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Glycerination as an alternative technique for preserving Vertebrata (Osteichthyes and Reptilia): a proposal for teaching purposes

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ABSTRACT. Biological specimens are essential for teaching, research, and extension, although they require effective preservation. The glycerination technique stands out for allowing specimens to be preserved dry, which saves costs and facilitates handling due to specimen malleability. We sought to establish an optimal glycerination protocol for fish and reptiles for teaching purposes. Several fixatives were tested, including formalin at different concentrations and Transeau solution. The fixation process involved injecting a 50% fixative and 50% double-distilled glycerin solution into the specimen, followed by immersion in the same fixative. After seven days animals were washed, partially dried, and subsequently immersed for 60 days in double-distilled glycerin with camphor. The specimens were then kept in air-conditioned environments with a dehumidifier during a 30-day drying period. A 3% formalin solution was found to be the most efficient, guaranteeing the mental 10 some of the specimens' colors, and their malleability. This study contributes to the optimization of glycerination preservation and effectively preserving fish and reptile specimens for teaching purposes while reducing handling risks.

Keywords: glycerin; fixation; animals; teaching.

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Introduction

Conservation techniques are designed to maximally preserve the morphological characteristics of animal specimens by maintaining their physical integrity (including color, tissue consistency, and flexibility). These conservation techniques can be achieved by various methods, such as chemical treatments with natural or industrialized reagents (Cury, Censoni, & Ambrósio, 2013), mechanical treatments that include manual desiccation, physical treatments that make use of temperature changes (either cold or heat), and biological treatments using specific microorganisms or insects (Rossi-Jr et al., 2013).

Traditionally, zoological collections employ fixative substances to preserve specimens, principally formaldehyde and ethyl alcohol. Formaldehyde has several disadvantages, including its toxicity and highly carcinogenic nature, unpleasant odor, the darkening of the specimens or parts thereof, increased specimen weight, and stiffness of the treated tissues; serious environmental problems must be considered when disposing of treated material (Karam, Cury, Ambrosio, & Mançanares, 2016). As indicated by Alves and Alciole (2012), formaldehyde has harmful effects when inhaled and absorbed through the respiratory tract, such as eye redness, tearing and burning, sneezing, coughing, throat and lung irritation, as well as tissue and DNA damage. The other widely used fixative, 70% alcohol, is highly flammable, and its volatile nature requires its frequent replacement (Papavero, 1994). These aspects highlight the need to consider safer and less environmentally harmful alternatives for specimen fixation and conservation.

Another alternative method for preserving specimens is the glycerination technique, which uses glycerin for the preparation and conservation of zoological material. Glycerin has an antiseptic action (resulting from its capacity for cell dehydration) that acts against fungi and gram-negative and gram-positive bacteria – providing superior preservation and more faithful morphological results when compared to formaldehyde. Glycerination employs a less aggressive chemical substance and better preserves the physical integrity of tissues in a manner that is closer to living specimens, including their color and mobility. The technique also

facilitates the identification of structures that are otherwise difficult to visualize in practical internal and external morphological studies by maintaining tissue consistency similar to that observed *in vivo* (Calamares-Neto & Colombo, 2015; Costa et al., 2021).

The use of safe and effective biological preservatives is essential for creating useful teaching and study collections with low costs, low toxicity, ease of maintenance, and high color and mobility preservation. The glycerination technique is viable because it allows dry handling and storage after completion, reduces costs and exposure to alcohol and formaldehyde during both conservation and replacement, and prolongs the useful lives of biological specimens (Costa et al., 2021). It is therefore essential to use alternative conservation techniques for animal specimen preservation that can increase their durability and maintain their malleability and natural colors while lowering exposure to toxic and/or carcinogenic chemicals (Silva, Dias, Tavares, Marques, & Furtado, 2008; Paiva et al., 2019).

A study of the application of the glycerination technique was presented by Costa et al. (2021) for crustaceans, although in light of the wide diversity of organisms found in biological collections, it will be necessary to examine its use with other groups. We therefore evaluated the glycerination technique for preparing zoological material [fish (Osteichthyes) and Reptilia (Testudines)] for educational and extension purposes, to determine: (i) the best fixative formulas; and (ii) additional techniques to preserve specimens that maintain the integrity of their morphological characteristics, including malleability and color.

Material and methods

The present study was carried out in the Zoology Laboratory of the Multidisciplinary Health Institute, Anisio Teixeira Campus, at the Federal University of Bahia (UFBA/CAT/IMS), in the municipality of Vitória da Conquista, Bahia State, Brazil. This work was approved by the Animal Use Ethics Committee (CEUA/IMS/CAT/UFBA) Protocol No. 105/2022. The fish were obtained directly from fishermen and were euthanasia using ice. The reptiles were donated by the Wild Animal Triage Center (CETAS) in Vitória da Conquista, Bahia, from animal trafficking seizures when animal deaths occurred for unknown reasons. A total of 56 specimens were used (32 fish and 24 reptiles). The selected fish groups were the species *Larimus breviceps* (Cuvier, 1830) and *Conodon nobilis* (Linnaeus, 1758); the reptiles were all young red-footed tortoises [*Chelonoidis carbonaria* (Spix, 1824)].

The actions of six different fixatives (treatments), and their effects on the quality of the specimens were analyzed: F1- 1% formaldehyde; F2 - 2% formaldehyde; F3 - 3% formaldehyde; F4 - 4% formaldehyde; F5 - 5% formaldehyde; and T - Transeau. The positive control (CP) was composed of individuals treated with the traditional method of zoological material conservation – fixed in 10% formaldehyde and preserved in 70% ethyl alcohol. The negative control (CN), in turn, included organisms without any fixative (the model closest to *in vivo*) that were kept frozen until analysis. The formaldehyde used as the fixative was buffered with sodium tetraborate.

The initial methodology of this work was proposed by Costa et al. (2021): before executing the tests with the different fixatives, the euthanized animals were thoroughly cleaned (using running water and neutral detergent until all sediment was removed) to minimize possible stains and unnatural irregularities in the individuals.

All of the specimens of the six different treatments were injected with a solution of 50% fixative and 50% double-distilled glycerin throughout their bodies. The organisms were then completely immersed in the different fixative solutions in containers with lids, with the animals occupying a maximum of 2/3 of the container volume, with the treatment solutions completely covering the specimens. The specimens all remained in the solutions for 7 days. After this period, they were washed in running water to remove excess fixative and set out to drain and partially dry.

Finally, the specimens were immersed in a double-distilled glycerin solution (with 1 g of camphor dissolved in 5 ml of absolute ethyl alcohol per liter of glycerin, to avoid the proliferation of fungi) for 60 days to become impregnated with glycerin. The specimens were subsequently removed from the glycerin, drained, and kept for 30 days in an air-conditioned environment (20 ºC) with a dehumidifier (Costa et al., 2021).

The specimen quality testing protocol used for evaluations for teaching purposes [adapted from the fish quality index proposed by Amaral and Freitas (2013)] was applied to the two animal groups examined in the present study, considering each of the five different fixatives tested, as well as the control groups. Fifteen variables were considered while evaluating the specimens: their opacity, scale integrity, rebound of the external muscles, peritoneum adhesion, external discoloration, anal orifice opening, malleability, eye transparency, eye orbit, eye shape, iris visibility, odor (putrefaction), morphology of the internal organs

(gastrointestinal tract), gill discoloration, and classroom exposure time (simulating the average time in which specimens are normally studied in practical classes – 4 hours). The variables: anal orifice opening, eye transparency, eye orbit, eye shape, and iris visibility were not evaluated in reptiles (Table 1).

After drying, the specimens were numbered from 1 to 56 and evaluated blindly and separately by two professors and two zoology researchers. Those professionals assigned values (from 1 to 4) to each of the specimens. Specimens with higher evaluations presumably appeared closer to their natural state (*in vivo* - CN).

Table 1. Variables used to evaluate the quality of fish and reptile specimens prepared using the glycerination technique based on an adaptation of the fish quality index proposed by Amaral and Freitas (2013). S. = scale, * Indicates variables not evaluated for reptiles

Variables	Criteria	S
Appearance (opacity)	Very bright	4
	Bright	3
	Slightly Opaque	2
	Opaque	1
Scale integrity	No detachment from the body	3
	Discreet detachment from the body	2
	Bald areas on the surface of the body and intense detachment	1
Return of external muscles	Quickly returns to the previous state when pressed	4
	Slow muscle recovery after pressure	3
	Depression left on the surface when pressed	2
	Not returning to initial state when pressed and/or the presence of fibrosis	1
Opening of the Peritoneum	Fully attached to the muscles	4
	Discreet muscle detachment	3
	Marked muscle detachment	2
	Peritoneum completely detached from the muscles	1
External discoloration	Absent	4
	Detectable	3
	Moderate	2
	Excessive	1
*Anal orifice	Completely closed	3
	Discreet orifice opening	2
	Significant orifice opening	1
Malleability	Very Malleable	3
	Malleable	2
	Hard	1
*Eye transparency	Clear and bright	4
	Loss of shine	3
	Slight opacity	2
	Completely opaque, no shine	1
*Eye orbit	Eyes completely filling the orbit	4
	Slight sinking into the orbit	3
	Sinking into orbit more pronounced	2
	Intense sinking into the orbit	1
*Eye shape	Flat	4
	Convex	3
	Concave	2
	Deformed	1
*Iris visibility	Visible	2
	Not visible	1
Odor	Complete absence of odor	3
	Slight presence of odor	2
	Presence of odor	1
Morphology of internal organs (gastrointestinal tract)	Integral	4
	Little changed	3
	Moderately changed	2
	Extremely changed	1
Gill discoloration	Absent	4
	Detectable	3
	Moderate	2
	Excessive	1
Exposure time in class (after 4 hours)	No Change	2
	With alteration and drying of the material	1

Data analysis was carried out in accordance with Costa et al. (2021), using the arithmetic mode, as that parameter evaluates the central tendency of the data set and therefore the value assigned with greater consistency by the evaluators. After calculating the modes of the evaluations, the final result value (RF) was calculated by summing the attributed scores of all of the aspects used to compare the fixative agents. This analysis was carried out using GraphPad Prism version 5 software.

After completing the experiments, the glycerinated animal specimens were incorporated into the Didactic Zoological Collection of the Zoology Laboratory of the *Universidade Federal da Bahia* - IMS/CAT. Storage receptacles with lids were used that contained hygienic mats moistened with an antiseptic solution (1 lt of 70% alcohol, with 2 ml of 2% iodine and 10 g of camphor diluted in 10 ml of absolute alcohol); small packages were also included containing silica and camphor to prevent fungal growth and specimen decomposition, as suggested by Costa et al. (2021).

Maintenance every 6 months is suggested for optimal conservation of the specimens, patting them down with a cloth dampened with the aforementioned antiseptic solution, followed by drying at room temperature. The hygienic mats and envelopes with silica and camphor, as above, should be periodically renewed.

Results

The analysis of the fixative solutions showed that for the variable opacity of appearance, the best results were achieved with CN and treatments F2, F3, and F5; those treated with Transeau, F1, and F4 evidenced a shiny appearance; the CP treatment evidenced a slightly opaque appearance (Table 2, Figure 1).

Table 2. Final results of the evaluation of preservation variables of fish specimens during the glycerination technique experiments. CN: Negative Control, CP: Positive Control, F1: 1 1% formaldehyde, F2: 2% formaldehyde, F3: 3% formaldehyde, F4: 4% formaldehyde, F5: 5% formaldehyde; and T: Transeau. RF: Final Result, being the sum of all of the evaluation criteria values. VM: Maximum value of each variable. The values used in the table correspond to the arithmetic mode.

Variables	VM	Controls		Treatments					
		CP	CN	T	F1	F2	F3	F4	F5
Appearance (opacity)	4	1	3	2	2	3	3	2	3
Scale integrity	3	1	3	1	1	2	3	3	2
Return of external muscles	4	3	4	2	1	4	1	1	4
Adhesion to the peritoneum	4	4	4	2	3	4	3	3	2
External discoloration	4	1	3	4	4	4	4	4	4
Anal orifice opening	3	3	3	1	2	1	2	3	2
Malleability	3	2	3	2	1	2	2	1	1
Odor	3	1	1	2	2	2	2	2	2
Eye transparency	4	1	3	2	2	2	2	2	1
Eye orbit	4	4	4	2	2	2	3	2	2
Eye shape	4	1	2	2	3	1	2	3	3
Iris visibility	2	1	2	2	2	2	2	2	2
Discoloration of the internal organs (gills)	4	1	4	2	1	3	1	1	2
Morphology of the internal organs	4	1	1	2	3	2	3	3	2
Exposure time in class (after 4 hours)	1	0	1	1	1	1	1	1	1

The best scale integrity was observed with CN and in treatments F3 and F4; the most inferior results were observed with CP and the T and F1 treatments (Table 2, Figure 1).

The best results for external muscles (fish only) were obtained in CN and fixatives F2 and F5; the poorest results were observed with treatments F1, F3, and F4 (Table 2).

The best results in terms of the evaluation of fish peritoneum adhesion were observed with CP and CN and the F2 treatment. None of the fixatives received an evaluation noting the peritoneum as completely detached from the muscles. Another variable that presented satisfactory results for almost all of the treatments was discoloration (with only CP showing very pronounced discoloration) (Table 2).

The best results for malleability were obtained with CP, and in treatments T, F2, and F3; stiffer parts were observed with F1, F4, and F5. Odor evaluations were consistent for most treatments, with T and F1 through F5 having a slight presence of fixative odor; the fixative odor of CP was more pronounced (Table 2).

In terms of eye transparency, treatments with T, F1, F2, and F3 resulted in slight opacity; the least favorable results were observed with CP and treatment F5, with very intense opaque eyes (Table 2). In terms

of eye orbit, the CP treatment showed the best results with the ocular orbit completely filled; the other treatments evidenced a sinking of the eye orbit. In terms of the shapes of the eyes, variations were observed among the different fish species. Finally, the analysis of iris visibility showed positive results in almost all treatments, with the sole exception of CP (Table 2).

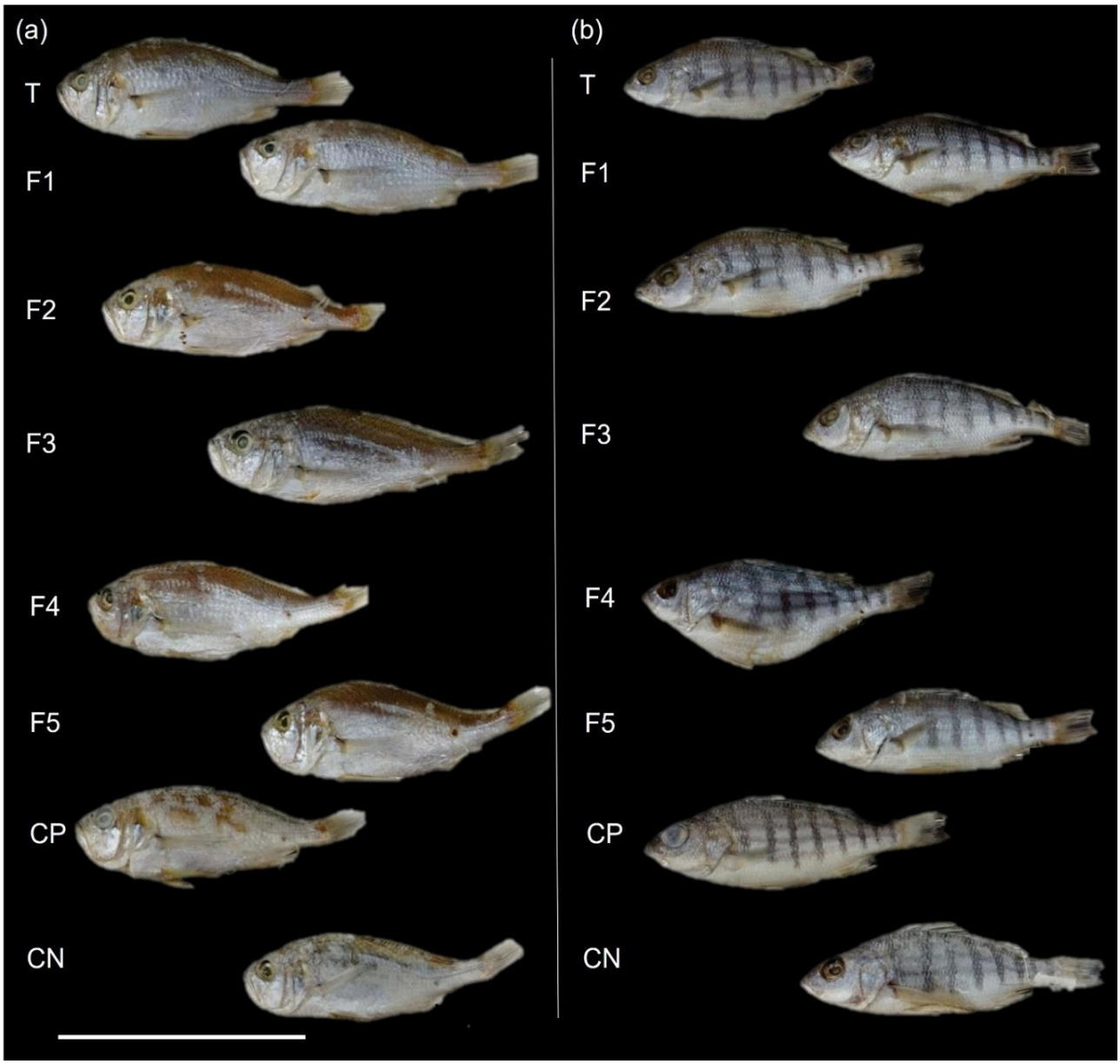

Figure 1. (a) *Larimus breviceps* and (b) *Conodon nobilis* specimens exposed to controls (CN, CP) and treatments (T, F1, F2, F3, F4, and F5). White bar: 5 cm scale.

The discoloration of the internal organs (gills) was most pronounced in the F2 treatment (Table 2, Figure 2), while the gills became darker or whitish in the CP and F1 treatments (Table 2, Figure 2).

In terms of the analyses of the morphologies of the internal organs (gastrointestinal), the specimens exposed to treatments F1, F3, and F4 presented the best results; the poorest result was observed in the CP group (Table 2). In terms of the analysis of exposure time in class, only the CP treatment evidenced specimen alterations and drying after 4 hours.

In summary, the formaldehyde fixatives at 2 and 3% concentrations evidenced the most positive results in relation to the variables analyzed, receiving RF values of 35 and 34 respectively. The 1% formaldehyde and Transeau fixatives scored 30 and 29 respectively, with both having poorer general appearances and marked scale detachment. The positive control scored a much lower value (25) and is considered inappropriate within the protocol proposed in this work.

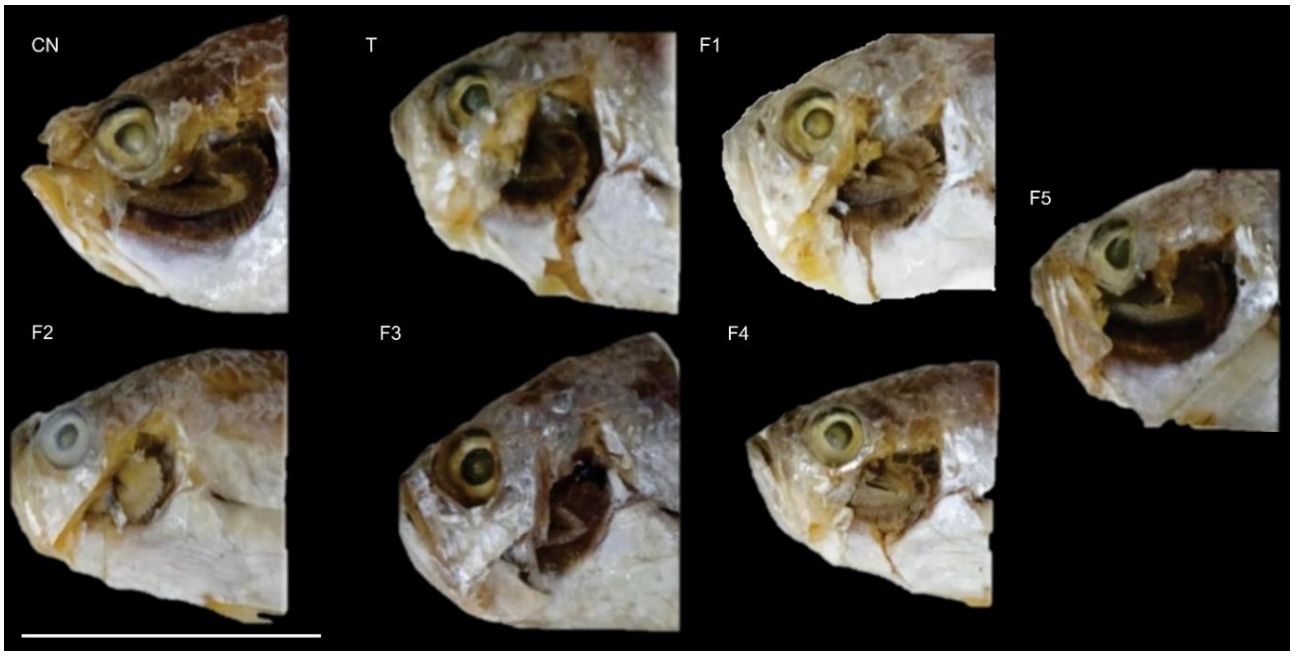

Figure 2. Discoloration of the gills of *Larimus breviceps* exposed to controls (CN) and treatments (T, F1, F2, F3, F4 and F5). White bar: 5 cm scale.

In terms of the variable opacity of appearance, the glycerination technique showed better results in reptiles with the F3 treatment, with the specimens appearing very shiny; the F2 and F4 treatments also resulted in good results, with the specimens evidencing a brilliant appearance. The most negative result, with an opaque appearance, was seen with the positive control and was closest to the negative control (Table 3, Figure 3).

The integrity of tortoise scutes was satisfactory for all of the fixatives analyzed, indicating that the glycerination process does not interfere with epidermal attachment (Table 3, Figure 3).

All of the specimens showed at least some external discoloration (Figure 03); the positive control and F5 treatment showed excessive discoloration. The F1 treatment showed the least specimen coloration alteration, with a limited detectable discoloration (Table 3, Figure 3).

Table 3. Variables evaluated and the final results for the reptile specimens during the glycerination technique experiments. CN: Negative Control, CP: Positive Control, F1: 1% formaldehyde, F2: 2% formaldehyde, F3: 3% formaldehyde, F4: 4% formaldehyde, F5: 5% formaldehyde, and T: Transeau. RF: Final Result, representing the sum of all of the evaluation criteria values. VM: Maximum value of each variable. The values used in the table correspond to the mode.

Variables	VM	Controls		Treatments					
		CP	CN	T	F1	F2	F3	F4	F5
Opacity of appearance	4	1	1	2	2	3	4	3	2
Scale integrity	3	3	3	3	3	3	3	3	3
External discoloration	4	1	4	2	3	2	2	2	1
Return of musculature	4	3	3	3	4	4	4	4	4
Malleability	3	1	3	2	3	2	2	2	1
Odor	3	1	1	2	1	1	2	1	1
Adhesion of the peritoneum	4	3	4	3	4	3	4	3	4
Morphology of internal organs (gastrointestinal tract)	4	4	4	1	3	3	3	1	2
Internal discoloration of organs	4	3	4	1	2	3	3	1	2
Exposure time in class (after 4 hours)	2	1	2	2	2	2	2	2	2
RF	35	21	29	21	27	26	29	22	22

None of the treatments showed a lack of muscle rebound when pressed, with the best results being observed in treatments F1, F2, F3, F4, and F5.

The malleability of the tortoises showed good results, with the F1 treatment evidencing very malleable specimens, although the specimens from the CN, F2, F3, F4, and T treatments were all classified as malleable the F5 and T treated specimens were characterized as stiffened, with greater rigidity (Table 3).

All of the fixatives had noticeable odors, with CP and the F1, F2, F4, and F5 treatments having the strongest odors and thus the most negative results. In terms of the variable adherence of the peritoneum, treatments

F1, F3, and F5 all stood out as being fully adhered to the muscles; the peritoneum evidenced slight detachment from the muscles with the CP, F2, F4, and T treatments. None of the specimens, however, presented a marked or complete detachment of the peritoneum from the muscles (Table 3).

Divergent values were observed regarding the analysis of internal organ morphology. CP was the only treatment in which the specimens evidenced fully intact organs, with results closest to the negative control; treatments F1, F2, and F3 showed little-altered morphologies, making it possible to differentiate their organs. Treatment F5 resulted in moderate changes in the internal morphologies of the organs, with some demonstrating wrinkling; treatments F4 and T evidenced the greatest degrees of internal morphology alteration (Table 3, Figure 4).

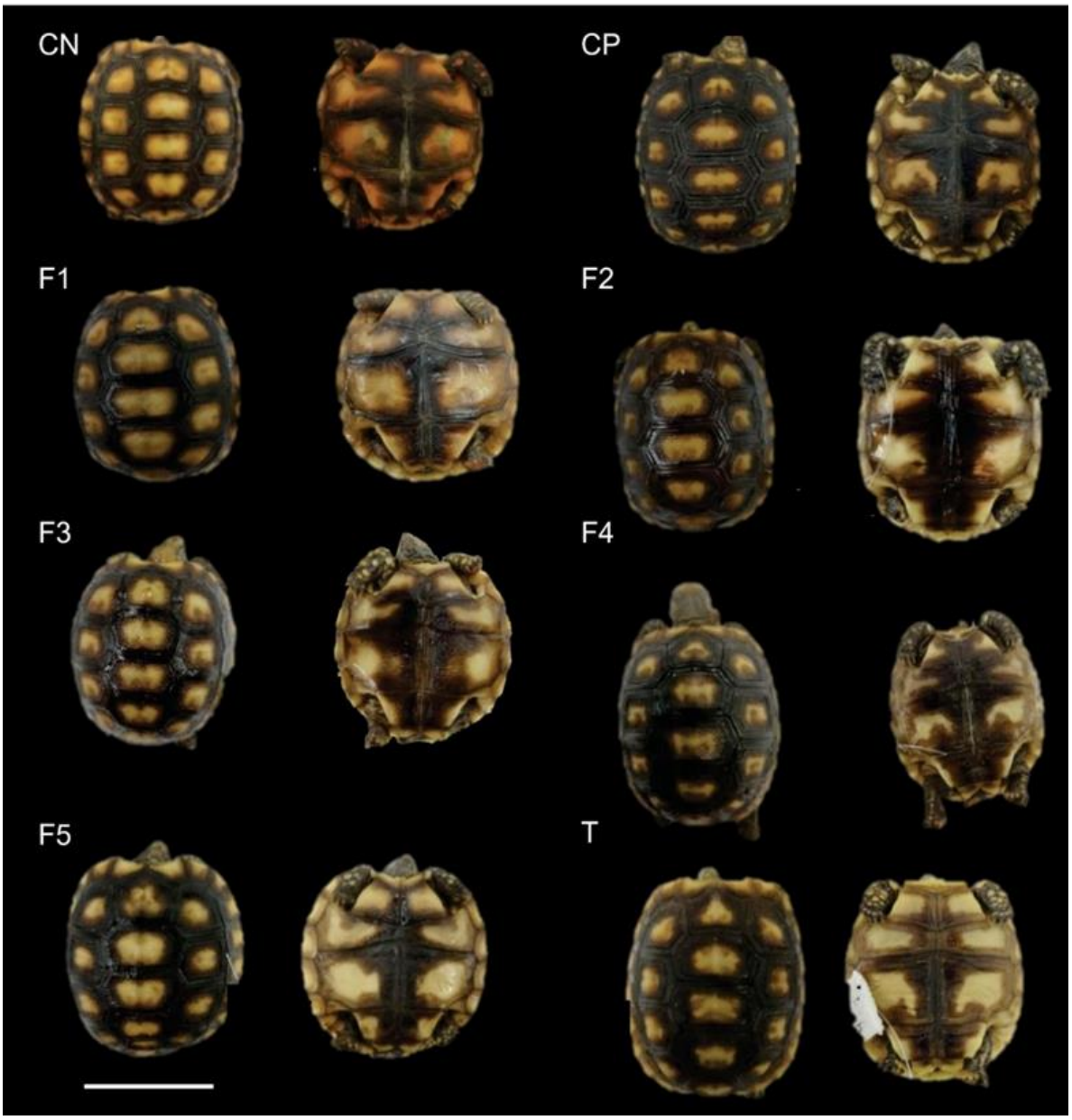

Figure 3. Dorsal and ventral view of *Chelonoidis carbonaria* exposed to the controls (CN, CP) and treatments (T, F1, F2, F3, F4 and F5). White bar: 5 cm scale.

The CP, F2, and F3 treatments showed the lowest rates of changes in the internal color of the organs, with detectable discoloration. Excessive discoloration was noted with the F4 and T treatments (Table 3, Figure 4).

The analysis of the exposure time variable revealed that the positive control was the only treatment that evidenced specimen alterations and dryness after prolonged exposure.

In summary, fixation in 3% formaldehyde appeared as the best fixative tested (RF = 29), with the same values as CN and few observable morphological changes – thus approximating *in vivo* animal specimens.

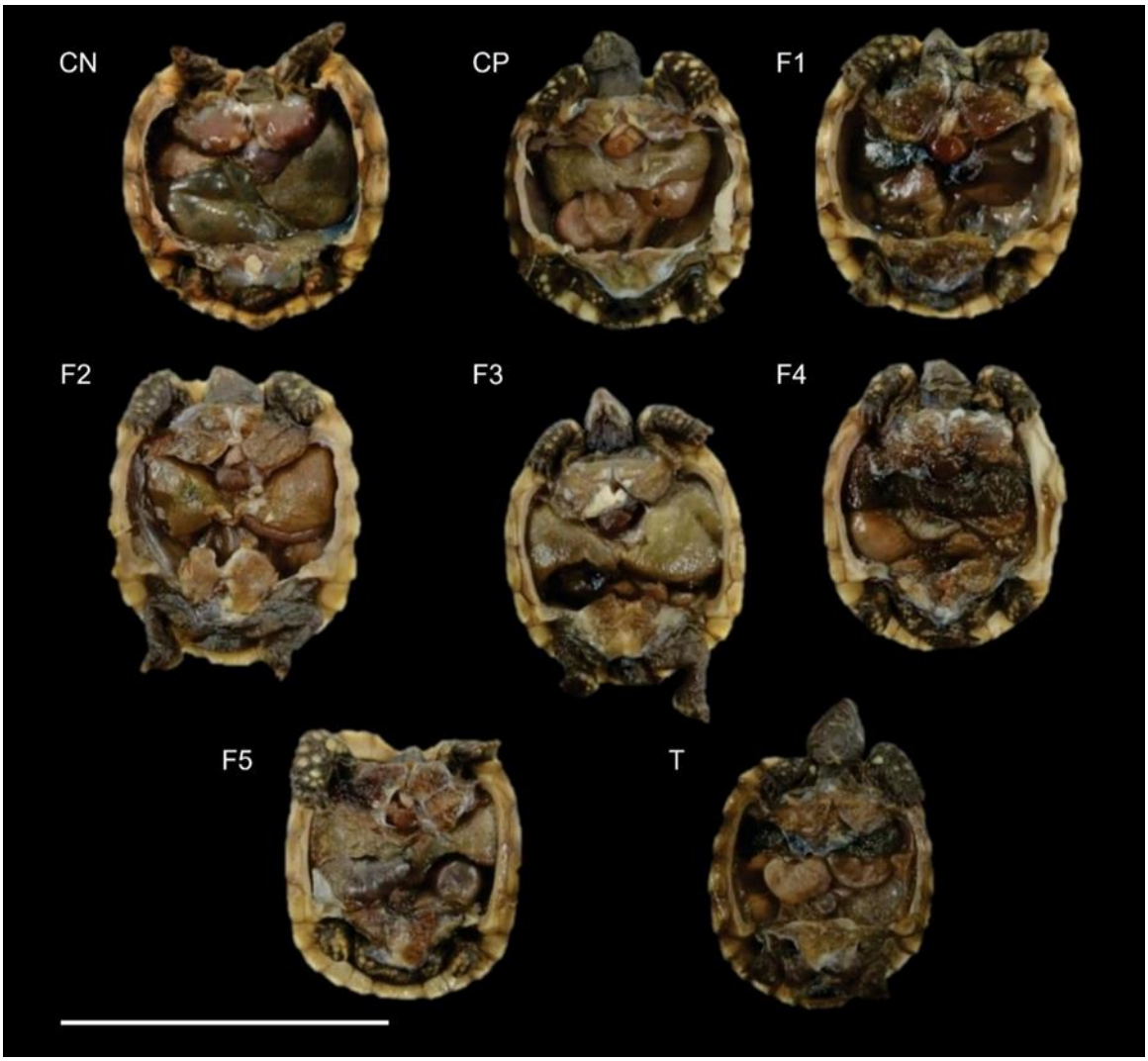

Figure 4. Internal morphology of *Chelonoidis carbonaria* exposed to the controls (CN, CP) and treatments (T, F1, F2, F3, F4 and F5). White bar: 5 cm scale.

Discussion

The results obtained in this study with fish and reptiles indicate the benefits of the alternative glycerination technique after treatment with formaldehyde. The quality of the glycerinated specimens was superior to the positive control – that is, specimens treated with 10% formaldehyde and subsequently preserved in 70% alcohol.

The glycerination technique examined here still uses formaldehyde in the fixation process, as it penetrates especially well into tissues, prevents the proliferation of pathogens, and does not allow the specimen material to deteriorate (Oliveira, Mindello, Martins, & Silva-Filho, 2013). However, glycerinated specimens are aesthetically superior to specimens preserved in formaldehyde, and glycerin is much less aggressive (Paiva et al., 2019). Glycerination is also a low-cost technique that allows for safer and easier manipulation of the specimens (Silva et al., 2008); glycerin is also an excellent preservative due to its antiseptic and antifungal action (resulting from its ability to dehydrate the cells of microorganisms) (Silva et al., 2008; Calamares-Neto & Colombo, 2015; Cury et al., 2013).

Specimens treated with low concentrations of formaldehyde (1%) showed the least external discoloration. Scaglia (2018) reported that high concentrations of formaldehyde distort and excessively discolor specimens, while Barbosa, Souza, Hermes, and Macedo, (2019) reported the ability of glycerin to preserve the natural colors of animal specimens, keeping them close to *in vivo* observations.

The positive control showed considerable lightening of organ colors, a property widely associated with high concentrations of formaldehyde (Karam et al., 2016); the other specimens examined, however, evidenced a darkening of their organs. According to Pereira (2019), glycerinated organs tend to darken over time, which

represents a disadvantage of this protocol; Oliveira et al. (2013) reported organ darkening and a loss of quality after only short storage periods.

Reductions in the masses of the organs during glycerination may sometimes represent an advantage, as those reductions can facilitate specimen handling (Silva et al., 2008). For small specimens, however, it may not be a welcome side effect and may be associated with shrinking, as was evident in treatments with 4 and 5% formaldehyde. The best results, however, were achieved by those authors using 10% formaldehyde, with excellent conservation of internal morphology. Studies have indicated that the inactivation of autolytic enzymes by formaldehyde is responsible for maintaining tissue morphology similar to that of *in vivo* animals (Karam et al., 2016).

The malleability data indicated that the higher the formaldehyde concentration, the greater the loss of malleability. According to Cury et al. (2013), the glycerin conservation technique preserves the specimens in terms of both tone and malleability, resembling *in vivo* individuals, and Freitas, Andrade, Baptista, Leite, and Assunção, (2020) and Godinho, Alvarenga, and Zofoli, (2021) reported that specimens preserved using that technique displayed malleability. Likewise, Karam et al. (2016) reported that glycerinated specimens show lower rigidity, differing from the rigidity of those preserved in formaldehyde. Regarding the opening of the anal orifice of fish, the muscles of specimens preserved with glycerin were well conserved, with no differences among the different treatments (except in CP), corroborating studies of Krung et al. (2011) who observed that formalized specimens demonstrated rigidity.

When analyzing the musculature of the specimens, it was observed that only the glycerinated specimens evidenced a rapid rebound of pressed musculature. Curry et al. (2013), examining bovine and equine kidneys, reported a low resistance of glycerinated samples to deformation, and they described the preserved organs as being "rubbery". This characteristic is also described as plasticity, maintaining organ morphology close to its original condition.

The evaluations of fish gill color gave unsatisfactory evaluations of preservation by glycerination (with the exception of the 2% formaldehyde trial) as that treatment resulted in gill darkening. Those organs also lose their original colors when treated with formaldehyde (Karam et al., 2016).

Several studies have indicated the high efficiency of glycerination when compared to the use of only formaldehyde, with the reduction (or absence) of odors (Silva et al., 2017; Barbosa et al., 2019; Fontoura, Mello, Gomes, & Mello, 2020; Lima et al., 2022). In the present study, this characteristic was noticeable with the 3% formaldehyde and Transeau treatments; the other glycerin treatments differed from the recurrent data in the literature.

Karam et al. (2016) noted the dryness of specimens preserved in 70% alcohol. In the current study, the positive control was preservation in 70% alcohol – and it was the only protocol that evidenced alterations in exposed specimens over time.

Among the treatments tested in the present study, 3% formaldehyde presented the best overall preservation characteristics, coming close to the results of the negative and *in vivo* animal controls. Therefore, the association of fixation in 3% formaldehyde with glycerin yields satisfactory results in terms of specimen appearance, odor, peritoneum adhesion, and low organ discoloration. Costa et al. (2021) likewise demonstrated with crustaceans (Crustacea: Decapoda) that 3% formaldehyde with glycerination yielded high scores for the above-mentioned variables, especially in terms of low external discoloration and the morphological states of the organs.

The second most effective treatment was with 1% formaldehyde, which was the most similar to the negative control and, consequently to the *in vivo* specimens due to their low formaldehyde contents, with only low morphological modifications. It is important to note, however, that the present study was only short-term, and that Przybysz and Scolin (2009) pointed out that low concentrations of formaldehyde might not provide satisfactory long-term conservation of anatomical specimens.

The positive control, together with Transeau, demonstrated the highest rates of specimen alteration. Karam et al. (2016) pointed out that formalization will alter the morphology of the specimens, with, for example, increased weight, rigidity, and discoloration. Transeau is composed of 6 parts water, 3 parts 95% ethyl alcohol, and 1 part 40% aqueous formaldehyde (Bicudo & Menezes, 2006) and is usually used as a fixative and preservative for planktonic groups (Barros et al., 2021). It was tested in the present study as a possible alternative for fixing reptiles, although the results showed high degrees of alteration of the specimens, and it was not aesthetically positive.

Despite the fact that among the glycerination techniques applied to fish, the F2 treatment demonstrated a more pronounced RF than the F3 treatment, we recommend the F3 treatment for both fish and reptiles because, when considering the duration of preservation (which was not analyzed in this study), higher concentrations of the fixative can guarantee the longer durability of biological specimens.

Conclusion

Glycerination protocols with fixation in 3% formaldehyde are recommended for fish and reptile specimen preservation. Our results highlight the importance of replacing classic conservation methods with glycerination protocols, which will reduce the use (and replacement) of toxic reagents in collections intended for teaching and allow them to be kept in a dry state. Furthermore, the coloring and characterization of the internal portions of the specimens are closer to their *in vivo* states, and those materials demonstrate excellent malleability (which facilitates handling) – resulting in excellent quality specimens for teaching and extension activities.

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