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Interspecific Differences in Treefrog Response to Artificial Light at Night and Spectral Manipulation

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Interspecific Differences in Treefrog Response to Artificial Light at Night and Spectral Manipulation

Understanding the effect of artificial light at night (ALAN) on biodiversity is a key research topic of the 21st Century. Evidence suggests that LED lighting may be particularly disruptive due to strong short-wavelength emissions. Spectral manipulation of LED lighting to reduce these emissions may mitigate some disturbance, although further research is required to assess its value in comparison with other techniques. The impact of LED lighting has been documented for many species, however, amphibians remain relatively under-studied. Amphibians may be particularly sensitive to the effects of ALAN due to specialised vision adapted for low-light environments and reduced mobility. Artificial lighting is known to affect many aspects of their biology, including reproduction, development, movement and foraging. Amphibians may be particularly vulnerable to LED lighting due to increased sensitivity to short-wavelength light. Despite this, the effect of spectral manipulation of existing light sources has not been tested for these species. This study was designed to explore the impact of LED lighting on anuran behaviour and assess the success of spectral manipulation as mitigation.

It was hypothesised that: illumination would delay emergence from daytime refugia, spectral manipulation would partially mitigate this delay and that two species would differ in their response to lighting. In a laboratory setting, emergence likelihood and response speed under typical "cool white" LEDs, filtered white LEDs (removal of wavelengths <500nm) and ambient dark conditions were compared for Cuban treefrogs (*Osteopilus septentrionalis*) and American green treefrogs (*Dryophytes cinereus*). Generalised linear mixed models (GLMMs) were constructed to examine the effect of eight explanatory variables on observed variation. Illumination affected Cuban treefrog behaviour, but not green treefrogs. Cuban treefrogs showed two diverse activity patterns, either emerging quickly or remaining immobile, demonstrating adaptive behavioural flexibility of this species. Greater resilience to evaporative water loss may explain why green treefrog activity was not influenced by artificial illumination. The filtered light source was no less disruptive than white light, suggesting that further research is required to develop reliable mitigation for amphibian habitats in order to minimise the effect of urbanisation on declining amphibian populations.

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<u>Chapter 1</u>

1: Ecological Light Pollution

The term "ecological light pollution" was first described by Longcore and Rich (2004), referring specifically to light which alters natural patterns of light and dark and has the potential to disturb interactions at many ecological levels (for example MacGregor *et al.* (2017)). Ecological light pollution which specifically effects the nocturnal environment is commonly referred to as ALAN. Artificial light at night (ALAN) is produced by a variety of sources but is predominately anthropocentric; often installed with the aim of being aesthetically pleasing (garden lighting), to advertise products (billboards) or increase neighbourhood safety (streetlighting, security lighting) (Gaston *et al.*, 2014). It may act to directly illuminate surfaces or scatter light skywards, increasing ambient illumination (skyglow) (Kyba *et al.*, 2011; Kyba *et al.*, 2012). Bennie *et al.* (2015) demonstrate that all ecosystems have experienced an increase in light pollution since 1996 and the worst affected are often of high conservation priority (such as mangrove, subtropical needleleaf and mixed forest). In fact, it has been found that almost two-thirds of Key Biodiversity Areas have artificially bright skies (Garrett *et al.*, 2020)

Natural cycles of light and darkness play a key role in gene expression (Hoadley *et al.* 2011), physiology (Walsh *et al.*, 2013) and behaviours such as daily movements (Beiswenger, 1977) biogeography (Tsakalakis *et al.*, 2018) and foraging (Wanless *et al.*, 1999). Through alteration of these diel light cycles ALAN may disturb these natural patterns (Gaston *et al.*, 2014), resulting in changes to behavioural patterns (Dias *et al.*, 2019), community structure (Pena *et al.*, 2008) and reduced reproductive ability (Touzot *et al.*, 2020). For example, in artificially lit environments blue tits (*Cyanistes caeruleus*) and great tits (*Parus major*) are reported to begin foraging earlier on winter mornings (Silva *et al.*, 2017) and common redshank (*Tringa totanus*) are reported to have enhanced night-time feeding ability (Dwyer *et al.*, 2013). An increased foraging period or improved foraging efficiency has the potential to increase the amount of food available to an individual which may have particularly strong benefits during harsh winters (Silva *et al.*, 2017). It is therefore important to identify the key methods by which natural light cycles are disturbed and to quantify the effects of ecological light pollution.

1.1: Skyglow

Skyglow arises when poorly designed lights emit a large portion of their light skywards which is then scattered throughout the atmosphere (Kyba *et al.*, 2012). The

deleterious effect of skyglow on astronomy (obscuring vision of stars) has long been recognised and quantified (Walker, 1970; Walker 1977), however, it is particularly difficult to quantify its ecological impacts (Kyba *et al.*, 2015). Perceived impacts include disruption of activity patterns (Brüning *et al.*, 2016) and nocturnal movements (Gathreaux, 1982; McLaren *et al.*, 2018; Beier, 1995), particularly when these are guided by celestial cues (Foster *et al.*, 2017). Additionally, Kupprat *et al.* (2020) have recently described that melatonin levels in Eurasian Perch (*Perca fluviatilis*) are reduced when exposed to low level (0.001-1lux) ALAN. It is therefore apparent that skyglow with a typical range of 0.03-0.55lux (Hänel *et al.*, 2018) may act to reduce immune system function, influence endocrine signals (including those driving reproduction (Brüning *et al.*, 2016)) and interfere with activity patterns; although it is acknowledged that the melatonin system of fish is understudied. These effects may also extrapolate to other species (Walsh *et al.*, 2013; Dimovski & Robert, 2018).

In general, the effect of skyglow on behaviour is largely understudied because most current research focuses on the impacts of direct illumination. These impacts may be considered similar, however, skyglow can affect areas far beyond the source, has the potential to mask celestial cues and is exacerbated by cloud cover (Kyba *et al.*, 2015). When it is considered that two thirds of the world's Key Biodiversity Areas are deemed to fall under artificially bright night skies (Garrett *et al.*, 2020) it becomes clear that the need to identify the ecological impacts of skyglow particularly is pressing.

1.2: Direct Illumination

The majority of current research focuses on the impacts of direct illumination produced by sources such as streetlamps, vehicles and security lighting (Gaston *et al.*, 2015), because these sources produce more intense light and are often associated with stronger impacts (Bennie *et al.*, 2016). Streetlighting is a dominant source of ALAN (Gaston *et al.*, 2014), as they are used widely to increase safety on roads at night. A trend in increasing the range of light wavelengths produced by these lamps (see Figure 1) has been observed during recent decades in the UK, with traditional low-pressure sodium (LPS) lights from the 1960's being replaced by high-pressure sodium (HPS) lamps to improve colour discrimination by humans (Gaston *et al.*, 2013; Gaston *et al.*, 2015). Light-emitting diodes (LEDs) are replacing existing lights, further increasing the breadth of wavelengths emitted and quickly becoming the lighting type of choice as they most closely represent the visible spectral radiance of daylight (Gaston *et al.*, 2015; Davies *et al.*, 2017).



Figure 1: Spectral Radiance of Common Streetlights. Spectral radiance of daylight (a), incandescent (b), low-pressure sodium (c), LED (d), mercury vapour (e), and fluorescent (f) light sources. From Gaston *et al.*, 2014).

1.3: Light-Emitting Diodies (LEDs)

Elvidge et al. (2010) describe LEDs as "solid-state light sources that generate light by electroluminescence" which differ from other commonly used light sources in the broad range of visible light wavelengths (typically 380-700nm for humans) produced (see Figure 1), minimal infrared output and their adaptability. The nature of LEDs allows manufacturers the opportunity to adjust proportions of red, green and blue (RGB) LEDs as well as manipulating phosphor coatings in order to produce light of different corrected colour temperatures (CCTs, unit=K). The popularity of LEDs can be largely attributed to their high energy efficiency, long lifetime, low heat output, customisability and broad output which acts to improve human vision at night (Gaston et al., 2014; Davies & Smyth, 2018). However, the broad range of wavelengths emitted by LEDs has the potential to affect multiple biological processes (Davies & Smyth, 2018) such as the "red" sensitive phytochrome response in plants (Bennie et al., 2016) and melatonin production (Brüning et al., 2016; Kupprat et al., 2020). Melatonin production is one process particularly sensitive to the short wavelength peak (see Figure 1) which is believed to be a particularly damaging characteristic of LED lighting (Davies & Smyth, 2018) since changing melatonin levels is linked to timing of seasonal reproductive cycles (Cleaver et al., 1991; Robert et al., 2015). All light suppresses melatonin production, however, blue light is acknowledged to be particularly disruptive for humans (West et al., 2011; Gabel et al., 2013), horses (Walsh et al., 2013) and marsupials

(Dimovski & Robert, 2018). Eurasian perch, however, seem most sensitive to longer wavelengths (Brüning *et al.*, 2016); though this trend is not observed for all fish species (Grubisic *et al.*, 2019). Many behaviours are also documented to be disturbed by exposure to short wavelength light in particular, including orientation (Cowan & Gries, 2009) and mating in moths (van Geffen *et al.*, 2015), sea-finding in turtle hatchlings (Rivas *et al.*, 2015) and emergence from roosts by bats (Spoelstra *et al.*, 2017). Fortunately, it is possible to manipulate the spectral output of LEDs and hence reduce the proportion of disruptive wavelengths emitted (Figure 2; Gaston *et al.*, 2015). This potential has led to an increasing body of research focused on identifying methods of mitigating the impacts of ALAN on organisms.



1.4: Mitigating ALAN Through Spectral Manipulation

A focus on researching the most disruptive wavelengths of ALAN is recommended by Gaston *et al.* (2015) with the target of advising the production of lighting units which cause minimal ecological damage whilst being functional for humans. It is generally considered that lights of a lower, "warmer" or "neutral" CCT are less disruptive to behavioural and ecological processes as these LEDs produce less blue light (see Figure 2). European Commission guidelines (2018) require that lights in urban green spaces and areas considered ecologically sensitive must have a CCT less than 3000K. Examples demonstrating the effectiveness of reducing short-wavelength output on mitigating behavioural and physiological effects of ecological light pollution are detailed below.

Figure 2: Emission Spectrum for Typical LED Streetlights. An emission spectrum is shown for typical 100W LED streetlights. The primary ("blue") emission at 450-460nm is clearly visible for both lighting types. Neutral white (4464K) light shows a reduced blue peak and greater emission of longer wavelength "red" light in comparison to the cool white bulb (6273K) (Elvidge *et al.*, 2010).

1.4.1: Mitigating Behavioural Effects

Previous studies have demonstrated decreased behavioural disturbance by longer wavelength lighting across a range of species (see Table 1 for examples). Whilst certain species may be acting in response to changes in behaviour of prey species, rather than reacting to the light condition (such as bats attracted to moths at streetlamps)(Spoelstra *et al.*, 2015), ALAN can still be considered to impact the ecology of these species as their behaviour deviates from the norm. A summary of existing literature demonstrating the success of this method for mitigating the ecological effects of ALAN can be found in Table 1.

The existing literature appears to be biased towards more common species which frequently occur around urban environments (such as invertebrates and bats); likely for the reason that they are often conspicuous and hence easy to observe. This taxonomic bias does not review the potential value of spectral manipulation for mitigating ecological light pollution at the community level. It is necessary to collect data for a broad range of species since spectral sensitivity varies between species groups (Davies et al., 2013) and response to light may also differ between subspecies (Anderson, 1972). In addition, the most studied species originate from Europe and North America, providing an unbalanced focus on temperate species. Whilst species inhabiting Mediterranean and temperate climates have experienced the greatest increases in light pollution (Bennie et al., 2015), the tropics may be particularly sensitive to disruption since they experience relatively stable light patterns (Longcore & Rich, 2004). Future research should therefore aim to identify whether spectral manipulation of light sources is successful at mitigating behavioural disturbance across a comprehensive range of species and provide a focus on locations which are predicted to experience particularly high increases in light pollution in the near future or are predicted to be especially sensitive to disturbance. This knowledge could play a key role in influencing development policy.

Furthermore, most studies to-date (Table 1) have tested the success of monochromatic red, green or blue LEDs for mitigating behavioural disturbance. Whilst these experiments can provide valuable information regarding the impacts of different parts of the visible light spectrum on a species' behaviour, the relevance of results is limited. These lights do not constitute a feasible alternative to existing LEDs because they provide low visual acuity for humans and hence are unlikely to be included in development plans (Davies & Smyth, 2018). Varying methods of modifying existing lights to reduce the proportion of highly disruptive

wavelengths (Newport *et al.*, 2014) (such as application of light filters) should be tested to determine their effectiveness. This approach to research would be more relevant to current development issues by helping to identify practical solutions to ecological light pollution.

Species	Observed Effect	Authors
Invertebrates		
Indian meal moth (<i>Plodia</i> interpunctella)	Significantly fewer males and mated females attracted to green, orange and red lights than blue	Cowan & Gries, 2009
Winter moth (<i>Operophtera</i> brumata)	Reduced disruption of mating behaviour on oak trees under red light	van Geffen et al., 2015
Nocturnal arthropods (various species)	Decreased attraction of various arthropod species to custom LEDs with higher emission peaks	Longcore et al., 2015
Beetles (Coleoptera spp.)	Amber LEDs did not significantly alter beetle abundance	Davies et al., 2017
Fish		
Sablefish (Anoplopoma fimbria)	Significantly more likely to be present at a bait illuminated by red light than white light	Widder et al., 2005
Tench	Juveniles significantly less disturbed (more active) under red light than white or blue	Owen et al., 2010
Reptiles		
Leatherback turtle (<i>Dermochelys</i> coriacea)	Reduced misorientation of hatchlings under orange and red lights. Least disorientation under orange lights	Rivas et al., 2015
Loggerhead turtle (Caretta caretta)	Red light had a lower decline in nesting attempts than yellow and orange and no significant effect on sea-finding behaviour of adults (but yellow and orange did)	Silva et al., 2017
Amphibians		
Common toad (Bufo bufo)	More likely to cross a road under red light than when under blue or green	van Grunsven et al., 2017

Table 1: Behavioural Effects of Long Wavelength LEDs. Observed behavioural effects of using long wavelength LED lighting (here defined as those with reduced short wavelength output) over LEDs which emit a greater proportion of short wavelengths are presented for a diverse array of species.

Mammals

Wood mouse (Apodemus sylvaticus)	Slightly decreased avoidance of red-lit areas	Spoelstra et al., 2015
Soprano pipistrelle (<i>Pipistrellus pygmaeus</i>)	More bats emerged under red light than blue. For one roost, there was no significant difference between the red condition and ambient control	Downs et al., 2003
Common pipistrelle (<i>Pipistrellus pipistrellus</i>)	Reduced attraction to red lights than white and green conditions	Spoelstra et al., 2015
Various bats (<i>Plecotus sp., Myotis sp.</i> and <i>Pipistrellus sp.</i>)	No difference in abundance between red light condition and ambient dark control	Spoelstra et al., 2017

1.4.2: Mitigating Physiological Effects

As previously mentioned, exposure to short wavelength light at night is known to suppress melatonin production in a range of species which may particularly affect hormone cycles of seasonal breeding species (Walsh *et al.*, 2013). When Tammar wallaby *(Macropus eugenii)* are exposed to white LEDs with a prominent short-wavelength peak melatonin production is significantly suppressed. However, animals exposed to amber LEDs with an emission peak beyond the upper limit of their visual sensitivity range, had melatonin concentrations which did not differ significantly from the natural, ambient control (Dimovski & Robert, 2018). This method may well be effective for other species, although the impact of ALAN on melatonin cycles is still understudied; particularly among amphibians and reptiles (Grubisic *et al.*, 2019).

Few studies have attempted to identify how changes in physiology may be mitigated by manipulating spectral output of light sources. We note that most studies discuss behavioural responses, perhaps on the basis that these changes are often more noticeable. Therefore, it is recommended that future studies analyse observations of physiological disruption and identify the value of spectral manipulation in their mitigation.

1.4.3: Potential of Spectral Manipulation

Despite the body of existing evidence, which demonstrates that ALAN is less disruptive when short wavelength light output is reduced, it is apparent that responses are not identical across all taxa (Zeale *et al.*, 2018). For example, mitigation success appears to vary particularly amongst invertebrates. Davies *et al.* (2017) report that whilst beetles are less sensitive to amber LEDs, spectral manipulation was not effective as mitigation for spiders; likely because the two groups are most sensitive to different wavelengths of light (Davies *et al.*, 2013). Additionally, results published by Pawson & Bader (2014) demonstrate that changing the CCT of LED lighting produced no effect on abundance of New Zealand's flying invertebrates. Interestingly, Longcore *et al.* (2015) have since demonstrated that lights of similar CCT can differ in their ecological impacts. They report that custom LEDs which produced reduced short wavelength emissions were less attractive to invertebrates than commercially available units with a similar colour temperature. Hence it is important to consider the emission spectra of units in addition to CCT when choosing LEDs. In order to produce similar light intensity to white LEDs, spectrally shifted units must produce a greater proportion of long-wavelength light (see Figure 2). Whilst the use of these units may mitigate impacts on some processes (such as melatonin suppression (Dimovski & Robert, 2018)), others which have increased sensitivity to longer wavelengths (for example photoperiod detection in plants (Bennie *et al.*, 2016) may experience increased disturbance (Davies *et al.*, 2013). In fact, it has been suggested that the most effective method of reducing ecological light pollution may be a combination of dimming units and reducing the time lights are switched on, rather than tweaking spectral output (Davies *et al.*, 2017). Despite this, it is valuable to understand which LED configurations are least disruptive to different species groups to enhance understanding of ecological processes and guide mitigation where other measures are not suitable.

In summary, the full potential of spectral manipulation cannot be assessed until it has been reviewed across a broader range of species. Spectral manipulation may not be equally effective for all taxa and sometimes reducing, rather than modifying disturbance may be necessary (Davies *et al.*, 2017); particularly where processes are sensitive to long-wavelength light (Bennie *et al.*, 2016). Finally, studies testing the effectiveness of spectral manipulation should ensure that their experimental conditions are relevant to the human requirements of ALAN to increase research value.

2: Amphibians and Light

Briefly, the clade Lissamphibia consists of three groups of organisms: Orders Anura (frogs and toads), Urodela (newts and salamanders) and Gymnophiona (caecilians). Whilst there is considerable variation across species, all amphibians share a dependence on water (if only for egg deposition), highly permeable skins (Buttemer, 1990) and ectothermic metabolism (Wells, 2007). Of the amphibians, frogs and toads inhabit the most diverse range of habitat (including desert, aquatic and arboreal habitat types) and demonstrate a diverse range of associated morphological adaptations. Urodeles are predominately either aquatic, terrestrial or arboreal specialists and caecilians, though under-studied, are understood to be predominately fossorial (Wells, 2007; Gower & Wilkinson, 2005). Most amphibians are nocturnal (Buchanan, 2006) and exhibit bimodal, light-dependent activity patterns throughout the night (Hailman, 1984; Buchanan, 1992). The impact of ALAN on amphibians is currently understudied (Perry *et al.*, 2008; Gaston *et al.*, 2013), but most is known about temperate anurans, likely because these species are often the most conspicuous. Moreover, little is

known about the light biology of caecilians, therefore the potential impacts of ALAN on this Order is unknown (Perry *et al.*, 2008).

Despite demonstration that amphibians use chemoreception and magnetoreception to navigate their environments, photoreception remains a key sense for most amphibians; though vision appears to be less important for caecilians (Wells, 2007; Mohun & Davies, 2019). The presence of two types of spectral rod photoreceptors in most Anurans and some Urodeles made the study of low-light vision a key topic in amphibian biology. The typical vertebrate rod has a spectral sensitivity maximum of approximately 500nm, whilst the second form, unique to amphibians, is most sensitive to wavelengths of 430nm (Denton and Wyllie, 1955; Donner & Reuter, 1962; Yovanovich et al., 2017). The ecological significance of this is clear when considering that most amphibians are nocturnal in nature (Buchanan, 2006). Sunset conditions have higher proportions of short-wavelength light so increased sensitivity to light at this end of the spectrum would provide a key advantage for animals navigating the nocturnal environment by vision; especially since some evidence suggests that amphibians are capable of discriminating colours at the absolute visual threshold (the lowest illumination detectable by a species) (Yovanovich et al., 2017). Additionally, this second rod could be particularly sensitive to the prominent short-wavelength peak characteristic of LEDs meaning that ALAN could be especially disruptive for this species group (van Grunsven et al., 2017).

Subsequent research by Yovanovich *et al.* (2019) suggests that visual sensitivity differs between species. The common toad (*Bufo bufo*) is noted to have more sensitive blue photoreceptors than the common frog (*Rana temporaria*), which is most sensitive to longer wavelengths. Additionally, common toads are reportedly more sensitive to Ultraviolet light than common frogs. Studying the spectral sensitivity of species may help to explain aspects of ecology, morphology and behaviour. For example, males of the moor frog (*Rana arvalis*) develop blue colouration during the breeding season (Ries *et al.*, 2008). This colouration has a large UV component so it may follow that moor frogs have increased sensitivity to short wavelength lighting to allow perception of this visual cue. It is therefore expected that response to ALAN will vary between species, highlighting the need for research across a range of taxa.

Amphibians deserve increased attention when ALAN is concerned. In fact, frogs have slower pupillary responses than birds and mammals, meaning they have a reduced ability to control the amount of light hitting the retina (Cornell & Hailman, 1984). This inability to

maintain constant retinal illumination may mean that amphibians are susceptible to being temporarily "blinded" at the onset of a sudden change in light intensity; for example, approaching car headlights (Mazarolle *et al.*, 2005). Cornell & Hailman (1984) suggest that it may take several hours for anuran eyes to fully adapt to a significant increase in light intensity, however, Buchanan (1993) reports that Cope's gray treefrogs (*Dryophytes chrysoscelis*) were able to adjust sufficiently to be able to capture prey 3-4 minutes after exposure to light. Length of the adaptation period is believed to be correlated with the intensity of the light source, with brighter lights disrupting vision for longer periods of time (Cornell & Hailman, 1984). The biological implications of this are discussed further in the next section.

To conclude, amphibians may be particularly sensitive to ALAN since they are adapted for navigating low-light conditions using a visual system which may be slow to adapt. Most research to date has focused on the impact of ALAN on amphibian reproduction, development, movement and foraging. These themes are explored further in the following section.

3: Research Themes

3.1: Reproduction & Development

3.1.1: Calling

Male anurans predominantly rely on the use of species-specific calls to attract females for mating (Wells, 2007). It is recognised that significantly fewer anurans call on bright, moonlit nights; presumably to avoid detection by predators which use a combination of visual and audible cues (Pena *et al.*, 2008). Contrary to expectations, Pena *et al.* (2008) reported that the Northern cricket frog (*Acris crepitans*) called more frequently on brighter nights. It is likely that this species takes advantage of reduced interspecific calling competition on moonlit nights as it is cryptic and hence harder for predators to detect.

The observed effect of moonlight intensity on calling frequency raised questions regarding the effect of ALAN at breeding pools. It has been observed that many North American species reduced calling intensity and frequency (Buchanan, 2006; Hall, 2016) and produced fewer multi-note calls under artificially lit conditions (Baker & Richardson, 2006). Where breeding pools are continuously exposed to high-intensity ALAN it is likely that reduced calling rate will result in lowered breeding success since calling is a key method of

mate selection (Ryan, 1980). A change in the community structure at such sites may occur should opportunistic species such as Northern cricket frogs (Pena *et al.*, 2008) achieve higher mating success in the absence of vocal competitors.

Additionally, Dias *et al.* (2019) have recently reported that populations of South American anurans may be disturbed when breeding wetlands are illuminated with highpressure sodium lighting. Whilst pulses of intense light only reduced calling at unlit sites, they report a trend in shortening of the calling season and a shift towards calling earlier in the year in wetlands illuminated by streetlights. Moreover, some species also called more frequently throughout the night, suggesting that ALAN may interfere with perception of time. It is thought that the observed changes may result in reduced mating success (Dias *et al.*, 2019) and juvenile survival, potentially leading to fitness decrease. Amphibian breeding is often highly seasonal and coincides with abiotic factors such as rainfall (Jensen *et al.*, 2003). If eggs were to hatch outside of wetter seasons, when water levels are lower, biotic factors (for example increased competition and reduced food availability) may, hypothetically, reduce survival rate of larvae. Where light pollution affects sites used by seasonal breeding anurans long-term, population level effects are likely, though we are not aware of an attempt to test these hypotheses.

3.1.2: Mating

Calling is not the only aspect of amphibian breeding influenced by ALAN. Under brighter conditions, female Túngara frogs (*Physalaemus pustulosus*) are reported to be less likely to choose a mate and to prefer closer, less appealing calls over more complex, distant sounds. This observed reluctance to move is thought to stem from higher perceived predation risk under brighter conditions (Rand *et al.*, 1997). However, it is important not to generalise that amphibians will always be less "choosy" under brighter conditions. For instance, Underhill and Höbel (2018) have demonstrated that mate choice of the Eastern gray treefrog (*Dryophytes versicolor*) was not affected by exposure to incandescent lighting. This species may be more resilient to light pollution as it is known to occupy urban wetlands (Simon *et al.*, 2009). Additionally, the individuals used in this study were collected from a pond adjacent to a university field station so may have habituated to light pollution prior to commencement of trials (Underhill & Höbel, 2018; Dias *et al.*, 2019).

There is some evidence to suggest that Túngara frogs may choose to create foam nests in sheltered locations when exposed to low-level ALAN in order to reduce predation risk

(Tárano, 1998). It is plausible that illumination, resulting in elevated perceived predation risk, may influence choice of oviposition site, but to the best of our knowledge (and that of Buchanan (2006)) this is the only published record.

A recent study has demonstrated that breeding males are also affected by artificial illumination. Under ALAN, common toads took longer to form amplexus with females and had reduced fertilisation success (25% lower under lights of 5lux) than dark controls (Touzot *et al.*, 2020). Reduced reproduction rates in illuminated habitats could have long-term effects on population fitness, particularly because this species is frequently found breeding in suburban ponds (Beebee, 1979). It is possible that similar effects will be observed in other species, particularly Bufonids, however, this topic requires further investigation.

3.1.3: Development

Whilst it is clear that ALAN can have instantaneous effects on behaviour of many amphibians, long-lasting effects on development and activity patterns are also evident. Dananay & Bernard (2018) observed that American toad larvae (*Anaxyrus americanus*) exposed to artificial lighting completed metamorphosis faster than under natural conditions; presumably due to increased perceived predation risk. Additionally, juveniles which were exposed to ALAN during the larval stage were marginally more active than control treatments. No carry-over effects of larval stage exposure on juvenile development were observed, although juveniles exposed to ALAN had reduced growth and did not seek refuge at night (Dananay & Bernard, 2018). The authors hypothesised that this change in activity patterns caused an increase in energy expenditure, thereby resulting in decreased growth rate. Recent evidence suggests that increased corticosterone concentration in juveniles exposed to ALAN during the larval stage may provide an alternative mechanism by which growth rate is reduced (Cope *et al.*, 2020). In a wild population, such changes could be expected to produce individuals with lower fitness levels which may be less fecund.

Further to this, it has also been found that tadpoles of the wood frog (*Lithobates sylvaticus*) exposed to ALAN are more susceptible to road salt (NaCl) run-off and parasitism by trematodes (May *et al.*, 2019). Additionally, tadpoles in this study were less active and had attained a greater size at metamorphosis than the control group. Since Warkentin (1992) observed that feeding rate did not differ under lit conditions for the green frog (*Rana clamitans*) it could be hypothesised that reduced energy expenditure resulting from lower activity levels may have allowed larvae to grow faster. Finally, findings from May *et al.*

(2019) also suggest that increased light intensity (at any time of day) results in slightly decreased hatching success of fertile embryos.

The above studies do not directly indicate reduced survival chance for young amphibians raised under artificially lit conditions, but suggest that exposure influences growth rate, activity levels and susceptibility to chemical contaminants and parasites. These effects are likely to contribute to decreased individual fitness and consequent reductions in population size and health in lit environments. Establishing the true effect of ALAN on development of amphibians may require researchers to study animals until maturation to ensure that carry-over effects can be identified.

3.2: Movement

3.2.1: Small-scale Movements

Phototaxis has long been a key research topic for amphibian biologists, largely because responses appear to vary between species (Jaeger & Hailman, 1971; Hailman & Jaeger, 1974). Briefly summarised, an extensive study (covering 127 species) by Hailman & Jaeger (1974) concluded that each anuran species has an optimal ambient illumination (OAI) representing their preferred ambient light intensity. They observed that frogs and toads would move away from lights brighter than their OAI but be attracted towards blue lighting when their surroundings were dimmer than their OAI. However, Recktenwald *et al.* (2014) report that leopard frogs (*Rana pipiens*) are only attracted to lights 20 times brighter than the ambient illumination and argue that an OAI would be maladaptive. Therefore, the introduction of ALAN sources several orders of magnitude greater than ambient foraging illuminations (Gaston *et al.*, 2014) is predicted to cause severe disturbance to natural behavioural patterns.

Even subspecies can differ in their behavioural responses to ALAN. Two subspecies of the long-toed salamander (*Ambystoma macrodactylum croceum* and *A. m. sigillatum*) are known to avoid lit conditions, however, *A. m. croceum* exhibits a stronger avoidance. Additionally, it has been demonstrated that large larvae of *A. m. sigillatum* become positively phototactic, although this behaviour reverts during metamorphosis (Anderson, 1972). Whilst it can be useful to understand phototactic behaviour, it is crucial to understand the underlying biological mechanism. For example, aquatic Eastern tiger salamanders (*Ambystoma tigrinum*) are more attracted to orange glowsticks, despite being most sensitive to wavelengths produced by the green and yellow options. In this case, it is probable that animals were

attracted towards the orange conditions as their colour mimics that of Eastern fairy shrimp (*Eubranchipus vernalis*), a favourite prey item (Liebgold & Carleton, 2020). Moreover, many terrestrial invertebrate species are commonly drawn to LED lights in particular (Davies & Smyth, 2018; Davies *et al.*, 2017), which may secondarily attract foraging amphibians to light sources. Perhaps the most valuable studies of attraction to light sources will be those conducted in-situ in a biologically relevant situation where biotic factors (such as prey abundance) can also be measured.

Constant nocturnal illumination has also been demonstrated to affect movement frequency. Common toads were less active at night in illuminated trials than under ambient conditions. Since foraging behaviour did not differ between conditions, it is hypothesised that exploratory behaviour was reduced owing to elevated perceived predation pressure (Touzot *et al.*, 2019). If species consistently move less in lit environments, larger scale movements such as dispersal and migration could become disrupted.

3.2.2: Habitat Preference

Artificial light at night also has the potential to modify perception of habitat quality in amphibians. For example, Cope's gray treefrog are significantly more likely to choose blue painted artificial refugia over brown or white (Cohen *et al.*, 2016). This attraction to objects which reflect blue light supports previous observations (Hailman & Jaeger, 1974) and could suggest that this species (and other hylids) may show preference for locations illuminated by "cool white" LEDs due to the large amount of blue light these units produce. The fact that this species uses colour vision to choose suitable habitat highlights the potential effect of coloured lighting on habitat choice. Results from Cohen *et al.* (2016) suggest that use of blue or "cool white" LEDs for advertising or enhancing the aesthetics or security of a site may increase the perceived attractiveness of a habitat. Whether this drive can cause frogs to choose unsuitable (or even hostile) habitat is currently unknown.

In another case, artificial illumination is shown to increase the probability that recently metamorphosed unisexual blue spotted salamanders (*Ambystoma laterale* \times *jeffersonianum*) will choose coniferous leaf litter upon leaving natal pools. When presented with a choice between deciduous and coniferous substrates they were more likely to choose coniferous leaf litter when it was illuminated than when it was dark. Since coniferous leaf litter is less favourable for this species, Feuka *et al.* (2017) have demonstrated that LED streetlighting may cause juvenile blue spotted salamanders to be more attracted to an

unfavourable habitat, potentially acting as an ecological trap (Battin, 2004). This result suggests positive phototaxis, however, the authors could not control for the effect of varying prey abundance. Importantly, this observation should not be generalised across all ambystomatid salamanders, following observations of strong negative phototaxis (*A. m. croceum* and *A. m. sigillatum*) by Anderson (1972). Conversely, wood frogs (*Rana sylvatica*) showed no preference and did not appear to respond to illumination.

Preference for lower quality habitat over nearby high-quality habitat may be caused by ALAN, causing an ecological trap. Ecological traps are often caused by human disturbance and may result in subsequent reduction in population fitness and size, or local extinction (Battin, 2004). Anthropogenic modification of habitat, such as creation of artificial crevices within buildings, modifying vegetation structure and increasing food availability is known to decrease reproductive output (Demeyrier *et al.*, 2016) and cause an increase in mortality across a range of species (Hawlena *et al.*, 2010; Lamb *et al.*, 2017). Since amphibians have reduced mobility, they may be particularly sensitive to mortality when drawn to artificially lit environments (such as roadside verges (Mazarolle *et al.*, 2005)), resulting in long-term population effects.

3.2.3: Migration

Artificial light is likely to influence large-scale daily and seasonal movements across a variety of species. For example, tadpoles of the American toads usually shelter in warmer depths of ponds at night, moving towards the surface as light levels increase during the morning; this is a crucial thermoregulatory behaviour. Beiswenger (1977) reports that light intensity is key in triggering morning activity and raising swimming and feeding rates. Larvae of several ambystomatid salamanders exhibit similar, though reversed, vertical migrations, rising to the surface to feed at night. For the marbled salamander (*Amybstoma opacum*) this behaviour is reported to be reduced on brighter nights (Anderson & Graham, 1967). Constant increased light intensity has the potential to disturb this daily movement towards the surface and may impede metabolic functions and hence growth rate.

Van Grunsven *et al.* (2017) demonstrated that migrating common toads crossing a road were more likely to avoid blue and green LED lighting than red. Whilst this species was most likely to cross under dark conditions, the longer wavelength light was less disruptive, presumably because this species is most sensitive to short-wavelength light (Yovanovich *et*

al., 2019). Construction of brightly lit corridors (such as roads) may, therefore, act to restrict migrations to breeding pools. Moreover, the tendency of amphibians to become immobile when encountering bright vehicle headlights on otherwise unlit roads may lead to increased mortality (Mazarolle *et al.*, 2005). Interestingly, mole salamanders (*Ambystoma maculatum* and *A. laterale*), spring peepers (*Pseudacris crucifer*) and ranid frogs (*Rana clamitans* and *R. sylvatica*) were more likely to remain immobile when approached by a vehicle from July-September than earlier in the year (May-June) when animals were likely migrating to breeding pools. American toads did not show this trend. It is hence possible that the effects of ALAN on amphibian movements may be seasonal. To the exclusion of bufonids, migration towards breeding pools may be more robust to ecological light pollution than late-summer migration (Mazarolle *et al.*, 2005). To date, this has only been observed for North American species, however, future research could identify whether a similar trend can be observed in Eurasian or tropical species.

3.3: Foraging

Most frogs are nocturnal predators which rely on specialised vision to detect and capture prey at low light levels (Buchanan, 1998; Buchanan, 2006; Yovanovich *et al.*, 2017). It has therefore been hypothesised that frogs may be particularly sensitive to the effects of ALAN on foraging (Martin *et al.*, 2018; Longcore & Rich, 2004). The ability of ALAN to directly influence foraging success was demonstrated by Buchanan (1993), who observed decreased ability of Cope's gray treefrog to detect and consume prey when exposed to sudden increases in ambient illumination. On average, frogs took approximately three or four times longer (depending on light intensity) to detect prey items following illumination than under ambient conditions. Slow pupillary adaption to increases in light intensity (Cornell & Hailman, 1984) may therefore impede or prevent foraging in environments prone to sudden increases in ambient illumination (Buchanan, 1993), for example roadside habitat (Mazarolle *et al.*, 2005).

Squirrel treefrogs (*Dryophytes squirellus*) are confirmed to be capable of detecting prey at very low illuminations ($6x10^{-5}$ lux, roughly equivalent to a cloudy night) but it is apparent that their ability to detect prey increases with ambient light intensity (Buchanan, 1998). Increased prey detection ability at higher ambient light intensities has also been documented for urodeles including red-backed salamanders ((*Plethodon cinereus*) Perry *et al.*, 2008). Low-level increases in ambient illumination resulting from distant lighting or skyglow may therefore also act to improve the ability of amphibians to continue foraging into

the darkest parts of the night when they are normally less active (Hailman, 1984; Buchanan, 1992).

Artificial lighting may, however, also indirectly affects foraging by interfering with activity patterns and perceived predation risk (Kurvers *et al.*, 2018). Many amphibians are documented to use refugia (both artificial and natural) in-situ as daytime retreats (Meshaka, 1996; Hoffmann, 2007; Piacenza, 2008; Hoffmann *et al.*, 2009) and certain species (such as red-backed salamanders) are known to only emerge several hours after sunset (Perry *et al.*, 2008). Unpublished data by Buchanan & Wise observed that fewer red-backed salamanders were observed on lit transects than dark ones. Based on their data, they hypothesised that ALAN may cause delayed onset of activity (Perry *et al.*, 2008), however, the fitness consequences of this disturbance are perhaps reduced when species are able to extend their foraging period into the middle of the night.

4: Mitigating for ALAN Impacts

It is important to have sufficient information prior to designing mitigation strategies. The success of spectral manipulation as mitigation for amphibians has only been documented for migrating common toads (van Grunsven *et al.*, 2017), however, the reliability of this study is questioned since these animals may have been more robust to disturbance during migration (Mazarollle *et al.*, 2005). Additionally, the practical applications of this study are limited since van Grunsven *et al.* (2017) did not test full spectrum lamps (such as Longcore *et al.*, 2015). Moreover, reducing the output of short wavelengths is not guaranteed to reduce disturbance. Other factors, such as attraction towards lights which mimic the colour of food sources may cause unexpected responses (Liebgold & Carleton, 2020). As such, there is currently insufficient evidence to be able to conclude whether spectral manipulation may be effective for mitigating the effects of ALAN on amphibians.

Behavioural responses to ALAN clearly differ between species, therefore, data for a model species may have little practical conservation value for another. Where the effect of spectral manipulation on a species is not known, reducing the illumination period and lighting intensity (Davies *et al.*, 2017) may be the most intuitive method of mitigation as the intensity of disturbance relative to the ambient light level is reduced. Arboreal species (such as hylids) which are likely to be active closer to lighting units (and hence exposed to higher light intensities) may benefit particularly from this approach. On the whole, the key to determining the optimal mitigation strategy for amphibians and influencing development policy will be

the publication of comparative studies using a variety of mitigation techniques. These studies should also expand on current knowledge, focusing on a broad range of species groups, identifying impacts on larvae and adults and making an effort to identify species which may be most vulnerable to increased ALAN intensity.

5: Summary

In summary, ALAN may provide the most resilient amphibian species with improved opportunities (Buchanan, 1998; Pena *et al.*, 2008), but the negative effects seem to be more prevalent (see Table 2). We are not aware of reports that ALAN may directly cause mortality, however, indirect mortality and population declines may occur due to light-induced immobility (Mazarolle *et al.*, 2005), fitness loss (Buchanan, 1993; Dananay & Bernard, 2018; Feuka *et al.*, 2017), decreased reproduction (Touzot *et al.*, 2020) and increased susceptibility to parasitism and pollutants (May *et al.*, 2019). Up-scaling observed effects to determine likely impacts on population fitness is challenging, as such population-level effects are often hypothetical and require evidencing. Fortunately, there is evidence that some anurans habituate to light pollution (Dias *et al.*, 2019) though it is not known whether all amphibians are so resilient. Spectral manipulation, reduction of light period and lighting intensity may prove successful for mitigating observed effects, however, no study has tested the success of relevant mitigation techniques for amphibians. These must provide adequate lighting for humans in order to secure their application in development schemes.

Table 2: Key Effects of ALAN on Amphibians. The observed effects of ALAN on amphibians discussed in Section 3 are summarised here. Where several studies have observed similar effects, only one key reference is provided.

Research Theme	Observed Effect
Reproduction & Development:	
	Reduced calling frequency and complexity (Baker & Richardson, 2006)
Calling	Change of chorus structure (Pena et al., 2008)
	Modification of calling season (Dias et al., 2019)
	Reduced choosiness (Rand et al., 1997)
Mating	Altered oviposition site preference (Tárano, 1998)
	Longer to form amplexus (Touzot et al., 2020)
	Reduced fertilisation chance (Touzot et al., 2020)
	Shorter metamorphosis (Dananay & Bernard, 2018)
Development	Increased juvenile activity & reduced growth rate (Dananay & Bernard, 2018)
Development	Increased larval susceptibility to parasites & salinity (May et al., 2019)
	Decreased hatching success (May et al., 2019)
Movement:	
Small-scale Movements	Phototaxis differs between species and across development stages (Anderson, 1972)
	Decreased nocturnal movement (Touzot et al., 2019)
	Attraction to "blue" habitats (Cohen <i>et al.</i> , 2016)
Habitat Frejerence	Increased likelihood to occupy sub-optimal habitat (Feuka et al., 2017)
	Disruption of daily larval migrations (Beiswenger, 1977)
Migrations	Fragmentation of habitat by roads (van Grunsven et al., 2017)
	Increased mortality on roads (Mazarolle et al., 2005)
Foraging:	Disruption following sudden illumination changes (Buchanan, 1993)
	Extended foraging into later parts of night (Hailman, 1984)
	Delayed foraging onset (Perry et al., 2008)

<u>Chapter 2</u>

1: Introduction

Now that 41% of amphibians are currently threatened with extinction (IUCN Red List, 2020) and many other species are in decline (Whitfield *et al.*, 2016) the need to identify population stressors is more pressing than ever. Urbanisation is believed to be a major driver in the decline of amphibian species (Whitfield *et al.*, 2016) and it is known to influence body size and condition of several species (Iglesias-Carrasco *et al.*, 2017). Increased urbanisation can be linked to elevated anthropogenic disturbance, including greater prevalence and intensity of ALAN sources (Gaston *et al.*, 2013). Managing light pollution may play a key role in facilitating amphibian presence in urban environments due to their reliance on vision and general nocturnal habit (Buchanan, 2006). Though LED lights often attract potential prey (Longcore *et al.*, 2015), negative effects of light pollution may play a significant role in reproduction, development, movement and foraging success (as identified in Chapter 1).

One area which has been overlooked is the impact of ALAN on emergence from places of shelter. It is well known that illumination may delay, or completely prevent emergence by some bat species (Boldogh *et al.*, 2007) due to perceived elevated predation risk which could have implications on available foraging time and consequently individual and population fitness (Duvergé *et al.*, 2000; Kurvers *et al.*, 2018; Perry *et al.*, 2008). North American treefrogs seek refuge during the day in a range of environments from tree holes to objects of artificial construction, such as PVC pipes. Nocturnal treefrogs, such as the Cuban treefrog (*Osteopilus septentionalis*) and the American green treefrog (*Dryophytes cinereus*), may commonly be found sheltering in these objects in urban locations throughout Florida (Goin, 1958; Delis *et al.*, 1996; Pham *et al.*, 2007; Piacenza, 2008; Glorioso *et al.*, 2010). It is necessary to better understand the relationship between amphibian activity and ALAN in order to safeguard the future of amphibian species in urbanising landscapes. Conservation of amphibians in urban spaces is also in the interest of public health, since they are key predators of mosquito larvae, a vector of human disease (Rubbo *et al.*, 2011).

Most amphibian studies focus only on determining the impact of ALAN, rather than testing the success of mitigation, which is particularly well documented for invertebrates (Davies et al., 2017; Longcore et al., 2015). Spectral manipulation of LED sources to reduce the proportion of short-wavelength light, whilst providing adequate visual acuity for humans, (Longcore et al., 2015) may be a particularly valuable mitigation technique for amphibians

due to their increased sensitivity to the shorter wavelengths of visible light (Denton and Wyllie, 1955; Donner & Reuter, 1962; Yovanovich et al., 2017). Whilst the literature suggests that reducing the short wavelength output of full-spectrum LEDs may reduce behavioural disturbance of amphibians, without comparing this method to standard LEDs it is not possible to demonstrate success of this mitigation technique.

Cuban and green treefrogs are nocturnal and commonly make use of urban habitats in Florida where they often occur sympatrically and are regularly being exposed to ALAN (Goin, 1958; Delis *et al.*, 1996; Pham *et al.*, 2007; Piacenza, 2008; Glorioso *et al.*, 2010). As such they were chosen as models for investigating the relationship between LED lighting and emergence behaviour. Whilst these species share similar ecologies, Cuban treefrogs are invasive and are more commonly found in urban habitats.

The native range of the green treefrog extends across most of the South-Eastern United States (US) (Hammerson & Hedges, 2017), whilst Cuban treefrogs are introduced on the US mainland, originating from Cuba (Heinicke *et al.*, 2011). Their distribution in the US is largely restricted to Florida (McGarrity & Johnson, 2009; Glorioso *et al.*, 2010; Hedges *et al.*, 2010) with some reports suggesting range expansion into neighbouring States (Vredenburg, 2009). Adaptations including a broad, adaptable diet (Glorioso *et al.*, 2010; Owen, 2005), scramble mating (Vargas Salinas, 2006) and high reproduction rates are believed to have facilitated dispersal of this species (Owen, 2005; Platenburg, 2007). High behavioural flexibility of a species is also recognised as facilitating invasion (Wright *et al.*, 2010). Additionally, Piacenza (2008) reports that Cuban treefrogs are more common in urban (presumably brighter) areas than green treefrogs. It therefore stands to reason that Cuban treefrogs may be able to adapt more readily to novel disturbances, such as ALAN.

2: Hypotheses

This study was designed to determine the effect of light pollution from LED sources on emergence behaviour of the Cuban treefrog (*Osteopilus septentrionalis*) and the American green treefrog (*Dryophytes cinereus*), and to explore the potential for spectral manipulation of existing light sources as mitigation for amphibians. We deduced that owing to differences in ecology Cuban treefrogs may show less dramatic behavioural differences between light treatments than green treefrogs (Gaston *et al.*, 2014; Piacenza, 2008). These results could provide important information on population ecology for both species, particularly in terms

of urban landscape use and time-partioning (Gaston *et al.*, 2014), and further scientific understanding of amphibian responses to ALAN.

Based on the existing literature, three hypothesises were constructed:

- Exposure to "cool white" LED lighting would significantly increase latency of a frog to emerge from and leave an artificial refuge when compared to an ambient dark condition due to the risks associated with emerging into a hostile environment (Kurvers *et al.*, 2018).
- The prominent short wavelength peak of this lighting will be responsible for the observed disturbance, hence reduction of short wavelength output was expected to partially mitigate behavioural effects.
- Owing to interspecific differences in behavioural flexibility, behavioural responses of the invasive Cuban treefrog would be more resilient to light pollution than native green treefrogs.

3: Methods

3.1: Study Species Husbandry

Twelve captive-bred individuals each of the Cuban treefrog (*Osteopilus septentrionalis*) and the American green treefrog (*Dryophytes cinereus*) were used in this study. Whilst the age of frogs was not known, it is reasonable to use published Snout-Vent Length (SVL) measurements (Duellman & Crombie, 1970; Redmer & Brandon, 2003) to conclude that all frogs were likely adults. The frogs were kept at Bangor University in a temperature-controlled room at 24 °C. Humidity was controlled using a portable humidifier, maintained at an ambient value of 65-75%RH (Hoffmann *et al.*, 2009; Buchanan, 1998). Study species were kept in separate vivaria (measuring 36x21x14cm) to prevent predation by Cuban treefrogs (Glorioso *et al.*, 2010). A substrate of coco fibre and moss was used to maintain humidity within the vivaria and a shallow water bowl offered to enable hydration.

Ambient lighting consisting of a warm white T8 fluorescent tube (~100 lux at 1m height in the centre of the room) was set on a twelve-hour timer (06:00-18:00 hours) to replicate natural lighting cycles. Supplementary lighting (Arcadia ProT5 HO unit with 2.4% UVB bulb) on an identical cycle was suspended above the housing tanks to better replicate high intensity daylight; we measured a mean light intensity of 2.84 Klux (range: 2.40-3.36 Klux) at the substrate level. Frogs were not exposed to LED lighting (except during trials) to ensure that habituation to this light type did not influence behavioural observations.

Frogs were individually identified using a combination of dorsal photographs and SVL measurements to control for the effects of repeated observation and presentation order. Both species were housed in pairs where one was noticeably larger for the duration of the experimental period to facilitate this and to ensure that frogs had adequate access to gut-loaded live crickets. SVL measurements were recorded using digital callipers and dorsal surface images were also taken for each frog using a DSLR camera whilst frogs were restrained under a transparent petri dish. This was a particularly useful technique for aiding identification since Cuban treefrogs exhibit diverse patterning and green treefrogs had some variation in spot pattern (when present).

Bangor University's "Research Project Ethical Issues Checklist" and "Preliminary Hazard Assessment Form" were submitted to and approved by the Health and Safety Manager and Animal Care Technician prior to collection of specimens. It was not deemed necessary for the project to be subjected to review by the university ethics committee.

3.2: Experimental Setup and Procedure

An experimental arena (30x30x60cm) was constructed from black acrylic with an artificial refuge made from PVC pipe (6cm diameter, 15cm height) present in the centre (see Figure 3). The use of PVC refugia is common in the in-situ study of treefrogs as they mimic natural cavities in which frogs seek refuge (Meshaka, 1996; Piacenza, 2008; Glorioso *et al.*, 2010). A hole approximately 5cm in diameter was drilled in the roof of the arena, directly above the refuge, to allow light to shine in. A plastic, trailing plant was attached to both the left and right walls and damp moss spread on the floor. The moss increased the humidity within the arena, ensuring similar experimental and husbandry conditions. Plastic plants were included in order to mimic natural habitat; providing cover for frogs to seek when leaving the refuge.



Figure 3: Diagram of Experimental Arena. A simplified diagram to represent the experimental arena is presented to highlight the position of the refuge in relation to lighting. Key components include a) lighting dome, b) plastic plants attached to walls, c) PVC refuge and d) moss covering the floor.

Three different lighting conditions were used (ambient dark, cool white LED, filtered white LED). Light intensity for each experimental condition was measured just above the entrance to the refuge using a light meter (Isotech ILM1337). The ambient dark condition (Condition 1) formed the control for this study, replicating natural low-light foraging illuminations for both species. As the room had adequately low light levels (<0.01 lux), no special considerations were necessary for this condition. The typical, white ALAN condition (Condition 2) was attained using a 6,500 K, dimmable LED spotlight (iDual G100 E27 iD75s) to maintain a mean light intensity of 170lux (range: 166-173 lux) at the entrance to the refuge. The final experimental condition, "filtered white LED" (Condition 3), was achieved by placing a 500nm longpass filter (Edmund Optics) beneath the light from the ALAN condition (see Table 3 for transmittance values) and adjusting the brightness to produce light of similar intensity (mean: 170lux, range: 167-171 lux).

Preliminary investigations of "cool white" LED streetlights in Bangor, Gwynedd, reported light intensities of up to 500lux at 2m above ground, although it was thought

unlikely that treefrogs would experience such intense light due to shading from vegetation. The target light intensity of 170lux was chosen largely due to the restrictions of available equipment, though it is important to note that this value falls within the range measured within tree canopies by Bennie *et al.* (2016). It was most appropriate to assign the white LED condition as the reference level rather than the dark control in order to allow comparison between white and filtered LED lighting.

Table 3: Transmittance of 500nm Filter. The percentage of light allowed to pass through the filter (transmittance) is shown for several wavelengths to demonstrate the ability of the filter to reduce wavelengths below 500nm. Wavelengths of 470nm or less were completely excluded by the filter, with 91.1% of all visible wavelengths equal to or greater than 560nm passing through. Data provided by Edmund Optics.

Wavelength (nm)	470	480	490	500	510	520	530	540	550	560
Transmittance (%)	0.00	0.01	5.8	40.0	71.3	84.0	88.3	90.0	90.8	91.1

The experimental arena and refuge were made from matte black acrylic to minimise behavioural bias caused by surface reflectance (Recktenwald *et al.*, 2014). Black-out fabric was used to cover all husbandry tanks in order to ensure that habituation to supplementary lighting conditions escaping into the room was reduced (Underwood *et al.*, 2017). Temperature (°C) and relative humidity (%RH) inside the experimental arena was also measured for each trial in order to control for their effects on frog behaviour. Trials were always carried out the day after food was offered, to ensure that varying levels of hunger did not influence behaviour.

Experimental trials began 2 hours after the lights were switched off, thereby ensuring full dark adaptation of frog pupils (Buchanan, 1998), and continued for four hours (20:00-00:00). A red LED headtorch was used by the observer to individually identify frogs whilst minimising behavioural impacts (Dananay & Benard, 2018; van Grunsven *et al.*, 2017). The use of red lighting was continuous but directed towards the study frog only when handling or when viewing the stop-clock. During the dark condition it was necessary to use the headtorch, directed away from the arena, to allow the observer to see whether the frog had left. This method was used consistently for both species, despite the fact that an infrared scope was also used during all green treefrog trials. Whilst this inconsistency may reduce the power of the results, it was necessary to improve the ability of the observer to determine when the trial had been completed in real-time, consequently allowing more trials to be conducted per night.

Once identified, the study frog was placed in a small, high humidity faunarium (with wet moss and a water bowl) where it was left for five minutes in order to standardise the hydration state. Following this, frogs were placed in the refuge and left for a further five minutes to adapt sufficiently to the light condition (Baker & Richardson, 2006) and minimise the effect of handling stress on results (Buchanan, 1993). A transparent cover was placed over the entrance of the refuge during this period to allow light penetration but prevent the frog from leaving. This cover reduced light intensity by 13lux, which was not considered a significant amount. Following the acclimatisation period, the cover was removed, and the time taken for the frog to begin emerging from the refuge (tip of snout passes rim) and to completely leave (vent passes the rim) was measured. These measurements are collectively referred to as "responses" throughout this paper. Once a frog had emerged from or left the refuge it was described as having "responded" to the trial. Both measurements were collected in order to observe potential latency to leave the refuge completely. All observation sessions were recorded using a modified GoPro Hero 4 to allow footage playback and analysis of data. Removal of the infrared filter and use of an IR torch (900nm, 60° beam) enabled the observer to record data during the ambient, low-light, control. This setup was standardised throughout all experimental trials. Whilst there is some evolutionary evidence that amphibians may be able to detect infrared light (Enright et al., 2015), infrared may be beyond the spectral sensitivity of amphibian eyes (Jaeger & Hailman, 1973, Yovanovich et al., 2019). Consequently, this technique is commonly used to observe amphibians in low-light conditions (Buchanan, 1993; Touzot et al., 2019). The frog was replaced in its holding tank after leaving the refuge, or if the cut-off time of 20 minutes had elapsed.

All frogs were observed under each light condition three times. A minimum 24-hour period was left between experimental trials in order to minimise stress from repeated handling and exposure on the individual. Frogs have been demonstrated to habituate to artificial light sources (Dias *et al.*, 2019), so it was necessary to control for the effects of habituation on repeated exposure to experimental stimuli (Blankenship *et al.*, 1979). Once a frog had been exposed to all three light conditions it was left in its husbandry tank for a week prior to commencement of repeat experiments to allow for spontaneous recovery. As such, animals were not repeatedly exposed to any one stimulus within a week, so it is unlikely that behavioural responses were affected by habituation. The order in which each individual was exposed to the light conditions was systematically decided to control for effects of presentation order bias on results (Perrill & Bee, 1996; McComb *et al.*, 2014; Morgan *et al.*,

2016). Since there are three conditions, six possible orders of presentation exist (see Table 4) meaning that each order was used twice for each species. In total, 216 trials were performed over 22.74 observer hours (not including habituation time).

Table 4: Presentation Orders. All possible orders in which conditions were presented (n=6) are shown for
Osteopilus septentrionalis (n=12), including repeat observations, where 1= ambient control, 2= ALAN
condition and 3= filtered ALAN. The same format was also applied for <i>Dryophytes cinereus</i> .

		Round One			Round Two			R	Round Thre	e	
ID	1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd
OS01	1	2	3		3	1	2		2	1	3
OS02	1	2	3		3	1	2		2	1	3
OS03	1	3	2		3	2	1		2	3	1
OS04	1	3	2		3	2	1		2	3	1
OS05	2	1	3	Wook	1	2	3	Wook	3	1	2
OS06	2	1	3	WEEK	1	2	3	Week	3	1	2
OS07	2	3	1		1	3	2		3	2	1
OS08	2	3	1		1	3	2		3	2	1
OS09	3	1	2		2	1	3		1	2	3
OS10	3	1	2		2	1	3		1	2	3
OS11	3	2	1		2	3	1		1	3	2
OS12	3	2	1		2	3	1		1	3	2

3.3: Data Analysis Procedure

Generalized Linear Mixed Models (GLMMS) were used to predict the effect of varying lighting type on the likelihood and speed of response. Where standardisation was not possible, other factors which may have influenced the response variable (confounding variables) were measured and included in models (see Table 5): date, observation number, time, temperature, humidity, body size and intensity of daytime lighting.

The first stage of the analysis involved assigning a binomial value for each trial (0= did not respond within twenty minutes or, 1= responded before the cut-off time). Next, all of the explanatory variables were standardised by subtracting the sample mean from the value and dividing this by the square root of sample variation (Schielzeth, 2010). Then, the effect of measured variables on the likelihood of a frog responding to a trial was investigated for both species using GLMMs with binomial error distributions in the R package lme4 (Bates *et al.*, 2018). Following this preliminary analysis, GLMMs with negative binomial error structures were used to determine how the measured variables influenced response speed. A negative binomial error distribution was used to account for overdispersion (Zuur *et al.*, 2010) since it was not appropriate to remove outlying values from the dataset. Only data for trials in which the frog responded was used because the highest value for the response variables (1200secs) represents failure to respond before the cut-off point. Inclusion of this value would violate an assumption of continuous variables as the difference between values would not have been equal. It was decided that assessing both the likelihood of response and the time taken to respond would provide a detailed insight of the effect of lighting on frog emergence.

Where variables were heavily correlated (for example light intensity and light condition) one was excluded from the analysis to ensure that this did not violate GLMM assumptions. Despite this, both observation number and date were retained in case they should provide differing insight. Since these variables were closely linked, they were not used together in a complex model as this would have violated model assumptions and may have biased the model rating. A mixture of single factor and multi-factor models (n=13) were constructed using the identified variables both independently and in biologically meaningful combinations (see Table 5). It was decided that functions such as "dredge" (package MuMin (Barton & Barton, 2015)) which create models using all possible combinations of factors would not be appropriate to use due to the risk of creating overly-complicated and undersupported models (Harrison et al., 2018). Since behavioural responses are known to vary between animals, individual ID was considered as a random effect in each model. Akaike's Information Criteria adjusted for small sample sizes (AICc) was used to compare models and rank them by explanatory power. The three models most heavily supported by this ranking were then explored further. This technique was chosen rather than excluding all models with delta AICc scores >10 as there is evidence that there can be some support for models with higher scores (Burnham et al., 2011).

Table 5: GLMM Candidate Models. A summary of the models (n=13) constructed for Generalized Linear Mixed Model (GLMM) analysis. Note that Individual ID was investigated as a random effect in all model constructions to control for repeated measures within individuals. This set of models was used to analyse data for two response variables (time taken to emerge from and to leave a refuge) across two species; the Cuban treefrog (*Osteopilus septentrionalis*) and the American green treefrog (*Dryophytes cinereus*).

Model No.	Name	Variables
1	Null	Random effect only (individual ID:1-12)
2	Date	Julian Date (Day of the year)
3	Observation No.	Observation number (1-108)
4	Time	Decimal time (hour of the day) when the experiment was started
5	Light Condition	Light condition (1,2 or 3)
6	Temperature	Temperature (degrees Celsius) at the end of habituation
7	Humidity	Humidity (%RH) at the end of habituation
8	SVL	Snout-vent length (mm)
9	Daytime Lighting	Daytime light intensity (Klux)
10	Temporal	Date + Time
11	Physiological	Temperature + Humidity
12	Physiological Size	Temperature + Humidity + SVL
13	Light Husbandry	Light condition + Daytime light intensity

In order to assess which variables best explained variation in the time taken to respond, the package AICcmodavg (Mazerolle, 2020) was used to extract β -estimates and 95% confidence intervals. In cases where the confidence intervals did not overlap zero it was assumed that the variable was likely to play a significant role in explaining observed variation of the behavioural responses.

4: Results

4.1: General Trends

Cuban treefrogs responded to approximately 74% of experimental trials (emerge= 76%, leave= 72%) whilst green treefrogs responded to 90% of all trials (see Appendix 1). Figure 4 demonstrates that both species were most likely to respond during the dark control, but this difference was smaller for green treefrogs (percentage of successes under dark conditions: Cuban treefrog- emerge: 39%, leave: 41%; green treefrog- emerge: 35%, leave: 35%). Additionally, success frequency was similar between both lit conditions, suggesting that the type of lighting was not important for either species (see Figure 4). Further analysis of response frequency data for individuals reveals that response likelihood varied within both species. For example, one Cuban treefrog (ID: OS04) did not respond to a single trial under artificial lighting (see Appendix 2). This result was not unusual; in total four individuals of this species (25% of the sample) failed to respond each time they were exposed to the filtered light condition. Conversely, no single green treefrog showed a complete aversion to one light condition, demonstrating a clear difference in sensitivity to the lit conditions between the two species. Moreover, every green treefrog which emerged from the refuge then proceeded to leave.



Figure 4: Success Frequency. Cuban treefrogs (*Osteopilus septentrionalis*) (n=12) and green treefrogs (*Dryophytes cinereus*) (n=12) were exposed repeatedly (n=3) to three different light conditions (1= dark, 2= white LED, 3= filtered LED). The frequency of frogs emerging from (stippled bars) and leaving (diagonal bars) a refuge within the twenty-minute observation period (termed "success") was recorded under each light condition.

The Cuban treefrog showed the greatest difference in response between the three light conditions (see Figures 5 and 6). Response time for this species was most varied under the dark control when frogs responded slowly (normally within 400 seconds). Under both lit conditions, Cuban treefrogs generally responded faster (responses peaked at 50 seconds) or, as Figure 4 suggests, failed to respond. On the other hand, response speed for green treefrogs showed little variation between light conditions. During the control, these frogs emerged

faster than Cuban treefrogs. These results, in combination with the above, imply that Cuban treefrogs were more likely to exhibit a bimodal "stay or flee" response when exposed to the lit conditions than green treefrogs which did not show a strong response to lighting.



Figure 5: Frequency Histograms- Emergence. Frequency histograms for time taken for Cuban treefrogs (*Osteopilus septentrionalis*, n=12) and green treefrogs (*Dryophytes cinereus*, n=12) to emerge from an artificial refuge under three light conditions (1= dark control, 2= cool white LED, 3= filtered LED) are shown. The cutoff point for these experiments was at 1200secs and each frog was exposed to each light condition three times.



Figure 6: Frequency Histograms- Leave. Frequency histograms for time taken for Cuban treefrogs (*Osteopilus septentrionalis*, n=12) and green treefrogs (*Dryophytes cinereus*, n=12) to leave an artificial refuge under three light conditions (1= dark control, 2= cool white LED, 3= filtered LED) are shown. The cut-off point for these experiments was at 1200secs.

4.2: GLMM Analyses- Response Likelihood

Cuban treefrogs were more likely to emerge (z=2.33, df=104, n=12, p<0.05) and leave (z=2.63, df=104, n=12, p<0.01) the refuge during the dark, than when exposed to the white LED. No significant difference was observed between the white and filtered LED conditions. Moreover, frogs were significantly more likely to emerge (z=2.138, df=105, n=12, p<0.05) and leave (z=3.01, df=105, n=12, p<0.005) the refuge during trials which began later in the evening. Decimal time can be considered an important factor in determining response for this species as it is independently responsible for 21% (emerge) and 46% (leave) of AICc weight (Table 6). According to both the top model summaries (Table 6) and model averaging analyses (Table 7), these factors were the only variables measured in this study which were likely to contribute significantly to variation.

Table 6: GLMM Top Model Summaries- Binomial. Thirteen GLMM candidate models (see Table 5) with binomial error structure were run in order to determine factors influencing the likelihood that two species, the Cuban treefrog (*Osteopilus septentrionalis*) and the American green treefrog (*Dryophytes cinereus*), would emerge from and leave an artificial refuge. Individual ID was considered as a random effect for all models. The summaries for each of top three models, as ranked by AICc values, are shown below. Note that every green treefrog which emerged from the refuge proceeded to leave within the experimental window so the results for both response variables are identical.

		Model	K	Delta AICc	AICc Weight	LL
		Light Condition	4	0.00	0.29	-46.21
	Emerge	Time	3	0.62	0.21	-47.60
O contentrionalic		Light Husbandry	5	1.57	0.13	-45.89
O. septentrionaus		Time	3	0.00	0.46	-46.34
	Leave	Light Condition	4	1.15	0.26	-45.83
		Temporal	4	2.14	0.16	-46.33
		Date	3	0.00	0.38	-23.96
	Emerge	Observation No.	3	0.41	0.31	-24.17
		Temporal	4	2.14	0.13	-23.96
D. cinereus	Leave					

The results for green treefrogs suggest that models "Date" and "Observation No." have the strongest effect on green treefrog response likelihood; together they are accountable for 69% of AICc weight (Table 6). This species was significantly less likely to respond during trials which took place later during the experimental period (julian date: z=-2.29, df=105, n=12, p<0.05; observation number: z=-2.24, df=105, n=12, p<0.05). The similarity of these effects is expected since these two factors were identified as being highly correlated. The GLMM analyses suggest that a combination of date and time was the third most

supported model, although time was not considered likely to have a significant effect by itself because the 95% confidence intervals overlap zero (Table 7). Unlike Cuban treefrogs, lighting type did not affect response likelihood of green treefrogs. Finally, it should be noted that the results for both response variables were identical for this species since every green treefrog which emerged proceeded to leave the refuge within the experimental window.

Table 7: Model Averaged Results- Binomial. A summary table produced when the effects of eight response variables were model averaged across thirteen Generalized Linear Mixed Models (see Table 5) with binomial error distributions. Light conditions are as follows, with condition 2 as the baseline for comparison: 1= dark control, 2= cool white LED, 3= filtered LED. Results are presented for two response variables, and across two species; the Cuban treefrog (*Osteopilus septentrionalis*) and the American green treefrog (*Dryophytes cinereus*). Every green treefrog which emerged from the refuge proceeded to leave within the experimental window. Where confidence intervals do not overlap zero (bold text), these explanatory variables are likely to have a significant effect on observed variation.

	0. se	ptentri	onalis	D. cinereus			
Response Variable	Explanatory Variable	β-estimate	SE	95% CI	β-estimate	SE	95% CI
	Julian Date	-0.15	0.30	-0.74, 0.43	-1.21	0.56	-2.31, -0.12
	Observation Number	0.16	0.28	-0.71, 0.39	-1.17	0.52	-2.20, -0.15
	Decimal Time	0.77	0.36	0.06, 1.47	0.14	0.78	-1.38, 1.66
	Light Condition 1	1.84	0.79	0.29, 3.38	1.11	1.09	-1.02, 3.23
Emerge	Light Condition 3	0.00	0.67	-1.31, 1.31	-0.46	0.96	-2.35, 1.43
Br	Temperature	0.32	0.31	-0.28, 0.92	0.52	0.50	-0.47, 1.50
	Humidity	-0.49	0.34	-1.15, 0.17	-0.26	0.47	-1.17, 0.66
	Snout-Vent Length	-1.08	0.75	-2.56, 0.39	0.48	1.61	-2.67, 3.64
	Daytime Light Intensity	0.61	0.79	-0.94, 2.17	-0.93	1.60	-4.08, 2.22
	Julian Date	-0.04	0.31	-0.64, 0.56			
	Observation Number	-0.12	0.27	-0.66, 0.42			
	Decimal Time	1.22	0.41	0.42, 2.01			
	Light Condition 1	2.08	0.79	0.53, 3.62			
Leave	Light Condition 3	-0.47	0.69	-1.83, 0.88			
Louve	Temperature	0.12	0.29	-0.45, 0.70			
	Humidity	-0.35	0.32	-0.97, 0.27			
	Snout-Vent Length	-1.07	0.83	-2.69, 0.55			
	Daytime Light Intensity	0.67	0.94	-1.17, 2.51			

4.3: GLMM Analyses- Response Speed

For Cuban treefrogs, the top model for both response variables (emerge and leave) was "Light Condition"; this accounted for 64% of AICc weight (Table 8). Further examination of the model output reveals that frogs emerged (z=3.88, df=73, n=12, p<0.001) and left (z=3.93, df=73, n=12, p<0.001) significantly quicker under the white LED condition (mean values: emerge= 86secs, leave= 123secs) than in the dark control (mean values:

emerge= 271secs, leave= 333secs). As with the binomial analyses, model averaging suggested that no significant difference was observed between the two light treatments (see Table 9). Daytime light intensity was included in the second-best model for both response variables ("Light Husbandry"), however, this was unlikely to cause a significant effect by itself as confidence intervals overlapped zero (Table 9). Although the two highest ranking models accounted for all of the AICc weight it is apparent that both decimal time and temperature were likely to have caused significant variation in response time. Table 9 demonstrates that these variables may have had negative interactions with both responses.

Table 8: GLMM Top Model Summaries. Thirteen GLMM candidate models (see Table 5) with negative binomial error structure were run for each of two response variables (time taken to emerge from and to leave an artificial refuge) across two species; the Cuban treefrog (*Osteopilus septentrionalis*) and the American green treefrog (*Dryophytes cinereus*). Individual ID was considered as a random effect for all models. The summaries for each of top three models, as ranked by AICc values, are shown below. *Both models have same AICc weight so are considered to share third place.

		Model	K	Delta AICc	AICc Weight	LL
		Light Condition	5	0.00	0.64	-455.15
	Emerge	Light Husbandry	6	1.16	0.36	-454.56
0 contentrionalis		Temperature	4	11.75	0.00	-462.17
0. septentitonuus		Light Condition	5	0.00	0.64	-477.77
	Leave	Light Husbandry	6	1.18	0.35	-477.18
		Time	4	11.22	0.00	-484.52
	Emerge	Null	3	0.00	0.17	-533.33
		Temperature	4	0.61	0.12	-532.55
		Daytime Lighting	4	0.73	0.12	-532.61
D. cinereus		Null	3	0.00	0.21	-581.31
	Ιοονο	Temperature	4	1.08	0.12	-580.76
	Leave	Daytime Lighting*	4	1.46	0.10	-580.95
		SVL*	4	1.55	0.10	-581.00

Table 9: Model Averaged Results. A summary table produced when the effects of eight response variables were model averaged across thirteen Generalized Linear Mixed Models (see Table 5) with negative binomial error distributions. Light conditions are as follows, with condition 2 as the baseline for comparison: 1= dark control, 2= cool white LED, 3= filtered LED. Results are presented for two response variables, and across two species; the Cuban treefrog (*Osteopilus septentrionalis*) and the American green treefrog (*Dryophytes cinereus*). Where confidence intervals do not overlap zero (bold text), these explanatory variables are likely to have a significant effect on observed variation.

	0. se	ptentri	onalis	D. cinereus			
Response Variable	Explanatory Variable	β-estimate	SE	95% CI	β-estimate	SE	95% CI
	Julian Date	0.04	0.12	-0.20, 0.29	-0.09	0.11	-0.30, 0.12
	Observation Number	0.12	0.12	-0.12, 0.36	-0.07	0.10	-0.27, 0.13
	Decimal Time	-0.34	0.14	-0.61, -0.06	-0.10	0.14	-0.37, 0.17
	Light Condition 1	1.08	0.28	0.54, 1.63	0.39	0.24	-0.08, 0.86
Emerge	Light Condition 3	-0.20	0.29	-0.78 ,0.37	0.12	0.25	-0.37, 0.61
g	Temperature	-0.40	0.14	-0.67, -0.13	-0.15	0.12	-0.38, 0.07
	Humidity	0.05	0.13	-0.21, 0.31	0.10	0.12	-0.14, 0.33
	Snout-Vent Length	0.12	0.23	-0.34, 0.58	0.10	0.25	-0.38, 0.58
	Daytime Light Intensity	-0.25	0.22	-0.68, 0.19	0.27	0.22	-0.16, 0.71
	Julian Date	0.09	0.12	-0.14, 0.32	-0.06	0.09	-0.23, 0.11
	Observation Number	0.17	0.12	-0.06, 0.40	-0.04	0.09	-0.21, 0.13
	Decimal Time	-0.36	0.13	-0.61, -0.10	-0.05	0.10	-0.25, 0.15
	Light Condition 1	1.06	0.27	0.53, 1.58	-0.05	0.20	-0.45, 0.34
Leave	Light Condition 3	-0.11	0.28	-0.66, 0.45	0.04	0.21	-0.37, 0.45
Leave	Temperature	-0.34	0.13	-0.59, -0.09	-0.11	0.10	-0.31, 0.09
	Humidity	0.10	0.13	-0.15, 0.35	0.04	0.10	-0.15, 0.23
	Snout-Vent Length	0.11	0.23	-0.33, 0.56	0.15	0.18	-0.21, 0.51
	Daytime Light Intensity	-0.23	0.20	-0.63, 0.17	0.15	0.17	-0.19, 0.49

None of the explanatory variables had strong effects on the response time of green treefrogs. The fact that null models (random intercept only) were most supported by the data suggests that individual choice was responsible for most of the observed variation (Table 8). Whilst temperature, daytime light intensity and body size were all included in the top-ranking models, there was no evidence in the model summaries or Table 9 to suggest that they were likely to play a significant role. It is clear that the factors driving variation in emergence behaviour differ greatly between these two species.

5: Discussion

5.1: General Trends

Regardless of the light condition, green treefrogs were more likely to respond to experimental trials than Cuban treefrogs. Moreover, every green treefrog which emerged from the refuge subsequently left within 20 minutes, whereas Cuban treefrogs would occasionally remain with the snout above top of the refuge or retreat following emergence. Cuban treefrogs are larger (Duellman & Crombie, 1970; Redmer & Brandon, 2003; Pham *et al.*, 2007) and are hence likely more conspicuous to predators than green treefrogs (Blanckenhorn, 2000). This may cause them to move less frequently and act more cautiously, sometimes retreating into the refuge following emergence. Additionally, Cuban treefrogs are noted to experience high levels of evaporative water loss (EWL) (Wygoda 1984; Roznik *et al.*, 2018), which they combat by using co-ossified skin on their heads (Trueb, 1966) to block entrances to small refugia (phragmosis) (Navas *et al.*, 2002; Roznik *et al.*, 2018). Although the refuge was too large for phragmotic behaviour to be effective, remaining stationary and lowering the head may reduce EWL from the ventral surface (Pough *et al.*, 1983). Furthermore, the Cuban treefrog has higher rates of water loss than the green treefrog (Wygoda, 1984; Roznik *et al.*, 2018), which may explain lower activity levels. Moreover, Barbeau & Lillywhite (2005) demonstrate that the green treefrog is able to combat high EWL through the wiping of lipid secretions, which may allow this species to remain more mobile than the Cuban treefrog (that is not documented to perform this behaviour (Roznik *et al.*, 2018)).

5.2: Other Variables

Evaporative water loss is known to increase with temperature (Buttemer, 1990); this may explain why Cuban treefrogs responded faster as temperature increased. Increased EWL may have driven this species to emerge quickly in order to seek a wetter environment. It is suggested that frogs may have been seeking bodies of water to soak in, rather than more humid environments since none of the models predicted that humidity (range: 43-68%RH) explained significant variation. Alternatively, temperature increase may directly influence frog activity levels. For other anurans, oxygen uptake (de Andrade & Abe, 1997) and jump performance (Walvoord, 2003; Köhler *et al.*, 2011) are known to initially increase with ambient temperature, however, the response of Cuban treefrogs to temperature is largely unknown. The effect of temperature on response speed should be interpreted with caution because variation during Cuban treefrog trials was minimal (range: 22.2-24.6°C); the observed correlation may therefore just be a spurious relationship. An examination of the relationship between EWL, activity levels and temperature would be required in order to interpret these findings with confidence.

During trials that began later in the evening Cuban treefrogs were more likely to respond and responded sooner. Few studies have documented timing of non-breeding activity in treefrogs, with the exception of Buchanan (1992). He reports that most squirrel treefrogs

(native to Florida) were active between 20:00-23:00, but did not observe increased activity as time progressed, as our results imply. Whilst the specific timing of general activity is not documented for the Cuban treefrog, observations of mating behaviour can be used as a correlate of activity level. Vargas Salinas (2006) reported that calling in this species began at 18:00 and increased until maximum intensity at 03:00-06:00. Moreover, females did not begin to arrive at breeding ponds until 21:00. These results provide some support to observations that this species would be more active during later trials, however, presentation order of trials may have biased the results. Despite the fact that presentation order of light conditions for individual frogs was determined systematically, no precautions were taken to control for the order trials took place within an evening. For example, we expect that repeatedly choosing to conduct dark trials later in the evening could imply that frogs were more likely to emerge as time of day increased. The fact that decimal time did not contribute to variation in green treefrog behaviour further supports this theory, as green treefrog responses were not influenced by the light condition.

It is unclear why green treefrogs were less likely to respond during trials that were conducted later in the year. All trials took place during the mating season for this species (Gunzbunger, 2006), however, since environmental conditions were standardised during the experimental period, change in reproductive drive is unlikely. Growth of frogs during experimental trials was not measured but is considered an unlikely explanation since statistical models suggested that SVL was not likely to have a significant effect on response likelihood. Habituation to the study procedure may have influenced this result, although a recovery period of seven days was considered sufficient, especially considering that a minimum 24-hour rest period was provided after each trial. A longer recovery period may have been necessary, however, spontaneous recovery of anuran behaviour is understudied. Alternatively, this observation may be consequent of anomalous data points, which this study may be particularly sensitive to due to comparatively small sample sizes.

Variation in behaviour between individuals was a key observation and was the only factor considered to explain variation in response time of green treefrogs. Moreover, according to the models, body size was not an important factor in determining individual differences in emergence behaviour. This was a surprising result since Kelleher *et al.* (2017) report that larger individuals of the Southern corroboree frog (*Pseudophryne corroboree*) showed greater exploratory tendencies, presumably because they had higher metabolic rates (Homyack *et al.*, 2010). Since the effect of body size appears to be of minimal importance in

explaining variation of response, observations are best explained as personality differences. Personality is succinctly summarised as "repeatable inter-individual differences in behaviour" by Roche *et al.* (2016). A controversial research topic, the relevance of studying personality is often argued, despite the fact that inter-individual differences in behaviour underpin the theory of natural selection (Dingemanse & Réale, 2005; Roche *et al.*, 2016) and are believed to be responsible for intra-specific variation in metabolic rate (Careau *et al.*, 2008). Findings from this study support previous observations that personality may influence frog boldness (Brodin *et al.*, 2013), activity and exploration (Wilson & Krause, 2012). Specifically, those observations are: persistent failure of an individual to respond during lit conditions, persistent failure of 25% of Cuban treefrogs to respond during the filtered light condition and suggestion that inter-individual variation was significant in determining speed of green treefrog responses. Frogs that persistently delay emergence from refugia may experience fitness loss consequent of reduced foraging time (Cooper & Frederick, 2007).

5.3: Effect of Light

Both species were most likely to respond during the dark control; this was expected since this condition best represents natural illuminations during active periods (Goin, 1958; Garton & Brandon, 1975; Vargas Salinas, 2006; Johnson, 2007). Despite being predominantly nocturnal, anecdotal evidence suggests that green treefrogs are occasionally active during the day (Goin, 1958). Tolerance of brighter environments may explain why this species did not adapt its emergence behaviour when exposed to artificial lighting, suggesting that green treefrogs may be resilient to ALAN intensities typical of streetlights. Whilst carrying out husbandry tasks during the day, we noted that green treefrogs were always more active than Cuban treefrogs, especially when food was provided. Statistical models also revealed that Cuban treefrogs were less tolerant of bright conditions than green treefrogs; revealing that this species emerged from and left the refuge significantly faster during the white LED condition. Adaptation of behaviour in illuminated conditions is well documented for a range of species. Some frogs are known to reduce movement, select mates quicker and call less frequently, when exposed to ALAN, implying perception of increased predation risk by visual predators in brighter environments (Rand et al., 1997; Pena et al., 2008; Bonachea & Ryan, 2011). On this basis, Cuban treefrogs may therefore have left the refuge quickly in favour of darker locations to decrease the chance of being noticed by visual predators, such as birds (Johnson, 2007), which have increased foraging ability in artificially lit conditions (Lebbin et al., 2007; Dwyer et al., 2013).

Although some Cuban treefrogs responded faster when illuminated by the white LED, response likelihood was generally lower than in the dark control; in fact, one individual never responded during any of the lit trials (see Appendix 1). Escape behaviour models predict a trade-off between fleeing from a hostile environment and the energetic costs of movement (Ydenburg & Dill, 1986). Cuban treefrogs were observed to either respond quickly, moving to darker areas of the arena, or to remain inside the refuge when exposed to artificial light. Although response to predation has not been studied for the Cuban treefrog, remaining stationary is a common approach for other North American hylids (Marchisin & Anderson, 1978) and may also enable this species to reduce EWL (Roznik et al., 2018). Furthermore, presence of co-ossified skin on the head may provide the Cuban treefrog with increased protection from predators, as in the casque-headed treefrog (Corythomantis greeningi), which uses its head to seal entrances to refugia (Jared et al., 2005). It may therefore be more energy efficient for a disturbed Cuban treefrog to remain stationary when predation risk is elevated, however, this persistent demonstration of this behaviour in response to ALAN may result in significant loss of foraging time where lighting is used for extended periods. There is clearly a fitness trade-off between leaving an unfavourable environment and remaining immobile (Cooper & Frederick, 2007); why then do Cuban treefrogs show two distinctly different behaviours in response to a novel stimulus, rather than an intermediate "compromise"?

Strong variation in response mode may demonstrate that Cuban treefrogs, a successful invasive (Owen, 2005; Rödder & Weinsheimer, 2009), exhibit greater behavioural flexibility. Behavioural flexibility is a key trait that allows invasive anurans, such as the cane toad (*Rhinella marina*), to successfully adapt to novel situations during range expansion and overcome unfavourable conditions (Webb *et al.*, 2014). In this study, Cuban treefrogs responded to illumination by either leaving the refuge quickly or by remaining stationary. In some cases, individuals consistently chose the same response. Wright *et al.* (2010) discuss that successful invasive species may show high inter-individual variation in behavioural responses when first exposed to novel stimuli in order to increase the probability that a founding population becomes established. Social learning, or natural selection, are suggested as mechanisms by which the most successful behaviour becomes more common within the population, facilitating growth and persistence of the population (adaptive flexibility hypothesis (Wright *et al.*, 2010)). Long-term studies examining the effect of ALAN exposure on light-naïve Cuban treefrogs may therefore expect to see increasing preference for a single behaviour type over time, representing emergence of an "optimal" behaviour which balances

fitness and predation risk, facilitating invasion of an unfavourable environment. Such results could demonstrate the timescale for behavioural adaptation of this species, which could be incorporated into theoretical models (such as Rödder & Weinsheimer (2009)) to predict rate of future range expansion.

Since this study was conducted in a laboratory environment it is possible to ascertain that behavioural responses to light conditions were not influenced by the attraction of prey to artificial lighting (Eisenbeis, 2006). This was particularly important to establish to ensure reliability of the comparison between the two lighting strategies.

5.4: Mitigation for Frogs

Application of the 500nm longpass filter did not have a significant effect on either response likelihood or response speed of the Cuban treefrog. Twenty-five percent of these frogs (n=4) failed to leave the refuge each time they were exposed to the filtered LED, whilst only one Cuban treefrog failed to respond each time it was exposed to the white LED (see Appendix 1). This suggests that they were able to distinguish between the two lit conditions. It was hypothesised that amphibians would be particularly sensitive to the short wavelengths produced by LEDs since they possess a second type of rod photoreceptor which is most sensitive to wavelengths of 430nm (Denton and Wyllie, 1955; Donner & Reuter, 1962; Yovanovich *et al.*, 2017). The longpass filter was efficient at removing wavelengths below 500nm (see Table 3), so it is surprising that it was not perceived as less disruptive than the white LED.

It is possible that the design of the experimental arena may have biased behavioural results by increasing perceived predation risk. In this study, the light shone directly into the refuge, potentially initiating a "stay or flee" response that may have suppressed normal behaviour. Testing whether frogs would emerge from a dark refuge into a lit environment may have reduced stress on the frogs and reported different behavioural responses, as frogs would have been able to choose whether to emerge into the light. However, it was decided to shine the light into the refuge to ensure that frog pupils had sufficiently adapted to the light level before the trial began (Buchanan, 1993; Baker & Richardson, 2006). Since green treefrogs consistently responded quickly, regardless of whether lighting was switched on, it can be presumed that behaviour of this species was not influenced by design of the experimental arena. Finally, the success of this mitigation technique cannot be judged for the green treefrog, since there was no evidence that the light condition (dark, white or filtered)

had a significant effect on behaviour. It is possible that this species possesses a tolerance threshold for ALAN which light intensities used in this study did not reach.

The results of this study cannot recommend the use spectral manipulation of LED lighting to reduce disruption of emergence from daytime refugia on Cuban treefrogs. It is suggested that the light intensity may be more important than colour, though light colour may play a greater role at lower light levels (van Grunsven *et al.*, 2017). Similar findings have been reported for invertebrates (Davies *et al.*, 2017), despite previous suggestions that spectral manipulation works for some species (Cowan & Gries, 2009; van Geffen *et al.*, 2015; Longcore *et al.*, 2015). A combination of dimming lighting and reducing the photoperiod duration may therefore be the most effective mitigation technique (Davies *et al.*, 2017), although including spectral manipulation in a mitigation plan could be valuable at some sites, such as sea turtle nesting beaches (Rivas *et al.*, 2015; Silva *et al.*, 2017). Further investigation is required to explore the success of this technique for mitigating behavioural disturbance across a range of amphibian species, in order to reduce the impact of ALAN on global amphibian declines.

6: Conclusions

Illumination of artificial refugia either caused Cuban treefrogs to emerge faster or to remain immobile but did not affect green treefrog behaviour. The adaptive flexibility hypothesis may explain expression of two diverse activity patterns by the Cuban treefrog and relate to its success as an invasive species. Lipid wiping may allow green treefrogs to reduce evaporative water loss and therefore be more active during brighter illuminations which simulate daytime conditions; ALAN may consequently cause minimal disturbance on nocturnal behaviour of this species. Extrapolating these findings to other species should be done with extreme caution (Nowakowski *et al.*, 2017). For example, despite being a close relative of the green treefrog, the squirrel treefrog may be less tolerant of ALAN since it is less active during the day (Goin, 1958). There is hence a pressing need for further studies to document the effects of ALAN on a range of amphibian species, particularly for urodeles, which are currently under-represented.

Reducing blue wavelengths typical of "cool white" LEDs did not influence behavioural responses to lighting for either species, suggesting that future research should focus on determining the effect of increasing light intensity on nocturnal behaviours of amphibians. As reported by Green *et al.* (2020), the IUCN acknowledges that approximately

75% of all amphibian species are threatened by habitat modification. Since urbanisation is likely to involve the lighting of amphibian habitat to increase neighbourhood safety, determining a lighting technique that minimises disturbance of mating and foraging behaviours should be a key research priority to ensure the conservation of global amphibian populations.

Reference List

Anderson, J.D., 1972. Phototactic behavior of larvae and adults of two subspecies of *Ambystoma* macrodactylum. Herpetologica, 28(3), pp.222-226.

Anderson, J.D. and Graham, R.E., 1967. Vertical migration and stratification of larval *Ambystoma*. *Copeia*, 1967(2), pp.371-374.

Baker, B.J. and Richardson, J.M.L., 2006. The effect of artificial light on male breeding-season behaviour in green frogs, *Rana clamitans melanota*. *Canadian Journal of Zoology*, 84(10), pp.1528-1532.

Barbeau, T.R. and Lillywhite, H.B., 2005. Body wiping behaviors associated with cutaneous lipids in hylid tree frogs of Florida. *Journal of Experimental Biology*, 208(11), pp.2147-2156.

Barton, K. and Barton, M.K., 2015. Package 'MuMIn'. Version, 1, p.18.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Scheipl, F., Grothendieck, G. and Green, P., 2018. Package 'lme4'. *Version*, *1*, p.17.

Battin, J., 2004. When good animals love bad habitats: ecological traps and the conservation of animal populations. *Conservation Biology*, *18*(6), pp.1482-1491.

Beebee, T.J., 1979. Habitats of the British amphibians (2): suburban parks and gardens. *Biological Conservation*, *15*(4), pp.241-257.

Beier, P., 1995. Dispersal of juvenile cougars in fragmented habitat. *The Journal of Wildlife Management*, 59(2), pp.228-237.

Beiswenger, R.E., 1977. Diel patterns of aggregative behavior in tadpoles of *Bufo americanus*, in relation to light and temperature. *Ecology*, 58(1), pp.98-108.

Bennie, J., Davies, T.W., Cruse, D. and Gaston, K.J., 2016. Ecological effects of artificial light at night on wild plants. *Journal of Ecology*, *104*(3), pp.611-620.

Bennie, J., Duffy, J.P., Davies, T.W., Correa-Cano, M.E. and Gaston, K.J., 2015. Global trends in exposure to light pollution in natural terrestrial ecosystems. *Remote Sensing*, 7(3), pp.2715-2730.

Blanckenhorn, W.U., 2000. The evolution of body size: what keeps organisms small?. *The quarterly review of biology*, 75(4), pp.385-407.

Blankenship, N., Peeke, H.V. and Figler, M.H., 1979. Retention and Recovery of Habituated Territorial Aggressive Behavior in the Three-Spined Stickleback (*Gasterosteus Aculeat* Us L.): the Roles of Time and Nest Reconstruction. *Behaviour*, 69(3-4), pp.171-182.

Boldogh, S., Dobrosi, D. and Samu, P., 2007. The effects of the illumination of buildings on house-dwelling bats and its conservation consequences. *Acta Chiropterologica*, 9(2), pp.527-534.

Bonachea, L.A. and Ryan, M.J., 2011. Simulated predation risk influences female choice in túngara frogs, *Physalaemus pustulosus. Ethology*, *117*(5), pp.400-407.

Brodin, T., Lind, M.I., Wiberg, M.K. and Johansson, F., 2013. Personality trait differences between mainland and island populations in the common frog (*Rana temporaria*). *Behavioral Ecology and Sociobiology*, 67(1), pp.135-143.

Brüning, A., Hölker, F., Franke, S., Kleiner, W. and Kloas, W., 2016. Impact of different colours of artificial light at night on melatonin rhythm and gene expression of gonadotropins in European perch. *Science of the Total Environment*, 543, pp.214-222.

Buchanan, B.W., 1992. Bimodal nocturnal activity pattern of *Hyla squirella*. *Journal of herpetology*, 26(4), pp.521-522.

Buchanan, B.W., 1993. Effects of enhanced lighting on the behaviour of nocturnal frogs. *Animal behaviour*, 45(5), pp.893-899.

Buchanan, B.W., 1998. Low-illumination prey detection by squirrel treefrogs. *Journal of Herpetology*, *32*(2), pp.270-274.

Buchanan, B. W. 2006. Observed and potential effects of artificial night lighting on anuran amphibians. Chapter 9 in: Rich, C. and T. Longcore (eds). *Ecological Consequences of Artificial Night Lighting*. Washington DC: Island Press, pp. 192-220.

Burnham, K.P., Anderson, D.R. and Huyvaert, K.P., 2011. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behavioral ecology and sociobiology*, *65*(1), pp.23-35.

Buttemer, W.A., 1990. Effect of temperature on evaporative water loss of the Australian tree frogs *Litoria caerulea* and *Litoria chloris*. *Physiological Zoology*, *63*(5), pp.1043-1057.

Careau, V., Thomas, D., Humphries, M.M. and Réale, D., 2008. Energy metabolism and animal personality. *Oikos*, *117*(5), pp.641-653.

Cleaver, B.D., Grubaugh, W.R., Davis, S.D., Sheerin, P.C., Franklin, K.J. and Sharp, D.C., 1991. Effect of constant light exposure on circulating gonadotrophin levels and hypothalamic gonadotrophin-releasing hormone (GnRH) content in the ovariectomized pony mare. *Journal of Reproduction and fertility. Supplement*, 44, pp.259-266.

Cohen, B.S., MacKenzie, M.L., Maerz, J.C., Farrell, C.B. and Castleberry, S.B., 2016. Color perception influences microhabitat selection of refugia and affects monitoring success for a cryptic anuran species. *Physiology & behavior*, *164*, pp.54-57.

Cooper Jr, W.E. and Frederick, W.G., 2007. Optimal time to emerge from refuge. *Biological Journal of the Linnean Society*, *91*(3), pp.375-382.

Cope, K.L., Schook, M.W. and Benard, M.F., 2020. Exposure to artificial light at night during the larval stage has delayed effects on juvenile corticosterone concentration in American toads, *Anaxyrus americanus*. *General and Comparative Endocrinology*, 295, p.113508.

Cornell, E.A. and Hailman, J.P., 1984. Pupillary responses of two *Rana pipiens*-complex anuran species. *Herpetologica*, 40(4), pp.356-366.

Cowan, T. and Gries, G., 2009. Ultraviolet and violet light: attractive orientation cues for the Indian meal moth, *Plodia interpunctella. Entomologia Experimentalis et Applicata*, *131*(2), pp.148-158.

Dananay, K.L. and Benard, M.F., 2018. Artificial light at night decreases metamorphic duration and juvenile growth in a widespread amphibian. *Proceedings of the Royal Society B: Biological Sciences*, 285(1882), p.20180367.

Davies, T.W., Bennie, J., Cruse, D., Blumgart, D., Inger, R. and Gaston, K.J., 2017. Multiple night-time lightemitting diode lighting strategies impact grassland invertebrate assemblages. *Global Change Biology*, 23(7), pp.2641-2648.

Davies, T.W., Bennie, J., Inger, R., De Ibarra, N.H. and Gaston, K.J., 2013. Artificial light pollution: are shifting spectral signatures changing the balance of species interactions?. *Global change biology*, *19*(5), pp.1417-1423.

Davies, T.W. and Smyth, T., 2018. Why artificial light at night should be a focus for global change research in the 21st century. *Global Change Biology*, 24(3), pp.872-882.

de Andrade, D.V. and Abe, A.S., 1997. Evaporative water loss and oxygen uptake in two casque-headed tree frogs, *Aparasphenodon brunoi* and *Corythomantis greeningi* (Anura, Hylidae). *Comparative biochemistry and physiology part a: physiology*, *118*(3), pp.685-689.

Delis, P.R., Mushinsky, H.R. and McCOY, E.D., 1996. Decline of some west-central Florida anuran populations in response to habitat degradation. *Biodiversity & Conservation*, *5*(12), pp.1579-1595.

Demeyrier, V., Lambrechts, M.M., Perret, P. and Grégoire, A., 2016. Experimental demonstration of an ecological trap for a wild bird in a human-transformed environment. *Animal Behaviour*, *118*, pp.181-190.

Denton, E.J. and Wyllie, J.H., 1955. Study of the photosensitive pigments in the pink and green rods of the frog. *The Journal of physiology*, *127*(1), p.81.

Dias, K.S., Dosso, E.S., Hall, A.S., Schuch, A.P. and Tozetti, A.M., 2019. Ecological light pollution affects anuran calling season, daily calling period, and sensitivity to light in natural Brazilian wetlands. *The Science of Nature*, *106*(7-8), p.46.

Dimovski, A.M. and Robert, K.A., 2018. Artificial light pollution: Shifting spectral wavelengths to mitigate physiological and health consequences in a nocturnal marsupial mammal. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 329(8-9), pp.497-505.

Dingemanse, N.J. and Réale, D., 2005. Natural selection and animal personality. *Behaviour*, 142(9-10), pp.1159-1184.

Donner, K.O. and Reuter, T., 1962. The spectral sensitivity and photopigment of the green rods in the frog's retina. *Vision Research*, 2(11), pp.357-372.

Downs, N.C., Beaton, V., Guest, J., Polanski, J., Robinson, S.L. and Racey, P.A., 2003. The effects of illuminating the roost entrance on the emergence behaviour of *Pipistrellus pygmaeus*. *Biological Conservation*, *111*(2), pp.247-252.

Duellman, W.E. and Crombie, R.I., 1970. *Hyla septentrionalis*. Catalogue of American Amphibians and Reptiles (CAAR): 92.1-92.4

Duvergé, P.L., Jones, G., Rydell, J. and Ransome, R.D., 2000. Functional significance of emergence timing in bats. *Ecography*, 23(1), pp.32-40.

Dwyer, R.G., Bearhop, S., Campbell, H.A. and Bryant, D.M., 2013. Shedding light on light: benefits of anthropogenic illumination to a nocturnally foraging shorebird. *Journal of Animal Ecology*, 82(2), pp.478-485.

Eisenbeis, G. (2006): Artificial night lighting and insects: attraction of insects to streetlamps in a rural setting in Germany. Chapter 12 in: Rich, C. and T. Longcore (eds). *Ecological Consequences of Artificial Night Lighting*. Washington DC: Island Press, pp. 281-304.

Elvidge, C.D., Keith, D.M., Tuttle, B.T. and Baugh, K.E., 2010. Spectral identification of lighting type and character. *Sensors*, *10*(4), pp.3961-3988.

Enright, J.M., Toomey, M.B., Sato, S.Y., Temple, S.E., Allen, J.R., Fujiwara, R., Kramlinger, V.M., Nagy, L.D., Johnson, K.M., Xiao, Y. and How, M.J., 2015. Cyp27c1 red-shifts the spectral sensitivity of photoreceptors by converting vitamin A1 into A2. *Current Biology*, *25*(23), pp.3048-3057.

European Comission, 2018. *EU green public procurement criteria for road lighting and traffic signals*. 10th December 2018. Brussels: European Comission.

Feuka, A.B., Hoffmann, K.E., Hunter Jr, M.L. and Calhoun, A.J., 2017. Effects of light pollution on habitat selection in post-metamorphic wood frogs (*Rana sylvaticus*) and unisexual blue-spotted salamanders (*Ambystoma laterale× jeffersonianum*). *Herpetological Conservation and Biology*, *12*(2), pp.470-476.

Foster, J.J., el Jundi, B., Smolka, J., Khaldy, L., Nilsson, D.E., Byrne, M.J. and Dacke, M., 2017. Stellar performance: mechanisms underlying Milky Way orientation in dung beetles. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1717), p.20160079.

Gabel, V., Maire, M., Reichert, C.F., Chellappa, S.L., Schmidt, C., Hommes, V., Viola, A.U. and Cajochen, C., 2013. Effects of artificial dawn and morning blue light on daytime cognitive performance, well-being, cortisol and melatonin levels. *Chronobiology international*, *30*(8), pp.988-997.

Garrett, J.K., Donald, P.F. and Gaston, K.J., 2020. Skyglow extends into the world's Key Biodiversity Areas. *Animal Conservation*, 23(2), pp.153-159.

Garton, J.S. and Brandon, R.A., 1975. Reproductive ecology of the green treefrog, *Hyla cinerea*, in southern Illinois (Anura: Hylidae). *Herpetologica*, *31*(2), pp.150-161.

Gaston, K.J., Bennie, J., Davies, T.W. and Hopkins, J., 2013. The ecological impacts of nighttime light pollution: a mechanistic appraisal. *Biological reviews*, 88(4), pp.912-927.

Gaston, K.J., Duffy, J.P., Gaston, S., Bennie, J. and Davies, T.W., 2014. Human alteration of natural light cycles: causes and ecological consequences. *Oecologia*, *176*(4), pp.917-931.

Gaston, K.J., Visser, M.E. and Hölker, F., 2015. The biological impacts of artificial light at night: the research challenge. *Phil. Trans. R. Soc. B*, *370*(1667), p. 20140133.

Gauthreaux, S.A., 1982. Age-dependent orientation in migratory birds. In: Papi F., Wallraff H.G. (eds) *Avian Navigation*. Berlin, Heidelberg: Springer, pp. 68-74.

Glorioso, B.M., Waddle, J.H., Crockett, M.E., Rice, K.G. and Percival, H.F., 2010. Diet of the invasive Cuban Treefrog (*Osteopilus septentrionalis*) in pine rockland and mangrove habitats in South Florida. *Caribbean Journal of Science*, 46(2–3), pp.346-355.

Goin, O.B., 1958. A comparison of the nonbreeding habits of two treefrogs, *Hyla squirella* and *Hyla cinerea. Quarterly Journal of the Florida Academy of Sciences*, 21(1), pp.49-60.

Gower, D.J. and Wilkinson, M., 2005. Conservation biology of caecilian amphibians. *Conservation biology*, 19(1), pp.45-55.

Green, D.M., Lannoo, M.J., Lesbarrères, D. and Muths, E., 2020. Amphibian population declines: 30 years of progress in confronting a complex problem. *Herpetologica*, *76*(2), pp.97-100.

Grubisic, M., Haim, A., Bhusal, P., Dominoni, D.M., Gabriel, K., Jechow, A., Kupprat, F., Lerner, A., Marchant, P., Riley, W. and Stebelova, K., 2019. Light pollution, circadian photoreception, and melatonin in vertebrates. *Sustainability*, *11*(22), p.6400.

Gunzburger, M.S., 2006. Reproductive ecology of the green treefrog (*Hyla cinerea*) in Northwestern Florida. *The American midland naturalist*, *155*(2), pp.321-328.

Hailman, J.P., 1984. Bimodal nocturnal activity of the western toad (*Bufo boreas*) in relation to ambient illumination. *Copeia*, 1984(2), pp.283-290.

Hailman, J.P. and Jaeger, R.G., 1974. Phototactic responses to spectrally dominant stimuli and use of colour vision by adult anuran amphibians: a comparative survey. *Animal Behaviour*, 22, pp.757-795.

Hall, A.S., 2016. Acute artificial light diminishes central Texas anuran calling behavior. *The American Midland Naturalist*, 175(2), pp.183-193.

Hammerson, G.A. & Hedges, B., 2017. Dryophytes cinereus (amended version of 2008 assessment). The IUCN Red List of Threatened Species. [Online]. http://dx.doi.org/10.2305/IUCN.UK.2017-1.RLTS.T55449A112713111.en. Accessed Jan 17, 2019.

Hänel, A., Posch, T., Ribas, S.J., Aubé, M., Duriscoe, D., Jechow, A., Kollath, Z., Lolkema, D.E., Moore, C., Schmidt, N. and Spoelstra, H., 2018. Measuring night sky brightness: methods and challenges. *Journal of Quantitative Spectroscopy and Radiative Transfer*, 205, pp.278-290.

Harrison, X.A., Donaldson, L., Correa-Cano, M.E., Evans, J., Fisher, D.N., Goodwin, C.E., Robinson, B.S., Hodgson, D.J. and Inger, R., 2018. A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ*, 6, p.4794.

Hawlena, D., Saltz, D., Abramsky, Z. and Bouskila, A., 2010. Ecological trap for desert lizards caused by anthropogenic changes in habitat structure that favor predator activity. *Conservation Biology*, 24(3), pp.803-809.

Hedges, B., Díaz, L., Ibéné, B., Joglar, R., Powell, R., Bolaños, F. and Chaves, G., 2010. *Osteopilus septentrionalis*. The IUCN Red List of Threatened Species. [Online]. <u>http://dx.doi.org/10.2305/IUCN.UK.2010-2.RLTS.T55811A11368202.en</u>. Accessed Jan 17, 2019.

Heinicke, M.P., Diaz, L.M. and Hedges, S.B., 2011. Origin of invasive Florida frogs traced to Cuba. *Biology letters*, 7(3), pp.407-410.

Hoadley, K.D., Szmant, A.M. and Pyott, S.J., 2011. Circadian clock gene expression in the coral *Favia fragum* over diel and lunar reproductive cycles. *PloS one*, 6(5), p.e19755.

Hoffmann, K.E., 2007. *Testing the influence of Cuban Treefrogs (Osteopilus septentrionalis) on native treefrog detection and abundance*. Doctoral dissertation. Gainesville: University of Florida.

Hoffmann, K.E., Johnson, S.A. and McGarrity, M., 2009. Interspecific variation in use of polyvinyl chloride (PVC) pipe refuges by hylid treefrogs: A potential source of capture bias. *Herpetological Review*. 40(4), pp.423-426.

Homyack, J.A., Haas, C.A. and Hopkins, W.A., 2010. Influence of temperature and body mass on standard metabolic rate of eastern red-backed salamanders (*Plethodon cinereus*). *Journal of Thermal Biology*, *35*(3), pp.143-146.

Iglesias-Carrasco, M., Martín, J. and Cabido, C., 2017. Urban habitats can affect body size and body condition but not immune response in amphibians. *Urban Ecosystems*, 20(6), pp.1331-1338.

IUCN Red List, 2020 [Online]. https://www.iucnredlist.org/. Accessed Sep 29, 2020.

Jaeger, R.G. and Hailman, J.P., 1971. Two types of phototactic behaviour in anuran amphibians. *Nature*, 230(5290), pp.189-190.

Jared, C., Antoniazzi, M.M., Navas, C.A., Katchburian, E., Freymüller, E., Tambourgi, D.V. and Rodrigues, M.T., 2005. Head co-ossification, phragmosis and defence in the casque-headed tree frog *Corythomantis greeningi*. *Journal of Zoology*, 265(1), pp.1-8.

Jensen, J.B., Bailey, M.A., Blankenship, E.L. and Camp, C.D., 2003. The relationship between breeding by the gopher frog, *Rana capito* (Amphibia: Ranidae) and rainfall. *The American midland naturalist*, *150*(1), pp.185-190.

Johnson, S., 2007. The Cuban Treefrog (Osteopilus septentrionalis) in Florida. Publication WEC, 218, pp.1-7.

Kelleher, S.R., Silla, A.J., Dingemanse, N.J. and Byrne, P.G., 2017. Body size predicts between-individual differences in exploration behaviour in the southern corroboree frog. *Animal behaviour*, *129*, pp.161-170.

Köhler, A., Sadowska, J., Olszewska, J., Trzeciak, P., Berger-Tal, O. and Tracy, C.R., 2011. Staying warm or moist? Operative temperature and thermal preferences of common frogs (*Rana temporaria*), and effects on locomotion. *The Herpetological Journal*, 21(1), pp.17-26.

Kupprat, F., Hölker, F. and Kloas, W., 2020. Can skyglow reduce nocturnal melatonin concentrations in Eurasian perch?. *Environmental Pollution*, 262, p.114324.

Kurvers, R.H., Drägestein, J., Hölker, F., Jechow, A., Krause, J. and Bierbach, D., 2018. Artificial light at night affects emergence from a refuge and space use in guppies. *Scientific Reports*, 8(1), pp.1-10.

Kyba, C.C., Ruhtz, T., Fischer, J. and Hölker, F., 2011. Cloud coverage acts as an amplifier for ecological light pollution in urban ecosystems. *PloS one*, *6*(3), p.e17307.

Kyba, C.C., Ruhtz, T., Fischer, J. and Hölker, F., 2012. Red is the new black: how the colour of urban skyglow varies with cloud cover. *Monthly Notices of the Royal Astronomical Society*, 425(1), pp.701-708.

Kyba, C.C., Tong, K.P., Bennie, J., Birriel, I., Birriel, J.J., Cool, A., Danielsen, A., Davies, T.W., Peter, N., Edwards, W. and Ehlert, R., 2015. Worldwide variations in artificial skyglow. *Scientific reports*, *5*, p.8409.

Lamb, C.T., Mowat, G., McLellan, B.N., Nielsen, S.E. and Boutin, S., 2017. Forbidden fruit: human settlement and abundant fruit create an ecological trap for an apex omnivore. *Journal of Animal Ecology*, 86(1), pp.55-65.

Lebbin, D.J., Harvey, M.G., Lenz, T.C., Andersen, M.J. and Ellis, J.M., 2007. Nocturnal migrants foraging at night by artificial light. *The Wilson Journal of Ornithology*, *119*(3), pp.506-508.

Liebgold, E.B. and Carleton, K.L., 2020. The Right Light: Tiger Salamander Capture Rates and Spectral Sensitivity. *Wildlife Society Bulletin*, 44(1), pp.68-76.

Longcore, T., Aldern, H.L., Eggers, J.F., Flores, S., Franco, L., Hirshfield-Yamanishi, E., Petrinec, L.N., Yan, W.A. and Barroso, A.M., 2015. Tuning the white light spectrum of light emitting diode lamps to reduce

attraction of nocturnal arthropods. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370(1667), p.20140125.

Longcore, T. and Rich, C., 2004. Ecological light pollution. *Frontiers in Ecology and the Environment*, 2(4), pp.191-198.

Macgregor, C.J., Evans, D.M., Fox, R. and Pocock, M.J., 2017. The dark side of street lighting: impacts on moths and evidence for the disruption of nocturnal pollen transport. *Global Change Biology*, 23(2), pp.697-707.

Martín, B., Pérez, H. and Ferrer, M., 2018. Effects of natural and artificial light on the nocturnal behaviour of the wall gecko. *Animal Biodiversity and Conservation*, *41*(2), pp.209-215.

May, D., Shidemantle, G., Melnick-Kelley, Q., Crane, K. and Hua, J., 2019. The effect of intensified illuminance and artificial light at night on fitness and susceptibility to abiotic and biotic stressors. *Environmental Pollution*, 251, pp.600-608.

Marchisin, A. and Anderson, J.D., 1978. Strategies employed by frogs and toads (Amphibia, Anura) to avoid predation by snakes (Reptilia, Serpentes). *Journal of Herpetology*, 12(2), pp.151-155.

Mazerolle, M.J., Huot, M. and Gravel, M., 2005. Behavior of amphibians on the road in response to car traffic. *Herpetologica*, *61*(4), pp.380-388.

Mazerolle M.J., 2020. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.3-1.

McComb, K., Shannon, G., Sayialel, K.N. and Moss, C., 2014. Elephants can determine ethnicity, gender, and age from acoustic cues in human voices. *Proceedings of the National Academy of Sciences*, *111*(14), pp.5433-5438.

McGarrity, M.E. and Johnson, S.A., 2009. Geographic trend in sexual size dimorphism and body size of *Osteopilus septentrionalis* (Cuban treefrog): implications for invasion of the southeastern United States. *Biological invasions*, *11*(6), pp.1411-1420.

McLaren, J.D., Buler, J.J., Schreckengost, T., Smolinsky, J.A., Boone, M., Emiel van Loon, E., Dawson, D.K. and Walters, E.L., 2018. Artificial light at night confounds broad-scale habitat use by migrating birds. *Ecology Letters*, 21(3), pp.356-364.

Meshaka, W.E., 1996. Retreat use by the Cuban Treefrog (*Osteopilus septentrionalis*): Implications for successful colonization in Florida. *Journal of Herpetology*, *30*(3), pp.443-445.

Mohun, S.M. and Davies, W.I.L., 2019. The evolution of amphibian photoreception. *Frontiers in Ecology and Evolution*, 7, p.321.

Morgan, K.V., Hurly, T.A., Martin, L. and Healy, S.D., 2016. Presentation order affects decisions made by foraging hummingbirds. *Behavioral ecology and sociobiology*, 70(1), pp.21-26.

Navas, C.A., Jared, C. and Antoniazzi, M.M., 2002. Water economy in the casque-headed tree-frog *Corythomantis greeningi* (Hylidae): role of behaviour, skin, and skull skin co-ossification. *Journal of Zoology*, 257(4), pp.525-532.

Newport, J., Shorthouse, D.J. and Manning, A.D., 2014. The effects of light and noise from urban development on biodiversity: Implications for protected areas in Australia. *Ecological Management & Restoration*, *15*(3), pp.204-214.

Nowakowski, A.J., Thompson, M.E., Donnelly, M.A. and Todd, B.D., 2017. Amphibian sensitivity to habitat modification is associated with population trends and species traits. *Global Ecology and Biogeography*, 26(6), pp.700-712.

Owen, J.L., 2005. The Cuban tree frog (Osteopilus septentrionalis): distribution, diet, and reproduction of an invasive species in the British Virgin Islands. Doctoral dissertation. Lubbock: Texas Tech University.

Owen, M.A., Davies, S.J. and Sloman, K.A., 2010. Light colour influences the behaviour and stress physiology of captive tench (*Tinca tinca*). *Reviews in fish biology and fisheries*, 20(3), pp.375-380.

Pawson, S.M. and Bader, M.F., 2014. LED lighting increases the ecological impact of light pollution irrespective of color temperature. *Ecological Applications*, 24(7), pp.1561-1568.

Pena, R., Granda, J. and Pierce, B., 2008. Effects of disturbance, position of observer, and moonlight on efficiency of anuran call surveys. *Applied Herpetology*, *5*(3), pp.253-263.

Perrill, S.A. and Bee, M.A., 1996. Responses to conspecific advertisement calls in the green frog (*Rana clamitans*) and their role in male-male communication. *Behaviour*, *133*(3-4), pp.283-301.

Perry, G., B. W. Buchanan, R. Fisher, M. Salmon, and S. Wise. 2008. Effects of night lighting on urban reptiles and amphibians. Chapter 16 in: J. C. Mitchell, R. E. Jung Brown and B. Bartholomew (eds) *Urban Herpetology: Ecology, Conservation and Management of Amphibians and Reptiles in Urban and Suburban Environments.* Salt Lake City: Society for the Study of Amphibians and Reptiles, pp.239-256.

Pham, L., Boudreaux, S., Karhbet, S., Price, B., Ackleh, A.S., Carter, J. and Pal, N., 2007. Population estimates of *Hyla cinerea* (Schneider)(Green Tree Frog) in an urban environment. *Southeastern Naturalist*, 6(2), pp.203-216.

Piacenza, T., 2008. Population densities of the Cuban treefrog, Osteopilus septentrionalis and three native species of Hyla (Hylidae), in urban and natural habitats of Southwest Florida. M.S. thesis. Tampa: University of South Florida.

Platenberg, R.J., 2007. Impacts of introduced species on an island ecosystem: non-native reptiles and amphibians in the US Virgin Islands. *Managing vertebrate invasive species: proceedings of an international symposium*. USDA/APHIS Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado. Aug 7-9, 2007. Fort Collins: National Wildlife Research Centre, pp.168-174.

Pough, F.H., Taigen, T.L., Stewart, M.M. and Brussard, P.F., 1983. Behavioral modification of evaporative water loss by a Puerto Rican frog. *Ecology*, 64(2), pp.244-252.

Rand, A.S., Bridarolli, M.E., Dries, L. and Ryan, M.J., 1997. Light levels influence female choice in túngara frogs: predation risk assessment?. *Copeia*, 1997(2), pp.447-450.

Redmer, M. and Brandon, R.A., 2003. *Hyla cinerea*. Catalogue of American Amphibians and Reptiles (CAAR): 766.1-766.13.

Ries, C., Spaethe, J., Sztatecsny, M., Strondl, C. and Hödl, W., 2008. Turning blue and ultraviolet: sex-specific colour change during the mating season in the Balkan moor frog. *Journal of Zoology*, 276(3), pp.229-236.

Rivas, M.L., Tomillo, P.S., Uribeondo, J.D. and Marco, A., 2015. Leatherback hatchling sea-finding in response to artificial lighting: Interaction between wavelength and moonlight. *Journal of Experimental Marine Biology and Ecology*, *463*, pp.143-149.

Recktenwald, E.W., Skorina, L.K., Neeb, C.N., Dudkin, E.A. and Gruberg, E.R., 2014. Light and shadow: Visual recognition of the stationary environment by leopard frogs. *Behavioural processes*, *107*, pp.127-132.

Rice, K.G., Waddle, J.H., Miller, M.W., Crockett, M.E., Mazzotti, F.J. and Percival, H.F., 2011. Recovery of native treefrogs after removal of nonindigenous Cuban treefrogs, *Osteopilus septentrionalis*. *Herpetologica*, 67(2), pp.105-117.

Robert, K.A., Lesku, J.A., Partecke, J. and Chambers, B., 2015. Artificial light at night desynchronizes strictly seasonal reproduction in a wild mammal. *Proceedings of the Royal Society B: Biological Sciences*, 282(1816), p.20151745.

Roche, D.G., Careau, V. and Binning, S.A., 2016. Demystifying animal 'personality' (or not): why individual variation matters to experimental biologists. *Journal of Experimental Biology*, 219(24), pp.3832-3843.

Rödder, D. and Weinsheimer, F., 2009. Will future anthropogenic climate change increase the potential distribution of the alien invasive Cuban treefrog (Anura: Hylidae)?. *Journal of Natural History*, *43*(19-20), pp.1207-1217.

Roznik, E.A., Rodriguez-Barbosa, C.A. and Johnson, S.A., 2018. Hydric balance and locomotor performance of native and invasive frogs. *Frontiers in Ecology and Evolution*, *6*, p.159.

Rubbo, M.J., Lanterman, J.L., Falco, R.C. and Daniels, T.J., 2011. The influence of amphibians on mosquitoes in seasonal pools: can wetlands protection help to minimize disease risk?. *Wetlands*, *31*(4), pp.799-804.

Ryan, M.J., 1980. Female mate choice in a neotropical frog. Science, 209(4455), pp.523-525.

Schielzeth, H., 2010. Simple means to improve the interpretability of regression coefficients. *Methods in Ecology and Evolution*, *1*(2), pp.103-113.

Silva, A.D., Diez-Méndez, D. and Kempenaers, B., 2017. Effects of experimental night lighting on the daily timing of winter foraging in common European songbirds. *Journal of Avian Biology*, 48(6), pp.862-871.

Simon, J.A., Snodgrass, J.W., Casey, R.E. and Sparling, D.W., 2009. Spatial correlates of amphibian use of constructed wetlands in an urban landscape. *Landscape Ecology*, 24(3), pp.361-373.

Smith, K.G., 2005. Effects of nonindigenous tadpoles on native tadpoles in Florida: evidence of competition. *Biological Conservation*, *123*(4), pp.433-441.

Spoelstra, K., van Grunsven, R.H., Donners, M., Gienapp, P., Huigens, M.E., Slaterus, R., Berendse, F., Visser, M.E. and Veenendaal, E., 2015. Experimental illumination of natural habitat—an experimental set-up to assess the direct and indirect ecological consequences of artificial light of different spectral composition. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1667), p.20140129.

Spoelstra, K., van Grunsven, R.H., Ramakers, J.J., Ferguson, K.B., Raap, T., Donners, M., Veenendaal, E.M. and Visser, M.E., 2017. Response of bats to light with different spectra: light-shy and agile bat presence is affected by white and green, but not red light. *Proceedings of the Royal Society B: Biological Sciences*, 284(1855), p.20170075.

Tárano, Z., 1998. Cover and ambient light influence nesting preferences in the Túngara frog *Physalaemus pustulosus*. *Copeia*, *1998*(1), pp.250-251.

Tennessen, J.B., Parks, S.E., Tennessen, T.P. and Langkilde, T., 2016. Raising a racket: invasive species compete acoustically with native treefrogs. *Animal Behaviour*, *114*, pp.53-61.

Touzot, M., Lengagne, T., Secondi, J., Desouhant, E., Thery, M., Dumet, A., Duchamp, C. and Mondy, N., 2020. Artificial light at night alters the sexual behaviour and fertilisation success of the common toad. *Environmental Pollution*, *259*, p.113883.

Touzot, M., Teulier, L., Lengagne, T., Secondi, J., Théry, M., Libourel, P.A., Guillard, L. and Mondy, N., 2019. Artificial light at night disturbs the activity and energy allocation of the common toad during the breeding period. *Conservation physiology*, *7*(1), p.coz002.

Trueb, L., 1966. Morphology and development of the skull in the frog *Hyla septentrionalis*. *Copeia*, 1966(3), pp.562-573.

Tsakalakis, I., Pahlow, M., Oschlies, A., Blasius, B. and Ryabov, A.B., 2018. Diel light cycle as a key factor for modelling phytoplankton biogeography and diversity. *Ecological Modelling*, *384*, pp.241-248.

Underhill, V.A. and Höbel, G., 2018. Mate choice behavior of female Eastern Gray Treefrogs (*Hyla versicolor*) is robust to anthropogenic light pollution. *Ethology*, *124*(8), pp.537-548.

Underwood, C.N., Davies, T.W. and Queirós, A.M., 2017. Artificial night-time light alters trophic interactions of intertidal invertebrates. *Journal of Animal Ecology*, *86*(4), pp781-789.

van Geffen, K.G., van Eck, E., de Boer, R.A., van Grunsven, R.H., Salis, L., Berendse, F. and Veenendaal, E.M., 2015. Artificial light at night inhibits mating in a Geometrid moth. *Insect Conservation and Diversity*, 8(3), pp.282-287.

van Grunsven, R.H., Creemers, R., Joosten, K., Donners, M. and Veenendaal, E.M., 2017. Behaviour of migrating toads under artificial lights differs from other phases of their life cycle. *Amphibia-Reptilia*, 38(1), pp.49-55.

Vargas Salinas, F., 2006. Breeding behavior and colonization success of the Cuban treefrog *Osteopilus septentrionalis*. *Herpetologica*, 62(4), pp.398-408.

Vredenburg, V., 2009. *Osteopilus septentrionalis: Cuban Treefrog*. Amphibiaweb. [Online]. http://amphibiaweb.org/species/1019. Accessed Jan 17, 2019.

Walker, M.F., 1970. The California site survey. *Publications of the Astronomical Society of the Pacific*, 82(487), p.672.

Walker, M.F., 1977. The effects of urban lighting on the brightness of the night sky. *Publications of the Astronomical Society of the Pacific*, 89(529), p.405.

Walsh, C.M., Prendergast, R.L., Sheridan, J.T. and Murphy, B.A., 2013. Blue light from light-emitting diodes directed at a single eye elicits a dose-dependent suppression of melatonin in horses. *The Veterinary Journal*, *196*(2), pp.231-235.

Walvoord, M.E., 2003. Cricket frogs maintain body hydration and temperature near levels allowing maximum jump performance. *Physiological and Biochemical Zoology*, *76*(6), pp.825-835.

Wanless, S., Finney, S.K., Harris, M.P. and McCafferty, D.J., 1999. Effect of the diel light cycle on the diving behaviour of two bottom feeding marine birds: the blue-eyed shag *Phalacrocorax atriceps* and the European shag *P. aristotelis. Marine Ecology Progress Series*, 188, pp.219-224.

Warkentin, K.M., 1992. Effects of temperature and illumination on feeding rates of green frog tadpoles (*Rana clamitans*). *Copeia*, 1992(3), pp.725-730.

Webb, J.K., Letnic, M., Jessop, T.S. and Dempster, T., 2014. Behavioural flexibility allows an invasive vertebrate to survive in a semi-arid environment. *Biology letters*, *10*(2), p.20131014.

Wells, K.D., 2007. The Ecology and Behaviour of Amphibians. Chicago: University of Chicago Press.

West, K.E., Jablonski, M.R., Warfield, B., Cecil, K.S., James, M., Ayers, M.A., Maida, J., Bowen, C., Sliney, D.H., Rollag, M.D. and Hanifin, J.P., 2011. Blue light from light-emitting diodes elicits a dose-dependent suppression of melatonin in humans. *Journal of applied physiology*, *110*(3), pp.619-626.

Whitfield, S.M., Lips, K.R. and Donnelly, M.A., 2016. Amphibian decline and conservation in Central America. *Copeia*, *104*(2), pp.351-379.

Widder, E.A., Robison, B.H., Reisenbichler, K.R. and Haddock, S.H.D., 2005. Using red light for in situ observations of deep-sea fishes. *Deep Sea Research Part I: Oceanographic Research Papers*, 52(11), pp.2077-2085.

Wilson, A.D. and Krause, J., 2012. Personality and metamorphosis: is behavioral variation consistent across ontogenetic niche shifts?. *Behavioral Ecology*, 23(6), pp.1316-1323.

Wright, T.F., Eberhard, J.R., Hobson, E.A., Avery, M.L. and Russello, M.A., 2010. Behavioral flexibility and species invasions: the adaptive flexibility hypothesis. *Ethology Ecology & Evolution*, 22(4), pp.393-404.

Wygoda, M.L., 1984. Low cutaneous evaporative water loss in arboreal frogs. *Physiological Zoology*, *57*(3), pp.329-337.

Ydenberg, R.C. and Dill, L.M., 1986. The economics of fleeing from predators. *Advances in the Study of Behavior*, *16*, pp. 229-249.

Yovanovich, C.A., Koskela, S.M., Nevala, N., Kondrashev, S.L., Kelber, A. and Donner, K., 2017. The dual rod system of amphibians supports colour discrimination at the absolute visual threshold. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1717), p.20160066.

Yovanovich, C.A., Grant, T. and Kelber, A., 2019. Differences in ocular media transmittance in classical frog and toad model species and its impact on visual sensitivity. *Journal of Experimental Biology*, 222(12), p.jeb204271.

Zeale, M.R., Stone, E.L., Zeale, E., Browne, W.J., Harris, S. and Jones, G., 2018. Experimentally manipulating light spectra reveals the importance of dark corridors for commuting bats. *Global change biology*, 24(12), pp.5909-5918.

Zuur, A.F., Ieno, E.N. and Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. *Methods in ecology and evolution*, 1(1), pp.3-14.

Appendices

Appendix 1: Response Likelihood. Cuban treefrogs (*Osteopilus septentrionalis*) (n=12) and green treefrogs (*Dryophytes cinereus*) (n=12) were exposed repeatedly (n=3) to three different light conditions (1= dark, 2= white LED, 3= filtered LED) under which the time taken to emerge from and leave a refuge was measured. The frequency of response (success or failure to emerge from and leave the refuge within a twenty-minute period) is shown for each light condition. The percentage of successes and failures is calculated for each species to highlight interspecific differences.

	Species		Dark	White	Filtered	Total
Emerge	O. septentrionalis	Success	32	25	25	82 (76%)
		Fail	4	11	11	26 (24%)
	D. cinereus	Success	34	32	31	97 (90%)
		Fail	2	4	5	11 (10%)
Leave	O. septentrionalis	Success	32	24	22	78 (72%)
		Fail	4	12	14	30 (28%)
	D. cinereus	Success	34	32	31	97 (90%)
		Fail	2	4	5	11 (10%)

Appendix 2: Frequency of Non-Completions. *Osteopilus septentrionalis* (ID prefix "OS", n=12) and *Dryophytes cinereus* (ID prefix "DC", n=12) were exposed to three different light conditions (1= dark, 2= white LED, 3= filtered LED) under which the time taken to emerge from and leave a refuge was measured. Each frog was exposed to each light condition three times. The frequency of failures to respond to a trial (emerge or leave the refuge) within a twenty-minute period are shown below for each frog.

	Emerge			Leave			
Frog ID	1	2	3	1	2	3	
OS01							
OS02							
OS03							
OS04		3	3		3	3	
OS05	1	2	1	1	2	1	
OS06	2	2	1	2	2	1	
OS07		2	3		2	3	
OS08							
OS09		1	1		2	3	
OS10	1	1	2	1	1	3	
OS11							
OS12							
DC01							
DC02							
DC03							
DC04							
DC05							
DC06	1	1	1	1	1	1	
DC07							
DC08							
DC09		2	2		2	2	
DC10							
DC11							
DC12	1	1	2	1	1	2	

Appendix 3: Light Effect Plots- Emerge. The effect of changing lighting type on the speed which Cuban treefrogs (*Osteopilus septentrionalis*) (a) and green treefrogs (*Dryophytes cinereus*) (b) emerge (seconds) from an artificial refuge is displayed using box and whisker plots. Three light conditions were used: 1= ambient, dark control, 2= white LED, 3= filtered white LED.



Appendix 4: Light Effect Plots- Leave. The effect of changing lighting type on the speed which Cuban treefrogs (*Osteopilus septentrionalis*) (a) and green treefrogs (*Dryophytes cinereus*) (b) leave (seconds) an artificial refuge is displayed using box and whisker plots. Three light conditions were used: 1= ambient, dark control, 2= white LED, 3= filtered white LED.

