HOKKAIDO UNIVERSITY

Title	THE NORMAL DEVELOPMENTAL STAGES OF THE POND SMELT, HYPOMESUS OLIDUS (PALLAS)
Author(s)	YAMADA, Juro
Citation	北海道大學水産學部研究彙報, 14(3), 121-126
Issue Date	1963-11
Doc URL	https://hdl.handle.net/2115/23183
Type	departmental bulletin paper
File Information	14(3)_P121-126.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

THE NORMAL DEVELOPMENTAL STAGES OF THE POND SMELT, *HYPOMESUS OLIDUS* (PALLAS)*

Juro YAMADA
Faculty of Fisheries, Hokkaido University

Hypomesus olidus is one of the important fishes in the Japanese freshwater fisheries industry. It was originally distributed in northern Japan. At present, however, artificial transplantations have brought this species to live in many lakes and ponds throughout the country. Today, in order to maintain the stock, efforts of protection or transportation of the fertilized eggs after artificial insemination are being continued in various places. Accordingly, a description of the normal developmental stages would be useful for persons who are engaging in the actual fishery and biological investigations of this species.

On the development of the pond smelt illustrated by a consecutive series of figures there is practically no report available, though Hamada (1961) made a gross observation on *H. olidus* and *H. sakhalinus*. On the other hand, detailed descriptions on closely related species, *Spirinchus lanceolatus* and *Plecoglossus altivelis*, were presented respectively by Hikita (1958) and Iwai (1962).

In this paper, photomicrographs obtained in series from the unfertilized eggs to the just hatched fry were arranged according to the advance of stages in development which were differentiated exclusively by changes of externally observable characters. In deciding the stages by such criteria, the description of development of *Fundulus* eggs by Oppenheimer (1937) was very helpful.

The writer is deeply indebted to Prof. S. Saito for his critical reading of the manuscript, and to Dr. K. Hamada who has given him much valuable advice.

Material and Method

Mature eggs were obtained from the fish caught at lake Onuma near Hakodate where the spawning takes place in early April. The eggs inseminated by the dry method were allowed to adhere to fine glass fibres and incubated in an aquarium in which water was constantly circulated by air bubbling. The water temperature

* According to the recent revision of McAllister (1963), the pond smelt in Japan (*Wakasagi*) is reported to be named *H. transpacificus nipponensis* as n. subsp., but the customary species name is used in this paper.

was regulated to fall within the range from 11°C to 12°C.* Observations of the unfertilized eggs and the course of germ disc formation were made on eggs bathed in M/7.5 Ringer's solution.

All photomicrographs were taken with living embryos by the use of a special microscope "Polyphoto" made by the Tiyoda Otical Co., Ltd.

The Stages of the Normal Development

1. *The unfertilized egg:* Fig. 1.

The mature egg measures 0.6–0.7 mm in diameter. Oil globules variable in number and size are buried in the light yellowed yolk mass. The whole egg surface is covered with a thin egg membrane, on the animal hemisphere of which overlaps another membrane, the adherent membrane. The structure of this membrane and the mechanism of adherence is the same as described in that of *Plecoglossus altivelis* (Kanoh, 1952; Iwai, 1962). These two membranes are connected with each other at the animal pole where the funnel-shaped micropyle is to be seen. Turning off inversely, the adherent membrane catches glass fibres to support the egg by transforming itself into the adherent stalk at the animal pole.

2. *Formation of the germ disc:* Fig. 2 (20 min.); Fig. 3 (1 hr.)

At fertilization, elevation of the egg membrane occurs to form the perivitteline space. After the elevation has completed the diameter of the egg increases to 0.8–0.9 mm. At the same time, the protoplasm begins to accumulate at the animal pole forming the germ disc. It finally supplies about one-third the volume of the whole egg within an hour after insemination.

3. *Two-celled ovum:* Fig. 4 (4 hrs.)

The first cleavage plane divides the germ disc vertically into two equal sized blastomeres.

4. *Four-celled ovum:* Figs. 5 a, b (5½ hrs.)

The second cleavage plane, also vertical, cuts the two blastomeres at right angles to the first plane to form four cells of equal size.

5. *Eight-celled ovum:* Fig. 6 (7 hrs.)

The two planes of the third cleavage are parallel to the first resulting in two rows of four cells each.

6. *Sixteen-celled ovum:* Fig. 7 (8½ hrs.)

The two planes of the fourth cleavage are parallel to the second, thus arises the blastoderm made up of irregularly arranged four rows of four cells each.

* It is reported that the daily water temperature of Lake Onuma during the spawning season varies extremely from 0°C to 18°C (Hamada, 1961), and that the optimum water temperature for the development of the pond smelt ranges from 7°C to 19°C (Yamamoto, 1937).

7. *Thirty-two-celled ovum:* Fig. 8 (10 hrs.)

The fifth cleavage plane divides the monolayered blastomeres horizontally. As the result, the blastoderm comes to have a stratified structure.

8. *Sixty-four-celled ovum:* Fig. 9 ($11^1/_2$ hrs.)

The sixth cleavage furrows can not be traced exactly but perhaps they are vertical. Also it is difficult to count the precise number of cells as the cleavages do not occur simultaneously.

9. *Morula:* Fig. 10 (13 hrs.)

The blastoderm takes a mulberry-like form being divided into many rather small cells.

10. *Early blastula:* Fig. 11 (16 hrs.)

The blastoderm is composed of still smaller cells than the former stage, although the general form is not changed.

11. *Late blastula:* Fig. 12 (21 hrs.)

The cells are divided so minutely that the blastoderm becomes somewhat opaque.

12. *Beginning of epiboly:* Fig. 13 (28 hrs.)

Epiboly, the downward movement of the periphery of blastoderm, begins at this stage. The blastoderm gradually undergoes flattening.

13. *Early gastrula:* Fig. 14 (31 hrs.)

As the epiboly proceeds covering the yolk, the cells of the blastoderm are invaginated at a part of the periphery where the gastrulation begins.

14. *Late gastrula:* Figs. 15 a, b (38 hrs.)

The blastoderm covers two-thirds of the yolk being flattened considerably. A partial thickening of the germ ring forms the embryonic shield.

15. *Appearance of the embryonic body:* Fig. 16 (44 hrs.)

The embryonic shield grows and is elongated on the yolk sphere. By this time the outline of the future embryo has clearly been established.

16. *Head fold:* Figs. 17 a, b (51 hrs.)

The anterior end of the embryonic shield expands making clear the cephalic region. The narrow dorsal keel forms the anlage of the spinal cord. The optic buds are vaguely visible on both lateral sides of the head. The yolk plug, uncovered part of the yolk surface, has become small.

17. *Optic vesicles:* Figs. 18 a, b (55 hrs.)

The optic buds have developed into the prominent optic vesicles. The spinal cord is clearly marked in the dorsal view.

18. *Beginning of somites formation:* Fig. 19 (64 hrs.)

Soon after the expansion of the optic vesicles, the differentiation of somites begins behind the cephalic region. In the figure, 5 pairs of somites can be counted.

The anterior end of the head increases its height. The Kuppfer's vesicle makes its appearance near the caudal end of the embryo.

19. *Closure of blastopore:* Fig. 20 (71 hrs.); Fig. 21 (76 hrs.)

The yolk plug disappears when the entire yolk surface is enclosed by the blastodermal cell layer which has now transformed into the extra-embryonic membrane. The number of somites has increased to 11–15.

20. *Optic cups:* Fig. 22 (83 hrs.)

The outer walls of the optic vesicles are folded inside and begin to form the optic cups. Twenty-one somites are shown in the figure. The three primary brain vesicles are barely distinguished.

21. *Auditory placodes:* Fig. 23 (91 hrs.)

The boundaries of the three primary brains, procencephalon, mesencephalon, and rhombencephalon or deuterencephalon become distinct. The auditory placodes appear in the deuterencephalic region. By this time 25 pairs of somites have been differentiated.

22. *Formation of lens:* Fig. 24 (98 hrs.)

The lens placode develops as an expansion of the outermost ectoderm; it separates from the latter and falls into the optic cup. At this stage, the embryo has just encircled the yolk sphere. Twenty-eight pairs of somites can be counted.

23. *Tail bud:* Fig. 25 (108 hrs.)

The caudal extremity of the embryo is budding from the yolk sphere. The Kuppfer's vesicle has disappeared and the number of somites has reached 31.

24. *Motility and heart beating:* Fig. 26 (119 hrs.); Fig. 27 (120 hrs.)

The embryos gain the ability of muscular contraction when they have had 36–38 pairs of somites. At the same time or just later, the heart, having yet a simple tubular structure, begins to beat. The optic lobes of the mesencephalon become visible evidently. The eye clearly shows the choroid fissure; the ear exhibits a vesicular structure.

25. *Pigmentation of retina:* Fig. 28 (148 hrs.); Figs. 29 a, b (151 hrs.)

The pigmentation in the retina appears; the somites differentiated number about 48–50. The oil globules in the yolk have been reduced in number showing a tendency to fuse into large ones. The embryo encircles the yolk sphere 1½ times. After this stage, the embryos are referred to as "eyed eggs" since their eyes are visible to the naked eye as black spots.

26. *Fin folds:* Figs. 30 a, b (168 hrs.)

The membraneous fins arise at the dorsal and ventral margins of the body. The fin folds are continuous around the caudal end. The somites are about 55 in number. Accordingly, the increase in number of somites is considered to have

ceased in this stage.*

27. *Pectoral fins:* Fig. 31 (175 hrs.)

The origins of the pectoral fins appear behind the ears. Melanophores and iridophores have deposited densely in the eyes which now become mobile.

28. *Hatching glands:* Figs. 32 a, b (191 hrs.)

Numerous hatching glands come to be distributed in the lateral space between the ear and the anus.

29. *Opening of the anus:* Fig. 33 (215 hrs.)

The lumen of the gut proceeds backward perforating so far a solid straight cord and finally reaches to the proctodeum where the anus opens.

30. *Pigmentation of the peritoneal wall:* Fig. 34 (245 hrs.)

Along the ventral wall of the alimentary canal limited from the yolk-sac to the anus, melanophores develop in a row. At this stage the embryo is so elongated that it is coiled twice within the egg capsule.

31. *Pre-hatching:* Fig. 35 (267 hrs.); Fig. 36 (289 hrs.); Fig. 37 (313 hrs.)

The organization of the body of the embryo has been completed. The embryos twist their body vigorously. The yolk has been greatly reduced in amount. The egg capsules become thin and the embryos are ready to hatch.

32. *Hatching:* Fig. 38 (314 hrs.)

The length of time required for bringing the inseminated eggs to the time of hatching was 14–17 days, but the greater part of the embryos hatched on the 16th day. Hatching takes place when the egg capsule is ruptured by the action of hatching enzyme. Usually the embryo sticks its posterior body out of the capsule; after a few seconds, the whole body slips out.

33. *The just-hatched fry:* Fig. 39 (315 hrs.)

The just hatched fry presents a slender transparent form and measures about 5 mm in total length. The fin folds are fully developed continuous from dorsal to ventral around the caudal part. The oval-shaped yolk-sac is rather small and contains a large oil globule in front. The mouth is mobile. The alimentary canal is a straight duct; its posterior half is larger than the anterior. Along the right side of the gut, posterior to the middle of the yolk-sac, there lies the pancreas, while the liver is at the left behind the yolk-sac. In front of the yolk-sac is the heart which consists of a ventricle and an auricle. The circulation can not be observed since the fetal blood is colourless and has no blood corpuscles (Yamada, 1959).

---

* The vertebrae of *H. olidus* from Lake Onuma are 54–57 in number (Hamada, 1961).

References

Hamada, K. (1961). Taxonomic and ecological studies of the genus *Hypomesus* of Japan. Mem. Fac. Fish., Hokkaido Univ. 9 (1), 1–55.

Hikita, T. (1958). On the development of long-finned smelt, *Spirinchus lanceolatus* (*HIKITA*). Sci. Rep. Hokkaido Fish Hatch. 13, 39–49.

Iwai, T. (1962). Studies on the *Plecoglossus altivelis* problems: Embryology and histophysiology of digestive and osmoregulatory organs. Bull. Misaki Mar. Biol. Inst., Kyoto Univ. 2, 1–101.

Kanoh, Y. (1952). Über das Ei und einige seiner Charakteristiks bei einem Japanischen Knochenfisch, *Ayu* (*Plecoglossus altivelis*). Jap. Jour. Ichth. 2 (4/5), 147–155.

McAllister, D. E. (1963). A revision of the smelt family, *Osmeridae*. Nat. Mus. Canada, Bull. 191, Biol. Ser. 71, 1–53.

Oppenheimer, J. M. (1937). The normal stages of *Fundulus heteroclitus*. Anat. Rec. 68 (1), 1–15.

Yamada, J. (1959). The structure of the yolk-sac and the absorption of the yolk in the pond smelt fry. Bull. Fac. Fish., Hokkaido Univ. 10 (3), 196–203.

Yamamoto, T. (1937). Influence of temperature on the embryonic development of the pond smelt, *Hypomesus olidus PALLAS*. Bull. Jap. Soc. Sci. Fish. 5 (5), 326–332.

Explanation of Plates

The magnification rate of all figures is 42 times with exceptions of Fig. 32 b (105×) and Fig. 39 (28×).

PLATE I

Fig. 1. Stage 1, Unfertilized egg

Fig. 2. Stage 2, Formation of the germ disc, elevation of the egg membrane (20 min.)

Fig. 3. Stage 2, Formation of the germ disc, one-celled ovum (1 hr.)

Fig. 4. Stage 3, Two-celled ovum (4 hrs.)

Figs. 5 a, b. Stage 4, Four-celled ovum (5½ hrs.)

a. Beginning of the second cleavage

b. Completion of the second cleavage

Fig. 6. Stage 5, Eight-celled ovum (7 hrs.)

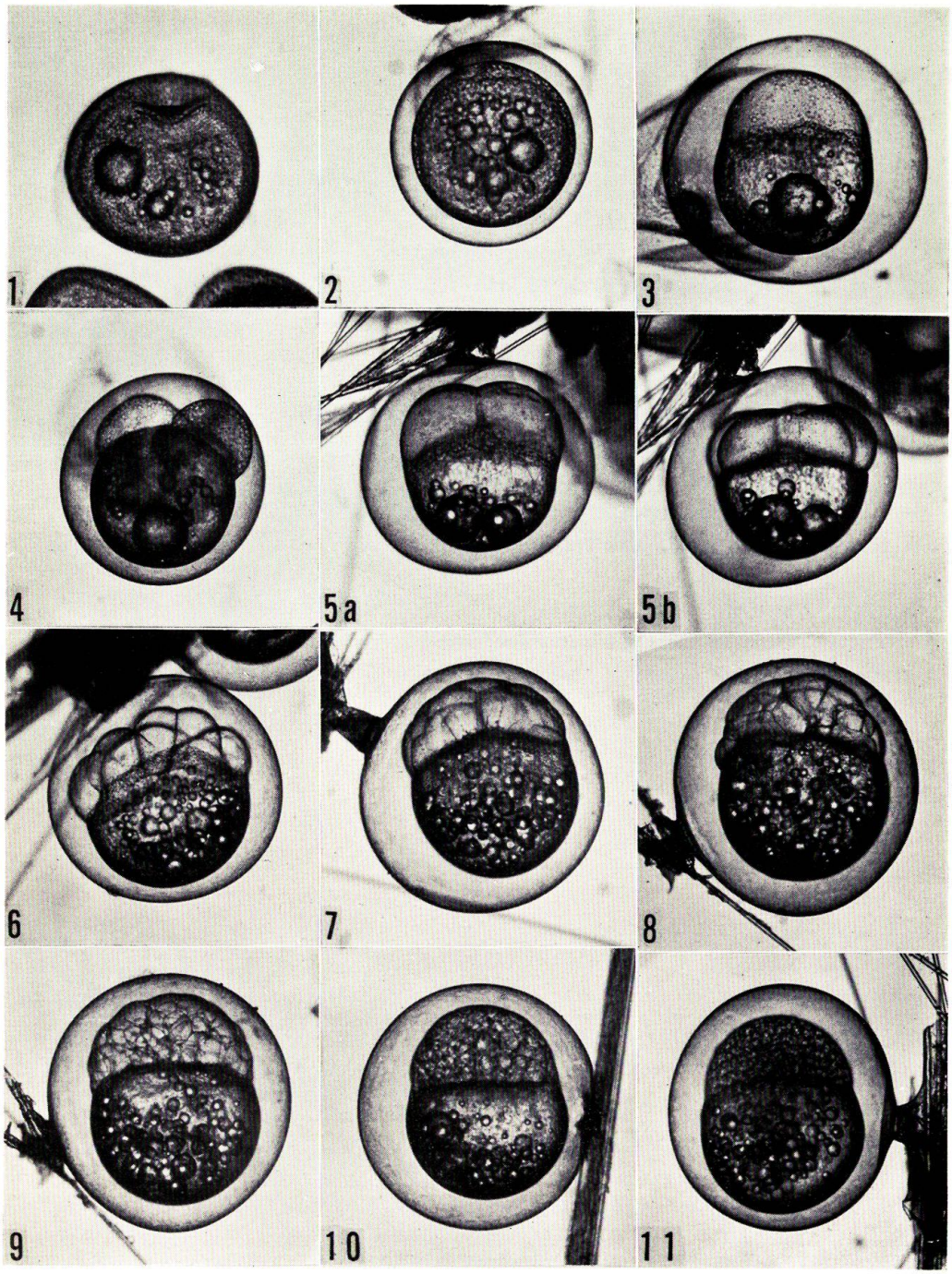
Fig. 7. Stage 6, Sixteen-celled ovum (8½ hrs.)

Fig. 8. Stage 7, Thirty-two-celled ovum (10 hrs.)

Fig. 9. Stage 8, Sixty-four-celled ovum (11½ hrs.)

Fig. 10. Stage 9, Morula (13 hrs.)

Fig. 11. Stage 10, Early blastula (16 hrs.)



J. Yamada: Normal developmental stages of *Hypomesus olidus*

PLATE II

Fig. 12. Stage 11, Late blastula (21 hrs.)

Fig. 13. Stage 12, Beginning of epiboly (28 hrs.)

Fig. 14. Stage 13, Early gastrula (31 hrs.)

Figs. 15 a, b. Stage 14, Late gastrula (38 hrs.)

a. Lateral view

b. Ventral view

Fig. 16. Stage 15, Appearance of the embryonic body (44 hrs.)

Figs. 17 a, b. Stage 16, Head fold (51 hrs.)

a. Lateral view

b. Ventral view

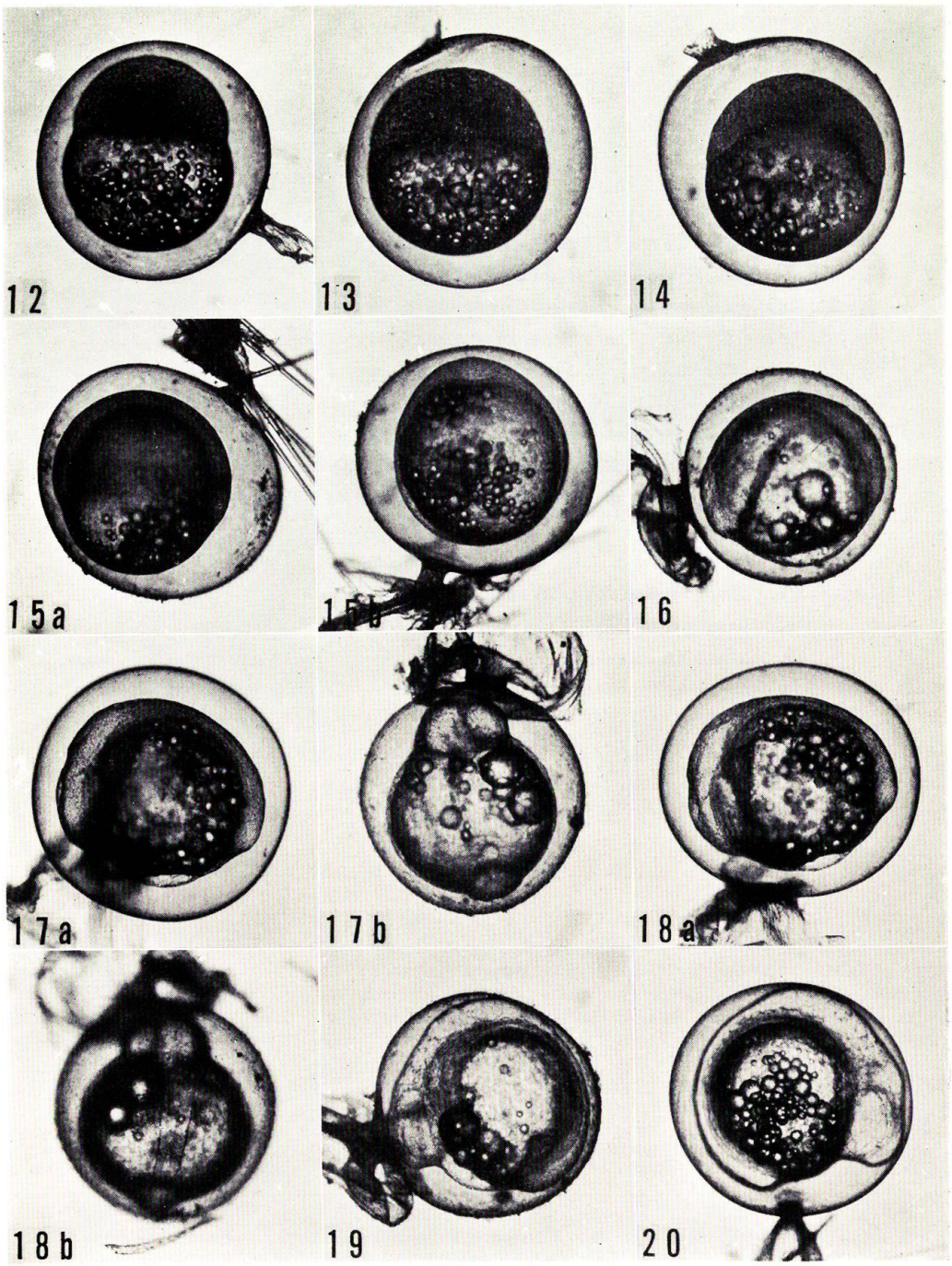
Figs. 18 a, b. Stage 17, Optic vesicles (55 hrs.)

a. Lateral view

b. Dorsal view

Fig. 19. Stage 18, Beginning of somites formation, 5 somites (64 hrs.)

Fig. 20. Stage 19, Closure of blastopore, 11 somites (71 hrs.)



J. Yamada: Normal developmental stages of *Hypomesus olidus*

PLATE III

Fig. 21. Stage 19, Closure of blastopore, 15 somites (76 hrs.)

Fig. 22. Stage 20, Optic cups, 21 somites (83 hrs.)

Fig. 23. Stage 21, Auditory placode, 25 somites (91 hrs.)

Fig. 24. Stage 22, Formation of lens, 28 somites (98 hrs.)

Fig. 25. Stage 23, Tail bud, 31 somites (108 hrs.)

Fig. 26. Stage 24, Motility, 36 somites (119 hrs.)

Fig. 27. Stage 24, Heart beating, 38 somites (120 hrs.)

Fig. 28. Stage 25, Pigmentation of retina, 48 somites (148 hrs.)

Figs. 29 a, b. Stage 25, Pigmentation of retina, 50 somites (151 hrs.)

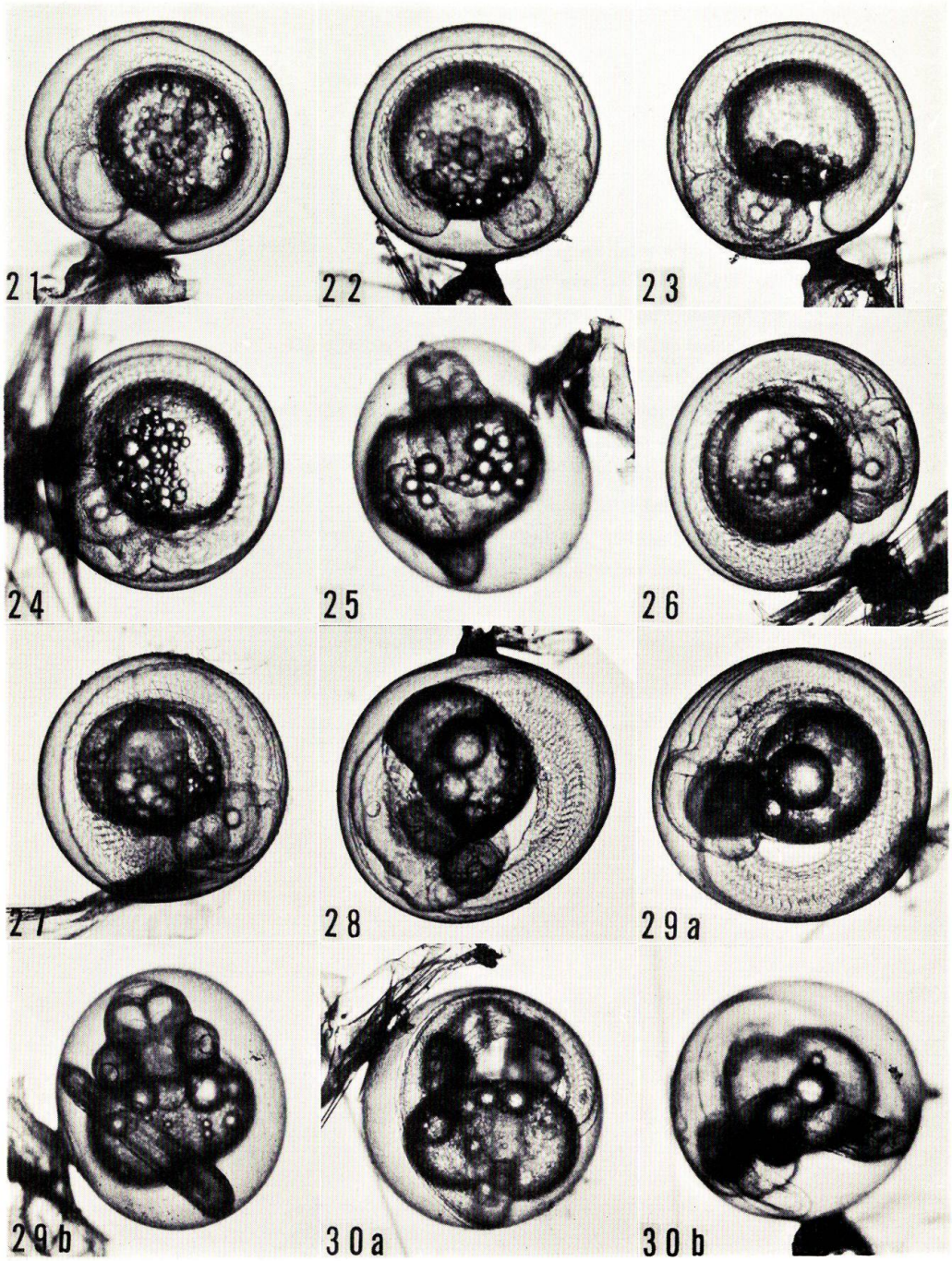
a. Lateral view

b. Ventral view

Figs. 30 a, b. Stage 26, Fin folds, 55 somites (168 hrs.)

a. Dorsal fin fold

b. Caudal fin fold



J. Yamada: Normal developmental stages of *Hypomesus olidus*

PLATE IV

Fig. 31. Stage 27, Pectoral fin (175 hrs.)

Figs. 32 a, b. Stage 28, Hatching glands (191 hrs.)

a. General view

b. Partial magnification of the lateral body side 165×

Fig. 33. Stage 29, Opening of the anus (215 hrs.)

Fig. 34. Stage 30, Pigmentation of the peritoneal wall (245 hrs.)

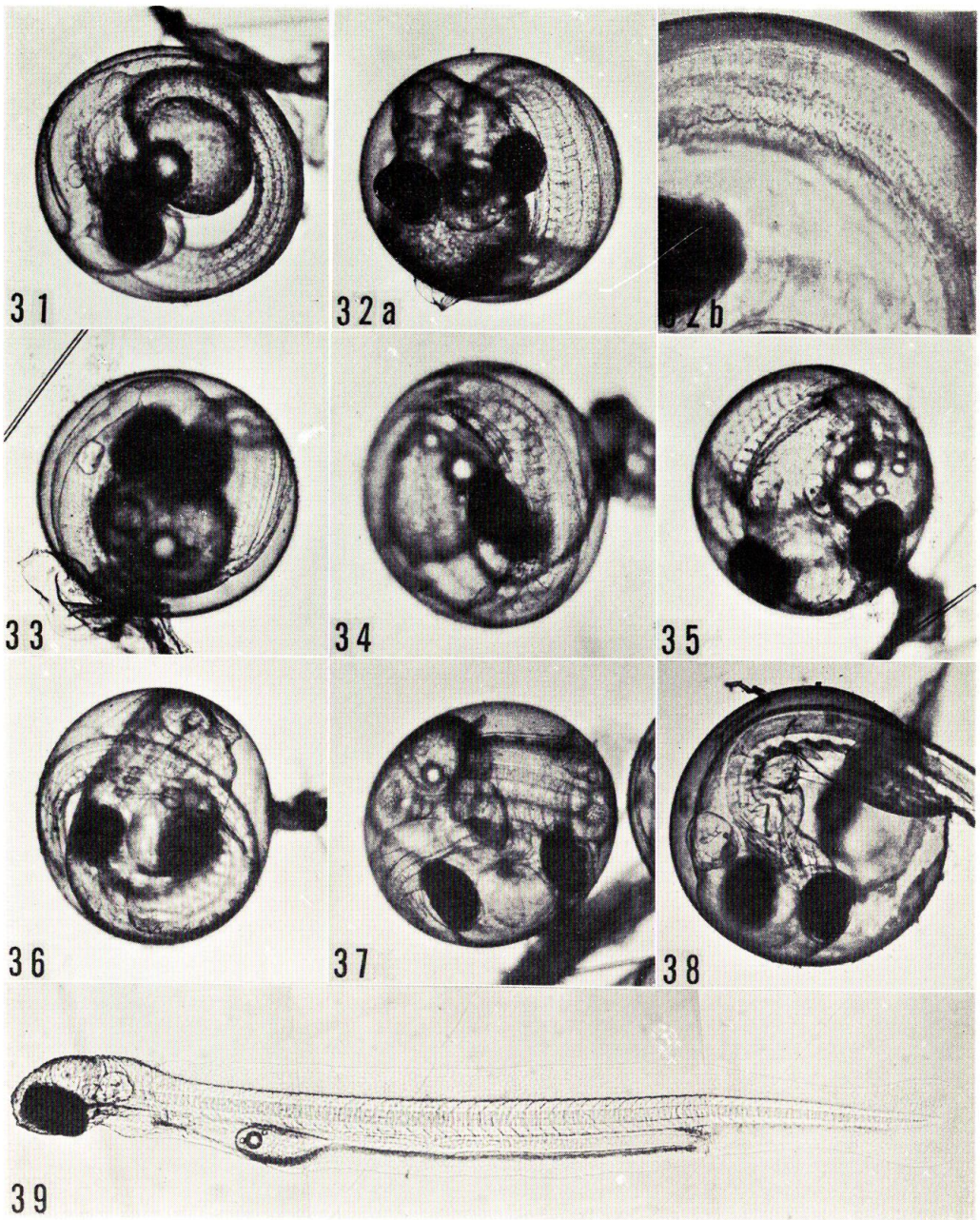
Fig. 35. Stage 31, Pre-hatching (267 hrs.)

Fig. 36. Stage 31, Pre-hatching (289 hrs.)

Fig. 37. Stage 31, Pre-hatching (313 hrs.)

Fig. 38. Stage 32, Hatching (314 hrs.)

Fig. 39. Stage 33, Just-hatched fry (315 hrs.) 28×



J. Yamada: Normal developmental stages of *Hypomesus olidus*