

PAPER • OPEN ACCESS

Hidden in plain sight: hyperspectral documentation of complex biofluorescence produced by the green sea urchin (*Strongylocentrotus droebachiensis*)

To cite this article: Thomas Juhasz-Dora *et al* 2024 *Methods Appl. Fluoresc.* **12** 025002

View the [article online](#) for updates and enhancements.

You may also like

- [Biomining: mineral formation by organisms](#)
Lia Addadi and Steve Weiner
- [Nutritional characteristics of sea urchin \(*Diadema setosum*\) in Bungus, West Sumatra Province](#)
R Karnila, D Iriani, S Md Shaarani *et al.*
- [Sea urchin-like CuO particles prepared using \$\text{Cu}_3\(\text{PO}_4\)_2\$ flowers as precursor for high-performance ethanol sensing](#)
Yanhua Sang, Guoshuai Song, Zhimin Gao *et al.*



EDINBURGH INSTRUMENTS

WORLD LEADING MOLECULAR SPECTROSCOPY SOLUTIONS

edinst.com

The advertisement features a red background with the Edinburgh Instruments logo on the left, which consists of a circular pattern of white dots. In the center, there are several pieces of laboratory equipment, including a large white and black spectrometer labeled 'FLS 1000' and a smaller white device labeled 'FSS'. The text 'EDINBURGH INSTRUMENTS' is written in white, bold, uppercase letters. Below the logo, the text 'WORLD LEADING MOLECULAR SPECTROSCOPY SOLUTIONS' is written in white, bold, uppercase letters. In the bottom right corner, the website 'edinst.com' is displayed in white text on a red rectangular background.

Methods and Applications in Fluorescence



PAPER

OPEN ACCESS

RECEIVED
8 September 2023

REVISED
9 January 2024

ACCEPTED FOR PUBLICATION
26 January 2024


PUBLISHED
7 February 2024

Original content from this work may be used under the terms of the [Creative Commons Attribution 4.0 licence](#).

Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.



Hidden in plain sight: hyperspectral documentation of complex biofluorescence produced by the green sea urchin (*Strongylocentrotus droebachiensis*)

Thomas Juhasz-Dora^{1,2} , Philip James³, Tor Evensen³ and Stein-Kato Lindberg³

¹ Bantry Marine Research Station, Gearhies, Bantry, Co. Cork P75 AX07, Ireland

² School of Biological, Earth and Environmental Sciences, University College Cork, Cork, T23 N73K, Ireland

³ Nofima AS, PO Box 6122, NO-9291, Tromsø, Norway

E-mail: tjuhasz-dora@ucc.ie

Keywords: hyperspectral imaging, fluorospectrometer, coelomic fluid, echinoderms, monitoring

Abstract

Biofluorescence in echinoderms is largely unexplored, and even though the green sea urchin *Strongylocentrotus droebachiensis* is a well-studied species, the presence and/or function of fluorescence remains very poorly understood. Hyperspectral imaging was conducted on adult sea urchins (N = 380) while fluorospectrometric analysis was conducted on sea urchin coelomic fluid (N = 30). Fluorescence was documented in both the spines and coelomic fluid of *S. droebachiensis*. Intact spines exhibited a low intensity green emission (~550–600 nm), while broken spines averaged a high emission peak in the green spectrum (~580 nm). Sea urchins produce a red exudate with a pronounced emission peak (~680 nm) with a shoulder peak (~730 nm). The sampled coelomic fluid exhibited high variability, with a majority exhibiting a low-level green fluorescence while pronounced emission peaks (N = 5) were found in the red spectrum (~680 nm). The complex fluorescence produced by *S. droebachiensis* warrants further investigation on its applicability for monitoring welfare of sea urchins in aquaculture facilities.

1. Introduction

Marine organisms produce biofluorescence through the uptake of photons from the spectrally restricted (blue-shifted) environment and emit a lower, less energetic wavelength light via fluorochromes present in their bodies [1]. Several families of invertebrates have been documented producing biofluorescence, with the two-step production of light in hydrozoan medusa *Aequorea Victoria* perhaps the most well-known. The chemiluminescent protein aequorin emits blue light (470 nm) in response to calcium ion bonding, which is subsequently reemitted by the Green Fluorescent Protein (GFP) as a green fluorescence (508 nm) [2–4]. Biofluorescence in echinoderms is largely unexplored and even though *Strongylocentrotus droebachiensis* is a well-studied species, the presence and/or function of fluorescence remains very poorly understood.

The green sea urchin *S. droebachiensis* is a commercially valued echinoid within the circumpolar

northern hemisphere [5]. The biomass of *S. droebachiensis* along the Norwegian coastline is estimated to be 80 billion individual animals/56,000 tons [6]. The poor quality of roe from wild caught urchins in Norway means there is a need for development of echinoculture methods in aquaculture facilities that can enhance the roe quantity and quality and ensure consistent quality for market consumption. An important limiting factor in echinoculture is the lack of background data and associated diagnostic techniques for monitoring their health and welfare. Clinical disease in sea urchins is poorly understood [7–9]. Echinoderms such as *S. droebachiensis* are susceptible to acute and chronic infections by microbial pathogens and parasitic nematodes [10]. In the northwest Atlantic, periodic mass mortalities caused by *Paramoeba invadens* can have impact on populations [10, 11]. Disease outbreaks and mass mortalities amongst wild echinoids are increasing over the last few decades [9, 12, 13]. In addition, there is very limited literature on how stress is manifested in sea urchin

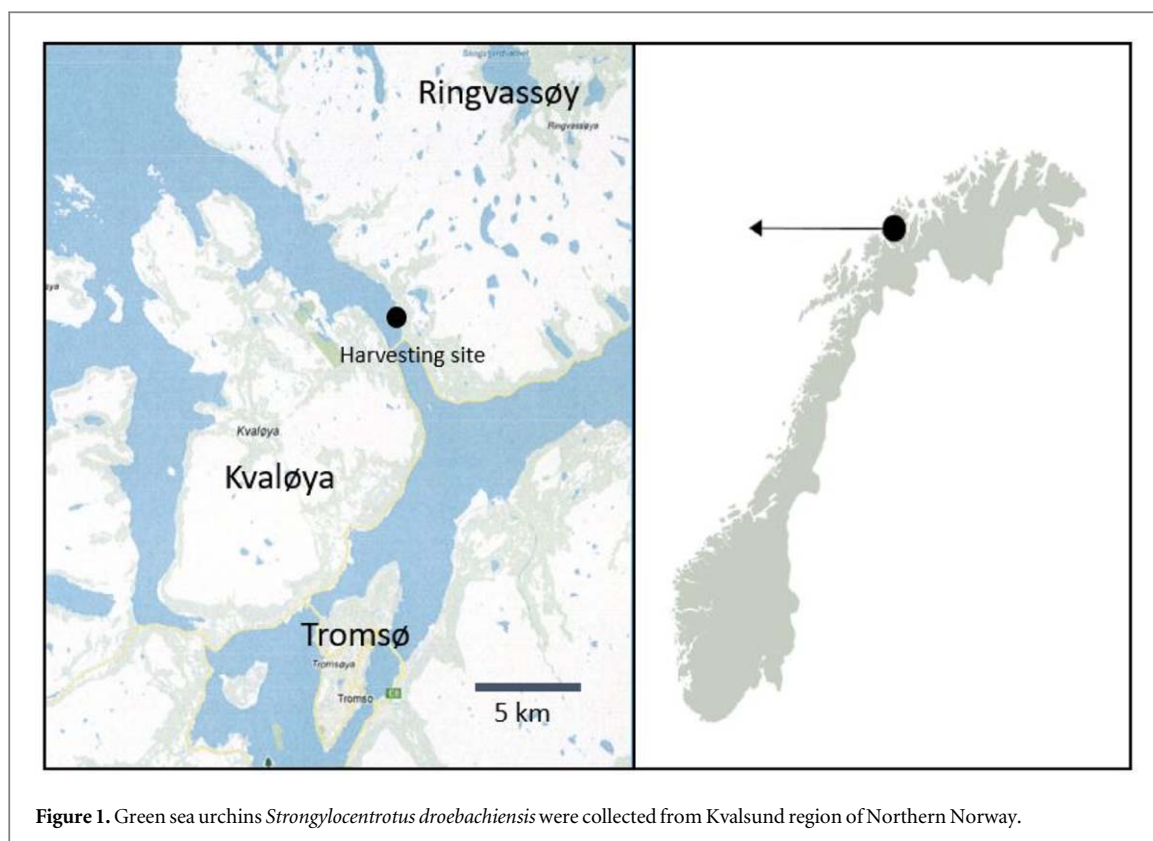


Figure 1. Green sea urchins *Strongylocentrotus droebachiensis* were collected from Kvalsund region of Northern Norway.

physiology and how such stress could be measured accurately within echinoderms. As aquaculture operations such as capture and captive holding are known to expose organisms to both acute and chronic stress [14], biological functions such as somatic growth and maintenance [15], reproduction [16], and immune response [17] typically suffer as the organisms' physiology reallocate resources to stress adaptation. Successfully monitoring sea urchin health and welfare is vital for future aquaculture activities as sea urchins are prone to sudden mortality events in aquaculture. Our study aims to evaluate biofluorescence in *S. droebachiensis* within a controlled setting and whether such fluorescence can be considered a dynamic process that can be monitored through hyperspectral or fluorospectroscopy technology as a non-invasive means of measuring the physiological status of *S. droebachiensis*

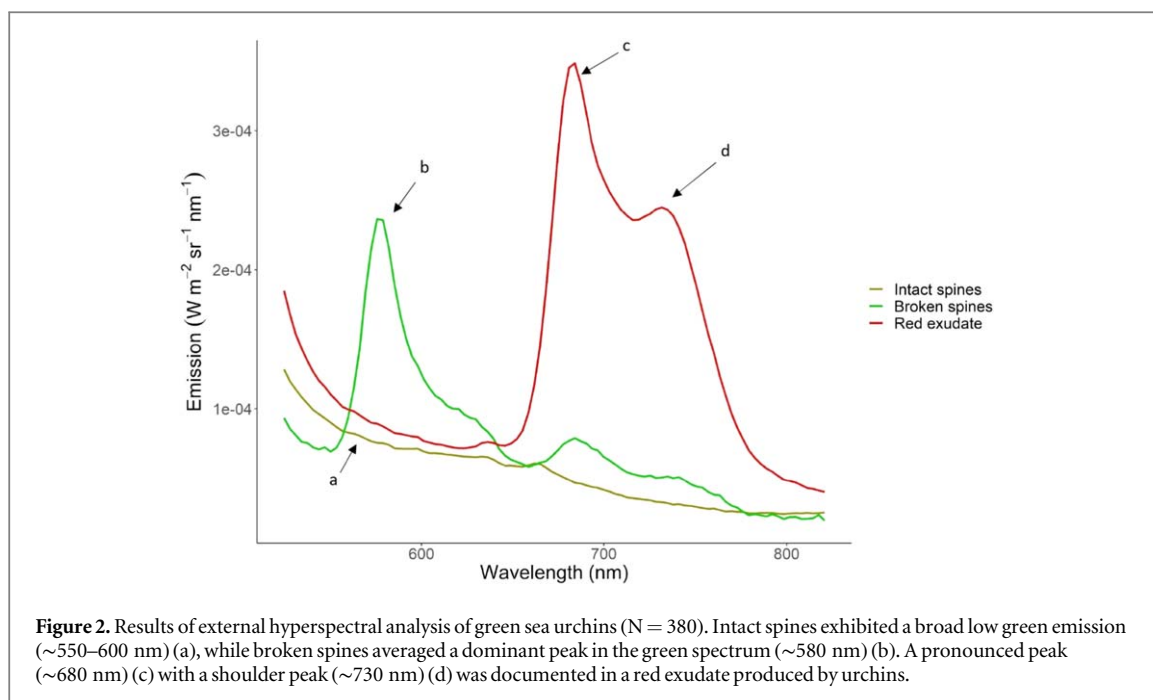
2. Materials and methods

Green sea urchins were collected in Kvalsund, Norway, ($69^{\circ} 45' 16.5312''$ N, $19^{\circ} 2' 2.0904''$ E) between 10–12/2022 (figure 1). Urchins were collected by free divers in net bags and placed in a seawater filled container (1,000 liters) within minutes of being removed from seawater for transport to the NOFIMA laboratory facilities (Tromsø, Norway). A total of 380 urchins were scanned on arrival at the facility. Of those animals, a random sample of 30 sea urchins were sampled for coelomic fluid for fluorospectroscopy.

For sampling, the sea urchins were randomly assigned a numbered square on a Styrofoam lid for scanning on a conveyor belt set at 44 mm s^{-1} to yield an along track resolution equal to twice the across track resolution. The conveyor belt was illuminated by the G5 XR30 Pro Radion LED lighting (Ecotech Marine Bethlehem, PA USA) in royal blue ($\sim 445 \text{ nm}$). Hyperspectral imaging was taken with the HySpex VNIR-1800 hyperspectral camera (Norsk Elektro Optikk AS, Oslo, Norway), with a spectral range from 400 to 1000 nm, a spectral resolution of 5.5 nm, and 1800 spatial pixels. The camera was mounted 1 m above the conveyor belt, resulting in a field of view of 300 mm. The spatial resolution across the track was 0.17 mm, with 1800 pixels across the conveyor belt. The images were spatially binned in the across track direction to yield square pixels with a resolution of 0.34 mm.

Red, green, and blue (RGB) photographs for illustrative purposes were taken using a digital single lens reflex (DSLR) camera (D5100, Nikon, USA/Nikon AF-S 60 mm f/2.8 G IF-ED Micro lens) and a yellow barrier filter (Tiffen 62DY15 62 mm Deep Yellow 12 Filter). A 20-liter photographic aquarium constructed of optic white glass with a moveable black acrylic background was used to take RGB photographs of sea urchins within sea water.

Coelomic fluid (2 ml) was extracted from the peristomal membrane of sea urchins using a 26-gauge hypodermic needle attached to a sterile syringe (BD microlance, Eysins, Switzerland). Each syringe was pre-loaded with 0.5 ml of pre-chilled citrate/EDTA anticoagulant based on a formula for crustaceans [18]



[0.45 M NaCl, 0.1 M glucose, 30 mM sodium citrate, 26 mM citric acid, 10 mM EDTA; pH 5.4]. Syringes containing coelomic fluid were placed on crushed ice prior to analysis. A Duetta fluorescence and absorbance spectrometer (HORIBA Scientific, Kyoto, Japan) was used to analyze hemolymph samples placed within a 3.5 ml fluorescence quartz cuvette (Science Outlet Inc., Weifang, China). The recorded fluorescence emissions were analyzed within the EZ Spec Software (HORIBA Scientific, Kyoto, Japan). The excitation wavelengths from 250 to 350 nm were recorded and emission wavelengths from 400 to 800 nm with a 0.5 nm spectral resolution.

Raw data files collected with hyperspectral scanning were radiometrically calibrated with HySpex Rad v2.5 (Norsk Elektro Optikk, AS, Oslo, Norway) to produce radiance values in units of $\text{Wm}^{-2}\text{sr}^{-1}\text{nm}^{-1}$. The radiometrically calibrated data was uploaded for analysis in Breeze hyperspectral imaging software (Prediktera, Umeå, Sweden) and each sea urchin was cropped out into a separate image. A 33×33 grid was then applied, and each square in the grid was treated as a sampling point to distinguish different spectral features in the sea urchins in a Principal Component Analysis (PCA) plot.

3. Results

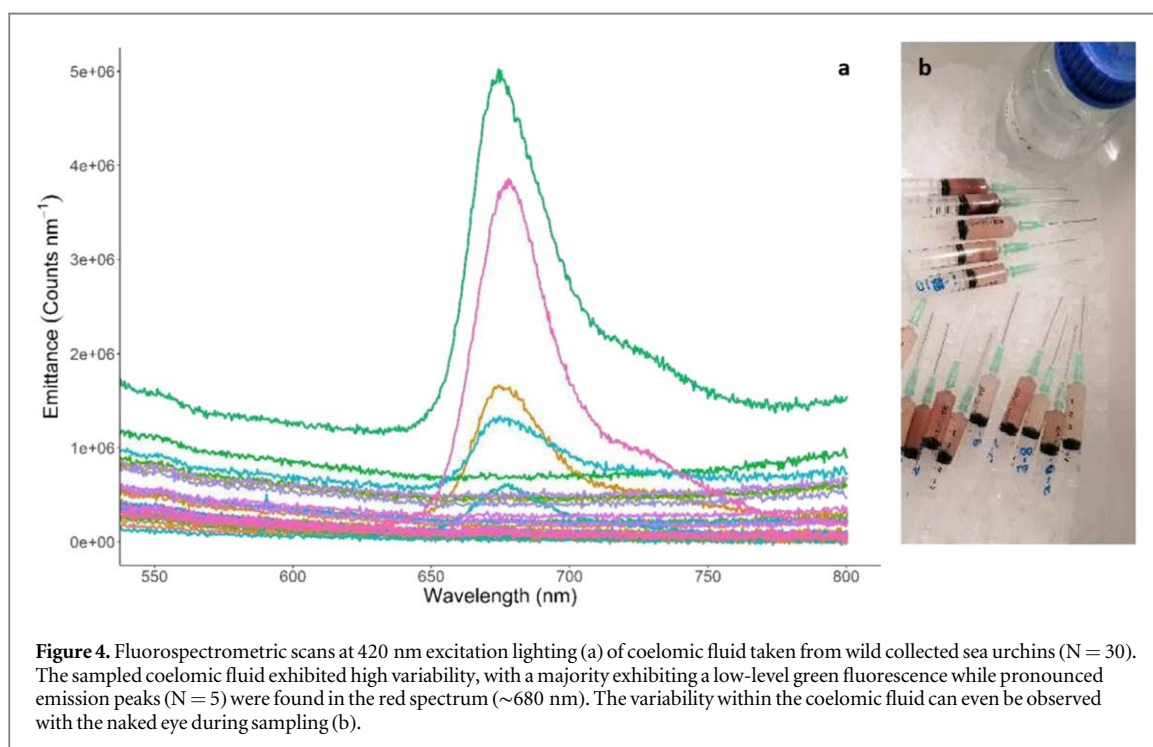
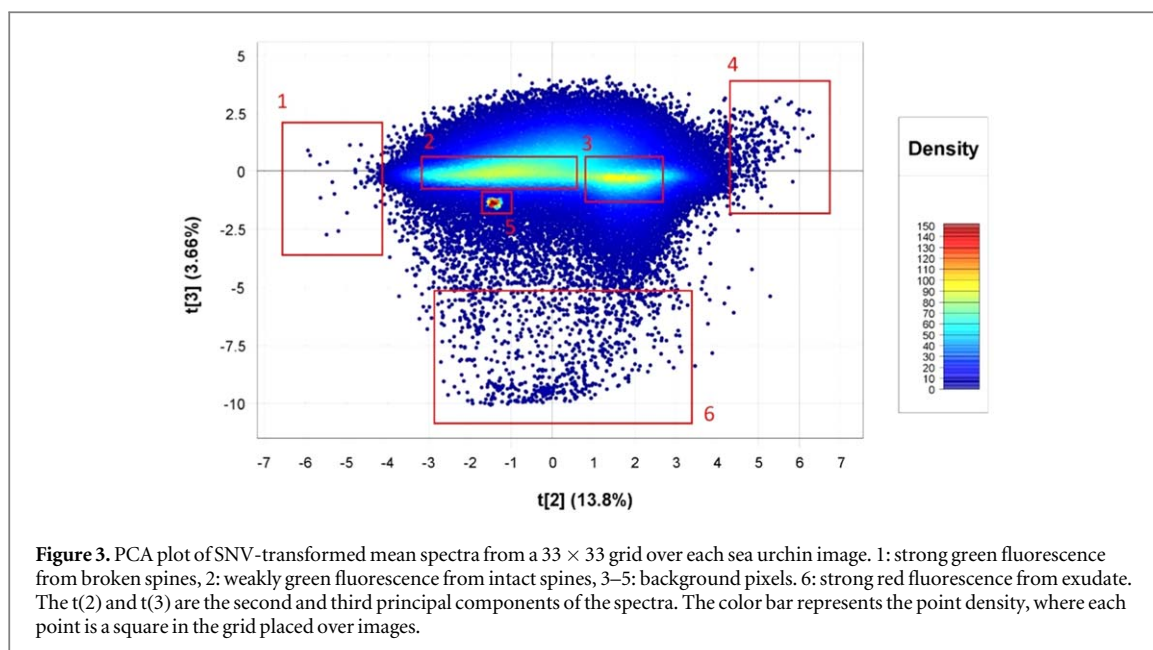
Fluorescence was documented in both the spines and coelomic fluid of *S. droebachiensis* (figure 2). Intact spines exhibited a low intensity green emission (~550–600 nm), while broken spines averaged a high emission peak in the green spectrum (~580 nm). Green sea urchins also produce a red exudate with a pronounced emission peak (~680 nm) with a shoulder peak (~730 nm). A PCA plot of the standard normal

variate (SNV) transformed mean spectra (figure 3) shows clusters of samples based on their similarity; strong clustering was detected within the strong green fluorescence from broken spines, the weakly green fluorescence from intact spines, and the strong red fluorescence from exudate.

The sampled coelomic fluid exhibited high variability, with a majority exhibiting a low-level green fluorescence while pronounced emission peaks (N = 5) were found in the red spectrum (~680 nm) (figure 4(a)). The variability within the coelomic fluid can even be observed with the naked eye during sampling (figure 4(b)). Photographs taken for illustrative purposes that depict fluorescence being produced by sea urchins can be observed within figure 5.

4. Discussion

S. droebachiensis produces a complex set of fluorescence within its anatomy, including a red exudate in likely response to external factors (i.e., handling). How this biofluorescence functions within the physiology of the animal is unknown. However, the documentation of fluorescence within the larvae of other echinoid species gives credence to the fact that fluorescence plays a larger role within echinoderms. Intense fluorescence in the larval urchin *Clypeaster japonicus* with unpublished data claiming weaker fluorescence emission in the larval stage of *Hemicentrotus pulcherrimus*, *Anthodiaris crassispina* and *Pseudocentrotus depressus* [19]. This same study notes the spines and epidermis of adult *C. japonicus* producing a fluorescence emission without providing the associated data. To the best of our knowledge, our study is the first to document multiple states of both external and internal fluorescence within an echinoderm species.



The variation in the coelomic fluid fluorescence documented during sampling is not surprising. The coelomic fluid, the echinoderm equivalent of blood, contains a dense population of immune cells known as coelomocytes [20]. These coelomocytes move about freely in all coelomic spaces, including the perivisceral coelomic cavities and the water-vascular system [20–22]. Physiological studies have investigated fluorescence in *S. droebachiensis* coelomocytes. A study conducted on immune cells known as red spherule cells (RSCs) documented strong, multi-color autofluorescence that is distinct from other coelomocytes. The original orange fluorescence in these RSCs is replaced by green fluorescence after a maximum excitation intensity (5–30 min)

[23]. Considered as a primary immune cell in sea urchins, RSC concentrations in coelomic fluid escalate with stressful conditions such as bacterial invasions [23, 24], migrating towards wounds or infections [23, 25–27]. Having migrated to these zones, the RSCs undergo a degranulation process that releases bactericidal substances [23, 28]. As the RSCs are comprised of spherical red pigmented granules [23, 29], it may be possible that the sampled coelomic fluids ($N = 5$) with a pronounced red spectrum (~ 680 nm) emission and the red exudate released by *S. droebachiensis* during this project is composed of degranulated RSCs.

The open water vascular system present in the green sea urchin may indicate the physio-pathological

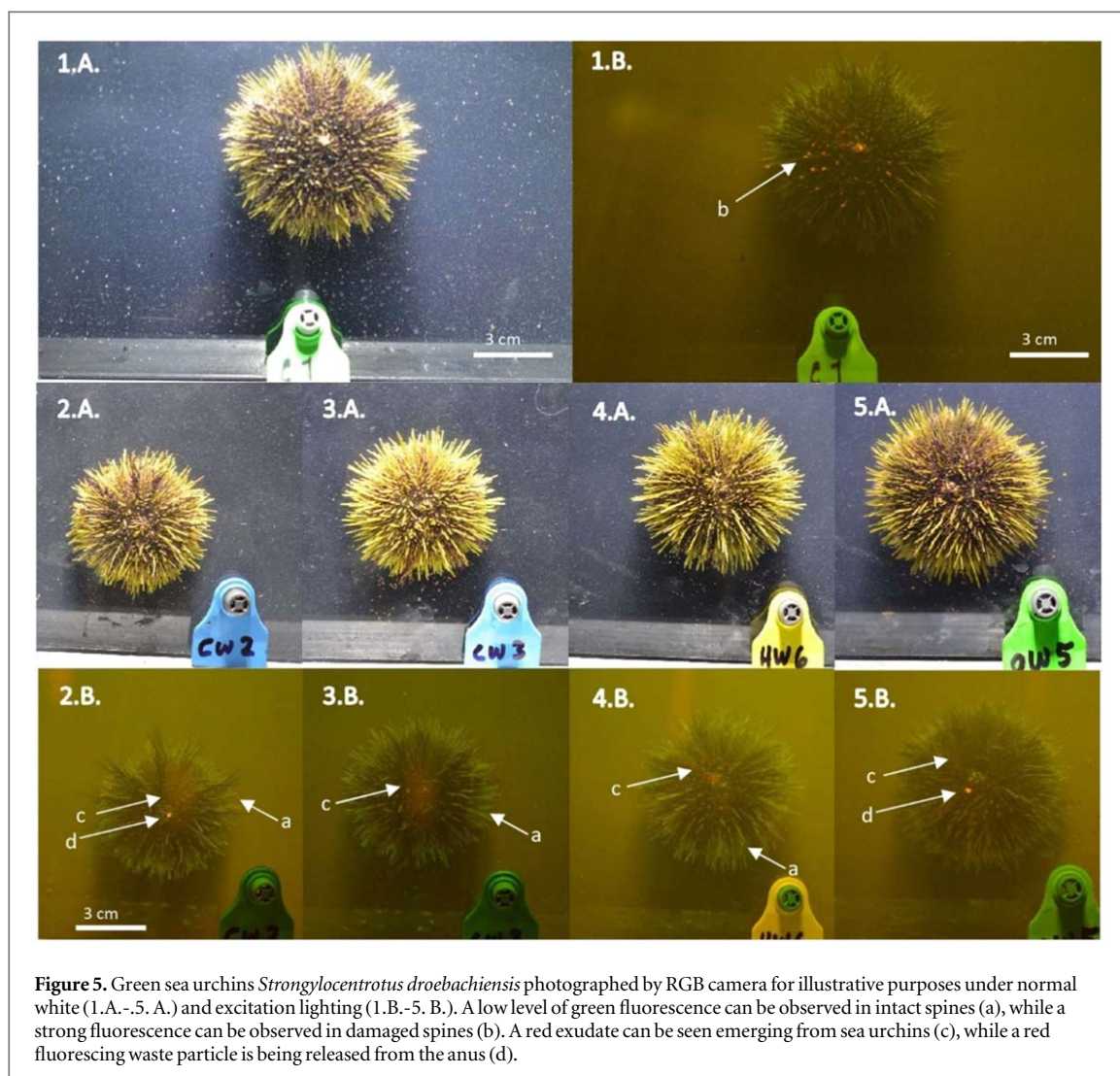


Figure 5. Green sea urchins *Strongylocentrotus droebachiensis* photographed by RGB camera for illustrative purposes under normal white (1.A.-5. A.) and excitation lighting (1.B.-5. B.). A low level of green fluorescence can be observed in intact spines (a), while a strong fluorescence can be observed in damaged spines (b). A red exudate can be seen emerging from sea urchins (c), while a red fluorescing waste particle is being released from the anus (d).

state of the sea urchin as it is in continuous contact with internal cells and tissues [20, 30]. As this open water vascular system circulates within the extremities of *S. droebachiensis* such as the spines and epidermis, the external and internal fluorescence detected during this study may reflect the physio-pathological state of the urchin. Welfare standards used to date for sea urchins are heavily dependent on behavior [9]. Using behavior for measuring individual well-being is difficult to assess quantitatively since it varies from time and with subject [31, 32]. Biofluorescence produced by *S. droebachiensis* has been documented in this study to vary in emission intensity and wavelength within the coelomic fluid and external anatomy (unbroken spines, broken spines), as well as the production of exudate through either fluorospectroscopy or hyperspectral imaging technology. The next step with this research is to understand how this physiological fluorescence in *S. droebachiensis* responds to long-term captive conditions in aquaculture as well as how acute stressors that can be experienced in aquaculture conditions such as abrupt temperature changes or out of water shipping impacts the species' physiological fluorescence. As sea urchins do not exhibit externally

visible signs of stress prior to permanent physiological impact(s), developing technology with this baseline data to monitor physiological changes will allow husbandry techniques to be developed that may minimize both acute and chronic stress within echinoderms in the aquaculture industry. The complex fluorescence documented in *S. droebachiensis* warrants further investigation on its applicability for monitoring the welfare of *S. droebachiensis* when in aquaculture.

Acknowledgments

This project has received funding from the European Union's Aquavita Project (818173). Further funding support was received from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 956697.

Data availability statement

The data that support the findings of this study are openly available at the following URL/DOI: <https://doi.org/10.5281/zenodo.8065657>.³³

Ethics statement

This study contains experimental procedures on non-cephalopod invertebrates performed in compliance with Norwegian national guidelines based on the European Union Directive 2010/63/EU for the protection of animals used for scientific purposes. The green sea urchin *S. droebachiensis* is not of conservation concern nor has any restrictions associated with their use in research.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ORCID iDs

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

References

- [1] Brahme A 2016 Comprehensive biomedical physics *Comprehensive Biomedical Physics*. **Vol 4** 10–11
- [2] Shimomura O 1979 Structure of the chromophore of Aequorea green fluorescent protein *FEBS Lett.* **104** 220–2
- [3] Shimomura O, Johnson F H and Saiga Y 1962 Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusa, Aequorea *J Cell Comp Physiol.* **59** 223–39
- [4] Macel M L, Ristoratore F, Locascio A, Spagnuolo A, Sordino P and D'Aniello S 2020 Sea as a color palette: the ecology and evolution of fluorescence *Zoological Lett.* **6** 9
- [5] Stephens R E 1972 Studies on the development of the sea urchin *Strongylocentrotus droebachiensis*: I. Ecology and normal development *Biol Bull.* **142** 132–44
- [6] Gundersen H, Christie H and Rinde E 2010 Sea urchins—from problem to commercial resource. Estimates of sea urchins as a resources and an evaluation of ecological gains by sea urchin exploitation *Norwegian Institute for Water Research. NIVA report no. 6001-2010* 1–19
- [7] Jangoux M 1984 Diseases of echinoderms *Helgolander Meeresunters* **37** 207–16
- [8] Jangoux M and Kinne O 1990 Diseases of echinodermata *Diseases of Marine Animals* **Vol. 3** 439–567
- [9] Francis-Floyd R 2020 Diagnostic methods for the comprehensive health assessment of the long-spined Sea urchin, *Diadema antillarum*: VM244/VM244, 05/2020 *EDIS* **2020**
- [10] Scheibling R E and Hatcher B G 2013 *Strongylocentrotus droebachiensis* *Developments in Aquaculture and Fisheries Science* (Elsevier) pp 381–412
- [11] Feehan C J, Johnson-Mackinnon J, Scheibling R E, Lauzon-Guay J S and Simpson A G B 2013 Validating the identity of *Paramoeba invadens*, the causative agent of recurrent mass mortality of sea urchins in Nova Scotia, Canada *Dis Aquat Organ.* **103** 209–27
- [12] Ward J R and Lafferty K D 2004 The elusive baseline of marine disease: are diseases in ocean ecosystems increasing? *PLoS Biol.* **2** e120
- [13] Feehan C J and Scheibling R E 2014 Effects of sea urchin disease on coastal marine ecosystems *Mar. Biol.* **161** 1467–85
- [14] Morgan K N and Tromborg C T 2007 Sources of stress in captivity *Appl Anim Behav Sci.* **102** 262–302
- [15] Lattin C R, Bauer C M, de Bruijn R and Michael Romero L 2012 Hypothalamus–pituitary–adrenal axis activity and the subsequent response to chronic stress differ depending upon life history stage *Gen Comp Endocrinol.* **178** 494–501
- [16] Lombardo M P, Thorpe P A and Colpetzer S 2010 Social environment affects beak color in captive male house sparrows *International Studies on Sparrows* **34** 8–17
- [17] Buehler D M et al 2008 Constitutive immune function responds more slowly to handling stress than corticosterone in a shorebird *Physiol. Biochem. Zool.* **81** 673–81
- [18] Söderhäll K and Smith V J 1983 Separation of the haemocyte populations of *Carcinus maenas* and other marine decapods, and prophenoloxidase distribution *Dev Comp Immunol* **7** 229–39
- [19] Nakamura S, Mikamori M, Hiramatsu M, Eura S, Takamoto H and Watanabe M 2001 Spectacular fluorescence emission in sea urchin larvae *Zoolog Sci.* **18** 807–10
- [20] Pinsino A and Matranga V 2015 Sea urchin immune cells as sentinels of environmental stress *Dev Comp Immunol.* **49** 198–205
- [21] Gliński Z and Jarosz J 2000 Immune phenomena in echinoderms *Arch Immunol Ther Exp (Warsz)* **48** 189–93
- [22] Smith L C et al 2010 Echinoderm immunity *Invertebrate Immunity* 260–301
- [23] Hira J, Wolfson D, Andersen A J C, Haug T and Stensvåg K 2020 Autofluorescence mediated red spherulocyte sorting provides insights into the source of spinochromes in sea urchins *Sci. Rep.* **10** 1149
- [24] Matranga V, Toia G, Bonaventura R and Müller W E G 2000 Cellular and biochemical responses to environmental and experimentally induced stress in sea urchin coelomocytes *Cell Stress Chaperones* **5** 113
- [25] Höbaus E 2020 Coelomocytes in normal and pathologically altered body walls of sea urchins *Echinoderms* (CRC Press) 247–9
- [26] Heatfield B M and Travis D F 1975 Ultrastructural studies of regenerating spines of the sea urchin *Strongylocentrotus purpuratus*: II. Cell types with spherules *J. Morphol.* **145** 51–71
- [27] Coffaro K A and Hinegardner R T 1977 Immune response in the sea urchin *Lytechinus pictus* *Science (1979)* **197** 1389–90
- [28] Gerardi P, Lassegues M and Canicatti C 1990 Cellular distribution of sea urchin antibacterial activity *Biol Cell.* **70** 153–7
- [29] Gibson A W and Burke R D 1987 Migratory and invasive behavior of pigment cells in normal and animalized sea urchin embryos *Exp. Cell. Res.* **173** 546–57
- [30] Smith V J 1981 Invertebrate blood cells *The Echinoderms* (Academic press) pp 514–62
- [31] Eisler R 1979 Behavioural responses of marine poikilotherms to pollutants *Philosophical Transactions of the Royal Society of London B, Biological Sciences* **286** 507–21
- [32] Lawrence J M and Cowell B C 1996 The righting response as an indication of stress in *Stichaster striatus* (Echinodermata, Asteroidea) *Marine & Freshwater Behaviour & Phy.* **27** 239–48
- [33] Juhasz-Dora T, James P, Evensen T and Lindberg S-K 2023 *Hidden in plain sight: hyperspectral documentation of complex biofluorescence produced by the green sea urchin (Strongylocentrotus droebachiensis)* (<https://doi.org/10.5281/zenodo.8065657>)