

Short communication

Fluorescent ornamentation in the Rhinoceros Auklet *Cerorhinca monocerata*

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Avian species have developed a range of markers for transmitting information, among them ornamented plumage, behavioural patterns and conspicuous bill structures. Members of the marine subfamily Fraterculinae have some of the most visibly noticeable ornaments among seabirds, and some species have been recently found to possess fluorescent properties in seasonally acquired bill plates. We examined a member of this subfamily, the Rhinoceros Auklet *Cerorhinca monocerata*, for fluorescence in the upper and lower mandibles as well as the namesake horn grown in preparation for mate selection. Fluorescence was noted primarily in the horn of adults, with greater variation present among individuals than between the sexes.

Keywords: biofluorescence, reproductive signalling, seabird, ultraviolet light.

Organisms exhibiting intersexual selection often display external cues intended to influence mate selection (Berglund *et al.* 1996). These features can act as markers of the reproductive status (Velando *et al.* 2001), underlying physiological condition (Loyau *et al.* 2005) or individual quality of potential mates (Hanssen *et al.* 2006). Selecting a reproductive partner with traits showcasing beneficial qualities should therefore lead to increased fitness through an improved ability to raise offspring (Andersson 1982). As monogamous species enter into a pair prior to the commencement of duties required to raise offspring successfully, external markers of

reproductive ability probably developed as a strategy for efficiently selecting appropriate partners before individual quality could be evaluated (Pagani-Núñez & Senar 2014). Among avian species, these cues can take the form of ornamented feathers (Andersson 1992), ritualized behaviours (DuVal 2007) or other highly developed features advertised during periods of reproductive activity (Curio 2004).

Several members of the subfamily Fraterculinae (Alcidae; hereafter 'Auks') have developed distinctly ornamented features grown during periods of reproductive activity. Auks are small to medium-sized seabirds distributed in cold waters of the northern hemisphere, and in some species both sexes can display oversized head and neck plumes as well as conspicuously marked bill plates during the breeding season (Jones & Montgomery 1992, Jones & Hunter 1999, Sorensen *et al.* 2010). These ornaments are thought to relate to individual quality but could also be used to identify mates in highly social species when congregating near burrows or interacting with conspecifics; their precise function remains uncertain in many cases (Seneviratne & Jones 2008).

In addition to conveying information through the visible light spectrum, some bird species are also able to detect cues transmitted through ultraviolet (UV) light (Chen *et al.* 1984). Indeed, recent investigations have found fluorescent properties in the bill plates of multiple auk species when exposed to ultraviolet light, namely the Crested Auklet *Aethia cristatella* (Wails *et al.* 2017) and Atlantic Puffin *Fratercula arctica* (Dunning *et al.* 2018). Fluorescence occurs as a result of the absorption of electromagnetic radiation at a certain wavelength followed by the re-emission of typically longer, lower energy wavelengths (Gruber & Sparks 2015). Often, this re-emission is highly visible to the naked eye as a bright, glowing hue. While this fluorescence is not universal among the taxon, it seems likely that other members of this subfamily may possess traits for fluorescence, especially species with other conspicuous forms of ornamentation.

To more fully understand the prevalence of bill fluorescence within the Fraterculinae, we examined the ornamental breeding structures of adult Rhinoceros Auklets *Cerorhinca monocerata* under UV light. We hypothesized that the keratinous horn grown by adults in preparation for mate selection would be a source of fluorescence given the role of auxiliary ornamental structures elsewhere in the family and the prominence of this feature in the species. Finally, we also investigated whether bill fluorescence differs between the sexes.

METHODS

Fieldwork was conducted on Southeast Farallon Island (37°42'N, 123°00'W), a rocky archipelago that contains an estimated breeding population of 4500 Rhinoceros Auklets (Warzybok *et al.* 2018). Birds were captured

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using a 30-mm nylon mist-net deployed at four locations with high auklet burrow densities. Netting sessions were conducted at dusk when Rhinoceros Auklets were most active, over the course of nine nights during the chick provisioning period from 17 June to 7 July 2018. A random sample of netted birds were photographed under UV light with a 4-watt T5 fluorescent bulb (253 nm) and a Canon 6D DSLR camera (40-mm focal length, ISO 8000, f4 at 1/3 s). The bill of each individual was framed in the centre, with the subject approximately 30 cm from the objective element of the camera lens. Care was taken to ensure the eyes of auklets were covered to avoid direct UV light exposure. Photographs were taken in near-complete darkness with no additional light source other than the UV light present, either outside post-nautical twilight or indoors in a dark room. A series of three photos were taken for each individual. Upon visual inspection of the photos, there existed no discernible differences in the pattern or extent of apparent fluorescence between images in a series. Therefore, the image with the least amount of distortion from 'shutter shake' given the slow shutter speed used was retained for analysis. In addition, morphometric measurements of bill depth, relaxed wing chord and mass were recorded for sexing.

Preliminary exposure to UV light revealed that fluorescence occurred in the upper mandible, lower mandible and, most notably, the horn. These regions of the bill emitted a distinct blue/green hue corresponding to the emission of longer wavelength visible light when irradiated with non-visible light in the UV spectrum. To quantify the amount of fluorescence in each of these bill segments, their total surface area and the total area of fluorescence (in pixels) were measured using the program ImageJ2 (Rueden *et al.* 2017), without the filtering of any specific colour channels. The total area of fluorescence was divided by the total surface area of each segment, providing a unitless measure of the proportion of bill fluorescence across individuals. Variation in the proportion of fluorescence was summarized by segment and by sex.

A linear discriminant analysis (LDA) incorporating bill depth, wing chord and mass was used to assign sex to each individual. The utilized function was molecularly validated for this population using blood samples taken from a subset of individuals in 2016 used in a previous study by Wilkinson *et al.* (2018). Accuracy of sexing Rhinoceros Auklets using this function has been validated at 100%; however, this number should be viewed with caution given the relatively few individuals used in the validation ($n = 10$).

Beta regression was used to test whether variability in the proportion of bill fluorescence was explained by the linear and interactive effects of sex and bill segment. We chose to use beta regression given the proportional nature of the response, which was bounded by 0 (no fluorescence) and 1 (total fluorescence). A set of candidate

models was fitted in R (R Core Team 2015) with the package betareg (Cribari-Neto & Zeileis 2010) and compared using Akaike's information criteria with sample size adjustments (AIC_c ; Burnham & Anderson 2002). The model with the lowest AIC_c was selected as the best fitting model. Normality and homogeneity of variance were confirmed visually with a QQ-plot and a plot of the residuals against the fitted values, respectively.

RESULTS

A total of 35 Rhinoceros Auklets were photographed, with sample of 20 males and 15 females based on estimates from the sexing LDA. All individuals showed some degree of bill fluorescence under UV light; however, the patterns of fluorescence in the three bill segments examined were highly variable across individuals (Fig. 1). Fluorescence was most prominent in the horn, with an average proportion (\pm sd) of 0.66 (\pm 0.13) for males and 0.61 (\pm 0.21) for females (Fig. 2). The gape and posterior side of the nares in the upper mandible, and the base of the lower mandible also exhibited some fluorescence; however, much of the upper and lower mandibles did not fluoresce (Table S1).

We found no evidence for a sex-specific difference in the amount of bill fluorescence. Modelling the proportion of fluorescence as a linear relationship with bill segment alone received the most support of the models tested (lowest AIC_c ; Table 1), confirming what is visually apparent, that the highest amount of fluorescence occurs in the horn. The addition of sex as a linear predictor increased the AIC_c value by 1, showing weak support for a possible difference in fluorescence by sex probably driven by slightly higher fluorescence of the lower mandible in males than females (Table 1). There was no evidence of overdispersion in the top model (residual deviance = 6.79, residual df = 102), and assumptions of normality and equal variance were met.

DISCUSSION

Here we provide the first evidence of fluorescence properties of another Fraterculinae, in the keratinous horn of breeding Rhinoceros Auklets, adding to the assessment of occurrence within this subfamily. It also augments our understanding of ornamentation and individual signalling in this relatively widespread species. Of particular note was the presence of variation in the amount of bill fluorescence between individual auklets. As causes for biofluorescence are often protein- or pigment-based (Gruber & Sparks 2015, Prötzel *et al.* 2018), it seems likely that horn composition influences expression between individuals. Horn growth occurs primarily from February to March, corresponding to the pre-breeding period of the annual cycle (Pyle 2008). During this

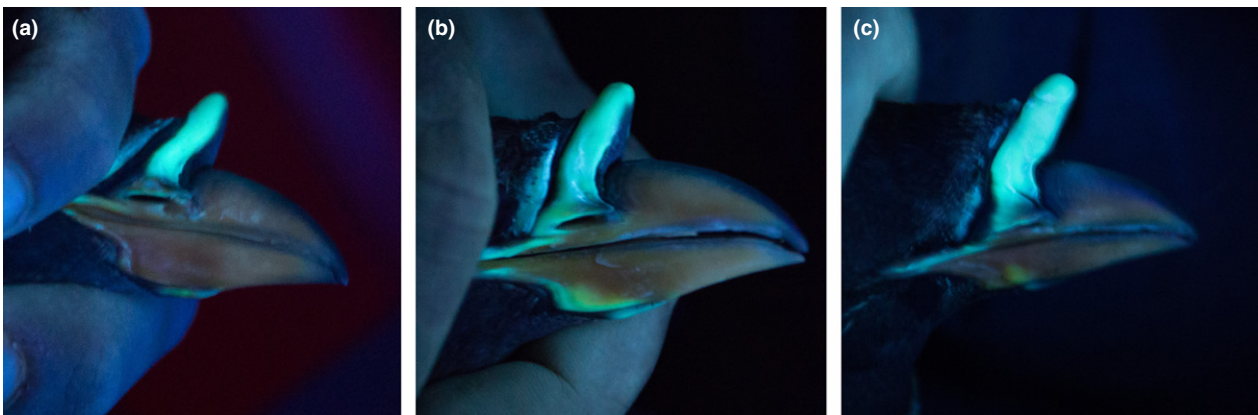


Figure 1. Photographs of three Rhinoceros Auklet bills under UV light, illustrating the amount of variation in the pattern of fluorescence in the upper and lower mandibles, and horn between individuals. Fluorescence is observed as a relatively narrow band in the horn only (a; female), in all three bill segments (b; male), and as a near fully fluorescent horn (c; female).

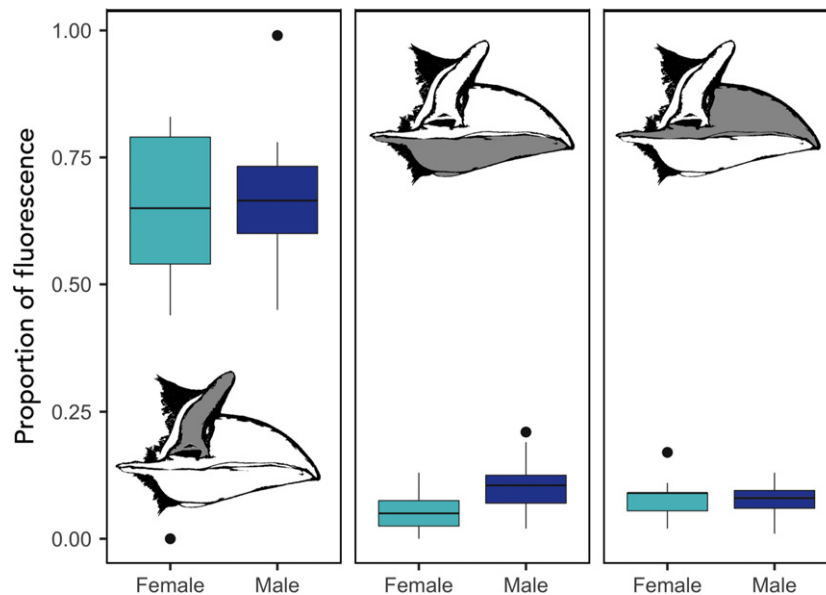


Figure 2. Boxplot showing the difference in the proportion of fluorescence across bill segments (horn, lower mandible and upper mandible; shaded in grey) and by sex. Shaded box indicates the interquartile range bounded by the 25th and 75th percentile, bold horizontal line the median, whiskers the maximum and minimum values, and dots the extreme outliers.

time, Rhinoceros Auklets have been shown to prey upon a higher proportion of euphausiids than in other parts of the year, when diet is almost exclusively piscivorous (Carle *et al.* 2014). However, it is unclear whether diet contributes to the expression of fluorescence in the horn or it is developed endogenously.

It should be noted that although the methods utilized here highlighted the presence of fluorescence under conditions optimized for human eyesight, the precise visual properties of the auklets themselves is not yet unclear. There is very scant information on the ocular structures

of seabirds generally, particularly in the Alcidae, and so it is unclear whether these species can detect fluorescence at differing wavelengths or at lower intensities than can be perceived by humans. However, numerous avian species (e.g. parrots) have been documented to possess UV-capable vision, making ornamental structures appear brightly coloured under natural illumination (Withgott 2000). We hypothesize that the presence of fluorescence itself is an indicator that it serves a biological role, but without knowing the detection abilities of the birds we cannot be certain that this is the case.

Table 1. Beta regression model selection results for testing the proportion of fluorescence (prop) as a function of relevant combinations of sex and bill segment. Models ranked by lowest ΔAIC_c , with corresponding number of parameters (K), raw AIC_c and ΔAIC_c values, and weights (ω_i).

Model	K	AIC	ΔAIC_c	ω_i
Prop ~ segment	4	-244.96	0	0.57
Prop ~ sex + segment	5	-243.95	1.01	0.34
Prop ~ sex * segment	7	-241.12	3.84	0.08
Intercept only (null)	2	-58.01	186.95	0
Prop ~ sex	3	-56.12	188.64	0

Despite this uncertainty, we suggest that ornamental fluorescence in Rhinoceros Auklets may aid in reproductive signalling, as has been proposed for other members of the subfamily (Wails *et al.* 2017). Previous investigations into the determinant factors affecting Rhinoceros Auklet ornament growth suggest that horn size may serve as different signals depending upon sex (Sorensen *et al.* 2010). Whereas female horn height was positively related to a diet of inshore fish, male horn height was predicted instead by overall body size (Sorensen *et al.* 2010). Females with an increased ability to forage for high-quality prey items may possess an underlying physiological condition more suited to the energetic demands of reproduction, and larger males may be better able to defend scarce nest burrows from competitors (Addison *et al.* 2008). Although we found no difference in the amount of fluorescence displayed between the sexes, it is possible that this adaptation serves to highlight the horn to conspecifics, with size acting as the ultimate signalling mechanism. This focusing of attention on a marker of individual quality may be especially pertinent given the crepuscular habits of Rhinoceros Auklets during mate selection, where interactions among individuals may take place under conditions of very low ambient light. It should be noted, however, that mutual sexual selection based on ornament size has not been shown experimentally for this species, as has been demonstrated elsewhere (Jones & Hunter 1993). However, several studies theorize that this phenomenon probably occurs in Rhinoceros Auklets, and we cautiously agree, although explicit experimental evidence in the published literature is lacking (Addison *et al.* 2008, Sorensen *et al.* 2010).

Horn fluorescence may also serve as a complementary signal of potential fitness regardless of ornament size. Previous experiments in the Psittacidae indicated that plumage fluorescence may act as a potentially honest signal of individual quality during mate selection (Arnold *et al.* 2002), but more recent investigations in the same system have indicated that other factors in the plumage may be of more relevance (Pearn *et al.* 2003, Berg & Bennett 2010). More experimentation is therefore

necessary to determine the precise functionality of fluorescence and mate choice in avian species generally, and certainly within the Fraterculinae. In addition, the discovery of a wider incidence of fluorescence in both marine and terrestrial vertebrate tetrapods has only recently begun, with many more species likely to be uncovered (Gruber & Sparks 2015, Taboada *et al.* 2017, Prötzel *et al.* 2018, Sloggett 2018). We subsequently encourage the investigation of fluorescence in other taxa to assess properly the rate of occurrence and to unravel the underlying development of this unique biological property.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Summary statistics of fluorescence found in the three bill segments examined in Rhinoceros Auklets separated by sex.