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# Significance of Retinal and The Pineal Photoreceptors in The Photoperiodic Regulation of Reproduction in The Bony Fish Channa Punctatus (Bloch)

KEYWORDS: Fish reproduction, Pinealectomy, Ovarian follicular kinetics, Pineal/retinal photoreceptors, Melatonin

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**ABSTRACT** *Most poikilotherms perceive photic signal through retinal and non-retinal photoreceptors. The photoreceptors present in the pineal organ are important in mediating the non-retinal photoreception in fish. The purpose of this study was to assess the significance of retinal and pineal photoreception in the regulation of reproduction in Channa punctatus. While the retinal pathway was disrupted by surgical enucleation (EN), the pineal pathway was cut off by pinealectomy (PX). Both, EN and PX led to a stimulation of ovarian function in fish held in light-dark (LD) 12:12 cycle. Highest stimulation was noticed in fish that underwent both EN and PX. However, when the EN and PX fish were challenged with short photoperiod (LD 06:18) their ovaries were inhibited. The results indicate that both retinal and the pineal photoreceptors are important in the photoperiodic regulation of reproduction. In addition, the findings also provide a clue for the involvement of some non-retinal and non-pineal photoreceptors in the photic signal transduction in this fish. Pineal gland is known to convert the physical photic signal into a chemical hormonal signal in the form of melatonin produced rhythmically. Since both, the lateral eyes and the pineal organ are known to produce melatonin the observed changes in ovarian function could be due changes in melatonin turnover under the experimental conditions.*

## INTRODUCTION

Photoperiod individually or in combination with temperature or other ecological factors(s) regulates the sexual periodicity in most fish species living in low, mid- and high latitudes (Vivien-Roels, 1985; Takemura et al., 2004). At low latitudes, where there is less seasonal variations in photoperiod and water temperature other factors may also be in the seasonal regulation of reproduction.

The pineal and retinal photoreceptors mediate the photoperiodic cues in many fish species (Falcon et al. 1992a, 1992b). Though, both the pineal organ and the eyes of fishes possess the circadian oscillator (Cassone, 1998), the former structure seems to be more important for the transduction environmental photoperiodic signals (Falcon, 1999). In addition to the pineal and retinal photoreceptors, there are non-retinal and non-pineal photoreceptors located elsewhere (Vollrath, 1981; Vigh and Vigh-Teichmann, 1999; Russell and Mark, 2002). The photoreceptors transmit the environmental photoperiodic information to the neuroendocrine system through neural links.

Several experimental studies indicated that the pineal organ translates the photoperiodic information into a hormonal message (Falcon et al., 1992b; Okimoto and Stetson, 1999; Amano et al., 2000), in the form of rhythmically secreted melatonin. Melatonin in turn transduces the of photic cues to the brain-pituitary-gonadal axis in fishes (Amano et al., 2000) and many other vertebrates (Reiter, 1984; Joshi et al., 1985). The pineal organ in fish is thus involved in the regulation of rhythmic physiological processes including reproduction (de Vlaming, 1974; Ralph, 1978).

Disruption of photic input to the neuroendocrine system by either pinealectomy or enucleation leads to alterations in physiological responses under experimental coniditions. Rasquim (1958) in Astyanax mexicanus, Pang (1967) in Fundulus heteroclitus, and Peter (1968) in Carassius auratus, failed to detect any effect of pinealectomy on gonads. These studies restricted to species inhabiting temperate regions were limited primarily to assess the effects of pinealectomy. The present study therefore was set up to assess the effects of disruption of pineal and ocular photoreception on ovarian follicular kinetics in a fish inhabiting tropical fresh waters. The objective was to assess the impact of the disruption of retinal and pineal pathways of photic signal transduction under experimentally altered photoperiodic regimen.

## MATERIAL AND METHODS

The fish, Channa punctatus (Bloch) were collected from Bheema River (N latitude $17^0$) during early November. They were sorted on the basis weight and size (average wt being 30g; length snout to tip of the tailfin 13 cm), and held in glass aquaria for two weeks prior beginning the experiment. During this period they were treated with chloromphenicol (5mg/litre of water) as prophylactic antibiotic.

Fish held in aquaria were then placed in chambers with facility for automatic regulation of photoperiod. During the course of experiment the animals were fed with live earthworm on alternate days and the water of the aquaria changed daily. The water temperature was maintained at 21 ± 1° C. For the experiment 48 fish were divided into six groups of 8 each; four of these groups held in LD 12:12 (light on at 06.00h and off at 18.00h) were either pinealectomised (PX), enucleated (EN), pinealectomised and enucleated (PX + EN) or sham operated to (SC) serve as control. The remaining two groups were held in LD 06:18 (light on at 06.00 and off at 12.00) were subjected t SC or PX.

Pinealectomy and enucleation were performed under anesthesia. Pineal gland was removed after making an incision in skin overlying the pineal window and carefully cutting and lifting part of the parietal bone to pull off the pineal. The bone was then replaced to seal the wound. Enucleation was performed by puncturing the eye ball with a fine sterilized needle; retina and the other ocular tissue were removed carefully. Following surgery chloromphenicol (5mg/litre) was added to the aquaria water, as an antibiotic.

Autopsies were performed two months post operation. Ovaries were dissected out and immediately weighed on an electronic balance (Sartorius) and fixed in Bouin's fluid. The tissues were later embedded in paraffin wax for histological study. Five micron thick sections were taken and stained in hematoxylin and eosin.

Follicular kinetics was studied in histological sections of the

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ovaries. The follicles were classified as previtellogenic, vitellogenic or atretic based on their histomorphology. All the eight fish from each group were used for this purpose. The counting of the follicles was done in 20 slides from anterior, middle and posterior segments of the ovary.

The statistical analysis was done using a computer program for ANOVA and Schiffe's pair wise comparison test. The Gonadosomatic index (GSI; ovarian weight per 100g body weight) was also calculated for each group for comparison.

**THEORY AND CALCULATION:**

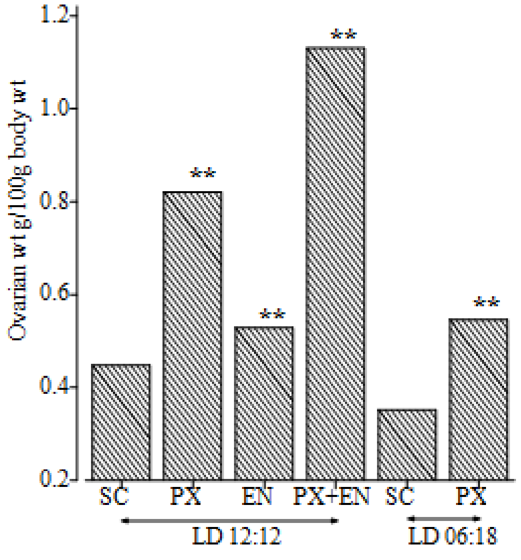
In most vertebrate species photoperiod regulates pineal / retinal melatonin secretion. Melatonin in turn modulates the physiological responses of organisms to changing photoperiods. The photoreceptors (whether retinal, pineal or others) thus form an interface photoperiodic environment and the organism. The question was to assess the relative importance of retinal or pineal photoreceptors in transferring the photic signal to the neuroendocrine reproductive axis in this species. Ovarian follicular dynamics was chosen as a parameter to assess the reproductive function following disruption of ocular / pineal photoreception.

**RESULTS**

Ovarian weight increased in PX fish held either in LD 12:12 or in LD 06:18 (Figure 1), however the increase was more appreciable (P<0.01) in the fish held in LD 12:12. Enucleated fish held in LD 12:12 also had bigger ovaries (P<0.01), but enucleation combined with pinealectomy resulted in highest increase (P<0.01) in ovarian weight (Enucleation was not performed in fish held in LD 06:18 for technical reason).

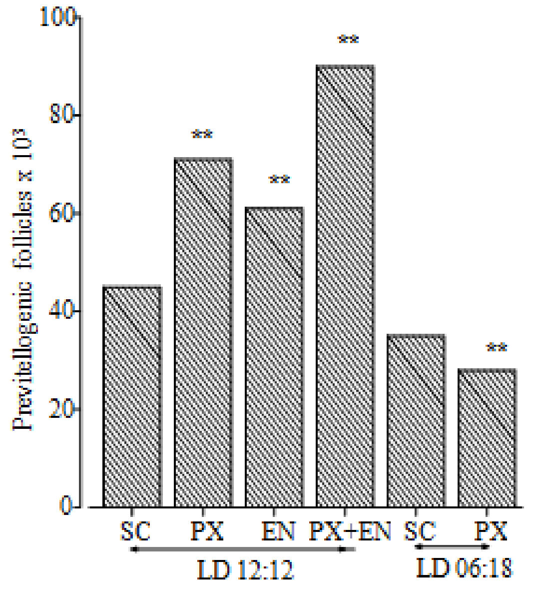
**Legend to Figure 1 to 4**
**Figure (1) Ovarian weights (GSI = ovarian weight / 100g body weight)**
**(2) Number of Previtellogenic Follicles (PVF)**
**(3) Vitellogenic Follicles (VF) and**
**(4) Atretic Follicles (AF) in sham operated control (SC), pinealectomised (PX), enucleated (EN) and pinealectomised + enucleated (PX+EN) fish held in either in LD 12:12. or LD 08:16. Values are means ± SE. **P<0.01 Vs controls.**

**Figure 1**



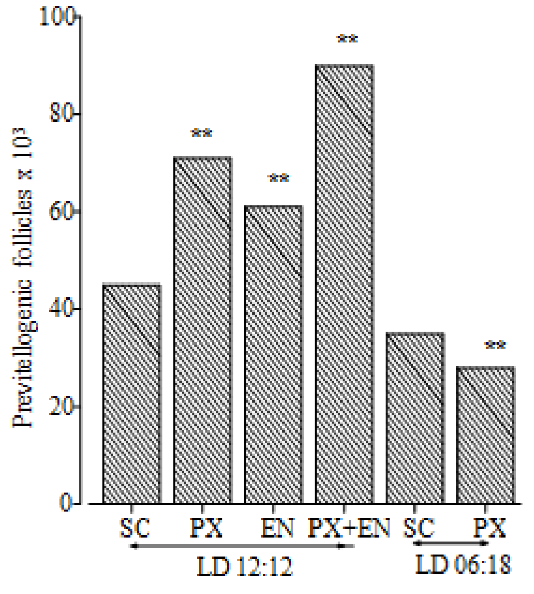

The study of follicular kinetics revealed an increase (P<0.01) in the number of previtellogenic follicles in PX, PX+EN groups held in LD 12:12 (Figure 2). However the PX and SC groups held in LD 06:18 had fewer previtellogenic follicles (P<0.01) compared either to the SC, PX or PX+EN groups of fish held in LD 12:12.

**Figure 2**



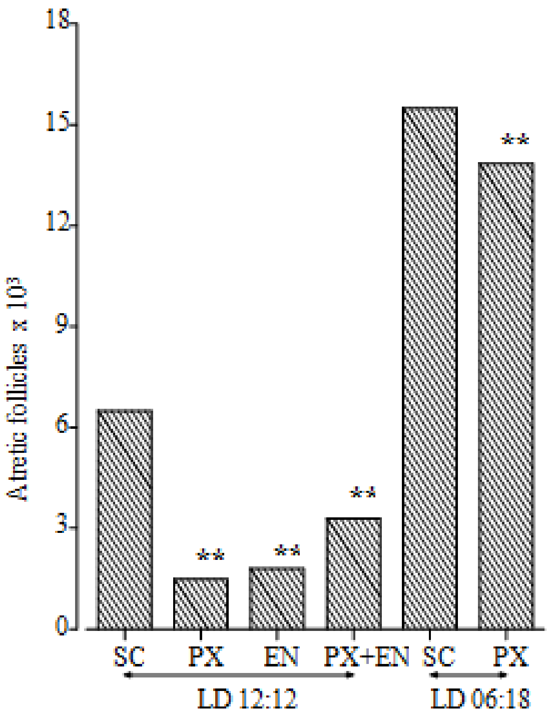

The number of vitellogenic follicles increased in the ovaries of PX+EN ((P<0.01 Figure 3) group held in LD 12:12. Ovaries of EN fish held in LD 12:12 had lesser (P<0.01) number of vitellogenic follicles. The number of vitellogenic follicles in the ovaries of both SC and PX group held in 06:18 decreased (P<0.01). In general the pattern of change in the number of vitellogenic follicles was similar to the pattern of GSI in these groups.

**Figure 3**



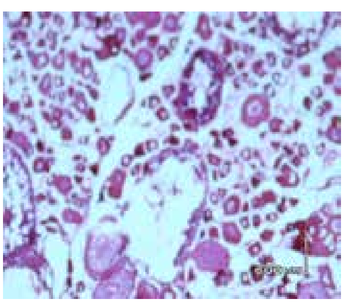

The number of atretic follicles increased (P<0.01) in the ovaries of SC and PX group held in LD 06:18 (Figure 4). The number of atretic follicles in the ovaries of both EN and PX+EN group held in 12:12 decreased (P<0.01). Ovaries of PX fish held in LD 12:12 had least number of atretic follicles (Figure 4).

Figure 4

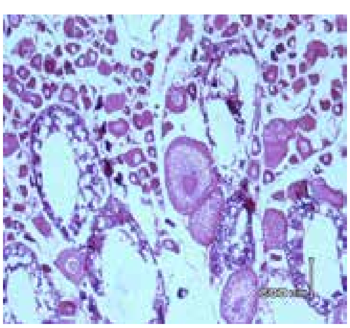



Histological observations corroborate the inferences drawn from gravimetric and follicular kinetic studies. Ovaries of control fish held in LD 12:12 contained predominantly previtellogenic follicles. The number of vitellogenic follicles increased in EN, PX and EN+PX fish (Figure 5. B-D). The control fish held in short photoperiod had regressed ovaries filled with only previtellogenic and atretic follicles. However the PX fish held in short photoperiod had previtellogenic, vitellogenic and atretic follicles (Figure 5 A-F).

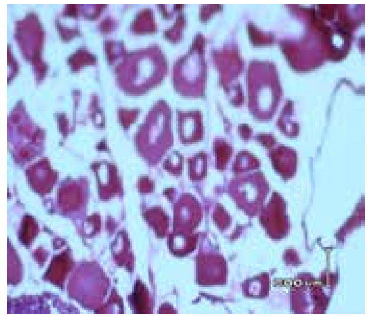
Figure 5 A-F



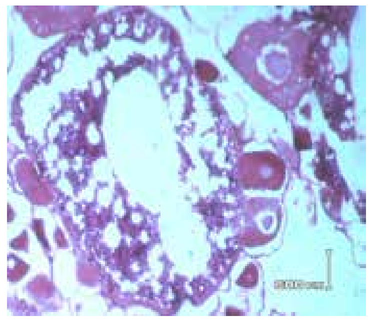
A



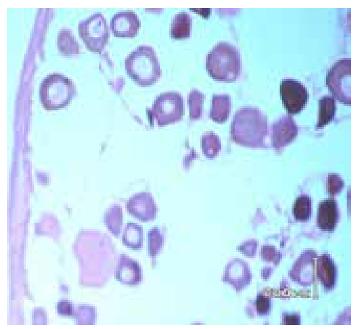
B



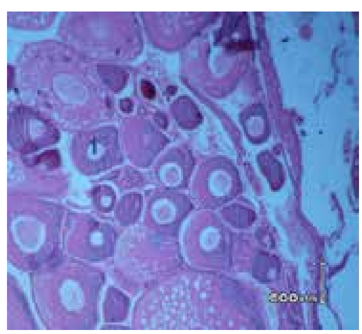
C



D



E



F

Legend to Figure 5: Photomicrographs of transverse sections of the ovary of the teleost fish Channa punctatus (Bloch) (A) sham operated control (SC), (B) pinealectomised (PX), (C) enucleated (EN) and (D) pinealectomised + enucleated (PX+EN) fish held in LD 12:12. (E) sham operated control (SC), (F) pinealectomised (PX) fish held in LD 08:16

**DISCUSSION**

Earlier work from this laboratory (Renuka and Joshi, 2010) confirms that Channa punctatus is a seasonally breeding fish, the gonads mature during spring and spawning usually coincides with the onset of monsoon. Exposures to long days during the preparatory stage induce early maturation of the ovaries. As noted in this study challenging the fish with short photoperiod (LD 08:16) retards ovarian maturation as evidenced by fall in GSI, decrease in the number of vitellogenic follicles and an increase in follicular atresia. Channa punctatus exhibits photoperiodicity in reproduction despite the limited seasonal variations in the duration of photoperiod they witness in this tropical region. Photoperiod is an important and dependable environmental cue for timing of reproduction on a seasonal basis in many species (Meier, 1975; Wingfield and Kenagy, 1991). However, in ectothermic vertebrates especially those inhabiting tropical regions, the importance of photoperiod in the regulation of reproduction has been a matter of debate (Saidapur and Hoque, 1995). In mammals the perception of the environmental photoperiodic information is primarily through retinal photoreceptors, in non mammalian vertebrates (especially in ectotherms) on the other hand photoperiodic information is perceived not only by the retinal photoreceptors but also by pineal and other photoreceptors (Falcon et al. 1992a, 1992b; Vigh and Vigh-Teichmann, 1999; Foster and Soni, 1998).

Results of the present study clearly indicate that elimination of pineal pathway of photoreception results in precocious maturation of ovaries in Channa punctatus held in LD 12:12. Even in fish held in short photoperiod disruption of pineal pathway seemed to stimulate the ovary as indicated by increased GSI vitellogenic follicles. However the significant increase in follicular atresia observed in this group needs to be explained. Pinealectomy in combination with enucleation further enhanced the GSI and the number of previtellogenic follicles, but the number of vitellogenic follicle however remained similar to those found in only pinealectomised group. Probably pinealectomy and enucleation affect dynamics of follicular kinetics at different levels, i.e. at the level of formation of previtellogenic follicles, recruitment of previtellogenic follicles to vitellogenic follicles or induction of atresia of follicles. It is clear that pinealectomy alone stimulates the ovaries, as indicated by enhanced GSI, increased number of vitellogenic follicles and decreased follicular atresia. Enucleation on the other hand seems to stimulate ovary in terms of GSI and formation of the pre-vitellogenic follicles but not their conversion to vitellogenic follicles. The combined effect of pinealectomy and enucleation is enhanced stimulation of the ovary as indicated by data on the gravimetric and follicular kinetics. The results do confirm that the pineal organ of Channa punctatus participates in the regulation of gonadal activity and the effect of pinealectomy on gonadal activity depends on the photoperiodic environment to which the fish are exposed.

In Poecilia reticulates, pinealectomy apparently accelerates sexual maturation (Pflung, 1954). Other investigators like Rasquim (1958), Peter (1968), suggested that pinealectomy has little influence on gonadal activity in teleosts. Pinealectomy altered gonadal activity in goldfish from late winter to early summer. From late July to early December, pineal removal had no obvious effects on gonadal activity in the fish (de Vlaming and Vodicnik, 1978).

Working on the catfish Heteropneustes fossilis Garg (1989) opined that the effects of removal of pineal or disruption of optic photoreception on ovarian function depended on the phase of the reproductive cycle and the photoperiodic regimen the fish are exposed. Other observations in the same study point to the involvement of non-pineal, non-retinal photoreceptors in the seasonal regulation of reproduction.

Jonathan and Malcolan (1981) also found that Fundulus heteroclitus could perceive environmental photoperiodic changes regardless of whether they were blinded, pinealectomised, or both. Pinealectomy of Heteropneustes fossilis held under normal photoperiod and ambient temperature produced season-dependent effects on ovarian activity (Joy and Khan, 1989).

In the present study the gravimetric data and the analysis of follicular kinetics suggest that eyes and pineal organ both are important in the transduction of photoperiodic information that regulates reproduction in this fish. It is hard to speculate if a particular photoreceptor-pathways is more important than the other in regulating physiological responses. Since the gonads respond even after the disruption of pineal and retinal pathways it is likely that some non-pineal and non-retinal photoreceptors provide the photoperiodic input to the neuroendocrine system. Further it is interesting to note that the effects of disruption of either pineal, retinal or both pathways of photoreception on reproduction vary and seem to affect different stages of follicular dynamics. The reason for such variation in the response remains to be elucidated.

Melatonin is involved in the transduction of photoperiodic information to the brain-pituitary-gonadal axis in teleosts (Amano et al., 2000) and many other vertebrates (Reiter, 1984; Joshi et al., 1985). While pineal and retinal photoreceptors transduce the photoperiodic signal through rhythmic melatonin secretion the pathway involved in signal transduction by other photoreceptors remains to be understood.

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