

Article

Catecholamine Involvement in the Bioluminescence Control of Two Species of Anthozoans

Laurent Duchatelet ^{1,*}, Constance Coubris ¹, Christopher Pels ¹, Sam T. Dupont ^{2,3} and Jérôme Malfefet ¹

¹ Marine Biology Laboratory, Earth and Life Institute, Université Catholique de Louvain, 1348 Ottignies-Louvain-la-Neuve, Belgium; constance.coubris@uclouvain.be (C.C.); christophe.pels@uclouvain.be (C.P.); jerome.malfefet@uclouvain.be (J.M.)

² Department of Biological & Environmental Sciences, University of Gothenburg, 451 78 Fiskebäckskil, Sweden; sam.dupont@bioenv.gu.se

³ Marine Environment Laboratories, International Atomic Energy Agency, MC-98000 Monaco, Monaco

* Correspondence: laurent.duchatelet@uclouvain.be

Abstract: Bioluminescence, the ability of living organisms to emit visible light, is an important ecological feature for many marine species. To fulfil the ecological role (defence, offence, or communication), bioluminescence needs to be finely controlled. While many benthic anthozoans are luminous, the physiological control of light emission has only been investigated in the sea pansy, *Renilla koellikeri*. Through pharmacological investigations, a nervous catecholaminergic bioluminescence control was demonstrated for the common sea pen, *Pennatulula phosphorea*, and the tall sea pen, *Funiculina quadrangularis*. Results highlight the involvement of adrenaline as the main neuroeffector triggering clusters of luminescent flashes. While noradrenaline and octopamine elicit flashes in *P. phosphorea*, these two biogenic amines do not trigger significant light production in *F. quadrangularis*. All these neurotransmitters act on both the endodermal photocytes located at the base and crown of autozooids and specific chambers of water-pumping siphonozooids. Combined with previous data on *R. koellikeri*, our results suggest that a catecholaminergic control mechanisms of bioluminescence may be conserved in Anthozoans.

Keywords: luminescence; sea pen; *Pennatulula*; *Funiculina*; adrenaline; noradrenaline; octopamine



Citation: Duchatelet, L.; Coubris, C.; Pels, C.; Dupont, S.T.; Malfefet, J. Catecholamine Involvement in the Bioluminescence Control of Two Species of Anthozoans. *Life* **2023**, *13*, 1798. <https://doi.org/10.3390/life13091798>

Academic Editor: Liudmila P. Burakova

Received: 2 August 2023

Revised: 20 August 2023

Accepted: 22 August 2023

Published: 23 August 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Bioluminescence, defined as the ability of live organisms to produce visible light via biochemical reactions, is an important ecological trait in marine ecosystems [1,2]. Among the benthic sessile organisms, the anthozoan class harbors the most abundant luminous species taxonomically spread over at least 17 different families from four different orders [3]. Among these orders (i.e., Actiniria, Zoantharia, Alcyonacea, and Pennatulacea), Pennatulacea contains the most studied bioluminescent anthozoan species. While physiological studies on the bioluminescence of these species remain anecdotal, the bioluminescence system was extensively documented in many species [3], sea pansy's (*Renilla reniformis*, *R. muelleri* and *R. koellikeri*) luminous systems being the more investigated (e.g., [4–10]).

The colonial Pennatulacea luminescence is produced by endodermal cells (i.e., photocytes) spread in the tissue of autozooid and siphonozooid polyps [11–13]. Bioluminescence biochemical reactions typically involve an enzyme (i.e., a luciferase) which catabolized the oxidation of a substrate (i.e., a luciferin) with or without the intervention of co-factors [14]. In some cases, pre-oxidized luciferin and luciferase are coupled in a protein complex called photoprotein, needing a cofactor to be activated and produce luminescence [14]. The luminous system of *R. reniformis* involves coelenterazine (i.e., the most widespread luciferin in the marine environment) and the activity of a coelenterazine-dependent luciferase (i.e., RLuc) in the presence of dissolved oxygen without other co-factors [5,7]. The RLuc was shown to share sequence homology with bacterial haloalkane dehalogenase

implying a potential horizontal gene transfer during evolution [15]. Homologous *RLuc*s are found in other Pennatulacea species but also in phylogenetically distinct species such as an echinoderm (i.e., *Amphiura filiformis*; [16]) and a tunicate (i.e., *Pyrosoma atlanticum*; [17]). Evidence tends to demonstrate a similar requirement of both coelenterazine and an *RLuc* homologous luciferase among benthic anthozoans [3,18]. Studies on the sea pansy and other Pennatulacea species also reveal the presence of luminescence-associated molecules such as a coelenterazine binding protein and a green fluorescent protein [19–24].

The ecological functions of anthozoan light production may provide misdirection for predators, aposematic signals, or burglar alarms [1], although there is no experimental evidence available clearly unveiling these hypotheses. To efficiently perform its ecological role, bioluminescence needs to be finely adjusted and controlled. Bioluminescent organisms possess a variety of control mechanisms to regulate their light emission. Such mechanisms allow organisms the switching of their luminescence on and off and/or the modulation of the physical characteristic of their light emission in terms of color, intensity, or angular distribution [1]. Physiological controls of light emission in marine metazoan species include (i) the direct neuronal and (ii) the hormonal control of the light organ [25,26]. Direct neural regulation is mainly found in intrinsic luminescent organisms and has been extensively studied in osteichthyes and echinoderms. Among the first studied species, *Porichthys* has been demonstrated as a catecholamine-controlled light emitter [27–31]. Most of the nervous molecules involved in the control of light emission belong to three groups: (i) simple amino acids (e.g., γ -aminobutyric acid (GABA), tryptamine or glutamate); (ii) classical neurotransmitters (e.g., serotonin, (nor)adrenaline, acetylcholine, purines, nitric oxide (NO) or octopamine (p-hydroxyphenylethanolamine)); and (iii) neuropeptides (e.g., SALMFamide neuropeptides S1 and S2) (e.g., [25,28–30,32–43]).

Previous work suggests some level of phylogenetic conservation in the control mechanisms of bioluminescence. Insects are assumed to mainly use octopamine as a neurotransmitter to control the light emission [32,33]. Acetylcholine is found to be the principal neurotransmitter in ophiuroids [38,44]. The main neurotransmitter of osteichthyes appears to be adrenaline [40–42,45]. Nevertheless, some closely related species do not always share common nervous light emission control mechanisms. Nitric oxide has a specific neuromodulator role in neural-induced luminescence since it modulates the light emission in species such as the krill *Meganyctiphanes norvegica*, the midshipman fish *Porichthys notatus*, the hatchetfish *Argyropelecus hemigymnus*, the pearlfish *Maurollicus muelleri* and probably other fishes such as the Myctophidae species [34,46]. Nervous control can either directly control the photocytes or indirectly control the light emission by regulating (i) structural photophore elements (e.g., optical filters, lenses, chromatophores) and (ii) indirect elements linked to photophores (e.g., muscles) [47,48]. Another hormonal physiological control is singularly found in luminescent elasmobranchs [26]. While the majority of bony fish have a nervous control of the emitted light (e.g., via adrenaline, noradrenaline), hormones rather than these neurotransmitters are used for the control of the light emission in luminous shark [26].

Among anthozoans, *Renilla* luminescence is demonstrated to be under an adrenergic control with either rapid flashes triggered at low concentrations (1 pM) or long-lasting glow and superimposed flashes at higher concentrations (1 to 10 mM) [49]. Calcium has also been demonstrated to be needed for the light emission in two anthozoan species, *Renilla reniformis* and *Veretillum cynomorium* [50,51]. No other studies on the bioluminescence physiological control have been published in Pennatulacea.

The aim of this study is to document the physiological control of bioluminescence in two sea pen species inhabiting similar soft sediment ecotypes. The phylogenetic conservation of bioluminescence control among taxa (e.g., octopamine in insects, acetylcholine in ophiuroid, adrenaline in bony fish) leads to the hypothesis that a common control mechanism is shared among anthozoans. As suggested from *Renilla*, this mechanism could have been co-opted from the nervous catecholaminergic control of contractile elements during the pennatulacean evolution [49].

The common sea pen *Pennatula phosphorea* lives in sandy and muddy sediments in the depth between 10 and 100 m. It is distributed throughout the North Atlantic and Mediterranean Sea, with some records from the Indo-Pacific, eastern Pacific, and Southern Oceans [52,53]. This anthozoan possesses a robust axis which may reach 40 cm in length with an emerged rachis up to 25 cm outside the sediment [53]. Autozooid polyps are fused in large triangular structures extending from the rachis in two alternating and opposing rows. This species exhibits rapid flash propagations of green light (maximum emission wavelength (λ_{\max}) = 510 nm) along the colony following mechanical stimulations [54–56]. Waves of light can be initiated up and down the colony in either direction, highlighting a non-polarized transmission system [56]. As with *Renilla*, the light emission is demonstrated to be under nervous control [11,56–58] but without the determination of the neurotransmitters involved. *Funiculina quadrangularis* is a much taller sea pen, exceeding 200 cm in length with approximately one quarter of its body embedded in the sediment [59]. This species is also distributed in fine muddy sediments along the coast of the north-eastern Atlantic Ocean. Each polyp emits a bright blue-luminescence (λ_{\max} = 485 nm) in response to a mechanical stimulus [3,60]. While both species may have preferences for different microhabitats within their respective ranges, they share overlapping distribution areas and generally coexist in the same geographic region (i.e., sympatric species).

Our results show that the light production of *P. phosphorea* and *F. quadrangularis* is mainly under a similar nervous adrenergic control. Two other biogenic amines, noradrenaline and octopamine, also play a neuroeffector role in *P. phosphorea* luminescence, but not in *F. quadrangularis*. The catecholamine precursor dopamine is not involved in the bioluminescence control of either species.

2. Materials and Methods

2.1. Specimen Collection

A total of 50 common sea pens, *P. phosphorea*, were collected in the Gullmarsfjord, Sweden, using a small one-meter aperture dredge at a 35 m depth in May and July 2022. Similarly, 4 tall sea pens, *F. quadrangularis*, were collected in May 2023. Animals were brought back to the Kristineberg Marine Research Station (University of Gothenburg, Fiskebäckskil, Sweden) and maintained in a dark cold room with fresh running deep-sea water pumped from the adjacent fjord. Pictures of the sea pens and their mechanically stimulated bioluminescence were taken with a Sony α 7S II camera.

2.2. Dissection

Specimens were anaesthetized by immersion in a MgCl_2 solution (183 mmol L^{-1} MgCl_2 , 9.9 mmol L^{-1} CaCl_2 , 27.7 mmol L^{-1} Na_2SO_4 , 20 mmol L^{-1} Tris; pH 8.2) for 30 min. Then, for each tested *P. phosphorea* specimen, (i) the rachis was divided into three equivalent portions defined as upper, middle, or lower, and weighed; (ii) pinnules were dissected, weighed, polyp numbers were counted, and the location on the colony (upper, middle, lower) was defined depending on attachment position on the rachis. Rachis of *F. quadrangularis* was divided into three equivalent portions defined as upper, middle, and lower. Then, each portion was subdivided into multiple 1.5 cm long portions and weighed. For both sea pen species, the peduncle (i.e., the portion of the colony settled in sediment) was also collected and weighed. Sea pen pinnules/rachis/peduncles were rinsed for 3 h in fresh running deep-sea water before pharmacological assays to remove excess of mucus that may impair pharmacological measures.

2.3. Evaluation of Luminescence Capability

Measurements of light emission were performed in a dark room using an FB12 tube luminometer (Tirtertek-Berthold, Pforzheim, Germany) calibrated using a standard 470 nm light source (Beta light, Saunders Technology, Hayes, UK). Light responses were recorded using FB12-Sirius PC Software (version 1) (Tirtertek-Berthold). Light emission was characterized as the total amount of light emitted (L_{tot}) over time, expressed in megaquanta. For *P. phosphorea* pinnules, considering the positive correlation between the numbers of

the polyp and the mass of pinnules (Spearman correlation, $R^2 = 0.78$, p -value < 0.0001 ; Figure S1A), all data were only standardized per unit of mass (g). For the other sea pen portions (i.e., *P. phosphorea* rachis and peduncle, and *F. quadrangularis* polyp-bearing rachis), data were also standardized per unit of mass (g).

2.4. KCl and Pharmacological Assays

Luminescence inductions were performed on pinnules, rachis or peduncle portions placed in a tube luminometer filled with 500 μ L of artificial seawater (ASW; 400 mmol L⁻¹ NaCl, 9.6 mmol L⁻¹ KCl, 52.3 mmol L⁻¹ MgCl₂, 9.9 mmol L⁻¹ CaCl₂, 27.7 mmol L⁻¹ Na₂SO₄, 20 mmol L⁻¹ Tris; pH 8.2). Then, the light emission was triggered with the addition of 500 μ L of an KCl depolarizing solution (400 mmol L⁻¹ KCl, 52.3 mmol L⁻¹ MgCl₂, 9.9 mmol L⁻¹ CaCl₂, 27.7 mmol L⁻¹ Na₂SO₄, 20 mmol L⁻¹ Tris; pH 8.2), and Lt_{tot} was recorded over 3 min [61].

For pharmacological assays, 6-hydroxydopamine hydrobromide (6-OHDA, 162957, Merck, Rahway, NJ, USA) was used as a terminal nervous system catecholamines releaser to confirm the catecholamine implication on the *P. phosphorea* and *F. quadrangularis* luminescence control. *P. phosphorea* pinnules, rachis and *F. quadrangularis* polyp-bearing rachis were placed in a tube luminometer filled with 500 μ L of ASW. Light emission was recorded for 30 min after the addition of 500 μ L of the 6-OHDA 10⁻⁶ mol L⁻¹ solution [49]. Effects of adrenaline ((\pm)-Epinephrine hydrochloride, E4642, Merck), noradrenaline (L-Norepinephrine hydrochloride, 74480, Merck), octopamine ((\pm)-Octopamine hydrochloride, O0250, Merck), and dopamine (Dopamine hydrochloride, H8502, Merck) applications on pinnules and rachis of *Pennatula* and rachis of *Funiculina* were recorded in the luminometer for 30 min. First, adrenaline, noradrenaline, octopamine, and dopamine were tested at a concentration of 10⁻⁶ mol L⁻¹. Dose-dependent response curves (10⁻³ to 10⁻⁶ mol L⁻¹) were then tested for adrenaline, noradrenaline and octopamine on *Pennatula*. For all experiments, the ASW application was used as mechanical (non-pharmacological) control.

2.5. Statistical Analysis

All statistical analyses were performed with R Studio (version 2023.03.1 + 446, 2022, R Studio Inc., Boston, MA, USA). Variance normality and equality were tested by the Shapiro–Wilk test and the Levene’s test, respectively. When these parametric assumptions were met, Student *t*-test and ANOVA coupled with Tukey’s test were used, respectively, to perform single or multiple comparisons between groups.

When log transformation did not provide normality and homoscedasticity, non-parametric Wilcoxon test and the Kruskal–Wallis test coupled with the Wilcoxon rank-sum test were used, respectively, to assess whether significant differences were present between two groups or multiple groups. Each difference was considered to be significant at a minimum p -value < 0.05 . Values were graphically illustrated with mean and standard error of mean (s.e.m).

3. Results

3.1. Bioluminescence Characterization

Upon visual observations and video recordings, the luminescence of *P. phosphorea* was confirmed as waves of green luminescence when mechanically stimulated, mainly emitted by autozooid polyps within pinnules (Figure 1A; Video S1). To document the luminescence abilities of the species, colonies were divided into various areas (Figure 1B). Original recordings of the KCl application presented a rapid repetition of flashes for at least 2 min on both pinnules or rachis portions (e.g., Figures 1C and S1B). Light outputs through KCl applications showed no statistical Lt_{tot} differences between pinnule sides (Student *t*-test, p -value = 0.99; Figure 1D). In addition, the Lt_{tot} from the upper part of the colony was significantly higher than either the middle area (Tukey’s test, p -value = 0.0001) or the lower area (Tukey’s test, p -value = 0.003) (Figure 1E). The Lt_{tot} from the middle and lower areas were not significantly different from each other (Tukey’s test, p -value = 0.18; Figure 1E). No differences between Lt_{tot} were observed between the different rachis portions (Kruskal–Wallis test, p -value = 0.65;

Figure 1F). The mean L_{tot} value for pinnules ($297.90 \pm 28.92 \times 10^9 \text{ q s}^{-1}$) was significantly higher than that for the rachis (Wilcoxon sum-rank test, p -value = 8.3×10^{-10}) and the peduncle (Wilcoxon sum-rank test, p -value $< 2 \times 10^{-16}$) portions (Figure 1G). The mean L_{tot} value from the rachis portion ($87.50 \pm 14.55 \times 10^9 \text{ q s}^{-1}$) was also significantly higher from the peduncle portion (Wilcoxon sum-rank test, p -value $< 2 \times 10^{-16}$), which displayed a mean L_{tot} value of $0.03 \pm 0.005 \times 10^9 \text{ q s}^{-1}$ (Figure 1G). Moreover, the peduncle part presented values comparable to L_{tot} values of the ASW control (mean $L_{tot} = 0.41 \pm 0.06 \times 10^9 \text{ q s}^{-1}$).

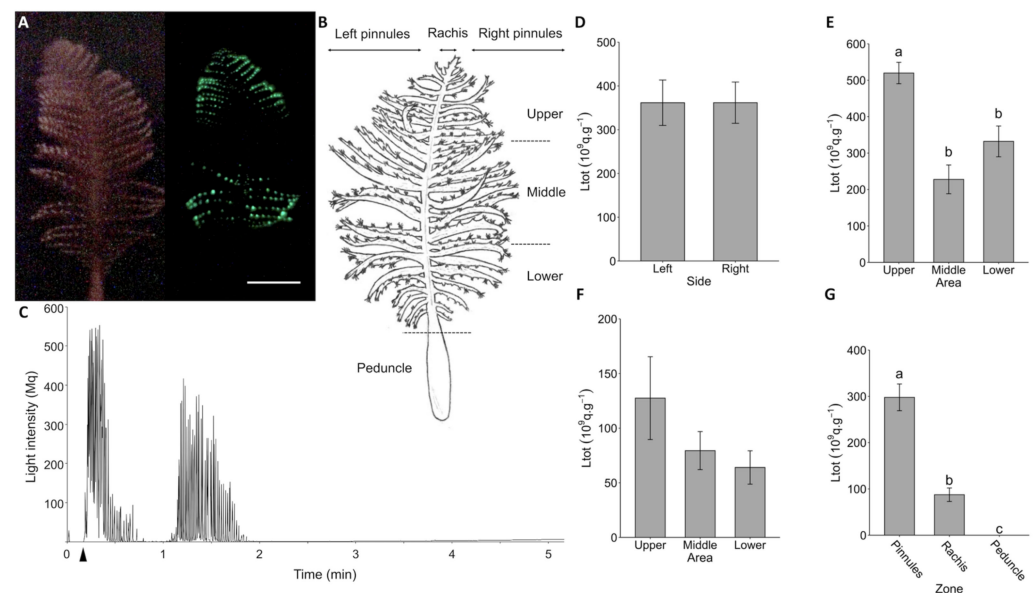


Figure 1. Luminescence capability analyses on *Pennatula phosphorea*. (A) Picture of *Pennatula* colony in dim light (left) and emitting waves of luminescence upon a mechanical stimulation (right). (B) Representation of a *Pennatula* colony showing the different experimental parts used (left/right—upper/middle/lower, pinnules, the rachis, and the peduncle). (C) Original recording of KCl application on pinnules. Arrowhead indicates the KCl application. Total amount of light (L_{tot}) produced by pinnules under KCl application depending on (D) the side of the colony ($n = 18$), (E) the position on the rachis ($n = 12$). L_{tot} produced by rachis under KCl application depending on (F) the rachis portion ($n = 16$). (G) Comparison between the L_{tot} produced by pinnules, rachis and peduncle parts of the colony under KCl application ($n = 50$). All L_{tot} values are expressed as $10^9 \text{ q s}^{-1} \text{ g}^{-1}$. Different lettering indicates statistical differences. Error bars correspond to s.e.m. Scale bar = 1 cm.

Similar experiments conducted on *Funiculina* confirmed the production of blue light waves upon mechanical stimulations (Figure 2A). To document the luminescence abilities of *Funiculina*, the colonies were divided into various areas (Figure 2B). The light was emitted by the polyps spread along the rachis. The original recording of the KCl application also showed a rapid repetition of flashes (e.g., Figure 2C). There were no L_{tot} statistical differences in connection to the location upon the colony (ANOVA, p -value = 0.4236; Figure 2D). As shown for *Pennatula*, the mean L_{tot} value of the polyp-bearing rachis ($L_{tot} = 316.6 \pm 81.5 \times 10^9 \text{ q s}^{-1}$) was significantly higher than that for the peduncle ($L_{tot} = 2.18 \pm 0.81 \times 10^9 \text{ q s}^{-1}$; pairwise Wilcoxon test, p -value = 3.168×10^{-5} ; Figure 2E). The peduncle part presented values comparable to L_{tot} values of the ASW control (mean $L_{tot} = 1.72 \pm 0.37 \times 10^9 \text{ q s}^{-1}$).

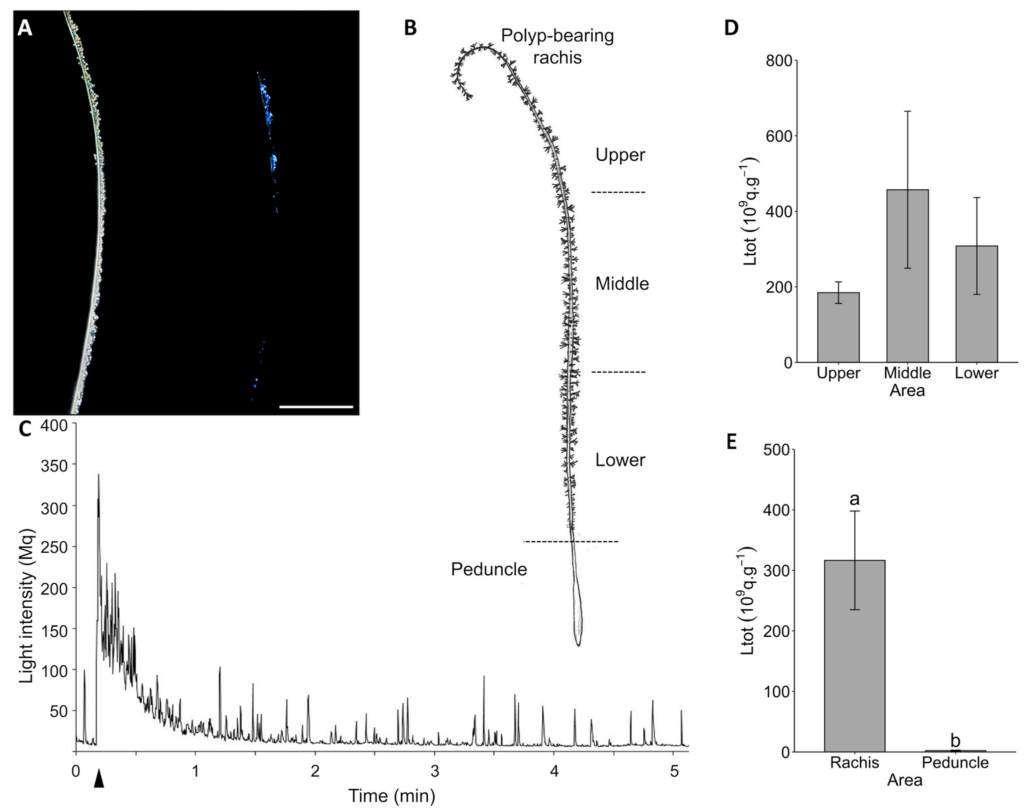


Figure 2. Luminescence capability analyses on *Funiculina quadrangularis*. (A) Picture of *Funiculina* colony in dim light (left) and emitting waves of luminescence upon a mechanical stimulation (right) (Dr. E. Infantes pictures). (B) Representation of a *Funiculina* colony showing the different experimental parts used (polyp-bearing rachis and peduncle). (C) Original recording of KCl application on polyp-bearing rachis. Arrowhead indicates the KCl application. (D) Total amount of light (Ltot) produced by polyp-bearing rachis portion under KCl application depending on the position along the colony ($n = 5$). (E) Comparison between the Ltot produced by polyp-bearing rachis ($n = 15$) and the peduncle ($n = 10$) parts of the colony under KCl application. All Ltot values are expressed as $10^9 \text{ q s}^{-1} \text{ g}^{-1}$. Different lettering indicates statistical differences. Error bars correspond to s.e.m. Scale bar = 1 cm.

3.2. Effects of 6-OHDA

Application of the 6-OHDA $10^{-6} \text{ mol L}^{-1}$ solution on *Pennatula* pinnules resulted in a drastic increase in light production ($L_{\text{tot}} = 153.46 \pm 41.38 \times 10^9 \text{ q s}^{-1}$) compared to the application of ASW ($L_{\text{tot}} = 3.51 \pm 0.65 \times 10^9 \text{ q s}^{-1}$) (pairwise Wilcoxon test, p -value $< 2.2 \times 10^{-16}$; Figure 3A). Similar results were observed for the rachis with a L_{tot} value of $105.82 \pm 21.98 \times 10^9 \text{ q s}^{-1}$ for the 6-OHDA $10^{-6} \text{ mol L}^{-1}$ application and of $0.41 \pm 0.05 \times 10^9 \text{ q s}^{-1}$ for the ASW application (Student t -test, p -value $< 2.2 \times 10^{-16}$; Figure 3B). Similarly, 6-OHDA $10^{-6} \text{ mol L}^{-1}$ application on *Funiculina* rachis resulted in an increase in luminescence production ($L_{\text{tot}} = 41.9 \pm 7.4 \times 10^9 \text{ q s}^{-1}$) compared to the ASW control ($L_{\text{tot}} = 1.6 \pm 0.3 \times 10^9 \text{ q s}^{-1}$) (Student t -test, p -value = 0.0002; Figure 3C).

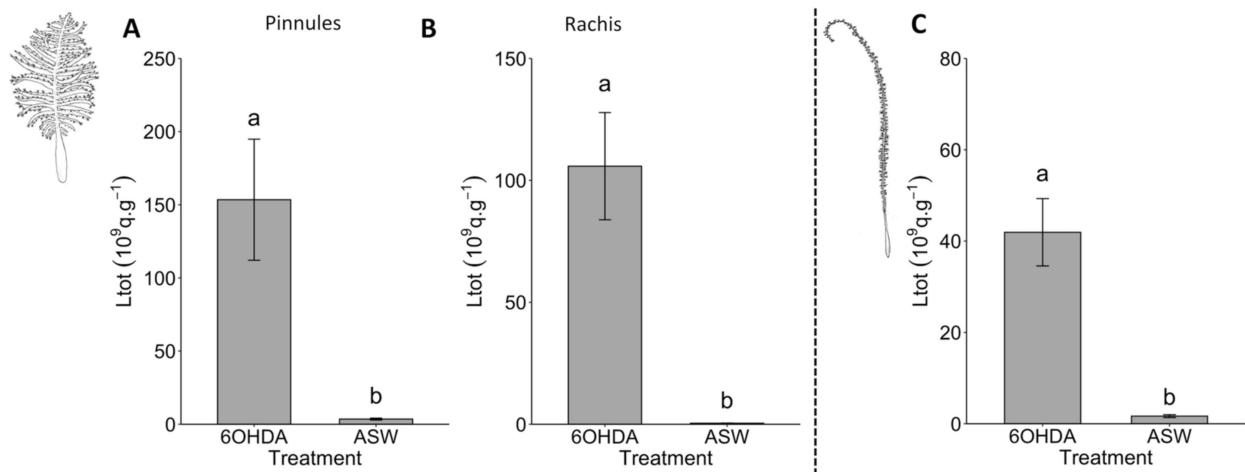


Figure 3. Effects of 6-OHDA 10^{-6} mol L⁻¹ on the luminescence of *Pennatula phosphorea* and *Funiculina quadrangularis*. Total amount of light (Ltot) produced after 6-OHDA application on (A) the pinnules, and (B) the rachis of *P. phosphorea*, and polyp-bearing rachis of *F. quadrangularis* (C). All Ltot values are expressed as 10^9 q s⁻¹ g⁻¹. Different lettering indicates statistical differences. Error bars correspond to s.e.m. Scale bar = 1 cm.

3.3. Effects of Catecholamines

Adrenaline, noradrenaline, and octopamine application at 10^{-6} mol L⁻¹ generated rapid flash series on the *Pennatula* pinnules that can last for 30 min. Each of those biogenic amines induced the light emission of *P. phosphorea* pinnules compared with the ASW control (pairwise Wilcoxon test, p -value < 0.05; Figure 4A), while they did not present statistical differences between each other (pairwise Wilcoxon test, p -value > 0.05; Figure 4A). Comparatively, dopamine at 10^{-6} mol L⁻¹ did not show statistical differences compared to the ASW application (pairwise Wilcoxon test, p -value = 0.13; Figure 4D) on pinnules.

On *Pennatula* rachis, similar results comparing the ASW control to the biogenic amines were obtained for adrenaline and octopamine (Tukey's test, p -value < 0.05), while noradrenaline did not present a significant difference with the ASW control (Tukey's test, p -value = 0.1215; Figure 4B). The response of the three biogenic amine applications did not present any statistical differences between each other (Tukey's test, p -value > 0.05; Figure 4B). Dopamine at 10^{-6} mol L⁻¹ did not elicit luminescence of the rachis, with a mean Ltot value significantly lower than the one of ASW (Student t -test, p -value = 0.0001; Figure 4E). Only adrenaline triggered luminescence flash production in *F. quadrangularis* compared to the ASW control application (Tukey's test, p -value < 2.2×10^{-6} ; Figure 4C), while noradrenaline and octopamine did not differ from the control (Tukey's test, p -value > 0.05; Figure 4C). Dopamine did not trigger light emission in *F. quadrangularis* (Student t -test, p -value = 0.5603; Figure 4F).

At higher concentrations (10^{-3} to 10^{-5} mol L⁻¹), adrenaline, noradrenaline, and octopamine application on *Pennatula* pinnules presented an increase in the total light emission compared to the 10^{-6} mol L⁻¹ applications (Figure 5; Table S1). At concentrations of 10^{-3} and 10^{-4} mol L⁻¹, noradrenaline and octopamine presented a higher response than adrenaline.

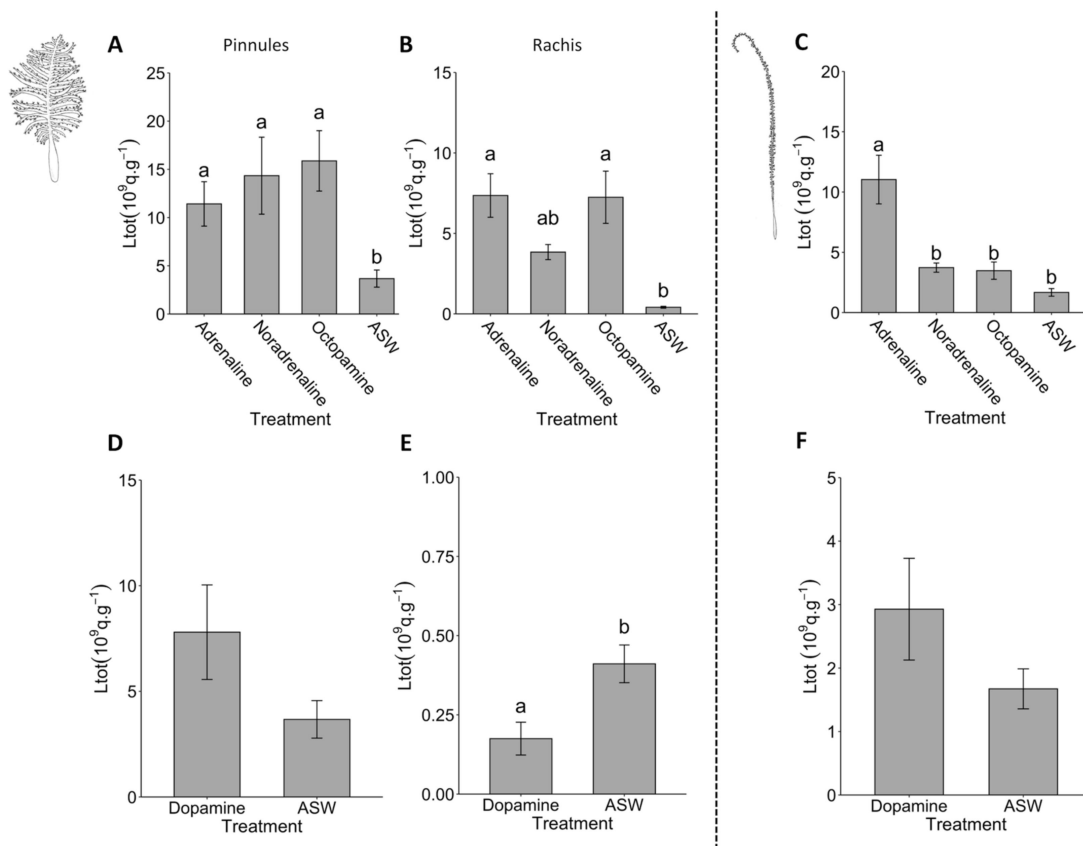


Figure 4. Pharmacological analyses on *Pennatula phosphorea* and *Funiculina quadrangularis* luminescence control. Comparison between the total amount of light (Ltot) produced by (A) pinnules ($n = 62$), and (B) the rachis ($n = 20$) of *P. phosphorea*, and (C) the rachis ($n = 12$) of *F. quadrangularis* after adrenaline, noradrenaline or octopamine applications at 10^{-6} mol L⁻¹. Comparison between the Ltot produced by (D) pinnules ($n = 20$), and (E) the rachis ($n = 20$) of *P. phosphorea*, and (F) the rachis ($n = 12$) of *F. quadrangularis* after dopamine application at 10^{-6} mol L⁻¹ and ASW control. All Ltot values are expressed as 10^9 q s⁻¹ g⁻¹. Different lettering indicates statistical differences. ASW: artificial seawater. Error bars correspond to s.e.m.

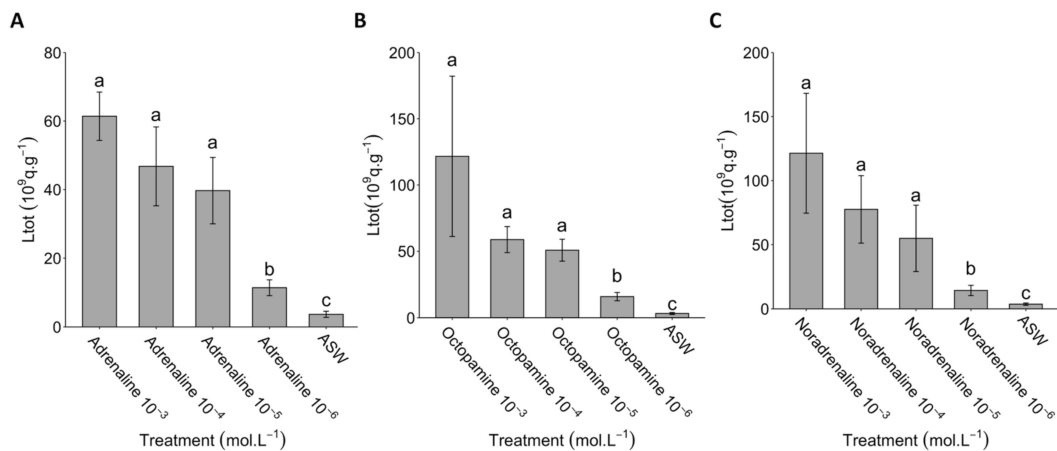


Figure 5. Dose-dependent response of catecholamines on *Pennatula phosphorea* bioluminescence. Total amount of light (Ltot) produced by pinnules after (A) adrenaline, (B) noradrenaline, and (C) octopamine applications at concentrations ranging from 10^{-6} to 10^{-3} mol L⁻¹. All Ltot values are expressed as 10^9 q s⁻¹ g⁻¹. Different lettering indicates statistical differences. ASW: artificial seawater. Error bars correspond to s.e.m.

4. Discussion

The common sea pen, *P. phosphorea* and the tall sea pen, *F. quadrangularis*, produced light after exposure to KCl. The light emission in response to KCl applications confirms not only the bioluminescence status of these species but also the direct involvement of a nervous light emission control [11,56–58]. In *Pennatulula*'s pinnules, the total light emission did not vary from left to right sides of the colony when the upper part produced a higher luminescence than middle or lower parts. The rachis did not show any statistical differences of total light emission depending on the upper/middle/lower position. Pinnules emitted a more intense luminescence than the rachis which reflected the in vivo observations where only pinnules luminescence has been observed (Video S1). *F. quadrangularis* emitted a homogenous light emission along the axis. For both species, the KCl application on the peduncle part of the colony failed to trigger any luminescence (exceptionally low values like the ASW control). As the function of bioluminescence in these two species is unknown, the biological significance of our observations remains to be clarified. However, as the peduncle is embedded within the sediment, the lack of bioluminescence is not surprising.

6-OHDA is a sympatholytic drug releasing terminal synaptic catecholamines. In both *P. phosphorea* and *F. quadrangularis*, 6-OHDA triggered luminescence, indicating the involvement of catecholamines in the nervous control of light emission. This is consistent with the results of a previous work on *R. koellikeri* [49].

In *Renilla*, only adrenaline induces a localized, phasic luminescence at 2×10^{-4} M [49]. This is consistent with our observations for *F. quadrangularis*. In *P. phosphorea*, the luminescence is shown to be under adrenaline, noradrenaline and octopamine control. An increase in the catecholamine concentration from 10^{-6} to 10^{-3} M results in an increase in the mean Ltot values. All these neurotransmitters induce clustered flashes spaced in time, suggesting a lag time before the next set of flashes. Adrenaline is thought to directly act through the mesogleal nerve net on the $\beta 1/\beta 2$ -like adrenergic receptors of the endodermal photocytes located at the base and crown of the autozooids and specific chambers of the water-pumping siphonozooids of the sea pansy, *Renilla* [35,62]. These catecholaminergic G protein-coupled receptors have been shown to interact with adenylate cyclase to regulate intracellular cAMP concentration [35,62]. The data presented here provide strong support to a similar adrenergic pathway for other luminous pennatulacean species, such as *P. phosphorea* and *F. quadrangularis*.

Noradrenaline and octopamine neurotransmitters failed to trigger any luminescent flashes in *Renilla* [49] and *Funiculina*. For *P. phosphorea*, noradrenaline at 10^{-5} and 10^{-6} mol L⁻¹ induced a similar response to adrenaline in terms of total light emission, while the mean response was higher at 10^{-3} and 10^{-4} mol L⁻¹, which suggests a better affinity with receptors (specific or unspecific). Our results also show that octopamine triggers a significant luminous response in *P. phosphorea*. The presence and localization of noradrenaline and its receptor has been detected by high-performance liquid chromatography and immunoreactivity within the mesogleal nerve net of *Renilla* [63]. Conversely, the monoaminergic octopamine molecule has not been detected in the tissues of *R. koellikeri* [63]. In metazoan, noradrenaline, mainly through adrenoceptors, and octopamine, via octopaminergic receptor binding, also act on the cAMP cell concentrations via interaction with membrane-bound adenylate cyclase enzyme [64,65]. The monoaminergic octopamine molecule is structurally close to noradrenaline and known to be involved in the luminescence control in other invertebrates [36], but it is also the main neuroeffector responsible for the light emission in insects [32,66–68]. While a study demonstrated the absence of octopamine and its receptors in nerve cells of *R. koellikeri* [63,69], more recent research identified two new aminergic-like G protein-coupled receptors (Ren1 and 2) homologous to mammalian monoaminergic receptors, with cAMP and Ca²⁺-mediated signalling [70,71]. Even if no specific biogenic amines have been identified as specific ligands, adrenaline, noradrenaline and/or octopamine could be assumed as neurotransmitter candidates binding these receptors. Therefore, a cellular pathway involving both noradrenaline and octopamine, their receptors, adenylate cyclase, and cAMP in the light emission control of *P. phosphorea* is here suggested. A similar catecholaminergic control involving both adrenaline and noradrenaline has been

demonstrated in bioluminescent bony fishes [40,42,72], highlighting the efficiency of the nervous catecholaminergic pathways to regulate the luminescence and the conservation of such mechanisms among both protostome and deuterostome lineages. However, further investigations are needed to understand the specific affinity of catecholamines with their respective receptors in these luminous anthozoans.

Catecholamines are derived from the tyrosine amino acid [73]. One of the biosynthesis pathways, passing through L-Dopa and dopamine, leads to catecholamines (e.g., noradrenaline and adrenaline; [73] for review). The dopamine failed to trigger light production in both tested species. To date, the dopamine has been described as a weak or non-triggering actor in metazoan luminescence [44,74,75], rather being involved as a light emission inhibitor in some cholinergic-dependent luminous brittle stars [38].

Calcium ions are known to trigger light emission in two anthozoans, *Renilla reniformis* and *Veretillum cynomorium* [50,51], where it is assumed to transiently enter in photocytes breaking down the link between the luciferin-binding protein and coelenterazine that becomes available for the bioluminescent reaction [50,51]. The present results do not provide clues on this aspect, but work is in progress to demonstrate the involvement of calcium ions in *P. phosphorea* and *E. quadrangularis* bioluminescence.

Our results demonstrate biogenic amines to be involved in the light emission regulation in two anthozoan species. Adrenaline acts as the main neuroeffector triggering luminescence in these two species. Further investigations are needed to fully apprehend the pathways and actors involved in the process from the binding of biogenic amines to their receptors till the light production. Based on literature and the presented results, we can assume the conservation of the catecholaminergic control of luminescence among pennatulaceans.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/life13091798/s1>, Video S1: Original video recording of the light emission of *Pennatula phosphorea* upon mechanical stimulation; Figure S1: (A) Spearman correlation between the number of polyp and the weight of pinnules in *Pennatula phosphorea*; (B) Original recording of KCl application on *P. phosphorea* rachis; Table S1: Statistical analyses of the dose response experiments for (A) adrenaline (Kruskal–Wallis test, p -value = 1.69×10^{-10} ; $n = 6$ from 10^{-3} to 10^{-5} ; and Wilcoxon test); (B) noradrenaline (Kruskal–Wallis test, p -value = 3.96×10^{-14} ; $n = 6$ from 10^{-3} to 10^{-5} ; and Wilcoxon test), and (C) octopamine (Kruskal–Wallis test, p -value = 1.857×10^{-12} ; $n = 6$ from 10^{-3} to 10^{-6} ; and Wilcoxon test).

Author Contributions: Conceptualization, L.D. and C.C.; methodology, L.D.; software, L.D. and C.C.; validation, L.D. and C.C.; formal analysis, L.D.; investigation, L.D.; resources, L.D., C.P. and S.T.D.; data curation, L.D. and C.C.; writing—original draft preparation, L.D. and C.C.; writing—review and editing, L.D., C.C., S.T.D. and J.M.; visualization, L.D. and C.C.; supervision, L.D.; project administration, L.D.; funding acquisition, J.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by an F.R.S.-FNRS grant (T.0169.20) awarded to the Université de Louvain—UCLouvain Marine Biology Laboratory and the Université de Mons Biology of Marine Organisms and Biomimetics Laboratory. The research leading to these results also received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement No 730984, ASSEMBLE Plus project.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding authors.

Acknowledgments: The authors acknowledge Ursula Schwarz captain of Alice’s vessel, and the skillful members of the Kristineberg Center (University of Gothenburg, Sweden) for their help during the *Pennatula* and *Funiculina* collections. The authors also thank M. Jacquet for his contribution to the study during his master thesis, E. Infantes for the original bioluminescent picture of *Funiculina*, and

J. Lindstrom for sharing original protocol data on anthozoans in early 2000. LD is researcher at the Université de Louvain—UCLouvain, CC is students under a FRIA fellowship, CP is Earth and Life Institute technician, SD is Senior Lecturer and Associate professor at the University of Gothenburg, and JM is Research Associate the Fund for Scientific Research of Belgium (F.R.S.-FNRS). This study is the contribution BRC#408 of the Biodiversity Research Center (UCLouvain) from the Earth and Life Institute Biodiversity (ELIV) and the “Centre Interuniversitaire de Biologie Marine” (CIBIM).

Conflicts of Interest: The authors declare no conflict of interest.

References

- Haddock, S.H.D.; Moline, M.A.; Case, J.F. Bioluminescence in the sea. *Annu. Rev. Mar. Sci.* **2010**, *2*, 443–493. [[CrossRef](#)]
- Martini, S.; Haddock, S.H.D. Quantification of bioluminescence from the surface to the deep sea demonstrates its predominance as an ecological trait. *Sci. Rep.* **2017**, *7*, 45750. [[CrossRef](#)]
- Bessho-Uehara, M.; Francis, W.R.; Haddock, S.H.D. Biochemical characterization of diverse deep-sea anthozoan bioluminescence systems. *Mar. Biol.* **2020**, *167*, 114. [[CrossRef](#)]
- Cormier, M.J. Studies of the bioluminescence of *Renilla reniformis*: I. Requirements for luminescence in extracts and characteristics of the system. *Biochim. Biophys. Acta* **1960**, *42*, 333–343. [[CrossRef](#)] [[PubMed](#)]
- Matthews, J.C.; Hori, K.; Cormier, M.J. Purification and properties of *Renilla reniformis* luciferase. *Biochemistry* **1977**, *16*, 85–91. [[CrossRef](#)] [[PubMed](#)]
- Lorenz, W.W.; McCann, R.O.; Longiaru, M.; Cormier, M.J. Isolation and expression of a cDNA encoding *Renilla reniformis* luciferase. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 4438–4442. [[CrossRef](#)] [[PubMed](#)]
- Loening, A.M.; Fenn, T.D.; Gambhir, S.S. Crystal structures of the luciferase and green fluorescent protein from *Renilla reniformis*. *J. Mol. Biol.* **2007**, *374*, 1017–1028. [[CrossRef](#)] [[PubMed](#)]
- Schenkmyerova, A.; Toul, M.; Pluskal, D.; Baatallah, R.; Gagnot, G.; Pinto, G.P.; Santana, V.T.; Stuchla, M.; Neugebauer, P.; Chaiyen, P.; et al. Catalytic mechanism for *Renilla*-type luciferases. *Nat. Catal.* **2023**, *6*, 23–38. [[CrossRef](#)]
- Titushin, M.S.; Markova, S.V.; Frank, L.A.; Malikova, N.P.; Stepanyuk, G.A.; Lee, J.; Vysotski, E.S. Coelenterazine-binding protein of *Renilla muelleri*: cDNA cloning, overexpression, and characterization as a substrate of luciferase. *Photochem. Photobiol. Sci.* **2008**, *7*, 189–196. [[CrossRef](#)]
- Hastings, J.W.; Morin, J.G. Calcium-triggered light emission in *Renilla*. A unitary biochemical scheme for coelenterate bioluminescence. *Biochem. Biophys. Res. Commun.* **1969**, *37*, 493–498. [[CrossRef](#)]
- Nicol, J.A.C. Observations on luminescence in *Renilla* (Pennatulacea). *J. Exp. Biol.* **1955**, *32*, 299–320. [[CrossRef](#)]
- Davenport, D.; Nicol, J.A.C. Observations on luminescence in sea pens (Pennatulacea). *Proc. R. Soc. B* **1956**, *144*, 480–496. [[CrossRef](#)]
- Wampler, J.E.; Karkhanis, Y.D.; Morin, J.G.; Cormier, M.J. Similarities in the bioluminescence from the Pennatulacea. *Biochim. Biophys. Acta* **1973**, *314*, 104–109. [[CrossRef](#)] [[PubMed](#)]
- Shimomura, O. *Bioluminescence: Chemical Principles and Methods*; World Scientific: Singapore, 2012.
- Delroisse, J.; Duchatelet, L.; Flammang, P.; Mallefet, J. Leaving the dark side? Insights into the evolution of luciferases. *Front. Mar. Sci.* **2021**, *8*, 673620. [[CrossRef](#)]
- Delroisse, J.; Ullrich-Lüter, E.; Blaue, S.; Ortega-Martinez, O.; Eeckhaut, I.; Flammang, P.; Mallefet, J. A puzzling homology: A brittle star using a putative cnidarian-type luciferase for bioluminescence. *Open Biol.* **2017**, *7*, 160300. [[CrossRef](#)] [[PubMed](#)]
- Tessler, M.; Gaffney, J.P.; Oliveira, A.G.; Guarnaccia, A.; Dobi, K.C.; Gujarati, N.A.; Galbraith, M.; Mirza, J.D.; Sparks, J.S.; Pieribone, V.A.; et al. A putative chordate luciferase from a cosmopolitan tunicate indicates convergent bioluminescence evolution across phyla. *Sci. Rep.* **2020**, *10*, 17724. [[CrossRef](#)] [[PubMed](#)]
- Cormier, M.J.; Hori, K.; Karkhanis, Y.D.; Anderson, J.M.; Wampler, J.E.; Morin, J.G.; Hastings, J.W. Evidence for similar biochemical requirements for bioluminescence among coelenterates. *J. Cell. Physiol.* **1973**, *81*, 291–297. [[CrossRef](#)]
- Inoue, S.; Kakoi, H.; Murata, M.; Goto, T.; Shimomura, O. Complete structure of *Renilla* luciferin and luciferin sulfate. *Tetrahedron Lett.* **1977**, *18*, 2685–2688. [[CrossRef](#)]
- Ward, W.W.; Cormier, M.J. Energy transfer protein in coelenterate bioluminescence. *J. Biol. Chem.* **1979**, *254*, 781–788. [[CrossRef](#)]
- Inouye, S. Expression, purification and characterization of calcium-triggered luciferin-binding protein of *Renilla reniformis*. *Protein Expr. Purif.* **2007**, *52*, 66–73. [[CrossRef](#)]
- Ogoh, K.; Kinebuchi, T.; Murai, M.; Takahashi, T.; Ohmiya, Y.; Suzuki, H. Dual-color-emitting green fluorescent protein from the sea cactus *Cavernularia obesa* and its use as a pH indicator for fluorescent microscopy. *Luminescence* **2013**, *28*, 582–591. [[CrossRef](#)] [[PubMed](#)]
- Kumar, J.S.Y.; Raghunathan, C.; Venkataraman, K. New records of octocorallia (order: Pennatulacea) from Indian waters. *Int. J. Appl. Biol. Pharm. Technol.* **2014**, *5*, 52–56.
- Kudryavtsev, A.N.; Krasitskaya, V.V.; Efremov, M.K.; Zangeeva, S.V.; Rogova, A.V.; Tomilin, F.X.; Frank, L.A. Ca²⁺-triggered coelenterazine-binding protein *Renilla*: Expected and unexpected features. *Int. J. Mol. Sci.* **2023**, *24*, 2144. [[CrossRef](#)] [[PubMed](#)]
- Case, J.F.; Strause, L.G. Neurally controlled luminescent systems. In *Bioluminescence in Action*; Herring, P.J., Ed.; Academic Press: London, UK, 1978; pp. 331–366.

26. Duchatelet, L.; Claes, J.M.; Delroisse, J.; Flammang, P.; Mallefet, J. Glow on sharks: State of the art on bioluminescence research. *Oceans* **2021**, *2*, 822–842. [[CrossRef](#)]
27. Nicol, J.A.C. Observations on photophores and luminescence in the teleost. *Porichthys*. *J. Cell Sci.* **1957**, *3*, 179–188. [[CrossRef](#)]
28. Baguet, F.; Case, J.F. Luminescence control in *Porichthys* (Teleostei): Excitation of isolated photophores. *Biol. Bull.* **1971**, *140*, 15–27. [[CrossRef](#)]
29. Anctil, M.; Case, J.F. Pharmacomorphological study of denervation induced by 6-hydroxydopamine in *Porichthys* photophores. *Cell Tiss. Res.* **1976**, *166*, 365–388. [[CrossRef](#)]
30. Christophe, B.; Baguet, F. Luminescence of isolated photocytes from *Porichthys* photophores: Adrenergic stimulation. *J. Exp. Biol.* **1983**, *104*, 183–192. [[CrossRef](#)]
31. Larivière, L.; Anctil, M. A comparative analysis of noradrenaline and adrenaline uptake in photophores of the midshipman fish, *Porichthys notatus*: Kinetics and pharmacology. *Comp. Biochem. Physiol. C* **1986**, *85*, 335–339. [[CrossRef](#)]
32. Hashemzadeh, H.; Hollingworth, R.M.; Voliva, A. Receptor for ³H-octopamine in the adult firefly light organ. *Life Sci.* **1985**, *37*, 433–440. [[CrossRef](#)]
33. Trimmer, B.A.; Aprille, J.R.; Dudzinski, D.M.; Lagace, C.J.; Lewis, S.M.; Michel, T.; Qazi, S.; Zayas, R.M. Nitric oxide and the control of firefly flashing. *Science* **2001**, *292*, 2486–2488. [[CrossRef](#)] [[PubMed](#)]
34. Krönström, J.; Dupont, S.; Mallefet, J.; Thorndyke, M.; Holmgren, S. Serotonin and nitric oxide interaction in the control of bioluminescence in northern krill, *Meganyctiphanes norvegica* (M. Sars). *J. Exp. Biol.* **2007**, *210*, 3179–3187. [[CrossRef](#)]
35. Awad, E.W.; Anctil, M. Identification of β -like adrenoceptors associated with bioluminescence in the sea pansy *Renilla koellikeri*. *J. Exp. Biol.* **1993**, *177*, 181–200. [[CrossRef](#)]
36. Vanderlinden, C.; Mallefet, J. Synergic effects of tryptamine and octopamine on ophiuroid luminescence (Echinodermata). *J. Exp. Biol.* **2004**, *207*, 3749–3756. [[CrossRef](#)]
37. De Bremaeker, N.; Baguet, F.; Thorndyke, M.C.; Mallefet, J. Modulatory effects of some amino acids and neuropeptides on luminescence in the brittlestar *Amphipholis squamata*. *J. Exp. Biol.* **1999**, *202*, 1785–1791. [[CrossRef](#)] [[PubMed](#)]
38. De Bremaeker, N.; Baguet, F.; Mallefet, J. Effects of catecholamines and purines on luminescence in the brittlestar *Amphipholis squamata* (Echinodermata). *J. Exp. Biol.* **2000**, *203*, 2015–2023. [[CrossRef](#)]
39. Dupont, S.; Mallefet, J.; Vanderlinden, C. Effect of β -adrenergic antagonists on bioluminescence control in three species of brittlestars (Echinodermata: Ophiuroidea). *Comp. Biochem. Physiol. C* **2004**, *138*, 59–66. [[CrossRef](#)] [[PubMed](#)]
40. Baguet, F.; Marechal, G. The stimulation of isolated photophores (*Argyrolepeucus*) by epinephrine and norepinephrine. *Comp. Biochem. Physiol. C* **1978**, *60*, 137–143. [[CrossRef](#)]
41. Baguet, F.; Christophe, B. Adrenergic stimulation of isolated photophores of *Maurolucis muelleri*. *Comp. Biochem. Physiol. C* **1983**, *75*, 79–84. [[CrossRef](#)]
42. Mallefet, J.; Duchatelet, L.; Hermans, C.; Baguet, F. Luminescence control of *Stomiidae* photophores. *Acta Histochem.* **2019**, *121*, 7–15. [[CrossRef](#)]
43. Gouveneaux, A.; Mallefet, J. Physiological control of bioluminescence in a deep-sea planktonic worm, *Tomopteris helgolandica*. *J. Exp. Biol.* **2013**, *216*, 4285–4289. [[CrossRef](#)] [[PubMed](#)]
44. Dewael, Y.; Mallefet, J. Luminescence in ophiuroids (Echinodermata) does not share common nervous control in all species. *J. Exp. Biol.* **2002**, *205*, 799–806. [[CrossRef](#)]
45. Anctil, M. Stimulation of bioluminescence in lanternfishes (Myctophidae). II. *Can. J. Zool.* **1972**, *50*, 233–237. [[CrossRef](#)]
46. Krönström, J.; Mallefet, J. Evidence for a widespread involvement of NO in control of photogenesis in bioluminescent fishes. *Acta Zool.* **2010**, *91*, 474–483. [[CrossRef](#)]
47. Hastings, J.W.; Morin, J.G. Bioluminescence. In *Neural and Integrative Animal Physiology*; Prosser, C.L., Ed.; Wiley-Liss: New York, NY, USA, 1991; pp. 131–170.
48. Paitio, J.; Oba, Y. Luminous fishes: Endocrine and neuronal regulation of bioluminescence. *Fish. Aquac. J.* **2023**. [[CrossRef](#)]
49. Anctil, M.; Boulay, D.; Larivière, L. Monoaminergic mechanisms associated with control of luminescence and contractile activities in the coelenterate, *Renilla köllikeri*. *J. Exp. Zool.* **1982**, *223*, 11–24. [[CrossRef](#)]
50. Henry, J.-P.; Ninio, M. Control of the Ca²⁺-triggered bioluminescence of *Veretillum cynomorium* lumisomes. *Biochim. Biophys. Acta* **1978**, *504*, 40–59. [[CrossRef](#)]
51. Charbonneau, H.; Cormier, M.J. Ca²⁺-induced bioluminescence in *Renilla reniformis*. Purification and characterization of a calcium-triggered luciferin-binding protein. *J. Biol. Chem.* **1979**, *254*, 769–780. [[CrossRef](#)]
52. Williams, G.C. Living genera of sea pens (Coelenterata: Octocorallia: Pennatulacea): Illustrated key and synopses. *Zool. J. Linn. Soc.* **1995**, *113*, 93–140. [[CrossRef](#)]
53. Edwards, D.C.B.; Moore, C.G. Reproduction in the sea pen *Pennatula phosphorea* (Anthozoa: Pennatulacea) from the west coast of Scotland. *Mar. Biol.* **2008**, *155*, 303–314. [[CrossRef](#)]
54. Panceri, P. Gli organi luminosi e la luce delle Pennatule. *R. C. Accad. Napoli* **1871**, *10*, 204–211.
55. Panceri, P. The luminous organs and light of the Pennatulae. *Quart. J. Micr. Sci.* **1872**, *12*, 248–254.
56. Nicol, J.A.C. Observations on the luminescence of *Pennatula phosphorea*, with a note on the luminescence of *Virgularia mirabilis*. *J. Mar. Biol. Assoc. UK* **1958**, *37*, 551–563. [[CrossRef](#)]
57. Nicol, J.A.C. Nervous regulation of luminescence in the sea pansy *Renilla köllikeri*. *J. Exp. Biol.* **1955**, *32*, 619–635. [[CrossRef](#)]

58. Nicol, J.A.C. Physiological control of luminescence in animals. In *The Luminescence of Biological Systems*; Johnson, F.H., Ed.; American Society for the Advancement of Science: Washington, DC, USA, 1955; pp. 299–319.
59. Greathead, C.F.; Donnan, D.W.; Mair, J.M.; Saunders, G.R. The sea pens *Virgularia mirabilis*, *Pennatula phosphorea* and *Funiculina quadrangularis*: Distribution and conservation issues in Scottish waters. *J. Mar. Biol. Assoc.* **2007**, *87*, 1095–1103. [[CrossRef](#)]
60. Herring, P.J. Observations on bioluminescence in some deep-water anthozoans. In *Coelenterate Biology: Recent Research on Cnidaria and Ctenophora, Proceedings of the Fifth International Conference on Coelenterate Biology, Southampton, UK, 10–14 July 1989*; Springer: Dordrecht, The Netherlands, 1991; pp. 573–579.
61. Mallefet, J.; Duchatelet, L.; Coubris, C. Bioluminescence induction in the ophiuroid *Amphiura filiformis* (Echinodermata). *J. Exp. Biol.* **2020**, *223*, jeb218719. [[CrossRef](#)]
62. Awad, E.W.; Anctil, M. Positive coupling of β -like adrenergic receptors with adenylate cyclase in the cnidarian *Renilla Koellikeri*. *J. Exp. Biol.* **1993**, *182*, 131–146. [[CrossRef](#)]
63. Pani, A.K.; Anctil, M.; Umbriaco, D. Neuronal localization and evoked release of norepinephrine in the cnidarian *Renilla Koellikeri*. *J. Exp. Zool.* **1995**, *272*, 1–12. [[CrossRef](#)]
64. Caron, M.G.; Lefkowitz, R.J. Catecholamine receptors: Structure, function, and regulation. *Recent Prog. Horm. Res.* **1993**, *1993*, 277–290. [[CrossRef](#)]
65. David, J.-C.; Coulon, J.-F. Octopamine in invertebrates and vertebrates. A review. *Prog. Neurobiol.* **1985**, *24*, 141–185. [[CrossRef](#)]
66. Carlson, A.D. Effect of adrenergic drugs on the lantern of the larval *Photuris* firefly. *J. Exp. Biol.* **1968**, *48*, 381–387. [[CrossRef](#)] [[PubMed](#)]
67. Carlson, A.D. Effect of drugs on luminescence in larval fireflies. *J. Exp. Biol.* **1968**, *49*, 195–199. [[CrossRef](#)] [[PubMed](#)]
68. Carlson, A.D. Neural control of firefly luminescence. *Advances in Insect Physiol.* **1970**, *6*, 51–96. [[CrossRef](#)]
69. Pflüger, H.-J.; Stevenson, P.A. Evolutionary aspects of octopaminergic systems with emphasis on arthropods. *Arthropod Struct. Dev.* **2005**, *34*, 379–396. [[CrossRef](#)]
70. Bouchard, C.; Ribeiro, P.; Dubé, F.; Anctil, M. A new G protein-coupled receptor from a primitive metazoan shows homology with vertebrate aminergic receptors and displays constitutive activity in mammalian cells. *J. Neurochem.* **2003**, *86*, 1149–1161. [[CrossRef](#)]
71. Bouchard, C.; Ribeiro, P.; Dubé, F.; Demers, C.; Anctil, M. Identification of a novel aminergic-like G protein-coupled receptor in the cnidarian *Renilla koellikeri*. *Gene* **2004**, *341*, 67–75. [[CrossRef](#)]
72. Guariépy, P.; Anctil, M. A pharmacological study of adrenergic and serotonergic mechanisms in the photophores of the midshipman fish, *Porichthys notatus*. *Comp. Biochem. Physiol. C.* **1983**, *74*, 341–347. [[CrossRef](#)]
73. Gallo, V.P.; Accordi, F.; Chimenti, C.; Civinini, A.; Crivellato, E. Catecholaminergic system of invertebrates: Comparative and evolutionary aspects in comparison with the octopaminergic system. *Int. Rev. Cell Mol. Biol.* **2016**, *322*, 363–394. [[CrossRef](#)]
74. Anctil, M. Cholinergic and monoaminergic mechanisms associated with control of bioluminescence in the ctenophore *Mnemiopsis leidyi*. *J. Exp. Biol.* **1985**, *119*, 225–238. [[CrossRef](#)]
75. Rigby, L.M.; Merritt, D.J. Roles of biogenic amines in regulating bioluminescence in the Australian glowworm *Arachnocampa flava*. *J. Exp. Biol.* **2011**, *214*, 3286–3293. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.