The Journal of Experimental Biology 213, 1852-1858

doi:10.1242/jeb.041947

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

Julien M. Claes* and Jérôme Mallefet

Laboratory of Marine Biology, Earth and Life Institute, Université catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium

*Author for correspondence (julien.m.claes@uclouvain.be)

Accepted 20 February 2010

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_{tot}; time from stimulation to L_{max}, TL_{max}), which confirms the multifunctional character of this shark's luminescence. L_{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_{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 α-melanocyte stimulating hormone (α-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,

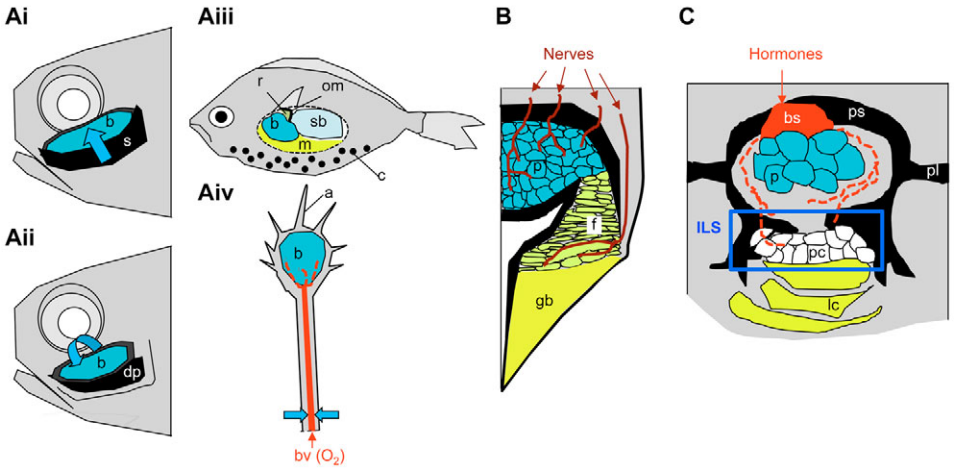

Fig. 1. Luminescence control mechanisms in fishes. (A) Symbiotic luminescence (bony fishes). (Ai) The luminescence from the bacterial photogenic organ (b) of *Photoblepharon palpebratus* is controlled by the movement (blue arrow) of a dark shutter (s), which acts as an inverse eyelid (modified from Howland 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 leiognathid (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 esca (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 (≥200 m) area of the Raunefjord (Norway) during two field sessions (February and June 2009), and transferred to Espeland Marine Station (Espegrend, Norway) where they were kept in two 1 m×1 m×1 m tanks placed in a dark cold room (6°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

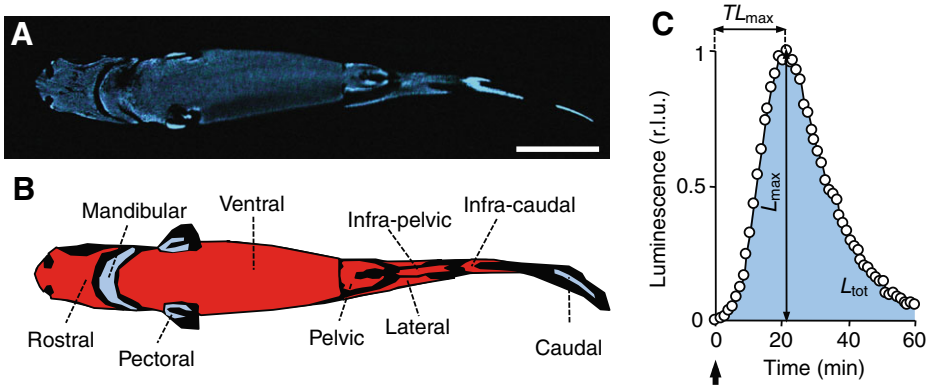

Fig. 2. (A) Ventral view of a spontaneously glowing specimen of the lantern shark *E. spinax* showing the heterogeneity of luminescence intensity present among the luminous zones producing the luminescent pattern. (B) Schematic drawing of the ventral part of *E. spinax* showing the different luminous 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_{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^{-1} NaCl, 3.2 mmol l^{-1} KCl, 5 mmol l^{-1} $CaCl_2$, 0.6 mmol l^{-1} $MgSO_4$, 1.6 mmol l^{-1} Na_2SO_4, 300 mmol l^{-1} urea, 150 mmol l^{-1} trimethylamine N-oxide, 10 mmol l^{-1} glucose, 6 mmol l^{-1} $NaHCO_3$; total osmolarity 1.080 mosmol l^{-1}; 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^2). 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^{-6} mol l^{-1}, 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, Mq s^{-1}), the total quantity of light emitted during the experiment (L_{tot}, in teraquanta per hour, Tq h^{-1}) 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^2). 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 luminometer, Berthold Simplicity software parameters were adjusted in order to obtain at least one point every minute.

Table 1. Hormone-induced luminescence parameters (L_{max}, L_{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_{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	**0.0126**
L_{tot}	14.41±4.56	83.34±22.21	3.04	**0.0103**
TL_{max}	14.01±1.61	20.15±3.29	1.68	0.1667
P_D (units cm^{-2})	3152±318	2614±147	–1.72	0.1034

L_{max}, maximum intensity of light emission; L_{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	**<0.0001**
Sex	1	1.25	0.2668
LZ × sex	5	2.06	0.0756
Melatonin			
L_{max}			
LZ	5	7.29	**<0.0001**
Sex	1	0.75	0.3900
LZ × sex	5	0.31	0.9032
L_{tot}			
LZ	5	10.17	**<0.0001**
Sex	1	0.10	0.7528
LZ × sex	5	0.52	0.7584
TL_{max}			
LZ	5	5.66	**0.0001**
Sex	1	3.14	0.0792
LZ × sex	5	1.19	0.3193
Prolactin			
L_{max}			
LZ	5	2.36	**0.0452**
Sex	1	0.12	0.7347
LZ × sex	5	1.13	0.3500
L_{tot}			
LZ	5	3.38	**0.0070**
Sex	1	23.06	**<0.0001**
LZ × sex	5	2.34	**0.0465**
TL_{max}			
LZ	5	4.67	**0.0007**
Sex	1	9.97	**0.0021**
LZ × sex	5	0.79	0.5599

P_D, photophore density; LZ, luminous zones.
Bold values indicate significant relationships.

Statistical analysis

Knowing that L_{max} and L_{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_{tot} of the luminescent response from the ventral zone (which has the lowest

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

	N	Intercept	Slope	R^2	*P*-value
Melatonin					
L_{max}	6	0*	1.22±0.17	0.9852	**<0.0001**
L_{tot}	6	0*	1.06±0.23	0.9650	**<0.0001**
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	**0.0003**
L_{tot}	9	0*	0.56±0.24	0.7733	**0.0008**
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.

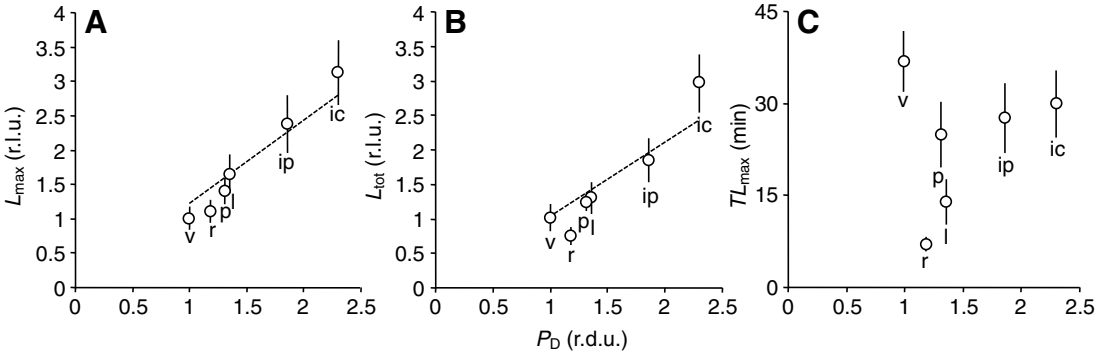

Fig. 3 Relationship between photophore density (P_D, in relative density units, r.d.u.) and light parameters of melatonin (MT)-induced luminescence from different luminous zones of *E. spinax* luminescent pattern: maximum intensity of luminescence (A), total light emitted during the experiment (B) and 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 ± 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

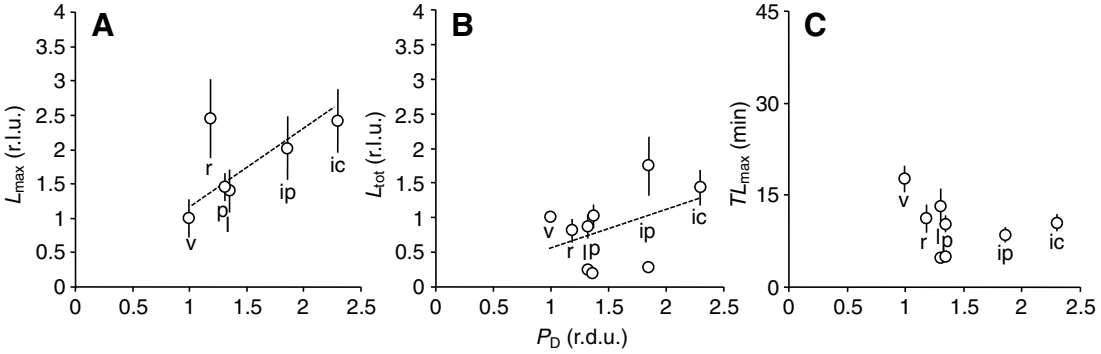

Fig. 4. Relationship between P_D (in relative density units, r.d.u.) and light parameters of prolactin-induced luminescence from different luminous zones of *E. spinax* luminescent pattern. Categories, values, regression lines, source and abbreviations as in Fig. 3. Two-way ANOVA showed a significant 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,

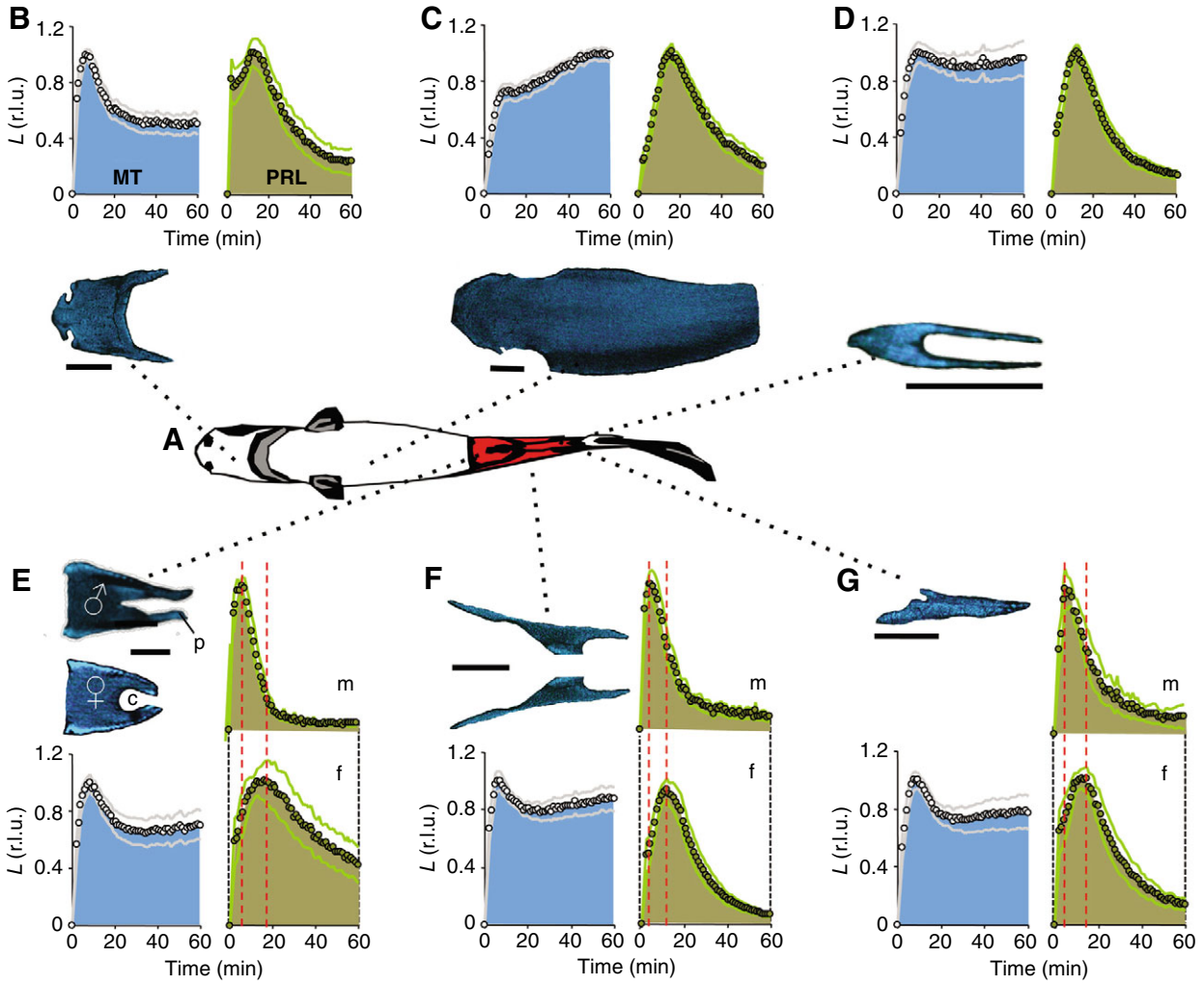

Fig. 5. Time course of luminescence (*L*) induced by MT and prolactin (PRL) for different luminous zones of *E. spinax*. Standardised luminescence curves (in relative units, r.l.u.) are shown (the luminescence curve of each single shark was standardised so that L_{max} value=1) from the luminescent pattern 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_{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, H_2O_2) and P_D accross *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_{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_{tot}	total light emission
MT	melatonin
P_D	photophore density
PRL	prolactin
TL_{max}	time between stimulation and maximum intensity of light emission
α-MSH	α-melanocyte stimulating hormone

ACKNOWLEDGEMENTS

This work was supported by a grant from the F.N.R.S. to J.M.C. and another grant (FRFC 2.4516.01) to J.M. We would like to acknowledge A. Aadnesen, manager of Espeland Marine Biological station, T. Sørlie for skilful help during field collections, and the two anonymous reviewers for their comments that greatly improved the earlier version of the manuscript. J.M. is research associate of FSR-F.N.R.S. Contribution to the Biodiversity Research Centre (BDIV) and to the Centre Interuniversitaire de Biologie Marine (CIBIM).

REFERENCES

Baguet, F. (1975). Excitation and control of isolated photophores of luminous fishes. *Progr. Neurobiol.* **5**, 97-125.

Baguet, F. and Christophe, B. (1983). Adrenergic stimulation of isolated photophores of *Maurolicus muelleri*. *Comp. Biochem. Physiol.* **75**, 79-84.

Baguet, F. and Marechal, G. (1978). The stimulation of isolated photophores (*Argyropelecus*) by epinephrine and norepinephrine. *Comp. Biochem. Physiol.* **60**, 137-143.
Bernal, D., Donley, J. M., Shadwick, R. E. and Syme, D. A. (2005). Mammal-like muscle power swimming in a cold-water shark. *Nature* **437**, 1349-1352.
Bertelsen, E. (1951). *The Ceratioid Fishes: Ontogeny, Taxonomy, Distribution and Biology*. Copenhagen, Denmark: A. F. Høst.
Branham, M. A. and Greenfield, M. D. (1996). Flashing males win mate success. *Nature* **381**, 745-746.
Buck, J. B. (1978). Functions and evolution of bioluminescence. In *Bioluminescence in Action* (ed. P. J. Herring), pp. 419-460. New York: Academic Press.
Claes, J. M. and Mallefet, J. (2008). Early development of bioluminescence suggests counter-illumination in the velvet belly lantern shark *Etmopterus spinax* (Squaloidea: Etmopteridae). *J. Fish Biol.* **73**, 1337-1350.
Claes, J. M. and Mallefet, J. (2009a). Hormonal control of luminescence from lantern shark (*Etmopterus spinax*) photophores. *J. Exp. Biol.* **212**, 3684-3692.
Claes, J. M. and Mallefet, J. (2009b). Ontogeny of photophore pattern in the velvet belly lantern shark *Etmopterus spinax*. *Zoology* **6**, 433-441.
Coehlo, R. and Erzini, K. (2008). Life-history of a wide-ranging deepwater lantern shark in the north-east Atlantic, *Etmopterus spinax* (Chondrichthyes: Etmopteridae), with implications for conservation. *J. Fish Biol.* **73**, 1419-1443.
Crane, J. M. (1965). Bioluminescent courtship display in the teleost *Porichthys notatus*. *Copeia* **2**, 239-241.
Demary, K., Michaelidis, C. I. and Lewis, S. M. (2006). Firefly courthip: behavioural and morphological predictor of male mating success in *Photinus greeni*. *Ecology* **112**, 485-492.
Denton, E. J., Herring, P. J., Widder, E. A., Latz, M. F. and Case, J. F. (1985). The role of filters in the photophore of oceanic animals and their relation to vision in the oceanic environment. *Proc. R. Soc. Lond. B. Biol. Sci.* **225**, 63-97.
Haddock, S. H. D., Moline, M. A. and Case, J. F. (2010). Bioluminescence in the sea. *Ann. Rev. Mar. Sci.* **2**, 443-493.
Haneda, Y. and Tsuji, F. I. (1976). The luminescent system of pony fishes. *J. Morph.* **150**, 539-552.
Harper, R. D. and Case, J. F. (1999). Disruptive counter-illumination and its anti-predatory value in the midshipman fish *Porychthys notatus*. *Mar. Biol.* **134**, 529-540.
Haygood, M. G. (1993). Light organ symbioses in fishes. *Crit. Rev. Microbiol.* **19**, 191-216.
Herring, P. J. (1982). Aspects of the bioluminescence of fishes. *Oceanogr. Mar. Biol. Ann. Rev.* **20**, 415-470.
Herring, P. J. (1988). Luminescent organs. In *The Mollusca: Form and Function* (ed. E. R. Trueman and M. R. Clarke), pp. 449-489. San Diego: Academic Press.
Herring, P. J. (2007). Sex with the lights on? A review of bioluminescent dimorphism in the sea. *J. Mar. Biol. Assoc. UK* **87**, 829-842.
Herring, P. J. and Morin, J. G. (1978). Bioluminescence in fishes. In *Bioluminescence in Action* (ed. P. J. Herring), pp. 273-329. London: Academic Press.
Howland, H. C., Murphy, C. J. and McCosker, J. E. (1992). Detection of eyeshine by flashlight fishes of the family Anomalopidae. *Vision Res.* **32**, 765-769.
Krönström, J., Holmgren, S., Baguet, F., Salpietro, L. and Mallefet, J. (2005). Nitric oxide in control of luminescence from hatchetfish (*Argyropelecus hemigymnus*) photophores. *J. Exp. Biol.* **208**, 2951-2961.
Munk, O. (1999). The escal photophore of ceratioids (Pisces; Ceratioidei) – a review of structure and function. *Acta Zool.* **80**, 265-284.
Munk, O. and Bertelsen, E. (1980). On the esca and its light-guiding structure in the deep-sea anglerfish *Chaenophryne draco* (Pisces, Ceratioidei). *Vidensk. Meddr. Dansk naturh. Foren.* **142**, 103-129.
Reif, W.-E. (1985). Function of scales and photophores in luminescent mesopelagic sharks. *Acta Zool.* **66**, 111-118.
Rivers, T. J. and Morin, J. G. (2008). Complex sexual courtship displays by luminescent male marine ostracods. *J. Exp. Biol.* **211**, 2252-2262.
Sasaki, A., Ikejima, K., Aoki, S., Azuma, N., Kashimura, N. and Wada, M. (2003). First evidence of bioluminescent signalling in the ponyfish, *Leiognathus elongatus*. *Env. Biol. Fish.* **66**, 307-311.
Tsuji, F. and Hill, E. (1983). Repetitive cycles of bioluminescence and spawning in the Polychaeta, *Odontosyllis phosphorea*. *Biol. Bull.* **165**, 444-449.
Warner, J. A., Latz, M. I. and Case, J. F. (1979). Cryptic bioluminescence in a midwater shrimp. *Science* **203**, 1109-1110.
Wilson, T. and Hastings, J. W. (1998). Bioluminescence. *Annu. Rev. Cell. Biol.* **14**, 197-230.
Wourms, J. P. (1977). Reproduction and development in chondrichthyan fishes. *Int. Comp. Biol.* **17**, 379-410.
Young, R. E., Kampa, E. M., Maynard, S. D., Mencher, F. M. and Roper, C. F. E. (1980). Counter-illumination and the upper depth of midwater animals. *Deep-sea Res.* **27**, 671-691.