Age, growth and reproductive biology of diamond-shaped squid Thysanoteuthis rhombus (Oegopsida:Thysanoteuthidae)

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Age, growth and reproductive biology of diamond-shaped squid *Thysanoteuthis rhombus* (Oegopsida: Thysanoteuthidae)*

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ABSTRACT: Age and growth of the large oceanic squid *Thysanoteuthis rhombus* were estimated by statolith increment analysis of 72 specimens [mantle length (ML) ranging from 25 to 805 mm] and reproductive biology was studied on 162 *T. rhombus* (ML 150 to 850 mm) captured in the eastern tropical Atlantic and southwestern Pacific between 1976 and 1990. The maximum ages found in our samples were observed in a mature female of 750 mm ML (305 d) and in a mature male of 770 mm ML (309 d). The life span of *T. rhombus* was estimated to be about 1 yr; males and females matured at age 6 to 8 mo. *T. rhombus* is one of the fastest-growing squids: by age 300 d, they reached 750 to 800 mm ML and 17 to 17.5 kg body weight (BW). The increase in both ML and BW during ontogenesis was best fitted by a logistic growth curve. *T rhombus* has high potential fecundity (up to 4.8 million oocytes), but a rather small maximum volume of oviducts (up to 140 000 eggs) and egg masses (35 000 to 75 000 eggs). It is suggested that *T rhombus* is an intermittent spawner with multiple filling and evacuation of oviducts. The main function of the (for squid) unusual behaviour of *T rhombus*, i.e. living in pairs consisting of a male and female, is reproduction and is optimal given the low density of *T rhombus* populations and low active movement of squids.

KEY WORDS: Statolith · Growth · Reproduction · *Thysanoteuthis rhombus* · Squid

INTRODUCTION

The diamond-shaped squid *Thysanoteuthis rhombus* Troschel, 1857 is the only species of the family Thysanoteuthidae. This squid is a non-abundant epipelagic inhabitant of warm tropical and partially subtropical waters (water temperature > 20 or 21°C, usually 23 to 26°C) of the World Ocean and the Mediterranean. The squid penetrates into the upper latitudes of subtropical and temperate waters (the Japan Sea, waters of South Africa and America) with warm currents (the Tsushima, Kuroshio, Agulhas and Brazil Currents, and the Gulf

Stream) (Arkhipkin et al. 1983, Roper et al. 1984, Nesis 1985). Nazumi (1975) observed that a sudden drop of temperature below 14 to 15°C in the depth range 0 to 100 m caused a decrease in activity and even death of squids in the Japan Sea. Because of this, *T. rhombus* were sometimes found dying in nearshore waters or stranded ashore at the outer edges of the species range (Pfeffer 1912, Barnard 1947, Bruggen 1962, Nishimura 1966, Morales 1981, Roeleveld & Pheiffer 1987). These strange-looking, large animals [max. mantle length (ML) 850 mm, max. body weight 24 kg] have attracted the attention of fishermen and have sometimes fallen into the hands of scientific investigators. Such was the case when several somewhat damaged *T. rhombus* became available for morphological and anatomical studies, more than 130 yr since the species' first description (as *Sepioteuthis major*) by Gray (1828, cited in Pfeffer 1912).

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*To the memory of the kind and marvellous smile of Prof. Gilbert L. Voss — a great man, scientist and teacher
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Since the end of the 1950s, frequent occurrences of *Thysanoteuthis rhombus* near the Japanese coast have led to the development of a local fishery, which has enabled scientists to collect data on length-frequency distribution, seasonal migrations and aspects of *T. rhombus* biology (Nishimura 1966, Nazumi 1975, Misaki & Okutani 1976, Osako & Murata 1983).

An analysis of dynamics in the length-frequency distribution of *Thysanoteuthis rhombus* during the fishing season in the Japan Sea led Nazumi (1975) to suggest that this species' life cycle lasts 1 yr. His suggestion was based mainly on estimated monthly increments in *T. rhombus* ML: squids >400 mm ML grew at a high rate, 90 to 120 mm ML mo^{-1}.

The reproductive biology of *Thysanoteuthis rhombus* is little known. The structure of the sexual system has been analyzed mainly on the basis of immature specimens (Vigelius 1881, Brock 1882, Pfeffer 1912, Brug-gen 1962, Nazumi 1975). In our first communication on *T. rhombus* reproductive biology (Nigmatullin et al. 1991), we described the morphology and anatomy of the sexual system of mature adults and found that it was characterized by rather primitive features with respect to type of gonad attachment and structure of the hectocotylus and mostly secondary characters, including small oviducts and very large oviductal glands and ovary. Unusual sausage-like egg masses of *T. rhombus* were found relatively often in the surface layers of the tropical World Ocean. Some of them were incubated until hatching (review in Sabirov et al. 1987).

In the present paper, we estimate age and growth rates of *Thysanoteuthis rhombus*, based on statolith increment studies, and describe the main features of its reproductive biology.

MATERIALS AND METHODS

Collection of squids. *Thysanoteuthis rhombus* were collected in the tropical Atlantic (136 specimens; ML 60 to 850 mm) and southeastern Pacific (26 specimens; ML 25 to 650 mm) between 1975 and 1990 on board various Soviet research vessels as a by-catch during research surveys of the Atlantic orange-back squid *Sthenoteuthis pteropus* and Pacific jumbo squid *Dosidicus gigas* (Fig. 1).

Juveniles (25 to 100 mm ML) were captured using zoological trawls (horizontal openings ranging from 16 to 24 m, vertical openings 6 to 8 m with 6 mm mesh) and an Isaacs-Kidd trawl at a depth ranging from 0 to 100 m.

Immature and mature adults (100 to 850 mm ML) were caught mainly during light drift stations at night using handle rods equipped with jiggers of various kinds, as well as jigger lines, scoop nets with an opening diameter ranging from 20 to 100 cm and hand harpoons. In addition, 152 hauls were made between 5 and 150 m depth at night in the eastern equatorial Atlantic using 2 kinds of 'twin' trawls towed simultaneously by 2 medium-size vessels (800 GRT). The horizontal openings were 68 and 86 m, the vertical openings were 10 and 15 m, respectively, and the mesh size was 40 mm. Forty *Thysanoteuthis rhombus* (50 to 800 mm ML) were analyzed from catches of twin trawls.

Analysis of each *Thysanoteuthis rhombus* individual included measurements of dorsal ML (to the nearest millimeter) and BW (to the nearest gram), determination of sex and maturity stage, noting the presence of mating traces on the buccal membrane of females, and determining the degree of stomach fullness (after Zuev
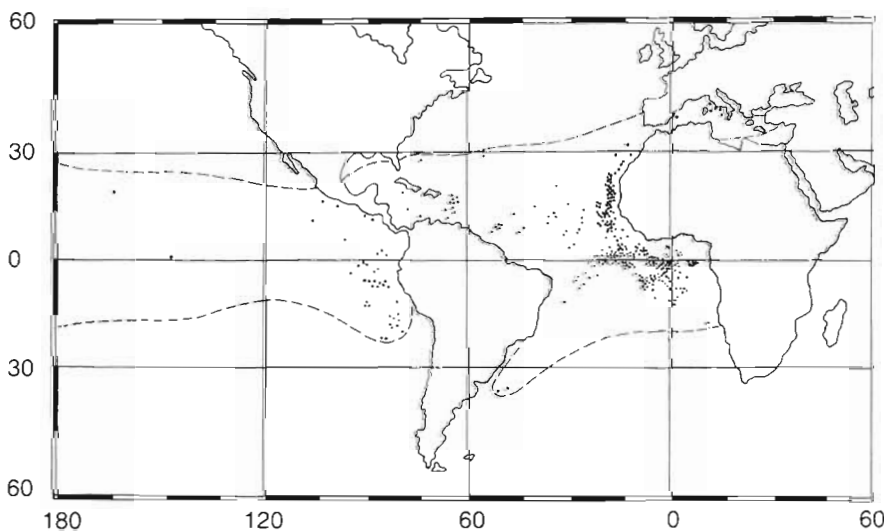

Fig. 1. Location of *Thysanoteuthis rhombus* samples collected in the tropical Atlantic and southeastern Pacific

et al. 1985). To examine ontogenetic, sexual and geo-graphical variations in *T. rhombus* morphology, 72 specimens were analyzed thoroughly according to the scheme of morphometrics proposed by Roper & Voss (1983).

Statolith analysis. Statoliths were extracted from 72 specimens of *Thysanoteuthis rhombus* (25 to 805 mm ML) captured in the eastern tropical Atlantic and southeastern Pacific between 1982 and 1988. Statoliths were stored in 96 % ethyl alcohol and processed in the laboratory. Terminology and measurement of statoliths followed Clarke (1978). Statoliths were ground on both sides on wet waterproof sandpaper (1000 grit) and embedded into Canada balsam. Patterns of statolith microstructure were examined using a light micro-scope (BIOLAM R-14) under 400× magnification. Growth increments were counted from the nucleus to the edge of the statolith dorsal dome using an ocular micrometer (as described in Dawe & Natsukari 1991).

In this study, it was impossible to validate the period-icity of increment formation in *Thysanoteuthis rhom-bus* statoliths. However, in structure and width of increments, *T. rhombus* statoliths resemble those of squid species for which validations have been obtained (review in Arkhipkin 1991). Only daily peri-odicity of statolith growth increments has been observed in squids of 3 large families: Loliginidae (*Loligo opalescens*, Hixon & Villoch 1984; *Alloteuthis subulata*, Lipinski 1986; *Sepioteuthis lessoniana*, Jack-son 1990), Ommastrephidae (*Illex illecebrosus*, Dawe et al. 1985; *Todarodes pacificus*, Nakamura & Sakurai 1991) and Enoploteuthidae (*Abralia trigonura*, Bigelow 1992) Considering these points we assume that the hypothesis '1 increment per day' is also true for *T rhombus*. The total number of growth increments within a statolith was considered to be the age of the squid in days. Hatching dates were back-calculated.

For construction of a group growth curves, we tested the fit of our ML and BW data to one of several sig-moidal curves (logistic, Gompertz and von Bertalanffy) using the iterative nonlinear least squares method. Using the formula for the best-fitted curve (logistic in our case), we calculated values ML and BW values for each 15 d interval starting at age 60 d. Daily growth rates (DGR, mm d^{-1} or g d^{-1}) and instantaneous growth rate (G) were calculated after Ricker (1958):

$$DGR = (W_2 - W_1)/T$$
$$G = (\ln W_2 - \ln W_1)/T$$

where W_1 and W_2 are ML or BW at the beginning and end of the time interval (T = 15 d).

Analysis of the sexual system. The sexual systems of 162 *Thysanoteuthis rhombus* (150 to 850 mm ML) were extracted during analysis on board ship and stored in 6 % formaldehyde for further analysis in the laboratory.

The ovaries, oviducts, and oviductal and nidamental glands in females and the testes, spermatophoric glands and Needham sacs in males were measured and weighed to the nearest 0.1 g after fixation. For determination of oocyte number in the ovary, we took 3 subsamples (50 mg) from the ovary surface, from the ovary core and from the intermediate layer. To avoid the effect of preservation, when the oocytes were not perfectly symmetrical we measured the diameters of oocytes with an ocular micrometer placed in a horizon-tal position (Clarck 1934). The average diameter of each phase of oocytes was estimated based on 50 oocytes of each female. Six oocyte phases were dis-tinguished according to Burukovskii et al. (1977). Oocyte number in the oviducts was assessed from counts of three 200 mg subsamples.

The gonadosomatic index (GSI) and maturity coeffi-cient (MI) were calculated for each specimen as

$$GSI = (GW/BW) \times 100$$
$$MI = (SSW/BW) \times 100$$

where GW is gonad weight, SSW is total weight of the sexual system and BW is body weight of the squid excluding weight of the stomach contents.

Potential fecundity (PF) was calculated as the sum of total oocyte number in the ovary and total egg number in the oviducts. Relative fecundity (RF, number of oocytes per gram) was estimated as

$$RF = PF/BW$$

In addition, we estimated the actual realized fecun-dity (ARF) as the total number of vitelline oocytes in the ovary and eggs in the oviducts, and relative actual realized fecundity (RARF) as

$$RARF = ARF/BW$$

The scheme used for analyzing the male sexual sys-tem is described in Nigmatullin et al. (1991). Number of spermatophores was determined after dissection of the spermatophoric gland and Needham sac. We as-sumed the total number of spermatophores both form-ing within the spermatophoric gland and accumulating within the Needham sac to be the ARF for males.

RESULTS

Age and growth

Statolith morphology

The statolith of adults is elongate (Fig. 2C). The dor-sal dome is developed and smooth. The lateral dome is not well-developed, and is prolonged and smooth. The rostral angle is approximately 90°. The rostrum is
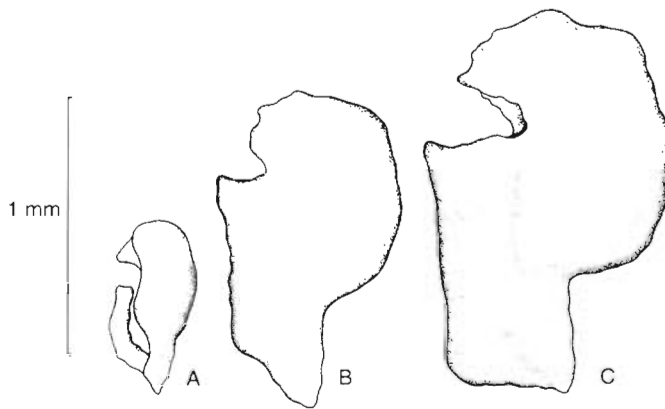
Fig. 2. *Thysanoteuthis rhombus*. Statolith morphology, posterior views. (A) Juvenile, 25 mm mantle length (ML), (B) immature female, 320 mm ML, (C) mature female, 770 mm ML

short, 27 to 30 % of total statolith length (STL). The dorsal spur is well-defined, arising out of the dorsal dome. The wing is broad and thick, its lower part curved at an angle of 70 to 80° to the main plane of the statolith. The statolith width is about 60 % of STL.

Statoliths of immature squid (ML 200 to 400 mm) were already at stage V (the 'definite' stage) (Arkhipkin & Murzov 1986), without upper and lower fissures. However, they are distinguished from those of large adults by their obtuse rostral angle and relatively narrow wing (Fig. 2B).

Statoliths of early juveniles (ML 27 mm) were characterized by a narrow wing, attaching to the lower part of the statolith body, and slightly curved rostrum (Fig. 2A).

Statolith microstructure

On the ground surface of a *Thysanoteuthis rhombus* statolith, 4 main growth zones could be distinguished (Fig. 3A). The nucleus lay under the statolith spur, was oval in shape and had a maximum diameter of 26 µm (SD = 2.82, n = 72) and minimum diameter 18 µm (SD = 2.45, n = 72). A dark stone-like concretion lay in the focus of the nucleus (Fig. 3B). The postnuclear zone was translucent, with growth increments that became wider in the direction of the dorsal dome and narrowed rapidly in the direction of the lateral dome (Fig. 3B). The total number of growth increments in the postnuclear zone varied from 21 to 28 (mean = 25.2, SD = 2.12). Their maximum width was 4.5 to 5 µm. Within the postnuclear zone, there were several (4 to 6) checks with 4 to 7 increments between them (Fig. 3B). Usually we could observe a prominent check between the postnuclear and dark zones. The dark zone was distinct and appeared

slightly brown in transmitted light. Growth increments in the dark zone were wide (4.8 to 5.7 µm) and numerous (56 to 79; mean = 67, SD = 4.5) (Fig. 3C). The width of growth increments gradually decreased in the transluscent peripheral zone, from 2.2–2.4 µm in the midportion to 1.0–1.1 µm near the statolith edge (Fig. 3D). Stress marks were not observed in the outer portion of the peripheral zone in the statoliths of large mature squid.

Statolith growth

Statoliths of early juveniles (25 to 27 mm ML) were rather large, with STL 0.67 mm (2.48 to 2.68 % ML). However, they grew very slowly during ontogenesis and increased only 2.5 times in length by the age of 330 d (1.55 mm in a mature male of 770 mm ML) (Fig. 4). Allometric growth of STL versus ML was strongly negative, with a low allometric coefficient (Fig. 5, Table 1).

Age and growth of squid

In our samples, the minimum age (60 d) was observed in early juveniles of 25 and 27 mm ML, and the maximum ages in a mature female of 750 mm ML (305 d) and a mature male of 770 mm ML (309 d). The largest specimen (mature male, 805 mm ML) had 288 growth increments within its statoliths.

Sexual dimorphism in the growth of *Thysanoteuthis rhombus* was not apparent (Figs. 6 & 7). Data on growth of both ML and BW during ontogenesis was best described by logistic growth curves; the parameters for these curves are shown in Table 1. These data show that *T. rhombus* is one of the fastest-growing squids. Individuals attained 90 mm ML at age 90 d, and 590 mm ML at age 240 d (Fig. 6). At age 300 d, they reached 750 to 800 mm ML and up to 17 to 17.5 kg BW (Fig. 7). However, growth rates were not uniform (Figs. 8 & 9). Daily rates of ML growth were low in early juveniles (1.2 to 1.5 mm d^{-1}), but they quickly attained maximum values by age 150 to 180 d (4.6 mm d^{-1}), then decreased to 0.97 mm d^{-1} at age 300 d (Fig. 8). Daily rates of BW growth showed a similar tendency, but with maximum values (177 g d^{-1}) occurring at age 240 d. By age 300 d, growth decreased to 51 g d^{-1}.

The instantaneous growth rate, G, of ML was maximum in early juveniles (0.022 to 0.025), gradually decreasing to 0.0012 by age 300 d. In contrast, G of BW was high and almost constant from age 60 to 150 d (between 0.037 and 0.039). After that it decreased sharply to 0.003 at age 300 d.
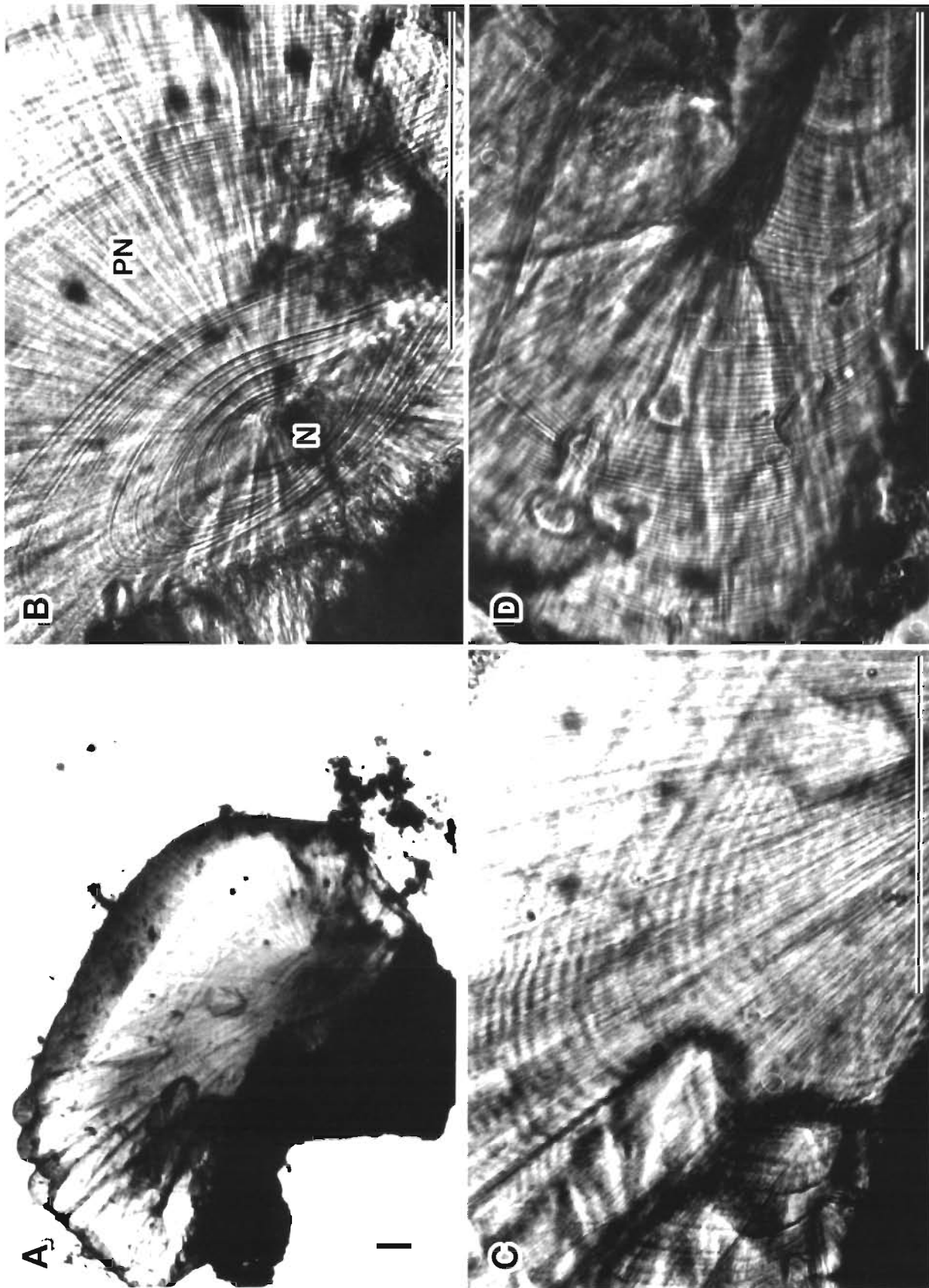
Fig. 3. *Thysanoteuthis rhombus*. Light micrographs of statolith of a mature female, 770 mm ML. (A) General view, (B) nucleus (N) and postnuclear zone (PN), (C) dark zone, (D) peripheral zone in the dorsal dome. Scale bars = 100 μm
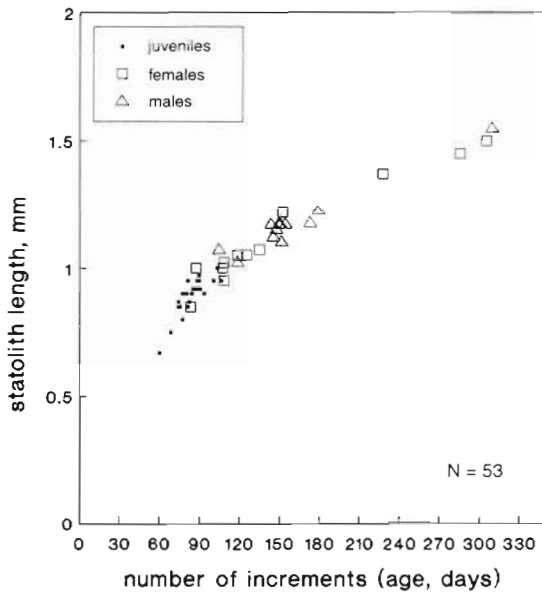
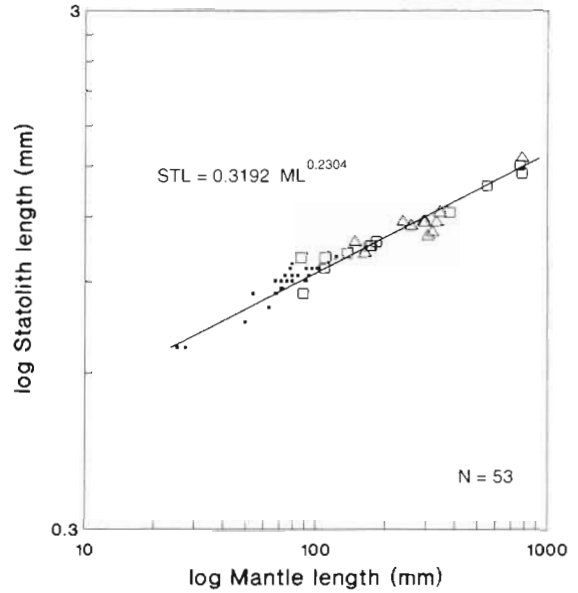Fig. 4. *Thysanoteuthis rhombus*. Relationship between number of increments in statoliths and statolith length

Fig. 5. *Thysanoteuthis rhombus*. Allometric growth of statolith length versus mantle length. Symbols as in Fig. 4

Reproductive biology

Size and age of maturation

Sex may be distinguished visually in squids greater than 100 to 120 mm in ML by the appearance of primordial nidamental glands on the coelomic coverings of females, and a primordial spermatophoric gland on the coelomic coverings of males. Calculated ages of these individuals ranged from 100 to 110 d. Physiological maturation (i.e. maturation of the gonad to maturity

stage III) was observed in males that had attained 250 to 350 mm ML (calculated age 150 to 170 d). In females, physiological maturation occurred later, at 500 to 620 mm ML (calculated age 215 to 240 d). Fig. 6 and Table 2 show the sizes and ages at which males and females reached functional maturity (stage V). The

Table 1. *Thysanoteuthis rhombus*. Parameters of the allometric growth curve STL = a ML^b based on total statolith length (STL, mm) at mantle length (ML, mm) relationships; and the logistic growth curve $Y(t) = K/\{1+[(K-Y(0)/Y(0)]\exp(-rt)\}$ based on mantle length (ML, mm) or body weight (BW, g) at age (t) relationships

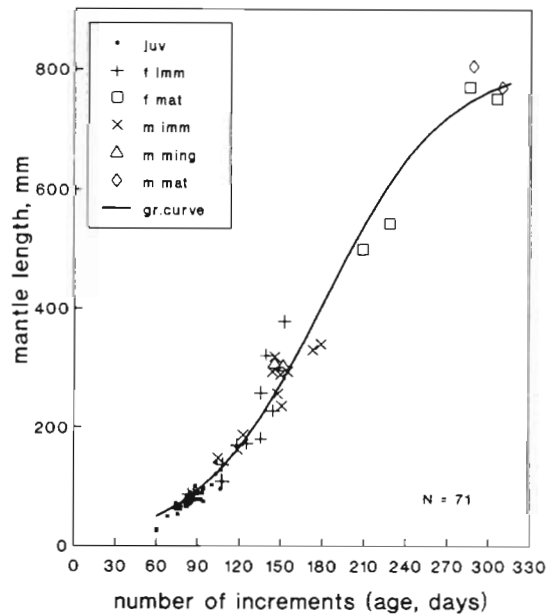| Parameter	Estimate	Asymptotic standard error	R^2
Allometric growth curve: STL versus ML			
a	0.3192	0.03411	0.79
b	0.2304	0.02045	
Logistic growth curve: ML versus age (t)			
r	0.02288	0.001009	0.9761
K	812	20.039	
$Y(0)$	13.22	1.704	
Logistic growth curve: BW versus age (t)			
r	0.03868	0.002815	0.9916
K	19020	517.8	
$Y(0)$	1.577	0.9711	

Fig. 6. *Thysanoteuthis rhombus*. Relationship between number of increments in statoliths and mantle length, showing fitted logistic growth curve (parameters in Table 1). juv: juveniles; m: males; f: females; imm: immature; ming: maturing; mat: mature
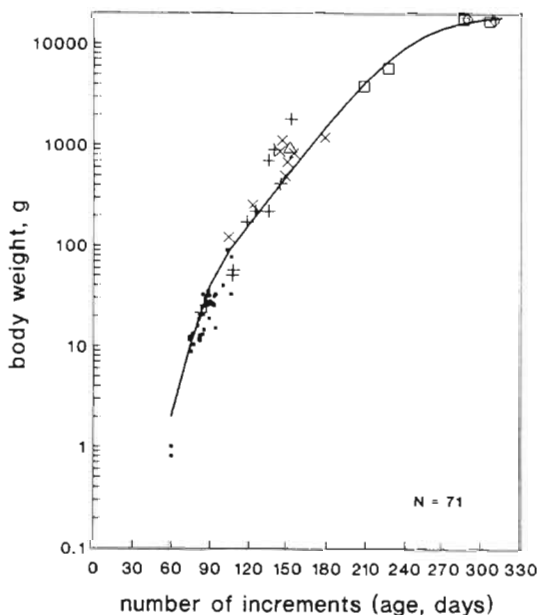
Fig. 7. *Thysanoteuthis rhombus*. Relationship between number of increments in statoliths and body weight, showing fitted logistic growth curve (parameters in Table 1). Symbols as in Fig. 6

Fig. 9. *Thysanoteuthis rhombus*. Instantaneous rate of growth of mantle length (ML) and body weight (BW) versus number of increments in statoliths

smallest mature male was 390 mm ML, whereas maturity in females began at 520 mm ML (age was not determined). The bulk of males were mature at 400 to 450 mm ML (calculated age 190 to 200 d) and of females at 550 to 650 mm ML (calculated age 230 to 250 d). All males larger than 450 mm and females larger than 650 mm were mature.
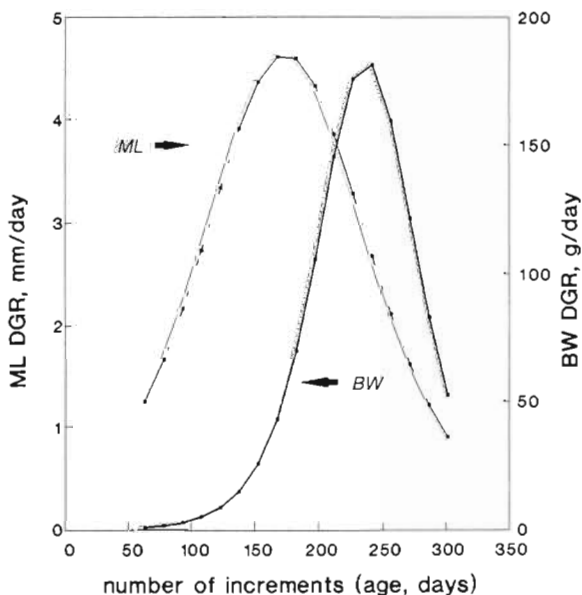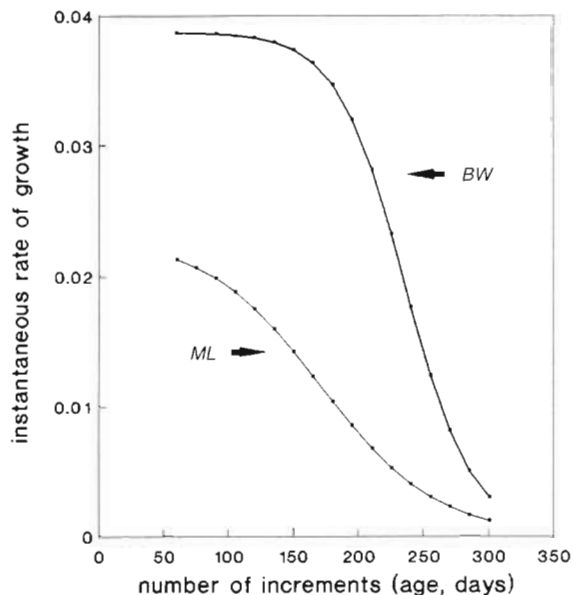
Female sexual system

In females of 100 to 150 mm ML, a pair of nidamental glands appeared as narrow and long stripes on the surface of the sexual coelom. The length of the nidamental glands in these individuals was

Fig. 8. *Thysanoteuthis rhombus*. Daily growth rates (DGR) of mantle length (ML) and body weight (BW) versus number of increments in statoliths

Table 2. *Thysanoteuthis rhombus*. Dynamics of maturation of males and females; values are numbers of individuals in each maturity stage

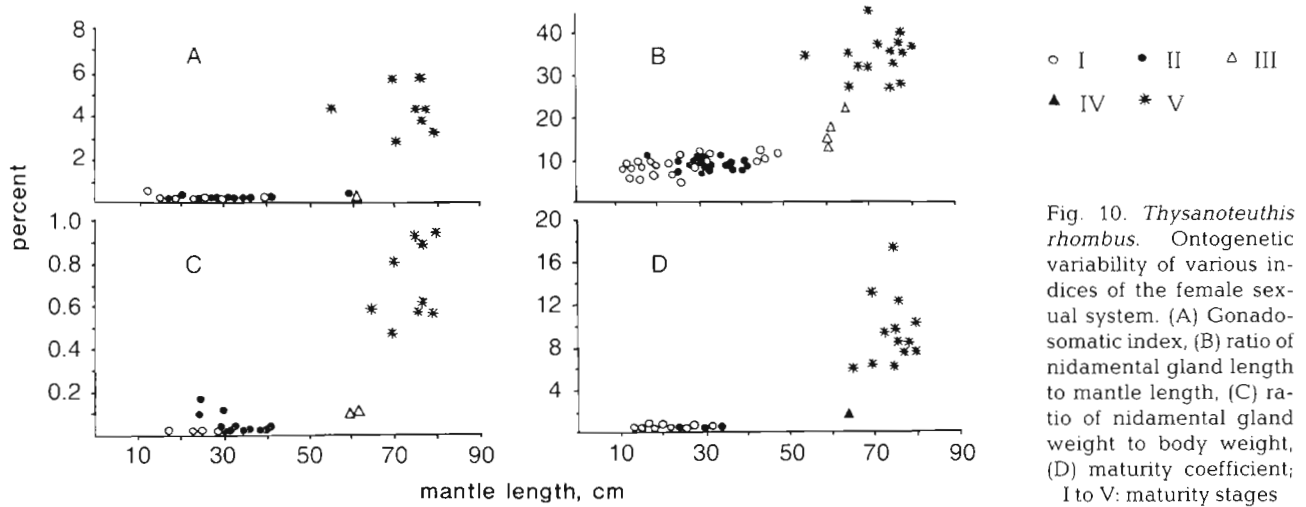| Mantle length interval (mm)	Females					Males				
	I	II	III	IV	V	I	II	III	IV	V
0										
100	9					11				
150	13					9				
200	9					7	1			
250	8	3				5	1	1		
300	6	7				3	5	1		
350	–	4				3	–	1	–	1
400	2	3				1	2	–	2	1
450	2									3
500										1
550					1					3
600		1	3	1	–					4
650					4					1
700					3					3
750					8					3
800					1					1
850										
Total	49	18	3	1	17	39	9	3	2	21
Grand total			88					74		

Fig. 10. *Thysanoteuthis rhombus*. Ontogenetic variability of various indices of the female sexual system. (A) Gonadosomatic index, (B) ratio of nidamental gland length to mantle length, (C) ratio of nidamental gland weight to body weight, (D) maturity coefficient; I to V: maturity stages

<10 mm (8 to 9 % ML); their weight was about 0.05 g (0.001 % BW) (Fig. 10B, C). Length of the nidamental glands at maturity stages I and II was 6 to 11 % ML; at stage III, 13 to 17 % ML; at stage IV, 21 % ML; and at stage V, 26 to 44 % ML. Maximum development of the nidamental glands was revealed in females with the highest maturity coefficient (MC).

MC increased with the progress of both ML and maturity stage, from 0.1 % BW at maturity stage I to 1.5 % BW at maturity stage IV. Mature females had a higher MC, up to between 6 and 17 % BW (Fig. 10D).

Ovary shape did not change during ontogeny, but ovary relative weight (GSI) increased considerably (Fig. 10A). The ovary of females at maturity stage IV was pale, without a mosaic structure after fixation and storage in formaldehyde. At maturity stage V, the ovary was yellowish-brown, with a mosaic structure.

In the ovary of mature females, oocytes were at various stages of ripening, exhibiting asynchrony in their development. It was possible to distinguish 6 size groups of oocytes (Fig. 11, Table 3) that corresponded to the following stages of oocyte development (after Burukovskii et al. 1977): Size group I corresponded to the second phase of provitellogenesis ('primary follicle'), oocytes were polygonal, and the centrally situated nucleus was oval and large. Group II corresponded to the third phase of provitellogenesis ('simple follicle'), oocytes were either oval or goblet-shaped, and the cytoplasm volume was increased in comparison to the preceding size group. Group III obviously corresponded to the intercalary period of oocyte development ('complicated follicle'); oocytes were leaf-like, of dark color, with numerous shallow longitudinal grooves together with follicle cells that protruded into the grooves. The nucleus was not visible. Group IV corresponded to the first and second phases of trophoplasmatic growth ('vacuolization and vitelline accumulation'). Oocytes were covered with
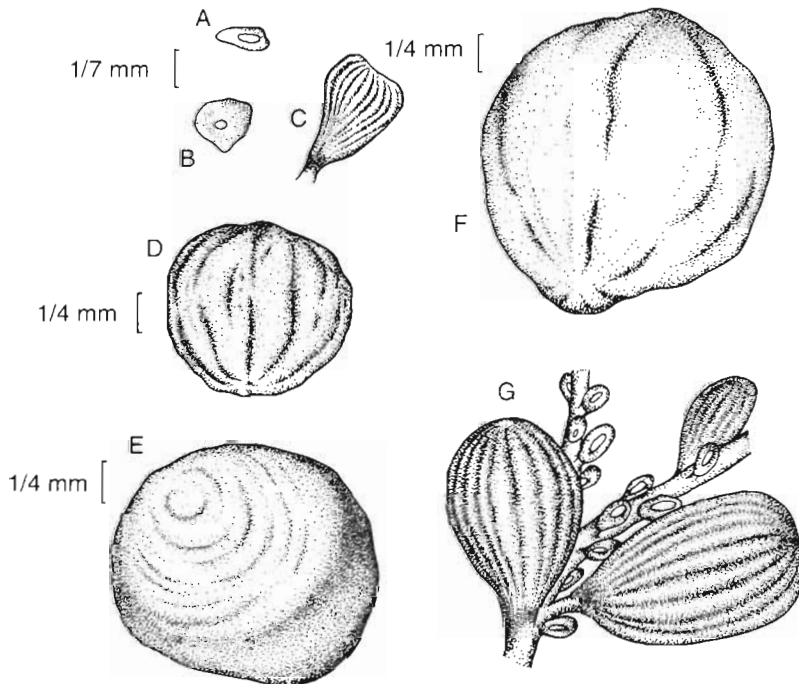

Fig. 11. *Thysanoteuthis rhombus*. Size groups of oocytes within the ovary. (A) Size group I, (B) group II, (C) group III, (D) group IV, (E) group V, (F) group VI, (G) blood vessel with various size groups of oocytes
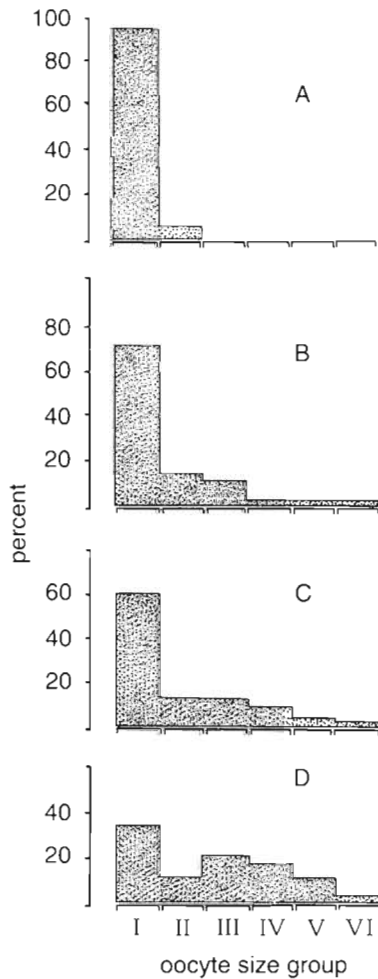
Fig. 12. *Thysanoteuthis rhombus*. Frequency distribution of different size groups of oocytes within ovary of: (A) immature female of 330 mm ML, (B) maturing female of 640 mm ML (MC = 1.6), (C) mature female of 730 mm ML (MC = 9.0), (D) mature female of 703 mm ML (MC = 12.9)

reticulate grooves, and dark in color. Group V corresponded to the third phase of trophoplasmatic growth ('expulsion of follicles'); oocytes were rounded and yellow, the reticulate grooves having almost disappeared. Group VI corresponded to the fourth phase of

Table 3. *Thysanoteuthis rhombus*. Diameter of different size groups of oocytes from the ovary of females

Size group	Diameter (mm)		
	Range	Mean	SD
I	0.07–0.25	0.15	0.032
II	0.1–0.3	0.19	0.052
III	0.3–0.5	0.38	0.047
IV	0.5–0.15	0.94	0.121
V	1.05–1.8	1.45	0.093
VI	1.5–1.8	1.68	0.046

the trophoplasmatic growth ('ripe oocyte'). Oocytes had a smooth surface and were oval and crimson-violet.

The proportion of oocytes in different size groups changed as the sexual system developed (Fig. 12). In immature females, oocytes of size group I dominated (95%); the remaining ones were of size group II. In maturing females, ripe oocytes were practically absent. In mature females, the relative numbers of oocytes in groups II and III were almost the same (10 to 15%), whereas the proportion of oocytes in groups IV and V increased with increasing MC. Females with maximum MC had oocytes of size groups IV and V in almost equal proportion to those of size groups II and III. The relative quantity of ripe oocytes in the ovaries was always low (<1%), because they were immediately transferred into the oviducts after their extrusion into the coelom.

Individual PF varied considerably (Table 4): from 2.3–5 million oocytes in immature females to 2.7–4.8 million oocytes in maturing and mature females. The female with maximum MC had the lowest PF (2.4 million). ARF increased from 300 000 vitelline oocytes in females with MC = 5.9% ML to 860 000 in females with MC = 12.9%.

Male sexual system

The testis in males <150 mm is a narrow, long strip hanging from the mesentery of the sexual coelom. In immature males <400 mm ML, the length of the testis varied significantly from 3–4 to 18–20% ML. In larger males, the relative length of the testis stabilized and became more or less uniform at 10 to 16% ML (Fig. 13A). In mature males, the testis became broader and more robust in shape, like an elongate oval. Weight of the testis increased from 0.01–0.1 to 0.2–0.6% BW (Fig. 13B). The same increase was observed in the weight of the spermatophoric complex of organs (Fig. 13C). MC increased with the progressing maturity state of males, with values of 0.15% BW in immature squid, 0.3% BW in maturing and 0.9 to 1.2% BW in mature males (Fig. 13D).

Formation of the spermatophores in different parts of the spermatophoric gland (SG) is similar to that in other squid and cuttlefish (Blanquaert 1925). Spermatozoa enter into the first section of the SG from the spermaduct and mix with its gelatinous secretions. The frequency of occurrence of developing spermatophores (% of the sampled male squid that contained spermatophores in a given section of their SG) varied in different sections of the SG, from 66% in sections II, III and IV, to 17% in section V, and 100% in section VI, as well as 34% in the spermatophoric duct. In sections II to IV, there occurs a

gradual and complete formation of all parts of the spermatophores from the sperm mass to the external coverings. In section V of the SG the spermatophore turns and quickly moves into section VI, where its coverings become resilient as a result of dehydration.

The spermatophores of *Thysanoteuthis rhombus* are large, ranging from 60–70 mm in males of 420 mm ML to 95–100 mm in males of 850 mm ML, the volume of their seminal reservoirs varying from 0.12–0.13 to 0.44–0.5 cm^3, respectively. Thus, the total volume of seminal fluid in all spermatophores of a single male may reach 2.4 to 9 cm^3.

In developing spermatophores, the cement body is 1.2 to 2 times larger and the ejaculatory tube is 1.5 to 2 times longer than those of ripe spermatophores. These parts of the spermatophore attain definite sizes when the spermatophore exits section VI of the SG.

The number of spermatophores located in different parts of the SG does not depend on the length of males. Usually, in both the SG and the spermatophoric duct there were 3 developing spermatophores (in 66% of males); in rare cases there were 2 or 4 spermatophores (17 and 17%, respectively). The number of developing spermatophores also does not depend on the total number of ripe spermatophores in the Needham sac: in a male of 695 mm ML there were 15 spermatophores in the Needham sac and 3 developing spermatophores in the SG; in a male of 850 mm ML, 11 and 4, respectively; in a male of 770 mm ML, 6 and 3; in a male of 420 mm ML, 10 and 3.

Table 4. *Thysanoteuthis rhombus.* Characteristics (in mm and g) of the sexual system of 1 maturing and 6 mature females (arranged in order of increasing maturity coefficient, MC, indicated in **bold**). NGL, nidamental gland length; OW, weight of ovary; OVW, weight of oviducts; OGW, weight of oviductal glands; SSW, total weight of sexual system; OVN, number of eggs in oviducts ($\times 10^3$); PF, potential fecundity ($\times 10^6$ oocytes); RF, relative potential fecundity (oocytes g^{-1} BW); ARF, actual realized fecundity ($\times 10^6$ oocytes); RARF, relative actual realized fecundity (oocytes g^{-1} BW); GSI, gonadosomatic index (%); MC, maturity coefficient (%)

Feature	Maturing female	Mature females					
		1	2	3	4	5	6
Mantle length (mm)	640	650	686	730	751	800	703
Body weight (kg)	9.1	10.4	12.3	16.8	10.5	13.9	12.5
NGL	140	220	212	260	235	280	308
OW	25.6	225	255	590	435	530	525
OVW	20.0	22.0	27.5	84.5	64.0	65.0	150
OGW	70	270	300	700	376	650	740
SSW	146	615	752	1509	971	1375	1590
OVN	–	22	25	74	72	62	140
PF	2.7	4.8	3.9	4.9	3.1	3.5	2.4
RF	291	457	316	292	299	251	194
ARF	0.036	0.3	0.325	0.7	0.64	0.7	0.86
RARF	3.8	28.5	26.5	42.0	59.7	50.3	69.7
GSI	0.3	2.0	2.0	3.5	4.2	3.8	4.3
MC	**1.6**	**5.9**	**6.1**	**9.0**	**9.3**	**9.9**	**12.9**

The total number of spermatophores in the Needham sac varied from 5 to 10, commonly 8 or 9. Therefore, the total number of spermatophores located in both the SG and the Needham sac is low and does not exceed 15 to 17. Probably, males form 15 to 20 spermatophores between any 2 matings. This assumption is supported by the total number of spermatangues found on the buccal membrane of females (not more than 20), which are the traces of the previous copulation.
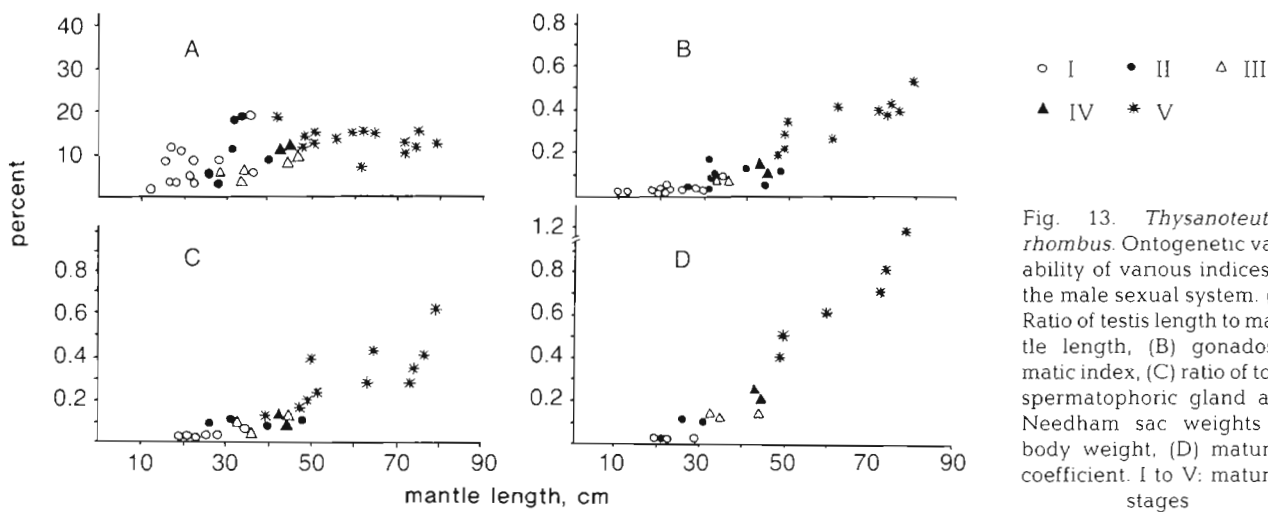

Fig. 13. *Thysanoteuthis rhombus.* Ontogenetic variability of various indices of the male sexual system. (A) Ratio of testis length to mantle length, (B) gonadosomatic index, (C) ratio of total spermatophoric gland and Needham sac weights to body weight, (D) maturity coefficient. I to V: maturity stages
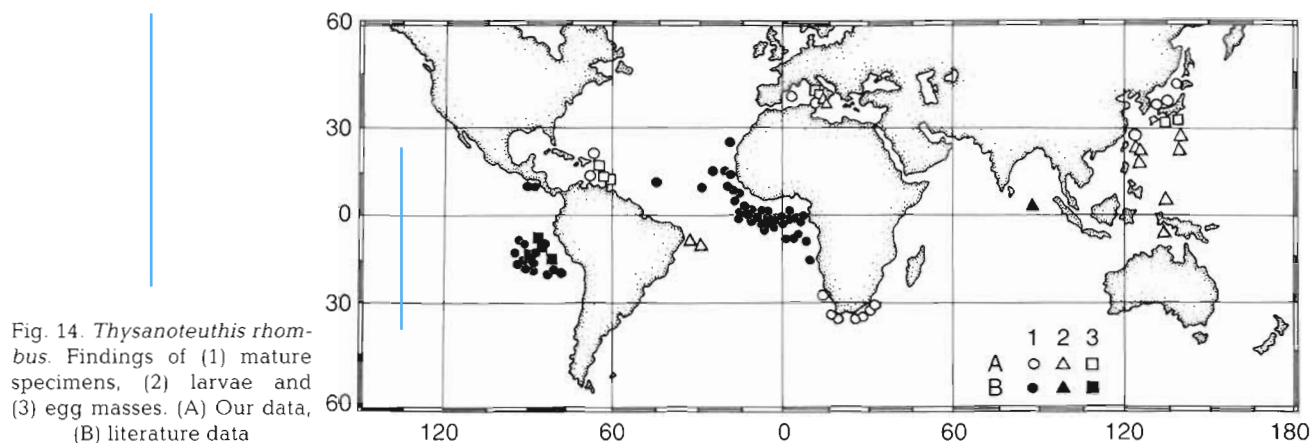
Fig. 14. *Thysanoteuthis rhombus*. Findings of (1) mature specimens, (2) larvae and (3) egg masses. (A) Our data, (B) literature data

Spawning grounds

Spawning grounds of *Thysanoteuthis rhombus* were identified as those areas in which egg masses, larvae and completely mature squid were found (Fig. 14). These coincided almost completely with the species' geographic range (except for nearshore peripheral regions). All occurrences of *T. rhombus* egg masses, larvae and mature squid in peripheral regions corresponded with strong warm currents such as the Agulhas or Kuroshio (Nishimura 1966, Nazumi 1975, Roeleveld & Pheiffer 1987).

Clearly, this squid does not reproduce in subtropical oligotrophic waters of the World Ocean, except in the Mediterranean Sea. *Thysanoteuthis rhombus* spawns throughout the year in tropical waters. In peripheral regions such as in the Mediterranean (Degner 1926, Sanzo 1929), near southern Japan (Yamamoto & Okutani 1975, Misaki & Okutani 1976) and in the southeast Pacific (Sabirov et al. 1987), spawning takes place during the warm seasons (summer and early autumn).

DISCUSSION

Age and growth

The statolith shape of *Thysanoteuthis rhombus* resembles that of shelf ommastrephids (i.e. *Illex coindeti*; Arkhipkin 1989) in its well-developed and smooth dorsal dome, relatively short rostrum and almost right rostral angle in adults. Statoliths of juveniles (<100 mm ML) are relatively large (2 to 2.68% ML), but owing to the strong negative allometry of statolith length versus mantle length, their relative sizes were very small in adults (up to 0.19% ML), whereas in oceanic ommastrephids statoliths of large adults were 1.7 to 2.3 times larger [i.e. in a mature *Dosidicus gigas* male of 724 mm ML, STL = 2.75 mm (0.38% ML), and in a mature

Sthenoteuthis pteropus female of 540 mm ML, STL = 1.75 mm (0.32% ML); our data]. Thus, in shape and possibly size, statoliths of *T. rhombus* resemble those of moderately active nektonic squids (*I. coindeti*), but not those of highly active oceanic nektonic squids such as *D. gigas* and *S. pteropus*.

Statocysts of *Thysanoteuthis rhombus* resembled those of non-buoyant oegopsids, but possessed unique characters. The statocyst volume of a *T. rhombus* adult of 800 mm ML (11.61 mm³) was one-seventh that in an omastrephid (*Ommastrephes bartrami*) of approximately the same size, 850 mm ML (73.22 mm³). The main differences in statocyst shape between *T. rhombus* and ommastrephids were the small knob-like anticrista 1 in *T. rhombus*, unlike the large rod of ommastrephids; and the presence of a large hamulus at the first turn of the crista, which is characteristic of *Sepia* but not of ommastrephids, and is possibly connected with the presence of long fins along the mantle of *T. rhombus*. It has been suggested that the statocyst of *T. rhombus* is less suited for rapid movement than in adult ommastrephids (Young 1989).

The shape of both the statocyst and statolith of *Thysanoteuthis rhombus* corresponds with its behavior and activity patterns. In contrast to other nektonic ommastrephid squids which are nictoepipelagic (Zuev et al. 1985), *T. rhombus* is a holopelagic species that makes diel migrations within the upper 100 to 200 m water layer (Roper et al. 1984, our data). The holopelagic lifestyle of *T. rhombus* is corroborated by its high oxygen requirements: the critical and threshold oxygen concentrations for *T. rhombus* juveniles were 2 and 3 times as high, respectively, as those for *S. pteropus* juveniles (Stolbov 1986). Despite its nektonic shape, *T. rhombus* is a low-activity migrant that undertakes long seasonal migrations in waters of warm peripheral currents. Therefore, *T. rhombus* may be characterized paradoxically as a planktonic nekteur. Underwater observations of *T. rhombus* behavior sup-

port this assumption (Nigmatullin 1987). During a major part of its life, *T. rhombus* does not actively move, but rather is propelled slowly by the undulation of its long triangular fins. However, when it encounters a sudden danger *T. rhombus* is capable of making one or several powerful contractions of its muscular mantle and thus escaping the dangerous situation. Earlier, O'Dor et al. (1991) found in squid that the costs of movement by means of fin undulation were much lower than those due to mantle contractions. Thus, the behavior of *T. rhombus* leads to a decrease in the costs of active metabolism, enabling the squid to maintain very high growth rates (Figs. 8 & 9).

Growth rates of *Thysanoteuthis rhombus* juveniles of 25 to 300 mm ML are strikingly high and close to the maximum known for other immature squid in this size range (Arkhipkin 1989, Jackson & Choat 1992). As a result, *T. rhombus* juveniles quickly pass through the size range (ML 50 to 150 mm) that is most vulnerable to active oceanic predators such as tunas, swordfish and squids, and thus reduce predation loss. There is no sexual dimorphism in the growth rates of *T. rhombus*. Males and females mature at age 6 to 8 mo and live for around 12 mo. Therefore, the longevity of *T. rhombus* is about 1 yr similar to the majority of large neritic and oceanic ommastrephids and loliginids (Arkhipkin 1989, Jackson & Choat 1992).

Reproductive biology

The sexual system of *Thysanoteuthis rhombus* may be characterized by a set of specific features such as structure and size of the oviducts and oviductal glands in females; size of the Needham sac, penis and spermatophores in males; and, in both sexes, the pattern of gonad attachment (Nigmatullin et al. 1991).

Copulation

Before spawning, the female and male mate in 'head to head' position. This is confirmed by the presence of spermatangues (mating traces) on the buccal membrane of females. During copulation, the male transfers the spermatophores to the female by the hectocotylus. In *Thysanoteuthis rhombus*, the fourth left arm is hectocotylized; 7 to 8 rows of suckers in the median of the arm shrink or even disappear. Several glands appear on the transformed region of the hectocotylus, which together with the dorsal protective membrane help in effective transportation and retention of the spermatophores (Nigmatullin et al. 1991). After ejaculation, spermatophores are attached to the surface of the female's buccal membrane. Nigmatullin et al. (1991) found from 3 to 6 sper-

matangues in females. Spermatozoa from the spermatangues are transported via an unknown pathway into the seminal receptacles located on the ventral part of the buccal membrane. It is noteworthy that *T. rhombus* accumulates approximately the same volume of seminal fluid in several large spermatophores as the most fecund ommastrephid, *Ommastrephes bartrami*, which produces up to 1700–1800 small spermatophores containing a total of 5 to 9 cm^3 of seminal fluid (Zalygalin et al. 1983).

Functioning of the female sexual system

Based on the structure of the sexual system and the number and types of oocytes within the ovary and oviducts of mature females, it is possible to propose a hypothesis describing the functioning of the sexual system of a *Thysanoteuthis rhombus* female. The total number of oocytes has formed in immature individuals by the end of maturity stage II. In the subsequent stages, the total amount of oocytes remains constant, but they start to develop asynchronously. As soon as the oocytes become ripe, they are released from the follicles into the sexual coelom and transferred to the oviducts, where they accumulate. Simultaneously with the accumulation of oocytes in the oviducts, the nidamental and oviductal glands are developing. When the oviducts have become completely filled with ripe eggs, a spawning takes place.

Egg mass structure

Until recently, a total of 21 egg masses of *Thysanoteuthis rhombus* had been found. All were found drifting in the surface water layer of the tropical Atlantic, northwest and southeast Pacific and the Mediterranean (review in Sabirov et al. 1987). The egg mass of *T. rhombus* is a dense, resilient, oblong cylinder with rounded tips (Fig. 15C). Their lengths varied from 600–800 to 1500–1800 mm and their diameters from 110–150 to 200–300 mm. These variable lengths may correspond with the dimensions of the female. In the outer layer of the mucous cylinder there are 2 rows of crimson-purple eggs, spirally arranged around the cylinder. The total number of eggs in each egg mass varies from 32 000 to 76 000 (Sabirov et al. 1987).

Egg mass formation

In accordance with the specific characters in the structure of the accessory glands and egg masses of *Thysanoteuthis rhombus*, we propose 2 hypotheses regarding egg mass formation (Fig. 15).
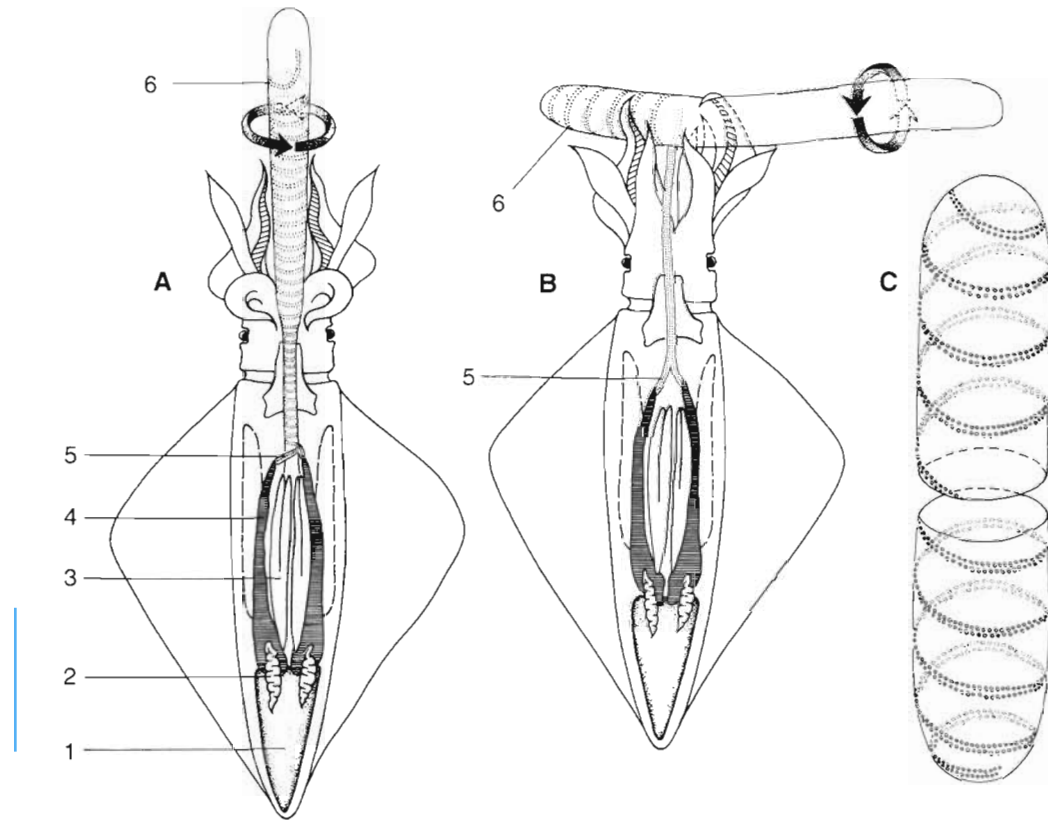
Fig. 15. *Thysanoteuthis rhombus.* Possible methods of egg mass construction by females. (A) Hypothesis 1, (B) hypothesis 2, (C) schematic diagram of egg mass. 1: Ovary; 2: oviduct; 3: nidamental glands; 4: oviductal glands; 5: secretion of oviductal glands with ripe eggs; 6: egg mass

The first hypothesis. At spawning, both oviductal and nidamental glands secrete simultaneously. Secretions of the nidamental glands appear in the mantle cavity and form a mucous, cylindrical egg mass which exits through the funnel (Fig. 15A). After extrusion into the water, the mucous cylinder quickly swells and becomes neutrally buoyant. The female seizes the cylinder with her arms and starts to rotate it around the longitudinal axis. This time each oviductal gland forms a thin mucous thread with 1 row of ripe eggs in it. Threads from both oviductal glands fuse together and attach to the surface of the cylinder within the mantle cavity. As a result of the cylinder rotation, the 2 threads of eggs are wound onto the cylinder in a double spiral. After the cylinder swells in the water, the distance between the eggs in the spiral and between the loops of the spiral increases. The consistency of the mucous cylinder and threads is approximately the same, so the boundaries between them are poorly visible. In our opinion, this hypothesis has one weak point: one tip of the egg mass must be without eggs or bear a straight line of them (instead of spiral), because the first part of the cylinder remains unrevolved. But this has not been observed in actual egg masses.

The second hypothesis. Females of *Thysanoteuthis rhombus* first produce a secretion of the nidamental glands like that of the ommastrephid *Todarodes pacificus* (Hamabe 1962). The secretion enters the water, and swells, and the female molds it into a cylinder (Fig. 15B). After that, the oviductal glands start to work, forming 2 mucous threads, each with 1 row of eggs. In the mantle cavity both threads fuse and develop into a single cord containing a double row of eggs. This cord exits into the water through the funnel, and the eggs are fertilized with spermatozoa from seminal receptacles located in the female's buccal membrane. The female then winds the cord of eggs onto the cylinder. As a result, the structure of the egg mass is uniform, as observed in natural egg masses of *T. rhombus*.

Reproductive strategy

Because of the large values of potential and realized fecundity, the rather small maximum volume of both oviducts in mature females (up to 140 000 eggs) and the small volume of individual egg masses (35 000 to

76 000 eggs), it is possible that *Thysanoteuthis rhombus* has multiple spawnings of the 'pulsate' type, i.e. with successive fillings and evacuations of the oviducts. During the spawning period, which lasts 2 to 3 mo, a female *T. rhombus* may produce about 8 to 12 egg masses if it utilizes all of its vitelline oocytes (average 600 000).

Thysanoteuthis rhombus appears to be characterized by a relatively low abundance and population density — 5 to 20 kg km^{-2} (Nigmatullin 1987). This seems to have led to a situation unique among squids, namely monogamy, where pairs consisting of a male and female of the same size remain together from their juvenile stage until death. We assume that the main function of this paired lifestyle is reproductive. Immature squids probably recognize the opposite sex by the sexual dimorphism in length of the third pair of arms: in males they are twice as long as in females (our data). This kind of behavioral strategy is optimal given the low density of *T. rhombus* populations and slow movement of squids (Nigmatullin 1987).

Thysanoteuthis rhombus displays a combination of characters of both K- and R-strategies. Among the features of the K-strategy are a relatively large size of eggs (1.8 mm in diameter; Sanzo 1929), embryogenesis in well-protected gelatinous egg mass, early escape from the pressure of oceanic predators, well-developed behavioral mechanisms and morphological adaptations for escaping predators, and large body dimensions. R-selection characters of *T. rhombus* include high fecundity, high growth rates, monocyclic reproduction and a short (1 yr) life span. Adaptations to peripheral, narrow ecological niches resulted in the specific lifestyle of *T. rhombus* and have enabled this species to survive despite the low density of its populations within the vast oceanic range.

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