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"Visual abilities of Golden-collared Manakins (*Manacus vitellinus*)"

verfasst von / submitted by

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LIST OF ABBREVIATIONS

A/C	air conditioning
CFF	critical flicker fusion frequency
cc, nc, wc	correct choice, no choice, wrong choice
DEE	daily energy expenditure
E	illuminance
FF	flicker frequency
FFF	flicker fusion frequency
GCM	Golden-collared Manakin (*Manacus vitellinus*)
HD	high definition
ID	identification
L	luminance
L1, L2	Light #1, Light #2
LED	light-emitting diode
n	sample size
ND	neutral density
p-value	probability value
±SD	standard deviation
STRI	Smithsonian Tropical Research Institute
UV	ultraviolet

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1. Introduction

Perceiving light reflected from objects is a primary method used by many animals to gain information about their environment. For flying birds a superior vision is the most important sense for safe flight. Additionally for many bird species good eye sight is relevant regarding sexual selection (e.g. colorful plumage and courtship displays). Bird species performing fast moving manoeuvres and complex courtship displays to attract mating partners are assumed to have well-adapted eyes and excellent visual acuity (e.g. several Manakin species (*Manacus sp.)* [Fusani et *al*., 2014]). It is suggested that the eyes of fast-moving animals, like flying birds, have considerably higher temporal resolution capabilities than those of slow moving animals like humans [Inger et *al*., 2014]. Visual abilities and the visual processing speeds of birds and other vertebrates have previously been studied using flicker fusion frequency experiments [Lisney et *al*., 2011; Healy et al., 2013; Boström et *al*., 2016]. The flicker fusion frequency (FFF) [Hz] is the stimulus frequency at which a flickering light stimulus is perceived as continuous. The critical flicker fusion frequency (CFF) [Hz] is the highest flicker fusion frequency at any light intensity [cdm^{-2}]. For example, on average, humans have a CFF of 50-60 Hz [Maddocks et *al*., 2001], chickens a CFF of 87.0 Hz at high light intensity [Lisney et *al*., 2011] and flying birds tend to have CFFs even well above 100 Hz [Maddocks et *al.*, 2001] (e.g. 100 Hz for pigeons (*Columba livia*) and up to 146 Hz for Pied flycatchers (*Ficedula hypoleuca*) at high light intensity [Healy et *al.*, 2013; Boström et *al*., 2016]). It has been demonstrated that high CFF correlates with high metabolic rate and small body size of vertebrates [Healy et *al.*, 2013].

The Golden-collared Manakin (GCM, *Manacus vitellinus*) is a passerine bird species of the *Pipridae* family, found in mature secondary woodland, forest borders and regenerating clearings in the Lowlands of Panama to northwestern Colombia. Previous studies have shown that these birds demonstrate a very complex, high-power-demanding courtship display [Barske et *al*., 2013]. To attract females, male Manakins perform competitive display dances within their courtship court. Theses displays, consisting of loud wing-snaps and rapid movements, are executed with very high accuracy and neuromuscular coordination [Fusani & Schlinger, 2012; Fusani et *al*., 2007 & 2014]. Thereby the female's choice of suitable mating partner appears to correlate with the male's motor skills. Favourable performance parameters are high speed, accuracy and frequency of specific display movements. Speed differences in a range of tens of milliseconds can be detected by females [Barske et *al*., 2011 & 2015, Fusani & Schlinger, 2012]. This suggests that GCM have high temporal resolution capabilities allowing them to process visual information very quickly. This leads to the primary focus of this study: what are the visual capabilities of *Manacus vitellinus*? Do these birds have particularly rapid visual processing which allows them to distinguish differences of a few milliseconds between the displays of different males? Answering to our research question may lead us to a better understanding of the complex courtship display of Golden-collared Manakins.

Our investigations were based on a previous FFF study conducted with chickens (*Gallus gallus domesticus*) [Lisney et *al.*, 2011]. We used an operant conditioning approach. Birds were trained and tested for their ability to distinguish a flickering light stimulus from a continuous one. We utilized calibrated full-spectrum LED-lights with six different light intensities and varying flicker frequencies (FF) as the light stimulus. The birds were individually put in an experimental cage. In every cage there were two separate closable feeders and a pair of LED-lights of which one produced a flickering and the other one a constant light stimulus. At different adjusted light conditions the birds were trained to be rewarded at either the flickering or the constant light stimulus by opening the appropriate feeder. The birds were allowed to become familiar with their surroundings and testing parameters before testing started. During the testing phase, flicker frequencies were increased (ranging from 20-200 Hz) at six different light intensities (with luminance ranging from 80 to 3000 cdm^{-2}) until the birds were unable to distinguish between the flickering and continuous stimulus.

It was expected that 1) GCM would demonstrate optimal visual performance (CFF) at medium to high light intensities in keeping with their natural habitat light conditions [see Appendix – Table A 1a-d] and 2) their CFF should be well above average (> 100 Hz) and close to the rapid vision of other fast flying passerine bird species such as flycatchers [Boström et *al*., 2016].

2. Material & Methods

The research project was conducted from March 4th to May 28th 2015 at a facility of the Smithsonian Tropical Research Institute (STRI) in Gamboa, Panama. Eight birds were caught from elected natural Manakin lekking sites in the forest using the mist-netting method. After acclimatization in specially designed experimental bird cages at the research facility, the daily training sessions for Operant Conditioning started. The birds were kept and trained individually to distinguish between a flickering light stimulus and a continuous one at various light intensities. Half of the birds were conditioned to be rewarded at the flickering light, the other half at the continuous light. Once a bird appeared familiar with the procedure and confidently performed the task of distinguishing the flickering light and the constant light during the stage of training, it entered the testing phase. During experimental sessions the flicker frequency was increased stepwise and a bird had to make correct choices in 4 out of 5 trials at a given FF and light intensity to proceed to a higher FF-level. Training and testing sessions took place during morning and afternoon hours according to a set schedule. Upon completion of the experimental period, each bird was marked with two colored leg rings and released back into the wild.

The continuous light stimulus (FF = 20 kHz) and the flickering light stimulus, with FF ranging from 20 to 200 Hz, were produced by two attached function generators. Six pairs of LED-lamps with 6 different light intensities, with luminance target values (L) of 80, 160, 350, 750, 1500 and 3000 cdm^{-2}, were used as light stimuli.
Next to six pairs of calibrated full-spectrum lights, two function generators and four specially designed experimental bird cages with accessories (feeders, decision-making perches, HD web-camera), the experiments required eight differently powered LED-lamps (8, 12 and 23 Watts), neutral density filter sheets (0.3 ND and 0.6 ND) and neutral card-boards for light adjustments in the research facility as well as in and around the cages, and a photometer. According to the protocol the experimental equipment was newly set up and adjusted before every experimental session.

2.1. Mist-netting & housing procedures

Mist-netting

The birds were caught from their natural environment using standard mist-netting methods during peak hours of Manakin activity in the early morning and early afternoon hours. Appropriate catching sites had to be elected and catching transects had to be prepared prior to catching. Straight transects of 6 meters close to the display ground and common flight routes of Manakin lekking sites were cut through the bush. To find an optimum location to cut transects, it was necessary to go out in the field in advance and check for the wing-snapping sounds of GCM and find the lekking sites and display courts of males. Due to a sexual plumage dimorphism in GCM, adult males can easily be distinguished from dull olive green colored females and juveniles. Adult males got a bright yellow collar and throat, a black cap and mostly black back with olive green on the center of the back, rump and lower underparts.

To create a court for their courtship display, the male GCM clear a small patch of forest ground from leaf litter. Typically the patch is positioned between several sticks arranged in a triangle. At a height of about 20 cm above the ground these sticks show obvious wear marks as well as the ground in between the sticks.

Once the transect was ready, the two poles which hold the net were put up at a distance of 6 meters to each other. The net has to be spanned tensely, not allowing any sagging, and it has to be free of leaves and branches so the birds cannot detect the net. Each pole had a string attached with which the poles were fastened to a stable tree. Then the net was left alone was checked at intervals of about 30 minutes.

Figure 1: GCM caught in a mist-net.

Figure 2: GCM after being removed from a mist-net.

When a bird was caught it was removed from the mist net as quickly as possible and placed in a cotton bag to minimize stress (see Figure 1 & 2). Once placed into a prepared cage at the research facility, the birds were left alone for two hours to feed from papaya junks. Birds that failed to eat within 2 hours were released.
The newly captured birds that successfully started feeding were let to rest for two days to allow them to acclimatize to captivity before training began. A total of eight birds were caught during the months of March and April at six selected catching sites (see Appendix – Table A 2).

The sample pool included two adult males, one juvenile male, four females and one sexually undetermined green bird. The sex determination was conducted at the end of the experimental period. Adult male birds were distinguished from the olive green coloured adult females and juvenile males and females by their bright yellow colouration of the collar plumage. Females and juvenile males were sexually determined using genetic tools after Griffith [Griffith et *al.*, 1998].

Housing

The eight individuals used in this study were kept separately in prepared bird cages within a windowed, air-conditioned room at the STRI facility in Gamboa, Panama. Since the windows of the facility were not air-tight the humidity in the room varied from 65 to 95%, depending on the outside weather conditions. Likewise, the room temperature also often significantly exceeded the temperature of 27 degrees Celsius set by the A/C.

Four birds at a time were kept individually in specially designed experimental cages. The equipment used during the experiments was removed from the cages when no experiments were taking place. The remaining birds were kept in two additional bird cages aside from the experimental setup for acclimatization purposes (see Figure 3).

The captured birds' diet consisted of papaya given twice daily, at 06h30 in the morning and following the experimental session at 17h30-18h00. The artificial room illumination, set with a clock timer, was switched on before the morning feeding session and turned off after the second feeding period at 18h30. This scheduled light adaptation in the facility was supposed to mimic the natural time of dawn and dusk in the local forest. During the acclimatization phase the birds were allowed to feed all day and had water containers available for bathing. Once the experimental phase started the birds were allowed to feed by themselves for 1 hour in the morning and then were food-deprived for 1.5 hour before starting the experiments to get maximum cooperation. It has been observed that shorter periods of food-deprivation resulted in little or no cooperation during training/ testing sessions.

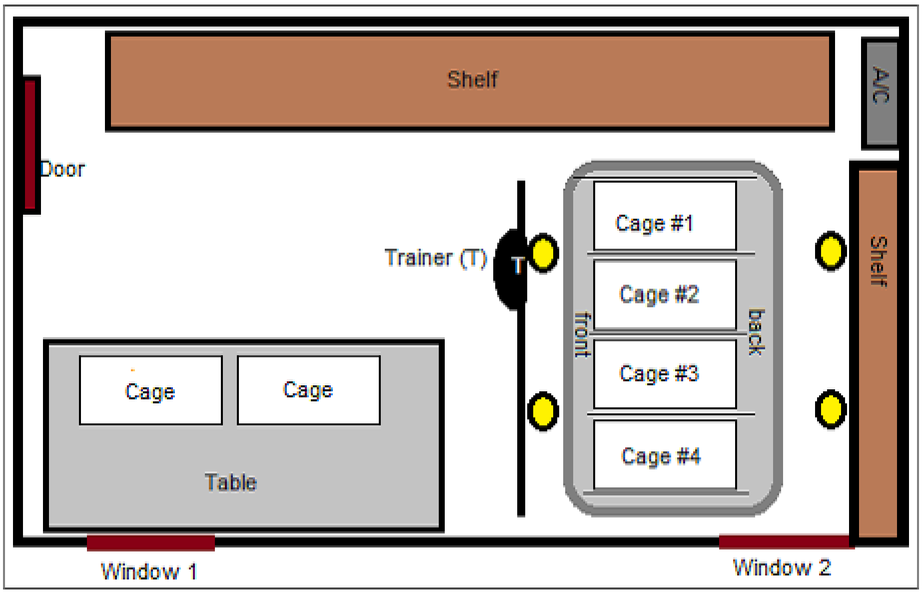

Figure 3: Design of the research facility (STRI building #152, Gamboa, Panama). The four experimental cages (cage #1-4, marked white) used for the experiments are set up in a row on a table by a window (window #2, marked red). Artificial lights for light condition adjustments are positioned above at the front and at the back of the cages (marked yellow). Two more non-experimental cages are set on an additional table by a window (window #1, marked red).

The experimental cages

The experimental cages were set up parallel to each other on a 2 m long table. Four cages were set up for the experiments with two additional cages used for housing of the non-experimental birds (see Figure 3). Each experimental cage featured one non-removable high perch in the back of the cage and one low one close to the front wall

and stimulus lights. The non-transparent front wall of each experimental cage had two slots for sliding in two closable feeders as well as two slots for a pair of experimental full-spectrum lights to be introduced above each feeder. The lid of the feeders was closed and opened from the outside via an attached string (see Figure 5). The cage floors were white.
Before an experimental session two “decision-making perches” (one in front of each feeder/ stimulus light) on a cork-board were introduced at the front of the cage, together with a pair of full-spectrum lights and the two closed filled feeders. At the back of the cage a HD web-camera was mounted to video-document each session. Additionally one neutral cardboard with the dimensions of the cage was positioned at each side of the cage for light adjustment reasons and to prevent visual distractions to the bird from the outside. The accessories were removed after every experimental session to signal that the session is over.

The experimental cage had the dimensions of 71.0 x 45.0 x 45.0 cm (length x width x height) (Figure 4). The high perch was positioned 53.5 cm away from the front wall at a height of 10.0 cm above ground, the low perch 14.0 cm away from the front wall at a height of 2.5 cm above ground. The two removable decision-making perches were attached to a cork board which was introduced beneath the feeders before every session and removed after. Thus, these perches were positioned at a height of 2.5 cm above ground, in a distance of 6.0 cm away from the front wall and 1.0 cm away from the border of the feeders. There was a distance of 7.0 cm between the two decision-making perches, each being 10.0 cm wide.
The two feeders were positioned in the front wall of the cage, extending 5.5 cm into the cage. The food tray of each feeder had a length of 5.0 cm, a width of 6.0 cm and a depth of 1.0 cm (see Figure 5). Each feeder was separately closed or opened quickly from the outside of the cage by pulling an attached string. Before every session the feeders were cleaned, filled with tiny fresh papaya pieces (~ 2 mm) and closed.
The two light stimuli L1 and L2 had a diameter of 3.5 cm each. They were positioned in the frontal cage wall with a distance of 3.5 cm to the ground and 15.0 cm between each other.

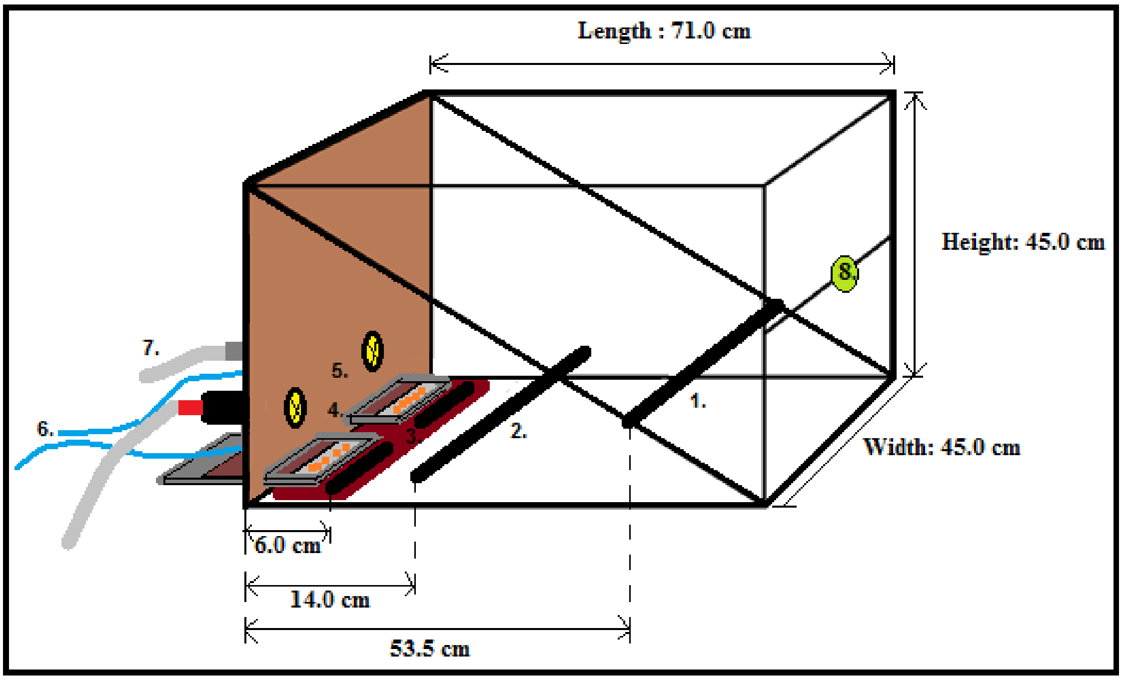

Figure 4: Design of an experimental cage. One high perch (1.), one low perch (2.), one pair of decision-making perches (3.) attached to a cork-board (marked red), one pair of closable feeders (4.) with attached strings (6., marked blue), one pair of calibrated full-spectrum lights L1 and L2 as light stimuli (5.), a pair of electric cables (7.) which connected the test lamps to the two function generators (one for flickering and one for constant light production) and a HD web-camera (8., marked green).

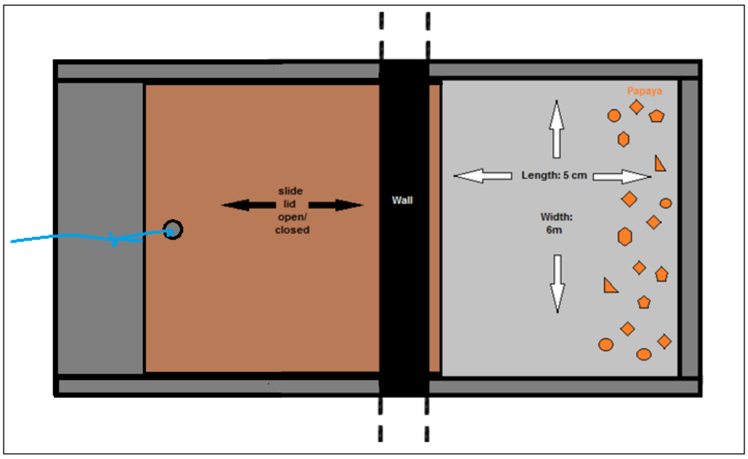

Figure 5: Design of a feeder. The food tray is on the inside of the cage, the lid of the feeder can be closed by the trainer from the outside by a string. The food tray has dimensions of 5 cm x 6 cm x 1 cm.

2.2. Experimental approach – Training and testing procedures

The experiments took place from March 7th to May 24st 2015. The experimental process for each bird was divided into two stages: the training period and the testing period.
The training and testing sessions were conducted from 07h30 to 12h30 and 13h30 to 17h30 daily, adjusted to the natural main activity hours of GCM and according to a consistent daily schedule as described in Table 1.
Using an operant conditioning approach, the birds were first trained individually to distinguish a flickering light stimulus from a continuous one. When a bird seemed familiar with the training procedure and constantly demonstrated correct choices in 4 out of 5 trials (or 8 out of 10) at a given light intensity and low flicker frequency it entered the second stage of the experimental process. Two to four birds at a time underwent training or testing and were permanently housed in the four prepared experimental cages.
Half of the sample birds were trained to be rewarded at the constant, non-flickering light stimulus, the other half of the sample birds were trained to be rewarded at the flickering, inconsistent light stimulus. In particular, the four individuals with the ID code CB012015, CB022015, CB072015 and CB082015 were rewarded at the constant light stimulus, the four individuals CB032015, CB042015, CB052015 and CB062015 at the flickering one (see Appendix – Table A 2)

The experiments followed a continuous daily schedule (see Table 1). It started with the removal of the food from the experimental cages of the first two birds (bird A and bird B) to be trained or tested at 07h30, one hour after introducing the food into the cages. The papaya-filled feeders and the stimulus lights were placed into their corresponding slots as well as the HD web-camera, the neural card-boards and the decision-making perches. The luminance target value (L) was measured with the Hagner© photometer and recorded.

Table 1: General daily schedule for training and testing procedures:

Time	Procedures for bird A and B	Procedures for bird C and D
06:30	morning feeding of bird A-D (papaya chunks)	
07:30	- food removal from bird A+B - introduction and adjustment of	

	experimental equipment (2 light stimuli, decision-making perches, feeders, HD camera, neutral cardboards)	
08:30	- adjustment of light conditions in and around the cages (4 LED lamps, ND filter sheets), verification with photometer	
09:00	- start of experiments with bird A+B - removal of experimental equipment after finishing experimental session	prior to 09h00 a.m.: - food removal from bird C+D - introduction and adjustment of experimental equipment
10:00		- adjustment of light conditions in and around the cages, verification with photometer
10:30		- start of experiments with bird C+D - removal of experimental equipment after finishing experimental session
11:30	- introduction and adjustment of experimental equipment - adjustment of light conditions in and around the cages, verification with photometer	
12:00	- start of experiments with bird A+B - removal of experimental equipment after finishing experimental session	
13:30		- food removal from bird C+D - introduction and adjustment of experimental equipment
14:30		- adjustment of light conditions in and around the cages, verification with photometer
15:00	prior to 15h00 p.m.: - food removal from bird A+B - introduction and adjustment of experimental equipment	- start of experiments with bird C+D - removal of experimental equipment after finishing experimental session
16:00	- adjustment of light conditions in and around the cages, verification with photometer	
16:30	- start of experiments with bird A+B - removal of experimental equipment after finishing experimental session	
17:30	evening feeding of bird A-D (papaya chunks)	

At 08h30, after 1 hour of food deprivation of bird A and B, the light conditions were adjusted accordingly to the light intensity to be applied. This was accomplished by placing neutral density filter sheets on the roof of the cage and using LED lamps mounted above the cages as described in Chapter 2.3. If necessary, their angle was adjusted, too. The illumination (E) at the stimulus lights was verified with the photometer. Birds A and B were then allowed to rest for 5 minutes before the experimental session started at 09h00. This was 1.5 hours after food deprivation. A session began when the light stimuli were illuminated.

Each session consisted of several trials at a given light intensity and FF. There were three possible responses during each trial. The birds could make a correct choice (cc) by correctly choosing the rewarding stimulus, an incorrect/wrong choice (wc) by choosing the non-rewarding stimulus or no choice (nc) by not choosing or responding. If the bird made a correct choice (cc) within 2 minutes (= 1 trial), the feeder was immediately opened to allow the bird to feed briefly (reward). The lights were switched off and the feeder then closed to begin the subsequent trial.
Likewise, if the bird made an incorrect choice within the 2 minutes (= 1 trial), the feeder remained closed (no reward) and the lights were switched off. As soon as the bird voluntarily moved back to the distal perch, the light stimuli was illuminated to begin the subsequent trial. A session ended when the bird did not move or made no choice during two consecutive trials. At the end of a session, the feeders, the light stimuli and the decision-making perches were removed.

At 11h30 the same procedure was repeated for birds A and B. After finishing the second experimental session, food was introduced into the cage again.

The food was removed from the other the experimental birds (C and D) just prior to 09h00.
At 10h00, after 1 hour of food deprivation, the experimental procedures were initiated. At 10h30, i.e. after 1.5 hours of food deprivation, the experimental session for birds C and D began and proceeded in an identical manner as with birds A and B.

In the afternoon two further experimental sessions took place. At 13h30 the food was removed from bird C and D. At 14h30, i.e. after 1 hour of food deprivation of birds C and D, the experimental protocol was repeated in an identical manner as in the morning session. The session was being started at 15h00.
At 15h00 the food of bird A and B is being removed. At 16h00, i.e. after 1 hour of food deprivation of birds A and B, the session was initiated. The afternoon session for birds A and B started at 16h30.

On the following day the experimental schedule was altered and the experimental sessions began with birds C and D in the morning and finished with birds C and D in the afternoon.

	Day 1	**Day 2**
Morning session	A+B C+D A+B	C+D A+B C+D
Afternoon session	C+D A+B	A+B C+D

Day 3 will be as day 1, day 4 will be as day 2 and so on.

Training (Operant Conditioning)

There were two test groups in the experimental phase. Birds of Group 1 were rewarded when choosing the constant light (c) correctly. Birds of Group 2 were rewarded when choosing the flickering light (f) correctly (see Appendix – Table A 2).

The calibrated stimulus lights (L1 and L2) were randomly connected to either the flickering-light-stimulus producing generator (variable FF [Hz]) or the continuous-light-stimulus producing generator (permanently set at 20 kHz).

The birds were habituated to the experimental equipment, including the stimulus lights being switched on and off and the feeders being opened and closed, at the beginning of the training period. At first only one light stimulus was introduced according to the test group the bird is in - Group 1 constant light stimulus or Group 2 flickering light stimulus (habituation FF: 20 Hz) - by randomly connecting L1 and L2 to the appropriate single generator.

The feeder at the correct stimulus light was opened half way at the time the light turned on to facilitate the habituation. It was opened fully and immediately when the bird landed on the correct decision-making perch or directly on feeder. The stimulus is then switched off.

During the habituation period, each bird had 4 minutes per trial to choose the correct light (rewarding). Once the test bird demonstrated no fear or apprehension of the feeder and stimulus light, the training began. Initially the correct feeder was partially opened when the bird approached on the low perch and fully opened when the bird landed on the feeder. As the bird progressed through the training period, the correct feeder was partially opened when the bird approached it at the correct decision-making perch in front of the feeder and fully opened when the bird landed on the feeder. At the end of the training period, the feeder door was opened only when the bird correctly approached and landed on the rewarding feeder.
Once the each bird consistently demonstrated correct choices with one stimulus (the rewarding one), the second light stimulus (non-rewarding) was added by randomly connecting L1 and L2 to the flicker-light generator and the constant-light generator. The habituation to the 2-light-stimuli situation was conducted according to the same protocol as with the single stimulus light.

Initially, the feeder corresponding to the correct stimulus light was partially opened when both lights (flickering and constant) switched on and completely opened when a correct choice was made by the bird. After each choice, both light stimuli were turned off. As the birds progressed through the training period, the feeder door was partially opened only when the bird approached the feeder appropriately and fully opened when a correct choice was made. By the end of the training period, the feeder was opened only when the bird correctly chose the rewarding feeder (see Figure 6).

The training period ended once the habituation to both light stimuli was accomplished and the bird was able to confidently choose the correct rewarding stimulus within a fixed 2 minute time period. The bird has been trained at the various light intensities and varying low flicker frequencies from 20 to 40 Hz. The test bird was ready to enter the test phase when it was able to confidently distinguish the flickering light (f) and the constant light (c), and made correct choices in 4 out of 5 trials (or 4 of 4 or 8 of 10 or 8 of 8 trials) at a given FF and light intensity.

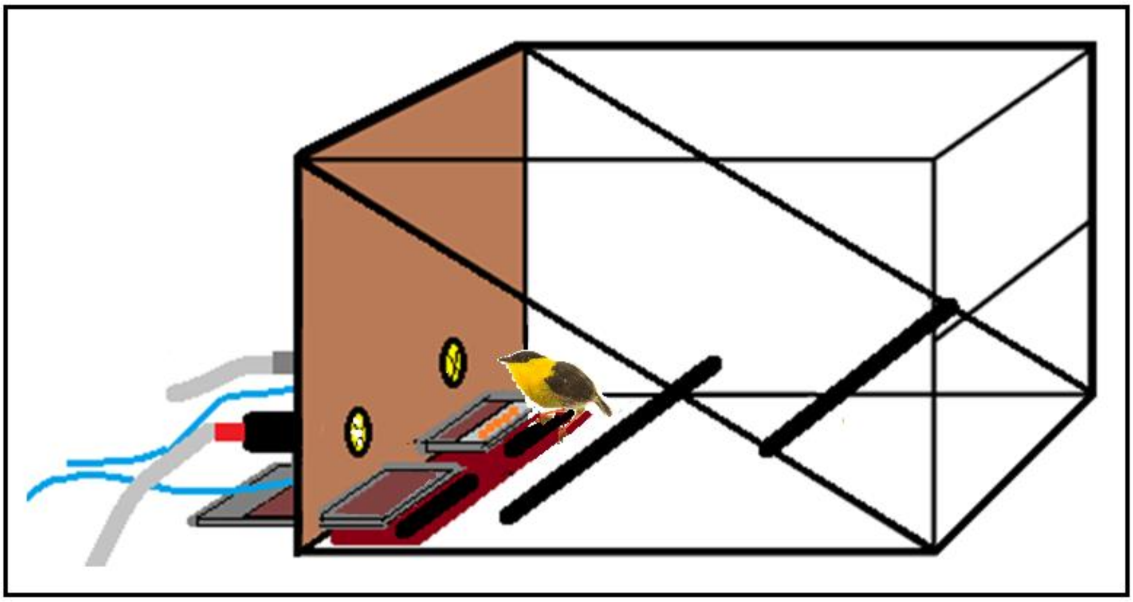

Figure 6: Operant conditioning – reward system. The bird makes a correct choice in an experimental trial and immediately gets rewarded by the operator by opening the correct feeder briefly.

Testing

The testing stage was entered when the test bird consistently demonstrated correct choices in 4 out of 5 trials (or 4 of 4 or 8 of 10 or 8 of 8 trials) at given low FF and light intensities over several training sessions.

During the testing phase, eight sample birds were tested at six different light intensities [cdm^{-2}] in combination with FFs [Hz] ranging from 20 Hz to 200 Hz at a given light intensity.

The testing of each bird started with the lowest FF= 20 Hz at any given light intensity and then proceeded with the next higher FF (increase in 20-Hz-steps). The birds proceeded to the next higher FF level at a given L according to the protocol used during their training. The bird was required to choose correctly in 4 out of 5 trials (or 4 of 4 or 8 of 10 or 8 of 8 trials) within a period of 2 minutes, at a given light intensity, before the next higher flicker frequency (FF) was tested. The FF was increased by 20 Hz intervals following each successful trial.

If the bird's choices were incorrect and it chose the non-rewarding light stimulus in more than 2 out of 5 trials at a given FF and L, the progression to the next higher FF was halted and testing returned to the last positively tested FF (i.e. with ≥ 4/5 (or ≥ 8/10) correct choices). In these birds, the FF was then increased in 5-Hz-steps with successful completion of each trial. If the bird again was only able to make less than 4 out of 5 correct choices during a trial, testing again returned to the last positively tested FF. The FF was then increased in 1-Hz-steps until the test bird repeatedly failed to get 4 out of 5 (or 4 of 4 or 8 of 10 or 8 of 8) correct choices at the given FF and light intensity. This was the point when the FFF is reached at a given light intensity.
The FFF for a given light intensity tested was confirmed with at least 8/8 or ≥ 8/10 correct choices in a test session. A "no choice" response from the bird was not recorded as a correct nor as a wrong/incorrect choice.

Example of testing protocol: given light intensity: 750 cdm^{-2}, given FF: 40 Hz:

- situation A: birds makes **≥ 4/5 correct choices at given FF**
  → proceed to next higher FF (= 60 Hz)
- situation B: bird makes **< 4/5 correct choices at given FF**
  → return to last positively tested FF (= 40 Hz) and increase FF in **5-Hz-steps**
  i.e. 40 Hz → 45 Hz → 50 Hz → ...
  → proceed in 5-Hz-steps until bird makes < 4/5 correct choices at a given FF (e.g. at 50 Hz)
    → return to last positively tested FF (= 45 Hz) and increase FF in **1-Hz-steps**
    i.e. 45 Hz → 46 Hz → 47 Hz→ ...
    → proceed in 1-Hz-steps until FFF is reached for this light intensity
  → confirm FFF with at least 8/8 or ≥ 8/10 correct choices

2.3. Experimental setup

2.3.1. Experimental lights & function generators

For the conduction of the experiments six pairs of calibrated full-spectrum lights were used as light stimuli. Each pair of lights had a different luminance target value (L in [cdm^{-2}]). Light pairs with the six following light intensities (with luminance target values (L)) were used for the experiments: 80 cdm^{-2}, 160 cdm^{-2}, 350 cdm^{-2}, 750 cdm^{-2}, 1500 $cdm^{-2}$and 3000 cdm^{-2}.
For the light intensities 750 cdm^{-2} and 1500 cdm^{-2} there was an extra backup pair of calibrated lights available since those light intensities come closest to the natural light conditions of the birds' habitat and were expected to be utilized more often (see Appendix – Table A3).

A stimulus light was composed of up to six single LEDs (combination of white and UV LEDs to match natural daylight as perceived by the birds) attached to the distal end of an aluminium tube (lamp shaft). The proximal end of the tube was covered by the lamps' head including an UV-transparent Perspex panel with an adjusted amount of round neutral density filters (with 0.3 ND and 0.6 ND) and diffusion filters behind it to suitably alter the illumination of a lamp (see Figure **7**). To produce one continuous and one flickering light stimulus at the same time two different function generators were used that were randomly connected to L1 and L2 via two electric cables (see Figure 8). Since single LEDs inside of the prepared calibrated test lamps began to malfunction at an early stage of the experiments due to high humidity, they were either replaced or the lamps used for testing of next higher or lower light intensities were modified and applied (see Appendix – Table A 4). Neutral density filters inside the functioning test-lamps were added or removed to adjust its illumination (E) to get a higher or lower luminance target value to be able to continue testing all different light intensities (e.g.: the 750-cdm^{-2}-lamps were used as 350-cdm^{-2} -lamps by adding one extra 0.3 ND filter). If the luminance (L) of a test lamp did not meet study parameters, it could be altered by adjusting the length of the lamps' aluminium tube. This was done by slightly pulling the lamp shaft out the lamps' head. This resulted in a lower luminance of the lamp. However, these adjustments cause the calibration of a lamp to deviate.

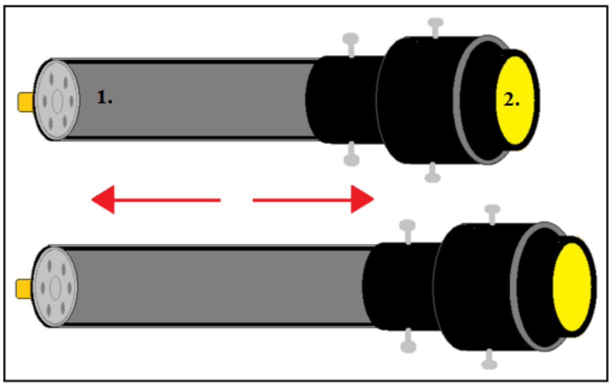

Figure 7: Design of a full-spectrum light and a possible adjustment of the lamps' luminance [cdm^{-2}]. The lamp includes contact points for a combination of white and UV LEDs at the distal end of the aluminium lamp shaft (1.). In the lamps' head there's an adjusted amount of ND and diffusion filters behind a transparent Perspex panel (2.). The luminance of a lamp can be adjusted by pulling the aluminium tube slightly out of the lamps' head**.**

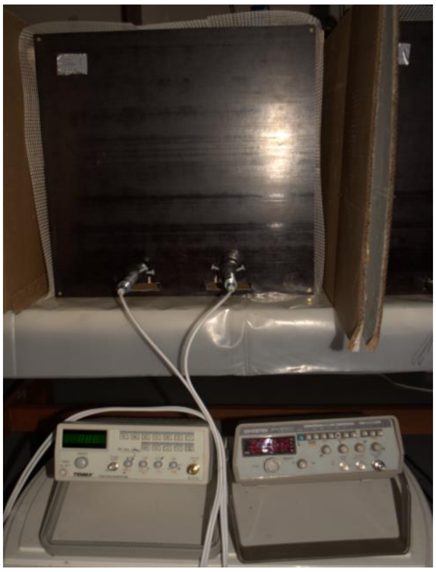

Figure 8: Two function generators (Models TENMA (bottom left) and GwInstek (bottom right)) attached to one full-spectrum light each. One generator produced a continuous light stimulus and the other one a flickering light stimulus.

To train and test for the CFF of Golden-collared Manakins it is necessary to produce light stimuli ((f) and (c)) with various flicker frequencies (FF) ranging from 20-200 Hz (f) and 20 kHz (c). For this purpose there were two function generators with DC output (see Figure 8) which were randomly attached to test light L1 and L2.
Generator #1 (TENMA, 3 MHz, Taiwan) was permanently set to a FF of 20 kHz for a constant light stimulus production. Generator #2 (GwInstek, 2 MHz, China) was variably set to a FF ranging from 20 to 200 Hz for a flickering light stimulus production.

During experimental sessions light stimuli with one chosen light intensity (luminance (L): 80, 160, 350, 750, 1500 or 300 cdm^{-2}) were applied in combination with flicker-frequencies (FF) ranging from 20 Hz to 200 Hz. The FF of the flickering stimulus (f) was increased in 20-Hz-intervals starting at 20 Hz and proceeding to 40, 60, 80, 100,120, 140, 160, 180 Hz and finally 200 Hz following the experimental protocol as described in the chapter 2.2. (Experimental approach – Testing). This scheme was used for each sample bird at each of the six light intensities. It was terminated when the test subject repeatedly failed to correctly distinguish the flickering from the continuous stimulus (< 4 out of 5 trials) at a given light intensity. After reaching this limit it was returned to the last positively tested FF. Then the FF was increased in 5-Hz-step until reaching another limit. Then a FF-increase in 1-Hz-steps was applied.

2.3.2. Experimental light conditions

To test six different light stimuli intensities the light conditions in the cage were adapted according to the luminance (L) of the pair of experimental lights. The illumination (E in [lux]) at the stimulus lights (L1 and L2) in the cage was set to be 10% of the luminance (L in [cdm^{-2}]) of the tested stimulus lights. For example, if the light intensity of 750 cdm^{-2} was tested, the illumination at the decision-making site in the cage was set to be 75 lux.

The laboratory room was equipped with two windows with natural lighting which changed the light conditions in the room and cages depending on the time of the day as well as the weather and light conditions (see Figure 9). Therefore the cages positioned closer to the window (cage #3 and #4) often had higher lux-values than the cages further away from the window. This was corrected by using more and/or darker neutral density filter sheets to adjust the light conditions at the stimulus lights in the cage.

To adjust the lighting conditions for the experiments so that the illumination (E) at the stimulus lights was 10% of the luminance (L) of the stimulus lights, we used the following tools:

I. Four LED-lamps with a combination of three different powers (8 Watts, 12 Watts or 23 Watts; model: Phillips Mini Twister (see Appendix – Table A5) according to the light conditions needed in the cage. From a distance of 1 meter away from the cages, the LED lights were mounted in pairs above the cage in the front (260.0 cm above the cage) and in the back (240.0 cm above the cage) as indicated in Figure 10.

We used five different LED lamp combinations combining either 4x 8 Watts for very dim conditions, 2x 8 Watts plus 2x 12 Watts, 2x 8 Watts plus 2x 23 Watts, 2x 12 Watts plus 2x 23 Watts or 4x 23 Watts for very bright conditions (see Appendix – Table A6). The stronger lights (12 W or 23 W) were positioned at the front of the cage and the dimmer ones at the back. The angles of the lamps (pointing up, straight or down) were adjusted as necessary.

The corresponding powers of incandescent light bulbs emitting the same light intensity as the LEDs we used for the experiments are as following: one 8-Watt-powered LED light corresponds to one 30-Watt-powered incandescent bulb, one 12-Watt-powered LED corresponds to a 50-Watt-powered incandescent bulb and one 23-Watt-powered LED corresponds to a 90-Watt-powered incandescent bulb.

II. Neutral density filter sheets on top of an experimental cage if needed. There were two different kinds of ND filter sheets available: 0.3 ND and 0.6 ND. They were applied in different amounts and combinations (see Appendix – Table A6). If necessary, one of the sheets was pulled down half way at the rear end of the cage to minimize light influx.

III. Neutral card-boards at both sides of a cage to prevent uneven light conditions in the cage.

To make sure the ambient light conditions of the stimulus lights are set correctly and the full-spectrum lights had the proper luminance target value, the luminance of the test-lamps (L in [cdm^{-2}]) and the illumination (E in [lux]) at the stimulus lights were measured prior to every experimental series with a calibrated photometer (model ‘Hagner© ScreenMaster’). To obtain the illumination measurements, the photometer was placed between the two light stimuli at the inner frontal cage wall.

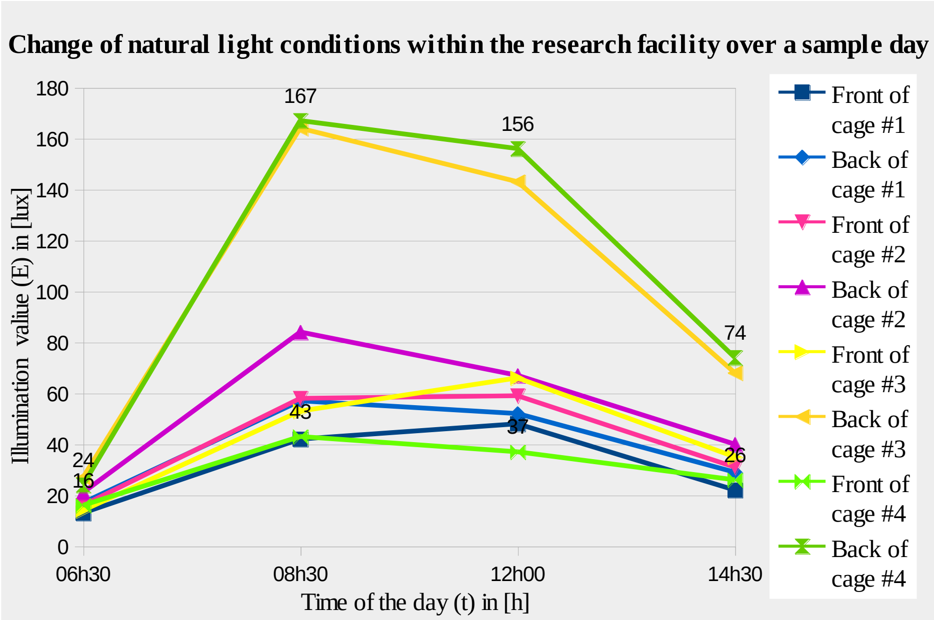

Figure 9: Changes of natural light conditions (illumination value (E) in [lux]) within the research facility over a sample day. Artificial lights have been turned off. Light conditions were measured with a photometer at the front and back of all four experimental cages at different times of a sample day. The cages closer to the window usually have higher lux-values than the cages away from the window. Additionally, the angle of the incoming light has an effect on light conditions at different locations in the cage.

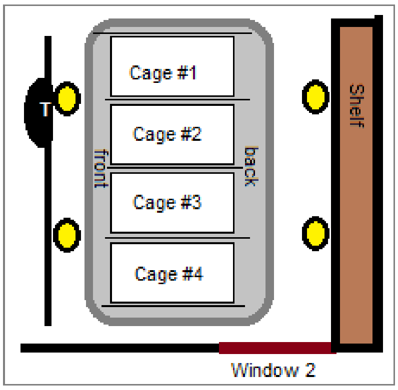

Figure 10: **Four differently powered LED lamps (marked yellow) as tools for adjustment of the ambient light conditions.** The LEDs were mounted above the cages in the back and in the front.

2.4. Data analysis

Originally the planned sample size was 24 birds but due to several time-consuming issues that have been faced during the experiments (see chapter 3.2.) the sample size was reduced to only 8 individuals. The small sample size plus the occasional lack of motivation of these test birds affected the amount of pertinent data collected. The $\geq$4/5-correct-choice-criterion with a p- value of $p<0.05$ was not always attainable.

Two methods of data analysis were applied to analyze the data set towards the flicker fusion frequencies (FFF) of the six light intensities tested and the CFF at any light intensity. The first method used only the data that fulfilled the $\geq$4/5-correct-choices-criterion (or 4/4 or 8/10 or 8/8) with a p-value of $p<0.05$. The second method also took results with a p-value of $p>0.05$ into account.

The p-value is a measure of statistical evidence. It is a number between 0 and 1. It describes the level of significance or likelihood of error of a scientific result and reflects the strength of evidence against the null hypothesis.
A small p-value ($p \leq 0.05$) indicates a 'significant' result, a strong evidence against the null hypothesis. This means the null hypothesis can be rejected and the alternative hypothesis can be accepted. A p-value of $p > 0.05$ indicates that the evidence against the null hypothesis is 'non-significant' and therefore not strong enough to reject the null hypothesis [Vickers, 2010].

Data analysis included only correct choices (cc) and incorrect/wrong choices (wc). No choices (nc) within a trial were not included in the data analysis.

3. Results

3.1. Results

Eight birds were trained during the experimental period and, of these, 7 individuals entered the testing stage. But not all of these birds delivered a FFF result for all six light intensities.

Due to the limited amount of sample size and test results two approaches for data analysis were applied. The first approach includes test results that fulfilled the ≥4/5-correct-choices-criterion only with a p-value of $p<0.05$. The second approach includes test results that fulfilled the ≥4/5-correct-choices-criterion with a p-value of $p<0.05$ as well as $p>0.05$.

Independent of the type of analysis approach applied the highest FFF at any light intensity found in one bird was 200 Hz at the lowest light intensity (80 cdm^{-2}). Three other birds had higher FFF at highest light intensities than at low or medium light intensities, i.e. up to 115 Hz at 1500 and 3000 cdm^{-2} (for more detail see Table 2 & 3 and Appendix – Table A7 & A8).

<u>Analysis approach #1:</u>

Only includes test results that fulfilled the ≥4/5-correct-choices-criterion (or 4/4 or 8/10 or 8/8) with a p-value of $p<0.05$

Applying this approach three birds (CB01, CB03, CB04) reached a FFF for only one light intensity, one bird (CB02) for three light intensities, two birds (CB06, CB07) for four light intensities and one bird (CB05) reached a FFF for all six light intensities as indicated in Table 2 (for more detailed information see Appendix – Table A7).

One bird (CB02) reached its highest FFF of 200 Hz (= CFF) at 80 cdm^{-2}, the lowest light intensity. Another time the same bird was only capable of mastering a FFF = 90 Hz at 80 cdm^{-2} but on the other hand got a second highest FFF of 115 Hz at 1500 cdm^{-2}.

Two other birds showed their best visual performance at highest light intensity of 3000 cdm^{-2} (CB05: CFF = 115 Hz, CB06: CFF = 105 Hz). Another bird (CB07) did not get to a FFF at 3000 cdm^{-2} but demonstrated its highest FFF of 90 Hz at 750 and 1500 cdm^{-2}.

On average the CFF of 103 Hz ±13.76 Hz (±SD) was reached at 3000 cdm^{-2} (n=4) and the lowest visual performance was demonstrated at low light intensities as indicated in Figure 11.

Table 2: Results of FFF experiments for GCM at six different light stimuli intensities – Approach #1 ($p<0.05$). Flicker fusion frequency [Hz] test results of seven individuals are shown. Individuals (CB01 – CB07) were tested at six given light intensities: 80 cdm^{-2}, 160 cdm^{-2}, 350 cdm^{-2}, 750 cdm^{-2}, 1500 cdm^{-2} and 3000 cdm^{-2}. Only test results that fulfilled the ≥4/5-correct-choices-criterion (or 4/4 or 8/10 or 8/8) with a p-value of $p<0.05$ are included. A birds' best visual performance at any light intensity tested (=CFF) is highlighted in bold.

Light intensity/ luminance target value [cdm^{-2}]	**80 cdm^{-2}**	**160 cdm^{-2}**	**350 cdm^{-2}**	**750 cdm^{-2}**	**1500 cdm^{-2}**	**3000 cdm^{-2}**
Bird ID	**Flicker fusion frequency (FFF) [Hz]**					
CB012015	-	80	-	-	-	-
CB022015	**200**	-	-	-	115	112
CB032015	-	50	-	-	-	-
CB042015	-	-	-	-	-	80
CB052015	67	87	89	90	91	**115**
CB062015	-	92	92	-	88	**105**
CB072015	40	-	76	**90**	**90**	-

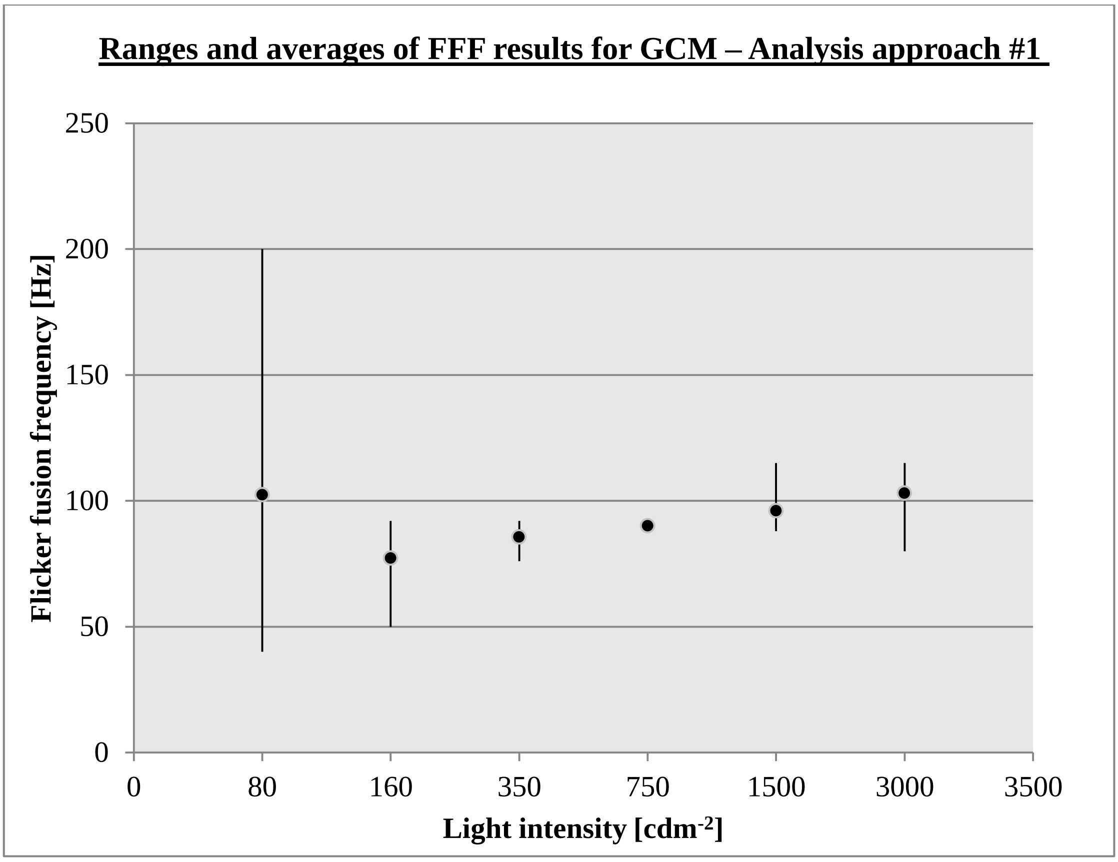

Figure 11: **Ranges and averages of FFF results for GCM at six different light stimuli intensities – Analysis approach #1.** Ranges and averages (filled circles) are shown. Only test results that fulfilled the ≥4/5-correct-choices-criterion (or 4/4 or 8/10 or 8/8) with a p-value of p<0.05 are included. Not all of the seven test individuals delivered a FFF result for each of the six tested light intensities: 80 cdm^{-2} (n_{80} = 3), 160 cdm^{-2} (n_{160} = 3), 350 cdm^{-2} (n_{350} = 3), 750 cdm^{-2} (n_{750} = 2), 1500 cdm^{-2} (n_{1500} = 4) and 3000 cdm^{-2} (n_{3000} = 4). On average the critical flicker fusion frequency CFF = 103 Hz ±13.76 Hz (±SD) was reached at 3000 cdm^{-2} while one individual demonstrated a CFF = 200 Hz at 80 cdm^{-2} (102.33 Hz on average ± 69.93 Hz (±SD)).

Analysis approach #2

This approach also includes test results that fulfilled the ≥4/5-correct-choices-criterion (or 4/4 or 8/10 or 8/8) with a p-value of p<0.05 as well as p>0.

Applying this approach two birds (CB03, CB04) reached a FFF for only one light intensity, one bird (CB01) for three light intensities, two birds (CB06, CB07) for five light intensities and two birds (CB02, CB05) reached a FFF for all six light intensities as indicated in Table 3 (for more detailed information see Appendix – Table A8).

The highest FFF (=CFF) found in one bird (CB02) was 200 Hz at 80 cdm^{-2}. Another time the same bird was only capable of reaching a FFF = 95 Hz at 80 cdm^{-2} ($p>0.05$) but on the other hand mastered a FFF of 115 Hz at 1500 cdm^{-2}.

Two other birds showed their best visual performance at 3000 cdm^{-2} (CB05: CFF = 115 Hz, CB06: CFF = 105 Hz). Two further birds did not reach a FFF for 3000 cdm^{-2} but demonstrated a highest FFF of 115 Hz at 1500 cdm^{-2} (CB01) and FFF = 96 Hz at 750 cdm^{-2} (CB07).

Within this approach the average CFF = 104.8 Hz ±10.77 Hz (±SD) was reached at 1500 cdm^{-2} (n=5) and the lowest visual performance was demonstrated on average at medium light intensities as indicated in Figure 11.

Table 3: Results of FFF experiments for GCM at six different light stimuli intensities – Approach #2 ($p< 0.05$ and $p> 0.05$). Flicker fusion frequency [Hz] test results of seven individuals are shown. Individuals (CB01 – CB07) were tested at six given light intensities: 80 cdm^{-2}, 160 cdm^{-2}, 350 cdm^{-2}, 750 cdm^{-2}, 1500 cdm^{-2} and 3000 cdm^{-2}. Test results that fulfilled the ≥4/5-correct-choices-criterion (or 4/4 or 8/10 or 8/8) with a p-value of $p<0.05$ *as well as p>0.05* are included. A birds' best visual performance at any light intensity tested (=CFF) is highlighted in bold.

Light intensity/ luminance target value [cdm^{-2}]	**80 cdm^{-2}**	**160 cdm^{-2}**	**350 cdm^{-2}**	**750 cdm^{-2}**	**1500 cdm^{-2}**	**3000 cdm^{-2}**
Bird ID	**Flicker fusion frequency (FFF) [Hz]**					
CB012015	-	80	-	80	**115**	-
CB022015	**200**	100	80	110	115	112
CB032015	-	95	-	-	-	-
CB042015	-	-	-	-	-	80
CB052015	70	87	89	90	110	**115**
CB062015	74	92	92	-	89	**105**
CB072015	50	80	76	**96**	95	-

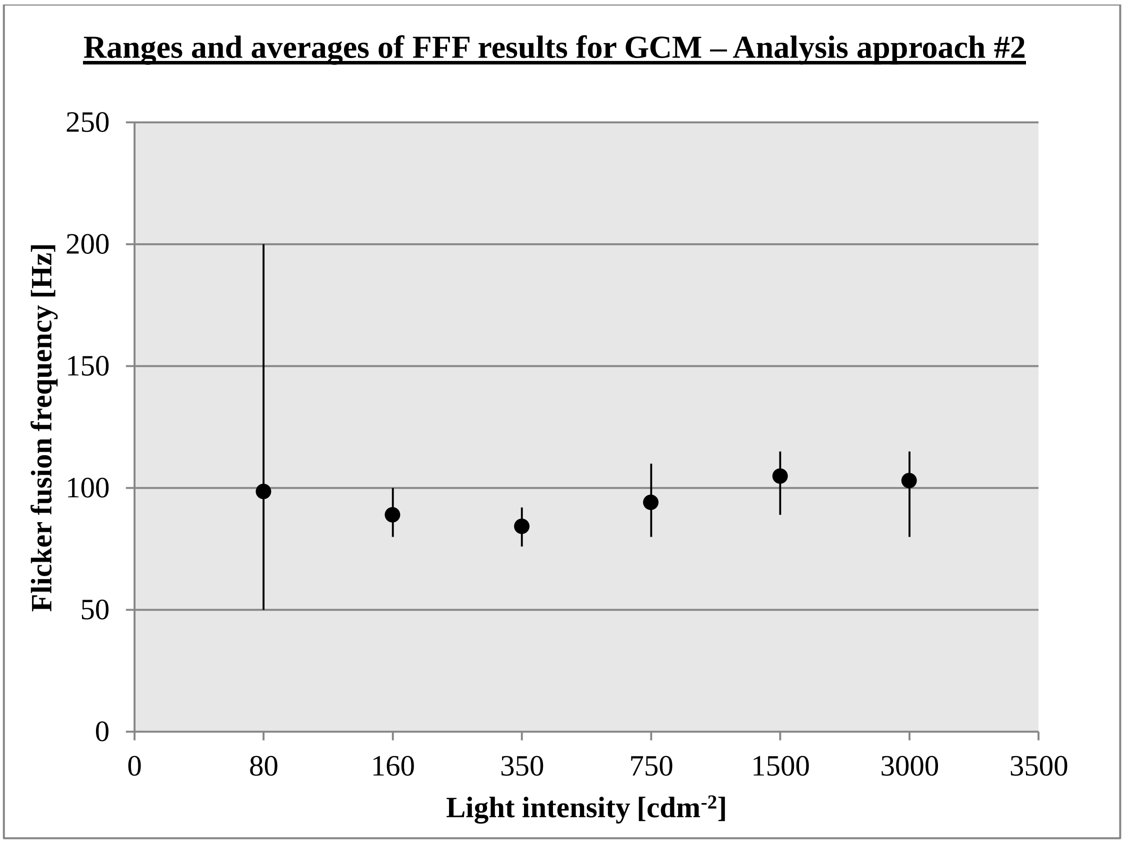

Figure 12: Ranges and averages of FFF results for GCM at six different light stimuli intensities – Analysis approach #2. Ranges and averages (filled circles) are shown. Included test results fulfilled the ≥4/5-correct-choices-criterion (or 4/4 or 8/10 or 8/8) with a p-value of <u>p<0.05 *as well as p>0.05*</u>. Not all of the seven test individuals delivered a FFF result for each of the six tested light intensities: 80 (n_{80} = 4), 160 (n_{160} = 6), 350 (n_{350} = 4), 750 (n_{750} = 4), 1500 (n_{1500} = 5) and 3000 cdm^{-2} (n_{3000} = 4). On average the critical flicker fusion frequency of CFF = 104.8 Hz ±10.77 Hz (±SD) was reached at 1500 cdm^{-2} while one individual demonstrated a CFF = 200 Hz at 80 cdm^{-2} (98.50 Hz on average ± 59.30 Hz (±SD)).

3.2. Challenges

Although seven birds entered the testing stage, the FFF for all six light intensities for every sample bird was not reached. During the experimental period concerns included a limited motivation or cooperation of the sample birds as well as malfunctioning of electrical instruments due to high humidity. The birds were kept in captivity for up to 3 months. The quality and quantity of the birds' involvement in the experiments was increasingly decreased.

Due to not air-tight windows in the research facility high temperatures and changing humidity with values up to 95% had a negative impact on the performance of electrical equipment (LEDs, photometer and generators).

These circumstances made it difficult to fulfill the set criterions (i.e. 4 out of 5 (or 4/4 or 8/10 or 8/8) correct choices in a session at a given FF and light intensity as well as 8 out of 10 (or $\geq$ 8/10) correct choices in a final test session to confirm the FFF at a given light intensity).

4. Discussion

Studies of flicker fusion frequency in vertebrates, including birds, have been previously published. It has been shown that slow moving animals tend to have critical flicker fusion frequency (CFF) below 100 Hz (e.g. chickens (*Gallus gallus domesticus*): average CFF = 87.0 Hz at 1375 cdm^{-2} [Lisney et *al*., 2011]) while fast moving and flying animals tend to have CFFs well above 100 Hz [Maddocks et *al*., 2001] (e.g. doves (*Columba livia*): average CFF = 100 Hz at high light intensity level [Healy et al., 2013]). A recent publication of blue tits and flycatchers revealed a CFF at light intensity 1500 cdm^{-2} of 131 Hz for Blue tits, 141 Hz for Collared flycatchers and 146 Hz for Pied flycatchers. These values are well above the visual abilities of humans (50 – 100 Hz depending on stimulus size) [Boström et *al*., 2016].

It is postulated that GCMs have rapid visual perception with an average CFF well above ordinary (>100 Hz). During courtship displays, the male birds perform complex high-speed displays and the female must be able to detect speed differences in a range of tens of milliseconds to allow preferential selection of males by the means of their motor skills [J.Barske et *al*., 2011 & 2015, Fusani & Schlinger, 2012]. The bird's best visual performance (CFF) is likely to be at the medium to high intensities found in the average light conditions of their natural habitat (see Appendix – Chart A 1a-d).

It has been determined that temporal perception of information is linked to the metabolic rate and body size in vertebrates suggesting that species with smaller size and higher metabolic rates manifest a more rapid visual perception (higher CFF) and behavioral response [Healy et *al.*, 2013]. The perception of visual flicker of small passerines is predicted to be up to 100 Hz [Healy et *al.*, 2013].

Despite the high-power-demanding male courtship displays, GC Manakins maintain a low daily energy expenditure (DEE) ('slow pace of life' classification) typical for tropical birds. On average, males use 1.2% of their DEE on courtship dances [Barske et *al.*, 2013]. It was postulated that the CFF for these bird was expected to be close to that of similar small fast-flying passerines such as flycatchers, but at a minimum well above average (>100 Hz).

Independent of the type of analysis approach applied, our flicker frequency experiments indicate that the GC Manakin (*Manacus vitellinus)* on average has its best visual performance (CFF) at high light intensities of 1500 and 3000 cdm^{-2}. Hence the data supports our first hypothesis.
Although one bird (CB02) mastered an outstandingly high FFF at the lowest light intensity, its validity may be questioned. The bird might have learned another cue. Another time the same bird was only capable of reaching a FFF well below its oddly high result for this light intensity. It demonstrated its second best visual performance at highest light intensities which is in accord to the CFF results of other birds and the average CFF.

Nevertheless it would appear that GCM, despite their fast courtship displays, do not have above-average rapid visual perception like found in flycatchers. According to our results they demonstrate a visual processing speed similar to doves, or only insignificantly above, only disproving our second hypothesis. When taking into account that GCM maintain a low DEE ('slow pace of life') it appears that the prediction of Healy et *al*. [2013] for small passerine birds, i.e. visual flicker of up to 100 Hz, is true.

Despite our data, the GCM may have visual abilities greater than our test results suggest. Although the high test scores of bird CB02 might indicate that this bird learned from another cue, it also may suggest that it is indeed capable of a higher visual performance.

Further flicker frequency studies in GC Manakins are required using a greater sample size (n>20). Such studies would confirm and validate the FFF results for all six light intensities. Correspondingly, improvements of the experimental methodology must be addressed including a revised reward system, a shorter captivity period as well as precautions for electrical instruments to avoid damage due to humidity.

The reward system used in this study originally has been developed for seed eating birds. To tailor the reward system to GCM in order to increase the quality of their involvement and shorten their period of captivity we need to include aspects of the behavioural ecology of this bird species.

The natural diet of GCM consists of small fruits and insects, plucked or snatched in short aerial sallies [Snow, 2017]. Thus, feeding pieces of papaya on a daily basis over several months, as done in our study, is a variable that could be better controlled. A regular varying diet, at least outside of experimental periods, might be advantageous in terms of their motivation and cooperation.

Additionally, the birds were captured during their mating season and housed individually. While the sample birds often showed a lack of motivation for participation in the experiments two male individuals occasionally engaged in display dances in their cages within and outside of experimental periods. Taking these observations into account, a possible improvement might be a reward system based on social contacts. In particular, one could avoid visual contact of the birds outside of experimental periods and use visual contact and social interactions as a reward for correct choices during experiments.

Atop these possibilities of improvement one main issue remains: the quantity of involvement of this small tropical bird species. Outside of courtship display periods, the Golden-collared Manakins generally maintain a low activity level demonstrated by their short feeding and attention periods during experiments. This issue must be addressed in future experiments by adapting the experimental setup accordingly.

5. Summary

This master thesis addressed the visual abilities of Golden-collared Manakins, a tropical small passerine bird species performing complex, high-speed courtship displays suggesting fast visual processing. The experiments were based on a similar FFF study conducted with chickens (*Gallus gallus domesticus*) [Lisney et al., 2011] using an operant conditioning approach.

It was expected that the Golden-collared Mankins would demonstrate optimal visual performance (CFF) at medium to high light intensities in keeping with their natural habitat light conditions and that their CFF should be, at a minimum, above average (well above 100 Hz) and close to the rapid vision of other fast flying passerine bird species such as flycatchers [Boström et *al*., 2016].

The results of this flicker fusion frequency study do indicate that the Golden-collared Manakin (*Manacus vitellinus)* has best visual performance (CFF) at high light intensities.

Nevertheless it appears that GC Manakins do not have above-average rapid visual perception like flycatchers have. The results rather demonstrate a visual processing speed similar to slower moving animals such as doves (up to 100 Hz) disproving the second hypothesis. When taking into account that GCM maintain a low DEE ('slow pace of life') it appears that the prediction of Healy et *al*. [2013] for small passerine birds, i.e. visual flicker of up to 100 Hz, is true.

Despite our data, the Manakin may have visual abilities greater than our test results suggest. Indications for higher visual performance might be the high test scores of one bird (FF = 200 Hz at 80 cdm^{-2} and 140 Hz at 160 cdm^{-2}).

To further investigate in this research question more flicker frequency studies in Golden-collared Manakins need to be done. Though improvements of the experimental methodology must be addressed including a revised reward system, a shorter captivity period as well as precautions for electrical instruments to avoid damage due to humidity. Furthermore a higher sample size (n>20) is required.

6. References

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7. Appendix

Table A 1a-d: Examples for light conditions at lekking sites: The light measurements demonstrate the natural light conditions at four different sample lekking sites named “TRUNK2015-01 “, “TRUNK2015-02 “, “ACAD2015-01” and “ACAD2015-2”. Measurements were taken during morning and afternoon hours within the courtship court in the forest. The illuminance [lux] was measured at a height of 20 cm above ground with the photometer pointing a) horizontally towards the sun and b) horizontally away from the sun, as well as at a height of 40 cm above ground with the photometer pointing c) vertically towards the sun and d) vertically away from the sun, towards the ground. Additionally, the luminance [cdm^{-2}] was measured using a white polystyrol plate (equivalent to the white floor of an experimental cage) positioned on the court ground as a white reference. Weather conditions varied from cloudy, partly cloudy to sunny as indicated below.

Table A 1a: Example for light conditions at lekking site “TRUNK2015-01”.

Site: **TRUNK2015-01,** Time: 07h10 a.m.							
position of photometer	horizont. – towards sun [lux]	horizont. – away from sun [lux]	vertical – towards sun [lux]	vertical - towards ground [lux]	polystyrol, horizont. – towards sun **[cdm^{-2}]**	polystyrol, horizont. – away from sun **[cdm^{-2}]**	polystyrol, vertical – towards ground **[cdm^{-2}]**
weather conditions							
cloudy	270	74	1600	9	101	189	481

Table A 1b: Example for light conditions at lekking site “TRUNK2015-02”.

Site: **TRUNK2015-02,** Time: 07h45 a.m.							
position of photometer	horizont. – towards sun [lux]	horizont. – away from sun [lux]	vertical – towards sun [lux]	vertical - towards ground [lux]	polystyrol, horizont. – towards sun **[cdm^{-2}]**	polystyrol, horizont. – away from sun **[cdm^{-2}]**	polystyrol, vertical – towards ground **[cdm^{-2}]**
weather conditions							
partly cloudy	542	1210*	1980	128	191	359*	455
cloudy	101	162	265	33			

*directed towards open area (trail)

Table A 1c: Example for light conditions at lekking site "ACAD2015-01".

Site: **ACAD2015-01,** Time: 14h00 p.m.							
position of photometer	horizont. – towards sun [lux]	horizont. – away from sun [lux]	vertical – towards sun [lux]	vertical - towards ground [lux]	polystyrol, horizont. – towards sun **[cdm^{-2}]**	polystyrol, horizont. – away from sun **[cdm^{-2}]**	polystyrol, vertical – towards ground **[cdm^{-2}]**
weather conditions							
cloudy	520	211			219		
partly cloudy	711	271	1600	114		68	
sunny					309	143	1854

Table A 1d: Example for light conditions at lekking site "ACAD2015-02".

Site: **ACAD2015-02,** Time: 14h35 p.m.							
position of photometer	horizont. – towards sun [lux]	horizont. – away from sun [lux]	vertical – towards sun [lux]	vertical - towards ground [lux]	polystyrol, horizont. – towards sun **[cdm^{-2}]**	polystyrol, horizont. – away from sun **[cdm^{-2}]**	polystyrol, vertical – towards ground **[cdm^{-2}]**
weather conditions							
sunny	5730	830	4630	570	1130	260	2900

Table A 2: Sample birds – additional information. Eight sample birds (male (m) and female (f)) were used for this study. They were caught at selected locations within a distance of about 30 km around Gamboa, Panama.

	Sex (m/f)	**Age** (adult/ juvenile/ unknown)	**Date of catch** (dd/mm/ yy)	**Location of catch**	**Date of release** (dd/mm/ yy)	**Reward (**constant/ **f**licker**)**
Bird ID						
CB012015	m	adult	05/03/ 15	Academy lek #1	07/05/ 15	c
CB022015	m	juvenile	05/03/ 15	Academy lek #1	11/05/ 15	c
CB032015	m	adult	10/03/ 15	Academy lek #2	15/04/ 15	f
CB042015	un-known	unknown (green bird)	10/03/ 15	Academy lek #2	30/03/ 15	f
CB052015	f	unknown	14/03/ 15	Trunk lek #1	28/05/ 15	f
CB062015	f	unknown	30/03/ 15	Summit lek #1	28/05/ 15	f
CB072015	f	unknown	04/04/ 15	Summit lek #2	28/05/ 15	c
CB082015	f	unknown	16/04/15	Chunga lek #1	27/05/15	c

Table A 3: Stimulus lights used in the experiment. There were 8 pairs of stimulus lights used during the experiments with 6 different light intensities (with luminance target value (L) in [cdm^{-2}]).

Lamp #	Lamp ID	Luminance target value [cdm^{-2}]	Remark
#01	L1 80A	80	
#02	L2 80A	80	
#03	L1 1500A	1500	replacement (May)
#04	L2 1500A	1500	replacement (May)
#05	L1 160A	160	
#06	L2 160A	160	
#07	L1 350B	350	malfunctioning
#08	L2 350B	350	malfunctioning
#09	L1 750A	750	malfunctioning & replacement (May)
#10	L2 750A	750	malfunctioning & replacement (May)
#11	L1 3000A	3000	
#12	L2 3000B	3000	
#13	L1 750B	750	malfunctioning
#14	L2 750B	750	malfunctioning
#15	L1 1500B	1500	malfunctioning
#16	L2 1500B	1500	malfunctioning

Table A 4: Adjustments of lamp ND filters in available lamps to obtain a different luminance target value. Due to malfunction of test lamps other functioning test-lamps were used for testing of next higher or lower light intensities. Therefor the functioning lamps were modified by adding or removing neutral density filters (0.3 and 0.6 ND) inside it.

Lamp ID	Original luminance target value [cdm^{-2}]	Adjustment	Adjusted luminance target value [cdm^{-2}]
L1 40A	40	removal of 1x 0.6 ND filter	80
L2 40A	40	removal of 1x 0.6 ND filter	80
L1 160A	160	addition of 1x 0.3 ND filter	80
L2 160A	160	addition of 1x 0.3 ND filter	80
L1 750A	750	addition of 1x 0.3 ND filter	350
L2 750A	750	addition of 1x 0.3 ND filter	350
L1 3000A	3000	addition of 1x 0.3 ND filter	1500
L2 3000A	3000	addition of 1x 0.3 ND filter	1500

Table A 5: LED lamp models used for adjustment of ambient light conditions.

Phillips – Mini Twister	8 W CDL
Phillips – Mini Twister	12 W CDL
Phillips – Mini Twister	23 W CDL

Table A 6: Combinations of differently powered LED lamps (8 W, 12 W and 23 W) and number (0-3) of ND filter sheets (0.3 ND or 0.6 ND) applied for every light intensity tested to obtain the illumination target value [lux] of the stimulus lights.

Tested light intensity/ Luminance [cdm^{-2}]	Illumination target value [lux]	Combination of differently powered LEDs (8, 12 and 23 Watts) [W]	Amount and type of applied ND filter sheets [0-3 filter sheets; 0.3 ND or 0.6 ND]
80	8	4x 8 W	1x 0.3 + 2x 0.6 (= 3x 0.3 + 1x 0.6) or 2x 0.3 + 2x 0.6 (= 3x 0.6) or 1x 0.3 + 3x 0.6 (= 3x 0.3 + 2x 0.6) or 2x 0.3 + 3x 0.6 or 3x 0.3 + 3x 0.6
		2x 8 W + 2x 12 W	3x 0.3 + 3x 0.6
160	16	4x 8W	3x 0.3 (= 1x 0.3 + 1x 0.6) or 2x 0.3 + 1x 0.6 (= 2x 0.6) or 3x 0.3 + 1x 0.6 (= 1x 0.3 + 2x 0.6) or 2x 0.3 + 2x 0.6 (= 3x 0.6) or 3x 0.3 + 2x 0.6 (= 1x 0.3, 3x 0.6) or 2x 0.3 + 3x 0.6 or 3x 0.3 + 3x 0.6
		2x 8 W + 2x 12 W	3x 0.6 or 3x 0.3 + 3x 0.6
350	35	4x 8 W	1x 0.3 or 2x 0.3 (= 1x 0.6) or 3x 0.3 (= 1x 0.3 + 1x 0.6) or 2x 0.6
			2x 0.3 (= 1x 0.6)

350	35	2x 8 W + 2x 12 W	or 3x 0.3 (= 1x 0.3 + 1x 0.6) or 2x 0.3 + 1x 0.6 (= 2x 0.6)
		2x 12 W + 2x 23 W	2x 0.3 (= 1x 0.6)
750	75	4x 8 W	none or 1x 0.3
		2x 8 W + 2x 12 W	1x 0.3
		2x 8 W+ 2x 23 W	1x 0.3
		2x 12 W + 2x 23 W	none
		4x 23 W	1x 0.3 or 2x 0.3 (= 1x 0.6) or 3x 0.3 (= 1x 0.3 + 1x 0.6)
1500	150	2x 12 W + 2x 23 W	none
		4x 23 W	none or 1x 0.3
3000	300	4x 23 W	none

Table A7: **Results of FFF experiments for GCM at six different light stimuli intensities – Approach #1 (p<0.05).** Flicker fusion frequency [Hz] test results of seven individuals are shown. Individuals were tested at six given light intensities: 80 cdm^{-2}, 160 cdm^{-2}, 350 cdm^{-2}, 750 cdm^{-2}, 1500 cdm^{-2} and 3000 cdm^{-2}. Only test results that fulfilled the ≥4/5-correct-choices-criterion (or 4/4 or 8/10 or 8/8) with a p-value of **p<0.05** are included. One additional result (put in brackets/ marked blue) is included although the test bird did not meet the ≥4/5-correct-choices-criterion but demonstrated 3x 2/2 correct choices with a p-value of **p<0.05**. A birds' best visual performance at any light intensity tested (=CFF) is highlighted in bold.

Bird ID/ individual tested	**Light intensity/ luminance target value [cdm^{-2}]**	**Flicker fusion frequency (FFF) [Hz]**	**Criterion ≥4/5 (or 4/4 or 8/10 or 8/8) correct choices**	**Comment**
CB012015	160	80	5/5	
CB022015	80	90	8/10	
		200	5/5	
	(160)	**(140)**	(2/2 + 2/2 + 2/2)	
	1500	115	5/5	
	3000	112	8/10	

CB032015	160	50	4/5 + 4/5	
CB042015	3000	80	5/5	
CB052015	80	67	4/5 + 4/5	
	160	87	4/5 + 4/5	
	350	89	5/5	
	750	90	4/5 + 4/5	
	1500	91	4/5 + 4/5	Plus another day: 2/3 + 4/5 cc
	3000	**115**	4/5 + 4/5	
CB062015	160	92	5/5	
	350	92	4/5 + 4/5	
	1500	88	4/5 + 4/5	
	3000	**105**	4/5 + 4/5	
CB072015	80	40	4/5 + 4/5	
	350	76	4/5 + 4/5	
	750	**90**	5/5	
	1500	**90**	4/5 + 4/5	

Table A8: **Results of FFF experiments for GCM at six different light stimuli intensities – Approach #2 (p<0.05 *as well as* p>0.05).** Flicker fusion frequency [Hz] test results of seven individuals are shown. Individuals were tested at six given light intensities: 80 cdm^{-2}, 160 cdm^{-2}, 350 cdm^{-2}, 750 cdm^{-2}, 1500 cdm^{-2} and 3000 cdm^{-2}. Test results that fulfilled the ≥4/5-correct-choices-criterion (or 4/4 or 8/10 or 8/8) with a p-value of **p<0.05 *as well as* p>0.05**. are included. One additional result (put in brackets/ marked blue) is included although the test bird did not meet the ≥4/5-correct-choices-criterion but demonstrated 3x 2/2 correct choices with a p-value of **p<0.05**. A birds' best visual performance at any light intensity tested (=CFF) is highlighted in bold.

Bird ID	Light intensity/ luminance target value [cdm^{-2}]	Flicker fusion frequency (FFF) [Hz]	Criterion ≥4/5 (or 4/4 or 8/10 or 8/8) correct choices	Comment
CB012015	160	80	5/5	
	750	80	4/5	
	1500	**115**	4/5	
CB022015	80	95 **200**	4/5 5/5	
	160	100 **(140)**	4/5 (2/2 + 2/2 + 2/2)	
	350	80	4/5	
	750	110	4/5	
	1500	115	5/5	
	3000	112	8/10	
CB032015	160	95	4/5	
CB042015	3000	80	5/5	

CB052015	80	70	4/5	
	160	87	4/5 + 4/5	
	350	89	5/5	
	750	90	4/5 + 4/5	
	1500	110	4/5	
	3000	**115**	4/5 + 4/5	
CB062015	80	74	4/5	
	160	92	5/5	
	350	92	4/5 + 4/5	
	1500	89	4/5	
	3000	**105**	4/5 + 4/5	
CB072015	80	50	4/5	
	160	80	4/5	
	350	76	4/5 + 4/5	
	750	**96**	4/5	
	1500	95	4/5	

Abstract

The aim of this study is to investigate the visual capabilities of Golden-collared Manakins (GCM, *Manacus vitellinus*), a passerine bird species of the Pipridae family. For flying birds a superior vision is the most important sense for safe flight but also relevant regarding attraction of mating partners and sexual selection. Male GCM demonstrate a very complex, high-power demanding and competitive courtship display to attract females. Females appear to favour male motor skills, i.e. high speed, accuracy and frequency of specific display movements. Speed differences in a range of tens of milliseconds can be detected by females.This suggests excellent visual acuity with high temporal resolution capabilities and particularly rapid visual processing speeds for GCM leading to the primary focus of this study. Using a food reward system, visual abilities and visual processing speeds of birds and other vertebrates have previously been studied by determining the flicker fusion frequency (FFF), i.e. the eye can no longer distinguish a flickering light from a continuous light stimulus of equal intensity at different luminances. Based on these FFF studies previously conducted, i.a. with chickens, we trained eight birds to discriminate a constant light stimulus from a flickering one, at six different light intensities between 80 and 3000 cdm^{-2}.
It is expected that 1) GCM demonstrate optimal visual performance at medium to high light intensities in keeping with their natural habitat light conditions and 2) their highest FFF at any light intensity tested (= CFF = Critical flicker fusion frequency) should be well above ordinary (> 100 Hz) and close to the rapid vision of fast flying passerine bird species such as flycatchers (up to 146 Hz). The highest FFF at any light intensity (CFF) found in one bird at 80 cdm^{-2} (lowest light intensity) was 200 Hz. Three other birds had higher FFF (up to 115 Hz) at highest light intensities of 1500 and 3000 cdm^{-2} than at low or medium light intensities. These results do indicate that the GCM has best visual performance at high light intensities. Nevertheless it appears that GCM do not have above-average rapid visual perception like flycatchers have. Still, there are indications that Manakins may have visual abilities greater than our first test results suggest but this remains to be retested.

Abstrakt

Das Ziel dieser Studie ist es, die visuellen Fähigkeiten von Golden-Collared Manakins (GCM, *Manacus vitellinus*) zu untersuchen, einer Sperlingsvogel-Art aus der Familie der Pipridae. Für fliegende Vögel ist ein ausgezeichnetes Sehvermögen der bedeutenste Sinn für den sicheren Flug in der Luft. Jedoch ist dieser auch relevant hinsichtlich dem Anlocken von Geschlechtspartnern und der sexueller Selektion. Männliche GCM demonstrieren zum Anlocken von Weibchen ein sehr komplexes, energetisch höchst anspruchsvolles und konkurrenzbetontes Balzen. Die Weibchen scheinen dabei jene Männchen zu bevorzugen, die herausragende motorische Fähigkeiten zeigen, d.h. spezifische Balzbewegungen mit besonders hoher Geschwindigkeit, Präzision und Frequenz vollführen. Dabei können die Weibchen Geschwindigkeitsunterschiede im Bereich von Zehntel-Millisekunden wahrnehmen. Dies suggeriert, dass GCM eine exzellente Sehschärfe besitzen mit hohem zeitlichem Auflösungsvermögen und besonders schnellen visuellen Verarbeitungsgeschwindigkeiten, was zum primären Fokus unserer Studie führt. Unter Verwendung eines Futter-Belohnungssystems wurden in vorangegangen Studien die visuellen Fähigkeiten und visuellen Verarbeitungsgeschwindigkeiten von Vögeln und anderen Vertebraten mittels Bestimmung der Flimmerverschmelzungsfrequenz (FFF) untersucht. Unter der FFF bei verschiedenen Helligkeiten versteht man jene Flimmerfrequenz, bei welcher das Auge einen flimmernden Lichtstimulus nicht mehr von einem konstanten Lichtstimulus von gleicher Lichtintensität unterscheiden kann. Basierend auf jenen, bisher z.B. mit Hühnern durchgeführten FFF-Studien haben wir 8 Vögel darauf trainiert, bei sechs verschiedenen applizierten Lichtintensitäten zwischen 80 und 3000 cdm^{-2}, einen flimmernden Lichtstimulus von einem konstanten zu unterscheiden.

Es wurde erwartet, dass 1) GCM eine optimale visuelle Leistungsfähigkeit bei mittleren bis hohen Lichtintensitäten demonstrieren, was sich mit den Lichtverhältnissen in ihrem natürlichen Habitat deckt, und 2) ihre höchste FFF bei jeglichen getesteten Lichtintensitäten (= CFF = kritische Flimmerverschmelzungsfrequenz) weit überdurchschnittlich (>100 Hz) liegt und der rapiden visuellen Wahrnehmung von schnell fliegenden Sperlingsvogel-Arten, wie den Fliegenschnäppern (bis zu 146 Hz), gleichkommt. Die höchste FFF bei jeglichen getesteten Lichtintensitäten (CFF) wurde bei einem Vogel bei 80 cdm^{-2} (niedrigste Lichtintensität) bei 200 Hz dokumentiert. Drei

weitere Vögel zeigten eine höhere FFF (bis zu 115 Hz) bei den höchsten Lichtintensitäten von 1500 und 3000 cdm^{-2} als wie bei niedrigen oder mittleren Lichtintensitäten. Diese Ergebnisse deuten darauf hin, dass GCM ihre beste visuelle Leistungsfähigkeit bei hohen Lichtintensitäten besitzen. Jedoch scheint es, dass GCM keine überdurchschnittlich schnelle visuelle Wahrnehmung wie die Fliegenschnäpper aufweisen. Dennoch gibt es Anzeichen dafür, dass Manakins möglicherweise höhere visuelle Fähigkeiten besitzen als unsere ersten Testergebnisse andeuten. Dies erfordert erneute Tests.