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From the Chairman

Welcome to the latest issue of the Veterinary Invertebrate Society Journal. Please bear with us while it is still in its larval form, pupation has been delayed due to environmental conditions. It has become quite a cliché to say we are in difficult times, but it is very true. As a voluntary society of veterinary and scientific professionals spare time for volunteering has been greatly reduced due to the need to cover front line work with reduced staffing. Therefore, as the chairman of the VIS I whole heartedly apologise to our members and friends for the delays and greatly reduced activity of the Veterinary Invertebrate Society. In asking for your understanding and continued support I am also giving my assurance that as a committee we have remained in touch and have been planning for the resurgence when it is appropriate to do so. But I anticipate we will remain in sub-dormancy throughout 2021 as we take stock of the impact of COVID-19 and start to plan for the future.

Since the last issue of the VISJ in May last year, we have hosted an online lecture and many of our members have still managed to produce scientific publications including reference books on exotic pet medicine and invertebrate histology, in addition to our main work. We are looking at detailing publications from our members, so it is easier to understand where our expertise lies and who to contact about particular specialties of invertebrate medicine. We hope to be able to bring this exciting content to life when it is safe and appropriate to gather once more. We have also been building our network and this has included a partnership through a memorandum of understanding with the Institute of Animal Technology (IAT) through which we hope to be able to bring our expertise to benefit invertebrates in the laboratory and for the VIS to gain knowledge of the needs of invertebrates in this setting.

One of the great operational changes during this pandemic has been the rise and greater acceptance of online communication. Our Twitter profile is growing slowly but we have much greater engagement with Facebook, with over a thousand followers and posts reaching over 50,000 people, far more than we could meet in person. Through Facebook in particular veterinary and scientific advice is dispensed, as many of the committee members are monitoring the site and thus able to direct the questions to the appropriate experts. We have also had a diverse range of questions and calls for help through our website contact page, including attending to over 300 ants found in an unclaimed package. I must stress we are not a rehoming organisation, in this instance that was the easiest thing to get the best care for the animals.

I am very pleased to be able to introduce you to the July 2021 issue of the Veterinary Invertebrate Society Journal, in this issue, we have an interesting article on scorpion heamolymph biochemistry which should be helpful to those that want to utilise heamolymph biochemistry as a diagnostic tool. We also have some summaries of some recent articles as well as letters covering invertebrate natural biology by Prof. Ross Cooper.

In the UK the COVID-19 vaccination program is rolling out well and a tentative return to scientific conferences looks likely in the Autumn. For us at the VIS we are aiming to get back to more usual operations in 2022 as many of us need to take a moment to recharge from the huge challenges of the last 18 months. We hope you are all also taking time out to recharge and look after yourselves as well as the invertebrates. I look forward to catching up with you all online in 2021 and hopefully many of you in person next year.

Thank you for your patience and taking the time to read our latest issue.

Steve Trim
Chair of the VIS committee.

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Summaries of Recent Articles in the Field of Invertebrate Medicine

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Gallini, S. H., Cole, S. D., & DiGeronimo, P. M. (2019). In vitro inhibition of shell flora by a commercial probiotic marketed for use in captive hermit crabs (Coenobita spp.). Journal of Exotic Pet Medicine, 31, 91-94.

This article discusses the effects of commercial probiotics on shell bacteria in terrestrial hermit crabs. Probiotics have been widely used in shrimp aquaculture but until now their actions have not been studied in terrestrial invertebrates. The study compares the action of probiotics in hermit crabs to its use in aquaculture. Commercial probiotics may be useful for improving the husbandry of captive hermit crabs. Currently, this research focuses on external shell bacteria, therefore further studies regarding internal bacteria are still needed.

Catanese, G. (2020). An emergency situation for pen shells in the Mediterranean: the Adriatic Sea, one of the last Pinna nobilis shelters, is now affected by a mass mortality event. Journal of Invertebrate Pathology, 107388.

Although the article is more focused on the ecological role of the animals and the impact of parasitic and bacterial disease in the wild it can be seen as a model for conservation and zoo settings. The pathogens identified can spread through sea currents therefore seashell farms can be affected which makes this paper a good starting point for further studies into treatment methods for the parasite *Haplosporidium pinnae* and the pathogenic *Mycobacterium* species identified.

Zhao, R. H., Gao, W., Qiu, L., Chen, X., Dong, X., Li, C., & Huang, J. (2020). A staining method for detection of Enterocytozoon hepatopenaei (EHP) spores with calcofluor white. Journal of Invertebrate Pathology, 107347.

This article focuses on using a staining method to test for the presence of Enterocytozoon hepatopenaei in the shrimp species *Penaeus vannamei*. The stain is used to detect the parasite in the faeces of the infected animal. This prevents the need to use time consuming PCR or hepatopancreas staining post-mortem techniques. It provides a quicker and less invasive testing method. This article focuses on shrimp in aquaculture, however it could be used in veterinary medicine within the zoo or exotics industry.

Sharma, D., & Ravindran, C. (2020). Diseases and pathogens of marine invertebrate corals in Indian reefs. Journal of Invertebrate Pathology, 107373.

This article is a review of the main diseases, including bacterial, viral or environmental, that can affect corals. The article is relevant for zoo and aquarium veterinarians, especially those in ocean conservation roles. Important references regarding the diseases and methods of treatment can also be found in the review.

Survey of Tarantula Owners being conducted by the Royal Veterinary College

Recently Adam Crowther, a student of the Royal Veterinary College, and Benjamin Kennedy, a member of this society, have started a study entitled: “Evaluating the demographic of tarantula owners and their perspective of the human-animal bond and tarantula health.”

This study aims to elucidate more about the tarantula owning community, as very little research has been done on the demographic of these invertebrate owners.

The study used an online survey which covered questions on the respondant, how the respondant feels about their tarantula and what health issues, if any, the respondant has encountered in their animals.

The researchers hope that this data will aid clinicians in providing better veterinary care for tarantulas. The survey has had over 1000 respondents and we are eagerly awaiting the results!

Below: image used to advertise the survey on social media

PEER REVIEWED ARTICLE

Evaluation of selected biochemical values in subadult emperor scorpion (Pandinus imperator) hemolymph

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Abstract

Emperor scorpions (Pandinus imperator) have become increasingly popular for private and public uses in the United States. This increased popularity is reflected in scorpions presenting more frequently for veterinary evaluation. Just as serum biochemical analysis is often needed for proper assessment of vertebrate patients, biochemical analysis of hemolymph is often required for proper assessment of invertebrate patients. Little is known, however, about emperor scorpion hemolymph analytes. This pilot study represents the first attempt to evaluate selected biochemical analytes in subadult emperor scorpion hemolymph using benchtop analyzers commonly found in a veterinary practice. Eleven emperor scorpions, all captive-bred subadults, were obtained from a commercial vendor and hemolymph was collected for biochemical analysis. The biochemical analytes measured in the study included alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine phosphokinase (CK), gamma glutamyl transferase (GGT), uric acid, urea nitrogen, glucose, magnesium, calcium, phosphorus, total protein, albumin, potassium, sodium, ionized calcium, pH, partial pressure of carbon dioxide (pCO2), and partial pressure of oxygen (pO2). This pilot study demonstrates that it is possible to use benchtop analyzers commonly found in veterinary practice to measure many of the hemolymph biochemical constituents of emperor scorpions, including calcium, potassium, phosphorus, total protein, pH, pCO2, pO2, and ionized calcium. A commercial analyzer or sample dilution may also be needed for some analytes such as sodium.

KEY WORDS
Biochemistry, *Pandinus imperator*, hemolymph, scorpion, arachnid, invertebrate

INTRODUCTION

Invertebrates comprise the majority of the world's fauna, and are popular as research, display, and pet animals. The most ancestral of these invertebrates are the scorpions (Stefoff, 2009). Scorpions are terrestrial chelicerate arthropods and members of the order Scorpiones, class Arachnida in which there are 16 extant families (Mullen and Sissom, 2018). Popular scorpions kept as pets and for zoological display are the emperor scorpions (*Pandinus imperator*) of the Scorpionidae family. Their popularity has grown to the point where their numbers in the wild are diminishing and are now listed with the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II (UNEP, 2019).

To protect the future of these scorpions, research to increase our understanding of their physiologic functions is required. In scorpions, metabolic activity, including venom production, is in direct relation to the hemolymph biochemical constituents (Moghadam et al., 2013). Reference values for blood biochemical and gas constituents have long been established for domestic species, but only a few limited attempts have been made for scorpions of any taxonomic group (Bricteux-Grégoire et al., 1963; Padmanabhanaidu, 1966; Bowerman, 1976; Sheldon et al., 2019).

These previous attempts focused on analyzing different organic and inorganic hemolymph constituents using relatively slow, technically involved procedures with equipment that would not be found in the average veterinary clinic or zoological hospital. The objective of this pilot study is to establish a preliminary data for establishing reference ranges for the biochemical components of emperor scorpion hemolymph using standard analytical machines that could be commonly found in general veterinary practice.

MATERIALS AND METHODS

Eleven (five male and six female) captive-bred, subadult emperor scorpions were obtained from an invertebrate importer in Arizona (Ken The Bug Guy -An Exotic Pet Shop, Tuscon, AZ, USA) and added to a zoological collection for display and educational purposes. All scorpions appeared to be healthy based on gross physical examination. In addition to a physical examination, hemolymph was collected for the facility entrance health assessment. This allowed an opportunity to formulate a study to evaluate the results of the hemolymph analysis. For one month prior to the study, the scorpions were housed individually in rectangular, 5.7 liter plastic storage containers in a rack system. Coconut fiber (Eco Earth®, Zoo Med Laboratories Inc., San Luis Obispo, CA, USA) was used for the substrate which was misted twice weekly with tap water. A small cardboard box was provided to serve as a hiding space. The temperature and humidity in the enclosures were maintained at approximately 23.9°C (75°F) and 80%, respectively. The scorpions had constant access to chlorinated tap water and were fed four or five adult crickets weekly. The crickets had access to varied fresh fruit and vegetables and fresh water until being offered to the scorpions.

Each scorpion was placed into a square, 3 liter plastic storage container that was modified into a gas anesthetic chamber. The container was modified by drilling a hole in one side and inserting an endotracheal tube adapter. Each scorpion was anesthetized with 5% isoflurane (Fluriso™, VetOne®, Boise, ID, USA) in oxygen at a flow rate of 2 L/min. Once the scorpion had lost its ability to right itself, it was removed from the anesthetic chamber and weighed. A low dose insulin syringe (½ cc) with an attached 28-ga, ½-inch needle was used to collect an intracardiac hemolymph sample. A volume of 0.3 - 0.4 ml was collected from each individual by inserting the needle at approximately a 45° angle between the fifth and sixth mesosomal tergites along the dorsal midline. This method was used to facilitate the rapid collection of a relatively large amount of hemolymph. Hemolymphostasis was assisted by applying a small amount of cyanoacrylate glue (GLUture®, Zoetis Inc., Kalamazoo, MI, USA) to the collection site. The scorpions were recovered in 100% oxygen, and recovery from anesthesia was uneventful in all scorpions.

Each sample was immediately analyzed using the Avian/Reptilian Profile Plus (ARPP) rotor for the VetScan Analyzer (Abaxis Inc., Union City, California 94587, USA). Results were collected for the following biochemical analytes: aspartate aminotransferase (AST), bile acid, creatine phosphokinase (CK), uric acid, glucose, calcium, phosphorous, total protein (TP), albumin, potassium, and sodium. In addition, each sample was also immediately analyzed using a CG8+ blood gas cartridge with the i-STAT analyzer (Abbott Laboratories, North Chicago, Illinois 60064, USA). The CG8+ cartridge measures pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), ionized calcium (iCa), sodium, potassium, glucose, and hematocrit. The i-STAT analyzer will also calculate base excess (BE), bicarbonate (HCO_3), total carbon dioxide (TCO_2), percent oxygen saturation (sO_2%), and hemoglobin (Hb) using formulae based on human physiology. Since BE, HCO_3, TCO_2, sO_2, and Hb were not actually measured, the results for these analytes were ignored. Results were collected for the following additional hemolymph values: ionized calcium (iCa), pH, sodium, potassium, glucose, pCO_2, pO_2, and hematocrit. Sodium levels for both the VetScan analyzer and i-STAT analyzer have a maximum measurable limit of 180 mmol/L. Since hemolymph sodium levels for arachnids were often greater than 180 mmol/L, an aliquot of each sample was placed in a lithium heparin microtainer (Micro tube LH, Sarstedt AG & Co., Nümbrecht , Germany) and shipped on an icepack to a commercial laboratory (Avian and Exotic Animal Clin Path Labs, Wilmington, OH) where the hemolymph pH, iCa, sodium, and potassium levels were tested within 24 hours using the EasyLyte analyzer (Medica Corporation, Bedford, Maryland 01730, USA). In four of the scorpions, the hemolymph sample was also analyzed using the Large Animal Profile (LAP) rotor for the VetScan analyzer. Results were collected for the following biochemical analytes: AST, alkaline phosphatase (ALP), CK, gamma glutamyl transferase (GGT), magnesium, calcium, phosphorous, TP, albumin, and urea nitrogen.

The distribution of each hemolymph biochemical parameter was evaluated for normality using the Shapiro–Wilk test, q-q plots, skewness, and kurtosis. For purposes of analysis, all non-normally distributed data was logarithmically transformed. To assess sex variability on hemolymph parameters, a Student's *t*-test on normally distributed data and a Wilcoxon rank sum test on non-normally distributed data. When no statistical difference was found between the sexes, the data was combined and evaluated for normality as previously described. The mean, standard deviation (SD), and minimum-maximum values are reported for normally distributed data. The median and minimum-maximum are reported for non-normally distributed data. A power analysis was performed when no difference was found between the sexes to assess the potential for a type II error. For those cases where no difference was found, the highest observed power was 0.95. Significance testing was set at p = 0.05. The statistical analyses were performed with Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA 98052, USA).

Table 1

Sex	Units	Mean	Median	SD	Min	Max	n	p
Male	g	14.0		2.4	11.6	17.1	5	0.01
Female	g		24.0		12.7	24.4	6	

Body weight of emperor scorpions in grams. The mean, standard deviation (SD), and minimum-maximum (Min, Max) values are reported for normally distributed data. The median and minimum-maximum are reported for non-normally distributed data. n = number of individuals, $p < 0.05$ is considered statistically significant.

RESULTS

The ARPP rotor results were marked by the analyzer with a very high lipemia index (3 on a scale of 0 to 3) for all samples analyzed. Only three of the samples had AST levels above the minimal detectable limit of 5 IU/L. Bile acid, uric acid, and albumin were below the detectable limits of the analyzer (35 µmol/L, 0.3 mg/dL, and 1.0 mg/dL respectively) in all eleven samples. Results for CK were not displayed for ten of the eleven samples, and the only sample with a displayed value was measured at 0 U/L. Only four of the eleven samples had glucose above the minimal detectable limit of 10 mg/dL and only two had calcium levels below the maximum measurable limit of 16 mg/dL. All of the sodium levels were above the maximum limit of 180 mmol/L.

The i-STAT CG8+ cartridge would not display values for hematocrit and potassium. All of the samples were below the detectable limits of glucose and above the detectable limits of sodium (20 mg/dL and 180 mmol/L, respectively). Two of the eleven hemolymph samples would not display results for pH and pCO_2. Only eight of the eleven samples had enough volume to run an electrolyte module on the EasyLyte analyzer at the commercial laboratory.

The LAP rotor was used to analyze four of the hemolymph samples and were

Table 2

Parameter	Units	Mean	Median	SD	Min	Max	n	p
AST [ARPP]	U/L		< 5		< 5	12	9	0.48
AST [LAP]	U/L	8.5		9.9	1	23	4	
ALP [LAP]	U/L	2.5		3.8	0	8	4	
CK [ARPP]	U/L		0		0	0	1	*
CK [LAP]	U/L		0		0	13	4	
GGT [LAP]	U/L		0		0	3	4	
Calcium [ARPP]	mg/dL				12.8	> 16	10	*
Calcium [LAP]	mg/dL	17.1		2.3	14.4	20	4	
iCa [i-STAT]	mmol/L	2.35		0.10	2.21	2.50	11	0.03
iCa [EasyLyte]	mmol/L	1.78		0.64	0.63	2.80	8	
Sodium [ARPP]	mmol/L				> 180	> 180	11	**
Sodium [i-STAT]	mmol/L				> 180	> 180	11	
Sodium [EasyLyte]	mmol/L	228.7		21.7	199.9	269.8	8	
Potassium [ARPP]	mmol/L	4.4		0.4	3.9	5.0	11	0.51
Potassium [i-STAT]	mmol/L				<>	<>	0	
Potassium [EasyLyte]	mmol/L		4.9		2.3	5.2	8	
Magnesium [LAP]	mg/dL	4.5		1.8	2.6	6.6	4	
Phosphorus [ARPP]	mg/dL		0.4		< 0.02	4.2	11	0.15
Phosphorus [LAP]	mg/dL	0.7		0.2	0.4	0.9	4	
Total Protein [ARPP]	g/dL	7.2		3.2	2.4	11.6	10	0.01
Total Protein [LAP]	g/dL	3.9		2.1	1.9	6.9	4	
Albumin [ARPP]	g/dL	0.0		0.0	0.0	0.0	11	0.12
Albumin [LAP]	g/dL		0.4		0.0	1.3	4	
pH [i-STAT]			7.406		7.380	7.818	9	0.24
pH [EasyLyte]			7.503		7.420	7.930	8	
pCO_2 [i-STAT]	mmHg		32.2		6.8	35.7	9	
pO_2 [i-STAT]	mmHg	201		74	89	372	11	

Selected hemolymph parameters of emperor scorpions. The mean, standard deviation (SD), and minimum-maximum (Min, Max) values are reported for normally distributed data. The median and minimum-maximum are reported for non-normally distributed data. n = number of individuals, $p < 0.05$ is considered statistically significant. AST = aspartate aminotransferase, ALP = alkaline phosphatase, CK = creatine phosphokinase, GGT = gamma glutamyl transferase, iCa = ionized calcium, pCO_2 = partial pressure of carbon dioxide, pO_2 = partial pressure of oxygen, ARPP = Avian/Reptilian Profile Plus rotor, LAP = Large Animal Profile rotor. * = no continuous variable value available from ARPP for corresponding LAP value, preventing comparison statistics from being performed for this analyte. ** = no continuous variable value available from ARPP or i-STAT for corresponding EasyLyte value, preventing comparison statistics from being performed for this analyte.

marked by the analyzer with a high to very high lipemia index (2 or 3 on a scale of 0 to 3) for all four samples. The calcium levels were detectable in all four samples. These same four samples were above the level of detectability with the ARPP rotor so comparative statistics were not able to be performed. The value for globulin proteins is not reported since its value is mathematically calculated on both the ARPP and LAP.

Significant differences were found in body weight between the sexes ($p = 0.01$; Table 1). There were no significant differences between the sexes for any hemolymph parameter analyzed ($p < 0.05$; Table 2). There was a significant difference between the i-STAT and EasyLyte analyzers for iCa ($p = 0.03$). There was a significant difference between ARPP and LAP for TP ($p = 0.01$). There were no significant differences between ARPP and EasyLyte for potassium ($p = 0.51$); between i-STAT and EasyLyte analyzers for pH ($p = 0.24$); and between ARPP and LAP for AST ($p = 0.48$) and phosphorus ($p = 0.15$).

DISCUSSION

All the scorpions survived the anesthesia and hemolymph sampling procedures. In order to obtain adequate sample sizes, volumes of hemolymph between 1% and 2% of body weight (1–2 ml/100 g body weight) were collected. In vertebrates, it is recommended that less than 1 ml of blood be collected per 100 g body weight so that no more than 10% of blood volume would be removed (Divers, 2019). Preliminary measurements of scorpion hemolymph volume has revealed an approximate 34% volume to body mass ratio (Gefen and Ar, 2004; Moghadam et al., 2013), thus a 0.3 ml sample from a 14 g scorpion did not constitute more than 6% of the total hemolymph volume. Similar studies in other arachnids have used volumes consistent with this study with no ill effects to the animal (Zachariah et al., 2007). When locust (*Chortoicetes terminifera*) larvae lost 25% of hemolymph volume, there was no change in osmotic pressure and the hemolymph volume rapidly returned to normal when the larvae were allowed to imbibe water (Djajakusumah and Miles, 1966). The level of hypovolemia that can be tolerated by scorpions is not known but all eleven scorpions appeared to tolerate this sample volume well without supplemental parenteral fluid therapy as all recovered from anesthesia without incident and continued to thrive for several months following the procedure.

All of the scorpions in this study were subadult. The hemolymph biochemical analyte values reported in this study may not be representative for all life stages. In mammals, differences in serum biochemical analyte values are commonly seen between the age groups (Elkhair 2016; Couch et al. 2017; Azimzadeh and Javadi 2020). Additional studies investigating potential differences between age groups of emperor scorpions are warranted, especially when establishing reference intervals.

Several analytes were not detectable with the benchtop analyzers. This could be due to hemolymph levels that were outside the detectable limits of the analyzer or due to lipemia interference since all of the hemolymph samples had a high lipemia index. The hemolymph of the emperor scorpion has been shown to contain a unique high-density lipoprotein, which probably contributed to the high lipemia index (Schenk et al., 2009). In mammals, lipemia results from increased concentration of triglyceride-rich lipoproteins in the blood, causing a cloudy/turbid appearance to the serum or plasma (Getahun et al., 2019). This high lipemia index is associated with light scattering effects and may increase absorbance during end point reactions and non-blanked reactions for some analytes (Getahun et al., 2019). An increase in lipemia index has been shown to cause biochemical analyzers to underestimate the serum levels of sodium, potassium, calcium, phosphorus, urate, TP, and ALP (Calmarza and Cordero, 2011; Sen et al., 2016). Since all of the animals tested in this study had a high lipemia index, future hemolymph studies in emperor scorpions should include cholesterol, triglycerides, and lipoproteins.

Interestingly, both AST and CK levels were below measurable levels for the VetScan analyzer. The VetScan analyzer lower limit for AST is 5 U/L and only 3 of 11 scorpion samples had AST levels greater than 5 U/L in this study when measured with the ARPP rotor. For CK, the analyzer was able to produce a result in only 1 of 11 scorpion hemolymph samples when using the ARPP rotor. The remaining hemolymph sample results produced error flags. Since lipemia can cause an interference in sample AST and CK levels (Jagannatha and Chandrakar, 2019), clearing the hemolymph sample with ultracentrifugation may be beneficial when evaluating scorpion hemolymph for AST and CK (Calmarza and Cordero, 2011). Of the four hemolymph samples where GGT and ALP were also measured, no interference flags were raised by the analyzer. The value of these tissue enzymes is uncertain as the origin of the enzymes in arachnid tissues have not been elucidated. Some work has been done evaluating LDH in multiple arachnid species and it appears that there may be variability in LDH isoenzymes between the tissue types (Long and Kaplan, 1968; Gleason et al., 1971; Padmaja et al., 2010). Additionally, it has been shown that arachnids do not possess AST and any results provided by the analyzers for AST may be due to the analyzer reagents cross-reacting with an enzyme similar to AST (Kennedy et al., 2019). Evaluation of scorpion tissue for multiple enzymes is warranted before these analytes can be useful clinically.

Hemolymph calcium levels were only able to be assessed on two of the eleven samples using the ARPP rotor. When the LAP rotor was used, however, all four of the four samples were able to be measured. Unfortunately, there was not enough hemolymph available to run duplicate rotors in all eleven scorpions and the four samples that were able to be read by the LAP gave an error on the ARPP, preventing comparison statistics from being performed for this analyte. Other arachnids have had calcium levels within the detectable limits of the ARPP rotors (Zachariah et al., 2007). Either emperor scorpions have higher calcium levels than the spiders evaluated by Zachariah et al. (2007), or the lipemia index present in the emperor scorpion hemolymph samples caused too much interference for proper measurement by the benchtop analyzer using the ARPP rotors.

The measured hemolymph calcium in this study was similar to what has been reported in several species of buthid scorpions (14.4 ± 1.6 mg/dL; Kimura et al., 1988; Moghadam et al., 2013). These values are lower than the more closely related Scorpionidae species. The northern Indian black scorpion (*Heterometrus fulvipes*) had hemolymph calcium levels reported to be 24.4 mg/dL (Padmanabhanaidu, 1966). Species variation could be a potential explanation of these differences. It is also possible that the emperor scorpions in this study may have been calcium deficient as the prey offered were not supplemented with vitamins or minerals and the northern Indian black scorpions studied by Padmanabhanaidu (1966) were wild caught. Further study into supplementing captive emperor scorpion prey with calcium is warranted.

The ionized calcium levels provided by the i-STAT analyzer had a statistically significant difference from the levels provided by the EasyLyte analyzer but these values were similar to other arachnids, which is higher than that reported in most vertebrates (Zachariah et al., 2007). In vertebrates, un-ionized calcium is generally bound to albumin (Raiti, 2019). Arachnids have negligible albumin levels in their hemolymph which may account for the higher levels of circulating ionized calcium. Further investigation is warranted as calcium may be bound to other circulating proteins in invertebrates (Kennedy et al., 2019).

In spiders and scorpions, the hemolymph concentration of calcium seems to depend on the hemolymph concentrations of sodium, potassium, and magnesium (Burton, 1984). The osmotic pressure of scorpion hemolymph, however, is due almost entirely to sodium and chloride with small organic molecules contributing only to a very limited extent (Jeuniaux, 1971).

Hemolymph sodium levels of the emperor scorpion were dramatically elevated compared with typical vertebrate serum levels but similar to levels previously reported in other scorpions like the northern Indian black scorpion and the fat tailed scorpion (*Androctonus australis*; Bricteux-Grégoire et al., 1963; Padmanabhanaidu, 1966). These hemolymph sodium levels, however, are much lower than one member of Buthidae, the Chinese scorpion (*Buthus martensi*), which has a reported hemolymph sodium concentration of 291.6 ± 5.8 mmol/L (Kimura et al., 1988). Interestingly, five other species of scorpions in the Buthidae family had hemolymph sodium levels reported between 4.6 - 118 mmol/L (Moghadam et al., 2013). This great disparity between the species (especially between taxonomic families) necessitates the need for developing species specific reference ranges.

There was not a statistically significant difference between the potassium levels measured by the ARPP rotor and the reference laboratory's EasyLyte analyzer ($p = 0.51$). This is consistent with reports that spectrophotometric methods were not statistically different from electrode technology (Hübl et al., 1994). Potassium levels could not be measured, however, with the i-STAT analyzer. Although both the i-STAT and the EasyLyte analyzers use ion-selective electrode potentiometry, the samples evaluated with the i-STAT analyzer were not diluted while the samples evaluated with the EastyLyte were diluted in order to obtain a sodium value. It is possible that the increased lipemia index of the undiluted sample may have interfered with the i-STAT analyzer's ability to read the potassium levels using electrode technology. The hemolymph potassium levels measured in the emperor scorpions were higher than those reported in spiders (1.6 – 2.8 mmol/L; Zachariah et al., 2007; Schartau and Leidescher, 1983; Punzo, 1982) and the northern Indian black scorpion (1.37 mmol/L; Padmanabhanaidu, 1966) but lower than reported the fat tailed scorpion (8.0 mmol/L; Bricteux-Grégoire et al., 1963), Chinese scorpion (6.7 ± 0.8 mmol/L; Kimura et al., 1988), and five species of Middle Eastern buthid scorpions (6.3 – 73 mmol/L; Moghadam et al., 2013).

The hemolymph magnesium levels were only measured in four of the emperor scorpions but had a mean value consistent with what has been reported in some buthid scorpions (Moghadam et al., 2013). However, the hemolymph magnesium level was much lower that what has been reported in the more closely related northern Indian black scorpion (25.35 mg/dL). It has been demonstrated that magnesium levels will increase in scorpion hemolymph after exercise (Paul et al., 1994). Therefore, the variability in the magnesium concentrations between the scorpions may not be species related but related to scorpion activity prior to sample collection. Further controlled studies are warranted to assess this hypothesis.

Hemolymph phosphorus levels measured by ARPP rotor did not differ significantly from the levels measured by the LAP rotor ($p = 0.15$). These values were much lower than what has been previously reported in other arachnids (Zachariah et al., 2007). The biochemical demand for inorganic phosphorus in scorpions is low as the majority of the hemolymph anion is reported to be chloride with a concentration approaching nearly 100% of all anions (Jeuniaux, 1971). Unfortunately, chloride is not an analyte measured by the Abaxis rotors or i-STAT cartridges used in this study. Additional research using alternate rotors/cartridges may prove to be beneficial.

Scorpion hemolymph proteins can be categorized as either respiratory or non-respiratory; and the respiratory protein, hemocyanin, is predominant (Ali et al., 1995; Menze et al., 2005). The total hemolymph protein measured by ARPP was statistically higher than that measured by LAP ($p = 0.01$). As the methodology in measuring total protein is the same for both rotors, timing of sample analysis from collection may be a factor since ARPP rotors were run prior to LAP rotors. It is possible that the proteins could have coagulated as a "clot" and been removed by the analyzer during sample processing with the LAP rotors, preventing a proper value to be measured. Additional studies regarding timing of sample analysis is warranted to confirm this hypothesis, but one supporting factor is the results from the ARPP were consistent with what has been reported in other scorpions (Moghadam et al., 2013). Albumin was not measured by the ARPP rotor which agrees with previous studies stating that albumin is not one of the protein components of scorpion hemolymph (Ali et al., 1995). It is uncertain as to why the LAP rotors reported a value for albumin. It is possible that the chemical reagents used to detect albumin in the LAP rotors may have cross-reacted with another hemolymph protein, reporting a false albumin level (Kennedy et al., 2019). Further studies using protein electrophoresis may be helpful in determining an underlying cause.

The hemolymph pH and gases of the emperor scorpion were similar to reports in other scorpions and some spiders (Angersbach, 1978; Zachariah et al., 2007; Moghadam et al., 2013; Sheldon et al., 2019). Because the animals were sampled after having been anesthetized with pure oxygen and isoflurane, this could have resulted in an artificial elevation in the partial pressure of oxygen, and reduction in partial pressure of carbon dioxide. This may also have affected an increase in pH.

Uric acid and urea nitrogen were not detected in the hemolymph of emperor scorpions in this study. Similar to terrestrial reptiles, scorpions crystallize uric acid, along with guanine, for conservation of water (Polis, 1990). The amount of uric acid, if present, in the scorpions in this study was below the detectable limits of the analyzer. Likewise, glucose was also not present in the hemolymph above the minimal detectable levels as well. Glucose is transported through insect hemolymph in the form of trehalose, a disaccharide (Nation, 2001), and presumably, this may also be the case in scorpions. In some species of scorpions (e.g. fat tailed scorpion), trehalose is the main sugar of hemolymph (Bricteux-Grégoire et al., 1963) but some species, such as southern Indian black scorpion (*Heterometrus bengalensis*), a nonidentified pentose is the only sugar in the hemolymph (Jeuniaux, 1971). Carbohydrate analysis of emperor scorpion hemolymph has shown a high-mannose-type-N-glycans to be the only carbohydrate present (Schenk et al., 2009).

CONCLUSIONS

This pilot study demonstrates that it is possible to use Abaxis and i-STAT benchtop analyzers to measure many of the hemolymph biochemical constituents of emperor scorpions. Alternate rotors and cartridges may be more appropriate over the Avian and Reptilian Profile and CG8+, respectively for accurate measurements of some analytes. A commercial analyzer or sample dilution may also be needed. Further study with a larger population of emperor scorpions is needed to facilitate the calculation of valid reference intervals of hemolymph parameters for this species. A larger population may also reveal other differences between the analyzers due to possible type II statistical error common in low population studies. Furthermore, additional studies to correlate hemolymph and tissue levels of the biochemical analytes are needed. The values reported in this study highlight the differences found among the various scorpion species, which suggests that hemolymph biochemistry references may be required on a species-by-species basis.

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SHORT COMMUNICATION

Rhinoceros beetle (*Oryctes monoceros*) in Zimbabwe – gardeners of the insect world

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The current preliminary investigation studied the rhinoceros beetle (Fig. 1) and its life in a 2-acre urban garden in Vainona, Harare, Zimbabwe in September during the hot season.

In Zimbabwean the scarab beetles [Kingdom – Animalia, Phylum – Arthropoda, Class – Insecta, Order – Coleoptera, Family – Scarabaeidae, Sub-family – e.g. Dynastinae – rhino beetles] include the dung and rhinoceros beetle (Fig. 1).

The beetles are the largest group of insects. Larvae and adults possess biting and chewing mouthparts. There are two main sub-orders of beetles: Adephaga and Polyphaga. The latter have coxae that are not fused to the thorax and the first visible abdominal segment remains undivided, e.g. the dung beetles. Other scarabs include the cane beetle and Christmas beetle. There are about 1,800 species of rhinoceros beetles in the world. The scarabs possess antennae that end in a club which may open out into a fan. Scarab larvae are fat and C-shaped. They live in the soil, dung and rotting vegetation (Cooper, 2010). Many occur in Africa. Their bodies are heavy, rounded, oval or almost oblong. They are majestically coloured in shiny hues of black, purple, blue, green, bronze or gold. They have short legs with flattened and broadened middle joints. Their short antennae are attached to a head, and their thorax may have horns. Tolerable temperatures include 22-30°C. Water should be provided although they tend to get most of their moisture from the food they eat. They are extremely strong in relation to their body weight. They can lift weights 850 times their own. Common species include: Atlas beetle (*Chalcosoma atlas*), Elephant beetle (*Megasoma elephas*), European rhinoceros beetle (*Oryctes nasicornis*), Japanese rhinoceros beetle (*Trypoxylus dichotomus*), Hercules beetle (*Dynastes hercules*), Ox beetle (*Strategus antaeus*), the Unicorn beetle (*Dynastes tityus*), and the Common rhinoceros beetle (*Xylotrupes gideon*). The rhinoceros beetle (*X. gideon*) makes loud hissing squeaks when in danger by rubbing its abdomen on the end of its wing covers. They are strong fliers and are attracted to lights. Males use their horns to push each other off branches when competing for a female. Horns and the beetle's strength allow it to push its way through decaying vegetation and to escape danger with ease. The adult beetle feeds on many shoots especially of Poinciana trees. Females lay about 50 eggs in decaying vegetation which hatch in three weeks. The larvae help to convert the matter into compost. It takes up to two years for them to reach maturity. A fully-grown larva forms a cell in the soil and lines it with faeces which then solidify into a waterproof layer. The larva pupates and the adult emerges after a month. It lives for 2-4 months. The world's largest rhinoceros beetle, *Dynastes hercules*, is 16 cm long and lives in South America. Adult rhinoceros beetles (*Oryctes monoceros*) can be very destructive to coconut, oil and date palm trees (Burton and Burton, 1980). They damage unopened fronds and palm meristems. The rhinoceros beetles have three instars as they moult thrice before their pupa stage. Most are extremely useful for recycling plant matter back into the soil. Adults feed on plant sap and fruit. However, they do not eat vast quantities of food. The larvae consume much rotting wood and compost. Rarely have they become a problem. This mostly occurs in patches of cultivated cane or palms in the jungle. Hiding under logs and in vegetation during the day helps them avoid predators. Horns can be used during competition when feeding (Montieth, 2007).

In this preliminary study an 8-foot walled gardened 2-acre property with numerous vegetation types ranging from Cyprus trees (A, 33% of total area), to banana, citrus and stone fruit trees (B, 28%), grassy-lawn areas (C, 38%) and an enormous compost heap (D, 10%), was used as the study habitat area.

Figure 1 Adult rhinoceros beetle (Oryctes monoceros)

The percentage distribution was mapped on survey charts and aerial photographs. Counts of beetles took place by vegetation location (A-D) over 7 days. Research enthusiasts were allocated to each location for 8 hours/day. They were shifted to a new location the next day. The investigation spanned 0800-1700 hr. The weather during the study was sunny and partly clouding with the ambient temperature of 23-27°C and R.H. 43-45% (Electronic weather station with probe). The results showed an abundance of adult movement in the mid-morning and late afternoon periods from 0900-1100 and 1600-1700hr, respectively. They presumably scurried from one section to another in search of food or a mate. Feeding was especially prevalent in section D (58%) whereas courtship was more abundant in sections A-C (57%) – the males were probably attracted by female pheromone emissions (Gries and Gries, 1994). In the more exposed areas particularly section C, there was a greater risk of predation from rodents, cats and dogs resident on the property. They are indeed a common garden invertebrate (Hurry and Hurry, 1991; Sayce, 1987; The Bundu Book of Birds, Insects and Snakes, 1981).

The rhinoceros beetle is ecologically important and as a consequence there is need to protect them from harmful activities like cultivation and digging (Cooper and Cooper, 2009i, ii & iii) as their larvae will be unearthed. They are also of veterinary interest as many are kept in glass tanks as pets. Watching any beetle including the rhinoceros beetle walking over grass or burrowing in the soil is immensely fascinating. Beetles should be recognised as essential for turning nutrients in the soil and consuming rotten vegetation and dung. More needs to be taught in school science curricula of the importance of beetles and entomological pursuits which need to attract more research funding. They are also of veterinary interest as a food source to wild animals.

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SHORT COMMUNICATION

River crabs (*Potamonautes unispinus*) in Zimbabwe – counts, behaviour & threats

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The current preliminary investigation studied the river crab (Fig. 1) and its life in urban streams in two vlei areas of Harare, Zimbabwe, namely Avondale Stream and Pomona Stream during the October rainy season. Both streams eventually drain into the Gwebi River.

The river crab [Kingdom: Animalia, Phylum: Arthropoda, Subphylum: Crustacea, Order: Decapoda, Suborder: Pleocyemata, Infraorder: Brachyura, Section: Eubrachyura, Subsection: Heterotremata, Superfamily: Potamoidea, Family: Potamonautidae] Potamonautes unispinus (Heterotremata: Potamonautidae) [crab (English), krap or kreef (Afrikaans), gakanje (chiShona) and umankala (Sindebele)] is migratory and emerges after the first rains. As the river flows it stimulates female crabs to move upstream. The Potamonautidae are a family of freshwater crabs and are widespread in tropical Africa. This family is made up of 18 genera and contains over 100 species. Fossil evidence suggests that the crabs evolved in the Late Miocene (Martin and Trautwein, 2003).

During dry spells crabs nocturnally migrate down stream beds. The benefits of migration include optimization of feeding, avoiding unfavourable physiological stressors, decolonisation of drought-lost territory, competition for space or cover, attenuated predation and disease. Dead grass and reeds are eaten in dambos. Suitable nursery areas are nurtured in the river. Intra-specific competition includes dispersal when newly submerged habitats emerge. Migration upstream may occur to avoid mammalian, avian and fish predators in perennial streams. Crabs are often seen in vlei streams and they scavenge for dead matter, eat insects, grasshoppers, worms, millipedes, small fish, etc. They burrow into the bank close to the water's edge where their claw marks can be observed. Locking its abdomen against the sternum makes it more mobile and protects the male genitalia. The female crab broods her fertilised eggs and larvae hatch in a protective sternal carapace as first crabs. Many small crabs later fall victim to predators, river flooding and pollution (Cooper, 2010). Crabs are covered with a hard chitinous calcified exoskeleton and have a single pair of claws and four pairs of legs. They have a pair of compound eyes and two antennae. Their body is divided up into the anterior cephalothorax and the posterior abdomen. A pair of maxillae draws a current of water over the gills for gaseous exchange. The gills also help in osmotic regulation. The mandible has a cutting edge. The crab has an open circulatory system (Van Rensburg et al., 1980). Crab leg articulation makes their sidelong gait more efficient. Despite male crabs fighting for territory, crab groups co-operate to provide food and protection for each other. Crabs can be caught by dangling a piece of meat or a millipede tied to a piece of string. The hungry crab grabs the bait and can then be pulled out of the water. Crabs may be kept in aquaria but are not easy to maintain as they may be cannibalistic (Cooper, 2008).

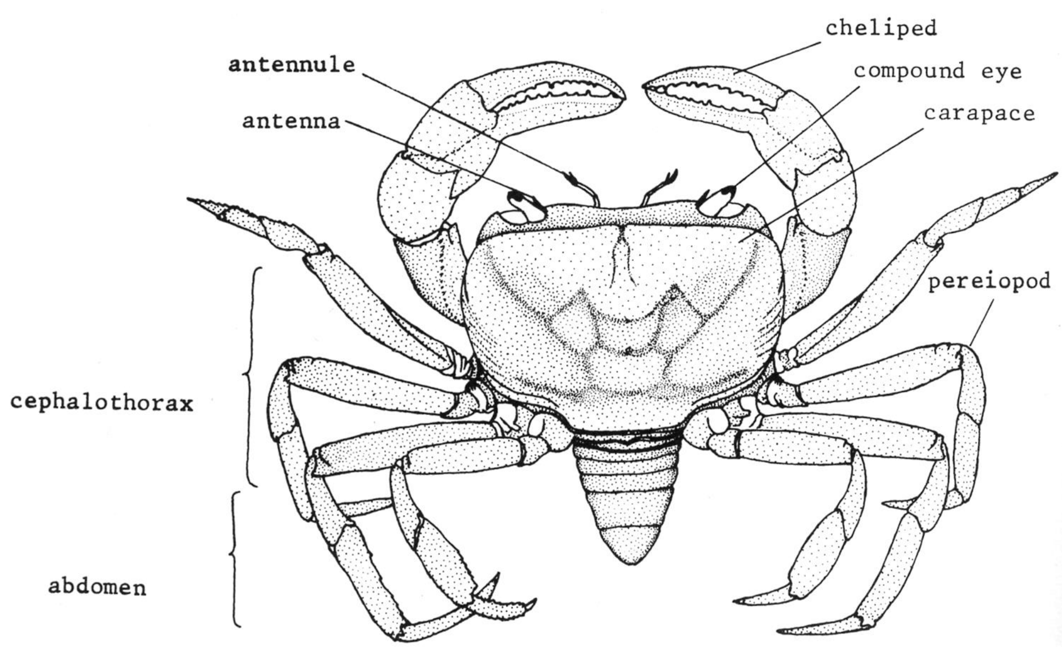

Figure 1 Schematic illustration of a river crab (ref. The Children's Illustrated Encyclopaedia of General Knowledge, 1957)

Figure 2 Polluted Avondale Stream – a large crab is in the centre

Figure 3 Pomona Stream water - silty

In the current study observations were made of crab numbers and the threats to their environment, including stream bank cultivation, pollution, overfishing and drought, were considered. The ambient temperature was 27°C, water at 10cm depth (21-23°C), R.H. 47% (Portable Electronic Weather Station with probe). Visual crab counts commenced from the bridges on Avondale West Road and Chatsworth Road 1 km into the vlei. Counts included 250 (Avondale) and 89 (Pomona). *En-route* stream bank cultivation of maize and pumpkins in hoed beds were observed: 34 (Avondale) and 12 (Pomona). The latter was attenuated due to marked flooding in the vlei. In some cases, the cultivation encroached too far on the bank resulting in parts of it collapsing causing the water to become rather muddy with the subsequent loss of silt. Pollution was especially noticeable in the Avondale Stream as the suburb, being part of greater Harare city, was fed by numerous storm drains, presumably along which plastics and other items of garbage were carried. Some sections had oil slicks on the water surface (Fig. 2).

There was significantly less pollution in the Pomona Stream, although it was silty from the clay soil (Fig.3). Greater biodiversity in this stream would assist crab migration during the rainy season through the puddles to other river tributaries and up stream in search of new territories. Their movement would contribute towards ecological niches and the crabs, being scavengers, would feed off dead fish (e.g. *Clarias gariepinus*) (Cooper, 2004) and other animals that they came across. The larger numbers of crabs in Avondale Stream were probably associated with more dead animals to feed off.

In conclusion, river crabs are an essential part of the food chain and the ultimate survivability of a riverine ecological niche. Where ever the conditions are suitable a crab will thrive and develop rapidly. Many river crabs prefer rocky streams and can migrate overland during the rainy season surviving as opportunistic scavengers and predators (Gratwicke, 2003). Dispersal of freshwater crabs within their natural habitats including rivers, streams, marshes and lakes occur due to their adaptation to fresh water involving direct development in the absence of free-living larval stages (Schmidt-Nielsen, 1997). Limitations to the dispersal of crabs include survivability out of water and avoidance of desiccation, particularly during drought. However, adverse human impacts via agriculture and pollution certainly have deleterious effects on river crab populations.

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OPINION

Termites (*Pseudacanthotermes*)

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Termites (order Isoptera) live (ca.8,000/m^2, total biomass: 1-5 to 22 g/m^2) in the tropical and sub-tropical regions (45-50º north and south of the Equator). Many feed on wood in various stages of decay, others eat herbivore dung, and others ear humus soil. They eat about one-third of the yearly dead wood, leaves and grass. Many are ecologically important as they bring minerals to the surface, increase soil aeration and moisture penetration, accentuate the recycling of vegetable matter, and provide food for mammals, birds and reptiles.

Each colony has one large white queen and a smaller king which are fed by the workers. The queen may live for 15 years and if removed the colony does not die. The queen is replaced. The workers also tend to the eggs and nymphs. The colony is guarded by soldiers (*Pseudacanthotermes militaris*), some of which can inflict a painful bite or powerful blows that can dismember ants in a single stroke. The Rhinotermitidae dwell in rotting trees and fallen branches. The Apicotermitinae nest underground and may dwell in the mounds of other termites. They recycle nutrients.

The Termitinae nest underground and make small mounds or live in other's mounds. The soldiers have biting or snapping mandibles. They feed on humus and wood. The Macrotermitinae cultivate fungus gardens in their nests and make mounds. The fungus gardens are made of spongy combs where fungi grow and provide the colony's food. The fungus, Termitomyces, breaks down faeces and termites eat it. Fungal hyphae are mixed with regurgitated food and fed to nymphs. Termites can survive the dry season by cultivating the fungus. They construct mud-covered tunnels to reach their food.

The Nasutitermitnae have soldiers with non-functional mandibles. Their heads are drawn out into snouts. Their frontal glands produce sticky and noxious chemicals that deter predators. They build small mounds and some are arboreal. Termites are extremely important ecologically for soil dynamics and provide food for countless animals and birds. The order, neatness and incredible complexity of a termite colony and the spectacular swarms of flying ants are fascinating. They may, however, be destructive due to their gnawing activities on untreated wood, furniture, books and

Termites (Macrotermes carbonarius). Photo Credit to Bernard DUPONT Wikimedia Creative Commons crops. They can also damage sugarcane, potatoes, yams and forests. Tree-resistant varieties, genetically engineered bacteria to express *Bacillus thuringiensis* endotoxins and fungal pathogens may be used to control termites. They are stimulated to emerge after the first rains in October-November. On earth the termites shed their wings and pair up. They may be attracted to street lights at night. They provide a rich feast for birds, chameleons, lizards, spiders and humans providing protein, energy, iron, phosphorus and calcium. Termites are also devoured by the aardvark and aardwolf. The former is exceedingly powerful and can use its claws to rip through the hardest termite mound. The latter lacks claws strong enough to dig into termite nests and is thus restricted to mopping up surface termites or digging them up in soft earth. Pangolins, armadillos, anteaters, sloth bears and echidnas also eat termites. Chimpanzees use sticks as tools to extract termites from their mounds. Some termites are eaten by other ants (all references).

As termites provide food for many animals they are therefore of Biological and Veterinary interest, and should be conserved.

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Guidelines for Authors – Veterinary Invertebrate Society Journal

Aims and Scope of Journal

The Veterinary Invertebrate Society Journal endeavours to be a point of reference for vets and academics for current and upcoming research into invertebrate medicine, welfare and conservation. There will be a focus on the practical application of veterinary principles and research to invertebrate species. This journal is an open access journal with no publication or submission fees. Prior to open publication, the journal is accessable by members of the Veterinary Invertebrate Society.

The journal accepts submissions of clinical case reports, original research papers, review articles and short communications.

Authorship

Authors should only be listed if they have made a significant contribution to the data and work involved in any submission. One author should be nominated as the corresponding author. The contact details of this author will be present alongside the published paper. As a rough guide contributors should have contributed at least 15% of the work to be an author or else should be recognized in the acknowledgements. Authors may be asked to produce a short biography for the membership to go alongside the article.

The journal has a philosophy of promoting young authors and researchers so welcomes submissions from university students.

Authors should disclose if they have any conflicts of interest in regard to data expressed or conclusions taken.

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The manuscript font should be “Ariel” of size 12 point with single spacing. Text should be aligned to the left. Formatting that may be used includes bold, italics, superscript and subscript. Avoidance of personal pronouns is expected (i.e. “We”, “I” and “Our”). American and British English may be used but it is expected that this be consistent throughout the manuscript. It is expected that submitted articles will be written in clear English and will be spell and grammar checked before submission.

The Harvard referencing style is used within this journal (e.g. Vega, F.E., 2008. Insect pathology and fungal endophytes. Journal of invertebrate pathology, 98(3), pp.277-279).

Abbreviations must be explained and elaborated on within the text. Numbers between one and nine should be expressed in words unless they are used with respect to units or mathematical symbols (e.g. 3 grams or 2%).

Articles should be submitted with a title page. This should include the manuscript title, authors and organisation affiliations of all authors.

Tables and figures should be referenced in the text and have a descriptive legend that can be understood alone.

Drugs should be stated by their generic names with the trade formulation in brackets with the manufacturer (e.g. meloxicam (Metacam, oral suspension for cats, Boehringer Ingelheim)). This nomenclature will also apply to diagnostic tests and laboratory equipment where appropriate.

Genus and species names should be italicised when scientific names are used. Due to the variable and dynamic taxonomic status of invertebrates, it is recommended to cite the species authority when discussing taxa as this will make it clear which species is being discussed.

Apart from opinion/end piece articles, all submissions should include a 250 word abstract.

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The maximum length of articles are expressed below.

Article Type	Word Length
Original Research Paper	3000
Opinion/End Piece Article	700
Short Communication	1000
Case Reports and Series	1500
Review Article	3000

The following articles types will be included in the journal:

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These articles are more informal and opinion-based though appropriate references are expected when factual discussion is undertaken. These articles are designed to inform the journal readership on a specific area within invertebrate medicine.

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Short communications should provide novel information on current research or clinical cases. They differ from original research papers in their scope and involvement. These submissions should typically involve some novel information or species which have not been reported on previously. New information should be placed in the context of existing literature. Headings should include: Short communication and references.

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Case reports will focus on a specific invertebrate species with a core clinical focus. This can involve a novel disease, treatment, diagnostic process or follow up of a previously reported presentation.

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A review should report on the current research and discussion around a specific disease or relevant clinical area. It is advisable that a specific invertebrate family or species is the focus of an article, as the entirety of invertebrates as a group can be diverse and challenging to discuss easily. Their format will be similar to original research papers

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