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Growth of the Medaka (V) – Formation and Development of Otoliths in the Inner Ear During Metamorphosis

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ABSTRACT

The purpose of the present study is to provide information on formation and development of otoliths in the inner ear during the period of growth of the medaka from larva to juvenile. Before hatching, there are already two kinds of otoliths i.e. utricular and saccular otoliths in the optic capsules. After hatching, lagenar otoliths newly form in the most posterior region of the membranous labyrinth. Lagenar otoliths in one side of the lateral inner ear appear first in larvae about 6 mm total length (TL). The first otoliths form morphologically as a conglomerate of small granules or spherules. Frequency of individuals with lagenar otoliths increases linearly in proportion to the body size from about 6 mm to 9 mm TL. In larvae more than about 5 mm TL each otolith increases in diameter proportional to body growth, although large saccular otoliths exhibit a slightly steeper rise in diameter in comparison with other otoliths. As a whole, otoliths also exhibit a slow increase in thickness proportional to body growth during metamorphosis.

Keywords: otolith formation, metamorphosis, larva, medaka

INTRODUCTION

In teleosts, the inner ears possess three pairs of otoliths, utricular, saccular and lagenar. These fish otoliths are generally involved in the detection of gravitational force and sound (Popper and Lu, 2000). Few investingators have studied morphological and physiological formation of otoliths in the inner ears of growing fish of the medaka *Oryzias* species. Also, the formation and development of otoliths corresponding to body size during the growth period from the larva (fry) to the juvenile have hardly been investigated. At hatching, larvae of the medaka (*Oryzias latipes*) already have two pairs of otoliths, i.e., utricular and saccular in the inner ears, but the size of these otoliths is still small. Just after hatching, lagenar otoliths in the most posterior region of the membranous labyrinths have yet to appear. The present author was conscious of the need to examine these events on development of otoliths during the growth period.

Little meticulous basic study has been conducted on the morphological changes in the otoliths in the medaka during the metamorphic period from larva to adult. Therefore, the present study addresses the issue of the changes in the early process of formation and development of otoliths during metamorphosis. Resolving this issue should contribute to clarifying the behavior of medaka.

MATERIALS and METHODS

Specimens of the medaka fish, *Oryzias latipes* (d-*rR* strain, 3 females and 2 males), used in the present study were reared in a rectangular glass aquarium (60×35×30 cm, about 60 liters of water) with an appropriate feeding regimen and temperature and light conditions (L14: D10, 26–28°C). During the rearing period, fish were fed four times a day a balanced diet containing one part each of shrimp powder and parched barley flour, supplemented with an equal volume of powdered diet for the juvenile goldfish (Ryukin). Immediately after hatching, larvae were fed the balanced diet in rainwater containing green algae in a rat PC (polycarbonate) cage (18×26×42 cm) and reared under the same breeding conditions as those of adult fish.

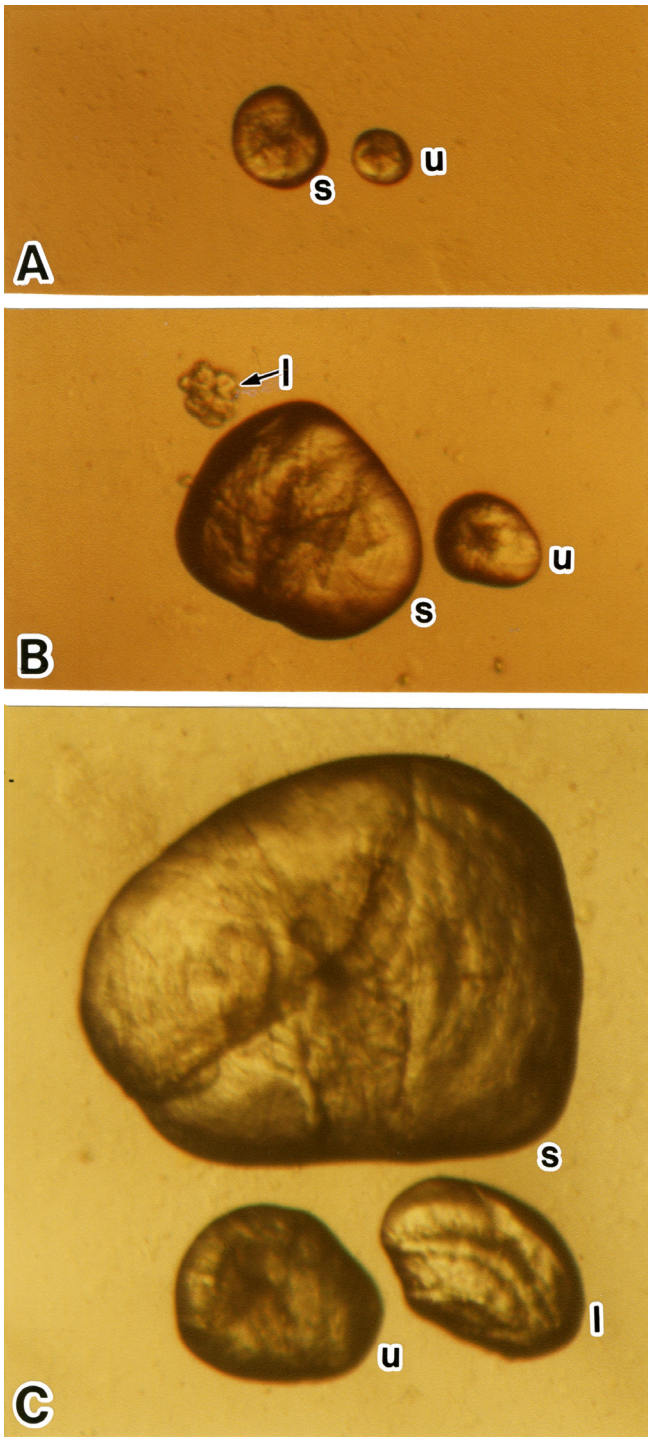

Fig. 1 Microphotographs of otoliths in growing medaka during metamorphosis.
A: Just after hatching, 4.8 mm TL. (x 138), B: 7.7 mm TL (× 135), C: 15.0 mm TL (× 113). l, lagenar otolith; s, saccular otolith (arrow, a conglomente of spherules); u, utricular otolith.

For collection of fertilized eggs, spawned females were netted, and clusters of chorion-hardened, fertilized eggs that hung from their urogenital pores were plucked off manually. Under a stereoscopic microscope (Olympus SZX12) with substage illumination, long attaching filaments on the chorion of each egg were carefully grasped with watchmaker forceps and cut off at their base with the blunt tip of a small glass rod as the egg was pulled away.

In the present study examining the developmental processes of otoliths in the inner ear, the stages of body growth are represented by total length (TL: the distance from the anterior tip of the lower oral jaw to the posterior edge of the caudal fin) rather than age. To examine otoliths, fish were anesthetized in a saline solution containing a mixture of 7 parts of phenylurethane and 3 parts of ethanol. Body size (TL) was recorded on deeply anesthetized fish (cf. Iwamatsu, 2006). Treated with 5% KOH for about 10 min in room temperature, the head was adequately rinsed once in 0.1 M phosphate buffer solution (pH 7.2) containing alizarin red. Then the head was carefully dissected, and the membranous labyrinths were separated as individually as possible using a pair of small scalpels under a stereoscopic microscope (× 50–63). The size (diameter) of each otolith isolated from a membranous labyrinth was measured precisely to the nearest micrometer line of a calibrated ocular micrometer ruled to 9 μm diameter. The mean values were plotted on section papers. Photomicrographs were made with an Olympus SZX12 microscope equipped with substage illumination and an Olympus automatic camera (Y-E330 super system). Growth stages of fish were assigned following the author's developmental criteria (Iwamatsu, 2004, Iwamatsu *et al.*, 2003).

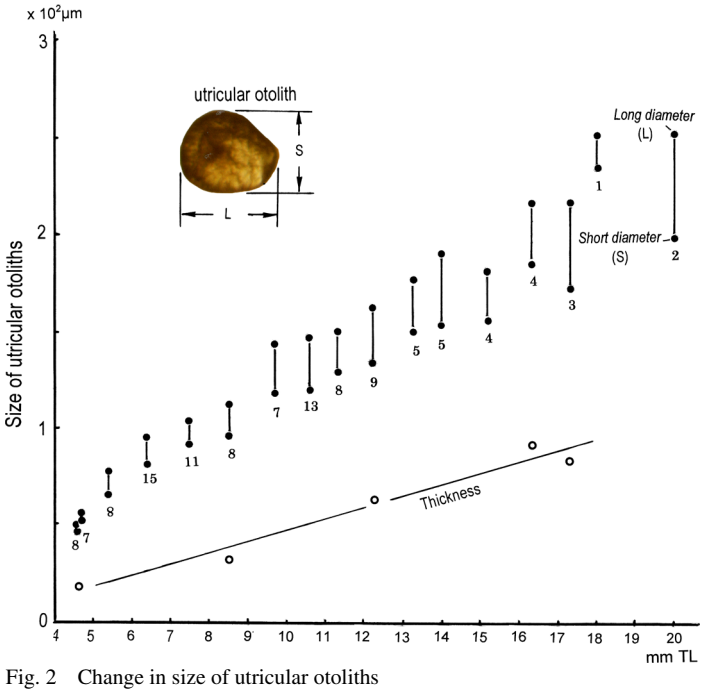

Fig. 2 Change in size of utricular otoliths during metamorphosis.

OBSERVATIONAL RESULTS and DISCUSSION

Two pairs of otoliths (utricular and saccular otoliths) appear respectively as a pair of granular aggregations in each ear vesicle (membranous labyrinth) in medaka embryos at stage 25 which form at 18 to 19 somites (Iwamatsu, 1994, 2004). In zebrafish, the otic cavity contains precursor minute particles or spherules, secreted from the apical portions of the epithelial cells of the inner ear (Riley *et al.*, 1997). Otolith growth starts as a nucleus of spherules aggregating at the top of a tether cilium (Pisam *et al.*, 2002; Clendenon *et al.*, 2009). Recently, it has been found that otolith development of the zebrafish is critically dependent on flow forces generated by beating cilia, although the mechanism by which flow controls otolith formation is not understood (Wu *et al.*, 2011). In medaka larvae just after hatching, both utricular and saccular otoliths (Fig. 1) are recognizable in the membranous labyrinths at the lateral sides of the hind brain in the intact head under a dissecting microscope. Small utricular otoliths are situated in the utriculus (utricle) at the anterior position of the semicircular canals and large saccular otoliths occupy the posterior positons to them. But at this stage, lagenar otoliths cannot be detected in the inner ears under a dissecting microscope. According to Ijiri *et al.* (2003), dysfunction of utricular otoliths causes a defect that affects the gravity-detection abilities of medaka. That is, utricular otoliths seem to have a function as sensors for balance.

In a short period from the time of hatching to about 5 mm TL, in which lagenar otholiths are yet to be observable, early utricular and saccular otoliths enlarge rapidly (Figs. 2 and 3). In growing larvae about 6 mm TL, a lagenar otolith is first found in either the right or left inner ear. In larvae about 6 mm TL, lagenar otoliths detectable first in the most posterior region of semicircular canals are extremely small (45 µm in long diameter). More than 9 mm TL, all larvae have a pair of lagenar otoliths in the inner ears. Yet the mechanisms by which the period of appearance of lagenar otoliths is controlled remain unclear. In some larvae about 7 mm, a lagenar otolith is recognized as a conglomerate (about 45 µm in diameter) of small granules

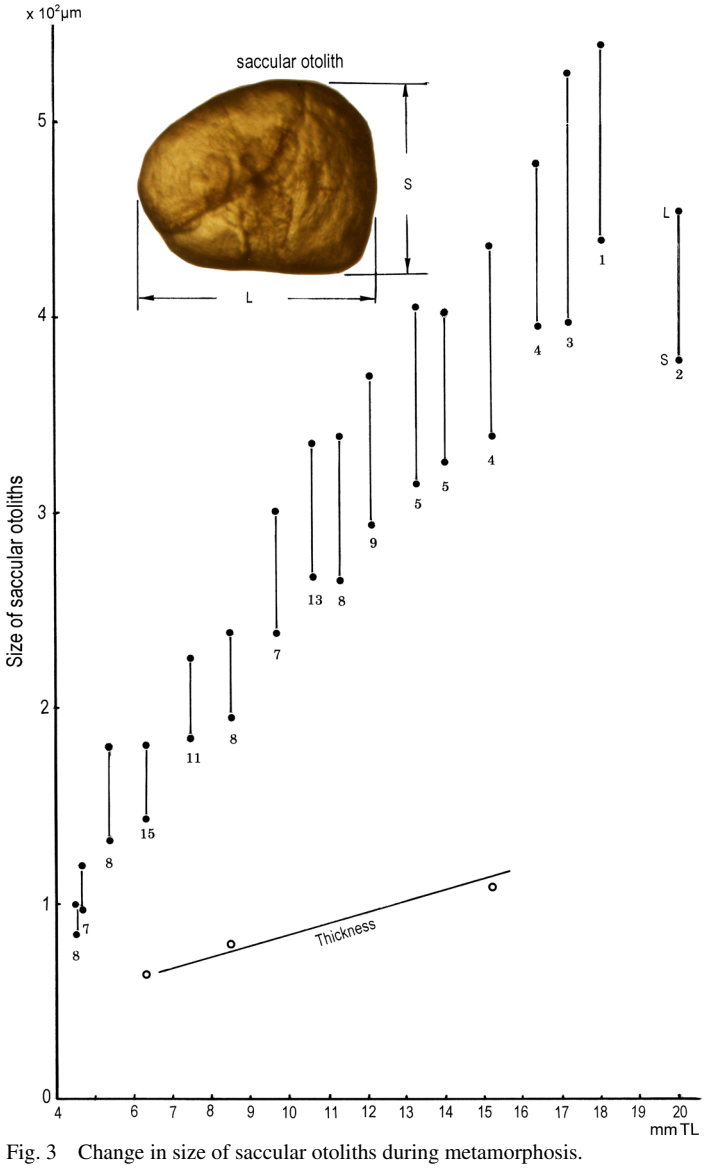

Fig. 3 Change in size of saccular otoliths during metamorphosis.

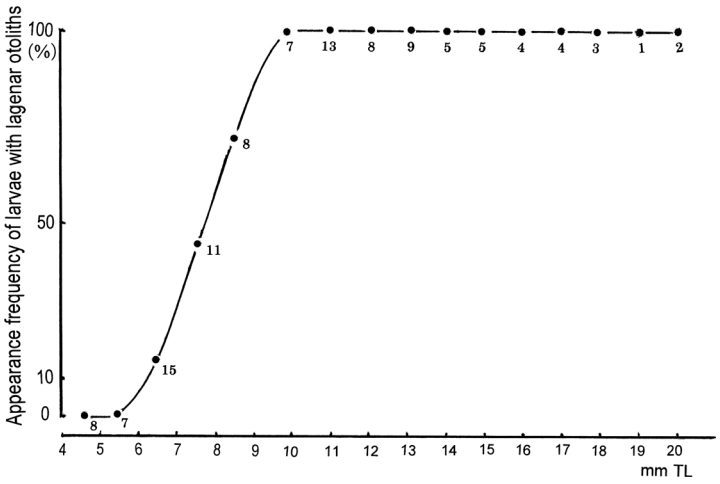

Fig. 4 Frequency of appearance of medaka larvae with lagenar otoliths after hatching. Numbers indicate the number of larvae examined.

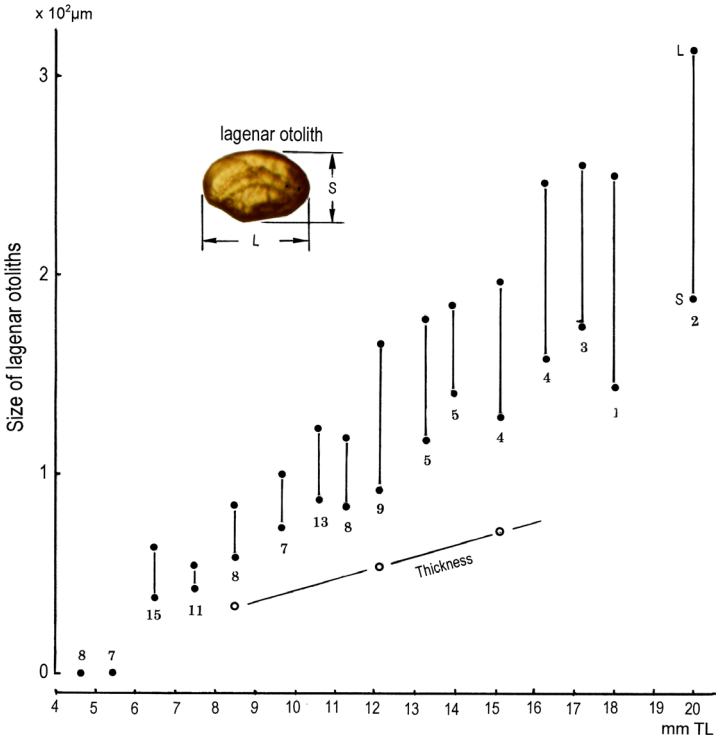

Fig. 5 Change in size of lagenar otoliths during metamorphosis.

or spherules (diameter 5–10 μm) (Fig. 1). The lagenar otoliths form in the same way as utricular and saccular otoliths form in embryos (Iwamatsu, 2004). Frequency of appearance of larvae with lagenar otoliths increases linearly from about 6 mm up to 9 mm TL (Fig. 4).

In medaka embryos, the zinc finger transcription factor *osterix,* which is expressed in the otic vesicle stage, is required for otolith formation (Renn and Winkler, 2014). According to Hughes *et al.* (2006), teleost otoliths are composed of calcium carbonate and an organic matrix made of glycoproteins, proteoglycans and collagens. The self-organizing properties of otolith formation could depend solely on the hydrodynamics mediated by cilia, diffusion and self-aggregation properties of the spherules (Wu *et al.*, 2011). The formation of minute spherules seems to be attributable to the binding of free calcium or calcium carbonate to calcium-binding proteins to coagulate or accumulate at small area in the membranous labyrinth of the inner ear.

When body size (TL) reaches about 9 mm, three kinds of utricular, saccular and lagenar otoliths are established in the inner ear. These otoliths appear on the macula of the membranous labyrinth in larvae. The size of these otoliths exhibits an enlargement in diameter and in thickness, proportional to that of body size during metamorphosis. The saccular otoliths which are anteroposteriorly long, at hatching are always larger in size (diameter) than other two otoliths (Fig. 3). In early small lagenar otoliths, the shape is already elliptic and flat in about 6 mm TL (Figs. 4 and 5).

In larvae 4.5 mm TL just after hatching, flat utricular otoliths (50–60 μm in long diameter) are 18–20 μm thick (Fig. 2). Larvae 12 mm TL have utricular otoliths about 160 μm in long diameter and about 63 μm in thickness. In the end (about 16.4 mm TL) of metamorphosis, the thickness of utricular otoliths becomes about 80 μm and exhibits a slow change, as that of other otoliths (Figs. 2, 3 and 5). These changes in thickness of otoliths during metamorphosis also exhibit in proportion to the increase in body size. Thus, we conclude that the size of otoliths which is closely correlated with that of body, seems to be a reliable way to estimate the fish age.

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