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A Summary of the Freezing, Thawing, Preservation, and Display Methodology Used on a Large Megamouth Shark, *Megachasma pelagios*

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Key words: megamouth shark, freezing, thawing, preservation, display

Abstract Freezing, thawing, preservation, and display methodology of a large megamouth shark, *Megachasma pelagios*, are summarized. The specimen was frozen (−30℃) from the evening of 29 November 1994 to 1 February 1995. Thawing required nine days, from 1-9 February 1995. Half-strength sea-water, with its temperature was held at 3℃ by circulating water, was used during the thawing process. After dissection on 9 February 1995, the abdominal cavity was stuffed with blankets and stitched closed to restore the shark's original profile. The specimen was fixed for 45 days in a tank that contained formalin at a concentration of 20%. The size of the acrylic display tank is 500cm × 150cm × 135cm. The liquid preservative within the acrylic display tank darkened slightly after about three months.

A stranded megamouth shark (*Megachasma pelagios*) was found on a tidal flat off Gannosu, Higashi-ku, Fukuoka, Japan, at 10:30 A.M. on 29 November 1994. This specimen is the seventh confirmed record of this species and it is the first female ever captured. Four of the seven specimens have been preserved. In addition to our specimen, the holotype is displayed in the Bishop Museum in Hawaii, one is in the Natural History Museum of Los Angeles County, and the third is at the Western Australia Museum (Taylor et al., 1983; Lavenberg and Seigel, 1985; Berra and Hutchins, 1991). More recently, two young megamouth sharks were found in the Atlantic Ocean (Séret, 1995; Amorim et al., 1995). One of them has been preserved at the Instituto de Pesca (Amorim et al., 1995).

The female megamouth shark was kept frozen from the evening of 29 November 1994, the day of its discovery, until 1 February 1995. The shark was thawed from 1 to 9 February 1995 and was dissected by the Megamouth Shark Research Project on 9 February 1995. After fixation, the specimen was moved to Marine World umino-nakamichi on 28 March 1995 and it was introduced to the public at the grand opening of Marine World.

The specimen is 4710mm long and its weight is 790kg. A detailed report on the freezing, thawing, and displaying of such a large specimen should be a useful reference in the event of similar cases in the future. We will describe the whole process from freezing to display for reference by whom it may concern.

Methods and Materials

Freezing

The recovered specimen was frozen on 29 November 1995 at a warehouse in Fukuoka and remained frozen until 1 February 1995. The specimen was carried into the warehouse on a cart

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and the freezing process was started immediately at −30℃. The freezing was performed well away from other articles to ensure uniform freezing. The body was covered with plastic sheets to provent direct contact with the chilling air. The total time from the shark's discovery to the initiation of the freezing process was about 8.5 hrs. The air temperature on the day of discovery ranged from 9.5-17.3℃. The temperature inside of the freezer was kept at −30℃ throughout the freezing process. Beginning on the third day, the shark was sprayed with water that froze to form a five-mm thick ice layer that prevented dehydration of the body surface (Fig. 1). The specimen was removed from the freezer and delivered to the Veterinary Hospital at Miyazaki University by a refrigerated car at −25℃ on 21 January 1995 so that X-rays of its vertebrae and dentition could be taken. No melting was observed during radiography due to cold weather and the heat capacity of the specimen itself, except for a minor portion of its thin fins. The specimen was returned to its original freezer the following morning. Some samples of skeletal muscle were extracted from the specimen for DNA studies after the radiography on 21 January and again on 1 February just before the thawing process. The extracted tissue was taken from the ventral portion of the caudal fin, a spot that is would not be noticeable when the specimen was placed on display.

Thawing

Thawing required nine days, from 1 to 9 February 1995. This thawing period was decided on after a series of consultations with specialists on freezing. Kashii Flower Park, where the thawing took place, is located near the warehouse (only a 10-min drive) where the shark was frozen. Transfer time from the warehouse was brief. We placed a 16-cubic-meter water tank (500cm × 180cm × 180cm), with a concrete panel and a canvas on it, at an annexed space near the dissection room to ensure minimal damage due to shifting of the specimen after thawing (Fig. 2). The frozen specimen was suspended on a stretcher in the water tank during thawing. Part of the specimen's body was exposed to the air due to its buoyancy.

We covered the shark with a blanket and sank it in the water with concrete blocks. We used half-strength sea-water to thaw the shark in an effort to dehydration or swelling due to osmosis. The temperature was held at 3℃ by circulating water in the thawing tank through a cooling machine. This kept the specimen fresh during the thawing process. In addition, we kept spraying cooling water from the upper area of the water tank to maintain a stable water temperature in the tank.

The water temperature was measured at one-minute intervals by a Time-Temperature Recorder (Alec Electronics Co., Ltd., Micro Data Recorder System, MDS-T). The water temperature went up by 2-3℃ every day for the first three days and then became stable at about 3℃ on 7 February 1995 (Fig. 3).

A temperature sensor (Anritsu Keiki Thermoprinter, AP-210) was inserted into the base of the caudal fin musculature 20cm under the skin that recorded hourly body temperature automatically. Due to mechanical trouble with the sensor, measurement was not started until 3 February. At that time, the body temperature was −5.1℃ and it increased by 2-3℃ daily for three days and became stable at 3℃ for the last three days before dissection of the shark (Fig. 4).

Preservation

After its dissection on 9 February 1995, the specimen's abdominal cavity was stuffed with blankets and stitched closed so that the shark's original profile was restored. The stitching work was completed at 1:30 A.M. on 10 February. It took 17 hrs for the dissection and stitching procedures to be completed. Prior to the stitching work, a hose was set into the abdominal cavity to fill it with an undiluted formalin solution. The hose intentionally extended out of the

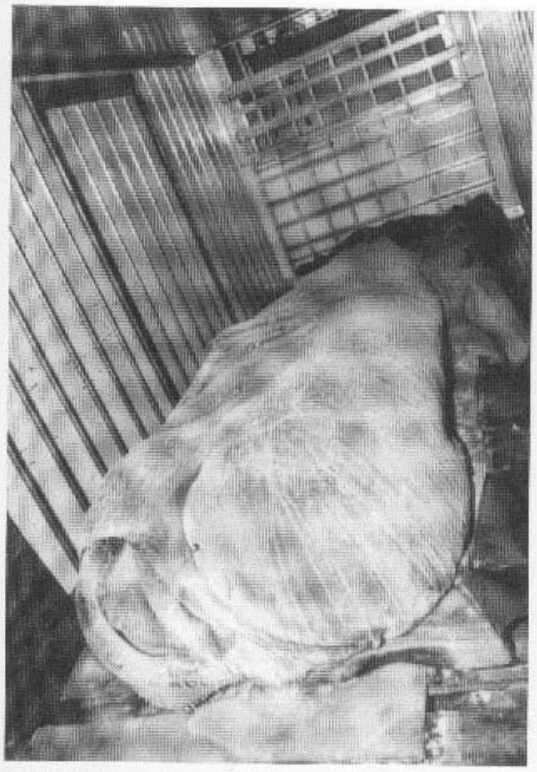

Fig. 1. Setting process of a 5-mm thick ice layer on the body surface of the megamouth shark, *Megachasma pelagios*.

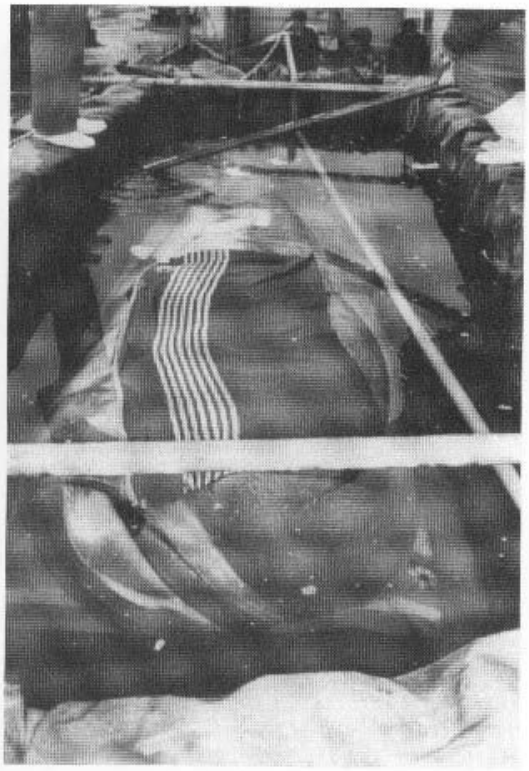

Fig. 2. The specimen in the thawing tank and the spraying pipe.

body about two meters (Fig. 5).

The dissected specimen was loaded again on the stretcher which was used during thawing and transferred to Marine World umino-nakamichi for formalin fixation. The journey took 30 min. The formalin fixation tank (500cm × 180cm × 180cm) was the same one used to thaw the specimen. Three layers of canvas and plastic sheeting were installed inside the tank and the top was closed with a zipper to avoid formalin leakage.

The specimen was loaded on a stretcher of trough-shaped PVC pipes 700mm in diameter. This device was used to restore the cylindrical shape of the megamouth shark during fixation. The diameter of 700mm was determined by the outer circumference of specimen's head. Portions of the pipe were cut off in advance to enable extension of the pectoral and pelvic fins from the pipe. In addition, sand bags were placed on the bottom of the tank to protect each fin and also to provide a solid foundation for the stretcher (Fig. 6).

At first, fresh water was placed into the fixation tank so that the specimen's position could be adjusted. Next, formalin was poured into the tank to a concentration of 20%. The total volume was 13.5 cubic meters and the depth was 1500mm.

An undiluted solution of formalin was injected into the shark's abdominal cavity via the preset hose and into its muscles via a syringe. Fixation was completed at 4:30 A.M. the following morning (10 February). Twenty-two hours passed from the removal of the shark from the thawing tank to its injection with formalin. The air temperature was about 5℃ which helped preserve the freshness of the specimen. Formalin soaking continued for 45 days.

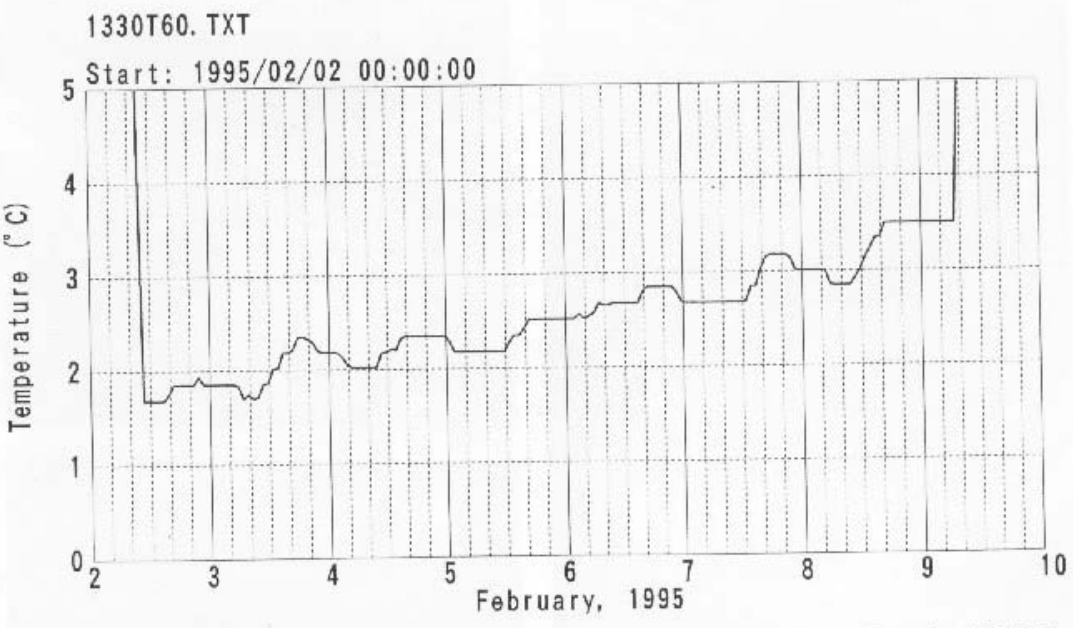

Fig. 3. Variation of water temperature in the thawing tank measured by a Time-Temperature Recorder (MDS-T).

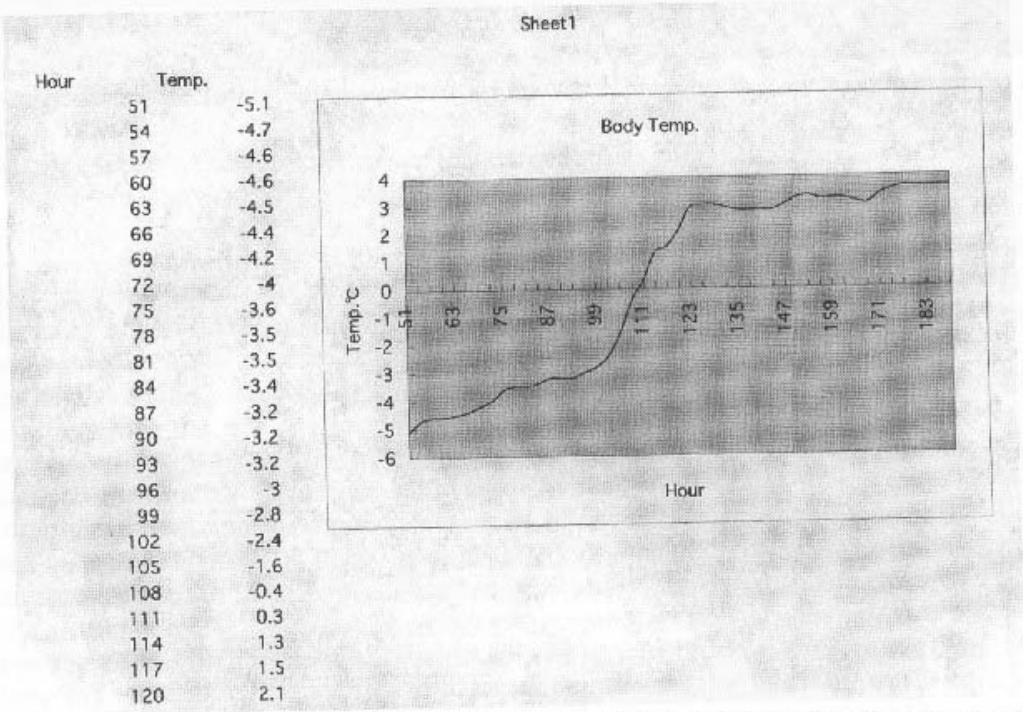

Fig. 4. Variation of megamouth's body temperature in the thawing tank measured by a temperature sensor (Thermoprinter, AP-210).

Display in Formalin

Three other megamouth specimens are displayed in the world. These three display cases are opaque on five sides. Thus, observation is only possible through one side. Therefore, this peculiar shark can never be fully appreciated. Therefore, we decided to make an acrylic tank so

Fig. 5. The hose for formalin supply, its abdominal cavity stuffed with blankets, and the stitching process on the megamouth shark, *Megachasma pelagios*.

that observation was available from every angle.

The size of the display tank is 500cm × 150cm × 135cm, in accordance with the body size of the specimen (Fig. 7). The thickness of the acrylic sheeting is 45mm and the total volume of the tank is 10 cubic meters. We made a supporting deck of 6318mm × 1608mm × 320mm, at an angle. The height of the deck was designed with consideration of the average eye angle of spectators and the display effect as well. Its width and length were determined in accordance with the relative regulations on floor load (1300kg/square meter). The supporting deck was covered by a man-made, stone-like material for decoration. An explanatory board rests on top of the display tank.

Physical expansion of the display tank after filling was controlled by 20-mm thick acrylic sheeting set at the upper portion of the tank and by four stainless bars of 10mm diameter set across the open area. The liquid preservative can be poured into the tank through a 200mm opening in the acrylic sheeting set over the tank. The specimen was transferred to the annexed pool on 28 March to be reloaded on transparent, half-cut, cylindrical pipes for display (Fig. 8).

Three pieces of pipes had been prepared. Two of them are 700mm in diameter × 650mm long and one is 300mm in diameter × 400mm long (Figs. 9A, B). The two 700-mm pipes were used to support the head and the abdomen, and the 300-mm pipe was used to support the tail. Because, the transparent, half-cut, cylindrical pipes had the same diameter as the PVC pipes used for fixation, the specimen was set on them as it. Six holes were drilled in the upper edges of the transparent, half-cut, cylindrical pipes so that the specimen could be hung from the upper side of the tank.

The specimen, set on trough-shaped PVC pipes, was placed into the acrylic display tank on 28 March 1995. The display tank, with the specimen in it, was moved to the entrance hall on the second floor at a planned position.

After positioning the display tank, supporting work followed. Fresh water was poured into the tank to a depth of 500mm (the maximum height allowed to maintain its structural strength without its cover) to float the specimen 150mm above the bottom of the tank. While floating the specimen, the three transparent pipes (which are tied by fishing line to the upper part of the tank through six holes) were properly positioned to cope with the specimen's proportions (Fig. 9C). Additional fishing lines hang the specimen from the upper side of the display tank by its

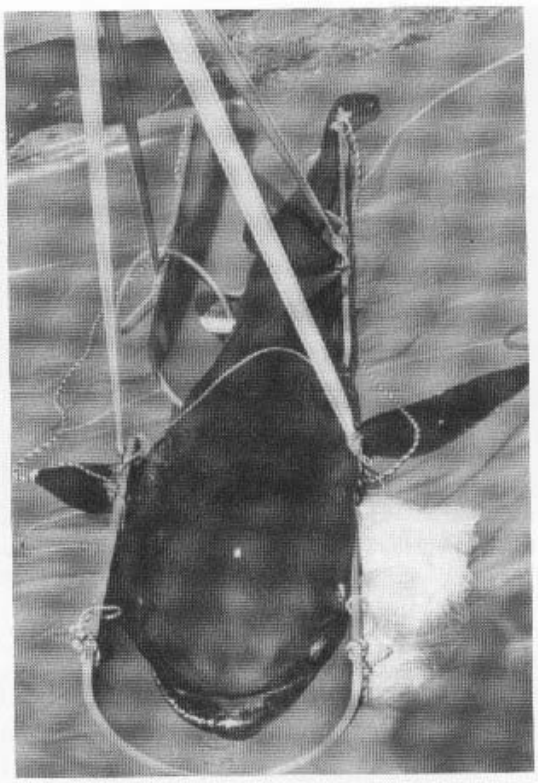

Fig. 6. PVC pipes used for formalin fixation of the megamouth shark, *Megachasma pelagios*.

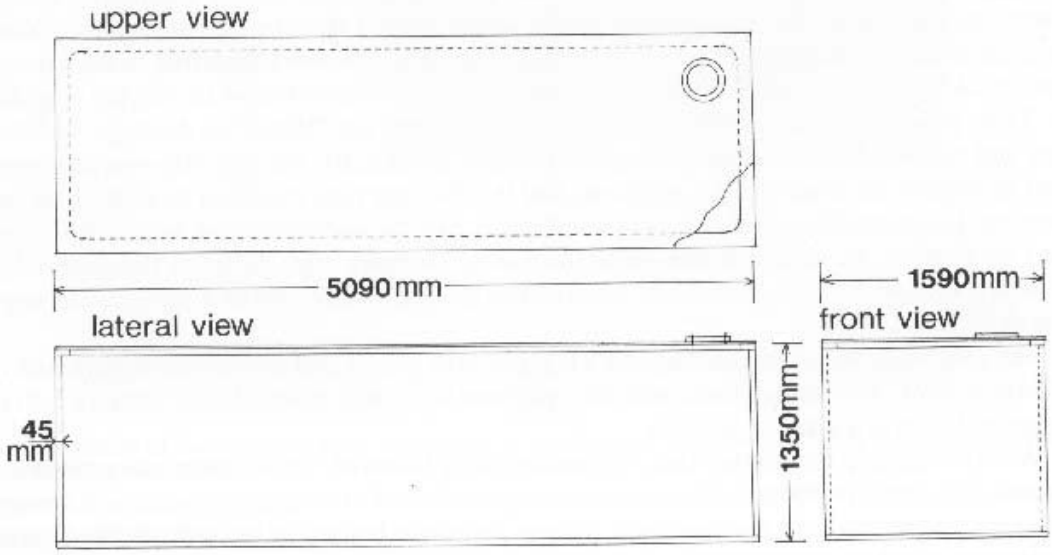

Fig. 7. Manufacturing blueprint of the display tank designed for the megamouth shark, *Megachasma pelagios*.

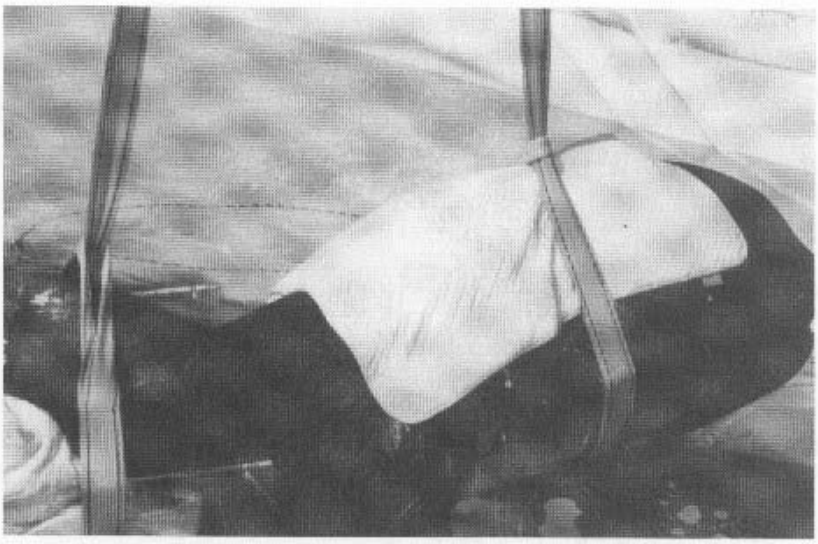

Fig. 8. Transferring of the megamouth specimen on transparent, half-cut cylindrical pipes.

back and fins and prevent folding.

After final positioning of the specimen within the tank, the lid was set into place and then the tank was filled with fresh water and formalin (maintaining a 20% formalin concentration). A small underwater pump was used for one full day to mix the liquid preservative uniformly.

The displayed specimen had minor wrinkles on its back, although the peculiar features of its head and its overall proportions were maintained, which makes it look like a living shark. We managed to keep the shark damage-free. The specimen can be seen from every angle. The procedure, as a whole, was well done. It has been highly evaluated for its display quality (Fig. 10).

About three months after the specimen was placed on public display, the liquid preservative darkened slightly. It seemed to have been caused by dyes leaching from the blankets stuffed in the specimen's coelom. Fortunately, this discoloration faded and the solution regained its original transparency after six months. Subsequent color changes and impurities were not observed as of April, 1996, and additional liquid preservatuve nor total exchange of the preservation fluid have been necessary. The condition of the specimen itself has been stable and no countermeasures need be taken, at least for the time being (although the original black coloration of its body has been fading gradually).

Results and Discussion

The freezing temperature of −30℃ was set by the freezer at the warehouse. The setting condition proved to be adequate because no effect on the body's appearance could be detected after thawing. The ice layer on the body surface was effective in protecting the specimen from dehydration.

During the thawing process, wrinkles appeared on the body surface due to dehydration, but no other substantial effect has been observed. The dissection seemed to be successful, though minor problems with its tissue were seen. It was confirmed that the internal organs and flesh were thawed uniformly and with a high degree of freshness. The diluted sea-water used for thawing was not replenished nor replaced during thawing. No discoloration of the water nor odor were observed. The quality of the water seemed to be adequate as well. The thawing of

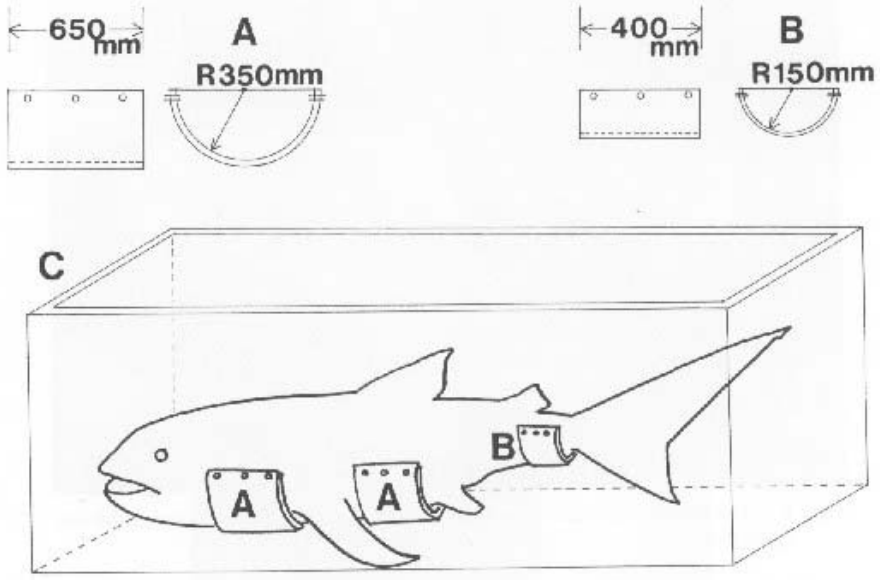

Fig. 9. Transparent MMA pipes for display of the megamouth shark, *Megachasma pelagios*. A, design of transparent MMA pipe for display (700-mm diameter); B, design of transparent MMA pipe for display (150-mm diameter); C, transparent MMA pipes for display of the specimen.

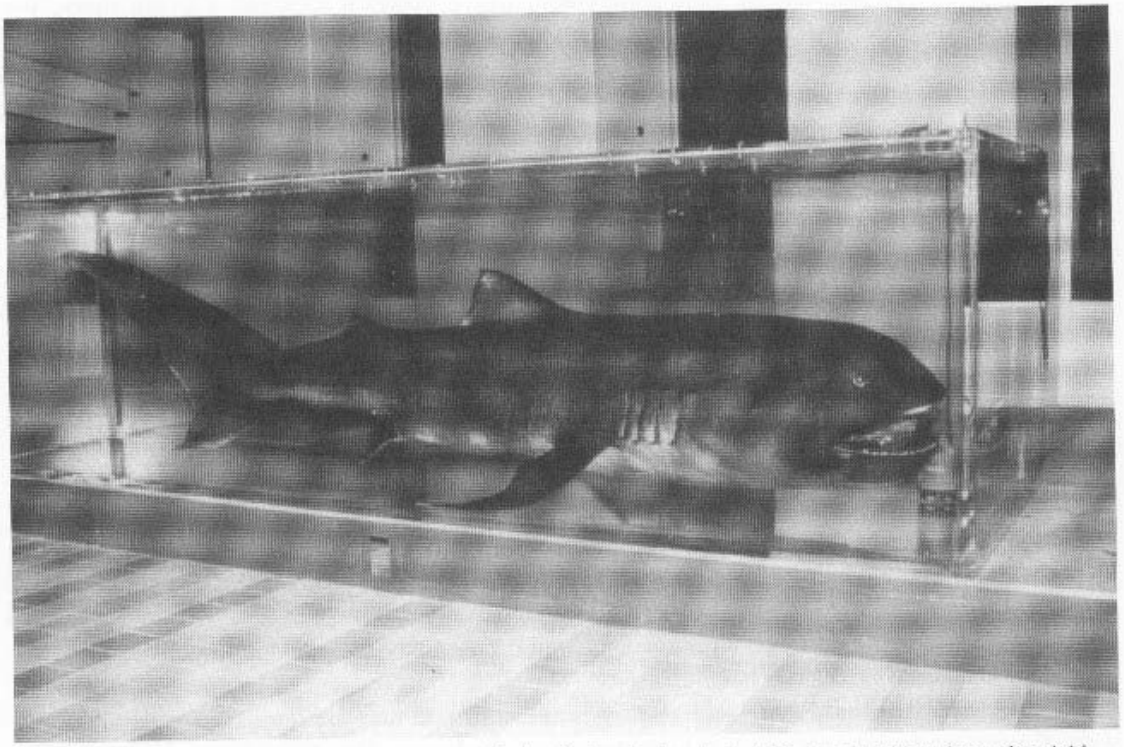

Fig. 10. The megamouth shark, *Megachasma pelagios*, in the display tank at Marine World umino-nakamichi.

large specimens should be performed by circulation of half-strength sea-water (or slightly less) at about 3℃. The duration of the thawing period should be based on the specimen's size (nine days were required for this specimen). Some countermeasures should have been taken in advance in consideration of the formalin fixation process. The fixation tank should be designed with consideration of the planned display. Finally, it was helpful that the hose used to supply formalin to the coelom was inserted into the body during the stitching process.

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