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A MIGRATION MECHANISM FOR TRANSFER OF SHARKS FROM ETHANOL TO AQUEOUS GLYCEROL SOLUTIONS

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ABSTRACT

This paper presents a scoping study on the impregnation of glycerol into formaldehyde-fixed and aqueous-ethanol-preserved sharks. The weight increase and solution density changes were monitored in sequential baths of aqueous glycerol. Positive results included a return of the colour of the specimens, improved flexibility, and removal of alcohol from the body of the specimen and significant improvement of the original profiles and skin textures of the specimens.

RÉSUMÉ

Cet article présente une étude préliminaire sur l'imprégnation du glycérol dans des requins fixés avec du formaldéhyde et conservés dans de l'éthanol aqueux. La hausse du poids et les variations de la densité de la solution ont été surveillées dans une série de bains de glycérol aqueux. Les résultats positifs incluent un retour de la couleur des spécimens, une meilleure flexibilité, l'élimination de l'alcool imbibé dans le corps du spécimen, ainsi qu'une amélioration significative des profils originaux et des textures de peau des spécimens.

RESUMEN

Este artículo presenta un estudio para analizar la impregnación de glicerol en tiburones fijados con formaldehído o bien conservados en soluciones acuosas de etanol. Se hizo un seguimiento del aumento de peso y los cambios de densidad de la solución en baños secuenciales de soluciones acuosas de glicerol. Entre los resultados positivos se incluía la recuperación del color de los especímenes, una mejora de la flexibilidad, la eliminación del alcohol del cuerpo del espécimen y una mejora significativa de los perfiles originales y las texturas de la piel de los especímenes.

INTRODUCTION

The impending demolition of the main building of the Western Australian Museum in Perth necessitated the removal of the 5.2 m, 700 kg megamouth shark from its present storage location in 10,000 litres of 70 vol% ethanol, since vibrations during demolition of the seven-storied reinforced concrete building and falling debris could have irreparably damaged the fibreglass-reinforced plastic exhibition container and its 12 mm-thick laminated glass cover. A second major factor dictating the relocation of the specimen was the impact of revised regulations associated with the Dangerous Goods Codes, which rendered the storage container non-compliant and the museum employee liable for a personal fine of \$250,000 and six months imprisonment. Present regulations exclude the continued use of large volumes of ethanol inside public buildings and even in close proximity to public open spaces, so a new storage environment had to be found. Prior to its insertion into the exhibition tank in 1996, the megamouth shark had been properly washed to remove the excess formaldehyde associated with the initial fixing process (Berra and Hutchins 1990, Burroughs et al. 2006). Previous studies have reported that the mechanism of removal of formaldehyde from small and large sharks showed linear kinetics with the logarithm of washing time (MacLeod 2008). Following a visit to Leiden and extensive discussions between the authors, it was decided that a possible solution to the alcohol problem would be to impregnate Megamouth with aqueous glycerol. This decision was based on more than 100 years of effective storage of preserved human tissue in 65 vol% glycerol following the principles of the Kaiserling method, enabling the conservation in natural colours (Kaiserling 1896, Edwards and Edwards 1959) with no visible bio-deterioration in formaldehyde-fixed tissue samples. With this knowledge, it was decided to trial the impregnation of small sharks with aqueous glycerol to determine the kinetics that will be observed in treating the massive shark in a custom-designed treatment and exhibition tank. In order to assure curatorial staff that the conservators were not about to destroy the precious specimen, experiments with two small sharks were conducted as a trial and this paper reports on the successful outcome of the experiment. The CEO of the Western Australian Museum has subsequently commissioned the building of a massive stainless steel tank lined with portholes at child and adult viewing positions, in which Megamouth is now undergoing treatment in the Maritime Museum in Fremantle while on public exhibition.

EXPERIMENTAL

A small hammerhead shark from near North West Cape, *Sphyma lewini* 27232.002, and a larger reef shark from the Kimberley region of Western Australia, *Carcharhinus amblyrhynchos* 28404.001, weighed 455 g and 2349 g respectively when they were removed from their nominal 70% ethanol storage environment. As part of their normal preparation as museum collection specimens, they had been gutted and injected with formaldehyde and left to soak in the 4 wt% formaldehyde for three days before being rinsed under running water for several hours and then placed in the storage drum. When recovered from the wide-necked polyethylene black drum, the sharks were fixed with bends in them that replicated the radius of the storage drums. The specimens were very stiff and the surfaces were highly wrinkled owing to shrinkage during storage. Unfortunately there were not photographs of the sharks at the time of their recovery, but records showed that they had been in storage for more than 12 years (Figure 1).



Figure 1a

Image of the sharks before treatment in glycerol solutions

Figure 1b

Sharks after 296 days of treatment in a final concentration of 65 vol% glycerol

The specimens were photographed, weighed, measured and placed in a 20 litre rectangular polyethylene treatment tub containing a 33% glycerol solution and were monitored for weight at approximately weekly intervals. In order to obtain reproducible data, it was necessary to “towel dry” the sharks from excess glycerol solution on the surface of the skin, which tended to retain the impregnating solution. The density of the soaking solution was monitored using an Anton Paar portable digital densitometer which typically had a reproducibility of $\pm 0.0002 \text{ g}\cdot\text{cm}^{-3}$. During the first two weeks of treatment, the density fell in a regular fashion until the specimens were manipulated and the cut abdomen area was “massaged” to remove accumulated solution. This caused a drop in density, after which the solution continued to fall in density at the same rate until the weight had plateaued after approximately two months. As the density fell, there was an increasingly strong smell of alcohol coming from the tub and this is consistent with the incoming glycerol solutions displacing the ethanol preservative. In order to assess the amount of exchange of bodily fluids, it was assumed that the fall in density was due to the mobilisation of ethanol into the aqueous glycerol solution and that the two liquids combined in a linear fashion with regards to their colligative properties. The plot of the rate of change of density in the initial phases of the impregnation had the best fit with the logarithm of immersion time (Figure 2). The estimated volume of their former ethanol storage solution released during the treatment was calculated using an initial density of $0.9162 \text{ g}\cdot\text{cm}^{-3}$, a glycerol solution volume of 27 litres with an initial density of the glycerol solution of $1.0937 \text{ g}\cdot\text{cm}^{-3}$, and this value came to 1700 ml of the ethanol storage solution being released from within the body cavities and from their muscles.

If the assumption that the densities of the impregnation liquor and the outgoing solution are additive (i.e., that the ethanol-water mixtures do not chemically interact with the glycerol-water mixtures) is correct, it is apparent from the increased weight of the sharks that there is a larger

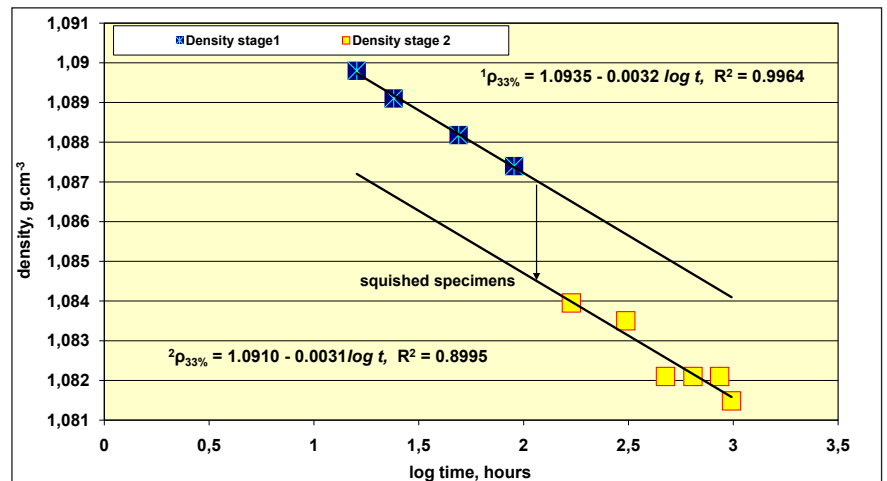


Figure 2

Plot of solution density versus square root of immersion time for the first two months

weight gain than that anticipated on the simple incorporation of the same volume of the glycerol solution replacing the alcohol. The mean ratio of the real weight increase over the calculated weight gain is 1.36 ± 0.16 , which indicates that the glycerol has a real affinity for the tissue and that it is likely that some form of chemical bonding or physical adsorption of the polyalcohol is taking place in the body of the sharks. During this initial impregnation period, it was noticed that the colour of the sharks had improved from being a washed out dull grey surface to being rich in brown and yellow hues as the glycerol solutions had effectively colour-saturated the surface of the alcohol-preserved sharks. It was also noted that the amount of shrinkage in the skin of the fish appeared to be diminishing (i.e., the fish were becoming plumper and the apparent selective uptake of glycerol by the sharks would be the underlying reason behind the change in physical dimension).

In the initial few months, the weights of the sharks were plotted as a function of linear time, square root of time and log time to see what format best suited the experimental results. The highest correlation coefficients (R^2) were found for plots of weight against the log of time. Data from the subsequent increasing glycerol concentration baths were also plotted against a range of time functions, but all the data best supported a linear relationship with the log of the total elapsed time. The rate of weight increase as a function of log time in the first treatment bath in the 33 vol% glycerol is seen in Figure 3. Inspection of the data in the graph shows that the hammerhead shark reaches its weight plateau after nine days ($\log t_{\text{hours}}, 2.34$), while it took the reef shark nearly 33 days ($\log t_{\text{hours}}, 2.91$) or 3.5 times the time to reach a steady weight. The reason for this is due to the fundamental differences in the surface area to volume ratio of the two sharks, for the hammerhead has a surface area to volume ratio of 540 cm^{-1} , while the reef shark has a value of 330 cm^{-1} (Figure 1a). The hammerhead shark is characterized by a thin body mass with a large surface area, whereas the reef shark has a much greater amount of flesh to its shape and form.

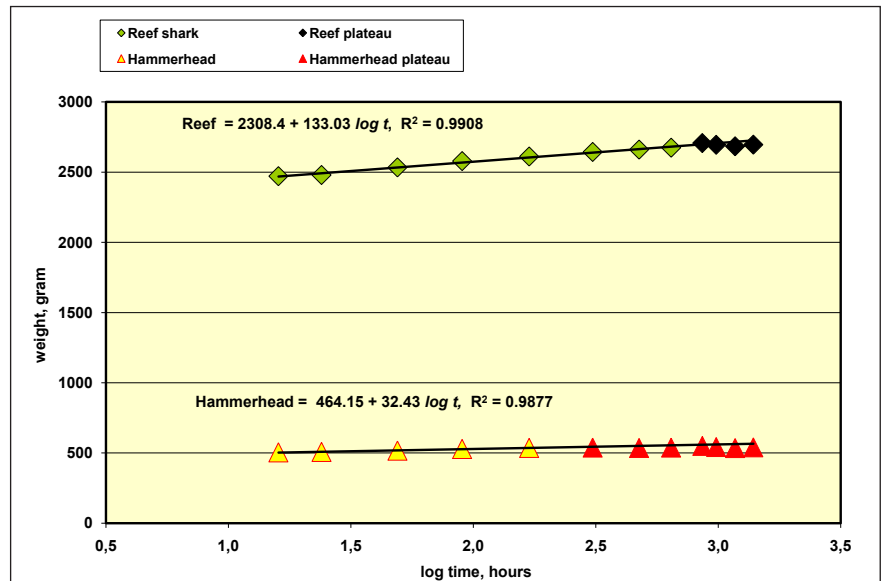


Figure 3

Plot of the weight of the reef and hammerhead sharks in 33 vol% glycerol

When the slopes of the weight vs. log time plots are compared, the reef shark increases its weight at 4.04 ± 0.54 faster than the hammerhead specimen. Estimates of the surface area of the sharks were made using standard outline tracing and the ratio of the reef shark to the hammerhead was 3.2, which indicates that the surface area of the sharks is a controlling factor in the uptake of the glycerol from solution. This method of assessing relative surface areas assumes that both specimens have the same thickness. Since the reef shark is thicker than the hammerhead shark, this method will underestimate its surface area and so the real ratio of the two sharks is going to be greater than the calculated value. Since the smaller shark had a higher surface to volume ratio, it achieved the plateau weight some weeks ahead of the larger shark. When each animal had attained a steady weight for 3–4 weeks, the concentration of the bath was increased in accordance with the standard procedure for stepwise increases in concentration of impregnating solution to minimise stress on the tissues of the specimen.

Once impregnation had begun, the colour of the sharks changed to reflect the saturated colour that the original specimens had before being immersed in the ethanol storage solutions for several years. Initially, the sharks were “rock hard” and had minimal flexibility, but after several months in the glycerol baths the gills became mobile and the degree of flexion of the tails and body significantly increased with the concentration of glycerol. The amount of skin wrinkles associated with dehydration in the ethanol decreased with the increasing amount of glycerol in solution as the specimens “plumped up”. One of the collapsed eyes in the hammerhead shark became fully turgid and looked as if the specimen had been freshly killed. A possible explanation for this phenomenon can be found in the properties of glycerol and the experience built up during 100 years of maintaining glycerol-preserved collections. Glycerol is a humectant, attracts water and promotes cell hydration. Furthermore, it has an extremely low vapour pressure which prevents loss of glycerol from the specimen

tissue due to evaporation and/or diffusion. In fact, in case of fluid loss by evaporation, it will lead to an increase of the alcohol concentration because only water is lost, leaving the antiseptic and (re)-hydrative properties of glycerol intact. Topping up with water is sufficient to restore the concentration without any risk of shrinkage damage to the specimen tissue. Because the vapour pressure of ethanol is significantly higher than that of water, fluid evaporation in ethanol-water solutions leads to a decrease in alcohol concentration and less antiseptic strength. A significantly higher concentration of ethanol has to be added to compensate for the concentration loss, which can lead to instant dehydration of the specimen tissue. Topping up jars with glycerol at the Leiden Museum of Anatomy overcame collapsed cavities of partially exposed specimens, as the solution restored their original shape and form within a few days of being re-immersed. With regard to cell hydration, glycerol has so-called regenerative properties.

It is likely that the tortuosity of the diffusion path through the shark skin may be the underlying cause of the linear dependence on the log of the immersion time, be it for either removal of formaldehyde or impregnation with glycerol. Shark skin is composed of a matrix of tooth-like structures called denticles or placoid scales. Each denticle has an outer enamel layer which covers a dentine and central pulp cavity. As sharks grow, the denticles remain the same size and become a characteristic of the species, as seen in Figure 4, which shows the surface of the reef shark (Figure 4a) and the hammerhead shark (Figure 4b) – the surfaces of the two sharks are clearly different, but the gross morphological features are similar (Naresh et al. 1997). The dark blue coloured areas appear to be pigment cells located under the skin layer.

RESPONSE OF THE SHARKS TO INCREASED CONCENTRATION OF GLYCEROL

The concentration of glycerol in the baths was increased in a stepwise fashion from 33, 45, 54 to 65 vol% and the sharks were monitored for their weight and kept in the solution baths until the weight had stabilised or plateaued. In order to get reproducible results, each shark had to be drained of excess solution for two minutes before being dried with a paper towel and weighed on a digital balance. The 45 vol% solution was found to have the greatest error associated with the line of best fit ($\pm 18\%$) and this was due to poor measurement technique, but subsequent solution baths had typical errors of $\pm 13\%$ in their slopes. When the rate of weight increase for each shark was plotted as a function of the concentration of the glycerol in the bath, it was found that the rates of increase were directly proportional to the concentration of glycerol in the solution. The equations for the reef shark and the hammerhead shark were as follows: $R_{\text{reef}} = 34.0 [\text{wt}\%] - 1240$, while the rate of weight gain for the hammerhead shark was $R_{\text{hammerhead}} = 8.3 [\text{wt}\%] - 307$, where the rates are in units of gram per log t and the R^2 values for the linear regressions were 0.9895 and 0.9953 respectively. In simple terms, this means that the rate of increase

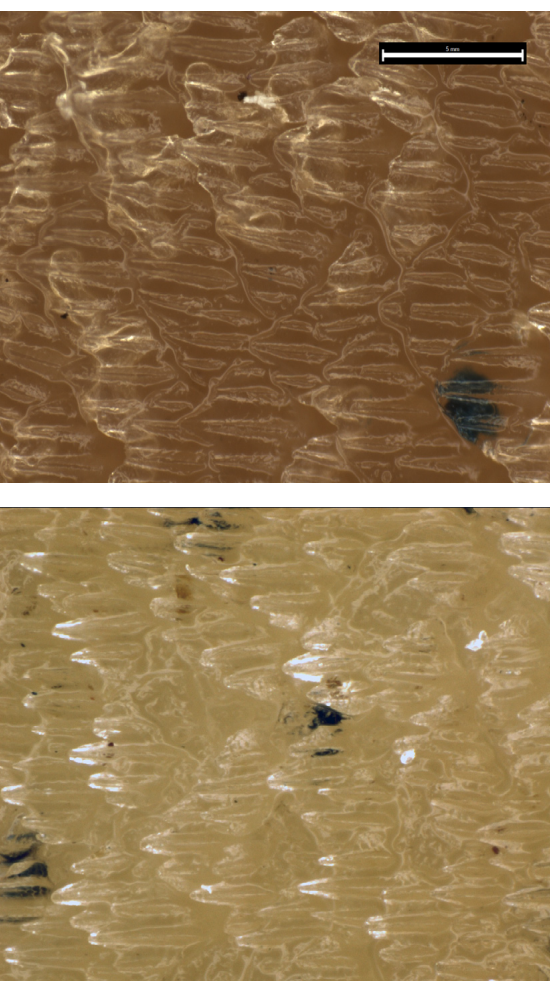


Figure 4a
Reef shark skin with 0.5 mm scale bar
showing denticles and pigmentation cells

Figure 4b
Hammerhead shark skin at same
magnification showing pigmentation cells

of the sharks' weight is 4.04 times faster for the reef shark than for the hammerhead shark. If this ratio is divided by the density of glycerol at 1.26 g.cm^{-3} , the ratio falls to 3.33, which compares favourably with the ratio of the surface areas of 3.21. The initial relative rates of increasing weight of the sharks in the 33vol% solution may have been artificially higher due to the impact of the rapid release of the previous aqueous ethanol storage solution. When variations in the slopes are taken into account, the mean ratio of the slopes is 4.0 ± 0.5 (against wt% glycerol), which strongly supports that the transport mechanism for the adsorption of glycerol into the specimens is related to their surface area.

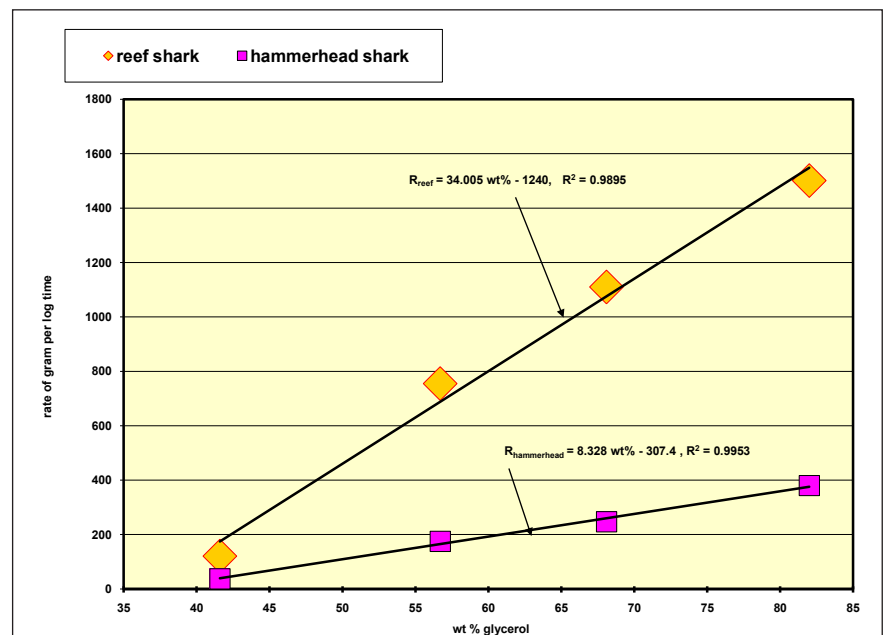


Figure 5
Rate of weight increase for the reef and hammerhead shark as a function of glycerol concentration

It had previously been noted that during the impregnation of the sharks with glycerol, the colour of the specimens improved and the amount of skin fold shrinkage diminished. Another measurable indicator of how the glycerol had mobilised the tissue was found in the measure of flexibility of the sharks. Initially, the amount of movement that the curator and conservator were able to extract from the sharks was very limited, with one holding down the head and the other bending the tail backwards towards being in line with the spine and not in a fixed radial curve dictated by the shape of the previous storage container. Owing to its greater surface area to volume ratio, the hammerhead shark showed rapid improvement in flexibility and the gills on both sharks could be readily moved back and forward. By the end of the treatment, it was possible to open the jaws of the sharks, whereas they had previously been “frozen solid” with the combination of formaldehyde and the alcohol solution. A measure of the improved flexibility of the specimens is seen in Figure 6, which plots the flexion of the sharks as a function of the glycerol concentration. The flex fraction is the ratio of the perpendicular distance of the tail under flexion to the length of the shark from the tip of the head to the rise of the tail

fin. The reef shark showed continuing improvement in flexibility with increasing glycerol concentration. The flex fraction for the hammerhead shark reached the maximum value of 1.0 at a glycerol concentration 54 vol%, while the reef shark continued to improve beyond the initial maximum value as the bend in the shark relaxed, which allowed for greater apparent mobility and hence a flex fraction greater than unity.

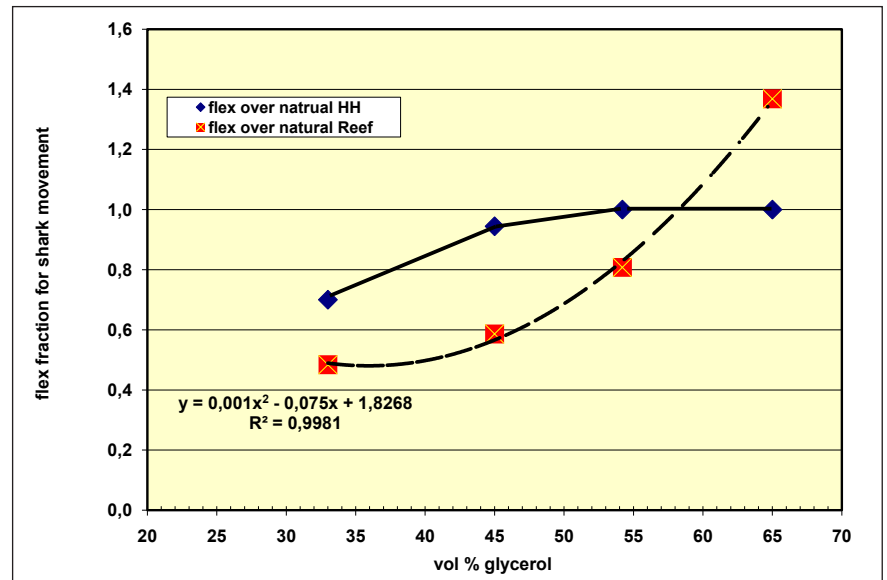


Figure 6

Plot of the changing flexibility of the hammerhead and reef shark with glycerol concentration

It is likely that residual formaldehyde coming from the sharks during the initial impregnation with the glycerol was responsible for preventing any outbreaks of mould and fungi in the 33 vol% bath, as there was a clearly discernable smell of formaldehyde, as well as that of the ethyl alcohol during the first two months of the treatment in the lowest concentration of glycerol.

CONCLUSION

The initial results from the experiments with the small sharks has awakened the interest of natural science curators, who have requested that the work be extended to trial terrestrial-based specimens such as snakes, lizards and a number of fur-covered marsupials. The trial with the sharks has convinced the fish curator and senior museum management to provide funds for the construction of a large stainless steel storage and treatment container with a number of glass porthole viewing areas that will permit the exhibition of Megamouth III during its impregnation in various glycerol solutions up to the final concentration of 65 volume percent. Data from the first six weeks of impregnation of the megamouth specimen show that the rate of decrease in the density of the 30% glycerol solution is -0.030 g.cm^{-3} , which is the same rate as observed with the small sharks in a similar concentration of glycerol. The tank has been designed to facilitate periodic weighing of the massive shark through a series of transponders attached through an integrated pulley support system, in order to assess the success

of the impregnation and to monitor the rate at which the glycerol is being absorbed into Megamouth.

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