

Reproductive biology of the golden cuttlefish *Sepia esculenta* (Cephalopoda, Sepiida)

Elizaveta V. Vlasova*, Rushan M. Sabirov, Alexey V. Golikov

***Corresponding author:** Elizaveta V. Vlasova, Department of Zoology, Kazan Federal University, Kazan 420008, Russia. E-mail: evlasova.uni@gmail.com

Table S1 Studied specimens of *Sepia esculenta*. ML – mantle length.

| Specimen code | Sex | Location | Data | Maturity stage | ML (mm) |
|---------------|---------|------------|------------|----------------|---------|
| 1-1 | Males | Ushimado | 15.03.2012 | V ₂ | 141 |
| 2-1 | | | 15.03.2012 | V ₂ | 147 |
| 3-1 | | | 15.03.2012 | V ₂ | 139 |
| 7-2 | | Mukaishima | 09.03.2007 | V ₂ | 154 |
| 3-2 | | | 09.03.2007 | V ₂ | 154 |
| 6-2 | | | 09.03.2007 | V ₂ | 153 |
| 4-2 | | | 09.03.2007 | V ₂ | 158 |
| 3-3 | | Iriomote | 05.05.2005 | V ₂ | - |
| 1-3 | | | 30.04.2005 | V ₂ | - |
| 22-3 | | | May-June | V ₂ | - |
| 33-3 | | | May-June | V ₂ | - |
| 28-3 | | | May-June | V ₂ | - |
| 31-3 | | | May-June | V ₂ | - |
| 25-3 | | | May-June | V ₂ | - |
| 23-3 | | | May-June | V ₂ | - |
| 27-3 | | | May-June | V ₂ | - |
| 6-3 | | | 17.05.2005 | V ₂ | - |
| 16-3 | | | 17.05.2005 | V ₂ | - |
| 11-3 | | | 17.05.2005 | V ₂ | - |
| 13-3 | | | 17.05.2005 | V ₂ | - |
| 7-3 | | | 17.05.2005 | V ₂ | - |
| 8-3 | | | 17.05.2005 | V ₂ | - |
| 1-1 | Females | Ushimado | 15.03.2012 | V ₂ | 140 |
| 3-1 | | | 15.03.2012 | V ₂ | 139 |
| 5-1 | | | 15.03.2012 | V ₂ | 137 |
| 6-1 | | | 15.03.2012 | V ₂ | 140 |
| 7-1 | | | 15.03.2012 | V ₂ | 142 |
| 8-2 | | Mukaishima | 09.03.2007 | V ₂ | - |

Table S2 Measurements of the female and male reproductive system parts in *Sepia esculenta*. ML – mantle length; SG – spermatophoric gland. The values are min–max (mean \pm SE).

| Measurements of the female reproductive system parts | | | Measurements of the male reproductive system parts | | |
|--|---------------------------------|---------------------------------|--|---------------------------------|----------------------------------|
| Character | Absolute length (in mm) | Relative length (in % of ML) | Character | Absolute length (in mm) | Relative length (in % of ML) |
| Oviduct length | 23.0–44.0 (32.2 \pm 3.43) | 16.8–31.0 (23.02 \pm 2.33) | Testis length | 19.0–48.5 (31.2 \pm 2.05) | 13.3–20.9 (17.5 \pm 1.20) |
| Oviducal gland length | 15.0–21.00 (18.0 \pm 1.10) | 10.7–14.8 (12.9 \pm 0.78) | Testis width | 19.0–37.0 (27.8 \pm 1.67) | 18.0–18.4 (18.3 \pm 0.14) |
| Right nidamental gland length | 35.0–48.0 (39.6 \pm 2.34) | 25.2–34.3 (28.4 \pm 1.60) | Sperm duct length | 17.0–35.0 (24.5 \pm 1.57) | 14.4–22.70 (18.9 \pm 2.43) |
| Right nidamental gland width | 20.0–22.0 (21.2 \pm 0.49) | 14.3–16.06 (15.2 \pm 0.36) | Part I of SG length | 7.0–19.0 (10.2 \pm 0.50) | 5.7–13.5 (8.34 \pm 1.18) |
| Left nidamental gland length | 35.0–45.0 (37.8 \pm 1.83) | 25.2–32.14 (27.1 \pm 1.29) | Part II of SG length | 8.0–19.0 (10.4 \pm 0.53) | 5.2–13.5 (7.8 \pm 1.15) |
| Left nidamental gland width | 20.0–28.0 (22.4 \pm 1.47) | 14.3–20.14 (16.1 \pm 1.06) | Part III of SG length | 8.0–16.0 (10.7 \pm 0.38) | 5.1–11.4 (7.2 \pm 0.84) |
| Right accessory nidamental gland length | 20.0–33.0 (28.0 \pm 2.49) | 14.4–23.6 (20.1 \pm 1.79) | Part IV of SG length | 12.0–29.0 (19.1 \pm 0.77) | 10.1–19.9 (13.4 \pm 1.18) |
| Right accessory nidamental gland width | 15.0–19.0 (17.6 \pm 0.87) | 10.8–13.87 (12.6 \pm 0.62) | Part V of SG length | 13.0–21.0 (16.5 \pm 0.39) | 8.4–14.2 (10.6 \pm 0.77) |
| Left accessory nidamental gland length | 22.0–30.0 (27.8 \pm 1.50) | 15.8–21.4 (19.9 \pm 1.04) | Part VI of SG length | 7.0–16.0 (12.2 \pm 0.40) | 7.1–9.5 (8.6 \pm 0.35) |
| Left accessory nidamental gland width | 14.0–18.0 (16.8 \pm 0.80) | 10.1–13.1 (12.03 \pm 0.57) | Spermatophoric sac length | 39.0–75.0 (56.02 \pm 2.08) | 29.8–42.48 (39.80 \pm 1.73) |
| | | | Penis length | 7.0–25.0 (14.3 \pm 1.10) | 5.04–13.6 (7.2 \pm 1.12) |

Figure S1 Correlations between mantle length and different parts of male reproductive system in *Sepia esculenta*. ML – mantle length; SG – spermatophoric gland.

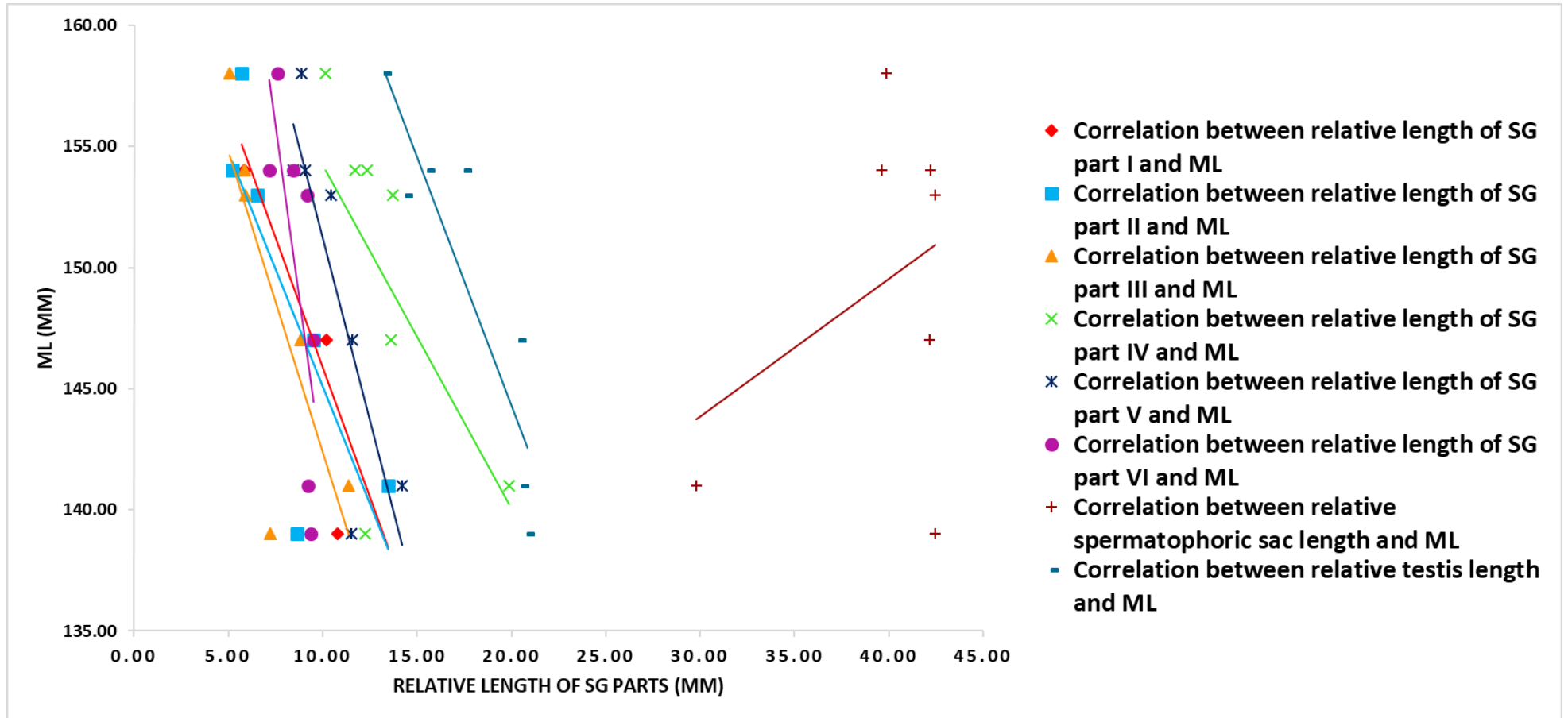


Table S3 Equations of correlations between mantle length and different parts of male reproductive system in *Sepia esculenta*. ML – mantle length; SG – spermatophoric gland. Significant p-values are in **bold**.

| Correlations | Equation |
|---|--|
| Correlation between relative testis length and ML | Relative testis length = $-0.40 \cdot \text{ML} + 76.50$; $r^2 = 0.811$; $p = \mathbf{0.0058}$; $n = 7$ |
| Correlation between relative length of SG part I and ML | Relative length of SG part I = $-0.40 \cdot \text{ML} + 68.02$; $r^2 = 0.85$; $p = \mathbf{0.0033}$; $n = 7$ |
| Correlation between relative length of SG part II and ML | Relative length of SG part II = $-0.34 \cdot \text{ML} + 58.75$; $r^2 = 0.659$; $p = \mathbf{0.0272}$; $n = 7$ |
| Correlation between relative length of SG part III and ML | Relative length of SG part III = $-0.28 \cdot \text{ML} + 52.35$; $r^2 = 0.493$; $p = 0.8$; $n = 7$ |
| Correlation between relative length of SG part IV and ML | Relative length of SG part IV = $-0.26 \cdot \text{ML} + 52.57$; $r^2 = 0.372$; $p = 0.15$; $n = 7$ |
| Correlation between relative length of SG part V and ML | Relative length of SG part V = $-0.24 \cdot \text{ML} + 45.95$; $r^2 = 0.714$; $p = \mathbf{0.0180}$; $n = 7$ |
| Correlation between relative length of SG part VI and ML | Relative length of SG part VI = $-0.09 \cdot \text{ML} + 22.61$; $r^2 = 0.522$; $p = 0.07$; $n = 7$ |
| Correlation between relative spermatophoric sac length and ML | Relative spermatophoric sac length = $0.23 \cdot \text{ML} + 5.80$; $r^2 = 0.129$; $p = 0.43$; $n = 7$ |

Figure S2 Correlation between number of spermatophores and ML. ML – mantle length.

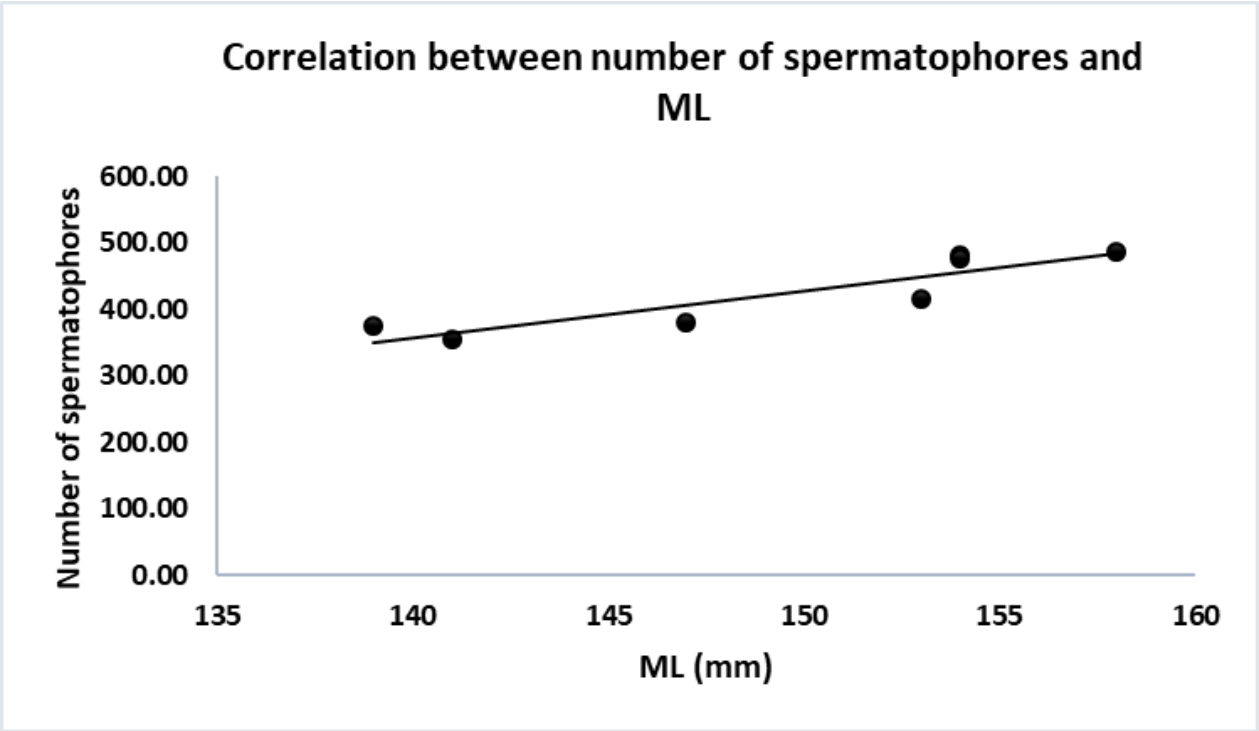


Figure S3 Correlation between number of spermatophores and weight of reproductive system.

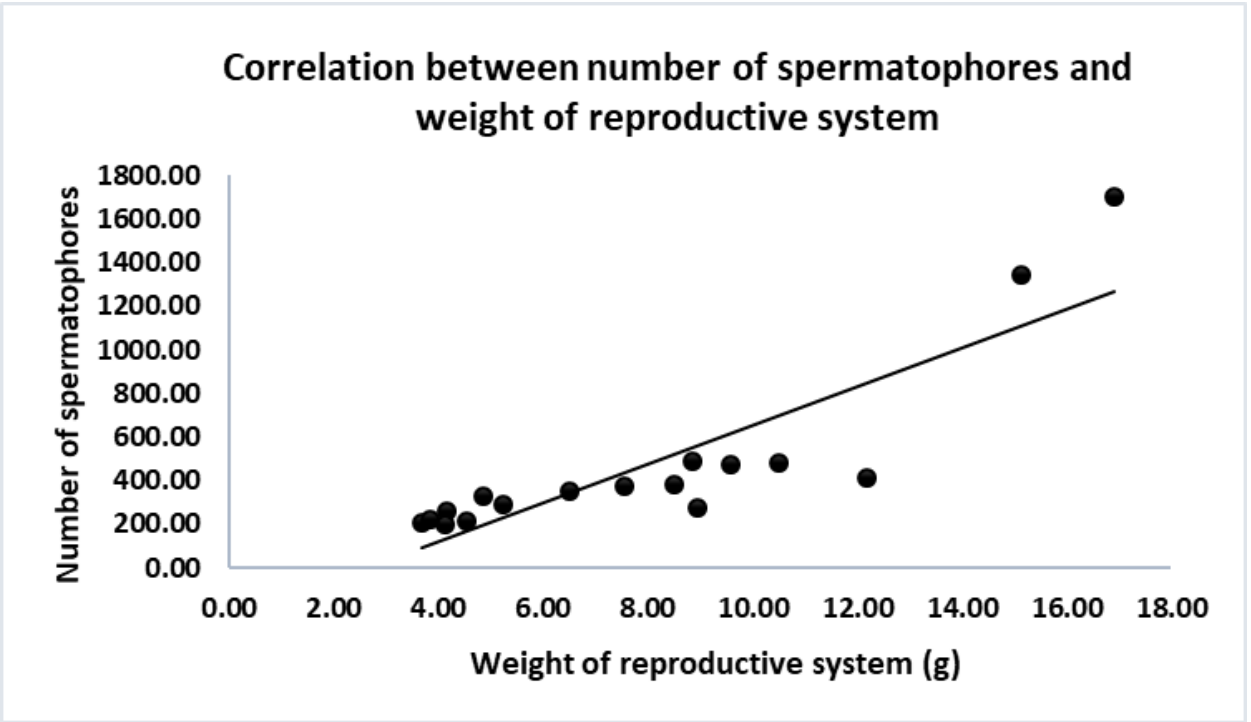


Figure S4 Correlation between number of spermatophores and total volume of sperm.

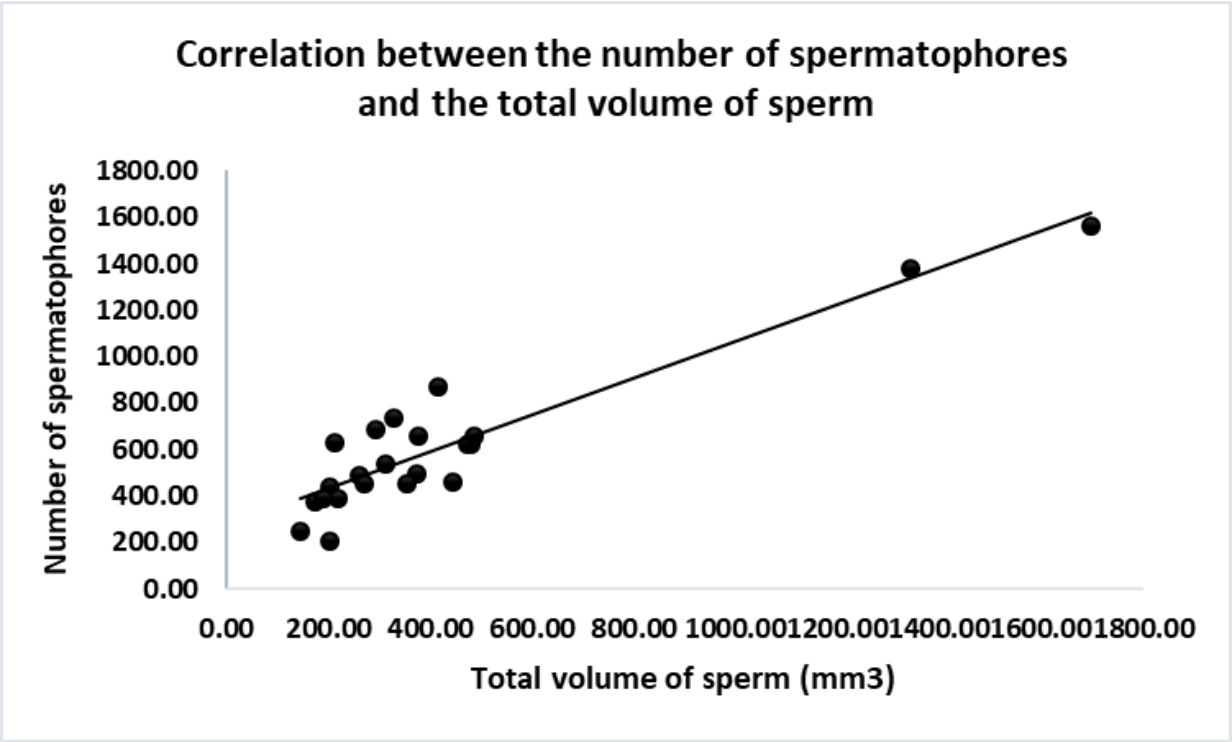


Figure S5 Correlations between absolute/relative spermatophore length and ML. ML – mantle length, SL – spermatophore length.

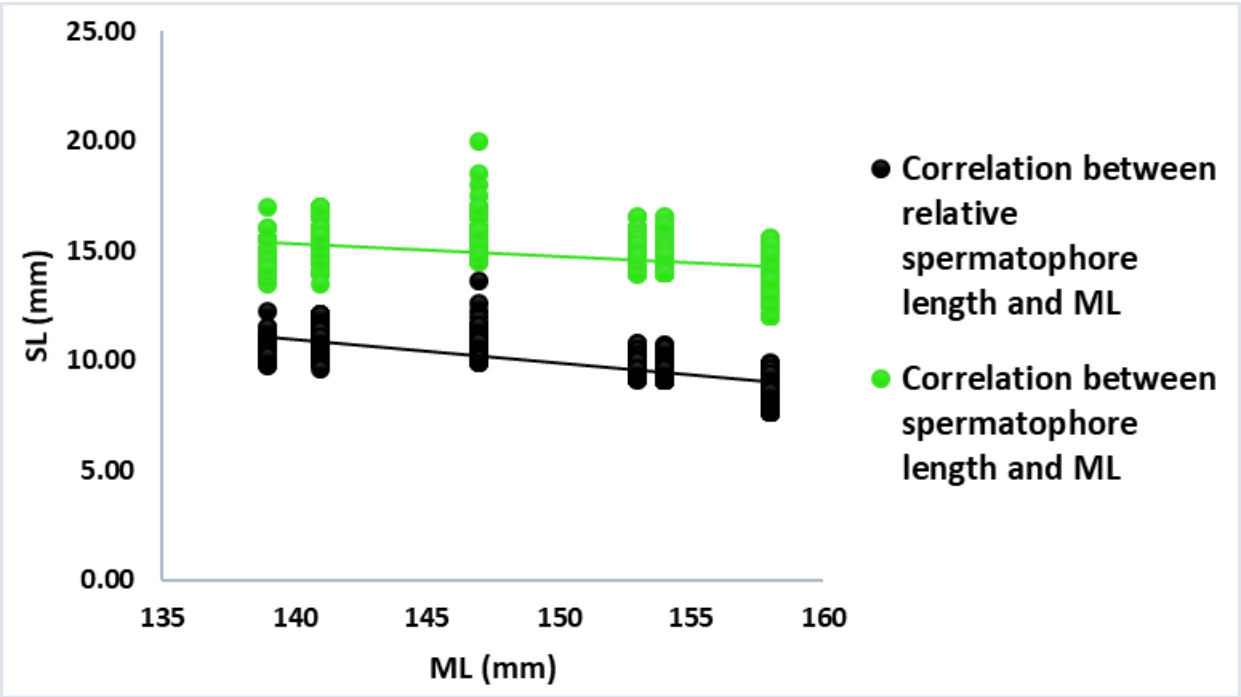


Figure S6 Correlations between spermatophore length and different parts of spermatophore. ML – mantle length, SL – spermatophore length.

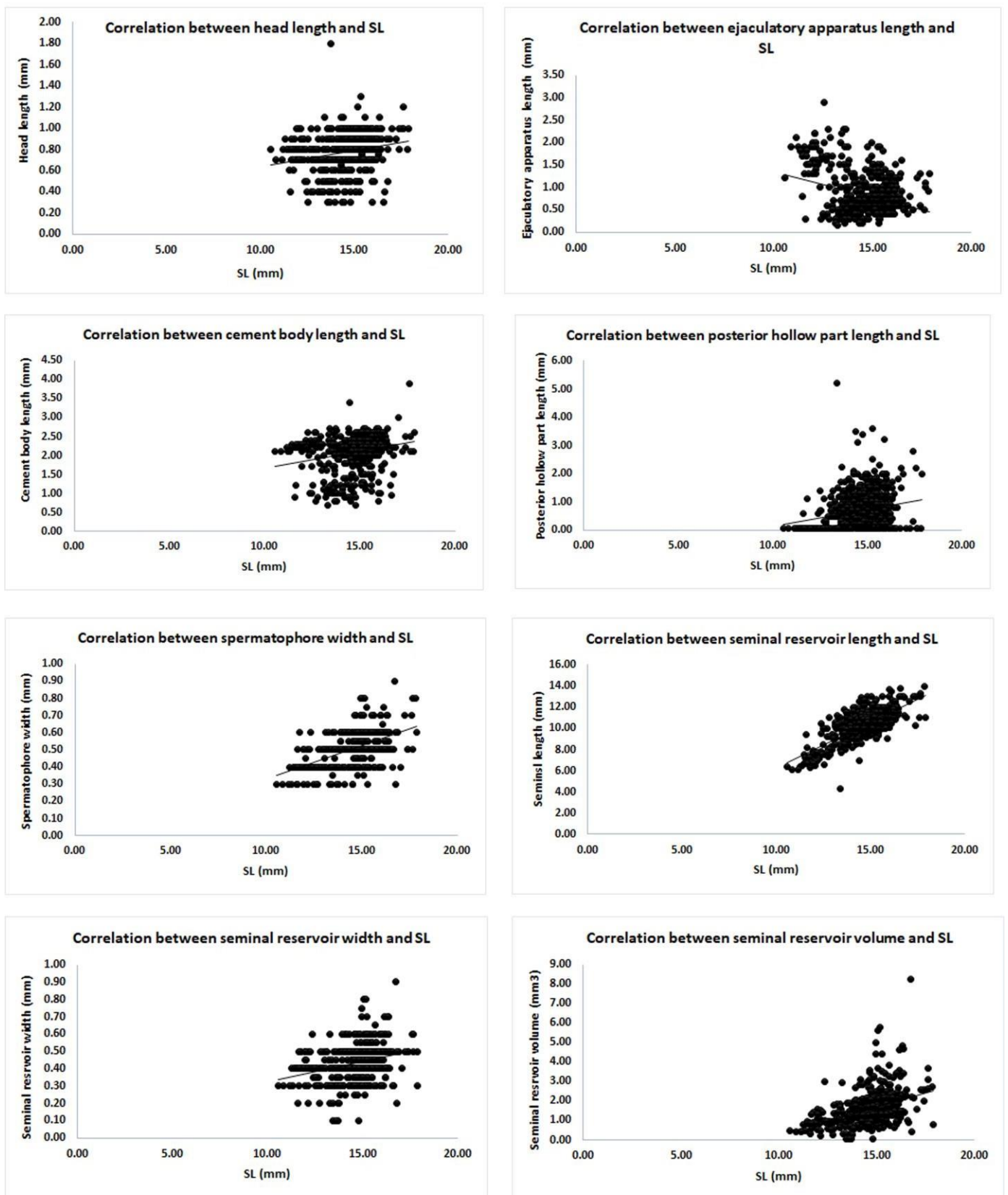


Table S4 Equations of correlations between mantle length and spermatophore length, and correlations between spermatophore length and different parts of spermatophore. ML – mantle length, SL – spermatophore length. Significant *p*-values are in **bold**

| Correlations | Equation |
|--|--|
| Correlation between spermatophore length and ML | Spermatophore length = $-0.06 \cdot \text{ML} + 23.45$; $r^2 = 0.233$; $p < 0.00001$; $n = 2570$ |
| Correlation between relative spermatophore length and ML | Relative spermatophore length = $-0.11 \cdot \text{ML} + 25.78$; $r^2 = 0.705$; $p < 0.00001$; $n = 2570$ |
| Correlation between head length and SL | Head length = $0.03 \cdot \text{SL} + 0.32$; $r^2 = 0.050$; $p < 0.00001$; $n = 752$ |
| Correlation between ejaculatory apparatus length and SL | Ejaculatory apparatus length = $-0.11 \cdot \text{SL} + 2.47$; $r^2 = 0.108$; $p < 0.00001$; $n = 752$ |
| Correlation between cement body length and SL | Cement body length = $0.09 \cdot \text{SL} + 0.78$; $r^2 = 0.057$; $p < 0.00001$; $n = 752$ |
| Correlation between seminal reservoir length and SL | Spermatophore reservoir length = $0.86 \cdot \text{SL} - 2.31$; $r^2 = 0.576$; $p < 0.00001$; $n = 752$ |
| Correlation between posterior hollow part length and SL | Posterior hollow part length = $0.12 \cdot \text{SL} - 1.04$; $r^2 = 0.042$; $p < 0.00001$; $n = 752$ |
| Correlation between Spermatophore width and SL | Spermatophore width = $0.04 \cdot \text{SL} - 0.05$; $r^2 = 0.242$; $p < 0.00001$; $n = 752$ |
| Correlation between seminal reservoir width and SL | Seminal reservoir width = $0.0234 \cdot \text{SL} + 0.0882$; $r^2 = 0.082$; $p < 0.00001$; $n = 752$ |

Table S5 Spermatophore length and number of spermatophores of the different *Sepia* spp. and *Sepiella* spp.

| Species | Spermatophore length (in mm) | Number of spermatophores |
|------------------------------|--|--|
| <i>Sepia esculenta</i> | 9.0–20.0 (15.0 \pm 0.02) (This study) | 146–1698 (422.7 \pm 79.92) (This study) |
| <i>Metasepia tullbergi</i> | 4.7–5.9 [92] | - |
| <i>Sepia bidhaia</i> | 2.9–3.2 (3.1 \pm 0.1) [53] | - |
| <i>Sepia braggi</i> | 2.4–4.4 (3.4 \pm 0.6) [53] | - |
| <i>Sepia brevimana</i> | 6–8 [93] | 200–300 [93] |
| <i>Sepia dollfusi</i> | 5.3–11.5 (8.0 \pm 1.27) [7] | 34–460 (224 \pm 99) [7] |
| <i>Sepia elegans</i> | 3.9–5.5 (mean 4.7) [16] | 167–486 (mean 370) [16] |
| <i>Sepia filibrachia</i> | 3.4–5.2 (4.5 \pm 0.7) [57] | - |
| <i>Sepia grahami</i> | 3.4–6.2 (4.5 \pm 1.1) [54] | - |
| <i>Sepia hedleyi</i> | 5.8–7.9 [55] | - |
| <i>Sepia koilados</i> | 6.3 [53] | - |
| <i>Sepia limata</i> | 2.1–3.2 (2.7. \pm 0.5) [53] | - |
| <i>Sepia mestus</i> | 2.7–4.0 (3.3 \pm 0.6) [57] | - |
| <i>Sepia mira</i> | 2.8–3.0 [52] | - |
| <i>Sepia officinalis</i> | 5–14 [8] | - |
| <i>Sepia opipara</i> | 6.6–17.1 [27] | - |
| <i>Sepia orbignyana</i> | 3.32–11.81 (7.95 \pm 1.49) [18] | 68–1055 (mean 485) [15] 32–597 (178 \pm 123) [18] |
| <i>Sepia pharaonis</i> | 5.3–11.5 (8.0 \pm 1.27) [7] | 38–530 (188 \pm 104) [7] 400–500 [93] |
| <i>Sepia plangon</i> | 4.9–9.7 [27] | |
| <i>Sepia prashadi</i> | 7–8 [93] | 600–800 [93] |
| <i>Sepia rhoda</i> | 3.2–6.1 (4.8 \pm 0.9) [53] | - |
| <i>Sepia rozella</i> | 8.1–14.0 [53] | - |
| <i>Sepia senta</i> | 4.8–6.5 (5.6 \pm 0.5) [51] | - |
| <i>Sepia sulcata</i> | 4.8–8.5 (6.0 \pm 1.5) [51] | - |
| <i>Sepia tanybracheia</i> | 4.2–4.9 (4.6 \pm 0.5) [53] | - |
| <i>Sepia vercoi</i> | 2.1–3.7 (3.2 \pm 0.6) [53] | - |
| <i>Sepiella mangkangunga</i> | 3.3–5.0 [56] | - |
| <i>Sepiella weberi</i> | 4.5–6.0 (SD 0.7) [56] | - |

Table S6 Some features of cuttlefish females reproductive biology.

| Species | Fecundity | Ripe oocyte diameter (in mm) | Ovulation pattern |
|--------------------------|---|---|-----------------------------------|
| <i>Sepia esculenta</i> | 1701–3719 (2945.1 ± 317.30) (this study) | 3.6–5.8 (5.3 ± 0.08) (this study) | group synchronous (this study) |
| <i>Sepia aculeata</i> | 206–1568 (mean 587) [6] | 1-5 (mean 3.4) [6] | - |
| <i>Sepiella inermis</i> | 216–354 [12] | 3.6 [12] | - |
| <i>Sepia pharaonis</i> | 75–1525 [7] 102–616 [15] | - | - |
| <i>Sepia dollfusi</i> | 30–273 [7] | - | - |
| <i>Sepia orbignyana</i> | 201–1532 [13] 77–806 (365 ± 179) [18] | 6.7–8.3 (mean 7.9) [13] 4.9–9.3 [18] | group synchronous [13] |
| <i>Sepia elegans</i> | 513–1190 (mean 985) [16] | 2.0–25.0 (mean 5.0) [16] | group synchronous [16] |
| <i>Sepia opipara</i> | 4473–22333 [27] | - | group synchronous [27] |
| <i>Sepia plangon</i> | 988–2781 [27] | - | group synchronous [27] |
| <i>Sepia rozzella</i> | 1198–4819 [27] | - | group synchronous [27] |
| <i>Sepia mestus</i> | - | 8.1–10.5 [57] | - |
| <i>Sepia filibrachia</i> | - | 2.7–5.2 [57] | - |
| <i>Sepia officinalis</i> | 3700–8000 (mean 5871) (in maturing and pre-spawning females) 1380-6200 (mean 3265) (in mature spawning females) [26] | 3.5–8.5 (mean 5.5 ± 1.29) [28] 6.45–7.53 [26] | group synchronous [28] |