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From the Research Institute of Wildlife Ecology

University of Veterinary Medicine Vienna

(Department head: O. Univ. Prof. Dr. rer. nat. Walter Arnold)

THE HEMOLYMPH COMPOSITION OF THE AFRICAN EMPEROR  
SCORPION (*PANDINUS IMPERATOR*)

&

SUGGESTIONS FOR THE USE OF PARENTERAL FLUIDS IN  
DEHYDRATED AFRICAN EMPEROR SCORPIONS

**MASTER THESIS**

by

Melinda de Mul

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1<sup>st</sup> Reviewer: Univ.Prof. Dr.med.vet. Tzt. Christian Walzer

2<sup>nd</sup> Reviewer: Ao.Univ.Prof. Dr.rer.nat. Thomas Ruf

3<sup>rd</sup> Reviewer: Ao.Univ.Prof. Dr.med.vet. Tzt. Franz Schwarzenberger

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# 1 Introduction

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The majority of living animals are invertebrates and they are gaining popularity as research, display and pet animals. Of all scorpion species, *Pandinus imperator* is one of the most popular invertebrates to be kept as a pet (FRYE, 2006; DOMBROWSKI and DE VOE, 2007). *P. imperator* belongs to the family of the Scorpionidae, which includes some of the world's largest living scorpions (SISSOM, 1990). It inhabits the tropical forests of Western Africa. *P. imperator* is currently listed with the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II (UNEP-WCMC, 2009).

Since more and more invertebrates are kept in captivity, an increasing number is presented as patients to veterinarians. Hence, the growing need for veterinarians to be capable to treat invertebrates. The most common reason for their presentation are husbandry-related problems (DOMBROWSKI and DE VOE, 2007).

Although the anamnesis and physical examination of scorpions are similar to that of dogs and cats, handling scorpions requires certain specific safety measures (e.g. a long-handled padded forceps is needed to manipulate the scorpion). Hemolymph can be easily withdrawn from an anaesthetised scorpion and the sample analysed with a standard analytical machine.<sup>1</sup> However, (physiological) reference values are lacking, which presents a major obstacle when interpreting hemolymph values (VAN WETTERE and LEWBART, 2007).

The present study attempts to establish reference values for the hemolymph of *Pandinus imperator*. Furthermore, the results were compared with the reported results of other scorpion species. In addition to this comparison, the parenteral use of perfusion fluids in dehydrated *P. imperator*, as described in literature, is discussed.

An anatomy and physiology section will follow, with special emphasis on the scorpion's fluid balance and finally the clinical approach of a fluid deficit in scorpions will be discussed.

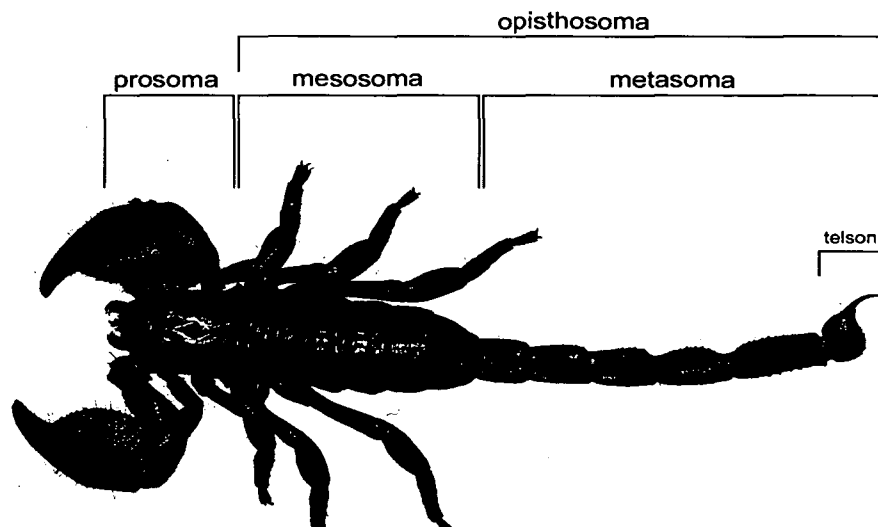
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<sup>1</sup> Specifics on the anaesthesia and the hemolymph withdrawal of *Pandinus imperator* can be found in the master thesis of Rita Krickl (KRICKL, 2008).

## Anatomy and Physiology

### *Morphology*

Scorpions have a hemocoel (i.e. a body cavity containing the visceral organs that is continuous with the circulatory system) and their body can be divided into three parts: 1) the *prosoma* (or cephalothorax) with the pincerlike pedipalps, chelicerae (mouthparts), four pair of walking legs, paired median eyes and the paired comblike pectines ventrally; 2) the *mesosoma*, which contains all organs, incl. digestive tract, hepatopancreas (or mid-gut gland), heart and booklungs with the spiracles (book lung openings) located ventrally; 3) the *metasoma* (or tail) with the anus and a bulbous telson containing paired venom glands (figures 1.1 and 1.2).



**Figure 1.1** Body structure of an adult *Pandinus imperator*, dorsal view: \* paired median eyes.

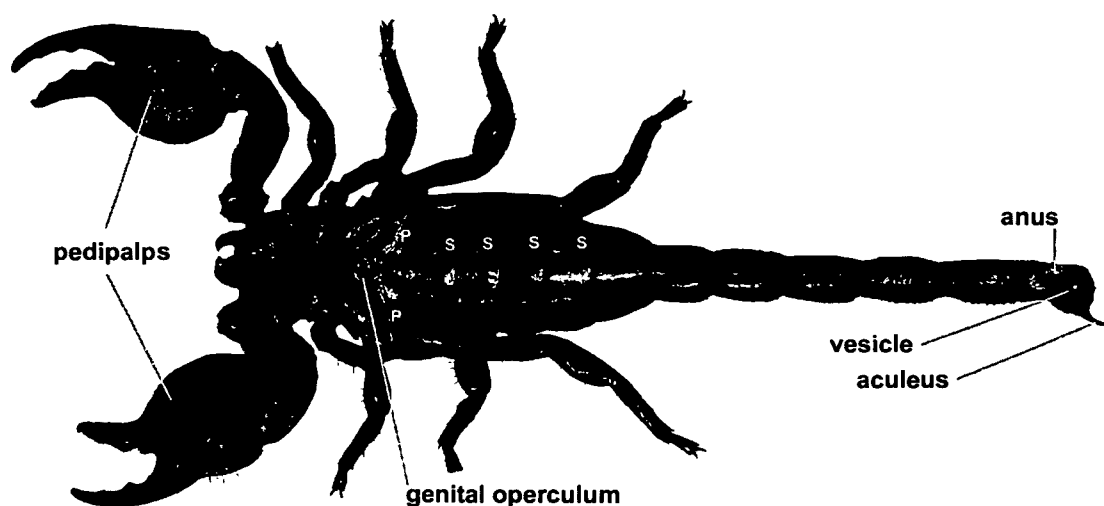
### *Integumentum*

Scorpions possess a segmented chitinous exoskeleton. Between the segments (or somites) there are intersegmental membranes, which are best noticed on the dorsal and lateral mesosoma. The cuticle is a noncellular, multilayered membrane overlying the epidermal cells (HADLEY, 1990). The outermost layer (the epicuticle) contains lipids (waxes), which restrict the water efflux (HADLEY, 1990). Furthermore, the epicuticle contains fluorescent material (HJELLE, 1990), which causes the scorpions to fluoresce when exposed to near-ultraviolet light (320-400 nm) (SISSOM et al., 1990).

### *Alimentary tract*

The alimentary canal is a simple tube, which runs from the chelicerae to the anus and lies just ventral to the heart (figure 1.3). The intestine gives off five pairs of ducts, which lead to the hepatopancreas (or mid-gut gland) (figure 1.3). The hepatopancreas accounts for 12-39% of the scorpion's body weight and fills out most of the mesosoma (WARBURG et al., 2002). The hepatopancreas produces digestive enzymes and stores nutrients (e.g. glycogen and lipids) (WARBURG et al., 2002). Apart from nutrient storage, the hepatopancreas also stores water, both as bulk water and as potential metabolic water source in the form of metabolic fuels (e.g. glycogen bound water) (GEFEN and AR, 2005). The hepatopancreas water stores are used to replenish lost hemolymph water upon desiccation to maintain both hemolymph volume and its osmotic concentration (GEFEN and AR, 2005; GEFEN, 2008).

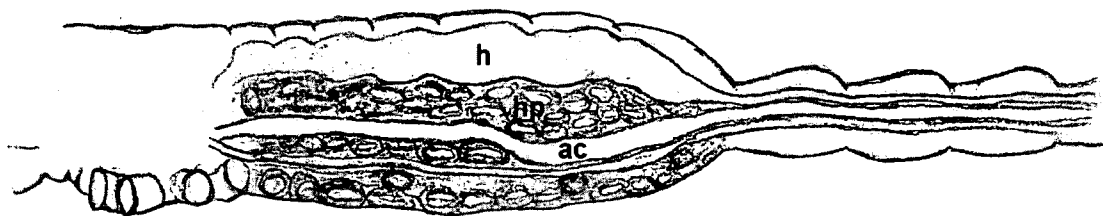
Scorpions feed by external grinding and digestion of prey. They ingest the soluble portion of the prey and compact the residue into 'feeding pellets'. The water obtained from the prey represents the most important source of fluid intake (YOKOTA, 1984). Experiments by YOKOTA (1984) with the scorpion *Paraructonus mesaensis* showed that almost all the potassium ingested was excreted, whereas most of the sodium and chloride were retained, possibly serving to expand hemolymph volume.



**Figure 1.2.** Body structure of an adult *Pandinus imperator*, ventral view: p, pectines; s, spiracles.

### *Respiratory system*

The respiratory organs are four pairs of book lungs, which are located in the mesosoma. The book lungs open to the surface (ventrally) through the spiracles (or stigmata). Each book lung is made up of ca. 150 lamellae (HJELLE, 1990). Ventilation of air is accomplished by rhythmic contractions and relaxations of the dorsoventral muscles and hemolymph flows within the hollow lamellae, where gaseous exchange takes place by simple diffusion (HJELLE, 1990).



**Figure 1.3.** Diagrammatic representation of an adult scorpion, longitudinal section, showing gross anatomy: ac, alimentary canal; h, heart; hp, hepatopancreas.

### *Cardiovascular system*

The scorpion heart is a tube like organ, which stretches through the whole mesosoma (dorsally) and lies within the pericardial sinus (pericardium) (figure 1.3). Seven pairs of lateral arteries arise laterally from the heart and supply the viscera. At the anterior end the heart continues as the anterior aorta. Several arteries branch off the anterior aorta to supply the appendages (chelicerae, pedipalps, legs), brain, eyes and rostrum. The book lungs are supplied by the unpaired supraneural artery, which also branches off the anterior aorta and runs ventromedian below the alimentary canal. HJELLE (1990) also describes the presence of a posterior aorta, which serves the metasomal muscles, the alimentary canal and terminates in the telson to serve the poison glands and muscles of the aculeus (sting). More details on the vascular anatomy of scorpions can be found in HJELLE (1990), and WIRKNER and PRENDINI (2007).

Contractions of the heart occur simultaneously throughout the myocardium and following one another in a regular uninterrupted sequence rhythmically (KANUNGO, 1957). KANUNGO (1957) describes the diastole and the systole of

isolated hearts from the scorpion *Palamnaeus bengalensis*. During diastole the heart shortens in length and the hemolymph flows in through the paired ostia. During systole, the heart extends lengthwise, the ostial valves close and the hemolymph is expelled at both ends. The hemolymph passes through the arterial system into the ventral sinus, from where it passes through the book lungs and is brought to the pericardial sinus via pulmonary veins, and enters the heart to be recirculated (HJELLE, 1990). KANUNGO (1957) observed changes in heart rate when exposing isolated hearts from *Palamnaeus bengalensis* to different temperatures and saline pH. At 25-27°C the heart rate was 50-54 beats per minute. The heart rate increased to 80-85 beats/min at 42°C and on cooling to 5°C decreased to 4-5 beats per minute. Below this temperature the heart ceased, but recovered when the temperature was raised (KANUNGO, 1957). The heart remained active between pH 6.1 and pH 6.5. With increase or decrease of the saline's pH beyond this range, depression of the heart rate occurred (KANUNGO, 1957).

#### *Hemolymph and hemocytes*

Hemolymph is the scorpion's equivalent of vertebrate blood and has a bluish colour, because of the copper containing respiratory pigment hemocyanin (i.e. a metalloprotein containing two copper atoms that bind a single oxygen molecule (O<sub>2</sub>)). Invertebrates do not have red or white blood cells, but hemocytes instead (DOMBROWSKI and DE VOE, 2007). The hemocytes are involved in phagocytosis and encapsulation of foreign material, and play a key role in coagulation (VAN WETTERE and LEWBART, 2007). RAVINDRANATH (1974) described the hemocytes of the scorpion *Palamnaeus swammerdami* in great detail. The scorpion's hemopoietic tissues consist of a series of cellular masses attached to the nerve cord in the region of the cephalothorax and the mesosoma (HJELLE, 1990). Hemolymph further contains organic (e.g. lipids and proteins) and anorganic components (e.g. electrolytes, copper and zinc). Specific information on the lipid composition of hemolymph can be found in EL-SALHY et al. (1981).

PADMANABHANAI DU (1966) found that the hemolymph volume of *Heterometrus fulvipes* ranged between 25-40ml/100g body weight (BW) in both sexes, with an average hemolymph volume of 34.5 ± 5.5ml/100g BW.

Hemolymph, hence, acts as transporter for gas, metabolites and waste products. It has hemostatic functions and facilitates hydrostatic extension to extremities (WILLIAMS, 1999).

### **Fluid balance of *Pandinus imperator***

Scorpions are small animals and as such have a relatively large ratio of surface area to body mass. Therefore, they can experience high rates of water loss (HADLEY, 1990). Scorpions rely on a combination of behavioral, morphological and physiological adaptations to limit their water loss under stressful conditions (HADLEY, 1970).

#### *Water-conserving mechanisms*

*P. imperator* inhabits burrows and hence avoids harsh surface conditions. Morphologically, the waxes in the scorpion's epicuticle make the scorpion's integumentum relatively impermeable and thus restrict cuticular water loss.

Scorpions have low metabolic rates, which results in reduced respiratory water loss and extend the time that scorpions can remain inactive during particularly stressful periods (HADLEY, 1974). GEFEN (2008) found that the scorpion *Smeringurus mesaensis* partially shifts to anaerobic catabolism, which allows closure of the book lung spiracles for longer duration, thus minimising respiratory water loss. GEFEN (2008) also showed that desiccated scorpions regulate their hemolymph volume and its osmotic concentration by mobilising water from the hepatopancreas to the hemolymph.

The principal nitrogenous excretory product of most scorpions is guanine (HADLEY, 1990 and references there in). This purine can be eliminated in crystalline form, which minimizes the concomitant loss of water (HADLEY, 1990). Apart from all these water-conserving adaptations, most scorpions respond to water loss by simply tolerating increased hemolymph osmolality until body fluids can be replenished (HADLEY, 1974).

#### *Water-regaining mechanisms*

The scorpion's most important source of water is their food. Scorpions are strictly carnivorous, feeding primarily on insects and arachnids whose body mass consists of between 50 and 80% water (HADLEY, 1990). Scorpions are able to imbibe water from moist substrate and standing water in their habitat, especially when dehydrated (HADLEY, 1990). In the absence of these water sources, scorpions rely on metabolic water.

## **Fluid deficit in scorpions**

### *Causes*

There are two main causes for fluid deficits in scorpions. In the first place, trauma leading to a cracked or damaged exoskeleton can result in serious hemorrhage. Because of the scorpion's semi-open circulatory system, a crack in the exoskeleton, trauma to a limb or retained bands of exoskeleton cutting into the scorpion's body can lead to loss of hemolymph (DOMBROWSKI and DE VOE, 2007). Secondly, improper husbandry (e.g. too high ambient temperature, too low relative humidity, lack of food or water) may cause the scorpion to lose excessive body fluids and its inability to replenish its water stores. In both cases the scorpion would be suffering from a fluid deficit, which can lead to the death of the animal.

### *Symptoms*

Literature does not describe specific symptoms by which scorpions suffering from a fluid deficit can be recognized. In general though, dehydrated arthropods may be lethargic or anorexic or have a shrivelled opisthosoma (BRAUN et al., 2006). Dysecdysis (i.e. difficulty with molting) may be caused by dehydration (DOMBROWSKI and DE VOE, 2007), but is by no means pathognomic for dehydration in scorpions. Loss of more than 1-5% body weight of hemolymph is deleterious, while lesser loss can generally be tolerated (WILLIAMS, 2002).

### *Diagnostics*

The assessment of a scorpion's hydration status is difficult. The techniques used in vertebrates (e.g., skin turgor, mucus membrane evaluation, globe position in the orbit, urine production) are impossible in scorpions. The anamnesis (e.g. history of a trauma) is of great importance and could serve as an indication of the scorpion's hydration status. However, the best measure of dehydration in invertebrates is probably the evaluation of serial body weight and hemolymph electrolyte levels (ZACHARIAH, 2007). Dehydration causes an increase in hemolymph osmolality (WARBURG et al., 1980; DEJOURS and AR, 1991; GEFEN and AR, 2003). Apart from an increase in hemolymph osmolality DEJOURS and AR (2003) observed that the hemolymph became viscous.

### *Treatment*

In case of hemorrhage from a crack in the exoskeleton or from a lost appendage, staunch the flow and repair the defect with a small amount of cyanoacrylate or paraffin wax (WILLIAMS, 2002; VISIGALLI, 2004; BRAUN et al., 2006; FRYE, 2006; DOMBROWSKI and DE VOE, 2007). Once the cause has been treated the scorpion can be encouraged to drink by placing its mouth in a shallow dish of water (BRAUN et al., 2006; DOMBROWSKI and DE VOE, 2007) or water can be dripped into the scorpion's mouthparts with a syringe. Many scorpions also accept prekilled prey as food if placed near their mouths (DOMBROWSKI and DE VOE, 2007).

Too severely debilitated scorpions may not be able to drink or eat actively and in such cases parenteral fluids can be administered. Fluids can be injected intracoelomic, intracardial, intrapericardial or into the ventral joint membrane (BRAUN et al., 2006). However, the side of fluid administration limits the volume one can inject. The pericardial, cardiac or intracoelomic injection allows a large fluid volume to be infused quickly, whereas injecting into the ventral joint membranes restricts the administration rate (BRAUN et al., 2006). Of course, the volume that one would like to administer depends on the scorpion's deficit. Nevertheless, the volume that can be administered depends first and foremost on the scorpion's size (i.e. body weight) and one should bear in mind that the exoskeleton has a certain expansion limit.

In the literature there is only limited information about the fluid volume, which can be safely administered to scorpions. FRYE (2006) suggests using sterile physiological half-strength dextrose saline in scorpions. He found that 1 injection of approximately 0.3 - 0.5 mL was sufficient to rehydrate emperor scorpions (FRYE, 2009). For dehydrated *arachnids* DOMBROWSKI and DE VOE(2007) recommend the use of 15 to 25 mL/kg (i.e. 15-25  $\mu$ L/g) 0.9% sodium chloride or lactated Ringer's solution. BRAUN et al. (2006) calculated that for a 30-gram *tarantula* with a 10% hydration deficit (this is approximately 2% to 4% of the spider's body weight) 0.9 mL of fluids is appropriate. However, VISIGALLI (2004) states that hemolymph *transfusion* provides the best opportunity in *tarantulas* to counteract shock, dehydration and hemolymph loss.

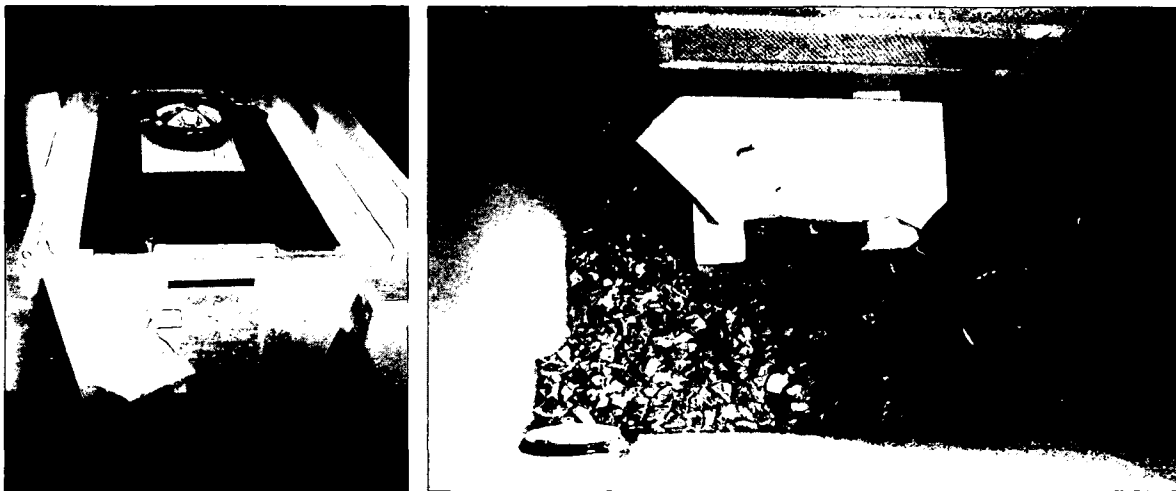
Finally, it is important to use a small-size hypodermic needle 30G (FRYE, 2009) and the temperature of the fluid administered should be close to the scorpion's ambient temperature, since a large amount (relative to the scorpion's body weight) of cold fluids may seriously decrease the scorpion's body temperature and cause immobility.

## 2 Materials & Methods

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### Animals

In the present study we used ten scorpions, four females and six males, of the species *Pandinus imperator*, belonging to the family Scorpionidae, class of the Arachnida. We selected this particular scorpion species for two main reasons: (a) its size, the scorpions used in the trials weighed an average of 17 grams, allowing us to take a sufficient amount of hemolymph for analysis, and (b) it is an increasingly popular invertebrate pet and thus commonly presented to veterinarians.



**Figure 2.1.** Scorpion housing. A transparent plastic container to house each scorpion individually, equipped with: a thermometer, hygrometer, 20-Watt halogen spot, ventilation grill, layer of fir bark, a water dish and a tile as burrow: a, outside view; b, inside view from above.

### Housing

The scorpions were housed individually in transparent plastic containers, which measured ca. 40x30x30cm (figure 2.1a). Ventilation grilles were build-in in the ceiling and a sidewall. As substrate a three-centimetre deep layer of fir bark was chosen to enable the scorpions to dig and to maintain sufficient humidity. Further a tile was added as burrow. A 20-Watt halogen spot (non-UV) provided additional light and heating. The light was placed in such a manner that a temperature gradient was present with an average temperature in the containers of 27°C. A time switch regulated a 10h photoperiod. Also, a thermometer and a hygrometer were placed

within the containers to monitor the temperature and humidity. Twice daily the bark substrate was sprayed with tap water to maintain humidity at an average of 70% (figure 2.1a and b).

### Feeding

The scorpions were fed one cricket weekly and the cricket was provided a piece of carrot to prevent starvation and therefore the possibility of attacking the scorpions, which may occur when no food source is available for the cricket (FRYE, 2006). A shallow ceramic water dish allowed for drinking. The scorpions were weighed once every two weeks to get an indication of their well-being.

### Scorpion immobilisation

To enable hemolymph withdrawal the scorpions were anaesthetised with 5% Isofluran (flow 1l/min) in an induction chamber, as illustrated in figure 2.2. With loss of the righting reflex the animals were taken out of the induction chamber and placed in ventral recumbancy. To ensure the animal's and handler's safety the scorpions' 5th tail segment (the one proximal to the telson) was grasped with a padded forceps and their prosoma and pedipalps were gently pressed to the table with a transparent piece of plastic, as shown in figure 2.3.

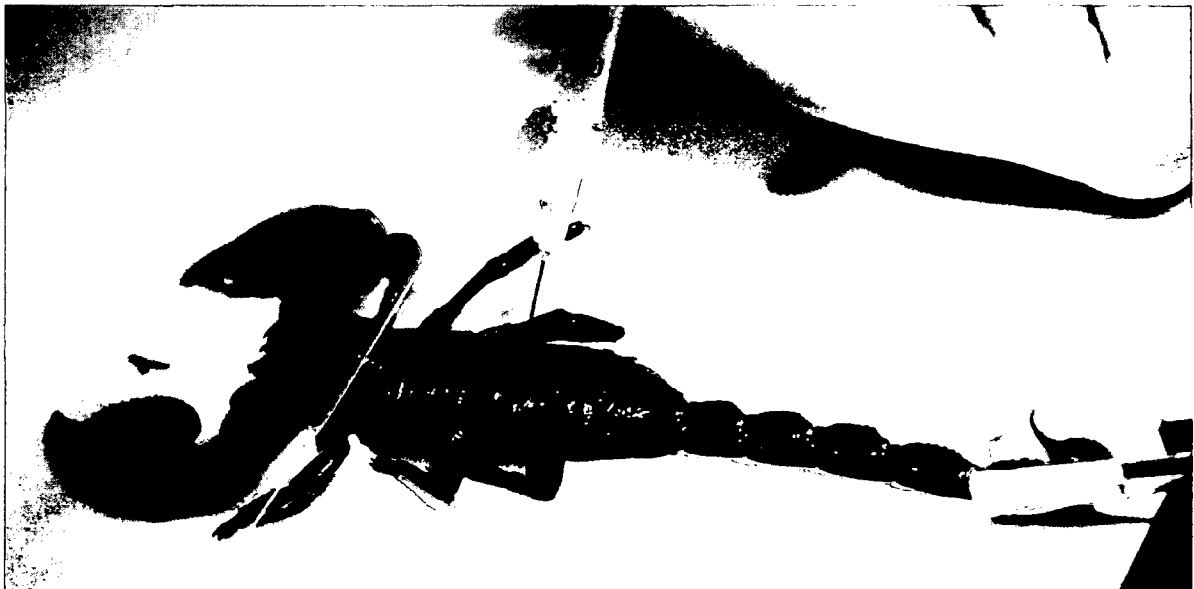


**Figure 2.2.** The induction chamber, consisting of two plastic bottle heads taped together and with one end connected to the anaesthesia unit.

### Hemolymph withdrawal

A 1 ml syringe armed with a 26G needle (0,45mm wide and 23mm long) was inserted from dorsal in a ca. 45° angle (rostroventral direction) through the intersegmental membrane (approx. 0,5 cm deep) between the 4th and 5th mesomal segments into the heart, as shown in figure 2.3.

An average of 2,3 % of the scorpion's bodyweight (this equals between 0,35-0,40 ml Hemolymph) was withdrawn and collected in a 1,3 ml Lithium-Heparin coated microtube (SARSTEDT) and stored in a fridge (at 7°C) before analysis the next day. Hemolymph was withdrawn twice (in March and June), with a thirteen-week interval, from all ten individuals.



**Figure 2.3.** Hemolymph withdrawal. The scorpion is restrained with a padded forceps and a transparent piece of plastic to allow safe hemolymph withdrawal.

### Hemolymph analysis

#### *Electrolytes and Osmolality*

The hemolymph samples were centrifuged at 1500 rpm (Hettich Universal centrifuge, cytospin cartridges) and 100 µl of supernatant was analysed with a Hitachi 911® Autoanalyzer (Roche Diagnostics, Vienna). Results were collected for the following biochemical analytes: sodium, chloride, potassium, calcium, phosphorus, magnesium and total protein. Furthermore the osmolality of the hemolymph samples was determined with a freezing point osmometer (Advanced® Micro osmometer Model 3mo).

### *Metallic elements*

To determine the levels of the metallic elements: copper, mangan, iron and zinc, 50 or 100 µl supernatant, depending on the supernatant quantity, was diluted 1:100 or 1:250 (depending on colour intensity) with bidistilled water. The diluted samples were analysed with flame atomic absorption spectroscopy (FAAS) (Atomabsorption-spectrophotometer Fa.: Perkin-Elmer Corp., D-7770 Überlingen, Typ 4100).

### **Statistics**

The statistical analysis was performed using R 2.6.0. (R DEVELOPMENT CORE TEAM, 2008). The distribution of each hemolymph analyte was tested for normality using the Shapiro-Wilk test. Non-normally distributed data were transformed with the Box Cox transformation. Paired t-tests were used to determine if there were differences in the hemolymph analytes between the samples collected in March and those collected in June. Since ten t-tests were carried out the p-values were adjusted for multiple comparisons with the Benjamini & Hochberg method (BENJAMINI and HOCHBERG, 1995). Correlations between variables were computed using Pearson's product-moment coefficient.

### 3 Results

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#### Differences between sampling times

The results of the hemolymph analyses are shown in Tables 3.1 and 3.2. There was a significant difference in hemolymph osmolality ( $P = 0.015$ ) between the two sampling times (Table 3.2). The mean hemolymph osmolality for March and June was  $472 \pm 23.3$  and  $451 \pm 20.5$  mOsm/L respectively. No significant differences between the March and June samples were found for any of the other hemolymph parameters (Table 3.1 and 3.2). Nor was there a significant difference between genders for any of the hemolymph parameters.

**Table 3.1.** Metallic element content (mg/L) of *P. imperator* hemolymph analyzed in ten animals on two occasions.

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	Mean	Standard deviation	Minimum-maximum
<b>Copper</b>			
March	126	44.2	52-183
June	116	36.2	47-173
<b>Zinc</b>			
March	28	8.9	18-41
June	32	7.1	22-42
<b>Iron*</b>			
March	<1	-	-
<b>Mangan*</b>			
March	<0.5	-	-

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\*Since hemolymph sample quantities were limited, only one dilution was prepared for analyses. For this reason mangan and iron were below the detection limit.

## Results

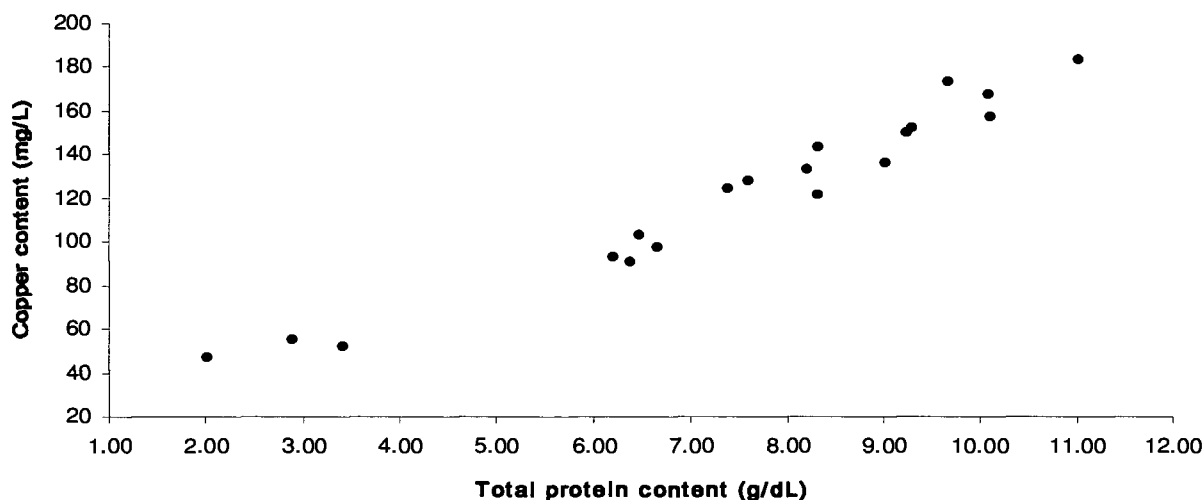
**Table 3.2.** Ionic composition (mmol/L), osmolality (mOsm/L) and total protein (g/dL) content of *P. imperator* hemolymph analyzed in 10 animals on two occasions.

	Mean	Standard deviation	Minimum-maximum
<b>Sodium</b>			
March	236	11.5	221-251
June	231	11.9	212-245
<b>Chloride</b>			
March	207	14.4	183-224
June	207	14.8	185-229
<b>Potassium*</b>			
March	7.9	8.8	3.8-31.9
June	6.0	4.2	3.9-17.7
<b>Calcium</b>			
March	5.53	1.01	3.70-6.74
June	5.68	0.83	4.33-6.89
<b>Phosphorus</b>			
March	0.20	0.13	0.05-0.38
June	0.32	0.25	0.06-0.80
<b>Magnesium*</b>			
March	1.72	0.38	1.25-2.51
June	1.75	0.41	1.37-2.53
<b>Osmolality</b>			
March	472	23.3	436-505
June	451	20.5	411-477
<b>Total protein</b>			
March	7.64	2.69	2.90-11.02
June	7.43	2.33	2.01-10.10

\* The calculated values include the outliers.

### Correlations

The data show a correlation between the hemolymph's total protein content and the copper content. The correlation in March ( $r = 0.996$ ,  $P < 0.001$ ) was more pronounced than in June ( $r = 0.952$ ,  $P < 0.001$ ). Figure 3.1 shows both data sets plotted together ( $r = 0.974$ ,  $P < 0.001$ ).



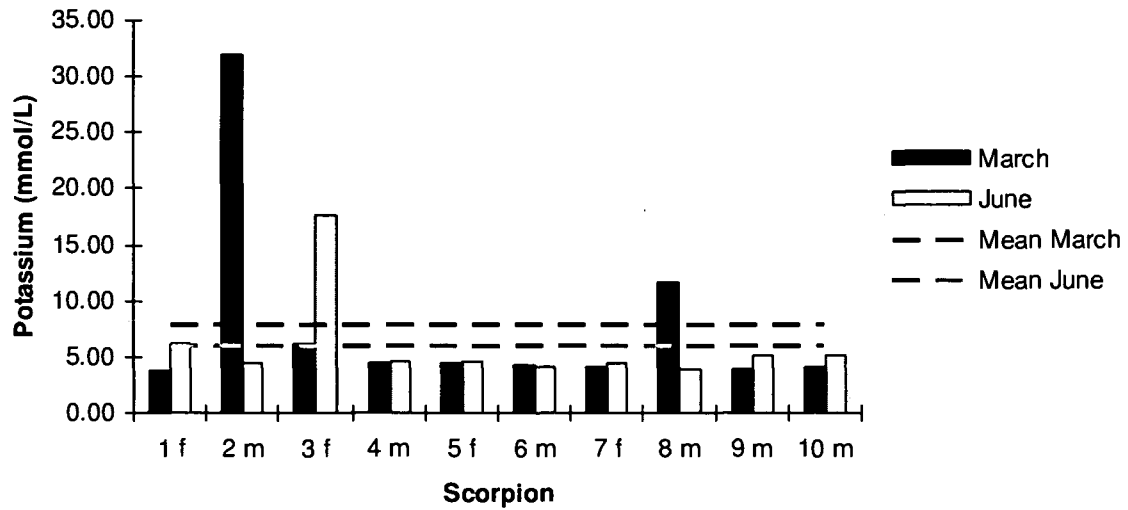
**Figure 3.1.** Hemolymph copper content plotted against the hemolymph's total protein content of *Pandinus imperator* in March and June ( $r = 0.974$ ,  $P < 0.001$ ).

### Distribution of the data

All hemolymph parameters were normally distributed except for potassium and magnesium. Several outliers in the potassium and magnesium data (figure 3.2 and 3.3) caused a right-skewed distribution.

#### *Potassium*

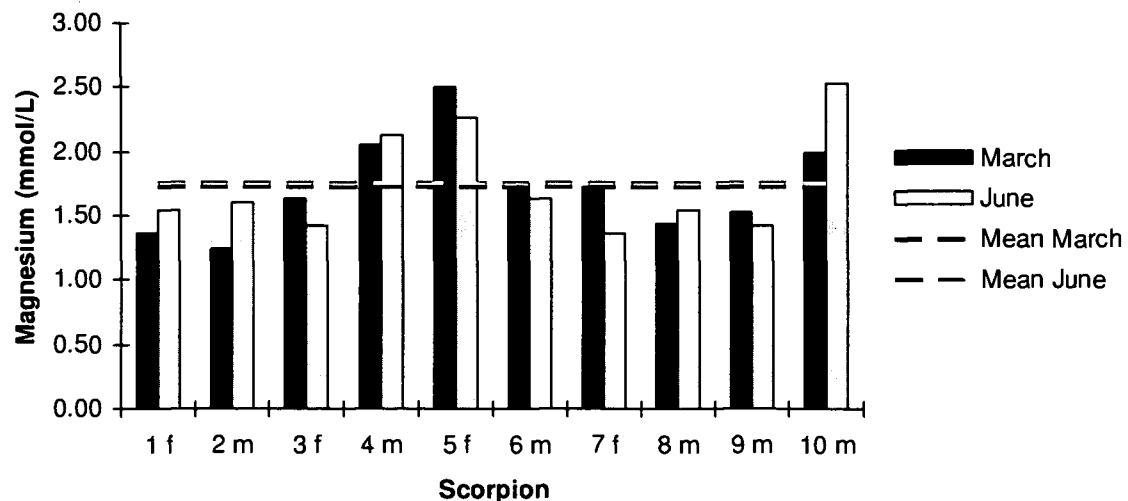
Figure 3.2 shows the potassium content of the hemolymph samples from all ten scorpions taken in March and June. The mean potassium content in March and June was  $7.9 \pm 8.8$  (mean  $\pm$  S.D.) and  $6.0 \pm 4.2$  mmol/L respectively. The hemolymph samples of scorpion 2m in March and 3f in June had a potassium content exceeding twice the standard deviation. With a potassium content of 31.9 mmol/L in March scorpion 2m had the highest potassium level of all scorpions at both sampling times. This potassium level was seven times higher than that of the sample withdrawn from the same scorpion in June. Scorpion 3f peaked with 17.7 mmol/L in June, a value triple that of the March sample.



**Figure 3.2.** Potassium content and mean potassium content of the hemolymph samples from all ten, female (f) and male (m), emperor scorpions taken on two occasions.

### *Magnesium*

The mean magnesium content of the hemolymph samples in March and June were  $1.72 \pm 0.38$  and  $1.75 \pm 0.41$  mmol/L respectively (figure 3.3). Results in figure 3.3 show that the magnesium content of the hemolymph samples from scorpion 4m, 5f and 10m was well above the mean magnesium content on both sampling times. In March scorpion 5f peaked with 2.51 mmol/L magnesium. This value exceeded twice the standard deviation.



**Figure 3.3.** Magnesium content and mean magnesium content of the hemolymph samples from all ten, female (f) and male (m), emperor scorpions taken on two occasions.

## 4 Discussion & Conclusion

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### Hemolymph composition and osmolality

The primary objective of this study was to determine the hemolymph composition of *Pandinus imperator*, regarding the electrolytes, the total protein content and the hemolymph osmolality. As shown in table 3.2, the hemolymph osmolality of *P. imperator* is mainly determined by the high sodium and chloride concentrations, the other electrolytes have little influence on the osmolality. It is worth noting that similar results have been found for *Heterometrus fulvipes* (PADMANABHANAIDU, 1966; cited from BURTON, 1984), *Ophisthophthalmus capensis* (ROBERTSON et al., 1982), *Scorpio maurus fuscus* and *Scorpio maurus palmatus* (WARBURG, 1986; GEFEN and AR, 2004), all belonging to the same family as *P. imperator*. GEFEN and AR (2004) state that sodium and chloride account for more than 93% of total hemolymph osmolality. Whereas PADMANABHANAIDU (1966) states that the high osmotic pressure observed in scorpions may not be entirely due to inorganic salts alone. Organic molecules such as amino acids, sugars and proteins may also contribute considerably to keep up a steady and constant osmotic pressure of the blood.

The ratio of sodium to chloride in *P. imperator* averages 1.12 - 1.14, this concurs with the ratio found by BURTON (1984). Sodium and chloride are present in scorpion's hemolymph in concentrations much higher than those in most insects and mammals (KIMURA et al., 1988). Therefore, the hemolymph osmolality is much higher than in many other terrestrial animals. The physiological significance of this is not yet clear (BURTON, 1984). BURTON (1984) suggests an advantage in that a high osmolality matches that of the juice of the scorpion's partly digested prey.

The present data show a significant difference in hemolymph osmolality between the two subsequent sampling times. It is unlikely that this difference is caused by the successive hemolymph withdrawal, since a thirteen-week interval would normally allow for a complete recovery. Although the scorpions were housed under similar environmental conditions (e.g. ambient temperature, relative humidity, water ad libitum, and allowed a four-week acclimatisation period), it is possible that the difference in hemolymph osmolality reflects a different hydration status of the animals or may be due to metabolic changes associated with the animal's nutrition (PADMANABHANAIDU, 1966; ZACHARIAH et al., 2007). In this regard it is worth noting that in the present study the time between the scorpions catching their prey

and hemolymph withdrawal differed among the scorpions sampled. Arguably, this might have caused a certain difference in the hydration and nutritional status, and consequently a difference in the scorpions' hemolymph osmolality.

This difference in the scorpions' hydration and nutritional status may also explain the wide ranges in total protein content, 2.90-11.02 for March and 2.01-10.10 g/dL for June, of *P. imperator's* hemolymph. After all in vertebrates the blood's total protein content is affected by the animal's hydration and nutritional status, hence, this might also be true for invertebrates. PADMANABHANAIDU (1966) found a variation in protein content from 5.12 to 8.75 g/dL for the scorpion *Heterometrus fulvipes* (Scorpionidae).

The hemolymph's mean total protein content of *P. imperator* was  $7.64 \pm 2.69$  and  $7.43 \pm 2.33$  g/dL for March and June respectively (Table 3.2). PADMANABHANAIDU (1966) measured similar levels for the scorpion *H. fulvipes*, namely  $7.35 (\pm 0.86)$  g/dL (Table 4.1; PADMANABHANAIDU, 1966) and found no differences between sexes.

The hemolymph's copper content correlated strongly with the total protein content ( $r = 0.996$  and  $r = 0.952$  for the March and June data respectively) (figure 3.1). This might indicate that most of the hemolymph's protein is actually hemocyanin. Kimura et al. (1988) suggests that the serum's copper concentration of *Buthus martensi* (Buthidae) may reflect the amount of hemocyanin. PADMANABHANAIDU (1966) concludes that out of the total protein present in the blood of *Heterometrus fulvipes* (Scorpionidae) about 90% may be due to hemocyanin alone. In addition, MARK et al. (1976, cited from SCHARTAU and LEIDESCHER, 1983) showed that there are mainly two protein fractions, namely hemocyanin (80% of the protein) and another protein (20%) in spiders, a species closely related to scorpions.

### **Interspecific differences**

Table 4.1 shows the hemolymph values of *Pandinus imperator* and five other species all belonging to the family Scorpionidae. There is some variation in the hemolymph values between the species. This variation may reflect a particular characteristic of the Scorpionidae family (KIMURA et al., 1988) or a true difference between species. GEFEN and AR (2004) found that the hemolymph osmolality of the scorpionids was generally more variable than that of buthids occupying the same habitats, supporting the idea of KIMURA et al. (1988) that the variation in hemolymph values may be a characteristic of the Scorpionidae family.

However, several other factors may have contributed to the variation in the data. For example: GEFEN and AR (2004) and WARBURG(1986) measured the hemolymph osmolality within 48 hours of capture, whereas the other authors, including the present study, measured the osmolality from hemolymph samples obtained from (wild caught) scorpions acclimatised to laboratory settings. The results of GEFEN and AR (2004) clearly show that feeding significantly increased the scorpion's hemolymph osmolality and decreased the variability. Thus feeding proved to have a major effect on hemolymph values. PADMANABHANAI DU (1966) and ROBERTSON et al. (1982) fed their scorpions (immature) cockroaches and withheld food and water 1 or 3 days, respectively, prior to hemolymph withdrawal. Whereas the scorpions used in the present study were fed crickets and had access to water prior to hemolymph withdrawal. Accordingly, this could mean that the scorpions had a different nutritional- and hydration status, which could result in a variation in the hemolymph values.

Several studies have been conducted to test the effect of starvation and desiccation on scorpion hemolymph composition and osmolality. WARBURG et al. (1980) found an increase in hemolymph osmolality when he dehydrated the scorpions at 37°C and 0% RH. He even found that the hemolymph osmolality in the mesic species, *S. m. fuscus* increased at a much greater rate than in the xeric species. Similarly, GEFEN and AR (2004) reported that the hemolymph osmolality from both *S. m. fuscus* and *S. m. palmatus* increased with ca. 23% in response to a 10% mass loss when desiccated at 30°C and 40-60% relative humidity (RH). Their data also showed that the contribution of sodium and chloride ions to the total hemolymph osmolality decreases with increasing total hemolymph osmolality. DEJOURS and AR (1991) found that the hemolymph osmolality increased in starved scorpions (*Leiurus quinquestriatus*). Evidently, desiccation and starvation influence the scorpion's hemolymph osmolality. This has to be considered with regard to the wild caught scorpions, which GEFEN and AR (2004) and WARBURG(1986) used, since these scorpions have an unknown hydration and nutritional status.

Furthermore, both GEFEN and AR (2004) and WARBURG et al. (1980) found significant seasonal differences in scorpion hemolymph osmolality. The data from WARBURG et al. (1980) show that hemolymph osmolality was low in winter and high in summer, whereas GEFEN and AR (2004) found the opposite with higher hemolymph osmolality in October- December. This could influence the values from the wild caught specimen.

**Table 4.1.** Ionic composition (mmol/L) and osmolality (mOsm/L) of Scorpionidae hemolymph

Species	Sodium	Chloride	Potassium*	Calcium	Magnesium*	Osmolality	References
<b>Pandinus imperator March</b> (n = 10)	235 ± 11.5	207 ± 14.4	7.9 ± 8.8	5.53 ± 1.01	1.72 ± 0.38	472 ± 23.3	Present report
<b>Pandinus imperator June</b> (n = 10)	231 ± 11.9	207 ± 14.8	6.0 ± 4.2	5.68 ± 0.83	1.75 ± 0.41	451 ± 20.5	Present report
<b>Scorpio maurus palmatus</b> (n = 189)	-	-	-	-	-	493 ± 53 <sup>†</sup> 562 ± 32 <sup>‡</sup>	GEFEN and AR (2004)
<b>Scorpio maurus fuscus</b> (n = 107)	-	-	-	-	-	511 ± 56 <sup>†</sup> 578 ± 49 <sup>‡</sup>	GEFEN and AR (2004)
<b>Scorpio maurus fuscus</b> (n = 25)	-	231	-	-	-	465 ± 8 <sup>§</sup>	WARBURG (1986)
<b>Opisthophthalmus capensis</b> (n = 21)	258 ± 5	255 ± 7	7.9 ± 5.4	-	-	519 ± 14	ROBERTSON et al. (1982)
<b>Heterometrus fulvipes</b> (n = 9-16)	209.7 ± 44.6	266.5 ± 56.1	1.37 ± 0.83	6.08 ± 1.07	10.43 ± 3.27	426	PADMANABHANAIDU (1966)
<b>Heterometrus swammerdami</b>	150-335	150-450	0.5- 3.0	5-10	5-25	-	PADMANABHANAIDU (1962; cited from KIMURA et al., 1988)

\* Values include outliers; <sup>†</sup> values measured within 48hr of capture; <sup>‡</sup> values measured after 14 days ad libitum feeding; <sup>§</sup> values measured within 24hr of capture.

Another factor possibly causing a variation in hemolymph values is the fact that PADMANABHANAI DU (1966) used different measurement methods, making it difficult to compare his data with the results of the present study.

Finally it is unclear whether the other authors used a single measurement per scorpion or serial samples to calculate their parameters' means. The latter makes it easier to establish reference ranges and exclude individual differences.

### Perfusion fluids for *Pandinus imperator*

The secondary objective of this study was to find an appropriate perfusion fluid for dehydrated *P. imperator*. Table 4.2 and 4.3 list fluids used in scorpions and spiders and compare the perfusion fluid's content with that of *P. imperator* hemolymph.

**Table 4.2.** Electrolyte content (mmol/L), lactate content (mmol/L), dextrose content (g/L), pH and osmolality of perfusion fluids, listed in literature, compared to hemolymph content and osmolality of *P. imperator*.

	Hemolymph <i>P. imperator</i> March Present study	Hemolymph <i>P. imperator</i> June Present study	KANUNGO (1957)	PADMANABHANAI DU (1967)	ZWICKY (1968)	AHEARN and HADLEY (1976)	RATHMAYER (1965)	Spider Ringer's solution SCHARTAU and LEIDESCHER(1983)	Tarantula ORS WOLLF (1993)
<b>Sodium</b>	235 ± 11.5	231 ± 11.9	111	147	185	281.5	220	190	220
<b>Chloride</b>	207 ± 14.4	207 ± 14.8	111	147	185	260	220	190	226
<b>Potassium</b>	7.9 ± 8.8*	8.0 ± 4.2*	4	2.0	15.0	8	5	2	5
<b>Calcium</b>	5.53 ± 1.01	5.88 ± 0.83	2.7	5	10.0	10	4	4	4
<b>Phosphorus</b>	0.2 ± 0.13	0.32 ± 0.25	-	-	-	-	-	1	-
<b>Magnesium</b>	1.72 ± 0.38*	1.75 ± 0.41*	-	5	-	20	1.1	4	2.7
<b>Dextrose</b>	-	-	-	3	20	-	-	-	-
<b>HCO<sub>3</sub></b>	-	-	-	-	-	1.5	3	-	3
<b>SO<sub>4</sub></b>	-	-	-	-	-	44	-	-	2.7
<b>pH</b>	-	-	6.3	7.3	6.3	7.4	7.3	-	-
<b>Osmolality</b>	472 ± 23.3	451 ± 20.5	229	309	415	550	450	391	461

\*Values include outliers. In red, fluids with an electrolyte content and osmolality similar to that of the *P. imperator* hemolymph and therefore theoretically suitable as perfusion fluids for dehydrated *P. imperator*.

KANUNGO (1957), PADMANABHANAI DU (1967), ZWICKY (1968) and AHEARN and HADLEY (1976) were the first to suggest a scorpion saline on the basis of the hemolymph composition of *Palamnaeus bengalensis*, *Heterometrus fulvipes*, *Urodacus novahollandiae* and *Hadrus arizonensis* respectively (Table 4.2). They determined their salines so as to maintain the heart and nerve activity (both *in situ* and isolated) of the scorpions. KIMURA et al. (1988) show that the scorpion's nerve and heart are quite invulnerable and therefore the maintenance of these activities cannot be a good criterion to determine a physiological saline (KIMURA et al., 1988).

**Table 4.3.** Electrolyte content (mmol/L), lactate content (mmol/L), dextrose content (g/L), pH and osmolality of commercially available perfusion fluids compared to hemolymph content and osmolality of *P. imperator*.

	Hemolymph <i>Pandinus imperator</i> March	Hemolymph <i>Pandinus imperator</i> June	0.9 % sodium chloride	Lactated Ringer's solution	Hartmann's solution	5 % dextrose in 0.45% sodium chloride
<b>Sodium</b>	235 ± 11.5	231 ± 11.9	154	130	131	77
<b>Chloride</b>	207 ± 14.4	207 ± 14.8	154	109	111	77
<b>Potassium</b>	7.9 ± 8.8*	6.0 ± 4.2*	-	4.0	5	-
<b>Calcium</b>	5.53 ± 1.01	5.68 ± 0.83	-	1.5	1	-
<b>Phosphorus</b>	0.2 ± 0.13	0.32 ± 0.25	-	-	-	-
<b>Magnesium</b>	1.72 ± 0.38*	1.75 ± 0.41*	-	-	-	-
<b>Dextrose (g/L)</b>	-	-	-	-	-	50
<b>pH</b>	-	-	5	6.5	6	± 5.6
<b>Osmolality</b>	472 ± 23.3	451 ± 20.5	310	272	278	405

\*Values include outliers.

More recent literature mainly list commercially available fluid products for the use in invertebrates, such as 0.9% sodium chloride and lactated Ringer's solution (DOMBROWSKI and DE VOE, 2007). PIZZI (2006) has used 0.9% sodium chloride, lactated Ringer's solution and Hartman's solution without problems to rehydrate

dehydrated spiders (Table 5). He further suggests the use of Spider Ringer's lactate from SCHARTAU and LEIDESCHER (1983)<sup>2</sup> (Table 4.2). WOLFF (1993) gives the recipe for an oral replacement solution (ORS) for tarantula (Table 4.2). The only indication for the use of a perfusion fluid in scorpions is from FRYE (2006). He suggests a physiological half-strength dextrose saline as rehydration therapy for scorpions (Table 4.3).

When comparing the hemolymph osmolality of *P. imperator* to the osmolality of the perfusion fluids (Table 4.2 and 4.3) one can easily see, that most of the listed fluids are hypotonic. Only the saline suggested by AHEARN and HADLEY (1976) is hypertonic (Table 4.2). A hypertonic perfusion fluid would cause further rehydration by extracting intracellular and interstitial fluids and is therefore not appropriate for the treatment of dehydration in *P. imperator*. The saline from ZWICKY (1986) contains much more potassium and calcium than is found in the hemolymph of *P. imperator*. Calcium plays an important role in the scorpion's muscle contraction (GILLY and SCHEUER, 1984) and potassium is found to inhibit transmural water transport in the scorpion's ileum (AHEARN and HADLEY, 1976 and 1977). Thus a perfusion fluid with an excessive amount of calcium and potassium may interfere with the scorpion's metabolism. Therefore, the saline used by ZWICKY (1968) is inappropriate as perfusion fluid for *P. imperator*.

The saline suggested by RATHMAYER (1965) for spiders and the tarantula ORS from WOLFF (1993) are isotonic to the *P. imperator* hemolymph. In addition to the osmolality, the electrolyte content of both fluids is very similar to the hemolymph electrolyte content of *P. imperator*. Based on the osmolality and electrolyte content, these fluids would be suitable as perfusion fluids for dehydrated *P. imperator*. However, the fluid suggested by WOLFF (1993) is an *oral* replacement solution and RATHMAYER (1965) has only used his saline for *in vitro* experiments on spider limbs, which could mean that both fluids might not be suitable for parenteral (*in vivo*) use in *P. imperator*.

All commercially available perfusion fluids listed in table 4.3 are hypotonic to the hemolymph of *P. imperator* and will therefore distribute themselves rapidly to the intracellular space. This makes them highly suitable for the treatment of dehydrated animals such as *P. imperator*. In order to administer them isotonicly 2.5% dextrose solution can be added to 0.9% sodium chloride (ratio 1:1), to lactated Ringer's

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<sup>2</sup> This comes sold as a research product (BRAUN et al., 2006).

solution (ratio 1:1.5) or to Hartmann's solution (ratio 1:1.5).

The pH of the commercially available perfusion fluids is 6.5 or lower. This seems rather acidic compared to the hemolymph pH of several scorpion species (table 4.4.). The hemolymph pH of the scorpions ranges from 6.3 to 7.51, with most scorpions having a hemolymph pH of 7.0 or higher. The Scorpionidae are only represented with one scorpion *Heterometrus fulvipes*. PADMANABBHANAIDU(1967) has measured a mean hemolymph pH of 7.32 in this species. Since the hemolymph pH of *P. imperator* was not measured in the present study and the hemolymph pH of *Heterometrus fulvipes* may not be representative for *P. imperator*, a clear statement about the suitability of the commercially available perfusion fluids in regard to their pH cannot be made. Probably small amounts of acidic perfusion fluids applied parenteral to dehydrated *P. imperator* specimen may not be harmful.

**Table 4.4.** The hemolymph pH of several scorpion species.

Species	pH	References
<i>Palamnaeus bengalensis</i>	6.3	KANUNGO (1955)
<i>Urodacus novahollandiae</i>	6.3	ZWICKY (1968)
<i>Centruroides gracillis</i>	7.0	BOWERMAN (1972)
<i>Leiurus quinquestriatus</i>	7.0	GILAI and PARNAS (1970)
<i>Leiurus quinquestriatus</i>	7.2 ± 0.025	DEJOURS and AR (1991)
<i>Hadrus arizonensis</i>	7.2 ± 0.04	BOWERMAN (1976)
<i>Buthus martensi</i>	7.2-7.3	KIMURA et al. (1988)
<b><i>Heterometrus fulvipes</i></b>	<b>7.32 ± 0.09</b>	PADMANABBHANAIDU (1967)
<i>Hadrus arizonensis</i>	7.4	AHEARN and HADLEY (1976)
<i>Paruroctonus mesaensis</i>	7.51 ± 0.04	BOWERMAN (1976)

## Conclusion

This study provides a first indication of the physiological values for certain hemolymph parameters of *Pandinus imperator*. Ultimately, it led to the conclusion that the hemolymph composition of *P. imperator* differs from that of other scorpion species. Furthermore, this study compared the hemolymph composition of *P. imperator* with: a) saline compositions reported in literature, and b) commercially available perfusion fluids. On the basis of this comparison the present study suggests using one of the commercially available perfusion fluids (e.g., 0.45% sodium chloride,

0.9% sodium chloride, lactated Ringer's solution or Hartmann's solution) for the parenteral treatment of *P. imperator* suffering from a fluid deficit. However, it is advisable to add 2.5% dextrose solution to these salines in order to administer the fluids isototically, and to warm the fluids to the scorpion's ambient temperature to prevent hypothermia and subsequent immobility.

Further studies are needed to measure the hemolymph pH of *P. imperator* and the *in vivo* response to the parenteral administration of: a) the fairly acidic commercially available perfusion fluids, b) Wollfs's tarantula oral replacement solution (ORS) (WOLLF, 1993), and c) Rathmayer's saline (RATHMAYER, 1965).

## 5 Summary

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An increasing number of invertebrates are kept in captivity, with the African emperor scorpion (*Pandinus imperator*) being one of the most popular invertebrates kept as a pet. Invertebrates are most often introduced to veterinarians with husbandry-related problems. Too high ambient temperature, too low relative humidity, lack of food or water, may cause the scorpion to dehydrate. Dehydration in scorpions can be measured by the evaluation of serial hemolymph electrolyte levels. However, (physiological) reference values for *P. imperator* are lacking, which presents a major obstacle when interpreting the hemolymph values.

The primary objective of this study was to determine the hemolymph composition of *P. imperator* and to compare it with that of other scorpion species described in literature. In the course of this study, hemolymph from ten anaesthetised emperor scorpions was withdrawn twice, with a thirteen-week interval. The hemolymph samples were analysed for their sodium-, chloride-, potassium-, calcium-, magnesium-, phosphorus- and total protein content using a Hitachi 911® Autoanalyzer (Roche Diagnostics, Vienna) and the hemolymph osmolality was measured with a freezing point osmometer (Advanced® Micro osmometer Model 3mo).

The results show that the *P. imperator's* hemolymph osmolality is mainly determined by the high sodium and chloride concentrations. Furthermore, the results show a statistically significant difference in mean hemolymph osmolality between the two sampling times (results for March and June were  $472 \pm 23.3$  and  $451 \pm 20.5$  mOsm/L, respectively). However, further studies are necessary to determine the possible biological relevance of these results. Considering the scarce amount of literature available on scorpion hemolymph composition and osmolality, it seems that the hemolymph composition and osmolality of *P. imperator* is comparable to that of other scorpion species.

Based on the composition and osmolality of *P. imperator* hemolymph, this study suggests using one of the following perfusion fluids: (a) 0.45% sodium chloride + 5% dextrose solution (ratio 1:1); or (b) 0.9% sodium chloride + 2.5% dextrose solution (1:1); or (c) lactated Ringer's solution + 2.5% dextrose solution (1:1.5); or (d) Hartmann's solution + 2.5% dextrose solution (1:1.5), for the parenteral treatment of *P. imperator* suffering from a fluid deficit. The efficiency of these solutions for counterbalancing a fluid deficit shall be analyzed in future studies. In addition, it is advisable to warm the fluids to the scorpion's ambient temperature to prevent hypothermia and subsequent immobility.

*Keywords: Pandinus imperator, emperor scorpion, dehydration, hemolymph, osmolality, electrolytes, perfusion fluid.*

## 6 Zusammenfassung

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Die Zahl der in Gefangenschaft gehaltenen Invertebraten steigt kontinuierlich, wobei der Afrikanische Kaiserskorpion (*Pandinus imperator*) eines der beliebtesten wirbellosen Haustiere darstellt. Folglich werden auch Tierärzte vermehrt mit Invertebraten konfrontiert. Die häufigsten Krankheiten resultieren aus nicht artgerechter Haltung. Eine zu hohe Umgebungstemperatur, zu geringe relative Luftfeuchtigkeit, sowie Nahrungs- bzw. Wassermangel können zur Dehydrierung des Skorpions führen. Diese kann durch Beurteilung serieller Hämolympfproben festgestellt werden. Für eine adäquate Interpretation der Hämolympfwerte fehlen jedoch (physiologische) Referenzwerte für den *P. imperator*.

Das primäre Ziel dieser Studie war es, die Hämolympfzusammensetzung von *P. imperator* zu bestimmen und mit der anderer Skorpionarten zu vergleichen. Im Rahmen dieser Studie wurde in einem dreizehnwöchigen Intervall zweimal Hämolymphe von zehn anästhesierten Kaiserskorpionen abgenommen und analysiert. Der Gehalt an Natrium, Chlorid, Kalium, Kalzium, Magnesium, Phosphor, sowie Totalprotein der Hämolympfproben wurde mit ein Hitachi 911® Autoanalyzer (Roche Diagnostics, Vienna) gemessen und die Hämolympfsmolalität wurde mit einem Gefrierpunktsosmometer (Advanced® Micro osmometer Model 3mo) bestimmt.

Die Ergebnisse zeigen, dass die Hämolympfsmolalität des Kaiserskorpions hauptsächlich durch die hohe Natrium- und Chloridkonzentrationen verursacht wird. Außerdem zeigen die Ergebnisse einen statistisch signifikanten Unterschied in den Mittelwerten der Hämolympfsmolalität zwischen den beiden Probenentnahmen (Ergebnisse für März und Juni waren jeweils  $472 \pm 23,3$  und  $451 \pm 20,5$  mOsm/l). Allerdings sind weitere Studien notwendig um die eventuelle biologische Relevanz dieser Ergebnisse zu prüfen. In Anbetracht der geringen vorhandenen Literatur über die Hämolympfzusammensetzung und -osmolalität des Skorpions, scheint es, dass die Hämolympfzusammensetzung und -osmolalität des Kaiserskorpions vergleichbar mit der anderer Skorpionarten ist.

Aufgrund der Zusammensetzung und Osmolalität der Kaiserskorpion-hämolymphe empfiehlt diese Studie folgende Perfusionsflüssigkeiten für die parenterale Flüssigkeitstherapie von Kaiserskorpionen mit einem Flüssigkeitsdefizit: (a) 0,45% Natriumchlorid + 5% Glukose-Lösung (Verhältnis 1:1); oder (b) 0,9% Natriumchlorid + 2,5% Glukose-Lösung (1:1); oder (c) Ringer-Laktat-Lösung + 2,5% Glukose-Lösung (1:1,5); oder (d) Hartmann-Lösung + 2,5% Glukose-Lösung (1:1,5). Die Effizienz dieser Perfusionsflüssigkeiten um einen Flüssigkeitsdefizit des Kaiserskorpions auszugleichen soll in zukünftige Studien geprüft werden. Außerdem müssen die Lösungen auf die Umgebungstemperatur der Skorpione erwärmt werden, um Hypothermie und daraus resultierende Immobilität der Skorpione zu verhindern.

Keywords: *Pandinus imperator*, Kaiserskorpion, Dehydratation, Hämolymphe, Osmolalität, Elektrolyte, Perfusionsflüssigkeit.

## 7 Samenvatting

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Steeds meer invertebraten, met de Afrikaanse keizerschorpioen (*Pandinus imperator*) als een van de populairste ongewervelde dieren, worden in gevangenschap gehouden. Als gevolg krijgen dierenarsten steeds vaker met invertebraten te maken. De meest voorkomende ziektes bij invertebraten worden veroorzaakt door verkeerde of ontoereikende huisvesting. Een te hoge omgevingstemperatuur, te lage relatieve luchtvochtigheid, voedsel- en/of watergebrek veroorzaken uitdroging van de schorpioen. Dehydratatie in schorpioenen kan door evaluatie van seriële hemolymfmonsters worden vastgesteld. Door het ontbreken van (fysiologische) referentiewaarden voor *P. imperator*, is de interpretatie van de hemolymfwaarden echter lastig.

Het primaire doel van deze studie was het bepalen van de hemolymfsamenstelling van *P. imperator* en deze te vergelijken met die van andere schorpioenensoorten. In het kader van deze studie, werd tweemaal, met een interval van dertien weken, hemolymfe van tien verdoofde keizerschorpioenen afgenomen. Het gehalte aan natrium, chloride, kalium, calcium, magnesium, fosfor en eiwit van de hemolymfmonsters werd met een Hitachi 911® Autoanalyzer (Roche Diagnostics, Vienna) gemeten en de hemolymphosmolaliteit met een vriespunt osmometer (Advanced® Micro osmometer Model 3mo) bepaald.

De resultaten laten zien, dat de hemolymfosmolaliteit voornamelijk wordt bepaald door de hoge natrium- en chloridconcentraties. Tevens, tonen de resultaten een statisch significant verschil in de hemolymfosmolaliteit tussen de twee afnames (de gemiddelde hemolymfosmolaliteit voor maart en juni waren respectievelijk  $472 \pm 23,3$  en  $451 \pm 20,5$  mOsm/l). Nadere studies moeten echter de biologische relevantie van deze resultaten bepalen. Ondanks het beperkte aantal publicaties over de Hemolymfsamenstelling en -osmolaliteit van schorpioenen, is het waarschijnlijk dat de Hemolymfsamenstelling en -osmolaliteit van *P. imperator* vergelijkbaar is met die van andere schorpioenensoorten.

Gebaseerd op de hemolymfsamenstelling en -osmolaliteit van *P. imperator* adviseert deze studie de volgende infuusvloeistoffen als parenterale therapie voor Keizerschorpioenen met een tekort aan lichaamsvocht: (a) 0,45% natriumchloride + 5% dextrose-oplossing (verhouding 1:1); of (b) 0,9% natriumchloride + 2,5% dextrose-oplossing (1:1); of (c) Ringer lactaat + 2,5 dextrose-oplossing (1:1); of (d) Hartmann oplossing + 2,5% dextrose-oplossing. De efficiëntie van deze infuusvloeistoffen om het te kort aan lichaamsvocht van *P. imperator* te compenseren moet echter in verdere studies onderzocht worden. Bovendien is het raadzaam de infuusvloeistof tot de omgevingstemperatuur van de schorpioen te verwarmen om hypothermie en de daar op volgende immobiliteit van de schorpioen te voorkomen.

Trefwoorden: *Pandinus imperator*, keizerschorpioen, uitdroging, hemolymfe, osmolaliteit, elektrolyten, infuusvloeistof.

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