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First Observation of Fluorescence in Marine Turtles

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ABSTRACT

In recent years, biofluorescence has been observed in an increasing diversity of animals. Biofluorescence has been primarily examined in cnidarians, and it is also known to occur in other marine animal phyla, including Ctenophora, Annelida, Arthropoda, and Chordata. Most recently, the phenomenon has been shown to be phylogenetically widespread and phenotypically variable in cartilaginous and ray-finned fishes. Here we report on the first observation of fluorescence in a marine tetrapod, sea turtles.

INTRODUCTION

Biofluorescence results from the absorption of electromagnetic radiation at one wavelength by an organism, followed immediately by its reemission at a longer, lower energy, wavelength. In clear ocean water, the light spectrum bandwidth progressively narrows with increasing depth, reaching a wavelength peak of 465 nm and a narrow bandwidth of ~20 nm at the maximum depth of penetration (Jerlov, 1968). The spectrally restricted (blue-shifted) illumination in clear ocean water provides unique lighting conditions for organisms to exploit fluorescence to produce visual contrast and patterns (Sparks et al., 2014; Gruber et al., in prep.).

Until recently, apart from photosynthetic pigments, most known biofluorescence resulted from GFP-like proteins, which comprise a protein superfamily (Shagin et al., 2004). Green fluorescence protein was originally discovered colocalized with photocytes of a biolumines-

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cent hydrozoan, *Aequorea victoria* (Shimomura et al., 1962), and converts blue bioluminescent light to green (Morin and Hastings, 1971). To date, the majority of these GFP-like proteins have been isolated from anthozoans (Pieribone and Gruber, 2006; Gruber et al., 2009; Sparks et al., 2014). Fluorescent proteins (FPs) are now known to be widespread in non-bioluminescent anthozoans, especially scleractinian corals (Matz et al., 1999; Gruber et al., 2008). Homologs to GFPs have also been found in nonluminous planktonic copepods (Shagin et al., 2004; Hunt et al., 2010), lancelets (Deheyn et al., 2007), and ctenophores (Haddock et al., 2010).

In recent years, biofluorescence has been observed in an ever-increasing number of marine animals, including crustaceans, polychaetes, cephalopods, and chordates (Mäthger and Denton, 2001; Mazel et al., 2004; Deheyn et al., 2007; Mehr et al., 2015). Our recent finding shows that biofluorescence is widespread and phenotypically variable in cartilaginous and bony fishes (Sparks et al., 2014). Within fishes, a new family of bilirubin-inducible FPs has recently been discovered, with novel members identified in both anguillid (Kumagai et al., 2013) and chlopsid eels (Gruber et al., 2015).

Marine organisms biofluoresce by absorbing the dominant ambient blue light via fluorescent compounds and reemitting it at longer, lower energy wavelengths, visually resulting in green, orange, and red fluorescence. This creates spectra not normally present at depth. Some fishes show strong interspecific variation in fluorescent emission patterns (e.g., the lizardfish genus *Synodus* and the goby genus *Eviota*) that has led to the hypothesis that biofluorescence functions as a form of species recognition or camouflage (Sparks et al., 2014). There are also many fishes that possess yellow intraocular (lenses or cornea) filters (see Heinermann, 1984), which potentially function as long-pass filters and enable enhanced perception of biofluorescence.

Biofluorescence also has been recently shown to play a role in the behavior of marine organisms. Biofluorescence at the tips of the tentacles in the hydromedusa *Olindias formosa* attract juvenile *Sebastes* rockfishes (Haddock and Dunn, 2015), whereas the red-eye wrasse, *Cirrhilabrus solorensis,* responds to red biofluorescence (Gerlach et al., 2014).

Here we report the presence of green and red fluorescence in both hawksbill (*Eretmochelys imbricata*) and loggerhead (*Caretta caretta*) sea turtles, the first observation of biofluorescence in a marine vertebrate other than fishes. The presence of this phenomenon in turtles raises intriguing questions as to its potential function.

MATERIALS AND METHODS

Specimens of *Eretmochelys imbricata* were observed and imaged using open-circuit SCUBA during the full-moon evening of July 31, 2015, near Nugu Island, Central Province, Solomon Islands, at a depth of 20 m. Underwater imaging was conducted using a Red Epic-M 4K camera (Red Digital Cinema, Irvine, CA) housed in an Aquatica Rouge housing (Montreal, Quebec, Canada). *Caretta caretta* was imaged in captivity at Mystic Aquarium (Mystic, CT) using the same camera and lighting configuration as for *E. imbricata*.

To excite a fluorescence response, the housing was fitted with custom-designed blue excitation lighting that provides high-intensity blue light (royal blue LEDs). The LED light was collimated to ensure its perpendicular incidence on the 415–485 nm excitation-filter surface (Semrock, Inc., Lake Forest, IL), minimizing the transmission of out-of-band energy. The ultrabright LEDs, collimating lenses, filters, and exit diffusers were contained in custom-made water- and pressure-proof housings and powered by NiMH Battery Packs (Ikelite Underwater Systems, Indianapolis, IN). To image and record biofluorescence, a 514 nm long-pass emission filter (Semrock, Inc., Lake Forest, IL) was embedded behind the sensor of the Red Epic-M 4K camera.

RESULTS

We serendipitously observed and imaged both green and red fluorescence in *E. imbricata* as the turtle swam directly in front of our blue excitation lights (415–485 nm) while we were examining fluorescent corals in the Solomon Islands. Fluorescent patterning in *E. imbricata* was observed as follows: green expression on the head (fig. 1A, B), and both green and red fluorescence on

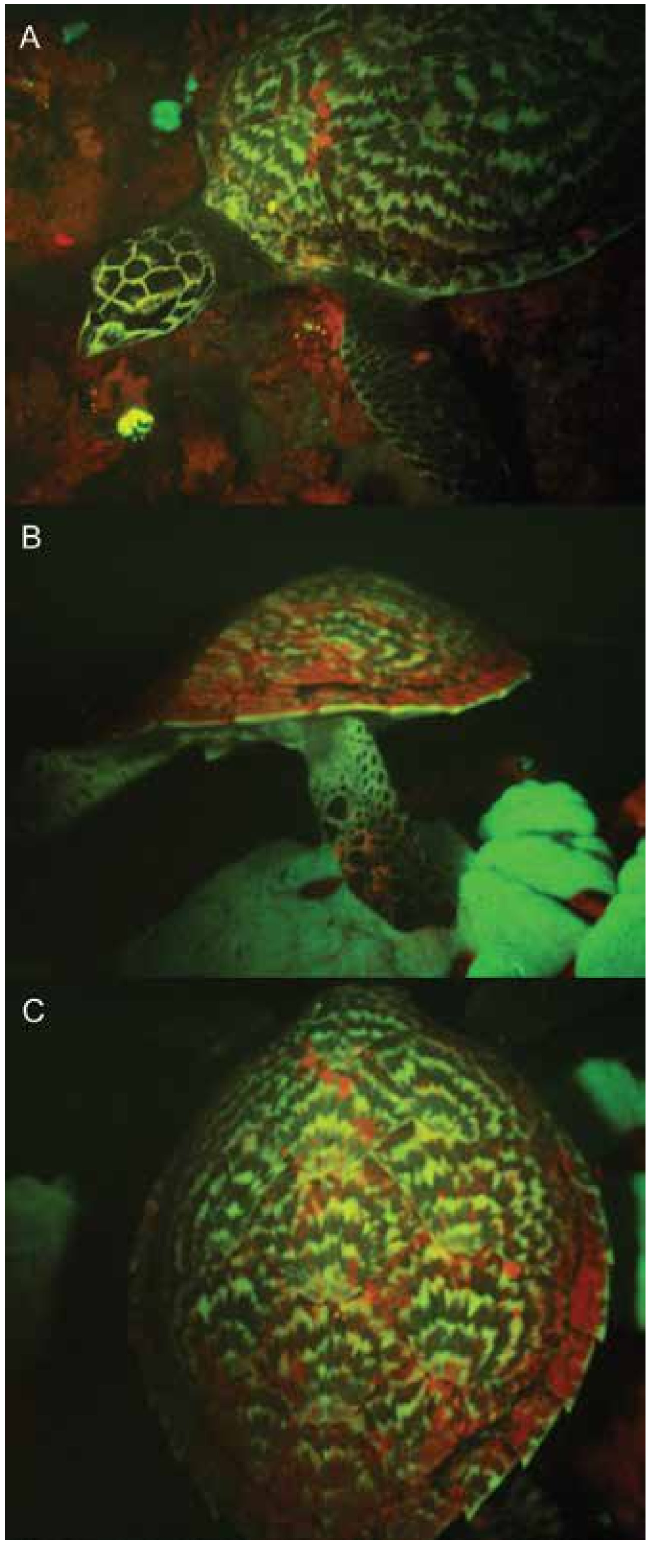

FIGURE 1. Fluorescent hawksbill sea turtle (*Eretmochelys imbricata*) imaged on coral reef at 20 m depth near Nugu Island, Solomon Islands, showing green and red fluorescence on: **A.** Head and anterior region of carapace. **B.** Hind flippers. **C.** Carapace (dorsal view).

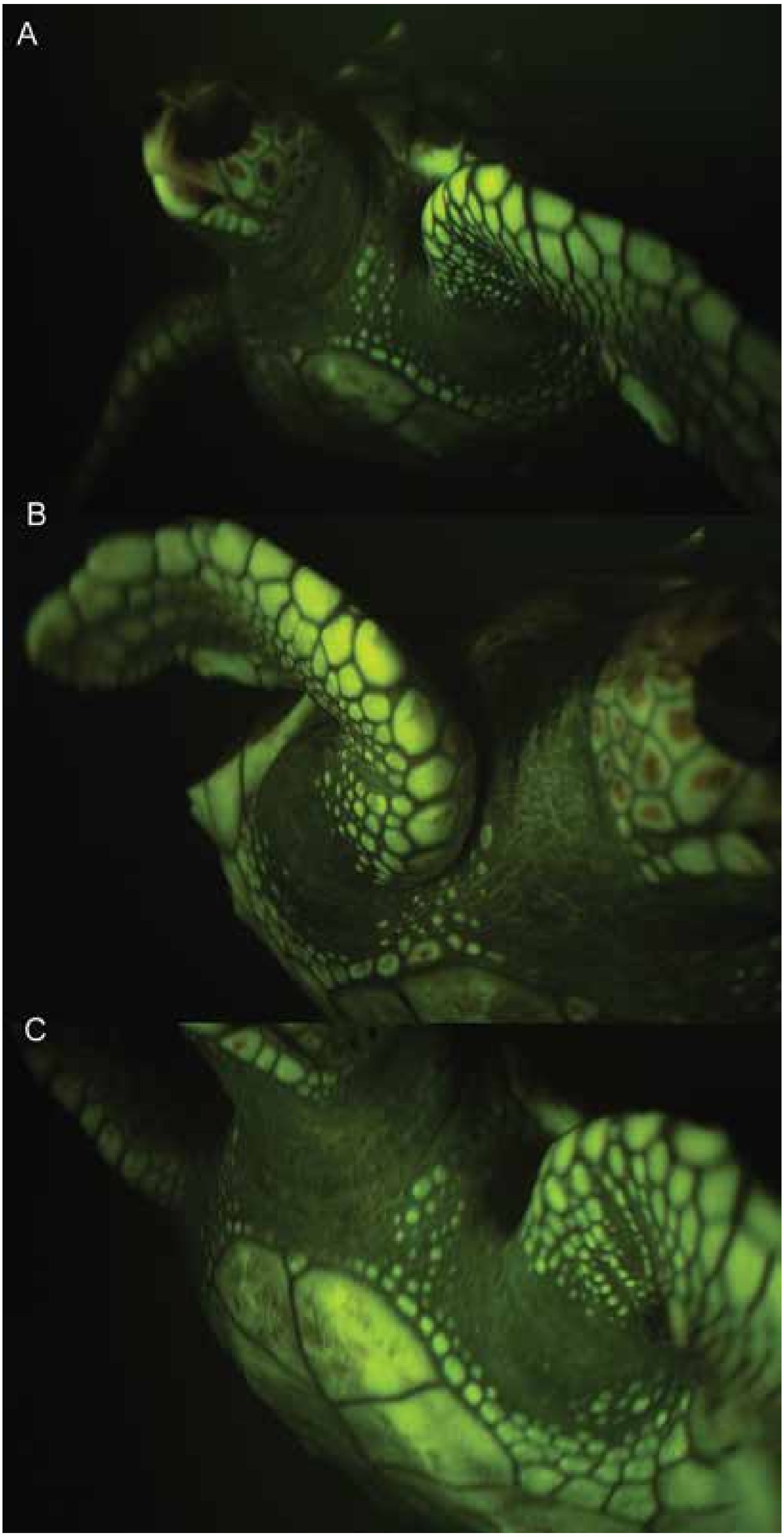

FIGURE 2. Fluorescent loggerhead sea turtle (*Caretta caretta*) imaged at Mystic Aquarium (Mystic, CT), showing green fluorescence on: **A.** Head and anterior region of body. **B.** Right flipper. **C.** Anterior region of plastron.

FIGURE 3. Female swell shark (*Celphaloscyllium ventriosum*) imaged under: **A.** fluorescent lighting (see Materials and Methods); **B.** white light.

the flippers (fig. 1B) and carapace (fig. 1A, C). We also had the opportunity to image a loggerhead sea turtle (*Caretta caretta*) in captivity at Mystic Aquarium (Mystic, CT), which exhibited primarily green fluorescence on its head and body (fig. 2A–C), flippers (fig. 2B), and plastron (fig. 2C).

DISCUSSION

During a night SCUBA dive during the full moon, we observed and imaged both green and red fluorescence in the hawksbill sea turtle (*Eretmochelys imbricata*) at 20 m depth, which is the first report of biofluorescence in a marine tetrapod. The turtle swam directly in front of the blue excitation lights while we were imaging biofluorescent corals. The fluorescent patterning we observed included green expression on the turtle's head (fig. 1A) and both green and red fluorescence on its flippers (fig. 1B) and carapace (fig. 1C). To confirm that fluorescence was more widespread in sea turtles, we imaged a loggerhead sea turtle (*Caretta caretta*) in captivity. The loggerhead sea turtle also exhibited bright fluorescence, in this case primarily green fluorescence on the head (fig. 2A), body (fig. 2A–C), and plastron (fig. 2C).

The diet of *E. imbricata* includes hard coral (*Physogyra lichtensteinii*), which is fluorescent, as well as sessile invertebrates such as sponges, zooanthids, soft corals, corallimorphs, ascidians, and mobile invertebrates (Obura et al., 2010). Whether the observed fluorescence is a result of the turtle's diet or is endogenously produced, is unknown. In addition, some of the red fluorescence noted on the carapace is likely the result of algal fluorescence. Male *E. imbricata* are noted to have more intense pigmentation than females (Marquez-M., 1970). In light of this sexually dichromatic pigmentation, it is interesting to speculate on a potential ecological/behavioral role for fluorescence in sea turtles. *Eretmochelys imbricata* is known to display exceptional navigation skills and periodic migrations from feeding to breeding habitats, yet no studies have been published on the visual acuity of this species. As we have observed for some sharks (fig. 3), the potential for fluorescent tissue to create greater contrast at depth, leads to questions regarding the role fluorescence plays in the behavior and biology of fluorescent marine organisms (Gruber at al., in prep.).

Although the Solomon Islands possess the largest rookery for hawksbill sea turtles in the oceanic South Pacific, globally *E. imbricata* is critically endangered and remains under threat from climate change, loss of nesting habitats, illegal trade, by-catch, and legal subsistence take (Spotila, 2004; Hamilton et al., 2015). Likewise, loggerhead sea turtles are listed as endangered for these same reasons (Spotila, 2004). The novel observation of biofluorescence in both hawksbill and loggerhead sea turtles highlights the urgency to understand and better protect and manage these endangered species.

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