

クロウミウマHippocampus kudaの卵生産様式の推定

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Short Paper

Estimating the Mode of Egg
Production in the Spotted Seahorse
Hippocampus kuda to Establish
Optimal Captive Breeding Conditions

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Abstract: This study investigated the mode of egg production in the spotted seahorse *Hippocampus kuda* to determine when females are receptive to mating. There were two distinct groups of oocytes in the ovary, the first a cohort of extremely small oocytes, and the second a cohort of oocytes that increased in size with time. Ovulation occurred once before spawning, and only oogonia and oocytes in early developmental phases remained in the ovary after spawning. The presence of a synchronous group of growing oocytes indicates that the mode of egg production in *H. kuda* is of the group-synchronous type.

Key words: *Hippocampus kuda*; Oogenesis; Syngnathidae; Spawning

Seahorses (Syngnathidae), well known for paternal care of eggs in a brood pouch (Wilson et al. 2001), are faced with overexploitation for the aquarium and traditional medicine trades (Vincent et al. 2011). Consequently, all species of seahorses are listed in Appendix II of CITES (Convention for the International Trade in Endangered Species of Wild Fauna and Flora), and interest in commercial aquaculture of these fish has increased markedly (Koldewey and Martin-Smith 2010). In recent years, many studies have been conducted to determine the most suitable environment for seahorse aquaculture. Most studies have focused on the influence of rearing conditions (e. g., food, stocking density, and the abiotic environment) on the survival and growth of juveniles, and on the maturation of brood stocks (Wong and Benzie 2003; Planas et al. 2013). On the other hand, the most suitable conditions for reproduction (e. g., gamete production, mating and egg

brooding) remains poorly understood.

In seahorse aquaculture, where stocking density is often unnaturally high compared to their natal environment (Foster and Vincent 2004), brood stocks may be exposed to an environment unsuitable for reproduction. For instance, high stocking density inevitably increases the frequency of intra- and intersexual encounters, resulting in intensive mating competition and courtship. Such a situation would increase the risk of injury or mating disruption, and reduce gamete production through stress-induction which leads to a reduction in the number and quality of offspring (Faleiro and Narciso 2013). For successful breeding in captive conditions, the rearing environment should be optimized based on the reproductive state of the brood stock (e. g., gamete production or mating phase), because suitable conditions may change with the course of the reproductive cycle. Thus, a proper estimation of the reproductive state is required for establishing the most suitable environment for each reproductive phase.

The spotted seahorse *Hippocampus kuda* Bleeker is widely distributed throughout the tropical to temperate Indo-Pacific, usually inhabiting seaweed beds in shallow waters (Kuitert 2003). They are listed as vulnerable in the International Union for the Conservation of Nature (IUCN) Red List of threatened animals, although little is known about their general biology both in the wild and aquaria. Field observations of southern Japanese populations suggest that they reach maturity at the age of 12 months and reproduce throughout the year (S. Kato personal observation). Lin et al. (2006) reported that the optimal reproductive performance (e. g., fecundity, gonad development speed and embryonic survival) of captive *H. kuda* is obtained in water temperatures of 26–28°C. In this study, the mode of egg production in *H. kuda* was investigated to establish when the female is receptive to mating. To achieve this, females were sampled at various intervals after spawning to follow the change in the developmental state of oocytes in the ovaries.

Adult males and females (four of each sex) were placed in a mating tank (45 × 90 × 45 cm) equipped with artificial holdfasts, a recirculating filtration system, and two 300W heaters to keep the water

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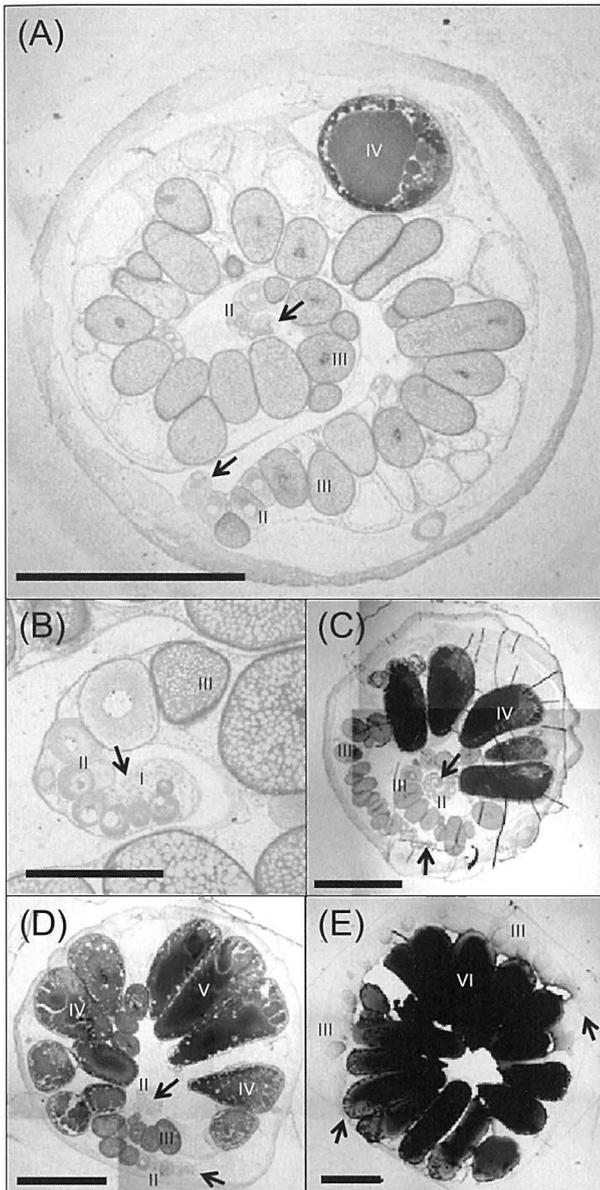


Fig. 1. Transverse section of the ovary of *Hippocampus kuda* sampled at (A) 4, (C) 8, (D) 12 days after spawning, and (E) soon before spawning. (B) An enlarged image of the germinal ridge. The scale bars represents 1 mm, except for B (200 μ m). Arrows indicate the position of the germinal ridge, and Roman numerals indicate the oocyte developmental stage.

temperature constant (26–28°C). The mating tank was established near a window facing south, and natural light adjusted to a 10L:14D light regime. Fishes were fed to satiation with frozen mysids (*Mysis* spp.) once a day. The occurrence of spawning was detected by either direct observation of copulation, including pre- and post-copulatory behaviour, or a qualitative change in the female genital papilla. After the confirmation of spawning, the females were kept in the tank until the predetermined day of sampling (i. e., 4, 8, or 12 days after spawning). Additionally, one immature female, and a female just before spawning, were sampled. The standard length (SL) of sampled females was measured as in Lourie et al. (1999), to the nearest 0.1 mm. Thereafter, these females were euthanized with an overdose of clove oil (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and their ovaries removed. The paired ovaries were fixed in Bouin’s fixative and then separated at the oviduct; one was used for histological observation, and the other for measurement of the size and number of oocytes and eggs. The preserved ovary was dehydrated in a series of ethanol and acetone treatments, and embedded in Spurr’s resin. Tissues were sectioned at a thickness of 2–3 μ m and stained with 1% toluidine blue in 0.1M sodium phosphate buffer (pH 7.4). The oocyte developmental state was staged by following the description of Begovac and Wallace (1988) for syngnathids’ (stage I, oogonia; stage II, oocyte in the primary growth phase; stage III, cortical alveoli formation phase; stage IV, vitellogenesis phase; stage V, maturation phase; and stage VI, mature egg). All oocytes and mature eggs removed from the ovary were photographed, and the number and size of oocytes and eggs as a projected area of each cell as an index of size were measured from digital images using the IMAGEJ program (National Institute of Health, USA).

The ovary of syngnathids consists of a rolled

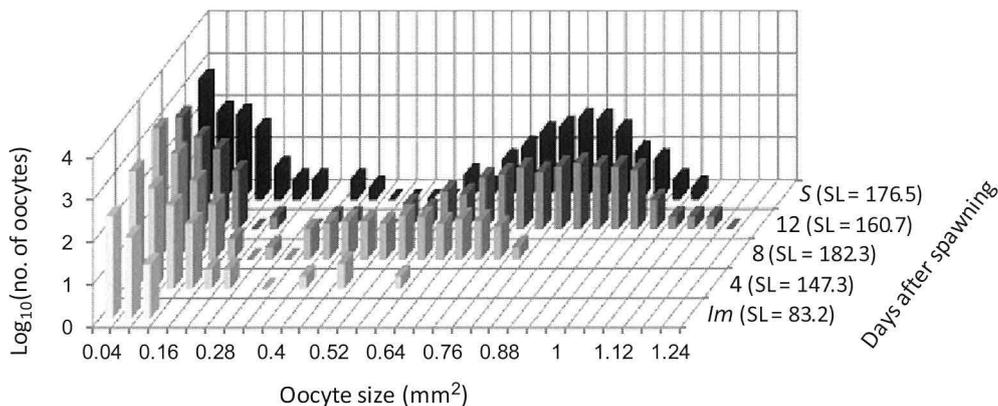


Fig. 2. Temporal changes in the size distribution of developing oocytes in the ovary of *Hippocampus kuda* in relation to the time from spawning. The number of oocytes was \log_{10} transformed. *im*, immature female; *s*, soon before spawning; SL, standard length in mm.

follicular sheet, which has stem cell compartments called the germinal ridge where the oogonia undergo proliferation, running along the entire length of the edge of the follicular sheet (Wallace and Selman 1981). Oocyte development starts at the germinal ridge, and developing follicles are arranged in sequence according to their development (Begovac and Wallace 1988). Distinct differences in ovarian structure have been reported in syngnathids. Some species have a single germinal ridge at one edge of the follicular sheet, whereas others have two germinal ridges, one at each edge of the sheet (reviewed in Sogabe et al. 2013). Transverse sections of the ovary of *H. kuda* showed the latter type of ovary (Fig. 1A, 1B). This type of ovary is also reported in the congeneric seahorses *H. erectus* (Selman et al. 1991) and *H. abdominalis* (Poortenaar et al. 2004), indicating that the ovarian type is shared among closely related species.

Temporal change in the size distribution of oocytes in the ovary of *H. kuda* is shown in Fig. 2. Only oogonia and oocytes in early developmental phases (stage I-III) were observed in the ovary of the immature female. In mature females, oocytes in the vitellogenesis phase (stage IV) were present in the ovary and these increased in size with time, whereas a cohort of extremely small oocytes (stage I-III) was observed irrespective of the timing of sampling (Fig. 1A, 1C, 1D, 1E). Oocytes in the maturation phase (stage V) first appeared in the ovary sampled at 12 days after spawning (Fig. 1D). On the day of spawning, all mature oocytes were ovulated into the ovary lumen, resulting in 349 mature eggs (stage VI), and only oocytes in early developmental phases (stage I-III) were distributed sparsely in the follicular sheet (Fig. 1E). The presence of a synchronous group of growing oocytes indicates that the mode of egg production in *H. kuda* is of the group-synchronous type (Wallace and Selman 1981), where the production of mature eggs proceeds synchronously and discontinuously. Therefore, female *H. kuda* cannot prepare mature eggs for a long period of time (>13 days) after spawning, and thus are unreceptive for mating during such a period.

This study has shown that the reproductive state of *H. kuda* females is divided into two distinct phases; an egg production and a mating phase. During the egg production phase, females are unable to mate even if a potential mate is available because there are no mature eggs in the ovary. Thus, intensive courtship from males would have a negative effect on female reproduction owing to the reduction of gamete production through stress-induction or energy allocation for coping with such a physical interaction, as suggested in Faleiro and

Narciso (2013). If it is possible to properly estimate the reproductive state of females, we are able to enhance offspring production efficiency by adjusting the rearing environment depending on the reproductive phase. Further studies should be conducted to clarify the determinant of egg production speed, the method to correctly identify the reproductive state, and then the best environment for each reproductive phase.

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