CURRENT KNOWLEDGE ON THE MELATONIN SYSTEM IN TELEOST FISH

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Abstract

Melatonin is a much conserved feature in vertebrates that plays a central role in the entrainment of daily and annual physiological rhythms. Investigations aiming at understanding how melatonin mediates the effects of photoperiod on crucial functions and behaviors have been very active in the last decades, particularly in mammals. In fish a clear-cut picture is still missing. Here we review the available data on (i) the sites of melatonin production in fish, (ii) the mechanisms that control its daily and annual rhythms of production and (iii) the characterization of its different receptor subtypes, their location and regulation. The *in vivo* and *in vitro* data on melatonin effects on crucial neuroendocrine regulations, including reproduction, growth, feeding and immune response, are also reviewed. Finally we discuss how manipulation of the photic cues impact on fish circannual clock and annual cycle of reproduction, and how this can be used for aquaculture purposes.

Key words: Fish, reproduction, photoperiod, melatonin, circadian, circannual

1. INTRODUCTION

Virtually all organisms have adapted their behaviors and functions to the daily and annual variations of the external cues. The alternation of light (L) and darkness (D), the 24 h LD cycle, is the most prominent and reliable of these cues (noise free signal) but others, such as temperature, food availability, rainfall or water salinity, may also shape the rhythms. In fish, larval development, locomotor activity, sedation, skin pigmentation, oxygen consumption, thermoregulation, food intake and shoaling behaviour are among a number of functions that display daily rhythms (Ekström and Meissl, 1997; Falcón *et al*., 2007a). Horizontal migration (salmonids), growth, immune system and reproduction are the main functions known to exhibit annual rhythms, particularly in fish living in temperate and arctic areas. In some cases, these daily and annual rhythms are just a passive, on/off, type of response to the variations in photoperiod and temperature. In other (most?) cases, however, they are driven by internal clocks that free-run with a period close to 24 h (circadian rhythms) or one year (circannual rhythms) under constant conditions. Organisms equipped with such time measurement systems are able to predict and anticipate environmental changes, so that the right event will occur at the right time. This is a major improvement if one considers the number of more or less inter-dependent events that cycle on a 24 h and on an annual basis, from molecules to organisms, and from populations to ecosystems. If there is quite a huge amount of information on the mechanisms underlying circadian rhythms very little is known on those mechanisms driving the circannual rhythms (Bradshaw and Holzapfel, 2007; Paul *et al*., 2008).

A circadian system comprises all the different components by which light enters the organism and is transformed into a timed nervous or hormonal signal. The core of the system is made of a clock machinery, whose autonomous activity is synchronized to the prevailing 24

h LD cycle by light perceived through specific light sensors; in turn, the clocks drive the production of rhythmic output signals. Melatonin is one major output of the vertebrates' circadian clocks, which conveys rhythmic information to the organism. The daily pattern of melatonin secretion is much conserved among vertebrates, in which the pineal organ produces melatonin at night; this results in blood and cerebrospinal fluid melatonin levels that are high at night and low during day. This constancy emphasizes the key role the hormone plays in vertebrates. However, the organization of the circadian system that controls this melatonin rhythm has changed dramatically in vertebrates. In mammals, the photic information is perceived through the eyes and conveyed, through a retino-hypothalalamic tract (RHT), to the suprachiasmatic nuclei of the hypothalamus (SCN), where the master clocks reside; from there, a multisynaptic pathway (hypothalamic paraventricular nuclei [PVN] $\rightarrow$ preganglionic neurons of the sympathetic nervous system $\rightarrow$ superior cervical ganglion [SCG]) connects the SCN to the pineal gland, the melatonin producing unit (Fig. 1) **(**Simonneaux and Ribelayga, 2003). In fish and other non mammalian vertebrates, the circadian system is organized as a network of more or less tightly interconnected circadian systems (Fig. 1) (Falcón *et al.,* 2007). In all cases, the pineal organ and retina occupy a central position in this circadian organization.

What are the neuro-anatomical and functional basis of the rhythms? How are they synchronized and entrained? How do they impact on overt rhythms? These are some of the questions of crucial interest for our understanding of both the regulation of basic fish physiological functions and the control of fish maintenance, growth and reproduction for aquaculture purposes. The present review summarizes our current knowledge on the organization of time measurement systems in fish (circadian axis), with special emphasis on melatonin as the time-keeping hormone that impacts on crucial physiological functions, including growth and reproduction.

2. THE SOURCES OF MELATONIN PRODUCTION

2.1. The pineal gland

2.1.1 Functional anatomy of the pineal organ

In most species investigated, the pineal organ appears as a vesicle attached to the roof of the diencephalon by a slender stalk; it is usually located below a window in the skull through which light enters. The vesicle is made of a pseudo-stratified epithelium that is opened to the cerebrospinal fluid (CSF); folliculated (as in birds) as well as compact (as in mammals) glands have also been described (Omura and Oguri, 1969; Ekström and Meissl, 2003). The pineal epithelium is made of true cone-like photoreceptor cells and of ependymal interstitial cells that contact the CSF in their most apical part (Falcón, 1999). The photoreceptor cells establish synaptic contacts with second order neurons that send their axons to the brain. The pineal organ thus resembles very much to a simplified retina, and the structural and functional analogies between the two organs have been extensively reviewed in the past (O'Brien and Klein, 1986; Ekström and Meissl, 1997; Falcón, 1999; Falcón *et al*., 2007b).

The pineal photoreceptors share more than structural analogies with the retinal cones (Ekström and Meissl, 1997; Falcón, 1999; Falcón *et al.*, 2007b). As true light sensitive photoreceptors, they have a similar composition in lipids and proteins of the phototransduction cascade (opsins, transducin, arrestin, cyclic nucleotide gated channel). And, their electrical response to light stimuli is similar: light induces a dose-dependent cell hyperpolarization that results in the inhibition of an excitatory neurotransmitter (aspartate or glutamate). In the pineal organ, the excitatory neurotransmitter reaches directly the ganglion cells, which send their axons to the brain. Thus, the signals that are conveyed to the brain

142 reflect mainly the response of the photoreceptor cells, *i.e*., the pineal organ is a luminance
143 detector that provides information on light intensity, spectral content and duration of day-
144 length. It is interesting that the pineal and retinal ganglion cells may target similar brain areas,
145 particularly in the thalamus and pretectum (Ekström and Meissl, 1997).

146 In addition to the excitatory neurotransmitter, the pineal and retinal photoreceptors
147 both produce melatonin at night, following cell depolarization (Falcón, 1999; Falcón *et al*.,
148 2007b).

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150 ***2.1.2. Nocturnal melatonin biosynthesis in the pineal photoreceptor cells***

151 Melatonin is synthesized from tryptophan taken up by the pineal cells (Fig. 2). Two
152 enzymatic steps allow the formation of serotonin from tryptophan: Tryptophan hydroxylation,
153 catalyzed by tryptophan hydroxylase (TpOH), allows the synthesis of hydroxytryptophan,
154 which is then decarboxylated by the aromatic aminoacid decarboxylase, leading to the
155 formation of serotonin. Another two enzymatic steps transform serotonin into melatonin: the
156 arylalkylamine *N*-acetyltransferase (AANAT) catalyses the formation of *N*-acetylserotonin,
157 and the hydroxyindole-*O*-methyltransferase (HIOMT) converts the *N*-acetylserotonin formed
158 into melatonin (Falcón, 1999; Falcón *et al*., 2007a,b). While serotonin levels are high during
159 the day and decrease at night, melatonin levels present a shifted pattern with elevated levels at
160 night and basal levels during the day (Falcón 1999; Bromage *et al.,* 2001). The nocturnal rise
161 in melatonin production by the pineal reflects an increase in AANAT activity, whereas
162 HIOMT activity remains steady throughout the LD cycle.

163 Teleost fish are special because unlike all other vertebrates, they possess two AANAT
164 genes, probably as a result of genome duplications (Falcón *et al*., 2007). And, the so-called
165 AANAT1 and AANAT2 display tissue specific distribution: AANAT1 is more specifically
166 expressed in the retina and brain, whereas AANAT2 is more specifically expressed in the

pineal organ. Recently it was found that more distant teleost even possess two AANAT1 (1a and 1b; Coon *et al*., 2006). This has implications in terms of photic regulation of melatonin production.

Light and circadian control of pineal AANAT2 activity and melatonin production

Light inhibits AANAT2 activity and melatonin release *in vivo* or *in vitro*. At night, photoreceptor depolarization allows calcium (Ca^{2+}) entry (through voltage-gated Ca^{2+} channels) and cyclic AMP (cAMP) accumulation (Falcón, 1999). Both contribute to increase AANAT2 amount and activity through phosphorylation of the AANAT2 protein. This process is reversed by illumination, which sequentially induces photoreceptor hyperpolarization, dephosphorylation and degradation of AANAT2 through proteasomal proteolysis, resulting in the decrease of melatonin production (Falcón *et al*., 2001). The light-induced decrease in AANAT2 activity and melatonin secretion is a dose-dependent process (Fig. 3; Migaud et al., 2006), as it is the case for the inhibition of the neurotransmitter release, and depends on the spectral composition of the light. In trout, inhibition is seen with the short wavelengths of the visible spectrum (Max and Menaker, 1992). This situation applies in salmonid fish in which the pineal photoreceptor cell integrates the light signal; this allows controlling the amount of melatonin produced in an on/off manner (Fig. 4; Falcón 1999; Falcón *et al.,* 2007; Iigo *et al*., 2007). In this scheme, AANAT2 messenger RNA (mRNA) is made available at night, when AANAT2 is allowed to increase, or is constitutively expressed. As a consequence, continuous light (LL) suppresses melatonin secretion, whereas constant darkness (DD) results in constantly high levels of melatonin secretion. But in a majority of teleost species the response to light is not as passive as in the case of salmonids. This is because the control in melatonin secretion involves a circadian clock system located within the photoreceptor cells themselves (Bolliet *et al*., 1996). Extensive discussion on how such a time-keeping mechanism operates and is entrained by light is available elsewhere (Cahill, 2002; Reppert and Weaver, 2002).

Briefly, the circadian clock machinery is based on a molecular feed-back loop consisting of two heterodimers, PER/CRY acting as repressors and BMAL/CLOCK acting as activators, and additional interlocking loops. BMAL/CLOCK heterodimers also drive the rhythmic expression of a number of genes including the *Aanat2*, which thus appears as a direct output gene of the circadian clock (Appelbaum *et al*., 2004, 2006; Zilberman-Peled *et al*., 2007). Transcription of *Aanat2* allows accumulation of AANAT mRNA later during the day and early at night making AANAT production possible as soon as night starts. Morning light resets the clock (Ziv *et al*., 2005) and inhibits AANAT activity and melatonin secretion (Fig. 3). The presence of such a circadian clock allows the system anticipating changes in the LD conditions. This explains that under LL only the AANAT2 mRNA rhythm is maintained, whereas under DD, the rhythms in AANAT2 mRNA abundance, AANAT2 activity and melatonin secretion are maintained for days (Falcón 1999; Falcón *et al.,* 2007; Martinez-Chavez *et al*., 2008).

The diversity of the responses to light among fish may reflect specific adaptations to their environment, where light may vary in terms of quantity (intensity), quality (spectral content) and duration (photoperiod) (Sumpter, 1992; Boeuf and Le Bail, 1999). Intensity and spectral composition are prone to daily variations, particularly underwater, depending on the time of day (dawn and dusk), weather conditions or moon phase. These parameters are likely to affect the amplitude of the melatonin signal through a direct control on AANAT2 protein amount and enzymatic activity. In contrast, photoperiod (duration) is considered as a "noise free" signal as it remains constant over the years and reflects seasonality depending on the latitude. This signal is integrated by the clock machinery. In tropical areas, the phase of the rhythm is locked to the 12L/12D cycle and displays remarkable stability (Martinez-Chavez *et al*., 2008), whereas in temperate regions, the phase is adjusted day after day (Ziv *et al*., 2005).

Temperature and melatonin biosynthesis

Fish are ectotherms and as such they are directly influenced by the external temperature, which fluctuates on a daily and seasonal basis. Studies have shown that temperature acts directly on the pineal organ to modulate melatonin secretion, through the regulation of AANAT2 activity (Benyassi *et al*., 2000; Coon *et al*., 1999; Falcón, 1999; Falcón *et al*., 1994, 1996; Zachmann *et al*., 1992). Interestingly, (i) there is a good correlation between the peak of AANAT2 response and the fish optimal physiological temperature (trout: 12°C, pike: 20°C, seabream: 27°C; zebrafish: 30°C); (ii) the response to temperature is an intrinsic property of the enzyme itself, because the same response curves were obtained when activities were measured from cultured pineal organ homogenates or recombinant AANAT2 enzymes. In the pike, temperature had no effect on the phase and period of the circadian rhythm) (Falcón *et al*., 1994). Thus, the concurrent action of photoperiod, that determines the duration of the melatonin signal, and of temperature, that determines its amplitude, provide accurate definitions of both the daily and annual cycles. Any change in temperature, related to husbandry conditions or global warming, may thus have dramatic consequences on the time-keeping system of fish.

Internal factors and melatonin biosynthesis

The role played by internal factors in the control of melatonin production has received little attention. The data available is limited to few fish species, so that no general rule can be extrapolated (review in Falcón *et al*., 2007). Among these factors is melatonin itself (high concentrations inhibit its own production), neurotransmitters/neuromodulators produced locally (adenosine, GABA) or out of the pineal (norepinephrine), and hormones (glucocorticoïds, sexual steroids). For some of these factors (melatonin, adenosine), this represents a fine-tuning internal mechanism; for others (steroids) they might reflect a feed-back loop within melatonin regulated processes. The case of norepinephrine deserves special attention. Indeed, in mammals, norepinephrine is the final link in the pathway that brings light

information from the eyes to the pineal gland, through the circadian clocks of the SCN (Klein *et al*., 1997): the nocturnal release of the neurotransmitter synergistically activates α1- and α2-adrenergic receptors to stimulate nocturnal melatonin secretion. This is because the mammalian pinealocyte has lost any direct photosensitivity and circadian clock properties; the photoperiodic and circadian control of melatonin secretion relies exclusively on the RHT/SCN/SCG pathway (Fig. 1). An intermediate situation is seen in birds, which possess both a direct and an indirect photosensitivity, and where light and norepinephrine acting through α2-adrenergic receptors, concomitantly inhibit daytime melatonin secretion. The existence of a norepinephrine control of melatonin secretion in some, but not all, fish species might reflect the existence of a convergent evolution of the pathways involved in the control of melatonin production. Three arguments favor this view. First, a norepinephrine control has been demonstrated in the pike pineal gland, which possesses both 'typical' as well as 'rudimentary' photoreceptor cells, but not in trout pineal gland, which has only 'typical' cone like photoreceptors (Falcón, 1999). Second, Migaud *et al*. (2007) has recently shown that at least three categories of fish may be distinguished depending on whether the photoperiodic control of melatonin production by the pineal gland relies on the pineal itself, or the eyes, or both. Third, in contrast to the situation observed *in vitro* (see above), trout pineal AANAT activity as well as serotonin and melatonin contents do oscillate on a circadian basis in vivo, *i.e*., in animals maintained under constant darkness (Ceinos *et al*., 2008). This would suggest that the circadian organization that controls pineal melatonin secretion is likely to depend on a retinal/brain pathway in trout as is the case in mammals. Future studies should aim at exploring this point.

2.2. The retina and melatonin production

There are two ways by which the retina is involved in melatonin production. The first might be, as mentioned above, through controlling pineal melatonin secretion, although this remains to be fully demonstrated. The second is through its own production. In most vertebrate species so far investigated, retinal melatonin is produced during darkness as is the case in the pineal organ. However, teleost fish appeared to behave differently. Indeed, a nocturnal retinal melatonin pattern, as seen in zebrafish and goldfish (Cahill et al., 1991; Cahill, 1996; Iigo *et al.,* 1997a), is not the general rule. In other species either no rhythm is detected, or the peak is seen at different times of the LD cycle, including day (Gern *et al.,* 1978; Besseau *et al.,* 2006; Iigo *et al.*, 1997b; Migaud *et al*., unpublished). In the sea bass, the phase of the retinal rhythm changed throughout seasons (Bayarri *et al*., 2003). These differences in the retinal patterns may be due to the fact that several retinal cell types (including photoreceptors, inter-neurons and ganglion cells) express the melatonin biosynthesis enzymes AANAT and HIOMT (Besseau *et al*., 2006; Vuilleumier *et al*., 2007). It is not known yet (i) to which extent these cell types contribute to the overall retinal melatonin content and (ii) if melatonin production by these cells is under photoperiodic control. In this regard it is interesting that non-visual photoreceptors have been identified in the inner nuclear and ganglion cell layers of the fish retina (Cahill and Besharse 1995; Foster and Bellingham 2004; Bellingham *et al*., 2006). Thus, the photoperiodic control of melatonin production would not be exclusive to photoreceptor cells as believed for long. Another explanation for the retinal/pineal differences may result from this unique feature of teleost fish, which express one or two AANAT1 (1a, 1b) genes in the retina (Begay *et al*., 1998; Falcon *et al*., 2003; Tosini and Fukuhara, 2003; Coon and Klein, 2006). Irrespective of these differences, the production by the retina is under circadian clock control in some but not all species (Iuvone *et al*., 2005), as is the case in the pineal organ.

292 **2.3. Plasma and cerebrospinal fluid (CSF) melatonin is from the pineal organ?**

293

294 The plasma melatonin rhythm shows a nocturnal surge. In higher vertebrates, it was
295 shown that melatonin produced by the pineal gland is directly released into the CSF through
296 the pineal recess resulting in CSF levels twenty times as high as in the blood (Tricoire *et al*.,
297 2002). However, because there are several sources of melatonin in the organism (retina,
298 pineal gland, intestine; Bubenik *et al*., 1997), this raises the question of knowing to which
299 extend each of these organs contributes to the plasma and CSF levels. The question is even
300 more relevant in species where retinal and pineal melatonin rhythms are not in phase. Early
301 studies have shown that pinealectomized fish loose the plasma melatonin rhythm, indicating
302 the pineal organ is the main source of plasma melatonin. The presence of a strong melatonin
303 deacetylase activity in retinal tissues of non mammalian vertebrates, including fish (Grace *et*
304 *al*., 1991), prevents melatonin from being released into the blood stream and conversely, of
305 melatonin from other sources to reach the retina. It is believed that the main role of retinal
306 melatonin is to serve local functions, including retinomotor movements, modulation of
307 neurotransmitter release or neuronal electrical activity (Besseau *et al*., 2006; Siu et al., 2006;
308 Ping *et al*., 2008; Sauzet *et al*., 2008). It is interesting that the retinal cells that express the
309 melatonin biosynthesis enzymes also express the melatonin receptors, further supporting the
310 idea of melatonin being an autocrine signal in the retina (Sauzet *et al*., 2008). In brief,
311 although non pineal tissues may contribute to the blood plasma levels, the circulating
312 melatonin levels reflect mainly the activity of the pineal organ circadian clocks synchronized
313 by the LD cycle.

314

315 **2.4. Extra-pineal and extra-retinal sources of melatonin in the fish brain?**

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317 Since the initial studies by von Frisch (1911) and Benoit (1935), morpho-functional
318 evidence has accumulated indicating the existence of a non-pineal, non-retinal
319 photosensitivity in the central nervous system. First, light penetrates deep into the brain
320 (Foster and Hankins 2002; Vigh *et al.,* 2002b). Second, ophthalmectomized / pinealectomized
321 fish still responded to photoperiodic stimuli (Davis *et al.,* 1986; Garg 1989; Day and Taylor
322 2005; Masuda *et al.,* 2005). Third, electrical recordings from non-pineal non-retinal origin
323 have been obtained from frog diencephalon and mesencephalon (Cadusseau and Galand,
324 1980, 1981). Fourth, components of the phototransduction cascade, including opsin and α-
325 transducin have been detected in discrete brain areas of lampreys, fish, frogs and lizards
326 (Foster *et al*., 1994; Yoshikawa *et al*., 1994; Garcia-Fernandez *et al*., 1997; Okano *et al*.,
327 2000; Philp *et al*., 2000a, b; Àlvarez-Viejo *et al*., 2004). These areas include ependymal cells
328 that border the third ventricle as well as cells located in the SCN and preoptic area (POA).
329 Because the pineal and retinal photoreceptors are cellular circadian systems, it is tempting to
330 speculate that all the photoreceptive cells of the fish organism possess a molecular clock.
331 Indeed, studies in the lizard *Podarsis sicula* have shown that blockage of opsin expression in
332 the cells bordering the IIIrd ventricle abolished circadian entrainment in pinealectomized and
333 eyectomized lizards (Pasqualetti *et al*., 2003). In zebrafish, clocks have been identified in
334 several extra-retinal/extra-pineal tissues, including brain, liver and heart (Cahill 1996; Cahill
335 1997; Kazimi and Cahill 1999; Whitmore *et al.,* 1999; Cahill 2002; Dekens *et al.,* 2003;
336 Vallone *et al.,* 2005). Thus, the circadian organization of non mammalian vertebrates,
337 including fish, might consist of a network of more or less powerful interconnected circadian
338 oscillators, located in distinct areas (Fig. 1). This is supported by previous findings showing
339 that pinealectomy induces species-dependent effects on the circadian activity rhythm of fish
340 and lizards (Kavalier, 1989), resulting in either (i) complete loss of circadian activity rhythm,
341 (ii) change of circadian period or (iii) splitting of the circadian rhythm into several

components. In other words, the pineal organ is a 'mandatory' component or just a more or less powerful element in the circadian network.

The demonstration that diencephalic cells of *Rana perezi* (including SCN cells) express *Aanat1* adds to this puzzling picture and leads to the tempting hypothesis that some parts of the brain concentrate photosensitivity, circadian clock function and entrainment of AANAT gene expression. The functional significance of *Aanat* expression in the brain is yet to be discovered. Kinetic studies indicated AANAT1 may catalyse the acetylation of both dopamine and serotonin, leading to the formation of acetyl derivates, with specific functions in the brain (Zilberman-Peled *et al*., 2006). Another possibility would be that these cells also produce melatonin; preliminary investigations suggested this could be the case.

2.5. Conclusions

In brief, there is extensive evidence demonstrating the complexity of the light perception system in fish which involves the 'conventional' photoreceptive organs (retina, pineal) as well as most probably deep brain photoreceptors, the nature of which awaits further characterization. The organization of the circadian system has changed dramatically during evolution. Obviously, the mammalian and teleost fish arms have followed different evolution patterns, the modalities of which are far from being understood. It is interesting that the system in some fish has features displaying resemblance with the mammalian system (Migaud *et al*., 2007). More information is needed in order to determine whether this reflects a convergent evolutionary trend between teleost fish and tetrapods. The coexistence of several circadian systems in teleost fish raises a number of questions relative to their respective roles and synchronization.

3. THE PINEAL TARGETS

3.1. The targets of the nervous message

The pineal organ exhibits bidirectional connections with the brain through pinealofugal (efferent) and pinealopetal (afferent) projections. These connections have been elucidated in different fish classes including agnatha (lampreys), Chondrichtyes and teleosts using anterograde and retrograde tract-tracing markers such as horseradish peroxidase, lysine-cobalt and DiI (Ekström *et al*., 1984; Jiménez *et al*., 1995; Yáñez and Anadón, 1998; Pombal *et al*., 1999; Mandado *et al*., 2001). Pinealofugal projections can be considered as neural outputs conveying photic information to the central nervous system. These studies have revealed the existence of an efferent tract that exits the pineal stalk to reach a number of structures including the *habenula*, ventral and dorsal thalamus, posterior commissure, periventricular *pretectum*, pretectal area, posterior *tuberculum*, paraventricular organ, posterior tuberal nucleus, dorsal synencephalon and *tegmentum* (Fig. 5). A controversy exists concerning the presence of pinealofugal terminal fields in the POA/anterior hypothalamus (SCN?), which is also a retino-recipient area. Terminal projections have been detected in the Atlantic salmon, goldfish, sole, sturgeon and skate (Confente and Muñoz-Cueto, unpublished; Holmqvist *et al*., 1992; Jiménez *et al*., 1995; Mandado *et al*., 2001; Yañez and Anadón, 1998), but not in dogfish, lamprey, rainbow trout, stickleback, eel, carp and sea bass (Ekström,1984; Ekström and van Veen, 1984; Hafeez and Zerihun, 1974; Mandado *et al*., 2001; Servili *et al*., 2005; Yáñez *et al*. 1993). The functional significance of the pinealofugal innervations remains enigmatic. The fish pineal organ also receives axon terminals originating from cells in the thalamic *eminentia, habenula*, dorsal thalamus, ventromedial thalamus,

periventricular pretectum, posterior commissure, posterior *tuberculum* and dorsal synencephalon (Ekström *et al*., 1994; Jiménez *et al*., 1995; Yáñez and Anadón, 1998; Pombal *et al*., 1999; Mandado *et al*., 2001; Servili *et al*., 2005). Some of these brain areas overlap with brain regions that also appear connected with the retina (ventral and dorsal thalamus, pretectal area, posterior *tuberculum*), revealing their importance in the integration of photoperiod information, and constituting a possible pathway for the exchange of information between the retina and pineal organ.

3.2. The targets of the hormonal message

Melatonin represents the main hormonal output of the pineal organ. Its involvement in the control of processes displaying daily or seasonal rhythms is widely accepted but not fully demonstrated yet. In fish, daily rhythms affected by the pineal organ and/or melatonin include locomotors activity, thermal preference, rest, food intake, vertical migration and shoaling, skin pigmentation, osmoregulation and metabolisms (including control of hypothalamic monoamines, hepatic lipids and glucose and plasma steroid levels); annual processes include smoltification (for migrating salmonids), growth and reproduction (Falcón *et al*., 2007). Studies that aimed at elucidating the role melatonin plays in fish first used photoperiod manipulations, pinealectomy or melatonin administration, however the responses to these treatments was variable as they were dependent on too many factors as discussed elsewhere (Mayer *et al*., 1997). Another strategy is to identify and characterize the melatonin receptors, and localize there sites of expression, from where it will be possible to investigate what functions they modulate and how.

3.2.1. Identification of the melatonin receptors

In fish the cloning and pharmaco-kinetic experiments using 2-[^{125}I]-iodomelatonin (^{125}IMel) have allowed the identification of three high affinity melatonin receptor subtypes, all belonging to the family of G-protein coupled seven transmembrane domains receptors, the MT1, MT2 and Mel1c (Falcón *et al*., 2007). Studies conducted mainly in mammals indicated that the melatonin receptors may be coupled to several intracellular pathways, the more common being the adenyl cyclase/cyclic AMP (cAMP) and the pholspholipase C/diacyglycerol/inositol tri-phosphates, pathways (Falcón *et al*., 2007). The full length cloning of the melatonin receptors has been obtained in trout (MT1), rabbitfish (MT1, Mel1c), seabass (MT1, MT2, Mel1c), sole (MT1, MT2, Mel1c) and pike (MT2) (Sauzet *et al*., 2008 and unpublished). More full length sequences predicted from the genome analysis of zebrafish, tetraodon (*T. biocellatus*) and fugu (*Sphoeroides maculatus*), are available from the databases. Functional studies indicated the fish MT2 receptor is negatively coupled to the cAMP pathway.

3.2.2. Tissue specific expression of the melatonin receptors

Fish MT1 and MT2 receptors are widely distributed in the nervous (retina, brain) and peripheral tissues, whereas Mel1c expression is mainly found in the skin and retina (Falcón *et al*., 2007; Sauzet *et al*., 2008).

Brain and pituitary

In the brain of lampreys, Chondrichtyes and Teleost fish, gene expression or ^{125}IMel binding are associated with areas that receive or integrate information from sensory organs (*e.g*., olfactive bulbs, telencephalon, diencephalon, optic tectum and cerebellum) and the receptors are mostly associated with areas receiving input from the retina and/or the pineal. Melatonin serves as yet unknown functions in the central nervous system of fish. However, it is noteworthy that fish diencephalic areas that bind melatonin may also express molecules of

442 the visual cascade (*e.g.*, opsins, transducin, arrestin) and receive nervous input from the eye or
443 the pineal or both (Ekström and Meissl, 1997; Alvarez-Viejo *et al*., 2004; Philp *et al*.,
444 2000a,b). This could suggest melatonin might well be the 'conductor' that phases photoperiod
445 related activities in a network of players (Fig. 1). Because, as suggested above, melatonin
446 might be synthesized locally, it could also be an autocrine regulator in these areas, as it
447 appears to be the case in the retina.

448 In addition, expression, binding and pharmacological studies have demonstrated that
449 melatonin receptors linked to inhibition of cAMP are present in the pituitary gland of pike,
450 trout and seabass (Gaildrat *et al*., 2002; Falcón *et al*., 2003, Sauzet *et al*., 2008).

451 *Retina*

452 Retinal melatonin is involved in the control of a number of retinal functions, including
453 melanosome aggregation in the pigment epithelium, rod outer segment shedding, cone
454 retinomotor movements, modulation of neurotransmitters release and electroretinogram
455 (Lundmark *et al*., 2006; O'Brien and Klein, 1986; Pautler and Hall, 1987). A recent study in
456 the seabass identified MT1 and MT2 expression in the three nuclear layers of the neural fish
457 retina (as is the case in other vertebrates) as well as in the retinal pigment epithelium (Sauzet
458 *et al*., 2008). It is interesting that in the neuronal retina, the receptors are expressed in the
459 same cell types that also express the melatonin biosynthesis enzymes, which indicates
460 melatonin is a true autocrine regulator in this organ.

461 *Peripheral tissues*

462 Expression of melatonin receptors or ^{125}IMel binding, have been detected in different
463 peripheral tissues, including kidney, intestine, blood cells, gonads and gills (Kulczykowska *et*
464 *al*., 2006; Park *et al*., 2006; Sauzet *et al*., 2008, and authors' unpublished data). The functional
465 significance of these receptors awaits further experimentation.

466

3.3. The photoneuroendocrine connexions in fish

The photic information can reach the pituitary directly through its hormonal messenger melatonin and pituitary melatonin receptors, as mentioned above. Upstream to the pituitary, at least four diencephalic areas appear as key components: the POA, SCN, lateral tuberal nucleus (LTN) and ventromedial thalamic nucleus (VTN). All four receive hormonal information (Ekström and Vanecek, 1992; Vernadakis *et al*., 1998; Herrera-Perez *et al*., 2007; Sébert *et al*., 2008), and some receive nervous information as well, from either the retina (POA, SCN) or the pineal organ (POA, VTN) (Ekström and Meissl, 1997; Mandado *et al*., 2001). In turn, POA and hypothalamic neurons send projections to the pituitary; dopamine and peptides released from these terminals regulate pituitary function (Batten *et al*., 1993, 1999; Cerdá-Reverter *et al*., 1999, 2000; Chiba, 1999; Kah *et al*., 1993; García-Robledo and Muñoz-Cueto, unpublished). The latter include pituitary adenylate cyclase activating peptide (PACAP), Neuropeptide Y (NPY), growth hormone-releasing hormone (GHRH), corticotrophin-releasing hormone (CRH) and gonadotropin-releasing hormones (GnRHs) (Batten *et al*., 1999; Montero *et al*., 2000; González-Martinez *et al*., 2002).

There is also indication that POA and hypothalamic neurons may impact on the light sensitive organs. This includes GnRH neurons that innervate both the retina and the pineal organ (Gonzalez-Martinez *et al*., 2004; Sakharkar *et al*., 2005; Servili *et al*., 2007; Wirsig-Wiechmann and Wiechmann, 2002), NPY and GHRH neurons that innervate the pineal (Blank *et al*., 1997; Subhedar *et al*., 1996). Of course the list is most certainly not exhaustive.

4. MELATONIN EFFECTS

Early studies dealing with the effects of photoperiod manipulation, pinealectomy and/or melatonin treatment led to conflicting conclusions regarding the role melatonin plays in neuroendocrine regulations (Mayer *et al*., 1997; Bromage *et al*., 2001; Boeuf and Falcón, 2002; Falcón *et al*., 2007). This is because these studies used different experimental procedures (the time of the year at which the experiments were done was crucial), different species or, within a same species, animals of different sex and historical status. However, evidence is now coming to light which indicates melatonin mediates the effects of photoperiod on several neuroendocrine functions.

4.1. Reproduction

The impact of melatonin on the seasonal cycle of reproduction has been largely investigated using photoperiod manipulations, pinealectomy and/or melatonin administration (Ekström an Meissl, 1997; Mayer *et al*., 1997). But the first unequivocal evidence that melatonin has indeed an effect came from an *in vitro* study in the Atlantic croaker (*Micropogonias undulatus*) (Khan and Thomas, 1996). In fish with fully developed gonads, low concentrations of melatonin stimulated *in vitro* LH release from pituitary cells in culture; *in vivo*, melatonin elicited significant elevations in plasma LH levels late during the photophase of the day-night cycle, when administered in the basal diencephalon. This suggested that melatonin acted both at the POA/hypothalamus and pituitary levels. This was recently confirmed in the eel where melatonin implants induced a decrease in LHβ and FSHβ expressions as well as in plasma levels of some sexual steroids (Sébert *et al*., 2008). This was partly achieved through an action on the POA dopaminergic cells, which were the only brain catecholaminergic cells showing increased tyrosine hydroxylase (TH) expression. However, what applies to eels might not be the rule for other fish. In cultured carp hypothalamus,

517 melatonin reduces dopamine levels, which would result in an increased LHβ secretion (Popek
518 *et al*., 2005). Further evidence that melatonin plays a significant role in the regulation of
519 annual testicular events was obtained in a sub-tropical fish species, carp, *Catla catla*
520 (Bhattacharya *et al*., 2007). Indeed, the authors of this study showed precocious testicular
521 maturation in both melatonin-treated fish and fish exposed to continuous darkness (DD)
522 during the preparatory phase and an inhibition of testicular function during the pre-spawning
523 and spawning phases. Results obtained by the same group also recently demonstrated that
524 melatonin can accelerate the action of the Maturational Inducting Hormone (MIH) when
525 added 4 h prior to MIH in the incubation medium (Chattoraj *et al*., 2005) and that serotonin
526 inhibits the actions of MIH, but also the actions of melatonin on the MIH-induced oocyte
527 maturation in carp (Chattoraj *et al*., 2008).
528
529 **4.2. Growth and feeding**
530
531 Available data indicate fish growth follows a seasonal pattern which varies as a
532 function of day-length (Boeuf and Falcón, 2002). Generally, larvae need a minimal light
533 intensity threshold to be able to develop and grow normally. Older fish (marine and salmonid
534 species) also react to photoperiod manipulations; long days generally stimulate growth in
535 diurnal fish species. The synergistic effect of "food availability" and "day length" is
536 important. Growth, food intake and digestion are related to specific behavioural rhythms and
537 to reproduction; and, a pineal (melatonin) control is thought to operate here (Ekström and
538 Meissl, 1997; Mayer, 2000; Porter *et al*., 1998; Underwood, 1989; 1999; Zachmann *et al.,*
539 1992; Zhdanova *et al.,* 2001). However, the results are often contradictory. For example, (i)
540 *i.p*. administration of melatonin to goldfish maintained under short - but not long -
541 photoperiod for several days accelerated weight gain and growth (De Vlaming, 1980); (ii)

melatonin implants increased weight in Atlantic salmon parr (*Salmo salar*; Porter *et al*., 1998) but reduced body weight and growth rate in trout (*Oncorhynchus mykiss*; **Taylor et al., 2005**). Regarding feeding, it has been shown that acute melatonin treatments generally result in a reduced food intake (De Pedro *et al*., 2008; Lopez-Olmeda *et al*., 2006; Pinillos *et al.,* 2001; Rubio *et al*., 2004); but, fish grow differently depending on the circadian time feeding (Spieler, 2001). Overall, these discrepancies might just reflect a seasonal regulation of feeding and growth. It has been shown that *in vitro*, cultured trout pituitary glands or cells released increasing levels of GH when challenged with physiological concentrations of melatonin (Falcón *et al*., 2003). However, inhibition of GH release was also observed under specific pharmacological conditions, suggesting a bimodal effect of melatonin on GH production. And, under conditions that stimulate GH secretion, melatonin also induced a sustained inhibition of PRL release (Falcón *et al*., 2003). GH and PRL are two closely related hormones that often act in an antagonistic manner (Nguyen *et al*., 2008). The effects of melatonin on growth may thus result from the differential impact the hormone has on GH and PRL, and perhaps on other pituitary hormones. In addition to a direct effect on the pituitary, melatonin might also modulate fish feeding and growth through controlling the production of releasing and inhibiting factors by neurons from the POA and hypothalamic nuclei (see above) as well as by targeting directly peripheral tissues. Thus, in the goldfish melatonin administration (i) inhibited food intake, but only after *i.p*., not intra-cerebral administration, excluding a centrally-mediated action (Pinillos *et al*., 2001), and (ii) reduced body weight gain and specific growth rate through modulating noradrenergic metabolism in the hypothalamus (De Pedro *et al*., 2008).

4.3. Immune system

It is well documented that seasonality affects the immune response of vertebrates (Zapata, 1992). In fish, adaptive immunity exhibits a seasonal cycle. This includes changes in resting antibody titer and response to antigenic challenge (Nakanishi, 1986), lymphoid system (Álvarez *et al*., 1998; Wojtowicz and Plytycz, 1997), number of circulating lymphocytes (Slater and Schreck, 1998), lysozyme activity and number of red and white blood cells (Morgan *et al*., in press). The involvement of the pineal organ and melatonin in the control of immunity remains to be clarified. But preliminary investigations in trout and sea bass indicate melatonin affects the expression of genes such as PRL, GH or proopiomelanocortin (POMC) (Falcón *et al*., 2001, and unpublished). GH and PRL are involved in the control of immunity in fish; and, the POMC gene encodes a protein precursor of active peptides including α- and β-melanocyte stimulating hormone, corticotropin hormone (ACTH), lipoprotein hormone and β-endorphine, which are involved in the control of stress, feeding and immunity.

4.4. Conclusions

In brief, although investigations on the relationships between the pineal organ and melatonin on the one hand, and the neuroendocrine system on the other hand, are just at their beginnings, it seems more and more evident that the time-keeping hormone impacts directly or indirectly on the production of pituitary hormones, thus affecting time regulated functions, including feeding, growth, reproduction and immunity. More studies are necessary to unravel the regulatory processes activated by melatonin on a daily and annual basis. Such investigations have great potential interest for the aquaculture industry.

5. IMPLICATIONS FOR AQUACULTURE

592

593 The question regarding the mechanisms driving the reproductive circannual (seasonal)
594 rhythms and how they are synchronized and entrained in teleost is a black box despite its
595 importance for aquaculture. Fish reproductive physiology shows an extraordinary close
596 adaptation to the cyclical variations of the environment; fish synchronize their spawning to
597 the period of the year most favorable for the survival of progeny. Accordingly, fish have
598 developed predictive mechanisms using photoperiod as a reliable environmental cue
599 (proximal factor) to anticipate and activate gametogenesis long before spawning (Carrillo *et*
600 *al*. 1993; Bromage *et al*. 2001). The period of reproduction of most temperate commercial
601 fish species important for aquaculture is restricted to only a few months of the year, when the
602 most appropriate environmental conditions are found; this guarantees the best chances of
603 progeny survival. However, the restriction of reproductive activity to a short annual window
604 is a problem for fish farmers that rely on year long supply of juveniles to satisfy an increasing
605 demand in fish. Early maturation during on growing is another major bottleneck leading to
606 losses due to deterioration of flesh quality, external appearance and poor growth
607 performances. Consequently, based on the basic understanding of the circadian axis and
608 photoperiodic entrainment of reproduction, regimes have been developed with great success
609 to manipulate the natural circannual rhythm of spawning in many temperate fish species.
610 Importantly, photoperiod could also be used to improve reproductive performances in tropical
611 fish species although these species do not experience significant annual photoperiodic
612 changes in their natural habitat (Campos Mendoza *et al*., 2004).

613

614 **5.1. Photic manipulation of fish physiology**

615

616 Lighting regimes aiming at suppressing the melatonin rhythmic signal or changing the

617 season is used throughout the industry to manipulate the timing of broodstock spawning,
618 smoltification and early maturation in a number of commercially important species (salmon,
619 trout, cod, sea bass, halibut….). Compression or extension of the seasonal light cycle results
620 in spawning advance or delay, respectively, in salmonid species (Bromage and Duston, 1986;
621 Bromage *et al*., 2001). Similarly, the time of first sexual maturation can also be modified by
622 photoperiod manipulations in teleosts (Randall *et al*., 1998; Rodríguez et al., 2001). In
623 salmonids, it is now evident that the increasing and decreasing components of the seasonally
624 changing day-length are responsible for the recruitment of fish into reproduction and
625 gonadogenesis, respectively (Endal *et al*., 2000; Bromage *et al*., 2001). This is in contrast
626 with findings in Atlantic cod (*Gadus morhua*) showing a phase shift control of reproduction
627 with decreasing day-length recruiting individuals into reproduction and increasing day-length
628 stimulating gonadogenesis (Hansen *et al*., 2001; Davie *et al*., 2007a,b). However, these
629 increasing and decreasing photoperiods can also be replaced by periods of constant day-length
630 (Carrillo et al., 1993; Bromage *et al*., 2001). Interestingly, continuous light can also fully
631 inhibit reproduction in cod (Davie *et al*., 2007a) and prevent precocity in juvenile male sea
632 bass (Begtashi et al., 2004). This is routinely used in fish farming especially to suppress or
633 decrease early maturation during the growth phase. For this purpose, continuous artificial
634 lighting is applied from either winter solstice in salmon or summer solstice in cod with the
635 aim to trick the fish in believing that they are in summer (salmon) during the winter time or
636 mask the autumnal decrease in daylength (cod). Continuous light treatments (LL) given either
637 at the pregametogenesis or during the gametogenesis to juvenile male sea bass were equally
638 effective in reducing the number of early maturing males suggesting light may have a direct
639 effect on the arrest of meiotic divisions of the germinal cells and thus on the gonadogenesis
640 (Felip *et al*., 2008). Besides, this work indicated that a potential photo-labile period may exist
641 in the sea bass located somewhere in the autumn between September and October. The recent

642 location of such a photo-labile period (Fig. 6; Carrillo et al., 2008) offers the possibility to
643 effectively suppress early maturation in sea bass by a two months exposure to continuous
644 light in otherwise natural photoperiod, reducing considerably the duration of the treatment
645 with equal effectiveness than the longer ones.

646 Bayarri et al., (2008) demonstrated that the presence of a photoperiod is necessary to
647 maintain the circadian variations in certain reproductive hormones, which are, at the same
648 time, necessary for the normal process of reproduction. However, when LL is applied and
649 suppression of daily rhythms of key hormones such as melatonin and LH in fish is produced,
650 gonadal development and maturation becomes full arrested (Fig. 7).

651 Light is also used in the salmon industry to manipulate the timing of smoltification by
652 using short day winter photoperiods in summer (Duston and Saunders 1992). A combination
653 of constant 1-3 months duration long days, in otherwise constant short days, applied at
654 different times of the year followed by an appropriate thermal manipulation has been proved
655 to be a reliable tool to obtain spawns every month of the year in some sea bass farms of the
656 Mediterranean area (Carrillo et al., 1995; Carrillo, unpublished results).

657

658 **5.2. Light sensitivity**

659

660 One major question concerning light manipulation in fish farming relates to the
661 duration and quality of the photic signal (Bromage *et al.,* 2001; Boeuf and Falcón 2002).
662 Duration varies along the annual cycle in a regular and predictable manner, whereas quality
663 varies in a less predictable manner. Thus, in order to optimize the rearing conditions and
664 eventually being able to manipulate the fish physiology one has to consider the respective
665 durations of day and night and, in both situations, light intensity, spectral composition and
666 orientation. It is important to emphasize that fish perceive light both from above (*via* the

667 pineal organ), the sides (*via* the eyes) and possibly through deep brain photoreceptors. In
668 addition, it is evident that the intensity and quality of light that reaches the pineal organ and
669 brain depends on the degree of absorbance through the skin and skull. The amount and quality
670 of light that crosses the pineal window varies from one species to another (Gern *et al*., 1992;
671 Migaud *et al*., 2006, 2007). Differences in light penetration through the skull range from 1 to
672 8% of simulated daylight in Teleost fish; and, long wavelengths (650–700 nm) are far more
673 effective at penetrating the skull than shorter wavelengths (400–450 nm). For example, in
674 terms of melatonin production the threshold of light intensity above which melatonin is
675 suppressed depends on the species, experimental conditions (*in vitro* or *in vivo*), light quality
676 and duration (Aoki *et al.,*, 1998; Bayarri *et al.,*, 2002; Migaud *et al*., 2006 and unpublished;
677 Oliveira *et al*., 2007; Vera *et al*., 2005). It depends also on the developmental stage/size of the
678 fish, temperature profiles and previously experienced photic conditions. All these factors
679 highlight the difficulties generally encountered when attempting to compare and review
680 existing data in fish.
681
682 **5.3. Circannual Rhythms and the Endogenous Control of Reproduction**
683
684 The demonstration that the seasonal cycle of reproduction is controlled by a time
685 keeping system requires long term and costly studies. An endogenous clock controlled rhythm
686 must satisfy a number of criteria in order to be defined as such. It should be observed for at
687 least 2 full cycles and with a period that approximates 12 months under constant conditions; it
688 should also be entrained by an environmental *zeitgeber* (*e.g*., photoperiod) and display
689 temperature compensation (Gwinner, 1986). The demonstration that a circannual endogenous
690 mechanism controls reproduction is of paramount importance to fully understand reproductive
691 physiology and eventually elaborate species depend strategies to optimize either growth or

692 reproduction. An endogenous control of reproduction has been reported in many species of
693 fish, in animals maintained under constant conditions of photoperiod, temperature, salinity,
694 etc… These include trout (Bromage *et al*., 1984; Duston and Bromage, 1991; Randall *et al*
695 1999), stinging catfish, *Heteropneustes fossilis* (Sundararaj *et al*., 1982), three-spined
696 stickleback, *Gasterosteus aculeatus* (Baggerman, 1980), sea bass, *Dicentrarchus labrax*
697 (Carrillo *et al*., 1993, Prat *et al*., 1999); Barbel, *Barbus barbus* (Poncin 1991) and Atlantic
698 halibut, *Hippoglossus hippoglossus* (Björnsson *et al*., 1998). A better understanding of these
699 circannual rhythms can help to improve broodstock management within the aquaculture
700 industry.
701
702
703 **6. CONCLUSIONS**
704
705 It is widely accepted that melatonin is a much conserved feature that plays a central role
706 in the entrainment of daily and annual physiological rhythms in vertebrates. Over the last
707 decades, a large amount of research has been carried out in fish, especially teleosts, to unravel
708 the puzzling roles of melatonin in fish and characterize inter-species differences. As a result,
709 the picture is becoming much clearer with regards to the control of the melatonin synthesis
710 (limiting enzyme AANAT, connection/projection…) and the identification of target tissues,
711 neurons and cells (melatonin receptor expression and localisation). However, the multiple and
712 complex effects of melatonin on fish neuroendocrine regulation still awaits a more complete
713 and precise understanding. Indeed, to date, although fish seasonality and photoperiodism has
714 been demonstrated in many fish species, only few direct evidences exists on how melatonin
715 acts on the Brain-Pituitary-Gonadal axis controlling reproduction, the somatotropic axis
716 controlling appetite, feed intake and growth, and behaviour, just to name a few examples, in

717 fish. With the recent "post-genomic" advances, it will now be feasible to identify and
718 characterize patterns of gene expression among the suite of genes that control circadian
719 rhythms and link them to physiological processes. Such a broad-view transcriptomics
720 approach is particularly appropriate for addressing basic questions concerning co-ordination
721 of activities within complex functional networks such as the melatonin system. One major
722 constraint that fish biologist have to address is the multitude of adaptations/organisations that
723 can be found in fish in contrast to mammals meaning that no generalized fish model can be
724 easily drawn. On the other hand, this can also be considered as a strength as comparative
725 studies within fish species can help to better understand evolutionary trends that have led to
726 the situation observed in mammals. Importantly, the study of the circadian axis and melatonin
727 is very relevant to the aquaculture industry. The large variability in regulatory systems,
728 sensitivities and responses observed among Teleosts means that species specific regimes will
729 have to be implemented in commercial set ups to improve and standardized husbandry
730 practices (use of light, handling, broodstock management, vaccination…) in fish aquaculture.
731 To do so, a better understanding and characterisation of circadian and circannual rhythms is
732 clearly needed.
733

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1195

FIGURE LEGENDS

Figure 1. Photoperiodic and circadian control of neuroendocrine functions. (a) Fish versus mammals. In mammals (i) a linear flow leads to the rhythmic production of melatonin. Nonvisual information from the retina reaches the SCN of the hypothalamus through the retinohypothalamic tract (blue arrow). The periodic signals enable synchronizing of the circadian activity of the SCN clocks, which, in turn, impact on the pineal gland through a multisynaptic pathway (blue arrows), thus controlling cyclical melatonin secretion. Melatonin feeds back to the SCN and acts on the pars tuberalis of the pituitary and other brain areas to modulate seasonal neuroendocrine functions. The situation is more complex in fish (ii): the photoneuroendocrine system seems to be organized as a network of independent and interconnected light-sensitive oscillatory units in the retina, the pineal and, perhaps, in the brain. The dashed blue arrow indicates a hypothetical connection. '?' in the brain indicates the hypothetical presence of brain circadian oscillators. (b) Photoneuroendocrine regulation in fish. Light (yellow arrows) impacts on photoreceptor cells of the pineal organ and retina, enabling synchronization of their internal molecular clocks. Light might also impact on other possible photosensitive and circadian structures in the ventral diencephalon (POA and hypothalamic area; yellow arrow with '??') and peripheral organs. In response to the photoperiodic information, the retina and the pineal organ elaborate two types of rhythmic information. The neural information (blue arrows) from the retina and pineal organ reach the ventral diencephalon through the retinohypothalamic and the pineal tracts, respectively. This information provides an indication of day length, as well as of subtle variations in ambient illumination. The hormonal information is relayed by melatonin (red arrows), the production of which reflects day length and season. In the retina, melatonin is an autocrine and/or paracrine factor, which is metabolized locally. Pineal melatonin is released into the

1221 cerebrospinal fluid and blood, and acts on specific targets through melatonin receptors (red
1222 filled circles). In the hypothalamus, melatonin might contribute to synchronizing the activities
1223 of circadian oscillatory units [SCN and others (depicted by '?')] and modulating the
1224 production of pituitary gland releasing factors. Melatonin receptors have been identified in
1225 areas that impact on pituitary function, including the POA, which also receives nervous input
1226 from both the pineal organ and the retina. Melatonin impacts on the pituitary gland itself to
1227 modulate the production of hormones. Taken from Falcón et al. (2007) with permission.
1228
1229 **Figure 2.** Melatonin biosynthesis pathway. See text for details. On the top right is indicated a
1230 photoperiodic 24 L (light) and D (dark) cycle with a schematic indication of the daily
1231 variations in the corresponding compound or enzyme activity. AAAD: aromatic amino-acid
1232 decarboxylase; AANAT: arylalkylamine *N*-acetyltransferase; HIOMT: hydroxyindole-*O*-
1233 methyltransferase; TP-OH tryptophan hydroxylase;
1234
1235 **Figure 3.** Irradiance response plots for Atlantic salmon, European sea bass and Atlantic cod
1236 derived from *in vitro* pineal gland studies (from Migaud *et al*., 2006, Migaud *et al*.,
1237 unpublished). Arrows indicate theorical *in vitro* thresholds between day and night.
1238
1239 **Figure 4.** Diagrammatic representation of the different melatonin profiles recorded in
1240 vertebrates. Examples of species which express such patterns of plasma melatonin for each
1241 profile are listed. Horizontal black bar denotes subjective dark period.

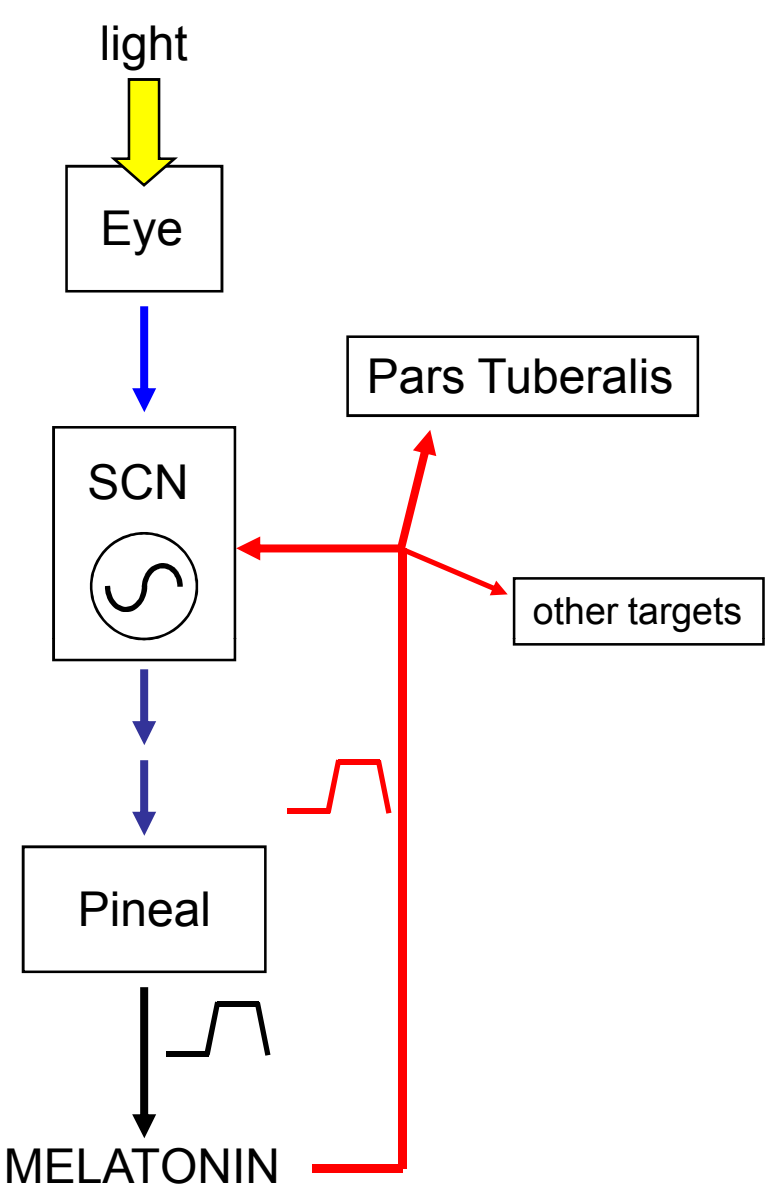
Figure 5. A. Pinealofugal projections from the sea bass pineal organ reaching the ventromedial thalamic nucleus (VM). DiI tract tracing analysis. **B.** Neurons from the ventromedial thalamic nucleus (VM) projecting to the sea bass pineal organ. DiI tract tracing analysis. **C.** Neurons from the ventromedial thalamic nucleus (VM) projecting to the sea bass pituitary. DiI tract tracing analysis. **D.** Tyrosine-hydroxylase (catecholaminergic) cells in the ventromedial thalamic nucleus (VM). Immunohistochemical study.

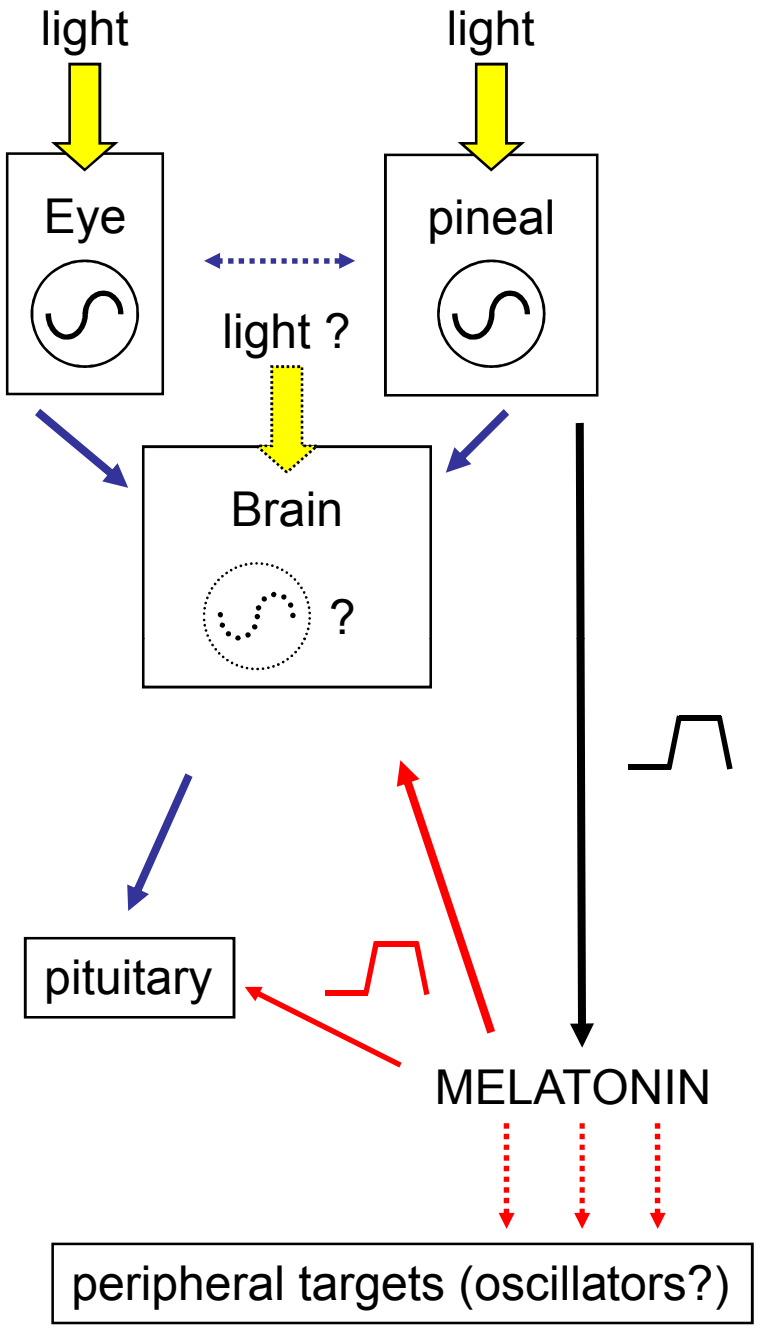
Figure 6. Screening of the sensitive period for inhibition of maturation of sea bass males at the first year of life by over-imposition of continuous light of different durations during the natural autumnal photoperiodic reduction. A sensitivity period is likely placed around August-September (Grey area). 2-4 = Carrillo et al., 2008. 5-6 = Felip et al., 2008; 7 = Begtashi et al. 2004.

Figure 7. Effects of continuous light (LL) exposure on the seasonal plasma LH daily rhythm of male sea bass during their first year of life. Suppression of the nocturnal peak of LH induced an immature state of gonad development and inhibited the appearance of precocious fish. After Bayarri et al., 2008. Chronobiol. Int. (*in press*).

Figure 8. Circannual rhythms of spawning in sea bass maintained under constant short photoperiod (9 hours light: 15 hours dark; 9HL: 15HD) (B) and constant long photoperiod (15HL: 9HD) (D) during several consecutive years. The spawning rhythms "free run" with a period of approximately a year. A constant long photoperiod of one month of duration applied during May, April or March, in otherwise constant short photoperiod, was used to entrain the endogenous annual clock. Progressive advances of the spawning time were obtained with early applications of long days and a return to the free running rhythm was observed after exposure to constant short days (C). The clock controlled circannual rhythm would be fully in phase with the ambient light cycle (A) as a result of ongoing re-entrainment by the seasonally-changing day length and consequently spawning time will occurs at the natural period of reproduction (dashed area). LP = long photoperiod; SP = short photoperiod. After Carrillo *et al*. (1995).

Figure 1


light
Eye
SCN
Pars Tuberalis
other targets
Pineal
MELATONIN


light
light
Eye
pineal
light ?
Brain
?
pituitary
MELATONIN
peripheral targets (oscillators?)

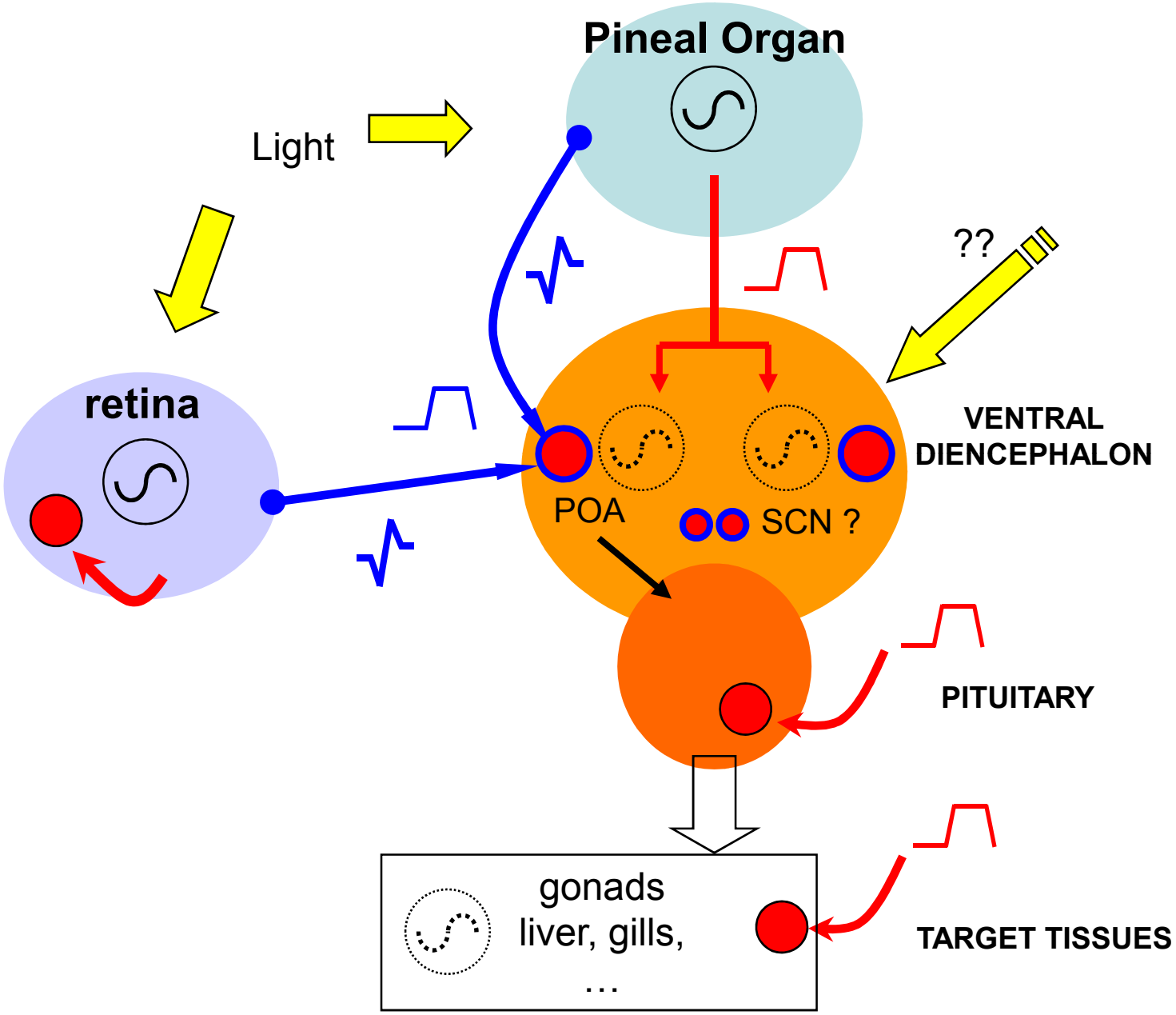

Pineal Organ
Light
??
retina
VENTRAL
DIENCEPHALON
POA
SCN ?
PITUITARY
gonads
liver, gills,
…
TARGET TISSUES

Figure 2-4

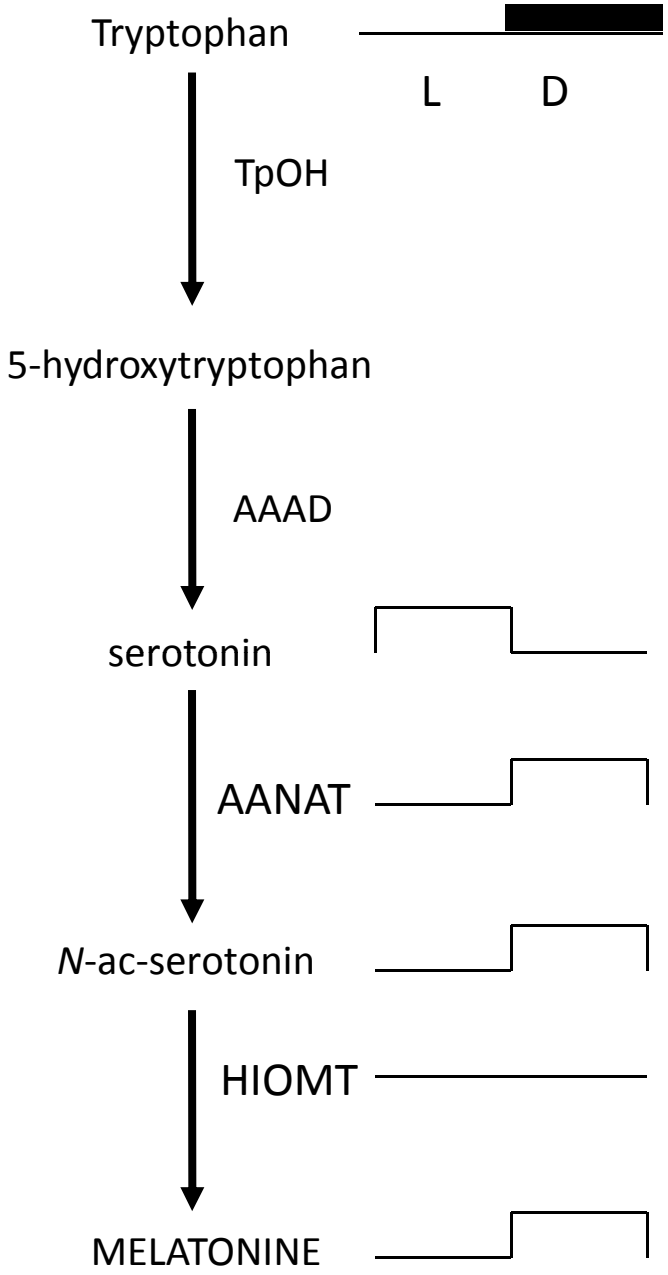


Figure 2

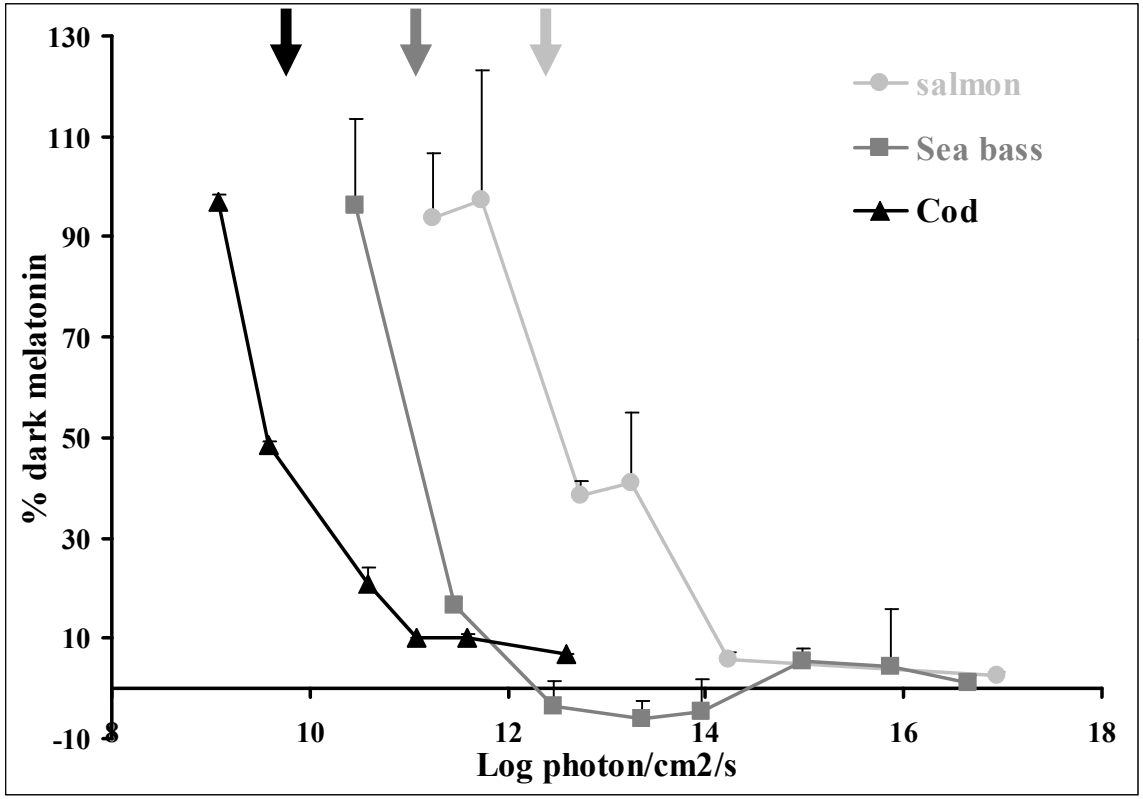
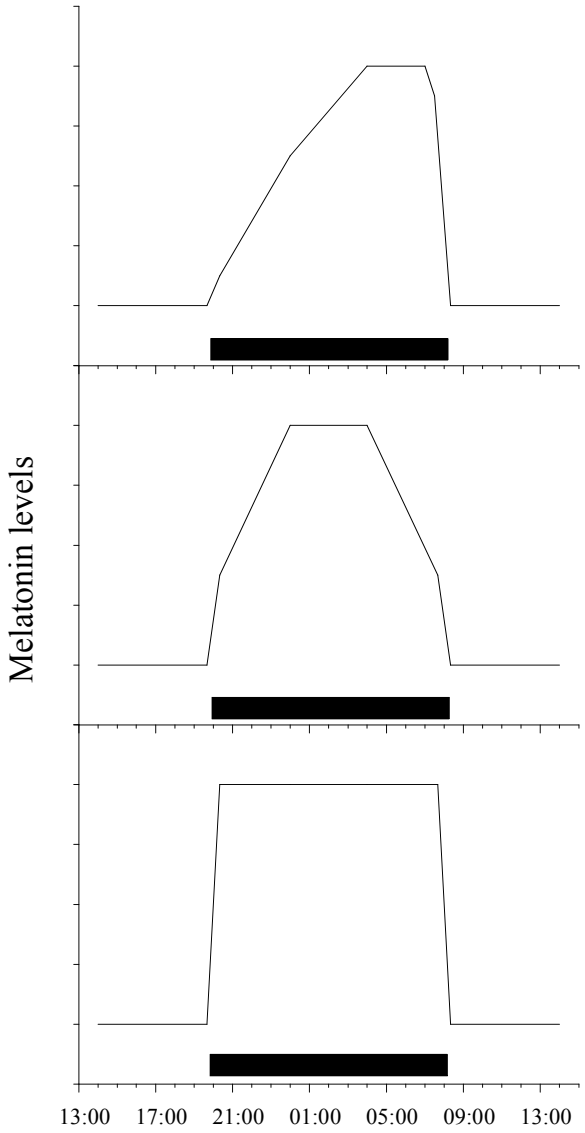

130
110
90
70
50
30
10
-10
% dark melatonin
8
10
12
14
16
18
Log photon/cm2/s
salmon
Sea bass
Cod

Figure 3



Description of rhythm

Type A: Discrete peak in late dark phase

Species: Syrian Hamster
Mongoloian Gerbil
House Mouse
Cod
Haddock

Type B: Discrete peak in mid dark phase

Species: Albino Rat
Eastern Chipmunk
Turkish Hamster
Human
Nile Tilapia

Type C: Prolonged peak through the majority of the dark pahse

Species: White - Foot Mouse
Djungarian Hamster
Domestic cat
Sheep
Atlantic salmon
Rainbow trout
Atlantic halibut

Figure 4

Figure 5

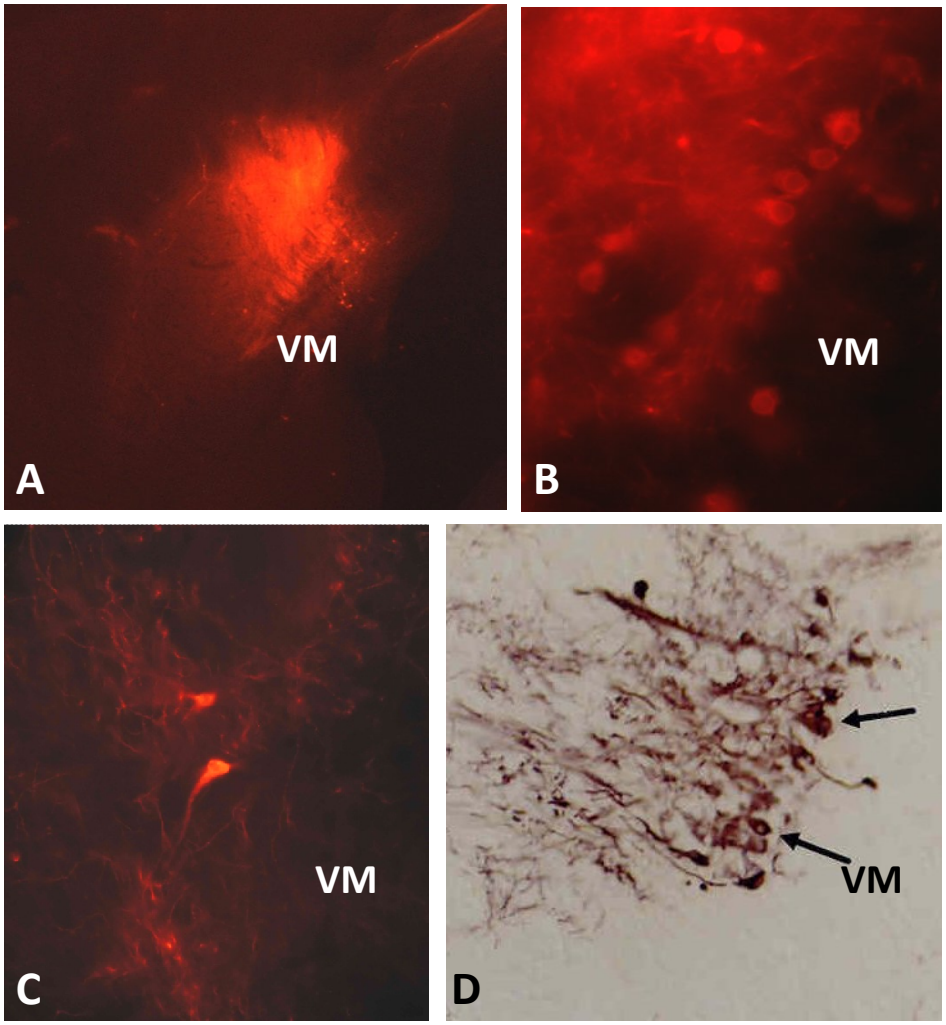


Figure 6

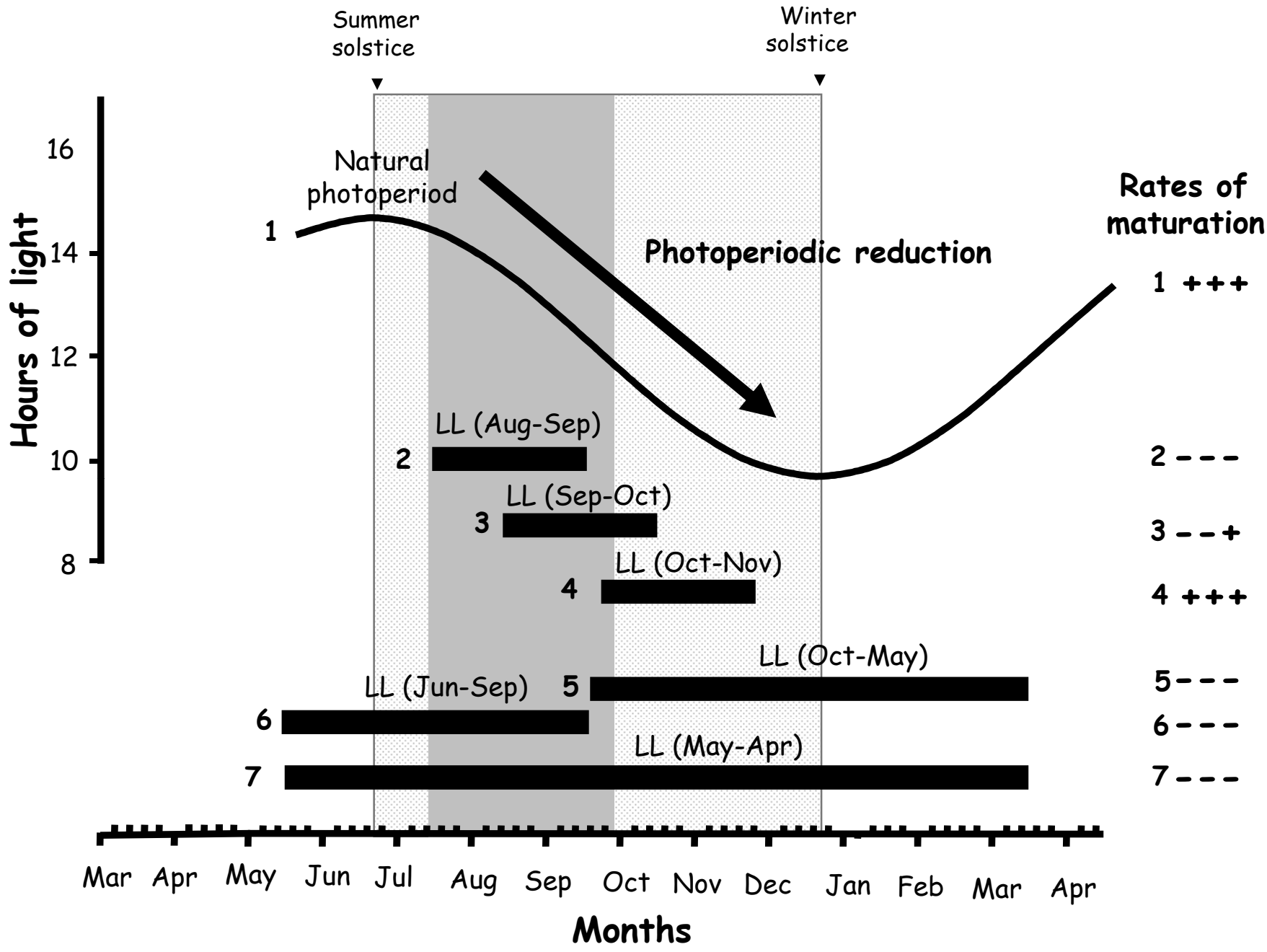

Summer solstice
Winter solstice
Natural photoperiod
Photoperiodic reduction
Rates of maturation
Hours of light
16
14
12
10
8
1
1 +++
LL (Aug-Sep)
2
2 - - -
LL (Sep-Oct)
3
3 - - +
LL (Oct-Nov)
4
4 +++
LL (Oct-May)
LL (Jun-Sep)
5
5 - - -
6
6 - - -
LL (May-Apr)
7
7 - - -
Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr
Months

Figure 7

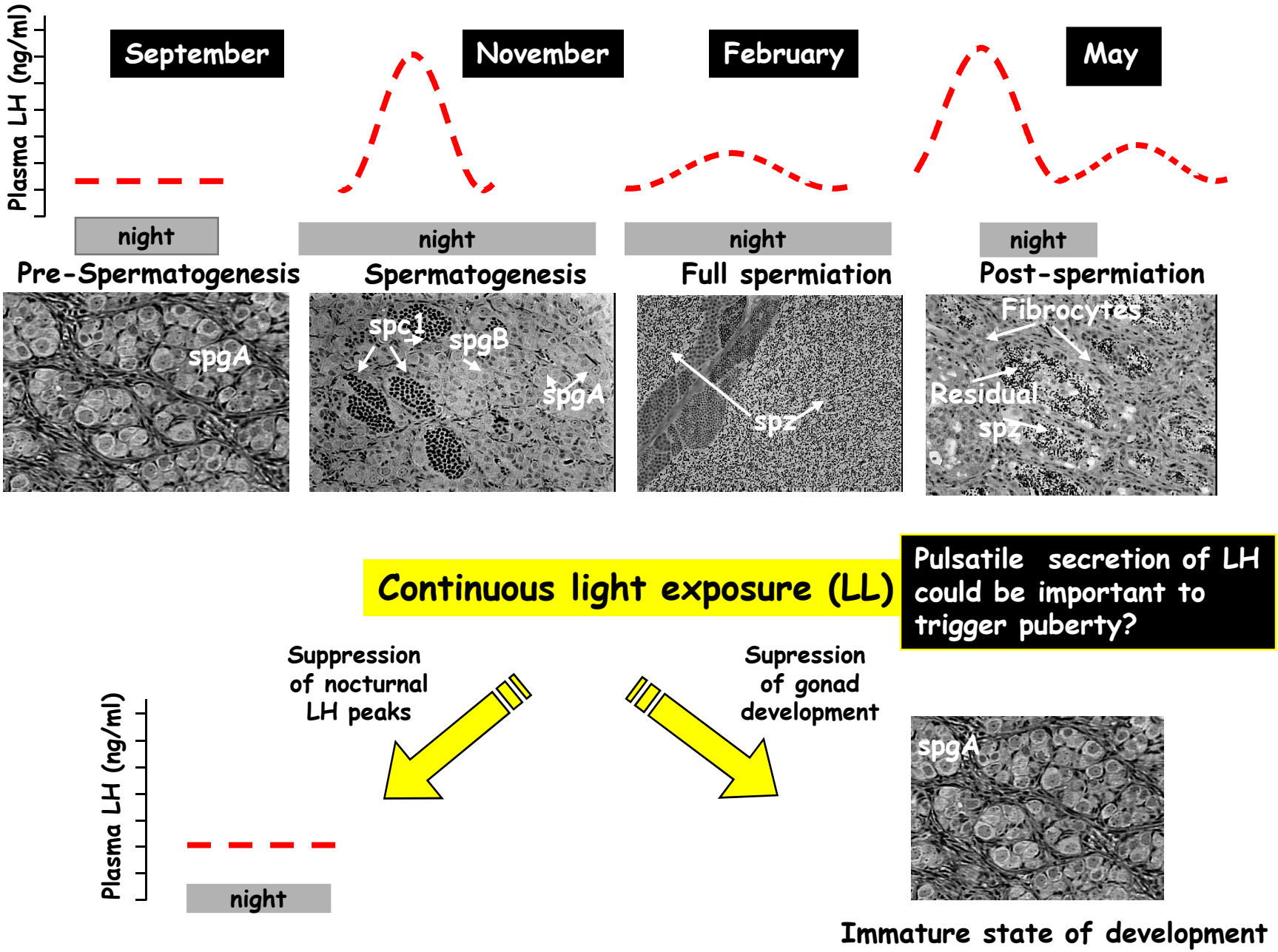
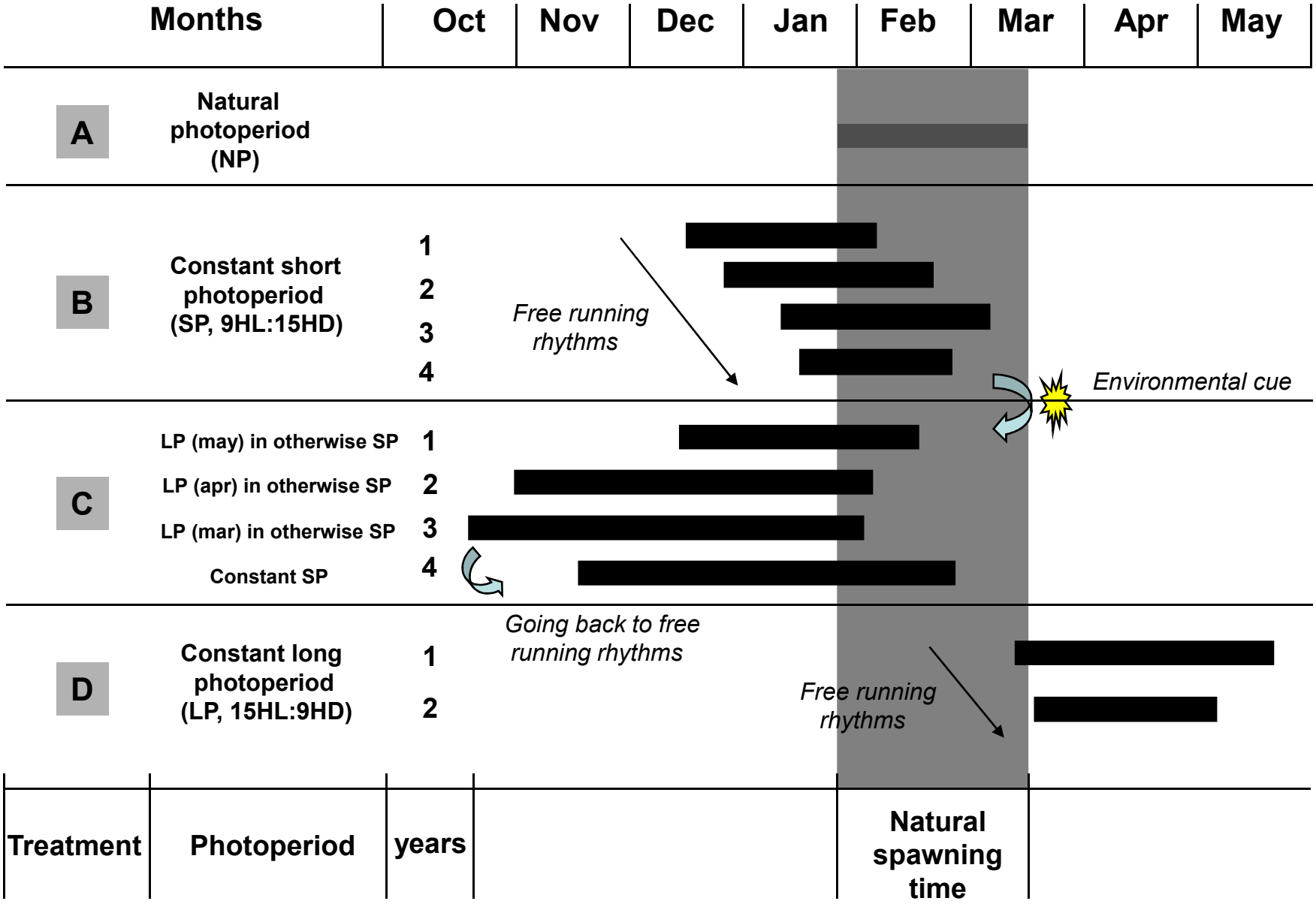

Plasma LH (ng/ml)
September
November
February
May
night
night
night
night
Pre-Spermatogenesis
Spermatogenesis
Full spermiation
Post-spermiation
spgA
spc1
spgB
spgA
spz
Fibrocytes
Residual
spz
Continuous light exposure (LL)
Pulsatile secretion of LH could be important to trigger puberty?
Suppression of nocturnal LH peaks
Supression of gonad development
Plasma LH (ng/ml)
night
spgA
Immature state of development

Figure 8


Months
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
A
Natural photoperiod (NP)
B
Constant short photoperiod (SP, 9HL:15HD)
1
2
3
4
Free running rhythms
Environmental cue
C
LP (may) in otherwise SP
LP (apr) in otherwise SP
LP (mar) in otherwise SP
Constant SP
1
2
3
4
Going back to free running rhythms
D
Constant long photoperiod (LP, 15HL:9HD)
1
2
Free running rhythms
Treatment
Photoperiod
years
Natural spawning time