In Vivo X-Ray Imaging in Biomechanics and Developmental Biology

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This paper outlines some of the ways that researchers in the field of vertebrate biomechanics use x-ray imaging. It focuses on various techniques used to study morphology and movement in diverse vertebrates including fish, amphibians, reptiles, birds, and mammals.

One technique that has been particularly valuable in recent decades is X-ray videography. Modified medical fluoroscopes are used for zoological research. In the best systems, the fluoroscope phosphor has a short half-decay time (2 ms or less). This allows a high-speed video camera, generally 250 frames per second (fps), to be connected to the image intensifier to catch the rapid movements of small animals.

X-ray videography is used primarily for studies of skeletal movement. For example, G.E. Goslow, K.P. Dial and F.A. Jenkins set up a wind tunnel in front of the x-ray machine at Harvard University, and trained birds to fly in the x-ray beam (Fig. 1). They discovered that the furculum (wishbone) of birds acts like a spring (Jenkins et al., 1988). Images in this 1988 study were recorded on high-speed, 16 mm film (200 fps) because it was the study was done before high-speed video supplanted film.

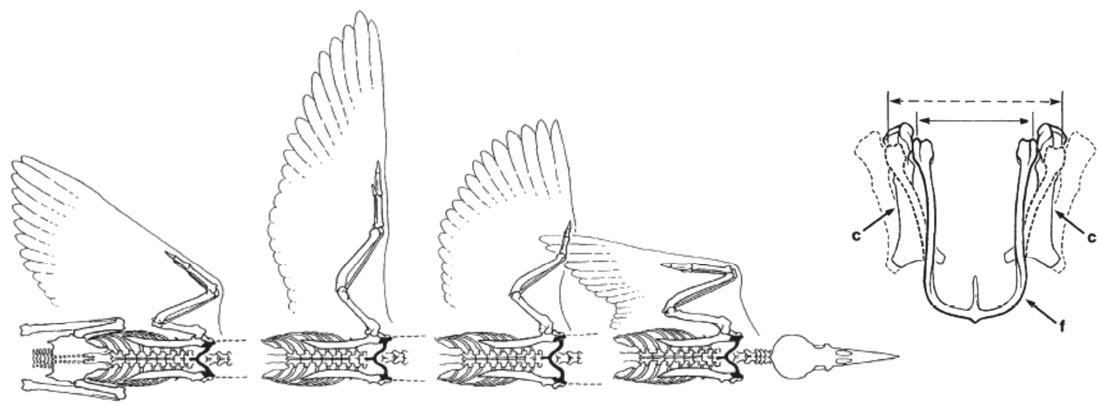

Figure 1. Drawings from an x-ray film of a European starling flying in a wind tunnel From (Jenkins et al., 1988). On the right is a drawing of the deformation of the wishbone.

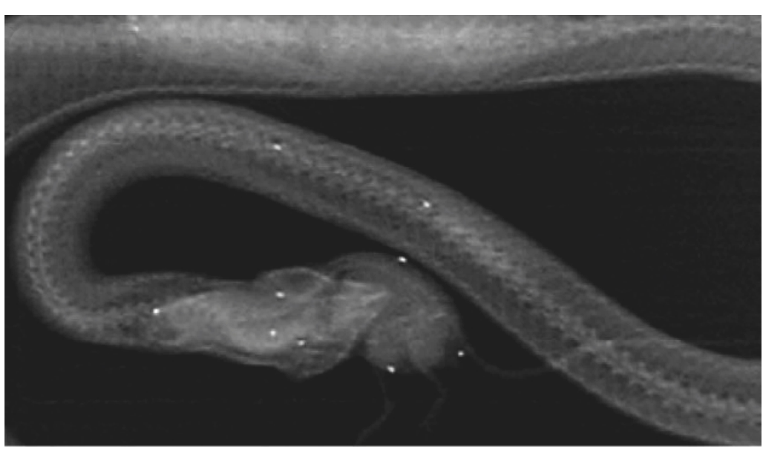

Figure 2. Pinesnake swallowing a rat. The white dots are lead balls marking the soft tissues of predator and prey. From Kley and Brainerd, 2002.

In some studies, high-speed recording is not required. N.J. Kley and I studied the biomechanics of snakes swallowing rats (Kley and Brainerd, 2002), in which the process was so slow that only 5 fps were required to study the swallowing movements (but we used standard 30 fps video and downsampled for analysis). In this study we used small lead balls to mark specific areas of the snake and the rat (white dots in Fig. 2). This is a useful technique for visualizing specific soft-tissue landmarks that have low radiographic contrast. Other radiographic contrast agents can be used, for example to image the circulatory system.

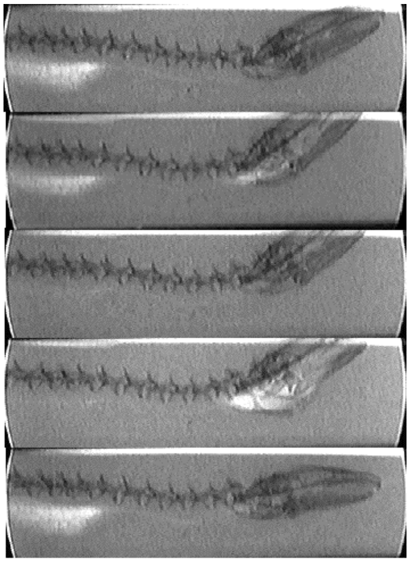

Figure 3. Lung ventilation in an aquatic salamander, *Amphiuma* (Brainerd, 1999). These are x-ray positive images.

In addition to studies of x-ray dense skeletal structures, x-ray video is also an effective tool for studying the biomechanics of breathing (Fig. 3). Lungs image well in x-ray because of their substantially lower x-ray density than surrounding tissues. I have used x-ray video to study the biomechanics of breathing in fishes, amphibians and reptiles. In this work, my colleagues and I discovered an air-breathing fish that uses aspiration breathing and a lizard that uses a mouth pump as an accessory breathing mechanism (Brainerd et al., 1989; Owerkowicz et al., 1999). D.M. Bramble and F.A. Jenkins used x-ray to study the breathing mechanisms of dogs that were trained to run on a treadmill placed in the x-ray beam (Bramble and Jenkins, 1993). They discovered that the forces applied to the lungs by locomotion increases gas exchange by mixing air within the lungs.

X-ray CT imaging is also gaining wide use in the field of vertebrate biomechanics. The 3D skeletal models produced by CT and particularly microCT form an excellent basis for biomechanical modeling. For example, E.R. Dumont and I.R. Grosse are using x-ray CT models of the skulls of bats as the basis for finite element models (Dumont and Grosse, 2004).

In the future, x-ray imaging could become much more important in studies of the development and ontogeny of vertebrates. Most of the current biomechanical research is on adult animals, and more work on the biomechanics of embryos and juveniles is needed. The high-resolution provided by APS synchrotron imaging may find a valuable application in imaging small life stages.

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