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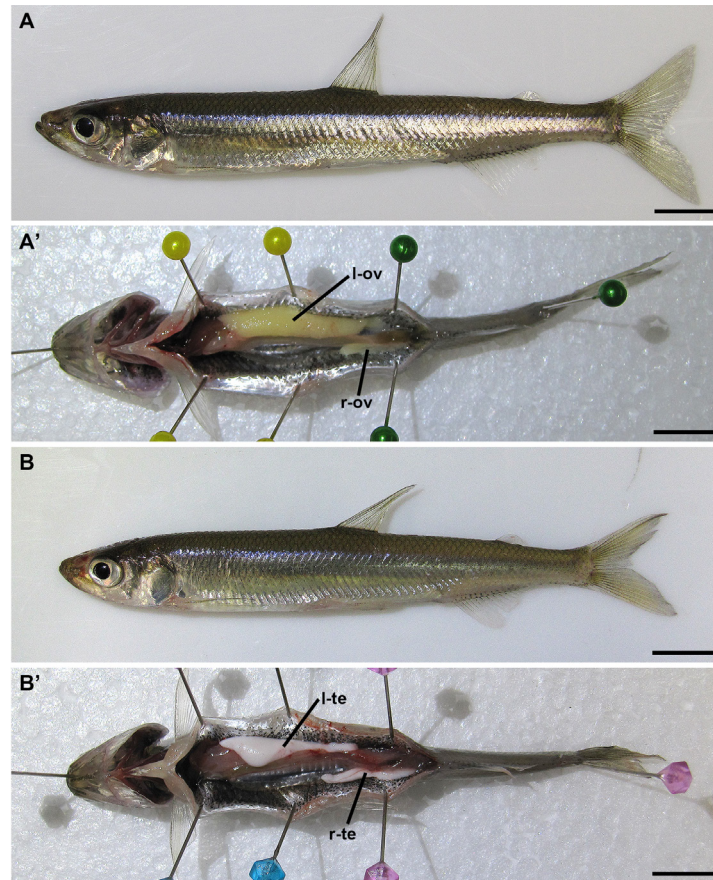
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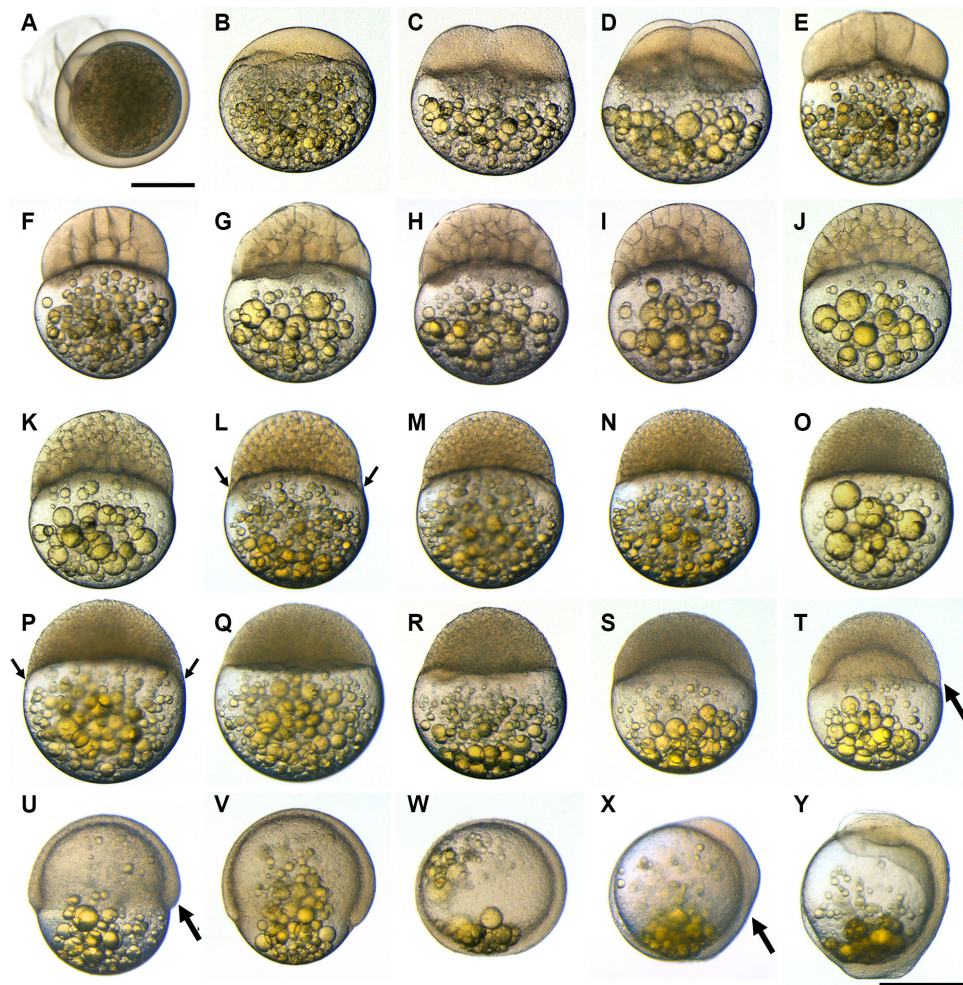
**Migration behavior of PGCs and asymmetrical gonad
formation in pond smelt *Hypomesus nipponensis***

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and ETSURO YAMAHA

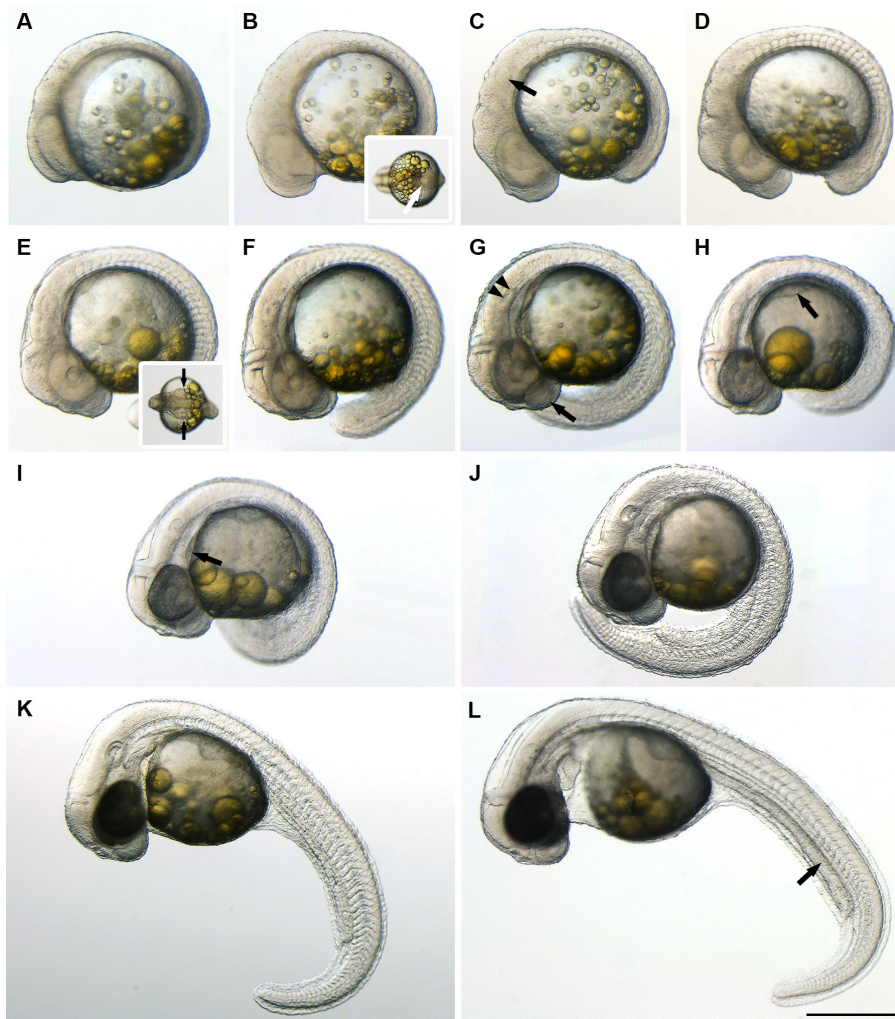
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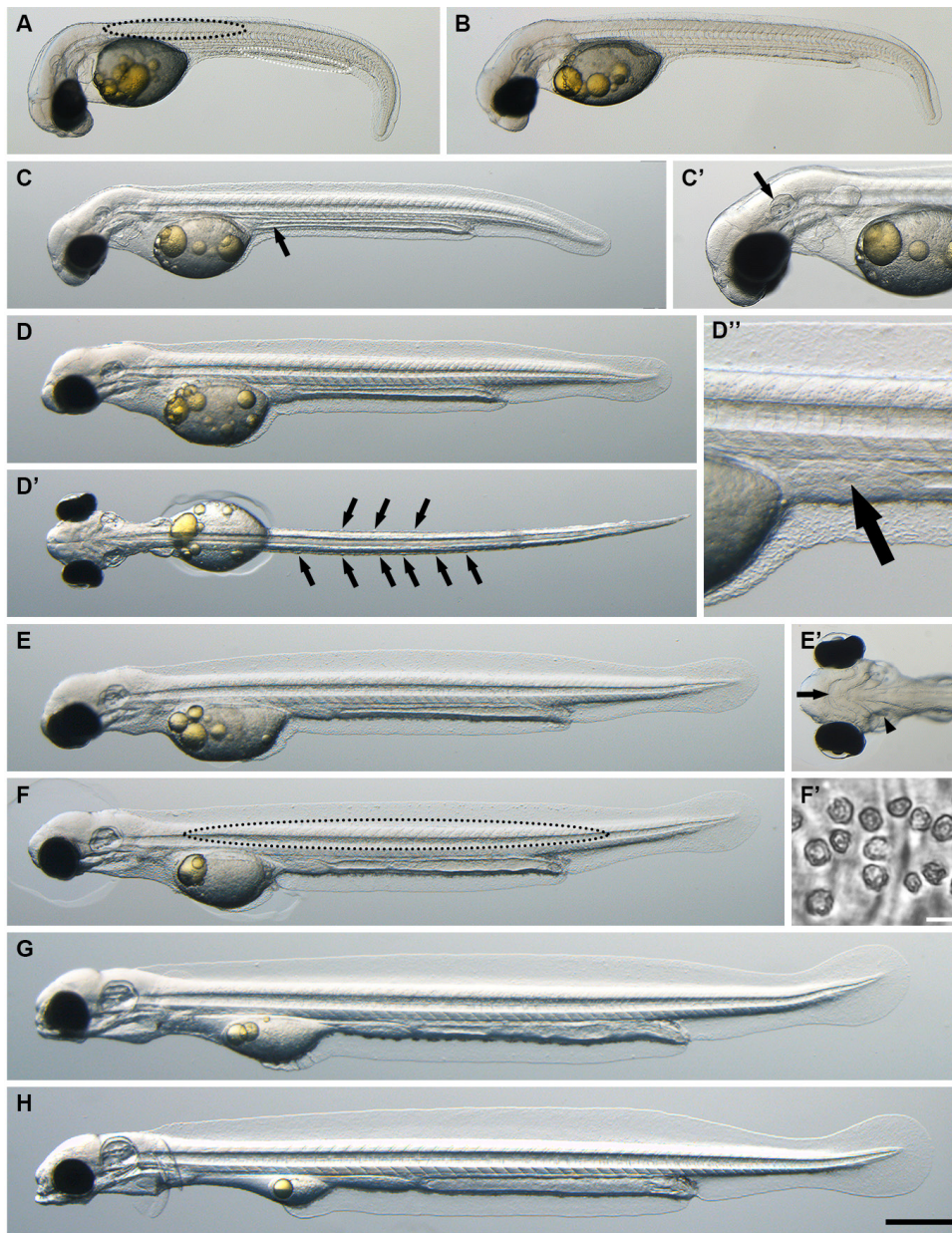
Suppl. Fig. S1. Asymmetrical gonadal growth in pond smelt. Pond smelt 4 months before the spawning season. (A,B) Side views; (A',B') Internal body cavity from the ventral side. (A) Female pond smelt. (A') Left ovary located in the anterior part of the body cavity is larger than the right gonad. (B) Male pond smelt. (B') Left testis located in the anterior part of the body cavity is larger than the right testis. l-ov: left-ovary, l-te: left-testis. r-ov: right-ovary, r-te: right-testis. Scale bars, 1,000 μm .



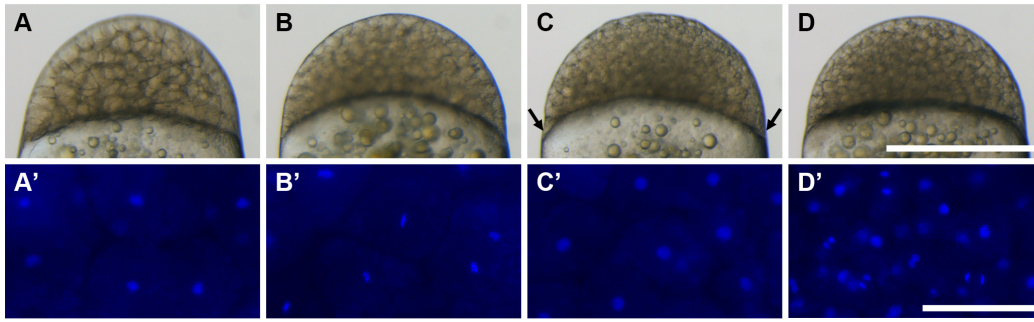
Suppl. Fig. S2. Embryonic development in pond smelt during fertilization, cleavage, blastula and gastrula period. (A) Fertilized embryo. The layered outer adhesive membrane can be observed. **(B)** 1-cell stage. Blastodisc forms. **(C)** 2-cell stage. **(D)** 4-cell stage. **(E)** 8-cell stage. **(F)** 16-cell stage. Two tiers of blastomeres are formed by latitudinal cleavage. **(G)** 32-cell stage. **(H)** 64-cell stage. **(I)** 128-cell stage. **(J)** 256-cell stage. **(K)** 512-cell stage. **(L)** 1k-cell stage. External yolk syncytial layer (E-YSL; Arrow) is observed. **(M)** 22.0 h post-fertilization (hpf). **(N)** 21.5 hpf. **(O)** 23.0 hpf. **(P)** Oblong stage. Arrow indicates the flattened boundary between the blastoderm and yolk cell. **(Q)** Sphere stage. **(R)** Beginning of epiboly stage. **(S)** Dome stage. **(T)** 30% epiboly stage. Arrow indicates presumptive dorsal region with thickened cells. **(U)** 50% epiboly stage. Epiboly is arrested for 2 h; subsequently, the germ ring and embryonic shield (arrow) form. **(V)** 70% epiboly stage. **(W)** 90% epiboly stage. **(X)** Embryonic body formation stage. Arrow indicates embryonic body. **(Y)** Bud stage. All embryos are side views. Animal pole is up and vegetal pole down. Scale bars, 500 μm .



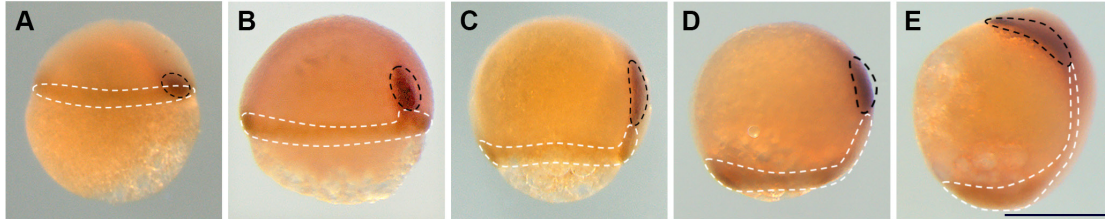
Suppl. Fig. S3. Embryonic development of pond smelt during the segmentation period. (A) 5-somite stage. Optic vesicle is formed. (B) 9-somite stage. Insert is vegetal view. Optic cup formation starts and Kupfer's vesicle (white arrow) is formed. (C) 15-somite stage. Optic cup is formed and otic placode (arrow) formation starts. (D) 22-somite stage. (E) 28-somite stage. Insert shows the animal pole view. Lenses (arrows) are formed. (F) 34-somite stage. Eye pigmentation starts and Kupfer's vesicle disappears. (G) 39-somite stage. One pair of otoliths (arrowheads) and nasal placodes (arrow) are formed. (H) 44-somite stage. Body twists clockwise strongly and intestinal bulb is formed at the 1st to 4th somite region (arrow). (I) 50-somite stage. Pectoral fin formation (arrow) is observed. (J) 54-somite stage. Caudal fin fold and intestinal tract become observable. (K) 58-somite stage. Median fin-fold is formed. (L) 65-somite stage. Lumen of intestinal tract (arrow) is visible. All embryos are side views. Scale bar, 500 μm .



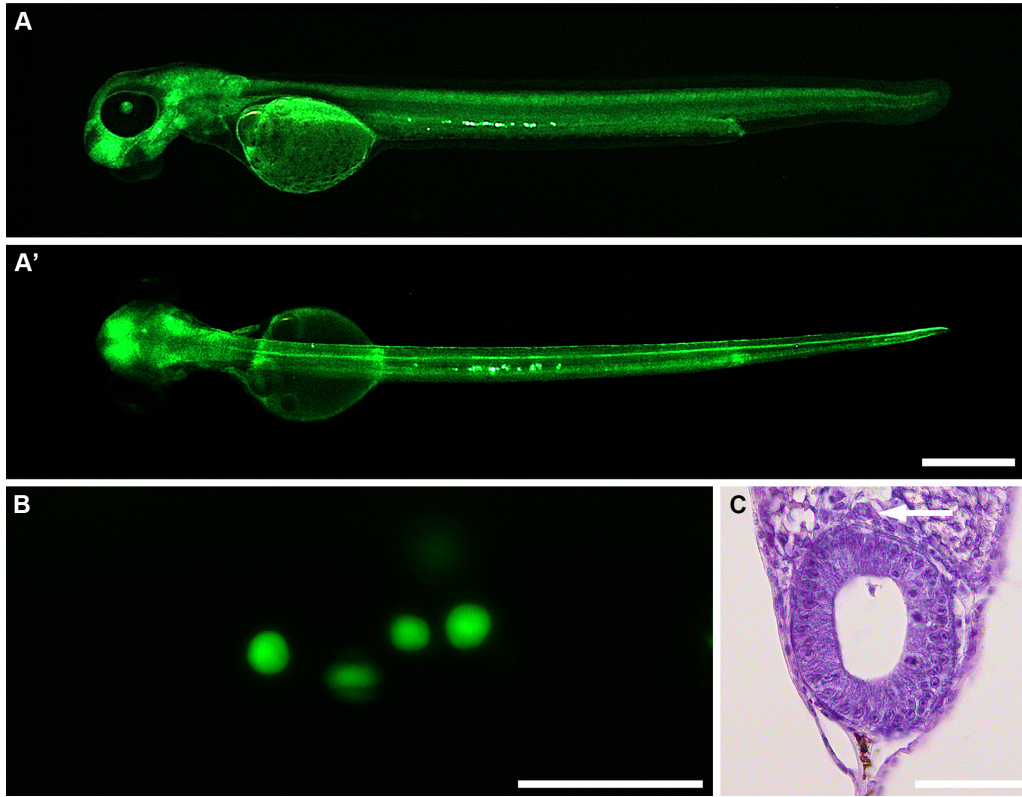
Suppl. Fig. S4. Embryonic development of pond smelt during pharyngula to hatching period. (A) 10.0 dpf. Hatching glands and melanophore are present. The hatching glands are positioned in the dorsal region of the middle body trunk and are highlighted by the black dotted line; melanophores are present in the lower part of the intestinal tract and highlighted by the white dotted line. (B) 11.0 dpf. (C) 12.0 dpf. The anterior part of the lumen of the intestinal tract (arrow) becomes larger. (C') Higher magnification view of the head region of a 12.0 dpf embryo. Semicircular canals (arrow) are visible. (D) 14.0 dpf. Pigmentation has increased in the lower part of intestinal tract. The intestinal bulb has moved to the posterior region of the yolk and occupies the 11th to 14th somite region. (D') Dorsal view of 14.0 dpf embryo. Arrows indicate lateral line primordium. (D'') Central part of trunk. Liver (arrow) is visible. (E) 15.0 dpf. (E') Dorsal view of head region of 15.0 dpf embryo. Mouth opening (arrow) and gill arch (arrowheads) are visible. (F) 16.0 dpf. Hatching glands increase in number and are located in the area indicated by the black spotted line. (F') Higher magnification view of hatching gland. Reflective granules are visible in the hatching glands. Scale bar denotes 10 μm . (G) 18.0 dpf. Anus opening is formed. Embryos start hatching from this stage. (H) 20.5 dpf. Hatched larvae with chorion. Almost all embryos have hatched by this time. Scale bar, 500 μm .



Suppl. Fig. S5. Transition of synchronous to asynchronous cleavage cycle during mid-blastula period and formation of yolk syncytial layer (YSL). (A,B,C,D) Light microscope images. (A',B',C',D') DAPI-stained blastomeres during the 512-cell to 2k-cell stage. (A,A') 17.0 hpf. 512-cell stage. Blastomeres are synchronous at S phase. (B,B') 17.75 hpf. 512-1k cell stage. Blastomeres are synchronous at metaphase. (C,C') 18.5 hpf. 1k-cell. Blastomeres are synchronous at S phase. YSL formation is underway (arrows). (D, D') 19.25 hpf. 1k-2k cell stage. Nuclei of the embryo are at different parts of the cell cycle, suggesting that cleavage is occurring asynchronously. Scale bars, (A-D) 500 μm ; (A' - D'), 50 μm .



Suppl. Fig. S6. Expression pattern of mesodermal marker genes *gsc* and *ntl* from 30% epiboly until embryonic body formation stage. (A) 30% epiboly. *gsc* (enclosed by black dotted line) is expressed in the presumptive dorsal region and *ntl* (enclosed by white dotted line) in the marginal region of the blastodisc. (B) 50% epiboly. The expression region of *gsc* and *ntl* separate, due to invagination. (C) 70% epiboly. *gsc* is expressed at the tip of invaginating cells. The position of *gsc* expressing region shows a similar position at 30% epiboly stage. (D) 90% epiboly stage. The position of *gsc* expressing region is similar to 30% epiboly stage. (E) Embryonic body formation stage. The expressing region of *gsc* shifts towards the animal pole region. Scale bar, 500 μm .



Suppl. Fig. S7. Morphological status of primordial germ cells (PGCs) in 14 dpf embryo. (A) Side view of 14 dpf embryo. PGCs are present in the 16th to 24th somite region. (A') Dorsal view of embryo in Fig. S1 (A). PGCs are arranged in a row. (B) Higher magnification of PGCs in 14 dpf embryo. PGCs are spherical and do not have protrusions. (C) Histological section of 14 dpf embryo stained with hematoxylin and eosin. PGCs are located on the intestinal tract. Arrow indicates PGC. Scale bars: (A,A') 1000 μm ; (B,C) 50 μm .

SUPPL. TABLE S1
DEVELOPMENTAL STAGES OF POND SMELT AT 10°C

Stage name	Time	Characteristics	Suppl. Fig.
Cleavage period			
Fertilization	0.5 hpf	Attached to matrix by layered adhesive membrane, forming perivitelline space	2A
1-cell	2.5 hpf	Formation of the blastodisc on the animal pole	2B
2-cell	5.0 hpf	Formation of the first cleavage furrow	2C
4-cell	6.5 hpf	Blastodisc forms by 2×2 array blastomeres	2D
8-cell	8.0 hpf	Blastodisc forms by 2×4 array blastomeres	2E
16-cell	9.5 hpf	The fourth cleavage plane divides the monolayered blastomeres horizontally	2F
32-cell	11.0 hpf		2G
64-cell	12.5 hpf	The number of blastomeres becomes uncountable by external appearance	2H
Early blastula period			
128-cell	14.0 hpf		2I
256-cell	15.5 hpf		2J
512-cell	17.0 hpf		2K
1k-cell	18.5 hpf	Midblastula transition and asynchronous cleavage begins, E-YSL formation	2L
	20.0 hpf	The number of blastomeres can not be predicted by the cell cycle	2M
	21.5 hpf		2N
	23.0 hpf		2O
Late blastula period			
Oblong	28.0 hpf	Ellipsoidal shape, the boundary of blastodisc and yolk becomes flat	2P
Sphere	32.0 hpf	Spherical shape is formed by stretching of blastodisc toward the yolk	2Q
Beginning of epiboly	38.0 hpf	Embryo becomes oval shaped and epiboly begins	2R
Dome	42.0 hpf	Bulging occurs	2S
30% epiboly	44.0 hpf	Epiboly proceeds and blastoderm covers 30% of yolk, beginning of embryonic shield formation	2T
Gastrula period			
50% epiboly	50.0 hpf	Germ ring and embryonic shield formation, epiboly is arrested for approximately 2 hours	2U
70% epiboly	58.0 hpf		2V
90% epiboly	64.0 hpf		2W
Embryonic body	72.0 hpf	Completion of the blastoderm covering on the yolk cell, embryonic body forms	2X
Bud	84.0 hpf	Embryonic body expands rapidly by drastic conversion and extension movement	2Y
Segmentation period			
5-somite	4.0 dpf	Beginning of somitogenesis, optic vesicle formation	3A
9-somite	4.5 dpf	Kupffer's vesicle formation, beginning of optic cup formation	3B
15-somite	5.0 dpf	Optic cup and lens placode formation, Otic placode formation starts	3C
22-somite	5.5 dpf		3D
28-somite	6.0 dpf	Lens formation	3E
34-somite	6.5 dpf	Beginning of eye pigmentation, disappearance of Kupffer's vesicle	3F
39-somite	7.0 dpf	Otoliths and nasal placode formation, tail part begins to bend to right side of the body, motility begins	3G
44-somite	7.5 dpf	Depression on the yolk due to formation of intestinal bulb. The tail part is actively moves	3H
50-somite	8.0 dpf	Pectoral fins formation, beginning of heart beating	3I
54-somite	8.5 dpf	Beginning of caudal fin fold and intestinal tract formation	3J
58-somite	9.0 dpf	Beginning of median fin fold formation	3K
62-somite	9.5 dpf	Completion of somitogenesis, lumen of intestinal tract becomes observable from outside	3L
Pharyngula period			
	10.0 dpf	Beginning of hatching glands formation at middle region of dorsal body trunk and pigmentation at lower part of intestinal tract	4A
	11.0 dpf	Many of hatching glands appear at dorsal body trunk	4B
	12.0 dpf	Semicircular canals formation, anterior part of gut's lumen enlarges	4C, C'
	14.0 dpf	Many pigments are formed at the lower part of intestinal tract, intestinal bulb moves to posterior region of yolk and liver formation can be observed. Formation of lateral line, tail part becomes straight	4D, D', D''
	15.0 dpf	Mouth opens and gill arch formation	4E, E'
	16.0 dpf	Weak peristaltic motion in a part of the intestinal tract, hatching glands with reflective granules	4F, F'
	18.0 dpf	Open anus, beginning of hatching and swimming	4G
Hatching period			
	20.5 dpf	More than half of embryos cultivated with chorion hatch	4H