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we had been discussing: access to the real artefacts, access to the processes of science.

After lugging an interesting specimen of fossil coral up the hill for the museum, we departed for Whitby Museum. This gave us a chance to see Kate Andrew's Conservation Award winning work on the wall mounted saurian collection. The restoration formed part of a project partly funded by the National Lottery. We heard of the complex and often painstaking processes involved in removing the extensive pyrite decay and historically applied lacquer coating on them. And admired the impressive results. The rest of this small museum is an interesting mix of many objects ranging from a large collection of Whitby jet to curiosities that form part of the local history.

Overall, the conference highlighted and illustrated the multi-faceted nature of access and its necessarily intimate relationship with social inclusion. The subject presents so many considerations for every museum, most of which too important to be neglected if the future of our museums and their collections is to be safeguarded. Answers to these problems are not available 'off the shelf' and a huge amount of work still needs to be carried out to facilitate maximised access to natural science collections. We need to act fast and with purpose if the future is to be grasped and used to our advantage. In response to this, anyone who is making an attempt to find solutions to access problems within museums should make it a priority to share this information with the rest of the profession.

This report was compiled by Kate Geddes from the papers given by the fourteen speakers at the conference, all of whom I would like to thank very much for helping to make it an enjoyable and thought provoking conference. I would like to extend a special thanks to all those involved in organising the conference.

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An Introduction To Molluscs

Curation, Conservation and Uses

Oxford University Museum of Natural History

Monday 31st January 2000

Following on from successful one day meetings on bones, botany and entomology, the lesser tackled subject of molluscs was decided as this years first meeting. This was a very well attended meeting, perhaps highlighting the need for information and training on some of the less high profile collections.

PRESERVATION AND CURATION OF MARINE MOLLUSCAN SPECIMENS

Once specimens have been collected in the field, careful procedures must be followed to preserve and store them as permanent biological collections with lasting scientific value. The care and maintenance of a biological collection is referred to as **'curation'**, and those who do this skilled task are **'curators'**. Clearly, the techniques required for preserving whole living animals are different from those necessary for dead shells, and are described separately below. There are, however, some similarities in the curation of both wet and dry material, which are discussed together.

It is often asked how many specimens should be collected to make a representative collection of a species? This depends upon many considerations, e.g. rarity, conservation considerations, specimen size, storage facilities, and the purpose of the collection. Clearly, for a thorough study of geographical distribution and morphological variation within a single species, large numbers of specimens and samples are desirable. At the opposite extreme, even a broken fragment of a very rare species may be a useful specimen. For a teaching collection, just a few shells may suffice. In general, museums should aim to house samples large and numerous enough to display the range of morphological and ontogenetic variation, to represent the geographical distribution and morphological variation across it, and to have material to spare for destructive dissection as required. For a common species of moderate size, a reasonable sample is 10 to 30 specimens from a locality.

1. Wet and dry collections

When making a collection of molluscs, there is always a dilemma about whether to keep live-collected specimens 'wet' (i.e. preserving both shell and soft parts in a fluid such as alcohol or formalin) or 'dry' (i.e. preserving shell alone, although as a compromise this may still contain the dried animal).

The decision must be made on the basis of the scientific importance of the specimen, the scientific purpose of the collection, and considerations of the practicality and economics of storage.

There are advantages and disadvantages of both wet and dry collections:

WET COLLECTION	Advantages	Disadvantages
	i. Preserves complete, intact specimens of maximum scientific value ii. Possible to dissect soft anatomy iii. DNA can be sampled from alcohol-fixed material	i. Bulky storage ii. Expensive containers iii. Laborious care (topping up of fluid required) iv. Acidity of preserving fluid may damage shells
DRY COLLECTION	Advantages	Disadvantages
	i. Convenience of storage (less bulky; cheaper containers; little long-term care necessary) ii. Easier to pick up and examine shells iii. If purpose of collection is for identification, record or teaching, then for most shelled molluscs only the shell is necessary for species-level identification	i. Scientific value is much reduced, since soft anatomy cannot be examined (but dried animals can be rehydrated, and their radulae can still be examined)

Therefore, most museums maintain both wet and dry collections, which serve different purposes. If the sample of a species is large, part may be dried and part kept wet.

2. Preservation of live-collected specimens

Preservation is often a three-stage process, involving narcotisation, fixation and long-term preservation and storage.

2.1 Narcotization

Most molluscs contract violently when placed in a chemical fixative. In order to preserve the body in a relaxed, lifelike manner it is therefore desirable (and humane) to first narcotise (i.e. relax and anaesthetise) the animal. Many recipes are available for narcotisation, and specialists on particular groups usually have their own favourites, developed through experience.

For marine molluscs, the best general narcotic is a solution of magnesium ions that is isotonic with seawater. A 7.5% (weight/volume) solution of **magnesium chloride** is ideal. The exact concentration is not critical; a satisfactory solution is easily prepared by dissolving one volume of hydrated $MgCl2$ crystals in 13 volumes of fresh water (**not** seawater). The time taken to achieve narcotisation varies from 1 to 12 hours according to the species. When the animal is fully extended, and shows no response if touched, narcotisation is complete. Most molluscs can safely be left in the narcotising solution overnight. However, some delicate forms may die and begin to decompose, so should be kept cool and fixed as soon as possible. Curiously, some molluscs appear to show partial recovery from narcotisation if left too long, and contract before finally dying. There are so many possible responses to narcotics that some trial and error are often inevitable. For bivalves and those gastropods that close the operculum very tightly, narcotisation is much more rapid if the valves or the operculum are first kept open by inserting a pin or small piece of wood while the animal is agape or crawling. For molluscs from brackish water, a more dilute solution must be used.

One alternative is to place the animals in a bowl of sea water and add several small crystals of menthol, but this is generally less satisfactory. If no chemical narcotic is available, animals can be kept overnight in a tightly stoppered container completely full of seawater, and some will relax as the oxygen is exhausted. Opisthobranchs can be cooled (**not** frozen) in a freezer before fixation.

2.2 Fixation

This is the initial phase of preservation, in which the fresh tissues are stabilised by coagulation of proteins or chemical reaction with them. For best results, the fixative should penetrate the tissue rapidly. Two fixatives are commonly used for molluscs: formalin and ethanol (often simply referred to as alcohol or spirit). There are advantages and disadvantages of both:

ETHANOL	Advantages	Disadvantages
	i. Not toxic to humans ii. Will preserve DNA (at concentrations above 90%) iii. Does not normally damage shells (must be buffered for long-term storage)	i. A slow fixative, therefore less suitable for large specimens ii. Poor preservation of tissues at the cellular level (for histology) iii. Large volume must be carried
FORMALIN	Advantages	Disadvantages
	i. A rapid fixative ii. Good preservation of tissues at cellular level iii. Can be carried in concentrated form and diluted with sea water as required	i. Dangerously toxic to humans ii. Destroys DNA iii. Is acidic unless buffered, so will corrode calcareous shells

Ethanol (sometimes known as ethyl alcohol; often sold cheaply as 'industrial methylated spirit', but see remarks on DNA samples below) can be used at concentrations of 70 to 100%. For general-purpose fixation, 80% is recommended. Concentrations above 90% make the tissues unacceptably firm for dissection, but are necessary for preservation of samples for DNA analysis. Dilution of 100% ethanol must be carried out with fresh water, not sea water. It is important that there is sufficient ethanol in the container to fix the tissue. Ideally the volume of ethanol should be ten times the volume of the tissue (remember that for many shelled molluscs the impermeable shell makes up most of the volume of the animal, so that containers can be filled one third full of animals and filled up with ethanol). The time taken for fixation to be completed depends on the size of the animals, but will not take longer than 10 days. For very large animals, some fixative can be injected into the tissues.

Formalin is the recommended fixative if the animals are required for dissection and/or histology. It is very important to remember that formalin is a highly toxic solution, and **must be handled with great care**. Ideally, it should only be handled when wearing latex gloves and eye protection. It is very damaging to eyes even when dilute, and must not be inhaled. There is sometimes confusion about the terminology and concentration of formalin solutions. The chemical molecule responsible for the fixation reaction is formaldehyde. This is a gas at ambient temperatures, and is sold commercially as a solution in water; this solution is called formalin. The strongest concentration available is a 40% solution of formaldehyde gas, and this is called 'concentrated formalin' or '100% formalin'. To use as a fixative, this concentrated solution must be diluted to one tenth of its strength, by mixing one volume with nine volumes of sea water (for freshwater or terrestrial molluscs, fresh water should of course be used instead). This 1:9 dilution produces 10% seawater formalin (which is a 4% solution of formaldehyde). Since formalin is acidic, this solution must be buffered. A common buffering agent is borax (di-sodium tetraborate); one large spoonful should be added to each litre of formalin. If this is not done, calcareous shells will quickly be damaged, their shiny surfaces becoming whitish and etched. Formalin is a more rapid fixative than ethanol, and fixation is complete after 5 days; only the very largest molluscs may require injection of formalin into the tissues to hasten the penetration of the fixative. Ideally, the volume of formalin should be 3 to 5 times that of the tissue to be fixed; containers can therefore be filled one half or two-thirds full of shelled molluscs, and filled up with formalin.

For specialist purposes, other fixatives can be used. Bouin's solution is the best fixative for histological samples (although formalin is adequate for most histological purposes). For electron microscopy, tissues should be fixed in 2.5% glutaraldehyde in a suitable buffer (e.g. phosphate, cacodylate, or filtered seawater). Glutaraldehyde is rather unstable, and should be kept refrigerated; it is also very poisonous.

The coloration of the living animal is usually lost during fixation and preservation. If possible, make notes on the appearance of the living animal, or take a photograph. This is especially important for opisthobranchs, in which colour patterns are often necessary for identification.

2.3 Long-term preservation

Although it is a superior fixative for most purposes, formalin is not suitable for long-term storage, because of its acidity and oxidising properties. Molluscan tissues left in formalin for periods of several years become translucent and gelatinous. Furthermore, as has been stressed, formalin is an unpleasant and dangerous solution. Therefore, formalin-fixed material is usually transferred to 70 to 80% ethanol for storage. Not only is ethanol less dangerous, but it

hardens tissues slightly, making them easier to dissect. The formalin-fixed material must first be thoroughly rinsed under running fresh water, before transfer to ethanol. (Some curators soak the material in fresh water for longer periods, but while removing the formalin this may damage the tissues; instead, for complete removal of formalin, the ethanol solution can be changed after several weeks). Even ethanol can become slightly acidic over time, and should be buffered with a small quantity of borax (0.5cm3 per litre), to prevent damage to shells. One significant disadvantage of ethanol for storage is that it evaporates quickly, so that containers must be of high quality in order to maintain a tight seal. All wet collections must be routinely monitored to check that fluid levels are maintained.

If ethanol is not available for long-term preservation, a 5% buffered formalin solution in sea water can be used, but is less satisfactory as explained above.

If material has been fixed in ethanol, the fluid should ideally be replaced before long-term storage.

2.4 DNA samples

A specialised (but increasingly common) use of biological collections is as a source of DNA samples. Any contact of the material with formalin will make extraction of DNA much more difficult or impossible. If molecular work is a requirement (or even if it is a distant possibility), fixation and storage must be carried out in ethanol. For the best preservation of DNA, fixation and storage must be carried out in ethanol of at least 90% concentration, and 100% is preferred. Rapid fixation is extremely important, and therefore shells must be gently cracked and thick tissues (e.g. foot muscle) sliced with a scalpel, to hasten penetration of the ethanol. Of course, these processes damage the specimen, so it may be preferable to fix material required for DNA studies separately from that required for anatomical work. Another source of damage to DNA molecules is methanol, which is often added to 'industrial alcohol' (or 'methylated spirit') to make it unsuitable for consumption. Although methylated sprit is suitable for fixation and storage of general-purpose collections, only pure ethanol should be used for material for DNA extraction. It is not necessary to preserve the whole animal for a DNA sample; if the animal is unique, or rather large, then a small piece (say 1 cm3) of foot can be removed for the DNA sample, and the rest preserved or dried separately, as a voucher specimen to confirm the identification of the sample.

3. Preservation of dry material and shells

There is an unfortunate tendency among some malacologists to dismiss collection of dead shells for scientific purposes, and to stress the overriding importance of making wet collections of living animals. It is of course true that intact, live-collected, wet-preserved specimens have the maximum scientific value. Nevertheless, it must be appreciated that collections can serve a variety of purposes, and that even dead shells can be scientifically useful. For rare species, or those from inaccessible habitats, only dead shells washed up on the shore may be available. As mentioned earlier, the shell on its own is usually adequate for species identification, and so may be useful for teaching and reference collections. Where live specimens are available, and can be gathered without undue environmental damage, this does make the best material for collections.

3.1 Treatment of dead shells

Empty shells should be washed in fresh water before drying, to avoid chemical deterioration during long-term storage (see 4.2).

3.2 Treatment of living specimens

If only the empty shell, or a dry specimen, is required, the soft tissues can be removed or dried. For removal, the specimens should be placed in cold fresh water and heated for 30 minutes (but not quite boiled). The tissues can then be extracted with a needle and forceps, and the shells left to dry. Alternatively, the shells may be frozen, and the soft parts removed with a powerful jet of water. Insects may assist in complete cleaning of shells. A preferable method is to fix the animals as described earlier (see 2.2), without previous narcotisation, and then dry them without removing the tissues. In this way a compromise is reached; the dry specimens are easily stored, but if the tissues are required for dissection or radula extraction, this can be achieved by rehydrating in detergent solution for 1 or 2 days (the results are, of course, less satisfactory than in the case of wet-preserved specimens). However, under tropical conditions dried animals may decompose unless stored in air-conditioned surroundings, so that complete removal of the soft parts may be necessary.

4. Curation of the collection

Curation includes all aspects of the storage, labelling, cataloguing and long-term care of the collection. The standard of curation again depends upon the function of the collection. The bare minimum is required for a simple reference or teaching collection. However, a large national museum is a repository of valuable scientific material that will be a resource for future generations of scientists; in this case curatorial care must be of the highest possible quality, to ensure the preservation of the specimens in good condition for many years to come.

4.1 Wet material

The most important requirement for wet material is that the containers used must be watertight, to reduce the need for topping up of fluid after loss by

evaporation. Slow evaporation is almost inevitable, but can be minimised. The cheapest containers of plastic, or glass jars with plastic lids, are convenient for short-term storage, but on a timescale of 10 to 20 years the plastic perishes and the fluid is lost. The best quality containers are of glass, with ground glass lids, sealed with petroleum grease, which require little or no attention for centuries, but such containers are very expensive. A compromise can be found in glass jars with metal or glass lids that are sealed with rubber washers (the rubber perishes eventually, and more long-lasting washers are made of neoprene). If the seal is poor, the opening can be covered with plastic film before screwing on the lid. Small containers are relatively more expensive; if only large jars are available, many small samples can be packed in individual glass tubes, plugged with cotton (or mineral) wool, and placed together in the jars. Most containers require regular (e.g. annual) checking to monitor possible evaporation. Since ethanol evaporates more quickly than water, topping up should be done with 95 to100% ethanol if this is available. Otherwise, after many years of slow evaporation and topping up with 80% ethanol, the ethanol solution will become too diluted, and must be entirely replaced. An alcohol meter is available to monitor the strength of ethanol. If wet specimens do dry out completely, they can nevertheless often be quite satisfactorily rehydrated by soaking for 24 hours in a solution of detergent in fresh water, before being returned to ethanol. Wet material should be stored in the dark if possible. A closed, cool cupboard also reduces evaporation. It is important that labels are not stuck only on the outside of containers, but are also included within them (see below).

4.2 Dry material

Dry material presents far fewer difficulties for long-term storage. Specimens can be stored in a range of plastic bags (those with a clip seal are best), plastic boxes, glass or plastic tubes. If tubes are used, these are best closed with a plug of mineral wool rather than a plastic stopper (the former permits ventilation, cushions the shells, and is more long-lasting). Cork stoppers should never be used (they are a source of acid, see below). Very small shells can be contained in gelatin capsules within tubes (such capsules are available for pharmaceutical use, but must be kept completely dry since they dissolve in water). In the long term, ordinary glass tubes (made of soda glass) may damage small shells, and inert plastic may therefore be the best material for small tubes. Shells should be kept in the dark to avoid fading of colours.

Dry shells are relatively robust, but one potentially serious problem can arise during long-term storage. Since shells are largely composed of calcium carbonate, they react with acid in the presence of moisture. There are several possible sources of acidic vapours in dry storage areas. Acidic residues may be present in wood (especially hardwoods; metal cabinets are best), hardboard (this should never be used in cabinet construction, since formic acid is used in its manufacture), cotton wool (this is bleached with acid; mineral wool is much better), cork stoppers, and even in some glass used for tubes (acid-free glass can be obtained). The process of attack is gradual, and affected shells appear dull, with a superficial whitish powdery deposit; when rubbed this deposit has a smell of vinegar (acetic and formic acid salts are constituents). Such shells are said to suffer from 'Byne's disease' (after its describer; no micro-organism is actually involved in this condition). In order to minimise the risk, all sources of acid vapours should be avoided. If this is not entirely possible (e.g. if the only available cabinets are of wood), then the cabinets should be well ventilated, the storage area should be of low humidity, and care should be taken to soak shells in fresh water before they are added to the collection (thus avoiding hygroscopic uptake of water from the atmosphere). To treat a shell affected by 'Byne's disease', wash thoroughly with soap and water, rinse well and dry, then rub with a little silicone oil.

Another problem that can cause deterioration in dry collections is attack by insects and fungi. The calcareous shells of molluscs are usually too robust for significant damage by these agents, but dried soft tissues, periostraca and ligaments of shells, labels and certain storage boxes are all highly susceptible. To avoid fungal damage the collection should be kept in conditions of low humidity. Where possible, air-conditioned storage is desirable in the tropics. Insects can be excluded by keeping all material in closed bags and tubes, but occasional checks for infestation are recommended, and in extreme cases fumigation with insecticide may be necessary. When introducing new material into the dry collection, it should first be thoroughly cleaned and checked for insect pests.

4.3 Labelling

Whatever the purpose of the collection, and whether material is wet or dry, it is essential to label each sample with adequate locality information. The degree of detail required will vary, but the very minimum is the name of the institution, the precise locality, and the date. The correct name of the species is the least important piece of information, since this can always be added or changed later. A record should be kept of any curatorial actions affecting the sample (e.g. examination on loan, publication of figure, re-spiriting, rehydration of dried material etc.). It is very important that if new labels are added to the collection, 'old' labels are retained with the sample and **never** thrown away, even if the identification is believed to be incorrect or the label is hand-written (in the future, these 'old' labels may be a useful source of

historical information, e.g. handwriting can be identified to show which scientists may have examined them). Here is an example of the most complete information on a museum label:

British Museum (Natural History)

Registration number 19993762

Littorinidae: *Littoraria scabra* (Linnaeus, 1758)

Locality: Kampong Uglam Hujung, Kudat, Sabah, Malaysia. 116°33'E, 7°12'N

Date: 3 September, 1998.

Habitat: On branches and trunks of *Rhizophora* mangrove trees, 1-2 m above ground, on edge of forest fringing small, sheltered, muddy bay. Uncommon. Live collected.

Collector: D.G. Reid

Notes: Specimen figured in D.G. Reid (1999) J. Moll. Stud. 65: 127, fig. 6. Sample fixed in formalin. Additional samples in 100% ethanol and in dry collection. Material borrowed on loan by R. Kilburn (Natal Museum) 9/1999.

The best quality of paper that can be afforded should be used (e.g. 100% rag paper, acid-free vegetable parchment, or a synthetic equivalent). Labels should be written in indelible ink, or printed. Labels should always be placed inside the containers with the specimens, never stuck on the outside (where they may eventually become detached and lost).

For the wet collection, labels should be written in indelible ink on waterproof paper, and left to dry thoroughly before immersion in ethanol inside the container. Photocopied labels are unsuitable for wet material, since the printing becomes detached from the paper. If waterproof labels are not available, Dymotape labels are useful for registration numbers inside the containers.

4.4 Registration system

When establishing a scientific collection, it is strongly recommended that a registration system is initiated at the outset. Each sample (otherwise known as a 'lot') should be given a unique registration number, and all the information on the label should be duplicated in a separate registration book, or in a computer database. For both wet and dry samples, this number should be written both on the label and on a separate small slip of paper enclosed within the container (if the original label becomes lost or damaged, this allows the information to be retrieved). In addition, it may be possible to inscribe larger dry shells individually with their registration numbers, using black ink. Do not rely only on an electronic database; a printed or hand-written copy should also be kept for additional security. Museums employ a variety of different numbering systems, for example starting each year with the date followed by a 4-figure field, e.g. registration numbers for 1999 run from 19990001 to 19999999 (this has the advantage of adding the information of the year in which the material reached the museum); it is also possible to simply start at number 1 and number sequentially (which will show the total number of lots in the collection). Complex numbering systems according to species identification or geographical locality are not recommended.

There are many advantages of a registration system: lost data can be retrieved; individual specimens or lots can be referred to (or figured) in publications and then easily retrieved by future workers; in large museums that operate a system of specimen loans, the registration number allows curators to monitor the destination and condition of each sample; in a computer database it is of course essential that each sample has a unique identifier (the species name may be changed according to revisions of taxonomy).

4.5 Arrangement of collection

This will depend largely upon considerations of space and economics. However, within these constraints some recommendations can be made. In almost all cases the collection should be arranged in standard systematic order of families (this order changes somewhat from time to time according to ideas about evolutionary relationships of the major groups, but if an up to date listing of molluscan families is followed (e.g. Vaught, 1989; Rosenberg, 1992) this arrangement can be adopted for convenience. Within families, an alphabetical arrangement of genera and species is usually suitable, with space at the end for unidentified members of each genus and family. Remember to leave sufficient space for future expansion of the collection. A single systematic sequence for all the material in the collection is not usually practicable. For example, wet material has special storage requirements, and is therefore stored separately from the dry collection. Type specimens are of particular scientific value, and for security reasons are sometimes stored separately in locked cabinets. Very large specimens may be too big to fit in the available cabinet drawers. In these cases, it is

strongly suggested that the general dry collection should act as a quick visual catalogue of the entire collection, by including in it empty boxes in their correct systematic position with labels indicating that large, wet or type specimens are stored elsewhere.

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The Conservation of Molluscan Collections

All Collections are open to the 'Nine Agents of Decay' (cited in MGC 1998). These have been defined by the Canadian Conservation Institute (CCI) as:

1: Direct Physical Forces e.g. dropping; wearing away.

2: Security e.g. risk of theft; vandalism; displacement through poor collection management.

3: Fire

4: Water e.g. flood; leaking building; fire suppression.

5: Pests e.g. insects; vermin; moulds; microbes; children!

6: Contaminants e.g. atmospheric pollutants; liquids; solids.

7: Light especially visible and UV.

8: Temperature

9: Relative Humidity

Ideally the Collection Environment will protect the specimens from these agents of decay. The environment can be usefully divided into two main compartments;

- The Storage area; the macro-environment.
- The Storage Units housing the collection; the micro-environment.

Mollusc Collections tend to fall into two broad categories. Either dry shell collections or fluid preserved 'wet' collections. Parts of the same specimen can be in both collection types. This is where the shell has been separated from the soft animal parts. It is also possible to dry the whole animal, rehydrating the soft body parts with Decon 90 if required, a practise used at the Natural History Museum.

Dry Collections

Dry Mollusc Collections can have a number of characteristic conservation problems such as Bynes Disease (a white crystalline growth on the surface of the shell), Cracking and Exfoliation. These problems can have a variety of causes, such as;

- Original preparation of the specimen.
- Subsequent remedial work.
- Neglect, poor custodial care.
- Environmental conditions; such as incorrect and/or fluctuating humidity and temperature; light – both visible and UV; Pollutants.
- Incorrect storage problems e.g. off-gassing from woods, MDF; decay of storage plastics; plasticisers in plastic clip bags migrating out and degrading.

Looking at some of the more specific problems associated with dry shell collections;

Bynes Disease (Tennant and Baird 1985; Von Endt et al 1996). Not a disease but a chemical reaction causing degradation of the shell;

- Characterised by a white or grey, water soluble crystalline efflorescence on the surface of the shell, which is usually a mix of calcium formate and calcium acetate.
- Affects mainly marine shells, but can be a problem in land shells (where its occurrence probably relates to the method of preparation).
- Caused by formic acid and acetic acid, off-gassing from storage furniture. The presence of hygroscopic salt residues in the shell is also considered to be contributory factor.

Shell cracking (Child and Buttler 1996)