

REHYDRATION OF DRIED-OUT SPECIMENS: A NEW APPROACH

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Abstract.—Different procedures are proposed in the literature for the rehydration of dried-out specimens. These procedures vary greatly in their efficiency and application. This work describes a new procedure that is inspired by the literature but that avoids heating the specimens. This method was applied to reconditioning dried-out specimens from a historical collection (Swiss freshwater fishes, bird brains, and bird eyes), stored at the Naturhistorisches Museum Bern in Switzerland. The procedure consists of five steps. The first step is the softening of hardened soft tissue with benzaldehyde and demineralized water. The second step is an indirect rehydration with water vapor. The third step is a chemically induced direct hydration using a trisodium phosphate solution that allows the specimen to swell in size before being washed with water to remove all additives. Finally, the rehydrated specimen is transferred into new preserving fluid. Because the dehydrating properties of ethanol as a preservative are problematic, this paper presents the results of an experimental case study using a glycerol solution as a preservation fluid.

Key words.—Fluid preservation, rehydration, dried-out specimens, cherry laurel, benzaldehyde.

INTRODUCTION

In November 2017, the Naturhistorisches Museum Bern (NMBE) received the private research collection of Paul Steinmann (1888–1953), a well-known Swiss hydrobiologist. His research on Swiss whitefish (Coregoninae) is presented in two of his most important works: *Schweizerische Fischkunde* (Steinmann 1948) and *Monographie der Schweizerischen Koregonen* (Steinmann 1950). These two publications are important for the well-known *Handbook of European Freshwater Fishes* (Kottelat and Freyhof 2007). Paul Steinmann's ichthyological collection is of high historical and scientific value. It includes a large number of specimens of freshwater fish, including one holotype, numerous paratypes, and many extinct species of *Coregonus*. The collection also contains eggs, developmental states, gonads, fins, scales, and parasites of fish specimens.

The value of this collection lies in its significance for Swiss cultural heritage, as it also includes older material from past pedagogical collections of institutions like the Swiss Federal Institute of Technology in Zürich and various fish specimens that date back to 1870. Therefore, any conservation treatment must take into consideration the collections historical value (see also Mulder 1997).

A typical challenge of natural history wet collections is the loss of preservation fluids. This can progress so far that most of the fluid has evaporated, leaving a dried-out specimen. Fluid loss happens especially due to poor-quality specimen containers that do not seal correctly in collections that are not properly monitored over time.

This was the case with Steinmann's collection. The collection was deposited for many years in a storage container outside the research facility of the Swiss Federal Institute of Aquatic Science and Technology (EAWAG), where it had been exposed to temperature changes (cold winters and hot summers) and where there had been no monitoring of fluid levels.

The reconditioning project of the Steinmann collection was intended to restore the fish specimens for further scientific study. In order to achieve this goal, the dried-out specimens had to be treated with a nondestructive but efficient rehydration method.

It is of great importance to note that the decision to rehydrate dried-out specimens is an irreversible intervention requiring reflection. A dehydrated specimen will likely be stable over decades (Simmons 2014) but is significantly less useful for morphological studies. Rehydrating may enable further studies but may also compromise the specimens condition. In this particular case, such risk was considered as acceptable.

It was decided to research rehydration methods that would be suitable for Steinmanns ichthyological collection. Several case studies describing rehydration processes are reported in the literature, most of which involved rehydration of small invertebrates. The procedures include soaking specimens in alkaline solutions (Van Cleve and Ross 1947, Vogt 2001), using a vacuum to facilitate exchange of the softening fluid and air trapped inside the specimen (Jeppesen 1988) and/or using heated surfactants to shorten the rehydration time (Banks and Williams 1972, Waterhouse and Graner 2009). All of these methods are listed in Simmons (2014: table 22). In all the above-mentioned procedures, the specimens are placed directly in the rehydrating fluid.

Wechsler et al. (2001) explain the importance and the most effective method of softening dried-out soft tissue before the specimen is reimmersed in any kind of fluid. The authors propose that the specimens first be placed in chopped cherry laurel leaves (*Prunus lauro-cerasus*) and afterward rehydrated in a 1% aqueous solution of trisodium phosphate. The treatment with cherry laurel leaves is often used in taxidermy as a means to soften skin on old mounts. This publication is complemented by Wennerstrand (2015), who studied the use of cherry laurel as a softening agent for archaeological leather objects in more depth. Wennerstrand (2015) claims that the active compounds in the leaves are benzaldehyde fumes and water vapor that are released when the leaves are crushed. An alternative treatment is presented by Wennerstrand (2015) that involves the use of pure benzaldehyde and water instead of cherry laurel leaves. The author argues that this method is not only less toxic but also much faster and less contaminating for the treated objects. However, this procedure was proposed and tested for the purpose of softening archaeological leather and not specimens from natural history collections.

Another idea, proposed by Singer (2014), consists of using an indirect hydration with water vapor to reverse the drying process. Specimens are not placed directly in a fluid but instead absorb water moisture in a closed environment. Because of its noninvasive properties and the lack of any chemicals, this method became the starting point for further experimentation presented in this study.

Review of the previous studies described above led to the development of the following hypotheses:

- The rehydration of dried-out specimens is a process that requires time. Heat speeds up the procedure but can damage specimens.
- To rehydrate a dried-out specimen, it appears important to first soften the soft tissue so it can absorb water.
- Softened soft tissue should be rehydrated gradually by using water moisture.
- To overcome wrinkles and fill the soft tissue with fluid, the already rehydrated cells should be hydrated with either an alkaline solution or a surfactant.
- Refixation of the specimen for its final conservation helps the specimen to remain in its regained rehydrated shape.

The present study aimed to take the best methods from the above-mentioned studies in order to propose a complete rehydration treatment for the whole Steinmann collection.

MATERIALS AND METHODS

Three collections of the NMBE were used in this study: the Steinmann collection (see above), a discarded EAWAG collection, and mounted anatomical specimens from the collection of Walter Kuenzi (NMBE director from 1952 to 1964). An inventory was carried out of this material, including photo documentation. The original Steinmann fish collection consisted of a total of 1,012 jars and other receptacles. The inventory showed that 56% of the jars contained well-preserved specimens, but the rest were in need of reconditioning (Neisskenwirth 2019). Of the total of 450 jars that needed reconditioning, 70 jars contained numerous specimens that had completely dried out.

The EAWAG collection consisted of 17 jars of dried-out fish specimens. It was deposited together with the Steinmann collection in the previously mentioned storage container outside the research facility. The EAWAG collection was used as experimental material for the Steinmann collection. The knowledge gained from the fish specimens of both collections was applied to the dried-out mounts of the Kuenzi collection. The anatomical specimens collected by Walter Kuenzi make up 13 dried-out mounts of bird brains and eyes. It dates back to 1917 with formaldehyde as original preservative fluid, similar to the Steinmann collection.

A series of fluid analyses and the documentation of the Steinmann collection showed that most of the fish specimens were stored in a 4% aqueous formaldehyde solution. An important goal of the reconditioning project of the Steinmann collection was to transfer all the specimens from their formaldehyde solution into ethanol, part of the new policy of the NMBE to avoid the use of hazardous preservatives and ensure the safety of staff and students doing research on the specimens from their collection. Therefore, it was necessary to find a fluid that enables the preservation of rehydrated specimens. Macleod and Van Dam (2011) successfully used glycerol solution as a preservative fluid. Because ethanol has a strong dehydrating effect and formaldehyde is a health hazard, glycerin was used in this study.

For the experiments in this study, a solution of trisodium phosphate was used based on Wechsler et al. (2001), who recommended a concentration of 1%. Van Cleve and Ross (1947) used a 0.5% or 0.25% solution, explaining that it would be less harmful to the treated specimens. This was tested on later applications and showed equally successful results. As such, a dosage of 0.5% trisodium phosphate was used for further treatments.

Chemicals used in this study included formaldehyde 37% solution, benzaldehyde for synthesis, disodium hydrogen phosphate anhydrous for analysis (EMSURE®; Sigma-Aldrich through Grogg Chemie) and sodium dihydrogen phosphate for analysis (EMSURE), trisodium phosphate dodecahydrate $\geq 98\%$, TECHNICAL and glycerol 85% (VWR through Grogg Chemie), thymol crystals (Grogg Chemie), and 99.8% absolute ethanol (Alcosuisse). All dilutions were performed with demineralized water obtained from the NMBE desalination plant.

Other materials used included pieces of polyethylene doormat “tropic green” from Siena Home (subsequently referred to as the “spacer”) and a bath mat (subsequently referred to as the “perforated rubber platform”), both purchased at a local hardware store. Glassware included a microwave dish from Duran Consumer Glass (subsequently referred to as the “borosilicate container”). Notes, photos, and measurements of weight during each step of the procedure helped to document changes and other observations.

Description of the Method

Softening the soft tissue.—The use of benzaldehyde was preferred over cherry laurel for several reasons. First, cutting and collecting leaves is time consuming. Second, choosing pure chemical products limits the number of hazardous fumes released during leaf chopping (Dierks 2016) and possible contamination with other substances contained in the leaves. Third, as the cherry laurel is required to be in direct contact with the specimens, undesirable chopped leaf residue could accumulate in small orifices and cavities of the specimens.

Specimens to be processed were placed in a borosilicate container with an airtight lid (see Fig. S1). It is important not to use plastic materials, as benzaldehyde will soften plastics. Furthermore, the container should be large enough to hold two Petri dishes and the specimen. One Petri dish was filled with 20 ml of concentrated benzaldehyde and the other with 20 ml of demineralized water. The container was closed and left for 24 hours at a room temperature of 20–24°C (it was observed that if the room was too cold, processing time was much longer). During this process, benzaldehyde slowly oxidizes to benzoic acid, a white crystalline substance. It should be noted that this substance is very dangerous to the respiratory system. To avoid exposure to possible suspended particles of the benzoic acid crystallization, a fume hood and personal protective equipment, including safety glasses, gloves, and a dust mask, are mandatory.

After 24 hours, the container was opened and checked for crystallization of benzoic acid (Fig. 1a). The specimens were turned over to expose the other side, and the container was closed for another 24 hours. The specimens were then taken out of the container and checked carefully for flexibility.

If the crystallization process expanded in the container and threatened to contact the specimen (Fig. 1b), the container was cleaned, and both the benzaldehyde and the water were replaced. The procedure was repeated until the specimen showed a notable flexibility. The duration and repetition of this treatment depend on the scale and skin thickness and size of the specimen and the length of time it has been dehydrated. Specimens smaller than 30 mm may skip this treatment because the steps that follow are able to soften the skin of small specimens without exposing them to unnecessary chemical reactions.

Rehydration with suspension over water (100% relative humidity).—The specimen was placed in a clean airtight container (as used in the previous step) on the perforated rubber surface, resting on spacers to avoid contact with the water (Fig. S2). The container was filled with demineralized water to a level dependent on the size of the container and height of the perforated rubber platform and spacers. To prevent fungal growth, thymol crystals were added to the water. The container was closed and set aside to allow the specimen to absorb water moisture for at least a week. A constant room temperature of 20–24°C was crucial, as a colder room can slow down the rehydration process. After this procedure, the specimen gained considerable weight and regained more of its original appearance (Fig. 2).

Specimen swelling.—To ensure that the rehydrated specimen swells in the next treatment, it must first be tested to determine whether it floats or sinks in demineralized water. If it floats, the specimen must again be treated with the previous step to absorb more water. If this is due to air trapped inside of the specimen, a vacuum pump can be used to remove the trapped air. For this, a container containing the specimen is filled with demineralized water and placed in the desiccator. The pump is activated until the vacuum gauge achieves



Figure 1. (a) Benzoic acid crystallization on Petri dishes after 24 hours of treatment. (b) Extreme benzoic acid crystallization after 4 days of exposure in a test of growth speed without specimens (© NMBE, F. Neisskenwirth).

−0.6 bar. The desiccator is then opened to equalize the pressure inside the specimen (Jeppesen 1988). This procedure can be repeated several times.

The rehydrated specimen was then immersed in an aqueous solution of 0.5% trisodium phosphate (Na_3PO_4) to induce swelling. The swelling process must be carried out with caution, as excessive swelling of the soft tissue is irreversible and may cause structural damage.

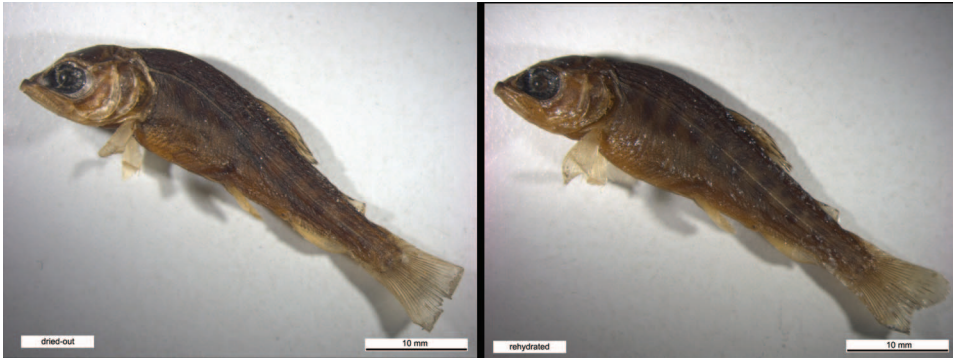


Figure 2. Specimen of juvenile *Salmo trutta* before (left) and after hydration through moisture (right). The first step was skipped because of the small size of the specimen (© NMBE, F. Neisskenwirth).

This damage typically happened only after the swelling process, and it was assumed that it was caused by the alkaline solution. If dried-out specimens already exhibit cracks, swelling is not recommended, as such treatment can lead to even greater damage to the specimens (Fig. 3). Delicate membranes are also very susceptible to the swelling process, and, as such, the swelling should be done under constant monitoring. Here again, a constant room temperature of 20–24°C is essential. If the temperature is too high, swelling will be much faster and may not be adequately controlled. During the present study, a doubling of the rate of swelling on a hot summer day of 27°C was noted.

Swelling in small specimens is usually complete after a few hours, but, in the case of larger specimens, this may take a few days. The eyes of fish specimens tend to bulge but will return to their normal shape after final storage (Fig. 4). Alkaline conditions can cause clearing of soft tissues, as proteins and lipids are leached from the specimens. Because of this, it is likely that the specimen will lose some of its original coloration, appearing more translucent.

After this final step, the specimen should be fully rehydrated and should have attained its desired shape. At this stage, it should be possible to stretch and articulate fins, tail, and jaw of treated fish specimens (Fig. 4).

Rinsing of additives with water.—It is important to rinse all the residual alkaline trisodium phosphate solution from the specimen to prevent further swelling and possible pH changes in the final preservation fluid. Specimens should be immersed in a water bath filled with demineralized water for 20–30 hours. The contaminated water should be changed twice during the process to extract all of the residual trisodium phosphate.

Transfer into preservative fluid.—One of the most difficult part of the process is keeping the rehydrated specimens shape in the final preservative solution. Ethanol has the negative effect of shrinking the specimen back into a dehydrated state because of its hygroscopic properties (Fig. 5). Experiments attempting different transfer steps into ethanol for two different species of dried-out fish specimens (*Telestes souffia* with softer skin and *Gymnocephalus cernua* with harder skin) resulted in visible dehydration through weight loss and shrinkage of the specimens after all transfer attempts. To overcome this, J. Simmons and S. Moore (pers. comm. 2018) recommend re-fixing the specimen in a 3.7% solution of aqueous buffered formaldehyde for one to three days, as this process will return the specimen to a more “balanced” state. To avoid decalcification and other possible damage caused by the



Figure 3. Damaged specimen of *Salmo trutta* before and after swelling (© NMBE, F. Neisskenwirth).

formaldehyde, a buffer of disodium hydrogen phosphate and sodium dihydrogen phosphate was added to the solution (subsequently referred to as “buffered formaldehyde”).

Afterward, the treated specimen was transferred from formaldehyde into ethanol using gradually increasing concentrations of 5–10% (e.g., 20–30–40–50–60–70–75% ethanol) with at least 1 day in each solution. The specimens refixed and transferred to ethanol in this way kept their shape better than those without refixing and with just three increasing ethanol transfers (Fig. S3).

Better results were obtained when ethanol was avoided, and the specimen was transferred directly into buffered formaldehyde. This because this preservative has no hygroscopic reaction with the specimens. The fish specimen was monitored for at least a week to see if any unwanted structural changes appeared.

An alternative method involves the use of glycerol solution as the final preservative (see A. van Dam, this volume). The specimen was transferred through three increasing glycerol solutions, 30–50–70%, and finally into 65% glycerol solution for final storage. The result of this method was notably better than transfer into ethanol. A negative impact on specimen shape was not observed in the present study after the transfer into glycerol. A side effect of this method was the light transparency of the specimens skin due to the alkaline swelling bath and the refractive index of glycerol (Fig. 6).

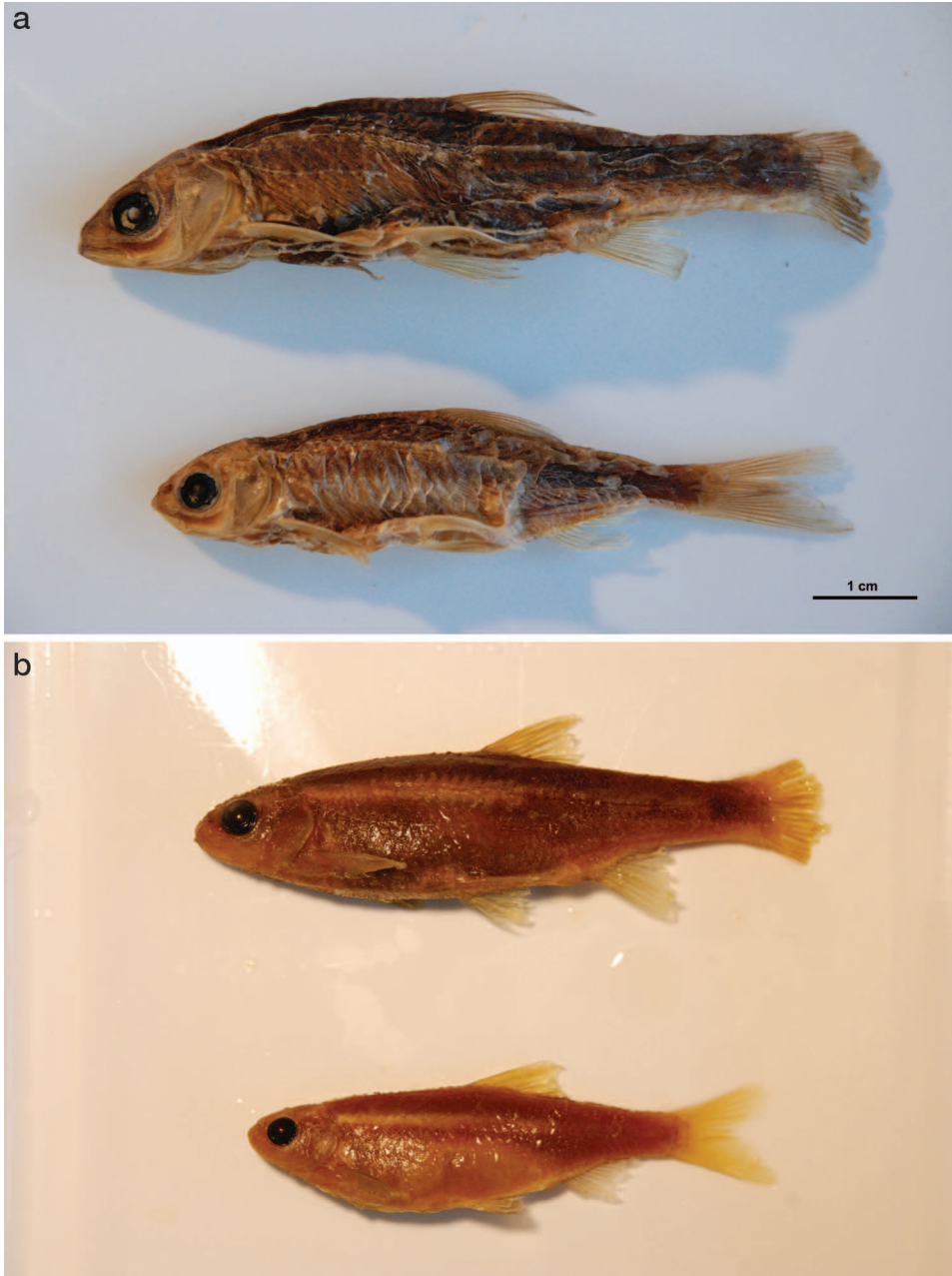


Figure 4. (a) Dried-out specimens of *Leucos aula* and (b) the same specimens after rehydration and swelling in a 0.5% trisodium phosphate solution. (© NMBE, F. Neisskenwirth).

Application of the Method to Other Vertebrates

The rehydration method described above on the Steinmann collection was tested with specimens from the Künzi collection in order to determine the protocols effectiveness on other vertebrates (bird brains and eyes).



Figure 5. Juvenile *Salmo trutta* specimen. Dried-out specimen (left), swollen up (middle), and after the transfer into a solution of 75% ethanol using gradually increasing concentrations of 40–60–75% (right) (© NMBE, F. Neisskenwirth).

Certain specimens in the Kūenzi collection had suffered a substantial loss of fluid due to leaking jar seals, leaving some of the specimens completely dried out. As these were voucher specimens from the dissertation of Dr. Kūenzi, it was decided to keep the collection in its original buffered formaldehyde preservation fluid. Given this decision, it was possible to rehydrate the specimens without fear of water loss. A remarkable outcome of this experiment was that the veins of the brain surface reappeared after the rehydration. There was also a significant increase in weight and a return of the original color of the anatomical specimens (Fig. 7).

DISCUSSION AND CONCLUSIONS

Simmons (2014) emphasizes that the results of rehydration methods obtained from certain vertebrate and invertebrate species could not be transferred to other groups. He points out that blind transfer of the methodology could fail and cause irreparable damage to the treated specimens.

Indeed, a key finding of the present study is that the specific characteristics of the soft tissues of different fish species and of the avian anatomical specimens have a significant influence on the effectiveness of rehydration and on the duration of this procedure. Based



Figure 7. Dried-out mount of eyes and brain of *Podiceps cristatus* (left), after the rehydration (middle), and after treatment and ready to be sealed for storage (right) (© NMBE, F. Neisskenwirth).

on the present study, it appears that large dried-out specimens can take much longer to rehydrate than smaller ones. The same is true for specimens with overly hardened soft tissue. Wechsler et al. (2001) state that the method of preservation—preserved with ethanol or fixed with buffered formaldehyde—also affects the treatment time. In the present study, this could not be demonstrated due to a lack of information on the original fluid in the dried-out jars.

Another observation of the present study is that partially dried-out fish specimens did not respond as successfully as completely dried-out fish specimens to the rehydration method. In these cases, gradual rehydration is recommended (Singer 2014). However, it was possible to transfer partially dried-out fish specimens directly into their final preservative (ethanol, glycerol, or buffered formaldehyde) without rehydration, depending on the state of dehydration of the specimens.

Further research on the methodology presented here is needed with respect to the interaction between preservative fluids and the weight loss of specimens associated with shrinkage. The weight loss of specimens after the swelling process in the present study was irreversible, even if the specimen was refixed with buffered formaldehyde immediately after successful swelling. The most significant weight loss and shrinkage in this study was caused by transfer into ethanol, while rehydrated specimens stored in glycerol showed the least weight loss after final transfer. In the limited findings of this study, glycerol and buffered formaldehyde solutions showed the best results. Glycerol has a much lower health risk than buffered formaldehyde solutions and therefore is considered the most suitable of all three preservation fluids used in this present study.

The method presented in this study was successful in achieving the desired flexibility of soft tissue in treated specimens for both fish and anatomical bird specimens. In addition to the improvement in the fish specimens of the Steinmann collection for morphological study, a substantial aesthetic enhancement was accomplished in the bird specimens in the Küenzi

collection. Although color changes occurred during the rehydration of the specimens, many of the anatomical details were visible or even restored to their original state.

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Résumé.—Différents protocoles sont proposés dans la littérature pour la réhydratation des spécimens desséchés. Leur efficacité et leur mise en oeuvre sont très variables. Ce travail vise à définir une nouvelle procédure qui s'inspire de la littérature tout en évitant de réchauffer les spécimens. Celle-ci a été testée sur des spécimens desséchés d'une collection historique (poissons deau douce, cervelles et yeux doiseaux), du Naturhistorisches Museum Bern en Suisse (NMBE). La procédure se déroule en cinq étapes. Premièrement, lassouplissement des tissus mous avec du benzaldéhyde et de leau déminéralisée. La deuxième étape est une réhydratation indirecte avec de la vapeur deau. Lors de la troisième étape, léchantillon est regonflé très délicatement grâce à une solution de phosphate trisodique, puis lavé à leau pour enlever les additifs. Enfin, le spécimen réhydraté est transféré dans un nouveau liquide de conservation. Les propriétés déshydratantes de léthanol étant problématiques, des tests utilisant du glycérol comme fluide de conservation ont été lancés.

Zusammenfassung.—Die verschiedenen in der Literatur beschriebenen Rehydrierungsverfahren für trockengefallene Präparate unterscheiden sich sehr stark in ihrer Effizienz und Anwendung. Die Verfahren wurden meist für spezifische Tierarten entwickelt und sind daher nicht für alle Weichgewebe anwendbar. Ziel dieser Arbeit ist es, ein neues Verfahren vorzustellen, welches sich zwar an der bereits existierenden Literatur orientiert, gleichzeitig aber eine Erwärmung der Präparate vermeidet. Das Verfahren wurde für die Aufarbeitung ausgetrockneter Exemplare aus einer historischen Fische Sammlung aus Schweizer Seen angewandt, welche sich im Naturhistorischen Museum Bern befindet. Das vorzustellende Verfahren besteht aus fünf Phasen: Die Aufweichung des Weichgewebes mit Benzaldehyd und Wasser. Einer indirekten Rehydrierung mit Wasserfeuchtigkeit. Aufquellen der Probe in einer Trinatriumphosphatlösung mit anschließender Wässerung zum Auswaschen aller Zusätze. Überführung des rehydrierten Präparates in neue Konservierungsflüssigkeit. Da die wasserentziehenden Eigenschaften von Ethanol in der Umsetzung problematisch waren, wurde eine experimentelle Fallstudie mit einer Glycerinlösung als Konservierungsflüssigkeit durchgeführt, die ebenfalls in dieser Studie vorgestellt wird.

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APPENDIX



Figure S1. Implementation of the specimen softening step. (© NMBE F. Neisskenwirth).



Figure S2. Example of used materials to suspend specimens over water (left). Closed container with specimen depicted in Fig.1 (right). (© NMBE F. Neisskenwirth).



Figure S3. Rehydrated specimens of *Telestes souffia* and *Gymnocephalus cernua* before and after the transfer in ethanol 75%. (© NMBE F. Neisskenwirth).