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SHORT REPORT

Photoluminescence in the bill of the Atlantic Puffin *Fratercula arctica*

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ABSTRACT
We report the discovery of photoluminescence in the ornamental bill of the Atlantic Puffin *Fratercula arctica*. The excitation and emission properties of the ceres of one dead and three live individuals were similar.

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The occurrence of photoluminescent ornamentation is well described in birds (Boles 1990, 1991, Pearn *et al.* 2001, Arnold *et al.* 2002). Such ornaments can serve important roles in enhancing signal reception and intensity, which can influence mate choice and inform sexual selection (Pearn *et al.* 2001, Arnold *et al.* 2002, Dobson *et al.* 2008). Here we report photoluminescent properties of specific portions of the Atlantic Puffin *Fratercula arctica* (hereafter Puffin) bill.

The Puffin's bill is a large, colourful ornament, which is subjected to a series of annual and life-history changes (Harris 2014) and is featured prominently in the Puffin's most obvious displays: head-jerking and billing (Figure 1, Cramp 1985). As the breeding season approaches, the hard, dermal plates (cere and lamellae) develop on the inner bill, and form the yellowish band around the 'blue-ish' proximal nasal and sub-nasal plates (Bureau 1878, Harris 2014). These seasonal morphological adaptations of the bill suggest an important role of bill morphology in breeding: either mate acquisition or foraging for offspring. The functional significance of this bill morphology has not yet been fully explored.

Here we have observed previously undescribed photoluminescence (apparent fluorescence) on the Puffin bill. Fluorescence and phosphorescence are both forms of photoluminescence and occur when an object absorbs energy from an excitation light (usually long-wave ultraviolet light), which raises an electron from its ground state to an excited state and longer wavelength light is released as that molecule transitions back to ground state (Needham 1974). The difference between these two forms of photoluminescence involves their extinction times (fluorescence continues for 10^{-8} s and phosphorescence continues for 10^{-4} s after excitation), due to how the excited molecule descends back to its ground state (Needham 1974). Although these photoluminescent properties are generally weak in natural systems, they have been documented in a range of organisms, and can be useful for a range of functions from predator deterrence (Andrews *et al.* 2007) to prey capture (Haddock *et al.* 2005). Here we document weak photoluminescence on the cere and the lamella (Figure 2) of the Puffin.

Photoluminescence was initially observed on recently deceased Puffins in late May 2010 on Machias Seal Island in the Bay of Fundy, Canada (44°30'08.3"N 67°06'04.5"W) and in early May 2016 on North Haven, Skomer Island, UK (51°44'08.8"N 5°17'47.0"W). Bill morphology (Harris 2014) indicated that the Canadian bird was an adult and the UK Puffin was a second-year bird; neither was sexed nor showed obvious signs of injury or cause of death. The presence of ornamental fluorescence in the Canadian Puffin was measured while fresh, while the UK bird was housed in a freezer maintained at −80°C. Each Puffin was photographed under a black light torch (Figure 1) to document the presence and

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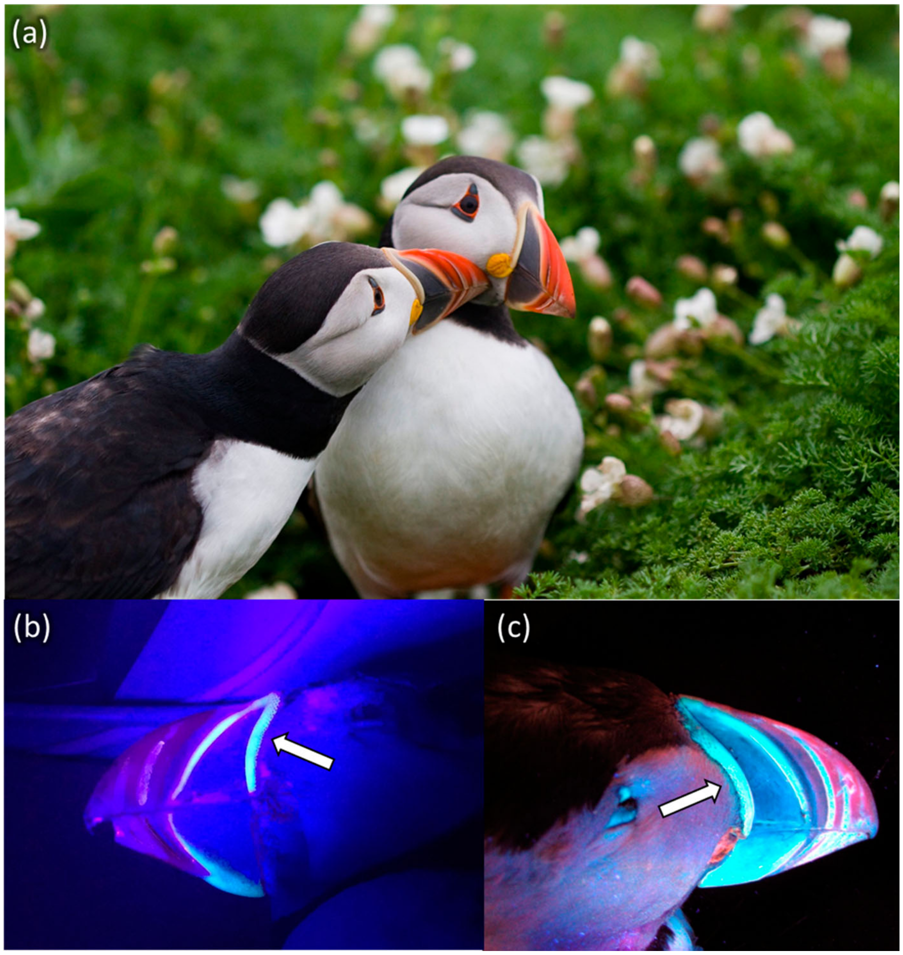

Figure 1. Atlantic Puffins engaged in (a) billing behaviour, which is associated with sexual signalling (Photo: T. Finch). We identified photoluminescence on the cere (arrow) and lamellae of Puffins found deceased in (b) UK and (c) Canada.

location of emission on the bill. The Canadian and UK Puffin samples were illuminated by a MiniMAX UV-5NF (reported lambda max = 365 nm; Spectroline, USA) and a Vansky 3 W 12-LED (reported lambda max = 395 nm; Shenzhen Wansikai Network Technology Company, Limited, Guangdong, China) black light, respectively. Figure 1(b)–(c) shows that luminescence was most apparent on the cere and the lamella surrounding the inner bill in both Puffins but also occurred in the grooves on the red distal part of the bill of the Canadian bird (Figure 1(c)).

We characterized the excitation and emission properties of photoluminescence of the bill of three live Puffins caught on 16 July 2018 on Petit Manan Island, Maine, USA (44°22′3.29″ N 67°51′51.7″ W) that is owned and managed by US Fish and Wildlife Service – Maine Coastal Islands National Wildlife Refuge. Additionally, we measured a single frozen Puffin to determine whether these properties were still present after death. The measurements were conducted on the cere, which was the largest fluorescent surface on the bill. We used a high-power UV black light (lambda max = 375 nm) mounted with a UV bandpass filter (peak transmission = 380 nm) approximately 2 cm from the cere (45° relative to the cere). The bandpass filter removed 88.8% of the excitation energy from the visible range (mean visible irradiance ± se = 0.002 ± 0.0001 μWatt cm^{-2}; mean visible irradiance behind the bandpass filter = 0.0002 ± <0.0001 μWatt cm^{-2}; Figure 2(a)). In order to protect the eyes of live Puffins measured for this study, we developed a specially shaped opaque eye-shield (or 'sunglasses') made from foam and waterproof neoprene, which were held in place around the head of the Puffins secured over the nasal saddle (online Figure S1). These sunglasses blocked 99.9% of the UV irradiance from the Puffins' eyes (mean UV irradiance ± se = 3.66 ± 0.75 μWatt cm^{-2}; mean UV irradiance behind glasses = 0.004 ± 0.0008 μWatt cm^{-2}). We then used a radiometrically calibrated field portable spectrometer (Jaz, Ocean Optics) mounted with a 200 μm slit and custom 600 μm fibre optic cables, held approximately

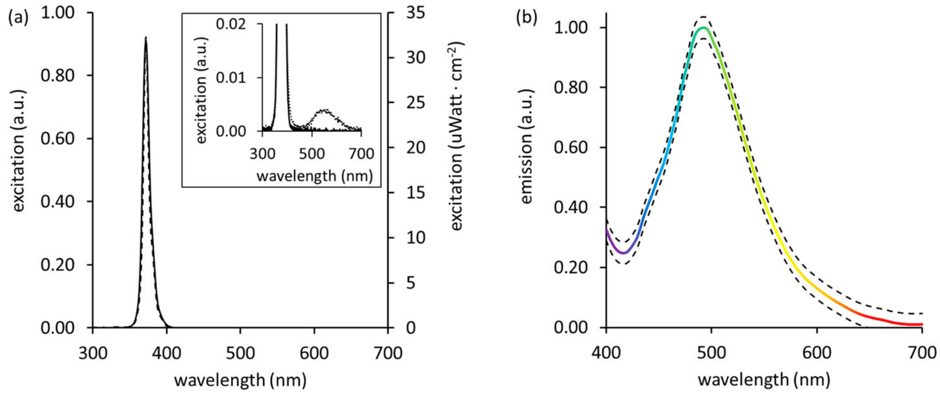

Figure 2. (a) The excitation light in arbitrary units measured relative to a white standard (solid) and in absolute irradiance (dashed) and the irradiance as measured through the protective glasses (dotted, essentially zero, see Methods). In addition, inset in (a) shows the excitation after blocking visible light with a UV band pass filter (solid) and the excitation prior to blocking visible light (dotted line). (b) The emission spectra of the ceres of three live puffins (solid) with approximate 95% confidence intervals (dashed). All emission spectra are normalized in arbitrary units (a.u.).

2 mm from the cere (90° relative to the cere), in a dark room. Integration time was set at 50 ms to maximize the signal to noise ratio. Excitation spectra were quantified both by examining the intensity (counts) against a non-fluorescent white standard (WS-SL-1) and by measuring absolute irradiance (Figure 2(a)). We found that all three live birds had similar wavelength at maximum emission (mean lambda max ± se = 492.0 ± 0.58 nm) and full width at half maximum (96.7 ± 1.8 nm). These values were similar to those obtained from a dead Puffin stored at −80°C for about 1 year (lambda max = 494 nm, fwhm = 82 nm).

Here we document photoluminescent properties of the adult Puffin bill during the breeding season. The bill fluorescence occurs within a region that undergoes seasonal development; these plates are formed prior to the breeding season and are discarded afterwards. Similarly, previous research has found that the ornamental bill plates of the Crested Auklet *Aethia cristatella* fluoresce under blue light (Wails *et al.* 2017); these plates, like the seasonal ornaments of the Puffin, are associated with the breeding cycle, suggesting that photoluminescence may serve a similar function for these two auk species. Although the function of bill luminescence in Puffins is currently unknown, a number of other seabirds use bill features for signalling purposes (Dobson *et al.* 2008, Wails *et al.* 2017).

Photoluminescent properties serve a number of important roles in nature, such as deterring predators (Andrews *et al.* 2007), luring underwater prey (Haddock *et al.* 2005), and enhancing signals (Arnold *et al.* 2002). Given that the photoluminescent regions are fully developed in the Puffins during the breeding season and shed over the non-breeding season, we expect that this property plays a role in displaying during the breeding season (territorial defence or mate attraction) or chick provisioning (either prey capture or provisioning). We strongly encourage future research on these properties to use freshly dead and museum specimens to identify the form of photoluminescence and to establish its prevalence, and potential association with natural and life-history characteristics, in auks and other seabirds. Although quantifying these properties on live birds is an essential first step, we were unable to distinguish between fluorescence and phosphorescence (two forms of photoluminescence) because measuring the extinction of emission spectra was unreliable on live birds. Regardless of form, this property could provide a benefit for provisioning chicks in dark subterranean nest sites (i.e. weak emission in the burrow, either with, or shortly after exposure to excitation from the sun may help chicks more easily orient toward parents' bills) or as an underwater lure (Haddock *et al.* 2005) because the excitation bands are present at the Puffin's known diving depths of 5–41 m (Spencer 2012, Shoji *et al.* 2015, Symons 2018). These photoluminescent properties were detected in some museum specimens of several other Alcidae species (Dunning & Bond, unpublished), suggesting that these properties are more pervasive than previously recognized. Therefore, we recommend further comparative investigation into the relationship between these photoluminescent properties and behaviour and ecology within the notably diverse alcid family.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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