

First Observation of Fluorescence in Marine Turtles

DAVID F. GRUBER^{1,2} AND JOHN S. SPARKS^{2,3}

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-

1 Baruch College and the Graduate Center, Department of Natural Sciences, City University of New York. Send reprint requests to D.F.G.

2 American Museum of Natural History, Sackler Institute for Comparative Genomics.

3 American Museum of Natural History, Department of Ichthyology, Division of Vertebrate Zoology.

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 Heinemann, 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, *Cirrhitilabrus 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

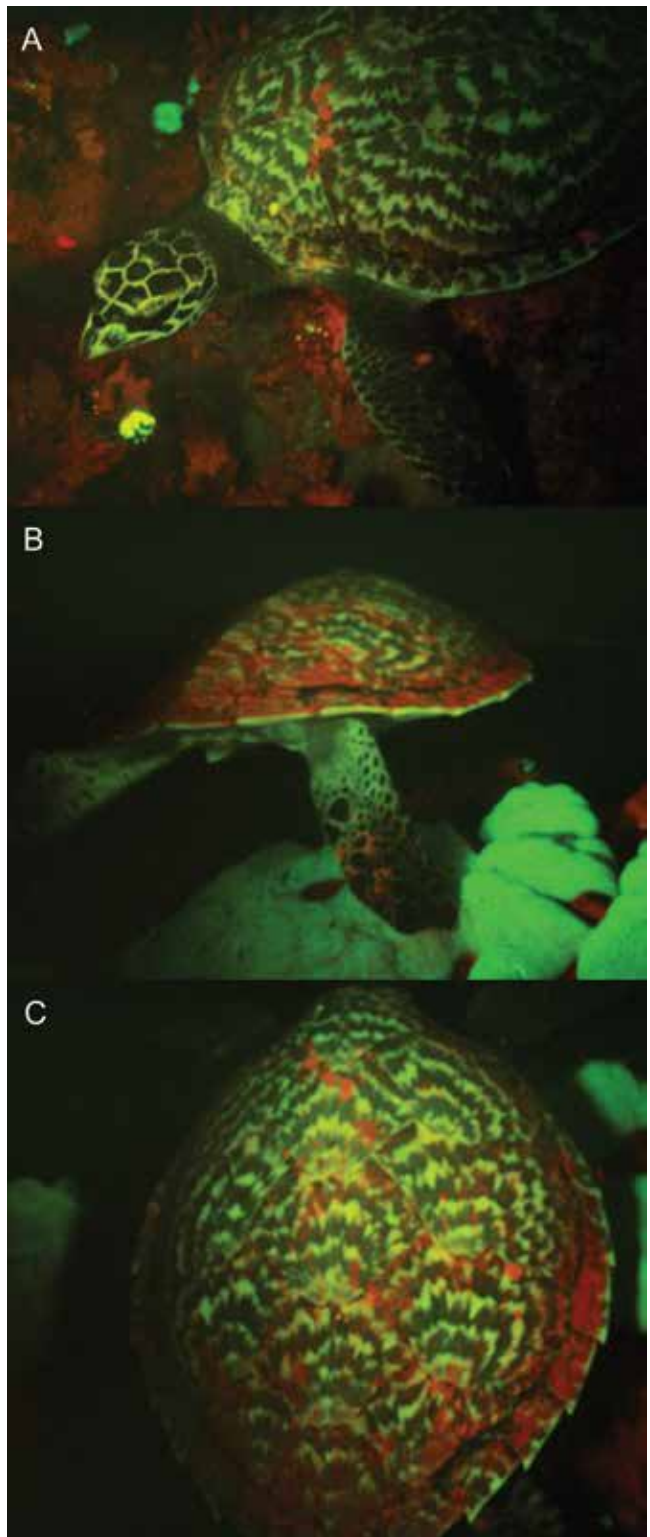


FIGURE 1. Fluorescent hawksbill sea turtle (*Eretmochelys imbricata*) imaged on coral reef at 20 m depth near Nugu Island, Solomon Islands, showing green and red fluorescence on: **A.** Head and anterior region of carapace. **B.** Hind flippers. **C.** Carapace (dorsal view).

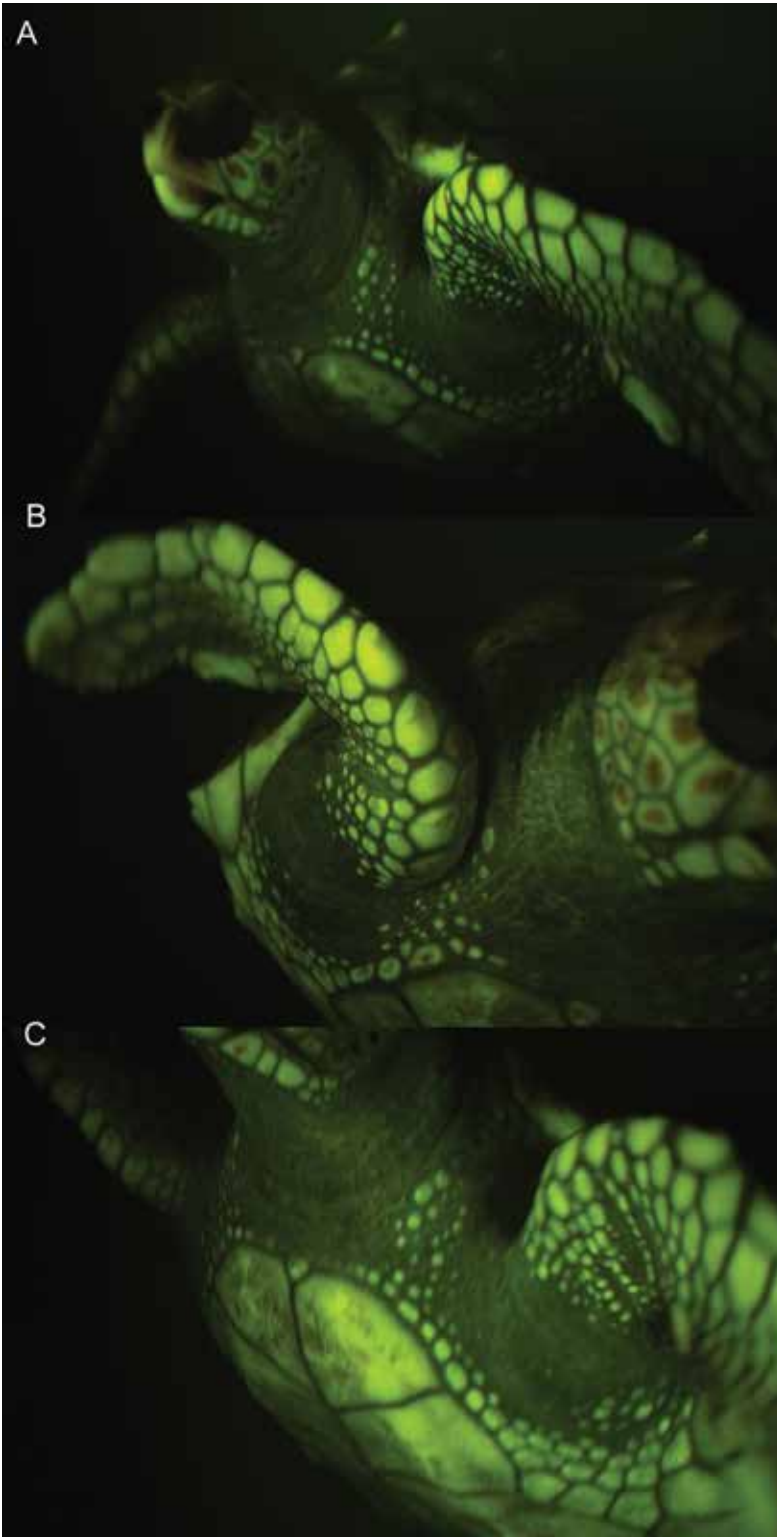


FIGURE 2. Fluorescent log-gerhead sea turtle (*Caretta caretta*) imaged at Mystic Aquarium (Mystic, CT), showing green fluorescence on: **A.** Head and anterior region of body. **B.** Right flipper. **C.** Anterior region of plastron.



FIGURE 3. Female swell shark (*Cephaloscyllium 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 et 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.

ACKNOWLEDGMENTS

These observations were made onboard a TBA-21 Academy Expedition. We are indebted to TBA-21 and Francesca von Habsburg for their very generous support. This study was made possible due to TBA-21 and the SCUBA diving and field assistance of Francesca von Habsburg, Markus Reymann, Eleonore von Habsburg, Gloria von Habsburg, and the crew of the R/V *Dardanella*. Jeanette Wyneken provided helpful comments related to sea turtle biology. We thank Vincent Pieribone and the John B. Pierce Laboratory, Yale University, for equipment design and manufacturing. We are grateful to Tracy Romano of Mystic Aquarium for allowing us access to image *Caretta caretta*. We gratefully acknowledge the Ray and Barbara Dalio Family Foundation and the National Science Foundation (DEB-1257555 to J.S.S. and CRPA-1007749 and MRI-1040321 to D.F.G.) for their support.

REFERENCES

- Deheyn, D.D., et al. 2007. Endogenous green fluorescent protein (GFP) in amphioxus. *Biological Bulletin* 213: 95–100.
- Gerlach, T., D. Sprengel, and N.K. Michiels. 2014. Fairy wrasses perceive and respond to their deep red fluorescent coloration. *Proceedings of the Royal Society of London B Biological Sciences* 281: 20140787.
- Gruber D.F., H.-T. Kao, S. Janoschka, J. Tsai, and V.A. Pieribone. 2008. Patterns of fluorescent protein expression in scleractinian corals. *Biological Bulletin* 215: 143–154.
- Gruber, D.F., et al. 2009. Novel internal regions of fluorescent proteins undergo divergent evolutionary patterns. *Molecular Biology and Evolution* 26: 2841–2848.
- Gruber, D.F., et al. 2015. Adaptive evolution of eel fluorescent proteins from fatty acid binding proteins produces bright fluorescence in the Marine Environment. *PLOS ONE* 10 (11): e0140972. [doi:10.1371/journal.pone.0140972]
- Gruber D.F., et al. In preparation. Biofluorescence in catsharks (Scyliorhinidae): assessment of elasmobranch fluorescence with relevance to visual ecology.

- Haddock, S.H.D., and C.W. Dunn. 2015. Fluorescent proteins function as a prey attractant: experimental evidence from the hydromedusa *Olindias formosus* and other marine organisms. *Biology Open* 4: 1094–1104. [doi:10.1242/bio.012138]
- Haddock, S.H.D., N. Mastroianni, and L.M. Christianson. 2010. A photoactivatable green-fluorescent protein from the phylum Ctenophora. *Proceedings of the Royal Society of London Series B Biological Sciences* 277: 1155–1160.
- Hamilton R.J., et al. 2015. Solomon Islands largest hawksbill turtle rookery shows signs of recovery after 150 years of excessive exploitation. *PLOS ONE* 10 (4): e0121435. [doi: 10.1371/journal.pone.0121435]
- Heinermann, P.H. 1984. Yellow intraocular filters in fishes. *Experimental Biology* 43: 127–147.
- Hunt M.E., M.P. Scherrer, F.D. Ferrari, and M.V. Matz. 2010. Very bright green fluorescent proteins from the pontellid copepod *Pontella mimocerami*. *PLOS ONE* 5 (7): e11517. [doi:10.1371/journal.pone.0011517]
- Jerlov, N.G. 1968. *Optical oceanography*. New York: American Elsevier Publishing.
- Kumagai A., et al. 2013. A bilirubin-inducible fluorescent protein from eel muscle. *Cell* 153: 1602–1611. [doi: 10.1016/j.cell.2013.05.038]
- Márquez-M., R. 1970. *Las tortugas marinas de Mexico*. Ph.D. dissertation, El Instituto Politecnico Nacional, Escuela Nacional de Ciencias Biologias, Casco de Santo Tomas, Mexico City.
- Mäthger, L.M. and E.J. Denton. 2001. Reflective properties of iridophores and fluorescent ‘eyespot’ in the loliginid squid *Alloteuthis subulata* and *Loligo vulgaris*. *Journal of Experimental Biology* 204: 2103–2118.
- Matz, M.V., et al. 1999. Fluorescent proteins from nonbioluminescent Anthozoa species. *Nature Biotechnology* 17: 969–973. [doi:10.1038/13657]
- Mazel, C.H., T.W. Cronin, R.L. Caldwell, and N.J. Marshall. 2004. Fluorescent enhancement of signaling in a mantis shrimp. *Science* 303: 51. [doi:10.1126/science.1089803]
- Mehr, S., et al. 2015. Transcriptome sequencing and annotation of the polychaete *Hermodice carunculata* (Annelida, Amphinomidae). *BMC Genomics* 16: 445. [doi:10.1186/s12864-015-1565-6]
- Morin, J.G. and J.W. Hastings. 1971. Energy transfer in a bioluminescent system. *Journal of Cellular Physiology* 77: 313–318.
- Obura D.O., A. Harvey, T. Young, M.M. Eltayeb, and R. von Brandis. 2010. Hawksbill turtles as significant predators on hard coral. *Coral Reefs* 29: 759.
- Pieribone, V. and D.F. Gruber. 2006. *A glow in the dark: the revolutionary science of biofluorescence*. Cambridge, MA: Harvard University Press.
- Shagin, D.A., et al. 2004. GFP-like proteins as ubiquitous metazoan superfamily: evolution of functional features and structural complexity. *Molecular Biology and Evolution* 21: 841–850.
- Shimomura, O., F.H. Johnson, and Y. Saiga. 1962. Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, *Aequorea*. *Journal of Cellular and Comparative Physiology* 59: 223–239.
- Sparks J.S., et al. 2014. The covert world of fish biofluorescence: a phylogenetically widespread and phenotypically variable phenomenon. *PLOS ONE* 9 (1): e83259. [doi: 10.1371/journal.pone.0083259]
- Spotila, J.R. 2004. *Sea turtles: a complete guide to their biology, behavior, and conservation*. Baltimore, MD: Johns Hopkins University Press.

All issues of *Novitates* and *Bulletin* are available on the web (<http://digitallibrary.amnh.org/dspace>). Order printed copies on the web from:

<http://shop.amnh.org/a701/shop-by-category/books/scientific-publications.html>

or via standard mail from:

American Museum of Natural History—Scientific Publications
Central Park West at 79th Street
New York, NY 10024

Ⓢ This paper meets the requirements of ANSI/NISO Z39.48-1992 (permanence of paper).