Review

PHOTOPERIOD, PINEAL PHOTORECEPTORS AND MELATONIN AS THE SIGNAL OF PHOTOPERIOD IN THE REGULATION OF REPRODUCTION IN FISH

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SUMMARY

Annual variation in photoperiod is the most regular phenomenon that has strong predictive value in the temporal organization of seasonal activities in biological world, especially reproduction. Carefully controlled studies demonstrated importance of photoperiods in the regulation of annual reproductive events in diverse groups of animals including fish. Apart from academic interests, study in fish has gained momentum because of its tremendous economic importance as food. Thus, for obvious reasons, efforts are continued to develop technologies for increasing fecundity and growth of fish in culture. As a result, photoperiodic manipulation has emerged as an effective tool of reproductive management in culture fisheries, and understanding the physiology of photoperiodic regulation of fish reproduction became the priority topic of research in different countries. The present paper reviews and summarizes the information gathered in recent years on the importance of photoperiod and the mechanism of photoperiodic signal transduction in the photo-neuroendocrine system in fish emphasizing the role of pineal organ, which is considered as an intermediary between the environment and the endocrine system in vertebrates.

Key words: Fish, pineal organ, photoperiod, photoreceptors, reproduction.

INTRODUCTION

Environment plays an important role in the regulation of reproduction in different animals including the fish (1). Among various components of the environment, annual changes in the duration of the solar day or photoperiod, which become marked from the temperate to the polar zones, has been proved to be the primary and regular variable that individually, or in combination with water temperature or any other environmental factor(s), impel the 'driving function' in determining the sexual periodicity in most of the fish species living in mid- and high latitudes (2-4). However, it does not exclude the possible role of supplementary information in the temporal adjustment of breeding in fishes at low latitudes, where there is less seasonal variations in photoperiod and water temperature (5).

Processing of environmental information is one of the most important physiological events in the regulation of reproduction in animals. Studies in different teleosts indicated that synchronization of physiological and environmental events is mediated through the system composed of sensors and circadian oscillators like the pineal organ, the lateral eyes and the suprachiasmatic nuclei of the hypothalamus (6). This circadian oscillator system among the fish species is located in the pineal organ and the eyes, among which the pineal organ is considered as the most important component for its responsiveness mechanisms to the changes in environmental light and darkness (7-8). In fish the entire system (the photodetector, the circadian clock and melatonin synthesizing enzymes) is located in the pineal organ (9). Several experimental studies indicated that the pineal organ is able to translate environmental information (photoperiod and temperature) into rhythmic messages (10-11), and the pineal hormone melatonin is the internal chemical messenger of environmental signal or *Zeitgeber* (6, 11-13) and controls a number of functions, especially reproduction, in vertebrates (14). There are evidences to suggest that the pineal organ and/or melatonin may play a role in the seasonal maturation of gonad in fish (15-17), and in transduction of photoperiodic information to the brain-pituitary-gonadal axis in teleosts (11). However, results of experimental studies in different fish are not identical. Other than melatonin, a number of molecules which are known to be involved in photo-signal transduction and are commonly found in vertebrate retinal photoreceptors have been detected in the pineal photoreceptors of different fishes. Such photoreceptor molecules include opsin, alpha- subunit of the G protein transducin, and S-antigen (10), of which opsin is known to mediate non-visual light-detection tasks (18).

The present review summarizes the data collected in recent years focusing on possible role of photoperiod, pineal photoreceptor molecules and melatonin in the regulation of reproduction in fish.

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ROLE OF PHOTOPERIOD IN THE REGULATION OF FISH REPRODUCTION

Annual fluctuations in the duration of light or photoperiod constitute one of the major and regular environmental variables, which appear to perform an important role in the regulation of or synchronization with the reproductive cycle in most fish species that breed at mid- and high- latitudes (3, 4, 15, 19). Recent studies with juvenile red sea bream (*Pagrus major*), Nile tilapia (*Oreochromis niloticus*), haddock (*Melanogrammus aeglefinus*), and black sea turbot (*Psetta maeotica*) revealed that gonadal growth was significantly lower in fish exposed to 24L:0D than others which were exposed to different photoperiodic schedules such as 20L:4D, 18L:6D, 16L:8D, 12L:12D, 0L:24D (20-23). In *O. niloticus*, during fingerling stage, mean female gonosomatic index (GSI) and mean oocyte size were significantly lower in fish maintained under constant light regime (24L:0D) than those of 20L:4D, 18L:6D treatments and control (24). But a conclusion on the use of photoperiods in the temporal organization of seasonal breeding in low-latitude fish in general suffered a set back from the lack of studies representing diverse groups of species. The role of photoperiod as a source of environmental cue in the regulation of reproduction in tropical fish has been considered by several workers, but the studies have been confined mostly to different air-breathing fish, like *Heteropneustes fossilis* (25), *Mystus tengara* (26), *Channa punctatus* (27-29), and *Clarias batrachus* (30-31) as it was easy to maintain them under laboratory conditions for experimental studies. Ovarian growth in *H. fossilis* was stimulated under the influence of long photoperiods, while retarded in short photoperiodic fish (32). An acceleration of gonadal growth was also noted

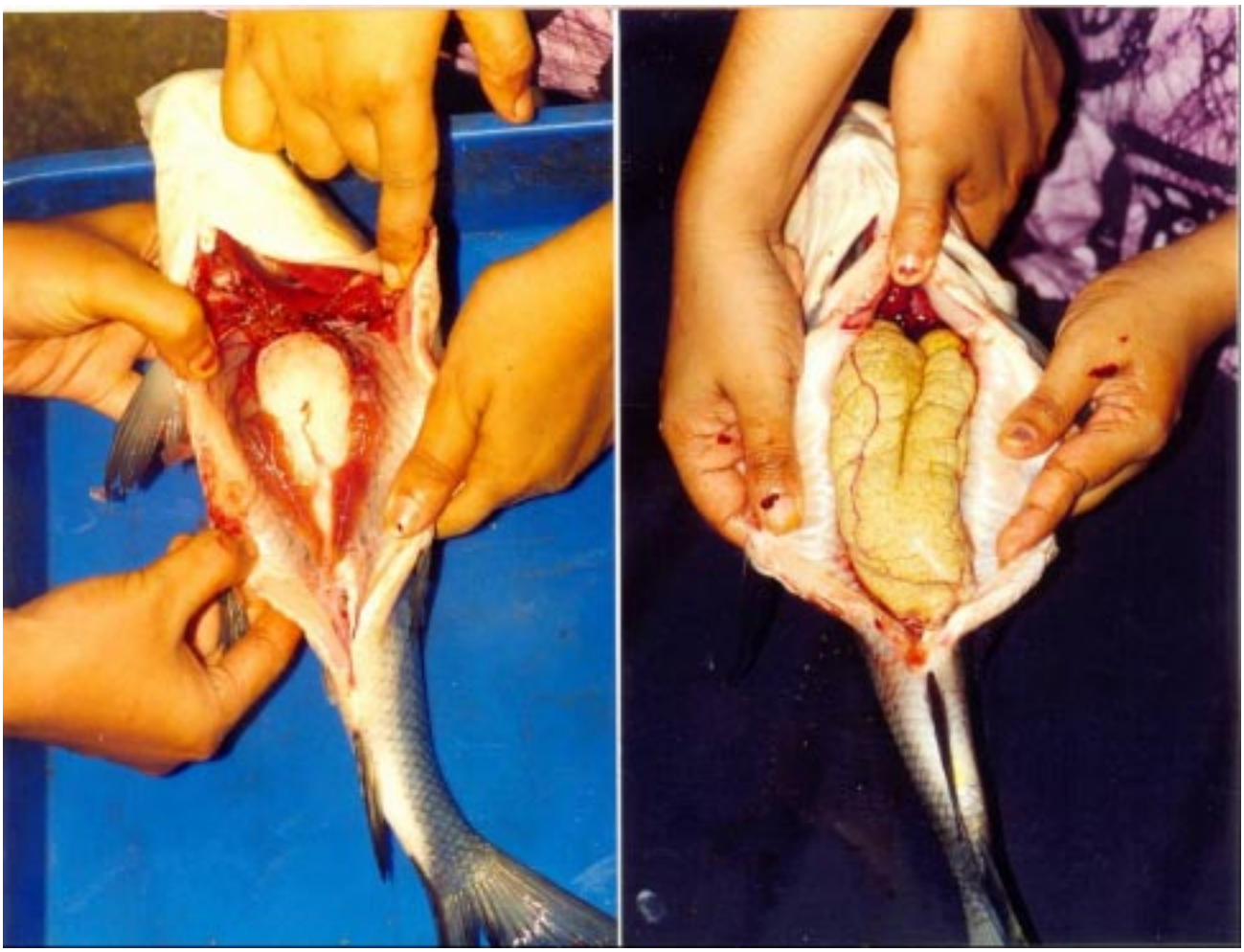

Fig. 1. Photographs of dissected abdomen of Indian major carp *Catla catla* showing precocious development of ovary (right photograph) under the influence of long photoperiods (16L: 08D) during the pre-spawning phase (April-May) compared to the ovary in respective control fish (left photograph) held under natural photoperiods.

in *M. tengara* (26) and in *C. punctatus* (28, 33) following exposure to different schedules of long photoperiods (14L:10D or 18L:6D) as well as to continuous light (LL). Short photoperiods, on the other hand, were found to be inhibitory to gonadal functions in several sub-tropical fish including *Cirrhina reba* (34), *H. fossilis* (32), and *C. punctatus* (28, 33). Nonetheless, the question remained open whether the effects were strictly short photoperiodic, or coupled with altered water temperature. A combination of long photoperiods and relatively high temperature regimen was found to be effective in causing maturation of gonad in the Indian murrel, *C. punctatus* (28, 33).

Until recently (35-38), none of the Indian major carps received proper attention for revelation of the influence of photoperiods on their reproductive performance. The study on both male (38), and female (35) free-living carps (*Catla catla*) clearly indicated role of photoperiods as possible environmental synchronizer of the seasonal gonadal growth. Experimental studies (36, 37) revealed that LP is stimulatory to only steroidogenic functions of the gonad during the preparatory phase (February-March), and stimulatory to both steroidogenic and gametogenic functions of the gonad during the pre-spawning phase (April-May), when precocious maturation of gonad occurred in LP subjects (Fig. 1). However, LP had no influence on reproduction during the spawning (July-August) and the post-spawning (September-October) phases. On the other hand, a schedule of SP was inhibitory to ovarian growth and maturation during the pre-spawning and the spawning phases, or of no influence on reproductive functions during the preparatory and the post-spawning phases of an annual cycle. The study provided the first evidence that photoperiod *per se* plays an important role in the seasonal maturation of gonad in a sub-tropical freshwater major carp (36, 37).

THE PHOTORECEPTOR MOLECULE IN FISH PINEAL ORGAN

A number of molecules involved in phototransduction and desensitization commonly found in vertebrate retinal photoreceptors have also been immunocytochemically localized in pineal photoreceptors. This includes opsin, alpha- subunit of transducin, S-antigen or arrestin and recoverin (10). Several studies suggest that the pineal organ of adult teleosts utilizes two or more photopigments.

Immunocytochemical detection of opsin proteins in the pineal organ of various species indicated that pineal photoreceptors contain retinal-like opsins, possibly structurally similar to both cone opsin(s) and rod opsin (39). The anti-opsin reactivity, characteristic of rods, was particularly strong in the lumina of the pineal stalks and the anti-visinin reactivity stained cones in the retina occurred in the end vesicle of the pineal organ in two Antarctic fishes (40). The degenerated photoreceptor outer segments were recognized in the pineal organ of the blind cave salamander (*Proteus anguinus*) exclusively by using antibody against the red-sensitive cone opsin (41).

The growing list of photoreceptive opsin molecules found in fish pineal includes parapinopsin, isolated from the channel cat fish *Ictalurus punctatus* (42) and is responsible for the UV-sensitivity as exhibited by the lamprey pineal and may be the UV photo-pigment in the fish pineal capable of forming a bi-stable pigment with a maximum absorption at 370nm in the UV spectrum (43). Vertebrate Ancient Opsin, forming a functional photo-pigment with a maximum absorption between 460nm and 500nm, was isolated from the pineal of Atlantic salmon (*Salmo salar*) (44) and zebra fish (*Danio rerio*) (45). A second isoform of VA Opsin was identified in another species of teleost (the common carp, *Cyprinus carpio*) (46). This second isoform of VA Opsin is characterized by a very long carboxyl terminus (79 amino acids) in comparison to salmon VA Opsin. Independently, this long isoform, together with the short form of VA Opsin, was identified in zebra fish (45). Another isoform of VA Opsin with medium carboxy- terminus has been isolated from a smelt fish (*Plecoglossus altivelis*) (47). Salmon VA Opsin forms a functional photo-pigmant at 451 nm when expressed *in vitro* and reconstituted with 11-cisretinal (48). Salmon VA Opsin shares 37-41% amino acid identity with the classical retinal opsins and 42% identity with chicken P-opsin (44).

Exorhodopsin, a rod-like opsin, was isolated from the zebra fish (49), the puffer fish (*Fugu rubripes*) and the Atlantic salmon (*Salmo salar*) (18). This pineal opsin shares only 74% identity with the rod-opsins from the retina of the same species, and phylogenetic analysis suggests that the pineal rod-like opsins and the retinal rod-opsins diverged early in the evolution of the teleost lineage (18). Yokoyama and Zhang (50) isolated a genomic sequence from the marine lamprey (*Petromyzon marinus*), termed lamprey P-opsin. It shares 46%- 48% identity with the P-opsins and 61%-65% with VA opsins. In view of the evolutionary position of the lampreys, it was proposed that lamprey P-opsin is the evolutionary precursor of the teleost VA-opsin family (46). Pinopsin, which possesses a maximum absorbance between 470 and 462 nm, was isolated from the pineal of the birds (chicken, pigeon), reptiles and amphibians (51). A recent study, adopting

Western blot, identified two closely spaced bands at the 39 kDa, and bands of 78 and 115 kDa opsins have been localized in the pinealocytes of carp *Catla catla* (52).

Several studies indicated importance of other photoreceptor molecules as well, but the information, compared to opsin, is frustratingly poor. Alpha- Transducin (43 kDa), as an important photoreceptor molecule, is known to couple light activation with stimulation of cGMP phosphodiesterase in the phototransduction pathway. The presence of the 43 kDa band in the fish pineal organ, which is absent in the mammalian pineal gland, raises the possibility that the fish pineal organ contains 43 kDa photoreceptive alpha-Transducin molecule and it is photosensory in nature (53). An antiserum against S-antigen or Arrestin**,** a protein that binds to photoexcited phosphorylated rhodopsin and largely terminates its action, has been found to label the whole cytoplasm of all photoreceptor cells in the pineal organ of Salmon (54). Recoverin, a photoreceptor-specific Ca^{++} binding protein was also detected in the pineal photoreceptors of *Xenopus laevis* and the pigeon (55).

Immunocytochemical studies of the developing teleosts (e.g., *Salmo salar*, Atlantic halibut) demonstrated Opsin proteins and other phototransduction molecules in the pineal organ during embryonic development (56, 57). In halibut pineal organ, the first phototransduction molecules to appear are opsins; they expressed at 70% of the embryonic period. Expression of opsins is followed by expression of alpha-Transducin and arrestin at 90% of the embryonic period, when putative melatonin-producing pineal photoreceptors also appear (57).

Although role of different photoreceptor molecules in the regulation of fish reproduction remains as a topic of speculation, the results of a recent study provide an inroad to that belief. In that study, expression of pineal opsin has been found to vary in relation to the reproductive status of carp (52). A gradual increase in expression of opsin was found in parallel with the gonadal development in a reproductive cycle, being highest during the spawning phase followed by a sudden fall in post-spawning phase, and rebound during the preparatory phase to repeat the cycle (Fig. 2). A diurnal peak of opsin was noted in the mid-day and fall at mid-night. Considering the data available at this time, it appears worthwhile to study the behavior of different photoreceptor molecules in the fish pineal under natural as well as diverse experimental conditions to find the possible role of these molecules in the photoperiodic regulation of fish reproduction.

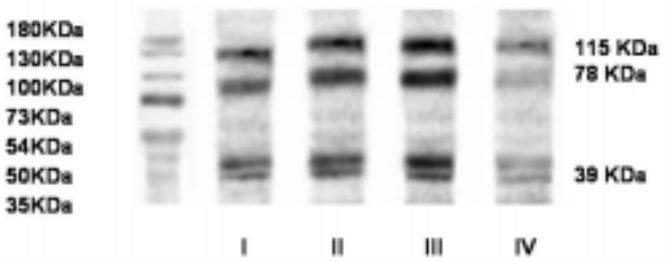

Fig. 2. Western blot analysis of opsin proteins in the pineal organ of *Catla catla* during the preparatory (I), pre-spawning (II), spawning (III) and post-spawning (IV) phases of the annual reproductive cycle.

TRANSDUCTION OF PHOTIC SIGNAL IN THE FISH PINEALOCYTES

Early electrophysiological studies showed that the spike discharges by the axons of the pineal tract are inhibited by light and increased in the dark (39, 58). Inhibition is directly related to the intensity of the stimulus. This response is triggered by the photoreceptors located presynaptically to the second order neurons. Like the retinal photoreceptors, the intracellular recordings have shown that the trout pineal photoreceptor is depolarized in the dark (-20/-30mV) and hyperpolarized in the light (-60/ -80mV) (59, 60). Hyperpolarization of the photoreceptor results in the inhibition of the release of an excitatory neurotransmitter, aspartate and/or glutamate (61).

In terms of intensity and duration, the response of the pineal photoreceptor is very similar to that of the retinal photoreceptor but the latency and recovery are much longer in the pineal (59, 62). Under prolonged illumination, the pineal photoreceptor cells maintain an intensity-related membrane potential and act as a mediator of gradual light intensity changes (39). The spectral sensitivity of the pineal photoreceptors indicated the presence of one or two photo-pigments with maximum sensitivity in the green range of the visible spectrum (39).

Pharmacological studies have further supported the idea that photoreceptor cells transduce the photic information in a way similar to that occurring in the retina. In trout pineal or dispersed trout pineal cells in culture, illumination reduces by 30-40% cGMP levels measured in the dark (6). A novel cGMP-gated channel was cloned from the trout pineal photoreceptors which exhibits a high sequence homology to cyclic nucleotide-gated 3 (CNG) channels from retinal cones (63). The effect of light was abolished in the presence of (a) pertussis toxin, which uncouples some GTP-binding proteins including transducin from their receptors after ADP-ribosylation, or (b)

phosphodiesterase inhibitors. This study suggested that light activates a phosphodiesterase through a GTP-binding protein, likely to be transducin (6). Finally, it has been shown that external Na^+ ions are essential for the generation of light response in lampreys (64). PCR amplification of trout pineal mRNAs, using specific oligonucleotide primers, indicated that the photoreceptors express a cGMP-gated channel which displays stronger analogies with the channels expressed in retinal cones than those expressed in retinal rods (63).

MELATONIN: THE ENDOCRINE SIGNAL OF PHOTOPERIOD

In fish as well as other vertebrates, melatonin is a conservative chemical signal of light-dark cycles of the environment in all the vertebrates (65, 66). In all the species investigated so far, melatonin production is high during the night time and low during the day time (67). The fish pineal, through the cyclical synthesis and release of melatonin, seems to be involved in the timing and control of a number of rhythmic functions, especially reproduction (68).

Biosynthetic pathway of melatonin

The biosynthesis of melatonin and other indoles occurs in photoreceptor cells. The general mechanism of biosynthesis of melatonin in fish appears to be identical to that in mammals (10). The pineal gland takes up tryptophan selectively from the blood (69) and utilizes the amino acid in the synthesis of melatonin and other indoles. The first step is the conversion of tryptohpan to 5-hydroxytryptophan, by *tryptophan hydroxylase* (TPOH) enzyme. 5-hydroxytryptophan is decarboxylated by the *aromatic amino acid decarboxylase* to produce serotonin or 5-hydroxytryptamine, which in the pineal gland may undergo different metabolic pathways: (a) oxidative deamination by *monoamine oxidase* (MAO-A and MAO-B) to produce 5-hydroxyindole acetaldehyde, which in turn may be converted to 5-hydroxyindole acetic acid or 5-hydroxytryptophol; and (b) N-acetylation by *N-acetyltransferase* (NAT) to produce N-acetylserotonin, which in turn is methylated by *hydroxyindole-O-methyl transferase* (HIOMT) to produce N-acetyl-5-methoxytryptamine or melatonin. *Monoamine oxidase* activity has been detected in the pineal photoreceptor cells in pike. The HIOMT-like immunoreactivity was associated with the cone-like photoreceptors in the fish (70).

Melatonin synthesizing enzymes: Daily variations in the expression and activities

Several indole-like compounds, including serotonin, N-acetylserotonin, and melatonin display light-dark fluctuations in the pineal organ of different fish (10). The pineal organ in goldfish, pike, trout, and white sucker releases melatonin rhythmically in low quantities during the day and high quantities during the night, when maintained in super-fusion or in the static culture under a 24 h LD cycle (71). In zebra fish, serotonin N-acetyltransferase-2 (zAANAT-2) is considered as the marker for development of pineal photoreceptors and circadian clock function (72). AANAT mRNAs exist as three transcripts in the trout, two transcripts in the pike, AANAT-1 and AANAT 2, and one transcript in the zebra fish (14, 73). A physiological day/night rhythm in pineal AANAT-2 protein exists in pike. The expression and activity of AANAT increases after lights off and decreases late at night and early in the morning as Mel secretion does (73). The abundance of AANAT-2 protein and activity decreases on exposure to light at midnight (9). The decrease in AANAT2 activity results from both decrease in AANAT2 expression and the light-dependent activation of enzyme proteolysis, as shown in sea bream and pike pineal organ culture (9). In trout there is no rhythmic expression of pineal AANAT mRNA. It has been noted further that in the pike, pineal MAO activity is higher at the end than at the beginning of the day .The abundance of TPOH mRNAs varies on a 24 hr basis in the pineal of pike and zebra fish but not in the trout pineal (14, 74). In contrast, HIOMT activity remains constant throughout the 24 h LD cycle in pike, salmon and trout pineal (10).

Diurnal and seasonal variations in the circulating melatonin

The existing knowledge on the rhythmic pattern of circulating melatonin in fish is chiefly based on data from the studies on temperate fish (17, 71, 75), in which circulating levels of melatonin were high during the night and decreased to basal level during the day. The nocturnal levels of circulating and pineal melatonin change on an annual basis as a consequence of seasonal variations in day length (76). Significant diel changes exist in the plasma melatonin of sea bass with nocturnal melatonin varying from144 pg/ml in summer to 23pg/ml in autumn (4). Circannual changes in pineal melatonin were recorded also in deep demersal fish, inhabiting regions outside the reach of sunlight, with significant differences existing between autumn and spring values of pineal melatonin (77). The only report on any low-latitude fish is based on the study on a carp *Catla catla* (78), which demonstrated a time-bound change in the concentrations of melatonin in a 24 hr cycle in each reproductive phase, but the pattern of the rhythm was not identical throughout the annual cycle. Like most of the vertebrates studied , a minimum diurnal value

of melatonin was recorded in the mid-day, *i.e.,* at 12.00 noon. However, the circulating level reached its peak during mid-night in each reproductive phase, other than the preparatory phase when the diurnal peak value of melatonin was recorded just before the onset of light (Fig. 3). Moreover, it was evident that the level of melatonin reached its seasonal peak during the post-spawning phase and nadir during the spawning phase (78).

Neuronal and intracellular regulation of melatonin synthesis

The studies on neuronal regulation of the mechanism of photic signal transduction in the biosynthesis of melatonin in the pineal organ revealed that cholinergic, adrenergic and dopaminergic mechanisms are involved in different ways in different species of fish. For example, *in vitro* studies of pike pineal indicated that nocturnal melatonin production was inhibited by alpha- adrenergic agonist and stimulated by beta- adrenergic agonist (10). In pinealocyte culture of rainbow trout (*Oncorhyncus mykiss*) and zebra fish (*Danio rerio*) cyclic application of adrenergic receptor agonists, norepinephrine, phenylephrine, clonidine and isoproterenol and dopamine or D1 receptor agonist had no effect on pineal melatonin secretion. Therefore, noradrenergic and dopaminergic mechanisms are not involved in signal transduction in trout and zebra fish (7, 58). The study on trout pinealocyte culture demonstrated that forskolin (adenylate cyclase activator) and cAMP analogues increase cAMP and NAT activity in light and dark (79). On the other hand, it was found that an inhibition of the hypothalamo-pituitary-dopaminergic metabolism may be a specific mechanism of melatonin action in the rainbow trout brain (80).

Extra cellular Ca^{++} ions have been shown to play an essential role for melatonin biosynthesis in rainbow trout pineal organ (81). In the rainbow trout and dogfish, putative cholinergic neurons were observed in most brain regions including the pineal photoreceptors (82). Cholinergic neurons and fibers were immunohistochemically localized in the pineal organs of lamprey and teleost fishes (83). In the trout pineal organ, acetylcholine constituted post-synaptic modulation of photoreceptor signals (84) and both muscarinic and nicotinic receptors were uniformly distributed throughout the pineal gland (85). A recent immunocytochemical study revealed the presence of

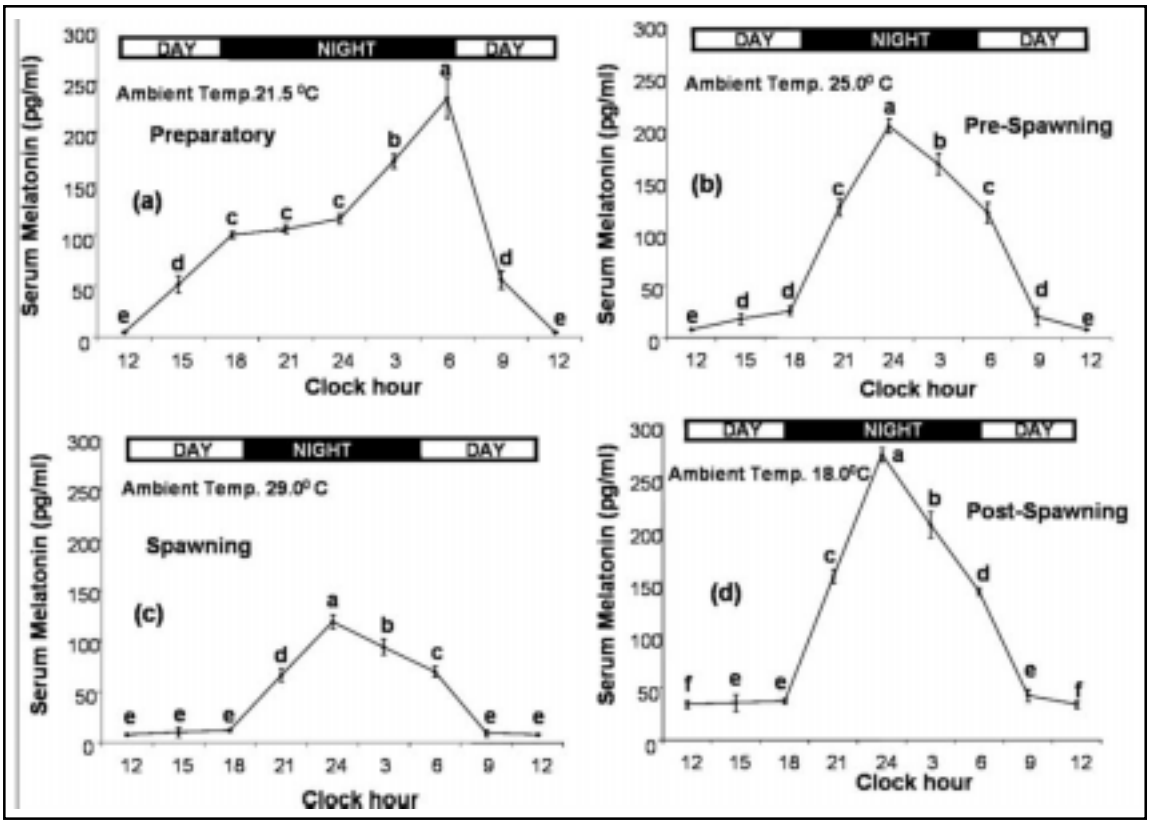

Fig. 3. Diagrammatic presentation of the diurnal profiles of serum melatonin (each data denotes mean±SE in vertical bars) in Indian major carp *Catla catla* during (a) preparatory, (b) pre-spawning, (c) spawning, and (d) post-spawning phases in an annual cycle. At each time point, at least 5 fish were sampled, and samplings were done for two consecutive years.

GABAergic perikarya and fibers in the pineal organ of the late stage embryos and adults of an elasmobranch (*Scyliorhinus canicula*). Thus, the regulatory mechanisms underlying rhythmic activity of pineal appear to be diverse among different teleosts (86).

MELATONIN AND FISH REPRODUCTION

The importance of melatonin as the most critical candidate involved in the mediation of photic effects on piscine reproduction has been emphasized in several studies. Melatonin is important in controlling the reproductive seasonality by stimulating the final stages of sexual maturation and by synchronizing the oocyte maturity with optimal timing of spawning (87). Melatonin has also been found to affect estradiol levels in mature carp females (88) and to indirectly influence the GtH II secretion *via* hypothalamic stimulatory (GnRH) centers (89, 90). In Atlantic croaker, *Micropogonias undulates*, melatonin influences LH secretion directly at the pituitary level and indirectly at the brain level (91). Melatonin has been considered as one of the candidates that mediate the transduction of photoperiodic information to the brain-pituitary-gonad axis in gonadal maturation of precocious male masu salmon (11). But the mechanism by which melatonin performs this function remains speculative. On the other hand, a study on the rainbow trout suggested that the pineal organ may not be the site of pacemaker that controls rhythms, and further research is required to study the involvement of other photoperiod-tranducing systems and melatonin (non-pineal origin) in the regulation and expression of circadian rhythm in this species (13). Likewise, a study in female three-spined stickleback (*Gasterosteus aculeatus*) indicated that a major part of the photoperiodic effects is mediated *via* mechanisms other than circulating melatonin (3). Another study in this species suggested the potential inhibitory effect of melatonin on reproduction in short photoperiod and stimulatory effect in long photoperiod (92). It was found that the nocturnal rise of melatonin was associated with the nocturnal peaks of plasma LH in both control and long photoperiodic groups of caged European sea bass (*Dicentrarchus labrax*) (17). On the basis of this study, it may be postulated that melatonin may possibly be acting at some other level of the hypothalamo-hypophyseal axis, serving as a signal for the brain (or particularly for the pituitary) to secrete specific gonadotropins (17).

EFFECTS OF EXOGENOUS MELETONIN ON FISH REPRODUCTION

Dose-dependent effects of melatonin on reproduction were studied by Singh and Lal (93) in catfish *Clarias batrachus.* These authors reported that melatonin administration prior to darkness, at the dose of 100 and 200 mg/fish, decreased testosterone levels, while at higher doses (400 mg/fish) increased testosterone levels. Administration of melatonin to *Clarias batrachus* (93) at lower doses of 25, 50 mg/fish significantly lowered the 17alpha-hydroxyprogesterone levels and at higher doses of 100, 200 and 400 mg/fish further reduced it to non-detectable levels. In *Fundulus similes*, implantation of melatonin induced asynchronous spawning in rainbow trout (94). The effects of (*ip*, 10 injections over 20 days) melatonin (75mg/100 gm BW) on gonadotropin secretion and ovarian activity were studied in *Heteropneustes fossilis* during the late preparatory to early prespawning phase showed significant reductions of plasma GtH and estradiol-17beta levels, the gonadosomatic index, frequency distribution of vitellogenic and postvitellogenic oocytes and ovarian and serum ^{32}P-labelled alkali-labile phosphoprotein (a marker of vitellogenic activity) and most of the oocytes were nonvitellogenic or had undergone atretic changes (95). *Intraperitoneal* injection of melatonin during the late light phase of the day-night cycle elicited a significant elevation in plasma GtHII levels during the dark phase in Atlantic croaker *Micropogonias undulates*, with fully developed gonads. Melatonin stimulated GtHII release during the late night and early and mid-dark phases. No significant effect of melatonin on GtHII release was observed in the regressed croaker. Melatonin injection into the third ventricle in the preoptic anterior hypothalamic area (POAH) of croaker with fully developed gonads resulted in an elevation in plasma GtHII concentrations, and a low concentration of melatonin stimulated *in vitro* GtHII release from the pituitary fragments of this fish with fully developed gonads suggesting that melatonin can influence GtHII secretion by acting at the level of POAH and also directly at the pituitary to stimulate GtHII release (91). Mazurais *et al.* (96), by studying the effects of melatonin on estrogen receptors (ER) and/or vitellogenin expression under *in vivo* and *in vitro* conditions, suggested that melatonin had no or little effect on estrogen receptor in the liver.

Feeding of pellets sprayed with melatonin (0.5 mg/kg body weight/day) to male masu salmon induced stimulatory effects on GSI, pituitary gonadotropin GtH-I content and testosterone levels when reared under LD 16:8 (11). In male sticklebacks administration of melatonin *via* water on a schedule aimed at mimicking short stimulatory photoperiod cycle did not inhibit maturation in fish kept at stimulatory long photoperiod (3). The inhibitory role of melatonin on gonadal activity has also been documented

in *Channa punctatus* (27, 97). It has been inferred that hypothalamic 5-HT may play a central role in photo-sexual mechanisms and mediate long photoperiodic effects on neuroendocrine-reproductive axis. The photoperiodic study on the same species revealed that a schedule of LD 16:8 is stimulatory whereas LD 8:16 regime is inhibitory to the testicular activity at 30^0C temperature (29). But the role of pineal in this phenomenon remains unknown. Melatonin administration in *H. fossilis* for 20 days at 6 to 8 hr after the onset of light phase inhibited vitellogenesis, induced follicular atresia and lowered the number of pituitary gonadotrophs during the pre-spawning phase and induced ovarian regression and lowering of pituitary gonadotrophs in the spawning phase (32). Nayak and Singh (98) reported melatonin as anti-steroidogenic in *C. batrachus*. In contrast, no effects of melatonin treatment on ovarian activity or on vitellogenin levels were observed in *H. fossilis* (99). Administration of melatonin at lower concentrations (25, 50 mg/fish) to *C. batrachus* significantly lowered the 17-alpha hydroxyprogesterone levels and higher doses (100, 200 and 400 mg /fish) further reduced it to non-detectable levels (93).

Modulation of the actions of gonadal steroids by melatonin in oocyte maturation

A recent study on carp oocytes (100) demonstrated, for the first time, that melatonin accelerates the action of 17alpha, 20beta-dihydroxy-4-pregnen-3-one (17alpha, 20betaDHP), the potent MIH in teleosts, and thereby stimulates oocytes maturation. In this *in vitro* study oocytes were incubated for 4 -, or 8 -, or 12 -, or 16 hr post- administration of MIH, and the effects of treatment on oocyte maturation were evaluated by considering the rate (%) of germinal vesicle breakdown (GVBD). Administration of melatonin along with MIH (at 0hr interval) or 2hr after addition of MIH did not result in any significant change in the rate of GVBD compared to that in a medium containing only MIH. However, incubation of oocytes with melatonin, especially 4hr prior to addition of MIH in the medium, led to an accelerated rate of GVBD in the oocytes. Further study revealed that pre-incubation with melatonin accelerated the action of MIH on the formation of a complex of two proteins (MPF), a regulatory component called cyclin B and the catalytic component protein kinase known as cyclin-dependant kinase, Cdk1. Moreover, determination of H1 *kinase* activity as an indicator of MPF activity in oocytes revealed that melatonin pre-incubation considerably increased MIH stimulation of histone H1 phosphorylation as compared to MIH alone (100). It appears possible that melatonin may modify or accelerate the action of MIH on piscine oocytes through an interaction between the receptors of melatonin and MIH. However, the matter remains speculative till further study.

Receptors of melatonin in relation to reproduction in fish

Types of melatonin receptors

Melatonin acts through different specific G-protein-coupled receptors which have been identified in various neural and peripheral tissues (101, 102). Radioreceptor assay techniques using 2-[^{125}I]-iodomelatonin ([^{125}I] Mel) as the radioligand have demonstrated two types of plasma membrane-associated melatonin binding sites, namely, ML1 and ML2 (103, 104). ML1 has a high affinity to [^{125}I] Mel and belongs to the G-protein-coupled receptor family, while ML2 with a low affinity to [^{125}I] Mel has been identified as quinone reductase2 (QR2). Studies employing molecular techniques revealed the existence of three different subtypes of melatonin receptors, namely, MT1, MT2 and Mel1c (101, 105, 106). MT1 and MT2 are widely distributed in vertebrates (101, 107). On the other hand, Mel1c has been cloned only in nonmammalian species such as zebra fish, clawed toad and chicken (101, 106).

MT1 is the principal melatonin receptor detected in the suprachiasmatic nuclei (108-110), the location of the master circadian clock system in mammals (111, 112). However, most of the piscine studies demonstrated melatonin receptors on the whole brain (113, 114). MT1 is expressed in the hypothalamic suprachiasmatic nuclei and in the hypophyseal pars tuberalis, where it is presumably involved in circadian and reproductive responses (109). Recently, MT1 and MT2 melatonin receptor expression has been found in the liver, kidney, retina and brain of the golden rabbit fish, *Siganus guttatus* (115, 116). The study on the same fish (116) demonstrated that melatonin receptors in the neural and peripheral tissues have closer identity to non-mammalian MT1 (82-92%) than to MT2 and Mel_{1c} (63-69%).

Diurnal variations of melatonin receptors and the actions of melatonin

Diurnal variations in the melatonin binding sites in the retina and whole brain (17, 111, 113, 117), and in the melatonin receptor gene expression in neural tissues (118, 119) were reported in several species. A day-night change in proportion of Mel receptor subtypes, Mel1a and Mel1b was evident in the optic tectum-thalamus of European sea bass (17). On the other hand, molecular aspects of diurnal variations in melatonin receptors have been reported in the chum salmon (*Oncorhynchus keta*)

for MT1 and MT2 (114). A high expression of MT1 mRNA with a day-night difference was observed in the whole brain, liver and kidney of the golden rabbit fish. The expression of MT1 mRNA in the cultured pineal gland also showed diurnal variations with high expression levels during night time suggesting that the increased expression level observed in the whole brain is partially of pineal origin. Alteration of light conditions in the pineal gland cultures resulted in changes in melatonin release into the culture medium as well as MT1 mRNA expression in the pineal gland. Takamura *et al.* (5) found that in the golden rabbit fish, the melatonin level in the blood circulation exhibits both diurnal and lunar (with an increase during the moon period) variations. Such a duality may imply that melatonin receptors are also involved in the regulation of daily and monthly activities through melatonin actions in this fish species. It is possible that circulating melatonin itself may have regulatory influence on melatonin receptors, as reported in the brain of goldfish (113).

The density and affinity of melatonin binding sites were higher at mid-day than at mid-night in sham-pinealectomized gold fish brain under light-dark cycles. The rhythms disappeared after pinealectomy or constant light exposure, both of which abolish plasma melatonin rhythms, indicating that the diel changes of melatonin binding sites in the goldfish brain are regulated by endogenous melatonin of pineal origin (120). Regarding reciprocal relationship between melatonin and melatonin binding sites, it was proposed that melatonin itself regulates melatonin receptors, because a reverse pattern of their daily variations was found in the brain of goldfish (113). An increase in the MT1 mRNA expression occurs during nighttime in the brain and the retina of the golden rabbit fish (116). The high expression of another type of melatonin receptor, Mel_{1b} or MT2 during day time has been noted in the liver and kidney of *Siganus guttatus* (116).

Melatonin and melatonin receptors positively play a role in the regulation of vitellogenesis, during which the yolk precursor protein (vitellogenin) is synthesized in the liver under the influence of estrogens followed by its active incorporation into the developing oocytes (121). Further support to this contention is available from the report that melatonin treatment and pinealectomy failed to induce gene expressions of estrogen receptors and vitellogenin in rainbow trout *Oncorhynchus mykiss* (96). Though melatonin binding sites have been detected in the ovaries of various vertebrates (122-125), it remains to be discovered whether or not the diurnal and seasonal profiles of MT1 receptor in the ovary of any fish are also under the direct control of circulating melatonin.

CONCLUSION

The pineal organ of fishes contains photoreceptor cells analogous to the cones of retina. These cells constitute a circadian system and contain the photoreceptive unit and the melatonin producing unit. This allows generation of a rhythm in the secretion of melatonin, which results in a high nocturnal production and low diurnal production. The rhythm is synchronized to the alteration of light and dark, the 24 hr LD cycle, but other factors from external (temperature) or internal (neurotransmitters) origin may modulate the shape of the oscillations. However, the available literature suggest that despite apparent homogeneity in the structural organization of the pineal organ in teleosts (10, 67), a wide range of variations exists in the mechanism involved in the regulation of reproduction in different fishes. The results of the response of gonadal activity to surgical removal of pineal organ under both natural and altered photothermal conditions indicates the possibility of pineal organ being an important component of the neuroendocrine-gonadal axis. The influence of exogenous administration of the hormone melatonin on gonadal activity points that the pineal organ may play an important role in the regulation of reproduction by acting as a transducer of the environmental cues. As the intensity of light influences the production of melatonin in the pineal, there is a possibility that the pineal photoreceptor molecules may have some seasonal and diurnal rhythms. A critical analysis of the existing literature clearly reflects an inadequacy of information relating to the influences of photoperiods on endogenous profiles of melatonin and gonadal growth, the nature and behavior of different pineal photoreceptor molecules in relation to biosynthesis of melatonin and photo-induced gonadal functions, and the photic signal transduction cascades involved in the synthesis of melatonin in relation to the annual reproductive events. Additionally, demonstration of the role of melatonin and its receptors on the ovary in the photoperiodic regulation of fish reproduction is an interesting topic of future research. Thus, it appears meaningful to undertake a study in more fish species inhabiting different hydro-realms on different pineal photoreceptor molecules and melatonin output in respect to the different reproductive strata and photo-schedules.

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