J. mar. biol. Ass. U.K. (1997), 77, 1213-1222
Printed in Great Britain

INTRASPECIFIC VARIATIONS OF BIOLUMINESCENCE IN A POLYCHROMATIC POPULATION OF *AMPHIPHOLIS SQUAMATA* (ECHINODERMATA: OPHIUROIDEA)

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Six colour varieties were recognized in an intertidal population of *Amphipholis squamata* from Normandy (France). Each variety exhibits its own capacity to produce light, the light emitted by the most luminous variety being 500 times more intense than that emitted by the least luminous one. Differences in luminescent capabilities observed between varieties do not seem to be due to differences in pigmentation or to be of exogenous origin. It was shown that the capability to produce light changes according to whether individuals are brooding or not, brooding individuals emitting a more intense light than non-brooding ones. This supports the defensive use of luminescence generally associated to ophiuroid production of light. However, light capabilities differ so much between *A. squamata* colour varieties that bioluminescence could also be associated with another role in the species.

INTRODUCTION

Luminescent echinoderms have been known for almost two centuries (Viviani, 1805). They belong to four of the five extant classes of the phylum as no luminescent echinoid species have ever been described (Herring, 1987). Although bioluminescence is widely distributed within echinoderms, ecological and biological investigations mostly considered two ophiuroid species, *Ophiopsila californica* and *O. riisei*. The light produced seems to be used for defence to deter predators (Basch, 1988; Grober, 1988a,b). The only biological factor mentioned to influence luminescence intensity is the pigmentation that usually shows various patterns along ophiuroid arms (Brehm & Morin, 1977).

Amphipholis squamata was first named *Asterias noctiluca* due to its luminous properties (Viviani, 1805). This ophiuroid is of small size, maximal arm length of adult individuals being 15 mm, for a disc diameter of 3·5 mm. The species is brooding (Fell, 1946) and shows a widespread geographic and bathymetric distribution, being found worldwide from the tidal zone down to 2000 m depth (Gage et al., 1983). *Amphipholis squamata* luminescence was recently investigated since it had been noted that 'dark' and 'clear' pigmented individuals were showing differences within intensity and neurophysiological control of light production (Deheyn et al., 1994; De Bremaeker et al., 1994, respectively). No information is available on biological and ecological aspects of the

luminescence, the major difficulty being that the species is of small size and that the light it produces is much weaker than that produced by species from the genus *Ophiopsila* (Brehm & Morin, 1977; D.D., personal observation).

Amphipholis squamata exhibits different coloration patterns (six at Roscoff, Brittany, France) and was described as polychromatic by Binaux & Bocquet (1971). The present work aims to determine light capabilities of *A. squamata* individuals from a single intertidal population in relation to their coloration pattern and brooding condition.

MATERIALS AND METHODS

Specimens of *Amphipholis squamata* (Delle Chiaje, 1828) were collected with forceps from under stones of a sandy-muddy beach at Langrune-sur-Mer (Normandy, France). Samplings were done from March to May 1994 along a transect that crosses the tidal zone perpendicularly to the coast. Seven stations were considered 40 m apart from each other, the upper and lower stations being, respectively, 1·7 and 0·2 m above the minimal sea level. Four samplings were performed at each station from March to May according to a procedure in which the sampling time (10 min), the sampled surface (~16 m^2) and the four collectors involved were identical each time. For each station, the number of ophiuroids was counted and their coloration noted (data from the four samplings were pooled in the results). Individuals were then transported to the Laboratory of Animal Physiology at the Université Catholique de Louvain and kept alive in a closed-circuit marine aquarium (12°C, 33‰ salinity) for light intensity measurements (maximum three days). Light:dark cycle in the aquaria were those of the natural population at the collecting period, i.e. 12:12 in March, 14:10 in April and 16:8 in May.

The arms are the only luminescent body parts of *A. squamata* (Brehm & Morin, 1977) and the five arms from a single individual produce light of the same intensity providing they are of the same length (Mallefet et al., 1992). Measurements of light intensity were carried out in a dark room at constant temperature (18°C). Individuals were anaesthetized by immersion (3 min) in a 3·5% (w/w) $MgCl_2$ solution in artificial sea-water without NaCl, and the diameter of their disc and the length of their arms were measured under a dissecting microscope. They were then placed in artificial sea-water (ASW) of the following composition (in mMol): 400·4 NaCl; 9·6 KCl; 52·3 $MgCl_2$; 9·9 $CaCl_2$; 27·7 Na_2SO_4; 20 Tris HCl buffer, pH 8·3. Arms were cut from the disc using a fine scalpel and isolated arms were rinsed (3 min) in ASW before being positioned oral side down in small glass chambers filled with 200 µl of ASW. Arms showing a constant decrease in diameter from the proximal to the distal segments were the only ones to be considered, i.e. arms that were not showing any regenerating stump. Chambers were placed onto a rotating circular support articulated to a photomultiplier (type IL 760, International Light). Luminescence was triggered by injection into the chambers of 200 µl of a 400 mM KCl solution in ASW where NaCl had been replaced by KCl. Final KCl concentration was thus 200 mM which is the optimal concentration for triggering light emission in *A. squamata* (Mallefet et al., 1992). In measuring bioluminescent capabilities of marine organisms, KCl is often used (Herring, 1974). It acts on photogenous tissues by depolarizing cellular membranes having as consequence, when used at optimal

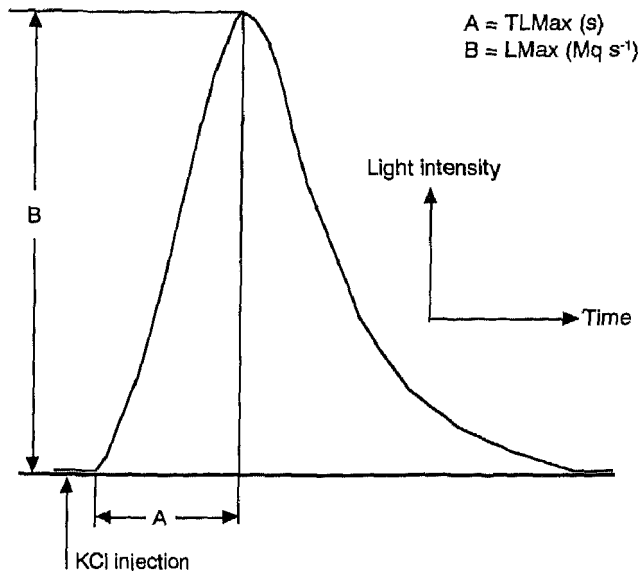

Figure 1. *Amphipholis squamata*: parameters of the light response. LMax, the maximum light intensity observed during the light response; TLMax; the time to reach the maximum light intensity.

concentration, to trigger the maximal emission of light, viz the emission that involves all the photogenic material into the photogenous reaction (Mallefet et al., 1992). The emitted light was detected by the photomultiplier phototube (type IP 21 with S 20 spectral sensitivity – maximal from 400 to 600 nm) and transformed into an electric signal. The signal was amplified (Amplifier-Radiometer type *IL 1700, International* Light) before being graphically recorded (Servogor S). Each month the system was calibrated using a tritium-phosphor light source (emission spectra maximal from 470 to 510 nm). The light intensity was calculated using the formula $I=119{\cdot}7\ e^{-0{\cdot}056t}$ where I is the intensity expressed in megaquanta per second (Mq s^{-1}) and t the age of the source from the 1 April 1971 expressed in years (Beta Light Source, Saunders & Roe, Nuclear Enterprise).

Light emission of *A. squamata* (spectra maximal emission from 480 to 520 nm with a peak at 510 nm; Brehm & Morin, 1977) is monophasic and occurs within 2–3 min following KCl addition (Mallefet et al., 1992). Two light emission parameters were considered (Figure 1): (1) the time expressed in seconds (TLMax) to reach the maximum light intensity and (2) the maximum light intensity (LMax) expressed in megaquanta per second and per millimetre of arm length (Mq s^{-1} mm^{-1} or LMax mm^{-1}). For practical reasons LMax was the only measured parameter referring to the quantity of photogenic material as it was demonstrated that in KCl stimulation LMax is linearly related to the total light produced (Vanhoutte, 1990). Analyses of variance (Nested ANOVA, arms being nested within ophiuroids) and multiple mean comparison tests (Tukey) were used to determine the significance of the observed differences; simple linear regression models were used to test relations between variables. All tests were carried out to a significance level (α) of 0·05 using Statview 4·0 and Systat 5·2·1 software.

Light emission parameters were measured on arms from adult individuals (disc diameter >1·7 mm; Emson & Whitfield, 1989) showing the different coloration patterns observed in the field, and on arms of both brooding or non-brooding individuals having the same coloration pattern. To determine the brooding condition, anaesthetized individuals were positioned aboral side down, their bursae were dissected under a microscope (there are ten bursae in a single disc, one pair per radius) and the presence or the absence of larvae and/or juveniles was noted. Larvae and juveniles found in bursae were counted and the juveniles diameters were measured. *Amphipholis squamata* can brood in one bursa both larvae and juveniles at the same time as the species shows a reproductive activity through all months of the year, with an intensification of the reproductive effort in February–March (Emson & Whitfield, 1989; Alva, 1996).

RESULTS

Coloration patterns and intertidal distribution

Individuals of *Amphipholis squamata* were found all along the transect. Their number increased progressively from the upper to the lower stations (Table 1). In each station ophiuroids of different coloration patterns were collected and six colour varieties were recognized. Orange individuals represent 3% of the population, beige individuals 34%, dark brown individuals 14%, grey individuals 33%, black individuals 11% and spotted individuals 5%. The proportion of each variety was generally maintained from one station to another indicating that they are distributed homogeneously across the tidal zone (Figure 2).

Table 1. Amphipholis squamata: *number of individuals of each variety at each station (number of samples = 4).*

Colour varieties	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Total
Orange	5	9	7	6	6	14	4	51
Beige	32	64	50	99	105	147	179	676
Dark brown	10	26	17	45	48	60	71	277
Grey	23	69	49	77	123	154	129	624
Black	3	20	10	37	51	77	67	265
Spotted	5	12	9	19	14	16	18	93
Total	78	200	142	283	347	468	468	1986

Light emission according to coloration pattern

Ophiuroids from different colour varieties differ in their capacity to produce light, the maximal intensity of the emitted light (LMax mm^{-1}) being statistically different from one variety to another ($P<0·0001$, calculated after logarithmic transformation of the N values as indicated by Zar, 1984 when heteroscedasticity occurs) (Table 2). However, this is not the case for the kinetic parameter since the TLMax values recorded in the orange, dark brown and grey varieties are not significantly different (Table 2).

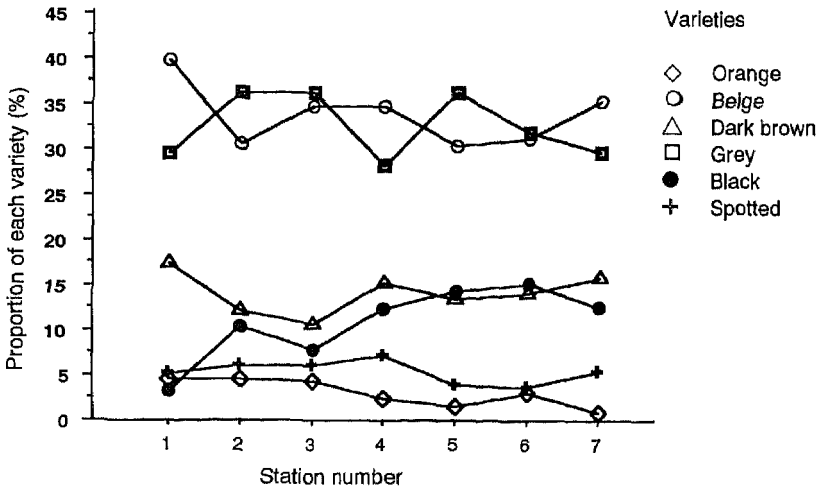

Figure 2. *Amphipholis squamata*: proportions of each variety at each station of the transect (pooled results of four samples).

Table 2. Amphipholis squamata: *values of luminescent properties for the six colour varieties.*

Colour varieties	Orange	Beige	Dark brown	Grey	Black	Spotted
LMax mm^{-1} ($Mq\ s^{-1}$)	2·27 * ±0·48	5·56 * ±0·64	24·07 * ±5·61	79·02 * ±11·60	793·36 * ±90·85	961·41 * ±174·74
TLMax (s)	8·63 ±1·09	6·58 * ±1·78	7·95 ±1·16	8·47 ±1·57	1·61 * ±0·15	10·53 * ±1·11
Number of stimulated arms[1]	N=31	N=33	N=33	N=30	N=31	N=28

[1], for each variety arms from seven individuals were stimulated, regenerating arms were not investigated; mean ±95% confidence limit; *, statistically different from each of the five other varieties; $P<0·0001$.

Light emission in brooding and non-brooding individuals

Only beige and black ophiuroids were investigated. They were both chosen because of their important differences in luminescent properties and their relative abundance in the population. Adult beige and black ophiuroids were divided into two categories depending on whether or not they were brooding larvae and/or juveniles. Brooding ophiuroids were those having at least one larva or juvenile in their bursae (brooding and non-brooding individuals were of the same size). Whatever the variety the light emitted by brooding individuals was always significantly more intense ($P<0·0001$) than that emitted by non-brooding ones (Figure 3A,C). It is noteworthy that significant differences in the kinetics of the luminescent responses were observed only with black ophiuroids which produced light faster when they were brooding (Figure 3B&D).

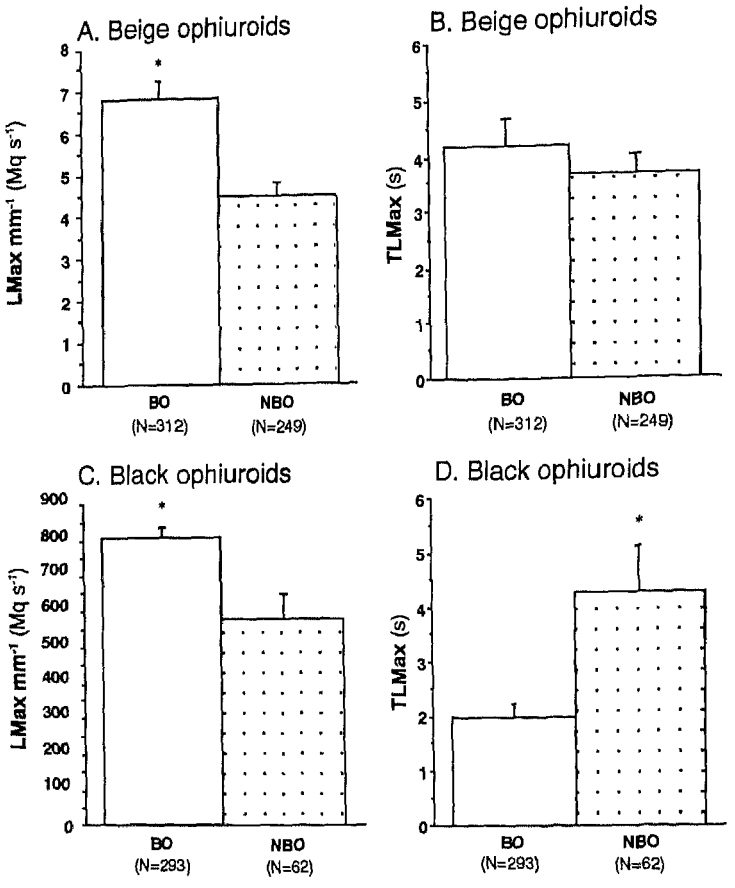

Figure 3. *Amphipholis squamata*: measurements of light intensity parameters in brooding (BO) and non-brooding ophiuroids (NBO) of beige (A,B) and black (C,D) varieties (N=number of stimulated arms) (mean value ±95% confidence limit; *, statistically different; $P<0{\cdot}0001$).

Using simple linear regression models it was shown that luminescence of brooding individuals is not influenced by the number of brooded larvae and juveniles found per ophiuroid. For the beige variety ($P>0{\cdot}08$, N=312) this number varies from 1 to 6, and for the black variety ($P>0{\cdot}28$, N=293) it goes from 1 to 12 (N is the number of values building the regression). *Amphipholis squamata*'s capability to produce light is neither influenced by the size (discal diameter) of its brooded juveniles. For the beige variety ($P>0{\cdot}11$, N=165) juveniles' discal diameter ranges from 0·1 to 0·95 mm, and for the black variety ($P>0{\cdot}37$, N=329) from 0·1 to 0·90 mm.

DISCUSSION

As first reported by Tortonese (1932), the species *Amphipholis squamata* displays various colorations. The different colour varieties (the orange, beige, dark brown, grey, black and spotted varieties) that were recognized in the intertidal population of Langrune-

sur-Mer (present study) correspond to those observed by Binaux & Boquet (1971) at Roscoff (Brittany, France). These authors named the varieties from A to F, with A variety corresponding to black individuals, B to beige ones, C to grey ones, D to spotted ones, E to dark brown ones and F to orange ones. We have also demonstrated that these varieties have different luminescent properties. Bioluminescence and pigmentation in coastal marine luminescent invertebrates have been a matter of discussion especially regarding the selective pressure to direct the light produced in a particular direction using pigments (Herring, 1990; Morin, 1983). This not not occur in luminous ophiuroids as produced light is diffused and restricted to dense areas of photocytes, no pigments having ever been involved in mechanisms that focus light (Herring, 1974, 1978, 1995). Pigments were shown to vary in density in relation to ambient light (they were so-called chromatophores by Hendler & Byrne, 1987), giving individuals the potential to stay cryptic within their habitat (Hendler, 1984; Hendler & Byrne, 1987). Such a mechanism provides individuals with the capability of avoiding predation, with arm autotomy the 'last line' of defence when attacked by predators (Basch 1988; Grober, 1988a, b, 1990). In addition to these cryptic and autotomic defensive strategies, luminous species show the capability to produce light of sufficient intensity to stun and frighten attacking predators. This provides a more worthwhile defensive capability to luminescent species, as they can for example deter predators before they trigger ophiuroid arm autotomy reaction (Basch, 1988; Grober, 1988a,b, 1990).This superimposition of defence strategies is illustrated in the genus *Ophiopsila*. Both luminescent and non-luminescent species are cryptic, arms showing banded patterns of pigmentation, the amount of pigments varying from segment to segment along the arms (the darker the bands the more pigments they have; a band groups together several brachial segments). As a consequence, arms of luminescent species appear to be made of bands lighting at different intensities: the darker the arm segment the weaker the light it produces. No functional, behavioural or ecological implications could have been associated with such patterns of luminescence (Brehm & Morin, 1977).

The situation is different in *A. squamata*. This species shows different coloration patterns that define six colour varieties with differently pigmented disc and arms (Binaux & Bocquet, 1971). Considering only arms (the only body part to be luminescent in the species, Brehm & Morin, 1977), pigmentation is not equally spread all over their surface nor all along their length, so that whitish – viz unpigmented- brachial bands occur within some varieties. It was demonstrated that individuals from one variety have the same capability to produce light (present study), and it was also noted that individual luminescence varies in intensity along arms with regard to pigmentation (Brehm & Morin, 1977; D.D., personal observation). Light intensity and pigmentation are related within each individual so that darker arm segments produce weaker light. Although this seems to be the general rule within each variety, it is not valid when individuals are from different colour varieties. Indeed, the present investigation has shown that individuals producing the most intense light are either from lightly or heavily pigmented varieties (i.e. the spotted or black varieties, respectively) and that individuals producing luminescence of weak intensity are either from heavily or lightly pigmented varieties (i.e. the dark brown or orange varieties, respectively).

Luminescence intensity depends on the amount of photogenic material that occurs in luminous organs or body areas (McCapra, 1990). When the material is endogenous to the species, individuals have similar capabilities to produce light; when it is exogenous, it is usually of dietary origin and the property of luminescence is consequently highly variable so that it can be facultative in such organisms as it depends on whether or not prey containing the photogenic material is present in the area (Hastings, 1983; McCapra, 1990). In *A. squamata* the photogenic material is presumably endogenous as: (1) individuals of the same colour variety do not show wide variations within their luminescent capabilities; (2) individuals of different varieties show their own luminescent capabilities and coexist in the same habitat; and (3) it is likely that they have the same diet as analyses of *A. squamata* gut contents have provided evidence that the species is suspension-feeding, deposit-feeding and omnivorous (Martin, 1968; Johnson, 1972; Emson & Whitfield, 1989).

Luminescence in *A. squamata* is under nervous control (Brehm, 1977), and individuals of the beige and black varieties differ in the nature of their cholinergic receptors and some of their neuropeptides (De Bremaeker et al., 1994; Mallefet et al., 1994; respectively). In addition it could also be hormonally regulated as brooding induces both the over development of the bursal-associated haemal system (Fell, 1946; Nisolle et al., 1990) and a significant increase in the light production of the considered individuals. It then seems that individuals of *A. squamata* are accumulating various biological differences – viz within pigmentation patterns, luminescent capabilities and neuro-physiological processes. This is highly schematic and in the present situation there is no way to say whether these differences are linked or not to each other. A possible answer could be given by studying various populations of *A. squamata* located in different geographical regions (the species is cosmopolitan). Are the same varieties found in different populations and are they always showing their own luminescent capabilities?

By now, investigations have begun to understand the evolutionary context of the occurrence of colour varieties in *A. squamata*. Colour varieties have been described in other echinoderms and the general trend is that they are genetically determined (Lewis & Storey, 1984; Growns & Ritz, 1994). Morphs, defined as genetically differentiated colour varieties, are usually found living in different (micro) habitats (for review see Growns & Ritz, 1994). When varieties are co-occurring in the same habitat as in asteroids genus *Othilia* (=*Echinaster*), it is proposed from biochemical and genetic analyses that genetic speciation is occurring (Tuttle & Lindahl, 1980). Therefore, one should not dismiss the speciation hypothesis to explain the differences that occur between *A. squamata* colour varieties.

Bioluminescence in ophiuroids is usually said to be a sacrificial lure (when associated with an autotomized arm) or an aposematic signal to mislead or deter predators (Basch, 1988; Grober, 1988a,b; respectively). Assuming these functions to be true, the fact that colour varieties show significant differences in light capability would mean that some individuals in the population would be less protected than others. This seems not to be the case as individuals producing the most intense light (i.e. those of the black and spotted varieties) are clearly not the most abundant in the studied population (Tables 1 & 2). However, one cannot totally exclude the possibility that luminescence in *A. squamata* may have a protective function as it appears that individuals always produce a more intense light when brooding whatever the photogenic capabilities of the their variety.

We thank N. De Bremaeker, A. Borges, E. Lamarque and G. Seghers for field assistance; Professor J. Avoine for providing facilities at the Marine Laboratory of Luc-sur-Mer (France). We are grateful to anonymous referees for their constructive comments. Research supported by a IRSIA grant to D. Deheyn, a UCL FDS grant to J. Mallefet and by a FRFC convention (ref. 6.231.85). Contribution of the 'Centre Interuniversitaire de Biologie Marine' (CIBIM). J.M. is a research associate of the National Fund for Scientific Research (FNRS, Belgium).

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Submitted 28 December 1995. Accepted 28 August 1996.