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Sequential assessment of prey through the use of multiple sensory cues by an eavesdropping bat

Rachel A. Page · Tanja Schnelle ·
Elisabeth K. V. Kalko · Thomas Bunge ·
Ximena E. Bernal

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Abstract Predators are often confronted with a broad diversity of potential prey. They rely on cues associated with prey quality and palatability to optimize their hunting success and to avoid consuming toxic prey. Here, we investigate a predator's ability to assess prey cues during capture, handling, and consumption when confronted with conflicting information about prey quality. We used advertisement calls of a preferred prey item (the túngara frog) to attract fringe-lipped bats, *Trachops cirrhosus*, then offered palatable, poisonous, and chemically manipulated anurans as prey. Advertisement calls elicited an attack response, but as bats approached, they used additional sensory cues in a sequential manner to update their information about prey size and palatability. While both palatable and poisonous small anurans were readily captured, large poisonous toads were approached but not contacted suggesting the use of echolocation for assessment of prey size at close range. Once prey was captured, bats used chemical cues to make final, post-capture decisions about whether to

consume the prey. Bats dropped small, poisonous toads as well as palatable frogs coated in toad toxins either immediately or shortly after capture. Our study suggests that echolocation and chemical cues obtained at close range supplement information obtained from acoustic cues at long range. Updating information about prey quality minimizes the occurrence of costly errors and may be advantageous in tracking temporal and spatial fluctuations of prey and exploiting novel food sources. These findings emphasize the sequential, complex nature of prey assessment that may allow exploratory and flexible hunting behaviors.

Keywords Multimodal cues · Foraging strategies · Prey palatability · Prey size · Predator flexibility · *Trachops cirrhosus*

Introduction

Foraging strategies of animals evolve to optimize detection, handling, and consumption of food (Krebs 1973). To survive, predators must find prey that is both of an appropriate size and palatable. To accomplish this goal, predators often use multiple sensory cues to detect and assess prey (e.g., Marimuthu and Neuweiler 1987; Kardong et al. 1997; von der Emde and Bleckmann 1998; Barber and Conner 2007; Roberts et al. 2007). Within-species prey availability and toxicity can vary both temporally and spatially (e.g., Hanifin et al. 1999; Clark et al. 2006; Saporito et al. 2007), and thus, predators are expected to exhibit behavioral flexibility to assess prey throughout the detection–capture–consumption sequence.

We conducted experiments to investigate prey assessment in the fringe-lipped bat, *Trachops cirrhosus*, a predator with flexible hunting strategies (Page and Ryan 2005). We hypothesized that information updating minimizes potentially lethal

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R. A. Page (✉) · E. K. V. Kalko · X. E. Bernal
Smithsonian Tropical Research Institute,
Apartado 0843-03092, Balboa,
Ancón, Panamá, Republic of Panama
e-mail: PageR@si.edu

T. Schnelle · E. K. V. Kalko · T. Bunge
Institute of Experimental Ecology, University of Ulm,
Albert-Einstein-Allee 11,
89069 Ulm, Germany

X. E. Bernal
Biological Sciences, Texas Tech University,
Box #43131, Lubbock, TX, USA







errors in prey quality assessment. *T. cirrhosus* feeds on a wide variety of prey, including frogs. It listens to species-specific frog mating calls as an initial cue to discriminate palatable from poisonous frogs and does not approach the calls of toxic toad species (Tuttle and Ryan 1981). *T. cirrhosus* has pronounced tubercles on its chin and lips (Supplementary Fig. 1), postulated to act as chemosensors enabling rapid prey palatability assessment before capture and consumption (Miller 1907). Using the calls of a palatable species to elicit bat approach, we presented bats with chemically manipulated and unmanipulated prey items. We offered bats three sympatric anuran species including a palatable and preferred prey species, the túngara frog, *Physalaemus (Engystomops) pustulosus* (Tuttle and Ryan 1981), and two poisonous toads, the large cane toad, *Rhinella marina* (*Bufo marinus*), and the small leaf litter toad, *Rhinella alata* (*Bufo typhonius*), both with toxins that are potentially lethal if ingested (Chen and Kovarikova 1967; Toledo and Jared 1995). Previous studies have highlighted frog mating calls as the key component of prey selection because

they offer reliable information about species identity (Tuttle and Ryan 1981). In this study, we examined the degree to which cues eliciting approach are modulated by subsequent cues detected later in the hunting sequence. We investigated the hypothesis that predators use multiple sensory cues to sequentially update their information about prey quality, with the prediction that predators respond to conflicting information with rapid and flexible reassessment throughout the hunting approach.

Materials and methods

Eight bats were captured with mist nets set along streams and ponds and tested in a 4.5×4×2.5-m outdoor flight cage on Barro Colorado Island, Panama from July to October 2007. Conditions in captivity followed Page and Ryan 2005. We offered bats six types of live prey (Table 1); three without experimental manipulation: (1) palatable and small, *P.*

Table 1 Prey items offered to bats. Snout-vent lengths are shown in parentheses (from Ibáñez et al. 1999). Drawings of anurans approximate their relative sizes. Anuran color indicates chemical cues: toxic toads (*R. marina*, dark gray; *R. alata*, light gray) and palatable frogs (*P. pustulosus*, white)

Unmanipulated prey (vary in size and toxicity)	Chemically manipulated prey (vary only in toxicity)
<p><i>P. pustulosus</i>: palatable and small</p> 	<p><i>P. pustulosus</i> coated with <i>R. marina</i> secretions (toxic)</p> 
<p><i>R. alata</i>: toxic and small</p> 	<p><i>P. pustulosus</i> coated with <i>R. alata</i> secretions (toxic)</p> 
<p><i>R. marina</i>: toxic and large</p> 	<p><i>P. pustulosus</i> coated with <i>P. pustulosus</i> secretions (palatable)</p> 

pustulosus, (2) toxic and small, *R. alata*, and (3) toxic and large, *R. marina*; and three with manipulation, all small: (1) toxic with secretion from a large species: *P. pustulosus* coated with *R. marina* secretions; (2) toxic with secretion from a small species: *P. pustulosus* coated with *R. alata* secretions; and as a control, (3) *P. pustulosus* coated with *P. pustulosus* secretions (palatable). Secretions were extracted from the toads by gently massaging their parotid glands and were applied immediately before each trial to the head and thoracic dorsum of the palatable frog. We were not able to precisely quantify the amount of secretion extracted, but we standardized the amount of secretion applied to the frogs by systematically coating the entire upper surface of the head and thoracic dorsum with a thin layer of toad secretion. We immobilized the prey by attaching a thin string to the frog's rear leg; we threaded this string through the screen and held it in place until the bat approached (Supplementary Video 1). Each prey type was offered to each bat once for a total of six trials per bat; trial order was randomized. Because the bats quickly stop responding if offered only unpalatable prey, we gave bats untreated *P. pustulosus* following trials with unpalatable prey. We controlled the food intake of the bats and conducted trials only when bats were motivated to feed. Testing for each bat was completed in a single night.

We broadcast túngara frog calls from a Dell Inspiron 3800 computer, a SA-150 Realistic amplifier, and a 40–1040 Radio Shack speaker beneath a screen landing platform at intensities approximating túngara calls in nature (Ryan 1983). We positioned the frogs on the screen directly above the speaker (Fig. 1). We used an infrared camcorder

(Sony DCR-HC17E PAL) and a high-speed camera (Optronis CamRecord 600 at 500 frames/s) to record capture and handling behavior. To minimize the bat's use of vision, we illuminated the flight cage with two 25-W red light bulbs and four infrared lights.

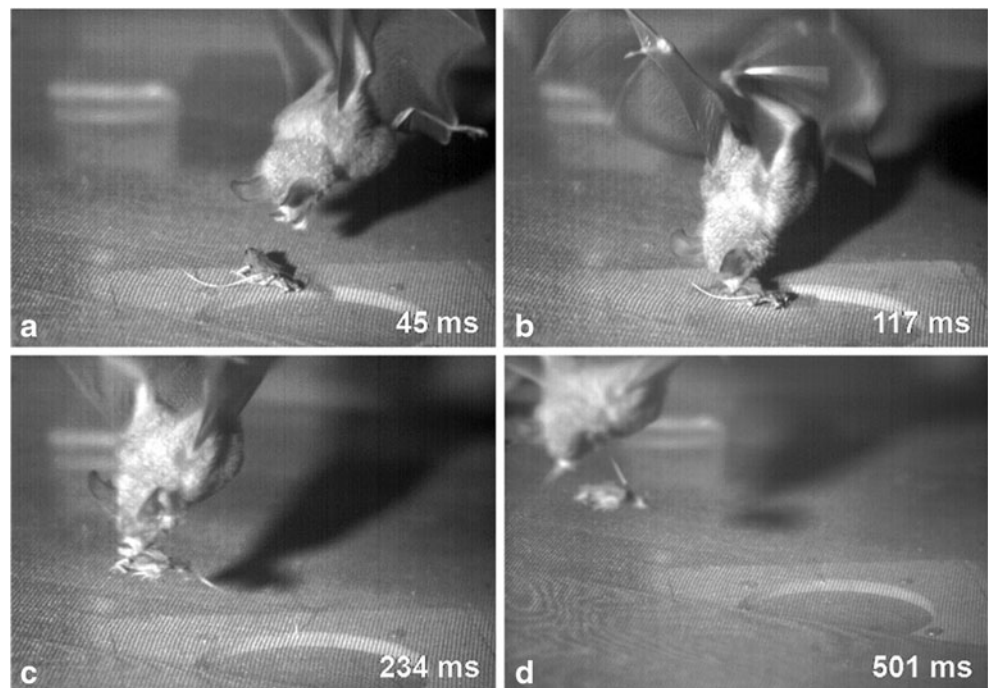
We classified the prey as “captured” when the bat bit into it and “consumed” if it was fully ingested. Differences in capture and consumption behavior among prey types were analyzed using Fisher exact tests.

After testing, each bat was released at the site of capture. All anurans that were not consumed were thoroughly washed in clean water and released. All such frogs recovered completely. Experiments were licensed and approved by the Smithsonian Tropical Research Institute (IACUC protocol number 2007-14-06-15-07).

Results

Small, palatable frogs were captured by all bats regardless of whether they were coated with poisonous or nonpoisonous extract (Fig. 2). Bats were as likely to capture túngara frogs that had been coated in toxins as those that were not coated with toxins (Fisher's exact test, $P=1$). Seven of eight bats captured the small but toxic leaf litter toad (Fig. 2a). In contrast, all bats circled above the large cane toad but returned to the perch without landing, with the exception of one bat that landed next to the toad and came into brief contact with it (Fig. 2a).

Fig. 1 Stills from high-speed video of a fringe-lipped bat capturing and subsequently dropping a túngara frog coated with toad parotid secretions. **a** Bat approaching prey in flight, **b** bat biting into prey's head, **c** bat flying back toward the perch with prey in its mouth, **d** bat dropping prey



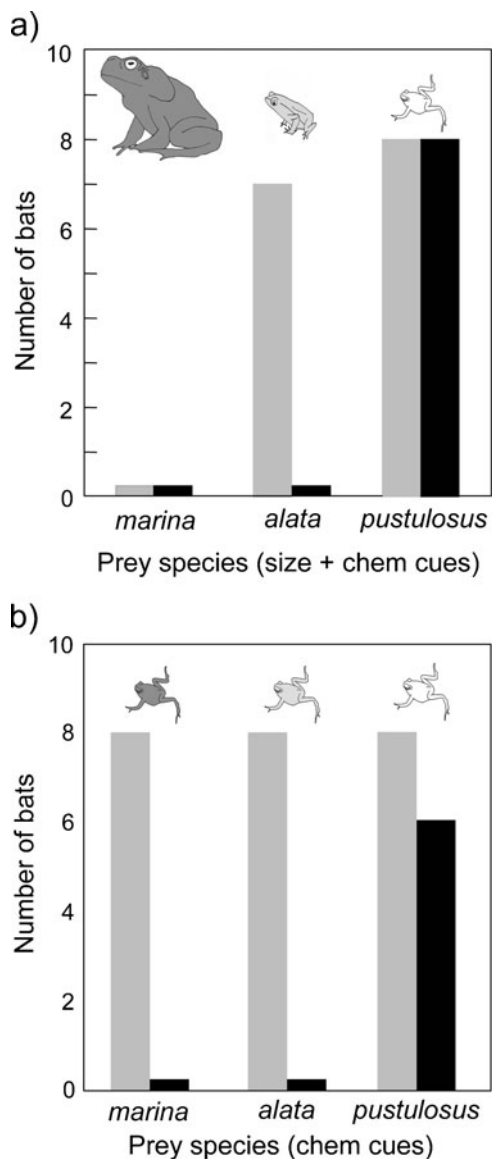


Fig. 2 Number of bats that captured (gray bars) and consumed (black bars) anuran prey that vary in **a** both size and toxicity or **b** toxicity only ($n=8$ bats). Size and color of anuran figures as in Table 1. Bats were as likely to capture túngara frogs that had been coated in toxins as those that were not (Fisher's exact test, $P=1$), but only consumed túngara frogs that were not coated with toxins, rejecting túngara frogs that were coated with toad toxins (Fisher's exact test, $P<0.001$)

Untreated túngara frogs and túngara frogs coated in túngara secretions were consumed, while túngara frogs coated with toad toxins were rejected by all bats (Fisher's exact test, $P<0.001$). Small, toxic toads were captured but dropped in flight. When bats chewed toad-toxin-coated túngara frogs prior to dropping them, there was a noticeable increase in salivary production. Careful examination of high-speed video sequences did not offer any evidence that the bats rubbed their tubercles against the skin of their prey prior to capture (Supplementary Video 1).

Discussion

Our study demonstrates that following initial assessment of prey, bats have the ability to use alternate sensory modalities to sequentially reassess prey at close range, and thus compensate for potentially deadly errors. We show that following initial prey assessment using sonic acoustic cues, fringe-lipped bats update information on prey size and prey palatability upon approach likely using first echolocation, then chemical cues.

In all cases, hunting was triggered by broadcast prey-emitted acoustic cues, here túngara frog advertisement calls. Following the initial approach, our evidence suggests bats used echolocation to assess prey size. All bats approached and captured *R. alata*, a toad similar in size to the palatable túngara frog, when it was placed on top of a speaker broadcasting túngara frog calls. In contrast, when presented in the same manner with the large cane toad, *R. marina*, the majority of bats aborted the hunting attempt prior to landing. *R. marina* is considerably larger not only than the túngara frog but also than the fringe-lipped bat. The parotid secretions of *R. marina* are not volatile and are secreted only when in contact with the predator (Toledo and Jared 1995), so it is unlikely that bats rejected *R. marina* on the basis of chemical cues secreted from the parotid glands. It is possible, however, that the bats smelled the toads at close range. We did not record echolocation calls in our experiment, but it is known that *T. cirrhosus* emit echolocation calls throughout the hunting approach (Barclay et al. 1981; Page unpublished data). We think it likely that the bats aborted their approaches to *R. marina* when they perceived its large size by echolocation, a secondary cue which overrode the initially positive sonic acoustic cue. While we minimized the potential for visual assessment of prey by conducting the experiments in red and infrared light, conditions under which bats are largely unable to see (Winter et al. 2003), we cannot be sure that bats did not have some access to visual information. Further experiments are necessary to determine the relative roles of echolocation and vision in bat assessment of prey.

When prey size was removed as a cue, *T. cirrhosus* used chemical cues to assess prey quality post-capture. All bats captured small frogs coated with toad toxins. Because assessing prey by touching it before capture can elicit flight or defensive behavior, it is possible that bats first capture and then assess because this is a more effective hunting strategy. We found no evidence from high-speed video that the bats use their tubercles for chemical classification of prey before or during capture; thus, observations from this study do not support the hypothesis that tubercles on the bats' chin and lips act as chemosensors. Because we used túngara frog calls to elicit the bats' approach, the bats in our study were expecting palatable prey. It is possible that bats approaching a novel prey cue, for instance the call of a frog with which they had no experience, would be more cautious and would rely to a greater extent on other sensory cues. Other

bat species in addition to *T. cirrhosus* have some degree of facial tubercles; more investigation is necessary to understand the possible sensory role of bat facial tubercles in prey assessment.

When prey was rejected after partial consumption, chewing was accompanied by extreme salivation, evidenced by saliva exuding from the mouth and frothing over the chin and lips. *T. cirrhosus*, together with other frog-eating bats, possess unique accessory submandibular salivary glands, which may protect them from toxins and allow them to reject poisonous prey post-capture without ill effects (Tandler et al. 1996, 1997).

T. cirrhosus are quick to learn novel associations between acoustic cues and prey quality, and readily show exploratory behavior in foraging (Page and Ryan 2005, 2006). This rapid learning could be facilitated by the ability to update information in the final stages of the hunting process, providing a rigorous multi-stage assessment system that may diminish the chance of error and allow bats to have flexible hunting strategies. Similar rapid learning and use of multiple sensory modalities for prey assessment is found in insectivorous bats that learn to associate the ultrasonic clicks emitted by tiger moths with noxious chemical cues, and then use these acoustic warning cues to make rejection decisions to avoid unpalatable prey (e.g., Surlykke and Miller 1985; Bates and Fenton 1990; Hristov and Conner 2005). Ratcliffe and Fullard (2005) show that the sensory cues predators use to assess prey quality depend on the predator's foraging mode: northern long-eared bats (*Myotis septentrionalis*) flexibly use moths' ultrasonic warning clicks to avoid noxious prey when capturing prey in flight, but when gleaning the same prey species from surfaces, the bats rely on chemical cues to reject noxious prey.

Our study suggests that last-instant prey assessment minimizes the occurrence of costly errors and may allow animals to exploit novel or changing food sources. Our results bring to light the sequential, complex nature of prey assessment foraging strategies that may allow exploratory and flexible hunting behaviors.

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