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Animal behaviour

Do I stay or do I go? Shifts in perch use by lizards during morning twilight suggest anticipatory behaviour

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Anticipatory behaviour is the expectation of a near-future event based on information processed in the past and influences an animal's tactical decisions, particularly when there are significant fitness consequences. The grass lizard (*Takydromus viridipunctatus*) perches on blades of grass at night which likely reduces the probability of predation by terrestrial predators such as snakes, rodents and shrews. During twilight (starting 30 min before sunrise), they move from above the grass to within grass clumps and this is thought to afford the lizard protection while reducing detection by avian predators. Here, we examined how lizards shift their behaviour as a function of visual detectability to their primary predator, the cattle egret (*Bubulcus ibis*). We show that the lizards shift from their perch site during twilight at the earliest time at which egrets depart communal roosts. At the same time, visual modelling shows a dramatic increase in the detectability of the lizards to the visual system of egrets. Therefore, anticipatory behaviour in response to environmental cues acts to reduce predation risk as lizards become more conspicuous and predators become more active. Grass lizard anticipatory behaviour appears to be finely tuned by natural selection to adjust to temporal changes in predation risk.

1. Introduction

Predation, one of the most important agents of selection, has driven the evolution of anti-predator traits and tactics and frequently results in a coevolutionary arms race between predator and prey [1,2]. Potential prey is predicted to adjust their behaviour in relation to perceived predation risk; this form of anticipatory behaviour is shaped by experience from the past and anticipation of near-future events [3]. Anticipatory behaviour entails responding to cues or past experience (e.g. consistent temporal events that may be threatening such as a predator or conspecific rival that travels the same path daily) and responding with appropriate behavioural decisions. For example, when predatory curly tailed lizards (*Liocephalus carinatus*) were introduced onto small islands in the Caribbean containing brown anoles (*Anolis sagrei*), anoles became more arboreal, reducing their probability of an encounter with the terrestrial curly tailed lizards [4]. Conversely, many animals adjust their behaviour to reduce predation risk even if a potential predator is not readily visible. For example, velvet geckos will trade-off temperature against predation risk if there are chemical cues of a predator (snake) in their environment [5].

A common anti-predator tactic in many species is the use of camouflage. In lizards, many species are cryptic and closely match their background or even change colour in response to a specific predator type (e.g. chameleons, [6,7]).

The degree to which an organism is conspicuous against its background will also dictate its behaviour and how closely it will allow a potential predator to approach. On the other hand, the degree to which an individual is cryptic also depends on the habitat light environment and may change as the amount of downwelling light changes. This can be particularly abrupt at dawn or dusk when ambient light levels change relatively rapidly. Potential prey that rely on crypsis are expected to change their tactics when habitat light conditions change sharply, but this is rarely considered.

Here, we used the green-spotted grass lizard (*Takydromus viridipunctatus*) and the cattle egret (*Bubulcus ibis*), a well-studied prey–predator system, to examine how lizards adjust their behaviour in relation to temporal changes in predation risk (anticipatory behaviour). *Takydromus viridipunctatus* is a diurnal lacertid lizard which mainly occurs in early successional grasslands (figure 1*a*). Our 7-year study with greater than 20 000 capture records showed that avian predators are a major threat to this small-sized lizard [8,9]. In particular, the mortality of this lizard was significantly correlated with cattle egret abundance in the same month, implicating this bird as the major diurnal predator of the lizard.

The grass lizard is highly specialized and is adapted to living in grasslands and uses grass clumps for refuge. Their slender, elongated body shape and extremely long tail (*ca* 2.5 times longer than the body) allows them to perch on the surface of blades of grass. The lizard is cryptic when hiding in, or even on, the grass, making detection by birds challenging. At night, they sleep on the surface of the grass (figure 1*a*; electronic supplementary material, figure S1), which is thought to be a behavioural adaptation against nocturnal predators on the ground (e.g. rodents, shrews or snakes) because the leaves cannot support the weight of these predators, thereby facilitating early detection of an approaching threat (reviewed in Mohanty *et al*. [10]).

Nevertheless, the lizards become more detectable during twilight as the light environment changes. At the same time, egrets leave communal roosts and begin foraging around daybreak. During this time, changing ambient light is predicted to reduce crypsis. We tested the prediction that lizards show anticipatory behaviour by abandoning their night-time perches prior to the onset of foraging by egrets, at a time (twilight) when they become significantly more conspicuous to the avian visual system and move to a habitat where they are less conspicuous and less accessible.

2. Material and methods

(a) Lizard activity patterns

We recorded activity patterns of the lizards in both non-breeding (September and October 2018) and breeding (May and June 2019) seasons at Hualin Experimental Forest (24.890° N, 121.567° E), a 92 ha protected region in New Taipei City, northern Taiwan. The habitat consisted of a mosaic landscape with secondary hardwood forest and grassland patches. We surveyed for sleeping lizards starting at midnight. Upon locating a lizard, we set up a camera (KeepGuard, KG780NV) *ca* 60 cm from the lizard and set it to record video 10 s min^{-1} from 1 h before, to 1 h after, sunrise.

From the camera footage, we scored behaviours as follows: (i) eyes open; (ii) head movement: head-turning or any body movements less than 1× snout–vent length (SVL); and (iii) body movement: movements more than 1 × SVL from the original position. If the focal lizard was disturbed either by a predator or by another lizard during the 2 h (4% of videos), the record was excluded from analyses. We standardized the time of a behaviour by relating it to the sunrise time of that date (i.e. how many minutes before or after sunrise), which was available from the Central Weather Bureau, Taiwan.

Sex was determined by the presence (males) or absence (females) of nuptial green spots on the lateral sides of the body. We acquired simultaneous air temperature continuously recorded every minute by Hualin Weather Station (located just within the grassland where the experiment was conducted) and Cyuchih Weather Station (3970 m from the site).

(b) Egret activity patterns

The cattle egret is a highly abundant avian predator widely distributed across the lizard's habitat. We focused our monitoring on the activity pattern of a nearby roosting colony (24.936°N, 121.713°E) consisting of more than 100 nesting pairs because this large sample was the most efficient way of quantifying egret activity. The time of sunrise, light environment and weather conditions were identical to the primary lizard study area. We surveyed the egrets from 1 h before to 1 h after sunrise in May and June 2019 (the breeding season of the lizards) and in October and November 2020 (the non-breeding season). We monitored the colony using binoculars and recorded the moment when every cattle egret departed the colony during twilight for their first foraging trip. Similarly, we standardized the time of egret departure referring to the sunrise time of that date (i.e. how many minutes before or after sunrise).

(c) Ambient light measurements

During the twilight period, downwelling light (surface power density, $\mu\text{W cm}^{-2}\,\text{s}^{-1}$) was measured every minute using a power meter (Newport 1935-C, CA, USA) with a photodiode detector (Newport 918D-SL-OD1R) at the exact moment that behavioural observations were made. The sample rate was 0.1 ms, with 10 000 samples averaged to produce a mean. The power meter was set to detect wavelengths of 416/478/542/607 nm, which indicated the maximum absorbance of the four cones: VS/SWS/MWS/LWS in v-type eyes of birds, including cattle egrets [11–13].

(d) Visual modelling of the avian predator

To model how conspicuous a lizard was in its environment to an egret, we quantified downwelling light (irradiance; vertical from the land surface) by measuring absolute irradiance ($\mu\text{W m}^{-2}\,\text{s}^{-1}\,\text{nm}^{-1}$) every minute using a spectrometer (Ocean Optics Flame UV–VIS model, FA USA) with an attached fibreoptic probe (Ocean Optics QP600-2-SR) and a cosine-correcting probe (Ocean Optics CC-3-UV) in conjunction with the power meter. We then measured the spectral reflectance of live lizards (figure 1*a*) and grasses in both the breeding (10 males and seven-females) and non-breeding (10 males and 10 females) seasons using a reflectance probe (Ocean Optics QR600-7-SR-125F) and a portable spectrometer (Ocean Optics Flame UV–VIS model). Finally, we used the receptor noise-limited (RNL) model [14–17] to estimate chromatic and luminance discrimination thresholds for known stimuli. When the light environment was dim, the photon shot noise was used when calculating the discrimination thresholds [17–19]. The contrast between lizards and the grasses was calculated using the magnitude of 'just noticeable difference' (JND) as the unit, where 1 JND indicates the detection threshold [20]. See electronic supplementary material for details.

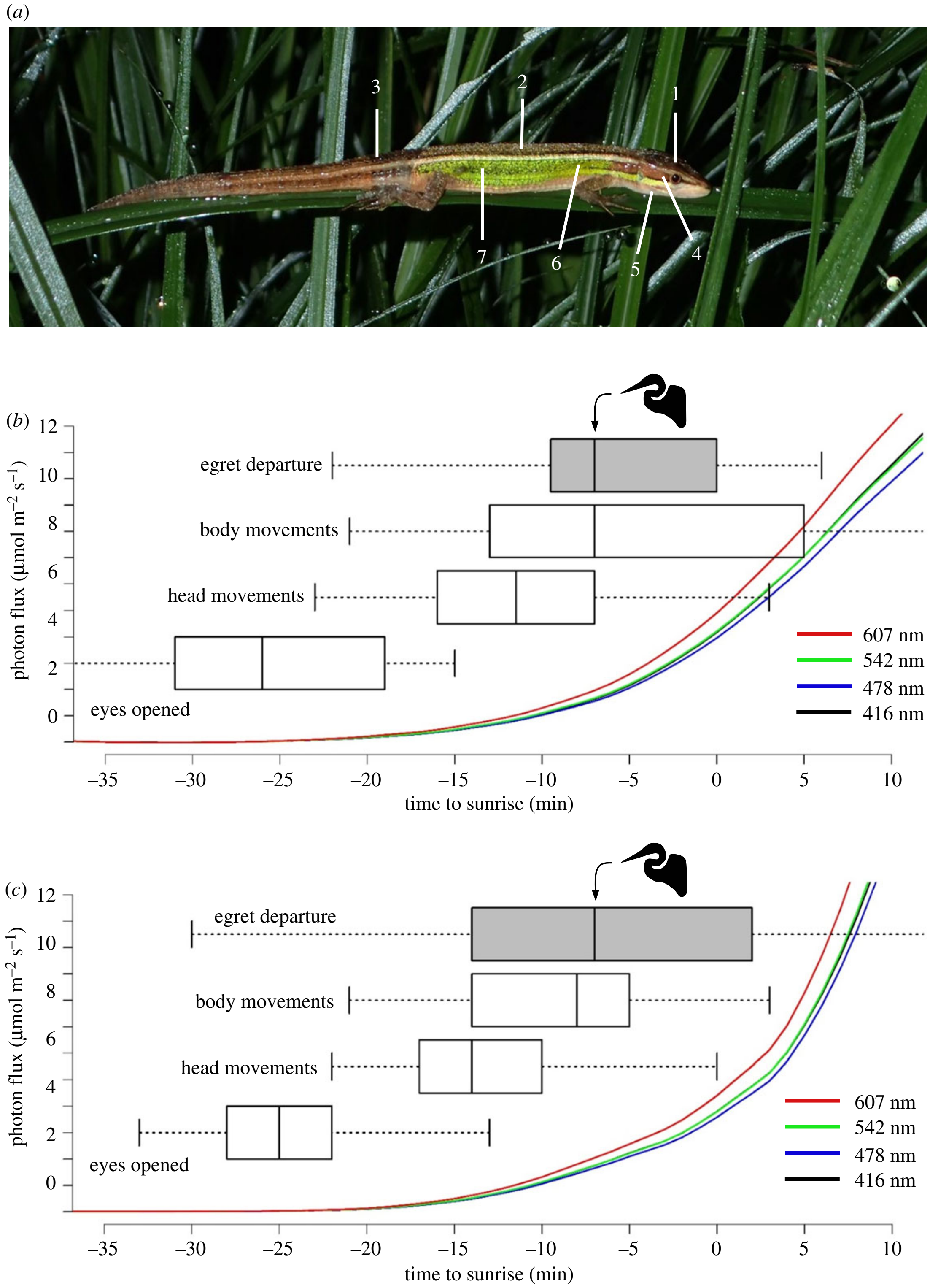

Figure 1. (*a*) A typical perching posture of a grass lizard at night, and the body regions where reflectance spectra were measured, including head, dorsum, tail, cheek, lower jaw, anterior flank and posterior flank. (*b*,*c*) The temporal behavioural sequence of grass lizards as they awoke, and the median time at which cattle egrets departed from their roosts in relation to ambient light of four wavelengths in the non-breeding season (*b*) and in the breeding season (*c*). The vertical lines in the boxplots are the medians of each behaviour.

3. Results

(a) Body movements of lizards congruent to egrets' departure

Unless disturbed, all the lizards showed a predictable sequence of behaviours: their eyes opened first, followed by head movements and then body movements (figure 1*b*,*c*). The majority (greater than 95%) of these behaviours occurred between 30 min before and 5 min after sunrise (hereafter referred as −30 min or +5 min, respectively).

The timings of their eyes opening were similar between the non-breeding (median = −26 min; figure 1*b*) and the breeding (median = −25 min; figure 1*c*) seasons ($p = 0.4355$). The timing of body movements was also similar: median of the non-breeding season = −7 min, while the median of the breeding season = −8 min ($p = 0.1946$). Body movements of the lizards precisely fitted the median of the first foraging

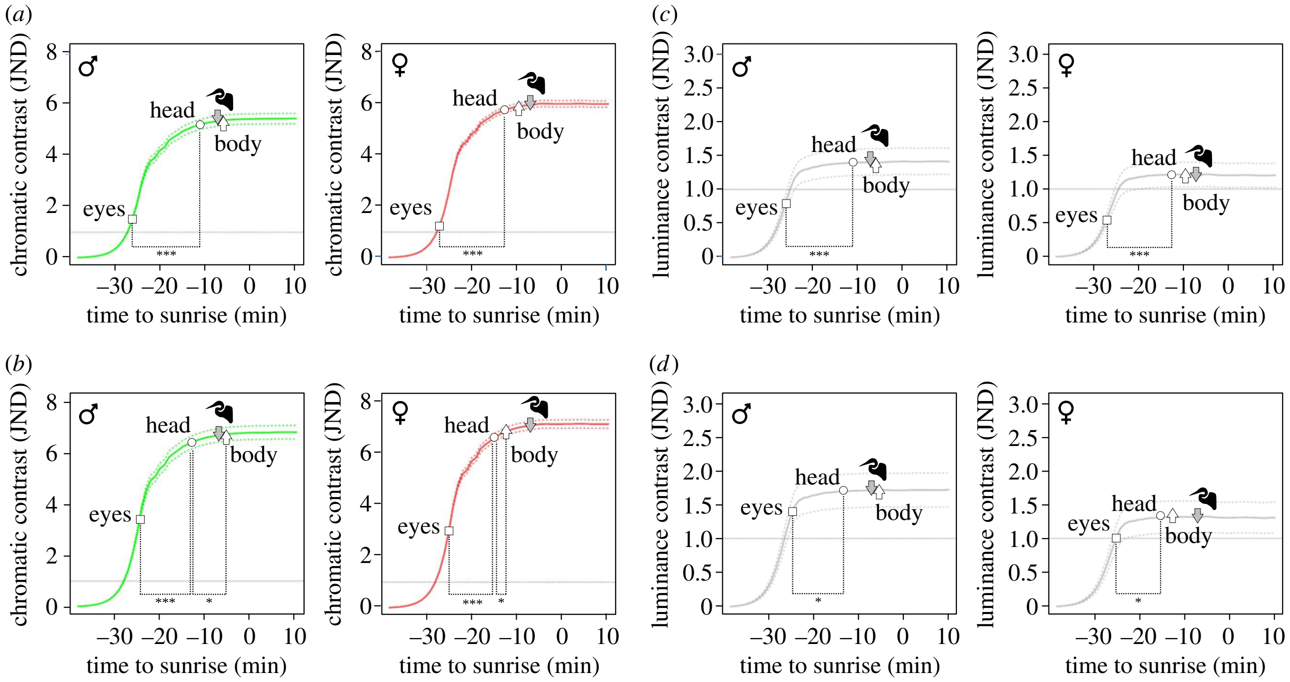

Figure 2. Chromatic (*a*,*b*) and luminance (*c*,*d*) contrast to grass (means and 95% confidence intervals; using JND as the unit) calculated by the RNL model [14] in the non-breeding season (*a*,*c*) and the breeding season (*b*,*d*). Females moved earlier than males in the breeding season because they were more vigilant during this period. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.005$.

behaviour of the egrets, occurring at −7 min in both seasons. Nevertheless, head movements (the second stage of the behaviours) occurred significantly earlier (−14 min) in the breeding compared to the non-breeding (−11.5 min) season ($p = 0.04961$). Furthermore, females initiated body movements significantly earlier (*ca* 7 min; $p = 0.0094$) than males, but only in the breeding season (electronic supplementary material, figure S2).

(b) Relationship between conspicuousness and behaviour

Although a total of seven lizard body regions (figure 1*a*) were measured (electronic supplementary material, figures S3 and S4), we focused on the contrasts of dorsal and lateral coloration to the grass background (electronic supplementary material, figure S5) because these areas are critical for visual predators. Males have green flanks during the breeding season and brown in the non-breeding season; while females remained brown all year round (electronic supplementary material, figure S5). Because the grass was green, females were more conspicuous against the grass than males in the breeding season. The remaining regions (head, dorsal, tail, cheek and lower jaw) did not show significant sex differences in chromatic contrast in either the breeding or non-breeding seasons (electronic supplementary material, figures S3 and S4).

The lizards woke up and initiated their movements at the period when visual contrast in the egrets' visual system increased. When chromatic contrast was modelled against an egrets' visual system (figure 2*a*,*c*), it increased dramatically during −35 to −15 min, then started to converge at around 5–7 JNDs after −15 min. Lizards opened their eyes when the chromatic contrasts obviously changed (−25 min), and then moved to the ground precisely at the moment when egrets began their earliest foraging (−7 min). This is also the moment when the chromatic contrasts became large enough ($\mathrm{JND} > 1$) for the egrets to detect the lizards. An identical pattern could also be found in luminance contrast (figure 2*b*,*d*), with the values converging at 1.2 × to 2.4 × JNDs.

4. Discussion

The most interesting finding from this study was the synchronous behaviours between lizards and egrets. In the early morning, the lizards opened their eyes when the ambient light conditions started to change (25 min before sunrise). This behaviour was triggered by ambient light instead of ambient temperature because temperature did not affect this behaviour (see electronic supplementary material). The lizards left their perch at about the same time that the egrets left their roost. Although this congruence is predictable given their predator–prey relationship, this study is the first temporal quantification of this link and supports the hypothesis that lizards are showing anticipatory behaviour.

Anticipatory behaviour is an interesting notion because it suggests decision-making based on anticipation or awareness of a near-future event [3]. When egrets depart their roosts at dawn, this constitutes a consistent and repetitive behaviour with the potential to act as a reliable source of information to the lizards, especially because it correlates with ambient light conditions, thereby providing a reliable environmental cue. By modelling chromatic and luminance contrast from the perspective of an avian predator, we demonstrated that within a short time interval, although ambient light increased gradually (figure 1), their conspicuousness increased dramatically (figure 2). The most crucial period was the half hour before sunrise, when the contrast between the lizard and the background increased from less than 1 JND (not detectable) to more than 5 JNDs (easily detectable), which led to this shift in strategy. This change in conspicuousness greatly increased the lizards' risk of predation, prompting the lizards to move into the grass clumps where they would be much harder to be detected and captured. This adaptive behaviour is similar to what we see in other systems when animals adjust their behaviour according to changing risk [1,21,22], although we are unaware of examples of switches in behaviour associated with diel changes in conspicuousness.

Moving from the top of a grass clump to into the grass clump is a low-cost behaviour with potentially significant fitness benefits, making the lizards less detectable and also less accessible. It is very unusual for diurnal lizards to emerge or waken during twilight, prior to sunrise and many hours before their activity peak. Most diurnal lizards only initiate activity after sunrise, because of the role of the light-sensitive parietal eye in determining circadian rhythms [23], although activity is also temperature-dependent [24]. This is, therefore, a rare example of a lizard species shifting habitat during morning twilight, prior to normal daily activity (see [25] for the few exceptions). Lizards are more commonly known for either selecting a single predator-safe refuge or perch, or, reacting to a direct predator threat and then seeking out either a refuge or a different microhabitat in which it may be more concealed [26,27]. In the case of brown anoles (*A. sagrei*), daily activity patterns differ between habitats with or without predators [22].

Interestingly, both males and females showed a higher alertness (head movement) in the breeding season compared to the non-breeding season (figure 1); possibly due to higher predation risk [8], or their higher residual reproductive value in this period [28]. Females also move into grass clumps earlier than males in the breeding season (electronic supplementary material, figure S2), and this could be explained by their higher contrast against the grass background (electronic supplementary material, figure S5). Apart from this, the lower mobility and higher residual reproductive value of a pregnant female in the breeding season are also possible explanations for their early awareness.

In summary, we describe a finely tuned predator–prey system in which potential prey show anticipatory behaviour by compensating for increased conspicuousness to predators at the moment predators initiate activity (during morning twilight). As the light environment changes during sunrise, the lizards become more conspicuous, which makes them vulnerable to a new suite of predators—particularly cattle egrets. Our observations suggest a system in which lizards have predator-specific anti-predator responses which shift in response to risk. Furthermore, this potential anticipatory behaviour by grass lizards makes it a novel example of the effects of predation pressure on decision-making and acts as a selection agent driving a predator–prey arms race.

Ethics. All the treatment procedures followed protocols approved by Institutional Animal Care and Use Committee (IACUC), National Taiwan Normal University (license no. 106023) and were performed in accordance with the local law of the Wildlife Conservation Act in Taiwan.

Data accessibility. The data are provided in electronic supplementary material [29].

Authors' contributions. C.-W.C. and S.-M.L. conceived the study; C.-W.C. and E.-C.Y. conducted the experiments; C.-W.C. and M.J.W. analysed the data; C.-W.C., M.J.W., E.-C.Y. and S.-M.L. wrote the manuscript. All authors approved the final version and agree to be held accountable for the content.

Competing interests. We declare we have no competing interests.

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