Toxicon 125 (2017) 19–23

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Relationship between food and venom production in the estuarine stonefish *Synanceia horrida*

Silvia Luiza Saggiomo*, Christine Zelenka, Jamie Seymour

Australian Institute of Tropical Health and Medicine, James Cook University, McGregor Road, Cairns Campus, Australia

ARTICLE INFO

Article history:
Received 6 October 2016
Received in revised form
11 November 2016
Accepted 16 November 2016
Available online 17 November 2016

Keywords:
Venom yield
Venom production
Starvation
Venom profile
Stonefish
Synanceia horrida

ABSTRACT

Background: The potential costs of venom production may be significant to many marine venomous taxa. In general, the parameters that influence the rate of venom production are poorly understood, but seem to be related to feeding frequency.
Methods: This study examines the effects of starvation on venom profile and venom yield on the estuarine stonefish (*Synanceia horrida*). In total, the venom of eight stonefishes was tested under two feeding regimes. Over a four week period, one of the two groups underwent an episode of suspended feeding, while the other was fed on a daily basis. The effect of time on venom replacement was determined by a paired T-test. ANOVA was performed to analyze differences in venom weight between fed and unfed treatments.
Results: Nutritional suspension was found to have a significant effect on the quantity of venom produced. SDS-PAGE gel and FPLC revealed that the components of the venom collected from both groups were similar, indicating that four weeks is an adequate time to regenerate key venom components but not replenish initial venom quantities.
Conclusions: Venom production was found to be affected by starvation.

1. Introduction

Venom is a widespread trait amongst marine taxa. Its evolution sometimes is related to prey acquisition (i.e. jellyfish) and/or defence (i.e. marine venomous fishes). This trait, although advantageous, comes as a trade-off between energetic production costs and the ultimate benefit it brings to the animal. The potential production costs, in turn, may be quite significant and venom is used in a conservative way in many different taxa, possibly as an attempt to minimise energetic expenditure on stock renewal (Hayes, 1993, 1995; Inceoglu et al., 2003). Likewise, the rate and quantity of venom being produced may be influenced by its metabolic production costs and also may depend on the necessity and the ecology of the animal producing it (Mirtschin et al., 2002; Kintner et al., 2005; Mebs, 2001). In general, however, the ecological parameters influencing the rate of venom production in venomous animals are poorly understood. Although concentrated focus is often maintained on variations in venom composition, venom yield remains a relevant feature to examine, as it may reveal a greater understanding of the general venom ecology of an organism as well as the consequences associated with envenomations.

An example of venomous marine taxa is the Synanceiidae family, which encompasses some of the most venomous fish species known to date (Haddad, 2016). Some of its members are the stonefishes (genus *Synanceia*), regarded as the most venomous fishes in the world (Russell, 1965; Tang et al., 2006). All species possess dark coloured skin covered in wart like projections (tubercles) and frequently embed themselves under rocky soils with their large pectoral fins (Endean, 1961). Naturally, they can camouflage themselves very effectively (Isbister and Caldicott, 2004), due to their coloration and shape, blending in with rocks, corals and seaweed (Auerbach, 1991). Stonefishes are bottom dwelling and remain motionless in the shallow tropical and warmer temperate waters, waiting for prey to swim by, moving only when disturbed in a clumsy and reluctant manner (Isbister and Caldicott, 2004; Cooper, 1991).

Stonefishes have 13 dorsal spines, closely associated with twin venom glands, used for defence and can deliver large quantities of venom from each dorsal spine when stepped upon or mishandled,

* Corresponding author. Tel.: +61 07 04421229.
E-mail addresses: silvia.saggiomo@my.jcu.edu.au (S.L. Saggiomo), christine.zelenka@my.jcu.edu.au (C. Zelenka), jamie.seymour@jcu.edu.au (J. Seymour).

http://dx.doi.org/10.1016/j.toxicon.2016.11.250
0041-0101/

as 100% of known envenomations occur in the hand and foot (Ngo et al., 2009). Venom is involuntarily expelled from both venom glands when the gland is depressed, releasing up to 10 mg of venom per spine (Ngo et al., 2009). With an estimated LD_{50} value of 0.36 μg/g in mice, it is predicted that only 6 depressed spines, containing a total of 18 mg of venom, has the potential to cause fatality in a 60 kg human (Garnier et al., 1995). Apart from this primary mode of defence of the stonefish, the epidermal tubercles that cover the animal's body release a milky substance (icthyocrinotoxin) when compressed (Cameron et al., 1981). Icthyocrinotoxin and the venom found in the venom glands are known to be different in both structure and function (Cameron et al., 1981), while icthyocrinotoxins possess distasteful and debilitating properties to predators (Cameron and Endean, 1973). Although investigations into the icthyocrinotoxins and the venom found in the dorsal spines of the animal have been conducted, an inadequate amount of information is known about these organisms. As such, inquiries into their general venom ecology and the factors influencing venom yield warrant further investigation.

This study aims to examine the effects of starvation on venom yield and profile in the estuarine stonefish *Synanceia horrida.* In general, nutrition provides the critical elements for an organism to maintain and produce required protein based body tissue, such as secretory glands and venom. Since the consumption of food is necessary for sustained maintenance, it is suspected that withholding nutritional resources would result in a reduction of venom production, thus influencing total venom yield. In addition to diminished venom quantities, a difference in the venom profile may also occur, as nutritional supplementation is thought to supply the raw materials needed to synthetize venom components.

2. Methods

2.1. Study species

Although *S. horrida* are known to be sexually dimorphic, external gender determination is impossible and as such, sex was not determined for any of the animals used in this study. Two groups of four individuals were housed in similar conditions and maintained normal feeding regimes for six months prior to milking. Individuals were offered prawns once daily until they rejected additional food. Initial milking of all eight stonefish occurred after six months and then fed for a subsequent four weeks and milked once more. All animals were then split into two groups, one group (n = 4, mean weight = 951 g, ranging from 453 g to 1619 g) fed daily as previously described and one group (n = 4, mean weight = 1096 g, ranging from 416 g to 1841 g) starved for four weeks. At the end of the fourth week both groups were milked for a second time.

2.2. Collection of venom from dorsal spines

Venom was extracted from all 13 dorsal spines of all eight

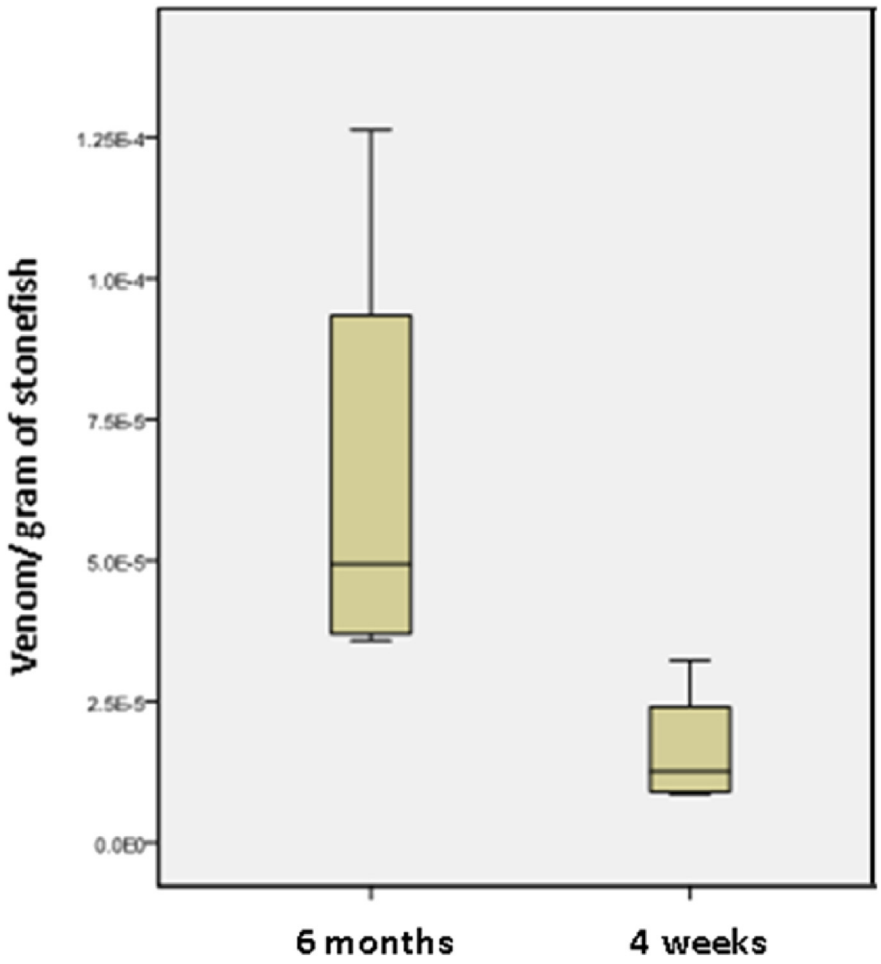

Fig. 1. Comparison of time effect between successive milkings on venom yield (mg of venom/g of stonefish) of *S. horrida* individuals (95%CI).