TENTATIVE Colossal Squid FIXING/PRESERVATION NOTES

Foreword

The following account is to be treated as preliminary only. It does not deal with the defrosting process of the colossal squid, dealing only with the process of fixation. However, the following must be strictly adhered to when defrosting the carcass, or damage to it will occur:

- All temptation to prise the specimen apart, especially in an attempt to disentangle the mantle from the head, arms and tentacles to accelerate defrosting, must be resisted. The slightest wrench can snap an arm, tentacle, pull series of suckers from the arms or sucker rings from the suckers, or fracture the skin.
- When handling the mantle great care must be taken not to exert too much pressure with your fingers, as hand and finger impressions remain that prove almost impossible to smooth over (remaining on the final fixed specimen).

Only when the animal is almost completely defrosted will we be in a position to appraise its condition, and this will determine what we do next with it (how it will be fixed). Based on footage and stills of this animal at the time of capture and on deck it would appear to be in excellent condition. However, having been folded over on itself to accommodate it in the $1.2m^3$ bin it is likely to have sustained some damage and organs within the mantle cavity and gladius (pen) are likely to have ruptured or broken, and this will affect how the specimen is handled.

If so then I have a few further recommendations:

- I do not seek to cut the mantle open along the ventral mid-line. Although this will expose the viscera and provide unrestricted access to alimentary and reproductive systems (in particular the stomach and digestive caecum, and to appraise the relative maturity of the individual), doing so will immediately destroy the specimen's structural integrity and aesthetic value.
- A similar amount of scientific information can be discerned from the specimen by making several strategic incisions through the ventral surface of the mantle. The digestive caecum (and contents; i.e. 'stomach contents') can be located and removed through a cut of ~ 20 cm length, and a smaller incision (or core sample) can be made in the posterior-ventral portion of the mantle through which ovarian or testis tissue can be removed, should samples be required. These incisions can then be sewn up, preserving the cosmetic value of the specimen.
- The oesophagus and intestine of large squid are almost invariably empty, so extensive dissection to examine their contents is unnecessarily destructive. I would not recommend any attempt to examine these by way of dissection on this specimen. An alternative approach is to use our (AUTs) endoscope and insert this down the oesophagus and up the intestine; the specimen would need to be completely defrosted for this to work. It is likely to tell us little, but would look good on camera (I am always concerned about cameras).

Precautionary or pre-fixation measures:

A simple test to determine whether squid tissue is ammoniacal is to run Ammonia Litmus paper over the tissue. The pH of formalin solutions fluctuates widely when fixing ammoniacal squid, more so than non-ammoniacal species, and as a consequence sucker rings and hooks tend to corrode rapidly (within hours). Having said this, on the earlier-fixed colossal squid we managed to monitor pH through the fixing process and adjust same as required, using large quantities of sodium bicarbonate (baking soda); I do not like using any borax on squid as it sends the tissue translucent.

It is assumed that the squid will be at all times immersed in water/brine solution, so that its weight is supported.

Should the colossal squid prove to be ammoniacal, and we have good reason to believe that this will be the case, as a precautionary measure normally I would:

1. Remove representative sucker rings and hooks from the arms and tentacle clubs prior to fixing. I do not believe that we will need to do this on this specimen, as I do not believe we will lose them. The only benefit this will provide is that hooks and sucker rings are readily available for descriptive purposes (publication), when access to this specimen at any other time might not prove possible (when on display).
2. Remove at least one statolith. However, as I believe the specimen will be ammoniacal there is a good chance that these will have dissolved during defrosting, or over the past year that the specimen has been frozen.
3. Collect pieces of arm tissue, score well, and place into a jar of 90% ETOH (DNA analyses). Only when this tissue sample has been removed should you proceed to step 4.
4. Inject the squid tissues with **4**% (relatively high as we will not get an opportunity to do this again, once immersed in formalin solution [below]) bicarbonate-buffered formalin solution using a 150cc syringe (or greater), with long screw-on needle (~ 150mm length). This introduces formalin into the tissues faster than it would otherwise penetrate if the specimen if immersed in formalin solution. Should resistance be met then care must be taken not to exert too much pressure on the syringe plunger: unsightly clots of formalin solution can form in the tissues; if the needle is blocked then the plunger can break, resulting in a high-velocity wash of formalin solution being shot backwards. Into each of the mantle, head and arms slowly inject the following volumes of 4% carbonate buffered formalin solution:
    - Mantle (dorsal): (note that the anatomy of colossal squid differs considerably from that of giant squid, so the following, a guide for giant squid will need to be modified for the colossal once the position of the digestive caecum has been determined) ~ 2 litres injected mid-dorsally, deep into the mantle, to reach the digestive gland (for giant squid); about 1 litre injected in the posterior-most portion of the mantle (~ mid-fin length) to reach either testis or ovarian tissue (for colossal squid and giant squid). Mantle (ventral): ~ 1 litre either side of the ventral mid-line, in the posterior third of the mantle to reach renal and cardiac tissue; and ~ 1 litre on the animals left hand side in the posterior quarter of the mantle to reach the digestive caecum (in the

event we have not made an incision to remove the contents of this caecum earlier).

- Head: dorsal and ventral mantle and head injections are required; ~ 1 litre of formalin solution needs to be injected into the cranium to fix oesophageal, nervous and buccal tissue.
- Arms: at distances of ~ 30 cm from the arm base, down each arm, ~ 300 ml of formalin solution, progressively decreasing the volume as the tips attenuate.

Fixing tank and formalin solution preparation

1. For an ~ 500 kg squid I would be comfortable with no less than a 4:1 formalin solution/squid ratio. Until the squid is defrosted it will be almost impossible to determine the most appropriate fixing tank size and dimension, and I believe one is being procured and there will need to be some compromise. My recollection is that the squid is in a bin of 1.2m x 1.2m x 1.2m, but does not fully fill the bin (perhaps it is 0.3m from the top), and as such the squid is probably of $1m^3$ dimension, requiring $4m^3$ of formalin solution (therefore the fixing bin volume should be no less than $5m^3$). Pre-mark levels inside on the tank wall to indicate volume. Assuming 4000 litres of formalin solution are required, add **80** litres of concentrated formalin (37%) to 4000 litres of 31ppt salt water to make a stock solution of **2**%, agitate well, record pH, buffer accordingly (to bring to 7), and continue to agitate.

2. Sodium bicarbonate (baking soda) is my buffer of choice, as opposed to using sodium borate (borax), calcium carbonate (shell) or marble chip. Borax tends to render the tissues translucent, whereas shell and marble chip are ineffective for rapid buffering during the first week of fixation. To fix a 500kg colossal squid I would expect to use anywhere up to 50kg of sodium bicarbonate through the entire fixing process (one month), but perhaps only 3kg on day 1 to neutralise the initial **2**% formalin solution.

The following is entire guesswork, based on dealing with *Architeuthis*. 24 hour (shift work) access must be available to this colossal squid. I would envisage monitoring of the pH over the first 24 hours from 1, 2, 3 and 4 hours after initial immersion in **2**% solution, and depending on fluctuations during this period, every 2 or 3 hours thereafter.

After 24 hours another 80 litres of formalin solution would be added to the fixing tank to bring the formalin solution to **4**%. Again, 24-hour access must be available to this colossal squid. The solution would need to be immediately buffered, and monitoring of pH would need to resume for a further 24 hours, from 1, 2, 3 and 4 hours after initial immersion in **4**% solution, and depending on fluctuations during this period, every 2 or 3 hours thereafter.

After 48 hours another 80 litres of formalin solution would be added to the fixing tank to bring the formalin solution to **6**%. Again, 24-hour access must be available to this colossal squid. Similarly, upon addition of the new formalin, the solution would need to be immediately buffered, and monitoring of pH would resume for a further 24 hours, from 1, 2, 3 and 4 hours after initial immersion in **6**% solution, and depending on fluctuations during this period, every 2 or 3 hours thereafter.

After 72 hours a final 80 litres of formalin solution would be added to the fixing tank to bring the formalin solution to **8**%. As earlier, 24-hour access is required, and upon addition of the new formalin, the solution would need to be immediately buffered, with pH monitoring for a further 24 hours, from 1, 2, 3 and 4 hours after initial immersion in **8**% solution, and depending on fluctuations during this period, every 2 or 3 hours thereafter.

After 96 hours (4 days fixing) the condition of the squid will be appraised, and should a further 80 litres of formalin be required it will be added. I believe that 6% formalin solution would suffice, but 8% is proposed as a precautionary measure; 10% is probably too strong. After 96 hours the pH would need to be regularly monitored, no less than twice each day (morning and evening) until such a time as it stabilises (probably 1–1.5 weeks after initial fixing).

Monitoring the squid and pH

The formalin solution in which the squid is immersed needs to be regularly monitored for the first 7 days, but particularly the first 72 hours. Although the squid was immersed into a neutral formalin solution, the pH can dive to 3 or 4 if left unchecked within the first 5 hours the animal has been immersed in it; if left unchecked for 24 hours all suckers rings are likely to be completely destroyed. Sodium bicarbonate must be added to the solution, either in powder or slurry form, with the entire solution then being manually agitated. Measure pH after the solution has been thoroughly agitated.

When adding bicarbonate (to excess) to the formalin solution, care must be taken not to let any settle on squid tissue. Should precipitation occur, especially on the tentacle clubs and within the club suckers, the tissues corrode. By gently gripping the anterior margin of the mantle, raising and lowering it, fresh formalin solution can be circulated throughout the mantle; this exercise should be repeated during every pH check (every 3 hours for the first 24 hours). Similarly, mucous deposits around the buccal membrane and base of arms need to be wiped free of the fixing squid, and the arm crown periodically agitated to ensure fresh formalin solution is exposed to the inside of the arms.

By the end of the first week the buffered, discoloured, oxidised squid and formalin solution is a disgusting yellow to red-brown soup. Monitoring of pH is best achieved with a digital meter, as litmus paper and coloured solutions no longer give interpretable results.

For best results (in the event the specimen is destined for display), the discoloured formalin solution should be replaced with fresh solution so as not to unduly discolour the squid; if not then no damage is done to it by leaving it in. However, the specimen must remain in the formalin solution for at least a month in order for it to fix thoroughly.

Preservation

Following the month in formalin solution the specimen needs to be thoroughly soaked and the mantle cavity gently flushed with low velocity water to remove residual formalin and miscellaneous grunge. Three water changes are recommended.