

### SOP: Induction of Stress via Randomized Stressors

Purpose: Induce stress in captive birds using unpredictable and randomized regimes of stressors.

Reference: Mike Romero's publications and personal communication.

Overview: There will be three different levels of stressors administered to birds; low, medium, high.

#### Protocol

**Low/Baseline Stressor regime** – This will be the daily required care for captive birds and, when scheduled, audio/video recording. These should be scheduled for a single session at exactly the same time each day and should be accomplished as quickly, quietly and efficiently as possible. Birds should be left alone in between these sessions. All care should be performed by the study personnel (not animal care staff).

**Medium and High Stressor regimes** – These regimes will include the daily required care for captive birds plus recording sessions, performed in the same manner and at the same time as with the low stressor regime. In addition, throughout the day, at any point from 30min prior to lights coming on to 2 hours after lights go off, one of the 7 stressors below should be deployed. The medium regime should have 3 stressors administered each day, while the high regime should have 6 stressors administered each day. The timing and particular stressor should be administered according to a predetermined, randomized schedule.

#### Stressors:

1. Human voice (reading aloud to the birds while in the room for 30 min, sit in one place, do not move around)
2. Radio (tuned to talk radio for 30 min)
3. Cage tapping (running something like a pencil across the bird cage every 1-2 min for 30 min, stay in place between tapping sessions)
4. Cage rolling (putting cages on a lab cart – or a cage rack with wheels – and gently rock back and forth, just enough to prevent comfortable perching, for 30 min. Note, remove waterers before doing this so you don't make a horrible mess, remember to replace when done)
5. Opening cage door and putting hand into the cage for 30 seconds every 2-6 min for 30 min total
6. Moving around the room randomly, approaching the cage and looming over the cage for 1 min periods over 30 min total
7. Tapes of predator sounds (hawk calls, cats meowing, etc.), for 30 min

Periodically through this study the stressor of 30min of handling for stress response blood collection will be added and can substitute for one of the other stressors on that day.

Regimes should be fully scheduled in advance and shared among study personnel and animal care staff. They should be posted outside holding rooms so that all know what needs to be done when.

Notes: Apply stressors as determined every day for required length of time for your study. Standard in Mike Romero's lab is 21 days of varying stressor applications. Many changes start to become evident after about 10 days, so this is considered the minimum number of days stressors must be applied (M. Romero, pers. comm.). Changes become more robust and statistically significant as stressors continue out to at least 21 days. (M. Romero, pers. comm.).