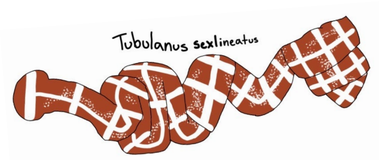
# Neurotoxins in Nemerteans: An ELISA Exploration for the Presence of Tetrodotoxin

Chayse Bono and Megan Schwartz




(P2-137)

## Introduction

Nemerteans are a phylum of understudied animals that use a specialized eversible organ, the proboscis, to subdue and paralyze their prey. Although a few protein venoms have been characterized from some species, several nemerteans are reported to contain tetrodotoxin (TTX), a potent neurotoxin likely acquired from environmental microbial sources. Previous studies suggest that TTX is concentrated in the epidermis and proboscis, implicating it in both defense and prey capture (Schwartz and Campbell, 2010).

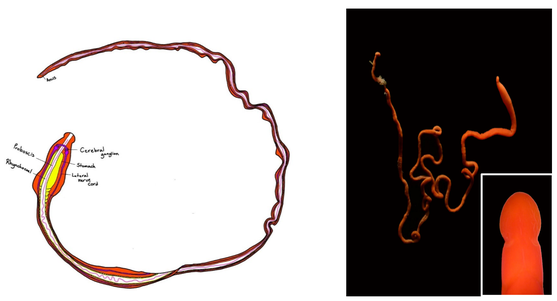





Figure 1: Illustration of nemertean anatomy of *Tubulanus ruber*, a brightly colorful nemertean with TTX in both its proboscis and epidermis.

In this pilot study we used a commercially available, competitive enzyme-linked immunosorbent assay (ELISA) to test epidermis and body wall tissue sections and dissected proboscides of several nemertean species obtained from intertidal habitats summer 2025 near Friday Harbor Laboratories, WA, USA for the presence of TTX.

## Materials and Methods

For each species the proboscis, anterior, midbody, and posterior tissues were dissected, preserved in 70% EtOH, homogenized, and centrifuged prior to ELISA processing if the specimen was large enough. Tetrodotoxin presence was assayed using the BioVenic TTX Competitive ELISA Kit (EK8F384). Nemertean tissue samples were extremely small (<0.5 mg), we modified the kit's steps. Most tissues were run in triplicate when sufficient material was available.

## Conclusions

1. ELISA-based screening reveals high concentrations of TTX in both epidermal + bodywall and proboscis tissues.
2. ELISA for TTX testing is a potent method with even the smallest of nemerteans.
3. Presence of TTX in both proboscis and epidermal + body wall tissues implicates its ecological roll for prey capture and antipredator defense.

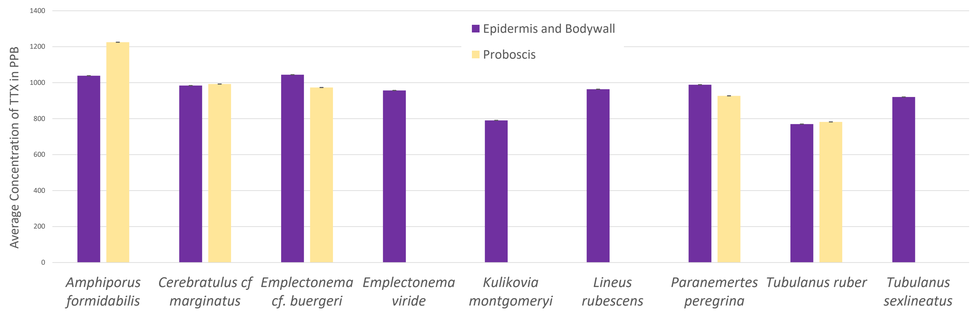



Figure 2: Average concentration of tetrodotoxin (TTX) found in each species broken down by tissue type. For most species two separate individuals and three tissue replicates were tested. Error bars indicate +/- 2 standard deviations.

Figure 3: Nemerteans used in this study from left to right, top to bottom: *E.* viride feeding on barnacles, *A. formadabilis* with closeup of anterior (inset), *L. rubescens, T. sexlineatus. E. cf. buergeri,* and *P. peregrina.*

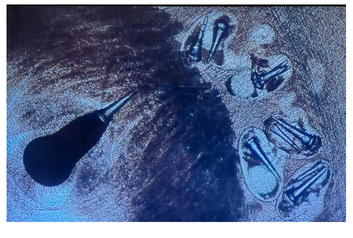
## Results and Discussion

Our study demonstrated that TTX presence and concentration varied among species and tissue types, with all species containing detectable TTX in both epidermis + bodywall and proboscis tissues. These results suggest that nemerteans employ TTX for both prey capture and defense and highlights some potential interspecific variability in toxin concentrations.

We were only able to use two TTX standards and small pieces of tissue for our first study, so these results represent relative patterns only. Nevertheless, we demonstrate the feasibility of applying commercial ELISA-based TTX screening to nemerteans.

Future work using fresh tissues, with a more precise analytical scale and multichannel pipettor will help to validate standards and clarify the concentration of TTX per mg of tissue.

Figure 4: Aposematically colorful *Kulikova montgomeryi* (left) and infaunal *Cerebratulus cf. marginatus* (right). Stylet and accessory stylet pouches of *A. formadabilis*.



## Acknowledgements

We would like to thank the Marine Invertebrate Zoology Class of Summer 2025 for help collecting nemerteans used in this study. Additionally, CB received a Mary Cline Fellowship that enabled this work.