

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

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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 (Imslund *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

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FIGURE 1 A selection of three juvenile lumpfish (*Cyclopterus lumpus*) displaying biofluorescence during laboratory photographic investigation. Limited visual variation and spatial coverage of green biofluorescence exist between individuals

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 subject 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 commercially 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 permitted 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^{-3} . Ambient blue lighting was utilised in the RAS to minimise stress as well as corneal damage to the large, sensitive eyes typical of the species. A light sedative dose of tricaine methane sulphonate was administered 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 $11.3 \pm 0.2 \text{ cm}$ and $62.9 \pm 2.0 \text{ g}$. 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 photographic 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, Hauppauge, 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 lumpfish, 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 fluorescence 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 snailfish (*Liparus* sp.) are lacking.

Lumpfish belong to the order Scorpaeniformes, of which Playtychephalidae (flatheads) and Sebastidae (rock perches) have representatives in the tropics known to biofluoresce (Sparks *et al.*, 2014). The fact that lumpfish are biofluorescent is not phylogenetically surprising.

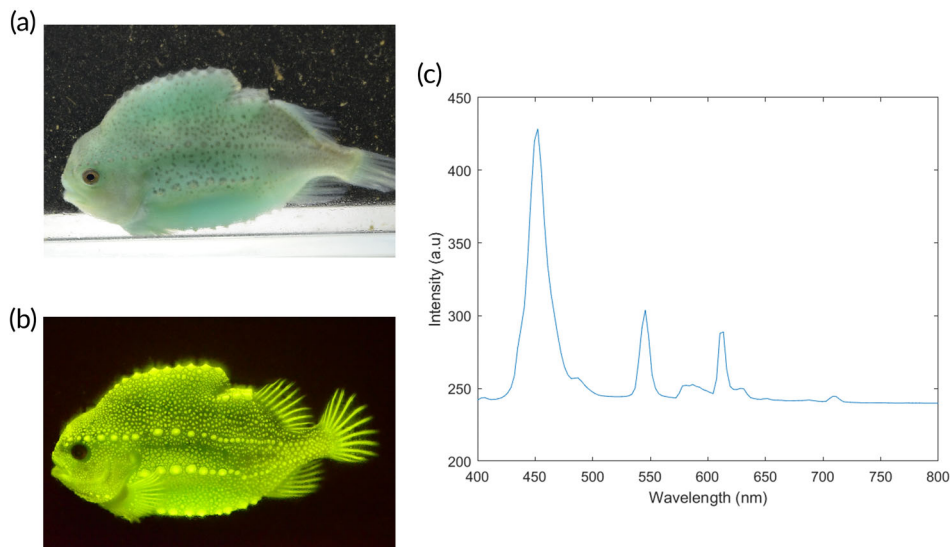


FIGURE 2 Juvenile lumpfish (*Cyclopterus lumpus*) displaying biofluorescence during laboratory photographic investigation. The lumpfish under (a) white light conditions and (b) fluorescing under royal blue (452 nm) excitation. Fluorescence levels transition in intensity from the high crest, longitudinal 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

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 *Tripterygion 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

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 Scorpaeniforme 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.

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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.

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