Provisional clinical chemistry parameters in the African Sharptooth catfish (*Clarias gariepinus*)

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ABSTRACT

Pollution affects aquatic systems worldwide and there is an urgent need for efficient monitoring. Fish are generally sensitive to their environment and are thus considered to be valuable bioindicator species. The African Sharptooth catfish (*Clarias gariepinus*) is particularly important in this respect because of its very wide distribution. In order to use *C. gariepinus* as a bioindicator species its baseline clinical chemistry must be defined. Existing data are scarce, and the objective of this work was therefore to establish clinical chemistry parameters for *C. gariepinus.* Blood was collected from male and female catfish and a number of clinical chemistry parameters were determined. Plasma protein values, but particularly those of plasma albumin, were found to be very low, approximately half the value for dogs, but similar to the values in Channel catfish (*Ictalurus punctatus*). Plasma urea values in Sharptooth catfish were found to be much lower than in dogs, but only marginally lower than in Channel catfish. Plasma creatinine in Sharptooth catfish, however, was only a quarter of that of dogs and one third of that found in Channel catfish. These findings may have implications for using urea and/or creatinine as an index of renal glomerular filtration, as is done in mammals. Plasma enzyme activity ranges were much lower in Sharptooth catfish than in dogs, particularly for alkaline phosphatase (ALP) and alanine aminotransferase (ALT). By comparison, Channel catfish have an even lower ALT activity range but an ALP range that is very similar to dogs. The implications for using these enzymes as markers for liver disease are not clear from these data, as factors such as plasma half-life and tissue distribution remain to be determined. The very low plasma thyroxine (T_4) levels have important implications for laboratory personnel, who will have to set up calibration and standardisation adaptations for the methods that are generally designed for human samples. Although the sample size was too small for reliable comparisons, it appeared that there was little difference in the parameters measured between male and female fish. The values obtained are a useful starting point for using *C. gariepinus* as a bioindicator species.

Key words: bioindicator, *Clarias gariepinus,* clinical chemistry, plasma enzymes, proteins, renal function, Sharptooth catfish, thyroxine, T_4.

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INTRODUCTION

Aquatic ecosystems are subject to pollution that may have dire effects[3,18]. Deleterious effects of pollution are often preceded by subtle molecular and cellular changes that can be detected in some inhabitants of these ecosystems[5,12,19]. Fish are suitable bioindicator animals because they respond with great sensitivity to changes in the aquatic environment[3,14,18,19].

The Sharptooth catfish (*Clarias gariepinus*) is one of the most important individual species in traditional freshwater fisheries in Africa[15]. It is widely distributed in Africa, where it occurs in almost any freshwater habitat, but favours floodplains, large sluggish rivers, lakes and dams[15]. The fish is omnivorous, feeding on fish, birds, frogs, small mammals, reptiles, snails, crabs and other invertebrates. It is also capable of feeding on seeds and fruit[15].

Numerous chemical pollutants have been detected in tissue samples from Sharptooth catfish collected in South Africa[7]. Anatomical and histological abnormalities of the reproductive organs have been observed in Sharptooth catfish collected from the Rietvlei and Marais Dams near Pretoria, South Africa[1]. These aberrations were similar to those attributed to chronic exposure to aquatic pollutants reported in fish in other parts of the world[2,18]. Controlled laboratory exposure of Sharptooth catfish to copper concentrations that were measured in the Olifants River, Kruger National Park, significantly altered their blood chemistry parameters[19].

Given its wide distribution, the African Sharptooth catfish could be a suitable bioindicator of pollution in a variety of freshwater aquatic ecosystems. However, to be able to detect molecular and cellular level changes in response to pollutants, prior knowledge of physiological baseline parameters is essential[5]. There is, unfortunately, a dearth of information on the clinical chemistry of the African Sharptooth catfish. The objective of this paper is, therefore, to define baseline clinical chemistry parameters of *C. gariepinus* that could be used as indicators of organ function or damage, to assess the impact of pollution in aquatic ecosystems.

MATERIALS AND METHODS

Experimental animals and facilities

Male ($n = 13$) and female ($n = 15$) African Sharptooth catfish (*Clarias gariepinus*) with body mass ranging from 600–1000 g were obtained from an earth dam at the University of Pretoria experimental farm. The fish were kept indoors in a 1500 tank containing municipal water. An external filter system was used to constantly circulate the water. The filter system was cleaned and at least a third of the water in the tank was replaced every week.

Commercial trout pellets were fed 3 times a week.

Collection of samples

After a depuration period of 1 month the fish were netted, removed from the holding tank and placed in a lateral position. Fish were not anesthetised for blood collection. Blood was collected from the central vein, located just below the spinal cord, caudal to the cloaca and using the lateral line as reference point. The blood was collected with heparinised (Heparin sodium-fresenius, Intramed) 2 m syringes (Omnifix 2 m , B Braun Melsungen AG) and 22 G needles (22GX1½′, Terumo).

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Table 1: **Summary of the Sharptooth catfish clinical chemistry data (95 % confidence and/or 2.5 to 97.5 % percentile reference ranges).**

		Approximate normal / Gaussian data			Non-normal data distribution or data unable to be normalised by log-transformation			Sexual difference		
Parameter	**Units**	**Lower 95 % CL**	**Mean**	**Upper 95 % CL**	**Lower 2.5 % percentile**	**Median**	**Upper 97.5 % percentile**	**Difference**	***P*-value *t*-test equal var.**	***P*-value Willcoxon Rank**
Length	mm				430	550	660	NS		0.190
TPP	g/	30.8	46.2	61.6				NS	0.799	
ALB	g/	8.1	12.35	16.7				Sig	0.036	
GLOB	g/	21.8	33.1	44.4				NS	0.670	
A/G ratio		0.21	0.37	0.54				Sig	0.014	
ALT	U/	0	12.7	29				NS	0.345	
ALP	U/	0.1	2.3	52				NS	0.849	
AST	U/				0	117	285	NS		0.700
CK	U/	200	2370	28052				NS	0.888	
GGT	U/	0	6.6	15.0				Sig	0.004	
LD	U/				0	450	878	NS		0.959
HBD	U/				0	131	306	NS		0.597
GLD	U/				0	17	55	NS		1.000
T Bil	µmol/				4.4	16.4	38.7	NS		0.919
Urea	mmol/	0.3	1.9	3.4				NS	0.442	
Creat	µmol/				0.0	20.5	29.0	NS		0.643
T_4	nmol/				0.0	4.5	7.5	NS		0.642

NS = no significant difference between male and female group data ($P \geq 0.05$).
Sig = significant difference between male and female group data ($P < 0.05$).

The blood was transferred to lithium heparin blood collection tubes (BD Vacutainer, Becton, Dickinson and Company) immediately after collection. Blood was centrifuged at 1500 g, the plasma collected and frozen in sterile vials (1.8 m cryotube vials, Nunc A/S). Samples were submitted to the Clinical Pathology Laboratory, Faculty of Veterinary Science, University of Pretoria for analysis.

Clinical chemistry

The NExCT™ clinical chemistry system (Schiapparelli Biosysytems) was used to quantify total plasma protein (TPP) (Biuret principle of Weichselbaum), total bilirubin (T Bil) (van den Bergh diazo principle), albumin (ALB) (BCG dye-binding method) and urea (enzymatic UV method) using NExCT™ reagent kits. Creatinine (kinetic Jaffe method), alkaline phosphatase (ALP) (pNP substrate and AMP buffer), aspartate aminotransferase (AST) (IFCC recommended kinetic UV method) and γ-glutamyltransferase (GGT) (optimised kinetic method of Szaz) were determined using ACE™ reagent kits and the ACE™ clinical chemistry system. Lactate dehydrogenase (LD) (optimised DGKC recommended method), alanine aminotransferase (ALT) (IFCC recommended kinetic UV method) and creatine kinase (CK) (NAC-activated DGKC recommended method) were determined by the Alfa Wassermann clinical chemistry systems (ACE® & NExCT™) using Alfa Wassermann reagents. Glutamate dehydrogenase (GLD) was quantified using Randox reagents (Randox Laboratories) (optimised DGKC recommended method). α-Hydroxybutyrate dehydrogenase (HBD) was determined on an automated clinical chemistry analyser (Roche/Hitachi Modular Analytics) (optimised DGKC recommended method). All enzyme activity assay results were obtained at 37 °C. Total thyroxine (T_4) was determined using a Coat-A-Count Total T_4 solid phase ^{125}Iodine Radioimmunoassay kit (Siemens Medical Solutions Diagnostics). Radioactivity was measured by an Auto-Gamma 5000 Series Gamma Counting system. Owing to sample quality and/or availability, only 15 sera (8 from male fish sand 7 from female fish) were assayed.

Statistical treatment of data

Using the statistical software package, NCSS 2000 Statistical System for Windows (Copyright J L Hintze, Kaysville, Utah, USA, 1998), the following statistical procedures were conducted:

- All data were inspected for outlier values by both the National Committee for Clinical Laboratory Science (NCCLS) recommended Dixon algorithm[8] as well as Horn's algorithm based on the Box-Cox transformation[16]. Only data detected as 'outliers' by both methods were then defined as true outliers. The data distributions were tested for 'normality' (or approximation to a Gaussian distribution).
- Where the distribution did not pass the test for normality (by exceeding the allowable D'Agostino limit for skewness), the data were subjected to a logarithmic transformation and the transformed data re-tested for normality.
- Where the logarithmic transformation did not yield a good approximation of normality or when the D'Agostino criterion for kurtosis was exceeded, non-parametric statistical methods (*vide infra*) were then applied to those data.
- The reference interval (range) for those data that met the criteria for approximate normality (both untransformed as well as log-transformed) was based on the 95 % confidence limit for 26 observations (mean ± 2.056 SD).
- The reference intervals (ranges) for data that were non-normal (even after log-transformation) were calculated according to the non-parametric method of Rumke and Bezemer[13], using the 2.5 % and 97.5 % percentile approximation.
- In order to determine whether there was a significant difference between male and female fish data for the various parameters, a Student's *t*-test comparison was performed on approximately 'normal' data and the non-parametric Wilcoxon Rank-Sum Test was used on 'non-normal' data.

RESULTS

Laboratory results

The clinical chemistry results of the Sharptooth catfish are summarised in Table 1 and also compared, in Table 2, with data from the Channel catfish (*Ictalurus punctatus*) and the domestic

Table 2: **Clinical chemistry data of the Sharptooth catfish compared with data from the Channel catfish and domestic dog.**

Parameter	Units	Recommended reference range in Sharptooth catfish			95 % confidence range in 22 acclimated Channel catfish[4] Derived from mean ± 2 SD			Reference range in domestic dogs[11]		
		Lower cutoff	Mean	Upper cutoff	Lower cutoff	Mean	Upper cutoff	Lower cutoff	Mid-range value	Upper cutoff
TPP	g/	34.4	46.2	58.0	22	34	46	54	64	75
ALB	g/	9.04	12.35	15.65	6	10	14	23	27	31
ALT	U/	0.1	12.7	25.2	0.0	2.6	5.8	10	60	109
ALP	U/	0.2	2.3	25.1	0.0	66	134	1	58	114
AST	U/	0	117	285	0.0	91	193	13	14	15
CK	U/	355	2370	15811	0.0	3156	6565	52	210	368
GGT	U/	0.0	6.6	13.2	0.0	2.4	6.4			
LD	U/	0	450	878	0.0	177	499	0	118	236
T Bil	µmol/	4.4	16.4	38.7	0.0	3.4	6.8	0.0	2.6	5.1
Urea	mmol/	0.6	1.9	3.1	1.39	2.96	4.53	2.9	6.5	10.0
Creat	µmol/	0.0	20.5	29.0	0.0	61.8	132.6	44.2	97.3	150.3
T_4	nmol/	0.0	4.5	8.8	15.6	25.4	35.2[4]	19.3	35.4	51.5

dog. Table 3 records the data for the plasma chemistry constituents where a statistically significant difference between the sexes was found.

Outlier rejection

The inspection of data for outlier data values revealed that:

- A female fish (B02) was very significantly different from the other fish in the study (being considerably smaller with a length of 24.7 cm, compared with the 95 % reference range of 38.1–69.8 cm). Furthermore, this fish had outlier values for plasma proteins (albumin and globulin) and total bilirubin as well as zero value results for ALT, ALP, AST, LD, HBD and creatinine. On the basis of these findings, this fish's data were excluded from the data used for further statistical treatment.
- A female fish (B31) had outlier values for ALT, AST, LD and HBD as well as a zero value for GGT. It was decided that this fish was probably not 'normal' (*i.e.* normal in terms of health) and should not be included in the calculation of reference ranges that purported to reflect clinical chemistry data in healthy catfish.
- Furthermore, the globulin value of Fish B01 and GGT value of Fish B11 were identified as outliers (by both the Dixon as well as Box-Cox approaches) and these data items were excluded from further statistical calculations. However, the rest of the data from these fish were retained in the data set.
- This left the data set with 13 male fish as well as 13 female fish with just 2 additional data points deleted (a globulin value in a male fish and a GGT value in a female fish).

Data distributions

Using the d'Agostino criteria for skewness and kurtosis, ALP, AST, CK, LD, HBD, GLD and T Bil were found to be too severely skewed to be accepted as 'approximately normal'. In addition, length, creatinine and T_4 were found to show more kurtosis than would be acceptable. Log-transformations were applied to the skewed data and ALP and CK were found to transform sufficiently well to approximate normality. Those 2 parameters were, thereafter, manipulated in their log-transformed state for statistical analysis and only the final data were transformed back to non-log (non-exponential) data. Consequently, length, AST, LD, HBD, GLD, T Bil, creatinine and T_4 data were handled by non-parametric statistical approaches.

Reference ranges

Table 1 reflects the 95 % confidence and/or 2.5 to 97.5 % percentile reference ranges computed by the methods described above.

DISCUSSION

Outliers

Although the view exists that outlier data derived from clinically normal subjects should be retained in a dataset that is intended to reflect the 'normal' or 'healthy' reference range, the NCCLS has accepted that it is often not possible to identify sub-clinical illness or pathology[9] and therefore recommends the elimination of outliers (using appropriate statistical techniques, *vide supra*) if their inclusion could lead to establishing a reference range that loses its diagnostic sensitivity through being too 'wide'. This is particularly a threat when dealing with relatively small numbers of subjects in the sample from the population, as is the case here. The NCCLS recommendation is that at least 120 subjects should be included in the sample. In this study only 28 fish were used and the authors accept that, as a consequence, the derived reference ranges can, at present, only be provisional and that, in time, they will be re-evaluated through the accumulation of more data from healthy catfish. In the interim, however, the authors believe that the proposed (derived) reference ranges will provide a useful set of criteria to assist in using the catfish as a bioindicator species.

Table 3: **Data for the plasma chemistry constituents where a statistically significant difference between the sexes was found (ALB, A/G ratio and GGT).**

Parameter	Units	Reference range for all fish in this study (both sexes)			95 % Confidence data for male Sharptooth catfish			95 % Confidence data for female Sharptooth catfish		
		Lower 95 % CL	Mean	Upper 95 % CL	Lower 95 % CL	Mean	Upper 95 % CL	Lower 95 % CL	Mean	Upper 95 % CL
ALB	g/	8.1	12.35	16.7	10.5	11.5	12.5	11.9	13.2	14.5
A/G ratio		0.21	0.37	0.54	0.30	0.34	0.38	0.37	0.41	0.46
GGT	U/	0.0	6.6	15.0	7.5	8.9	10.2	1.5	4.3	7.0

The elimination of the 1st female fish (B02) from the study can be justified on the basis that this was a very small specimen compared with the other fish (being only half the average length). Whether this fish's small size was attributable to age or disease is not known (it did not appear to be clinically ill and no age determinations were made). Furthermore, the finding that this fish had the lowest TPP, albumin and globulin values and zero values for 5 plasma enzyme activities as well as the creatinine concentration, constitute reasonable grounds for believing that this specimen was not a typical member of an otherwise reasonably homogeneous group of healthy fish.

The elimination of the other female fish (B31) is justified on the basis that it represented 4 of the 7 identified 'outlier' parameter values in the dataset. These 4 relatively high plasma enzyme values constitute reasonable grounds for believing that, although it was not clinically ill, this fish may have harboured a subclinical illness.

Reference ranges

The authors could find no previous data on clinical chemistry reference ranges (with the exception of T_4, see below) for this species and therefore it is difficult to comment on whether these data meet any specific expectations.

A somewhat disturbing feature is the number of zero activity results for enzyme data. With ALT, ALP and GGT this is perhaps not surprising as the ranges are quite low and the modal values are not far from zero (20, 8 and 9 U/ , respectively). However, it is surprising to find zero values for AST, LD and HBD, as the ranges are set much higher and the modal values are, relatively speaking, very high (316, 630 and 250 U/ , respectively). In addition, the finding of zero enzyme activity values appears to recur within the same fish (plasma sample) and could suggest that there may have been some sample or sample-handling effect. Illustrating this is the finding that each zero activity in LDH (and there are 4 such results) is correlated with at least 2 other enzyme zero activity results. In the 30-year laboratory experience of one of the authors (FR), this is a very unusual finding and could suggest the presence of inhibitors or analytical problems. This reservation should be borne in mind when using these recommended reference ranges for *C. gariepinus,* because low plasma enzyme activities may not bear any relationship to pathology. High enzyme activities, however, would nevertheless suggest increased release from damaged cells, increased enzyme induction or delayed removal.

T_4 levels were very much lower in this study than those reported in the same species[6] during the breeding phase. However, they were similar to, but still lower than, the values found during the quiescent phase in the previous study.

Reports on clinical chemistry parameters in other species of fish include a limited number on catfish, although not *Clarias* spp. One study, on Channel catfish (*Ictalurus punctatus*)[4] is possibly the most substantial in terms of range of chemistry parameters as well as number of fish sampled. When the data on Channel catfish from that publication are compared with the data in this study, there are some very striking differences and similarities. When, in addition, these data from fish are compared with those from domestic mammals (the canine range being used for illustrative purposes), there are further striking issues that beg to be addressed in future studies. These data are reflected in Table 2.

Sharptooth catfish appear to have a slightly higher TPP range than Channel catfish (differing by some 10 to 12 g/). Compared with most mammals, however, this range is still 10 to 20 g/ lower. The difference between the 2 species of catfish appears to lie in the globulin level, as the fish do not differ substantially in terms of ALB. The ALB ranges found in both Sharptooth catfish and Channel catfish are substantially lower than that found in mammals (6 to 15 g/ compared with 23 to 30 g/). In mammals a concentration below 15 g/ is consistent with fluid loss from the plasma into body cavities (such as ascitic transudate) and interstitium (oedema). However, it is well-known that plasma protein concentrations in fish are lower than in higher vertebrates[5].

A striking difference in clinical chemistry parameters between these 2 species of catfish and dogs relates to the renally cleared nitrogenous waste products, plasma urea and creatinine. The catfish urea values appear to lie around the 1 to 5 mmol/ level, whereas mammals tend to have plasma urea values in the 3 to 10 mmol/ range. Plasma urea is a fair and commonly used indicator of glomerular filtration (GFR) in dogs[11] but could be a relatively poor (insensitive) index in Sharptooth catfish. Ip and co-workers[10] contended in an intriguing study that ammonia detoxification in *C. gariepinus* does not follow the classical 'urea cycle' (Krebbs Hensleit cycle) and that instead *C. gariepinus* 'actively excretes' ammonia. The literature in this regard, however, appears somewhat contradictory, according to these authors, and reports on urea cycle enzyme activity in related species such as *C. batrachus* are not always in agreement, with specimens from India studied apparently having a functional urea cycle[10].

The level of plasma creatinine, a sensitive and reliable index of GFR in mammals[11], suggests that it may not be as useful in Sharptooth catfish. However, Channel catfish appear to have a serum creatinine range that is similar to that found in mammals and it is reasonable to assume that, in that species, it would be an equally useful index of GFR. In fish creatine predominates over creatinine. Creatine is excreted through the kidneys, not the gills, and forms more than half of the urinary nitrogen excretion. It should be mentioned that GFR in fish is affected by the salinity of the water in which the fish are kept, which would affect the usefulness of creatine as an index of GFR.

Although there are considerable differences in the plasma enzyme activities between Sharptooth catfish and Channel catfish, it is difficult to evaluate their potential usefulness (in either species) as appropriate markers of tissue (organ) damage. In mammals, an enzyme such as Sorbitol (Iditol) dehydrogenase can reflect very severe hepatic pathology when it is elevated above 5 U/ [17], whereas CK in cattle for instance, can rise to values in the thousands (even tens of thousands) as a marker of myopathy. This demonstrates that the actual numeric value of plasma enzyme activity is very poorly correlated with the degree of tissue damage. Consequently, the fact that plasma ALT in Channel catfish is approximately one third to one fourth of that found in Sharptooth catfish and the plasma ALT activity in the latter species is about 20 % of what is commonly encountered in carnivores, may not offer any useful information about the potential usefulness of this enzyme as a marker of hepatocellular pathology. In mammals, the transaminases ALT and AST as well as the bile-duct-associated ALP and GGT tend to be useful markers of hepatocellular pathology and biliary pathology respectively[11]. There are, however, important differences in their respective sensitivity. ALT is more liver specific than AST in carnivores than it is in ruminants, while plasma AST activity is almost the opposite, being more sensitive in ruminants. This difference is probably related to the difference in the preferred metabolic pathway for gluconeogenesis between carnivores and ruminants. The ability to anticipate the relative usefulness of a serum enzyme activity is confounded by another factor, namely its plasma half-life. A good illustration of this is seen in carnivores where, in dogs, the half-life is long

enough (2 to 3 days) to allow ALT to sensitively reflect, among other conditions, hepatobilliary pathology. In cats, however, the half-life is only about 6 hours, and consequently the plasma activity only reflects very severe and sustained release (severe, ongoing pathology) but is generally insensitive to milder pathology[11]. The very substantial difference in ALP activity between Channel and Sharptooth catfish may therefore reveal very little about its relative sensitivity as a marker of hepatobilliary pathology.

The above observations imply that, over and above the establishment of reference ranges (the focus of this study), an additional aspect of plasma clinical chemistry in catfish, especially in relation to enzyme activity, would involve studies on tissue activity and plasma half-lives as well as empirical studies of enzyme activities during or associated with specific, known organ pathology.

T_4 levels were one-ninth of those found in dogs. As dogs already have relatively low levels when compared with humans, this places a special responsibility on investigators to inform the laboratory of this fact so that appropriate calibration and standardisation adjustments can be made to the assay method (generally developed for human samples) to avoid invalid results.

Sex differences

Statistically significant differences between the sexes were found for some of the plasma chemistry constituents (Table 3; see also Table 1 for the *P*-values). However, the use of only 13 specimens per sex raises doubt as to whether these data accurately represent sex-specific reference ranges. The finding that, of the 16 clinical chemistry parameters, only ALB and its derived A/G ratio as well as GGT showed statistically significant sex differences and that these are relatively small (Table 3) suggests that, until more data are available, the use of a single reference range for both sexes may be a prudent and reasonable approach.

CONCLUSIONS

The results from this study may serve as an important starting point for other studies but may also, in the short term, prove to be of value in monitoring the health of farmed African Sharptooth catfish. In this case, these parameters could also be used as biomarkers to assess the impact of pollution in their aquatic system.

In addition, the data from this study were all obtained from fish that had been kept in fairly stable conditions. In the study on Channel catfish referred to above, which compared serum analytes of normal and acutely stressed channel catfish[4], it was reported[4] that laboratory-acclimatised and production pond catfish exhibited differences in most serum chemistry parameters measured. The authors concluded that perceived normal serum chemistry ranges are influenced by the diet, season, and environmental stress. It is likely that this applies to *C. gariepinus* as well, and it is therefore important that future studies on Sharptooth catfish clinical chemistry also attempt to define the effect of age, growth stage, season and environmental conditions in this species to improve the usefulness of clinical chemistry parameters when this fish species is used as a bioindicator.

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REFERENCES

1. Barnhoorn I E, Bornman M S, Pieterse G M 1999 Histological evidence of intersex in feral sharptooth catfish (*Clarias gariepinus*) from an estrogen-polluted water source in Gauteng, South Africa. *Environmental Toxicology* 19: 603–608
2. Colborn T, Dumanoski D, Myers J P 1996 *Our Stolen Future.* Dutton Books, New York
3. Colborn T, Thayer K 2000 Aquatic ecosystems: harbinger of endocrine disruption. *Ecological Applications* 10: 949–957
4. Ellsaesser C F, Clem L W 1987 Blood serum chemistry measurements of normal and acutely stressed catfish. *Comparative Biochemistry & Physiology.* Part A: *Comparative Physiology* 88: 589–594
5. Evans D H, Claiborne J B 2006 *Physiology of fishes.* CRC Press Taylor & Francis Group, Boca Raton, Florida
6. Gupta B B P, Premabati Y 2002 Differential effects of melatonin on plasma levels of thyroxine and triiodothyronine levels in the air-breathing fish, *Clarias gariepinus,* during breeding and quiescent periods. *General and Comparative Endocrinology* 129: 146–151
7. Heath R G M, Claassen M 1999 An overview of the pesticide and metal levels present in populations of the larger indigenous fish species of selected South African rivers. *Water Research Commission Report,* #428/1/99
8. Horn P S, Feng L, Li Y, Pesce A J 2001 Effect of outliers and nonhealthy individuals on reference interval estimation. *Clinical Chemistry* 47: 2137–2145
9. Horn P S, Pesce A J 2003 Reference intervals: an update. *Clinica Chimica Acta* 334: 5–23
10. Ip Y K, Subaidah R M, Liew P C, Loong A M, Hiong K C, Wong W P, Chew S F 2004 African Sharptooth catfish *Clarias gariepinus* does not detoxify ammonia to urea or amino acids but actively excretes ammonia during exposure to environmental ammonia. *Physiological and Biochemical Zoology* 77: 242–254
11. Latimer K S, Mahaffey E A, Prasse K W 2003 *Duncan and Prasses's veterinary laboratory medicine* (4th edn). Iowa State University Press
12. Rodriguez-Cea A, Linde Arias A R, Fernandez de la Campa M R, Costa Moreira J, Sanz-Mendel A 2006 Metal speciation of metallothionein in white sea catfish, *Netuma barba* and pearl cichlid, *Geophagus brasiliensis,* by orthogonal liquid chromatography coupled to ICP-MS detection. *Talanta* 69: 963–969
13. Rumke C L, Bezemer P D 1972 Method for the determination of normal values. II. New method. *Nederlands Tijdschrift voor Geneeskunde* 116: 1559–1568
14. Siroka Z, Drastichova D 2004 Biochemical markers of aquatic environment contamination-Cytochrome P450 in fish. A review. *Acta Veterinaria Brno* 73: 123–132
15. Skelton P, 2001 *A complete guide to the freshwater fishes of southern Africa.* Southern Book Publishers, Halfway House
16. Solberg E H, Lahti A 2005 Detection of outliers in reference distributions: performance of Horn's algorithm. *Clinical Chemistry* 51: 2326–2332
17. Travlos G S, Morris R W, Elwell M R, Duke A, Rosenblum S, Thompson M B 1996 Frequency and relationships of clinical chemistry and liver and kidney histopathology findings in 13-week toxicity studies in rats. *Toxicology* 107: 17–29
18. Van der Oost R, Beyer J, Vermeulen N P E 2003 Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology* 13: 57–149
19. Van Vuren J H J, Van der Merwe M, Du Preez H H 1994 The effect of copper on the blood chemistry of *Clarias gariepinus* (Clariidae). *Ecotoxicology and Environmental Safety* 29: 187–199