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Note

First record of planktonic egg masses of the diamond squid, *Thysanoteuthis rhombus* Troschel, in the Sea of Japan

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Abstract: This paper provides the first evidence that *Thysanoteuthis rhombus* spawns in the Sea of Japan. Five planktonic egg masses were collected in the southern Sea of Japan during 29 October to 24 November 2004 and transported to onshore laboratories for observation. Part of each egg mass was reared in indoor, aerated tanks supplied with filtered running seawater. The egg masses (ca. 60–120 cm in total length and 13–15 cm in diameter) were cylindrical with rounded ends and consisted of a resilient, transparent gelatinous core with a pair of egg rows forming spiral loops around the core. The embryos had dome-shaped mantles covered with many chromatophores and slowly rotated inside the spherical egg capsules. Hatching was observed in all the egg masses 3–10 days after collection. Morphological characteristics of the egg masses, eggs and embryos, especially the large number of chromatophores present in the early embryonic stages, agreed with the descriptions in previous studies. Near the collections sites, surface water temperature and salinity ranged 18–22 °C and 33.3–33.7, respectively, both of which were lower than the optimum conditions for *T. rhombus* spawning. The early embryonic stages of the egg masses at collection such as cleavage and slow currents at the sea surface suggest the egg masses were spawned near the collection sites. Increased abundance of *T. rhombus* in the Sea of Japan and increased sampling efforts were proposed as two possible causes for the egg-mass discoveries.

Key words: Diamond squid, *Thysanoteuthis rhombus*, Sea of Japan, Egg mass

The diamond squid, *Thysanoteuthis rhombus* Troschel, is a large oegopsid squid (maximum mantle length (ML)=100 cm) distributed worldwide in tropical and subtropical waters (Nigmatullin & Arkhipkin 1998). Around Japan, it occurs at higher latitudes than in other regions due to the existence of the Tsushima Current (Fig.1), which transports it northeastward from southern spawning areas into the Sea of Japan (Nishimura 1966). Nazumi (1975) and Okiyama (1995) suggested that this might be a "one-way" migration, with no spawning occurring in the Sea of Japan, but details of this migration are poorly known.

Thysanoteuthis rhombus spawns large, gelatinous, planktonic egg masses (Nigmatullin & Arkhipkin 1998), which have been mistaken as pyrosome colonies (Berrill 1966), and is one of the few oceanic cephalopods whose egg masses are commonly observed (Okutani 1982). In Japan, egg masses occur near the Okinawa Islands (Suzuki et al.1979, Okutani 1982, Watanabe & Segawa 1998, Watanabe et al. 1998), off the Pacific coast of Honshu (Misaki & Okutani 1976) and near the Ogasawara (Bonin) Islands and Izu Islands (Watanabe et al. 1998, Ando et al. 2004). This paper provides the first evidence that *T. rhombus* also spawns in the Sea of Japan.

During 29 October to 24 November 2004, five egg masses were collected off Hyogo and Shimane prefectures in the southern Sea of Japan (Fig.1, Table 1), which is a major fishing ground for *T. rhombus* in Japan (Miyahara & Gorie 2004, Bower & Miyahara 2005, Miyahara et al. 2005). One egg mass (EM1) was caught in a large, inshore, stationary trap net, and the other four (EM2-5) were found floating just beneath the sea surface at different locations and collected using a landing net or a bucket (Fig.1, Table 1). All egg masses were transported either immediately (EM1-3 and 5) or on the day after collection (EM4) to onshore laboratories using a 20- or 80-*l* tank filled with seawater. At the laboratories of the Hyogo Tajima Fisheries Technology Institute (for EM1-4) and the Shimane Prefectural Fish Hatching Center (for EM5), the egg masses were measured in seawater, and their characteristics, such as the shape, color, and arrangement of eggs, were recorded. Eggs were removed from the egg masses using tweezers and forceps, and the morphological characteristics of the live eggs and embryos were observed using a stereomicroscope (Olympus SZX12). The egg diameter (ED, diameter of the egg chorion) and ML of developing embryos were mea-

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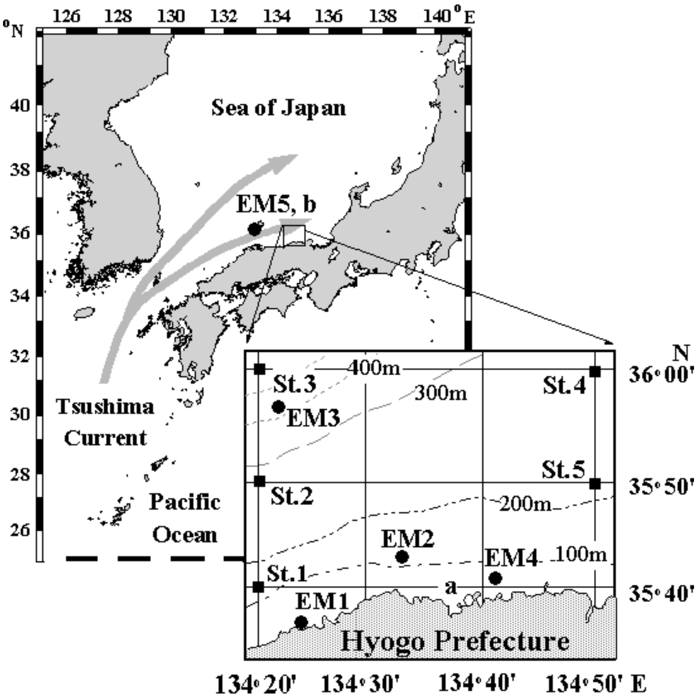

Fig. 1. Sites where five diamond squid *Thysanoteuthis rhombus* egg masses (EM1-5) were collected and oceanographic stations (St.1–5) in the Sea of Japan. Approximate isobaths are also shown. Observations on the egg masses, eggs, embryos and hatchlings were made at the Hyogo Tajima Fisheries Technology Institute (a, for EM1–4) and the Shimane Prefectural Fish Hatching Center (b, for EM5).

Table 1. Collection time, date and characteristics of five egg masses (EM1-EM5) collected in the Sea of Japan in 2004

	EM1	EM2	EM3	EM4	EM5
Collection date	29 Oct	29 Oct	2 Nov	5 Nov	24 Nov
Lat. (N)	35°37′	35°43′	35°56′	35°41′	36°05′
Lon. (E)	134°24′	134°33′	134°21′	134°41′	132°50′
Egg mass, total length (cm)	80	90–120	90	60–80	60
diameter (cm)	13	14	15	13	13
Distance (mm) between eggs	—[a]	3	3	2	—[a]
Color of eggs	purple	purple	light pink	light pink	light pink
Total number of eggs	800–1,000	18,000–23,000	23,000	16,000–22,000	—[a]
Diameter of egg chorion (mm) at collection	1.8±0.12 (30) [b]	2.0±0.09 (30) [b]	1.5±0.04 (30) [b]	1.5±0.05 (30) [b]	1.0–1.1 (6) [c]
Developmental stage at collection [d]	15–18	24–25	6–9	4–6	4–5
Number of days from collection until hatching	6–7 [e]	3–4 [e]	7–8 [e]	7–8 [e]	8–10 [f]
Hatchling mantle length (mm)	1.5±0.09 (20)	1.5±0.10 (20)	1.3±0.11 (10)	1.4±0.08 (20)	1.5–1.6 (6)

a: Not examined
b: Mean±standard deviation (sample number)
c: Range of mean (sample number)
d: Described in Watanabe et al. (1996), in which stages 4–10 are in cleavage, 11–15 are in segregation of the germ layers and growth of the blastoderm, 16–26 are in organogenesis and 27–34 are post-hatching.
e: Incubated at water temperature 20.5–21.5 °C
f: Incubated at water temperature 18.0–18.5 °C

sured using a video micrometer (Olympus VM-60). Part of each egg mass was reared in an indoor, aerated, 30-*l* tank in a water bath with a thermostat (EM1-4) or a 500-*l* tank (EM5) supplied with filtered running seawater (water exchange rate=35 L d^{-1} for EM1-4 and 1,700 L d^{-1} for EM5). The seawater was collected from near each laboratory and maintained at 20.5–21.5 °C (salinity: 32.85–33.37) for EM1-4, and 18.0–18.5 °C for EM5 (salinity not measured). The eggs were observed every morning, and the embryos were assigned to the developmental stages described in Watanabe et al. (1998) for *T. rhombus* and in Watanabe et al. (1996) for the Japanese common squid *Todarodes pacificus* Steenstrup. To determine

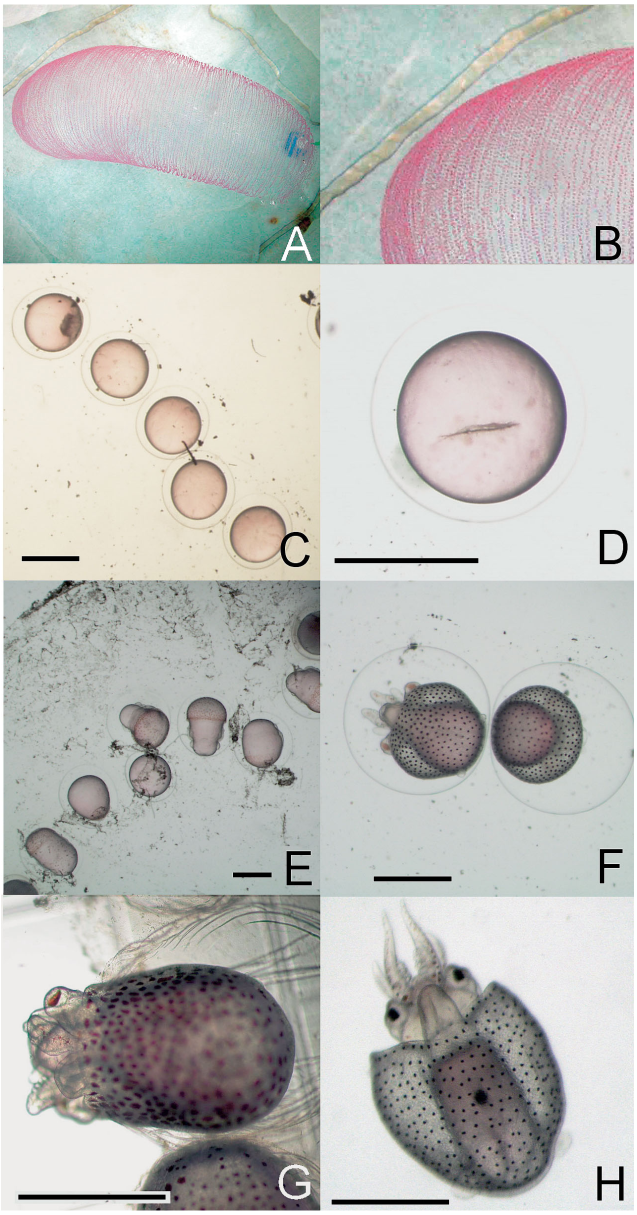

Fig. 2. Egg mass (A–B), eggs (C–D), embryos (E–G) and a hatchling (H) of *Thysanoteuthis rhombus*. A and B: Cylindrical egg mass (EM5) consisted of a resilient transparent gelatinous core and many light-pink eggs. Eggs were densely arranged in a pair of rows making spiral loops. The mass measured 60 cm in total length and 13 cm in diameter. C: Photomicrograph of eggs (from EM3) on the day after collection. Eggs were at developmental stages 6–7 of Watanabe et al. (1996). D: Eggs (from EM4) on the day after collection at stage 4 of Watanabe et al. (1996). E: Embryos (from EM1) on the day of collection at stages 16–18 of Watanabe et al. (1996). Dome-shaped mantles were covered with many chromatophores. Eye premordia and arm buds were visible on the cephalic region. F: Embryos (from EM2) on the day of collection at stages 24–25 of Watanabe et al. (1996). Pigmented eyes, a pair of tentacles with some chromatophores and suckers, a funnel, and a large internal yolk sac were visible. G: Hatching embryo (from EM5), 9 days after collection. H: Hatchling (from EM2), 4 days after collection. Scale bars = 1 mm.

the oceanographic conditions near the spawning area, water temperature and salinity were measured from 0 to 150m depths using an STD instrument (Alec Electronics, model AST1000) during 28–29 October 2004 at five stations close to where EM1-4 were collected (Fig.1).

The egg masses (Table 1, Fig. 2) were cylindrical with rounded ends and consisted of a resilient, transparent gelatinous core with a pair of egg rows forming spiral loops around the core. The egg masses measured ca. 60–120 cm in total length and 13–15 cm in diameter. Each egg mass contained 16,000–23,000 eggs except for EM1, which was damaged in the trap net and had only 800–1,000 eggs. EM1 was light brown due to detritus embedded in the surface of the core, but the other egg masses were colorless except for the purple or light-pink eggs. At the time of collection, the eggs were in different developmental stages between egg masses (Table 1, Fig. 2). Eggs in EM1 and EM2 were in the organogenesis stages (Figs. 2E, 2F). The embryos had dome-shaped mantles covered with many chromatophores and slowly rotated inside the spherical egg capsules. Eye primordial and arm buds were visible in EM1, and pigmented eyes, a pair of tentacles with some chromatophores and suckers, a funnel, and a large internal yolk sac were visible in EM2. Eggs in EM3-5 were in the early cleavage stages when they were collected (Figs. 2C, 2D), suggesting they were collected just after they were spawned.

Hatching was observed in all the egg masses (Table 1, Figs. 2G, 2H). The eggs reared from EM1 and EM2 hatched 6–7 and 3–4 days after collection, respectively. The embryos from EM3–5 entered the early organogenesis stages 3–4 days after collection and hatched 7–10 days after collection; both of these development periods are longer than in previous reports (Sabirov et al. 1987, Watanabe et al. 1998, Ando et al. 2004). In all egg masses, the ED increased before hatching, and hatchling MLs were 1.3–1.6 mm (Table 1).

Morphological characteristics of the egg masses, eggs and embryos in our study, especially the large number of chromatophores present in the early embryonic stages, were very similar to the descriptions in previous studies (Sabirov et al. 1987, Nigmatullin et al. 1995, Watanabe et al. 1998, Guerra et al. 2002, Ando et al. 2004). In our live hatchlings, the chromatophores were usually retracted and appeared as small dots (Fig. 2H), whereas in specimens fixed in formalin, they are generally expanded (Guerra et al. 2002).

At the oceanographic stations near the collection sites for EM1-4, surface water temperature and salinity were 20–22 °C and 33.3–33.7, respectively, and the thermocline occurred near 50–70 m depths (Fig. 3). The water temperature at the time of collection for EM5 was 18 °C. Currents at the sea surface in the collection area were slow (0.15–0.26 m s^{-1}) during late October to early November 2004 (The 8th Regional Coast Guard Headquarters 2004), which suggests the egg masses, particularly EM3-5, were spawned near the collection sites. Nigmatullin & Arkhipkin (1998) reported the optimum temperature and salinity for spawning are >23–24 °C and 34–35 psu, respectively, both of which were higher than those observed in this study.

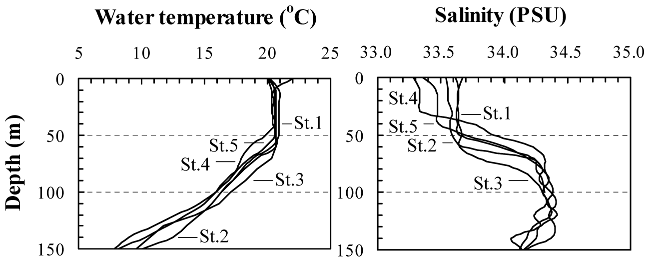

Fig. 3. Vertical profiles of water temperature (°C) and salinity at five stations near the egg-mass collection sites. See Fig. 1 for the location of the stations.

We propose two possible causes for our egg-mass discoveries: (1) Increased abundance of *T. rhombus* in the Sea of Japan associated with oceanographic conditions: Annual catches of *T. rhombus* in the Sea of Japan ranged from 0 to 600 tons from the early 1960s through 1994, but have reached 1000–3700 tons since 1995 (Bower & Miyahara 2005, Miyahara et al. 2005). This recent increase in catches has been due in part to increased biomass associated with warmer temperatures and other environmental changes in the Sea of Japan (Miyahara et al. 2005). The abundance of many squids is strongly influenced by environmental conditions (Dawe & Warren 1993, Bakun & Csirke 1998, Dawe et al. 2000, Rodhouse 2001, Waluda et al. 2004), and spawning areas presumably change as these conditions change (Sakurai et al. 2000). (2) Increased sampling effort: The Hyogo Tajima Fisheries Technology Institute initiated *T. rhombus* egg-mass search trials in 2001 and since then has distributed posters to fishermen and fishery institutes asking them to gather information and egg masses. All egg masses except EM5 were discovered by local fishermen who had been asked to help in this search.

Thysanoteuthis rhombus spawns throughout the year in tropical waters, but only during warm seasons in its peripheral spawning regions (Nigmatullin et al. 1995). Our observations took place in October and November in the Sea of Japan, but mature females (> 59–61 cm ML, Nazumi 1975, Takeda & Tanda 1998) are caught through most of the fishing season from August to February in this sea (Miyahara & Gorie 2003), which suggests that spawning might also occur here during the warm months of August and September. Our observations shows that hatching can occur at temperatures below the optimum range described by Nigmatullin & Arkhipkin (1998), though this results in an increased development period. Further investigations on the relation between environmental conditions and spawning by *T. rhombus* are needed to clarify its early life history and to determine the survival of young that are spawned in the Sea of Japan.

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