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Development of Free Neuromasts in Larvae of Cyprinid Fish

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Synopsis

The distribution, morphology, and sensory cell polarity of free neuromasts in developing larvae of two cyprinid fish, pale chub, *Zacco platypus*, and Japanese dace, *Tribolodon hakonensis* were examined. Few free neuromasts were found in newly hatched larvae of pale chub, and no neuromasts were found in such larvae of Japanese dace. The prelarvae of both species remain in the spawning bed in the river and emerge from the bed at the postlarval stage. The neuromasts of both species developed rapidly in the prelarval stage, and the apical surface of neuromast organs were close to their mature form when the postlarval stage was reached. Accordingly, the prelarvae of both species seemed to prepare free neuromasts for survival after emergence from the spawning bed.

All neuromasts contained sensory hair cells of opposing polarity, which shows the orientation of maximum sensitivity. The polarity of hair cells coincided with the minor axis of the outline of the neuromast area and the direction in which the cupulae bent. In the trunk, the polarity of hair cells of most neuromasts was in the antero-posterior axis of the body, but a few had dorso-ventral polarity. In the head, the polarity of hair cells was arranged on lines tangential to concentric circles about the eyes.

Introduction

During ontogeny, fish ordinary lateral-line develops from free neuromasts on the larval body surface. There are many morphological studies on the development of free neuromasts[1-10], but few studies have closely examined changes in the prelarval stage[11-13]. This study describes neuromast morphology and the relationship between hair cell polarity and the outline of the neuromast area in early larvae of two species of cyprinid fish, pale chub, *Zacco platypus*, and Japanese dace, *Tribolodon hakonensis*, by scanning electron microscopy (SEM).

Larval behavior seems to have a close connection with the development of sensory organs[5,7]. Larvae of both species show marked changes in their behavior during growth. Adult fish lay their eggs in a spawning bed in a river, and after hatching, the prelarvae remain in the bed, emerging when they become postlarvae[14,15]. The morphological development of free neuromasts is discussed here in relation with the larval behavior of the two species.

Materials and Methods

Larvae of pale chub and Japanese dace were reared from artificially fertilized eggs obtained

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from mature wild fish. Mature pale chub were collected from the Tomio River in Nara Prefecture, and mature Japanese dace were collected from the Nakaoku River, a branch to the Yoshino River in Nara Prefecture. The larvae were given artificial feed and the water temperature was kept at 18-28°C for pale chub and 18-20°C for Japanese dace. For observations by SEM, the larvae were preserved in Karnovsky's fixative. Specimens were dehydrated in graded ethanol, critical-point dried using liquid CO_2, sputtered with gold, and examined under a JEOL JSM T-200 microscope.

The number and distribution of free neuromasts were examined in 31 pale chub and 28 Japanese dace larvae. Morphological changes on the apical surface of neuromast organs were measured, in the order from front to rear, in ten free trunk neuromasts with sensitivity in the antero-posterior orientation.

Results

Growth of larvae and distribution of free neuromasts

Pale chub

Newly hatched larvae were unpigmented on their entire body surface, and eyes were recognizable in outline only. Prelarvae one to two days after hatching moved on the bottom in the aquarium. The prelarvae assembled at pebbles, under which they put their heads. An air bladder was seen in three-day-old larvae. At four days after hatching, larvae began to swim horizontally above the bottom of the aquarium. At seven days of age, the larvae had completely resorbed the yolk, attaining the postlarval stage.

The changes in the distribution and number of neuromasts during larval development are shown in Figure 1 and Table 1, respectively. Newly hatched larvae of pale chub had few free neuromasts, but the number of neuromasts increased rapidly on both the head and trunk during prelarval growth. At three days, 63 free neuromasts were recognized in one side of a larva, and the number continued to increase during postlarval growth. On the head, the neuromasts were located around the eyes, between the nostrils, in the opercular and occipital regions, and on the lower jaw. The distribution of the neuromasts on the head was almost symmetrical (Plate I A).

Table 1. Number of free neuromasts on one side of larval fish.

Days after hatching	Pale chub (*Z. platypus*)		Japanese dace (*T. hakonensis*)	
	Head	Trunk	Head	Trunk
0	1	5	0	0
1	14	23	2	11
3	34	29(3)*	11	42(1)
7	40	32(5)	37	55(3)
11			44	63(6)
15	49	44(6)	55	67(7)
29			66	73(8)
35	79	61(11)		

* The number of trunk neuromasts includes the numbers in parentheses, showing the numbers of trunk neuromasts on the dorsal surface.

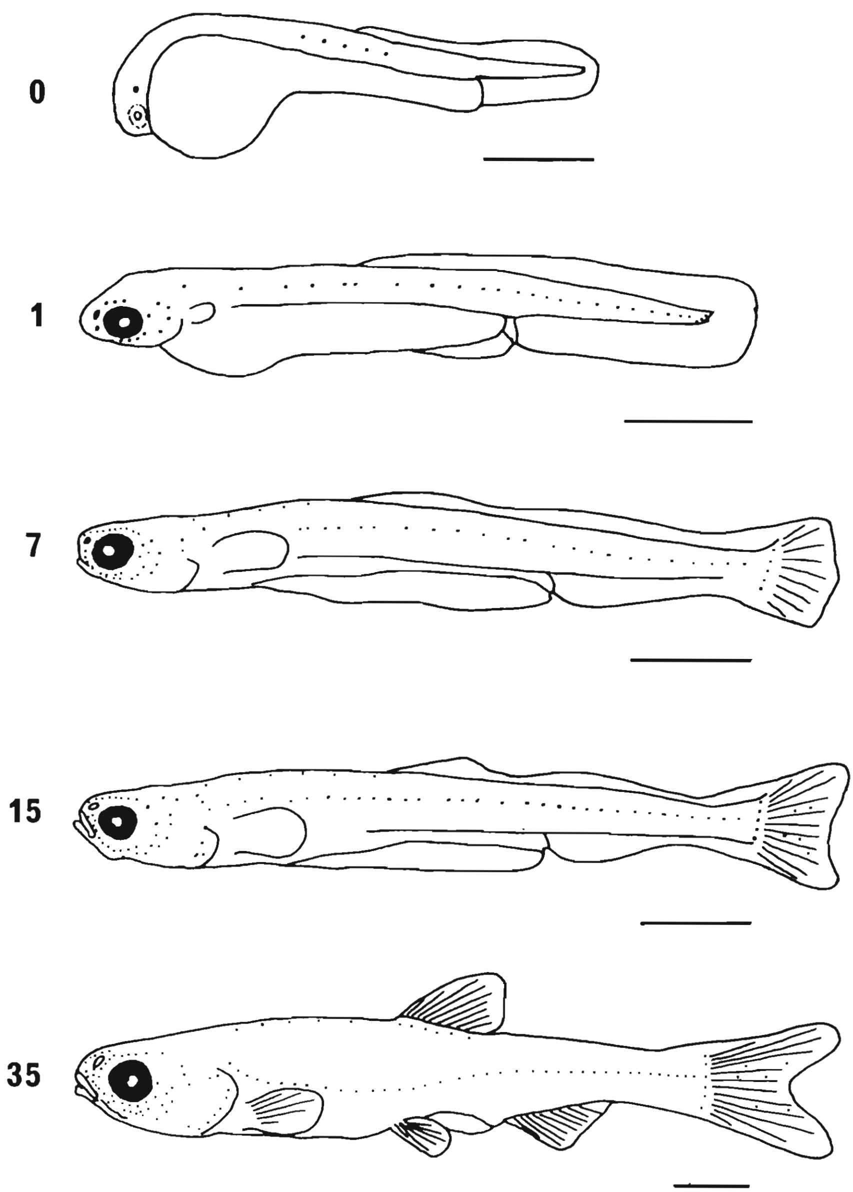

Fig. 1. Distribution of free neuromasts (black spots) at different stages of larval growth of pale chub, *Z. platypus*. Numerals show the day after hatching. Scale bars, 1 mm.

On the trunk, the neuromasts were arrayed along the middle of the flank, except that neuromasts were few dorsal to the pectoral fin until larvae were seven days old (Plate I B). The trunk neuromasts were distributed asymmetrically on the right and left side of larvae inspected from above by phase-contrast microscopy. Neuromasts also appeared on the dorsum of the trunk and on the caudal fin (Plate I C). At 35 days, there was one neuromast per segment along the middle

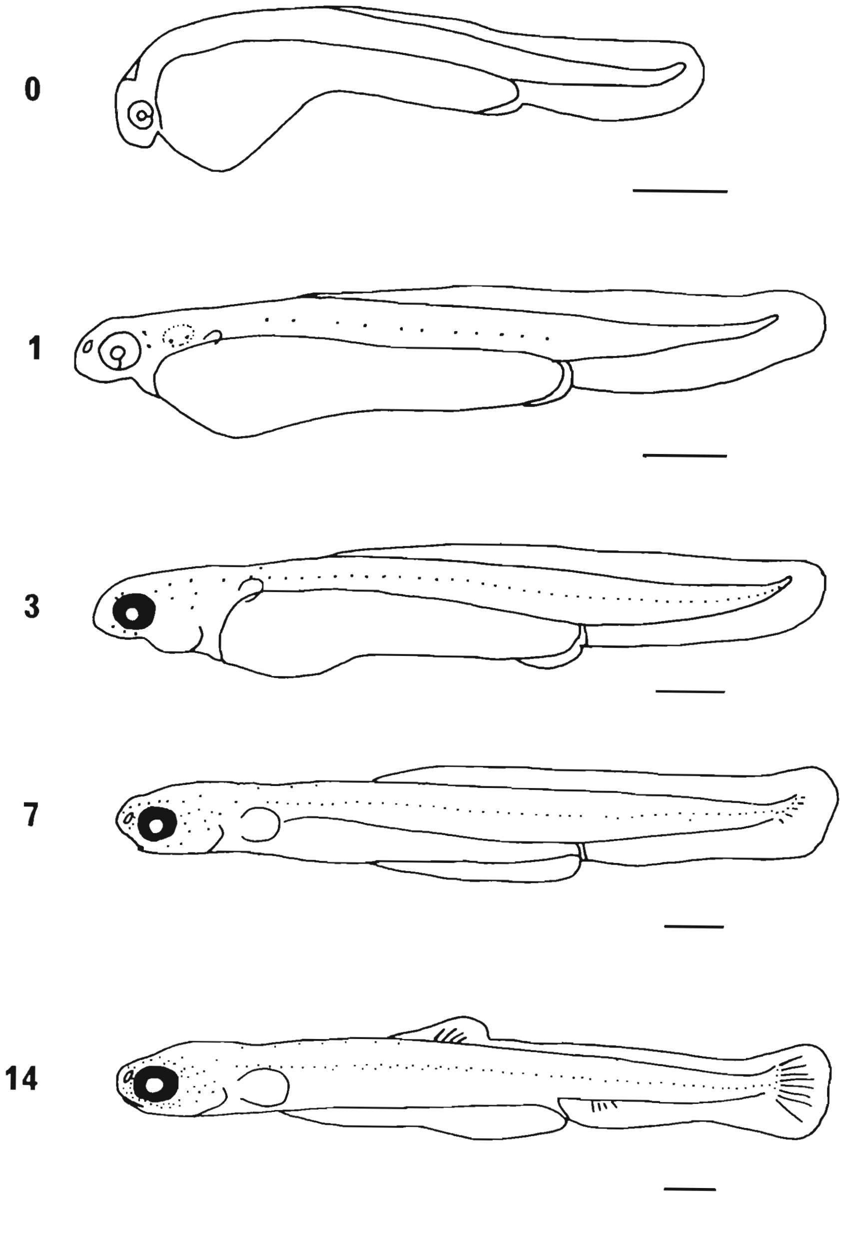

Fig. 2. Distribution of free neuromasts (black spots) at different stages of larval growth of Japanese dace, *T. hakonensis*. Numerals show the day after hatching. Scale bars, 1 mm.

of the trunk ; there were almost the same number of neuromasts as there are lateral scales in adult fish.

Japanese dace

Newly hatched larvae of Japanese dace showed poor general development. Pigment was not observed on the body surface, and the eyes were recognizable in outline only. The prelarval behavior in the aquarium was similar to that of pale chub. The prelarvae assembled at pebbles, under which they put their heads. At seven days after hatching, the air bladder had differentiated, and the larvae swam horizontally above from the bottom of the aquarium. The larvae reached the postlarval stage at seven to eight days after hatching.

Newly hatched larvae of Japanese dace had no free neuromasts on either the head or the trunk, but many neuromasts appeared rapidly during prelarval growth (Fig. 2, Table 1). The regions of distribution of free neuromasts were almost the same as those of pale chub, but the number of neuromasts on the trunk was 50% more than that of pale chub. At three days of ages, neuromasts on the trunk were already distributed with one per segment along the middle of the trunk. Neuromasts appeared on the dorsum of the trunk and on the caudal fin also. At 29 days, there were almost the same number of trunk neuromasts as lateral scales in the adult fish.

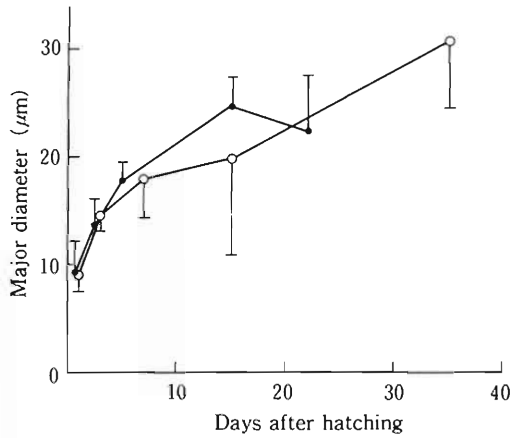

Fig. 3. Changes in the major diameter of the neuromast area (the apical surface of the free neuromasts). Each value is the mean±SD of ten free neuromasts with antero-posterior orientation on the trunk. Open circles, pale chub ; solid circles, Japanese dace.

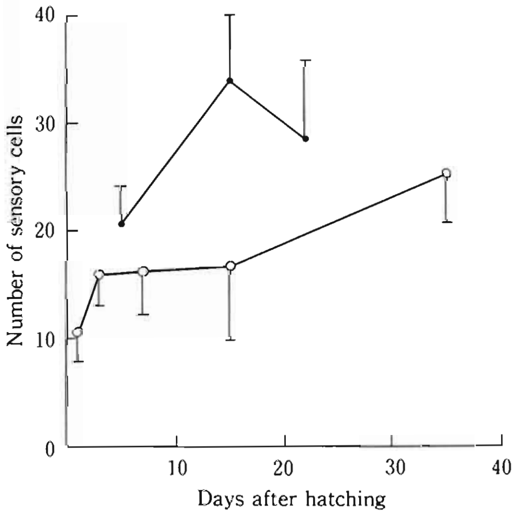

Fig. 4. Changes in the number of sensory cells per free neuromast with larval growth. Each value is the mean±SD of ten free neuromasts on the trunk. Open circles, pale chub ; solid circles, Japanese dace.

Development of free neuromasts

Neuromasts appeared as a small round shape with a few hair cells in both species (Plate II A, B). The major diameter of the neuromast area, defined as the apical surface of a free neuromast, increased rapidly at the prelarval stage but increased slowly at the postlarval stage (Fig. 3). The number of sensory hair cells increased rapidly in the prelarval stage in pale chub (Fig. 4). The neuromasts in five-day-old larvae of Japanese dace had more than 20 hair cells. In both species, the number of hair cells increased during postlarval growth (Fig. 4).

The neuromast area changed from a round to a lozenge shape (Plate II C, D). The changes in the shape were calculated as the degree of narrowness in the outline of neuromast area (Fig. 5), calculated as the ratio of the length (L) to the width (W) of the neuromast area. The ratio increased in the prelarval stage and was almost unchanged in the postlarval stage. The ratio reflected the development of a cupular shape[12]. Fully developed cupulae were found on seven-day-old larvae of both species (Plate III, A, B).

Each sensory cell of a neuromast had one kinocilium and many stereocilia (Plate II C, D). The length of sensory hairs in pale chub seven days old was 6 to 9 μm for kinocilia and about 1.5 μm for long stereocilia (measured by SEM). In Japanese dace five days old, these length were 8 to 10 μm and about 2 μm, respectively.

The number of free neuromasts increased during larval growth (Table 1) ; newly added neuromasts were small and round and looked like those shown in Plate II A.

Hair cell polarity

The orientation of the kinocilium in relation to the stereocilia indicates the polarity of the hair cell, since the hair cell is excited only if the hair bundle is displaced in the direction from the stereocilia towards the kinocilium[4,16,17]. Every neuromast of both species contained a mixture of hair cells of opposing polarity in approximately equal numbers (Plate IV). The orientation of neuromast cell polarity coincided with the minor axis of the neuromast area and the direction in which the blade-shaped cupulae bent (Plate III). The orientation of neuromast polarities of both species showed a similar distribution with more variability on the head than on the trunk (Figs. 6, 7). The orientation of head neuromasts formed lines tangential to concentric circles about the eye. On the lower jaw, the orientation was from side to side, perpendicular to the fish axis. On the trunk of both species, all neuromasts that appeared initially had an antero-posterior orientation. Neuromasts with a dorso-ventral orientation on the trunk were first found in three-day-old larvae of pale chub and in seven-day-old larvae of Japanese dace. On the trunk, the neuromasts with an antero-posterior orientation were predominant in both species. On the caudal fin, all neuromasts had an antero-posterior orientation. On the dorsum of the trunk, the neuromasts from the occipital to the dorsal fin were arranged from side to side, perpendicular to the fish axis, and the ones at the base of the dorsal fin had an antero-posterior orientation.

Discussion

Free neuromasts were hardly observed in newly hatched larvae of both species, but many neuromasts which appeared after hatching grew rapidly during the prelarval stage. Thereafter, the major diameter of the neuromast area and the number of sensory cells in a neuromast continued to increase gradually in the postlarval stage, but the ratio (L/W) of neuromast area already reached a constant at the beginning of the postlarval stage in seven days after hatching in both species. The change in the ratio reflected the development of cupular shape[12], and fully developed cupulae appeared on many free neuromasts in both species at the beginning of the postlarval stage. Therefore, many free neuromasts of both species seemed to be almost complete-

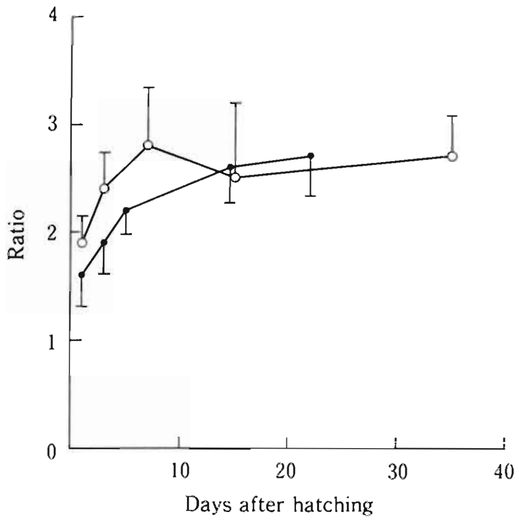

Fig. 5. Changes in degree of narrowness in neuromast area during larval growth. The ratio is calculated as the proportion of longitudinal length to the width in the outline of neuromast area. Each value indicates the mean ± SD of ten free neuromasts on the trunk. Open circles, pale chub; solid circles, Japanese dace.

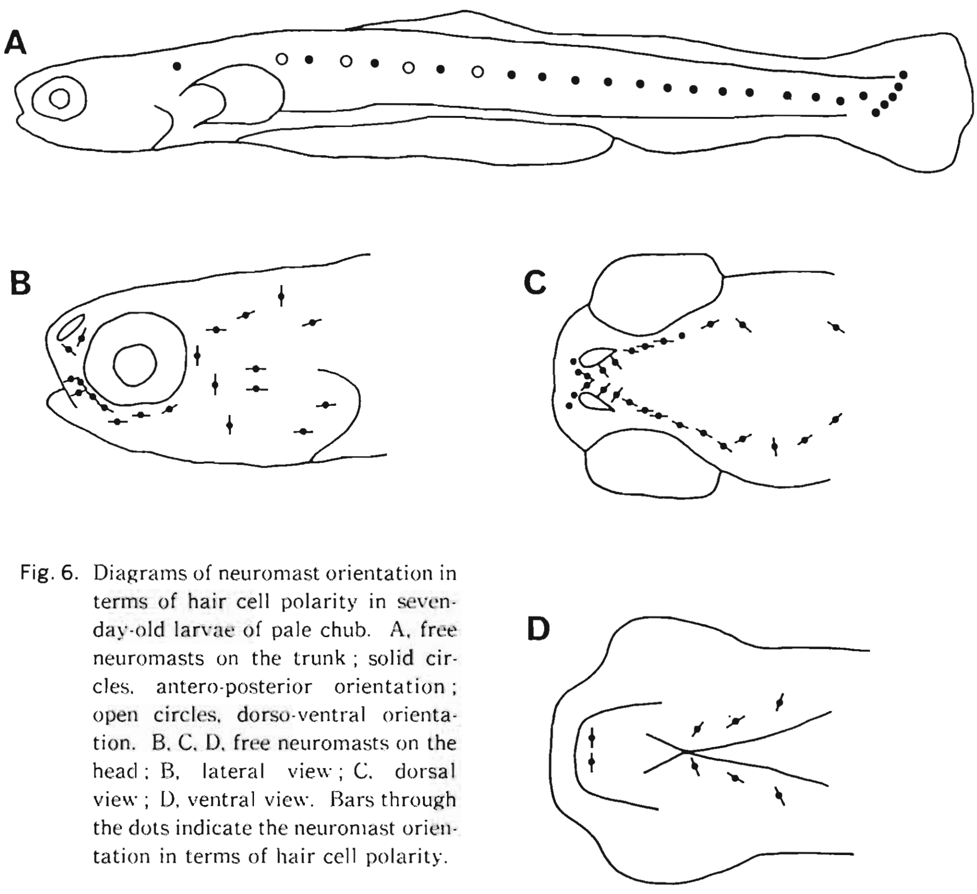

Fig. 6. Diagrams of neuromast orientation in terms of hair cell polarity in seven-day-old larvae of pale chub. A, free neuromasts on the trunk; solid circles, antero-posterior orientation; open circles, dorso-ventral orientation. B, C, D, free neuromasts on the head; B, lateral view; C, dorsal view; D, ventral view. Bars through the dots indicate the neuromast orientation in terms of hair cell polarity.

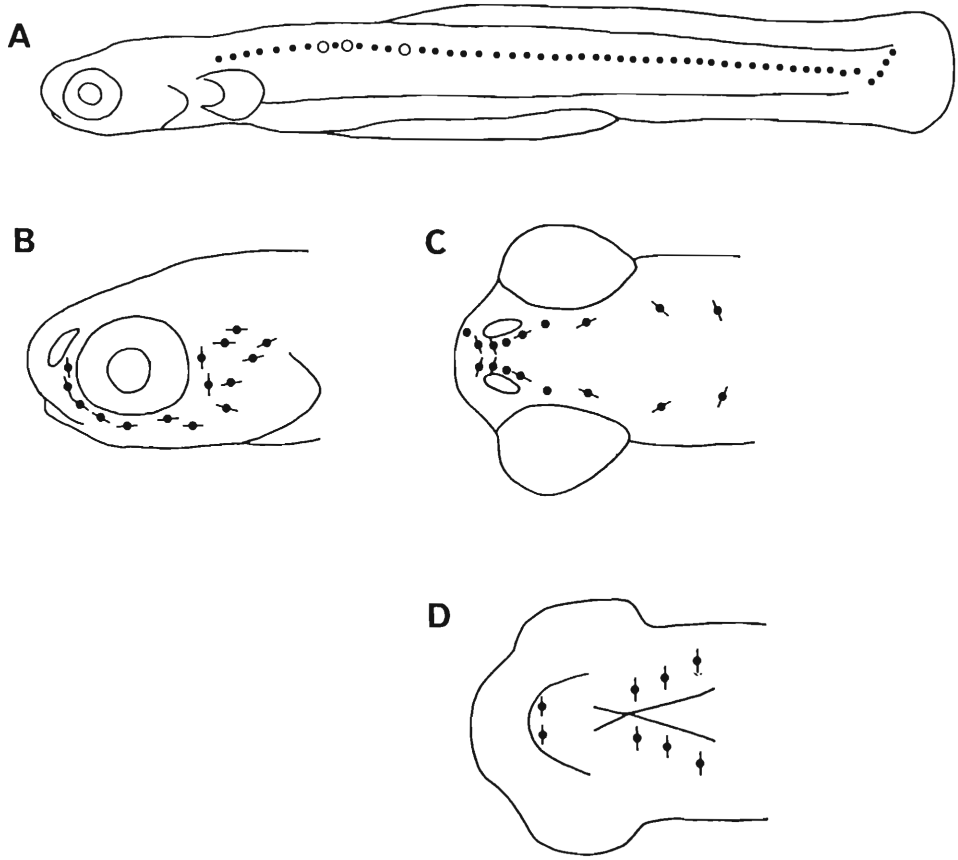

Fig. 7. Diagram of the distribution of neuromast orientation in terms of hair cell polarity in seven-day-old larvae of Japanese dace. For symbols and letters, see the legend of Fig. 6.

ly developed at the start of the postlarval stage. Free neuromasts are important mechanosensory organ that have sensitivity to water flow. Both species studied here inhabit rivers, where larvae must seek weak water flow. Therefore, the rapid development of free neuromast of larvae inside of the spawning bed is needed for rheotaxis in the river. In contrast, larvae of *Gnathopogon elongatus caerulescens* and *Plecoglossus altivelis* are active starting in the prelarval stage, and even newly hatched larvae have well-developed neuromasts[10,18-20]. The timing of ontogenetic development of free neuromasts and the acquisition of behavior appear to be part of each species' adaptation for survival.

The cupulae of free neuromasts are easily broken by mechanical contact. Considering the time when free neuromasts develop, it is evident that cupulae of both species would develop in the spawning bed. Therefore, there is a question whether the cupulae are uninjured by touching sand or pebbles in the bed. The cupulae of pale chub regenerate after being shed[21] ; even if the cupulae of both species are broken in the spawning bed, the cupulae will regenerate completely within a day.

Free neuromasts with dorso-ventral polarity on the trunk have been found in juvenile cichlids also, and the neuromasts appeared in an orthogonal orientation to presumptive canal neuromasts with antero-posterior polarity[9]. Free neuromasts with a dorso-ventral orientation on the trunk have been found in early stages of fish ontogeny in some species such as herring[4] and anchovy[22]. The role of dorso-ventral neuromasts is unknown, but neuromasts of this type appeared when the larvae came out from the spawning bed. When the larvae swim horizontally above from the bottom, they may need to sense vertical stimuli to fish axis, in addition to horizontal stimuli.

The predominant distribution of trunk neuromasts with antero-posterior orientation was similar to finding for larvae of herring[4] and anchovy[22]. Trunk neuromasts would be expected whether their functions to measure the fish's motion or to aid in selection of weak water flow in the river. The complex orientation of free neuromasts on the head may be associated with shoaling or feeding.

In pale chub, few free neuromasts were found in the portion dorsal to the pectoral fin until the age of seven days. Dijkgraaf[23] pointed out as examples of adaptation that the anterior part of trunk lateral line in adult fish is displaced towards the dorsal side, to avoid the area of disturbance caused by the pectoral fin. It seems that development of free neuromasts on the anterior part of the trunk may be related to the growth of the pectoral fin in pale chub.

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オイカワおよびウグイ仔魚の成長に伴う遊離感丘の形態変化

向井幸則・小林　博

摘　　　　要

オイカワおよびウグイ仔魚は河川における産卵床内でふ化，発育し，後期仔魚期が始まる頃に産卵床から浮上する。本研究は両仔魚の遊離感丘の形態変化を，走査電子顕微鏡を用いて調べた。遊離感丘はふ化直後のオイカワにはわずかに認められ，ウグイには全くなかった。しかし，両種ともに前期仔魚期に急速に増加した。また，遊離感丘の遊離面の形態およびクプラもこの時期に顕著に発達し，後期仔魚期が始まる段階で多くの遊離感丘はほぼ完成していた。この急速な発達は，両仔魚が河川に生息するための適応と考えられた。一方，遊離感丘の刺激受容方向は感覚細胞の極性から求められ，それは遊離面の輪郭の短軸の方向，つまりクプラがたわみ易い方向と一致していることが判明した。刺激受容方向からみると，体側では体軸の前後からの刺激を受容する遊離感丘が多く，背腹方向のものはわずかであった。頭部では，多くの遊離感丘の刺激受容方向は，眼を中心とした同心円の接線方向にあった。

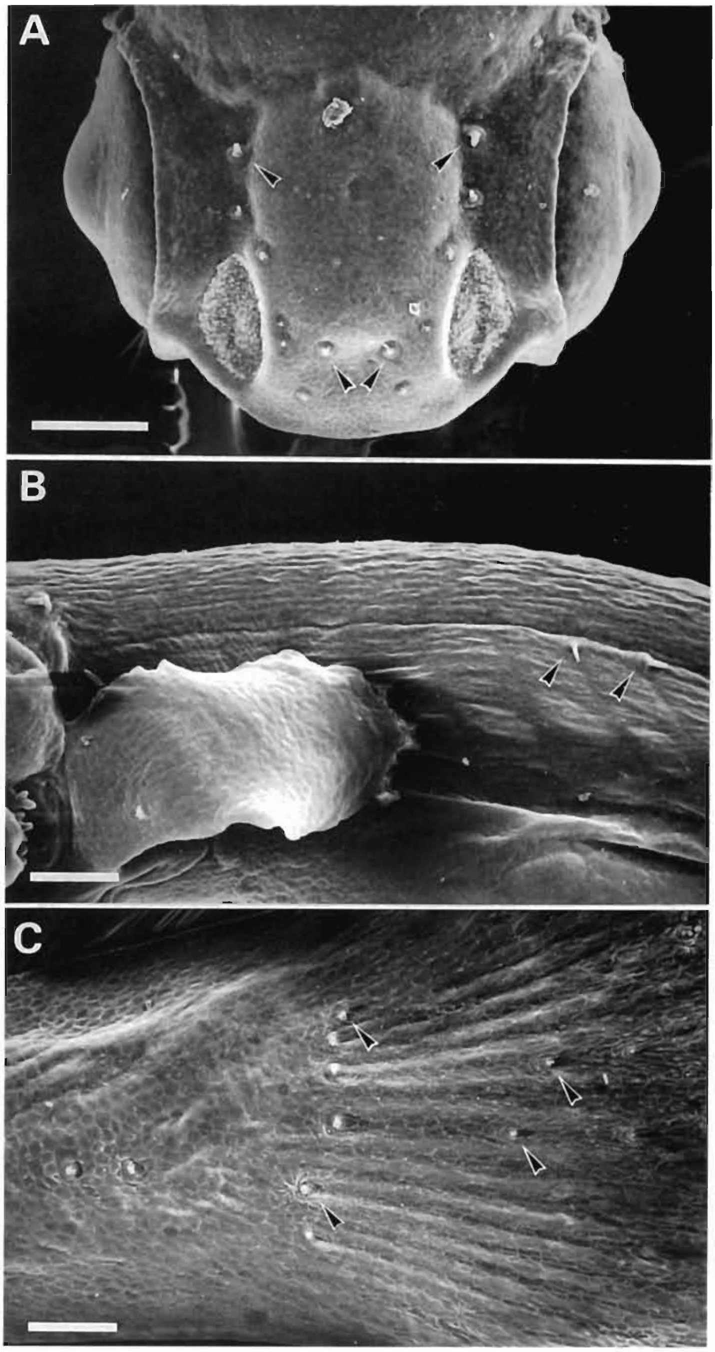

Plate I. Scanning electron micrographs showing free neuromasts of pale chub. A, Dorsal view of the head of a three-day-old larva. B, Trunk of a seven-day-old larva. The free neuromasts above the pectoral fin cannot be seen in this micrograph. C, Caudal fin of a 15-day-old larva. Arrow heads indicate free neuromasts. Scale bars, 100 μm.

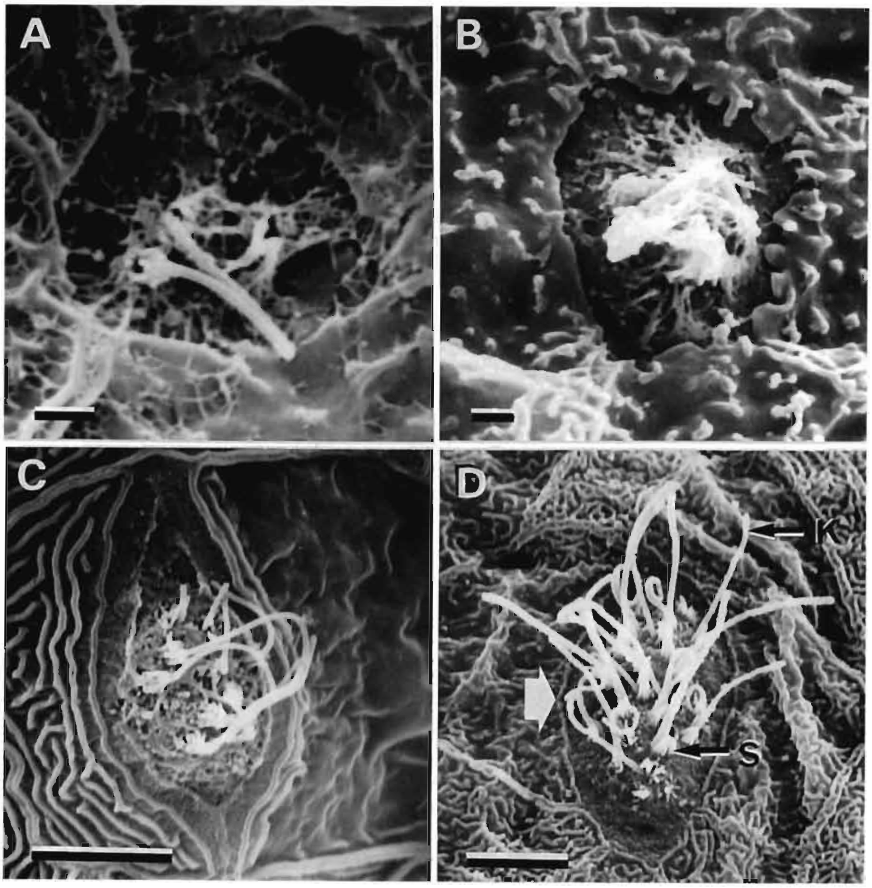

Plate II. Scanning electron micrographs showing development of free neuromasts. A, neuromast of a newly hatched larva of pale chub. B, neuromast of one-day-old larva in Japanese dace. C, neuromast of seven-day-old larva in pale chub. D, neuromast of five-day-old larvae in Japanese dace. Thick white arrow, outer edge of the neuromast area ; K, kinocilium ; S, stereocilia. Scale bars are 1 μm in A and B, and 5 μm in C and D.

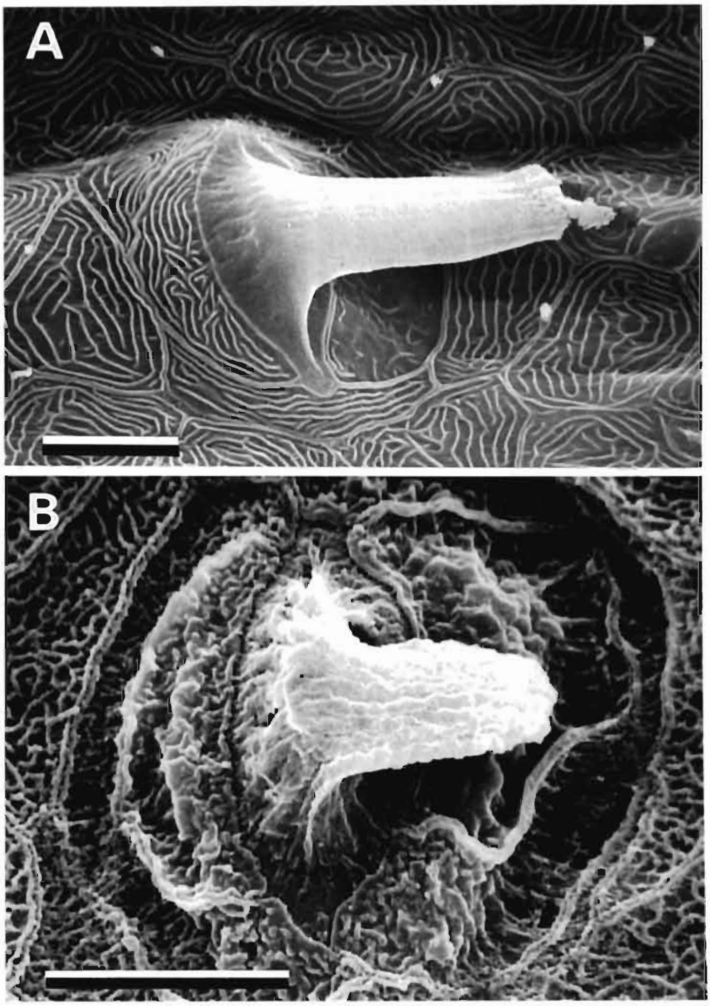

Plate III. Scanning electron micrographs showing fully developed cupulae of free neuromasts. A. Cupula of seven day-old larva in pale chub. B. Cupula of seven-day-old larva in Japanese dace. Scale bars, 10 μm.

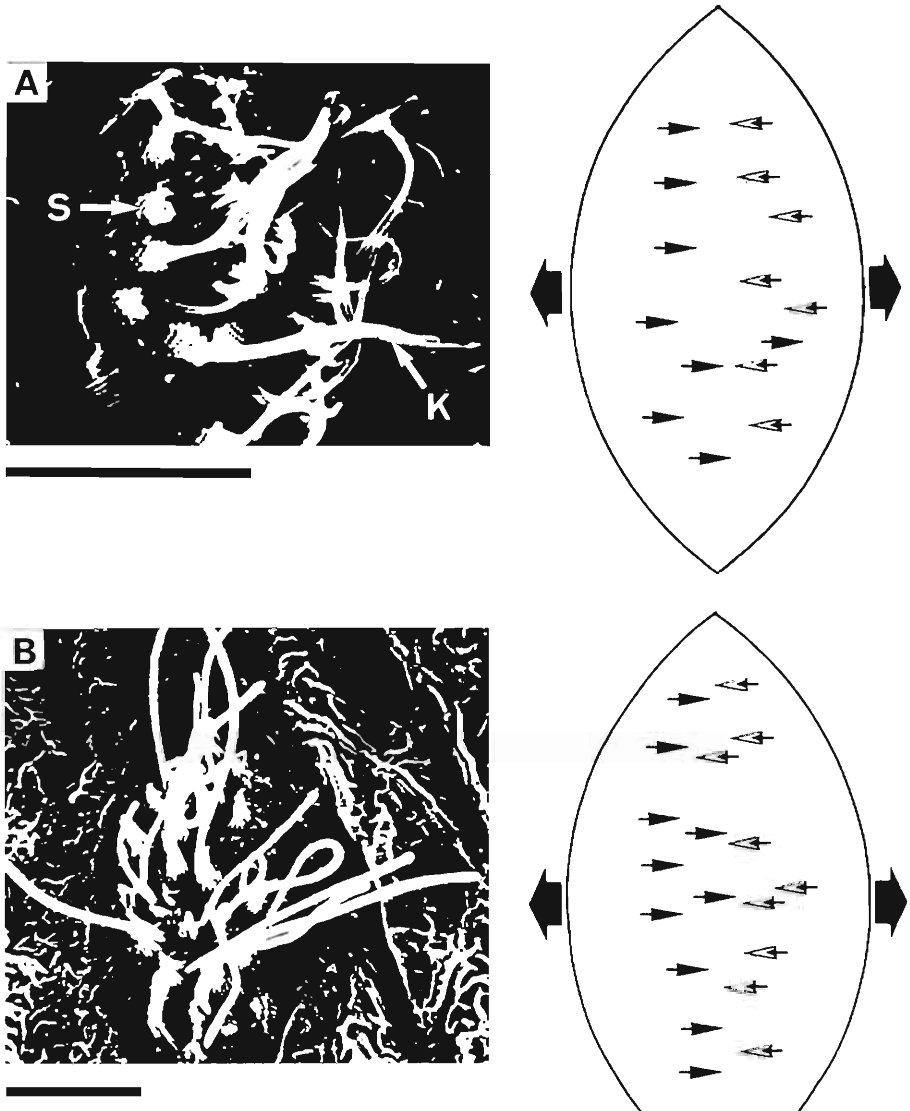

Plate IV. Scanning electron micrographs and diagrams showing hair cell polarity. A. free neuromast on the trunk of three-day-old larva in pale chub. B. Free neuromast on the trunk of five-day-old larva in Japanese dace. K. kinocilium ; S. stereocilia. Thin arrows in diagrams indicate the polarities of hair cells. Thick black arrows indicate the orientation of physiological sensitivity of the neuromasts. Scale bars, 5 μm.