

Protocol

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Date of Visit: June 16, 2014-August 22, 2014

Title: 24-hour Adult Fish Recording, developed for *Astyanax mexicanus*

Rational: In order to quantify sleep behavior in adult fish, a high throughput system is needed so that optimal and standard conditions can be achieved. This protocol allows for 30 adult fish to be recorded over a 24 hour period of time.

Recording Stage Setup:

- I. Six of 8.7 L rectangular tanks with 4 opaque even partitions are placed on individual shelves in a 14 hour light, 10 hour dark room environment.
- II. Recording stages aligned vertically are illuminated with IR LED source (Infrared 850 nm 5050 LED Strip Light) from the backside. IR LED remains on during 24 hour recording.
- III. Two of USB webcams (LifeCam Studio 1080p HD Webcam, Microsoft) with fitted zoom lens (Zoom 7000, Navitar) and IR high-pass filters (Optical cast plastic IR long-pass filter) to block visible light are set 2.0 m away from the rectangular tanks. Videos were captured with a video capturing software, Virtualdub (V.1.10.4) with a mpeg2 codic.

Recording

- I. Fish were acclimated in recording tanks for 5 days prior to actual recording.
 - a. 5 individuals housed per tank
 - b. Light/Dark cycle is the same as normal conditions (14 hr Light, 10 hr Dark)
- II. On the day 5, recording begins one hour post zeitgeber time (ZT 1).
 - a. Virtualdub settings
 - i. Turn off audio recordings
 - ii. Set stop conditions to stop after 86,400 seconds (= 24 hrs).
 - iii. Adjust zoom/focus/iris to allow video to be recorded at 15 frames/sec.

Recording analysis:

- I. Video analysis using Ethovision XT 7.1 (Noldus, IT): will have to be done in two trials or based off the number of video files to be analyzed at once.
 - a. Arena Settings
 - i. Fifteen square arenas per video to be manually adjusted based on their shapes on videos
 - b. Tracking parameters for Detection
 - i. Detection: Dynamic differentiating
 - ii. Detection: brighter than background
 - iii. Brightness contrast: 20-255
 - iv. Frame weight: 15
 - v. Video Sample Rate: 15 frames/second
 - vi. No pixel smoothing.

Data Quantifying

1. Export data into text files to extract the data of the interest.
2. PERL script and EXCEL Macro used to quantify data developed by Dr. Yoshizawa