First Report of Biofluorescence in Arctic Snailfishes and Rare Occurrence of Multiple Fluorescent Colors in a Single Species

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ABSTRACT

Biofluorescence has recently been reported to be phylogenetically widespread and phenotypically variable across bony fishes, and is most common in tropical coral reef lineages. Here we provide the first documentation of prominent biofluorescence in Arctic fishes including two juvenile specimens of Liparis gibbus (variegated snailfish) collected from the coastal waters of Eastern Greenland, as well as an adult L. tunicatus (kelp snailfish) collected in the Bering Strait off of Little Diomede Island, AK. Observations of L. gibbus were made during nighttime dives within kelp forests in iceberg habitats in Southeastern Greenland in August 2019. The juvenile L. gibbus specimens exhibit both green (523–530 nm) and red (674–678 nm) biofluorescence on discrete anatomical areas, which provides a rare example of multiple fluorescent colors emitted from a single individual. Notably, the adult L. tunicatus emitted only red fluorescence in a bilaterally symmetrical pattern of discrete red dots and blotches. Potential weak green biofluorescence was also noted in a flatfish (Hippoglossoides platessoides) collected in Greenland, but in no other Arctic species. As the distribution and function of biofluorescence in marine fishes is further examined, this report adds context to its widespread geographical and environmental distributions, and shows that, although rare, biofluorescence does occur in Arctic fish lineages.

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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). Marine organisms biofluoresce by absorbing the dominant ambient blue light via a variety of fluorescent compounds and reemit it at longer wavelengths, visually resulting in green, orange, and red fluorescence.

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., 2016). Many fishes also possess yellow intraocular (lenses or cornea) filters (see Heinermann, 1984), which potentially function as long-pass filters and enable enhanced perception of biofluorescence. Biofluorescence has been shown to be widespread and phenotypically variable in both cartilaginous and bony fishes (Sparks et al., 2014), and some lineages exhibit strong interspecific variation in fluorescence-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 (Sparks et al., 2014).

Biofluorescence has recently been shown to play a role in the behavior of marine organisms, including both invertebrates and vertebrates, establishing a potential functional role for this poorly studied phenomenon. For example, fluorescence in corals has been hypothesized to enhance visual contrast in the monochromatic marine environment (Lukyanov et al., 2000; Mazel and Fuchs, 2003; Gruber et al., 2008), whereas fluorescence at the tips of the tentacles in the hydromedusa Olindias formosa has been shown to attract juvenile Sebastes rockfishes (Haddock and Dunn, 2015).

Fluorescence may also play a critical role in specific visually guided behaviors in marine fishes (Gerlach et al., 2014; Gruber et al., 2016). For example, red-eye wrasse, Cirrhilabrus solorensis, have been shown to both perceive and respond to their red biofluorescence (Gerlach et al., 2014). In the catshark species Cephaloscyllium ventriosum and Scyliorhinus retifer, green fluorescence has been shown to enhance contrast of the sharks' mottled pigmentation pattern, a feature that becomes more pronounced with a spectrally narrowing range of sunlight with depth, making these sharks more visible to conspecifics at depth (Gruber et al., 2016). Fluorescence in marine fishes is particularly common and morphologically variable in cryptically patterned lineages (Sparks et al., 2014), providing additional support for the hypothesis that fluorescence serves a visual function in the marine environment. In addition, sexually dimorphic fluorescence patterning has also recently been observed in multiple lineages of marine bony and cartilaginous fishes (Sparks et al., 2014; Gruber et al., 2016). Based on these and other studies, it appears that biofluorescence in marine fishes functions in many of the same ways as bioluminescence, specifically for communication, predator avoidance, or prey attraction (Davis et al., 2014; Gruber et al., 2019).

In some anthozoans, pigments homologous to fluorescent proteins (FPs) constitute up to 14% of the soluble protein content (Leutenegger et al., 2007). In marine chlopsid eels, the FPs
represented up to 20% of total soluble protein based on native gel electrophoresis analysis (Gruber et al., 2015; Guarnaccia et al., in prep). Further studies are needed to determine the metabolic cost of producing these FPs in such high amounts.

Within fishes, the biochemical mechanism for producing biofluorescence has to date been elucidated in both anguillid (Kumagai et al., 2013) and chlopsid eels (Gruber et al., 2015), where it has been shown to be produced by a family of bilirubin-inducible FPs. In catsharks, fluorescence has been attributed to a brominated tryptophan-kynurenine small molecule metabolite (Park et al., 2019), as opposed to a fluorescent protein (FP). Biochemical studies to identify and characterize the fluorescent molecules of additional lineages of marine fishes, including lizardfishes and other eel lineages, are ongoing (D.F.G., J.S.S. and G.P. Gaffney, unpub. data; Guarnaccia et al., in prep).

Oceanic light regimes vary depending on the turbidity and primary production in the region, with oligotrophic oceanic systems having a narrow blue bandwidth at ~467 nm (Jerlov, 1968). In addition, Arctic systems have been shown to be slightly UV shifted (Aas et al., 2002). With increasing latitude approaching the poles, there is a corresponding increase in seasonal variation of the night-day ratio, with winter experiencing almost total darkness and, in contrast, nearly continuous sunlight in summer. This highly seasonal light regime provides significantly more time in some months for daylight biofluorescence, especially compared with the winter months of near total darkness, when fluorescence would not be functional. As a result, we hypothesized that fluorescence should be less common in high-latitude Arctic waters, where it would not be emitted, and therefore not functional, during long periods of darkness. Previous studies have shown that fluorescence in marine fishes is most common in tropical regions, becoming far less common and less widely phylogenetically distributed in temperate regions (Sparks et al., 2014). Our hypothesis was confirmed in that we found marine fluorescence to be quite rare in the Arctic, in both invertebrate and vertebrate lineages. Moreover, we found that fluorescence was rare or absent in members of widely distributed groups in which fluorescence was widespread and common across lineages in tropical and temperate regions (e.g., multiple lineages of scorpionfishes, flatfishes).

Classification and General Biology of Snailfishes (Liparidae)

Liparidae is a diverse family of cottoid (scorpaeniform) fishes comprising approximately 420 species arrayed within 32 genera. Liparids are characterized by their asquamate, elongate bodies, and by a sucking disk formed by modified pelvic fins. Liparidae is bipolar in distribution, with members of the widespread genus Liparis (~60 spp.) restricted to the northern hemisphere. Liparids inhabit the intertidal zone to the extreme depths of the Mariana Trench, and exhibit a greater depth range than any other family of fishes (Wang et al., 2019). They are among the most common hadal vertebrate species, and Pseudoliparis swirei and the undescribed “ethereal snailfish” are currently recognized as the deepest-dwelling vertebrates (8076 and 8178 m, respectively) (Linley et al., 2016; Wang et al., 2019).
Liparis gibbus, the variegated snailfish, is a benthic species occurring in the Arctic, North Atlantic, and the Northeast Pacific oceans (Greenland, Canada, Russia, Svalbard, and southeastern Alaska). It generally occurs between 100–200 m depth, has an average length of 11 cm (max. 52 cm), and typically feeds on amphipods and crabs (Scott and Scott, 1988; Eschmeyer and Herald, 1999; Evans, 2003). Liparis tunicatus, the kelp snailfish, occurs in the Arctic and Northwest Atlantic oceans, ranging from the Bering Strait to Labrador, Canada (Leim and Scott, 1966), at depths up to 620 m, but is generally found in less than 100 m (Robins and Ray, 1999). It is a benthic species commonly found among kelp that feeds on crustaceans, particularly amphipods (Scott and Scott, 1988). Arctic seas are inhabited by only ~270 species of fishes (Møller et al., 2010; Mecklenburg et al., 2011), with 15 endemic marine fish species, comprising mostly sculpins, snailfishes, and eelpouts (Christiansen and Reist, 2013).

MATERIALS AND METHODS

The juvenile Liparis gibbus specimens (AMNH 277096, \( n = 2 \), 18 and 19 mm SL; fig. 1) were collected within a kelp forest on August 26, 2019 in Sermilik Fiord, near Qaattu (65.788, -37.879) in eastern Greenland, at a depth of approximately 15 m (fig. 2). One specimen was preserved in 10% buffered formalin for morphological studies and the other was preserved in RNA Stabilization Solution (RNAlater, Qiagen) for transport back to the laboratory for downstream genomic analyses. Fishes were collected via SCUBA using a handnet, following the application of quinaldine to temporarily immobilize the specimens for capture. This study was approved and carried out in strict accordance with the recommendations in the Guidelines for the Use of Fishes in Research of the American Fisheries Society and the American Museum of Natural History’s Institutional Animal Care and Use Committee (IACUC). Institutional abbreviations are as listed in Leviton et al. (1985) and Sabaj Pérez (2010).

The Liparis gibbus specimens were photographed and filmed under blue fluorescence excitation light using a Red Digital Cinema Gemini 5K S35 CMOS camera with Royal Blue LED lights collimated to ensure perpendicular incidence on the scientific grade 450/70 nm interference filter surface (Semrock, Inc., Lake Forest, IL), thereby minimizing the transmission of out-of-band energy. To image and record biofluorescence, a scientific-grade 514 nm long-pass emission filter (Semrock, Inc.) was embedded in front of the sensor of the camera. For still fluorescent photography, individual fish specimens were placed in a narrow photographic tank and held flat against a thin plate glass front. Fluorescent macro images were produced using Nikon D800 or D4 DSLR cameras outfitted with a Nikon 60 or 105 mm macro lens in a dark room by covering the flashes (Nikon SB910) with blue interference bandpass excitation filters (Omega Optical, Inc., Brattleboro, VT; Semrock, Inc., Rochester, NY). Longpass (LP) emission filters (Semrock, Inc.) were attached to the front of the camera lens to record fluorescence. Multiple LP filter pairs were tested to best capture the fluorescence-emission spectrum (e.g., a 514 nm LP filter was utilized to cap-
ture green fluorescence, whereas a 561 nm LP filter was used to image longer-wavelength fluorescence [orange and red], and block emitted green fluorescence). The adult *L. tunicaetus* specimen was scanned for fluorescence by Jay Orr (NOAA Fisheries Service, Seattle, WA) using a blue Sola LED excitation light (Light and Motion, Marina, CA) and plexiglass long-pass filter.

Emission spectra were recorded using an Ocean Optics USB2000+ miniature spectrometer (Dunedin, FL) equipped with a hand-held fiber optic probe (Ocean Optics ZFQ-12135). Excitation spectra were achieved during illumination with Royal Blue LED lights collimated to ensure perpendicular incidence on the scientific grade 450/70 nm interference filter surface. Emission spectra were recorded by placing the fiber optic probe proximate to specific anatomical parts of the individual fish specimen exhibiting biofluorescence. This was repeated several times for each specimen and each anatomical region to ensure the accuracy of measurements.

We sequenced the transcriptome of a juvenile variegated snailfish, *Liparis gibbus*, specimen # GRLD_JSS_19_12 (fig. 1). Total RNA was extracted from musculature on the dorsal half of the specimen. Illumina NovaSeq 2×150 bp sequencing and initial bioinformatic analyses were performed by Genewiz (South Plainfield, NJ). The NGS run yielded 281,412,170 reads (84,424 megabases) with a mean Q score of 35.71 (92.79% of bases ≥ Q30). Trimmomatic v0.36 was used to trim adapters and quality-trim the raw data. Trinity v2.5 was used to assemble the transcriptome de novo with a minimum contig length of 200 bp.
FIG. 2. (Top) Aerial view of iceberg-filled habitat in Sermilik Fiord, eastern Greenland, near where specimens of *Liparis gibbus* were collected. The authors can be seen underwater in center of image. (Bottom) The authors collecting specimens on iceberg shown in top image.
RESULTS

Genomic Analyses

Two fragments of mitochondrial 16S were located in the sequenced transcriptome. A 153 bp fragment was a 100% match to *Liparis fabricii*. A 213 bp fragment also matched *Liparis ochotensis* across all positions from 1465–1677 in the 16S sequence. Using these data, we were able to identify the small juvenile specimens as members of the genus *Liparis* and final identification as *L. gibbus* was provided by Jay Orr (NOAA Fisheries Service, Alaska Fisheries Science Center, Seattle, WA), based on an examination of pigmentation pattern and external morphological features.

**Description of Fluorescence Pattern in Juvenile *Liparis gibbus***

Two distinct fluorescence-emission peaks, one in the green and the other in the red portion of the spectrum, were observed in the juvenile *Liparis gibbus* specimens (AMNH 277096) (compare fig. 3A–C with D–F), depending on the specific anatomical region scanned, an uncommon occurrence among marine fishes where generally only a single fluorescent color is emitted. The two juvenile *L. gibbus* specimens scanned for fluorescence exhibited multiple fluorescence-emission colors, green and red, and an identical fluorescent pattern (see fig. 1C, D and Supplemental Video S1, available online at https://doi.org/10.5531/sd.sp.48). The eyes were a bright fluorescent green (523 nm; figs. 1C, 3C), and large portions of both the dorsal and ventral body surfaces, as well as the posterior flank, exhibited numerous small discrete green fluorescent markings (527–530 nm), creating an overall speckled pattern (figs. 1C, D, 3A, B). The head, anterior body dorsally, and a broad region surrounding the pelvic sucking disk ventrally are also peppered with discrete bright green fluorescent markings of the same wavelength range (figs. 1C, D, 3A). There are some scattered red fluorescent markings on the body (674–678 nm), both dorsally and ventrally, but they are not nearly as numerous as the green markings (figs. 1C, D, 3D). The pectoral fins and immediate surrounding lateral body surfaces are fluorescent red (675 nm; figs. 1C, D, 3F), and the ventral pelvic sucking disk was solid fluorescent red (674 nm; figs. 1D, 3E).

**Description of Fluorescence Pattern in Adult *Liparis tunicatus***

The adult specimen of *L. tunicatus* (UW uncat.) exhibited only red fluorescence, comprising a bilateral pattern of discrete red spots and blotches that was identical on both the left and right sides (fig. 4). No green fluorescence was observed, in contrast to that of *L. gibbus*, in which the eyes and large portions of the body exhibited bright green fluorescence. An identical bilateral pattern of discrete red spots and blotches on the left and right head, flank, and fins clearly indicates intrinsic fluorescence and not extrinsic algae on the epidermis, which would be distributed in a random pattern, of this adult specimen of *Liparis tunicatus*. It is uncommon to find differences in fluorescence-emission spectra/colors between juvenile and adult individuals of the same or closely related species.
DISCUSSION

Our prior studies on marine fishes show that the emission of multiple colors of fluorescence in a single species or individual is rare (Sparks et al., 2014; Gruber et al., 2016). For example, we previously reported that the sand stargazer (*Gillellus uranidea*) has two colors of biofluorescence, green and red, which can be observed simultaneously (Sparks et al., 2014: fig. 1K), similar to what we observed for the juvenile specimens of *Liparis gibbus* (fig. 1C and D; Supplemental Video S1). In the juvenile *L. gibbus* specimens we observed both green and red fluorescence in a single individual, with the eyes bright fluorescent green (523 nm emission peak), the dorsal and ventral body surfaces, head, and posterior flank covered primarily in small, discrete, bright green fluorescent spots (527–530 nm emission peak), with some isolated scattered red fluorescent markings on the body (674–678 nm emission peak), the pectoral fins and surrounding lateral surfaces are fluorescent red (675 nm emission peak), and the ventral pelvic sucking disk a solid fluorescent red (674 nm emission peak). In addition, we have shown that both chlopsid eels and reef (shallow water) lizardfishes exhibit both green and red fluorescence in a single species (Sparks et al., 2014: fig. 1H, I). However, in both chlopsid eels and reef lizardfishes, the two fluorescent colors cannot be seen simultaneously. For example, if we excite with high-energy blue light in these lineages, and use a long-pass filter (LP) to block out only the blue excitation light (i.e., using a 514 nm LP filter), we see only green fluorescence (Sparks et al., 2014: fig. 1H). To observe the red fluorescence in these lineages, we needed to use a LP filter that also filters out the emitted green fluorescence (i.e., using a 561 nm LP filter) (Sparks et al., 2014: fig. 1I). That is likely due to the fact that in both chlopsid eels and reef lizardfishes the fluorescent molecules/proteins are widely distributed, covering the entire fish (e.g., the fluorescent protein is widely distributed internally in the musculature as well in chlopsid eels, see Gruber et al., 2015: fig. 3b), such that one fluorescent color will obscure the other, unless filtered out using a scientific grade LP filter. Conversely, in the sand stargazer...
(Gillellus uranidea), the green and red fluorescent molecules/proteins are more discretely distributed (i.e., they do not overlap in distribution), enabling both colors to be viewed simultaneously using only a single LP filter (Sparks et al., 2014: fig. 1K).

The two Liparis species imaged in this study are relatively closely related (Orr et al., 2019), and based on our studies of fluorescence pattern variation within other bony fish genera, the results related to fluorescence emission that we observed should apply across the genus (i.e., red fluorescence only in adults vs. both green and red fluorescence in juveniles). However, we fully acknowledge and caution that further study of additional species is needed to confirm whether these results are generalizable across the diverse snailfish genus Liparis. In our studies of marine fishes to date, we have documented variation in fluorescence-emission colors and patterns between juveniles and adults within specific lineages, including marine anguilliform eels (no fluorescence in juveniles vs. strong fluorescence in adults) and surgeonfishes (strong green fluorescence in juveniles vs. no fluorescence in adults) (Sparks et al., 2014: fig. 1N; Gruber et al., 2015). Admittedly, we have not been able to comprehensively sample juveniles and adults across a broad range of cartilaginous and bony fishes, due to the difficulty in both collecting and identifying juvenile representatives to compare with adult specimens.

Finally, we note that the phylogenetic distribution of fluorescence at high latitudes is notably muted compared to tropical and temperate locals (see Sparks et al., 2014). We note a conspicuous lack of fluorescence in Arctic members of lineages whose members are, for the most part, highly fluorescent elsewhere (in temperate and tropical localities), including scorpionfishes and flatfishes. For example, none of the sculpin species (e.g., Myxocephalus spp.) that we collected and scanned in Greenland exhibited any observable fluorescence, whereas flatfishes (e.g., Hippoglossoides platessoides) were at most very weakly fluorescent (vs. strongly fluorescent in the tropics and temperate waters for both groups). We acknowledge that more comprehensive surveys for fluorescence in the Arctic are warranted, such that these generalizations can be corroborated.

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