First record of biofluorescence in lumpfish (Cyclopterus lumpus), a commercially farmed cleaner fish

Thomas Juhasz-Dora1,2 | Jonathan Teague3 | Thomas K. Doyle2,4 | Julie Maguire1

1Bantry Marine Research Station, Cork, Ireland  
2School of Biological, Earth and Environmental Sciences, University College Cork, Cork, Ireland  
3Interface Analysis Centre, School of Physics, University of Bristol, Bristol, UK  
4Science Foundation Ireland Research Centre for Energy, Climate and Marine, Environmental Research Centre, University College Cork, Cork, Ireland

Correspondence  
Thomas Juhasz-Dora, Bantry Marine Research Station, Gearhies, P75 AX07 County Cork, Ireland.  
Email: tjuhasz-dora@ucc.ie

Funding information  
H2020 Marie Sklodowska-Curie Actions, Grant/Award Number: 956697; Horizon 2020; European Union

Abstract  
This study is the first known observation of biofluorescence in the lumpfish (Cyclopterus lumpus). Individual lumpfish were illuminated with blue excitation lighting for photography with both hyperspectral and filtered multispectral cameras. All photographed juvenile lumpfish (n = 11) exhibited green biofluorescence. Light emissions were characterised with two peaks observed at 545 and 613 nm, with the greatest intensity along the tubercles of the high crest and the three longitudinal ridges. Further research on the dynamics of biofluorescence through the lifecycle of this species is required.

KEYWORDS  
cleanerfish, hyperspectral imaging, temperate species

Biofluorescence has been documented in a wide variety of terrestrial and aquatic species, including the duck-billed platypus, springhare, butterflies, parrots, scleractinian corals and over 100 species of fish (Olson et al., 2021; Sparks et al., 2014). When an organism biofluoresces, light absorbed by the organism is remitted in lower energy wavelengths (Sparks et al., 2014). As light interacts with water in the marine environment, it is attenuated at different rates according to wavelength. This variance is a result of scattering from the water itself or by suspended particles within the water body or absorption by particles (Jerlov, 1968). Marine organisms absorb the ambient blue light (450–495 nm) present in their environment generally via fluorescent compounds, reemitting green (495–570 nm), orange (590–620 nm) and red (620–750 nm) fluorescence into their environment (Gruber & Sparks, 2021; Vaccani et al., 2019). The shorter wavelengths (blue, green) can penetrate to deeper depths, whereas longer wavelengths (orange, red) are quickly attenuated in depths >15 m (Kirk, 2011). The unique lighting conditions produced by this spectrally restricted (blue-shifted) illumination allow marine organisms to exploit fluorescence to produce visual contrast and patterns (Gruber et al., 2016; Gruber & Sparks, 2021; Sparks et al., 2014). Biofluorescence in marine teleost fish may function for communication, predator avoidance or prey attraction in otherwise cryptic species (Gruber & Sparks, 2021; Vaccani et al., 2019; Sparks et al., 2014). Biofluorescence appears particularly common and phenotypically variable in tropical fish communities (Anthes et al., 2016; Gerlach et al., 2016; Gruber & Sparks, 2021). Limited published research exists on the presence or function of biofluorescence within temperate zone fish lineages outside the Arctic Ocean (Gruber & Sparks, 2021).

The lumpfish (Cyclopterus lumpus) are found in temperate waters (4–12°C) and are produced commercially for controlling sea lice (Lepeophtheirus salmonis) in Atlantic salmon (Salmo salar) farming operations in North Atlantic countries (Imsland et al., 2014; Staven et al., 2021). Lumpfish are unique in having a rough, scaleless skin with a variable colour pattern that is sexually dimorphic in adult specimens. The species has also been documented to change colour for camouflage (Powell et al., 2018). The body is covered with multiple rows of
knobby protuberances called tubercles, with the most pronounced
tubercles found along three longitudinal ridges along the length of
the body (Patel et al., 2019; Powell et al., 2018). The integrity and function
of fish skin is critical to their health and welfare as it is constantly sub-
ject to osmotic pressures and mechanical abrasion in the environment
(Imsland et al., 2014; Patel et al., 2019; Staven et al., 2021). Although
lumpfish are in high demand for biological control on salmon farms, an
effective method for understanding subclinical stress in this species
through non-invasive methods that focus on the skin has yet to be
determined. In this study, the authors document biofluorescence in
juvenile lumpfish and characterise the type of biofluorescence
observed under aquaculture conditions. This observation adds to the
growing literature on biofluorescence in marine fishes but notably
describes the first known documentation of biofluorescence in a com-
mercially produced fish species. Juvenile lumpfish (n = 11) of c.
90 days old were photographed in December 2021 at the Bantry
Marine Research Station located in Bantry, County Cork, Ireland. The
juvenile lumpfish utilised for this initial investigation were reared in an
enclosed semi-static recirculation aquaculture system (RAS) as permit-
ted by the Irish aquaculture licence T5/314. The fish were reared in
10,000 l tanks stocked at a maximum stocking density of 40 kg m
Ambient blue lighting was utilised in the RAS to minimise stress as
well as corneal damage to the large, sensitive eyes typical of the spe-
cies. A light sedative dose of tricaine methane sulphonate was admin-
istered to the fish prior to measurements. Each fish photographed
was measured for weight and length to ensure that all the fish utilised
were of similar size class as means to compare fluorescence without
the influence of size-based aggression. Fish were measured using total
length (TL) from the tip of the snout to the caudal tip of the caudal fin
and weighed via a scientific digital scale (Precia Molen BJ 6100G) in
grams. The study group averaged 11.3 ± 0.2 cm and 62.9 ± 2.0 g. Fish
were returned unharmed to the same grow-out tank as a group.

During photography, lumpfish were placed in a photographic
aquarium constructed of optic white glass. Filtered sea water from the
RAS was used to minimise stress during photography. The photo-
graphic aquarium was placed in a portable foldable photography light
box within a dark room to minimise external light contamination. The
aquarium was then illuminated with the Ecotech G5 XR30 Pro Radion
full spectrum LED light. The biofluorescence was recorded under royal
blue spectrum (emission peak of 452 nm) (spectrum outlined in
Figure 1c) using a DSLR camera (D5100, Nikon, Melville, NY, USA/
Nikon AF-S 60 mm f/2.8G IF-ED Micro lens). A yellow barrier filter
(Tiffen 62DY15 62 mm Deep Yellow 15 Filter, Hauppage, NY, USA)
was utilised to block reflected excitation wavelengths between
440 and 460 nm for the purpose of capturing RGB images of the
fluorescence.

To spectrally characterise the fluorescence emission of a lump-
fish, hyperspectral data were captured using a snapshot hyperspectral
imager (IQ, Specim, Oulu, Finland) from a distance of 30 cm under the
same excitation source described earlier. Hyperspectral data were
analysed in ENVI (Harris Geospatial Solutions, Boulder, Co, USA)
where regions of interest were used to average the spectra of 1000
individual pixels to generate an average spectrum.

All juvenile lumpfish photographed (n = 11) through the yellow
barrier filter exhibited visible green biofluorescence. Limited visual
variation and spatial coverage exist between the biofluorescence of
individuals (Figure 1).

Light emissions were most intense on the tubercles of the high
crest and the three longitudinal ridges. A diffuse biofluorescence was
observed on skin on the lower head, operculum and ventral zone to
the caudal fin. The small tubercles on the scaleless skin exhibit slightly
lower emissions. A diffuse fluorescence was observed on the soft rays
of the pectoral, dorsal, caudal and anal fins (Figure 2b). Using the
hyperspectral data gathered, the specific wavelength of the fluores-
cence was characterised with two peaks observed at 545 and 613 nm
(Figure 2c).

This study is the first known observation of biofluorescence
occurring in the lumpfish C. lumpus. This is also the first known study
to document biofluorescence in fish produced within a commercial
aquaculture operation. Biofluorescence has been well documented in
over 100 species in tropical fish communities (Anthes et al., 2016;
Gerlach et al., 2016; Gruber & Sparks, 2021; Michiels et al., 2008);
nonetheless, published data on temperate fish lineages outside snail-
fish (Liparus sp.) are lacking.

Lumpfish belong to the order Scorpaeniformes, of which Playty-
chephilidae (flatheads) and Sebastidae (rock perches) have representa-
tives in the tropics known to biofluoresce (Sparks et al., 2014). The
fact that lumpfish are biofluorescent is not phylogenetically surprising.
Biofluorescence in temperate marine species such as the lumpfish is understudied, with the geographically closest published study documenting two juvenile specimens of the variegated snailfish Liparis gibbus in eastern Greenland and an adult kelp snailfish Liparis tunicatus collected in the Bering Strait off Alaska (Gruber & Sparks, 2021). Further research is needed to understand the role of biofluorescence in temperate oceans.

Lumpfish are generally considered a solitary species outside of seasonal spawning aggregations (Powell et al., 2018). Nonetheless, close resting aggregations regularly occur in aquaculture (Powell et al., 2018). As one of the documented functions of biofluorescence in marine vertebrates and invertebrates is communication (Gruber & Sparks, 2021; Meadows et al., 2014; Michiels et al., 2008), lumpfish behaviour at sea may be more social than is currently accepted. Biofluorescence may be used to communicate territorial claims to other lumpfish through their lifecycle. Hatchery-produced juveniles can be aggressively territorial in the first 3 months post hatching (Treasurer et al., 2018), with studies documenting multi-year fidelity to specific territories in adults (Powell et al., 2018). The concentrated biofluorescence found at the high crest and ridges may advertise to conspecifics at a distance.

Lumpfish undergo remarkable morphological changes as they mature into adults (Treasurer et al., 2018). How such sexually dimorphic life stages of C. lumpus affect biofluorescence is currently unknown. Biofluorescence has been documented to vary between life stages in marine organisms. Sexually dimorphic fluorescence patterning has been observed in multiple lineages of marine bony and cartilaginous fishes (Gruber et al., 2016; Sparks et al., 2014). Profound biofluorescence dimorphism has been documented between juvenile and adult Liparis snailfish of the Arctic Ocean (red in adults, green and red in juveniles) (Gruber & Sparks, 2021; Powell et al., 2018). Laboratory studies with male red-eye wrasse (Cirrhilabrus solorensis) found strong behavioural responses to red biofluorescence in patterns documented only in mature males (Gruber & Sparks, 2021; Gerlach et al., 2014), whereas the manipulation of red and blue wavelengths in experimental tanks was able to induce intraspecific aggressive interactions in the Mediterranean rainbow wrasse Coris julis (Braun et al., 2014). Males of the cryptic fish Tripterygon delaisi regulate their biofluorescent signals to receptive females, which perceive intraspecific signalling through red-fluorescent irises (Kalb et al., 2015; Wucherer & Michiels, 2014).

How juvenile lumpfish utilise their green biofluorescence in their natural habitat is unknown and warrants further research. The green biofluorescence documented in C. lumpus has been scientifically documented in other marine species. Sparks et al. (2014) reported green fluorescence covering the entire body of varying fish species, whereas Vaccani et al. (2019) documented the green fluorescence concentrations around the head, eyes and upper trunk of the diurnal seahorse Hippocampus reidi. Green fluorescence may be related to prey attraction, as has been documented through the attraction of juvenile Sebastes rockfish to green hydromedusa tentacles under excitation lighting (Haddock & Dunn, 2015; Vaccani et al., 2019). The green fluorescence of the catshark species Cephaloscyllium ventriosum and Scyliorhinus retifer enhances the mottled pigmentation pattern on the skin as depth increases, making these sharks more visible to conspecifics at depth (Gruber et al., 2016; Gruber & Sparks, 2021). The epibenthic-pelagic lifecycle of lumpfish likewise seasonally varies from nearshore spawning to depths recorded to 868 m (Coad & Reist, 2004; Kennedy et al., 2016; Stein, 1986).

Anatomical studies are needed to determine whether yellow intraocular corneal filters exist in C. lumpus as documented in other marine species (Sparks et al., 2014). Yellow intraocular corneal filters are believed to function as long-pass filters for enhanced perception of biofluorescence produced by fluorescent proteins found in tissue or through metabolism of specific small molecule metabolites as found in two species of catsharks (Park et al., 2019; Gruber et al., 2015; Kumagai et al., 2013). The sensitivity of lumpfish to lighting outside of the blue spectrum in commercial production as viewed by the author alludes to such a structure existing within the C. lumpus cornea. Staven et al. (2021) notes that blue-green colouration typically found

**FIGURE 2** Juvenile lumpfish (Cyclopterus lumpus) displaying biofluorescence during laboratory photographic investigation. The lumpfish under (a) white light conditions and (b) fluorescing under royal blue (452 nm) excitation. Fluorescence levels transition in intensity from the high crest, longitudinal tubercle lines, and finally to the shallow tubercles on the skin. (c) The average spectra of the fluorescence show three main peaks, the excitation peak at 452 nm and two fluorescence peaks one at 545 and 613 nm.
in lumpfish skin is regulated by the antioxidant biliverdin, a metabolic breakdown product of haemoglobin. This is similar to the study by Kumagai et al. (2013), who found the fatty acid binding protein, UnaG, in the Japanese eel (Anguilla japonica). This fluorescent protein produces an oxygen-independent green fluorescence when excited by heme metabolite bilirubin. Lumpfish tissue should be analysed if its biliverdin can induce excitation as in Japanese eels. The authors hope that future in-depth studies will reveal the full complexity of biofluorescence in this temperate Scopaeiforme fish.

AUTHOR CONTRIBUTIONS
Conceptualisation, T.J. J.T.; methodology, T.J., J.T.; data/analysis, T.J., J. T.; fish specimens, T.J.; review and editing, T.D. J.M; supervision, T. D. J.M; funding acquisition, J.M. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGEMENTS
This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 956697. Open access funding provided by IReL.

CONFLICTS OF INTEREST
The authors declare no conflict of interest.

COMPLIANCE WITH ETHICAL STANDARDS
This study followed the “Ethical Principles in Animal Research” guidelines adopted by the Health Products Regulatory Authority (HPRA) of Ireland. No additional permitting was required under these guidelines for this study. Lumpfish used in this study were produced under Irish aquaculture licence T5/314. Fish were returned to the aquaculture unit after photography.

ORCID
Thomas Juhasz-Dora https://orcid.org/0000-0003-3447-7395

REFERENCES
Anthes, N., Theobald, J., Gerlach, T., Meadows, M. G., & Michiels, N. K. (2016). Diversity and ecological correlates of red fluorescence in marine fishes. Frontiers in Ecology and Evolution, 4, 126.
Braun, C., Michiels, N. K., Siebeck, U. E., & Sprenger, D. (2014). Signalling function of long wavelength colours during agonistic male-male interactions in the wrasse Coris julis. Marine Ecology Progress Series, 504, 277–286.
Coad, B. W., & Reist, J. D. (2004). Annotated list of the Arctic marine fishes of Canada (p. 2674). Winnipeg, Canada: Fisheries and Oceans Canada.
Gerlach, T., Sprenger, D., & Michiels, N. K. (2014). Fairy wrasses perceive and respond to their deep red fluorescence coloration. Proceedings of the Royal Society B: Biological Sciences, 281(1787), 1–7.
Gerlach, T., Theobald, J., Hart, N. S., Collin, S. P., & Michiels, N. K. (2016). Fluorescence characterisation and visual ecology of pseudocheilinid wrasses. Frontiers in Zoology, 13(1), 1–8.
Gruber, D. F., Gaffney, J. P., Mehr, S., DeSalle, R., Sparks, J. S., Platisa, J., & Pieribone, V. A. (2015). Adaptive evolution of eel fluorescent proteins from fatty acid binding proteins produces bright fluorescence in the marine environment. PLoS One, 10(11), e0140972.
Gruber, D. F., Loew, E. R., Deheyne, D. D., Akkaynak, D., Gaffney, J. P., Smith, W. L., ... Sparks, J. S. (2016). Biofluorescence in catsharks (Scyliorhinidae): Fundamental description and relevance for elasmobranch visual ecology. Scientific Reports, 6(1), 1–16.
Gruber, D. F., & Sparks, J. S. (2021). First report of biofluorescence in Arctic Snaillfishes and rare occurrence of multiple fluorescent colors in a single species. American Museum Novitates, 2020(3967), 1–12.
Haddock, S. H., & Dunn, C. W. (2015). Fluorescent proteins function as a prey attractant: Experimental evidence from the hydromedusa Olindias formosus and other marine organisms. Biology open, 4(9), 1094–1104.
Imsland, A. K., Reynolds, P., Eliassen, G., Hangstad, T. A., Nytrø, A. V., Foss, A., ... Elvegård, T. A. (2014). Assessment of growth and sea lice infection levels in Atlantic salmon stocked in small-scale cages with lumpfish. Aquaculture, 433, 137–142.
Kirk, J. T. O. (2011). Light and Photosynthesis in Aquatic Ecosystems (3rd ed.). Cambridge: Cambridge University Press.
Kumagai, A., et al. (2013). A bilirubin-inducible fluorescent protein from eel muscle. Cell, 153, 1602–1611.
Meadows, M. G., Anthes, N., Dangelmayer, S., Alwany, M. A., Gerlach, T., Schulte, G., ... Michiels, N. K. (2014). Red fluorescence increases with depth in reef fishes, supporting a visual function, not UV protection. Proceedings of the Royal Society B: Biological Sciences, 281(1790), 20141211.
Michiels, N. K., Anthes, N., Hart, N. S., Herler, J., Meixner, A. J., Schießenbaum, F., ... Wucherer, M. F. (2008). Red fluorescence in reef fish: A novel signalling mechanism? BMC Ecology, 8(1), 1–14.
Olson, E. R., Carlson, M. R., Ramanujam, V. S., Sears, L., Anthony, S. E., Anich, P. S., ... Martin, J. G. (2021). Vivid biofluorescence discovered in the nocturnal springhare (Pedetidae). Scientific Reports, 11(1), 1–8.
Park, H. B., Lam, Y. C., Gaffney, J. P., Weaver, J. C., Krivoshik, S. R., Hamchand, R., et al. (2019). Bright green biofluorescence in sharks derives from bromo-kyurenine metabolism. iScience, 19, 1291–1336. https://doi.org/10.1016/j.isci.2019.07.019.
Patel, D. M., Bhide, K., Bhide, M., Iversen, M. H., & Brinchmann, M. M. (2019). Proteomic and structural differences in lumpfish skin among the dorsal, caudal and ventral regions. Scientific Reports, 9(1), 1–13.
Powell, A., Pooley, C., Scalamacchia, M., & Garcia de Leaniz, C. (2018). Review of lumpfish biology. Cleaner Fish Biology and Aquaculture Applications, 6, 90–113.
Sparks, J. S., Schelly, R. C., Smith, W. L., Davis, M. P., Tchernov, D., Pieribone, V. A., & Gruber, D. F. (2014). The covert world of fish biofluorescence: A phylogenetically widespread and phenotypically variable phenomenon. PLoS One, 9(1), e83259.
Staven, F. R., Nordeide, J. T., Gjest, M., Andersen, P., Patel, D. M., & Kristensen, T. (2021). Behavioural and physiological responses of lumpfish (Cyclopterus lumpus) exposed to Atlantic salmon (Salmo salar) sensory cues. Aquaculture, 544, 737066.
Stein, D. L. (1986). Cyclopteridae. In P. J. P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen, & E. Tortonese (Eds.), Fishes of the North-Eastern Atlantic and the Mediterranean (pp. 1269–1274). Paris: UNESCO Vol. III. (Ref. 4701).
Treasurer, J. W., Noble, C., Puvanendran, V., Planellas, S. R., & Iversen, M. H. (2018). Cleaner fish welfare. In J. W. Treasurer (Ed.), Cleaner fish biology and aquaculture applications (pp. 221–257). UK: 5M Publications. Sheffield.

Vaccani, A. C., Freret-Meurer, N. V., Bertoncini, Á. A., & Santos, L. N. (2019). Shining in the dark: First record of biofluorescence in the seahorse Hippocampus reidi. PLoS One, 14(8), e0220561.

Wucherer, M. F., & Michiels, N. K. (2014). Regulation of red fluorescent light emission in a cryptic marine fish. Frontiers in Zoology, 11(1), 1–8.

How to cite this article: Juhasz-Dora, T., Teague, J., Doyle, T. K., & Maguire, J. (2022). First record of biofluorescence in lumpfish (Cyclopterus lumpus), a commercially farmed cleaner fish. Journal of Fish Biology, 101(4), 1058–1062. https://doi.org/10.1111/jfb.15154