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Author(s)	Watanabe, Kumi; Sakurai, Yasunori; Segawa, Susumu et al.
Citation	American Malacological Bulletin, 13(1/2), 73-88
Issue Date	1996
Doc URL	https://hdl.handle.net/2115/35243
Type	journal article
File Information	sakurai-23.pdf

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Development of the ommastrephid squid *Todarodes pacificus*, from fertilized egg to rhynchoteuthion paralarva

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Abstract: The present study establishes for the first time an atlas for the normal development of *Todarodes pacificus* Steenstrup, 1880, from fertilized egg to rhynchoteuthion paralarva. In the course of the study, observations on embryogenesis and histological differentiation in *T. pacificus* were made for consideration of the developmental mode of the Oegopsida, which is a specialized group with a reduced external yolk sac. It appears that differentiation of the respiratory and digestive organs is relatively delayed in the Oegopsida, with reduction of the yolk sac as well as the egg size. These characters could be related to a reproductive strategy for paralarval dispersion in the open ocean.

Key words: *Todarodes pacificus*, oceanic squid, development, rhynchoteuthion, yolk sac

The pelagic squid, *Todarodes pacificus* Steenstrup, 1880, is distributed all around Japan and its neighboring waters, extending from the northern part of the Kurile Islands south to Hong Kong (Okutani *et al.*, 1987; Okutani, 1995). This squid is one of the most commercially important cephalopods in Japan. Knowledge of the early life history of *T. pacificus* would provide basic information for fishery biology. It would also be invaluable in the analysis of the phylogeny of the Cephalopoda by clarifying the embryogenesis of an oegopsid species that has seldom been previously pursued by researchers.

There are only a few embryological studies on the Oegopsida, despite the many embryological studies on the Myopsida (*e. g.* Naef, 1928; Arnold, 1965, 1990; Segawa, 1987; Segawa *et al.*, 1988; Baeg *et al.*, 1992) and Sepioidea (*e. g.* Naef, 1928; Yamamoto, 1982). Soeda (1952, 1954) and H. Hayashi (1960) observed the embryonic development of *Todarodes pacificus* from artificially inseminated eggs, and described only a part of the development because embryonic development became abnormally arrested. Hamabe (1961, 1962) described and illustrated the embryonic development of *T. pacificus* without defining developmental stages. Naef (1928) proposed the developmental stages for one ommastrephid species (as ommastrephid Y), subsequently identified as *Illex coindetii* (Verany, 1837) (Boletzky *et al.*, 1973), but the stages were separated by large intervals and applied only after organogenesis. Recent observations of the embryonic development of *I. illecebrosus* were made by O'Dor *et al.* (1982) without providing an atlas of stages.

The development of a technique of artificial fertilization for ommastrephid squids (Sakurai and Ikeda, 1992; Ikeda *et al.*, 1993; Sakurai *et al.*, 1995) has made it possible to examine the embryonic development and early life stages of *Todarodes pacificus*. In the present study, embryogenesis and histological differentiation of *T. pacificus* were observed in artificially fertilized eggs, and a complete atlas of developmental stages was established for the first time. Comparisons are made with several other species of the Oegopsida (Ommastrephidae, Enoploteuthidae, and Thysanoteuthidae), Myopsida, and Sepioida (Watanabe, unpub.) to characterize the developmental mode of oceanic oegopsids.

MATERIAL AND METHODS

Artificial Fertilization and Cultivation

Fertilized eggs of *Todarodes pacificus* were obtained by artificial fertilization using the method described by Sakurai *et al.* (1995). Female squids were captured in the waters near Hakodate, Hokkaido, and maintained in a tank at the Usujiri Fisheries Laboratory of Hokkaido University until they reached maturity. Ova were obtained by dissecting the oviducts of a sacrificed female. Sperm masses were dissected from the seminal receptacles in the labral area of the same female and suspended in filter-sterilized, aerated seawater. After the sperm-seawater

mixture was added to the ova, freeze-dried oviducal gland powder, which had been dissolved in seawater beforehand, was added and mixed with the ova in a petri dish. The eggs were divided into groups of 15-20 per petri dish (60 mm in diameter) filled with filter-sterilized, aerated seawater. The seawater in each petri dish was changed twice daily. The fertilized eggs were incubated at 17°, 20°, and 23°C, on the advice of Sakurai *et al.* (1996) who reported that the peak of embryonic survival rates occurred between 14.7° and 22.2°C. At each temperature unit, about 1,000 eggs were incubated for morphological and histological observations.

Observation of Development and Determination of Stages

Observations were made of developing embryos under a light microscope and an atlas of developmental stages was prepared. Regular stages were identified mainly from embryos incubated at 20°C and supplementary observations at either 17° or 23°C were made for stages 9, 10, 11, 19, 20, 21, and 25.

To discriminate the developmental stages, the criteria established by Naef (1928) for ommastrephid Y and Arnold (1965, 1990) for *Loligo* were used. In the following description in the Results section, Arabic stage numerals in brackets represent the stages of Arnold (1965, 1990) and Roman stage numerals represent the stages of Naef (1928).

Developmental stages were defined to seven days after hatching, because many organs were differentiated after hatching. Measurements and means were taken on at least ten live animals.

Observation of Hatchlings from an Egg Mass

In addition to artificially fertilized eggs, newly emerged hatchlings from an egg mass spawned in a tank at the Usujiri Fisheries Laboratory of Hokkaido University on 30 September 1994 (Bower and Sakurai, 1996) were investigated. Eggs within the large spherical egg mass were surrounded by a large quantity of nidamental and oviducal gland jelly.

Histological Observation

Histological sections were prepared by fixing the specimens in Bouin's fixative, embedding in paraffin, sectioning, and staining with haematoxylin and eosin.

RESULTS

ATLAS OF DEVELOPMENT

Ova were ovoid in shape, and measured *ca.* 0.83 x 0.70 mm before fertilization. Following fertilization, the egg shape became spherical, measuring *ca.* 0.74 mm in diameter. The embryos took 90-95 hr at 20°C to develop from fertilization to hatching *in vitro* and the mean mantle length of hatchlings was 0.95 mm.

After hatching, paralarvae were maintained for up to *ca.* seven days without being fed while the internal yolk was completely absorbed. Paralarval mantle length measured 1.25 mm on the seventh day. The proboscis started to grow on the second day after hatching. The length of the proboscis from the anterior edge of the eyes was 0.27 mm on the third day and 0.49 mm on the seventh day, respectively. Arms III were not yet differentiated seven days after hatching.

In artificially fertilized eggs, hatching occured at stage 26, whereas eggs that developed within the egg mass spawned in the tank hatched at stage 28.

Fertilization and Meiosis (Figs. 1 and 5)

Stage 1 (Stage 1 of Arnold), 10 min after insemination: Fertilization. Following fertilization, perivitelline space expands and egg shape changes from ovoid to spherical. Micropyle is visible at the animal pole.

Stage 2 (Stage 2), 30 min: First maturation division. First polar body appears close to the animal pole.

Stage 3 (Stage 3), 1.5 hr: Second maturation division. Second polar body appears next to the first. Polar bodies are visible until stages 12 or 13. Blastodisc appears and increases in size. The blastodisc area is more transparent than the ooplasmic area.

Cleavage (Figs. 1 and 5)

Stage 4 (Stage 4), 2.6 hr: First cleavage. The furrow occurs at the center of the blastodisc running beneath the polar bodies and extending to the equator of the egg.

Stage 5 (Stage 5), 3.7 hr: Second cleavage. Second furrow occurs across the first one at right angle and divides the first cells into anteriorly, where the polar bodies are situated, and posteriorly located segments.

Stage 6 (Stage 6), 4.5 hr: Third cleavage. The cells are divided differently in the anterior half and the posterior half. In the posterior half, the furrow slants, almost parallel to the first one, while in the anterior half it extends radially pulling the second one forward.

Stage 7 (Stage 7), 5.0 hr: Fourth cleavage. Inner four cells are established as blastomeres while the surrounding 12 cells remain contiguous at their outer margins as blastocones.

Stage 8 (Stage 8), 5.5 hr: Fifth cleavage; 32 cells (14 blastomeres and 18 blastocones).

Stage 9 (Stage 9), 4.5 hr at 23°C: Sixth cleavage. The cells divide asynchronously and finally become 64 cells. A group of eight very small cells exists near the cen-

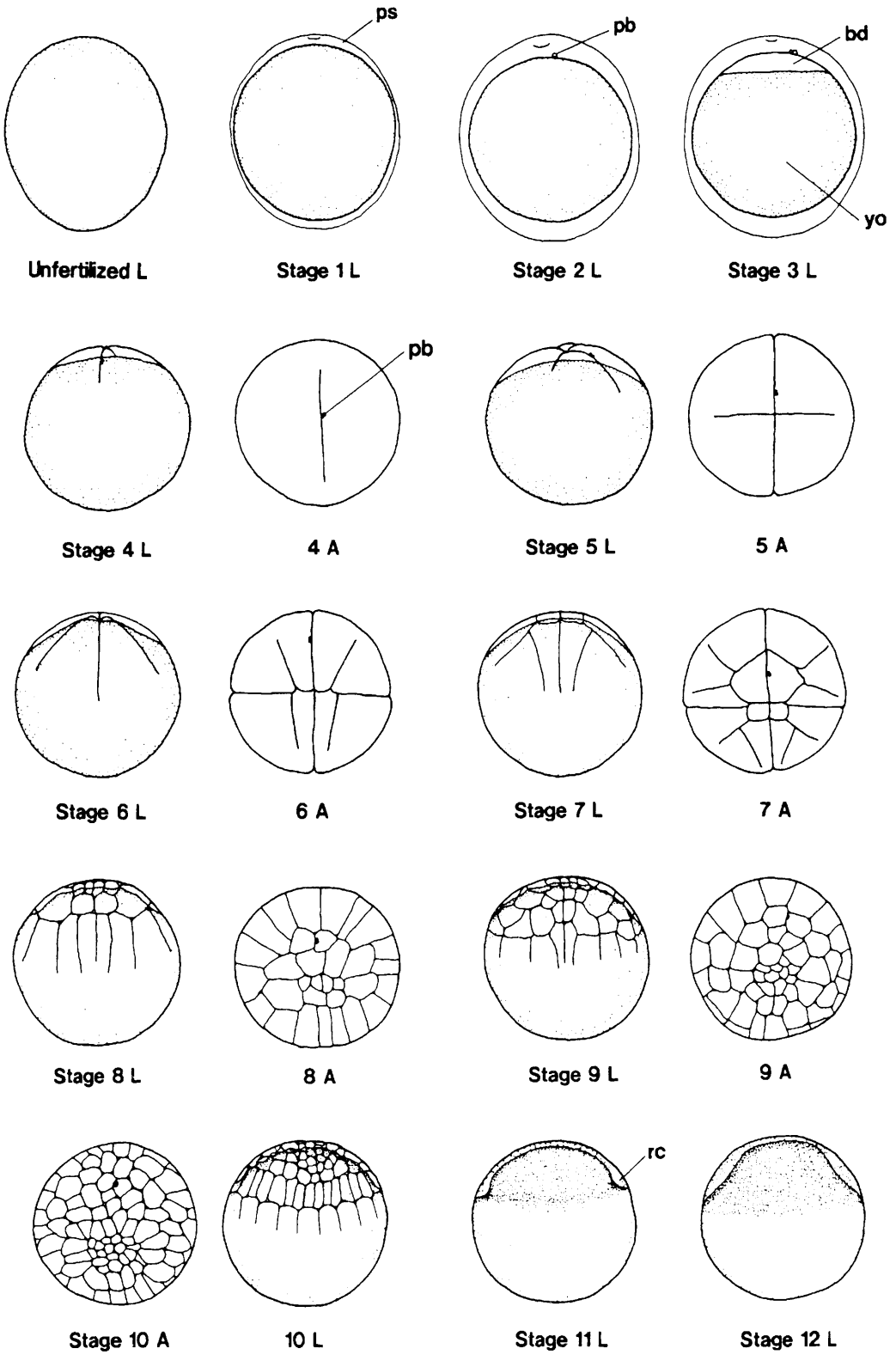

Fig. 1. Development of *Todarodes pacificus*, from fertilized egg to Stage 12 embryo. A or L with stage number indicates apical or lateral view. Egg membrane is omitted from Stage 4. Scale bar = 0.5 mm. (bd, blastodisc; pb, polar body; ps, perivitelline space; rc, ring-shaped group of cells; yo, yolk).

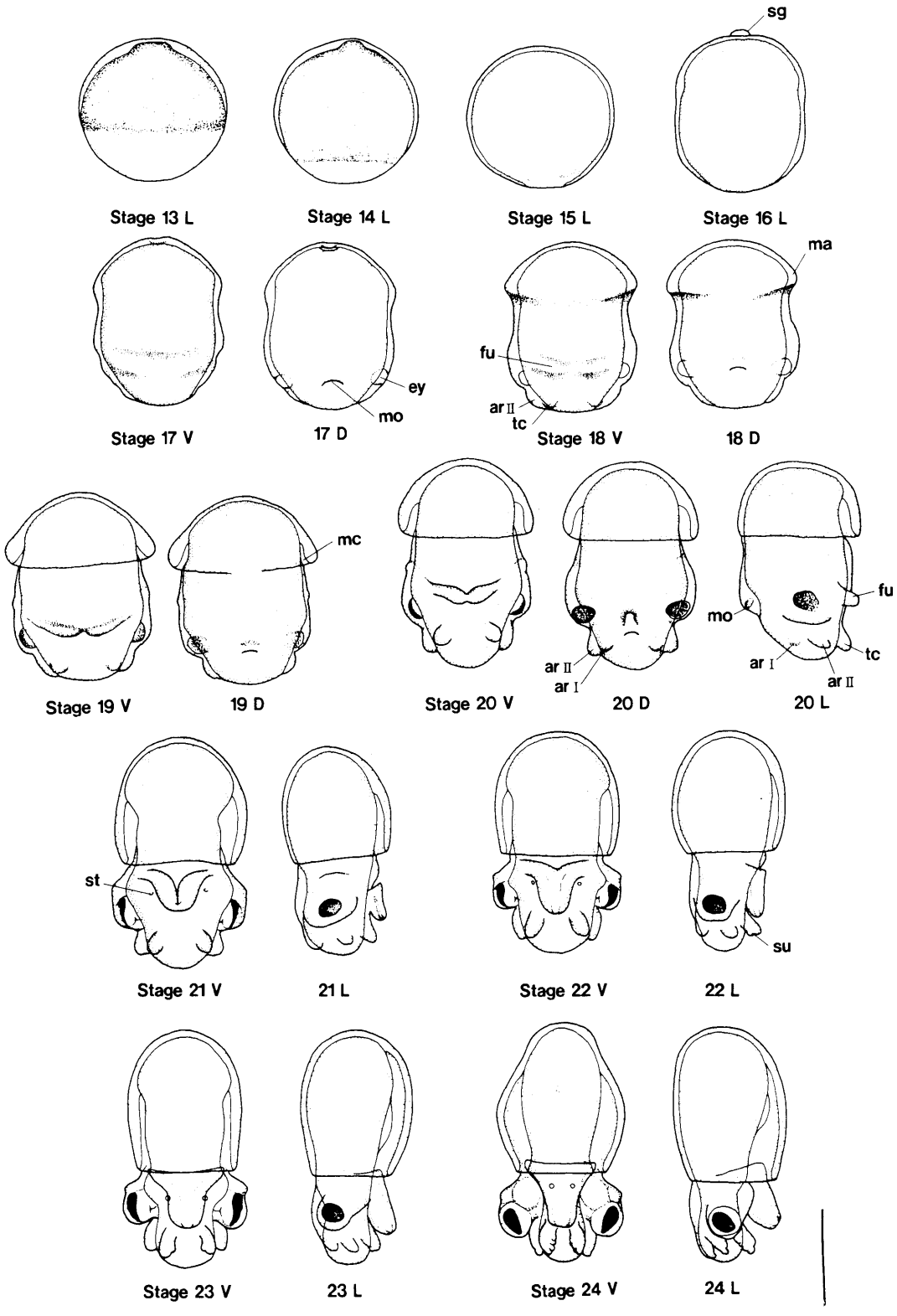

Fig. 2. Development of *Todarodes pacificus*, from Stage 13 to Stage 24 embryo. D, L, or V with stage number indicates dorsal, lateral, or ventral view, respectively. Egg membrane is omitted. Scale bar = 0.5 mm. (ar, arm; ey, eye; fu, funnel; ma, mantle; mc, mantle cavity; mo, mouth; sg, shell gland; st, statocyst; su, sucker; tc, tentacle club).

ter; this was designated as the future shell gland by Arnold (1990).

Stage 10 (Stage 9+), 5.0 hr at 23°C: Cleavage continues asynchronously. The group of very small cells remains recognizable centrally.

Segregation of the Germ Layers and Growth of Blastoderm (Figs. 1, 2, and 5)

Stage 11 (Stage 10), 5.8 hr at 23°C: The cell size becomes smaller and almost uniform. A ring-shaped group of cells appears around the yolk mass below the margin of the blastoderm, which is caused by marginal superposition of the single early layer of the germ layer, as described by Boletzky (1988) and Arnold (1990).

Stage 12 (Stages 11-15), 19.0 hr at 17°C: The inner layer spreads toward the animal pole below the outer layer. The yolk mass becomes papilla-like in the center of the blastoderm at the animal pole. The edge of the blastoderm expands toward the vegetal pole and covers almost half of the egg.

Stage 13 (Stages 11-15), 19.5 hr: The blastoderm covers approximately two-thirds of the egg. The papilla-like yolk apex gradually becomes smaller with the formation of the inner germ layer.

Stage 14 (Stages 11-15), 26.5 hr: The blastoderm covers approximately three-quarters of the egg.

Stage 15 (Stages 11-15), 33.0 hr: The inner layer of the blastoderm closes at the vegetal pole and the papilla-like yolk apex disappears. Both layers of blastoderm nearly (but not quite) close at the vegetal pole.

Organogenesis (Figs. 2, 3, 5, 6, and 8)

Stage 16 (Stage 16), 42 hr: The embryo expands vertically. Major organ primordia appear as thickenings, with the primordium of the shell gland especially evident as a projection.

Stage 17 (Stage VIII of Naef), 50 hr: Invaginations of the shell gland and the mouth begin. The thickenings of the major organs progress. Primordia of eyes are visible.

Stage 18 (Stage VIII+), 54 hr: Elevation of mantle, funnel, eyes, tentacle clubs, and arms II begins. The shell gland is closed.

Stage 19 (Stage X), 40 hr at 23°C: Formation of mantle margins begins at ventral side. The funnel folds on each side rise clearly, but fusion in the midline has not yet commenced. Primordia of tentacle clubs and arms II are clearly visible.

Stage 20 (Stage X+), 43 hr at 23°C: Faint primordia of arms I appear. Retina pigmentation begins. Mantle cavity spreads into dorsum. Anterior parts of the funnel folds unite by margin. A row of faint chromatophores is first visible on the ventral and dorsal mantle margins. Embryo revolves around the vertical axis.

Stage 21 (Stage X++), 46 hr at 23°C: Funnel folds are fusing. Statocyst invagination begins. Optic stalks increase in height. Two rows of chromatophores are visible on the anterior mantle surface.

Stage 22 (Stage XII), 68 hr: Funnel tube is established and is not covered by the mantle. First sucker primordia appear on the tentacle clubs. Three rows of chromatophores are visible on the mantle.

Stage 23 (Stage XII+), 72 hr: Mantle covers the posterior margin of the funnel. Four rows of chromatophores are visible on the mantle.

Stage 24 (Stage XIV), 78 hr: Cephalic organs, such as eyes and optic ganglia, are concentrated. Yolk is transferred from yolk sac of cephalic region to mantle cavity. Four sucker primordia on the tentacle clubs and one sucker primordium on each arm are clearly visible. Positions of the suckers are different on right and left tentacle clubs. The chromatophores on the mantle become larger and more distinct.

Stage 25 (Stage XIV+), 67 hr at 23°C: A gutter is formed at the posterior apex of inner yolk sac by esophagus. Lens primordia are first visible. Primordia of ventral organs, such as gills, branchial hearts, systemic heart, stomach, caecum, and anal knoll, become prominent as three swellings. Bases of the two tentacle clubs come together in the midline. Hatching can occur by external stimulation.

Stage 26 (Stage XVI), 92 hr: Hatching. Primary lids cover the eye vesicles.

Post-Hatching (Figs. 3, 4, 7, and 8)

Stage 27 (Stage XVI+), 4 hr after hatching: Yolk sac at cephalic region is contracting. The gutter of the posterior apex of inner yolk sac deepens.

Stage 28 (Stage XVIII), 1 day after hatching: Yolk sac at cephalic region almost disappears. Fin primordia appear on the apex of the mantle. Primordia of arms IV are first visible. Statoliths are evident in both statocysts. Head chromatophores are first visible. Systemic heart is pulsating. Hatching from egg mass spawned in tank occurs at this stage.

Stage 29 (Stage XVIII+), 2 days after hatching: A stalk on the base of the tentacles begins to elongate, forming the so-called proboscis. The right and left club suckers, which were at different positions from stage 24 onwards, are combined together in a symmetrical pattern. Ink begins to be concentrated in ink sac. Primodia of gills and branchial hearts are evident. Digestive gland and salivary gland are visible.

Stage 30 (Stage XX), 3 days after hatching: Inner yolk sac has contracted and separated from the posterior end of the mantle. The eyes are finally covered by primary

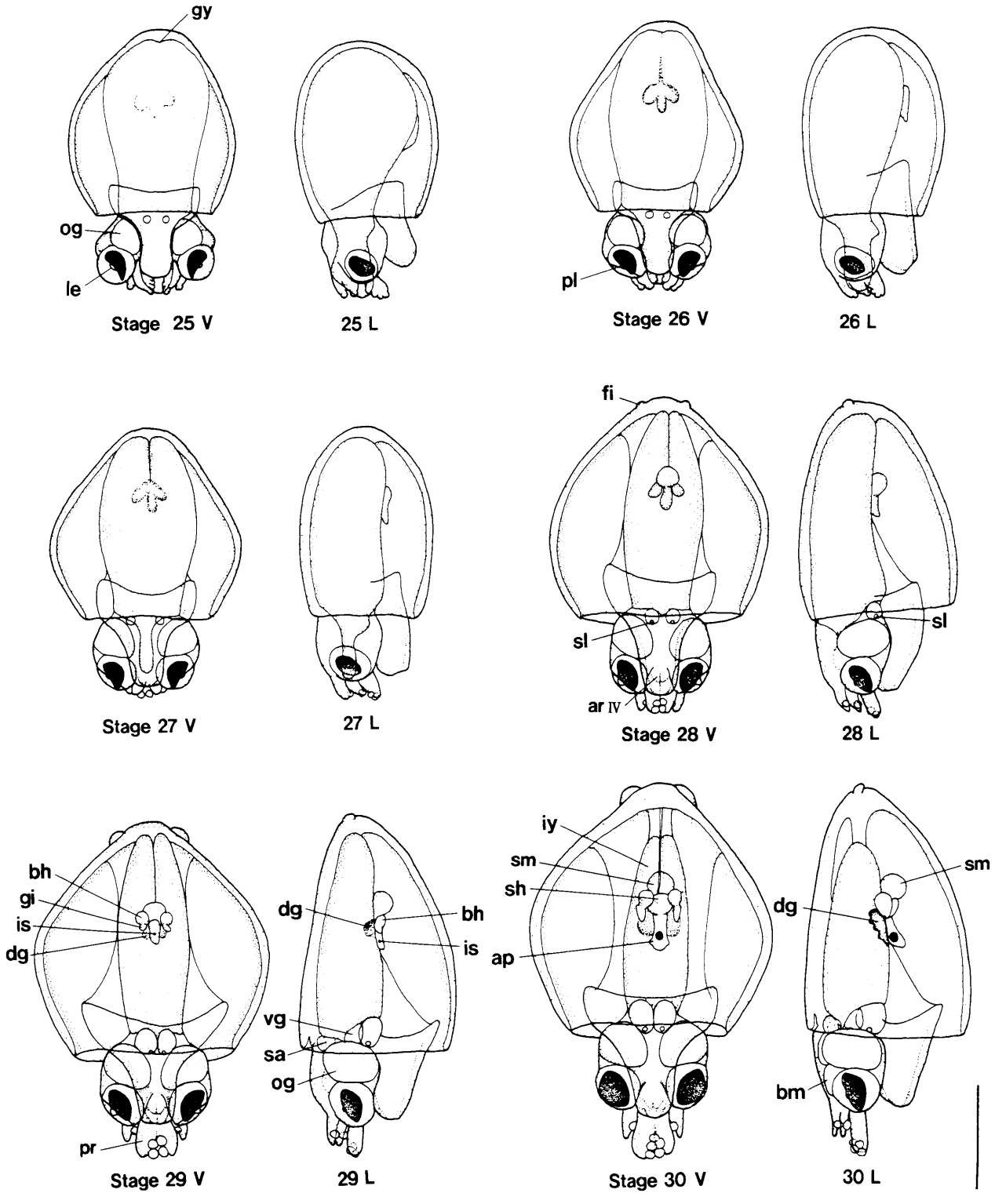

Fig. 3. Development of *Todarodes pacificus*, from Stage 25 embryo to Stage 30 rhynchoteuthion paralarva. L or V with stage number indicates lateral or ventral view. Egg membrane is omitted. Scale bar = 0.5 mm. (ap, anal papilla; ar, arm; bh, branchial heart; bm, buccal mass; dg, digestive gland; fi, fin; gi, gill; gy, gutter of inner yolk sac; is, ink sac; iy, inner yolk sac; le, lens, og, optic ganglion; pl, primary lid; pr, proboscis; sa, salivary gland; sh, systematic heart; sl, statolith; sm, stomach; vg, visceral ganglion).

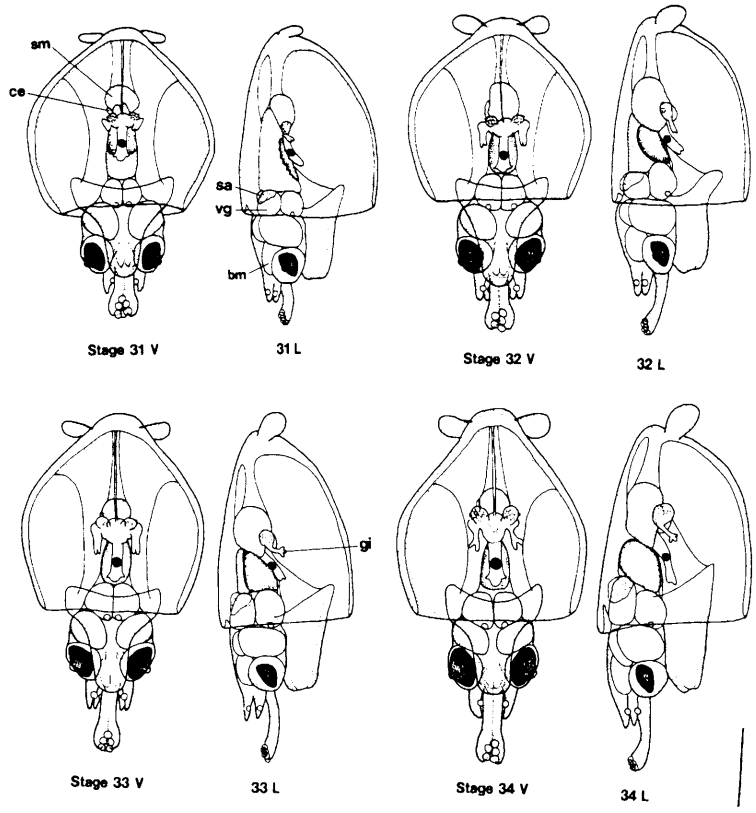

Fig. 4. Development of *Todarodes pacificus,* from Stage 31 to Stage 34 rhynchoteuthion paralarva. L or V with stage number indicates lateral or ventral view. Scale bar = 0.5 mm. (bm, buccal mass; ce, caecum; gi, gill; sa, salivary gland; sm, stomach; vg, visceral ganglion).

lid except protruded lens. Stomach is visible. Intestine grows and anal papillae are evident.

Stage 31, 4 days after hatching: Inner yolk sac becomes smaller and its posterior part lies behind the stomach in ventral view. Caecum is visible. Digestive gland grows larger. Ink sac is filled with ink. The stomach shows peristaltic movement. The suckers of the proboscis have primordia of chitinous rings.

Stage 32, 5 days after hatching: Posterior part of the inner yolk sac lies behind the systemic heart in ventral view. Digestive gland becomes larger. The proboscis stretches and contracts with the suckers moving.

Stage 33, 6 days after hatching: Branched gills are visible.

Stage 34, 7 days after hatching: Yolk almost consumed.

HISTOLOGICAL OBSERVATION

Histological observations of the digestive and respiratory organs revealed that they were still immature at the stage of hatching (Figs. 9A-B). From stage 28, the radula sac began to differentiate and started to secrete chitinous material (rs, Fig. 9C). Radula teeth were well developed and extended along the entire length of the ribbon by stage 29 (rs and rt, Fig. 9D). Upper and lower plates of the jaw also developed at stage 29 (uj and lj, Fig. 9D).

Rudiments of the digestive gland were histologically distinct from the surrounding mesoderm as a pair of monolayered saccular organs at stage 26, the lumina extending from the primary alimentary canal. From about stage 28, the stomach rudiment became distinct as a saccular body (sm, Fig. 9A). High vascularization in the digestive gland started at stage 29, and the lumen of the structure of the originally sac-like organ became complex. From stage 31, the caecum was differentiated, but the stomach and caecum were not clearly divided internally. The digestive duct appendages (the so-called pancreas) were histologically distinct from the digestive gland (the so-called liver) at stage 31. Differentiation of glandular cells in the digestive gland was conspicuous at stage 32. The space occupied by the digestive gland in adults was largely occupied by yolk in the hatchling. Fusion of the pair of rudimentary digestive glands began at stage 34 when the yolk was almost completely absorbed.

The rudiments of the salivary gland were histologi-

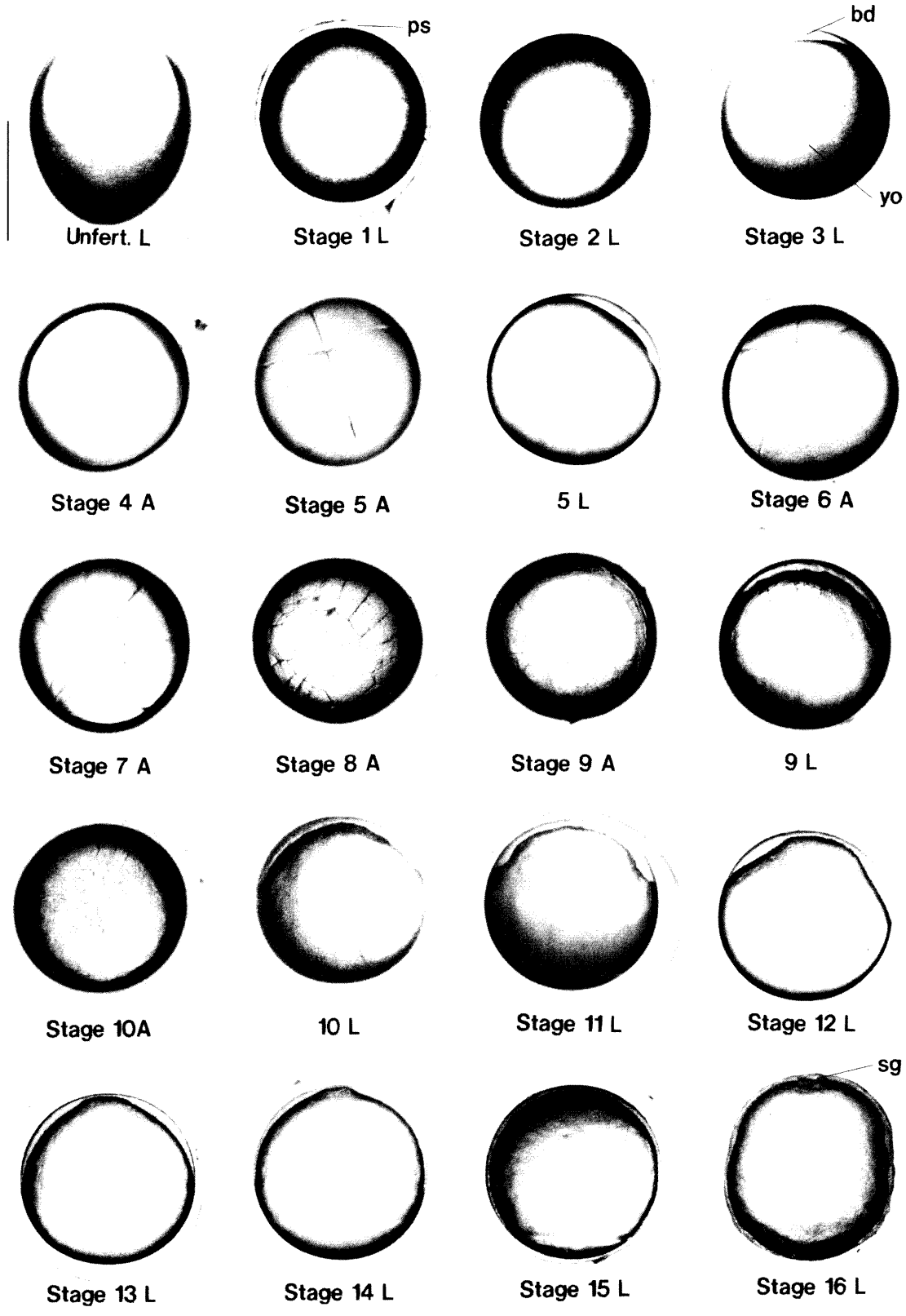

Fig. 5. Unfertilized egg and Stages 1-16 in the development of *Todarodes pacificus*. A or L with stage number indicates apical or lateral view. Scale bar = 0.5 mm. (bd, blastodisc; ps, perivitelline space; sg, shell gland; yo, yolk).

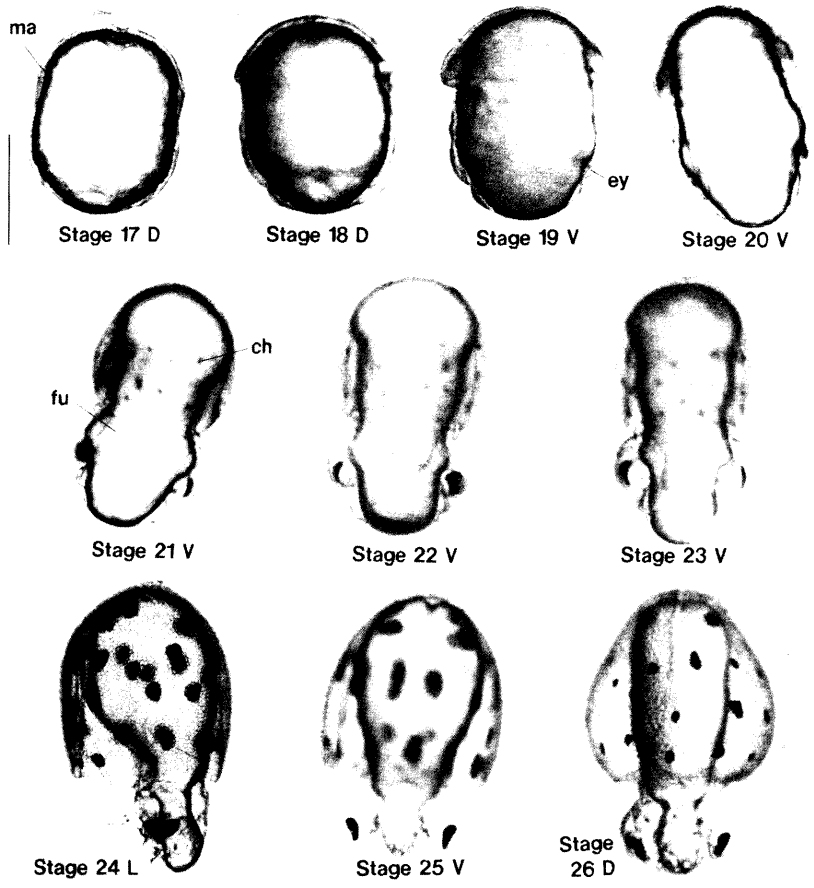

Fig. 6. Stages 17-26 in the development of *Todarodes pacificus*. D, L, or V with stage number indicates dorsal, lateral, or ventral view, respectively. Scale bar = 0.5 mm. (ch, chromatophore; ey, eye; fu, funnel; ma, mantle).

cally distinct as a monolayered sac-like papilla at stage 28 (sa, Fig. 9E). Glandular cells started to differentiate, and secretion of granules (stained by eosin) was observed in the lumen of salivary glands at around stage 32 (sa, Fig. 9F).

Todarodes pacificus has no distinct external yolk sac, but a dome-shaped external yolk sac was seen at late embryonic stages. No blood sinus was observed in the dome-shaped external yolk sac. The blood space, which includes primodia of the branchial and systemic hearts, and major vessels, such as the aorta posterior and vena cava, began to appear at stage 26. At the same time, the pericardial cavity developed.

The rudiments of gills became visible at stage 28 (gi, Fig. 10A), and lamellae of the gills became distinct at stage 30 (gi, Fig. 10B). The development of gills was so slow that only three rudimentary lamellae were observed at stage 34.

A functional Hoyle's organ became visible at stage 25 just before hatching (stage 26), and quickly degenerated at stage 27 just after hatching. In contrast, a functional, active Hoyle's organ was still visible at stage 28 in hatchlings taken from the egg mass.

DISCUSSION

For comparative studies on cephalopod development, common criteria of developmental stages must be established. Most previous embryological investigations (*e. g.* O'Dor *et al.*, 1982; Segawa *et al.*, 1988; Arnold and O'Dor, 1990; Baeg *et al.*, 1992) have been based either on the stages of Naef (1928) for *Sepia officinalis* Linné, 1758, *Loligo vulgaris* Lamarck, 1798, and ommastrephid Y, or on the stages of Arnold (1965) for *L. pealeii*. There is correspondence between Naef (1928) for *L. vulgaris* and Arnold (1965) for *L. pealeii* (Segawa *et al.*, 1988). It is, however, impossible for all of the stages of *Loligo* to be applied to *Todarodes pacificus*, due to differences in the sequence of differentiation of major features, such as gills, branchial hearts, caecum, and stomach (Fig. 11). Because Naef (1928) never provided any early developmental stages for

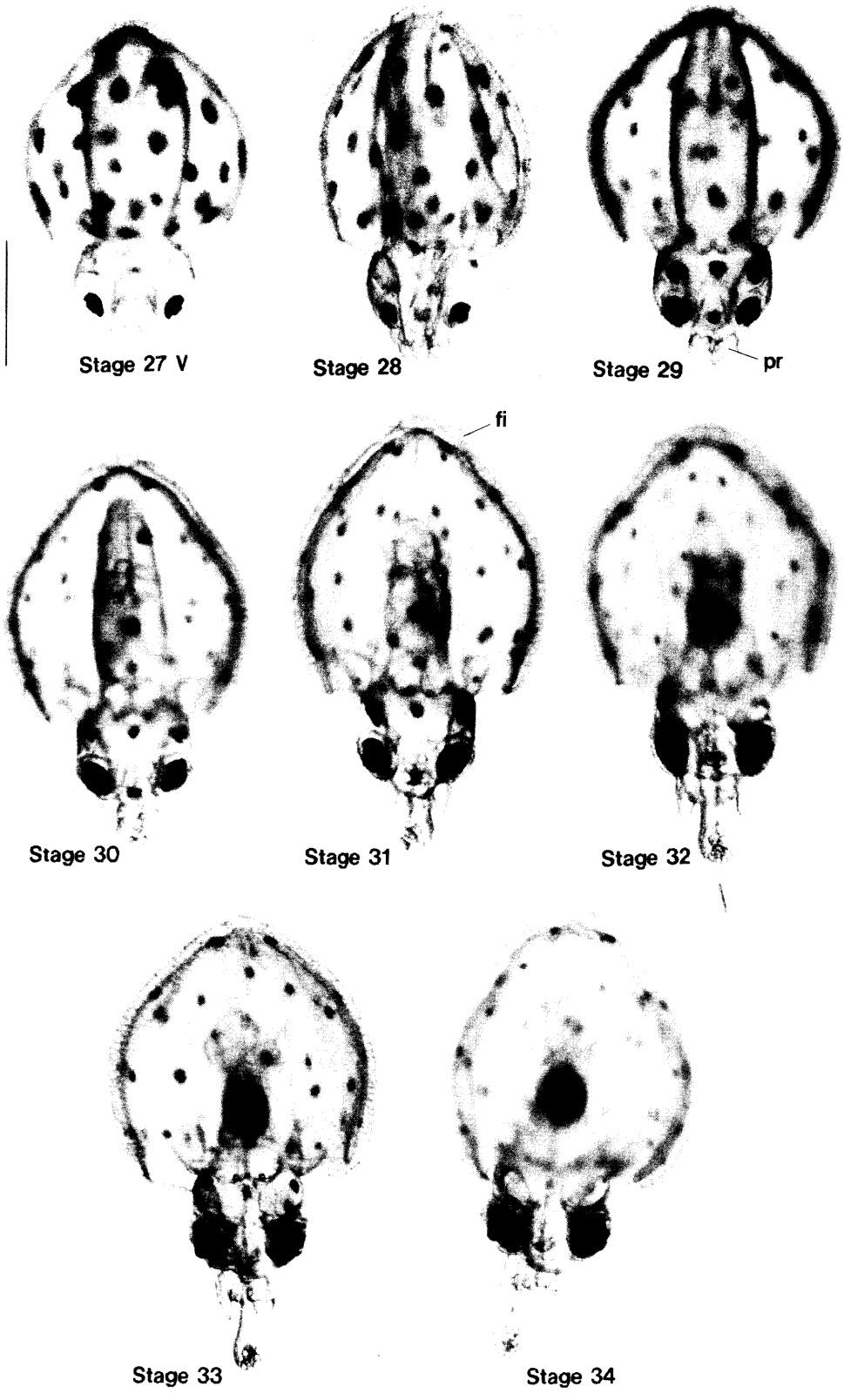

Fig. 7. Stages 27-34 in the development of *Todarodes pacificus*, ventral view. Scale bar = 0.5 mm. (fi, fin; pr, proboscis).

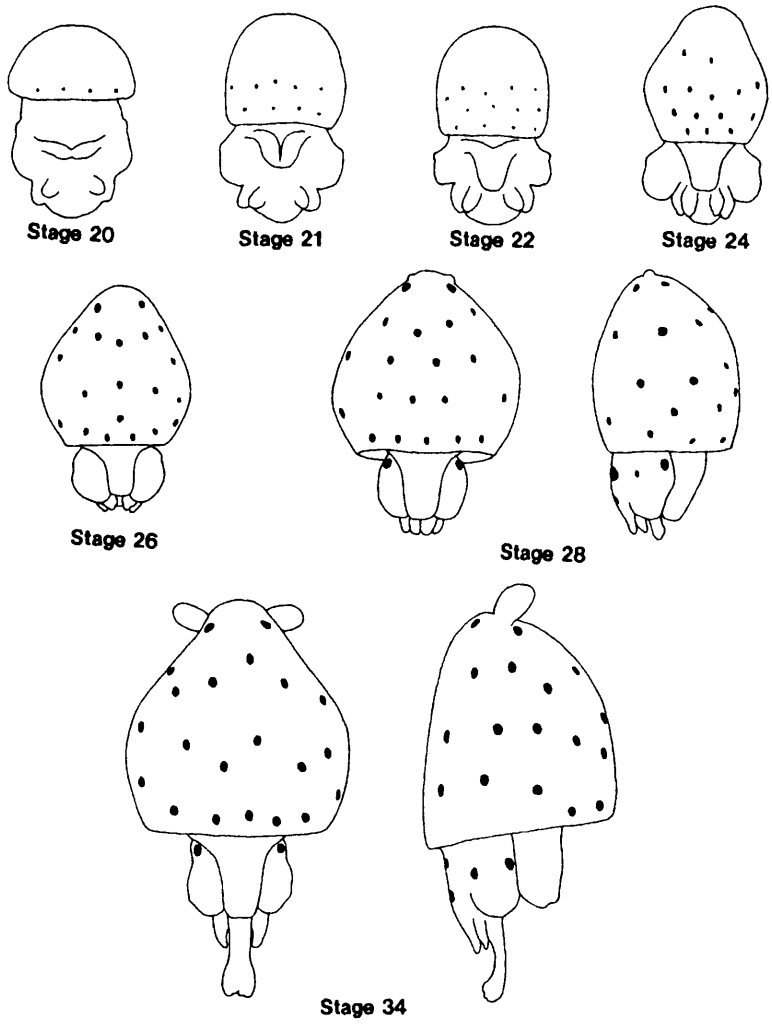

Fig. 8. Sequence of chromatophore development (Stages 20-34).

Ommastrephidae, the criteria of Arnold (1965) were used in the present study. The criteria for the phase of cleavage of *Loligo* (Arnold, 1965) are applicable to *T. pacificus*, although there are some differences in the blastoderm formation: the cleavage furrows reach the equator in eggs of *T. pacificus*, whereas the furrow length of *Loligo* represents only a small fraction of the egg perimeter.

There are differences between artificially fertilized eggs and eggs from the egg mass spawned in the tank. The eggs from the egg mass hatched approximately two stages later than artificially fertilized eggs. A key difference during development between artificially fertilized eggs and those from the egg mass in the present study was that the latter were enwrapped by nidamental and oviducal gland jelly. The observation of the development of Hoyle's organ suggests that hatching can occur between stages 25 and 28, and the timing of hatching can vary slightly, depending on external conditions of the eggs. It is possible that the presence of nidamental and oviducal gland jelly could somehow delay hatching.

In Naef's (1928) description of the developmental stages of an ommastrephid squid *(Illex coindetii)*, hatching occurs at stage XX. *I. illecebrosus* observed by O'Dor *et al.* (1982) reportedly hatched at the same stage XX of Naef. However, *Todarodes pacificus* hatched at stage XVI from artificially fertilized eggs and at stage XVIII from the egg mass. The newly hatched *T. pacificus* described by Hamabe (1962) appeared to be at stages XIV or XVI although he did not define any developmental stages. It is not certain whether this difference in hatching stage is due to a difference among species or to an environmental factor.

Hamabe (1962) assumed that arms III appear earlier than arms IV, based on an observation on hatchlings two days old (1.1 mm mantle length [ML]) and planktonic par-

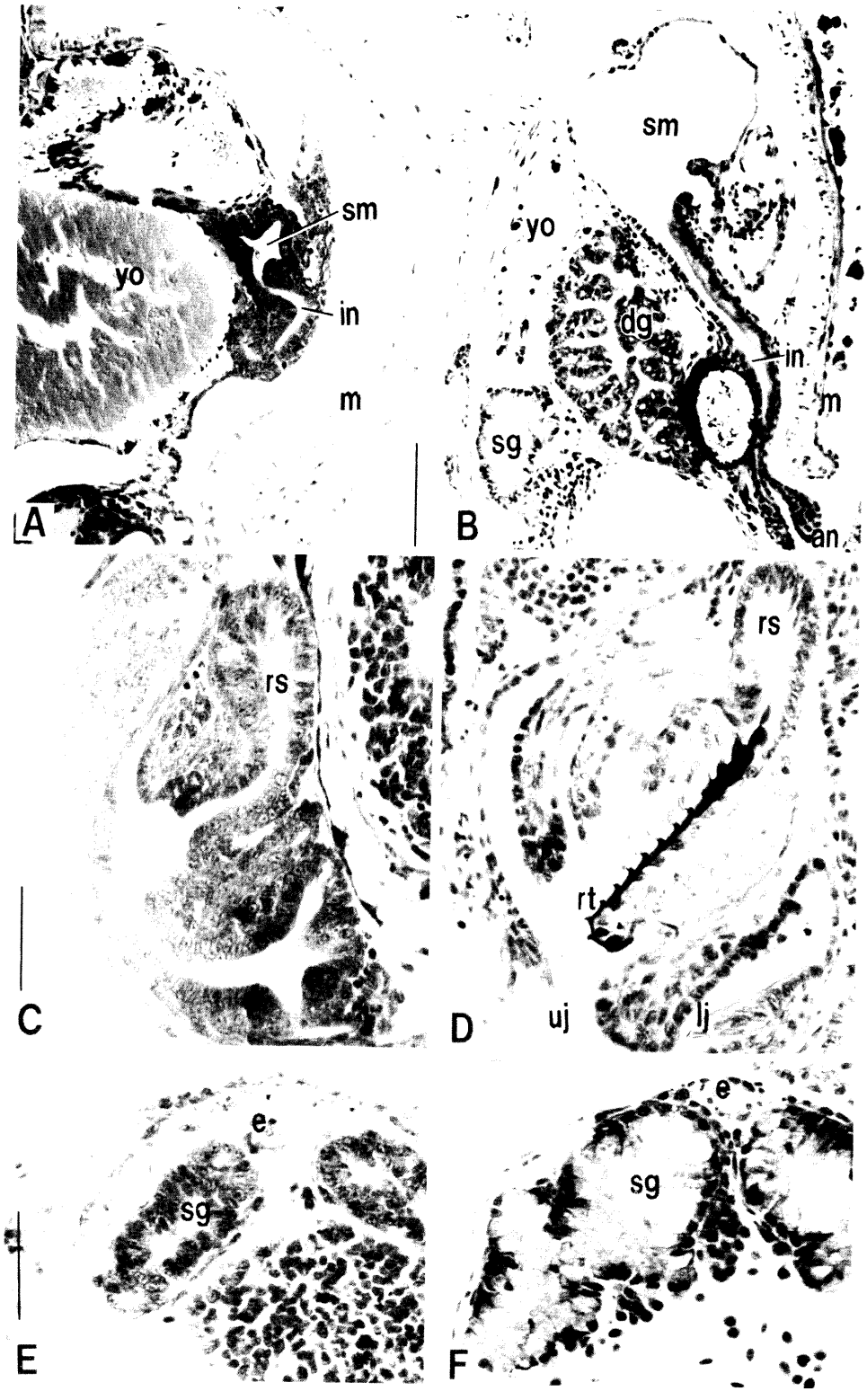

Fig. 9. Histological differentiation of digestive organs of *Todarodes pacificus* around hatching stage. A-B. Alimentary canal, longitudinal sections at Stages 28 (A) and 32 (B). C-D. Buccal mass, longitudinal sections at Stages 29 (C) and 31 (D). E-F. Salivary gland, cross-sections at Stages 30 (E) and 32 (F). Scale bars = 100 μm (A-B); 50 μm (C-F). (an, anus; dg, digestive gland (so-called liver); e, esophagus; in, intestine; lj, lower jaw; m, mantle; rs, radula sac; rt, radula teeth; sg, salivary gland; sm, stomach; uj, upper jaw; yo, yolk).

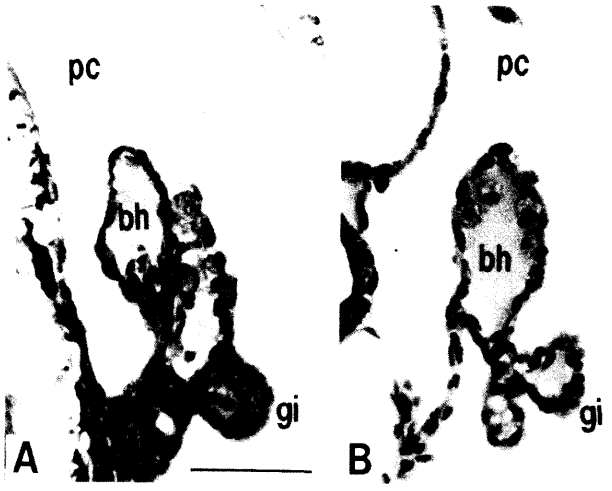

Fig. 10. Histological differentiation of the gills of *Todarodes pacificus*, longitudinal sections at Stages 28 (A) and 31 (B). Scale bar = 50 μm. (bh, branchial heart; gi, gill; pc, pericardial cavity).

alarvae (3-5 mm ML) captured from the sea. Hamabe could have mistaken arms IV for arms III, due to lack of information on intermediate specimens between the hatchling (1 mm ML) and later stage paralarvae (3 mm ML). The arms III appear and increase in size rapidly and outgrow arms IV, I, and II, while arms IV grow very slowly.

In *Todarodes pacificus*, the differentiation of digestive and respiratory organs is postponed until posthatching stages (Fig. 11). A similar tendency has been observed in other oegopsid squids, such as *Abraliopsis* sp. (see Arnold and O'Dor, 1990), *Illex illecebrosus* (see O'Dor *et al.*, 1982), and *Watasenia scintillans* (see S. Hayashi, 1995). In the Sepioidea and Myopsida, development of the digestive organs is almost completed long before hatching (Naef, 1928), and hatchlings are able to capture prey in the same manner as the adults, although a large quantity of yolk is still stored in the internal yolk sac (Boucaud-Camou *et al.*, 1985; Vecchione, 1987). On the other hand, although little is known about paralarval feeding of the Oegopsida, *T. pacificus* is estimated to start feeding at about stage 32 (five days after hatching) when the digestive organs are well differentiated. At this stage, the stomach exhibits peristaltic movement, and secretion of enzymes is observed in the salivary glands. *T. pacificus* could live on yolk absorption for a few days after hatching, and could immediately shift to exogenous feeding as soon as the yolk is absorbed. O'Dor *et al.* (1985) have suggested *Illex* rhynchoteuthion paralarvae have the capacity for suspension feeding. It is likely that small premature paralarvae of oceanic squids (Table 1) have a special paralarval feeding habit, given such

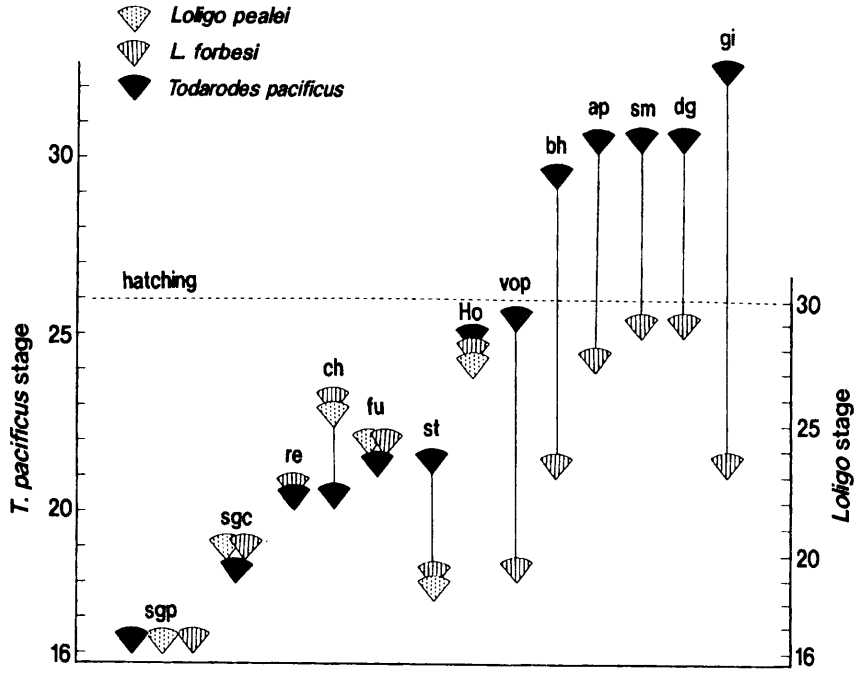

Fig. 11. The sequence of appearance of major features in *Loligo pealeii* (*fide* Arnold, 1965), *L. forbesi* (*fide* Segawa *et al.*, 1988), and *Todarodes pacificus* in the present study. For comparison, two stages are used for major criteria: stage 16 at commencement of organogenesis and the stage of hatching. (ap, anal papilla; bh, branchial heart; ch, chromatophore; dg, digestive gland; fu, funnel; gi, gill; Ho, Hoyle's organ; re, retina; sgc, closure of shell gland; sgp, primordium of shell gland; sm, stomach; st, statocyst; vop, primordium of ventral organ as three swellings, such as gills, branchial hearts, systemic heart, stomach, caecum, and anal knoll).

Table 1. Summary of egg size, number of eggs per spawning, and embryonic development time of 13 species of cephalopods. (–, no data).

Taxa	Egg size (mm)	Number of eggs per spawning	Days required for hatching (plus water, temperature, °C)	Hatchling size (mm ML)	Reference*
Sepia lycidas Gray, 1849	29.0 x 12.5	–	–	–	1
S. latimanus Quoy and Gaimard, 1832	28.5 x 22.3	30-40	about 40	13-14	1, 2
S. esculenta Hoyle, 1885	17.5 x 13.0	500	40 (18°C)	–	1
Sepiella japonica Sasaki, 1929	9.5 x 7.0	100s	35 (21°C)	3.4-4.3	3
Euprymna scolopes Berry, 1913	–	200	18 (24°C)	1.6	4
Idiosepius paradoxus Ortmann, 1881	1.5 x 1.3	–	16 (20°C)	1.16-1.22	5
Sepioteuthis lessoniana Lesson, 1830	5.8 x 5.6	500-1,000	25 (25°C)	5-7	6
Loligo pealeii LeSueur, 1821	1.6 x 1.0	–	–	1.6	7
L. bleekeri Keferstein, 1866	2.7 x 2.6	1,200-2,000	64-67 (18°C)	3.0-3.3	8, 9
L. forbesi Steenstrup, 1856	3.3 x 3.0	–	68-75 (25°C)	4.3-4.9	10
Watasenia scintillans (Berry, 1911)	1.5 x 1.2	2,000	5 (18°C)	1.4	11
Illex illecebrosus (LeSueur, 1821)	0.9 x 0.7	100,000	9 (21°C)	1.1	12
Todarodes pacificus Steenstrup, 1880	0.8 x 0.7	200,000†	4 (20°C)	0.95	13

*1, Okutani, 1978; 2, Corner and Moore, 1980; 3, Yamamoto, 1982; 4, Arnold *et al.*, 1972; 5, Natsukari,1970; 6, Segawa, 1987; 7, Boletzky and Hanlon, 1983; 8, Baeg *et al.*, 1992; 9, Natsukari and Tashiro, 1991; 10, Segawa *et al.*, 1988; 11, S. Hayashi, 1995; 12, O'Dor *et al.*, 1982; 13, Present study, and Bower and Sakurai,1996 (†).

a rapid shift from endogenous to exogenous feeding.

In *Todarodes pacificus,* development of the gills is remarkably delayed in spite of their later importance as vital organs. In embryos with a large external yolk sac, *viz.* the Myopsida and Sepioidea, the gills are fully developed long before hatching, and oxygen-rich blood fills the sinus of the external yolk sac before final development of the gills (Boletzky, 1987). In such embryos, the external yolk sac already functions as a pump before the development of the systemic heart and branchial hearts in later organogenesis (Boletzky, 1989). In contrast, *T. pacificus* embryos have no blood sinus in the external yolk sac and the final development of the gills is very late. The embryos and hatchlings of *T. pacificus* should achieve dermal respiration through the surface tissues by the final development of gills as was assumed by Arnold and O'Dor (1990).

In conclusion, the paralarval stages of *Todarodes pacificus* are characterized by special developmental patterns in digestive and respiratory systems as well as by some external morphological characters, such as formation of a so-called proboscis. A developmental process in which hatching occurs before the completion of some important organs could characterize the Oegopsida which produces small eggs, have a short embryonic developmental time, and small hatchlings as compared with the Myopsida and Sepioidea (Table 1).

In the Cephalopoda, egg size and fecundity vary among species (Table 1). In general the Sepioidea and Myopsida tend to produce fewer but proportionally larger eggs and developmental time is longer, as compared with the Oegopsida. The Sepioidea and Myopsida embryos have a large external yolk sac at later embryonic stages, and the yolk therein is gradually transferred into the internal yolk sac during stages 25-30 of Arnold (1965). In this process, the shape of the internal yolk sac becomes rather complicated. On the other hand, although embryos of *Todarodes pacificus* do not have such a distinct external yolk sac, they do have a dome-shaped external yolk sac, and a small-scale transfer of yolk is observed during stages 24-28. Observations of morphological changes in the yolk sac from nine species of the families Sepiidae, Sepiolidae, Loliginidae, Enoploteuthidae, Ommastrephidae, and Thysanoteuthidae (Watanabe, unpub.), show three families belonging to the Oegopsida obviously have no distinct external yolk sac except for a dome-shaped external yolk sac. And from this observation, it is clear that each taxon has its own pattern of morphological change in the internal yolk sac. The patterns of the morphological changes of the Oegopsida are simpler than those of the Sepioidea and Myopsida. The only morphological change of the internal yolk sac in *T. pacificus* is the formation a gutter by the esophagus, in contrast to several gutters formed by blood vessels as well as the alimentary canal in the Sepioidea and Myopsida. This morphological change of the internal yolk sac could play a dominant role in the development of circulatory organs, because the sinus surrounding the internal yolk sac differentiates a network of vessels (Boletzky, 1989). The morphological change of the yolk sac is reflect-

ed by the location of organs around the yolk sac in the embryonic phase, including the vessels. In approaching a phylogeny of cephalopods from an embryological point of view, comparative study of morphological change of the yolk sac could be important.

From such a comparative point of view, the Oegopsida including *Todarodes pacificus* which produce a large number of small eggs are specialized among the Recent cephalopods by the presence of a reduced external yolk sac, and delayed differentiation of some organs, such as the respiratory and digestive organs. These characters could reflect a reproductive strategy for paralarval dispersion in the open ocean.

ACKNOWLEDGMENTS

We thank the staff of Usujiri Fisheries Laboratory of Hokkaido University for their kind technical assistance in the maintenance of the squid. We also thank Mr. J. Bower, Hokkaido University, for providing hatchlings for study and for his revision of the English, and Dr. Y. Ikeda, Kyoto University, for his technical assistance with artificial fertilization.

LITERATURE CITED

Arnold, J. M. 1965. Normal embryonic stages of the squid, *Loligo pealii* Lesueur. *Biological Bulletin* 128(1):24-32.

Arnold, J. M. 1990. Embryonic development of the squid. *In: Squid as Experimental Animals*, D. L. Gilbert, W. J. Adelman, Jr., and J. M. Arnold, eds. pp. 77-90. Plenum Press, New York.

Arnold, J. M. and R. O'Dor. 1990. In vitro fertilization and embryonic development of oceanic squid. *Journal of Cephalopod Biology* 1(2):21-36.

Arnold, J. M., C. T. Singley, and L. D. Williams-Arnold. 1972. Embryonic development and post-hatching survival of the sepiolid squid *Euprymna scolopes* under laboratory conditions. *The Veliger* 14(4):361-364.

Baeg, G. H., Y. Sakurai, and K. Shimazaki. 1992. Embryonic stages of *Loligo bleekeri* Keferstein. *The Veliger* 35(3):234-241.

Boletzky, S. v. 1987. Ontogenetic and phylogenetic aspects of the cephalopod circulatory system. *Experientia* 43:478-483.

Boletzky, S. v. 1988. Cephalopod development and evolutionary concepts. *In: The Mollusca, Vol. 12. Paleontology and Neontology of Cephalopods,* M. R. Clarke and E. R. Trueman, eds. pp. 185-202. Academic Press, London.

Boletzky, S. v. 1989. Recent studies on spawning, embryonic development, and hatching in Cephalopoda. *In: Advances in Marine Biology, Vol. 25,* J. H. S. Blaxter and A. J. Southward, eds. pp. 85-115. Academic Press, London.

Boletzky, S. v. and R. T. Hanlon. 1983. A review of the laboratory maintenance, rearing and culture of cephalopod molluscs. *Memoirs of the National Museum of Victoria* 44:147-187.

Boletzky, S. v., L. Rowe, and L. Aroles. 1973. Spawning and development of the eggs, in the laboratory, of *Illex coindetii* (Mollusca: Cephalopoda). *The Veliger* 15:257-258.

Boucaud-Camou, E., M. Yim, and A. Tresgot. 1985. Feeding and digestion of young *Sepia officinalis* L. (Mollusca: Cephalopoda) during post-hatching development. *Vie et Milieu* 35(3/4):263-266.

Bower, J. R. and Sakurai, Y. 1996. Laboratory observations on *Todarodes pacificus* (Cephalopoda: Ommastrephidae) egg masses. *American Malacological Bulletin* 13(1/2):65-71.

Corner, B. D. and H. T. Moore. 1980. Field observations on the reproductive behavior of *Sepia latimanus*. *Micronesia* 16(2):235-260.

Hamabe, M. 1961. [Experimental studies on breeding habit and development of the squid, *Ommastrephes sloani pacificus* Steenstrup. Early embryonic development and morphological features of the larvae immediately after hatching.] *Zoological Magazine* 70(12):408-420. [In Japanese with English title and abstract].

Hamabe, M. 1962. [Embryological studies on the common squid, *Ommastrephes sloani pacificus* Steenstrup, in the southwestern waters of the Sea of Japan.] *Bulletin of Japan Sea Regional Fisheries Research Laboratory* 10:1-45. [In Japanese with English title and abstract].

Hayashi, H. 1960. [Development of the squid, *Ommastrephes sloani pacificus.*] *Bulletin of Faculty of Fisheries, Nagasaki University* 9:43-47. [In Japanese with English title and abstract].

Hayashi, S. 1995. [Fishery biological studies of the firefly squid, *Watasenia scintillans*, in Toyama Bay.] *Bulletin of Toyama Prefectural Fisheries Research Institute* 7:1-128. [In Japanese with English title and abstract].

Ikeda, Y., Y. Sakurai, and K. Shimazaki. 1993. Fertilizing capacity of squid *(Todarodes pacificus)* spermatozoa collected from various sperm storage sites, with special reference to the role of gelatinous substance from oviducal gland in fertilization and embryonic development. *Invertebrate Reproduction and Development* 23(1):39-44.

Naef, A. 1928. Die Cephalopoden. *Fauna Flora Golf Neapel* 35(2):1-357.

Natsukari, Y. 1970. [Egg-laying behavior, embryonic development and hatched larva of the pygmy cuttlefish, *Idiosepius pygmaeus paradoxus* Ortmann.] *Bulletin of the Faculty of Fisheries, Nagasaki University* 30:15-29. [In Japanese with English title and abstract].

Natsukari, Y. and M. Tashiro. 1991. Neritic squid resources and cuttlefish resources in Japan. *Marine Behaviour and Physiology* 18:149-226.

O'Dor, R. K., N. Balch, E. A. Foy, R. W. M. Hirtle, D. A. Johnston, and T. Amaratunga. 1982. Embryonic development of the squid, *Illex illecebrosus*, and effect of temperature on development rates. *Journal of North-west Atlantic Fishery Science* 3:41-45.

O'Dor, R. K., P. Helm, and N. Balch. 1985. Can rhynchoteuthion suspension feed? *Vie et Milieu* 35(3/4):267-271.

Okutani, T. 1978. [Studies on early life history of decapodan Mollusca - VII. Eggs and newly hatched larvae of *Sepia latimanus* Quoy and Gaimard.] *Venus* 37(4):245-248. [In Japanese with English title and abstract].

Okutani, T. 1995. *Cuttlefish and Squids of the World in Color.* National Cooperative Association of Squid Processors, Tokyo. 185 pp.

Okutani, T., M. Tagawa, and H. Horikawa. 1987. *Cephalopods from Continental Shelf and Slope around Japan.* Japan Fisheries Resource Conservation Association, Tokyo. 194 pp.

Sakurai, Y, J. R. Bower, Y. Nakamura, S. Yamamoto, and K. Watanabe. 1996. Effect of temperature on development and survival of *Todarodes pacificus* embryos and paralarvae. *American Malacological Bulletin* 13(1/2):89-95.

Sakurai, Y. and Y. Ikeda. 1992. [Laboratory rearing methods of *Todarodes pacificus* for the ecological study of life cycle.] Proceedings of the Squid Stock and Fisheries Oceanic Conditions. *National Research Institute of Far Seas Fisheries* (1993):51-69. [In Japanese with English title].

Sakurai, Y., R. E. Young, J. Hirota, K. Mangold, M. Vecchione, M. R.

Clarke, and J. Bower. 1995. Artificial fertilization and development through hatching in the oceanic squids *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis* (Cephalopoda: Ommastrephidae). *The Veliger* 38(3):185-191.

Segawa, S. 1987. Life history of the oval squid, *Sepioteuthis lessoniana* in Kominato and adjacent waters central Honshu, Japan. *Journal of the Tokyo University of Fisheries* 74(2):67-105.

Segawa, S., W. T. Yang, H. J. Marthy, and R. T. Hanlon. 1988. Illustrated embryonic stages of the eastern Atlantic squid *Loligo forbesi*. *The Veliger* 30(3):230-243.

Soeda, J. 1952. [On the artificial insemination and the early cleavage of the ovum in oegopsid Cephalopoda, *Ommastrephes sloani pacificus*, Surume-ika.] *Bulletin of the Hokkaido Regional Fisheries Laboratory* 5:1-15. [In Japanese with English title and abstract].

Soeda, J. 1954. [A study on the fertilization of the egg of the squid, *Ommastrephes sloani pacificus* (Steenstrup), Surume-ika.] *Bulletin of the Hokkaido Regional Fisheries Laboratory* 11:1-6. [In Japanese with English title and abstract].

Yamamoto, M. 1982. [Normal stages in the development of the cuttlefish, *Sepiella japonica* Sasaki.] *Zoological Magazine* 91:146-157. [In Japanese with English title and abstract].

Vecchione, M. 1987. Juvenile ecology. *In: Cephalopod Life Cycles, Vol. 2*, P. R. Boyle, ed. pp. 61-84. Academic Press, London.

Date of manuscript acceptance: 12 April 1996