

The effect of phenylpropanolamine on the development of the lateral line system, heart rate, and morphology of *Danio rerio* larvae.



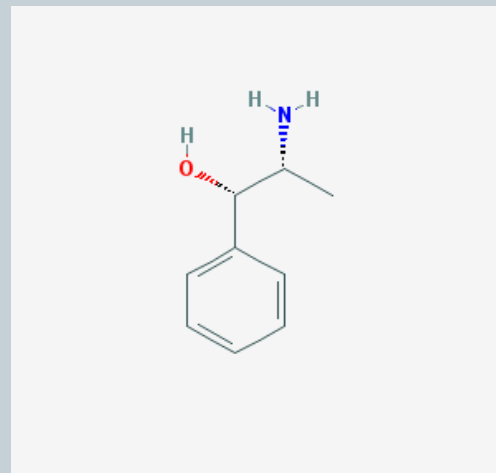
CASSIE OLSON



Phenylpropanolamine (PPA)



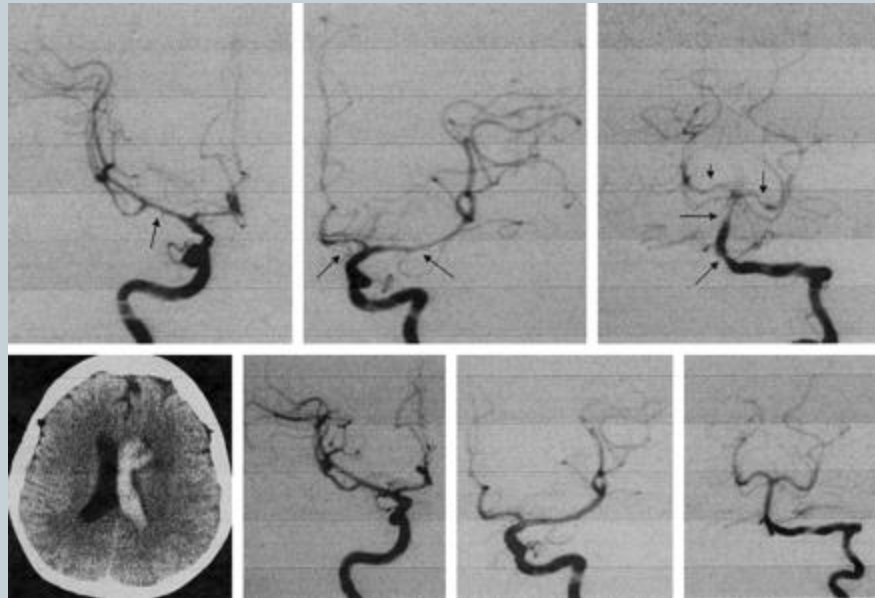
- Sympathomimetic amine
- Binds to α_1 - and α_2 -adrenoreceptors (Flavahan 2005)
- Causes the release of norepinephrine (Flavahan 2005)
- Vasoconstrictor
- Over-the-counter cough and cold medications and appetite suppressants



Phenylpropanolamine (PPA)



- Removed from market in 2000 due to causing stroke.
- Remains in veterinary medicine (Claeys et al. 2011).
- May still be present in homes (Delorio 2002).



Cantu et al. 2003

Previous Research

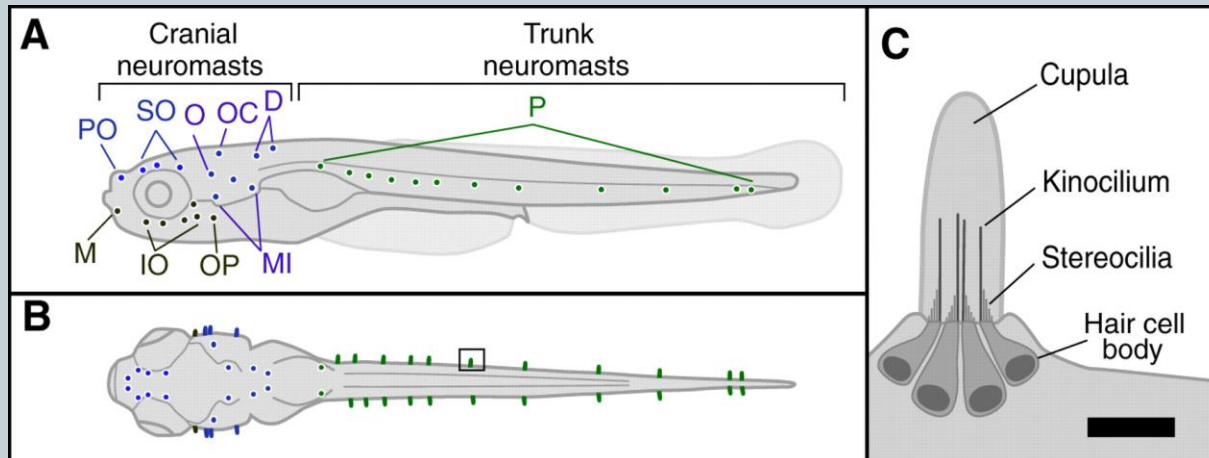


- Effects of PPA on development have been studied by Anna Galle (2013).
 - Significant effect on touch response of developing zebrafish embryo.
- Effects on the developing lateral line system have not yet been studied.

Lateral Line System



- Lateral line system
 - Used to sense and escape the strike of predators (McHenry et al. 2009) and also for schooling.
 - Stimulation causes escape response.
- Structure
 - Neuromasts dispersed over the length of the body.

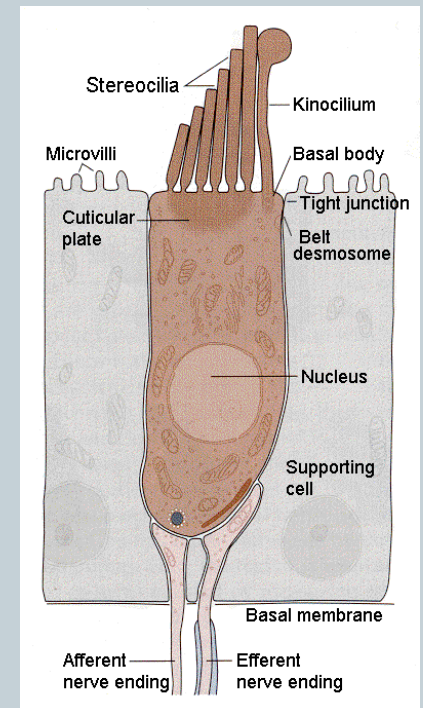
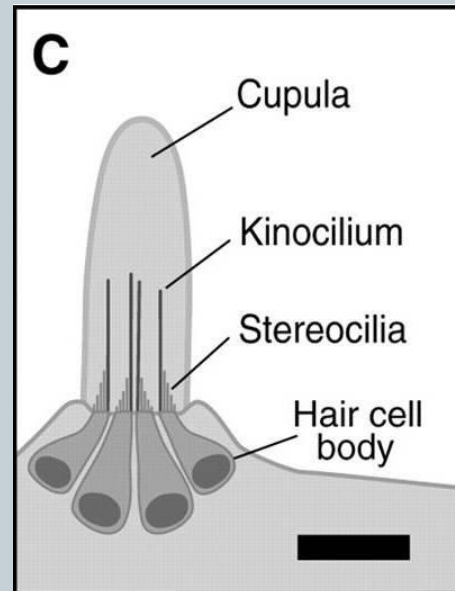


McHenry et al. 2008.

Relevance



- Hair cells in neuromasts of the lateral line system are similar to hair cells in the mammalian ear.
 - Hair cells in the ear have vestibular and auditory functions.
- Drugs that affect the hair cells in neuromasts may also affect the hair cells in the ear.



To learn more...



- Effects of PPA on the development of the lateral line system of *Danio rerio* (zebrafish).
- If development is affected, there should be a delayed or absent escape response.
- Hypothesis:
 - Higher concentrations of PPA would have a greater affect on the lateral line system and therefore cause a delayed or absent escape response.

Experiment



- Incubated zebrafish embryos in various concentrations of PPA
- Gathered data on reaction time by stimulation of the lateral line
- Heart rate recorded before and after recovery
- Morphology examined

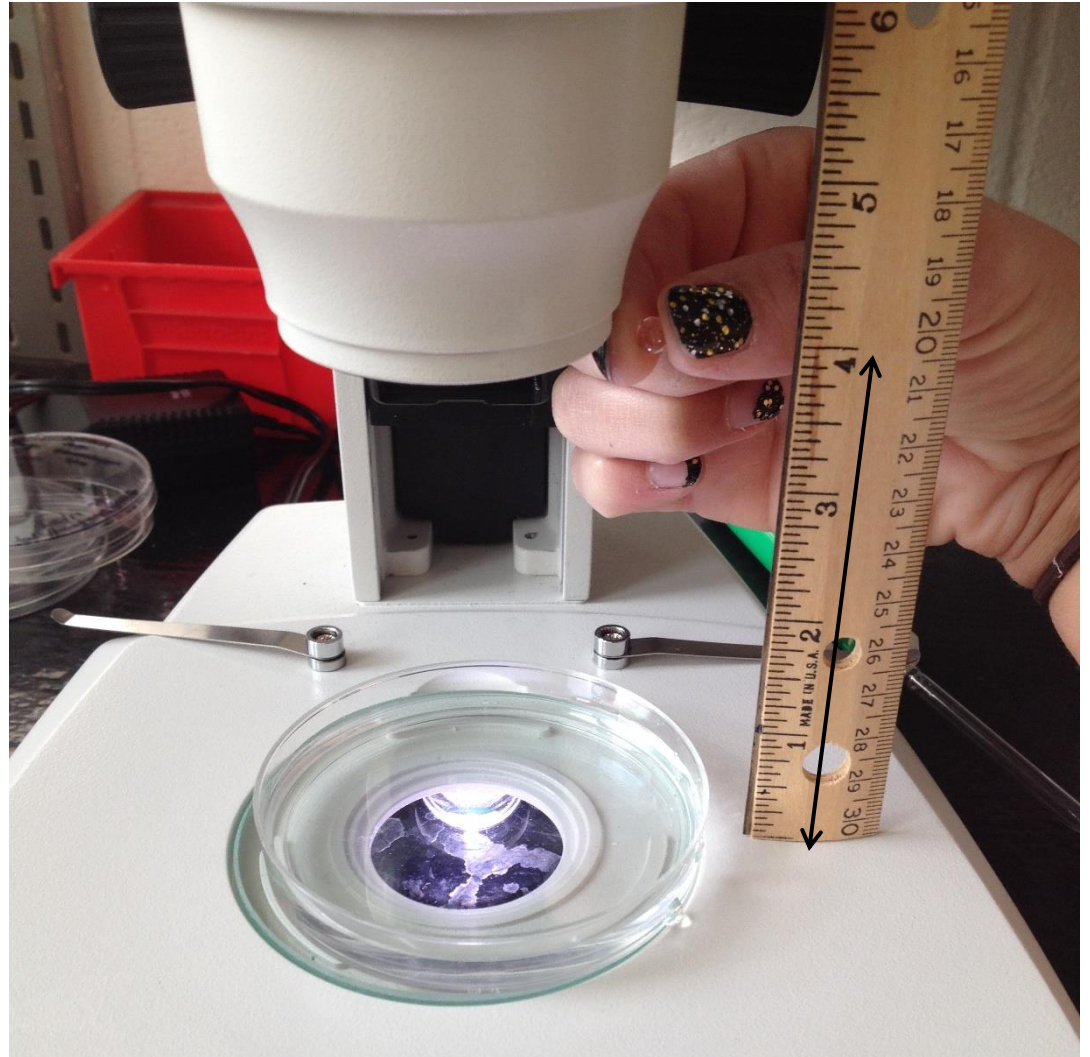
Methods



- Reaction assay
 - Embryos incubated at 28°C
 - PPA solutions
 - ✦ Concentrations (mM): 0.25, 0.50, 0.75, 1.0, 1.5
 - Exposed 20 embryos to each PPA solution at 18 hpf
 - Incubated until 5 dpf at 28°C
 - Recorded survival
 - Assayed larvae by stimulating lateral line system
 - Video of response



- Bead=0.2310 g
- Dropped bead from 4 inches above dish
- Response videotaped
- Reaction time determined
- Two-tailed t-test
- Regression analysis



Assay set-up

Video of Response



Results: Survival



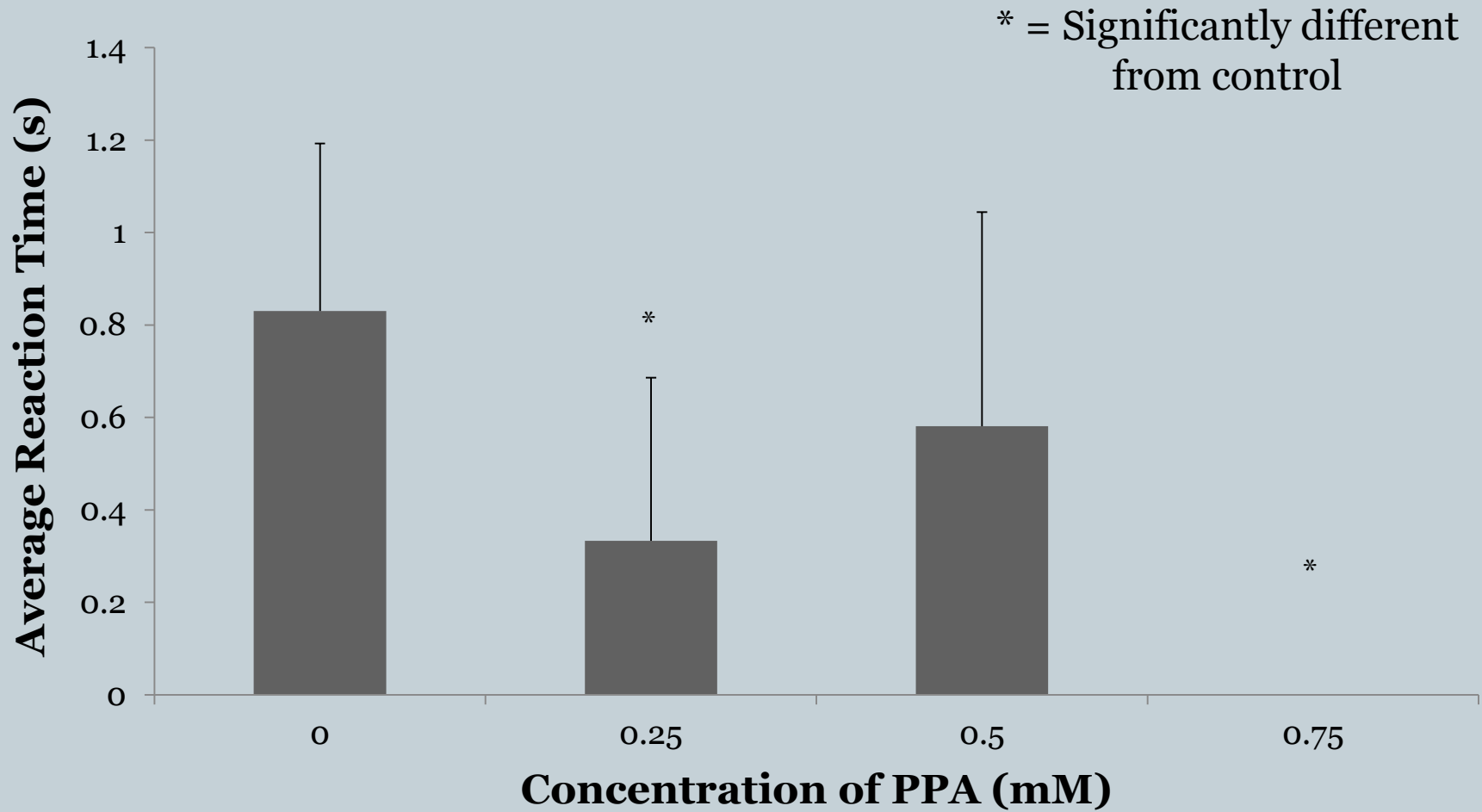
- Survival rate of larvae incubated in various concentrations of PPA at 5 dpf

	Control	0.25 mM	0.50 mM	0.75 mM	1.00 mM	1.50 mM
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Percent Survival

(%)	100	95	100	85	0	0
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Results: Reaction

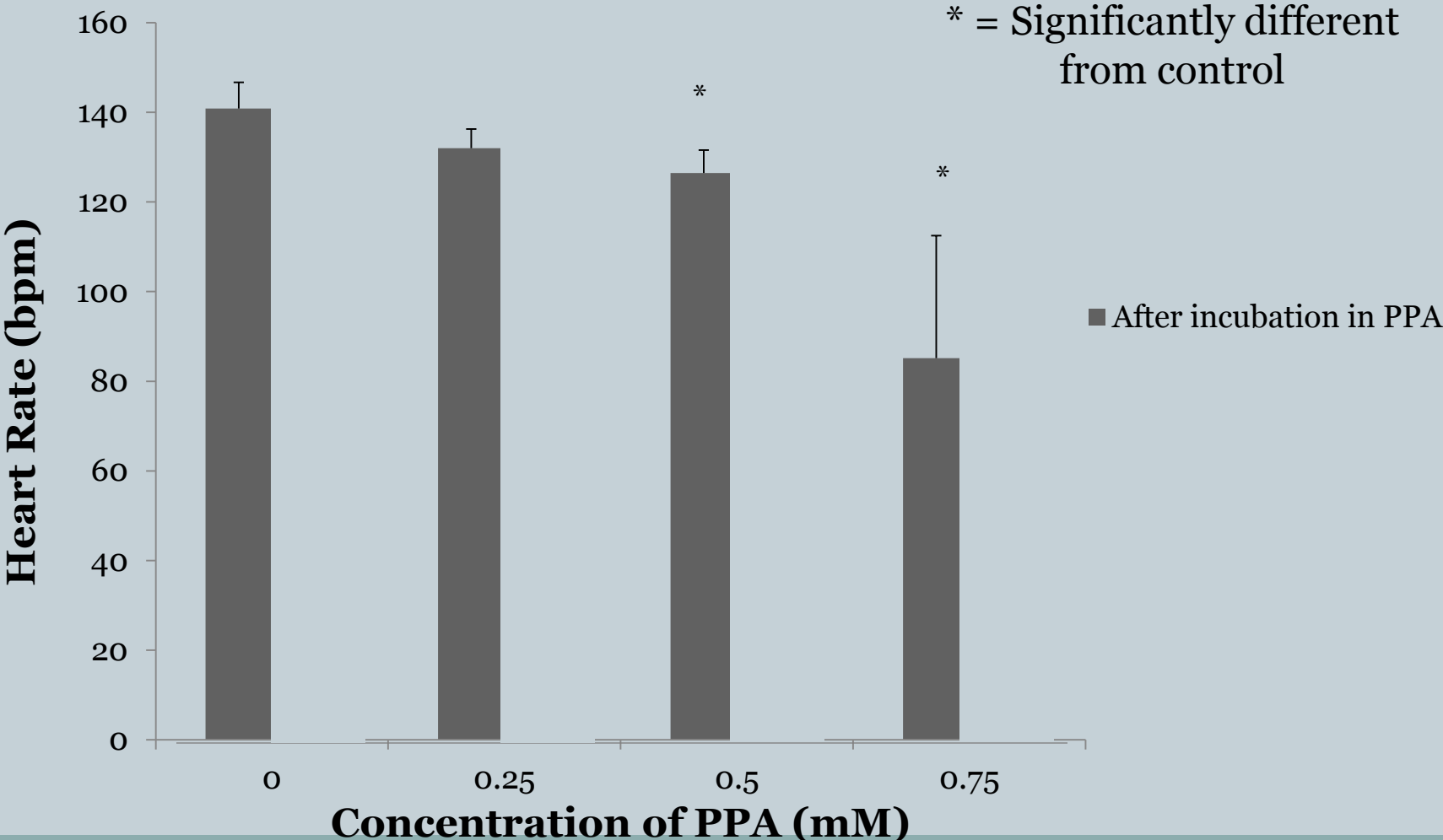


Methods

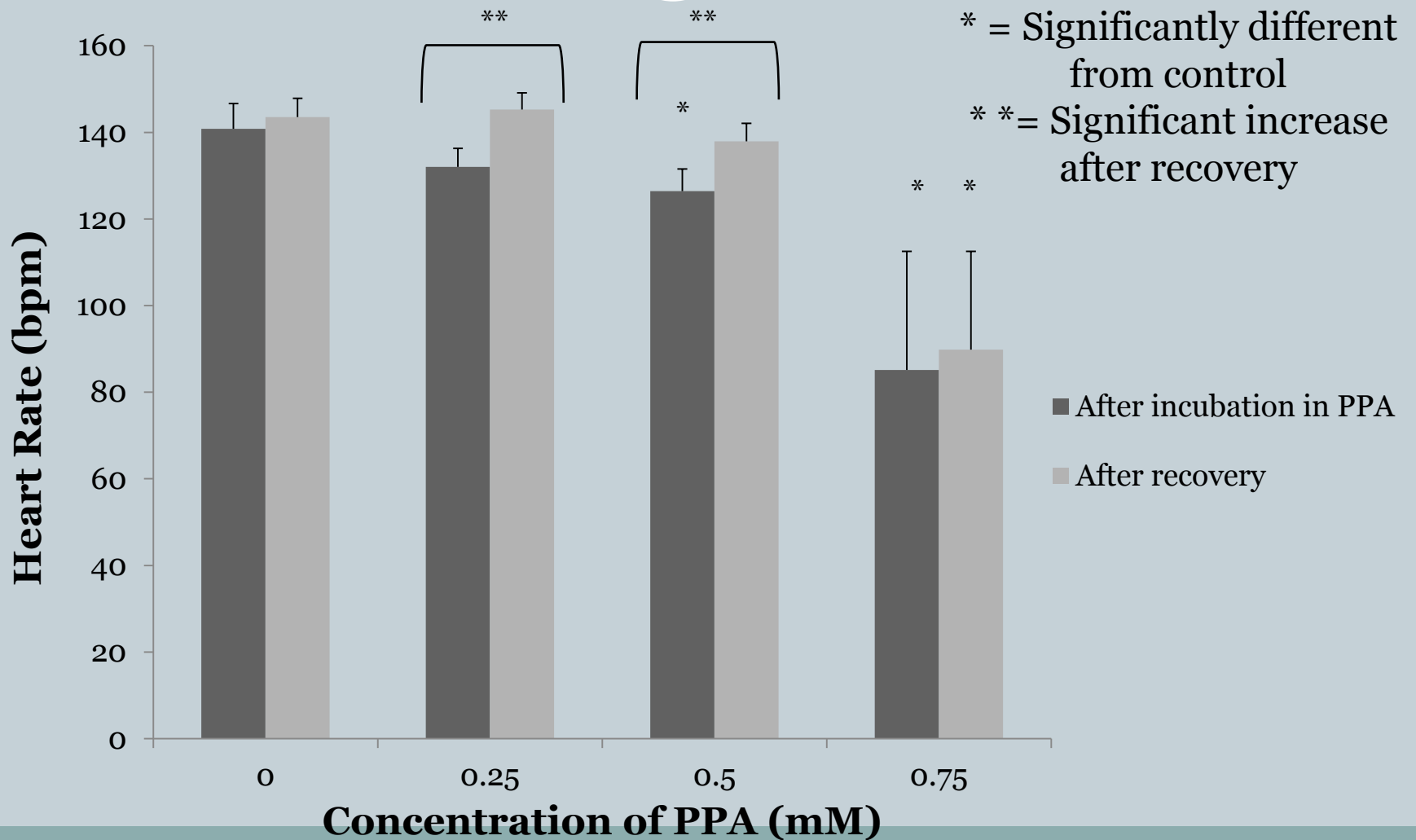


- **Recovery Assay**
 - Exposed 10 larvae to 0.25, 0.50, and 0.75 mM PPA at 18 hpf
 - At 5 dpf, five larvae from each solution were treated with tricaine to slow movement
 - Photos
 - Heart rate recorded after exposure to PPA
 - Remaining five larvae returned to 1X egg water and incubated at 28°C overnight
 - Treated with tricaine
 - Heart rate recorded after recovery
 - Two-tailed t-test

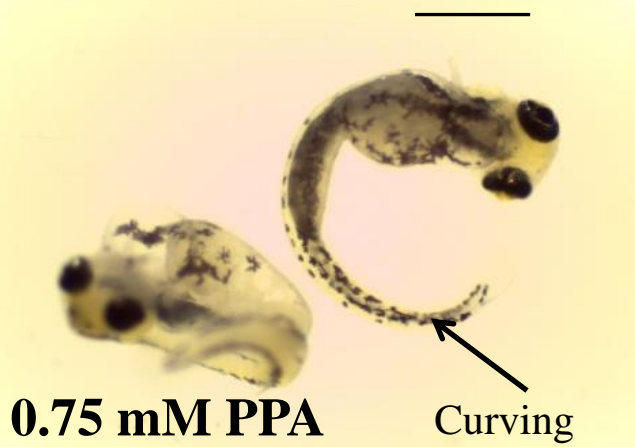
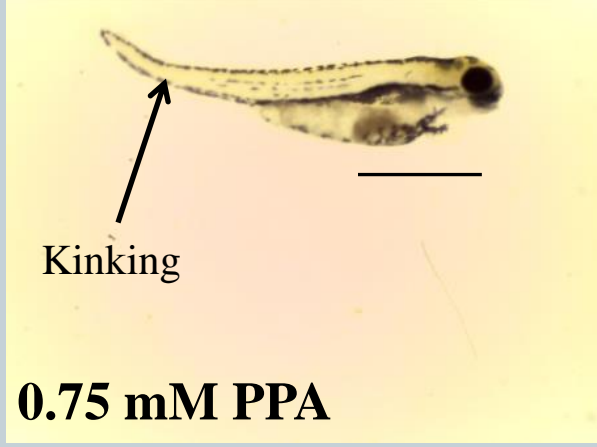
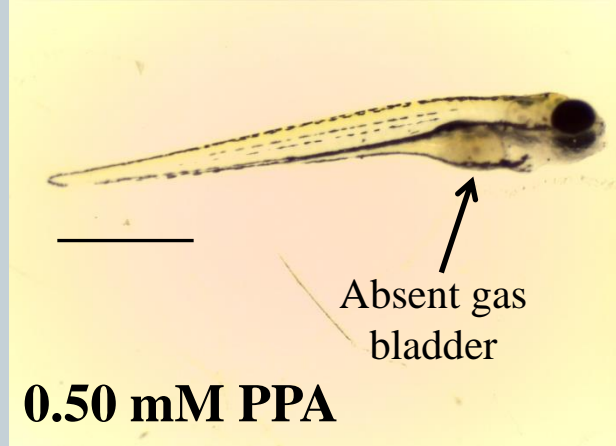
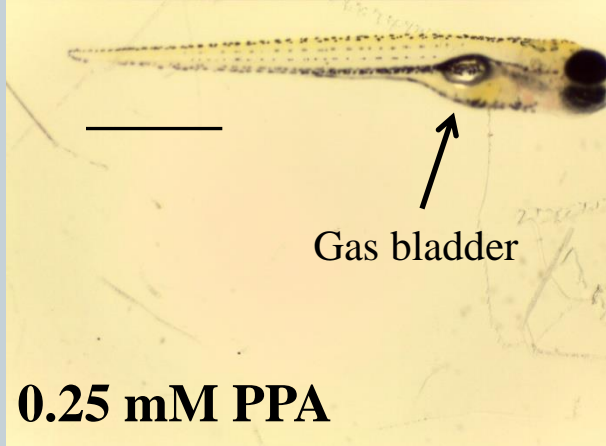
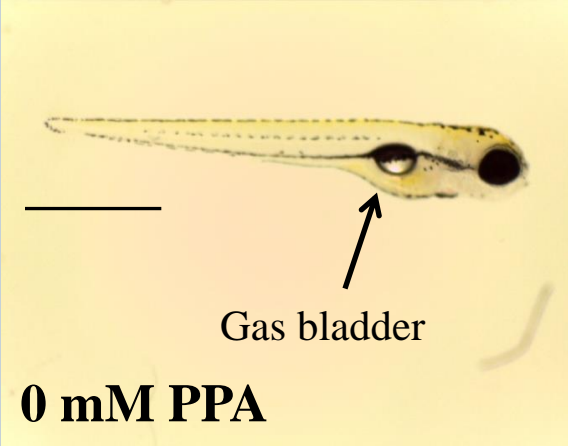
Results: Heart Rate After Incubation in PPA



Results: Heart Rate After Recovery



Morphology



Scale bar, 1 mm

Discussion

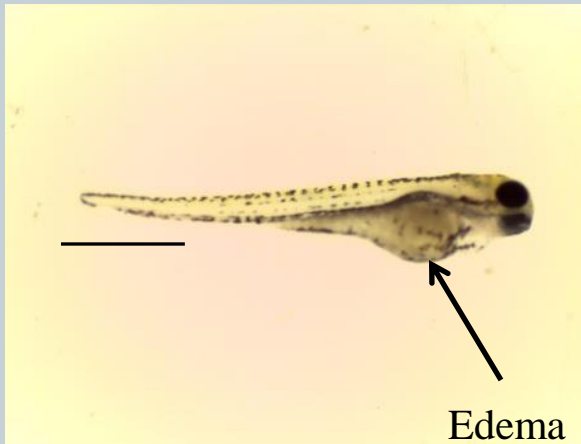


- Lateral line stimulation reaction time data inconclusive
 - 0.75 mM PPA larvae did not react yet had 85% survival
- Heart rate is important for assessing cardiac function
 - 0.25 mM PPA- no significant effect
 - 0.50 and 0.75 mM PPA- significant effect
 - ✦ Consistent with Claeys et al. 2011
- Recovery and heart rate
 - 0.25 and 0.50 mM larvae experienced significant recovery
 - 0.75 mM larvae experienced no significant recovery
 - ✦ 0.75 mM PPA too toxic for larvae
 - ✦ Possible permanent developmental damage

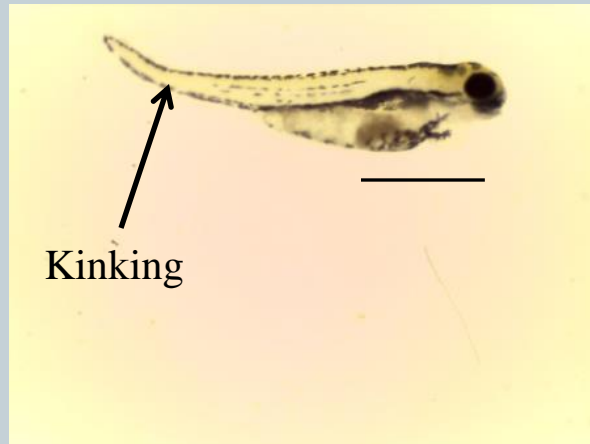
Discussion



- Morphology
 - 0.75 mM larvae demonstrated most morphological differences
 - ✦ Over all edema, and kinking/ curving of tail



Edema



Kinking



Curving

Future Studies



- More accurate assay to eliminate human error
 - Determining reaction time
- Gather more data
- Morphological effects
- Concentration of PPA where larvae lose ability to react and recover
 - Between 0.50 and 0.75 mM PPA according to this research

Acknowledgements



- Dr. Barbara Sisson
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Questions?

