Wavelength of light mimics the effects of the duration and intensity of a long photoperiod in stimulation of gonadal responses in the male blackheaded bunting *(Emberiza melanocephala)*

Vinod Kumar*, Sangeeta Rani and Shalie Malik

Department of Zoology, University of Lucknow, Lucknow 226 007, India

In male blackheaded bunting, we have investigated whether stimulatory effects of the duration and intensity of a long photoperiod can be mimicked by the wavelength of light used. The growth and development of testes, considered as the index of the photoperiodic effects, were examined at periodic intervals over a period of seven weeks. Two light wavelengths, one short (blue, 450 nm) and the other long (red, 650 nm), were used in different combinations of photoperiod and intensity in two sets of experiments. In the first set of experiments, the gonadal response to a 14 h photoperiod of blue light (14L:10D; L = 0.241 W/m^2) was compared with that to a 12 h photoperiod of red light (12L:12D; L = 0.057 W/m^2). The second set of experiments was identical to the first, except that it used high intensity of light (14L:10D, L-blue light = 1.46 W/m^2; 12L:12D, L-red light = 0.219 W/m^2). There was a similar photoperiodic induction of testicular growth under two different lighting conditions in both sets of experiments. This clearly suggests that long light wavelengths mimic the effects of the duration and intensity of a long photoperiod in stimulation of gonadal responses in the male blackheaded bunting *(Emberiza melanocephala)*.

IN 1939, Burger[1] showed that, regardless of the light intensity used, a 10.5 h light per day (10.5L:13.5D – a photoperiod less than the critical day length, CD, for photoperiodic induction) did not stimulate spermatogenesis in the European starling. This finding implicates that the light intensity cannot act as 'substitute' for CD in stimulation of the photoperiodic responses. However, light intensity may produce synergistic photoperiodic effects in the sense that at high light intensity a photoperiod is interpreted as being longer than at low light intensity[2,3]. Also, at the same light intensity, a stimulatory photoperiod is interpreted as being 'stronger', resulting into faster rate and greater magnitude of the gonadal response, if the wavelength of light used is long (e.g. red light) than if it is short (e.g. blue or green light). This is clearly evident from studies on a number of birds, including ducks[4], Japanese quail[5] and blackheaded bunting[6,7]. However, whether the wavelength of light can mimic the effects of the duration and/or light intensity of a long photoperiod in stimulation of the physiological responses (e.g. gonadal growth and development) is not yet established.

The blackheaded bunting (*Emberiza melanocephala*) is a seasonal, highly photosensitive migratory emberizid finch in which day length (photoperiod) regulates seasonal responses. Photostimulation occurs when the light period extends into the photoinducible phase (ϕ_I begins 11.5 h after dawn – the onset of light) of the circadian rhythm of photoperiodic photosensitivity (CRPP) which is suggested in mediating the photoperiod-induced seasonal responses (e.g. body fattening and weight gain and gonadal growth and development) in this species[8–11]. Thus, in buntings, CD lies close to 12 h and day lengths ≥ 12 h are photostimulatory[12]. However, compared to a typical long photoperiod like 14L:10D, 15L:9D or 16L:8D, the gonadal response to a 12L:12D photoperiod is slower and weaker. In a 4-week photoperiodic treatment at high light intensity (≥ 500 lux), the growth of testes in buntings under 12L:12D remains partial and significantly lower ($P < 0.001$) than under 15L:9D (ref. 12) or 16L:8D in which it is full (Kumar *et al.*, unpublished). The difference between gonadal response to 12 h and 14 h or longer photoperiods is more pronounced at relatively low light intensities and/or short wavelengths of light. For example, after 4 weeks of exposure (i) to white light at 100 lux light intensity, testes recrudesce in a 13 h, but not in a 12 h photoperiod (refs 6 and 13), and (ii) to blue light (450 nm) at 1.46 W/m^2 light intensity, testes were significantly ($P < 0.05$) smaller in 12L:12D (mean testis volume, $TV = 3.11 \pm 0.78$ mm^3) than in 14L:10D (mean $TV = 24.49 \pm 6.71$ mm^3) (Kumar and Rani, unpublished). Clearly, as evident from other studies as well[6,7], the response of blackheaded bunting to day length is influenced by changes in wavelength and intensity of light. This feature of the bunting's photoperiodic response system was taken to examine whether the long wavelengths of light can mimic the effects of the duration and light intensity of a longer photoperiod in the stimulation of photoperiodic responses. In the present study, we have compared the gonadal growth and development in male buntings exposed to 14L:10D of blue light with those exposed to 12L:12D of red light with a difference of about 4 to 6 folds in the intensity of light.

Groups of photosensitive birds ($n = 8$ or 9) were exposed to programmed light:dark (L:D) cycles for seven weeks. Two light wavelengths, one short (blue, 450 nm) and the other long (red, 650 nm), were used in different combinations of photoperiod and intensity in two sets of L:D cycles. In the first set of L:D cycles, the gonadal response to 14L:10D (L-blue light = 0.241 W/m^2) was compared with that to 12L:12D (L-red light = 0.057 W/m^2). The second set of L:D cycles was identi-

*For correspondence. (e-mail: DRVKUMAR@LW1.VSNL.NET.IN)

cal to the first, except that it used high intensity of light (14L:10D, L-blue light = 1.46 W/m^2; 12L:12D, L-red light = 0.219 W/m^2). Different intensities of coloured illumination were obtained by covering the fluorescent tubes with coloured cinemoid filters (Rosco Filters, Blanchard Works, Kangley Bridge Road, Sydenham, London, England). It is known that in buntings held under long photoperiods (e.g. 13L:11D), the difference in gonadal response to short (green, 528 nm) and long (red, 654 nm) light wavelengths found at 40 lux (Kumar *et al.*, unpublished) disappears at 100 lux[6], probably because of the 'saturation effects' of light on the photoperiodic response system. Therefore, in the present experiments, we have used relatively weak light intensities in order to exclude any such possibility.

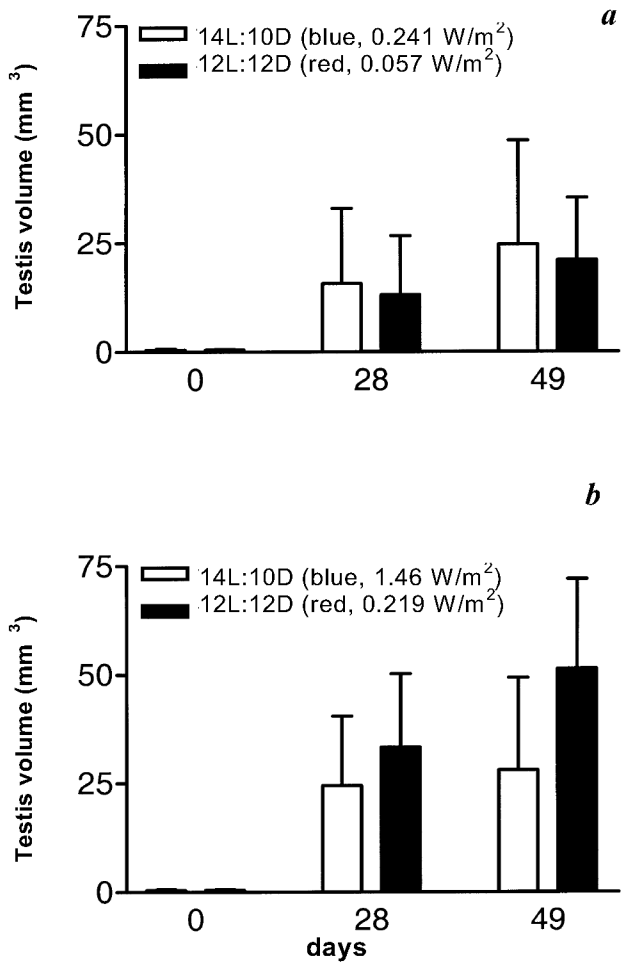

Figure 1. Photoperiodic gonadal response of photosensitive male blackheaded buntings exposed to 14L:10D (blue light, 450 nm) and 12L:12D (red light, 650 nm) at two different combinations of light intensities, ***a***, 0.241 W/m^2 blue: 0.057 W/m^2 red and ***b***, 1.46 W/m^2 blue: 0.219 W/m^2 red. For recording the changes in testis size, birds were examined at the beginning, after 4 weeks and at the end of seven weeks. The intervals of observation were decided in view of the fact that 4 weeks exposure to stimulatory photoperiods of white light induces full gonadal growth in buntings[12], and that seven weeks of exposure to such lighting conditions will be sufficient to address upon the question raised[7]. Each bar represents mean for 8 to 9 birds, and the vertical line on it (error bars) indicates the confidence interval at 95%. Note in (***b***) that on day 49, red light appears to have induced larger response than blue light, but the difference between the two means remains statistically insignificant ($P = 0.0834$, Student's '*t*'-test; $n = 8$).

All birds were housed in groups of 4 to 5 per cage (45 × 25 × 25 cm) and provided food and water *ad libitum*. The size of testes, considered as the index of the photoperiod-induced response, was recorded by laparotomy as testis volume (*TV*) at the beginning, after 4 weeks, and at the end of seven weeks of the photoperiodic treatment. The dimensions of left testis were measured and *TV* was calculated from $4/3\pi ab^2$, where *a* and *b* denote half of the long and short axes, respectively. The data were plotted as mean (± confidence interval, CI), and the means of two groups on selected days were compared using Student's '*t*'-test. Significance was taken at $P < 0.05$.

This is the first finding suggesting that the action on the photoperiodic response system of light wavelengths can be compensatory in certain photoperiodic situations. The results (Figure 1) are quite significant in showing that long light wavelengths can mimic the stimulatory effects of the duration and intensity of a longer photoperiod of short wavelengths. A 12 h (weakly inductive) photoperiod at long light wavelength produced testis response similar to, or somewhat larger than (Figure 1 *b*) that induced by a longer 14 h (strongly inductive) photoperiod at short light wavelength, even though there was a 2 h difference in duration and a 4 to 6-fold difference in light intensity between the two L:D cycles.

There can be one or both of the following reasons for a similar photoperiodic induction of gonadal growth and development under two different lighting conditions. (1) The number of photons reaching diencephalon per unit time differs with the wavelength applied. This may be because long light wavelengths scatter to a lesser extent by the biological tissues than short wavelengths[14] and/or because the former penetrate several times more effectively into the diencephalon than the latter[15]. (2) CRPP in the blackheaded bunting responds to light in a wavelength-dependent manner, as do circadian rhythms of locomotor activity in the species of mammals[16,17]. Then, it can be argued that at a given light intensity, long light wavelengths in 12L:12D induce phase changes (e.g. phase advance) in CRPP such that a larger portion of the ϕ_i, corresponding to the portion of ϕ_i under 14L:10D in short light wavelengths, is illuminated. However, this remains purely speculative since the characteristics of the CRPP cannot be monitored directly. Assuming that CRPP in bunting responds to light wavelengths such as proposed above, it would be of interest to examine whether wavelength-induced phase changes result in alteration of CD for photoperiodic induction. We intend to explore this possibility in our future set of experiments on this species.

1. Burger, J. W., *J. Exp. Zool.*, 1939, **81**, 333–341.
2. Bissonnette, T. H., *Physiol. Zool.*, 1931, **4**, 542–574.
3. Bentley, G. E., Goldsmith, A. R., Dawson, A., Briggs, C. and Pemberton, M., *J. Biol. Rhythms*, 1998, **13**, 148–158.
4. Benoit, J., *Ann. NY Acad. Sci.*, 1964, **117**, 204–216.
5. Oishi, T. and Lauber, J. K., *Am. J. Physiol.*, 1973, **225**, 880–886.
6. Kumar, V. and Rani, S., *Physiol. Behav.*, 1996, **60**, 625–631.
7. Rani, S. and Kumar, V., *ibid.*, 2000, **69**, 277–283.
8. Kumar, V., Jain, N., Singh, B. P. and Kumar, B. S., *Reprod. Nutr. Dev.*, 1993, **33**, 143–150.
9. Tewary, P. D. and Kumar, V., *J. Comp. Physiol. B*, 1981, **144**, 201–203.
10. Tewary, P. D. and Kumar, V., *Curr. Sci.*, 1984, **53**, 113–114.
11. Kumar, V., Jain, N. and Follett, B. K., *J. Comp. Physiol. A*, 1996, **179**, 59–64.
12. Kumar, V. and Tewary, P. D., *ibis*, 1983, **125**, 305–312.
13. Kumar, V. and Rani, S., *Curr. Sci.*, 1999, **77**, 283–285.
14. Seliger, H. H. and McElroy, W. D. (eds), in *Light: Physical and Biological Action*, Academic Press, New York, 1965.
15. Hartwig, H. G. and Veen, T. van, *J. Comp. Physiol. A*, 1979, **130**, 277–282.
16. Joshi, D. and Chandrashekaran, M. K., *ibid.*, 1985, **156**, 189–198.
17. Boulos, Z., *Physiol. Behav.*, 1995, **57**, 1025–1033.

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1. Burger, J. W., *J. Exp. Zool.*, 1939, **81**, 333–341.
2. Bissonnette, T. H., *Physiol. Zool.*, 1931, **4**, 542–574.
3. Bentley, G. E., Goldsmith, A. R., Dawson, A., Briggs, C. and Pemberton, M., *J. Biol. Rhythms*, 1998, **13**, 148–158.
4. Benoit, J., *Ann. NY Acad. Sci.*, 1964, **117**, 204–216.
5. Oishi, T. and Lauber, J. K., *Am. J. Physiol.*, 1973, **225**, 880–886.
6. Kumar, V. and Rani, S., *Physiol. Behav.*, 1996, **60**, 625–631.
7. Rani, S. and Kumar, V., *ibid.*, 2000, **69**, 277–283.
8. Kumar, V., Jain, N., Singh, B. P. and Kumar, B. S., *Reprod. Nutr. Dev.*, 1993, **33**, 143–150.
9. Tewary, P. D. and Kumar, V., *J. Comp. Physiol. B*, 1981, **144**, 201–203.
10. Tewary, P. D. and Kumar, V., *Curr. Sci.*, 1984, **53**, 113–114.
11. Kumar, V., Jain, N. and Follett, B. K., *J. Comp. Physiol. A*, 1996, **179**, 59–64.
12. Kumar, V. and Tewary, P. D., *ibis*, 1983, **125**, 305–312.
13. Kumar, V. and Rani, S., *Curr. Sci.*, 1999, **77**, 283–285.
14. Seliger, H. H. and McElroy, W. D. (eds), in *Light: Physical and Biological Action*, Academic Press, New York, 1965.
15. Hartwig, H. G. and Veen, T. van, *J. Comp. Physiol. A*, 1979, **130**, 277–282.
16. Joshi, D. and Chandrashekaran, M. K., *ibid.*, 1985, **156**, 189–198.
17. Boulos, Z., *Physiol. Behav.*, 1995, **57**, 1025–1033.

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Isolation of *Yersinia enterocolitica* and *Yersinia intermedia* from wastewaters and their biochemical and serological characteristics

Indrajit Sinha, Indu Choudhary and J. S. Virdi*

Microbial Pathogenicity Laboratory, Department of Microbiology, University of Delhi South Campus, Benito Juarez Road, New Delhi 110 021, India

***Yersinia enterocolitica*, an important food- and water-borne enteric pathogen was isolated from wastewater samples collected in and around the national capital territory of Delhi. All *Y. enterocolitica* isolates belonged to biotype 1A. Among these, O:6,30–6,31 was the predominant serotype. Serotypes O:10-34; O:6,31; O:15; O:41, 42 and O:41, 43 were also isolated. Except for two non-agglutinable isolates belonging to biotype 1 and 2, all *Y. intermedia* isolated were of serotype O:40 (biotype 4). Majority of the *Y. intermedia* isolates were rhamnose negative, representing the rare biotype. This study reports occurrence of *Y. enterocolitica* and *Y. intermedia* in wastewaters in India and highlights the need for further surveillance studies on *Y. enterocolitica* in understanding the global epidemiology of such emerging pathogens.**

YERSINIA enterocolitica, an important food- and water-borne gastrointestinal agent is regarded as an emerging pathogen worldwide[1]. In India, the first outbreak of gastroenteritis due to *Y. enterocolitica* was reported recently from the North Arcot district of Tamil Nadu and the organism was isolated from well water used to dilute the buttermilk consumed by the affected individuals[2]. Earlier, in India, *Y. enterocolitica* has been isolated from the stools of diarrhoeic patients[3–5], pigs[6,7], pork[8] and fresh buffalo milk[9]. Serological evidence of porcine yersiniosis has been reported from Bangalore[10]. In an another study however, *Y. enterocolitica* could not be isolated from diarrhoeic stools of farm animals, namely cows and buffaloes[11]. There has been no systematic study on the prevalence of water-borne *Y. enterocolitica* in India. This study reports the occurrence of *Y. enterocolitica* and *Y. intermedia* in wastewater from Delhi. Data on the serological and biochemical characteristics of these isolates are also presented.

The study was carried out in the national capital territory of Delhi. Wastewater samples were collected over a period of one and half years, from five sewage treatment plants (50 samples), three major drains (15 samples) and from the river Yamuna (7 samples) (Figure 1). The sewage treatment plants located at Okhla (capacity 150 million gallons per day, MGD), Keshopur (86

Figure 1. Map showing study area and sampling sites.

*For correspondence. (e-mail: micro@dusc.ernet.in)