Reproductive Behavior and Mating Strategy of Sepia esculenta Postprint

Authors: Wang Liang, Zhang Xiumei, Ding Pengwei, Liu Tianyu, Chen Siqing

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Abstract

In June 2014, continuous observation and recording of the reproductive process of Sepia esculenta were conducted in indoor large-scale tanks using a camera system. Through qualitative and quantitative comparative analysis, the behavioral characteristics during its reproductive process, including swimming, predation, courtship, fighting, mating, and egg-laying, were analyzed. The results showed that Sepia esculenta swimming primarily relies on the reaction force generated by funnel jet propulsion, with relatively weak sustained swimming ability. During the reproductive period, Sepia esculenta continues to feed, being able to detect Litopenaeus vannamei within a range of 20-38 cm, with an attack distance of 7-24 cm, and can complete the capture of shrimp within 2.1-6.1 s with extremely high success rate. Exogenous nutrition provides continuous energy support for asynchronous gonad development, batch spawning, and complex reproductive behaviors. Sepia esculenta exhibits obvious courtship behavior, and size difference is an important factor affecting courtship. Specifically, male parents tend to select females of similar or slightly smaller size, while female parents prefer larger males (larger size facilitates winning in fights). A single mating in Sepia esculenta lasts 125-398 s. Males exhibit obvious sperm removal behavior and territoriality. After mating, males accompany the female within a range of 3-24 cm, preventing other cuttlefish from approaching, and will mate again after an average accompaniment period of 61 min. Sperm removal, accompaniment, and multiple mating are key behavioral foundations for male Sepia esculenta to effectively increase paternity contribution. The research results indicate that Sepia esculenta adopts a polygynandrous promiscuous mating strategy, with both sexes exhibiting multiple mating phenomena, which can effectively improve the reproductive success of both males and females and the genetic diversity of fertilized eggs.

Full Text

Reproductive Behavior and Mating Strategy of *Sepia esculenta*

WANG Liang[1], ZHANG Xiumei[1,2,*], DING Pengwei[1], LIU Tianyu[1], CHEN Siqing[3]

[1]College of Fisheries, Ocean University of China, Qingdao 266003, China
[2]Function Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266072, China
[3]Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266071, China

Abstract

Sepia esculenta Hoyle is widely distributed in Chinese coastal waters and represents one of China' s most important economic species due to its taste and high nutritional value. This organism can reach up to 200 mm in mantle length during its short lifespan of one year or less. Mature individuals migrate to inshore areas to mate and spawn during the breeding period, which occurs from June to July in Xuejiadao, Qingdao, China. Reproductive behavior and mating strategy, including mate selection and sperm competition, determine the quality (e.g., dorsal mantle length or body weight of newly hatched larvae) and quantity of offspring, and play an important role in generating genetic diversity. Here, sexually mature *S. esculenta* were captured using a basket trap off the coast of Xuejiadao, Qingdao, China, between May and June of 2014. The reproductive behaviors of *S. esculenta*, including swimming, predation, courtship, battle, mating, and egg-laying, were analyzed using both qualitative and quantitative comparisons after observation in an indoor pool with an image pickup system. The results revealed that the swimming power of *S. esculenta* was mainly linked to the funnel and could not be sustained for long periods of time. *S. esculenta* continued to feed on *Litopenaeus vannamei* ((10.4±0.6) cm in total length) within a distance ranging from 20 to 38 cm and could rapidly catch prey (in 2.1–6.1 s), illustrating that it utilized the energy from feeding for the non-synchronous ovarian (testicular) development, batch spawning, and complex reproductive behaviors. Before mating, *S. esculenta* demonstrated obvious courtship behavior. The males tended to choose females of similar or slightly smaller sizes, whereas the females tended to choose larger males. The males competed for females and the winner had an opportunity to mate with the female. The male would begin by lightly touching the cheek of the female, and also maintained a head-to-head position during the mating. Before ejaculation, the males moved arm III to remove the sperm masses attached to the residual buccal membranes from previous matings. After sperm removal, the male held spermatophores ejected through its funnel with the base of hectocotylized left arm IV and then pressed the coiled arm on the ventral portion of the female' s buccal membrane. A successful mating lasted a relatively long time (125–

398 s), but the mating process could easily be interrupted by interference from other individuals nearby, especially other males. The male continued to guard the spawning female (staying 3–24 cm from the female), and repeated matings occurred at an average interval of 61 min during the mate guarding process. Fertilized female *S. esculenta* individuals began to lay eggs approximately 4.5 min after mating. The eggs were relatively large (long axis diameter: 16.4–20.0 mm; short axis diameter: 10.5–12.6 mm). Although males involved in guarding females delayed their search for other mates, they may have reduced the probability that the female would remate with other males. Sperm removal, guarding, and repeated mating could effectively improve the male success rate. The mating strategy of *S. esculenta* was promiscuity, and repeated instance of mating, in both males and females, could effectively improve the reproductive success rate and level of genetic diversity.

Keywords: *Sepia esculenta*; swimming; predation; courtship; mating; sex selection; promiscuity; sperm competition

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Introduction

Cephalopods are characterized by rapid growth, short life cycles, and fast generational turnover, making the reproductive period a critical stage in their life history [1-2]. Mating and other reproductive behaviors and strategies not only determine the quantity and quality of offspring in cephalopods but also significantly impact their genetic diversity levels. Scholars have reported distinct courtship, mating, and spawning behaviors in various cephalopod species including *Sepiella maindroni*, *Sepiotheuthis australis*, *Sepia officinalis*, *Sepia lycidas*, and *Sepia pharaonis* [3-9]. These species exhibit interspecific differences in swimming ability and mating intervals. *Sepia esculenta* belongs to the phylum Mollusca, class Cephalopoda, order Sepioidea, family Sepiidae. It is primarily distributed in the Yellow and Bohai Seas of China, south of Hokkaido in Japan, the southwestern waters of Korea, and the Philippine archipelago [10]. Previous studies have reported on the reproductive migration [11], broodstock domestication [12], and development of suitable spawning substrates [13-15] of *S. esculenta*. However, most reports on *S. esculenta* reproductive behavior have been qualitative descriptions of courtship, battle, and mating body characteristics [13,15-17], while systematic studies on mating strategies such as territoriality, mating systems, and sperm competition during reproduction remain lacking. This study used *S. esculenta* as the research object, conducting continuous observation and recording of its reproductive process in indoor large tanks using a camera system in May-June 2014. Through qualitative and quantitative comparative analysis, we examined behavioral characteristics including swimming, mating, and spawning during reproduction, as well as energy utilization, mate selection, and sperm competition strategies during the breeding period, aiming to enrich research content on *S. esculenta* reproductive behavior and provide scientific basis for stock enhancement, release, and resource conservation.

1. Experimental Materials

Sexually mature *S. esculenta* broodstock were captured using a ground cage net in the waters around Xuejiadao, Qingdao (35°59 N, 120°19 E) between May and June 2014. The broodstock were temporarily held in rectangular tanks (5.5 m × 2 m × 1 m) at Jintan Beach Aquatic Products Co., Ltd. in Qingdao for 48 hours.

2. Experimental Process and Conditions

From the holding tanks, we randomly selected 10 individuals in good condition with few and inconspicuous dorsal mantle injuries: males weighing (420±98) g with mantle length (14.1±1.5) cm, and females weighing (400±72) g with mantle length (13.5±1.6) cm. These were transferred to experimental tanks (2.2 m × 1.2 m × 0.7 m). Two white sea cucumber seedling baskets (43 cm × 38 cm × 30 cm) were placed at the bottom of the tank as spawning substrates. Seawater temperature was maintained at 20–23°C, pH 7.7–7.8, with 24-hour circulation. Dissolved oxygen saturation was 100%. Live *Litopenaeus vannamei* ((10.4±0.6) cm, n = 10) were provided as prey. The light cycle followed natural light conditions.

Reproductive behaviors were continuously observed and recorded from 8:00 –12:00 and 18:00–22:00 for 10 days. An HDR-AS100VR portable camera (Sony, Japan) and an ROV3.0 underwater camera system were used for continuous recording at 20 frames/s. The portable camera' s zoom function enabled individual-focused sampling [18]. Videos were recorded for backup analysis. Spawning quantities were collected and measured using vernier calipers (0.01 cm precision) to measure long and short axis diameters.

3. Data Processing and Analysis

Continuous video recordings were edited using Ulead Video Studio 15 software. Typical behavioral segments of swimming, predation, courtship, battle, mating, and spawning were randomly extracted (4 females, 4 males, 10 minutes each). Each sample was analyzed frame-by-frame. Reaction distances were measured using the known dorsal mantle length of *S. esculenta* as a scale. Predation speed, mating duration, and other temporal parameters were calculated based on embedded video timestamps. Data were processed using Photoshop CS6 for illustrations and SPSS 18.0 for statistical analysis, calculating means and standard deviations for distance, length, and time data across individuals.

1. Swimming Behavior

S. esculenta swimming primarily relies on the reactive force of funnel jet propulsion. The funnel can rotate freely to assist in changing swimming direction. Fins maintain body balance and assist swimming and direction changes through varying amplitude and frequency. Swimming behavior mainly includes forward and

backward movement. Forward swimming involves the funnel jetting water backward while fins swing rapidly to assist. Backward swimming includes normal backward swimming and rapid backward escape, with the latter often accompanied by ink ejection. During rapid backward escape, the funnel jets water forward, fins remain stationary, and the direction cannot be changed. When turning right, the head and arms first turn right, the right fin' s swing amplitude and frequency decrease, the left fin' s amplitude and frequency increase, and the funnel jets water backward to the left; left turning shows the opposite pattern.

S. esculenta has weak sustained swimming ability. Continuous swimming duration ranges from 34–240 s ((127±60) s, n = 40), covering distances of 0.3–1.0 m ((0.6±0.16) m, n = 40). After swimming, individuals typically rest for 5–150 s ((49±33) s, n = 40) in a low-frequency fin 摆动 state with slight funnel jetting. During swimming, arms are naturally extended and close together. Body color changes frequently occur during swimming, which are instinctive colorations adapted to different environmental conditions, beneficial for predation and predator avoidance.

2. Predation Behavior

Video analysis revealed that *S. esculenta* continues feeding during the breeding period. It can detect *Litopenaeus vannamei* moving within 20–38 cm ((29±5) cm, n = 40). Predation behavior consists of four stages: (1) **Attention**: The first pair of tentacles are raised high and move continuously as the cuttlefish slowly approaches the shrimp, with body color showing patchy patterns; (2) **Positioning**: The cuttlefish positions itself 7–24 cm ((16±4) cm, n = 40) from the prey; (3) **Attacking**: Tentacles rapidly eject to grasp the prey; (4) **Seizure**: Arms firmly secure the shrimp. The entire predation process is extremely rapid, taking only 2.1–6.1 s ((4.0±1.2) s, n = 40) from detection to capture, with extremely high success rates. After successful capture, *S. esculenta* typically swims to a corner of the tank to chew and swallow. Consuming one shrimp generally takes over 1 minute, after which the cuttlefish often rests. Body color changes from dark to light during feeding. Feeding rates significantly decrease during late breeding stages, with some individuals ceasing feeding entirely.

3. Courtship Behavior

Male *S. esculenta* are behaviorally active during courtship, with dorsal mantles displaying thick horizontal stripes and dense small spots. Females are relatively passive, accepting, avoiding, or rejecting pairing attempts. Females' dorsal mantles show larger spots, with indistinct horizontal stripes only faintly visible on both sides.

3.1 Chasing and Pairing Behavior When a male selects a female, its body color becomes bright and it chases the female within a range of 11–54 cm ((30±10) cm, n = 20). If the female is unwilling to mate, she continuously evades. After chasing for 21–74 s ((52±14) s, n = 20), the male rests briefly

before continuing pursuit or changing target females. If the female is receptive, they swim together for a distance.

3.2 Territorial Behavior Male *S. esculenta* frequently engage in intrasexual combat to compete for mates. Successful males guard females by swimming around them at distances of 3–24 cm ((13±6) cm, n = 20). When other males approach the female, the guarding male' s body color becomes exceptionally bright as a warning. If the intruding male is smaller or unintentionally approaches, its body color darkens. If the intruder continues approaching, the guarding male tilts its body to protect the female, raises its first pair of arms, and confronts the intruder, sometimes even ejecting ink to intimidate. If confrontation fails, the two males whip each other' s mantles with their arms or even leap out of the water for physical collisions. Larger males typically win fights (94.4%, n = 18). When size differences are substantial, fights become extremely intense, lasting up to 27–86 s ((55±17) s, n = 20). Larger males have significantly higher winning probabilities and obtain mating opportunities. Some large males even forcibly mate with non-paired females, though mating duration is shorter.

4. Mating Behavior

The mating process in *S. esculenta* is complex and consists of five stages: (1) The male uses its arms to lightly touch the female' s head. If unwilling, the female dodges and flees rapidly; if receptive, she remains stationary. The male spreads its arms to envelop half of the female' s head. (2) When the female opens her arms, the male immediately moves to envelop her entire head. (3) The male uses its third pair of arms to scrape off spermatophores previously attached to the female' s buccal membrane (sperm removal behavior). (4) The hectocotylized left arm IV holds spermatophores ejected through the funnel and presses them onto the ventral portion of the female' s buccal membrane. (5) The male' s mantle compresses forcefully twice to attach the spermatophores to the buccal membrane. The entire process uses a head-to-head mating position.

A successful mating lasts 125–398 s ((281±75) s, n = 20), with most time spent on sperm removal (2–4 s, (3.0±0.6) s, n = 20). Males remain highly vigilant during mating, monitoring surroundings to prevent intrusion by other males. When intruders approach within 21–43 cm ((34±6) cm, n = 16), males quickly release the female and engage in combat. Small males may use camouflage, slowly approaching unpaired females with faded dorsal stripes and large spots on the mantle, waiting for opportunities to mate.

5. Guarding Behavior

After mating, males continue to guard females at distances of 3–24 cm ((13±6) cm, n = 20) to prevent other males from approaching. Repeated matings occur at average intervals of 23–82 min ((61±15) min, n = 20). If guarding males lose

fights, they leave (<1 min) and intruding males quickly attempt to mate with the female.

6. Spawning Behavior

After mating, females swim to quiet corners near spawning substrates to rest for 3.2–5.3 min ((4.5±0.6) min, n = 20) before spawning. During spawning, the female approaches the substrate with arms close together, pauses for several seconds 3–5 cm away, then uses the tips of arms 1, 2, and 3 to cross and grasp the substrate. Using arm pressure and funnel jetting, she attaches eggs to the substrate. This process lasts 6.2–7.9 s ((6.9±0.5) s, n = 20). Females typically spawn one egg at a time, then rest in corners for 4.3–6.8 min ((5.3±0.6) min, n = 20) before spawning again. Eggs are relatively large (long axis: 16.4–20.0 mm ((18.2±1.3) mm, n = 50); short axis: 10.5–12.6 mm ((11.3±0.7) mm, n = 50)). Each female produces approximately 50 eggs per day initially, decreasing to about 30 eggs per day in later breeding stages. Final eggs are smaller and mostly lack tertiary egg membranes. Females preferentially attach eggs to the middle-lower portion of spawning substrates in alternating left-right patterns. When substrate coverage is low, eggs show distinct regional distribution; when coverage is high, females spawn on existing egg layers.

1. Swimming Behavior

Sepia species share similar locomotion patterns [6-7]. Jet propulsion provides the main propulsive force for *S. esculenta*, while fins assist in changing swimming direction and maintaining balance. *S. esculenta* has poorer swimming ability than *Sepia pharaonis*, requiring rest after 127 s of continuous swimming, whereas *S. pharaonis* can swim continuously for 417 s [7]. Frequent body color changes during swimming are instinctive responses to environmental conditions, beneficial for predation and predator avoidance.

2. Predation Behavior

Adult *S. esculenta* prefers feeding on xanthid crabs (*Xanthidae*), *Squilla oratoria*, and *Trachypenaeus curvirostris* [10], classifying it as an active predator. The attack distance of mature broodstock is 7–24 cm, approximately 0.5–1.7 times mantle length. While this distance increases with mantle length, it may be related to allometric tentacle growth [1]. *S. esculenta* predation is rapid and highly successful, enabled by a well-developed visual system [13] for accurate prey detection and highly developed tentacle striated muscles [24] for rapid ejection and secure grasping. Unlike *Octopus tankahkkee* which ceases feeding during breeding [9], *S. esculenta* continues feeding to support its prolonged breeding period and batch spawning pattern [13]. Feeding rates decrease significantly during late breeding stages, likely due to energy expenditure from reproductive activities.

3. Courtship

During sexual maturity and breeding, *S. esculenta* exhibits obvious courtship behavior similar to other *Sepia* species [25]. Males actively chase females while females passively accept, avoid, or reject pairing. Experimental results show males prefer females of similar or slightly smaller size, likely because pursuing larger females consumes more time and energy. Females more readily accept larger males, which correlates with larger males' higher winning probability in fights. Size is an important factor in mate selection [26-27]. Males frequently engage in intrasexual combat for mates, with fights in *Sepia* species being more frequent and intense than in octopuses [28]. Combat begins with display of body stripes and pigment spots, escalating to arm contact and body collision. Victorious males gain better access to females, and large males may even forcibly mate with non-paired females, allowing dominant individuals multiple mating opportunities and greater reproductive success.

4. Mating

Unlike the distance mating of *Octopus vulgaris* and *O. cyanea* where males show extreme territoriality [29], *S. esculenta* employs head-to-head mating in a relatively gentle process. However, if other individuals intrude, males quickly release females to engage in combat, whereas *O. cyanea* can mate while using non-hectocotylized arms to repel intruders [28]. This indicates *S. esculenta* adopts a promiscuous mating system.

Both sexes mated multiple times with multiple partners in our experiments, differing from Han' s [16] report of polyandry. This discrepancy likely relates to sample sizes—in Han' s study, the male:female ratio was 2:18, with intense male-male competition influencing sexual selection. Multiple mating by males deposits more sperm, while for females, although multiple mating consumes substantial time and energy and carries risks of injury, disease, or parasitism, it reduces fertilization failure risk from insufficient sperm and enhances reproductive success and genetic diversity [31], similar to patterns in *Apis mellifera capensis* [32] and *Rapana venosa* [33].

S. esculenta exhibits sperm removal behavior during mating, differing from *Sepia officinalis* and *S. apama* which use funnel jetting to wash away sperm [34-35]. Instead, *S. esculenta* uses its third arm pair to scrape spermatophores from previous males off the female' s buccal membrane. Sperm removal is a crucial strategy in sperm competition [36]. Mating duration in *S. esculenta* is longer than in *Sepia pharaonis* [7] and *Sepiella maindroni* [3], providing more time for sperm removal, which correlates positively with removal efficiency [37] and enhances paternity success.

Post-mating guarding occurs in many cephalopods including *S. officinalis* [34], *S. maindroni* [3], and *S. pharaonis* [7]. The 3—24 cm guarding distance represents the male' s territory. While guarding delays searching for additional mates, it effectively prevents other males from removing the guarder' s spermatophores

during subsequent matings. *S. esculenta* has longer intervals between repeated matings (61 min) compared to *S. pharaonis* (<15 min) [7], suggesting frequent mating in *S. pharaonis* may compensate for shorter mating durations (<150 s).

5. Spawning Behavior

S. esculenta spawns on substrates, similar to *Sepiotheuthis australis* [4] and *S. officinalis* [5], but differing from *O. vulgaris* which prefers spawning on nest walls [8]. Average fecundity in *S. esculenta* is much lower than in *O. vulgaris*, likely because *S. esculenta* females are smaller ((400±72) g) and produce much larger eggs (16.4–20.0 mm × 10.5–12.6 mm vs. 2.4–2.7 mm × 1.0–1.3 mm). This strategy relates to the absence of parental care in *S. esculenta*. Larger yolk and thicker tertiary egg membranes improve hatching rates, and newly hatched juveniles (5–7 mm) adapt quickly with high survival rates, unlike *O. vulgaris* juveniles which experience high mortality. Unlike *S. lycidas* which mixes eggs on the same strand [6], *S. esculenta* females have separate spawning areas, preventing sediment burial and increasing water contact area to improve hatching success. Females preferentially attach eggs to the middle-lower portion of substrates in alternating patterns.

2. Mating Strategy of *Sepia esculenta*

Reproduction is central to animal life history and a primary factor in species continuation and diversity formation [38]. In sexually reproducing species, successful mating is key to reproductive success, and the strategies employed represent adaptive features evolved over long periods.

Energy Sources: *S. esculenta* has asynchronous ovary and testis development [13], with mature eggs developing and being released in batches. This batch spawning extends hatching time sequences, benefiting juvenile dispersal and survival, but requires substantial energy investment. Complex reproductive behaviors also demand energy support. Similar to *O. vulgaris* [39], *S. esculenta* continues feeding during breeding to supplement energy for reproductive activities. In contrast, *Octopus defilippi* relies on stored reserves for sexual maturation and reproduction, showing gradual atrophy of digestive glands and other organs [40]. Studies on Argentine shortfin squid (*Illex argentinus*) also show biochemical composition changes in female tissues after spawning begins [41]. Further research is needed on energy reserve utilization in *S. esculenta* during breeding.

Mating Strategies: Both sexes invest substantial time, energy, and resources during breeding [42-43] and select mates based on secondary sexual characteristics to improve reproductive success [44]. Similar to three-spot seahorses (*Hippocampus trimaculatus*) [45], size is an important factor in *S. esculenta* mate selection. Studies on red swamp crayfish (*Procambarus clarkii*) and pine wood nematodes (*Bursaphelenchus xylophilus*) show that mating status also influences mate choice—virgin females prefer virgin males [46-47]. Whether *S. esculenta* can distinguish mating status and shows similar preferences requires further in-

vestigation. Understanding these reproductive patterns is crucial for broodstock cultivation and seed production in *S. esculenta*.

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Note: Figure translations are in progress. See original paper for figures.