**Changes in Color, Body Dimensions, and Biomass**

## Preservation alters specimens by dehydration and chemical removal of colors and constituent parts.

## Color changes may result from chemical or physical alterations, or both. The changes may involve the loss, acquisition, or change of color. Color is conferred chemically by pigments, and structurally by interference, light scattering, or refraction. Fluid preservatives will both extract pigments and cause structural changes. For example, lipochromes (responsible for yellows, oranges and reds) are alcohol-soluble carotenoids (Pettingill 1970). Fry (1985) described how the loss of color (yellow) due to submersion in alcohol in some bird specimens led to an erroneous description of it as a new subspecies; Fry confirmed the color loss by subjecting dry specimens to submersion in alcohol. Figure 4 shows color changes in a live *Hamptophryne* in the Amazon of Peru and the same specimen post-preservation. Green-to-blue changes in most vertebrates, such as the specimen of *Phyllomedusa vallianti* shown in figure 5, occur when xanthophores (responsible for yellow) are leached out by a preservative and the remaining iridophores are altered by dehydration, which affects the interference of light. Specimens are often darkened by exposure to formaldehyde, a condition commonly referred to as formaldehyde brown or formaldehyde gray. Although much of this discoloration is due to the interaction of acidic formaldehyde, metal trays or tags, and the specimens, studies have also shown that specimens become darker the longer they are left in formaldehyde (see *Unwanted Effects of Formaldehyde* above).

Stenjener’s instructions for field preservation of amphibians and reptiles recommended the use of a color standard due to the loss of colors in preservative solutions (Stenjener 1891), a suggestion ignored by most field workers since. Fluid preserved specimens are chemically and physically altered by the fixative and preservative. The most obvious alterations are dehydration, shrinking, swelling, and color changes. There is a reasonable literature on the overall dimensional changes (tables 3 and 4), but little research has been carried out on the specific effects of fixatives and preservatives on the particular tissues involved. We do know, for example, that elastin (a fibrillar protein in connective tissue and blood vessels) swells with exposure to formaldehyde and alcohol but does not go into solution (Mukherjee and Hoffman 1971).

Many of the early descriptions of fluid preserved specimens proclaim them to be perfectly preserved, but often the writer was describing the preserved animal without having seen a living specimen of the species, or was making a subjective comparison to other preservation methods available (e.g., dehydration). An example of a comment on a color change that affects the usefulness of the specimen is the note by Bliss (1872) concerning the unexpected appearance of a diagnostic vermillion spot on an alcohol preserved cyprinoid fish that was not present when the fish was alive; dissection demonstrated that the color was a true pigmentary color, probably visible only when the fish is in reproductive mode or preserved in alcohol. Prum et al. (1994) noted differences in structural color of greens in bird skin preserved in formaldehyde vs. gluteraldehyde (which came closer to preserving the color of the tissues in life). As a consequence of the color changes during preservation, there have been many attempts to concoct a preservative that will retain colors. Probably the best know of these is Kaiserling’s solution (and it is important to note that there are many variations of the recipe for Kaiserling’s solution in the literature). The original Kaiserling’s solution was composed of potassium acetate, potassium nitrate, formaldehyde, and water (Kaiserling 1896); Kaiserling himself published several variations on it, as did others (Craig 1914, Edwards and Edwards 1959). Craig (1914) recommended a mixture of formaldehyde, potassium nitrate, and potassium acetate as a better color preserver for anatomical specimens than Kaiserling’s solution. Various published methods proposed to preserve color in fishes were reviewed by Borodin (1930) and all found to be unsatisfactory. At the same time, Borodin complained about the damage that formaldehyde does to scales and bones (presumably this was because Borodin was using inappropriately buffered or unbuffered formaldehyde). Based on the ingredients in British-made industrial methylated spirits (IMS) used for few jars of fishes from the Red Sea that had retained much of their color, Borodin ultimately recommended a formula consisting of 30 parts alcohol, 2 parts formaldehyde, 1½ parts wood tar, and 66 ½ parts water saturated with common salt for the preservation of color in fishes. One of the more creative attempts to preserve color was based on experiments conducted at the Colombo Museum (Sri Lanka), using coconut oil and carbolic acid with glycerin (Haly 1892). More recently, an antioxidant (BHT, or butylated hydroxytoluene, sold under the trade name Ionol or Ionol-40), was advocated to preserve colors (Smith 1995, Waller and Eschmeyer 1965, White and Peters 1969). Windsor (1971) recommended the use of a 50% solution of ammonium sulfate to preserve color in frogs, although he noted that the preservative caused excess dehydration of the specimens.

Bliss, R. 1872. Appearance of colour in fish kept in alcohol. *Popular Science Review* 11: 335-336.

Borodin, N.S. 1930. Effective method of preserving fishes in natural color. *Museum News* 8(6):11-12.

Craig, H.K. 1914. A new method of preparing museum specimens. *Journal of the American Medical Association* 62(116):1241-1242.

Edwards, J.J. and M.J. Edwards. 1959. *Medical Museum Technology*. Oxford University Press, London, 172 pages.

Fry, C.H. 1985. The effect of alcohol immersion on the plumage colours of bee-eaters. *Bulletin of the British Ornithologists' Club* 105(2):78-79.

Haly,A. 1892. A medium for preserving the colours of fish and other animals. *Nature* 45:212.

Kaiserling, C. 1896. Über die conservirung von sammlungspräparaten mit erhaltung der natürlichen farben. *Berliner Klinische Wochenschrift* 33(35):775-777.

Mukherjee, D.P. and A.S. Hoffman. 1971. Physical and mechanical properties of elaastin. Pages 219-241 in Elden, H.R. (editor). *Biophysical Properties of the Skin*. John Wiley and Sons, Inc., New York, vii + 645 pages.

Nieuwland, J.A. and A.D. Slavin. 1928. Preservation of monotropa and similar plants without discoloration. *Proceedings of the Indiana Academy of Science* 38:103-104.

Pettingill, O.S. 1970. *Ornithology in Laboratory and Field*. 4th edition. Burgess Publishing Company, Minneapolis, Minnesota, xvii + 524 pages.

Prum, R.O., R.L. Morrison, and G.R. Ten Eyck. 1994. Structural color production by constructive reflection from ordered collagen arrays in a bird (*Philepitta castanea*: Eurylaimidae). *Journal of Morphology* 222:61-72.

Scully, F.J. 1937. Preservation of plant material in natural colors. *Rhodora* 39:16-19.

Smith, D.G. 1995. Preservation of color in larval fishes. *Curation Newsletter* 11:5-6.

Stejneger, L. 1891. Directions for collecting reptiles and batracians. *Bulletin of the United States National Museum* number 39:1-13.

Waller, R.A. and W.N. Eschmeyer. 1965. A method for preserving color in biological specimens. *Bioscience* 15(5):361.

White, D.A. and E.J. Peters. 1969. A method of preserving color in aquatic vertebrates and invertebrates. *Turtox News* 47(9):296-297.

Windsor, D.A. 1971. Ammonium sulfate as a preservative which does not remove color from frogs. *Copeia* 1971(2):356-357.