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Planktonic egg masses of the diamond-shaped squid *Thysanoteuthis rhombus* in the eastern Atlantic and the Mediterranean Sea

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Eight planktonic egg masses of the diamond-shaped Thysanoteuthis rhombus observed from 1995 to 2000 are described. Four were found in the western Mediterranean and the others were found off the Canary Islands. The egg masses from the Canary Islands are the first records for the eastern Atlantic. All were found near the surface at the end of summer and the beginning of autumn. The planktonic egg masses were dense, resilient oblong cylinders with rounded tips ranging from 80 to 130 cm in length and between 15 and 20 cm in diameter. Egg capsule dimensions ranged from 2.8 to 3.4 mm and total length of the newly hatched paralarvae was between 2.5 and 2.8 mm. Each egg mass contained an estimated 24 100–43 800 eggs. Some new characters that should help identification of the paralarvae, such as arm formulae, presence of an incipient keel-shaped membrane on some arms, and the type and chromatophore pattern are given. Ecological factors influencing the presence and distribution of these egg masses are discussed.

INTRODUCTION

The diamondback squid *Thysanoteuthis rhombus* Troschel, 1857 is a large oegopsid squid, which reaches up to 85 cm mantle length and 24 kg total body weight (Nigmatullin *et al.*, 1995), found in tropical and subtropical waters world-wide, including the Mediterranean Sea. Its distribution and migration is related to surface circulation of oceanic currents (Nigmatullin and Arkhipkin, 1998). This species is characterized by low population densities and appears to have a unique social organization for squids; they live throughout their life cycle in couples of one male and one female of similar size (Nigmatullin and Arkhipkin, 1998; Bello, 1999). There are several observations of diamond-shaped squids swimming or stranded in couples (Guerra, 1992; Bello, 1999). This species has one of the fastest growth rates among squids. Its life span is estimated to be about 1 year, and males and females mature at an age ranging from 6 to 8 months (Nigmatullin and Arkhipkin, 1998). Although females have a high potential fecundity (up to 4.8 million oocytes), their egg masses contain from 35 000 to 75 000 eggs. This indicates that spawning is intermittent with multiple episodes of oocytes filling the oviducts followed by their evacuation (Nigmatullin and Arkhipkin, 1998; Rocha *et al.*, 2001).

Thysanoteuthis rhombus is one of the few species of oegopsid cephalopods in which planktonic egg masses are known. To date, a total of 24 egg masses have been recorded world-wide. These were found drifting in the surface layers of the tropical Atlantic Ocean, the north-west and southeast Pacific Ocean and the Mediterranean Sea (Sabirov *et al.*, 1987; Guerra and Rocha, 1997; Watanabe *et al.*, 1998). Egg masses of this species are gelatinous, oblong cylinders with rounded tips, and their maximum length and diameter ranged from 60 to 180 cm and from 10 to 30 cm, respectively. Each egg mass contains a double spiral, composed of one row of eggs each, and embedded in the surface layer of the mass (Misaki and Okutani, 1976; Suzuki *et al.*, 1979).

According to Mangold and Boletzky the presence of *T. rhombus* in the Mediterranean is rare (Mangold and Boletzky, 1988). However, it is collected as a by-catch in

several pelagic fisheries in Tunisian waters, more frequently in those undertaken near the coast (Ezzeddine-Najai, 1996). Although its geographical distribution in the eastern Atlantic waters ranges from the English Channel to the Cape of Good Hope (Roper *et al.*, 1984), it was not previously recorded in either the Iberian waters or the Canary Islands (Guerra, 1992).

This paper provides data on eight *T. rhombus* egg masses observed in the western Mediterranean and the Canary Islands. A detailed description of the paralarvae and estimates of egg numbers per egg mass were made for three egg masses, emphasizing those characters that may be used to identify the early stages of development of this species.

METHOD

The first egg mass (No. 1) was observed from the research vessel 'Toftevaag' in the western Mediterranean Sea (37°11.85′N 1°31.15′E) on August 27, 1995. This egg mass was subjected to a preliminary study (Guerra and Rocha, 1997). The associated fauna was composed of drifted neuston, mainly medusa stages of different species. The fragile consistency of the egg mass led to its fragmentation during collection. However, two eggs in an early stage of development, one embryo within its egg capsule, two paralarvae within the egg capsules and 32 paralarvae in the water column were obtained. These samples were fixed in 5% formalin for 1 day and preserved in 70% ethanol. Eggs and paralarvae (mantle length and total length) were measured to the nearest 0.1 mm using a stereomicroscope equipped with a camera lucida.

The second egg mass (No. 2) was observed at the surface from the R/V 'Toftevaag' in the western Mediterranean Sea (36°31.74′N 3°48.87′W) on August 9, 2000. It was filmed for 2 min with a digital 3CCD video camera. A sample of this egg mass was preserved in 70% ethanol. Maximum length and diameter of this egg mass were 80 cm and 18 cm respectively. Eggs containing almost fully developed paralarvae were measured.

The crew of the R/V 'Toftevaag' also reported observations of two additional egg masses of *T. rhombus* floating in the western Mediterranean Sea. These were observed on July 17, 1998 (1 m total length) at 36°32.50′N 2°29.46′W and July 4, 1996 (more than 1 m total length) at 37°22.8′N 1°09.40′W.

The third egg mass (No. 3) was caught in Montaña Pelada Bay, Tenerife (28°17′N 16°31′W) on 14 October 2000. This was found floating near the surface in association with macroplankton, mainly jellyfishes. It consisted of a gelatinous mass of 130 cm total length, with maximum and minimum widths of 20 cm and 15 cm respectively. The whole mass was photographed and a sample was removed and maintained under rearing conditions. The paralarvae hatched in the aquarium and survived for 2 days. All paralarvae were preserved in 70% ethanol for further analysis.

Another two egg masses of *T. rhombus* of similar characteristics were observed off Tenerife (Point Masca 28°01′N 16°42′W and Point Vizcaíno 28°18′N 16°53′W) and an additional one in the Orchilla harbour (27°43′N 18°9.5′W), El Hierro (Canary Islands) in October 2000.

RESULTS

All egg masses observed were dense, resilient and cylindrical with round ends (Figures 1a–d and 2d). Measurements and estimates of egg numbers in the three egg masses collected are shown in Table I. The egg capsules are purplish in colour and are disposed in two rows spirally arranged in the outer surface of the cylinder (Figure 2d). The diameter of the egg capsules that contain fully developed embryos ranged from 2.8 to 3.4 mm (Figure 2e). Considering the measurements of each egg mass, the maximum diameter of the egg capsules, an estimate of the surface of each egg mass and the fact that the surface occupied for the eggs is approximately half of the total surface of the egg mass, we estimated a total number of eggs per egg mass ranging from 24 100 to 43 800 (Table I).

The average dorsal mantle length of the paralarvae was 1.8 ± 0.2 mm (n = 132) and its total length varied from 2.5 to 2.8 mm. Most of the paralarvae observed had the head inside the mantle cavity, showing only the arms and the tentacles externally (Figure 1e,f). The mantle of the paralarva is oval, short and blunt posteriorly. The anterior margin of the mantle is concave on both dorsal and ventral sides, more so ventrally. The fins are subterminal, small (0.54 × 0.4 mm) and paddle-shaped, and the fin length is 6.6% of the mantle length (Figure 2 a,b). Paralarvae have broadly separated, slightly protruding eyes with four reddish chromatophores at their base (Figure 2b). Paralarvae also have a funnel locking cartilage (Figure 2c) with a short, broad transverse groove and a long relatively wide, longitudinal groove. Each tentacle has 16–18 reddish chromatophores disposed in one row and three more at the base. Tentacles comprise about 60% of the mantle length, and are stouter and slightly longer than the longest arm (II). The tentacular club is not differentiating, but there are between 13 and 14 suckers on the one-third of tentacle length. The distal portion of the tentacle avoids armatures, and terminates in an acute tip. Arm length is one-third of mantle length. Arms III and IV are rudimentary. The arm formulae is II . I . IV . III (dorsolateral arms > dorsal > ventral > ventrolateral). Developed arms have small suckers arranged in two rows. An

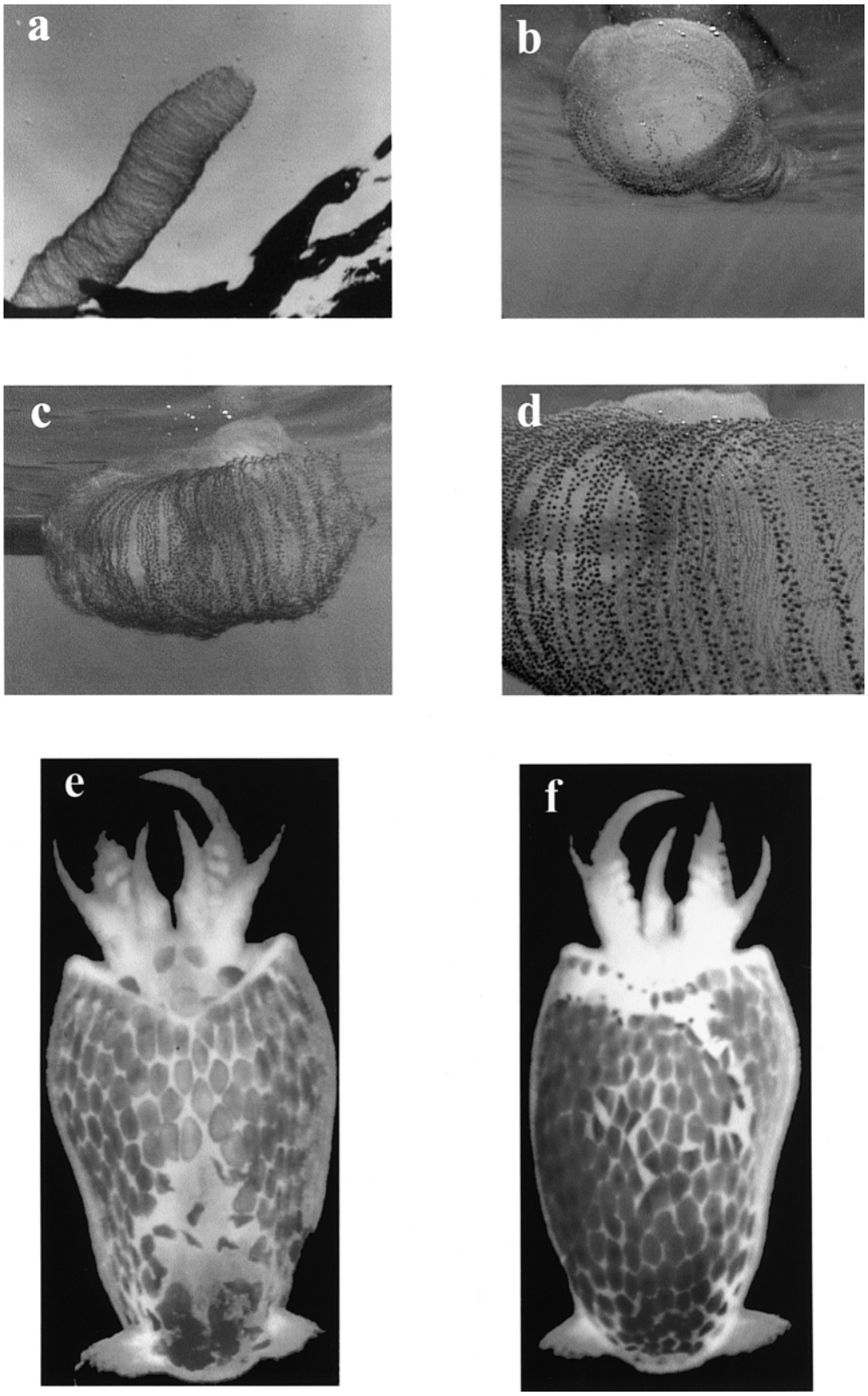

Fig. 1. Different perspectives of a floating planktonic egg mass of *Thysanoteuthis rhombus* (length 100 cm and width 20 cm) collected in the western Mediterranean Sea (36°31.74′N 3°48.87′W) on August 9, 2000 (**a–d**); Dorsal (**e**) and ventral (**f**) views of a newly hatched paralarvae (mantle length 1.5 mm) showing the expanded chromatophores in the mantle and the head inside the mantle. I–IV, arms; t, tentacle.

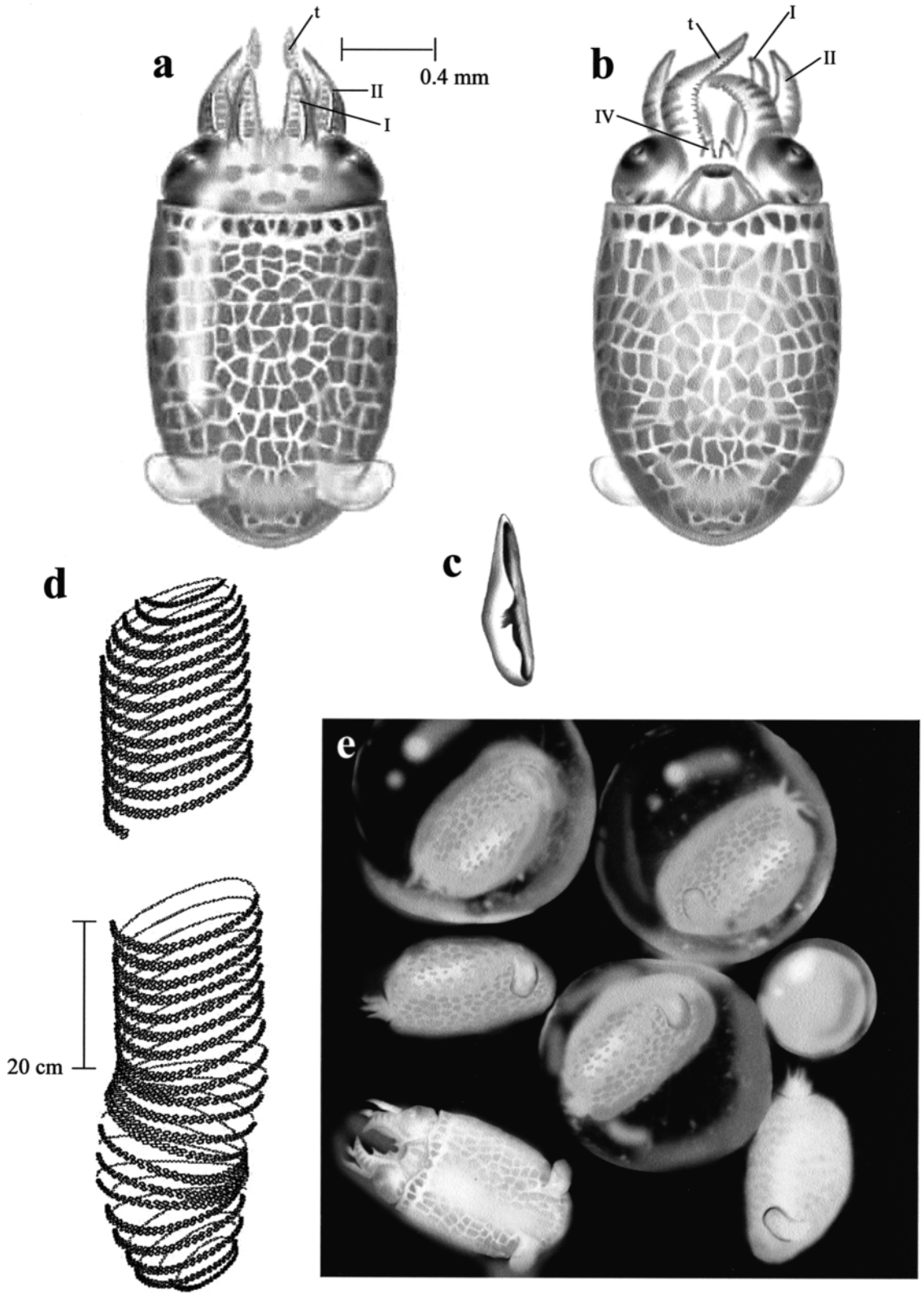

Fig. 2. Dorsal (**a**) and ventral (**b**) view of a paralarvae of *Thysanoteuthis rhombus* showing the shape and disposition of the chromatophores in the head, mantle, arms and tentacles; funnel locking-cartilage (**c**); schematic view of a floating egg mass showing the eggs disposed in spiral pairs (**d**); egg without developed embryos, paralarvae within the egg capsule and newly hatched (**e**).

Table I: Dimensions and total number of eggs contained in each egg mass studied

Egg mass	Length (cm)	Width (cm)	Area (cm^2)	Total no. of eggs
1	100	20	6280	33 400
2	80	18	4522	24 100
3	130	20	8164	43 800

incipient swimming keel membrane was present on arms I and II. A trabeculate protective membrane was absent in arms and tentacles.

The paralarvae have two types of chromatophores: the first, large and reddish densely concentrated on dorsal, lateral and ventral sides of the mantle (Fig. 1e), and the second, yellow chromatophores on the dorsal sides of the arms. Dorsally, at the anterior end of the mantle, there is a line of 16 chromatophores, and there are 150 more posterior to these forming a mosaic. Ventrally, at the anterior end of the mantle, there is a line of 16–20 chromatophores and there are about 250 posteriorly. On the dorsal part of the head, there are seven chromatophores, two of them at the attachment of the first pair of arms, another two situated laterally and the remaining ones are very close to and ventral to the others. On the arms, there are single lines of three to five chromatophores. The fins lack chromatophores.

DISCUSSION

The observations of *T. rhombus* reported here are the first records for the eastern Atlantic and the second for the Mediterranean Sea. The first egg mass observed in the Mediterranean was reported by Sanzo in the Strait of Messina (Sanzo, 1929). Our observations extended the possible spawning area far west in the Mediterranean Sea, as indicated by Guerra and Rocha (Guerra and Rocha, 1997). The Canary Islands records are the first sightings of egg masses in these waters, where adults had already been found (Nigmatullin *et al.*, 1995). These new records may be the result of an increase in abundance, as proposed by Ezzeddine-Najai (Ezzeddine-Najai, 1996) in Tunisian waters, or an increase in sampling effort.

The egg masses from the Mediterranean Sea were captured or observed near the surface in the zone where the inflow of Atlantic water is high due to the proximity of the Strait of Gibraltar (Speich *et al.*, 1996). The first egg mass of this species was observed by Sanzo (Sanzo, 1929) in the Strait of Messina (Mediterranean Sea) where there are strong currents (Astraldi *et al.*, 1996). The Atlantic egg masses reported here were located in surface waters around the Canary Islands, an area characterized by intense mesoscale structures (eddies) and where the Islands present obstacles to the trade winds and to the Canary Current flowing towards the equator past them (Barton *et al.*, 1998, 2000). Elsewhere, egg masses of *T. rhombus* have occurred in regions with strong warm currents, such as the Kuroshio, the Peru counter current and the Equatorial counter current (Yamamoto and Okutani, 1975; Nigmatullin *et al.*, 1995). Therefore, as in the Pacific, in the western Mediterranean and the central-east Atlantic the species seems to spawn in waters associated with strong currents.

Nigmatullin *et al.* indicated that *T. rhombus* spawns throughout the year in tropical waters, but during the warm season (summer and early autumn) in peripheral regions (Nigmatullin *et al.*, 1995). Our observations support this pattern. The egg masses observed in the western Mediterranean and central-east Atlantic have a shape and a size which coincide with those illustrated by others (Sanzo, 1929; Misaki and Okutani, 1976; Suzuki *et al.*, 1979).

The egg numbers estimated here for each egg mass of *T. rhombus* are similar to the range estimated by Sabirov *et al.*, which were from 32 000 to 76 000 eggs (Sabirov *et al.*, 1987). The eggs number estimated for egg mass No. 1 is considerably lower than that given by Guerra and Rocha, since these authors considered that the whole surface of the cylindrical egg mass was occupied by eggs (Guerra and Rocha, 1997).

The sizes and body shapes of the paralarvae found coincide with those given by Clarke and Stephen (Clarke, 1966; Stephen, 1992). We agree with Watanabe *et al.* that the arms III on hatchling of this species are very primordial and small-bud-shaped (Watanabe *et al.*, 1998), and that the previous interpretations on the length of these arms (Misaki and Okutani, 1976; Guerra and Rocha, 1997) were misconceptions. In the case of Guerra and Rocha this was due to a misinterpretation of arms II and III instead of arms I and II. The estimated numbers of chromatophores in the dorsal and ventral mantles of hatchlings given in this study agree with those from Watanabe *et al.* (Watanabe *et al.*, 1998).

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