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Vertebral Anomalies in the Cyprinid Fish, *Tribolodon hakonensis*

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Abstract Vertebral number and the incidence of vertebral anomalies in newly hatched *Tribolodon hakonensis* collected from the Nagara River and incubated at various water temperatures (15°～25°C) were examined. The vertebral number of the fish incubated at 22°C was lower than that at high (25°C) and low temperatures (15°C, 18°C). The frequencies of vertebral anomalies in 11～18 mm SL fish incubated at 18°C and at 25°C were significantly higher than those at 22°C. The frequencies in natural populations were basically similar to those at 17°～22°C.

The commonest type of vertebral anomalies in hatchery-reared fish was a helical suture of the centra. The incidence of helical sutures of vertebrae located in the middle part of vertebral column was higher than in the other regions, in 11～18 mm SL fish reared for two months after hatching. The frequencies of fused vertebrae and the incidence of fused vertebrae throughout the vertebral column in 18～30 mm SL fish reared for 3 and 4 months after hatching were similar to those with helical sutures in 11～18 mm SL hatchery-reared fish. The centra showing helical sutures in 11～18 mm SL fish were fused together during the next period, 18～30 mm SL. Therefore, helical sutures are one cause for fused vertebrae in *T. hakonensis*.

It has long been known that a higher incidence of gross malformations and vertebral anomalies is found in laboratory- and hatchery-reared fish (Gabriel, 1944; Houde, 1971; Suzuki et al, 1973; Komada, 1978a, 1980a). In addition, the incidence of vertebral anomalies in newly hatched larvae depends on the water temperature and other conditions during incubation (Gabriel, 1944; Alderdice et al., 1958; Seymour, 1959; Garside, 1959). Komada (1978a, b, c, 1979, 1980b) reported on the growth of centra, morphological changes of vertebrae, variations of vertebral length and "base-line" frequencies and type of anomalies in natural and laboratory-reared cyprinid fish, *Tribolodon hakonensis*. In this study, the number of myotomes and vertebrae and the frequencies of vertebral anomalies in newly hatched *T. hakonensis*, in the Nagara River and at a hatchery were counted and measured to determine the influence of water temperature during early development and to establish "base-line" frequencies of vertebral anomalies and the time of fused vertebrae during juvenile stage.

Materials and methods

On 21 June 1977 and 25 May 1978, 200 and 370 individuals of *Tribolodon hakonensis*, 10～20 mm SL, were collected from the Nagara River, respectively. An additional total of 1000 fish, 8～20 mm SL, were collected from the Nagara River with a spoon net periodically from 1 May to 6 July, 1979. The spawning period of this fish was from the middle of April to late May, and their hatching period was from late April through early June in the Nagara River. The water temperature in the Nagara River in middle and late April was 13°～15°C, 14°～16°C in early May, 16°～18°C in the middle of May, 19°～21°C in late May and 20°～22°C in June.

About 1000 eggs obtained by artificial insemination of two pairs of adult fish from the Nagara River on 14 May were incubated and reared in a hatchery at different temperatures ranging from 15°C to 25°C and at control (17°～22°C) throughout the embryonic and larval-juvenile periods using zooplankton and artificial foods. The standard length (SL) of these newly hatched fish was measured.

All of the specimens were stained with 1.0% KOH solution of 0.1% alizarin red S. The number of myotomes and vertebrae of these specimens were counted. In case of fused vertebrae, the number of vertebrae was decided by a count of arch elements (Ford, 1937;

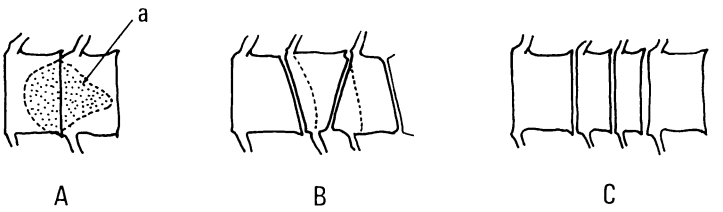

Fig. 1. Types of abnormal vertebrae in juvenile *Tribolodon hakonensis*, 14.0 mm SL. A: Fused vertebrae having calcified substance. B: Helical sutures of centra. C: Individually compressed (shortened) vertebrae. a, calcified substance stained with alizarin red S.

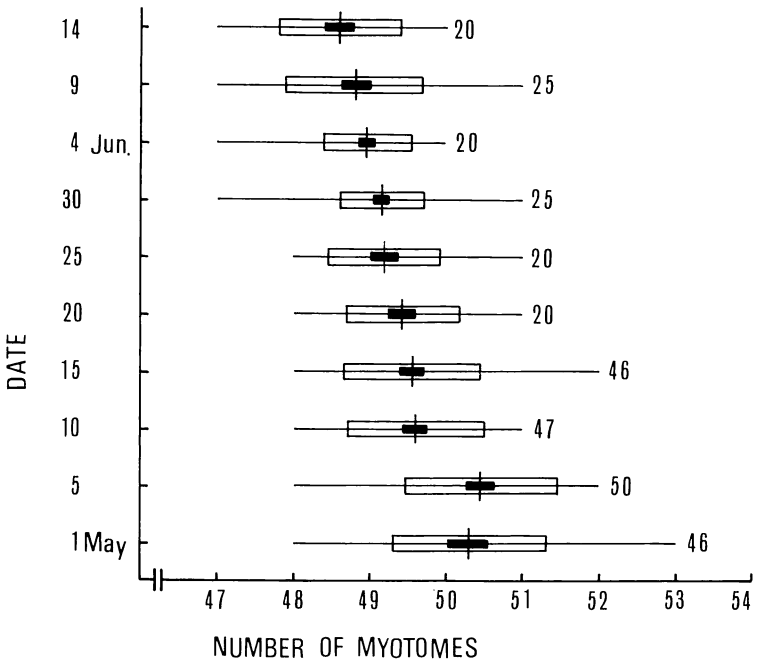

Fig. 2. Number of myotomes of 10～12 mm SL *Tribolodon hakonensis* in the Nagara River. Horizontal line, range of measurements; vertical line, arithmetical mean; solid black; standard errors on either side of mean; open rectangle, standard deviation on either side of mean. Number to the right represents number of specimens.

Gabriel, 1944). The stained samples were examined for vertebral anomalies: fused vertebrae with calcified substance, helical sutures of centra, compressed or shortened vertebrae, abnormal vertebrae with one or two additional spines (Gabriel, 1944; Dawson, 1964, 1966, 1971; Dawson and Heal, 1976; Komada, 1980a) not discernible from external examination (Fig. 1). The compressed or shortened vertebrae were judged on the basis of the standard length of vertebrae (Komada, 1978b).

Results

The number of myotomes in specimens (10～12 mm SL) collected from the Nagara River in early May was approximately 0.7 more than in those in the middle of May, approximately 1.2 more than in those in late May, and approximately 1.5 more than in those in June (Fig. 2). The average number of vertebrae of 12～13 mm SL fish collected from the Nagara River in early May was about 49.3 and those in middle May and June were 48.8～48.4 (Table 1). The water

temperature in the Nagara River in middle and late April was 13°~15°C, 14°~16°C in early May, 16°~18°C in the middle of May, 19°~21°C in late May and 20°~22°C in June.

A few eggs (about 20%) hatched at 25°C in the hatchery. The fish incubated at 22°C showed approximately 0.7 fewer vertebrae than eggs kept at 25°C and approximately 0.4 fewer vertebrae than those kept at 18°C. These differences were significant at $0.001<p<0.01$ and at $0.01<p<0.05$ in t-tests, respectively. Also the vertebral number of fish incubated at 15°C was clearly larger than that of those incubated at 18°C (t-test, $0.01<p<0.05$). The average number of vertebrae in fish held at 17° to 22°C (control) was similar to that at 18°C (Table 2). Namely, an intermediate temperature (22°C) produced a lower average number of vertebrae than extreme high (25°C) or low temperature (15°C, 18°C).

The frequencies of vertebral anomalies (fused vertebrae with calcareous deposit, helical suture, compressed or shortened vertebrae) in 11~18 mm SL fish incubated at 18°C and at 25°C were significantly higher than those held at

Table 1. Vertebral counts (including urostyle) of *Tribolodon hakonensis* collected from the Nagara River in 1979 (12~13 mm SL).

Date	No. of fish examined	Vertebral number (Mean±SD)
5 May	20	49.30±0.60
20 May	46	48.79±1.01
4 Jun.	40	48.52±0.83
19 Jun.	46	48.42±0.65

Table 2. Vertebral counts (including urostyle) of *Tribolodon hakonensis* reared at various water temperatures. Significant level determined by t-test.

Temperature (°C)	No. of fish examined	Vertebral number (Mean±SD)	P
15±1	20	49.40±0.60	<0.05
18±1	40	49.05±0.47	
22±1	40	48.67±0.65	<0.05
25±1	17	49.41±0.84	<0.01
17~22 (control)	40	48.90±0.43	

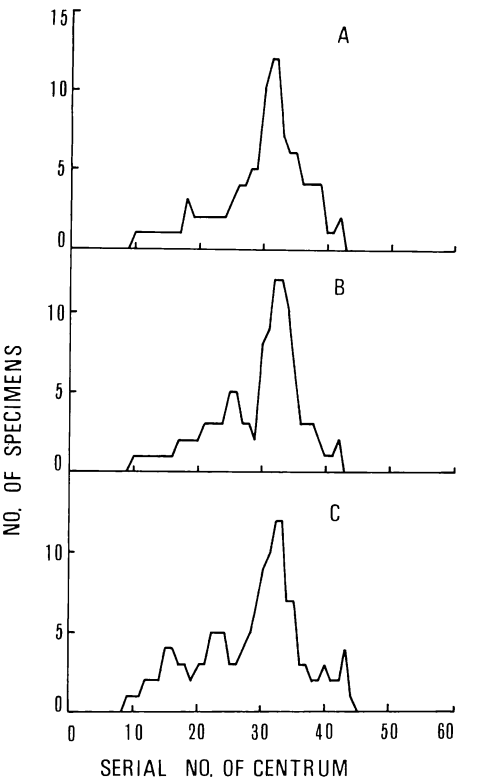

Fig. 3. Incidence of helical sutures of centra (A) and fused vertebrae (B, C) throughout the vertebral column in hatchery-reared *Tribolodon hakonensis*. A: 11~18 mm SL fish reared for two months. B: 17~25 mm SL fish reared for 3 months. C: 22~30 mm SL fish reared for 4 months.

22°C and at 17°~22°C (x^2-test, $0.01<p<0.05$) (Table 3). On the other hand, the frequencies of these anomalies in fish from the Nagara River were similar to those incubated at 22°C and at 17° to 22°C (Table 4). There was no difference of the frequency of vertebral anomalies in fish from the Nagara River between May and June.

The commonest type of vertebral anomalies in hatchery-reared fish was helical sutures of the centra. The helical sutures apparently occurred more frequently in fish incubated at 18°C and at 25°C than at 22°C and at 17° to 22°C (x^2-test, $p<0.05$). Helical sutures were seen only in 11~18 mm SL fish kept for two months after hatching. And the incidence of spiral sutures of centra located in the middle part of the column was higher than in the other regions

(Fig. 3). However, not a single instance of helical sutures has been found in 229 fish (17～41 mm SL) kept for 3, 4 and 8 months after hatching. In 229 specimens, 17～41 mm SL, the frequencies of fused vertebrae were basically similar to those of helical sutures in 11～18 mm SL fish kept for two months after hatching. These abnormal vertebrae were individually fused in sizes above 18.0 mm SL, and some vertebrae were fused together so that separate centra could not be distinguished in sizes above about 25 mm SL. These abnormal vertebrae had 2～4 additional spines (Fig. 4). Furthermore, the incidence of fused vertebrae throughout the vertebral column in 18～30 mm SL fish kept for 3 and 4 months after hatching were similar to those of helical sutures in 11～18 mm SL fish kept for two months (Fig. 3). Therefore, it is considered that the centra showing helical sutures in 11～18 mm SL specimens were partially fused together in the next period. In this case, the separate centra could be distinguished at first, and then the separate centra could not be distinguished (Fig. 4; Table 5).

The frequency (32%) of individually compressed and shortened vertebrae in 26～41 mm SL fish kept for 8 months after hatching in the aquarium was significantly higher than in 11～18 mm SL fish kept for two months (x^2-test, $p<0.001$). In these deformed fish, the layer of connective tissue attaching the abnormal vertebrae disappeared. Frequencies of discernible morphological deformities found in fish kept for 4 and 8 months at the hatchery were 0.63% and 12.9%, respectively. But, there was not a morphological deformity in 1390 fish collected from the Nagara River and in 433 fish reared at a hatchery for 1 and 2 months at various water temperatures in this study. Spinal curvature was the type of malformation occuring in hatchery-reared fish.

Morphological changes of fused vertebrae having calcareous deposits (calcified substance) stained with alizarin red S (Fig. 1) were not traced, because the number of specimens showing these abnormal vertebrae were few.

The frequencies of abnormal vertebrae showing duplication of haemal or neural spines on the last two vertebrae before the urostyle in fish (11～20 mm SL) collected from the Nagara

Table 3. Frequencies of abnormal vertebrae in *Tribolodon hakonensis* reared at various temperatures (11～18 mm SL).

Temperature (°C)	No. of fish examined	Abnormal vertebrae							
		A*		B*		C*		Total	
		n	(%)	n	(%)	n	(%)	n	(%)
18±1	126	8	(6.35)	33	(26.19)	1	(0.79)	42	(33.33)
22±1	80	1	(1.25)	5	(6.25)	1	(1.25)	7	(8.75)
25±1	17	2	(11.75)	4	(23.53)	0	(0.00)	4	(35.29)
17～22	154	1	(0.65)	9	(5.84)	1	(1.65)	11	(7.14)

* As for A, B and C, see legends of Fig. 1.

Table 4. Frequencies of abnormal vertebrae in *Tribolodon hakonensis* collected from the Nagara River (11～20 mm SL).

Date	No. of fish examined	Abnormal vertebrae							
		A*		B*		C*		Total	
		n	(%)	n	(%)	n	(%)	n	(%)
1977 21 Jun.	200	11	(5.50)	2	(1.00)	1	(0.50)	14	(7.00)
1978 25 May	370	12	(3.24)	1	(0.27)	1	(0.27)	14	(3.78)
1979 30 May	70	1	(1.43)	1	(1.43)	0	(0.00)	2	(2.85)
4 Jun.	70	4	(5.71)	1	(1.43)	0	(0.00)	5	(7.14)
14 Jun.	50	1	(2.00)	1	(2.00)	0	(0.00)	2	(4.00)
6 Jul.	630	13	(2.06)	7	(1.11)	2	(0.30)	22	(3.49)

* As for A, B and C, see legends of Fig. 1.

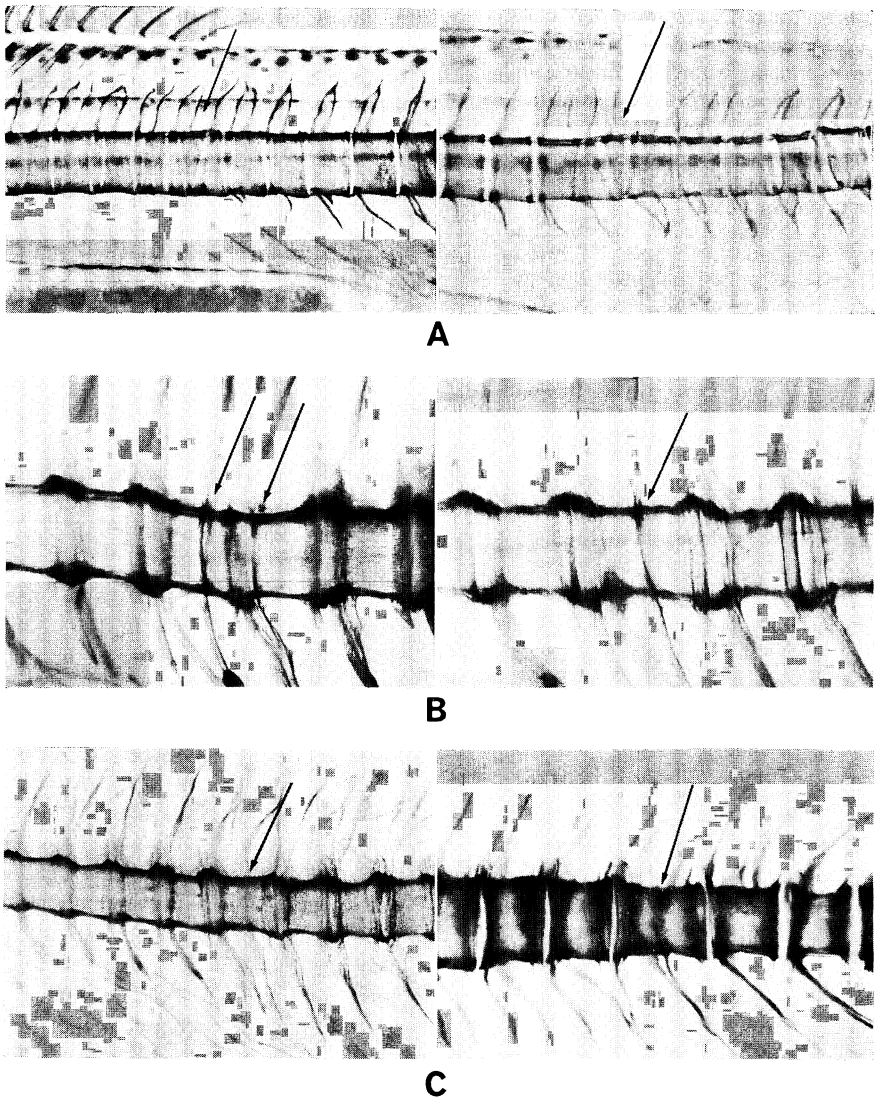

Fig. 4. Helical sutures of centra and fused vertebrae (arrows) in hatchery-reared *Tribolodon hakonensis*. A: Helical sutures of centra in 13 mm SL fish. B: Fused vertebrae in 20 mm SL fish. C: Fused vertebrae in 24 mm SL fish.

Table 5. Frequencies of abnormal vertebrae in *Tribolodon hakonensis* reared in hatchery at 18°C.

Reared period (months)	No. of fish examined	SL (mm)	Abnormal vertebrae									
			A*		B*		C*		D*		Total	
			n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
2	56	11～18	1	(1.79)	21	(37.50)	1	(0.79)	0	(0.00)	22	(39.29)
3	38	17～25	1	(2.63)	0	(0.00)	1	(2.63)	15	(39.47)	17	(44.74)
4	160	22～30	6	(3.75)	0	(0.00)	8	(5.00)	47	(29.38)	61	(38.13)
8	31	26～41	0	(0.00)	0	(0.00)	10	(32.26)	8	(25.81)	18	(58.06)

* A, B, C: See Fig. 1, for legend; D: Fused vertebrae with 2～4 additional spines.

Table 6. Frequencies of abnormal vertebrae with one or two additional spines on the last two vertebrae before the urostyle in *Tribolodon hakonensis* collected from the Nagara River (11～20 mm SL).

Date	No. of fish examined	Frequency	
		n	%
1977 21 Jun.	200	58	29.00
1978 25 May	370	110	29.73
1979 30 May	70	23	32.86
4 Jun.	70	16	22.86
14 Jun.	50	8	16.00
6 July	630	210	31.90

Table 7. Frequencies of abnormal vertebrae with one or two additional spines on the last two vertebrae before the urostyle in *Tribolodon hakonensis* reared at hatchery (11～18 mm SL).

Temperature (°C)	No. of fish examined	Frequency	
		n	%
18±1	126	25	19.84
22±1	80	13	16.25
25±1	17	4	23.53
17～22	154	29	18.83

River in May and June were 16.0～33.0% (Table 6). The percentages of occurrence of these vertebral anomalies in hatchery-reared fish at 18°C, 22°C, 25°C and 17° to 22°C were 19.8%, 16.3%, 23.5% and 18.8%, respectively (Table 7). These frequencies did not differ significantly from those in natural populations.

Discussion

It has long been known that the incidence of vertebral anomalies in newly hatched larvae depends on the water temperature and other conditions during incubation. The frequencies of vertebral anomalies in *Tribolodon hakonensis* incubated at 18°C and at 25°C were significantly higher than those in fish incubated at 22°C. The common type of abnormality was spiral sutures of the centra. Gabriel (1944) reported that the helical sutures apparently occurred only at a high temperature (24.5°C) in *Fundulus heteroclitus* and there was not a single instance of this anomality in 700 embryos raised at 18.8°C and 13.5°C; the mean vertebral number increased from 32.72 at 24.5°C to 33.09 at 18.8°C and 33.22 at 13.5°C. In *T. hakonensis*, the frequencies of spiral sutures in fish incubated at either high (25°C) or low temperature (18°C) were significantly higher than that at 22°C. The vertebral number of fish incubated at 22°C were clearly fewer than those at 18°C and 25°C. Furthermore, the frequencies of helical sutures in 10～20 mm SL fish collected from the Nagara River were clearly lower than those incubated at 18°C and 25°C. The vertebral number of juvenile fish in this study and of young and adult fish (Komada, 1980b) was similar to that of the fish incubated at 22°C. In fish collected from the rivers, the incidence of shortened and fused vertebrae located posterior in the caudal region and near the transition from abdominal to caudal vertebrae were higher than those in the other regions, and, in laboratory-reared fish, the rates of occurrence of deformed vertebrae throughout the column were nearly equal (Komada, 1979). But, in 11～18 mm SL fish incubated at the hatchery, the incidence of helical sutures of centra located in the middle part of the vertebral column was higher than those in the other regions. Such centralization of helical sutures at the middle part of the column may be associated with the torsion of the spinal column. However, Gabriel (1944) reported that in *Fundulus heteroclitus*, since the centra were apparently determined, the spiral suture was probably not caused by the torsion itself at a stage earlier than that at which pronounced embryonic torsion set in. These facts suggest that the increase in the frequencies of spiral sutures at high or low water temperatures may be similar to the effect of temperature upon the number of vertebrae in fishes during early development.

The helical sutures of the centra partially disappeared and the fusion of vertebrae occurred during the juvenile stage, 20～30 mm SL. The abnormal vertebrae have 2～4 additional spines and a separate centrum could not be distinguished. In the 24 mm SL fish, the neural and haemal spines become heavier and the neural and haemal postzygapophysis started to grow on the posterior end of each centrum (Komada, 1978c). This result suggests that the helical suture may cause the occurrence of fused vertebrae in fishes.

On the other hand, the frequencies of indi-

vidually compressed and shortened vertebrae in 26～41 mm SL fish kept for 8 months in the hatchery were similar to those of laboratory-reared fish (Komada, 1979). The length and the diameter of centra located at the middle part of the vertebral column became longer than those in the other regions in 35～40 mm SL fish (Komada, 1980b). Komada (1979) suggested that these vertebral anomalies were probably caused by abnormal growth ratios of centra of the spinal column in *Tribolodon hakonensis*.

The type of malformation occurring in hatchery-reared fish was spinal curvature. This type was recognized in natural populations (Komada, 1978a). On the other hand, the type of malformations occurring in hatchery-reared ayu, *Plecoglossus altivelis*, were more numerous than in natural populations (Komada, 1980a). Few eggs hatched at 25°C and more than half of the larvae were humpbacked in ayu (Komada, 1977). The types of malformations occurring in hatchery-reared ayu (Komada, 1980a) were more numerous than those in hatchery-reared *T. hakonensis*. Furthermore, the frequencies of malformations in the former were higher than those in the latter. These facts may be associated with the physiological and phylogenetic differences between the two species. Finally, the frequencies of abnormal vertebrae having one or two additional spines on the last two vertebrae before the urostyle in *T. hakonensis* both collected from the Nagara River and reared in the hatchery were basically similar to those in *Fundulus heteroclitus* (Gabriel, 1944). Vertebral anomalies of this common kind appear to be equal under all environmental conditions in fishes.

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Literature cited

Alderdice, D. F., W. P. Wickett and J. R. Brett. 1958. Some effects of temperature exposure to low dissolved oxygen levels in Pacific salmon eggs. J. Fish. Res. Bd. Canada, 15(2): 229～249, figs. 1～8.

Dawson, C. E. 1964. A bibliography of anomalies of fishes. Gulf Res. Rep., 1: 308～399.

Dawson, C. E. 1966. A bibliography of anomalies of fishes. Supplement 1. Gulf Res. Rep., 2: 169～176.

Dawson, C. E. 1971. A bibliography of anomalies of fishes. Supplement 2. Gulf Res. Rep., 3: 215～239.

Dawson, C. E. and E. Heal. 1976. A bibliography of anomalies of fishes. Supplement 3. Gulf Res. Rep., 5: 35～41.

Ford, E. 1937. Vertebral variation in teleostean fishes. J. Mar. Biol. Asoc., 22(1): 1～60, figs. 1～18, pls. 1～16.

Gabriel, M. L. 1944. Factors affecting the number and form of vertebrae in *Fundulus heteroclitus*. J. Exp. Zool., 95: 105～145, figs. 1～25.

Garside, E. T. 1959. Some effects of oxygen in relation to temperature on the development of lake trout embryos. Can. J. Zool., 37: 689～698, figs. 1～4.

Houde, E. D. 1971. Developmental abnormalities of the flat fish, *Achirus lineatus*, reared in the laboratory. Fish. Bull., 69(3): 537～544, figs. 1～8.

Komada, N. 1977. Influence of temperature on the vertebral number of the ayu, *Plecoglossus altivelis*. Copeia, 1977 (3): 572～573.

Komada, N. 1978a. Studies on abnormal growth of skeleton in common dace, *Tribolodon hakonensis*. (1) Frequencies of morphological abnormalities of common dace kept in the aquarium. J. Growth, 17(1): 28～35, figs. 1～11. (In Japanese).

Komada, N. 1978b. Studies on abnormal growth of skeleton in common dace, *Tribolodon hakonensis*. (2) Variation of vertebral length. J. Growth, 17(2, 3): 39～45, fig. 1. (In Japanese).

Komada, N. 1978c. Studies on abnormal growth of skeleton in common dace, *Tribolodon hakonensis*. (3) Vertebral variation with size of body. J. Growth, 17(4): 72～75, figs. 1～3. (In Japanese).

Komada, N. 1979. Studies on abnormal growth of skeleton in common dace, *Tribolodon hakonensis*. (4) Incidence of vertebral anomalies of natural and reared fish. J. Growth, 18(2, 3): 49～59, figs. 1～6. (In Japanese).

Komada, N. 1980a. Incidence of gross malformations and vertebral anomalies of nature and hatchery *Plecoglossus altivelis*. Copeia, 1980(1): 29～35, figs. 1～8.

Komada, N. 1980b. Growth of vertebral centra in the cyprinid fish, *Tribolodon hakonensis*. Japan. J. Ichthyol., 26(4): 351～356, figs. 1～5. (In Japanese).

Seymour, A. 1959. Effects of temperature upon formation of vertebrae and fin rays in young chinook salmon. Trans. Amer. Fish. Soc., 88: 58～69, figs. 1～7.

Suzuki, K., H. Kishimoto and Y. Tanaka. 1973. Head deformity in tunas kept in the aquarium. Japan. J. Ichthyol., 20 (2): 113～119, figs. 1～4. (In Japanese).

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自然河川産および人工孵化ウグイ稚魚における脊椎骨異常の発現

駒田格知

ウグイ稚魚における脊椎骨異常の発現率および発現機序について検討した．18°C および 25°C 恒温条件下で人工孵化した稚魚の脊椎骨数は 22°C の時よりも明らかに増加していた．一方，18°C および 25°C で人工孵化した稚魚（標準体長 11～18 mm）における脊椎骨異常の発現頻度は 33～35% で 22°C の時の約 4 倍であつた．しかし，22°C の場合には約 9% で自然河川の場合とほぼ同じであった．主要な脊椎骨異常は椎体の螺旋状縫合であり，その出現頻度は 18°C および 25°C で孵化した稚魚において 24～26% で 22°C の時の約 4 倍であった．脊柱のほぼ中央部に集中しているこれらの異常椎体は，体長 18 mm 以上（孵化後 3 ケ月以上）に成長すると螺旋状縫合が部分的にゆ合し始め，始めはその縫合線が明瞭に確認されるが，やがてそれも消失して他よりも長く 2～4 本の過剰棘を有する脊椎骨となる．体長約 24 mm 以上に達すると螺旋状縫合は全くみられず，前時期における螺旋状縫合と同程度の頻度で，しかも同じ場所にゆ合椎体が認められた．すなわち，椎体の螺旋状縫合の出現条件は脊椎骨数の増減の原因となる条件と深く関連しているものと考えられ，さらに，これら螺旋状縫合は稚魚期におけるゆ合椎体の発現の主要な誘因の一つであることが判明した．

(501-02 岐阜県本巣郡穂積町高野 1851 岐阜歯科大学解剖学教室)