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CHARACTERIZATION OF ACETYLCHOLINE-INDUCED LUMINESCENCE IN *AMPHIPHOLIS SQUAMATA* (ECHINODERMATA : OPHIUROIDEA)

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Abstract. *Amphipholis squamata* is a luminescent polychromatic ophiuroid species. The population from Normandy (France) exhibits six different coloration patterns (orange, beige, dark brown, grey, spotted and black). Each variety of ophiuroid exhibits a different pattern and intensity of luminescence as induced by acetylcholine. The luminescence of dark brown and of black specimens was investigated over six months. A significantly higher intensity of luminescence was observed in February for both varieties.

Key words : *Amphipholis squamata*, echinoderm, ophiuroid, luminescence, acetylcholine.

INTRODUCTION

For almost two centuries (VIVIANI, 1805 ; HARVEY, 1952) it has been known that echinoderms are able to produce light. There are many luminous echinoderms, but only in ophiuroids have *in situ* observations been possible and rapid flashing reported. Most studies were conducted on two species, *Ophiopsila californica* (Clark) and *O. riisei* (Lütken). The most common explanation for the role of bioluminescence in ophiuroids is defence against potential predators. The light is used either to stun by intense flashes (BASCH, 1988), to warn by aposematism (GROBER, 1988a,b), or to lure by leaving behind one or more autotomized luminescent arms (GOTTO, 1963).

Amphipholis squamata (Delle Chiaje, 1828) is a small cosmopolitan viviparous ophiuroid, which was first described as luminous in 1805 by Viviani. This species is polychromatic ; six different coloration patterns have been described (BINAUX & BOCQUET, 1971). A recent study has shown these varieties to exhibit different luminescent capabilities (DEHEYN *et al*., 1997) and seasonal variations (DEHEYN, 1998) in response to KCl stimulation. Given that the luminescence in *A. squamata* is under cholinergic nervous control (DE BREMAEKER *et al.* 1993 ; 1996), the aim of this study was to investigate the acetylcholine-induced luminescence in the different colour varieties, and the variation of this light production over six months for two of these varieties.

MATERIAL AND METHODS

Specimens of *A. squamata* were collected intertidally at Langrune-sur-Mer (Normandy, France). Two sets of experiments were conducted. First, experiments were designed to investigate light emission according to coloration pattern ; specimens were collected in June 1997. Next, experiments were performed to study the luminescence variation over a six month period ; specimens were collected from October 1996 to May 1997. A recent study (DEHEYN *et al.*, 1997) showed that brooding and non-brooding ophiuroids exhibited a difference in intensity of light production, hence only brooding adult specimens (disc diameter > 1.6 mm ; EMSON & WHITFIELD, 1989) were used for our experiments.

Specimens were maintained for one day in open-circuit marine aquaria at the marine station in Luc-sur-Mer, and then transferred to our laboratory in Louvain-la-Neuve, Belgium. Luminescence measurements were investigated no more than two days following the transfer, to ensure the luminescence performance was not affected (DUBUISSON, unpubl.). During these two days, specimens were kept in a closed-circuit marine aquarium under field conditions of temperature and salinity.

The arms are the only luminescent parts of *A. squamata* (BREHM & MORIN, 1977). After anaesthetization of the ophiuroid by immersion in artificial sea water (ASW) containing 3.5 % w/w $MgCl_2$, arms were removed and placed in small chambers containing 200 µl ASW with the following composition (in mM) : 400.4 NaCl ; 9.6 KCl ; 52.3 $MgCl_2$; 9.9 $CaCl_2$; 27.7 Na_2SO_4 ; 20 Tris HCl ; pH 8.3. The arms were stimulated by application of exogenous acetylcholine (Ach) (Sigma Chemical Co.) at a final concentration of 10^{-3}M.

Light emission was monitored with either a PM 270 D photomultiplier connected via an amplifier IL500 (International Light, USA) to a chart recorder (Servogor S, Germany), or a 1250 Bioorbit luminometer (Bioorbit, Finland) connected to a computer.

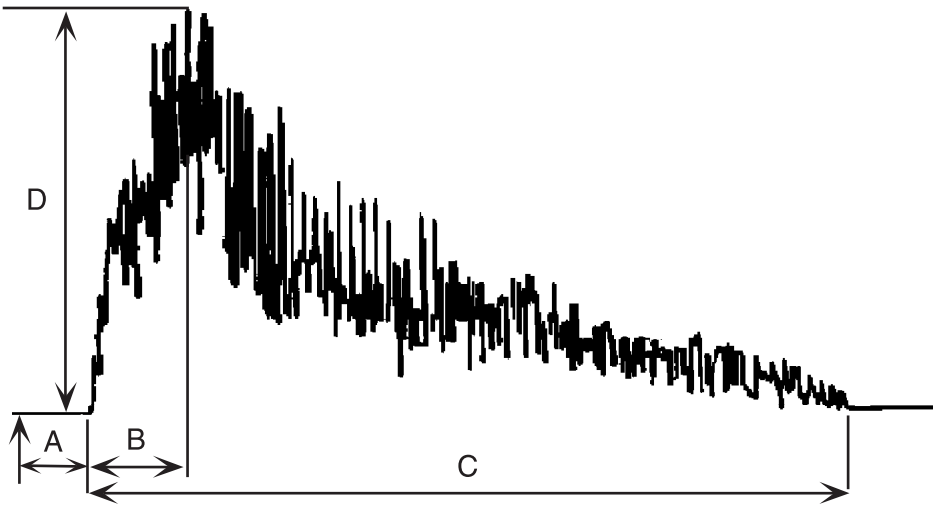

Fig. 1. – *Amphipholis squamata*: parameters of ACh-induced luminescence. – (A) TL: the latency time expressed in seconds; (B) TLmax: the time to reach the maximal light intensity in seconds; (C) Tresp: the total response time in seconds; (D) Lmax: the maximal light intensity in Mq/s/mm.

The parameters used to characterize the luminescence were (Fig. 1): (i) the latency time (TL) expressed in seconds, (ii) the time expressed in seconds to reach the maximal light intensity (TLmax), (iii) the total response time in seconds (Tresp) and (iv) the maximal light intensity (Lmax) expressed in Megaquanta/sec per millimetre of arm length (Mq/s/mm). Statistical analyses were performed using analysis of variance (anova); each mean value is expressed with its standard error of mean (mean ± s.e.) and number of preparations (n).

RESULTS

Light emission according to coloration pattern

The pattern (Fig. 2) and the parameters (Table 1) of luminescence of ophiuroids varied from one variety to another.

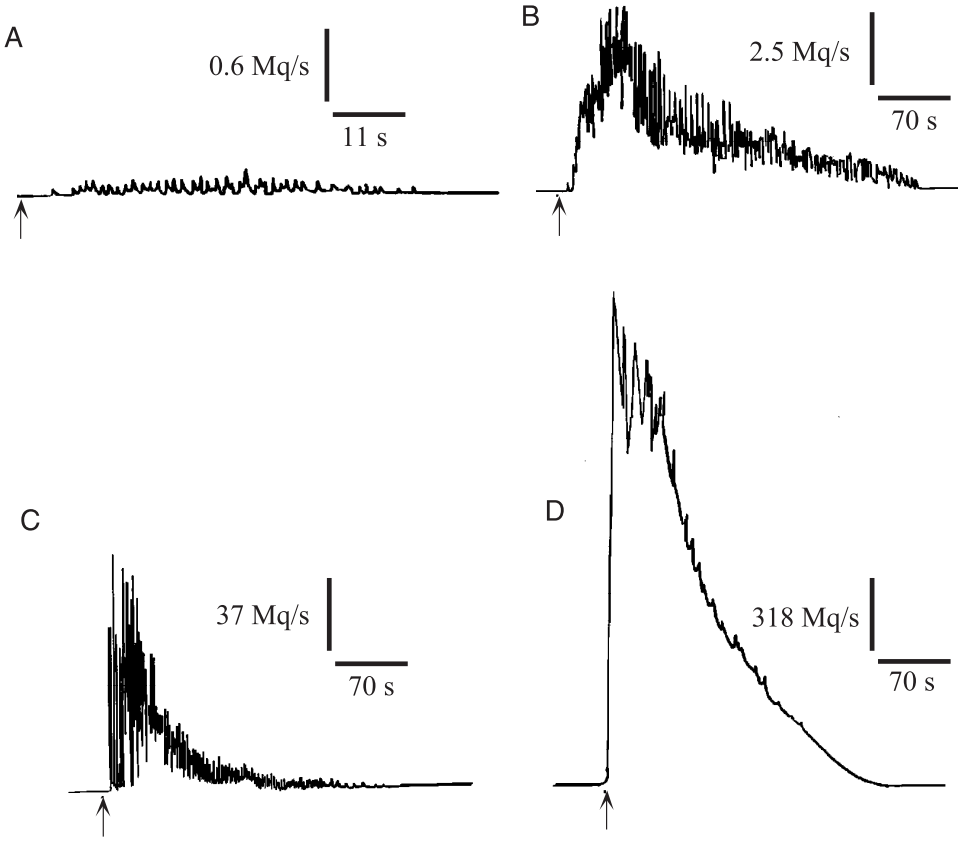

Fig. 2. – Luminescence patterns of ophiuroids from different colour varieties in response to ACh stimulation. – (A) orange specimens, (B) dark brown specimens, (C) spotted specimens, (D) black specimens. Arrow: injection of ACh.

Orange and beige specimens : only 33 % and 50 % of stimulated arms, respectively, emitted light in response to ACh. In addition, the maximal light production (Lmax), which was similar in orange and beige specimens, was significantly lower than for the other varieties ($P < 0.01$, calculated after logarithmic transformation of the n values as indicated by Zar, 1984 when heteroscedasticity occurs) (Table 1). Although the latency time (TL : 9.05 ± 1.57 s) and total response time (Tresp : 40.05 ± 3.21 s) of light emission for orange specimens was significantly different ($P < 0.01$) from the TL (4.46 ± 0.65 s) and Tresp (133.13 ± 9.29 s) of beige specimens, the luminescence pattern for both varieties was similar. The original recording, represented in Fig. 2A, showed a succession of individual flashes of very low intensity.

Dark brown and grey specimens : nearly all of the stimulated arms emitted light ($n = 28$ and 29, respectively). The parameters (Table 1) and the pattern of luminescence (Fig. 2B) were very similar for both types of specimens, except that Lmax was significantly higher ($P < 0.01$) for the grey specimens (2.53 ± 0.37 Mq/s/mm) than for the dark brown (0.72 ± 0.07Mq/s/mm). The luminescence pattern was a series of superimposed light flashes of increasing amplitude that reached a maximal value and then decreased in magnitude.

Spotted specimens : all of the stimulated arms emitted light. The latency time and maximal light production were, respectively, shorter and higher than for the other varieties ($P < 0.01$) except for the black specimens. The luminescence pattern (Fig. 2C) consisted of a series of individual flashes of increasing amplitude that reached a maximal value and then decreased in magnitude.

Black specimens : all of the stimulated arms emitted light. The luminescent response was very fast with a significantly shorter TL and Tlmax, whereas Lmax was significantly higher than for the other varieties ($P<0.01$) (Table 1). The luminescence pattern (Fig. 2D) was monophasic-like, although several peaks of decreasing intensity were observed.

TABLE 1

Values of luminescent parameters for the six colour varieties of Amphipholis squamata. *For each variety, arms from six specimens were stimulated, giving a total of 30 stimulated arms per variety (mean ± s.e.; *= P< 0.05, **= P< 0.01; n= number of responding arms).*

Colour varieties	**Orange**	**Beige**	**Dark Brown**	**Grey**	**Spotted**	**Black**
TL	9.05 **	4.46	4.11	4.17	2.4 **	0.76 **
(s)	± 1.57	± 0.65	± 0.48	± 0.27	± 0.35	± 0.05
TLmax	28.40	20.0	21.4	22.77	18.77	1.68 **
(s)	± 6.66	± 5.08	± 4.83	± 5.02	± 2.25	± 0.23
Lmax	0.03 **	0.08 *	0.72 *	2.53 **	13.14 **	238.26 **
(Mq/s/mm)	± 0.006	± 0.01	± 0.07	± 0.37	± 2.42	± 38.53
Tresp	40.05 **	133.13 *	169.96 *	168.48 *	140.17 *	148.73 *
(s)	± 3.21	± 9.29	± 10.10	± 3.31	± 5.95	± 19.26
n	10	15	28	29	30	30

Variation in luminescence performance over six months

Only dark brown and black ophiuroids were investigated. They were both chosen because of their differences in luminescent properties and their relative abundance in the population.

Dark brown specimens exhibited Lmax values which did not significantly vary between the months October, December 1996 and January, March, April, May 1997. Lmax values ranged from 0.13±0.03 Mq/s/mm in December 1996 to 0.66 ± 0.31 Mq/s/mm in January 1997. Conversely, a peak of significantly higher intensity of light production ($P < 0.01$) was observed in February 1997, reaching a value of 5.04±0.54 Mq/s/mm (Fig. 3A).

Black specimens showed Lmax values which varied from 336.4 ± 57.2 Mq/s/mm in October 1996 to 138.1 ± 37.1 Mq/s/mm in April 1997. A peak of significantly higher inten-

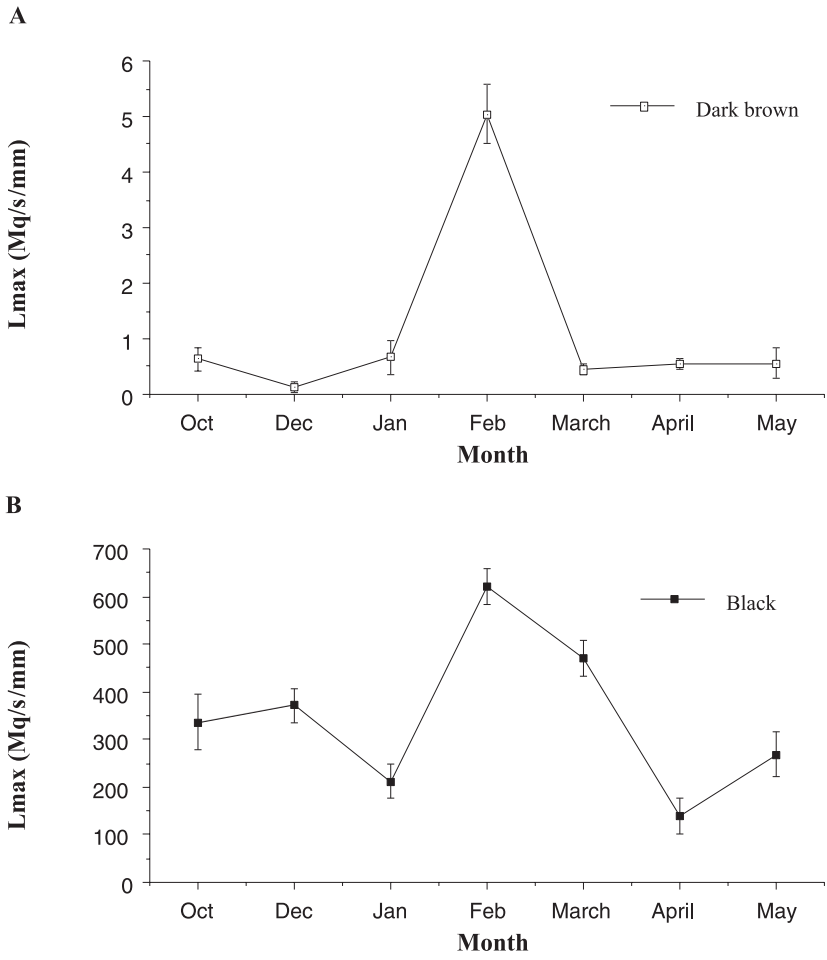

Fig. 3. – Variation of light intensity (Lmax in Mq/s/mm) from October 1996 to May 1997 of (A) dark brown specimens, (B) black specimens.

sity of light production ($P < 0.01$) was observed in February 1997, reaching a value of 621.8 ± 37.6 Mq/s/mm (Fig. 3B). The Lmax value for March 1997 was not significantly different from the Lmax value for October and December 1996.

It is noteworthy that the pattern of light emission varied through the year : with an increasing Lmax we usually observed a reduced latency time, a shorter TLmax and superimposed flashes which, for the black specimens in periods of maximal light emission, led to a monophasic curve.

DISCUSSION

Light emission according to coloration pattern

Ophiuroids of each colour variety differed in their luminescence parameters. The latency time was the shortest for black specimens and the longest for orange specimens. The following ranking was observed : black < spotted < grey = dark brown = beige < orange. TLmax was significantly shorter for black specimens whereas the other varieties exhibited similar values (black << orange = beige = dark brown = grey = spotted). The total light response time was significantly shorter for orange specimens and longer for grey and dark brown specimens (orange << beige = spotted = black < grey = dark brown). The maximal light production differed significantly from one variety to another. The orange and beige specimens exhibited the lowest levels of luminescence, followed in order of intensity by dark brown, grey, and spotted specimens, with a maximal intensity reached by black specimens (orange = beige < dark brown < grey < spotted << black).

Compared with the results obtained using KCl stimulation (DEHEYN *et al.*, 1997), ACh-induced luminescence differed in many ways from KCl-induced luminescence. Firstly, the pattern of light emission induced by ACh did vary from one variety to another whereas the pattern of KCl-induced luminescence was a monophasic curve, similar for all varieties. Secondly, the maximal amplitude of luminescence was lower for ACh-induced luminescence. And finally, like the results obtained with ACh, the maximal light production induced by KCl differed significantly from one variety to another, but the spotted specimens showed the highest intensity for KCl-induced luminescence while it was the black specimens for ACh-induced luminescence.

So far, the most common role described for bioluminescence in ophiuroids is defence against predators (BASCH, 1988 ; GROBER, 1988a,b ; GOTTO, 1963). Assuming this function to be true for *A. squamata*, the fact that colour varieties show significant differences in light intensities would mean that some individuals in the population would be more protected than others. This seems not to be the case, as individuals producing the most intense light (spotted and black varieties) have been shown not to be the most abundant in the population (DEHEYN *et al.*, 1997).

The difference of light emission between polychromatic ophiuroids may be explained either by a variable amount of luminescent tissue in the arm, or by a different quantity of substrate and/or enzyme for the light reaction in the luminescent cells. Conversely, pharmacological studies showed that there were differences in the subtype of cholinergic

receptors and in the neuromodulation of luminescence between dark brown and black specimens (De Bremaeker *et al.,* 1996 ; Mallefet *et al.*, 1994). These results support the view that there are distinctive functional differences in the neuromodulatory control of luminescence between dark brown and black specimens.

Variation in luminescence performance over 6 months

Both dark brown and black specimens of *A. squamata* displayed variation of luminescence performance over the investigated period of the year ; the light production in response to ACh stimulation was significantly higher in February. Since only brooding adult specimens were considered, the variation of luminescence performance could not be related to sexual maturity.

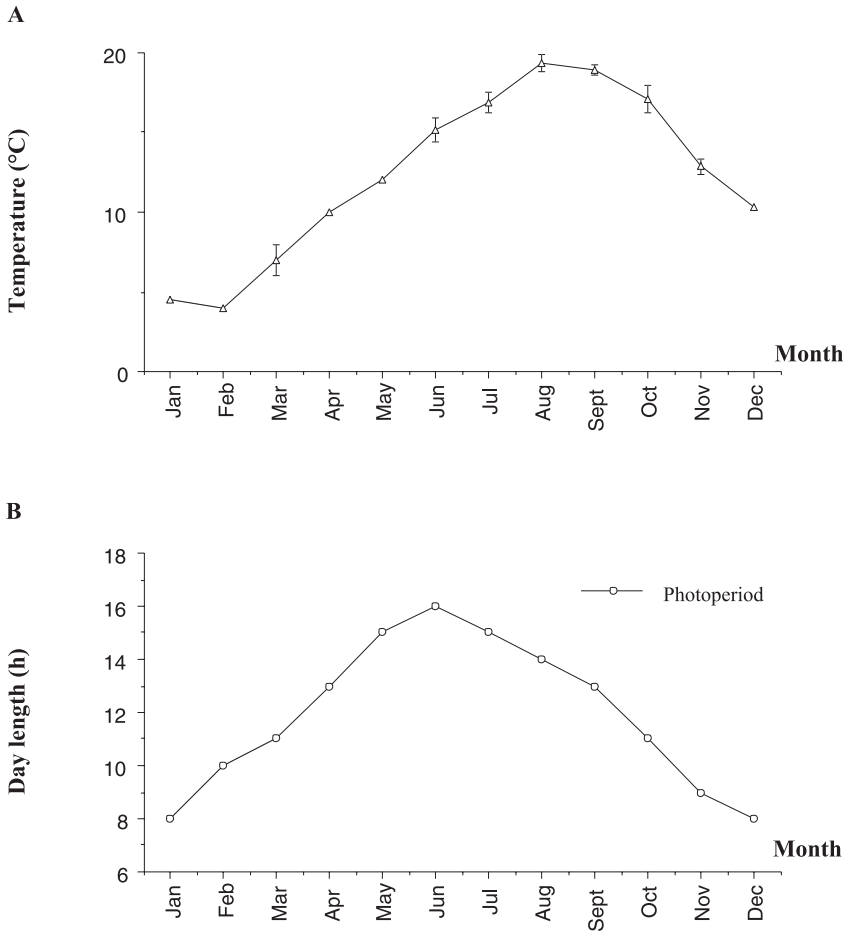

Fig. 4. – Seasonal variations from January 1997 to December 1997 of (A) water temperature, (B) photoperiod.

Other factors could affect luminescence. In this study, three abiotic field parameters were measured : surface salinity, surface temperature and photoperiod. Salinity was not significantly different from one month to another, ranging from 32.4 to 33.5 ‰ (not shown). Hence this factor was not likely to affect luminescence. Conversely, temperature and photoperiod exhibited large variations (Fig. 4A-B) ; temperature and photoperiod being low (4 °C ; 10 :14, Light : Dark) in February when a peak of luminescence was observed. These two factors may influence directly or indirectly the luminescence. This hypothesis agrees with recent field investigations and laboratory experiments on the effect of exogenous factors on the luminescence induced by KCl depolarisation, which showed that salinity had no main effect whereas the temperature and photoperiod significantly affected the KCl-induced luminescence (DEHEYN, 1998).

Seasonal photoperiod changes are known to guide the reproductive cycle of many organisms, including the echinoderms (BAY-SCHMITH & PEARSE, 1987 ; BOULAND & JANGOUX, 1988 ; XU & BARKER, 1990). Although *A. squamata* produce fertile gametes all year (Fell, 1946 ; Emson & Whitfield, 1989), the reproductive effort varies according to season, and all the gonads reach complete sexual maturity in winter. The maximal reproductive effort thus coincides with the coldest months, *i.e.* February-March for northern temperate locations (JONES & SMALDON, 1989 ; EMSON & WHITFIELD, 1989 ; EMSON *et al.* 1989 ; ALVA, 1996). As a consequence, the seasonal variation of the luminescence in *A. squamata* could be influenced by an endogenous factor guided, by the photoperiod, causing the luminescence performance to increase in winter.

Seasonal variation of light production could be due to seasonal changes in the level of neuromodulators. Seasonal changes in the levels of monoamines (serotonin, dopamine and noradrenaline) occur in invertebrates and vertebrates (SPAFFORD & PENGELLEY, 1971 ; YORK & TWAROG, 1973 ; STEFANO & AIELLO, 1975). In the invertebrate bivalve mollusc *Mytilus edulis*, these monoamines were higher during the summer and lower during the winter (STEFANO & CATAPANE, 1977). In two echinoderm species, the starfish *Asterias amurensis* and the sea urchin *Strongylocentrotus intermedius*, seasonal changes of catecholamine levels have also been reported. The levels of dopamine were higher in the winter and tryptamine higher in the summer (KHOTIMCHENKO & DERIDOVICH, 1988). Preliminary pharmacological studies have shown that catecholamines inhibited ACh-induced luminescence in *A. squamata*. Further studies will investigate the neuromodulatory processes occuring in *A. squamata* luminescence.

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