The Influence of Anthropogenic Activities on Macro-Invertebrates Assemblage and Water Quality in the Crocodile River (East) Mpumalanga, South Africa

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Abstract
Biological indicators such as macro-invertebrates and water quality parameters can give an overall overview of what is happening in a river catchment. The aim of the study was to determine the influence of anthropogenic activities on macro-invertebrates assemblage and water quality using multivariate analysis and to determine the present ecological state of the river using the Macro-Invertebrates Response Assessment index. The South African Scoring System Version 5 (SASS 5) was used to collect macro invertebrates. Water quality samples were collected using a polyethylene bottle and analysed by Mpumamanzi Laboratory in Nelspruit and Water lab in Pretoria. From the results obtained it was evident that anthropogenic activities along the Crocodile River play a role in water quality deterioration and the subsequent distribution of macro-invertebrates during high and low flow conditions. The main anthropogenic activities contribute to the influence of macro-invertebrates community and water quality are agricultural activities in the upper reaches and a combination of industrial, domestic, mining and agricultural activities in the middle and lower reaches of the Crocodile River.

Keywords
macro-invertebrates, water quality, anthropogenic activities

1. Introduction
Anthropogenic activities as well as ecological processes contributes to the macro-invertebrates status of aquatic ecosystem. Pollutants from various sources like domestic sewage, pesticides, and fertilizer disturb the river system and increase the level of nutrients which give rise to algal bloom and extensive growth of aquatic weeds (Bagade & Belagali, 2010). According to Girbert and Wendy (2003); Kunwar et al. (2005) effluent from different activities flowing to the river also contributes to main pollutants to
the water resource in a catchment, causing serious ecological and sanitary problems. The aquatic biodiversity of the world is changing and getting depleted alarmingly fast because of extinctions caused by habitat loss, pollution and other anthropogenic activities (Moyle, 1995). In South Africa, the River Health Program which is source of information regarding the overall ecological status of river system was developed.

The River Health Program uses biological communities such as macro-invertebrates, fish and vegetation to characterise the response of the aquatic environment to multiple disturbance. The rational is that the integrity or health of the biota inhabiting the river ecosystems provides a direct and integrated measure of the health of the river (Karr & Chu, 1997). According to Brooks et al. (2006) sustainable biological diversity is a priority of nature conservation in terrestrial and fresh water environment. Thus, the assessment of biological diversities in freshwater play an important role as the basis for nature protection. Biological indicators such as macro-invertebrates have been used to assess the biological integrity of stream ecosystems because they exhibit a wide variation of response to pollutants and have been thoroughly studied in flowing river to assess water quality and complement physico-chemical surveys (Hawkes, 1979; Shutes, 1985).

Macro-invertebrates are measured using a recognized biomonitoring method such as SASS (Dickens & Graham, 2002). Biomonitoring measures such as SASS and Average Score Per Taxon (ASPT) score are related to each ecological class. The SASS protocol was designed by (Chutter, 1998) and currently is in its fifth stage of development. SASS makes use of the natural sensitivity or tolerance to adverse water quality of the wide variety of benthic invertebrates in a river, aggregating the effects of water quality over time. It provides an ideal system to measure the response of aquatic fauna to general water quality conditions in a river. The SASS method produces three different and complimentary scores SASS Score, Number of Taxa and Average Score Per Taxa (ASPT) and it was design for running water and it should not be used to set reserve of ephemeral rivers and standing waters (Dallas, 2000; Dickens & Graham, 2002).

The Crocodile River is a perennial river which has rifles, runs and pool biotopes and the SASS protocol has been tested or used in many pilot or case studies in the river. Some of the macro-invertebrates that are likely occurring in the Crocodile River includes: Heptagenidae, libellulidae, Culicidae, Ceratopogonidae, Gomphidae, Oligochaeta, Beatitidae, Hydropsychidae, Turbellaria, Coenagrionidae, Leptophlebiidae, Perlidae, Trichorythidae, Aeshnidae, Elmidae, Psephenidae, Thiaridae, Tabanidae, Simuliidae, Hirudinidae, Prospisomatidae, Potamonautidae, Nauocoridae, Nepidae, Oligoneuridae, Chlorocyphidae, Validate, Gyrinidae, Bulinnae, Ancylidae, Athericidae, Corbicullidae and Leptoceridae and they live in different places in the water body, e.g., some live in water surface, some in the water itself, others in sediment or on bottom or on submerged rocks, logs and leaf litter, and identification of these macro-invertebrates can indicate whether the river is in the poor or good state.

The protection of the environment requires tools that can be used to assess environmental conditions as well as for setting ecological objectives to ensure proper and sustainable management of the resource.
Thus, the biomonitoring program was designed to monitor the health of the river systems in South Africa (Roux et al., 1999). The health of the river system can be defined as the state at which a river catchment can support goods and services to people who depended on it. The aim of the study was to determine the influence of anthropogenic activities on macro-invertebrates assemblage and water quality using multivariate analysis and to determine the present ecological state of the river using the Macro-Invertebrates Response Assessment index (Thirion, 2008). The results of this study contribute to the understanding of the relationship between macro-invertebrates and physico-chemical parameters and how they complement each other. Thus, it will close the gap where only physico-chemical parameters are used in monitoring the river ecosystem. Moreover, the study assist in the understanding of the impact of anthropogenic activities to the river and its biota.

2. Materials and Methods

2.1 Study Area

The Crocodile River (east) is in Mpumalanga Province in the northern east of the republic of South Africa. It is a relatively larger river basin with a total length of approximately 320 km and draining a catchment area of about 10,450 km² (Figure 1).

![Figure 1. Map Displaying the Study Area and Sampled Sites in the Crocodile Catchment](image)

2.1 Macro-Invertebrates

Macro-invertebrates were collected at the sites indicated on the map in Figure 1 during low and high flow conditions in the year 2013. During the study a South African Scoring System Version 5 (SASS5) was used as a method to collect macro-invertebrates (Dickens & Graham, 2002).
2.2 Water Quality
Thirty six water quality samples were collected from twelve sites of the study area during low flow period (June-August, 2012) and high flow (December-March, 2013). Each site was visited three times during the survey and a polyethylene bottle was used to collect the water quality samples and in situ measurements were taken using an YSI Multi meter (HQ40d). Certain water quality variables such as Ammonium, Chloride, Nitrates-Nitrites, Sulphates, Phosphate, Manganese and Sodium were analyzed by Mpumamanzi Laboratory in Nelspruit and Waterlab in Pretoria.

2.3 Data Analysis
The analysis of macro-invertebrates data focused on quantifying the spatial and temporal variation in species richness and abundance, and identification of environmental variables explaining variation across the study sites. The Statistical Product and Service Solutions (SPSS) were used to test the differences in species richness and abundance across sites during low flow and high flow sampling. The multivariate statistical analysis was performed using CANOCO version 4.5. A Macro-invertebrates Response Assessment Index (MIRAI) was performed to determine the ecological status of the river (Thirion, 2008). Other statistical analysis like Detrended Correspondence Analysis (DCA) and Redundancy Analysis (RDA) were performed to further identify the strongest gradient of assemblage composition independent of the environmental variables and to evaluate the variability in the assemblages structure in relation to the measured environmental factors respectively.

3. Results
3.1 Macro-Invertebrates
A total of 6396 individuals belonging to 54 macro-invertebrates families were collected in the Crocodile River and its tributaries during low and high flow seasons (Table 1 and Table 2: Appendix). In this study insect a constituted 95% of all the macro-invertebrates sampled during the survey (Figure 2).
The class insecta was the largest class by having 6091 individuals and Hirundea was the least of the macro-invertebrates assemblages sampled with 14 individuals. Figure 3 also shows the order contributions of the insecta class. Insecta class includes families such as Ephemeroptera and Plecoptera.

The Order-level distribution pie graph indicated that Ephemeroptera, Plecoptera and Tricoptera Families constituted 44% of the Insecta in the study. These families are sensitive to change in water quality and habitat in the riverine system therefore their presence is more significant to any river.
3.2 Univariate Analysis for Macro-Invertebrates

The Margalef Species Richness Index (R) ranges between 1.296 (site CR8) and 5.086 (site KR1) during low flow condition and from 2.321 (site CR7) to 4.506 (site CR2) during high flow condition. The evenness component (E) varied from 0.6581 at site CR10 to 0.9225 at site CR5 during low flow condition and from 0.4692 at site KR1 to 0.8845 at site CR2 during high flow condition. The Shannon Diversity values ranges between 1.665 (site CR8) and 2.876 (site KR1) during low flow condition and 1.491 (site KR1) and 2.773 (site CR2) during high flow condition (Table 1).

Table 1. Number of Macro-Invertebrates’ Individuals Collected and the Univariate Analyses Test Results for the Crocodile River and Its Tributaries

| Site names | Number of Individuals | Margalef’s Richness | Pielous Evenness | Shannon Diversity |
|------------|-----------------------|---------------------|-----------------|------------------|
|            | L H                   | L H                 | L H             | L H              |
| CR1        | 321 200               | 3.964 3.812         | 0.8108 0.6373   | 2.506 1.998      |
| CR2        | 132 366               | 4.066 4.506         | 0.7004 0.8845   | 2.255 2.773      |
| CR3        | 412 268               | 4.829 4.152         | 0.7884 0.6956   | 2.627 2.266      |
| CR4        | 189 219               | 4.082 3.434         | 0.7261 0.605    | 2.277 1.781      |
| CR5        | 426 135               | 4.485 3.469         | 0.9225 0.7978   | 2.892 2.466      |
| CR6        | 82 -                  | 2.496 -             | 0.8393 -        | -                |
| CR7        | 417 67                | 4.281 2.321         | 0.9174 0.642    | 2.701 1.739      |
| CR8        | 376 222               | 1.296 2.53          | 0.8006 0.8182   | 1.665 2.269      |
| CR9        | 209 303               | 3.5 4.492           | 0.8433 0.6492   | 2.567 2.090      |
| CR10       | 424 396               | 3.009 3.141         | 0.6581 0.7414   | 1.938 2.221      |
| ER1        | 229 178               | 4.632 3.313         | 0.7562 0.6431   | 2.434 1.893      |
| NR1        | 92 252                | 3.074 4.423         | 0.7131 0.8557   | 2.061 2.605      |
| KR1        | 345 136               | 5.089 3.936         | 0.8826 0.4692   | 2.876 1.491      |

3.3 The K-Dominance Curve for Macro-Invertebrates Assemblage

The K-dominance curve (Figure 4) indicated that 60% of the total community structure was dominated by family Thiaridae at site KR1 during low flow condition.
Figure 4. Ranked Species K-Dominance Curves for the Macro-Invertebrate Communities Collected at the Sites on the Elands and Crocodile Rivers during High and Low Flow Conditions

The dominance curve further indicated that sites (CR3, CR4, CR9 and ER1) during low flow and (CR10 and NR1) during high flow condition were dominated by families which contributed above 40% of the total community structure. These dominant families include the Simuliidae at ER1, Baetidae at CR3 and CR4, Gomphidae at CR9 and Pleida at CR10.

3.4 The RDA Trip Lot with Environmental Variable

In RDA plot the length of the arrow is related to the strength of the correlation. In general, the longer the arrow, the more highly related that variable was to families composition and the approximation correlation is positive when the angle is acute and negative when the angle is larger than 90 degrees (Figure 4). The distance between the sampling sites in the diagram indicated the similarity of their macro-invertebrates community as they were measured by their Euclidean distance. The RDA trip lot for both flow regimes indicated similarity of sites in the Crocodile River and its tributaries due to similarity of macro-invertebrates Families. A positive correlation between macro-invertebrates taxa such as Chironomidae, Gomphidae and Libellulidae at sites CR7L, CR8L, CR8H, CR9L, CR10L, ER1H and ER1L with chlorine and sodium was observed.
Families such Physidae, Hydracarina, Ceratopogonidae, Thiaridae, Hirudinae and Corbicullidae at sites CR9H, KR1H, CR5H correlated positively with physico-chemical such as salinity, total dissolved solid, electrical conductivity, magnesium and sulphate.

3.5 The Ecological Condition (Macro-Invertebrates Response Assessment Index)

The Macro-Invertebrates Response Assessment Index (Figure 5) indicated that the upstream of the Kwena Dam was in Ecological Category B Class (Largely natural with few modifications) for both sites CR2 and CR3 during low flow condition. Site CR1 which is upstream of these sites was in ecological category C class (moderately modified).
Site CR4 had an ecological category C Class (moderately modified) for both flow conditions, while sites CR5 and CR6 had an Ecological B class. The Crocodile River remain in an Ecological Category C Class (moderately modified) from downstream of the Nelspruit town (sites: CR7, CR8, CR9 CR10) until it confluence with the Komati River. The Nels River which is a tributary of the Crocodile River was in an Ecological C Class (moderately modified). The Elands (ER1) and the Kaap River (KR1) had an Ecological Category B Class (largely natural with few modifications) and B/C class (largely natural with little modification) respectively.

3.6 Water Quality

The physical and chemical variables analyzed for water quality indicated that site CR3 had a higher concentration of nitrate compared with all the sites sampled in winter with a mean value of 7.3 mg/L and a mean value 2.4 mg/L in summer. A high concentration of phosphate at site CR7 with a mean value of 0.7 mg/L was recorded in winter. Ammonium recorded a mean value of 0.1 mg/L in winter at sites CR4, CR6, CR9 and CR10. Nitrate and phosphate had high concentrations at site CR10 in summer with mean values of 4.1 mg/L and 0.4 mg/L respectively. Water quality constituents such as total dissolved solids, electrical conductivity and salinity in the Crocodile River increased with the river flow distance downstream during summer months (Table 2 and Table 3). At site ER1 a high concentration of TDS with a mean value of 528 mg/L and Salinity with a mean value of 0.358 (ppt) both in summer were also recorded (Table 2). Chloride recorded a mean value of 47.3 mg/L in winter. The middle part of the Crocodile River system was characterized by the presence of solid waste and industrial effluent or run off, sewage discharge and domestic run off contributing to increase in nutrients as observed in this study. Salinity in the middle part of the Crocodile River was higher compared to the upper reaches and was associated with the various activities taking place in this area. At site KR1 high concentration of salinity with a mean value of 0.315 (ppt), TDS with a mean value of 460 mg/L was recorded during summer months (Table 2). A high concentration of electrical conductivity with a mean value of 58.8 S/m−1 was measured during winter month (Table 3).

| Physico-chemical variables | CR1 | CR2 | CR3 | CR4 | CR5 | CR6 | CR7 | CR8 | CR9 | CR10 | ER1 | KR1 |
|---------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|
| Cl (mg/l)                 | -   | -   | -   | 17  | -   | -   | 19  | 26  | 38.3| 47.3 |     | 17.9|
| EC (mS/m)                 | 8.8 | 9.6 | 13.1| 14.7| 22.9| 24.7| 21.5| 17.1| 42.9| 48.4 | 57.96| 58.8|
| Mg (mg/l)                 | 40.0| 5.0 | 5.0 | 4.4 | 10  | 5.0 | 0.025| 16.7| 20  | 15.5 | 27.5 |     |
| Na (mg/l)                 | 2   | 5.3 | -   | -   | -   | -   | -   | -   | -   | 45.1 | 30.8 |     |
| NH4-H (mg/L)              | -   | 0.025| 0.025| 0.1 | 0.025| 0.1 | -   | -   | 0.1 | 0.1  | 0.025| 0.06|
| NO3 (mg/L)                | -   | 0.025| 0.025| 0.1 | 0.025| 0.2 | -   | -   | -   | 0.1  | 0.6 | -   |
| pH                        | 7.7 | 7.7 | 7.7 | 7.8 | 7.6 | 7.5 | 7.7 | 7.6 | 7.9 | 8.0  | 8.1 | 8.2 |
Table 3. Mean Values for Water Quality Results Sampled in the Crocodile River and Its Tributaries during High Flow Condition (December-March 2012)

| Physico-chemical variables | CR1 | CR2 | CR3 | CR4 | CR5 | CR6 | CR7 | CR8 | CR9 | CR10 | ER1 | KR1 |
|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|
| Cl (mg/L)                  | 1.7 | 3.2 | 1.5 | 2.0 | 17.1| 13.1| 11.9| 11.9| 39.9| 104.9| 52.6| 11.7|
| EC (mS/m⁻¹)                | 5.8 | 11.4| 11.4| 12.6| 23.9| 19.8| 18.2| 16.9| 33.5| 84.1 | 55.7| 35.5|
| Mg (mg/L)                  | 4.0 | 9.7 | 5.5 | 7.6 | 10.9| 5.3 | 10.8| 6.3 | 22.1| 27.5 | 21.6| 19.3|
| Na (mg/L)                  | 2   | 2.8 | 1.9 | 4.0 | 16.2| 12.9| 11.5| 11.6| 22.9| 86.5 | 46.4| 15.0|
| NH₄ (mg/L)                 | 0.025| 0.025| 0.05| 0.025| 0.025| 0.04| 0.025| 0.025| 0.025| 0.0025| 0.025| 0.25|
| NO₃ (mg/L)                 | 0.025| 0.08 | 2.4 | 0.025| 0.025| 0.2 | 0.025| 0.19 | 0.43 | 4.1 | 0.0025| 0.5 |
| pH                         | 7.8 | 7.5 | 7.3 | 7.7 | 8.1 | 7.8 | 7.6 | 8.1 | 7.9 | 8.1 | 8.2 |
| PO₄ (mg/L)                 | 0.005| 0.005| 0.005| 0.005| 0.005| 0.008| 0.009| 0.01 | 0.02 | 0.4 | 0.007| 0.02|
| SO₄ (mg/L)                 | 1.5 | 1.5 | 1.5 | 2.2 | 27.1| 19.4| 18.8| 16.2| 38.4| 25.4 | 85.8| 36.2|
| Salinity (ppt)             | 0.0428| 0.0656| 0.0705| 0.066| 0.138| 0.152| 0.127| 0.139| 0.227| 0.299| 0.358| 0.315|
| TDS (mg/L)                 | 60.5| 96.9| 104| 93.9| 205| 228| 192| 206| 335| 438 | 528| 460|
| Temperature (°C)           | 16.9| 16.6| 17 | 14.1| 17.2| 15.2| 19.6| 20 | 21.8| 21.9| 16.4| 20.2|

4. Discussion

Polluted habitats are poor in species richness as it reduces both species diversity and abundance and only few individuals can survive in pollution. A study conducted by Karr et al. (1985); Soto-Galera et al. (1998); Allen et al. (1999); Waite and Carpenter (2000) found that pollution effect reflected decreasing richness at communities. The univariate analysis of macro-invertebrates in the Crocodile River catchment indicated that there was a variation of macro-invertebrates richness, evenness and diversity during both flow conditions which were attributed to change in habitat complexity or substrate complexity and water quality. During the current study sites, such as CR3 and CR4 which are in an area dominated by agricultural activities (e.g., cattle farming, crop and trout farming) were observed to have higher concentration of nitrates and ammonium respectively. The higher concentration of nitrates and ammonium was attributed to trout feeds effluent and fertiliser application to crop farming at this area. Macro-invertebrates distribution at CR3 and CR4 were poor during low flow condition which was attributed to change in habitat in the river. The lowest macro invertebrates richness was found at sites CR7 and CR8 during high and low flow respectively. These sites CR7 and CR8 were situated...
downstream of Nelspruit and Kanyamazane town respectively receiving effluent from these towns. The lower macro-invertebrates richness was attributed to poor habitat at this area. Moreover, water quality played a role in the richness as higher concentration of Phosphate was measured at site CR7 downstream of the Nelspruit town. The higher concentration of phosphate was attributed to sewage, industrial and agricultural effluent. At sites CR10, a higher concentration of nitrates and phosphate were measured during high flow condition. The higher concentration of this physico-chemical parameter where attributed to effluent from sugar cane farming and effluent from the Malelane town during rainy seasons. Distribution and diversity of macro-invertebrates were recorded to be low during low flow condition which was attributed to poor habitat caused by poor flow condition, absence of overhanging vegetation on the river. The higher concentration of total dissolved solid and salinity at sites ER1 and KR1 which are tributaries of the Crocodile River during high flow was attributed to effluent coming from Ngodwana saw mill, orchards farms effluent and mining industries respectively. Deteriorating water quality parameters such as TDS, Nitrate, and Phosphate and Electrical conductivity (Soko & Gyedu-Ababio, 2015), were among the major factors contributing to the disappearances of some families in the Crocodile River. A study conducted by Kleynhans (1999) concluded that the middle section of the Crocodile River had high nutrient concentration. The K-dominance curve indicated the dominance of family Beatidae at sites CR3 and CR4 which was attributed to good habitat and water quality as this family is intolerant to impacted water quality, while the presence of family Pleidae at site CR10 was attributed to poor water quality as this family is more tolerant to impacted water quality. The deterioration of water quality at site CR10 was attributed to the discharge of effluent from the Malelane town and run-offs from agricultural activities. The dominance of single families at other sites was attributed to poor habitat, such as gravel, sand, mud and aquatic vegetation. The RDA trip lot of macro-invertebrates and environmental variables showed similarities of sites which were attributed to the presence of similar macro-invertebrates assemblage occurring at those sites, while the separation of sites such as CR1, NR1, CR4 and CR8 was due to the change in macro-invertebrates assemblage as results of change in habitat availability and water quality. The correlation of macro-invertebrates taxa at sites CR9, KR1 and CR5 with environmental variables such as Chloride, salinity, total dissolved solids, electrical conductivity and sulphate during high flow condition within the river was an indication that macro-invertebrates within the river were distributed based on their water quality tolerance or preference. The presence of agricultural activities, settlements and industries played a role to the change in macro-invertebrates assemblages in the river. The Macro-Invertebrates Response Assessment Index calculated for the Crocodile River and its tributaries indicated that river is in a B to C class ecological category. The lower ecological category class at site CR1 and CR4 was attributed to poor habitat diversity for macro-invertebrates in the upper reaches. The same results were found in a study conducted by (Roux et al., 1999). The modification of the mainstem of the Crocodile River at sites CR7, CR8, CR9 and CR10 were attributed to impairment of water quality (effluent from sugar cane, orchards farm and industrial activities), sedimentation in the river caused by river banks instability which results
in engraving of riffles, rapids, aquatic vegetation in the river. Thus, results in absence of sensitive macro-invertebrates in the river.

5. Conclusion
The present study shows that macro-invertebrates communities are characterised by temporal and spatial changes in its population and distribution pattern seems to be fully governed by the physicochemical and hydrological characteristics of the environment. The higher concentration of phosphates, salinity, total dissolved solid, ammonium and nitrates in areas mostly dominated by anthropogenic activities such as crops, orchards, tobacco, sawmill, sugar cane, towns and the lower diversity and distribution of macro-invertebrates in such areas was an evidence that indeed anthropogenic activities have a negative impact on macro-invertebrates and water quality in the Crocodile River. The deterioration of the Ecological Class and the dominance of single family in area mostly manifested by both agricultural and industrial activities (sites CR6-CR10) was also a proof that anthropogenic activities influenced water quality and macro-invertebrates communities. Agricultural run-offs, industrial and sewage run-offs in the mainstem and mining seepage at site KR1 (dominated by single family Thiaridae) were the source of pollution and change in macro-invertebrates communities in the Crocodile River and its tributaries.

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### Appendix A

**Expected Taxa Sampled during the High Flow Condition in the Crocodile River and Its Tributaries**

| Taxon                        | CR1H | CR2H | CR3H | CR4H | CR5H | CR6H | CR7H | CR8H | CR9H | CR10H | ER1H | NR1H | KR1H |
|------------------------------|------|------|------|------|------|------|------|------|------|-------|------|------|------|
| **Class: Turbellaria**       | 4    | 1    | 1    | 6    | 3    | 0    | 0    | 6    | 27   | 1     | 0    | 0    |      |
| **PHYLUM: ANNELIDA**         |      |      |      |      |      |      |      |      |      |       |      |      |      |
| **Class: Oligochaeta**       | 1    | 2    | 1    | 3    | 20   | 0    | 0    | 0    | 3    | 1     | 4    | 1    |      |
| **Class: Hirudinae**         | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 0    | 10   | 2     | 0    | 0    | 0    |
| **ORDER: DECAPODA**          |      |      |      |      |      |      |      |      |      |       |      |      |      |
| Family Potamonautidae        | 1    | 6    | 0    | 1    | 4    | 0    | 0    | 0    | 0    | 1     | 0    | 0    |      |
| Family Tayside               | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 6     | 0    | 0    | 7    |
| Family Palaeonidae           | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0     | 0    | 0    | 0    |
| Taxon Hydracarina            | 0    | 0    | 1    | 0    | 2    | 22   | 0    | 0    | 16   | 32    | 1    | 0    | 8    |
| **ORDER: PLECOPTERA**        |      |      |      |      |      |      |      |      |      |       |      |      |      |
| Family Notonemourida         | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0     | 0    | 0    | 0    |
| Family Perlidae              | 4    | 1    | 2    | 0    | 9    | 0    | 1    | 0    | 25   | 0     | 10   | 0    | 1    |
| **ORDER: EPHEMEROPTERA**     |      |      |      |      |      |      |      |      |      |       |      |      |      |
| Family Baetidae              | 43   | 74   | 74   | 92   | 6    | 8    | 2    | 19   | 18   | 21    | 15   | 9    | 21   |
| Family Caenidae              | 28   | 21   | 10   | 9    | 4    | 2    | 9    | 0    | 2    | 0     | 3    | 12   | 0    |
| Family Heptageniida          | 35   | 80   | 25   | 21   | 6    | 0    | 0    | 12   | 0    | 0     | 4    | 35   | 7    |
| Family Leptophlebiida        | 0    | 0    | 18   | 15   | 0    | 0    | 0    | 0    | 0    | 0     | 4    | 10   | 8    |
| Family Oligoneurida          | 6    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0     | 4    | 10   | 8    |
| Family Prospistomatida       | 4    | 1    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0     | 0    | 0    | 0    |
| Family Telagonodida          | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0     | 0    | 0    | 0    |
| Family Trichorythrida        | 11   | 37   | 15   | 3    | 0    | 0    | 0    | 0    | 0    | 0     | 0    | 112  | 0    |
| **ORDER: ODONATA**           |      |      |      |      |      |      |      |      |      |       |      |      |      |
| Family Chlorocyniphiida      | 0    | 0    | 0    | 0    | 0    | 2    | 0    | 0    | 0    | 1     | 0    | 12   |      |
| Family Coenagronida          | 0    | 0    | 3    | 3    | 0    | 0    | 0    | 0    | 0    | 2     | 0    | 0    | 1    |
| Family Aeshnida              | 4    | 16   | 30   | 3    | 7    | 0    | 0    | 0    | 0    | 1     | 5    | 0    |      |
| Family Corduliida            | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0     | 0    | 0    | 1    |
| Family Gomphidae             | 2    | 11   | 5    | 2    | 12   | 0    | 9    | 0    | 19   | 3     | 15   | 5    | 4    |
| Family Libellulida           | 0    | 0    | 2    | 0    | 2    | 3    | 3    | 6    | 2    | 2     | 9    | 0    | 0    |
| **ORDER: HEMIPTERA**         |      |      |      |      |      |      |      |      |      |       |      |      |      |
| Family Corixidida            | 1    | 4    | 8    | 8    | 0    | 0    | 0    | 0    | 15   | 0     | 0    | 0    |      |
| Family Gerridae              | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0     | 0    | 0    | 0    |
| Family            | TRICOPTERA | COLEOPTERA | DIPTERA      | MOLLUSCA  |
|-------------------|------------|------------|--------------|-----------|
| **PHYLUM: TRICOPTERA** |            |            |              |           |
|                  |            |            |              |           |
| **ORDER: TRICOPTERA** |            |            |              |           |
| Family Naucorinae   | 0          | 0          |              |           |
| Family Notonectidae | 1          | 0          |              |           |
| Family Pleida       | 0          | 0          |              |           |
| Family Velida       | 0          | 1          |              |           |
| **ORDER: COLEOPTERA** |            |            |              |           |
| Family Ecnomidae    | 0          | 0          |              |           |
| Family Hydropsychida| 16         | 7          |              |           |
| Family Philoptamida | 0          | 4          |              |           |
| Family Hydroptilida | 0          | 0          |              |           |
| Family Leptocerida  | 0          | 1          |              |           |
| Family Petrothrinida| 0          | 0          |              |           |
| **ORDER: DIPTERA**  |            |            |              |           |
| Family Athericida   | 0          | 0          |              |           |
| Family Ceratopogonida| 0          | 2          |              |           |
| Family Chironomida  | 9          | 75         |              |           |
| Family Culicida     | 1          | 1          |              |           |
| Family Muscida      | 0          | 0          |              |           |
| Family Psychodida   | 0          | 0          |              |           |
| Family Simulida     | 10         | 8          |              |           |
| Family Syrphida     | 0          | 0          |              |           |
| Family Tabanida     | 0          | 1          |              |           |
| Family Tipulida     | 2          | 8          |              |           |
| Family Ancylida     | 0          | 0          |              |           |
| **PHYLUM: MOLLUSCA** |            |            |              |           |
| Family Physida      | 0          | 0          |              |           |
| Family Planorbinae  | 0          | 5          |              |           |
| Family Thiarida     | 0          | 0          |              |           |
| Family Viviparida   | 0          | 0          |              |           |
### Appendix B

**Expected Taxa Sampled during the Low Flow Condition in the Crocodile River and Its Tributaries**

| Taxon                        | CR1L | CR2L | CR3L | CR4L | CR5L | CR6L | CR7L | CR8L | CR9L | CR10L | ER1L | NR1L | KR1L |
|------------------------------|------|------|------|------|------|------|------|------|------|-------|------|------|------|
| **Class: Turbellaria**       | 1    | 1    | 10   | 0    | 9    | 0    | 0    | 0    | 0    | 88    | 0    | 0    | 0    |
| **PHYLUM: ANNELIDA**        |      |      |      |      |      |      |      |      |      |       |      |      |      |
| Class: Oligochaeta           | 0    | 0    | 15   | 9    | 7    | 0    | 6    | 4    | 1    | 0     | 0    | 2    | 8    |
| Class: Hirudinea             | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0     | 0    | 0    | 1    |
| **ORDER: CRUSTACEA**         |      |      |      |      |      |      |      |      |      |       |      |      |      |
| Potamonautidae               | 12   | 9    | 1    | 10   | 113  | 0    | 0    | 1    | 0    | 1     | 11   | 9    |
| Atyidae                      | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 5    | 2    | 0     | 0    | 1    |
| **ORDER: PLECOPTERA**        |      |      |      |      |      |      |      |      |      |       |      |      |      |
| Perlidae                     | 0    | 1    | 1    | 9    | 72   | 0    | 4    | 0    | 0    | 3     | 6    | 8    |
| **ORDER: EPHEMEROPTERA**     |      |      |      |      |      |      |      |      |      |       |      |      |      |
| Baetidae                     | 114  | 9    | 116  | 107  | 50   | 0    | 4    | 8    | 16   | 3     | 10   | 13   | 12   |
| Caenidae                     | 15   | 8    | 65   | 0    | 2    | 0    | 14   | 15   | 12    | 50    | 0    | 0    | 3    |
| Heptageniida                 | 9    | 9    | 78   | 8    | 5    | 0    | 4    | 2    | 5     | 6     | 5    | 8    |
| Leptophlebiidae              | 5    | 14   | 7    | 3    | 0    | 0    | 0    | 0    | 0     | 4     | 4    | 8    |
| Oligoneuridae                | 0    | 0    | 9    | 4    | 0    | 0    | 0    | 0    | 0     | 0     | 1    | 0    |
| Polymitarcyidea              | 0    | 0    | 0    | 0    | 5    | 0    | 0    | 0    | 0     | 0     | 0    | 0    |
| Prosopistomatidae            | 99   | 4    | 0    | 0    | 0    | 0    | 0    | 0    | 0     | 0     | 0    | 0    |
| Teloganodidae                | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0     | 0     | 0    | 0    |
| Trichorythidae               | 2    | 8    | 27   | 1    | 0    | 0    | 0    | 0    | 0     | 0     | 9    | 1    |
| **ORDER: ODONATA**           |      |      |      |      |      |      |      |      |      |       |      |      |      |
| Family:                      |      |      |      |      |      |      |      |      |      |       |      |      |      |

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| Family               | 5  | 2  | 8  | 10 | 10 | 0  | 8  | 70 | 1  | 9  | 4  | 2  | 10 |
|---------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Chlorocypidae       |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Chlorolestidae      |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Coenagrionidae      |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Aeshnidae           |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Cordulidae          |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Gomphidae           |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Libellulidae        |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **ORDER: HEMIPTERA**|    |    |    |    |    |    |    |    |    |    |    |    |    |
| Belostomatidae      |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Corixidae           |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Gerridae            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Naucoridae          |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Nepidae             |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Notonectidae        |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Pleidae             |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Veliidae            |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **ORDER: TRICHOPTERA**|    |    |    |    |    |    |    |    |    |    |    |    |    |
| Hydropsychidae      |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Philopotamidae      |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Hydroptilidae       |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Leptoceridae        |    |    |    |    |    |    |    |    |    |    |    |    |    |
| **ORDER: COLEOPTERA**|    |    |    |    |    |    |    |    |    |    |    |    |    |
| Dytiscidae          |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Elmidae             |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Gyrinidae           |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Hydraenidae         |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Hydrophilidae       |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Psephenidae         |    |    |    |    |    |    |    |    |    |    |    |    |    |

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| ORDER: DIPTERA                        |                |
|--------------------------------------|----------------|
| Family: Athericidae                  | 0 0 0 1 0 0 4 0 0 0 0 0 0 0 0 0 |
| Family: Ceratopogonida               | 0 3 0 0 0 0 0 0 2 0 4 0 1 0 0 0 |
| Family: Chironomida                  | 2 19 29 1 19 0 148 88 22 15 12 1 0 0 0 |
| Family: Culicidae                    | 1 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 |
| Family: Ephydridae                   | 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 |
| Family: Muscidae                     | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| Family: Simulidae                    | 0 1 1 0 20 0 157 0 2 7 98 0 1 0 0 |
| Family: Tabanidae                    | 16 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| Family: Tipulidae                    | 5 0 1 0 0 0 0 0 4 4 1 0 2 0 0 8 |
| Family: Tipulidae                    | 0 5 1 10 0 0 13 0 0 0 14 15 1 0 0 0 |

| FAMILY: MOLLUSCA                     |                |
|--------------------------------------|----------------|
| ORDER: GASTROPODA                   |                |
| Family: Ancylidae                   | 11 0 1 1 34 0 0 0 0 0 0 1 0 0 0 0 |
| Family: Bulininae                   | 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 |
| Family: Lymnaeidae                  | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |
| Family: Physidae                    | 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 |
| Family: Planorbinae                 | 0 0 0 0 0 0 0 0 6 0 0 0 0 0 0 0 |
| Family: Thiariae                     | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 237 0 |
| Family: Viviparidae                 | 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 |

| CLASS: BIVALVIA (PELECYPODA)        |                |
|--------------------------------------|----------------|
| Corbiculidae                         | 0 0 0 3 0 0 0 0 1 4 0 0 1 0 0 0 |