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# Is the Megamouth Shark susceptible to mega-distortion? Investigating the effects of twenty-two years of fixation and preservation on a large specimen of *Megachasma pelagios* (Chondrichthyes: Megachasmidae)

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**ABSTRACT –** On 18 August 1988, the third reported Megamouth Shark (*Megachasma pelagios* Taylor, Compagno and Struhsaker, 1983) became stranded on a beach in Western Australia. The 5.15 m TL male shark (WAM P.29940–001) was preserved at the Western Australian Museum (WAM) and put on public display. Many deep water chondrichthyan species, including *M. pelagios*, possess soft and flabby muscle tissue and a poorly calcified skeleton. Such species are prone to distortion through dehydration due to the fixation and preservation process. Such distortions may result in taxonomic and nomenclatural issues. The WAM Megamouth Shark was recently relocated, providing an opportunity to remeasure the specimen to allow a comparison with measurements taken of the specimen when it was fresh, and to assess the extent of change to these measurements after 22 years of preservation. We found that with the exception of the pelvic-fin, that we hypothesise is the fleshiest part of *M. pelagios* with the least skeletal support, the specimen has suffered little to no distortion over this time. However, there has been some degradation of teeth and denticles that is most likely due to the acidity of the formalin solution.

**KEYWORDS:** megamouth, long-term preservation, dimensional changes, ethanol preservation, formaldehyde fixed.

## INTRODUCTION

The Megamouth Shark, *Megachasma pelagios* Taylor, Compagno and Struhsaker, 1983, is a highly distinctive species with a patchy circumtropical and warm temperate distribution. Records and sightings of this enigmatic shark were few in the first three decades after the species was first discovered in 1976, and it was known from only 14 specimens at the turn of the century (Compagno 2001; Burgess 2010). However, since 2001 an additional 36 specimens have been recorded, with the 50th specimen reported in June 2010 (Burgess 2010). Many recent specimens have been retained and are reported as being prepared for public display and/or deposition in an ichthyological collection.

On 18 August 1988, the third recorded Megamouth Shark specimen was found stranded on a beach 50 km south of Perth, Western Australia (Berra and Hutchins 1990, 1991; Hutchins 1992; Last and Stevens 1994, 2009; MacLeod 2008). The 5.15 m TL male shark (WAM P.29940–001) was transported to Perth, then frozen and put on brief display before being preserved. Over six years later on 25 January 1995 it was placed on permanent public display at the Perth site of the Western Australian Museum (WAM), in a below-ground polyester resin glass-topped case, with the upper glass surface of the case level with the ground (Figure 1). Recent renovations at the Perth site necessitated the relocation of the Megamouth Shark specimen. The shark was removed from the below-ground case and relocated to the Western Australian Maritime Museum in Fremantle, approximately 20 km away. There it was placed in a custom made stainless steel tank with viewing portholes, for continued public display.

The Megamouth Shark possesses soft and flabby muscle tissue and its skeleton is poorly calcified (Compagno 1988, 1990; Smale et al. 2002; Taylor et al. 1983; BAH personal observation of dissection of Megamouth #17), and similar observations were noted


FIGURE 1 Photo of Megamouth #3 (WAM P.29940–001) on public display between 25 January 1995 to 22 September 2010, in ethanol solution in the glass-topped tank at Western Australian Museum (Perth). Image: Western Australian Museum.

by Berra and Hutchins (1991) during preservation of Megamouth #3. It is a well known phenomenon that such chondrichthyan species are prone to distortion as an artefact of fixation and preservation (Jones and Geen 1977; and see below). The distortion observed in some taxa has led to nomenclatural and taxonomic issues, resulting in junior synonyms arising for an already available and valid species name. An example illustrating this issue is *Hexatrygon bickelli* Heemstra and Smith, 1980, a distinctive six-gilled stingray originally described from South Africa. The species uses its long, flexible snout to probe sediments for prey (Hennemann 2001; Last and Stevens 2009; L.J.V. Compagno personal communication). The snout lacks a rostral cartilage and is filled with an acellular gelatinous matrix (Heemstra and Smith 1980). Further *Hexatrygon* specimens were collected from the South China Sea (Zhu et al. 1981, 1982), Taiwan (Shen and Lui 1984; Shen, 1986a, b), Japan (Ishihara and Kishida 1984), Indonesia (Stehmann and Shcherbachev 1995), and Australia (Last and Stevens 1994, 2009). The highly variable shape of the snout is to some extent a result of ontogeny and individual variation, but desiccation of the gelatinous matrix through fixation and preservation has a distorting effect that led to four new species being described, all on the basis of snout morphology (Ishihara and Kishida 1984; Stehmann and Shcherbachev 1995) and all of which are currently recognised as junior synonyms of *H. bickelli* (Compagno et al. 2005; Eschmeyer 2010; P.C. Heemstra personal communication).

Although taxonomic issues of the kind illustrated above are unlikely to apply to such a distinctive species as the Megamouth Shark, the effects of fixation and preservation on the WAM specimen are a potential issue that warrant further investigation. The preservation and fixation of specimens of *Megachasma pelagios*, besides the WAM specimen, have only been documented with any detail for a limited number of specimens (Taylor et al. 1983; Takada et al. 1997), and to the best of our knowledge, there have been no reports on potential morphological distortions resulting from fixation and preservation of *M. pelagios*. At the time of this study, the WAM Megamouth Shark specimen had been fixed in formalin for nearly 6½ years and subsequently in alcohol preservative for 15¾ years, giving a total time of 22¼ years since first fixation. Berra and Hutchins (1990) provided a comprehensive list of morphometric measurements for this specimen prior to fixation. The purpose of this study is to compare those measurements with new ones taken by the authors during the relocation of the Megamouth Shark and to determine the effects, if any, of more than 20 years of preservation.

## MATERIALS AND METHODS

### PRESERVATION AND PUBLIC DISPLAY

Detailed accounts of the fixation and preservation of the specimen are provided by Berra and Hutchins (1990, 1991), Hutchins (1992) and MacLeod (2008), and are summarised here. Details of the history of preservation and public display since 1992, including the recent move, are also provided to give a complete history of treatment and movement of the specimen. The shark was snap frozen on the afternoon of its discovery on 18 August 1988. According to bystanders it had died on the beach that morning. On 21 August 1988, three days after its discovery, the frozen shark was briefly put on public display for three hours under shade and then returned to the freezer. On 15 September 1988, a month after first capture, a pit lined with plastic swimming pool liner was filled with water and the specimen was placed in it, after being weighed at a public weighing station while frozen (690±20 kg; Berra & Hutchins 1990), and allowed to thaw over a weekend. About half of the water was removed from the pit, and Berra and Hutchins took the morphometric measurements (as per Compagno 1984; Table 1).

The body musculature was injected on 19 September 1988 with 20 l of approximately 30% formalin using 130 mm x 2 mm needles attached to 20 ml syringes. Forty litres of approximately 50% formalin was pumped into the body and body cavity using a 1300 mm x 10 mm needle. The pit was drained and clean fresh water was added to cover the specimen by approximately 30 cm. The water volume in the pit was estimated and full strength formalin (40% formaldehyde in solution) was added. The formalin strength was tested on 21 October 1988 at 8.25% formalin. On 13 January 1989, the formalin concentration had dropped to 4.25% and additional full strength formalin was added to produce a final concentration of 11.75% formalin (Berra and Hutchins 1990). Owing to concerns regarding residual amounts of formaldehyde in large sharks leaching into ethanol-based storage solutions, Megamouth #3 was washed in fresh water. Experiments with a small shark (*Hemigaleus australiensis* White, Last and Compagno 2005, WAM P.26190–002) had established that the release of formaldehyde was linearly related to the logarithm of the washing time (MacLeod 2008). Megamouth #3 was immersed in tap water for 41 days from 30 November 1994 to 3 January 1995, and removed from the temporary preservation pit on 25 January 1995, at which time the formalin concentration was measured at 0.032% (MacLeod 2008). The liver and intestines were also removed at this time, to minimise the amount of liver oil leaking from the specimen and detracting from the display (MacLeod 2008). The shark was then placed into a custom built glass-topped fibre

FIGURE 2 Lifting Megamouth #3 from the truck in the custom stretcher outside the Western Australian Maritime Museum, Fremantle, Western Australia (22 September 2010). Image: B.A. Human.

TABLE 1 Morphometric and proportional measurements of Megamouth #3 (*Megachasma pelagios* WAM P.29940.001) taken when fresh (original) and after 22 years of preservation (current study), and percentage difference between the two studies. Proportional measurements of the holotype (BPBM 22730) are provided for comparison. [a] from Berra and Hutchins (1990), [b] % current study-% original study, [c] from Taylor, Compagno and Struhsaker (1983), [d] – compared with original TL measurement.

| | Actual measurement (mm) | | Proportional measurement (%TL) | | | |
|---|---|---|---|---|---|---|
| Morphometric | Original[a] | Current study | Original[a] | Current study | Percentage difference[b] | Holotype (% TL)[c] |
| Total length (TL) | 5150 | 5090 | 100.0 | 98.8[d] | -1.2 | |
| Fork length | | 3890 | | 76.4 | | |
| Precaudal length | 3430 | 3380 | 66.6 | 66.4 | -0.2 | 69.3 |
| Pre-second dorsal-fin length | 2720 | 2700 | 52.8 | 53.0 | 0.2 | 56.7 |
| Pre-first dorsal-fin length | 1670 | 1680 | 32.4 | 33.0 | 0.6 | 34.5 |
| Body length | | 2015 | | 39.6 | | |
| Interdorsal-fin space | 640 | 650 | 12.4 | 12.8 | 0.4 | 13.2 |
| Second dorsal-fin–caudal-fin length | | 2125 | | 41.7 | | |
| Dorsal-fin–caudal-fin space | 430 | 435 | 8.3 | 8.5 | 0.2 | 8.9 |
| Preanal-fin length | | 3009 | | 59.1 | | 63.5 |
| Prepelvic-fin length | 2510 | 2520 | 48.7 | 49.5 | 0.8 | 50.9 |
| Prepectoral-fin length | 1390 | 1340 | 27.0 | 26.3 | -0.7 | 24.9 |
| Snout–vent length | | 2645 | | 52.0 | | 51.5 |
| Trunk length | | 1235 | | 24.3 | | |
| Pectoral-fin–pelvic-fin space | | 910 | | 17.9 | | |
| Pelvic-fin–anal-fin space | 370 | 360 | 7.2 | 7.1 | -0.1 | 7.4 |
| Vent–caudal-fin length | 2450 | 2510 | 47.6 | 49.3 | 1.7 | 48.5 |
| Pelvic-fin–caudal-fin space | 715 | 610 | 13.9 | 12.0 | -1.9 | |

| Morphometric | Actual measurement (mm) | | Proportional measurement (%TL) | | | |
|---|---|---|---|---|---|---|
| | Original[a] | Current study | Original[a] | Current study | Percentage difference[b] | Holotype (% TL)[c] |
| Anal-fin–caudal-fin space | 215 | 150 | 4.2 | 2.9 | -1.3 | 5.2 |
| Head height 2 | | 480 | | 9.4 | | |
| Head width 2 | | 630 | | 12.4 | | |
| Interorbital space | 550 | 530 | 10.7 | 10.4 | -0.3 | |
| Head height | | 1590 | | 31.2 | | |
| Trunk height | | 645 | | 12.7 | | 14.3 |
| Trunk width | | 350 | | 6.9 | | |
| Girth | 1790 | | 34.8 | | | 40.4 |
| Abdomen height | 640 | 635 | 12.4 | 12.5 | 0.1 | |
| Abdomen width | | 250 | | 4.9 | | |
| Tail height | 420 | 450 | 8.2 | 8.8 | 0.6 | 9.9 |
| Tail width | | 230 | | 4.5 | | |
| Caudal peduncle height | 280 | 300 | 5.4 | 5.9 | 0.5 | 5.3 |
| Caudal peduncle width | | 110 | | 2.2 | | 2.4 |
| Head length | 1320 | 1290 | 25.6 | 25.3 | -0.3 | 26.5 |
| Prebranchial length | 1090 | 1085 | 21.2 | 21.3 | 0.1 | 19.1 |
| Prespiracular length | 935 | | 18.2 | | | 10.1 |
| Preorbital length | 350 | 425 | 6.8 | 8.3 | 1.5 | 5.4 |
| Eye length | 60 | 62 | 1.2 | 1.2 | 0 | 1.3 |
| Eye height | 40 | 50 | 0.8 | 1.0 | 0.2 | 1.2 |
| Spiracle length | | 10 | | 0.2 | | 0.1 |
| Eye-spiracle length | | 260 | | 5.1 | | 3.9 |

| Morphometric | Actual measurement (mm) | | Proportional measurement (%TL) | | | |
|---|---|---|---|---|---|---|
| | Original[a] | Current study | Original[a] | Current study | Percentage difference[b] | Holotype (% TL)[c] |
| Intergill length | 320 | 260 | 6.2 | 5.1 | -1.1 | |
| First gill slit height | 220 | 215 | 4.3 | 4.2 | -0.1 | 5.9 |
| Second gill slit height | 225 | 225 | 4.4 | 4.4 | 0 | 5.8 |
| Third gill slit height | 225 | 230 | 4.4 | 4.5 | 0.1 | 5.9 |
| Fourth gill slit height | 210 | 220 | 4.1 | 4.3 | 0.2 | 5.7 |
| Fifth gill slit height | 200 | 215 | 3.9 | 4.2 | 0.3 | 5.2 |
| Preoral length | 60 | 43.5 | 1.2 | 0.9 | -0.3 | 1.5 |
| Prenarial length | 105 | 155 | 2.0 | 3.0 | 1 | 2.2 |
| Mouth length | 450 | 570 | 8.7 | 11.2 | 2.5 | 6.1 |
| Mouth width | 580 | 630 | 11.3 | 12.4 | 1.1 | 18.5 |
| Nostril width | 30 | 32 | 0.6 | 0.6 | 0 | 0.7 |
| Internarial space | 400 | 410 | 7.8 | 8.1 | 0.3 | 7.6 |
| Outer internarial width | | 455 | | 8.9 | | |
| Anterior nasal flap length | 7 | 10 | 0.1 | 0.2 | 0.1 | |
| Pectoral-fin length | | 460 | | 9.0 | | 10.2 |
| Pectoral-fin anterior margin | 990 | 960 | 19.2 | 18.9 | -0.3 | 18.8 |
| Pectoral-fin base | 330 | 260 | 6.4 | 5.1 | -1.3 | 5.9 |
| Pectoral-fin height | 870 | 855 | 16.9 | 16.8 | -0.1 | |
| Pectoral-fin inner margin | | 230 | | 4.5 | | 4.3 |
| Pectoral-fin posterior margin | | 650 | | 12.8 | | 13.8 |
| Pectoral-fin radial length | | 690 | | 13.6 | | |
| First dorsal-fin length | | 535 | | 10.5 | | |

| Morphometric | Actual measurement (mm) | | Proportional measurement (%TL) | | | |
|---|---|---|---|---|---|---|
| | Original[a] | Current study | Original[a] | Current study | Percentage difference[b] | Holotype (% TL)[c] |
| First dorsal-fin anterior margin | 280 | 435 | 5.4 | 8.5 | 3.1 | 9.3 |
| First dorsal-fin base | 500 | 460 | 9.7 | 9.0 | -0.7 | 9.1 |
| First dorsal-fin height | 250 | 250 | 4.9 | 4.9 | 0 | 5.1 |
| First dorsal-fin inner margin length | 80 | 70 | 1.6 | 1.4 | -0.2 | 1.8 |
| First dorsal-fin posterior margin length | 295 | 285 | 5.7 | 5.6 | -0.1 | 5.9 |
| Pelvic-fin length | | 340 | | 6.7 | | 5.5 |
| Pelvic-fin anterior margin length | 330 | 320 | 6.4 | 6.3 | -0.1 | 5.9 |
| Pelvic-fin base | 320 | 220 | 6.2 | 4.3 | -1.9 | 4.6 |
| Pelvic-fin height | 185 | 250 | 3.6 | 4.9 | 1.3 | 5.7 |
| Pelvic-fin inner margin length | 35 | 90 | 0.7 | 1.8 | 1.1 | 0.9 |
| Pelvic-fin posterior margin length | 195 | 185 | 3.8 | 3.6 | -0.2 | 4.1 |
| Clasper outer length | 360 | 370 | 7.0 | 7.3 | 0.3 | 8.0 |
| Clasper inner length | 560 | 560 | 10.9 | 11.0 | 0.1 | 12.3 |
| Clasper base width | 70 | 60 | 1.4 | 1.2 | 0.2 | 1.1 |
| Second dorsal-fin length | | 285 | | 5.6 | | |
| Second dorsal-fin anterior margin | 240 | 200 | 4.7 | 3.9 | -0.8 | 4.4 |
| Second dorsal-fin base | 255 | 210 | 5.0 | 4.1 | -0.9 | 4.3 |
| Second dorsal-fin height | 100 | 100 | 1.9 | 2.0 | 0.1 | 2.3 |
| Second dorsal-fin inner margin length | 75 | 75 | 1.5 | 1.5 | 0 | 1.8 |
| Second dorsal-fin posterior margin length | 155 | 150 | 3.0 | 2.9 | -0.1 | 3.5 |
| Anal-fin length | 145 | 140 | 2.8 | 2.8 | 0 | |
| Anal-fin anterior margin | 155 | 150 | 3.0 | 2.9 | -0.1 | 4.4 |

| Morphometric | Actual measurement (mm) | | Proportional measurement (%TL) | | | |
|---|---|---|---|---|---|---|
| | Original[a] | Current study | Original[a] | Current study | Percentage difference[b] | Holotype (% TL)[c] |
| Anal-fin base | 80 | 90 | 1.6 | 1.8 | 0.2 | 3.6 |
| Anal-fin height | 80 | 60 | 1.6 | 1.2 | -0.4 | 1.7 |
| Anal-fin inner margin length | 65 | 55 | 1.3 | 1.1 | -0.2 | 1.5 |
| Anal-fin posterior margin length | 85 | 70 | 1.7 | 1.4 | -0.3 | 1.8 |
| Dorsal caudal-fin margin | 1730 | 1655 | 33.6 | 32.5 | -1.1 | 32.4 |
| Preventral caudal-fin margin | 720 | 690 | 14.0 | 13.6 | -0.4 | 14.0 |
| Upper postventral caudal-fin margin | 1210 | 1190 | 23.5 | 23.4 | -0.1 | 27.4 |
| Lower postventral caudal-fin margin | 430 | 395 | 8.3 | 7.8 | -0.5 | 8.5 |
| Caudal-fin fork width | 450 | 410 | 8.7 | 8.1 | -0.6 | 10.6 |
| Caudal-fin fork length | 480 | 460 | 9.3 | 9.0 | -0.3 | 6.1 |
| Subterminal caudal-fin margin | 85 | 85 | 1.7 | 1.7 | 0 | 1.3 |
| Subterminal caudal-fin length | | 70 | | 1.4 | | |
| Terminal caudal-fin margin | 105 | 85 | 2.0 | 1.7 | -0.3 | 2.2 |
| Terminal caudal-fin lobe length | | 85 | | 1.7 | | 3.1 |
| First dorsal-fin midpoint–pectoral-fin insertion | | 270 | | 5.3 | | |
| First dorsal-fin midpoint–pelvic-fin origin | | 655 | | 12.9 | | |
| Pelvic-fin midpoint–first dorsal-fin insertion | | 615 | | 12.1 | | |
| Pelvic-fin midpoint–second dorsal-fin origin | 415 | 0 | 8.1 | 0 | -8.1 | |
| Second dorsal-fin origin–anal-fin origin | | 430 | | 8.4 | | |
| Second dorsal-fin insertion–anal-fin insertion | | 230 | | 4.5 | | |

FIGURE 3 Megamouth #3 about to be lifted off the stretcher and placed into the custom made stainless steel tank (22 September 2010). Image: B.A. Human.

reinforced polyester tank (Figure 1), which was filled with carbon (to remove coloured components) and a cotton wool pre-filter (to remove oil droplets). However, the pump system became inoperable after four years (MacLeod 2008). The specimen was on display in an undercover, open sided gazebo from 25 January 1995 until the recent move.

On 22 September 2010, the glass top of the tank was cut open in order to remove Megamouth #3 for relocation. The tank was drained and the shark was placed on a purpose built metal-framed stretcher with canvas slings. A crane was used to extract Megamouth #3 from the tank and place it on a flat-bed truck, and the specimen was covered in water soaked coarse woven hessian for the relocation. The journey from the Perth site to the Fremantle site took approximately 1 hour. Megamouth #3 was removed from the truck and brought inside for inspection, measurements, and placement into the new tank that had been partially filled with a 30% glycerol solution (Figure 2). It was while the tank was being filled and the shark was on the stretcher that the majority of morphometric measurements used in this study were collected (Figure 3). A few measurements could not be collected (mostly of the head) because of the way the specimen was lying on the stretcher. A number of other measurements/samples were taken at this time by other researchers, including skin

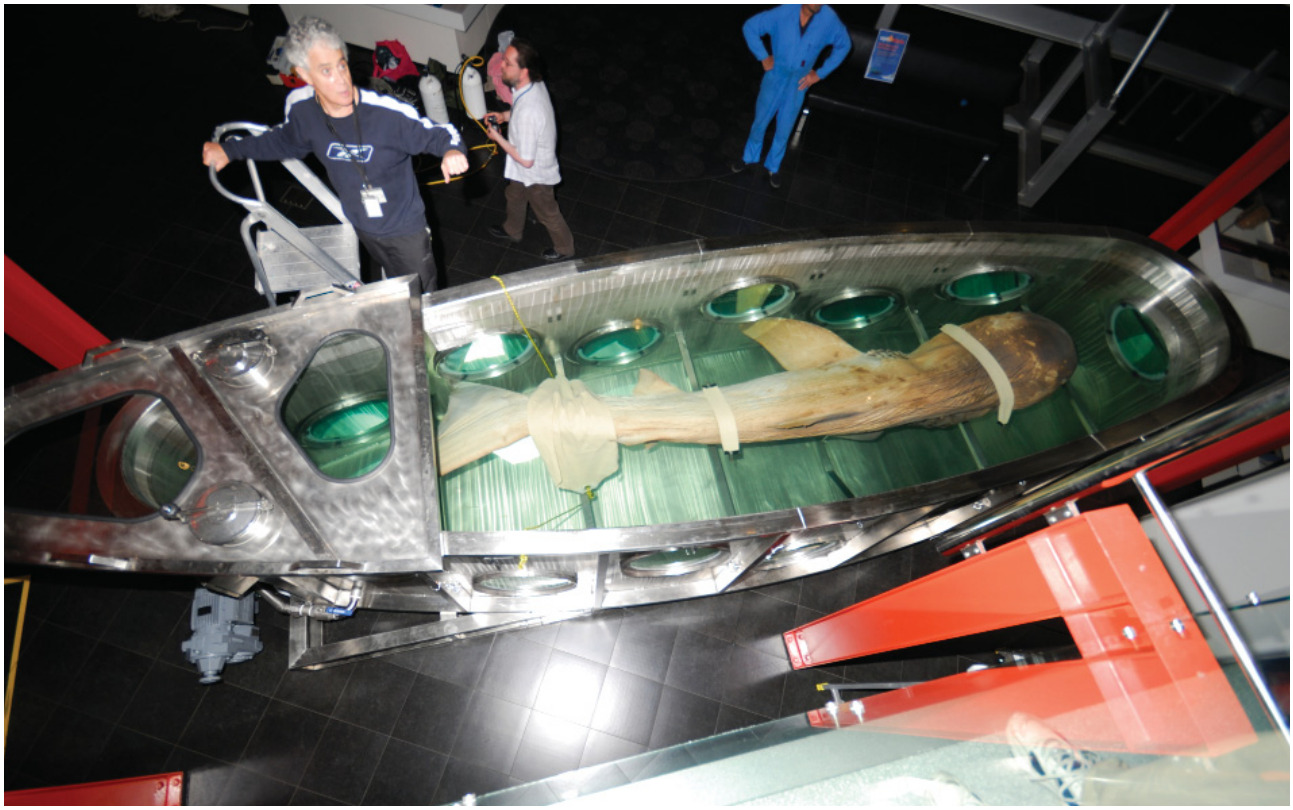

FIGURE 4 Overhead view (with lids removed) of the newly constructed stainless steel tank for public viewing of Megamouth #3 at the WA Maritime Museum. This photo was taken shortly after the specimen had been lowered into the glycerol solution (22 September 2010). Image: B.A. Human.

FIGURE 5 View of the head of Megamouth #3 through one of the portholes (details as for figure 4). Image: B.A. Human.

chromametric readings, denticle and tooth samples, and lateral line and electrosensory pore mapping (Kempster 2011; MacLeod unpublished data). The data collection and documentation took approximately one hour.

Megamouth #3 was then placed into a custom built 304 stainless steel (high grade) tank 560 cm L x 188 cm H x 183 cm W (maximum width in the middle of the tank) with 50 cm diameter portholes and a filtration pump, containing a 30% by volume glycerol solution, for above ground viewing, and which also provided good access for research purposes (Figures 4–5). A number of slings were used to position Megamouth #3 in the tank to prevent it floating due to the density difference between the ethanol in its tissues and the glycerol solution, and as a result of a large amount of air entering the body and oral cavities. On 30 September 2010, the specimen had settled into the glycerol solution and personnel entered the tank to adjust the slings. At this time the remaining morphometric measurements that could not be taken previously were recorded, and a number of body morphometrics remeasured to ensure accuracy because the shark had not been lying completely flat on the stretcher.

The use of a glycerol solution as a preservative is not a standard curatorial procedure, but was chosen due to occupational health and safety restrictions regarding ethanol and formalin solutions, and favourable results obtained during trials with glycerol as a preservative on chondrichthyan specimens (MacLeod and van Dam 2011). On 20 December 2010, approximately 2000 l of glycerol solution was removed from the Megamouth #3 display tank and 100% glycerol was added to the remaining solution to increase the concentration of glycerol to 45%, in accordance with a plan to incrementally increase the glycerol concentration to 65% by volume by December 2012. This not only increased the glycerol concentration, but also reduced the levels of ethanol and formalin that had been leaching from the specimen (MacLeod 2008; MacLeod and van Dam 2011).

In October 2010, shortly after the shark was placed in the tank, yeast and mould contamination developed in the glycerol solution, and a filamentous slime and black spots of mould appeared, resulting in the glycerol solution becoming opaque and the clarity of the display being severely compromised. The infection probably occurred during the placement of the hessian over the specimen to avoid dehydration during the journey from Perth to Fremantle. Approximately two weeks after the infection was noticed, a bactericide, kathon (methylisothiazolinone), was added to the glycerol solution. The filtration pump was initially run only after-hours to reduce noise pollution in the public gallery, and is now operating at night with the 10 micron filter providing clear storage solutions and as of December 2011 the infection has not returned. The suspended dead contaminants were removed from the solution through successive use of 100 µm, 50 µm and 10 µm filters. A further benefit of running the pump at night is that periodic circulation of the glycerol solutions assists in the even penetration of the consolidant.

### TEETH AND DENTICLES

Scanning electron micrographs of the teeth and denticles were taken using a back-scattered secondary electron image mode in a Phillips XL40 Controlled Pressure cell. The samples were cut with a scalpel and placed directly into the SEM chamber and pumped down with no coating applied to the tissues.

### MORPHOMETRICS

The morphometrics were taken according to Compagno (1984, 2001) and Human (2006). Berra and Hutchins (1990) acknowledged the help of A.J. Bass during the measuring process, who is well acquainted with measuring sharks (Bass 1973; Bass et al. 1973, 1975a, b, c, d, 1976). Likewise, the senior author has extensive experience taking morphometric measurements of sharks, including previous experience measuring *Megachasma pelagios* (Human 2006, 2007a, b; Smale et al. 2002). Morphometric measurements from Berra and Hutchins (1990) and from the current study are given in Table 1, as well as percentage (of total length) differences between the two studies. The majority of measurements were taken using a standard tape measure, and the smallest dimensions measured with digital calipers. The crane hoist was used to measure the weight of the shark.

## RESULTS

### TEETH AND DENTICLES

Scanning electron micrographs show the degradation of a patch of denticles (Figure 6) that is most probably due to prolonged exposure to the acidic formalin solution. Figure 7 shows the upper section of a tooth taken from Megamouth #3. Modern elasmobranchs (subcohort Neoselachii) possess a triple-layered enameloid tooth morphology, with a thin outermost shiny layered enameloid, a thick middle layer of parallel bundled enameloid, and an innermost layer of tangled bundled enameloid which surrounds a dentine core (Reif 1977, 1980; Compagno 1988, 2001; Rees and Cuny 2007). It is hypothesised that the acidic nature of the formalin solution has etched away the shiny layered enameloid, leaving the parallel bundled enameloid exposed, observed as parallel striations in Figure 7 (M. Siversson, personal communication).

### MORPHOMETRICS

It was not possible to weigh the specimen accurately in this study. The crane used to relocate the specimen measured the weight of the shark at 600 kg, however the accuracy of the crane was ±100 kg (BAH with crane operator, personal communication).

Most of the measurements (actual and proportional) corresponded well to those of Berra and Hutchins (1990), indicating that despite 22 years of preservation, little distortion has occurred in the specimen. The difference in measurements between the two studies were within 1% of each other for the majority of the morphometrics (Table 1). The measurements total

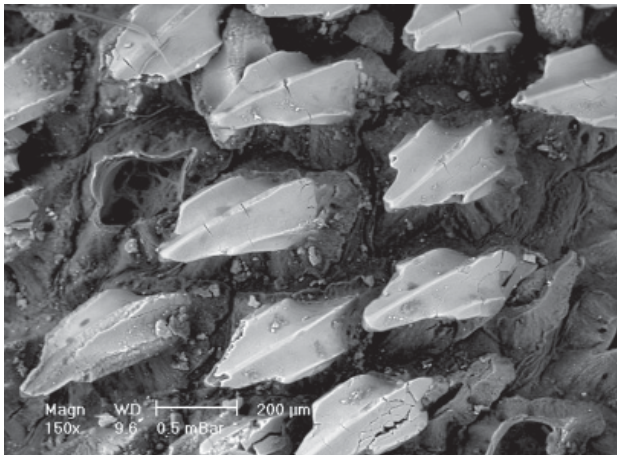


FIGURE 6 Scanning electron micrograph of a patch of denticles taken from Megamouth #3 during the recent relocation, showing the damage likely to have been caused by prolonged exposure to formalin solution. Image: M. Verral.

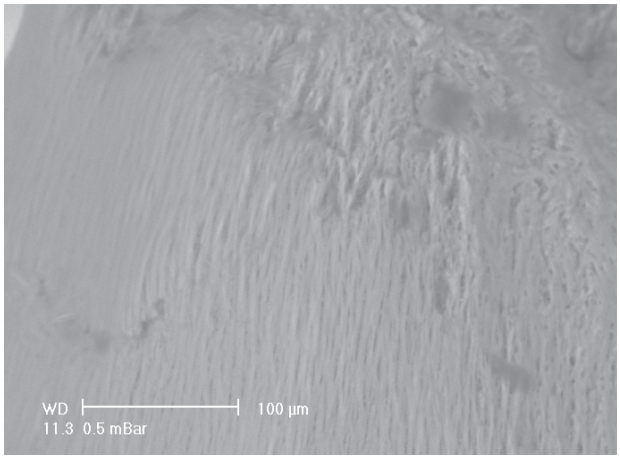


FIGURE 7 Scanning electron micrograph of the upper section of a tooth taken from Megamouth #3 during the recent relocation, showing the damage likely to have been caused by prolonged exposure to formalin solution. Image: M. Verral.

length, vent-caudal–fin length, pelvic-fin–caudal-fin space, and dorsal caudal-fin margin showed a slightly greater than 1% difference between the two studies, but these are relatively long measurements and we regard this as acceptable when considering the likelihood of inconsistency in measuring a specimen as large as this. For instance, if a specimen of this size is lying with a slight curvature, then that will translate into a significant margin of error in any long body measurements.

The smaller measures of anal-fin–caudal-fin space, preorbital length, prenarial length, mouth length, mouth width, pectoral-fin base, first dorsal-fin anterior margin, pelvic-fin base, pelvic-fin height, pelvic-fin inner margin, and pelvic-fin midpoint–second dorsal-fin origin were more variable between the two studies. Again, the differences may reflect discrepancies in the method of measurement rather than distortional effects, despite authors in both studies being highly experienced in taking morphometric measurements of sharks. An exception may be the pelvic fin, which had many different morphometric measurements between the studies which may indicate physical distortion.

A number of morphometrics were collected in this study that were not recorded by Berra and Hutchins (1990) and are compared to the proportions recorded for the holotype (Taylor et al. 1983), which is included here as a reference point for these additional measurements. All of the morphometric proportions recorded for this study corresponded very closely to the proportional measurements of the holotype and most differed much less than 5% between this specimen and the holotype. The notable exception is mouth length, which was measured when Megamouth #3 was already immersed in glycerol and thus difficult to measure, therefore probably reflecting error on our part. Mouth width is also much wider in the holotype compared with the measurements taken both in this study and by Berra and Hutchins (1990), possibly reflecting individual variation in this proportion. There were discrepancies of greater than 5% for the measurements of pectoral-fin base length and anal-fin–caudal-fin space between the current study and Berra and Hutchins (1990), and these most likely reflect differences in measurement technique and/or error due to the positioning of the shark.

## DISCUSSION

Megamouth #3 has suffered little distortion despite being in formalin for over six years and in ethanol for nearly 16 years. In sharks that have been otherwise carefully preserved, as is the case here, one could argue that fins are probably the features that are most vulnerable to distortion and this could be so for the pelvic-fin in this study. That the other fins show no apparent signs of distortion may reflect the fact that they are relatively well supported by skeletal elements. Being a lamnoid shark, *Megachasma pelagios* possesses plesodic pectoral fins, in which the distal fin radials extend to the margins of the fin web (Compagno 1988, 1990; Taylor et al. 1983; BAH personal observation of dissection of Megamouth #17). With the exception of the pectoral fin, all other fins of Megamouth Sharks are relatively small, and therefore are well supported by skeletal elements and are not fleshy. The pelvic fin has the least skeletal support in Megamouth Shark (BAH persernal observation of dissection of Megamouth #17), which is the most probable explanation for the pelvic fin showing some distortion whereas the other fins do not. White et al. (2004) similarly argued that fin dimensions for the Megamouth Shark should be relatively invariable.

One apparent consequence of the long-term storage in the formaldehyde solution is that the denticles show signs of significant chemical attack (Figure 6) and the teeth had become etched (Figure 7), which is consistent with long-term storage in acidic solutions (M. Siversson

personal communication). Whilst the prolonged fixation time has apparently resulted in little damage to the body of the shark, it seems that the acidic solution has affected the teeth and the denticles.

Another consequence, most likely due to the previous display method, is that the colouration of the skin has notably faded (MacLeod 2008; MacLeod and van Dam 2011). In the original display, sunlight fell directly onto the left side of the body of the shark, until side panels were erected at either end of the display gazebo (MacLeod 2008), resulting in the skin colour becoming very pale (Figures 3–5). Glycerol trials on ethanol preserved shark specimens by MacLeod and van Dam (2011) showed that the skin of those specimens appeared darker following treatment. The fading in those specimens, however, resulted from ethanol preservation rather than the effects of bleaching by the sun. It is likely that the colour changes brought about through treatment with aqueous glycerol are due to colour saturation of the surface i.e. the glycerol solutions fully wet the shark skin and allow its natural colour to be viewed. This appears to be the underlying reason why the 'colour' of Megamouth #3 has been returned. Halfway through the glycerol impregnation treatment, the colour saturation of the skin brought about by the removal from ethanol appears to have brought back some of the darker colour that had been bleached out. Final colour measurements, with a chromameter, will be done at the end of the treatment program and the effectiveness of the preservative on restoring colour will then be assessed.

The use of glycerol as a preservative for fishes is a new approach that will reduce occupational health and safety risks and hazards. A more detailed examination of the use of glycerol on formalin preserved shark specimens is provided by MacLeod and van Dam (2011), and continued observation of the WAM Megamouth Shark will determine the long term suitability of glycerol as a preservation fluid. Some human tissues at the University of Leiden Medical Museum have been preserved in glycerol solution for more than 100 years and so the method can be regarded as being suitable for museum specimens.

Despite *Megachasma pelagios* being a relatively flabby-bodied shark, the current specimen shows very little distortion from fixation and preservation. Although the skeleton is poorly calcified (Compagno 1990; Taylor et al. 1983; BAH personal observation of dissection of Megamouth #17), it is apparently effective in preventing distortion of preserved specimens, as is demonstrated by the majority of morphometric measurements remaining unchanged in Megamouth #3. The only parts of the shark seemingly fleshy enough to allow morphometric distortion from fixatives and preservatives were the pelvic fins.

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