

Investigating Toxicity and Differential Protein Expression in the *Aedes aegypti* Mosquito Larvae as an In Vivo Bioassay for Chemical and Biological Weapon Agents



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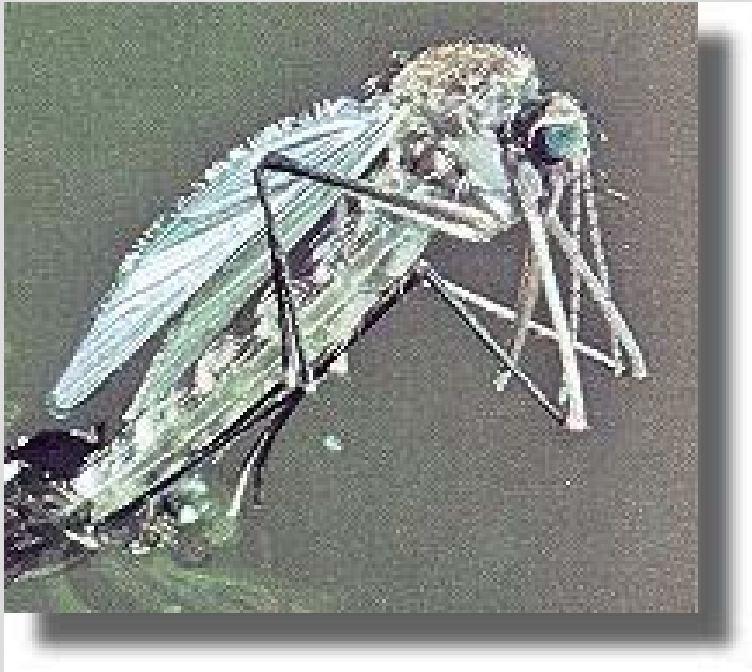
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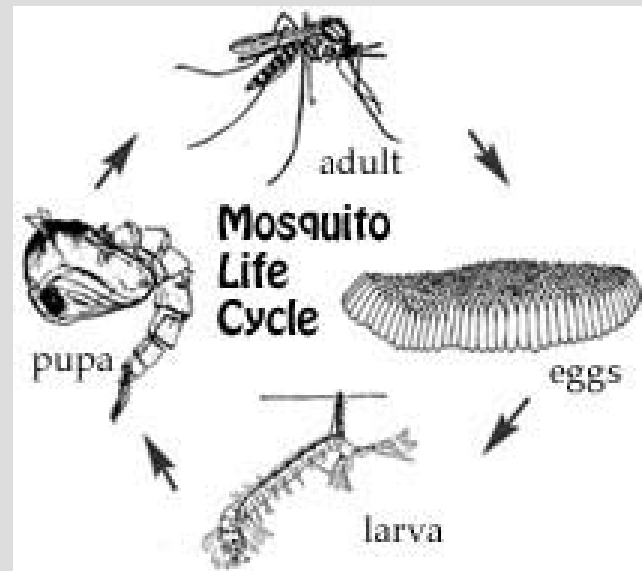
Mosquito Biology



- Aquatic
- Holometabolous
- Hematophagic
- Vectors for parasites, viruses and some bacteria
 - 300 million to 500 million cases, 1 million deaths resulting from Malaria each year
 - 50 million cases of Dengue each year
 - 200,000 cases, 30,000 deaths resulting from Yellow Fever Virus each year

Life Cycle

- Holometabolous
 - Larvae or juveniles do not resemble adults
 - Separate life stages
- Egg (up to 6 months)
- Larvae (10-21 days)
- Pupae (2-6 days)
- Adults (1-? Days)



Worldwide Distribution

- Every continent except Antarctica
- Non-flowing, stagnant water (old tires, rain gutters, bird baths)
- Marshes, swamps, and lakes
- Opportunist



Using Insects As Bioreporters

- Response to chemical, biological or physical insult can be measured using many methods
 - Genetic (gene duplication, transcriptional regulation)
 - Translational/post-translational modification (protein/enzyme alteration)
 - Physical appearance and LD50 data

Beaty BJ, Mackie RS, Mattingly KS, Carlson JO, Rayms-Keller A.

The midgut epithelium of aquatic arthropods: a critical target organ in environmental toxicology.

Environ Health Perspect. 2002 Dec;110 Suppl 6:911-4.

Mattingly KS, Beaty BJ, Mackie RS, McGaw M, Carlson JO, Rayms-Keller A.

Molecular cloning and characterization of a metal responsive *Chironomus tentans* alpha-tubulin cDNA.

Aquat Toxicol. 2001 Oct;54(3-4):249-60.

Molecular and genetic ecotoxicologic approaches to aquatic environmental bioreporting.

Environ Health Perspect. 1998 Dec;106 Suppl 6:1395-407.

What Sets this Detection Method Apart from Others ?

- Currently, detection systems for biological warfare agents utilize PCR (polymerase chain reaction) to amplify genetic material specific to an agent, or Antibodies that recognize proteins specific for known agents
- Current detection systems for chemical warfare agents utilize physical and chemical properties that are specific for known agents
- In the IAD system, we are measuring the **response of an organism** to an agent, or class of agents. Inherent in this, is that our antibodies detect proteins that are in the insect, and their translational/post-translational alteration after exposure to an environmental sample
- So far, the proteins that are responsive to insult with chem/bio agents are agent and dose specific

Mortality in *Aedes aegypti* Larvae Exposed to Select Agents (2 Hour Exposure)

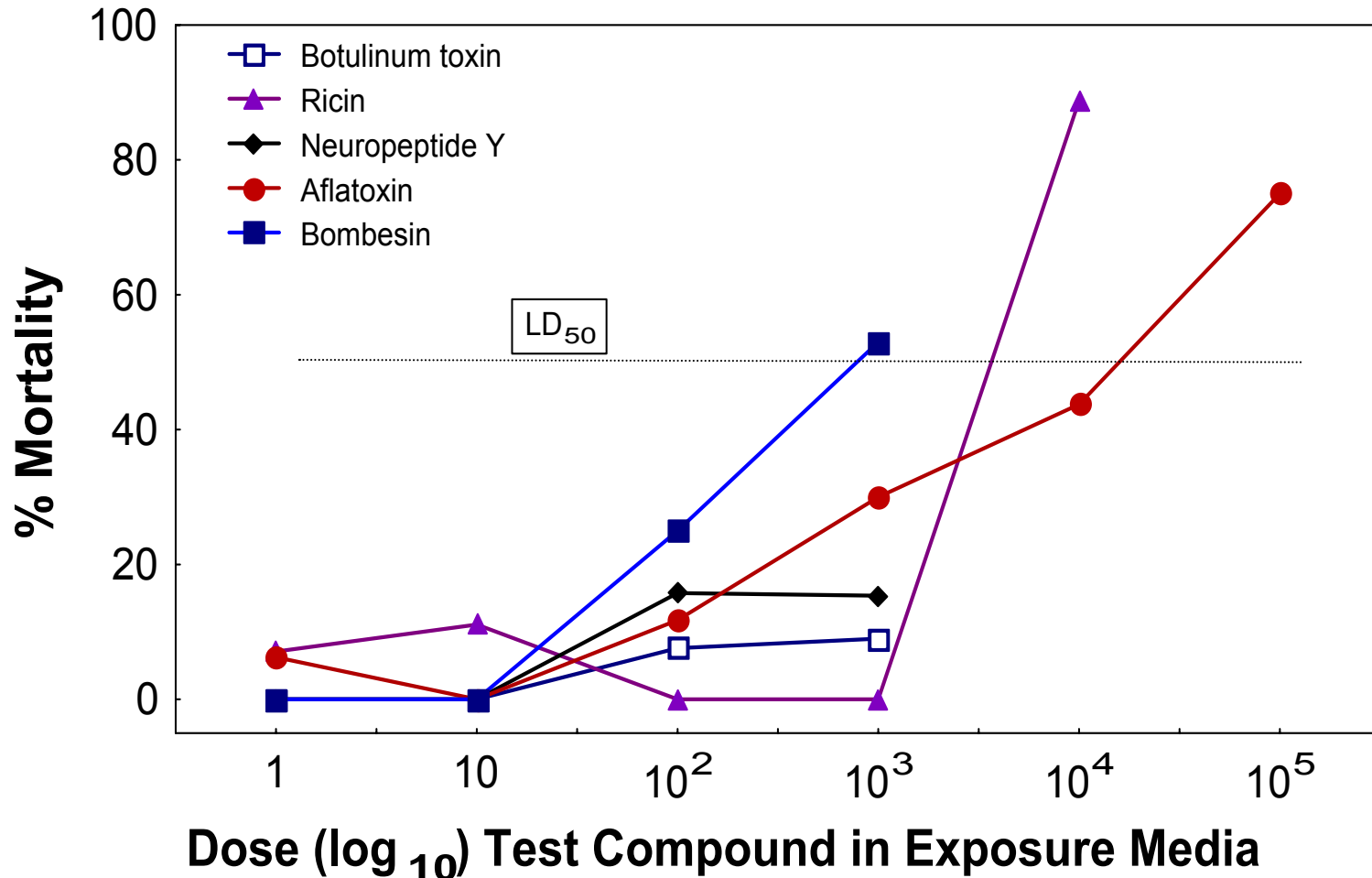


Figure 1. LD₅₀ Studies in *Aedes aegypti* L4 Larvae Exposed to Select Agents. Groups of larvae were exposed to log-fold increases of select agents for 2 hours. Thereafter, the percent of live and dead larvae were determined.

LDH Activity in the Exposure Media Following Treatment with Select Agents

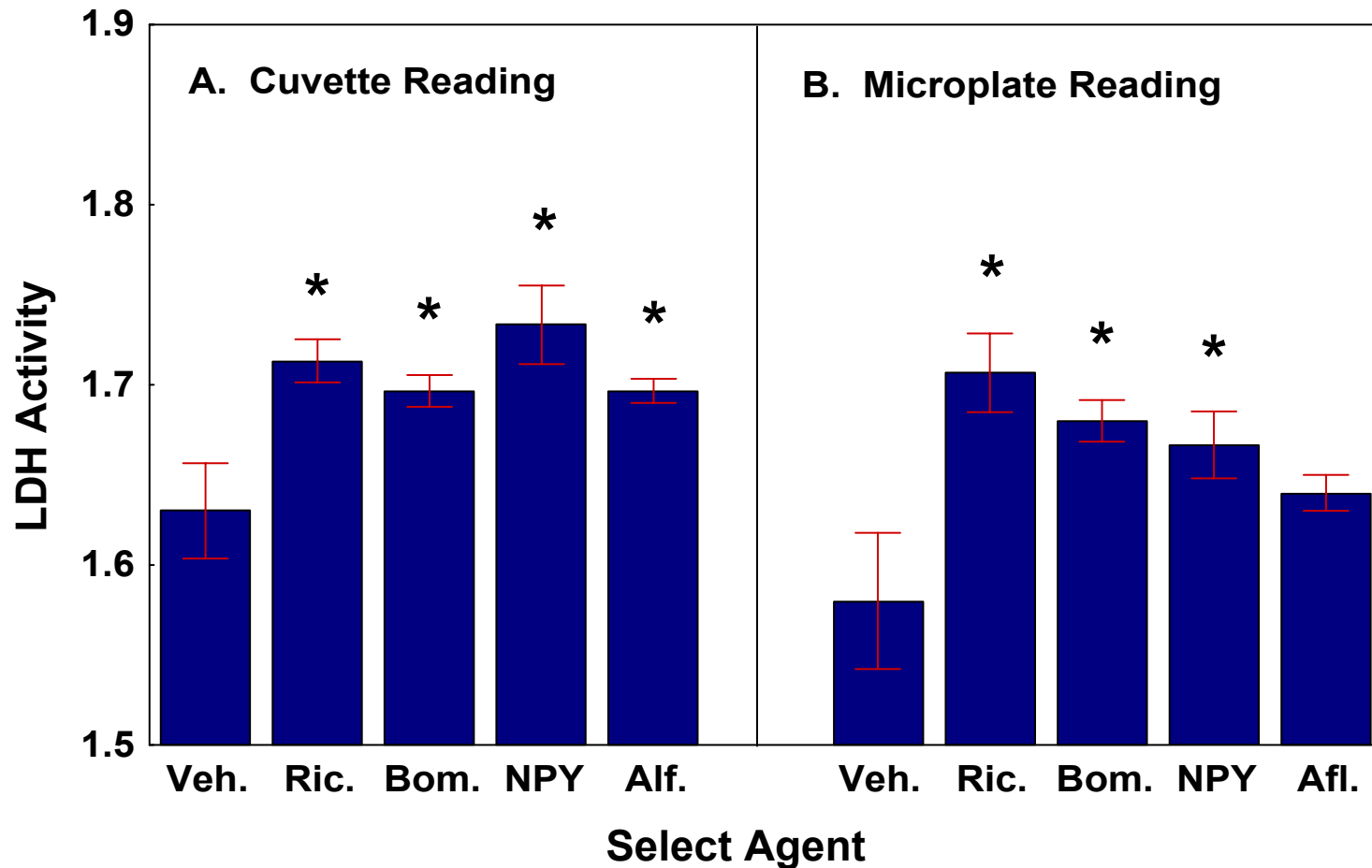
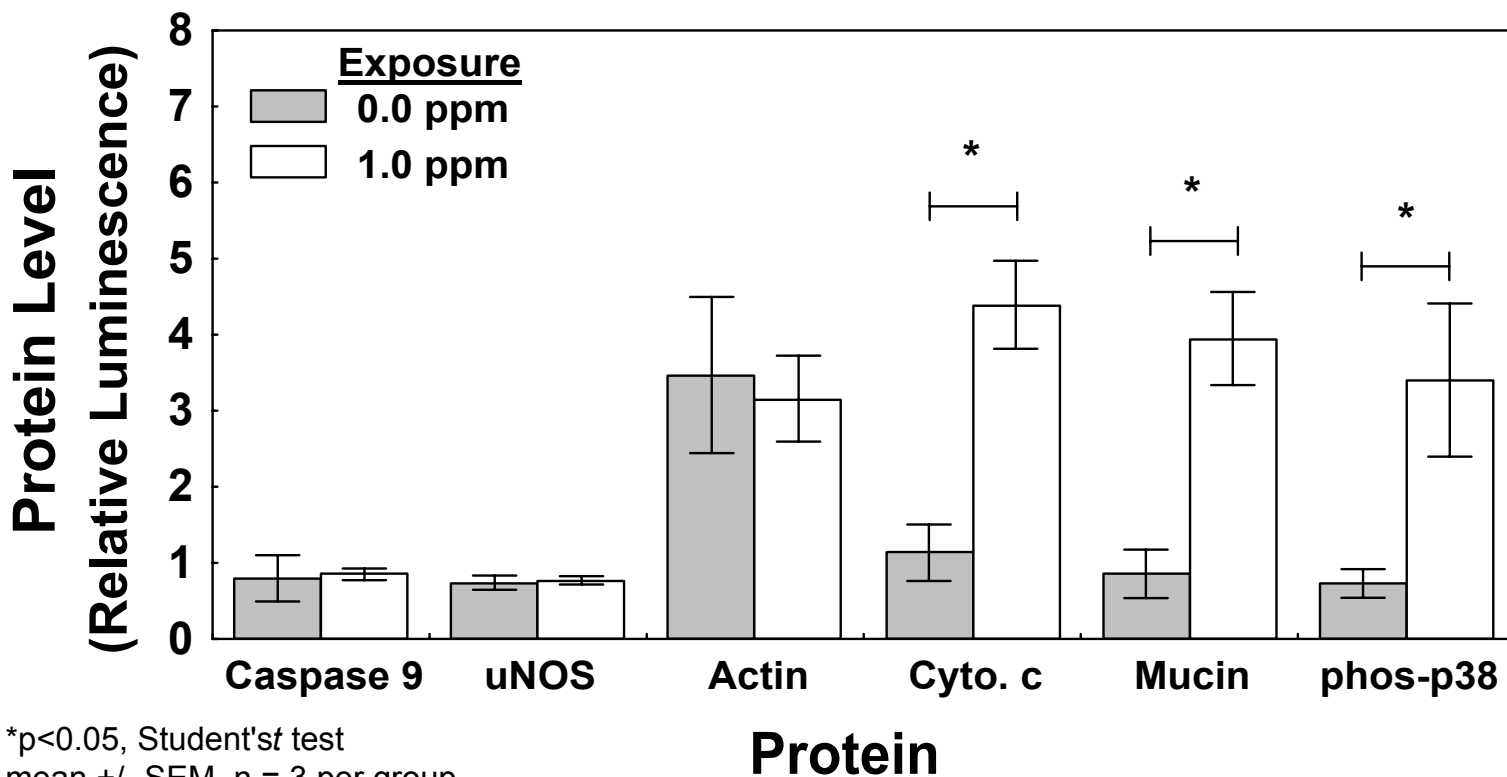


Figure 2. LDH Activity in the Exposure Media 1-HOUR Following Exposure to a High Dose of Select Agent. L3/L4 larvae were exposed to a high dose of select agent (see Table 1 for doses and agents) for 1 hour. Thereafter, the lactate dehydrogenase (LDH) activity in the exposure media was determined using A) a cuvette-type spectrophotometer or B) a microplate reader.

Proteins Modified by Post-Translational Modification are Targets of Interest

Toxicity 'Biosignature' for n-Octane

**Selective Protein Alteration Following
10 Minute Exposure to n-Octane**



Dose-Dependent Induction of Phosphorylated p38 After 10 Minute Exposure to TCE

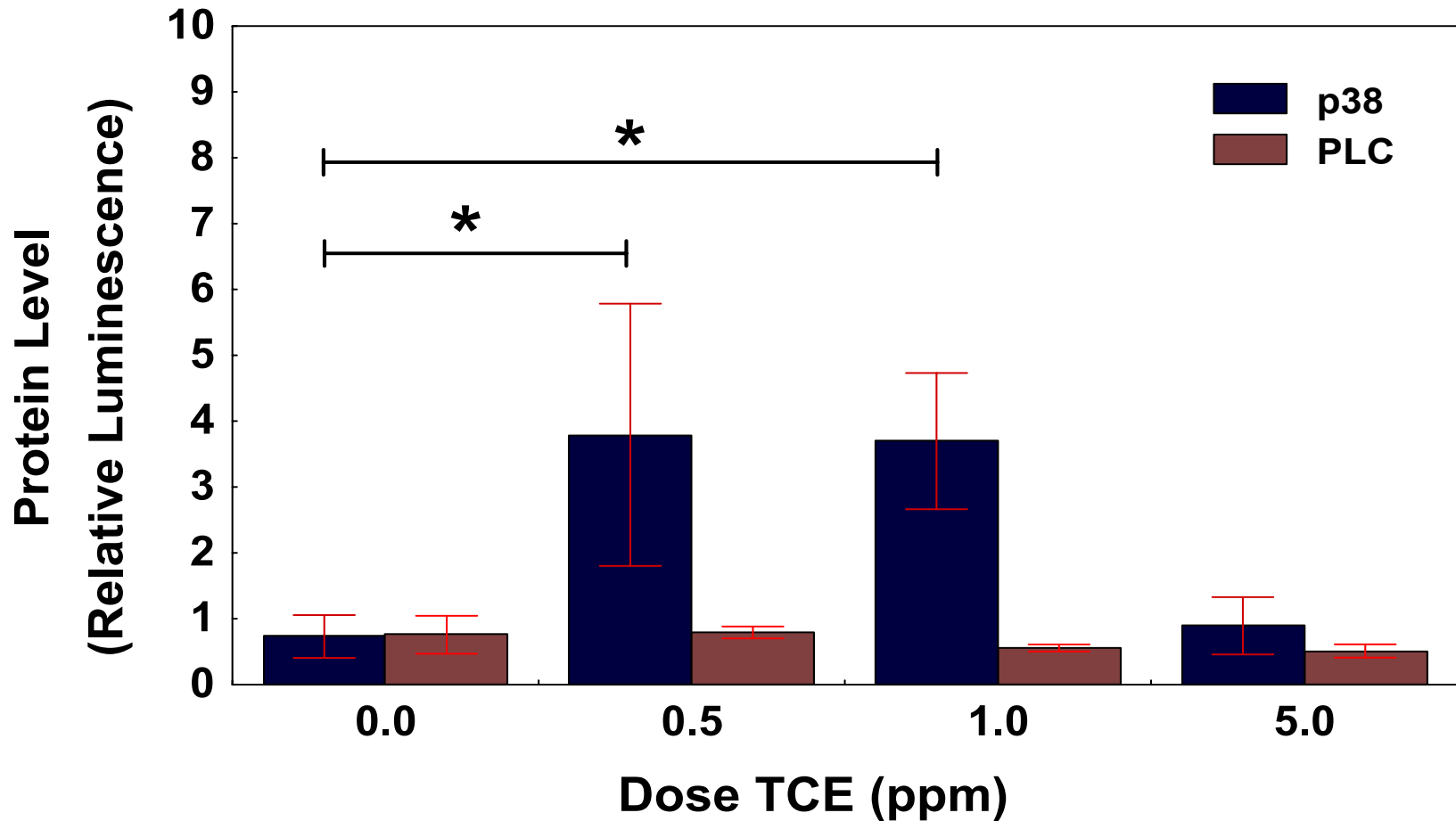
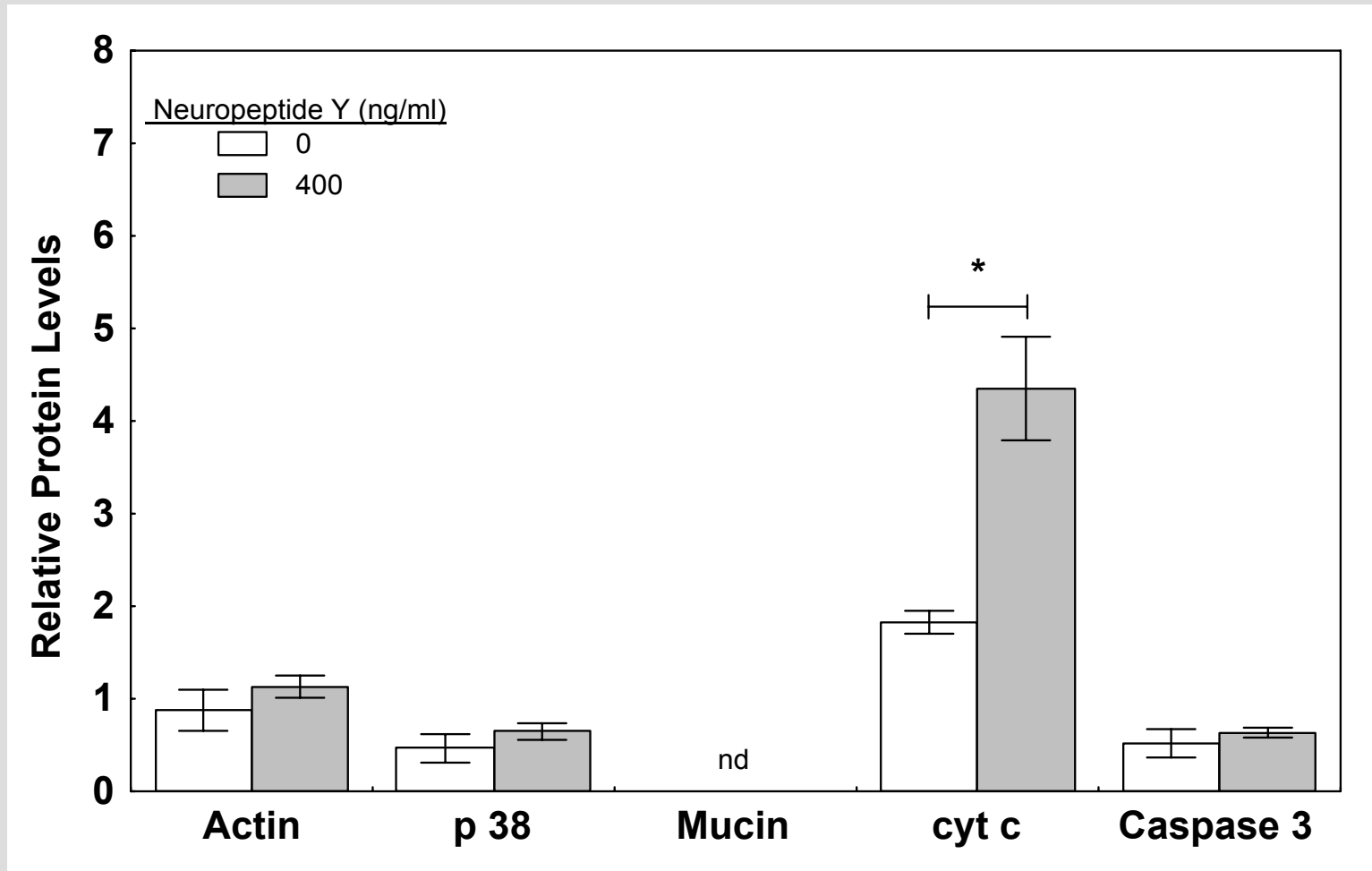


Figure 3. Larvae were exposed to 0.0, 0.5, 1.0 or 5.0 ppm trichloroethylene (TCE) for 10 minutes. Thereafter, whole-larvae were homogenized in lysis buffer and total protein was extracted. Levels of phosphorylated p38 and phospholipase C γ 1 (PLC) were immediately quantified using an HRP-based ELISA. Values indicated are relative protein levels from control or TCE-treated larvae (mean \pm SEM, n = 3 per group).

Biosignature for Neuropeptide Y in *Aedes aegypti*



*p<0.05; n = 3 per group
 mean ± SEM
 Student's *t* test
 nd = not determined

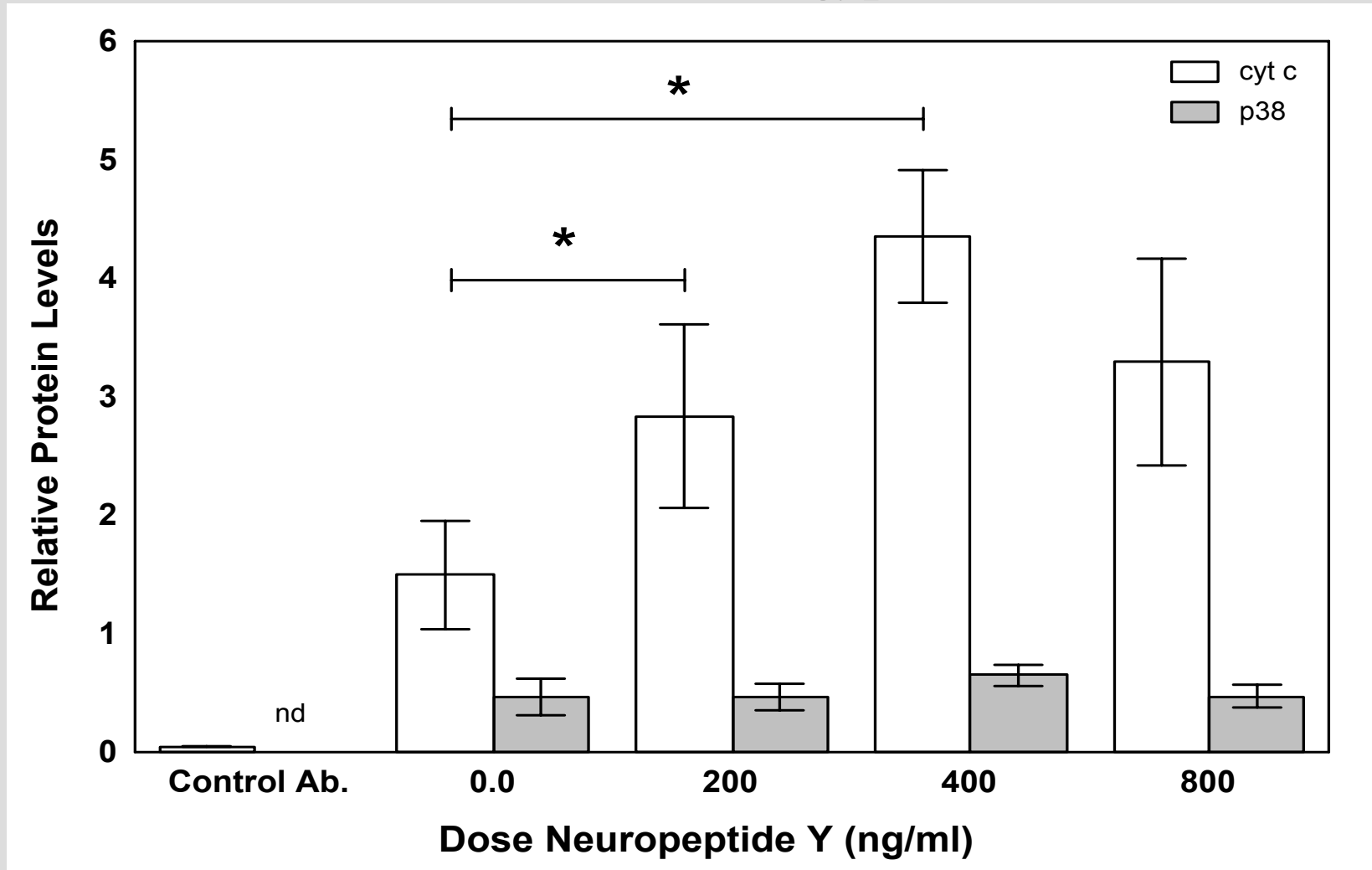
Subsequent Study

Exposure	Mucin Levels
Control (n=3)	23567 ± 4611
Neuropeptide Y (n=3)	36092 ± 2669

*p<0.05 ↑

Figure 4. NPY 400ng/ml t=30min

Cyt c and p38 Levels Following Exposure to Neuropeptide Y in *Aedes aegypti*



* $p < 0.05$; $n = 3$ per group

mean \pm SEM

One-way ANOVA, LSD *post hoc*

Control Ab. = isotype control antibody

Figure 5. Dose Response $t=30$ min

Ricin (1mg/ml media 18h Exposure)

$$y = 768 * 0.022533 * \text{normal}$$

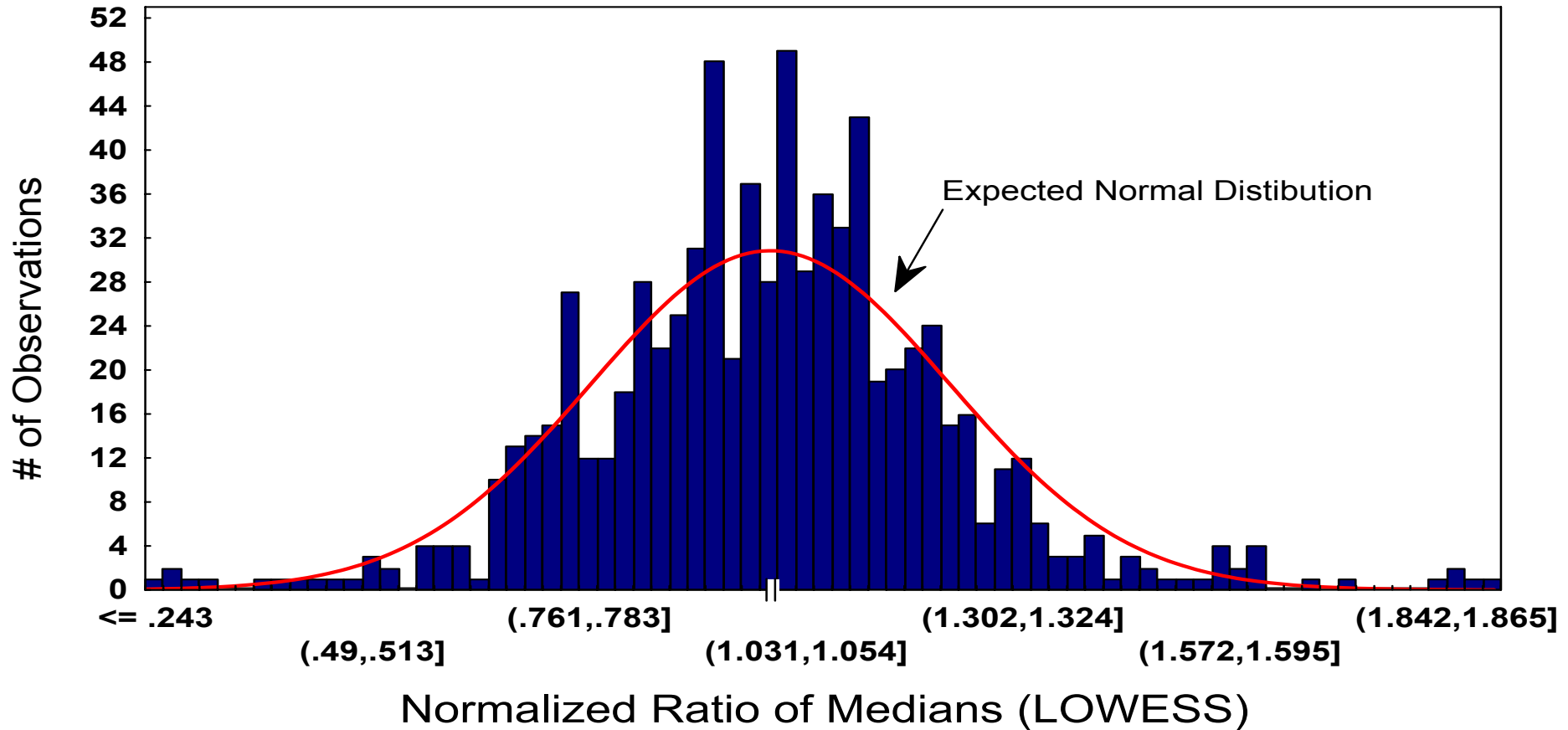
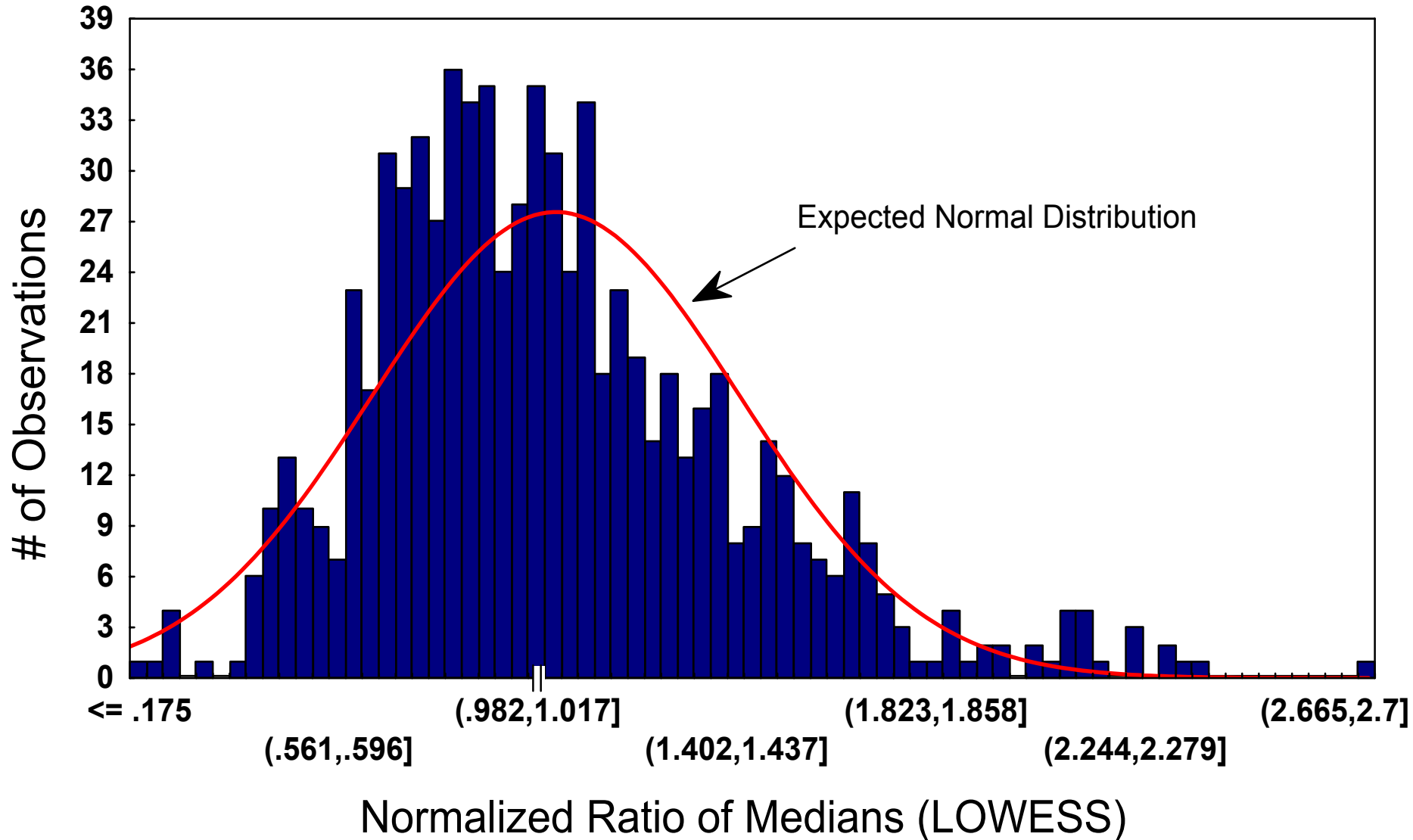


Figure 6 & 7. Histogram of normalized ratios of median intensities of cy3 (control) and cy5 (treatment) proteins following ricin or Bacillus anthracis exposure. L3/L4 larvae were exposed to B. anthracis/ricin or vehicle-control (water) exposure media.

B. anthracis (10^5 spores/ml 18hr Exposure)

$$y = 764 * 0.035067 * \text{normal}$$



Future Issues

- To obtain a better estimate, or biosignature of the “total effect”, our focus is to shift to the Antibody Microarray format more thoroughly
- To date, experiments designed to measure the LDH present in the media surrounding treated mosquitoes have produced meaningful, statistically significant data. We intend to continue with this line of investigation, including metabonomics, as it adds to the credibility of our hypothesis that *Aedes aegypti* is susceptible to insult with CBW materials
- In collaboration with Dr. Stephen Higgs and the group at UTMB we will utilize our assay system to analyze mosquito proteins isolated from groups “treated” with a variety of viral agents

Conclusion

With the advent of microarray technology, the ability to produce “biosignature” data is greatly increased. However, multiple exposures and subsequent data mining to compare results across single experiment boundaries are needed. In concert with the LDH assay, in the form of a system that samples exposure media in real-time, detection of CBW agents using *in vivo* protein bioreporters is foreseeable. However, the limitations of using a systems to auto-detect chem/bio agents without a user/operator include high false alarm rates and environmental contamination issues. Short of this, we have shown that *Aedes aegypti* larvae are susceptible to insult with a variety of threat agents as well as toxic industrial chemicals. Furthermore, we have demonstrated that chem/bio agents produce a unique profile of protein alteration in mosquitoes that is measurable.

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