

# Sexual selection in a tropical toad: Do female toads choose brighter males in a species with rapid colour change?

Katrina M. Gardner | Daniel J. Mennill  | Lincoln M. Savi | Nicole E. Shangi |  
Stéphanie M. Doucet

Department of Integrative Biology,  
University of Windsor, Windsor, ON, Canada

## Correspondence

Katrina M. Gardner and Daniel J. Mennill,  
Department of Integrative Biology,  
University of Windsor, 401 Sunset Ave,  
Windsor, ON N9B3P4, Canada.  
Emails: katrina.ms.gardner@gmail.com;  
dmennill@uwindsor.ca

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## Abstract

Conspicuous visual signals play an important role in animal communication, both within and between species. Bright, colourful signals allow animals to discriminate between individuals and can inform behavioural decisions and social interactions. In many instances, conspicuous colouration appears to have evolved through sexual selection via female mate choice or male–male competition. Male Neotropical yellow toads, *Incilius luetkenii*, display bright-yellow colouration during their brief explosive mating events and then revert to a cryptic, female-like, brown colour following amplexus and fertilization. Recent research has revealed that the yellow colouration of males may serve as a sex identification signal and that males remain yellow in the presence of both males and females but darken without conspecific stimulus. However, there is considerable variation in the brightness of yellow colouration in courting males, ranging from dull olive-green to vibrant lemon-yellow. The function of this variation remains unknown. In this study, we conducted two-choice model presentation trials to test one mechanism of sexual selection that may drive this variation: intersexual selection. Specifically, we set out to determine whether females differentiate between males based on their colouration. We presented females with two hyper-realistic robotic model toads, with one model painted to match a bright-yellow male and the other a dull-yellow male. Using several metrics of choice, we found that females did not show a preference for bright or dull colouration. Our research suggests that variation in the intensity of male breeding colouration is not driven by female choice in this species. Our study is consistent with the idea that there may be limited opportunity for female choice to influence male-trait expression in explosive breeders. Future research will address whether intrasexual selection may influence colour variation in male yellow toads.

## KEYWORDS

*Bufo luetkenii*, dynamic sexual dichromatism, explosive breeding, female mate choice, *Incilius luetkenii*, mate choice, robofrog, yellow toad

## 1 | INTRODUCTION

For many animals, conspicuous visual signals play a vital role in social and sexual communication (Smith & Harper, 2003). Bright, colourful signals allow animals to discriminate between individuals and can influence behavioural decisions and social interactions. Such signals may convey meaningful information about an individual's quality (Doucet & Montgomerie, 2003; Sheldon et al., 2003; Zhao et al., 2018), physiological state (Mészáros et al., 2017; York et al., 2016), reproductive state (Galván et al., 2016; Kodric-Brown, 1998), social status (Gerald, 2001; Martín et al., 2007) or other information. Conspicuous visual signals arise from both natural and sexual selection pressures and serve diverse functions across taxa (Andersson, 1994; Bradbury & Vehrencamp, 2011).

When conspicuous signals are displayed by just one sex, sexual selection is often invoked as the driving force of these traits (Darwin, 1871). In many animals, the functions of such signals have been well-established. Male long-tailed widowbirds (*Euplectes progne*), for example, develop extremely long tail feathers that are attractive to conspecific females (Andersson, 1982). Likewise, male white-tailed deer (*Odocoileus virginianus*) grow large antlers that are used both to defend resources against other males and attract females (Morina et al., 2018). For sex-role-reversed gulf pipefish (*Syngnathus scovelli*), females display colour ornaments that attract males (Paczolt & Jones, 2010). In many cases, sexually dimorphic conspicuous visual signals arise through mate choice for elaborate traits (most often female choice for elaborate male traits; Andersson, 1994; Darwin, 1871). However, multiple factors can influence a female's choice of breeding partner (e.g. intrasexual competition, Yang et al., 2019; population density, Wells, 1977; time constraints, Real, 1990), therefore, studies often first investigate whether females exhibit preference for a particular elaborate trait with controlled experiments.

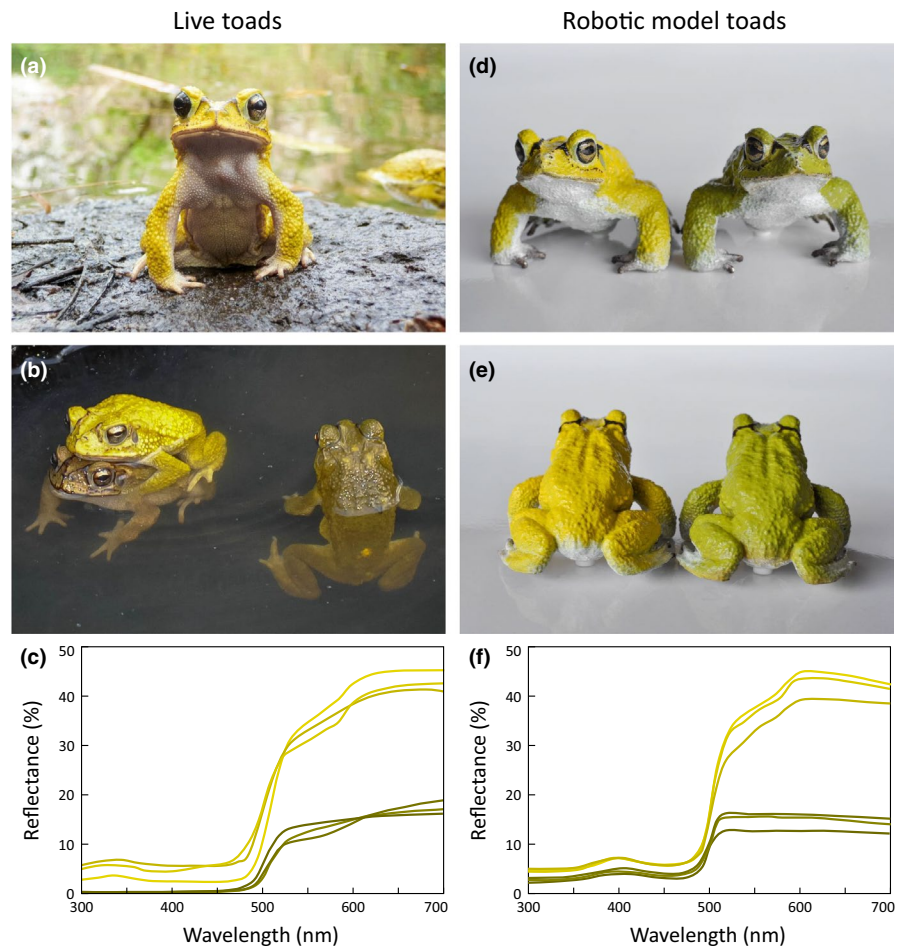
Anurans exhibit a great diversity of conspicuous visual traits, derived through various forms of both natural and sexual selection pressures, making them a compelling taxon for investigating the function and evolution of these traits. Many mate-choice studies in anurans focus on acoustic signals and studies assessing female mate choice for conspicuous visual signals in anurans are relatively rare, despite these elaborate visual traits being widespread throughout the group (Hödl & Amézquita, 2001). Colour is one notable conspicuous visual trait in anurans that has received scant empirical attention within the context of sexual selection (Reynolds & Fitzpatrick, 2007; Rojas, 2017), perhaps due to the nocturnal nature of most frogs (Duellman & Trueb, 1986). The few experiments that have investigated mate choice of elaborate colouration show that females of several species do exhibit colour preferences for male sexual signals. Female European tree frogs (*Hyla arborea*) prefer males with brighter and more chromatic vocal sacs and flank stripes (Gomez et al., 2009); female spadefoot toads (*Scaphiopus couchii*) prefer males with brighter colour (Vásquez & Pfennig, 2007); and female strawberry poison frogs (*Oophaga pumilio*) exhibit colour-assortative mate choice in lab

experiments (Summers et al., 1999). These studies suggest that conspicuous visual signals may play an important role in anuran mate choice. Sexually dimorphic-colour signals in frogs may be ontogenetic (colour changes that occur on a permanent basis, known in both sexes) or dynamic (colour changes that occur on a temporary basis; known only in males; Bell & Zamudio, 2012). Dynamic sexual dichromatism, and the evolution of temporary colour signals in males, is known from many frog species that breed in large aggregations, suggesting that these signals are important for understanding sexual selection in anurans (Bell et al., 2017).

In this study, we investigate female choice for conspicuous male colouration in the Neotropical yellow toads (*Incilius luetkenii*). Yellow toads are a Central American anuran species, distributed from southern Chiapas, Mexico throughout Northwestern Costa Rica (Savage, 2002). They inhabit regions characterized by two distinct seasons: Approximately six months of abundant rainfall from May to November followed by six months of pronounced drought (Campos & Fedigan, 2013). They are explosive breeders (Ulloa et al., 2019), concentrating their mating activities during a very short time period, typically for just a few hours during one or two days of the year. Yellow toads exhibit dynamic sexual dichromatism, a form of dimorphism in which males and females differ in colour or pattern for a limited time (Doucet & Mennill, 2010): males of this species display conspicuous colouration for only a few hours during their very short breeding event. As the first heavy rains of the wet season begin to fall, yellow toads emerge from aestivation to gather around newly formed ponds and streams to breed explosively for a few short days (Doucet & Mennill, 2010; Savage, 2002). It is at this time that males undergo a dramatic shift in colouration from a dull brown, similar to females of this species, to a vibrant yellow (Figure 1). Males gather around the edge of the pond and produce loud calls (Rehberg-Besler et al., 2017). Females, presumably attracted by the calls, approach the breeding aggregation. The initiation of amplexus is poorly studied (we usually detect females after they are in amplexus). Amplexus can last several hours; if the male is able to fend off usurpers by kicking them away, or if the pair hides under rocks or logs, the pair will eventually enter the water for egg-laying and fertilization (Doucet & Mennill, 2010). Male colour gradually reverses while in amplexus. Although the precise function of this rapid colour change remains unknown, recent studies have begun to shed some light on this unique phenomenon. Recent findings indicate that sex identification is one important function of the temporary colour dimorphism: when presented with a brown and yellow clay model, male yellow toads attempted to amplex the brown female-like model much more often (Rehberg-Besler et al., 2015). Similar patterns have been observed in other species (Sztatecsny et al., 2012). The ability to rapidly discriminate between the sexes may prevent injury and costly errors in mate-finding in a species with explosive breeding and scramble competition.

When male yellow toads undergo their dramatic colour change, they exhibit considerable variation in brightness (Doucet

**FIGURE 1** Comparison of live Neotropical yellow toads (*Incilius luetkenii*) and 3D printed robotic model toads. (a) Photograph of a male-yellow toad in a typical posture. (b) Photograph of three toads showing a bright-yellow male (upper left), in amplexus with a brown female (lower left), near a dull-yellow male (right). (c) Reflectance spectra of three bright male toads (upper three curves) and three dull-male toads (lower three curves; data from Doucet & Mennill 2010). (d, e) Photographs of 3D printed male-yellow toads used in this experiment, showing a bright model beside a dull model. (f) Reflectance spectra of three bright 3D printed model toads (upper three curves) and three dull 3D printed model toads (lower three curves), which closely match the reflectance spectra of the live toads in C [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



& Mennill, 2010), and the function of this variation remains unknown. A recent investigation has revealed that the brightness of a male's yellow colouration is influenced by the presence of conspecifics, whereby subjects held in the presence of males or females during a breeding event were brighter than those housed in the absence of conspecifics (Gardner et al., 2019). No study has yet investigated whether variation in male colour might also be a sexual signal used in male–male competition or female mate attraction. Although the opportunity for female choice may be limited in explosive breeding systems (i.e. systems where animals mate during short time windows, sometimes limited to just a few hours per year), females of some species still exhibit preference for male traits under certain conditions (Jennions & Petrie, 1997; Székely et al., 2018; Yang et al., 2019).

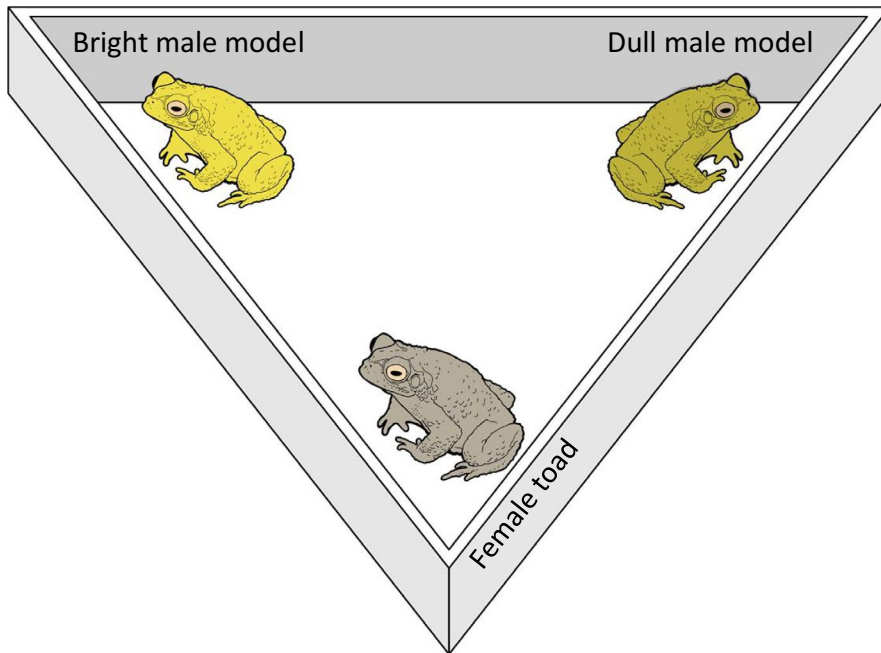
Here, we investigate whether female yellow toads exhibit a preference for bright male colouration. We studied female choice in a free-living population of yellow toads by placing a female in a two-choice arena where she was introduced to two hyper-realistic three-dimensional (3D) printed robotic models: one bright-yellow male model and one dull-yellow male model. We used reflectance spectrometry to paint models that were representative of the brightest and dullest 10th percentiles of males in breeding colouration (Figure 1). If female mate choice influences male colour variation in this species, we predicted that females would show a preference

for brighter males, approaching the brighter models more quickly, and spending more time near the brighter models.

## 2 | METHODS

### 2.1 | General methods

We conducted this experiment over two days in the spring of 2018 (May 16 and 17) in Sector Santa Rosa of the Area de Conservación Guanacaste (10°53' N, 85°37' W) immediately following the first heavy rains of the wet season. On both days, there was a large aggregation of breeding males at our study pond. Many adult females approached the pond and engaged in amplexus during the morning and egg-laying during the afternoon. Mating was restricted to those two days in 2018. We began trials at approximately 0,600 hr each day, sampling a total of 74 females (45 on May 16, 29 on May 17). We collected subjects by hand at the periphery of the breeding pond and transported them in buckets from the breeding pond to the trial site, 50 m away. We immediately placed them into a clear acclimation chamber within a two-choice triangular experimental arena (Figure 2). We gave each female approximately two minutes to acclimate to the arena (Derex et al., 2011; Gomez et al., 2011; Stange et al., 2017), during which time she was able to



**FIGURE 2** We exposed female yellow toads (*Incilius luetkenii*) to two robotic male stimuli, one bright in colour and one dark in colour, to determine whether females selectively interact with males based on their colouration. The arenas were equilateral triangles with each side measuring 150cm in length and 60cm in height. After a two-minute acclimation period in a clear acclimation chamber (located where the female is shown in the figure), a female could interact freely with the stimuli, making a “choice” when she entered the 50cm by 50cm choice zone in each corner of the arena [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

view both stimuli but remained confined to the acclimation chamber. Females typically made one or more hops around the acclimation chamber and then remained still. Then, we released the female from the acclimation chamber, and she was able to freely move about the arena and interact with the two models. Trials lasted five minutes, and we conducted up to four trials simultaneously in four identical triangular arenas. We conducted trials between 0,600 and 1,430 hr on May 16 and 0,600 and 1,030 hr on May 17 (by these times, nearly all toads were in amplexus or had dispersed away from the breeding pond). At the end of each trial, we measured the female's mass (to the nearest g) and snout-vent-length (to the nearest mm), and then we released females back at the edge of the breeding pond, at which time they resumed normal mating activities.

This study was approved by the University of Windsor Animal Care Committee under AUPP 16-10 and the government of Costa Rica (MINAE); the study was performed in accordance with institutional and national guidelines for the care of animals. We made the assumption that our sampling technique (manually capturing 74 female toads at the periphery of the breeding pond) provided us with a sample that was representative of the broader population, and that this group was not pre-disposed to choose bright or dull model toads by our capture technique.

## 2.2 | Robotic model design

We created male toad models through a combination of techniques including photogrammetry, digital sculpting, 3D printing, and spectrally referenced painting. We took 11 photographs of a wild male toad from different angles and processed them in Agisoft Photoscan (Agisoft LLC), a photogrammetry software package that generates three-dimensional measurements from two-dimensional data.

The resulting 3D model was lacking an underside, toes and detailed warts, so we manually sculpted these in the software ZBrush (Pixologic Inc., Los Angeles, CA.) using the photographs for reference. We also modified the model such that it was perfectly symmetrical using the mirror and weld tool within ZBrush. We exported the model as a wavefront object file, a common 3D object file used by 3D modelling or printing software. The 3D model is available online at [savimade.com](http://savimade.com).

We printed eight identical copies of the 3D toad model on a Tevo Tarantula 3D printer (TEVO 3D Electronic Technology Co., Ltd). This is a fused-deposition-modelling type 3D printer that melts and fuses plastic filament into precise shapes through a moving, heated print-head. We sliced the models for printing using Cura 3.2.1 software and printed life-size models in polylactic acid plastic with an outer shell thickness of 1.2 mm, an internal infill of 10%, and with 0.1 mm per layer height to maximize the resolution capabilities of the 3D printer and to minimize horizontal layer-lines on the surface of the models. The complex geometry of the toad models required us to use support material as a scaffolding to support the model during the printing process. When printing concluded, we removed the support material and prepared the models for painting by coating them with grey Krylon colour master primer.

We used reflectance spectrometry to match the paint colour on the models to reflectance data, measured between 300 and 700 nm, collected from live male yellow toads in a previous study (Doucet & Mennill, 2010), using a portable spectrometer (model: JAZ-COMBO; Ocean Optics, Dunedin, FL). We determined the average 90th and 10th percentiles of male breeding colouration from the samples in Doucet and Mennill (2010) and, through trial and error, found combinations of acrylic paints and a layering technique that matched these colours when applied to swatches of our 3D printed substrate. We painted the models with acrylic paints in layers using an airbrush. We painted the bright models by first applying a layer of burnt umber,

then multiple layers of primary yellow, a layer of mixed primary red and primary yellow, and then a final layer of bright yellow. We painted the dull models first with a layer of burnt umber, and then several layers of bright yellow. For both model types, we painted the eyes of each model by hand with black and gold paint, and we painted the cranial crest using black paint (see Figure 1).

We created robots from the 3D printed models in order to add life-like motion to the models, because the movement has been shown to encourage responsiveness in other studies (de Luna et al., 2010; Rehberg-Besler et al., 2015; Sztatecsny et al., 2012). We affixed each 3D printed model to a servomotor connected to a microcontroller board (Arduino Uno) that we programmed to rotate on the model's central axis. This produced a movement pattern similar to the small movements we have observed from live toads at the mating pond. We programmed the models to rotate to a random angle position from  $-60$  to  $60$  degrees where  $0$  degrees corresponds to the model facing towards the back of the arena with its dorsum facing towards the female. We placed each model toad in the arena so that the dorsum of the model was always facing towards the female's starting apex within the triangular arena (Vásquez & Pfennig, 2007). The two servo motors within each arena (one controlling the movement of a bright model, and the other a dull model) were powered by a common microcontroller, with power delivered by a portable battery pack (Anker PowerCore). The microcontroller controlled both servomotors, so that both the bright model and dull model moved at the exact same times with the exact same movements, so that movement differences between the two models could not influence female responses to the two models. To ensure that any side-bias in female responses did not influence our results, we changed whether the bright or the dull model was on the left or right side after each round of trials. To ensure that any subtle variation across the 8 models did not influence our results, we switched the models between the four different arenas after each round of trials.

Male yellow toads produce loud calls which are understood to serve in attracting females to the site of the breeding aggregation (Rehberg-Besler et al., 2017). We have not observed males producing calls when females are in close range, as we would expect if male vocalizations were important in female mate choice. Therefore, we did not simulate vocalizations in this experiment, allowing us to focus on colour differences between the two differently coloured robotic toads.

### 2.3 | Quantifying female choice

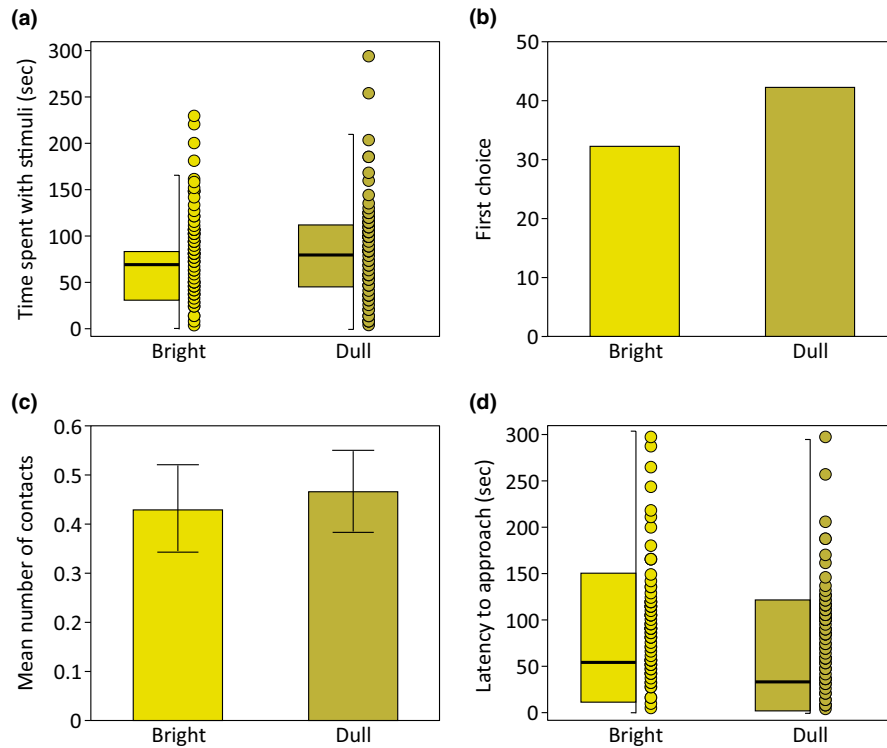
One observer (NS) watched the videos and scored female movement within the arena. The observer noted the movements and the position of the female relative to a predetermined choice zone for each model (a 50 cm by 50 cm area from the corner of the apparatus containing each stimulus), as well as the number of contacts the female made with each model. The observer was blind to the hypothesis, we were testing and the goals of the experiment, and she recorded each female's response as pertaining to either the left or right model,

rather than the bright versus dull model. We quantified choice in our analyses as the total time a female spent with each model, the first model that the female approached, the number of times the female made contact with each model, and the latency to approach each model. Portions of videos, showing female activities within the arenas, are included in Appendices S1 and S2.

We compared female reactions to the bright versus dull model with paired *t* tests for our continuous response variables (time spent with stimuli, latency to approach stimuli, number of contacts with stimuli) and a binomial test for our binary response variable (first stimulus approached; JMP version 15, SAS Institute). To explore whether other factors might influence female behaviour, such as size of the female, day of breeding or time of day, we further analysed our data with four Generalized Linear Models (GLM) where our response variables were time spent with the bright versus dull model (calculated as the amount of time spent with the bright model minus the time spent with the dull model for each female), latency to approach the bright versus dull model (calculated as the latency to approach the choice zone of the bright model minus latency to approach the choice zone of the dull model), number of contacts with the bright versus dull model (calculated as the difference between the number of contacts with the bright model versus number of contacts with the dull model), and whether the female first approached the bright or dull model (we used a binomial fit instead of a normal fit for this variable). The main effects in each GLM were female size (snout-to-vent length), trial day (the first or second day of the breeding aggregation), and trial start time; we first ran these GLMs with all interaction terms, but no interaction terms showed a pattern with female choice, and therefore, we present the main effects alone. We consider snout-vent-length (SVL) to be a proxy for female age because these features are correlated in females of several anuran species (Höglund & Säterberg, 1989; Cog & Miaud, 2004; Guarino & Andreone, 2014; Otero et al., 2017;  $n = 73$  due to missing SVL for one female). All analyses were conducted in JMP 15 (SAS Institute).

## 3 | RESULTS

When presented with robotic models of bright-yellow and dull-yellow males, female yellow toads approached one or both of the model toads in all 74 trials, made physical contact with the model toads in 38 of the 74 trials, and were even observed backing up to the male models, as though to initiate amplexus. Females did not, however, exhibit a preference for bright versus dull robotic model toads. Females spent similar lengths of time with both bright and dull model toads (Figure 3a; *t* test:  $t_{73} = -1.38$ ,  $p = .17$ ). Females were similarly likely to first approach the bright versus dull model toad (Figure 3b; binomial test:  $p = .90$ ). Across the 74 trials, 38 females made contact with one or both of the model toads, but females made contact with bright versus dull model toads a similar number of times (Figure 3c; *t* test:  $t_{73} = -0.23$ ,  $p = .82$ ). Females showed a similar latency to approach the bright versus dull model toad (Figure 3d; *t* test:  $t_{73} = 1.24$ ,  $p = .22$ ).



**FIGURE 3** Female yellow toads (*Incilius luetkenii*) did not exhibit a preference for either the bright male or dull robotic male model toad. (a) Boxplots of time spent with the models show that females spent similar lengths of time with both bright and dull models. (b) Bar plots of the total number of trials where females first approached the models show that females were similarly likely to approach both bright and dull models first. (c) Bar plots of the mean number of contacts made with the models show that females made a similar number of contacts with both the bright and dull models (error bars show standard error). (d) Boxplots of the latency to approach the models show that females took a similar amount of time to approach both the bright and dull models. In (a) and (d), boxplots show the median (thick black bar), 1st and 3rd quartiles, and the point value of each individual [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

To investigate whether female reactions to bright and dull model toads varied with female size, the day of the breeding season (day 1 versus 2), and time of day, we ran four generalized linear models with our four response variables: the time females spent with the bright versus dull model, whether females first approached the bright versus dull model, the number of contacts females made with the bright versus dull model, and the latency to approach the bright versus dull model. We found no evidence that these features influenced female reaction to the bright versus dull robotic model toads (Table 1).

## 4 | DISCUSSION

Male yellow toads exhibit considerable variation in breeding colouration, ranging from dull olive-green to vibrant lemon yellow, but it is a dynamic sexual ornament present for only a few days of the year during their explosive breeding events at the start of the rainy season (Doucet & Mennill, 2010). We set out to determine whether female yellow toads selectively interact with males based on their colouration using a model presentation experiment. When given the opportunity to interact with a bright versus dull robotic model toad, female yellow toads did not respond differently on the basis of colouration. Females approached the models in most trials and made physical contact with males in more than half of the trials, but

showed no propensity to differentiate between robotic male toads that varied only in their colouration. Therefore, our results provide no evidence that females differentiate between males on the basis of colour, and our study offers no support for the hypothesis that female yellow toads choose males on the basis of their colour.

Several anuran species that exhibit prolonged breeding periods (i.e. non-explosive breeders) have provided evidence that females choose more visually conspicuous males. For example, in a model presentation experiment, female squirrel tree frogs (*Hyla squirella*) showed preference for males with larger lateral body stripes (Taylor et al., 2007). Similarly, when female túngara frogs (*Physalaemus pustulosus*) were presented with video playback of calling males, they preferred males who inflated their conspicuously pigmented vocal sacs in synchrony with an acoustic stimulus over static male callers (Rosenthal et al., 2004). Further, female strawberry poison-dart frogs courted the brightest males (Dreher et al., 2017). Given that female choice for male visual signals has been demonstrated in prolonged breeders, but not in explosive breeders, our findings are consistent with the idea that female mate choice is not a strong selective agent for male sexual traits in explosive breeding anurans (Wells, 1977, 2007). Unlike in prolonged breeders, reproductive pairings in explosive breeders often occur after physical struggles between males; this may leave little opportunity for active female choice, although the possibility

**TABLE 1** Results of four Generalized Linear Models (GLM) analysing of the responses of female yellow toad (*Incilius luetkenii*) to paired presentations of bright versus dull 3D printed models ( $n = 73$ ), showing the results of the intercept (which includes zero for all four models, indicative of no preference for bright or dull models) and the effect tests for female size, sampling day and time of day

	Estimate	SE	$\chi^2$	$p$
Time spent with bright minus dull stimulus				
Intercept	-78.69	116.68	0.45	.58
Female size	0.72	1.31	0.30	.58
Sampling day	-9.07	20.89	0.19	.66
Time of day	2.3e-4	1.2e-3	0.04	.85
First choice (bright or dull stimulus)				
Intercept	2.39	2.92	0.68	.41
Female size	-0.01	-0.03	0.11	.74
Sampling day	-0.78	0.53	2.29	.13
Time of day	-4.3e-5	3.0e-5	2.05	.15
Number of contacts with bright minus dull stimulus				
Intercept	0.30	1.37	0.05	.83
Female size	-0.001	0.01	0.01	.93
Sampling day	0.25	0.26	1.01	.32
Time of day	-9.5e-6	1.4e-5	0.45	.50
Latency to approach bright minus dull stimulus				
Intercept	56.69	155.73	0.13	.72
Female size	-0.52	1.75	0.09	.77
Sampling day	8.86	27.89	0.10	.75
Time of day	1.8e-5	1.6e-3	0.01	.99

remains that females could exhibit choice under certain conditions (for example by approaching a specific male in the breeding aggregation, by refusing to lay eggs while in amplexus with an undesirable mate, or by exhibiting post-copulatory cryptic mate choice). Further comparative investigations between species of prolonged and explosive breeders are required to verify the function of sexually dimorphic-visual traits in these species.

Yellow toads exhibit substantial variation in the brightness of their yellow colour, and we designed our robotic models to provide a highly contrasting pair of bright and dull males; we used spectrometry to verify that they matched the colour of the brightest 10th percentile and dullest 10th percentile of males from a previous investigation (Doucet & Mennill, 2010). Yet it is possible that females within our experimental design did not choose or express preference for male colouration due to some limitation of our study. Animals vary in their ability to distinguish colour across taxa, and even within species. For example, the visual threshold for colour discrimination in some anurans varies for different tasks, such as mate choice and prey capture (Yovanovich et al., 2017). Furthermore, colour vision capabilities may vary under different light conditions. In our study, we did not have control over ambient light, because our choice apparatus was set up in the field near

the site of the annual mating aggregation of our study population. On the first day of our experiment, the sky was overcast, and on the second day, the sky was sunny. Although light environment may play a role in discerning colour differences, we still did not see female choice or preference for colour on the sunnier day. Moreover, we conducted this study in the natural environment of yellow toads and during the breeding event, so a toad's vision would presumably be optimally adapted for use under these conditions, making it unlikely that light environment influenced these female choice results.

One explanation for the lack of differences in response to the bright versus dull models is that females may not have recognized the 3D printed models as yellow toads. We find this explanation unlikely. Multiple females were seen backing up to the models during trials, possibly in an attempt to initiate amplexus. Furthermore, when we placed models in the aggregation of toads at the seasonal pond, we observed males approach and touch them (*personal observation*). Additionally, when male yellow toads were presented with simple clay models in a previous experiment, they attempted to amplex with brown female-like models more often than yellow male-like models (Rehberg-Besler et al., 2015), demonstrating an ability to discriminate even crude representations of toads within this species. Finally, several other anuran species were shown to respond to model stimuli in experiments (e.g. Gomez et al., 2011; de Luna et al., 2010; Narins et al., 2003; Szatecsny et al., 2012; Taylor et al., 2007).

In conclusion, we did not find evidence for female preference for bright-yellow male colouration or dull-yellow colouration in Neotropical yellow toads. Precopulatory intersexual selection does not appear to be a driving variation of yellow colouration in males of this species. Our findings support what might be expected of explosively breeding animals, a group in which trait variation is often hypothesized to be the result of intrasexual struggle rather than active intersexual mate choice (Wells, 1977, 2007). We postulate that sexual selection may still influence yellow colouration in male yellow toads, and future studies should seek to determine whether this trait serves as a visual signal during male-male competitive interactions and whether colour is indicative of some aspect of male quality. Our findings contribute to our understanding of the functions and evolution of conspicuous colouration and dynamic sexual dichromatism in anurans.

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#### ORCID

Daniel J. Mennill  <https://orcid.org/0000-0001-9314-6700>

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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