DIGITIZING NORTH AMERICA'S FISHES

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INTRODUCTION

In the past decade, the ability to construct digital models of natural history specimens has become faster, cheaper, and easier for researchers. This in part has led to the modern movement to create models of representatives of all vertebrate species and make these data freely available to other researchers and the general public. The openVertebrate project (or "oVert") is leading this charge by using micro computed tomographic (μCT) scanners at universities across the United States to synchronously tackle this ambitious initiative. But what happens to a specimen in a museum before a digital model of that specimen is published on the internet? Here we provide a window into some of the steps involved in this process and focus on what is involved in scanning specimens of fishes.

METHODS

Preparing specimens for μCT scanning

The process of CT scanning may take several hours to complete. Since most preserved fishes are stored in alcohol, there is a risk that the specimen will desiccate over the course of the scan period. To mitigate this risk, each specimen is wrapped in cheesecloth that has been dampened with alcohol (Fig. 1A). Individually wrapped fishes are put together into a kind of cheesecloth "fish burrito" and placed into a 3D printed, cylindrical scanning vessel to maximize the efficiency of the scan time (Fig. 1B).

Processing μCT scan data

The CT scanning process uses X-rays to create a series of radiographs of the specimens, from which 3D structure and density are calculated. The results of these calculations are exported as a series of 2D cross sectional images that can be "stacked" onto one another to reveal the 3D layout of the objects in the CT scan (Fig. 1C). The individual specimens making up the fish burrito can then be digitally isolated using freely available image-processing software such as ImageJ (Reuden et al. 2017) and its extension, Fiji (Schindelin et al. 2012) (Fig 1D–E).

Analyzing and publishing μCT scan data

Digitally isolated specimens can be further processed to isolate individual bones, create models for 3D printing (Fig 1F), measure anatomical structures with extreme (i.e., micron-scale) precision, or many other applications. All specimens that are scanned as part of the oVert effort are made publicly available through the online repository, MorphoSource.org. While the initial CT scan and reconstruction can take several hours to complete, this process is largely automated. Digital isolation and analysis are mostly manual, but free software such as Fiji (mentioned above) and 3D Slicer (Kikinis et al. 2014) enables users to perform the necessary tasks to go from an image stack of CT data to a 3D model ready for printing in less than an hour.

DISCUSSION

The digitization of natural history specimens provides a non-invasive means of creating permanent, indestructible models of these specimens that can be copied and freely distributed globally. This grants researchers, educators, and members of the general public access to a vast diversity of specimens, with the only prerequisite being a connection to the internet. The data being generated towards this end are suitable for a wide range of applications, including high-precision descriptions of anatomy and 3D printing. These data have been used to describe the differences in skull shape of intertidal sculpins and how their morphology relates to specific habitats (Buser et al. 2018). Evans et al. (2018) used CT data to describe the differences in shape found in the extremely sexually dimorphic heads of electric knifefishes, in which males grow elongate jaws and teeth to engage in male-male combat for access to females, much like the jaws and teeth found in breeding male salmon. The use of CT data has also facilitated the study of how the impressive jaws and teeth of piranhas are not only used to dismember prey items, but in some species specialized to pluck scales off of the bodies of other fishes (Kolmann et al. 2018). Recent techno-

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Adam Summers (Twitter: @Fishguy_FHL) earned a master's in biology at New York University and a PhD at the University of Massachusetts. He is a professor in the Department of Biology and in the School of Aquatic and Fisheries Sciences at the University of Washington. With students and collaborators he has published more than 140 articles in scientific journals on abstruse subjects including the heads of hammerhead sharks, the properties of skeletons, and the difficulties of eating hard prey.

Thaddaeus Buser (Twitter: @Cottus_rex) is a Ph.D. Candidate at Oregon State University studying the biology and evolution of sculpin fishes. Growing up in the Pacific northwest, he has spent much of his life outdoors, peering into lakes, streams, and tide pools. Thaddaeus has always been drawn to fishes, especially the "ugly" ones.

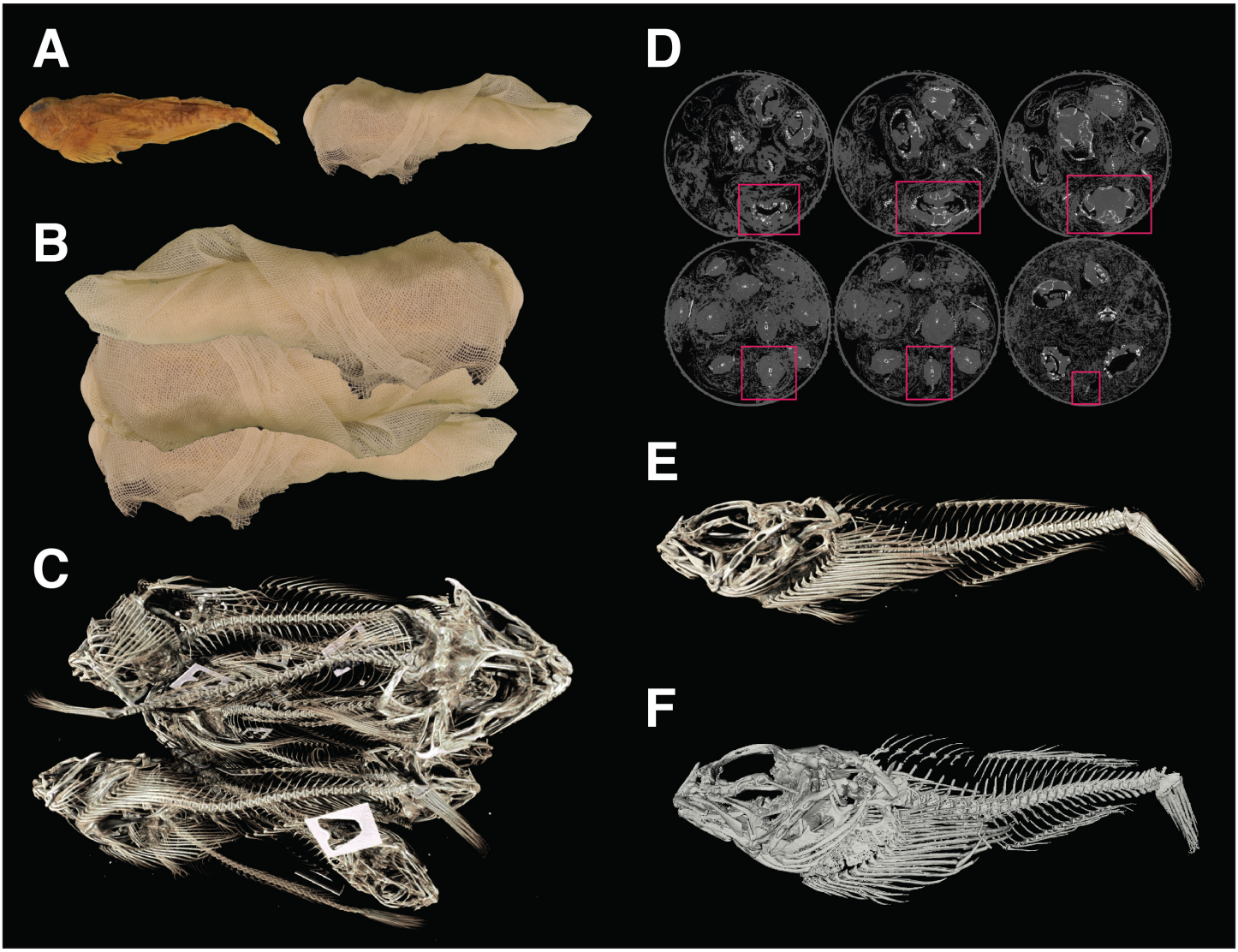

Figure 1: The steps of CT scanning, from original specimen to 3D model of its skeleton. A: original specimen of the sculpin species *Artediellus scaber* (Oregon State University Fish Collection specimen number OS 11482, 69.7 mm SL), before and after being wrapped in alcohol-soaked cheesecloth. B: multiple cheesecloth-wrapped specimens, ready to be placed into a cylindrical vessel for CT scanning. Radio-opaque tags are added to each fish prior to scanning to aid in identification of each skeleton. C: Reconstruction of the CT scanned container of specimens. Note the tag in the shape of the letter "A" which had been added to the specimen of *A. scaber*. D: a series of cross sectional images from the CT reconstruction with a red box drawn around the areas of the slice that run through the specimen of *A. scaber*. Programs such as Fiji allow users to crop the entire series of images simultaneously in order to isolate the specimen of interest. E: Reconstruction of the isolated specimen of *A. scaber*. F: 3D model of the specimen of *A. scaber*. This model can be 3D printed, used for measuring anatomical structures, or many other applications. See text for further details.

logical innovations have even enabled the study of the movement of the internal jaws of Grass Carp (*Ctenopharyngodon idella*) while they are feeding. Gidmark et al. (2014) used a combination of X-ray video motion capture (analogous to the technology used in cinema, but with X-ray emitters and detectors rather than film cameras) combined with CT reconstructions to animate the bones of the skull of a Grass Carp while it feeds (video here: http://movie.biologists.com/video/10.1242/jeb.096248/video-1). Since carps lack teeth on their oral jaws, most of their food processing takes place within their mouths and is therefore extremely difficult to observe. Using CT technology thus shed new light on a fundamental aspect of carp biology.

The CT data generated in these and other studies are freely available on the online repository Morphosource.org (see Figure 2 for examples). This website also hosts the data generated by the openVertebrate project, and is thus a fantastic, free resource for interested scientists and laypersons alike around the world. The CT data for fishes is also hosted at the Virtual Natural History Museum website (vnhm.de), which allows users to see reconstructions of the CT data for any fish species that has been scanned. Because the VNHM website hosts the scan, users can see the skeleton as a rotatable object, zoom in and out to examine the anatomy, and play with visualization parameters of the reconstruction, all using just their web browser.

Figure 2: Reconstructions of the skeletons of several North American freshwater fishes, from CT data available on MorphoSource.org. From top: Olympic Mudminnow (*Novumbra hubbsi,* MorphosourceID 51317), Walleye (*Sander vitreus,* MID 57862), Bleeding Shiner (*Luxilus zonatus,* MID 30971), Three-spine Stickleback (*Gasterosteus aculeatus,* MID 70344), Yellow Perch (*Perca flavescens,* MID 57861), and Allegheny Pearl Dace (*Margariscus margarita,* MSID 30975).

REFERENCES

Buser, T.J., B.L. Sidlauskas, and A.P. Summers. 2018. 2D or not 2D? Testing the utility of 2D vs. 3D landmark data in geometric morphometrics of the sculpin subfamily Oligocottinae (Pisces; Cottoidea). The Anatomical Record 301(5):806–818.

Evans, K.M., M.J. Bernt, M.A. Kolmann, K.L. Ford, and J.S. Albert. 2018. Why the long face? Static allometry in the sexually dimorphic phenotypes of Neotropical electric fishes. Zoological Journal of the Linnean Society XX:1–17.

Gidmark, N.J., J.C. Tarrant, and E.L. Brainerd. 2014. Convergence in morphology and masticatory function between the pharyngeal jaws of grass carp, *Ctenopharyngodon idella*, and oral jaws of amniote herbivores. Journal of Experimental Biology 217(11):1925–1932.

Kikinis, R., S.D. Pieper, and K.G. Vosburgh. 2014. 3D Slicer: a platform for subject-specific image analysis, visualization, and clinical support. *In*: Intraoperative imaging and image-guided therapy (pp. 277–289). Springer, New York, NY.

Kolmann, M.A., J.M. Huie, K. Evans, and A.P. Summers. 2018. Specialized specialists and the narrow niche fallacy: a tale of scale-feeding fishes. Royal Society Open Science 5(1):171581.

Rueden, C.T., J. Schindelin, M.C. Hiner, B.E. DeZonia, A.E. Walter, E.T. Arena, and K.W. Eliceiri. 2017. ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics 18(1):529.

Schindelin, J., I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, and J.Y. Tinevez. 2012. Fiji: an open-source platform for biological-image analysis. Nature Methods 9(7):676.