ABUNDANCE OF EPIPHYTIC INVERTEBRATES ON EMERGENT MACROPHYTES IN A LENTIC SYSTEM (GOREANGAB DAM) WINDHOEK NAMIBIA

BY:

BENJAMIN N. KONDOWE: 200703871

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Supervisor

MR. L. KANDJENGO

Department of Fisheries and Aquatic Sciences, Faculty of Agriculture and Natural Resources,

University of Namibia

Windhoek, Namibia.

Declaration

I hereby declare that this work is the product of my own research efforts, undertaken under the supervision of Mr. L. Kandjengo and has not been presented elsewhere for the award of degree. All the sources have been duly and appropriately acknowledged.

Candidate signature________________________________ Date____________________

Benjamin N. Kondowe: 200703871

Certification

This is to certify that this report has been examined and approved for award of degree of Bachelor of Science in Fisheries and Aquatic Sciences of the University of Namibia.

Supervisor____

Head of Department____

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Dedication

This work is dedicated to my family for always being there for me and giving encouraging advices tirelessly. Special dedication to my late father who always wanted me to be educated for me to become a better citizen, it is sad that you did not live to see what you always wanted to see in your last born, may your soul continue to rest in internal peace. Special dedication also to my Mum, I shall always cherish you for being so loving and supportive, this work is one of your uncountable acts. To the whole family i love you all as much as you love me.

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Abstract

The study was done in order to identify, quantify and look at the factors affecting the occurrence of microinvertebrates and macroinvertebrates in the vegetated area of Goreangab dam. Invertebrates were quantitatively collected from two species of plants (grass); D. eagyptium and C. longus Sample collection was done on three different sites in Goreangab dam from 7th June to 2nd October, 2010, with an exception of August where no samples were collected. From all the sampling Chironomid larvae of the ***Pseudosmittia*** *species (orthocladius) were the only species collected and D. eagyptium has a higher abundance than pointed leaf grass. In terms of seasonal variation the differences were not significant enough though winter dominated at two sites (site 3 and site 2) while spring on registered higher figures at the oulet. However season influences the abundance of invertebrates at a given macrophyte type i.e. for D. eagyptium in summer a mean of 37.3 was recorded then winter the mean was 54.3 where as C. longus mean for summer was 29.8 and winter the mean was 26.7. Water parameter like temperature and pH were also recorded during each sampling.*

Introduction

Epiphytic invertebrates that are associated with macrophytes are important in aquatic ecology for both lotic and lentic water systems because they are a nutritious source of food for fish and they help in regulating algal populations since these invertebrates consume algae (Schriver *et al*., 1995). Macrophytes can be either emergent or submerged, with emergent macrophytes being plants that are rooted in sediment and whose growth habit results in plant protruding above the water surface, whereas submerged macrophytes on the other hand are those that are rooted and grow within water column and do not protrude above water surface (Mike, 2000). Some of the common examples of epiphytic invertebrates are Chironomids that form part of the feed for fish and to some extent they are used as bait by fishermen (Azumi *et al*., 2004). Chironomids are insect larvae that look like worms and generally are red, green, white or yellow in colour. Distribution and abundance of epiphytic invertebrates is depended on the structure of the habitat i.e. some of the habitat could be a complex stem or leaves (Stephene *et al*., 2007). The abundance of these invertebrates is influenced by seasonal and environmental cycles, as this influences the hydrology that in turn results in fluctuation of the water level in a lake or dam (Vannote and Sweeney, 1980; Ward, 1992 as referenced by Stephene *et al*., 2007). Therefore the fluctuating levels of the water will determine whether the macrophyte are submerged or not and that in turn has an effect on the abundance of the invertebrates. Goreangab dam is an example of a lentic water system surrounded by plants on its shores that harbor epiphytic invertebrates as the plant grow in the water or when they bend and get submerged in the water.

Chironomidae

Chironomidae are insect larvae that belong to the phylum Arthropoda, order Diptera (William and Ferrington, 2008). Chironomidae are holometabolous, meaning they undergo egg, larval, pupal, and adult stages, in addition morphologically; the larval and adult stages are very different (William and Ferrington, 2008). Chironomids are the major invertebrates found in freshwater environments, both lentic and lotic, with the larval stages being completed in aquatic environments (John, 2001). The larvae have parapods at both anterior and posterior end that are used for attaching to a substrate and sometimes parapods are used as legs for movement on the substrate (William and Ferrington, 2008). The pupal stage is characterized by full development of body tissues plus wings, then the pupae swims to the water surface. When the wings are fully developed which also signifies the adult stage, the insect flies out of the water to terrestrial environments (Azumi *et al*., 2004). These larvae are used in water quality studies because the presence of ***Pseudosmittia*** species is associated with polluted water as they are able to survive under very low oxygen level environment because they are able to respire anaerobically in the absence of oxygen (John and Epler, 2001). Previous investigations by Beatrix and Ernst (1988) reported that larvae of *Chironomus thummi* degrade glycogen anaerobically forming ethanol, meaning these chironominids make use of alcoholic fermentation to release energy. That implies aquatic organisms like fish would still have food in absence of other zooplankton that cannot withstand low level of oxygen. Larvae of chironomids feed on settling organic particles, then predators like fish feed on these larvae, hence chironomids play a big role in the material cycle of aquatic ecosystems (Azumi *et al*., 2004). The study of invertebrates is generally neglected because of the tedious task of identifying them despite their ecological importance (William and Ferrington, 2008).

Lentic water system

Lentic water system refers to the water body that is enclosed and the water is stagnant meaning there is no flowing water, for example lakes, ponds and dams. However there is an inflow to the system through the running water resulting from rains and in most cases such water brings in nutrients that are used in the production of the system (Brunke and Gonser 1997).

Literature review

Existence of living organisms in aquatic environments such as dams is dependent on physical and chemical factors. These factors include; type of macrophyte, water level and temperature fluctuations, and seasonal variations. Phyla such as cnidarians, nematodes, annelids, arthropods and mollusks are some of the invertebrates that occur in lentic system (Christopher *et al*., 2004).

Macrophytes

Different macrophyte types such as emergent and submerged macrophytes exist in aquatic environments and they harbour epiphytic invertebrates. Morphological make up of macrophyte in terms of size and structure dictates the number of invertebrates' available (Donelle and Michael, 1999). For instance, plants with small stems have a smaller surface area than plants with bigger stems, (height being that same) meaning the latter should have a higher abundance than the former (Raffaelli *et al*., 2000). The organism's body size will also influence their abundance by limiting the parts a given species can occupy on the macrophyte. Smaller organism utilizes macrophytes better, because they can go through crevices and can for instance cover either sides of a leaf (Raffaelli *et al*., 2000). The macrophytes type dominating in the water body would signify status of water in terms of turbidity (Christopher *et al*., 2004). Presence of submerged macrophytes is associated with clear water while emergent (floating) macrophytes are associated with turbid water (Christopher et al., 2004). When water is turbid, light is not able to penetrate deeper to reach submerged plants since particles responsible for the turbidity of the water reflects light rays back and therefore only emergent plants will flourish. Dominance of emergent macrophytes is therefore not a very good sign as it signifies eutrophication of the water, (Christopher *et al*., 2004).

Water level

The water level does vary in any freshwater body with time of the year as a result of evaporation and precipitation. When the water level is too high, epiphytic invertebrates are forced to move to shallower waters until the optimum water level is achieved as water evaporates. But when too much water evaporates it would also be a problem because the invertebrates are exposed to high temperatures that may also kill them through desiccation or heat stroke (Peter, 1990). But at some given water level after evaporation, seeds in the sediments are able to germinate because there is enough sunlight reaching them, thereby increasing the macrophyte community that increases the abundance of epiphytic invertebrates due to availability of space (Van der Valk, 1978 as quoted by Van Geest, 2005). In this regard evaporation does not always bring negative effects, but high rate of evaporation is undesirable.

Seasonal variation

During winter, numbers of invertebrates are generally high because the dam is well enriched with nutrients brought in through runoff during summer and to some extent autumn runoff. In addition, the water is generally clear in winter meaning there is high illumination increasing the amount of dissolved oxygen through photosynthesis since the photic zone is deeper enough (Stephene, *et al*., 2007). When there is a high rate of photosynthesis, it means phytoplankton is flourishing, thereby providing enough food for the invertebrates (Stephene, *et al*., 2007).

Spring is associated with low numbers of invertebrates as a result of evaporation of water leaving behind high concentration of nutrients such as nitrogen (Christopher *et al*., 2004). Excess nutrient build up in the dam might lead to eutrophication of the dam resulting in algal bloom. An increase in algal biomass is associated with depletion of oxygen since there is an increase in oxygen demand by algae for respiration, hence there will be insufficient oxygen for other aquatic organisms like invertebrates and fish (Stephene *at al*., 2007).

Spring is associated with turbid water in the dam especially when there are strong winds that bring about upwelling of nutrients from the bottom of the dam carrying along fine particles of sediments that contributes to the total suspended solids in the water (Christopher et al., 2004). Suspended solids in the water scatter the light rays coming from the sun. Light rays are important as they are the source of chemical energy that facilitates the process of photosynthesis which contributes significantly to the total dissolved oxygen in the water (Reece and Campbell, 2005).

Problem statement

Aquatic invertebrates play an important role in maintaining a stable ecological structure as they have specific niche, being food for fish and preying on algae hence absence of invertebrates will lead to unregulated algal biomass plus carnivorous fish will suffer due to lack of food. Presence of some invertebrates act as indicator of the well being of the water i.e. they determine water quality, for instance presence of tubifex worms and chironomids usually entails that the water is dirty while stoneflies signifies clean water (Coops and Havens, 2005). In addition water quality with regard to turbidity and eutrophication can be evaluated based on the macrophyte type dominating in a water body. Pollution at the dam is also accelerated through human activities for example during spring and summer there is a high number of people at the dam since the dam is used for recreational purposes as mentioned in the introduction, and these people contribute to the organic matter running into the dam because some empty their waste into the dam despite effort by the municipality of keep our environment clean. The dam area is also used for boat cruises as well as for research purposes by educational institutions due to its close proximity to the city adding to pollution from oil used by the boats.

Findings of this kind of research will help Fisheries officers and Environmental Health Personnel in advising the society and government in regulating the activities in and around such water bodies. Health status of Goreangab dam is crucial because it is used as recreational park and people have to be informed if there is any danger coming into contact with the water from the dam.

Material and Methods

Study area

Goreangab dam (*Fig. 1*) came into reception in 1969 and is located west of Windhoek; whereit borders the Katutura and Khomasdal settlements. The dam has a maximum depth of about 12 meters close to the dam wall. The primary aim of the dam is to reclaim used water from households and purify it for reuse, since water is a scarce resource in the semi-arid Namibia (Law, 2003). Purified water from the dam supplements the water that is extracted from sub-surfaces reservoirs like boreholes.

The above sampling sites were chosen because of the activities that take place there and they are;

- Site 1: Inlet of purified water from the Gammams wastewater purification plant,
- Site 2: Recreational area characterized by a lot of organic pollution from revelers who frequent the dam
- Site 3: Settlement area characterized by organic and chemical waste from household uses

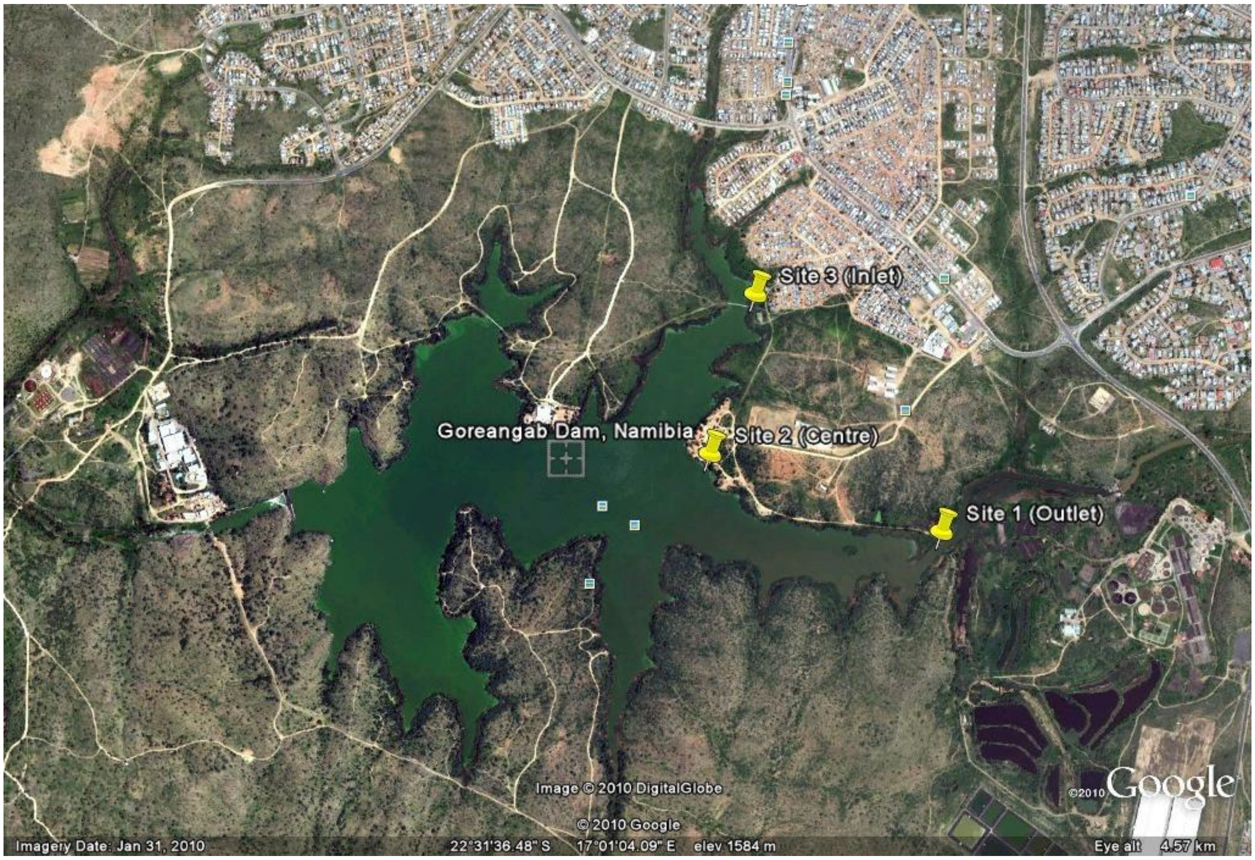

Figure 1: Goreangab dam aerial map.

Invertebrate sampling

Invertebrates were sampled at the three different sites along the shore of the dam and three samples of each species of plant were collected at each. The plants were cut about one centimeters into the sediments, and then were put in collecting bottles that were filled with 10 percent formalin for preservation. The bottles were labeled according to site, date and time of collection. Sampling was done monthly with a spacing of four weeks between one sampling and the other and from June until October, so that season factor could be observed if it affects the abundance of epiphytic invertebrates. Other parameters such as temperature and pH were measured during the collection and from each site. Two macrophyte types, namely *Dactyloctenium aegyptium* and *Cyperus longus* as identified by the National Botanic Research

Institute of Namibia (NBRI), were selected prior to sampling based on their difference in structure and morphology, for example *D. aegyptium* that has larger surface area since it is highly branched compared to *C. longus* that grow thin with less leaves.

Below are photographs of the two macrophytes.

D. aegyptium *C. longus*

Figure 2: Macrophyte type.

Laboratory analysis

Invertebrates were scraped off from the macrophyte into a petri dish using a sharp needle and the ones in the water were sieved into a basin using a sieve of mesh size 0.250 mm, then transferred into petri dish. Samples were studied under a dissecting microscope for detailed examination of their morphology but in some cases compound microscope was used for samples that were too small to be seen clearly under a dissecting microscope. At a magnification of 100* immersion oil was used to enhance contras. Photographs were taken using a digital camera that was pressed to the eye piece of the microscope. The invertebrates were then identified at least to genus level and counted using a book by Michael Quigley (1977), titled invertebrates of streams and rivers identification key.

Hypothesis

- There are significant differences in abundance of epiphytic invertebrates among the three sites.
- There are significant differences in abundance of epiphytic invertebrates between the two seasons (winter and spring) at the three sites.
- There are significant differences in the abundance of epiphytic invertebrates between the two macrophyte types at the three sites
- There is significant interaction between location and season
- There is a significant interaction between site and season

There is significant interaction among location, site and season

Data analysis

Genstat 7.1 software was used to test the hypotheses listed above by analyzing the data using General ANOVA at 5% level of significance.

Results

The identification key that consisted of pictures with descriptions of organisms was used and photographs of the samples were taken for future reference. The interest for the study was on invertebrates that inhabit macrophytes, but sometimes other organisms like copepods were present in the water sample. This was possible because water from the dam was used for keeping and preserving the macrophyte samples containing the invertebrates.

As shown in Table 1 below, there were no significant differences in mean invertebrate counts among the sites (p=0.079)

Table 1: means for sites.

Site	Mean Invertebrate count
Outlet	30.1
Centre	38.1
Inlet	42.9
Overall mean	37.0
P-value	0.079
s.e.d.	5.13
l.s.d	11.17
Cv%	27.7

The results presented in Table 2 shows that *D. aegyptium* has significantly higher invertebrate counts (45.8) than *C.longus* (28.2), P=0.001.

Table 2: table of means for location (macrophyte type)

Location	Mean Invertebrate count
D.aegytpium	45.8
C. longus	28.2
Overall mean	37.0
p-value	0.001
s.e.d	4.19
l.s.d	9.12
Cv%	27.7

There are no significant differences in mean invertebrate counts between the season (p=0.124) (As shown in Table 3). The mean count for summer was 33.6 while that for winter was 40.5

Table 3: Table of means for Season	Mean Invertebrate count
Winter	40.5
Spring	33.6
Overall mean	37.0
p-value	0.124
s.e.d	4.19
l.s.d	9.12
Cv%	27.7

Based on the literature, the chironomidae species collected were identified to be ***Pseudosmittia sp*****, as seen in *Fig 2* below (image as viewed under a microscope and taken using a digital camera (**Michael, 1977)**.**

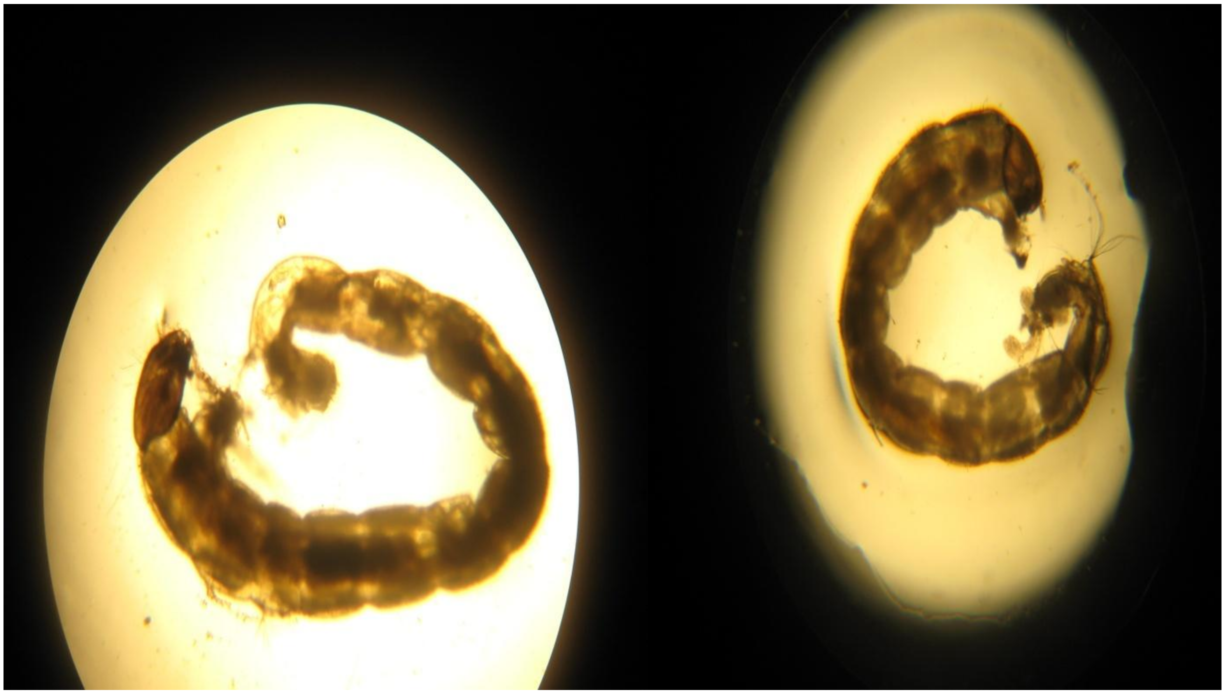

Figure 3: Photographs of ***Pseudosmittia*** *species.*

INVERTEBRATE COUNT FOR SEASON AND LOCATION

There is a significant interaction (p=0.033) between season and location. It is evident that in both seasons *D. aegyptium* has a higher count of invertebrates than the pointed grass. In spring the invertebrate counts are not very different, but in winter the *D. aegyptium* (54.3) has a higher count than the *C. lontus* (26.7). Graph drawn using appendix 4.

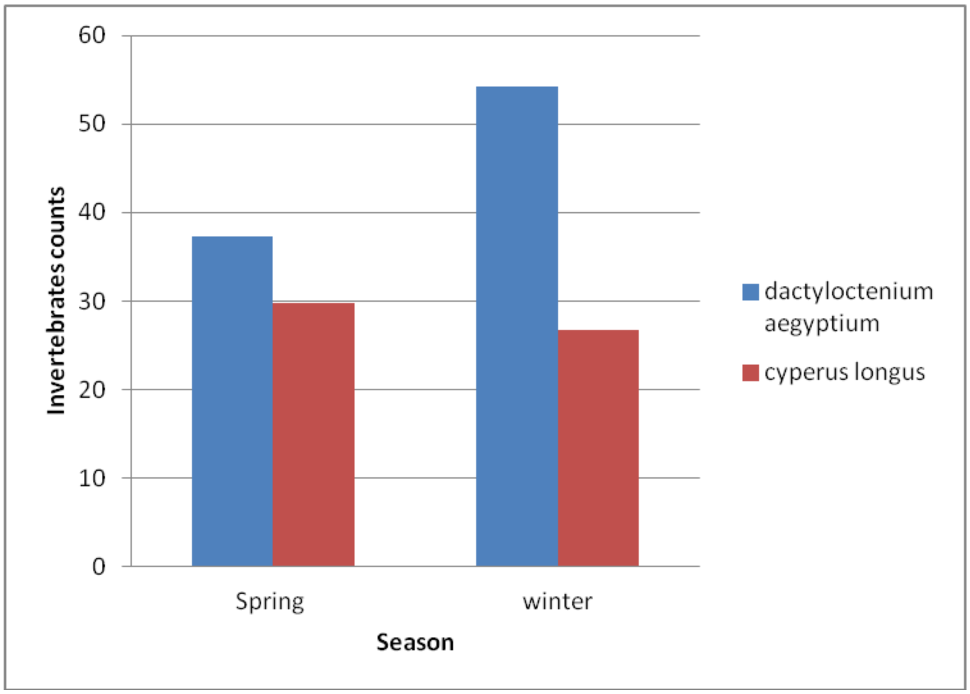

Figure 4: Invertebrates count on macrophyte against season.

INVERTEBRATE COUNT FOR SEASON AND SITE

There is no significant interaction (p=0.135) between season and site. For the site 2 and site 3, the invertebrate counts are high in winter, but the pattern is reserved for the site 1, instead the invertebrate counts are higher in spring (32.8) than in winter (27.5) but the difference is not statistically significant. Appendix 5, was used to draw the graph below.

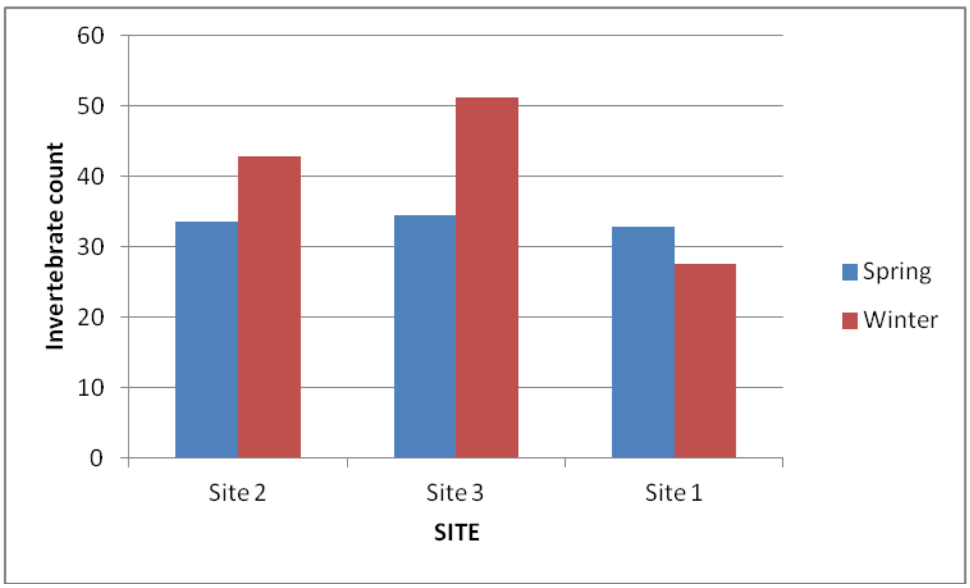

Figure 5: Invertebrate count with regard to season against site

INVERTEBRATE COUNT FOR SITE AND LOCATION

There is no significant interaction (p=0.221) between site and macrophyte type. *D. aegyptium* consistently high invertebrate counts across all the sites with inlet having a count of 56.5 compared to pointed grass at 29.2 at the same site. Appendix 6 was used to draw graph below.

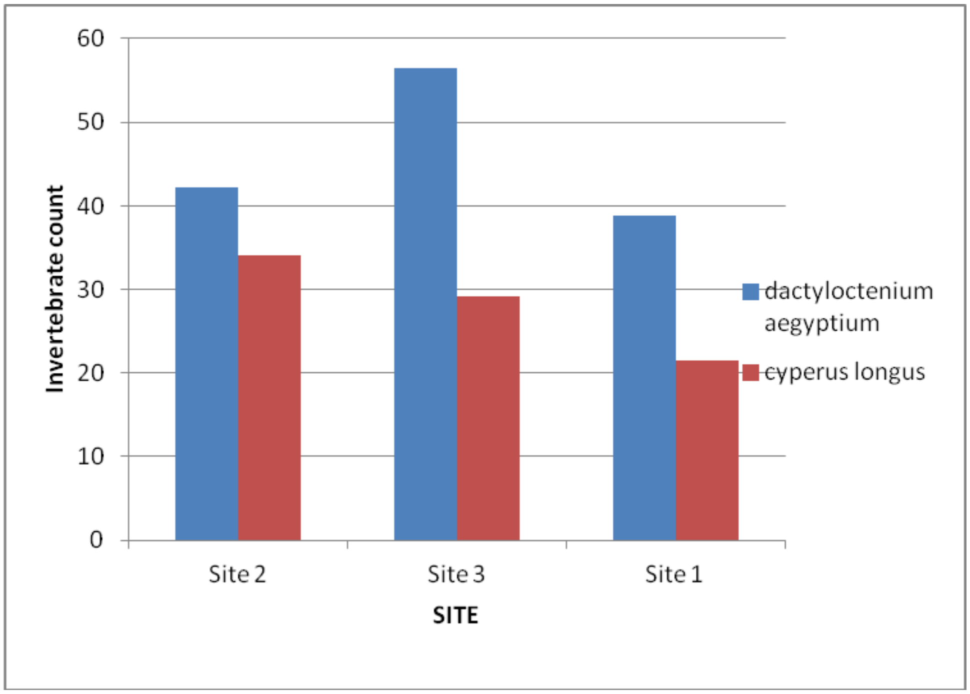

Figure 6: Invertebrate count (with regard to macrophyte type) against site

The results show no diversity in terms of epiphytic invertebrates from all the three sites as only one species was identified during the whole project as shown in Appendix 1. However, as shown in appendix 1, site 3 registered the highest value (70) of invertebrates for the sampling that took place on 7th June, 2010 while lowest figure of 41 invertebrates was recorded from site 2 on 4 September, 2010.

Discussion

The research project was aimed at identifying and quantifying the invertebrates associated with the two macrophyte speciee, *D. aegypteum* and *C. longus* as well as to look at the abundance of these invertebrates among the three chosen sites. In addition, factors that affect invertebrates' abundance, such as macrophyte type, season and site were studied as to how they affect abundance of epiphytic invertebrates.

Variations in invertebrate count are not significantly different among the sites (p=0.079), this would suggest uniformity in factors like nutrient load at the three sites because all the three sites have similar vegetation type. Statistically, no significant differences exist between winter and summer, (p=0.124) table 2. Spring is a transition between winter and summer, therefore it is not well pronounced in Namibia as it has variations in temperature such that sometimes it gets too cold inducing temperatures similar to winter. For example the lowest temperature was recorded in spring (17.9 ^{o}C) appendix 2, suggesting the minimal differences in invertebrates count between the two seasons, winter (40.5) and spring (33.6), table 2.

Macrophytes showed significant differences, (p=0.001) table 3. *D. aegyptium* generally has a big surface area because it forms a dense branch system as opposed to *C. longus* that is straight with a few leaves close to the water surface. Surface area is directly proportional to number of individuals on a substrate such that the bigger the area the more individuals are likely to be found since there is enough space to accommodate organisms, (McAbendroth et al., 2005). This is evident in the two given macrophyte *D. aegyptium* (45.8) versus *C. longus* (28.2). *D. aegyptium* lies on the floor of the dam meaning a higher percentage if not all the plant is in water, whereas for *C. longus* almost 40% of the plant is emergent. Therefore, *D. aegyptium* has an added advantage because chironomids prefer substrate that is submerged in water according to Azumi *et al*., (2004).

There is no interaction between site and macrophyte type (macrophyte type) (p=0.221) *fig. 4*, as the sites have similar type of vegetation is available. *D. aegyptium* has consistently high invertebrate counts across all the sites with site 3 having a count of 56.5 compared to *C. longus* at 29.2 at the same site. Meaning abundance on a particular macrophyte type is not influenced by site where the macrophytes grow.

Results show that there is a significant correlation (p=0.033) between season and macrophyte type It is evident that in both seasons *D. aegyptium* has a higher count of invertebrates than the *C. longus*. In spring the invertebrate counts are not very different, but in winter the *D. aegyptium* (54.3) has a higher count than the *C. longus* (26.7). Results for seasons and macrophyte type are significantly different (p=0.033). During spring, the water level drops as a result of evaporation since the temperatures are high, this was evident during sampling period as the shore line receded. *C. longus* were more affected because they grow tall, meaning they were exposed to excessive heat that kill algae found on the macrophyte reducing the food available to the invertebrates since algae is one of the major source of food for the invertebrates hence reduced numbers of invertebrate count (Azumi *et, al*., 2004). In *fig. 5*, *D. aegyptium* dominate for both seasons winter (54.3) and spring (37.3) as opposed to *C. longus*, winter (26.7) and spring (29.8).Season and site do not influence each other (p=0.135). This means invertebrates counts are independent of seasonal variations at all the three sites. However by looking at *fig. 6,* one would notice that centre and inlet sites, have high invertebrate counts in winter, but the pattern is reversed for outlet site, instead the invertebrate counts are higher in spring (32.8) than in winter (27.5) but the difference is not statistically significant. This would due to favourable conditions for the growth of macrophytes satisfying the shelter needs of invertebrates.

Conclusion

It is therefore important to investigate the occurrence of organisms, factors that affect their existence and the ecological importance of organisms. For example macrophyte type affect the abundance of organisms, meaning vegetation occurring in an area has an effect on the population of some organisms in this case chironomidae. From the study epiphytic invertebrates prefer submerged macrophytes because *D. aegyptium* (54.3) has a higher count than the *C. longus* (26.7) which is emergent macrophyte. They also used in preminarly study of water quality.. Turbid water for instance is an indication of polluted water with a lot of suspended solids. When the water is turbid emergent macrophyte dominate in that water body because light is reflected by suspended solids depriving submerged macrophytes of light for photosynthesis. If there is no photosynthesis going on in green plant it will eventually die as no more food is being produced for the growth and survival of the plant hence absence of submerged macrophytes in turbid waters (Christopher *et al*., 2004). Clear water support submerged macrophytes by allowing enough light penetrate the water column to facilitate photosynthesis (Christopher *et al*., 2004). Goreangab dam is dominated with emergent macrophytes entailing the risk of pollution of the dam. Chironomidae can help in the study of water quality because these organisms are associated with heavily polluted areas that would work as an alarm for more advanced studies to be done on a specific water body.

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Appendices

Appendix 1: Data collection form

#	Date	Site	season	Species	Location	InvertCount
1	07/06/2010	Inlet	winter	***Pseudosmittia***	pointed_ grass	33
2	07/06/2010	Inlet	winter	***Pseudosmittia***	lawn grass	70
3	07/07/2010	Inlet	winter	***Pseudosmittia***	pointed_ grass	35
4	07/07/2010	Inlet	winter	***Pseudosmittia***	lawn grass	67
5	04/09/2010	Inlet	Spring	***Pseudosmittia***	pointed_ grass	31
6	04/09/2010	Inlet	Spring	***Pseudosmittia***	lawn grass	51
7	02/10/2010	Inlet	Spring	***Pseudosmittia***	pointed_ grass	18
8	02/10/2010	Inlet	Spring	***Pseudosmittia***	lawn grass	38

9	07/06/2010	Centre	Winter	***Pseudosmittia***	pointed_ grass	27
10	07/06/2010	Centre	Winter	***Pseudosmittia***	lawn grass	61
11	07/07/2010	Centre	Winter	***Pseudosmittia***	pointed_ grass	27
12	07/07/2010	Centre	Winter	***Pseudosmittia***	lawn grass	56
13	04/09/2010	Centre	Spring	***Pseudosmittia***	pointed_ grass	30
14	04/09/2010	Centre	Spring	***Pseudosmittia***	lawn grass	11
15	02/10/2010	Centre	Spring	***Pseudosmittia***	pointed_ grass	52
16	02/10/2010	Centre	Spring	***Pseudosmittia***	lawn grass	41
17	07/06/2010	Outlet	Winter	***Pseudosmittia***	pointed_ grass	21
18	07/06/2010	Outlet	Winter	***Pseudosmittia***	lawn grass	35
19	07/07/2010	Outlet	Winter	***Pseudosmittia***	pointed_ grass	17
20	07/07/2010	Outlet	Winter	***Pseudosmittia***	lawn grass	37
21	04/09/2010	Outlet	Spring	***Pseudosmittia***	pointed_ grass	26
22	04/09/2010	Outlet	Spring	***Pseudosmittia***	lawn grass	55
23	02/10/2010	Outlet	Spring	***Pseudosmitti***	pointed_ grass	22

				a		
24	02/10/2010	Outlet	Spring	***Pseudosmittia***	lawn grass	28

Appendix 2: water parameter

Date	Site	Temperature	pH
07|06|10	1	18.1	8.0
	2	18.3	8.7
	3	18.0	7.12
07|07|10	1	24.89	7.21
	2	25.0	7.0
	3	23.33	6.87
04/09/10	1	18.9	6.48
	2	18.6	6.69
	3	19.9	6.12

02/10/10	1	22.5	9.23
	2	22.6	8.68
	3	17.9	8.68

Appendix 3: Computer output:

***** Analysis of variance *****

```
November 2010 12:41:01
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                 ________________________________________

                 GenStat Teaching Edition 1
                 GenStat Procedure Library Release PL15.2
                 ________________________________________

   1  %CD 'C:/Users/s/Documents'
   2  "Data taken from File: F:/PROJECTS 2010/Ben data 2010.xls"
   3  DELETE [Redefine=yes] _stitle_: TEXT _stitle_
   4  READ [print=*;SETNVALUES=yes] _stitle_
   8  PRINT [IPrint=*] _stitle_; Just=Left

 Data imported from Excel file: F:\PROJECTS 2010\Ben data 2010.xls
  on: 8-Nov-2010 12:41:30
  taken from sheet ""Sheet3"", cells A2:F25

   9  DELETE [redefine=yes] Number,Site,season,Species,Location,InvertCount
  10  UNITS [NVALUES=*]
  11  VARIATE [nvalues=24] Number
  12  READ Number
```

```
    Identifier   Minimum      Mean   Maximum    Values   Missing
        Number     1.000     12.50     24.00        24         0

  14  FACTOR [modify=yes;nvalues=24;levels=3;labels=!t('Centre','Inlet','Outlet')\
  15  ;reference=1] Site
  16  READ Site; frepresentation=ordinal

    Identifier    Values   Missing    Levels
          Site        24         0         3

  18  FACTOR [modify=yes;nvalues=24;levels=2;labels=!t('summer','winter')\
  19  ;reference=1] season
  20  READ season; frepresentation=ordinal

    Identifier    Values   Missing    Levels
        season        24         0         2

  22  FACTOR [modify=yes;nvalues=24;levels=1;labels=!t('Spaniotoma')\
  23  ;reference=1] Species
  24  READ Species; frepresentation=ordinal

    Identifier    Values   Missing    Levels
       Species        24         0         1

  26  FACTOR [modify=yes;nvalues=24;levels=2;labels=!t('dactyloctenium aegyptium',\
  27  'cyperus longus');reference=1] Location
  28  READ Location; frepresentation=ordinal

    Identifier    Values   Missing    Levels
      Location        24         0         2

  30  VARIATE [nvalues=24] InvertCount
  31  READ InvertCount

    Identifier   Minimum      Mean   Maximum    Values   Missing
   InvertCount     11.00     37.04     70.00        24         0

  33
  34  "General Treatment Structure (no Blocking)."
  35  BLOCK "No Blocking"
  36  TREATMENTS Site*Location*season
  37  COVARIATE "No Covariate"
  38  ANOVA [PRINT=aovtable,information,means,%cv; FACT=32; FPROB=yes;
PSE=diff,lsd,means;\
  39   LSDLEVEL=5] InvertCount
9..............................................................................

***** Analysis of variance *****

Variate: InvertCount

Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Site                       2      664.3      332.2    3.16  0.079
Location                   1     1855.0     1855.0   17.63  0.001
season                     1      287.0      287.0    2.73  0.124
Site.Location              2      361.3      180.7    1.72  0.221
Site.season                2      500.3      250.2    2.38  0.135
Location.season            1      610.0      610.0    5.80  0.033
Site.Location.season       2      576.3      288.2    2.74  0.105
Residual                  12     1262.5      105.2
Total                     23     6117.0
```

* MESSAGE: the following units have large residuals.

units 14	-15.0	s.e. 7.3
units 16	15.0	s.e. 7.3

***** Tables of means *****

Variate: InvertCount

Grand mean 37.0

Site	Centre	Inlet	Outlet
	38.1	42.9	30.1

Location	*dactyloctenium aegyptium*	*cyperus longus*
	45.8	28.2

season	summer	winter
	33.6	40.5

Site	Location	*dactyloctenium aegyptium*	*cyperus longus*
Centre		42.2	34.0
Inlet		56.5	29.2
Outlet		38.8	21.5

Site	season	summer	winter
Centre		33.5	42.8
Inlet		34.5	51.2
Outlet		32.8	27.5

Location	season	summer	winter
dactyloctenium aegyptium		37.3	54.3
cyperus longus		29.8	26.7

	Location	*dactyloctenium aegyptium*		*cyperus longus*	
Site	season	summer	winter	summer	winter
Centre		26.0	58.5	41.0	27.0
Inlet		44.5	68.5	24.5	34.0
Outlet		41.5	36.0	24.0	19.0

*** Standard errors of means ***

Table	Site	Location	season	Site Location
rep.	8	12	12	4
d.f.	12	12	12	12
e.s.e.	3.63	2.96	2.96	5.13

Table	Site season	Location season	Site Location season
rep.	4	6	2
d.f.	12	12	12
e.s.e.	5.13	4.19	7.25

*** Standard errors of differences of means ***

Table	Site	Location	season	Site Location

```
rep.                      8          12          12           4
d.f.                     12          12          12          12
s.e.d.                 5.13        4.19        4.19        7.25

Table                  Site    Location        Site
                     season      season    Location
                                             season
rep.                      4           6           2
d.f.                     12          12          12
s.e.d.                 7.25        5.92       10.26

*** Least significant differences of means (5% level) ***

Table                  Site    Location      season        Site
                                                       Location
rep.                      8          12          12           4
d.f.                     12          12          12          12
l.s.d.                11.17        9.12        9.12       15.80

Table                  Site    Location        Site
                     season      season    Location
                                             season
rep.                      4           6           2
d.f.                     12          12          12
l.s.d.                15.80       12.90       22.35


***** Stratum standard errors and coefficients of variation *****

Variate: InvertCount

   d.f.          s.e.         cv%
     12         10.26        27.7

  40  APLOT [RMETHOD=simple] fitted,normal,halfnormal,histogram
  41  AGRAPH [METHOD=means]
```

Appendix 4: table of mean for season in relation to macrophyte type

	dactyloctenium aegyptium	*cyperus longus*
Summer	37.3	29.8
Winter	54.3	26.7

Appendix 5: table of means for season in relation to site

	summer	winter
centre	33.5	42.8

inlet	34.5	51.2
outlet	32.8	27.5

Appendix 6: table of means for macrophyte type

	dactyloctenium aegyptium	*cyperus longus*
centre	42.2	34
inlet	56.5	29.2
outlet	38.8	21.5