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MORPHOLOGICAL CHANGES WITH GROWTH IN THE PARALARVAE OF THE DIAMONDBACK SQUID *THYSANOTEUTHIS RHOMBUS* TROSCHEL, 1857

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ABSTRACT: *Thysanoteuthis rhombus* paralarvae ranging from 1.0 to 15.0 mm in dorsal mantle length (DML) are described, based on specimens collected from western Australian waters. Paralarvae smaller than 3.0 mm DML possessed a round mantle with many chromatophores, long tentacles, and small fins. At 3.0–6.0 mm DML, paralarval arms grew rapidly and a primordial protective membrane developed simultaneously. By 15.0 mm DML, the shape of the mantle had become similar to that of an adult, and fins occurred along the entire length of the lateral mantle. Although arm suckers were present, they were not prominent and were absent from the distal one-third of the arms in specimens smaller than 6.0 mm in DML. The development of the protective membranes and the relatively long arms in the post-larval stage may be adaptations for floating in ocean currents rather than for active swimming, and suggest that *T. rhombus* might have a long planktonic phase. The development of beak rostra and the disappearance of lip cilia occurred at 6.0–8.0 mm DML, which may reflect a change in feeding habitat and may define the end of the paralarval stage in *T. rhombus*.

INTRODUCTION

The diamondback squid *Thysanoteuthis rhombus* Troschel (Family Thysanoteuthidae) is a large nektonic squid that occurs in tropical and subtropical waters worldwide (Okutani, 1995; Nigmatulin and Arkhipkin, 1998). Several studies have reported and reviewed the biology and fisheries science of *T. rhombus* (Nishimura, 1966; Nazumi, 1975; Nigmatulin *et al*, 1991; Nigmatulin and Arkhipkin, 1998). Other studies have described the embryos and paralarvae of *T. rhombus* (Issel, 1920; Sanzo, 1929; Yamamoto and Okutani, 1975; Guerra and Rocha, 1997), and it is one of the few oceanic squids whose egg masses have been described (Sanzo, 1929; Misaki and Okutani, 1976; Suzuki *et al*., 1979; Guerra and Rocha, 1997; Guerra *et al*., 2002). Some studies have reported on the morphology of hatchlings based on oceanic egg masses, but the paralarval stages have been described only from oceanic specimens (Watanabe *et al*., 1998; Billings *et al*., 2000).

A reconsideration of paralarval morphology in cephalopods has been necessary since the term "paralarva" was proposed by Young and Harman (1988). *T. rhombus* is a relatively inactive migrant (Nigmatulin and Arkhipkin, 1998). This study describes in detail the morphology of the paralarval stages of *T. rhombus* based on several characters previously unobserved with optical and scanning electron microscopy (SEM). The purpose of this research was to explore the functional morphology of the paralarval phase of the inactive migrant, *T. rhombus*.

MATERIALS AND METHODS

The specimens used for this study were collected during seven survey cruises in western Australian waters by the R/V *Shoyo-Maru* (Fig. 1, Table 1). Samples were collected with (1) oblique hauls of the upper 50 or 100 m using a bongo net (0.7 m in mouth diameter, 0.5 mm mesh) and (2) surface horizontal tows using a large larva net (2 m in mouth diameter, 0.5 mm mesh, Table 1). Samples were fixed on board the ship with 10% formalin or 70% ethanol and later preserved in 40% isopropanol or 70% ethanol.

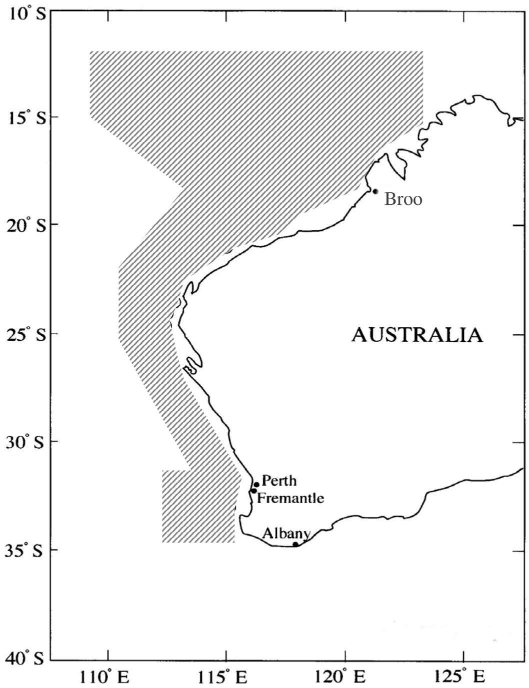

Figure 1. Sampling area in this study.

A binocular optical microscope with a micrometer was used for observations and measurements. The measurements, indices, and terminology presented in this study follow those of Roper and Voss (1983). The specimens used for SEM observations were dehydrated in graded ethanol and t-butyl alcohol, freeze-dried, and coated with gold. Mouth parts, tentacles, arms, and suckers were observed and measured with two SEM models – a JEOL JSM-100 and a TOPCON SM-200.

DESCRIPTIONS

A total of 126 *T. rhombus* paralarvae with DMLs ranging from 1.0 to 15.0 mm were collected at 99 stations.

The mantles of specimens of 1.4 mm DML were rather thin, sac-shaped, and contained about 200 chromatophores in the dorsal and ventral mantle regions (Fig. 2A). In 4.2 mm DML specimens, the mantle appeared dome-shaped (Fig. 2C). However, 7.0 mm DML paralarvae had cylindrical mantles that tapered abruptly in the posterior direction (Fig. 2D). The mantle became spindle-shaped and similar to that of an adult in 15.0 mm DML specimens (Fig. 2E). Specimens of 2.4, 4.2, 7.0 and 15.0 mm DML had mantle width indices (MWI) of 91.7, 73.8, 58.6 and 54.0% respectively.

In 1.4 mm DML specimen, the fins were very small, round, and located somewhat anteriorly to the mantle's posterior end (Fig. 2A). The fins were separated from each other and had a fin length index (FLI) of 15%. In 4.2 mm DML specimens, the fins grew longitudinally, had a FLI of 31%, and reached the mantle's posterior end (Fig. 2C). At 15.0 mm DML, the FLI was 86%, and the fins extended to almost the entire length of the lateral mantle (Fig. 2E).

At 1.4 mm DML, the paralarval head was trapezoid with small, stalked eyes (Fig. 2A). In 7.0 mm DML specimens, the head was subcubic and the eyes had become sessile (Fig. 2D).

For 1.4 mm DML paralarvae, the arm formula was I = II > III = IV. Arm II was about 35% of the DML, and two suckers with chitinous rings

Table 1. Period and towing methods by cuise.

Cruise	Period	Bongo net	Larva net
1	8 Jun.- 12 Mar., 1987	Oblique tow (50-0 m)	Surface horizontal tow
2	13 Dec., 1987 - 12 Jun., 1988	Oblique tow (50-0 m)	Surface horizontal tow
3	7 Dec., 1988 - 12 Jun., 1989	Oblique tow (50-0 m)	Surface horizontal tow
4	8 Oct. - 4 Dec., 1989	Oblique tow (50-0 m)	Surface horizontal tow
5	19 Nov., 1990 - 7 Jun., 1991	Oblique tow (50-0 m)	Surface horizontal tow
6	22 Dec., 1991 - 18 Feb., 1992	Oblique tow (100-0 m)	
7	28 Dec., 1992 - 24 Feb., 1993	Oblique tow (100-0 m)	

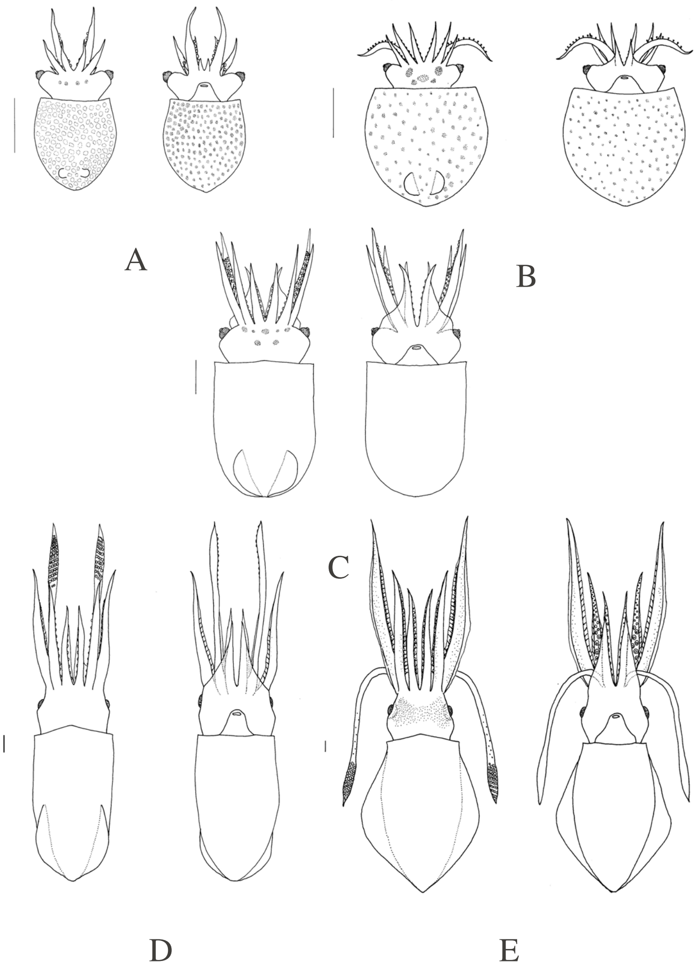

Figure 2. Dorsal and ventral views of paralarvae of *Thysanoteuthis rhombus*. A. 1.4 mm DML. B. 2.4 mm DML. C. 4.2mm DML. D. 7.0 mm DML. E. 15.0mm DML. Scale bars = 1.0 mm.

occurred on the proximal ends of arms I and II (Fig. 3A). Arms III and IV were bud-like without suckers. In 2.4 mm DML specimens, arms II and III had increased in length, the arm formula had changed to II > I > III > IV, and the protective membrane of arm IV had become observable (Fig. 2B). In 2.9 mm DML specimen, four large suckers with chitinous rings had developed proximally and twelve small suckers without chitinous rings occurred in the middle, but there were no suckers on the distal tip of arm II. In 3.4 mm DML, four large suckers with chitinous rings occurred proximally and twenty small suckers without chitinous rings were evident in the middle, but no suckers had developed on the distal tip of arm II (Fig. 3E and Fig. 3F). At 4.2 mm DML, the protective membranes of arms I–III had developed and were clearly differentiated; the arm formula had changed to II > III > I > IV, and arm II had grown to 60% of DML (Fig. 3C). By 7.0 mm DML, the arm formula became III > II > I > IV, and arm III equaled the mantle in length. At 9.6 mm DML, suckers had developed on the distal tips of the arms (Fig. 3D). The length of arm III had increased to 120% of DML in 15.0-mm DML specimens.

The 1.4 mm DML specimens had tentacles that were 60% of the DML longer than the arms (Fig. 2A). Tentacular suckers were arranged in

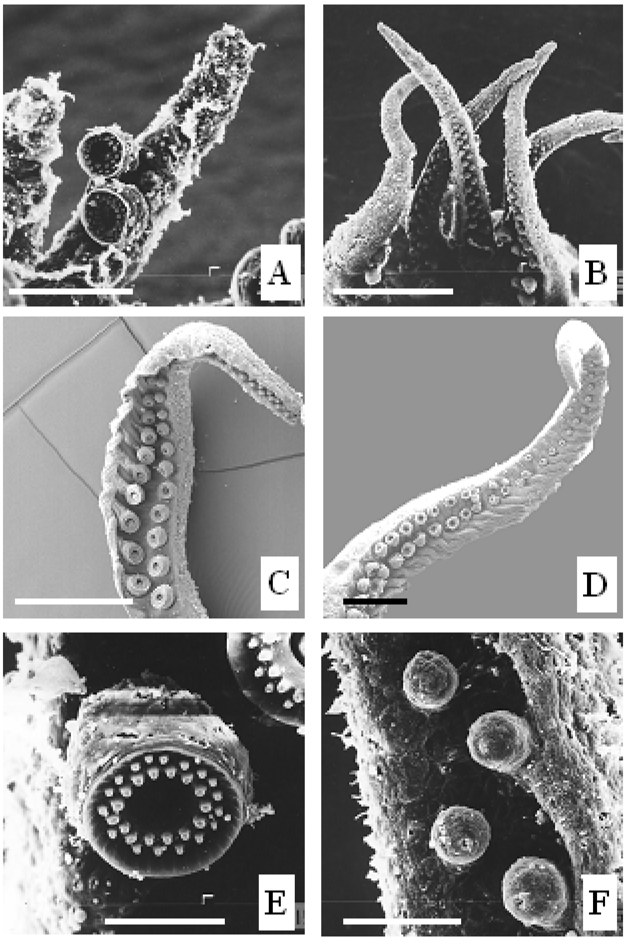

Figure 3. Scanning electron micrographs in *Thysanoteuthis rhombus*. A. Arm II, 1.4 mm DML. Scale bar = 0.1 mm. B. Arm I-III, 2.9 mm DML. Scale bar = 0.5 mm. C. Arms II, 5.4 mm DML. Scale bar = 0.5 mm. D. Arm II, 9.6 mm DML. Scale bar = 0.5 mm. E. Proximalsucker of Arm II, 3.3 mm DML. Scale bar = 0.05 mm. F. Medial suckers of Arm II, 3.3 mm DML. Scale bar = 0.05 mm.

two transverse and four longitudinal rows on the proximal half of a tentacle. Additionally, the distal portions of tentacles terminated in acute tips (Fig. 4A). At a paralarval size of 2.9 mm DML, the tentacular club occupied 70% of the total tentacle length and had expanded slightly, with suckers arranged in eight transverse and four longitudinal rows. At a DML of 5.4 mm, sixteen transverse and four longitudinal rows of tentacular suckers occurred on the manus, whereas five pairs of suckers occurred in double rows on the carpus (Fig. 4C). In 9.6 mm DML specimens, the tentacular suckers were arranged in seventeen transverse and four longitudinal rows on the manus, but six pairs occurred in double rows on the carpus (Fig. 4C).

The tips of the upper and lower beaks of 2.9 mm DML paralarvae were not pointed (Fig. 5A). Minute dentitions were present on the cutting edge of the lower beak, and cilia occurred around the entire lip (Fig. 5A). In 3.4 mm DML paralarvae, the lower beak's rostrum was developed slightly, but minute dentition remained on its cutting edge (Fig. 5B). In 5.4 mm DML specimens, the lower beak remained blunt, but beak serrations had become smooth and lip cilia had disappeared (Fig. 5C). At 15.0 mm DML, the rostra of the upper and lower beaks of the paralarvae had become pointed (Fig. 5D).

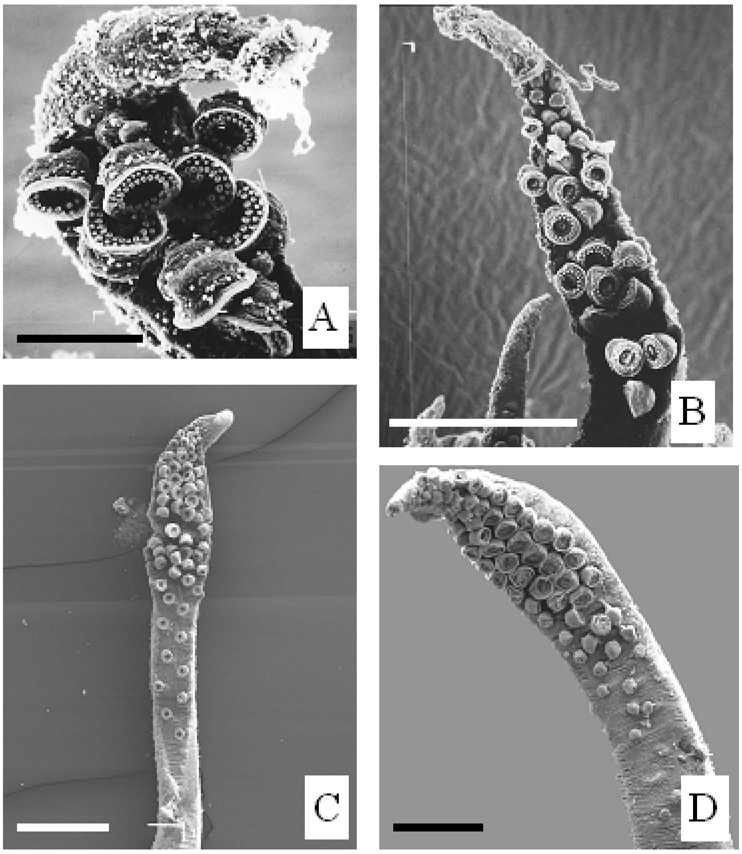

Figure 4. Scanning electron micrographs of tentacle in *Thysanoteuthis rhombus*. A. 1.4 mm DML. Scale bar = 0.1 mm. B. 3.4 mm DML. Scale bar = 0.5 mm. C. 5.4 mm DML. Scale bar = 0.5 mm. D. 9.6 mm DML. Scale bar = 0.5 mm.

DISCUSSION

This study is the first to describe *T. rhombus* paralarvae, ranging in size from 1.4 mm to 15.0 mm DML, in terms of both conventional and several new characters, such as the structure of suckers, beaks and lip cilia. Previously, morphological changes that occur with the growth of paralarvae and juvenile *T. rhombus* collected from the sea have been described for specimens with DMLs of 1.1 to 18.0 mm. Issel (1920) described paralarvae with DMLs that ranged from 4.5 to 18.0 mm. Sanzo (1929) studied paralarvae with DMLs up to 4 mm. Yamamoto and Okutani (1975) also studied paralarvae with DMLs that ranged from 1.1 to 9.9 mm. The morphological changes that occur in conventional characters such as the mantle, fins, and the protective membrane of the arms and tentacles with growth have been commonly observed, as in this study.

The morphology of hatchlings has also been described during observation of embryonic development. Watanabe *et al*. (1998) showed that hatchlings have only one or two suckers near the base of arms I and II, and that in 4- to 5-day-old paralarvae the distal portion of tentacles terminates in acute tips. In this study, paralarvae specimens with DMLs ranging from 1.4 to 7.0 mm also had arms and tentacles with acute tips. However, the arrangement of arm suckers was unique as compared to that in the paralarvae of other oceanic squids, such as ommastrephidae and onychoteuthidae

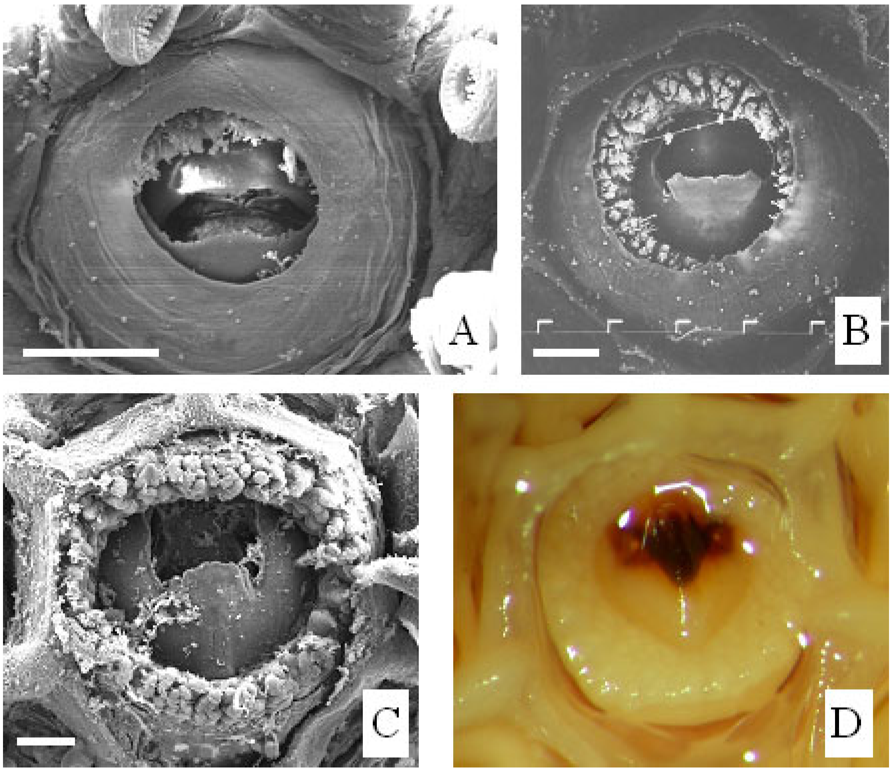

Figure 5. Scanning electron micrographs and biological micrographs of mouth part in *Thysanoteuthis rhombus*. A. 2.4 mm DML. B. 3.4 mm DML. C. 9.6 mm DML. D. 15.0 mm DML. 0000Scale bars = 100ìm

(Wakabayashi, 2002). Although the developmental stages of arm suckers can vary with arm position in ommastrephid and onychoteuthid paralarvae, in *T. rhombus* paralarvae arm sucker development clearly occurs in two separate stages. The proximal suckers of *T. rhombus* develop with chitinous rings, whereas the middle suckers are bud-like in shape and have no chitinous rings. As the chitinous rings in suckers are important for prey capture (Hanlon and Messenger, 1996), the bud-shaped suckers lacking chitinous rings may play no role in prey capture.

Watanabe *et al*. (1998) also reported that the upper and lower beaks of paralarvae did not fit each other, and that denticles developed on the cutting edges of the lower beak in 4- to 5-day-old specimens. In this study, serrated beaks changed to pointed beaks by the time that paralarvae had grown to 5.0–10.0 mm DML. Furthermore, *T. rhombus* paralarvae with DMLs ranging from 1.4 to 6.0 mm had lip cilia. Vidal and Haimovici (1998) suggested that in the paralarvae of *Illex argentinus* Rhynchoteuthion, filaments on the lips of the buccal mass may represent an adaptation for the manipulation and ingestion of microorganisms or small pieces of enriched mucus. In this study, morphological changes in the beak and the disappearance of lip cilia of *T. rhombus* occurred between 4.0 and 8.0 mm DML, and may reflect changes in their feeding behavior. Additionally, the simultaneous development of arm suckers in *T. rhombus* may reflect a change in feeding behavior during this phase.

These results and those of previous studies indicate that the paralarval stage of *T. rhombus* can be divided into three distinct phases. Phase one (1.0–3.0 mm DML) is characterized by adaptations to floating, including a round mantle, small fins, and long tentacles. Phase two (3.1–8.0 mm DML) is marked by a change in feeding behavior, as suggested by the presence of suckers with chitinous rings, differentiated tentacles, pointed beaks, and the disappearance of lip cilia. In phase three (8.1–15.0 mm DML) the shape of the paralarvae becomes similar to that of adults but they still possess long tentacles and arms.

Nigmatulin and Arkhipkin (1998) divided the ontogenetic stage of *T. rhombus* into two phases: the pre-paralarval phase from 1–1.3 to 3 mm ML, analogous to phase one (1.0–3.0 mm DML) of *T. rhombus* paralarvae described in this study, and the paralarval phase from 3–18 mm ML, which is similar to phases two and three of *T. rhombus* paralarvae described above. The paralarval phase described by Nigmatulin and Arkhipkin (1998) could be divided further into two additional phases based on the morphological changes that occur in the paralarvae relative to feeding behavior.

The paralarval phase of *Sthenoteuthis oualaniensis* terminates with the separation of the proboscis at 7.0–8.0 mm ML (Harman and Young, 1985). The paralarval phase of *T. rhombus* includes a wide range of DMLs as compared to that of *S. oualaniensis* (Fig. 6). *T. rhombus*' unique paralarvae grow arms and tentacles for a longer time, which may allow the paralarvae to utilize water flow for feeding before the suckers that are used for prey capture develop. The distribution and migration of *T. rhombus* depend on the surface circulation of oceanic waters (Nigmatulin and Arkhipkin, 1998). These results indicate that *T. rhombus* has developed efficient and independent evolutionary strategies to survive as an oceanic squid, as evidenced by its unique paralarval phase of development.

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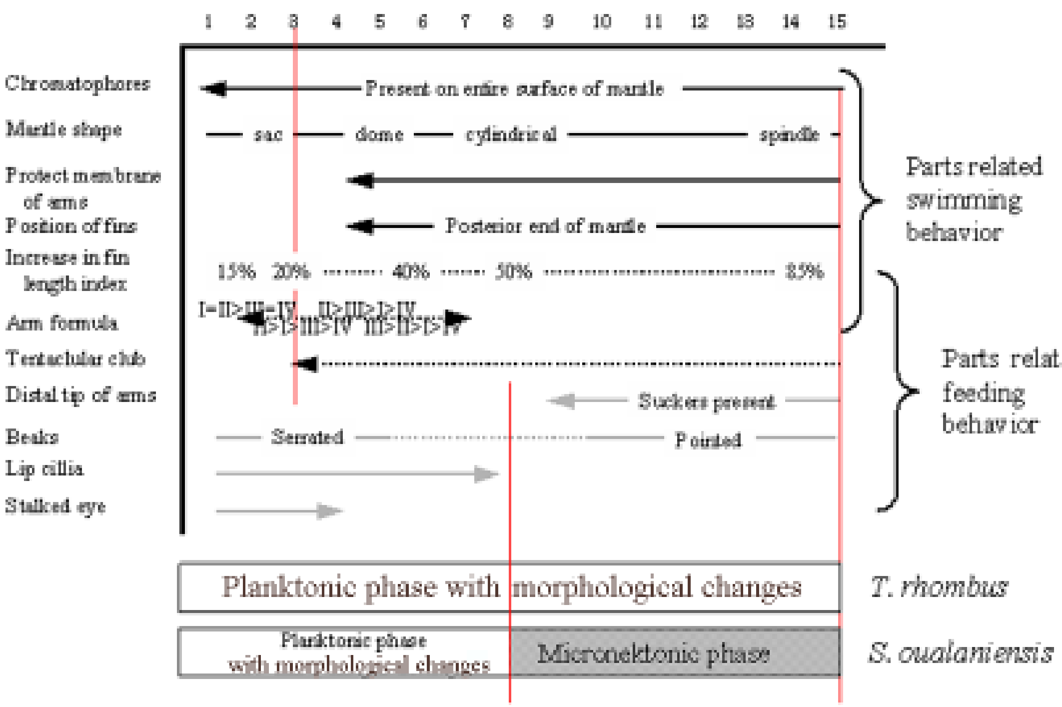

Figure 6. Morphological changes with growth of *T. rhombus* and comparison with tipical oceanic squids *Sthenoteuthis oualaniensis*.

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