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Running head: ***Photography for studies of moult***

Photography is an efficient method to study avian moult

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Methods to obtain moult data from wild birds have not changed much over the last century and most studies still depend on checking museum specimens or capturing birds. Here we assess the applicability of systematic photography for detecting and scoring moult in adult Black Skimmers from southern Brazil. Moult data from photographs have a high within- (R_{GLMM} = 0.98) and between- (R_{GLMM} = 0.97) observer repeatability and show very good fit to current moult Underhill-Zucchini models (R^2 = 0.75). Photography has advantages of being less invasive, requiring less equipment and human effort, being feasible in areas where captures may not be possible, and causing less disturbance so enhancing the number of sampled individuals.

Keywords: non-invasive moult assessment, Underhill-Zucchini moult model, feathers, Black Skimmers

Moult in birds is an evolutionary strategy of feather renewal that influences flight efficiency, thermoregulation and seasonal appearance, and therefore has fitness consequences at key stages in birds' lifecycles, such as breeding and migration (Newton 2009). Assessing the moult process can provide better understanding of individuals' choice and use of resources and thus also of breeding, migratory and foraging strategies (Newton 2009). Methods to obtain moult data from wild birds did not change much in the last century and largely depend on scoring feathers or verifying the presence/absence of moult of museum specimens and/or captured birds (e.g. Newton 1966, Underhill & Zucchini 1988, Newton & Rothery 2009, Scherer *et al.* 2013, Morrison *et al.* 2015).. However, such data may also be acquired with other methods such as photography, a method that has been used to study moult in marine mammals (McConkey *et al.* 2002).

Opportunistically taken photographs of birds in moult have been used to complement information based on conventional methods (Snyder *et al.* 1987, Ryan 2013, Zuberogoitia *et al.* 2016). Keijl (2011) suggested that photography would be a promising way to study moult in pelagic seabirds that are difficult to catch. Bugoni *et al.* (2015) studied seabird moult by catching birds at sea, and they also presented photographs to show whether feathers were moulted or not. González-Solís *et al*. (2011) used photographs from websites to confirm the moulting patterns described in the literature to determine what feathers to use for stable isotope analysis. However, few studies have yet used photography as a systematic method to study moult nor compared its performance with other methods. We took photographs of Black Skimmers (*Rynchops niger*) from southern Brazil during the moulting period and scored their moult from the photographs. Here we assess the repeatability of photographic moult scoring and compare its performance in typically used moult models to data acquired from the same and other species using traditional methods. This study thus explores the value of systematic use of photography as a method to study moult in birds.

METHODS

We studied Black Skimmers on the Island of Santa Catarina in southern Brazil during the moulting period. From October 2015 to April 2016, photographs of flocks were taken with a Canon© EOS Rebel T1i SLR camera using fast shutter speed (≥1/4000 s) and a 75-300mm lens during two sessions each month in the estuary of Ponta das Canas (27°24'26"S, 48°25'41"W). Each session lasted two hours and involved walking systematically along two parallel 650 m long line transects 100 m apart from each other, covering all habitats suitable for Black Skimmers. Limiting each session to two hours was intended to minimize the chances that individuals were photographed more than once during the same session. We took 2,054 photographs with most of them containing one bird, yet photographs could contain up to 130 individuals with open wings while flying, landing, taking off or stretching (Fig. 1A-D). Whenever possible we viewed the upper-wings of birds, although under-wings were also suitable (Fig. 1B).

The 2,054 photographs contained a total of 2,278 skimmers and we could record a moult score for 1,418 individuals, representing 62% of all birds detected. We used the traditional scoring system allocating a score between 0 and 5 for each of the ten primary feathers (Fig. 1E, Newton 1966, Underhill & Zucchini 1988). Moult differs from incidental feather replacement by having the same pattern on both wings (Pyle 2008). Because moult pattern is similar for both wings (Pyle 2008) the score was given to the more visible wing. Old feathers scored as 0 were recognised by full length, dull colour and at least some wear (Fig. 1E). Feathers missing or in small pin stage were scored as 1 while feathers in large pin or brush stage grown up to a third of their full length when compared to old feathers were scored as 2. Feather brushes grown half of their full length were scored as 3. Feathers grown to half to three-quarters of their full length were scored as 4, and feathers grown more than three-quarters of their full length with bright colour and no wear were scored as 5 (Fig. 1E). The moult scores of all individual primaries of one wing were summed and then divided by the maximum score possible (= 50). The resulting moult index ranged between 0 and 1 and was used as response variable in the moult model.

To test within- and between-observer repeatability of moult scoring we re-sampled 20 randomly selected photographs containing on average 4.5 individuals per photograph that could be scored, yielding a total of 91 moult indices. For the within-observer repeatability the second scoring was performed around five months after the first scoring by the same person without checking any information relating to those photos. To assess the between-observer repeatability the same 20 photographs were also scored by three additional people that had no previous information on any of the photographs. Each of the within- and the between-observer repeatability was tested using a general linear mixed model (R_{GLMM}) with original scale, 100 bootstraps and 100 permutations using the *rptR* package (Nakagawa & Schielzeth 2010) in R 3.2.4 (R Core Development Team 2016). As moult indices were not normally distributed we used a logarithmic transformation and applied a Bland-Altman plot of estimates against each other using the *MethComp* package in R 3.2.4 (Carstensen *et al.* 2013). The Bland-Altman plots the mean difference of the indices against the average value of these indices to test significance of bias between measures. It also considers the limits of agreement to assess how much variation occurs within the 95% interval of one scoring compared to the other. An ideal within- or between-observer repeatability is expected to present a mean difference of zero and all estimates within the limits of agreement (Bland & Altman 1999).

From the moult indices derived from the photographs we estimated the duration and timing of moult by plotting the moult index (response variable) against date and applying the Underhill-Zucchini (UZ) model that uses a likelihood approach to estimate timing and duration of moult in a population assuming independent observations (Underhill & Zucchini 1988) implemented in the *moult* 2.0 package in R 3.2.4 (Erni *et al.* 2013). Date was considered the number of days from 30th September (1st October = day 1) when the photograph was taken. We specified type 3 data considering only individuals in moult, therefore excluding individuals scored as 0 and 1 (Underhill *et al.* 1990), so that 519 moult indices were considered in this analysis.

We compared our photographic moult indices with conventionally derived indices from captures from other studies. The comparison between the methods was based on standard error values from the UZ models and the R^2-values of the estimated moult trajectories. The model explains variation in the moult index in relation to date, but additional variation may occur due to individual differences in moult dynamics, for example due to age, sex or body condition. Assuming different populations have a similar composition, any differences in the accuracy of scoring between methods could introduce additional variation. Thus, if the R^2-value from the photographic moult scoring falls within the range of models using conventional data, it is unlikely that the photographic method has introduced additional variation. We compared our photographic moult indices with capture data for the same species in Scherer *et al.* (2013). Raw data were obtained from the authors for 58 Black Skimmers mist-netted at Lagoa do Peixe (31º21’18”S, 51º03’03”W) in southern Brazil during the non-breeding seasons between October 2010 and April 2012. We also compared the R^2-value of our data set to those from other studies that analysed temporal variation in moult using the same scoring principle,) although unfortunately few studies published the performance of their data (Underhill & Zucchini 1988, Newton & Rothery 2009).

RESULTS

The within-observer repeatability showed photography allows for consistent scoring of primary moult (R_{GLMM} = 0.983 ± 0.015 (SE), 95% confidence interval = 0.903 – 0.995, P = 0.01). The mean difference between the two repeated moult indices by the same observer was -0.02, limits of agreement: -0.20, 0.17 (Fig. 2A). The between-observer repeatability was also high (R_{GLMM} = 0.969 ± 0.062, 95% confidence interval = 0.939 – 0.986, P = 0.01) with a mean difference between the four observers’ indices of -0.009, limits of agreement: -0.012, 0.006 (Fig. 2B). The number of moult indices out of the limits of agreement was higher for the between- than for the within-observer repeatability (Fig. 2).

The photographic moult indices provided a larger sample size and showed a better fit to the UZ model than the data collected from the nearby Black Skimmer population scored conventionally in the hand. The UZ models applied to data from Scherer *et al*. (2013) provided estimates of timing and duration of moult with large standard errors (duration = 270.2 ± 182 days; mean start date = 5 ± 92.2). Our estimates for duration and mean start date of moult based on the UZ model (Fig. 3) had narrower standard errors and fell within the band of estimates derived from the conventionally collected data by Scherer *et al*. (2013): duration = 194.2 ± 6.5 days, mean start date = 28 ± 4.5. Comparing across the few studies that reported a fit of the model relating moult index to date, the R^2-value of our photographic study is within the range found in studies using birds in the hand (Table 1).

DISCUSSION

We found that photography is a convenient method to study moult reliably and remotely, and yields results that are comparable in accuracy to results from studies handling birds. Data based on photographs allowed us to determine the timing and duration of Black Skimmers' primary moult in southern Brazil. The species starts moulting primary feathers in October and their complete moult takes around 194 days from austral spring to summer.

Advantages of systematic photography include its feasibility in areas and situations where birds in flight can be readily photographed but their capture might be difficult, for instance due to intense human disturbance, type of landscape, and license restrictions on capturing birds. Although these factors can make captures more difficult, they do not affect photography to a similar extent. There are, however, some limitations in systematic photography. Though the method works for flight feathers, recording moult of body feathers is much more difficult because those feathers are normally hidden. Photographs do not normally allow individual recognition as capturing and marking individuals does, unless the study species was already marked in another season or has distinct natural markers such as specific bill or iris patterns. Photography cannot be applied to all birds and conditions since data depend on a clear view of at least one open wing. Nonetheless, systematic photography can be adapted, for example using bait and playback to attract and photograph birds in certain positions. Photography may reduce disturbance to birds, thus enhancing sample sizes that can be obtained. This method also needs less equipment and less fieldwork effort compared to catching birds.

This is the first study we are aware of systematically using photographs to assess moult in birds and evaluate the method for within- and between-observer repeatability. The repeatability was high in both cases. The mean difference being almost zero, with most differences between the measurements within estimated narrow limits of agreement, suggests that moult of primary feathers can be consistently scored by the same or different observers using photography. However, as in any other scoring method depending on human observations, variability in results exists and might be related to the observer's experience in detecting feathers moulting in a certain species. A further strength of the photographic approach is that it can provide a permanent archive available for future research uses.

Considering the studies that made R^2-values available, photographic data yielded a similarly good fit as conventional studies, suggesting that the photographic method has not introduced significant additional variation to the intra-individual variation present in such data sets. However, further evaluations of the photographic method on other species and populations would be desirable.

Conventional moult scoring of birds in the hand (Scherer *et al.* 2013) and our photographic results indicated Black Skimmers in southern Brazil began their primary moult in October and that primary moult lasted from austral spring to summer. The results indicate a consistent pattern for the species in southern Brazil which is clearly distinct from the timing of moult observed in North America where moult occurs during boreal autumn to spring (Pyle 2008).

Snyder *et al.* (1987) and Keijl (2011) advocate the use of photographs to assess moult scores and we show the potential value of systematic photography for the study of moult. We believe this method could also be extended to assess moult centres in secondaries as well as timing and duration of the moult of secondaries, tertials and rectrices. Studies on single populations could be extended to cost effective studies of geographic variation in moult patterns in widespread species. Moreover, photographs can benefit from associations with the citizen science movement to cover wider geographic areas. The photographic method can also be carried out in association with other imaging techniques such as thermal imaging cameras to monitor stress levels under challenging conditions (Jerem *et al.* 2015).

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Table 1: R^2 values related to studies using photography (this study) and examining individuals in the hand (Underhill & Zucchini 1988, Newton & Rothery 2009and Scherer *et al.* 2013) to score moult in birds. Sampling size refers to the total number of moult scores analysed. Type data refers to classification in Underhill *et al.* (1990) in which type 2 requires moult scores of all sampled individuals; type 3 only includes individuals in moult; and type 5 uses scores of the population pre-moulting and in moult.

	Species	**sample size**	**type data**	**R^2 value**
This study	Black Skimmer (*Rynchops niger*)	519	3	0.755
Scherer *et al.* (2013)[1]	Black Skimmer (*Rynchops niger*)	53	3	0.246
Underhill & Zucchini (1988)	Sanderling (*Calidris alba*)	164	3	0.847
Newton & Rothery (2009)	European Goldfinch (*Carduelis carduelis*)	108	5	0.966

[1] R^2-value calculated from raw data provided by the authors.

Figure 1: Black Skimmers flying with fully open wings (A, B) give a good view of moulting patterns yet birds landing or taking off (C), or even flying at some other angles (D) can also allow for moult scoring of primaries. Detail of Black Skimmer's right wing and the moult scoring system used in this study (E). Primaries are identified from inner (P1) to the outermost feather (P10 in this species). Double counting the primaries from inner to outermost feather and vice-versa plus special attention to gaps between feathers is recommended because pins can be hidden. Old feathers have a dull colour, some wear and often lighter edges such as the P4 to P10 shown in (E); thus, these seven feathers were scored as 0. New feathers are brighter, darker, and have no wear, such as the P1 shown in (E); thus, scored as 5. P2 shown in (E) is half-grown compared to old feathers and scored as 3 whereas the P3 shown in (E) is less than a third of the full length of old feathers and thus scored as 2. Note that primaries with scores of 2 or 3 (P2 and P3 in this example) are partially visible while feathers with score 1 are hardly seen yet the gap of a missing feather can be detected. Thus, the moult score of the right wing of this individual shown in (E) is 10. This sum is then divided by the maximum possible score (= 50) to result in the moult index of 0.2 (= 10/50) used in the analysis. Photo by BVP.

Figure 2: Bland-Altman plots of within- (A) and between-observer repeatability (B) of the photographic moult index . Horizontal solid and dotted lines are the mean difference and limits of agreement, respectively. The within- (mean = -0.02, limits of agreement = -0.20, 0.17) and the between-observer differences (mean = -0.009, limits of agreement = -0.012, 0.006) are based on logarithmically transformed data.

Figure 3: Photographic moult index in relation to date (day 1 is October 1) based on photographs of moulting Black Skimmers with open wings. The line represents the estimated moult trajectory beginning at the mean start date based on Type 3 data (Underhill & Zucchini 1988).


A
P10
P1
P1
P10
B
P10
P1
P1
P10
C
P10
P1
P1
P10
D
P10
P1
P1
P10
E
P4
P3
P2
P1
S1
P10
P9
P8
P7
P6
P5
P4
P3
P2
P1
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
Tertials

Figure 1.

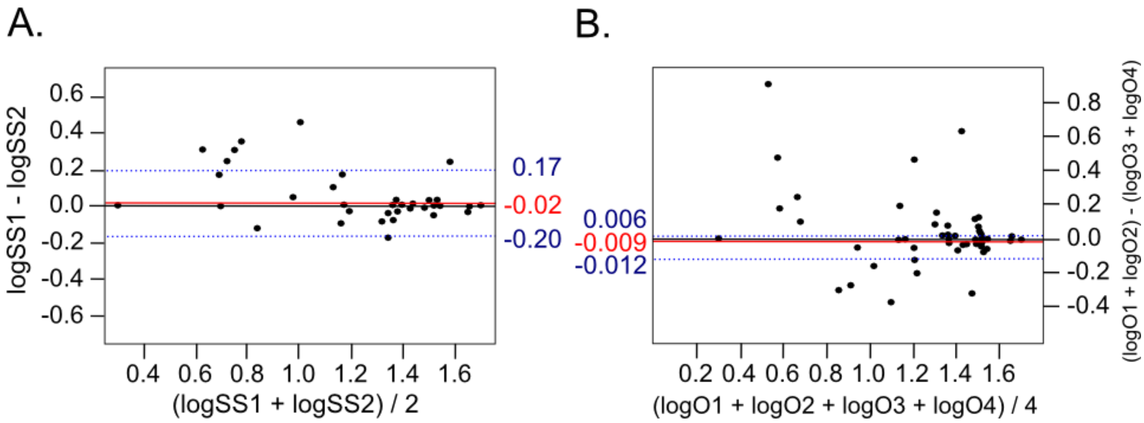

Figure 2.

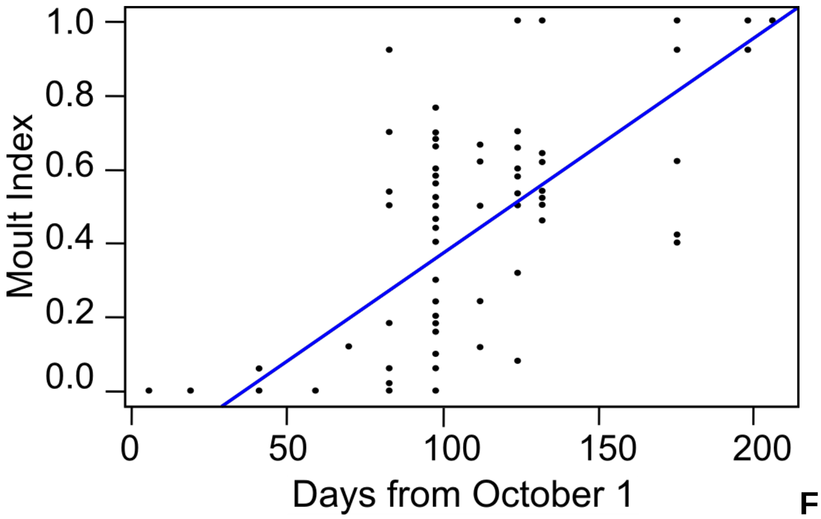

Figure 3.