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Embryonic Development and Larvae of *Gymnocanthus herzensteini* Jordan and Starks

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Abstract The eggs of *Gymnocanthus herzensteini* were fertilized and cultured for the study of their embryonic and larval development. The eggs were demersal and adhesive. The yolk was light orange in color with a single large oil globule, sometimes accompanying a few small oil globules, and contained a mass of many small granular materials. Melanophores and xanthophores appeared on the embryonal body after the mid-stage of embryonic development, but not on the yolk and oil globule. Hatching occurred between 42 and 49 days after fertilization at the water temperature 5.5°–6.8°C.

The newly hatched larvae averaged 5.83 mm in body length with 41–43 pairs of somites. The prominent characteristic of larvae lay in the pigmentation pattern of melanophores and xanthophores on the nape, the crown of the head, the body cavity, and especially on the ventral margin of the tail portion. After thirty-nine days, the larvae reached an average body length of 7.84 mm. The pigmentation pattern remained essentially unchanged except the appearance of melanophores on the lower jaw and the opercle region. Various body parts were measured to examine the changes in body form in the larval stage.

Gymnocanthus herzensteini is the sculpin commonly distributed in the coastal waters of Hokkaido. In the Funka Bay area the species is not important commercially, although the fish are caught by various fishing gears all year round. It is used for human consumption and the bait on long line to catch octopus as well as the food for mink. The author succeeded in the artificial fertilization and rearing of the species in 1968. The present paper reports observations on embryonic and larval development, and changes in body form of early larval stage.

Methods and procedure

In Funka Bay the spawning season of the species continues from early January to early March when water temperatures are about 4°–7°C. On January 20th, 1968 the eggs and milt were taken from a female and a male caught by the bottom gill net for the Alaska pollack at about 75 m in depth in the coastal water of Shikabe at the entrance of Funka Bay, Hokkaido. The body length of the mature fish was about 25 cm. The eggs fertilized by the ordinary wet method were rinsed thoroughly in sea water containing chloromycetin at 50 p.p.m., and transferred to the laboratory in a thermos jar. Water temperature was about 6°C at fertilization and nearly 4°C in the container during transport.

Upon arrival at the laboratory, about eight hours after fertilization, the eggs were transferred from the jar to 10-liter glass vessels of seawater placed in a tank of fresh water. They were reared at an average temperature of 6.0°C with the range of 5.5°–6.8°C and at an average chlorinity of 18.7‰ with the range of 18.5–19.1‰. During incubation a slight stream of air was bubbled continuously in the container, and the sea water was changed frequently to keep the eggs free from contamination. After hatching, the larvae were transferred to 10-liter glass vessels and reared at an average water temperature of 6.0°C

with the range of 5.7°–6.5°C without aeration or change of water. The nauplii of *Artemia salina* were provided as food for the larvae.

Microscopic observations were made at frequent intervals on the living eggs and larvae anaesthetized with 5 mg percent solution of MS 222-Sandoz to determine the stage of development. Furthermore, measurements were taken under a microscope with a micrometer eyepiece to determine the body length, the anteanal length, the head length, the eye diameter and the diameter of auditory vesicle for the study of changes in body form. The head length is the distance from the snout to the posterior margin of gill measured along the horizontal axis of the body because of the indistinctness of the opercle margin. The tail length was calculated by subtracting the anteanal length from the body length, similarly the trunk length by subtracting the head length from the anteanal length. After those observations and measurements, the eggs and larvae were preserved in 4% formalin.

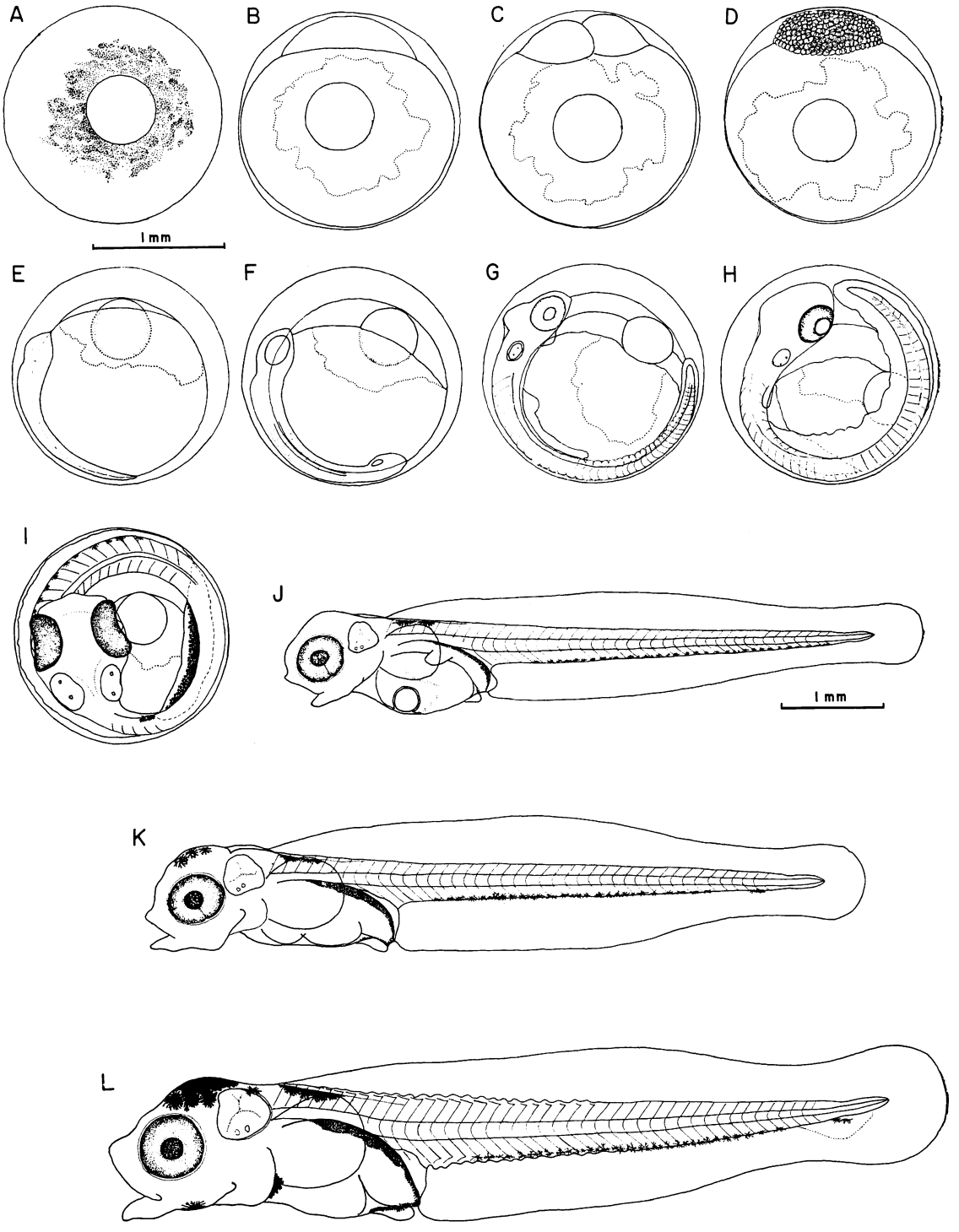
Eggs and embryonic development

The eggs of *Gymnocanthus herzensteini* are spherical in shape with the range from 1.64 to 1.73 mm in diameter. The chorion is very thin, colorless, slightly opaque but fairly transparent after fertilization, and no conspicuous structure was seen on its surface. The yolk is light orange in color. There is always a large clear oil globule, about 0.52 mm in diameter but some eggs have a few small oil globules in addition to the large one. Many small granular materials form a mass in the yolk on the same side as the oil globules (Fig. 1–A).

The eggs are demersal and adhere to one another and to glass surface but not so solidly as do those of *Hexagrammos otakii* (Hamai and Kyushin, 1966) or *Hemitripterus villosus* (Kyushin, 1968), both carrying a thick chorion.

The first observation was made eight hours after fertilization, when the blastodisc reached its full development (Fig. 1–B). The first cleavage was completed four hours later, dividing the blastodisc into almost equal blastomeres (Fig. 1–C). Subsequent cleavages followed at approximately four and a half hour intervals and the eggs progressed in the early morula stage forty-eight hours after fertilization (Fig. 1–D).

The eggs reached the blastula stage at approximately on the 69th hour, when the blastodermal cap was a lenticular dome with a granular texture. They reached the gastrula stage by the 93rd hour. The blastoderm showed a marginal thickening of the germinal ring and yet had a slightly granular appearance. At the 138th hour, the germinal ring was almost equatorial in position. At the 183rd hour, it exceeded two-thirds of the yolk, and the embryo was discernible. At the 204th hour, the blastopore completely closed and the optic vesicles became outlined in the cephalic region of the embryo (Fig. 1–E). No constriction of the yolk by the germinal ring was observed until the closure of the blastopore. At the 217th hour, the optic vesicles were well defined, Kupffer's vesicle had finally appeared near the posterior end of the embryo, and 2–3 pairs of somites were counted in the middle part of the embryo. At the 264th hour, the embryo encircled about a half of the circumference around the yolk sphere, but there was no differentiation of the lens or the auditory vesicle, and 10–11 pairs of somites were apparent (Fig. 1–F). By the 312nd hour, Kupffer's vesicle had disappeared, and the tail had started to grow free from the yolk sac. At the 362nd hour, the embryo extended about two-thirds of the circumference around the yolk sphere with the oil globule on the opposite side protruding beyond the yolk sphere. The lenses and the auditory vesicles were now clearly completed, the heart was indicated by a slight bulge below the nape and 25–26 pairs of somites were counted. One day after this stage, the heart was pulsating slowly and regularly but no action of the embryo was


A
B
C
D
1 mm
E
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observed. The embryo at the 455th hour showed some movements occasionally twitching its tail, and the intestine was differentiated below the ventral side of the embryo. At the 464th hour, the embryo extended about three-quarters of the circumference around the yolk sphere, the melanophores could be seen with a proper light on the optic vesicles, a transparent, faint fin fold appeared on the dorsal and the ventral side of the embryo around the tail, and the somites were differentiated to about 40 in number (Fig. 1–G). The increase in number of somites from the 217th hour stage to this last stage was proportional to the time elasped; the relationship is expressed in the linear equation, $S = 0.152t - 29.322$, where S is the number of somites and t is the hours after fertilization (Fig. 2).

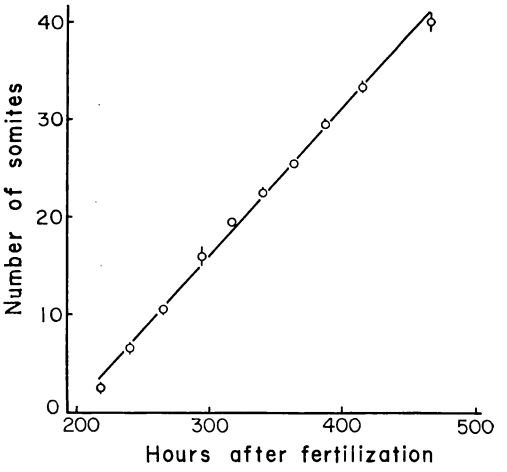

Fig. 2. Linear relation of the increasing number of somites in the embryos of *Gymnocanthus herzensteini* referred to the growth of embryos expressed in time elasped after the fertilization of eggs.

At the 598th hour, the embryo had grown large to allow the tip of the tail reaching the snout, thus body encircling the yolk sac (Fig. 1–H). The twitching movement of the embryo became vigorous, and the blood was observed running in the blood vessels below the notochord. At about the 650th hour, the mouth began to differentiate, the eyes became increasingly dark and the patches of minute nodules were clearly observed on the crown of the head, the snout and the lower jaw. At this stage the black minute stellate chromatophores were apparent on the dorsal side of the body cavity. By the 888th hour after fertilization, the body was increasing in bulk and the head had become greatly widened (Fig. 1–I). One or two black stellate chromatophores were evident on the nape of the embryo, and xanthophores distributed from the crown to the nape. The stellate melanophores appeared in one row along the ventral margin of the body. The dorsal side of the body cavity became completely black by the heavy pigmentation. Hatching occurred firstly at 1008 hours after fertilization and continued for the next eight days.

Larvae

The average body length of the newly hatched larvae was 5.83 mm with a range of 5.57 to 6.12 mm (Fig. 1–J). The body of a larva tapered gradually from the nape to the tail with a short trunk. The vent was situated at about one-third of the body length from the snout. The yolk sac was from 0.86 to 1.08 mm in its horizontal diameter. The oil globule, measuring about 0.25 mm in diameter, was located at the anterior part of the yolk. The number of somites of the larvae was 41–43 (the number of vertebrae of the species is 37–39). The transparent fin fold

Fig. 1. Drawings of the eggs under development and the larvae in early stages of *Gymnocanthus herzensteini*: Figures A to D and I are top view, and all the rest lateral view. A, Mature egg before fertilization; B, 8 hours after fertilization, showing protoplasmic germ disc; C, 12 hours, 2-cell stage; D, 48 hours, morula stage; E, 204 hours, blastopore closed; F, 264 hours, embryo in nearly half-circle; G, 464 hours, embryo in about three-quarters circle; H, 598 hours, embryo forming nearly a circle; I, 888 hours, nearly ready to hatch out; J, Newly hatched larva, 5.79 mm long in body length; K. 17 days old larva, 6.59 mm; L, 39 days old, 7.55 mm.

arose from the nape and extended to the vent surrounding the tail. The most remarkable taxonomic characteristic lay in the pigmentation pattern—two or three black dendritic chromatophores on the nape, heavy pigmentation of melanophores on the dorsal side of the body cavity, and a row of ventral stellate chromatophores, starting from 4th–6th somite posterior to the vent and extending toward the tail. These ventral melanophores varied from 23 to 37 in number. Furthermore, xanthophores were well established on the crown and the dorsal side of the body cavity. Some larvae had one or two stellate melanophores on their crown. At this stage, no other body portion showed pigmentation.

The newly hatched larvae were able to swim just after hatching by fluttering motion of their tail and fanning of their pectoral fins. Four days after hatching, some larvae began to take *Artemia* nauplii and swam actively near the bottom of the rearing vessel.

Seventeen days after hatching, the larvae measured 6.64 mm in average body length with a range of 6.33 to 6.83 mm (Fig. 1–K). The yolk was almost entirely consumed, and some larvae had small oil globules at the anterior part of the body cavity. The incipient pectoral rays were clearly defined. The pigmentation pattern remained essentially the same as in the newly hatched larvae except that the crown of the head and the nape portion had become much more pigmented.

About thirty-nine days after hatching, the larvae had attained an average body length of 7.84 mm with a range of 7.22–8.51 mm (Fig. 1–L). The number of somites was nearly the same as that of the larvae at hatching. The bases of dorsal and anal rays appeared as wavy structures dorsally and ventrally at the midpart of the body, but there was no sign of ray formation. The pigmentation areas on the crown and the nape were more expanded than in the preceding stage. The ventral isolated melanophores, varying from 29 to 44 in number, became dendritic and pushed out into the ventral fin fold. A few melanophores were apparent on the lower jaw and the opercle region.

None of the larvae died during the first fifteen days after hatching, but a high mortality followed during the yolk absorption; consequently, the surviving fish thirty-nine days after hatching were very few in number. They were measured and preserved.

Changes in body form

The changes in body form at the early larval stage were examined by the allometric equation, i.e. $\log y = \log b + k \log x$, where x is the body length and y is the length of body part. Plotting these measurements on logarithmic graph paper, a linear relationship is

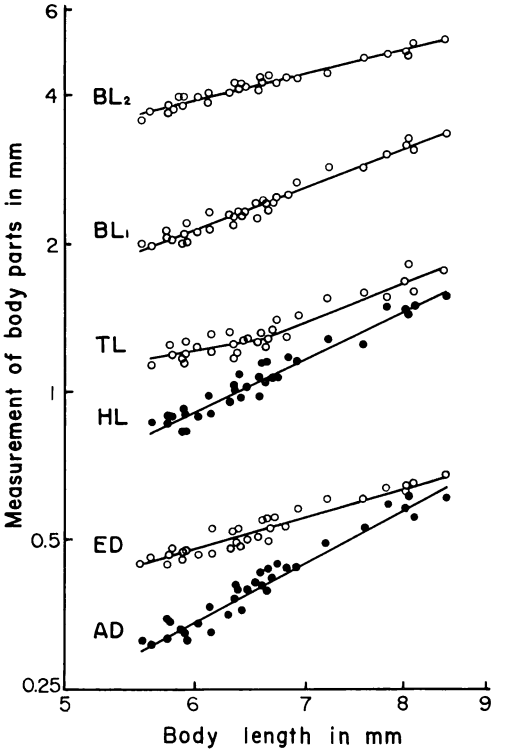

Fig. 3. Fitted straight lines to show the relative growth in the 6 body parts of *Gymnocanthus herzensteini* in early larval stage. The calculated values of constants in allometric equation of each line are presented in Table 1. BL_2, tail length; BL_1, anteanal length; TL, trunk length; HL, head length; ED, eye diameter; AD, auditory vesicle diameter.

Table 1. Values of equilibrium constant k and constant log b in the allometric equation calculated to show the relative growth in the 6 body parts of larval fish, *Gymnocanthus herzensteini*. See Fig. 3 for the designation of each body part. Figures in parentheses are 95% confidence intervals.

Relation	N	k	log b	Remarks
BL_1—BL	34	1.324 (1.22–1.43)	−1.697	
BL_2—BL	34	0.809 (0.75–0.87)	0.532	
TL —BL	20	0.598 (0.31–0.89)	0.822	Before inflection
	13	1.247 (0.65–1.85)	−1.646	After inflection
HL —BL	33	1.614 (1.45–1.78)	−3.138	
ED —BL	34	0.984 (0.87–1.10)	−1.031	
AD —BL	34	1.813 (1.63–2.00)	−4.311	

apparent in each regression except the trunk length (Fig. 3). Two constants in the allometric equation are shown in Table 1. Equilibrium constant, k, of 1.32 for the anteanal length, 1.61 for the head length and 1.81 for the diameter of auditory vesicle all show tachyauxesis. This constant is 0.81 for the tail length, this shows bradyauxesis. The eye diameter gives an equilibrium constant of 0.98 and shows isauxesis. In the case of the trunk length, there is an appreciable growth inflection when the larvae reach a length of about 6.61 mm: relative growth shifts from strong bradyauxesis before inflection to isauxesis thereafter. The body length at the inflection coincides well with that at the end of the yolk absorption period. Such a growth inflection of the trunk length was also observed in the rearing experiment of the greenling (Hamai and Kyushin, 1966).

Acknowledgments

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ツマグロカジカ, *Gymnocanthus herzensteini* Jordan and Starks の胚発生および仔魚 久新健一郎

1968 年 1 月 20 日，北海道噴火湾口鹿部村沿岸で漁獲された本種成熟魚を用い，湿導法により人工受精を行ない，卵および孵化仔魚を水温 5.5°～6.8°C の静水海水中で飼育した．卵は球形沈性粘着卵で，卵径は 1.64～1.73 mm，大型油球 1 個をもち，油球側に顆粒物質群がある．卵黄は淡橙色である．胚発生は一般の硬骨魚卵のそれと大差ない．受精後 42 日目から孵化を開始した．孵化直後の仔魚は体長 5.57～6.12 mm，筋節数 41～43 で，活発に遊泳する．孵化後約 15 日で卵黄を吸収し，39 日で体長 7.22～8.51 mm に達した．黒色素胞は頭頂部，頭後方背部，腹腔部，尾部下側部に，また，黄色素胞は頭頂部，腹腔部にそれぞれ観察された．仔魚期の体形の変化をアロメトリー式で検した．体長に対して優比成長を示す体部分は肛門前体長，頭長および耳胞径であり，等比成長の部分は眼径，劣比成長の部分は尾長である．胴長は卵黄吸収時に変移点をもち，劣比成長から等比成長に変わる．

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