Partial text:

An evaluation of video playback using *Xiphophorus helleri*

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(Received 8 February 1999; initial acceptance 13 April 1999; final acceptance 20 September 1999; MS. number: A8267R)

Video playback is being increasingly used as a technique for behavioural research. The importance of critically evaluating the effectiveness of video playback is clear, as available video technology is not designed for nonhuman visual systems. We discuss several aspects concerning the perception of video images that could lead to inconclusive or erroneous results. Researchers should verify that behaviour observed in response to video playback is comparable to behaviour observed in response to live animals. We conducted such a verification using live and video playback methods to measure female response to swords of varying lengths in the green swordtail, *Xiphophorus helleri*. Using both methods, female response appeared to be an increasing function of male sword length. Females did not differ in their response to live and video versions of noncourting, noninteractive males, however, females tended to prefer video playbacks of males with longer swords, a result that has also been found in experiments using live males. These results suggest that females express the same qualitative mating preference, but not necessarily the same quantitative preference, for sword length when viewing video stimuli. Several methodological factors that may contribute to an apparent difference in the strength of the preference are discussed. Despite these differences, both methods produced comparable results; female response to sworded males tended to increase as sword length increased. These experiments demonstrate that video playback is an effective method to measure female preferences accurately in *X. helleri* and provide an example of how video playback can be evaluated in other species.
human trichromatic spectral sensitivities are perceived differently by animals with different spectral sensitivities (Fleishman et al. 1998). Many birds and fish are sensitive to spectra in the ultraviolet wavelengths (Bennett et al. 1994) and thus standard video monitors that do not emit UV radiation will exclude signals at wavelengths that may be important to test subjects. For example, female starlings rank males differently depending on whether UV wavelengths are present or absent (Bennett et al. 1997).

Another potential problem with video playback is flicker fusion. The critical frequency of fusion (CFF) is a measure of how rapidly a light must flash before the observer will perceive it as a single continuous light, rather than a flashing image. Video systems in the U.S.A. flicker at 60 Hz; therefore, if a test subject’s CFF is above 60 Hz, it may perceive the video images as flashing. A rigorous evaluation of video playback is essential to evaluate the impact that these factors may have on test subjects (see Fleishman et al. 1998 for discussion).

A few studies have qualitatively compared the responses of test subjects to live and video stimuli (Clark & Uetz 1990; Evans & Marler 1991; Kodric-Brown & Nicoletto 1997). For example, Clark & Uetz (1990) found no difference in the response of jumping spiders, Maevia inclemens, to live prey and a simultaneously presented video image of the prey. Kodric-Brown & Nicoletto (1997) found no difference in the response of female guppies, Poecilia reticulata, to live males behind one-way glass and video male stimuli. In these experiments, the only manipulated variable was stimulus type: either live animals or video images. An experiment designed to examine the response of test subjects to manipulated stimuli using both live and video techniques would be useful because it would determine how the use of video images influences responses to a second manipulated aspect. In this paper, we examine the response of female green swordtails, Xiphophorus helleri, to a set of manipulated video stimuli varying in the degree of a male trait previously found to be a target of intersexual selection. We interpret this data set based on a similar set of data collected using live males.

The green swordtail is a sexually dimorphic poeciliid. Males possess a complex character known as the sword, which consists of elongation of a specific set of ventral caudal rays, a lower black stripe, an upper black stripe, and green and/or orange pigmentation between the black stripes (Basolo 1996). It has been demonstrated that female green swordtails prefer males with longer swords (Basolo 1990) and males of greater body size (Basolo 1998b). In addition to male morphology, females express preferences for courtship displays (Rosenthal et al. 1996). Male courtship behavior varies temporally within and between individuals in both display rate and expressed behaviors (Clark et al. 1954). The visual system of X. helleri does not appear to be sensitive to UV radiation (Rush 1995). The maximum CFF for green swordtails is 43.1 Hz (Crozier & Wolf 1939), which is substantially lower than the flicker frequency of U.S. video monitors (60 Hz). These aspects of the visual system, together with the presence of behavioral and morphological variation in males, makes the use of video playback an attractive method for testing behavior in the green swordtail.

We tested the effectiveness of video playback as a tool for measuring female mating preferences by comparing the responses of female green swordtails to live and video stimuli. First, we measured the response of females to live males behind one-way glass versus video images of the same males to determine whether females respond to video images of males in a manner comparable to their response to live males. Second, we measured the response of females to video stimuli of males that varied in sword length to obtain a preference function for sword length. Female preference functions describe the variation in the strength of the preference for a male trait in relation to variation in the trait (Basolo 1995; Wagner et al. 1995).

We then plotted the video preference function against a preference function obtained in another experiment using presentations of live males in which sword length was surgically manipulated (Basolo 1998a). We examined whether females express similar preference functions based on sword length when viewing either live or video stimuli. Similar responses to live and video stimuli would suggest that video playback is an appropriate method to evaluate female preferences for this species.

METHODS

Subjects

Subjects were virgin female descendants of wild-caught X. helleri collected in May 1994 from Savannah Stream near mileage marker 28 on the Western Highway, Belize. Fish were maintained in the laboratory on a 16:8 h light:dark cycle with broad spectrum fluorescent lighting and an average water temperature of 25°C. Fish were fed three times per day; live brine shrimp nauplii in the morning, liver paste in the afternoon, and TetraMin® fish flakes in the evening. Females were housed as pairs in 6-litre tanks. Males were housed in 38-litre tanks, with four males separated from one another by clear glass dividers.

Experiment 1

Video stimuli preparation

We prepared video stimuli in the afternoon the day before testing by recording feeding behavior in high-quality, 5-video cassettes using a professional-quality Panasonic VCR (AG-1980), Panasonic monitor (CT-1384 Y), and Panasonic videocamera (S100 HS). Two filtered 40-W Power Twist Vita-Lights® were used to provide broad-spectrum lighting and a sheet of light blue paper was used as a backdrop. We placed a male in a Plexiglas recording tank (30.5 × 20.3 × 30.5 cm) with the longest side of the tank facing the camera. We adjusted the focal length of the camera so that a ruler temporarily placed inside the center of the tank appeared as actual size on the monitor. After a 5-min acclimation period, we added brine shrimp nauplii to the tank and recorded the male for 25 min. During taping, the male swam about the tank feeding on brine shrimp. The recording of courtship
behaviour would be impractical, as the behaviour would not be directed at the camera and thus would not be directed towards a female viewing the video stimulus. We also recorded a separate 25-min control video stimulus of the recording tank containing only brine shrimp. We used 18 different males as stimuli in this experiment.

**Presentations**

Because live males will court females they can see (Farr 1980), and females prefer males that court noncourting males (Rosenthal et al. 1996), we prevented live males from seeing females using one-way glass. We placed two pieces of one-way glass on opposites sides of a glass test tank (30.5 × 30.5 × 30.5 cm) and black cloth along the back of the test tank. We placed two polarizing filters rotated at 90° on top of the tank to darken the inside of the test tank so that a female test subject in the tank could observe the live and video presentations through the one-way glass, but live males could not see into the test tank. We placed two pieces of tape vertically on the front surface of the test tank, marking two end sections, each measuring 10.0 cm, and a central section measuring 10.5 cm. For test sessions using live males, we placed the recording tank with the blue background 3.8 cm away from one of the test tank sides. In video presentations, a monitor replaced the recording tank. One-way glass remained on the test tank for both live and video trials. We placed a piece of opaque plastic on each side of the test tank to prevent females from viewing the stimuli prior to the initiation of the trials. Live males were not observed to court during presentations.

Females viewed both a live male and a video version of the same male in balanced random order on successive days between 0800 and 1000 hours. Each female was acclimated in the test tank overnight. In the morning, the female was fed brine shrimp 20 min before testing. For live presentations, we began the trials by placing a male in the recording tank, starting the VCR, adding brine shrimp to the recording tank and removing the blinds. For video presentations, we began the trials by starting both VCRs and removing the blinds. In both live and video tests, we played the video of the empty tank with brine shrimp opposite the male stimulus. Trials were viewed remotely via a video camera connected to a monitor outside the test room. Data recording began when the female had entered all three sections of the test tank. We observed each female for 10 min, and recorded the time the female spent in each section of the tank. We also measured female response, defined as the amount of time spent by the female oriented towards the male (either video or live), within three body lengths of him (Basolo 1995). Immediately after the trial, we replaced the blinds and switched the positions of the empty tank video and the male stimulus. After an interval of 10 min, we conducted a second trial. These two trials together constituted a single session. When a session was completed, the female was returned to her home tank. We conducted a second session with the same female in the same manner the next day using the alternate stimulus (live or video). A female was considered to have a side bias if she spent more than 960 s (80%) on one side of the tank during a session. Females showing side biases were omitted from the analysis. We tested a total of 23 females; three live trials and two video trials were omitted due to side bias. Males from omitted trials were re-used in future trials with different females. Each female was tested only once in each condition (live and video). We analysed the difference in female response between live and video sessions (live–video) using a Wilcoxon paired-sample test.

**Experiment 2**

**Video stimuli preparation**

We used one male with a fully developed sword as the stimulus for all video presentations. This male had a standard length of 43.3 mm (measured from the anterior-most point to the point just posterior to the caudal fin vein) and a sword length of 40.4 mm (measured from the insertion of the fin rays at the base of the caudal fin to the distal tip of the sword). To record courtship behaviour, we placed the male and a female in the recording tank described for experiment 1. After a 10-min acclimation period, we recorded courtship behaviour in 30-min intervals. We used courtship behaviour of the male that was directed towards the camera and unobstructed by the female. We included three clips of typical courtship behaviours in the primary video stimulus: lateral presentation (Hemens 1966; Ryan & Causey 1989; Basolo 1990), backward swim (Basolo 1990; Rosenthal et al. 1996) and sigmoid flex (Clark et al. 1954). In addition to these clips, we also included a clip of the male turning to change direction (from left to right).

We imported the video clips into a Macintosh 7600/120 computer equipped with a Targa 2000 PCI video board using Adobe Premiere. We then imported the clips into Macromedia Director to generate individual frames. We exported the frames as PICT files and erased the background in each frame in Adobe Photoshop. We imported the PICT files into Director where the frames were linked to form a 12-s looping sequence. We used a morphing program (Gryphon Morph) to create transition frames in places where the beginnings and the ends of clips required matching. We overlaid the frames of courtship behaviour on a light blue background. In the final stimulus, the male performed a backwards swim, then moved across the screen displaying a lateral presentation, turned around, and finished with a sigmoid flex. The video loop started and ended at the same point on the screen, so the male appeared to move continuously.

We manipulated sword length by scaling (up or down within the natural range of sword length variation) the part of the sword extending beyond the distal margin of the caudal fin using Adobe Photoshop. When this part of the sword was scaled, all four components of the sword (elongation, upper stripe, lower stripe and internal coloration) were manipulated in a consistent fashion. The short sword stimulus had a sword length of 28.5 mm (70% of original sword length) and the long sword stimulus had a sword length of 54.0 mm (133% of the original sword length). We transferred these video stimuli and the original intermediate sword stimulus to high-quality, S-video cassettes for video playback.
To control for the potential effect of schooling behaviour on the measurement of female response to the male stimulus, we constructed a female video sequence and presented it opposite the male stimulus in all video presentations. We made the female stimulus in the same manner as the male stimulus. To make the video stimulus, we used two clips of a female (standard length 43.3 mm) swimming across the recording tank and two clips of the female turning around. In the completed video, the female appeared to swim back and forth across the screen at the same speed as the male stimulus. The female stimulus contained no described courtship behaviours.

**Video playbacks**

Test subjects were virgin females maintained as in experiment 1. We measured female response in a single stimulus design, which has recently been suggested as a more effective method for evaluating female preferences than traditional two-stimulus designs (Wagner 1998). We presented the three sword length stimuli in random order to each female. Females viewed one stimulus per day on consecutive days between 0800 and 1800 hours. The time of day at which an individual female was tested was held constant across sessions. We placed a monitor adjacent to each side of the test tank described in experiment 1. For this experiment, we did not use one-way glass and polarizing filters. We positioned blinds between the tank and monitors before each trial. Prior to testing, females were fed brine shrimp, and then placed in a glass tank (30.5 x 20.3 x 30.5 cm) with a live male to stimulate female sexual behaviour. The male and female were separated from each other by a clear divider. We allowed the female to view the male for 10 min, and then placed her in the test tank to acclimate for 30 min. We began the trial by starting the VCRs and removing the blinds. We made observations as in experiment 1. After the trial, we switched the male and female video stimuli, and conducted a second trial after a 10-min intertrial interval. Females deemed to have a side bias by the criterion used in experiment 1 were not included in the analysis. We tested a total of 21 females on each of the three stimuli; four females were omitted from analysis due to a side bias (two females had a side bias during the short-sword presentations, one female had a side bias during the intermediate-sword presentation and one female had a side bias during the long-sword presentations). Each female was used only once in each condition.

We used Proc MIXED in SAS to test for the effects of sword video, order of presentation and order x sword interaction, while blocking by individual females. We treated order of presentation and sword video stimulus as fixed factors and individual female as a random effect. We compared the least squared means for each video stimulus with data collected in a previous experiment using live males (Basolo 1998a). We compared female response to live and video stimuli using a three-factor mixed model analysis of variance (ANOVA) using individual females, sword length and stimulus method. We compared the strength of preference for live males and video images by testing the interaction between stimulus method (live or video) and sword length.

**RESULTS**

**Experiment 1**

We used nonparametric comparisons because these data were not normally distributed. Females responded more to the male stimulus than to the video of the empty tank, regardless of whether they were viewing live males (Wilcoxon paired-sample test: $Z_{17}=3.68, P<0.001$) or video images of males (Wilcoxon paired-sample test: $Z_{17}=2.24, P<0.02$). There was no significant difference between female response to live males and video males (Wilcoxon paired-sample test: $Z_{17}=-1.15, P=0.25$; Fig. 1). In addition, female response did not differ between the first and second trials (Wilcoxon paired-sample test: $Z_{18}=-0.36, P=0.72$). The power to detect a difference of $30 \text{s}$ at $a=0.05$ was 0.86. The minimum significant difference is based on the least significant difference calculated in another experiment (unpublished data).

**Experiment 2**

Averaged across all three male video stimuli, females responded more to male videos than to the female video (paired $t_{16}=8.47, P<0.0001$; Fig. 2). We examined the residuals to verify the assumptions for parametric tests. There was no significant difference in variance between the sword-video groups (Hartley’s $F_{\text{max}}=2.36, \text{NS}$), no significant autocorrelation (Durbin–Watson $D=2.72, \text{NS}$) and no significant evidence for non-normality ($W=0.98, P=0.84$). There was no effect of order of presentation on female response in the video sessions ($F_{2,26}=0.09, P=0.91$), nor was there a significant order x sword-video interaction ($F_{4,26}=0.51, P=0.73$). There was a significant effect of sword video on female response ($F_{2,26}=6.37, P<0.006$). We calculated and compared the least squared means for each of the three sword-length stimuli (Table 1). We plotted the video preference function with a
preference function obtained using live males corrected for the effect of male standard length (Fig. 3). There was a significant interaction between sword length and stimulus method (live or video), indicating that the slopes of the preference functions differed significantly ($F_{1,33}=7.65, P<0.01$).

**DISCUSSION**

There was no difference in female response to live males behind one-way glass and unmanipulated video stimuli in experiment 1. This outcome is similar to work with female *P. reticulata*, in which there was no difference in female response to live males behind one-way glass and video playbacks of males (Kodric-Brown & Nicoletto 1997). In numerous studies, female response to noncourting males is lower than that for both live courting males (Farr 1980; Kodric-Brown & Nicoletto 1997) and video images of courting males (Rosenthal et al. 1996). Our findings of a low level of female response in experiment 1 are consistent with previous studies using video playback. Clark & Uetz (1990) demonstrated that jumping spiders do not discriminate between live prey and the video image of the prey. Evans & Marler (1991) demonstrated that video playbacks of active, vocalizing chickens were just as effective at eliciting alarm calls from roosters in the presence of a predator. These types of studies demonstrate that test subjects will respond to video stimuli, but provide little detailed information about how subjects will respond to trait variation in live and video presentations. To our knowledge, only one study has compared response to variation in a trait using live and artificial stimuli. Schmitt et al. (1993) compared female response to natural vibrations and computer-generated vibrations in the spider *Cupiennius salei*. In this experiment, two groups of females were tested with either natural or computer-generated stimuli in which the interseries duration of vibrations was varied systematically. The key difference between Schmitt et al.’s work using vibrational signals and previous studies is that subjects were tested for response to variation using cally indistinguishable between live and video males. The aim of experiment 1 was to evaluate whether females respond in the same way to noncourting, noninteractive live and video males, not whether they express the same mating preferences. In experiment 2, we examined the expression of female mating preferences to video playbacks. The possibility of a schooling preference was controlled for by presenting a female video stimulus at one end of the tank and a male video stimulus at the opposite end. In experiment 2, females responded more to the male video stimuli than to the female video stimuli. This suggests that the females responded to males as a potential mating partner, not just as a conspecific.

The results from experiment 2 suggest that the female preference for a male in green swordtails is an increasing function of sword length; these results are in agreement with those previously found for live males varying in sword length. Previous studies have concentrated on determining the presence or absence of an effect of a trait on behaviour, rather than the effect of variation in the trait on a behavioural response. Clark & Uetz (1990) demonstrated that jumping spiders do not discriminate between live prey and the video image of the prey. Evans & Marler (1991) demonstrated that video playbacks of active, vocalizing chickens were just as effective at eliciting alarm calls from roosters in the presence of a predator. These types of studies demonstrate that test subjects will respond to video stimuli, but provide little detailed information about how subjects will respond to trait variation in live and video presentations. To our knowledge, only one study has compared response to variation in a trait using live and artificial stimuli. Schmitt et al. (1993) compared female response to natural vibrations and computer-generated vibrations in the spider *Cupiennius salei*. In this experiment, two groups of females were tested with either natural or computer-generated stimuli in which the interseries duration of vibrations was varied systematically. The key difference between Schmitt et al.’s work using vibrational signals and previous studies is that subjects were tested for response to variation using

![Figure 2. Female response to male and female video playback stimuli used in experiment 2.](image2)

![Figure 3. Preference functions for live (Basolo 1998a) and video stimuli. • One female’s response to a live male with an artificially attached sword (adjusted for body size); ◦ the average ± SE response of 17 females to one of three video stimuli.](image3)

**Table 1. Comparisons of least squared means**

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<thead>
<tr>
<th>Comparison</th>
<th>t</th>
<th>df</th>
<th>P</th>
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<tr>
<td>Long sword versus short sword</td>
<td>3.6</td>
<td>16</td>
<td>0.002</td>
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<tr>
<td>Long sword versus intermediate sword</td>
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<td>16</td>
<td>0.057</td>
</tr>
<tr>
<td>Short sword versus intermediate sword</td>
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<td>16</td>
<td>0.153</td>
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manipulated natural and artificial stimuli. Our work considers the effectiveness of video playback when there is trait variation in the length of the sword.

Although the same qualitative female response was obtained with both live and video presentations, there are apparent differences between female response to live and video stimuli. The overall level of female response appears to be greater, and the strength of preference for sword length appears to be stronger (steeper slope) in live presentations than in video presentations. This is not a surprising outcome, however, as in the live presentations, a male could visually interact with a female by moving back and forth along the side of the tank, while in video presentations, a video male moved back and forth across the screen independent of a female's behaviour. In addition, a live male could escalate courtship behaviour based on a female's response, possibly inducing a greater response by the female. Thus, one drawback when using current video playback methods to examine female mating preferences is that they do not allow for interactions between the test subject and the video stimulus. As courtship is a dynamic interaction between signaler and receiver, the video stimuli may not have elicited the full response from our female test subjects. In experiments where live male guppies could interact with females (Kodric-Brown & Nicoletto 1997), female response to live stimuli was significantly greater than to video stimuli. In several poeciliids, females prefer males that court over noncourting males (Farr 1980; Ryan & Wagner 1987). Although male courtship behaviour was included in the video stimulus, the interaction between the stimulus male and female in live experiments may account for some of the difference found between the two preference functions for sword length. Schmitt et al. (1993) also found a difference in the strength of preference for interseries duration between natural and artificial call bouts. The authors concluded that data collected with artificial stimuli would lead to qualitatively different conclusions regarding male vibrational behaviour than data collected with natural stimuli. However, despite the lack of visual interaction in our video sessions with green swordtails, the same qualitative response was found with both live and video presentations. In the future, experimenters planning on using digitally manipulated video stimuli to quantify behaviour may wish to consider developing interactive video sequences to simulate natural signaler-receiver interactions.

There were several additional differences between the video presentations conducted in experiment 2 and the live presentations which may partially account for the difference in the strength of the two preference functions. In video presentations, females were tested three times as opposed to only once in the live tests. Retesting of females could have resulted in a difference in female response due to habituation to video stimuli across trials or between sessions. However, there was no significant difference between the first and second trials of test sessions, nor was there a difference in female response across test sessions. Another potential factor influencing response is that females could have habituated to the video loops of repeated behaviours within the 10-min trials. This type of habituation would not have been detected in our analysis.

Because we used only one exemplar as a base stimulus in experiment 2, the generality of these results may be somewhat limited. Previous discussions of bird song playback experiments have pointed out that the use of one individual for all playback stimuli may not represent the variation present in the population for the trait of interest (Kroodsma 1989a, b). To date, the majority of video playback experiments conducted using manipulated videos have used a single exemplar (Rosenthal, in press). Researchers planning experiments that will involve the use of video playback should consider how this methodology will affect their conclusions. Currently, the creation and manipulation of video images is an extremely time-consuming process, so addressing the issue of external validity in video playback experiments is nontrivial. Advances in technology may soon help overcome this obstacle. Despite using one exemplar, we can still infer from our results that the long-sword video stimulus elicited greater responses from females (McGregor et al. 1992). This is consistent with previous manipulations of sword length using live males.

Our results make a convincing case that female X. helleri respond to live males and video stimuli in a qualitatively similar manner. The difference in the strength of preference between live and video stimuli may be partially accounted for by the lack of behavioural interactions between males and females imposed by present technology. Despite the differences between the video and live presentations, however, females preferred the long-sword stimulus over the short-sword stimulus. Our results demonstrate that video playback can produce results that are qualitatively similar to the results obtained using live presentations. These experiments also illustrate many of the advantages and disadvantages of using video playback. With further refinement, such as interactive video stimuli, video playback techniques may also become quantitatively comparable to live presentations.

Acknowledgments

The authors thank W. Wagner, Jr, for help in experimental design, D. Clark, L. Fleishman, J. Wise for discussions of video playback, R. Diaz-Uriarte for statistical advice, A. Asbury, K. Benson, A. Goudy & K. Delaney, C. Marler, W. Wagner, Jr, and two anonymous referees for comments on the manuscript. This research was supported by a UNL Layman grant, NSF grant RPG 9309211, NSF grant IBN9629318, NIH grant MH55288-01 to A.L.B. and a Grant-in-aid from Sigma Xi to B.C.T. The research presented here was described in Animal Research Protocol Nos 94-09-077 and 97-09-056, approved on 26 September 1997 by the Institutional Animal Care and Use Committee of the University of Nebraska, Lincoln.

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