SDJV PROJECT #148: MEASURING PENTOSIDINE IN SKIN BIOPSY SAMPLES TO RELIABLY AGE SCOTERS –(Agreement F16AC00145)

~Summary Report 2019~

Lucas Savoy
Biodiversity Research Institute
276 Canco Road
Portland, Maine 04103
lucas.savoy@briloon.org
Tele: 207-839-7600, ext. 104

Project Investigators:
Lucas Savoy, Biodiversity Research Institute, Portland, Maine 04103;
lucas.savoy@briloon.org; Tel: 207-839-7600, ext 104
Jesse Fallon, Virginia Tech, Blacksburg, VA 24061; jfallon@vt.edu; Tel: 304-685-8762
Hillar Klandorf, West Virginia University, Morgantown, West Virginia 26506

Project co-Investigators:
Alicia Berlin-Wells, USGS Patuxent Wildlife Research Center, Laurel, MD
Glenn Olsen, USGS Patuxent Wildlife Research Center, Laurel, MD
Arnold Schouten, Dry Creek Waterfowl, Port Angeles, WA
Sandy Smith, DVM, NW Wilderness Veterinary Services, Port Angeles, WA
Chuck Cerbini, Toledo Zoo, Toledo, OH
Nick Tiberio, Livingston Ripley Waterfowl Conservancy, Litchfield, CT

Project Description:

Recent declines in many of North America's 15 sea duck species populations have caused concern among wildlife managers and conservationists on the sustainability of their populations. The reasons for these declines are not currently understood. Since 1999, the Sea Duck Joint Venture (SDJV) partnership has learned a vast amount about sea duck ecology and management (Savard et al. 2015). However, many aspects are still lacking or remain unknown for some species. In particular, reliable population indices and survival estimates for most sea duck species remain inadequately studied. The SDJV has recently identified the surf scoter (*Melanitta perspicillata*) as a priority species to investigate annual survivorship and longevity, in an effort to improve surf scoter harvest assessment.

Currently, data available to evaluate surf scoter annual survival and longevity is extremely limited and consists of recovery and recaptures of banded birds through relatively small-scale capture efforts. The initiation of larger and longer-term capture and banding efforts of surf scoters would provide insightful, information but has its limitations due to the costs and effort required to perform sea duck banding projects and the dependency on hunters reporting banded birds. Alternative or parallel studies are needed to enhance our knowledge of wild surf scoter annual survivorship and longevity.

Current aging techniques (i.e., plumage, bursal measurements) for scoters can reliably age individuals up to 3-years of age (Carney 1992; Iverson et al. 2003). Most scoter species are believed to be long-lived (9-18 years; Mallory 2015), and often do not begin breeding until two or three years of age. The ability to age live in the hand and hunter harvest scoters beyond 3 years would greatly enhance our knowledge of survivorship, longevity, and improve harvest assessments.

Pentosidine is a naturally-occurring protein crosslink that forms in all animals, including birds, and is accumulated and stored in skin collagen, throughout an individual's lifetime (Sell et al. 1996). Previous studies have successfully measured pentosidine concentrations among several wild and captive bird species (Chaney et al. 2003; Fallon et al. 2006a; Cooey et al. 2010; Rattiste et al. 2015; Dorr et al. 2017) (Table 1). Pentosidine accumulates more rapidly in short-lived bird species and at a more gradual rate in longer-lived species (Fallon et al. 2006a; Cooey 2008). Cooey et al. (2010) refined a technique to safely and efficiently collect a small skin sample from live birds.

Table 1. Previous studies assessing pentosidine concentrations in birds.

Common name	**Scientific name**	**n**	**Age range (months)**	**Sampling location**	**Reference**
California gull	*Larus callifornicus*	17	48-288	foot webbing	Chaney et al. 2003
Black vulture	*Coragyps atratus*	28	unk	breast, patagium	Cooey et al. 2010
Monk parakeet	*Myiopsitta monachus*	105	1-60	breast, patagium	Cooey et al. 2010
Double-crested cormorant	*Phalacrocorax auritus*	36	12-60	breast	Dorr et al. 2017
Double-crested cormorant	Phalacrocorax auritus	19	unk	breast	Fallon et al. 2006
Ruffed grouse	*Bonasa umbellus*	52	<1 - 120	breast	Fallon et al. 2006
Common gull	*Larus canus*	47	24-360	patagium	Rattiste et al. 2015

Pentosidine can accumulate at varying rates and levels between species with differing life histories (Fallon et al. 2006b), indicating a species specific or potentially a genus specific age index is required for accurately aging scoters. A quantitative relationship can be formulated using pentosidine concentrations in the skin of known-aged scoters and comparing to the age of the individual in years. Previous studies have successfully determined the quantitative relationship in double-crested cormorants (*Phalacrocorax auritus*) ($Y=0.1914x + 6.6701$; $r^2=0.93$, (Fallon et al. 2006b; Cooey 2008), ruffed grouse (*Bonasa umbellus*) ($Y=0.0059x^2 + 0.1551x + 11.636$, $r^2=0.87$, (Fallon et al. 2006a, b), and a composite of 29 species of wild birds ($Y=0.2047x + 7.4725$, $r^2 = 0.73$, (Chaney et al. 2003).

Several captive bird facilities in the US contain known-aged surf scoter and white-winged scoters (*Melanitta fusca*) and from a wide range of ages spanning from hatch year through 17 yrs in surf scoters and hatch year through 18 yrs in white-winged scoters (Figure 1). We collected skin samples from known-aged captive surf scoter and white-winged scoter to evaluate the technique of measuring pentosidine to establish a scoter age index curve. The development of an accurate scoter age index curve would establish a novel wildlife management tool which could be used to age wild scoters, either through live-capture and sampling efforts or the use of parts collections, such as hunter shot birds or wings collected through the annual flyway harvest surveys. The major goal of this study is to provide a quantitative age index model for scoters to be utilized in subsequent sampling efforts of live or harvested scoters, to evaluate longevity and survivorship parameters in wild North American scoters.

Objectives:

1) Utilize captive rearing facilities to collect skin biopsy samples from known-aged captive surf scoter and white-winged scoter.
2) Measure pentosidine concentrations in scoter skin samples from known-aged individuals through laboratory analysis.
3) Compare the quantitative age index curve between surf scoters and white-winged scoters to determine if results are comparable among scoter species.
4) Establish a quantitative scoter age index tool for wildlife managers and researchers to utilize in evaluating surf scoter and white-winged scoter survival rates, longevity, and harvest assessments through the subsequent sampling of live, deceased (carcasses), or wings (USFWS Wing Bee/hunter harvest) of unknown-aged scoters.

Methods:

Skin Sample Collection

We collected small skin samples from known-aged live surf scoters and both live and frozen deceased known-aged white-winged scoters among three waterfowl rearing programs: 1) Dry Creek Waterfowl (Port Angeles, WA), 2) U.S. Geological Survey Patuxent Wildlife Research Center (PWRC) (Laurel, MD), and 3) Livingston Ripley Waterfowl Conservancy

(Litchfield, CT). The sampling occurred between March-September 2016 and March 2017. Surf scoters were sampled solely at PWRC while white-winged scoters were sampled at all three facilities. From carcasses, we collected a small skin sample using sterile scalpel blades and forceps from three locations: 1) patagium, 2) breast, and 3) shoulder in order to compare pentosidine concentrations from multiple locations. From live birds, we collected a skin sample from the patagium only using a Sklar Tru-Punch© disposable 6mm biopsy punch using similar sterilized methods described in Cooey et al. (2010). Prior to sampling live birds, lidocaine was injected under the skin near the sampling location to alleviate discomfort derived from skin sampling. The sampling area was damped with isopropyl alcohol to disinfect the area and expose the skin. The disposable biopsy punch was placed on the exposed area and rotated to collect the skin sample. The incision location was closed with either tissue glue or sutures. Each collected skin sample was placed in a sterile vile and frozen (-25°C) immediately until analysis (Cooey 2008). Frozen samples were shipped overnight for analysis to West Virginia University (WVU), Morgantown, West Virginia.

Laboratory Analysis

Skin samples were prepared by removing adipose tissue and subdermal layers followed by mincing. Then depilation (5 ml of 2:1 chloroform: methanol solution for 18 h on an agitator in a 4°C cold room, rehydration using 2-3 ml of 1:1 methanol: distilled water solution for 2 h at 20°C, acid hydrolysis in 1 ml of ml of nitrogen flushed 6N HCl per 10 mg skin incubated 18 h at 110°C. This is followed by acid evaporation in a centrifuge/dryer, a second rehydration with 500 µl distilled water, and filtering in a centrifuge tube filter at 4,000 rpm for 10 minutes. Collagen content is then determined through spectrophotometric hydroxyproline analysis using a DU 640 spectrophotometer (Beckman Coulter, Fullerton, California) at a 564 wave length. Pentosidine concentrations are then determined through reverse-phase high performance liquid chromatography.

Results:

We collected a total of 81 skin samples from 62 individuals (29 surf scoters and 33 white-winged scoters) (Table 2) of known-age among three captive facilities: 1) Livingston Ripley Waterfowl Conservancy, 2) USGS Patuxent Wildlife Research Center, and 3) Dry Creek Waterfowl. Among these samples, 10 white-winged scoters were previously deceased and from those individuals, we collected skin samples from three distinct areas: 1) patagium, 2) breast, and 3) shoulder. Our intention is to compare pentosidine concentrations between differing locations on the bird, as previous studies have found stronger correlations at different sampling areas, depending on species.

Table 2. Summary of tissue samples collected from captive surf scoter and white-winged.

Species	n	Age Range (yrs)
SUSC	29	0.4 – 13.7
WWSC	33	0.3 – 18.2
Totals	**62**	**0.3 – 18.2**

We compared results among the three tissue sampling locations (i.e., breast, patagium, shoulder) (Figures 1-3). Skin samples collected from the breast (r=0.94) and shoulder (r=0.79) provided the best predictors of age in scoters (Figures 1 and 2). Samples collected

from the patagium (Figure 3) resulted in a weak correlation (r=0.26) between pentosidine concentrations and scoter age.

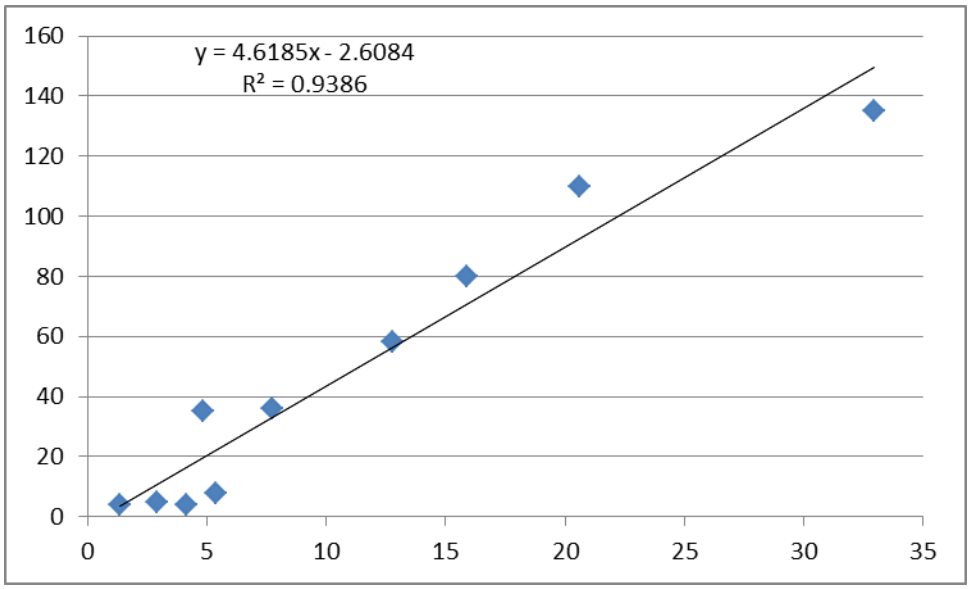

Figure 1. Pentoside concentrations in relation to age (months) from skin samples collected from the breast of white-winged scoters.

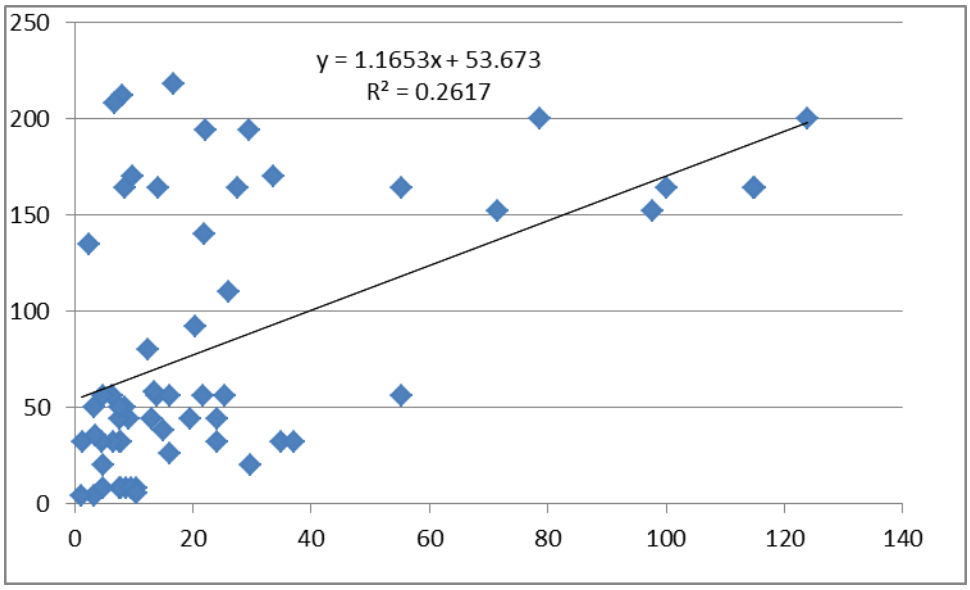

Figure 2. Pentoside concentrations in relation to age (months) from skin samples collected from the patagium of white-winged and surf scoters.

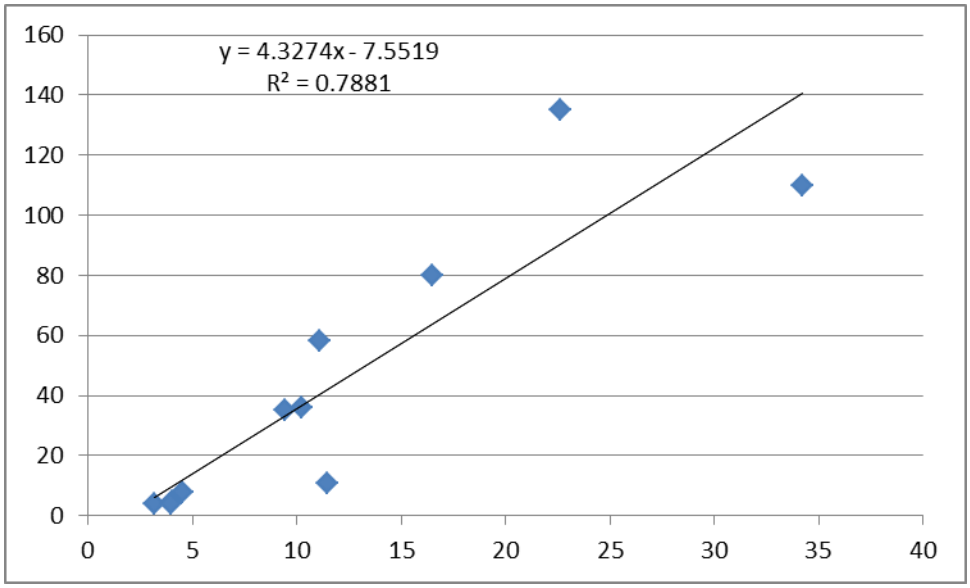

Figure 3. Pentoside concentrations in relation to age (months) from skin samples collected from the shoulder of white-winged scoters.

Discussion:

The reason for the weaker correlation observed in patagium samples is unclear. Several previous studies determined samples collected from a bird's patagium were strong predictors of an individual's age. Due to this assumption, we focused on collecting samples from this area on all live scoters, since sampling of multiple areas on live birds is not advisable. The deceased scoters provided an opportunity to compare samples from multiple sampling locations of each bird. Our preliminary findings suggest samples collected from the breast and shoulder are the best predictors of a scoter's age and continued sampling of scoters should focus on acquiring additional samples from these areas. All surf scoter sampling was performed on live birds, and therefore, samples were only collected from the patagium. Further sampling of known-aged captive scoters is needed to increase sample size and strengthen confidence in statistical analyses in developing a scoter age curve using skin samples measured for pentosidine concentrations.

The captive facilities still maintain both live and newly deceased scoters and are willing to continue collaborations. Although the same individuals we previously sampled in 2016 and 2017 exist in captivity and would be available to utilize again, those birds would be 3-4 years older in 2020, providing potentially new age classes and an opportunity to compare pentosidine concentrations in the same individuals over time.

Providing additional sampling and results continues to show strong correlations between skin samples and an individual's age, the next step would be sample and analyze wild, unknown-aged scoters to determine their ages and assess the population age structure. Utilizing hunter harvest scoters would allow for samples to be collected from both the breast and shoulder areas and wings submitted to the annual Wing Bee would facilitate collection of shoulder samples. Combined, wildlife managers could begin assessing scoter population indices and survival estimates.

Literature Cited:

Carney SM (1992) Species, age and sex identification of ducks using wing plumage. US Department of the Interior, US Fish and Wildlife Service, Washington, D.C.

Chaney R, Blemings K, Bonner J, Klandorf H (2003) Pentosidine as a measure of chronological age in wild birds. Auk 120:394–399. doi: 10.1642/0004-8038(2003)120[0394:PAAMOC]2.0.CO;2

Cooey CK (2008) Development and Evaluation of a Minimally Invasive Sampling Technique to Estimate the Age of Living Birds. Ms Thesis. West Virginia University, Morgantown, WV.

Cooey CK, Fallon JA, Avery ML, et al (2010) Refinement of biomarker pentosidine methodology for use on aging birds. Human-Wildlife Interact 4:304–314.

Dorr BS, Stahl RS, Hanson-Dorr KC, Furcolow CA (2017) Using pentosidine and hydroxyproline to predict age and sex in an avian species. Ecol Evol. doi: 10.1002/ece3.3388

Fallon JA, Cochrane RL, Dorr B (2006a) Interspecies comparison of pentosidine accumulation and its correlation with age in birds. Auk 123:870–876.

Fallon JA, Radke WJ, Klandorf H (2006b) Stability of pentosidine concentrations in museum study skins. Auk 123:148–152.

Iverson SA, Esler D, Boyd SW (2003) Plumage Characteristics as an Indicator of Age Class in the Surf Scoter. Waterbirds 26:56–61.

Mallory ML (2015) Site Fidelity, Breeding Habitats, and the Reproductive Strategies of Sea Ducks. In: Ecology and conservation of North American sea ducks. pp 337–364.

Rattiste K, Klandorf H, Urvik J, et al (2015) Skin pentosidine and telomere length do not covary with age in a long-lived seabird. Biogerontology 16:435–441. doi: 10.1007/s10522-015-9564-1

Savard J-PL, Derksen DV, Esler D, Eadie JM (2015) Ecology and Conservation of North American Sea Ducks, Vol. 46. CRC Press.

Sell, D.R., M.A. Lane, W.A. Johnson, E.J. Masoro, O.B. Mock, K.M. Reiser, J.F. Fogarty, R.G. Cutler, D.K. Ingram, G.S. Roth, and V.M. Monnier. 1996. Longevity and the genetic determination of collagen glycoxidation kinetics in mammalian senescence. Proceedings of the National Academy of Sciences USA 93:485-490.