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[Short Communication]

Changes in the Distributions of Chemical Elements in Regenerating Scales of Carp, *Cyprinus carpio*, Studied by SRXRF Imaging

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ABSTRACT—Two-dimensional distributions of calcium, phosphorus, strontium, and zinc in regenerating scales of carp, *Cyprinus carpio*, were observed over a period of 90 days by utilizing synchrotron radiation-excited X-ray fluorescence (SRXRF) imaging. On the 15th day, these elements were highly accumulated in the part that is exposed to the ambient water, and the accumulated amount decreased linearly toward the basal edge through the focal area of the scale. As the regeneration progressed, this characteristic pattern changed such that the amount of each element in the rim part, which was exposed to the ambient water, obviously increased, while the amount in the basal edge decreased. However, the relative amounts of phosphorus, strontium, and zinc with respect to calcium in the regenerating scale remained almost constant during the regeneration. In addition, it was found that the diameter of, and the number of ridges in, the regenerating scales increased rapidly during the first 15 days, and increased at a slower pace later on.

INTRODUCTION

Fish scales can be regenerated in a short period of time. An investigation of the fine structure of regenerating scales and their associated cells revealed that the deep scleroblasts are responsible for the formation of the basal plate, while marginal scleroblasts increase the diameter of the osseous layer (Sire and Géraudie, 1984). The formation of the matrix of a regenerating scale was also found to be different from that of a normal scale in that scleroblasts, which produce the collagen fibrils that form the basal plate, are prismatic in the former and very flat in the latter (Zylberberg *et al.*, 1992).

The effects of environmental changes on the inorganic composition of the calcified structures of fish, such as scales, have been studied by various methods (Moreau and Barbeau, 1983; Sauer and Watabe, 1989). Since the growth of the regenerating scale is much faster than that of the normal scale, the intake of the constituent elements of the regenerating scale must be quite rapid and would reflect the environmental conditions. However, little is known about the process of the accumulation of chemical elements and their distributions in the regenerating scale. In particular, it is important to study the distribution in relation to morphology of the regenerating scale.

Synchrotron radiation-excited X-ray fluorescence (SRXRF) imaging (Iida and Gohshi, 1991) is an effective method for non-destructive two-dimensional analysis to obtain information on constituents of animal tissues. This method has also shown promise in the field of biomineralization (Okoshi *et al.*, 1991), and has recently been used to demonstrate the distributions of chemical elements in the normal scales of red sea bream, *Chrysophrys major* (Ishikawa *et al.*, 1991). Non-destructive two-dimensional imaging is particularly appropriate for clarifying the process of regeneration because the growth of the regenerating scale is mostly two-dimensional.

In this study, we have tried to clarify the changes in the distributions of the chemical elements, calcium, phosphorus, strontium, and zinc, during regeneration of the scale of carp, *Cyprinus carpio*, by SRXRF imaging. Calcium and phosphorus are the most important consisting elements of scale. Strontium is a bone-seeking element and its concentration is strongly correlated with that of calcium in fresh water fish (Rosenthal, 1957). Investigation of the distribution of zinc is important because zinc is thought to behave in fish body in the similar way as cadmium and mercury (Sorensen, 1991). In addition,

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morphological measurements of the regenerating scale have been carried out in order to investigate the growth rate.

MATERIALS AND METHODS

Sampling and morphological measurements

Six carp, *Cyprinus carpio*, were used for the morphological study. The average weight of the fish was 30.8 ± 4.4g. The fish were maintained in a 50 liter tank at a temperature of 25 ± 1°C, and were fed with commercial pellets at a rate of 1% body weight per day. At the beginning of this study, three scales at fixed positions A, B, and C, behind the head and above the lateral line on the left side of the fish body were surgically removed. The regenerating scales at positions A, B, and C were taken on days 15, 30, and 90, respectively, for morphological and X-ray studies. After the regenerating scales were washed with distilled water, the number of ridges on the basal edge was counted and the regeneration ratios were determined by light microscopy. The morphology of a regenerating scale is shown in Fig. 1. The regeneration ratio (%) was obtained by dividing the diameter of the regenerating scale by the diameter of the original scale, where the diameter is the distance between the cranial and the caudal edge.

X-ray detection and imaging

Measurements were made on beam line 4A of the Photon Factory at the National Laboratory for High Energy Physics. SRXRF imaging was performed on the regenerating scales from one of the six fish used for the above morphological measurements. The specimens were first dried completely before being attached to a Mylar membrane backed by a plastic frame. The specimens were kept in a vacuum of 10^{-2} torr. Synchrotron radiation was monochromatized by a Si(111) double crystal monochromator. The energy of incident X-rays was 18 keV. The angle between the incident beam and the sample surface was 45°. The incident X-ray intensity during the measurements was monitored by an ionization chamber and was used to normalize fluorescent X-ray intensities. Since the variation in the incident intensity during the whole measurement was less than 5%, the data shown below are those before the normalization. The fluorescence X-rays were detected by a Si(Li) solid state detector. Two-dimensional analyses were carried out by mounting scale samples on an X-Z stage under the following conditions: beam size = 300 mm × 300 mm, step size = 150 mm/step, counting time = 5 sec/pixel.

RESULTS AND DISCUSSION

Fifteen, 30, and 90 days after the original scales were removed from six carp, the regeneration ratios of the regenerating scales as the mean ± SD were 82.6 ± 3.3%, 96.0 ± 2.9%, and 100.3 ± 2.4%, respectively, and the number of ridges on the regenerating scales as the mean ± SD were 13.4 ± 0.6, 22.0 ± 2.1, and 29.2 ± 3.1, respectively. These data show that the diameter and the ridge number of the regenerating scales increased rapidly until the 15th day, and then continued to increase but at a slower rate. At the last stage of regeneration, the ridges became as clear and solid as those of normal scales.

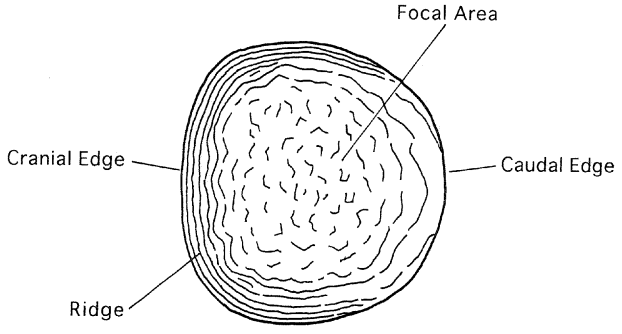

Fig. 1. Illustration of the morphology of a regenerating scale of carp.

The X-ray fluorescence spectrum from the whole area of the regenerating scales is shown in Fig. 2. The main elements observed were calcium, phosphorus, strontium, and zinc. The distribution patterns of the above-mentioned elements in the regenerating scale are shown in Fig. 3. For mapping the distribution of the chemical elements, the peak areas of the Kα and Kβ lines of calcium and phosphorus, and the Kα lines of strontium and zinc were used. It should be noted that for each of the studied elements, the highest amount was observed in the region that was exposed to the ambient water. The amount decreased linearly toward the basal edge through the focal area of the scale. As the regeneration progressed, this characteristic pattern changed in such a way that the amounts of the elements in the rim part, which was exposed to the ambient water, obviously increased, while the amounts in the basal edge decreased. To clarify this trend, the distribution of calcium along the central line from the cranial edge to the caudal edge was measured (Fig. 4). The ratios of the integrated intensity of phosphorus, strontium and zinc lines with respect to the calcium line were approximately 0.07, 0.04, and 0.02, respectively. There were no distinct changes in any of these ratios during the regeneration process.

It was found that the growth of the regenerating scale of carp is most rapid in the first 15 days after descaling. This finding is consistent with observations of the calcium intake of regenerating scales in goldfish, *Carassius auratus* (Mugiya, 1980).

The main elements observed in the regenerating scale of carp were calcium, phosphorus, strontium, and zinc. This

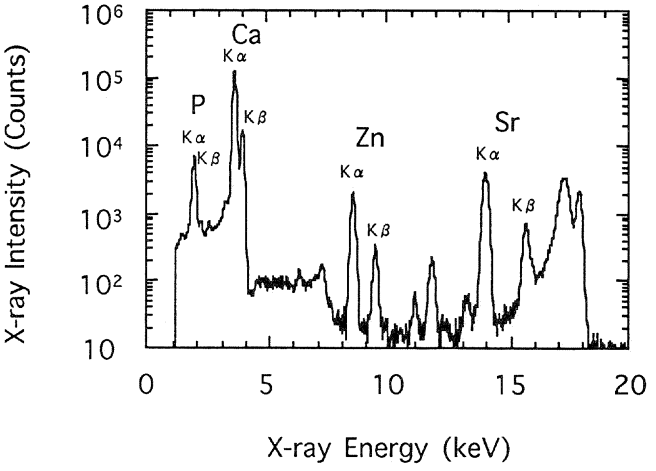

Fig. 2. The fluorescence spectrum of a regenerating scale on the 90th day. Spectrum was taken by scanning the X-ray beam over the whole area for 7000 seconds.

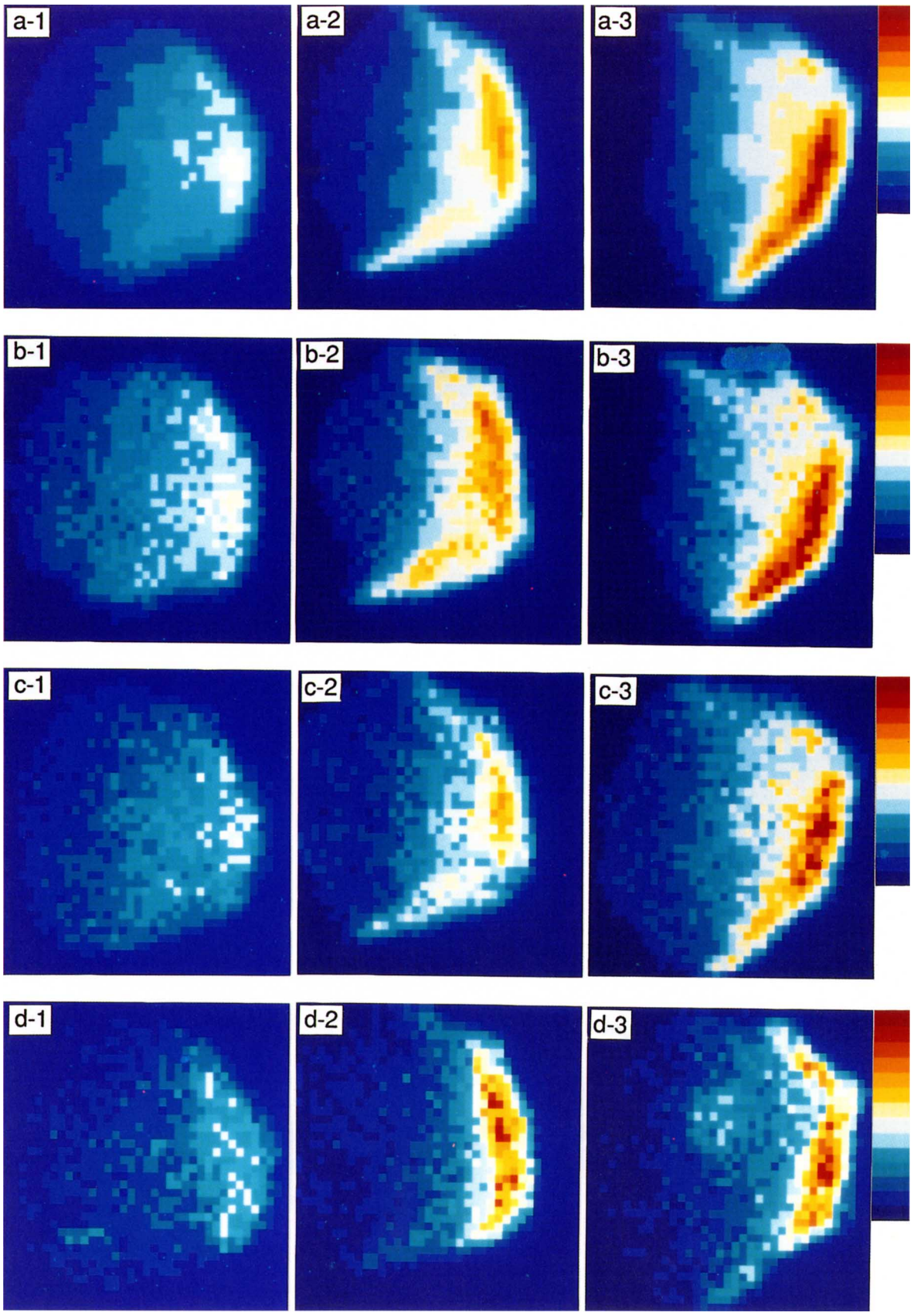

Fig. 3. The distribution patterns of calcium (**a**-**1**~**3**), phosphorus (**b**-**1**~**3**), strontium (**c**-**1**~**3**) and zinc (**d**-**1**~**3**) in regenerating scales on the 15th (**1**), 30th (**2**), and 90th day (**3**). The maximum intensities of calcium, phosphorus, strontium and zinc were 4089, 277, 173 and 98, respectively. The left side is the basal edge of the regenerating scales. The highest intensity corresponds to the red region. The fields of view are 5.55 mm × 5.55 mm.

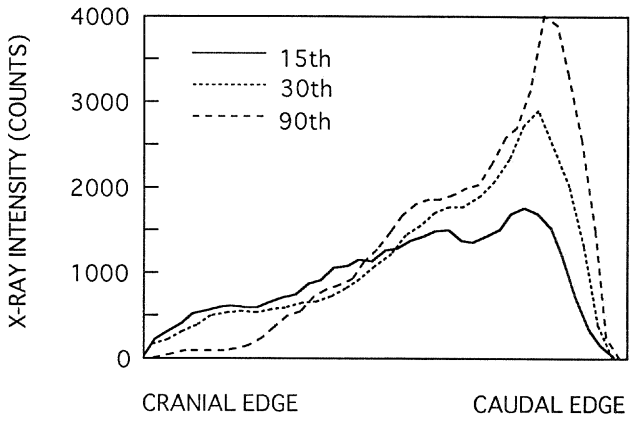

Fig. 4. The distribution of calcium along the central line from the cranial edge to the caudal edge of the regenerating scales on the 15th, 30th, and 90th day.

result is fairly consistent with the previous studies on the normal scale of sockeye salmon, *Oncorhynchus nerka* (Lapi and Mulligan, 1981) and red sea bream (Ishikawa *et al.*, 1991).

The present results show for the first time that the above-mentioned elements are unevenly distributed over the regenerating scale and are accumulated mainly in the part which is exposed to the ambient water.

Compared with the distributions of calcium and strontium in normal scales of red sea bream (Ishikawa *et al.*, 1991), the distribution patterns in the present study are different. The distributions in the scale of red sea bream are patterned mostly along the grooves. However, the distributions are also peaked in the exposed part.

It is suggested that the accumulation of calcium in the scales occurs not directly from the ambient water but via the blood (Ichii and Mugiya, 1983). The sources of calcium for regenerating scale are the bones and other scales in the fish body as well as the ambient water and diet (Weiss and Watabe, 1978; Mugiya, 1980). From a physiological point of view, it is interesting to note that before the regenerating scale grows to full size, the elements are transported rapidly into the part of the scale that is exposed to the ambient water. High accumulations of these elements in the descaled and exposed portion may be interpreted as some kind of protection for the fish body.

The present results show that trace elements in regenerating fish scales, as shown by SRXRF imaging, accumulated mainly in the part that is exposed to the ambient water.

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