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AN INVESTIGATION OF THE HEMOTOXICITY OF THE DUVERNOY'S GLAND SECRETION OF THE NORTHERN WATER SNAKE (*Nerodia sipedon)*

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Abstract

Daniel J. Ranayhossaini
Schreyer Honors College Senior Thesis

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Abstract: The northern water snake (*Nerodia sipedon*) preys primarily on fish and other aquatic organisms in an environment where constriction is no longer an effective method of killing prey. Previous research has linked the Duvernoy's gland to toxin production. Salivary swabs and Duvernoy's gland secretions from *N. sipedon* were collected and compared through biochemical techniques. Salivary swabs were quantified by a Bradford protein assay. It was established that there was between 5 and 10 ug of protein per salivary swab. The proteins from the salivary swabs were then subjected to a SDS-PAGE that yielded proteins of molecular weights 150, 59, 24, 22, 19, and 17 kDa. The 59 kDa protein was observed in much greater amounts in samples directly from the Duvernoy's gland. Hemolytic activity was observed though light microscopy of erythrocytes from *Notropis atherinoides* mixed with salivary samples from *N. sipedon*. Invaginations and cup-shaping of erythrocytes mimicked the toxic action of cyclodextrins, indicating that the toxic component of the saliva may not be a protein as originally thought. Future research will investigate this possibility.

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Acknowledgments

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Introduction

The search for novel biologically active compounds for pharmaceutical production has recently extended to the voluminous class of chemicals collectively known as venoms. Traditionally regarded as highly dangerous and potentially lethal, new research indicates the existence of venoms that have little effect on mammals yet are detrimental to other organisms. For example, *Xenodon merremii* produces a toxin that has negligible effects on pigeons and rabbits, yet proves toxic for tree frogs (Mackessy, 2002). It stands to reason that snakes may have specialized their venoms to the degree that the venom has an observable effect only on prey species. Thus, these snakes may have gone unnoticed as venom producing species due to the lack of evidence of toxicity in humans. The active components of weak or selectively toxic venoms can be identified, characterized, and their phylogenetic relationships clarified using a combination of biochemical, molecular, and statistical techniques. Snakes that produce venoms with minimal effect on human physiology may provide a new route for pharmaceutical discovery; the function of these venoms may also provide valuable insight into the evolutionary history of snakes and their relationship to other saurian reptiles (Barlow, 2009). As such, I believe the nonvenomous status of certain snakes should be reconsidered; my research project was designed to find evidence for a venom in a traditionally nonvenomous snake, *Nerodia sipedon*.

Three types of venomous snakes are commonly recognized; solenoglyphous, proteroglyphous, and opisthoglyphous. Solenoglyphs posses the most complex mechanisms for delivering venom, a pair of hollow hinged fangs at the front of the

mouth for envenomation. Characteristic members of this group are the vipers and pitvipers. Proteroglyphs possess a set of fixed front fangs to deliver venom. The elapids exhibit the key characteristics of proteroglyphs. Opisthoglyphs posses grooved, enlarged teeth at the rear of the maxilla that may deliver venom toxic to animals many times larger than the usual prey. This category includes venomous members of the colubrid family (Pough, 1999). Included in the colubrid family along with definitively venomous opisthoglyphs are many snakes considered to be nonvenomous even though they possess indisputable venoms. I have inferred that the oversight to classify these snakes as opisthoglyphs is likely based on their comparatively weak venom or the lack of a distinctive delivery system.

The Duvernoy's gland is an organ present behind the eye of certain snakes that has been shown to produce a venom in multiple species (Rodriguez-Robles, 1994 and Lumsden, 2004). This modified salivary gland is connected to the oral cavity by distinct ductwork. Musculature used for contracting the Duvernoy's gland may not always be present (Jansen, 1983). The common garter snake, *Thamnophis sirtalis* instead possesses a unique anatomical arrangement of the integument, musculature, and skull so that indirect muscle contraction will lead to increased secretion from the Duvernoy's gland (Jansen, 1983). The toxicity of Duvernoy's gland secretions (DGS) is highly variable between and within species, although it is clear that these secretions do indeed function as venoms in many colubrids (Rodriguez-Robles, 1994).

In an effort to clarify the classification of species within venomous families, Kardong and Lavin-Murcio (1993) identified alternate venom delivery mechanisms by measuring the rate of venom output from different species of snakes. This work

revealed the presence of two distinct venom delivery systems, termed high and low pressure. High pressure systems involve visible fangs and mechanical structures to force the venom through the fang into the predator or prey. Low-pressure systems are much simpler and Duvernoy's gland secretions seep into the oral cavity with little muscular contribution (Kardong, 1993). As opisthoglyphs do not posses a hollow fang for venom delivery, it is likely that they use a low pressure system for venom delivery.

The myriad of compounds that comprise a venom consist mainly of proteins of the families of metalloproteases, amino acid oxidases, disintegrin, and phospholipase A2 (Fox, 2008). Although most venom action definitively occurs via enzymes, it is conceivable that other chemicals may be active within the venom. Cyclodextrins are macrocyclic structures formed by glucosyl residues joined by alpha-1,4 linkages (Sajadi, 2007). Toxicity of cyclodextrins can vary depending on the venue through which the cyclodextrin encounters a cell. Both alpha-cyclodextrin and gamma-cyclodextrin have been tested for toxicity when ingested and have shown no negative effects on test organisms (Waalkens-Berendsen, 2004; Munro, 2004). However, shape distortion and hemolysis of human erythrocytes has been observed as a result of contact with concentrations as low as 2mM alpha, beta, or gamma cyclodextrin (Tetsumi, 1982). The cyclodextrins in this experiment caused the release of cholesterol, phospholipids, and proteins from the membranes of lysed erythrocytes (Tetsumi, 1982). The toxicity of cyclodextrins to unprotected cells was further supported through an experiment where sea urchin embryo's were allowed to develop in a solution of 0.01M alpha-cyclodextrin. This resulted in delayed

attachement of the archenteron during early embryonic development, possibly due to cyclodextrin acting as an inhibitor for the synthesis N-glycosidicically linked glycoproroteins or other extracellular matrix polysaccharaides (Sajadi, 2007).

Since the distinction between types of venom delivery systems and the notion of toxic DGS was confirmed, a number of snake species have been identified that produce toxins in the Duvernoy's gland, including specimens of the genus *Boiga, Telescopus,* and *Trimorphodon* (Fry, 2004). It is estimated that nearly 40% of colubrids produce some type of venom (Mackessy, 2002). However, not all colubrids with Duvernoy's glands use the gland to produce venom and there is even disagreement over whether the secretion is consistently used for prey capture within species. Despite possessing a potent toxin comparable to that produced by the clearly venoumous elapids, bites from the brown treesnake, *Boiga irregularis,* have no effect on prey mortality and the snake relies on constriction to kill its prey (Fry 2004; Rochelle, 1993). My project sampled DGS from northern water snakes (*Nerodia sipedon,* family Colubridae), in an effort to increase the number of selectively toxic venom producing snakes characterized and to determine the primary means *N. sipedon* uses for killing its prey.

Nerodia sipedon is a common sight on many lakes and streams in the Eastern United States. It can grow to impressive sizes in excess of 1.3 meters. Its diet consists of fish, crustaceans, amphibians, young turtles, and the occasional small mammal (Shaffer, 1999). *N. sipedon* emerges from winter brumation in late April and begins looking for a mate shortly thereafter (Shaffer, 1999). Early spring is the easiest time to capture *Nerodia* specimens as low water temperatures slow muscle

activity and locomotion in ectotherms, and the lack of aquatic vegetation makes spotting the elusive creature easier. As summer continues, increasing amounts of aquatic vegetation make search and seizure extremely difficult and other methods for capture must be used.

N. sipedon consumes most of its prey in an aquatic, and therefore low friction, environment; thus constriction is an ineffective means of killing prey. To complicate this dilemma, most fish and amphibians have a mucous coating on the epidermis and lack substantial limb structure. These morphological characteristics allow prey to easily slide out from the coils of the snake. This line of evidence led me to hypothesize that *N. sipedon* might use a weak salivary venom as an alternative to constriction to kill prey. Indeed, an interesting reaction takes place when a human is bitten by the snake. Perforations resulting from the bite usually bleed more profusely than the bite of a comparably sized colubrid snake (Ranayhossaini, personal observation, Behler, 1979, Shaffer, 1999).

The apparent anticoagulant properties of *N. sipedon* DGS suggest the possibility of using this venom to treat ailments in the circulatory system. Many snake venoms are already under research for their potential as pharmaceuticals. The Malaysian pit viper, *Calloselasma rhodostoma*, is a subject of intense research for reducing blood clots in stroke patients and has led to the development of the drug Viprinex (Laino, 2007). A 21 amino acid sequence purified from a protein present in the venom of Australian taipan, *Oxyuranus s. scutellatus* is being researched by Houston based Ophidia Products as a pharmaceutical that may increase healing rates for minor cuts and scrapes (Lipps, 2004). This drug, termed Oxynor, expresses

mitogenic activity in mouse eukaryotic cells and has been shown to decrease the amount of time needed for wound closure in murine systems (Lipps, 2004). The venoms of certain colubrids, including *N. sipedon*, may provide another avenue for pharmaceutical development due to their inherent weakness; weak venoms have less potential for systemic tissue damage that makes more powerful venoms notorious. Thus, whereas venom from *C. rhodostoma* and *O. scutellatus* must be chemically altered to be safe for medical use, an inherently weak venom might require little or no chemical modification to be safe for medical treatments.

The presence of an anticoagulant in the saliva of *N. sipedon* provides strong indication for a toxic secretion being produced by the Duvernoy's gland. My thesis project was designed to investigate this possibility; as such, my work had three objectives:

1. Demonstrate that the DGS of *N. sipedon* is hemolytically active
2. Determine the toxicity of DGS in *N. sipedon*
3. Characterize the DGS in *N. sipedon* using biochemical techniques

The first and second objectives were accomplished by mixing DGS with fish erythrocytes and quantifying hemolysis. Biochemical techniques were used to accomplish the third objective.

Methods

Acquisition of specimens of N. sipedon

Traditionally, search and seizure is the predominant method by which snakes are captured for study; however, the aquatic habitat of *N. sipedon* makes this inordinately difficult as the reptile can rapidly retreat to inaccessible depths. Search and seizure is only practical in early spring shortly after the snakes emerge from burmation. As summer progresses and temperatures increase, growing aquatic vegetation impedes search and seizure methods. To address this problem, a method of trapping live specimens was devised. Local anglers consider *N. sipedon* to be a nuisance as it regularly enters minnow traps to consume their bait. This predictable behavior was used to capture most specimens of *N. sipedon* used in this study. Commercially available minnow traps consisting of a cylinder formed from 1mm fiberglass or galvanized wire with inward facing funnels on each end were employed for this purpose. The traps were modified by using camouflaged Styrofoam as a float to maintain approximately 75% of the trap under water (Figure 1). This ratio prevented the drowning of captured reptiles while ensuring the survival of captured fish and amphibians. All captured animals were handled in accordance with Penn State Institutional Animal Care and Use Committee (IACUC) protocol #25549. In concordance with the Pennsylvania Fish and Boat Code, a scientific collector's permit was also acquired for this study (Ref. #327).

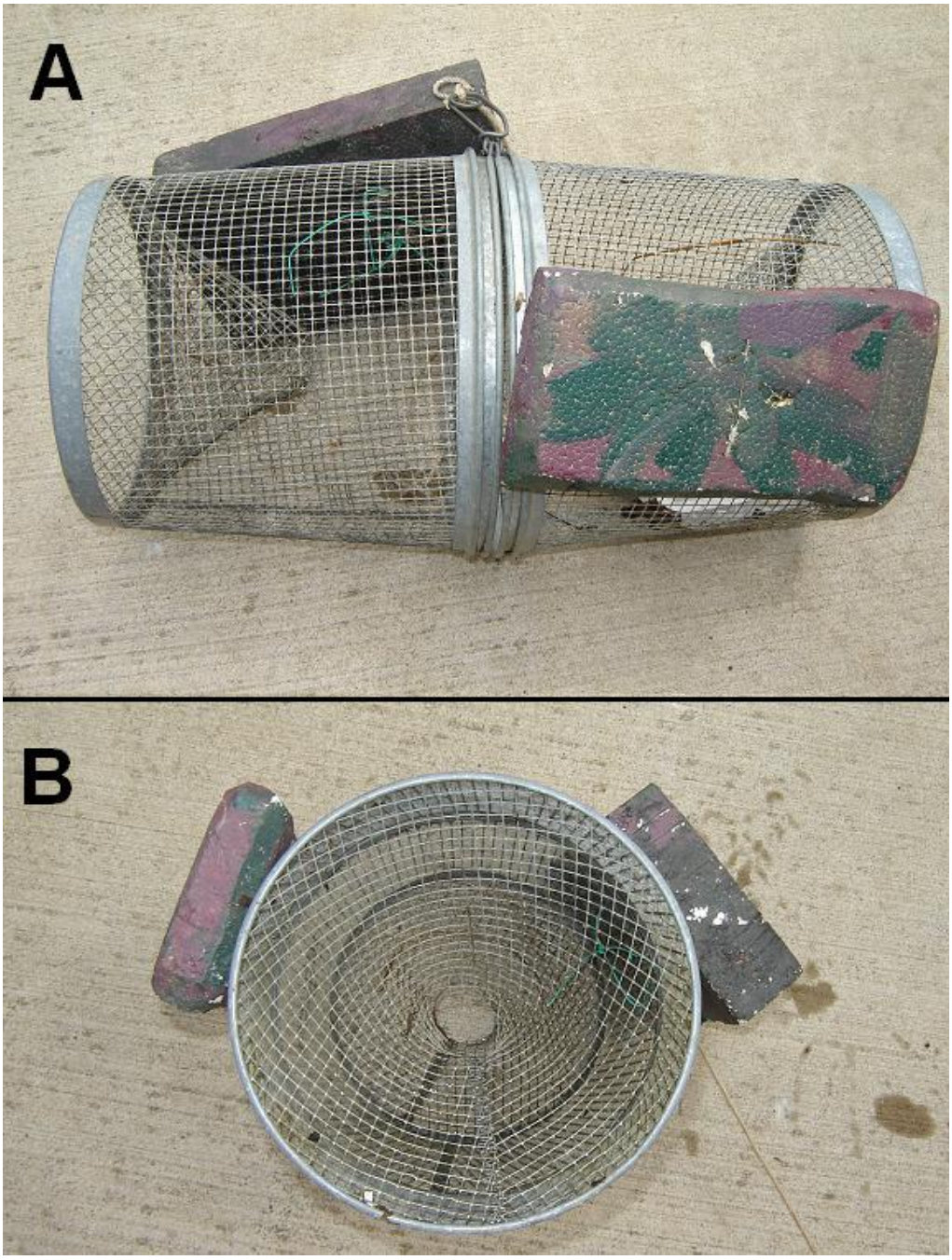

Figure 1: Example of trap used for capturing specimens of *N. sipedon*. Side view "A" highlights camouflage pattern on floats as well as the conical shape of the ends of the trap. Face view "B" shows the entry point into the trap and the position of the floats at the 2 and 10 o'clock positions

Once a specimen of *N. sipedon* was captured, it was removed from the trap and immediately prepared for acquisition of salivary secretions and DGS. No chemical sedatives were used to ensure the maximum release of DGS by the reptile and to lessen the chance of harming the animal. *N. sipedon* was immobilized by placing the posterior 2/3 of its body within a length of PVC pipe and then grasping the cervical vertebrae immediately posterior the cranium to restrain the snake and prepare to acquire DGS (Figure 2).

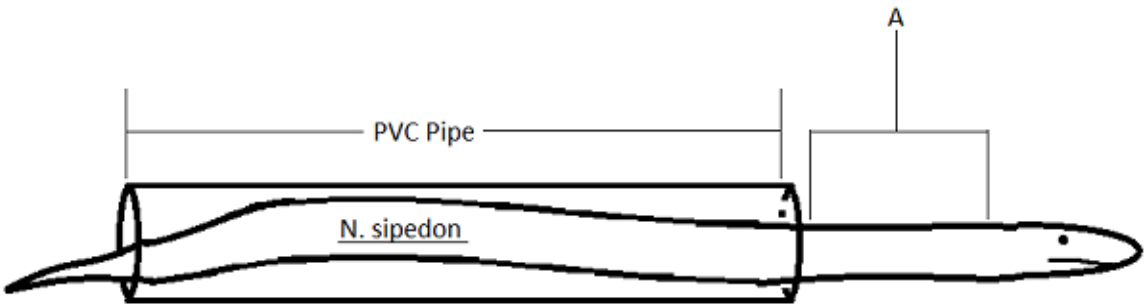

Figure 2: Diagram showing the methods used to restrain *N. sipedon* for acquiring DGS. The location marked "A" indicates where the snake was grasped with a hand.

Acquisition of DGS and saliva samples

Upon restraining the snake, the animal was persuaded to open its mouth through gently applied pressure from a sterile swab. A second swab was placed parallel to the lateral axis of the animal in the most posterior region of the mouth. This second swab was used to hold the mouth open as DGS extraction occurred. The entire interior surface of the snake's mouth was then sampled with a sterile cotton swab to remove any blended salivary secretions present; this swab was retained for

analysis. After removing extraneous salivary secretions from the oral cavity, a micropipette was placed over the rearmost maxillary fang and used to apply gentle vacuum and extract DGS. Samples retrieved using cotton swabs and those pippetted were stored in microcentrifuge tubes at -20°C to be transported back to the lab for protein extraction.

Protein extraction

Cotton swabs used to collect saliva from *N. sipedon* were suspended in 500 µl of extraction buffer containing 100mM Tris (pH 8.0), 10mM EDTA, 0.1% Tween-20, and 1mM dithiothreitol. Pipetted samples were suspended in the same manner. Samples suspended in extraction buffer were then precipitated by adding 40 µl of 70% ethanol and holding the solution at 0°C. Protein precipitate from salivary swabs were then suspended in 100 µl of buffer containing 100mM Tris (pH 8.0), 10mM EDTA, and 0.1% Tween-20. Protein precipitate from pipetted samples was suspended in 25 µl of a similar buffer.

Protein quantification

To determine protein concentration, 150µl of Bradford Quick Start dye reagent was added to 150µl of suspended salivary samples. Absorbance at 595nm was determined through spectrophotometric assay and compared to a BSA standard curve to determine concentrations. Reagents were purchased from Bio-Rad laboratories, Hercules, CA.

Electrophoresis

Samples of both salivary swabs and pipetted samples were precipitated from the extraction buffer and suspended in a sample running buffer of 100mM Tris pH 6.8, 2% SDS, 5% β-mercaptoethanol, and 15% glycerol. Samples were equlibrated in concentration to create a 25 μl solution containing 1 mg/ml of protein. These samples were placed in a Bio-Rad ReadyGel consisting of polymerized acrylamide. Standard proteins were: hen egg white conalbumin type 1 (76,000 Da), bovine serum albumin (66,200 Da), bovine muscle actin (43,000 Da), rabbit muscle glyceraldehyde 3-phosphate dehydrogenase (36,000 Da), bovine carbonic anhydrase (31,000 Da), soybean trypsin inhibitor (21,500 Da), and equine myoglobin(17,500 Da). Running buffer solution (Laemmli buffer, 10X) contained 24g Tris, 115.2g glycine, and 20 ml 20% SDS diluted to 4L with DIH_2O. Gels were subjected to 220V DC for 60 minutes to separate proteins.

Gel Staining

Gels were stained using SilverXpress® Silver Staining Kit provided by Invitrogen, Carlsbad, CA.

Determination of Osmomolarity

Osmomolarity of samples was determined using a Vapro® 5520 vapor pressure osmomometer manufactured by Wescor, Inc, Logan, Utah. Temperature, pressure, and air movement changes were kept to a minimum during the course of osmomolarity determinations.

Determination of Hemolytic Activity

A 100µl sample of fresh blood was collected by inserting a heparinized needle 1mm deep on the ventral surface underneath the operculum of *Notropis atherinoides* (emerald shiner). This blood was added to 100µl of hemolytic assay buffer (100mM Tris (pH 8.0), 10mM EDTA, 0.1% Tween-20) containing either 0, 2, 4, 8, or 10 µg of salivary proteins. This was repeated with extracts of DGS and compared to standards of extraction buffer containing no proteins. The blood/buffer mixture was then spread on a microscope slide and allowed to dry. Slides were stained using the Wright-Giemsa method with using fast green methyl alcohol fixative, xanthene dye, and thiazine dye mixture (Thermo Fisher Scientific Inc., Waltham, MA). Using light microscopy, one hundred erythrocytes were counted on each slide and the percentage of damaged erythrocytes was noted.

Results

Sample acquisition

A total of 15 snakes were sampled throughout the course of the experiment. During sampling of snakes, a novel morphological characteristic of the mouth was observed. Beside the rearmost maxillary fang, a small pocket was observed between the jaw and labia of every snake sampled (Figure 3). Each snake yielded approximately 3µl of DGS.

Protein concentrations

Bradford protein assay yielded protein concentrations shown in Table 1. Repeatedly, the amount of DGS isolated was comparable to that or exceeded the amount of protein in the entire mouth (mean ±variance: DGS 0.204±0.01μg μl^{-1}; salivary secretion 0.103±0.005μg μl^{-1}). Although not statistically significant, the raw sample isolates were much lower in volume for DGS compared to salivary swabs, indicating that the secretions coming from the DGS had a much higher protein concentration than the complex in the saliva (n=5, df=4, p=0.26, paired t-test).

Electrophoresis

SDS-PAGE indicated that the salivary swab contained a complex mixture of at least six proteins with molecular weights of 59, 47, 32, 28, 21, and 18 kDa. Pipetted DGS samples contained at least 3 proteins of weight 59, 21, and 18 kDa (Table 2). The 59 kDa protein appeared in concentrations much higher than any other present on the gel (Figure 4), indicating that it may be the primary secretory product of the Duvernoy's gland.

Osmolarity

The osmolarity of both DGS (421.8±297.2 mmol kgl^{-1}) and salivary secretion (445.4±7586.3 mmol kgl^{-1}) samples were significantly higher than the osmolarity of the fish blood (312 mmol/kg; one tailed t-test, df=9, p=0.043). Because the samples exhibited osmolarities greater than that of the fish blood, any effect observed due to osmotic effects should cause crenation of the cells, not distention or hemolysis.

Hemolytic assay

Control of hemolytic assay buffer added to fish erythrocytes showed no significant hemolytic activity or damage to erythrocytes (DGS and salivary secretion combined; mean ± 95% confidence interval = 20.31±9.41 cells lysed; control = 6 cells lysed; 95% confidence interval for secretion did not include value for control buffer; $p<0.05$, df =11). β-hemolysis (complete hemolysis) was observed in erythrocytes with added snake salivary compliments and DGS. Curiously, erythrocytes exposed to snake saliva and DGS developed large vacuoles within their cells and became severely distended (Figure 5). The correlation between number of cells lysed and micrograms of protein added was marginally significant ($p=0.075$, $n=6$, $R^2=0.4658$) for salivary swabs and Duvernoy's secretions ($p=0.058$, $n=6$, $R^2=0.3140$); the variation in hemolytic response was high, thus obscuring the relationship between micrograms of protein added and number of cells lysed. Slope for DGS was 1.652 cells/microgram and for saliva was 1.652 cells/microgram, indicating that there was no significant difference between the number of cells lysed in the DGS and salivary samples (Figure 6).

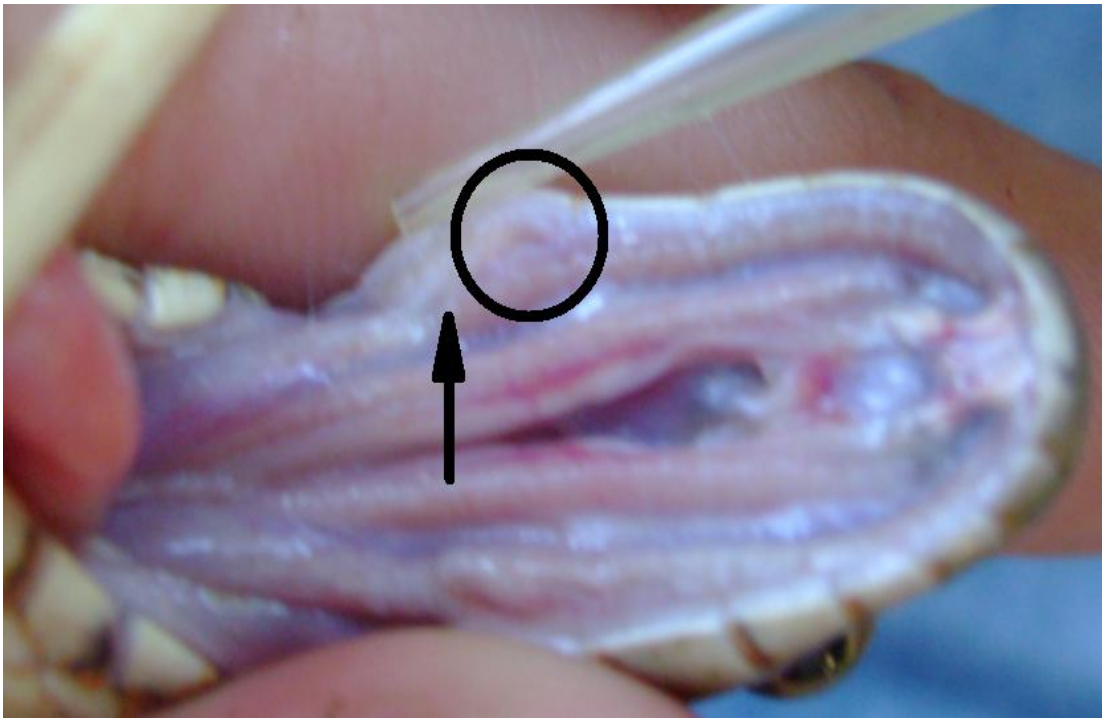

Figure 3: Interior of the mouth of *N. sipedon*. Rearmost maxillary fang is identified by the arrow. Unique pocket observed in *N. sipedon* but observed to be absent in *Lampropeltis mexicana*, *Elaphe guttata*, and *Pituophis melanoleucus.* It is possible that this could be a product of evolution that functions as a primitive or vestigial venom gland.

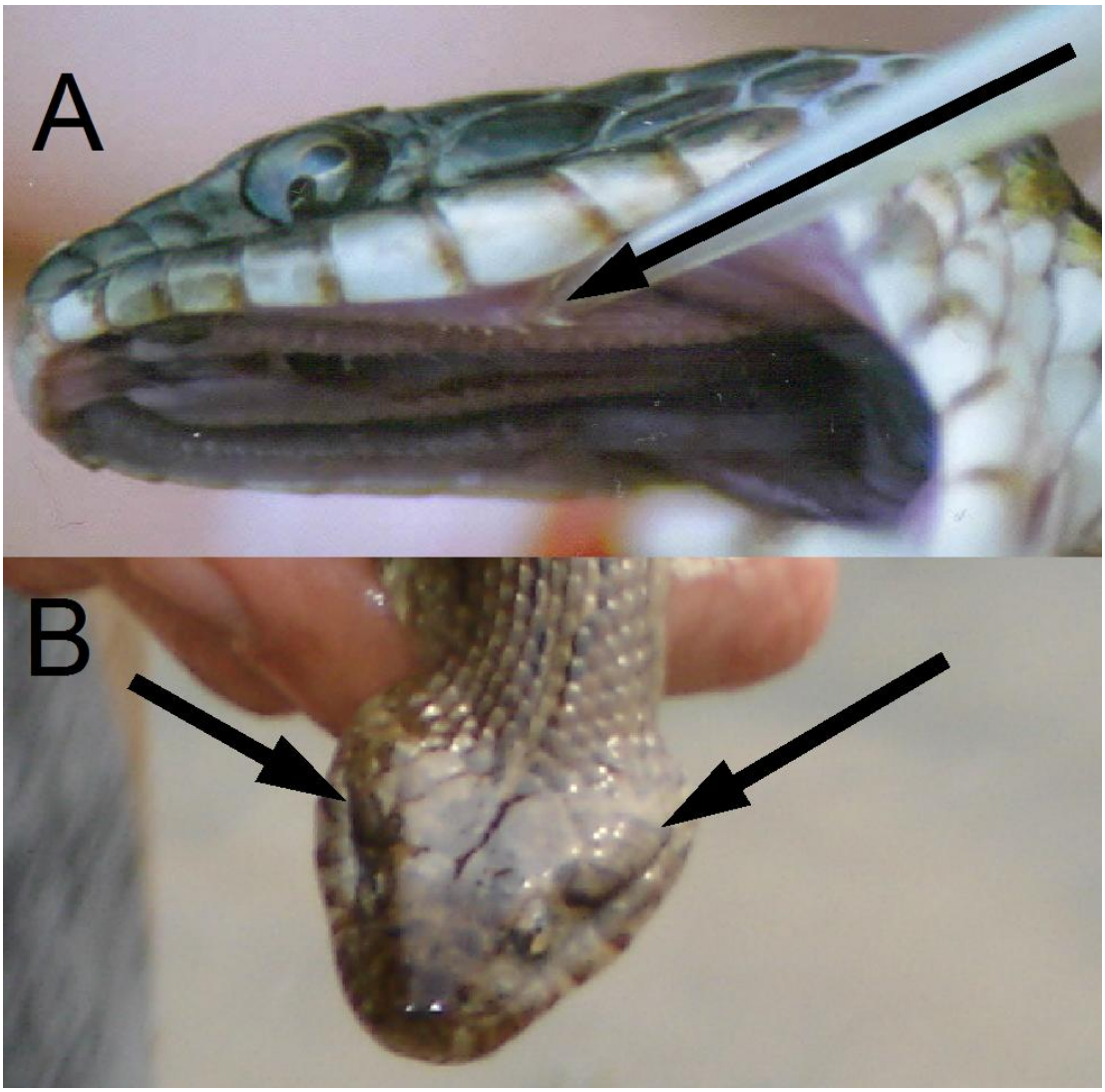

Figure 4:: Morphological characteristics of *N. sipedon*. In "A", the rearmost maxillary fang is noticeably larger than any other fang present in the jaw, possibly due to a function related to predatory activities. Frame "B" highlights significant engorgement of Duvernoy's glands in a captured snake. Also note the outward flare of the jaws in "B", giving the snakes head a triangular appearance.

Sample	µg/µl Protein	Total µg Protein Collected	Osmolarity (mmol/kg)
Snake 5 Saliva	0.17	87	355
Snake 5 DGS	0.09	9.2	414
Snake 5 Saliva Sample 2	0.13	65	409
Snake 5 DGS Sample 2	0.11	11	423
Snake 7 Saliva	0.06	32	550
Snake 7 DGS	0.2	20	450
Snake 8 Saliva	0.06	32	387
Snake 8 DGS	0.31	31	404
Snake 9 Saliva	0.22	110	526
Snake 9 DGS	0.31	31	418

Table 1: Summary of properties of tested salivary and DGS samples. Protein concentration was determined through a Bradford protein assay. Micrograms of protein was calculated by comparing absorbance data at 595nm to a standard curve derived from bovine serum albumin.

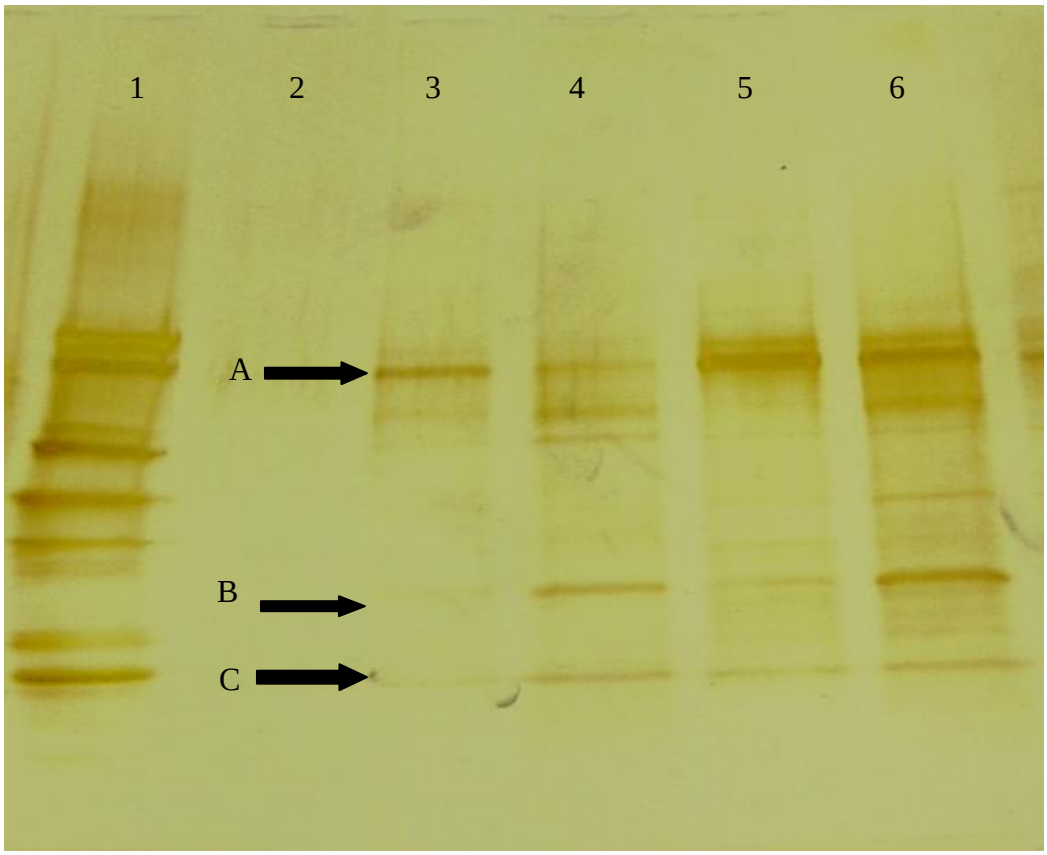

Figure 5: Silver stained SDS-PAGE of salivary proteins. The 59 kDa protein "A" appears to be the primary constituent of DGS. Proteins B (21 kDa) and C (18 kDa) also appear to be present in DGS, although in markedly lower concentrations. Salivary swabs contain many more proteins than DGS. Lane 3 exhibits proteins of 59, 21, and 18 kDa, Lane 4 exhibits proteins of 59, 47, 32, 21, and 18 kDa, Lane 5 exhibits proteins of 59, 21, and 18 kDa, and Lane 6 exhibits proteins of 59, 47, 32, 28, 21, and 18 kDa.

Lane1: Standards
Lane 2: Blank
Lane 3: DGS from snake 5
Lane 4: Salivary swab from snake 5
Lane 5: DGS from snake 7
Lane 6: Salivary swab from snake 7

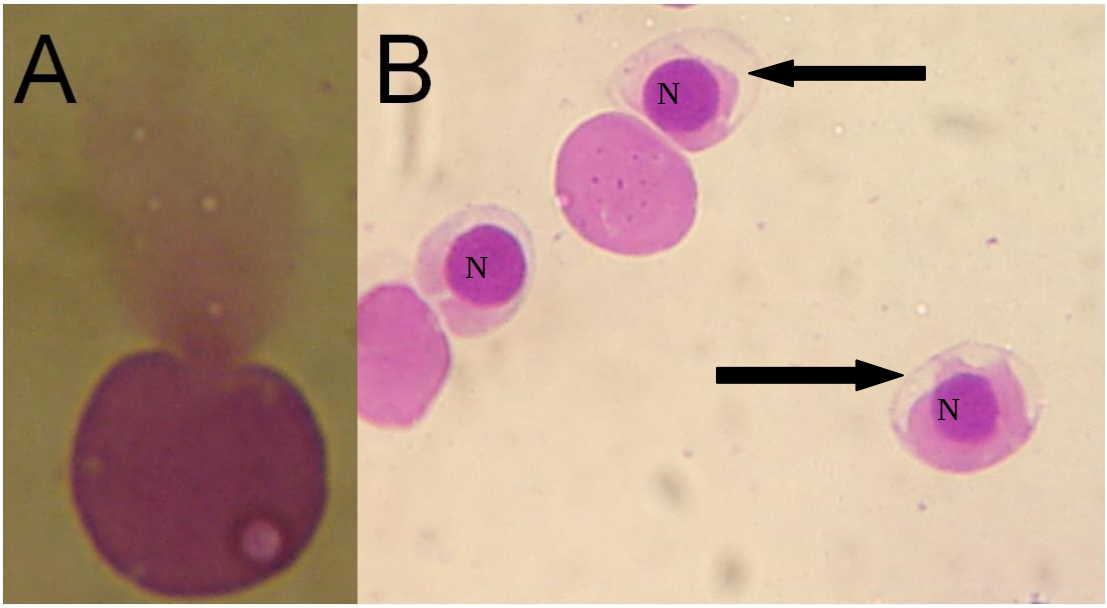

Figure 6: Light microscopy of erythrocytes from *Notropis atherinoides* exposed to proteins present in saliva and DGS of *Nerodia* sipedon. "A" indicates β-hemolysis. "B" shows presence of large vacuoles (indicated by arrows) and distention of erythrocytes. Nucleus in erythrocytes is identified by "N".

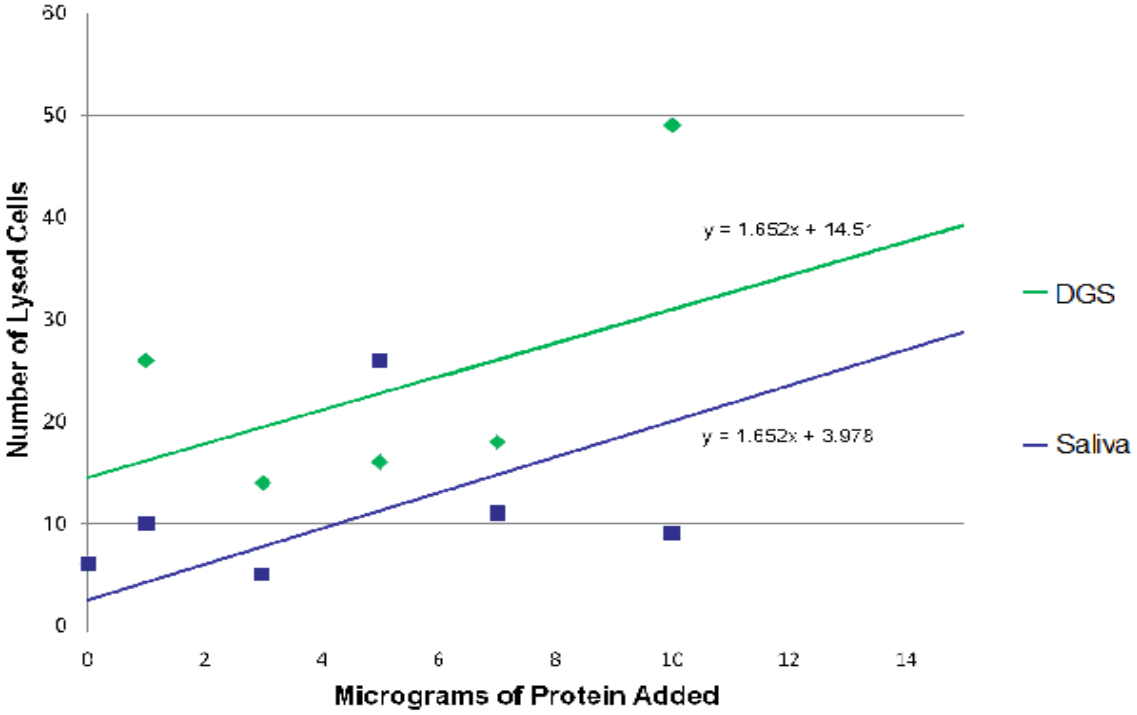

Figure 7: Linear regression analysis of the number of erythrocytes from *N. atherinoides* lysed from exposure to DGS secretions or salivary samples from *N. sipedon*.

Discussion

Several results from this study suggest the presence of hemolytic agents in the DGS of *N. sipedon.* In the course of procuring salivary and DGS samples for analysis, a number of morphological features on *N. sipedon* were noticed that appear to be underdeveloped envenomation structures. These characteristics include a pocket along the distal side of the jaw, a rearmost maxillary fang noticeably larger than other fangs in the mouth, and engorgement of Duvernoy's glands in agitated snakes. The observed pocket was verified to be absent in other colubrid specimens (*Lampropeltis mexicana*, *Elaphe guttata*, and *Pituophis melanoleucus*). It was also observed that this pocket retained significant amounts of salivary secretions, most likely those produced by the Duvernoy's gland. This observation suggests that the structure may represent a form of venom storage gland. The increased size of the rearmost maxillary fang is notable in that it is a unique characteristic also observed in a number of other colubrid snakes widely regarded as venomous (Pough, 1999). Engorgement of the Duvernoy's gland during agitation may be analogous to the preparation of venom glands for use against a predator. Another possibility is that this engorgement, which was observed along with a flattening and triangulation of the head, may serve to discourage predators by making the snake appear as if it is one of its dangerously venomous relatives. Current evidence suggests that colubrid snakes shared a common venomous ancestor with front-fanged snakes before evolutionary specialization favored speed and narrow bodies in the colubrids (Pough, 1999). Under this assumption, these structures are more likely evolutionary relics of a venom delivery system rather than new developments in the morphology of the snake.

Distinct differences in protein composition between salivary and Duvernoy's gland secretions were observed through SDS-PAGE. Duvernoy's secretions were clearly much simpler in protein composition and similar protein yields were achieved despite distinct differences in the amount of raw sample collected from the snake. Protein extracts representing the salivary complement of proteins and DGS were added to erythrocytes from *N. atherinoides* and hemolytic activity was observed. This hemolytic activity was had a marginal significant difference when compared to controls and increases in activity were observed to correlate with increased protein concentrations. Through linear regression analysis, it was shown that there was no significant difference between the hemolytic activities of DGS versus salivary proteins (Figure 7). Large variances in data may have caused this and could theoretically be remedied through an increased sample size. Through the course of the experiment, the development of large vacuoles within erythrocytes with distention of cellular membranes was observed as a result of administration of salivary proteins and DGS from *N. sipedon*. This observation prompted further investigation and the discovery that the addition of α-cyclodextrin to human erythrocytes causes similar effects (Irie, 1982). It has also been observed that α-cyclodextrin inhibits the ability of cell to cell adhesion (Sajadi, 2007). The formation of vacuoles as a result of cyclodextrin could theoretically result in cell lysis if the vacuoles were permitted to become large enough. Also, inhibition of cell to cell adhesion by cyclodextrins could be an explanation for the apparent anticoagulant activities of a bite from *N. sipeon*. During hemolytic assays, cells with vacuoles were not considered "lysed", possibly resulting in the high variation and insignificance of the observed numbers of lysed

cells. In addition, the majority of analytical techniques used in the course of this experiment were directed at a protein component of the saliva. If the toxic component was cyclodextrin based, analytical methods would have not elucidated any data on cyclodextrin composition and purification techniques would have largely removed most of the cylclodextrins from the tested samples. Further research will investigate the possibility of a cyclodextrin component to the DGS of *N. sipedon* and its hemotoxicity.

Conclusion

Conclusive evidence for hemolytic activity of the Duvernoy's gland secretions of *Nerodia sipedon* against erythrocytes *Notropis atherinoides* was not found in this study. Despite this, significant morphological and biochemical characteristics have been observed that continue to support the possibility of a hemolytic agent being present in the DGS of *N. sipedon*. Among these include enlarged rearmost maxillary fangs, engorged Duvernoy's glands, and significant protein composition differences between DGS and saliva. A protein of 59 kDa was found to be the primary component of DGS secretions of *N. sipedon.* Morphology of *N. atherinoides* erythrocytes after addition of DGS secretions changed such that hemolysis, membrane distention, and formation of large vacuoles was observed. These morphological distinctions along with the observed evidence of anticoagulant activity of the snake's saliva suggest the possibility that cyclodextrins may be present within the DGS of *N. sipedon* and may cause significant hemolysis.

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Curriculum Vita of Daniel Ranayhossaini

Academic Experience:

Penn State Erie Biology Major
-Molecular & Cell Biology & Biochemistry Option
Chemistry Minor
Graduation: May 2010

University of Pittsburgh School of Medicine
Interdisciplinary Biomedical Graduate Program – Entering Class of 2010

Professional Experiences:

Independent Research (2005 to present)
- Created an ongoing independent research project regarding hemotoxicity of saliva of *Nerodia sipedon* (2007)
- Presented findings at 2008 and 2009 Penn State Erie Sigma Xi

Engineering Intern at Pennsylvania Transformer Technology Inc. (2008-2009)
- Liaison between Pennsylvania Transformer Technology and Huntsman Advanced Materials
- Determined the best new material to use for core insulation with regard to chemical resistance and strength
- Determined the best new epoxy for binding insulation to core steel
- Interpreted gas chromatograms of transformer oil to measure levels of contamination and dissolved gas emissions

Molecular Biology Research Experience
- Submitted potato cDNA from phage clone libraries into EST database of NCBI (2009)
- Compared genetic variations between tomato strains using DNA microarrays (2009)
- Assisted in research on the production of antifungal phytoalexins in maize and sorghum (2005)

Penn State Erie School of Science/Learning Resource Center (2007-2010)
- Chemistry lab instructor
- Tutor in chemistry, biology, math, and physics
- Teaching Assistant for introductory and molecular biology courses

Honors & Activities:

- Vice-President of βββ Biological Honors Society
- Penn State Erie Chancellors Scholarship
- Penn State Erie Honors Program
- Frank W. Preston Memorial Scholarship
- Received "Runner-up" award for best presentation at Sigma Xi Research conference (2009)