CHAPTER 10
Museum Techniques in Preservation

When we search in the past about the preservation of natural history, it is nature that stands as the first museum as well as the curator. Nature did the first-ever clearing of massive fleshy creatures; stored specimens on various racks of the planet's crust, in rocky and icy jars; used inventive preservatives like amber that kept the specimens untainted for millions of years. Later, when people began digging for natural history at the dawn of modern science all the valuable collections that nature preserved over the vast geological time scale became the major tools for taxonomy and systematic studies as well as, recently, for species conservation measures. Modern science followed that path of nature to preserve specimens of organisms in a more sophisticated manner for the future. Thus numerous natural history museums were established over the last few centuries that now accommodate millions of specimens collected from across the planet.

The three major steps of museum techniques can be broadly named as collection, fixation and preservation. All these steps are equally important in which sloppiness in any of them will result in the loss of valuable collections. For those people who are involved in museum practices, it is important to understand the historical, scientific, cultural, aesthetic and conservational values of the specimens they handle and curate.

The process of collection

The process of sample preservation begins at the site of specimen collection. First of all, note the exact location from where the sample is being collected. Nowadays the location can be recorded to the most accurate point by using a GPS instrument, along with the name of the place if it is collected secondarily from the land. For marine specimens, most often, this is the case since the majority of samples has been collected from the shores or landing centres. For samples collected from the fish landing centres, we can get the most accurate location of the fishing grounds recorded in the GPS system of the fishing vessels, along with the depth up to which they operated the nets. This is of utmost importance if the collected species has certain taxonomical importance or conservation status. While in the case of specimens washed ashore, for eg. a seabird, we can only record the land location even though the species might have an original distribution somewhere else.

Multiple specimens should be collected if available instead of just one or two as a representative of the whole catch. In the case of some species which has only seasonal landings or are caught

accidentally, specimens should be collected in such a quantity that is enough to serve taxonomical and biological analysis in the future. Specimens collected from the field can be temporarily kept in plastic bottles and carry bags. For comparatively big specimens, large containers or iceboxes should be needed for easier and better transportation. Specimens vulnerable to fast deterioration, e.g. jellyfishes or engraulid fishes like anchovies and herrings can be initially treated at least mildly with a fixative. Instead of putting the specimen directly on ice which could change the colour of the specimen at the points of contact with ice, ice boxes filled with ice-cold water is necessary for retaining the freshness of the samples (Motomura and Ishikawa, 2013).

If the specimen is intended for molecular taxonomy studies it is most appropriate to take tissue samples in alcohol from the collection site itself for better preservation of the genetic materials. In this process, utmost care should be taken to avoid contamination of the study sample by other specimens from the location. Prior to taking the tissue sample of a specimen from a trash landing, the specimen should be washed to clear any unwanted particles to avoid genetic contamination.

Specimens brought into the lab can be studied in detail to corroborate the species identity. After noting down the essential morpho-meristic characteristics of the samples they can be prepared for fixation and then preservation in the museum.

The process of fixation

Fixation is the process of stiffening or stabilizing ("chemically freezing") the cell contents of an organism into insoluble components through cross-linking proteins (Clyde et al., 1983; Martin, 2016). Specimens must be preserved as fast as possible after collecting the specimen to avoid any deterioration. Live specimens can be collected and narcotized by using anaesthetics such as MS-222 before fixation, which gives better results for long term preservation. Fixation increases the staining quality of the specimen by raising the refractive index (Martin, 2016). Different types of fixatives are used often selectively for different types of specimens based on their tissue characteristics. Generally, the fixatives are either aldehydes or alcohol.

Formalin: Formalin (40 per cent formaldehyde) is the most commonly used fixative in biological museums. It is found to be more efficient in maintaining the colour and shape of the specimen (Musial et al., 2016). The degree of concentration of formalin needed for fixation depends on the size of the specimens. Usually, a solution of one part formalin and nine-part seawater or distilled water is used for fixation. For small marine animals, a mild concentration of 5 per cent is enough for fixing. In the case of larger, bulky specimens, a maximum of 10 per cent of formalin is needed to inject into different parts of the body. The alimentary system of large fish can be removed or otherwise can be injected formalin. Large hypodermic needles must be required for safely injecting formalin into animals having tough skins like that of sharks. The fixed animal or tissue needs to be kept as such for 24 to 48 hours in the case of smaller animals while several days are required for sizeable specimens before washing out the excess formalin for further preservation.

Since it is highly toxic, safety precautions should be taken prior to using it. Face masks, safety glasses, gloves and proper ventilation is necessary while dealing with the formalin fixation process.

Bouin's Fixative: This is one of the best-known fixatives for whole animals as well as tissues. It is a combination of picric acid (150 ml), 40 per cent aqueous formalin (50ml) and glacial acetic acid (10ml). It has rapid penetration properties and will preserve well the soft and delicate structures of the animal. But it is not preferred for specimens having calcium structures owing to its high acidity. At least ten times the volume of Bouin's solution is required over the volume of the specimen for fixation and the specimen should be kept for 4 to 24 hours depending on the size of the sample (Clyde et al., 1983).

Alcohol: Although it is widely preferred for the long term storage of specimens, alcohol is not an ideal fixative especially for certain groups of animals such as cephalopods. Alcohol fixes the tissue by means of its hygroscopic activity which is well below the efficacy of formalin which is known for protein denaturation of the tissue. In addition, alcohol is very slow in action and has poor penetration makes the specimen brittle, so even if it is used as a fixative in the field in an emergency (the concentration must be 70 to 75 percentage), the specimen must be transferred later to buffered formalin for better fixation. At 70% concentration alcohol is an effective biocide, below which it is not. Above the 70% limit, it will dehydrate the sample.

Paraformaldehyde: Paraformaldehyde is a convenient and economical solution for the fixation of specimens. A 10 per cent solution (35 g of paraformaldehyde in 1.0 L of water) of paraformaldehyde is suitable for the fixation of fishes. A base (e.g. sodium hydroxide or sodium carbonate) should be added to the water and then boil it before adding paraformaldehyde to avoid precipitation and polymerisation.

The process of Preservation:

In contrast to fixatives, a preservative is used only to store the specimen without any degradation of the already fixed tissue. Thus fixatives should be milder and suitable in their chemical properties, otherwise, over time the preservative itself causes the eventual deterioration of the specimen. Moreover, the long term preservation in museums of the biological specimens, where they might be frequently used for academic and research needs, should be kept in non-toxic or less toxic preservatives.

The commonly used preservatives are alcohol (ethyl or isopropyl) and formalin. In some cases, alcohol is more suitable than formalin or any other preservative due to the tissue characteristics of the animals such as the cephalopods. However, alcohol has some serious disadvantages too as it might render the specimen brittle over time as a result of dehydration. Also, the chances of alcohol getting evaporated from poorly sealed containers demand careful and regular curatorial examinations and maintenance. Specimens having a high water content will drastically reduce the alcohol level. Compared to ethyl alcohol, isopropyl alcohol has some advantages of being more cost-effective, can be used in greater dilutions and is less volatile. Nonetheless, it is noxious and relatively unpleasant. The dilution of alcohol should be done carefully as impurities in the water cause precipitations in the preservation media and will damage the specimens.

Formalin is used widely as a preservative mainly because of its low cost. However, as mentioned earlier it is highly toxic to those who are continuously exposed to it. Thus, maintaining thousands of specimens preserved in formalin raises health risks to the workers or professionals in a museum. Importantly, it is also detrimental to valuable specimens due to the demerits already mentioned above. However, it is important to note that certain specimens need

to be preserved in formalin, e.g. cephalopods because other preservatives are not efficient for their storage. Planktonic samples are also usually fixed and preserved in formalin. But there are studies that suggest the preservation of ichthyoplankton in 70% ethanol for valuable morphological, anatomical and molecular studies (Schnell et al., 2016). If the specimens are indented not for research purposes only but for displaying to the public also, preserving the natural colours is very much desirable. Studies suggest that alkaline fixatives and preservatives should be avoided and a pH between 6.3 to 7 is most appropriate for pigment retention (Taylor, 1981).

Glycerine can be used instead of formalin for the preservation of specimens since it gives the double benefit of not being toxic and preserving the morphology, flexibility and colour of the specimen. Glycerination thus enables the frequent handling of the specimens for academic purposes (Costa et al., 2021). Glycerin has antifungal and antibacterial properties. Costa et.al (2021) found that glycerine is most suitable for the preservation (after fixation with 4% formalin or 70% ethyl alcohol) of crustaceans samples as it maintains the in vivo states of colour and malleability of the animal.

Buffers: In biological specimen preservation buffer chemicals are inevitable in order to save the storage life of the valuable specimens. Fixation or preservation of samples using the two most widely used agents such as formalin and alcohol progressively develops a low pH in the medium as a result of interaction with the breakdown products like proteins and fatty acids from the specimen. The formation of formic acid due to the oxidation of formalin degenerates the calcified parts such as bones, carapace and cuttlebones of the organisms. Thus buffers such as calcium carbonate, sodium borate (borax) and hexamine can be used for efficient preservation. Marble chips or marble powder can be used as calcium carbonate buffer. Borax, which is easily soluble, is more used as a buffer in formalin preservation. However, care should be taken not to add excess borax as it causes the clearing of tissues. Hexamine or hexamethylenetetramine is more desirable than borax and calcium carbonate buffer in formalin media as it constantly maintains a pH near neutrality. It acts as a mild base, an anti-oxidant and a remover of acid in formalin solution.

Only labels make the sample in a collection useful. Without the details of its origin or date of collection, a sample is less attractive for research purposes. Ideally, a label must contain all the details such as the taxonomic position of the specimen, place, date and method of collection, name of the collector, the accession number exactly the same as those in the museum catalogue, date of deposition in the museum, if possible, as well as the medium of preservation. Labels can be fixed on a convenient part of the container for easy examination by anyone interested, and it will not mask the specimen inside. Tagging of the specimens with essential details is appropriate and beneficial and it must be done in the field itself if time and situations permit. This is especially important when collecting a particular species from various places in a single day. In such cases, a specific number of samples of a single species can be assigned as a lot tied together with a single label exhibiting their common field data.

The preservation, storage and regular maintenance of the specimens is a major aspect of museum techniques. It requires knowledge, skills as well as instinctive abilities while dealing with valuable specimens. Specimens can be stored in various types of containers according to availability, suitability and needs. Tough sealing of each container is indispensable since the contact with air will initiate physical, chemical and biological changes and processes in the specimens as well as the preservation media. Once a specimen is stored, periodic topping up or

replacement of the preservative is essential to check pH alterations, remove breakdown products and retain the exact dilutions of the preservative. Systematic documentation of the techniques of fixation, preservation and maintenance of specimens, at least that of supreme importance, like an endangered species or of type specimens is imperative for future reference. Label the containers carrying information regarding the specimens along with the type of preservative used.

Topping up the preservative is most recommended than whole replacement as it can damage specimens and containers in addition to resulting in ill-health to those who are involved. Transferring of the specimen must be done in occasions of inescapable reasons such as the case of acidification of the preservative or inappropriate preservation technique, health and safety concerns, or for research and educational purposes.
(https://conservation.myspecies.info/node/33).

Suggested Reading:

- Clyde, F., & Sweeney, J. (1983). Techniques for fixation, preservation, and curation of cephalopods.
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