In ovo sexing as a management tool for the Southern Screamer (*Chauna torquata*) AZA Species Survival Plan®

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History of screamers in AZA

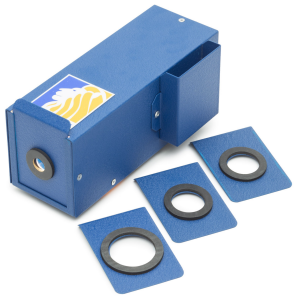
- Monogamous pair bonds, cooperative parenting
- Most commonly housed in 1.1 pairs or as singletons
- Generally long-lived (late 20s-late 30s)
- Skewed sex ratio 61.45.9
- Historically low chick survivability (~40%)
  - Impaction
  - GI issues

Why *in ovo* sexing?

- Limited amount of space and resources available
- Better planning regarding placement and management
- There is a need for facilities to try same sex and bachelor groups

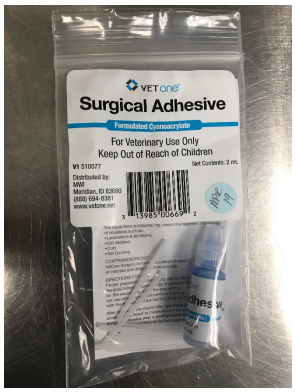
Materials Used

- Lyon High-Intensity LED candler or LED flashlight
- Fine point Sharpie® or pencil
- Dremel® Flexible Shaft Model 225 rotary hand tool
- 2mm diamond wheel point bit



Materials Used

- Tegaderm™
- Dilute 5% chlorhexadine diacetate solution (Nolvasan)
- 0.5mL insulin syringe with a 28.5 gauge needle
- Vet One™ surgical adhesive
- Sterile gauze
- Saline solution

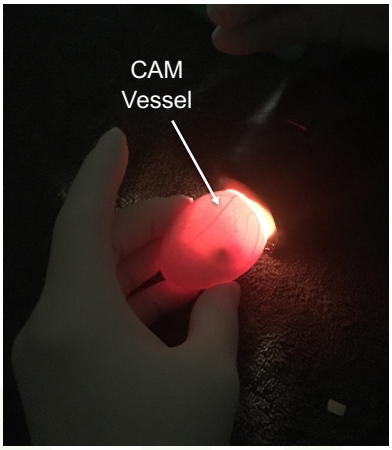


Prior to the procedure

- Estimated incubation window using 42-46 days
- Scheduled for halfway mark in development
- Pulled eggs from parents ~day 20, replaced with resin dummy eggs

Selecting a site

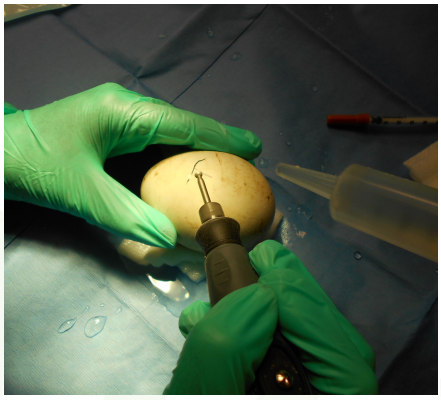
- Prominent chorioallantoic membrane (CAM) vessel
- Away from air cell, embryo
- Clean selected area with dilute Nolvasan prior to markup
- If doing a blind stick, trace with Sharpie® or pencil
- If drawing blood while illuminated by LED light, no need to trace



Making a window

Light, circular dremeling over desired area.

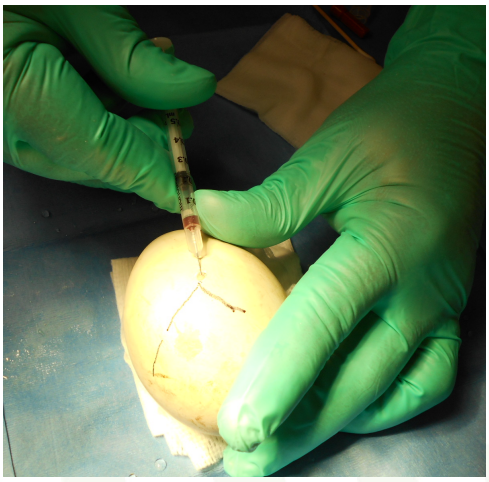
Making a window



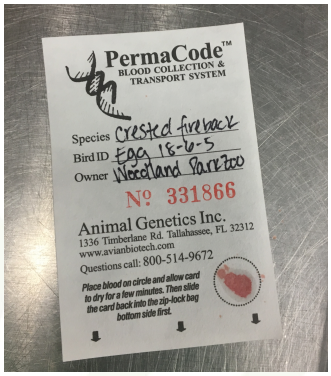
- Saline solution to rinse away debris and prevent overheating
- Stop once through cuticle, taking care not to rupture membranes
- Hole just needs to be slightly larger than the needle (~2mm) – take into account that the blood is drawn at an angle

The blood draw

- Shallow angle
- In line with markings or blood vessel, if candling
- Do not push down on plunger after a draw attempt – can inject air accidentally
- Hold egg at angle so insertion site is below midline – reduces potential for introduction of air



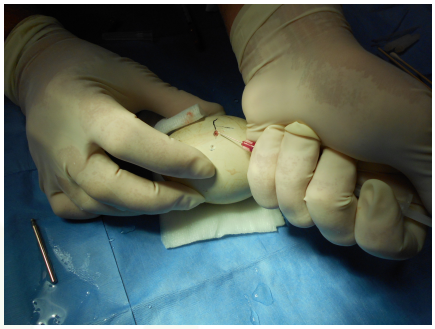
Preparing the sample



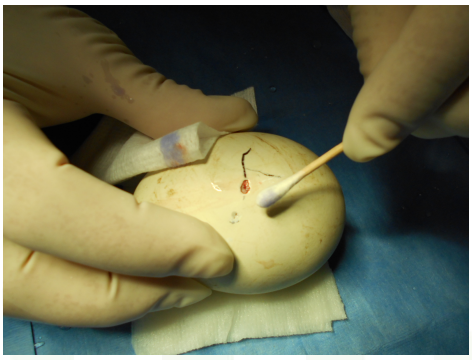
Don't be greedy!

- Pure blood is great, but pink, blood-tinged fluid provides plenty of DNA
- Just need a drop
- For screamers, drew ~.03cc
- Submitted to Avian Biotech in Florida

Patching things up

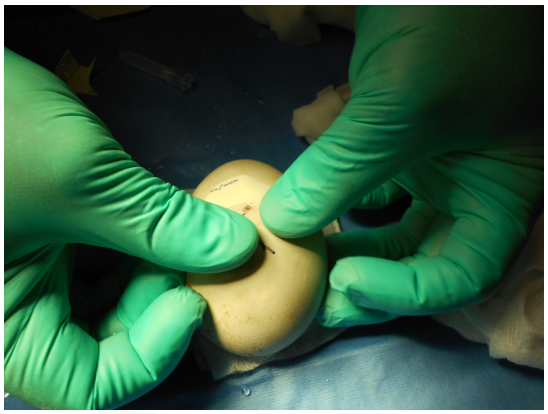


Apply drop of Vet One™ surgical adhesive, clean up excess.



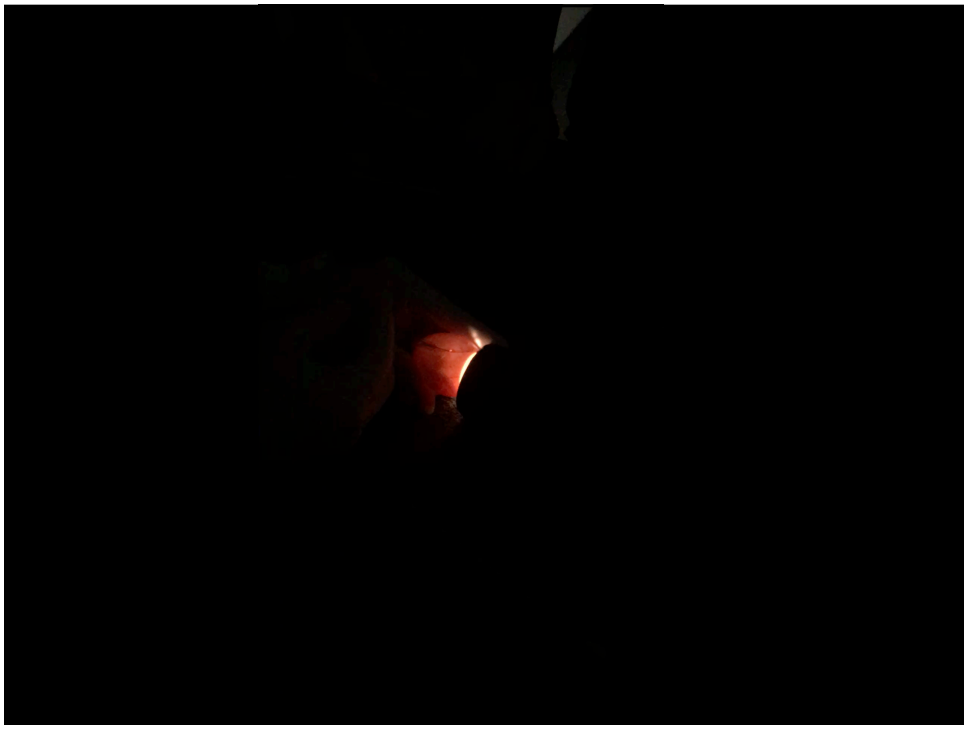
Patching things up

Cut Tegaderm™ to size and apply to blood draw site, keeping potential air cell drawdown and where the chick will potentially externally pip in mind.



Patching things up

- Artificial incubation to avoid puncturing and introduction of harmful bacteria
- Rcom Max20
- 36.9ºC/98.4ºF
- 60% relative humidity
- Hand-turned 3-5x/day
- Returned to parents once vocalizing/internally pipped



What happens to the untargeted sex?

- Humanely euthanized
  - A large hole was created in the shell overlying the air cell of each egg, the eggs were placed within a chamber, and they were continuously exposed to carbon dioxide gas for > 3 hours before placing in the refrigerator
- Full necropsies were performed on the embryos

Acknowledgements

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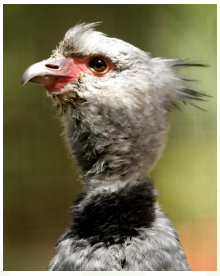

Recommended Resources

Dutton, C. J. and A. Tieber. A modified protocol for sex identification of *in ovo* avian embryos and its application as a management tool for endangered species conservation programs. *Journal of Zoo and Wildlife Medicine* **32**,176-180 (2001).

Jensen, T., Mace, M. & Durrant, B. Sexing of mid-incubation avian embryos as a management tool for zoological breeding programs. *Zoo Biology* **31,** 694–704 (2011).

Kjelland, M. E., Blue-Mclendon, A. & Kraemer, D. Determining air cell location and embryo development in opaque shelled eggs. *Avian Biology Research* **5,** 99–102 (2012).