Study of the luminescence in the black brittle-star *Ophiocomina nigra*: toward a new pattern of light emission in ophiuroids*

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Abstract

The black brittle star *Ophiocomina nigra*, common in the English Channel, is known to produce mucus when attacked. This mucus, already known for its antifouling capabilities and its role in the feeding and the locomotion behaviours of the brittle star, also emits weak light. We describe and characterize this emission of bioluminescence, thanks to a chemical triggering by hydrogen peroxide. It appears that the light emitted is 1000 times less intense than the light emitted by other brittle star species (*Ophiopsila aranea* and *Amphipholis squamata*). The luminous capabilities are homogeneously spread along the arms of the brittle star, what goes against the use of bioluminescence as a sacrificial lure. The mechanical stimulation of arms before chemical triggering strongly enhances the luminous capabilities of the brittle star. Luminous mucus emission can be associated with other defensive function, such as a smoke screen effect or a burglar alarm, but these two functions require intense light emissions. The fact that the luminous component is excreted outside the body might be in favour of the aposematic use of light, *i.e.*, as a signal to warn predators of the toxicity or unpalatability of its prey.

Key words: Bioluminescence, ophiuroid, *Ophiocomina nigra*, ethology, aposematism

Introduction

Bioluminescence, *i.e.*, the emission of light by living organisms, is widespread in the marine environment (for a review see Haddock 2010). Actually, luminous organisms exist in at least 13 phyla, from bacteria to fishes, encompassing cnidarians, annelids, molluscs or echinoderms. The abundance of luminous organisms and the diversity of light emissions patterns let suppose that this capacity has originated at least 40 times independently in the course of evolution (Hasting 1983), and confirms the important evolutionary advantage to posses this ability.

Patterns of light emission can be described in terms of duration or spectral characteristics. Emissions durations are commonly classified in three categories: bright short flashes, during less than 2 seconds, slow glows, during more than 5 seconds, and permanent emission (Morin 1983). Concerning the spectral characteristics, most of luminous marine organisms emit blue-green light, with peak of maximal intensity around 450–490 nm, which spreads well in the sea water whose spectral absorbance is around 460 nm. Benthic and coastal organisms tend to emit at higher wavelength, around 510 nm, in a greener coloration, probably due to the waters turbidity in these habitats (Herring 1983).

With 66 luminous species known the Ophiuroidea are the largest class of luminous echinoderms. It represents 55 % of all luminous echinoderms, but this high proportion is also due to recent research activity in this class. Ophiuroids are commonly green-emitters, although four blue emitting species are also known, namely 2 new species discovered during a recent deep-sea cruise. Luminous ophiuroids can be found in every type of biotope (soft of rocky bottoms), and occur from the intertidal zone down to deep-sea, mostly in temperate and sub-tropical waters. Until now, all brittle stars are known to emit short and intense flashes after disturbance (for a review see Mallefet 2009) associated with a defensive function (Herring 1995), *e.g.*, to deter a predator (Grober 1988a), lure it (Deheyn *et al.* 2000) or even warn it against toxicity like brightly coloured insects (Grober 1988b; Jones & Mallefet 2010).

In this study, we will explore a completely new luminous pattern in ophiuroids, the one of the black brittle star, *Ophiocomina nigra*, known to emit a mucus of weak luminous intensities when attacked (Mallefet, pers. obs.). *O. nigra* is a common brittle star in the English Channel. Its distribution stretch throughout the North-west Atlantic coasts from Norway to the Azores, and the Mediterranean Sea. It is a medium-sized ophiuroid, with a central disc measuring about 25 mm of diameter, and arms up to 5 times this size. Its typical coloration is the dark brown or black, but white individuals occur in every population. *O. nigra* lives in dense beds (more than 100 individuals per square meter), on shallow rocky bottoms, with a low water flow. This species lives often associated with *O. fragilis* (Mortensen 1927; Moyse & Tyler 1990; Picton 1993; Stöhr & O'Hara 2007). The mucus, produced at the arm level when the brittle star is attacked, is known for its anti-fooling properties (Ball & Jangoux 1990, 1996). It could also play a role in the locomotion and feeding behaviour of the species. In this study, we describe the luminous capabilities of *O. nigra*, with a view to highlighting the function of bioluminescence in this species.

Material and Methods

Sampling. Individuals of the brittle star species studied, *O. nigra,* were collected in Roscoff (Brittany, France), by scuba divers, in 2009. Back in the lab, brittle stars were maintained in a 400 litres artificial sea water closed circuit aquarium. The room temperature was maintained to 14°C, and brittle stars were kept under a 12:12 day-night cycle. Animals were fed once a week with *Artemia* and krill.

Luminous pattern. The emission of light of *O. nigra* was recorded without stimulation, and then induced by a chemical stimulation with hydrogen peroxide at different concentrations (0.05–0.1 M at final concentrations). Whole arms, discs, and portions of arms were stimulated in a luminometer, which allows recording all the kinetic parameters of the luminous response: the maximal intensity of light emitted (Lmax), the total amount of light emitted (Ltot), the time at the maximal intensity (T_{Lmax}), and the latency between the hydrogen peroxide injection and the beginning of the luminous response (TL).

Before the sampling of arms, brittle stars were immersed into a 3.5 % $MgCl_2$ anaesthetic solution. Six non-anaesthetised arms were also stimulated at each peroxide concentration, to control a possible effect of the anaesthesia on the luminous capabilities of the brittle star.

The presence of a proximal-distal arm gradient in luminous capabilities of the brittle star was also investigated. Some species are known to emit more light at the distal part of their arms (*e.g.*, *Amphi-*
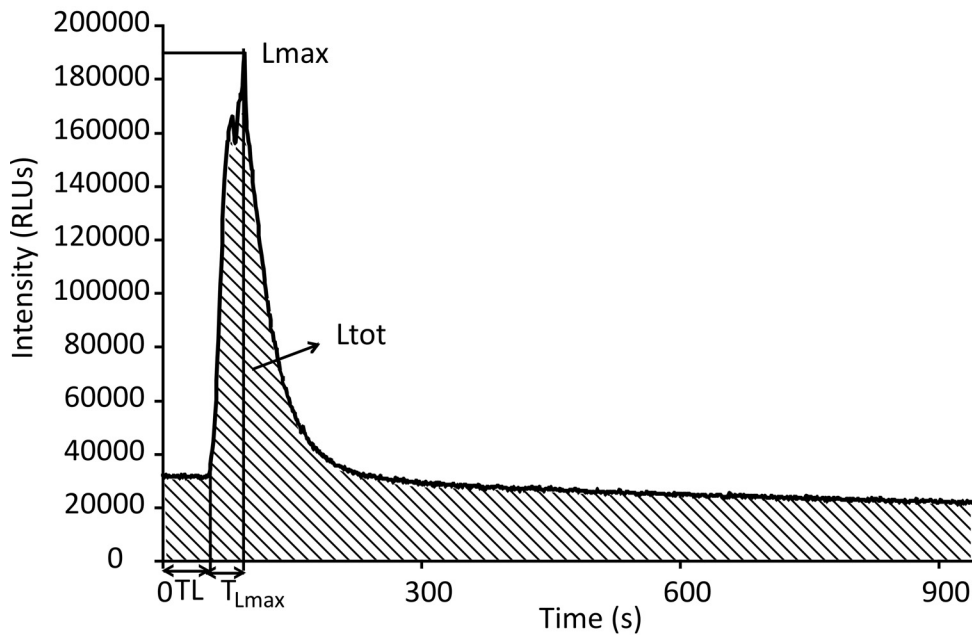
FIGURE 1. Temporal record of the light emitted during stimulation of an anaesthetised arm of *O. nigra* with hydrogen peroxide at 0.1M. (Lmax=maximal intensity of light emitted, Ltot=total amount of light emitted, T_{Lmax}=time between the beginning of the response and the peak of maximal intensity, TL=time between the injection of hydrogen peroxide and the beginning of the response).

pholis squamata, Deheyn *et al.* 2000). Arms of *O. nigra* were cut in three parts of the same size: the proximal, median and distal parts and the luminous capabilities of these parts were recorded.

Finally, since we know that the light is emitted in mucus released when the brittle star is disturbed, we recorded the luminous response of previously mechanically stressed arms. Arms were mechanically stressed during 5 minutes using small forceps, supposed to mimic a crab claw, in order to enhance the release of mucus before the chemical stimulation.

Results

Figure 1 represents the temporal record of the light emitted during an anaesthetised arms triggering with hydrogen peroxide at 0.1 M. Maximal intensity (Lmax) is around 18000 RLUs (Relative Light Units). Six replicates were conducted, and mean maximal intensity of light (Lmax) reaches 126408 ± 33469 RLUs (Relative Light Units), 14.8 ± 0.68 seconds after the stimulation (= time of latency, TL), 3.8 ± 0.54 seconds (T_{Lmax}) after the beginning of the response. Since each luminometer possess its own sensitivity, results have to be transformed in standard units for comparison. After calibration with a radioactive source, we obtain results in Megaquantas . In this case, Mean Lmax reaches the intensity of 5.74 ± 1.52 Mq/s, while Ltot (the total amount of light emitted) reaches 206.04 ± 59.19 Mq. Finally, these results were standardized by the weight of tissue (Fig. 2). The mean weight of a brittle star used in this work is 256.12 ± 22.07 mg (n=36).

Results for non-chemically stimulated arms, on Fig. 2A, show that the total amount of light emitted, as well as the maximum intensity of light emitted are significantly lower without chemical stimulation compared to the results obtained with a hydrogen peroxide stimulation. ($F_{1,29}=12.6543$, p=0.0042). Maximal intensity of light emitted reaches 0.0015 ± 0.0001 Mq.s^{-1}.mg^{-1} while total amount of light
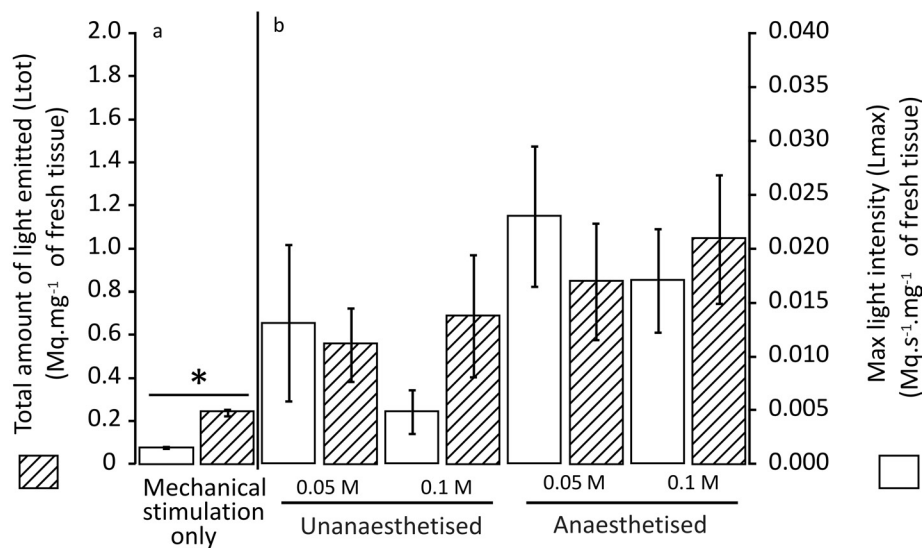
FIGURE 2. A: Total amount of light emitted and maximal light intensity reached for non chemically stimulated *O. nigra* arms (n=6). Both values are significantly lower than values obtained with chemically stimulated arms (Fig. 2B) (* P < 0,05). B: Chemical induction of a luminous response on arm of *Ophiocomina nigra* (n=6 for each treatment): Effect of the hydrogen peroxide concentration and of the anesthesia. (* P < 0,05). No significant differences appear between anaesthetised and non-anaesthetised brittle stars. Error bars represent SE.

emitted reaches 0.2398 ± 0.0152 Mq.mg^{-1}.

Looking at Fig. 2B, we can see that the anaesthesia does not significantly affect the amount of light emitted ($F_{1,11} = 1.070$, p=0.3143), neither the maximal intensity of light emitted ($F_{1,11} = 0.1894$, p=0.6657) (Fig. 2), which convinced us to always use the anaesthesia for the following experiments. Moreover, the response does not differ using different hydrogen peroxide concentrations, neither for the total amount of light emitted ($F_{1,11} = 0.2501$, p =0.6204) nor for the maximal intensity ($F_{1,11} = 2.95$, p=0.1012) (Fig. 2).

The luminous capabilities are evenly distributed along the arms of the brittle star. The amount of light was standardized by the weight of the arm parts since proximal, medium and distal parts of the arms differ in thickness and thus in weight. Mean weight for proximal, medium and distal part of the arm are respectively 141.16 ± 11.72, 77.86 ± 10.71, 30.93 ± 10.64. Standardized amount of light emitted does not significantly differ among the proximal–median or distal part ($F_{2,5} = 0.030$, p=0.9704) (Fig. 3).

Finally experiments on mechanically stressed arms show that they emit significantly more light than non-stimulated ones ($F_{1,23} = 15.2623$, p=0.0008) and reach significantly higher maximal intensities ($F_{1,23} = 9.65$, p=0.0051) (Fig. 4).

Discussion

Characterization of the luminous capabilities indicates that *O. nigra* emits a weak light compared to other brittle star species. This light is in average 1000 times less intense than in other species (Pilet 2006). The light emitted by *O. nigra* is weak, far from intense flashes classically described as anti-predatory defences (Morin 1983).

The luminous capabilities are evenly distributed along the arm suggesting that the function of a sacrificial lure is unlikely in this case. This function presupposes that the brittle star autotomize the
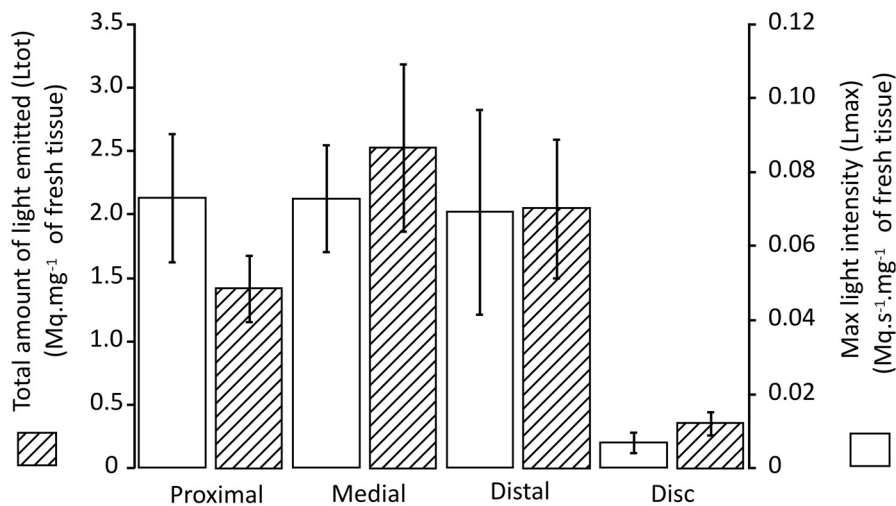
FIGURE 3. Comparison of the luminous capabilities between proximal, median and distal part of the arm (n=6). Error bars represent SE.

luminous distal part of its arm when attacked, to distract its predator (Morin 1983; Deheyn *et al.* 2000). To be effective, the distal part of the arm would need to be brighter than the rest of the prey.

Despite the weak intensity, the luminous capabilities of the species rise when the brittle star is mechanically stressed. The light is emitted by the mucus, released when the brittle star is attacked.

Mucus emission of *O. nigra* is known for more than 50 years now. Mucus analyses have shown antifouling (Bavington 2004) and anticoagulant capabilities (Fontaine 1964). Moreover, mucus seems to play an important role in locomotion (Smith 1937; Fontaine 1964; Ball & Jangoux 1990) and feeding (Fontaine 1964, 1965). Mucus adhesives properties would explain the great capacities of *O. nigra* climbing vertical wall and staying fixed on it. It has also been suggested that mucus in *O. nigra* could act as a web to catch prey (Fontaine 1964, 1965).

The mechanical triggering of mucus release also speaks in favour of an anti-predation function (Fontaine 1964). Moreover, mucus acidity (pH≈1) encourage this hypothesis since it can thus act as a chemical repellent, especially against fishes. Mucus viscosity would, on the other hand, disrupt predation by slow predators such as Asteroidea (Fontaine 1964). In this context, bioluminescence of *O. nigra* mucus would complete this anti-predatory system.

Luminous mucus release is often associated as defensive functions: in particular with the "smoke screen" effect, the "burglar alarm" effect or the aposematism (Morin 1983; Young 1983; Haddock 2010). Mucous clouds are emitted to blind a predator in the first case (Morin 1983; Widder 2001; Haddock *et al.* 2010) or to attract a secondary predator in the second one (Robison 1992; Robison *et al.* 2003). Luminous mucus is known in many ctenophores (Hamner *et al.* 1975; Harbison *et al.* 1978; Harbison & Miller 1986), in crustacean decapods such as Oplophoridae, Pandalidae, Thalasso-carididae, and in the Penaeidae (Herring 1985), as well as in the freshwater molluscs *Latia neritoides* (Bowden 1950).

However, to be effective, these functions need a strong intensity of the light emitted, which is not the case with *Ophiocomina nigra*. The aposematic use of bioluminescence, *i.e.*, as a signal to warn predators of the toxicity or unpalatability of its prey, stays the most probable function. This function has already been highlighted in Lampyridae larvae (De Cock & Matthysen 2001, 2003) and in two brittle star species, *Ophiopsila riseii* (Grober 1988b)—however this study is still the subject of controversy (Guilford & Cuthill 1989; Grober 1989)—and *Ophiopsila aranea* (Jones & Mallefet 2010).
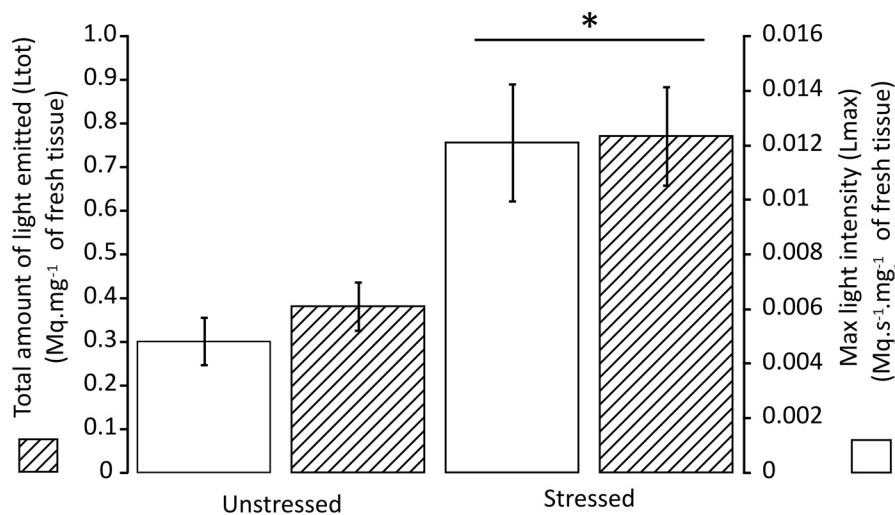
FIGURE 4. Effect of a mechanical stress on the luminous capabilities of the brittle star *Ophiocomina nigra* (n=6 for each treatment). (** P < 0,01). Error bars represent SE.

Both *Ophiopsila* species emit green intense flashes, while the firefly larvae emits slow glows, a pattern closer to this of *Ophiocomina nigra*.

It is also possible that light in the case of *O. nigra* could help to attract prey. Weak light in the marine environment is known to attract prey as decapods (Nicol 1959). Many deep sea fishes also use luminous appendices to attract prey in the dark (Nicol 1967).

In the future, it will be primordial to provide evidence on predators or prey sensitivity to *O. nigra* luminous signal before tackling the question of the precise function of its bioluminescence.

Acknowledgments

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