Laboratory observations on *Todarodes pacificus* (Cephalopoda: Ommastrephidae) egg masses

John R. Bower and Yasunori Sakurai

Faculty of Fisheries, Hokkaido University, 3-1-1 Minato-cho, Hakodate, Hokkaido, 041 Japan

Abstract: Two egg masses of the ommastrephid squid *Todarodes pacificus* (Steenstrup, 1880) are described. Immature squid were collected from inshore waters of southern Hokkaido, Japan, and maintained in a raceway tank where they matured, mated, and spawned. Both gelatinous masses were spherical and nearly neutrally buoyant. The larger mass measured 80 cm in diameter and contained approximately 200,000 eggs. The egg-mass surface layer effectively prevented crustaceans, protozoans, and bacteria from infesting the masses. Paralarvae hatched after 4-6 days at 18-19°C and actively swam at once, with many individuals swimming at the surface. Both masses disintegrated soon after hatching. Paralarvae died approximately 6-7 days after hatching, presumably due to starvation.

Key words: reproduction, eggs, squid, *Todarodes*, Cephalopoda

The ommastrephid squid *Todarodes pacificus* (Steenstrup, 1880) is a commercially important resource in Japan, occurring throughout Japanese coastal waters (Okutani, 1983; Murata, 1989, 1990). Many studies have focused on the fishery biology of *T. pacificus* (Murata et al., 1971, 1973; Tashiro et al., 1972; Araya, 1976; Murata, 1978); little, however, is known about reproduction. Ommastrephid squids, where known, generally produce large numbers of small eggs, encapsulated in gelatinous masses (O’Dor et al., 1982a). Few records exist of naturally spawned eggs or egg masses from oceanic cephalopods (Sabirov et al., 1987; Lapitkhovsky and Murzov, 1990), and there have been no observations of *Todarodes pacificus* egg masses in the natural habitat. [Egg masses found near the Kuril Islands and south of Japan, and attributed to *T. pacificus* by Akimushkin (1963) were presumably misidentified (Kir Nesis, pers. comm.).] All information of this critical period of the life cycle comes from laboratory observations of spawning by captive *T. pacificus*.

Spawning in captivity by *Todarodes pacificus* was first observed by Hamabe (1961a), who suggested that egg masses of *T. pacificus* are normally demersal, and either attached to the sea bottom or deposited in crevices. Hamabe kept spawning females in small barrels anchored on the sea bottom at depths of 5-20 m and obtained 15 egg masses, with each mass containing 300-4,000 eggs. Hamabe (1963) further described broken and incomplete masses spawned in small (volume < 0.13 m³) laboratory tanks.

Observation of the spawning of a captive *Todarodes pacificus* female by one of us (YS) and our colleague Y. Ikeda in 1991 revealed that *T. pacificus* can produce gelatinous egg masses that resemble those of captive *Illex illecebrosus* (LeSueur, 1821) (O’Dor and Balch, 1985). This paper describes the second such spawning by captive *T. pacificus* and is the first published description of a complete egg mass spawned by this species under laboratory conditions.

MATERIALS AND METHODS

On 1 September 1994, 20 immature squid (mean dorsal mantle length (DML) ca. 20 cm) were collected with automatic jigging machines and by hand jigging from the inshore waters of Tsugaru Strait, southern Hokkaido, Japan, and transferred to the Usujiri Fisheries Laboratory, Hokkaido University. The squid were maintained in a filtered, recirculating raceway tank (5.5 m in length, 2.5 m in width, 1.2 m in depth, and 13,000 I in capacity). The maintenance procedure followed that described by Sakurai et al. (1993). The squid were maintained for 25 days at a mean temperature of 17.3°C (range 15.8-18.5°C) while they matured and mated, and were fed a daily diet of frozen Pacific saury [*Cololabis saira* (Brevoort, 1850)], and Japanese anchovy [*Engraulis japonicus* (Temminck and Schlegel, 1846)]. The maturity and condition of the squid were monitored daily.
On 16 September, when mature eggs were first observed in the oviducts, all but four ripe females were removed from the tank. Water circulation was weakened and aeration was turned off to prevent possible damage to egg masses. Two females spawned incomplete egg masses and died on 24 September, leaving two mature females. The DML of the two remaining females measured 26.5 cm and 27.0 cm. Two egg masses were discovered on the morning of 25 September. The masses were maintained a mean temperature of 18.7°C (range 18.3-19.2°C). To facilitate the moving, viewing, and photographing of the masses, the smaller mass was held in a plankton-net container with a plastic window, and the larger mass was held in a gill net (mesh size 49 mm in stretch length) suspended from the surface. Both masses were photographed with a 35-mm camera and videotaped with a Sony CCD-V5000 video camera and an Olympus endoscope. Distances between eggs and fertilization rates within each mass were determined from the videotaped recordings. The mean inter-egg distance was used to estimate the total number of eggs in each mass.

Daily observations were made of the paralarvae. Feeding was not attempted. Individuals were removed daily for future scanning electron microscopic and statolith analysis. Paralarvae were maintained at a mean temperature of 18.8°C (range 18.4-19.2°C).

RESULTS

Spawning females

About two days before spawning, the two females stopped feeding and often rested on the tank bottom. While resting, the females' chromatophores flashed rapidly over the entire body surface in a characteristic, incandescent pattern that is indicative of imminent spawning (YS, pers. obs.). This behavior continued through spawning. Both females died within 12 h after spawning. Postmortem examination of the oviducts revealed neither female spawned all of her eggs. The 27-cm and 26.5-cm DML females had approximately 110,000 eggs and 93,000 eggs, respectively, remaining in the oviducts after spawning. The anterior ends of the nidamental glands from both females were attenuated and slightly translucent.

Egg masses

Both spherical egg masses were nearly neutrally buoyant and found floating near the surface. Both were attached to fragments of previously spawned incomplete masses floating at the surface. The two layers of the egg mass described by Hamabe (1961a, 1963) were clearly visible. Externally, the masses were covered with a jellylike secretion, presumably from the nidamental gland, and the interior of the masses, which contained the eggs, consisted of a jelly presumably secreted by the oviducal gland.

The larger egg mass measured 80 cm in diameter and contained approximately 200,000 eggs (Fig. 1A). More than 90% of the eggs within the mass were fertilized, with localized areas of unfertilized eggs evident within the mass. Infertile eggs were translucent. One side of the mass was damaged, and the jelly forming the surface layer was missing in this area; eggs from the inner core appeared to exude from this area. The smaller egg mass measured 40 cm in diameter and contained approximately 21,000 eggs. Percent fertilization of eggs within this mass was approximately 95%. All developing embryos within both stationary masses underwent development in a vertical position, with the tail pointed upwards. Eggs were positioned 0.4-2.0 cm apart throughout the inner mass. The chorion surrounding each egg expanded to diameters of 1.9-2.3 mm before hatching.

Examination of the egg-mass surface layer revealed that the outer nidamental-gland jelly was effective in preventing crustaceans, protozoans, and bacteria present in the tank from infesting the egg masses (Fig. 1B). Sections of the larger mass where the outer jelly was damaged or missing were quickly infested by bacteria and protozoans. Fragments of nidamental-gland jelly with attached eggs, presumably from the large mass or a previous failed spawning, were also seen floating at the surface. The buoyancy of these fragments was due to embedded air bubbles from the tank's water circulation system. Most of these eggs were quickly infected and died.

The small and large egg masses completely disintegrated six and seven days after spawning, respectively (Fig. 2). After disintegration, pieces of the surface-layer jelly were found on the bottom of the tank. Several pieces with a few unhatched eggs attached and with embedded air bubbles were found floating at the surface.

Paralarvae

Hatching occurred 4-6 days after spawning at ca. 19°C. This development rate was similar to that described by Hamabe (1961b) for Todarodes pacificus (4-5 days at 15-20°C). The DML of hatching paralarvae measured 1.1 mm. At hatching, paralarvae appeared to swim vertically out of the egg masses. Once free, they swam freely in the tank, with many at the surface. No paralarvae appeared to remain within either mass after hatching.

Two general swimming patterns were seen. Many paralarvae followed a general pattern of slow ascent from midwater in the tank until they reached the surface, where they swam in circular or random patterns at the surface. These paralarvae spent a long period swimming at the surface, after which they would cease swimming and sink from the surface. Another pattern seen in several paralar-
Fig. 1. Egg masses of *Todarodes pacificus*. A. The floating 80-cm-diameter spherical egg mass spawned in a laboratory tank. Positive buoyancy was due to attached fragments from previously spawned masses floating at the surface. Note the two dead spawned females on the tank bottom. Scale bar = 50 cm.

B. Top surface of the 40-cm-diameter egg mass. Crustaceans, protozoans and bacteria visible on the outer nidamental-gland jelly layer of the egg mass could not infest the egg mass. Scale bar = 5 mm.
Fig. 2. Time series showing the 80-cm-diameter egg mass held in a gill net suspended from the surface. Scale bar = 50 cm (A-F). A. 2 days after spawning. B. 3 days after spawning. C. 4 days after spawning. D. 5 days after spawning. E. 6 days after spawning. F. 7 days after spawning. Arrows indicate outline of egg mass.
vaes was a slow ascent to the surface, cessation of swimming once the surface was reached, followed by immediate sinking. Feeding by the paralarvae was not observed. Paralarvae swam with rapid mantle contractions of ca. 125 beats per minute (normal adult rate = 75 beats per minute). While swimming at the surface, many paralarvae had unidentified particles stuck to their mantle surface. Two paralarvae were also seen swimming with mucus strands approximately 2 cm in length trailing from the mantle surface.

No pattern of phototaxis was seen; paralarvae were found at the surface in uniform numbers both day and night. When we shined lights directly on the paralarvae at the surface while photographing, however, the paralarvae avoided the strong light by sinking in a somersault motion. Paralarvae at the surface displayed positive rheotaxis when a weak flow was generated from a bubbling stone. Paralarvae died approximately 6-7 days after hatching, presumably due to starvation.

**DISCUSSION**

**Spawning females**

The observed resting behavior on the bottom by mature females before spawning appears to support Hamabe's (1961a, 1963) suggestion that Todarodes pacificus is a demersal spawner. The female observed by Hamabe spawned while resting on the bottom of a barrel. Such resting behavior has been reported in other neritic ommastrephids (Bradbury and Aldrich, 1969; Boletzky et al., 1973; O’Dor and Balch, 1985; Vecchione and Roper, 1991; Young, 1995). However, while spawning could occur near the bottom in shallow inshore waters, T. pacificus likely spawns at the same depths when over deep waters, which would ensure that the nearly neutrally buoyant egg masses are maintained in the warm surface layer, where temperatures are adequate (above 12°C; Sakurai et al., 1996) for normal embryonic development. The delicate nature of the masses indicates that they are not attached to the sea bottom, as Hamabe proposed.

Our findings suggest that captive post-spawning females die with many eggs still remaining within their oviducts. Such incomplete spawning is common in captive, spawned females (Ikeda et al., 1993). In November 1994, a pre-spawning female (DML = 26.8 cm) had ca. 304,000 eggs within her oviducts (JB, pers. obs.). This count is comparable to the figures of Soeda (1956), who estimated the fecundity of Todarodes pacificus to be between 320,000 and 470,000 eggs. Future fecundity estimates of T. pacificus must consider that females do not necessarily spawn all eggs before dying.

**Egg masses**

Our observations demonstrate that Todarodes pacificus can produce nearly spherical egg masses up to 80 cm in diameter, with ca. 200,000 eggs. Hamabe (1961a) obtained 15 small egg masses, with each mass containing 300–4,000 eggs, however, the small size of the barrels used during his experiment (barrel length = 50 cm; barrel inner diameter = 33 cm) presumably prevented the formation of a complete mass.

Egg masses formed by Todarodes pacificus resemble those formed by the ommastrephid Illex illecebrosus (Durward et al., 1980; O’Dor and Balch, 1985). Percent fertilization within the T. pacificus masses, however, was significantly higher than the maximum of 40% reported for I. illecebrosus egg masses (O’Dor et al., 1980). The main difference during spawning between these species is the manner in which fertilization occurs. During egg-mass formation by T. pacificus, sperm from the seminal receptacles, located on the buccal membrane, must pass through the nidamental gland jelly and mix with the oviducal jelly and eggs in the inner layer of the egg mass. An uneven flow of sperm from the seminal receptacles to the egg mass could account for the localized variability in fertilization rates within the large egg mass. In contrast, I. illecebrosus has no seminal receptacles, and fertilization occurs when females form a mixture of concentrated jelly (nidamental and oviducal), eggs and broken spermatophores within the mantle cavity (Durward et al., 1980).

A notable difference in embryonic development rates was found between Todarodes pacificus eggs that developed within an egg mass and those reared by artificial fertilization. Hatching from the egg masses occurred 4-6 days after spawning at ca. 19°C. This developmental rate was approximately one day longer than that for T. pacificus paralarvae reared by artificial fertilization at the same temperature (Sakurai et al., 1996). O’Dor et al. (1982b) also reported delayed hatching from Illex illecebrosus egg masses. The longer developmental period within egg masses suggests that animals reared by artificial fertilization might hatch at a premature stage. Watanabe et al. (1996) confirmed that artificially fertilized T. pacificus eggs hatched approximately two developmental stages earlier than eggs within the egg masses (stage criteria defined by Watanabe).

The enveloping oviducal-gland and nidamental-gland jellies of the egg masses presumably reduce mechanical stimulation of developing embryos, a cause of premature hatching in some cephalopods (Choe, 1966).

**Paralarvae**

Durward et al. (1980) suggested that ommastrephid paralarvae might feed on microorganisms and plankton that colonize the egg mass. We saw no evidence of any feeding by the paralarvae within the egg mass after
hatching, however the longer developmental time within the egg mass indicates greater opportunity for developing embryos to absorb organics from the oviducal gland jelly.

Hatching paralarvae swim upward immediately, with many animals found concentrated at the surface. Much biological emphasis has been placed on the surface film of organic matter in the sea (e.g. Sieburth et al., 1976). Dissolved and particulate organic matter concentrations are significantly higher in the thin layer at the sea surface than in bulk seawater (Liss, 1975; Hunter and Liss, 1981). The possibility that cephalopods can use dissolved organic matter as a nutrition source has been proposed by Hanlon et al. (1991). Several studies have published evidence in support of this hypothesis (Castille and Lawrence, 1978; Vecchione and Hand, 1989). Further investigation of uptake of dissolved organics is needed, especially in the case of ommastrephid paralarvae.

ACKNOWLEDGMENTS

We appreciate reviews of the manuscript by Richard E. Young, Ron O’Dor, Michael Vecchione, John Arnold, and two anonymous reviewers. We thank Kir Nesis for his helpful communication regarding misidentified egg masses. We also thank Youetsu Arashida and Kiyoshi Nomura for their assistance during the maintenance experiment.

LITERATURE CITED


Hamabe, M. 1961b. [Experimental studies on breeding habit and development of the squid, Ommastrephes sloani pacificus Steenstrup.] Zoological Magazine 70(11):408-419. [In Japanese with English summary].


Date of manuscript acceptance: 21 May 1996