Cah. Biol. Mar. (2013) 54 : 729-734

Why do brittle stars emit light? Behavioural and evolutionary approaches of bioluminescence

Alice JONES and Jérôme MALLEFET
Marine biology laboratory, Catholic University of Louvain, 3 Place Croix du Sud, Box L7.06.04,
B-1348 Louvain-la-Neuve, Belgium. Fax: + 32 (0)10 47 34 76, E-mail: alice.jones@uclouvain.be

Abstract: In this study, we investigated the functions of bioluminescence (the production of light by living organisms) in five brittle star species. Bioluminescence is a widespread phenomenon in the marine environment, and is especially abundant in the class Ophiuroidea. It is assumed that light in marine invertebrates mainly plays a role of defense against predation, and many mechanisms of defense have been proposed for brittle stars. We investigated the potential functions of startle effect (use of light to deter predator), use of light to advertise a predator that the prey is toxic (aposematic signal) and attraction of a secondary predator (function usually called "burglar-alarm effect"). Predatory experiments, involving one or two predators from different trophic levels allowed us determining benefits of the light emission for several brittle star species. We clearly demonstrated that the three functions cited behind are used in brittle stars. It is clear now that brittle stars use a wide variety of defensive mechanisms involving light.

Résumé : *Pourquoi les ophiures émettent-elles de la lumière ? Approches comportementales et évolutionnistes de la bioluminescence.* Cette étude porte sur les fonctions de la bioluminescence (la production de lumière par des organismes vivants) chez cinq espèces d'ophiures. La bioluminescence est un phénomène courant dans le milieu marin, et il est particulièrement abondant dans la classe des ophiuridés. Il est admis que la bioluminescence joue un rôle de défense contre la prédation chez les invertébrés benthiques. Plusieurs mécanismes de défense ont été suggérés pour les ophiures. Nous avons investigués les fonctions potentielles de "startle effect" (répulsion du prédateur par la lumière), d'utilisation de la lumière pour prévenir le prédateur que sa proie est toxique (signal aposématique) et d'attraction d'un prédateur secondaire (fonction communément appelée "burglar-alarm") ont été testées. Des expériences de prédation, faisant intervenir des prédateurs d'un ou deux niveaux trophiques, nous ont permis de situer les bénéfices associés à l'émission de lumière pour plusieurs espèces d'ophiures. Les trois fonctions citées plus haut ont pu être clairement démontrées chez les ophiures. Il est maintenant clair que les ophiures utilisent une grande variété de mécanismes de défense impliquant la lumière.

Keywords: Bioluminescence • Ophiuroid • Aposematism • Burglar-alarm • Ethology

Introduction

Bioluminescence, the emission of visible light by living organisms (for a review see Haddock et al., 2010), is a common feature in echinoderms, especially in brittle stars. In this class, at least 77 luminous species are known (Mallefet, 2012), of 222 species tested, with a total of 2,128 species and subspecies described (Stöhr & O'Hara, 2012). This abundance of luminous species, discovered recently (Mallefet, 2012) suggests that light production has important functions in these organisms. Functions of bioluminescence can be divided in three categories: intraspecific communication, help with predation, and defense against predation. Studies suggest that the function of bioluminescence in brittle stars involve defense against predation (Herring, 1995), such as startle effect (deterrence of predators by intense flashes), sacrificial lure (autotomy of a luminous distal part of arms when attacked), aposematism (use of a conspicuous signal to advertise predators that the prey is toxic or unpalatable), or burglar alarm effect (use of light to attract secondary predator).

We investigated the bioluminescence functions in five luminous ophiuroids with prey-predator experiments: *Amphiura arcystata* (H.L. Clark, 1911), *Amphipholis squamata* (Delle Chiaje, 1828), *Ophiocomina nigra* (Abildgaard, in O.F. Müller, 1789), *Ophiopsila aranea* (Forbes, 1843) and *Ophiopsila californica* (A.H. Clark, 1921). These species differ in their luminous pattern and colour. While *O. aranea* and *O. californica* are known to emit intense green flashes, the luminous signal of *A. squamata* is far lower. *Ophiocomina nigra* emits very weak luminous mucus when disturbed (Fontaine, 1964). The last species, *A. arcystata,* emits a blue light. Finally, a sixth species, *Ophiothrix fragilis* (Abildgaard, in O.F. Müller, 1789) was used as non bioluminescent control.

As predator, we chose the crab *Carcinus maenas* (Linnaeus, 1758). This species is common in both the North Sea and in the Mediterranean Sea. Moreover, this species was interesting because it is known to prey on echinoderms (Muntz et al., 1965). Crabs were used sighted or blinded, to compare behaviour between crabs able or unable to see bioluminescence. A secondary predator, the fish *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817), common in the Mediterranean Sea, was used for burglar alarm experiments, because it preys on crabs (pers. Obs.).

Many experiments are conceivable to asses if bioluminescence is involved in defensive functions in ophiuroids. If light is used to deter predator, bioluminescent prey should suffer lower predation than non bioluminescent ones when bioluminescence is visible, meaning during the night and with sighted crabs. If light is used to advertise predators that the ophiuroid is toxic or unpalatable (aposematic signal), it means that, on one hand, ophiuroids are unpalatable and, on the other hand, predator are able to associated the ophiuroid unpalatability with its luminous signal. Finally, if light is used to attract a secondary predator, it means that the primary predator (the crab) should be more predated by the secondary predator (the fish) when a luminous ophiuroid is present. It means also that the ophiuroid should be less predated when the fish is present.

Our experiments on ophiuroids can then be listed as a set of questions:

- Does ophiuroid bioluminescence deter predators?
- Does ophiuroid bioluminescence advertise predators?
  (i) Are ophiuroids unpalatable?
  (ii) Are predators able to associate unpalatability and luminous signal?
- Does ophiuroid bioluminescence attract a secondary predator?

Material and Methods

Sampling

Brittle stars were collected between 2007 and 2012 in France and in California. *Ophiopsila aranea* was collected at ARAGO marine station at Banyuls-sur-mer (France) using SCUBA, and *O. nigra* and *A. squamata* were collected in North France, Wimereux, by trawl and directly on the shore, respectively. The non-bioluminescent control species *O. fragilis* was also collected in Wimereux by trawl. *Ophiopsila californica* and *A. arcystata* were collected in Santa Barbara campus, University of California, by SCUBA. Predators, crabs *Carcinus maenas* and the fish *Diplodus vulgaris* were collected in Wimereux and Banyuls, respectively.

Does ophiuroid bioluminescence deter predators?

To determine the potential deterrent effect of light for predators, experiments were run on the five luminous brittle star species. Crabs *C. maenas* were used as predators. For each test a brittle star and a crab were put in contact in an aquarium of 60 l, with a stone in the center to provide shelter. Each experiment lasts 12 hours, and was repeated six times during the day, and six times during the night with each species. The ophiuroids were weighed before and after to estimate the amount of tissue lost during the experiment. This index (weight of brittle star eaten) was standardized by the weight of the predator and called "predation rate". Experiments were repeated during a day/night cycle, to highlight a possible influence of the bioluminescence on the predatory behaviour of the crabs in the dark, when it is the most clearly seen. We also ran this experiment with blinded crabs. Experiments were repeated with blinded crabs six times during the day and six times during the night. Crabs were blinded by painting the distal part of the eyestalk black, with nail polish, as used in other studies (for example see Duffy et al., 2006).

Does ophiuroid bioluminescence advertise predators?

(i) Are ophiuroids unpalatable?

Aposematism is the use of light or bright colors to warn a predator that the prey is toxic or unpalatable. Since association between a conspicuous signal and unpalatability is required for this to work, we investigated the palatability of the brittle star species. Tissues (arms) of the ophiuroids were mixed with agar to form blocks. Blocks of agar mixed with fish pieces were also made, to verify that agar itself has no deterrent effect since fish is the common crab diet in the laboratory. Each block was provided to a crab for 30 min, in a small aquarium of 10 l. Each block was weighed before and after the experiment, in order to evaluate the amount of agar eaten. This index, standardized by the weight of the predator, was called consumption rate. The handling time of the block by the crab, and the consumption rate, were recorded as indicators of palatability (the longer and the more the block is handled and consumed, the more palatable is the species).

Does ophiuroid bioluminescence advertise predators?

(ii) Are predators able to associate unpalatability and luminous signal?

To study aposematism, we used the species *O. nigra* and the species *O. fragilis* as non bioluminescent control. Crabs were placed in contact repeatedly with the ophiuroid, to see if the crab would avoid the ophiuroid after the emission of light, which would suggest aposematic use of the bioluminescence. One brittle star and one crab were put on a small aquarium (10 l) during four successive trials of 30 minutes. Between two trials, the brittle star was removed for 20 minutes. The consumption of brittle star was recorded for each trial. Experiments were run six times during the day and six times during the night with both ophiuroid species, to distinguish the effect of the bioluminescence. Indeed, bioluminescence is visible for the crabs only at night. Experiments were also run during the day to verify that no other deterrent mechanisms were involved in the avoidance learning.

Does ophiuroid bioluminescence attract a secondary predator?

Finally, we studied burglar alarm effect on *O. aranea* (using *O. fragilis* as non-bioluminescent control species). We put in contact brittle stars and predators from two different trophic levels (crabs *C. maenas* as primary predator, and fish *D. vulgaris* as secondary one). Different prey-predators combinations were tested to assess the predation on brittle stars by crabs, fishes and both predators together. Predation rate on crabs by the fishes, in the presence or absence of luminous brittle stars, was also tested. Predatory experiments took place in a 100 l aquarium, during 12 hours. Each prey-predator combination was repeatedly tested six times during the day, and six times during the night.

Statistical analysis

All analyses were performed in JMP 8 or 10, using analysis of variance and multiple mean comparison test (HSD Tukey). Significance was always tested with the probability of 0.05 ($P < 0.05$).

Results

Does ophiuroid bioluminescence deter predators?

Figure 1 illustrates the general effect of bioluminescence as a way of defense against predation. Comparisons between luminous and non-luminous species, as well as blinded and sighted crabs allow us to estimate the efficiency of light as a defense against predation on a day/night cycle. We observed that with sighted crabs, the predation rate is always significantly higher during the day than during the night, with luminous brittle star species. With the non-luminous species, we observed the opposite result, although without significant differences. Predation rates significantly increase with the blind crabs during the night, which indicates that vision of bioluminescence is efficient to reduce predation.

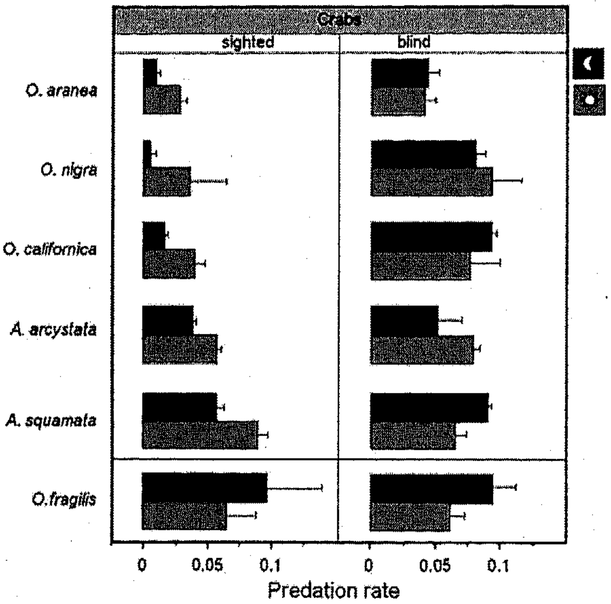

Figure 1. Predation rates for blind or sighted crabs, during day and night. HSD Tuckey, different letters indicate strongly different means, n = 6 for each combination.

Does ophiuroid bioluminescence advertise predators?

(i) Are ophiuroids unpalatable?

Figure 2 shows the consumption rate of agar blocks made with different brittle star species. Consumption significantly differs according to the species ($F_{1,47} = 20.195$, $p < 0.001$). Lower consumption rate are observed in four species: *O. aranea*, *O. californica*, *O. nigra* after mechanical stress and *A. arcystata*.

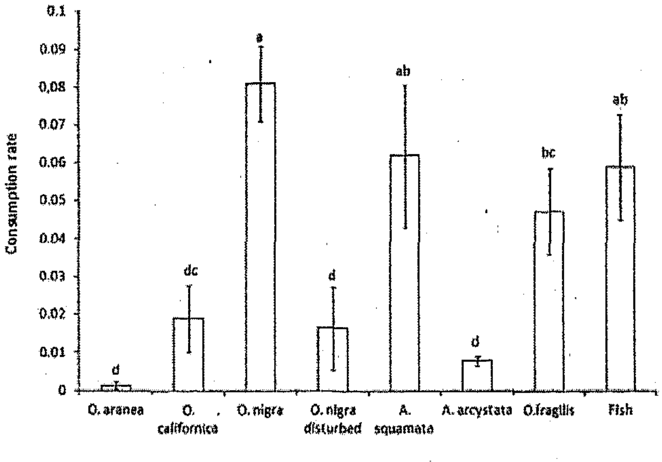

Figure 2. Consumption rates of agar blocks. HSD Tuckey, different letters indicate strongly different means, n = 6 for each combination.

Does ophiuroid bioluminescence advertise predators?

(ii) Are predators able to associate unpalatability and luminous signal?

During successive trials, it appears that the predation rate is higher for *O. fragilis* than for *O. nigra*, whatever the trial or the period (experiment ran during day or night) ($F_{1,94} = 10.267$, $p = 0.002$). Multiple mean comparisons test shows that strong differences between predation in the successive trials appear in two combinations: with the species *O. nigra* during the night, and with the species *O. fragilis* during the day. For *O. nigra* during the night, the consumption of brittle star is significantly higher during the first trial than during the two last trials, where the consumption is null. For the combination *O. fragilis*/day, predation rate is higher for the third trial. No differences between trials appear for *O. nigra* during the day or for *O. fragilis* during the night (Fig. 3).

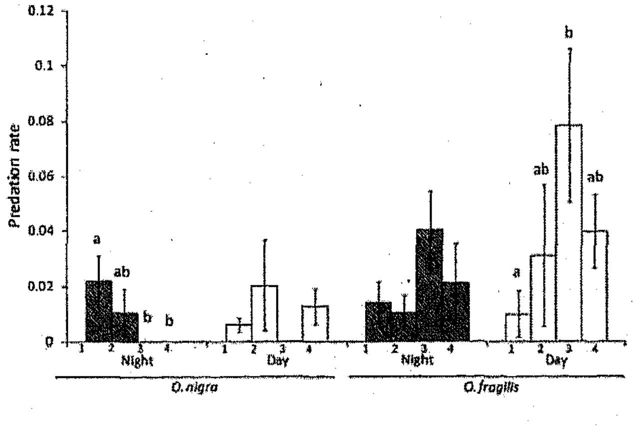

Figure 3. Predation rates during successive trials. HSD Tuckey, different letters indicate strongly different means, n = 6 for each combination.

Does ophiuroid bioluminescence attract a secondary predator?

Figure 4A. shows predation rate on crabs, for different prey-predator combination: crab-fish; crab-fish-luminous brittle star and crab-fish-non luminous brittle star, for experiments in day and night period. During the day, crabs are more predated for combinations including a brittle star. No significant differences appear between combination with the luminous or the non-luminous ophiuroid. During the night, predation on crabs increases significantly in the combination with the luminous brittle star, but not with the non-luminous species.

Results for predation suffered by brittle stars (Fig. 4B) show no significant differences between combinations (brittle star-crabs; brittle star-fish; brittle star-crab-fish) neither during the day nor during the night.

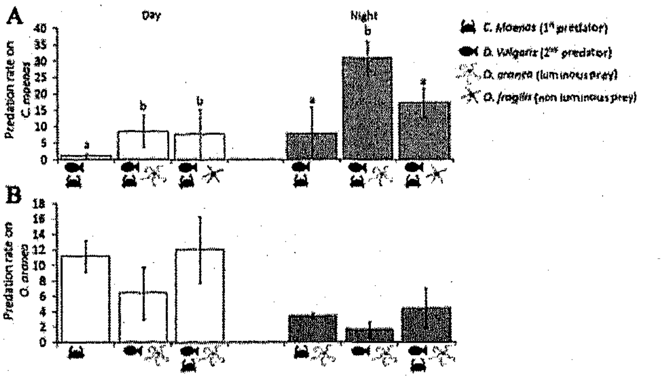

Figure 4. A. Predation on the primary predator (crab *C.maenas*). HSD Tuckey, different letters indicate strongly different means n = 6 for each combination). **B.** Predation on the brittle stars (ophiuroid *O. aranea*). HSD Tuckey, different letters indicate strongly different means n = 6 for each combination.

Discussion

Does ophiuroid bioluminescence deter predators?

Predation experiments were undertaken to address questions of the function of the bioluminescence in ophiuroids. Brittle stars are vulnerable to predation at night with sighted crabs. This is uncommon since crabs are commonly nocturnal predators. In contrast, this difference in predation rate and consumption disappears with blinded crab. Hence the predation experiments strongly suggest that the five luminous species exhibit a visual signal of defense against their predators. The bioluminescence is thus most likely involved in the defense against predation in these species. During the day, there was no difference in predation of luminous brittle stars and the control species

O. fragilis. In addition, predation by blinded crabs does not differ between day and night. These results indicate that defense against predation in these ophiuroids are based on vision. No other anti-predatory strategies such as chemical defenses seem sufficient to deter predators without the bioluminescence.

Does ophiuroid bioluminescence advertise predators?

(i) Are ophiuroids unpalatable?

The palatability tests delivered interesting and surprising results. The palatability of *O. aranea*, *A. arcystata* and *O. californica* was low. Concerning *O. nigra*, the palatability is strongly modified by the release of mucus which has a strong deterrent effect on the crabs. In absence of the released mucus, the tissue of *O. nigra* is eaten as much as tissue of *O. fragilis* or fish. We can conclude that the mechanical stress induces the release of mucus, and that this mucus includes both a visual way of defense, the bioluminescence, and a deterrent component. These data support the hypothesis of the aposematic use of bioluminescence. 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 riseii* Lütken, 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 millipeds (Marek et al., 2011) emit slow glowing light, which gets closer to the pattern of *O. nigra* (De Cock & Matthysen, 2001). Evolution of aposematic signals requires precise conditions concerning appearance of the signal as well as its maintenance (Leimar et al., 1986). Only low value prey (i.e. toxic or unpalatable prey) can evolve aposematic display. Regarding these palatability tests, this seems to be the case with mechanically stimulated *O. nigra*. Secondly, aposematic prey should suffer a lower predation rate than cryptic species. Predation experiments showed that also this condition is fulfilled for *O. nigra*. Finally, aposematic displays should allow the avoidance learning of the predator.

Does ophiuroid bioluminescence advertise predators?

(ii) Are predators able to associate unpalatability and luminous signal?

When a crab is repeatedly in contact with *O. nigra*, during the night, its consumption of brittle star significantly decreases along the trials. It seems that the crab actually avoids the prey, after two trials of 30 minutes. In contrast, this difference does not appear during the day, when bioluminescence is not visible. The consumption does not decrease with the control brittle star *O. fragilis*, which proves that the decrease in the first case cannot be explained by the satiety of the crab. Avoidance learning also occurs with *Ophiopsila aranea* and *Ophiopsila riseii*, but after only one trial (Grober, 1988; Jones & Mallefet, 2010), which suggests that their strong signal may be more effective. The use of selective light signals as aposematic signals includes undeniable advantages. Actually, the light is emitted only after disturbance, for *O. aranea* and *O. riseii* as well as for *O. nigra*. The prey remains cryptic when not disturbed, and does not suffer the costs of an increased detectability by naïve predators, which is a well-known problem in the explanation of the evolution of aposematic display (Eisner & Grant, 1981; Guilford & Dawkins, 1993; Speed & Ruxton, 2005). Thus *O. nigra* has an important advantage over the two other species. Actually, the signal and the deterrent component are both present in the sticky mucus excreted outside the body when attacked. Here *O. nigra* takes advantage of its defensive mechanism deterring its predator at the earlier stage of their interaction. The emission of luminous mucus, after disturbance, seems to be an advantageous strategy for aposematic displays. However patterns differ strongly among brittle stars with luminous emission, aposematic use of light seems to include many evolutionary advantages, and this function of bioluminescence may have been underestimated in the past. Further investigations, with other brittle star species, would allow generalizing the function of light for these organisms and tackling the question of the light function conservation in this class of echinoderms.

Does ophiuroid bioluminescence attract a secondary predator?

Our observations concerning interactions between luminous brittle stars, crabs and fishes support the burglar alarm hypothesis. Indeed, during the night, the predation on crab by the fish is higher if *O. aranea* is present. This clearly indicates that light emitted by *O. aranea* can advertise the presence of the crab to a predatory fish. During the day, we observe that predation rate on the crab increases when a brittle star is present, no matter whether the brittle star is luminous or not. Since bioluminescence is not visible during the day, it simply indicates that hunting ophiuroids makes the crab less cryptic during the day hence more available for the fish.

We also recorded predation rates on *O. aranea*. In this case, we do not observe any difference between combinations. The predation rate on the brittle star is lower during night than day, but does not decrease when predators of the two levels are present, contrary to what is predicted by the theory, i.e. lower predation rate on the luminous brittle star due to higher predation on the primary predator. These results support the use of bioluminescence as burglar-alarm signal for *O. aranea*, but we suggest that the benefits of this function might be visible at the level of the population not for the single individual. Studies of the genetic structure of the Banyuls *O. aranea* population should in the future allow us to determine if a kin-selected function of bioluminescence is conceivable for this species. The use of light as burglar alarm signal has also been highlighted in dinoflagellates (Mensinger & Case, 1992; Abrahams & Townsend, 1993), and suggested by Robison (1992) in the holothurian species *Enypniastes eximia* (Théel, 1882).

In conclusion, this study illustrates the diversity of mechanisms involved in the use of light as a defense against predation in brittle stars. This diversity should explain why this feature is so widespread in the class Ophiuroidea. Moreover, besides the defensive function, it would be very interesting to study other roles of bioluminescence (such as help for predation or intraspecific communication…) in this class to increase the information concerning the function of bioluminescence in brittle stars.

Acknowledgements

This project was financially supported by the ASSEMBLE programme of the European Community, grant agreement no. 227799, for the access to the Marine Station Arago of Banyuls-sur-Mer. The authors also thank the University of Santa Barbara for collaboration in the sampling of *O. californica*. This work was supported by a grant from de FNRS-FRIA to A. Jones. J. Mallefet is a research associate of the FNRS. This is a contribution to the Biodiversity Research Center (BDIV).

References

Abrahams M.V. & Townsend L.D. 1993. Bioluminescence in dinoflagellates - a test of the burglar alarm hypothesis. *Ecology*, **74**: 258-260.

De Cock R. & Matthysen E. 2001. Do glow-worm larvae (Coleoptera : Lampyridae) use warning coloration? *Evolutionary Ecology*, **13**: 619-639.

De Cock R. & Matthysen E. 2003. Glow-worm larvae bioluminescence (Coleoptera : Lampyridae) operates as an aposematic signal upon toads (*Bufo bufo*). *Behavioral Ecology*, **14**: 103-108.

Duffy E., Penn D., Botton M., Brockmann H. & Loveland R. 2006. Eye and clasper damage influence male mating tactics in the horseshoe crab, *Limulus polyphemus*. *Journal of Ethology*, **24**: 67-74.

Eisner T. & Grant R. 1981. Toxicity, odor aversion, and olfactory aposematism. *Science*, **213**: 476-476.

Fontaine A.R. 1964. The integumentary mucous secretions of the ophiuroid *Ophiocomina nigra*. *Journal of the Marine Biological Association of the United Kingdom*, **44**: 145-162.

Grober M. 1988. Brittle-star bioluminescence functions as an aposematic signal to deter crustacean predators. *Animal Behaviour*, **36**: 493-501.

Grober M. 1989. Bioluminescent aposematism - A reply to Guilford & Cuthill. *Animal Behaviour*, **37**: 341-343.

Guilford T. & Cuthill I. 1989. Aposematism and bioluminescence. *Animal behaviour*, **37**: 339-341.

Guilford T. & Dawkins M. 1993. Are warning colors handicaps? *Evolution*, **47**: 400-416.

Haddock S., Moline M. & Case J. 2010. Bioluminescence in the sea. *Annual review of Marine Science*, **2**: 443-493.

Herring P.J. 1995. Bioluminescent echinoderms: unity of function in diversity of expression? In: *Echinoderm Research 1995* (R. Emson, A.B. Smith & A.C. Campbell eds). A.A. Balkema: Rotterdam.

Jones A. & Mallefet J. 2010. Abstracts of the 16th International Symposium on Bioluminescence and Chemiluminescence (ISBC 2010). *Luminescence*, **25**: 155–156.

Leimar O., Enquist M., Sillentullberg B. 1986. Evolutionary stability of aposematic coloration and prey unprofitability - A theoretical analysis. *American Naturalist*, **128**: 469-490.

Mallefet 2012. Luminous brittle stars: an updated list. *Abstract of 14th International Echinoderm Conference*, Brussels, 122 pp.

Marek P., Papaj D., Yeager J., Molina S. & Moore W. 2011. Bioluminescent aposematism in millipedes. *Current Biology*, **21**: 680-681.

Mensinger A. & Case J. 1992. Dinoflagellate luminescence increases susceptibility of zooplankton to teleost predation. *Marine Biology*, **112**: 207-210.

Muntz L., Ebling F. & Kitching J. 1965. The ecology of lough ine. *Journal of Animal Ecology*, **34**: 315-329.

Robison B. 1992. Bioluminescence in the benthopelagic Holothurian *Enypiastes e+ximia*. *Journal of the Marine Biological Association of the United Kingdom*, **72**: 463-472.

Speed M. & Ruxton G. 2005. Aposematism: what should our starting point be? *Proceedings of the Royal Society B-Biological Sciences*, **272**: 431-438.

Stöhr S. & O'Hara T. 2012. World Ophiuroidea Database. Available online at http://www.marinespecies.org/ophiuroidea. Consulted on August 2012.