



Evidence of seasonal variation in bioluminescence of *Amphipholis squamata* (Ophiuroidea, Echinodermata): effects of environmental factors

D. Deheyn^{a,*}, J. Mallefet^b, M. Jangoux^{a,c}

^aMarine Biology Laboratory, Brussels Free University (ULB), Brussels, Belgium

^bAnimal Physiology Laboratory, Louvain Catholic University (UCL), Louvain-la-Neuve, Belgium

^cMarine Biology Laboratory, Mons-Hainaut University (UMH), Mons, Belgium

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Abstract

The bioluminescence of *Amphipholis squamata* was assessed from freshly collected individuals for 16 successive months, and from individuals maintained in the laboratory under various experimental conditions of salinity, temperature and photoperiodic regime. Field investigations showed that bioluminescence intensity and kinetics varied seasonally, with the light produced being brighter and faster in winter and summer. The seasonal variation was not correlated with changes of ambient salinity. However, it was correlated with changes in temperature, the luminescence being brighter and faster in coldest and warmest seasons, and with the changes of photoperiod, the luminescence being brighter and faster in seasons with shortest and longest day length. Laboratory investigations also demonstrated that luminescence was not affected by salinity conditions. Conversely, luminescence was affected by temperature, the light production being brighter and faster in warmer conditions (in agreement with field observations) and dimmer and slower in colder conditions (in disagreement with field observations). Light production was also affected by photoperiod since experimental changes of natural light:dark regime caused the bioluminescence to decrease. Considering that photoperiod guides the biology of *A. squamata* and that reproduction takes place during coldest months in the species, an endogenous factor of neurophysiological nature linked to the ophiuroid reproductive cycle is proposed to induce the luminescence to peak in winter. This was confirmed by the fact that seasonal variation of luminescence was different between adult and juveniles, the latter showing no winter peak of luminescence. It is suggested that the luminescence normally associated with defense could also be part of an intraspecific visual signal related to individuals aggregating for reproduction during winter. © 2000 Elsevier Science B.V. All rights reserved.

*Corresponding author. Present address: Marine Biology Research Division, Scripps Institution of Oceanography, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0202, USA. Tel.: +1-858-534-6579; fax: +1-858-534-7313.

E-mail address: ddeheyn@ucsd.edu (D. Deheyn)

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1. Introduction

Bioluminescence is the production of visible light by living organisms (Hastings and Morin, 1991). In the marine environment, the light production can be influenced by exogenous factors such as salinity, temperature or photoperiodic regime (Tett, 1969; Hastings, 1986; Shimomura, 1986; Latz, 1995). Because of these environmental effects, luminescence may have seasonal variations which might be consistent with changes in the use of the luminous signals (Tett, 1972; Christophe and Baguet, 1985; Fischer and Fischer, 1995).

The varied functions of luminescence are associated with visual signals of inter- or intraspecific communication (Morin, 1983; Herring, 1990). Interspecific functions are related to predation and/or defense, the luminescence being used to attract prey, to frighten a potential predator or warn it by aposematism, or to avoid detection by countershading camouflage. Intraspecific functions are related mainly to courtship, mating and territoriality behaviour. In many organisms bioluminescence serves multiple functions, e.g. a luminescent signal can be produced at any time for defense but only at the time of reproduction for mating (Mauchline, 1960; Tett, 1972; Christophe and Baguet, 1985; Fischer and Fischer, 1995). Whether the function is inter- or intraspecific depends on the nature of the receptor individual (emitter–receptor conspecifics or not), and on the period of the emitter biological cycle (period of foraging, sexual maturity, nursing or schooling). The luminous function is likely to change with context of luminescence production, such as in copepods that normally produce light for defense in response to mechanical stimulation, but during the mating period they also produce light spontaneously in response to visual stimulation from conspecifics (Mauchline, 1960; David and Conover, 1961; Morin and Cohen, 1991).

Echinoderms include numerous luminescent species for which the luminescence is believed to be produced for the sole purpose of defense (Herring, 1995). *Amphipholis squamata* is a luminescent ophiuroid in which the function of luminescence has been little commented on, probably because of the small size of individuals (see below). The effect of environmental conditions on bioluminescence is not known, and the only factor which may have an influence is the individual's brooding condition, the luminescence becoming brighter and faster when embryos or juveniles are housed in the bursa (Deheyn et al., 1997).

The present study aimed to determine whether the bioluminescence of *A. squamata* displayed a seasonal variation correlated with that of salinity, temperature and photoperiodic regime in the field. The environmental factors considered were each assessed separately for their influence on luminescence in the laboratory. Possible factors causing the luminescence to vary seasonally are suggested after comparison between field and experimental observations, and function(s) of the luminescence are discussed taking into consideration both the seasonal variation of light production and the ophiuroid reproductive cycle.

2. Materials and methods

2.1. Collection and maintenance

Specimens of *Amphipholis squamata* (Delle Chiaje, 1828) were collected by hand in the intertidal population of Langrune-sur-Mer (Normandy, France), fine forceps being used for the collection as the ophiuroid is of small size (disc diameter of ca. 3 mm and arm length of ca. 17 mm for adult specimens). The individuals were maintained for 3 days in open-circuit marine aquaria in the nearby located marine station of Luc-sur-Mer. There they were screened first for size and shape, those used being the adults (disc diameter > 1.6 mm) with four or five intact arms (without regenerating stumps). They were then screened for colour variety since six colour varieties with their own luminescence are known from the population (Deheyn et al., 1997). In the present study, only individuals of the black variety were used as they are abundant and highly luminescent (Deheyn et al., 1997).

2.2. Field seasonal variation in bioluminescence

Individuals were collected monthly from January 1993 to April 1994. At least 15 individuals were tested for light production each month. For luminescence measurements (see below), individuals were transferred to the Louvain-la-Neuve University (UCL, Belgium) in 30-l containers filled with seawater with algae (*Ulva* sp., *Enteromorpha* sp., *Sargassum* sp.) in which the ophiuroid could take refuge. Luminescence was investigated within 2 days following the transfer. During these 2 days individuals were kept under simulated field conditions of salinity, temperature and photoperiodic regime; they were placed in closed-circuit aquaria filled with seawater taken from the field at the time of collection, and containing stones under which the individuals could hide. Temperature and salinity data for the area were provided by IFREMER (French Institute for the Study of the Sea). Values of field photoperiod we considered were the light:dark regime (L:D) of the middle of each month (data from the French Official Tide Table).

2.3. Effects in the laboratory of environmental factors on bioluminescence

The effects of salinity, temperature and photoperiod were investigated independently in separate aquaria. The seawater salinity (in PSU, Practical Salinity Unit) or temperature were modified for each aquarium by 2 PSU/day or 2°C/h until the individuals were under the appropriate saline and thermal conditions (the tested conditions were considered as 'realistic' as they could occur in the field), whereas changes in the photoperiod were done in one step from the field to the final conditions. Only individuals with five intact arms were used in experiments. They were acclimated for 3 weeks in the various experimental conditions and the seawater, continuously filtered and aerated, was checked every other day for pH (7.8), nitrite (<0.1 mg/l), nitrate (<0.1 mg/l), salinity and temperature. Liquid food designed for marine invertebrates (Liquifry Marine Interpet) was added once a week to the seawater. After the 3-week period, the individuals' luminescence was measured (see below). The results were then compared

with the bioluminescence from 6 to 15 individuals from the field measured immediately prior to each experiment.

2.3.1. Salinity

The effects of salinity were tested in December 1994 on batches of six individuals under four saline conditions (27, 30, 33 and 36 PSU), while temperature and photoperiod were maintained constant similar to field values (9°C, 8:16 h L:D).

2.3.2. Temperature

The effects of temperature were investigated on three samples collected in April and October 1994, and in February 1995. For each sample batches of seven to 12 individuals were exposed to four temperatures (8, 12, 16 and 20°C), while salinity and photoperiod remained constant for the three assays (32 PSU, 12:12 h L:D).

2.3.3. Photoperiodic regime

The effects of photoperiod were tested in June 1994 (16:8 h L:D) and November 1994 (9:15 h L:D). For each sample, the effect was tested on batches of six individuals transferred to three aquaria under constant light:dark regime (6:12 h, 12:12 h and 18:6 h). Each aquarium was isolated from the external light and illuminated using a cool white fluorescent tube (OSRAM 18W/25, 400–620 nm) whose light was reduced through a translucent PVC sheet. Temperature and salinity during the experiments remained constant for the two assays (12°C, 32 PSU).

2.4. Bioluminescence measurement

Arms are the only luminescent body parts in *A. squamata* and the luminescence was measured from individuals with at least four intact arms using 200 mM KCl stimulation to maximize the bioluminescence production (Mallefet et al., 1992). The measurement was always done for each of the arms following the same procedure (Deheyn et al., 1997). Individuals were anaesthetized in a 3.5% w/w MgCl₂ seawater solution and their arms measured (length, in mm) before being separated from the disc using a fine scalpel. Temporal differences in arm length were tested for significance to determine whether it could contribute to seasonal variation of luminescence. The 10 bursa of the disc were dissected under a binocular microscope and the presence vs. absence of embryos and/or juveniles was noted. The brooding/non-brooding ratio of individuals was considered as possibly contributing to the seasonal variation of luminescence as it is known that brooding individuals produce a higher luminescence (Deheyn et al., 1997). Juveniles were extracted from the bursa and the disc diameter and arm length measured. *A. squamata* is a simultaneous hermaphrodite with 10 testes and 10 ovaries that can in turn produce fertile gametes all year around (Fell, 1946; Emson and Whitfield, 1989). However, the ophiuroid reproductive effort varies according to season and reaches complete and maximal (the 20 gonads together) sexual maturity in winter (Jones and Smaldon, 1989; Alva, 1996). Therefore it was common to find large variability within sizes of juveniles every month. Only those with a disc diameter between 0.6 and 0.8 mm were considered for luminescence measurements (Deheyn and Jangoux, 1999). The light

produced by a juvenile was measured from only one of its arms, because the individual then has similar length arms with presumably equivalent luminous capability (Mallefet et al., 1992). All the luminescence studies were carried out in a dark room at 18°C using as light detector an IP21-S20 phototube connected to an IL 760 photomultiplier and an IL 1700 radiometer. The light output was transformed into an analog voltage that was graphically recorded (Sevorgor S), and every month and prior to the measurements, the experimental set-up was calibrated using a tritium-phosphor source, the light production of which was of known intensity and in the similar spectral distribution of *A. squamata* luminescence (Deheyn et al., 1997).

Two parameters were used to characterize ophiuroid bioluminescence after KCl stimulation: the maximal luminescence intensity and the kinetic parameter (Deheyn et al., 1997). The maximal light intensity (LMax) indicates the maximal flux of quanta energy associated with the emitted photons. It is expressed in megaquanta produced per second normalized per millimetre of arm (Mq/s/mm). LMax represents the maximal luminescence energetically possible for the arm, and thus for the individual. The kinetic parameter (TLMax) is the time taken for the arm to reach maximal light intensity. TLMax is expressed in seconds (s) and is linked to the interaction between the reactives (including cofactors) leading to the light production. KCl induces instantaneous and maximal depolarization of all cells including the luminescence producing cells (Mallefet et al., 1992; Deheyn et al., 1996), and KCl stimulation thus guarantees that the entirety of the luminous material available is consumed in the luminescence reaction and that stimulation occurs as fast as chemically possible, the time of diffusion and transduction through tissues being minimized.

2.5. Statistical analyses

Statistical analyses were based on a significance level (α) of 0.05, using Statview 4.0, SuperAnova and Systat 5.0 software. Nested analysis of variance (arms being nested within individual) and multiple means comparison analyses (Tukey) were used to test the significance of differences between the monthly measurements of luminescence and those of arm length, between individuals in determined experimental conditions of salinity, temperature and photoperiod, and between experimental individuals and the field controls.

The seasonal variations of salinity, temperature, photoperiod and luminescence were analysed for periodicity by testing their agreement with the theoretical model of periodic regression (Halberg et al., 1987):

$$Y = \mu + \delta \times \sin(\phi \times \text{Month} - \lambda)$$

where μ is the mean of the variable, δ the amplitude of the fluctuation, ϕ the angular frequency of the periodic variation, and λ the maximal value the sinusoidal function can reach.

The parameters were estimated using the nonlinear procedure of Quasi-Newton applied to the original values without transformation so that fitness of the models, indicated by the R^2 corrected for non-linear systems (Zar, 1996), integrated both the

within-each-month variation and the variation from month to month. Calculated curves thus represented expression of the between-month variation, i.e. the seasonality of the variation. For each of the factors the periodic regression of the model was fitted with the original dataset (the modeled curves were superimposed on the original values).

Correlation between the seasonal variation of luminescence intensity and kinetics, and those of salinity, temperature and photoperiod was tested using the ρ correlation coefficient of Spearman (Conover, 1980). The correlations were determined from paired comparisons of $N = 160$ values calculated by each of the models in which the variable 'month' was made to increase gradually (with step 0.1) from 0 to 16.

3. Results

3.1. Field seasonal variation in bioluminescence

In total, the luminescence of 1727 arms from 393 individuals was measured. Each month 72 to 164 arms were stimulated from 15 to 37 individuals, respectively (Table 1). Arms were of similar length within each month and from one month to another, except in July and August 1993 when arms were significantly shorter ($P < 0.0001$; Table 1). Individuals used were either brooding or non-brooding adults, both being present at all

Table 1

Amphipholis squamata. Number (N) of individuals investigated from January 1993 to April 1994, with their arm length (mean values $\pm 95\%$ confidence limit) and with details of the number of brooding and non-brooding specimens

Month	Ophiuroids			
	N	Arm length	N_{Brooding}	$N_{\text{Non-brooding}}$
1993				
January	15	8.6 \pm 0.6	11	4
February	27	8.4 \pm 0.4	21	6
March	26	9.4 \pm 0.4	20	6
April	23	8.9 \pm 0.5	22	1
May	18	9.2 \pm 0.5	16	2
June	20	9.1 \pm 0.6	20	–
July	37	5.6 \pm 0.3*	15	22
August	22	6.8 \pm 0.5*	7	15
September	26	8.1 \pm 0.3	9	17
October	32	8.6 \pm 0.4	9	23
November	21	7.7 \pm 0.4	12	9
December	29	9.1 \pm 0.4	10	19
1994				
January	24	9.2 \pm 0.4	11	13
February	30	9.2 \pm 0.4	18	12
March	27	8.8 \pm 0.4	18	9
April	16	8.7 \pm 0.5	16	–
Total	393	8.4 \pm 0.1	235	158

* Significantly different with $P < 0.0001$.

months, except in June 1993 and April 1994 (only brooding individuals). Brooding individuals were more abundant from February to April 1993, non-brooding ones from August to October 1993 (Table 1).

Arms always produced luminescence in response to KCl and the luminescence differed among months ($P < 0.0001$), both in light intensity and light kinetics (Fig. 1A). The variation was identical for non-brooding and brooding individuals (data not shown) except that luminescence of the brooders was always brighter and faster than that of the non-brooding, as already reported by Deheyn et al. (1997).

LMax values (luminescence intensity) showed temporal variations that were seasonal, being high from January to March and from July to September and, alternatively, low from April to June and from October to December (Fig. 1A). Seasonality of the LMax variation was statistically supported based on the significant fit of a periodic regression (Fig. 2A):

$$\text{LMax} = 983.4 + 378.3 \times \sin(1.069 \times \text{Month} - 4598.9)$$

$$\text{with } R_{\text{corr}}^2 = 0.202, P < 0.0001, N = 1727.$$

Periodicity of the LMax variation (fixed by the angular factor) appears to be about 6 months. Temporal variation of the luminescence intensity varies alternatively from lower to higher values in the same range around the mean intensity ($\text{LMax} = 983.4 \pm 378.3$ Mq/s/mm, i.e. sinus function ± 1), yet the model also considers that the luminescence intensity may reach extreme values up to 4598.9 Mq/s/mm. The significance of the model indicates that altogether 20.2% of the LMax variation was due to the factor 'season'.

TLMax values (luminescence kinetics) also showed seasonal variation, being low in February–March and in August–September and, by contrast, high in January, May–June and from October to December (Fig. 1A). Seasonality of the TLMax variation was confirmed as statistically significant by the periodic regression (Fig. 2A):

$$\text{TLMax} = 3.0 + 1.6 \times \sin(1.082 \times \text{Month} - 17.1)$$

$$\text{with } R_{\text{corr}}^2 = 0.192, P < 0.0001, N = 1727.$$

Periodicity of the TLMax variation appears to be about 6 months. Temporal variation of the luminescence kinetics varies around the mean value ($\text{TLMax} = 3.0 \pm 1.6$ s), yet the model considers that higher values up to 17.1 s can also be reached. The model significance indicates that altogether 19.2% of the TLMax variation was due to the factor 'season'.

The pattern for TLMax was opposite that of the LMax curve and the two parameters were linked by an exponential decay relationship (Fig. 3). According to the relation, TLMax would be 6.1 s for LMax=0 before it decreased to 1.3 s for the higher LMax values (Fig. 3). Yet, only 17% of the TLMax variation was linked to the LMax variation ($R_{\text{corr}}^2 = 0.17$) and various other factors of endogenous or exogenous (environmental) origin could therefore contribute to the observed TLMax variation.

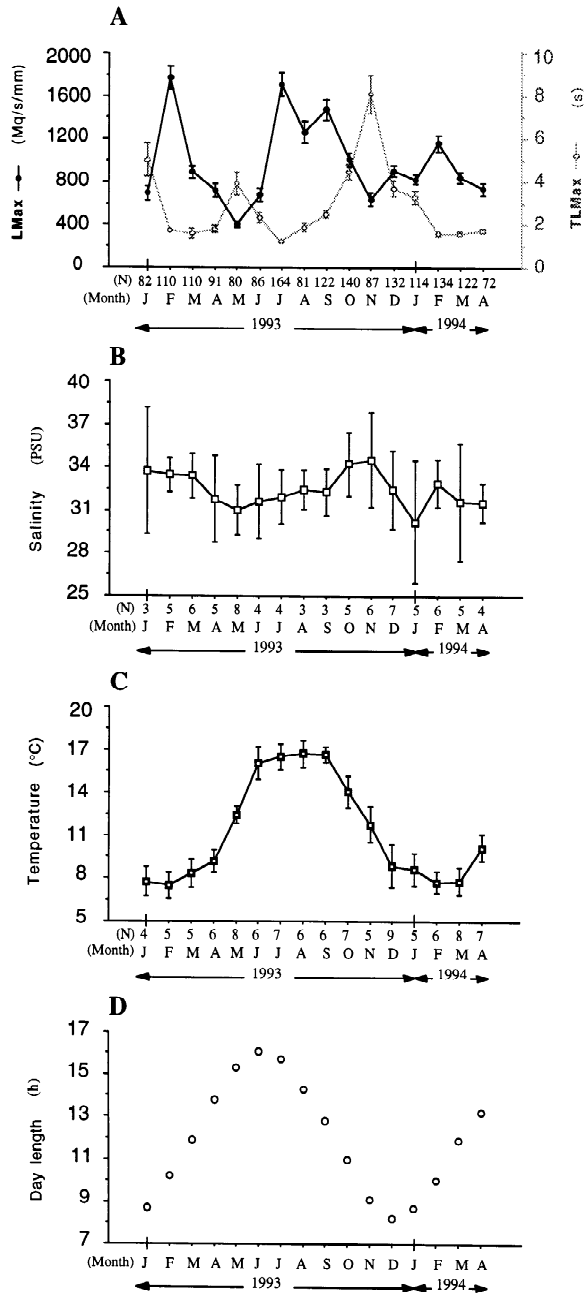


Fig. 1. *Amphipholis squamata*: seasonal variation from January 1993 to April 1994 of (A) luminescence intensity (LMax in Mq/s/mm; black circle) and kinetics (TLMax in s; grey circle), (B) ambient salinity, (C) ambient temperature and (D) photoperiodic regime (mean values \pm 95% confidence limit, except for D). *N* is the number of measurements.

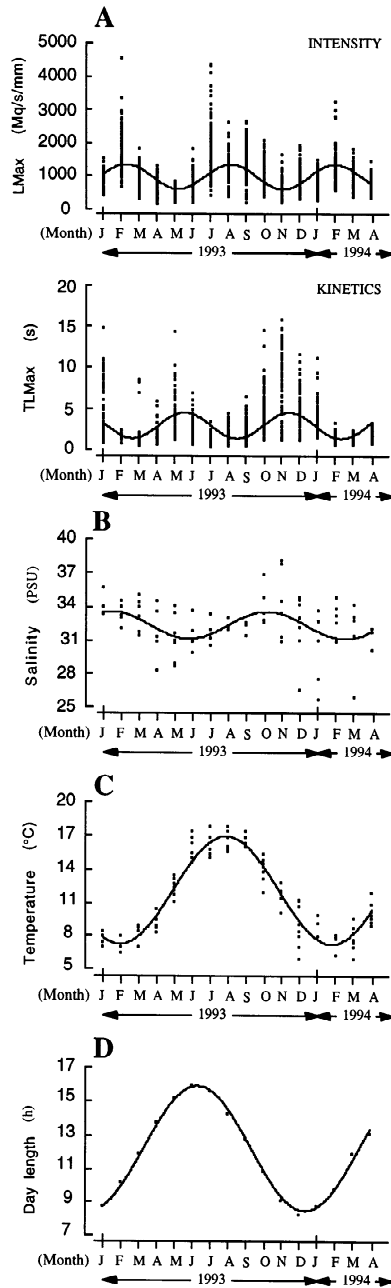


Fig. 2. *Amphipholis squamata*: periodic regressions fitting the seasonal variation from January 1993 to April 1994 of (A) luminescence intensity (LMax) and kinetics (TLMax), (B) ambient salinity, (C) ambient temperature and (D) photoperiodic regime; (original dataset shown).

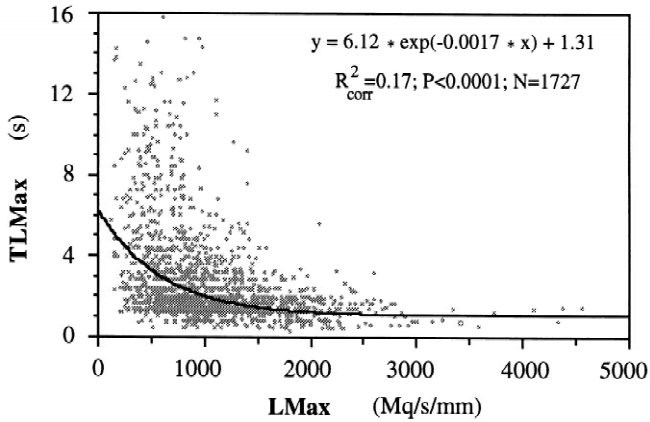


Fig. 3. *Amphipholis squamata*: exponential relation decay between luminescence intensity (LMax) and kinetics (TLMax) calculated from values of the 16 investigated months together (original dataset shown).

The field salinity, temperature and photoperiod were measured during the period of investigation (Fig. 1B–D) and seasonal variation in each of these environmental factors were fitted significantly by the same model of periodic regressions as previously used (Fig. 2B–D). Salinity was not significantly different between months as variability was high within each set of measurements, the salinity ranging from 30 to 34 PSU with at some time high values that exceeded 36 PSU in January and November 1993, and low values that fell down to around 26 PSU in January 1994 (Fig. 1B; see range of confidence limit). Seasonality of the salinity variation was modeled significantly by the periodic regression (Fig. 2B):

$$\text{Salinity} = 32.5 + 1.3 \times \sin(0.72 \times \text{Month} - 45.6)$$

$$\text{with } R_{\text{corr}}^2 = 0.161, P < 0.0001, N = 79.$$

No significant correlation was found between the variations in salinity and those in LMax ($\rho = 0.043$, $P = 0.604$) and TLMax ($\rho = -0.070$, $P = 0.392$). Yet, it was observed that months of the highest salinity (January and November 1993) were also months with the highest TLMax values (Fig. 1A and B).

Temperature and photoperiod each varied significantly between months ($P < 0.0001$; Fig. 1C and D). Temperature in the field was lowest (around 8°C) from January to March, and highest (around 18°C) from July to September (Fig. 1C). Seasonal variation of the temperature was modeled significantly by a periodic regression (Fig. 2C):

$$\text{Temperature} = 12.2 - 4.9 \times \sin(0.53 \times \text{Month} - 20.1)$$

$$\text{with } R_{\text{corr}}^2 = 0.910, P < 0.0001, N = 100.$$

Periodicity of the temperature variation appears to be about 12 months. The seasonal

variation of luminescence was significantly correlated with that of temperature (LMax: $\rho = -0.306$, $P = 0.0001$; TLMax: $\rho = 0.291$, $P = 0.0003$), and seasons with brightest and fastest luminescence coincided with months of lowest and highest temperatures (Fig. 2A–C).

The photoperiod changed significantly between months ($P < 0.0001$) and the values of field light:dark regime were lowest in winter (8:16) and highest in summer (16:8) (Fig. 1D). Seasonal variation of the photoperiod was modeled significantly (Fig. 2D):

$$\text{Day length} = 12.2 - 3.80 \times \sin(0.52 \times \text{Month} - 17.4)$$

$$\text{with } R_{\text{corr}}^2 = 0.996, P < 0.0001, N = 16.$$

Periodicity of the photoperiod variation appears to be about 12 months. The seasonal variation of luminescence was significantly correlated with that of photoperiod (LMax: $\rho = -0.272$, $P = 0.007$; TLMax: $\rho = 0.226$, $P = 0.005$), and the luminescence intensity decreased along with day length from August to November while both the luminescence and photoperiod increased together from December to February (Fig. 2A–D).

Juveniles were also assessed for seasonal variation of luminescence. They were taken every month from field brooding individuals, and from one to 32 juveniles each month were large enough for luminescence measurement (see Section 2.4) (Fig. 4). Light was always produced after stimulation and the bioluminescence was significantly different between months. LMax values were lowest from February to April and highest from August to October (Fig. 4). Seasonality of the LMax variation was confirmed as fitted significantly by a periodic regression (Fig. 4):

$$\text{LMax}_{\text{Juv}} = 53.12 + 37.06 \times \sin(0.542 \times \text{Month} - 179.3)$$

$$\text{with } R_{\text{corr}}^2 = 0.169, P < 0.0001, N = 105.$$

Periodicity of the LMax variation appears to be about 12 months for the ophiuroid

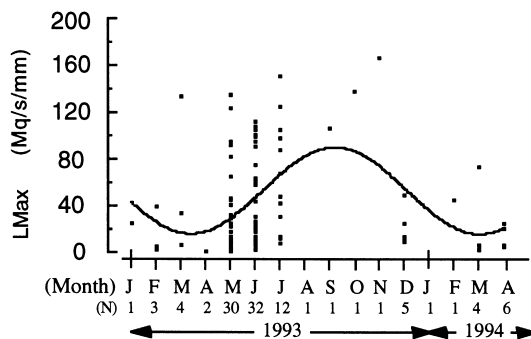


Fig. 4. *Amphipholis squamata*: periodic regression fitting the seasonal variation of luminescence intensity (LMax) from January 1993 to April 1994 for brooded juveniles. N is the number of brooded juveniles stimulated to produce luminescence (original dataset shown).

when it is juvenile. Temporal variation of the luminescence intensity varies in the same range around the mean intensity ($L_{Max} = 53.12 \pm 37.06$ Mq/s/mm), yet the model also considers that the luminescence intensity may reach higher values up to 179.3 Mq/s/mm. The significance of the model indicates that altogether 16.9% of the L_{Max} variation was due to the factor 'season'. Seasonal variation in juveniles was significantly correlated with temperature, highest luminescence coinciding with warmest field conditions ($\rho = 0.610$, $P < 0.0001$) (compare Fig. 2C and Fig. 4).

3.2. Effects in the laboratory of environmental factors on bioluminescence

Individuals survived the transfer to, and the maintenance in, various experimental conditions of salinity, temperature and photoperiod. The well-being of experimental individuals was indicated by the fact that only a few showed observable signs of stress (i.e. autotomized arms). Salinity, temperature and photoperiod had significant effects on luminescence ($P < 0.0001$) and these effects were different for each of the investigated factors.

3.2.1. Salinity (Fig. 5)

Salinity did not have a significant effect on luminescence except at 36 PSU, L_{Max} being reduced and TL_{Max} increased in comparison to that of field-collected specimens and to the other experimental conditions. For experimental salinities ranging from 27 to 33 PSU, L_{Max} was high and TL_{Max} low, as was found for field-collected specimens (Fig. 5).

3.2.2. Temperature (Fig. 6)

The effect of temperature was similar for the 3 months investigated: the luminescence was dimmer and slower at lower temperatures, and brighter and faster at higher

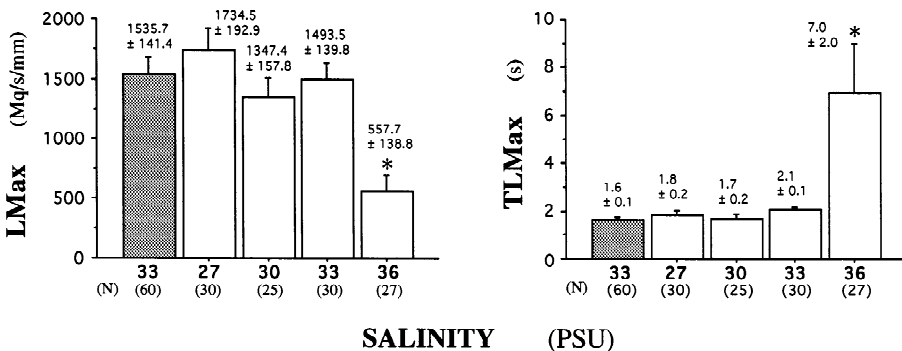


Fig. 5. Salinity effect on *Amphipholis squamata* luminescence: mean values ($\pm 95\%$ confidence limit) of luminescence intensity (L_{Max}) and kinetics (TL_{Max}) in various conditions of salinity (27, 30, 33 and 36 PSU). The experiment took place in December 1994. Grey bar for field-collected individuals measured prior to the experiment. *Means significantly different from the grey bar (nested ANOVA). N is the number of stimulated arms. Experimental temperature was 9°C and photoperiod 8:16 h L:D.

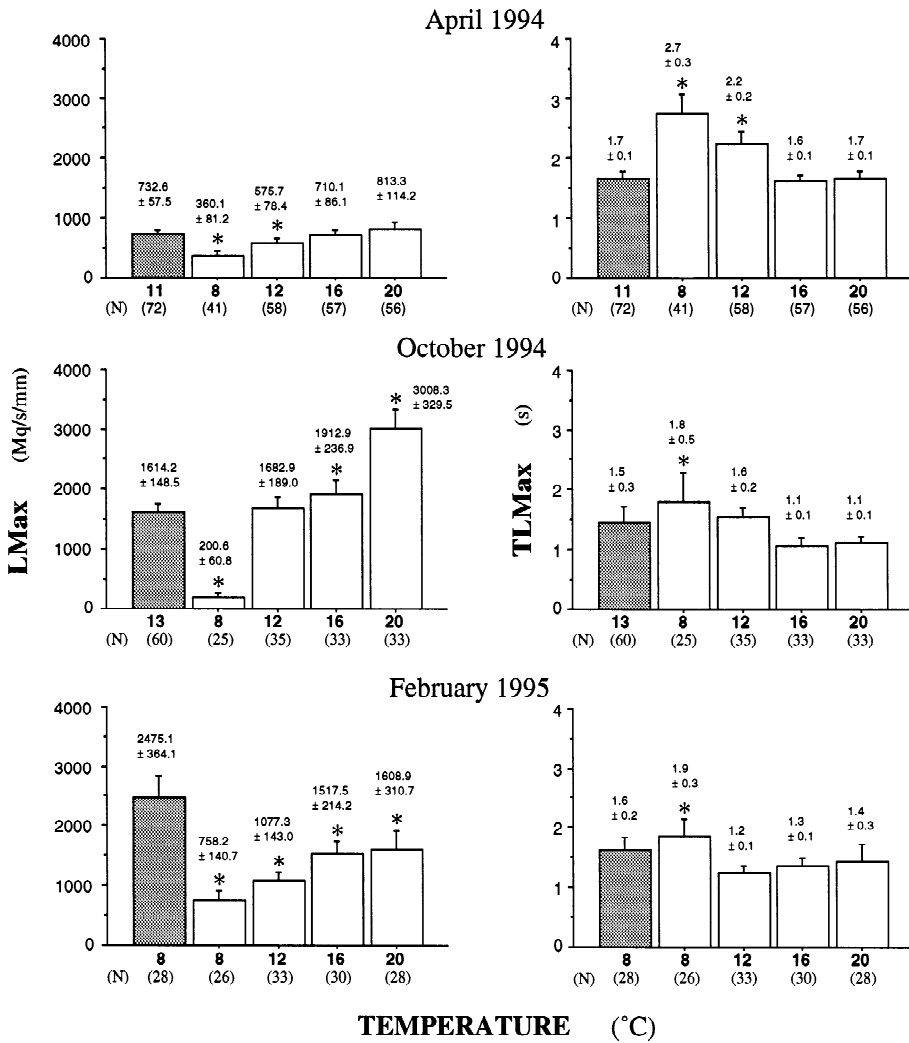


Fig. 6. Temperature effect on *Amphipholis squamata* luminescence: mean values ($\pm 95\%$ confidence limit) of luminescence intensity (LMax) and kinetics (TLMax) in various conditions of temperature (8, 12, 16 and 20°C). The experiments took place in April 1994, October 1994 and February 1995. Grey bar for field-collected individuals measured prior to the experiment. *Means significantly different from the grey bar (nested ANOVA). *N* is the number of stimulated arms. Experimental salinity was 32 PSU and photoperiod 12:12 h L:D for the three assays.

temperatures. Increase in temperature thus induced an increase of LMax and decrease of TLMax. It must be noted, however, that the level of luminescence intensity was different among the investigated months, e.g. LMax at 8°C was about 360, 200 and 760 Mq/s/mm in April 1994, October 1994 and February 1995, respectively. By contrast, luminescence kinetics were more consistent and with reference to the cited example,

TLMax was about 2.7, 1.8 and 1.9 s, respectively (Fig. 6). It must also be noted that after 3 weeks acclimation, the luminescence of experimental individuals became different from field-collected specimens, even when maintained at temperatures close to the natural condition (except from the October 1994 experiment; compare grey and white bars in Fig. 6). This was the case in April 1994 when at 12°C, LMax was lower and TLMax higher than for field-collected specimens at 11°C ($P < 0.0001$). In February 1995, experimental LMax at 8°C was lower than for field-collected specimens, also at 8°C ($P < 0.0001$), whereas TLMax was more consistent between field-collected and laboratory-maintained specimens (Fig. 6).

3.2.3. Photoperiodic regime (Fig. 7)

Results were similar for the 2 months investigated. Photoperiod had an effect on bioluminescence and the effect was directly related to the similarity of the experimental photoperiod to that in the field. Experimental LMax and TLMax were similar to field

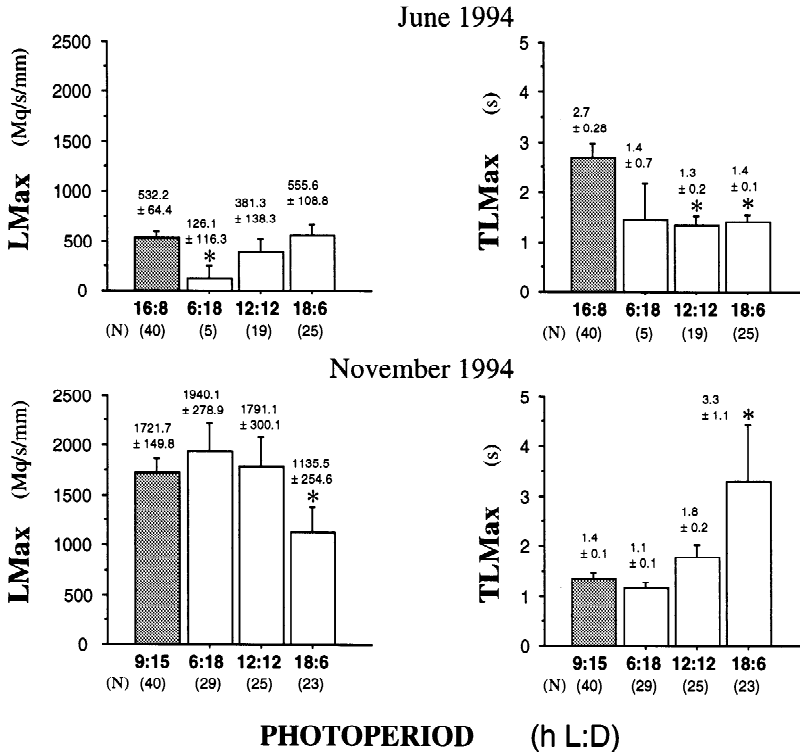


Fig. 7. Photoperiod effect on *Amphipholis squamata* luminescence: mean values (±95% confidence limit) of luminescence intensity (LMax) and kinetics (TLMax) in various conditions of light:dark regime (6:18 h, 12:12 h and 18:6 h). The experiments took place in June 1994 and November 1994. Grey bar for field-collected individuals measured prior to the experiment. *Means significantly different from the grey bar (nested ANOVA). N is the number of stimulated arms. Experimental salinity was 32 PSU and temperature 12°C for both assays.

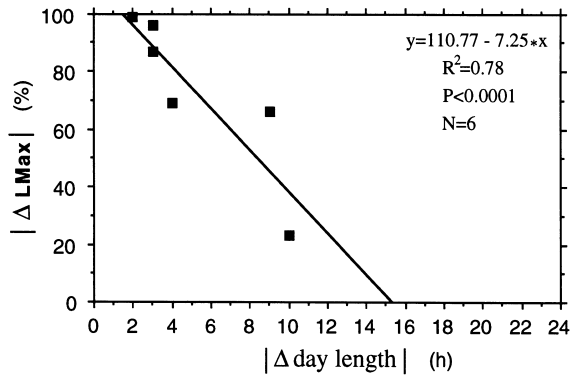


Fig. 8. Photoperiod effect on *Amphipholis squamata* luminescence: linear regression between changes of luminescence intensity and changes of photoperiod. Changes of luminescence are shown by the absolute difference of LMax between the field-collected and the experimental individuals (y , in %); changes of photoperiod are shown by the absolute difference of day length between the field and the experiment (x , in h).

values for light:dark regimes most similar to the natural one (except for TLMax in June 1994). Conversely, the greater the difference between experimental and natural regimes, the lower the LMax and the higher the TLMax. Because the months investigated were opposite for the photoperiodic regime, the luminescence variations in given experimental light:dark regimes were opposite for the 2 months investigated. For instance, in comparison to the natural photoperiod, LMax was decreased at 6:18 h and unchanged at 18:6 h in June 1994 (16:8 h natural L:D) whereas it was unchanged at 6:18 h and decreased at 18:6 h in November 1994 (9:15 h natural L:D). Considering y the absolute value of the relative expression in % of LMax level and x the absolute difference of light:dark regime expressed in hours of day length between the field and the laboratory, and grouping together results from the 2 months investigated, the relation between y and x was expressed by a linear regression (Fig. 8). It showed that the greater was the difference between experimental and natural regimes the lower was the LMax. LMax in experimental conditions remained equivalent to the field one ($y = 100\%$) when the difference in photoperiodic regimes was less than 1.5 h, LMax being decreased till no more luminescence produced ($y = 0$) when the difference in photoperiodic regimes was increased to 15.3 h (Fig. 8).

4. Discussion

The luminescence of *Amphipholis squamata* in the field displayed a seasonal variation being brighter and faster in winter and summer, dimmer and slower in spring and autumn. The seasonal variation was not due to a difference in size of individuals because arms length was similar among months. Only adult individuals were investigated and the seasonal variation was not due to a difference in the ratio of brooding/non-brooding individuals. Both brooding and non-brooding were considered in the luminescence measurements (and both displayed seasonal variation in light production) and only when

brooding individuals were present (June 1993 and April 1994) was luminescence intensity the lowest. Therefore, changes in brooding condition did not contribute to the seasonality of luminescence because brooding individuals are known to produce highest luminescence (Deheyn et al., 1997).

Among the other possible factors likely to affect luminescence, we investigated salinity, temperature and photoperiod. Salinity had no major effect on the luminescence. Yet, high saline conditions (>36 PSU) should be considered as a possible factor making the light production slower. Indeed this was observed under experimental conditions (Fig. 5) and in the field because the slowest luminescence occurred when the salinity was among the highest (January and November 1993) (Fig. 1A and B). Temperature had a significant effect on bioluminescence, the light production being brighter and faster when temperature was higher. Accordingly, luminescence intensity in field-collected specimens was higher in summer. Yet, luminescence also peaked in winter when field temperature was lowest, contra-indicating a temperature effect at that season, thus suggesting other factor(s) influence the luminescence. Photoperiod could be a factor having such an influence because it was shown under experimental conditions that changes of light:dark regime greater than 1.5 h affected individuals whose bioluminescence was then reduced (Figs. 7 and 8). Effect of photoperiod also appeared through the temperature experiments done in the laboratory. In that case, experimental photoperiod was maintained constant at 12:12 h L:D whereas natural photoperiod was 14:10 h L:D in April, 11:13 h L:D in October and 10:14 h L:D in February. Luminescence intensity compared between the field-collected and the laboratory-maintained specimens for temperature closest to the natural value (grey vs. white bars; Fig. 7) showed that LMax were similar in October 1994 (similar photoperiods) but different in April 1994 and February 1995 with then experimental LMax values lower. In those cases, field and experimental photoperiods were different with more than the threshold value of 1.5 h, which could be the possible factor causing the luminescence decrease. Difference in the photoperiods could also explain that the range of experimental luminescence from 8 to 20°C was different among the investigated months, with LMax ranging from 400 to 800 Mq/s/mm in April 1994, from 200 to 3000 Mq/s/mm in October 1994, and from 800 to 1600 Mq/s/mm in February 1995, while the range of TLMax values was more consistent (Fig. 6). Yet, such an effect could as well be related to the different physiological condition of individuals among the investigated months.

Photoperiod as a key factor affecting luminescence was also supported by the fact that a significant correlation was found between the seasonal variation of luminescence and that of photoperiod, which showed that LMax increased with day length in winter (Fig. 2A–D). Considering this with the fact that photoperiod is a main factor guiding the physiology of organisms (Vernberg and Vernberg, 1972), it is suggested that photoperiod would act on the ophiuroid light production capability not directly, but by acting first on an endogenous factor of neurophysiological nature which would in turn influence bioluminescence. It already has been suggested that physiological condition of the ophiuroid could modulate the light production, as brooding (either a juvenile or a parasite) increases the ophiuroid luminous capability (Deheyn et al., 1997, 1998).

Natural change of photoperiodic regime guides the reproductive biology of many organisms including echinoderms (Bay-Schmith and Pearse, 1987; Bouland and

Jangoux, 1988; Xu and Barker, 1990). This is also the case for *A. squamata* in which, for example, maturity of the testes is correlated with the photoperiod in winter, the testes maturity index being lowest in November–December and highest in February–March (Alva, 1996). The reproductive cycle usually goes along with a seasonal variation of the individual endogenous (physiological and/or neurophysiological) condition (Calow, 1979; Voogt et al., 1986; Khotimchenko and Deridovich, 1988). Therefore, it is believed that the winter peak of luminescence occurring in *A. squamata*, while correlated to the low temperature and the increasing day length of the season, was in fact induced by a particular endogenous condition of individuals. The reproductive cycle of *A. squamata* can be summarized as brooded juveniles that grow during spring and are released in summer, after which the gonads go through the maturity process from autumn to the early winter (Emson and Whitfield, 1989; Jones and Smaldon, 1989; Alva, 1996). The reproductive effort is then maximal and followed by breeding that thus coincides with the coldest months of the year, i.e. February–March for northern temperate locations (Alva, 1996). This is exactly when luminescence is also highest, and it seems therefore that the seasonal variation of luminescence of *A. squamata* originates from the alternate influence of two factors, an exogenous factor (temperature) causing the luminescence to increase in summer, and an endogenous factor linked to the reproductive cycle and guided by the photoperiod that causes the luminescence to increase in winter. This hypothesis was supported by the single peak seasonal variation of luminescence observed in brooded juveniles (Fig. 4). Their luminescence intensity increased only in summer with no peak in winter as for adult individuals, which could be due to the fact that juveniles do not have changes of physiology in relation to the reproductive cycle.

Luminescence is associated with several inter- and/or intraspecific functions whose efficiency increases with the luminescence intensity. Luminous signals are brighter when associated with defense, territoriality or courtship of sexual partners, dimmer when used for camouflage (Morin, 1983; Hastings and Morin, 1991; Latz, 1995). Luminous signals are rarely associated with one single function, and the latter possibly changes according to the physiological condition linked to the reproductive cycle of the emitter and/or according to nature of the receptor organism (conspecific or not, potential predator or not) (Herring, 1990; Hastings and Morin, 1991; Seliger, 1993). It is common for luminous organisms to display seasonal variation in luminescence, and the function of the light produced can change according to the intensity of light production, thus according to the season (Tett, 1969, 1972; Christophe and Baguet, 1985; Fischer and Fischer, 1995). This suggests that seasonality in the luminescence of *A. squamata* could have particular and possibly different functions in summer and winter (periods of highest bioluminescence). Luminescence in ophiuroids is associated with defense behaviour to deter predators (Basch, 1988; Grober, 1988a,b), and *A. squamata* may use the bioluminescence for defense (see Deheyn et al., 1999), which would be useful especially in summer when potential predators (crustaceans and fishes) are most active. In winter, organisms are less active and the predation low on *A. squamata* (Jones and Smaldon, 1989; Alva and Jangoux, 1990). Hence, one can question whether the luminescence is produced for defensive purpose during the cold season.

Echinoderms are known to be sensitive to light due to a diffuse dermal photosensitivity (Millot, 1957; Stubbs, 1982; Yoshida et al., 1984). Assuming the photosensitivity

could be developed enough to detect luminescence, luminous echinoderms could use light signals for intraspecific communication, as already suggested for some luminous ophiuroids (after the ‘group effect’ reported by Basch (1988)) and holothuroids (Gutt and Piepenburg, 1991). However, this goes against the assumption of Herring (1995) that luminescence in echinoderms would be associated only to interspecific signals and not intraspecific ones. *A. squamata* is a brooding species that produces very few gametes (only one oocyte a time per ovary; Fell, 1946). Cross-fertilization may only be successful when sexual partners are close (Fell, 1946; Johnson, 1972; Deheyn and Jangoux, 1999). Nothing is known about the behavioural process of reproduction in the species, but one might suppose that luminescence could help individuals to aggregate at the time of reproduction to increase the chance of genetic exchange, such as suggested for the luminous elaspod holothurians (Gutt and Piepenburg, 1991).

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