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Methods for Preparing Dry, Partially Articulated Skeletons of Osteichthyans, with Notes on Making Ridewood Dissections of the Cranial Skeleton

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We describe methods for preparing dry skeletons of virtually any osteichthyan species with a well-ossified skeleton, including very large specimens (e.g., > 1 m *Megalops atlanticus*). Our approach differs from those conventionally used to prepare skeletons of tetrapods in that (1) fairly complete dissection of the specimen is required at the outset of processing; and (2) we use an alcohol dehydration step to rapidly dry the specimen. Similar techniques can be used to prepare well-calcified chondrichthyan skeletons. We also outline the steps for making Ridewood dissections of the skull. Dry, partially articulated skeletons prepared by these methods can be stored indefinitely in acid-free containers in an environmentally controlled space (21 C ± 3 C; Rh = 40% ± 5%) in pest-proof specimen cases. Although a truism of anatomical research is that you cannot learn everything from studying one specimen or one type of preparation, partially articulated dry skeletons are useful for research ranging from phylogenetic investigations to age and growth analyses to functional morphology, making them of great and lasting value to any collection.

MORPHOLOGICAL research typically depends on new preparations of study specimens. Many advances in preparation techniques have aided comparative osteological studies of fishes, such as improvements for clearing and double staining bone and cartilage in whole specimens (e.g., Dingerkus and Uhler, 1977; Springer and Johnson, 2000) or means for studying disarticulated bones of small fishes (e.g., Mayden and Wiley, 1984). However, much remains to be learned from the study of partially articulated dry skeletons. A partially articulated dry skeleton is one in which preparation is stopped prior to removal of ligaments attaching adjacent skeletal elements to each other. Stopping the preparation leaves the elements in their natural relationships and yields a preparation that is both aesthetic and easy to study. Hildebrand (1968) explained two basic methods for preparing such skeletons. The first depends on stopping the cold-water maceration of a specimen before all ligaments are removed by bacterial action (muscles, nerves, blood vessels, and soft connective tissues tend to be degraded and removed first). Cold-water maceration is practical for well-ossified tetrapods, but it does not work reliably for preparing articulated skeletons of any but the very largest fishes. Thus, we use the second method, which employs insects (carpet beetles, family Dermestidae) to eat and thereby remove soft tissues attached to the skeleton (Hall and Russell, 1933; Jones, 1970; and Sommer and Anderson, 1974). It is still necessary to monitor progress and eventually to stop the action of the insects, because some articulations in fish skeletons, such as attachments between ribs and the vertebral column, are prone to disarticulate.

Here, we summarize techniques developed while field collecting and preparing thousands of dry, partially articulated fish skeletons. Many were perfected at the Dauphin Island Sea Lab, Alabama, during our 20 years of sampling skeletons of marine fishes obtained during fishing tournaments. Most skeletal materials collected at Dauphin Island are held at the American Museum of Natural History.

MATERIALS AND METHODS

The balance of this article consists of subsections that trace the processing of specimens from acquisition to storage in a collection. We include information on specimen handling; tools, tags, and measurements; dissection and disarticulation; washing and dehydrating; dermestid processing; degreasing; and storage and postprocessing.

Specimen handling.—To yield the best end product, good technique during specimen acquisition and dissection is essential. Fishes must be kept on ice (or frozen) prior to dissection to avoid any trace of decay, which can cause skel-

etal disarticulation during processing. Flies must not be allowed to lay eggs on specimens. Specimens should be identified as completely as possible before dissection because identification typically requires examination of features such as color patterns that may be lost during processing.

Tools, tags, and measurements.—Tools for dissecting fishes for skeletal studies include fish measuring boards and cloth measuring tapes; fillet knives and means for keeping them sharp; serrated hunting knives for skinning large specimens (e.g., Cutco brand hunting knives, which are ideal for cutting through the tough skin of large actinopterygians and elasmobranchs); scalpel handles and blades; single-edged razor blades; coarse and fine scissors; blunt probes; small-toothed forceps; spoons for quick removal of trunk muscle; pliers for skinning; and rongeurs (sold by surgical supply companies and especially helpful for removing tough connective tissues and skin). We photograph specimens before dissection and also retain tissue samples.

Assign a unique field number before dissection and field tag the specimen. We make our field tags from heavy waterproof card stock (dissection specimen tags from Carolina Biological Supply Co.) cut to ~38 mm long by ~19 mm wide, with a ~3 mm round hole punched and centered ~10 mm from one end. We loop a length of sturdy cotton string (30–50 cm) through the hole and tie a standoff knot ~2 cm from the tag. As each specimen is measured and prepared for processing, we use a #7 Kohinor disposable India ink pens to write its field number on a tag slightly dampened with water to help the ink soak into the tag. At this point, a photograph of the left lateral side of each specimen, with the field tag visible, may be taken. Because each specimen will be disarticulated into at least two parts, it is not practical to write out the remaining tags until the dissection is complete and the total number of tags needed for that specimen is known. Depending on the dissection strategy adopted, a 50 cm specimen may eventually require as many as nine field tags (1: gill arches; 2: left side of the skull + neurocranium; 3: right side of the skull; 4: scleral ossicles; 5: trunk + caudal region; 6: dorsal fin[s]; 7: anal fin; 8: pelvic fin; and 9: pectoral girdle). Therefore, prepare enough blank tags before beginning any large-scale collecting effort.

Before dissection, record external measurements (total length, fork length, standard length, prepectoral fin length, prepelvic fin length, predorsal fin length, preanal fin length, pre orbital length, and head length; see Grande and Bemis, 1998). During dissection, soft anatomical details such as sex, reproductive condition, stomach contents, or parasite load can be recorded. Preserve (e.g., in a vial of 95% ETOH or by freezing in liquid nitrogen) a sample of tissue for later extraction of genetic markers (e.g., DNA for sequence analyses). If the specimen is rare, then consider preserving internal organs in formaldehyde. Skins can be saved for studies of scale anatomy.

In the field, dissection waste is best disposed of by burying it in a pit, ~1.5 m deep. Temporarily cover the pit with sheets of plywood that have been covered by plastic tarps. At the end of the day, cover with soil and any waste added that day.

Dissection and disarticulation.—Proper dissection begins with careful skinning of the specimen. To do this, make incisions to either side of the dorsal fin and extend each cut anteriorly and posteriorly, cutting carefully around the base of the dorsal fin(s). At the back of the skull, continue this skin incision ventrally on each side of the body. Continue to cut the skin along the posterior edge of each pectoral girdle, working toward the ventral midline of the body. Here, the right and left incisions will meet. At the caudal fin, turn the skin incision ventrally, and cut as cleanly as possible along the bases of the caudal fin rays. Then continue the incision forward toward the cloaca, carefully flanking the anal fin. At the cloaca, the incision will meet the contralateral incision. By following these steps, the skin can then be carefully peeled away from the underlying muscles (a pair of pliers or rongeurs is helpful for this step for large specimens), leaving two flaps of skin attached in the ventral midline. To complete skinning, carefully cut around the base of the pelvic fin and peel the skin away in one piece. Any muscle that remains attached to the skin usually can be scraped away using the edge of a spoon. If the skin has scales, then it can be tagged and dried either by alcohol immersion or by placing it in front of a fan (adequate in most cases) or by mounting it onto a herbarium sheet and pressing it in a plant press until dry. Such skins allow study of scale characteristics and variation on the body. Once the skin is removed, eviscerate the specimen.

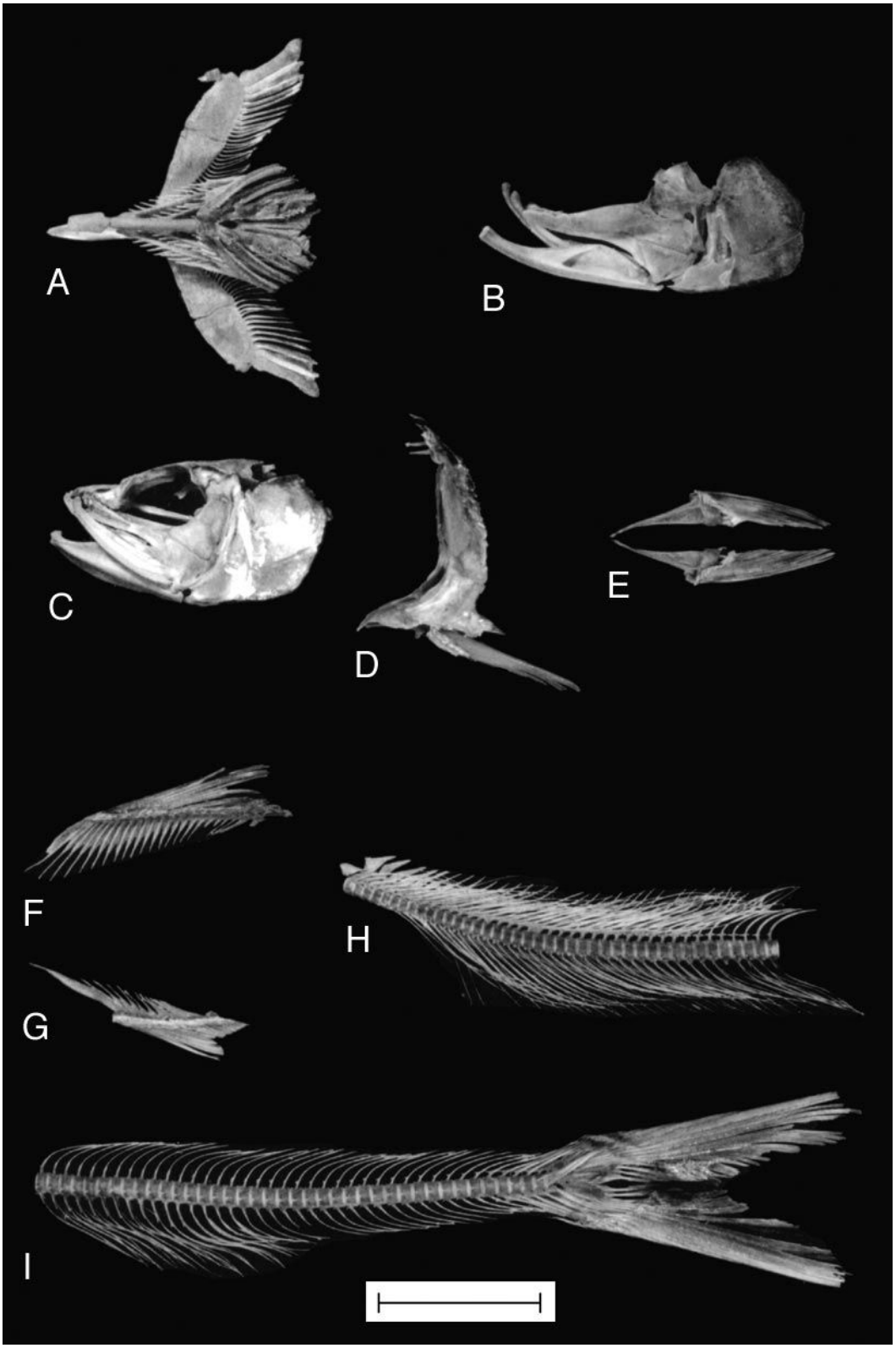
The next step is to remove the trunk and fin muscles. The goal is to leave intact the vertebral column, ventral ribs, and fin bases as well as any intermuscular bones. For some osteichthyans, such as tarpons (e.g., *Megalops atlanticus*), this can be difficult because several series of intermuscular bones are embedded in the trunk

muscles. In certain cases (e.g., many eels), it is impractical to keep the intermuscular bones intact, and these bones may be completely removed from the rest of the axial skeleton, particularly if they are only connected to the rest of the skeleton by thin ligaments that will be eaten by the dermestid beetles. Therefore, proper study of intermuscular bones (e.g., Patterson and Johnson, 1995) must be based on alcohol stored or cleared and stained specimens.

Match cutting tools to the size of the specimen and to the toughness of its connective tissues. Spoons can be used to scrape away the trunk muscle to expose the vertebral column and median septum. Whenever possible, avoid cutting or breaking through the median septum because it helps to keep the vertebral column and fin bases intact. Open the body cavity and remove the viscera by transecting the esophagus and hepatic veins. It is important to carefully and completely remove the swimbladder (a pair of rongeurs can be indispensable for this step). The swimbladder often has close connections with the ribs and vertebrae, and if it is not completely removed at this point, then it can leave stubborn dense connective tissue on the bones. Such tissue can become hardened during dehydration, and there is a risk that the other portions of the skeleton will be completely disarticulated by the beetles before this tougher tissue is consumed. Next, wash the specimen in cold water and scrub it with brushes to remove blood and mucous. It is important to remove the kidneys, dorsal aorta, and posterior cardinal veins as completely as possible, because these retroperitoneal structures lie closely adjacent to or even surrounded by skeletal elements and can permanently stain the skeleton if they are not removed. If the specimen is very large, then it is a good idea to take the additional step of removing muscle and connective tissue from each rib.

At this point, make decisions about partially disarticulating the axial skeleton. We follow the general ichthyological convention of keeping the left anatomical side of the specimen as intact as possible for future study and illustration. If the specimen is longer than will be convenient to store in collection cases, then divide it into smaller lengths, working carefully to separate adjacent vertebrae and fin supports in such a way that no skeletal elements will be damaged or lost (this can be, as in billfishes, which have complex articulations between the vertebrae; patience usually is rewarded). For larger fishes, we commonly separate the dorsal and anal fins from rest of the skeleton, working carefully so that none of the fin radials or neural or hemal arches becomes damaged during dissection. We commonly remove the pectoral and pelvic girdles for processing as separate pieces; some prefer to remove the pectoral girdle on only one side (typically the right side) of the body. To remove the pectoral girdle, locate the posttemporal bones at the back of the skull and carefully separate them from the rest of the skull. The stout ligaments connecting the pectoral girdle to the neurocranium (Baudelot's ligaments) often are so strong that they must be specifically cut before the pectoral girdle can be removed. If the pelvic girdle is freely floating in the trunk muscles (as, for example, in tarpons, salmonids, clupeids, and catfishes), then it can be easily removed for processing. If the pelvic girdle is closely associated with the pectoral girdle (as in most percomorphs), then we usually leave it in place in contact with the pectoral girdle. It is helpful to work patiently to remove as much muscle, skin and connective tissue as possible from each of the disarticulated pieces of the skeleton, because this speeds dehydration, air-drying and dermestid processing. Each section of the skeleton is then tagged with the specimen's field number and processed separately, with the pieces being reaggregated at the time of cataloging/accessioning. We find it useful to note on our data sheet how many sections were prepared from each specimen so that we can easily track each portion through its further processing.

Preparation of the cranial skeleton begins with removal of the eyes; be sure to wash, tag, and save any scleral ossifications that may be present. Invariably for osteichthyans larger than 25 cm, we follow the additional steps for making a Ridewood dissection of the cranial skeleton (Figs. 1, 2). This dissection technique is named for anatomist Walter G. Ridewood who published five important papers on the cranial osteology of teleostean fishes in 1904 and 1905 (Ridewood, 1904a,b,c; 1905a,b). In the first of these papers, Ridewood (1904a:36–37) outlined a useful way of disarticulating the skull of teleostean fishes, citing his frustrations of trying to make meaningful studies of a skull that had not been disarticulated at all or of sorting through boxes with completely disarticulated skulls. Our method is only slightly modified from that of Ridewood (e.g., he proposed removing the left suspensorium and cheek, whereas we remove these structures on the right side of the body; he also suggested removal of the infraorbital bones as a separated unit, whereas we generally leave them associated with the suspensorium and jaw unit). We emphasize that there is no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single correct way to disarticulate an osteichthyan skull and that every specimen is unique. If sufficient numbers of specimens of the same species are available, then use different ways to disarticulate the skull to gain different perspectives on cranial anatomy.

To make a Ridewood dissection, cut the (usually) thin skin between the mandible and the hyoid arch on each side of the head, then cut the ligament connecting the hyoid arch to the inner surface of the palate and disarticulate the joint between the interhyal and symplectic. Next, make a transverse cut in the mucosa that lines the roof of the oral cavity and, working posteriorly, pull the mucosa away from the parasphenoid bone. Blunt dissect deep to this mucosa to locate the dorsalmost gill arch elements (e.g., pharyngobranchials) and separate them from their connections to the base of the skull (these connections are chiefly muscular, and the separation often can be made by gently pulling on the gill arches while pushing on the pharyngobranchials with a finger). Once the gill arches are completely separated from the base of the skull, cut the skin and muscles attaching them to the pectoral girdle, and cleanly separate them from the rest of the specimen. Remove the urohyal with the gill arches; because its only connection to the rest of the skeleton is typically via long ligaments and muscles, it usually will become disarticulated by the end of processing. The gill arches are then tagged, washed, and prepared as a unit; some further work usually is required to clean them of muscles and blood vessels before they can be dried for further processing.

Once the gill arches have been removed from the specimen, it is easy to complete a Ridewood dissection. Beginning at the posterodorsal corner of the right operculum, cut the muscles linking it to the skull. Then work forward to separate the right hyomandibula from its articulation with the otic region of the braincase (work the joint back and forth to help loosen this articulation). Cut the symphysis between the left and right lower jaws and carefully cut between the left and right premaxillae. Continue this last cut into the oral mucosa, separating carefully between the right palatal bones and median structures such as the vomer and parasphenoid. Work the joints between the palatal complex and the neurocranium to loosen and eventually completely disarticulate the right palatal complex from its anterior contact with the braincase. It is also necessary to separate the right infraorbital series from its connections to the skull roof (different groups of osteichthyans require slightly different solutions; hence there is no one correct way). Also cut the premaxillary and mandibular symphyses such that the entire right cheek and jaws of the specimen can be removed, preserving the left side of the head for surface views of the dermatocranium and enabling close study of the internal surface of the dermatocranium and braincase on the right side (Fig. 1). Ridewood dissections facilitate all further steps in processing of the specimen by making it easier to properly clean and dehydrate the skeleton. For example, in a large osteichthyan (i.e., $>$ 15 kg), it is important to completely remove the mucosal lining of the oral cavity and to skin the maxilla, premaxilla, and lower jaws as thoroughly as possible before dehydration, because the skin in these areas can prove resistant to dermestids and may be difficult to remove by hand once it has dried.

Fig. 2. A partially articulated specimen of a large Atlantic tarpon, *Megalops atlanticus*, in a suitable box ready for the dermestid colony. Each piece has a tag with specimen's field number. This specimen was prepared using the Ridewood dissection method; the inner surface of the right cheek and jaws is visible below and the gill arches can be seen on the left.

Washing and dehydrating.—After dissection, thoroughly wash the specimen in cold water (hot water can cause thin bones to deform or disarticulate), carefully working with an appropriately sized toothbrush or scrub brush to remove blood, mucous, and remaining soft tissue. Tie

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Fig. 1. A dry, partially articulated skeleton of an adult ladyfish, *Elops saurus* prepared using the Ridewood dissection method, in which the gill arches (A) and right side of the head (B) were dissected away from the rest of the skull (C). To ensure thorough cleaning, the paired (D, E) and median fins (F, G) were isolated, and the vertebral column was separated into abdominal (H) and caudal (I) regions. Scale bar = 5 cm.

field tags to each separate portion of the specimen, and let it drip dry for ~1 h to prevent dilution of the alcohol that will be used to dehydrate it. Again, prevent flies from laying eggs on the specimen. The tag's string must be tied tightly around a portion of the skeleton from which it cannot be dislodged during processing; trim the tag ends closely such that they do not become tangled with other specimens during further preparation.

Dehydrate the tagged specimen by immersing it in 99% isopropanol or 95% ethanol for two or more days, depending on its size. We prefer to use two or more dehydration baths. The first bath invariably becomes contaminated with much of the water removed from the tissues of specimens, and the subsequent baths more fully complete the dehydration. Remove the specimen from the last bath, and place it in front of a fan to rapidly evaporate the alcohol. At this point, if the specimen has been dried properly, it can be stored in an airtight barrel or other container for an extended period or shipped from a field site to the collection. It is important to fully dehydrate specimens prior to sealing them in a shipping container, because any moisture trapped in the container can lead to mold formation on the specimens. Mold not only discolors the bones but also discourages dermestids from cleaning the skeleton. Once it has been received at the collection, a specimen can be stored dry or immediately processed.

Dermestid processing.—Place each specimen in a cardboard or enamel tray, cover it with a piece of non-absorbent cotton to provide a place for the beetle larvae to pupate, and put it into a dermestid beetle colony. Note that dermestids will eventually eat cardboard trays; therefore, specimens in cardboard trays must be monitored. The many ways that a dermestid colony can be established are described elsewhere (Hall and Russell, 1933, Russell, 1947, Jones, 1970, and Sommer and Anderson, 1974). Specimens can be sprayed with water to encourage beetles to initiate cleaning, but it is important not to over wet fish specimens because too much moisture can cause molds to grow. The specimen may need to remain in the colony for a few days, weeks, or for many months. Larval dermestids are more efficient cleaners than are the adults, and it can take two months or more for a newly established deremestid colony to increase to the point at which processing becomes rapid; specimens placed into such an active colony must be watched carefully to prevent unwanted disarticulation. Sommer and Anderson (1974) described how formalin can be selectively brushed onto ligaments and other tissues to prevent further processing by the dermestids; we have used this method to successfully prepare fishes with intact swimbladders to demonstrate swimbladder-ear connections.

When the beetles have cleaned the skeleton of all adhering soft tissues, the cotton should be removed, and the specimen exposed to light, which will cause beetles and larvae to leave the skeleton. This can be hastened if a new specimen for processing is placed next to the old one, as the dermestids will depart for the new food source. Then remove the skeleton from the colony and freeze it to kill remaining beetles and larvae.

Use tweezers and paintbrushes to remove dead beetles, shed skins, and frass (beetle waste) from the skeleton. Be careful not to disturb fragile articulations because it is better at this point to leave a few beetles in a hard-to-reach spot than to break a bone or loosen an articulation. We sometimes use compressed air to blow off the skeleton, although this is not essential.

Degreasing.—Soak the specimen (overnight for large skeletons; a few minutes for delicate ones) in full strength household ammonia for degreasing. Then, wash the specimen in a slow stream of running water to remove ammonia and dissolved grease. Dry the specimen in front of a fan, arranging it in position for permanent storage (e.g., with median fins erect).

For large skeletons or exceptionally greasy ones, further degreasing may be needed. Soak such specimens in trichloroethylene in a closed metal or glass container until the bones are free of grease. Trichloroethylene can eventually damage bones and it is a cancer suspect agent and known mutagen; thus, it must be handled with care, in a fume hood or outdoors. After degreasing, air dry the specimen in a fume hood or outdoors in front of a fan.

Storage and postprocessing.—Store the specimen in an acid-free cardboard box. For short-term storage, materials can be put into a heavy polyethylene bag sealed using a bag sealer to limit access by insects; a drawback of polyethylene bag storage is that the plastic eventually breaks down. Thus, storage in acid-free cardboard boxes is preferable. The specimen should be stored in a pest-proof cabinet in an environmentally controlled space (21 C ± 3 C; Rh = 40% ± 5%).

Sturgeons, paddlefishes, lungfishes, coelacanths, and other osteichthyans with extensive bone and cartilage in the skeleton of adults can

pose special problems for skeletal preparation (see Hilton and Bemis, 1999). We follow the same basic steps outlined above but keep careful track of progress during dermestid preparation. It usually proves necessary to remove such specimens from the dermestid colony at a relatively early stage and to complete the cleaning by hand.

A final preparation objective concerns postprocessing of specimens to reveal additional anatomical information. For example, it may be desirable to more extensively disarticulate a specimen that has been through the normal preparation steps. This may be needed to study closely the articulations between the individual skeletal elements and can easily be done by manual dissection or by cold-water maceration. Specialized postprocessing may also be needed to examine details related to other anatomical systems (e.g., the circulatory system, sense organs, nerves, etc.). For example, dry partially articulated specimens can be sawed with a bandsaw in various planes to reveal additional structural details.

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