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**Juvenile Intersexuality in a Cyprinid Fish, the Sumatra
Barb, *Barbus tetrazona tetrazona***

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Abstract

The process of sex differentiation and development of the gonad in the Sumatra barb, *Barbus tetrazona tetrazona*, reared in laboratory conditions was studied histologically in the present study. Gonads of all juvenile fish were initially provided with ovarian structures, which were characterized by the development of germ cells into meiotic prophase and auxocyte stages and by the formation of ovarian cavity, during the period from 20 to 25 days after hatching. The ovary continued to grow steadily in part of the fish on successive days, whereas in other fish it remained in ill-developed states in which sparsely distributed oocytes, a relatively large number of gonial germ cells and compact stromal tissue with increased somatic cells were notable. In gonads of the latter type, testicular development began with clustering of gonial germ cells to form primordial seminal lobules, which indicated definitely the differentiation of spermatogonia, and subsequent initiation of spermatogenesis in some of them. Concomitant degenerative changes of oocytes accompanied in some cases by marked infiltration of somatic cells over the stromal tissue were also prominent in these gonads. Intersexual gonads with developing male germ cells and degenerating oocytes thus occurred apparently within 45 and 50 days after hatching. The transformation of gonads from ovarian to testicular nature occurring in future males seemed to be completed rapidly, since no ovarian characteristics remained in the testes of the fish examined later than 60 days after hatching, though residual ovarian cavities were occasionally retained in the testis of adult males. Thus true gonochorism with a nearly 1:1 sex ratio was established following the intersexual stage in the Sumatra barb.

Comparatively many species of teleost fishes belonging to the family Cyprinidae are known to have some lability in expression of their gonadal sex, often showing the occurrence of intersexual features such as ovo-testes and testis-ova even in the adult stage (Atz, 1964). Among them, goldfish *Carassius auratus* (Stromsten, 1931; Takahashi and Takano, 1971), zebrafish *Brachydanio rerio* (Takahashi, 1977), rose bitterling *Rhodeus ocellatus ocellatus* (Shimizu, 1979) and common carp *Cyprinus carpio* (Davis and Takashima, 1980) have been shown to display the lability also during the process of gonadal sex differentiation as juvenile intersexuality in which gonads of future males are initially provided with ovarian characteristics before they develop into histologically defined testes. It seems likely that the juvenile intersexuality may in some cases reflect on the abnormal intersexuality found in the gonad of adult fishes. There may generally occur, however, different modes of juvenile intersexuality in different cyprinids studied so

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far, the development of female characteristics in future testes being rather slight and sporadic in the goldfish and the carp, while more prominent and constant in the zebrafish and the rose bitterling. Anyway, information regarding intersexuality in juvenile fishes is still too scanty in amount to relate the phenomenon to the occurrence of abnormal intersexuality in adults.

The present paper deals with histological studies on a type of juvenile intersexuality in a domestic cyprinid fish, the Sumatra barb *Barbus tetrazona tetrazona*. In a related species of barb, *Barbus stigma*, the appearance of an ovum in a mature testis has been recorded by Sathyanesan (1957).

Material and Methods

Juvenile males and females of the Sumatra barb, *Barbus tetrazona tetrazona*, were obtained from a commercial dealer and were subsequently raised to maturation in the laboratory. Each mature female was mated with a mature male in separate breeding aquaria with bottom vegetation as spawning substrate. In some cases, culsters of finely teared vinyl strips were submerged and served as an artificial spawning bed in small glass aquaria without soil. The parents were removed after spawning from the aquarium in which eggs and fry were kept successively. When fry began to swim freely, they were fed a sufficient amount of boiled egg yolk suspended daily in the rearing water. The feed was gradually replaced for several successive days by powdered compound food for carp-culture which was solely given to juvenile fish afterwards. Constantly aerated rearing water was regulated at about 25°C under natural light conditions, and was renewed with well-aerated and temperature-regulated water generally once a week throughout the present study.

Fry and juvenile fish obtained from 6 separate broods were preserved, at various intervals for up to 90 days after hatching, *in toto* in Bouin's fluid. After measuring the body length on fixed specimens, the trunk region with gonads and associated structures in position was sectioned serially at 7–10 μm in thickness, and stained with Delafield's hematoxylin and eosin for histological observations.

Observations

The ovary of the Sumatra barb is a paired organ lying along the dorsolateral sides of the body cavity (Fig. 15). It has a narrow ovarian cavity of a typical parovarian type on the dorsal side of each ovary directly ventral to the coelomic wall. The paired ovaries are fused with each other at their caudal end to connect their ovarian cavities with a short, single median oviduct. The testis is also paired and consists of numerous seminal lobules containing germ cells at various stages of maturation (Fig. 16), showing a structure of the unrestricted spermatogonial testis-type (Grier, 1981). At their caudal proximity, the two testes join to form a flat network of complicatedly anastomosed, sterile lacunae, which appears to function as a sperm reservoir. The sperm reservoir converges caudally and leads eventually to a short, single median sperm duct to open into the urethra near the urogenital sinus. In the region of the testis proper, a single, or a main, sperm duct is not distinct, and mature spermatozoa are accumulated in some series of anastomosed small lumina lying all along the testis at its proximal margin and in

the neighbouring dorsolateral coelomic wall to be transported caudally to the sperm reservoir (Fig. 16).

In the present study, the process of sex differentiation and structural development of the gonads and associated structures was studied histologically. Distribution of gonadal sexes among the specimens of two representative broods examined at various days after hatching is shown in Table 1.

Table 1. Distribution of gonadal sexes at various days after hatching in two different broods of Sumatra barb, *Barbus tetrazona tetrazona*.

Days after hatching	Body length* (mm)	Gonadal sex**					No. of fish examined
		I	♀ ₁	♀ ₂	♂	♂	
5	4.5	4					4
11	5.0	8					8
17		5		3			8
20	6.8	1		8			9
25				8			8
30	7.8		3	4			7
35			4	5			9
40	9.3		4	3	1		8
46			1	3	2		6
51	12.6		3	1	4		8
(Total)							75
50	12.7		5		1	4	10
70	15.6		5			4	9
90	20.3		9			9	18
(Total)							37

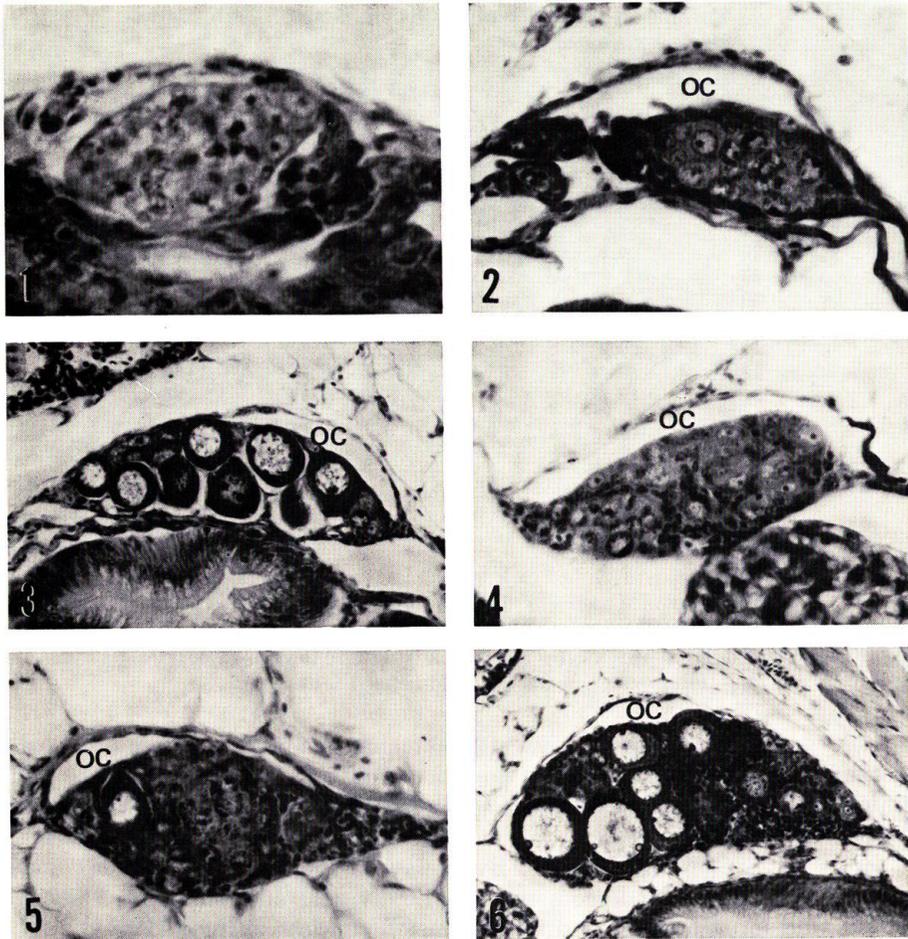
* Measured on specimens fixed in Bouin's fluid.

** No. of fish having sexually indifferent gonads (I), gonads with well-developed oocytes (♀₁), gonads with ill-developed oocytes (♀₂), intersexual gonads (♂), and defined testes (♂).

At least during the first 10 days after hatching, genital ridges were observed to be localized at the level near the posterior half of the gall bladder. They contained a few gonial germ cells packed by a thin layer of capsular somatic cells and were short in length, while no genital ridges were formed on the dorsal coelomic wall caudal to that germinal region. By 15 days of age, germ cells in most gonads were frequently seen to undergo active mitotic divisions and, moreover, those in a few cysts thus formed were found to show meiotic nuclear changes (Fig. 1). At 20 days of age, the germinal part of gonads increased its length considerably, but still no genital ridges were found to occur on the coelomic wall caudal to that part. Cysts of germ cells in meiotic prophase developed especially in the anterior region of gonads of almost all the specimens observed. By that age, in addition, the enlarged gonads started to lean laterally toward the dorsal coelomic wall and had aggregations of stromal cells along their distal border. Opposite to the stromal aggregations, the coelomic wall also developed a narrow, ridge-like thickening of epithelial cells which soon fused with the confronted stromal masses of the

gonad to eventually form a cavity, viz. the ovarian cavity, on the dorsal side of the gonad (Fig. 2).

By 25 days of age, gonads of all the fish examined had cysts of germ cells in meiotic prophase and ovarian cavities in the process of formation, and some specimens among them possessed a few oocytes in the first growth phase (auxocytes) together with an increased number of cysts of younger germ cells, thus becoming

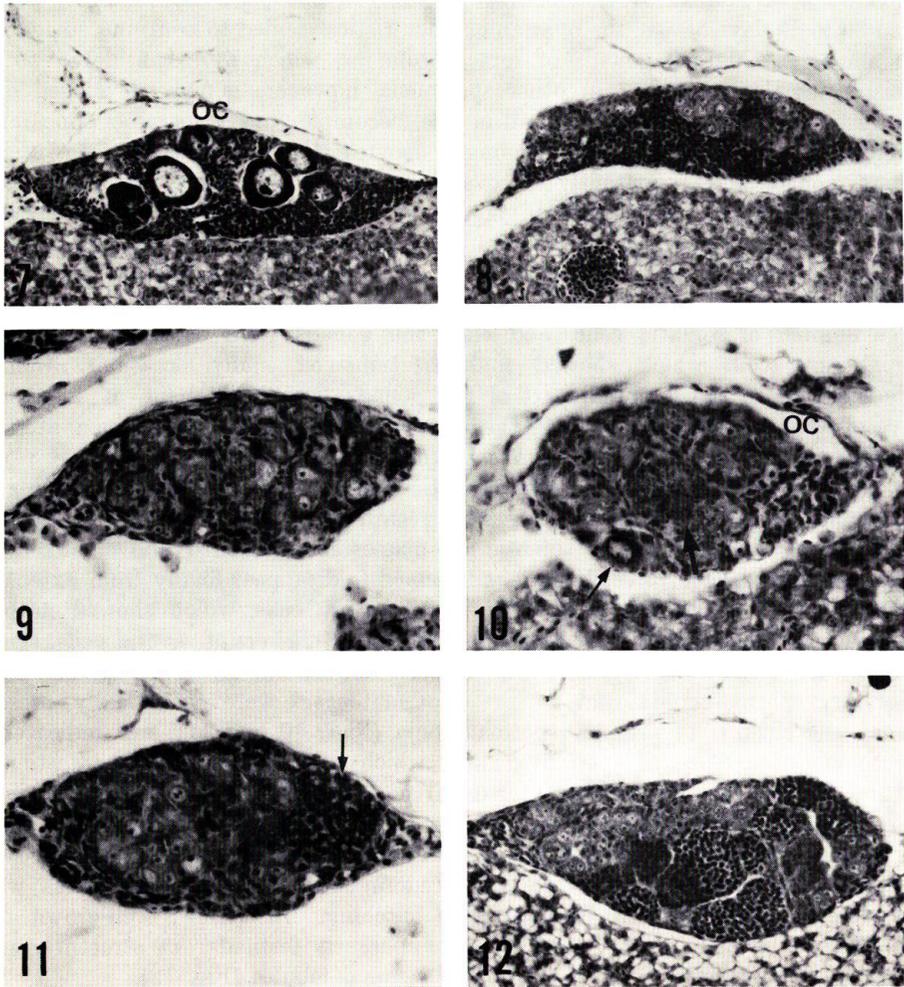


Figs. 1-6. Frontal sections of gonads of juvenile Sumatra barb in the ovarian phase. OC, ovarian cavity. Fig. 1. Gonad with germ cell cysts in meiotic prophase, 17 days after hatching. $\times 760$. Fig. 2. Gonad at the initiation of formation of ovarian cavity, 20 days after hatching. $\times 400$. Fig. 3. Ovary of a possible female, 40 days after hatching. $\times 280$. Fig. 4. Gonad showing retarded development of oocytes and abundant presence of gonial germ cells, 40 days after hatching. $\times 410$. Fig. 5. Gonad with ill-developed female germ cells, 46 days after hatching. $\times 380$. Fig. 6. Gonad suffered by extensive infiltration of somatic cells, 40 days after hatching. $\times 210$.

provided with evident ovarian characteristics. In fish observed during a period from 30 to 40 days of age, the ovarian characteristics seemed to continue steady development in enlarged gonads of some specimens, which were considered to be decisive females (Fig. 3). In other specimens, however, gonads developed only poorly and remained relatively small in size, accompanying a sporadic appearance of small auxocytes and cysts of younger oocytes (Figs. 4 and 5). In a few others, gonads were apparently of ovarian nature, but their auxocytes were generally smaller in number when compared with those in ovaries of decisive females, and the stromal tissue was found to be infiltrated by masses of somatic cells at various parts of the gonads (Fig. 6). It was shown further in the gonads of the latter two cases that their stromal tissue appeared compact with a relatively large number of somatic cells, and that the gonads were considerably rich in gonial germ cells which showed multiplications on many occasions. Genital ridges caudal to the germinal part of the gonads were still solid at about 40 days of age, though in some cases they were furnished with a flat lumen at their anterior region continuous to the ovarian cavity.

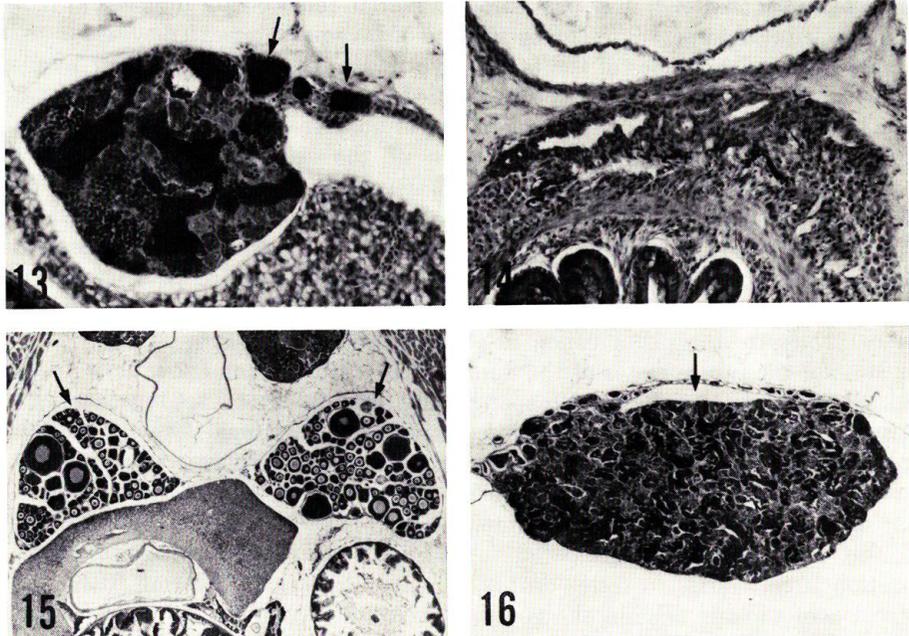
By 45 and 50 days of age, characteristic changes were observed proceeding in the gonads of specimens other than those regarded as decisive females. In such ovaries as those infiltrated by somatic cell masses as mentioned above, auxocytes advanced their degenerative changes and seemed to disappear finally from extensive regions of the gonads (Figs. 7 and 8). Gonial germ cells, which showed mitoses frequently, were partitioned with somatic cells into clusters of several cells, representing the formation of primordial seminal lobules (Fig. 9). Large cysts of secondary spermatogonia and of first spermatocytes were occasionally present among the clusters of primary spermatogonia (Figs. 10 and 11) and indicated a precocious occurrence of spermatogenesis which in turn disclosed the testicular nature of these gonads. Thus intersexual gonads with degenerating auxocytes and younger oocytes dispersed in definite testicular tissues made a prominent appearance during the period between 45 and 50 days after hatching. Changes of gonads from predominantly ovarian to predominantly testicular structures were considered to begin within 40 days after hatching. In a few, well-grown fish examined at 50 days of age, gonads were apparently testicular in structure, with many spermatogenetic cysts in developing seminal lobules (Fig. 12).

From 60 days of age onwards, males and females were easily distinguishable by histological inspections of their gonads, establishing a sex ratio of nearly 1:1. In ovaries, formation of ovarian lamellae progressed along their dorsal border from the cranial region caudalwards, and some oocytes were observed to have entered into vitellogenesis in 90-day-old females (Fig. 15). The oviduct system caudal to the ovary proper had completed its differentiation by that time. In testes, neither auxocytes nor cysts of younger germ cells were present, and ovarian cavities formed in the ovarian phase of these testes also disappeared in many cases, though the cavity was rarely retained even in the testis of adult males (Fig. 16). Testes with mature spermatozoa in developing seminal lobules were present in most of the 70-day-old males. The lobule lumina were confluent, especially in the caudal region of the testis, to several short cavities localized at the testicular hilum. These cavities, which lacked any germinal component on their walls, were anastomosed with each other to form a system of longitudinal ducts leading to the



Figs. 7-12. Frontal sections of gonads of juvenile Sumatra barb in the intersexual and testicular phases. OC, ovarian cavity. Fig. 7. Gonad with degenerating oocytes surrounded by hyperplastic stromal cells, 51 days after hatching. $\times 210$. Fig. 8. Gonad of the same specimen as above, showing gonial germ cells mounted in hyperplastic stroma and disappearance of oocytes, 51 days after hatching. $\times 210$. Fig. 9. Gonad with gonial germ cells partitioned in primordial seminal lobules, 51 days after hatching. $\times 330$. Fig. 10. Gonad with a cluster of secondary spermatogonia (thick arrow), degenerating auxocyte (thin arrow) and residual ovarian cavity, 51 days after hatching. $\times 250$. Fig. 11. Gonad with a cluster of first spermatocytes (arrow), 51 days after hatching. $\times 380$. Fig. 12. Developing testis with advancing spermatogenesis, 50 days after hatching. $\times 250$.

caudal sperm duct. Anastomosed cavities with mature spermatozoa frequently extended in distribution also to the lateral coelomic wall close to the testicular hilum (Fig. 13). They were likely to originate from stromal elements of the testis



- Fig. 13. Defined testis with mature spermatozoa in seminal lobules, 70 days after hatching. Note luminal structures (arrows) with spermatozoa in the testicular hilum and its neighbouring coelomic wall. $\times 190$.
- Fig. 14. Anastomosed lacunae, or sperm reservoir, occurring at the caudal proximity of bilateral sperm ducts, 90 days after hatching. $\times 150$.
- Fig. 15. Ovaries at the initiation of vitellogenesis, 90 days after hatching. Arrows indicate ovarian cavities. $\times 20$.
- Fig. 16. Testis of an adult male. Note the presence of residual ovarian cavity (arrow) lined by sperm-transporting longitudinal lumina along its dorsal border. $\times 50$.

which were potentiated to have central lumina as did the somatic cells composing the wall of seminal lobules.

In contrast to the prominent development of testes from 50 days of age onwards, the sperm duct system caudal to the testis proper exhibited no obvious development even at 70 days of age, assuming still a shape of sterile solid ridges running on the dorsal coelomic wall. However, they were prolonged further caudally and joined with each other to form a single flat mass of somatic cells attached on the dorsal wall of the rectum at the level of the anterior border of the urinary bladder. At 90 days of age, the bilateral genital ridges of some males had a narrow lumen, though they were still solid in their posterior region, and the single median mass of somatic cells of the genital ridge simultaneously showed irregular lacunae, indicating a differentiation of the sperm reservoir (Fig. 14). A single median sperm duct caudal to that region was still solid and primordial in nature even by that time.

Discussion

The present study indicates that the gonad of males of the Sumatra barb,

Barbus tetrazona tetrazona, may pass through ovarian followed by intersexual phases during their juvenile period. The occurrence of juvenile intersexuality seems to be not rare among fish species of the family Cyprinidae, though only limited numbers of species of cyprinids have been studied so far. Ovarian characteristics represented particularly by the development of oocytes in gonads of future males appear to be more intensified in the cases of the zebrafish, *Brachydanio rerio* (Takahashi, 1977) and the rose bitterling, *Rhodeus ocellatus ocellatus* (Shimizu, 1979) than in the cases of the goldfish, *Carassius auratus* (Stromsten, 1931; Takahashi and Takanao, 1971) and the common carp, *Cyprinus carpio* (Davis and Takashima, 1980), the Sumatra barb in the present study being of an intermediate type between the two. It seems likely that a relatively prolonged course of gonadal sex differentiation may obscure the phenomenon in the goldfish and the carp.

Most of the future testes of the Sumatra barb are characterized, prior to their eventual differentiation, by having sporadically distributed oocytes together with a relatively large number of gonial germ cells in compact stromal tissue. Degeneration of these oocytes is always seen to be preceded by hyperplastic development of somatic cells in the stroma. Similar phenomenon has been reported to occur in other cyprinid fishes with juvenile intersexuality (Takahashi, 1977; Shimizu, 1979) and also in an anabantid, the paradise fish *Macropodus opercularis* in a phase of transition from ovaries to testes during the juvenile period (Schwier, 1939). The activation of somatic cells in the gonadal stroma may possibly be implicated not only in causing degenerative changes of oocytes but in inducing the clustering of gonial germ cells to form primordial seminal lobules in which spermatogenesis occurs successively, thus signifying decisive testicular differentiation through the intersexual phase. In some cases, extensive infiltration of masses of somatic cells was found over the stromal tissue of gonads with a fair number of growing oocytes. The somatic cells were generally smaller in size and more roundish in shape when compared with ordinary stromal cells of the gonad. Although the origin of these peculiar somatic cells could not be determined in the present study, they undoubtedly play an essential role in causing degeneration of oocytes during the intersexual phase of future testes. The significance of somatic elements of gonads in testicular differentiation in fishes has been emphasized also by other authors (Harrington, 1975), and should be substantiated by further studies including experimental ones.

Ovarian nature in developing gonads of male Sumatra barb is exhibited also by the presence of a cavity along the dorsal border of gonads. The cavity is formed at the same time and through the same process as the ovarian cavity constructed in decisive ovaries, but never develops to act as a route of sperm transport in differentiated testes. On the contrary, the cavity is often seen to disappear as the testis grows in young males. A sperm transporting system is formed newly in the stromal tissue as a longitudinal system of anastomosed lumina at the hilum of the testis. These are also the cases for male gonads with juvenile intersexuality found in the zebrafish (Takahashi, 1977) and the rose bitterling (Shimizu, 1979), though in the zebrafish, residual ovarian cavities are retained in adult testes and seem to function at least as a part of the sperm duct system. In the goldfish, too, a homolog of ovarian cavity exists, though only rudimentarily, in the hilar region of adult testes (Takahashi and Takano, 1972). The behavior of such sex-specific somatic structures as the ovarian cavity might reflect the degree of a lability of

gonadal sexes in originally gonochoristic species of teleosts.

In one brood of the Sumatra barb observed in the present study, a distinct delay in body growth occurred in fry and juvenile fish, which was accompanied by a prolongation of the sexually indifferent stage in their gonads. In many of the future males of that brood, sexually indifferent gonads were observed to differentiate directly into testes following remarkable proliferation of stromal somatic cells, without showing evident signs of juvenile intersexuality. The exact cause of the delay in body growth is not clear in this case. However, the fact reminds the present writers of the induction of "primary males" in a simultaneous hermaphroditic cyprinodont, *Rivulus marmoratus*, by experimental control of environmental factors (Harrington, 1967), though environmental influences on sex differentiation of the gonad in fishes are still a matter for debate (Reinboth, 1980). On the other hand, references to earlier investigations reveal some discrepancies as to the occurrence of juvenile intersexuality in the same species of teleosts: in the rainbow trout, *Salmo gairdneri*, juvenile intersexuality was noted by Mřsić (1930) but not by Padoa (1937), and in the guppy, *Poecilia reticulata*, it was described by Dildine (1936) but was denied by Miyamori (1964). It is quite uncertain at present whether or not a lability of sex determining mechanism may differ in degree genetically in different broods or individuals of the Sumatra barb as well as the fishes cited above.

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