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Article

#### Structure of the Zebrafish Locomotor Repertoire Revealed with Unsupervised Behavioral Clustering

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#### Introduction

- Objective: To explore zebrafish larvae's locomotor repertoire through unsupervised behavioral clustering
- Motivation: Revealing underlying organization of motor behavior in zebrafish helps in understanding neural circuit function and behavior in other organisms
- Why I chose this: aligns with my interest in data-driven approaches for understanding animal behaviors and neural functions



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## Methods & Results

#### Video recording

- Movements of thousands of zebrafish larvae were recorded using a real-time, high-speed video tracking system
- Performed in 9 different behavioral contexts by varying acoustic and visual stimuli

# Stim. 1 Stim. 2 Stim. N Behav. 1 Behav. 2 Behav. N

#### Nine behavioral contexts:

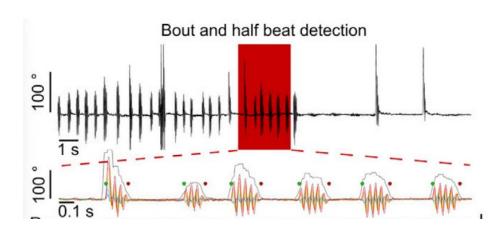
- 1. Forward OMR
- 2. Directional OMR
- 3. Acoustic startle
- 4. Dimming
- 5. Spontaneous Swimming
- 6. Prey capture
- 7. Photoaxis

Library of stimuli

- 8. Expanding spot
- 9. Social Context

#### **Detection of Behavior**

- Complex behaviors can be constructed from a set of basic motor patterns
- Zebrafish swim in sequence of bouts alternating with passive movement



#### Data size:

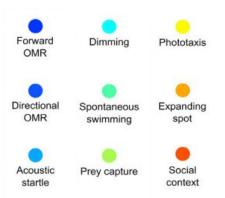
- 11M half beats
- 2M bouts
- 10k+ sequences

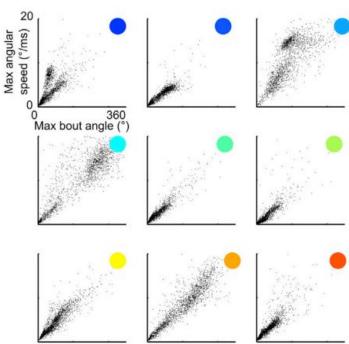
Half beats side-to-side tail bending movement

Bouts
Alternate sequence of half beats

## Transforming Kinematic data

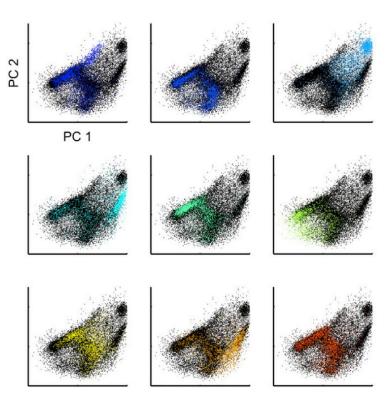
- Transformed time-series data of bouts to individual data points of 73 kinematic parameters such as:
  - Max angular speed
  - Forward distance
  - Bout end angle





## Dimension reduction using PCA

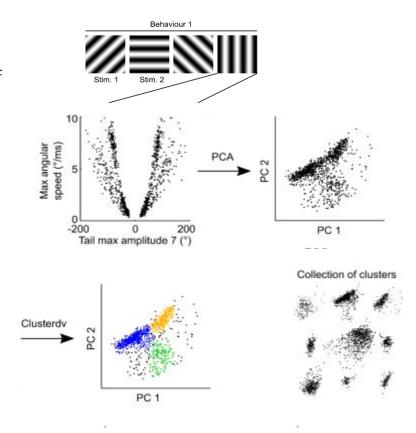
- Reduced from d=73 to 4 dimensions
- Shows clustered structure
  - Regions of high density are evident
  - Each behavioral context associated with a distinct distribution
- Kinematic features represented by this space
  - PC 1: Angular speed and tail amplitude
  - PC 2: Second half beat amplitude



## Clustering: Stage 1

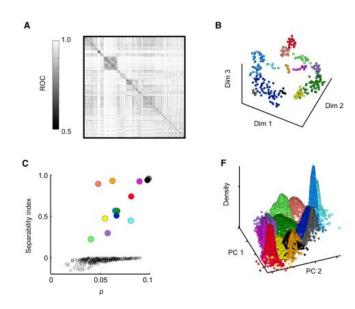
- Clustering performed to identify the basic set of swim types
- Processed in two stages to correct biases in sampling behaviors with different counts
- Divided data into sets based on stimulus parameters to observe rarer swim types
- Identified clusters in each set using **clusterdv**
- Whole set of clusters pooled to form a library

Clusterdv unsupervised estimation of density-based clusters across datasets

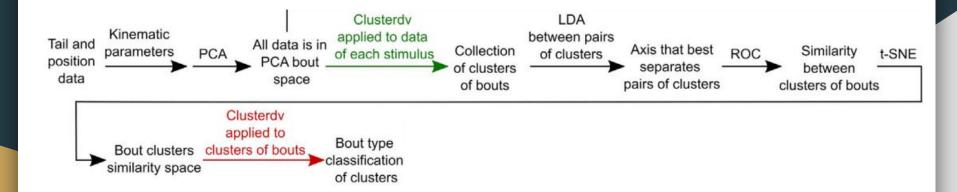


## Clustering: Stage 2

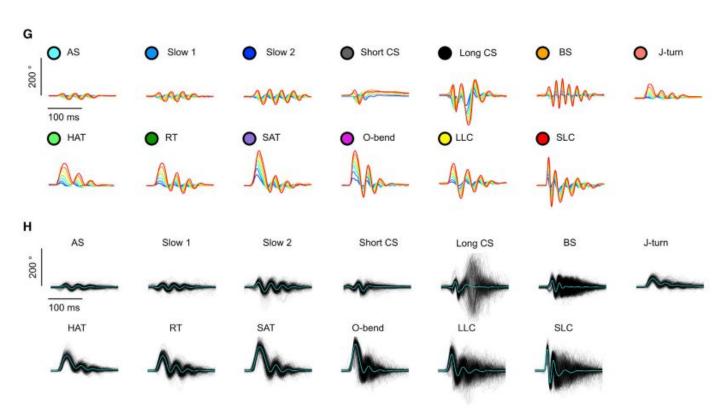
- A: Similarity of clusters quantified along maximum separable axis
- B: Used this to embed them into 3D space using t-SNE
- C: Clusterdv applied again to identify 13 groups
- F: Density map of bouts in the top-2 PC space



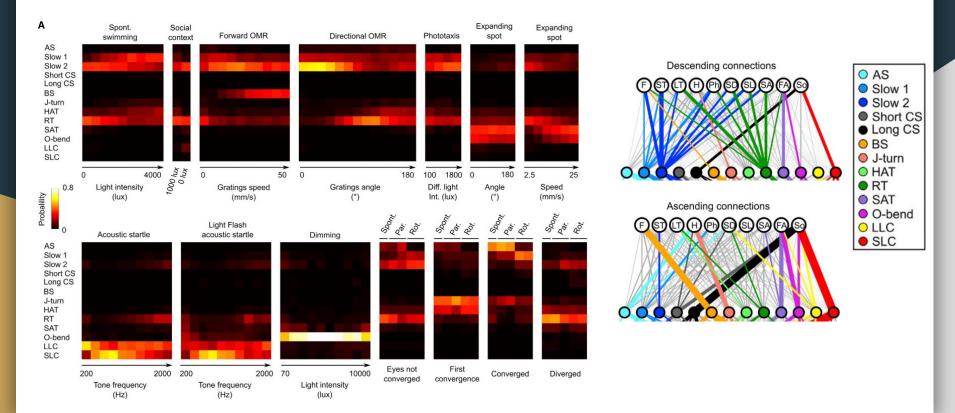
## Analysis pipeline



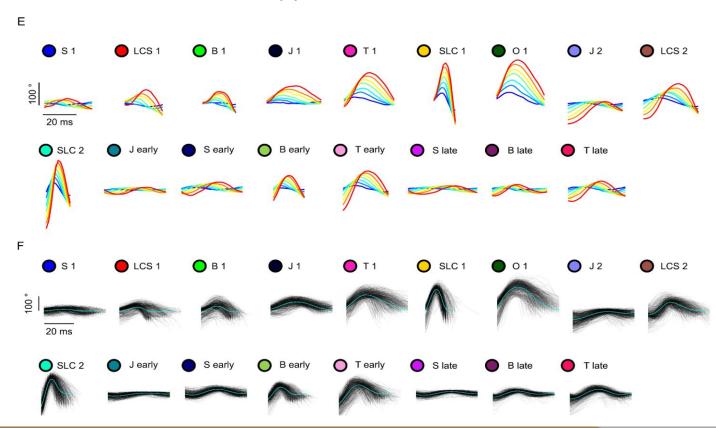
## Thirteen Bout Types



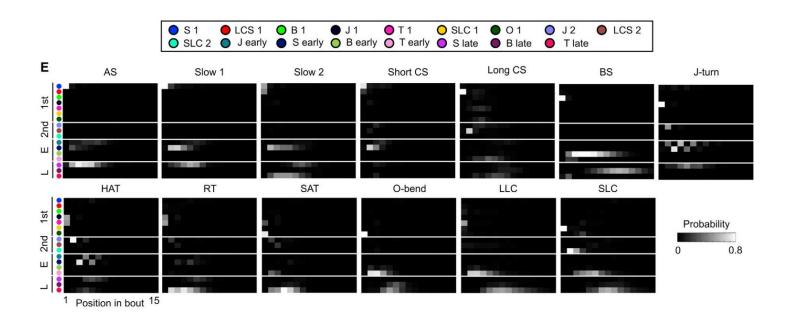
#### Bout types across behaviors



#### Seventeen Half beat types

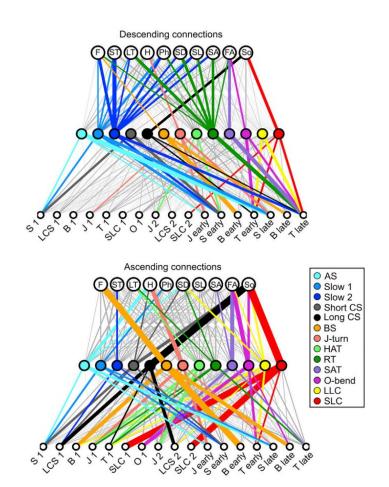


#### Half beat across bouts



#### Behavioral Hierarchy

- Zebrafish locomotor behavior has a hierarchical temporal organization
- Categories at one level consist of different, overlapping combinations of lower level



# Discussion

#### Discussion

- A fundamental question in ethology: distinct movement categories versus a smooth continuum
  - distinct categories evidence:
     multiple peaks in the distribution of certain kinematic parameters
  - smooth continuum evidence:
     kinematic parameters vary in a
     continuous fashion sometimes

- Unsupervised methods provide an unbiased way to analyze behavior and enable the discovery of new features
- This analysis captured categories previously described as well as identified novel ones

# Questions?