Comparing consistency of stress and anxiety-related behaviors across time in zebrafish (*Danio rerio*)

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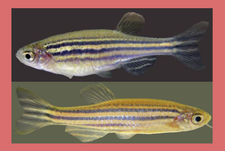
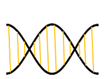


Introduction

- Animals are frequently faced with stressors in their environments that they must overcome
- Two common stress coping styles or 'personalities'[1]

Stress coping style	Proactive ("Bold")	Reactive ("Shy")
Physiological stress response	Low	High
Behavioral Flexibility	Low	High
Exploratory Behaviors	High	Low

- Distinct personalities have consistent and repeatable phenotypes across time and contexts
- Here, we use selectively bred lines of proactive and reactive zebrafish to determine the consistency and repeatability of stress and anxiety-related behaviors
- These selectively bred lines of zebrafish have been shown to have distinct[2,3]:



Genetic Backgrounds

Latency to feed
https://www.cam.ac.uk/research/

Exploration of novelty
http://www.noldus.com/animal-behavior-research/

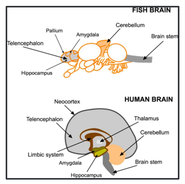
Antipredatory behavior

- Females display higher stress and anxiety related behaviors[3,4]

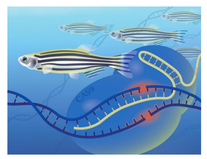
Unknowns:
- Are the zebrafish 'personalities' repeatable across time?
- Are selectively bred zebrafish more repeatable than a wild caught strain?
- Are some individuals more consistent than others?
- Does sex influence consistency or repeatability?

Objectives
1. Assess the repeatability of personality across time
2. Assess the consistency of individuals across time
3. Identify any sex specific effects on repeatability or consistency

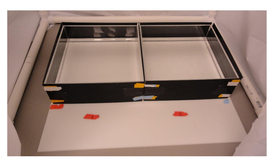
Danio rerio: An emerging translational model[5]



Comparative anatomy and physiology to humans
http://www.fishpain.com/fish-and-pain-brain-structures.htm

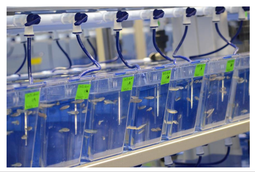


Fully sequenced and easily manipulated genome
https://phys.org/news/2015-06-role-zebrafish-larger-scale-gene.html



High throughput behavioral assays

Transparent/rapidly developing embryos
http://xmaslectures.imascientist.org.uk/profile/rachaelinglis/



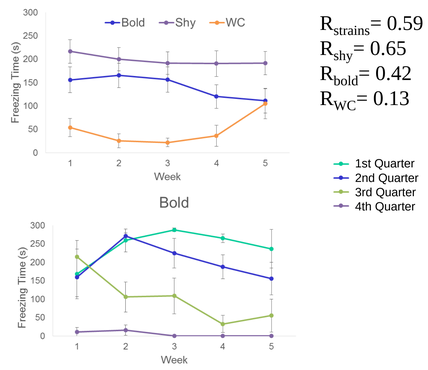
High space and cost efficiency
https://speakingofresearch.com/tag/zebrafish/

Results

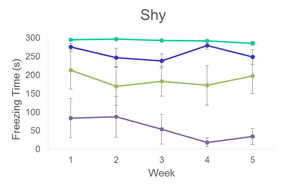
STRAIN EFFECTS:

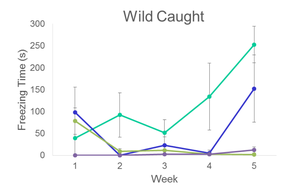
- Significant main effect of strain on time frozen with significant differences between each strain on post-hoc analysis (LSD)

$F_{2,57} = 12.14$, $p < .001$ Bold/Shy: $p_{one\text{-}tail} < 0.05$ Bold/WC: $p < 0.01$ Shy/WC: $p < 0.001$



$R_{strains} = 0.59$
$R_{shy} = 0.65$
$R_{bold} = 0.42$
$R_{WC} = 0.13$



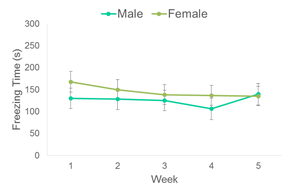


- Shy fish spent significantly more time frozen than bold and wild caught fish
- Personalities were repeatable (R= 0.59) with selectively-bred individuals more consistent

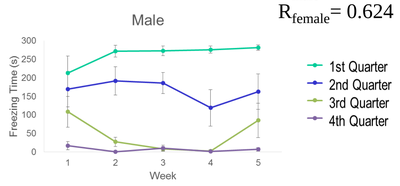
SEX EFFECTS:

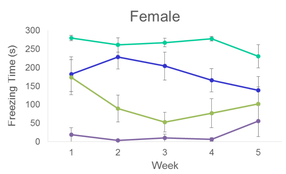
- No significant differences in freezing time between sexes

$F_{1,57} = 0.034$ $p = 0.854$



$R_{male} = 0.516$
$R_{female} = 0.624$





- Males and females were undistinguishable in freezing behavior
- Both sexes were relatively consistent

Summary

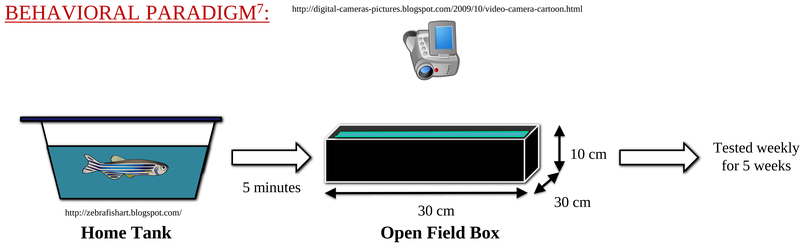
- **Each of the three lines displayed significantly different freezing behaviors**
- **Overall the zebrafish personalities were quite consistent at R= 0.59**
- **Selectively bred zebrafish are more repeatable than a wild caught strain with shy being the most consistent at R= 0.65**
- **Though males and females did not significantly differ in freezing behavior, they were relatively consistent with females having a slightly higher repeatability**

Methods

SUBJECTS:

	Proactive F10	Reactive F10	Wild Caught F0
Male	7	11	11
Female	15	9	5

BEHAVIORAL PARADIGM[7]:



ANALYSIS:

- Trials recorded and analyzed via Noldus EthoVision XT video tracking software
- "Freezing behavior" or amount of stationary time serves as a proxy for fear and anxiety

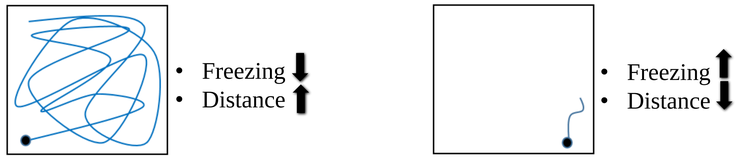


Figure 2. Predicted open field test results.

- Differences between strain and sex were analyzed with a general linear model repeated measures tests using SPSS software
- Repeatability and consistency was determined using the following equation[7]:
  R = Repeatability (ranges from 0-1, with 1 being the most consistent)
  VAR_{AI} = Variation across individuals or strain
  VAR_{WI} = Variation within an individual

$$R = \frac{VAR_{AI}}{VAR_{AI} + VAR_{WI}}$$

References

1) Overli O, *et al.* (2007) *Neurosci and Biobehav Reviews* 31(3):396-412.
2) Wong RY, *et al.* (2012) *Behaviour* 149:1205-1240.
3) Wong RY, *et al.* (2015) *BMC Genomics* 16(1):425.
4) Li K, *et al.* (2016) *Cell* 167:60-72.
5) Stewart AM, *et al.* (2014) *Trends in Neuroscience* 37(5):264-278.
6) Godwin J, *et al.* (2012) *Zebrafish Protocols for Neurobehavioral Research* 181-189.
7) Biro PA and Stamps JA (2015) *Animal Behaviour* 105:223-230.

Acknowledgements

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