UNIVERSIDADE DA CORUÑA

UiO

# Grao en Bioloxía

## Memoria do Traballo de Fin de Grao

**Does diet influence the venom composition of lacewings?**

**¿Influye la dieta en la composición del veneno de las crisopas?**

**Inflúe a dieta na composición do veleno das crisopas?**

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**ABSTRACT**

This study examines how nutrition affects lacewings' molecular composition, considering their diverse diets including aphids, insect eggs, and small insects. Experiments with lacewing larvae and varied diets were conducted, analysing molecular profiles using advanced techniques. Really few diet-based differences were observed in lacewings' molecular makeup. This work clarifies the intricate connection between nutrition and lacewings' molecular features, advancing our understanding of ecological dynamics and enhancing their efficiency as biological pest control agents. Overall, this research highlights the importance of considering diet-mediated effects in studying lacewings' molecular ecology.

**KEY WORDS**

Lacewing, larvae, Chrysopidae, venom extraction, venom composition, mass spectrometry, diet.

**RESUMEN**

Este estudio examina cómo la nutrición afecta a la composición molecular de las crisopas, considerando sus dietas diversas que incluyen áfidos, huevos de insectos e insectos pequeños. Se realizaron experimentos con larvas de crisopa y dietas variadas, analizando perfiles moleculares mediante técnicas avanzadas. Se observaron muy pocas diferencias significativas en la composición molecular de las crisopas basadas en su dieta. Este trabajo aclara la conexión compleja entre la nutrición y las características moleculares de las crisopas, mejorando

nuestra comprensión de las dinámicas ecológicas y mejorando su eficiencia como agentes de control biológico de plagas. En general, esta investigación resalta la importancia de considerar los efectos mediados por la dieta al estudiar la ecología molecular de las crisopas.

**PALABRAS CLAVE**

Crisopa, larva, Chrysopidae, extracción de veneno, composición del veneno, espectrometría en masa, dieta.

**RESUMO**

Este estudo examina como a nutrición afecta a composición molecular das crisopas, considerando a súa dieta diversa que inclúe áfidos, ovos de insectos e insectos pequenos. Realizáronse experimentos con larvas de crisopa e dietas variadas, analizando perfís moleculares mediante técnicas avanzadas. Observáronse moi poucas diferenzas na composición molecular das crisopas baseadas na súa dieta. Este traballo aclara a conexión complexa entre a nutrición e as características moleculares das crisopas, mellorando a nosa comprensión das dinámicas ecolóxicas e aumentando a súa eficiencia como axentes de control biolóxico de pragas. En xeral, esta investigación destaca a importancia de considerar os efectos mediados pola dieta ao estudar a ecoloxía molecular das crisopas

**PALABRAS CLAVE**

Crisopa, larva, Chrysopidae, extración de veleno, composición do veleno, espectrometría en masa, dieta.

## ABBREVIATIONS AND SYMBOLS

-MALDI: Matrix Assisted Laser Desorption/Ionization

-DTT: Dithiothreitol

-IAA: Indole-3-acetic acid

-TPCK: L-(tosylamido-2-phenyl) ethylchloromethylketone

-LC-MS: Liquid chromatography – Mass spectrometry

-mL: Millilitre

-µL: Microlitre

-mM: Millimolar

-mg: Milligram

-µg: Microgram

-ºC: Degrees Celsius

# 1. Introduction

Green lacewings are the second largest family of insects in the order Neuroptera, with approximately 6,000 known species today (Encyclopedia of Life), grouped into 92 genera and 7 suborders: Hemerobiiformia, Myrmeleontiformia, Nevrothiformia, Nymphidae, Psychopsidae, Rapismatidae and Chrysopidae. In the suborder Chrysopidae there are three subfamilies: Apochrysinae, Chrysopinae y Nothochrysinae (NCBI). Neuropterans constitute an order of insects with complete metamorphosis (holometabolism) and endopterygote development, meaning that after hatching, the eggs must go through larval and pupal stages before reaching adulthood. Among their notable characteristics is a distinct and somewhat primitive venation in a reticulate pattern, with two pairs of membranous wings (Neuroptera, from the Greek neûron, "nerve," and ptéron, "wing"; "wings with nerves"). They have a forward or downward-facing head, a narrow thorax, and generally long abdomen. They include commonly known insects such as green lacewings and antlions (Valencia et al., 2006). This is a highly diverse group with records dating back to the Permian period (over 250 million years ago), although most fossils are limited to wing fragments, making taxonomic assignments challenging.

Neuroptera are distributed worldwide and are recognized as important biocontrol agents. As a result, their habitats are highly varied, with different species found in almost all biomes or ecosystems, including areas with diverse climates. Notable habitats include leaf litter zones, areas near rivers or sandy areas, underground habitats, dead tree trunks, vegetation, and caves. Habitat types and shapes can determine and influence the diversity, abundance, and distribution of lacewings in forests (Bozdoğan, 2020)

Within the suborder Chrysopidae, the subfamily Chrysopinae is characterized by being medium-sized insects that range in colour from green to light brown, with green or golden eyes and antennae of various lengths. This is one of the most important entomophagous families within Neuroptera, as there are currently 15 genera that can serve as biocontrol agents (Amarasekare, 2020).

They communicate through vibrations in their abdomens that are transmitted through the leaves they sit on. Females use this system to attract males, even when they are just a few centimeters away.

Reproduction is usually sexual, with internal fertilization. Males inject sperm into the female's reproductive system. Depending on the species, eggs are laid using an ovipositor. The eggs are usually deposited at the end of a long, rigid but flexible thread, resembling a rod, with the other end attached to vegetation to remain inconspicuous to potential predators. Mating normally occurs in the first stages of adult life and they start laying eggs a few days after that (New, 1975)

The larvae of all species and the adults of some genera are carnivorous and feed on a variety of herbivorous insects such as aphids, scale insects, mealybugs, mites, and other soft-bodied insects found on leaves.

However, green lacewing larvae, like cannibals, also attack the eggs and larvae of their siblings. They are sympatric, heterospecific predators. To prevent early-hatched larvae from attacking unhatched eggs or larvae, the female lays each egg on a long stalk. When offspring are exposed to elevated heterospecific predation risk, parents will stop parental care and cannibalize their offspring. The eggs are oval and measure approximately 0.5 mm in length. Fresh eggs are green and turn brown as they mature and are ready to hatch. When eggs are destroyed, the egg stalks turn white and remain attached (Law, 2013).

In the larval stage, green lacewings are voracious predators. They are often called "aphid lions" because they are capable of attacking and quickly killing a large number of aphids and other arthropod pests. They also feed on nectar, pollen, and other small insects. They are highly mobile and have a chewing mouthpart, but also possess piercing and sucking mouthparts that are less developed. They produce digestive enzymes, which they administer to their prey and then suck out the contents of the prey's body cavity (Amarasekare, 2020).

The larvae of Neuroptera are venomous predators of other insects. They employ active hunting and ambush strategies, including pitfall traps. Their venom paralyzes prey and also serves a liquefying function. The venom originates from a gland in the maxilla called the venom gland. (Walker, 2018).

They can be aquatic (although not prevalent) but are mostly terrestrial. Each larva has a pair of large sickle-shaped mandibles that they use to pierce the body of their prey and suck out the bodily fluids. (Amarasekare, 2020).

They are highly active individuals and fast runners (Valencia et al., 2006), as their larvae have the ability to move more than 30 meters in search of prey. They typically have three larval stages, with the last stage forming a cocoon for pupation. They overwinter as larvae, prepupae, or adults. Diapause and temperature influence their development, longevity and reproductive patterns. Discrete generations and diapause can make difficult phenology and predation patterns (New, 1975).

As for adult green lacewings, they are green in colour and have golden eyes. They have two pairs of green wings that are held or folded. Adults measure between 17 and 19 mm in length, depending on the species. They are not strong fliers and are often found near aphid colonies. Adults fly at night and are often attracted to nighttime lights. They can live for 30 to 40 days. Newly emerged adults mate and lay eggs within 4 to 5 days. It takes approximately one month to complete the life cycle, from newly laid eggs to the emergence of adults (Amarasekare, 2020).

Due to these characteristics, their larvae are used in biological control programs in different crops, such as olive trees. Currently, many countries are interested in rearing and releasing green lacewings in biological control programs in agricultural systems (Redolfi, 2014). There’s an increase in implementing sustainable agricultural practices, preserving natural habitats, and controlled introduction of these insects. There’s also a benefit of utilizing neuropterans as part of biologican control strategies, reducing reliance on chemical pesticides (Stelzl, 1999)

## 2. Objectives

### General Objective

Determining the influence of diet on the molecular composition of green lacewing venom.

### Specific objectives

- ✓ Evaluate the effects of different artificial diets on the optimal development of green lacewings under controlled conditions
- ✓ Compare the molecular composition of venom from laboratory-cultivated green lacewings based on the type of diet.

## 3. Materials and methods

The present study was conducted in Oslo, Norway at the CEES of the UIO over a period of two months, from July 01, 2022, to September 01, 2022.

This research is based on an experimental methodology to understand the influence of different diets on the venom composition produced by green lacewings under controlled conditions, which allowed us to determine the most suitable parameters for conducting this study.

We start with a batch of adults stored individually in Eppendorf tubes. We begin to separate them by sex. Once we have the adults separated in males and females, we use tweezers to put one of each in their new enclosure. This enclosure consists of a circular plastic container with a lid made of tulle, in order to let the samples breathe, and secured with a circular plastic cap **(Figure1).** Once both specimens are in the enclosure we wait for them to start mating, so that the female can start laying eggs.

**Figure 1**: Lacewings enclosure.

A monitoring process was then carried out for approximately 5 days to verify that the females were laying eggs. After three days, small green eggs could be observed scattered throughout the enclosure where the pairs were kept. It is important that once the females start laying eggs, the containers are moved to a separate room from the rest of the adults, as the presence of larvae can affect egg laying. Freshly laid or infertile eggs have a green colour, and it is necessary to wait until they turn greyish before transferring them to plates. Greyish eggs indicate that they are about to hatch **(Figure 2).**

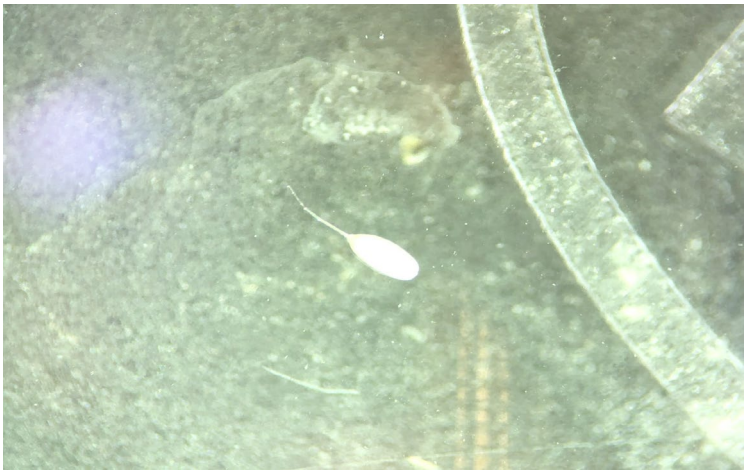

**Figure 2**: Lacewing egg about to hatch.

After 6-7 days, when there were enough greyish eggs, their collection began. It is necessary to collect the eggs before hatching as the larvae of this species are cannibalistic. The eggs were collected with tweezers and directly put in plates individually.

A sample of eight groups of *Chrysoperla carnea* (Stephens 1836) larvae was used, from which nine eggs were obtained per family, totalling seventy-two individuals. Each larval family was divided into three groups of three, each receiving a specific diet for one month, until they reached the appropriate size to extract the venom sample and perform the analysis of its molecular composition. These groups in each family received the following types of diets:

- Group A: Diet with flies
- Group B: Diet with aphids
- Group C: It was the control group; it received a diet based of moth eggs

Diets used in the different groups are detailed in Table 1.

**Table 1. Diet administered to the *Chrysoperla carnea larvae sample***

| Sample | Diet | Quantity |
|---|---|---|
| **Group A** | Flies | 1 |
| **Group B** | Aphids | 5-7 |
| **Group C** | Moth eggs | 1 tsp |

We start putting food in the plates once the eggs hatch. For the control group we deposit the moth eggs with a spatula. For the aphids we get them from a pea plant by lightly shaking its leaves, and we keep them in a separate plate. For the flies we have a big plastic container with food made of oats, honey and water at the bottom **(Figure 3).** Before getting the flies we lightly shake the container so all of them go to the bottom and can't escape, and we grab them individually with some tweezers to deposit them in the plate with the larva.

**Figure 3:** Flies enclosure.

It is necessary to check the larvae daily because when they reach a good size (about 1 or 2 days before they start forming their cocoon to enter the adult phase), all their food needs to be removed in order to proceed with milking the following day. This ensures that when they are hungry at the time of being attacked, they produce more venom, thus making the collection more efficient.

In some cases, when waiting too long to remove the food, upon checking the condition of the larvae the next day, they were already in a cocoon, and therefore cannot be milked, resulting in a small number of samples being lost.

Once the larvae have been starved for at least 24 hours they are ready to milk. For this process we need to grab them with some tweezers and secure them with a magnet since they move fast. Once that's done the larva gets slightly poked so they can start to attack and produce venom **(Figure 4).** To induce them to a defensive state in which they produce venom, we use a hose connected to a $CO_2$ cylinder, which has a lever to regulate the gas output. We lightly gas them until they start producing venom, this can't be for too long since they will pass out.

The drops of venom are recollected in a pipette tip and then dissolved in 5 µl of water in an Eppendorf tube. They must be put in ice as soon as possible and then preserve them in the freezer until is time for the analysis. **(Figure 5)**

**Figure 4**: Larva ready for the venom extraction.

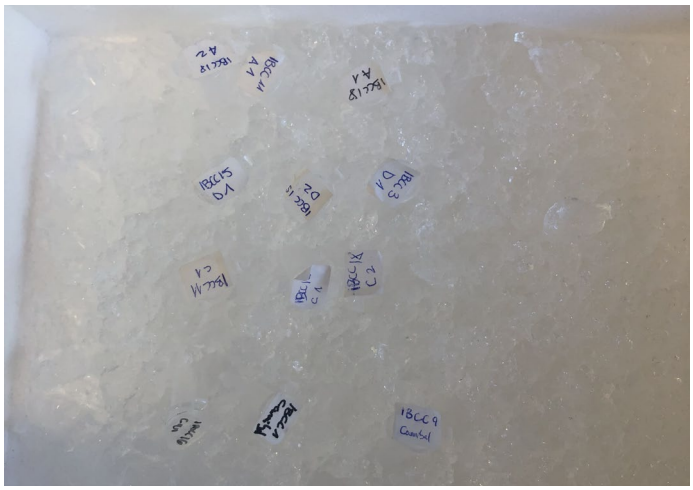

**Figure 5**: Samples stored in ice to prevent protein denaturation.

For the evaluation of the molecular composition of venom from laboratory-cultivated green lacewings, mass spectrometry (MS) was used for peptide identification. This is an analytical technique applied to assess compounds of organic, inorganic, or biological nature, providing qualitative and/or quantitative information for analysis (López et al., 2020).

We employed DTT to break the disulfide bonds that link the proteins together and then reacted them with IAA to prevent the reformation of oxidized cysteines, also known as cystines.

In this regard, the Cold Spring Harb protocol (2011) was employed to determine trypsin digestion of proteins in solution. For tandem mass spectrometry, digestion is typically performed using the protease trypsin, which cleaves on the carboxy-terminal side of lysine (K) and arginine (R) residues, except for KP and RP sites. The modified trypsin (treated with TPCK) used in this protocol is a serine endopeptidase prepared by treating trypsin with L-(tosylamido-2-phenyl) ethylchloromethylketone (TPCK) to inactivate any remaining chymotryptic activity. TPCK acetylates the amino groups of lysine residues to limit autolysis. Modified trypsin cleaves KP and RP bonds at a much slower rate than other amino acid residues. This method is described below.

1. The protein sediment is dissolved in a 100 mM ammonium bicarbonate and 5% acetonitrile solution.
2. The exact volume to resuspend a protein sediment should be considered ºarbitrary. Sometimes it may be necessary or not to add more buffer to completely solubilize a sediment. Trypsin is a fairly robust enzyme, but its activity is optimal at pH 8. Ammonium bicarbonate forms a mildly alkaline buffer when dissolved in $H_2O$ (pH 7.7) and is commonly used to buffer trypsin digestions. It is a simple volatile buffer that can be removed by lyophilization. Low concentrations of acetonitrile (<10%) facilitate trypsin proteolysis compared to ammonium bicarbonate buffer alone.
3. Check the pH of the resuspended protein solution by placing less than <1µL on the second square of a pH 0-14 paper strip.
4. Add 1/10 of the volume of 50 mM DTT. Incubate the reaction for 5 minutes at 65°C.
5. Add 1/10 of the volume of 100 mM IAA. Incubate the reaction in the dark for 30 minutes at 30°C.
6. Modified sequencing-grade trypsin is added as follows:
    a. For the digestion of a 20 µl summary of silver-stain detectable proteins, add 2 µl of 100 ng/µl common trypsin

b. To digest Coomassie detectable proteins, add 1-5 µl of 1 µg/µl common trypsin.

c. If the total protein amount in the sample is known, add trypsin to achieve a final substrate:trypsin ratio of 50:1.

7. The reaction is incubated overnight at 37°C.
8. Stop the reaction by adjusting the pH of the solution below pH 6.
9. Quickly freeze the digest with dry ice and store the digested proteins at -20 °C.
10. Before using in mass spectrometry, the digestion product should be centrifuged at >15,000 g for 5 min. If a pellet is detected, transfer the supernatant to a new tube.

Once the proteins were transformed into a linear structure, we utilized trypsin to cleave them into smaller peptides. This was necessary because the intact proteins were too large to efficiently fragment, thus impeding the acquisition of sequence information. Trypsin specifically cleaves proteins after arginine and lysine residues, except when followed by a proline. As a result, we gain some initial sequence information when matching sequences to peptide fragments. Furthermore, the newly digested tryptic fragments possess at least two charges (N-terminus and K/R), enabling easy detection and improved fragmentation in the MS.

Following digestion, we performed a sample purification process to eliminate excessive salts and any substances that could disrupt the LC-MS analysis. To accomplish this, we utilized zip tips, which are essentially pipette tips containing a reversed-phase C18 column at the tip. We employed a stepwise gradient approach to bind, cleanse, and elute our digested proteins:

I. Initially, we activated the C18 resin by using a high concentration of acetonitrile. The term "C18" denotes an alkyl chain composed of 18 carbon atoms that covers the resin's surface.
II. Since peptides exhibit an affinity for C18 under hydrophilic conditions, we equilibrated the tip using a low concentration of acetonitrile.

III. Subsequently, we bound our peptides by repeatedly flushing the sample through the tip.
IV. To desalt and cleanse the peptides, we passed them through a clean solution of low acetonitrile. Finally, we eluted the peptides into a new tube by repeatedly flushing them (pipetting up and down) using a high concentration of acetonitrile.

Essentially, this process mirrors the LC (liquid chromatography) step of LC-MS, where peptides are flushed onto a lengthy C18 column and gradually eluted (for subsequent MS analysis) by utilizing an acetonitrile concentration that gradually increases. However, since our peptides would not bind to the C18 column in the LC when subjected to high acetonitrile concentrations, we must remove the acetonitrile before injecting the peptides into the LC. Luckily, acetonitrile is volatile, which is why we utilized vacuum centrifugation to evaporate the samples prior to redissolving the peptides in the same solution employed at the very beginning of our LC-MS solvent gradient.

From the four groups with the different diets, control, aphids, flies and cannibal, we chose three larvae of each to analyze the venom. After this we will obtain the amino acid sequences of all transcriptome contigs identified in the LC-TIMS-MS/MS data. In this excel we obtained 573 rows with results for the 12 samples in which we could observe the protein ID, protein group, accession, -10lgP, the coverage of each sample, the area sample, peptides, PTM, average mass and a short description. To select which proteins we are going to use we have to take a look at the columns named “Accession”. In that column we’ll only choose proteins that begins with “TRINITY”. This is a proper name used to designate specific protein sequences or a set of related protein sequences. Each of these has an additional number that is used to organize and catalog the protein sequences. In addition to the Excel file, we obtained a FASTA file format with all the sequences named “TRINITY”, which, once the selection of the most remarkable ones is made, we will pass through different databases. We’ll run them through three different programmes, UniProt, InterProScan and SignaIP. To select the sequences, we’ll take a look at the presence or absence of the “TRINITY” depending on the group, and selecting what seems unusual. We divided it int five groups, the ones present only in the control, then only in aphids’

diet, only in flies diet, only in cannibal diet and finally the ones common and abundant in all of them.

## 4. Results

Over a period of approximately 20 days, daily checks were conducted on the larvae to assess their condition and replenish their food as needed. The larvae fed with the control diet exhibited the fastest growth and were also the healthiest. The larvae fed with aphids showed relatively rapid growth and displayed slightly more aggression compared to the control group. However, in the case of larvae fed with flies, there was an increase in mortality. Despite being predators, the majority of larvae did not feed on the flies, even when they were already dead. Additionally, the type of food influenced the external appearance of the larvae. The control larvae had a yellowish colour **(Figure 6),** those feeding on aphids appeared green **(Figure 7)**, and those fed with flies had a dark coloration. **(Figure 8)**

**Figure 6:** Larva with control diet showing a yellowish appearance.

**Figure 7**: Larva on the aphids' diet showing a greenish appearance.

**Figure 8**: Larva on the flies' diet showing a black appearance.

Furthermore, due to issues with moisture scarcity, a significant number of larvae dried out, resulting in a significantly reduced sample size for venom collection.

An additional group of cannibalistic larvae was studied. These larvae exhibited higher aggression levels and it was observed that they released a greater amount of venom during milking. The coloration of these cannibalistic larvae varied between green and darker shades depending on whether they consumed unfertilized eggs or other larvae.

We obtained a summary of the spectral matching between LC-TIMS-MS/MS data and the transcriptome of the same species, along with the presence of added contaminants. We also obtained the amino acid sequences of all transcriptome

contigs identified in the LC-TIMS-MS/MS data. Accessions beginning with "TRINITY" can be searched to retrieve the corresponding sequences. The results have been filtered to maintain a false discovery rate of less than 1%. In addition, any entries with a coverage below 9% have been removed to ensure relative confidence in those containing two or fewer tryptic peptides.

We got an excel with 573 proteins. After examining the presence or absence of these proteins, a selection is made of those that are present in a single group or that stand out in terms of quantity compared to the rest. **(Appendix 1)**

Once these proteins, in our case 25, are selected, their sequences are extracted in FASTA format and subjected to a series of analyses. The analyses include running the sequence through Uniprot to check for any similar sequences known from elsewhere. Interproscan is used to identify any recognizable domains within the sequence. Lastly, SignalP is utilized to search for predicted signal peptides, which provide insights into whether the protein/peptide is likely to be a secreted protein/peptide, a fragment, or a cytosolic protein.

These proteins were selected based on the following criteria: they only appeared in the control diet, they only appeared in the diet with flies, they only appeared in the diet with aphids, they only appeared in the cannibal diet, or they appeared in all diets.

**Table 2: Sequences chosen for each group**

| Control | Aphids | Flies | Cannibal | Common |
|---|---|---|---|---|
| TRINITY_DN789 | TRINITY_DN16815 | TRINITY_DN4316 | TRINITY_DN3466 | TRINITY DN_849 |
| TRINITY_DN1172 | TRINITY_DN600 | TRINITY_DN1687 | TRINITY_DN1182 | TRINITY_DN0 |
| | TRINITY_DN26 | TRINITY_DN3679 | TRINITY_DN2672 | TRINITY_DN10234 |
| | TRINITY_DN6651 | TRINITY_DN1335 | | TRINITY_DN48 |
| | | TRINITY_DN1415 | | TRINITY_DN92 |
| | | TRINITY_DN15306 | | TRINITY_DN1012 |
| | | TRINITY_DN30 | | TRINITY_DN3000 |
| | | TRINITY_DN645 | | TRINITY_DN42 |

These proteins were subjected to the before mentioned analyses. In Interproscan, results were obtained for all of them except for three: TRINITY_DN6651, TRINITY_DN3000, and TRINITY_DN15306.For the remaining proteins, we obtained common domains that already exist, which provide us with information about the type of protein obtained. **(Appendix 2)**

**Table 3: Results of the chosen sequences after running them through InterproScan**

| nº | Name | Molecular Function | nº | Name | Molecular Function |
|---|---|---|---|---|---|
| 1 | HATPase_C_sf | ATP binding | 12 | Peptidase_S1_PA | Serine type endopeptidase activity |
| 2 | Fumarylacetoacetase | Catalytic activity | 13 | P-loop_NTPase | GTP binding |
| 3 | WH1 | G- Protein-coupled glutamate receptor | 14 | P-loop_NTPase | GTP binding |
| 4 | Annexin | Calcium binding | 15 | His_PPase_superfam | |
| 5 | Kazal_dom_sf | Protein binding | 16 | Saposin-like | Lipid metabolic process |
| 6 | Colicin | Pore-forming activity | 17 | Lipid_transp_b-sht_shell | Lipid transport |
| 7 | BGBP_N_sf | Hydrolase activity | 18 | NAD(P)-bd_dom_sf | Glucose metabolic process |
| 8 | IsoPropMal_DH-like_dom | Isocitrate dehydrogenase activity | 19 | Tropomyosin | |
| 9 | PyrdxIP-dep_Trfase | Cystathionine gamma-lyase activity | 20 | Ubiquitin-like | Ubiquitin protein ligase binding |
| 10 | Chemosensory protein | | 21 | LRR_dom_sf | Protein binding |
| 11 | CGCT-like_sf | Gamma- glutamylcyclotransferase activity | 22 | ApoLp-III | Lipid transport |

# 5. Discussion

As mentioned in the study from Walker, (2018) the source of the venom responsible for prey paralysis by neuropteran larvae is not clear from current literature There are also suggestions that the alimentary canal might be the source of paralysing venom produced by larvae. To make poison use clear the maxillary gland and digestive tract contents of larval neuropterans should be investigated utilizing transcriptomics and proteomics to identify which toxins are actually injected into prey to aid in capturing and isolate and characterize the toxins from both tissues.

In the context of New (1975) review on Chrysopidae and Hemerobiidae, the phenomenon of cannibalism among individuals of these insects is discussed. This behaviour could be influenced by environmental factors, such as prey availability, and it could have significant implications for population structure and dynamics. Since we observed that cannibalistic larvae produced more amount of

venom and also were the most aggressive ones, this could mean there's a potential selection of the most aggressive larvae

Also in a study carried out by Rothschild et al. (1973), demonstrated that these larvae can store toxic cardenolides that they acquired from their prey, in this case a type of aphid. We found some unique protein in the group of larvae that were feeding on aphids so this could support Rothschild's hypothesis.

According to the obtained results, we can observe that the presence or absence of sequences is very similar across all groups from different diets. We can see that among the sequences common to all groups, TRINITY_DN92 is the most abundant. This appears to be a type of ubiquitin, which, when analyzed in Uniprot, shows an 86.6% match with an existing sequence. Additionally, we can observe that the proteins common to all groups are primarily involved in lipid transport. In the remaining groups, we find a set of proteins that are quite different from each other. Furthermore, when these sequences were passed through Uniprot, we obtained low percentages of matches, except for a couple of exceptions.

Among these exceptions, we found TRINITY_DN789 from the control group, which showed an 88.8% match to Heat shock protein 83 found in the organism *Tribolium castaneum.* Another exception was TRINITY_DN3466 from the cannibal diet group, with a 96.5% match to Elongation factor 1-alpha found in the organism *Asbolus verrucosus.* From the cannibal group, we also identified TRINITY_DN1182, with a 95.2% match to Ras-related protein found in the organism *Cryptotermes secundus*. For the remaining proteins, the match percentage ranged from 25% to 80%.

We could also find strong variation between larvae in the same group, specifically in the group feeding on flies. This could be similar as the previous mentioned Rothschild et al. (1973), that the larvae can acquire some elements from their preys, and since the flies were from different batches, this could've affected the venom in the lacewing larvae.

## 6. Conclusions

1.1 The diet followed didn’t strongly affect the molecular composition of venom in lacewing larvae, but there still were changes.

1.2 This could mean that the animal could be involved in the venom composition

1.3 The majority of changes were observed in larvae that had followed a diet with flies, suggesting that this could be due to variations in the flies’ diet.

1.4 All groups responded positively to the different diets except for the one with flies that had a high mortality rate which could mean that, that wouldn’t be a feeding option in their natural habitat.

1.5 The cannibalistic experiment suggested a possible selection based on aggressiveness.

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# 8.Appendix 1

## Sequences chosen from the Excel

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 213 | 354 | TRINITY_DN849_c0_g2_i1.p1 | 193.97 | 3 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 2 | 1 | 0 | 2 | 0 |
| 213 | 355 | TRINITY_DN849_c0_g2_i2.p1 | 193.97 | 3 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 2 | 1 | 0 | 2 | 0 |
| 258 | 286 | TRINITY_DN789_c2_g1_i1.p1 | 164.92 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 288 | 3887 | TRINITY_DN16815_c0_g1_i1.p1 | 66.78 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 301 | 1809 | TRINITY_DN4316_c0_g1_i1.p1 | 83.03 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| 301 | 1810 | TRINITY_DN4316_c0_g1_i2.p1 | 83.03 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| 315 | 3597 | TRINITY_DN600_c0_g2_i2.p1 | 64.42 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 315 | 3598 | TRINITY_DN600_c0_g2_i4.p1 | 64.42 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 315 | 3599 | TRINITY_DN600_c0_g2_i1.p1 | 64.42 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 315 | 3600 | TRINITY_DN600_c0_g2_i3.p1 | 64.42 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 316 | 1164 | TRINITY_DN1687_c0_g1_i1.p1 | 146.19 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| 316 | 1165 | TRINITY_DN1687_c0_g1_i2.p1 | 146.19 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| 316 | 1166 | TRINITY_DN1687_c0_g1_i3.p1 | 146.19 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| 317 | 4532 | TRINITY_DN3679_c0_g1_i7.p1 | 86.15 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| 317 | 4533 | TRINITY_DN3679_c0_g1_i4.p1 | 86.15 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| 317 | 4534 | TRINITY_DN3679_c0_g1_i9.p1 | 86.15 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| 317 | 4535 | TRINITY_DN3679_c0_g1_i1.p1 | 86.15 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| 324 | 3806 | TRINITY_DN1335_c0_g1_i7.p1 | 80.32 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 324 | 6068 | TRINITY_DN1335_c0_g1_i6.p1 | 80.32 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 324 | 6069 | TRINITY_DN1335_c0_g1_i2.p1 | 80.32 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 324 | 6070 | TRINITY_DN1335_c0_g1_i4.p1 | 80.32 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 156 | 950 | TRINITY_DN0_c0_g2_i1.p1 | 85.64 | 4 | 4 | 0 | 4 | 0 | 4 | 4 | 4 | 0 | 4 | 0 | 4 | 4 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 264 | 132164 | TRINITY_DN26_c0_g1_i64.p1 | 111.66 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 264 | 162217 | TRINITY_DN26_c0_g1_i17.p1 | 111.66 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 264 | 370465 | TRINITY_DN26_c0_g1_i65.p1 | 111.66 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 264 | 370547 | TRINITY_DN26_c0_g1_i47.p1 | 111.66 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 264 | 370640 | TRINITY_DN26_c0_g1_i40.p1 | 111.66 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 264 | 370723 | TRINITY_DN26_c0_g1_i23.p1 | 111.66 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 140 | 102411 | TRINITY_DN10234_c0_g1_i1.p1 | 139.9 | 5 | 5 | 5 | 5 | 5 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 5 |
| 249 | 718 | TRINITY_DN3466_c0_g1_i4.p1 | 194.49 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 249 | 719 | TRINITY_DN3466_c0_g1_i2.p1 | 194.49 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 249 | 720 | TRINITY_DN3466_c0_g1_i5.p1 | 194.49 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 249 | 721 | TRINITY_DN3466_c0_g1_i7.p1 | 194.49 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 249 | 722 | TRINITY_DN3466_c0_g1_i1.p1 | 194.49 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 249 | 818 | TRINITY_DN3466_c0_g3_i1.p1 | 194.49 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 249 | 819 | TRINITY_DN3466_c0_g3_i2.p1 | 194.49 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 323 | 4915 | TRINITY_DN1182_c0_g1_i3.p1 | 81.43 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 323 | 4916 | TRINITY_DN1182_c0_g1_i2.p1 | 81.43 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 323 | 4917 | TRINITY_DN1182_c0_g1_i1.p1 | 81.43 | 5 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 196 | 4158 | TRINITY_DN48_c0_g1_i8.p1 | 142.14 | 7 | 0 | 7 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 3 | 3 |
| 196 | 4166 | TRINITY_DN48_c0_g1_i6.p1 | 142.14 | 7 | 0 | 7 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 3 | 3 |
| 196 | 4225 | TRINITY_DN48_c0_g1_i5.p1 | 142.14 | 7 | 0 | 7 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 3 | 3 |
| 239 | 247 | TRINITY_DN1172_c1_g1_i2.p1 | 175.95 | 8 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| 239 | 248 | TRINITY_DN1172_c1_g1_i6.p1 | 175.95 | 8 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| 239 | 249 | TRINITY_DN1172_c1_g1_i3.p1 | 175.95 | 8 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| 239 | 250 | TRINITY_DN1172_c1_g1_i1.p1 | 175.95 | 8 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| 103 | 27824 | TRINITY_DN92_c0_g1_i45.p1 | 254.28 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 0 | 9 | 9 | 0 | 9 | 9 |
| 103 | 27804 | TRINITY_DN92_c0_g1_i44.p1 | 254.28 | 13 | 12 | 12 | 12 | 12 | 12 | 12 | 0 | 12 | 12 | 0 | 13 | 13 |
| 103 | 27815 | TRINITY_DN92_c0_g1_i50.p1 | 254.28 | 13 | 12 | 12 | 12 | 12 | 12 | 12 | 0 | 12 | 12 | 0 | 13 | 13 |
| 103 | 17102 | TRINITY_DN92_c0_g1_i20.p1 | 254.28 | 17 | 16 | 16 | 16 | 16 | 16 | 16 | 0 | 16 | 16 | 0 | 17 | 17 |
| 103 | 27792 | TRINITY_DN92_c0_g1_i2.p1 | 254.28 | 17 | 15 | 15 | 15 | 15 | 15 | 15 | 0 | 15 | 15 | 0 | 17 | 17 |
| 117 | 266 | TRINITY_DN1012_c0_g1_i4.p1 | 269.54 | 12 | 6 | 0 | 3 | 0 | 9 | 6 | 9 | 9 | 12 | 0 | 3 | 6 |
| 117 | 267 | TRINITY_DN1012_c0_g1_i2.p1 | 269.54 | 12 | 6 | 0 | 3 | 0 | 9 | 6 | 9 | 9 | 12 | 0 | 3 | 6 |
| 117 | 268 | TRINITY_DN1012_c0_g1_i3.p1 | 269.54 | 12 | 6 | 0 | 3 | 0 | 9 | 6 | 9 | 9 | 12 | 0 | 3 | 6 |
| 117 | 269 | TRINITY_DN1012_c0_g1_i5.p1 | 269.54 | 12 | 6 | 0 | 3 | 0 | 9 | 6 | 9 | 9 | 12 | 0 | 3 | 6 |
| 235 | 255 | TRINITY_DN2672_c0_g1_i9.p1 | 242.33 | 12 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| 235 | 256 | TRINITY_DN2672_c0_g1_i27.p1 | 242.33 | 12 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| 235 | 257 | TRINITY_DN2672_c0_g1_i29.p1 | 242.33 | 12 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| 235 | 258 | TRINITY_DN2672_c0_g1_i11.p1 | 242.33 | 12 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| 129 | 3883 | TRINITY_DN3000_c0_g1_i1.p1 | 117.05 | 13 | 13 | 13 | 13 | 0 | 13 | 13 | 13 | 13 | 13 | 0 | 13 | 13 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 242 | TRINITY_DN1415_c0_g1_i1.p1 | 162.56 | 13 | 0 | 0 | 13 | 0 | 0 | 0 | 13 | 13 | 13 | 0 | 0 | 0 |
| 231 | TRINITY_DN15306_c0_g1_i1.p1 | 119.39 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 0 | 18 | 0 | 0 | 0 |
| 98 | TRINITY_DN42_c0_g1_i2.p1 | 244.83 | 19 | 19 | 19 | 19 | 0 | 19 | 12 | 19 | 19 | 19 | 19 | 19 | 12 |
| 98 | TRINITY_DN42_c0_g1_i3.p1 | 244.83 | 19 | 19 | 19 | 19 | 0 | 19 | 12 | 19 | 19 | 19 | 19 | 19 | 12 |
| 192 | TRINITY_DN30_c0_g1_i3.p1 | 230.94 | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 27 | 0 | 0 | 0 |
| 276 | TRINITY_DN6651_c0_g3_i1.p1 | 189.15 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 32 | 0 |

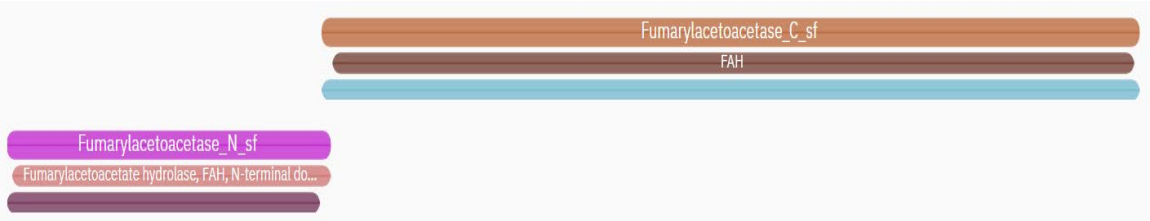
## 9. Appendix 2

**Results obtained from InterProScan for each protein sequence selected**.

These are all the common domains present in the protein sequences. Numbers explained in **Table 3.**

1. TRINITY_DN789


2. TRINITY_DN1172



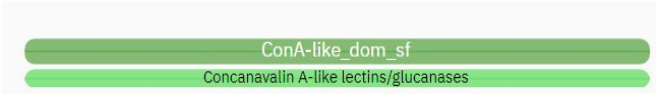

3. TRINITY_DN16815


4. TRINITY_DN600

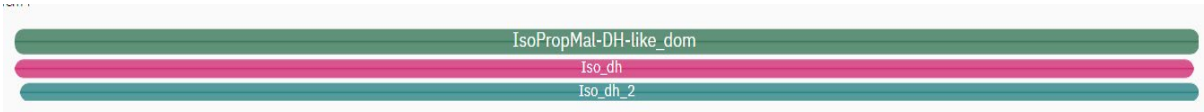

5. TRINITY_DN26


6. TRINITY_DN4316

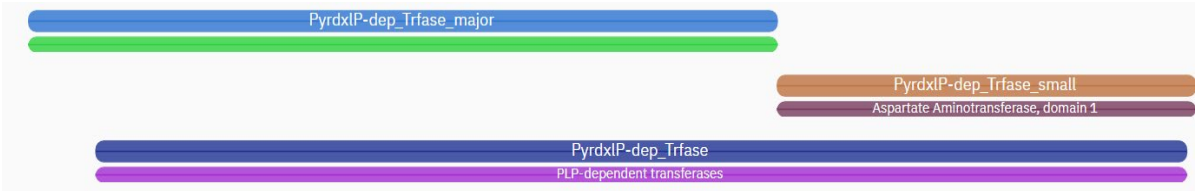

7. TRINITY_DN1687




8. TRINITY_DN3679




9. TRINITY_DN1335



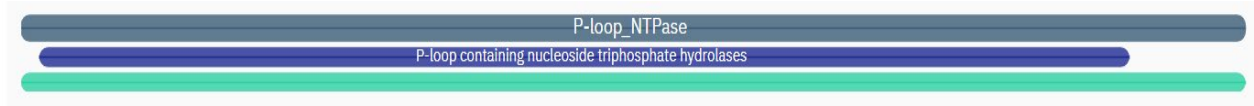

10. TRINITY_DN1415


11. TRINITY_DN30

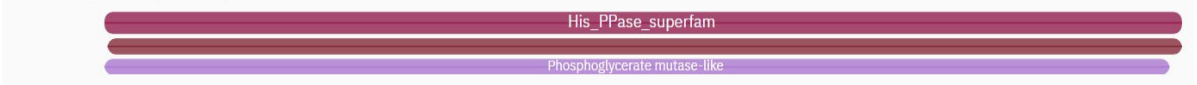

12. TRINITY_DN645


13. TRINITY_DN3466


14. TRINITY_DN1182

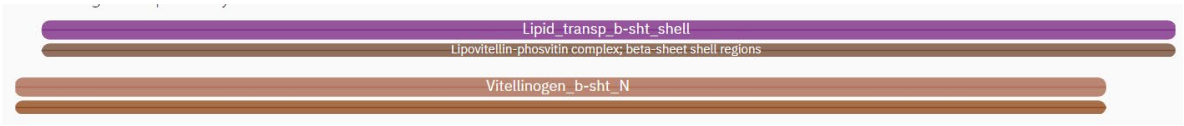



15. TRINITY_DN2672

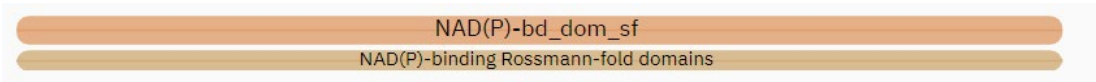


16. TRINITY_DN849

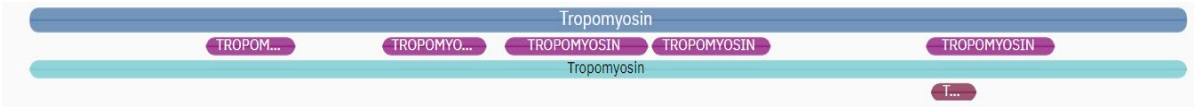

17. TRINITY_DN0




18. TRINITY_DN10234

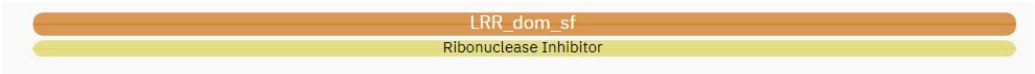



19. TRINITY_DN48




20. TRINITY_DN92


21. TRINITY_DN1012




22. TRINITY_DN42