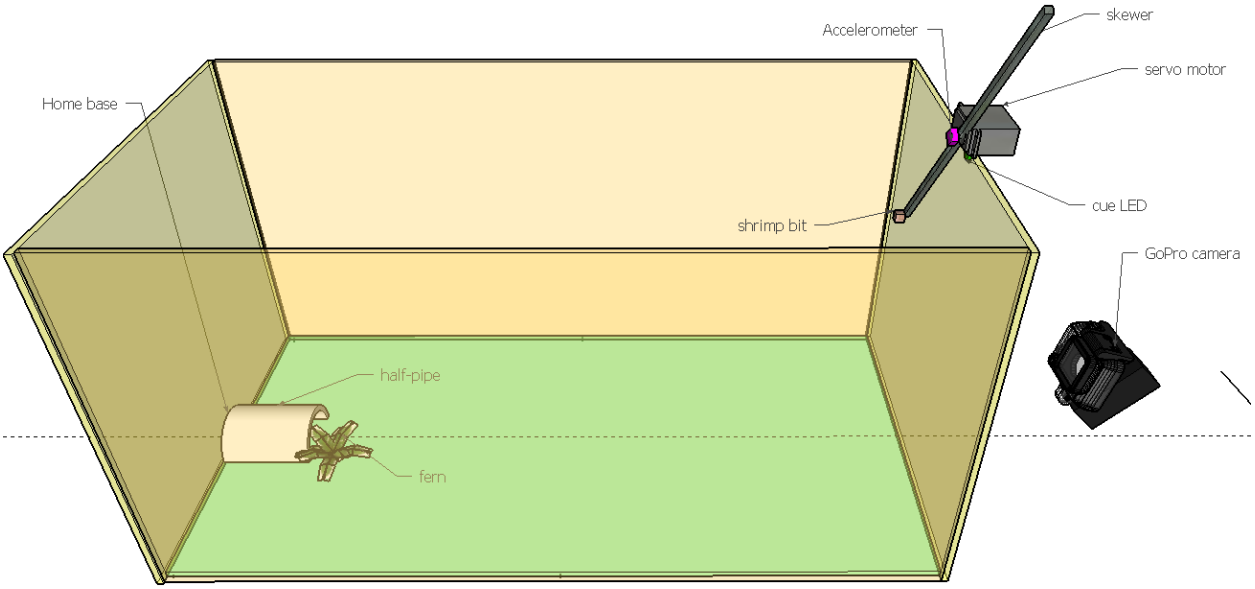
Intelligent Systems Lab: Experiment Plan

Title:

"Prediction of Prey Behavior in Cuttlefish"

Assay:



Behavior Training Sessions:

Foraging/Habituation:

Description: 30 min long, prey is stationary and has varying availability, cuttlefish become habituated to the experimental tank and to catching food from a motorized skewer.

Animals: naive cuttlefish on food deprivation (see detailed schedule below)

Assay: Cuttlefish enters experimental tank, triggers start of session by crossing the midline of the corridor to be in the half of the experimental tank that contains the “home base”. Skewer with food item (shrimp) starts out of the water. Green cue LED turns on, and 2 seconds later motorized skewer rotates into the water until pointed vertically down (perpendicular to floor), then “wiggles” in place by oscillating +/-10 deg then pausing. If cuttlefish does not catch food on the skewer, skewer rotates back to start position and waits for a random interval before cue LED turns on and skewer enters water again. This random interval is determined by a random exponential timer: the mean duration between food availability is set to a fixed 30 seconds to which a random waiting time with a mean duration of 30 seconds is added (so on average food will be available once per minute). If cuttlefish catches the food on the skewer, skewer is rotated back to start position and reloaded with another piece of shrimp. Catch event is notated by human observer via overhead camera view. Next trial starts when cuttlefish returns to “home base”. Cue LED stays on while food is available.

Progression:

- Level 1: experimental tank habituation (hand fed inside experimental tank)
- Level 2: prey available on motorized skewer for 10 seconds at a time

Patterned Movement:

Description: 30 min long, prey sweeps through water at varying speeds.

Animals: habituated via the Foraging session, food deprived

Assay: Cuttlefish enters experimental tank, triggers start of session by entering the "home corner". Skewer with food item (shrimp) starts out of the water. Green cue LED turns on, and 2 seconds later the prey begins to move in a "sweep wiggle" - skewer rotates at varying speeds into and out of the water while also wiggling in a fashion similar to the foraging/habituation behavior. If cuttlefish does not catch food on the skewer, skewer rotates back to start position and waits for a random interval before cue LED turns on and skewer enters water again, this time moving in the opposite direction. The random interval between food availability is determined by a random exponential timer: the mean of duration between availability intervals is set to a fixed 30 seconds, to which a random waiting time with a mean duration of 30 seconds is added (so on average food will be available once per minute). If cuttlefish catches the food on the skewer, skewer is rotated back to start position and reloaded with another piece of shrimp. Next trial starts when cuttlefish returns to "home base". Each trial alternates the side on which the skewer enters the water (left versus right).

Progression:

- Level 1: prey takes 12 seconds to sweep one cycle
- Level 2 (optional): prey takes 6 seconds to sweep one cycle

Causal Movement:

Description: 30 min long, prey movement is signaled by a behavioral code

Animals: completed Patterned Movement session, food deprived

Assay: Cuttlefish enters experimental tank, triggers start of session by entering the "home base". Skewer with food item (shrimp) starts pointing vertically up. Green cue LED turns on, and 2 seconds later the skewer begins to rotate into the water. Prey performs a "causal movement" followed by the corresponding "effect movement".

Causal movements and their corresponding effects:

- skewer enters water and rotates to point vertically down and pauses → skewer exits water back the way it came
- skewer enters water and does not pause → skewer continues in the same direction to exit water

If cuttlefish does not catch food on the skewer, skewer rotates back to start position and waits for a random interval before cue LED turns on and skewer enters water again. The random interval between food availability is determined by a random exponential timer: the mean of duration between availability intervals is set to a fixed 30 seconds, to which a random waiting time with a mean duration of 30 seconds is added (so on average food will be available once per minute). If cuttlefish catches the food on the skewer, skewer is rotated back to start position and reloaded with another piece of shrimp. Next trial starts when cuttlefish returns to "home base". Each trial alternates the side on which the skewer enters the water (left versus right).

Progression:

- Level 1: prey moves at 1 rotation per 5 seconds and pauses for 3 seconds
- Level 2 (optional): prey moves at 1 rotation per 5 seconds and pauses for 1 second

Reactive Movement:

Description: 30 min long, prey avoids cuttlefish

Animals: completed Causal Movement session, food deprived

Assay: Cuttlefish enters experimental tank, triggers start of session by entering the "home base". Skewer with food item (shrimp) starts pointing vertically up. Green cue LED turns on, and 2 seconds

later the motorized skewer begins to move the food into the water. Using visual feedback from the overhead camera, prey wiggles until approached by the cuttlefish, then moves to avoid being near cuttlefish. If cuttlefish does not attack food on the skewer, skewer stays in the water and wiggles. If cuttlefish catches the food on the skewer, skewer is rotated back to start position and reloaded with another piece of shrimp. Next trial starts when cuttlefish returns to "home base". Each trial alternates the side on which the skewer enters the water (left versus right).

Progression:

- Level 1: Prey moves at half the speed of approaching cuttlefish
- Level 2: Prey moves at same speed as approaching cuttlefish

Schedule: [Start Date – Saturday, September 6th, 2014]

Group A (older animals)	***Week 1: 9/6 – 9/12*** Sat: Food deprivation Sun: Food deprivation Mon: Foraging, L1 Tues: Foraging, L2 Wed: Foraging, L2 Thurs: Foraging, L2 Fri: Free food	***Week 2: 9/13 – 9/19*** Sat: Food deprivation Sun: Food deprivation Mon: Patterned Mvmt, L1 Tues: Patterned Mvmt, L1 Wed: Patterned Mvmt, L1 Thurs: Patterned Mvmt, L1 Fri: Free food	***Week 3: 9/20 – 9/26*** Sat: Food deprivation Sun: Food deprivation Mon: Patterned Mvmt, L1 Tues: Patterned Mvmt, L1 Wed: Patterned Mvmt, L1 Thurs: Patterned Mvmt, L1 Fri: Free food	***Week 4: 9/27 – 10/3*** Sat: Free food Sun: Free food Mon: Free food Tues: Free food Wed: Free food Thurs: Free food Fri: Free food
Data Analysis, CCU Student Retreat, CISS talk	***(Week 4: 9/27 – 10/3)*** Thurs: CCU Student retreat Fri: CCU Student retreat	***Week 5: 10/4 – 10/10*** Animals fed free food daily Sat: CCU Student retreat Mon: CISS talk, assess progress so far		
Group B (older animals)	***Week 6: 10/11 – 10/17*** Sat: Free food Sun: Free food Mon: Free food Tues: Free food Wed: Free food Thurs: Free food Fri: Free food	***Week 7: 10/18 – 10/24*** Sat: Food deprivation Sun: Food deprivation Mon: Causal Mvmt, L1 Tues: Causal Mvmt, L1 Wed: Causal Mvmt, L1 Thurs: Causal Mvmt, L1 Fri: Free food	***Week 8: 10/25 – 10/31*** Sat: Free food Sun: Free food Mon: Free food Tues: Free food Wed: Free food Thurs: Free food Fri: Free food	
Group C (juvenile animals)	***Week 10: 11/1 – 11/8*** Sat: Food deprivation Sun: Food deprivation Mon: Foraging, L1 Tues: Foraging, L2 Wed: Foraging, L2 Thurs: Foraging, L2 Fri: Free food	***Week 10: 11/9 – 11/15*** Sat: Free food Sun: Free food Mon: Free food Tues: Free food Wed: Free food Thurs: Free food Fri: Free food		

Experiment Protocol

0. Subjects

Group A

Tank(s): Loop 1 Tub 2 Left-front; Loop 7 Tub 1 Left-front

Animal #s: L1-H2013-01 ("Dora"), L1-H2013-02 ("Scar"), L1-H2013-03 ("Ender"), L7-H2013-01 ("Old Tom"), L7-H2013-02 ("Plato"), L7-H2013-03 ("Blaise")

Age: Hatched June 2013

Notes: This group began training for this protocol at about age 15 months (September 2014). At the time of starting this group, the older animals were starting to reach the point where some of them were refusing food (just a symptom of age).

Group B

Tank(s): Loop 1 Tub 1 Left-front top; Loop 1 Tub 2 Left-front top

Animal #s: L1-H2014-01, L1-H2014-02, L1-H2014-03, L1-H2014-04, L1-H2014-05, L1-H2014-06_group

Age: Hatched June 2014

Notes: This group began training for this protocol at about age 5 months (November 2014). During the first 4 days of training (Nov 3-6, 2014) the animals appetites and food intake dramatically decreased, and so we decided to stop training them in the protocol. On the morning of Friday Nov 7, 2014, L1-H2014-04 was found dead. We then decided to spend one week feeding the cuttlefish in their home tubs, then another week hand feeding the cuttlefish in the experimental setup. On the morning of Tuesday Nov 11, 2014, one of the cuttlefish in the L1-H2014-06_group was also found dead. After investigating possible causes of death, we concluded that the stress of the experiment weakened the immune systems of the young cuttlefish and made them more susceptible to rising toxins in their hometub water. Toxins in the home tub increase during the early stages of the cuttlefish life cycle at the MBL because while cuttlefish are young and very small, hometubs are only siphoned, not scrubbed down like they are when the cuttlefish are bigger and less likely to ink themselves during a tub cleaning.

1. Prepare Prey (food reward)

1.0

- Remove frozen shrimp from freezer and defrost, @~9am
- Chop defrosted shrimp into ~5mm^2 chunks

2. Prepare Experimental Tank

2.0

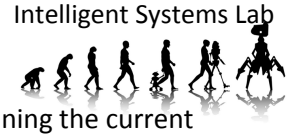
- Siphon out any debris in the holding tank
- Place half-pipe hut and artificial fern plant in "home base" area. Rinse these items at the end of each day.

2.1

- Plug in holding tank LEDs and turn on
- Connect overhead Point Grey camera usb to recording computer. Open the Point Grey FlyCap2 GUI to set camera to Resolution: 1280x960; Pixel Format: Y8; Frame Rate: 70
- Turn on GoPro camera, place inside waterproof case, position and secure case to holding tank

2.2

- Connect the arduino to the recording computer. Open the appropriate file for the current assay, update any new variables, and upload updated code to arduino board.



- Test that the prey-on-motorized-skewer and cue LED are working by running the current Bonsai workflow and the arduino code, then save that data in the appropriate folder in /dailytests.
- Place bowl of shrimp bits near the motorized skewer

3. Prepare the Animals

3.0 Subject order

- The first cuttlefish will go into the tank around 10am
- Cuttlefish will be tested in the same order every day

Order of cuttlefish:

Group A

- L1-H2013-01
- L1-H2013-02
- L1-H2013-03
- L7-H2013-01
- L7-H2013-02
- L7-H2013-03

Group B

- L1-H2014-01
- L1-H2014-02
- L1-H2014-03
- L1-H2014-04
- L1-H2014-05
- L1-H2014-06_group

4. Running a trial

4.0 Week 1: Foraging

4.0.0

- Go to /MBL/animals and open the excel file that corresponds to the cuttlefish ID. Enter date, assay type, and start time. Save and close excel file.

4.0.1

- Start recording on the GoPro camera. Every 3 animals, remove the GoPro and recharge for 30 minutes. During this break, also backup and label the video files.

4.0.2

- Open the serial monitor in Arduino. Watch the movement tests to make sure everything is working as expected.
- Open the Bonsai workflow **tank_foraging**. Start the workflow.

4.0.3

- Wait for the camera to start recording (around 10 seconds) before putting cuttlefish into the tank. Trial starts when the cuttlefish enters the “home base”.
- **Note the start time for each cuttlefish** (if you forget, use the timestamp on the data files).

4.0.4

- After 30 minutes, end the trial by stopping the workflow. Stop the GoPro camera. Remove cuttlefish from the tank.

4.0.5

- Reset session by:
    - restoring skewer to start configuration

- charge/backup GoPro if 3 trials have passed
- copy serial monitor output for each animal

4.0.6

- When all cuttlefish have been run:
  - rinse “home base” half-pipe and fern with fresh water, leave out to dry
  - throw away any leftover shrimp
  - remove GoPro camera and case from holding tank; remove camera; rinse case and leave out to dry
  - turn off holding tank LEDs

4.1 Week 2: Patterned Movement

4.1.0

- Place a piece of shrimp on the motorized skewer.

4.1.1

- Right-click on the **Windows PowerShell** icon in the taskbar and select **prepare_patterned_session**. When the terminal window opens, enter current cuttlefish ID and hit enter.

4.1.2

- The Bonsai workflow **tank_patterned** will initialize. Start the workflow.

4.1.3

- Wait for the arduino to initialize and for the camera to start recording (around 10 seconds) before putting cuttlefish into the tank. Trial starts when the cuttlefish enters the “home base”.
- **Note the start time for each cuttlefish** (if you forget, use the timestamp on the data files).

4.1.4

- After 30 minutes, end the trial by stopping the workflow. Remove cuttlefish from the tank.

4.1.5

- Reset tank by:
  - restoring skewer to start configuration
  - rinsing the “home base” half-pipe and fern with fresh water

4.1.6

- When all cuttlefish have been run:
  - remove experimental tank from holding tank and leave out to dry
  - rinse “home base” half-pipe and fern with fresh water, leave out to dry
  - throw away any leftover shrimp
  - remove GoPro camera and case from holding tank; remove camera; rinse case and leave out to dry
  - turn off holding tank LEDs

4.2 Week 3: Causal Movement

4.2.0

- Place a piece of shrimp on the motorized skewer.

4.2.1

- Right-click on the **Windows PowerShell** icon in the taskbar and select **prepare_causal_session**. When the terminal window opens, enter current cuttlefish ID and hit enter.

4.2.2

- The Bonsai workflow **tank_causal** will initialize. Start the workflow.

4.2.3

- Wait for the arduino to initialize and for the camera to start recording (around 10 seconds) before putting cuttlefish into the tank. Trial starts when the cuttlefish enters the “home base”.
- **Note the start time for each cuttlefish** (if you forget, use the timestamp on the data files).

4.2.4

- After 30 minutes, end the trial by stopping the workflow. Remove cuttlefish from the tank.

4.2.5

- Reset tank by:
    - restoring skewer to start configuration
    - rinsing the “home base” half-pipe and fern with fresh water

4.2.6

- When all cuttlefish have been run:
    - remove experimental tank from holding tank and leave out to dry
    - rinse “home base” half-pipe and fern with fresh water, leave out to dry
    - throw away any leftover shrimp
    - remove GoPro camera and case from holding tank; remove camera; rinse case and leave out to dry
    - turn off holding tank LEDs

4.3 Week 4: Reactive Movement

4.3.0

- Place a piece of shrimp on the motorized skewer.

4.3.1

- Right-click on the **Windows PowerShell** icon in the taskbar and select **prepare_reactive_session**. When the terminal window opens, enter current cuttlefish ID and hit enter.

4.3.2

- The Bonsai workflow **tank_reactive** will initialize. Start the workflow.

4.3.3

- Wait for the arduino to initialize and for the camera to start recording (around 10 seconds) before putting cuttlefish into the tank. Trial starts when the cuttlefish enters the “home base”.
- **Note the start time for each cuttlefish** (if you forget, use the timestamp on the data files).

4.3.4

- After 30 minutes, end the trial by stopping the workflow. Remove cuttlefish from the tank.

4.3.5

- Reset tank by:
    - restoring skewer to start configuration
    - rinsing the “home base” half-pipe and fern with fresh water

4.3.6

- When all cuttlefish have been run:
    - remove experimental tank from holding tank and leave out to dry
    - rinse “home base” half-pipe and fern with fresh water, leave out to dry
    - throw away any leftover shrimp
    - remove GoPro camera and case from holding tank; remove camera; rinse case and leave out to dry
    - turn off holding tank LEDs

5. Data Hygiene

5.0 Point Grey Camera

5.0.0

- extract time stamps for the start of each trial
- extract time stamps for the moment tentacles "go ballistic" (tentacles initially exit mouth in slow, controlled fashion, then suddenly move much more quickly, akin to the release of a coiled spring)
- extract time stamps for moments when cuttlefish "orients" to the prey (an "orient" = rapid change in body pattern or orientation)

5.0.1

- make 6 second video clips where each frame is cropped and aligned to be tightly centered on the cuttlefish; center these clips (in time) to:
    - cue LED turns on
    - moment tentacles go ballistic
    - start of an "orient"

5.1 GoPro Camera

5.1.0

- using time stamps from Point Grey Camera, make video clips centered (in time) to:
    - cue LED turns on
    - moment tentacles go ballistic
    - start of an "orient"

5.2 Data Plots

5.2.0

- number of orients, tentacle shots, and successful catches per session
- order and timing of tentacle shots, labeled as "misses" and "catches"

5.2.1

- from cropped and aligned video clips:
    - average cuttlefish mantle luminance at each frame
    - spatial frequency of cuttlefish body pattern at each frame
    - contrast of cuttlefish body pattern at each frame

5.2.2

- ethograms:
    - tentacle shot sequence
    - "orients" sequence

6. Open Lab Notebook

6.0 Video Dataset

6.0.0

- Point Grey:
    - full video dataset: http://bit.ly/28JG5Rv
    - cropped and aligned video clips: http://bit.ly/28Jo0EK