The Pineal Gland and Photoperiodism in Fish

Jack Falcón, Biology of Aquatic Organisms and Ecosystems (BOREA), MNHN, CNRS 7208, SU, IRD 207, UCN, UA, National Museum of Natural History, Paris, France

José A Muñoz-Cueto, Department of Biology, Faculty of Marine and Environmental Sciences and INMAR Research Institute, Marine Campus of International Excellence (CEIMAR), The European University of the Seas (SEA-EU), University of Cádiz, Cádiz, Spain

Abstract

Fish reproduction is a rhythmic process occurring at particular times of day and year. Photoperiod is the most predictable and powerful environmental synchronizer of reproduction and entrains biological clocks, which allow anticipating the variations of this environment. Temperature is another rhythmic factor used by fish to adjust their metabolic and physiological processes. The pineal organ plays a key role in transducing photoperiod and temperature cycles and controlling the daily and annual reproductive rhythms. We review here how the pineal photoreceptors integrate photo-thermo-periodic information, and produce rhythmic neural and neuroendocrine messengers, including melatonin, which modulate the daily and seasonal production of brain/pituitary/gonad hormones involved in fish reproduction.

Glossary

Photoperiod Alternation of light (L) and darkness (D) or LD cycle, during the 24 h daily cycle.

Circadian Clock The machinery of a circadian clock consists of core clock genes and proteins interconnected by a transcription/translation feedback loop, robustness and stability of which is reinforced by auxiliary molecular mechanisms. A circadian clock functions autonomously with a period of 24 ± 4 h (circa = approximately; dian = day) and needs to receive input signals from photoreceptors to synchronize their period to the 24 h LD cycle.

Circadian System A circadian system is made of an input machinery to the clock (*e.g.*, phototransduction unit), a circadian clock machinery acting as a pacemaker, and an output machinery that delivers one or several rhythmic messages to the rest of the organism.

Circannual Clock A molecular machinery that functions on a yearly base.

Non-Visual Opsins Opsins found in photoreceptive (central and/or peripheral) cells not involved in vision.

Pinealofugal Neurons The population of neurons, the axons of which form the pineal tract that innervates different brain areas and conveys the rhythmic neural message exiting from the pineal organ.

Temperature Compensation A property of the circadian clock, the period of which remains constant over a wide range of temperatures.

Visual Cycle   The process that replenishes the molecule retinal for its use in the phototransduction process. Retinal binds to most opsins. Upon capture of a photon, 11-cis retinal photo-isomerizes into all-trans retinal starting the phototransduction cascade. All-trans retinal is ejected from the opsin protein and the photoreceptor, captured by retinal pigment epithelium (RPE)-like cells where it is refreshed into 11-cis retinal.

Whole-Genome Duplication (WGD)   A process that leads to chromosomal duplication of all the genes of one individual. Two rounds of WGD occurred early during vertebrates' evolution. A 3rd round occurred at the origin of the fish lineage, and a 4th is specific to salmonids.

Zeitgeber   Timekeeper; a factor capable of synchronizing and entraining biological clocks.

Key Points

- The circadian system is made of (1) master clocks, (2) signals that provide input to the clocks (eyes, pineal gland, deep-brain and skin photoreceptors) and (3) signals that are produced rhythmically under control by the core clock mechanism.
- The daily and seasonal light/dark and temperature cycles are considered the strongest and more reliable synchronizers of biological rhythms, including reproductive rhythms.
- The pineal organ of fish plays a key role in transducing photo-thermo-periodic information and controlling the daily and annual reproductive rhythms.
- Neural (pinealofugal fibers) and neuroendocrine (melatonin) signals of pineal origin modulate directly and indirectly hormonal secretion along the brain-pituitary gonads axis to control fish reproduction.

Introduction

In most living species the timing of periodic events, including reproduction, is synchronized by the cyclic changes of the environment, mainly the daily and annual changes in **photoperiod** and temperature. This allows harmonization and anticipation of thousands of precisely timed biochemical, physiological and behavioral processes, including all aspects of reproduction. Other factors may interact with photoperiod and temperature depending on the geographical location (*e.g.*, food availability, rainfall, water salinity, monsoons, feeding, social cues, lunar or tidal cycles) (Falcón and Muñoz-Cueto, 2024). Photoperiod acts at different timescales from daily to once in a life; and, in seasonal breeder fish may display one or two spawning windows, with months of preparation preceding, and a phase of arrest following, the window. Equatorial species tend to spawn throughout the year. Depending on the species, spawning may occur in the morning or afternoon or most often at night (**Fig. 1**). These daily fluctuations rely on **circadian clocks**. In turn, the clocks produce rhythmic output signals that inform the rest of the organisms on the environmental fluctuations. Although less investigated, circannual clocks seem also to be at work, and it is suspected that the circadian and circannual clocks cooperate in the timing and entrainment of the reproductive cycle. In brief, photoperiod, acting through the circadian and circannual systems, appears as a main synchronizer of internal biological clocks to determine at which time of the day and year spawning occurs (Falcón and Muñoz-Cueto, 2024). Daily and annual fluctuations are observed at all stages of the brain-pituitary-gonads (BPG) axis (Oliveira and Sánchez-Vázquez, 2010; Paredes *et al.*, 2019). This axis controls
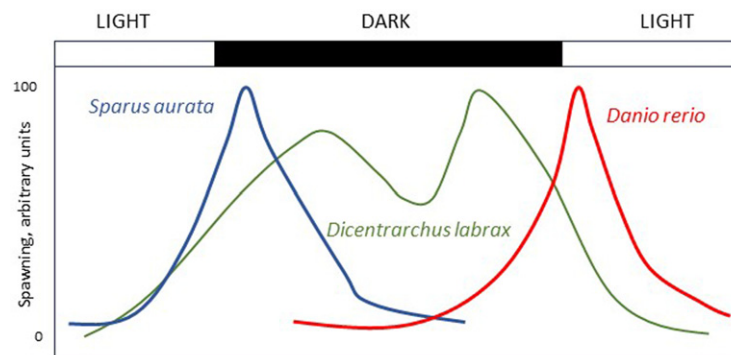
Fig. 1   **Spawning daily rhythm**. Fishes spawn at different times of the 24 h Light/Dark cycle depending on the species, *e.g.*, dusk in the Mediterranean seabream (*Sparus aurata*), nighttime in the European sea bass (*Dicentrarchus labrax*) and dawn in the zebrafish (*Danio rerio*). Modified from Falcón and Muñoz-Cueto (2024).

reproduction from molecules to physiological regulations (including development and maturation of the gonads) and behaviors (courtship, mating, spawning) (Kah *et al.*, 2010; Kulczykowska *et al.*, 2010).

While the 24 h light/dark (LD) cycle is considered the strongest and more reliable synchronizer of biological rhythms, temperature cycles are also able to entrain the circadian system. The LD cycle causes a daily temperature cycle with water temperature increasing after sunrise and decreasing following sunset. Temperature is important for ectotherms, including fish, because their body temperature depends on the water temperature. Daily temperature cycles and drastic temperature variations can set the phase of the circadian rhythms. Daily thermocycles are involved in the control of hatching rhythms, sex differentiation, gonad development and sexual steroids production in several fish species. Seasonal temperature changes contribute to triggering the onset and subsequent development of gametogenesis and to positioning spawning at specific times of the year (Falcón and Muñoz-Cueto, 2024).

The pineal organ (or pineal gland or *epiphysis cerebri*) occupies a central position in the circadian organization of vertebrates. In fishes, the pineal is made of cells that are both photo- and thermo-receptors. Integration of the photoperiod and temperature information synchronizes the daily and seasonal secretion of the time-keeping hormone melatonin. In a majority of cases this process involves a circadian molecular machinery located within these same cells, which thus constitute full cellular **circadian systems**. The fish pineal has for long been suspected to control the rhythmic aspects of reproduction (Falcón and Muñoz-Cueto, 2024). We summarize below our current knowledge on the functional organization of the pineal organ, the place it occupies in the fish circadian organization and its role in setting the daily and seasonal rhythms of reproduction.

The Circadian System in Fish

The circadian system is made of (1) master clocks, (2) signals that provide input to the clocks (eyes, pineal gland, deep-brain and skin photoreceptors) and (3) signals that are produce rhythmically under control by the core clock mechanism. The circadian system provides information on daily and calendar time to the rest of the organism through controlling a myriad of downstream rhythmic processes; this allows anticipating the cyclic variations of the environment (Falcón and Muñoz-Cueto, 2024). Studies in a small species, the zebrafish, indicate that all fish cells are photoreceptive and possess a molecular clock machinery (Whitmore *et al.*, 2000). These multiple circadian clock systems are organized in a network of more or less potent oscillatory units. While the LD cycle is the main synchronizer of the clocks' activity, other factors, including temperature, food availability, water composition, social cues, rainfall, tides, and lunar cycles, may also contribute.

The pineal gland occupies a central position in this system by virtue of its location, at the interface between the environment and the organism, and its photo- and thermo-sensibilities. In addition, it appears to be itself a master oscillator or a partner of interconnected master oscillators in a majority of species (Underwood, 1989; Falcón and Muñoz-Cueto, 2024). While in mammals, the master circadian clock is located in the suprachiasmatic nuclei of the hypothalamus (SCN), in fish "*the circadian organization … clearly involves a number of components including the pineal, the eyes, and the SCN. However, it is becoming increasingly apparent that the relative roles that these sites play between species can vary*" (Underwood, 1989).

Functional Organization of the Fish Pineal Organ

The pineal is usually shaped as a vesicle that occupies a mid-line position above the cerebral hemispheres. It is located below the skull in a so-called 'pineal window' where the bone is thinner and translucent, and the skin above is less pigmented (**Fig. 2**A-C). The pineal is connected to the epithalamus by a slender stalk (**Fig. 2**E). In flatfishes, it locates in the upper pigmented side, where both eyes are also placed after metamorphosis. In many species the pineal lumen communicates with the third ventricle (3rd V) and filled with cerebrospinal fluid (CSF) (**Fig. 3**), although a range of anatomical situations may exist, from a purely vesicular to a fully glandular organ highlighting the existence of a great diversity among the ∼33,000 existing species of fishes (**Fig. 2**D-G) (Falcón and Muñoz-Cueto, 2024; Kulczykowska *et al.*, 2010).

The pineal epithelium resembles a simplified retina, made of photoreceptor cells, neurons, glial cells, macrophages and retinal-pigmented epithelium (RPE)-like cells (**Fig. 3**) (refs in: Kulczykowska *et al.*, 2010; Falcón and Muñoz-Cueto, 2024). The *photoreceptor cells* express one (or two) **non-visual opsins** (pinopsin, parapineal opsin, exorhodopsin, vertebrate ancient opsins, melanopsin), which diversified after the teleost-specific **whole-genome duplication (WGD)** event. While displaying a cone shape, only a minority of the photoreceptor's population expresses cone specific genes; the majority expresses rod-specific genes. In addition, a cell type expressing melanopsin, and displaying a neuronal-like rather than a photoreceptor-like shape, sends projections to the brain (see refs. in Falcón and Muñoz-Cueto, 2024). Photoreceptor cells establish synaptic connections among them, with **interneurons** or with **pinealofugal neurons**; the latter send their axons to the brain *via* the pineal tract (**Fig. 6**). *Glial (interstitial) cells*, which occupy the whole height of the pineal epithelium, isolate photoreceptors and neurons from the extra-pineal spaces (**Fig. 3**). They incorporate elements released from the blood vessels that surround the organ, allowing their distribution to other cell components of the pineal epithelium and/or their release into the pineal lumen. In addition to a nutritive role, they also recycle outer segments material from photoreceptor cells, as *macrophages* also do. Glial cells are indispensable to maintain the integrity and survival of photoreceptors. Finally, the *RPE-like cells* contribute to photoreceptor development and function, including the **visual cycle**.
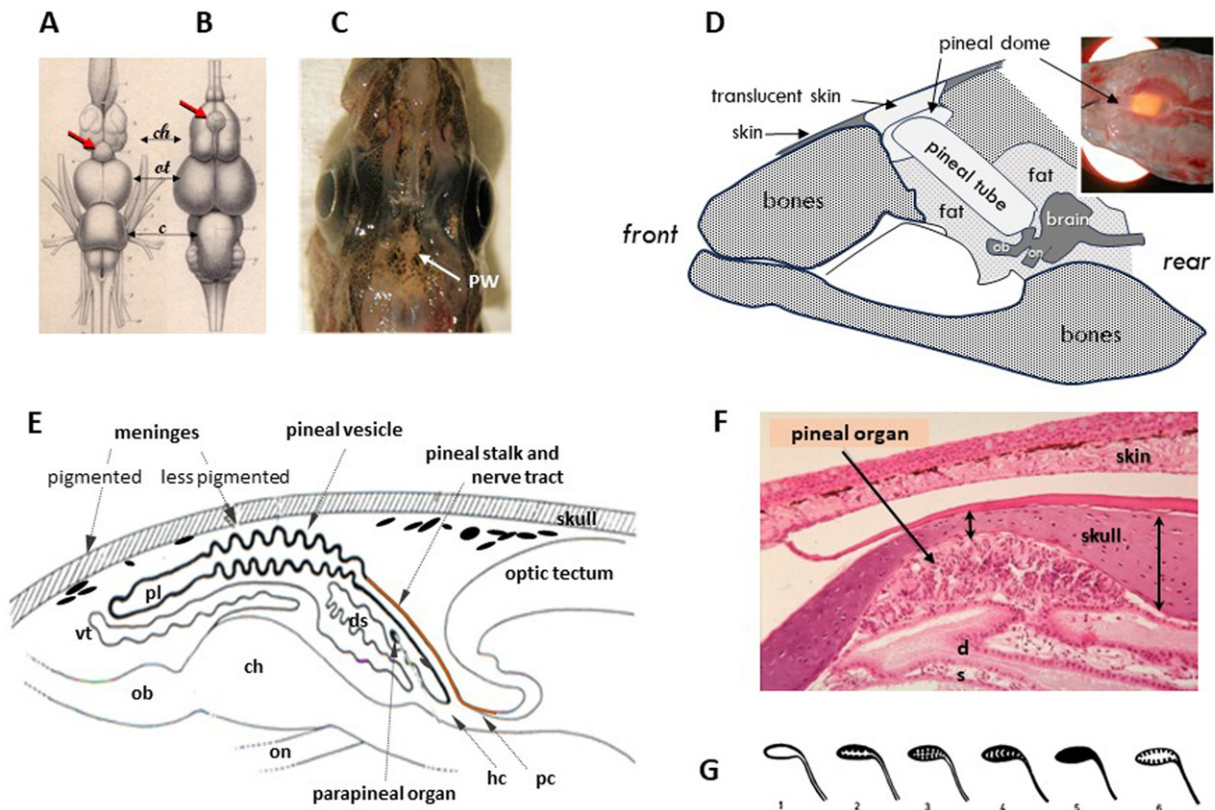
Fig. 2 **Anatomy of the fish pineal organ. A, B**. Dorsal views of the brains of the conger, *Conger vulgaris* (**A**) and stripped red mullet, *Mullus surmulletus* (**B**) showing the central location of the pineal organ (red arrows) above the cerebral hemispheres (*ch*). *ot*, optic tectum; *c*, cerebellum. **C**. Dorsal view of the head of the Arctic cod (*Boreogadus saida*) showing the pineal window (PW), an area where the meninges are less pigmented, located in between the two lateral eyes. **D**. Schematic drawing of a sagittal section through the head of the bluefin tuna, *Thunnus thynnus*; the brain is located deep into the head, and a translucent cartilaginous structure (the pineal tube) allows light to reach the pineal gland. This pineal tube is covered by a translucent cartilage, the pineal dome, covered itself by thin translucent and less pigmented skin. **E**. Schematic drawing of a sagittal section through the brain of the northern pike *Esox lucius*. The pineal vesicle is connected to the brain between the habenula (hc) and posterior (pc) commissures by the pineal stalk. The large pineal vesicle covers the cerebral hemispheres (ch) and olfactory bulbs (ob). The pineal lumen (pl), filled with cerebrospinal fluid, opens into the 3rd ventricle. ds, dorsal sac; on, optic nerve; vt, *velum transversum*. **F**. Eosin staining of a sagittal section through pineal area of the trout, *Oncorhynchus mykiss*. The pineal tissue is located in a pit, formed by the bone skull becoming substantially thinner in this area. **G**. Diagrammatic presentation of the 6 types of pineal epithelia in teleosts: 1, flat; 2: folded; 3, convoluted; 4, small space; 5, compact; 6, intermediate. From Falcón and Muñoz-Cueto (2024), with permission.

## The Pineal Gland at the Interface Between The Environment and the Organism

Light, temperature and internal factors modulate the production of two photoreceptor cells messengers, an excitatory neurotransmitter and a neurohormone.

An Excitatory Neurotransmitter: Glutamate?

The excitatory neurotransmitter (likely to be glutamate) accumulates in the synaptic vesicles observed within the photoreceptor end-pedicle(s). Typically, the photoreceptor exhibits a resting potential between -20 to -40 mV in the dark, which allows the release of the neurotransmitter at the synaptic connections between the end-pedicles of photoreceptors and dendrites of neurons. Illumination induces electrical responses from photoreceptor cells (early receptor potentials, cell hyperpolarization and mass slow potentials [or pinealograms, EPG]). The photoreceptor hyperpolarization may reach -80/-90 mV, is intensity- and duration-dependent over a range of several log units. This induces a graded inhibition of the neurotransmitter release and a hyperpolarization of the neuronal population monitored by an inhibition of the spike discharges from the pineal tract. The great majority of the neuronal responses are 'achromatic', some may be chromatic, *i.e.*, UV light induces a long-lasting inhibition, whereas light of longer wavelengths causes excitation. For extensive review on this matter see Kulczykowska *et al.* (2010) and Falcón and Muñoz-Cueto (2024).

Temperature also modulates the pineal electrical responses in the trout, *Oncorhynchus mykiss*. The neuronal discharges are strongly diminished at temperatures above 20°C, while they are insensitive to light at temperatures below 10°C or 15°C depending on the neuron population (Tabata *et al.*, 1993).
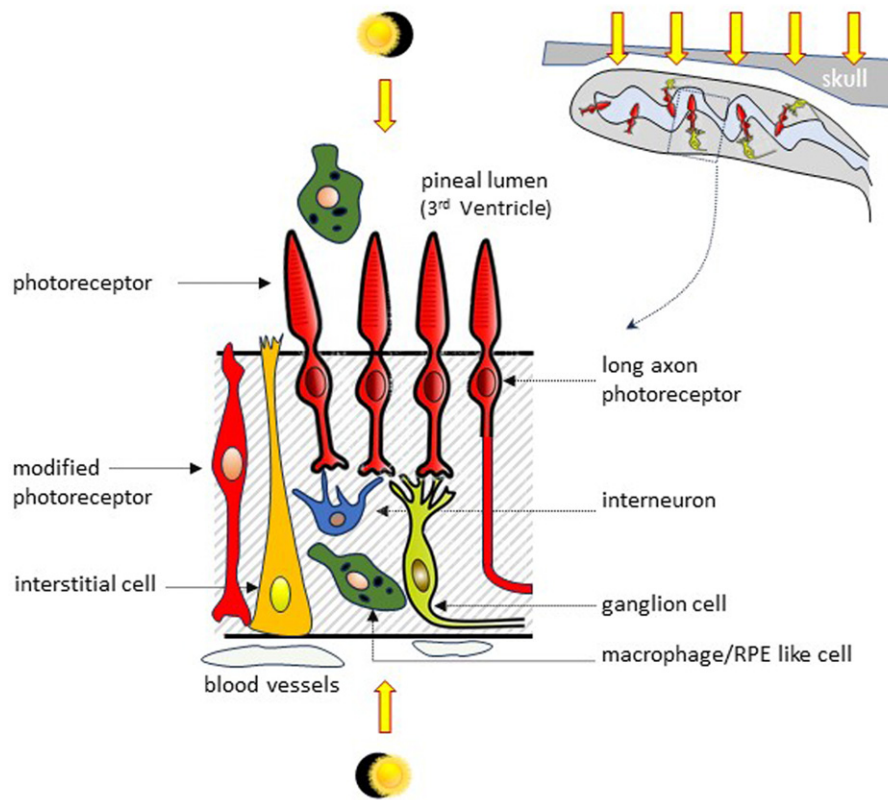
Fig. 3 The different cell types of the pineal epithelium. The arrows show the directions of the light input on these cells depending on their position in the epithelium as shown in the upper drawing. From Falcón and Muñoz-Cueto (2024), with permission.

A Neurohormone: Melatonin

Photoreceptors produced melatonin at night (Falcón and Muñoz-Cueto, 2024). The synthesis of melatonin from serotonin involves two enzymes (**Fig. 4**): the arylalkylamine *N*-acetyltransferase (Aanat) and the acetylserotonin *N*-methyltransferase (Asmt). The former catalyzes the conversion of serotonin into *N*-acetylserotonin, the latter ensures *O*-methylation of *N*-acetylserotonin to produces melatonin. Fish possess two main families of Aanat, Aanat1 and Aanat2, as a result of several rounds of WGD (see above). Aanat1 and isoforms (Aanat1a and Aanat1b) are found in the retina, brain and peripheral tissues; conversely, only one form of Aanat2 has been retained by fishes, which is mainly expressed in the pineal gland. Similarly, Asmt is mainly expressed in the pineal and retina, which thus appear as the main sources of melatonin. However, pineal melatonin is a neurohormone released into the blood stream that surrounds the organ and the CSF. In contrast, retinal melatonin is used and metabolized *in situ* and thus acts in an auto/paracrine manner.

Light and circadian modulation of melatonin production

Elevated during nighttime, melatonin secretion is inhibited by light (**Figs. 4** and **5**). The shape of the daily oscillations may vary from a species to another. In a few species, the eyes contribute partially (seabass, cod) or totally (catfish, tilapia) to controlling pineal melatonin secretion, indicating the existence of a neural pathway that connects the retina to the pineal. In the majority of the species investigated, the melatonin response is driven by intrinsic circadian clock machinery located within the pineal photoreceptor cell (**Fig. 4**) (Falcón and Muñoz-Cueto, 2024). The 24 h LD cycle synchronizes the clocks and consequently the melatonin secretion rhythm. Under constant darkness the clocks free-run and so does melatonin production (**Fig. 5**). A circadian machinery is apparently not functional in lampreys, salmonids and flatfish (**Fig. 5**). **Fig. 4** summarizes our current understanding of the photoperiodic regulation of melatonin secretion by the pineal photoreceptor cell.

Temperature modulation of melatonin production

Temperature modulates the amplitude of the nocturnal melatonin surge in a species-specific manner. The response somehow reflects the aerobic scope of the fish; this is also the case of the spike responses from the pineal nerve (*c.f.* An Excitatory Neurotransmitter: Glutamate?) (**Fig. 4**). The complexity of the response to temperature has been detailed and discussed elsewhere (Falcón and Muñoz-Cueto, 2024). In brief, temperature may act in different ways. The first way targets the Aanat2 enzyme itself: Its kinetics display a temperature dependency (not observed with the Aanat1 isoforms). The second way involves thermo-receptors that belong to a family of Ca^{2+} channels, the transient-receptor-potential vanilloid (Trpv) (**Fig. 4**). They are present within the cell
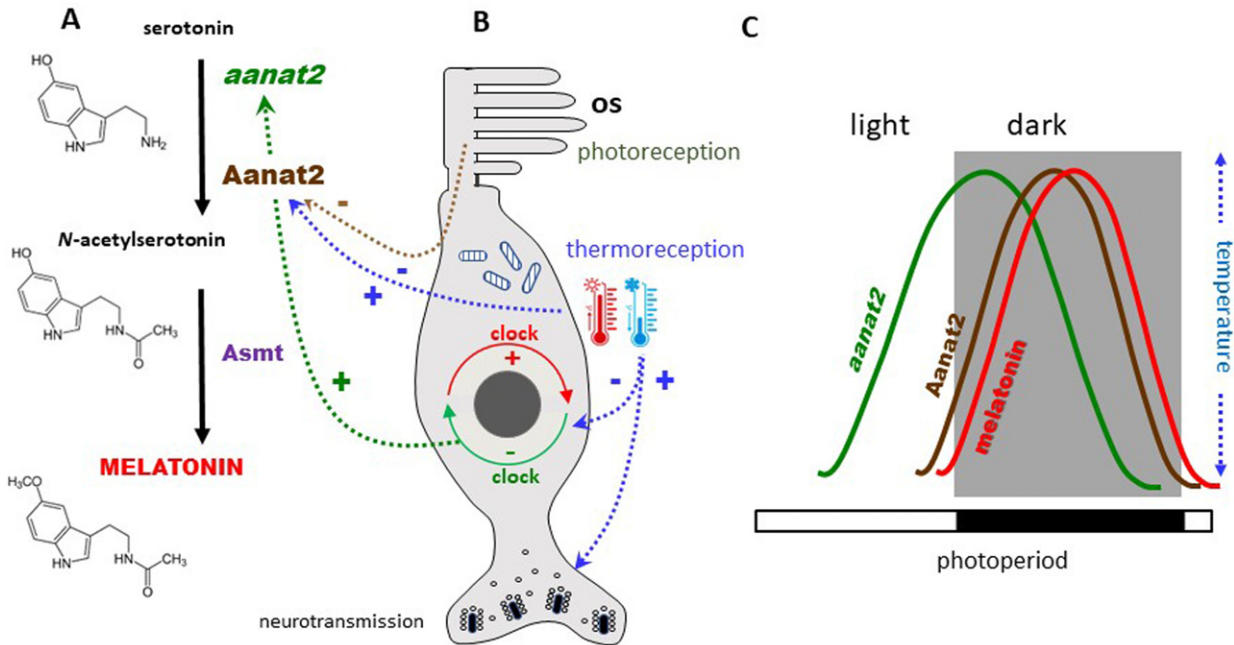
Fig. 4   Photoperiod and temperature control of melatonin synthesis in the pineal photoreceptor cell. **A**. Melatonin synthesis from serotonin involves two enzymatic steps in the dark: (1) The arylalkylamine *N*-acetyltransferase protein (Aanat2 in brown) is stabilized due to an intracellular accumulation of Ca^{2+} and cyclic AMP induced by the photoreceptor depolarization; Aanat2 catalyzes the acetylation of serotonin, which produces *N*-acetylserotonin. (2) The latter is then converted to melatonin by the acetylserotonin *O*-methyltransferase (Asmt). **B**. Upon illumination, phototransduction, within the photoreceptor outer segment (os), induces a cascade of events from cell hyperpolarization that induces depletion of intracellular Ca^{2+} and cyclic AMP, followed by Aanat2 protein degradation (brown dotted line). Photoperiod also synchronizes the circadian clock (when present), consisting of a loop of interacting positive (red) and negative (green) components. One of these components activates *aanat2* expression and thus accumulation of *aanat2* mRNA (green dotted line), which then decreases progressively as the clock runs. The blue dotted arrows indicate that temperature impacts on Aanat2 protein kinetics (determined by the aminoacid sequence of the enzyme), or indirectly *via* calcium dependent thermoreceptors. **C**. Schematic presentation of the daily profiles of *aanat2* mRNA (green), Aanat2 protein (brown) and melatonin production (red). Photoperiod dictates the duration of the melatonin secretion rhythm and temperature controls its amplitude. Details in, and from, Falcón and Muñoz-Cueto (2024) with permission.

membrane of the photoreceptor cell population. The third way is *via* the circadian clock (when present), *i.e.*, only in species in which *aanat2* expression is clock controlled. In brief, photoreceptors are also thermo-receptors, and they use the same intracellular pathway to modulate melatonin production in response to light and temperature variations (Fig. 4). Photoperiod controls the duration of the nocturnal melatonin signal while temperature controls the amplitude. This provides a strong internal indication of both daily and calendar time.

Internal factors modulate the pineal signals

Several internal factors may modulate the pineal productions. Melatonin and GABA (the neurotransmitter of the pineal inter-neurons) modulate the production of both the nervous and melatonin signals, while adenosine (produced locally), catecholamines and steroids (Estradiol 17β [E2], glucocorticoids) affects only melatonin secretion. Afferent fibers carrying peptidergic information have been evidenced in the pineal of several fish representatives, including neuropeptide Y and two peptides involved in fish reproduction, gonadotropin-releasing hormone (GnRH) and gonadotropin-inhibitory hormone (GnIH) (Muñoz-Cueto *et al.*, 2017, 2020; Falcón and Muñoz-Cueto, 2024).

The Targets of the Pineal Messengers

The pineal nerve and its projections

The axons from the pineal second-order neurons and from central projecting photoreceptor cells constitute the pineal tract that innervates specific central areas (Fig. 6). They enter the brain through the habenular commissure, and the rostral and caudal parts of the posterior commissure (PC) and extend bilaterally, innervating the habenula, preoptic area (POA), prethalamus, thalamus, periventricular hypothalamus, periventricular, central and superficial pretectal regions, posterior tuberculum, as well as medial and dorsal mesencephalic tegmentum and optic tectum. Most of these areas also receive retinal projections, and/or express melatonin receptors (Fig. 6). Furthermore, some of these pinealo- and/or retino-recipient areas belong to neuroendocrine centers containing hypophysiotropic cells such as the habenula, POA, prethalamus/thalamus, periventricular hypothalamus, posterior tuberculum,
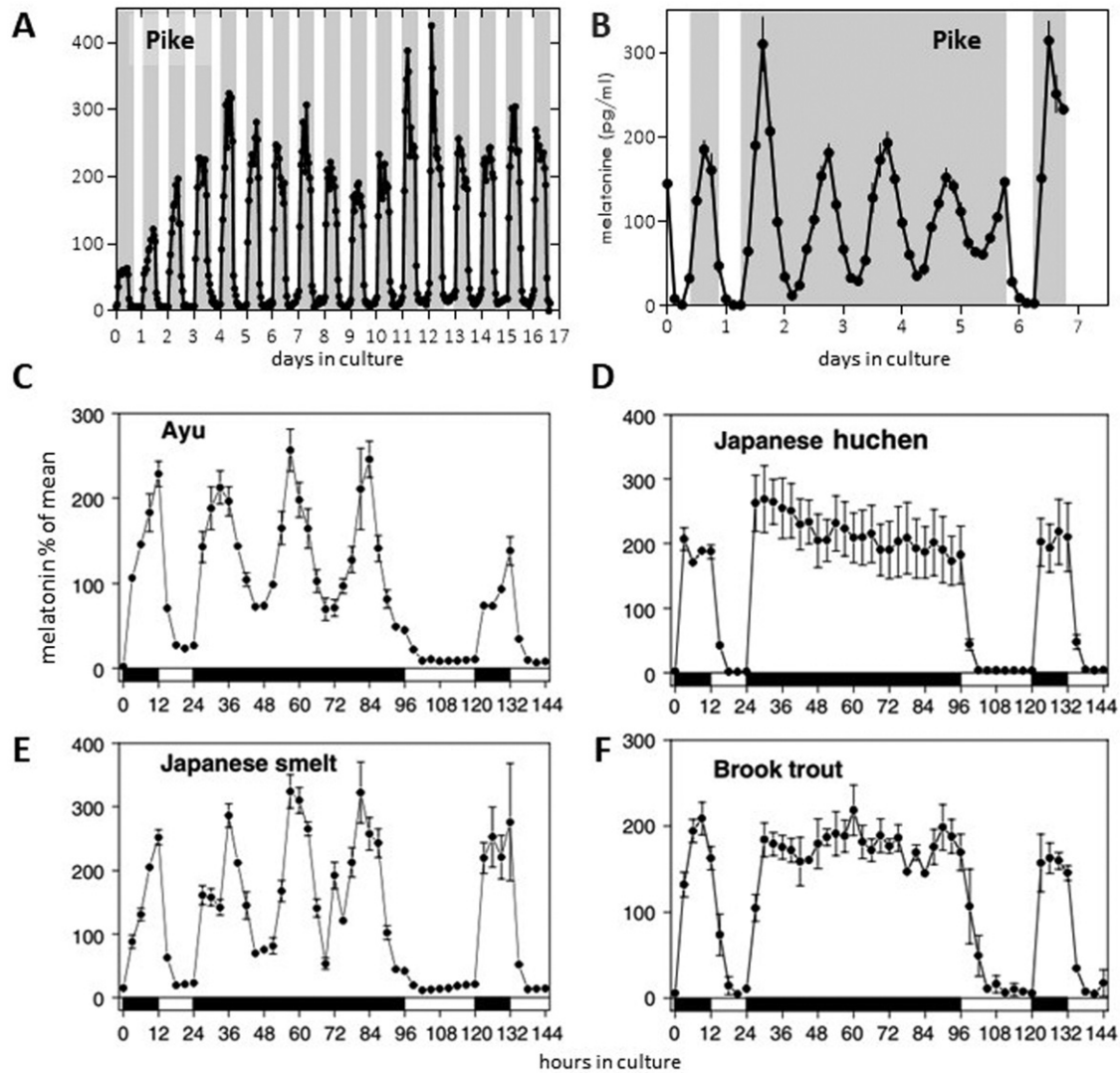
Fig. 5 **Photoperiodic and circadian control of melatonin secretion**. In all species investigated, isolated superfused fish pineal glands produce melatonin rhythmically in a high at night low during day pattern, as is the case *in vivo*. **A**. The rhythm is sustained for several weeks (northern pike, *Esox lucius*). **B, C, E**. In culture, pineal photoreceptor cells (**B**, pike), or pineal glands (**C**, Ayu, *Plecoglossus altivelis*, and **E**, Japanese smelt, *Hypomesus nipponensis*) maintain the rhythmic pattern under DD, indicating a circadian clock control. The dampening of the rhythm in **B**, suggests desynchronization of the individual cellular oscillators, which resynchronize again upon restoring the LD condition. **D, F**. No such circadian clock operates in salmonids (*e.g.*, Japanese huchen, *Hucho perryi*, and brook trout, *Salvelinus fontinalis*) so that the melatonin response to the LD is an on/off type of response. From Falcón and Muñoz-Cueto (2024), with permission.

dorsal mesencephalic tegmentum (Fig. 6) (for details and references see Falcón and Muñoz-Cueto, 2024). In fishes, these areas are involved in reproduction, food intake, feeding behavior and/or metabolism. The pineal fibers that reach these areas could represent one pathway through which the photic information conveyed by the pineal organ reaches the neuroendocrine centers, thus contributing to the daily and/or seasonal synchronization of reproduction and other hormonally controlled rhythmic processes.

The melatonin receptors

Four main melatonin receptor subtypes have been characterized to date in fishes: MT1 (Mel1a), MT2 (Mel1b), MT3 (Mel1c) and Mel1d. Several paralogs of MT1 and MT2 have been retained after the teleost-specific and salmonids-specific WGDs; the other two always appear as a single copy or are lost (refs. in: Maugars *et al.*, 2020; Falcón and Muñoz-Cueto, 2024). The receptors belong to the G-protein-coupled receptors (GPCR) family and are generally (not only) coupled to inhibition of the intracellular messenger cAMP *via* a Gi protein. They display a ubiquitous distribution, being found in the pineal gland, retina, brain (olfactory bulb, telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon), pituitary, and peripheral tissues (gills, heart, spleen and blood cells, adipose tissue, skin, kidney, liver, intestine, muscle, testis and ovary) (Fig. 6). The subtypes expressed and
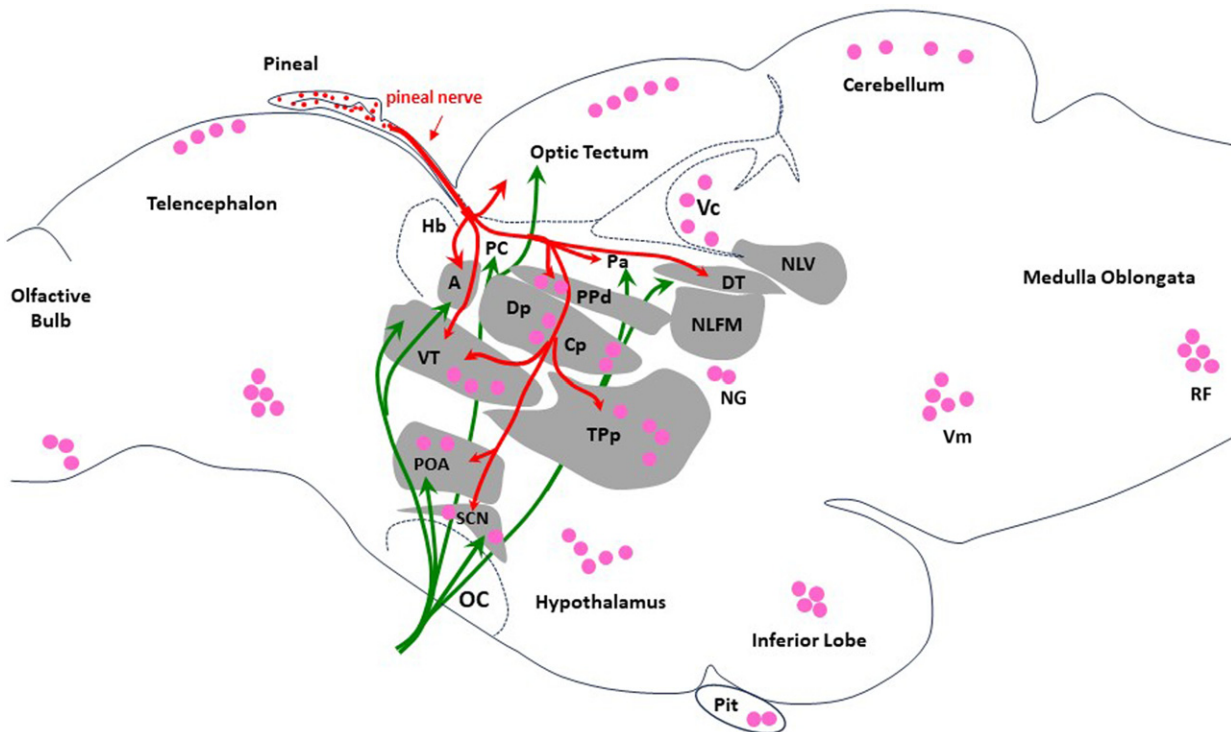
Fig. 6 **Pineal and retinal targets in the fish brain.** The drawing is a schematic presentation of the pineal (red arrows) and optic (green arrows) nerve projections, together with the areas where the melatonin receptors have been identified (pink dots). A, anterior prethalamic nucleus; Cp, central posterior thalamic nucleus; Dp, dorsal posterior thalamic nucleus; DT, dorsal tegmental nucleus; Hb, habenula; NFLM, nucleus of the medial longitudinal fascicle; NG, *nucleus glomerulosus*; NLV, lateral nucleus of the valvula; OC, optic chiasm; Pa, paracommissural pretectal nucleus; PC, posterior commissure; Pit, pituitary; PPd, dorsal periventricular pretectal nucleus; POA, preoptic area; RF, reticular formation; SCN, suprachiasmatic nucleus; TPp, periventricular nucleus of the posterior tubercle; Vm, trigeminal motor nucleus; Vc, valvula of the cerebellum; VT, ventral prethalamus. From Falcón and Muñoz-Cueto (2024), with permission.

relative abundance depend on the species and tissue considered, as well on sex and age (with a developmental profile paralleling that of sexual maturity) (Falcón and Muñoz-Cueto, 2024). Abundance of mRNA, number of binding sites (B_{max}) and their affinity for melatonin, may display daily, lunar and annual variations (refs. in Falcón and Muñoz-Cueto, 2024). While the LD oscillations in mRNA abundance display different profiles from a species to another, the B_{max} is usually high during the day and low at night. The daily and seasonal variations are under melatonin as well as circadian and circannual control in some tissues. The lunar and annual variations are correlated to oocyte maturation and spawning (B_{max}), or development and reproductive status (mRNA). They are also modulated by temperature and salinity. In the zebrafish, the response to temperature seems to reflect the thermal preferences of the fish, as is the case for melatonin secretion (*c.f.* Temperature modulation of melatonin production).

The Reproduction System

During the breeding season, the levels of androgens (males), estrogens (females) and progestins (both sexes) rise, allowing gonad maturation and spawning behavior. This is under the control of the brain-pituitary-gonads (BPG) axis (**Fig. 7**). One main component of the BPG axis is GnRH, which stimulates the synthesis and release of gonadotropins: follicle-stimulating hormone (Fshβ) and luteinizing hormone (Lhβ), which in turn regulate steroidogenesis. Sex steroids are necessary for gametogenesis and final gamete maturation, also exerting diverse positive/negative feedback effects on tissues throughout the body, including the reproductive brain and pituitary (**Fig. 7**).

In distant teleost (perciforms), three isoforms of GnRH exist: the hypophysiotropic GnRH1 in the ventral telencephalon/POA/hypothalamus, GnRH2 in the midbrain tegmentum, and GnRH3 mainly in the terminal nerve area (Muñoz-Cueto *et al.*, 2020). In early teleost (salmonids and cyprinids) only GnRH2 and GnRH3 isoforms have been identified, with the latter mainly assuming the non-redundant roles of GnRH1. GnRH stimulation of gonadotropins secretion may be opposed by dopamine (DA) inhibition in some, but not all, teleost fish (Kulczykowska *et al.*, 2010; Zohar *et al.*, 2010). Both the GnRH (stimulatory) and DA (inhibitory) neurons are mainly located in the POA.

Other neuropeptides are also involved in the control of reproduction acting upstream or downstream GnRH1/3 and DA (Zohar *et al.*, 2010) (**Fig. 7**):
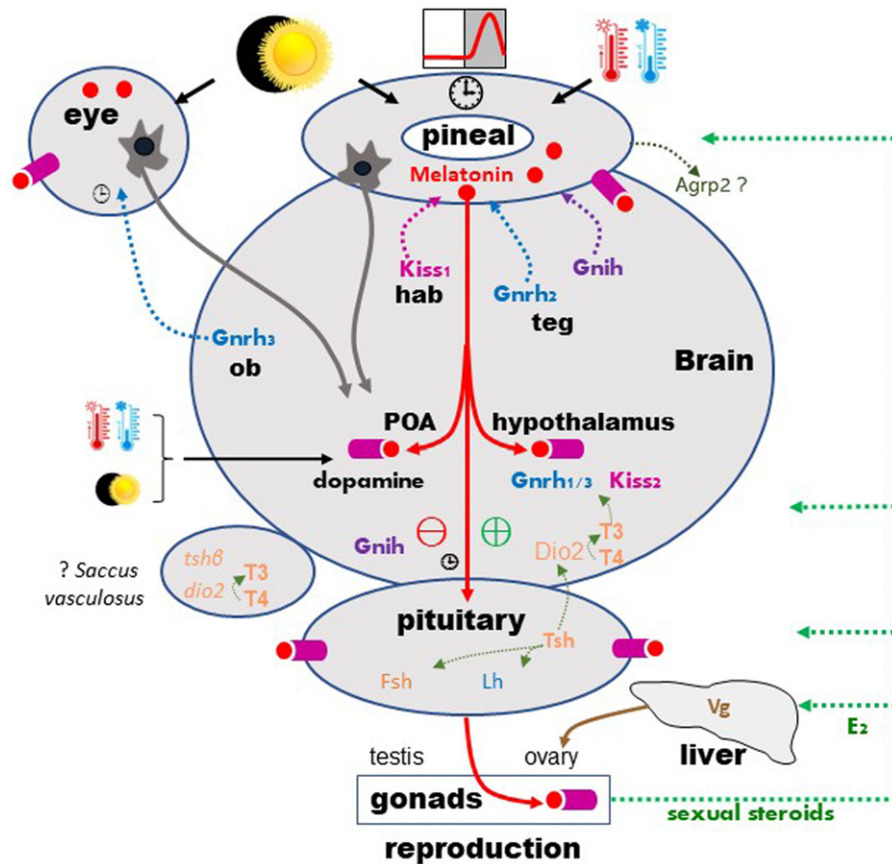
Fig. 7 **Schematic representation of the photo- and thermo-periodic regulation of the brain-pituitary-gonads (BPG) axis**. The photoperiodic (retina and pineal) and photo-thermo-periodic (pineal) information reach the neuroendocrine axis through non-visual retinal and pineal nerve fibers (gray arrows) and pineal melatonin (red circles) released into the cerebrospinal fluid and circulation. Melatonin acts through specific receptors (purple boxes) found at all levels of the BPG axis. Deep brain thermoreceptors and photoreceptors might also operate in the basal telencephalon. Melatonin effects have been reported on the main preoptic (POA) and hypothalamic stimulatory (GnRH1/3, Kiss2, ⊕) and inhibitory (GnIH, dopamine, ⊖) factors known to control fish pituitary gonadotropes (Fsh and Lh cells). At the pituitary level melatonin also modulates the production of adeno-hypophysial hormones, including those involved in the control of reproduction, Fsh, Lh and Tsh. Tsh from the pituitary may regulate Fsh and Lh productions *via* the pituitary folliculostellate cells and/or the brain deiodinase-2 (Dio2), which allows production of T3 (triiodothyronine) from T4 (thyroxine). Fsh and Lh act on the gonads (ovary and testis) to control the progress of gametogenesis and steroidogenesis. Feedback of gonadal steroids operates on the pineal gland, reproductive brain and pituitary (green dotted lines). Estradiol (E2) also acts on the liver of female fish to promote the synthesis and release of vitellogenin (Vg), which reaches the ovary through the vascular system and contributes to the progression of vitellogenesis. Moreover, nerve projections (colored dotted lines) from the habenula (hab), containing Kiss1-producing cells, and mesencephalic tegmentum (teg), containing GnRH2 and GnIH neurons, reach the fish pineal gland, whereas the retina receives GnRH3 fibers originating from cells in the olfactory bulbs (ob). The *saccus vasculosus* (SV) contains opsin proteins, Tsh and Dio2; it might also act as a sensor of seasonal changes in day length in those fish species that possess such a structure. The pineal gland also produces AgRP2 (agouti-related peptide-2), although its function remains unknown. ⊕ circadian clock machinery. For details and references see text. From Falcón and Muñoz-Cueto (2024), with permission.

- *GnIH*, belongs to the RFamide peptide family (also known as LPXRFamide); it has been reported to either inhibit or stimulate gonadotropins. In sea bass, GnIH peptides interact functionally with GnRH and Kisspeptin (Kiss) neurons and reduce GnRH1/2 and Kiss1/2 mRNA levels (Muñoz-Cueto *et al.*, 2017). An inverse relationship characterizes GnIH and GnRH mRNA abundances during the reproductive cycle of the carp. In this species, three GnIH receptor subtypes are detected at all stages of the BPG axis, which also display seasonal variations in mRNA abundance. In the zebrafish, LPXRFamide antagonizes the Kiss2 activation of Kiss receptors (see refs. in Muñoz-Cueto *et al.*, 2017; Wang *et al.*, 2022).
- *kiss1* and *kiss2* genes are expressed, respectively, in the habenula/medio-basal hypothalamus, and hypothalamus (see refs. in Wang *et al.*, 2022). In some fish species, Kiss2 acts on the hypothalamus (targeting GnRH neurons) and the pituitary, in a stage- and sex-dependent manner. This results in the upregulation of GnRH1, Fshβ and Lhβ expressions and secretion for the latter two. Kiss and Kiss receptors are also present in the gonads but their roles remain enigmatic (see refs. in Wang *et al.*, 2022).
- *Neurokinins b and f (NKb and NKf)* stimulate Fshβ and Lhβ levels in the tilapia, zebrafish and goldfish pituitaries as well as *fsh* and *lh* mRNA and Lhβ release in the striped-bass; it also inhibits *kiss2* expression and stimulates *gnrh* expression (see review of Trudeau, 2022).

- *Secretoneurins* (SN) stimulate the synthesis and release of Lh from both goldfish pituitary cells and stimulate hypothalamic *gnrh3* and pituitary *lhβ* and *fshβ* mRNA levels in the orange-spotted grouper, *Epinephelus coioides* (for a review on this matter see Trudeau, 2022).

The Pineal Gland, Melatonin and Reproduction

The pineal gland is involved in modulating the activity of the BPG axis, the components of which generally display daily and annual variations. While the role of the nervous message remains obscure, more information is available regarding melatonin. Pinealectomy and/or melatonin administration (*i.c.v.*, *i.p.*, *i.m.*, implants, water) experiments provided strong indication that the pineal impacts the BPG axis. The reported effects vary depending on the species investigated, gender, time of the day and year, modes and amounts of melatonin administered, etc. (Falcón and Muñoz-Cueto, 2024). In general, melatonin has anti-gonadal properties, acting at all stages of the BPG axis.

Melatonin Effects in the Brain

In vivo melatonin administration impacts the main brain factors known to control pituitary gonadotrophs, *i.e.*, GnRH, Kiss, GnIH, and DA (**Fig. 7**) (see refs. in Cowan *et al.*, 2017; Ciani *et al.*, 2021; Falcón and Muñoz-Cueto, 2024). Administration of melatonin into the 3rd ventricle (near the POA and hypothalamus) decreased the GnRH-induced Lh release from the pituitary of Atlantic croaker, *Micropogonias undulatus*. In the European sea bass, *Dicentrarchus labrax*, melatonin inhibits the transcript levels of *gnrh1* and *gnrh3*, and of three GnRH receptor genes (*gnrhr-II-1c, -2a* and *-2b* subtypes), as well as of *kiss1* and *kiss2*, respectively 1 and 3 months after receiving a melatonin implant; this treatment also inhibited testicular maturity (see refs. in Wang *et al.*, 2022). In the same species, drastically reducing melatonin levels by pinealectomy induced increased diencephalic *kiss2* mRNA transcripts in August and decreased midbrain/hindbrain *gnih* transcripts in March. In the damselfish, *Chrysiptera cyanea*, fed melatonin pellets, short- and long-term reductions of *gnrh1* and *kiss2* mRNA abundance were accompanied by reduced *fshβ* and *lhβ* transcript levels, lower gonado-somatic index (GSI) and an increased oocyte atresia. In masu salmon, *Oncorhynchus masu*, fed melatonin pellets, pituitary GnRH and Lh levels, plasma testosterone (T) and GSI were diminished. In female *Heteropneustes fossilis*, *i.p.* administration of melatonin reduced *gnrh2* and *kiss2* mRNA. In contrast, in the zebrafish, melatonin administration increased transcripts levels of *kiss1*, *kiss2* and *gnrh3* in the brain and of *lhβ* in the pituitary. Parallel to the inhibition of the GnRH/Kiss system, melatonin stimulates *gnih* mRNA levels, as shown in the Nile tilapia, resulting in a decrease of *fshβ* and *lhβ* mRNA levels in the pituitary, and of E2 and 11-ketotestosterone (11-KT) in the plasma. Again, the data obtained in the zebrafish were in the opposite direction because *in vitro* treatment of whole zebrafish brains by melatonin reduced *gnih* mRNA.

In other fish, brain melatonin and DA display daily and annual rhythms that are $180°$ out of phase. Melatonin may inhibit the effects of DA, thus releasing the inhibition exerted by the latter on pituitary gonadotropins. Melatonin thus provides fish with a dual "DA$^{(-)}$/GnRH$^{(+)}$" control of reproduction in the brain (**Fig. 7**). The melatonin/DA relationship is however complex as shown in the European carp, *Cyprinus carpio*, where the hypothalamic DA is increased by melatonin in immature fish, and decreased during the spawning period of mature fish (Kulczykowska *et al.*, 2010).

Melatonin Effects in the Pituitary

Direct effects of melatonin on the fish pituitary have also been observed, in agreement with the evidence that melatonin receptors are expressed in the pituitary of some fish species (refs. in Cowan *et al.*, 2017; Ciani *et al.*, 2021; Falcón and Muñoz-Cueto, 2024). In the Atlantic croaker, Lh*β* release was stimulated in pituitaries challenged with melatonin in a time- and dose-dependent manner. In the medaka, melatonin reduced *fshβ*, *tshβ* and somatolactin (*sl*) transcript levels, while in the European sea bass, the mRNA levels of all pituitary hormone genes were affected by melatonin challenges *in vitro* (Falcón *et al.*, 2021; Falcón and Muñoz-Cueto, 2024). However, the effects varied depending on the time of the year and reproductive status of the fish, as well as on previous adaptation to low or high water salinity: in February (spawning phase), *fshβ* and growth hormone (*gh*) mRNA levels were inhibited, those of proopiomelanocortin (*pomc*) were increased, and those of *tshβ* displayed a biphasic response (increased at the picomolar, and decreased at the nanomolar, range of concentrations). In August (arrest phase), the effects on *fshβ*, *tshβ* and *gh* where less pronounced; they were inhibitory on *pomc*; and stimulatory on *lhβ* mRNA.

The effects of melatonin on Tsh deserve attention as thyroid hormones are known to be involved in seasonal reproduction of fish, as is the case in birds and mammals (Nakane and Yoshimura, 2019; 60061 this volume). In birds, light sensitive CSF-contacting neurons transmit the photoperiodic information to Tsh cells of the *pars tuberalis* of the pituitary. Once induced and secreted, Tsh increases expression of deiodinase 2 (*dio2*), which catalyzes the conversion of thyroxine (T4) into triiodothyronine (T3). T3 then controls morphological changes of GnRH cell, a process that regulates GnRH secretion. Such a mechanism seems operative in the coronet cells of the *saccus vasculosus* in the Masu salmon (**Fig. 7**). However, other mechanisms must also operate because a significant number of fish do not possess a *saccus vasculosus*, as is the case of the Atlantic salmon, in which Tsh from the pituitary activates Dio2 in midbrain, optic tectum and hypothalamus to catalyzing the production of T3. In the medaka, Tsh acts locally on gonadotropic cells *via* the folliculostellate cells of the pituitary (**Fig. 7**). Thus, multiple mechanisms operate in fish,

either through the *saccus vasculosus* and/or the pituitary not excluding the possible involvement of CSF contacting deep-brain photoreceptors (Falcón and Muñoz-Cueto, 2024).

Melatonin Effects in the Gonads

The gonads are also a target for melatonin in fish, and a potential source of melatonin as well (refs. in: Cowan *et al.*, 2017; Ciani *et al.*, 2021; Takahashi and Ogiwara, 2021; Falcón and Muñoz-Cueto, 2024). The *aanat* and *asmt* mRNA, melatonin itself and its receptors are present in the gonads (Takahashi and Ogiwara, 2021), and the hormone is also detected in seminal plasma (Félix *et al.*, 2023). However, measurement of Aanat and Asmt enzyme activities are needed before concluding that melatonin is synthetized in the gonads. Whatever it might be, melatonin may have pro- or anti-gonadal effects depending on its concentration, the species, sex, season and reproductive status (Falcón and Muñoz-Cueto, 2024; Takahashi and Ogiwara, 2021). In the gonads, melatonin affects gametogenesis, sperm production, folliculogenesis (and cytoskeleton rearrangement in the follicular cells), maturation and ovulation (Takahashi and Ogiwara, 2021). Both the direct action of melatonin on the ovary and its indirect effects *via* the BPG axis seem to operate to promote oocyte maturation and ovulation (Takahashi and Ogiwara, 2021). The hormone was shown to modulate steroidogenesis, in both testis and ovary, including E2, 11-KT, 17α,20b-dihydroxy-4-pregnen-3-one (DHP; a maturation-inducing hormone) and prostaglandin E2 (PGE2). It also increases the expression of two membrane progesterone receptor isoforms (*mprα* and *mprβ*) in the zebrafish follicles (see reviews of: Takahashi and Ogiwara, 2021; Falcón and Muñoz-Cueto, 2024).

A constant communication between the brain/pituitary complex and the gonads allows the activity of the different components of the BPG axis to be synchronized at all stages of the life cycle (**Fig. 7**). Thus, steroids released by the gonads exert a feed-back control at different levels of the brain-pituitary axis. They also feed-back on the liver where they control Vg production (**Fig. 7**). The three major points of gonadal feedback regulation are the pituitary, the DA neurons of the POA and the pineal gland, where E2 and T modulate melatonin secretion. Steroids also modulate GnRH expression and content through Kiss neurons in the brain (see refs. in Wang *et al.*, 2022).

Conclusions

As emphasized elsewhere (Falcón and Muñoz-Cueto, 2024), fishes represent by far the largest group of vertebrates (more than 33,000 species), with a long evolutionary history and a great diversity in habitats (*i.e.*, pelagic, estuarine, shallow water, benthic, from tropics to polar regions). Accordingly, they have been exposed to a variety of environmental conditions (light intensity and spectral composition, photoperiod, temperature, salinity, water turbidity, food availability, pressure…), and have developed a great variability of sensory, physiological, and behavioral adaptations to meet different ecological challenges. As a consequence, they exhibit the most diverse modes of reproduction among vertebrates in terms of reproductive strategies, modes of fertilization, mating, parental care, or secondary sexual characteristics. And, they have developed different strategies to achieve a precise timing of reproduction, a mandatory process to ensure the best survival of a healthy progeny. **Fig. 7** summarizes our current knowledge on the topic; it focusses on the important role played by the pineal gland and melatonin in transducing the photo-thermo-periodic information that regulates the rhythmic aspects of reproduction along the BPG axis. The process is complex for a number of reasons: (i) melatonin impacts all stages of the BPG axis, (ii) it may have pro-or anti-gonadal effects, (iii) the effects depend on the species and, within the same species, on (iv) the previous history of the fish, development, aging, gender, time of day or year, concentration of melatonin, type of melatonin receptor(s) expressed, etc... In addition, the brain distribution of the melatonin receptors is large and overlaps with areas receiving nervous signals from the retina and the pineal itself. This apparently redundant convergence of photic- and thermic information in brain centers that regulate neuroendocrine functions -including reproduction-, underlines the importance of the periodic information for regulating these processes (Falcón and Muñoz-Cueto, 2024). Crucial information is still lacking concerning (i) the melatonin receptors (cellular localization and regulation [abundance, rhythms, homo- and/or hetero-dimerization, transduction pathways, clearance]), (ii) possible extra-pineal sites of melatonin synthesis and melatonin clearance, (iii) involvement of other non-visual photoreceptive areas (deep-brain photoreceptors, *saccus vasculosus* when present) and their interaction with the pineal gland. In the meantime and considering the extremely few number of species investigated compared with the huge amount of fish representatives, we believe that generalizations should be made with caution, and conclusions provided on a case-by-case basis.

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