

Information Panel

The Embryonic Development of the Hawaiian Bobtail Squid (*Euprymna scolopes*)

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INTRODUCTION

A staging series based on easily distinguishable morphological features is a basic and necessary tool for developmental studies. It provides a consistent reference for comparisons between independent studies, negates the need to know when fertilization occurred, allows correlation of the phase of development with the time of development (to facilitate collection of embryos at specific stages), and allows comparisons between species. Given the growing interest in Hawaiian bobtail squid (*Euprymna scolopes*) as a contemporary cephalopod developmental system, this article provides a detailed survey of *E. scolopes* embryogenesis from cleavage through hatching under controlled environmental conditions, including detailed descriptions of externally visible morphological features that are easily distinguished in either live or freshly fixed embryos under a dissecting microscope. Photomicrographs are also provided to aid in the accurate and rapid staging of *E. scolopes* embryos.

RELATED INFORMATION

This article supplements an earlier account of *E. scolopes* embryogenesis (Arnold et al. 1972). Descriptions of readily observable morphological features at each specific stage of development are presented in Table 1. For a more extensive discussion on the background, husbandry, and potential uses of *E. scolopes* as model organisms, see **The Hawaiian Bobtail Squid (*Euprymna scolopes*): A Model to Study the Molecular Basis of Eukaryote-Prokaryote Mutualism and the Development and Evolution of Morphological Novelties in Cephalopods** (Lee et al. 2009a).

For the initial observations of developmental stages reported here, a single egg mass containing 114 embryos was used, and ~10 embryos were sampled from the mass each day. The frequency of observations varied with the stage of development: During early cleavage, observations were made every 2 h; during gastrulation, observations were made every 3 h; during early organogenesis, observations were made every 6 h, and during later stages of development, observations were made once per day. To establish the reproducibility of the morphological descriptions of each stage and to measure the rate of development for *E. scolopes* at a constant temperature of 24°C, four additional egg masses (108, 118, 201, and 123 embryos) were collected and sampled. The stages of sampled embryos were recorded once per day, from Stage 10 through hatching. For details on the methods used, see **Culture of Hawaiian Bobtail Squid (*Euprymna scolopes*) Embryos and Observation of Normal Development** (Lee et al. 2009b).

STRUCTURE OF THE EGG MASSES AND EGGS

As is typical of cephalopod reproduction, egg deposition and fertilization occur simultaneously in *E. scolopes*. Females deposit eggs during the evening hours on the underside of the artificial substrate

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Table 1. Morphological features of *Euprymna scolopes* developmental stages at 24°C

Stage	Key morphological features	Time ^a
1	Fertilized egg , without polar bodies ^b	—
2	First maturation division. First polar body formed	20 min
3	Second maturation division. Second polar body formed; blastodisc formed	40 min
4	First cleavage: The first cleavage furrow begins at the center of the blastodisc at the animal pole. Cleavage is incomplete; it divides the blastodisc but not the underlying yolk mass. The first cleavage furrow marks the plane of bilateral symmetry, establishing the future right and left halves of the embryo.	8 h
5	Second cleavage: The second cleavage furrow forms obliquely at the midpoint of the first furrow, producing two large and two smaller cells. The second division divides the left and right halves of the embryo into a larger anterior and a smaller posterior half. By the second cleavage, the three body axes (anteroposterior, dorsoventral, left-right) are established. The strong contraction of the second furrow produces a central gap (typical of large cephalopod eggs [von Boletzky 1988a]) between the four daughter cells, exposing the underlying yolk mass.	10 h
6	Third cleavage: The angle formed between the third and second cleavage furrows is slightly oblique, and divides the two larger anterior cells into two equal parts. The posterior region of the cleavage furrow is parallel to the first cleavage plane and results in two smaller regions adjacent to the first furrow. Through this stage, the embryo is a syncytium.	12 h
7	Fourth cleavage: This produces the first four blastomeres of the embryo. The other 12 cells remain cytoplasmically connected at their outer margins, forming the syncytial blastocones.	14 h
8	Fifth cleavage: 32-cell stage	16 h
9	Sixth and seventh cleavage: 64- and 128-cell stages, respectively	18-20 h
10	Formation of the blastoderm: At the end of cleavage, the future embryo is represented by the blastoderm (a single layer of blastomeres surrounded by lines of blastocones) at the animal pole. The blastoderm gives rise to the embryo proper and the external yolk sac.	1 d
11	Gastrulation and formation of the germ layers: Blastomeres at the periphery of the blastoderm are covered over by adjacent interior blastomeres. The submerged blastomeres form a ring of mesendoderm at the periphery of the blastoderm (von Boletzky 1988a,b; Fioroni 1990). The single cell-layered yolk papilla is visible at the center of the blastoderm.	2 d
12	Blastoderm expansion: The blastoderm expands over the yolk by division of the outer layer of blastomeres at the lateral periphery. The mesendoderm spreads toward the center of the blastoderm beneath the outer layer, creating the embryonic germ layers, decreasing the size of the yolk papilla in the process.	3 d
13	Blastoderm encompasses 30% of yolk mass: The blastoderm continues to expand through cell divisions and covers ~30% of the surface of the egg. The yolk papilla at the animal pole continues to decrease in diameter with the development of the inner germ layers.	4 d
14	Blastoderm covers 40% of yolk mass	5 d
15	Blastoderm covers ~50% of the surface of the egg	6 d
16	Gastrulation nearly complete: The blastoderm covers ~60% of the egg surface.	7 d
17	Early organogenesis: The blastoderm covers 80%-90% of egg, with only a small plug of yolk at the vegetal pole. The embryo rotates freely within the chorion by moving the cilia on the external yolk sac (von Boletzky 1971). The eye primordia appear as two bilaterally positioned oval ectodermal thickenings; the shell gland primordium first becomes visible as a slight oval depression at the former animal pole.	8 d
18	Eye placodes and arm band primordia visible; shell gland invaginates; stomodeum appears: The blastoderm completely encloses the external yolk mass. Organ primordia are slightly more apparent. Eye ectodermal placodes thicken, elevate, and position bilaterally toward the anterior. The shell sac border elevates slightly as the oval shell gland primordium invaginates. The mantle primordium is first visible as a thickening around the shell gland at	9 d

(continued)

Table 1. *Continued*

	the former animal pole. Arm primordia arise as two thickened bands of tissue located laterally at the “equator” of the embryo. The mouth first appears as a crescent-shaped placode (stomodeum) between the eye primordia. The funnel complex primordia are present on the posterior surface as two pairs of bilateral thickened placodes, the dorsal and ventral funnel folds.	
19	Optic vesicles begin to form; shell gland oval; five discrete arm pairs discernible; mantle thickens; stomodaeum invaginates; statocyst and gill primordia visible; anal papilla present: The organ primordia are more readily apparent. The eye ectodermal placodes internalize as the annular folds thicken around the periphery, forming the optic vesicle. The elliptically shaped shell gland continues to invaginate, but remains open. The mantle primordium thickens and lengthens. Each thickened band of the arm primordia divides into five discrete pairs of arm buds (numbered I-V from anterior to posterior). The arm buds are more closely spaced at the posterior surface; there is a large gap between the first pair of anterior arm buds. The stomodeum invaginates. The dorsal and ventral funnel folds thicken. Statocyst primordia appear as two shallow circular depressions located laterally on the posterior surface between the dorsal and ventral funnel folds. Paired gill primordia appear as thickened placodes on the posterior surface below the ventral edge of the mantle primordium. The anal papilla appears at the midline between the gill primordia.	10 d
20	Optic vesicle 50%-90% complete; fin primordia appear; salivary pit visible; statocysts invaginate; medial edges of dorsal and ventral funnel folds grow toward midline: Internalization of the eye vesicle continues; the annular fold closes around the periphery of the placode. Shell gland invagination continues; the edges of the shell sac begin to close. The paired fin primordia appear as thickened crescent-shaped placodes on the mantle lateral to the outer edges of the shell gland. Each arm bud is more distinct. The stomodeum continues to invaginate; the salivary pit is visible as a secondary depression within the mouth. The dorsal and ventral funnel folds elevate and the medial edges of each pair grow toward the midline. Statocyst primordia begin to invaginate. The paired gill primordia and anal papilla appear as small nubs.	11 d
21	Optic vesicles complete; retina lightly pigmented; optic lobe primordia visible; shell sac closing; sucker primordia visible; ventral funnel folds fused at midline: Formation of the eye vesicles is complete. The retinas are pigmented light orange. Paired optic lobe (optic ganglia) primordia are evident as masses of tissue located posteroventrally to the eye vesicles. The diamond-shaped opening of the shell sac starts to close, with fusion lines radiating from the four points. The distal edges of the fin primordia begin to separate from the mantle. The first sucker primordia are evident on the arms. The region surrounding the stomodeum thickens and elevates along the dorsal and lateral edges. The salivary pit shifts toward the dorsal edge of the stomodeum. The ventral funnel folds fuse at the midline, forming a continuous fold. The edges surrounding the statocysts thicken as invagination continues.	12 d
22	Optic lobes prominent; lens primordia visible; shell sac closed; fin edges separate from mantle; mantle covers one-third of the gills; dorsal and ventral funnel folds fused along length: Pigmentation of the retina increases (light orange). The optic lobes become prominent masses comprising lateral bulges on either side of the head. The lens primordia are visible within the eye vesicles. The shell sac is completely closed. The fins have separated from the mantle. The mantle increases in length; the ventral edge covers one-third the length of the gills. The number of suckers increases. The mouth is still positioned on the anterior surface between arm pair I. The salivary pit is no longer visible from the anterior surface as the stomodeum grows deeper and the dorsal edge thickens. The dorsal and ventral funnel folds fuse to each other along their lengths; the funnel complex appears as a “W” when viewed from the posterior. Statocysts have completely invaginated.	13 d
23	Retina orange and cup-shaped; mantle covers one-half of the gills; medial edges of dorsal and ventral funnel folds fused to form funnel tube; external yolk sac separated from embryo; internal yolk sac single-lobed: The retina is cup-shaped and the lens is visible as a clear rod in the center of the eye vesicle. The folds of the iris have occurred (Arnold 1965; Baeg et al. 1992), but are difficult to see. The fins are rounded and larger. The mantle covers one-half the length of the gills. The brachial crown contracts, decreasing	14 d

(continued)

Table 1. *Continued*

	the distance between arm pair I as they migrate toward each other, dorsal to the mouth. The medial edges of the dorsal and ventral funnel folds fuse ventrally at the midline, forming the beginning of the funnel tube. The external yolk sac is well separated from the embryo, and the animal-vegetal axis flexes such that the posterior surface of the embryo will come to lie near the posterior surface of the external yolk sac. The single lobe of the internal yolk sac is visible within the mantle from the anterior surface.	
24	Retina dark orange; mantle covers gills totally; mouth ventral within brachial crown; medial margins of funnel tube fused 50%-90%; branchial heart contractions; organ of Hoyle visible: Pigmentation of the retina increases (dark orange). Fins are well separated from the mantle, which completely covers the gills and anal papilla; the funnel retractor muscles are still visible under the ventral edge of the mantle. Brachial crown contraction is complete: Arm pair I meets at the midline, and the mouth is now located ventrally, completely within the brachial crown. The median margins of the funnel folds continue to fuse from ventral to dorsal; the fusion is 50%-90% complete. The internal yolk sac appears bilobed. Branchial hearts, located at the base of the gills, start to contract irregularly. The organ of Hoyle (hatching gland) first appears at the dorsal tip of the mantle.	15 d
25	Iris clearly visible; mantle covers dorsal edge of funnel (small triangular opening remains); gill filaments visible; primary lid forming; internal yolk sac bilobed; organ of Hoyle arrow-shaped; external yolk sac longer than body: The iris is visible and pigmented dark orange. The lens is spherical. The fins are separated from the mantle, with only the median margins still connected. The ventral mantle edge covers the dorsal portion of the funnel, but a small triangular opening remains. Contractions of the mantle can occur. Gill filaments are visible through the mantle. The statocysts are completely developed with statoliths. The two lobes of the internal yolk sac increase in size and extend toward the dorsal end of the mantle. The arrow-shaped organ of Hoyle is prominent and the terminal spine located at its tip is evident. The external yolk sac remains longer than the body of the embryo. The primary lid of the eye begins to develop as dorsal tissue extensions from arm pairs III and IV.	16 d
26	Retina reddish; mantle completely covers dorsal portion of funnel; primary lid nearly complete; internal yolk sac with four lobes; chromatophores first visible: Pigmentation of the retina deepens from dark orange to red. Pigmentation of the iris also increases. The mantle completely covers the dorsal margin of the funnel. A second pair of small lobes forms laterally from the internal yolk sac. The primary lid is nearly complete. A few light orange chromatophores become visible on the mantle and the head.	17 d
27	Late developmental stages: The eyes can move freely in their orbits. The second pair of lobes of the internal yolk sac increases in size. Dorsal chromatophores become evident and the total number of chromatophores increases.	18 d
28	Primary lid complete; iridophores present on eye; external yolk sac about size of head; olfactory tubercles present: The embryo possesses the characteristics of the adult form, having the appearance of a miniature adult. The primary lid completely covers the eyes, and pale yellow patches of iridophores (which reflect light) are present on the eye. The external yolk sac has been depleted further and is comparable in size to the head of the embryo. Olfactory tubercles are present as two bilaterally positioned circles on the ventral surface of the head, under the optic lobes (Arnold 1965; Baeg et al. 1992). In practice, these are very difficult to see (they are mostly obscured by the ventral edge of the mantle), but can be clearly distinguished using tubulin probes.	19 d
29	Ink sac visible under posterior mantle; external yolk sac nearly depleted: Pigmentation of the ink sac is visible from the posterior surface within the mantle cavity as a black ovoid area at the dorsal-most end of the funnel tube. The external yolk sac is nearly depleted and is mostly obscured by the arms. Hatching can occur prematurely if the embryos are disturbed.	20 d
30	Hatching stage: The small external yolk sac is dropped and the organ of Hoyle becomes depleted upon hatching. The internal yolk sac persists and is slowly consumed during the first day after hatching.	21 d

^aApproximate time post-fertilization at 24°C.

^bNot observed.

provided. The eggs are laid singly and are subsequently repiled on top of each other, forming a large, compact mass several eggs thick, comprised of 50-250 embryos (Fig. 1). Each ovoid egg is ~2.0 mm in length by 1.5 mm in width and is encased in three protective coats. The innermost protective case is the chorion, a thin translucent acellular membrane that tightly surrounds the developing embryo and large yolk mass. The chorion is surrounded by layers of spirally wound “jelly” produced by the nidamental glands. As the chorion expands during development, the spiral layers of jelly become compressed such that, at the end of development, the thickness of the jelly layer is very much reduced. The outermost coat consists of a resilient opaque material to which sand grains adhere, providing camouflage for the egg mass. The diameter of each egg capsule, including the protective layers, is ~4 mm.

DEVELOPMENTAL STAGING OF *E. SCOLOPES*

Development is a continuous process of change with time, and the stage descriptions here represent static sections of embryogenesis at discrete times based on a morphological consensus from many different embryos. Arnold et al. (1972) used a 30-stage system based on *Loligo pealei* development (Arnold 1965) in the initial descriptions of embryogenesis in *E. scolopes*. This staging scheme has since been used widely to describe the development of many cephalopods (Arnold et al. 1972; Hunter and Joseph 1975; Baeg et al. 1992; Sakurai et al. 1995), with minor adjustments to accommodate differences in the developmental timing of particular organs between different species. Subtle variations in the timing of the appearance of specific organs or developmental features in different individuals can be expected (Baeg et al. 1992; Naef 2000). Times listed in Table 1 for each stage represent the estimated time after fertilization and egg deposition, based on an estimated 8 h between fertilization and first cleavage at 24°C. For purposes of experimental embryology, it is worth noting that in a systematic survey of 664 embryos, 11% displayed morphological abnormalities.

For all embryological descriptions presented here, the morphological axes of the embryo (rather than the functional axes of the adult) are used (Fig. 2). The embryonic mouth is anterior, the funnel is posterior, the mantle is dorsal, and the arms are ventral. The mouth-funnel axis of a cephalopod embryo corresponds to the anteroposterior axis of other molluscs (Brooks 1880).

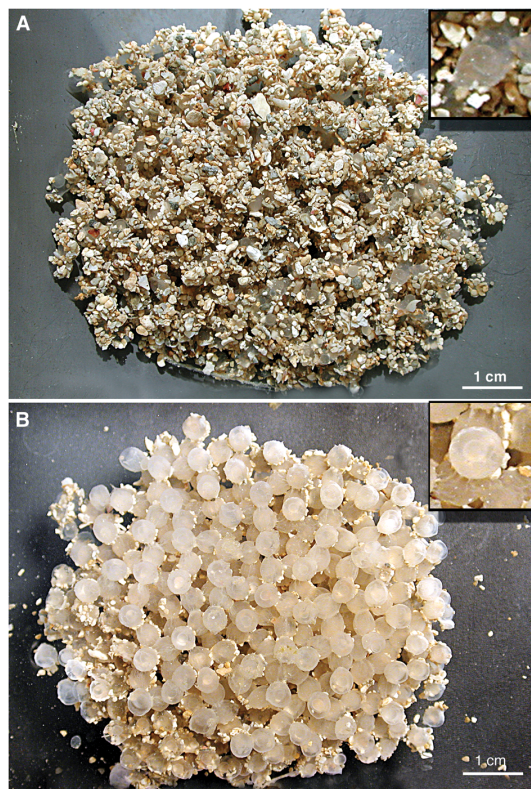


FIGURE 1. (A) Large mass of *E. scolopes* embryos deposited in captivity on a polyvinyl chloride (PVC) pipe offered as a substrate. The female squid covers the embryos with sand (which adheres to the surface of the mass) as a means of camouflage. (Inset) A single embryo. (B) Same clutch of embryos as in A after being removed from the PVC pipe to show the underside of the embryo mass without the sand covering. Individual embryos within their protective jelly layers and outer capsules are visible. (Inset) One such embryo, enlarged. Each embryo in its capsule adheres to the adjacent embryos in the clutch.

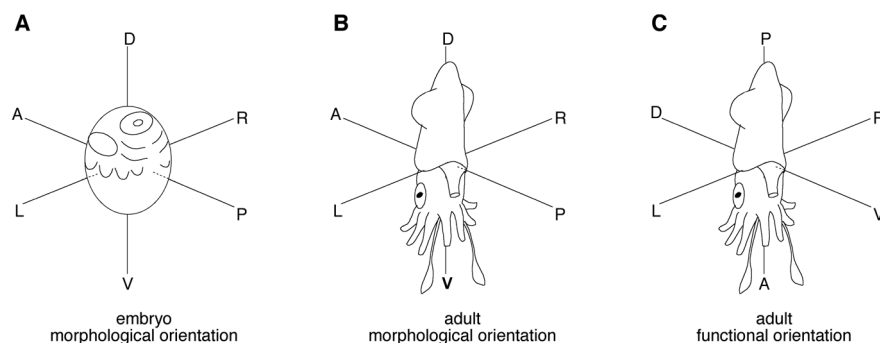


FIGURE 2. Physiological and morphological body axes in cephalopods. The morphological body axes of cephalopods are homologous to the body axes of other mollusks. In the morphological orientation, the location of the embryonic mouth is anterior (A), the funnel posterior (P), the mantle dorsal (D), and the arms ventral (V). (A) The morphological axes of a cephalopod embryo. (B) The morphological axes of an adult cephalopod. (C) The functional axes of an adult cephalopod.

The development of *Euprymna* can be roughly divided into four main phases from early cleavage to hatching. The initial cleavages generate a blastodisc at the animal pole of the yolk and set up the future morphological axes of the animal (Fig. 3). The first three divisions do not cleave the yolk and thus generate an eight-cell syncytium. Obviously, this might have practical consequences for the purpose of generating transgenic animals or for marking cells for cell lineage studies. The fourth division

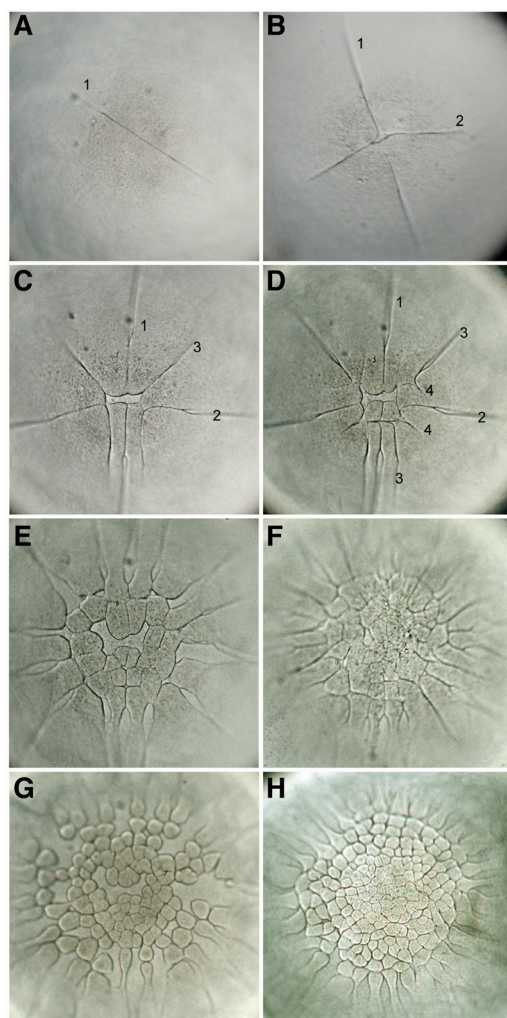


FIGURE 3. *E. scolopes* cleavage stages. All panels are views of the animal pole. Cleavage furrows are numbered. (A) Stage 4: Formation of the first cleavage furrow (1), marking the plane of bilateral symmetry. (B) Stage 5: Formation of the second cleavage furrow (2) oriented slightly obliquely to the first furrow. (C) Stage 6: Formation of the third cleavage furrow (3). Note the large central gap exposing the underlying yolk, and the unequal division of the posterior regions. (D) Stage 7: Formation of the fourth cleavage furrow (4). This division produces the first four blastomeres, located in the posterior region. The other 12 cells remain cytoplasmically connected at their outer margins, forming the syncytial blastocoel. (E) Stage 8: Fifth cleavage (32-cell stage). (F) Stage 9: Sixth cleavage (64-cell stage) and (G) seventh cleavage (128-cell stage). (H) Stage 10: Early blastoderm formation. The future embryo is comprised of a blastoderm at the animal pole of the egg, consisting of a single layer of blastomeres surrounded by lines of blastocoel.

generates the first blastomeres of the embryo. The 12 peripheral blastomeres remain connected via cytoplasmic bridges, creating syncytial blastocones.

The early developmental phase (Stages 1-10, up to the establishment of the blastoderm) takes up ~4%-5% of the total development and is followed by gastrulation and the generation of the germ layers (Fig. 4). The blastoderm continues to expand laterally over the yolk by cell division and covers the entire yolk mass by ~40% of development (Stage 18). Gastrulation is nearly complete around Day 7 (Stage 16) and gives way to early organogenesis (Fig. 5).

By Stage 18 (35%-40% development), organ primordia become visible in the form of localized ridges, depressions, or thickenings. Initially, the shell sac, eye placodes, and mantle become visible, followed by a rapid succession of additional organ primordia. By 50%-60% development, arm buds, fins, and the eye vesicles are complete and the latter begin to accumulate pigment. The eyes and optic lobes soon become the prominent feature of the developing embryo, and by ~70% development (Stage 24), the eyes are morphologically distinct and appear dark orange (Fig. 6). During the second half of development, organ primordia continue to grow and differentiate (Fig. 7). By Stage 27, the eyes can move freely and chromatophores are ubiquitous. At this point, the embryos assume the characteristics of miniature adults and can hatch prematurely if perturbed.

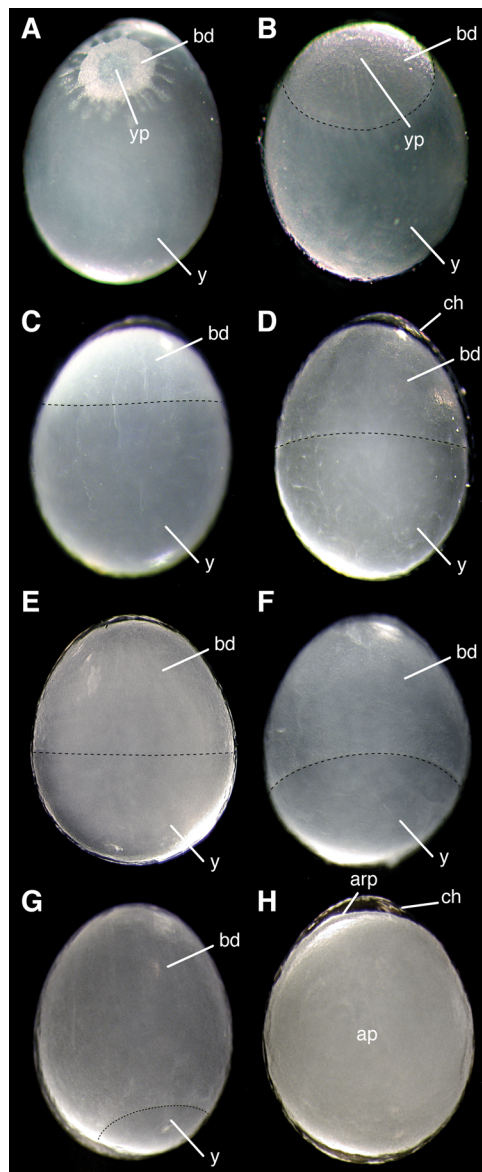


FIGURE 4. Gastrulation in *E. scolopes*. (A-G, lateral views; H, view from animal pole). (ap) Animal pole; (arp) arm primordia; (bd) blastoderm; (ch) chorion; (yp) yolk papilla; (y) yolk. (A) Stage 11: Initiation of germ layer formation. (B) Stage 12: Expansion of the blastoderm and mesendoderm. (C) Stage 13: ~30% of the yolk surface is covered by the blastoderm. (D) Stage 14: ~40% epiboly. (E) Stage 15: The blastoderm covers ~50% of the surface of the egg. (F) Stage 16: The blastoderm covers ~60% of the egg surface. (G) Stage 17: The blastoderm covers 80%-90% of egg, with only a small plug of yolk at the vegetal pole of the egg. Gastrulation is nearly complete and organogenesis begins. Eye and shell gland primordia are first visible. (H) Stage 18: The blastoderm completely encloses the external yolk mass. The brachial (arm) crown primordia are apparent as a band of tissue at the equator of the embryo.

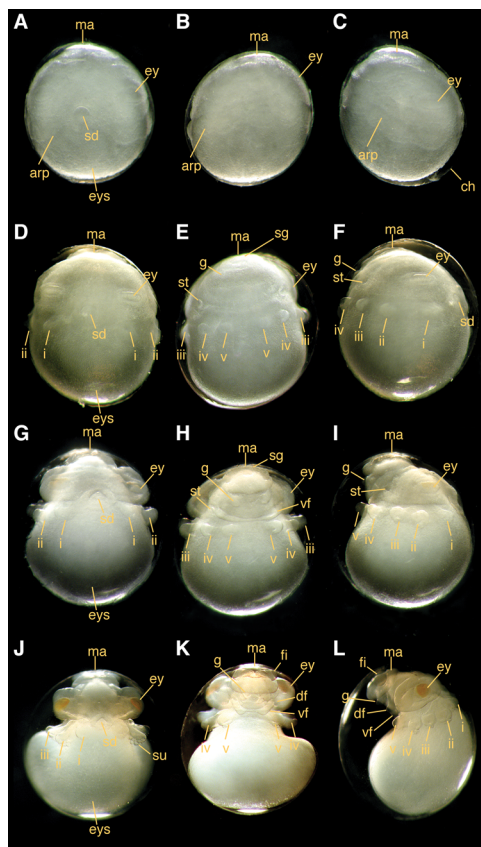


FIGURE 5. Early organogenesis in *E. scolopes*. All lateral views are of the right side of the embryo (i.e., the anterior is to the right). (A-C) Stage 18: (A) anterior, (B) posterior, and (C) lateral views. (D-F) Stage 19: (D) anterior, (E) posterior, and (F) lateral views. (G-I) Stage 20: (G) anterior, (H) posterior, and (I) lateral views. (J-L) Stage 21: (J) anterior, (K) posterior, and (L) lateral views. Morphological features are labeled: (i) arm I; (ii) arm II; (iii) arm III; (iv) arm IV; (v) arm V; (arp) arm primordia; (ch) chorion; (df) dorsal funnel fold; (ey) eye; (eys) external yolk sac; (fi) fin; (g) gill; (ma) mantle; (sd) stomodeum; (sg) shell gland; (st) statocyst primordium; (su) sucker; (vf) ventral funnel fold.

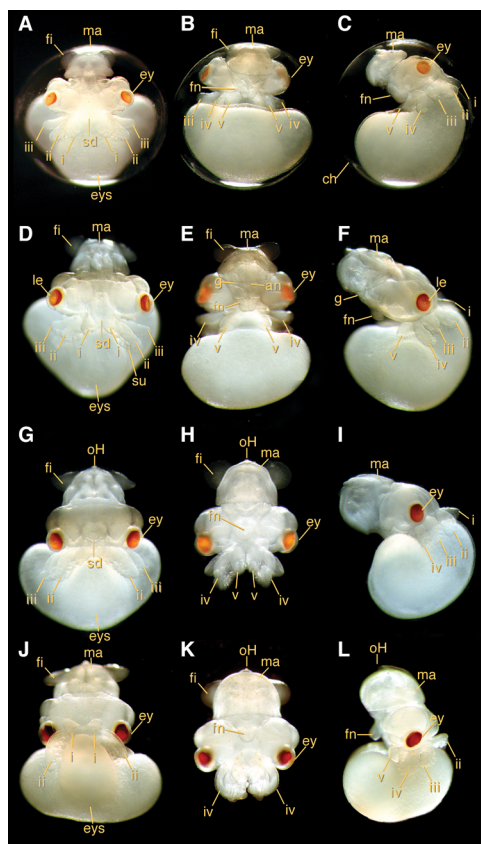


FIGURE 6. Late organogenesis in *E. scolopes*. All lateral views are of the right side of the embryo (i.e., the anterior is to the right). (A-C) Stage 22: (A) anterior, (B) posterior, and (C) lateral views. (D-F) Stage 23: (D) anterior, (E) posterior, and (F) lateral views. (G-I) Stage 24: (G) anterior, (H) posterior, and (I) lateral views. (J-L) Stage 25: (J) anterior, (K) posterior, and (L) lateral views. Morphological features are labeled: (i) arm I; (ii) arm II; (iii) arm III; (iv) arm IV; (v) arm V; (an) anal papilla; (ch) chorion; (ey) eye; (eys) external yolk sac; (fi) fin; (fn) funnel; (g) gill; (le) lens; (ma) mantle; (oH) organ of Hoyle; (sd) stomodeum; (su) sucker.

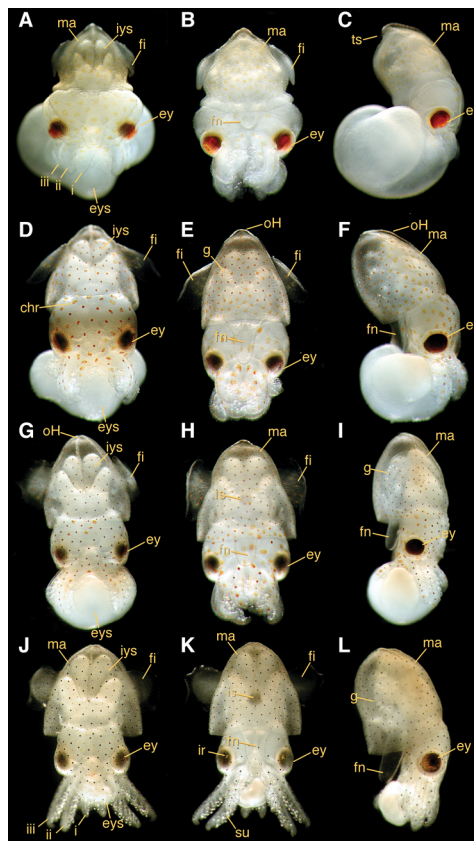


FIGURE 7. Later developmental stages in *E. scolopes*. All lateral views are of the right side of the embryo (i.e., the anterior is to the right). (A-C) Stage 26: (A) anterior, (B) posterior, and (C) lateral views. (D-F) Stage 27: (D) anterior, (E) posterior, and (F) lateral views. (G-I) Stage 28: (G) anterior, (H) posterior, and (I) lateral views. (J-L) Stage 29: (J) anterior, (K) posterior, and (L) lateral views. Morphological features are labeled: (i) arm I; (ii) arm II; (iii) arm III; (chr) chromatophore; (ey) eye; (eys) external yolk sac; (fi) fin; (fn) funnel; (g) gill; (ir) iridophore; (is) ink sac; (iys) internal yolk sac; (ma) mantle; (oH) organ of Hoyle; (su) sucker; (ts) terminal spine.

HATCHING

Hatching from the chorion occurs once most of the external yolk sac has been reabsorbed. The intra-chorionic fluid likely contains a tranquilizing substance that prevents the squid from hatching prematurely (Marthy et al. 1976). The normal stimulus for hatching is not known. However, during later stages of development, the embryo can be induced to hatch by mechanical stimulation. Hatching begins with increased mantle contractions and movement of the fins of the embryo (Fig. 8). The

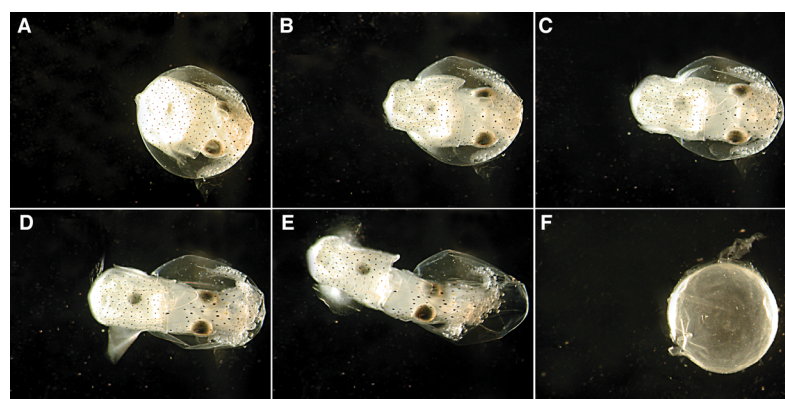


FIGURE 8. Hatching sequence of *E. scolopes*. The protective jelly layer and outermost capsule have been removed to facilitate observation. Hatching from the chorion occurs once the external yolk sac has been reabsorbed. (A) The embryo attaches to the inner surface of the chorion and presses the organ of Hoyle and the terminal spine in direct contact with the chorion. (B) The terminal spine is extended, perforating the chorion and the enzyme-resistant outermost protective coat and forming an opening through which the embryo can pass. (C-E) Mantle contractions and fin undulations expel the hatchling through the opening in the chorion (F).

embryo attaches its arms, via the suckers, to the inner surface of one side of the chorion and extends its body by contracting the mantle and extending the arms so the dorsal end of the mantle contacts the opposite side of the chorion. This presses the organ of Hoyle and the terminal spine directly against the chorion. The organ of Hoyle releases proteolytic enzymes that digest the chorion (Arnold and Singley 1989). The terminal spine is extended by muscle contractions and mechanically perforates the chorion and the enzyme-resistant outermost protective coat, forming an opening through which the embryo can pass. The embryo uses mantle contractions and fin undulations to expel itself through the opening in the chorion.

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