Effect of 12hrs Phase Delay in Light-Dark Cycle, Subsequent Enucleation Followed by Exogenous Melatonin Treatment on Air-Breathing Activity Rhythm in Clarias Batrachus

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Abstract

It has been aimed to examine the effect of 12hrs phase delay in light-dark cycle, subsequent enucleation followed by exogenous melatonin treatment on air-breathing activity rhythm in Clarias batrachus. Following acclimation, seven Clarias batrachus approximately of the same size were transferred to chronocubicles. They were initially maintained under LD 12:12 regime (lights on 06:00hrs) and considered as control group. The air-breathing activity was monitored with the help of PC based event recorder for 7days. Then, the phase delay of 12hrs in light dark cycle was maintained by reversing the light schedule with the help of automatic timer for consecutive 10 days. Subsequently the animals were enucleated and left in the similar photoperiod for next ten days and then they were given melatonin treatment (2µg/fish) daily at the initiation of subjective dark hour for another ten days. The air–breathing activity was recorded with the help of infrared photoswitches connected to a microprocessor based event recorder. The results obtained by actograms depicted the occurrence of entrained rhythm with the timings of light on/off in air-breathing activity under LD 12:12 regime. Later, the activity pattern obtained from the actogram revealed that the air-breathing activity rhythm is quickly entrained with the timings of lights on/off as phase delayed by 12 hrs and the activity was found to be increased and decreased during the experimental dark and light hours of the LD regime, respectively. Further, after enucleation under phase delay by 12hrs, the activity curve graph showed that the air-breathing activity rhythm is similar as obtained under phase delay, showing increased activity during the experimental dark hour in most of the individuals. Then, individuals after receiving daily treatment of melatonin under the phase delayed by 12 hrs showed occurrence of the two peaks in air-breathing activity rhythm at 12:00 hrs and 24:00 hrs in most of the enucleated.

Keywords: Air-breathing behaviour, Blinding, Enucleation, Catfish, Circadian-rhythm, Melatonin, Phase delay.

Introduction

The mechanisms and effects of photoperiod controlled rhythmicity are best known for vertebrates. In most fish species investigated, photoperiod acts on the pineal organ and retina both secreting melatonin during the night (Sumpter, 1990). Randall and coworkers (1991) have reported that in salmonid species, the duration of the elevated levels of melatonin at night compared to lower levels during the day reflects the length of the night.

Photoperiod provides the most reliable external signal in nature indicating the time of the year (Gwinner 1986), and with its stability and predictability, photoperiod acts as a proximal cue that signals cyclic changes before they occur (Brett 1979). Directional changes in photoperiod, that is, the decrease or increase in day lengths in relation to the previous experienced photoperiod, has been suggested as cues in some processes (Randall *et al.* 1991; Bromage *et al.* 1993), but also the actual length of the day (or night) or a combination between the two may be involved. In addition, it has been suggested that the organism's circadian system and zeitgeber determine the phase relation between entrained circadian rhythms and their entraining periodic signals (Aschoff, 1965; Hoffman, 1969).

The present study was aimed to examine the effect of 12hrs phase delay in light-dark cycle, subsequent enucleation followed by administration of melatonin on air-breathing activity rhythm in a fresh water catfish, *Clarias batrachus*.

Materials and Methods:

Collection and care of animals

Live *Clarias batrachus* of mixed sex (40-50 g body weight) were procured locally and kept in the stock aquaria under the laboratory conditions (10 days) for proper acclimation. During the period of acclimation, water inside the aquaria was renewed every alternate day. Fishes were fed pieces of small dry fishes locally available in the market *ad libitum*. (Sahu, 2008, 2019, 2020).

Surgery

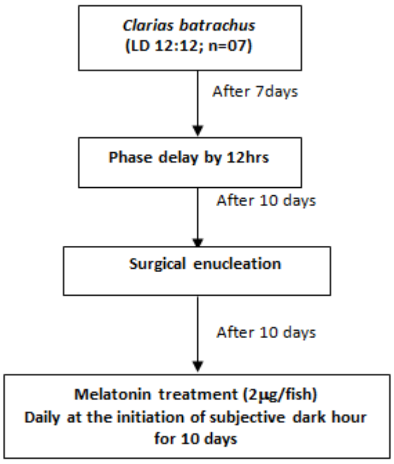
Enucleation:

The bilateral ocular enucleation was performed with the help of fine surgical instrument.

After anesthetizing the animals by keeping them in ice-tray, an incision was made around the eyeball. The eyeball was pulled out carefully from the socket and the connections were cut with scissors (Chiba *et al.*, 1993; Khan and Joy, 1990).

Experimental Protocol:

Following acclimation seven C*larias batrachus* approximately of the same size were transferred to chronocubicles. They were initially maintained under LD 12:12 regime (lights on 06:00hrs) and considered as control group. The air-breathing activity was monitored with the help of PC based event recorder for 7days. Then, the phase delay of 12hrs in light dark cycle was maintained by reversing the light schedule with the help of automatic timer for consecutive 10 days. Subsequently the animals were enucleated and left in the similar photoperiod for next ten days and then they were given melatonin treatment (2μg/fish) daily at the initiation of subjective dark hour for another ten days. Enucleation was performed as earlier described (Chiba *et al.*, 1993; Khan and Joy, 1990). The experimental protocol was as follows:



Activity recording and data collection

Each animal was kept in a specially designed glass aquaria inside the chronocubicles for recording of air-gulping/breathing activity. A porous plate was fixed just below the surface of water with a single large space, available in the centre from where the fish can come to gulp the atmospheric air. The infrared photoswitches were fixed in such way that a beam of infrared ray lies in the middle of this space. Air-gulping/breathing activity was monitored and recorded by using a PC-based event recorder (Vision Automation, Pune). This device employs the principal of IR beam interruptions to record air-gulping/breathing activity effectively. Whenever fish came to the surface for gulping air it intrupted the infrared beam. This signal was amplified and recorded by a microprocessor based event recorder. The two types of data files were prepared, viz; graphical (for obtaining actograms) and numerical (to obtain number of gulps per hour for statistical analysis) with the help of such event recorder. In addition, hourly recording of climatic variables such as water temperature, room temperature and humidity was also done simultaneously on daily time scale. Light schedule was maintained with the help of automatic timers (Sahu, 2008, 2019, 2020).

Statistical analysis

The data, expressed in number of gulps/hour and were subjected to single cosinor method at τ = 24hr (Nelson *et al.*, 1979). A rhythm was characterized by three parameters, viz., the mesor (M, rhythm adjusted mean), the amplitude (A, half of the difference between the minimum and maximum of the best fitting cosine function) and the acrophase (ϕ, the time of maximum of this cosine function with local midnight as ϕ reference). The ϕ was obtained with its 95% confidence limit if a rhythm was detected with regards to the considered τ in that the amplitude differed from zero (non null amplitude test) with $P<0.05$. A power spectrum method was also employed for detecting prominent period in air-gulping/breathing activity of the fishes under LD 12:12 (De Prins *et al.*, 1986) Student's *t*-test (Bruning & Kintz, 1977), ANOVA (Snedecor & Cocharan, 1994) and Duncan's Multiple range test (Duncan, 1955) were applied whenever required (Sahu, 2008, 2019, 2020).

Results

Activity under LD 12:12

The activity pattern emerged from the actogram depicted the occurrence of entrained rhythm with the timings of light on/off in air-breathing activity (activity elevated during dark and declined during light hours) under LD 12:12 regime (Fig.1a and b). Further, results of cosinor analysis revealed a statistically significant 24-h rhythm in 6 out of 7 (85.7%) studied individuals (Table1, Fig.3). The acrophases of the activity in most of the individuals were located during the dark hour of the light-dark cycle (Fig. 4a). In addition, the period (τ) of the activity rhythm was prominent 24-h in all the studied individuals except Fish #04 (Table 3).

Activity under phase delay:

The visual analysis of the actograms revealed that the air-breathing activity rhythm is quickly entrained with the timings of lights on/off as phase delayed by 12 hrs (Fig.1a and b) and the activity was found to be increased and decreased during the experimental dark and light hours of the LD regime, respectively.

Results of cosinor analysis depicted a statistically significant 24-h rhythm in air-breathing activity in 4 out of 7 (57.1%) individuals. In addition, most of the individuals, the acrophases of the activity were located in experimental dark hour (Fig. 4b). However, the period (τ) of the activity was not equal to 24-h in most of the individuals (Table 3).

Further, daily mean of the activity was slightly decreased after phase delayed by 12hrs, as compared to control group (Fig 5). In addition, amplitude (Fig 7) and phase angle (Fig 8) of peak of the activity with reference to lights on was decreased as compared with individuals under LD 12:12 (Control group). However, statistical validation was obtained only for phase angle.

Activity after enucleation under phase delay

To study the activity pattern, the activity curve graphs have been prepared by using the numerical data file obtained by the PC based event recorder. After enucleation under phase delay by 12hrs, the activity curve graph (Fig 2a and b) showed that the air-breathing activity rhythm is similar as obtained under phase delay, showing increased activity during the experimental dark hour in most of the individuals.

Results of cosinor analysis depicted that still 57.1% (4 out of 7 individuals) individuals have retained a statistically significant 24-h rhythm in their air-breathing activity (Table1; Fig.3) and in most of the individuals the peaks of the activity were located during the experimental dark hour (Fig.4c). The results of the spectral analysis revealed that in 5 out of 7 enucleated individuals maintained under phase delay, the period (τ) of the activity was not equal to 24-h (Table 3).

The daily mean of the air-breathing activity was highest as compared to other studied groups, however, statistical validation could not be obtained (Fig 5). In addition, rhythm adjusted mean i.e., mesor and amplitude of the activity were also increased with statistical validation after enucleation under phase delay by 12hrs as compared with the individuals under LD 12:12 and phase delay (Fig.6 and Fig.7). However, phase angle of peak of activity with reference to lights on was significantly decreased as compared to the individuals under LD 12:12 (Fig.8).

Activity after enucleation under phase delay and melatonin receiving:

Results of activity curve graph showed occurrence of the two peaks in air-breathing activity rhythm at 12:00 hrs and 24:00 hrs in most of the enucleated individuals after receiving daily treatment of melatonin under the phase delayed by 12 hrs (Fig.2 a and b).

Results of cosinor analysis revealed a statistically significant 24-h rhythm in their air-breathing activity rhythm in 57.1% (4 out of 7 individuals) (Table1; Fig.3). However, period of the activity i.e., τ was not equal to 24-h in almost all the studied individuals. Further, peaks of the activity of 24-h rhythm in all the individuals occurred during the experimental dark hour of the LD regime (Fig.4d).

Table1 Results of cosinor analysis of air-breathing activity in *Clarias batrachus* at τ=24h

Group	Animal Code	P[a]	Mesor ± SE[b]	Amplitude ± SE[c]	Acrophase[d](95%CL)
IG	F#01	<0.001	0.26 ± 0.02	0.28 ± 0.09	0.14 (1.29, 22.98)
	F#02	<0.01	1.30 ± 0.09	1.49 ± 0.41	0.46 (1.22,23.69)
	F#03	0.0005	123.4± 1.84	48.04 ± 3.39	2.05 (4.73, 23.5)
	F#04	0.2166	1.06 ± 0.03	8.16	23.96
	F#05	0.0001	25.96 ± 1.74	10.72 ± 2.15	21.33 (23.63, 19.05)
	F#06	0.1647	2.05 ± 0.45	1.20 ± 1.02	18.91 (18.05, 19.77)
	F#07	<0.001	0.78 ± 0.05	1.02 ± 0.07	0.31 (1.25, 23.33)
PD	F#01	0.9223	75.34 ± 1.35	3.03	13.30
	F#02	0.11	1.009	0.01	17.62
	F#03	0.04	45.96 ± 2.06	7.38 ± 7.17	11.38 (16.49, 6.34)
	F#04	<0.001	9.17 ± 0.63	5.38 ± 2.21	21.96 (23.57, 20.36)
	F#05	0.813	21.14 ± 1.29	0.85	1.86
	F#06	<0.001	8.01 ± 0.30	2.14 ± 1.05	19.73 (21.59, 17.87)
	F#07	<0.001	27.56 ± 1.04	6.38 ± 4.69	16.67 (20.03, 13.31)
PDEG	F#01	<0.001	142.24 ± 1.82	66.08 ± 2.11	11.54 (12.73,10.35)
	F#02	0.3654	1.003 ± 0.004	0.008	17.79
	F#03	<0.001	53.96 ± 1.17	29.41 ± 4.05	12.27 (12.81, 11.74)
	F#04	<0.001	302.48 ± 7.18	75.03 ± 2.81	11.07 (12.37, 9.78)

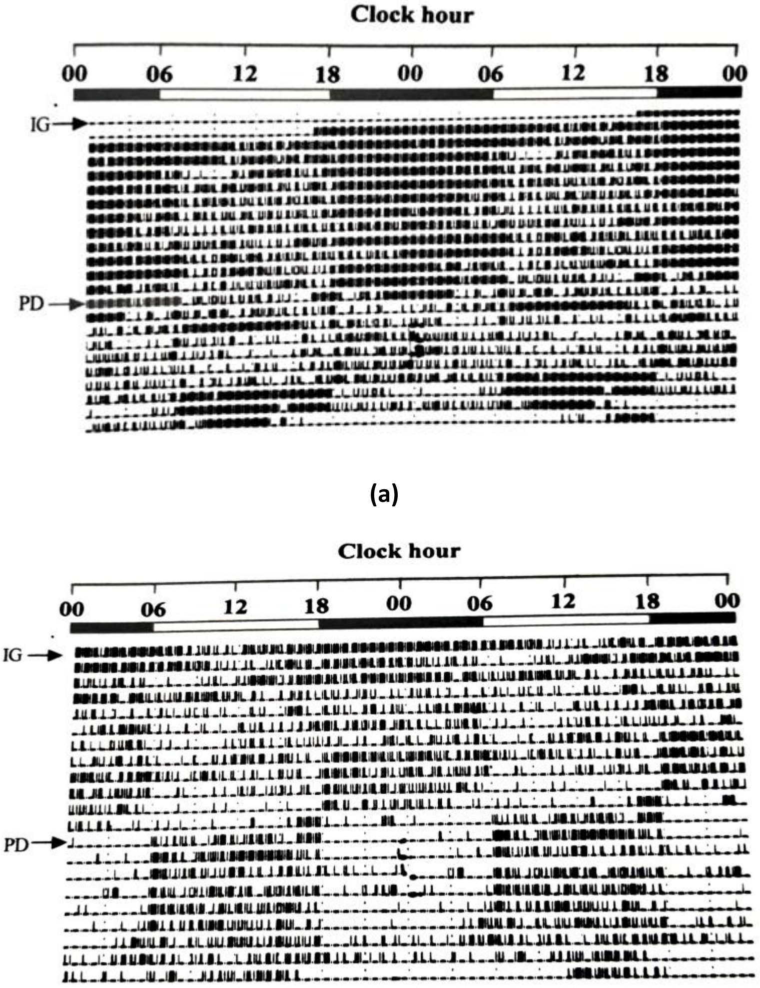
	F#05	<0.001	4.98 ± 0.13	0.96 ± 1.47	15.94 (19.14, 12.75)
	F#06	0.0001	35.98 ± 1.67	3.98 ± 2.29	18.34
	F#07	0.1745	175.54 ± 2.85	59.41 ± 0.65	19.28
PDEMG	F#01	0.03	57.06 ± 2.67	9.88 ± 2.39	16.76 (21.57, 12.05)
	F#02	0.1018	79.25 ± 4.38	13.39	14.04
	F#03	0.003	60.71 ± 1.79	8.43 ± 1.15	10.79 (13.93,7.59)
	F#04	0.1422	96.03 ± 5.66	15.60	10.47
	F#05	<0.001	20.27 ± 1.30	19.08 ± 4.55	16.11 (17.02, 15.2)
	F#06	<0.001	23.36 ± 1.56	16.01 ± 5.32	17.72 (19.07, 16.37)
	F#07	0.001	14.62 ± 0.52	9.29 ± 1.85	12.88

[a] From F test of null amplitude rejection hypothesis.
[b] Rhythm-adjusted mean of best-fitting cosine function ± 1 standard error.
[c] Half of the difference between maximum and minimum of best- fitting cosine function ± 1standard error.
[d] Timing of maximum in best-fitting cosine function with 95% confidence limit.
IG = Intact
PD = Phase dealy
PDEG = Phase dealy + Enucleated
PDEMG = Phase dealy + Enucleated + Melatonin treated

Table2 Results of cosinor analysis of air-breathing activity in *Clarias batrachus* at τ =12h

Group	**Animal Code**	**P[a]**	**Mesor ± SE[b]**	**Amplitude ± SE[c]**	**Acrophase[d] (95% CL)**
IG	F#01	0.246	0.26 ± 0.01	0.05	5.16
	F#02	0.419	1.30 ± 0.09	0.17	11.11
	F#03	0.07	121.90 ± 1.95	28.74	1.32
	F#04	0.2166	1.06 ± 0.03	8.16	23.96
	F#05	0.0001	25.96 ± 1.74	10.72 ± 2.15	21.33 (23.63, 19.05)
	F#06	0.1647	2.05 ± 0.45	1.20 ± 1.02	18.91 (18.05, 19.77)
	F#07	<0.001	0.78 ± 0.05	1.02 ± 0.07	0.31 (1.25, 23.33)
PD	F#01	0.5147	75.47 ± 1.35	8.73	8.11
	F#02	0.01	1.009 ± 0.0045	0.018 ± 0.01	5.61 (7.62, 3.60)
	F#03	0.5244	46 ± 2.07	3.33	0.84
	F#04	0.3027	9.17 ± 0.64	1.41	8.33
	F#05	0.813	22.03 ± 2.19	0.85	1.92
	F#06	<0.001	8.56	2.14 ± 1.74	19.73 (21.59, 17.87)
	F#07	0.4135	27.58 ± 1.35	4.58	4.58
PDEG	F#01	0.7699	142.60 ± 2.07	6.21	5.07
	F#02	0.3589	1.004 ± 0.004	0.008	5.77
	F#03	0.06	54.05 ± 1.39	4.67	0.68
	F#04	0.3077	302.72 ± 7.43	16.11	2.82
	F#05	0.3181	4.89 ± 0.13	0.29	4.84
	F#06	0.6575	35.83 ± 1.51	1.03	8.07
	F#07	0.002	175.2 ± 1.95	58.75	17.36
PDEMG	F#01	0.4187	57.07 ± 2.68	4.98	6.96
	F#02	0.2721	79.19 ± 4.39	10.06	4.60
	F#03	0.4879	60.28 ± 1.80	3.06	9.32
	F#04	0.125	95.72 ± 5.65	16.27	2.19
	F#05	0.0001	20.27 ± 1.30	18.25	5.51
	F#06	0.7581	24.28 ± 1.45	16.75	21.65
	F#07	0.001	14.79 ± 0.71	11.5 ± 0.02	7.85

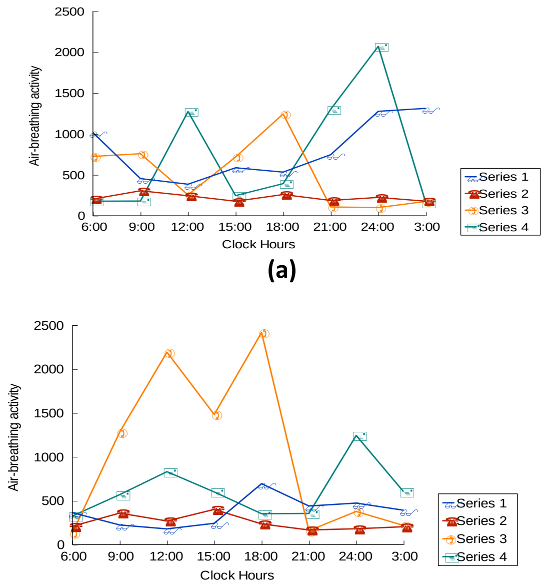
Please see legend to Table 1



(a)

(b)

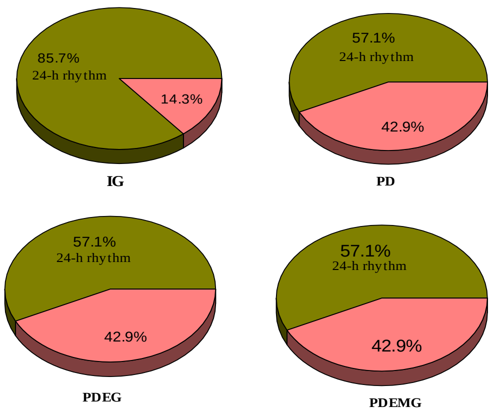
Figure 1a &b Actogram record in double plot (48hrs) showing air-breathing activity of *Clarias batrachu;* IG= Intact Group, PD=Phase delay



(a)

(b)

Figure 2 (a) and (b) Activity curve graph based on numerical data
Series1 = Intact
Series 2 = Phase delay
Series 3 = Phase delay + Enucleation
Series 4 = Phase delay + Enucleation + Melatonin treated



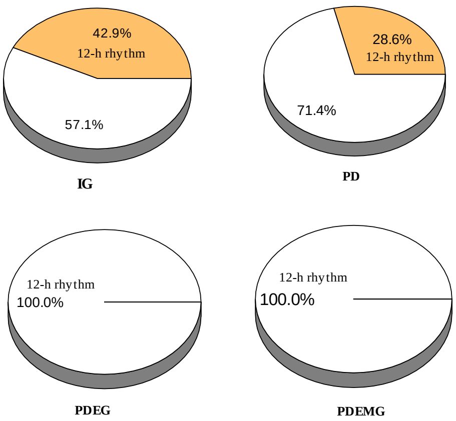
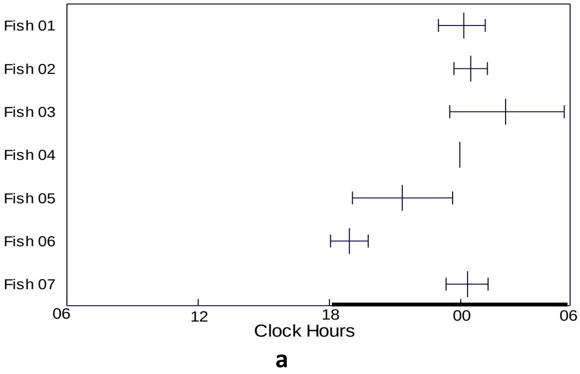
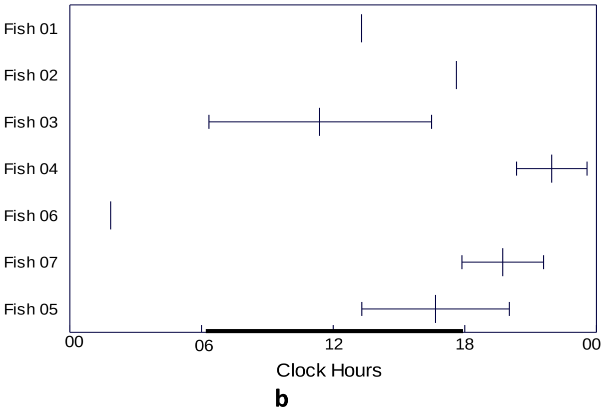


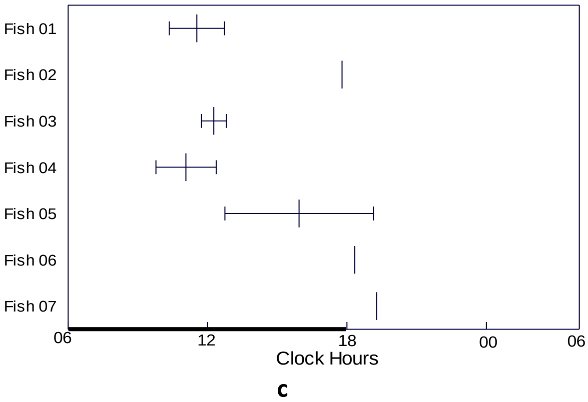
Figure 3 Occurrence of 24-h and12-h rhythm in air-breathing activity in

Intact (IG), Phase delay (PD), Phase delay + Enucleated (PDEG),

Phase delay + Enucleated + Melatonin Treated (PDEMG) *Clarias batrachus* (Results based on cosinor analysis)







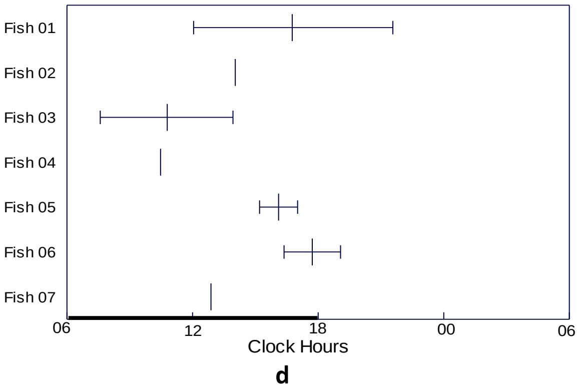


Figure 4 Peak map for air-breathing activity of *Clarias batrachus* (a) Intact (b) Phase delay (c) Phase delay +Eucleated

(d) Phase delay + Enucleated + Melatonin treated

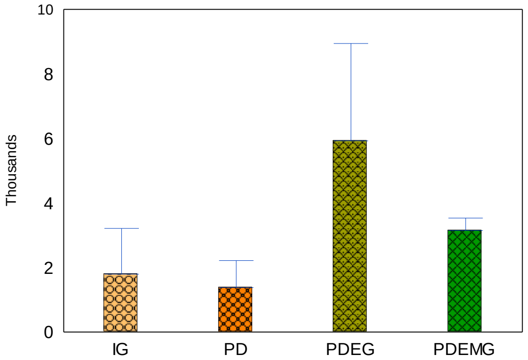


Figure 5 Daily mean of air-breathing activity in *Clarias batrachus*

Please see legend to Fig.3

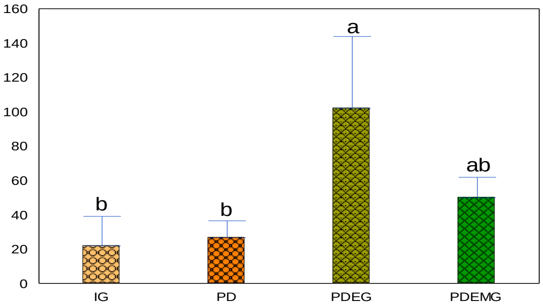


Figure 6 Mesors (rhythm-adjusted mean) of air-breathing activity rhythm in the *Clarias batrachus.* Please see legend to Fig. 3

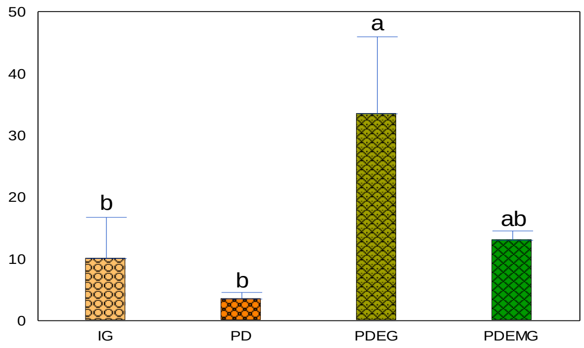


Figure 7 Amplitude of the 24-h air-breathing activity in the *Clarias batrachus.* Please see legend to Fig. 3

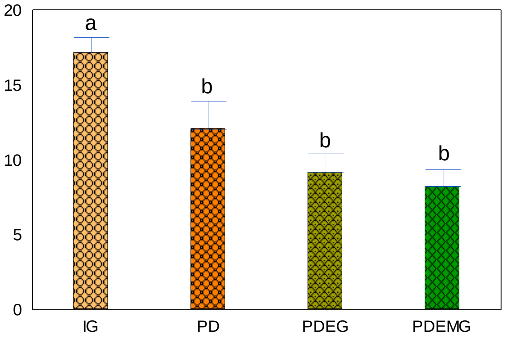


Figure 8 Mean of Phase angle of the air-breathing activity with reference to lights on in the *Clarias batrachus*. Bars having dissimilar alphabets differ with statistical validation. Please see legend to Fig.

Table 3 Results of power spectrum analysis showing period (τ) of air-breathing activity in *Clarias batrachus*

Animal Code	**IG**	**PD**	**PDEG**	**PDEMG**
F#01	24	20.3	24	17.6
F#02	24	12	24	16.5
F#03	24	18.85	12	9.42
F#04	26	16.5	17.45 (24)*	29.33
F#05	24	24	12.8	17.5
F#06	24	12.57	27.42	26.4
F#07	24	19.2	19.2	18.85

* Second peak
Please see legend to Table1

Table 4 Results of phase angle of peak of air-breathing activity with reference to lights on in *Clarias batrachus*

Animal Code	IG	PD	PDEG	PDEMG
F#01	18.14	7.30	5.54	11.76
F#02	18.46	11.62	11.79	8.04
F#03	20.05	5.38	6.27	4.79
F#04	17.96	15.96	5.07	4.47
F#05	15.33	19.86	9.94	10.11
F#06	11.91	13.73	12.34	11.72
F#07	18.31	10.67	13.28	6.88

Please see legend to Table 1

Discussion

Diurnal periodicity in activity characterized by maximum surfacing frequency in the dark phase and minimum in the light phase of LD photo regime has been reported by several workers (Pandey, 1987; Devi, 1987; Singh, 1992; Srivastava, 1992; Srivastava et al., 1993; Gupta, 1998; Maheshwari, 1998; Yadu & Shedpure, 2002a, b; Shedpure & Yadu, 2002; Tikariha & Shedpure, 2002) in *Clarias batrachus.*

In general, it has been documented that the endogenous self-sustained oscillations among organisms have been found to be entrained to the exogenous oscillation (Zeitgeber) with a specific phase angle difference (Weidmann, 1970, Schwassmann, 1971). Further, phase differences depends on zeitgebers as the period of entraining cycle, ratio of light-dark hour, relative intensities of light-dark phases and the rate of transition between light-dark levels (Schwassmann, 1971). In addition, if zeitgeber has a period outside the range of entrainment, rhythms may free run and do not coupled or synchronized with the zeitgeber (Schwassmann, 1971). Organisms usually take several period to become re-entrained in a sudden shift of the zeitgeber's phase and resynchronization does not occur immediately in most species (Singaravel et al., 1996).

A phase shift in environmental light-dark cycle results after a transient lag period in a corresponding phase shift of their circadian rhythm in various vertebrate species (Aschoff, 1969; Pittendrigh, 1981; Moore-Ede et al., 1982). The results of present study showed the existence of synchronized air-breathing activity rhythm in *Clarias batrachus* with the timings of lights on/off under LD 12:12. However, in contrast to reports available on other vertebrates (Singaravel et al., 1996; Aschoff, 1969; Pittendrigh, 1981; Moore-Ede et al., 1982) present findings depicted that after 12hrs phase delay in light-dark cycle the air-breathing activity rhythm in *Clarias batrachus* was quickly re-entrained with the new timings of lights on/off and the activity was found to be increased and decreased during the experimental dark and light hours of the LD regime, respectively. The peaks of the activity were located during the experimental dark hour in most of the individuals. The cosinor rhythmometry revealed a statistically significant 24-h rhythm in air-breathing activity in most of the studied individuals under LD 12:12 and in 4 out of 7 individuals under phase delay by 12hrs. Further, the period of the air-breathing activity was prominent 24-h in almost all the individuals under LD 12:12 but not equal to 24-h in most of the individuals under phase delay. These results also corroborate with our earlier findings in which we have noticed quick re-entrainment of air-gulping activity rhythm in *Clarias batrachus* in 3 successive phase delay by 4h of light-dark cycle (Yadu and Shedpure, 2002 b) and 12-h phase delay of LD cycle (Tikariha, 2005). Similarly, circadian rhythm of swimming activity in juvenile pink salmon, *Oncorhynchus gorbuscha* has been shown to re-entrain after only one transient cycle, subjected to phase delayed LD cycle (Beugnon, 1982).

The dual role of the endogenous melatonin in the circadian timing system has been suggested by Armstrong (1989). First as an internal zeitgeber, melatonin may act to maintain coupling between individual oscillators and appropriate phase relationship between the various body rhythms. Second, it may modulate the photic sensitivity of the SCN circadian pacemaker, facilitating or inhibiting its responsiveness to changes in light intensity. Further, endogenous circadian oscillator that synchronises rhythmic cycles of rhythmic activities with the photoperiodic cycle in various vertebrates including fishes is known to be located in the pineal organ, the hypothalamus (suprachiasmatic nucleus i.e.,SCN) and the eyes (Menaker, 1985; Chiba *et al.*, 1993; Janik *et al.*, 1990; Ebihara *et al.,* 1984; Reiter, 1993; Shedpure, 2000). In addition, in reptiles and birds, blinding (bilateral ocular enucleation) causes various effects on circadian activity rhythms which are similar to those caused by pinealectomy (Underwood and Menaker, 1976; Underwood, 1981; Ebihara *et al*., 1984; Underwood and Siopes 1984; Foa, 1991). However, data from catfish suggested that the eyes and the pineal contribute to synchronize the behavioural rhythms by a light:dark cycle. In addition, other photoreceptors and oscillators also contribute (Tabata *et al*., 1988) for the same. Further, Bayarri and coworkers (2003) reported that even though sea bass eyes do not directly contribute to plasma melatonin, the pineal organ, which unlike that of mammals is a direct photoreceptor in fish, requires light information from the lateral eyes to normally secrete melatonin into the bloodstream (Bayarri *et al*., 2003_) In addition to the effects of light and dark adaptation, many cellular phenomena in the vertebrate retina are now known to be regulated by a circadian clock (Reme *et al.* 1991; Cahill & Besharse, 1995), a type of biological oscillator that is intrinsic to neural tissue and that has persistent rhythmicity with a period of approximately 24 h in the absence of external timing cues (e.g. constant darkness) (Block *et al.* 1993).

After enucleation under phase delay by 12hrs, the activity curve graph showed that the air-breathing activity rhythm is similar as obtained in intact individuals under phase delay. The activity increased during the experimental dark hour in most of the individuals. Results of cosinor analysis depicted that still 57.1% (4 out of 7 individuals) individuals have retained a statistically significant 24-h rhythm in their air-breathing activity and in most of the individuals the peaks of the activity were located during the experimental dark hour. The results of the spectral analysis revealed that in 5 out of 7 enucleated individuals maintained under phase delay, the period (τ) of the activity was not equal to 24-h. These results corroborate with our findings of earlier chapters which show desynchronized rhythm of air-breathing activity with τ ≠ 24-h after enucleation. Further, Gothilf and his coworkers (1999) have reported that melatonin is synthesised in retinal photoreceptor cells, where melatonin is thought to play a paracrine role in adaptation to light and darkness. The daily mean of the air-breathing activity was highest as compared to other studied groups, however, statistical validation could not be obtained. In addition, rhythm adjusted mean i.e., mesor and amplitude of the activity were also increased with statistical validation after enucleation under phase delay by 12hrs as compared with the intact individuals under LD 12:12 and phase delay. However, phase angle of peak with reference to lights on was significantly decreased as compared to the individuals under LD 12:12.

Studies in various mammalian species have led to the hypothesis that an endogenous circadian mechanism controls pineal melatonin content and can adapt the melatonin rhythm to reflect shifts in the prevailing light-dark schedule (Yellon et al., 1982). Earlier studies on the circadian locomotor activity rhythm in the field mouse *Mus booduga* (Sharma *et al.,* 1999) have demonstrated that a single dose of melatonin shifts the onset of activity in a phase-dependent manner. Golombek and his colleagues (1998) have reported that golden hamster took 6-9 days to re-entrain after a 6 hour phase advance in the photoperiod but the treatment of melatonin accelerated the rate of re-entrainment.

Present study revealed that after melatonin administration in enucleated individuals under phase delay, the activity curve graph showed occurrence of the two peaks in air-breathing activity rhythm at 12:00 hrs and 24:00 hrs in most of the individuals. Results of cosinor analysis revealed a statistically significant 24-h rhythm in air-breathing activity in 57.1% (4 out of 7 individuals). However, period of the activity i.e., τ was not equal to 24-h in almost all the studied individuals. Further, peaks of the activity of 24-h rhythm in all the individuals occurred during the experimental dark hour of the LD regime. The daily mean was higher than the intact individuals under LD12:12 after melatonin treatment. These finding corroborate with our earlier finding (Tikariha, 2005) that daily light and dark hour means of the activity were increased after melatonin treatment under phase delay. Results from Duncan's multiple range test documented that mesor and amplitude of the air-breathing activity were increased but phaseangle of acrophase decreased after melatonin treatment.

It has been unequivocally accepted that the circadian systems can be entrained both by photic as well as nonphotic time cues. In general, the endogenous self-sustained oscillations among organisms have been found to be entrained to the exogenous oscillation (zeitgeber) with a specific phase angle difference (Weidmann, 1970; Schwassmann, 1971).

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