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Research Article

Estimating the Effects of Cyanide Level on Freshwater Fishes Using Landmark-Based Morphometrics Analysis

Julie S Berame*, Rex M Andante and Rex Bomvet D Saura

College of Education, Biology Department, Caraga State University, Philippines

***Corresponding Author:** Julie S Berame, College of Education, Biology Department, Caraga, State University, Philippines.

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Abstract

The study aimed to determine the presence of cyanide levels of freshwater fishes in *Channa striata*, *Oreochromis niloticus* and *Clarias batrachus*, and described the allometry and sex dimorphism of *Chevron Snakehead.* The physicochemical parameter levels of Cyanide, Copper, Lead, Zinc and Iron in nine (9) stream tributaries of Agusan Marsh was evaluated and compared to the DENR freshwater quality standards. The research employed Cyanide Detection Tests, length-weight relationship, land-marking and relative warp analysis using tps software and PAST-Hotellings test. The result revealed that 82% were positive in cyanide, found in all two (2) samples *O. niloticus*, in *C. batrachus* four (4) is positive only one is negative, three (3) *C. striata*, were positive and only one (1) is negative. Negative allometric were observed to 15 male and 15 female *Channa striata* showed b value is 1.0798, strong positive correlation with r^2 = 0.8908 and significant with p=0.00001. No shape variation among male and female C. *striata*. Hotelling's test where T^2 = 6.168, F = 1.1774, P = 1, means no significant sexual dimorphism for the body shape of the *C. striata*. Moreover, lead, copper, zinc and iron and cyanide in water revealed in detectable levels and physico-chemical water quality of the streams were affected. Thus, landmark based geometric analysis were useful in describing sexual dimorphisms among fish species. In addition, freshwater fishes were sensitive and susceptible to bio accumulation of cyanide and growth affected by the water quality.

Keywords: Cyanide Levels; Sex Dimorphism; Allometry; Landmark-Based Geometric Morphometrics; Physico- Chemical; Freshwater Fishes

Introduction

Caraga Region have soundly ecosystem dome for organisms to live. It is considered as one of the bio-diversified areas and abundant in fishes and endemic species of amphibians and reptiles [1]. Pertaining to industrial mining, Caraga Region, Philippines has several registered large scale mining companies based on the data from Mines and Geosciences Bureau-Surigao as of 2015, not only large scale mining but active small scale mining operations also existed region wide. Since 1970' to harvest gold but a minute sized amount of cyanide can be deadly to humans and even smaller amounts can be lethal to fishes once contaminated water [2]. Impact of cyanide to aquatic organisms like fish lost its equilibrium with too much mucous discharge on its gill filaments and skin [3,28]. Increase water temperature, minor reduction of pH and dissolved oxygen, disturbs fitness and stress to fishes [4,5,6]. Wildlife death likely occurs near mines processing copper gold due to formations of copper-cyanide complexes which is toxic even in birds and bats [7]. In general, water pollutants like decayed plants can affects fish body growth and shape because of biological oxygen demand in decomposition process lowers dissolved oxygen level in water resulting fish ability to survive. Similarly fish accumulation of toxins can be transfer to other trophic level as they are eaten [8]. Cyanide instigated severe initial suppression of specific growing proportion [9]. American study in lake fishes contaminated with metals was impaired sense of smell, resulting to lessen from escaping predator that the problem could distress fish density [10]. Furthermore, cases of cyanide leak from mining in Bunawan, Agusan Del Sur had been reported in the newspaper [11]. Thus, this study was conducted and evaluated the presence of cyanide in water and three (3) selected common fish commodity such as Chevron Snakehead (*Channa striata*), Nile Tilapia (*Oreochromis niloticus*) and catfish (*Clarias batrachus*) including base metals and its physicochemical analysis in nine (9) selected stream tributaries of Agusan Rivers. In addition, this study attempted to described

the morphology of the Chevron Snakehead (*Channa striata*) as to allometry and compare its shape variations in relation to their sex differences using geometric morphometric landmark based using morphometrics software. This provide significant information on the occurrence of four base metals, cyanide and water quality of stream tributaries of Agusan Rivers and accumulation of cyanide in the fish tissue of Chevron snakehead, Nile tilapia, and catfish samples which included the allometry and body shape variation of *Channa striata* relative sex differences and provide awareness for the environmental and public health sectors, mining companies and local government for monitoring and formulation of policy and regulation to minimize the entry of toxic pollutants into water bodies.

Materials and Methods

Study area

The study was conducted in the Municipality of Bunawan, Agusan Del Sur, Philippines in academic year 2017-2018 shown in figure 1 with their respective coordinates.

Figure 1: Map of study stations with coordinates in Bunawan, Agusan Del Norte.

The ten (10) sampling stations for water samples were Magsagangsang Creek, Lucad Creek, Simulao-lower stream, Bunawan Brook River, Saugon creek, Bagnan creek, Simulao River-upper stream, Singanan Creek, Koo Creek and Masapia falls. The water samples were collected and tested in four (4) coordinated areas per sampling station.

Collection and sampling procedures for water analysis

Collection procedures for water samples was adopted and modified sampling [8,20]. The water samples were collected through depth of 1-50 cm of the water column of each sampling sites. About 0.5 L of water samples only was collected in each stations using graduated cylinder, a total of ten (10) bottles with 0.5 L water samples each with only one replicate. The graduated cylinder will be rinsed with distilled water four times before using over again. The samples were carefully transferred to the prepared reused 0.5 L PET (polyethylene terephthalate) plastic bottles of nature spring to avoid bubbles formation. The bottles was sealed tightly and labeled with information as to area, station, and date of collection and photograph for documentation. Then all samples were placed in the bucket with splitter and ice for preservation.

Collection, data gathering and preparation for fish biota samples

Freshwater fish samples such as Nile Tilapia, chevron snakehead and Catfish was obtained from the local fisher folks Bunawan, Agusan Del Sur. About 1.3 kilogram of each fish types was obtained as prescribed by the BFAR 13 for cyanide analysis and another thirty (30) samples of Chevron snakehead for landmark based geometric morphometric analysis were obtained. All Fish samples were washed with tap water then after were rinsed with distilled water. The weight of each fish samples of three (3) species was determined using digital weighing scale. All samples for cyanide analysis were subject to photograph for documentation using iPhone 5s camera.

Hereafter, the fresh samples of fish was placed in a zip lock and labeled with information as to area, station, and date of collection and placed in the bucket with ice and frozen. Moreover, the thirty (30) Chevron snakehead sample collected for geometric morphometric analysis were separately placed in a bucket with ice for geometric morphometric analysis.

Analytical procedure for cyanide and base metals. The collected water samples were immediately brought to Philsaga Mining Corporation, Assay Laboratory for Cyanide and Base metals Analysis. The prepared fish samples were delivered to Philippine Bureau of Fisheries and Aquatic Resources (BFAR) Central Office-Manila thru

BFAR Caraga for the cyanide detection and analytical procedure. The physico- chemical analyses were done using the Hach HQ40D.

Fish sample preparation for Land-marking

The prepared fish samples the length of each fish samples was determined by measuring the fish from its snout down to the caudal fin using tape measure and weighed using digital weighing scale. The length and weight were recorded. The individual fish was applied with 10% formaldehyde to harden the fins and manual manipulation was done to make it more visible and wider. The left flank of each fish taken using cannon eos 1200 D for land-marking. Samples was dissected individually to determine the sexes and the sorted as to male and female.

Land-marking selection and digitization

The images of fish sample of each sex were sorted and replicated into two (2). Then the images were subjected to tpsUtil64 to create tps files images for digitization. A total of 16 landmarks were digitized using tpsDig2 software. The location of the landmarks and anatomical descriptions of each sex were represented in figure 2 and table 1. Assigning landmark were based on stickleback fish model of Anyfish.com.

Figure 2: Landmark points of female (A) and male (B) *Channa striata*.

Shape analysis

The saved files of digitized samples were analyzed through TPS relative warp where Relative warp score and deformation grid and PCA retrieved. Further analysis for sexual dimorphism was done using PAST software for the discriminant analysis and generations of histogram and discriminant plot.

Coordinates	Location
1	Snout Tip
2	End of head length
3	anterior junction of the dorsal fins
4	Posterior junction of the dorsal fins
5	Dorsal insertion of caudal fin
6	Midpoint of caudal border of hypural plate
7	Ventral insertion of caudal fin
8	Posterior insertion of anal fin
9	Anterior insertion of anal fin
10	Dorsal base of pelvic fin
11	anterior border of ectocoracoid bon
12	anterior border of ectocoracoid bon
13	Anterior margin through midline of orbit
14	Posterior margin through midline of orbit
15	Dorsal end of operculum
16	Dorsal base of pectoral fin

Table 1: Description of the landmark points in the body shape of *Channa striata*.

Data analysis and consolidation of data collected

Ecological aspect of the sampling was described through ocular and observation to the ecological profile of the sampling sites. The allometry determination was determined using the equation W=aL b (Pauly, 1983). At

where:

W= Weight of fish (g)

L = Total length of fish (cm)

a = Constant (intercept)

b = The length exponent (slope)

The result was interpreted using linear regression values [12] presented in table 2 and Pearson Correlation was used for r2 values and coefficient of determination with their corresponding interpretation shown in table 3. Furthermore, the results of the cyanide analysis was analyzed as negative or positive only and the concentration of cyanide in water of each sampling were compare only if it is above or below to the maximum contamination level.

Results and Discussion

The sampling area were surrounded of agricultural land like rice fields, rubber tree and falcate plantation, compose of shrub to trees and coconut. East areas of Bunawan observed high to low range of mountains with perceived landscape of mining activities and during the actual survey fishing were observed. The fish caught by local fisher folks either for family consumption.

b value	Interpretation
3.0	Isometric growth- no change of body shape as an organism grow
<3.0	Negative Allometric- body shape slenderer as it increases in length
>3.0	Positive Allometric- - body shape relatively stouter as it increases in length

Table 2: Corresponding interpretation of the b values for linear regression (Riedel., *et al*, 2007) b value Interpretation.

Coefficient of Determination (r^2)	Interpretation
+.70 or higher	Very strong positive relationship
+.40 to +.69	Strong positive relationship
+.30 to +.39	Moderate positive relationship
+.20 to +.29	weak positive relationship
+.01 to +.19	No or negligible relationship
0	No relationship [zero order correlation]
-.01 to -.19	No or negligible relationship
-.20 to -.29	weak negative relationship
-.30 to -.39	Moderate negative relationship
-.40 to -.69	Strong negative relationship
-.70 or higher	Very strong negative relationship

Table 3: The values of (r2) coefficient of determination with their corresponding interpretation.

Allometry

The thirty (30) individual samples of chevron snakehead (*Channa striata*) were sorted by sexes and measured the total length and then weighed and recorded.

The length ranged from 26.0 cm – 37.5 cm with 32.6 cm average mean for male while female ranged from 29.0 cm to 35.5 cm with 32.3 cm average mean. The weight ranged from 218.66 g - 315.37 g with 273.7 g average mean for male while 243.89 g – 320.55 g for female with 284.2 g average mean.

The results of Length- weight relationship of fifteen (15) male and 15 female *Channa striata* collected in the month of October were and presented in graph as shown in figure 3 Furthermore, the length and weight relationship of combined sexes of *Channa striata* were determined as shown in figure 4. The graph shown that b value result is 1.0798 which means negative allometric, correlation coefficient or r2 is 0.8908 means strong positive correlation, p value is 0.00001 which means that there is significant association of length of the collected samples.

Figure 3: Split Scattered Plot Diagram of Log Total Length and Log Total weight of Male and Female *Channa striata*.

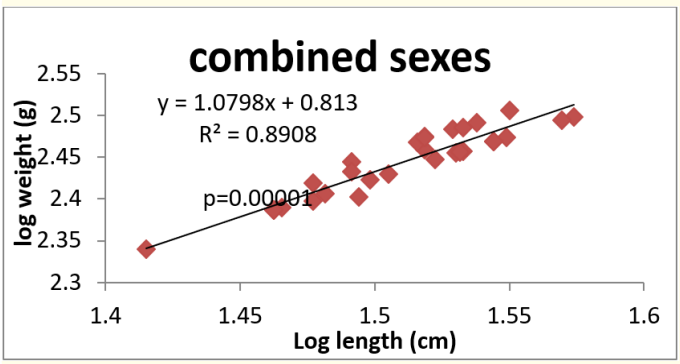

Figure 4: Scattered Plot Diagram of Log Total Length and Log Total weight of *Channa striata.*

In general, all samples of *Channa striata* data revealed negative allometric which means that the growth of fish become slender as it is increase in length. The correlations coefficients revealed significant associations; this entails that as length increase the weight of fish also increase but in more slender [29,30]. Similar studies on the allometric growth was observed in chevron snakehead tributaries of Lake Kilobidan, Agusan Marsh, Philippines in terms of length-weight relationship that there was no significant difference amid male and female *Channa striata* and in terms of their

condition factor was poor indicated 1.50 and 1.49 for and female respectively [13]. Another study of *Channa striata* collected in different river results showed that in Ganga River displayed isometric growth b equal to 3.012, in Yamuna b is 2.18 and in Gomti b value is 2.86 revealed a negative allometric shape of growth [31].

In addition r2 was 0.99, 0.94 and 0.97 from different river respectively which length and weight were highly correlated [14]. Length-Weight Relationship of *Channa striata* is isometric b value is 3.0 and larger individuals having negative allometric relationship b less than 3, represents possible agelinked variation in growth form [15]. Fish allometry affected by fish habitat and nutrition availability in the area.

Sexual dimorphism

Relative warp analysis using tpsRelw ver.1.69 was used to determined differences in shape through deformation grid among specimens in term of sexes. The sample does not include pooled data analysis.

The PCA of each sex were shown in figure 5 and figure 6 which include Histogram between deformation grid of the negative relative warp which is on the left side and positive relative warp on right side of each Principal Component. The shape variation is less among species which means that shape differences also less base on 16 landmarks. Based on the cumulative report retrieved relative warp analysis, both of the specimen male or female have the same percentage of Principal components., PC1 is 33.15%, PC2 = 21.98 %, PC3 = 11. 35%, PC4 = 8.56 %, and PC5 8.08% as shown in Figure 5 and 6 respectively.

The mean thin spline models illustration as express by landmark showing the same grid formation of sixteen (16) anatomical land mark points of male and female as shown in figure 7. This implied that no anatomical landmark points were affected. Furthermore, discriminant analysis using PAST software revealed that there is no sexual dimorphism between male and female as presented in figure 8. The graph showed the discriminant plot that overlaps which mean same shape differences between male and female *Channa striata* sample. Hotellings T2 test results revealed no significant sexual dimorphism for the body shape of the *Channa striata* Hotelling's T2 = 6.168, F = 1.1774, P = 1.

Similar result of sexual dimorphism for the shape has no significant or shape were the same of the mandible both in T. truncatus

Figure 5: Five (5) Principal components (PC) implied grid and histogram of individual shape in Channa striata male species.

Figure 6: Five (5) Principal components (PC) implied grid and histogram of individual shape in Channa striata female species.

Figure 7: Mean thin plate spline models illustrating the same grid of Male and Female *Channa striata*.

Figure 8: The discriminant plot of sexual dimorphism of Male and Female *Channa striata*.

[16]. The important difficult concerns why the sexes are frequently so alike in size this might tell to sperm competition, size buffering effects, and many other features [17]. However, Chevron snakehead female are larger than male [18].

In general, negative allometry was observed in the samples that as fish grow in length it's become slender. Some factors may affect fish growth [6], cyanide had various distinct special effects on growing cichlids, outcome in erratic time of contact and with the level tried cyanide upheld reduction in fish growth. Cyanide can disturb fitness and stress to fishes [4]. Agency for Toxic Substances and Disease Registry provide results of the toxicity level Cyanide and its effects and fund out that, at 0.05 mg/dL is toxic to blood, 0.3 100 and higher were deadly while in hydrogen cyanide at 546 ppm with 10 minutes exposure and 110 ppm after one hour exposure are both lethal.

At the same time, people who eat small amounts of cyanide compounds in a short time may decease unless they rapidly take remedy therapy. Sex dimorphism is the differences in general characteristics of external appearance [32,33], shape and sizes among species Fishes were recognized on behalf of enormous phenotypic malleability evolutionary characters relative ecological physiognomies and this was studied on the fish *Mesopristes cancellatus* in Tagoloan River using tps relwarp revealed significant sex dimorphism variation of body shape based on landmark generated [19].

The software was very useful in learning intraspecific variation, or perhaps to examine micro-evolution or distinguish among kind which may be interpreted in terms of environment, genetic differences, sexual dimorphisms, instars or year classes and Principal components analysis (PCA) is a method that produces hypothetical variables or components, accounting for as much of the variation in the data as possible [20] wider uses of these findings can link to evolutionary biology, organization, and preservation [21].

Landmark-based geometric morphometrics is a powerful approach to quantifying biological shape, shape variation, and co-variation of shape with other biotic or abiotic variables or factors [34,35]. The resulting graphical representations of shape changes were visually attractive and instinctive [22]. Physicochemical, cyanide and four based metals. The water parameters such as Dissolved Oxygen, pH, Salinity, Total Dissolve Solids, Total suspended solids, conductivity and resistivity were determined presented in table 4. The cyanide, Copper, Lead, Zinc and Iron of the water samples from 10 stations shown in table 5. The results were compared to DENR standards as presented in table 6. During the sampling proper water temperature were observed from 25°C to 28.5°C in the sampling stations. From the Table 4, the range of DO was 4.8 ppm of Saugon creek to 7.50 ppm of Magsagangsang creek. The pH level ranges from 6.25 and 7.0 of Bagnan creek and Koo creek respectively.

The salinity is ranging from 500 ppm observed from Magsagangsang creek to 1700 ppm recorded from Saugon creek. The range of TDS is 503 ppm to 1674 ppm Magsagangsang and Saugon respectively. The conductivity is ranging from 1003 μS/cm recorded in Magsagangsang creek to 3250 μS/cm which observed from Saugon creek with minimum resistivity which 307 Ω·m and high resistivity of 997 Ω·m observed in Magsagangsang creek.

Stations	Parameters						
	DO ppm	*pH	Saline ppm	TDS Ppm	*TSS ppm	Conductivity µS/cm	Resistivity Ω·m
Magsagang-sang Creek	7.5	6.25	500	503	9.8	1018	980
Lucad Creek	6.9	6.36	620	618	21	1245	802
Simulao River-(lower)	5.4	6.32	1350	1335	13.8	2617	382
Bunawan Brook River	5.3	6.63	1590	1569	16.8	3060	327
Saugon creek	4.8	6.72	1700	1674	24.2	3250	307
Bagnan creek	6.0	6.5	480	494	15.6	1003	997
Simulao River-(upper)	7.3	6.48	131	1292	22.4	2541	393
Singanan Creek	5.1	6.6	750	746	12.6	1494	668
Koo Creek	6.1	7.0	570	564	9.2	1147	877
Masapia Falls	6.2	6.75	550	551	17.8	1113	895

Table 4: Parameter concentration level of 10 stations collected.

*Results given by Philsaga Mining Corp. Assay Lab of Bunawan, Agusan del Sur, Philippines.

Stations		Base Metals			
	CN^- (ppm)	Cu (ppm)	Pb (ppm)	Zinc (ppm)	Iron (ppm)
Magsagangsang Creek	<0.01	0.02	0.02	0.02	<0.01
Lucad Creek	<0.01	0.01	0.15	0.03	2.32
Simulao River-(lower)	0.01	<0.01	0.02	<0.01	0.41
Bunawan Brook River	0.01	0.01	<0.01	<0.01	0.41
Saugon River	0.01	<0.01	<0.01	<0.01	0.47
Bagnan River	0.01	0.01	<0.01	<0.01	2.34
Simulao River-(upper)	0.02	<0.01	0.02	0.01	0.3
Singanan Creek	0.01	<0.01	0.02	<0.01	1.05
Koo Creek	0.01	<0.01	0.01	<0.01	0.43
Masapia Falls	<0.01	<0.01	0.02	<0.01	0.19

Table 5: Cyanide and Four Base Metals concentrations of ten (10) stations collected.

P Parameters	Present Data	DENR
DO, ppm	4.8--7.50	5
pH	6.25 --7.0	6.5-9.0
Salinity, ppm	500 -- 1700	nd
TDS, ppm	503—1674	96-100
TSS, ppm	9.2--24.20	80
Cyanide, ppm	<0.01-- 0.02	0.1
Cu, ppm	<0.01-- 0.02	0.02
Pb, ppm	<0.01-- 0.15	0.02
Zn, ppm	<0.01-- 0.03	1.5
Fe, ppm	<0.01---2.34	2
Conductivity µS/cm	1003---3250	nd
Resistivity	307---997	nd

Table 6: Range and Comparison of parameters concentrations to DENR standards.

*Legend: < presence, nd- no data

The result of the Laboratory analysis of all water samples in terms of cyanide and base metals concentrations was presented in the Table 5. Based on the report and result, all waters samples from the ten (10) stations of nine (9) stream tributaries were positive of cyanide but varied in detectable concentrations from <0.01 to 0.02 ppm. less than 0.01 (<0.01) it doesn't mean not detected but interpreted as presence of cyanide. On the other hand, the four base metals have the lowest concentrations of <0.01 ppm Copper recorded upto 0.02 ppm of Magsagangsang creek. Lead and zinc top concentrations observed at Lucad creek and Bagnan River observed with 2.34 ppm Iron.

Furthermore, the water parameters such as Dissolved Oxygen, pH, salinity, and total Dissolve Solids, Total suspended solids, conductivity and resistivity. Including cyanide, Copper, Lead, Zinc and Iron were compared with the DENR freshwater quality standards as shown in Table 6. Comparison revealed that Saugon dissolve oxygen was lower at 4.8 ppm, while salinity, total dissolved and suspended solids were the highest and exceed the acceptable level as well as the conductivity was the highest inversely to resistivity relatively low compare to other area. However, all sampling area reached beyond the normal level of total dissolved solids. pH 6.25 ppm in Magsagangsang creek deviates minute level of pH to the accepted value. In Lucad creek the Lead (0.15 ppm) and Iron (2.32 ppm) exceed above acceptable and also the Iron (2.34 ppm) level in Bagnan. Cyanide and Copper and Zinc ranged from below detectable and equal to the accepted value.

Total dissolved solids is relatively high and exceeded DENR standards because the day before sampling there was low pressure and weather conditions were cloudy to rainy during sampling. High runoff of sediments due to wet weather conditions contribute to high dissolved and suspended solids in fact water in most area were turbid through ocular observation except in Masapia Falls. The turbidity it is a relative increase of TDS, TSS, [23] which also increase salinity and conductivity, this was observed from the water sample. The target analyses which is cyanide is very low in concentration observed may be because of the rainy weather conditions since it falls to wet season in the country that rises water level in water and affected the concentration level of cyanide and other four base metals or two hand way either diluted due increase in volume or more added other metal complexes [36]. The four base metals like Pb and Cu is a primary pollutant while Zn and Fe were secondary pollutants that should be monitor otherwise adverse effect color to water and organisms. Lead should be 0.015 mg/L maximum concentration limits because potentially affects metal development to infants and children, deficits attention and learning, for adults it is kidney related problems [24].

Cyanide detection in fish sample

Three (3) freshwater fish species such as Nile Tilapia weighed 1,147.54 g with two (2) individual, Chevron snakehead weighed 1100.08 g with four (4) individual and catfish weighed 1358.78 g with five (5) individuals collected in the month of October with a total of 11 fish sample was sent to BFAR Manila for Cyanide Detection Test or CDT. The analysis used Ion Selective Electrode reference to American Public Health Agency methods no. 4500 with a Limit of Detection 1.209 E-2 mg/Kg and results revealed that Cyanide were found positive in all Tilapia samples, in three (3) samples of chevron snakeheads and in four (4) Catfish as shown in table 7.

FISH	N	Test parameters	Test methods	Specifications	Results
Nile Tilapia	2	Cyanide	ISE-APHA 4500	Limit of Detection 1.209 E-2 mg/Kg	positive
Chevron Snakehead	3	Cyanide	ISE-APHA 4500	Limit of Detection 1.209 E-2 mg/Kg	positive
Catfish	4	Cyanide	ISE-APHA 4500	Limit of Detection 1.209 E-2 mg/Kg	positive

Table 7: CDT results of fish samples positive in cyanide.

*N-total number of samples.

In table 8 showed the fish sample negative with cyanide, one (1) in chevron snakehead sample and one (1) Catfish sample. Furthermore, the result revealed that 82% were positive in cyanide, 18% is negative shown in figure 9.

Fish samples	N	Test parameters	Test methods	Specifications	Results
Chevron Snakehead	1	Cyanide	ISE-APHA 4500	Limit of Detection 1.209 E-2 mg/Kg	negative
Catfish	1	Cyanide	ISE-APHA 4500	Limit of Detection 1.209 E-2 mg/Kg	negative

Table 8: CDT results of fish samples negative in cyanide.

Figure 9: Percentage of fish samples positive in cyanide.

Accordingly, "positive means cyanide (CN-) concentrations in fish is greater than 0.20 mg/L. Traces means cyanide (CN-) concentrations from 0.05 to 0.20 mg/L. Cyanide levels greater than zero (0) and less than 0.05 mg/L are scored BDL-below detectable limits. However, zero (0) values are considered to be negative [4]. While most prosecutions deal with fish that tested as being Positive, any level of cyanide found in the fish is an indication that the fish were exposed to cyanide coming from anthropogenic sources.

This indicates the fish samples accumulate detectable concentration of cyanide from water since cyanide was also detected in water samples. Fishes are most cyanide-delicate group of aquatic organisms [5]. In water cyanide can rise water temperature, minor reduction of pH and dissolved oxygen disturbs fitness and stress to fishes [4]. There are reports that even cyanide concentrations as low as 0.01-0.1 mg/L lethal to sensitive animals species present in waters. Sodium cyanide (NaCN) evidently accumulated to water species and absorption factors for various aquatic organisms [25].

In this study, landmark based geometric analysis revealed no shape differences of male and female *Channa striata* and also observed negative allometric growth which so far accumulated cyanide as well as other fish samples such as catfish and Nile tilapia due to cyanide contamination of water from nine (9) stream tributaries of Agusan Marsh. This implies that fishes greatly affected water quality and other ecological disturbance. Similarly, an analysis on Ibis fish (*Ambassis interrupta*) collected from Masao River, Butuan City, Philippines revealed the two (2) factors sides and interaction had significant differences with ($P<0.0001$) which influence by prevailing factors like existence of fish ponds, use of feeds and publics home lengthways Masao River affecting water chemistry of river that might also distress general phenotype of the fish [26]. Fluctuating asymmetry of fishes were susceptible near ecological changes that possibly manifested the size and forms which analyzed by utilizing Geometric Morphometric which vital in recognizing body differences and similarities of species in the same taxon [27].

Conclusion

Therefore, the study concluded that pollutants such lead, copper, zinc and iron and cyanide were already in detectable levels and contaminated water quality in stream tributaries of Agusan Marsh. Further, landmark based geometric morphometric were a useful tool in comparing the shape variation of *Channa striata* relative to sex dimorphism. The relative warp analysis similarly provided first five principal component analysis revealed no significant differences with $p = 1$ which means the body shape of male and female were same in shape likewise provided a strong positive correlations of length – weight relationship and both sexes have negative allometry. Also, a positive accumulation of cyanide in *Channa striata*, *Oreochromis niloticus*, and *Clarias batrachus* samples were observed thru cyanide detection tests. Thereby bio accumulation of cyanide pertains to the detectable levels of cyanide and growth of fish affected by primary and secondary pollutants such as base metals in the fish habitat.

This study suggests to LGU in the monthly monitoring as to cyanide analysis in water and fish and provide strict implementation of cyanide waste disposal in mining industries.

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