

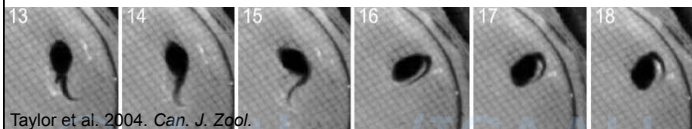
Effects of the anesthesia agents benozocaine
and tricaine methanesulfonate (MS-222) on
immune parameters in Cuban tree frog
(*Osteopilus septentrionalis*) tadpoles

B.F. Sears, P. Snyder, J.R. Rohr



Introduction

- Anesthetics
 - Medical procedures
 - Reduce handling/traveling stress
 - **Eliminate behavioral confounds**
- Anti-parasite behavior
 - Free-swimming trematode cercariae
 - Complementary to immune defense
 - Can study by “removing” behavior during an infection



Taylor et al. 2004. *Can. J. Zool.*

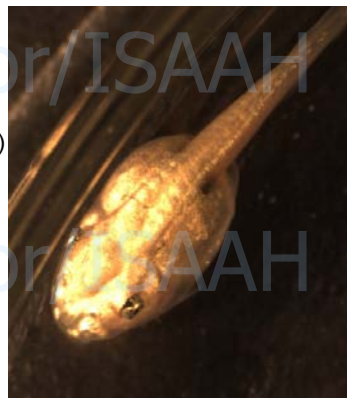
Introduction

- MS-222 immunosuppressive?
 - May adversely affect neutrophil function (Azuma et al. 2000, Palic et al. 2006)
 - Reduced cytotoxic cell activity in seabream (Cuesta et al. 2003)
 - Increases serum lysozyme activity in chinook salmon (Cho & Heath 2000)
- Benzocaine immunosuppressive?
 - Decreased complement activity (Ortuño et al. 2002)

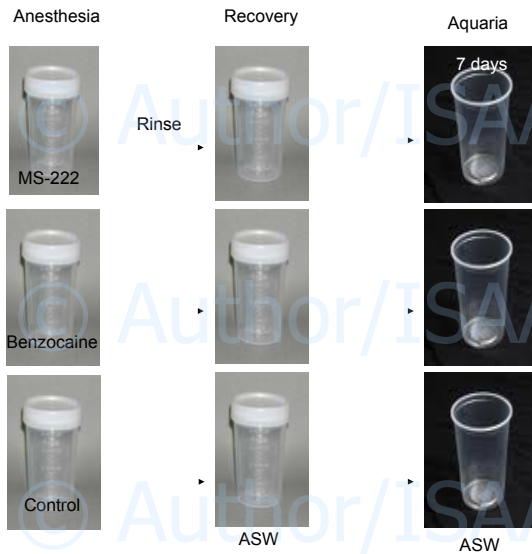


Materials and Methods

- Cuban tree frog tadpoles (*Osteopilus septentrionalis*)
- Treatments
 - 0.1% MS-222 (Koprivnikar et al. 2006)
 - 0.005% benzocaine (Vanable 1985)
 - Artificial spring water (control)
 - “stressed” and “unstressed” controls
- Exposure to induce 10 min anesthesia
 - 10 min MS-222
 - 14 min benzocaine



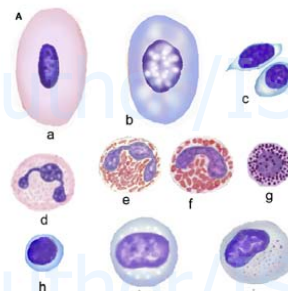
Materials and Methods



Materials and Methods: Experiment 1

- Blood collection
 - 0, 2, 6, 12, 24, 48, 72, 168 hours post-exposure (n=5 per sampling)
 - Giemsa and benzidine-stained
- Leukocytes/5,000 RBC counted
 - Eosinophils and basophils combined
- Results analyzed using Statistica

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A, Blood cells of amphibians. Erythrocyte (a), polychromatic erythrocyte (b), thrombocytes (c), neutrophil (d), heterophil (e), eosinophil (f), basophil (g), small lymphocyte (h), monocyte (i), and azurophil (j). B, Heterophils. *Buffo arenarum* (Anura). Courtesy of Mariana Cabagna, from (Claver & Quaglia 2009).

Results: Experiment 1

Univariate results	Degr. of Freedom	logThrombo SS	logThrombo MS	logThrombo F	logThrombo p	LogLymph SS	LogLymph MS	LogLymph F	LogLymph p
Intercept	1	193.7473	193.7473	4807.905	0	169.2677	169.2677	3617.058	0
Treatment	2	0.5171	0.2586	6.416	0.00254	0.3159	0.158	3.376	0.038843
Hour	1	0.0322	0.0322	0.8	0.373551	0	0	0	0.990465
Treatment* Hour	2	0.2752	0.1376	3.414	0.037482	0.0527	0.0263	0.563	0.571836
Error	85	3.4253	0.0403			3.9778	0.0468		
Total	90	4.0678				4.3685			

LogNeutro SS	LogNeutro MS	LogNeutro F	LogNeutro p	LogE+B SS	LogE+B MS	LogE+B F	LogE+B p
127.2323	127.2323	1958.505	0	92.47372	92.47372	918.0868	0
0.0601	0.03	0.463	0.631261	0.10372	0.05186	0.5149	0.599437
0.0603	0.0603	0.928	0.338076	0.12657	0.12657	1.2566	0.265462
0.1156	0.0578	0.89	0.414435	0.08669	0.04335	0.4303	0.651692
5.5219	0.065			8.56157	0.10072		
5.7682				8.9123			

Results: Experiment 1

Thrombocytes

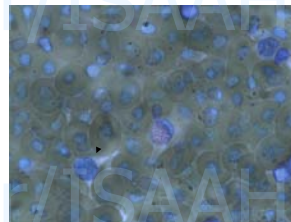
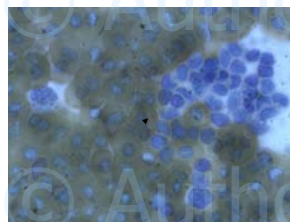
Fisher's LSD

Treatment	Control	Benzocaine	MS-222
	1.8587	1.8853	1.7386
Control		0.602342	0.023055
Benzocaine	0.602342		0.00583
MS-222	0.023055	0.00583	

Lymphocytes

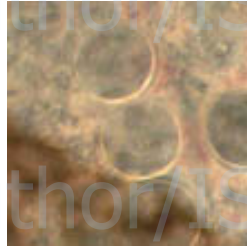
Fisher's LSD

Treatment	Control	Benzocaine	MS-222
	1.7236	1.7931	1.6435
Control		0.209693	0.155356
Benzocaine	0.209693		0.00893
MS-222	0.155356	0.00893	



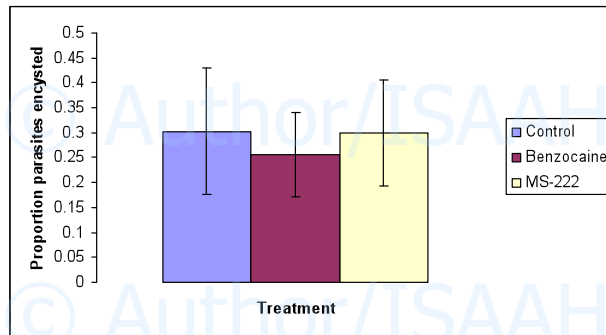
Materials and Methods: Experiment 2

- What if numbers of some leukocyte classes don't change, but their function does?
- Same procedures as Experiment 1
- Exposed to trematode cercariae (15 or 30) post-recovery
 - Tadpoles should have regained anti-parasite behaviors
 - Any increase in infection intensity should be a physiological effect of anesthesia
- Parasites quantified



Results: Experiment 2

- Student's t-test
 - No significant difference in parasite encystment between control, benzocaine, or MS-222 treatments ($p > 0.05$)



Discussion

- Where are the monocytes?

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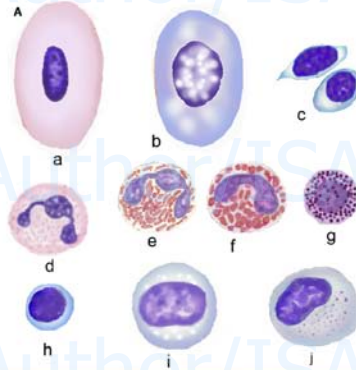
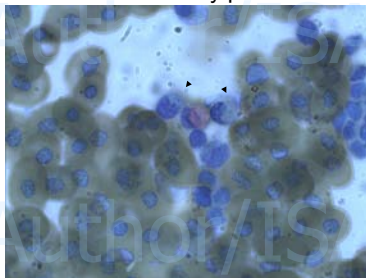


Figure 3. A. Blood cells of amphibians. Erythrocyte (a), polychromatic erythrocyte (b), thrombocytes (c), neutrophil (d), heterophil (e), eosinophil (f), basophil (g), small lymphocyte (h), monocyte (i), and azurophil (j). B. Heterophils. *Bufo arenarum* (Anura). Courtesy of Mariana Cabagna.

Discussion

- Benzocaine does not appear to immunocompromise tadpoles
- Effect of MS-222 on thrombocytes raises red flags
 - Peroxidase, complement receptors
 - Phagocytic? (Meseguer et al. 2002)
 - →contribute to inflammatory processes



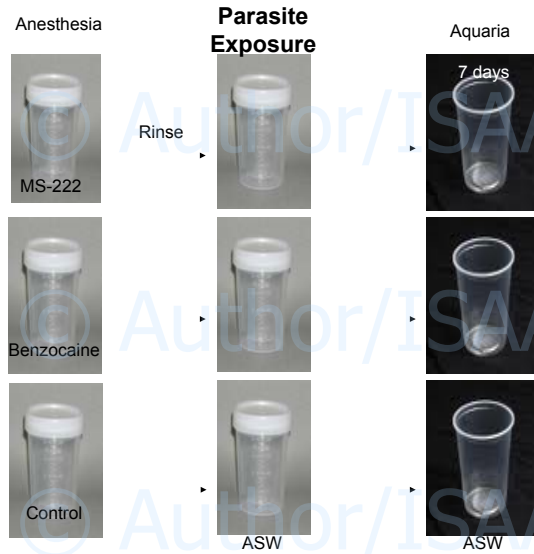
Discussion

	MS-222	Benzocaine
Cost	Expensive \$4/g	Cheap! <\$2.50/g
Concentration required	More	Less
Induction/ Recovery	Predictable	Variable*

Future Directions

- Perform experimental infections in anesthetized and control tadpoles
- How does investment in behavior affect investment in immunological defense?

Future Directions



Acknowledgements

- Undergraduate assistants
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Questions?

