method of Eberhard & Gutiérrez (1991), where a partial regression equation was fitted to determine whether the relationship between ML and SL was nonlinear (see Iwata & Sakurai, 2007; Apostólico & Marian, 2017).

#### RESULTS

Of 145 females examined, all were fully mature and had sperm in their seminal receptacles on the buccal membrane. Eighty females (55.2%) had spermatangia attached only around the seminal receptacle on the buccal membrane, five females (3.5%) had spermatangia attached only on the oviduct, 25 females (17.2%) had spermatangia both around the seminal receptacle and on the oviduct and 35 females (24.1%) had no spermatangia on either site despite having sperm in the seminal receptacle.

Of the 105 females with spermatangia around the seminal receptacle, two had only the cement bodies (with an adhesive head) of the spermatangia remaining, but all 103 remaining females had only drop-like spermatangia present (Fig. 1B). The oviduct in this species has a wide membrane around the terminal opening and spermatangia were usually found attached to this membrane (Fig. 1C, D). Of the 30 females (16.6%) with spermatangia on the oviduct, five had only cement bodies remaining attached, so that the morphology of individual spermatangia could not be elucidated, 24 had only ropetype spermatangia attached and one female had only drop-type spermatangia on the oviduct (Fig. 1C, D).

ML of the mature males ranged from 115 mm to 428 mm (n =287). SL was related to body size, with larger males having longer spermatophores (Fig. 2). Clear spermatangium dimorphism was observed among males, with 160 males displaying only rope-type spermatangia and 68 displaying only drop-type spermatangia, while no individuals displayed a mixture of drop-type and ropetype spermatangia (Fig. 2). Spermatangium dimorphism also could be related to male size, with larger males (ML 147-428 mm) displaying rope-type spermatangia and smaller males (ML 115-200 mm) displaying drop-type spermatangia. Males with ML of 147-200 mm had either one or the other type of spermatangia (n = 98; 56 drop-type and 42 rope-type; Fig. 2). As 12 of these 98 individuals did not have intact spermatophores in their storage organs, SL could not be measured, although spermatangium type could be determined due to the presence of ejaculated spermatangia within the storage organ. For the remaining 86 males in the 147-200 mm ML range (51 with drop-type and 35 with rope-type spermatangia), SL was compared with spermatangium morphology: spermatophores producing rope-type spermatangia were significantly longer than spermatophores producing drop-type spermatangia (ANCOVA,  $F_{(1, 82)} = 70.1, P < 0.001$ ).

A logistic regression analysis demonstrated a significant relationship between ML and spermatangium morphology (logistic regression model:  $\chi^2_2 = 167.79$ , P < 0.001; ML: z = 6.037, P < 0.001; Fig. 3). The estimated ML below which there was >50% probability of being a male producing drop-type spermatangia and above which there was >50% probability of being a male producing rope-type spermatangia was 175.9 mm.

The partial regression analysis confirmed dimorphism among males with rope-type spermatangia; the slope of the relationship changes at ML of 273 mm (at ML < 273 mm: SL = 0.044 ML + 4.165, at ML > 273 mm: SL = 0.022 ML + 10.213;  $R^2 = 0.932$ , P < 0.001). The allometric slope was less steep in larger males than in smaller males, suggesting that large males allocate fewer resources to each spermatophore. SL was significantly correlated with ML in males producing drop-type spermatangia of all sizes (SL = 0.035 ML + 4.554,  $R^2 = 0.517$ , P < 0.001).



Figure 1. Spermatophore attachment sites on body of female *Loligo reynaudii*. A. Sperm transfer sites (white arrowhead) in mantle cavity used by consort males in male-parallel mating. B. Spermatangia (white arrowheads) attached on buccal membrane around seminal receptacle (black arrowhead). C. Attached spermatangia (white arrowhead) on membrane lip of oviduct opening. D. Wide membrane on oviduct opening (white arrowhead).



1.0 Probability to produce rope-type spermatangia 0.8 0.6 0.4 0.2 0.0 0 300 150 200 250 350 400 Mantle length (mm)

**Figure 2.** Relationship between individual mantle length and average length of 10 spermatophores. Males having rope-type spermatangia are indicated by a closed circle and drop-type spermatangia by an open circle.

**Figure 3.** Logistic regression between mantle length and the probability of being a rope-type spermatangium (drop-type = 0, rope-type = 1).

#### DISCUSSION

Alternative male mating tactics are a well-known phenomenon in a range of taxa (Oliveira et al., 2008) and in cephalopods have been recorded in loliginid squid (Drew, 1911; Hanlon, 1996; Hanlon et al., 1997, 2002; Jantzen & Havenhand, 2003; Wada et al., 2005), cuttlefish (Nornan, Finn & Treganza, 1999; Hall & Hanlon, 2002; Naud et al., 2004) and octopus (Huffard, Caldwell & Boneka, 2008). Alternative mating tactics have contrasting sperm competition risks and this can lead to strategic sperm usage among males (Parker, 1990). Loliginid squid also show a rare characteristic in having discrete sperm transfer sites associated with each mating tactic-inside the female's mantle cavity for male-parallel mating and around the buccal area for head-to-head mating (Drew, 1911). These discrete sperm transfer sites differ in sperm competition risk and fertilization environment, and morphological and physiological adaptations are shown by the spermatophores, spermatangia and sperm placed in these sites (Iwata & Sakurai, 2007; Iwata et al., 2011, 2015; Marian, 2012; Hirohashi et al., 2013, 2016a, b; Apostólico & Marian, 2017). Dimorphism among males in spermatophore and spermatangium size and shape has been reported previously in two species of the Loliginidae, Heterololigo bleekeri distributed in the western Pacific Ocean (Iwata & Sakurai, 2007; Iwata et al., 2015) and Doryteuthis plei distributed in the southwestern Atlantic Ocean (Apostólico & Marian, 2017). Spermatophore size can be related to the sperm allocation strategy associated with sperm competition risk for each mating tactic: the number of spermatozoa within a single spermatophore is five-fold greater for consort males than for sneaker males in H. bleekeri (Iwata et al., 2011) and sperm mass volume is greater in consorts than in sneakers in D. plei (Apostólico & Marian, 2017). In the present study, both spermatophore size and spermatangium shape dimorphism were related to size among males in Loligo reynaudii; in addition, a clear relationship between the different spermatangium types and the two different spermatophore deposition sites on the females was seen, which we hypothesize to relate to male mating tactic. Combining the findings here from male and female L. reynaudii samples with the morphological/behavioural classifications defined in previous studies of H. bleekeri and D. plei (Iwata & Sakurai, 2007; Apostólico & Marian, 2017), we can determine small males producing drop-like spermatangia as sneaker males that attach their spermatophores around the female's seminal receptacle and large males producing ropelike spermatangia as consort males that attach their spermatophores to the female's oviduct. The results of our study therefore suggest that dimorphism associated with alternative mating tactics may be a common phenomenon in loliginid squid worldwide.

In previous studies of squid, males were classified into a morphological type according to a switch point in the relationship between SL and ML (Iwata & Sakurai, 2007; Apostólico & Marian, 2017). However, the SL/ML relationship in *L. reynaudii* males is continuous; body sizes of sneaker and consort males overlap, meaning that mating type cannot be assigned based on the relationship between SL and ML. However, spermatophores in loliginid squid display dimorphism not only in spermatophore size, but also in the morphology of the spermatangia produced (Iwata *et al.*, 2015). Spermatangial morphology in *L. reynaudii* was also found here to show discrete dimorphism with two distinct shapes: 'rope-like' (associated with consort sperm deposition site) and 'drop-like' (associated with sneaker-sperm deposition site), with no individuals possessing both types of spermatangia. Therefore, we were able to classify individual male mating tactic based on spermatangium morphology.

The oviduct opening in female *L. reynaudii* has a wide membranous lip and spermatophores transferred into the mantle cavity during male-parallel copulations were attached to the surface of this membrane in all cases, suggesting that the membrane serves as a receptor for spermatophores. In *H. bleekeri*, consort males attach spermatophores to the inside wall of the oviduct and the oviduct opening lacks a membrane lip (Iwata & Sakurai, 2007). However, a similar membrane structure has been observed in *D. plei* and males in this species attach spermatophores to the membrane (Marian, 2012). The membrane lip in these two species is not similar to a seminal receptacle, which is designed to keep spermatozoa alive for a prolonged storage period, but rather is likely to function as a receptor for spermatangia for short periods only, during active spawning events. In the present study, among females with spermatangia on the oviduct opening, 16.6% had only the cement body remaining, which supports the interpretation of short-term storage.

The relationship between SL and ML in D. plei displayed two switch points: one related to smaller body size, distinguishing sneaker and consort males, and the second related to the size range of consort males, suggesting the possibility of three alternative mating tactics in that species (Apostólico & Marian, 2017). In both L. reynaudii and D. plei, the relationship between SL and ML is continuous, but the slope becomes less steep for the larger size ranges in consort males, indicating that the largest males allocate relatively fewer resources to each spermatophore and suggesting that they experience lower sperm-competition risk. The existence of three male mating tactics is known in isopods (Shuster & Wade, 1991) and some lizards (Sinervo & Lively, 1996). In these cases, mating tactics of males are genetically determined (Shuster & Sassaman, 1997; Sinervo & Zamudio, 2001) and the three tactics are thought to be evolutionarily stable through a mechanism of all tactics conferring equal fitness (Shuster & Wade, 1991) or through frequency-dependent selection not dissimilar to the rock-paperscissors model (Sinervo & Zamudio, 2001). In such a genetically determined mating system, it is expected that the tactic adopted by each male will be fixed both morphologically and behaviourally. In cephalopods however, mature body size is largely determined by temperature and food availability (e.g. Jackson & Moltschaniwskyj, 2002) and so if mating tactics were genetically determined in squid then any consistency between size and mating tactics would be low. An alternative system, consisting of two distinct tactics (harem-holders and sneakers), but with a third group of males employing a mixture of each tactic according to circumstance, is known in the reindeer Rangifer tarandus (Pintus et al., 2015). A similar system has been suggested for the oval squid Sepioteuthis lessoniana, where males exhibit three distinct mating behaviours and may change their mating tactics according to the relative body size of rival males: male-parallel, male-upturned and sneaking (Wada et al., 2005). However, the mating position of the S. lessoniana sneaker is identical to the male-upturned and so there are only two sperm transfer sites, one on the oviduct opening for male-parallel mating and the other on the buccal membrane for male-upturned and sneaking (Wada et al., 2005). Although to date no observation of a third male mating behavioural tactic has been made in L. reynaudii, it might be possible that males with intermediate body size use both consort and sneaker mating tactics flexibly according to the situation they face in male-male competition and female choice, with a consort switching to sneaking or vice versa. Our results included a rare case (1 in 25) where sneaker-type spermatangia were attached to the oviduct opening, suggesting a sneaker mating in the male-parallel position (a consort tactic); this is not consistent with the occurrence of two types of consort male and as males appear to have fixed spermatangial types adapted to their specific mating position and deposition site, it is unlikely that such tactic switches will be very successful.

An alternate hypothesis would be the presence of a further previously undescribed mating tactic, which utilizes the male-parallel mating position but with a different level of sperm competition risk. In male peacock wrasse, *Symphodus tinca*, four types of mating tactic have been observed: 'nest owners' who build a nest with seaweed and guard the nest and spawned eggs; 'satellites' who have a small body size and spawn by rushing into nest owners' nests whenever a female spawns; 'interceptors' who have a wide range

of body sizes and intercept females about to spawn, away from a nest where no parental care would be provided, and 'pirates' who are the largest males and parasitize nesting males by fighting with nest owners and taking over high-quality nests, then abandoning the nest to the original owner after the spawning peak (van den Berghe, 1988). Although L. reynaudii do not build a nest or provide parental care after spawning, consort males guard females during spawning (Hanlon et al., 2002). Paternity analysis in this species has revealed that consort males present at the time of egg-string deposition sometimes do not sire any offspring (21% of broods sampled, Naud et al., 2016), suggesting that other large males have intercepted and mated with the female guarded by the consort males and that such displacement of consort males is not rare. As in the case of peacock wrasse, perhaps the largest consort males produce relatively small spermatophores. These males may behave as pirates, making less effort to guard females and simply taking over females just before spawning, and so experiencing less sperm competition risk. The reason for the presence of a switch point in the relationship between ML and SL among consort males is still elusive and further morphological and behavioural observations will be necessary to understand fully the mating system for this species.

#### ACKNOWLEDGEMENTS

The research was funded by a European Commission Marie Curie Actions International Incoming Fellowship (IIF) to Y.I., the South African Squid Management Industrial Association (SASMIA) to W.H.H.S., and Rhodes University to W.H.H.S. We thank Brian Godfrey for help in fieldwork.

#### REFERENCES

- APOSTÓLICO, L.H. & MARIAN, J.E.A.R. 2017. Dimorphic male squid show different gonadal and ejaculate expenditure. *Hydrobiologia*. DOI:10.1007/s10750-017-3145-z.
- BURESCH, K.C., MAXWELL, M.R., COX, M.R. & HANLON, R.T. 2009. Temporal dynamics of mating and paternity in the squid *Loligo* pealeii. Marine Ecology Progress Series, **387**: 197–203.
- DREW, G.A. 1911. Sexual activities of the squid, Loligo pealii (Les.). Journal of Morphology, 22: 327–359.
- EBERHARD, W.G. 1996. Female control: sexual selection by cryptic female choice. Princeton University Press, Princeton, NJ.
- EBERHARD, W.G. & GUTIÉRREZ, E.E. 1991. Male dimorphisms in beetles and earwigs and the question of developmental constraints. *Evolution*, **45**: 18–28.
- EMLEN, D.J. 1997. Alternative reproductive tactics and male-dimorphism in the horned beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Behavioral Ecology and Sociobiology*, **41**: 335–341.
- GROSS, M.R. 1996. Alternative reproductive strategies and tactics: diversity within sexes. *Trends in Ecology and Evolution*, **11**: 92–98.
- HALL, K.C. & HANLON, R.T. 2002. Principal features of the mating system of a large spawning aggregation of the giant Australian cuttlefish *Sepia apama* (Mollusca: Cephalopoda). *Marine Biology*, **140**: 533–545.
- HANLON, R.T. 1996. Evolutionary games that squids play: fighting, courting, sneaking, and mating behaviors used for sexual selection in *Loligo pealei. The Biological Bulletin*, **191**: 309–310.
- HANLON, R.T., MAXWELL, M.R. & SHASHAR, N. 1997. Behavioral dynamics that would lead to multiple paternity within egg capsules of the squid *Loligo pealei*. *Biological Bulletin*, **193**: 212–214.
- HANLON, R.T. & MESSENGER, J.B. 1996. Cephalopod behaviour. Cambridge University Press, Cambridge.
- HANLON, R.T., SMALE, M.J. & SAUER, W.H.H. 2002. The mating system of the squid *Loligo vulgaris reynaudii* (Cephalopoda, Mollusca) off South Africa: fighting, guarding, sneaking, mating and egg laying behavior. *Bulletin of Marine Science*, **71**: 331–345.

- HIROHASHI, N., ALVAREZ, L., SHIBA, K., FUJIWARA, E., IWATA, Y., MOHRI, T., INABA, K., CHIBA, K., OCHI, H., SUPURAN, C.T., KOTZUR, N., KAKIUCHI, Y., KAUPP, U.B. & BABA, S.A. 2013. Sperm from sneaker male squids exhibit chemotactic swarming to CO<sub>2</sub>. *Current Biology*, 23: 775–781.
- HIROHASHI, N., IIDA, T., SATO, N., SAUER, W.H.H. & IWATA, Y. 2016a. Complex adaptive traits between mating behaviour and postcopulatory sperm behavior in squids. *Reviews in Fish Biology and Fisheries*, 26: 601–607.
- HIROHASHI, N., TAMURA-NAKANO, M., NAKAYA, F., IIDA, T. & IWATA, Y. 2016b. Sneaker male squid produce long-lived spermatozoa by modulating their energy metabolism. *Journal of Biological Chemistry*, **291**: 19324–19334.
- HUFFARD, C.L., CALDWELL, R.L. & BONEKA, F. 2008. Mating behavior of *Abdopus aculeatus* (d'Orbigny 1834) (Cephalopoda: Octopodidae) in the wild. *Marine Biology*, **154**: 353–362.
- IWATA, Y., MUNEHARA, H. & SAKURAI, Y. 2005. Dependence of paternity rates on alternative reproductive behaviors in the squid *Loligo* bleekeri. Marine Ecology Progress Series, 298: 219–228.
- IWATA, Y. & SAKURAI, Y. 2007. Threshold dimorphism in ejaculate characteristics in the squid *Loligo bleekeri*. Marine Ecology Progress Series, 345: 141–146.
- IWATA, Y., SAKURAI, Y. & SHAW, P. 2015. Dimorphic sperm-transfer strategies and alternative mating tactics in loliginid squid. *Journal of Molluscan Studies*, 81: 147–151.
- IWATA, Y., SHAW, P., FUJIWARA, E., SHIBA, K., KAKIUCHI, Y. & HIROHASHI, N. 2011. Why small males have big sperm: dimorphic squid sperm linked to alternative mating behaviours. *BMC Evolutionary Biology*, 11: 236.
- JACKSON, G.D. & MOLTSCHANIWSKYJ, N.A. 2002. Spatial and temporal variation in growth rates and maturity in the Indo-pacific squid *Sepioteuthis lessoniana* (Cephalopoda: Loliginidae). *Marine Biology*, **140**: 747–754.
- JANTZEN, T.M. & HAVENHAND, J.N. 2003. Reproductive behavior in the squid *Sepioteuthis australis* from South Australia: interactions on the spawning grounds. *Biological Bulletin*, **204**: 305–317.
- LIPINSKI, M.R. 1994. Differences among basic biological parameters in a population of chokka squid *Loligo vulgaris reynaudii* (Cephalopoda: Loliginidae) sampled by three methods. *South African Journal of Marine Science*, 14: 281–286.
- MARIAN, J.E.A.R. 2012. Spermatophoric reaction reappraised: novel insights into the functioning of the loliginid spermatophore based on *Doryteuthis plei* (Mollusca: Cephalopoda). *Journal of Morphology*, **273**: 248–278.
- NAUD, M.J., HANLON, R.T., HALL, K.C., SHAW, P.W. & HAVENHAND, J.N. 2004. Behavioural and genetic assessment of reproductive success in a spawning aggregation of the Australian giant cuttlefish, *Sepia apama. Animal Behaviour*, 67: 1043–1050.
- NAUD, M.J., SAUER, W.H.H., MCKEOWN, N.J. & SHAW, P.W. 2016. Multiple mating, paternity and complex fertilization patterns in the chokka squid *Loligo reynaudii*. PLoS One, 11: e0146995.
- NORNAN, M.D., FINN, J. & TREGANZA, T. 1999. Female impersonation as an alternative reproductive strategy in giant cuttlefish. *Proceedings of the Royal Society of London. Series B*, 266: 1347–1349.
- OLIVEIRA, R.F., TABORSKY, M. & BROCKMANN, H.J. 2008. Alternative reproductive tactics: an integrative approach. Cambridge University Press, Cambridge.
- PARKER, G.A. 1970. Sperm competition and its evolutionary consequences in the insects. *Biological Reviews*, 45: 525–567.
- PARKER, G.A. 1990. Sperm competition games: sneaks and extra-pair copulations. Proceedings of the Royal Society of London. Series B, 242: 127–133.
- PARKER, G.A. 1993. Sperm competition games: sperm size and sperm number under adult control. *Proceedings of the Royal Society of London. Series B*, **253**: 245–254.
- PEREZ, J.A.A., AGUIAR, D.C. & OLIVEIRA, U.C. 2002. Biology and population dynamics of the long-finned squid *Loligo plei* (Cephalopoda: Loliginidae) in southern Brazilian waters. *Fisheries Research*, **58**: 267–279.
- PINTUS, E., UCCHEDDU, S., RØED, K.H., GONZALÉZ, J.P., CARRANZA, J., NIEMINEN, M. & HOLAND, Ø. 2015. Flexible mating tactics and associated reproductive effort during the rutting season in male reindeer (*Rangifer tarandus* L. 1758). *Current Zoology*, **61**: 802–810.

- SAUER, W.H.H., ROBERTS, M.J., LIPINSKI, M.R., SMALE, M.J., HANLON, R.T., WEBBER, D.M. & O'DOR, R.K. 1997. Choreography of the squid's "nuptial dance". *Biological Bulletin*, **192**: 203–207.
- SHAW, P.W. & SAUER, W.H.H. 2004. Multiple paternity and complex fertilisation dynamics in the squid *Loligo vulgaris reynaudii*. *Marine Ecology Progress Series*, 270: 173–179.
- SHUSTER, S.M. & SASSAMAN, C. 1997. Genetic interaction between male mating strategy and sex ratio in a marine isopod. *Nature*, **388**: 373–377.
- SHUSTER, S.M. & WADE, M.J. 1991. Equal mating success among male reproductive strategies in a marine isopod. *Nature*, **350**: 608–610.
- SINERVO, B. & LIVELY, C.M. 1996. The rock-paper-scissors game and the evolution of alternative male strategies. *Nature*, **380**: 240–243.

- SINERVO, B. & ZAMUDIO, K.R. 2001. The evolution of alternative reproductive strategies: fitness differential, heritability, and genetic correlation between the sexes. *Journal of Heredity*, **92**: 198–205.
- SMALE, M.J., SAUER, W.H.H. & ROBERTS, M.J. 2001. Behavioural interactions of predators and spawning chokka squid off South Africa: towards quantification. *Marine Biology*, **139**: 1095–1105.
- SNOOK, R.R. 2005. Sperm in competition: not playing by the numbers. Trends in Ecology and Evolution, 20: 46–53.
- VAN DEN BERGHE, E.P. 1988. Piracy as an alternative reproductive tactic for males. *Nature*, **334**: 697–698.
- WADA, T., TAKEGAKI, T., MORI, T. & NATSUKARI, Y. 2005. Alternative male mating behaviors dependent on relative body size in captive oval squid *Sepioteuthis lessoniana* (Cephalopoda, Loliginidae). *Zoological Science*, **22**: 645–651.