BIO342 F2018 CICHLID DOMINANCE LAB

Before Lab:

1) Read this handout
2) Read Martin and Bateson page 75 – 79

   Consider how you could use the different methods of intra- and inter-observer reliability with the experimental design we are doing in class. This will become more obvious once you are working with the data, but try to imagine how you would have to adapt the data collection protocol to use the different methods?

3) Read Martin and Bateson page 131 – 134

   This is rather advanced for the first week of class. We will discuss methods to measure dominance hierarchies in lab **during week 2**. After the discussion, each group will have to settle on a method to use for their own data analysis. It will be interesting to see if different methods give different results. There will be additional resources in lab. The data collection method that we use, may lend itself to certain methods better than others. This is meant to demonstrate that data collection is part of a behavioral protocol that also requires trouble shooting.

Background:

The African cichlid fish, *Astatotilapia burtoni* (formerly: *Haplochromis burtoni*) has become an important model system to study the mechanisms underlying socially mediated behavioral change. In this species, 20-30% of males are dominant, slow growing, brightly colored and actively defend territories for mating. The remaining subordinate males school and display cryptic coloration, while experiencing faster growth (Hofmann et al 1999). Subordinate males show little aggression and territoriality, and, importantly, have regressed gonads and thus are not reproductively active (Fernald 1977). These behavioral and physiological characters are plastic and influenced by the immediate social environment, such that an individual male switches between the dominant and subordinate phenotypes several times during its life depending upon its relative ability to obtain and maintain access to a territory through encounters with other males. Environmental conditions, availability of territorial shelters, relative body size and physiological condition influence the probability of acquiring and maintaining a territory. The phenotypic switch occurs over a timescale of minutes to days to weeks in both the field and the laboratory (Fig. 1 reviewed in(Maruska 2015).

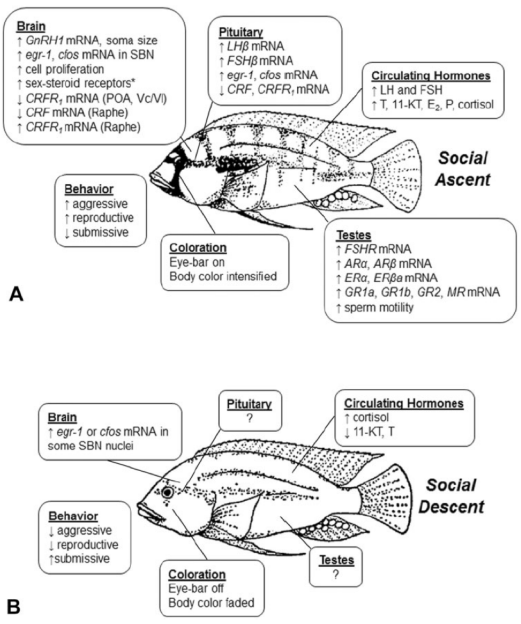


Fig. 1 Summary of rapid phenotypic changes in *Astatotilapia burtoni* males that are ascending (A) or descending (B) in social rank. Changes that occur within the first 24 h of males rising or falling in rank are shown. These adaptive phenotypic changes while some cellular changes and changes in level of gene expression along the brain–pituitary–testes axis occur more slowly (days to weeks) (Taken from Maruska 2015, Figure 4).

Female *A. burtoni* are thought to behave much like subordinate males under normal conditions in the field, i.e., they school and feed with other females, juveniles, and subordinate males (Fernald 1977, Fernald & Hirata 1977a, Fernald & Hirata 1977b). Once gravid (full with mature eggs), a female will spawn with dominant males, after which she incubates the fertilized eggs in her buccal cavity for several weeks without eating (Fig. 2). Upon release of the fry a female may defend a territory and continue to exhibit maternal care for a short period (Renn et al 2009).

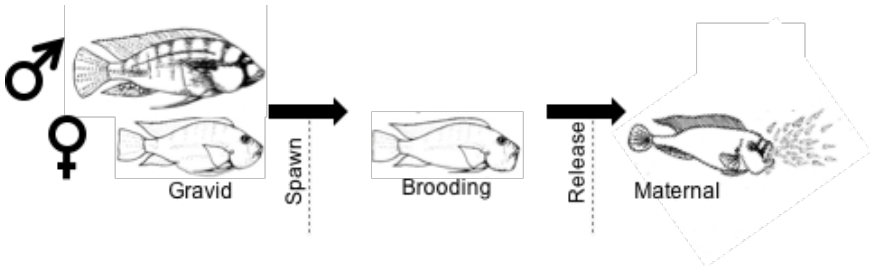


Fig. 2 Summary of the female reproductive stages in A. burtoni. The females lose body mass while brooding yet inhibit feeding behavior even if fry are removed (Mrwoka 1984)

Females territorial behavior can also be induced experimentally by removing all males from a tank(O'Connell et al 2013, Renn et al 2012). Under these conditions one female (or two if there are sufficient territories provided) will become aggressive, acquire dark eye-bars, defend territories, with vigorous chasing, threat displays and border threats, and even court and spawn with other females. This artificial manipulation suggests that females possess neural circuitry similar to males and may form female dominance hierarchies within the school that have been overlooked by researchers. At the physiological level, the behavioral changes associated with artificial female aggression are accompanied by the increase in circulating levels of androgens (Renn et al 2012; O'Connell 2013).

In the next experiment, we aim to determine whether androgen levels are correlated with aggressive behavior (as a proxy for dominance rank) among females in a more normal social setting. Considerable evidence from the Renn Lab suggests that females do in fact have a social dominance hierarchy, though convincing evidence for physiological correlates is lacking (Ivanov, 2018; Bremmer, 2010; Guo personal comm.). We will also measure Estradiol levels as a proxy to determine what stage of reproductive cycle the female is in (Fig 3).

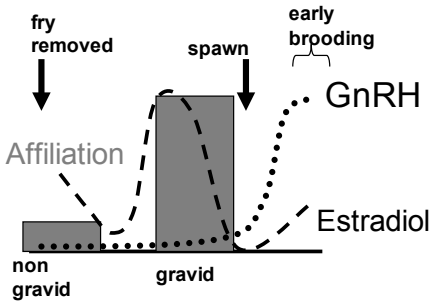


Fig. 3 Physiological reproductive cycle of female *A. burtoni*. Neuropeptide (GnRH) and hormone (Estradiol) cycles underlie changes in behavior (Affiliation).

Bio342 Cichlid Lab Overview

This lab requires both sections to collaborate.

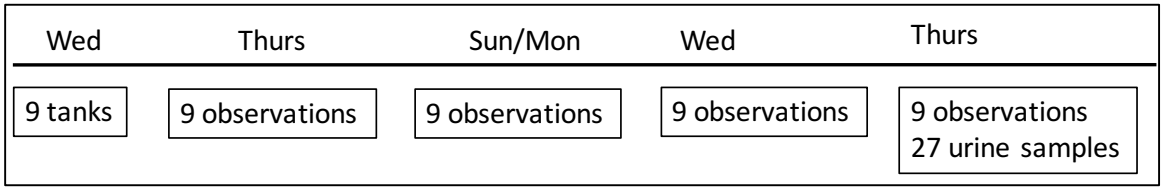
Wed Lab week 1– Groups of 2 each set up a tank (n=9)

Thurs Lab week 1– Groups of 2 will observe 2 tanks each and check inter-observer reliability.

Mid Week observations – 1 student from each Wednesday group and 1 student from each Thursday group will do an observation on Sunday or Monday.

Wed Lab week 2– Groups of 2 observe 2 tanks each and check inter-observer reliability.

Thurs Lab week 2– Groups of 2 each observe a tank, collect holding water samples for hormone analysis and clean up.



Before you leave lab in week 1, be sure you know when you will be coming in to do mid-week observation.

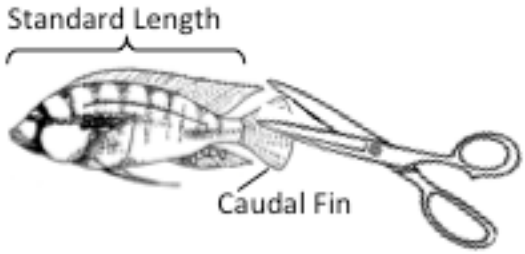
Sunday or Monday

1 student from each Wednesday group and 1 student from each Thursday group will check in on the fish, feed them, and do a single observation and enter the data in the class dataset.

The hormone measures are non-invasive and all animal protocols for this lab are IACUC approved but we are required to keep a record of animal health and check on the animals throughout the week.

Tank Set Up

1. Working in groups of two, obtain a 5 gallon tank (rat cages).
2. Each tank will be assigned a number (1-9)
3. Divide each tank with both a clear perforated divider about ¼ of the distance form one end to create a small end and a large end.
4. Fill each tank with prepared "Lake Tanganyika" water above the perforations.
5. Add enough gravel to cover the bottom of the tank.
6. Add a single pot shard to serve as shelter each side of the divider
7. Each tank MUST be provided with a bubble filter in the smaller side.
    i. Connect the "house air" hose to the top of the bubble filter which must be submerged.
8. Obtain 3 female *A. burtoni* of roughly the same size using a Tupperware container filled with water to transport each.
9. Weigh each fish and measure standard length of each fish (nose to caudal peduncle).
10. The class should coordinate to group 3 females of the closest size.
11. Among your three fish, flip a coin to decide which fish will have its fin clipped on the top or on the bottom to allow individual identification.
12. Each fish will have a unique ID of Tank#_T/N/B. (based on whether it is the one with the tail fin clipped).
13. Using small dissecting scissors, make a small clip in the tail.
14. Be sure all of this information is recorded in your lab notebook.
15. Put the 3 females in the large end of the observation tank.
16. Get one male *A. burtoni* that is slightly larger than the females and put him on the small end of the observation tank.
17. <u>The tank must be covered with a lid, these fish jump!</u>
18. Attach an animal Care log to the lid. (date, health, initials of observer, if fed)
19. Go to Bio342Google Drive and enter the information on the meta_data tab of the google form "Cichlid_Lab_Class_data"
20. On the Behavior_Data tab, sign up for observations on one of the days (Sunday or Monday). Wednesday students will be paired with a Thursday student who will have experience with observations.



Behavioral Observation

Later in the semester we will work with more detailed ethograms and methods for observing animals and collecting behavioral data, but often, simple is better.
Given 3 females in a tank and a goal to identify which one is dominant, we will simply observe who picks on who. We 3 have tagged each fish with a unique fin clip (top, bottom, none). We will simply record the number of times (in 15 minutes) that top attacks bottom, top attack none etc. We will also include an entry for social behavior with no obvious dominance interaction. Make a data collection table like the following in your lab notebook.

Tank#	Observer	Time	T>N	T>B	N>T	N>B	B>T	B>N	Social

Behavioral Observation Protocol

1. While it only requires 1 student to observe a tank, having two students do this will allow us to check inter observer reliability.
2. Allow the fish to acclimate for 5 minutes to acclimate to your presence.
3. DO NOT LOOM at the fish during the observation.
4. Note the time of day in your lab notebooks.
5. Start the 15-minute observation period.
6. Record behavior into your lab notebook using tick marks for each specific encounter for 15 minutes.
7. Tally your tick marks for each specific behavior.

Inter Observer Reliability

1. Using excel, JMP, or R measure enter the number of each behavior events observed by the two paired observers.
2. Calculate a Pearson Correlation coefficient (r) noting the degrees of freedom.
3. Discuss any discrepancy.
4. Decide which observation data you consider "best" and enter these into the class data set.
5. Your lab notebook should reflect the above steps and logic for your decision.

Hormone Sample Collection

1. Get 3 empty hormone sample bottles.
2. Get 3 500 ml beakers filled with 300ml clean "Lake Tanganyika" water.
3. Label these with Fish ID.
4. Note the time.
5. Without disrupting the barrier, gently lift away the territory potshard and use a net to capture your female fish.
6. Place the fish in its beaker of water.
7. Place the Beaker in a bucket to limit visual distraction.
8. Note the time and wait 30 minutes
9. During this time, you can return the male to the main tank and begin to clean up.
10. After 30 minutes, measure and weigh your females and enter that data into the google sheet.
11. Transfer holding water to labeled hormone bottles with a funnel.
12. Hormone samples can be stored in at -20C.
13. Once fish are removed from the tank, put the animal care log in one of the lab notebooks.

If there is time we may begin to "concentrate the hormones on columns". See "EIA_water_instructions_20151112.pdf" for instructions.

Calculating Dominance Hierarchies

Calculating Dominance Hierarchies is not simple. We will discuss this in class. There are two papers available on the lab website that will be useful and should be read before week 2 of this lab (Bayly et al 2006, Gammell et al 2003).

Lab WriteUp Due week 3 before lab:

Unlike Intro Bio, there is greater flexibility in what your lab write-ups.

1) Working with a partner, **Download** the class dataset and create a figure that presents behavioral data from all of the observations (Thursday, Mid week, Wednesday, Thursday). (DO NOT MESS with the raw class dataset)
    a. Take notes in your lab notebook as you work (include draft figures is you want).
    b. You will decide whether to use JMP, Excel, or R.
    c. You will decide if you want to use this raw data or calculate an index of dominance according to one of the methods we discuss in lab.
    d. You will decide how to visualize the data.
    e. To the extent possible, use color, symbols, etc. to make the visualization as clear as possible, but this is raw data, it is likely to be messy.
    f. Use this figure to decide which animals you will include in your hormone analysis.
    g. Write a good figure legend for this figure.
    h. Also write one paragraph of text that explains how you decided which animals to use in the future hormone analysis.
2) Create a second figure that includes only those animals that you decide we should use for the hormone analysis.
    a. This figure should summarize data from only the last two observations. (This is likely to involve averages, box plots or some other summary unlike the above figure that is closer to the raw data.)
    b. Write a good figure legend for this figure.
3) Save your document as a .doc or .pdf named "Cichlid_Lab_LastNamesOfBothStudents" and upload it to Moodle.
4) Please attend office hours Friday or by special appointment Monday if you want help with all of this.

Literature cited:

Bayly KL, Evans CS, Taylor A. 2006. Measuring social structure: A comparison of eight dominance indices. *Behavioural Processes* 73: 1-12

Fernald RD. 1977. Quantitative behavioural observations of Haplochromis burtoni under semi-natural conditions. *Anim. Behav.* 25: 643-53

Fernald RD, Hirata NR. 1977a. Field study of Haplochromis burtoni : Quantitative behavioral observations. *Anim. Behav.* 25: 964-75

Fernald RD, Hirata NR. 1977b. Field Study of Haplochromis burtoni: Habitats and co-habitants. . *Environmental Biology of Fishes.* 2: 299-308

Gammell MP, De Vries H, Jennings DJ, Carlin CM, Hayden TJ. 2003. David's score: a more appropriate dominance ranking method than Clutton-Brock et al.'s index. *Animal Behaviour* 66: 601-05

Hofmann HA, Benson ME, Fernald RD. 1999. Social status regulates growth rate: Consequences for life-history strategies. *Proc. Natl. Acad. Sci. U. S. A.* 96: 14171-76

Maruska KP. 2015. Social Transitions Cause Rapid Behavioral and Neuroendocrine Changes. *Integrative and Comparative Biology* 55: 294-306

O'Connell LA, Ding JH, Hofmann HA. 2013. Social Status Predicts how Sex Steroid Receptors Regulate Complex Behavior across Levels of Biological Organization.

Renn SCP, Carleton JB, Magee H, Nguyen MLT, Tanner ACW. 2009. Maternal care and altered social phenotype in a recently collected stock of Astatotilapia burtoni cichlid fish. *Integrative and Comparative Biology* 49: 660-73

Renn SCP, Fraser EJ, Aubin-Horth N, Trainor BC, Hofmann H. 2012. Females of an African cichlid fish display maletypical social dominance behavior and elevated androgens in the absence of males. *Hormones and Behavior* 61: 496-503

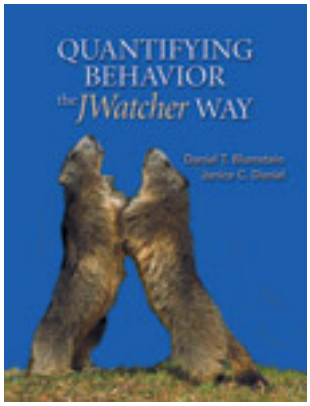
Ivanov, D. 2018 Reed Senior Thesis

Bremmer, E. 2010 Reed Senior Thesis

Appendix:
If you pursue research with *A. burtoni* for your independent project the following ethogram may be useful.

A. burtoni, lives in muddy rivers and crocodile-infested, reed-filled ponds around Lake Tanganyika. Therefore, only two field studies of behavior have been conducted (Fernald and Hirata 1977a; 1977b).

Behavior	**Description**
Flee:	Swim rapidly away from another male.
Chase:	Rapidly pursue fleeing opponent.
Bite:	Mouth open & closing within a few millimeters of opponent's side.
Threat:	May include spread opercula, lowered chin or beating of the tail with dorsal and spread pelvic fins while either facing the opponent or sideways to the opponent.
Jaw lock	Head on Confrontation between two males may be at the site of their common border, may include mouth to mouth contact.
Court:	sideways quivering in front of a female with anal fin spread and/or swim in front of female toward spawning pit, ostentatiously wagging tail.
In nest:	Entering the spawning pit, often emerging with large mouthfuls of gravel.
Sift/Feed:	Collect mouthfuls of gravel outside of the spawning pit and sift through in search of food particles.
Free swim:	Oriented locomotion.
Still:	Motionless.
Other:	Any movement or posture not described above.
Out of sight	Any time that the animal cannot be reliably observed.
Eyebar on	Display of black vertical bar across eye



If you need to collect timing information to calculate durations or create a time budget for your Indpependne project we can use the JWatcher even recorder.