Effects of enhanced lighting on the behaviour of nocturnal frogs

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Abstract. Biologists studying anuran amphibians usually assume that artificial, visible light does not affect the behaviour of nocturnal frogs. This assumption was tested in a laboratory experiment. The foraging behaviour of grey treefrogs, *Hyla chrysoscelis,* was compared under four lighting conditions: ambient light (equivalent to bright moonlight, 0.003 lx), red-filtered light (4.1 lx), low-intensity 'white' light (3.8 lx), and high-intensity 'white' light (12.0 lx). The treatments were chosen to correspond to standard methods of field observation of frog behaviour. The foraging behaviour of frogs in the four treatments was observed using infra-red light that was invisible to the frogs. The ability of the frogs to detect, and subsequently consume prey was significantly reduced under all of the enhanced light treatments relative to the ambient light treatment. Thus, the use of artificial light, within the visible spectrum of the frogs' eyes, can influence the outcome of nocturnal behavioural observations. These results lead to the recommendation that anuran biologists use infra-red or light amplification devices when changes in frogs' visual capabilities may influence the conclusions drawn from a study.

Studies of the behaviour of nocturnal animals frequently have been complicated by the use of artificial light during observations (Mistry & McCracken 1990). Researchers of the behaviour of nocturnal anurans frequently have assumed that the use of artificial light did not affect the patterns of behaviour studied. I surveyed the journal *Animal Behaviour* from 1981 through 1990 for studies of the behaviour of adult, nocturnal frogs. Of the 36 papers found, over 58% did not report the type(s) of light used during the studies. Of the remaining papers, 37% employed unfiltered incandescent 'white' light, 16% used 'white' light of lowered intensity, and 31% used red filters or bulbs. The assumption that these methods do not affect the behaviour of nocturnal frogs has not been tested, and current evidence concerning visual physiology suggests that it may be invalid.

Hailman & Jaeger (1974, 1976) and Jaeger & Hailman (1976) proposed that each species of frog has an optimum ambient illumination (for a given adaptational state) at which the frogs' visual discriminability is greatest. Although some species of frogs exhibit extraordinary latitude in retinal response to a range of illuminations (Sustare 1977), many species are active only in a narrow range (< 4 log units) of environmental illuminations (Jaeger et al. 1976; Jaeger & Hailman 1981; Hailman 1982, 1984) and may exhibit certain patterns of

behaviour (e.g. foraging and calling) only within a portion of that range (Jaeger & Hailman 1981). A shift of illumination to a level above or below this range may cause the cessation or modification of a particular behaviour.

Shifts in relative light intensity may also affect frogs' visual capabilities (Jaeger & Hailman 1973, 1976; Hailman & Jaeger 1978; Hartman & Hailman 1981). Rapid shifts in light intensity may cause pupillary constriction or dilation and photopigment bleaching and migration (Muntz 1977). The period of adaptation to the new stimulus may be prolonged even if the shift in the intensity of the light stimulus is as small as one log unit of intensity (Cornell & Hailman 1984). Rapid shifts in light intensity may temporarily impair the vision of the frog and may, therefore, affect the behaviour of the frog if the frog's behaviour is not independent of its visual capabilities.

A typical method of field observation of nocturnal frogs involves the use of a battery powered electric light. Many frogs of the genus *Hyla* are most active at ambient light intensities of 10^{-5} to 10^{-3} lx (overcast starlight to bright moonlight: personal observation). The use of artificial light (minimum of 1-10 lx) will cause the frogs' eyes to experience rapid shifts of large magnitude away from the previous adaptational state, and thus probably impairs the vision of the frogs being observed.

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Some researchers have attempted to ameliorate these effects by changing the spectral properties of the light (using red filters) or slightly decreasing the absolute intensity of the light source by using only diffuse light peripheral to the central beam of the source.

The purpose of my study was to determine whether the rapid shifts in light intensity that frogs often experience during behavioural observations affect their subsequent behaviour. Additionally, I attempted to determine whether changing the spectral properties or decreasing the illumination of the light source could ameliorate these potential effects. I used male grey treefrogs, *Hyla chrysoscelis* (Anura: Hylidae) for this experiment because they are common test subjects in behavioural studies (Morris 1989; Ritke & Semlitsch 1991). The results from tests with grey treefrogs probably are applicable to other species of frogs as well, because grey treefrogs exhibit a form of phototactic response and spectral sensitivity that is common to a majority of the species that have been tested (Jaeger & Hailman 1973; Hailman & Jaeger] 974).

METHODS

Twenty adult males of *H. chrysoscelis* were collected from a chorus in St Martin Parish, Louisiana, on 22 February 1991. The frogs were returned to the laboratory and maintained on a natural light cycle at 25-28°C as described in Buchanan & Jaeger (in press). All tests were performed between 22 March and] April 1991, from 2030 to 2330 hours at 25·5-28°C.

Experimental Apparatus

I observed the behaviour of the frogs using an IR Scientific model EL T infra-red viewer with a 75-mm Cosmicar lens and 6-V incandescent light source with a 900-nm pass filter. Infra-red light ($>$ 750 nm) is beyond the region of spectral sensitivity of the frogs' eyes and is, therefore, invisible to the frogs (Jaeger & Hailman 1973). Observations were made at a distance of 2-m from behind an opaque curtain. Illuminations were measured at a 45° angle to the back wall of the test chamber at a distance of 0·50 m using a Science and Mechanics model 102 super-sensitive photometer.

The two chambers (habituation and test) used during the experiment were in light-safe positions and were separated from each other and the observation area by a series of opaque curtains. The test chamber was a wooden box (40 x 40 x 50 cm), painted grey with grid lines every 10 cm on the floor and a transparent acrylic front and ceiling. The transparent ceiling isolated the test area from the area containing the light sources and photometer probe above, thereby retaining the frogs in the test area while allowing light to pass into the chamber and return to the photometer probe. Ambient light in the test chamber was provided from a rheostat-controlled 6- V, 25-mA incandescent bulb embedded in the centre of the top of the chamber. The light for the experimental treatments was provided by a 6-V, PR-12, 50D-mA incandescent bulb mounted in a Rayovac headlamp reflector that could be fitted with various filters and was operated from a remote position. I used combinations of ground-acrylic filters to create the two 'white' light treatments and red acetate film (transmittance not determined) combined with ground-acrylic filters to create the 'red-filtered' treatment. The test chamber contained a habituation cup that I raised from a remote position at the beginning of a test, causing it to recess into the ceiling of the test chamber.

The habituation chamber was identical to the test chamber in size and ambient illumination. Individual frogs were habituated to the ambient illumination of the test chamber while in the habituation chamber. Individual habituation cups were provided for each frog and were formed from inverted, transparent glass bowls (90-mm diameter, 40-mm height) that rested on thin ($<$ 1-mm) plastic pads (130-mm diameter). The plastic pads allowed the pad, cup and frog to be moved from the habituation chamber to the test chamber without handling the frog directly.

Experimental Procedure

Individual frogs were tested under each of four lighting conditions: ambient + infra-red light (JR, 0.003 lx), red-filtered + infra-red light (R, 4.1 lx), low-intensity 'white' + infra-red light (WL, 3.8 lx) and, high-intensity 'white' + infra-red light (WH, 12.0 lx). These levels of intensity and spectral properties were chosen to correspond with current techniques of nocturnal behavioural observations (e.g. Robertson 1986; Howard 1988). Before beginning the experiment, I compared the light output of three commonly available battery-operated headlamps. The range of maximum illuminations on

brown and green vegetation at 2 m for the three headlamps was 6-45 lx depending on the headlamp and the background. The minimum 'peripheral' illumination that was useful for detailed behavioural observations at 2-m was approximately 1-5 lx depending on the headlamp and the background. The level of ambient illumination for the experiment (0.003 lx) corresponds to bright moonlight and represents the upper level of illuminations at which many hylid frogs are normally active (personal observation).

The frogs were assigned randomly to two test groups that were tested on alternate nights. I randomized test order among individuals within nights and individuals' exposure order among nights for the four treatments. The frogs were not fed for 48 h prior to testing. Gut clearance time is less than 48 h at 25°C (unpublished data) and hunger levels were comparable among frogs and among testing dates for individuals. I placed the frogs into the habituation chamber at 1830 hours and allowed them to habituate to the ambient light conditions for a minimum of 2 h prior to testing. I initiated a test by moving a frog (within its habituation cup) to the test chamber. After the habituation cup and frog were in place in the test chamber, I added five 2-week-old crickets, *Acheta domestic us* (about 0·25-0·35 g total mass), to the test chamber and allowed the frog to habituate to the test chamber for 5 min. During this period, I frequently observed frogs attempting to capture prey that were apparently visible to the frogs through the transparent habituation cup. I simultaneous]y raised the habituation cup (leaving the frog sitting on the plastic pad in the centre of the chamber) and turned on the light source for a given treatment while observing the behaviour of the frog through the infra-red viewer. Individual tests were terminated after 10 min or when all of the prey had been consumed.

Response Variables

I used two measures to compare the evenness of prey availability among the light treatments. This comparison was important because I had predicted that changes in light intensity may affect the behaviour of the frogs; similarly, cricket behaviour might have been affected, and subsequent changes in prey availability might have biased the outcome of the experiment. The total number of prey encountered by a frog (Number of Prey) during a test was a measure of the evenness of opportunities for prey capture over the test period. A prey encounter was defined as a cricket being within 10 cm of a frog. Although only five crickets were present in the test chamber during a test, each prey encounter was assumed to be an independent event so that the total number of encounters could be analysed statistically. The time to the first prey encounter (Encounter) was used to compare the initial reaction of crickets to each light treatment. If differences were detected in prey availability among the treatments, then potential differences among treatments in the frogs' foraging performance could not be attributed solely to the effect of the experimental variables.

I analysed three indices of frog foraging performance. I considered time to first orientation towards prey (Orient) to be the most sensitive indicator of the initial effect of changes in light intensity on the visual capabilities of the frogs. An orientation toward prey was defined as a frog moving its head or entire body so that forward movement by the frog would cause physical contact between the frog and the cricket. Time to first prey capture attempt (Attempt) represents the time required for a frog to detect and attempt to consume a prey item. A prey capture attempt was defined as a lunge (with the mouth open) in the direction of a prey item. Time to first prey capture (Capture) was considered to be the best measure of a frog's actual foraging performance and represented the minimum time required for a frog to capture and consume a prey item. A successful prey capture was defined as a prey capture attempt followed immediately by the observed ingestion of the prey item.

Statistical Analysis

Friedman's test and non-parametric Tukey-type multiple comparisons (Zar 1984) were used to compare prey availability and the foraging performance of frogs among all four treatments and between each pair of treatments. The non-parametric ana]ysis was chosen over parametric analogues because not all of the variables exhibited normal distributions or homoscedasticity. Multiple statistical comparisons using related data (e.g. response variables from the same animal) may inflate the overall type I 'family' error rate (Miller 1981) when statistically significant correlations exist among the related variables; 'statement' error rates (Miller 1981) remain constant regardless of dependency

Table I. Spearman rank correlation of the five response variables reported in this study

	Number of prey	Encounter	Orient	Attempt
Encounter	- O.34027*			
Orient	-0·19585	0·18506		
Attempt	-0'11217	0·21337	0·72268*	
Capture	-0,10148	0·21837	0'72056*	0·92041*

*Statistically significant correlation at $r > 0.22855$.

(W.J. Conover, personal communication). Because the over-reduction of alpha, using Bonferonni's inequality (Snedecor & Cochran 1980) to decrease the probability of committing a type I error, inflates the overall type II statistical error rate (Miller] 98]), I adjusted alpha using a value that was sensit1ve to the reuse of data and to the variable-specific probability that related response variables would increase the type I error rate as follows: adjusted $\alpha = \alpha/(c+x)$ where $a=0.05$, c is a constant and equals the number of analyses in which the data were involved according to Bonferonni's inequality (Snedecor & Cochran 1980) and x equals the total number of statistically significant correlations that a particular response variable shares with related response variables. I performed Spearman rank correlations among the response variables (two-tailed, $\alpha = 0.05$; Zar 1984) and used these values to assess the adjustment to alpha for the separate Friedman's tests. This technique has been suggested to be overly conservative (W. J. Conover, personal communication) for tests where type I error is of concern and should not be used when type II error sensitivity is required. An alternative to this technique is to choose a single response variable to report. However, as in this experiment, the existence of strong correlations among response variables does not mean that each correlated variable is not individually biologically meaningful.

RESULTS

Statistically significant correlations were detected between Encounter and Number of Prey, Attempt and Orient, Capture and Orient, and Capture and Attempt (Table I) and alpha was adjusted (reduced) accordingly for the appropriate Friedman's tests (see Methods).

Friedman's tests failed to find differences among treatments for either Number of Prey ($X^2 = 2.715$,

$P > 0.25$, $a = 0,025$: Fig. 1a) or Encounter ($\chi^2 = 5.7$, P>0.05, a=0.0025: Fig. 1b) suggesting that prey availability and activity were equal across the treatments. Thus, any differences found among the treatments for measures of treefrog foraging performance may be attributed directly to the effects of the treatments and not to differential activity of the prey.

Treefrog foraging performance was significantly affected by rapid shifts in the intensity and/or spectral properties of the light in the test chamber. Treefrogs required significantly more time to detect prey under all of the experimental treatments (R, WL, WH) relative to the control treatment (JR, Orient: X2=27·77, P<0.001, 0=0·0167) although no differences were found among the experimental treatments (Fig. lc, Table II). The time to the first prey capture attempt was significantly greater under all of the experimental treatments relative to the control treatment (Attempt: $\chi^2 = 26.75$, P<0.001, $\alpha=0·0167$), but no differences were detected among the experimental treatments (Fig. 1d, Table II). The time to first successful prey capture was greater under the two 'white' light treatments (WL, WH) relative to the control (Capture: $\chi^2 = 20.84$, P<0.001, $\alpha = 0.0167$) but, the 'red' light treatment (R) did not differ from the control. No differences were detected among the experimental treatments (Fig. 1e, Table II).

DISCUSSION

The results of this experiment demonstrate that the behaviour of nocturnal frogs can be influenced by the method of observation. Rapid increases in light intensity (3-4 log units), comparable to those experienced during observational studies, substantially reduced the foraging performance of the frogs. The reduction in foraging performance was
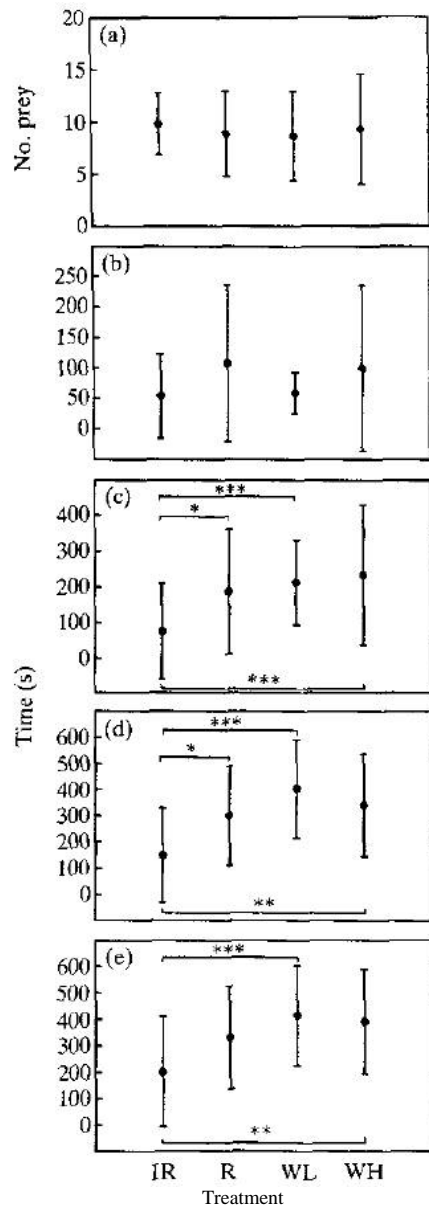
Figure 1. Mean (±SD) number of prey items captured (a) and mean (±SD) time in seconds to first prey encounter (b); to first orientation towards prey (c); to first prey capture attempt (d); and to first successful prey capture (e) for treefrogs under four light treatments: IR: ambient light; R: red-filtered light; WL: low-intensity 'white' light; WH: high-intensity 'white' light. Asterisks denote significant differences between treatments based on Tukey-type multiple comparisons (*$P < 0.01$, **$P < 0.005$, ***$P < 0.001$, $\alpha = 0.05$).

probably due to the over-stimulation of the frogs' visual systems and the corresponding pupillary

constriction, photopigment bleaching, and photoreceptor migration (Muntz 1977; Cornell & HaHman 1984). Thus, the frogs' vision was probably temporarily impaired and they were less able to detect and subsequently consume prey. The commonly employed technique of using diffuse, peripheral light to observe frogs does not ameliorate this effect, as the shift in intensity was only slightly less (1 log unit) than that of the maximal stimulus in this study, and still represents a shift in intensity from the ambient illumination of about 3 log units, which was sufficient to depress foraging performance.

I was unable to detect any lessening of the 'intensity' effect when using red-filtered light except when actual prey capture success was considered. The ability of the frogs to detect prey was reduced to the same extent in the red light treatment as it was in the 'white' light treatments. Colour vision has been demonstrated in a number of anuran taxa, and the spectral sensitivity exhibited is nearly identical to that of humans (Muntz 1962; Chapman 1966; Hailman & Jaeger 1974; Jaeger & Hailman 1976) except that the same spectral sensitivity may also occur at lower ambient illuminations when frogs are dark-adapted (Hartman & Hailman 1981). Thus, it is unlikely that the behaviour of nocturnal frogs would remain unaffected by any light within the spectral or intensity ranges of vision of a human observer when the use of that light causes a substantial shift in the frog's perceived illumination.

Cornell & Hailman (1984) found that it may take minutes or hours for a frog's eyes to readapt to a new ambient illumination. In my experiment, the time required for frogs to detect prey increased from an average of just over 1 min in the control treatment to 3-4 min in the various experimental treatments (Fig. lc). Thus, under conditions of shifting intensities of illumination, some form of habituation period is necessary before observed behaviour can be considered to be normal relative to behaviour under the initial conditions. Additionally, it is impossible at this time to predict the effect of shifts in absolute light intensity. It may be that certain patterns of behaviour are exhibited only at specific levels of illumination (Hailman & Jaeger 1974; Jaeger & Hailman 1976, 1981) and that increasing the perceived ambient illumination above that level may inhibit the activity of the animal being observed. Thus, under some conditions, even employing extended habituation periods may prove inadequate to avoid disrupting the 'normal'

Table II. Tukey-type multiple comparisons *(q)* among treatments for response
variables where significant overall differences were found with Friedman's test

Treatment	Orient		Attempt		Capture	
	q	*P*	*q*	*P*	*q*	*P*
IR versus R	4,59	<0'01*	4,59	<0'01*	3,55	>0·05
IR versus WL	6'58	<0'001*	7·1	<0'001*	6·0	<0·001
IR versus WH	6·15	<o·oor*	4·9	< 0·005*	4·9	<0'005
R versus WL	1·99	>0'2	2·5	>0·2	2'5	>0·2
R versus WH	*J'56*	>0'5	0,3	>0·5	*J'39*	>0·5
WL versus WH	0-43	>0'5	2·	>0·2	1·1	>0·2

IR: Ambient light; R: red-filtered, low-intensity light; WL 'white' low-intensity
light; WH: 'white' high-intensity light.
*Statistically significant difference at ıı = 0·05.

activity of the study animal. Recent increases in the availability of 'night-vision' devices should allow for the observation of the behaviour of nocturnal animals with no more disturbance than might be caused during observations of diurnal organisms.

The most important conclusion to be drawn from this study is that investigators of nocturnal anuran behaviour should consider carefully what effect a change in the visual capabilities of their study animals may have on the outcome of a particular study. The potential role of visually mediated behaviour in frogs that are active at night has generally been ignored, probably due to the success of the study of acoustically mediated behaviour. Many species of frogs are active only at very low environmental illuminations (Hailman 1982. 1984), and their visually mediated behaviour patterns predominantly occur at light levels well below those humans perceive as total darkness (Larsen & Pedersen 1982). It may be that the paucity of in formation on nocturnal visually mediated behaviour in frogs is due to the methods of observation and not the lack of such behaviour.

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