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The venoms of the lesser (*Echiichthys vipera*) and greater (*Trachinus draco*) weever fish– A review

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ABSTRACT

In comparison with other animal venoms, fish venoms remain relatively understudied. This is especially true for that of the lesser *Echiichthys vipera* and greater weever fish *Trachinus draco* which, apart from the isolation of their unique venom cytolysins, trachinine and dracotoxin, respectively, remain relatively uncharacterised. Envenomation reports mainly include mild symptoms consisting of nociception and inflammation. However, like most fish venoms, if the venom becomes systemic it causes cardiorespiratory and blood pressure changes. Although *T. draco* venom has not been studied since the 1990's, recent studies on *E. vipera* venom have discovered novel cytotoxic components on human cancer cells, but due to the scarcity of research on the molecular make-up of the venom, the molecule(s) causing this cytotoxicity remains unknown. This review analyses past studies on *E. vipera* and *T. draco* venom, the methods used in the , the venom constituents characterised, the reported symptoms of envenomation and compares these findings with those from other venomous Scorpaeniformes.

1. Piscine venoms

Although over half of all living venomous vertebrates are fish (Smith and Wheeler, 2006), their venoms remain relatively understudied (Church and Hodgson, 2002; Ziegman and Alewood, 2015; Smith et al., 2016). Piscine venoms are hypothesised to have evolved on 18 separate occasions (Smith et al., 2016), with the evolution of the fish venom glands being hypothesised to be from the thickening and aggregation of epidermal cells near the spine apparatus that originally secreted anti-parasitic skin toxins (Harris and Jenner, 2019). Venomous fish are mainly distributed among the catfish (Siluriformes), six clades of spiny rayed fish (Acanthomorph), toadfish (Batrachoidiformes), scorpionfish (Scorpaeniformes and Scorpaenoidei), surgeonfish, scats, and rabbitfish (Perciformes: Acanthuroidei), saber-toothed blennies (Perciformes: Blennioidei), jacks (Perciformes: Percoidei), and stargazers and weevers (Perciformes: Trachinoidei) (Smith and Wheeler, 2006).

Most venomous fish (~95%) harbour spines which contain antero-lateral grooves on their surface that encompass the venom glands (Sivan, 2009; Smith et al., 2016). Only a small proportion of venomous fish have evolved fangs (2%), cleithral spines (2%) and opercular/sub-opercular spines (1%) (Smith et al., 2016). Most fish venoms are used defensively (~95%) (Gomes et al., 2011; Smith et al., 2016); however, in some cases (~2%) they are used in predation to incapacitate prey (Smith et al., 2016). All types of fish venom apparatus lack musculature, therefore they cannot voluntarily control the secretion of their venom (Ziegman and Alewood, 2015). Thus, envenomation occurs when mechanical pressure is applied to the spine, breaking the integumentary sheath surrounding the spines, allowing venom to seep from the venomous cells into the victim (Sivan, 2009; Borges et al., 2018).

A characteristic fish venom symptom is that the immense pain inflicted is disproportionate to the size of the envenomation injuries, which are commonly small puncture wounds (Church and Hodgson, 2002). Fish venoms can cause cardiovascular, neuromuscular, oedematic and cytolytic symptoms with cardiovascular activity noted as the main effect after envenomation (Church and Hodgson, 2002; Sivan, 2009). The venoms are composed of a cocktail of toxins, enzymes,

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bioactive peptides and non-proteinaceous components such as serotonin, norepinephrine and acetylcholine (Ziegman and Alewood, 2015). Many fish venoms act in similar ways and have similar components, evidenced by the cross-reactivity of stonefish (*Synanceia trachynis*) anti-venom which can neutralise certain fish venoms (Church and Hodgson 2002, 2003; Sivan, 2009; Gomes et al., 2011). However, even though the venom components are similar between species, venoms often contain a lethal toxin unique to that species (Church and Hodgson, 2002; Ziegman and Alewood, 2015).

2. Trachinidae

Weever fish (family Trachinidae) are venomous benthic fish which comprise nine extant species, split between two genera (*Trachinus* and *Echiichthys*). The two main venomous species of weever fish are the greater weever (*T. draco*) and the lesser weever (*E. vipera* - also referred to as *Trachinus vipera*). Unlike most venomous marine animals which are found in the Indo-pacific (Fenner, 2004), weevers are mainly found in temperate regions throughout the Mediterranean, North, Celtic and Irish Seas, expanding in some cases to the Atlantic coast of North Africa (Dehaan et al., 1991) (Fig. 1).

Weever fish typically are to be found on sandy substrata, against which they are well camouflaged. Frequently, they will partially bury themselves within the substrate, only revealing their anterior and dorsal portions of the body (Fig. 2A). These exposed anterior and dorsal body portions possess venomous dorsal and opercular spines, with one individual spine situated on each operculum and three spines on the dorsal fin (Ziegman and Alewood, 2015) (Figs. 2a and 3).

Weevers, like other venomous fish e.g. stonefish, have convergently evolved dorsal spines housing multiple anterolateral grooves that contain venom glands, lined on the interior of the spine encompassing large glandular cells (Skeie, 1962b; Perriere and Michel, 1986; Smith et al., 2016) (Fig. 2B). It should be noted that many previous authors have studied the anatomy and histology of the venom apparatus and glands from *T. draco* and *E. vipera* (Allman, 1840; Byerley, 1849; Schmidt, 1874; Gressin, 1884; Parker, 1888; Borley, 1907). The venom gland cells are surrounded by supporting cells in the connective tissue (Fig. 2b) which are derived from epidermal cells (Perriere and Goudey-Perriere, 1989). The method of venom secretion from the weever venom glands has been reported as holocrine (Skeie, 1966). Unlike certain other venomous fish, weevers also possess opercular spines which do not differ in their histology from dorsal spines (Skeie, 1966). Most human envenomation cases result from being stung in the limbs, most often during handling by fishermen (mainly *T. draco*) or after having been stepped on in the shallow intertidal zone (Padin et al., 2018). Stepping injury is more common for *E. vipera* as it migrates to very shallow inshore waters in warmer months. In agreement with this, the probability of being stung by *E. vipera* increases with both increasing sea and air temperature (Padin et al., 2018). Approximately 10–12 cases of weever envenomation per year are reported in Denmark and Italy (Cain, 1983), 15 per 100,000 inhabitants along the south eastern Spanish coast (Strempel et al., 2009) with recent reports of 1005 cases in Northern Spain during one summer (equating to 0.3% of all bathers) (Padin et al., 2018). During the period 2003–2004, 17 cases of weever injury among fishermen were reported along the eastern Mediterranean coast (PMID: 19070287). Although the cases of envenomation are relatively frequent, there remains a paucity of contemporary information concerning the composition and activity of venom from the weever family. Prior to the most recent study by Fezai et al. (2017) the last research note on weever fish venom (*T. draco*) was published in 1992

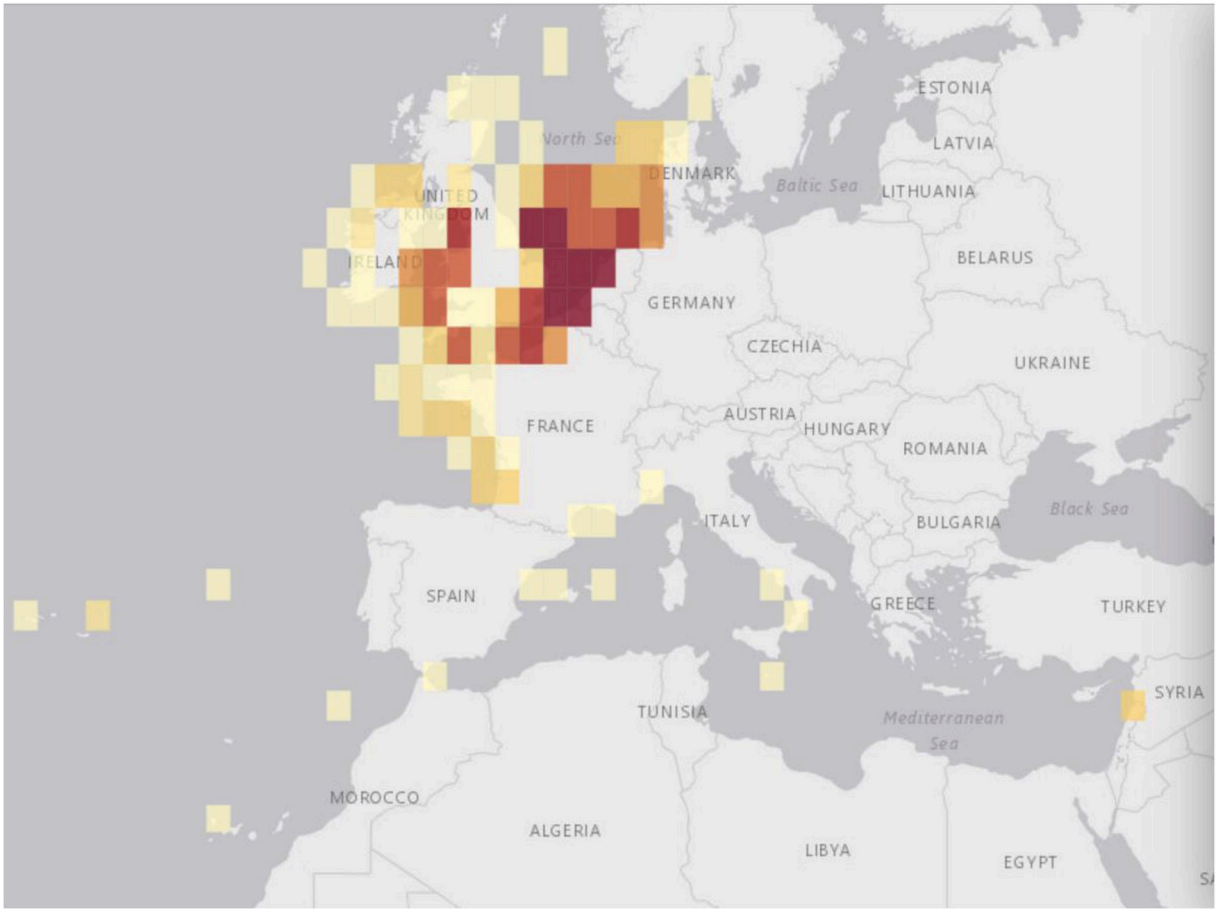

Fig. 1. Native distribution of *E. vipera*. Map taken with permission from OBIS (2015). Darker shade represents a higher abundance. *Colour should be used in print*.

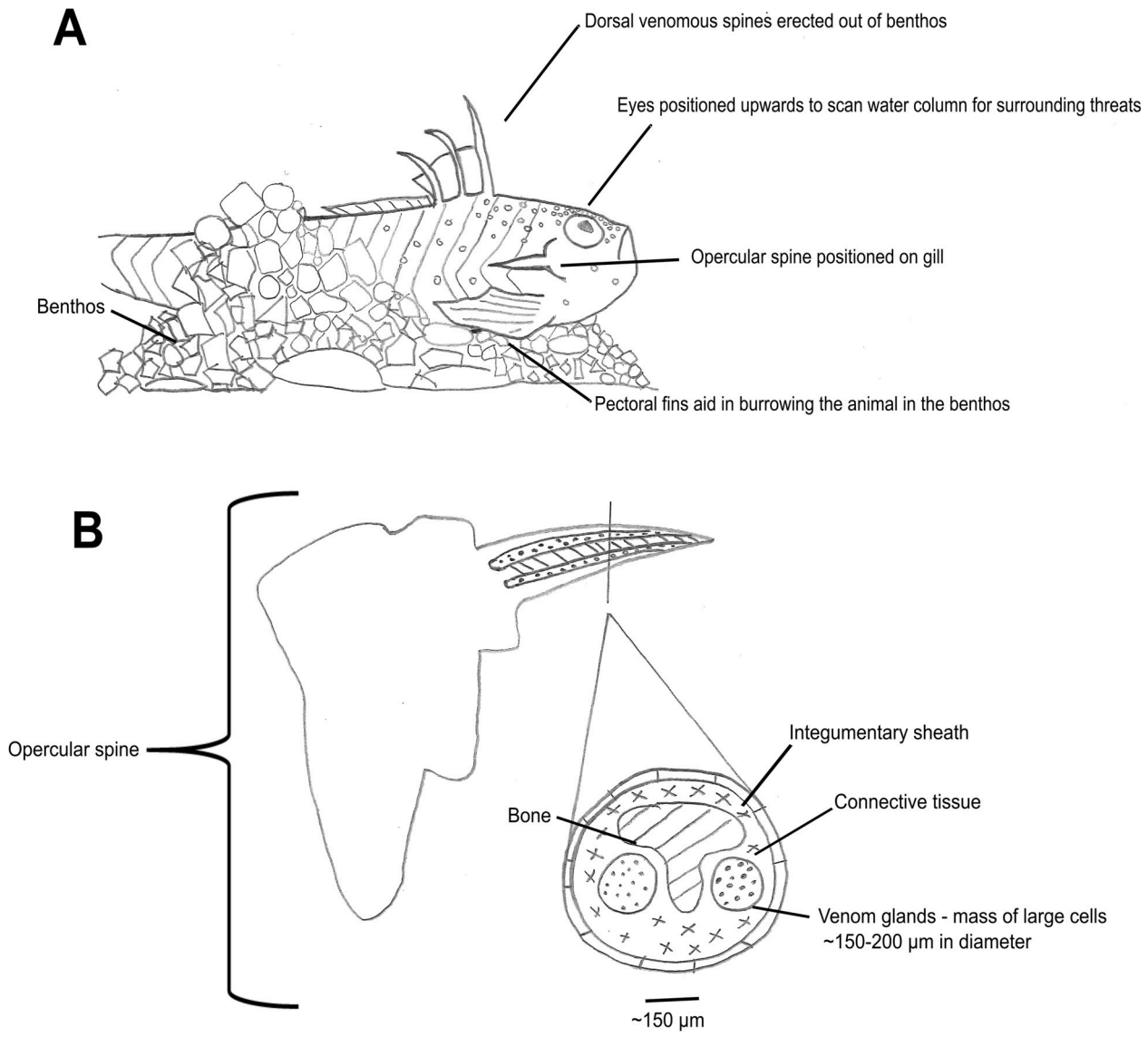

Fig. 2. Sketch A depicts the typical concealment and observation behaviour of a weever fish. Sketch B is based on histological photos and description of the venom gland architecture by Perriere and Michel (1986) in the opercular spine of *E. vipera.* Author's sketches.

(Chhatwal and Dreyer, 1992b).

3. Symptomology and treatment of envenomation

Commonly reported symptoms of *E. vipera* human envenomation include local pain, abdominal pain, inflammation, nausea, necrosis and vomiting (Skeie, 1966; Dehaan et al., 1991; Bonnet, 2000; Borondo et al., 2001; Chinellato and Savelli, 2015). *T. draco* envenomation also causes an inflammation reaction with a subsequent oedema occurring at the wound site (Dekker, 2001; Halpern et al., 2002; Łopaciński et al., 2009). The pain from a weever fish sting has been described as feeling more excruciating than that experienced from a stingray envenomation (Russell and Emery, 1960), although this comparison is challenging to conceptualise without personal experience. If the deposition of venom becomes systemic, weever fish envenomation can cause disturbances in cardiac rhythm (including increased heart rates and heart failure) and respiratory distress in mammals, from mice to humans (Evans, 1907; Russell and Emery, 1960; Carlisle, 1962; Skeie, 1962b; Russell, 1983; Borondo et al., 2001; Chinellato and Savelli, 2015).

Other reported symptoms include dermal fibrosis and hyperalgesia (Davies and Evans, 1996; Mulcah et al., 1996; Chinellato and Savelli, 2015), and Secondary Raynaud's Phenomena (reduced blood flow due to artery spasms, causing a white/blue colouration accompanied with a feeling of numbness or pain) (Evans, 1907; Carducci, et al., 1996; Mayser, et al., 2003), with the affected area being rendered useless from days to weeks after envenomation.

Only one human fatality has been attributed to weever envenomation (Borondo, et al., 2001). Post-mortem examination of the victim found a haematic coagulation within the epidermis near the puncture wound, a 5 cm haematoma within the left leg, several oedemas within the alveoli and fluid in the lungs. Before death the victim experienced respiratory distress, palpitations and vomiting, ultimately resulting in cardiorespiratory arrest.

Stonefish *Synanceia trachynis* anti-venom (SFAV) can be used to neutralise the symptoms for some piscine envenomation cases (Church and Hodgson, 2002; Sivan, 2009; Ziegman and Alewood, 2015) e.g. scorpionfish *Scorpaena plumieri* (Gomes et al., 2011), lionfish (*Pterois volitans*) and the soldierfish (*Gymnapistes marmoratus*) (Church and Hodgson, 2003), however it does not universally cross-react against all fish venoms. For instance, bullrout *Notesthes robusta* venom shows no

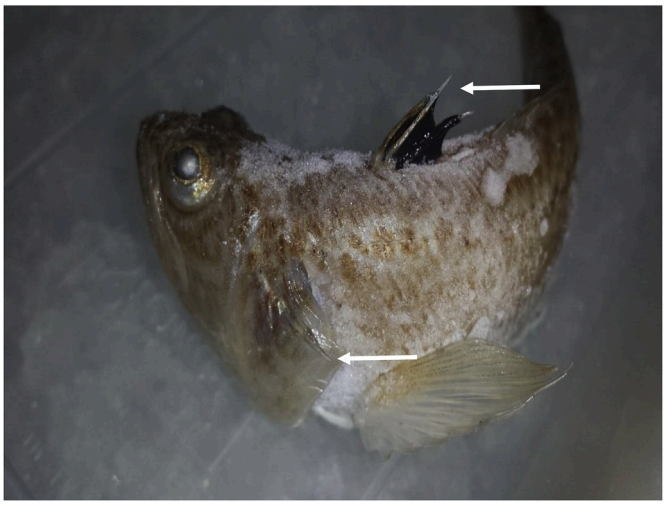

Fig. 3. Frozen specimen of an adult *E. vipera* collected from the Celtic Sea in November 2015. White arrows point to dorsal and opercular spines, respectively. *Colour should be used in print*. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

antigenicity to SFAV (Hahn and O'Connor, 2000). Common treatment for victims of most piscine envenomations (including weevers) is hot water immersion (39°C–45 °C) for 20–90 min, as most piscine venoms are thermolabile, thus relieving the pain (Russell, 1983; Diaz, 2015; Ziegman and Alewood, 2015; Barnett et al., 2017). Another treatment involves administering painkillers or local anaesthetics to the victim in order to relieve the nociceptive symptoms (Ziegman and Alewood, 2015), for example some authors have reported lidocaine as an effective treatment for reducing the symptoms of weever envenomation (Zammit, 1974).

4. Comparison of *Trachinus draco* and *Echiichthys vipera* venoms

A seminal study on the bioactivity of *T. draco* and *E. vipera*, pooled the venom samples from both species in order to increase sample size (Russell and Emery, 1960) due to the similarity in the anatomies of the respective venom organs (Halstead and Modglin, 1958; Russell and Emery, 1960). Whether the pooling of venom in this study (Russell and Emery, 1960) has led to the inaccurate recording of bioactivity in *E. vipera* or *T. draco* venom is unclear. This study consisted of testing the chemistry of the venom and the mode of action of the venom in a range of non-human mammalians, recordings cardiovascular effects including disturbances in cardiac rhythm, ischemic injury, and respiratory distress. For the chemical studies analysis of the spines from six *T. draco* individuals were combined with the spines from 86 *E. vipera* individuals, whereas in the biological assays the authors did not specify whether they used venom from *E. vipera* opercula and dorsal spines or the venom from the spines of both *E. vipera* and *T. draco* (Russell and Emery, 1960). After venom injection into small mammals, a "staircase effect" on blood pressure (falling, then stabilising, then falling) was observed, with both changes to venous and arterial blood pressure. In addition, heart rate changes and shallowing of respiration were noted in the animals, with some fatalities. The mode of action was reported to be similar to that of stingray venom. The bioactive effects of the venom are mainly targeted to the cardiorespiratory system, similar to those other fish venoms (Church and Hodgson, 2002; Sivan, 2009; Ziegman and Alewood, 2015), however it is unclear which individual venom was responsible for the bioactivity recorded in Russell and Emery (1960) study.

4.1. *Trachinus draco* venom

Studies on the venom of *T. draco* date back to 1849, where Byerley published a paper on the anatomy of the venom glands in the opercular and dorsal spines in *T. draco* (Byerley, 1849). Phisalix (1899) demonstrated that *T. draco* venom elicited paralysis, local swelling, necrosis and, ultimately, death in guinea pigs. Evans (1907) found *T. draco* venom to be haemolytic on roach, perch, guinea-pig, sheep, ox, horse and human blood. Later, Evans attributed the haemolytic action to a complement-like substance and amboceptor within the venom (Evans, 1910). Similar to the biological activity reported in the study by Russell and Emery (1960), Evans (1907) observed respiratory, blood pressure and cardiac changes in both cats and rabbits after *T. draco* injection. De Marco (1936) hypothesised the venom to contain neurotoxic properties following observations of increased potassium permeability in a frog nervous system.

Skeie (1962a) was the first author to use quantitative titrations of *T. draco* venom and attribute them to the biological results produced in mice and guinea pigs. Mice were intravenously injected with crude venom (venom extracted from the glands of a venomous species without any subsequent purification) where the LD_{100} after 24 h was 0.4 μL. Skeie (1962a) also noted that the opercular glands contained the most venom as opposed to the dorsal spines in *T. draco*. Skeie (1962a) also used these titrations on chick fibroblasts where crude *T. draco* venom also had an LD_{100} of 0.4 μL. However, the occurrence of multiple vacuoles in the cytoplasm and profuse cell granulation was witnessed in much lower titrations (1:1024 dilution) of *T. draco* venom.

Skeie (1962b) also conducted *in vivo* bioassays on mice, guinea pigs and rabbits using *T. draco* venom. As witnessed in Russell and Emery (1960) Skeie (1962b) recorded similar blood pressure, respiration and cardiac changes after administering *T. draco* venom at lethal doses. Autopsies revealed all animals exhibited frothy discharge in the respiratory systems and contained heart anomalies with the left ventricle being devoid of blood whereas the right was full (Skeie, 1962b) indicating that the venom may have elicited heart failure, possibly due to high catecholamine levels (the so-called catecholamine storm), which has been reported for jellyfish, scorpion and spider envenomations (Abroug, et al., 2015; Weisel-Eichler and Libersat, 2004). Skeie (1962b) attributed the oedema symptoms produced by the venom to be to the high content of hyaluronidase present (38,600 IU/mL). Antigenicity to the venom was also witnessed with the disappearance of necrotic symptoms after repeated sub-lethal injections in mice (Skeie, 1962b).

Skeie (1962c) was the first study to purify the lethal fraction within *T. draco* venom. Skeie (1962c) isolated the fraction by performing paper electrophoresis on the crude venom which revealed three fractions. Density and gradient zone electrophoresis was subsequently performed to further separate the fractions and these fractions were then used in toxicity bioassays (Skeie, 1962c). One of the six peaks found in the density and gradient zone electrophoresis contained seven fractions with five of these fractions containing 85% of the toxicity found in the crude venom (Skeie, 1962c). In the same study, the immunity of animals against *T. draco* venom was tested and after 60 days of venom injections, antigenicity against *T. draco* venom was achieved with lethal doses causing no reaction in mice (Skeie, 1962c).

Haavaldsen and Fonnum (1963) first confirmed venom components specific to *T. draco*, by using paper chromatography to reveal the presence of histamine and catecholamine, and then using a photofluorimetric method to further classify the catecholamines as adrenaline and noradrenaline. Venom homogenates were then tested for their cholinesterase activity and the venom was discovered to contain acetyl- and butyryl-cholinesterases (Haavaldsen and Fonnum, 1963). Even though venoms are a main source of non-synaptic acetylcholinesterase, the function of cholinesterase during envenomation is unclear (Ziegman and Alewood, 2015; Ahmed et al., 2016). In snake venoms it has been hypothesised that the presence of soluble acetylcholinesterase may be to hydrolyse acetylcholine in muscles disrupting the transmission at the

neuromuscular junction of the prey, potentially leading to paralysis (Ahmed et al., 2016).

The 1990s saw renewed interest in weever venom. Chhatwal and Dreyer (1992a) showed that *T. draco* venom activated neurons, thus supporting the neurotoxin hypothesis proposed by De Marco (1938). The crude venom used in the study (Chhatwal and Dreyer, 1992a) had an LD_{50} of 1.8 μg/g in mice and had an LD_{50} 0.075 μg/mL on rabbit erythrocytes. Chhatwal and Dreyer (1992a) concluded that the crude venom was not proteolytic because it did not hydrolyse azocaesin.

Subsequently, Chhatwal and Dreyer (1992b) isolated a unique protein toxin (dracotoxin) from crude *T. draco* venom using 50% ammonium acetate precipitation of the crude venom followed by gel filtration using high performance liquid chromatography to separate the venom components. Chhatwal and Dreyer (1992b) calculated the molecular weight of dracotoxin by using SDS- polyacrylamide gel electrophoresis and by calculating the retention time of the gel filtration column in the high-performance liquid chromatography. Dracotoxin had a molecular weight of 105 kDa and possessed neuronal membrane depolarising activities, causing haemolysis of rabbit erythrocytes with an EC_{50} of 0.003 μg/mL; 25 times more potent than the crude venom (Chhatwal and Dreyer, 1992b). Both crude *T. draco* venom (Chhatwal and Dreyer, 1992a) and dracotoxin (Chhatwal and Dreyer, 1992b) have potent cytolytic effects on rabbit and rat erythrocytes, but no impact on the erythrocytes of humans. Chhatwal and Dreyer (1992b) suggested that the haemolytic action was due to the interaction of dracotoxin with glycophorin in the rabbit erythrocyte membrane.

4.2. *Echiichthys vipera* venom

The first scientific interest in *E. vipera* venom dates back to 1840 when Allman (1840) described the histology of the opercular spines (Zammit, 1974). Following the study by Russell and Emery (1960), Carlisle (1962) conducted chemical and bioactivity assays on *E. vipera* venom. Carlisle separated the *E. vipera* venom into a dialysable and a non-dialysable fraction. Paper chromatography showed the dialysable fraction to contain both 5-hydroxytrypatmine (serotonin) and a histamine releaser. Bioactivity assays using both himself and a goby fish (*Gobius ruthensparri*) proved the dialysable fraction to be the non-lethal fraction and the main pain producing fraction of the venom. Carlisle concluded that the high levels of 5-hydroxytrypatmine (ranging from 0.1 to 2% of venom dry weight), which were some of the highest to be found in a living animal (Carlisle, 1962), to have a primary effect of inducing pain. In fish venoms 5-hydroxytrypatmine is hypothesised to be a major contributor to their nociceptive activity (Ziegman and Alewood, 2015). Contrastingly, no 5-hydroxytrypatmine was found in *T. draco* venom extracts (Haavaldsen and Fonnum, 1963), similar to *S. verrucosa* venom where the absence of 5-hydroxytrypatmine has also been reported (Garnier et al., 1996). Paper electrophoresis revealed that the non-dialysable fraction contained two separable albumins and a neutral amino mucopolysaccharide (Carlisle, 1962). However, Carlisle (1962) concluded that the lethal constituents found in the non-dialysed fraction (two albumins and an amino-polysaccharide) may represent a combined protein complex in the native venom and not necessarily separate constituents, as there was no evidence in the study supporting them being separate complexes. The non-dialysable fraction was lethal to *G. ruthensparri* and elicited respiratory distress and an increased heart rate in Carlisle upon self-injection. Due to the absence of haemolysis on citrated human blood, Carlisle (1962) concluded that the main bioactivity of the non-dialysed fraction was neurotoxicity.

Perriere (1985) analysed the enzyme presence within *E. vipera* venom and recorded the activity of: alkaline phosphatase; acid phosphatase; phosphoamidase; arylamidase; an ATPase; proteases; esterases; β glucuronidase; and, cytochrome oxidase - see Table 1 (Perriere and Goudey-Perriere, 1999). Enzymes are a common component of other fish venoms, allowing the deterioration of biological structures in the victim that may inhibit the spread of the venom itself (Ziegman and Alewood, 2015). Interestingly though, there was no presence of any hyaluronidase activity which is found in many Scorpaeniform venoms (Ziegman and Alewood, 2015). Hyaluronidase is a spreading factor and degrades hyaluronate, allowing venom to move through the victim's tissue quicker (Khoo, 2002). Hyaluronidase is present at higher quantities in stonefish venom than in snake venoms (Poh et al., 1992). Fish venom hyaluronidase has a low similarity to hyaluronidases found in other animals (<50%) however, there is a high homology in Scorpaeniformes with >72% identity between stonefish venom hyaluronidase (*S. horrida* and *S. verrucosa*) and that of lionfish (*P. volitans* and *P. antennata*) (Ziegman and Alewood, 2015).

Table 1
The venom components and their respective bioactivity within *E. vipera* and *T. draco* venoms.

Species	Venom constituents discovered	Bioactivity	Reference
E. vipera	Trachinine	Cytolysin	Perriere et al., (1988); Fezai et al., (2016)
	5-hydroxytrypatmine	Nociception	Carlisle, (1962)
	Histamine releaser	Inflammation	Carlisle, (1962)
	Enzymes (alkaline phosphatase; acid phosphatase; phosphoamidase; arylamidase; an ATPase; proteases; esterases; β glucuronidase; and, cytochrome oxidase)	Unknown	Perriere, (1985); Perriere and Goudey-Perriere (1987)
T. draco	Dracotoxin	Haemolysin, Cytolysin	Chhatwal and Dreyer, (1992b)
	Cholinesterases (acetylcholinesterase and butyrylcholinesterase)	Unknown	Haavaldsen and Fonnum, (1963)
	Hyaluronidase	Spreading factor	Skeie, (1962b)
	Histamine	Inflammation	Haavaldsen and Fonnum, (1963)

Proteases are abundant within fish venoms (Ziegman and Alewood, 2015), but as with many fish venom components, not many proteases from fish venoms have been isolated (Campos et al., 2016). Exceptions include Sp-GP from *Scorpaena plumieri* venom which exhibits gelatinolytic activity (Carrijo et al., 2005) and the Natterin protease family from *Thalassophryne nattereri* venom which exhibit kininogenase activity and degrade collagen, contributing to many symptoms witnessed after envenomation including necrosis, inflammation, nocieception and oedema (Magalhães et al., 2005).

Alkaline phosphatase and acid phosphatase were also found in the venoms of *Scatophagus* argus (Sivan et al., 2010), *G. marmoratus* and *S. horrida* (Hopkins and Hodgson, 1998). Although their full biological roles are not well known, in snake venoms acid and alkaline phosphatases are thought to play a role in liberating purines which produces a multi-toxin, possibly aiding in prey immobilisation upon envenomation (Dhananjaya and D'Souza, 2011). In addition, in bee venom, acid phosphatase is a potent allergen and histamine releaser (Grunwald et al., 2006). As with *E. vipera* venom (Perriere and Goudey-Perriere, 1987), arylamidase has been reported to be one of the components of the enzyme active fraction in honeybee venom, although its biological function upon envenomation is unknown (Zolfagharian et al., 2015).

Nucleosidases such as ATPase have been observed in snake venoms (Sales and Santoro, 2008; Dhananjaya and D'Souza, 2010) and their role is thought to help exacerbate the effects of the myotoxins also present in the venom (Caccin et al., 2013). Myotoxins produce ATP which nucleosidases then convert to ADP, ADMP and Adenosine (Caccin et al., 2013). High concentrations of adenosine cause hypotensive, anti-coagulant and paralysing effects *in vivo*, contributing to the pharmacological responses seen in snake envenoming (Caccin et al., 2013). Future research should aim to isolate and conduct bioassays on the

enzymes present in *E. vipera* venom, in order to understand whether venom bioactivity can be attributed to certain venom constituents e.g. natterins in *Thalassophryne nattereri* venom (Magalhães et al., 2005).

Perriere et al. (1988) used gel electrophoresis to isolate two lethal fractions from *E. vipera* venom (a migratory and a non-migratory fraction); although this cannot necessarily be taken to suggest two separate active constituent fractions as they may combine to form a single protein. The crude venom used for separation in the gel electrophoresis was denatured, thus the proteins within the venom were not isolated in their native form. The migratory fraction was identified as trachinine, with an LD_{100} of <100 μg/kg in mice. The molecular weight of trachinine was determined by comparing the mobility of trachinine in different percentage acrylamide gels (5, 6 and 7%) to the mobility of different bovine serum albumin polymers (monomer, dimer, trimer and tetramer) in these same gels, following the method by Hedrick and Smith (1968) (Perriere et al., 1988). This same method (Hedrick and Smith, 1968) was used to determine the molecular weight of Verrucotoxin from *Synanceia verrucosa* venom (Garnier et al., 1995). Trachinine was hypothesised to comprise four identical subunits, each of 81 kDa, giving a combined molecular mass of 324 kDa (Perriere, et al., 1988). However, it should be noted that this electrophoresis of trachinine was performed under denaturing conditions. The second non-migratory fraction was an aggregate of proteins ranging from 40 to 92 kDa (40, 48, 54, 61, 75, 84 and 92 kDa), however no further identification was conducted. No further analysis on the biological activity or structure was conducted on trachinine due to its high lability, losing toxicity within 1 h at room/-body temperature (Perriere, et al., 1988).

After a pause in the research of *E. vipera* venom, Fezai et al. (2016, 2017) measured the pharmacological potential of semi-purified *E. vipera* venom on human erythrocytes and colon carcinoma cells. *E. vipera* venom was semi-purified by first extracting the dorsal spines, and then sonicating the spines to release the cell constituents (Fezai et al., 2016, 2017). A dialysis cellulose membrane with an 8 kDa cut-off was then used to dialyse the venom and the dialysate was then filtered through a 0.22 μm membrane, lyophilised, and stored at - 20 °C until further use (Fezai et al., 2016, 2017). The semi-purified *E. vipera* venom (500 μg/mL) triggered eryptosis by cell shrinkage, calcium entry and phosphatidylserine translocation to the cell surface (Fezai et al., 2016). Eryptosis is the death of erythrocytes by an apoptotic-like pathway (Repsold and Joubert, 2018). Furthermore, the *E. vipera* venom caused cell death of the colon carcinoma cells (HCT116 and RKO) by triggering the mitochondrial intrinsic pathway of apoptosis (Fezai et al., 2016). These findings may explain necrotic symptoms following envenomation by *E. vipera* (Dehaan et al., 1991). Additionally, colon carcinoma cells were arrested in the G1 phase of the cell cycle when exposed to semi-purified *E. vipera* venom, inhibiting their proliferation (Fezai et al., 2016). This venom also affected cell motility and reduced the migration of treated colon cancer cells through inducing marked changes in the cell shape and size with a huge reorganization of actin and tubulin (cytoskeletal proteins) (Fezai et al., 2017). Other venoms which show components with apoptotic and inhibitory cell proliferation mechanisms such as bee venom (Son et al., 2007) and snake venom (Vyas et al., 2013) have been hypothesised to be novel targeted cancer therapies. In addition to cancer, venoms with apoptotic bioactivity have been shown to target other autoimmune diseases such as rheumatoid arthritis. For example, bee venom was shown to inhibit the proliferation of human rheumatoid synovial fibroblasts after 24 h through the caspase 3 activated apoptosis (Hong et al., 2005). Since the induction of apoptosis is one of the most important functions of anti-cancer therapies (Vyas et al., 2013), these studies (Fezai et al., 2016, 2017) highlight the possible pharmaceutical potential of *E. vipera* venom and its constituents.

Table 2
Toxins isolated from piscine venoms. Adapted from Ziegman and Alewood (2015) and Church and Hodgson (2002).

Species	Common name	Toxin	Molecular Weight	Bioactivity of toxin	Citation
Scorpaena guttata	California scorpionfish		50–800 kDa toxin	N/A	Schaeffer et al. (1971)
Echiichthys vipera	Lesser weever	Trachinine	324 kDa	N/A	Perriere et al. (1988)
Synanceia horrida	Estuarine stonefish	Stonustoxin	148 kDa	Haemolysis, oedematic	Poh et al. (1991)
Trachinus draco	Greater weever	Dracotoxin	105 kDa	Haemolysis, neuronal activation	Chhatwal and Dreyer (1992b)
Plotosus canius	Grey eel catfish	Toxin-PC	15 kDa	Neuromuscular blocking activity in cardiac tissues	Auddy et al. (1995)
Synanceia verrucosa	Reef stonefish	Verrucotoxin	322 kDa	Haemolysis, cardioactive	Garnier et al. (1995)
		Neoverrucotoxin	166 kDa	Haemolysis	Ueda et al. (2006)
		Cardioleputin	46 kDa	Cardioactive	Abe et al. (1996)
Synanceia horrida	Estuarine stonefish	Trachynilysin	158 kDa	Cardioactive	Colasante et al. (1996)
Notesthes robusta	Bullrout	Nocitoxin	169.8–174.5 kDa	Nociceptive, haemolysis	Hahn and O'Connor (2000)
Scatophagus argus	Spotted scat	SA-HT	18 kDa	Haemorrhagic, oedema, muscle contraction/relaxation, capillary permeability	Karmakar et al. (2004)
Thalassophryne maculosa	Cano toadfish	TmC4-47.2	Unknown	Myotoxic	Sosa-Rosales et al. (2005)
Hypodytes rubripinnis	Redfin velvetfish	Karatoxin	110 kDa	Cytolytic, mitogenic, chemotactic	Nagasaka et al. (2009)
		[a]	160 kDa	N/A	Kiriake et al. (2013)
Scorpaena plumieri	Spotted scorpionfish	Plumieribetin	14 kDa	Weakens cell-collagen contacts, reduces cell spreading, alters actin cytoskeleton	de Santana Evangelista et al. (2009)
		Sp-CTx	121 kDa	Neurotoxic, cardiotoxic, inflammatory, vaso-relaxant, haemolytic	Andrich et al. (2010)
		SP-CL 1-5	16.8–17 kDa	Haemolytic, biphasic vasoactivity	Andrich et al. (2015)
Pterois volitans	Red lionfish	[a]	2 subunits, both ~75 kDa	N/A	Kiriake and Shiomi (2011)
Pterois antennata	Spotfin lionfish	[a]	2 subunits, both ~75 kDa	N/A	Kiriake and Shiomi (2011)
Thalassophryne nattereri	Toadfish	Nattectin	15 kDa	Hemagglutination	Lopes-Ferreira et al. (2011)
Cathorops spixii	Madamango sea catfish	Wap65	54 kDa	Pro-inflammatory action	Ramos et al. (2012)
Pterois lunulata	Luna lionfish	[a]	160 kDa	N/A	Kiriake et al. (2013)
Inimicus japonicus	Devil stinger	[a]	160 kDa	N/A	Kiriake et al. (2013)

[a] Unnamed toxin similar to stonefish (based on SNTX and VTX).

4.3. Trachinine and dracotoxin

In piscine venoms most of the large proteinaceous toxins display cytolytic/haemolytic activity and function by forming pores within cell membranes, leading to apoptosis and other effects (Ziegman and Alewood, 2015) (Table 2). Weever fish (Family: Trachinidae) form part of the order Scorpaeniformes, which also contains some of the world's most dangerous fish including stonefish and scorpionfish (Smith, et al., 2016; Malacarne, et al., 2018). The cytolysins from certain species of Scorpaeniformes have been shown to have genetic and protein domain similarity (Chuang and Shiao, 2014) and are hypothesised to be evolutionarily related (Malacarne, et al., 2018). Thus, unsurprisingly, it has been hypothesised (Ziegman and Alewood, 2015) that dracotoxin and trachinine may be related to the cytolysin toxins found in other Scorpaeniformes due to their similar structure and biological activities.

Most cytolysisns within piscine venoms are around 150 kDa and possess one alpha and one beta subunit (Andrich et al., 2010; Ziegman and Alewood, 2015). However, like verrucotoxin (VTX), trachinine's structure and hypothesised molecular weight is much larger than other conventional fish venom haemolysins, with both toxins being tetramers and 322 kDa and 324 kDa in size, respectively (Garnier, et al., 1995). It is possible that this stark difference in the molecular weight of trachinine compared with other haemolysins (including dracotoxin) is due to the method used to estimate the molecular weight. Both VTX and trachinine had their molecular weight predicted using the same method by Hedrick and Smith (1968) and coincidentally are the haemolysins that are hypothesised to have the largest size (Perriere et al., 1988; Garnier, et al., 1995). Contrastingly, the molecular weight of dracotoxin was confirmed with high performance liquid chromatography (Chhatwal and Dreyer, 1992b). Thus, it is recommended that future studies use methods such as column chromatography to isolate trachinine and confirm its molecular weight.

Dracotoxin possesses severe haemolytic activity on rabbit erthyrocytes (Chhatwal and Dreyer, 1992b). This biological activity is similar to that of Verrucotoxin (Garnier, et al., 1995), neoverrucotoxin (Ueda, et al., 2006), Stonustoxin (Poh et al., 1991), Sp-CTX (Andrich, et al., 2010) and Nocitoxin (Hahn and O'Connor, 2000) which all also possess haemolytic activities.

Dracotoxin was reported have membrane depolarising activities which did not involve sodium channels or potassium channels (Chhatwal and Dreyer, 1992b). The mode of action of how dracotoxin elicits membrane depolarisation and/or haemolysis was not investigated, however it may be due to pore formation in cell membranes as reported for several cytolysins from fish venoms (Desong et al., 1997; Ouanounou et al., 2002; Church et al., 2003; Andrich et al., 2010).

As with dracotoxin, the mode of action of trachinine was not studied, therefore it is recommended that future work should conduct bioassays of dracotoxin and trachinine on cells with and without osmoprotectants to check for pore-formation activity of the toxins (see Desong et al., 1997; Ramírez-Carreto et al., 2019). In conjunction with bioassays, future work should also focus on investigating the genetic similarities between both trachinine and dracotoxin, and other venomous fish toxins from the order Scorpaeniformes to help us understand their functions.

5. Piscine venom stabilisation

Throughout the last century high lability has been agreed by authors as a common problem with fish venoms (Evans, 1910; Malacarne, et al., 2018) and therefore makes their pharmacological activities hard to investigate. Furthermore, their mucus-rich composition makes it difficult to separate proteins within the venom (Baumann et al., 2014). Thus, the high lability of fish venoms presents a major bottleneck for research, slowing the throughput of venom prospecting. This is especially true in the case of *E. vipera* venom, which has been speculated to be one of the most fragile fish venoms (Perriere and Goudey-Perriere, 1999).

Despite the known loss of crude bioactivity, freezing is a commonly practised method of stabilising crude fish venoms (Skeie, 1962a; Chhatwal and Dreyer, 1992a; Ueda et al., 2006; Prithiviraj, 2012). However, other methods such as ammonium sulphate precipitation have been shown to maintain the toxicity of *T. draco* crude venom for several weeks if kept at 4 °C, preserving the bioactivity as optimally as freezing (Chhatwal and Dreyer, 1992a). Furthermore, ammonium sulphate and acetone precipitation has been used to remove mucus contamination from the venom of blue-spotted stingray (Baumann, et al., 2014).

Isolating and preserving the individual toxins present within piscine venoms is just as challenging as preserving the crude venom bioactivity, due to their difficulty in handling and extreme lability out of their native form (Malacarne, et al., 2018). Nevertheless, recently Malacarne et al. (2018) investigated the optimal preservation of fish venom toxin Sp-CTx from *Scorpaena plumieri* venom, and concluded that if it was kept at – 80 °C or −196 °C in the presence of 10% glycerol at pH 7.4 its biological activity can remain for up to 60 days. Similar to Malacarne et al. (2018), Perriere et al. (1988) reported an increase in trachinine's shelf life if preserved in glycerol (50%) from 1 h to 24 h. Future research should aim to use methods from Perriere et al., 1988, Chhatwal and Dreyer (1992a) and Malacarne et al., 2018 to further extend the shelf life of both dracotoxin and trachinine.

6. Conclusions

Previous studies on *E. vipera* venom have been infrequent, with a 20+ year gap between the some of the most recent publications (Perriere and Goudey-Perriere, 1989; Fezai et al., 2016, 2017), whilst *T. draco* venom has not been studied since the 1990's (Chhatwal and Dreyer, 1992b). During this gap the field of venomics has developed significantly, allowing more rigorous and detailed dissection of piscine venoms using complementary bioactive and proteomic studies, resulting in an increase in the discovery of many bioactive peptides. In the scorpion fish *Scorpaena plumieri,* venomics has allowed toxins in both the venom and skin mucus to be directly related to their biological activity (Borges et al., 2018), whereas in stonefish (*Synanceia horrida)* it has allowed an expansion in discovered venom constituents from only hyaluronidase and Stonustoxin (SNTX), to a full transcriptome of putative venom constituents where only 0.4% had previously been associated with piscine venoms (Ziegman et al., 2019), further emphasising the untapped source of toxins and proteins. As molecules within venoms are bioactive and often novel, many venoms are attracting interest for their pharmaceutical properties, with certain venom molecules being synthesised into drugs. As recent findings from Fezai et al. (2016, 2017) demonstrate the cytotoxic bioactivity of *E. vipera* venom on human cancer cells, and as it is still unclear what components are present within *E. vipera* or its relative *T. draco* venom, complementary proteomic analysis and venom gland transcriptomics (see Xie et al., 2019) should be a focus for future research.

Ethical statement

The research that prompted the forming of this review was approved by the Newcastle University Ethics in Research Committee and was conducted in compliance with the UK Animals (Scientific Procedures) Act, 1986.

Declaration of competing interest

The authors have no conflicts of interest.

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