

Aposematic use of bioluminescence in *Ophiopsila aranea* (Ophiuroidea, Echinodermata)

Jones A. & Mallefet J. (Marine Biology laboratory, UCL, Belgium) Université catholique de Louvain, Marine Biology Laboratory, Bâtiment Kellner, Place croix du Sud, 3, Louvain-la-Neuve, 1348, Belgium.

Bioluminescence i.e. the production of light by living organisms occurs in many ecosystems all around the world, mostly in the marine environment. It concerns at least 13 phyla, from bacteria to fishes, and has three main functions: the defense against predators, the help for predation, and the intraspecific communication^[1]. The abundance of luminous species in the class of ophiuroids (66 luminous species known on 175 tested and on a total of 2278 species described^[2]) suggests that luminescence should play an important ecological role for these organisms. In this study, we tested if light emission in *Ophiopsila aranea*, a common ophiuroid found in the Mediterranean is used as an aposematic display, thus to prevent potential predators that their prey is unprofitable. Aposematic function of bioluminescence has already been suggested in *Ophiopsila risei*^[3] but this study stirred up controversy because it seemed that conditions for maintaining and evolution of aposematic display were not fulfilled^[4]. Consequently, we tested the four conditions required^[5] and it appeared that in *O. aranea* three out of four are fulfilled. (i) Palatability tests show that the brittle star is unprofitable for two crustacean predators (the Shore crab *Carcinus maenas* and the Hairy crab *Pilumnus hirtellus*). (ii) The brittle star suffers a lower predatory rate than another cryptic brittle star species (*Ophiura ophiura*). Moreover, the bioluminescent species was preyed during the day (when bioluminescence was not visible for predators) as much as the night, while *Ophiura ophiura* was mostly preyed at night, which is the common behaviour of nocturnal predators such as crabs (Fig. 1). (iii) Finally, the luminescence allows the avoidance learning of the predator. Indeed, the handling time of the brittle star by predators decreased significantly during successive putting in touch trials (Fig. 2). On the contrary, the handling time remained constant during experiments with the non bioluminescent species and with block of agar mixed with *O. aranea* (to test the effect of the brittle star's taste without

bioluminescence) and a control block of agar mixed with fish. Our hypothesis is reinforced by behavioural observations indicating that predatory behaviours occurred significantly more during the first trial. Only the fourth condition i.e. predators should show an initial reluctance to attack aposematic prey, was not verified with *O. aranea*. It can be easily explained by the fact that *O. aranea* emits light only after disturbance, and remains cryptic the rest of the time. Predators cannot therefore see the aposematic display before attacking the prey. This kind of facultative aposematism could be more advantageous than classic aposematic displays because preys do not suffer the costs of a higher probability of detection, which is a well-know problem in the explanation of the evolution of aposematic signals^[6]. Our results clearly support the aposematic use of bioluminescence in *O. aranea*.

References

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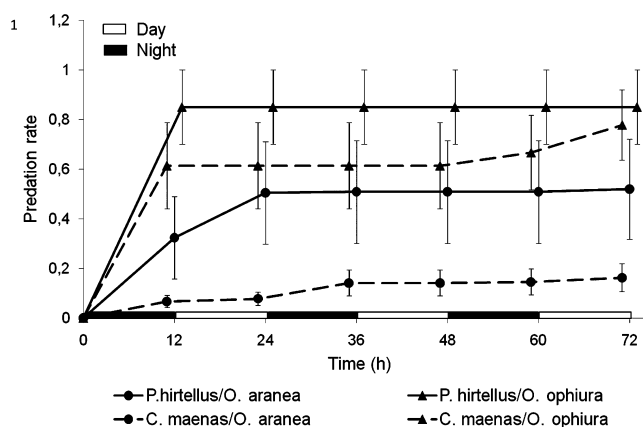


Figure 1. Predation rates for each crab and ophiuroid species during the 72 hours long experiment ($n = 6$). Day and Night periods are indicated in the horizontal bar under the chart.

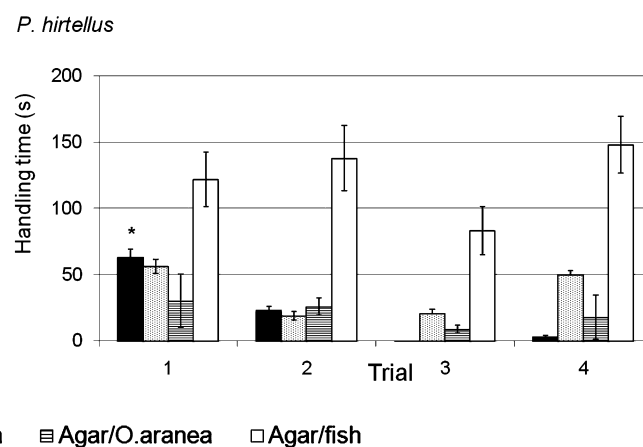
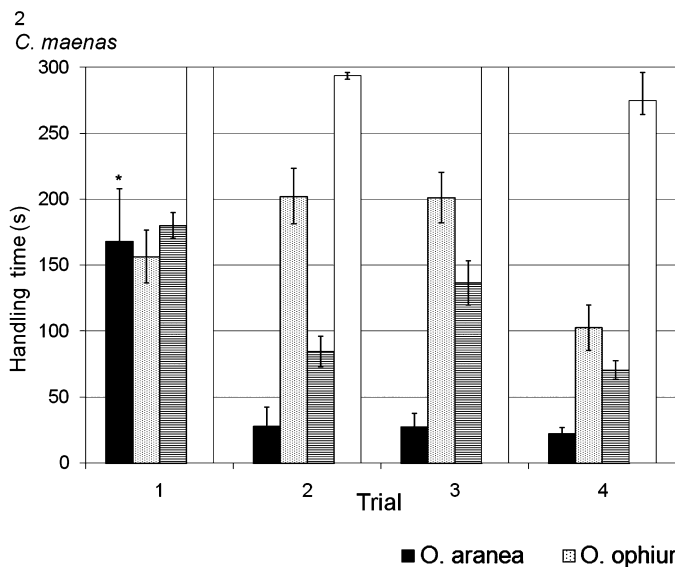


Figure 2. Handling times by crabs during successive putting in touch trials of 30 minutes ($n = 6$). * p -val < 0.05.

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First description of the luminous system of the velvet belly lantern shark *Etmopterus spinax* (Chondrichthyes: Etmopteridae)

Renwart M. & Mallefet J. (Marine Biology Laboratory, UCL, Belgium)

Catholic University of Louvain (UCL), 3, Place Croix du Sud, bt 4, Louvain-la-Neuve, 1348, Belgium

In the deep-sea, bioluminescence is more present in bony fishes (70%) than in cartilaginous fishes (6%)^[1]. From literature, it is known that among sharks, many deep-sea species are able to produce light. Nevertheless, until recently, available data were anecdotic or mainly limited to morphological information. Recently a multidisciplinary research program (morphological, physiological, behavioural) was focused on the velvet belly lantern shark, *Etmopterus spinax*^[2,3]. The aim of the present work was to document the nature of the luminous reaction involved in *E. spinax* luminescence.

Juveniles, adults and embryos of *E. spinax* were captured by long line lowered on the bottom of a fjord near Bergen (Norway).

Various tissues were collected and stored at -80°C before assays for luminous compounds.

Free coelenterazine and a specific luciferase-like activity have been detected in most tissues of the shark. In embryos, coelenterazine is present in the yolk sac. During embryogenesis, the yolk sac decreases in size and the coelenterazine is progressively absorbed by the growing embryo. Nevertheless, no increase of the luciferin concentration has been observed in the tissues of the embryos. At the end of the development, specific luciferase-like activity was detected in the embryo, indicating that they could be able to produce light before birth. This is in concordance with Claes & Mallefet (2) who observed a luminous embryo. In free-swimming specimens, coelenterazine has been found in all tissues tested, with the higher response in the photophores of new-borns (10–20 cm total length) (Fig 1). The presence of the luciferin in the digestive tract of the shark suggests an alimentary acquisition of this compound as it has been suggested for other fishes^[4]. Luciferase-like activity has been detected in the different tissues with the higher response in the photophores of mature sharks (>30 cm TL) (Fig 2). Besides the somatic tissues, tests have been carried out on the gonads, for males and females independently. While coelenterazine concentration doesn't change in males, mature females show a decrease of luciferin, reaching a lower concentration than mature males. This decrease could reveal a maternal transfer of coelenterazine to embryo via the yolk sac. A similarly hypothesis was already suggested for teleost fishes^[5]. Our results provide the first information on the luminous system of a shark: the presence of a luciferin/luciferase-like reaction, coelenterazine being the luciferin. Two mechanisms for coelenterazine acquisition are suggested (maternal transfer

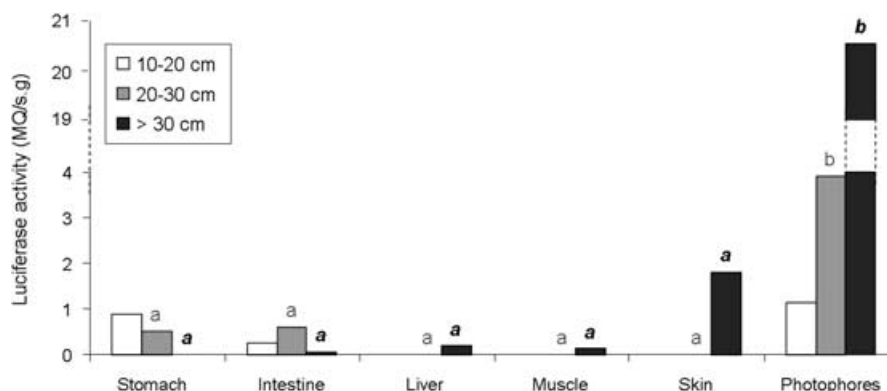


Figure 1. Coelenterazine concentration in the tissues, for each age classes: new-borns (10–20 cm TL); juveniles (20–30 cm TL) and matures (>30 cm TL).

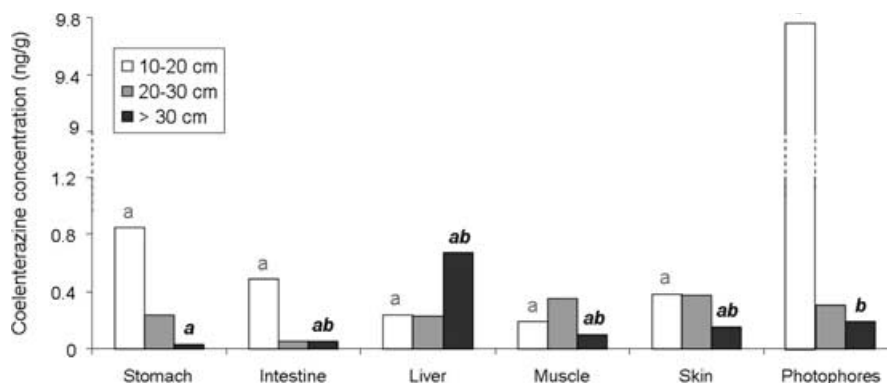


Figure 2. Luciferase activity in the tissues, for each age classes: new-borns (10–20 cm TL); juveniles (20–30 cm TL) and matures (>30 cm TL).

The reproductive strategies of Ophiuroidea differ widely and some lack a clear reproductive periodicity in temperate and tropical shallow waters (Falkner et al., 2013; Hendler, 1991; Strathmann & Rumrill, 1987). It is somewhat difficult to isolate gametes from brittle stars and there is not a common protocol that works across species. See chapter "Temnopleurus as an emerging echinoderm model" by Yaguchi for detailed methods on culturing *A. filiformis*, a species gaining popularity as a model. Ophiuroids can reproduce asexually by a process called fissiparity (splitting across the plane of the disc; c.f. Mladenov & Emson, 1984; Mladenov, Emson, Colpit, & Wilkie, 1983), but most species reproduce sexually. Although most species are dioecious, some are hermaphrodites. Several further genera with disputed taxonomic placement, e.g. *Amphylimna*, *Ophiopsila*, *Ophiolimna*, *Ophioconis*, were studied especially and their revised placement is proposed. The following genera are excluded from the family Ophiacanthidae: *Amphylimna*, *Ophiocymbium*, *Opiodaces*, *Ophiodelos*, *Ophiologimus*, *Ophiophrura*, *Ophioprium* and *Ophiosparte*. Oegophiurid groove spines are suggested to be homologous with the tentacle scales of the remaining Ophiuroidea. It is suggested that the family Ophiomyxidae thus may be related to some crown Oegophiurida that had already acquired fused vertebrae. The higher ophiuroid taxonomy, based on the genital plate patterns, is critically analyzed in the light of the present data. Aposematic use of bioluminescence has already been demonstrated for firefly larvae (De Cock & Matthysen, 2001 & 2003, for millipedes (Marek et al., 2011) and in the brittle star species *Ophiopsila aranea* (Jones & Mallefet, 2010) and *Ophiopsila risei* (Atken, 1859 (Grober, 1988), however controversy remains for this study (Guilford & Cuthill, 1989; Grober, 1989). The two brittle star species emit green intense flashes, a very different luminous pattern than *O. nigra*, but the Lampyridae larvae and millipedes (Marek et al., 2011) emit slow glowing light, which gets closer to the patt