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Color perception influences microhabitat selection of refugia and affects monitoring success for a cryptic anuran species

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ABSTRACT

Perceptual-biases are important for understanding an animal's natural history, identifying potential ecological traps, and for developing effective means to monitor individuals and populations. Despite research demonstrating anurans having a positive phototactic response towards blue colors, we do not yet understand if color cues are used functionally beyond sexual selection. The aim of our study was to determine if color cues are used in selecting microhabitat, and if anuran's blue-positive phototactic response could increase selection of artificial PVC refugia used to monitor cryptic camouflaging anuran species. We captured 32 Cope's Gray Treefrogs and placed them in mesh enclosures with three PVC tubes painted blue, brown, and white. Concurrently, we placed blue, brown, or unpainted white PVC tubes in stratified arrays around a treefrog breeding pond, and counted the number of occasions treefrogs occupied different colored PVC tubes. In the confined choice experiment, treefrogs selected blue tubes (48.3%) significantly more often than brown (28.5%) or white (23.2%) tubes. Our field experiment mirrored these findings (52.0% of capture events in blue, 29.0% in brown, and 19.0% in unpainted white tubes). Our results suggest color influences Cope's Gray Treefrog microhabitat selection, and they utilize color vision when choosing refugia. We demonstrate simple, small changes based on perceptual-biases can induce behaviors that may in turn have large impacts on sampling techniques used in monitoring and inventorying. Incorporating non-traditional physiological measures into animal inventorying and monitoring programs can be used in the future to improve conservation efforts.

1. Introduction

Behavior is the most proximate and labile way in which animals interact with their environment. Behaviors manifest and change through the interaction of environmental stimuli and an animal's perceptive and cognitive capacities, and behaviors determine the influence of animals on communities and ecosystem processes. Animal behavior is also an important part of conservation [6]. Behaviors are at the core of some of the most pressing conservation issues of the early twenty-first century. For example, mitigating human-wildlife conflicts, detecting and eradicating invasive species, and monitoring populations often rely on successfully exploiting or manipulating animal behavior [17].

Animals rely on adaptations in sensory modalities and perception to glean the saliency of innumerable cues and make informed decisions in uncertain environments. These inherited perceptual biases have evolved in response to the specific challenges of their ancestral environments and aid individuals in choosing mates [12], selecting habitats to live or forage in [37,44], and other innately adaptive behaviors [7]. For example, adult guppies are attracted towards orange coloration, the color of highly preferred fruity forages [42]. By understanding animal sensory biases and cognitive processes at the root of behaviors, we can purposefully alter cues to shape responses and produce wanted behaviors to augment conservation efforts [17].

A fundamental activity for conservation and management is the inventory and monitoring of rare or camouflaging species and estimation of species occupancy and population abundance [30]. Effective inventory and monitoring, and common population estimation techniques (i.e., capture-recapture) require accounting for and maximizing detection of individuals [29,30]. For some taxa, exploiting attentional or sensory biases can increase detection of individuals [4]. For example, exploiting chemosensory biases of animals by baiting traps or monitoring stations with food or pheromones can increase detection [16]. However, some species are presumed to not respond to chemical or gustatory cues, and therefore, wildlife biologists are often limited in their abilities to enhance detection of those taxa.

Amphibians are high priorities for global conservation, and their populations are widely viewed as sentinels of environmental change [5,39,45,46]. However, many amphibians have notoriously low or highly variable detection and capture probabilities, which can reduce the sensitivity to detect population changes and compromise study inferences [26,27,32]. Common amphibian monitoring approaches include

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call surveys for frogs, which can provide coarse estimates of abundance, or more labor-intensive approaches such as drift fences for migratory, aquatic-breeding species. For non-migratory terrestrial and arboreal species, techniques for capture and detection are generally passive and yield low capture rates. For example, manmade refugia such as coverboards and pipes are provided as attractive habitat for non-migratory terrestrial amphibians [9,20] and treefrogs [2,48] respectively. It is assumed the effectiveness of artificial cover is determined solely by the quality of the microhabitat relative to the natural habitat including the availability of natural refugia and animal refuge fidelity [2,22,31]. To our knowledge, there has been no effort to evaluate sensory biases of terrestrial or arboreal amphibians with regards to microhabitat selection and use of artificial cover.

Use of artificial arboreal refugia is a minimally invasive, low cost technique for capturing treefrogs. During the day, treefrogs naturally seek out refugia, such as shedding bark and tree cavities, to rest, retain moisture and hide from predators [10,33,34,36,41]. Arboreal tubes are presumed effective primarily because they mimic the natural microhabitat of moist cavities that treefrogs occupy. The most common artificial refuge is polyvinyl chloride (PVC) pipes [2,31], though others have used black acrylonitrile–butadiene–styrene pipes [22,23]. Our experience and those reported by others [22,31] suggest PVC pipes can be effective at capturing larger numbers of treefrogs in pine dominated habitats, particularly in the southeastern Coastal Plains, but are relatively ineffective in hardwood and mixed hardwood forests of the Piedmont. PVC tubes generally are left white unless painted to disguise their presence. Although toxicity of paint applied to PVC tubes may be a concern, treefrogs only occupy the inside of these tubes, which remained unpainted. To our knowledge, no study has found the use of painted PVC tubes is associated with acute or chronic toxicity [16]. Johnson [22] suggests darker arboreal tubes might be attractive because they better mimic the dark conditions within natural refugia; however, no study has directly compared if color affects selection of these microhabitats when anurans seek refugia.

Anurans have a visual system that is highly sensitive in scotopic (dark) and mesopic (low light) lighting conditions [1,13], which they rely on for numerous searching and foraging behaviors [13,43]. This low-light system is based on unique amphibian "green" rods and typical vertebrate "red" rods, providing anurans with the ability to process color or light-intensity in scotopic conditions [25,28]. Anurans also possess three cone receptors, which require more energy to activate and provide visual information in photopic (well-lit) conditions [13,24]. Thus, depending on light level, anurans could use their rod or cone receptors to extract color and brightness parameters to make decisions. Anurans tend to seek refugia the hour before and up to sunrise [3,47], when light conditions quickly change. Such changes could necessitate the use of either receptor-type [11]. Previous studies have suggested anurans avoid white, bright hues, and prefer to approach hues in the blue portion of the visible spectrum [8,18,35]. This suggests that the decision to use white PVC may reduce the attractiveness of PVC pipes as refugia to treefrogs, and the use of blue or the removal of white could increase treefrog attraction to this artificial microhabitat.

We were interested in examining if the blue-positive phototactic response of anurans was functionally used in microhabitat selection. We hoped that using this perceptual-bias could affect anuran refuge selection and hence be used to improve monitoring efforts. Therefore, we used the Cope's Gray Treefrog (*Hyla chrysoscelis*) to investigate the functional significance of color on microhabitat selection. Specifically, we examined whether selection of artificial PVC refugia was affected by color consistent with reported amphibian color sensory biases. If color plays a role in the selection of microhabitats, we hypothesized that Cope's Gray Treefrogs would occupy blue-colored tubes more often when choosing between blue, white, and non-white colored tubes. If color is not used functionally for anuran microhabitat selection, we would not detect any differences in occupancy when treefrogs could select to occupy the aforementioned tubes.

2. Materials and methods

2.1. Captive study

Between June and August 2012, we captured 32 Cope's Gray Treefrogs and placed each individually in 660-L nylon mesh cages (Reptarium®, Dallas Manufacturing Company, Dallas, Texas, USA) situated inside a hardwood forest. Each enclosure contained a small dish of water, *ad libitum* access to commercial feed crickets and three 60 cm long PVC tubes with an inside diameter of 3.81 cm. Of the three PVC tubes in the enclosure, one was painted white, one blue, and one brown along the outside of the tube. We measured the spectral quantities (hereafter reported as Hue, Saturation, Light) and relative brightness of each color on the PVC tube using a SVC HR-1024i spectroradiometer (Spectra Vista Corporation, Poughkeepsie, NY, USA) under the same fluorescent lighting conditions (Fig. 1). We painted the PVC with Rust-Oleum® Flat White (240°, 10.0%, 96.1%), Rust-Oleum® Leather Brown (25°, 75.9%, 31.0%), and Rust-Oleum® Flat Blue (209°, 94.0%, 54.5%). Although the PVC was originally white, the white tube was painted to remove bias introduced by paint. We allowed the painted tubes to dry for 3 days to remove paint odors. We capped the bottom of each tube and drilled a drainage hole 15 cm from the bottom so that each tube could hold a small amount of water [2]. PVC tubes were vertically placed with the opening facing up and randomly attached to either the north, south, east, or west side of the cage, with no more than one tube per side. Each day between 1200 and 1600 we checked the cage and recorded the color and position of the PVC tube occupied by the treefrog. Afterward we removed the frog by tapping the PVC tube in a downward position, washed each tube in a 5% nitric acid solution to remove any organic odors, rinsed each tube twice with distilled water, refilled with water, and placed the tubes back in the cage in a randomly determined orientation. We placed the frog

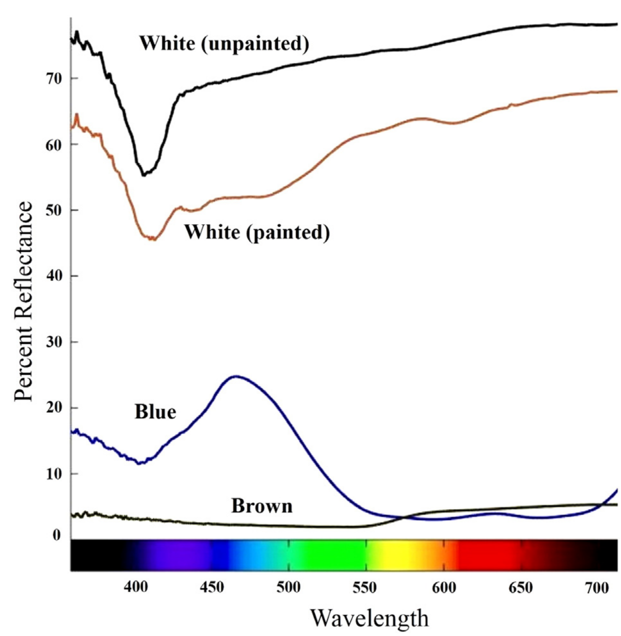

Fig. 1. Relative reflectance as a measure of brightness of the four colored PVC tubes used in experiments to test if color cues were used functionally for Cope's Gray Treefrog's decision on what artificial refuge to occupy. PVC tubes were painted white [white (painted)], blue, and brown in a confined choice experiment. Concurrent with the confined choice experiment, we conducted a field study around a 0.4 ha treefrog breeding pond. To facilitate comparison to the current methodology which uses unpainted PVC tubes, we only painted the blue and brown tubes, and left the white tubes unpainted [white (unpainted)] in the field study. Measurements were taken with a SVC HR-1024i spectroradiometer under the same fluorescent lighting conditions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)