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THE EMBRYONIC DEVELOPMENT AND LARVAL STAGES OF *HEMITRIPTERUS VILLOSUS* (PALLAS)

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Hemitripterus villosus (PALLAS) is a cottid fish commonly distributed in the coastal waters of Hokkaido and relatively abundant in Funka Bay during the spawning season. Although this species is a commercially important cottid fish, there are no contribution on its biology or its early development.

In 1964 and 1965 the author succeeded in the artificial fertilization and rearing of this species and the present paper reports observations made in 1965 on the embryonic and larval development, changes in body form, and survival of eggs and larvae.

Before going further, the author wishes especially to acknowledge Prof. Dr. Ikuso Hamai, Faculty of Fisheries, Hokkaido University, for his critical review of the manuscript and his kind advice, and to Mr. Yasuji Kanno for his valuable help in many ways.

Methods and procedure

In Funka Bay this species spawns from mid-October to early November. According to the author's observations, spawning occurs on rocky sea bottom in water about 10 to 30 m in depth and with a temperature of 15° to 18°C.

On October 25 and 30, 1965 artificial fertilization was carried out by the ordinary dry method. Both ripe females and males were caught by gill nets in the coastal waters off Shikabe at the entrance of Funka Bay. The fertilized eggs were rinsed thoroughly in chloromycetin sea water of 50 p.p.m. to remove the remnants of milt and other contaminants. The eggs were kept in a 10-liter glass vessel of sea water placed in a tank of fresh water. The eggs were incubated at temperatures averaging between 12.07° and 13.53°C (Table 1). During incubation, a continuous stream of air was bubbled vigorously in the container and the water changed frequently to keep the eggs free of contamination. Microscopic observations were made at frequent intervals to determine the stage of development.

Newly hatched larvae were transferred to 10-liter glass vessels and reared at water temperatures averaging 11.56° to 11.83°C without aeration or change of water. One hundred ten to one hundred thirty larvae were kept in each rearing vessel and beginning two days after hatching the small fish were fed various sizes of living *Artemia salina* every other day. Every effort was made to keep the vessels clean by use of a glass siphon to remove the dead *Artemia salina* and other dirt which

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Table 1. Samples and water temperatures during

Date of fertilization	Experiment	Color of yolk	Body length of adult fish (mm)	Number of eggs cultured	Egg diameter (mm)	
					Range	Average
25th Oct. 1965	A	Light reddish-orange	Female 306 Male 270	1219	4.45~ 4.64	4.58
	B	Light yellow	Female 298 Male 304	976	4.33~ 4.52	4.41
30th Oct. 1965	C	Ditto	Female 310 Male 280	857	4.52~ 4.72	4.61
	D	Ditto	Female 340 Male 280	802	4.45~ 4.61	4.54

accumulated on the bottom. To observe larval development, the material was anaesthetized with 5 mg percent solution of MS 222-Sandoz; detailed observations were made on material preserved in 5 percent formalin. Furthermore, several body parts of the larvae, that is, the anteanal length (BL_1), the tail length (BL_2), the head length (HL), the snout length (SL), the eye diameter (ED) and the body height at the anus (BH), were measured on the anaesthetized material to study changes in body form. The distance from the posterior margin of the eye to that of the opercle (EO) was calculated by subtracting the snout length plus the eye diameter from the head length; similarly the trunk length (TL) was determined by subtracting the head length from the anteanal length (Fig. 1).

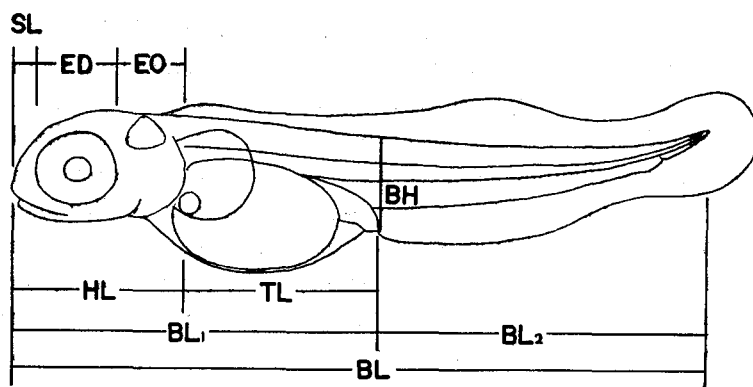


Figure 1. A diagram of the various measurements made of the larvae

During the rearing experiment, dead eggs and dead larvae were removed daily from the vessels, counted and preserved.

The number of ovarian eggs in a ripe female

The length composition of adult fish taken during the spawning season forms a skewed frequency distribution with a single mode. The females range in size from

the incubation period

Water temperature (°C)		Incubation time (hours)	Percentage of hatched egg	Body length of newly hatched larvae (mm)		
Range	Average			N	Range	Average
9.8~ 15.6	13.43	2495	24.4	15	13.02~ 14.37	13.80
9.8~ 15.6	13.53	2447	16.1	14	13.41~ 14.68	14.10
9.9~ 15.5	12.12	2398	67.0	35	14.05~ 15.16	14.69
10.2~ 14.0	12.07	2422	95.0	34	13.89~ 14.96	14.29

24 to 40 cm with the mode at 29.5 cm. The size of the males range from 21 to 36 cm with a mode of 25.5 cm (Table 2).

The ovaries of a female are very fat weighing from 120 to 580 g—17 to 40 percent of the body weight. In contrast, the male has small testes weighing from 5 to 18 g—only 0.6 to 1.9 percent of the body weight. The number of ovarian eggs vary from 2250 to 11170 for body lengths between 249 and 383 mm (Table 3). The relationship between the number of ovarian eggs and the body length can be expressed by the equation, $E=21.47 \times 10^{-6} \cdot L^{3.374}$, where E is the number of ovarian eggs and L is the body length in mm. The correlation coefficient between the two variables is +0.926 (with the 95% confidence interval from 0.892 to 0.950). Based on measurements of 30 eggs from each female, the average diameter of mature eggs ranges from 4.31 to 4.90 mm for material preserved in 5 percent formalin. The

Table 2. Frequency distribution of the body lengths of mature fish

Body length (cm)	Female	Male	Total
21~22		3	3
22~		15	15
23~		33	33
24~	2	47	49
25~	15	66	81
26~	58	57	115
27~	104	53	157
28~	142	34	176
29~	175	20	195
30~31	165	14	179
31~	100	4	104
32~	88	4	92
33~	51	3	54
34~	21	2	23
35~	9	1	10
36~	7		7
37~	4		4
38~	1		1
39~40	1		1
Total	943	356	1299

Table 3. The number of ovarian eggs

N	Body length (mm)		Egg number	
	Range	Average	Range	Average
20	249~ 270	259	2250~ 4000	2970
28	270~ 300	287	2770~ 5790	4110
22	300~ 330	315	4700~ 8250	6250
25	330~ 360	341	5610~ 8990	7370
7	360~ 383	371	7570~11170	9850

correlation coefficient between average diameter of eggs and body length is $+0.543$ (with the 95% confidence interval from 0.332 to 0.703). The diameter of the egg is proportional to the body length.

Eggs

The eggs of *Hemitripterus villosus* are demersal and adhere solidly to one another and to the glass surface after coming in contact with sea water. Eggs are spherical in shape but are slightly flattened at their point of contact (Pl. 1, Fig. 2). They are relatively opaque because of the heavy corion, light milk-white in color; conspicuous sculptures could not be seen on the surface of the corion. The average diameter of 50 eggs in each sample ranges from 4.41 to 4.61 mm and the corion is about 150μ in thickness. A striking characteristic of the eggs is the light yellow or light reddish-orange color of the yolk. It is also unusual that a large number of irregularly shaped granules are randomly scattered in the yolk. Clear oil globules, about 120 to 160 in number form a mass on the upper side of the yolk. There is considerable variation in the diameters of the oil globules, which ranging in size from 50 to 260μ (Pl. 1, Fig. 1). During the course of development, the oil globules gradually decrease in number by merging with one another until they finally form a large single globule during late embryonic development.

Embryonic development

Since there is almost no difference in embryonic development between samples from the different experiments, the following description is based on observations of sample from C (Table 1).

Eight hours after fertilization, the small blastodisc, lenticular in shape, is fully formed. The perivitelline space is very narrow, about 50μ in width (Pl. 1, Fig. 2). At about the 11th hour, the blastodisc first divides into two almost equal blastomeres (Pl. 1, Fig. 3). Subsequent divisions occur at approximately three and a half hour intervals, each reducing the size of the individual cells. By the

21st hour after fertilization, the 16-cell stage is complete and most of the eggs have cells nearly equal in size and form, but irregular in position (Pl. 1, Fig. 4). The embryonic development reaches the morula stage in about 38 hours (Pl. 1, Fig. 5), and the blastula stage in about 55 hours. The blastodermal cap at this stage is very similar to the blastodisc in size and shape when viewed from the side.

At about the 80th hour after fertilization, the gastrula stage is reached and shortly thereafter a germinal ring is visible (Pl. 1, Fig. 6). The germinal ring is so vague that observation of the epibolic growth is difficult without careful manipulation of the light source. At about the 140th hour, the germinal ring is almost equatorial in position and a short embryo is distinguishable (Pl. 1, Fig. 7). At the 190th hour, the blastopore begins to close, the optic vesicles are well defined but yet without lenses, Kupffer's vesicle has finally appeared and 8-9 pairs of somites can be counted (Pl. 1, Fig. 8). No constriction of the yolk by the germinal ring was observed until closure of the blastopore.

At about the 218th hour after fertilization, the lenses are indistinctly observed in the optic vesicles, about 14-15 pairs of somites can be counted and shortly before this stage, the blastopore has closed. At about the 240th hour, the embryo extends about a quarter of the circumference around the yolk sphere, the lenses are now clearly complete, the auditory vesicles are distinguished on the nape of the embryo, about 23-24 pairs of somites can be counted and Kupffer's vesicle has disappeared (Pl. 1, Fig. 9). At this stage, the number of oil globules have decreased to about 30. At the 274th hour after fertilization, the heart is pulsating slowly and regularly, 32-33 pairs of somites are formed, and the embryo is observed to occasionally move spasmodically. The increase in number of somites from the 190th hour stage to this stage is proportional to the time elapsed. By about the 342nd hour, the head of the embryo has increased greatly in width and by now the body extends about half the distance around the yolk sac.

At about the 403rd hour after fertilization, the first melanophores can be seen with proper light on the marginal region of the optic vesicles. At about the 434th hour, a blood vessel appears along the yolk sac below the cephalic region. The pectoral fins are now easily seen, a faint fin fold extends around the body from the dorsal area to the anus and 40-42 pairs of somites are apparent (Pl. 1, Fig. 10).

Because of the vigorous movement and the complicated structure of the embryo, further embryonic development becomes difficult to observe in any detail. For these reasons, subsequent observations were made on the embryo carefully extracted out of the egg.

At about the 604th hour after fertilization, melanophores become visible as minute spots between the posterior end of the auditory vesicle and the mid-region of the body; a few stellate melanophores, although small in size, are seen on the

yolk sac in the vicinity of the pectoral fins but nowhere else on the embryo. The jaws are developed, a large oil globule about 700μ in diameter and a few small oil globules are seen below the cephalic region and the intestine is almost completely formed as a long tubule on the ventral side of the embryo. At this stage a patch of minute nodules appears on the crown of the head, the snout and the lower jaw (Pl. I, Fig. 11, Pl. II, Fig. 13). At about the 640th hour, a few stellate or dendritic, brownish-black chromatophores are well defined on the crown of head and the dorsal region of the nape. The black pigment spots, remaining quite small, increase in number in the mid-region of the body and the pigmentation spreads to the posterior part of the embryo.

At about the 1133rd hour after fertilization, the embryo has grown so that by now the tip of the tail reaches the snout completely, encircling the yolk sac (Pl. I, Fig. 12, Pl. II, Fig. 14). The optic vesicles are very dark, and the black minute spots on the body region have developed into stellate or dendritic pigments, brownish-black in color. A marked increase of chromatophores is especially apparent on the intestine. No pigment spots appear even at this stage on the lower jaw nor on the posterior part of the tail. Blood can be seen to course through the blood vessel spread over the yolk sac and through blood vessels in other parts of the body. Quite minute structures can be observed along the fin fold except in its marginal region. There is no difference in the number of somites during development from the 434th hour to this stage. From this stage until the time of hatching, only a few changes occur; namely an increase in the size of the embryo and heavier pigmentation.

The vigorous twitching movement of the embryo ceases completely as the embryo approaches hatching and the eggs immediately before hatching become almost opaque in appearance; the reason may be that the perivitelline fluid jellies and turns a light, cloudy white. Hatching occurs first at about the 2184th hour after fertilization and continues for the next 16 days. The incubation time from fertilization to 50 percent of hatching is 2398 hours or about 100 days. As high as 67 percent of the eggs hatched. There is no appreciable difference in incubation time between the eggs fertilized on October 25th and those fertilized on October 30th except differences due to water temperature (Table 1).

Larvae

Owing to the large size of the eggs and the long period required for incubation, the newly hatched larvae are comparatively large and well-developed. Immediately after hatching the larvae swim about actively by a fluttering of their tails and are able to balance their bodies by fanning their pectoral fins. For about 10 days after hatching they congregate towards the light in the rearing vessel due to their phototactic behaviour. No conspicuous feeding behaviour, such as a

rapid, forward-darting movement or a pecking action at the wall and the bottom of vessel, was observed throughout the larval stage. However, the larvae begin to bite each other occasionally, when development approaches the end of the yolk-sac stage. Cannibalism was rarely observed.

The average body length of newly hatched larvae is 14.69 mm with a range of 14.05 to 15.16 mm (Pl. II, Fig. 15). The body of a larva is thick, gradually tapering from the nape to the tail, the tail begins to turn upwards. The head, eyes and auditory vesicles are relatively large. The vent is slightly posterior to the midpoint of the body and completely opened. The yolk sac is ovoid, measuring about 3.9 mm along its horizontal axis and about 1.9 mm in depth. The oil globule lies at the anterior end of the yolk sac. The large mouth is functional with many sharp larval teeth on each side of both the upper and lower jaws.

The fin fold has its dorsal origin just posterior to the auditory vesicle and extends continuously around the posterior half of the body to the vent. It is highest about two-thirds of the body length measured from the tip of the head. Furthermore, the ventral fin fold extends from slightly beyond the vent to the posterior edge to the yolk sac. As observed in the embryonic stage 1133 hours after fertilization, quite minute structures are densely distributed over the fin fold except in the marginal region.

The larva has 40-42 pairs of somites. The pectoral fins located just posterior to the auditory vesicles, are fan-like in shape and have 9-11 incipient rays. The caudal thickening has 10-12 incipient rays. The bases of the dorsal and anal soft rays appear as wave-like structures extending posteriorly from the midpart of the body; there is no sign of ray formation dorsally or ventrally. At this stage the pelvic buds can be observed on the ventral side of the nape.

The pigmentation is quite heavy; the main body, the crown of the head and the dorsal region of the body cavity are covered with closely spaced, brownish-black dendritic chromatophores. This pigmentation pattern gives the larva a dark amber appearance. The caudal region has no pigment from about the 31st to 35th somite to the tip of the tail, and is light yellow in color. The fin fold is colourless and without pigment. The eyes are conspicuously dark. A few pigment spots in which the dendritic is brownish-black and the dotted black, are well defined on the opercle.

Twenty days after hatching, the larvae attain an average body length of 15.57 mm with a range of 15.24 to 16.03 mm (Pl. II, Fig. 16). At this stage the yolk is almost entirely absorbed and only some of the specimens have an oil globule at the anterior part of the body cavity. The dorsal and anal incipient rays are clearly defined. However, there is a considerable variation in the number of rays from specimen to specimen; the dorsal fin has 6-12 spiny rays and 7-12 soft rays; the anal fin has 7-13 soft rays. The differentiation of dorsal spines begin at the

anterior end of the dorsal fin fold and proceeds posteriorly. The dorsal and anal incipient soft rays develop from their respective bases. Although there is little increase in number, the caudal fin fold now contains 13-14 rays at this stage. The number of somites could not be determined because of the conspicuous pigmentation of the body. The pigmentation pattern remains essentially the same as in the preceding stage except that the chromatophores can be seen extending into the surface of the dorsal and ventral fin folds.

Twenty-nine days after hatching, the larvae reach an average body length of 16.11 mm with a range of 15.40 to 16.75 mm (Pl. II, Fig. 17). At this stage the dorsal fin fold has 9-16 spines and 11-12 soft rays, the ventral fin fold 12-14 rays, caudal fold 14-16 rays and the pectoral fin 16-18 rays. Compared with the preceding stage, few changes in the pigmentation pattern are evident except that the pigmented areas of the fin fold have somewhat expanded and the pectoral fins have some pigment along their rays.

Although very few in number, all the surviving larvae were preserved thirty days after hatching for further study of the larval development.

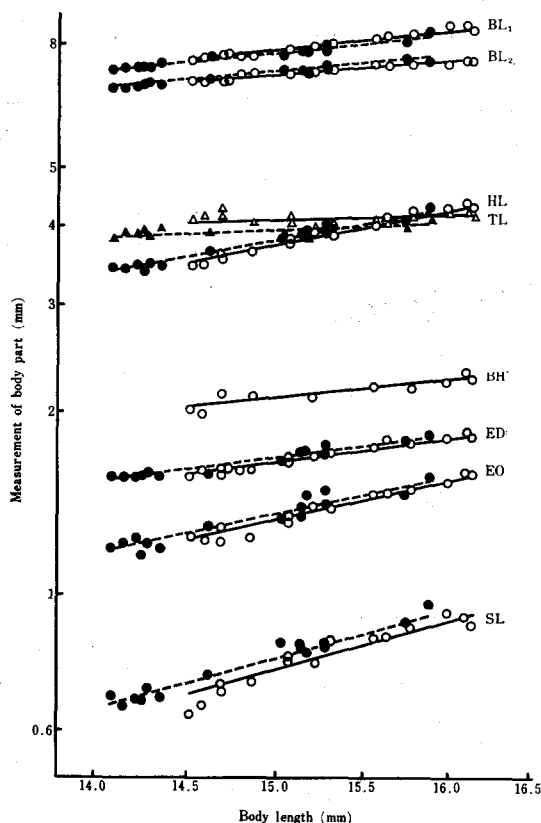


Figure 2. Relative growth of the body parts plotted against body length

Open circles, open triangles, full lines: experiment C

Closed circles, closed triangles, dotted lines: experiment D

Changes in body form

Relative growth measurements are used for interpretation of changes in body form during the course of the larval development.

Logarithmic values of these measurements are plotted in Figure 2 to show the relationship between various body parts and body length. In each regression a single liner relationship is readily apparent; then, the equilibrium constant k and the constant $\log b$ of the allometric equation, $\log y = \log b + k \log x$, where x is the body length and y is the length of body part, can be calculated by the method of least squares (Table 4). Plotting relative growth against body length, the equilibrium constant is 1.13 or 1.08 for the anteanal length, 1.97 or 1.79 for the head length, 2.85 or 2.80 for the snout length, 1.32 or 1.24 for the eye diameter and

Table 4. Equilibrium constant k and another constant $\log b$ in the allometric equation, and comparison of k and the adjusted mean value between experiments

Relation	Experiment	k	F-test	$\log b$	Adjusted mean (μ)	F-test
BL ₁ -BL	C	1.130* (1.06~1.20)	$\left. \begin{array}{l} F = 0.80 \\ df = 1,155 \\ P > 0.20 \end{array} \right\}$	- 0.823	7896	$\left. \begin{array}{l} F = 25.92 \\ df = 1,156 \\ P < 0.001 \end{array} \right\}$
	D	1.083* (1.01~1.16)		- 0.633	7800	
BL ₂ -BL	C	0.863*** (0.79~0.94)	$\left. \begin{array}{l} F = 0.43 \\ df = 1,155 \\ P > 0.20 \end{array} \right\}$	0.250	7140	$\left. \begin{array}{l} F = 29.38 \\ df = 1,156 \\ P < 0.001 \end{array} \right\}$
	D	0.900*** (0.82~0.98)		0.101	7235	
TL - BL	C	0.341*** (0.14~0.55)	$\left. \begin{array}{l} F = 0.28 \\ df = 1,144 \\ P > 0.20 \end{array} \right\}$	2.188	4116	$\left. \begin{array}{l} F = 27.22 \\ df = 1,145 \\ P < 0.001 \end{array} \right\}$
	D	0.422*** (0.20~0.65)		1.836	3969	
HL - BL	C	1.967* (1.77~2.16)	$\left. \begin{array}{l} F = 1.63 \\ df = 1,144 \\ P > 0.20 \end{array} \right\}$	- 4.642	3784	$\left. \begin{array}{l} F = 4.50 \\ df = 1,145 \\ 0.01 < P < 0.05 \end{array} \right\}$
	D	1.787* (1.58~1.99)		- 3.884	3836	
SL - BL	C	2.847* (2.25~3.45)	$\left. \begin{array}{l} F = 0.02 \\ df = 1,144 \\ P > 0.20 \end{array} \right\}$	- 9.012	766	$\left. \begin{array}{l} F = 4.95 \\ df = 1,145 \\ 0.01 < P < 0.05 \end{array} \right\}$
	D	2.798* (2.33~3.27)		- 8.787	797	
ED - BL	C	1.321* (1.18~1.46)	$\left. \begin{array}{l} F = 0.62 \\ df = 1,155 \\ P > 0.20 \end{array} \right\}$	- 2.299	1663	$\left. \begin{array}{l} F = 9.60 \\ df = 1,156 \\ 0.001 < P < 0.01 \end{array} \right\}$
	D	1.243* (1.11~1.38)		- 1.967	1685	
EO - BL	C	2.293* (1.99~2.59)	$\left. \begin{array}{l} F = 0.88 \\ df = 1,143 \\ P > 0.20 \end{array} \right\}$	- 6.451	1346	$\left. \begin{array}{l} F = 0.67 \\ df = 1,144 \\ P > 0.20 \end{array} \right\}$
	D	2.085* (1.76~2.41)		- 5.580	1356	
BH - BL	C	1.066** (0.79~1.34)	$n = 41$	- 1.126		

Figures in brackets represent 95% confidence interval of k .

* : tachyauexis, ** : isauexis, *** : bradyauexis

2.29 or 2.09 for the distance from the posterior margin of the eye to the end of the opercle; they all demonstrate tachyauexesis. However, this constant is 0.86 or 0.90 for the tail length and 0.34 or 0.42 for the trunk length, i.e. these parts show bradyauexesis. Body height gives an equilibrium constant of 1.07 and is approximately isauexesis. Growth of the head and parts in the head tend to be more rapidly than that for other parts of the body; the center of growth in the allometry along the horizontal body axis seems to be in the head region.

Comparing samples from experiments C and D, the equilibrium constants for every body part are not significantly different, but the adjusted means of the relative growth lines for each body part are significantly different; only exception is for the distance from the posterior margin of the eye to the end of the opercle (Table 4). This experiment, in which the larvae from both experiments C and D were kept at the same constant water temperatures, the author's previous report¹⁾ on the rearing of greenling at different water temperatures, and the contribution made by Martin (1946)²⁾ all suggest that the equilibrium constant of relative growth is modified by environmental conditions.

The size of various parts of the body at given body lengths was computed from the allometric equation; the percentages of body length are shown in Table 5. As an illustration for experiment C, at a body length of 14.7 mm (the average body

Table 5. Size of the body parts computed from the allometric equation at given body lengths

Relation	Experiment	Body length (mm)			
		14.3	14.7	15.5	16.5
BL ₁ - BL	C		7.69 (52.3)	8.17 (52.7)	8.76 (53.1)
	D	7.38 (51.6)	7.61 (51.8)	8.06 (52.0)	
BL ₂ - BL	C		7.01 (47.7)	7.33 (47.3)	7.74 (46.9)
	D	6.92 (48.4)	7.09 (48.2)	7.44 (48.0)	
TL - BL	C		4.08 (27.8)	4.16 (26.8)	4.25 (25.8)
	D	3.89 (27.2)	3.94 (26.8)	4.02 (25.9)	
HL - BL	C		3.60 (24.5)	4.00 (25.8)	4.52 (27.4)
	D	3.49 (24.4)	3.67 (24.9)	4.03 (26.0)	
SL - BL	C		0.72 (4.9)	0.83 (5.4)	0.99 (6.0)
	D	0.69 (4.8)	0.74 (5.1)	0.86 (5.6)	
ED - BL	C		1.61 (11.0)	1.73 (11.2)	1.88 (11.4)
	D	1.58 (11.1)	1.64 (11.1)	1.75 (11.3)	
EO - BL	C		1.27 (8.7)	1.44 (9.3)	1.66 (10.0)
	D	1.22 (8.5)	1.29 (8.8)	1.44 (9.3)	
BH - BL	C		2.07 (14.1)	2.19 (14.1)	2.34 (14.2)

Figures in brackets represent percentage of the size of various body parts to the body length.

length of samples from experiment C at hatching), the anteanal length is 7.69 mm (52.3 percent of the body length), the tail length 7.01 mm (47.7 percent), the trunk length 4.08 mm (27.8 percent), the head length 3.60 mm (24.5 percent), the snout length 0.72 mm (4.9 percent), the eye diameter 1.61mm (11.0 percent), the distance from the posterior margin of the eye to the end of the opercle 1.27 mm (8.7 percent) and the body height 2.07 mm (14.1 percent). In the five body parts which exhibit tachyauaxis, viz, the anteanal length, the head length and so forth, these percentages gradually increase with growth of the larvae. On the contrary, the percentages for both tail and trunk length decrease with an increase in body length owing to bradyauaxis. The body height demonstrates isauaxis, consequently its percentage remains almost equal during the growth of the body from 14.7 to 16.5 mm.

Survival of eggs and larvae

Survival curves for eggs from the different experiments are given in Figure 3. The important feature to be noted in these curves is the similarity between experiments: an increase in mortality during the gastrula stage and at hatching and a marked decrease in mortality from closure of the blastopore to the time just prior to hatching. Therefore, under laboratory conditions the eggs were most susceptible to environmental factors during the early and the late embryonic periods. However, mortality rates at the critical stages differ between experiments and the percentage of hatch differs decidedly from experiment to experiment. The percentage of deformed larvae at hatching was 3.7 for experiment A, 1.3 for experiment B, and 0 for experiments C and D. It is probable that the differences in the percentage of hatch and in the percent deformed larvae among the experiments cannot be attributed to water temperature, with a maximum average difference of only about 1.5°C (Table 1), but to other factors such as the maturity of eggs, aggregation, etc.

Survival curves for the larvae are shown in Figure 4. These curves generally show an accelerated increase in mortality rate at and after the end of the yolk-sac stage. The mortality rate in experiment D began to increase about the 10th day after hatching. Then, the majority of larvae with no food starved to death by the 16th day, and those larvae that were fed did not live for more than 24 days after hatching, although they survived beyond the yolk-sac stage. The survival of larvae from experiment C that were fed was relatively high compared with that from experiment D, and a few larvae were still alive 29 days after hatching even though there was a continuous, high mortality. The larvae in both experiments C and D were reared at almost identical water temperatures, fed the same food, and cultured by use of the same techniques; therefore, the phase differences in the survival curves between experiments for larvae that were fed is attributed to the

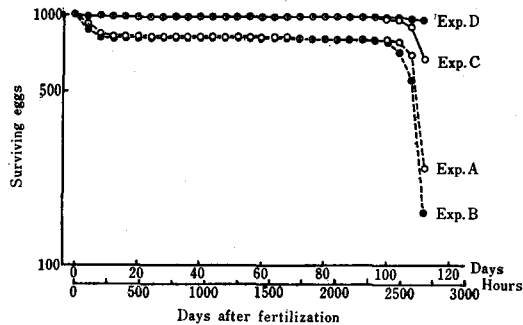


Figure 3. Survival curves during the incubation period for each experiment, expressed as the original number is 1000

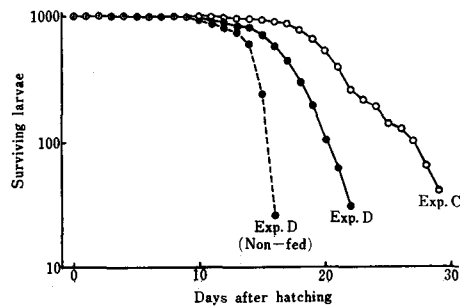


Figure 4. Survival curves for the larvae, expressed as the original number is 1000

size of larvae at hatching and essentially to the size of the eggs; this was pointed out by Blaxter (1963)³⁾ in his rearing experiments of herring larvae.

The dead larvae contained little or no food in their stomachs even though there ample supply of *Artemia salina* was available to them. This fact suggests that *Artemia salina* was not a suitable food for the larvae of this species.

Summary

Hemimtripterus villosus (PALLAS) is a cottid fish commonly found in the coastal waters of Hokkaido. The spawning season extends from mid-October to early November on rocky sea bottom, where the water temperature is from 15° to 18°C.

The present paper describes the normal embryonic and larval development of this species, changes in its body form, and its survival, based upon rearing experiments.

On October 25 and 30, 1965 eggs and milt were obtained from ripe adults taken by gill nets in the coastal waters of Funka Bay, off Shikabe and artificial fertilization was carried out by the ordinary dry method.

The eggs are demersal and adhesive. They are spherical in shape and large

size, averaging 4.41–4.61 mm in diameter. They are relatively opaque because of the heavy corion, and with a yolk of light yellow or light reddish-orange color. A prominent characteristic is the large number of minute granular materials randomly distributed in the yolk. The yolk contains many oil globules, about 120–160 in number, which unite into a single globule during late embryonic development. There are no essential differences between the embryonic development of this species and those of other teleostean eggs.

The time required for hatching is about 100 days at a water temperature of 12.1°–13.5°C. The newly hatched larvae average 13.8–14.7 mm in body length and are well-developed. The larvae are able to swim actively immediately after hatching; they are thick and have 40–42 pairs of somites. The large mouth is functional with many larval teeth on the jaws and the vent is completely open. A most striking characteristic is a heavy pigmentation on the main body, the nape and in the body cavity. A larva, twenty-nine days after hatching, has an average body length of 16.11 mm. Compared with a newly hatched larva, few changes are evident except for the slightly heavier pigmentation, the disappearance of yolk material by absorption and a rapid differentiation of the fin-rays.

A method for determining relative growth has been used to interpret changes in body form in the larval stages. The equilibrium constant in the allometric equation varies from 0.34 to 2.85 for different body parts. The anteanal length, the head length, the snout length, the eye diameter and the distance from the posterior margin of the eye to the end of the opercle demonstrate tachyauexis; consequently, their percentages to body length increase with the growth of the larvae. The tail length and the trunk length exhibit bradyauexis; their percentages to body length decrease with an increase in body length. The body height demonstrates isauexis and its percentage to body length remains almost constant throughout the larval stage. In the growth gradient, the center of growth along the horizontal body axis seems to be in the head region.

In the embryonic period, an increased mortality during the gastrula stage and at hatching was apparent in the laboratory. The mortality rate in the larval stage increased rapidly at and after the end of the yolk-sac stage even though ample *Artemia salina* were available for food. The larvae were not carried long after absorption of the yolk.

References

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Explanation of Plate I

Figure 1. Mature unfertilized egg, surface view

The irregularly shaped granular materials are omitted for the sake of clarity in all other figures.

Figure 2. Protoplasmic germ disc, 8th hour after fertilization, lateral view

Figure 3. 2-cell stage, 11th hour, surface view

Figure 4. 16-cell stage, 21st hour, surface view

Figure 5. Morula stage, 38th hour, surface view

Figure 6. First gastrula stage, 80th hour, surface view

Figure 7. Germinal ring attains to an equatorial position, 140th hour, lateral view

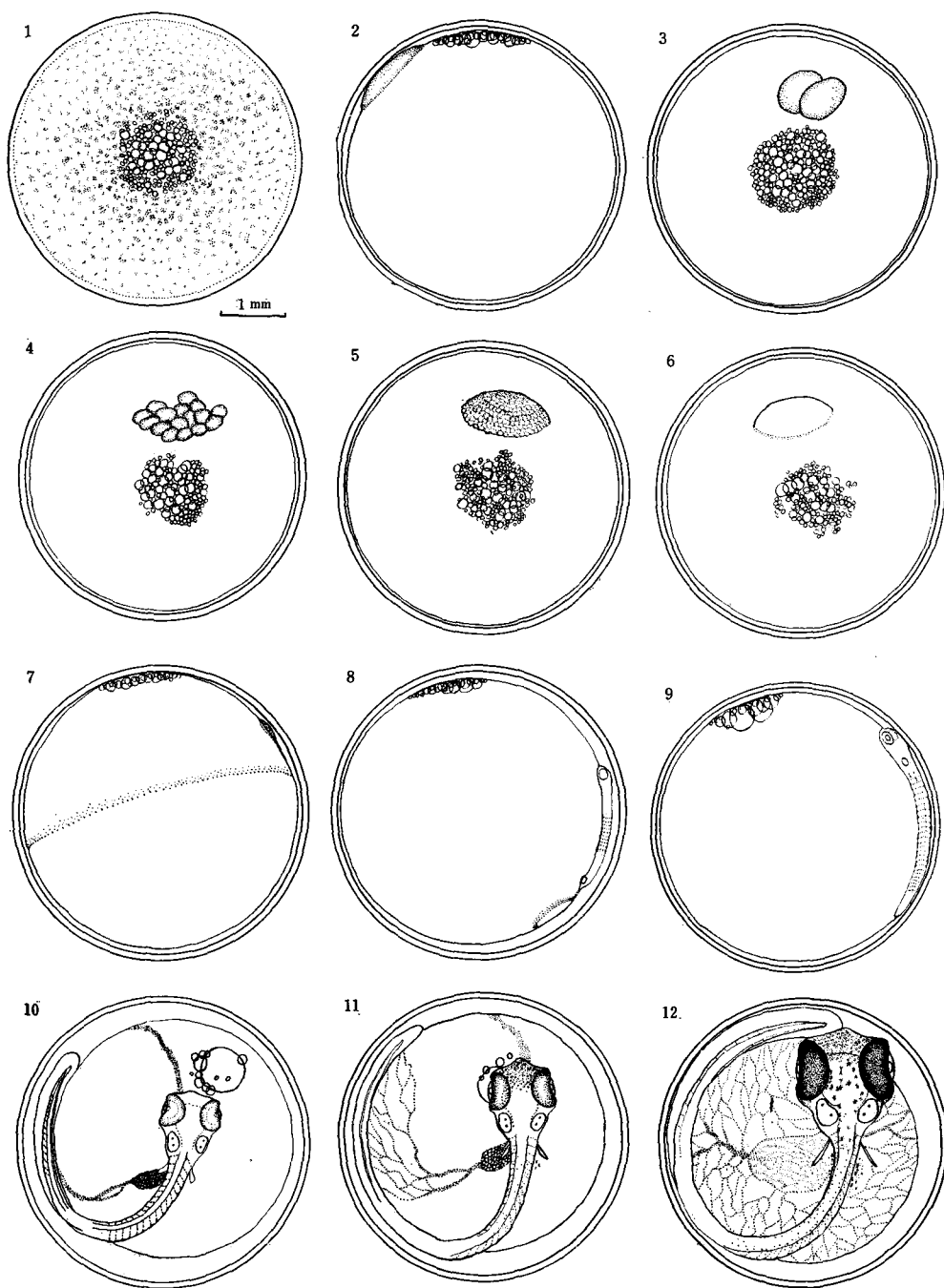
Figure 8. Egg shortly before closure of the blastopore, 190th hour, lateral view

Figure 9. Embryo approximately a quarter of circle, 240th hour, lateral view

Figure 10. Egg at 434th hour, a blood vessel appears around the yolk sac, surface view

Figure 11. Appearance of melanophores on the embryo and on the yolk sac, 604th hour, surface view

Figure 12. The tip of the tail reaches the snout encircling the yolk, 1133rd hour, surface view



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Explanation of Plate II

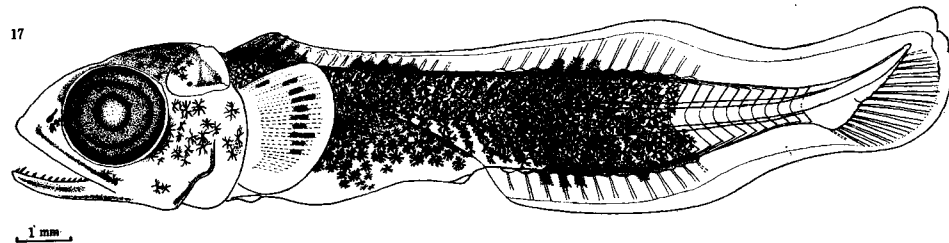
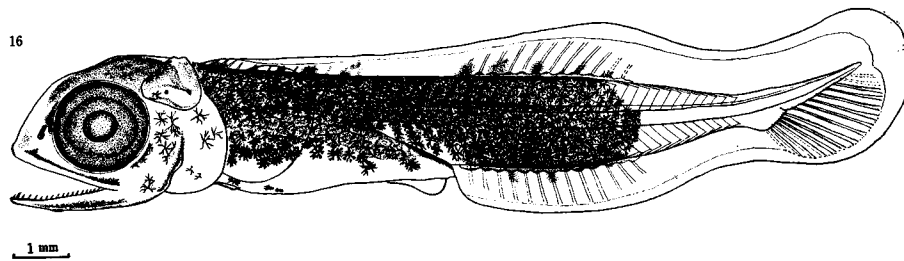
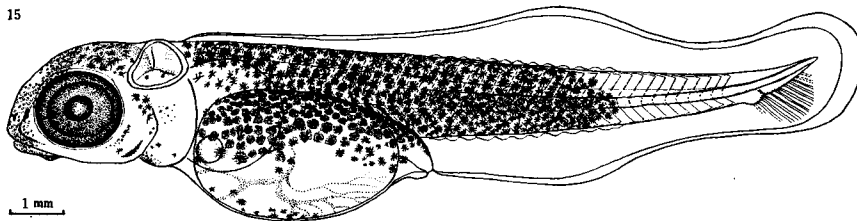
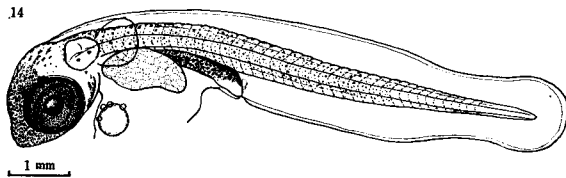
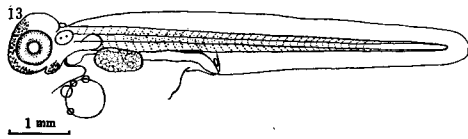
Figure 13. Embryo extracted out of the egg at the same stage as Fig. 11, lateral view
The yolk sac was removed when extracted.

Figure 14. Embryo extracted out of the egg at the same stage as Fig. 12, lateral view
The yolk sac was removed when extracted.

Figure 15. Larva just hatched, body length 14.78 mm

Figure 16. Larva twenty days old, body length 15.57 mm

Figure 17. Larva twenty-nine days old, body length 16.52 mm



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