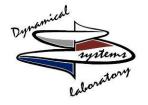




# Modeling the zebrafish animal model

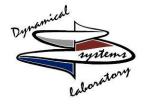
### Maurizio Porfiri Department of Mechanical and Aerospace Engineering New York University Polytechnic School of Engineering Brooklyn, NY 11201 http://faculty.poly.edu/~mporfiri/index.htm mporfiri@nyu.edu







- Zebrafish as an animal model for preclinical research
- NYU Polytechnic facility for zebrafish research
- Our unique engineering-based tools for zebrafish research
- An authentic data-driven model of fish behavior
- A jump persistent turning walker model to describe zebrafish swimming
  - Characteristics of zebrafish swimming behavior
  - Reproducing natural behavior through stochastic differential equations
- Current practice in modeling fish social behavior
- Beyond uniform noise, adapting the Vicsek and vectorial network models to include a refined turn rate model
  - Computational analyses of the models
  - A closed-form solution of the linearized vectorial network model
- Conclusions

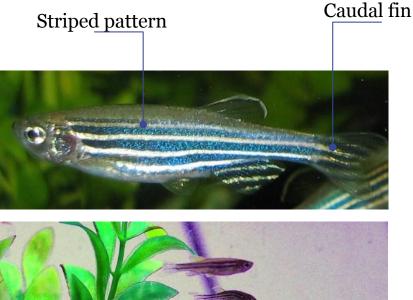






# Why are zebrafish so popular in laboratory experiments?

- Reduced costs
- Ethical considerations
- Ease in stocking and maintenance
- High reproduction rate
- Short intergeneration time
- Elevated homologies at genetic and neural levels with humans
- Availability of sophisticated genetic tools
- Highly social behavior
- Transparent embryos











Zebrafish are used for the investigation of several functional and dysfunctional processes:

- Emotions (fear and anxiety)
- Executive functions (learning, memory, and cognition)
- Effect of psychoactive compounds on individual and social behavior (alcohol, caffeine, LSD, cocaine...)







# NYU Polytechnic facility



### Our vivarium:

- Computer-controlled vivarium with separate ten gallon tanks
- Large 100 gallon tank
- Nine ten to twenty gallon housing tanks
- Several small tanks for animal breeding
- Stocking density at no more than one fish per liter
- Temperature and acidity maintained at 25°±1°C and 7.2 pH
- Illumination controlled according to a 10 hours light / 14 hours day circadian rhythm





# NYU Polytechnic facility



### Our experimental facilities include:

- Large tanks for studying interactions of fish and robots
- Smaller partitioned tanks for behavioral preference tests
- Water channel for studying swimming hydrodynamics
- High speed cameras, from 60 to 1,000 frames per second
- Laser doppler velocimetry for local flow measurements
- Planar and stereoscopic particle image velocimetry systems for flow measurements

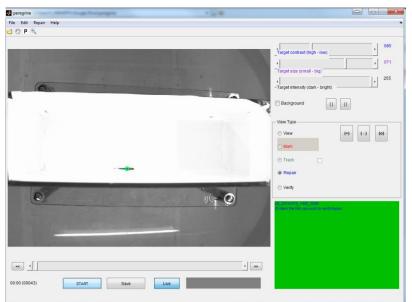


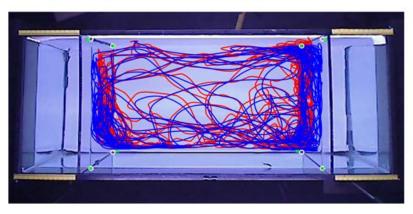


### **Engineering-based tools**

### Video acquisition system:

- In-house developed video-tracking platform (peregrine)
  - After background subtraction, fish blob centroid is utilized for measurement
  - Kalman-filtering is used to estimate the position and velocity
  - Post-processing is used to score locomotory patterns
- Peregrine tracks single or multiple fish in two and three dimensions, keeping their identities
- Peregrine has been recently updated to extract fish shape





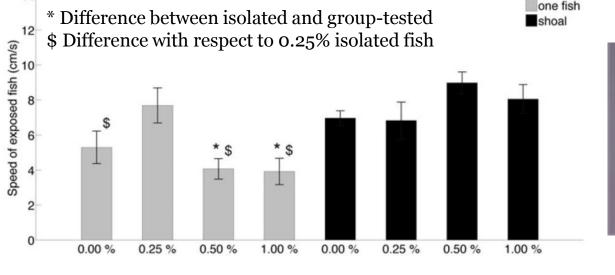


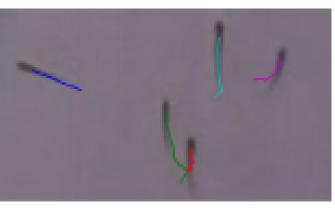




### Analysis of the effect of ethanol administration on social behavior:

- A treated individual swims alone or with a group of untreated subjects, whose identities are preserved using peregrine
- Ethanol-exposed subjects swim faster when group-tested than in isolation
- Untreated subjects also swim faster when the treated individual is exposed to intermediate ethanol concentrations







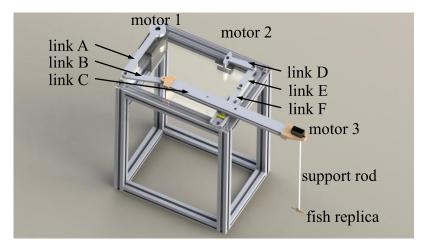
# **Engineering-based tools**

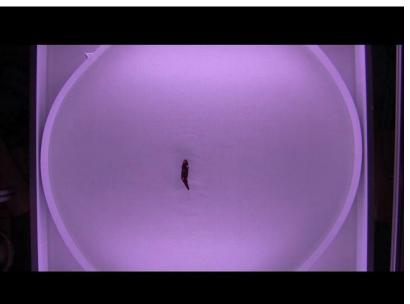


### **Robotics-enabled platforms:**

- Rapid prototyping is used to customize robotic replicas
- Robots of ten centimeters in length can swim autonomously in the tank using sensory feedback from an external camera, coupled with a lighter version of peregrine
- Zebrafish-sized replicas are externally maneuvered in two or three dimensions using custom-made manipulators
- Computer animations are integrated to offer alternative stimuli

[Entropy, 2014; TMECH, 2013]







# Engineering-based tools



### Comparing robotic predators, animated images and live predators:



- Zebrafish are tested in the preference setup
- The red tiger oscar is used as a predator, which elicits a less intense avoidance response than sympatric predators to avoid a ceiling effect
- Robotic stimulus elicits a similar response to the live predator
- Zebrafish display more consistent inter-individual response with the robotic predator
- Computer animation does not produce a similar response to the live predator

[Zebrafish, 2015]





### In-silico experiments:

- Computer simulations may offer new means to better analyze and interpret data, predict select experimental outcomes, and posit new directions for research
- In the context of the 3Rs, *In-silico* experiments may:
  - decrease the number of animal subjects needed for experiments (reduce)
  - quantify variables of interest in animal behavior without increasing animal discomfort (refine)
  - perform pilot trials preceding animal experiments (replace)

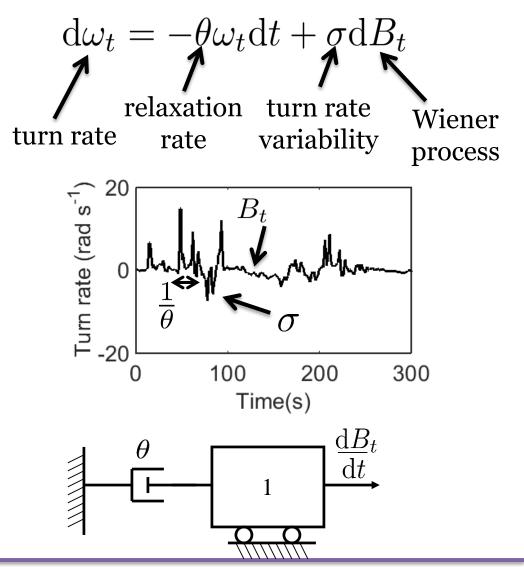






### Persistent turning walker (PTW):

- The first data-driven model of spontaneous fish movement was derived by Gautrais et al. 2009
- This approach allows for calibrating all the model parameters from experimental data using a maximum likelihood estimation
- The model focuses on the turn rate, whose dynamics is described by a Ornstein-Uhlenbeck stochastic differential equation (SDE)







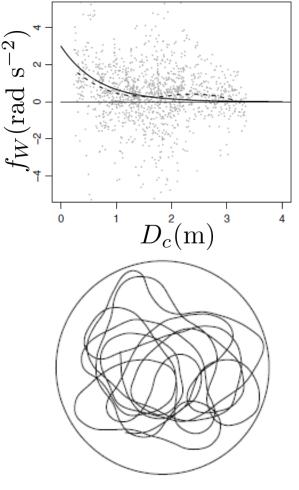
### Wall interaction:

• The PTW provides a versatile methodology to study fish locomotion in the presence of fixed boundaries and obstacles

$$d\omega_t = \theta(f_W - \omega_t)dt + \sigma dB_t$$

$$f$$
wall interaction
function

- $f_W$  is calibrated based on the proximity to a wall  $D_c$
- The framework can be adapted to study the effect of social interactions with predators or conspecifics



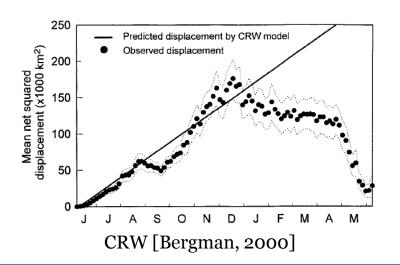
[Gautrais et al., 2009]

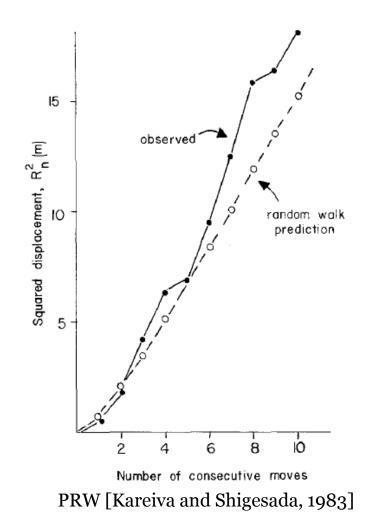




Correlation in successive animal steps beyond fish:

- A persistent random walker (PRW) has been proposed for insect displacement
- Correlated random walk (CRW) models have been considered to study caribou movement









Zebrafish are special, they turn really fast...

# A jump persistent turning walker to model zebrafish locomotion

# , Jump persistent turning walker 🍟 NYU

### Turn rate model for zebrafish [JRSI, 2015]:

- Extends the PTW model to capture the complex swimming movements of zebrafish
- The model contemplates the possibility of sudden jumps in the fish turn rate
- Jump diffusion processes are commonly used to model:
  - sudden changes in stock prices and interest rates in finance
  - considerable deviations in particle kinematics in statistical physics
  - dispersal of micro-organisms and foraging of animals in biology

Fast zebrafish turn maneuvers in successive video frames of 60 frames per second

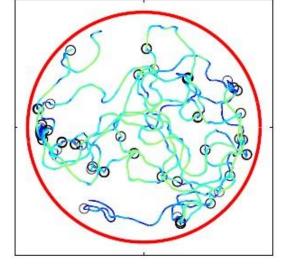


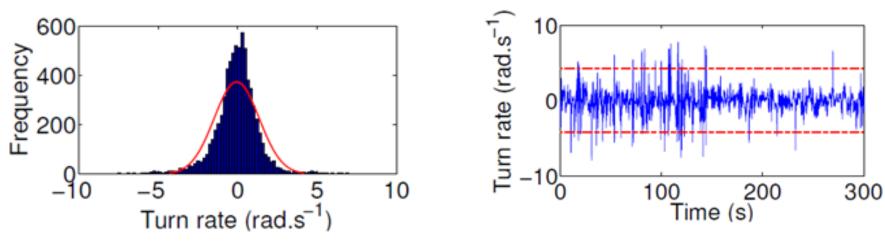


# Jump persistent turning walker 🌾 NYU

### Zebrafish turn rate probability distribution:

- Fish trajectories show sharp turns
- Time series of the turn rate indicate jumps followed by regular oscillations
- The distribution of the turn rate is leptokurtic, with fat tails and peakedness, whereby a Gaussian is not appropriate





Jump persistent turning walker 🦞 NYU

### Zebrafish turn rate SDE:

• Stochastic mean reverting jump diffusion process:

 $\mathrm{d}\omega_t = -\theta\omega_t \mathrm{d}t + \sigma \mathrm{d}B_t + \mathrm{d}J_t$ 

- The model captures large deviations in turn rate in an otherwise wellbehaved random process as the underlying PTW
- The jump term is modeled as a compound Poisson process of the form

$$J_t = \sum_{j=1}^{\nu_t} Y_j$$

- $Y_j$ , j = 1, 2, ... are independent and identically distributed (i.i.d.) Gaussian random variables
- The number of jumps  $\nu_t$  is a counting process, where for r < t,  $\nu_t \nu_r$  is a Poisson random variable with parameter  $\lambda(t-r)$

Jump persistent turning walker 🌾 NYU

### Discrete time approximation:

• A continuous time solution is obtained from Itô's integral formula and discretized to obtain at time step k

 $\omega(k) = \omega(k-1)e^{-\theta\Delta t} + \sigma\sqrt{\frac{\Delta t}{2\theta}}\left(1 - e^{-2\theta\Delta t}\right)\varepsilon(k) + \gamma\left(\nu(k\Delta t) - \nu((k-1)\Delta t)\right)\zeta(k)$ 

- $\Delta \nu = \nu(k\Delta t) \nu((k-1)\Delta t)$  is a Poisson process with parameter  $\lambda \Delta t$ , and  $\varepsilon$  and  $\zeta$  are standard Gaussian processes
- The conditional expectation of the process without jumps is  $\omega(k-1)e^{-\theta\Delta t}$
- The mean-reverting effect induces a drift towards zero
- The conditional variance of the process without jumps is  $\frac{\Delta t}{2\theta} (1 e^{-2\theta\Delta t})$
- The discrete time approximation convergences weakly to the continuous time SDE

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### Likelihood function for parameter estimation:

- We assume at most one jump in a time interval  $\Delta t$  (true for small sample rate, in our experiment  $\Delta t \simeq 1/24$ s)
- The Poisson process can be approximated by a Bernoulli-distributed random variable  $\nu(k\Delta t) \nu((k-1)\Delta t) \sim \mathcal{B}(\lambda\Delta t)$
- The conditional likelihood probability density function is

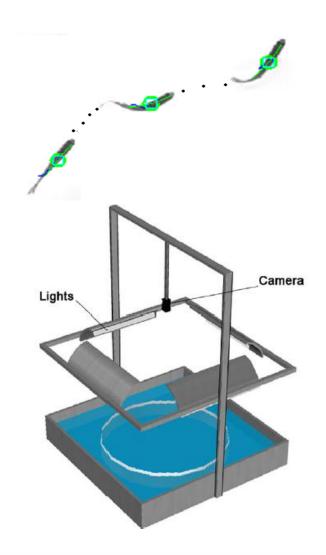
$$\begin{aligned} f_{\theta,\sigma,\lambda,\gamma}^{(k)}(\omega(k)|\omega(k-1)) &= (1-\lambda\Delta t)\phi\left(\omega(k);\omega(k-1)e^{-\theta\Delta t},\frac{\sigma^2\Delta t}{2\theta}\left(1-e^{-2\theta\Delta t}\right)\right) \\ &+\lambda\Delta t\phi\left(\omega(k);\omega(k-1)e^{-\theta\Delta t},\frac{\sigma^2\Delta t}{2\theta}\left(1-e^{-2\theta\Delta t}\right)+\gamma^2\right) \end{aligned}$$

- $\lambda \Delta t$  is the probability of a jump
- $\phi\,$  is the Gaussian probability density function
- The parameters  $\theta$ ,  $\sigma$ ,  $\lambda$ , and  $\gamma$  are estimated using my maximizing a Loglikelihood function based on experimental data over a window
- The PTW is a particular case of JPTW

# Jump persistent turning walker **Y** NYU

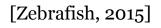
### Experimental data for validation:

- Data are from the control condition in the ethanol study in ACER, 2014
- The experimental dataset consists of trajectories of single zebrafish swimming in a large shallow water tank of 90 cm diameter
- A high-resolution wide-angle camera (GoPro Hero 3) is mounted 150 cm above the water surface to observe the fish
- Ten experimentally naive fish are recorded for five minutes at 24 frames per second
- Fish motion is tracked using peregrine



### Data processing:

- Data where excess freezing and wall following are observed in fish locomotion are excluded (two out of ten subjects)
- Data are post-processed using a Daubechies wavelet filter to remove oscillations due to fish tail beat that occur at about 2 Hz and the full 5 minute videos are considered
- The turn angle is computed based on a central difference approximation of the fish heading from the velocity
- The turn rate is obtained by dividing the turn angle  $\Delta \theta_i \simeq 1/2 \Delta \phi_i$  by the sampling rate



Turn rate reconstruction from full-shape tracking or heading yield equivalent results

 $V \cdot \theta$ 



 $\Delta \theta$ 

 $V_{i-}$ 



Jump persistent turning walker **Y** NYU systems tory

### Model calibration:

- Unfortunately, no closed-form solution exists
- Model parameters are estimated using a maximum likelihood estimation method based on the Loglikelihood
- The optimization algorithm is implemented using the MATLAB global optimization toolbox

PTW			JPTW				
ID	$\theta(s^{-1})$	$\sigma(\text{rad s}^{-1})$	$\theta(s^{-1})$	$\sigma(\text{rad s}^{-1})$	$\gamma(\text{rad s}^{-1})$	$\lambda(s^{-1})$	
1	1.582	3.260	1.470	3.001	3.064	0.011	
2	1.249	1.435	1.013	1.145	1.565	0.015	
3	1.642	2.559	1.307	1.976	2.323	0.023	
4	1.603	2.673	1.460	2.491	2.725	0.012	
5	2.101	3.751	1.779	3.390	3.451	0.012	
6	1.708	2.617	1.299	2.197	2.422	0.016	
7	1.726	2.973	1.594	2.665	2.777	0.012	
8	1.870	3.611	1.688	3.415	3.206	0.012	
Mean $\pm$ std	$1.685 \pm 0.245$	$2.860 \pm 0.732$	$1.451 \pm 0.245$	$2.535 \pm 0.765$	$2.692 \pm 0.594$	$0.014 \pm 0.004$	

# Jump persistent turning walker "NYU

### Goodness of fit:

• To compare the goodness of fit of the models, a likelihood ratio test (LRT) is used over the whole experimental window

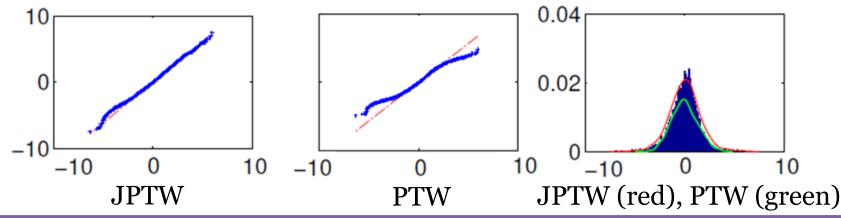
LRT ~ Loglikelihood\_JPTW –Loglikelihood\_PTW

- A chi-square test is used to assess the improvement of JPTW with respect to PTW
- The values for the LRT for the eight fish datasets suggest rejection of the null hypothesis, whereby including jumps in the fish turn rate model allows for more accurately representing live zebrafish motion
- One-way ANOVA tests are used to compare the model parameters of PTW and JPTW
- ANOVA results suggests that the baseline parameters of the PTW are not changed by including jumps

# Jump persistent turning walker **NYU**

### Model validation:

- A quantile-quantile plot (Q-Q plot) is used to compare the simulated probability distribution with real fish turn rate probability distribution (when two dataset distributions are aligned, Q-Q plot is a straight line)
- JPTW simulated data are aligned with zebrafish turn rate datasets
- JPTW model enables a robust interpretation of the components of zebrafish turn rate as opposed to PTW where jumps are discarded
- Improvement from JPTW is explained by burst-and-coast swimming style of zebrafish



zebrafish

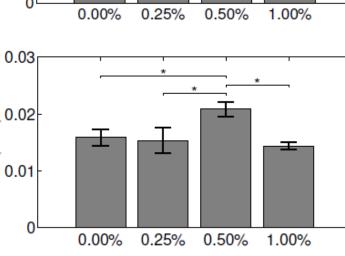
 $\lambda({
m s}^{-1})$ 

• Ethanol modulates both the relaxation rate and the jump frequency

• Likely, the increase in these parameters is related to the disinhibitory effects of ethanol, which translate in hyperactivity and novelty seeking

# Analysis of the effect of ethanol on model parameters:

- Experimental data from ACER, 2014 are used for the analysis
- The model is successful in explaining the dynamics of ethanol-treated zebrafish



[IJBC, 2015]





Extension of JPTW to include time varying speed (Collaborative: work with Prof. Mario di Bernardo):

speed

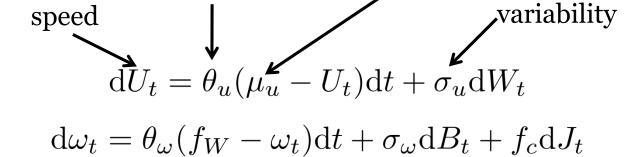
relaxation rate

Jump persistent turning walker **Y** NYU

speed

• Coupled SDEs:

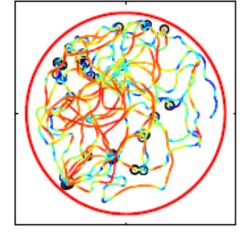
systems

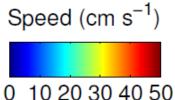


long term

mean of speed

Coupling function  $f_c = f_c(U_t, \omega_t)$  modulates the occurrence of jumps as a function of the speed and turn rate



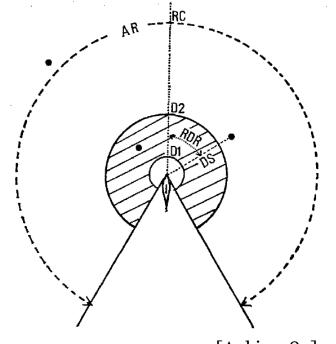


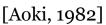
[A preliminary version without jump terms is in JMB, 2015]

# Modeling fish social behavior

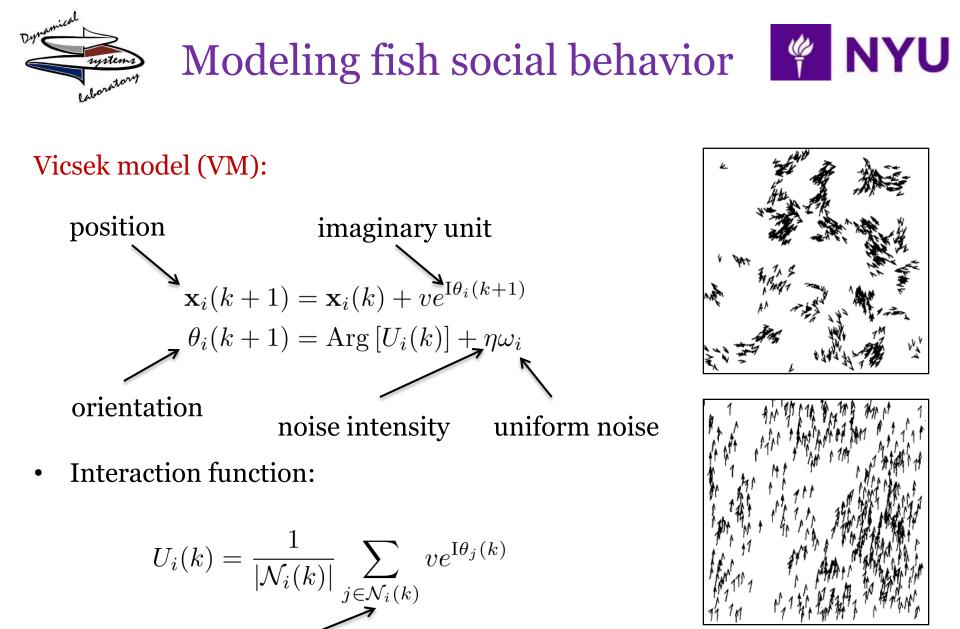
### Classical Aoki's model:

- Fish are treated as self-propelled particles at a speed independent of the group
- Fish heading is adjusted in time as a function of fish-to-fish interactions
- Interactions are based on physical distances and the rules posit alignment, attraction, and repulsion
- Simulation results often demonstrate exhibit swarming, milling, and other patterns exhibited by social groups
- The model has several tuning parameters and is difficult to analytically treat





**VINYU** 



[Vicsek et al, 1995]

set of connected neighbors

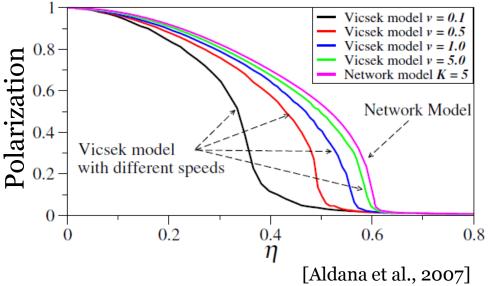


# Modeling fish social behavior

### Vectorial network model:

- The model is completely metricfree
- Neighbors are randomly selected from the whole group based on random mixing
- The model is expected to correspond to the VM for large speed

$$\theta_i(k+1) = \operatorname{Arg} \left[ U_i(k) \right] + \eta \omega_i$$
$$U_i(k) = \frac{1}{|\mathcal{N}_i(k)|} \sum_{j \in \mathcal{N}_i(k)} v e^{\mathrm{I}\theta_j(k)}$$



• Coordination is measured through the polarization:

$$\operatorname{Pol} = \lim_{k \to \infty} \operatorname{E} \left[ 1/N \left| \sum_{i=1}^{N} e^{\operatorname{I} \theta_{i}(k)} \right| \right]$$

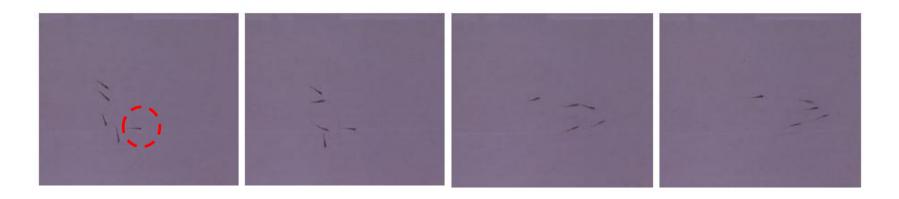
NYU





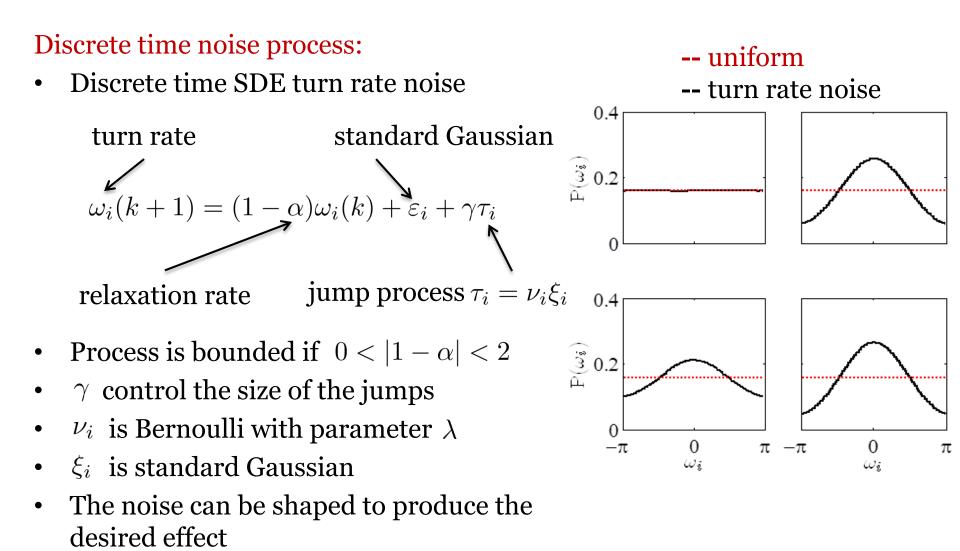
### Adapting existing models to zebrafish [IJNS, under review]:

- The traditional VM and VNM models are augmented with the noise process shaping the JPTW
- Each zebrafish is associated with a self-propelled particle that
  - travels at a constant speed in a square domain with periodic boundary conditions
  - updates its heading to align with geographically proximal neighbors for the VM and with randomly selected group members for the VNM













#### Model with turn rate noise:

$$VM-\omega \begin{cases} \mathbf{x}_{i}(k+1) = \mathbf{x}_{i}(k) + ve^{I\theta_{i}(k+1)} \\ \theta_{i}(k+1) = \operatorname{Arg} \left[ \frac{1}{|\mathcal{N}_{i}(k)|} \sum_{j \in \mathcal{N}_{i}(k)} ve^{I\theta_{j}(k)} \right] + \eta \omega_{i}(k) \\ \omega_{i}(k+1) = (1-\alpha)\omega_{i}(k) + \varepsilon_{i} + \gamma \tau_{i} \end{cases}$$

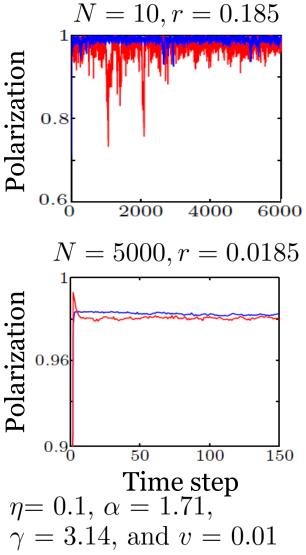
$$\text{VNM-}\omega \begin{cases} \theta_i(k+1) = \text{Arg}\left[\frac{1}{|\mathcal{N}_i(k)|} \sum_{j \in \mathcal{N}_i(k)} v e^{\mathrm{I}\theta_j(k)}\right] + \eta \omega_i(k) \\ \omega_i(k+1) = (1-\alpha)\omega_i(k) + \varepsilon_i + \gamma \tau_i \end{cases}$$





### Group coordination in VM:

- Groups of small size are more affected by jumps in the turn rate
- For small group size, a large turn in the orientation of a single individual can disorganize for the entire group
- Jumps in the heading of an individual can propagate through the group
- Groups of large size are less affected by jumps of a subset of particles
- Large groups achieve coordination faster when compared to small group size

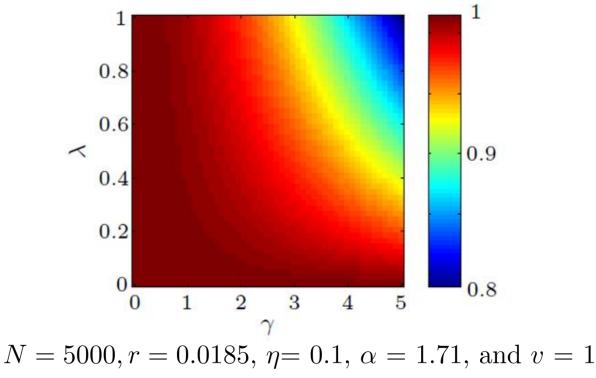






### Effects of jumps on group coordination in VM:

- The polarization is a decreasing function of jump parameters  $\lambda~$  and  $~\gamma~$
- The jump frequency  $\lambda\,$  affects the group coordination more than the jump amplitude  $\gamma\,$

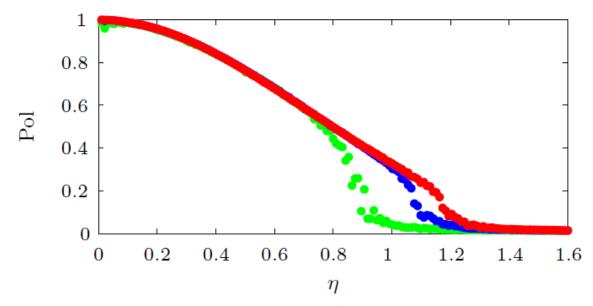






#### Phase transition in the VM:

• For large groups, a phase transition is observed and its location depends on the radius of interaction



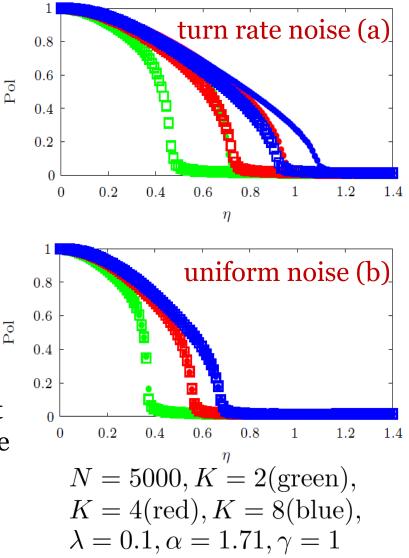
r = 0.0185 (green), r = 0.0375 (blue), and r = 0.05 (red) corresponds approximately on average to K = 2, 5, and 8 connected neighbors





### Neighbors' selection in the VNM:

- Neighbor selection affects the polarization in the VNM:
  - (a) VNM with turn rate noise
  - (b) traditional VNM with uniform noise
  - (dots) an individual is always included in its K selected neighbors
  - (squares) connected neighbors are randomly chosen
- When each individual always selects itself, the phase transition is attained at a higher noise in the VNM with turn rate
- The traditional VNM with random selection of neighbors is not affected by neighbor selection

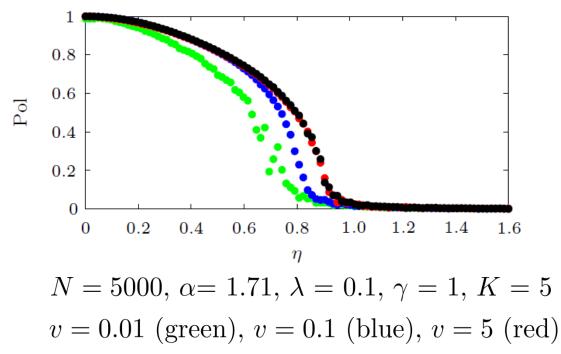






### Comparison of VM and VNM for different speeds:

- For high speeds, there is random mixing of the individuals
- The polarization of the VM with turn rate noise (colors) converges to the polarization of the VNM with turn rate noise (black) where each individual includes itself in its subset of connected neighbors







### Linearized VNM:

• Linear approximation for small perturbation with respect to a common heading

$$\psi_i(k+1) = \frac{1}{K} \sum_{j=1}^K \psi_{i_j}(k) + \eta \omega_i(k)$$
$$\omega_i(k+1) = \omega_i(k)(1-\alpha) + \varepsilon_i + \gamma \tau_i$$

by defining  $\psi_i(k) = \theta_i(k) - \theta_0$  where  $\theta_0$  is the synchronized state We define  $\psi(k) = [\psi_1(k), \dots, \psi_N(k)]^T$  and  $\omega(k) = [\omega_1(k), \dots, \omega_N(k)]^T$ 

$$\psi(k+1) = W\psi(k) + \eta\omega(k)$$
$$\omega(k+1) = \omega(k)(1-\alpha) + \varepsilon + \gamma\tau$$

where W is a random variable defined such that its rows are i.i.d. vectors with K randomly selected entries taking value1/K, while any other entry equals 0





### Mean square behavior of the linearized VNM:

- The linearized VNM is a linear consensus problem over so-called numerosity-constrained networks with additive uniform noise  $\omega_i$ , numerosity K 1, weight 1/K, and graph Laplacian matrix  $K(I_N W)$
- We set  $\xi(k) = \psi(k) \overline{\psi}$  to investigate the mean square behavior of the stochastic system through the autocorrelation matrix  $\Xi(k) = E\left[\xi(k)\xi(k)^{T}\right]$
- By analyzing the deterministic dynamics of the autocorrelation, we find
  - the asymptotic convergence rate in the absence of noise:

$$r_a = \sup_{||\xi(0)|| \neq 0} \lim_{k \to \infty} \left( \frac{\mathrm{E}[||\xi(k)||^2]}{||\xi(0)||^2} \right)^{\frac{1}{k}}$$

- the steady state mean square deviation (for a stable system):

$$\delta_{\infty} = \lim_{k \to \infty} \mathbf{E} \left[ \|\xi(k)\|^2 \right]$$





Analytical solution of the linearized VNM adapted from Abaid and Porfiri, 2012:

• The asymptotic convergence factor of the consensus protocol in the absence of noise is:

$$r_a = 1 - \frac{(K-1)\left(N + K(N^2 - N - 1)\right)}{K^2(N-1)^2}$$

• In the presence of noise, for  $r_a < (1 - \alpha)^2 < 1$ , the mean square deviation converges to a finite value

$$\delta_{\infty} = \eta^2 \frac{\mu(N-1)}{1-r_a}$$

with

$$\mu = \frac{1 + \lambda \gamma^2}{1 - (1 - \alpha)^2} \left( 1 + 2 \frac{(1 - \alpha) \left( 1 - \frac{N(K - 1)}{K(N - 1)} \right)}{1 - (1 - \alpha) \left( 1 - \frac{N(K - 1)}{K(N - 1)} \right)} \right)$$

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### Closed-form expression for the polarization:

• Using the steady state deviation from the linearized model

Pol 
$$\simeq 1 - \eta^2 \frac{\mu}{2N} \frac{K^2 (N-1)^3}{(K-1) (N + K(N^2 - N - 1))}$$

• Proof: recall Pol = 
$$\lim_{k \to \infty} E\left[\frac{1}{N} \left| \sum_{i=1}^{N} e^{I\theta_i(k)} \right| \right]$$

then substitute  $\theta_i(k) = \theta_0 + \psi_i(k)$ , i = 1, ..., N, in the expression of Pol, use a Taylor expansion of cosine and sine in  $e^{I(\cdot)}$ , and consider a first order expansion of the square root to find  $Pol \simeq 1 - 1/2N\delta_{\infty}$ 





### Analytical predictions:

• For a small noise intensity, the analytical expression provides a good approximation for the polarization (other salient parameters are kept fixed)

$\eta$	0.0	0.02	0.05	0.10	0.19
$\operatorname{Pol}_{(K=2)}^{\operatorname{num}}$	1	0.99973	0.99752	0.99011	0.96115
$\operatorname{Pol}_{(K=2)}^{\operatorname{an}}$	1	0.99973	0.99760	0.99040	0.96160
Error	0	0.00000	0.00008	0.00029	0.00045
$\operatorname{Pol}_{(K=5)}^{\operatorname{num}}$	1	0.99973	0.99755	0.99024	0.96159
$\operatorname{Pol}_{(K=5)}^{\operatorname{an}}$	1	0.99973	0.99760	0.99040	0.96162
Error	0	0.00000	0.00005	0.00016	0.00003
$\operatorname{Pol}_{(K=8)}^{\operatorname{num}}$	1	0.99972	0.99751	0.99010	0.96094
$\operatorname{Pol}_{(K=8)}^{\operatorname{an}}$	1	0.99973	0.99755	0.99019	0.96077
Error	0	0.00001	0.00004	0.00010	0.00017

• For larger noise intensities, the analytical prediction fails to capture the plateau effect observed in the numerical simulations



## Conclusions



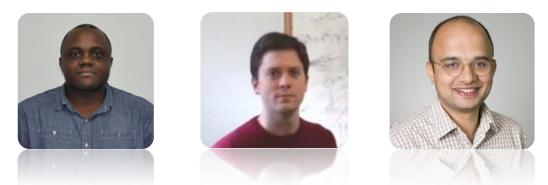
- Zebrafish may be the third millennium mouse and we are trying to contribute technological and modeling tools to study their behavior
- A valid model for zebrafish motion can aid the design of *in-silico* experiments, complementing preclinical animal research
- Zebrafish swimming is composed of **burst-and-coast** events that require a special treatment in a data driven model
- A stochastic mean reverting jump diffusion model is used to accurately describe zebrafish as a jump persistent turning walker (JPTW)
- The JPTW model can be included in traditional models of collective behavior, such as the Vicsek and the vectorial network models
- We still need to validate the model of zebrafish schooling and we are working on that
- A lot of work is needed to expand the framework to study the effect of robotic stimuli, predators, walls...







# Thank you!



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