

Dominance and eyebar activation:

Can eyebar alterations reverse social rank in male *A. burtoni* cichlids?

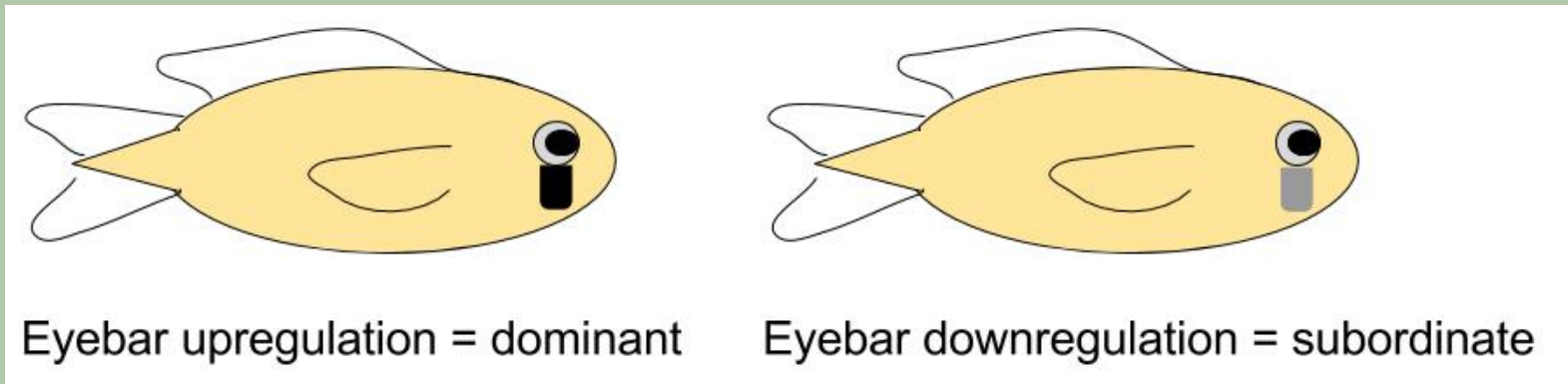
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Reed College Bio 342, Fall 2015

QUESTION: After a naïve aggressive encounter, can the resulting dominance hierarchy between two male cichlids be reversed by artificially enhancing the subordinate fish's eyebar salience?

BACKGROUND:

- Male cichlids (*Astatotilapia burtoni*) known for aggressive defense of resources [3]
- Dominance signaled through eyebar changes [3]



- Eyebar regulation controlled by internal/external processes that are sensitive to social rank
 - Sex steroid and gene expression changes [3]
 - Territory mound size [2]



Male cichlids engaging in aggressive open-mouth biting

(http://www.nbcnews.com/id/37261088/ns/technology_and_science-science/t/fish-fear-their-own-reflections/)

- Individual rank recognition based eyebar [1]
- Darkening male barn swallow birds patches (dominance) leads to increased aggression [4]

HYPOTHESIS: Darkening the subordinate fish's eyebars will reverse the dominance hierarchy in fish pairs.

EXPERIMENTAL DESIGN:

Repeated measures experimental design across conditions

	Negative control	Positive control	Experimental
Day 1	1. Behavioral test 2. No eyebar poking/injection	1. Behavioral test 2. Subordinate eyebar poking	1. Behavioral test 2. Subordinate eyebar injection
	↓	↓	↓
Day 2	3. Behavioral test	3. Behavioral test	3. Behavioral test

Behavioral test = 10 min; partner fish unfamiliar Day 1, familiar by Day 2
 Eyebar poking/injection = 10-15 min each side of fish

Expected behavioral results pre- and post-manipulation

	Eyebar score	Aggression score
Dominant (Day 1/2)	↑ / ↓	↑ / ↓
Subordinate (Day 1/2)	↓ / ↑	↓ / ↑

MATERIALS:

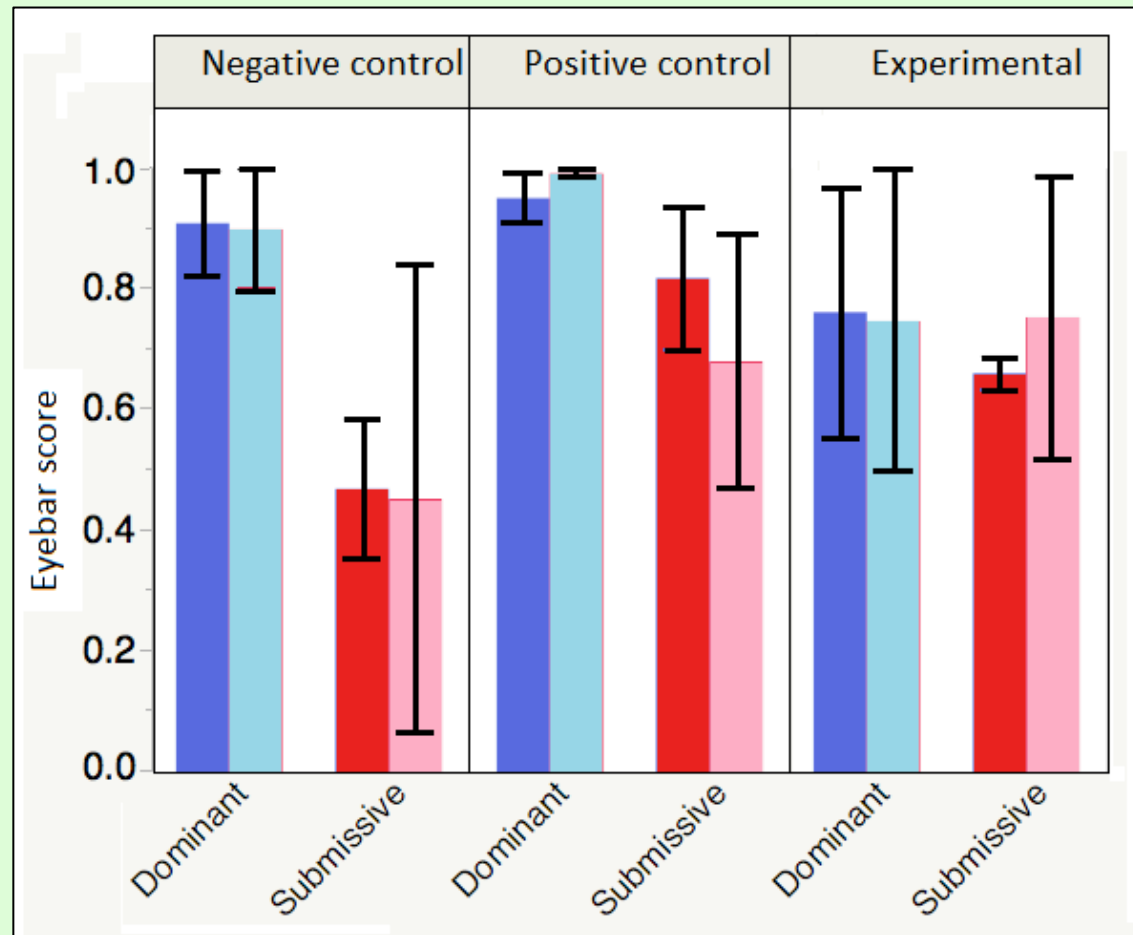
- Standard fish tanks set-up
- Visible Implant Elastomer (VIE) tagging supplies
- Size/color-matched lab, wild, mixed stock male *A. burtoni* cichlid fish (n=12)

MEASUREMENTS:

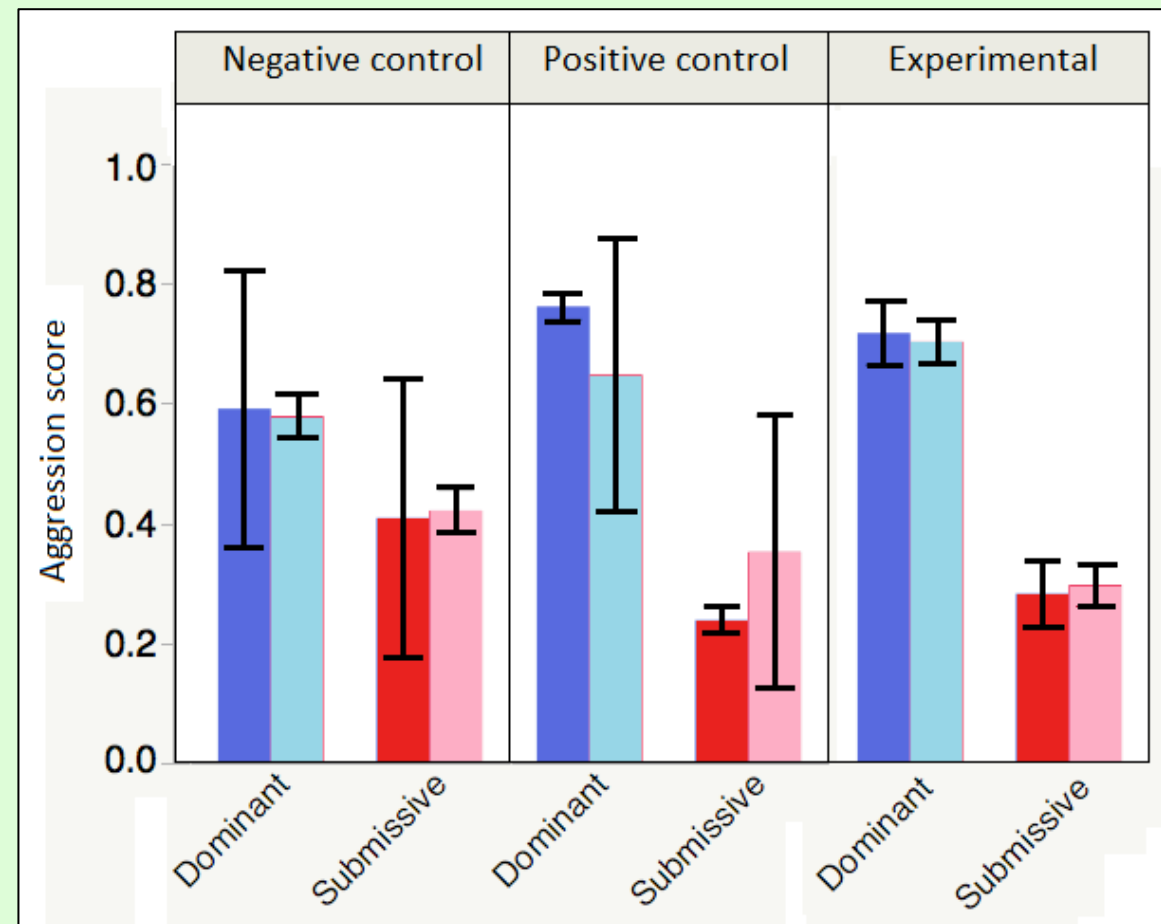
- Eyebar score (% total session duration with light/dark eyebar)
- Aggression score (% open-mouth barrier biting frequency within session accounted for by fish)

Results:

Eyebar upregulation not reversed by eyebar manipulation



Aggression not reversed by eyebar manipulation



Mean eyebar scores and aggression scores as percentages (pooled across 2 fish, \pm SEM, $n=12$) before the eyebar manipulation (dark blue and dark red bars) were unchanged after the eyebar manipulation (light blue and light red bars).



Injecting a subordinate experimental fish with VIE tagging material behind the eyebar on both sides.



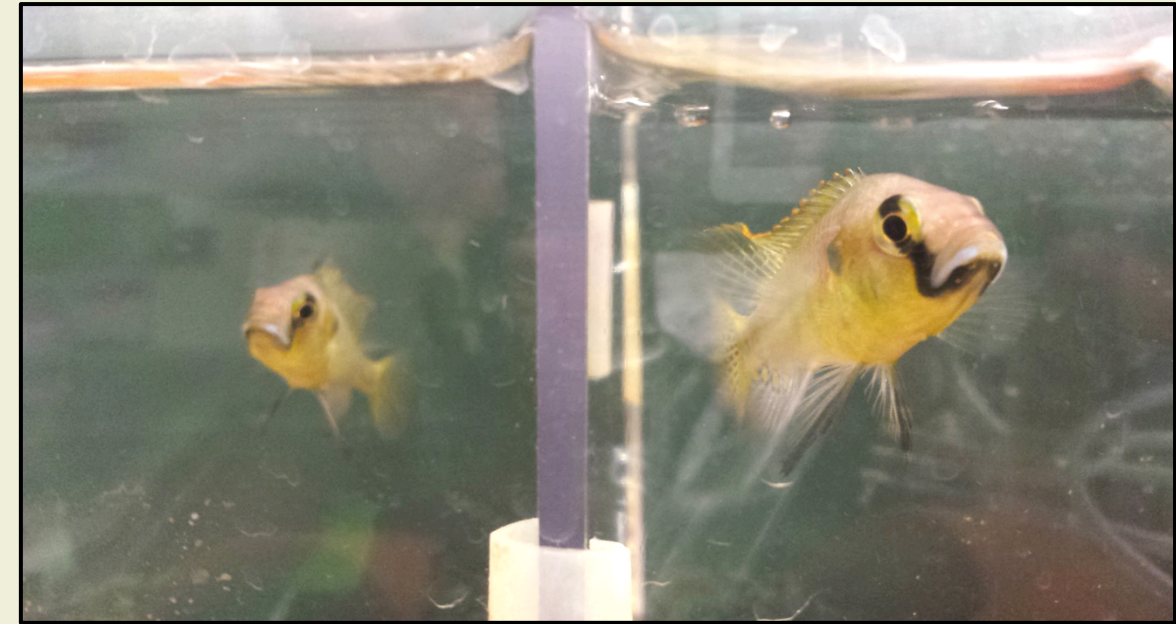
Fish before (A) and after (B) eyebar manipulation

CONCLUSIONS:

- The results did not support our hypothesis - no observed social rank change after eyebar manipulation
 - Dominance hierarchies unclear in many cases
 - Eyebar may not be the primary dominance signal
- Stress from poking/tagging the subordinate fish did not affect eyebar activation and aggression

FUTURE DIRECTIONS:

- Use fish with clearly established social rank
- Include second positive control/experimental condition to match time out of water for both fish
- Study eyebar manipulations in the presence of other variables that confer dominance (territory size, mates)
- Measure hormone levels from water



(Image from Klein and McKean's male cichlid experimental set-up, Fall 2015)

References:

1. Grosenick, L., Clement, T. S., & Fernald, R. D. (2007). Fish can infer social rank by observation alone. *Nature*, 445(7126), 429-432.
2. Magalhaes, I. S., Croft, G. E., & Joyce, D. A. (2013). Altering an extended phenotype reduces intraspecific male aggression and can maintain diversity in cichlid fish. *PeerJ*, 1, e209.
3. Maruska, K. P. (2015). Social transitions cause rapid behavioral and neuroendocrine changes. *Integrative and comparative biology*, icv057.
4. Safran, R. J., Adelman, J. S., McGraw, K. J., & Hau, M. (2008). Sexual signal exaggeration affects physiological state in male barn swallows. *Current Biology*, 18(11), R461-R462.

Acknowledgements:

We thank Sofia Claesson for helping with experimental set-up, the Reed college biology stockroom and Renn lab for providing fish equipment, Northwest Marine Technology, Inc. for providing tagging materials, and Suzy Renn for her advice and assistance in the planning and completion of this project and providing fish subjects.