A deepwater fish with ‘lightsabers’ – dorsal spine-associated luminescence in a counterilluminating lanternshark

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We report the discovery of light organs (photophores) adjacent to the dorsal defensive spines of a small deep-sea lanternshark (Etmopterus spinax). Using a visual modeling based on in vivo luminescence recordings we show that this unusual light display would be detectable by the shark’s potential predators from several meters away. We also demonstrate that the luminescence from the spine-associated photophores (SAPs) can be seen through the mineralized spines, which are partially translucent. These results suggest that the SAPs function, either by mimicking the spines’ shape or by shining through them, as a unique visual deterrent for predators. This conspicuous dorsal warning display is a surprising complement to the ventral luminous camouflage (counterillumination) of the shark.

In the permanent darkness of the deep-sea a surprising diversity of organisms, from bacteria to fish, have evolved the capability to produce a visible light1,2. This bioluminescence comes from a chemical reaction1–3 that occurs either in the exterior environment or within cells; these photogenic cells (photocytes) are often embedded in organs called photophores that can reach a high degree of sophistication, including reflectors, light guides, wavelength-specific filters and optical lenses4–6. Numerous adaptive benefits have been suggested for bioluminescent glows and flashes (light emission, < 2 s), all falling in the three functional categories essential to an organism’s survival: predator evasion, food acquisition and reproduction1–3,7.

In the mesopelagic zone (200–1000 m), many animals produce a continuous ventral glow to match downwelling sunlight and conceal their silhouette from organisms swimming beneath them, a process called counterillumination2,8,9. Conversely, dorsal glows are rarely observed in this environment, probably because they are highly detectable against the darker background of deeper waters when viewed from above. As a consequence, such conspicuous displays are usually of a predatory nature, produced by ‘anatomical fishing lures’ that attract prey from a distance2,7. Probably because of the stark difference in the apparent roles of dorsal and ventral luminescence, dorsal and ventral photogenic organs are rarely observed to glow concomitantly in the same mesopelagic organism10. Here, however, we report a particular exception where the dorsal glows of a deepwater shark might actually complement the counterilluminating luminescence produced by its ventral photophores.

The velvet belly lanternshark, Etmopterus spinax, is a diminutive shark (~ 60 cm in total length) that spends most of its time at mesopelagic depths11. Like other members of the Etmopteridae family, E. spinax harbour defensive mineralized spines in front of its dorsal fins and has a ventral surface covered by thousands of tiny (< 200 μm in diameter) photophores12,13. Stimulated by melatonin or prolactin, these organs produce a long lasting blue-green luminescence13. This self-produced light, the chemistry of which remains unknown, provides this lanternshark with a counterilluminating camouflage14 and perhaps aids in intraspecific communication15,16.

When investigating this species’ bioluminescence, we were surprised to observe glows from the frontal edges of the dorsal fins, forming conspicuous arcs immediately behind the spines (Fig. 1). This dorsal luminescent display seems in direct conflict with the camouflage role of the ventral photophores. Here we document the structural basis of this intriguing display and investigate its functional significance. We examined the histology and light emission of the dorsal photophores and determined the 3D structure and spectral transmittance of their associated spines. Using these structural and in vivo luminescence data, ocular measurements from local fishes and marine mammals and a recent theory for visual detection in pelagic habitats17, we then determined the detection
range of the luminescence produced by the spine-associated photophores (SAPs) in the shark’s environment, characterizing their in situ performance and suggesting their ecological role.

**Results**

**Structural basis.** SAPs form two to four vertical rows on both sides of the dorsal fins, at a distance of about 1–3 mm behind the finspines (Fig. 2a). These organs appear structurally similar to ventral photophores18, each organ comprising a pigmented sheath covered by one or several lens cells, and enclosing a cluster of photogenic cells (photocytes) containing fluorescent vesicles (Fig. 2b,c). Considering the relative orientation of lenses and photocytes, SAPs emit most of their light laterally, forming sickle-shaped glows on the sides of the leading edges of the dorsal fins, mimicking the curve of the spines anterior to them. Given this intimate association of photophores and spines (Fig. 2d) and that dorsal fins have been shown to rotate during swimming (Fig. 2e), the interaction of SAP luminescence and spines by measuring the spectral transmittance of these structures. The spines could hypothetically transmit about 10% of the SAP luminescence if the dorsal fins were to rotate laterally during swimming (Fig. 2e).

**Functional significance.** Ventral photophore and SAP luminescences are long-lasting and their intensities are significantly correlated (Fig. 3a), suggesting a common hormonal control for these photogenic structures. This information, combined with local environmental data and the visual theory developed by Nilsson et al.17 allowed us to calculate the depth at which the ventral photophores provide an optimal counterilluminating camouflage for the brightest of our specimens (Fig. 3b). This theoretical depth should reflect the preferred depth of the shark in its natural habitat. Using Nilsson et al. ’s theory again as well as the spine transmittance data and ocular anatomy of local fishes and marine mammals, we determined that, at theoretical counterillumination depth, SAPs can emit a close range luminescent signal, both around and through the spines, that would be detectable by most of the shark’s potential predators—but not its main prey—from several meters away (Fig. 3c).

**Discussion**

In the mesopelagic zone, where most animals—including* E. spinax*—have converged on camouflage strategies20, the dorsal long-lasting luminescence produced by the shark’s SAPs appears to be a paradoxical and risky display. As a consequence, a strong adaptive benefit of these intriguing photogenic structures might be expected.

We explored the functional significance of SAP luminescence using an integrative method involving structural and performance data and a recent theoretical visual modeling17. This novel approach appears particularly useful to test performance hypotheses involving deep-sea animals like *E. spinax*, which live in a largely inaccessible environment and demonstrate a physiological fragility that considerably restricts their use in behavioral experiments. The validity of our modeling was confirmed by the similarity between the theoretical counterillumination depth (164–254 m deep, depending on surface light conditions), which suggests the occurrence depth of the shark, and the actual capture depth of our specimens (170–230 m). At this theoretical counterillumination depth, the particular window of detection distances of SAP luminescence would not be effective in prey attraction: our calculations predict that the main prey of the shark (the pearside fish* Maurolicus muelleri*)21 can only
detect SAPs from short distance away (~1.5 m); conversely potential predators can detect them at several meters. This strongly suggests that dorsal bioluminescence in the lanternshark serves as a "predators-eyes-only" signal and therefore somehow functions as an aposematic (warning) beacon to deter approaching predators (or conspecifics), albeit without jeopardizing prey capture.

The exact message of this aposematic signal remains unclear and intriguing. Given the particular spatial organisation and close association of SAPs with the defensive dorsal finspines, and the fact that the finspines are capable of transmitting a portion of SAP luminescence (probably thanks to a reduced enameloid covering compared to the spines of other non-bioluminescent squalid sharks), we propose that SAPs evolved as a means of highlighting the spines themselves.

Dorsal mineralized finspines, sometimes linked to venomous glands, are found in chimaeras and a number of sharks, while stingray barbs can be easily whipped into defensive position in the event of a threat, the dorsal anchoring of the finspines largely restricts their use to risky post-capture defensive events as a means of rendering the soft tissue in the mouth of a biting predator.

SAP "advertisement" of these spines—by luminescent strips mimicking spine shape and/or shining through the spines when the fins rotate—would be particularly advantageous, since it would allow the shark to signal to potential predators from a distance the danger of the stinging appendages, maybe preventing attacks before they happen. The high density of E. spinax individuals present in Norwegian fjords limit the time between encounters with this species, perhaps facilitating a predator's avoidance learning through a succession of unsuccessful predation events.

Bioluminescent aposematism has been experimentally demonstrated in some terrestrial invertebrates and suggested for a number of marine animals including cnidarians, molluscs, annelids, crustaceans, echinoderm and teleost fish; this, however, is the first suggestion of this behaviour in a cartilaginous fish.

Transmission of bioluminescent signals by mineralized structures, such as the spines of the velvet belly lanternshark, are rare in nature, having only been observed in several organisms including opisthopterid fishes and a marine snail (Hinea brasiliensis). Further examination of E. spinax's spines may provide insights into evolutionary co-opting of structural biomaterials for optic functions.

Although SAPs appear structurally and physiologically similar to the other photophores harbored by the velvet belly lanternshark, their dorsal glow represents a unique bioluminescent display. This "abnormal" luminescence might also be found in other lanternsharks (Etmopteridae), which harbor similar dorsal finspines and ventral photophores. Even though the function of these organs remains hypothetical, they clearly demonstrate that warning luminescence and counterillumination, two apparently contradictory strategies, could surprisingly operate in a single organism at the same time.

**Methods**

**Sharks.** Adult sharks were collected using longlines in the Raunefjord (Norway) between December 2009 and October 2012, transferred to Espeland Marine Station and housed in tanks placed in a dark cold room. Our protocol, including fish sacrifice, was in accordance with national guidelines for experimental fish care (fish handling approval #1664 was given by the National Committees for Research Ethics of Norway).

Photographs of spontaneous luminescence were taken in complete darkness with a Canon 7D camera (whole shark, sensitivity 6400 ISO, objective 20 mm; dorsal fins, 12800 ISO, 60 mm; both with an aperture of 2.8 and an exposure time of 30 s). For visibility purpose, a post adjustment of brightness and contrast was applied to the entire pictures using Adobe Photoshop. Other pictures were taken with the same camera in natural light.

**Luminescence recording.** A Berthold FB12 luminometer coupled to an optical fibre was used to record in vivo intensity of dorsal and ventral continuous luminescence from several live specimens according to Claes et al.. Values were corrected for fiber absorption and angular losses. Ventral photophore spacing/density and mean intensity of specimens was estimated according to Claes and Mallefet; by counting the photophores present in standard (0.25 cm²) ventral skin patches under a binocular microscope.

**Spine structure.** Dorsal finspines were removed from dead specimens wrapped in plastic wrap with a small amount of fluid for hydration, and packed tightly with paper towels into plastic tubes for micro-CT scanning (1174 scanner, SkyScan®, Kontich, Belgium). The X-ray source was set at 50 kVp (peak kilovoltage) and 800 μA; 220 projections were acquired over an angular range of 360 degrees. Samples were scanned with an isotropic voxel size of 16.8 μm, an integration time of 4500 ms, and a 0.25 mm aluminum filter to decrease beam-hardening effects. Scans were reconstructed using commercial software (NRecon® SkyScan® software, version 1.6.1.2) then visualized in 3D and segmented virtually in multiple planes using Amira software (Mercury Computer Systems) to examine spine structure and morphology.

**Histology.** Skin tissues containing SAPs from dorsal fins of freshly dead specimens were excised and fixed in a 3.5% formaldehyde solution for two weeks. Tissues were progressively dehydrated (50, 70, 2 × 90%, 1 hour each), placed 1 h in 100% butanol and left overnight in 100% butanol at 60°C. Tissues were then submerged in paraffin wax for different periods (12 h, 1 h and 3 h) at melting temperature (58°C). Sections were performed with a metal knife microtome, stained using Masson’s trichrome, and photographed under a light/fluorescence microscope (Leitz Diaplan, Leitz, Wetzlar, Germany).

**Spine transmission.** The second dorsal finspines of freshly dead specimens were removed and their anterior-posterior spectral transmittance was measured near the tip, at 75% of the exterior spine length, using an Ocean Optics S2000 spectroradiometer. Broadband white light from an Ocean Optics PX-2 pulsed xenon lamp was delivered to the caudal surface of the spine via an optical fibre terminated with a quartz lens. Light transmitted anteriorly by the spine was collected via a second quartz lens coupled to an optical fibre connected to the spectroradiometer.

**Visual modeling.** The visibility ranges of both ventral and dorsal glows were calculated according to the theory developed by Nilsson et al.. This range depends on the intensity of down-welling daylight and thus on depth in the sea. Etmopterus spinax was assumed to occur at ‘counterillumination depth’ where its silhouette, ...
concealed by ventral photophores, is not detectable from below8. This depth was determined using the ventral photophore spacing (0.234 mm) and mean intensity (2.1 \pm 10^9 \text{ photons s}^{-1}) of the brightest shark in our dataset as inputs in the equation 7 in Supplemental Information from Nilsson et al.22. We adapted Nilsson et al.’s theory, originally developed for clear oceanic waters, by setting the beam attenuation and back-scattering coefficients to 0.3 m \text{ m}^{-1} and 0.0385 m \text{ m}^{-1}, respectively, to agree with the turbid green waters of the fjords. The visibility range of SAP luminescence, modeled as a point source seen against a black background (the dorsal part of the shark’s body), was then calculated at this counterillumination depth. The SAP luminescence intensity of the brightest shark (6.87 \text{ photons s}^{-1}) was then calculated at this counterillumination depth. The SAP luminescence intensity of the brightest shark in our dataset as inputs in the equation 7

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Author contributions
J.M.C. and J.M. collected experimental animals, took photographs and recorded in vivo luminescence measurements. J.M.C. performed the histological sections of the dorsal fins. The micro-CT scans were performed by M.N.D. while N.S.H. made the finspiral spectral transmittance measurements. D.E.N. conducted the visual modeling. J.M.C. and M.N.D. wrote the main manuscript. J.M.C. prepared the figures. All authors reviewed the manuscript.

Additional information
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