

**CARDIOTOXIC EFFECTS OF VENOMS FROM *Chironex fleckeri* AND
Chiropsalmus sp. ON AN INVERTEBRATE MODEL**

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ABSTRACT: Extracted venoms from two species of cubozoan, *Chironex fleckeri* (*C. fleckeri*) and *Chiropsalmus* sp., were injected into the freshwater crayfish *Cherax quadricarinatus* (*C. quadricarinatus*). By means of a Vascular Doppler, the cardiac parameters heart rate and contraction amplitude were recorded, with the theoretical total heart output calculated from these two recordings. *Cherax quadricarinatus* reflects prey items utilized by these two species in the wild and so comparisons of venom action on this animal have greater ecological relevance than those of previous mammalian models used. While the results of this study reflect previous research showing that the overall potency of *C. fleckeri* venom is greater than that of *Chiropsalmus* sp. venom, the action of the venoms on the heart muscle is found to be different. *Chironex fleckeri*, which has the most potent venom out of these two species, is actually less effective in reducing cardiac activity than *Chiropsalmus* sp., which may be a direct reflection of the variation in primary prey utilized by each species.

KEY WORDS: cubozoan, venom, cardiac function, *Cherax quadricarinatus*, *Chironex fleckeri*, *Chiropsalmus* sp.

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INTRODUCTION

Previous studies into venom from *Chironex fleckeri* have shown cardiac effects such as bradycardia produced in an assortment of predominantly vertebrate models (10, 11, 13), with comparable bradycardia having also been reported for *Chiropsalmus* sp. venom, likewise tested on vertebrate models (13).

Separated venom fractions from both species have been shown to elicit varying effects on both vertebrate models and isolated invertebrate muscle preparations (9, 10, 11, 12, 13), however, no direct comparisons of these two complete venom preparations have been conducted, particularly in reference to prey type targeted by these species. Previous cardiac toxicity trials have been based predominantly on rats, mice and guinea pig models (10, 11, 12, 17) and although these experimental animals may be relevant for insight into human envenoming, they do not feature as part of the natural predatory habits of cubozoans. In fact, for all the research into the venom of cubozoans, no study has investigated the changes that occur in the cardiac muscles of a relevant prey type after envenoming.

This study aims to investigate the venom from mature specimens of both *Chironex fleckeri* and *Chiropsalmus* sp. on a whole invertebrate animal model similar to prey items utilized by both species in the wild. It serves to examine the venom action from an ecological perspective and compare this to previous venom research predominantly focused on venom actions on terrestrial vertebrates and isolated muscle preparations.

MATERIALS AND METHODS

There are only two identified species of common large, multi-tentacled cubomedusae (*Chiropsalmus quadrigatus* and *Chironex fleckeri*) from the family Chiropodidae in Australian waters (1). The original description of *C. quadrigatus* reports the gonads to be 'cox comb' in shape (14). *Chiropsalmus* sp. present in Australia does not have 'cox comb' gonads but has a cluster of eight grape-like gonads within the bell, suggesting it is probably a different species from that originally described by Haeckel. Therefore the cubozoan usually termed *C. quadrigatus* in Australia is referred to as *Chiropsalmus* sp. in this study.

Mature specimens of *Chironex fleckeri* and *Chiropsalmus* sp. were collected from Mission Beach and Palm Cove in North Queensland with the tentacles of the animals

removed and their nematocysts extracted and lyophilized as per Bloom (3). Venom from lyophilized nematocysts was extracted as per Carrette and Seymour (5). The highest concentration of venom used for both species caused death in less than 1 minute while the lowest concentrations used still resulted in death of experimental animals in less than 7 minutes. These concentrations were selected to cover the range of real time to death of prey by naturally envenomed animals based on field observations.

Cardiac effects of extracted venom were examined on the freshwater crayfish *Cherax quadricarinatus*. Venom was intramuscularly injected into the 2nd abdominal segment of *C. quadricarinatus* specimens, and sound files of heart activity were collected via a vascular Doppler and Avisoft SAS lab Pro software (6).

Sound files of heart activity, recorded using a Vascular Doppler, were collected for an initial period of 20 seconds before the needle was introduced for injection of venom. Heart activity recorded in this first 20 seconds was used as a control period with which cardiac activity after venom injection was compared. Heart rate was measured as beats per 20-second intervals from the start of experimentation until time of cardiac death. Cardiac amplitude was measured as the height of the sound file peaks (6) and again was recorded at each consecutive 20-second interval until asystole. Total cardiac output (heart rate x heart amplitude) was additionally calculated from these two measurements.

The initial relative heart rates and cardiac amplitudes of *C. quadricarinatus* varied between animals, so data were transformed to allow for comparisons. The initial 20 seconds of cardiac activity before injection (control period) was defined as initial heart rate and amplitude and set at 100%. Each subsequent recording was then converted to a percentage of this initial control period. These transformed data were then combined to calculate the theoretical total heart output over a 20-second interval.

As time to death between experimental animals also varied with venom concentrations, time to cardiac standstill was also converted to a percentage. Actual time of cardiac death was seen as a time period of 100% with each proceeding time interval calculated as a percentage of this. These data points, recorded as percentages, were then grouped into time categories ranging from one to ten (category 1: 0%-10%, category 2: 10%-20%, etc.) to allow for comparative analysis.

These data transformations allowed trends of heart rate, amplitude and output to be directly compared independent of venom concentration, species of cubozoan used, and individual cardiac activity of experimental animals.

RESULTS

A larger dosage of venom was required to produce a similar time of death in the test animals *Cherax quadricarinatus* when using *Chiropsalmus* sp. venom as compared to *Chironex fleckeri* venom (Figure 1). However, the heart rate of injected *C. quadricarinatus* decreased more rapidly with the introduction of *Chiropsalmus* sp. venom than after injection of *C. fleckeri* venom (Figure 2). Just prior to death, heart rates of animals injected with *C. fleckeri* venom were approximately 75% of their original rate compared to the 50% decrease in heart rate for animals injected with *Chiropsalmus* sp. venom. *Chiropsalmus* sp. venom also had a more pronounced effect on cardiac amplitude with test animals recording only 6% of initial amplitude just prior to death compared to 23% for animals injected with *C. fleckeri* venom (Figure 3).

Total theoretical cardiac output, as calculated from rate and amplitude, reflected this trend. Just prior to death, after *C. fleckeri* venom injection, the theoretical percentage of total heart output for *C. quadricarinatus* was calculated to be approximately 12% of its original level compared to animals injected with *Chiropsalmus* sp. venom, which are at an estimated 1% of their original level (Figure 4).

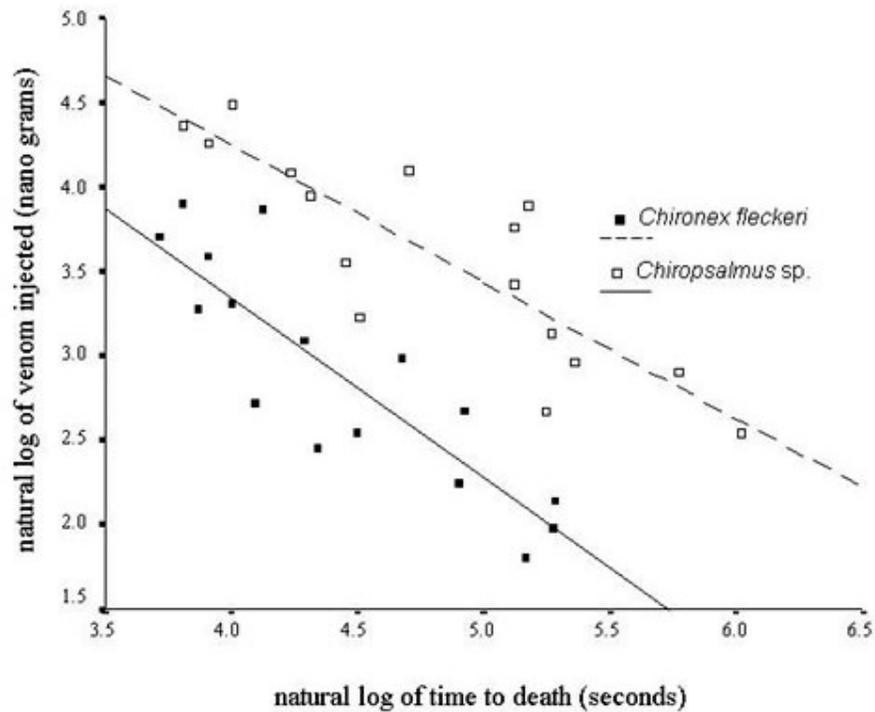


Figure 1: Natural log of venom concentration over natural log of time to death for *Chironex fleckeri* and *Chiropsalmus* sp.

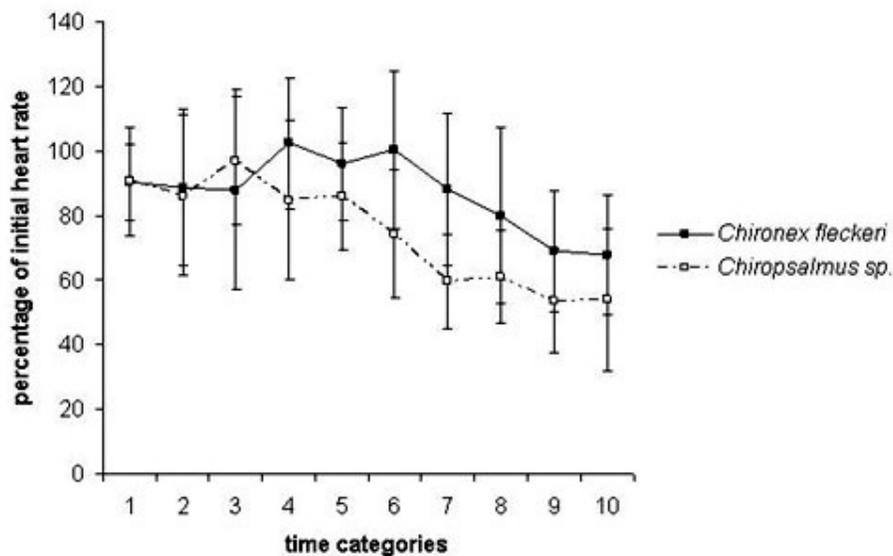


Figure 2: Percentage of initial heart rate over time category for animals envenomed with *Chironex fleckeri* and *Chiropsalmus* sp. venom (95% confidence limits displayed).

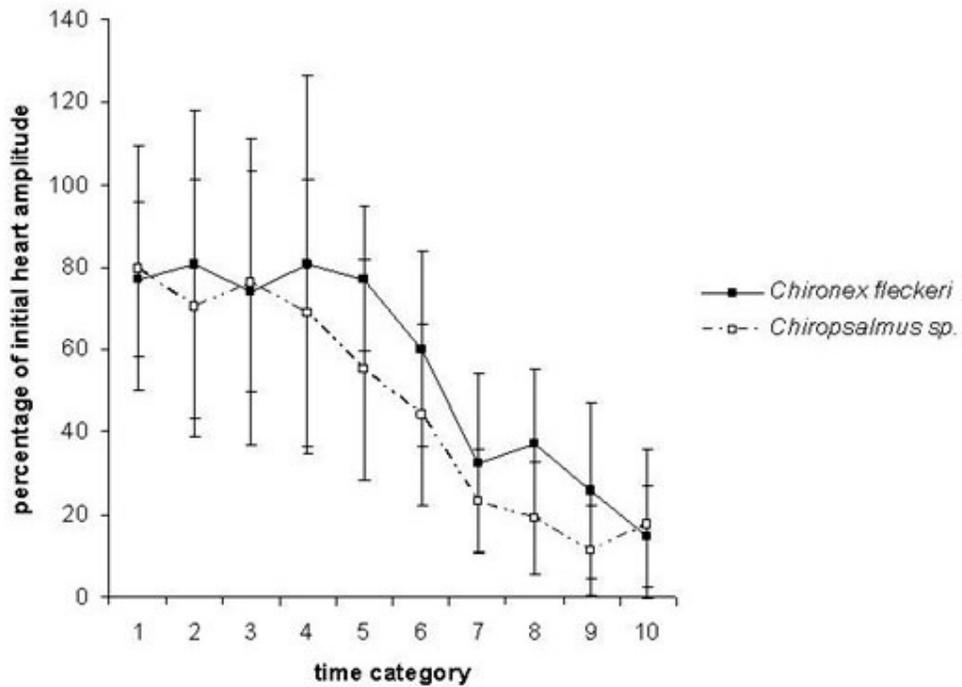


Figure 3: Percentage of initial heart amplitude over time category for animals envenomed with *Chironex fleckeri* and *Chiropsalmus sp.* venom (95% confidence limits displayed).

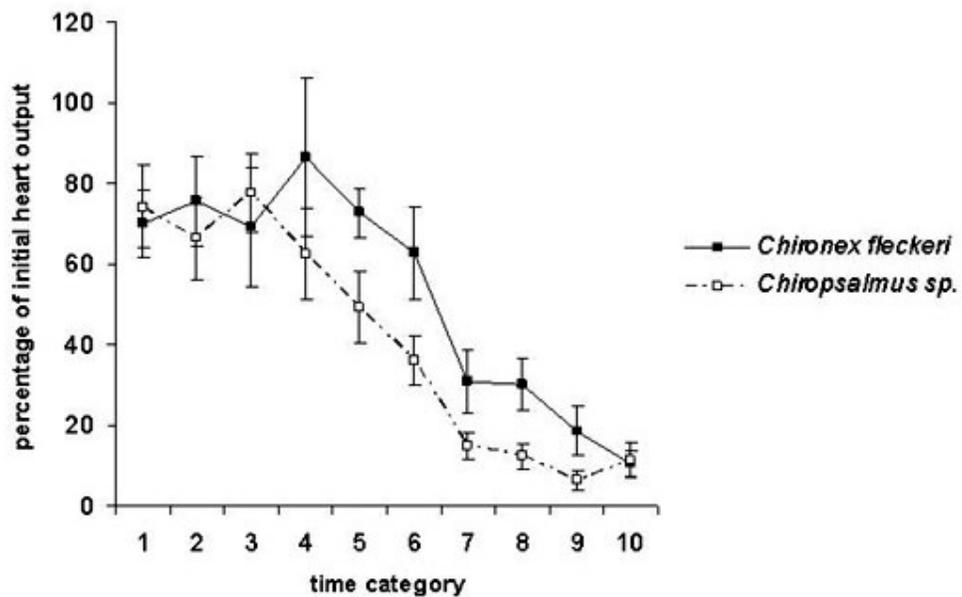


Figure 4: Percentage of initial heart output over time category for animals envenomed with *Chironex fleckeri* and *Chiropsalmus sp.* venom (95% confidence limits displayed).

DISCUSSION

The relative heart rate in experimental animals decreased at a distinctly slower rate after injections of *C. fleckeri* venom than that of animals injected with *Chiropsalmus* sp. venom. Bradycardia has previously been displayed in whole vertebrate and isolated heart preparations for both these species' venoms (10, 11, 13, 17), however this observation of differences in bradycardia produced by the venom of these two species of cubozoans has not been documented before. The relative amplitude of heart contractions also displayed a more pronounced decrease in animals injected with *Chiropsalmus* sp. venom compared to those injected with *C. fleckeri* venom. Previous studies have noted the reduction in heart amplitude for vertebrate models and isolated hearts with venom from both these species (10, 13, 17), however, any comparisons of the two species again has not been documented. Heart rate and heart amplitude change combined are reflected in the overall output or effectiveness of the heart and as was the trend for these two recordings, cardiac output also decreased over time at a relatively greater rate in *Chiropsalmus* sp. than in *C. fleckeri* injected specimens.

Higher dosage levels of *Chiropsalmus* sp. venom are required to produce death in the same amount of time as *C. fleckeri* venom, supporting previous research of reduced rates of toxicity in *Chiropsalmus* sp. (13, 15). However, in regards to the specific action of each venom on the invertebrate model examined, it would appear that as far as effectively reducing or hampering cardiac function, *Chiropsalmus* sp. venom is more efficient. The functional ability of heart rate and heart amplitude are dramatically hampered after injection of *Chiropsalmus* sp. venom, with implications for the total cardiac output, much more than in specimens injected with *C. fleckeri* venom. This perhaps is suggestive that the mode of action of these two venoms is different.

In an attempt to isolate the components contributing to cubozoan venoms, various authors utilizing different methods of venom extraction have investigated the extracts molecular weights (2, 3, 7, 8, 9). Not only has variation been observed between these two species but also between authors investigating the same species, which has been suggested to be an artefact of the different extraction methods utilized and the exposure of venom to different media after extraction (9).

One study investigating the composition of both species of venom however, using the same extraction method, found a distinctly higher number of protein components [20] in *C. fleckeri* venom compared to that of *Chiropsalmus* sp. [8] (16). Within this study, investigation into the components of these venoms using FPLC and SDS-PAGE analysis has indicated that the prominent proteins in these two species of cubozoans are also dramatically different in size (16). Taking these variations into account and those of the difference in nematocyst composition displayed between species (4), it is not surprising that venom extracted from these two species should display a variation in response when comparisons are made on the same whole animal model. As these venoms are quite complex in nature with a number of different protein components, a number of different processes contributing to prey death may be occurring. Some form of amplitude reduction is displayed from both venoms, however the heart rate variation between species of venoms may indicate a difference in the target actions on this invertebrate heart.

As these two species have distinctly different prey types when mature, but similar prey types when young, with *Chiropsalmus* sp. feeding on invertebrates and *C. fleckeri* shifting to a predominance of vertebrate prey (4), it is perhaps not surprising that the species observed to be predominantly an invertebrate feeder (*Chiropsalmus* sp.) is more efficient in inhibiting the cardiac function of an invertebrate heart.

Future directions for this study would have to include a similar comparison of cardiac action for fish, which feature as the predominant prey for *C. fleckeri* (4). This would give an indication as to whether the reverse action is displayed on such a model, with *C. fleckeri* more effective in decreasing cardiac function of their predominant vertebrate prey than *Chiropsalmus* sp.

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