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Short Note

Visualization of skeletal structure in Sakhalin taimen, *Hucho perryi*, using *in vivo* three-dimensional micro X-ray computed tomography

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Abstract—Visualization of skeletal structure was examined in Sakhalin taimen (*Hucho perryi*) larva using in vivo three-dimensional micro X-ray computed tomography (3D micro X-ray CT) imaging. Clear 3D images of not only the vertebra, pleural rib, and otolith but also non-skeletal tissues, such as eye, gill, and alimentary canal were obtained. Deformation of caudal fin in the larva was also observed. These images provided precise morphological characteristics of *Hucho perryi*. 3D micro X-ray CT, therefore, would be a useful technique to investigate fish physiology and behavior research regarding fish growth and life history.

Key words: Sakhalin taimen, *Hucho perryi*, 3D micro X-ray CT, skeletal structure, visualization

Introduction

Sakhalin taimen (*Hucho perryi*), a typical species of salmonid (Yamashiro 1965), is the largest salmonid in the western Pacific Ocean (Gritsenko et al. 1974) and with distribution restricted to Sakhalin, the Southern Kuriles, and eastern Siberia (Kimura 1966). In Japan, *Hucho perryi* is the largest freshwater fish and found only in Hokkaido. It is now seriously endangered and close to extinction. The reasons for this situation are thought to be water pollution, indiscriminate fishing, and habitat loss such as deterioration from river construction such as weirs and dams. For the protection of the rare fish, ecological studies, such as the spawning, habitat, and migration of *Hucho perryi* have been increased (Edo et al. 2000; Fukushima et al. 1994; Sagawa et al. 2002; Suzuki et al. 2008). Skeletal tissues such as bone and otolith, and their morphology and constituents provide information about growth and environmental conditions of the fish (Radtke 1989; Arai 1996). Details of inner body morphology of the fish, however, were unknown. Three-dimensional micro X-ray computed tomography (3D micro X-ray CT) is a simple and useful way to investigate the distribution of skeletal tissues. Recently, the technology has been developing toward compact and high resolution imaging, and various types of systems have been introduced. In particular, *in vivo* 3D micro X-ray CT for experimental animals offers the possibility of obtaining skeletal information using a very short imaging time which avoids harm to the fish and is thus a suitable destruction-free method of analysis for the study of rare species. This application of the technology will bring new possibilities in related research. In this study, we tried to visualize of skeletal structure in *Hucho perryi* larva using in vivo 3D micro X-ray CT imaging.

Materials and Methods

A *Hucho perryi* larva was offered by the Shibetsu Salmon Museum in Hokkaido. The specimen was 0+fry and the fork length was 37 mm. The specimen was exposed to the contrast medium iopamidol (Iopamiron 300; Nihon Schering, Osaka, Japan) which was added to the rearing water 24 hours before CT imaging in order to enhance the image (Fig. 1). The final concentration of the contrast medium was 1.4%. On CT imaging, the specimen was rinsed with deionized water

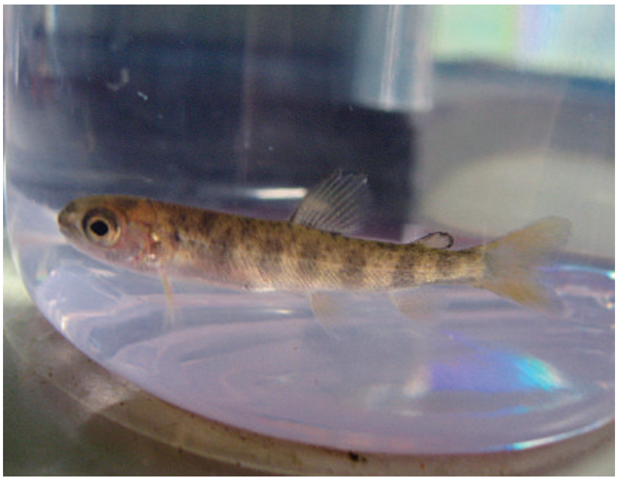

Fig. 1. *Hucho perryi* larva exposed with contrast medium.

Fig. 2. 3D micro X-ray CT sample stage.

to avoid any influence from the contrast medium adhering to the fish body. After the rinse, specimen was anesthetized and placed in a syringe on the sample stage (Fig. 2). The specimen was scanned using 3D micro X-ray CT (R_mCT System: Rigaku Corporation) in which the X-ray source was biased at 43 kV and the anode current was set to 200 μA. The scanning time was 17 seconds and 2 minutes. For visualization, obtained data were reconstructed and rendered using image processing software (i-VIEW-R; J. Morita Mfg. Corp). Field of view (FOV) is variable from ϕ5 mm by 5 mm to ϕ64 mm by 64 mm. Voxel size of FOV 5 mm and FOV 64 mm are 10 µm and 133 µm, respectively.

Results and Discussion

This experiment clearly showed the skeletal structure in *Hucho perryi* larva in high resolution images. Several representative slice images are shown in Fig. 3–5. These images provided views of skeletal tissue such as vertebra and pleural rib. In the image of the head, the otolith (lapillus, sagitta, and asteriscus) and the relation of surrounded tissues were clearly visible (Fig. 4). Lower jaw, cranium, and opercle were also seen. In a more detailed view, centrum and neural spine were also clearly observed. In addition, non-skeletal tissues such as eye, gill, and alimentary canal were also seen. In the image of the caudal fin, slight deformation of caudal skeleton was observed. Stills from a 360 degree view of the *Hucho perryi* larva head are shown in Fig. 6. This rotation view provided a 3D distribution of a variety of tissues and organs and made it easy to grasp their locations.

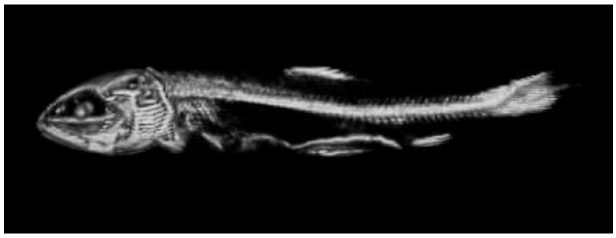

Fig. 3. Image of skeletal whole body of *Hucho perryi* larva.

We could obtain clear 3D images of the skeletal morphology of *Hucho perryi* larva. These images provided detailed views of skeletal structures from diverse angles. In addition, cross section images showed the precise location and size of inner skeletal and related tissue structures. Using micro X-ray CT, Neues et al. (2007) investigated skeletal deformation in Medaka (*Oryzias latipes*) and clearly described various patterns of alteration. 3D micro X-ray CT measurement in this study enable us to obtain 3D images of a live fish. The 3D micro X-ray CT examination took in only two minutes in this method, so far though taking picture was necessary for long time such images. Therefore it is possible to take 3D images while living and no specimen. It will be thought that it is also possible to observe growth of the individual fish if this method is used. So it is effective not only for basic research such as fish physiology including fish growth, but also applied research such as life history investigation of rare species. Recently, telemetry using a data logger has developed as a useful method to record movement of the animals in the water. Especially, acceleration signal recorded by the data logger appeared to be the main features associated with prey capture events as suggested by Naito (2007) and observed by Suzuki et al (2009). For successful use of this application of the data logger, a precise view of skeletal structure is required to accurately attach the data logger in a location that will keep it stable (Tanoue, H., personal communication). Therefore, a method that obtains detailed views of inner skeletal structure is useful for studying rare species such as *Hucho perryi*. There is further potential for research using 3D micro X-ray CT in that by changing indicators of density, it is possible to show not only skeletal tissue but also internal organs.

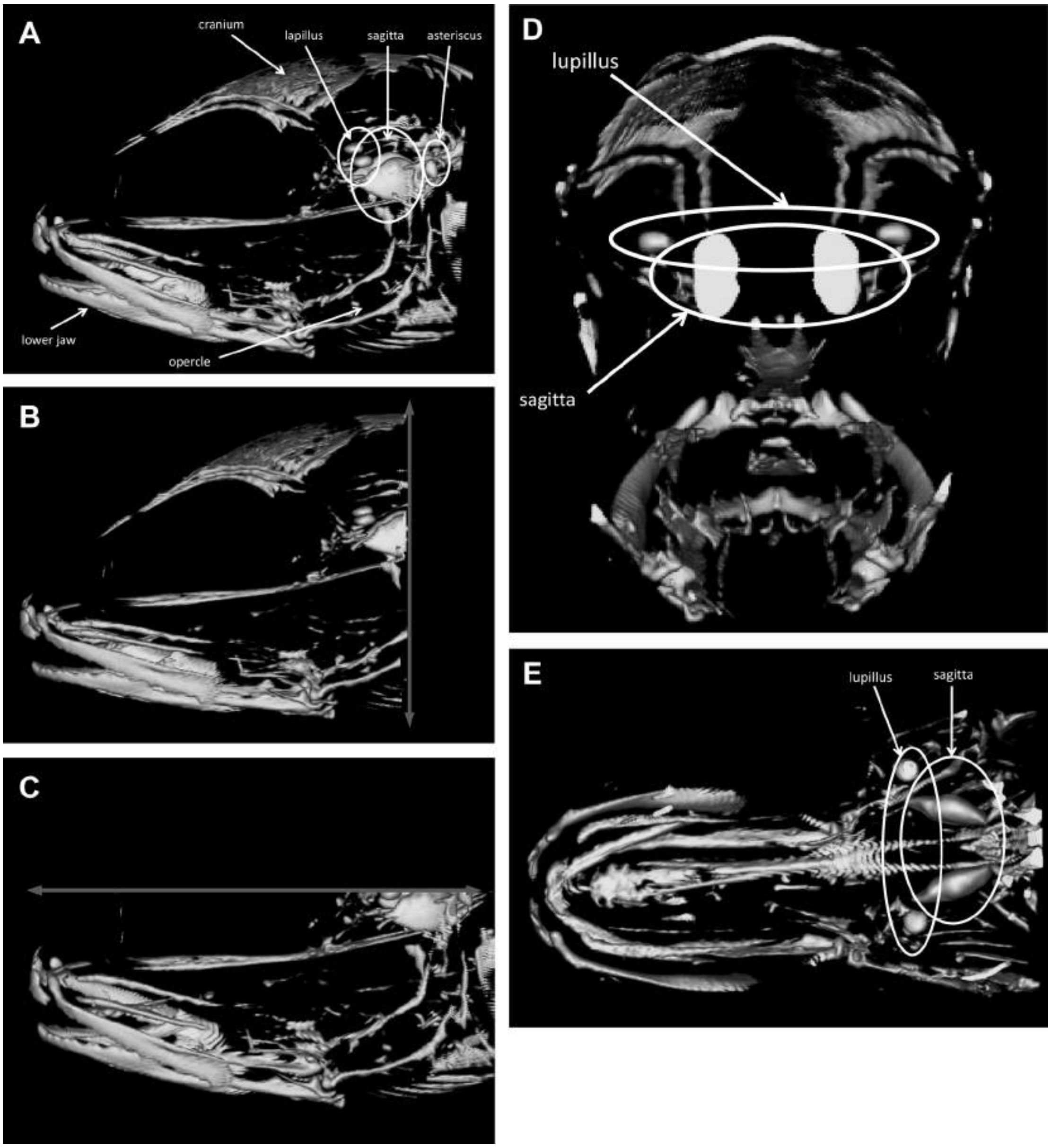

Fig. 4. Vertical and horizontal cross section images of the head. (D) is a vertical image at the arrow in (B) and (E) is a horizontal image at the arrow in (C).

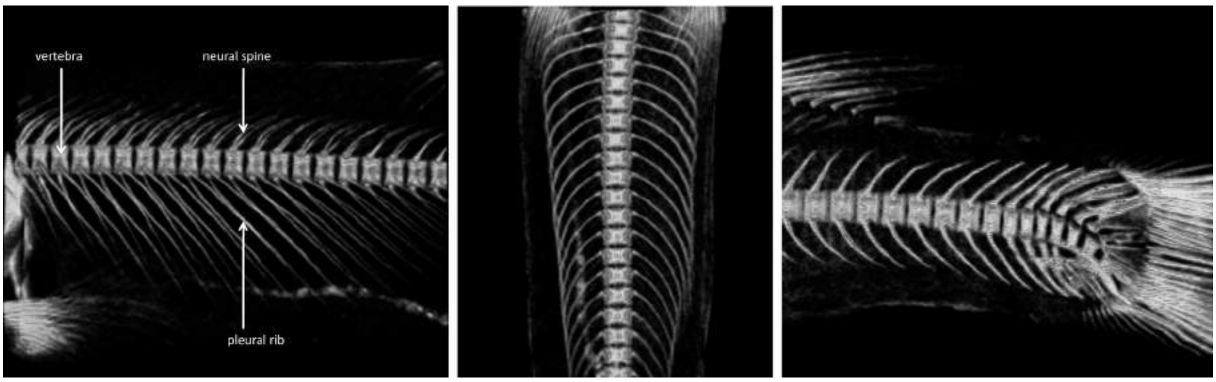

Fig. 5. Still of a vertebra and a caudal fin. Slight deformation of caudal skeleton can be seen.

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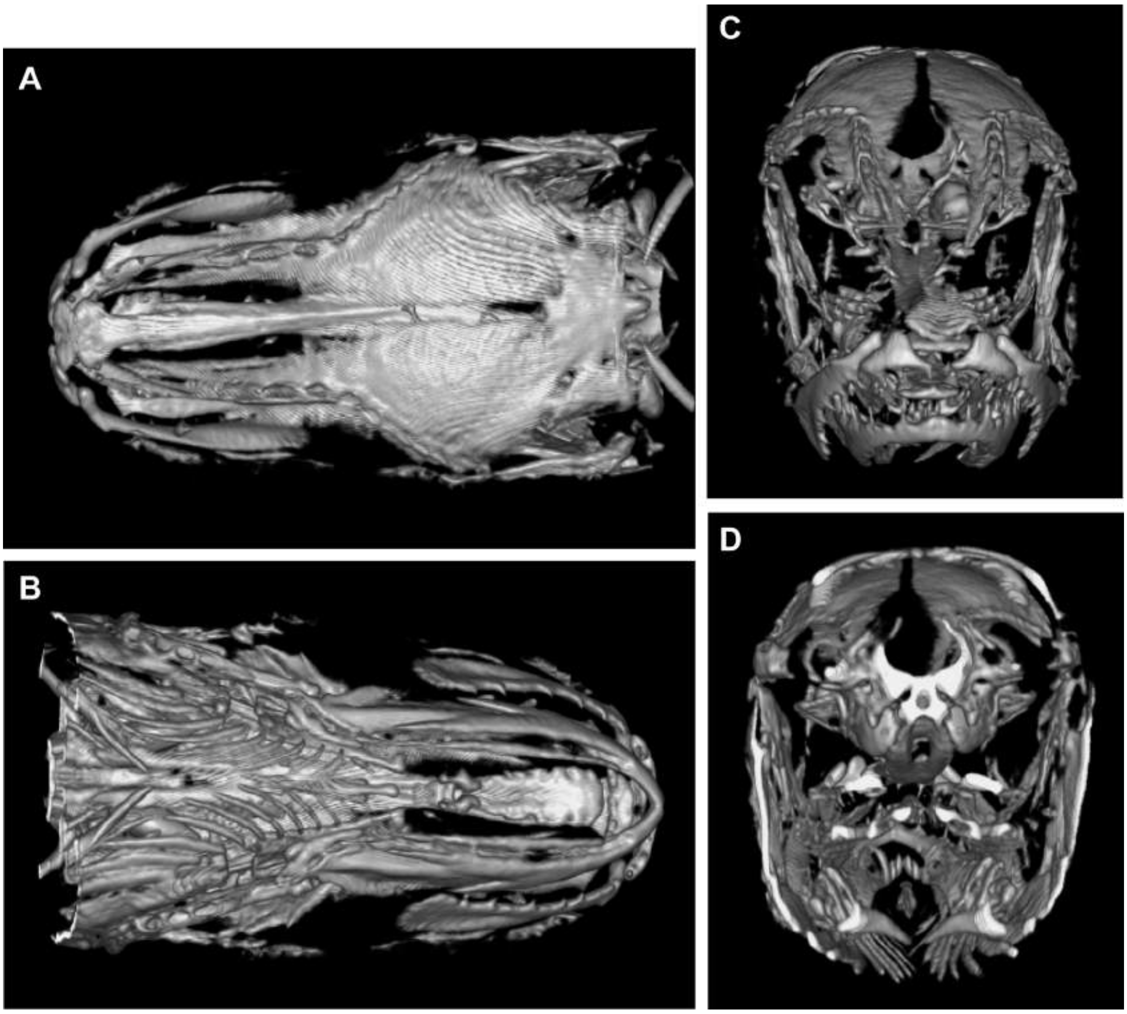

Fig. 6. Rotation view of *Hucho perryi* larva head (seen from above (A), below (B), front (C), and rear (D) sides).

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