

## Functional physiology of lantern shark (*Etmopterus spinax*) luminescent pattern: differential hormonal regulation of luminous zones

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### SUMMARY

Lantern sharks are small deep-sea sharks that harbour complex species-specific luminescent photophore patterns. The luminescent pattern of one of these sharks, *Etmopterus spinax*, is made up of nine luminous zones. Previous experiments revealed that in the largest of these zones (ventral zone), photophores are under hormonal control, light being triggered by both melatonin (MT) and prolactin (PRL). In this study, we analysed the luminescent responses to MT and PRL in five other luminous zones from 12 female and eight male *E. spinax* specimens. The results showed that all luminous zones respond to both hormones, with each zone having its own kinetic parameters (maximum light intensity,  $L_{\max}$ ; total light emitted,  $L_{\text{tot}}$ ; time from stimulation to  $L_{\max}$ ,  $TL_{\max}$ ), which confirms the multifunctional character of this shark's luminescence.  $L_{\text{tot}}$  and  $L_{\max}$  were found to be directly dependent on the photophore density ( $P_D$ ) of the luminous zone, while  $TL_{\max}$  varied independently from  $P_D$ . In addition, we demonstrate a sexual dimorphism in the luminescent response to PRL, with male specimens having smaller  $L_{\text{tot}}$  and  $TL_{\max}$  in the luminous zones from the pelvic region. As this region also harbours the sexual organs of this species, this strongly suggests a role for the luminescence from these zones in reproduction.

Key words: bioluminescence, chondrichthyes, melatonin, photophores, prolactin, sexual dimorphism.

### INTRODUCTION

The vast majority of deep-sea organisms rely on luminescence for numerous functions including predation avoidance, predation and intraspecific communication (Buck, 1978; Wilson and Hastings, 1998; Herring, 2007). The possession of photogenic organs (photophores) is a common feature in many of these organisms, including crustaceans, molluscs and fishes (Haddock et al., 2010). These photophores are divided into two types: extrinsic photophores, which shelter symbiotic luminous bacteria, and intrinsic photophores, which are self-luminescent, containing an endogenous luminescent system (Herring, 1982). To bring an adaptive advantage to their owner, photophores should be precisely controlled as the efficiency of luminescent behaviours depends on the timing and the physical characteristics of luminescence (Warner et al., 1979; Denton et al., 1985; Branham and Greenfield, 1996; Harper and Case, 1999; Demary et al., 2006; Rivers and Morin, 2008).

A variety of luminescence control mechanisms exist in fishes (for reviews, see Herring and Morin, 1978; Herring, 1982; Claes and Mallefet, 2009a), which are the only luminous vertebrates. Photogenic organs with bacterial symbionts in bony fishes are controlled mechanically *via* mobile dark shutters, rotation into a dark pocket or chromatophores, and/or physiologically, by controlling the physicochemical characteristics (osmolarity, glucose,  $O_2$  availability, etc.) of the bacterial growth medium (Bertelsen, 1951; Herring and Morin, 1978; Herring, 1982; Haygood, 1993; Munk, 1999) (Fig. 1A). In contrast, intrinsic photophores are under neural control (mostly by adrenergic nerves) in bony fishes but appear to be under hormonal control in Chondrichthyes (Baguet, 1975; Baguet and Marechal, 1978; Baguet and Christophe, 1983; Claes and Mallefet, 2009a) (Fig. 1B,C). In addition to these 'luminescence switches', accessory optical structures (including reflectors, light guides, optical filters and lenses) are often present

and allow a physical modulation of the light emitted by photophores (Herring and Morin, 1978; Munk and Bertelsen, 1980; Herring, 1982; Denton et al., 1985).

The velvet belly lantern shark, *Etmopterus spinax* (Linnaeus 1758), is a small deep-sea shark endowed with numerous photophores forming a complex luminous pattern organised in nine different types of luminous zones (Claes and Mallefet, 2008) (Fig. 2A,B). Recent studies suggest that this pattern is involved in camouflage by counterillumination as well as in intraspecific behaviours such as cooperative swimming/hunting and even sexual signalling (Claes and Mallefet, 2008; Claes and Mallefet, 2009b).

Luminescence from the ventral luminous zone of *E. spinax* is controlled by hormones involved in the physiological control mechanism of Elasmobranch (sharks and rays) skin pigmentation: melatonin (MT) and prolactin (PRL), which trigger light emission, as well as  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH), the application of which prevents MT- and PRL-induced luminescence (Claes and Mallefet, 2009a). As the *E. spinax* luminescent pattern is believed to be involved in more than one function (Claes and Mallefet, 2008; Claes and Mallefet, 2009b), the question arises whether functionally different luminous zones present different control mechanisms.

Through MT and PRL application on the different luminous zones that produce the luminescent pattern of the velvet belly lantern shark, *E. spinax*, the specific goals of this study were (i) to determine whether all the luminous zones respond to hormonal application, and if this is the case then (ii) to show whether different hormonal luminescent responses are observable among the different luminous zones, (iii) to investigate a potential sexual dimorphism in the luminescent responses to hormones, which would be a clue for the existence of sexual signalling, (iv) to investigate the effect of photophore density ( $P_D$ ) on the luminescence kinetic parameters,

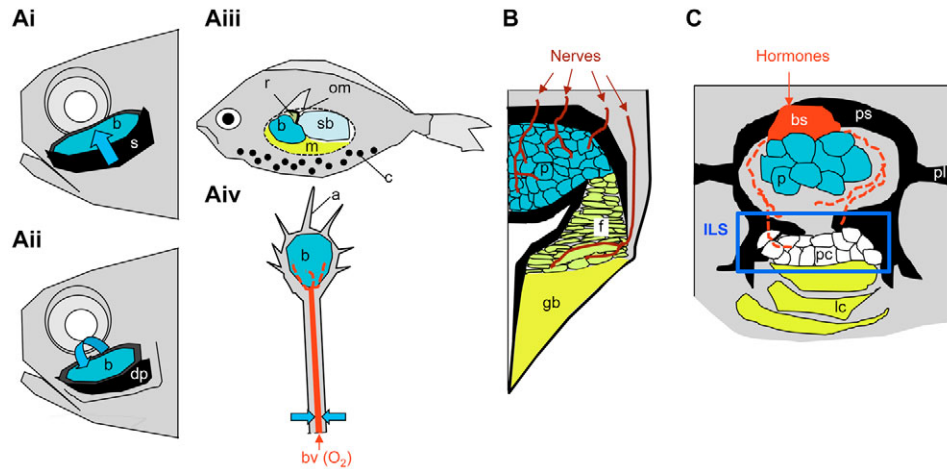


Fig. 1. Luminescence control mechanisms in fishes. (A) Symbiotic luminescence (bony fishes). (Ai) The luminescence from the bacterial photogenic organ (b) of *Photoblepharon palpebratus* is controlled by the movement (blue arrow) of a dark shutter (s), which acts as an inverse eyelid (modified from Howland et al., 1992). (Aii) The luminescence from the bacterial photogenic organ (b) of *Anomalops katoptron* is controlled by the movement (blue arrow) of the whole photogenic organ, which can rotate downward into a dark pocket (dp) (modified from Howland et al., 1992). (Aiii) Light organ system of a leionathid (modified from Haneda and Tsuji, 1976). The bacterial photogenic organ (b) opens laterally into the oesophagus and its light emission is transmitted to the ventral surface by a translucent muscle (m). Expansion of chromatophores (c) on the ventral surface prevents the light from being emitted to the outside. (Aiv) A modulation (blue arrows) of the blood supply to the bacterial photogenic organ (b) of a ceratioid anglerfish could allow control of its luminescence by varying the amount of oxygen ( $O_2$ ) available for the chemiluminescent reaction (Bertelsen, 1951; Munk, 1999). (B) Photophore section of the hatchetfish *Argyropelecus hemigymnus* (intrinsic luminescence; bony fishes). Like other luminous bony fishes endowed with intrinsic photophores, *A. hemigymnus* exhibits a neurally controlled luminescence (modified from Krönström et al., 2005). (C) Photophore section of the lantern shark *Etmopterus spinax* (intrinsic luminescence; cartilaginous fishes). The photophore luminescence from *E. spinax* is hormonally controlled (modified from Claes and Mallefet, 2009a), probably via movement of the pigmented cells (pc) comprising the iris-like structure (ILS, blue rectangle). Dashed red lines represent hypothetical movement of the blood inside the photogenic organ. The luminous tissue is shown in blue. a, appendage; bv, blood vessel; bs, blood sinus; f, filter; gb, gelatinous body; lc, lens cell; om, opaque membrane; p, photocyte; pl, pigmented layer; ps, pigmented sheath; r, guanine reflector; s, swimbladder.

and (v) to discuss the results in relation to the functional ecology of this shark species.

## MATERIALS AND METHODS

### Experimental animals

Twelve adult female and eight adult male living specimens of the velvet belly lantern shark *E. spinax* (30–51 cm total length) were caught by longlines lowered in a deep ( $\geq 200$  m) area of the Raunefjord (Norway) during two field sessions (February and June 2009), and transferred to Espeland Marine Station (Espesgrend,

Norway) where they were kept in two  $1\text{ m} \times 1\text{ m} \times 1\text{ m}$  tanks placed in a dark cold room ( $6^\circ\text{C}$ ) until use. Captive animals were killed by a blow to the head before the start of experimentation, following the local rules for experimental fish care.

### Photophore preparations

In this work, photophore preparations consisted of standard (0.55 cm diameter) circular skin patches dissected out from *E. spinax* specimens [following the method of Claes and Mallefet (Claes and Mallefet, 2009a)]. Since we wanted to test the effects of MT and

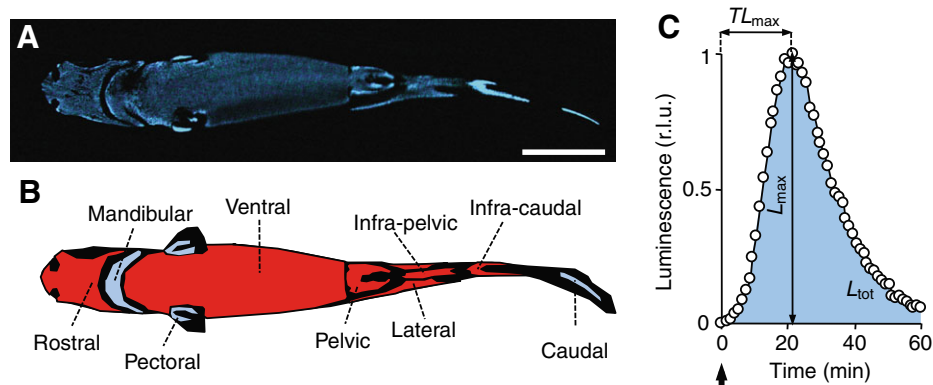


Fig. 2. (A) Ventral view of a spontaneously glowing specimen of the lantern shark *E. spinax* showing the heterogeneity of luminescence intensity present among the luminous zones producing the luminescent pattern. (B) Schematic drawing of the ventral part of *E. spinax* showing the different luminous zones giving rise to the luminescent pattern of this shark with their associated nomenclature (Claes and Mallefet, 2008). Zones tested in this study are presented in red while others are presented in blue. (C) Typical 1 h luminescence emission curve (in relative units, r.l.u.) with associated light parameters:  $L_{\max}$ , maximum intensity of light emission;  $L_{\text{tot}}$ , total quantity of light emitted during a given period;  $TL_{\max}$ , time from stimulation to  $L_{\max}$ . Scale bar in A, 5 cm.

PRL separately, we selected all the luminous zones that were large enough to give two separate but similar patches, which correspond to six out of the nine zones described previously (Claes and Mallefet, 2008) (Fig. 2B).

These skin patches were placed in a shark saline [292 mmol l<sup>-1</sup> NaCl, 3.2 mmol l<sup>-1</sup> KCl, 5 mmol l<sup>-1</sup> CaCl<sub>2</sub>, 0.6 mmol l<sup>-1</sup> MgSO<sub>4</sub>, 1.6 mmol l<sup>-1</sup> Na<sub>2</sub>SO<sub>4</sub>, 300 mmol l<sup>-1</sup> urea, 150 mmol l<sup>-1</sup> trimethylamine N-oxide, 10 mmol l<sup>-1</sup> glucose, 6 mmol l<sup>-1</sup> NaHCO<sub>3</sub>; total osmolarity 1.080 mosmol l<sup>-1</sup>; pH 7.7 (Bernal et al., 2005)]. For each shark, photophore densities present in the six investigated luminous zones was estimated using a binocular microscope (Leitz Diaplan, Oberkochen, Germany) by counting the number of photophores present in one of the two patches and by dividing this number by its surface area (0.2375 cm<sup>2</sup>). Skin patches were then stored in saline at 6°C until use (<2 h; maximum survival time of skin patches was more than 12 h).

### Luminometry

The same procedure was applied for each luminous zone of the 20 *E. spinax* specimens. Skin patches were transferred to a 96-well microplate (each well containing 100 µl saline) from a luminometer (Berthold Orion, Pforzheim, Germany) calibrated with a standard 470 nm light source (Beta light, Saunders Technology, Hayes, UK). These patches were stimulated by a single application of either MT (Tocris Bioscience, Ellisville, MO, USA) or PRL (Sigma, St Louis, MO, USA) at a final concentration of 10<sup>-6</sup> mol l<sup>-1</sup>, which is the concentration eliciting the highest luminescent response in the ventral zone, i.e. the concentration to which the luminous tissue is most sensitive (Claes and Mallefet, 2009a). The experimental procedure was performed at room temperature (18°C).

The luminescent responses were recorded for 1 h after the stimulation and were characterised using different kinetic parameters (Fig. 2C): the maximum intensity of light emission ( $L_{\max}$ , in megaquanta per second, Mqs<sup>-1</sup>), the total quantity of light emitted during the experiment ( $L_{\text{tot}}$ , in teraquanta per hour, Tqh<sup>-1</sup>) and the time to reach  $L_{\max}$  from the stimulation time ( $TL_{\max}$ , in min). Light parameters were standardised by skin surface area (in cm<sup>2</sup>). In order to compare the luminous response of six different luminous zones to the two hormones, a total of 12 skin patches were dissected out from each animal and stimulated simultaneously. Then, according to the number of experiments run at the same time in the

Table 1. Hormone-induced luminescence parameters ( $L_{\max}$ ,  $L_{\text{tot}}$  and  $TL_{\max}$ ) and photophore density for the ventral luminous zone of male and female specimens of *Etmopterus spinax*

	Males (N=8)	Females (N=12)	Student's t-test	
			t-value	P-value
Melatonin				
$L_{\max}$	15.28±3.44	22.74±3.05	1.24	0.2293
$L_{\text{tot}}$	40.32±6.96	58.16±12.41	1.02	0.3218
$TL_{\max}$	45.61±6.49	31.17±6.75	-1.47	0.1594
Prolactin				
$L_{\max}$	10.04±3.43	50.31±13.41	2.91	<b>0.0126</b>
$L_{\text{tot}}$	14.41±4.56	83.34±22.21	3.04	<b>0.0103</b>
$TL_{\max}$	14.01±1.61	20.15±3.29	1.68	0.1667
$P_D$ (units cm <sup>-2</sup> )	3152±318	2614±147	-1.72	0.1034

$L_{\max}$ , maximum intensity of light emission;  $L_{\text{tot}}$ , total quantity of light emitted during a given period;  $TL_{\max}$ , time from stimulation to  $L_{\max}$ ;  $P_D$ , photophore density.

Data for males and females are means ± s.e.m.

Bold values indicate significant sexual differences.

Table 2. Results of two way ANOVA performed on the hormone-induced luminescence parameters

	d.f.	F-value	P-value
$P_D$			
LZ	5	88.98	<b>&lt;0.0001</b>
Sex	1	1.25	0.2668
LZ × sex	5	2.06	0.0756
Melatonin			
$L_{\max}$			
LZ	5	7.29	<b>&lt;0.0001</b>
Sex	1	0.75	0.3900
LZ × sex	5	0.31	0.9032
$L_{\text{tot}}$			
LZ	5	10.17	<b>&lt;0.0001</b>
Sex	1	0.10	0.7528
LZ × sex	5	0.52	0.7584
$TL_{\max}$			
LZ	5	5.66	<b>0.0001</b>
Sex	1	3.14	0.0792
LZ × sex	5	1.19	0.3193
Prolactin			
$L_{\max}$			
LZ	5	2.36	<b>0.0452</b>
Sex	1	0.12	0.7347
LZ × sex	5	1.13	0.3500
$L_{\text{tot}}$			
LZ	5	3.38	<b>0.0070</b>
Sex	1	23.06	<b>&lt;0.0001</b>
LZ × sex	5	2.34	<b>0.0465</b>
$TL_{\max}$			
LZ	5	4.67	<b>0.0007</b>
Sex	1	9.97	<b>0.0021</b>
LZ × sex	5	0.79	0.5599

$P_D$ , photophore density; LZ, luminous zones.

Bold values indicate significant relationships.

luminometer, Berthold Simplicity software parameters were adjusted in order to obtain at least one point every minute.

### Statistical analysis

Knowing that  $L_{\max}$  and  $L_{\text{tot}}$  parameters of luminescent responses to MT and PRL vary according to the time of year (Claes and Mallefet, 2009a), and as our specimens were obtained at two different times (February and June), the results were standardised for each specimen by dividing the values obtained for these two parameters in the different luminous zones by the value of  $L_{\max}$  and  $L_{\text{tot}}$  of the luminescent response from the ventral zone (which has the lowest

Table 3. Relationship between hormone-induced luminescence parameters ( $L_{\max}$ ,  $L_{\text{tot}}$  and  $TL_{\max}$ ) and photophore density among the different luminous zones of *Etmopterus spinax*

	N	Intercept	Slope	R <sup>2</sup>	P-value
Melatonin					
$L_{\max}$	6	0*	1.22±0.17	0.9852	<b>&lt;0.0001</b>
$L_{\text{tot}}$	6	0*	1.06±0.23	0.9650	<b>&lt;0.0001</b>
$TL_{\max}$	6	15.34±17.19	5.35±30.44	0.0561	0.6514
Prolactin					
$L_{\max}$	6	0*	1.15±0.34	0.9378	<b>0.0003</b>
$L_{\text{tot}}$	9	0*	0.56±0.24	0.7733	<b>0.0008</b>
$TL_{\max}$	8	14.13±14.84	-2.78±9.80	0.0744	0.5133

\*These curves do not have an intercept because no light can be emitted when no photophores (photophore density=0) are present in the tissue. Bold values indicate significant relationships.

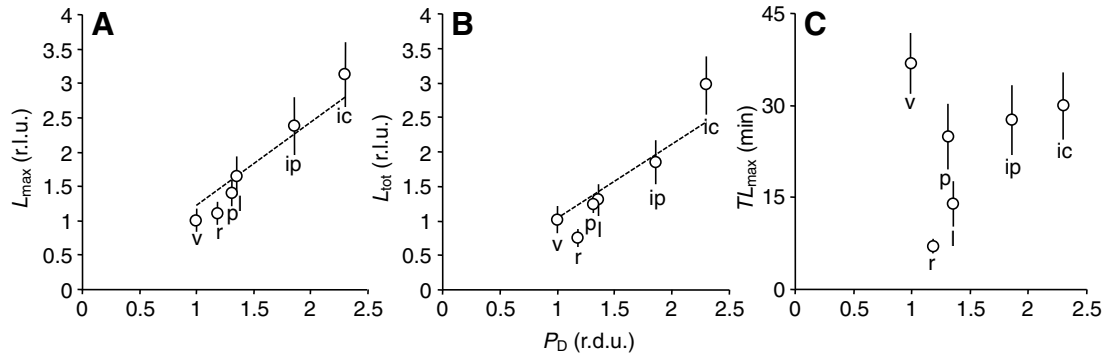


Fig. 3 Relationship between photophore density ( $P_D$ , in relative density units, r.d.u.) and light parameters of melatonin (MT)-induced luminescence from different luminous zones of *E. spinax* luminescent pattern: maximum intensity of luminescence (A), total light emitted during the experiment (B) and time from stimulation to  $L_{max}$  (C). Each value is shown with its s.e.m. and  $L_{max}$  and  $L_{tot}$  values are presented in relative light units (r.l.u.). For the three light parameters, two-way ANOVA showed a significant difference among the luminous zones but not among sexes (see Table 2). Dashed lines represent regression lines of the data. Results of the regressions are shown in Table 3.  $N=20$  replicates per luminous zone. ic, infra-caudal; ip, infra-pelvic; l, lateral; p, pelvic; r, rostral; v, ventral.

$P_D$ ). In order to perform coherent comparisons, the  $P_D$  of the skin patches was also standardised using ventral density as the reference. No such standardisation was applied for  $TL_{max}$ , which was found to be constant (for both hormones) over the year (Claes and Mallefet, 2009a). Control Student's *t*-tests were performed between sexes for (absolute) luminescence parameters and  $P_D$  in the ventral luminous zone.

Two-way analyses of variance (ANOVA) were performed to determine the effect of sex and luminous zone on the three different luminescence parameters and the  $P_D$ . If a sexual difference was detected, each luminous zone was investigated for sexual differences using Student's *t*-test. In addition, a linear regression was performed to identify a potential link, across the different luminous zones, between the mean values of the light parameters of hormone-induced luminescence and the mean  $P_D$ .

Statistical analyses (ANOVA, Student's *t*-tests and linear regressions) were performed with the software SAS/STAT 1990 (SAS Institute Inc., Cary, NC, USA). Each mean value is expressed with its standard error (means  $\pm$  s.e.m.) and  $N$  is the number of skin patches used for a specific treatment (which corresponds to the number of shark specimens tested as one skin patch from each individual was used in each treatment).

## RESULTS

Control Student's *t*-tests showed that the ventral luminous zones of male and female *E. spinax* specimens used in this study are rather uniform in terms of their  $P_D$  and their MT-induced luminescence kinetic parameters (Table 1). Sexual differences were, however, observed for the luminescence induced by PRL; females showed higher  $L_{max}$  and  $L_{tot}$  than males (Table 1). This difference might reflect the fact that males and females were not tested at the same time of year (all females were tested in February while 6 out of the 8 males were tested in June), as seasonal variations in the  $L_{max}$  (and  $L_{tot}$ ) of hormonally induced luminescence has already been detected in this species (Claes and Mallefet, 2009a). This seasonal variation in  $L_{max}$  (and  $L_{tot}$ ) confirms the need, for these two light parameters, to use the standardisation detailed above in the following analyses. Two-way ANOVA revealed a significant difference in  $P_D$  among the different luminous zones but failed to show any effect of sex on this variable (Table 2).

## Melatonin

Application of MT always evoked light emission in all the skin patches from the different luminous zones tested. Two-way ANOVA detected highly significant differences ( $P<0.001$ ) among the light

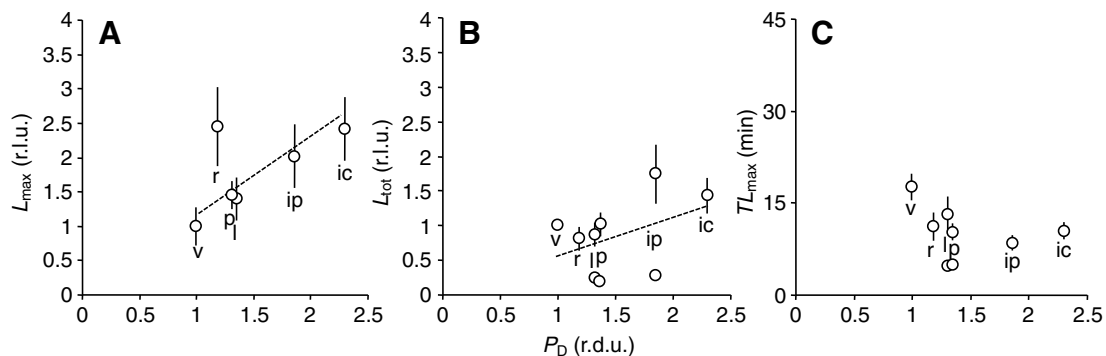


Fig. 4 Relationship between  $P_D$  (in relative density units, r.d.u.) and light parameters of prolactin-induced luminescence from different luminous zones of *E. spinax* luminescent pattern. Categories, values, regression lines, source and abbreviations as in Fig. 3. Two-way ANOVA showed a significant difference among the luminous zones in the three light parameters but sexual differences were only found in  $L_{tot}$  and  $TL_{max}$  (see Table 2). Student's *t*-test showed that females have significantly higher  $L_{tot}$  for lateral, pelvic and infra-pelvic luminous zones ( $P<0.05$ ) and significantly higher  $TL_{max}$  for lateral and pelvic luminous zones.  $N=20$  replicates per luminous zone except in sexually dimorphic zones where  $N=12$  for females and  $N=8$  for males.

parameters of luminescence from these luminous zones (Table 2). These ANOVA failed, however, to detect any sexual effect on the MT-induced luminescence (Table 2).

Linear regression analysis revealed a significant relationship between  $P_D$  and the kinetic parameters  $L_{max}$  and  $L_{tot}$  of the MT-induced luminescence (Fig. 3A,B; Table 3). No relationship was found between  $P_D$  and  $TL_{max}$  (Fig. 3C; Table 3).

**Prolactin**

Application of PRL always evoked light emission in all the skin patches from the different luminous zones tested. Two-way ANOVA detected significant differences ( $P < 0.05$ ) among the luminescence kinetic parameters of these luminous zones as well as sexual differences in  $L_{tot}$  and  $TL_{max}$  (Table 2).

Student's *t*-tests confirmed sexual differences in  $L_{tot}$  in lateral ( $t_{12,3}=4.89, P=0.001$ ), pelvic ( $t_{11,3}=3.90, P<0.01$ ) and infra-pelvic ( $t_{11,5}=3.39, P<0.01$ ) luminous zones, and sexual differences in  $TL_{max}$  in lateral ( $t_{17,3}=3.31, P<0.01$ ) and pelvic ( $t_{12,8}=2.82, P<0.05$ )

luminous zones. Linear regression analysis revealed a significant relationship between  $P_D$  and the kinetic parameters  $L_{max}$  and  $L_{tot}$  of the PRL-induced luminescence (Fig. 4A,B; Table 3). No relationship was found between  $P_D$  and  $TL_{max}$  (Fig. 4C; Table 3).

Standardised mean profiles of MT- and PRL-induced luminescence time courses from the different luminous zones investigated in this study (Fig. 5) clearly demonstrate that luminous zones respond differently to PRL and MT. While no difference was detected between male and female *E. spinax* specimens in response to MT, some zones (lateral, pelvic and infra-pelvic zones) showed sexual differences in response to PRL, with male sharks showing proportionally quicker light responses than females for these zones.

**DISCUSSION**

**Physiology**

The luminescent pattern of the lantern shark *E. spinax* is complex and appears to be involved in different functions (Reif, 1985; Claes and Mallefet, 2008; Claes and Mallefet, 2009a; Claes and Mallefet,

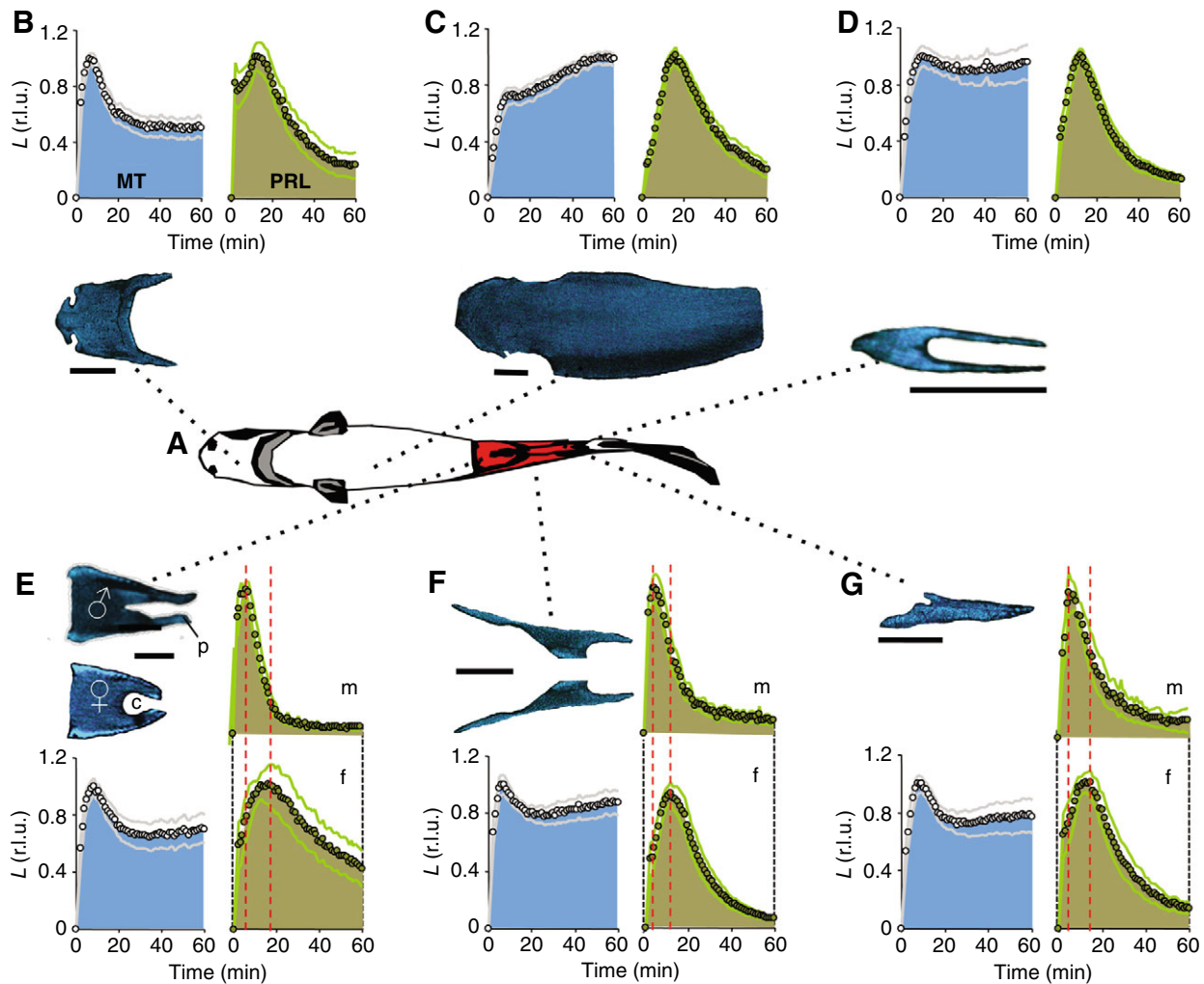


Fig. 5. Time course of luminescence ( $L$ ) induced by MT and prolactin (PRL) for different luminous zones of *E. spinax*. Standardised luminescence curves (in relative units, r.l.u.) are shown (the luminescence curve of each single shark was standardised so that  $L_{max}$  value=1) from the luminescent pattern after application (time=0) of MT (left; blue panel of each pair) or PRL (right; brown panel of each pair). Grey and green lines represent error limits. (A) Schematic drawing of the ventral part of *E. spinax* showing the different luminous zones of the luminescent pattern of this shark: black, non-luminous tissue; grey, zones that were not tested; red, zones that react differently after application of PRL according to sex; white, zones that did not show such a dimorphism in luminescence response. (B) Rostral luminous zone. (C) Ventral luminous zone. (D) Infra-caudal luminous zone. (E) Pelvic luminous zone. (F) Lateral luminous zone. (G) Infra-pelvic luminous zone. Dashed vertical red lines in E–G allow comparison of the sexual difference in  $TL_{max}$  observable in these zones in male (m) and female (f) specimens. Scale bars in B–G, 2 cm.

2009b). As bioluminescent behaviours generally imply kinetic differences in light emission or even do not occur at the same time, we asked whether the different functional areas of *E. spinax* have similar or different luminescence control mechanisms. A recent study showed that luminescence from the ventral luminous zone of this shark may be triggered by MT and PRL (Claes and Mallefet, 2009a). If other luminous zones of this shark are controlled by hormones as well, another question arises: how does a signalling molecule that diffuses in the blood throughout all parts of the organism efficiently control a multifunctional luminescent pattern such as the one of *E. spinax*?

In this study we showed that all luminous zones investigated so far respond to application of either MT or PRL by glowing for variable time courses (all other zones of the luminous pattern also responded to both hormones but their small size prevented them from being tested quantitatively; J.M.C. and J.M., unpublished data). The observed difference in the light parameters  $L_{\max}$  and  $L_{\text{tot}}$  of the different luminous zones certainly reflects the difference in  $P_D$  present in this zone. Not surprisingly, zones with higher photophore densities emitted more light than others after hormone application. This relationship is quite simple to understand: when MT- or PRL-containing blood reaches a specific luminous zone, all the photophores present in this zone are stimulated to glow. Direct observation of spontaneous luminescence from living specimens of *E. spinax* confirmed that brighter zones are the zones displaying the highest photophore densities (Claes and Mallefet, 2009b). A direct relationship was found between chemically induced luminescence intensity (via oxygen peroxide,  $\text{H}_2\text{O}_2$ ) and  $P_D$  across *E. spinax*'s luminous zones (Claes and Mallefet, 2009b). In this case, however, the relationship mainly reflected the fact that the amount of luminous substrate available for the chemiluminescent reaction in a given luminous zone is linked to the number of photophores in a given surface area (Claes and Mallefet, 2009b), suggesting that each photophore might contain a similar amount of luminous substrate. However, as the variation in  $TL_{\max}$  between the different luminous zones of *E. spinax* does not appear to be linked to a difference in  $P_D$ , the hormonal luminescence control present in this shark may act differently according to the part of the luminescent pattern that is stimulated. This heterogeneity in the timing of the light emission reflects a difference in the sensitivity of the luminous zones to both hormones, indicating that additional control mechanisms might be present. The observed sexual differences in response to PRL, which also do not seem to be linked to a difference in  $P_D$ , is another indication that the hormonal luminescence control mechanism of *E. spinax* is more complex than previously thought, and approaches in selectivity the neural luminescence control mechanism of bony fishes.

### Ecology

The luminous zones of *E. spinax* do not appear at the same time during embryogenesis (Claes and Mallefet, 2008) and present different surface area and  $P_D$  scaling patterns throughout ontogeny. These differences lead to the establishment of a complex heterogeneous luminescent pattern in mainly adult but also subadult individuals (Claes and Mallefet, 2009b). This luminous pattern heterogeneity appears to actually represent a trade-off between the continuously useful function of camouflage and periodic intraspecific functions such as cohesive swimming/hunting and sexual signalling which would only be useful in larger sharks (Reif, 1985; Claes and Mallefet, 2009b).

The results of this study (which focused on adult individuals of *E. spinax*) agree well with these hypotheses as they show that

hormones controlling the luminescence from photophores of the *E. spinax* ventral luminous zone are able to not only trigger light in other luminous zones but also, when applied at the same concentration, make them luminesce proportionally to their  $P_D$ , thus conserving the heterogeneity of the luminescent pattern. Moreover, this study adds a new step in the complexity of the luminescent pattern as it shows that the kinetics of the luminescent response to PRL and MT also varies independently from the  $P_D$  of the zone. The rate of light emission is very different from one zone to another, thus reinforcing the idea of the multifunctionality of the luminous pattern. Similar versatility in the luminescence emission has been found in lanternfishes (myctophids) which feature a caudal organ that flashes rapidly and is believed to be used in intraspecific communication, and slow glowing ventral photophores involved in camouflage (Young et al., 1980; Herring, 2007). In addition, the data presented here bring, for the first time, evidence of a clear dimorphism in the response to PRL between males and females of *E. spinax*. Interestingly, this dimorphism is only apparent in the pelvic region, where the sexually dimorphic organs of sharks, i.e. male's claspers and the cloaca of the female, are present (Wourms, 1977). This dimorphism strongly supports the involvement of luminescence from this region in reproduction, as already suggested (Claes and Mallefet, 2009b). For example, the differences in  $L_{\text{tot}}$  and  $TL_{\max}$  found in this study might reveal the presence of a luminescent courtship display between male and female *E. spinax* specimens. Such luminous sexual signals have already been documented/suggested in other marine animal species including polychaetes (Tsuji and Hill, 1983), crustaceans (Rivers and Morin, 2008), squids (Herring, 1988) and bony fishes (Crane, 1965; Sasaki et al., 2003; Herring, 2007). Since this sexual dimorphism is only observed in response to PRL, it should be more pronounced when luminescence response to PRL are higher, which appears to be in April, at the winter–spring transition (Claes and Mallefet, 2009a). In the Mediterranean, *E. spinax* is believed to mate in winter (when the proportion of active males is higher), with the female storing the sperm until fertilisation, which occurs in summer (Coehlo and Erzini, 2008). It can therefore be expected that similar specific timing may exist in Norwegian velvet belly lantern sharks with mating occurring at a specific time of year, and that possessing a more pronounced luminescent dimorphism at this period increases sexual signalling and reproductive success.

### LIST OF ABBREVIATIONS

$L_{\max}$	maximum intensity of light emission
$L_{\text{tot}}$	total light emission
MT	melatonin
$P_D$	photophore density
PRL	prolactin
$TL_{\max}$	time between stimulation and maximum intensity of light emission
$\alpha$ -MSH	$\alpha$ -melanocyte stimulating hormone

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