Proc. Indian natn. Sci. Acad. **B57** No. 5 pp 339-346 (1991)

Annual Modulation of Diel Motor Activity Rhythm of the Dusk Active Loach *Nemacheilus evezardi* (Day): A Correlation between Day Length and Circadian Parameters

J BISWAS

Department of Bioscience, Ravishankar University, Raipur 492 010 (*M.P.*)

(Received on 21 March 1991; after revision 27 May 1991; Accepted on 14 June 1991)

An investigation was made into the rhythmic behaviour in motor activity of a dusk active loach, *Nemacheilus evezardi* over the diurnal as well as annual time scale. Analysis of data clearly reveals circannual periodicity in circadian mesors and amplitudes. A negative correlation was obtained between circadian amplitude and day length indicating that the rhythm becomes flattened during summer. That there is a concomitant decrease in the level of activity is evidenced by a (significant) negative correlation between mesor and day length.

Key Words: Circadian rhythm, Circannual rhythm, Locomotor activity, Hill stream loach

Introduction

It has been unequivocally accepted that the occurrence of rhythms in motor activity is one of the basic properties of living organisms. Besides the behaviours of flying and terrestrial creatures many reports on fishes strengthen this statement (Neave 1964, Royce et al. 1968, Muller 1969, Eriksson 1972, 1973, Favorite et al. 1976, Kavaliers 1978, Thorpe 1978, Godin 1981, Helfman 1981, 1986, Biswas 1990, Biswas et al. 1990 a,b.). The day/night, as well as the seasonal variations in the environment act as synchronizers for the rhythmic behaviour of the living organism, with respect to its diurnal or nocturnal activity (Halberg 1960, Bunning 1973, Mayersbach 1976, Palmer 1976, Pittendrigh & Daan 1976, Scheving 1976, Thorpe 1978, Aschoff 1981, Pati et al. 1987, Marimuthu 1984, Kanoje, et al. 1989). The rhythms having different periodicities free-run with different periodicities in the absence of the synchronizers. In nature, such conditions are of rare occurrence. In fishes, reports as to this are very scarce (Muller 1969, Erikson 1972).

Most of the available reports regarding the motor activity of fishes are based on their migratory behaviour. Essentially the rhythms described in these studies represent group rhythms where the social interaction might play an important role in the process of rhythm synchronization. Nevertheless, in fishes intra- and inter-individual variations in the diel or circadian rhythm of activity have been reported (Eriksson 1978, Muller 1978). Reports are also available which suggest, that individuals of fishes with schooling of shoaling tendency show more perfect circadian activity rhythms that persist longer and are less variable under constant conditions than those of solitary-living fish (Muller 1978, Kavaliers 1980 a,b).

For the present study a hill stream loach *Nemacheilus evezardi* (Day) have been chosen as an experimental model. It is basically a bottom dweller that shows schooling behaviour. Occasionally the fish undertakes up and down or vertical movements. Our previous report shows, that these fishes use to conceal themselves under stones for most of the time of day and emerge at evening hours only. This suggests that this loach is a dusk active creature (Biswas et al. 1990a). It is an annual breeder, its fingerlings are noticed during each of the post-spring months (Biswas 1990). Here, attempts have been made to understand the characteristics of the group motor activity rhythm exhibited by this loach, along a circannual time scale. It is also tried to correlate the circadian parameters with day length to known that being an aquatic creature, whether this particular loach has any adjustment with the ever changing day and night cycle or not.

Materials and Methods

Maintenance of fish: Nemacheilus evezardi were collected from tributaries of the Kanger Riverine system, (18°52′40″ N 81°55′30″ E) and transported to our place, Raipur (21°14′ N and 81°38′ E). The stock aquaria containing the fishes were kept in the animal quarters of our department, exposed to natural day length and to the ambient temperature at Raipur, which remains at 28°C ± 1°C throughout the year. The water was renewed three or four times per month, but at irregular moments. This was strictly followed in order to eliminate the chance of entrainment of the circadian rhythm by a fixed timing of water renewal. The normal tap water was used for the above purpose and the water quality remained essentially the same. Fishes were fed *ad libitum* with pieces of chopped goat and/or chicken liver.

Experimental protocol: On 3rd week of every month, six healthy and mature fishes, approximately of the same body size and weight (i.e. 3.5 ± 0.25 cm; 4.00 ± 0.50 mg respectively) were selected randomly from the stock aquaria and were let loose by twos in the three experimental aquaria (base area of 250 cm each). The water was filled in each aquarium up to a height of 10 cm. Further conditions as to temperature, place, feeding and water renewal schedules were the same as above.

Procedures of Recording Swimming Activity

The investigated loach uses to swim parallel to the base, i.e. it is a bottom dweller. To record the horizontal swimming activity the base of the aquarium was divided into 40 squares of 6.25 cm^2 each. Individual fishes were observed for 10 min at each time point, and the distance swum by each was calculated and expressed as the distance travelled in meters per hour.

Further, the fishes occasionally exhibit a vertical swimming bout also. This vertical swimming activity was recorded as the number of upward swimming bouts per hour. Since height of the water level inside each aquarium was known, it was also possible to calculate the vertical distance swum by each fish, by multiplying the mean number of up and down movements/hr with twice the depth of the jar. In this way by adding horizontal and vertical movement, the total swimming activity of these fishes have been calculated. Zig-zag movements are almost nil.

After completion a period of accilimation (one week) in the experimental jar, the swimming activity was recorded for each fish. To observe the swimming activity during the night period, very dim indirect red light (from beneath the experimental jar) were used to which the loaches seemed to be insensitive. The study was continued over a period of 12 consecutive months. Each month the swimming activity was recorded over a span of 48 hr at 4-hr intervals beginning at 09.00 hr of the first day.

Statistics: A square root transformation was performed on each datum (datum $= \sqrt{\text{datum} + 1}$) especially because at certain time points the swimming activity was found to be zero. Then the means and standard errors were computed for the data obtained during different months. Thus, 12 time series were obtained for each month. Employing a least-squares method, the best fitting 24 hr cosine curve was computed for each time series (Nelson et al. 1979). The acrophase (time of peak value) of this cosine was used for establishing the phase-angle of the 24 hr rhythm of the swimming activity with reference to local mid-night. The acrophases were conventionally expressed in negative degrees, where midnight = 0° and each hour after midnight was −15°. The mesor (rhythm adjusted mean) and amplitude (one half of the peak to trough difference) of the fitted cosine were also obtained. These computations provided a quantitative description of the circadian rhythm in swimming activity for a group of six fishes.

Cosine curves were fitted to the data on circadian mesors, amplitudes and acrophase yielding circannual mesor, amplitude and acrophase. In this case December 22 was taken as the circannual phase reference, where midnight of December 22 = 0° and each month thereafter was approximately −30°.

Further, linear correlations were performed between circadian mesor or amplitude values obtained during different months and day length.

Results

Results are presented in tables 1-3 and figures 1, 2. Excluding April, a statistically significant circadian rhythm is detected every month (table 2). The circadian mesor recorded throughout the year is found to vary in a periodic manner. A statistically significant circannual rhythm in circadian mesors of this fish is detected ($P < 0.001$). In addition, a statistically significant circannual periodicity in circadian amplitudes is also recorded ($P < 0.003$). Furthermore, both circadian mesor and amplitude values of this fish exhibited negative correlation with the length of day (circadian mesor V_s day length : $r = -0.61$, $P < 0.05$; circadian amplitude V_s day length : $r = -0.74$, $P < 0.01$). This indicate that, in general during the winter months, when duration of night length is longer, both the levels and the extent of rhythmicity in motor activity of this fish are greater (figure 1, 2).

The peak values, i.e. acrophase timings in motor activity were observed in the evening hours, i.e. in between −257° to −328° (i.e. from 15.8 hr to 21.52

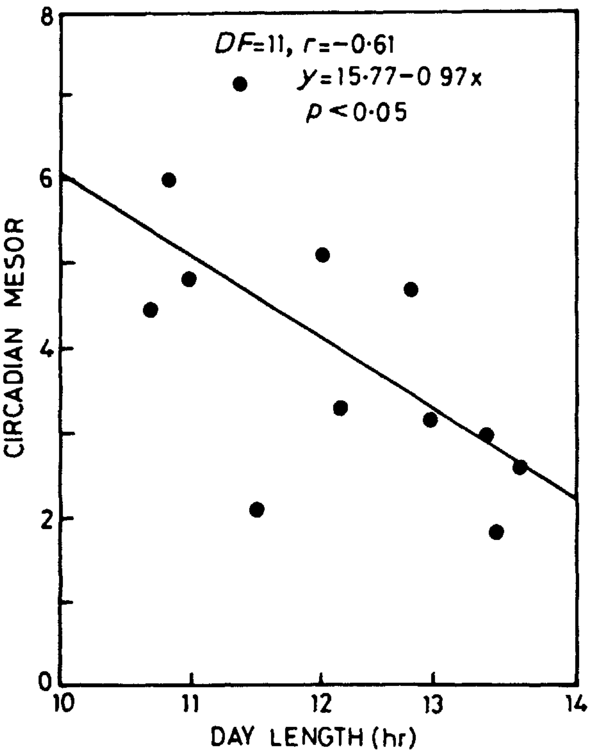

Figure 1 Linear regression of circadian mesor in relation to the day length

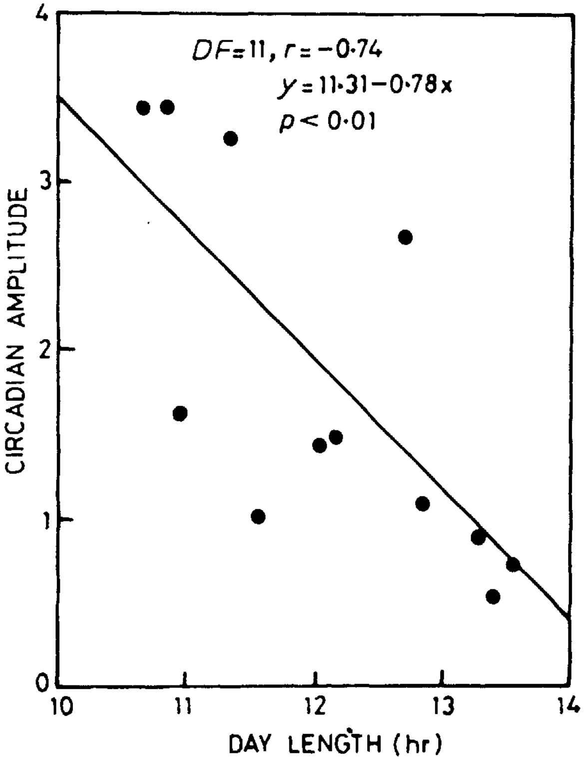

Figure 2 Linear regression of circadian amplitude in relation to the day length

Table 1 *Annual record of circadian rhythm in motor activity of fish* ***Nemacheilus evezardi*** *(each value represents Mean ±1 S E of six individuals)*

Month	Day	Time Clock 09.30	13.30	17.30	21.30	01.30	05.30
June	D_1	1.82±0.44	2.10±0.36	2.0±0.32	2.64±0.81	2.61±0.76	3.08±0.52
	D_2	1.7 ±0.42	2.0±0.32	5.0±0.93	3.32±0.71	1.50±0.27	2.0±0.45
July	D_1	1.70±0.24	4.76±1.48	5.24±1.02	4.68±0.81	3.32±0.54	2.95±0.76
	D_2	1.89±0.37	1.74±0.34	2.64±0.48	2.02±0.37	1.23±0.14	2.45±0.65
Aug.	D_1	3.33±1.2	1.9±0.39	9.82±1.86	7.17±1.15	4.34±0.56	4.05±0.85
	D_2	3.25±1.4	2.14±0.81	7.25±2.94	6.21±1.19	4.28±1.40	1.91±5.00
Sep.	D_1	6.17±0.47	2.98±0.47	6.83±1.12	6.26±0.79	2.96±0.65	3.77±0.51
	D_2	5.08±0.89	2.40±0.48	7.44±2.11	8.28±2.31	4.62±1.49	3.99±1.23
Oct.	D_1	1.83±0.40	5.85±2.30	9.76±1.67	12.55±0.32	4.78±0.32	5.04±1.01
	D_2	3.81±1.04	8.95±2.30	8.54±1.51	10.06±2.30	8.27±2.42	8.06±1.97
Nov.	D_1	3.95±1.07	9.52±2.06	11.17±1.96	6.55±2.03	4.41±1.33	3.16±0.95
	D_2	2.96±0.74	6.68±2.05	9.06±1.98	6.66±1.45	3.34±1.30	3.99±0.88
Dec.	D_1	1.35±0.25	8.13±2.53	6.75±1.45	5.38±0.57	3.91±1.37	2.03±0.22
	D_2	1.4 ±0.40	5.76±2.15	8.11±2.81	7.07±1.36	2.36±0.81	1±0
Jan.	D_1	1.79±0.32	11.44±3.25	4.12±0.88	6.22±0.96	5.66±0.88	4.41±0.89
	D_2	3.63±0.96	4.12±1.24	6.25±0.98	4.02±1.08	4.18±0.51	1.68±0.44
Feb.	D_1	1±0	1.13±0.13	2.36±0.48	3.68±1.01	2.52±1.27	1.10±0.01
	D_2	2.60±1.09	1.20±0.20	2.62±0.97	2.87±0.70	3.22±0.92	1±0
Mar.	D_1	2.75±1.15	6.12±2.77	6.06±1.83	4.65±0.82	3.78±1.56	1.59±0.46
	D_2	3.28±1.53	3.67±1.36	2.52±0.66	2.10±0.55	1.28±0.28	1.56±0.31
Apr.	D_1	1.17±0.17	2.44±0.40	2.08±0.18	4.89±1.49	4.83±1.97	3.45±1.31
	D_2	1.67±0.34	3.48±1.40	4.84±2.09	3.79±0.92	2.03±0.50	3.28±0.67
May	D_1	1.32±0.23	1.53±0.43	2.25±0.19	1.46±0.22	1±0	1±0
	D_2	1.52±0.34	1.79±0.30	2.47±0.42	3.22±0.60	1.82±0.40	1.97±0.36

hr), a statistically significant circannual rhythm in circadian acrophase values is not detectable (table 3).

Discussion

This is the first report that documents circadian rhythm in motor activity of a tropical loach *Nemacheilus evezardi*. Excluding the month of April, a significant group rhythm is detected every month. However, the absence of circadian rhythm during summer is not a curious factor for fishes, as the apparent free-running activity rhythms in the brown trout *Salmo tratta* (Muller 1969) and the brook charr *Salvelinus fontanalis* (Eriksson 1972) have already reported for arctic fishes, earlier. Could the absence of significant circadian rhythm reflect the same? The low amplitude value for April and its adjacent months might support this.

The record of a complete year shows a compact acrophase spread. The maximum activity of this fishes is observed during evening hours, i.e., at the early part of the dark phase (table 2). This is in accordance with our previous finding (Biswas et al. 1990b), about the dusk activeness of this loach. Several other fishes an also been known to be crepuscular (see review : Helfman 1986). It has been well documented that a light level of 10-100 lux have a great influence in the initiation or

Table 2 *Rhythmometric summary* of circadian variations in total swimming activity of fish, over a period of one year*

Month	Pr[a]	P[b]	Mesor[c] ± SE[d]	Amplitude[e] ± SE[d]	Acrophase[f] ± SE[d]
June	11	0.018	2.54 ± 0.18	0.74 ± 0.25	−291 ± 20
July	9	0.034	2.91 ± 0.23	0.86 ± 0.32	−275 ± 21
August	25	0.001	4.65 ± 0.38	2.61 ± 0.54	−301 ± 12
September	9	0.033	5.07 ± 0.38	1.42 ± 0.53	−297 ± 21
October	24	0.001	7.29 ± 0.50	3.27 ± 0.70	−305 ± 12
November	30	0.001	5.95 ± 0.44	3.42 ± 0.62	−257 ± 10
December	33	0.001	4.44 ± 0.41	3.43 ± 0.59	−268 ± 10
January	9	0.036	4.79 ± 0.43	1.60 ± 0.6	−258 ± 22
February	18	0.004	2.11 ± 0.21	1.02 ± 0.3	−327 ± 17
March	11	0.029	3.28 ± 0.38	1.48 ± 0.54	−239 ± 21
April	7	0.082	3.16 ± 0.33	1.07 ± 0.47	−328 ± 25
May	15	0.004	1.78 ± 0.11	0.52 ± 0.15	−284 ± 17

*Cosinor rhythmometry has been performed on square root-transformed; data $y = \sqrt{y+1}$
[a]Percent of total variability contributed by the fitted curve; [b]From an F test of null amplitude rejection hypothesis; [c]Rhythm adjusted mean of cosine function (Mesor) [d]standard error; [e]Half of the difference between maxima and minima of cosine function (Amplitude); [f]Time of maximum in fitted cosine function, with local mid-night as reference (360° = 24 hr) (Acrophase).

Table 3 *Rhythmometric summary on circannual variation* (360° = 365.25*d*) *in circadian rhythm characters in motor activity*

	Parameters	Number of observation	PR[a]	p[b]	V[c] ± SE[d]	A[e] ± SE[d]	ϕ[f] ± SE[d]
Fish	Mesor	12	77	0.001	4.02 ± 0.25	1.98 ± 0.36	−310 ± 10
	Amplitude	12	72	0.003	1.80 ± 0.19	1.26 ± 0.26	−323 ± 12
	Acrophase	12	67	0.707	228.7 ± 8.52	10.27 ± 12.11	−187 ± 67

For explanations, see legend for Table 2
Midnight of December 22 of previous year has been taken as acrophase reference.

cessation of swimming activity in many diurnal or nocturnal fishes (Hobson 1972, Helfman 1981, Helfman et al. 1982, Howick & O'Brien 1983). This intensity range commonly occurs about 10 min. after dusk and before dawn. This behaviour of fishes may be related to avoidance of predatory pressure, and intense light and heat. The twilight behaviour may be directly related to and limited by the speed of photomechanical movements involved during dark adaptation of fish eye (Munz & McFarland 1973, McFarland et al. 1979).

Significant circannual rhythms in circadian mesors and amplitudes are documented for this fish, apparently with a common peak during the month of November (table 3). Furthermore, a negative correlation is obtained between circadian amplitudes and day length which clearly indicates that the rhythms is flattened during the summer months. The high amplitude values during the winter season suggest that the sudden disappearance of the sun from the horizon induce the sudden increasement in the motor activity with a concomittant increase in the level of activity. The latter is supported by a significant negative correlation between circadian mesors and day length for the present fish. Here the observed amplitude and mesor values of motor activity could not be suggested as temperature oriented because the temperature is found to fluctuate with in a range of 2.5°C only through out the year. However, the limited motor activity during the summer could also be the reflexion of a post-spawning effect in this loach, i.e. to compensate the exhaustion produced by high energy demand processes, like spermatogenesis/oogenesis/oviposition etc. Finally it could be concluded that, although the annual records of acrophase (peak) timings of motor activity failed to show any definite fashion, but, the intensity as well as duration of motor activity of this loach were seen to be tightly synchronized with the ever changing natural day length conditions.

Acknowledgement

The author gratefully acknowledges the help extended to him by the Director and Staff members of the Forest Department (Kanger Valley National Park), Jagdalpur, during the course of this work.

References

Aschoff J 1981 *Handbook of Behavioural Neurobiology*, Vol 4 *Biological Rhythms. The Language of Science* (ed. F A King). (New York, London: Plenum Publishing Corporation)

Biswas J 1990 *Biospeleology: the Behavioural and Physiologic Adaptations in a Cavernicole*, Ph.D. Thesis, Ravishankar University, Raipur, India

— —, Pradhan R K and Pati A K 1990a Studies on burying behaviour in epigean and hypogean fish : an example of behavioural divergence; *Mem. Biospeleol.* **17** 33-41

— —, Pati A K and Pradhan R K 1990b Circadian and circannual rhythms in air gulping behaviour of cave fish; *J. Interdisc. Cycle Res.* **21** 257-268

Bunning E 1973 *The Physiological Clock* (New York: Springer-Verlag)

Eriksson L O 1972 Die Jahresperiodik augen-und pinealorganloser Bachsaiblinge *Salvelinus fontinalis* Mitchill; *Aquilo. Ser. Zool.* **13** 8-12

— — 1973 Spring inversion of the diel rhythm of locomotor activity in young sea-going brown trout, *Salmo trutta trutta* L., and Atlantic Salmon, Salmo Salar L., *Aquilo. Ser. Zool.*, **14** 68-79

— — 1978 Nocturnalism versus diurnalism - dualism within fish individuals; in *Rhythmic Activity of Fishes*, pp. 69-89 ed. J E Thorpe (New York: Academic Press)

Favourite F, Dodimead A J and Nasu L 1976 Oceanography of the subarctic pacific region 1960-71; *Int. North Pac. Fish. Comm. Bull.* **33** 1-187

Godin J G J 1981 Circadian rhythm of swimming activity in juvenile pink Salmon (*Onchorhychus gorbuscha*); *Mar. Biol.* **64**, 341-349

Halberg F 1960 Temporal coordination of physiologic function; *Cold Spr. Hard. Symp. Quant. Biol.* **25** 289-310

Helfman G S 1981 Twilight activities and temporal structure in a freshwater fish community; *Gan. J. Fish. Aquat. Sci.* **38** 1405-1419

— — 1986 Diel distribution and activity of American ells (*Anguilla rostrata*) in a cave-spring; *Can. J. Aquat. Sci.*, **43** 1595-1605

— —, Meyer J L and McFarland W N 1982 Ontogeny of twilight migration patterns in grunts (Pisces : Haemulidae). *Anim. Behav.* **30** 317-326

Hobson E S 1972 Activity of Hawaian reef fishes during evening and morning transitions between daylight and darkness; *Fish. Bull. U.S.* **70** 715-740

Howick G L and O'Brien W J 1983 Piscivorous feeding behaviour of large mouth bass : an experimental analysis; *Trans. Am. Fish. Soc.*, **112** 508-516

Kanoje R S, Pati A K and Biswas J 1989 Sunset related timings of flight activity rhythm of tropical bats in Kanger Valley National Park; *76th Indian Sci Congre*, Madurai, p. 160

Kavaliers M 1978 Seasonal changes in the circadian period of the lake chub *Couesius plumbeus*; *Can. J. Zool.* **56** 2591-2596

— — 1980a Social groupings and circadian activity of the killifish, *Fundulus heteroclitus*; *Biol. Bull. Mar. biol. Lab., Woods Hole.* **158** 69-76

— — 1980b Circadian locomotor activity rhythms of the burbot, *Lota lota*; seasonal differences in period length and effect of pinealectomy; *J. Comp. Physiol.* **136** 215-218

Marimuthu G 1984 Seasonal changes in the precision of the circadian clock of a tropical bat under natural photoperiod; *Oecologia (Berlin)* **61** 352-357

Mayersbach H V 1976 Time - A key in experimental and practical medicine; *Arch. Toxicol.* **36** 185-216

McFarland W N, Ogden J C and Lythgoe J N 1979 The influence of light on the twilight migrations of grunts; *Environm. Biol. Fish.* **4** 9-22

Muller K 1969 Die Tages-und Jahresperiodik der Bachforelle (*Salmo tratta* L.) am Polarkreis; *Aquilo. Ser. Zool.* **8** 50-62

— — 1978 Locomotor activity of whitefish-shoals (*Coregonus lavaretus*); in *Rhythmic Activity Fishes* pp. 225-233 ed J E Thorpe (New York: Academic Press)

Munz F W and McFarland W N 1973 The significance of spectral position in the rhodopsins of tropical marine fishes; *Via. Res.* **13** 1829-1874

Neave F 1964 Ocean migration of pacific salmon; *J. Fish. Res. Bd. Can.* **21** 1227-1244

Nelson W, Tong Y L, Lee J K and Halberg F 1979 Methods for cosinor-rhythmometry; *Chronobiologia.* **6** 305-323

Palmer J D 1976 *An Introduction to Biological Rhythms* (New-York, San Francisco, London: Academic Press)

Pati A K, Pradhan R K, Pathak V K, Saini S K and Biswas J 1987 Circadian rhythms in nonmammalian vertebrates; *Biome.* **2** 58-69

Pittendrigh C S and Daan S 1976 A functional analysis of circadian pacemakers in nocturnal rodents: Entrainment: Pacemaker as clock; *J. Comp. Physiol.* **106** 291-331

Royce W F, Smith L S and Hartt A C 1968 Models of oceanic migrations of Pacific salmon and comments on guidance mechanisms; *U.S. Fish. Wildl. Serv. Fish. Bull.* **66** 441-462

Scheving L E 1976 The dimension of time in biology and medicine chronobiology; *Endeavour* **35** 66-77

Thorpe J E 1978 *Rhythmic Activity of Fishes* (London, New York, San-Francisco: Academic Press)