EPA
United States
Environmental Protection
Agency

# Dysmorphology and Locomotor Activity Assessment In Larval Zebrafish (*Danio Rerio)*

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*This presentation does not necessarily reflect official US EPA policy.*

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Center for Computational Toxicology and Exposure/ Biomolecular and Computational Toxicology Division/Advanced Experimental Toxicology Models Branch
Research Triangle Park, North Carolina

50 EPA at
Progress for a Stronger Future

**To screen and prioritize chemicals for developmental neurotoxicology, the United States Environmental Protection Agency is utilizing:**

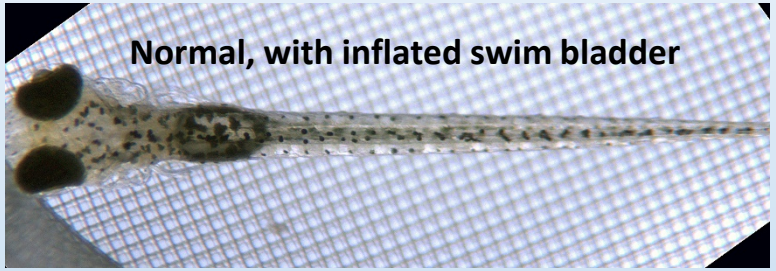
- non-mammalian models
- higher throughput testing approaches
- behavioral  endpoints

**Careful consideration of confounders must be considered:**

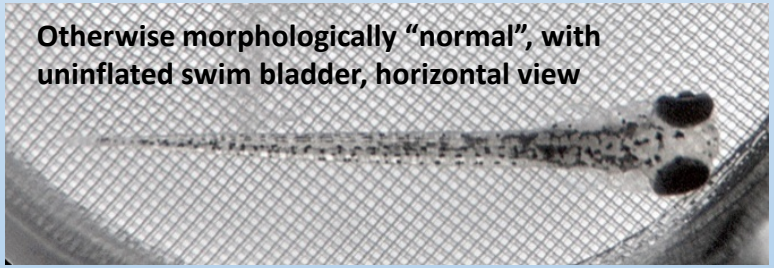
- rarely addressed in behavioral publications
- morphological assessment

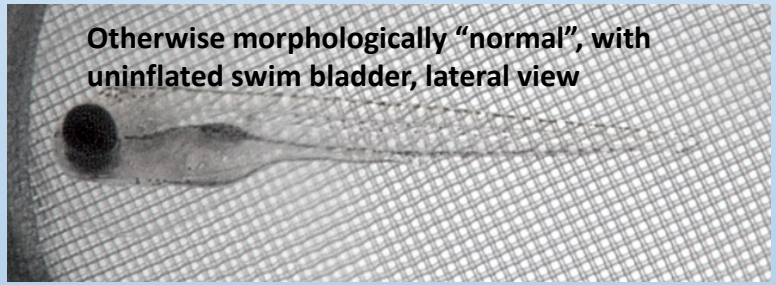
# Morphological Assessment of Zebrafish Larvae at 6 Days Post Fertilization

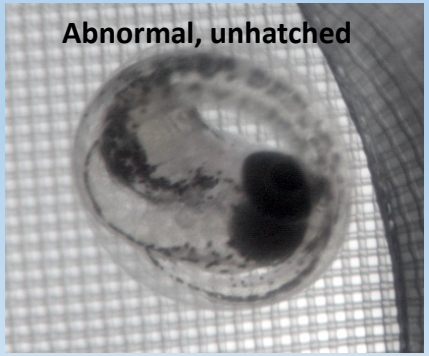
## Normal larvae:

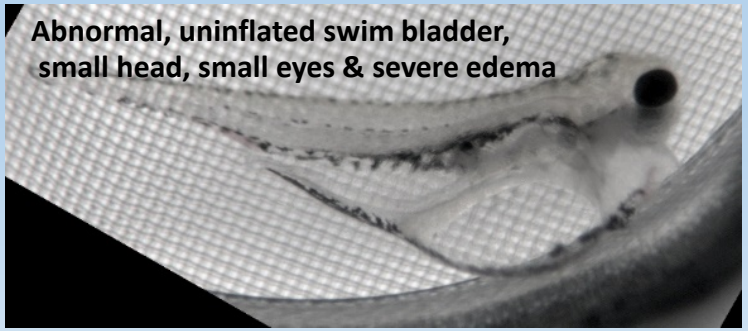



## Common malformations:



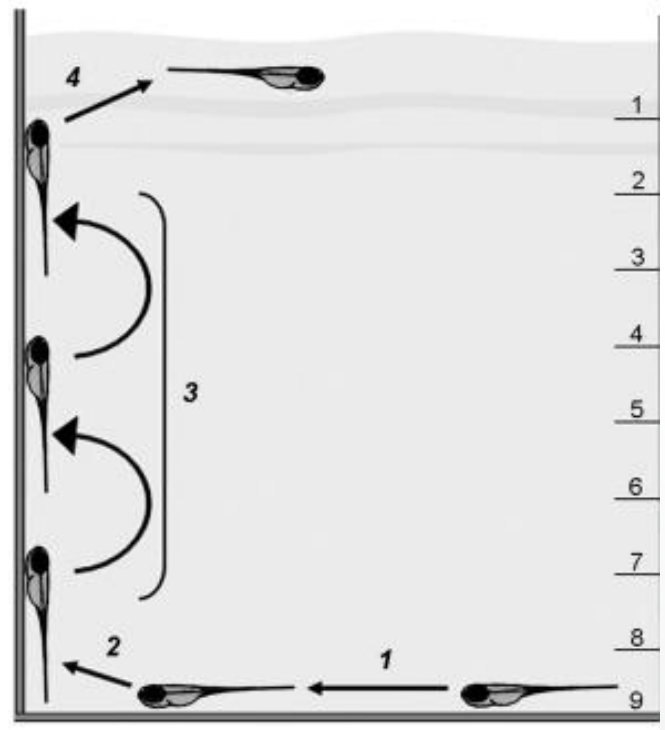




**The swim bladder (posterior) maintains buoyancy and without it larvae will expend more energy to move through the water column, thus it is considered essential for survival.**

- Develops as a two chamber organ. The first chamber, the posterior swim bladder, develops about 3 days post hatch.

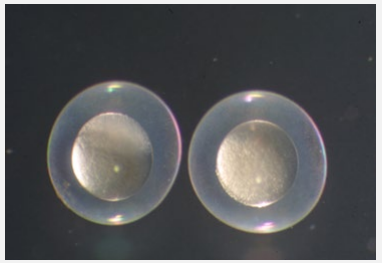
- Zebrafish larvae require a breath of air to inflate the first swim bladder chamber.



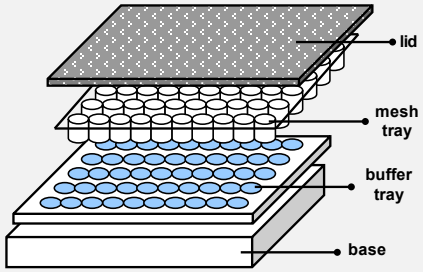

**Zebrafish larvae, 6 days post fertilization with first chamber of swim bladder inflated**

Lindsey et al. 2010. From inflation to Floatation: Contribution of the Swimbladder to Whole-Body Density and Swimming Depth During Development of the Zebrafish (*Danio rerio*) *Zebrafish* 7(1)

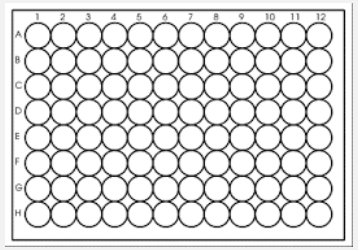
# EXPERIMENTAL METHODOLOGY



**Embryos Washed**
- 1- 2 hours post fertilization
- 0.06% bleach

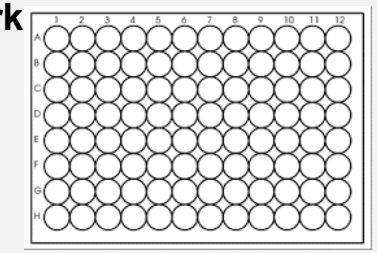



**Embryos Plated**
- 6 to 8 hours post fertilization
- 1 embryo / well
- 10% Hanks' buffer



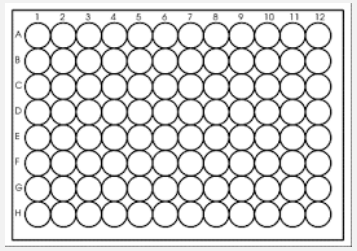
**Embryos Treated**
- days post fertilization 0 and 3 only
- Control (0.4 % dimethyl sulfoxide) or chemical

14:10 light : dark 26ºC



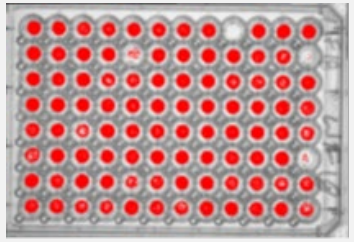
**Depuration**
- 5 days post fertilization
- Embryos changed to 10% Hanks' buffer

14:10 light : dark 26ºC



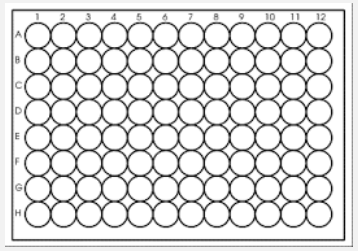
**Solution Change**
- 6 days post fertilization
- 10% Hanks' buffer

26ºC

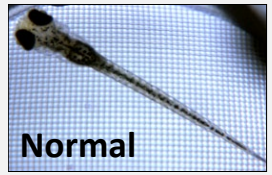


**Behavioral Testing**
- 6 days post fertilization
- 20 min. basal dark
- 40 minute light
- 40 minute dark

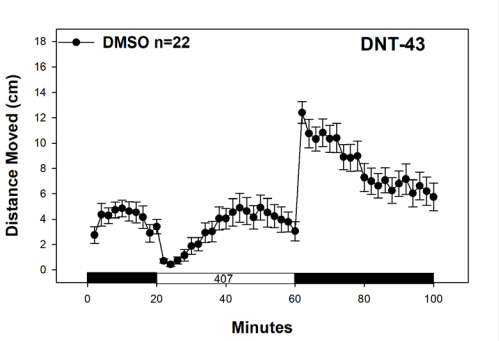
26ºC



**Assessment Post Test**
- 6 days post fertilization
- Gross morphology and survival

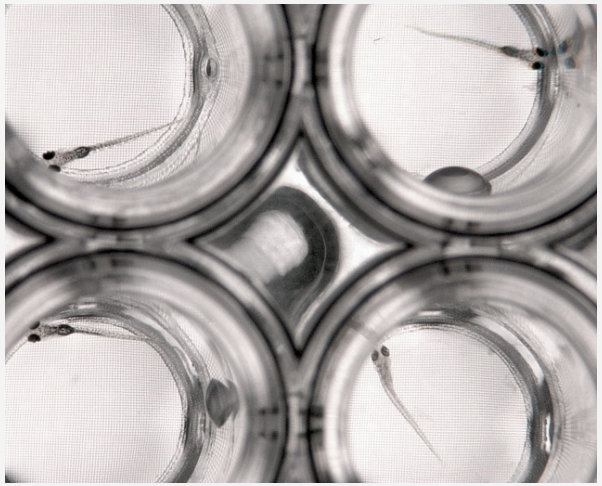


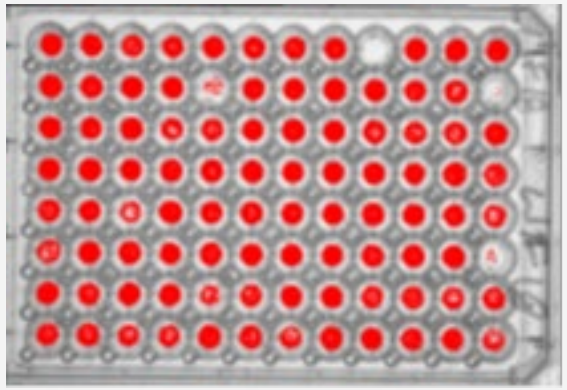


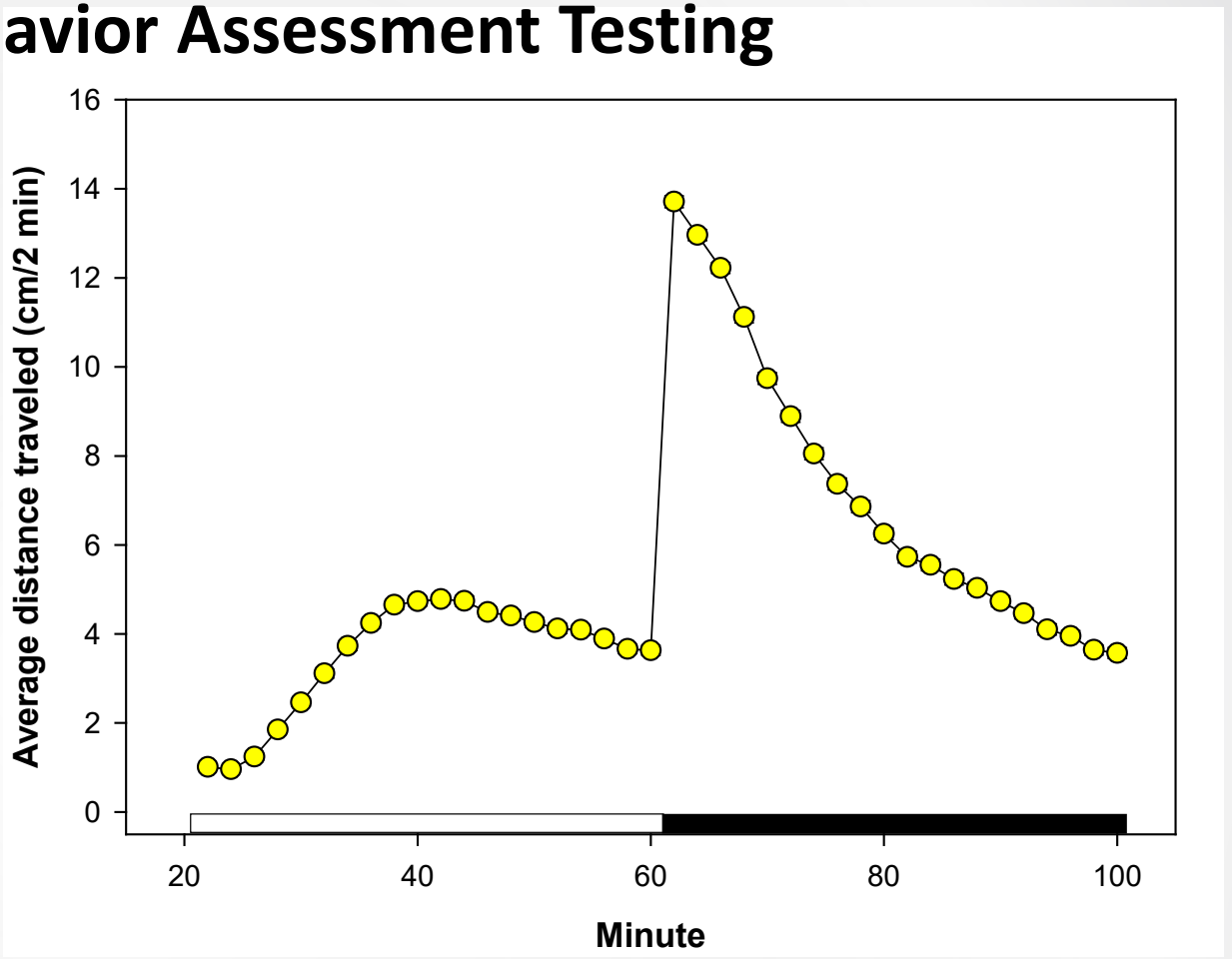



**Tracking of behavior and data analysis**

# Locomotor Behavior Assessment Testing



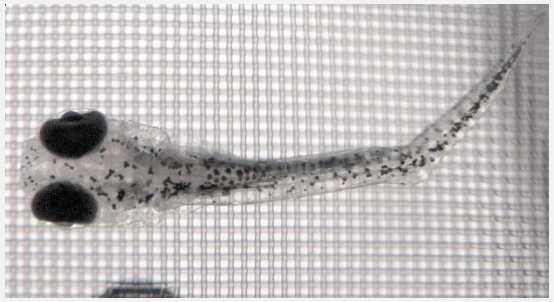




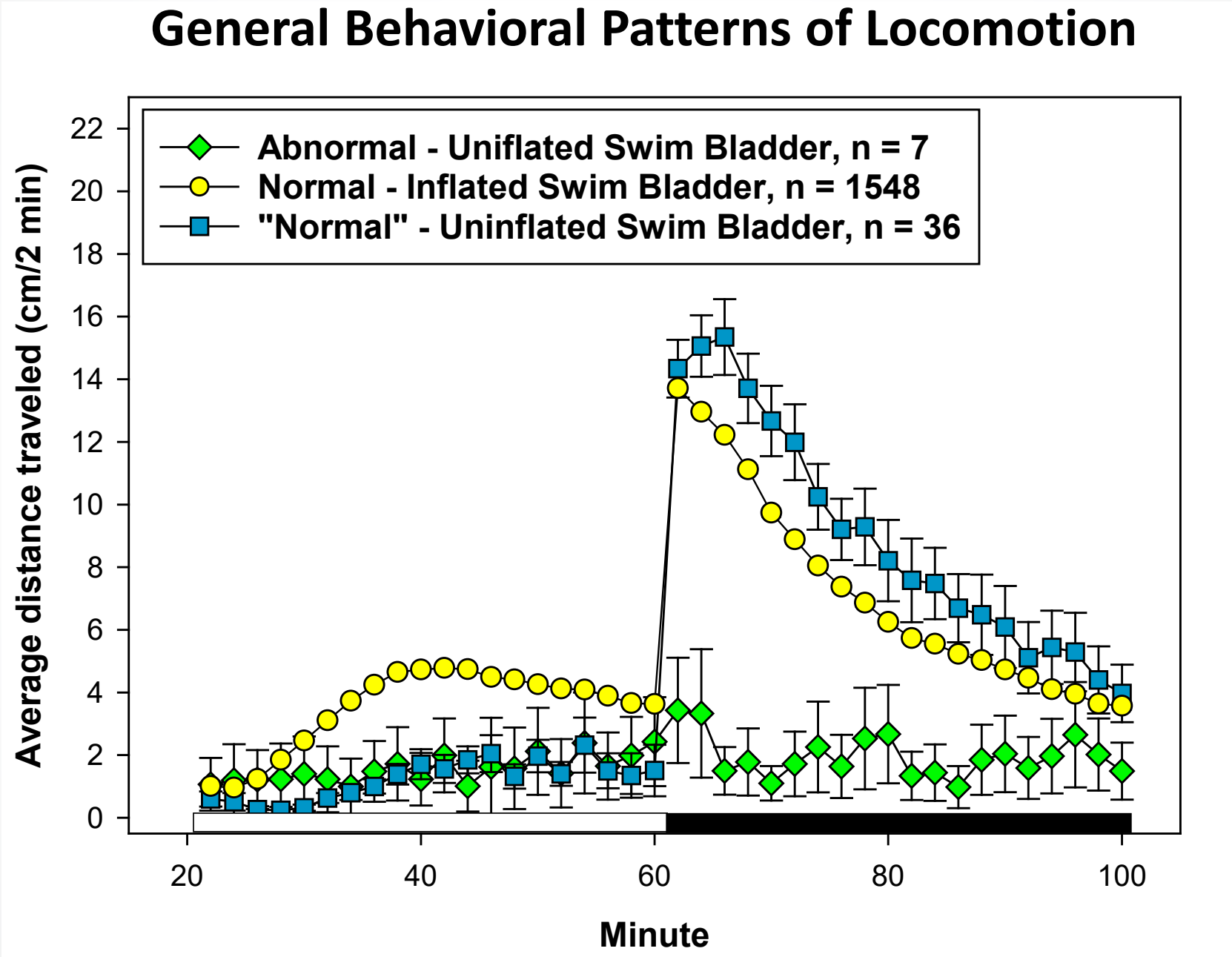

- All testing was performed on 6 days post fertilization larvae in the same 96 well plates where they were dosed and reared
- Luminance of the light phase of testing was ~ 18 lux visible light
- All testing occurred between 1230 and 1630h

- For testing, plates were transferred to the light box of the Noldus Tower Tracking System (Noldus Information Technology, Leesburg, VA) and held for a 20 min basal dark acclimation period, followed by 40 minutes of light, followed by 40 minutes of dark
- Fish movement (locomotion) was tracked from videos using Ethovision Software Version 13 (Noldus )

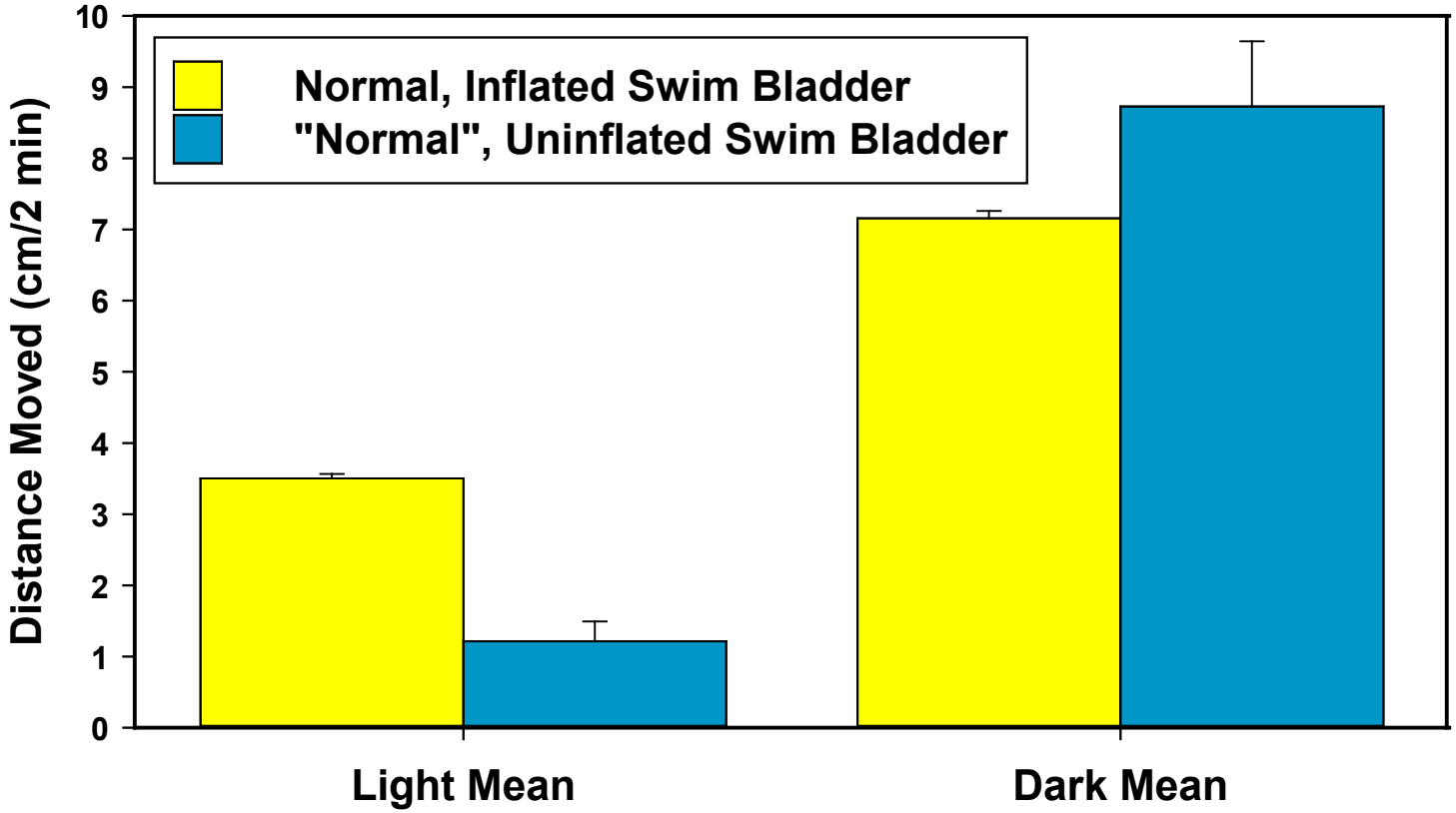
# Data on 1591 CONTROL zebrafish larvae were examined:

| Example | # of larvae | Condition of larvae at 6 days post fertilization |
|---|---|---|
|  | 7 | Morphologically Abnormal with Uninflated Swim Bladder |
|  | 36 | Otherwise Morphologically “Normal” with Uninflated Swim Bladder |
|  | 1548 | Morphologically Normal with Inflated Swim Bladder |

# General Behavioral Patterns of Locomotion

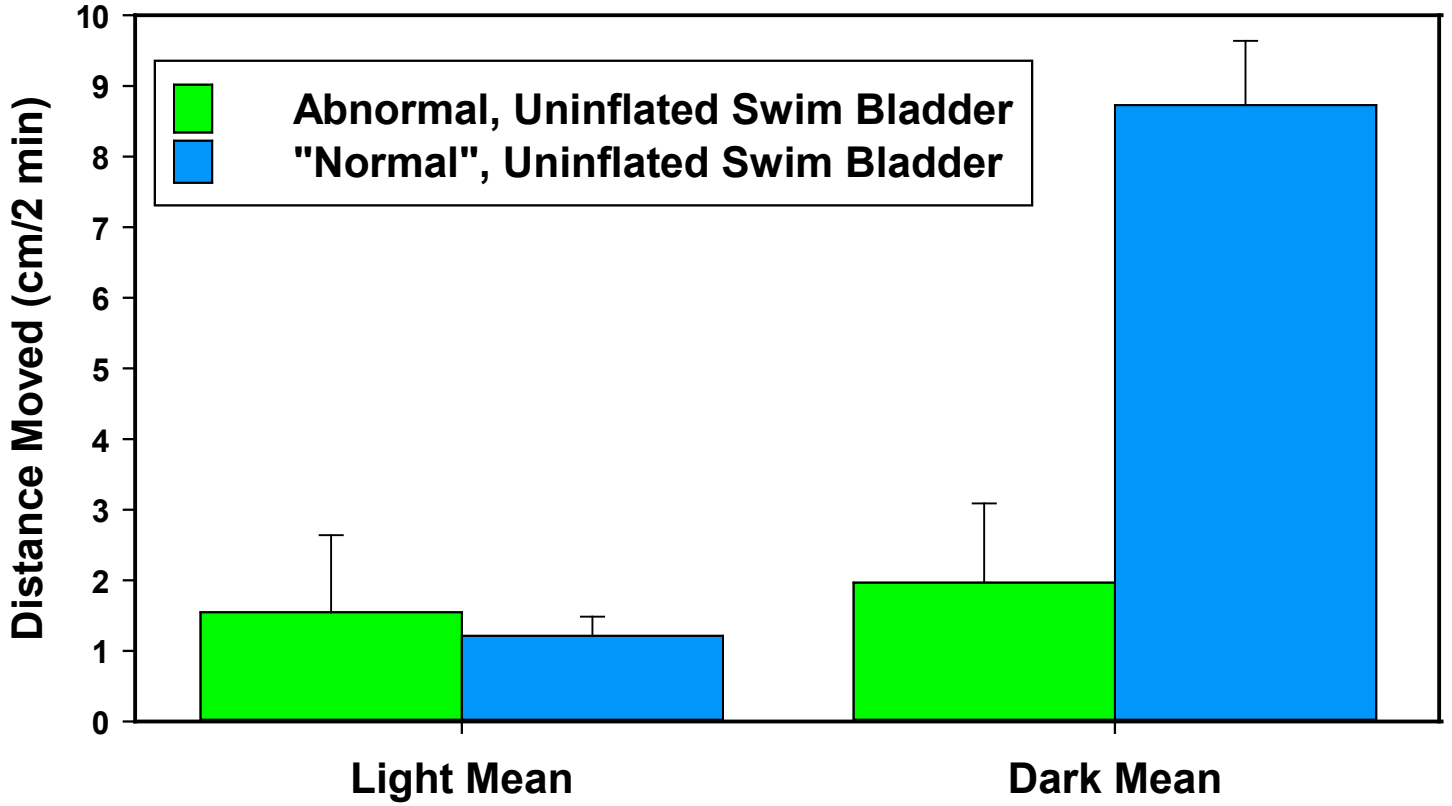


# In otherwise morphologically “normal” larvae, swim bladder inflation status made a difference in locomotor activity level in both the light and the dark phases

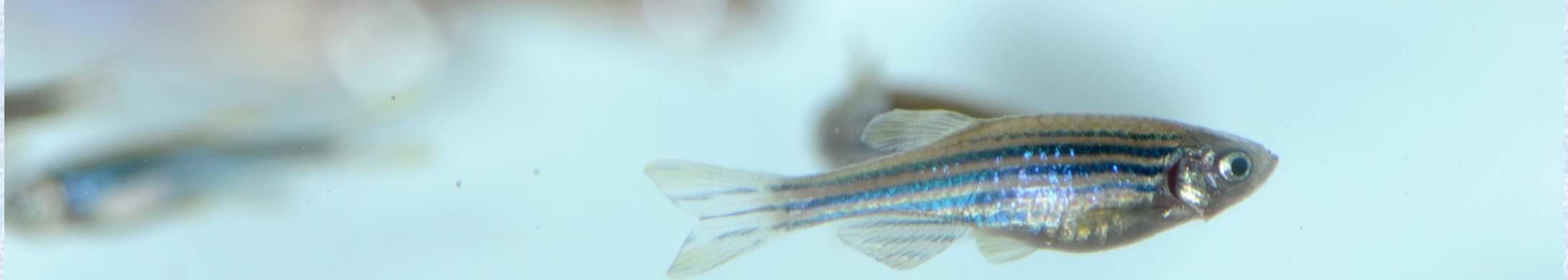



- Otherwise morphologically “normal” larvae with uninflated swim bladders (blue bars) showed 3 times less activity in the light phase than normal larvae with inflated swim bladders (yellow bars).
- Conversely, in the dark phase, otherwise morphologically “normal” larvae with uninflated swim bladders (blue bars) had slightly more activity than normal larvae with inflated swim bladders (yellow bars).

# Morphology status of zebrafish larvae with uninflated swim bladders made a difference in locomotor activity in the dark phase but not in the light phase




- There was no difference in activity between abnormal zebrafish larvae with uninflated swim bladders (green bars) and otherwise morphologically “normal” zebrafish larvae with uninflated swim bladders (blue bars) in the light phase.
- However, in the dark phase, abnormal zebrafish larvae with uninflated swim bladders (green bars) had 4.5 times less activity than otherwise morphologically “normal” zebrafish larvae with uninflated swim bladders (blue bars).



# SUMMARY AND CONCLUSIONS

- **Both swim bladder inflation and dysmorphology profoundly affect behavior in zebrafish larvae and therefore are significant confounding variables in the locomotor behavioral assay.**

- **These data illustrate the importance of morphological assessments and reporting in larval zebrafish behavior testing and the need to control for these variables.**

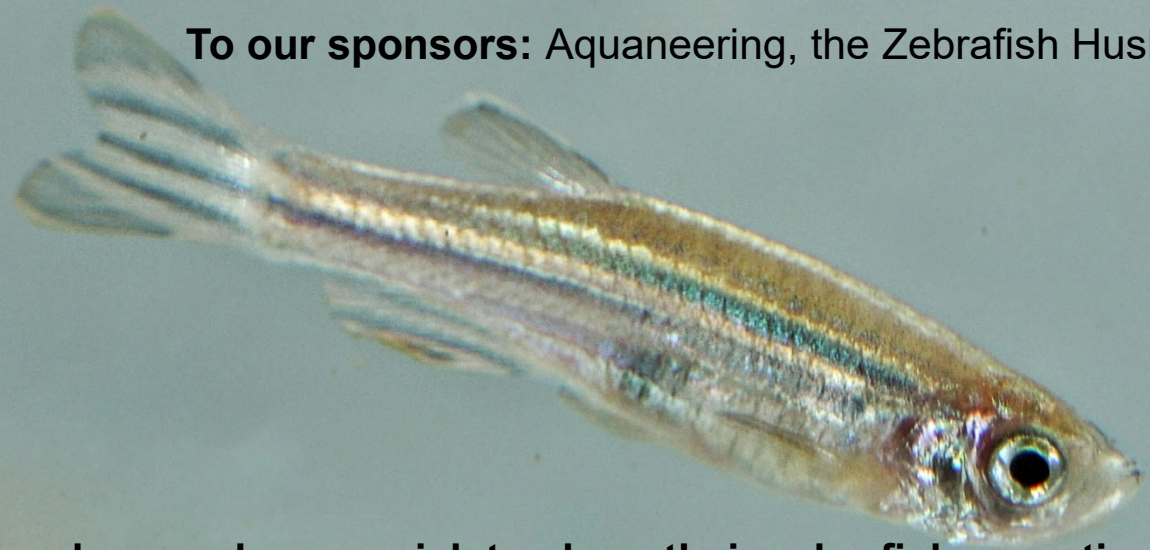
- **As human health and ecotoxicology rely more heavily on behavioral assessments in zebrafish and other non-mammalian species, it is essential that consideration of confounders be carefully addressed.**

# THANK YOU!

**To my ORISE & EPA collaborators:** Matthew Waalkes, David Korest, Bridget Hill, Kimberly Jarema, Deborah Hunter & Stephanie Padilla

**To the Animal Colony Care & Veterinary contract staff:** Donald Holman, Guilermo Orozco, Femi Yerumo, Clark Kridler, Jenelle Dunn, Kimberly Wingate & Leslie Jarrell

**To our sponsors:** Aquaneering, the Zebrafish Husbandry Association & Tecniplast



**And to those who are always quick to share their zebrafish expertise & advice:** Christian Lawrence, Jim Burris, Jr., Susan Farmer, Michelle Altemara & Marc Tye