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Scale Growth and Squamation Chronology for the Laboratory-Reared Hermaphroditic Fish *Rivulus marmoratus* (Cyprinodontidae)

Eun-Ho Park and Seung-Hwi Lee

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Abstract Scale morphology, growth and the squamation chronology are described for the hermaphroditic fish *Rivulus marmoratus* reared in the laboratory. The scales are round or oval shaped cycloid type, and their sizes are about 0.3–1.0 mm in diameter. The number of ridges increases more rapidly relative to the body growth of the fish in early stages, but this increase is proportionate to growth subsequently. Three loci of scale development have been identified. The scales first appeared on the center of the parietal region at 8 days after hatching. The second locus of scale formation was on the lateral line of the posterior end of the caudal peduncle. A third locus was later observed on the lower right corner of the operculum. The final squamation was completed at 6 weeks after hatching.

The oviparous cyprinodontid fish *Rivulus marmoratus* Poey inhabiting the brackish waters throughout the Caribbean is the only known vertebrate that naturally exhibits functional hermaphroditism with internal self-fertilization (Harrington, 1963). This species has received special attention not only because of interest in reproductive biology (Harrington, 1967, 1968, 1971, 1975) but also because of its potential as a laboratory animal for the study of experimental carcinogenesis (Park and Kim, 1984a).

R. marmoratus has several desirable attributes as an experimental animal for educational and research purposes in fish biology: A given population of this species is genetically homozygous due to functional hermaphroditism (Kallman and Harrington, 1964). The generation time is only 4–6 months; every day a mature individual lays several eggs which are large enough (1.8 mm, diameter) to handle, and they develop normally at room temperature outside the body (Harrington and Crossman, 1976) in 14 days thus enabling us to examine detailed developmental sequences through their transparent chorion. In addition, the fish are small (3–5 cm), hardy and easy to cultivate in the laboratory.

The early life history including sex determination of this species has been meticulously documented (Harrington, 1961, 1963, 1967, 1968, 1971, 1975; Harrington and Kallman, 1968). Little study, however, has been conducted on the detailed anatomy of this remarkable species. As part of an investigation of the comparative anatomy of this species, the scale morphology, growth and development were studied to accumulate biological information on this species. This information may serve as a baseline in normal morphogenesis of scales during larval and juvenile stages of *R. marmoratus*.

Materials and methods

Laboratory culture. The parental stock of *R. marmoratus* was obtained from the Zoology Institute and Museum, University of Hamburg, Federal Republic of Germany in 1981. Adult fish were kept singly in 20 *l* glass aquarium filled with 15 *l* of tap water. The salinity of water was adjusted to 15‰ with crude salts. All fish were kept in a room where the temperature was adjusted at 25±1°C and which was illuminated by fluorescent lamps for 14 hours daily.

Larvae and fish younger than 1 month were fed brine shrimp (*Artemia salina*) nauplii (eggs from Metaframe, Calif.), and adult fish were nourished with chicken liver, *Drosophila* and earthworm.

Sampling and analysis. Fertilized eggs were separated from parent fish and incubated in 1 *l* glass chambers. The hatched larvae were examined under a low-power microscope and any which appeared grossly abnormal were removed. The normal larvae were cultivated in homogeneous age groups for analysis. The fish density was

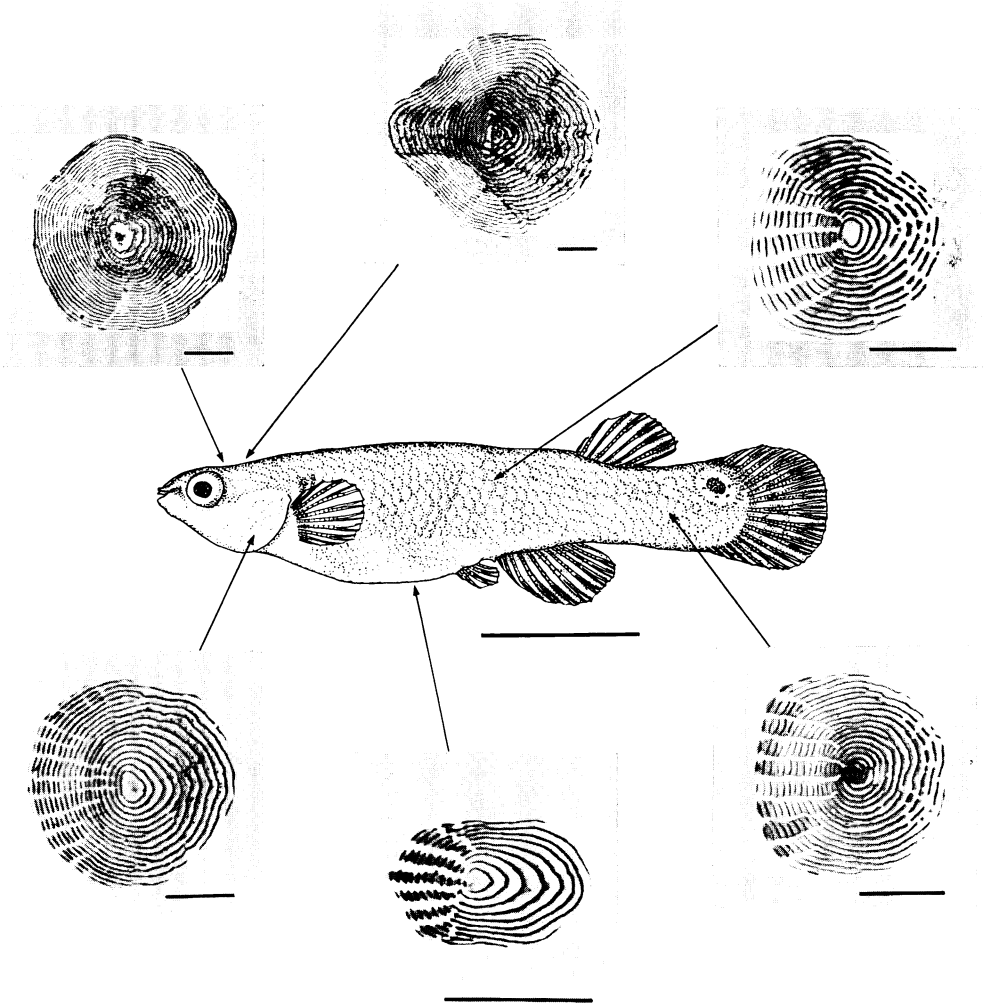

Fig. 1. Photomicrographs of scales from different locations of the skin in *Rivulus marmoratus* adult. Bar for fish=1 cm, bars for scales=0.2 mm.

maintained identically (1 fish/*l*) in experimental groups to avoid density dependent growth inhibition.

Fish were sampled at appropriate intervals to analyze scale development until 12 weeks after hatching. They were starved for 1 day prior to being sacrificed to empty the alimentary canal, and fixed in 10% neutral formalin. The fixed specimens were bleached with alkaline H_2O_2 to remove body pigments which obscure scale structures. Scales were stained with alizarin red S and cleared on whole fish according to the method previously described (Park and Kim, 1984b).

Ten fish were examined in each age group for analysis of scale growth pattern and squamation chronology. Scale morphology was examined from ten 3-month-old adult specimens.

Results

Scale morphology. The scales of adult *R. marmoratus* were the typical cycloid type ranging from round to oval shape, and their sizes were about 0.3–1.0 mm in diameter (Fig. 1). The smallest scales were oval and distributed on the ventral side of the trunk. The largest round scale has an anterior field medially, with concave anterolateral margins and found only on the parietal region. The remaining body surface was covered with medium sized round scales (Fig. 1).

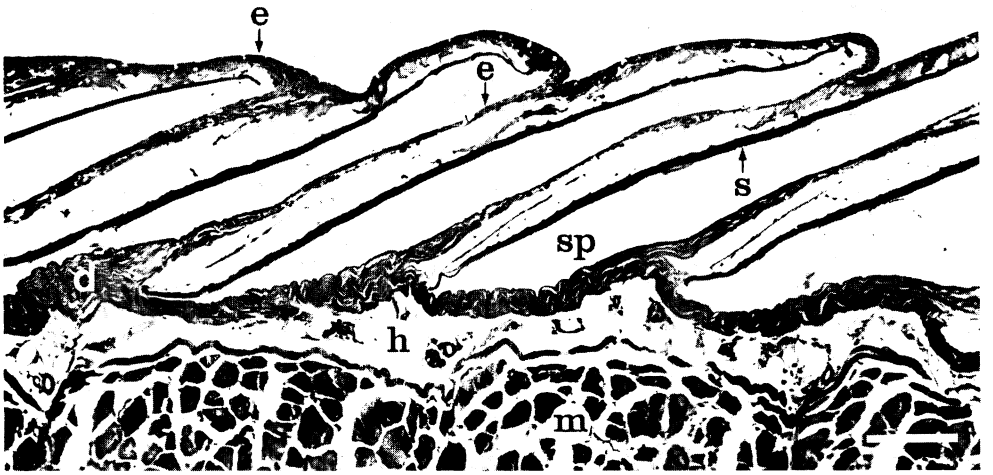

Fig. 2. Photomicrograph of longitudinal section of *Rivulus marmoratus* skin showing scale imbrication. Bar=100 μm. e, epidermis; d, dermis; h, hypodermis; m, muscle; s, scale; sp, scale pocket.

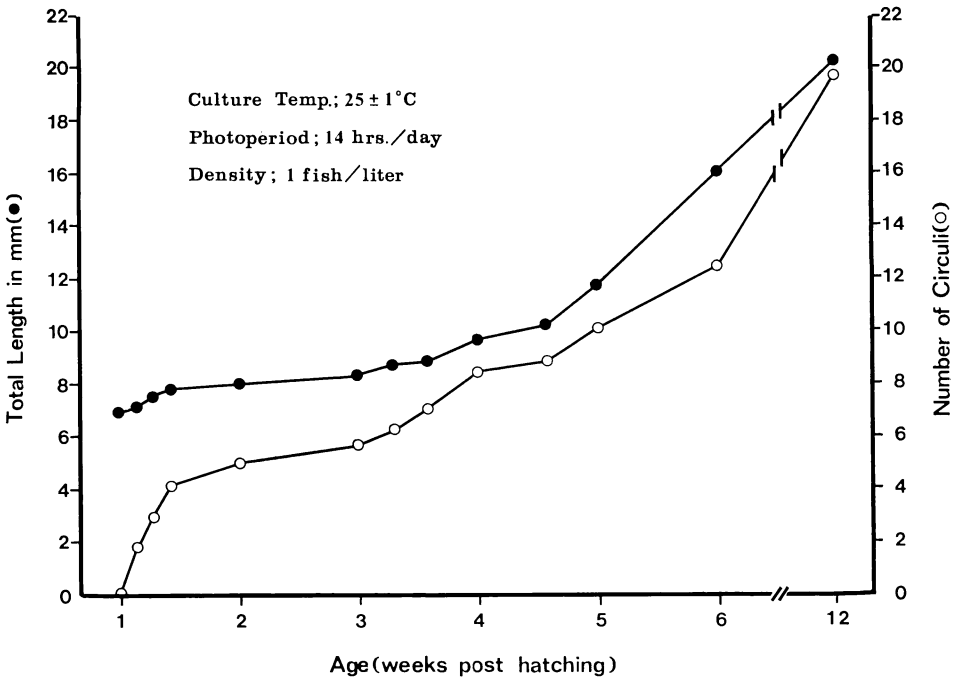

Fig. 3. Diagram showing the increase of circuli number on scales from the parietal region against age and total length of *Rivulus marmoratus* during 12 weeks after hatching. Each point represents the mean for 10 specimens.

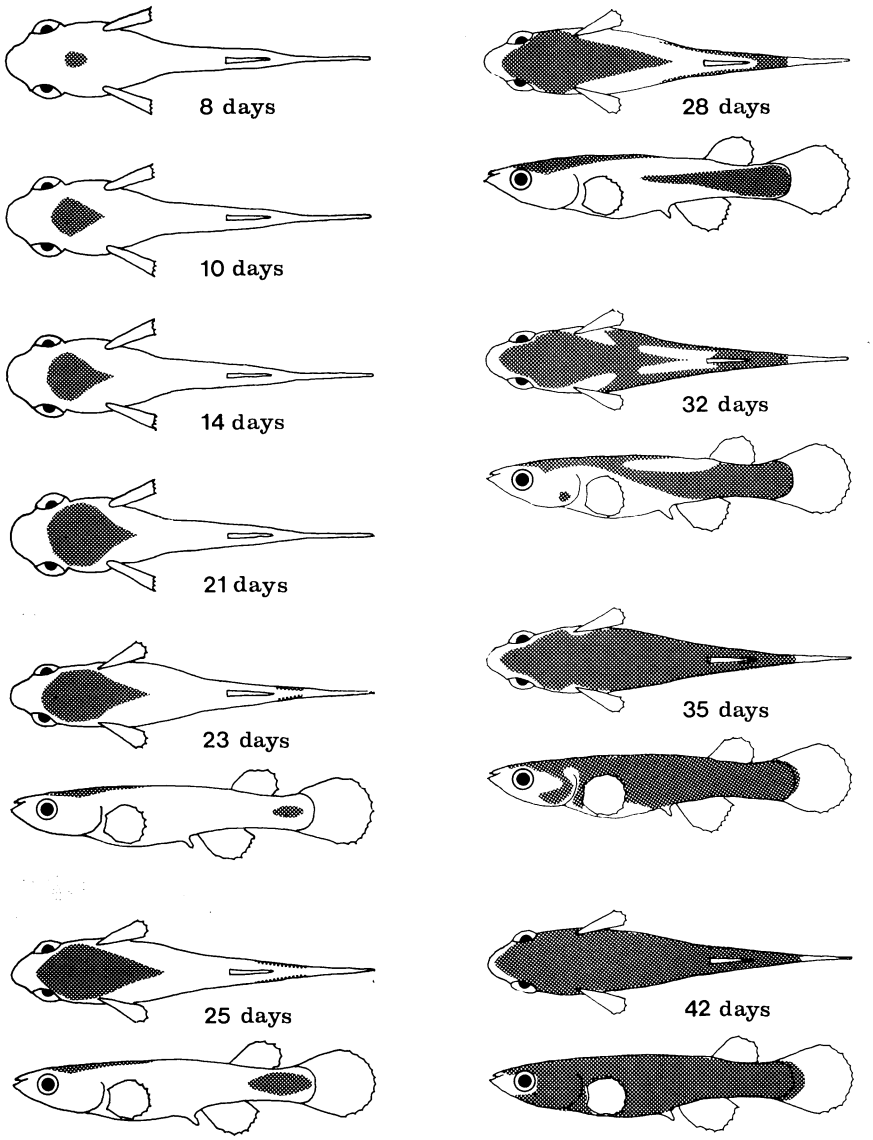

Fig. 4. Diagrammatic representation of sequence of squamation in *Rivulus marmoratus* after hatching. 8 days, $\bar{x}=7.09\pm0.28$ mm; 10 days, $\bar{x}=7.77\pm0.18$ mm; 14 days, $\bar{x}=8.01\pm0.31$ mm; 21 days, $\bar{x}=8.28\pm0.32$ mm; 23 days, $\bar{x}=8.74\pm0.36$ mm; 25 days, $\bar{x}=8.78\pm0.27$ mm; 28 days, $\bar{x}=9.71\pm0.57$ mm; 32 days, $\bar{x}=10.21\pm0.56$ mm; 35 days, $\bar{x}=11.73\pm0.94$ mm; 42 days, $\bar{x}=16.1\pm1.28$ mm. $\bar{x}$=average total length$\pm$S.D.

The scales were imbricated with the posterior field exposed; the anterior and lateral fields were embedded in the dermis. Histologically, they lay obliquely in a scale pocket surrounded by dermal tissue, which in turn were covered by an epidermal layer and a heavy layer of mucus (Fig. 2). Clear ridges (circuli) were visible in every scale, and they were discontinuous at the radii on the anterior field (Fig. 1).

Scale growth. The scales from a restricted area on the parietal region where the fisrt scales appeared were carefully examined and the number of ridges counted against the age and size (total length) of the fish. Fig. 3 shows the results ob-

tained. Each point represents the average number of ridges found for at least ten scales from each age group of fish. There was a relatively high rate of scale growth (ridge increase) during the early stage of fish growth, and then a steady decline when the number of ridges increased proportionately to the growth of the fish.

No annuli were formed even in eight fish older than 2 years in the laboratory.

Squamation. The origin of scales and the squamation for *R. marmoratus* are illustrated in Fig. 4. The initial site of scale formation was near the center of the parietal region. The first scale appeared on the center of the junction of the frontal and parietal bones at 8 days after hatching (average total length; 7.09±0.28 mm). No scales were observed on fish younger than 8 days. Scale development progressed concentrically to cover the entire head surface. The second locus of squamation was midlaterally at the posterior end of the caudal peduncle. Scale formation on this site proceeded anteriorly along a midlateral line, and met with the scales from the first locus on the dorsal region of the trunk. At 32 days after hatching, the scales of a third locus developed on the lower right corner of the operculum. Squamation here progressed dorsally and anteriorly, finally connecting with those from the first locus. At this time (5 weeks post hatching), the squamation was nearly completed except in the anterior region of the operculum and the central region of the belly. Scale formation was completed at 6 weeks post hatching. Nearly the entire body surface was completely scaled. The average size of specimens at this stage was 16.1± 1.28 mm, and the number of scales along their lateral line was counted to be 41–51 ($\bar{x}\pm$S.D.= 46.1±2.3, n=102).

The degree of squamation in fish within the same age groups was somewhat variable among individuals. The larger fish were observed to show more developed squamation than smaller ones.

Discussion

The present study is the first description of the morphology, growth and developmental sequence of the scales in *R. marmoratus*. The gross morphology and histological arrangement of scales are similar to other teleosts (Harder, 1975). Scale growth has been extensively studied to determine the age, life history and rate of growth of the fishes. The pattern of scale growth versus the growth of *R. marmoratus* is also similar to that of other fishes investigated (Wallin, 1957).

Most studies on squamation chronology have been made with species of the families Carangidae (Berry, 1960), Catostomidae (White, 1977), Centrarchidae (Siefert, 1965; Conley and Witt, 1966; Cooper, 1971), Clupeidae (Chaptoton, 1967), Coregonidae (Hoagman, 1970), Cyprinidae (Ward and Leonard, 1954; McCrimmon and Swee, 1967; Andrews, 1970; Armstrong, 1973), Esocidae (Franklin and Smith, 1960), Hexagrammidae (Fukuhara and Fushimi, 1984), Megalopidae (Harrington, 1958), Pomatomidae (Silverman, 1975), Salmonidae (Elson, 1939; Neave, 1943; Brown and Bailey, 1952; Warner and Havey, 1961) and Sciaenidae (Priegel, 1966). No report has been, however, published for any member of the family Cyprinodontidae.

The squamation pattern of *R. marmoratus* differs from that of other species. Although the location and number of loci where the squamation initiates vary among species, the single locus on the caudal peduncle has been reported in most species and may be the most commonly occurring pattern (Conley and Witt, 1966; Priegel, 1966; Andrews, 1970; White, 1977). Two loci have been found in the zebrafish, *Brachydanio rerio* (Armstrong, 1973) and in the greenling, *Hexagrammos otakii* (Fukuhara and Fushimi, 1984), one on the anterior portion of the trunk and the other on the caudal peduncle. In the black crappie (*Pomoxis nigromaculatus*), four loci of scale development have been identified; squamation first appears at the posterior of the anal fin and anterior of the pelvic fins. A fourth locus is the most anterior region of the lateral line (Cooper, 1971).

Even though the loci may vary, squamation usually progresses along the lateral line and then onto the dorsum and venter. This pattern is fairly consistent among the many species investigated. *R. marmoratus* is unusual in the number of loci (3), the squamation pattern, and the parietal region as the initial site of scale development.

Individual variation in the degree of scale development within the same age groups of *R. marmoratus* suggests that scale development is correlated with both size and age of fish. Scale development in the zebrafish is related to length

rather than age (Armstrong, 1973). Age as a main factor in determining squamation has been, however, suggested in the black crappie (Ward and Leonard, 1954; Cooper, 1971).

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(Department of Biology, College of Natural Sciences, Hanyang University, Seoul 133, Korea)

雌雄同体性メダカ目魚類 *Rivulus marmoratus* の鱗の生長と形成過程

Eun-Ho Park • Seung-Hwi Lee

実験室で飼育した雌雄同体性メダカ目魚類 *Rivulus marmoratus* の鱗の形質，生長および発生過程について観察した．鱗は円形又は長円形の円鱗でその大きさは直径にして 0.3–1.0 mm である．隆起線は魚の成長の初期段階でその数を急激に増すが，その後は成長と相対的な増加をした。鱗は 3 つの部位から発生した。まずふ化後 8 日目の仔魚の頭頂部，ついで尾柄部側線上の後方，そして鰓蓋下方部から出現した．鱗の形成はふ化後 6 週間で完了した．