CONSERVATION OF A MUSEUM MEGAMOUTH SHARK SPECIMEN BY CHANGING ITS PRESERVATIVE FROM AQUEOUS ETHANOL TO AQUEOUS GLYCEROL

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Abstract.—The Western Australian Museum's iconic 5.2-m megamouth shark (*Megachasma pelagios*) was relocated 20 km from Perth to the Maritime Museum in Fremantle for treatment in an exhibition gallery. A 70% ethanol solution was diluted to 16% before the glass lids of the fiberglass tank were removed to facilitate removal of the heavy shark. A custom-made stainless steel storage and exhibition tank containing 8,000 L of 30% glycerol solution was prepared inside the exhibition space prior to the arrival of the specimen. Portholes in the top sections provided access points to record the density of the solution using a digital densitometer. The density fell linearly with the logarithm of the immersion time. Equilibration was achieved after the solution showed no change in density for a period of 2 months. To increase the glycerol concentration, 2,000 L of the solution were decanted into storage tanks before the same volume of pure glycerol was added. This process was repeated four times to reach a final level of 62% during the 2.5-year conservation program. The color and flexibility of the shark improved and dehydration wrinkles from ethanol storage were significantly reduced.

Key words.—aqueous glycerol, color measurement, conservation, flexibility, megamouth shark, preservative.

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Introduction

On 18 August 1988, the third recorded megamouth shark specimen (WAM P.29940–001) was found stranded on a beach 50 km south of Perth, Western Australia. Following initial documentation, it was transported to Perth, then frozen and put on brief display before being preserved with formaldehyde (Berra and Hutchins 1990). After thawing from its frozen state, the shark was fixed in a plastic-lined pit 6.5 m long, 3 m wide, and 1.5 m deep in a 4% formalin solution and kept in that state for 18 months, pending funding for an exhibition tank. Excess formaldehyde was removed by washing the shark for 1 month in fresh water. Analysis of the wash solution confirmed that the release of formaldehyde followed logarithmic kinetics, i.e., the concentration of formaldehyde increased linearly with the log of the washing time (MacLeod 2008). Before being moved to the initial exhibition site in Perth, the shark was opened under the abdomen and 60 kg of viscera were removed before it was padded with spun Dacron and stitched together. This approach was similar to that taken by Takada et al. in their treatment of a female megamouth, which was the ninth recorded specimen (Takada et al. 1997). The shark was supported on a special stainless steel exhibition mount, to which it was secured with surgical staples, in a 10,000-L tank containing 70% ethanol, and was exhibited in this format for 13 years. Despite its scientific significance as the third specimen recorded in the world, funding shortfalls for the exhibition tank meant that it was not on public view until 25 January 1995 (MacLeod 2008). The megamouth exhibition was extraordinarily popular with museum visitors. The iconic status of Megamouth III ensured that it was left on exhibition when staff and objects were relocated from the main collection storage and administration building of the Western Australian Museum in Perth in 2004 (MacLeod 2007). The relocation was necessary because the 1970s building had major

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asbestos contamination, which placed the staff and collections at risk. Because the belowground tank was only 15 m from the former operational headquarters of the museum, the impending demolition of the building necessitated the relocation of the 5.2-m shark. Because there was no facility to house the iconic shark in Perth, the Chief Executive Officer instructed the author to develop a new treatment method, with the work to be conducted in a special tank inside the Western Australian Maritime Museum, 20 km away in Fremantle, to ensure that the specimen would be available for public exhibition during the redevelopment of the Perth site. The relocation of staff and collections to an off-site storage and research facility in 2004 had seen the disappearance of the iconic 25-m-long blue whale from public view, and the museum director was adamant that a second icon should not "disappear" (MacLeod 2007). Dangerous goods regulations in Western Australia preclude the use of 70% ethanol inside public buildings, so the megamouth display was located 5 m outside the main museum building, with the top of the exhibition tank at ground level. Because shock waves from the planned demolition of the building would have fractured the fiberglass resin tank and released the ethanol, which would have created a fire hazard, a safe alternative storage medium had to be found.

Over time, some of the storage solution leaked through hairline cracks in the fiberglass tank and the tank was topped up with 70%, rather than 100% ethanol; this resulted in a final storage solution of only 54% ethanol. Evaporation of the alcohol was minimized by the high-quality mastic seals on the glass lids and a goose-neck copper expansion pipe, which allowed the tank to be filled with virtually no air gap. The goose neck also allowed the solution to expand with increases in ground temperature during annual seasonal variations without any buildup of pressure on the laminated toughened glass.

Rationale for Choosing a Stepwise Glycerol Impregnation

During a visit to the Leiden Medical Museum in The Netherlands, 100-year-old glycerol-impregnated specimens of human hearts and other organs were observed that appeared to be in excellent condition. The stability of the formalin-fixed human tissue stored in aqueous glycerol solutions overcame curatorial concerns that the rare shark would be less stable than in 70% ethanol. To further allay curatorial reservations, a trial impregnation experiment was conducted on small sharks that had been fixed with formalin and stored for 12 years or more in 70% ethanol. Starting with a 35% v/v glycerol solution, the concentration was increased to 45%, then 54%, before a final concentration of 65%. Positive benefits included partial removal of dehydration shrinkage lines, return of color to the specimens, and remarkable improvement in the flexibility of the jaws and fins. The small shark experiment demonstrated that impregnation with aqueous solutions of glycerol is an effective method for replacing ethanol as the preserving liquid (MacLeod and van Dam 2011). The presence of three hydroxyl groups in glycerol (1,2,3-trihydroxy propane) makes it an ideal organic reagent with which to impregnate specimens because it is strongly absorbed by the structural cellular components. The advantages of glycerol principally lie in its capacity to form hydrogen bonds with the organic materials in cellular tissues. Because glycerol is a common industrial chemical, tables of data on its physical properties over a wide range of concentrations are readily available in standard handbooks (Wolf et al. 1974). Owing to the high degree of intramolecular bonding, glycerol solutions have a much higher flash point than aqueous ethanol solutions, and present a much lower fire hazard rating. The flash point of a 70% ethanol solution is

16.6°C, whereas an aqueous glycerol solution must reach a concentration of 77.4% before it will flash at 190°C. Because the standard glycerol concentration used for specimen storage is 65%, it is well below the amount needed to achieve a flash point. Thus, the proposed final concentration of glycerol in the new megamouth tank was insufficient to enable the solution to burn (www.aciscience.org/docs/physical_properties_of_glycerol_and_its_solutions.pdf). There are no health and OSH issues associated with aqueous glycerol solutions, because the glycerol is USP grade, i.e., approved for use in the USA for the preparation of pharmaceutical medicines for human consumption.

Opening the Original Tank

The inherently hazardous operation of opening the 15-mm-thick sealed glass lid, which consisted of two silicone sealant-joined sections, on 10,000 L of 70% ethanol to enable pumping of the bulk of the solution, was achieved by cutting the smaller glass section away from the support base and slicing the interface between the black, chemically resistant silastic and the overlying glass (Shalk 2010). The smaller heavy glass plate was slid 10 cm along the top of the tank to enable an engineering mount with 60-mm-diameter ball valves and a 100-mm internal diameter "elephant trunk" to be inserted into the tank. The combination of the ball valves and the hose diameter allowed the pump on the solvent tanker to safely pump thousands of liters of alcohol in 10 minutes. Explosion risks were minimized by conducting these operations during the cool of the late evening when the temperature was 12°C, some 4.6°C below the flash point of the alcohol. The screened chemical hoses, pump, and tanker were all grounded to prevent any sparks. Seven thousand liters of ethanol, containing traces of formaldehyde, were pumped into a chemical tanker. The remaining volume was diluted with tap water and the specimen left overnight. Testing of the alcohol solution before disposal confirmed that the formaldehyde levels in the tank were only 0.14% after 13 years of having the shark on exhibition. Tests on the soil underneath the tank showed that there was no measurable amount of ethanol, so the pit could be safely filled in. The tap water dilution brought the alcohol content down to 16%, with a flash point of 42°C. The conservation and curatorial team arrived before 7 AM, when the temperature was 12°C, which provided a safe margin of 30°C for the ethanol to be exposed to the air.

A team of 10 workers raised the 15-mm-thick larger glass panel from the tank and safely removed it. Museum staff, wearing respirators and personal protective equipment, then lay on boards above the tank and cut through the stainless steel sutures that held the tail and the head of the shark at the preferred display angles. The tank still contained 3,000 L of solution, which supported the shark. Corrosion of the stainless steel left brown rust marks on the skin of the shark, but owing to the mottled natural coloring of the shark, these marks were not readily visible to the public during the previous years of exhibition. A set of six woven straps were placed underneath the shark and attached with rope to a 6-m spreader bar. The strapping had been prepared in such a fashion as to avoid any direct contact with the massive pectoral fins and the protruding claspers. A mobile crane was used to elevate the shark out of the tank and carefully move it to a nearby 4-t truck on a custom-made cradle that had woven straps tied into position to ensure that the shark was evenly supported (Human et al. 2012). During the recovery operation it was readily apparent that our initial projections of how the shark would fare out of solution were inaccurate, because they had been modeled on the behavior and the musculature of more common shark species. The fixed specimen was weak in the abdominal and tail regions, which meant that extra care had to be

taken when moving the specimen onto the truck and into the treatment tank in Fremantle. This apparent weakness might be a reflection of imperfect fixing. The shark was protected from rapid drying by wrapping in a layer of hessian, which was then covered with a heavy-duty black polyethylene builders' membrane (0.2 mm thickness) and sealed with duct tape.

New Tank

A custom-made 9,000-L (14-gauge) stainless steel exhibition and treatment tank was prepared and pressure tested prior to the relocation step. The cost of the tank, its support cradle, and fitted portholes was US$40,000. The tank was designed to withstand a hydrostatic load of approximately 12 t and was laterally stiffened with three 50 × 50 × 2 mm cross braces and six vertical stiffeners that were each 100 × 50 × 2 mm. The tank was initially filled with 8,000 L of 30% (USP grade 99.5%) glycerol, because this is similar in osmotic pressure to 70% ethanol and would avoid undue cellular stress. Museum management required the iconic shark to be conserved on public view, inside a climate-controlled space. The tank has a series of glass portholes in the side and at the front to allow for easy viewing. All the portholes on the sides of the tank are located by stainless steel lugs, which were welded in place, and the glass was sealed with a Dow Corning® neutral-cure silicone resin. The top of the tank has three adjoining stainless steel lids with large glass windows that are held in position by 5-mm neoprene gaskets with adhesive on both sides to secure them to the stainless steel, allowing the shark to be viewed from the mezzanine walkway adjacent to and 50 cm above the top of the tank. In addition, there are two inspection ports on each lid; this arrangement facilitates easy access of a digital densitometer for regular solution sampling. When additional glycerol was required, the lids were removed and multiple lots of glycerol were decanted from 200-L drums (cost per drum US$350). The drums were laid horizontally on the tines of a forklift and the bungs were eased open to allow a steady stream of liquid glycerol to enter the treatment tank from a height of 40 cm. The initial 30% solution was prepared by decanting 1,200 L of glycerol into approximately 6,800 L of water. Any cleaning operations to remove debris from the tank bottom were conducted simultaneously with increases in the glycerol solution and in full view of the museum-going public. The sight of museum conservators and fish curators wading in the tank provided significant opportunities for public engagement (Figure 1).

Each lid section has four lifting handles through which woven straps were threaded for easy movement to and from the top of the tank, using an extension arm of a 1-t forklift. The spreader bar used in loading the shark was stored in the space underneath the tank. Full engineering drawings of the tank are available on the Western Australian Museum's website: http://museum.wa.gov.au/research/research-areas/materials-conservation/megamouth-design-treatment-tank. The treatment of the shark was monitored by regular measurement, both of the solution density with an Anton Paar digital densitometer (which measures the density to four decimal places) and the temperature. Recording both variables is essential in order to correct densities to a standard temperature, because the density of aqueous glycerol solutions typically varies by ± 0.0004 g/cm^3/°C. One advantage of the treatment being carried out inside a modern public museum gallery is the fact that the building is temperature controlled 24 hours a day. This level of control was not available in the historic buildings on the Perth museum site. During the 2.5-year treatment program the mean solution temperature was 21.8 ± 1.4°C.

Figure 1. Megamouth (*Megachasma pelagios)* being loaded into its new stainless steel treatment and exhibition tank, Western Australian Maritime Museum in Fremantle, 22 September 2011.

Control of Microbiological Activity

After several weeks of immersion in the unstirred 30% initial solution, major growths of an *Aspergillus versicolour* complex and a heavy mixed growth of mixed yeast species were readily apparent (Arthur 2010). These outbreaks occluded the views of the specimen

from the 10 portholes. The solution became smelly and there was a risk of damage to the specimen. This problem was overcome by application of the biocide Kathon CG, which is a mixture of methylchloroisothiazolinone used in conjunction with the parent methylisothiazolinone. This treatment has proven to be effective in managing microbiological activity in the impregnation of waterlogged organic archaeological materials with polyethylene glycol solutions and consolidants, which are otherwise an active soup for microflora (Graves 2004).

A biocide dosage rate of 2.5×10^{-3} wt. % or 25 parts per million (ppm) (5 L of solution) killed all the mold and algae within 72 hours. Mycological analysis of the growths was consistent with the microorganisms originating from the old hessian wrapping. Gross exposure of staff to the treatment solution was managed by wearing fishing waders when working in the tank, but realignment of the megamouth had to be done with bare hands because the glycerol solutions made it very slippery to wet synthetic gloves. Skin contamination was removed by careful washing of arms and hands after working in the tank. No biocide was initially added to the glycerol solutions in the shark tank because the trial experiment with the small sharks did not suffer from mold; however, it is likely that there was sufficient formaldehyde in the small sharks to kill anthropogenic yeasts, molds, and other fungi. Previous collection management procedures involved rinsing fixed sharks for 2 hours under running tap water before placing them into 70% ethanol, but this is clearly insufficient to remove the excess fixative, because analysis of the storage solutions showed that they contained up to 800 ppm formaldehyde. The current short-term exposure limit (15 minutes) is 2 ppm (https://www.osha.gov/OshDoc/data_General_Facts/formaldehyde-factsheet.pdf) (MacLeod 2008). A second reason for the lack of microbiological activity in the glycerol solutions containing the small sharks might be that the air in the collection storeroom is highly filtered so that contaminants need to be less than 1 μm to get through the heating, ventilation, and air conditioning system at the museum collection and research center. The air-handling units at the maritime museum use 20-μm mesh filters. It is strongly recommended that all materials covering a specimen should be sterile and that a biocide be added to the impregnating glycerol solutions. Swabbing the waders worn by scientific staff in the tank with ethanol would eliminate the contamination risk from this potential source.

Removal of the dead algae was achieved using a standard swimming pool sweep system that was connected by a hose to a high-volume electric pump that aspirated the debris and the solution through a filter into a storage tub alongside the treatment tank. After preliminary filtration and restoration of the glycerol solution to the tank, the solution remained turbid. To overcome this problem, a stainless steel mount was constructed as an extension of the stainless steel frame to take a 1.5 horsepower chemical pump designed for handling viscous solutions (Rotoflow model ME16R). High-pressure reinforced rubber tubing was connected from the lower 40-mm-diameter ball valve at the head of the tank and run under the base to the pump, which had an outlet connected to a 40-mm-diameter inline filter (Davey Filter Pure model W20J40, Davey Products Pty. Ltd., Scoresby, Australia, www.davey.com.au.). The hose ran from the filter to an upper ball valve, which allowed the return of the solution to the tank. Future designs should take into account improved orientation of inlet and outlet valves so that there is better mixing of the glycerol solutions. The filter was checked and backwashed each week for a period of 6 weeks, and then the size of the filter mesh was reduced from 100 μm to 50 μm. During this time the clarity of the solution noticeably improved. After 2 months at 50 μm,

the filter was changed to the final size of 10 μm, which ensures optical clarity of the treatment solution (Davey 2012).

Modeling the Absorption of Glycerol

Understanding the mechanism of absorption of glycerol into a specimen can lead to an improvement in collection management through a better understanding of the physical chemistry of the reaction. A model that produces the same logarithmic time dependence as has been observed for the removal of formaldehyde and absorption of glycerol in the shark is based on the adsorption of gases onto a reactive surface. In the gas phase, increasing the partial pressure of the gas will increase the amount of absorption and in the treatment tank the equivalent is the increasing concentration of glycerol, which leads to increased rates of absorption (Denbigh 1964). The shark skin is reactive where there are no denticles; this is akin to the gas adsorption process on physically reactive surfaces. The fraction θ of the covered surface is given by the ratio of $^{m}/_{M}$, where m is the total number of molecules adsorbed and M is the number of sites. Part of the driving force for the free energy change associated with the absorption of glycerol into the shark tissue is the lower energy that the glycerol molecules have when they are hydrogen-bonded with the proteins in the shark musculature. The free energy of adsorption is defined by Equation 1 (Table 1).

In Equation 1, ΔH is the heat given off through the adsorption of glycerol and s is the entropy per mole of the absorbed glycerol—the superscript ^{0} for entropy and free energy μ refer to a standard 1M glycerol solution. Inspection of Equation 1 shows that the energy driving adsorption is dependent on the logarithm of the fraction of the surface responding to the adsorption process. It is not surprising that the kinetics of glycerol absorption and desorption of formaldehyde from the megamouth follow linear trends with the logarithm of treatment time.

Treatment of Megamouth III

Monitoring the Impregnation Progress

Following the successful impregnation treatment of a 450g small hammerhead *(Sphyma lewini)* and a 2,400g Kimberley reef shark (*Carcharhinus amblyrhynchos*) using serial increases in glycerol concentration, it was decided to adopt a similar stepwise addition program of increasing glycerol concentrations for the megamouth. In addition to improvement in the flexibility of the small sharks, the change in the specimens from being pasty white to having their natural color restored by the glycerol treatment was deemed to be a desirable improvement in the overall condition of the megamouth shark. Whereas it was possible to regularly remove the smaller sharks and weigh them to gauge the adsorption rate of the glycerol and to simply add more glycerol to the 40-L half-filled treatment tub, the delicate nature of the megamouth shark and the high cost of cranes precluded this monitoring method. Comparison of the weighing data and changes in density with the small shark experiment showed that the changes in density provided a good indicator of the progress of the impregnation experiment (MacLeod and van Dam 2011). Because the solution in the treatment and exhibition tank was at 95% capacity, addition of more glycerol necessitated pumping out between 1,000 to 2,000 L of the equilibrated solutions and then adding USP glycerol from 200-L drums.

Plots of the density (ρ) of the initial impregnating solution against the log of time (t) showed a gradual fall as glycerol impregnated the shark and replaced the ethanol in cell tissues in the process. The initial rate of decrease of the density in 30% v/v glycerol was

0.0307 ± 0.0001 g/cm^3 per log time interval; see Equation 2 in Table 1. During the first 4 months of impregnation there was a strong smell of alcohol emanating from gaps in the rubber gasket underneath the tank lids, which led to some public complaints. Complaints were minimized by conducting experimental work, such as topping off the glycerol, before public opening hours. Concerns from the Dangerous Goods consultants about the amount of alcohol coming out of the shark were addressed through a risk assessment process. Once the first treatment had plateaued, the alcohol content was reduced by decanting 2,000 L of the treatment solution into Schutz chemical storage tanks and replacing it with 1,500 kg of pure glycerol. The very high viscosity of pure glycerol meant that despite vigorous initial stirring, it took several hours of pumping the solution before concentration gradients were eliminated.

Because the 30% glycerol solution was significantly denser than the ethanol solution from which the shark had come, the specimen had to be restrained in the treatment tank. A series of straps across the specimen kept it submerged in the treatment solution, because the natural tendency was for the shark to float to the surface. Although the solution was pumped and filtered each night for 16 hours, the configuration of the inlet and outlet ports was not optimal for mixing. Measurements at the monitoring ports above the tail and above the head showed small differences in density, on the order ± 0.0004 g/cm^3 (compared with a reproducibility of ± 0.0002), which were consistent with the observed rates of fall at the different ends of the shark. The tank was not designed for circulation and filtration of the solution, which is now considered a design defect. The tail area of the shark equilibrated more rapidly than the head, owing to the significantly higher surface area to volume ratio. During these phases, the different equilibration rates made the shark look as though it was rising to the surface of the tank.

Recycling the old megamouth treatment solutions is a cost-effective method of preserving other aquatic specimens. For example, the first 2,000 L of the equilibrated initial "30%" solution that was drained off was used to begin the treatment of a 150 kg sunfish (*Mola mola*) that had been preserved in 10% formalin. It was washed for 4 weeks in fresh tap water and then placed directly into the glycerol solution without any storage in 70% ethanol (MacLeod et al. 2015). Subsequent aliquots of old megamouth solutions have been stored for the continued treatment of the sunfish. The first 16 months of treatment saw a 40% increase in the weight of the sunfish.

After 88 days of equilibration, the first stage in 30% glycerol was completed and the glycerol concentration was increased. The addition of 1,200 L of glycerol to the megamouth tank increased the concentration to 45% glycerol. A visiting child shone his flashlight into the mouth of the shark to see inside the large gape. There were cries of, "Mummy, it's got bricks inside." Correspondence with the previous preparator confirmed four house bricks, weighing a total of 10 kg, had been placed deep inside the buccal cavity to ensure that the mouth remained open during its exhibition in the alcohol. Relaxation of the jaw in the glycerol had increased the gape and so revealed the bricks, which were removed at the next concentration change.

During the first 5.5 weeks in the 45% solution, the density fell rapidly (see Figure 2 for the diagonal stripes running from upper left to lower right of the squares labeled "Equation 3") at the rate of 0.18 g/cm^3/log t, where t is the number of treatment hours from the beginning of the impregnation (Table 1, Equation 3), and then at a more gradual rate of 0.023 g/cm^3/log t as the glycerol impregnated the specimen (Table 1, Equation 4). Once the second solution had plateaued after another 7 weeks, the density began to slowly increase as displaced ethanol began to evaporate over another 10 weeks.

Table 1. Equations relating to the adsorption rates of glycerol by Megamouth III. n.a. indicates not applicable.

Equation no.	Property	Equation	R^2
1	Free energy of glycerol adsorption	$\mu - \mu^0 = \Delta H - T(s - s^0) + 2.303\ RT \log \{^{\theta}/_{1-\theta}\}$	n.a.
2	Rate of density change in 30%	$\rho = 1.0885\ (0.0002) - 0.0031\ (0.0001) \log t$	0.9917
3	Rapid rate of density fall in 45%	$\rho = 1.7402\ (0.0328) - 0.1800\ (0.0097) \log t$	0.9886
4	Steady rate of density fall in 45%	$\rho = 1.2039\ (0.0066) - 0.0230\ (0.0019) \log t$	0.9744
5	Log (rapid density fall rate [R]) vs. glycerol concentration	$\log R = -4.65(0.33) + 0.072\ (0.007)\ \%\ v/v$	0.9752
6	Normalized rate of increase in weight of small sharks	$\text{Weight}_{kg/cm2/\log t} = 0.0718\ \%\ v/v - 2.077$	
7	Calculated weight of megamouth (MM)	$\text{MM Weight}_{kg} = 395 + 22.72 \log t$	
8	Rate of density fall for MM vs. glycerol concentration	$\rho_{megamouth} = 0.0476 - 0.1630\ \%\ v/v_{glycerol}$	
9	Rate of density fall for small sharks vs. glycerol concentration	$\rho_{small\ sharks} = 0.0072 - 0.0344\ \%\ v/v_{glycerol}$	
10	Calculation of colour change (ΔE)	$\Delta E = \{(L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2\}^{0.5}$	

The scatter of the data was too great to obtain a meaningful regression equation. At this point the solution had plateaued, so another boost in the glycerol concentration was necessary. The analyses of the density changes for the first two impregnation baths are summarized in Table 1.

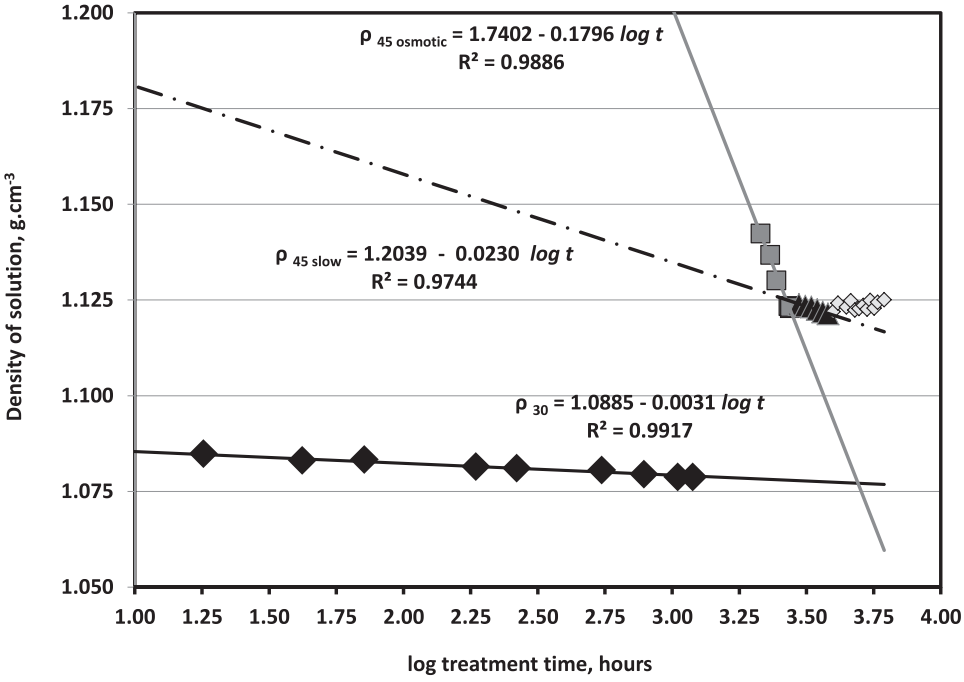

Figure 2. Density of shark impregnation solutions as a function of the logarithm of treatment time (hours) for 30% glycerol, showing linear decrease in density with log of treatment time, and 45% glycerol, with rapid and slow falls in density.

Effects of Changing Viscosity on Rapid Fall in Density as Glycerol is Increased

The rapid drop in density on increasing the glycerol concentration was initially thought to be consistent with the shark responding to the increased osmotic pressure of the impregnating solution. There was no perceived rapid fall in density for the 30% solution because the osmotic pressure of a 30% glycerol solution is similar to the pressure exerted by the aqueous ethanol solution from which the shark had come. As the glycerol concentration was progressively increased, it was noted that there was always a rapid decrease in density followed by a significantly slower rate of decrease as general diffusion of the glycerol into the specimen took place. At the end of the impregnation treatment, the data showed that the logarithm of the rate of the rapid fall in density (R) was directly dependent on the concentration of glycerol (Equation 5, Table 1 and Figure 3). The advantage of finding a linear relationship between the logarithm of the rapid rate of decreasing density (log R) and the concentration of glycerol (Equation 5, Table 1) is that it becomes much easier to check that the specimen and the solutions are behaving in a systematic fashion. An average error of ± 8% in the slope and intercept values of Equation 5 provides a high degree of confidence that predictions of the rapid-fall kinetics are reliable. It is possible that the increased viscosity of the new glycerol solutions increases the effective contact of the solution with the shark's skin and so provides a boost to the rate of adsorption of the glycerol, which gradually tapers off as the solution and the shark adjust to the changed environment. Each time liquid glycerol was added to increase the concentration, the team entered the tank and conducted a physical examination of the shark, which involved checking on the flexibility of the fins, gills, and tail. This work confirmed that there was no deterioration of the specimen and that the flexibility of fins, gills, and jaw increased with the glycerol concentration.

Modeling Glycerol Impregnation for the Megamouth

Before the treatment of the megamouth began, data from the previous small shark experiment were used to extrapolate the glycerol uptake rate from small surface areas to a specimen 15 times larger. The small reef shark weight increase was normalized by dividing the observed rate of kg/log time (kg/log t) by the surface area cm^2, as shown by Equation 6 in Table 1.

With an estimated surface area of 75,000 cm^2 or 7.5 m^2 for the megamouth shark, it was predicted that the initial rate would be 5.8 kg/log t, which implies an increase of 23.1 kg during the 42 days of treatment in the 30% v/v solution. It was not desirable from a curatorial perspective, nor practical or commercially feasible, to regularly weigh the specimen, so another method of estimating its changing weight was developed to test the validity of this approach.

The changes in density of the 30% glycerol solution were used to estimate the weight increase of the megamouth during the initial impregnation as the cellular contents of the shark changed from aqueous ethanol to aqueous glycerol. After correction for the weight loss due to the outward diffusion of the former storage solution it was estimated that the megamouth would increase in weight according to Equation 7 (Table 1).

The intercept value of 395 equates to the estimated initial mass of the 5.2-m-long shark. This calculation provides for a greater rate of glycerol uptake, by a factor of 3.9 times, than expected from the reef shark observations. This implies a weight increase of 90.9 kg during the 30% impregnation time between 6 minutes and 1,000 hours or 42 days. When all the density data from the four impregnation steps were used, the slope of Equation 7 remained the same, but the intercept, or initial weight, was estimated to be 10 kg lighter.

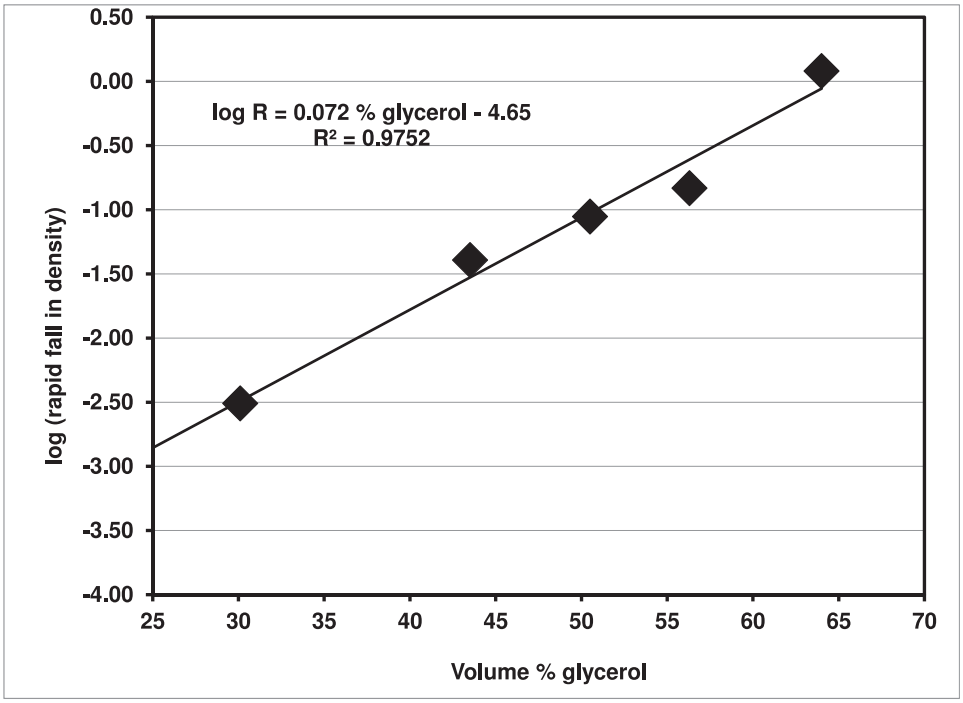

Figure 3. Logarithm of the rate of rapid fall in density (g/cm^3/log time that occurred after the addition of glycerol, against the bulk glycerol concentration.

At the end of the treatment, the shark was weighed by a digital scale attached to the crane hook while being suspended on the woven support slings attached to the spreader bar. The final weight of the shark was 115 kg heavier than the initial 369 kg. The density data for all the solutions had predicted an overall increase of 110 kg, whereas the prediction for the initial 30% solution was a 91 kg increase in weight. In comparison, the hammerhead shark increased in weight by 14% and the reef shark increased in weight by 19%. The bulk of the increase in weight took place in the first impregnation bath.

When the impregnation data from all the solutions were reviewed, it was found that the slopes of the decrease in density vs. log time plots became more negative as a function of the increasing glycerol concentration, as given by Equation 8 (megamouth) and Equation 9 (small sharks), illustrated in Figure 4.

The rate of decreasing density of the megamouth solution with increasing glycerol concentration is given by Equation 8 (Table 1).The corresponding equation for the combined treatment of the reef and hammerhead sharks is given by Equation 9.

The rate of change of the density of the solutions based on glycerol concentration is 4.7 times greater for the megamouth than for the small reef and hammerhead sharks. This compares favorably with the initial data from the 30% solution, which estimated that the megamouth would be 3.9 times more sensitive than the small sharks. Based on the larger surface area to volume ratio of 0.33 cm^{-1} for the small sharks compared with 0.23 cm^{-1}, it was expected that the megamouth would be less sensitive than the smaller sharks. Inspection of the skin of the reef shark and of the megamouth (Figure 5) shows that the denticles on the megamouth skin are like mesa formations sitting above the plain of the

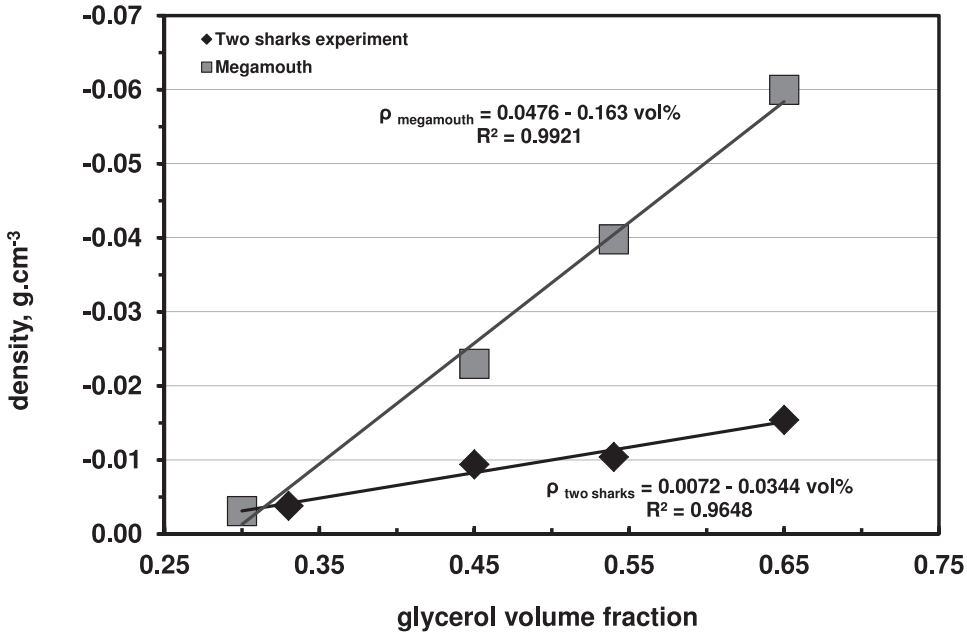

Figure 4. Plot of the rates of fall of the density of the glycerol solutions, g.cm^{-3}/log time, during treatment of Megamouth III (*Megachasma pelagios)* and for the Kimberley reef shark (*Carcharhinus amblyrhynchos*) and hammerhead shark *(Sphyma lewini)* as a function of the glycerol concentration.

surface, whereas the reef shark has a structure like a knight's armor. Each of the reef shark denticles is lozenge- (diamond-) shaped with five vertical ribs, the high points of which are spaced at distances of approximately 40, 70, 70, and 40 μm apart. The upper and lower sections of each denticle are overlapped by sections of three other denticles.

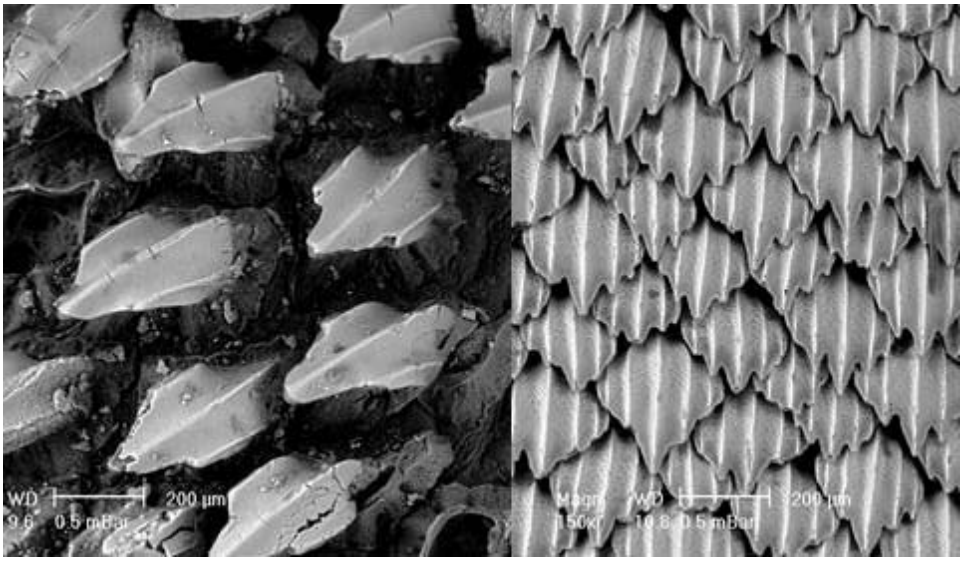

Figure 5. SEM images of the denticles on Megamouth III and the Kimberley reef shark (*Carcharhinus amblyrhynchos* WAM P.28404.001); full width of combined image is 2.14 mm.

This overlapping network allows the denticles to move over each other, and there are small areas of skin surface that are not covered by the plate like structures. The greater surface roughness or rugosity of the megamouth skin might play a very significant role in the response to changing glycerol concentrations. The model of surface active and inactive sites was confirmed by the observed logarithmic kinetics, so it is not unexpected that the rate at which the glycerol is absorbed is dependent on the net available surface area. The relative rates of absorption of glycerol shown in Equations 8 and 9 explain why the complete impregnation process for the megamouth took 2 years rather than the initially estimated 2.5 years.

Rates of glycerol uptake in the tail and head.—Quantification of the impact of relative surface area to volume ratios on the uptake of glycerol was gauged by comparing the rate at which the tail fell during impregnation in the 45% solution. The rate of descent of the different parts of the shark were assessed by using a plastic pole inserted through one of the inspection ports in the lid and marking the depth reached before the pole touched the specimen. The tail fell at a rate of 145 mm/log t (Figure 6), and it was apparent that the tail equilibrated faster than the head, owing to its much larger surface area to volume ratio, because it plateaued many weeks before the head. For the 55% treatment bath, the relative rates of adsorption of the glycerol were monitored by recording the distance of the tail and the head from the inspection ports at the top of the treatment tank. It took an additional 3 months for the tail to reach a plateau and 2 more months before the head reached equilibrium. The slopes of the depth vs. log time were 1,455 mm/log t for the tail and 228 mm/log t for the head; i.e., the rate of head descent was seven times slower than the tail. The implication of this for treatment of other species is that the rate of impregnation of glycerol is very sensitive to the surface to volume ratio of the specimen. The much greater rate of adsorption for the tail at the higher glycerol concentration (55%) is also reflected in the rate of change of the density with increasing glycerol concentration as shown in Figure 6.

Despite assurances from the author that the glycerol solutions presented no risk to the public and to museum staff, an audit required independent chemical analysis of the treatment solution towards the end of the treatment. The results showed that at a glycerol concentration of 56.4% v/v the solution also contained 0.42 wt. % ethanol and 5×10^{-4} wt. % or 0.12 ppm of formaldehyde. The formaldehyde and the ethanol came from residual material leached from the shark and at those concentrations they did not present a health hazard. It is estimated that the megamouth shark released about 100 L of ethanol during the overall treatment. The Chemistry Centre of WA could not quantify the amount of the Kathon biocide (methylisothiazolone), but calculations from the volume added to the tank at the end of the treatment program indicated an average concentration of 100 ppm (West 2012).

Chromameter assessment of color changes during glycerol treatment of sharks.—The pasty white-grey color observed in the small sharks appears to be mainly due to the dehydration of the skin through long immersion in aqueous ethanol, and not due to sunlight bleaching reactions. The reef and hammerhead sharks did not experience prolonged exposure to daylight and their "color" came back with rehydration of the sharkskin through the use of the aqueous glycerol treatment. In the case of the megamouth, the diminution of color in the 70% ethanol was exacerbated by sun-bleaching, which is why an improved shelter that gave protection from the easterly morning and westerly evening light was erected (Human et al. 2012). To test the supposition that the recovery of color was due to hydration by glycerol, a set of nine

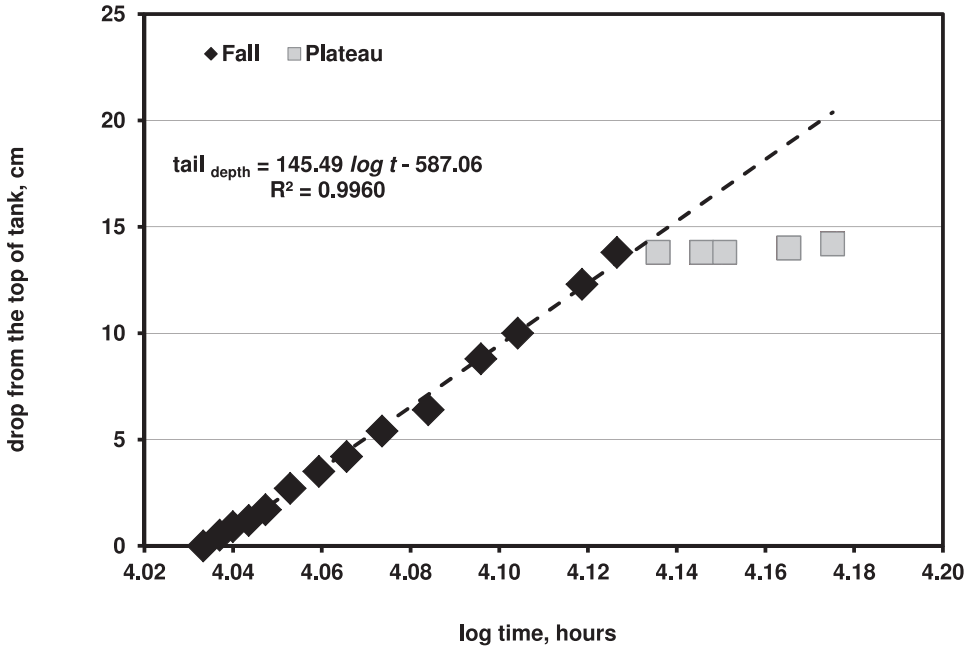

Figure 6. Plot of the fall of the tail in 52 vol.% glycerol showing linear uptake of glycerol as a function of the log of the treatment time until equilibrium was reached after 3 months in that solution.

measurement points were recorded on the specimen when it arrived in Fremantle and was still in an alcoholic impregnated state. Natural scientists and archaeologists tend to use Munsell color charts to characterize specimen colors, because the color books can be conveniently taken into the field and used on board a dive support vessel or at a base camp where the materials are processed. In a controlled laboratory situation, the use of the Commission Internationale de L'Eclairage (CIE) color space system measured by a chromameter is the preferred method, because it minimizes errors associated with faded reference colors and eliminates human error. Color readings of the shark were repeated at the 55% glycerol treatment stage using standard CIELAB color measurements on a Minolta Chromameter. The L*a*b* system uses three dimensional coordinates for every known color with positive L* values as measures of whiteness and negative L* values for shades of black. Positive a* values are in the red spectrum and negative a* values are green, and positive b* values are for yellow and negative values denote shades of blue. Color change is measured according to the distance in the three dimensions between color points and is defined by the formula given in Equation 10 (Table 1).

The measurement of the color of the shark head at two points (1 and 9) within 1 cm of each other had a reproducibility of $\Delta E \pm 0.26$, which means that any greater color difference is significant (Figure 7).

To determine the reproducibility of the measurement points across the shark, a series of color differences were mapped and compared with the untreated reference points. These values had ΔE differences within 2.3 ± 0.7 of each other. This experimental error is acceptable, particularly when noting that the glycerol measurements were done in the treatment tank while holding the shark in a position that would enable access to areas on the pectoral fins and the claspers for measurement. The areas of the shark that were

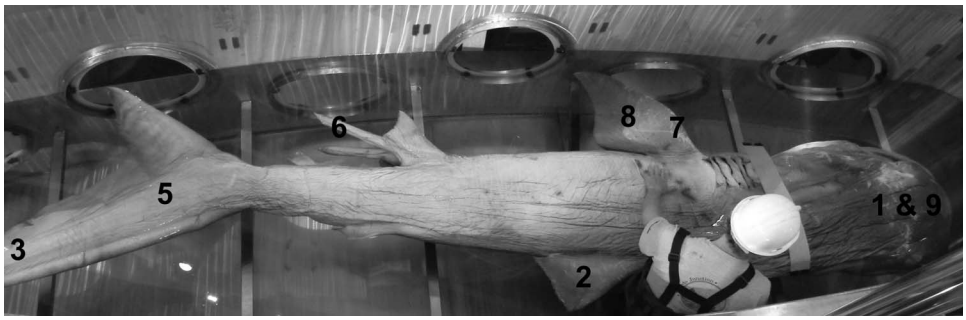

Figure 7. Color measurement points on the megamouth shark after removal from 70% ethanol and after 15 months in glycerol solutions. The color has darkened and the skin has lost the blanched look associated with prolonged ethanol storage.

initially measured were opportunistic and chosen for ease of access to the specimen on its cradle before immersion into the 30% glycerol solution. The locations are described in Table 2. Immediately prior to firing the chromameter trigger, the shark skin was patted dry with a lint-free paper towel. Apart from location 6, where the original point could not be relocated, all the measurements were recorded with relative ease. Glycerol impregnation resulted in a fall of mean L* values by 21 $\pm$ 11, a sign that the specimen became darker after taking up glycerol from solution. The mean increase in the a* value was 10 $\pm$ 2, indicating that the specimen became increasingly red, and the mean increase in b* was 23 $\pm$ 5, which indicated it had become more yellow. The combination of the increased a* and b* values led to the shark appearing to be more brown in hue, which approached the original color when found on the beach 26 years ago. It is likely that the color changes brought about through treatment with aqueous glycerol are due to color saturation of the surface, i.e., the glycerol solutions fully wet the shark skin and allowed its natural color to be viewed. This appears to be the underlying reason for why the "color" of Megamouth III improved, other than in areas of direct sun bleaching.

Conclusion

The trial impregnations of small reef and hammerhead sharks with preservative solutions demonstrated that the gradual changes in formaldehyde-fixed sharks stored in 70% ethanol through sequential baths of glycerol resulted in well-preserved specimens. The forced relocation of the Museum's megamouth specimen necessitated a complex

Table 2. Location of color measurement on the megamouth specimen.

Spot	Location	Measurement
1	Head (midpoint between eyes)	27.5 cm between eyes, 36 cm back from mouth
2	Dorsal fin	27 cm from top, 2 cm in from edge
3	Upper section of the tail	60 cm along tail
4	Tip of tail	13 cm from end, 4 cm down on inside edge
5	Peduncle	Midpoint of tail, 12 cm inside from edge
6	Clasper (top)	18.5 cm from proximal end of proper left clasper, approximately 2 cm in from edge
7	Pectoral fin	49 cm along fin, approximately 2 cm in from edge
8	Bottom fin	49 cm along fin, approximately 16.5 cm in from edge
9	Head (midpoint between eyes)	(As per spot 1 but approximate measurement only)

protocol including the dilution of the storage environment with water to raise the flash point from 16.6°C for the 70% ethanol solution to 42°C for the diluted 16% solution to allow for safe handling of the shark and the disposal of 10,000 L of alcohol. A custom-made 9,000-L stainless steel tank with viewing portholes became the new treatment and exhibition location for the shark in the Fishing Gallery at the Western Australian Maritime Museum. Despite the challenges of conserving a massive shark while on public view and the use of more than 10.5 t of glycerol, the migration experiment was completed only 2 months over the projected duration of 2.5 years. During the first weeks in a higher glycerol concentration above 30%, the density rapidly decreased due to changes in the physical chemistry of the impregnating solution. The increased viscosity of the solutions appeared to change the wettability of the shark skin, leading to increases in the rate of adsorption. The logarithm of the rate of density decrease increased linearly with glycerol concentration.

Experience with the outbreak of yeasts and molds in the initial 30% solution demonstrated the need to use sterile packing materials when transporting sharks from the original storage environment to a new glycerol impregnation facility. The use of low doses of the biocide Kathon is recommended as a method of suppressing biological activity at moderate glycerol concentrations. These issues point out a design defect because the inlet and outlet ports in the tank were not optimized for installation of a circulation and filtration system. It is recommended that the original design be reconfigured to take into account the need for a circulation system, because this is likely to improve the rate of impregnation and lead to shorter treatment times. During an impregnation program it is necessary to have some form of restraint system to keep the specimen immersed in solution, otherwise the stepwise increase in density of the glycerol concentration will cause the shark to float and be exposed to air. Over the 2.5-year treatment program the shark increased in weight by 31% or 115 kg as glycerol was absorbed into the specimen. During this process, significant quantities of aqueous ethanol were removed from the shark and replaced by glycerol, which engorged the shrunken tissue and "restored" the color of the specimen by changing the wettability of the surface.

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