Investigating the use of propylene phenoxetol preservation methods in natural history museums

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Abstract

Fluid preservation of organic material requires an initial fixing to prevent tissue breakdown and decomposition, commonly achieved using an aqueous solution of formaldehyde, or using ethanol. Post-fixation, specimens are frequently preserved in solutions of either 4% to 10% formalin, or 70-80% ethanol. Specialist fluids include Steedman's method using propylene phenoxetol (PP), developed for use with small marine invertebrates. Steedman's method was subsequently applied to a much wider range of zoological collections with varying reports of success. In more recent years the use of Steedman's and PP has been questioned and it is now considered inappropriate for long-term storage. Despite this, an audit of the fluid-preserved collections at the Cole Museum of Zoology (REDCZ) showed that many specimens preserved in PP remain in good condition after almost twenty years.

A survey was distributed via the Natural History Collections and Natural Sciences Collections Association mailing lists to determine the variety of preservation fluids used in museum collections worldwide. Only half of the 35 respondents were aware of the recent recommendation to monitor or remove Steedman's from their collections, and only two institutions had already followed this advice. Follow-up interviews with survey participants revealed wider systemic issues that prevent a deeper knowledge of fluid-preserved collections or ability to take appropriate action. These included a lack of financial resources, staffing gaps leading to a loss of institutional knowledge, a lack of suitable laboratory workspaces, and limited to no access to beneficial technology.

Keywords: Steedman, propylene phenoxetol, fluid preservation, zoology museum, fixation, preservation

Introduction

The role of a caretaker of fluid-preserved biological collections is to maintain their specimens in as good a condition as possible, for as long as possible, and to maximise the ways in which these collections can be used for teaching, research, and display.

Techniques used to preserve animal specimens in fluid have not changed drastically from those developed over a hundred years ago (Simmons, 2020). The preservation of whole animals or tissue samples requires an initial fixing to prevent tissue breakdown and decomposition. This is commonly achieved by immersing the specimen into a 4% solution of formaldehyde (10% Formalin – see Appendix II: Formulae) which hardens the

tissues by denaturing or cross-linking chains of proteins. The fixation process takes time, which is variable depending on the type and size of the specimen that is being fixed. Once fixed, specimens are then commonly preserved in aqueous solutions of either 2.5% formaldehyde (5% Formalin), or 70-80% ethanol (Simmons, 2014). Other preservation methods may be used for specific purposes such as tissue clearing, or the stabilisation of particularly delicate specimens (Harris, 1990).

The discovery of formaldehyde (a 37% aqueous solution of formaldehyde gas) as a fixative in the 1890s (Blum, 1893) was enthusiastically espoused by the fluid-preservation community, but it took more than eighty years to discover that long-term exposure to this compound could have not just irritant and sensitising, but also potentially carcinogenic effects on those working with it (Chemical Industry Institute of Toxicology, 1979). Phenoxetol, in varying forms, has been suggested several times as a safer alternative to the use of formaldehyde-based preservatives (Tandon *et al*., 2014, Frølich *et al*., 1984), but in the long term it has not proved to be universally reliable, and has therefore not been widely accepted in the same way that formaldehyde was.

Zoological specimens which have been fixed in formaldehyde, and preserved in either formaldehyde or ethanol, tend to lose much, or almost all, of their natural colour. This can make taxonomic and morphological research difficult, if not impossible. While periodic attempts to maintain colour in fluid-preserved collections have been made (Harris, 1990), it is generally held to be extremely difficult because of the biochemical processes taking place between the specimen and the preservation medium (Stoddart, 1989). Fluid-based preservation methods designed to retain the colours of skin and tissue were developed in the late 19th century by pathologists, including Leonhard Jores and Carl Kaiserling (Jores, 1896, Kaiserling, 1897). Several studies claimed that while good colour had been maintained using these methods for 15-20 years (Jores, 1896), colour degradation attributed to haemolysis had later been observed in those same specimens (Pulvertaft, 1950). However, these methods were developed for specimens where colour was mostly related to haemoglobin. This limits their usefulness in a wide-ranging zoological collection which contains animals that use different respiratory pigments such as haemocyanin or chlorocruorin.

The latter half of the twentieth century saw a resurgence in the development of new methods with the aim of improving colour retention in fluid-preserved specimens. These included work with hydrosulphites (Wentworth, 1957), a survey of potentially useful antioxidants (Gerrick, 1968), and Hugh Steedman's method based on propylene phenoxetol (PP) that was originally designed for preserving marine zooplankton (Owen and Steedman, 1956).

During the 1960s, propylene phenoxetol came into use as a preservation method in some UK museum collections, and Steedman introduced the addition of propylene glycol to propyelene phenoxetol in 1976. (Moore, 1997). However, by the end of the 1980s, some specimens were found to have been insufficiently well preserved by Steedman's method (Crimmen, 1989). In 2022 it was recommended that smaller institutions holding fluid-preserved biological collections should no longer use Steedman's Post-Fixation Preservative as a long-term preservation solution, and that larger institutions and those with the facilities to do so, should monitor their Steedman's specimens monthly for signs of deterioration (Neumann *et al*., 2022).

There are several different phenoxetol-based preservation methods that may be used in fluid-preserved museum collections (Neumann *et al*., 2022), but the one used by the Cole Museum of Zoology (REDCZ) from the early 2000s to the present is the formula given in chapter five of *Care and Conservation of Natural History Collections* (Carter and Walker, 1999) (Table 1).

Table 1: Steedman's Fixation and Preservation Formulae

Steedman's Fixative	**Steedman's Post-Fixation Preservative**
100ml propylene phenoxetol dissolved in 500ml propylene glycol	50ml of propylene phenoxetol dissolved in 500ml of propylene glycol
Add 500ml formaldehyde 37% (Formalin)	
Dissolve 110ml of concentrate in 890ml of distilled water or saline	Dissolve 110ml of concentrate in 890ml distilled or deionized water
pH 6.8-7	pH 7-7.4

There are two parts to the Steedman's process – the first is a fixation concentrate which is made up of propylene phenoxetol, propylene glycol, and formalin, in distilled water or saline solution. The second part is Steedman's Post-Fixation Preservative, which is what is present in the Cole Museum's collection. This does not contain either ethanol or formalin. Instead, it makes use of propylene phenoxetol for its antibacterial and anti-fungal properties, and propylene glycol as a humectant. This keeps the specimens softer and less brittle than other preservatives, which was particularly important to Steedman as he was working primarily with zooplankton and other small marine invertebrates.

Rather than being restricted only for use with similarly small and delicate specimens, Steedman's methods began to be applied to a much wider range of zoological collections. In the early days of its use, the Natural History Museum in London was aware of problems with specimens that had not been properly fixed in formaldehyde before being treated with phenoxyethanol (Nakanishi *et al*., 1969) – something that Steedman was very clear about in his subsequent work (Steedman, 1976). In more recent years the use of Steedman's post-fixation fluid has been questioned, and propylene phenoxetol in general is now considered inappropriate for the long-term storage of fluid-preserved specimens, particularly those of a large size (Neumann *et al*., 2022). This is partly due to the unexpected decomposition of a large (~200 gallons) tank of fishes at the Natural History Museum in London, which had been kept in Steedman's post-fixation preservation fluid (Crimmen, 1989). The Cole Museum currently holds 52 specimens preserved in Steedman's propylene phenoxetol-based preservation solution. Some of these are large fish and densely muscled mammals, which have remained in good condition since their transfer into PP during the early 2000s. It is also being used successfully in modern collections of marine specimens, including the Discovery Collections at the National Oceanography Centre.

The purpose of this paper is to re-examine the use of Steedman's and PP in natural history collections and to determine why the Cole Museum's specimens preserved in PP remain in good condition after almost twenty years in that solution. Those in other museums deteriorated after less than fifteen years (Crimmen, 1989), so this is an ideal time to be carrying out an in-depth investigation into the condition of these specimens. The working hypothesis is that because almost all of the Cole Museum's Steedman's specimens spent up to six decades in other preservation fluids before their transfer to PP, this may have improved their longevity compared with specimens that were both fixed and preserved solely by Steedman's methods.

In order to understand whether the Cole Museum's experience with Steedman's was representative of museum collections overall, a survey was developed to determine the prevalence of Steedman's, and its primary ingredient propylene phenoxetol, in fluid-preserved museum collections across the world (Appendix). This was distributed via the Natural History Collections (NHColl) and Natural Sciences Collections Association (NatSCA) mailing lists, and a link to the online survey was also provided via a QR code at the 2024 NatSCA conference.

Cole Museum survey

The Cole Museum's fluid-preserved collection numbers around two and a half thousand specimens, and 52 of those are currently in Steedman's Post-Fixation Preservative. Additionally, there is a Teaching Collection of around one and a half thousand jars, most containing multiple specimens. In 2007-8 there was an extensive programme of moving many of these specimens out of formaldehyde and into Steedman's, which was probably carried out to make them safer for study and maintenance by undergraduate students. Steedman's specimens make up around 29% of the Teaching Collection, so the total number of Steedman's-preserved specimens across both of the Cole Museum's fluid -preserved collections is approximately 500 jars. Following the SPNHC best practice recommendation, an assessment of the fluid-preserved collections was carried out (Neumann *et al*., 2022).

Cole Museum survey: results

Given that Steedman's preservation methods were developed for use with marine zooplankton, it was expected that marine specimens would be represented in greater numbers in this fluid (Table 2). In fact, mammals represented the largest individual class with a total of 13 specimens. The Steedman's collection overall is made up of 30 vertebrates (including 12 fishes), and 22 invertebrates. This represented a much wider taxonomic range than originally anticipated, which reflects the diversity of the Cole Museum's comparative anatomy collection.

Table 2: Steedman's-preserved specimens, by phylum and class

Phylum	Class	Number of specimens
Chordata	Actinopterygii	5
	Amphibia	2
	Ascidiacea	1
	Aves	1
	Chondrichthyes	6
	Dipnoi	1
	Mammalia	13
	Reptilia	1
Cnidaria	Anthozoa	5
	Myxosporea	1
	Scyphozoa	1
Echinodermata	Asteroidea	2
	Echinoidea	1
Mollusca	Bivalvia	1
	Gastropoda	4
Nematoda	Chromadorea	1
Porifera	Calcarea	1
	Demospongiae	2
Platyhelminthes	Cestoda	1

Most of the Cole Museum's Steedman's specimens have been moved through several different preservation fluids over many decades. Table 3 shows the progression of changes in fluid at two periods of recorded changes – the early 1960s, and the early 2000s. From at least the 1970s until the 2010s, the museum and teaching collections were maintained by technical staff with little oversight by the academic curators. This was because the specimens were seen purely as a teaching collection maintained by teaching technicians. There is no documentation to explain the reasoning behind the decision to transfer large numbers of specimens out of one fluid and into another, or the process by which this was carried out, but archives show that there was a trend during the 1960s for moving specimens out of spirit and into formaldehyde. More recently there was a short period, in 2007-8, of transferring formaldehyde specimens into Steedman's because of Health and Safety concerns. This appears to have been trialled on the Teaching Collection, which now has more than four hundred jars containing Steedman's specimens.

Examples of good and poorly preserved specimens in Steedman's

The general guidelines for Steedman's are that it should not be used for large specimens, and that it

Table 3: Progression of changes in preservation method of the Cole Museum's Steedman's-preserved specimens. Where the strength of a solution is not given, this was not documented in the archives.

Original Preservative 1909-1953	1960s Preservative	2000s Preservative	2024 Preservative	Number of specimens
Not recorded	Not recorded	Steedman's	Steedman's	1
Formaldehyde 2.5%	Formaldehyde 2.5%	Steedman's	Formaldehyde 2.5%	3
Formaldehyde 2.5%	Formaldehyde 2.5%	Steedman's	Steedman's	13
Formaldehyde & glycerol	Formaldehyde & glycerol	Steedman's	Steedman's	1
Methyl salicylate (Oil of Wintergreen)	Methyl salicylate (Oil of Wintergreen)	Steedman's	Paraffin	1
Spirit 70%	Formaldehyde 2.5%	Steedman's	Formaldehyde 2.5%	2
Spirit 70%	Formaldehyde 2.5%	Steedman's	Steedman's	29
Propylene Phenoxetol 1%	Propylene Phenoxetol 1%	Steedman's	Steedman's	1
Dry	Unknown	Steedman's	Steedman's	1

Figure 1a: Rhesus monkey, Macaca mulatta, *REDCZ 2957; 1b: Brown trout,* Salmo trutta, *REDCZ 2188; 1c: Pigeon,* Columbia livia, *REDCZ 2861 Images © The Cole Museum of Zoology*

is inappropriate for densely muscled animals (Moore, 1997). Simon Moore suggests that dense muscle tissue may form a barrier which prevents phenoxetol from entering a specimen, thereby creating only a surface level of preservation. This would allow the specimen to deteriorate from the inside out (Moore, 1997).

While many of the Cole Museum's Steedman's specimens are not large or densely muscled, there are also examples of such specimens that have remained in good condition. Figures 1a and 1c are dissections of the superficial muscles of a rhesus monkey's leg (REDCZ 2957), and a pigeon (REDCZ 2861). Figure 1b is a brown trout with the ovary dissected to show the eggs (REDCZ 2188), and this specimen is a relatively large one at 41 cm tall and 16.4 litres in volume. Despite being both large and densely muscled, there are no signs of turbidity or tissue degradation in these specimens which might suggest they are at immediate risk of damage due to their preservation method, although it is possible that unseen deterioration is taking place inside the specimens. All three were originally preserved in 'Spirit 70%' in the 1930s and 40s, changed to formaldehyde during the 1960s, and moved into Steedman's in 2007-8. It seems likely that the decades which these specimens spent in formaldehyde are a contributing factor to the stability and good condition of the Cole Museum's Steedman's-preserved specimens, in part due to the residual formaldehyde that remains even after the fluid has been changed (Waller and Simmons, 2003).

The trout in Figure 1b, for example, was originally preserved in 70% spirit in 1931. It was changed over to 2.5% formaldehyde in 1965, and then moved into Steedman's at some point prior to 2020. This is the most recent point at which there is documentation about the specimen's conservation treatment. A deteriorating jar seal was replaced, and the fluid was topped up following the resulting evaporation. The loss of preservative fluid due to evaporation can also cause deterioration of specimens, which may then be incorrectly attributed to the preservative itself.

However, not all of the Cole Museum's specimens that were transferred into Steedman's are currently in good condition. A long-eared bat (REDCZ 109 (duplicate)) (Figure 2) that was stored in Steedman's post-fixation preservative, has unexpectedly deteriorated. Although records show that the bat was in good condition in October of 2021, two months later, in December 2021, it was discovered that the most delicate areas of tissue, including the wings, tail, and long ears, had become detached and fallen to the bottom of the jar. The bat was removed from its jar as carefully as possible, and the fallen tissue was retained along with a sample of the fluid. It was re-fixed with 4% formaldehyde and preserved in 2.5% formaldehyde, and this appears to have prevented any further tissue loss.

Figure 2: Long-eared bat, Plecotus auritus*, REDCZ 109 (3/3 with this number), following re-fixation with 5% formaldehyde and preservation in 2.5% formaldehyde Image © The Cole Museum of Zoology, 2024*

The sudden degradation of this specimen is unlikely to be related to dense muscle, since it was the thin wing membranes that have been lost. An alternative hypothesis is that specimen breakdown is related to the lipophilic properties of phenoxetol. Andries van Dam has suggested that phenoxetol migrates towards the lipid-containing parts of the specimen, leaving the surrounding fluid unbalanced, and the less fatty parts of the specimen (e.g. the fins of The Natural History Museum's fishes, and the wings and ears of the Cole Museum's long-eared bat) open to microbial attack (van Dam, 2003). However, there was no evidence of microbial attack to these specific areas of the bat, and van Dam's hypothesis does not address the other signs of deterioration that were seen in the Natural History Museum's specimens (Crimmen, 1989). Checking the fluid sample retained from the bat for phenolic compounds might give an indication as to what extent the propylene phenoxetol had broken down (Carter, 2024). Fourier Transform Infrared (FT-IR) Spectroscopy will be performed on samples to verify this.

The two other issues that have been noted with the Cole Museum's Steedman's specimens are particulates recorded in the fluid, and detachment from their glass backing plates. Five specimens had become detached from their glass mountings. Four of these had originally been tied to the backing plate with an undocumented type of thread, and they were re-attached in 2007 or 2008, with nylon monofilament (REDCZ 738, 2298, 2340 and 2802). It is not clear how long these specimens had been preserved in Steedman's before their detachment and whether this had caused any softening of the tissues which allowed the original mounting thread to tear through the specimens, or whether it was

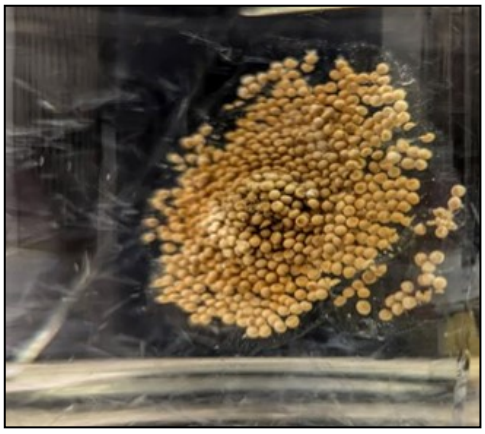

Figure 3: Eggs of albino common frog, Rana temporaria*, REDCZ 2298 Image © The Cole Museum of Zoology*

more likely to be a problem caused by using monofilament thread to mount already soft specimens. It is possible that using a softer and more flexible material, such as cotton or linen thread, could have prevented or minimised this damage. However this would be more visible, which is why monofilament is often preferred.

The fifth specimen, the eggs of an albino frog (REDCZ 2298) were originally fixed in 2.5% formaldehyde (in 1932) and were later moved into Steedman's. In 2007 a long-overdue audit noticed that they had all fallen from the glass plate to the bottom of the jar following the failure of the adhesive used. They were likely to have been attached with gelatine, which forms an effective adhesive when used with formaldehyde-preserved specimens (Carter and Walker, 1999). After the move into Steedman's, the attachment probably weakened. They were reattached with gelatine in 2007 and transferred back into formaldehyde. They remain attached in 2024.

Fixation in fluid-preserved specimens has always been synonymous with firmness, so soft tissue in specimens is generally taken to mean loss of structural integrity and therefore inadequate fixation (Simmons, 2014). Two specimens were removed from Steedman's because they were thought to be improperly fixed due to the softness of the tissues (REDCZ 18, and 2340). These were re-fixed with 5% formaldehyde, and have remained preserved in 2.5% formaldehyde. Specimen REDCZ 18 is one of the Cole Museum's earliest specimens to be accessioned, in 1909. It was originally in 'Spirit 70%' but was transferred to formaldehyde along with many other specimens during the 1960s. As with the albino frog eggs (REDCZ 2298), it was moved from Steedman's into formaldehyde in 2007, when it was also re-

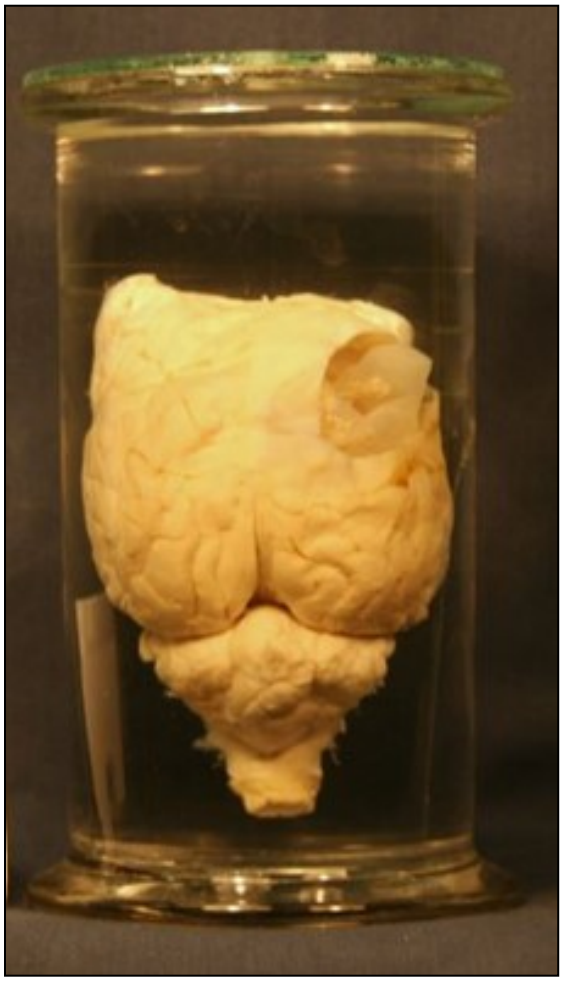

*Figure 4: REDCZ 18, a tapeworm cyst (*Taenia multiceps*) inside the brain of a sheep, following conservation in 2007 Image © The Cole Museum of Zoology*

fixed. The photograph in Figure 4 was taken immediately after this conservation work had been carried out.

This example shows that it is possible that Steedman's can soften previously fixed specimens, as well as keeping them soft when used as a primary preservation method (Frølich *et al*., 1984). It has also been used to keep rehydrated specimens in good condition (Carter and Walker, 1999). Research has not yet been undertaken to determine whether the softness of Steedman's specimens is caused by a modification or even a reversal of the fixation process. These are areas that could benefit from further investigation to determine whether, following appropriate fixation, Steedman's could still be used successfully in the longer term in specimens where softness is a desirable quality. Historic methods of mounting these specimens would also need to be revisited, in order to prevent future damage.

Fluid collections survey

A survey was developed to determine whether the Cole Museum's experiences with Steedman's as a preservation solution were reflected in fluid-preserved collections in other institutions (Appendix). Distributed via the Natural History Collections (NHColl) and Natural Sciences Collections Association (NatSCA) mailing lists, and a QR code at the 2024 NatSCA conference, approximately 1,000 respondents should have been reached through these requests to fill in the survey. During the allotted time period, only 35 individuals completed the survey on behalf of their respective museums. The results therefore represent a qualitative overview of self-selected participants.

Steedman's Post-Fixation Preservative is known by several different names and acronyms. These include Preserving Fluid, PP (propylene phenoxetol), PFP (Post-Fixation Preservative), and 1% (the concentration of propylene phenoxetol in the solution), amongst others. In order to avoid confusion, respondents were asked about Steedman's by name, and about PP as its characteristic ingredient. For the purposes of this analysis, the two have been combined. Other preservation methods were also included in the survey, as these may form the basis of further research about some of the less common preservatives used in fluid-preserved museum collections.

Fluid collections survey: results

The use of Steedman's was most common in European natural history collections, but the 19 respondents from this location made up the largest part of the survey results which has skewed them in this direction. Institutions with multiple types of collections, including comparative anatomy, pathology, and herbaria, were less likely to be aware of Steedman's as a preservation method. Respondents in the United States and Canada were almost entirely unfamiliar with Steedman's or PP as preservation methods, as Steedman's publications were not widely available outside of Europe and the UK. However, these results are an extremely small sample, so this may not represent the wider situation (Figure 5).

Twenty-one respondents were aware of Steedman's, and 13 of these used this preservation method in their own collections (Table 4). Opinions of the efficacy of Steedman's were mixed, with five negative responses, and one very negative. There were five neutral responses, and

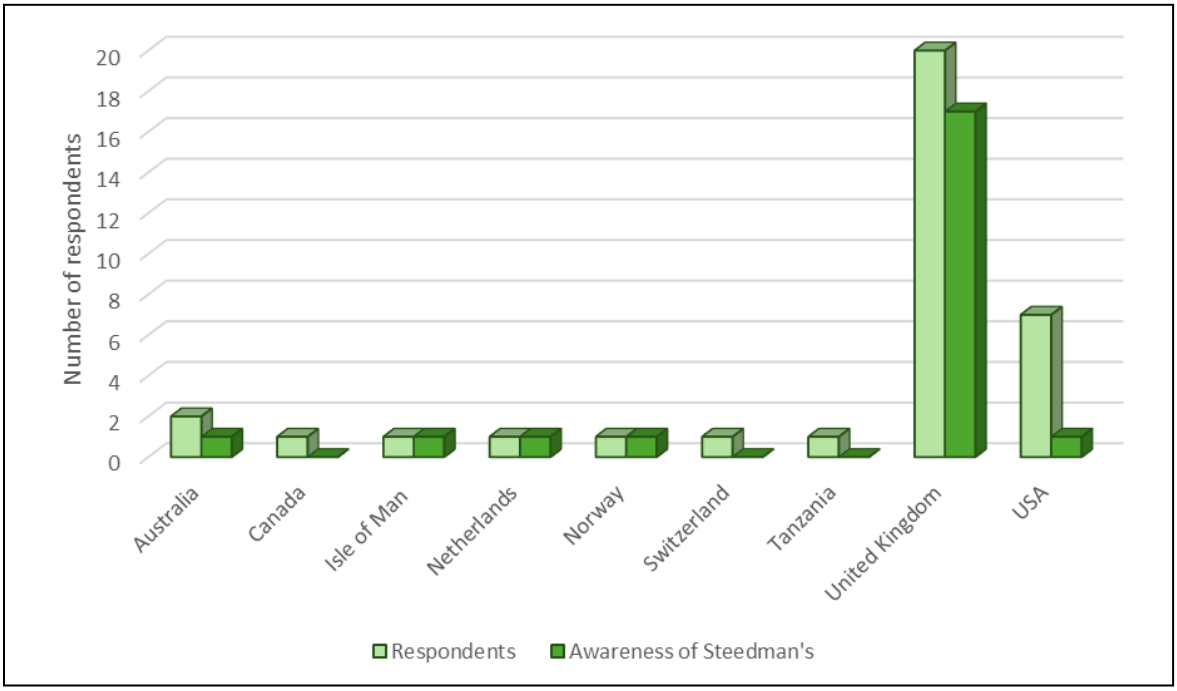

Figure 5: Survey respondents' awareness of Steedman's, by country

	Currently	Historically	No	Don't know	No response
Formaldehyde	26	5	3	0	1
Ethanol / IMS / other spirit	34	1	0	0	0
Steedman's Post-fixation Preservative	5	3	11	10	6
Propylene Phenoxetol (sometimes labelled PP or 1%)	3	6	11	9	6
Propylene Glycol	4	2	9	11	9
Liquid paraffin	2	1	17	7	8
Glycerol	19	1	7	6	2
Glycerol & water	12	0	10	7	6
Jores / Jories	0	1	14	11	9
Kaiserling	6	3	11	10	5
Other	7	3	7	10	8

Table 4: Breakdown of fluids used in natural sciences collections

just two people considered it to be an effective preservative.

Eighteen respondents were aware of the recent recommendation to monitor or remove Steedman's from their collections wherever possible (Neumann *et al*., 2022), and half of these were either currently using or had historically used this method in their collections. Two institutions had carried out the removal process already, three were undecided, and two were making plans to do so in the future. One museum was happy with the quality of their Steedman's specimens and saw no pressing reason to remove it from their collections, and one other institution had purposely moved specimens into Steedman's

rather than out of it. These were both collections of marine invertebrates. The remaining nine respondents did not use Steedman's as a preservative solution in their current collections. Steedman developed his methodology for the study and preservation of marine zooplankton, so it is unsurprising that the institutions whose specimens have been the most successfully preserved in Steedman's are those who hold dedicated collections of siphonophores. These were generally described as being in good condition, although their delicate nature makes them easily damaged by the trawling methods used for their collection. The problem areas illustrated by individual specimens from the Cole Museum were not apparent at other museums – not because their specimens were all in pristine condition, but because a similar audit had not been carried out.

Fluid collections survey: discussion

The survey responses indicated that a variety of preservation methods were used by different types of fluid-preserved biological collections, often for specific purposes. Glycerol, for example, is used not only for tissue clearing and alizarine preparations, but also for the preservation of teeth. Kaiserling is commonly (but not exclusively) used in pathology collections, who may also use methyl salicylate (oil of wintergreen) and turpentine. Fluid-preserved botanical specimens are often stored in Kew Mix, and sometimes temporarily transferred into formaldehyde-free Copenhagen Mix for work that requires handling by researchers. Entomology collections may use Güell & Mendel's Beetle Relaxing Fluid for preservation as well as preparation (Mendel, 1993), and propylene glycol is sometimes used as an additive to ethanol, to prevent embrittlement in small arthropod specimens (See Appendix 2 for formulae). When looking more widely at the range of preservation methods in use, it becomes clear that there is a great deal of nuance in the ways in which these methods can be used, and there may be discrepancies in the ways in which they are understood.

Confusion can also be caused where the same preservation method is known by multiple names. As well as Steedman's being also known as "Post-Fixation Preservative", and "Preserving Fluid", it may also be known only by its component ingredients. For example, three people responded that they had a combination of propylene phenoxetol and propylene glycol in their collections, in addition to specimens that were known to be preserved in Steedman's. This suggests that more people may have Steedman's in their collections than are aware of it, because they know the ingredients but not the name; they might have an alternative name for the same methodology; or the preservation details might not be included in their records. The same is true of Jores' solution: 12 museums said that they had specimens stored in glycerol and water, which is the third stage of preservation by Jores' 1913 method (Jores, 1913), but no respondents were aware of the name of Jores being associated with this methodology. Depending on the availability of documentation and shared knowledge, names associated with fluids can become lost, and they may become known by their constituent parts instead. This type of inconsistency of naming can be confusing both within and between collections.

Given that all respondents had said that they could answer questions about the preservation fluids used in their collections, there was a notable lack of response in some areas. There was a higher degree of certainty when it came to commonly used preservation methods such as formaldehyde and ethanol or other alcohol-based solutions, but less frequently used preservation fluids had a much higher rate of "don't know" or no response answers. There was a mixed response to the survey questions relating to the use of Steedman's. Since fewer than 25% of respondents whose collections included Steedman's were aware of the recommendations to monitor or remove specimens from this preservative, decisions regarding its continued use are largely related to other issues.

The same concerns came up repeatedly, regardless of the size of the institution. Museums are fundamentally under-resourced in terms of both staff, and money (Atkinson, 2024). New staff may be brought in or reassigned to look after sometimes already-problematic fluid collections without access to handover or specialist training, and comprehensive catalogues may not be available, increasing the amount of detective work that needs to be done before changes can be carried out safely. Where this kind of documentation is lacking, it becomes impossible to know which specimens may be at risk. Facilities and expertise to fill these knowledge gaps may not be available.

Individual interviews carried out following the survey indicated that there was frequently a lack of historical and contemporary documentation about the fluids used. Many collections are not fully catalogued and, where they are, there is not always information about the preservation method

assigned to the individual specimens. Sometimes this may be caused by inadequate historical record -keeping, and sometimes by a lack of handover from previous staff, often caused by a gap in funding for personnel. More than one institution shared that they hold collections covering a wide range of zoological specimens that came into their collections between the 1960s and 1980s. Unfortunately, there were no accompanying records to determine which fluids these specimens were preserved in. They are assumed to be in propylene phenoxetol, as it was gathering popularity in the UK during this time, and they are not preserved in either ethanol or formaldehyde. Without documentation the only way to confirm this safely is by chemical testing, and the technology to do this at a detailed level may not be easily or affordably available.

Collections management plans may differ within the same institution, depending on the types of specimens involved and the resources available. Where Steedman's-preserved specimens had been removed from collections, this was generally carried out as an ad-hoc process rather than as a large pre-planned project. When speaking with respondents who stated that they currently or had previously used Steedman's, it became clear that many Steedman's specimens were pre-existing in their collections, and that the curators and conservators who currently care for them would not necessarily advocate for its continued use.

This was not only to do with the degradation of specimens, awareness of the recommendation to remove the fluid from collections (Neumann *et al*., 2022), or the logistical difficulties associated with carrying out that task. There were also considerations concerning ease of use, particularly when compared with ethanol. Steedman's is made up from a concentrate, the ingredients for which need to be sourced and appropriately stored. This takes time, and requires both lab preparation and storage space that may not be available. Ethanol is also easier to use where volunteers carry out much of the topping-up of specimens, particularly when it is purchased pre-diluted to a 70% strength, as less rigorous training may be required. For additional convenience some institutions may be able to buy ethanol or IMS in bulk – or they may even have it readily available on tap. Factors such as these can make the decision to remove Steedman's from fluid-preserved collections perhaps a more pragmatic one than expected. A lack of time and resources for testing historic fluids, particularly in larger collections where this would be an enormous amount of work, suggests that this situation is unlikely to change. Another factor is the expense of specialised equipment relating to fluid-preserved collections, particularly items such as an alcohol density meter, that may be beyond the budget of smaller institutions. that may not be required by an institution on a permanent basis. A system similar to the Library of Things (Library of Things, 2024) might be a means of allowing equipment to be shared, or budgets could be pooled between collections that are relatively local to one another.

Where new staff are taking over fluid-preserved collections without prior training or experience in that area, they may have concerns about safety, particularly when dealing with preservation methods that are less well known than ethanol and formaldehyde. There are also concerns relating to the toxicity of exposure to formaldehyde, and the potential hazards of unidentified chemicals within a collection. Training for fluid preservation has always been difficult to access simply because of its scarcity. Institutions may lack the necessary space and equipment to host such a course themselves, or they may not have the financial means to support staff travel and accommodation as well as the cost of the training itself. This is not to say that such courses are not valuable – quite the opposite. But the fact that they are inaccessible to many means that there is space for additional solutions. Training in how to audit and catalogue a collection, for example, could be provided as a way for those new to wet collections to understand what they have, and how to start planning for their care.

A related issue was being able to find the right information on the subject of caring for fluid-preserved collections. This is not to say that information is unavailable – research is constantly evolving, and new work is frequently published. There is also a dedicated fluid preserved collections conference which was first held in 2018, and which is taking place again in November 2024. This situation could be remedied by making sure that people know where to look. For those who are new to wet collections, the amount of in-depth literature can be overwhelming. A resource dedicated to working with fluid-preserved specimens, such as a web page with a library of links to existing publications divided into themes, would make it a lot easier to find specific information. However, resources such as this require constant maintenance, the resources for which may not be available. This could also be backed up with a dedicated group for those working with fluid-preserved collections. While the NatSCA mailing list is an excellent resource, and an extremely helpful and knowledgeable one, it

may still be perceived as intimidating to ask what might feel like an embarrassingly basic question. There is also shame and embarrassment around having specimens that have deteriorated, even where facilities to investigate the potential causes may not have been available. This can lead to an unwillingness to ask for help from others with experience in the field, for fear of being seen as insufficiently knowledgeable. Sometimes the simplest solution becomes taking the path of least resistance and working with broad spectrum preservation methods, even though this may not be the optimal solution for the long-term safety of individual specimens. Talking openly about the difficulties, and perceived 'failures' of working with fluid-preserved collections can help colleagues to understand that these are complex specimens to care for, and that many of the issues faced are more common than we may think.

Conclusion

Where it is desirable to retain or create softness in a specimen, Steedman's may be used successfully. However, this application requires further research to differentiate between specimens that have remained pliable, and those which are insufficiently fixed. It is also possible to maintain larger and non-marine specimens in Steedman's for at least twenty years, but these specimens should be appropriately formaldehyde-fixed, and perhaps preserved in formaldehyde for an extended period of time before being transferred to Steedman's.

Beyond the original questions of the prevalence of Steedman's in fluid-preserved collections and how it is being used, there are wider issues which affect the type and scale of work that can be carried out. Lack of resources, including money, staff, suitable workspaces, or beneficial technology, prevents appropriate action from being undertaken. This can range from the ability to carry out a project of removing a fluid like Steedman's from an entire collection, to the kinds of monitoring and testing that would allow institutions to build a comprehensive record of the fluids present in their specimens. Without knowing which preservation fluids form the basis of these collections, it becomes extremely difficult to take care of them in the most appropriate way. Potential solutions such as audit training, simplified access to information, and shared equipment could be a beneficial way forward.

It transpires that the Cole Museum of Zoology is extremely fortunate to have a comprehensive manuscript catalogue from its inception in 1909 through to the late 1960s, with additional paper and digital records from the early 2000s onwards. Even so, we still face decades of lost information about our own specimens. Without that recorded infrastructure in place fluid collections are always going to be at risk, as new members of staff may take over without handover or training, and institutional knowledge is lost.

A larger data set would give a clearer picture of the number and types of collections who are using Steedman's or propylene phenoxetol-based preservation methods with their fluid-preserved specimens. This would enable a fuller understanding of the issues that wet collections face, not only regarding Steedman's, but also in terms of the barriers preventing necessary changes to these collections from being carried out. To that end, the survey has been re-opened, and all institutions with fluid-preserved collections are encouraged to respond.

Survey: Fluid Preservation Methods in Biological Collections
https://app.onlinesurveys.jisc.ac.uk/s/reading/fluid-preservation-methods-in-biological-collections

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Appendix 1: Survey

Fluid Preservation Methods in Biological Collections

The purpose of this survey is to determine the frequency of use of different preservation methods in fluid-preserved biological collections. Emphasis is placed on Steedman's Post-Fixation Preservative (Steedman's), sometimes known as PP, or 1% propylene phenoxetol. However, as the purpose of this survey is to gain an overview of methods used, it is requested that you fill in this survey even if this is something that is not used at your institution. The data collected will form part of my PhD research.

Project Description

The aim of fluid-preserved biological collections is to maintain their specimens in as good a condition as possible, for as long a period of time as possible, and to maximise the ways in which these collections can be used for teaching, research, and display. During the 1960s and 1970s a solution known as Steedman's Post-Fixation Preservative became popular, and was widely used in the preservation of these collections. However, by the end of the 1980s, some specimens were found to have been insufficiently preserved by this method, and in 2022 it was recommended that institutions holding fluid-preserved biological collections should no longer use Steedman's Post-Fixation Preservative as a long-term preservation solution. An assessment of the Cole Museum of Zoology's fluid-preserved museum and teaching collections showed that while we do hold specimens preserved by this method, the majority of these remain in excellent condition.

About the survey

No sensitive, impertinent, or distressing questions will be asked, and there is no risk of harm to either participant or researcher.

The data collected will form part of Claire Smith's PhD research, and will be securely stored for five years.

Your participation is voluntary, and your disclosure of identifying details including your name and email address are optional.

Data Protection

The organisation responsible for protection of your personal information is the University of Reading (the Data Controller). Queries regarding data protection and your rights should be directed to the University Data Protection Officer at imps@reading.ac.uk, or in writing to: University of Reading, Information Management & Policy Services, Whiteknights House, Pepper Lane, Whiteknights, Reading , RG6 6UR, UK.

The University of Reading collects, analyses, uses, shares and retains personal data for the purposes of research in the public interest. Under data protection law we are required to inform you that this use of the personal data we may hold about you is on the lawful basis of being a public task in the public interest and where it is necessary for scientific or historical research purposes. If you withdraw from a research study, which processes your personal data, dependent on the stage of withdrawal, we may still rely on this lawful basis to continue using your data if your withdrawal would be of significant detriment to the research study aims. We will always have in place appropriate safeguards to protect your personal data.

The categories of personal data collected are:

- Name of participant
- Email address of participant
- Name of the participant's workplace or institution

- City/town and country of the workplace or institution

These details are collected to enable follow-up contact, where consent is granted for this, and for the analysis of data by location. It is possible to opt out of these questions.

Data will be stored for 5 years as password protected electronic files on the computer of Claire Smith, with paper copies stored for 5 years in the locked office of Amanda Callaghan in the HLS Building, at the University of Reading. You can find out more about your rights on the website of the Information Commissioners Office (ICO) at https://ico.org.uk.
The University of Reading's Data Protection policies can be found at the following link: https://www.reading.ac.uk/imps/data-protection.

Consent

Please tick below to indicate your agreement with the following statements:

1. I understand the purposes of the project.

2. I understand what information will be collected about me, what it will be used for, who it may be shared with, how it will be kept safe, and my rights in relation to my data.

3. I understand that participation is entirely voluntary and that I have the right to withdraw from the project any time, and that this will be without detriment.

4. I understand that the data collected from me in this study may be preserved and made available in anonymised form, so that they can be consulted and re-used by others. This information will be used in a PhD thesis and may be shared with various committees, workshops or presentations, and may contribute towards research publications.

5. I understand that this data will be securely stored for five years, after which it will be deleted. This project has been reviewed by the University of Reading Research Ethics Committee, and has been given a favourable ethical opinion for conduct.

1. I give consent for my data to be used as described above: * ☐

Questions marked * are required.

About your Collections

2. What type of collection do you have at your institution? *

☐ Natural History

☐ Pathology

☐ Biological (other)

☐ None of the above

3. Do you have fluid preserved specimens at your institution? *

- [ ] Yes
- [ ] No
- [ ] Don't know

4. Are you able to answer questions about the types of preservation fluids used at your institution? *

- [ ] Yes
- [ ] No

If you are not able to answer questions about the types of preservation fluids used at your institution, please pass on this survey to other colleagues in the field who work with biological collections.

If you would like to discuss this research further, please contact:

Claire Smith: claire.smith@reading.ac.uk
Professor Amanda Callaghan: a.callaghan@reading.ac.uk

https://app.onlinesurveys.jisc.ac.uk/s/reading/fluid-preservation-methods-in-biological-collections

5. Do you currently have, or have you historically had, specimens stored in any of the following preservatives? *

You may choose multiple responses for each fluid type.

	Currently	Historically	No	Don't know
Formalin	☐	☐	☐	☐
Ethanol / IMS / other Spirit	☐	☐	☐	☐
Steedman's Post-fixation Preservative	☐	☐	☐	☐
Propylene Phenoxetol (sometimes labelled PP or 1%)	☐	☐	☐	☐
Propylene Glycol	☐	☐	☐	☐
Liquid paraffin	☐	☐	☐	☐
Glycerol	☐	☐	☐	☐
Glycerol & water	☐	☐	☐	☐
Jores / Jories	☐	☐	☐	☐
Kaiserling	☐	☐	☐	☐
Other	☐	☐	☐	☐

6. Please add which preservation fluids you use, if they are not listed above:

About your Collections

If you do not know about the balance of different preservation fluids that make up your collection, please skip this question and move on to question 8.

7. Please estimate the % of each type of preservation fluid in your collection

Please indicate ONE response for each fluid type.

	Don't know	up to 20%	up to 40%	up to 80%	over 80%
Formalin	☐	☐	☐	☐	☐
Ethanol / IMS / other Spirit	☐	☐	☐	☐	☐
Steedman's Post-fixation Preservative	☐	☐	☐	☐	☐
Propylene Phenoxetol (sometimes labelled PP or 1%)	☐	☐	☐	☐	☐
Propylene Glycol	☐	☐	☐	☐	☐
Liquid paraffin	☐	☐	☐	☐	☐
Glycerol	☐	☐	☐	☐	☐
Glycerol & water	☐	☐	☐	☐	☐
Jores / Jories	☐	☐	☐	☐	☐
Kaiserling	☐	☐	☐	☐	☐
Other	☐	☐	☐	☐	☐

Knowledge of Steedman's

8. Have you heard of Steedman's / propylene phenoxetol as a preservation fluid? *

☐ Yes

☐ No

9. Which of these options most closely reflects your experience of the use of Steedman's / propylene phenoxetol as a preservation fluid? *

Please indicate ONE response.

☐ Very Positive

☐ Positive

☐ Neutral

☐ Negative

☐ Very negative

☐ I have not used Steedman's / propylene phenoxetol

10. Are you aware of the recent recommendation to remove Steedman's / propylene phenoxetol from fluid-preserved collections? *

Neumann et. al., 2022, *Best Practices in the Preservation and management of Fluid-Preserved Biological Collections* (SPHNC, Chicago, pp66-68)

- [ ] Yes
- [ ] No

11. Are you planning to make any changes to your Steedman's / Propylene Phenoxetol preserved specimens? *

Please indicate ONE response.

- [ ] We have already removed Steedman's from our collection
- [ ] Yes, because of the recommendation above
- [ ] Yes, because we have experienced negative results in our own collection
- [ ] Yes, because we are aware of negative results in other collections
- [ ] Undecided / we do not have a plan either way
- [ ] No, because we are happy with our Steedman's specimens as they are
- [ ] No, but we plan to in the future
- [ ] No, but we are closely monitoring our Steedman's specimens
- [ ] No, we do not have sufficient resources to make this kind of change

12. Have any of your Steedman's / Propylene Phenoxetol specimens ever been transferred into or out of another preservation fluid?

- [ ] Yes - out of Steedman's / PP into another fluid
- [ ] Yes - into Steedman's / PP from another fluid
- [ ] No
- [ ] Don't know

Location details

13. Please provide the name of your institution

_____

14. In which town / city is your institution based?

_____

15. In which country is your institution based? *

_____

Online or digital catalogue

16. Do you have an online or digital version of your catalogue that I would be able to access? *

☐ Yes

☐ No

17. If yes, please provide access details for your online or digital catalogue:

_____

18. Are you willing to be contacted by email, to answer follow-up questions and/or provide access to a digital catalogue? *

☐ Yes

☐ No

Contact information

19. Your name:

_____

20. Your email address:

_____

Thank you for taking the time to participate in this research.

Please pass on this survey to other colleagues in the field who work with biological collections:

https://app.onlinesurveys.jisc.ac.uk/s/reading/fluid-preservation-methods-in-biological-collections

If you would like to discuss this research further, please contact:

Claire Smith: claire.smith@reading.ac.uk
Professor Amanda Callaghan: a.callaghan@reading.ac.uk

Appendix II: Formulae

Formalin

- A saturated solution of 37% formaldehyde gas in water.
- Fixation strength
    - ◊ 10% Formalin solution = 4% formaldehyde
- Preservation strength
    - ◊ 5% Formalin solution = 2.5% formaldehyde

Kaiserling

This is a three-step process, but most survey respondents who used Kaiserling in their collections were using only the preservative step to top up existing specimens. There are many iterations of the Kaiserling process, but the most widely cited was Pulvertaft's modification to remove the arsenious acid (Pulvertaft, 1950).

- 30% Glycerine
- 10% Sodium acetate (B.P.)
- 0.5 % Formalin
- adjust solution to pH 8

Steedman's Fixative (1 litre)

- Concentrate:
    - ◊ 100ml propylene phenoxetol dissolved in 500ml propylene glycol
    - ◊ Add 500ml formaldehyde 37% (Formalin)
- Fixative:
    - ◊ Dissolve 110ml of concentrate in 890ml of distilled water or saline

Steedman's Post-Fixation Preservative (1 litre)

- Concentrate:
    - ◊ 50ml of propylene phenoxetol dissolved in 500ml of propylene glycol
- Preservative:
    - ◊ Dissolve 110ml of concentrate in 890ml distilled water

Kew Mix: fixative for botanical specimens

- 5% formaldehyde
- 5% glycerol
- 53% industrial methylated spirit
- 37% water

Copenhagen Mix: study preservative for botanical specimens

- 70% industrial methylated spirit
- 28% water
- 2% glycerol

Güell & Mendel's Beetle Relaxing Fluid

- Ethyl alcohol (96%), 405ml
- Distilled water, 300ml
- Ethyl acetate, 167ml,
- Ether, 168ml
- Glacial acetic acid, 1ml