Current Zoology 58 (6): 791–796, 2012

Response of brown anoles *Anolis sagrei* to multimodal signals from a native and novel predator

Omar L. ELMASRI, Marcus S. MORENO, Courtney A. NEUMANN, Daniel T. BLUMSTEIN*

Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA 90095-1606, USA

Abstract Multiple studies have focused on the importance of single modalities (visual, auditory, olfactory) in eliciting anti-predator behavior, however multiple channels are often engaged simultaneously. While examining responses to multiple cues can potentially reveal more complex behavioral responses, little is known about how multimodal processing evolves. By contrasting response to familiar and novel predators, insights can be gained into the evolution of multimodal responses. We studied brown anoles' (*Anolis sagrei*) response to acoustic and visual predatory cues of a common potential predator, the great-tailed grackle *Quiscalus mexicanus* and to the American kestrel *Falco sparverius*, a species found in other populations but not present in our study population. We observed anole behavior before and after a stimulus and quantified rates of looking, display, and locomotion. Anoles increased their rate of locomotion in response to grackle models, an effect modulated by grackle vocalizations. No such response or modulation was seen when anoles were presented with kestrel stimuli. This suggests that the degree of sophistication of anole response to predators is experience dependent and that relaxed selection can result in reduced anti-predator response following loss of predators [*Current Zoology* 58 (6): 791–796, 2012].

Keywords Anti-predator behavior, Multimodal risk assessment, Brown anoles

Studies examining the response of prey to predator signals have shown prey capable of sophisticated risk assessment (Caro, 2005). Most studies focus on the importance of single modalities: visual (Curio, 1975), acoustic (Hauser and Wrangham, 1990) and olfactory (Berger et al., 2001), and their role in predatory risk assessment. Vocalizations from predators have elicited responses in a variety of taxa including primates, birds, bats, insects, and rodents (Blumstein et al., 2008). While unimodal experiments are useful for isolating behavioral responses to specific components of a signal or cue, multiple channels are often engaged simultaneously (Partan and Marler, 1999).

By examining the interplay between multiple signals, multimodal experiments can potentially reveal more complex behavioral response. To date, most studies have focused on multimodal communication (Partan and Marler, 2005), specifically on signals used in conspecific communication and have shown that a variety of decisions can be improved using multiple channels. However, conspecific signals may be intercepted and used by heterospecifics as cues of predation risk (Bradbury and Vehrencamp, 2011). Prey species have been shown to increase their response to predator cues using multiple channels (Peckarsky, 1980; Lohrey et al., 2009; Blumstein and Munoz, 2012).

Partan and Marler (1999, 2005) outlined a framework to study multimodal intraspecific communication. Munoz and Blumstein (2012) have extended this framework by applying it specifically to predatory risk assessment. Multiple modalities can be integrated in various combinations. Enhancement is due to the integration of multiple modalities resulting in heightened response, whereby the animal responds more to the combination of multiple modalities than to individual modalities. Conversely, antagonism refers to when a response is reduced due to a potential excess of information. Other possible results include dominance, whereby one modality cues much greater response than other modalities. In multimodal situations, an equal response is expected for the dominant cue and the combination of the dominant cue with any other cue. Redundancy refers to when multiple signals are used by the organism to assess the same information. In modulation, one modality primes response to another, resulting in an increased or decreased response to the combined modalities as compared to individual modalities. Emergence is when a combination of modalities leads to an

Received Feb. 4, 2012; accepted Feb. 21, 2012.

∗ Corresponding author. E-mail: marmots@ucla.edu

entirely new result (Partan and Marler, 2005). Using different senses allows for insurance should one sensory channel be blocked by the environment.

To better understand the importance of multiple modalities in risk assessment, we studied brown anoles *Anolis sagrei*. Anoles are potentially able to respond to visual (Leal and Rodriguez-Robles, 1997) and acoustic (Huang et al., 2011) stimuli and are able to differentiate between a wide range of bird calls based on perceived threat level (Cantwell and Forrest, 2010[1]). Therefore, they are a suitable species for studying multimodal risk assessment. We exposed anoles to various combinations of visual and acoustic signals from a native predator (great-tailed grackle *Quiscalus mexicanus*) to gain a better understanding of the complex interplay between the sensory channels involved in predator recognition. While multimodal studies may reveal the level of sophistication in signal interpretation, they do not illuminate the degree to which experience is important for the proper performance of anti-predator behavior.

Using various channels potentially leads to better risk assessment and may allow animals to better allocate time to important activities like foraging, vigilance, and reproduction. Engaging in excessive anti-predator behavior can decrease fitness. Lima and Dill (1990) suggested that animals deal with predation uncertainty by using simple 'rules' that reflect their evolutionary history of predation. These rules allow a default response to a potential threat and may be innate in that animals are able to perform the behavior correctly without any previous experience. However, in cases where prey species are isolated from specific predators, maintenance of anti-predator behavior is non-functional and costly (Berger et al., 2001). Thus, it may be informative to examine recently isolated populations to gain insight into the flexibility of multimodal perception. Prey are capable of learning about predators and increasing the effectiveness of their response through experience (Magrath et al., 2011), a useful trait for species regularly expanding their range such as brown anoles.

By moving into an area where predators are absent, selection for anti-predator behavior may be relaxed (Lahti et al., 2009). Most previous studies focused on somewhat discrete behaviors (Lahti et al., 2009). By contrast, multimodal perception involves the integration of multiple sensory systems. Thus, it is of interest to see how a complex integrated trait - multimodal perception - fares under relaxed selection.

American kestrels *Falco sparverius* prey on a variety of anoles in other locations (Wetmore, 1916; Cruz, 1976; McLaughlin and Roughgarden, 1989) and brown anoles have been shown to respond specifically American kestrel vocalizations (Huang et al., 2011). Brown anoles are native to Cuba and the Bahamas and have successfully spread both north to mainland USA and south and west throughout the Caribbean (Williams, 1976; Campbell, 1996). Kestrels breed in the northeastern Caribbean and they are infrequently spotted on the coast of the Yucatan Peninsula during winter migrations (Sullivan et al., 2009). Our study site was an isolated island with no resident kestrels and lacked any reported sightings of migratory kestrels (Sullivan et al., 2009). Thus, our site provided a unique opportunity to study anoles' potential loss of predator discrimination in a multimodal context. Studies have shown anoles to be capable of learning (Punzo, 1985), however it is unclear what role learning plays in predator recognition in this species. We hoped to determine the role experience plays in predator recognition by exposing anoles to various combinations of acoustic and visual signals from kestrels and grackles.

1 Materials and Methods

1.1 General Methods

Subjects were studied between 10–24 October 2011, on Calabash Caye (17°16′58″N, 87°48′39″W), a location with abundant anoles. We walked along trails and beaches locating experimental subjects, moving at least 5 m between individual trial locations to avoid carry-over effects. While we collected data for any sized anole, we focused our analyses on anoles larger than 3.8 cm (snout-vent length; average deviation from 50 size estimates = 0.47 cm). On average (± *SE*), experimental subjects were 7.5 ± 0.854 cm snout-vent length. Experiments were conducted between 06:30–17:30 h, in temperatures between 25–37 ºC and only during periods of limited wind (< 3 on the Beaufort scale). If a predator flew by during an experiment, the experiment was terminated.

Three observers walked slowly searching for anoles. Upon locating a test subject, all observers sat next to each other to be equidistant from the subject. One observer estimated the ground distance to the subject (m)

[1] Cantwell LR, Forrest TG, 2010. Response of *Anolis sagrei* to acoustic calls from predatory and non-predatory birds. Proceedings of 24th National Conference on Undergraduate Research (NCUR). University of Montana, Missoula (April 15–17): 153–160.

and recorded other environmental variables such as perch height (m), percent cloud cover, and anole snout-vent length (cm). Following a 120 s habituation period, the second observer performed a 90 s focal observation, dictating behavioral transitions on to a microcassette recorder. This was then divided into a 30 s baseline observation period and a post-stimulus 60 s experimental observation period. Expanding on Huang et al. (2011), our ethogram included the following behaviors: look (head fixed in position, or movement of head to side), push up (flex two or four legs to raise body), dewlap (extension of throat flap), hop (locomote by jumping), tail wag (move tail), walk (locomote using all four legs), run (locomote rapidly using all four legs), and out of sight (subject was out of sight). For analysis, these behaviors were later grouped into three basic behavioral categories: looking (look), displaying (push up, dewlap, tail wag), and locomoting (hop, walk, run). Anoles were abundant on the island, and by working on approximately 2 km of trails it is unlikely that many individuals were studied more than once. Any trial during which conspecifics were visible within < 2 m, or any trial interrupted by the presence of any bird (predatory or not) was stopped. While we believe our design was sufficient to study multimodal perception for the predators we selected (see for example Lehtonen et al., 2012). However, future studies might benefit from the addition of controls that include additional novel (non-predatory) acoustic and visual stimuli to gain a better understanding of the specific attributes responsible for response.

1.2 Do anoles respond to a native predator?

After the initial 30 s baseline focal observation, the third observer presented one of three stimuli: grackle model only (2 different exemplars: we modified commercially available-Ashland™, Covington, KY-black bird models (18 × 7.5 cm + a 14 cm tail) by adding tail feathers, painting the head and shoulder region with blue acrylic paint, and painting the eyes with yellow acrylic paint), 2 second grackle vocalization only (7 different exemplars taken from Peterson Field Guides - Eastern/Central Bird Songs compact disc), or grackle model and vocalization. Sound Studio (v4.1) was used to normalize the volume (to 95% of RMS peak amplitude) and fade vocalizations in and out for 10 ms to eliminate possible anole response due to abruptness of the onset of sound. The recordings were broadcast at 84-86 dB SPL (measured 1 m from the speaker using a RadioShack digital sound-level meter weighting A, peak response). Visual models were presented at ground level but in direct line of sight with focal individuals for the duration of the trial. In all treatments, stimuli (model, speaker or both) were covered by a camouflaged cloth, which we removed at the end of the baseline period. Focal trials were recorded and subsequently scored and analyzed using JWatcher (version 1.0; Blumstein and Daniel, 2007).

We calculated the difference in rates of behavior from the baseline using our three behavioral categories. We split the post-stimulus 60 s focal period into two 30 s time bins in order to analyze the difference in rate from baseline over time. A two factor mixed ANOVA treated the stimuli as a between-subjects factor and time as a repeated-measure factor.

One factor ANOVAs were applied to the potential confounding variables recorded by the first observer to determine if these variables differed across the three experimental conditions.

1.3 Do anoles respond to a novel predator?

After the initial 30 s baseline focal observation, the third observer presented one of three stimuli: kestrel model only (two different exemplars: hand carved life-sized wood figurines from Unique Carved Wood Birds - La Quinta, CA), 2 second kestrel vocalization only (5 different exemplars taken from Peterson Field Guides - Eastern/Central Bird Songs), or kestrel model and vocalization. Sound Studio (v4.1) was again used to normalize the volume and fade vocalizations in and out. The recordings were broadcast at 84-86 dB SPL (measured 1 m from the speaker). Visual models were presented at ground level on axis with focal individuals for duration of the trial. In all treatments, stimuli (model, speaker or both) were covered by a camouflaged cloth, which we removed at the end of the baseline period. Previous studies have shown brown anoles respond to visual models of kestrels constructed of Styrofoam and plywood (Simon, 2007). Focal trials were recorded and subsequently scored and analyzed using JWatcher (version 1.0; Blumstein and Daniel, 2007).

We calculated the difference in rates of behavior from the baseline using our three behavioral categories. We split the post-stimulus 60 s focal period into two 30 s time bins in order to analyze the difference in rate from baseline over time. A two factor mixed ANOVA treated the stimuli as a between-subjects factor and the two 30 s time bins as a repeated- measures factor.

One factor ANOVAs were applied to the potential confounding variables recorded by the first observer to determine if these variables differed across the three experimental conditions. All analyses were conducted in

SPSS v.20 (IBM 2012) with our alpha set to 0.05.

2 Results

2.1 How anoles respond to a common predator

From experiments conducted on 111 anoles (acoustic n = 40; visual n = 32; acoustic + visual n = 39), the rate at which anoles locomoted was influenced by stimulus (Fig. 1). We found no main effect of time ($F_{1, 108}$ = 0.698, P = 0.405) or treatment ($F_{2, 108}$ = 1.38, P = 0.256), yet there was a significant interaction between time and treatment ($F_{2, 108}$ = 3.44, P = 0.036). Anoles significantly (t = 2.01, P = 0.047) increased rates of locomotion to the visual stimulus in the second 30-second time bin but not to other stimulus combinations (P > 0.412). Baseline rates of looking (P > 0.147) and display (P > 0.176) behaviors did not change significantly in response to stimulus presentation. Our results were not confounded by: wind ($F_{2, 108}$ = 2.74, P = 0.068), distance to observer ($F_{2, 108}$ = 1.19, P = 0.307), distance to speaker ($F_{2, 108}$ = 0.725, P = 0.487) snout-vent length ($F_{2, 108}$ = 2.522, P = 0.085), or perch height ($F_{2, 108}$ = 1.30, P = 0.278).

2.2 How anoles respond to a novel predator

From experiments conducted on 122 anoles (acoustic n = 40; visual n = 40; acoustic + visual n = 42) we found no main effect of time (P > 0.099) or treatment (P > 0.415), and no significant interaction between time and treatment (all P-values > 0.239) (Fig. 1). Baseline

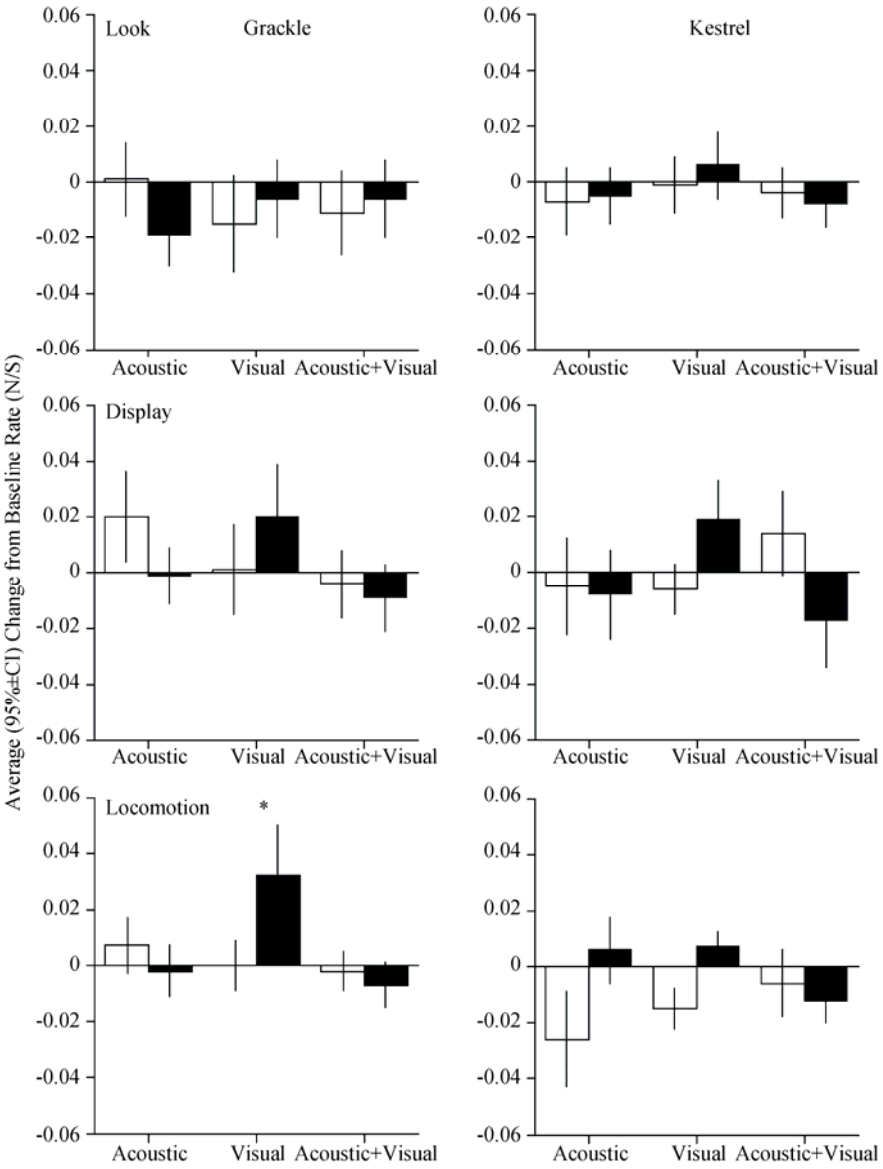

Fig. 1 Average (± 95% CI) Change from Baseline Rate (N/S) of looking, displaying, and locomotion for brown anoles in response to grackle and kestrels vocalizations, models, and the combination of model and vocalization

rates of looking ($P > 0.436$), display ($P > 0.233$) and locomotion ($P > 0.155$) did not change significantly in response to stimulus presentation. These results were not confounded by snout-vent length ($F_{2, 119} = 0.122$, $P = 0.885$), distance to observer ($F_{2, 119} = 0.573$, $P = 0.566$), distance to speaker ($F_{2, 119} = 0.431$, $P = 0.651$), wind ($F_{2, 119} = 2.18$, $P = 0.118$), or perch height ($F_{2, 119} = 0.769$, $P = 0.466$).

3 Discussion

Our results suggest that there is a more complex integration of predator cues in multiple modalities with a familiar predator. Anoles are highly visual (Fleishman et al., 1997), so it was not unexpected that they responded to a visual stimulus. Our finding that anoles increased locomotion rates parallels observations by Wunderle (1981) who observed anoles avoiding grackles and other avian species by moving to the opposite side of large tree branches and trunks. It is also unsurprising that this result was observed in the second 30 s time bin since, rather than fleeing, anoles often initially engage in tonic immobility and freeze for a period of time (Gallup, 1973). However, the response to the visual stimulus was modulated by the presentation of an acoustic stimulus, which was a surprising multimodal result. Modulation occurs only between non-redundant signals, implying that anoles treat the visual and acoustic cues as different messages, with the acoustic cue's message altering the anoles response to the visual cue.

Other studies have also shown that anoles respond to predator sounds (the American kestrel in particular [Huang et al., 2011]), so it is interesting that our population did not respond to the acoustic stimulus alone. This suggests that anole anti-predator response is to some degree experience dependent. Magrath et al. (2009) demonstrated that fairy-wrens only respond to heterospecific alarm calls of sympatric species, regardless of call similarity, implying that anti-predator behavior relies on experience. We found anoles failed to respond to any combination of kestrel visual or acoustic stimuli, further evidence that learning plays an important role in lizard anti-predator behavior.

Even though brown anoles and American kestrels evolved together, anole expansion to a region lacking kestrels resulted in either the loss of anti-predator behavior or an upward shift in the response threshold to kestrels. This finding is not consistent with the multi-predator hypothesis (Blumstein, 2006), which would have predicted predator discrimination to persist as long as there is some predation risk. Our finding is consistent with previous findings that report a loss of anti-predator behavior on islands (Blumstein and Daniel, 2005). The maintenance of costly anti-predator behavior can be lost following a loss of predators due to strong selection against no longer functional behavior (Blumstein et al., 2004; Lahti et al., 2009). This commonly occurs when a species is introduced to an island, which can lead to founder effects (Blumstein and Daniel, 2005). Our population was not devoid of predators and anoles maintained an anti-predator response to the local avian predator, an interesting finding considering that they failed to respond to a similar predator from their evolutionary past and given that they have been shown to respond to kestrels elsewhere.

Brown anoles are widely recognized as an invasive species (Losos et al., 1993; Campbell, 2000), a strong indicator of their ability to adjust to novel environments (Sol et al., 2002). A species capable of rapidly adjusting to new surroundings may be expected to show a high level of anti-predator plasticity. Future studies should attempt to tease apart the proximate causation of these anti-predator responses in a multimodal framework. Experiments using laboratory-reared anoles from our population with no exposure to the native or novel predator could potentially help distinguish if relaxed anti-predator behavior is the result of lack of experience or a genuine evolutionary response. Anoles are ideal subject for such studies because of their widespread distribution and ability at colonizing new areas, as well as their high activity levels and proven capacity to use multiple modalities.

Acknowledgements For partial support we thank the UCLA Department of Ecology and Evolutionary Biology and the UCLA Office of Instructional Development. We thank Kenneth Gayle, Neil Losin, Kathy Molina, and Matthew Patelle for assistance and support, and three anonymous reviewers for astute comments. Research was conducted under permits issued by the Belize Department of Fisheries (000023-11) and UCLA ARC Protocol #2000-147-31.

References

Berger J, Swenson JE, Persson IL, 2001. Recolonizing carnivores and naive prey: Conservation lessons from Pleistocene extinctions. Science 291: 1036–1039.

Bird DM, Palmer RS, 1988. Handbook of North American Birds: Buteos, Golden Eagle; Family Falconidae: Crested caracara, falcons. Family Accipitridae (concluded), Vol. 5 New Haven, CT: Yale University Press.

Blumstein DT, 2006. The multi-predator hypothesis and the evolutionary persistence of anti-predator resistance. Ethology 112: 209–217.

Blumstein DT, Cooley L, Winternitz J, Daniel JC, 2008. Do yellow-bellied marmots respond to predator vocalizations? Behavioral Ecology and Sociobiology 62: 457-468.

Blumstein DT, Daniel JC, 2005. The loss of anti-predator behaviour following isolation on islands. Proceedings of the Royal Society of London Series B: Biological Sciences 272: 1663–1668.

Blumstein DT, Daniel JC, Springett BP, 2004. A test of the multi-predator hypothesis: Rapid loss of anti-predator behavior after 130 years of isolation. Ethology 110: 919–934.

Bradbury JW, Vehrencamp SL, 2011. Principles of Animal Communication. 2nd edn. Sunderland, Massachusetts: Sinauer Associates, Inc.

Campbell TS, 1996. Northern range expansion of the brown anole *Anolis sagrei* in Florida and Georgia. Herpetological Review 27: 155–157.

Campbell TS, 2000. Analysis of the effect of an exotic lizard *Anolis sagrei* on a native lizard *Anolis carolinensis* in Florida, using islands as experimental units. Ph.D. Dissertation, Department of Ecology and Evolutionary Biology, University of Tennessee.

Caro T, 2005. Anti-predator Defenses in Birds and Mammals. Chicago, IL: University of Chicago Press.

Cruz A, 1976. Food and foraging ecology of the American kestrel in Jamaica. The Condor 78: 409–412.

Curio E, 1975. The functional organization of anti-predator behaviour in the pied flycatcher: A study of avian visual perception. Animal Behaviour 23: 1–115.

Fleishman LJ, Bowman M, Sanders D, Miller WE, Rury MJ et al., 1997. The visual ecology of Puerto Rican anoline lizards: Light and spectral sensitivity. Journal of Comparative Physiology 181: 446–460.

Gallup GG Jr, 1973. Simulated predation and tonic immobility in *Anolis carolinensis*. Copeia 3: 623–624.

Hauser MD, Wrangham RW, 1990. Recognition of predator and competitor calls in nonhuman primates and birds: A preliminary report. Ethology 86: 116–130.

Huang B, Lubarsky K, Teng T, Blumstein DT, 2011. Take only pictures, leave only...fear? The effects of photography on the West Indian anole *Anolis cristatellus*. Current Zoology 57: 77−82.

Lahti DC, Johnson NA, Ajie BC, Otto SP, Hendry AP et al., 2009. Relaxed selection in the wild: contexts and consequences. Trends in Ecology and Evolution 24: 487–496.

Leal M, Rodriguez-Robles JA, 1997. Signaling displays during predator-prey interactions in a Puerto Rican anole *Anolis cristatellus*. Animal Behaviour 54: 1147–1154.

Lehtonen TK, McCrary JK, Meyer A, 2012. Introduced predator elicits deficient brood defense behaviour in a crater lake fish. PLoS ONE 7(1): e30064.

Lima SL, Dill LM, 1990. Behavioral decisions made under the risk of predation: A review and prospectus. Canadian Journal of Zoology 68: 619–640.

Lohrey AK, Clark DL, Gordon SD, Uetz GW, 2009. Anti-predator responses of wolf spiders (Araneae: Lycosidae) to sensory cues representing an avian predator. Animal Behaviour 77: 813–821.

Losos JB, Marks JC, Schoener TW, 1993. Habitat use and ecological interactions of an introduced and a native species of *Anolis* lizard on Grand Cayman, with a review of the outcome of anole introduction. Oecologia 95: 525–532.

Magrath RD, Pitcher BJ, Gardner JL, 2009. Recognition of other species' aerial alarm calls: Speaking the same language or learning another? Proceedings of the Royal Society of London B: Biological Sciences 276: 769–774.

McLaughlin JF, Roughgarden J, 1989. Avian predation on anolis lizards in the northeastern Caribbean: An inter-island contrast. Ecology 70: 617–628.

Munoz NE, Blumstein DT, 2012. Multisensory perception in uncertain environments. Behavioral Ecology doi:10.1093/beheco/arr220

Partan SR, Marler P, 1999. Communication goes multimodal. Science 283: 1272–1273.

Partan SR, Marler P, 2005. Issues in the classification of multimodal communication signals. American Naturalist 166: 231–245.

Peckarsky BL, 1980. Predator-prey interactions between stoneflies and mayflies: Behavioral observations. Ecology 61: 932–943.

Punzo F, 1985. Neurochemical correlates of learning and role of the basal forebrain in the brown anole *Anolis sagrei* (Lacertilia: Iguanidae). Copeia 1985: 409–414.

Simon V, 2007. Not all signals are equal: Male brown anole lizards *Anolis sagrei* selectively decrease pushup frequency following a simulated predatory attack. Ethology 113: 793–801.

Sol D, Timmermans S, Lefebvre L, 2002. Behavioural flexibility and invasion success in birds. Animal Behaviour 63: 495–502.

Sullivan BL, Wood CL, Iliff MJ, Bonney RE, Fink D et al., 2009. eBird: A citizen-based bird observation network in the biological sciences. Biological Conservation 142: 2282–2292.

Wetmore A, 1916. Birds of Puerto Rico. U.S. Department of Agriculture Bulletin 326: 1–140.

Williams EE, 1976. West Indian anoles: A taxonomic and evolutionary summary. Breviora 440: 1–21.

Wunderle, JM, 1981. Avian predation upon anolis lizards on Grenada, West Indies. Herpetologica 37: 104–108.