

Washing formaldehyde from fixed spirit specimens: a mechanism for the preservation of *Megamouth III*

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Abstract

The discovery in August 1988 of a rare and massive deep-sea dwelling shark, *megachasma pelagios*, or megamouth presented the Western Australian Museum with a unique opportunity to study and exhibit the massive 5.2 m, 700 kg shark. In order to ensure that the formalin-preserved specimen was safe to handle and install in a custom-made polyester and fibreglass exhibition tank, it was essential to establish how long it would take to reduce the amount of formaldehyde in the shark to a safe level. Tests on a small shark established that formaldehyde diffuses from the preserved specimen in accordance with the logarithm of the washing time. The same mechanism controlled the release of formaldehyde on *Megamouth III* and this facilitated a compliant installation procedure. Hazards associated with the exhibition of more than 10,000 L of 70% aqueous ethanol and methods for purification of old storage solutions are reported. The extended washing resulted in a safe amount of formaldehyde being found in the storage solution even after 13 years on exhibition. Conservation challenges and proposed new display methods are also discussed.

Keywords: formaldehyde extraction, megamouth III, washing mechanism, sharks

Introduction

In August 1988 inspector Derek Blackman from the Western Australian Fisheries Department alerted the museum to the appearance of a 'large creature on the beach, I've never seen anything like it before—it appears to be a cross between a shark and a whale'. Ichthyology curator Barry Hutchins immediately organised to visit the site at Mandurah, 50 kilometres south of Perth. The large sharklike animal lay forlornly on the beach on the northern side of the ocean entrance to Mandurah inlet, with its long grey tail protruding from the surrounding crowd. The creature had the fins and gill slits normally expected of a shark, but a short, almost puglike snout that gave it the appearance of a baby whale. Other notable features were its wide mouth, designed for feeding on plankton, a bathtub-shaped lower jaw for gulping in large volumes of water, and a silvery to silvery-black lining to its mouth. The creature had rows of minute teeth presumably used to prevent larger food items like jellyfish from falling out of its mouth. It was a fine example of the megamouth shark, scientifically

known as *megachasma pelagios*. *Megamouth III* had apparently been sighted by surfboard riders the previous day. As it seemed intent on beaching itself, the surfers had tried to coax it into deeper water. Their efforts were obviously unsuccessful as it was found the next morning stranded on the beach and, although still alive, died a short while later (Hutchins 1992). Owing to the rarity of the specimens of this species and to assist researchers in identifying the specimens a Roman numeral suffix is added to indicate the chronological order in which the specimens were reported and identified. This form of nomenclature is extremely rare.

The Western Australian Museum specimen was recovered with the assistance of the Mandurah Shire Council, which quickly provided a frontend loader, a truck, and the necessary manpower. A ditch was dug adjacent to the shark, lined with concrete reinforcing mesh, upon which the megamouth was then rolled. Slings were placed around the wire mesh, attached to the scoop of the frontend loader, and the 700-kilogram shark was then lifted onto the back of the truck for the trip to a deep freezer in Perth. Public interest

was sufficiently high to warrant a special showing of *Megamouth III* that weekend. The frozen specimen was placed on a flat-bed trailer and viewed by almost 4,000 people over several hours in a congested museum car park. It was subsequently preserved in formalin, awaiting display.

Biology of *Megachasma Pelagios*

The first specimen, *Megamouth I*, which defined the species, was 4.5-metres long and was inadvertently collected off Hawaii from a depth of 165 m when it became entangled in a parachute sea anchor being used by a United States Navy research vessel. The small sharp teeth had snagged in the parachute's fabric. The scientific description, published in 1983, was so unusual that it was placed in a new family of sharks, the *megachasmidae* (anonymous, 1991). *Megamouth II* was the same length as the first recorded specimen and was recovered in November 1984 from a depth of only 38 metres and is now exhibited at the Natural History Museum, Los Angeles.

The feeding habits of the megamouth were established in 1990 by attaching an ultrasonic transmitter and depth sensor to *Megamouth VI*. The logged data showed that the shark was a vertical migratory feeder, spending the daylight hours at a depth of about 170 m before ascending at dusk to around 12 m below the surface, where it remained throughout the night. This vertical migration is obviously triggered by light changes, but may also be a response to the movement of the planktonic animals upon which it feeds. The euphausiid shrimps that make up part of megamouth's diet are known to migrate daily from deep waters to the surface (Lavenberg and Seigel 1985).

The museum's *Megamouth III* is a male specimen with its characteristic two claspers which are used for delivery of spermatophores during mating; these often result in surface damage as the sharks hold onto each other with their mouths and this was apparent in the museum specimen. Other wounds found on the body

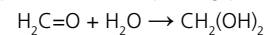


Figure 1. *Megamouth III*, on the beach at Mandurah. The wound above the gills was probably made by the Cookiecutter Shark, a small mid-water oceanic species that preys on large, slow-swimming marine animals. Photo: Barry Hutchins

were circular and craterlike, and they are believed to have been caused by the cookiecutter shark (*isistius rasilensis*), a small mid-water oceanic animal that attaches to its prey with the help of suckorial lips and a codified pharynx. Then, with a twist of its body, the cookiecutter easily removes a conical lug of flesh with its large sawlike teeth. The slow-swimming megamouth would be easy prey for the active cookiecutter shark.

Preservation of *Megamouth III* with formaldehyde

The use of formaldehyde solutions for preservation of anatomical and tissue specimens has been well documented since the seminal works by Ferdinand Blum in 1893. Within three years a further 50 references to the reaction of formaldehyde with organic tissues were reported (Rumph & Williams 1986). Formaldehyde (HCHO or H₂CO) is an effective preservative of organic tissues as this strong nucleophilic reagent initially reacts with primary amines, such as lysine, and thiols, such as cysteine and purine bases of nucleic acids. These reactions reach equilibrium in 1–2 days which renders the tissue much less prone to biological decay. Formaldehyde dissolves in water to form the highly reactive methylene glycol:



This chemical is characterised by a rapid diffusion into tissue. Contrary to most diffusion-controlled reactions, the rate of diffusion of formaldehyde is inversely proportional to the temperature of the specimen. A recent review has shown that washing the test samples results in the labile adducts of formaldehyde with some of the nucleic acids being readily removed. However, the secondary reaction involves cross-linking with proteinaceous materials which results in covalent and irreversible bonding to the tissue. These reactions take roughly one month to equilibrate (Helander 1994). A good review of the reactions involving formaldehyde and organic tissue is given in by Kiernan (2005).

Normally treatment of large aquatic zoological specimens, including massive grouper and sharks, consists of photographic documentation followed by preservation of the jaws or the skeletons of the animals once they have been dissected. Following removal of the gross flesh of the fish the remaining material is buried in sand mounds for periods of 18-24 months to complete the defleshing process before being washed and re-articulated using stainless steel wires to hold the skeletons together. This regime has been developed for a mixture of pragmatic, environmental and human safety reasons including the desire to reduce the amounts of formaldehyde used in specimen preparation, and the subsequent disposal of large

amounts of contaminated wash solutions. Apart from the cost of the ethanol preserving solutions there are significant costs associated with the construction of suitable storage vessels, since they must comply with the Dangerous Goods Code of Australia.

Owing to the rarity of *Megamouth III* and its cartilaginous nature it was decided to directly inject the body cavity with 20 L of 12% by weight (wt%) formaldehyde using 130 mm needles, 2 mm diameter. A massive 1300 mm needle, 10 mm in diameter, was used to pump about 40 litres of 20 wt% formaldehyde deep into the body and body cavity before it was placed in a treatment tank. Details of the challenges of monitoring the amount of formaldehyde in the treatment solution are reported by Berra and Hutchins (1990).

In order to assist the highly mobile formaldehyde to diffuse through the tough shark skin, a series of incisions were made in the soft underbelly of the animal before it was placed in the formaldehyde solution for eight weeks in the treatment tank (Fox et al. 1985) at a nominal concentration of 4 wt% formaldehyde. The 'tank' consisted of a large pit, 6.5 m long, 3 m wide and approximately 1.5 m deep, which was padded with cardboard and then lined with a double layer of 0.2 mm plastic swimming-pool liner. The 'tank' was constructed at the museum's off-site collection store in East Fremantle. The two layers of the pool liner ensured that the formaldehyde did not leach into the active groundwater table. Evaporation was minimised by placing another sheet of plastic over the top of the tank (Berra and Hutchins 1990). The water table at the site chosen for construction of the tank was more than 8 m below the base of the tank, and the goethite-rich yellow sand and the limestone rich ground would have trapped any material that accidentally spilled from the containment system. The local council was aware of the museum activities and the site managers were aware that it was the museum's responsibility to ensure that full site remediation, if needed, was at our cost. In the event there was no detected contamination at the end of the project.

Modelling removal of formaldehyde

Discussions with curatorial staff indicated that the normal amount of time given to remove formaldehyde varied between a few minutes for small fish to one to two days of rinsing for very large specimens. Since the author's experience with desalination of artefacts from shipwrecks had demonstrated

that the vast majority of processes are controlled by diffusion processes (North & Pearson 1978, MacLeod 1987, MacLeod and Davies 1987), it was decided to establish the kinetics for removal of formaldehyde before beginning the final stages of treating *Megamouth III*. Suspicions about the inadequacy of previous washing regimes for removing formaldehyde were confirmed through analysis of a number of shark and large fish storage solutions. The results of the analyses of four 40 L tubs containing sharks are shown in Table 1.

The very high concentration of formaldehyde found in three of the four storage containers demonstrated that the traditional approach for removing formaldehyde was inadequate and would ultimately result in storage solutions that were inherently toxic and could not be exhibited with any degree of responsibility. Formaldehyde levels of less than or equal to 100 mg per litre are generally regarded as being acceptable. The results of the analyses alarmed the ichthyology department staff and led them to revise their specimen preparation and handling methods for their spirit (75% ethanol) collections. The risks of exposure of museum staff working with wet specimens to varying levels of ethanol, methanol and formaldehyde has recently been reviewed (Burroughs et al. 2006). In order to establish the mechanism for controlling the release of formaldehyde from preserved sharks, the specimen stored in the worst solution environment was chosen to act as a model for developing appropriate treatment methodologies.

Washing the shark *Hemigaleus microstoma*

Owing to the high solubility of formaldehyde in water (370 g/L) and because of the safety concerns associated with other solvent systems, the shark was washed in deionised water. The 4.4 kg shark from drum P. 26190-002 was placed in an 80 L washing tub,

Table 1. Fish specimens in 70% ethanol storage solutions with formaldehyde concentration

Tag number	Species	Solution date	[HCHO] mg/L
P 26087-021	<i>Cephalopholis aurantia</i> , Golden Rock cod, Family Serranidae	22/05/1978	210
P 30320-047	<i>Cheilinus chlorurus</i> , Floral Maori Wrasse, Family Labridae	26/08/1991	780
P 83-001	<i>Coris auricularis</i> , Western King Wrasse, Family Labridae	1913	1170
P. 26190-002	<i>Hemigaleus microstoma</i> , Weasel Shark, Family Hemigaleidae, 759 mm overall length, 4.4 kg	Not known	1250

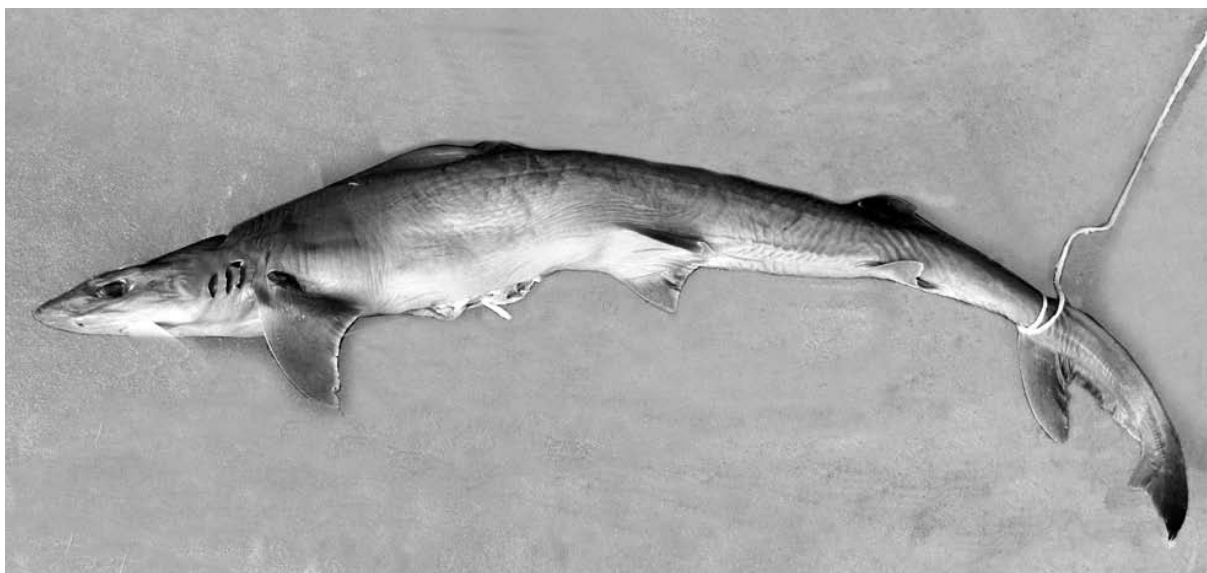


Figure 2 (above). Shark used for the test washing experiment, *Hemigaleus microstoma*. Figure 3 (right). Plot of formaldehyde concentration vs. log of the washing time in hours

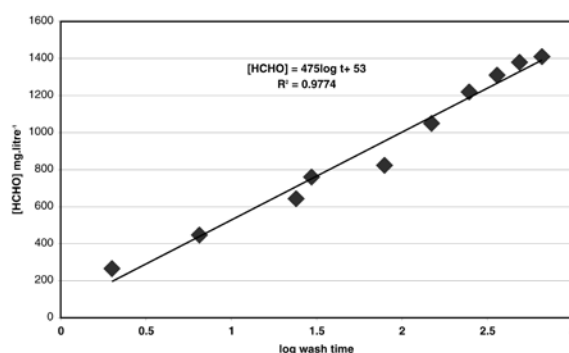
with a sealed lid to minimise any evaporative loss of the formaldehyde and the treatment was followed for 27 days until the formaldehyde levels appeared to have reached a plateau.

All of the formaldehyde concentrations were determined by reacting the sample with 2,4 dinitrophenylhydrazine and then analysing the derivative via high-performance liquid chromatography at the Chemistry Centre of Western Australia. A total of 13 readings of the formaldehyde concentration were made and the results are illustrated graphically in figure 3. Analysis of the washing data showed that the formaldehyde concentration increased linearly with the logarithm of the washing time, expressed as hours, according to the equation given below:

$$[\text{HCHO}]_{\text{Hemigaleus microstoma}} = 475 \log t + 53 \quad 1$$

The fit of the data was remarkably good with an R^2 (square of the correlation coefficient of the linear regression) of 0.9746 for 10 data sets with standard errors of ± 51 in the intercept and ± 26 in the slope of the $\log t$ plot, as shown in Figure 3. The intercept of equation 1 is essentially zero, taking the standard error into account, which means that there was little surface contamination of the shark with adventitious formaldehyde and that the preservative was being removed from the animal itself.

It is noted that the washing experiment involved the use of a shark that had been stored in a 70% ethanol/2% methanol solution for several years, which presumably had equilibrated at a formaldehyde concentration of 1250 mg/litre (see Table 1). The most important aspect of the experiment was that



the release of formaldehyde (during washing) was not linear but instead a logarithmic function of time. Recent experience with desalination of ceramics after chemical intervention using phosphoric acid solutions to remove concretions has demonstrated that the chloride release rate was linearly dependent on the logarithm of the washing period (MacLeod 2008). A paper by Rumph and Williams compared the efficiency of water and ethanol at removing formaldehyde from immersion fixed muscle tissues. Increasing the ethanol concentration from 20% to 40% resulted in a linear increase in the extraction of formaldehyde, but the difference between the best extraction, obtained in 40% ethanol, was only 0.2% better than water (Rumph and Williams, 1986), so it was concluded that there was little advantage to be had by washing in either isopropanol, ethyleneglycol or aqueous ethanol compared with water. The dependence of the formaldehyde kinetics on the log of the washing time is consistent with a chemical reaction, rather than diffusion, which is the factor controlling the release of the formaldehyde from the preserved specimen.

During the 27 days of the experiment, the 80 litres of washing solution reached a maximum concentration of formaldehyde of 1880 mg/litre which equates to a total of 150.40 g being extracted. Given that the shark weighed 4.40 kg, the concentration of formaldehyde

removed came to 3.58 wt% which is very close to the initial 4 wt% formaldehyde solution that was used to fix the specimen. The large amount of formaldehyde removed provides clear evidence that the previous washing regime was totally inadequate at removing the toxic preservation medium. Further research is required to determine how much residual formaldehyde is needed to ensure that the specimen does not undergo biological decay in the aqueous ethanol storage environment. Once such levels are determined over a wide variety of species it may be possible to revise washing and storage regimes that will lead to less chance of exposure of staff to the toxic effects of formaldehyde.

Washing of *Megamouth III*

Given that the shark-washing experiment had shown that a large amount of formaldehyde was extracted with prolonged washing, it was deemed to be essential to conduct routine monitoring of the concentration of formaldehyde in the wash solution of *Megamouth III*. The specimen had been fixed in a 16,400 L solution of 4 wt% formaldehyde, and the massive amount of used formaldehyde had to be trucked away for disposal, which was effected by burning the waste in a concrete manufacturing kiln. After removal of the fixing solution, the tank was filled with tap water and the specimen was washed from 30 November 1994 until 3 January 1995. The shark was removed at this relatively early washing time owing to concerns that removal of 'too much' of the formaldehyde from the specimen might make it less stable in the ultimate preserving solution of 70% ethanol. Sampling of the wash solutions contained in the massive tank was difficult; the method involved the author walking out across the treatment tank on a mild steel beam, leaning over and stirring the solution with a length of timber before taking the sample.

Owing to the imperfect nature of mixing of the wash solutions before each sample was taken, the data from the washing of *Megamouth III* is associated with larger errors than the washing of the small shark. It is likely that the increasing ambient temperatures combined with some leakage may have contributed to the experimental errors. Despite the difficulties, the results of the early stages of the washing process for *Megamouth III* showed that it followed a similar linear dependence of the formaldehyde concentration on the logarithm of the wash time (hours), in accordance with Equation 2, $[\text{HCHO}]_{\text{Megamouth III}} = 205 \log t - 171$ 2

The R^2 for the linear regression relationship shown in Equation 2 was 0.9493, and the slope of the log time plot had an error of ± 47 , and the intercept error was ± 91 , which is consistent with the scatter

of the data and the difficulties of sampling. Owing to the logarithmic nature of the washing equations the intercept value occurs when $\log t$ is zero which is at one hour. Equation 1 for the small shark predicts a zero formaldehyde concentration at two hours and Equation 2 predicts almost seven hours for *Megamouth III* which is consistent with the much greater solution volume associated with washing the giant-sized specimen.

The final concentration of formaldehyde in the wash solution from *Megamouth III* was 430 mg/L in an estimated solution volume of 16,400 L. This concentration amounts to 7052 g of formaldehyde or approximately 1.10 % had been removed in just over one month of washing. Prior to going on exhibition the shark was opened under the abdomen and the viscera were removed. The amount of tissue removed amounted to approximately 60 kg of liver and related intestines. If the liver had been kept it was likely that the tank solution would become occluded with globules of fat and oils. The final weight of the specimen was approximately 640 kg.

It is interesting to note that despite the huge differences in the size of the specimens, the sharks had apparently similar formaldehyde release rates. The similarities in release rates may be due to the similar physiology of the skin and could also be affected by the very similar mass to wash volumes ratios, which for the small shark was 18:1 and for *Megamouth III* was 23:1. Without a detailed knowledge of the surface areas of the specimens, however, it is not possible to get more information about the physical nature of the washing reactions and the relative release rates of the sharks.

Exhibition of *Megamouth III*

In order for the specimen to be safely viewed by the public at the main Perth site of the Western Australian Museum, a custom-made fibreglass tank was provided by a corporate sponsor and appropriate support facilities, including fibre-optic lighting, a shade cover and an ethanol solution treatment plant were constructed adjacent to the tank, which was located between the Old Gaol and the main exhibition and administration building in Francis Street.

Testing of old yellow oily 70% ethanol solutions from other shark storage tubs by the Chemistry Centre of Western Australia had shown that placing cotton wool swabs in a detachable bulbous PVC pipe fitting that led into the main filter was sufficient to capture any globules of fat that are characteristically released over time from large marine specimens. Beneath this removable 'oil filter' was 25kg of activated carbon in the form of carbonised copra, as supplied to the gold mining industry, which has been shown to remove the yellow-coloured materials



Figure 4. Preparation of tank for specimen support structure and installation of safety glass cover.

which develop over time in large sharks and fish stored in aqueous ethanol solutions.

The massive shark was placed on custom-designed 316-stainless steel supports that allowed it to be displayed to the greatest effect, showing both claspers and the open mouth when viewed through the glass top. Although 316-stainless steel is expensive, its high chromium content (16–18.5%), nickel (10–14%) and molybdenum (2–3%) ensures that it will not corrode in the storage environment. The storage tank was approximately $6.2 \times 1.5 \times 1.1$ m and was connected with polyvinylchloride (sewer quality) pipe to the ethanol storage and treatment plant located adjacent to the display in a purpose built brick building. Once *Megamouth III* had been properly supported and several thousand litres of water had been removed from the exhibition tank, the massive safety glass cover was sealed onto the top of the tank structure with Dow Corning 795 Building Sealant, which is resistant to swelling from ethanol vapours. The tank was thereby prepared for the delivery of 7,000 L of pure ethanol. A sufficient volume of water was left in the tank to ensure that the specimen was uniformly supported, since complete removal of all the fluid would have resulted in pressure points on the specimen that may have caused considerable damage. Another reason pure ethanol was used to make up the 70% solution in the tank was that it was 25% cheaper to use the pure alcohol rather than having had to pay the industrial chemists to mix it to the specified level.

The ethanol was delivered to the museum loading bay at the Francis Street site late in the evening of 25 January 1995, since safety precautions demanded that pumping such a large volume of ethanol at a public facility meant that the works had to be conducted out of hours. Although there was some element of risk in moving the specimen directly from water into 70%

ethanol, the rate of change was minimised by the fact that the pumps kept the incoming ethanol fully mixed with the existing volume of water. Sequential increases in the ethanol concentrations were not practical owing to the volumes of solution, the regulations that prevented sending such solutions down the sewer, and the additional cost. If 20% incremental steps had been taken, this would have used an additional 12,000 L of ethanol at a cost of \$30,000, and the charges by

Cleanaway contractors for the waste solutions would have amounted to an additional \$15,000. The advice from the ichthyology curator was that the specimen would not be damaged by the single step transition of going from water to 70% ethanol over the space of five hours. Since oxygen has a much higher solubility in ethanol than in aqueous ethanol (Shchukarev and Tolmacheva 1968), the venting mixture coming from the top of the tank was a mixed oxygen-ethanol vapour which created a pleasant sensation for Jamie Stuart and the author as they supervised the delivery operation. Prior to gaining endorsement from museum management to exhibit *Megamouth III*, all the necessary permits were obtained from the Dangerous Goods section of the Mines Department.

For the first six months of its exhibition period, the plumbing worked well and the solution remained bright and clear. Since the tank was not thermostatically controlled, concern had been expressed that the storage container might shatter the glass as the amount of 70% ethanol solution expanded as the terrestrial temperature increased from 15°C to 25°C. In order to determine the level of risk to the storage tank, the density of 70% ethanol was measured using a Parr Precision digital densitometer (DMA02D). The storage solution had a density of 0.8908 g.cm^{-3} at 15°C and 0.8831 g.cm^{-3} at 25°C. This means that an increase from 10°C is equivalent to a volume expansion of 87 L for the 10,000L storage tank. Given that the optimal exhibition of the specimen was attained with a full exhibition tank, a gooseneck expansion joint was connected to allow any volume variation to be accommodated while maintaining the integrity of the exhibition tank.

After two years the solution level in the tank was noticed to be falling and some leaks appeared in the plastic piping, so the purification facility was shut down.



The aqueous ethanol had also leaked into the fibre-optic lighting system and so it was shut down, as there was little call for the specimen to be lit during evening exhibition openings. Topping up of the storage solution was affected through the bung-hole that had been cut into the glass to allow gases to escape during the initial filling operation.

Conservation challenges with the exhibition

The initial structure used a 'dog bone' girder to suspend an elegant cover over the shark, enabling the visitor to walk over the heavy glass top of the tank and for children to lie down on top of it. Engineers supervising the installation and exhibition had assured the conservators that there would be no direct sunlight on the specimen, and so the design was endorsed. The top of the tank was protected by a powder-coated mild steel skirt that held the sponsors names, as shown in Figure 5a. The initial design was a brilliant success with students and visitors delighting in getting so close to the specimen.

The light sensitivity of natural science specimens in ethanol solutions has been reported by Macgregor and Planchot (2005) and others (see for example Horie 1989; Carter and Walker 1999). After a few years it was noticed that *Megamouth III* had lost some of the original grey-black colouration around its head and so a more shaded structure was built, as shown in Figure 5b. Despite difficulties in attracting additional funding for modifications of the initial structure to provide better



The exhibition tank as initially opened (Figure 5a, left) and as it exists in 2008, some 13 years later (Figure 5b, above).

protection from sunlight damage, the team persisted until the canopy was redesigned and an open-sided structure with closed ends was erected. Colourbond panels also provided shelter for school groups and a support structure for the interpretive panels that told the story of the shark. Concerns by safety managers about the very small risk of a child breaking the protective top of the tank resulted in the installation of the fence, which significantly reduces the visual impact of the very rare specimen.

In order to establish the present level of risk, should the tank spring a leak and release 10,000 L of aqueous ethanol into the ground and create a potential environment hazard, it was necessary to establish how much formaldehyde had leached out of *Megamouth III* into the storage solution. Analysis of the formaldehyde content of the tank on 5 March 2008 showed that the mean value was 58.5 ± 6.4 mg/L after 13.1 years in the storage solution. This indicates that the bulk of the formaldehyde had been extracted during the initial washing period and that the present solution does not represent any major health hazard. Given that the long-term stability of the fibreglass tank will begin to be compromised in the next seven years, it is planned to relocate the specimen into a custom-built stainless steel tank, with viewing ports at the head end and two ports at the sides to show the claspers and the tail section, in addition to the glass top. The tank will consist of an upper flange plate with a machined groove to take a Viton® O-ring, to provide an airtight and ethanol vapour-tight seal, and reinforced stiffened sides with approved lifting lugs that can be hidden from view when it is on exhibition in the new museum to be built on the present museum site in the Perth CBD.

During the valuation of the collections of the WA Museum it was noted that no value could be assigned to *Megamouth III* since it was such a rare specimen that it was deemed to be simply irreplaceable and of inestimable value. When the specimen is transferred to a new storage and exhibition tank some conservation work will be done

on the faded skin to restore the original colour.

Conclusion

The traditional methods for removal of formaldehyde residues from massive fish specimens such as grouper, sharks and wrasse has been shown to result in high levels of residual formaldehyde in the aqueous ethanol storage solutions. Washing fixed specimens for one to two days is clearly inadequate and generally results in potentially toxic working environments for the curators and technicians managing a spirit collection when accessing old collection materials. Modelling the removal of formaldehyde from a shark recovered from an old storage solution showed that the process was controlled by a pseudo first-order chemical reaction. This mechanism resulted in the formaldehyde levels in solution increasing linearly with the logarithm of the washing or storage time. Removing the preservative formaldehyde from *Megamouth III* took more than a month of washing in a storage tank of tap water. The formaldehyde removal from *Megamouth III* had the same kinetics as those observed for a smaller shark. The washing treatment has been demonstrated to be effective as the residual levels of formaldehyde in solution after 13 years of storage on exhibition are of the order of 50 mg/L.

Acknowledgments

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References

- Anonymous 1991. 'Megamouth reveals a phantom shark's realm', *National Geographic*, vol. 179, no. 3, p. 136.
- Berra, TM & Hutchins, JB 1990, 'A specimen of Megamouth Shark, *Megachasma pelagios* (Megachasmidae) from Western Australia', *Records of the Western Australian Museum*, vol. 14, pp. 651–6.
- Burroughs, GE, Makos, K, Hawks, C, & Ryan, TJ 2006, 'Exposure of museum staff to formaldehyde during some wet specimen activities', *Collection Forum*, vol. 20, no. 1–2, pp. 49–54.
- Carter, D & Walker, A 1999, *Care and conservation of natural history collections*, Oxford Butterworth-Heinemann.
- Fox, CH, Johnson, FB, Whiting, J & Roller PP 1985, 'Formaldehyde fixation', *Journal of Histochemistry and Cytochemistry* vol. 33, no. 8, pp. 845–53, & no. 9, pp. 177–9.
- Horie, CV (Ed.) 1989, *Conservation of natural history specimens—spirit collections*, The University of Manchester and the Manchester Museum.
- Helander, KJ 1994, 'Kinetic studies of formaldehyde binding in tissue', *Biotechnic & Histochemistry* no. 6.
- Hutchins, JB 1992, 'Megamouth: gentle giant of the deep', *Australian Natural History* vol. 23, no. 12, pp. 910–7.
- Kiernan, J 2005, 'Preservation and retrieval of antigens for immunochemistry—methods and mechanisms. 1. Effects of formaldehyde fixation', *The Cutting Edge*, National Society for Histotechnology, winter edition, pp. 5–9.
- Lavenberg, RJ & Seigel, JA 1985, 'The Pacific's mega mystery—Megamouth', *Terra* vol. 23, no. 4, pp. 30–1.
- Macgregor, C & Planchot, M 2005, 'The effects of light on spirit preserved natural science specimens', *Preprints of the AICCM Objects SIG Symposium*, Melbourne.
- MacLeod, ID 1987, 'Stabilization of corroded copper alloys: a study of corrosion and desalination mechanisms', *ICOM Committee for Conservation*, Sydney, pp. 1079–85.
- MacLeod, ID 2008, 'The legal consequences of inadequate conservation for contract maritime archaeology', *Preprints of the ICOM–CC Triennial Meeting*, New Delhi, India, September, pp. 217–23.
- MacLeod, ID & Davies, JA 1987, 'Desalination of glass, stone and ceramics recovered from shipwreck sites', *ICOM Committee for Conservation*, Sydney, pp. 1003–7.
- North, NA & Pearson, C 1978, 'Washing methods for chloride removal from marine iron artefacts', *Studies in Conservation*, vol. 23, pp. 174–6.
- Rumph, PF & Williams, JC 1986, 'A comparison of the efficiency of water and ethanol at removing formaldehyde from immersion fixed muscle tissues', *Anatomia, Histologia, Embryologia: Journal of Veterinary Medicine*, Series C, vol. 15, no. 3, pp. 269–76.
- Rumph, PF & Williams, JC 1988, 'Efficiency of phenoxyethanol at removing formaldehyde from immersion fixed muscle tissue', *Anatomia, Histologia, Embryologia: Journal of Veterinary Medicine*, Series C, vol. 17, no. 3, pp. 226–31.
- Shchukarev, SA & Tolmacheva, TA 1968, 'Solubility of oxygen in ethanol-water mixtures', *Journal of Structural Chemistry*, vol. 9, no. 1, pp. 16–21.

Biography

Ian MacLeod manages the care and conservation of the collections of the Western Australian Museum and has nearly 30 years of conservation experience in treating materials from historic shipwrecks. He has a deep awareness of the spiritual presence on rock art sites, on shipwreck graves and on land sites where the dead lie in a troubled sleep. He has specialised in metals and rock art conservation and developed in situ conservation methods for preservation of iron shipwreck materials in the ocean.