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Localization Error and Search Costs during Mate Choice in Túngara Frogs, *Physalaemus pustulosus*

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Abstract

Search costs can have profound influences on female choice, causing females to become less choosy or sample less of the diversity of available mates. Predator foraging strategies, however, determine exactly how search time affects predator encounter rates. Ambush predators are more likely to be encountered by females traveling longer distances to evaluate males, but evaluation time is unlikely to influence encounter-rate with this type of predator. Actively searching predators, however, may be more likely to be encountered by females employing longer travel times and evaluation times. In this study, we examine the effects of perceived search costs on both temporal and spatial aspects of the search behavior of female túngara frogs, *Physalaemus pustulosus*. Females were collected from natural choruses and presented with conspecific calls at a distance of 50, 115, or 180 cm from their release point. Assays were conducted in either darkness or simulated full moon light levels. Longer starting distances caused longer choice latencies, but choice latency was considerably lowered under higher light conditions. Females spent considerably less time moving under higher light conditions; however, light levels did not affect path length. Females were more likely to leave the release point with more accurate orientation to the sound source under higher light conditions. We demonstrate that females can respond to perceived search costs by altering spatial and temporal aspects of female search behavior. The overall emphasis of females on reducing time spent moving and increasing movement speed indicates that predation by actively searching predators represents a stronger cost to females than ambush predators.

Introduction

While females of many taxa exhibit strong preferences for a myriad of sexual ornaments and signals, being choosy about potential mates is not without its costs as evaluating males requires an investment of time and females must often move among males to evaluate them, increasing their chances of encountering predators along the way. Searching among potential mates then represents a tradeoff, where the benefits of additional sampling of the distribution of available mates must be weighed against additional costs incurred by moving between males (Real 1990). When search costs are high, females should theoretically respond by reducing overall search times, whether by evaluating fewer males, evaluating each male for less time, or discriminating less between males (Pomiankowski 1987; Real 1990; Crowley et al. 1991). In fact, increased search costs can cause females to become less choosy or even reverse their preferences for normally attractive mates (Evans et al. 2004; Schwartz & Hendry 2006, 2007; Dunn & Whittingham 2007), increase thresholds of mate attractiveness for mating (Demary et al. 2006; Su & Li 2006; Vélez & Brockmann 2006), reduce search times (Karino et al. 2000, deRiviera et al. 2003; Kim et al. 2007), and reduce mate sampling (Karino et al. 2000).

In situations where females encounter ambush predators rather than active searching predators, search time per se may be less of a factor in determining encounter rates than travel distance. In this case, female spatial paths may be shorter and more direct under increased predation risk, even though choice latency and movement speed are unaffected. In situations where females are likely to encounter actively searching predators, females should minimize time in the chorus to reduce encounter rates; thus, females should spend less time evaluating males and move very quickly to the chosen male, but the length or directness of spatial paths used to reach males may be unaffected. Thereby, predation strategy can potentially influence how females respond to perceived search costs. In this study, we examine how females respond to perceived risk incurred during longer travel times to males; particularly how distance to stimulus and light levels influence spatial paths, choice latency, localization, travel time, and movement speed during phonotaxis in túngara frogs, *Physalaemus pustulosus*.

The túngara frog is a small leptodactylid frog common throughout Middle America, from the Yucatán of Mexico to the Llanos of Venezuela (Ryan et al. 1996). Breeding takes place in the rainy season, from May to November in Panama, and males assemble in choruses at seemingly any available body of shallow water, often in extremely ephemeral puddles. Females can produce 3–4 clutches per season and visit choruses when they are ready to breed. Except in rare cases of high population density, females are mostly unimpeded by males as they move through a chorus (Ryan 1985).

Female choice is driven by preferences for several properties of the male acoustic advertisement signal, which consists of a downward frequency sweep or 'whine'. Males can produce a simple, 'whine-only' call or a complex call, a 'whine' with one to seven additional 'chucks' (Ryan & Rand 1993). Females strongly prefer complex calls to simple calls, and prefer lower dominant frequencies. All males appear to be capable of producing chucks, but doing so presents a tradeoff, as complex calls are also more attractive to frog-eating bats (Ryan et al. 1982) and corethrellid flies (Bernal et al. 2006).

Female túngara frogs encounter many predators while moving through choruses of males. The smoky jungle frog, *Leptodactylus pentadactylus*, and the cane toad, *Bufo marinus*, both share choruses with túngara frogs and largely act as ambush predators of frogs moving in the chorus (Ryan et al. 1981). On the other hand, cat-eyed snakes, *Leptodeira septeptrionalis*, juvenile fer-de-lance, *Bothrops asper*, ctenid spiders, and *Philander* opossums actively move throughout choruses, feeding on calling males and females visiting the chorus. Given the suite of predators they encounter, it is not surprising that females respond strongly to cues of predation risk during mate evaluation. Females are less likely to travel longer distances for otherwise more attractive males under high light conditions, presumably because higher light levels experienced on full moon nights improve detection by visual predators (Rand et al. 1997). Overall, females choose males significantly faster at higher light conditions, in both the lab and natural choruses (Baugh & Ryan 2010a; Bonachea and Ryan, unpublished data).

Methods

We collected amplexed pairs of túngara frogs from choruses in Gamboa, Panama near facilities of the Smithsonian Tropical Research Institute (9°07.0′N, 79°41.9′W) in September and October of 2009. These frogs were then brought back to the lab, tested, and released back at their original capture sites with their original mates. Prior to release, we gave each female a unique toe-clip combination to prevent retesting. In any test involving light levels, we first dark adapted females by holding them in a dark cooler for one hour (Cornell & Hailman 1984; Fan et al. 2001).

Female Phonotaxis

We observed the phonotaxis behavior of twenty female túngara frogs in 1.8 m × 2.7 m sound attenuating chamber (Acoustic Systems, Austin, TX, USA). A female was held under a plastic funnel for 3 min at a distance of 50, 115, or 180 cm from a speaker broadcasting either simple calls or complex calls. Stimulus intensities were measured as 88, 82, and 78 dB SPL (re. 20 μ P) at the release points, respectively. We then released the female and recorded her movements using a wide-angle video camera and infrared light source (Fuhrman Diversified, Inc., Seabrook, TX, USA) mounted on the ceiling of the acoustic chamber. Optomotor studies showed that the females are not sensitive to the IR light being emitted by this source (Cummings et al. 2008). A female was scored as having made a choice when she entered a 10 cm zone around either speaker. A female failed to make a choice if she did not leave the start zone after 5 min, stayed stationary for longer than 2 min, or failed to enter the choice zones after 15 min. We repeated these tests under total darkness in the human visible spectrum and at 0.28 lux light conditions (produced using Current USA Lunar light LEDs and measured using a Extech 403125 Light meter). These conditions are slightly brighter than levels we recorded at choruses on full moon nights (0.23 lux ± 0.01 SE), and optomotor studies show that the frogs are able to see at this light level (Cummings et al. 2008).

Analysis of Phonotaxis

From the video recordings, we calculated several temporal features of phonotaxis behavior: latency to first movement, choice latency, and travel time (choice latency – latency to first movement). Videos of the phonotaxis behavior were broken into JPEG single-frame image stacks using Virtual Dub v1.9.8 (http://www.virtualdub.org). From those image stacks, we were able to produce a single composite image of the spatial path taken by females (Fig. 1) using the Extended Depth of Field plugin (Forster et al. 2004) for ImageJ (Rasband 1997–2009). From the composite image, we calculated spatial path length and initial exit angle (Fig. 2). We corrected path lengths for female body length using snout-vent length measurements taken prior to release, allowing us to calculate an average movement speed for each female (path length corrected for snout-vent length (SVL)/total time spent moving to the sound source).

We used full factorial general linear models with Type III sum of squares to assess the effects of light levels, stimulus distance, and call complexity on all measurements of phonotaxis behavior: choice latency, latency to first movement, travel time, path length, initial exit angle, and movement speed.

Results

Stimulus distance, call complexity, and light levels all had strong effects on temporal aspects of female phonotaxis behavior. Females chose significantly faster when listening to complex calls than when listening to simple calls ($F = 15.689$, $p < 0.001$; Fig. 3) and also chose significantly faster, overall, under higher light conditions ($F = 12.846$, $p < 0.001$); however, light level had a considerably stronger effect when females were presented with only simple calls (light by stimulus interaction: $F = 6.353$, $p = 0.012$). Females left the starting zone considerably faster when presented with the complex call rather than the simple call ($F = 9.067$, $p = 0.003$, Fig. 4). Overall, choice latency increased with stimulus distance ($F = 5.926$, $p = 0.003$).

Females exhibited considerably shorter travel times under higher light levels ($F = 21.007$, $p < 0.001$; Fig. 5), although this effect was much weaker at longer stimulus distances ($F = 5.425$, $p = 0.005$). Overall, females had longer travel times when they started at farther distances to the stimulus ($F = 6.570$, $p = 0.002$), but this effect was considerably more noticeable when females were presented with complex calls ($F = 5.319$, $p = 0.006$). If we correct travel time for stimulus distance, we see no effect of starting position on this corrected travel time.

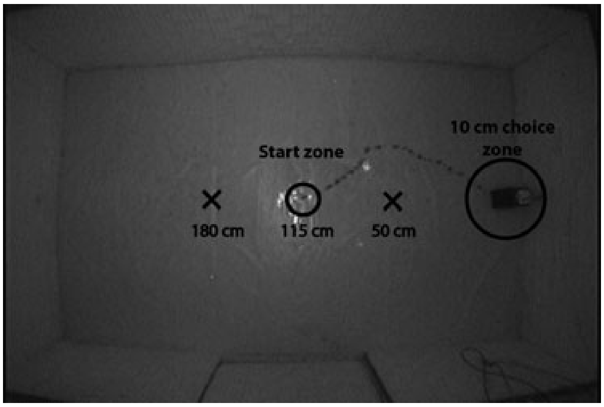

Fig. 1: Example composite image of a phonotaxis spatial path of female túngara frogs. Composite images were produced by merging single-frame still images from video recordings using the Extended Depth of Field plugin for ImageJ (see methods).

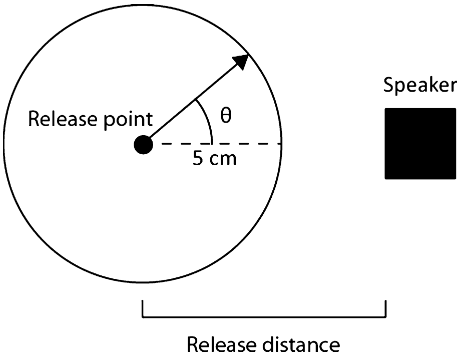

Fig. 2: Exit angle error, defined as the angular deviation between the true direction of a speaker broadcasting a stimulus and the bearing females took while moving towards the stimulus. Bearings were recorded at the point females exited the 5 cm radius release zone.

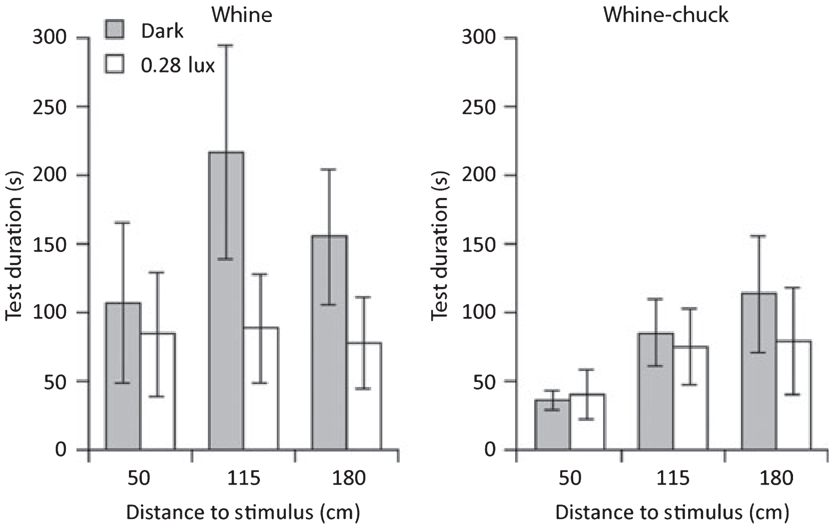

Fig. 3: Choice latencies for túngara frogs released at 50, 115, or 180 cm from a speaker presenting either "whine-only" simple calls, or "whine-chuck" complex calls. Tests were conducted under either dark conditions or under 0.28 lux of simulated moonlight. Error bars represent 95% confidence limits, n = 20 females in each test.

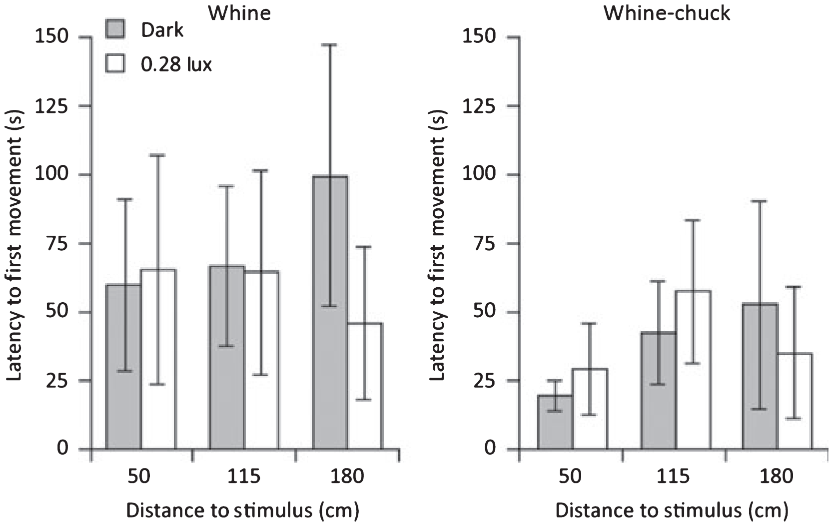

Fig. 4: Latency to first movement for túngara frogs released at 50, 115, or 180 cm from a speaker presenting either "whine-only" simple calls, or "whine-chuck" complex calls. Tests were conducted under either dark conditions or under 0.28 lux of simulated moonlight. Error bars represent 95% confidence limits, n = 20 females in each test.

Overall, females were more likely to exit the starting zone at angles smaller relative to the true direction of the speaker under higher light conditions ($F = 8.855$, $p = 0.003$; Fig. 4–6). Although the accuracy of exit angles tended to improve with longer distances to the stimulus, this effect was not significant ($F = 1.882$, $p = 0.155$). Path lengths increased with stimulus as expected and were, in all cases, significantly longer than the most direct possible path ($F = 204.611$, $p < 0.001$; Fig. 7). Stimulus distance had no effect on the magnitude of the difference between spatial path and the minimum possible spatial path ($F = 1.911$, $p = 0.1730$). As females differed in total body size (snout vent lengths of 2.1–3.4 cm), we corrected path length for SVL and calculated an average movement speed. Females moved significantly faster under higher light levels ($F = 463.308$, $p < 0.001$; Fig. 8).

Discussion

Females responded to perceived search costs by altering many aspects of phonotaxis behavior. Similar to previous studies with túngara frogs, we found that females chose significantly faster under higher light conditions and in response to complex calls (Baugh & Ryan 2010a; unpublished data).

Female túngara frogs often pause for an extended period of time before leaving the start zone. While it seems likely that females are evaluating male signals at the start before determining which direction to move in, we know from updating tests that females continue to assess male signals while moving towards a speaker (Baugh & Ryan 2010b). Longer travel times and higher light levels did not influence how long females remained at the release point, although females did leave sooner when presented

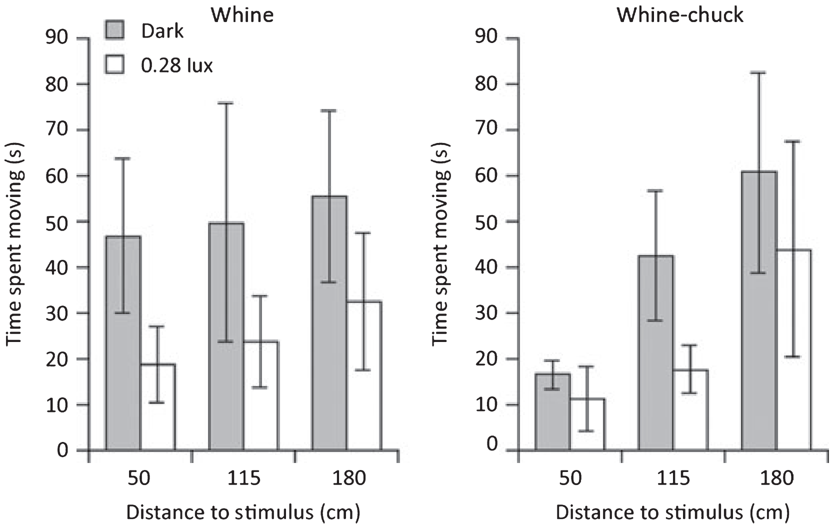

Fig. 5: Movement time for túngara frogs released at 50, 115, or 180 cm from a speaker presenting either whine-only simple calls, or "whine-chuck" complex calls. Tests were conducted under either dark conditions or under 0.28 lux of simulated moonlight. Error bars represent 95% confidence limits, n = 20 females in each test.

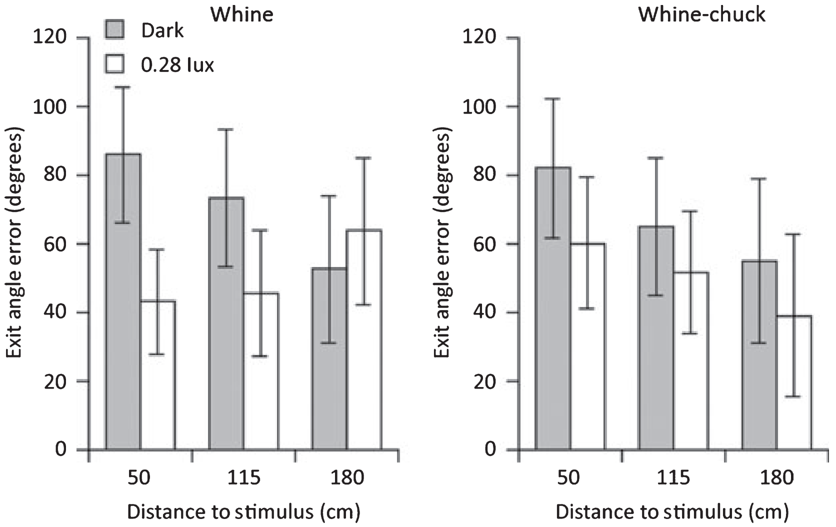

Fig. 6: Exit angle error (illustrated in Fig. 4-2) for túngara frogs released at 50, 115, or 180 cm from a speaker presenting either "whine-only" simple calls, or "whine-chuck" complex calls. Tests were conducted under either dark conditions or under 0.28 lux of simulated moonlight. Error bars represent 95% confidence limits, n = 20 females in each test.

with the complex call. This only supports a well established function of the 'chuck' component in increasing attractiveness and motivating females. Differences in choice latency were instead driven by movement speed, with females moving considerably faster and thus taking less time to reach the speaker under higher light levels. In a natural chorus, this would reduce the time females spent moving between males, reducing encounter rates with actively searching predators, but not necessarily ambush predators.

Although path lengths were, in all cases, significantly longer than the minimum possible path length (the distance to the speaker), we found no evidence that the magnitude of this difference varied at all with perceived search costs. This suggests that females do not respond to increased search costs by taking more direct paths. Likely, female search paths are more strongly determined by constraints imposed by their sensory system on localization of sound sources than tradeoffs in search strategies. However, we did find that females had more accurate initial-orientation to the sound source at higher light levels. It is possible that light improved the females' ability to see the speaker and thus orient to it, but previous studies strongly contradict this as females do not seem to recognize the speaker as the sound source (Farris et al. 2005; Taylor et al. 2008). In both these studies, localization was not impaired by the presence of additional speakers or visual models of males. Instead, we suggest that females are indeed responding to light

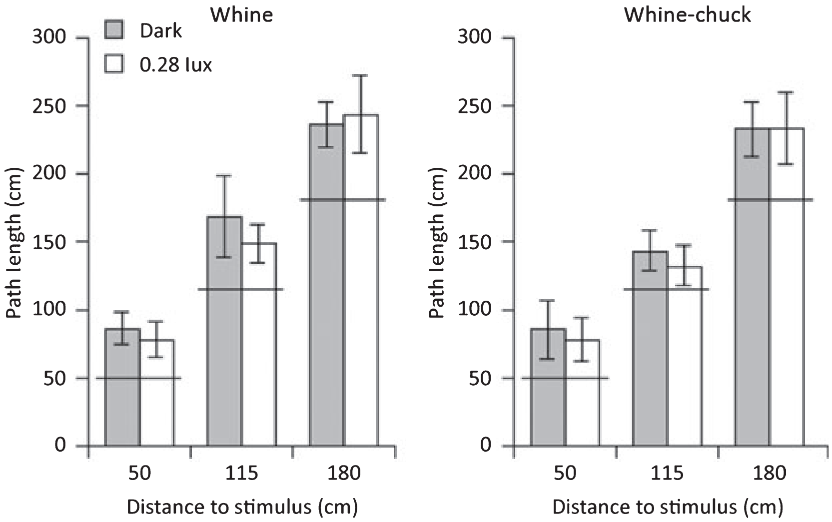

Fig. 7: Total path lengths for túngara frogs released at 50, 115, or 180 cm from a speaker presenting either ''whine-only'' simple calls, or ''whine-chuck'' complex calls. Tests were conducted under either dark conditions or under 0.28 lux of simulated moonlight. Error bars represent 95% confidence limits. Solid horizontal lines indicate the minimum path length needed to reach the stimulus, n = 20 females in each test.

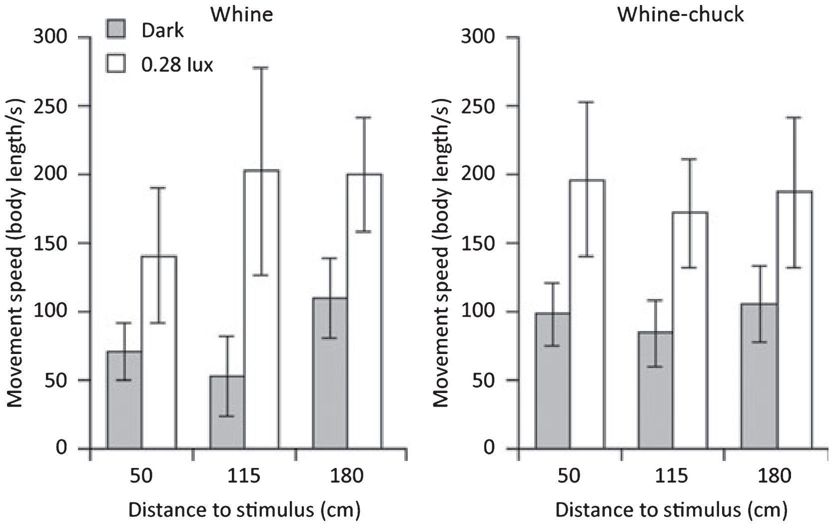

Fig. 8: Movement speed (path length corrected for body length / movement time) for túngara frogs released at 50, 115, or 180 cm from a speaker presenting either ''whine-only'' simple calls, or ''whine-chuck'' complex calls. Tests were conducted under either dark conditions or under 0.28 lux of simulated moonlight. Error bars represent 95% confidence limits, n = 20 females in each test.

levels with more accurate initial paths, but this contributes little to the length of spatial paths at these distances. We also find no evidence that females are better at localizing complex calls than simple calls. While enhanced localizability of complex calls has been suggested as a potential reason why females so strongly prefer complex calls, there remains, to date, no compelling evidence to support this within túngara frogs, although frog-eating bats (Page & Ryan 2008), but not blood-sucking flies (Bernal et al. 2006), are able to localize complex calls more accurately than simple calls.

Our results further support the role of predation risk in determining the search strategies of females. We specifically highlight ways females can alter temporal aspects of search strategies to minimize encounter rates with actively foraging predators. The results of this study suggest that responses to search costs can vary with the foraging strategy of predators, and that any study of female search strategies should incorporate temporal components of search strategies in addition to spatial paths.

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League, and the Society for the Study of Amphibians and Reptiles. This document is available at http://www.asih.org/pubs/herpcoll.html. We also thank Justin Touchon for his assistance in using Extended Depth of Field, and the anonymous reviewers who provided valuable comments for improving this manuscript.

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