

**The venom apparatus of the Weever fish (*Trachinus vipera*).**

**By**

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## **KEYWORDS**

Trachinus vipera

Trachindae

Opercular

Spine

Weever

Venom

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## INTRODUCTION

"Cruel spines defend some fishes, as the Goby fond  
Of sands and rocks, the scorpion, swallows fleet,  
Dragons and Dog fish, from their prickly mail  
well named the spinous. These in the punctures sharp,  
A fatal poison from their spines inject." (Oppian)

There are four species of weever fish belonging to the family Trachinidae, all contain the venomous spines, the toxicity of which varies between the individual species, with the Lesser weever (*Trachinus vipera*) the most toxic of all. Members of the Trachinidae family can be found from the North sea on the shores of Northwest Africa and throughout the whole of the Mediterranean sea and the black sea. There are three other species of weever: *Trachinus draco*, the great weever; *Trachinus radiatus* and *Trachinus araneus*, the last two are collectively known as the southern weevers.

Although the first documented study of the venom and associated structures of the weever fish family (Trachinidae) wasn't until 1840 (this was my Allman). The venomous nature of the weever was known about as far back as 50 AD in the "Greek Herbal" of Discorides (circa 50 AD.) (translated by gunther 1934). Under the subject Drakon Thalassois (*Trachinus draco*) is written:

"The sea dragon, being opened and soe applied is a cure for ye hurt  
doone by his prickles"

Indicating that the venomous nature of the Trachinus family was known about, whether this cure actually works is as yet unproved. The poet Oppian, (as quoted by Drummond (1840)) actually wrote poems about fish with spines including the members of the Weever family. Although in this poem he groups the weevers under a different name (Dragons) and includes other fish that do not process a venom of any kind.

The presence of venom considered to be a virus before 1840, was thought by the vast majority of scientists to be confined to the dorsal spines of the fish. It wasn't until Allman (1840) had the misfortune of being stung by the Opercular spine of the fish and recording the reaction that followed, that this theory was questioned. Allman wrote in a letter dated August 20 1840,

"On the 9th August 1839, I was wounded near the top of the thumb by *Trachinus vipera*, which had just taken in a seine of herrings, sand eels & co. The wound was inflicted by the spine attached to the gill covers during my attempt to seize the fish"

He then goes on to describe the pain sensation and symptoms experienced in the hand and wrist. Contained within the letter is a short description of the Opercular spine. Allman (1840) found the spine was grooved from the tip to the posterior edge of the bony part of the operculum. He also found a small pulpy mass which he suggested could possibly be of a glandular nature. Many scientists since have described the Opercular spine varying in detail but all essentially agreeing on the grooved structure and the presence of the small pulpy mass and in some cases describing this in detail with regards to position and structure. Parker (1888) describes the structure of the glands and possible method of venom delivery in two species of weever fish, *T.draco* and *T.vipera*.

In his paper he suggests that a possible method of venom delivery is that the cells simply burst and their contents pass up the groove in the spine to the exterior. This idea of the cells bursting is also mentioned by Skeie (1962) who describes these glands in detail. He also describes the secretion of toxin as that of the Holocrine type (without a venom duct, toxin passes directly from the cells to the spinal groove). The cellular contents were exuded as a "crude toxin which distinctly presented itself as a granular, stainable pulp.

In another paper Skeie (1962b) concentrates on the venom itself, mentioning that the toxicity of the secretion removed from the venom glands was varied having a toxicity between 2000 and 10.000 DML (number of lethal doses for mice) per ml when injected intravenously on white mice weighing between 16-18 grams. (Skeie:1962b) He further goes on to mention that generally speaking the venom of the weever has a similar toxicity to various species of common viper, *Vipera aspis*, *Vipera ammodytes* and *Vipera berus*.

The venom of the weever consists of 3 main constituents: Proteins, Carbohydrates and Lipids. Skeie (1962b) found that only a small fraction of the protein contained toxic properties and also showed that an anti-venom could be developed because survival test animals such as rabbits and mice showed signs of being immune to the toxin after treatment.

Studies have been carried out since 1962 on the structure and function of the venom glands on all members of the weever fish family. These scientists have found the same structures. Venom studies have also confirmed the previous work, only differing in the techniques and use of different test animals.

The first time weever fish anti-venom was produced in sufficient quantities for general use, was in 1968. Since this time anti-venom has been manufactured in the Institute for the Control and Research of Immuno-biological substances, by Dr. Dubravka Matic-Piantanida. The use of such an anti-venom is confined to those patients who have reacted unfavourably to the normal treatment of bathing the wound in hot water.

The aim of this study is to investigate the structure of the venom apparatus and method of venom delivery in *T. vipera* associated with the opercular spine of the species.

## METHODS

The Weeverfish chosen for the study was the lesser weever, *Trachinus vipera*. All the specimens used were caught in the Irish sea and surrounding waters around Anglesey by trawl and were preserved by one of two methods. The venom glands were then dissected out when required and put through a number of processes. These processes varied according to the method of study. A number of techniques were used, such as Light microscopy and electron microscopy to study the nature and structure of the venomous spines along with the associated venom glands. In this study all of the observations were carried out on the opercular spine.

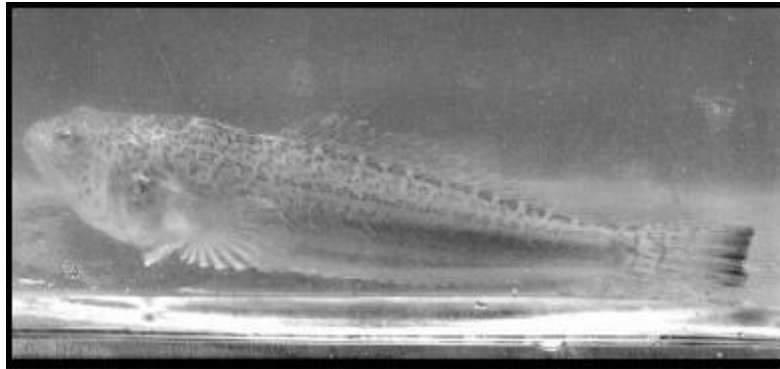


Fig. 1 A photograph of the lesser Weever (*T.vipera*). This particular specimen was 12.6cm long.



Fig 1a. A photograph of the head region of the lesser Weever (*T.vipera*), show the position of the opercular spine (arrowed). This particular specimen was 12.6 cm long with an opercular spine length of 1.1cm.

### Preservation Techniques.

Two preservation solutions were used during the study;

10% Formalin/Sea water Solution (Light Microscope)  
Glycerine Alcohol Solution (All other Studies)

The Weever fish specimen was placed in a screw top jar containing one of the above solutions and then left for a period of at least 24 hours before any dissection took place. The weever fish specimens used for these process were dead when they were captured using a trawl net. But any fish that had been in untreated sea water for longer than two hours was not used, due to the tissue structure would have been affected by natural breakdown processes and of no use for microscopy study.

### **Fixation of tissue.**

The relevant areas of the Weeverfish were dissected out and placed in a fixative. The tissue samples were then left for a period of time (time depended on the fixative used ).

FOR THE RECIPES OF FIXATIVES USED SEE FIXATIVES PAGE.

### **Decalcification.**

Decalcification was carried out using a commercially available solution (CALEX). The instructions supplied with the solution were carried out to the letter although the fixatives and preservatives were the ones described above and not those recommended in the text.

### **Light Microscopy Technique (Outline)**

All the slides used in this paper were prepared in a similar way, (general outline described below). Only the staining methods used deferred, all other stages were the same in every case.

#### Stage 1: Wax embedment.

1) After fixation and decalcification the tissue samples were dehydrated using various strength alcohol solutions until the tissue samples were in 100% alcohol.

2) 100% alcohol-Toluene (1 hour)

3) Toluene/wax solution in a 1:1 ratio (1.5 hours)

4) Wax 1 (100% wax (1.5 hours))

5) Wax 2 (100% wax (1.5 hours))

6) Embed in wax and allow to set.

#### Stage 2: Sectioning

The tissue samples were cut into slices (5 microns) using a Microtome and placed onto glass slides. The sections were allowed to spread using an Albumen/Glycerol solution. The slides were then left to dry out on a hot plate.

#### Stage 3: Removal of wax from sections.

Before staining all wax was removed from the sections. The glass slides were inspected before they were allowed to enter the next stage to insure all the wax was removed from the slide.

1) Immerse slide in HistoClear (5 mins)

2) Transfer slide to absolute alcohol (5 mins)

3) Transfer to 90% alcohol (5 mins)

#### Stage 4: Removal of mercury from slides.

This stage was only carried out if the fixative used was SUSA (see FIXATIVES)

#### Stage 5: Staining

For methods see STAINING

The tissue samples were dehydrated to the same alcohol concentration of the first step of a particular stain chosen.

### Stage 6: Treatment of slides after staining.

The slides were dehydrated using various alcohol solutions until the slide was placed in 90% alcohol and then the following steps were carried out.

- 1) Transfer slide into 100% alcohol (2 mins). This step was carried out at least twice for each slide.
- 2) Transfer to HistoClear. At this stage the HistoClear was checked for any signs of clouding (presence of water in the slide) if this was seen the slide was transferred back into 100% alcohol.

### Stage 7: Mounting.

The slides were left in the HistoClear and only removed one at a time for mounting. DPX was placed onto the slide (covering the stained section) onto which a cover slide was then placed. The slide was then allowed to dry (at least overnight).

### Stage 8: Examination

The slides were then placed under a light microscope and examined in the usual way.

### **Frozen Sectioning.**

One of the slides was prepared in a slightly different manner. Instead of embedding the material in wax as before, the material was frozen using liquid nitrogen. The chosen material was placed onto a drop of embedding fluid on top of a hollow metal specimen holder. This was then placed into a flask of liquid nitrogen and allowed to cool down (slowly) until the material become frozen solid. The holder and specimen was then placed in a cryostat and sectioned. The sections were then stained.

### **Electron Microscopy: Technique**

The electron microscope used was a Scanning electron microscope. The microscope was used to assess the form and structure of the opercular spine in order to relate this to the method of venom delivery. The steps below detail the spine preparation.

#### **Step 1: Flesh removal**

The opercular spine was dissected free from the surrounding tissue taking care not to damage the spine structure in anyway. The spine along with the surrounding tissue was placed in a glass beaker along with 50 mls of distilled water which had approximately 2 grams of Potassium Hydroxide dissolved in. The tissue in the solution was heated gently to start the process of flesh removal, taking care not to allow the solution to boil. Once most of the flesh was removed from the spine. The spine was placed in a fresh solution and allowed to stand overnight to insure that all the flesh was removed from the spine.

#### **Step 2: Mounting and coating in gold.**

The spine was placed onto the mount using double-sided cellotape and coated in gold. The machine used for this process was a Sputter-coater.

## RESULTS

The venom apparatus of the weever fish (*T.vipera*) was found to consist of two components: The venom Gland component (glandular cells and other accessory structures), and the hard component consisting of the opercular spine, all of which is encased in a membrane sheath. This sheath protects these structures from environmental substances and acts as an integral part of the entire venom system.

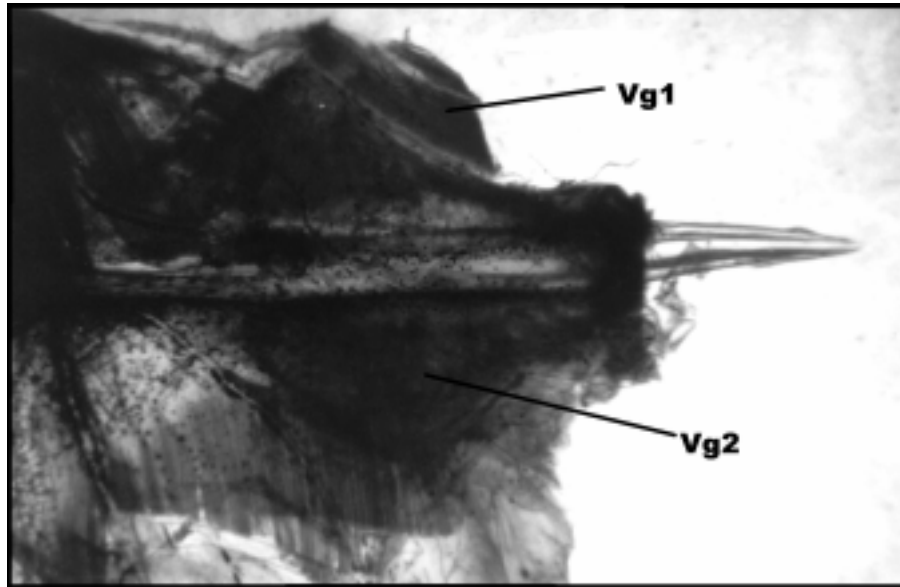


Fig 1c. A photograph of the opercular spine and accessory structures of *T.vipera*. The photograph shows the membrane, which surrounds the spine and associated structures. The venom glands can also be seen, One attached to the base of the spine (Vg2) and the other attached to the top of the spine (Vg1)  
Mag x16

The venom gland is not one gland but two. The glands are found in the basal grooves of the opercular spine and short way up the lateral groove, which runs along the length of the spine extending from the basal groove and terminating a short distance from the spine tip. The only difference between the two glands is that the gland on the bottom is slightly larger than the gland at the top. The venom gland consists of large globular cells containing the toxin and other cellular components, surrounded by fibres. This structure is surrounded by an outer membrane sheath.

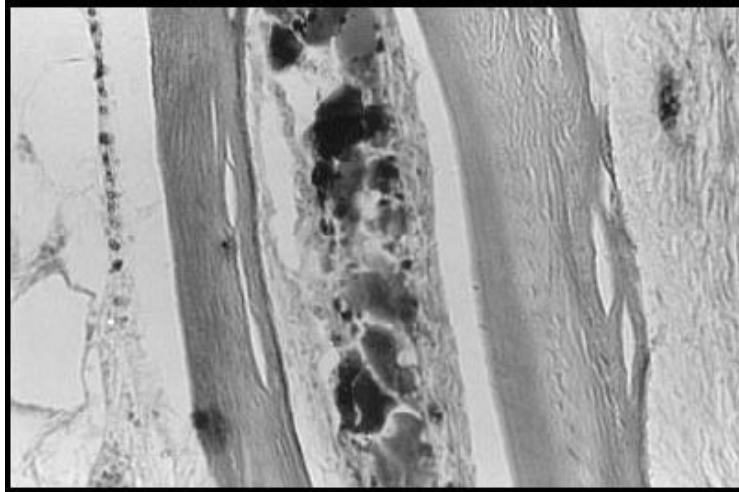


Fig 2 A photomicrograph of the venom gland in the basal groove of the opercular spine of the lesser weever (*T. vipera*). The venom gland is in the centre of the picture surrounded by the edge of the basal groove. Bromophenol blue. Mag x160.

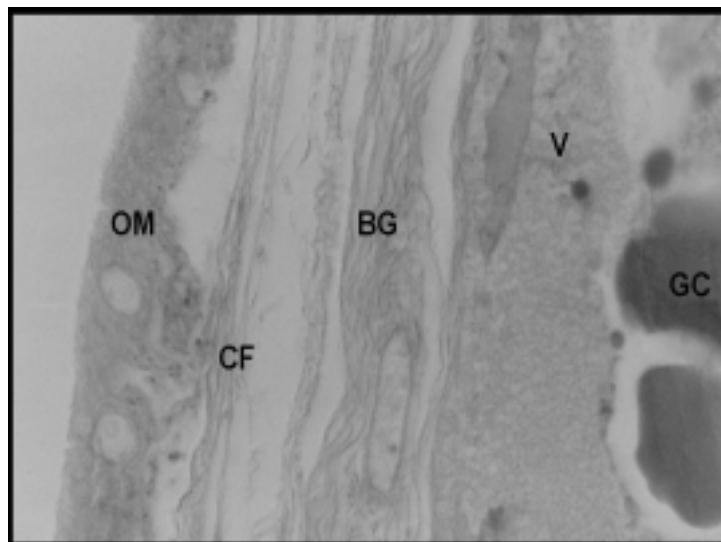


Fig 3. A photomicrograph detailing the distinct regions of the venom gland. Venom gland cells (GC), the Venom surrounding these glandular cells (V) and Basal groove or the 'hard components' of the spine (BG), the Glandular connective tissue layer (CF). This layer is thought to be a strong fibrous layer and the outer membrane (OM). Bromophenol blue. Mag x800

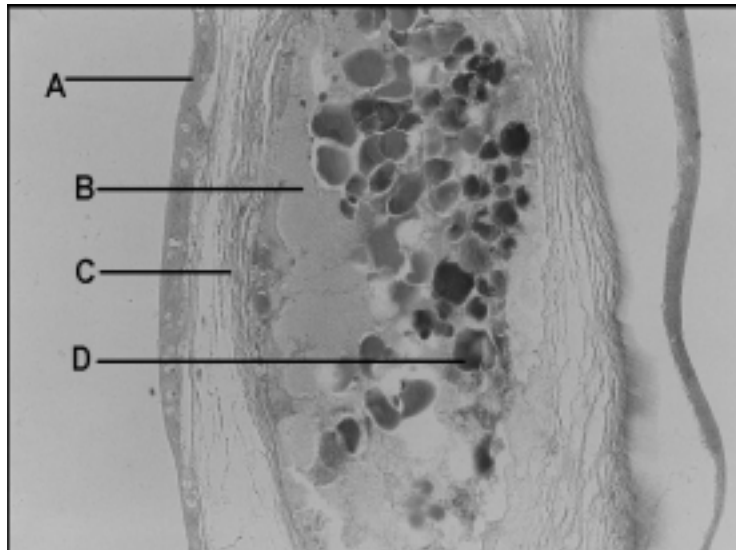


Fig 4. A photomicrograph of the venom gland (D) contained within the basal groove (C) and surrounded by the outer membrane (A). The glandular appearance of the venom toxin can be clearly seen (B). This toxin may have been leaked by the glandular cells damaged during preparation, or alternately the cells could be surrounded by the toxin as part of their normal function.  
Bromophenol blue. x200.

The venom of the Weeverfish after staining was found to give a grained appearance, this grained appearance was repeated whether the venom was contained within the glandular cells or on the outside. As the stain used was Bromophenol blue (A stain specifically for protein) the toxin could be protein based.

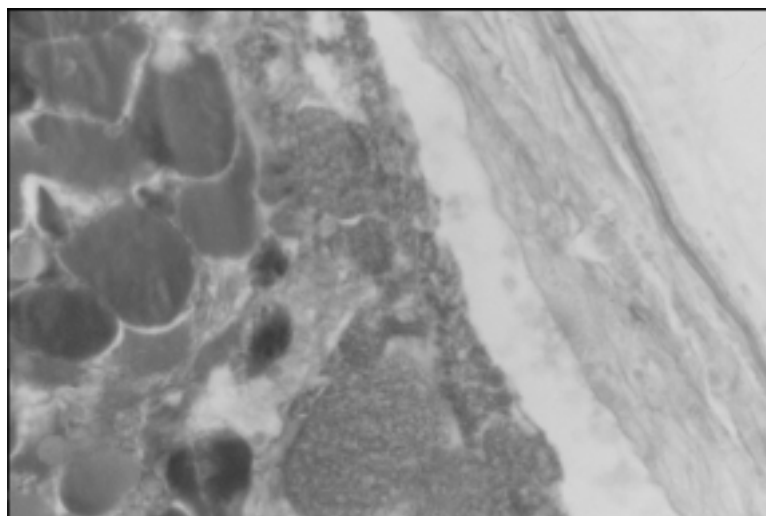


Fig 4a. A photomicrograph of the venom gland of *T.vipera*. The individual glandular cells can be clearly seen along with the granular appearance of the venom itself. The outer edge of the basal groove can also be seen.  
Bromophenol blue x400

The outer membrane surrounding the entire venom gland structure is a complex structure consisting of various cell types. These cells could have a maintenance function as well as a protective outer layer which is an integrate part of the entire venom system.

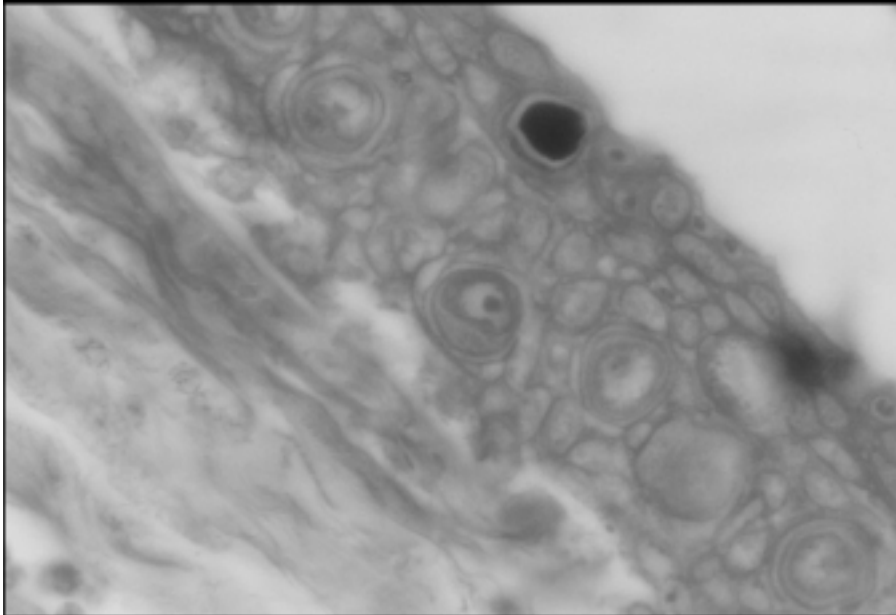


Fig 5. A photomicrograph of the membrane clearly showing the large globular cells on the underside of the membrane. The exact function of these cells is unknown but they may have a secretary function. The function of the large dark cell on the outer edge is also unknown, but these cells only appear intermediately along the outer edge of this cellular layer and are not as numerous as the cells on the underside. These cells could be some form of chemical receptor or just be pigments in the outer layer. Bromophenol blue. x1000 (Oil Emerson).

The hard part of the venom apparatus was found to consist of three distinct regions:

- 1) The opercular spine which had a longitudinal groove running along its length terminating a short distance from the tip.
- 2) Two Basel grooves that connected onto the spinal groove.
- 3) Opercular plate, where the muscles that raised and lowered the spine are attached. On the dorsal side no specific attachment point was found although a raised structure was observed that could serve as a muscle attachment point, on the ventral side a raised structure was found which the ventral muscle was attached to.

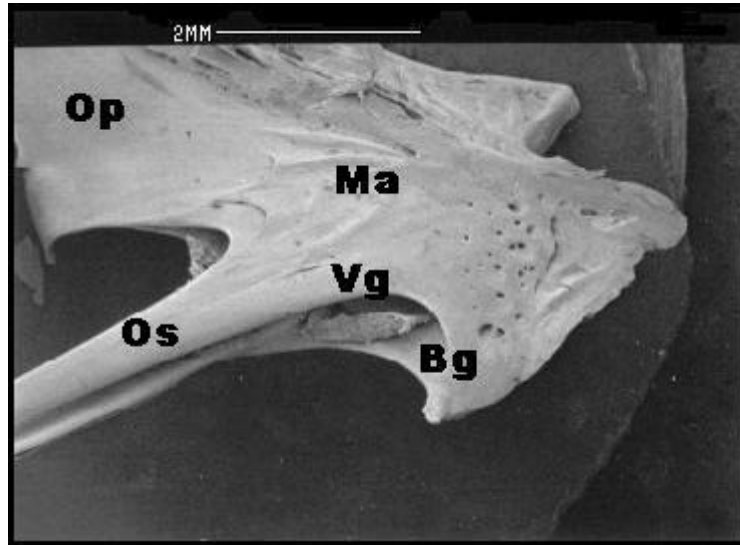


Fig 6. An Electron micrograph of the opercular structure (dorsal side) of the Weeverfish (*T.vipera*), showing the possible site of muscle attachment (Ma), the Basal grooves (Bg) with a remnant of the venom gland (Vg) still visible. Several holes can be seen within the opercular spine (Os) and the opercular plate (op). The function of these holes is unknown. They could be present on the spine for the passage of blood vessels etc. or could be caused by the flesh removal process damaging the spine structure.

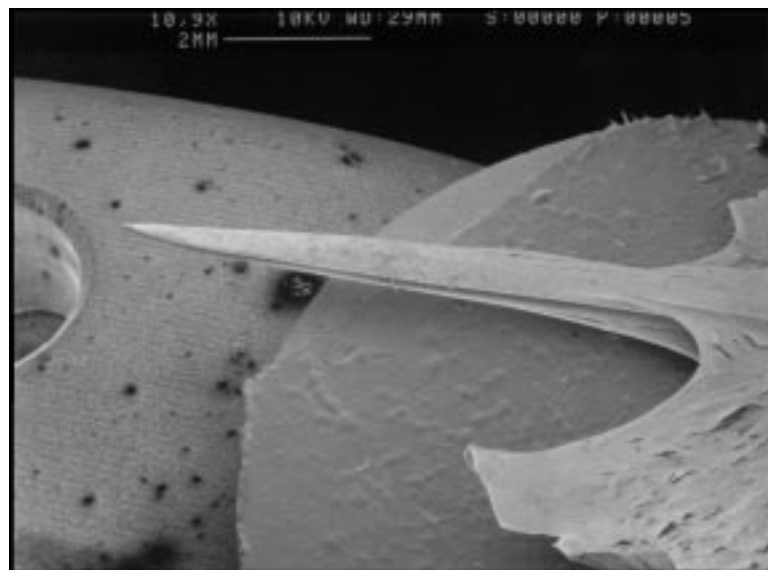


Fig 7. An Electron micrograph of the groove on the opercular spine of *T.vipera*. This groove runs along the entire length of the spine. The groove enters the basal part of the spine where the venom gland is located. The venom travels along this groove on both sides of the spine, which allows passage to the outside of the structure.

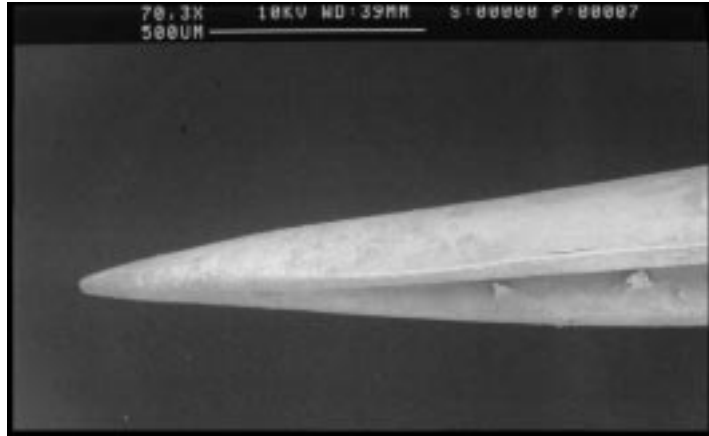


Fig 8. A scanning electron micrograph of the tip of the Opercular spine of the Lesser weever (*T.vipera*). The photo shows the narrowing of one of the longitudinal grooves as it nears the tip of the Opercular spine.

The longitudinal groove narrows towards the end of the spine terminating in a point. The opercular spine was found to have a layered structure (fig 10), similar to that of a laminated board. One of the spines was found to have a possible deformity in the Basel groove. (Fig 9 ).The Basel groove on the left of the structure is slightly deformed, in that it has a strut vertical splitting the basal groove in two.

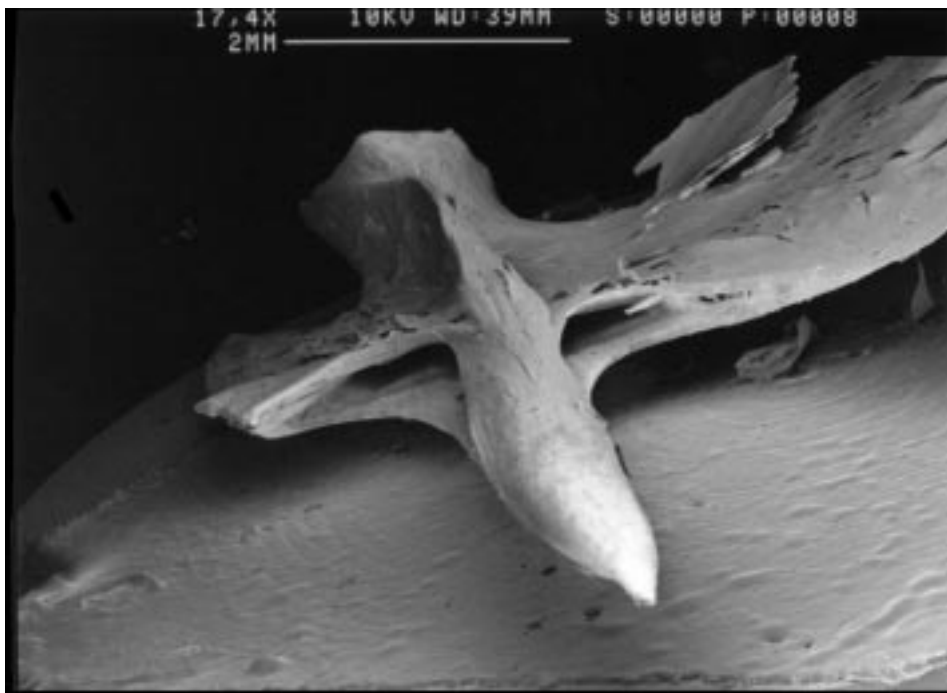


Fig 9. An scanning electron micrograph of the ventral side of the Opercular structure from *T.vipera* showing the basal grooves either side of the opercular spine. The raised structure at the back of the opercular spine is the point of attachment where the ventral muscle was found to be attached. The opercular plate is heavily damaged possibly due to the strong alkali solution that was used to remove the flesh associated with this structure, indicating the spine could be calcium based. The basal groove on the left is also slightly deformed as a strut crosses the basal groove, splitting the groove into two.

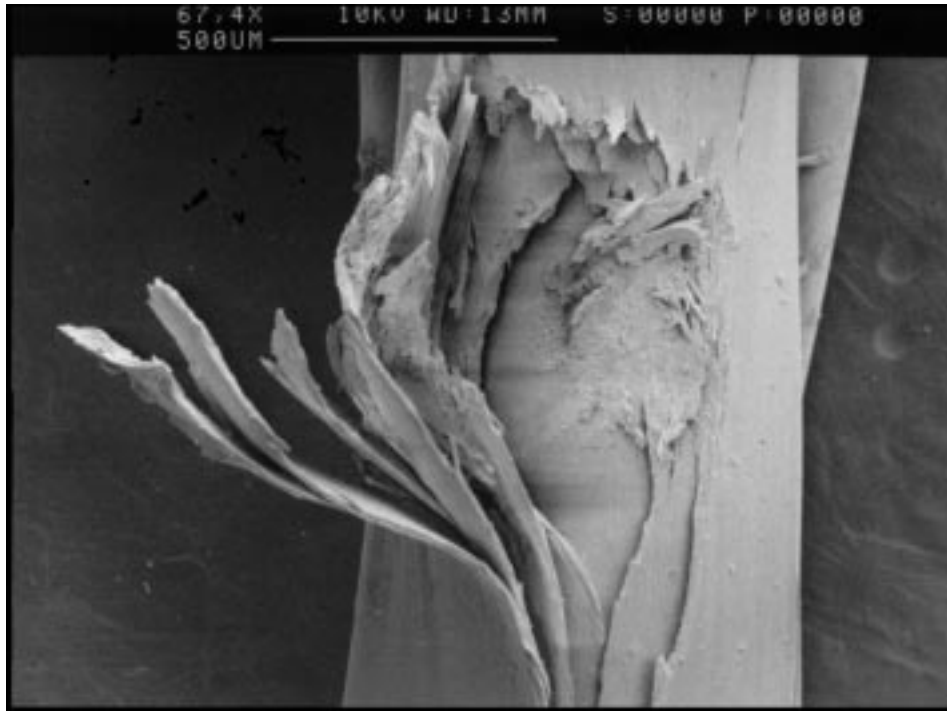


Fig 10 A scanning electron micrograph of part of the opercular spine of the lesser weever (*T.vipera*). The photo shows part of the spine, which was damaged during preparation exposing the internal structure of the spine. The spine is made up of thin layers rather like the structure of a laminated board.

## DISCUSSION

All the components of the Weeverfish venom gland come together to form a very effective defence mechanism. Although this project has described these components in detail. The way in which all this components work together resulting in the efficient delivery of the toxin is as yet unexplained. The Weeverfish has been known to actively strike at other organisms in order to do this a variety of processes have to be initiated. By elevating the opercular plate the weever exposes the opercular spine and therefore increasing the chances of the other organism being "stung".

One possible method of venom delivery is the opercular spine is first raised to increase the amount of spine exposed. This is achieved by the interaction of two muscles attached to the opercular plate (fig 11). These two muscles work antagonistically (one raising the spine the other lowering the spine). As the spine is raised the outer membrane is pulled down the shaft of the spine. This is due to the membrane being non-elastic which exposes the spine tip. The amount of tip exposed depends on the angle at which the opercular is raised from the body.

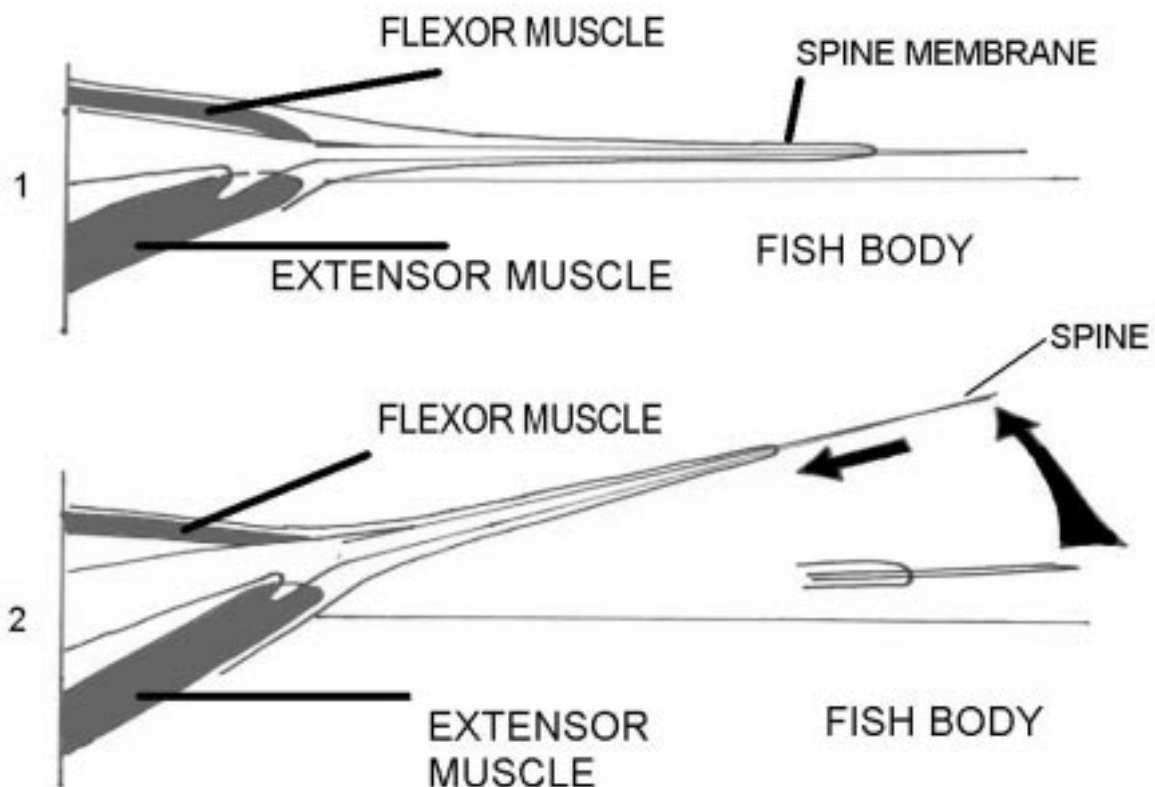


Fig 11. A diagram of how the interaction between two sets of muscles would raise the opercular spine away from the body of the weeverfish.

The outer Membrane moving down the shaft results in the build up of pressure directed onto the glandular mass, pressing the cells into the basal groove ( when relaxed the venom gland extends outside of the basal groove) . The resultant pressure forces some of the cells into the basal groove. This resultant pressure bursts some of the cells forcing their cell contents up along the spinal groove. Glandular cells that are forced up the spine still intact are burst as they near the spine tip (fig 8), due to the narrowing of the groove. The toxin and other cellular contents are passed to the outside.

This could be of advantage to the Weever if other organisms could detect the venom in the water and be repelled by it, therefore leaving the Weever alone, eliminating the risk of damage to the individual if the predator attacked. This idea of the Weever being able to give a chemical as well as a visual warning (exposure of the spines, dorsal spines especially as they have a black membrane) is purely speculative and requires further investigation.

Although actual venom excretion wasn't seen. The action of another body pressing onto the opercular spine and surrounding membrane would have a similar effect, resulting in the venom being passed through the spinal groove into the puncture wound made by the tip of the spine and into the host animal. The spine is very important as the toxin is most effective when injected intravenously and has very different effects depending on how the venom enters the body. There is no evidence in the literature concerning the effects of the toxin introduced into the body by other means, absorption by the skin etc. The site of the venom injection is also important due to the venom activity on various organs within the body.

In contrast to Skies (1962) suggestion that the cells simply burst releasing their contents into the spinal groove,

Another possible method of venom injection involves the cells remaining intact actively secreting the toxin into the spinal groove. Two possible methods of venom injection using this principle are described below:

#### **Method 1**

The spine could be 'pre-charged' - That is the glandular cells could secrete venom into the groove and by capillary action the venom would travel up the groove and remain in the groove until the spine was used. Once the spinal groove was full the cells would stop secreting toxin and therefore preventing waste. The spine would act rather like a hypodermic syringe, which would be used to inject the venom into the predator.

#### **Method 2**

A slight modification of method 1 would involve the cells continually secreting the toxin into the groove resulting in some toxin loss. No sensory cells were seen around the tip of the spine indicating that the mechanism employed by these fish for toxin injection isn't of the muscular type, injection of venom by muscles pressing onto the glandular mass, as this would require some form of trigger.

All these methods of venom injection are based on what was found require further investigation to prove or disprove these theories.

#### **Spine structure and form**

The Opercular spine is made up from numerous layers (fig 10). This laminated structure could have many advantages over a similar sized solid spine. This structure gives the spine added strength and durability, due to the presence of individual layers, which enable the spine to bend slightly under pressure and any damage, would be layer by layer. If the spine was solid the entire spine could snap under the same pressure and the Weeverfish would be defenseless until the entire spine was replaced which undoubtedly would take longer than replacing a few cracked layers. This structure could also be related to growth, the laying down of different layers each year, and may be interpreted as growth rings. The spinal layers could be laid down by the outer membrane covering the entire structure as pockets were seen along the inner most edge of the membrane which could have a secretory function (fig 5), this is speculative and requires further investigation. The general structure of the spine is in the form of a large Opercular plate connected to which is a spine that has a groove running from the Basal groove terminating a short distance from the tip. This structure is the same as other scientists (Allmen:1840, Skeie:1962) found in previous studies, although they do not mention the laminated structure of the spine. This basic structure was also seen in other members of the weever family by Parker (1888) again with no mention of the internal structure.

The number of holes seen in the opercular plate could be due to the preparation process (artificial) or could be part of the spines structure, maybe acting as nerve or nutrient channels which serve the spine in some way or feed into the base of the venom gland supplying nutrients to this structure as they are seen directly behind the basal groove.

This investigation hasn't added any new information to that already known about the Weeverfish but has reconfirmed many of the points raised by other scientists (Allman:1840,Skeie:1962) related to the structure of the opercular spine and associated venom glands.To my knowledge none of the previous authors mentioned the internal structure of the spine, which I found to be layered from my investigations. This discovery was due to accidental damage during the preparation which exposed the internal structure of the spine.

This project also contains two possible methods of venom delivery not mentioned by previous authors (Allman:1840, Skeie:1962) along with one detailed description of one particular method based on the principle mentioned by Skeie (1962) of the glandular cells bursting releasing the toxin into the spinal groove. The ideas contained within this project are just speculations and due to time constraints on this project will remain so until further investigations into the venom delivery, spine formation prove or disprove these ideas.

## SUMMARY

The points raised in this project concern the structure of the Opercular spine and associated venom glands of the Lesser Weever fish (*T. vipera*). The methods used were basic histological methods combined with the use of a scanning electron microscope. The venom gland was found to be made up of two parts situated at the base of the Opercular spine and a short way up the spine. Both venom glands were a mass of large globular venom cells which contained the venom surrounded by the outer membrane. The Opercular spine was also found to be made up of two distinct parts; the spine itself and a large Opercular plate with various muscle attachment points along with two basal grooves which accommodate the venom glands. The spine was found to have a layered structure similar to that of a laminated board. The protein stain used (Bromophenol blue) indicated that the venom was protein based due to the uptake of the stain both inside the glandular cells and outside (exuded cell contents due to the damage during preparation of slides). The spine also stained blue indicating the spine contained some element of protein.

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## **Appendix A**

### **Staining Methods**

#### Bromophenol blue stain for protein

- 1) Take to 95% alcohol
- 2) Stain 15 Mins 100 mg Bromophenol blue in 100mls 95% alcohol
- 3) 20 mins in 0.5% acetic acid
- 4) Rinse for 3 mins in running water
- 5) Dehydrate and mount

#### Bromophenol blue stain on frozen sections

The tissue sections were placed on a glass cover slide and the staining procedure is as follows:

- 1) 5 min in 100 mg Bromophenol blue in 95% alcohol
- 2) 1 min in 0.5% acetic acid
- 3) Rinse in water
- 4) 95% alcohol 1 min
- 5) 100% alcohol 1 min
- 6) Xylene 1 min
- 7) The glass cover slips were mounted onto glass slides by placing a drop of DPX onto a glass slide which the cover slip was placed. The resultant slide was left to dry (at least overnight)

All Alcohol used in the staining processes was Industrial Mentholated Spirits (I.M.S)

## **Appendix B**

### **Fixatives**

#### SUSA

Acetic acid (Glacial) 4ml  
Formalin 20ml  
Mercuric chloride 4.5gms  
Sodium chloride 0.5gms  
Trichloacetic acid 2.0gms  
Distilled water 80 ml

Mercury is removed before any staining procedures. The de-waxed slides are immersed in a solution of 0.5% iodine in 70% alcohol for 1 minute followed by immersion in 5% sodium thiosulphate for 1 minute. The slides are then washed in running water for 3 minutes.

#### 10% FORMALIN SOLUTION

10 ml Formalin  
90 ml of either Sea water or Distilled water

## **Appendix C**

CAL-EX (Decalcifying solution)  
Fisher Scientific company  
Chemical Manufacturing Division  
Fair lawn  
New Jersey  
U.S.A

TISSUE TEK II  
OCT COMPOUND (Embedding medium for frozen tissue sections)  
Lab – Tek Division  
Miles Laboratories  
Naperville  
Illinois  
60540