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Occurrence and Formation of Vertebral Anomalies in the Cyprinid Fish, *Zacco platypus*

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Abstract In order to clarify a developmental mechanism of vertebral anomalies, the incidence and the morphology of abnormal vertebrae in juvenile cyprinid fish, *Zacco platypus*, both wild-caught and laboratory-reared specimens were examined. The frquencies of vertebral anomalies in juvenile fish (8.0～33.0 mm in standard length (SL)) collected from the Ibi River were higher than those from either the Yahagi River or the Toki River. In juvenile fish taken from the Ibi River, the frequency of vertebrae joined by abnormal calcium deposits was about 7 times as great as that of helical sutures of centra and about 14 times as great as that of shortened vertebrae. Out of the 4,564 fish (8.0～19.0 mm SL) taken from the Ibi River, 273 (5.98%) specimens had abnormal calcium deposits stained with alizarin red S alongside of vertebral column. In 273 fish having abnormal calcium deposits, 171 (62.6%) specimens showed abnormal calcium deposits extending from one centrum to the next one, and 13 (4.8%) specimens showed them extending to the two adjoining centra. In these defective vertebrae, the separate centra could be distinguished. And the other fish (32.6%) showed the abnormal calcium deposit attaching to a single centrum. The vertebrae joined by abnormal calcium deposits were seen only in fish ranging from 8.0～19.0 mm SL; not a single instance of this anomaly has been found in fish ranging from 19.0～25.0 mm SL. The frequencies and the incidence of the fused vertebrae throughout the vertebral column in fish ranging from 19.0～25.0 mm SL were similar to those of vertebrae joined by abnormal calcium deposits in fish ranging from 8.0～19.0 mm SL. These results may suggest that the vertebrae joined by abnormal calcium deposits extending to the adjoining centra in specimens ranging from 8.0～19.0 mm SL were fused together during the next period, 19.0～25.0 mm SL. Therefore, abnormal calcium deposits extending to the adjoining vertebrae are one cause for fused vertebrae in *Zacco platypus*. The frequencies of this type of vertebral anomaly in fish taken from the Ibi River were higher than those from either the Yahagi River or the Toki River.

Malformations involving individual vertebra are not rare and have been reported to occur in natural populations of both fresh and marine fishes. Generally, a higher incidence of these abnormalities has been found to occur in laboratory-reared fish and has been correlated with the water temperature and other unfavourable conditions during ontogeny (Gabriel, 1944; Alderdice et al., 1958; Garside, 1959; Seymour, 1959; Houde, 1971; Suzuki et al., 1973; Komada, 1978a, b, 1979, 1980, 1981a). Komada (1981a, b, 1982a) reported growth of centra, variations in vertebral length and incidence of malformations in the cyprinid fish, *Zacco platypus* Temminck et Schlegel reared in a laboratory. In this study, the frequencies of vertebral anomalies in newly hatched *Z. platypus* in the Ibi River, the Yahagi River and the Toki River and in laboratory-reared fish were measured to clarify the time-occurrence and the developmental mechanism of fused vertebrae and shortened vertebrae during juvenile stage.

Materials and methods

A total of 4,924 larval and juvenile cyprinid fish, *Zacco platypus*, 8.0～33.0 mm in standard length (SL) was collected from the Ibi River, Gifu Prefecture, by spoon net from 1978 to 1982, and 1,684 juvenile fish (10.0～26.0 mm SL) were collected from the Yahagi River, Aichi Prefecture, using the same gear in 1978, 1980 and 1981. An additional total of 1,380 fish (12.0～26.0 mm SL) were collected from the Toki River, Gifu Prefecture, in 1980 and 1981. About 500 larval and juvenile fish (8.0～15.0 mm SL) taken from the Ibi River on September. 19, 1979 were reared in an aquarium ($60 \times 30 \times 35$ cm^3) and fed artificial foods during culture.

All of the specimens were stained with 1.0% KOH solution of 0.1% alizarin red S, and cleared

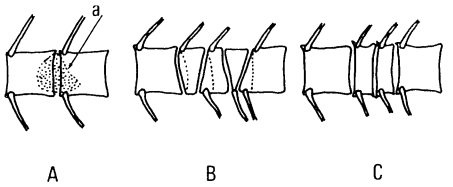

Fig. 1. Three types of abnormal vertebrae in the cyprinid fish, *Zacco platypus*, about 12.0 mm SL. A, vertebrae joined by abnormal calcium deposit extending to the adjoining centrum (Type A); B, helical sutures of centra (Type B); C, shortened vertebrae (Type C). a, abnormal calcium deposit.

through a series of aqueous glycerin containing 1.0% KOH, beginning with 20.0% thence through 50.0%, 80.0% and finally to 100% glycerin. The stained samples were examined for vertebral anomalies: vertebrae joined by abnormal calcium deposits extending to the adjoining centrum, helical sutures of centra, compressed or shortened vertebrae, fused vertebrae and abnormal vertebrae with one or two additional spines (Fig. 1). The compressed or shortened vertebrae were judged on the basis of the standard length of vertebrae in the same species (Komada, 1981b).

Results

There was no discernible morphological deformity among larval and juvenile fish (8.0～33.0 mm SL) collected from the Ibi River, the Yahagi River and the Toki River.

The stained specimens showed that minor anomalies included vertebrae joined by abnormal calcium deposits, helical sutures of centra and shortened vertebrae. The frequencies of such vertebral anomalies in juvenile fish taken from the Ibi River were significantly higher than those from either the Yahagi River or the Toki River (χ^2-test, $p<0.001$). In fish taken from the Ibi River, the frequency of defective vertebrae joined by abnormal calcium deposits was about 7 times as great as that of helical sutures of centra, and about 14 times as great as that of shortened vertebrae. The percentage of occurrence of this type of vertebral anomaly in fish taken from the Ibi River was significantly higher than those from either the Yahagi River or the Toki River (χ^2-test, $p<0.001$). Although collections were made in the same area, the frequencies of this type of anomaly in individual collections were variable (Table 1).

Out of the 4,564 fish (8.0～19.0 mm SL) taken from the Ibi River, 273 (5.98%) specimens had abnormal calcium deposits stained with alizarin red S alongside of their vertebral column (Table 2). The positions of these abnormal calcium deposits on the centra are shown in Figs. 2, 3 and 4. Out of the 273 specimens having abnormal calcium deposits, 171 (62.6%) specimens showed the abnormal calcium deposits extending

Table 1. Frequencies of vertebral anomalies in the juvenile fish from the Ibi River, the Yahagi River and the Toki River, 8～33 mm SL.

Locality	Date	Standard length (mm)	n	Vertebral anomalies						Total	
				A*		B*		C*			
				n	(%)	n	(%)	n	(%)	n	(%)
Ibi R.	1978	13～29	588	20	(3.40)	2	(0.34)	1	(0.17)	23	(3.91)
	1979	8～33	2,367	60	(2.53)	12	(0.51)	3	(0.13)	75	(3.17)
	1980	13～22	577	19	(3.29)	3	(0.52)	4	(0.69)	26	(4.51)
	1981	10～18	794	52	(6.55)	7	(0.88)	3	(0.38)	62	(7.81)
	1982	8～25	600	20	(3.33)	2	(0.33)	1	(0.17)	23	(3.83)
	Total		4,926	171	(3.47)	26	(0.53)	12	(0.24)	209	(4.24)
Yahagi R.	1978	11～26	218	1	(0.46)	1	(0.46)	2	(0.92)	4	(1.83)
	1980	10～24	346	1	(0.29)	3	(0.87)	0	(0.00)	4	(1.16)
	1981	10～20	1,120	2	(0.18)	16	(1.43)	5	(0.45)	23	(2.05)
	Total		1,684	4	(0.24)	20	(1.19)	7	(0.42)	31	(1.84)
Toki R.	1980	12～23	400	1	(0.25)	4	(1.00)	1	(0.25)	6	(1.50)
	1981	12～26	980	1	(0.10)	14	(1.43)	1	(0.10)	16	(1.63)
	Total		1,380	2	(0.14)	18	(1.30)	2	(0.14)	22	(1.59)

* A,B,C, see legends of Fig. 1.

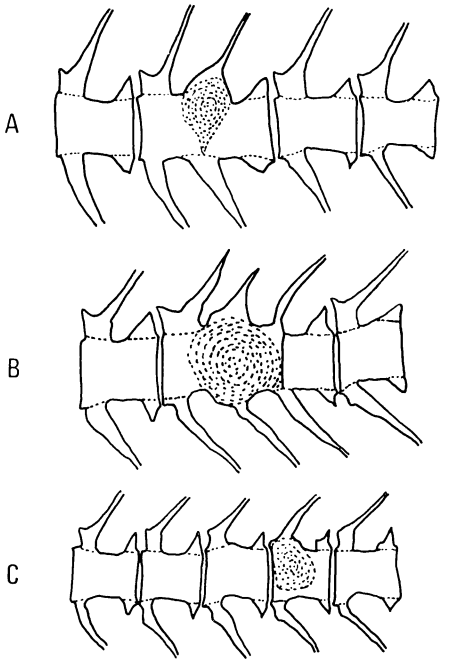

Fig. 2. Positions of abnormal calcium deposit on the centra in the juvenile A, extending to the adjoining centrum; B, extending to the two adjoining centra; C, localized alongside of a single centrum.

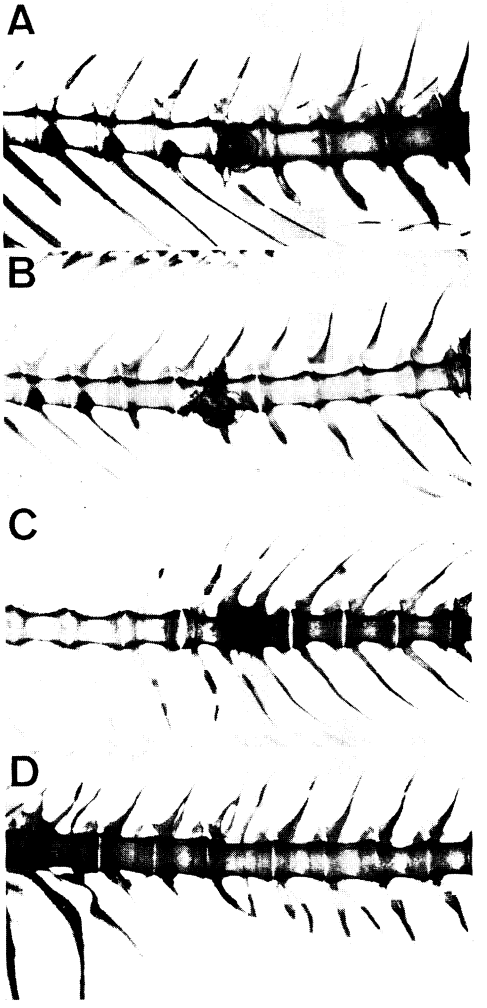

Fig. 3. Types of abnormal vertebrae in the juvenile. A, vertebra with abnormal calcium deposit localized on a single centrum; B, two vertebrae joined by abnormal calcium deposit; C, three vertebrae joined by abnormal calcium deposit; D, clear fused vertebrae.

from one centrum to the next one and 13 (4.8%) specimens showed those extending to the next two. In these defective vertebrae, the separate centra could be clearly distinguished. The other specimens (32.6%) showed abnormal calcium deposits attaching to a single centrum. In these cases, the abnormal calcium deposits were in contact with the side of the centrum. Furthermore, the incidence of defective vertebrae

Table 2. Frequencies of vertebral anomalies in each size group of fish from the Ibi River from 1978 to 1982.

		Standard length (mm)					
		8～10	10～13	13～16	16～19	19～22	22～25
No. of specimens		280	1,055	2,061	1,168	183	142
Type A anomaly*	n (%)	13 (4.64)	62 (5.88)	71 (3.44)	25 (2.14)	0 (0.00)	0 (0.00)
Type B anomaly*	n (%)	2 (0.71)	10 (0.95)	11 (0.53)	3 (0.26)	0 (0.00)	0 (0.00)
Type C anomaly*	n (%)	0 (0.00)	0 (0.00)	0 (0.00)	6 (0.51)	3 (1.64)	3 (2.11)
Fused vertebrae	n (%)	0 (0.00)	1 (0.09)	8 (0.39)	20 (1.71)	11 (6.01)	9 (6.34)

* See legends of Fig. 1.

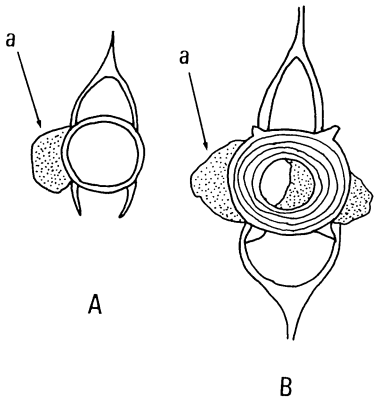

Fig. 4. Frontal view of vertebrae with abnormal calcium deposit. A, 12.8 mm SL; B, 16.6 mm SL. a, abnormal calcium deposit.

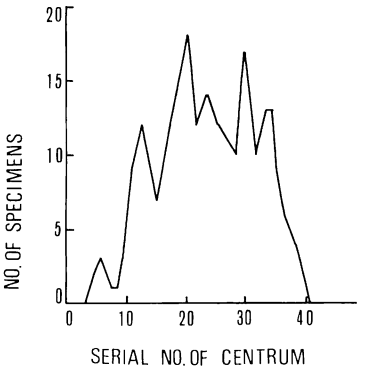

Fig. 5. Incidence of vertebrae joined by abnormal calcium deposits on the vertebral column of fish ranging from 9.0~19.0 mm SL in 1978 and 1979 from the Ibi River.

joined by abnormal calcium deposits located in the middle part of vertebral column was higher than that in the other region (Fig. 5). This type of vertebral anomaly was seen only in fish ranging from 8.0~19.0 mm SL; not a single instance of this anomaly was found in 325 fish ranging from 19.0~25.0 mm SL. The frequencies of fused vertebrae in the 325 specimens, 19.0~25.0 mm SL, were basically similar to those of vertebrae joined by abnormal calcium deposits in fish ranging from 8.0~19.0 mm SL (Table 2). In fish ranging from 19.0~25.0 mm SL, the defective vertebrae showed poorly developed arches, centra and spines. The fused vertebrae were individually compressed and fused together so that separate centra could not be distinguished, although the neural and hemal spines were distinct and the number of spines was not reduced. The surface of these defective vertebrae was rough.

On the other hand, the defective vertebrae joined by abnormal calcium deposits were seen only 13.0~23.0 mm SL fish kept for 2.0~8.5 months, and not a single instance of this type of vertebral anomaly has been found in 102 fish, 21.0~35.0 mm SL, which were kept for 9.0~11.0 months in an aquarium (Table 3). The incidence of fused vertebrae throughout the vertebral column in fish ranging from 19.0~25.0 mm SL taken from the Ibi River and in fish ranging from 14.0~35.0 mm SL reared in an aquarium is shown in Fig. 6. Generally, the incidence of fused vertebrae located in the middle part of vertebral column was higher than in other regions. The incidence of fused verte-

Table 3. Frequencies of vertebral anomalies in the fish reared in an aquarium for 2~11 months.

Reared period (months)	Standard length (mm)	No. of specimens	Vertebral anomalies								Total	
			A*		B*		C*		D*			
			n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
2.0	13~20	197	2	(1.02)	2	(1.02)	0	(0.00)	0	(0.00)	4	(2.03)
4.5	14~21	28	0	(0.00)	1	(3.57)	1	(3.57)	0	(0.00)	2	(7.14)
5.5	14~20	34	1	(2.94)	1	(2.94)	0	(0.00)	0	(0.00)	2	(5.88)
6.5	14~20	31	1	(3.23)	0	(0.00)	1	(3.23)	0	(0.00)	2	(6.45)
7.5	14~23	25	1	(4.00)	0	(0.00)	0	(0.00)	1	(4.00)	2	(8.00)
8.5	15~21	23	1	(4.35)	0	(0.00)	0	(0.00)	1	(4.35)	2	(8.70)
9.0	21~26	17	0	(0.00)	0	(0.00)	2	(11.76)	1	(5.88)	3	(17.65)
10.0	23~35	36	0	(0.00)	0	(0.00)	1	(2.78)	2	(5.56)	3	(8.33)
11.0	25~35	49	0	(0.00)	0	(0.00)	1	(2.04)	2	(4.08)	3	(6.12)

* A, B, C, see legends of Fig. 1; D, fused vertebrae with 2~4 additional spines.

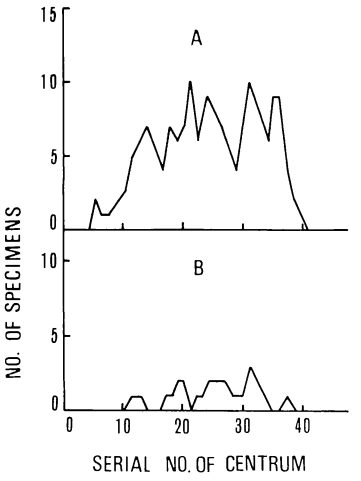

Fig. 6. Incidence of fused vertebrae with abnormal calcium deposits on the vertebral column. A, juvenile from the Ibi River from 1978 to 1980; B, juvenile reared in an aquarium.

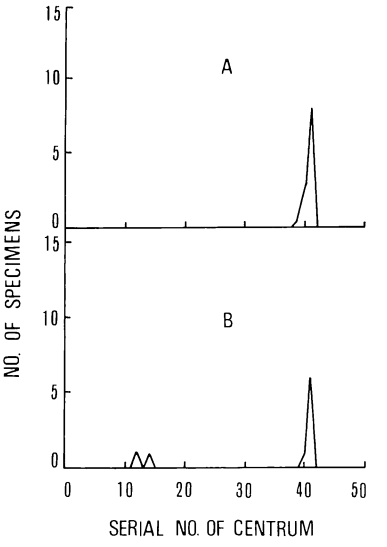

Fig. 7. Incidence of shortened vertebrae on the vertebral column. A, juvenile from the Ibi River from 1978 to 1980; B, juvenile reared in an aquarium.

brae throughout the vertebral column in fish ranging from 19.0～25.0 mm SL was basically similar to that of defective vertebrae joined by abnormal calcium deposits for fish ranging from 8.0～19.0 mm SL.

Frequencies of helical sutures of centra in juvenile fish taken from the Ibi River ranged from 0.33% to 0.88% (Table 1). In juvenile fish from the Yahagi River and the Toki River, the frequencies were 0.46～1.43%, about two times as high as that for fish from the Ibi River. Helical sutures were seen only in fish ranging from 8.0～19.0 mm SL; not a single instance of this anomaly has been found in fish ranging from 19.0～25.0 mm SL collected from the Ibi River (Table 2). Furthermore, there were no helical sutures in fish ranging from 14.0～35.0 mm SL kept for 6.5～11.0 months in an aquarium (Table 3). In these abnormal fish, ossification of vertebrae throughout the spinal column was not yet sufficient.

Frequencies of shortened vertebrae in fish ranging from 16.0～25.0 mm SL taken from the Ibi River ranged from 0.51% to 2.11%, but there was no shortened vertebrae in fish ranging from 8.0～16.0 mm SL (Table 2). The incidence of shortened vertebrae throughout the spinal column in fish taken from the Ibi River and in reared fish is shown in Fig. 7. Generally, the rate of occurrence of shortened vertebrae posteriorly in the caudal vertebrae clearly was higher than that in other regions.

The frequencies of abnormal vertebrae showing duplication of hemal and neural spines on the last two vertebrae before the urostyle in fish from the Ibi River, the Yahagi River and the Toki River were 46.0%, 36.8% and 38.1%, respectively (Table 4).

In juvenile fish which were collected from the Ibi River and then kept for 2～11 months in an aquarium, frequencies of vertebral anomalies ranged from 2.04% to 17.65%. These percentages of occurrence of vertebral anomalies in reared fish were basically similar to those in juvenile fish collected from the Ibi River.

Discussion

The types of malformed fish characterized as spinal curvature, hump-backed, dumpy, pug-headness and twisted lower jaw have been noted frequently among fishes of various species. The frequencies of these malformations in many species of fishes in nature were 0.03～0.31% (Patten, 1968; Dahlberg, 1970; Scherer, 1973; Moore and Rosey, 1974; Moore and Hixon, 1977; Komada, 1980). In this study, the frequencies of gross malformations in *Zacco platypus* from the Yahagi River, Ibi River and Toki

River were similar to those in the other natural populations reported by the other investigators. The types of deformities seen in the present species from the rivers were similar to those described by the others. The frequencies of fused vertebrae and shortened vertebrae, judged on the basis of the standard length of vertebrae (Komada, 1981a) in juvenile fish from the Yahagi River, Ibi River and Toki River, were 0.0～ca. 8.0%. These frequencies of vertebral anomalies were similar to those for the ayu, *Plecoglossus altivelis*, (Komada, 1978a, 1980) and for the dace, *Tribolodon hakonensis*, (Komada, 1979) in natural populations.

Generally, a higher incidence of gross malformations is found in laboratory- and hatchery-reared fish. The frequencies of deformities in *Zacco platypus* reared for 90～310 days in the aquarium (Komada, 1981a) were higher than those in natural populations examined in this study (χ^2-test, $p<0.001$). The common type of deformities in reared fish was pugheadness, which was not seen in the Yahagi River collections. But, in this species, the frequencies of vertebral anomalies in reared fish were basically similar to those in natural populations. This result suggests that the rearing conditions in the aquarium may be favorable for this species.

The vertebrae joined by abnormal calcium deposits were seen only in fish ranging from 8.0～19.0 mm SL, and these anomalies disappeared and the fusion of vertebrae occured during the next period (19.0～25.0 mm SL). The defective vertebrae have 2～4 additional spines and a separate centrum could not be distinguished. The frequencies and the incidence of fused vertebrae throughout the vertebral column in fish ranging from 19.0～25.0 mm SL were similar to those of vertebrae joined by abnormal calcium deposits for fish ranging from 8.0～19.0 mm SL. These results suggest that the vertebrae joined by the abnormal calcium deposits extending to the next one or two centra in fish ranging from 8.0～19.0 mm SL were fused together in the next period, 19.0～25.0 mm SL. Namely, the abnormal calcium deposits extended to the adjoining centra may cause the occurrence of fused vertebrae in *Zacco platypus*. But, when the abnormal calcium deposits attached to a single centrum, the fusion of vertebrae did not occur.

On the other hand, the frequency of vertebrae joined by the abnormal calcium deposits in fish taken from the Ibi River was clearly higher than those from either the Yahagi River or the Toki River. Although collections were made in the same area of the river, the frequencies of this type of vertebral anomaly in individual collection were variable. Gabriel (1944) and Seymour (1959) suggested that a higher incidence of

Table 4. Frequencies of abnormal vertebrae with one or two additional spines on the last two vertebrae before urostyle in the fish from the Ibi River, the Yahagi River and the Toki River.

Locality	Date	Standard length (mm)	No. of specimens	Frequencies	
				n	(%)
Ibi R.	1978	13～ 29	588	280	47.62
	1979	8～ 33	2,367	1,131	47.78
	1980	13～ 22	577	254	44.02
	1981	10～ 18	794	325	40.93
	1982	8～ 25	600	276	46.00
	Total		4,926	2,266	46.00
Yahagi R.	1978	11～115	547	201	36.75
	1979	60～125	234	84	35.90
	1980	10～ 24	346	123	35.55
	1981	10～ 20	1,120	418	37.32
	Total		2,247	826	36.76
Toki R.	1980	12～ 23	400	145	36.25
	1981	12～ 26	980	381	38.88
	Total		1,380	526	38.12

abnormalities may be associated with embryonic development at unfavourable water temperatures. Alderdice et al. (1958) found an association between vertebral anomalies and lower oxygen levels during egg incubation. Garside (1959) reported that deformed embryos of lake trout, *Salvelinus namaycush*, were found in all lots incubated in hypoxial conditions, and the frequency and severity of deformities appeared to diminish between 7.0°C and 2.3°C. Furthermore, Komada (1982b) reported that the helical sutures of centra apparently occur more frequently in the cyprinid fish *Tribolodon hakonensis*. Considering these facts the formation of abnormal calcium deposits may also be attributed to unfavourable environmental conditions during ontogeny in *Zacco platypus*.

Komada (1982b) reported that the helical sutures of centra induces a fusion of vertebrae in the dace. The present results support the previous report. Furthermore, the present findings and the previous results (Komada, 1982a) may suggest that the occurrence of shortened vertebrae is associated with the growth ratio of centra during the winter, and the time-occurrence of this type of anomaly is in the juvenile stage, 15.0～25.0 mm SL.

Komada (1982b) reported that the abnormal vertebrae having one or two additional spines on the last two vertebrae before the urostyle may appear equally at all environmental conditions in fishes. In this study, the frequencies of this type of vertebral anomaly in *Zacco platypus* from the Ibi River were clearly higher than those from the Yahagi River. Furthermore, as stated above the frequencies of fused vertebrae with abnormal calcium deposits in juvenile fish from the former were significantly higher than those from the latter. It appears that the occurrence of this type of vertebral anomaly may be associated with the environmental conditions during ontogeny in this species.

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オイカワ稚魚における脊椎骨異常の出現

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オイカワの稚魚期における脊椎骨異常の発現状況および発現機序について検討した．揖川産オイカワ稚魚（標準体長 8.0～33.0 mm）における脊椎骨異常の発現頻度は，矢作川や土岐川産稚魚よりも明らかに高かった．この場合の揖斐川産稚魚における主要な異常は，カルシウム塩の異常沈着物によって2個又は3個の椎体が連結されている異常椎体であり，椎体の螺旋状縫合の約7倍，短縮椎体の約 14 倍の出現頻度であった．全脊椎骨を通じて，カルシウム塩の異常沈着物が認められた稚魚は，4,564 尾の揖斐川産稚魚（体長 8.0～19.0 mm）中 273 尾（5.98%）であり，このうち 171 尾（62.6%）でこの異常沈着物が2個の椎体の側面に共通的に付着し両者を連結し，13 尾（4.8%）では3個の椎体を連結していた．この時，個々の椎体の間隙は明瞭であった．このような異常連結椎体は体長 8.0～19.0 mm の時期にのみ認められ，それ以後は明瞭なゆ合椎体を呈していた．しかし，これらの椎体の表面は滑らかではなく，神経棘や血管棘の発育も非常に悪かった．また，体長 19.0 mm～25.0 mm の稚魚におけるゆ合椎体の発現頻度および発現位置は，体長 8.0～19.0 mm 稚魚におけるカルシウム塩異常沈着物によって連結された異常椎体のそれらと同じであった．以上の結果から，オイカワ稚魚期における椎体側面でのカルシウム塩の異常沈着による椎体の連結がゆ合椎体の発現誘因の一つであることが示唆された．この型の異常椎体の発現頻度は河川によって異なっていた．

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