

Photic induction of locomotor activity is correlated with photic habitat in *Anolis* lizards

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Abstract A variety of ecologically important behaviors, including circadian rhythms and seasonal reproduction, are influenced by non-visual responses to light, yet very little is known about the relationship between photic habitat and non-visual photoreception. Puerto Rican *Anolis* lizards have diverged into multiple photic niches, making them a good model for non-visual photosensory ecology. We investigated the photic induction of locomotor activity, a non-visual response to light, in four species of *Anolis* comprising two pairs of closely related, ecomorphologically similar species whose microhabitats differ in solar irradiance. We developed a device for continuous, automated detection and recording of anole locomotor activity, and used it to characterize activity under 12:12 h light–dark cycles. Next, we administered a series of 2-h light pulses during the dark period of the light–dark cycle and measured the increase in locomotor activity relative to baseline dark activity. Five different irradiances (ranging from very dim to daytime levels) were given to each individual lizard on separate nights. As expected, light caused an irradiance-dependent increase in locomotor activity in all four species. The responses at the highest irradiances were significantly greater in species occupying relatively more shaded habitats, suggesting that non-visual photoreception may be adapted to habitat light in *Anolis* lizards.

Keywords Non-visual photoreception · Circadian · *Anolis* · Visual ecology · Masking

Introduction

The timing of behavioral activities defines an important dimension of ecological niche (Hutchinson 1957; Gotelli and Graves 1996; Kronfeld-Schor and Dayan 2003). Because of the regular daily changes in solar irradiance, an animal's temporal niche (the distribution of general behavioral activity across the 24-h day) is often highly correlated with its photic habitat (the spectral composition and intensity of light to which the organism is exposed). However, independent of time of day, mean irradiance and spectral distribution vary substantially across habitats and are important factors in the structure of some ecological communities (Rand 1964; Endler 1993; Leal and Fleishman 2002). Photic cues play a central role in the timing of behavioral activities, yet little is known about the evolutionary relationship between photic habitat and the photic control of activity.

The photic environment contains both visual information (images) and “non-visual” information (e.g., irradiance or “brightness,” the perception of which does not require image formation), which are conveyed through distinct image-forming and non-image forming sensory pathways (Fu et al. 2005). Visual adaptations to habitat light have been described in many organisms (Walls 1942; Lythgoe 1984; Warrant 2004; Collin 2010), and the visual system influences the evolution of visual communication signals such as body coloration (Endler 1992; Endler et al. 2005; Maan and Seehausen 2010). In contrast, very little is known about the ecology and evolution of non-visual photoreception, yet such responses to light are essential for a variety of ecologically important behaviors, including the photoperiodic control of seasonal reproduction and the synchronization of circadian rhythms.

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The timing of behavioral activity is determined by both internal and external factors. The internal circadian clock generates activity rhythms even in the absence of external stimuli, but under natural conditions this clock is synchronized to the 24-h day by environmental cues such as the light–dark cycle. External factors also influence the timing of behavior independently of the circadian rhythm, an effect known as *masking* because it can obscure the temporal activity pattern dictated by the circadian clock. Masking is prevalent in natural settings, where the presence of predators, mates, feeding opportunities, and weather conditions exert a major influence on the temporal distribution of activity (Reebs 2002; Orpwood et al. 2006; Levri et al. 2007; Fraker 2008; Valeix 2009). Thus, while the circadian clock sets a basic temporal framework, the role of masking is to “fine-tune” (Page 1989) the timing of behavior, allowing for flexibility and opportunistic responses in variable environments. There is some evidence to suggest that masking responses are evolutionarily more labile than the circadian clock (Ziv et al. 1993; Marques and Waterhouse 1994; Kronfeld-Schor et al. 2001). Light, in addition to being one of the most salient and reliable cues for synchronization of the circadian clock, also has well-known masking effects on behavior (Mrosovsky 1999; Gerrish et al. 2009; Chiesa et al. 2010; Fernandez-Duque et al. 2010; Narendra et al. 2010). Light can induce behavioral activity or it can inhibit it, largely dependent upon whether the animal is nocturnal or diurnal. This effect is irradiance-dependent, varies among species, and is fairly well-understood mechanistically (Mrosovsky 1999; Hattar et al. 2003). Photic masking, therefore, provides a useful behavioral output for asking questions about the “non-visual” photoecology of animals.

Anolis lizards provide an ideal system for studying the relationship between photic niche and non-visual photoreception. The *Anolis* genus has undergone extensive and repeated adaptive radiations in the West Indies, with similar patterns of habitat specialization occurring independently on each of the Greater Antillean islands. Adaptation to distinct ecological niches has produced “ecomorphs” (Williams 1972), sets of species that are similar in morphology, ecology, and behavior. Habitats are partitioned along three major axes: perch type (height and diameter), prey size, and thermal preferences (Losos 2009). In addition, habitats can be categorized into at least four distinct photic niches, listed in order of increasing irradiance: full shade, partial shade, canopy, and no shade (Fleishman et al. 1997; Loew et al. 2002). Of the Greater Antilles radiations, the Puerto Rican clade has produced the clearest and most extensive divergence in photic habitat (Rand 1964; Williams 1983). The phylogeny for this group is well resolved (Nicholson et al. 2005; see also Fig. 1) and indicates that photic habitat preferences have arisen independently. Importantly, photic

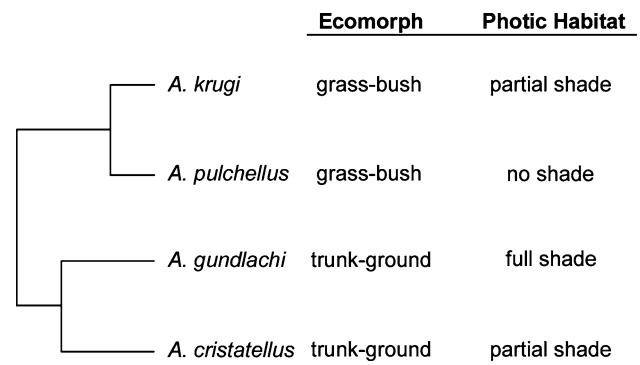


Fig. 1 Phylogeny, ecomorph classifications, and photic habitats of Puerto Rican *Anolis*. Cladogram was constructed by pruning the phylogeny in Nicholson et al. (2005) and depicts the phylogenetic relationships among the species used in the current study. Branch lengths are not quantitative, but shorter branch lengths indicate more recent divergence. Photic habitats are based on descriptions in Loew et al. (2002) and Fleishman et al. (1997)

niche is largely independent of temporal niche in the *Anolis* genus—all species are exclusively diurnal. This makes it possible to correlate photic habitat with the photic control of activity, while avoiding confounds due to the temporal distribution of activity.

The goal of the current study was to determine if photic masking of locomotor activity is correlated with irradiance of the photic habitat. We used four species of Puerto Rican anoles comprising two pairs of closely related, ecomorphologically similar photic niche variants: (1) *Anolis cristatellus* and *Anolis gundlachi*, and (2) *Anolis pulchellus* and *Anolis krugi*. Figure 1 shows the phylogenetic relationships, ecomorph classifications, and photic habitats of these species. Of the trunk-ground ecomorphs, *A. cristatellus* is found in partially shaded habitats, while *A. gundlachi* occupies fully shaded forests. Of the grass-bush ecomorphs, *A. pulchellus* lives in open areas with little to no shade, while *A. krugi* is more common near partially shaded forest edges. Since anoles are diurnal, we expected that light would induce locomotor activity. To assess masking, we administered a 2-h pulse of light during the dark phase of a light–dark cycle and recorded locomotor activity. Five different irradiances of light were tested in the same individual on separate nights. We hypothesized that within each pair of photic niche variants, the species belonging to the relatively more shaded niche (*A. gundlachi*, *A. krugi*) would be more sensitive to the masking effects of light.

Methods

Animals and housing conditions

Adult male anoles were captured from Puerto Rico in January–February and May–June 2010 and transported to

the University of Virginia, where all behavioral experiments were conducted. Lizards were housed individually in plastic cages equipped with a perch and a thin carpet floor substrate. Since male anoles are known to be aggressive, partitions were placed between cages to prevent the lizards from seeing each other and exhibiting aggression. Room temperature was maintained at $27.8 \pm 0.5^\circ\text{C}$ (*A. cristatellus*, *A. krugi*, *A. pulchellus*) or $24.4 \pm 0.5^\circ\text{C}$ (*A. gundlachi*). The thermal optimum of *A. gundlachi* is lower than the other species (Rand 1964; Huey and Webster 1976), and mortality was high when we attempted to keep them at the higher temperature. Relative humidity was kept at $60 \pm 10\%$. A 12:12 h light–dark (LD) cycle was provided by overhead fluorescent room lighting. Additional light was provided by fluorescent bulbs (enriched in ultraviolet wavelengths, Repti-Sun 10.0, Zoo Med Laboratories, Inc.) suspended from the top of the cage ($\sim 400 \mu\text{W}/\text{cm}^2$). Lizards were misted with water daily and were fed live crickets, dusted with vitamin powder, twice per week. Feeding and watering took place during the light period, but at randomly distributed times each day to avoid circadian entrainment to these stimuli.

Behavioral experiments were conducted between April and December 2010. For these experiments, animals were transferred to a different room with the same temperature and humidity as the regular housing rooms. Procedures for the provision of food and water were also identical to those used in regular housing. Lizards were housed in individual specialized enclosures (see description below and Fig. 2) for the duration of the behavioral recording period (~ 3 weeks). For the LD cycle, light was provided by fluorescent bulbs (see above) suspended over the enclosures. Since reptile circadian rhythms are extremely sensitive to temperature (entrainment can occur to thermocycles with amplitudes as low as 0.9°C ; see Hoffmann 1969), we took measures to minimize temperature fluctuation in the enclosures due to the fluorescent bulbs turning

off and on. An additional set of bulbs was wrapped with opaque black plastic, excluding light but allowing heat transfer to occur. These bulbs were positioned adjacent to the LD bulbs and set to turn on/off in anti-phase to the LD cycle. A fan was used to circulate the air in the room and provided an additional means of maintaining uniform temperature. Temperature in the activity enclosures was monitored continuously with iButton dataloggers (Maxim Integrated Products), and at selected times during the light and dark periods with a thermocouple probe, and was found to vary by 0.6°C .

Behavioral recording apparatus

In captivity, anoles exhibit very little spontaneous locomotor activity. Therefore, it was necessary to design a system with a high level of sensitivity for the continuous detection and long-term recording of locomotor activity (see Fig. 2). We utilized accelerometers (ADXL103, Analog Devices) to detect vibrations resulting from locomotor movement within specialized enclosures ($23 \times 23 \times 30 \text{ cm}^3$) constructed from cellulose acetate film ($125 \mu\text{m}$ thick), a transparent material that transmits vibrations originating from any position within the enclosure, which was mounted on soft cushions. Accelerometers, attached to the floor of each enclosure, produced electrical signals that were proportional to acceleration (vibration) of the enclosure. The signals were amplified and filtered such that the device reliably detected anole locomotor activity (running, walking, jumping, stepping of legs without bodily motion, and rapid neck flicking), but omitted vibrations resulting from irrelevant sources (e.g., human speech) from the recording. Signals were converted into a digital format and recorded on a computer using custom-written software in Matlab (The Mathworks, Inc., Natick, MA). Acceleration was continuously monitored at 20-millisecond intervals and the presence of a supra-threshold acceleration was examined once per second. When supra-threshold accelerations were detected, a time stamp was left in the computer memory with an accuracy of one second. These time-stamped records constituted locomotor activity data counts.

Experimental design

Our system allowed us to record from up to eight animals simultaneously. We conducted six separate experimental trials with 6–8 animals each ($N = 45$). Four trials included *A. cristatellus* ($n = 10$), *A. krugi* ($n = 9$), and *A. pulchellus* ($n = 10$), with 2–4 animals from each species in each trial. Since a different temperature was used for *A. gundlachi* ($n = 8$), a separate trial was conducted for these animals. Lizards were transferred to the activity enclosures where

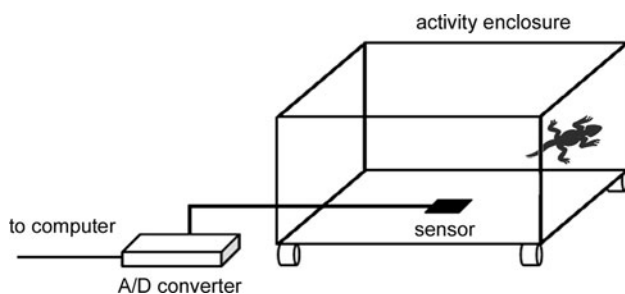


Fig. 2 Cartoon diagram of the activity recording system. Lizards were kept in transparent activity enclosures made from thin plastic film. An accelerometer was attached to the floor of each enclosure and served as a vibration sensor. Analog signals from the accelerometers were converted to digital signals and sent to a computer, where they were recorded

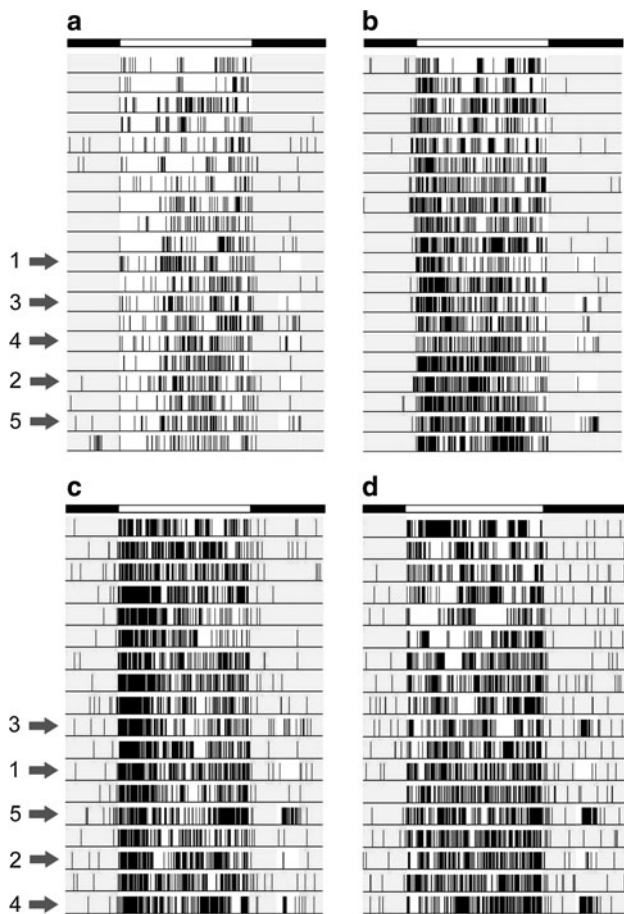


Fig. 3 Individual activity records of four lizards given 2-h light pulses during the night. Actograms depict locomotor activity across the 24-h day; each row represents 1 day of activity, with activity indicated by vertical black lines. The white/black bar above each actogram represents the light–dark cycle. In addition, dark periods are represented by light gray shading on the activity record. A representative record from each species is shown (a *A. pulchellus*, b *A. krugi*, c *A. cristatellus*, and d *A. gundlachi*). The first several days show baseline activity under the light–dark cycle. Light pulses were given on five separate nights, as indicated by the gray arrows. Numbers next to the arrows indicate the irradiance used, with 1 being the lowest and 5 being the highest (see text). The time of the light pulse is shown by the unshaded region within the gray-shaded dark period

they were kept under a normal 12:12 LD cycle for about 1 week. Following this initial period, a series of light pulses were administered during the dark period, at zeitgeber time (ZT) 15–17, with ZT 12 defined as the time of lights-off. The lighting protocol is depicted in Fig. 3. A total of 5 light pulses of varying irradiances were administered on separate nights. Each pulse began 3 h after the end of the normal light period and lasted for 2 h. In order to minimize the influence of aftereffects (long-lasting effects of the light pulse, i.e., effects seen after the 24-h period in which the pulse was given), pulse nights were alternated with non-pulse nights (12 h of darkness). Five

different irradiances (spanning six orders of magnitude) were used for the light pulses: 3.7×10^{-3} , 5.6×10^{-2} , 2.9×10^{-1} , 3.1, and $4.5 \times 10^2 \mu\text{W}/\text{cm}^2$. For convenience, we will refer to these irradiances in the text as 1, 2, 3, 4, and 5, respectively (with ‘1’ being the lowest and ‘5’ being the highest irradiance). Standard fluorescent light bulbs were used to administer light pulses (Sylvania Cool White 4100K 34W 48”). The five different irradiances were achieved by wrapping the bulbs with neutral density filters. The order in which the five different light pulses were given was arbitrarily selected and varied among trials. In order to avoid increased temperature due to the light pulse, the opaque plastic-wrapped bulbs used to maintain temperature during the dark period (see “Animals and housing conditions”) were set to turn off during the light pulse.

Data analysis

Actograms were constructed in order to visualize the patterns of activity (see Fig. 3). For each individual lizard, activity counts were summed into 1-min bins. Binned activity was used to calculate, for each individual, (1) mean photophase (L) activity under baseline LD conditions, (2) mean scotophase (D) activity under baseline LD conditions, (3) percentage of the total LD baseline activity occurring during the dark period (%D), (4) mean ZT 15–17 activity under baseline LD conditions, (5) total activity during each of the five light pulse treatments (ZT 15–17), and (6) mean activity during the pulse time (ZT 15–17) on non-pulse days during the treatment period (aftereffects). Positive masking (increased activity during the light pulse) was defined as the ratio of (5) to (4), calculated within individuals at each pulse irradiance level. To avoid undefined ratios due to an individual having zero counts during baseline ZT 15–17 conditions, a constant (0.1) was added to all activity counts. To assess potential aftereffects, the ratio of (6) to (4) was also computed.

All statistical analyses were carried out using the statistical program SAS (version 9.1.2). Species differences in baseline activity parameters (numbers 1–4 above) were analyzed with one-way analysis of variance (ANOVA) followed by Tukey’s multiple pairwise comparisons. Activity counts were \log_{10} -transformed to meet the assumptions of normality and homogeneity of variance. Masking responses (fold change in activity relative to baseline ZT 15–17) were compared using a mixed-model ANOVA with two fixed main effects (‘species’ and ‘pulse treatment’) and ‘species \times pulse treatment’ interaction. In addition, since each individual lizard received all five light pulse treatments, individuals were treated as blocks (as in a randomized block design). This was achieved by including ‘individual (species \times trial)’, that is, “individual of a given species in a given trial” as a random covariate in the

model, allowing for the reduction of noise due to individual variation in masking responses. Orthogonal pairwise contrasts were specified post hoc for significant main effects to provide comparisons between pairs of photic niche variants (i.e., within-ecomorph comparisons) at each treatment level.

Results

General activity patterns

Representative actograms are shown in Fig. 3. As expected, all species showed a clear diurnal pattern of activity. Figure 4 shows mean baseline activity for L, D, ZT 15–17, and % D. Significant species differences were found for L ($p < 0.0001$), D ($p < 0.0001$), and ZT 15–17 ($p = 0.0087$). In general, trunk-ground ecomorphs had higher activity levels than grass-bush ecomorphs. Post hoc comparisons revealed significant pairwise differences ($\alpha = 0.05$), as follows. For both L and D activity, each trunk-ground species (*A. cristatellus*, *A. gundlachi*) had higher activity levels than either grass-bush species (*A. pulchellus*, *A. krugi*). Within a given ecomorph, species did not differ

in L or D activity. For ZT 15–17 baseline activity, both trunk-ground species had greater activity levels than *A. krugi*. Importantly, species did not differ in percent dark activity ($p = 0.6048$), indicating that, although overall activity levels differed among ecomorphs, the relative proportions of activity in the light versus dark were the same. Moreover, photic niche variants of same ecomorph type did not differ significantly in any of the measured parameters for baseline activity, facilitating intra-ecomorph comparisons of masking responses.

Positive masking in response to light pulses

As expected, light given during the dark period induced locomotor activity. Positive masking responses (fold change in activity relative to baseline ZT 15–17) are plotted in Fig. 5. In general, light pulse treatments caused large (up to ~800-fold) increases in locomotor activity. Aftereffects were detectable and were greater in *A. krugi* (11.29 ± 3.94 , mean fold increase \pm SEM) than in other species (*A. cristatellus*: 2.64 ± 1.20 ; *A. gundlachi*: 1.75 ± 0.59 ; *A. pulchellus*: 1.87 ± 0.83), but overall these effects were small compared to the induction of activity by light. In the mixed-model ANOVA, we found a significant main

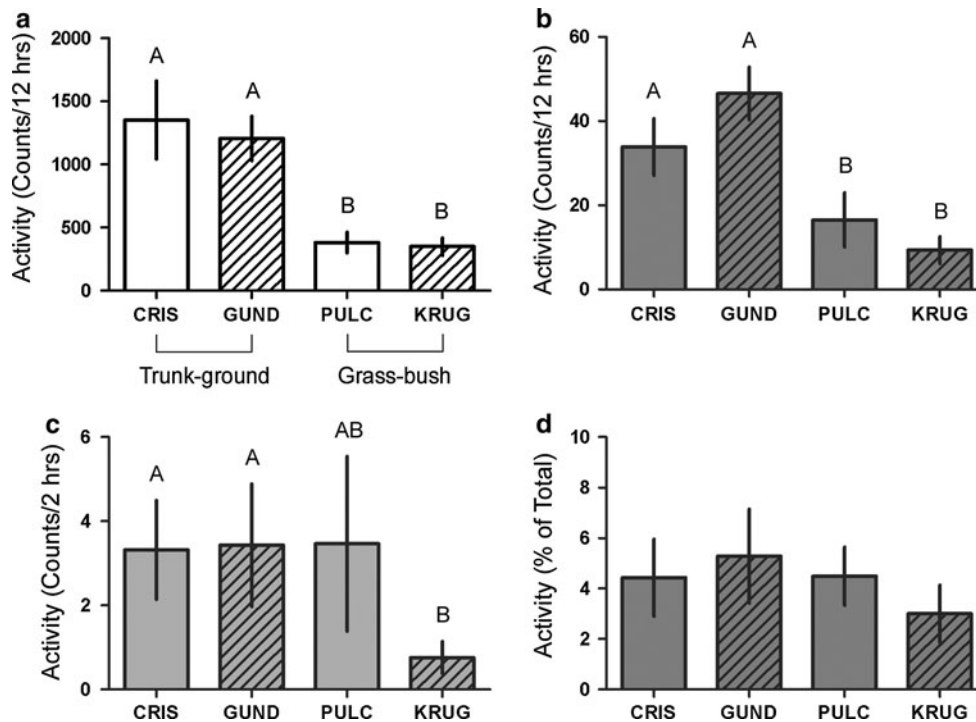


Fig. 4 Baseline activity under 12:12 h light–dark cycles. Mean activity counts during the light period (a), the dark period (b), and ZT 15–17 (c) are plotted for each species (CRIS = *A. cristatellus*, GUND = *A. gundlachi*, PULC = *A. pulchellus*, KRUG = *A. krugi*). Ecomorph classifications are indicated in (a). Light and dark activities are shown in separate graphs due to the large differences in scale (note the y-axes). The percentages of activity occurring during the

dark period are shown in d. Error bars represent standard error of the mean. Significant differences (Tukey’s multiple pairwise comparisons following one-way ANOVA, $\alpha = 0.05$) in activity levels are shown by upper-case letters above each bar (bars with the same letter are not significantly different from each other). There were no significant differences for % dark activity

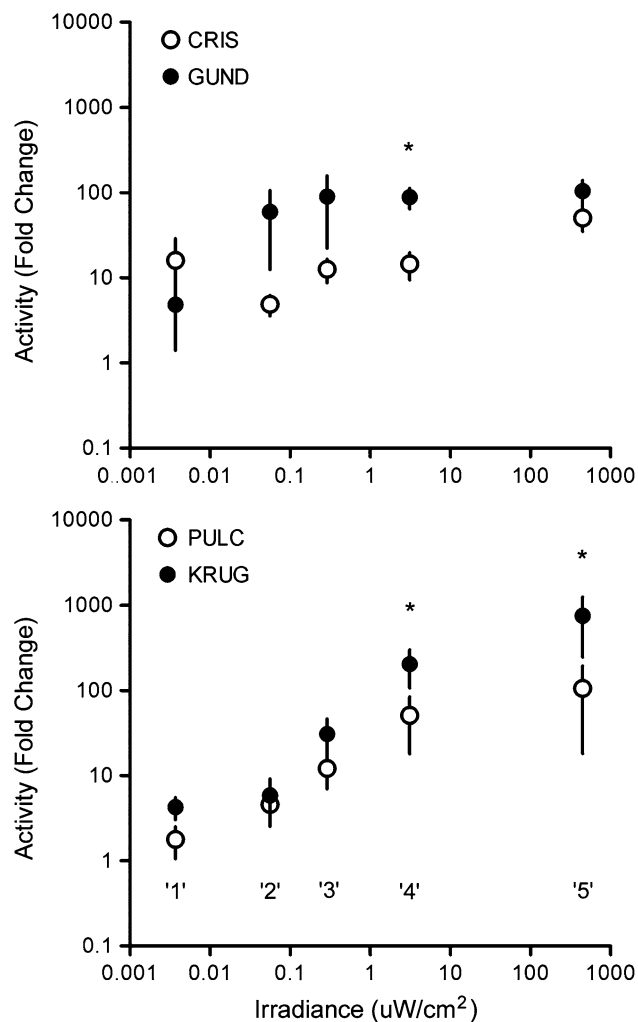


Fig. 5 Induction of activity during the night by light pulses. Increase in locomotor activity (fold change) during the light pulse period (ZT 15–17) is plotted against pulse irradiance. Fold change was calculated relative to individual mean baseline ZT 15–17 activity. Irradiances are represented by numbers 1–5 (as per the text and Fig. 3), shown below actual irradiance in $\mu\text{W}/\text{cm}^2$. Trunk-ground (*upper panel*) and grass-bush ecomorphs (*lower panel*) are plotted separately. Species names are abbreviated as in Fig. 4. Error bars represent standard error of the mean. Significant differences (orthogonal comparisons following two-way ANOVA, $\alpha = 0.05$) between pairs of species at a single irradiance are indicated by asterisks

effect of light pulse treatment ($p < 0.0001$) and a significant 'treatment \times species' interaction ($p = 0.0329$), but no significant main effect of species ($p = 0.2833$). These results indicate that light treatments induced activity in an irradiance-dependent fashion in all species and that species differed in the irradiance–response relationship. Within ecomorphs, responses differed significantly at some treatment irradiances. Post hoc analyses revealed significant differences between *A. cristatellus* and *A. gundlachi* at irradiance level '4' ($p = 0.0268$), as well as between *A. krugi* and *A. pulchellus* at irradiance levels '4' ($p = 0.0311$) and '5' ($p = 0.0468$).

Discussion

In this study, we compared photic masking of locomotor activity among four species of *Anolis* lizards. As expected for diurnal animals, light given at night induced locomotor activity in all species. The magnitude of the response differed among species and was dependent on the irradiance of light, with higher irradiances eliciting greater amounts of activity. The most important finding was that, within pairs of ecomorphologically similar species, masking responses were greater in the species occupying the more shaded habitat. This difference was most pronounced at higher treatment irradiances. We were able to detect these differences despite the large individual variation in masking responses (see error bars in Fig. 5). In nature, photic masking serves two main functions: (1) fine-tuning the timing of activity, ensuring an appropriate activity phase under a variety of conditions, and (2) modulating overall activity levels. Although we administered light pulses during the subjective night, the observed masking responses are likely to correlate with potential masking responses at other times of the day. Assuming that photic masking is equally important among shade-dwelling and sun-dwelling species, the differences in photosensitivity suggest that photic masking—a non-visual response to light—is adapted to the photic habitat. Whether this results from heritable variation in masking pathways or developmental plasticity remains to be determined.

Activity levels

Overall activity levels differed between ecomorphs, but not between species within ecomorph categories (see Fig. 4). Although activity per se was not the main focus of our study, it is important to consider activity levels in relation to the analysis of masking responses. Recorded activity levels, of course, are influenced by the behavioral recording equipment, which was developed specifically for long-term automated recording of locomotor activity in small (~ 1 – 10 g) animals. The activity sensor threshold was set at the same level for all activity recording enclosures. Considering the size differences among ecomorphs (mean body masses are ~ 1.5 – 2.5 and 7 – 8 g for grass-bush and trunk-ground species, respectively; Losos 1990), differences in detection may account for some of the observed variation in general activity levels. Whether the amount of activity exhibited in the behavioral enclosures is indicative of activity levels in nature is unknown, but it is important to emphasize that species of the same ecomorph type did not differ in baseline activity levels (Fig. 4). In analyzing masking responses, we focused on intra-ecomorph comparisons to avoid confounding photic niche with known behavioral differences among ecomorphs. Since there were no differences in baseline activity within ecomorphs, the

comparisons of masking responses (calculated relative to baseline ZT 15–17 activity levels) were not confounded by general differences in activity. Furthermore, masking responses were normalized against baseline ZT 15–17 activity within individuals.

Experimental versus natural light levels

Across the day, natural light levels span a range of approximately 6–8 orders of magnitude, mostly due to the dramatic changes at sunrise and sunset (Lythgoe 1979). The irradiances of the light pulse treatments (spanning 6 orders of magnitude) correspond to natural light levels, although caution must be used in such comparisons since the spectral distribution of natural light differs from the fluorescent lighting used in our experiments. Species differed significantly in their response to the brightest light pulses ('4' and '5'), which roughly correspond to natural light levels when the sun is at the horizon (sunrise/sunset) or slightly above it, depending on microhabitat (see below). Photoc masking is most likely to influence the timing of behavior during these light transition periods, at least theoretically. To avoid premature arousal in the dark hours of early morning, clock-timed locomotor activity in diurnal animals is typically a bit delayed with respect to dawn. Actual arousal time is fine-tuned by photic masking such that it occurs upon exposure to early morning sunlight, at some critical irradiance threshold. For the species used in this study, habitat irradiances differ by up to 2–3 orders of magnitude, with *A. pulchellus* inhabiting the most brightly illuminated environment (daytime irradiance $\sim 10^3 \mu\text{W}/\text{cm}^2$) and *A. gundlachi* the least illuminated (daytime irradiance $\sim 10^0 \mu\text{W}/\text{cm}^2$) (unpublished data; see also Fleishman et al. 1997). Light pulse '4' corresponds to habitat irradiances ranging from deep twilight (e.g., in *A. pulchellus* habitat) to sunrise/sunset levels (e.g., in *A. gundlachi* habitat). Light pulse '5' corresponds to sunrise/sunset levels in brightly illuminated habitats, but exceeds typical daytime light levels in more shaded habitats. Thus, the differences we observed among species may reflect different thresholds for photic masking during the early morning and/or evening, in accordance with differences in irradiance levels in the microhabitat. Interestingly, twilight periods are also thought to be critical in photoentrainment of the circadian clock (i.e., the adjustment of its period), which may be influenced by the same set of photoreceptors involved in masking responses (Peirson et al. 2009). A full description of the progressive changes in irradiance in the microhabitats of various *Anolis* species, along with observations of the times at which anoles transition between rest and arousal, would be useful in providing a natural context within which to understand photic masking and would lead to more refined investigations of non-visual sensory ecology.

In addition to the role of masking in fine-tuning the timing of activity onset and offset, light also modulates overall levels of activity. The dimmest light pulses are similar in total irradiance to moonless starlight/dim moonlight (light pulse level '1'), moonlight ('2'), and full moonlight/deep twilight ('3'). These light pulses had moderate effects on activity (up to \sim tenfold increase) in *A. cristatellus*, *A. krugi* and *A. pulchellus*, and large effects in shade-dwelling *A. gundlachi* (up to \sim 100-fold increase). Although *Anolis* are typically inactive at night, these results suggest that nocturnal light sources could play a role in modulating behavior. For example, the induction of locomotor activity at night could be important for predator-avoidance behaviors, such as the choice of sleeping perch or the fidelity to a given perch: a lizard illuminated by the moon might move to a different perch to avoid being spotted by nocturnal predators.

Thermal considerations

As with most measures of reptile performance, photic masking is likely to be temperature-dependent, and this dependency could vary among species. In *Anolis*, photic habitats are generally correlated with thermal preferences. Preferred daytime body temperatures for the species used in this study are, approximately: 30°C *A. cristatellus*, 26°C *A. gundlachi*, (Hertz et al. 1994), 32°C *A. pulchellus*, and 29°C *A. krugi*, (Rand 1964). Behavioral testing temperatures (24.4°C for *A. gundlachi*, 27.8°C for all others) were within the range of temperatures voluntarily selected by animals during the day in the field (Rand 1964); i.e., testing conditions were permissive of "normal" activity levels. While variation in thermal optima did not drive any large differences in baseline activity levels, the photic induction of locomotor activity may vary according to thermal preferences and experimental temperatures.

In our experiments, it is possible that in addition to being a photic stimulus, the light pulses provided a temperature cue (0.6°C increase, see "Methods"), that the animals responded to by increasing their activity. The threshold for thermal masking responses in anoles is unknown, and while we cannot exclude the possibility that a temperature cue contributed to the induction of locomotor activity, it is not sufficient to explain our findings. As can be seen in the actograms (Fig. 3), most animals responded to the light pulse within \sim 15 min, whereas the temperature records indicate that any increase due light (+0.6°C max) took \sim 45–60 min.

Non-visual photosensory ecology

Circadian synchronization and masking are both influenced by specialized non-image forming photoreceptors that

function as irradiance detectors (Peirson et al. 2009). Some of these non-visual photoreceptors are found in the eyes, but in non-mammalian vertebrates they can be found in other photoreceptive organs such as the pineal gland. Nocturnal species tend to be more sensitive to photic masking and photoentrainment of the circadian clock than diurnal animals, but virtually nothing more is known about the sensory ecology of non-visual photoreception. In contrast, visual ecologists have uncovered a number of photoreceptor adaptations that facilitate vision in particular photic environments (Walls 1942; Lythgoe 1979, 1984; Peirson et al. 2009). Our findings may point to another set of adaptations, those associated with irradiance detection, facilitating the divergence of species in multiple photic niches. In the last few years, numerous advances have been made in understanding the physiology of non-visual photoreception (Do and Yau 2010), laying the groundwork for future investigations of their function with respect to environmental conditions.

The parietal eye and pineal organ of lizards are photosensitive and could play a role in mediating behavioral masking to light. In anoles, the pineal gland contains a circadian clock that controls locomotor activity rhythms via the hormone melatonin, which is produced by the pineal during the night (Hyde and Underwood 2000). Although melatonin entrains the rhythm in locomotor activity, it probably does not directly control activity levels. Melatonin administration inhibits activity under some conditions (Hyde and Underwood 2000), but on the other hand, high melatonin levels have been observed during the late afternoon, when animals are still active (Underwood and Hyde 1989). Furthermore, light given at night does not strongly suppress melatonin levels in *Anolis* species (Underwood 1986; Moore and Menaker 2011), as it does in many other vertebrates. In contrast, the current data demonstrate that locomotor activity is strongly induced by light given at night. Thus, melatonin does not appear to be directly involved in the masking responses we observed.

In summary, the direct photic induction of locomotor activity, a non-visual response to light, is correlated with photic niche. Such a correlation suggests that the fine-tuning of activity time by light is adapted to the photic habitat. *Anolis* lizards, with their extensive radiation into different photic niches and their usefulness as a model system for integrative biology, provide an excellent opportunity to explore the functional relationships between non-visual photoreception, behavior, ecology, and evolution.

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References

- Chiesa JJ, Aguzzi J, Garcia JA, Sarda F, de la Iglesia HO (2010) Light intensity determines temporal niche switching of behavioral activity in deep-water *Nephrops norvegicus* (Crustacea: Decapoda). *J Biol Rhythm* 25:277–287
- Collin SP (2010) Evolution and ecology of retinal photoreception in early vertebrates. *Brain Behav Evol* 75:174–185
- Do MT, Yau KW (2010) Intrinsically photosensitive retinal ganglion cells. *Physiol Rev* 90:1547–1581
- Endler JA (1992) Signals, signal conditions, and the direction of evolution. *Am Nat* 139(Suppl):S125–S153
- Endler JA (1993) The color of light in forests and its implications. *Ecol Monogr* 63:2–27
- Endler JA, Westcott DA, Madden JR, Robson T (2005) Animal visual systems and the evolution of color patterns: sensory processing illuminates signal evolution. *Evolution* 59:1795–1818
- Fernandez-Duque E, de la Iglesia H, Erkert HG (2010) Moonstruck primates: owl monkeys (*Aotus*) need moonlight for nocturnal activity in their natural environment. *PLoS One* 5:e12572
- Fleishman LJ, Bowman M, Saunders D, Miller WE, Rury MJ, Loew ER (1997) The visual ecology of Puerto Rican anoline lizards: habitat light and spectral sensitivity. *J Comp Physiol A* 181:446–460
- Fraker ME (2008) The influence of the circadian rhythm of green frog (*Rana clamitans*) tadpoles on their antipredator behavior and the strength of the nonlethal effects of predators. *Am Nat* 171:545–552
- Fu Y, Liao HW, Do MT, Yau KW (2005) Non-image-forming ocular photoreception in vertebrates. *Curr Opin Neurobiol* 15:415–422
- Gerrish GA, Morin JG, Rivers TJ, Patrawala Z (2009) Darkness as an ecological resource: the role of light in partitioning the nocturnal niche. *Oecologia* 160:525–536
- Gotelli NJ, Graves GR (1996) Null models in ecology. Smithsonian Institution Press, Washington
- Hattar S, Lucas RJ, Mrosovsky N, Thompson S, Douglas RH, Hankins MW, Lem J, Biel M, Hofmann F, Foster RG, Yau KW (2003) Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature* 424:76–81
- Hertz PE, Fleishman LJ, Armsby C (1994) The influence of light intensity and temperature on microhabitat selection in two *Anolis* lizards. *Funct Ecol* 8:720–729
- Hoffmann K (1969) Zum Einfluss der Zeitgeberstärke auf die Phasenlage der synchronisierten circadianen Periodik. *Z Vergl Physiol* 62:93–110
- Huey RB, Webster TP (1976) Thermal biology of *Anolis* lizards in a complex fauna: the *Cristatellus* group on Puerto Rico. *Ecology* 57:985–994
- Hutchinson GE (1957) Concluding remarks. In: Cold spring harbor symposia on quantitative biology, vol 22, pp 415–427

- Hyde LL, Underwood H (2000) Effects of melatonin administration on the circadian activity rhythm of the lizard *Anolis carolinensis*. *Physiol Behav* 71:183–192
- Kronfeld-Schor N, Dayan T (2003) Partitioning of time as an ecological resource. *Annu Rev Ecol Evol Syst* 34:153–181
- Kronfeld-Schor N, Dayan T, Elvert R, Haim A, Zisapel N, Heldmaier G (2001) On the use of the time axis for ecological separation: diel rhythms as an evolutionary constraint. *Am Nat* 158:451–457
- Leal M, Fleishman LJ (2002) Evidence for habitat partitioning based on adaptation to environmental light in a pair of sympatric lizard species. *Proc Biol Sci* 269:351–359
- Levri EP, Lunnen SJ, Itle CT, Mosquea L, Kinkade BV, Martin TG, DeLisser MA (2007) Parasite-induced alteration of diurnal rhythms in a freshwater snail. *J Parasitol* 93:231–237
- Loew ER, Fleishman LJ, Foster RG, Provencio I (2002) Visual pigments and oil droplets in diurnal lizards: a comparative study of Caribbean anoles. *J Exp Biol* 205:927–938
- Losos JB (1990) Ecomorphology, performance capability, and scaling of West-Indian *Anolis* lizards—an evolutionary analysis. *Ecol Monogr* 60:369–388
- Losos JB (2009) Lizards in an evolutionary tree: the ecology of adaptive radiation in anoles. University of California Press, Berkeley
- Lythgoe JN (1979) The ecology of vision. Oxford University Press, New York
- Lythgoe JN (1984) Visual pigments and environmental light. *Vis Res* 24:1539–1550
- Maan ME, Seehausen O (2010) Mechanisms of species divergence through visual adaptation and sexual selection: perspectives from a cichlid model system. *Curr Zool* 56:285–299
- Marques MD, Waterhouse JM (1994) Masking and the evolution of circadian rhythmicity. *Chronobiol Int* 11:146–155
- Moore AF, Menaker M (2011) The effect of light on melatonin secretion in the cultured pineal glands of *Anolis* lizards. *Comp Biochem Physiol A* 160:301–308
- Mrosovsky N (1999) Masking: history, definitions, and measurement. *Chronobiol Int* 16:415–429
- Narendra A, Reid SF, Hemmi JN (2010) The twilight zone: ambient light levels trigger activity in primitive ants. *Proc R Soc B* 277:1531–1538
- Nicholson K, Glor R, Kolbe J, Larson A, Hedges S, Losos JB (2005) Mainland colonization by island lizards. *J Biogeogr* 32:929–938
- Orpwood JE, Griffiths SW, Armstrong JD (2006) Effects of food availability on temporal activity patterns and growth of Atlantic salmon. *J Anim Ecol* 75:677–685
- Page TL (1989) Masking in invertebrates. *Chronobiol Int* 6:3–11
- Peirson SN, Halford S, Foster RG (2009) The evolution of irradiance detection: melanopsin and the non-visual opsins. *Phil Trans R Soc Lond B* 364:2849–2865
- Rand AS (1964) Ecological distribution in anoline lizards of Puerto Rico. *Ecology* 45:745–752
- Reebs SG (2002) Plasticity of diel and circadian activity rhythms in fishes. *Rev Fish Biol Fisher* 12:349–371
- Underwood H (1986) Light at night cannot suppress pineal melatonin levels in the lizard *Anolis carolinensis*. *Comp Biochem Physiol A* 84:661–663
- Underwood H, Hyde LL (1989) The effect of daylength on the pineal melatonin rhythm in the lizard *Anolis carolinensis*. *Comp Biochem Physiol A* 94:53–56
- Valeix M (2009) Does the risk of encountering lions influence African herbivore behaviour at waterholes? *Behav Ecol Sociobiol* 63:1483–1494
- Walls GL (1942) The vertebrate eye and its adaptive radiation. Cranbrook Institute of Science, Bloomfield Hills
- Warrant E (2004) Vision in the dimmest habitats on earth. *J Comp Physiol A* 90:765–789
- Williams EE (1972) The origin of faunas. Evolution of lizard congeners in a complex island fauna: a trial analysis. *Evol Biol* 6:47–89
- Williams EE (1983) Ecomorphs, faunas, island size, and diverse endpoints in island radiations of *Anolis*. Harvard University Press, Cambridge
- Ziv Y, Abramsky Z, Kotler BP, Subach A (1993) Interference competition and temporal and habitat partitioning in two gerbil species. *Oikos* 66:237–246